Peptide modification by incorporation of α-trifluoromethyl α-amino acids via trifluoromethyl-substituted acylimines*

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Abstract

α-Trifluoromethyl-substituted α-amino acids can be introduced into the C-terminal position of peptides via acylimines obtained on reaction of trifluoropyruvates with N-protected α-amino acid amides and dipeptide amides, respectively.

Introduction

The development of new effective synthetic routes to α,α-dialkyl amino acids [4], e.g. α-trifluoromethyl-substituted α-amino acids (TFM amino acids) [5–8], and of methods for incorporating this class of non-natural amino acids into peptides is of current interest because of the conformational restrictions they induce and which promote the helical structure in peptides [9, 10].

TFM amino acids show a broad and interesting spectrum of biological activity. Some exhibit antibacterial and antihypertensive properties [11, 12], others are enzyme inhibitors (suicide inhibitors), especially for pyridoxal phosphate-controlled processes, e.g. transamination and decarboxylation reactions [13]. The presence of TFM amino acids in peptides should retard degradation by peptidases [4], improve transport rates *in vivo* because of the highly lipophilic character of the trifluoromethyl group and increase permeability of certain 'body barriers' such as the blood–brain barrier. The trifluoromethyl group is also attractive since it is relatively non-toxic and somewhat more stable than the mono- and difluoro–methyl analogues [14].

Experimental

^{13}C and ^{19}F NMR spectra were recorded with a Bruker AM 360 spectrometer at 90 and 339 MHz or with a Bruker AC 250 spectrometer at 62.5 and 235

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MHz. As reference standard, TMS was used for ^{13}C NMR spectra (internal) and trifluoroacetic acid for ^{19}F NMR spectra (external). Mass spectra were recorded with electron ionization (EI, 70 eV) on a Varian MAT CH5 instrument and with chemical ionization (CI) on a Varian MAT M 1125 instrument. Melting points (not corrected) were determined using a Tottoli apparatus (Büchi SMP-20). Elemental microanalyses were carried out with a Heraeus CHN-Elemental Analyzer.

*General procedure for the synthesis of N-protected 2-amino-2-hydroxy-3,3,3-trifluoropropionic acid methyl esters (**3**) and (**7**)*

Methyl trifluoropyruvate (**2**) (10 mmol) was added with stirring to a suspension or a solution of **1** (10 mmol) [or **6** (10 mmol)] in dry ether or methylene chloride (100 ml). The mixture was stirred at room temperature for 1–7 d. Completion of the reaction could be ascertained from ^{19}F NMR analysis. The resulting solution was washed with water (3–5×50 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give **3b–3g**. Compounds **3** can be used without purification for further reactions. In the case of **3a**, a colourless solid precipitated out of the solution and was separated by filtration. Recrystallization from chloroform acetone (1:2) gave **3a** as a colourless crystalline solid.

Satisfactory C (±0.68%), H (±0.23%) and N (±0.41%) analyses were obtained.

*General procedure for the synthesis of dipeptide and tripeptide methyl esters (**5**) and (**9**) containing 3,3,3-trifluoroalanine (see Table 2)*

Trifluoroacetic anhydride (15 mmol) and quinoline (30 mmol) were simultaneously added to a vigorously stirred solution of **3** (15 mmol) [or **7** (15 mmol)] in dry ether (100 ml) at −78 °C. After stirring for an additional 10 min the precipitated quinolinium trifluoroacetate was removed by filtration at low temperature under inert gas. The solution of **4** (or **8**) was then added to a stirred suspension of $NaBH_4$ (20 mmol) in dry ether (50 ml) at −78 °C. After 2 h at −78 °C, the mixture was allowed to warm up to room temperature. Excess $NaBH_4$ was carefully hydrolyzed with cold 1 N HCl. The organic layer was washed with H_2O (3×50 ml), dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by column chromatography on SiO_2 (eluent Et_2O) or by recrystallization from $CHCl_3$ or $CHCl_3$/hexane.

Satisfactory C (±0.45%), H (±0.17%) and N (±0.14%) analyses were obtained.

*General procedure for the synthesis of dipeptide methyl esters **10** containing 2-trifluoromethyl-substituted α-amino acids (see Table 3)*

A solution of the Grignard reagent (15 mmol) was added dropwise to a stirred solution of **4** (15 mmol) in dry ether at −78 °C. After 2 h at −78 °C, the reaction was allowed to warm up to room temperature. The mixture

was quenched with cold 1 N HCl. The organic layer was separated and washed with H_2O. The aqueous layers were twice extracted with ether (75 ml). The combined organic layers were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by column chromatography on SiO_2 (eluent Et_2O).

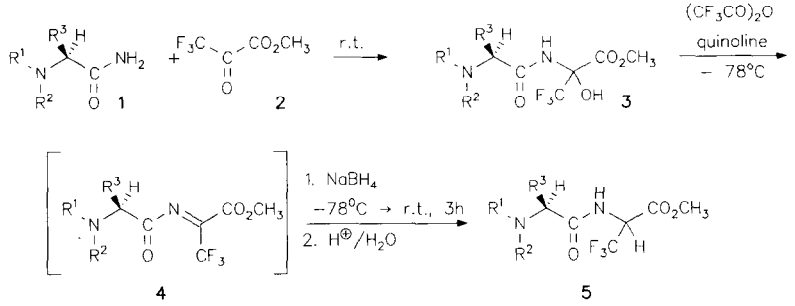
Satisfactory C (±0.38%), H (±0.07%) and N (±0.32%) analyses were obtained.

Results and discussion

In a preceding paper we described a methodology for the N-terminal introduction of TFM amino acids into peptides via TFM Leuchs anhydrides [1]. TFM amino acids are readily available from 3,3,3-trifluoropyruvates [5,6,8]. We have shown that this route is excellently suited to the introduction of urethane protecting groups [8, 15, 16]. The concept of amidoalkylation has been applied by others to the synthesis of fluorine free α-amino acid derivatives [17–20]. In this paper we report an adaptation of this strategy for the synthesis of di- and tri–peptides containing C-terminal TFM amino acids.

The reaction of *N*-benzoyl (Bz-) amino acid amides, *N*-benzyloxycarbonyl (*Z*-) amino acid amides [21] or *Z*-dipeptide amides [22] with methyl 3,3,3-trifluoropyruvate (**2**) at room temperature gives the [1:1] adducts **3** (Scheme 1, Table 1). The acylimines **4** are generated *in situ* by the dehydration of **3** with trifluoroacetic anhydride/quinoline in ether at −78 °C. Careful exclusion of moisture is essential to obtain satisfactory yields. The reactions may be monitored by ^{19}F NMR spectroscopy. Quinolinium trifluoroacetate is removed by filtration under inert gas at low temperature. In contrast to the analogous urethane-protected imines [8, 15], the acylimines **4** were found to be unstable at room temperature. However, the thermal stability of compounds **4** increases with the size of the R^3 substituent.

A solution of freshly prepared **4** in ether was added to a suspension of $NaBH_4$ (2 equiv.) in ether at −78 °C with vigorous stirring. Reduction of the C=N bond of the acylimine **4** gave the 3,3,3-trifluoroalanine containing



Scheme 1.

TABLE 1

N-protected 2-amino-2-hydroxy-3,3,3-trifluoropropionic acid methyl esters (**3**) and (**7**)

No.	R^1	R^2	R^3	Melting point (°C)	Yield (%)	Formula (m.w.)	^{19}F NMR δ (ppm)	^{13}C NMR (C–CF_3) δ (ppm)
3a	Bz	H	H	158 (dec.)	79	$C_{13}H_{13}F_3N_2O_5$ (334.26)	−0.3[b] (s, CF_3)	80.1 (q)[b] (J=31 Hz)
3b[a]	*Z*	H	CH_3	oil	70	$C_{15}H_{17}F_3N_2O_6$ (378.31)	−2.4/−2.5[c] (s, CF_3)	81.4 (q)[c] (J=32 Hz)
3c[a]	*Z*	H	$CH(CH_3)_2$	oil	89	$C_{17}H_{21}F_3N_2O_6$ (406.36)	−1.4/−1.5[d] (s, CF_3)	80.6/80.9 (q)[d] (J=33 Hz)
3d[a]	*Z*	H	$CH_2C_6H_5$	44 (dec.)	99	$C_{21}H_{21}F_3N_2O_6$ (454.41)	−1.5/−1.6[d] (s, CF_3)	80.5/80.8 (q)[d] (J=33 Hz)
3e[a]	*Z*		$-(CH_2)_3-$	48 (dec.)	83	$C_{17}H_{19}F_3N_2O_6$ (404.35)	−1.8/−1.9[d] (s, CF_3)	80.7/80.9 (q)[d] (J=33 Hz)
3f[a]	*Z*-Leu	H	CH_3	58–60	98	$C_{21}H_{28}F_3N_3O_7$ (491.47)	−1.4/−1.5[d] (s, CF_3)	80.7 (q)[d] (J=36 Hz)
3g[a]	*Z*-Gly	H	$CH_2C_6H_5$	48–50	99	$C_{23}H_{24}F_3N_3O_7$ (511.46)	−2.4/−2.5[c] (s, CF_3)	81.2/81.4 (q)[c] (J=32 Hz)
7[a]	(see formula)			105	98	$C_{11}H_9F_2O_6$ (436.19)	−2.4/−3.7[c] (m, $3CF_3$)	81.2/81.3 (q)[c] (J=32 Hz)

[a]Mixture of two diastereoisomers.
[b]DMSO-d_6.
[c]Acetone-d_6.
[d]$CDCl_3$.

TABLE 2

Di- and tri-peptides synthesized containing 3,3,3-trifluoroalanine in the C-terminal position

No.	Yield (%)	M.p. (°C)	Formula (m.w.)	DE[g] (%)	^{19}F NMR δ (ppm) (CH–CF_3)	^{13}C NMR δ (ppm)	
						(C*H*–CF_3)	(*C*F_3)
5a	35	168 (dec.)	$C_{13}H_{13}F_3N_2O_4$ (318.26)	–	5.3 (d)[d] (J=7.3 Hz)	54.3 (q)[d] (J=32 Hz)	123.9 (q)[d] (J=282 Hz)
5b[b]	34	148	$C_{15}H_{17}F_3N_2O_5$ (362.31)	10	5.5 (d)[d] (J=8.5 Hz)	54.3 (q)[d] (J=32 Hz)	123.9 (q)[d] (J=282 Hz)
5c[b]	55	162	$C_{17}H_{21}F_3N_2O_5$ (390.36)	52	8.4 (d)[c] (J=7.3 Hz)	52.9 (q)[c] (J=31 Hz)	122.8 (q)[c] (J=282 Hz)
5d[b]	38	168	$C_{21}H_{21}F_3N_2O_5$ (438.41)	29	5.4[d] (J=8.5 Hz)	54.2 (q)[d] (J=32 Hz)	123.9 (q)[d] (J=282 Hz)
5e[a]	26	108–111	$C_{17}H_{19}F_3N_2O_5$ (388.35)	47	6.7 (br.)[f]	54.2 (q)[f] (J=32 Hz)	123.8 (q)[f] (J=281 Hz)
5f[b]	56	203–205	$C_{21}H_{28}F_3N_3O_6$ (475.47)	59	8.3 (d)[c] (J=8.5 Hz)	53.2 (q)[c] (J=32 Hz)	122.9 (q)[c] (J=282 Hz)
5g[b]	32	167	$C_{23}H_{24}F_3N_3O_6$ (495.47)	57	8.3 (d)[c] (J=8.5 Hz)	53.2 (q)[c] (J=32 Hz)	122.8 (q)[c] (J=282 Hz)
					(CH–CF_3) (C(CF_3)$_2$)		
9[a]	49	104	$C_{11}H_9F_9N_2O_5$ (420.19)	–	6.0/6.1 (d)[e] (J=8.0 Hz) −1.7 (m_c), −2.3 m_c)	54.3/54.4 (q)[e] (J=32 Hz)	123.9 (q)[e] (J=281 Hz)

[a]Mixture of two diastereoisomers.

[b]One diastereoisomer isolated (optical purity: >95%).

[c, d, e]See footnotes b, c and d in Table 1.

[f]CD_3CN.

[g]DE determined by ^{19}F NMR spectroscopy of the crude products.

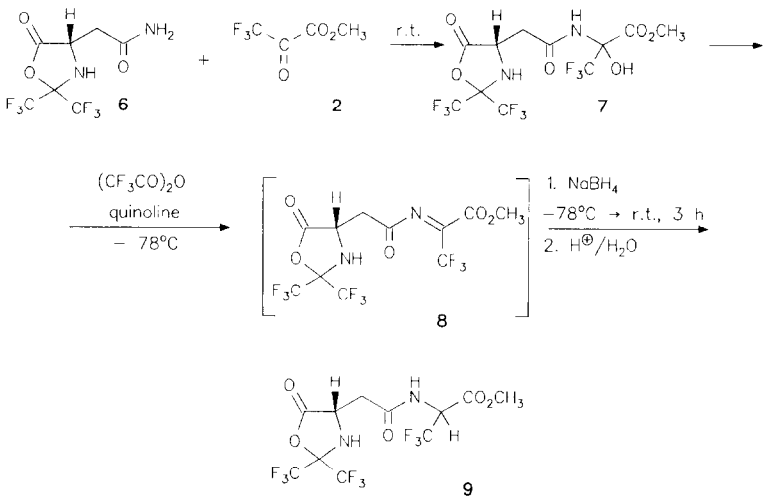
di- and tri–peptides **5**. The results are summarized in Table 2. In some cases (e.g. **5b**, **5f** and **5g**) the major diastereoisomer could be isolated by crystallization. In other instances (**5c**), the diastereoisomers were separated by HPLC (solvent: MeCN/H_2O 45:55; column: RPC 18; flow: 1 ml min.

By this route, the introduction of TFM amino acids at the ω-carboxy group of aspartic acid can be achieved starting from the hexafluoroacetone-protected asparagine derivative **6** (Scheme 2). Compound **9** represents the first isopeptide derivative of aspartic acid containing 3,3,3-trifluoroalanine (Table 2).

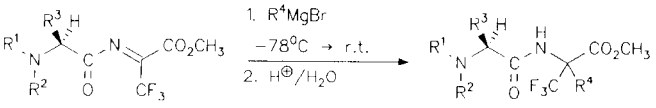
Of the few peptides containing 3,3,3-trifluoroalanine known to date, some exhibit interesting properties. They have been found to act as sweeteners [23] and as inhibitors of the murcine synthesis [24], and have been applied for the treatment of emphysemes [25].

The reaction of acylimines **4** with Grignard reagents (1 equiv.) at −78 °C in dry ether gave dipeptides **10** (Scheme 3, Table 3). The reactions studied so far showed little stereoselectivity, producing both diastereoisomers in approximately equal proportions.

The above strategy offers a preparatively simple synthetic route to small peptides containing TFM amino acids with variable side-chains in the C-



Scheme 2.



4, R^1 = Z, R^2 = H
R^3 = CH_3, $CH_2C_6H_5$

10a, R^4 = CH_3
10b, R^4 = $CH_2C_6H_5$

Scheme 3.

TABLE 3

Dipeptides synthesized containing TFM amino acids in the C-terminal position

No.	Yield (%)	M.p. (°C)	Formula (m.w.)	^{19}F NMR δ (ppm) (CR^4–CF_3)	^{13}C NMR δ (ppm)	
					(CR^4–CF_3)	(CF_3)
10a[a]	35	oil	$C_{16}H_{19}F_3N_2O_5$ (376.34)	2.6/2.8 (s)[c]	61.5 (q)[c] (J=29 Hz)	124.3 (q)[c] (J=285 Hz)
10b[a]	29	52	$C_{28}H_{27}F_3N_2O_5$ (528.53)	5.7/5.9 (s)[b]	67.0 (q)[b] (J=27 Hz)	125.1 (q)[b] (J=288 Hz)

[a]Mixture of two diastereoisomers.
[b, c]See footnotes c and d in Table 1.

terminal position, by addition of carbon as well as heteronucleophiles [26] to trifluoromethyl-substituted acylimines.

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