4-Ethoxy-1,1,1-trifluoro-3-buten-2-one as a New Protecting Reagent in Peptide Synthesis

M.G. Gorbunova, I.I. Gerus,* S.V. Galushko, V.P. Kukhar
Institute of Bioorganic and Oil Chemistry Ukrainian SSR, Academy of Sciences, Kharkoyskoe sh., 50, SU-252160 Kiev, USSR

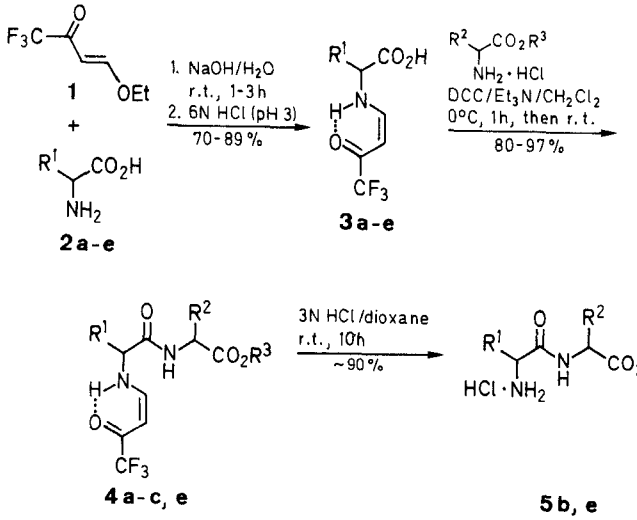
The 4,4,4-trifluoro-3-oxo-1-butenyl group is proposed as a suitable protecting group for the protection of the N-H terminal of amino acids in peptide synthesis. Amino acids react with 4-ethoxy-1,1,1-trifluoro-3-buten-2-one to give the *N*-protected amino acids, the protecting group can be removed by acidic hydrolysis. The formation of peptide bonds using on *N*-4,4,4-trifluoro-3-oxo-1-butenyl protected amino acids occurs without racemization.

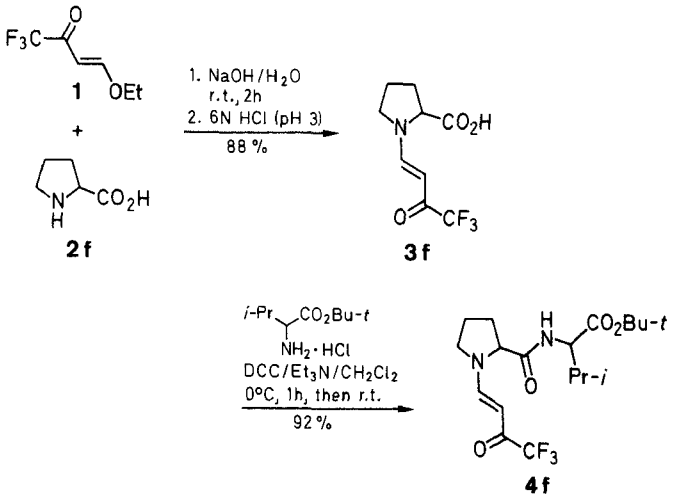
During the investigation of 4-alkoxy-1,1,1-trifluoro-3-buten-2-ones, in particular 4-ethoxy-1,1,1-trifluoro-3-buten-2-one (**1**), which readily available fluorine containing 1,3-dicarbonyl compound derivatives formed during the vinyl ether trifluoroacylation reaction,[1] we found that these ketones react with primary and secondary amines to give 4-amino-1,1,1-trifluoro-3-alken-2-ones in good yield.[2] We have now extended our investigations to the suitability of the 4,4,4-trifluoro-3-oxo-1-butenyl group as an *N*-terminal protecting group in peptide synthesis. Although the 3-oxoalkenyl and related groups have been used for *N*-terminal protection[3] in amino acids, they have significant disadvantages. For example, introduction of this protecting group is achieved by the reaction of amino acids with 1,3-dicarbonyl compounds with heating.[4] Fluorine-containing 1,3-dicarbonyl compounds have not previously been used for the *N*-protection of amino acids however we expect them to possess improved properties.

N-4,4,4-Trifluoro-3-oxo-1-butenyl protected amino acids **3a–e** are easily prepared by the reaction of **1** with an L-amino acid in aqueous sodium hydroxide (containing one equivalent of base). The reaction is complete after 0.5–3 h at room temperature. Compounds **3a–e** are isolated in high yield after acidification of the reaction mixture (Table).

The protected amino acids **3a–e** are colorless or slightly yellow crystalline compounds, which can be stored at 0 °C for a long time. Previously, we reported the reaction between **1** and primary amines to give products with in the *cis*-configuration, which are held by hydrogen bonding, while the reaction of **1** with secondary amines affords *trans*-β-amino α,β-unsaturated ketones.[2] The 4,4,4-trifluoro-3-oxo-1-butenyl protecting group in the compounds **3a–e** in possesses a *cis* configuration in non-polar solvents. This was confirmed by the ^{1}H-NMR spectra which showed a *cis* coupling constant (J = 7 Hz) for the olefin protons. The chemical shifts of the olefin protons of the protecting group are uninfluenced by the nature of the amino acid fragment R^1 and appear at δ = 5.4 (d, α-H) and at δ = 7.3 (dd, β-H).

The reaction of **1** with L-proline (**2f**) gave the protected amino acid **3f**, in high yield. In the ^{1}H-NMR spectrum of **3f** are two doublets for the olefin protons at – 78 °C δ = 5.3 (α-H) and at δ = 8.2 (β-H) with a coupling constant of J = 12.5 Hz, which is characteristic for *trans*-4-amino-1,1,1-trifluoro-3-buten-2-ones[2] (Table).





2–5	R^1	R^2	R^3
a	Me	H	Et
b	*i*-Pr	H	Et
c	$CH_2CH_2CO_2Bu$-*t*	*i*-Pr	*t*-Bu
d	$CH_2CH_2CO_2Me$	–	–
e	Bn	Bn	Me

The 4,4,4-trifluoro-3-oxo-butenyl group was easily removed from the amino acid derivatives **3a, b, f** by a dioxane/hydrochloric acid mixture (1 : 1) at room temperature. Starting concentration of the acid can be varied from 1 N to 6 N. Increasing the acid concentration (from 1 N to 6 N) shortens the reaction time from 12 to 4 hours.

The amino acid derivatives **3a–c, e, f** were used in peptide synthesis with various amino acid esters and 1,3-dicyclohexylcarbodiimide (DCC) as an activator according to the known method.[5] The corresponding *N*-4,4,4-trifluro-3-oxo-1-butenyl derivatives of the dipeptides,

Table. *N*-4,4,4-Trifluoro-3-oxo-1-butenyl Derivatives of Amino Acids **3a–f** and Dipeptides **4a–c, e, f**

Product	Yield (%)	$[\alpha]_{578}^{20}$ ($c = 1$, MeOH)	mp (°C)	Molecular Formula[a]	IR (KBr) ν (cm^{-1})	^{1}H-NMR ($CDCl_3$/TMS) δ, J (Hz)
3a	88	−17.6	102	$C_7H_8F_3NO_3$ (211.1)	1740, 1652, 1555	1.62 (d, 3H, J = 7.5), 4.18 (dq, 1H, $J \cong$ 7.5), 5.5 (d, 1H, J = 7.2), 7.15 (dd, 1H, J = 7.2, 13.5), 9.45 (br s, 1H), 10.4 (br s, 1H)
3b	89	−87.7	89	$C_9H_{12}F_3NO_3$ (239.2)	1738, 1657, 1558	–
3c	75	−89.9	114	$C_{13}H_{18}F_3NO_5$ (325.3)	1752, 1740, 1653, 1556	–
3d	70	−105.0	101	$C_{10}H_{12}F_3NO_5$ (283.2)	1755, 1745, 1655, 1555	2.15 (m, 2H), 2.4 (m, 2H), 3.6 (s, 3H), 4.25 (t, 1H, J = 7), 5.4 (d, 1H, J = 7.5), 7.3 (dd, 1H, J = 7.5, 13), 8.9 (br s, 1H), 10.4 (br s, 1H)
3e	72	−249.2	152	$C_{13}H_{12}F_3NO_3$ (287.2)	1752, 1653, 1564	3.0 (dd, 1H, J = 15.5, 9.3), 3.35 (dd, 1H, J = 15.5, 4.5), 4.12 ("td", 1H, J = 9.3, 4.5), 5.21 (d, 1H, J = 7.5), 6.56 (dd, 1H, J = 7.5, 13.1), 7.0–7.4 (m, 5H), 10.3 (br s, 1H)
3f[b]	88	−215.5	103	$C_9H_{10}F_3NO_3$ (237.2)	1770, 1650, 1560	2.05 (m, 2H), 2.3 (m, 2H), 3.3–3.5 (m, 2H), 4.4 (t, 1H, J = 7), 5.34 (d, 1H, J = 12.5), 8.15 (d, 1H, J = 12.5), 10.4 (br s, 1H)
4a	89	+0.8	124	$C_{11}H_{15}F_3N_2O_4$ (296.2)	1766, 1700, 1665, 1595, 1568	1.2 (t, 3H, J = 7), 1.5 (d, 3H, J = 7.8), 4.0 (m, 2H), 4.2 (q, 2H, J = 7), 4.3 (m, 1H), 5.4 (d, 1H, J = 7), 6.3 (br s, 1H), 7.1 (dd, 1H, J = 13, 7), 10.1 (br s, 1H)
4b	80	−44.5	120	$C_{13}H_{19}F_3N_2O_4$ (324.3)	1750, 1685, 1657, 1580, 1562	–
4c	94	−22.6	152	$C_{22}H_{35}F_3N_2O_6$ (480.5)	1730, 1715, 1673, 1652, 1580, 1562	–
4e	82	−116.5	123	$C_{23}H_{23}F_3N_2O_4$ (448.4)	1730, 1690, 1655, 1570, 1553	–
4f	91	+15.7	162	$C_{18}H_{27}F_3N_2O_4$ (392.4)	1760, 1685, 1650, 1580, 1560	–

[a] Satisfactory microanalyses obtained: C ±0.30, H ±0.17, F ±0.32.

[b] ^{13}C-NMR (50.327 MHz, $CDCl_3$/TMS): δ = 23.3 (β-CH_2), 29.4 (γ-CH_2), 48.5 (δ-CH_2), 64.8 (α-CH), 90.0 (α-CH=), 117.7 (q, J = 289 Hz, CF_3), 155.3 (β-CH=), 172.9 (CO_2H), 177.5 (q, J = 33 Hz, $COCF_3$).

4a–c, e, f, were isolated in high yield. They are stable, colorless crystalline compounds. There are various attributes that it is necessary for a protecting group in peptide synthesis to posess; in particular, easy and selectivity of the deprotection step is of great importance. The removal of the *N*-4,4,4-trifluoro-3-oxo-butenyl protecting group from dipeptides occurs under the same conditions as that of the deprotection of protected amino acids **3a–f**. Yields of the dipeptides ester hydrochlorides exceed 90%. The 4,4,4-trifluoro-3-oxo-1-butenyl group is the vinylog of the known trifluoroacetyl protecting group. *N*-Trifluoroacetyl amino acid derivatives have been shown to have a tendency towards racemization. A variation on the Weygand's test for determination of the extent of racemization using gas chromatography, consists in recognition of D-phenylalanine containing peptide formation in accordance with the reaction:

Z-Leu-Phe + Val-OBu-*t* → Z-Leu-Phe-Val-OBu-*t*,

various activators can be used. To clarify whether racemization of *N*-4,4,4-trifluoro-3-oxo-1-butenyl derivatives has occurred during peptide synthesis, we have synthesized dipeptide *rac*-**4e** starting from racemic *N*-4,4,4-trifluoro-3-oxo-1-butenylphenylalanine **(3e)** and peptide L,L-**4e** starting from L-**2e**. The ^{19}F-NMR spectrum of **4e** has a singlet at δ = 77.5 from by the trifluoromethyl group. In the spectrum of the *rac*-**4e** there are two singlets with close chemical shift values ($\Delta\delta$ = 0.01), belonging to the trifluoromethyl groups of the diastereoisomeric dipeptides. After removal of the 4,4,4-trifluoro-3-oxo-1-butenyl group dipeptides L,L-**5e** and *rac*-**5e** were examined by HPLC. It was shown that the dipeptide L,L-**5e** (L-Phe-L-Phe-OMe) contains not more than 0.5% of the D-isomer (D-Phe-L-Phe-OMe) as admixture. Therefore, we can claim that 4,4,4-trifluoro-3-oxo-1-butenyl) group does not promote racemization during peptide bond formation process in the presence of DCC even if no nucleophilic additions are used.[6]

In conclusion we consider 4-ethoxy-1,1,1-trifluoro-3-buten-2-one **(1)** to be a suitable reagent for the *N*-terminal protection of amino acids in peptide synthesis. The advantages of **1** are that it is readily available, the protection and deprotection steps are simple, and free from racemization, stability of the crystalline *N*-4,4,4-trifluoro-3-oxo-1-butenyl derivatives.

All the amino acids and hydrochlorides of amino acid esters used in the synthesis are from "Reanal", DCC is from "Fluka". 4-Ethoxy-1,1,1-trifluoro-ethoxyvinyl-3-buten-2-one **(1)** was prepared accord-

ing to literature procedure.[1] The hydrochlorides of the ester dipeptides **5b, e** and of the amino acids **2a, b, f** after removal of the 4,4,4-trifluoro-3-oxo-1-butenyl group are hydroscopic solids homogeneous under HPLC conditions. The isomeric composition of dipeptide **5e** was determined by reversed phase HPLC (LKB, column 150 × 3.3 mm Separon SGX.C_{18}, 5 mm, mobil phase MeOH/0.1% v/v TFA (7:3) flow 0.3 mL/min, k = 1.3 for L,L **5e** and k = 2.2 for D,L **5e**, detection at 220 nm). Observed rotations at 578 nm were obtained at 20 °C using a Polamat-A polarimeter and IR spectra were obtained using a Specord M-80 spectrophotometer. NMR spectra were obtained using a Bruker WP 200 MHz spectrometer.

***N*-4,4,4-Trifluoro-3-oxo-1-butenyl Amino Acids 3a–f; General Procedure:**

To a solution of **2** (14 mmol) in 1 N NaOH (14 mL) is added **1** (2.35 g, 14 mmol) and the mixture is stirred 1–3 h at r.t. (22 °C) until the solution is homogeneous. The solution is acidified using 6N aq HCl to pH 3.0, is extracted with Et_2O (3 × 15 mL). The etheral layer is dried ($MgSO_4$), hexane (5 mL) is added and the solvent is removed by evaporation. The solid is crystallized from hexane/Et_2O (5:1) (Table).

Dipeptides 4a–c, e, f; General Procedure:

Hydrochloride of the amino acid ester (5.2 mmol) and Et_3N (0.72 mL, 5.1 mmol) are added with stirring to the solution of Tfav amino acid (**3a–c, e, f**; 5 mmol) in CH_2Cl_2 (17 mL). To the cooled (−10 °C) mixture DCC (1.35 g, 6.5 mmol) is added. After 1 h at 0 °C, the mixture is allowed to warm to r.t., filtered and the solvent is evaporated. The residue is dissolved in Et_2O/EtOAc (1:1) (30 mL). The solution is washed with 1 N HCl (2 × 20 mL), H_2O (2 × 40 mL), 1 N $NaHCO_3$ (2 × 20 mL), 25% NaCl (2 × 30 mL) and dried ($MgSO_4$). The solvent is evaporated and crude product is crystallized from Et_2O/hexane (1:1) (Table).

Removal of the 4,4,4-Trifluoro-3-oxo-1-butenyl Protecting Group; General Procedure:

The Tfav amino acids **3a, b, f** and dipeptides **4b, e** (0.5 mmol) are dissolved in dioxane/3 N HCl (2:5, 10 mL). The mixture is allowed to stand for 10 h at r.t., then extracted with Et_2O (2 × 50 mL), the H_2O layer evaporated. The hygroscopic, solid hydrochlorides amino acids **2a, b, f** and esters of dipeptides **5b, e** are obtained.

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(1) Hojo, M.; Masuda, R.; Kokuryo, Y.; Shioda, H.; Matsuo, S. *Chem. Lett.* **1976**, 499.
(2) Gerus, I.I.; Gorbunova, M.G.; Vdovenko, S.I.; Yagupol'skii, Yu.L.; Kukhar, V.P. *Zh. Org. Khim.* **1990**, *26*, 1877.
(3) Greene, T.W. *Protective Groups in Organic Chemistry*, John Wiley & Sons, New York, 1981, p. 278.
(4) Hiskey, R.G.; Southard, G.L. *J. Org. Chem.* **1966**, *31*, 3582.
(5) Boisonnas, R.A.; Guttmann, St.; Jaquenoud, P.-A. *Helv. Chim. Acta* **1960**, *43*, 1349.
(6) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.