# Fluorinated amino acids and peptides. Synthesis of 3,3-difluoro-2-amino acids, peptides and cyclodipeptides incorporating 3,3-difluoro-2-aminobutyric acid or 3,3-difluorophenylalanine residues in their structures

A. I. Ayi*, M.-L. Martos-Alcaniz, R. Condom, P. R. T. Frogier and R. Guedj

*Laboratoire de Chimie Bio-Organique, Université de Nice-Sophia Antipolis, Parc Valrose, F-06034 Nice Cedex (France)*

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## Abstract

Convenient syntheses have been developed for *N*-9-fluorenylmethyloxycarbonyl-DL-2-amino-3,3-difluoro-butanoic acid and *N*-9-fluorenylmethyloxycarbonyl-DL-3,3-difluoro-phenylalanine which have been successfully introduced into peptide syntheses. Several fluorinated peptides have been obtained either by active ester coupling methods, mixed anhydride synthesis or with the aid of dicyclohexylcarbodiimide (DCC) in presence of 1-hydroxybenzotriazole (HOBt). The method has been applied to the preparation of seven hitherto unpublished representative 3-(1,1'-difluoroalkyl)-2,5-dioxopiperazines for biological purposes and structural chemistry studies.

## Introduction

Amino acids and peptides are derivatives of primary importance, both as enzymatic inhibitors [1–4] and as tools in the study of protein function or structure [5–7]. Although some recent studies have demonstrated the interesting roles of peptides with a monofluorinated amino acid residue included in their structure [8–11], there has been little effort to study the preparation of these compounds. On the other hand, only one limited route has been suggested in the literature for the preparation of dipeptides incorporating a 3,3-difluoro-2-amino acid residue [10]. The lack of convenient routes to mono- or di-fluorinated peptides is probably due to the difficulty of access to the starting fluorinated amino acids. Indeed, until recent years, the procedures described for the synthesis of 3-fluoro-amino acids were quite involved (use of highly toxic reagents) and/or not really suitable. Early work in our laboratory [12–14] involved the synthesis and biological evaluation of several 3-fluoro- and 3,3-difluoro-amino acids or their derivatives.

Because we found that 3,3-difluoro-amino esters could be prepared in good yields, it seemed to us of interest to evaluate their reactivity in the synthesis of peptides. Furthermore, 2,5-dioxopiperazines have been known

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for a long time [15], and over the years have served in structural chemistry as models in peptide structure studies and conformational analysis of the piperazinedione ring.

The aim of the present study is to report new routes to these important classes of organic compounds, peptides including a 3,3-difluorinated-2-amino acid residue in their structure, together with some 3-(1,1'-difluoroalkyl)-2,5-dioxopiperazines.

## Experimental

$^1H$ and $^{19}F$ NMR spectra were recorded on $CDCl_3$ or DMSO-$d_6$ solutions using Bruker spectrometers, models AC 200 E(200 MHz), WP-90 (84,67 MHz) and/or WP-80 (80 MHz). Signal positions are given in ppm, with tetramethylsilane or trichlorofluoromethane as internal standards. Infrared (IR) spectra were measured on KBr discs using a Bruker FT-IFS spectrometer. Microanalyses were performed by Le Service de Microanalyse du C.N.R.S., Vernaison, France. Silica gel chromatography was performed on 70–230 Grade 60 mesh ASTM silica gel (Merck) and TLC on silica gel 60 F-254 glass plates (Merck).

The solvents employed were freshly distilled from the following materials:ethyl acetate–phosphorus pentoxide; methylene chloride–calcium chloride; dimethyl formamide–calcium oxide; tetrahydrofuran–potassium hydroxide and lithium aluminium hydride. Unfluorinated $N^{\alpha}$-Boc amino acids were purchased from Fluka Chemie AG (Switzerland); pyridinium polyhydrofluoride and all other reagents were purchased from Aldrich Chemical Co. and used without further purification.

3,3-Difluoro amino esters were obtained by the method we have previously described [12].

*General procedure for the synthesis of alkyl* $N^{\alpha}$*-Fmoc fluoro amino esters*

Alkyl 3,3-difluoro-2-amino ester was dissolved in dioxan and *N*-methylmorpholine (1 equiv.) and treated with 9-fluorenylmethyl chloroformate (FmocCl) at 0 °C and then at room temperature for 6–18 h. The product was isolated after extraction with ethyl acetate and recrystallization from diethyl ether.

*N-9-fluorenylmethyloxycarbonyl-DL-2-amino-3,3-difluoro-butanoic methyl ester*

$\delta_H$ ($CDCl_3$): 7.71–7.16 (m, 8H, Fmoc), 6.3 (d, 1H, $^3J_{HH}=8$, NHFmoc), 4.54–4.1 (m, 4H, $CHCF_2$ and $CH_2CH$ (Fmoc)), 3.78 (s, 3H, $OCH_3$), 1.66 (t, 3H, $^3J_{FH}=18.7$, $CH_3CF_2$); $\delta_F$: −98.74 (dm, 2F, $^3J_{FCH_3}=18.7$, $^3J_{FH}=12.9$); *m/z* 178 ($C_{13}H_9^+$, 100%), 94 ($C_3H_6F_2N^+$, 10%), 65 ($CH_3CF_2^+$, 67%).

*N-9-fluorenylmethyloxycarbonyl-DL-3,3-difluoro-phenylalanyl methyl ester*

$\delta_H$ ($CDCl_3$): 7.78–7.31 (m, 13H, Phe and Fmoc), 5.64 (d, 1H, $^3J_{HH}=9.9$, NH (Fmoc)), 5.07 (q, 1H, $^3J_{FH}=11.7$, $^3J_{FH}=12.9$, $^3J_{HH}=9.9$, $CHCF_2$), 4.11–4.43 (m, 3H, $CH_2CH$(Fmoc)) 3.72 (s, 3H, $OCH_3$); $\delta_F$: −102.68 and −104.08 (2dd, $^2J_{FF}=248$, $^3J_{FH}=12$, $^3J_{FH}=14$); *m/z* 438 ($M^+$, 2%), 178 ($C_{13}H_9^+$, 100), 127 ($C_6H_5CF_2^+$, 46).

*General procedure for the synthesis of* $N^{\alpha}$*-Fmoc fluoro amino acids (1, 2)*

$N^{\alpha}$-Fmoc fluoro amino ester (10 mmol) in 40 ml of a mixture of dioxan and water (1/1 v/v) was treated with 30 ml of an aqueous hydrochloric acid solution (6 M) under reflux for 5 h. The solvents were evaporated and the solid residue dissolved in water. The pH was adjusted by the addition of aqueous diluted ammonia. The resulting mixture was washed with diethyl ether. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The organic layers were dried with $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude solid residue was purified by crystallization in $CHCl_3$/n-hexane.

*N-9-fluorenylmethyloxycarbonyl-DL-2-amino-3,3-difluoro butanoic acid (1) (nc)*

Yield 71%; $\delta_H$ (DMSO-$d_6$): 7.67–7.2 (m, 8H, Fmoc), 6.01 (d, 1H, $^3J_{HH}=9.1$, NHFmoc), 4.58 (m, 1H, $^3J_{FH}=12.6$, $^3J_{HH}=9.1$, $CHCF_2$), 4.38–4.09 (m, 3H, $CH_2CH$ (Fmoc)), 1.64 (t, 3H, $^3J_{FH}=18.9$, $CH_3CF_2$); $\delta_F$: −90.10 and −98.10 (2dm, $^2J_{FF}=246.3$, $^3J_{FCH_3}=18.9$, $^3J_{FH}=12.9$). Analysis. Calcd. C, 63.16; H, 4.74; F, 10.52%; found: C, 63.12; H, 4.76; F 10.49%.

*N-9-fluorenylmethyloxycarbonyl-DL-3,3-difluoro-phenylalanine (2) (nc)*

Yield 67%; $\delta_H$ (DMSO-$d_6$): 7.78–7.27 (m, 13H, Phe and Fmoc), 5.65 (d, 1H, $^3J_{HH}=9.6$, NH (Fmoc)), 5.14 (m, 1H, $^3J_{FH}=11.8$, $^3J_{FH}=16.8$, $^3J_{HH}=9.6$, $CHCF_2$), 4.4–4.1 (m, 3H, $CH_2CH$ (Fmoc)); $\delta_F$: −98.3 and −100.1 (2dd, $^2J_{FF}=247$, $^3J_{FH}=16.8$, $^3J_{FH}=11.8$). Analysis: Calcd. C, 68.08; H, 4.52; F 8.97%; found: C, 68.12; H, 4.49; F 8.95%.

*General procedure for the synthesis of peptides (3–11)*

The coupling of the fluorinated amino esters with the $N^{\alpha}$-protected unfluorinated amino acids or peptides was achieved via the cyanomethyl or *p*-nitrophenyl ester (method A) [15, 16], with the aid of dicyclohexylcarbodiimide in presence of 1-hydroxybenzotriazole (method B) [17] or by a mixed anhydride synthesis (method C) [18]. The products were purified by chromatography on silica gel (eluent methylene dichloride–ethyl acetate).

*N-(t)-butyloxycarbonyl-glycyl-DL-3,3-difluoro-phenylalanyl methyl ester (3) (nc)*

10 mmol of *N*-t-butyloxycarbonyl-glycine cyanomethyl ester in distilled ethyl acetate were added to 10 mmol of racemic 3,3-difluoro-phenylalanine

methyl ester and the mixture was stirred at room temperature for 72 h. The product was purified by recrystallization in diethyl ether. Yield 72%; $\delta_H(CDCl_3)$: 7.39 (m, 5H, Phe), 7.08 (d, 1H, $^3J_{HNCH}=8.9$, CONH (Phe)), 5.32 (ddd, 1H, $^3J_{FH}=13$, $^3J_{HNCH}=8.9$, $CHCF_2$), 5.14 (t, 1H, $^3J_{HNCH}=1.6$, NHBoc), 3.78 (m, 2H, $CH_2$ (Gly)), 3.67 (s, 3H, $OCH_3$), 1.42 (s, 9H, $(CH_3)_3C$); $\delta_F$: −102.29 and −103.81 (2dm, $^2J_{FF}=247$, $^3J_{FH}=13$). Analysis: Calcd. C, 54.83; H, 5.96; F, 10.2%; found: C, 54.85; H, 5.92; F, 10.17%.

Compound (**3**), was obtained in the same manner starting from the *N*-t-butyloxycarbonyl-glycine *p*-nitrophenyl ester (method A). Compound (**4**) was obtained by the above methods.

*N-t-butyloxycarbonyl-glycyl-DL-2-amino-3,3-difluoro-butanoic methyl ester (4) (nc)*

Yield 53%; $\delta_H$ ($CDCl_3$): 6.96 (d, 1H, $^3J_{HNCH}=9.5$, NHCO), 5.13 (m, 1H, NHBoc), 5.04 (dt, 1H, $^3J_{FH}=12.4$, $^3J_{HCNH}=9.5$, $CHCF_2$), 3.92 (dd, 1H, $^2J_{HH}=16.9$, $^3J_{CHNH}=5.80$, $CH_2{}^1$(Gly)), 3.84 (dd, 1H, $^2J_{HH}=16.9$, $^3J_{HNCH}=5.80$, $CH_2{}^2$(Gly)), 3.83 (s, 3H, $OCH_3$), 1.72 (t, 3H, $^3J_{FCH_3}=19.8$, $CH_3$), 1.48 (s, 9H, $(CH_3)_3C$); $\delta_F$: −98.67 and −98.8 (2dm, $^2J_{FF}=245$, $^3J_{FH}=19.8$, $^3J_{FH}=12.4$). Analysis: Calcd. C, 46.45; H, 6.5; F 12.25%; found: C, 46.5; H, 6.52; F, 12.1%.

*N-t-butyloxycarbonyl-L-tyrosyl-DL-2-amino-3,3-difluoro-butanoic methyl ester (5) (nc)*

To a mixture of 10 mmol of *N*-t-butyloxycarbonyl-tyrosine, 10 mmol of 2-amino-3,3-difluoro-butanoic methyl ester and *N*-methylmorpholine (1 equiv.) in 10 ml of DMF was added hydroxybenzotriazole (1 equv.). The mixture was stirred and cooled to 0 °C and then 1.1 equiv. of dicyclohexylcarbodiimide was added. The resulting mixture was stirred at room temperature for 12 h and 0.5 ml of acetic acid was added. The dicyclohexyl urea which precipitated was filtered off. The crude compound obtained after extraction with ethyl acetate was purified by chromatography on silica gel. Yield 60%; $\delta_H(CDCl_3)$ **5a**: 7.54 (m, 1H, NHCO), 6.88 (2d, 4H, Tyr), 5.25 (m, 2H, NHBoc and $CHCF_2$), 4.41 (m, 1H, CH(Tyr)), 3.71 (s, 3H, $OCH_3$), 3.0 (m, 2H, $CH_2$(Tyr)), 1.6 (m, 3H, $^3J_{FH}=18.8$, $^3J_{FH}=12.9$, $CH_3$); $\delta_F$: −97.67 and −97.82 (2dm, $^2J_{FF}=248$, $^3J_{FH}=18.8$, $^3J_{FH}=12.9$); **5b**: $\delta_F$: −98.7 and −100.2 (2dm, $^2J_{FF}=248$, $^3J_{FH}=18.8$, $^3J_{FH}=12.9$). Analysis: Calcd. C, 54.8; H, 6.29; F, 9.12%; found: C, 54.91; H, 6.25; F, 9.1%.

Compounds (**3–6**) were obtained in the same manner (method B).

*N-t-butyloxycarbonyl-L-proly-DL-2-amino-3,3-difluoro-butanoic methyl ester (6) (nc)*

Yield 64%; $\delta_H$ ($CDCl_3$) **6a**: 6.82 (d, 1H, $^3J_{NHCH}=9.6$, NHCO), 5.0 (dt, 1H, $^3J_{HF}=13.3$, $^3J_{NHCH}=9.6$, $CHCF_2$), 4.33 (m, 1H, CH(Pro)), 3.81 (s, 3H, $OCH_3$), 3.48 (m, 2H, $CH_2{}^{\delta}$(Pro)), 2.15 (m, 2H, $CH_2{}^{\beta}$(Pro)), 1.90 (m, 2H, $CH_2{}^{\gamma}$(Pro)), 1.69 (t, 3H, $^3J_{CH_3F}=18{,}8$, $CH_3$), 1.47 (s, 9H, $(CH_3)_3C$). $\delta_F$: −96.67 and −98.63 (2md, $^2J_{FH}=248$, $^3J_{CH_3F}=18.8$, $^3J_{HF}=13.3$); **6b**: $\delta_H$: 6.85 (d,

1H, $^3J_{NHCH}=9.6$, NHCO), 5.0 (dt 1H, $^3J_{FH}=13.3$, $^3J_{NHCH}=9.6$, $CHCF_2$), 4.33 (m, 1H, CH(Pro)), 3.81 (s, 3H, $OCH_3$), 3.48 (m, 2H, $CH_2^{\delta}$(Pro)), 2.15 (m, 2H, $CH_2^{\beta}$(Pro)), 1.90 (m, 2H, $CH_2^{\gamma}$(Pro)), 1.72 (t, 3H, $^3J_{CH_3F}=18.8$, $CH_3CF_2$), 1.47 (s, 9H, $(CH_3)_3C$); $\delta_F$: −97.70 and −99.24 (2dm, $^2J_{FH}=248$, $^3J_{CH_3F}=18.8$, $^3J_{FH}=13.3$); *m/z* 351 (M+H, 100%), 251(34), 94(4). Analysis: Calcd. C, 51.4; H, 6.91; F, 10.85%; found: C, 51.5; H, 6.87; F, 10.8%.

*N-9-fluorenylmethyloxycarbonyl-glycyl-DL-3,3-difluoro-phenylalanyl methyl ester (7) (nc)*

To a mixture of 10 mmol *N*-9-fluorenylmethyloxycarbonyl-glycine and 10 mmol of *N*-methylmorpholine in dry THF cooled to −15 °C, were added with stirring, 11 mmol of isobutyl chloroformate (10 mmol) and 3,3-difluoro-phenylalanine methyl ester (10 mmol) dissolved in 5 ml of DMF. The reaction mixture was allowed to warm to room temperature with continuous stirring and kept for 5 h at this temperature. The resulting solid residue was removed by filtration. The crude compound, obtained after extraction with ethyl acetate, was purified by chromatography on silica gel and recrystallized (diethyl ether). Yield 70%; $\delta_H(CDCl_3)$: 7.7–7.3 (m, 13H, Phe and Fmoc), 6.58 (d, 1H, $^3J_{NHCH}=8.9$,m NH(Phe)), 5.42 (m, 1H, $^3J_{NHCH}=8.5$, NH (FMoc)), 5.24 (ddd, 1H, $^3J_{FH}=13$, $^3J_{NHCH}=8.9$, $CHCF_2$), 4.25 (m, 2H, $CH_2$ (Fmoc)), 4.12 (m, 2H, $CH_2$(Gly), 3.61 (s, 3H, $OCH_3$); $\delta_F$: −101.89 and −102.76 (2dm, $^2J_{FF}=246$, $^3J_{FH}=13$). Analysis: Calcd. C, 65.58; H, 4.89; F, 7.68%; found: C, 65.6; H, 4.85; F, 7.65%.

Compounds (**8–11**) were obtained in the same manner (method C).

*N-t-butyloxycarbonyl-L-phenylalanyl-DL-3,3-difluoro-phenylalanine methyl ester (8) (nc)*

Yield 68%; $\delta_H$ ($CDCl_3$) **8a**: 7.22 (m, 10H, Phe); 6.82 (d, 1H, $^3J_{NHCH}=9.3$, CONH(Phe(F)$_2$); 5.29 (ddd, 1H, $^3J_{FH}=12.5$, $^3J_{NHCH}=9.3$, $CH^{\alpha}CF_2$); 4.97 (m, 1H, NHBoc); 4.34 (dd, 1H, $^3J_{HH}=6.4$, $CH^{\alpha}$); 3.64 (s, 3H, $OCH_3$); 3.0 (m, 2H, $^2J_{HH}=13.9$, $^3J_{HH}=6.4$, $CH_2^{\beta}$); 1.4 (s, 9H, $(CH_3)_3C$ Boc); $\delta_F$: −106.2 and −107.8 (2dm, $^2J_{FF}=248$, $^3J_{FH}=12.5$); **8b**: $\delta_F$: −107.3 and −108.9; *m/z* 463 (M+H, 9%), 127 ($C_7H_5F_2$, 65%). Analysis. Calcd. C, 62.33; H, 6.1; F, 8.22%; found: C, 62.4; H, 6.08; F, 8.2%.

*N-t-butyloxycarbonyl-L-phenylalanyl-DL-2-amino-3,3-difluoro-butanoic methyl ester (9) (nc)*

Yield 66%; $\delta_H$ ($CDCl_3$) **9a**: 7.24 (m, 5H, Phe), 7.05 (d, 1H, $^3J_{HNCH}=9.3$, CONH), 5.14 (m, 1H, HNBoc), 4.96 (m, 1H, $^3J_{HF}=12.9$, $^3J_{HNCH}=9.3$, $CHCF_2$), 4.40 (m, 1H, CH(Phe)), 3.77 (s, 3H, $OCH_3$), 3.13 and 2.99 (2dd, 2H, $^2J_{HH}=13.9$, $^3J_{HH}=7.6$, $^3J_{HH}=6.5$, $CH_2$(Phe)), 1.65 (t, 3H, $^3J_{CH_3F}=18.8$, $CH_3$), 1.39 (s, 9H, $(CH_3)_3C$); $\delta_F$: −96.96 and −98.63 (2dm, $^2J_{FF}=247$, $^3J_{FH}=18.8$, $^3J_{FH}=12.9$); **9b**: $\delta_H$: 7.24 (m, 5H, Phe), 7.05 (d, 1H, $^3J_{HNCH}=9.3$, CONH), 5.14 (m, 1H, NHBoc), 4.98 (m, 1H, $^3J_{HF}=13.8$, $^3J_{HNCH}=9.3$, $CHCF_2$), 4.40 (m, 1H, CH(Phe)), 3.76 (s, 3H, $OCH_3$), 3.12 and 3.05 (2dd, 2H, $^2J_{HH}=13.9$, $^3J_{HH}=7.6$, $^3J_{HH}=6.5$, $CH_2$(Phe)), 1.57 (t, 3H, $^3J_{CH_3F}=18.8$, $CH_3$), 1.40 (s,

9H, $(CH_3)_3C$); $\delta_F$: −97.06 and −98.49 (2dm, $^2J_{FF}=247$, $^3J_{FH}=18.8$, $^3J_{FH}=13.8$). Analysis. Calcd. C, 56.99; H, 6.54; F, 9.49%; found: C, 57.1; H, 6.5; F, 9.45%.

*N-t-butyloxycarbonyl-L-alanyl-DL-2-amino-3,3-difluoro-butanoic methyl ester (**10**) (nc)*

Yield 62%. Analysis: Calcd. C, 48.14; H, 6.84; F, 11.72%; found: C 48.2; H 6.82; F 11.7%; $\delta_H$ ($CDCl_3$) **10a**: 7.14 (d, 1H, $^3J_{HNCH}=9.3$, CONH), 5.05 (d, 1H, $^3J_{HNCH}=6$, BocNH), 5.01 (td, 1H, $^3J_{HF}=12$, $^3J_{HNCH}=9.3$, $CHCF_2$), 4.47 (m, 1H, CH(Ala)), 3.81 (s, 3H, $OCH_3$), 1.71 (t, 3H, $^3J_{CH_3F}=18.8$, $CH_3$), 1.45 (s, 9H, $(CH_3)_3C$), 1.38 (d, 3H, $^3J_{HH}=7.1$, $CH_3$(Ala)); $\delta_F$: −97.20 and −98.59 (2dm, $^2J_{FF}=247$, $^3J_{FH}=18.8$, $^3J_{FH}=12$); **10b**: $\delta_H$: 7.17 (d, 1H, $^3J_{HNCH}=9.3$, CONH), 5.05 (d, 1H, $^3J_{HNCH}=6$, BocNH), 5.01 (td, 1H, $^3J_{HF}=12$, $^3J_{HNCH}=9.3$, $CHCF_2$), 4.51 (m, 1H, CH(Ala)), 3.81 (s, 3H, $OCH_3$), 1.69 (t, 3H, $^3J_{CH_3F}=19$, $CH_3$); 1.45 (s, 9H, $(CH_3)_3C$), 1.35 (d, 3H, $^3J_{HH}=7.1$, $CH_3$(Ala)); $\delta_F$: −97.21 and −99.09 (2dm, $^2J_{FF}=248$, $^3J_{FH}=18.8$, $^3J_{FH}=12$); *m/z* 325 (M+H, 17%); 94 ($C_3H_6F_2N$, 33%).

*N-t-butyloxycarbonyl-L-alanyl-DL-3,3-difluoro-phenylalanyl methyl ester (**11**) (nc)*

Yield 74%; $\delta_H$ ($CDCl_3$) **11a**: 7.45 (m, 5H, Phe), 7.23 (m, 1H, CONH), 5.33 (ddd, 1H, $^3J_{FH}=12.7$, $^3J_{NHCH}=9.6$, $CHCF_2$), 5.11 (d, 1H, $^3J_{NHCH}=7.5$, NHBoc), 4.21 (m, 1H, CH(Ala)), 3.68 (s, 3H, $OCH_3$), 1.46 (s, 9H, $(CH_3)_3C$), 1.24 (d, 3H, $^3J_{CH_3F}=6.4$, $CH_3$(Ala)); $\delta_F$: −101.72 and −104.58 (2dm, 1F, $^2J_{FF}=248$, $^3J_{FH}=9$, $^3J_{FH}=12.7$); **11b**: $\delta_H$: 7.45 (m, 5H, $C_6H_5$), 7.23 (m, 1H, CONH), 5.33 (ddd, 1H, $^3J_{FH}=12.7$, $^3J_{NHCH}=9.6$, $CHCF_2$), 5.01 (d, 1H, $^3J_{NHCH}=7.6$, NHBoc), 4.11 (m, 1H, CH(Ala)), 3.68 (s, 3H, $OCH_3$), 1.44 (s, 9H, $(CH_3)_3C$), 1.20 (d, 3H, $^3J_{CH_3CH}=6.1$, $CH_3$(Ala)); $\delta_F$: −102.39 and −103.77 (2dm, $^2J_{FF}=247$, $^3J_{FH}=13$, $^3J_{FH}=12$). Analysis: Calcd. C, 55.95; H, 6.26; F, 9.83%; found: C, 56.0; H, 6.24; F, 9.8%.

*General procedure for the synthesis of peptides (**12–15**)*

The peptides **12–15** were synthesized starting from unfluorinated amino esters or peptides and $N^{\alpha}$-Fmoc 3,3-difluoro amino acids by methods B or C as described above. They were isolated in good yields after purification by column chromatography or recrystallization.

*N-9-fluorenylmethyloxycarbonyl-DL-3,3-difluoro-phenylalanyl-glycyl methyl ester (**12**) (nc)*

Yield 80%; $\delta_H$ ($CDCl_3$). 7.7–7.3 (m, 13H, Phe and Fmoc), 6.62 (m, 1H, CONH), 5.87 (d, 1H, $^3J_{NHCH}=8.9$, NHFmoc), 5.03 (m, 1H, $^3J_{FH}=14$, $^3J_{FH}=8.4$, $^3J_{NHCH}=8.9$, $CHCF_2$), 4.27 (m, 3H, $CH_2CH$(Fmoc)), 4.26 (q, 2H, $OCH_2$), 4.20 (m, 2H, $CH_2$(Gly)), 1.31 (t, 3H, $CH_3$); $\delta_F$: −101.75 and −104.12 (2dm, $^2J_{FF}=250$, $^3J_{FH}=14$, $^3J_{FH}=8.4$). Analysis: Calcd. C, 65.58; H, 4.89; F, 7.68%; found: C, 65.6; H, 4.9; F, 7.65%.

*N-9-fluorenylmethyloxycarbonyl-DL-3,3-difluoro-phenylalanyl-L-methionyl methyl ester (**13**) (nc)*

Yield 80%; $\delta_H$ ($CDCl_3$) **13a**: 7.8–7.2 (m, 13H, Phe and Fmoc), 6.85 (d, 1H, $^3J_{NHCH}=6.8$, CONH), 5.86 (d, 1H, $^3J_{NHCH}=8.5$, NHFmoc), 5.01 (m, 1H, $CHCF_2$) 4.71 (m, 1H, CH(Met)), 4.25–4.09 (m, 3H, $CH_2CH$(Fmoc), 3.75 (s, 3H, $OCH_3$), 2.43 (m, 2H, $SCH_2$(Met)), 2.01 (s, 3H, $SCH_3$); 1.98 (m, 2H, $CH_2$(Met)); $\delta_F$ −100.49 and −103.73 (dm, $^2J_{FF}=249$, $^3J_{FH}=13.5$, $^3J_{FH}=9.6$); **13b**: $\delta_H$: 7.8–7.2 (m, 13H, Phe and Fmoc); 6.75 (d, 1H, $^3J_{NHCH}=8.2$, CONH), 5.82 (d, 1H, NHFmoc), 4.38 (m, 1H, $CHCF_2$), 4.72 (m, 1H, CH(Met)), 4.25–4.09 (m, 3H, $CH_2CH$(Fmoc)), 3.75 (s, 3H, $OCH_3$), 2.43 (m, 2H, $SCH_2$(Met)), 2.01 (s, 3H, $SCH_3$), 1.98 (m, 2H, $CH_2$(Met); $\delta_F$: −100.70 and −104.29 (dm, $^2J_{FF}=250$, $^3J_{FH}=15$, $^3J_{FH}=9.6$). Analysis: Calcd. C, 63.37; H, 5.32; F, 6.68%; found: C, 63.4; H, 5.3; F, 6.6%.

*N-9-fluorenylmethyloxycarbonyl-DL-2-amino-3,3-difluoro-butanoyl-glycyl-L-phenylalanyl-L-methionyl methyl ester (**14**) (nc)*

The reaction led to two diastereoisomers which were identified by $^{19}F$ NMR spectroscopy performed on the crude product. Yield 50%; *m/z* 711 (M+H); $\delta_H$ ($CDCl_3$); N-*9*-fluorenylmethyloxycarbonyl-*DL-2*-amino-*3,3*-difluoro-butanoyl: 7.7–7.1 (m, 8H, Fmoc), 4.2 (m, 3H, $CH_2CH$(Fmoc)), 3.62 (m, 1H, $CF_2CH$), 1.55 (t, 3H, $CH_3CF_2$); glycyl: 5.87 (d, 1H, $^3J_{HNCH}=8.4$, NH), 3.9 (dd, 1H, $^2J_{HH}=16.2$, $^3J_{HNCH}=6.4$, $CH_2$), 3.7 (dd, 1H, $^2J_{HH}=16.2$, $^3J_{HNCH}=6.4$, $CH_2$); *L*-phenylalanyl: 8.03 (d, 1H, $^3J_{HNCH}=8.4$, NH), 7.25 (m, 5H, $C_6H_5$), 4.92 (m, 1H, CH), 3.1–2.8 (m, 2H, $CH_2$); *L*-methionyl methyl ester: 8.06 (d, 1H, $^3J_{HNCH}=7.9$, NH), 4.5 (m, 1H, CH), 3.65 (s, 3H, $OCH_3$), 2.4 (dd, 2H, $SCH_2$), 2.05 (s, 3H, $SCH_3$), 2.0–1.9 (m, 2H, $CH_2$); $\delta_F$: two AB systems centred at −93.1 and −96.3 after irradiation of the protons.

*N-9-fluorenylmethyloxycarbonyl-DL-2-amino-3,3-difluoro-butanoyl-glycyl-L-phenylalanyl-L-leucyl methyl ester (**15**)*

Yield 55%; *m/z* 693 (M+H); $\delta_H$ ($CDCl_3$): N-*9*-fluorenylmethyloxycarbonyl-*DL-2*-amino-*3,3*-difluoro-butanoyl: 7.7–7.1 (m, 8H, Fmoc), 4.2 (m, 3H, $CH_2CH$(Fmoc)), 3.62 (m, 1H, $CF_2CH$), 1.55 (t, 3H, $CH_3CF_2$); glycyl: 5.87 (d, 1H, $^3J_{HNCH}=8.4$, NH), 3.9 (dd, 1H, $^2J_{HH}=16.2$, $^3J_{HNCH}=6.4$, $CH_2$), 3.7 (dd, 1H, $^2J_{HH}=16.2$, $^3J_{HNCH}=6.4$, $CH_2$); *L*-phenylalanyl: 8.03 (d, 1H, $^3J_{HNCH}=8.4$, NH), 7.25 (m, 5H, $C_6H_5$), 4.92 (m, 1H, CH), 3.1–2.8 (m, 2H, $CH_2$); *L*-leucyl methyl ester: 8.06 (d, 1H, $^3J_{HNCH}=7.9$, NH), 4.5 (m, 1H, CH), 3.65 (s, 3H, $OCH_3$), 1.65 (m, 3H, $CH_2CH$), 0.89 (m, 6H, $(CH_3)_2$); $\delta_F$: two AB type multiplets at −93.5 and −96.7 after irradiation of the protons.

*Removal of the protecting groups*

*Cleavage of the t-butyloxycarbonyl group*

To a stirred solution of 1 mmol of the fully protected peptides **3–6** and **8–11** in 5 ml of ethyl acetate, was added 0.2 ml of concentrated hydrochloric acid. The mixture was stirred at the room temperature for 45 min and the solvent was evaporated under reduced pressure. The resulting hydrochloride salt was recrystallized from ether or methanol/ether.

*Elimination of the 9-fluorenylmethyloxycarbonyl group*

To a stirred solution of 1 mmol of the peptides **7** and **12–15** in 5 ml of dichloromethane, was added 2 mmol of diethylamine. The mixture was stirred at room temperature for 2 h and the solvent and the amine were evaporated under reduced pressure. The resulting residue was taken up in a hydrochloric acid solution (1 N) and extracted with ethyl acetate. The aqueous layer was neutralized by the addition of a 10% solution of ammonia and extracted with ethyl acetate (3×20 ml). The organic layers were dried over $MgSO_4$ and the product, obtained after evaporation of the solvent, was dissolved in ethyl ether. To this solution, was added at 0 °C, 5 ml of a saturated solution of dried HCl in anhydrous ethyl ether. The ethyl ether and the excess HCl gas were evaporated. The resulting solid residue was recrystallized.

*Hydrolysis of* O*-alkyl ester groups*

When *N*-t-butyloxycarbonyl *O*-alkyl ester difluorinated peptides were treated with hydroxylated bases (NaOH, KOH and LiOH) under various reaction conditions (time, temperature and concentration), defluorination occurred. In some cases, the desired products were obtained in about 10–15% yield. By contrast, *N*-9-fluorenylmethyloxycarbonyl *O*-alkyl ester difluorinated peptides reacted with hydrochloric acid solution (6 N) under reflux to provide the difluorinated peptide acids, after extraction with ethyl acetate, in good yields.

*General procedure for the synthesis of difluorinated cyclodipeptides*

A solution of 1–2 mmol of $N^{\alpha}$-Boc difluorinated dipeptide in 20–40 ml of formic acid was stirred at room temperature for 2 h. Excess formic acid was eliminated by evaporation *in vacuo* (bath temperature below 30 °C). The crystalline solid obtained was dissolved in s-butanol (20–40 ml) and toluene (10–20 ml). The mixture was heated under reflux for 5 h. During this time the solvent level was kept constant by periodic addition of i-butanol. At the end, the reaction mixture was allowed to cool to room temperature. The solid which precipitated was collected by filtration, washed with cold s-butanol and recrystallized from methanol.

*Cyclo[Gly-DL-Phe (F2)] (**16**) (nc)*

This compound was obtained in racemic form. IR (KBr): 3205, 3095, 1707, 1668 $cm^{-1}$. $^{19}F$ NMR: $\delta -102.4$ (dd, $F_a$, $^2J_{F_aF_b}=245$ Hz, $^3J_{F_aH}=13.7$); $-97.7$ (dd, $F_b$, $^2J_{F_aF_b}=245$ Hz, $^3J_{F_bH}=9.3$). Analysis: Calcd. C, 55.00; H, 4.19; F, 15.82%; found: C, 55.40; H, 4.02; F, 16.10%.

*Cyclo[D-Ala-DL-Phe (F2)] (**17**) (nc)*

The mixture contained the two diastereoisomers identified unambiguously by $^{19}F$ NMR spectroscopy. IR (KBr): 3202, 3096, 1666, 1450 $cm^{-1}$. $^{19}F$ NMR: **17a**: $\delta -101.7$ (dd, $F_a$, $^2J_{F_aF_b}=246$ Hz, $^3J_{F_aH}=13.1$ Hz); $-95.1$ (dd, $F_b$, $^2J_{F_aF_b}=246$ Hz, $^3J_{F_bH}=6.9$ Hz); **17b**: $\delta -101.9$ (dd, $F_a$, $^2J_{F_aF_b}=245$ Hz, $^3J_{F_aH}=13.5$ Hz); $-97.6$ (dd, $F_b$, $^2J_{F_aF_b}=245$ Hz, $^3J_{F_bH}=9.3$ Hz). Analysis: Calcd. C, 56.69; H, 4.75; F, 14.94%; found: C, 56.42; H, 4.58; F, 14.81%.

*Cyclo[L-Phe-DL-Phe (F2)] (**18**) (nc)*

After recrystallization, the two diastereoisomers were characterized by their spectrometric properties. IR (KBr): 3198, 3096, 1668, 1450 $cm^{-1}$. $^{19}F$ NMR: **18a**: $\delta$ −102.4 (dd, $F_a$ $^2J_{F_aF_b}$ = 245 Hz, $^3J_{F_aH}$ = 13.2 Hz); −96.7 (dd, $F_b$, $^2J_{F_aF_b}$ = 245 Hz, $^3J_{F_bH}$ = 7.8 Hz); **18b**: $\delta$ −101.3 (dd, $F_a$, $^2J_{F_aF_b}$ = 247 Hz, $^3J_{F_aH}$ = 13.2 Hz), −94.7 (dd, $F_b$, $^2J_{F_aF_b}$ = 247 Hz, $^3J_{F_bH}$ = 6.9 Hz). Analysis: Calcd. C, 65.44; H, 4.88; F, 11.57%; found: C, 64.86; H, 4.73; F, 11.37%.

*Cyclo[Gly-DL-Abu (F2)] (**19**) (nc)*

This cyclodipeptide was obtained in racemic form after purification. IR (KBr): 3200, 3092, 1700, 1660, 1458 $cm^{-1}$. $^{19}F$ NMR: $\delta$ −99.6 (dqd, $F_a$, $^2J_{F_aF_b}$ = 241 Hz, $^3J_{F_aH^\alpha}$ = 20.0 Hz, $^3J_{F_aH^\omega}$ = 20.2 Hz); −94.2 (dqd, $F_b$, $^2J_{F_aF_b}$ = 241 Hz, $^3J_{F_bH^\alpha}$ = 8.3 Hz, $^3J_{F_bH^\omega}$ = 20.2 Hz). Analysis: Calcd. C, 40.45; H, 4.52; F, 21.33%; found: C, 40.66; H, 4.67; F, 20.58%.

*Cyclo[D-Ala-DL-Abu (F2)] (**20**) (nc)*

Two diastereoisomers were isolated from the reaction mixture. IR (KBr): 3200, 3067, 1690, 1472 $cm^{-1}$. $^{19}F$ NMR: **20a**: $\delta$ −99.4 (dqd, $F_a$, $^2J_{F_aF_b}$ = 241, $^3J_{F_aH^\alpha}$ = 20.4, $^3J_{F_aH^\omega}$ = 19.4 Hz); −94.3 (dqd, $F_b$, $^2J_{F_aF_b}$ = 241 Hz, $^3J_{F_bH^\alpha}$ = 6.3 Hz, $^3J_{F_bH^\omega}$ = 19.9 Hz), **20b**: $\delta$ −99.8 (dqd, $F_a$, $^2J_{F_aF_b}$ = 241 Hz, $^3J_{F_aH^\alpha}$ = 20.0 Hz, $^3J_{F_bH^\omega}$ = 19.5 Hz); −94.3 (dqd, $F_b$, $^2J_{F_aF_b}$ = 241 Hz, $^3J_{F_aH^\alpha}$ = 8.7 Hz, $^3J_{F_aH^\omega}$ = 19.6 Hz). Analysis: Calcd. C, 43.75; H, 5.24; F, 19.77%; found: C, 44.05; H, 5.35; F, 19.35%.

*Cyclo[L-Phe-DL-Abu (F2)] (**21**) (nc)*

By the above procedure two isomers were obtained. IR (KBr): 3200, 3065, 1682, 1470 $cm^{-1}$. $^{19}F$ NMR: **21a**: $\delta$ −97.5 (dqd, $F_a$, $^2J_{F_aF_b}$ = 242 Hz, $^3J_{F_aH^\alpha}$ = 18.7 Hz, $^3J_{F_aH^\omega}$ = 18.7 Hz); −92.4 (dqd, $F_b$, $^2J_{F_aF_b}$ = 242 Hz, $^3J_{F_bH^\alpha}$ = 7.0 Hz, $^3J_{F_bH^\omega}$ = 19.9 Hz); **21b**: $\delta$ −99.2 (dqd, $F_a$, $^2J_{F_aF_b}$ = 241 Hz, $^3J_{F_aH^\alpha}$ = 19.4 Hz, $^3J_{F_aH^\omega}$ = 19.4); −94.3 (dqd, $F_b$, $^2J_{F_aF_b}$ = 241 Hz, $^3J_{F_bH^\alpha}$ = 7.2 Hz, $^3J_{F_bH^\omega}$ = 19.5 Hz). Analysis: Calcd. C, 58.20; H, 5.26; F, 14.16%; found: C, 57.93; H, 5.51; F, 14.32%.

*Cyclo[L-Tyr-DL-Abu (F2)] (**22**) (nc)*

After crystallization, the two diastereoisomers were identified. IR(KBr): 3422, 3195, 1666 1486 $cm^{-1}$. $^{19}F$ NMR: **22a**: $\delta$ −97.3 (dqd, $F_a$, $^2J_{F_aF_b}$ = 242 Hz, $^3J_{F_aH^\alpha}$ = 19.4 Hz, $^3J_{F_aH^\omega}$ = 19.6 Hz); −92.8 (dqd, $F_b$, $^2J_{F_aF_b}$ = 242 Hz, $^3J_{F_bH^\alpha}$ = 7.1 Hz, $^3J_{F_bH^\omega}$ = 19.8 Hz); **22a**: $\delta$ −99.6 (dqd, $F_a$, $^2J_{F_aF_b}$ = 241 Hz, $^3J_{F_aH^\alpha}$ = 20.0 Hz, $^3J_{F_aH^\omega}$ = 19.6 Hz); −94.6 (dqd, $F_b$, $^2J_{F_aF_b}$ = 241 Hz, $^3J_{F_bH^\alpha}$ = 7.0 Hz, $^3J_{F_bH^\omega}$ = 19.6 Hz). Analysis: Calcd. C, 54.92; H, 4.96; F, 13.36%; found: C, 54.98; H, 5.30; F, 13.65%.

## Results and discussion

3,3-Difluoro-amino esters were prepared as previously described by Wade and Guedj [12]. In all cases studied, the racemic 3,3-difluoro-2-amino acid

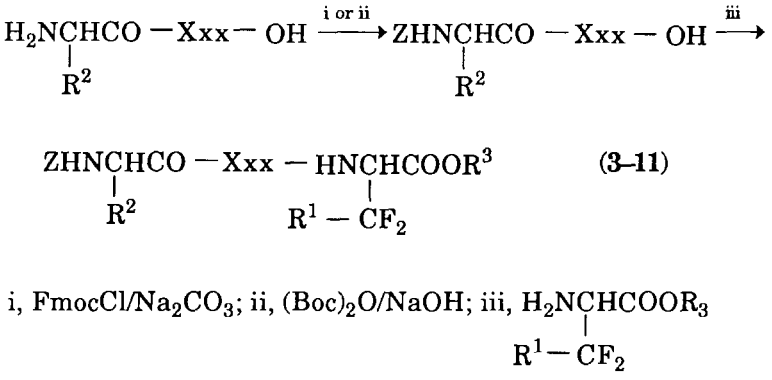
or ester, reacted under mild conditions with an unfluorinated optically active amino ester of $N^{\alpha}$-protected amino acid (or peptides) to give the corresponding fluorinated peptides in moderate to good yields. The products were identified by IR, $^{19}F$ NMR and $^{1}H$ NMR spectroscopy, and by mass spectral elemental analyses (FAB). No particular effort was made to separate the two different stereoisomers in the reaction mixture, since they could be clearly detected by $^{19}F$ NMR spectroscopy.

*Synthesis of the difluorinated peptides*

Two approaches were considered for the preparation of the peptides containing the 3,3-difluoro-2-amino acid residue. First, when the fluorinated residue was the last amino acid of the peptide chain, the compounds could be obtained in a one- or two-step procedure through initial formation of the $N^{\alpha}$-protected unfluorinated amino acids or peptides, and then coupling with 3,3-difluoro-2-amino esters by liquid-phase methods (Scheme 1): by cyanomethyl or *p*-nitrophenyl ester methods [16, 17]; with the aid of dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole [18]; or by mixed anhydride synthesis [19].

Thus, 2-carboxyester-1-azirines were allowed to react immediately after their preparation with hydrogen fluoride in pyridine solution, and the 3,3-difluoro-2-amino esters obtained in good yields [12] were coupled with the $N^{\alpha}$-Boc or $N^{\alpha}$-Fmoc unfluorinated amino acids in appropriate solvents. Although, the presence of two fluorine atoms in the molecules alters the reactivity of *ethyl 3,3-difluoro-2-amino-butyrate* and *ethyl 3,3-difluorophenylalanate*, [20] no particular modification in the coupling reactions was required (see experimental section). Various peptides (**3–11**) were obtained in moderate to good yields after column chromatography or recrystallisation.

Secondly, when the fluorinated residue was to be incorporated in other positions, the key synthetic step was the preparation of the $N^{\alpha}$-protected difluoro amino acid. This could be accomplished by blocking the amino functional group of the 3,3-difluoro-2-amino esters (prepared by the ring



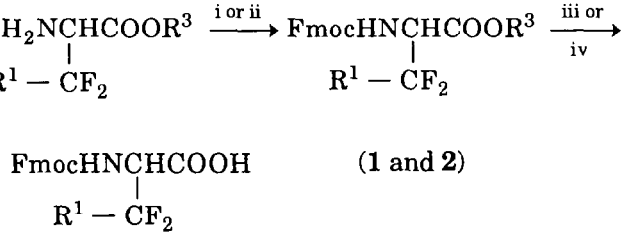

Scheme 1. Preparation of ZNHCH($R^2$)CO–Xxx–HNCH($CF_2R^1$)COOR (FmocCl = 9-fluorenylmethyl chloroformate; $(Boc)_2O$ = di-t-butyl-dicarbonate).

opening of 2-carboxyalkyl-1-azirines with HF in pyridine solution), with either Fmoc or Boc followed by acid hydrolysis of saponification on the ester function respectively (Scheme 2).

All attempts to prepare Boc-$N^{\alpha}$-3,3-difluoro-2-amino acids from the corresponding N-protected 3,3-difluoro-2-amino esters by reaction with bases (NaOH, KOH and LiOH) under various reaction conditions (time, temperature and concentrations) failed. The crude reaction mixtures were complex and composed of unfluorinated compounds ($\alpha,\beta$-unsaturated acids) together with very small amounts of the desired products.

By contrast, when Fmoc-$N^{\alpha}$-3,3-difluoro-2-amino esters were allowed to react with hydrochloric acid in a mixture of water and dioxan under reflux, the Fmoc-$N^{\alpha}$-3,3-difluoro-2-amino acids were obtained in good yield (*i.e.* 70%) after an acido–basic treatment followed by recrystallization. The successful preparation of *N*-9-fluorenylmethyloxycarbonyl-DL-2-amino-3,3-difluoro-butanoic acid [Abu(F2)] **1** and *N*-9-fluorenylmethyloxycarbonyl-DL-3,3-difluoro-phenylalanine [Phe(F2)] **2** allowed their incorporation into peptide structures. Subsequent coupling with the unfluorinated amino esters or *O*-ester peptides by liquid-phase methods as above yielded the desired peptides (**12–15**).

In both synthetic approaches, the reactions gave the fluorinated peptides in moderate to good yields after purification on silica gel chromatography and recrystallization. The good yields obtained of the peptides confirmed the feasibility of our approaches for the incorporation of a few fluorinated amino acids in peptide synthesis, even though the fluorine atoms and the $\beta$ position, by altering the p$K_a$ values of the amino and the carboxyl groups [20], could affect the course of the coupling reaction [21]. Apparently, the reactivities of these groups are not sufficiently decreased to prevent the incorporation of the 3,3-difluoro amino esters or acids by standard methods. In addition, the formation of only two diastereoisomers, identified by $^{19}F$ NMR spectroscopy (performed on the reaction mixtures before purification, in all cases), suggested that no significant epimerisation occurred during the coupling reactions at the asymmetric centers by the methods employed. Removal of amine protecting groups (*i.e.* Boc and Fmoc) in the peptides




i, FmocCl/*N*-methylmorpholine; ii, $(Boc)_2O$; iii, $H_3O^+$, reflux; iv, $HO^-$

Scheme 2. Preparation of $N^{\alpha}$-Fmoc difluoro amino acids.

was achieved in the usual manner and their hydrochloric salts isolated without the loss of any fluorine. The acid resistance of the Fmoc group permitted the cleavage of alkyl esters by acid-catalyzed hydrolysis.

*Preparation of fluorinated dioxopiperazines*

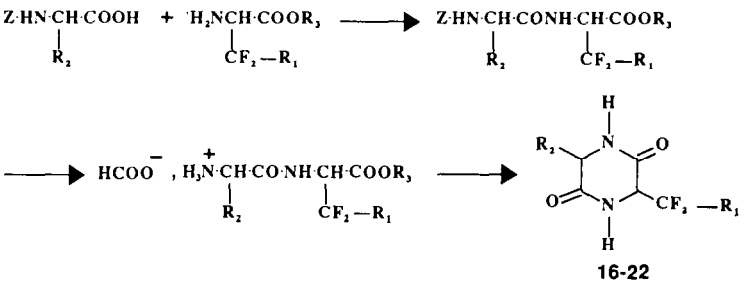
Numerous methods have been reported for the synthesis of cyclodipeptides [22–25]. One procedure described for the preparation of dioxopiperazines is the heating of the formate salts of the linear dipeptide alkyl esters in a mixture of toluene and s-butanol. This route readily leads to the products in good yields without racemisation [25]. We have tried this method which included as the first step the synthesis of the fluorinated dipeptides as described in Scheme 3.

The amino protecting group (*e.g.* Boc) of the peptides **3–5**, **8**, **10** and **11** was cleaved with formic acid at room temperature and the resulting salts, obtained after evaporation of the excess formic acid *in vacuo*, were dissolved in a toluene/s-butanol (1/1 v/v) mixture. Cyclisation was achieved by heating the solution under reflux for 5 h. The yields of fluorinated dioxopiperazines (**16–22**) after purification ranged from 56% to 80%. The $^{1}H$ and $^{19}F$ NMR spectra of the crude reaction mixtures for compounds **17**, **18**, **20–22** showed that the products were composed of two diastereoisomers the ratios of which could be determined by $^{1}H$ NMR spectroscopy (Table 1).

*NMR spectral data of cyclo-[Gly-D,L-Phe(F2)] (**16**) and cyclo-[Gly-D,L-Abu(F2)] (**19**)*

Several naturally occurring 2,5-dioxopiperazines have been studied by various physical methods. The skeletal conformations of cyclic dipeptides have been intensively investigated in the solid state by X-ray crystallography and in solution by many spectroscopic methods including $^{1}H$ and $^{13}C$ NMR and IR spectroscopy [26a].

The synthesis of the first difluoro dioxopiperazines allowed the study of their conformations in solution. $^{1}H$ and $^{19}F$ NMR spectra were measured in DMSO-$d_6$. $^{1}H$ parameters obtained are collected in Table 1. They were virtually constant over a temperature range of 15–60 °C, indicating a temperature independence of the skeletal conformations of the 2,5-dioxopiper-




Scheme 3. Synthesis of 3-(1,1'-difluoroalkyl)-2,5-dioxopiperazines.

TABLE 1

$^1$H NMR characteristics of the difluoro cyclodipeptides

| No. | Fluorinated residue | | | | Other | Unfluorinated residue | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\delta_{H\alpha}$[a] | $\delta_{NH}$[b] | $J_{CHNH}$[a] | $\delta_{H\gamma}$ | | $\delta_{H\alpha}$ | $\delta_{NH}$ | $J_{CHNH}$ | $\delta_{H\beta}$ |
| 16 | 4.44 (ddd) | 8.76 (d) | 4 | – | Phe 7.5 (m) | 3.14 (s) and 3.42 (d) | 8.28 (d) | <0.5 4 | |
| 17a | 4.54 (ddd) | 8.65 (d) | 3.6 | – | Phe 7.5 (m) | 3.76 (qd) | 8.33 (d) | 2.8 | 0.74 (d) |
| 17b | 4.46 (ddd) | 8.67 (d) | 4.6 | – | | 3.24 (q) | 8.4 (s) | <0.5 | 1.13 (d) |
| 18a | 4.24 (ddd) | 8.66 (d) | 3.5 | – | Phe 7.2/7.5 (2 m) | 3.52 (d) | 8.41 (d) | 4.8 | 2.77/3.1 (2dd) |
| 18b | 4.6 (ddd) | 8.71 (d) | 2.8 | – | Phe 7.5 (m) | 3.9 (d) | 8.15 (s) | <0.5 | 1.95/2.64 (2dd) |
| 19 | 4.13 (ddd) | 8.67 (d) | 3.8 | 1.71 (t) | – | 3.59 (dd) and 3.85 (d) | 8.42 (d) 8.4 (s) | 5.5 <0.5 | |
| 20a | 4.21 (ddd) | 8.66 (d) | 2.6 | 1.74 (t) | – | 3.88 (dd) | 8.33 (d) | 2.9 | 1.29 (d) |
| 20b | 4.15 (ddd) | 8.80 (d) | 4.2 | 1.72 (t) | – | 3.96 (q) | 8.4 (s) | <0.5 | 1.24 (d) |
| 21a | 4.10 (ddd) | 8.50 (d) | 2.9 | 1.41 (t) | Phe 7.5 (m) | 4.11 | 8.42 (d) | 5 | 2.98/3.0 (2dd) |
| 21b | 3.90 (ddd) | 8.52 (d) | 3.48 | 1.68 (t) | Phe 7.5 (m) | 4.27 | 8.54 (s) | <0.5 | 2.9/3.21 (2dd) |
| 22a | 4.10 (ddd) | 8.42 (d) | 2.8 | 1.39 (t) | Tyr 6.88 (dd) | 4.15 (dd) | 8.31 (d) | 3.5 | 2.76/2.98 (2dd) |
| 22b | 3.87 (ddd) | 8.46 (d) | 3.2 | 1.67 (t) | Tyr 7.1 (dd) | 4.17 (dd) | 8.44 (s) | <0.5 | 2.78/3.11 (2dd) |

[a]Chemical shifts are given on the $\delta$-scale (ppm) and interaction constants in Hz.

[b]Chemical shifts of NH protons are strongly dependent upon concentration in DMSO-$d_6$. All spectra were measured at the same concentration, *i.e.* $2\times10^{-2}$ M.

azines rings and the conformer populations of the side-chains of the seven cyclic fluorinated dipeptides. The coupling constants $^3J_{CHNH}$ permitted the calculation of the dihedral angles between $C^\alpha-N-H$ and $H-C^\alpha-N$ planes using different equations proposed by Davies and Khaled [26] and by Bystrov *et al.* [27]. Skeletal conformations of cyclodipeptides in solution are often assumed to have planar amide bonds. Under these assumptions, the conformation of 1,4-disubstituted 2,5-dioxopiperazines is expressed by a single angle $\beta$ which is the complementary angle between the two amide planes [28]. This latter angle indicates the degree of folding of the ring (Fig. 1).

On the other hand, the boat conformations should be expected in these cases as shown in Fig. 2. Moreover, the value of the $\beta$ parameter has been shown to depend linearly on the difference in the chemical shifts of the geminal hydrogen substituents of Gly in cyclic dipeptides of the c-Gly-L—Xxx type [27]. On these basis, we found that the $\beta$ values for c-Gly-Phe(F2) and c-Gly-Abu(F2) at 298 K were approximately $-27°$ and $-21°$, testifying to the predominance of the ring conformer in which the two $C^\beta$ atoms approach each other above the 2,5-piperazinedione ring (Fig. 2). The difference in the chemical shifts of the geminal hydrogen substituents of Gly in the cyclic peptides can be attributed to an anisotropic effect of the phenyl group or the fluorine atom of the second residue. The values suggested the preponderance of the 2,5-dioxopiperazine ring conformers, with the amino acid side-chain ($PhCF_2$ and $AbuCF_2$) (preferentially in a quasi-axial position

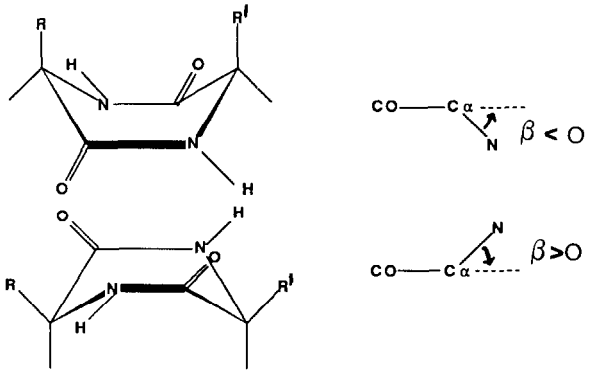



Fig. 1. Definition of the angle $\beta$ of boat-type conformations of 2,5-dioxopiperazines.

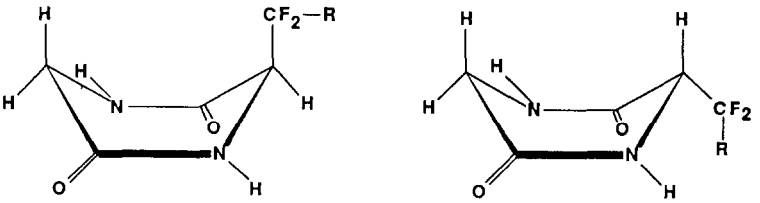



Fig. 2. Conformations of the dioxopiperazines ring: (a) $CF_2R$ pseudo-axial; (b) $CF_2R$ pseudo-equatorial.

['*flagpole*' boat-type conformation, Fig. 2(a)] as previously found for similarly unfluorinated cyclodipeptides [27, 28]. The same conclusions can be drawn for the other difluorinated cyclodipeptides, but it is desirable in these cases to identify each stereoisomer before the development of the NMR analysis of cyclic dipeptides. This work is under current investigation.

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