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# Synthesis of $CF_3$-containing tetrapeptide surrogates via Ugi reaction/dipolar cycloaddition sequence

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ABSTRACT

A convenient synthetic pathway to novel functionalized tetrapeptides containing the 1,2,3-triazole moiety is described. The target molecules were obtained by the reaction of fluorinated α-amino acids or α-aminophosphonates with azidopeptides via Cu(I)-catalyzed Huisgen cycloaddition reaction (click chemistry). The synthesized tetrapeptides may find important applications in biomedical chemistry as potential drugs.



## 1. Introduction

Peptides and proteins (polypeptides) are one of the most important classes of natural compounds. Being the fundamental in all organisms, they play an essential role in all processes within the cell. Short peptides are extremely important biogenic regulators having many functions. For example, glutathione is very simple tripeptide (γ-Glu-Cys-Gly), which acts as an antioxidant, detoxificant, anti-aging agent, protecting cells from damage.[1] Very interesting types of endogenous opioid tetrapeptides are endomorphin-1 (Tyr-Pro-Trp-Phe-$NH_2$) and endomorphin-2 (Tyr-Pro-Phe-Phe-$NH_2$). Endomorphins are natural fighters of stress and pain having the highest affinity and specificity to opioid μ-receptor.[2] Other tri- and tetrapeptides are also highly active small molecules exhibiting a broad variety of biological properties (regulation of immune system, hormone functions, wound healing, and skin remodeling activity, use as ACE inhibitors, etc.).

Synthetic α-amino acids play an important role in the area of peptide research and are extensively incorporated into biologically active peptides to restrict their conformational flexibility, enhance proteolytic stability, increase selectivity and improve pharmacokinetics and bioavailability properties of potential drugs.[3] Due to the extraordinary characteristics of fluorine, such as size, electronegativity, polarization, and the energy of its chemical bonds, fluorine chemistry has generated high level of interest over the recent decades.[4] Indeed, the introduction of fluorine atom(s) into a molecule often improves the therapeutic profile of potential biological agents, explaining why nowadays more than 20% of pharmaceutical agents and 40% of agrochemical compounds in the market feature at least one fluorine atom in their structure.[5] The advantages of peptides modified by trifluoromethyl-containing amino acids include enhanced proteolytic stability, affinity for lipid bilayer membranes, as well as stabilization of secondary supramolecular structures owing to the ability of the fluorine atom to form hydrogen bonds.[6,7] In addition, fluorinated compounds are extensively used as advanced materials.[8]

At the same time, α-aminophosphonates are structural mimics of α-amino acids. Some of these compounds exhibit very high potency inhibiting the enzymes that are involved in the metabolism of the corresponding amino acids. These compounds have already been found to display antibacterial, antiviral, anticancer, and some other types of bioactivity.[9]

On the other hand, the replacement of the native peptide bond (CONH) in peptidomimetic structures by different functions or atoms has been used for the optimization of their biological profiles.[10] Thus, we have previously reported effective approaches to

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the synthesis of trifluoromethyl peptidomimetics, such as depsipeptides by a metal carbene insertion reaction into COOH-group of *N*-protected amino acids[11] and by the Passerini reaction with $CF_3$-carbonyl compounds.[12] Now, we are interested in the synthesis of fluorinated pseudopeptides bearing a triazole ring as a peptide bond mimic. These heterocycles function as rigid linking units that can mimic the atom placement and electronic properties of a peptide bond without the same susceptibility to hydrolytic cleavage.[13–16]

The application of 1,2,3-triazole has been reported in peptide bond bioisosteres, peptide nanotubes,[14] β-turn mimics,[17] protease inhibitors,[18] cyclopeptide analogues,[19] and peptide chain analogues.[20] Therefore, the development of efficient synthetic methodologies for the preparation of new peptidomimetics containing fluorinated α-amino acids and α-aminophosphonates is of current interest. The copper-catalyzed 'click' reaction[21] is a method that can quickly and easily generate large libraries of different 1,2,3-triazole derivatives. This approach includes the ligation of azides and terminal alkynes. Moreover, due to its outstanding chemoselectivity and mild conditions, the reaction has been especially used in bioconjugate chemistry.[22]

Herein, we present a convenient synthetic pathway to novel functionalized tetrapeptides comprising triazole moiety as a linker connecting fluorinated α-amino acids and α-aminophosphonates with functionally substituted azidopeptides.

## 2. Results and discussion

The introduction of α-$CF_3$-α-AA into peptides by conventional methods of peptide chemistry is not a trivial task. Due to the low nucleophilicity of the amino-function and steric effects of the $CF_3$-group in α-trifluoromethyl α-amino acids the modification of standard methods for the peptide synthesis is required.[23] Until now only H-(α-$CF_3$)-Gly-OMe and H-(α-$CF_3$)-Ala-OMe were incorporated in the C-terminal position of peptides using the standard methodology. Often the classical activation methods for the amino-group of amino acids with more bulky substituents in the side chain are not suitable or lead to significant epimerization of the adjacent non-fluorinated amino acid. Therefore, click-methodology renders a good possibility to overcome this problem (Fig. 1).

The synthesis of the starting α-alkynyl-α-$CF_3$-α-amino acids and α-aminophosphonates **2a**–**f** was accomplished via the addition of sodium acetylide or allenylmagnesiumbromide to electrophilic trifluoromethylpyruvate imines and α-iminophosphonate under mild conditions.[24,25]

In recent years azides have become of increasing importance in organic chemistry especially for the synthesis of peptides due to their advantages, such as less steric hindrance and greater solubility when compared with amides and carbamates.[26] In spite of the fact that many synthetic approaches have been reported for the formation of α-azido acids, a convenient pathway to different azidopeptides by the Ugi four-component reaction[27] has been only recently suggested and it was demonstrated that these azidopeptides can be used quite effectively for the decoration of biomolecules by peptide residues.[28]

On the first stage of our investigation we decided to link tripeptide **1** with different α-trifluoromethyl-α-amino acid (α-$CF_3$-AA) derivatives **2a**–**d** to obtain modified peptides **3a**–**d**. We found that the click reaction of acetylenes **2a**–**d** and azidotripeptide **1** in biphasic mixture $CH_2Cl_2/H_2O$ proceeds regioselectively in high yields to afford conjugates **3a**–**d** after reflux (Scheme 1, Table 1). It should be noted that the click reaction of tripeptide **1** with propargyl-containing α-$CF_3$-AA derivatives required more prolonged reaction time (5 h) compared to their ethynyl analogues (3 h). Such a difference in the reactivity correlates with the electronic properties of acetylenes; alkynes bearing electron-withdrawing groups usually are more reactive in cycloaddition reactions.[29]

Then we conjugated α-propargyl-α-$CF_3$-AA Boc-protected derivative **2c** with various tripeptides using 1,3-dipolar Huisgen cycloaddition reaction under the same conditions (Scheme 2, Table 2). We have demonstrated that the variation of substituents in starting azidopeptides does not essentially affect the yield of the products. In all cases desired tetrapeptides **4a**–**f** have been obtained in high yields.

Next, we decided to expand this methodology to phosphorus analogues of amino acids, namely, aminophosphonates. Earlier we established that the generation of Cu(I) in situ from $CuSO_4$ and sodium ascorbate in a water–alcohol medium is the most preferred procedure for reactions of such type with acetylene-containing aminophosphonates.[24b] We found that the cycloaddition of

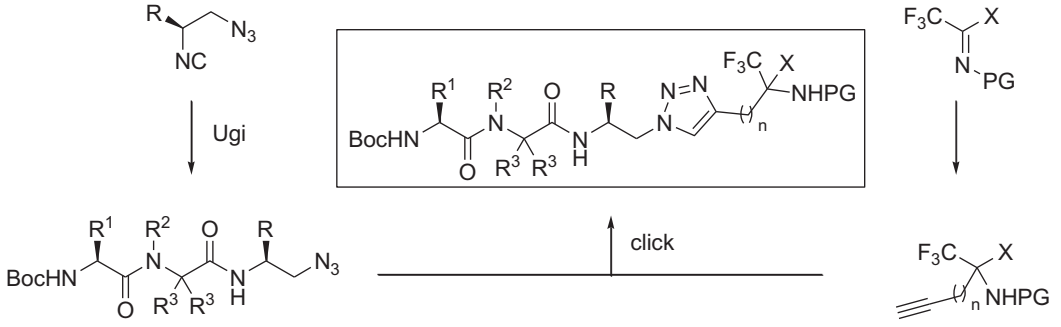
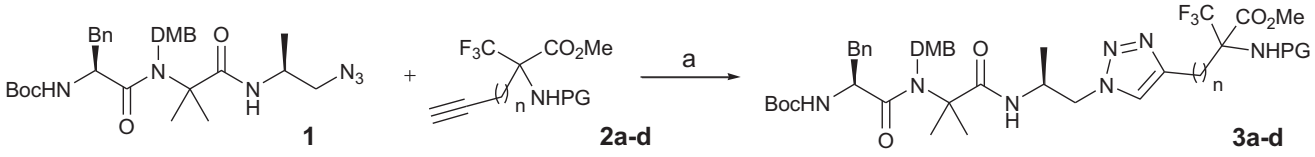



**Fig. 1.** The strategy for the synthesis of tetrapeptides.




**Scheme 1.** Synthesis of α-$CF_3$-α-amino acid containing tetrapeptides **3**. Conditions: (a) sodium ascorbate (40%), $CuSO_4 \cdot 5H_2O$ (10%), $CH_2Cl_2/H_2O$ (10:1), 40 °C, 3–5 h.

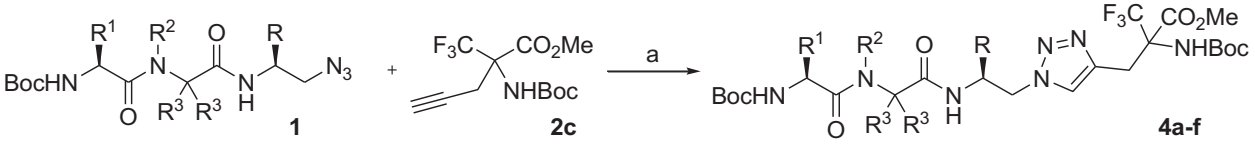
**Table 1**
α-$CF_3$-α-amino acid containing tetrapeptides **3** obtained via Scheme 1

| Entry | PG | *n* | Product | Yield[a] % |
|---|---|---|---|---|
| 1 | Boc | 0 | **3a** | 86 |
| 2 | Cbz | 0 | **3b** | 87 |
| 3 | Boc | 1 | **3c** | 90 |
| 4 | Cbz | 1 | **3d** | 90 |

[a] Isolated yields after purification by column chromatography.

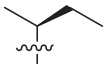
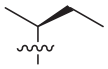
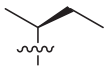
in good yield. Classical Pd-catalyzed hydrogenation in methanol was successfully applied for the deprotection of the amino-function of **5n** to afford the corresponding aminophosphonate **7** (Scheme 4).

Thus, the possibility of selective deprotection of obtained $CF_3$-peptides opens a convenient route for subsequent elongation of peptide chain and synthesis of higher $CF_3$-peptides.




**Scheme 2.** Synthesis of α-$CF_3$-α-amino acid containing tetrapeptides **4**. Conditions: (a) sodium ascorbate (40%), $CuSO_4 \cdot 5H_2O$ (10%), $CH_2Cl_2/H_2O$ (10:1), 40 °C, 5 h.

**Table 2**
α-$CF_3$-α-amino acid containing tetrapeptides **4** obtained via Scheme 2

| Entry | R | $R_1$ | $R_2$ | $R_3$, $R_4$ | Product | Yield[a] % |
|---|---|---|---|---|---|---|
| 1 |  | *i*-Pr | PMB | Me | **4a** | 95 |
| 2 | *i*-Pr | Me | DMB | H | **4b** | 73 |
| 3 | Me | H | DMB | H | **4c** | 74 |
| 4 | Bn | *i*-Pr | PMB | Me | **4d** | 85 |
| 5 | Me |  | DMB | Me | **4e** | 70 |
| 6 | Bn |  | PMB | Me | **4f** | 97 |

[a] Isolated yields after purification by column chromatography.

ethynyl- and propargyl-containing aminophosphonates **2e**,**f** to various azides **1** proceeded at 90 °C and led to completion within 2 h in all cases affording the corresponding tetrapeptides **5a**–**n** in good yields (Scheme 3, Table 3).

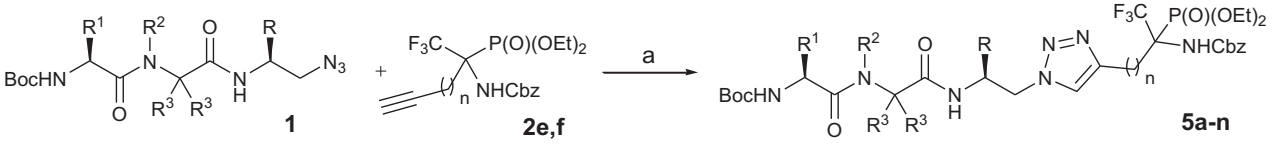
## 3. Conclusions

In conclusion, we have developed a convenient and simple method for the synthesis of α-$CF_3$-containing tetrapeptide surrogates based on copper-catalyzed 1,3-dipolar cycloaddition of azidopeptides to acetylenes having ester of phosphonates functionalities. The synthesized tetrapeptides may find important applications in biomedical chemistry as potential drugs.

## 4. Experimental section

### 4.1. General information

All solvents used in reactions were freshly distilled from appropriate drying agents before use. All other reagents were recrystallized or distilled as necessary. Reactions were performed under an atmosphere of dry nitrogen. Analytical TLC was performed with Merck silica gel 60 $F_{254}$ plates. Visualization was accomplished by UV light and spraying by $Ce(SO_4)_2$, solution in 5% $H_2SO_4$ or aqueous potassium permanganate ($KMnO_4$). Flash chromatography was carried out using Merck silica gel 60 (230–400 mesh ASTM). Optical rotations were measured on a Perkin–Elmer 341 polarimeter at 589 nm. High-resolution mass spectra (HRMS) were measured on a Micr OTOF II (Bruker Daltonics) spectrometer. IR spectra were measured on a Fourier




**Scheme 3.** Synthesis of α-$CF_3$-α-aminophosphonate containing tetrapeptides **5**. Conditions: (a) sodium ascorbate (30%), $CuSO_4 \cdot 5H_2O$ (5%), *t*-BuOH/$H_2O$ (1:1), 90 °C, 2 h.

It should be noted that in all cases the desired products have been isolated as a mixture of diastereomers because starting acetylenes contained a stereogenic center and were used in racemic form. The purification of the final triazoles **3a**–**d**, **4a**–**f**, **5a**–**n** has been achieved by means of column chromatography on silica gel.

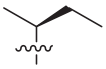
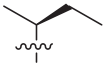
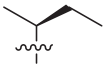
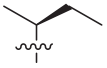
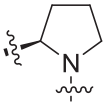
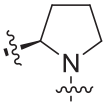
Both protecting groups of the amide and the amine functions can be easily removed from the target conjugates, either in an orthogonal mode or in one step. Thus, heating of the conjugate **3a** in TFA led to the product **6** containing free amide and amine groups

spectrometer Nicolet 6700. NMR spectra were recorded on a Bruker AV-300, AV-400 or AV-600 spectrometer operating at 300 MHz, 400 MHz or 600 MHz, respectively (TMS) for $^1H$; 100 or 151 MHz for $^{13}C$, 188 or 282 MHz for $^{19}F$ ($CF_3COOH$); 121 MHz for $^{31}P$ ($H_3PO_4$).

### 4.2. General procedure for the synthesis of triazoles 3 and 4

The corresponding peptide **1** (1 mmol), $CuSO_4 \cdot 5H_2O$ (25 mg, 0.1 mmol) in 0.5 mL of $H_2O$ and sodium ascorbate (79 mg,

**Table 3**
α-$CF_3$-α-aminophosphonate containing tetrapeptides **5** obtained via Scheme 3

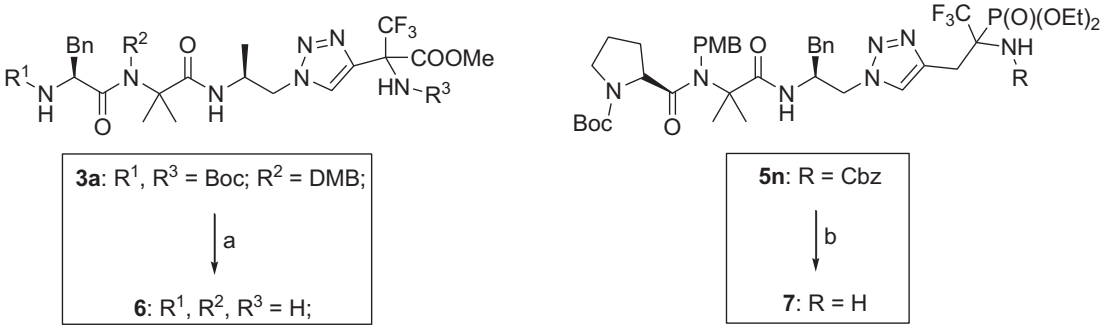
| Entry | R | $R_1$ | $R_2$ | $R_3$, $R_4$ | *n* | Product | Yield[a] % |
|---|---|---|---|---|---|---|---|
| 1 |  | *i*-Pr | PMB | Me | 0 | **5a** | 77 |
| 2 |  | *i*-Pr | PMB | Me | 1 | **5b** | 72 |
| 3 | Bn |  | PMB | Me | 0 | **5c** | 74 |
| 4 | Bn |  | PMB | Me | 1 | **5d** | 60 |
| 5 | Bn | *i*-Pr | PMB | Me | 0 | **5e** | 57 |
| 6 | Bn | *i*-Pr | PMB | Me | 1 | **5f** | 77 |
| 7 | H | Me | PMB | Me | 0 | **5g** | 65 |
| 8 | H | Me | PMB | Me | 1 | **5h** | 69 |
| 9 | Me | H | DMB | H | 0 | **5i** | 63 |
| 10 | Me | H | DMB | H | 1 | **5j** | 66 |
| 11 | Me | Bn | DMB | Me | 0 | **5k** | 68 |
| 12 | Me | Bn | DMB | Me | 1 | **5l** | 65 |
| 13 | Bn |  | PMB | Me | 0 | **5m** | 60 |
| 14 | Bn |  | PMB | Me | 1 | **5n** | 73 |

[a] Isolated yields after purification by column chromatography.

Physical and spectroscopic data, including NMR, high-resolution mass analysis, and elemental analysis of compounds **3b**–**d** and **4a**–**f** are available in Supplementary data.

### 4.3. General procedure for the synthesis of triazoles 5

To a solution of the corresponding acetylene **2e**,**f** (1 mmol) in the mixture of *t*-BuOH/$H_2O$ (1:1 ratio) (3 mL) was added a solution of corresponding azide **1** (1 mmol) in the mixture of *t*-BuOH/$H_2O$ (2 mL), sodium ascorbate (30 mol %), and 0.5 M copper sulfate pentahydrate (5 mol %). The reaction mixture was stirred at 90 °C, 2 h until the completion of the reaction monitored by TLC. After evaporation of the solvent under reduced pressure, water (5 mL) was added to a residue and the aqueous layer was extracted twice with ethyl acetate (2×10 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane/acetone).




**Scheme 4.** Deprotection of conjugates **3a** and **5n**. Conditions: (a) TFA, reflux, 2 h, 67%; (b) $H_2$, Pd/C, MeOH, rt, 62%.

0.4 mmol) in 0.5 mL of $H_2O$ were added successively to a solution of the corresponding acetylene **2a**–**d** (1 mmol) in 10 mL of $CH_2Cl_2$. The reaction mixture was stirred at 40 °C for 3 h for **3a**,**b** and 5 h for **3c**,**d** and **4a**–**f**. The solvent was removed in vacuo and the residue was purified by column chromatography (ethyl acetate or $CH_2Cl_2$/MeOH).

*4.2.1. N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl-$N^2$-[(2,4-dimethoxyphenyl)methyl]-$N^1$-[(1S)-2-[4-[1-[[(1,1-dimethylethoxy)carbonyl]amino]-2,2,2-trifluoro-1-(methoxycarbonyl)ethyl]-1H-1,2,3-triazol-1-yl]-1-methylethyl]-2-methylalaninamide (mixture of diastereomers, ~1:1) (**3a**).* Yield (0.742 g, 86%) as a white solid, mp 88–89 °C; $R_f$ ($CH_2Cl_2$/MeOH 25/1) 0.4; $[\alpha]_D^{20}$ −8.9 (*c* 3.0, MeOH); $\nu_{max}$ (Nujol) 3410, 1760, 1740, 1715, 1675, 1650 $cm^{-1}$; $\delta_H$ (400 MHz, DMSO-$d_6$, 80 °C) 1.09–1.13 (m, 3H, $CH_3$), 1.21–1.27 (m, 9H, $3CH_3$–Boc, 6H, $2CH_3$), 1.38 and 1.40 (both s, 9H, $3CH_3$–Boc), 2.61–2.71 (m, 1H, $CH_2Ph$), 2.86–2.97 (m, 1H, $CH_2Ph$), 3.72 (s, 3H, $OCH_3$), 3.77 (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 4.20–4.29 (m, 1H, CH), 4.33–4.54 (m, 3H, 2 $CH_2$, 1H, CH), 4.67–4.77 (m, 1H, $CH_2$), 5.65 (br s, 1H, NH), 6.44–6.48 (m, 1H, $CH_{Ar}$), 6.56–6.58 (m, 1H, $CH_{Ar}$), 6.78–6.90 (m, 1H, NH), 6.97–7.06 (m, 2H, $CH_{Ar}$), 7.11–7.20 (m, 3H, $CH_{Ar}$), 7.34–7.38 (m, 1H, $CH_{Ar}$), 7.63–7.73 (m, 1H, NH), 8.33 (br s, 1H, CH–triazole); $\delta_F$ (188 MHz, $CDCl_3$) 3.44 and 3.66 (both s, 3F, $CF_3$); $\delta_C$ (100 MHz, $CDCl_3$) 17.0, 17.2, 22.4, 22.6, 24.1, 24.2, 28.0, 28.2, 39.7, 39.9, 41.8, 41.9, 45.7, 53.1, 53.2, 53.5, 53.7, 55.2, 55.3, 62.6, 62.7, 64.6–65.3 (m), 79.5, 80.9, 98.4, 98.5, 104.2, 118.2, 123.1 (q, $^1J_{C-F}$=286.9 Hz, $CF_3$), 125.6, 126.7, 128.1, 128.4, 129.6, 136.6, 136.7, 138.8, 138.9, 153.5, 156.8, 156.9, 160.1, 160.2, 165.5, 173.2, 174.7; HRMS (ESI): $MNa^+$, found 886.3952. $C_{41}H_{56}F_3N_7O_{10}$ requires 886.3938.

*4.3.1. N-(tert-Butoxycarbonyl)valyl-$N^1$-[1-({4-[1-{[(benzyloxy)carbonyl]amino}-1-(diethoxyphosphoryl)-2,2,2-trifluoroethyl]-1H-1,2,3-triazol-1-yl}methyl)-2-methylbutyl]-$N^2$-(4-methoxybenzyl)-2-methylalaninamide (mixture of diastereomers, ~1:1) (**5a**).* Yield (66.1 mg, 77%) as a white solid, mp 88 °C; [Found: C, 55.91; H, 7.01; N, 10.11$C_{44}H_{65}F_3N_7O_{10}P$ requires C, 56.22; H, 6.97; N, 10.43%]; $R_f$ (hexane/acetone 1.5/1) 0.3; $[\alpha]_D^{25}$ −49.7 (*c* 1.8, $CHCl_3$); $\nu_{max}$ (Nujol) 3370, 1755, 1685, 1518, 1256, 1170 $cm^{-1}$; $\delta_H$ (600 MHz, DMSO-$d_6$, 70 °C) 0.74–0.77 (m, 6H, $2CH_3$), 0.86–0.89 (m, 3H, $CH_3$ in *i*-Pr), 0.95 (dd, 3H, $^3J_{H-H}$=6.7 Hz, 2.5 Hz, $CH_3$ in *i*-Pr), 1.15–1.23 (m, 9H, $3CH_3$, 1H, CH in *sec*-Bu), 1.31 (br s, 3H, $CH_3$), 1.36 (s, 9H, $3CH_3$–Boc), 1.52–1.61 (m, 2H, $CH_2$ in *sec*-Bu), 1.92–1.97 (m, 1H, CH in *i*-Pr), 3.75 (s, 3H, $OCH_3$), 3.87–3.94 (m, 1H, CH), 3.99–4.11 (m, 4H, $OCH_2$, 1H, CH), 4.50 (d, 2H, $^3J_{H-H}$=6.7 Hz, $CH_2$), 4.61 (dd, 1H, $J_{AB}$=18.1 Hz, 3 Hz, $CH_2$–triazole), 4.72 (d, 1H, $J_{AB}$=17.7 Hz, $CH_2$–triazole), 5.01 (d, 1H,

$J_{AB}$=12.6 Hz, CH), 5.04 (d, 1H, $J_{AB}$=12.4 Hz, CH), 6.22 (br s, 1H, NH–CH (*sec*-Bu)), 6.65–6.69 (m, 1H, NH–Cbz), 6.90 (d, 2H, $^3J_{H-H}$=8.7 Hz, $CH_{Ar}$), 7.31–7.37 (m, 7H, $CH_{Ar}$), 7.43 and 7.47 (br s, 1H, NH–Boc), 8.14 and 8.16 (both s, 1H, CH–triazole); $\delta_F$ (282 MHz, DMSO-$d_6$) 8.73 and 8.91 (both s, 3F, $CF_3$); $\delta_P$ (121 MHz, DMSO-$d_6$) 12.49 and 12.69 (both s); $\delta_C$ (100 MHz, DMSO-$d_6$) 11.7, 11.8, 15.4, 15.5, 16.5 (d, $^3J_{P-C}$=5.5 Hz), 18.2, 19.8, 23.0, 23.1, 24.9 (m), 28.5, 31.1, 36.8, 37.1, 46.2, 50.6, 50.7, 53.6, 53.8, 55.5, 56.9, 61.4 (dq, $^1J_{P-C}$=150.3 Hz, $^2J_{C-F}$=30.1 Hz), 62.5, 62.6, 64.3 (t, $J_{P-C}$=6.6 Hz), 64.6 (t, $J_{P-C}$=6.1 Hz), 66.3, 66.4, 78.5, 114.1, 124.2 (q, $^1J_{C-F}$=290.8 Hz, $CF_3$), 126.2, 126.4, 128.1, 128.3, 128.8, 131.9, 136.9, 138.3 (m), 154.1 (m), 155.9, 158.6, 172.5, 174.2.

Physical and spectroscopic data, including NMR, high-resolution mass analysis, and elemental analysis of compounds **5b**–**n** are available in Supplementary data.

### 4.4. Synthesis of 6

A solution of **3a** (0.05 g, 0.0058 mmol) in trifluoroacetic acid (0.7 mL, 9.1 mmol) was refluxed for 2 h. The reaction mixture was diluted with DCM (5 mL) and neutralized with aqueous saturated solution of sodium bicarbonate until violet color disappeared. Layers were separated and the aqueous layer was extracted with DCM (2×10 mL). The combined organic extract were dried ($Na_2SO_4$) and evaporated. The residue was purified by column chromatography ($CH_3CN/NH_3$).

*4.4.1. L-Phenylalanyl-N$^1$-[(1S)-2-[4-[1-amino-2,2,2-trifluoro-1-(methoxycarbonyl)ethyl]-1H-1,2,3-triazol-1-yl]-1-methylethyl]-2-methylalaninamide (mixture of diastereomers, ~1:1) (**6**).* Yield (20 mg, 67%) as a yellow oil; $R_f$ ($CH_3CN/NH_3$ 9/1) 0.4; $[\alpha]_D^{20}$ −1.4 (*c* 1.0, MeOH); $\nu_{max}$ (Nujol) 3350, 1750, 1660 $cm^{-1}$; $\delta_H$ (400 MHz, $CDCl_3$) 1.15 (d, 3H, $^3J_{H-H}$=6.6 Hz, $CH_3$), 1.44 (s, 6H, $3CH_3$), 3.16–3.21 (m, 1H, $CH_2Ph$), 3.53–3.56 (m, 1H, $CH_2Ph$), 3.85 and 3.86 (both s, 3H, $OCH_3$), 3.87–3.91 (m, 1H, CH), 4.30–4.59 (m, 2H, $CH_2$, 1H, CH), 7.05 and 7.13 (both d, 1H, $^3J_{H-H}$=7.5 Hz, NH), 7.18–7.24 (m, 3H, $CH_{Ar}$), 7.28–7.35 (m, 2H, $CH_{Ar}$), 7.61 (s, 1H, NH), 7.88 and 7.92 (both s, 1H, CH–triazole); $\delta_F$ (188 MHz, $CDCl_3$) 0.85 and 0.91 (both s, 3F, $CF_3$); $\delta_C$ (100 MHz, $CDCl_3$) 17.2, 17.4, 24.6, 25.0, 25.2, 25.4, 39.8, 40.2, 45.5, 54.0, 54.3, 56.2, 56.3, 56.9, 63.1, 64.8–65.6 (m), 123.8 (q, $^1J_{C-F}$=285.1 Hz, $CF_3$), 124.9, 127.0, 128.7, 129.3, 136.9, 137.3, 141.4, 167.8, 174.3, 174.4; HRMS (ESI): $MNa^+$, found 536.2201. $C_{22}H_{30}F_3N_7O_4$ requires 536.2209.

### 4.5. Synthesis of 7

To a solution of Cbz-protected aminophosphonate **5n** (0.41 g, 0.48 mmol) in methanol (10 mL) 10% Pd/C (0.025 g, 5 mol %) was added and slow stream of hydrogen was bubbled at room temperature. When TLC indicated no starting material (about 5 h), mixture was filtered and the solvent was evaporated to dryness. The residue was purified by column chromatography (hexane/acetone).

*4.5.1. 1-(tert-Butoxycarbonyl)prolyl-N$^1$-(2-{4-[2-amino-2-(diethoxyphosphoryl)-3,3,3-trifluoropropyl]-1H-1,2,3-triazol-1-yl}-1-benzylethyl)-N$^2$-(4-methoxybenzyl)-2-methylalaninamide (mixture of diastereomers, ~2:1) (**7**).* Yield (0.25 g, 62%) as a white solid, mp 65–67 °C; [Found: C, 56.11; H, 6.73; N, 11.35$C_{40}H_{57}F_3N_7O_8P$ requires C, 56.40; H, 6.74; N, 11.51%]; $R_f$ (hexane/acetone 1/1) 0.3; $[\alpha]_D^{25}$ +18.8 (*c* 0.8, $CHCl_3$); $\nu_{max}$ (Nujol) 3382, 1706, 1668, 1515, 1252, 1162 $cm^{-1}$; $\delta_H$ (300 MHz, $CDCl_3$) 1.31–1.55 (m, 21H, $7CH_3$), 1.71–1.79 (m, 1H, $CH_2$–Proline), 1.89–2.09 (br m, 1H, $CH_2$–Proline, 2H, $CH_2$–Proline), 2.69–2.78 (m, 1H, $CH_2$–Ph), 3.14–3.58 (m, 1H, $CH_2$–Ph, 4H, $2CH_2$), 3.87 (s, 3H, $OCH_3$), 4.24–4.62 (br m, 4H, $2OCH_2$, 2H, $CH_2$–triazole, 2H, $CH_2$, 2H, 2CH), 6.61 (m, 2H, $NH_2$), 6.98 (d, 2H, $^3J_{H-H}$=7.3 Hz, $CH_{Ar}$), 7.28–7.41 (m, 7H, $CH_{Ar}$), 7.47–7.50 and 7.67–7.71 (br s, 1H, CH–triazole), 7.94 and 8.15 (both s, 1H, NH); $\delta_F$ (282 MHz, $CDCl_3$) 5.97 and 6.15 (both s, 3F, $CF_3$); $\delta_P$ (121 MHz, $CDCl_3$) 17.64 and 17.91 (both s); $\delta_C$ (100 MHz, $CDCl_3$) 16.3–16.4 (m), 23.0, 23.1, 23.5, 23.8, 24.4, 24.6, 27.7, 27.8, 28.6, 30.6, 31.6, 37.5, 37.6, 46.6, 46.7, 47.2, 47.6, 51.3 (m), 51.6, 51.8, 55.3, 57.2, 57.3, 62.4, 62.5, 60.0 (dq, $^1J_{P-C}$=153.7 Hz, $^2J_{C-F}$=26.5 Hz), 63.4 (d, $^2J_{P-C}$=5.5 Hz), 64.2 (d, $^2J_{P-C}$=6.6 Hz), 79.6, 79.7, 114.3, 123.5 (q, $^1J_{C-F}$=283.4 Hz, $CF_3$), 124.5, 127.2–127.4, 126.7–126.9, 128.6, 128.7, 129.2, 129.3, 130.6, 130.7, 137. 3, 137.4, 140.7, 140.8, 154.7, 158.8–158.9 (m), 174.5, 174.7, 174.9, 175.0.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.11.037.

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