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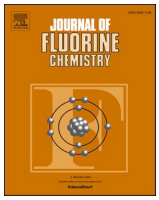

Synthesis of hexafluorovaline-containing di- and tripeptides

Maria Cristina Bellucci [a], Carola Romani [b], Monica Sani [c], Alessandro Volonterio [b,c,*]

[a] Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, via Celoria 2, Milano 20131, Italy
[b] Department of Chemistry, Materials, and Chemical Engineering "G. Natta", Politecnico di Milano, Via Mancinelli 7, Milan 20131, Italy
[c] Consiglio Nazionale delle Ricerche, Istituto di Scienze e Tecnologie Chimiche "G. Natta" (SCITEC), Via Mario Bianco 9, Milan 20131, Italy

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ABSTRACT

A new strategy for the synthesis of peptides incorporating racemic hexafluorovaline (hfVal) is presented. The synthetic pathway relies on the anti-Michael addition of benzyl amine derivatives to ad hoc prepared β-bis-trifluoromethyl-acryloyl-α-amino esters which proceeds in mild condition, high yields, even if with low stereocontrol. The following elaboration of the intermediates, namely deprotection of the benzyl moiety and coupling with α-amino esters allowed us to synthetize the targeted tripeptides in four overall synthetic steps, resulting in a synthetic pathway more favorable respect to those appeared in literature based on the synthesis and isolation of racemic Boc-hfVal-OH (eight synthetic steps).

1. Introduction

Fluorinated peptides and proteins represent an intriguing class of biomolecules that have garnered significant attention [1,2]. These peptide/proteins, basically composed of amino acids with fluorine substituents, offer a unique set of properties such as enhanced stability through protein degradation [3], enhanced bioavailability [4], and activity [5]. Indeed, the unique properties of fluorine, such as its similar in size to hydrogen (making it a bioisoster) but significantly higher electronegativity, enable it to form stronger and less polarizable bonds with carbon, with an opposite dipole moment compared to C—H bonds [6]. These characteristics make fluorine atom impactful when introduced into peptides, as it can bring about significant alterations in their physicochemical properties. By incorporating fluorine, peptides can gain increased resistance to enzymatic degradation, enhanced membrane permeability, and improved binding affinity to biological targets. Moreover, fluorine's unique electronic properties can modulate the peptide's conformational dynamics, potentially influencing its interaction with biomolecular partners [7].

Another important feature of molecules containing fluorine is that fluorine atoms are spin-active rendering ^{19}F NMR a valuable tool to investigate the behavior of fluorinated bio(macro)molecules and substrates [8]. Indeed, since fluorine is totally absent in biological systems, the incorporation of fluorinated amino acids in designed position of proteins and bioactive peptides has been exploited to study the folding properties and interactions of the (macro)molecules and to shed light on the mechanism of numerous enzymes, both in vitro and in vivo [9].

Fluorine can be incorporated into a peptide sequence through fluorinated surrogates of the peptide bond [10–13] or using non-natural amino acids having fluorine atoms in the side chain [14,15]. Among those amino acids, particularly interesting resulted to be highly fluorinated analogues of hydrophobic amino acids, such as hexafluoroleucine (hfLeu) and hexafluorovaline (hfVal). Indeed, the incorporation of extensively fluorinated amino acids in proteins has been intensively investigated in order to shed light on the importance of the "fluorous effect" on the stabilization of protein structure and function [2].

However, despite the interest on the synthesis of peptides and proteins incorporating highly fluorinated amino acids, there are only few examples of peptides incorporating hfVal with respect to those incorporating hfLeu. This is probably due to the challenge on the synthesis of enantiomerically pure hfVal due to its predisposition for racemization [16,17], whereas different methodologies for the preparation of enantiomerically pure hfLeu have been appeared in literature [18–21]. Actually, racemic hfVal has been incorporated in angiotensin II inhibitors [22], in an analogue of a natural precursor for the biosynthesis of penicillin [23], and used to replace Val residue in cyclic decapeptide gramicidin S [24]. The synthetic pathway exploited in these works foresaw the synthesis of racemic N-Boc protected hfVal through anti-Michael addition of gaseous ammonia to bis-β-trifluoromethyl acrylic ester, followed by its incorporation into the peptidic sequence through standard coupling/deprotection protocols (Chart 1). Although successful, the synthetic strategy is quite challenging due to the number

* Corresponding author at: Department of Chemistry, Materials, and Chemical Engineering "G. Natta", Politecnico di Milano, Via Mancinelli 7, Milan 20131, Italy.
E-mail address: alessandro.volonterio@polimi.it (A. Volonterio).

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of steps (5 steps only for the synthesis of Boc-hfVal-OH), the use of gaseous reactants such as hexafluoroacetone and ammonia, and very low temperatures in two steps. Herein we present an alternative and general strategy for the preparation of small peptides incorporating racemic hfVal, which encompasses only four synthetic steps (Chart 1), namely coupling of α-amino ester to 4,4,4-trifluoro-3-(trifluoromethyl) crotonic acid followed by anti-Michael addition of benzyl amines, N-de-benzylation and coupling of the resulting free amine with a N-protected amino acid. All the reaction occurred in mild conditions (room temperature, no gaseous reactant) and yielded the final tripeptides in good yields providing a straightforward alternative route to the synthesis of peptides incorporating hfVal.

2. Result and discussion

For several years, our research group has been dedicated to the study of the properties of fluorinated peptidomimetics exploiting the high reactivity of α,β-unsaturated carbonyl building blocks bearing a trifluoromethyl group in α or β position for their synthesis [25,26]. Actually, the presence of such highly electronegative trifluoromethyl group renders the carbon-carbon double bond much more electron-poor, thus much more reactive toward nucleophiles, and in particular N-nucleophiles such as α-amino esters [27]. Lately, we have developed a methodology for the synthesis of peptide-sugar conjugates [28] and glycomimetics [29] incorporating hfVal exploiting a multi-component process where a crucial step relies on an intramolecular aza-*anti*-Michael addition to an α,β-unsaturated carbonyl intermediate bearing two trifluoromethyl groups in β position. In the frame of this project, we sought to exploit the same key reaction to prepare peptides incorporating hfVal in a more straightforward way compared to the strategies appeared in literature, basically avoiding the challenging synthesis of racemic BocNH-hfVal-OH. Accordingly, we have synthetized four fluorinated Michael acceptors **3a-d** by coupling 4,4,4-trifluoro-3-(trifluoromethyl)crotonic acid **2** to, respectively, hydrophobic H-Val-OMe, H-Phe-OMe, H-Ala-O*t*Bu, and H-Arg(Pbf)-OMe bearing a polar side chain [30] (Scheme 1).

Since the planned strategy foresaw the intermediate synthesis of dipeptides incorporating N-deprotected hfVal, we decided to study the anti-Michael addition to Michael acceptor **3a-d** with benzyl amine derivatives that could be easily de-benzylated through catalytic hydrogenation.

As evidenced in the previous works, the diastereoselectivity in the reaction could rely on various factors, including the solvent, the type of the base, the R side chain of the α-amino acid esters in the Michael acceptor, the structure of the *N*-nucleophile, and the temperature. To refine these variables with the attempt to enhance diastereoselectivity, we conducted a set of experiments employing L-Val derivative **3a** as representative Michael acceptor and (*S*)-*p*-methoxybenzyl amine as nucleophile (Table 1). First, we investigated the influence of the solvent by performing the reaction at room temperature (rt) in solvents having increasing polarity such as toluene, DCM, and ethanol, in the presence of the same base, i.e. TEA (entries 1–3, Table 1). To our surprise and in contrast with the results we obtained in former studies [25,26], the reaction was slightly more diastereoselective in polar solvent such as ethanol (d.e. 43 %, entry 3, Table 1) compared to DCM (d.e. 28 %, entry 2, Table 1) and toluene (d.e. 13 %, entry 1, Table 1). Moreover, in low polar solvent the reaction was much slower resulting in only 53 % yield after one night (12 h). The base had no influence on the diastereoselection of the reaction as evidenced by performing the reaction in presence of three different tertiary amines such as TEA, DIPEA, and DABCO (entries 3–5, Table 1, respectively). However, the presence of a base was mandatory since we did not detect the formation of any products after 12 h by performing the reaction in absence of tertiary amines (entry 6, Table 1). The same result was obtained by performing the reaction at 0 °C, demonstrating that the anti-Michael addition to adducts of type **3** is not as easy as the addition to N-(α-trifluoromethyl) acryloyl-α-amino esters or trans-2-trifluoromethyl-1-nitroethene due to electronic and/or steric concerns. (entry 7, Table 1). This is also evidenced by the fact that Michael acceptor **3a** was not reactive with poor *N*-nucleophile such as α-amino esters. Indeed, by treating **3a** with S-phenyl glycine methyl ester in the best reaction condition, i.e. EtOH in the presence of TEA at rt, we recovered the unreacted starting materials after 12 hour (entry 8, Table 1).

Next, we investigated the influence of the stereogenic center on the *N*-nucleophile by reacting acceptor **3a** with (*R*)−1-phenylethan-1-amine. Rather surprisingly, we did not evidence a strong match/mismatch since the reaction lead to the formation of a mixture of diastereoisomers **4b** with similar diastereoisomeric excess (d.e. 31 % versus 43 %) (entry 9, Table 1). This result was corroborated by the fact that the reaction performed with achiral benzyl amine gave quite the same diastereoselectivity (d.e. 28 %) producing dipeptides **4c** (entry 10, Table 1). In light of these results, we have studied the influence of the R^1 group on stereochemical outcome of the reaction using benzyl amine as model *N*-nucleophile and EtOH/TEA as solvent/base. As expected, less bulky R^1 group compared to isoPr group on valine of **3a** gave lower diastereoselectivity. Actually, reaction performed with Phe-Michael

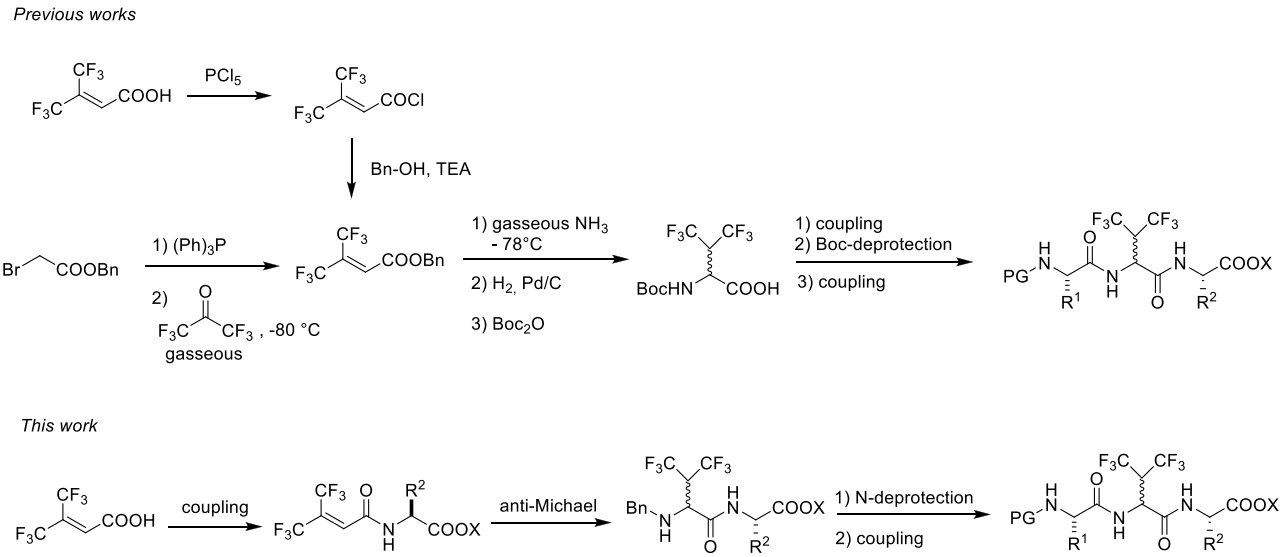
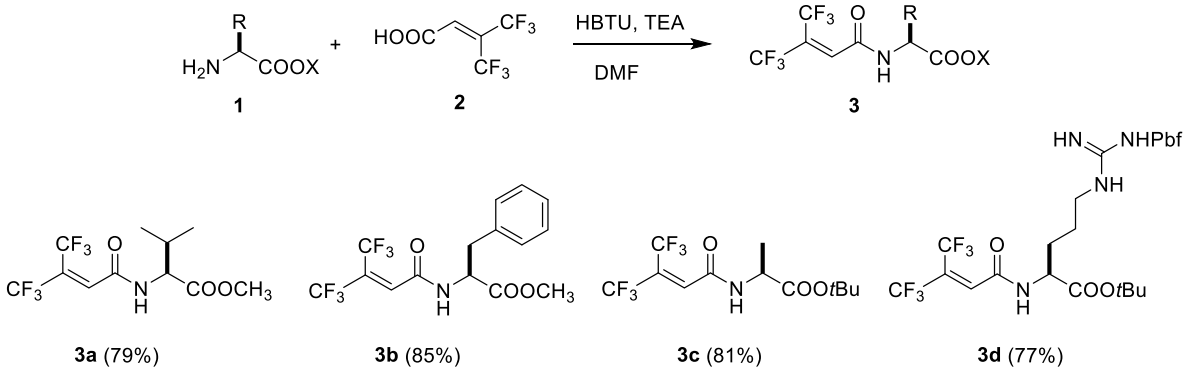


Chart 1. Synthetic pathways for the preparation of hfVal-containing tripeptides.



Scheme 1. Synthesis of Michael acceptors 3a-d.

acceptor (R^1 = Bn) **3b**, Ala-Michael acceptor (R^1 = Me) **3c**, and Arg-Michael acceptor (R^1 = -$(CH_2)_3$–NHC(=NH)NHPbf) **3d** produced an equimolar mixture of the two diastereoisomers **4d-f**, respectively (entries 11–13, Table 1). In order to avoid the deprotection step or to have a protecting group that would have been much easier to be removed, we tried the reaction using ammonia and trityl amine, respectively, however with disappointing results in both cases. Actually, the reaction with ammonia produced the formation of the desired mixture of diastereoisomers **4g** in equimolar ratio, but in very low yield along with unidentified by-products (entry 14, Table 1), whereas trityl amine did not react at all even after 12 h probably due to steric concerns (entry 15, Table 1). Finally, methyl amine reacted smoothly with Michael acceptor **3b** affording compounds **4h** as an equimolecular mixture of stereoisomers in very high yields (entry 16, Table 1). It is worth noting that adduct like **4h** can be used as building blocks for the preparation of *N*-methylated hfVal-containing peptidomimetics [31].

Next, we tackled the transformation of the *N*-benzyl adducts **4** into the desired tripeptides incorporating racemic hfVal as outlined in Scheme 2.

Gratifyingly, the catalytic hydrogenation performed on substrates **4f, h** worked very nicely (atmospheric pressure, 2 h) producing H-hfVal-Phe-OMe **5** and H-hfVal-Ala-O*t*Bu **7** dipeptides, respectively, in high yields. Analogously, the following coupling with CbzNH-Leu-OH promoted by HBTU afforded orthogonally protected, thus prompt for further elongation, CbzNH-Leu-hfVal-Phe-OMe **6** and CbzNH-Leu-hfVal-Ala- O*t*Bu **8** tripeptides, respectively, in good yields. Moreover, coupling with FmocNH-Phe-OH in the same condition yielded tripeptide FmcNH-Phe-hfVal-Ala- O*t*Bu **9** which is a suitable precursor for a possible synthesis of longer peptides through SPPS.

In summary, we have outlined a novel, simpler pathway for the synthesis of peptides incorporating racemic hfVal which avoids the challenging synthesis and recovery of racemic Boc-NH-hfVal-OH as described in previous works. This achievement was made possible by leveraging the anti-Michael addition of benzyl amine derivatives to β-bis-trifluoromethyl-acryloyl-amino esters, resulting in high yields and poor stereocontrol, followed by de-benzylation through catalytic hydrogenation and coupling. The procedure is operationally straightforward, utilizing readily accessible and inexpensive reagents. It operates under mild conditions and yields compounds suitable for combinatorial applications, making it particularly appealing for potential implementation in solid phase synthesis.

3. Experimental procedures

3.1. Materials

Commercially available reagent-grade solvents were employed without purification. All amino acids are of L-configuration. TLC were run on silica gel 60 F254 Merck. Visualization of the developed chromatogram was achieved with UV light and ceric ammonium molybdate (CAM) or ninhydrin stains. Flash chromatography (FC) were performed with silica gel 60 (60–200 μm, Merck). ^{1}H-, ^{13}C-, and ^{19}F NMR spectra were run at 400. Chemical shifts are expressed in ppm (δ), using tetramethylsilane (TMS) as internal standard for ^{1}H and ^{13}C nuclei (δ_H and δ_C = 0.00), while C_6F_6 was used as external standard (δ_F −162.90) for ^{19}F. ESI mass spectra were performed by a Bruker Esquire 3000+ instrument equipped with a MS detector composed by a ESI ionization source and a Single Quadrupole mass selective detector or by an Agilent Technologies 1200 Series HPLC system equipped with a DAD and a 6120 MS detector composed by an ESI ionization source and a Single Quadrupole mass selective detector. Optical rotations were measured on a Propol Digital Polarimeter with a sodium lamp.

3.2. Synthesis of Michael acceptors 3a-d. Typical procedure

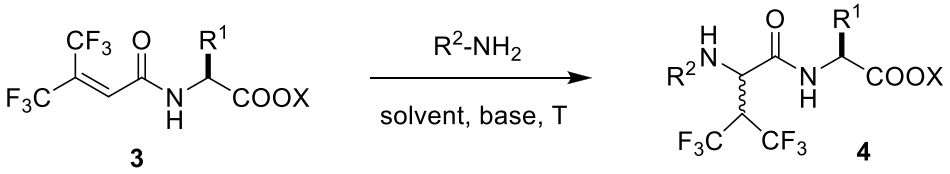
To a stirred solution of 4,4,4-trifluoro-3-(trifluoromethyl)crotonic acid **2** (0.72 mmol, 150 mg) in DMF (3.0 mL) solid HBTU (0.86 mmol, 328 mg) was added at 0 °C. After 10 min a solution of H-Val-OMe hydrochloride **1a** (0.86 mmol, 144 mg) and TEA (0.86 mmol, 120 μL) in DMF (2 ml) was added, the temperature left to reach rt and the solution stirred overnight. The solution was diluted with AcOEt and brine and extracted three times with AcOEt. The collected organic phases were washed three times with brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the crude was purified by FC (hexane/AcOEt 9:1) affording 183 mg (79 %) of the pure Michael acceptor **3a**.

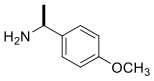
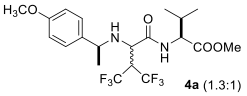
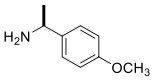
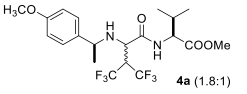
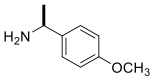
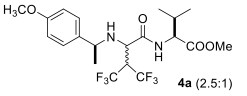
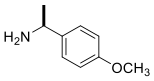
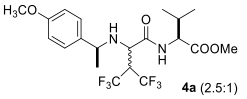
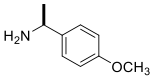
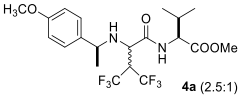
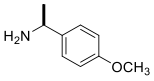
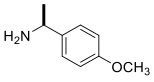
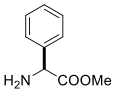
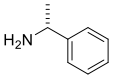
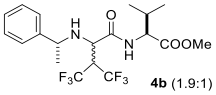
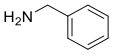
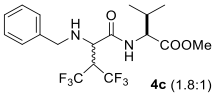
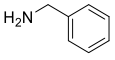
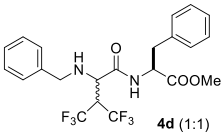
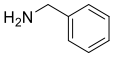
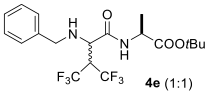
methyl (4,4,4-trifluoro-3-(trifluoromethyl)but-2-enoyl)-L-valinate **3a:** R_f 0.41 (20:80 AcOEt:hexane); $[\alpha]_D^{20}$ +3,2 (*c* = 0.96, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (br s, 1H), 7.02 (br s, 1H), 4.63 (dd, *J* = 8.8 and 4.8 Hz, 1H), 3.73 (s, 3H), 2.21–2.17 (septet, *J* = 6.8 Hz, 1H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 161.6, 135.8 (q, *J* = 3.0 Hz) 125.2 (septet, *J* = 33.3 Hz), 119.8 (q, *J* = 276.5 Hz),118.7 (q, *J* = 276.5 Hz), 57.4, 52.2, 31.2, 18.5, 17.5; ^{19}F NMR (470 MHz, $CDCl_3$): δ −60.3 (q, *J* = 4.7 Hz), −65.3 (dq, *J* = 9.4 and 1.8 Hz); MS (ESI) *m/z* 344.2 $[M+Na]^+$; Anal. Calcd for $C_{11}H_{13}F_6NO_3$: C 41.13, H 4.08, N 4.36, found: C 41.15, H 4.010 N 4.36.

methyl (4,4,4-trifluoro-3-(trifluoromethyl)but-2-enoyl)-L-phenylalaninate **3b**: yield 85 %; R_f 0.45 (20:80 AcOEt:hexane); $[\alpha]_D^{20}$ +7,1 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21–7.18 (m, 3H), 7.02–7.00 (m, 2H), 8.84 (br s, 1H), 6.53 (d, *J* = 7.6 Hz, 1H), 4.91–4.86 (m, 1H), 3.65 (s, 3H), 3.03 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3, 161.0, 135.3, 135.1, 129.1, 128.7, 127.4, 125.7 (septet, *J* = 33.3 Hz), 120.3 (q, *J* = 277.5 Hz), 119.9 (q, *J* = 276.7 Hz), 53.3, 52.5, 37.7; ^{19}F NMR (470 MHz, $CDCl_3$): δ −60.3 (q, *J* = 9.4 Hz), −65.2 (dq, *J* = 9.4 and 1.6 Hz); MS (ESI) *m/z* 392.0 $[M+Na]^+$; Anal. Calcd for $C_{15}H_{13}F_6NO_3$: C 48.79, H 3.55, N 3.79; found: C 48.78, H 3.55, N 3.80.

tert-butyl (4,4,4-trifluoro-3-(trifluoromethyl)but-2-enoyl)-L-alaninate **3c**: yield 81 %; R_f 0.32 (20:80 AcOEt:hexane); $[\alpha]_D^{20}$ +12,1 (*c* = 1.00,

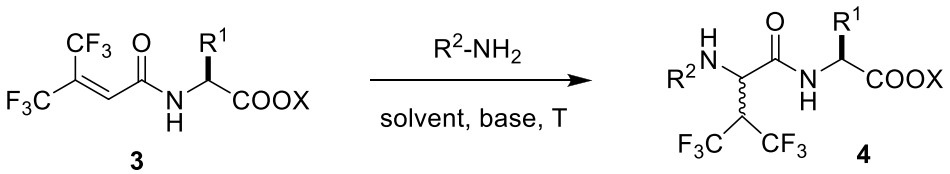
Table 1
Anti-Michael addition of amines to Michael acceptors 3.

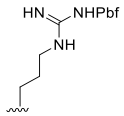
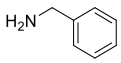
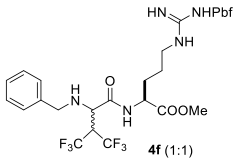
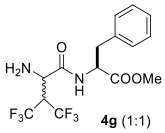
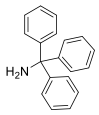
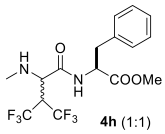


Entry	R^1	R^2-NH_2	solvent	base	T (°C)	Product	d.e.[a] (%)[b]	Yield (%)[c]
1	isoPr		toluene	TEA	25	 **4a** (1.3:1)	13	53[d]
2	isoPr		DCM	TEA	25	 **4a** (1.8:1)	28	81
3	isoPr		EtOH	TEA	25	 **4a** (2.5:1)	43	85
4	isoPr		EtOH	DIPEA	25	 **4a** (2.5:1)	43	87
5	isoPr		EtOH	DABCO	25	 **4a** (2.5:1)	43	85
6	isoPr		EtOH	/	25	n.r.[d,e]	/	/
7	isoPr		EtOH	/	0	n.r.[d,e]	/	/
8	isoPr		EtOH	TEA	25	n.r.[d,e]	/	/
9	isoPr		EtOH	TEA	25	 **4b** (1.9:1)	31	91
10	isoPr		EtOH	TEA	25	 **4c** (1.8:1)	28	90
11	Bn		EtOH	DIPEA	25	 **4d** (1:1)	0	86
12	Me		EtOH	DIPEA	25	 **4e** (1:1)	0	92

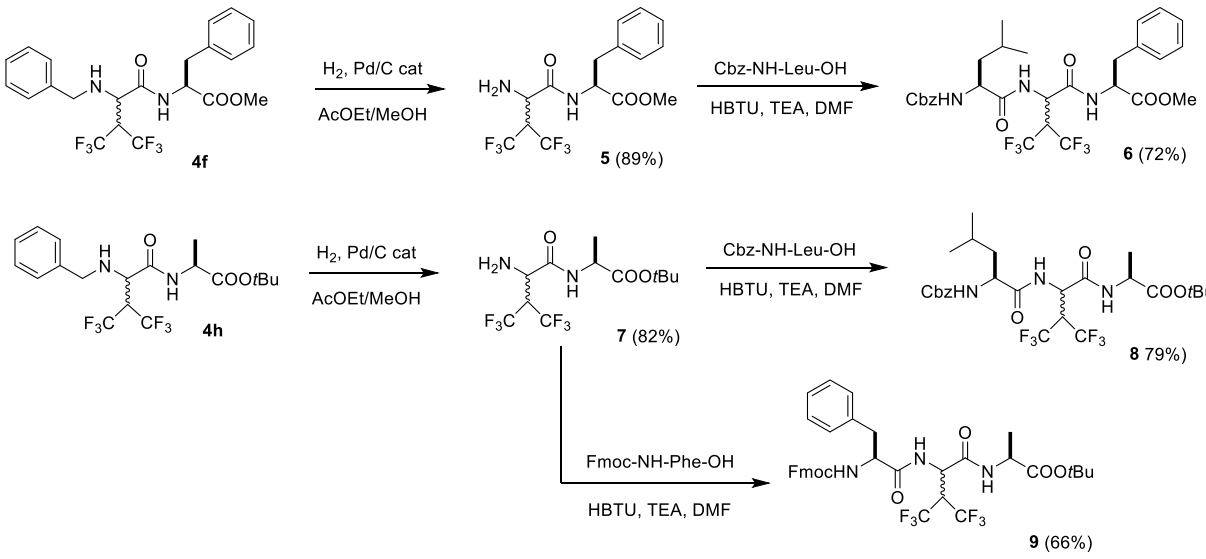
(*continued on next page*)

Table 1 (*continued*)



Entry	R^1	R^2-NH_2	solvent	base	T (°C)	Product	d.e.[a] (%)[b]	Yield (%)[c]
13			EtOH	DIPEA	25	 **4f** (1:1)	0	74
14	isoPr	NH_3[f]	EtOH	DIPEA	25	 **4g** (1:1)	0	24
15	isoPr		EtOH	DIPEA	25	n.r[d,e]	/	
16	Bn	H_2N—CH_3	EtOH	DIPEA	25	 **4h** (1:1)	0	82

[a] Diastereoisomeric excess.
[b] Determined with the aid of ^{19}F NMR spectroscopic analysis.
[c] Overall yield of purified diastereoisomeric mixture.
[d] No reaction occurred.
[e] Reaction carried out overnight (12 h).
[f] A 30 % aqueous solution of ammonia was used.



Scheme 2. Synthesis of hfVal-containing peptides.

$CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.02 (br s, 1H), 6.78 (d, $J = 7.2$ Hz, 1H), 4.55 (quintet, $J = 7.2$ Hz, 1H), 1.47 (s, 9H), 1.41 (d, $J = 7.2$ Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3, 160.7, 135.7 (q, $J = 3.0$ Hz), 125.4 (septet, $J = 33.3$ Hz), 119.2 (q, $J = 276.7$ Hz), 119.8 (q, $J = 276.7$ Hz), 82.8, 48.9, 27.8, 18.1; ^{19}F NMR (470 MHz, $CDCl_3$): δ −60.2 (q, $J = 9.4$ Hz), −65.2 (dq, $J = 9.4$ and 1.6 Hz); MS (ESI) m/z 358.2 $[M+Na]^+$; Anal. Calcd for $C_{12}H_{15}F_6NO_3$: C 42.99, H 4.51, N 4.18; found: C 43.00, H 4.50, N 4.20.

methyl Nω-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-N²-(4,4,4-trifluoro-3-(trifluoromethyl)but-2-enoyl)-L-argininate **3d**: yield 77 %; R_f 0.26 (95:05 DCM/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.0 Hz, 1H), 7.08 (s, 1H), 6.30–6.28 (br s, 3H), 4.56–4.51 (m, 1H), 3.69 (s, 3H), 3.20–3.18 (m, 2H), 2.94 (s, 2H), 3.31 (s, 3H), 3.29 (s, 3H), 2.07 (s, 3H), 1.88–1.86 (m, 1H), 1.81–1.79 (m, 1H), 1.60.1,57 (m, 2H), 1.45 (s, 6H); ^{19}F NMR ($CDCl_3$, 376 MHz) δ – 62.1 (q, J = 6.8 Hz, 3F), 67.0 (q, J = 6.8 Hz, 3F); ^{13}C NMR (101 MHz, CDCl3) δ 169.7, 169.4, 160.0, 157.1, 154.3, 136.4, 134.0, 130.3, 122.9, 117.9 (q, J = 276.7 Hz), 115.8, 84.7, 58.5, 50.6, 50.5, 41.2, 38.7, 27.0, 26.6, 23.3, 19.1, 17.2, 15.8, 12.2, 10.4, the carbon bonding the two CF_3 groups did not appear due to low intensity; ESI *m/z* 631.3 [M + H, (100)]+; Anal. calcd. for $C_{25}H_{32}F_6N_4O_6S$: C 47.62, H 5.12, N, 8.88; found: C 47.63, H 5.11, N, 8.88.

3.3. Anti-Michael addition to acceptors 3a-d. Typical procedure

To a stirred solution of Michael acceptor **3a** (100 mg, 0.31 mmol) in EtOH (or any other organic solvent, see Table 1, 2.0 mL), TEA (or any other organic base, see Table 1, 0.34 mmol, 47 μL) followed by benzyl amine (or any other amines, see Table 1, 0.34 mmol, 37 μL) were added at a given T (see Table 1). After the completion of the reaction (TLC monitoring, typically 3 h) the solvent was evaporated and the crude purified by FC affording 115 mg of **4c** (87% yield).

methyl (4,4,4-trifluoro-2-(((S)−1-(4-methoxyphenyl)ethyl)amino)−3-(trifluoromethyl)butanoyl)-L-valinate **4a,** major diastereoisomer: R_f 0.49 (20:80 AcOEt:hexane); $[\alpha]_D^{20}$ + 15,8 (c = 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.18 (d, J = 9.2 Hz, 1H), 7.26 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 4.42–4.35 (m 1H), 3.38 (dd, J = 8.8 and 5,2 Hz, 1H), 4.03–3.97 (m, 1H), 3.80 (s, 3H), 3.77 (s, 3H), 3.64–3.61 (m, 1H), 2.13–2.08 (m, 1H), 1.98 (d, J = 9.6 Hz, 1H), 1.43 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 7.2 Hz, 3H), 0.81 (d, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 170.0, 159.3, 134.9, 128.3, 123.7 (q, J = 282.5 Hz), 123.4 (q, J = 282.8 Hz), 114.3, 57.7, 56.4, 55.6, 55.3, 47.7 (quintet, J = 27.3 Hz), 31.1, 24.3, 18.7, 17.8; ^{19}F NMR (470 MHz, $CDCl_3$): δ – 61.4 (quintet, J = 11.3 Hz, 3F), - 64.2 (quintet, J = 11.7 Hz, 3F); MS (ESI) *m/z* 495.2 [M+Na]$^+$, 473.2 [M + H]$^+$; Anal. Calcd for $C_{20}H_{26}F_6N_2O_4$: C 50.85, H 5.55, N 5.93, found: C 50.86, H 5.55, N 5.93.

methyl (4,4,4-trifluoro-2-(((S)−1-(4-methoxyphenyl)ethyl)amino)−3-(trifluoromethyl)butanoyl)-L-valinate **4a,** minor diastereoisomer: R_f 0.45 (20:80 AcOEt:hexane); $[\alpha]_D^{20}$ + 10,3 (c = 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.34 (d, J = 9.2 Hz, 1H), 7.23 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 4.56 (dd, J = 9.2 and 4.4 Hz, 1H), 4.05–4.00 (m, 1H), 4.03–3.97 (m, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 3.67–3.65 (m, 1H), 2.23–2.25 (m, 1H), 2.04 (br s, 1H), 1.44 (d, J = 6.4 Hz, 3H), 0.97 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 170.0, 159.3, 135.3, 127.8, 114.1, 57.4, 57.0, 52.3, 52.2, 31.2, 22.2, 18.8, 17.5, the carbons of the two CF_3 groups and the carbon bonding the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, $CDCl_3$): δ – 61.1 (quintet, J = 11.7 Hz, 3F), - 64.8 (quintet, J = 11.3 Hz, 3F); MS (ESI) *m/z* 495.3 [M+Na]$^+$; Anal. Calcd for $C_{20}H_{26}F_6N_2O_4$: C 50.85, H 5.55, N 5.93, found: C 50.87, H 5.56, N 5.91.

methyl (4,4,4-trifluoro-2-(((R)−1-phenylethyl)amino)−3-(trifluoromethyl)butanoyl)-L-valinate **4b:** R_f 0.45 (20:80 AcOEt:hexane); ^{1}H NMR (400 MHz, $CDCl_3$), major diastereoisomer δ 8.16 (br d, J = 8.8 Hz, 1H), 7.39–7.28 (m, 5H), 4.56 (dd, J = 8.8 and 4.4 Hz, 1H), 4.10–4.05 (m, 1H), 3.89–3.87 (m, 1H), 3.79 (s, 3H), 3.69–3.67 (m, 1H), 2.34–2.30 (m, 1H), 2.24 (br s, 1H), 1.45 (d, J = 6.4 Hz, 3H), 1.03 (d, J = 6.8 Hz, 3H), 1.01 (d, J = 6.8 Hz, 3H); ^{1}H NMR (400 MHz, $CDCl_3$), minor diastereoisomer δ 8.31 (br d, J = 9.2 Hz, 1H), 7.39–7.28 (m, 5H), 4.41 (dd, J = 9.2 and 4.8 Hz, 1H), 4.38–4.32 (m, 1H), 4.06–4.03 (m, 1H), 3.76 (s, 3H), 3.61–3.59 (m, 1H), 2.21–2.16 (m, 1H), 1.96 (br d, J = 9.2 Hz, 1H), 1.45 (d, J = 6.4 Hz, 3H), 0.94 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) major diastereoisomer δ 171.6, 170.2, 143.0, 128.9, 128.0, 126.6, 57.6, 56.9, 55.9, 48.8 (q, J = 27.3 Hz), 31.2, 22.7, 19.1, 17.7, the carbons of the two CF_3 groups did not appear due to low intensity; ^{13}C NMR (101 MHz, $CDCl_3$) minor diastereoisomer δ 171.6, 169.7, 142.5, 128.8, 127.9, 127.3, 57.5, 56.9, 55.7, 48.8 (q, J = 27.3 Hz), 31.3, 24.4, 18.7, 17.6, the carbons of the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, $CDCl_3$): major diastereoisomer δ – 61.3 (quintet, J = 11.3 Hz, 3F), - 64.7 (quintet, J = 11.3 Hz, 3F); ^{19}F NMR (470 MHz, $CDCl_3$): minor diastereoisomer δ – 61.3 (quintet, J = 11.7 Hz, 3F), - 64.1 (quintet, J = 11.7 Hz, 3F); MS (ESI) *m/z* 443.2 [M + H]$^+$; Anal. Calcd for $C_{19}H_{24}F_6N_2O_3$: C 51.58, H 5.47, N 6.33, found: C 51.59, H 5.49, N 6.33.

methyl (2-(benzylamino)−4,4,4-trifluoro-3-(trifluoromethyl)butanoyl)-L-valinate **4c**: R_f 0.41 (20:80 AcOEt:hexane); ^{1}H NMR (400 MHz, $CDCl_3$), major diastereoisomer δ 8.10 (d, J = 8.8 Hz, 1H), 7.31–7.27 (m, 5H), 4.37 (dd, J = 9.2 and 5.2 Hz, 1H), 4.18–4.15 (m, 1H), 3.96 (d, J = 13.2 Hz, 1H), 3.77 (d, J = 13.2 Hz, 1H), 3.68–3.64 (m, 1H), 3.67 (s, 3H), 2.12–2.09 (m, 1H), 2.10 (br s, 1H), 0.80 (dd, J = 6.8 Hz both, 6H); ^{1}H NMR (400 MHz, $CDCl_3$), minor diastereoisomer δ 8.25 (d, J = 8.8 Hz, 1H), 7.31–7.27 (m, 5H), 4.43 (dd, J = 9.2 and 4.4 Hz, 1H), 4.18–4.15 (m, 1H), 3.90 (d, J = 12.4 Hz, 1H), 3.81 (d, J = 12.4 Hz, 1H), 3.69 (s, 3H), 3.68–3.64 (m, 1H), 2.12–2.09 (m, 1H), 2.16 (br s, 1H), 0.85 (dd, J = 6.8 Hz both, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) mixture of diastereoisomers δ 171.8, 171.6, 169.4, 169.3, 137.9, 137.8, 128.9, 128.8, 128.6, 128.5, 128.0, 127.9, 57.7, 57.6, 57.5, 57.3, 53.7, 53.3, 52.2, 52.1, 48.2 (septet, J = 27.3 Hz), 31.2, 31.0, 18.9, 18.8, 17.7, 17.5, the carbons of the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, $CDCl_3$): major diastereoisomer δ – 61.4 (quintet, J = 14.1 Hz, 3F), – 64.7 (quintet, J = 14.1 Hz, 3F); ^{19}F NMR (470 MHz, $CDCl_3$): minor diastereoisomer δ – 61.3 (quintet, J = 11.7 Hz, 3F), – 64.9 (quintet, J = 11.7 Hz, 3F); MS (ESI) *m/z* 452.1 [M+Na]$^+$; Anal. Calcd for $C_{18}H_{22}F_6N_2O_3$: C 50.47, H 5.18, N 6.54, found: C 50.48, H 5.18, N 6.55.

methyl (2-(benzylamino)−4,4,4-trifluoro-3-(trifluoromethyl)butanoyl)-L-phenylalaninate **4d**: R_f 0.45 (20:80 AcOEt:hexane); ^{1}H NMR (400 MHz, $CDCl_3$), mixture of two diastereoisomers δ 8.12 (d, J = 8.4 Hz, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.25–7.03 (m, 20H), 4.82–4.74 (m, 2H), 4.14–4.09 (m, 2H), 3.85–3.64 (m, 4H), 3.68 (s, 3H), 3.65 (s, 3H), 3.57–3.54 (m, 2H), 3.18–3.11 (m, 2H), 3.02–2.97 (m, 2H), 1.86–1.81 (br s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) mixture of two diastereoisomers δ 171.4, 171.3, 169.4, 169.2, 138.0, 137.9, 135.8, 135.5, 129.13, 129.10, 128.74, 128.71, 128.6, 128.5, 128.4, 127.8, 127.7, 127.3, 127.2, 57.5, 57.4, 53.5, 53.4, 53.2, 52.4, 52.3, 48.2 (quintet, J = 28.3 Hz), 38.0, 37.7, the carbons of the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, $CDCl_3$): mixture of two diastereoisomers δ – 61.4 (quintet, J = 14.1 Hz, 3F), – 61.5 (quintet, J = 14.1 Hz, 3F), – 64.8 (m, 6F); MS (ESI) *m/z* 477.2 [M + H]$^+$; Anal. Calcd for $C_{22}H_{22}F_6N_2O_3$: C 55.46, H 4.65, N 5.88, found: C 55.48, H 4.64, N 5.89.

tert-butyl (2-(benzylamino)−4,4,4-trifluoro-3-(trifluoromethyl)butanoyl)-L-alaninate **4e**: R_f 0.35 (20:80 AcOEt:hexane); ^{1}H NMR (400 MHz, $CDCl_3$), mixture of two diastereoisomers δ 8.20 (d, J = 8.0 Hz, 1H), 8.07 (d, J = 7.6 Hz, 1H), 7.29–7.18 (m, 10H), 4.35 (quintet, J = 6.8 Hz, 1H), 4.28 (quintet, J = 7.2 Hz, 1H), 4.20–4.05 (m, 2H), 3.92 (dd, J = 13.2 and 3.2 Hz, 1H), 3.86 (dd, J = 12.8 and 4.0 Hz, 1H), 3.78 (dd, J = 12.8 and 8.0 Hz, 1H), 3.72 (dd, J = 12.8 and 5.6 Hz, 1H), 3.62–3.57 (m, 2H), 2.00 (br s, 1H), 1.90 (br s, 1H), 1.41 (s, 9H), 1.39 (s, 9H), 1.28 (d, J = 7.2 Hz, 3H), 1.21 (d, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) mixture of two diastereoisomers δ 171.6, 171.4, 169.0, 168.8, 138.2, 138.1, 128.8, 128.7, 128.69, 128.60, 127.8, 82.1, 57.6, 57.5, 53.6, 53.4, 48.9, 48.2 (quintet, J = 27.3 Hz), 27.9, 27.8, 18.3, 18.2, the carbons of the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, $CDCl_3$): mixture of two diastereoisomers δ – 61.4 (m, 6F), – 64.8 (m, 6F); MS (ESI) *m/z* 443.5 [M + H]$^+$; Anal. Calcd for $C_{19}H_{24}F_6N_2O_3$: C 51.58, H 5.47, N 6.33, found: C 51.60, H 5.46, N 6.35.

methyl N²-(2-(benzylamino)−4,4,4-trifluoro-3-(trifluoromethyl)butanoyl)-Nω-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-L-argininate **4f**: R_f 0.24 (95:5 DCM:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$), mixture of two diastereoisomers δ 8.41 (br s, 1H), 8.35 (d, J = 7.6 Hz, 1H), 7.25–7.19 (m, 10H), 6.21–6.18 (br s, 6H), 4.49–4.46 (m, 2H), 3.78–3.73 (m, 4H), 3.64 (s, 3H), 3.62 (s, 3H), 3.35–3.33 (m, 4H),

3.13–3.11 (m, 4H), 2.89 (s, 4H), 2.50 (s, 6H), 2.43 (s, 6H), 2.34–2.31 (br s, 2H), 2.03 (s, 6H), 1.80–1.78 (m, 2H), 1.67–1.64 (m, 2H), 1.53–1.51 (m, 4H), 1.41 (s, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) mixture of two diastereoisomers δ 172.2, 172.1, 158.8, 156.3, 138.5, 138.3, 132.7, 132.2, 128.59, 128.57, 128.3, 128.2, 127.5, 124.7, 117.6, 86.4, 53.6, 53.4, 52.5, 52.4, 43.2, 40.6, 28.6, 19.2, 17.9, 12.4, the carbons of the two CF_3 groups and the carbon bonding the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, $CDCl_3$): mixture of two diastereoisomers δ – 66.2 (br s, 6F), – 66.4 (d, J = 9.4 Hz, 6F); MS (ESI) *m/z* 738.4 $[M + H]^+$; Anal. Calcd for $C_{32}H_{41}F_6N_5O_6S$: C 52.10, H 5.60, N 9.49, found: C 52.09, H 5.62, N 9.49.

methyl (4,4,4-trifluoro-2-(methylamino)−3-(trifluoromethyl)butanoyl)-L-phenylalaninate **4h**: R_f 0.18 (20:80 AcOEt:hexane); ^{1}H NMR (400 MHz, $CDCl_3$), mixture of two diastereoisomers δ 8.10 (d, J = 8.4 Hz, 2H), 7.31–7.29 (m, 6H), 7.14–7.11 (m, 4H), 4.89–4.83 (m, 2H), 4.14–4.11 (m, 2H), 3.75 (s, 3H), 3.73 (s, 3H), 3.48 (s, 1H), 3.39 (s, 1H), 3.24 (dd, J = 14.0 and 5.6 Hz, 1H), 3.18–3.06 (m, 3H), 2.51 (s, 3H), 2.40 (s, 3H), 1.65 (br s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) mixture of two diastereoisomers δ 171.6, 171.4, 169.3, 169.2, 135.8, 135.6, 129.2, 129.1, 129.0, 128.7, 128.6, 127.2, 127.1, 60.4, 60.3, 53.3, 53.2, 52.5, 52.3, 48.3 (septet, J = 27.3 Hz), 38.0, 37.8, 36.4, the carbons of the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, $CDCl_3$): mixture of two diastereoisomers δ – 61.6 (m, 6F), – 65.3 (septet, J = 9.4 Hz, 3F), – 65.2 (septet, J = 9.4 Hz, 3F); MS (ESI) *m/z* 423.4 $[M+Na]^+$; Anal. Calcd for $C_{16}H_{18}F_6N_2O_3$: C 48.01, H 4.53, N 7.00, found: C 48.00, H 4.55, N 7.01.

3.4. Hydrogenation of compounds 4. Typical procedure

To a stirred solution of Bn-NH-hfVal-Phe-OMe **4f** (100 mg, 0.21 mmol) in a 1:1 mixture of AcOEt/MeOH (6 mL), a catalytic amount of Pd/C was added and the mixture stirred under atmospheric pressure of H_2 for three hours at rt. The mixture was filtrated through a Celite pad, washed with MeOH and the solvents evaporated under reduced pressure. The crude was purified by FC affording 81 mg of H-hfVal-Phe-OMe dipeptide **5** (89 % yield).

methyl (2-amino-4,4,4-trifluoro-3-(trifluoromethyl)butanoyl)-L-phenylalaninate **5**: R_f 0.40 (20:80 AcOEt:hexane); ^{1}H NMR (400 MHz, $CDCl_3$), mixture of two diastereoisomers δ 7.95 (br d, J = 8.0 Hz, 1H), 7.91 (br d, J = 9.2 Hz, 1H), 7.20–7.18 (m, 6H), 7.06–7.03 (m, 4H), 4.79–4.73 (m, 2H), 4.25–4.23 (m, 2H), 3.81–3.79 (m, 2H), 3.66 (s, 3H), 3.65 (s, 3H), 3.11 (dd, J = 13.6 and, 5.6 Hz, 1H), 3.05 (d, J = 6.0 Hz, 2H), 3.02 (dd, J = 13.6 and 6.8 Hz, 1H), 1.75 (br s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) mixture of two diastereoisomers δ 171.5, 171.4, 169.2, 135.7, 135.6, 129.1, 129.0, 128.7, 128.6, 127.2, 53.6, 53.4, 52.4, 51.9, 51.8, 48.3 (septet, J = 29.3 Hz), 37.9, 37.8, the carbons of the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, $CDCl_3$): mixture of two diastereoisomers δ – 61.5 (m, 6F), – 65.9 (septet, J = 9.4 Hz, 3F), – 66.0 (septet, J = 9.4 Hz, 3F); MS (ESI) *m/z* 387.2 $[M + H]^+$; Anal. Calcd for $C_{15}H_{16}F_6N_2O_3$: C 46.64, H 4.18, N 7.25, found: C 46.64, H 4.19, N 7.27.

tert-butyl (2-amino-4,4,4-trifluoro-3-(trifluoromethyl)butanoyl)-L-alaninate **7**: R_f 0.33 (20:80 AcOEt:hexane); ^{1}H NMR (400 MHz, $CDCl_3$), mixture of two diastereoisomers δ 8.09 (br s, 2H), 4.44 (quintet, J = 7.2 Hz, 2H), 4.43–4.30 (m, 2H), 1.91 (br s, 4H), 1.49 (s, 9H), 1.48 (s, 9H), 1.41 (d, J = 7.2 Hz, 3H), 1.39 (d, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) mixture of two diastereoisomers δ 171.8, 171.6, 169.0, 168.8, 123.9 (q, J = 284.8 Hz), 82.2, 82.1, 52.0, 49.0, 48.9, 48.4 (septet, J = 26.3 Hz), 27.9, 27.8, 18.3, 18.2; ^{19}F NMR (470 MHz, $CDCl_3$): mixture of two diastereoisomers δ – 61.1 (m, 6F), – 65.9 (septet, J = 9.4 Hz, 3F), – 66.0 (septet, J = 9.4 Hz, 3F); MS (ESI) *m/z* 352.1 $[M + H]^+$; Anal. Calcd for $C_{12}H_{18}F_6N_2O_3$: C 40.91, H 5.15, N 7.95, found: C 40.89, H 5.16, N 7.96.

3.5. Coupling of H_2N-hfVal-dipeptides with Cbz-NH-Leu-OH. Typical procedure

To a solution of Cbz-Leu-OH (100 mg, 0.27 mmol) in DMF (2 mL) solid HBTU (102 mg, 0.27 mmol) was added at 0 °C. After 10 min a solution of H_2N-hfVal-Phe-OMe dipeptide **5** (93 mg, 0.24 mmol) and TEA (0.27 mmol, 37.5 μL) in DMF (1 mL) was added at the same temperature. The temperature was left to reach rt and the solution was stirred overnight. The solution was diluted with AcOEt and washed with brine four times. The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent was removed in vacuo. The crude was purified by FC (hexane/AcOEt 9:1) affording 109 mg (72 %) of the pure CbzNH-Leu-hfVal-Phe-OMe tripeptide **6**.

methyl (2-((S)−2-(((benzyloxy)carbonyl)amino)−4-methylpentanamido)−4,4,4-trifluoro-3-(trifluoromethyl)butanoyl)-L-phenylalaninate **6**: R_f 0.43 (20:80 AcOEt:hexane); ^{1}H NMR (400 MHz, $CDCl_3$), mixture of two diastereoisomers δ 7.27–7.29 (m, 16H), 7.03–7.01 (m, 4H), 6.76 (br d, J = 10.4 Hz, 2H), 5.30–5.25 (m, 2H), 5.10–4.85 (m, 4H), 4.72–4.68 (m, 2H), 4.27–4.24 (m, 2H), 4.08–4.06 (m, 2H), 3.62 (s, 3H), 3.61 (s, 3H), 3.09–2.96 (m, 4H), 1.64–1.60 (m, 4H), 1.48–1.46 (m, 2H), 0.86–0.84 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) mixture of two diastereoisomers δ 170.9, 166.7, 135.8, 135.5, 129.1, 129.0, 128.7, 128.6, 128.5, 128.4, 128.0, 127.9, 127.1, 67.8, 67.5, 60.4, 54.2, 54.0, 52.4, 39.6, 37.6, 37.5, 24.7, 22.8, 21.6, 21.0, 14.2, the carbons of the two CF_3 groups and the carbon bonding the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, $CDCl_3$): mixture of two diastereoisomers δ – 61.5 (septet, J = 9.4 Hz, 3F), – 61.6 (septet, J = 9.4 Hz, 3F), – 66.2 (septet, J = 9.4 Hz, 3F), – 66.4 (septet, J = 9.4 Hz, 3F); MS (ESI) *m/z* 634.7 $[M + H]^+$, 656.6 $[M+Na]^+$; Anal. Calcd for $C_{29}H_{33}F_6N_3O_6$: C 54.98, H 5.25, N 6.63, found: C 54.99, H 5.27, N 6.62.

tert-butyl (2-((S)−2-(((benzyloxy)carbonyl)amino)−4-methylpentanamido)−4,4,4-trifluoro-3-(trifluoromethyl)butanoyl)-L-alaninate **8**: R_f 0.36 (20:80 AcOEt:hexane); ^{1}H NMR (400 MHz, $CDCl_3$), mixture of two diastereoisomers δ 7.67–7.64 (br m, 2H), 7.26–7.19 (m, 11H), 7.07 (br s, 1H), 6.92 (br d, J = 10.4 Hz, 1H), 6.84 (br d, J = 8.4 Hz, 1H), 5.37–5.28 (m, 4H), 5.10–5.01 (m, 3H), 4.88 (d, J = 12.0 Hz, 1H), 4.39–4.35 (m, 4H), 4.16–4.13 (m, 1H), 3.96–3.93 (m, 1H), 1.69–1.54 (m, 4H), 1.48–1.46 (m, 2H), 1.37 (s, 9H), 1.36 (s, 9H), 1.29 (d, J = 7.2 Hz, 3H), 1.23 (d. J = 6.8 Hz, 3H), 0.89–0.83 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) mixture of two diastereoisomers δ 172.8, 171.8, 170.9, 166.6, 166.4, 156.9, 135.7, 135.6, 128.7, 128.6, 128.4, 128.3, 127.9, 127.8, 82.1, 81.9, 67.6, 67.3, 55.7, 55.4, 53.1, 49.5, 49.4, 48.0, 47.7 (septet, J = 27.3 Hz), 40.0, 39.9, 24.8, 24.2, 23.1, 22.8, 22.5, 22.1, 21.6, 21.3, 17.6, 17.3, the carbons of the two CF_3 groups and the carbon bonding the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, $CDCl_3$): mixture of two diastereoisomers δ – 61.63 (septet, J = 9.4 Hz, 3F), – 61.65 (septet, J = 9.4 Hz, 3F), – 66.1 (br m, 3F), – 66.4 (septet, J = 9.4 Hz, 3F); MS (ESI) *m/z* 622.5 $[M+Na]^+$; Anal. Calcd for $C_{26}H_{35}F_6N_3O_6$: C 52.08, H 5.88, N 7.01, found: C 52.07, H 5.90, N 7.00.

tert-butyl (2-((S)−2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)−3-phenylpropanamido)−4,4,4-trifluoro-3-(trifluoromethyl)butanoyl)-L-alaninate **9**: yield 66 %; higher diasteroisomers R_f 0.45 (20:80 AcOEt:hexane); $[\alpha]_D^{20}$ +26,1 (c = 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, dmso-d_6) δ 8.74 (d, J = 10.4 Hz, 1H), 8.30 (d, J = 6.8 Hz, 1H), 7.88–7.86 (d, J = 7.6 Hz, 2H), 7.79 (d, J = 8.0 Hz, 1H), 7.64–7.61 (m, 2H), 7.40–7.26 (m, 9H), 5.33 (dd, J = 10.0 and 3.6 Hz, 1H), 4.65–4.59 (m, 1H), 4.49–4.45 (m, 1H), 4.16–4.13 (m, 4H), 2.93 (dd, J = 14.0 and 4.4 Hz, 1H), 2.79 (dd, J = 14.0 and 10.0 Hz, 1H), 1.37 (s, 9H), 1.23 (d, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, dmso-d_6) δ 173.0, 171.4, 166.9, 156.6, 144.2, 144.1, 141.1, 138.1, 129.7, 128.5, 128.4, 128.09, 128.07, 127.5, 126.7, 125.7, 125.6, 120.5, 81.1, 79.6, 66.2, 56.3, 49.5, 47.3, 47.0, 37.2, 28.0, 27.9, 17.2, the carbons of the two CF_3 groups and the carbon bonding the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, dmso-d_6): δ −61.2 (quintet, J = 14.1 Hz), −64.3 (quintet, J = 9.4 Hz); MS (ESI) *m/z* 722.4 $[M + H]^+$; Anal. Calcd for $C_{36}H_{37}F_6N_3O_6$: C 59.91, H 5.17, N 5.82; found: C 59.90, H 5.19, N 5.82. lower diasteroisomers R_f 0.43

(20:80 AcOEt:hexane); ^{1}H NMR (400 MHz, dmso-d_6) δ 8.42 (d, J = 7.2 Hz, 1H), 8.28 (d, J = 10.0 Hz, 1H), 7.87 (d, J = 7.6 Hz, 2H), 7.86–7.84 (m, 1H), 7.64–7.62 (m, 2H), 7.41–7.20 (m, 9H), 5.34–5.24 (m, 1H), 4.45–4.42 (m, 2H), 4.16–4.13 (m, 4H), 3.07–3.03 (m, 1H), 2.82–2.79 (m, 1H), 1.39 (s, 9H), 1.27 (d, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, dmso-d_6) δ 172.4, 171.3, 166.2, 156.6, 156.3, 144.1, 141.1, 129.7, 128.5, 128.0, 127.5, 126.7, 125.7, 120.5, 81.2, 66.2, 49.4, 47.4, 47.0, 37.0, 27.9, 17.3 the carbons of the two CF_3 groups and the carbon bonding the two CF_3 groups did not appear due to low intensity; ^{19}F NMR (470 MHz, dmso-d_6): δ −61.1 (quintet, J = 9.4 Hz), −63.9 (quintet, J = 9.4 Hz).

CRediT authorship contribution statement

Maria Cristina Bellucci: Writing – original draft, Methodology. **Carola Romani:** Writing – review & editing, Methodology. **Monica Sani:** Writing – review & editing, Methodology. **Alessandro Volonterio:** Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

Authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jfluchem.2024.110315.

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