PEPTIDE SYNTHESIS WITH α-(DIFLUOROMETHYL)-SUBSTITUTED α-AMINO ACIDS

Thomas MICHEL[a], Beate KOKSCH[b1], Sergei N. OSIPOV[c], Alexander S. GOLUBEV[c], Joachim SIELER[d] and Klaus BURGER[b2,*]

[a] *Jenapharm, Otto-Schott-Strasse 15, D-07745 Jena, Germany*

[b] *Department of Organic Chemistry, University of Leipzig, Johannisallee 29, D-04103 Leipzig, Germany; e-mail: [1] koksch@organik.chemie.uni-leipzig.de, [2] burger@organik.chemie.uni-leipzig.de*

[c] *Institute of Organoelement Compounds, Russian Academy of Sciences, Vavilov str. 28, GSP-1, V-334, 117813 Moscow, Russia*

[d] *Department of Inorganic Chemistry, University of Leipzig, Linnéstrasse 3, D-04103 Leipzig, Germany*

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Methodology for the incorporation of α-(difluoromethyl)-substituted α-amino acids into N- and C-terminal positions of dipeptides as well as into position 2 of tri- and tetrapeptides is described.

Keywords: Peptides; α,α-Disubstituted amino acids; α-Fluoroalkyl α-amino acids; Peptide coupling reagents; Fragment condensation; Fluorinated compounds.

Incorporation of α,α-disubstituted amino acids into key positions of peptides is an efficient strategy to retard proteolytic degradation and to stabilize secondary structure elements[1]. α-(Fluoroalkyl)-substituted α-amino acids are a special class of α,α-disubstituted amino acids[2]. A fluoroalkyl group in α-position of an amino acid exerts considerable polarization effects on neighboring substituents. However, this effect strongly depends on the number of fluorine atoms present in the α-(fluoroalkyl) substituent as can be seen on comparison of pK_a (NH_2) values for alanine 9.8, 3-fluoroalanine 9.8, 3,3-difluoroalanine 8.4 and 3,3,3-trifluoroalanine 5.3 [3]. The remarkable gap of Δ = 3.1 between 3,3,3-trifluoro- and 3,3-difluoroalanine should result in major differences with respect to reactivity and molecular properties.

Recently it was disclosed that hydrophobic interactions are far more essential in ligand/receptor interactions than assumed, while the influence of

hydrogen bonding was overestimated[4]. The mechanism of hydrophobic interactions, however, was never elucidated in detail[5]. Side chain phenyl groups play an essential role in ligand/receptor interactions. When the aromatic system interacts with the side chain of Val, Leu, Ile and Ala, the aromatic π-system functions as a hydrogen bond acceptor. These interactions are denoted as CH/π interactions, a concept which has been recently established by Nishio *et al.*[6] In the series CF_3, CHF_2, CH_2F, CH_3, only the CHF_2 and CH_2F groups can react as both hydrogen bond donor and hydrogen bond acceptor[7], with the CHF_2 group being the most potent hydrogen bond donor in this series[8]. Furthermore, fluoroalkyl groups may act as coordinative sites in metal complexes.

Recently, we demonstrated that the incorporation of α-(trifluoromethyl) amino acids (α-Tfm amino acids) in peptides not only improves proteolytic stability and induces secondary structure motifs, but also improves lipophilicity[9] enhancing *in vivo* absorption and improving permeability through certain body barriers. ^{19}F NMR spectroscopy is an efficient tool for conformational studies of fluorine-containing peptides and for the elucidation of metabolic processes[10]. The spectra can be recorded even in water and under cell-like conditions.

The most convenient synthesis of α-Tfm amino acids is based on an amidoalkylation of carbon nucleophiles with highly electrophilic acylimines of 3,3,3-trifluoropyruvate[11]. This route enables the synthesis of α-Tfm amino acids with orthogonal protective groups[12]. A slightly modified approach provides access to homochiral α-Tfm amino acids *via* alkylation of *in situ* formed homochiral cyclic α-(trifluoromethyl)-substituted acylimines with C-nucleophiles[13] and *via* alkylation of trifluoromethyl-substituted acylimines with homochiral carbanions[14]. Analogously, α-(difluoromethyl) α-amino acids (α-Dfm amino acids), the virtually unknown α-(*c*hlorodifluoromethyl) and α-(bromodifluoromethyl) α-amino acids were obtained *via* addition of C-nucleophiles to acylimines of corresponding partially fluorinated pyruvates[15].

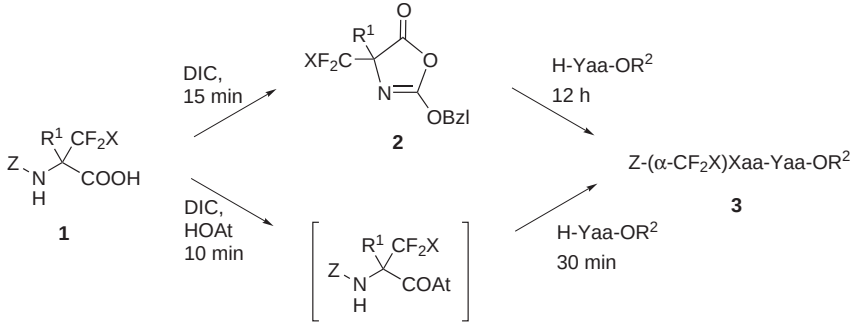
The low nucleophilicity of the amino group and the bulkiness of the trifluoromethyl group of α-Tfm amino acids requires modification of standard protocols for peptide synthesis[2]. Only H-(α-Tfm)Gly-OMe and H-(α-Tfm)Ala-OMe [16] can be coupled by classical methodology obtaining reasonable yields. However, for α-Tfm amino acids with bulkier side chains, all classic activation methods examined so far have turned out to be unsuccessful or resulted in substantial epimerization of the adjacent non-fluorinated amino acid[17].

To circumvent the above synthetic problems, we decided to introduce α-Dfm amino acids for peptide modification to improve nucleophilicity, while retaining the positive profile of the fluoroalkyl group. On the basis of the pK_a (NH_2) values, we expected the nucleophilicity to be of the order of α-methyl-substituted α-amino acids, exhibiting steric strain similar to α-aminoisobutyric acid (Aib). Therefore, we tested the efficiency of a series of currently used coupling methods for incorporation of α-Dfm amino acids into the N- and C-terminal position of peptides.

RESULTS AND DISCUSSION

N-Terminal Incorporation

Incorporation of α-Dfm amino acids into the N-terminal position of peptides can be successfully achieved either by activation of Z-protected α-Dfm amino acids with diisopropylcarbodiimide (DIC) *via* oxazolones (**1**→**2**→**3**) or on activation with DIC/1-hydroxy-7-azabenzotriazole (HOAt) *via* the step of an activated ester (Scheme 1). On activation of Boc-protected α-Dfm amino acids with DIC, *N*-carboxyanhydrides (NCAs) are formed *via* retro ene reaction from oxazolones[11c,18].



SCHEME 1
Incorporation of α-Dfm amino acids into N-terminal position of peptides (X = H, Cl, Br)

Attempts to convert Z-protected α-Dfm amino acids on treatment with DIC to symmetric anhydrides[19] failed, only the corresponding oxazolones were obtained. This type of intramolecular cyclization is favored in the presence of an additional α-substituent (geminal dimethyl effect)[20]. Epimerization *via* oxazolones is a major problem in peptide synthesis[21]. This problem is irrelevant with α,α-disubstituted α-amino acids because of

the absence of an α-proton. While dipeptide formation *via* ring opening of oxazolones with amino acid esters (H-Yaa-OR) **2**→**3** is a slow process and needs reaction times of 12–14 h at room temperature, carboxylic group activation with DIC/HOAt results in quantitative formation of the HOAt ester (^{19}F NMR analysis) within about 10 min, which is then reacted *in situ* with amino acid esters at room temperature to give the dipeptide ester in excellent yields within another 30 min. Under these reaction conditions, oxazolone formation could not be observed. In Table I some representative examples are summarized.

Compared with the oxazolone route, carboxylic group activation of Z-protected α-Dfm amino acids in the presence of additives is a more efficient coupling method, characterized by shorter reaction times and slightly higher yields. This protocol is not limited to peptide synthesis in solution.

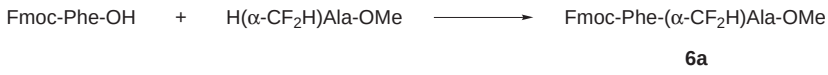
C-Terminal Incorporation

As expected, incorporation of α-Dfm amino acids into the C-terminal position of peptides is more difficult than into the N-terminal position. Therefore, we tested a series of currently used peptide coupling techniques, like the acid fluoride and acid chloride method, which seem to be exceptionally well suited for incorporation of sterically hindered amino acids into peptides[22]. Furthermore, we tested reagents like bromotris(pyrrolidin-1-

TABLE I
Yields of dipeptides with α-Dfm amino acids in N-terminal position

No.	Dipeptide	Method	Yield, %
3a	Z-(α-CF_2H)Ala-Gly-O^{t}Bu	DIC	73
3b	Z-(α-CF_2H)Ala-Phe-O^{t}Bu	DIC	75
3b	Z-(α-CF_2H)Ala-Gly-O^{t}Bu	DIC/HOAt	90
3c	Z-(α-CF_2H)Ala-Pro-O^{t}Bu	DIC/HOAt	90
4a	Z-(α-CF_2Cl)Ala-Gly-O^{t}Bu	DIC	78
4b	Z-(α-CF_2Cl)Ala-Phe-O^{t}Bu	DIC	83
4c	Z-(α-CF_2Cl)Ala-Pro-O^{t}Bu	DIC/HOAt	93
4d	Z-(α-CF_2Cl)Phe-Gly-O^{t}Bu	DIC	85
5	Z-(α-CF_2Br)Ala-Phe-O^{t}Bu	DIC	86

yl)phosphonium hexafluorophosphate (PyBroP), [(7-azabenzotriazol-1-yl)oxy]tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyAOP), [(dimethylamino)(1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl)methylidene]dimethylammonium hexafluorophosphate *N*-oxide (HATU) and *N*,*N*,*N*′,*N*′-tetramethylfluoroformamidinium hexafluorophosphate (TFFH). The coupling reagents were tested synthesizing the model peptide Fmoc-Phe-(α-Dfm)Ala-OMe (**6a**) (Scheme 2). A standard protocol was used: H-(α-Dfm)-Ala-OMe (0.38 g, 2.5 mmol), (ethyl)diisopropylamine (DIEA) (0.33 g, 2.5 mmol) and activated Fmoc-Phe-OH (5.0 mmol) were stirred in DMF at room temperature for 12 h. The progress of the reactions was controlled by ^{19}F NMR spectroscopy and RP-HPLC. The results obtained are summarized in Table II.



SCHEME 2
Synthesis of model peptide **6a** using different activation strategies

Conventional carbodiimide methodology with additives such as HOBt or active esters like pentafluorophenyl esters gave unsatisfactory results in the standard coupling reaction. Moderate to respectable yields were obtained

TABLE II
Yields (%) of Fmoc-Phe-(α-Dfm)Ala-OMe **6a** using different coupling methods. Yields were determined by ^{19}F NMR spectroscopy

Method	Yield (%) at reaction time (h)			Method	Yield (%) at reaction time (h)		
	0.5	3	12		0.5	3	12
Fmoc-Phe-F	55	85	>95	TFFH	<5	15	60
Fmoc-Phe-Cl	65	>95		MA; ^{t}BuOCOCl	5	55	75
Fmoc-Phe-OPfp	<5	<5	15	TBTU	5	45	70
HBTU	<5	25	40	HATU[a]	75	>95	
BOP	<5	30	55	PyBroP	90	>95	
DIC/HOBt	<5	<10	55	DIC/HOAt	45	60	>95
PyBOP	<10	35	80	PyAOP	45	70	>95

[a] Second activation after 30 min.

with the first-generation uronium salt and phosphonium salt coupling reagents, *e.g.* [(benzotriazol-1-yl)(dimethylamino)methylidene]dimethylammonium hexafluorophosphate *N*-oxide (HBTU), [(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate (BOP), [(benzotriazol-1-yl)oxy]tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyBOP), *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU). The mixed anhydride activation with isobutyl chloroformate gave acceptable yields as well as the activation with bromotris(pyrrolidin-1-yl)phosphonium hexafluorophosphate, were described to give good to excellent yields even in coupling reactions of two amino acids with high steric requirements[22]. Yields higher than 95% were obtained for the above mentioned model reaction within 3 h. On activation with DIC/HOAt the dipeptide **6a** was formed in nearly quantitative yield. Using HATU the reaction was complete (^{19}F NMR analysis) already after 1 h at room temperature, when a second activation was performed after 30 min.

Acid chlorides have been already successfully applied to coupling reactions of α,α-disubstituted amino acids. However, their application is limited to amino acid derivatives containing no acid labile protecting groups[23]. Another problem using the acid chloride method arises from the tendency of amino acids to be converted to oxazolones in the presence of tertiary amines[24]. A racemization test of the model peptide obtained from Fmoc-Phe-Cl using the method of Bayer *et al.*[25] was performed. After hydrolysis of the peptide with DCl/D_2O, the *N*-(trifluoroacetyl)-protected isopropyl phenylalaninate was synthesized by standard protocols and analyzed by chiral GC-MS (columns precoated with Chirasil-Val[26]). The loss of stereochemical integrity was shown to be less than 0.15%[27]. A serious drawback for the acid chloride strategy is their incompatibility with Boc- and -O^{t}Bu-protection. Therefore, it can not be applied to the highly efficient Fmoc/-O^{t}Bu strategy.

In contrast, the corresponding acid fluorides do not suffer from these limitations (Scheme 3). The acid fluorides are generally obtained from the corresponding acids on reaction with cyanuric fluoride and pyridine[28] or with (diethylamino)sulfur trifluoride (DAST) in the absence of a base[29]. Fmoc-protected amino acid fluorides can be used in the case of -O^{t}Bu, Boc-

SCHEME 3
C-Terminal incorporation of α-Dfm amino acids *via* Fmoc-amino acid fluorides

and trityl-protected amino acid derivatives[30]. Another advantage of acid fluorides compared with the corresponding acid chlorides is their higher stability to moisture and their low cyclization tendency to give oxazolones in the presence of tertiary amines[31].

Carpino *et al.* introduced TFFH [32], a reagent for the *in situ* preparation of acid fluorides from N-protected amino acids. However, it has been demonstrated that the application of the *in situ* technique resulted in lower yields of the model peptide **6a**.

Acyl transfer reactions to H-(α-Dfm)Ala-OMe with amino acids with high steric requirements such as Leu, Val and Aib turned out to be problematic. Of the five methods tested (PyBroP, DIC/HOAt, 7-azabenzotriazol-1-yloxy-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyAOP), HATU, Fmoc-Xaa-F), only PyBroP and Fmoc-Xaa-F (Xaa = Phe, Leu, Val) gave the dipeptides in good to excellent yields (Table III). However, experiments to synthesize Fmoc-Aib-(α-Dfm)Ala-OMe using the coupling methodology have been unsuccessful so far.

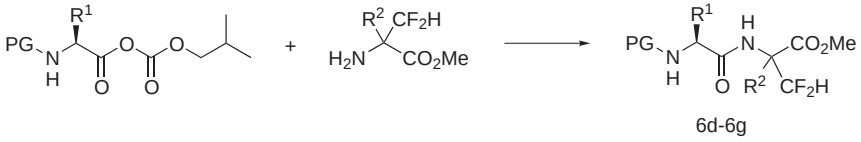
Although, giving only about 65–70% yields, the mixed anhydride method using isobutyl chloroformate as coupling reagent is attractive for the synthesis of Boc- and Z-protected dipeptides with (α-Dfm) amino acids

TABLE III
Synthesis of Fmoc-, Z- and Boc-protected dipeptides with (α-Dfm)Ala and (α-Dfm)Phe in C-terminal position

No.	Product	Coupling reagent/Yield, %	
		PyBroP	Fmoc-Xaa-F
6a	Fmoc-Phe-(α-Dfm)Ala-OMe	>95[a]/75[b]	95[a]/81[b]
6b	Fmoc-Leu-(α-Dfm)Ala-OMe	80[a]/58[b]	>95[a]/72[b]
6c	Fmoc-Val-(α-Dfm)Ala-OMe	65[a]/47[b]	80[a]/62[b]
		Isobutyl chloroformate	
6d	Z-Phe-(α-Dfm)Ala-OMe	67[b]	
6e	Z-(*R*)-Phe-(α-Dfm)Ala-OMe	65[b]	
6f	Boc-Tyr(O^{t}Bu)-(α-Dfm)Ala-OMe	72[b]	
6g	Boc-Gly-(α-Dfm)Phe-OMe	67[b]	

[a] Yield determined by ^{19}F NMR spectroscopy; [b] isolated yield.

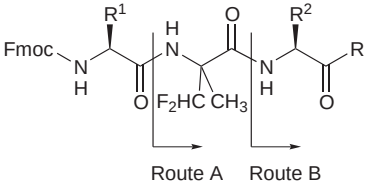
in C-terminal position because of its simplicity and inexpensive reagents (Scheme 4). Several solvent mixtures THF/DMF, ethyl acetate/DMF and CH_2Cl_2/DMF (7 : 1–10 : 1) can be used. The presence of DMF results in higher yields and shorter reaction times (12–16 h) due to better solubility of the amino acid derivatives.



SCHEME 4
C-Terminal incorporation of α-Dfm amino acids *via* mixed anhydride method

The resulting dipeptides **6a**–**6g** carry orthogonal protective groups. Therefore, they are valuable building blocks for peptide synthesis useful in fragment condensation.

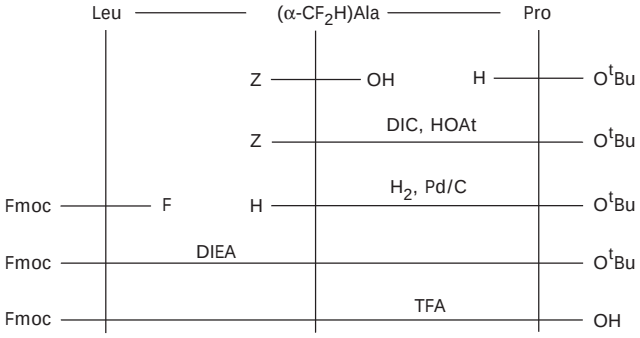
Tripeptides having (α-CF_2H)-Xaa or (α-CF_2Cl)-Xaa in position 2 are readily obtainable from Z- and Boc-protected dipeptides **3**–**5** (Scheme 5).



SCHEME 5
Two different strategies for the synthesis of tripeptides with α-Dfm amino acids in position 2

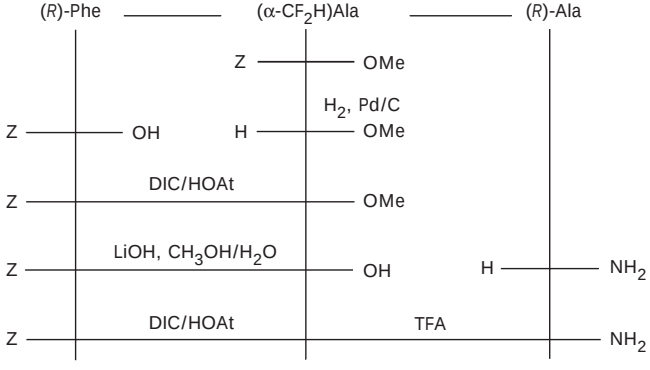
The Z-protecting group was cleaved by hydrogenolysis in the presence of Pd/C. Then, the dipeptide ester was reacted at room temperature under inert gas with the corresponding Fmoc-amino acid fluorides in a solvent mixture of CH_2Cl_2/DMF (2 : 1) in the presence of DIEA (Route A, Scheme 6). Finally, the *tert*-butyl ester was deblocked with 50% TFA in CH_2Cl_2 to give the Fmoc-protected tripeptides.

In a second approach (Route B, Scheme 7) we started from dipeptides **6**. After cleavage of methyl ester with LiOH in a CH_3OH/H_2O mixture (3 : 1) at 5 °C, [2+1]- and [2+2]-fragment condensations with amino acid amides and dipeptide amides on treatment with DIC/HOAt provide ready access to tri- and tetrapeptides with α-Dfm amino acids in position 2 (Table IV).



SCHEME 6
Route A, synthesis of **7a/1**

When α-(fluoroalkyl) α-amino acids are introduced in peptide synthesis as racemic mixtures or peptide fragments as diastereomer mixtures, pairs of diastereomers are obtained which can be readily separated by flash chromatography and medium pressure liquid chromatography (MPLC). For example, separation of the diastereomer mixtures of dipeptides **3c**, **4c**, **6d** and **6e** and of tripeptides **7a**–**7c** and **8** has been successfully performed[18b]. The homochiral pairs of tripeptides **7a/1**, **7a/2**–**7b/1**, **7b/2**–**7c/1**, **7c/2** and **8/1**, **8/2** obtained by flash chromatography or MPLC of the diastereomer mixtures are identical to those obtained *via* homochiral reagents (*e.g.* **3a/1**→**7a/1** and **3a/2**→**7a/2**).



SCHEME 7
Route B, synthesis of **7c/1**

TABLE IV
Synthesis of tri- and tetrapeptides with α-CF_2H and α-CF_2Cl amino acids in position 2

No.	Product	Educt	Yield %
7a	Fmoc-Leu-(α-Dfm)Ala-Pro-O^{t}Bu	Z-(α-Dfm)Ala-Pro-O^{t}Bu	
7b	Z-Phe-(α-Dfm)Ala-Ala-NH_2	Z-Phe-(α-Dfm)Ala-OMe	75
7c	Z-(*R*)-Phe-(α-Dfm)Ala-(*R*)-Ala-NH_2	Z-(*R*)-Phe-(α-Dfm)Ala-OMe	85
8	Fmoc-Leu-(α-CF_2Cl)Ala-Pro-O^{t}Bu	Z-(α-CF_2Cl)Ala-Pro-O^{t}Bu	
9	Boc-Tyr(O^{t}Bu)-(α-Dfm)Ala-Phe-Phe-NH_2	Boc-Tyr(O^{t}Bu)-(α-Dfm)Ala-Phe-OMe	78

The absolute configuration of the (fluoroalkyl)-substituted amino acids was determined for **7a/1** Fmoc-Leu-**(*R*)-(α-Dfm)Ala**-Pro-O^{t}Bu, for **7c/1** Z-(*R*)-Phe-**(*R*)-(α-Dfm)Ala**-(*R*)-Ala-NH_2 and for **8/1** Fmoc-Leu-**(*S*)-(α-CF_2Cl)Ala**-Pro-O^{t}Bu. The configuration of the α-fluoroalkyl α-amino acid can be identified directly from the X-ray structure[33], since there are two independent chiral reference centers present in the molecule (Table V). In the case of **7a/1** the chiral reference centers are (*S*)-Leu and (*S*)-Pro. The *SRS*-configuration was determined for **7a/1**. Since **7a/1** was synthesized from **3a/1**, the latter has *RS*-configuration. Consequently, **3c/2** is *SS* and **7a/2** is *SSS*-configured. A similar argumentation was applied to elucidation of the stereochemistry of dipeptides **4c/1**, **4c/2** and **6e/1** and **6e/2**.

Molecular structure of peptide **8/1** is shown in Fig. 1. The same atom numbering was chosen for **7a/1** and **8/1**. A comparison of the torsion angles shows that the α,α-disubstituted amino acid has the same conformation in both peptides (Table VI).

Compounds **7a/1** and **8/1** were found to be isomorphic (Table V). The structure of both peptides differs only in the type of halogen substituents. Despite the fact that **8/1** bears an additional chlorine atom, the difference of the cell volumes was shown to be only 28.8 Å^3. According to the volume increment system of Immirizi and Perini[33], the difference in volumes between a hydrogen atom (6.9 Å^3) and a chlorine atom (26.7 Å^3) is 19.8 Å^3. Therefore, the difference between the cell volumes should be 80 Å^3 calculating with four formula units within the space group Cc. Intermolecular hydrogen bonds between the atoms N(1)–H(1N)–O(1) and N(2)–H(2N)–O(2) were found to be a special feature of these strctures. Figure 2 shows the intermolecular hydrogen bonds along the monoclinic axis *b*, corresponding

TABLE V
Crystal data for **7a/1**, **7c/1**, and **8/1**

Parameters	**8/1**	**7a/1**	**7c/1**
Formula	$C_{34}H_{42}ClF_2N_3O_6$	$C_{34}H_{43}F_2N_3O_6$	$C_{24}H_{38}F_2N_4O_5 \cdot CH_3OH$
M	662.2	627.7	522.55
Temperature, K	223	223	213
Crystal system	monoclinic	monoclinic	monoclinic
Space group	*C*2	*C*2	$P2_1$
Unit cell dimension, Å			
a	39.5751(6)	39.236(5)	12.52760(10)
b	5.9819(10)	5.9509(8)	8.2386(2)
c	14.6786(3)	14.764(2)	14.3409(3)
β, °	102.51(1)	102.65(1)	112.510(1)
V, Å^3	3392.4(1)	3363.6(7)	1367.4
Z	4	4	2
D_{calc}, g cm^{-3}	1.296	1.240	1.269
μ (MoKα), mm^{-1}	0.171	0.092	0.100
2θ, °	54.4	50.0	54.0
Number of reflections collected	9006	7740	12118
Number of unique reflections	6069	4493	5012 (R_{int} = 0.0317)
Number of observed reflections	5596	2526	3960
Data used/parameters refined	6069/415	4493/414	5012/463
R_1, wR_2(obs)	0.0456, 0.1146	0.0923, 0.1434	0.0375, 0.0788
Goddness-of-fit on F^2	1.116	1.099	1.079
Largest difference peak and hole, e Å^3	0.386 and –0.255	0.224 and –0.284	0.206 and –0.207

data are given in Table VI. Obviously, strong intermolecular hydrogen bonds dominate the molecular structure and cause the isomorphism of both peptides. Remarkably, in α-(CF_2Cl)-substituted peptide **8/1**, we could

TABLE VI
Torsion angles of **7a/1** and **8/1**

Amino acid	Angle	Fmoc-Leu-Xaa-Pro-OtBu Xaa = (*R*)-(α-CF_2H)Ala	Fmoc-Leu-Xaa-Pro-OtBu Xaa = (*S*)-(α-CF_2Cl)Ala
Phe	Φ^{+1}	–93.3	–98.3
	Ψ^{+1}	–28.3	–26.9
Xaa	Φ^{+2}	41.8	49.3
	Ψ^{+2}	58.3	48.6–78.0
Pro	Φ^{+3}	–75.3	
	Ψ^{+3}	–8.7	–8.3

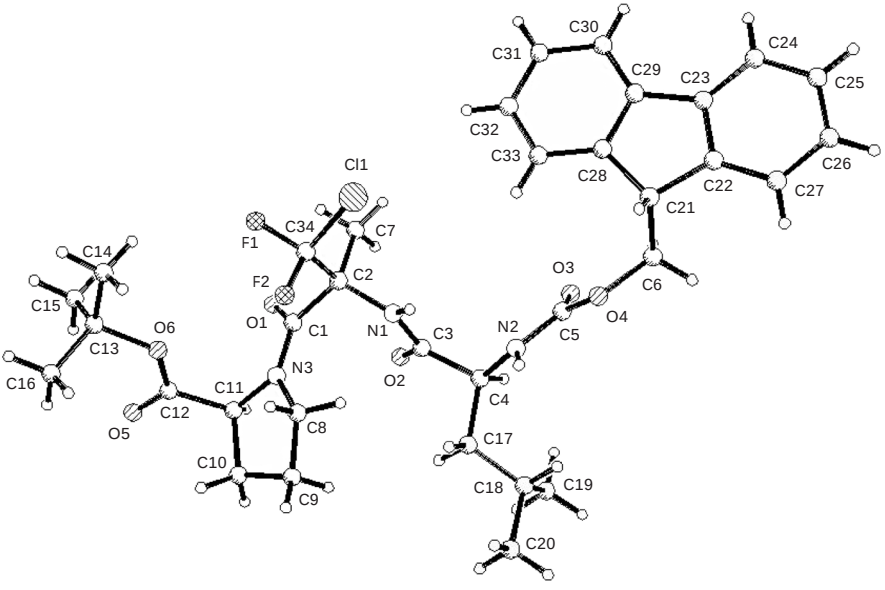


FIG. 1
Molecular structure of Fmoc-Leu-(*S*)-(α-CF_2Cl)Ala-Pro-O^{t}Bu (**8/1**)

find a weak intramolecular hydrogen bond between the atoms C(7)–H(7B)–F(1) as well as an intermolecular hydrogen bond between C(15)–H(15A)–F(2) (Table VII).

TABLE VII
Strong hydrogen bonds for **7a/1** and **8/1** (Å and °)

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	<(DHA)
Peptide **7a/1**				
N(1)–H(1N)···O(1)a	0.90	2.42	3.134(8)	136.2
N(2)–H(2N)···O(2)a	0.90	2.12	2.870(7)	140.5
N(2)–H(2N)···O(2)	0.90	2.45	2.823(8)	104.9
Peptide **8/1**				
N(1)–H(1N)···O(1)a	0.77	2.52	3.126(3)	136.8
N(2)–H(2N)···O(2)a	0.75	2.17	2.917(3)	171.0
N(2)–H(2N)···O(2)	0.77	2.47	2.827(3)	109.8
C(7)–H(7B)–F(1)b	0.94	2.44	2.80	101.9
C(15)–H(15A)–F(2)b	0.97	2.52	3.34	141.9

Symmetry transformations used to generate equivalent atoms: a) $x, y - 1, z$; b) $x, 1 + y, z$.

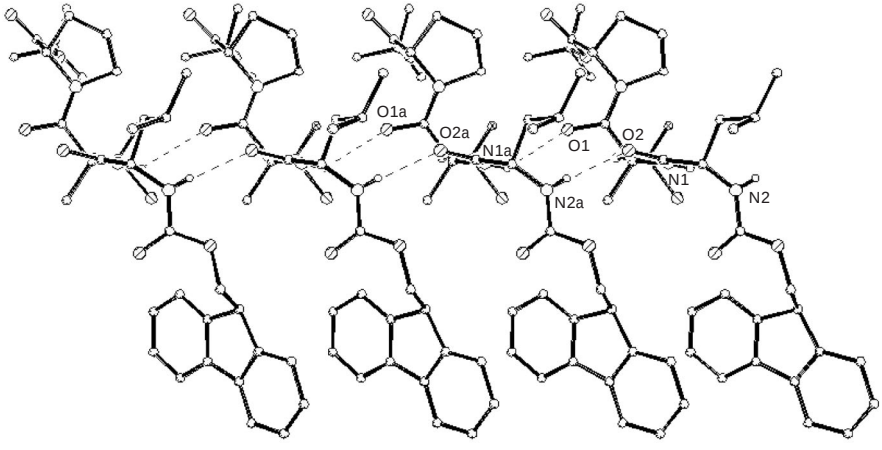


FIG. 2
Strong intermolecular hydrogen bonds of **8/1**; for hydrogen bond parameters, see Table VI

The crystal structure of **7c/1** is shown in Fig. 3. This peptide structure is characterized by five strong intermolecular hydrogen bonds between the carbonyl and amino groups (Fig. 4). Data are given in Table VIII.

TABLE VIII
Hydrogen bonds for **7c/1**

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	<(DHA)
N(1)–H(1NA)···O(71)a	0.77(4)	2.20(4)	2.936(3)	159(4)
N(4)–H(4N)···O(5)b	0.80(3)	2.09(3)	2.875(3)	168(3)
N(1)–H(2NB)···O(1)c	0.87(3)	2.15(3)	2.885(4)	143(3)
O(99)–H(99O)···O(3)d	0.76(5)	2.14(5)	2.806(3)	146(4)
N(2)–H(2N)···O(71)	0.89(3)	2.14(4)	3.012(3)	165(3)
N(3)–H(3N)···O(99)	0.82(3)	2.10(3)	2.918(3)	172(4)

Symmetry transformations used to generate equivalent atoms: a) $-x + 1, y - 1/2, -z$; b) $-x + 2, y + 1/2, -z$; c) $-x + 1, y + 1/2, -z$; d) $x, y + 1, z$

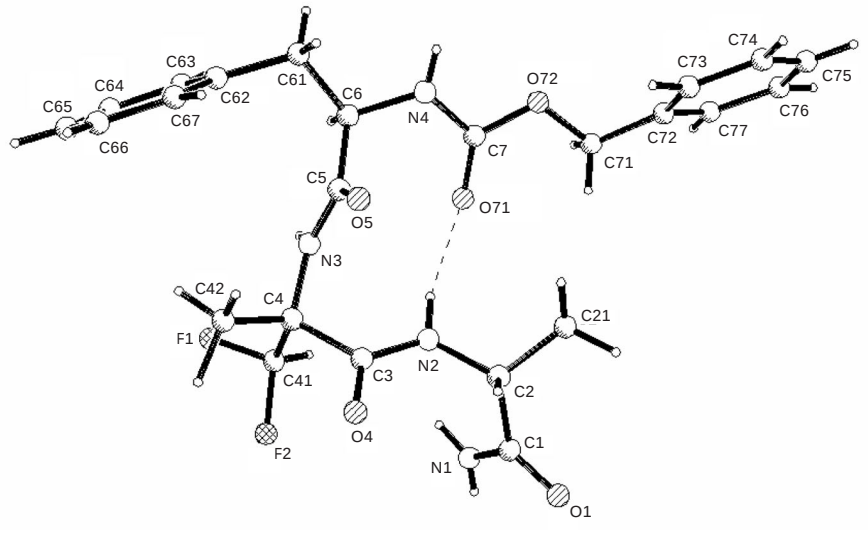


FIG. 3
Molecular structure of Z-(*R*)-Phe-(*R*)-(α-Dfm)Ala-(*R*)-Ala-NH_2 (**7c/1**)

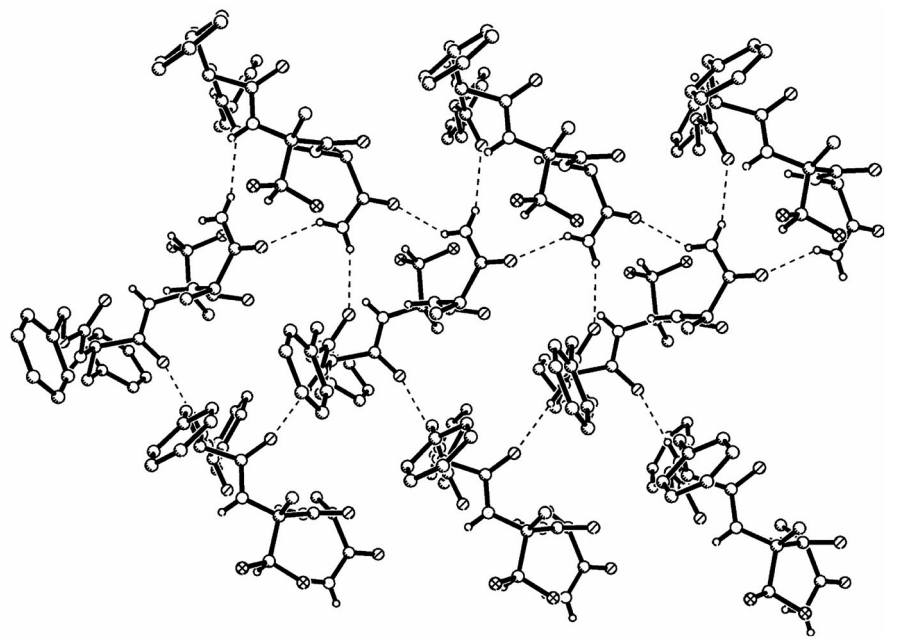


FIG. 4
Strong intermolecular hydrogen bonds of **7c/1**, for hydrogen bond parameters, see Table VIII

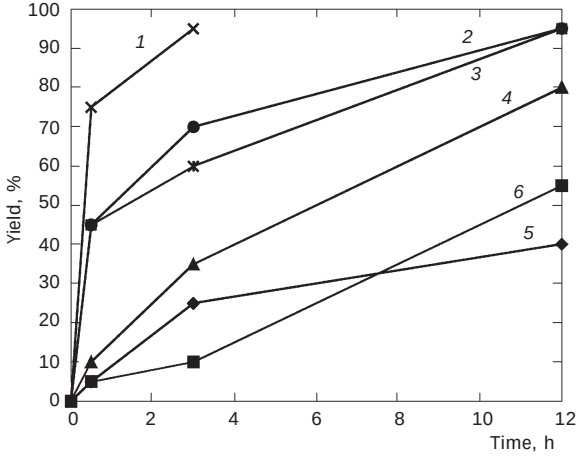


FIG. 5
Comparison of the efficiency of currently used peptide coupling reagents. *1* HATU, *2* PyAOP, *3* DIC/HOAt, *4* PyBOP, *5* HBTU, *6* DIC/HOBt

Conclusion

(α-Dfm)Ala as well as (α-Tfm)Ala represent chiral Aib surrogates. Therefore, both of these amino acids themselves and their peptides are of current interest as building blocks for the synthesis of modified peptides and peptaiboles[34]. We tested some currently used peptide coupling reagents for the N- and C-terminal incorporation of α-(CF_2H), α-(CF_2Cl) and α-(CF_2Br) amino acids into peptides (Fig. 5). We found that N-terminal incorporation can be performed without any problems using the reagent combination DIC/HOAt, while for C-terminal incorporation the reagent combinations DIC/HOAt, HATU, PyBroP and PyAOP are superior to DIC/HOBt, HBTU and PyBOP. Amino acid fluorides and chlorides gave excellent yields in C-terminal peptide coupling reactions of α-Dfm amino acids. The application of α-(fluoroalkyl) amino acids in the solid phase synthesis is reported elsewhere.

EXPERIMENTAL

General

^{1}H NMR spectra were recorded at 200 and 300 MHz with Me_4Si as internal standard. ^{13}C NMR spectroscopy was performed at 50, 75 and 100 MHz. ^{19}F NMR spectra were obtained at 188 and 282 MHz with trifluoroacetic acid as external standard, downfield shifts being designated as positive. Chemical shifts (δ) are given in ppm, coupling constants (*J*) in Hz. Mass spectra were obtained using EI ionization at 70 eV. Optical rotations were measured with a Schmidt and Haensch Polartronic-D polarimeter; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Melting points were determined on a Boetius apparatus. CCD diffractometer (AXS Bruker) was used for X-ray measurements (MoKα radiation, 0.71069 A at room temperature).

All reactions were routinely monitored by ^{19}F NMR spectroscopy, TLC or HPLC. Analytical TLC was performed using Merck precoated silica gel 60F-254 plates (0.25 mm). For flash chromatography, silica gel 60 (30–60 μm) was used with hexanes/ethyl acetate as solvent systems.

Organic solvents were dried and distilled prior to use.

General Procedure for Incorporation of α-(Difluoromethyl)-Substituted Amino Acids into N-Terminal Position of Peptides

Method A: Activation with DIC. To a solution of a Z-protected α-(difluoromethyl) amino acid (2 mmol) in a 2 : 1 mixture of CH_2Cl_2/DMF (7 ml) DIC (0.27 g, 2.1 mmol) was added and stirred at room temperature for 15 min, then the corresponding amino acid ester (3 mmol) and DIEA (0.35 g, 2.7 mmol) in a 2 : 1 mixture of CH_2Cl_2/DMF (7 ml) were added. After stirring for 12 h, the reaction mixture was acidified with 10% citric acid to pH 3–4. The solution was partitioned between H_2O (20 ml) and CH_2Cl_2 (30 ml). The organic layer was washed with 10% citric acid (20 ml), brine (20 ml), 1 M $NaHCO_3$ (20 ml), brine (20 ml) and

dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded a mixture of two diastereomers. The crude product was purified by recrystallization from $CHCl_3$/hexanes or by flash chromatography (SiO_2, EtOAc/hexanes).

Method B: Activation with DIC/HOAt. A solution of a Z-protected α-(difluoromethyl) amino acid (2 mmol) in a 2 : 1 mixture of CH_2Cl_2/DMF (7 ml), was stirred with DIC (0.27 g, 2.1 mmol) and HOAt (0.29 g, 2.1 mmol) at room temperature for 5 min. A solution of the corresponding amino acid ester (3 mmol) and DIEA (0.35 g, 2.7 mmol) in a 2 : 1 mixture of CH_2Cl_2/DMF (7 ml) was added to the vigorously stirred reaction mixture in one portion. After 15–20 min stirring at room temperature, the products were isolated; for work-up see above. The spectral and analytical data are identical to peptides obtained by method *A*.

tert-Butyl *N*-{*N*-[(Benzyloxy)carbonyl]-(*RS*)-2-(difluoromethyl)alanyl}glycinate (**3a**)

Method A: Yield 0.57 g (73%). MS (FAB, *m/z*), calculated for $C_{18}H_{24}F_2N_2O_5$: 386.39; found: 409.2 $[M + Na]^+$. M.p. 114–116 °C. R_F 0.22 (AcOEt/hexanes 1 : 3). ^{1}H NMR (200 MHz, $CDCl_3$): 1.47 (s, 9 H); 1.70 (s, 3 H); 3.90 (dd, $^2J_{HH}$ = 18.5, $^3J_{HH}$ = 4.9, 1 H); 3.98 (dd, $^2J_{HH}$ = 18.5, $^3J_{HH}$ = 4.9, 1 H); 5.10 (s, 2 H); 5.97 (s, br, 1 H); 6.39 (t, $^2J_{HF}$ = 56.2, 1 H); 6.71 (s, br, 1 H); 7.36 (s, 5 H). ^{13}C NMR (50 MHz, $CDCl_3$): 18.6; 28.4; 42.9; 61.6 (t, $^2J_{CF}$ = 23.0); 67.6; 83.2; 114.7 (t, $^1J_{CF}$ = 249.0); 128.6; 128.8; 129.1; 136.3; 155.5; 168.8; 169.0. ^{19}F NMR (188 MHz, $CDCl_3$): –52.20 (dd_{ABX}, $^2J_{FF}$ = 280.0, $^2J_{HF}$ = 56.0, 1 F); –50.91 (dd_{ABX}, $^2J_{FF}$ = 280.0, $^2J_{HF}$ = 56.0, 1 F).

tert-Butyl *ambo-N*-{*N*-[(Benzyloxy)carbonyl]-2-(difluoromethyl)alanyl}phenylalaninate (**3b**)

Method B: Yield 0.86 g (90%). MS (MALDI-TOF), calculated for $C_{25}H_{30}F_2N_2O_5$: 476.52; found: 499.18 $[M + Na]^+$; 515.14 $[M + K]^+$. M.p. 114–120°C. R_F 0.46 (AcOEt/hexanes 1 : 3). ^{1}H NMR (200 MHz, $CDCl_3$): 1.41/1.42 (s, 9 H); 1.58/1.62 (s, 3 H); 3.12 (d, $^3J_{HH}$ = 6.0, 2 H); 4.74 (m, 1 H); 5.08 (s, 2 H); 6.02 (s, br, 1 H); 6.36/6.37 (t, $^2J_{HF}$ = 50.4, 1 H); 6.76 (m, 1 H); 7.10–7.35 (m, 10 H). ^{13}C NMR (50 MHz, $CDCl_3$): 18.5; 28.3; 38.1/38.2; 54.5; 61.5 (t, $^2J_{CF}$ = 22.0); 67.5; 83.2; 114.6 (t, $^1J_{CF}$ = 250.0); 127.6; 128.5/128.6; 128.8; 128.9; 129.1; 130.0/130.1; 136.2/136.4; 155.4; 168.3/168.4; 170.3/170.4. ^{19}F NMR (188 MHz, $CDCl_3$): –50.58/–50.92 (dd_{ABX}, $^2J_{FF}$ = 288.0, $^2J_{HF}$ = 50.0, 1 F); –51.71/–51.03 (dd_{ABX}, $^2J_{FF}$ = 288.0, $^2J_{HF}$ = 50.0, 1 F).

tert-Butyl *ambo-N*-{*N*-[(Benzyloxy)carbonyl]-2-(difluoromethyl)alanyl}prolinate (**3c**)

Method B: Yield 0.77 g (90%). MS (MALDI-TOF), calculated for $C_{21}H_{28}F_2N_2O_5$: 426.46; found: 449.4 $[M + Na]^+$; 465.5 $[M + K]^+$. Separation of the diastereomers: MPLC; eluent: AcOEt/hexanes 2 : 9.

Diastereomer 1 (**3c**/**1**): M.p. 140 °C. $[\alpha]_D^{25}$ –94.0 (*c* 1, CH_2Cl_2). R_F 0.40 (AcOEt/hexanes 1 : 2). ^{1}H NMR (300 MHz, $CDCl_3$): 1.44 (s, 9 H); 1.55 (s, 3 H); 1.72 (m, 2 H); 1.85 (m, 1 H); 1.96 (m, 1 H); 3.43 (m, 1 H); 3.63 (m, 1 H); 4.42 (m, 1 H); 5.12 (m, 3 H); 6.36 (t, $^2J_{HF}$ = 56.2, 1 H); 7.35 (m, 5 H). ^{13}C NMR (50 MHz, $CDCl_3$): 16.6; 25.8; 28.1; 28.3; 47.5; 61.1 (t, $^2J_{CF}$ = 19.0); 61.6; 67.7; 81.6; 114.6 (t, $^1J_{CF}$ = 249); 128.9; 129.0; 129.1; 136.4; 155.2; 167.7; 171.7. ^{19}F NMR (282 MHz, $CDCl_3$): –52.44 (dd_{ABX}, $^2J_{FF}$ = 280.0, $^2J_{HF}$ = 56.0, 1 F); –53.50 (dd_{ABX}, $^2J_{FF}$ = 280.0, $^2J_{HF}$ = 56.0, 1 F).

Diastereomer 2 (**3c**/**2**): M.p. 132–133°C. $[\alpha]_D^{25}$ –24 (*c* 1, CH_2Cl_2). R_F 0.32 (AcOEt/hexanes 1 : 2). ^{1}H NMR (300 MHz, $CDCl_3$): 1.44 (s, 9 H); 1.79 (s, 3 H); 1.81 (m, 2 H); 1.95 (m, 2 H); 3.57

(m, 1 H); 3.68 (m, 1 H); 4.38 (m, 1 H); 5.11 (s, 2 H); 5.78 (s, 1 H); 6.23 (t, $^2J_{HF}$ = 56.2, 1 H); 7.35 (m, 5 H). ^{13}C NMR (50 MHz, $CDCl_3$): 17.4; 26.1; 28.2; 28.3; 48.0; 62.5 (t, $^2J_{CF}$ = 19); 62.6; 67.5; 81.6; 115.4 (t, $^1J_{CF}$ = 249); 128.7; 129.0; 129.1; 136.5; 155.1; 167.5; 171.6. ^{19}F NMR (282 MHz, $CDCl_3$): –49.65 (dd_{ABX}, $^2J_{FF}$ = 280.0, $^2J_{HF}$ = 56.0, 1 F); –52.00 (dd_{ABX}, $^2J_{FF}$ = 280.0, $^2J_{HF}$ = 56.0, 1 F).

tert-Butyl *ambo-N*-{*N*-[(Benzyloxy)carbonyl]-2-(chlorodifluoromethyl)alanyl}glycinate (**4a**)

Method A: Yield 0.66 g (78%). MS (FAB; *m/z*), calculated for $C_{18}H_{23}ClF_2N_2O_5$: 420.84; found: 443.4 $[M + Na]^+$. M.p. 83–85 °C. R_F 0.27 (AcOEt/hexanes 1 : 3). ^{1}H NMR (200 MHz, $CDCl_3$): 1.47 (s, 9 H); 1.95 (s, 3 H); 3.90 (dd, $^2J_{HH}$ = 18.4, $^3J_{HH}$ = 4.8, 1 H); 4.01 (dd, $^2J_{HH}$ = 18.4, $^3J_{HH}$ = 4.8, 1 H); 5.10 (s, 2 H); 6.00 (s, br, 1 H); 6.66 (t, $^3J_{HH}$ = 4.8, 1 H); 7.36 (s, 5 H). ^{13}C NMR (50 MHz, $CDCl_3$): 18.2; 28.4; 43.2; 67.1 (t, $^2J_{CF}$ = 25.0); 67.7; 83.3; 128.7; 128.8; 129.1; 129.7 (dd_{ABX}, $^1J_{CF}$ = 301.0, 303.0); 136.3; 154.7; 166.5; 168.7. ^{19}F NMR (188 MHz, $CDCl_3$): 16.32 (d_{AB}, $^2J_{FF}$ = 163.0, 1 F); 17.30 (d_{AB}, $^2J_{FF}$ = 163.0, 1 F).

tert-Butyl *ambo-N*-{*N*-[(Benzyloxy)carbonyl]-2-(chlorodifluoromethyl)alanyl}-phenylalaninate (**4b**)

Method A: Yield 0.85 g (83%). MS (FAB, *m/z*), calculated for $C_{25}H_{29}ClF_2N_2O_5$: 510.96; found: 511.6 $[M + H]^+$. M.p. 89–93 °C. R_F 0.10 (AcOEt/hexanes 1 : 10). ^{1}H NMR (200 MHz, $CDCl_3$): 1.39/1.41 (s, 9 H); 1.84/1.90 (s, 3 H); 3.05 (m, 2 H); 4.72 (m, 1 H); 5.08 (s, 2 H); 6.05/6.09 (s, 1 H); 6.62/6.69 (d, $^2J_{HH}$ = 7.2/7.2); 7.25 (m, 10 H). ^{13}C NMR (50 MHz, $CDCl_3$): 18.1/18.3; 28.3; 38.1; 54.9; 67.0/67.2 (t, $^2J_{CF}$ = 25.0); 67.6; 83.2/83.3; 127.6; 128.7; 128.8/128.9; 129.0; 129.1; 129.8 (t, $^2J_{CF}$ = 304); 136.3; 136.4; 154.7/154.8; 166.0; 170.3/170.4. ^{19}F NMR (188 MHz, $CDCl_3$): 16.36/16.54 (d_{AB}, $^2J_{FF}$ = 165.0, 1 F), 17.54/17.61 (d_{AB} = 165.0, 1 F).

tert-Butyl *ambo-N*-{*N*-[(Benzyloxy)carbonyl]-2-(chlorodifluoromethyl)alanyl}prolinate (**4c**)

Method B: Yield 0.87 g (93%). MS (MALDI-TOF), calculated for $C_{21}H_{27}ClF_2N_2O_5$: 460.90; found: 483.0 $[M + Na]^+$; 498.9 $[M + K]^+$. Separation of the diastereomers: MPLC; eluent: AcOEt/hexanes 2 : 9.

Diastereomer 1 (**4c/1**): M.p. 148 °C. $[\alpha]_D^{25}$ –54 (*c* 1, CH_2Cl_2). R_F 0.21 (AcOEt/hexanes 1 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): 1.43 (s, 9 H); 1.60–2.00 (m, 4 H); 1.93 (s, 3 H); 3.38 (m, 1 H); 3.74 (m, 1 H); 4.36 (m, 1 H); 5.13 (s, 2 H); 5.66 (s, 1 H); 7.37 (m, 5 H). ^{13}C NMR (APT; 75 MHz, $CDCl_3$): 19.4; 25.8; 27.7; 27.8; 48.3; 61.6; 67.4; 67.7 (t, $^2J_{CF}$ = 23.0); 81.2; 128.5; 128.6; 129.8 (t, $^1J_{CF}$ = 301.0); 135.8; 154.0; 163.8; 171.1. ^{19}F NMR (282 MHz, $CDCl_3$): 17.07 (d, $^2J_{FF}$ = 164.0, 1 F); 18.50 (d, $^2J_{FF}$ = 164.0, 1 F).

Diastereomer 2 (**4c/2**): M.p. 162–164 °C. $[\alpha]_D^{25}$ –74 (*c* 1, CH_2Cl_2). R_F 0.16 (AcOEt/hexanes 1 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): 1.44 (s, 9 H); 1.55–2.00 (m, 4 H); 1.86 (s, 3 H); 3.52 (m, 1 H); 3.61 (m, 1 H); 4.38 (m, 1 H); 5.13 (s, 2 H); 5.44 (s, br, 1 H); 7.36 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$): 18.8; 25.6; 27.8; 27.9; 47.8; 61.9; 66.1 (t, $^2J_{CF}$ = 23.0); 67.4; 81.2; 128.4; 128.5; 128.6; 130.5 (t, $^1J_{CF}$ = 304.0); 135.9; 154.1; 164.2; 171.1. ^{19}F NMR (282 MHz, $CDCl_3$): 18.19 (s, br, 2 F).

tert-Butyl *N*-{*N*-[(Benzyloxy)carbonyl]-(*RS*)-2-(chlorodifluoromethyl)phenylalanyl}-glycinate (**4d**)

Method A: Yield 0.85 g (85%). MS (MALDI-TOF), calculated for $C_{24}H_{27}ClF_2N_2O_5$: (496.94); found: 519.06 $[M + Na]^+$; 535.04 $[M + K]^+$. M.p 118–119 °C. R_F 0.19 (AcOEt/hexanes 1 : 6). ^{1}H NMR (200 MHz, $CDCl_3$): 1.51 (s, 9 H); 3.26 (d, $^2J_{HH}$ = 13.8, 1 H); 3.94 (d, $^3J_{HH}$ = 4.4, 2 H); 4.36 (d, $^2J_{HH}$ = 13.8, 1 H); 5.04 (d, $^2J_{HH}$ = 12.4, 1 H); 5.24 (d, $^2J_{HH}$ = 12.4, 1 H); 6.37 (s, br, 1 H); 6.76 (m, 1 H); 7.20 (m, 5 H); 7.37 (s, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$): 28.1; 33.7; 43.0; 67.0; 70.4 (dd, $^2J_{CF}$ = 24.0, 26.0); 83.3; 127.5; 128.3; 128.4; 128.6; 129.5 (dd_{ABX}, $^1J_{CF}$ = 301.0, 308.0); 130.0; 133.1; 136.2; 154.2; 164.5 (d, $^3J_{CF}$ = 4); 168.0. ^{19}F NMR (188 MHz, $CDCl_3$): 16.76 (d_{AB}, $^2J_{FF}$ = 169.0, 1 F); 20.60 (d_{AB}, $^2J_{FF}$ = 169.0, 1 F).

tert-Butyl *ambo-N*-{*N*-[(Benzyloxy)carbonyl]-2-(bromodifluoromethyl)alanyl}-phenylalaninate (**5**)

Method A: Yield 0.96 g (86%). MS (FAB, *m/z*), calculated for $C_{25}H_{29}BrF_2N_2O_5$: 555.41; found: 555.5 $[M + H]^+$. M.p. 98–99 °C. R_F 0.49 (AcOEt/hexanes 1 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): 1.39/1.42 (s, 9 H); 1.84/1.91 (s, 3 H); 3.11 (m, 2 H); 4.74 (m, 1 H); 5.10 (s, 2 H; 5.96/6.03 (s, br, 1 H); 6.56/6.63 (d, $^3J_{HH}$ = 6.9/7.2, 1 H); 7.10–7.40 (m, 10 H). ^{13}C NMR (50 MHz, $CDCl_3$): 17.9/18.1; 27.9; 37.8; 54.5; 67.2; 67.6/67.7 (t, $^2J_{CF}$ = 22.0); 82.9/83.0; 123.7/123.8 (t, $^1J_{CF}$ = 315.0); 127.2; 128.2; 128.3/128.4; 128.5/128.6; 129.5; 129.6; 135.7; 135.8/135.9; 155.0; 165.3; 169.7/169.8. ^{19}F NMR (282 MHz, $CDCl_3$): 22.67/22.93 (d_{AB}, $^2J_{FF}$ = 165.0, 1 F); 24.26/24.28 (d_{AB}, $^2J_{FF}$ = 165.0, 1 F).

General Procedure for Incorporation of α-(Fluoroalkyl)-Substituted Amino Acids into C-Terminal Position of Peptides (**6**, **7**)

Method A: Fmoc-amino acid fluoride method. A solution of Fmoc-protected amino acid fluoride (2.5 mmol) in a 2 : 1 mixture of CH_2Cl_2/DMF (7 ml) was added to the corresponding C-protected α-(fluoroalkyl)-substituted amino acid or dipeptide (1 mmol) and DIEA (0.33 g, 2.5 mmol) in a 2 : 1 mixture of CH_2Cl_2/DMF (7 ml) and stirred at room temperature for 16 h. The reaction mixture was acidified with 10% citric acid to pH 3–4 and extracted with CH_2Cl_2 (3 × 10 ml). The combined organic layer was washed with 10% citric acid (10 ml), brine (10 ml), 5% $NaHCO_3$ (10 ml) and brine (10 ml), then dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude products were purified by recrystallization from $CHCl_3$/hexanes or by flash chromatography (SiO_2, EtOAc/hexanes).

Method B: Mixed anhydride method. A solution of the N-protected amino acid (2.05 mmol) in a 10 : 1 mixture of AcOEt/DMF (7 ml) was cooled to –30 °C and neutralized with *N*-methylmorpholine (NMM; 0.2 g, 2 mmol). Isobutyl chloroformate (0.3 g, 2.20 mmol) was added at –15 °C. After 10 min at –15 °C, a solution of a precooled α-(fluoroalkyl)-substituted amino acid or peptide ester (1 mmol) in a 10 : 1 mixture of AcOEt/DMF (5 ml) was added. The reaction mixture was stirred at –15 °C for 2 h and then warmed up to room temperature After 12–16 h stirring at room temperature peptides were isolated. Work-up procedure, see method *A*.

Methyl *ambo*-*N*-{*N*-[(Fluoren-9-ylmethoxy)carbonyl]phenylalanyl}-2-(difluoromethyl)-alaninate (**6a**)

Method A: Yield 0.42 g (81%); 1 : 1 mixture of two diastereomers. MS (FAB, *m/z*), calculated for $C_{29}H_{28}F_2N_2O_5$: 522.55; found: 523.5 $[M + H]^+$; 545.5 $[M + Na]^+$. M.p. 140–143 °C. R_F 0.20 (AcOEt/hexanes 1 : 3). Racemization test: 0.13% (±0.01%) (*R*)-Phe. ^{1}H NMR (300 MHz, $CDCl_3$): 1.48/1.51 (s, 3 H); 3.02/3.11 (m, 2 H); 3.78/3.79 (s, 3 H); 4.20 (m, 1 H); 4.42 (m, 1 H); 4.45 (m, 2 H); 5.28 (s, br, 1 H); 6.18/6.20 (t, $^2J_{FH}$ = 56.0, 1 H); 6.30/6.42 (s, 1 H); 7.15–7.83 (m, 13 H). ^{13}C NMR (75 MHz, $CDCl_3$): 16.3; 38.2; 47.2; 53.2; 55.9/56.0; 61.0/61.2 (t, $^2J_{CF}$ = 23/23); 67.2; 112.8/113.2 (t, $^1J_{CF}$ = 249/249); 120.0; 125.0; 127.1; 127.8; 128.9; 129.4; 136.0; 141.4; 143.6/143.7; 155.9; 169.2; 171.0. ^{19}F NMR (282 MHz, $CDCl_3$): –52.72/–52.42 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F); –50.57/–50.51 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F).

Methyl *ambo*-*N*-{*N*-[(Fluoren-9-ylmethoxy)carbonyl]leucyl}-2-(difluoromethyl)-alaninate (**6b**)

Method A: Yield 0.35 g (72%); PyBroP: 0.28 g (58%); 1 : 1 mixture of two diastereomers. MS (MALDI-TOF), calculated for $C_{26}H_{30}F_2N_2O_5$: 488.53; found: 511.2 $[M + Na]^+$; 527.1 $[M + K]^+$. Oil. R_F 0.43 (AcOEt/hexanes 1 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): 0.94 (m, 6 H); 1.57 (s, 3 H); 1.60 (m, 3 H); 3.76/3.78 (s, 3 H); 4.22 (t, $^3J_{HH}$ = 6.6, 1 H); 4.24 (m, 1 H); 4.44 (m, 2 H); 5.13 (d, $^3J_{HH}$ = 7.2, 1 H); 6.25/6.26 (t, $^2J_{FH}$ = 56.2, 1 H); 6.96 (s, 1 H); 7.27–7.80 (m, 8 H). ^{13}C NMR (75 MHz, $CDCl_3$): 16.2/16.4 (s/t, $^3J_{CF}$ = 4); 22.0, 22.8; 24.6; 40.8/40.9); 47.2; 53.1; 53.2; 60.9 (t, $^2J_{CF}$ = 22.0); 67.2; 112.7/112.8 (t, $^1J_{CF}$ = 247.0, 247.0); 120.0; 124.7/125.0; 127.1; 127.6/127.8; 141.3; 143.6/143.7; 156.4; 169.5/169.6; 172.4. ^{19}F NMR (282 MHz, $CDCl_3$): –53.00/–52.79 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F); –50.54/–50.52 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F).

Methyl *ambo*-*N*-{*N*-[(Fluoren-9-ylmethoxy)carbonyl]valyl}-2-(difluoromethyl)-alaninate (**6c**)

Method A: Yield: 0.29 g (62%); PyBroP: 0.23 g (47%); 1 : 1 mixture of two diastereomers. MS (MALDI-TOF), calculated for $C_{25}H_{28}F_2N_2O_5$: 474.50; found: 497.0 $[M + Na]^+$; 513.0 $[M + K]^+$. Oil. R_F 0.34 (AcOEt/hexanes 1 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): 0.96 (m, 6 H); 1.59 (s, 3 H); 2.15 (m, 1 H); 3.78/3.80 (s, 3 H); 4.04 (m, 1 H); 4.23 (t, $^3J_{HH}$ = 6.6, 1 H); 4.44 (m, 2 H); 5.32/5.35 (s, br, 1 H); 6.17/6.28 (t, $^2J_{FH}$ = 56.0, 1 H); 6.65 (s, 1 H); 7.29–7.80 (m, 8 H). ^{13}C NMR (75 MHz, $CDCl_3$): 16.4/16.5; 17.7; 19.0; 30.9/31.0; 47.2; 53.1; 60.1; 60.7/61.0 (t, $^2J_{CF}$ = 22.0, 22.0); 67.2; 112.7 (t, $^1J_{CF}$ = 247); 120.0; 125.0; 127.1; 127.8; 141.3; 143.7/ 143.8; 156.4; 169.4/169.5; 171.3. ^{19}F NMR (282 MHz, $CDCl_3$): –52.87/–52.48 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F); –50.63/–50.53 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F).

Methyl *ambo*-*N*-{*N*-[(Benzyloxy)carbonyl]phenylalanyl}-2-(difluoromethyl)-alaninate (**6d**)

Method B: Yield 0.29 g (67%); 1 : 1 mixture of diastereomers. MS (FAB, *m/z*), calculated for $C_{22}H_{24}F_2N_2O_5$: 434.44; found: 435.4 $[M + H]^+$. Separation of the diastereomers by flash chromatography, eluent: AcOEt/hexanes 1 : 3.

Diastereomer 1 (**6d/1**): M.p. 79–80 °C. $[\alpha]_D$ +6 (*c* 1, CH_2Cl_2). R_F 0.16 (AcOEt/hexanes 1 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): 1.45 (s, 3 H); 3.04 (d, $^2J_{HH}$ = 7.2, 2 H); 3.76 (s, 3 H); 4.47 (m,

1 H); 5.08 (s, 2 H); 5.43 (d, $^2J_{HH}$ = 8.2, 1 H, NH); 6.17 (t, $^2J_{HH}$ = 55.6); 6.60 (s, 1 H, NH); 7.15–7.40 (m, 10 H). ^{13}C NMR (75 MHz, $CDCl_3$): 16.1 (t, $^3J_{CF}$ = 3.0); 38.2; 53.1; 55.9; 60.9 (t, $^2J_{CF}$ = 23); 67.3; 112.8 ($^1J_{CF}$ = 247.0); 127.2; 128.0; 128.3; 128.6; 128.8; 129.4; 136.1; 156.1; 169.4; 171.0. ^{19}F NMR (282 MHz, $CDCl_3$): –53.01 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F); –50.10 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F).

Diastereomer 2 (**6d/2**): M.p. 84–86 °C. $[\alpha]_D$ –11 (*c* 1, CH_2Cl_2). R_F 0.12 (AcOEt/hexanes 1 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): 1.48 (s, 3 H); 3.05 (d, $^3J_{HH}$ = 7.2); 3.76 (s, 3 H); 4.48 (m, 1 H); 5.09 (s, 2 H); 5.42 (d, $^3J_{HH}$ = 7.2, 1 H, NH); 6.19 (t, $^2J_{FH}$ = 56.0, 1 H); 6.72 (s, 1 H, NH); 7.15–7.35 (m, 10 H). ^{13}C NMR (100 MHz, $CDCl_3$): 15.7 (t, $^3J_{CF}$ = 3.0); 37.7; 52.7; 55.5; 60.5 (t, $^2J_{CF}$ = 23); 66.8; 112.4 (t, $^1J_{CF}$ = 247.0); 126.8; 127.6; 127.9; 128.2; 128.4; 129.0; 135.6; 155.7; 169.0 (d, $^3J_{CF}$ = 4.0); 172.4. ^{19}F NMR (282 MHz, $CDCl_3$): –52.60 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F); –50.41 (dd_{ABX}, $^2J_{CF}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F).

Methyl *ambo*-*N*-{*N*-[(Benzyloxy)carbonyl]-(*R*)-phenylalanyl}-2-(difluoromethyl)-alaninate (**6e**)

Method B: Yield 0.29 g (67%); 1 : 1 mixture of diastereomers. MS (FAB, *m/z*), calculated for $C_{22}H_{24}F_2N_2O_5$: 434.44; found: 435.4 $[M + H]^+$. Separation of the diastereomers by flash chromatography, eluent: AcOEt/hexanes 1 : 3.

Diastereomer 1 (**6e/1**): M.p. 79–81 °C. $[\alpha]_D$ –4 (*c* 1, CH_2Cl_2). R_F 0.16 (AcOEt/hexanes 1 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): 1.47 (s, 3 H); 3.02 (dd, $^2J_{HH}$ = 14.0, $^3J_{HH}$ = 7.3, 1 H); 3.11 (dd, $^2J_{HH}$ = 14.0, $^3J_{HH}$ = 6.4, 1 H); 3.78 (s, 3 H); 4.43 (m, 1 H); 5.08 (d, $^2J_{HH}$ = 12.0, 1 H); 5.12 (d, $^2J_{HH}$ = 12.0, 1 H); 5.29 (m, 1 H, NH); 6.16 (t, $^2J_{FH}$ = 56.5, 1 H); 6.39 (s, br, 1 H, NH); 7.20–7.40 (m, 10 H). ^{13}C NMR (75 MHz, $CDCl_3$): 16.2 (t, $^3J_{CF}$ = 3.0); 38.2; 53.1; 55.9; 60.9 (t, $^2J_{CF}$ = 23.0); 67.3; 112.8 (t, $^1J_{CF}$ = 247); 127.2; 128.0; 128.3; 128.6; 129.4; 136.1; 156.1; 169.3 (d, $^3J_{CF}$ = 3.0); 171.0. ^{19}F NMR (282 MHz, $CDCl_3$): –52.83 (dd_{ABX}, $^2J_{FF}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F); –50.59 (dd_{ABX}, $^2J_{FF}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F).

Diastereomer 2 (**6e/2**): M.p. 85–86 °C. $[\alpha]_D$ +12 (*c* 1, CH_2Cl_2). R_F 0.12 (AcOEt/hexanes 1 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): 1.49 (s, 3 H); 3.08 (m, 2 H); 3.78 (s, 3 H); 4.43 (m, 1 H); 5.08 (d, $^2J_{HH}$ = 12.3, 1 H); 5.13 (d, $^2J_{HH}$ = 12.3, 1 H); 5.29 (m, 1 H, NH); 6.19 (t, $^2J_{FH}$ = 56.0, 1 H); 6.44 (s, br, 1 H, NH); 7.18–7.40 (m, 10 H). ^{13}C NMR (75 MHz, $CDCl_3$): 16.1; 38.3; 53.1; 56.0; 61.0 (t, $^2J_{CF}$ = 23.0); 67.2; 112.8 (t, $^1J_{CF}$ = 249.0); 127.2; 128.0; 128.3; 128.6; 128.7; 129.4; 136.1; 156.1; 169.3 (d, $^3J_{CF}$ = 3); 171.1. ^{19}F NMR (282 MHz, $CDCl_3$): –52.49 (dd_{ABX}, $^2J_{FF}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F); –50.52 (dd_{ABX}, $^2J_{FF}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F).

Methyl *ambo*-*N*-{*N*-[(Benzyloxy)carbonyl]-O-*tert*-butyltyrosinyl}-2-(difluoromethyl)-alaninate (**6f**)

Method B: Yield 0.34 g (72%); mixture of diastereomers. MS (Maldi-TOF), calculated for $C_{23}H_{34}N_2F_2O_6$: 472.53; found: 495.4 $[M + Na]^+$; 511.4 $[M + K]^+$. Oil. R_F 0.25 (AcOEt/hexanes 1 : 3). ^{1}H NMR (300 MHz, $CDCl_3$): 1.32 (s, 9 H); 1.42 (s, 9 H); 1.48/1.50 (s, 3 H); 2.99 (m, 2 H); 3.78 (s, 3 H); 4.30 (m, 1 H); 5.03 (m, 1 H, NH); 6.18/6.20 (t, $^2J_{FH}$ = 56.0, 1 H); 6.55/6.62 (s, 1 H, NH); 6.93 (d, J_{HH} = 8.3, 2 H); 7.10 (dd, J_{HH} = 1.9, 8.3, 2 H). ^{13}C NMR (APT; 75 MHz, $CDCl_3$): 16.0; 28.2; 28.8; 37.3; 52.9; 55.4/55.5; 60.8 (t, $^2J_{CF}$ = 24.0); 78.3; 80.3/80.4; 112.7/112.8 (t, $^1J_{CF}$ = 247.0); 124.3; 129.8; 131.1/131.2; 154.4; 155.6; 169.4/169.5; 171.6. ^{19}F NMR (282 MHz, $CDCl_3$): –47.51/–47.26 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F); –45.08/–45.00 (dd_{ABX}, $^2J_{FC}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F).

Methyl *N*-{*N*-[(*tert*-Butyloxy)carbonyl]glycyl}-(*RS*)-2-(difluoromethyl)-phenylalaninate (**6g**)

Method B: Yield 0.26 g (67%). MS (FAB, *m/z*), calculated for $C_{18}H_{24}N_2F_2O_5$: 386.39; found: 387.4 $[M + H]^+$. Oil. R_F 0.16 (AcOEt/hexanes 1 : 3). ^{1}H NMR (200 MHz, $CDCl_3$): 1.42 (s, 9 H); 3.31 (d, $^2J_{HH}$ = 13.5, 1 H); 3.76 (m, 2 H); 3.81 (d, $^2J_{HH}$ = 12.8, 1 H); 3.86 (s, 3 H); 5.05 (s, br, 1 H, NH); 6.51 (t, $^2J_{HH}$ = 55.5); 6.93 (s, 1 H, NH); 7.03–7.07 (m, 2 H); 7.27 (m, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): 28.2; 34.8 (d, $^3J_{CF}$ = 3.0), 44.7; 53.1; 66.3 (t, $^2J_{CF}$ = 22); 80.4; 112.8 (dd_{ABX}, $^1J_{CF}$ = 250.0); 127.5; 128.6; 129.8; 133.6; 155.9; 168.3 (d, $^3J_{CF}$ = 3.0); 170.0. ^{19}F NMR (188 MHz, $CDCl_3$): –50.50 (dd_{ABX}, $^2J_{FF}$ = 281.0, $^2J_{HF}$ = 56.0, 1 F); –49.37 (dd_{ABX}, $^2J_{FF}$ = 281.0, $^2J_{HF}$ = 56.0, 1 F).

tert-Butyl *ambo-N*-(*N*-{*N*-[(Fluoren-9-ylmethoxy)carbonyl]leucyl}-(*R*)-2-(difluoromethyl)-alanyl)prolinate (**7a/1**)

To a solution of (*R*)-H-(α-CF_2H)Ala-Pro-O^{t}Bu (0.29 g, 1 mmol), prepared from **3c/1**, in a 2 : 1 mixture of CH_2Cl_2/DMF (5 ml) Fmoc-Leu-F (0.90 g, 2.5 mmol) and DIEA (0.32 g, 2.5 mmol) were added at room temperature. The mixture was stirred for 16 h. Then the pH was adjusted to 3–4 on addition of citric acid (10% aqueous solution) and the mixture was extracted with CH_2Cl_2 (3 ×). The combined organic layer was washed with citric acid, with saturated NaCl solution, with H_2O, and finally dried with anhydrous $NaSO_4$. After evaporation of the solvent *in vacuo*, **7a/1** was purified by flash chromatography. MS (MALDI-TOF), calculated for $C_{34}H_{43}F_2N_3O_6$: 627.72; found: 650.6 $[M + Na]^+$; 666.6 $[M + K]^+$. Yield 0.49 g (78%). M.p. 201–202 °C. $[\alpha]_D^{25}$ –77 (*c* 1, CH_2Cl_2). R_F 0.53 (AcOEt/hexanes 1 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): 0.95 (m, 6 H, Leu H); 1.44 (s, 9 H); 1.45–2.00 (m, 7 H); 1.60 (s, 3 H); 3.54 (m); 4.19 (m, 2 H); 4.42 (m, 3 H); 5.12 (d, $^3J_{HH}$ = 7.4, NH); 6.34 (s, 1 H, NH); 6.40 (t, 1 H, $^2J_{HF}$ = 56.2); 7.30–7.80 (8 H). ^{13}C NMR (50 MHz): 15.8 (t, $^3J_{CF}$ = 3.0); 22.0; 22.8; 24.6; 25.6; 27.9; 28.0; 40.9; 47.1; 47.2; 53.4; 60.6 (t, $^2J_{CF}$ = 22.0); 61.2; 67.4; 81.2; 113.7 (t, $^1J_{CF}$ = 249.0); 120.1; 125.0; 127.2; 127.8; 141.3; 143.7; 156.4; 166.6; 171.3; 172.1. ^{19}F NMR (282 MHz, $CDCl_3$): –52.80 (dd_{ABX}, $^2J_{FF}$ = 282.0, $^2J_{HF}$ = 56.0, 1 F); –51.68 (dd_{ABX}, $^2J_{FF}$ = 282.0, $^2J_{HF}$ = 56.0, 1 F).

tert-Butyl *ambo-N*-(*N*-{*N*-[(Fluoren-9-ylmethoxy)carbonyl]leucyl}-(*S*)-2-(difluoromethyl)-alanyl)prolinate (**7a/2**)

Synthesis from (*S*)-H-(α-CF_2H)Ala-Pro-O^{t}Bu (0.29 g, 1 mmol), prepared from **3c/2** and Fmoc-Leu-F (0.90 g, 2.5 mmol). Yield 0.48 g (77%). M.p. 178–180 °C. $[\alpha]_D^{25}$ –44 (*c* 1, CH_2Cl_2). R_F 0.46 (AcOEt/hexanes 1 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): 0.94 (m, 6 H); 1.45 (s, 9 H); 1.40–2.15 (m, 7 H); 1.73 (s, 3 H); 3.66 (m, 2 H); 4.21 (m, 2 H); 4.40 (m, 3 H); 5.35 (m, 2 H, NH); 6.31 (t, $^2J_{HF}$ = 56.0, 1 H); 7.26–7.77 (8 H). ^{13}C NMR (75 MHz, $CDCl_3$): 16.6; 21.8, 22.9; 24.7; 25.7; 29.9; 27.9; 41.2; 47.1; 47.7; 53.8; 62.2 (t, $^2J_{CF}$ = 22.0); 62.2; 67.3; 81.5; 114.4 (t, $^1J_{CF}$ = 248.0); 120.0; 125.0; 127.1; 127.8; 141.3; 143.7; 156.3; 166.7; 171.2; 172.4. ^{19}F NMR (282 MHz, $CDCl_3$): –50.89 (dd_{ABX}, $^2J_{FF}$ = 282.0, $^2J_{HF}$ = 56.0, 1 F); –48.95 (dd_{ABX}, $^2J_{FF}$ = 282.0, $^2J_{HF}$ = 56.0, 1 F).

ambo-N-(*N*-{*N*-[(Benzyloxy)carbonyl]phenylalanyl}-(*S*)-2-(difluoromethyl)alanyl)-alaninamide (**7b/1**)

A solution of the N-protected dipeptide ester (2 mmol) in methanol (3 ml) after addition of a solution of $LiOH/H_2O$ (0.42 g, 10 mmol) in a 2 : 1 H_2O/CH_3OH mixture (12 ml) was stirred at 5 °C for 15 h. The reaction mixture was concentrated *in vacuo*, adjusted to pH 3–4 by addition of dilute citric acid, and extracted with ether (3 × 25 ml). After drying with anhydrous $MgSO_4$, the organic solvent was evaporated *in vacuo*. The crude compound was directly used for the next reaction step. To a solution of the crude N-protected dipeptide in a 2 : 1 solvent mixture of CH_2Cl_2/DMF (7 ml), DIC (0.27 g, 2.1 mmol) and HOAt (0.29 g, 2.1 mmol) were added at room temperature. After 10 min H-Ala-NH_2 (0.18 g, 2 mmol) was added. The reaction mixture was stirred until the starting material was consumed (^{19}F NMR analysis). Then the reaction mixture was adjusted to pH 3–4 by addition of dilute citric acid and extracted with CH_2Cl_2 (3 × 35 ml). The combined organic layer was washed successively with dilute citric acid, concentrated NaCl solution, $NaHCO_3$ solution (5%) and finally with water. After drying with anhydrous $MgSO_4$, the organic solvent was evaporated *in vacuo*, the peptides were purified by recrystallization from $CHCl_3$/hexanes or by flash chromatography.

7b/1 was synthesized from Z-Phe-(*S*)-(α-CF_2H)Ala-OMe (**6d/1**) (0.87 g, 2 mmol) and H-Ala-NH_2 (0.18 g, 2 mmol). MS (MALDI-TOF), calculated for $C_{24}H_{28}N_4F_2O_5$: 490.50; found: 513.0 $[M + Na]^+$; 529.0 $[M + K]^+$. Yield 0.64 g (65%). M.p. 184 °C. $[\alpha]_D$ +8 (*c* 1, CH_3OH). R_F 0.10 ($CHCl_3/CH_3OH$ 20 : 1). ^1H NMR (300 MHz, $CDCl_3$): 1.38 (d, $^3J_{HH}$ = 7.3, 3 H); 1.43 (s, 3 H); 2.95 (dd, $^3J_{HH}$ = 8.3, 14.0, 1 H); 3.12 (dd, $^3J_{HH}$ = 6.8, 14.0, 1 H); 4.32 (m, 2 H); 5.01 (d, $^2J_{HH}$ = 12.1, 1 H); 5.10 (d, $^2J_{HH}$ = 12.1, 1 H); 5.66 (m, 2 H, 2 NH); 6.22 (t, $^2J_{FH}$ = 56.0, 1 H); 6.76 (s, 1 H, NH); 7.06 (s, 1 H, NH); 7.19 (d, $^2J_{HH}$ = 6.1, 1 H, NH); 7.26–7.33 (m, 10 H). ^{13}C NMR (75 MHz, $CDCl_3$): 17.2 (t, $^3J_{CF}$ = 3.0); 21.1; 37.3; 49.5; 57.4; 61.4 (t, $^2J_{CF}$ = 21.0); 67.5; 113.8 (t, $^1J_{CF}$ = 249); 127.5; 128.0; 128.5; 128.7; 129.0; 129.1; 135.5; 135.8; 156.9; 168.9; 172.6; 174.9. ^{19}F NMR (282 MHz, $CDCl_3$): –51.42 (d, $^2J_{FH}$ = 56.0, 1 F); –51.37 (d, $^2J_{FH}$ = 56.0, 1 F).

ambo-N-(*N*-{*N*-[(Benzyloxy)carbonyl]phenylalanyl}-(*R*)-2-(difluoromethyl)alanyl)-alaninamide (**7b/2**)

7b/2 was synthesized from Z-Phe-(*R*)-(α-CF_2H)-Ala-OMe (**6d/2**) (0.87 g, 2 mmol) and H-Ala-NH_2 (0.18 g, 2 mmol). Yield 0.70 g (72%). M.p. 152–153 °C. $[\alpha]_D$ –8.3 (*c* 1, CH_3OH). R_F 0.08 ($CHCl_3/CH_3OH$ 20 : 1). ^1H NMR (300 MHz, $CDCl_3$): 1.36 (d, $^3J_{HH}$ = 7.14, 3 H); 1.58 (s, 3 H); 2.95 (dd, $^3J_{HH}$ = 8.7, 14.2, 1 H); 3.12 (dd, $^3J_{HH}$ = 5.7, 14.2, 1 H); 4.30 (m, 1 H); 4.41 (m, 1 H); 5.00 (d, $^3J_{HH}$ = 12.1, 1 H); 5.13 (d, $^3J_{HH}$ = 12.1, 1 H); 5.53 (s, 1 H, NH); 5.58 (s, 1 H, NH); 5.94 (t, $^2J_{FH}$ = 55.1, 1 H); 6.89 (s, 2 H, NH); 7.18–7.35 (m, 10 H).^{19}F NMR (282 MHz, $CDCl_3$): –53.20 (dd_{ABX}, $^2J_{FF}$ = 274.0, $^2J_{FH}$ = 55.0, 1 F); –49.96 (dd_{ABX}, $^2J_{FF}$ = 274.0, $^2J_{FH}$ = 55.0, 1 F).

N-(*N*-{*N*-[(Benzyloxy)carbonyl]-(*R*)-phenylalanyl}-(*R*)-2-(difluoromethyl)alanyl)-(*R*)-alaninamide (**7c/1**)

7c/1 was synthesized from Z-(*R*)-Phe-(*R*)-(α-CF_2H)Ala-OMe (**6e/1**) (0.87 g, 2 mmol) and (*R*)-H-Ala-NH_2 (0.18 g, 2 mmol). MS (MALDI-TOF), calculated for $C_{24}H_{28}N_4F_2O_5$: 490.50; found: 513.0 $[M + Na]^+$; 529 $[M + K]^+$. Yield 0.86 g (88%). M.p. 166–168 °C (decomp.). $[\alpha]_D$ –7.4 (*c* 1, CH_3OH). R_F 0.12 ($CHCl_3/CH_3OH$ 20 : 1). ^1H NMR (300 MHz, $CDCl_3$): 1.35 (d,

$^3J_{HH}$ = 7.4, 3 H); 1.43 (s, 3 H); 2.95 (m, 1 H); 3.12 (dd, $^3J_{HH}$ = 7.6, 14.0, 1 H); 4.33 (m, 2 H); 4.99 (d, $^2J_{HH}$ = 12.3, 1 H); 5.07 (d, $^2J_{HH}$ = 12.3, 1 H); 5.88 (s, 1 H, NH); 6.18 (s, 1 H, NH); 6.22 (t, $^2J_{FH}$ = 56.0, 1 H); 6.80 (s, 1 H, NH); 6.93 (s, 1 H, NH); 7.15–7.34 (m, 11 H, 10 H^{Ar}, NH). ^{19}F NMR (282 MHz, $CHCl_3$): –51.82 (dd_{ABX}, $^2J_{FF}$ = 278.0, $^2J_{FH}$ = 56.0, 1 F); –50.97 (dd_{ABX}, $^2J_{FF}$ = 278.0, $^2J_{FH}$ = 56.0, 1 F).

N-(*N*-{*N*-[(Benzyloxy)carbonyl]-(*R*)-phenylalanyl}-(*S*)-2-(difluoromethyl)alanyl)-(*R*)-alaninamide (**7c/2**)

7c/2 was synthesized from Z-(*R*)-Phe-(*S*)-(α-CF_2H)Ala-OMe (**6e/2**) (0.87 g, 2 mmol) and (*R*)-H-Ala-NH_2 (0.18 g, 2 mmol). Yield 0.80 g (82%). Oil. $[\alpha]_D$ +9 (*c* 1, CH_3OH). R_F 0.07 ($CHCl_3$/CH_3OH 1 : 20). ^{1}H NMR (300 MHz, $CDCl_3$): 1.39 (d, $^3J_{HH}$ = 7.1, 1 H); 1.63 (s, 3 H); 2.95 (dd, $^3J_{HH}$ = 8.6, 15.0, 1 H); 3.12 (dd, $^3J_{HH}$ = 5.2, 13.0, 1 H); 4.31 (m, 1 H); 4.43 (m, 1 H); 5.01 (d, $^2J_{HH}$ = 12.1, 1 H); 5.14 (dd, $^3J_{HH}$ = 12.1, 1 H); 5.45 (s, 1 H, NH); 5.56 (s, 1 H, NH); 6.94 (t, $^2J_{FH}$ = 56.0, 1 H); 6.82 (s, 1 H, NH); 6.94 (s, 1 H, NH); 7.19 (d, $^3J_{HH}$ = 6.2, 1 H, NH); 7.26–7.34 (m, 10 H). ^{19}F NMR (282 MHz, $CDCl_3$): –53.29 (dd_{ABX}, $^2J_{FF}$ = 273.0, $^2J_{FH}$ = 56.0, 1 F); –49.90 (dd_{ABX}, $^2J_{FF}$ = 273.0, $^2J_{FH}$ = 56.0, 1 F).

tert-Butyl *ambo-N*-(*N*-{*N*-[(Fluoren-9-ylmethoxy)carbonyl]leucyl}-(*S*)-2-(chlorodifluoromethyl)-alanyl)prolinate (**8/1**)

8/1 was synthesized from Fmoc-Leu-OH (0.71 g, 2 mmol) and (*S*)-H-(α-CF_2Cl)Ala-Pro-O^{t}Bu (**4c/1**) (0.65 g, 2 mmol). MS (MALDI-TOF), calculated for $C_{34}H_{42}N_3ClF_2O_6$: 662.17; found: 684.4 $[M + Na]^+$; 700.4 $[M + K]^+$. Yield 0.83 g (63%). M.p. 158–159 °C. $[\alpha]_D^{25}$ –54 (*c* 0.5, MeOH). R_F 0.33 (AcOEt/hexanes 1 : 2). ^{1}H NMR (300 MHz, $CDCl_3$): 0.91 (m, 6 H); 1.43 (s, 9 H); 1.25–2.00 (m, 7 H); 1.94 (s, 3 H); 3.53 (m, 1 H); 3.69 (m, 1 H); 4.21 (m, 2 H); 4.42 (m, 3 H); 5.19 (d, $^3J_{HH}$ = 6.3, 1 H, NH); 7.11 (s, 1 H, NH); 7.25–7.80 (m, 8 H). ^{19}F NMR (282 MHz, $CDCl_3$): 17.41 ($^2J_{FF}$ = 165.0, 1 F); 19.00 ($^2J_{FF}$ = 165.0, 1 F).

tert-Butyl *ambo-N*-(*N*-{*N*-[(Fluoren-9-ylmethoxy)carbonyl]leucyl}-(*R*)-2-(chlorodifluoromethyl)-alanyl)prolinate (**8/2**)

8/2 was synthesized from Fmoc-Leu (0.71 g, 2 mmol) and (*R*)-(α-CF_2Cl)Ala-Pro-O^{t}Bu (**4c/2**) (0.65 g, 2 mmol). Yield 0.76 g (57%). M.p. 178 °C (decomp.). $[\alpha]_D^{25}$ –143 (*c* 1, MeOH). R_F 0.33 (AcOEt/hexanes 1 : 2). ^{1}H NMR (300 MHz, $CDCl_3$): 0.95 (m, 6 H); 1.44 (s, 9 H); 1.40–2.00 (m, 7 H); 1.85 (s, 3 H); 3.54 (m, 2 H); 4.21 (m, 2 H); 4.42 (m, 3 H); 5.14 (d, $^3J_{HH}$ = 7.4, 1 H, NH); 6.72 (s, 1 H, NH); 7.25–7.80 (m, 8 H). ^{13}C NMR (APT; 75 MHz, $CDCl_3$): 18.4 (t, $^3J_{CF}$ = 3.0, CH_3); 22.0; 22.8; 24.6; 25.8; 27.8; 28.0; 40.5; 47.1; 47.7; 53.9; 61.8; 65.8 (t, $^2J_{CF}$ = 22.0); 67.3; 81.2; 122.1 (t, $^1J_{CF}$ = 308.0); 120.1; 125.0; 127.1; 127.9; 141.4; 143.7; 156.5; 163.7; 171.1. ^{19}F NMR (282 MHz, $CDCl_3$): 18.55 ($^2J_{FF}$ = 162.0, 1 F); 18.98 ($^2J_{FF}$ = 162.0, 1 F).

ambo-N-[*N*-(*N*-{*N*-[(*tert*-Butoxy)carbonyl]-*O*-*tert*-butyltyrosinyl}-2-(difluoromethyl)alanyl)-phenylalanyl]phenylalaninamide (**9**)

Synthesis from *ambo-N*-Boc-O^{t}Bu-Tyr-(α-CF_2H)Ala-OMe (0.95 g, 2 mmol) (**6f**) and H-Phe-Phe-NH_2 (0.62 g, 2 mmol). MS (MALDI-TOF), calculated for $C_{40}H_{51}N_5F_2O_7$: 751.87; found: 774.3 $[M + Na]^+$; 790.3 $[M + K]^+$. Yield 1.17 g (78%); 1 : 1 mixture of two diastereomers. Oil. R_F 0.22 ($CHCl_3$/CH_3OH 20 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): 1.13/1.16 (s, 9 H);

1.32/1.35 (s, 9 H); 1.54/1.58 (s, 3 H); 2.65–3.63 (m, 6 H); 4.03 (m, 1 H); 4.78 (m, 1 H); 4.91 (m, 1 H); 6.18 (t, $^2J_{FH}$ = 56.0, 1 H); 6.57 (s, 1 H, NH); 6.62 (s, 1 H, NH); 6.84 (s, 1 H, NH); 6.95–7.40 (m, 16 H, H^{Ar}, NH); 7.44 (s, 1 H, NH). ^{19}F NMR (282 MHz, $CDCl_3$): –52.55/-52.91 (dd_{ABX}, $^2J_{FF}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F); –51.21 (dd_{ABX}, $^2J_{FF}$ = 282.0, $^2J_{FH}$ = 56.0, 1 F).

Data Collection and Structural Refinement for **7a/1**, **7c/1** and **8/1**

Crystallographic data are given in Table I. The data (λ MoKα = 0.71073 Å) were collected with a CCD diffractometer (AXS Bruker). All observed reflections are used for determination of the unit cell parameters. Empirical absorption correction was carried out with SADABS [35]. Structures were solved by direct methods. The structures were refined anisotropically for all non-hydrogen atoms using program SHELX97 [36]. Hydrogen atoms were refined in calculated positions.

CCDC 187347 (for compound **7a/1**), CCDC 187349 (for compound **7c/1**) and CCDC 187348 (for compound **8/1**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

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