Dialkyl (1-ethoxycarbonyl-2,2,2-trifluoro-1-phenylsulfonylaminoethyl)-phosphonates

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A number of fluorine-containing α-amino phosphonates of the general formula $(RO)_2P(O)C(CF_3)(COOC_2H_5)NHSO_2Ph$ were obtained and found to irreversibly inhibit four serine hydrolases: acetyl- and butyrylcholinesterases, neuropathy target esterase, and carboxylesterase.

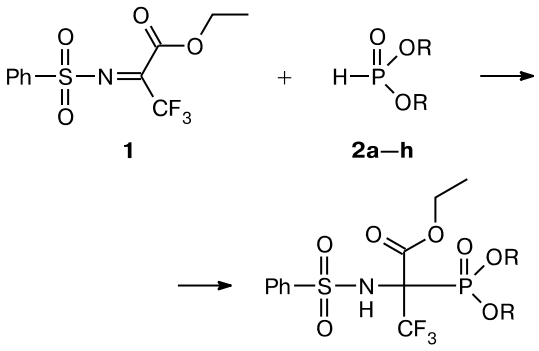
Key words: organofluorine compounds, α-amino phosphonates, serine hydrolase inhibitors.

α-Amino phosphonic acids and their esters, which are structural analogs of α-amino carboxylic acids, have been thoroughly studied as biologically active compounds.[1,2] Reviews dealing with the synthesis and biological activity of fluorinated phosphonates contain almost exhaustive data for 2007,[3,4] including fluorinated derivatives of α-amino phosphonic acids. The latter can inhibit various enzymes.[3] For instance, it has been shown that some fluorinated esters of α-amino fluorophosphonic acids and phosphine oxides act as inhibitors of cholinesterases[5,6] and thrombin,[7] in contrast to their nonfluorinated analogs.

Earlier, we have obtained a number of fluorinated α-amino phosphonates of the general formula $(RO)_2P(O)C(CF_3)_2NHSO_2Ph$ and demonstrated[8,9] that they irreversibly inhibit serine hydrolases *via* phosphorylation of serine at the active center of the enzymes with cleavage of the P—C bond. Here we describe the synthesis of new fluorinated representatives of α-amino phosphonates (Scheme 1) and present preliminary data on their reactions with four serine hydrolases that are essential for the physiological effects of organophosphorus compounds (OPC): acetylcholinesterase (EC 3.1.1.7, AChE, target for the acute toxicity of OPC), neuropathy target esterase (EC 3.1.1.5, NTE, target for the OPC induced delayed neuropathy, OPIDN), butyrylcholinesterase (EC 3.1.1.8, BChE), and carboxylesterase (EC 3.1.1.1, CE), which are secondary scavenger targets. As potential serine hydrolase inhibitors, the phosphonates studied have a distinctive feature: their molecules contain no typical leaving group (F, SAlk, OAr, *etc.*) characteristic of anticholinesterase compounds.

Reactions between equimolar amounts of sulfonylimine **1** (derived from ethyl trifluoropyruvate) and dialkyl phosphites **2a—h** gave the corresponding fluorinated α-amino phosphonates **3a—h** in 60—90% yields.

Scheme 1



R = Me (**a**), Et (**b**), Pr (**c**), Pr^i (**d**), Bu (**e**), Bu^i (**f**), n-C_5H_{11} (**g**), i-C_5H_{11} (**h**)

Phosphonates **3a—h** are crystalline solids. Their compositions and structures were proved by elemental analysis and ^1H, ^{19}F, and ^{31}P NMR spectroscopy. The ^1H NMR spectra of phosphonates **3a—h** show a characteristic doublet at δ 6.9—9.2 (NH, $J_{H,P}$ = 8.0 Hz). The ^{19}F NMR spectra contain a doublet at δ 9—12 (CF_3, $J_{F,P} \approx 5$ Hz). Accordingly, the ^{31}P NMR spectra show quartets at δ 10—12 ($J_{P,F} \approx 5$ Hz).

Kinetic studies of human erythrocyte acetylcholinesterase, hen brain neuropathy target esterase, horse serum butyrylcholinesterase, and pig liver carboxylesterase, the details and analysis of which would be published elsewhere, showed that phosphonates **3a—h**, like previously examined compounds containing two trifluoromethyl groups at the α-C atom,[8,9] act as irreversible inhibitors of all the above esterases with the inhibition kinetics typical of organophosphorus compounds. The bimolecular inhibi-

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tion constants k_i (L mol^{-1} min^{-1}) range from $6.8 \cdot 10^2$ (**3d**) to $6.4 \cdot 10^5$ (**3e,f**) for AChE, from $8.9 \cdot 10$ (**3d**) to $3.9 \cdot 10^5$ (**3g**) for NTE, from $8.3 \cdot 10^2$ (**3a**) to $6.8 \cdot 10^5$ (**3e**) for BChE, and from $2.7 \cdot 10^3$ (**3d**) to $6.8 \cdot 10^5$ (**3e**) for CE. Apparently, the presence of two strong electron-withdrawing substituents (CF_3 and COOEt) at the α-C atom makes the P—C bond more labile as demonstrated earlier for α-amino phosphonates containing two CF_3 groups[8,9] and the irreversible inhibition of serine hydrolases, which is evident from the kinetic data, is due to the phosphorylation of serine by compounds **3a—h** because of the cleavage of the P—C bond. An acute toxicity assay revealed that the α-amino phosphonates in question are not very toxic for the warm-blooded: LD_{50} for **3b** is 200 mg kg^{-1} (mice, intraperitoneal injection).

To sum up, we obtained novel fluorinated α-amino phosphonates, which can originate a new type of irreversible serine hydrolase inhibitors containing no typical leaving group, as distinct from known anticholinesterase agents.

Experimental

^{1}H, ^{19}F, and ^{31}P NMR spectra were recorded on a Bruker DPX 200 instrument (200.13, 188.29, and 81 MHz, respectively) with $SiMe_4$ as the internal standard (^{1}H) and with CF_3COOH (^{19}F) and H_3PO_4 (^{31}P) as the external standards. Melting points were determined in glass capillaries.

Ethyl 3,3,3-trifluoro-2-phenylsulfonyliminopropionate (**1**) was prepared according to a known procedure.[10] Dialkyl phosphites (Aldrich) were used as purchased.

Synthesis of phosphonates 3a—h (general procedure). An appropriate dialkyl phosphite **2a—h** (5 mmol) in anhydrous benzene (20 mL) was added to a solution of sulfonylimine **1** (5 mmol) in anhydrous benzene (20 mL). The reaction mixture was refluxed for 2 h, the solvent was removed, and the residue was recrystallized from hexane—benzene (10 : 1). The ^{1}H, ^{19}F, and ^{31}P NMR spectra of the compounds obtained are given in Table 1.

Ethyl 2-dimethoxyphosphoryl-3,3,3-trifluoro-2-(phenylsulfonylamino)propionate (3a). Yield 71%, m.p. 82—84 °C. Found (%): C, 37.46; H, 4.21; N, 3.06. $C_{13}H_{17}F_3NO_7PS$. Calculated (%): C, 37.24; H, 4.09; N, 3.34.

Ethyl 2-diethoxyphosphoryl-3,3,3-trifluoro-2-(phenylsulfonylamino)propionate (3b). Yield 60%, m.p. 77—78 °C. Found (%): C, 43.98; H, 4.82; N, 3.24. $C_{15}H_{21}F_3NO_7PS$. Calculated (%): C, 40.27; H, 4.73; N, 3.13.

Ethyl 2-di-*n*-propoxyphosphoryl-3,3,3-trifluoro-2-(phenylsulfonylamino)propionate (3c). Yield 72%, m.p. 80—82 °C. Found (%): C, 42.78; H, 5.18; N, 3.06. $C_{17}H_{25}F_3NO_7PS$. Calculated (%): C, 42.95; H, 5.30; N, 2.95.

Ethyl 2-diisopropoxyphosphoryl-3,3,3-trifluoro-2-(phenylsulfonylamino)propionate (3d). Yield 91%, m.p. 152—153 °C.

Table 1. ^{1}H, ^{19}F, and ^{31}P NMR spectra of fluorinated α-amino phosphonates **3a—h**

Compound	Chemical shifts δ and coupling constants J (Hz)		
	^{1}H	^{9}F (d, $J_{F,P}$)	^{31}P{H} (q, $J_{P,F}$)
3a[a]	1.19 (t, 3 H, $\underline{CH_3}CH_2$, $J_{H,H}$ = 7.0); 3.79 (d, 6 H, CH_3O, $J_{H,P}$ = 11.4); 4.13 (m, 1 H, CH_2O); 4.29 (m, 1 H, CH_2O); 7.55 (m, 3 H, H_{Ar}); 7.92 (m, 2 H, H_{Ar}); 9.18 (d, 1 H, NH, $J_{H,P}$ = 7.7)	10.36 (4.7)	12.37 (4.7)
3b[b]	1.33 (t, 3 H, $\underline{CH_3}CH_2OC(O)$, $J_{H,H}$ = 7.0); 1.37 (t, 6 H, $\underline{CH_3}CH_2OP(O)$, $J_{H,H}$ = 7.0); 4.14—4.46 (m, 6 H, CH_2O); 6.28 (d, 1 H, NH, $J_{H,P}$ = 11.9); 7.47—7.65 (m, 3 H, H_{Ar}); 7.92—8.02 (m, 2 H, H_{Ar})	12.29 (5.2)	10.28 (5.0)
3c[a]	0.92 (t, 6 H, $\underline{CH_3}CH_2CH_2$, $J_{H,H}$ = 7.0); 1.19 (t, 3 H, $\underline{CH_3}CH_2O$, $J_{H,H}$ = 7.0); 1.66 (m, 4 H, $CH_3\underline{CH_2}CH_2$); 3.90—4.14 (m, 5 H, CH_2O); 4.29 (m, 1 H, CH_2O); 7.54 (m, 3 H, H_{Ar}); 7.94 (m, 2 H, H_{Ar}); 9.18 (d, 1 H, NH, $J_{H,P}$ = 8.0)	10.48 (4.9)	9.88 (5.0)
3d[b]	1.12 (m, 12 H, $(\underline{CH_3})_2CH$); 1.22 (t, 3 H, $\underline{CH_3}CH_2O$, $J_{H,H}$ = 7.0); 4.00—4.30 (m, 2 H, CH_2O); 4.75 (m, 1 H, CHO); 6.68 (d, 1 H, NH, $J_{H,P}$ = 8.0); 7.58 (m, 3 H, H_{Ar}); 7.92 (m, 2 H, H_{Ar})	11.76 (4.9)	7.62 (4.8)
3e[a]	0.92 (t, 6 H, $\underline{CH_3}CH_2CH_2$, $J_{H,H}$ = 7.0); 1.10 (t, 3 H, $\underline{CH_3}CH_2O$, $J_{H,H}$ = 7.0); 1.26 (m, 4 H, $CH_3\underline{CH_2}CH_2$); 1.56 (m, 4 H, $CH_3CH_2\underline{CH_2}$); 3.90—4.18 (m, 5 H, CH_2O); 4.29 (m, 1 H, CH_2O); 7.56 (m, 3 H, H_{Ar}); 7.90 (m, 2 H, H_{Ar}); 9.24 (d, 1 H, NH, $J_{H,P}$ = 8.0)	9.31 (5.1)	8.64 (5.0)
3f[b]	0.96 (m, 12 H, $(\underline{CH_3})_2CH_2CH$); 1.24 (t, 3 H, $\underline{CH_3}CH_2O$, $J_{H,H}$ = 7.0); 1.86 (m, 2 H, $\underline{CH}CH_2$); 3.86—4.05 (m, 4 H, CH_2OP); 4.10 (m, 1 H, CH_2OC); 4.30 (m, 1 H, CH_2OC); 6.98 (d, 1 H, NH, $J_{H,P}$ = 8.0); 7.56 (m, 3 H, H_{Ar}); 7.94 (m, 2 H, H_{Ar})	9.02 (5.2)	9.92 (5.2)
3g[a]	0.88 (t 6 H, $\underline{CH_3}CH_2CH_2$, $J_{H,H}$ = 7.0); 1.12 (t, 3 H, $\underline{CH_3}CH_2O$, $J_{H,H}$ = 7.0); 1.26 (m, 8 H, $CH_3\underline{CH_2}\underline{CH_2}$); 1.55 (m, 4 H, $\underline{CH_2}CH_2O$); 3.83—4.16 (m, 5 H, CH_2O); 4.24 (m, 1 H, CH_2O); 7.49 (m, 3 H, H_{Ar}); 7.88 (m, 2 H, H_{Ar}); 9.04 (d, 1 H, NH, $J_{H,P}$ = 8.0)	10.52 (4.9)	10.03 (5.1)
3h[a]	0.89 (m, 12 H, $(\underline{CH_3})_2CH$); 1.14 (t, 3 H, $\underline{CH_3}CH_2O$, $J_{H,H}$ = 7.0); 1.45 (m, 4 H, $\underline{CHCH_2}$); 1.64 (m, 2 H, $CH\underline{CH_2}$); 3.86—4.37 (m, 6 H, $CH_2OP + CH_2OC$); 7.52 (m, 3 H, H_{Ar}); 7.93 (m, 2 H, H_{Ar}); 9.00 (d, 1 H, NH, $J_{H,P}$ = 7.7)	10.43 (5.0)	9.86 (5.0)

[a] DMSO-d_6. [b] $CDCl_3$.

Found (%): C, 42.69; H, 5.11; N, 3.18. $C_{17}H_{25}F_3NO_7PS$. Calculated (%): C, 42.95; H, 5.30; N, 2.95.

Ethyl 2-di-*n*-butoxyphosphoryl-3,3,3-trifluoro-2-(phenylsulfonylamino)propionate (3e). Yield 70%, m.p. 64—66 °C. Found (%): C, 45.56; H, 6.03; N, 3.04. $C_{19}H_{29}F_3NO_7PS$. Calculated (%): C, 45.33; H, 5.81; N, 2.78.

Ethyl 2-diisobutoxyphosphoryl-3,3,3-trifluoro-2-(phenylsulfonylamino)propionate (3f). Yield 90%, m.p. 95—99 °C. Found (%): C, 45.15; H, 5.66; N, 2.57. $C_{19}H_{29}F_3NO_7PS$. Calculated (%): C, 45.33; H, 5.81; N, 2.78.

Ethyl 2-di-*n*-pentyloxyphosphoryl-3,3,3-trifluoro-2-(phenylsulfonylamino)propionate (3g). Yield 68%, m.p. 85—87 °C. Found (%): C, 47.37; H, 6.33; N, 2.82. $C_{21}H_{33}F_3NO_7PS$. Calculated (%): C, 47.45; H, 6.26; N, 2.64.

Ethyl 2-diisopentyloxyphosphoryl-3,3,3-trifluoro-2-(phenylsulfonylamino)propionate (3h). Yield 84%, m.p. 98—100 °C. Found (%): C, 47.69; H, 6.03; N, 2.42. $C_{21}H_{33}F_3NO_7PS$. Calculated (%): C, 47.45; H, 6.26; N, 2.64.

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