Brief Communications

Anticholine esterase activity of phosphonium bis-zwitterions based on 2-cyanoacrylates

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Phosphonium bis-zwitterions based on bis-2-cyanoacrylates are weak reversible inhibitors of choline esterases with a diverse anticholine esterase effect. This can find use in additional classification of choline esterases.

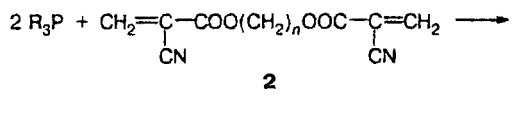
Key words: bis-2-cyanoacrylates, phosphorylation, phosphonium zwitterions, anticholine esterase activity, enzymes, inhibition.

It has previously been found[1] that phosphonium betaines with the formula $(R^1)_3P^+CH_2C^-(CN)COOR^2$ (**1**) (R^1 = Pr, Et_2N; R^2 = Me, Et; see Ref. 2), synthesized from 2-cyanoacrylates, are relatively weak reversible inhibitors of choline esterases.

It is known[3] that bisammonium and bisphosphonium compounds exhibit a higher reversible-type inhibition effect on choline esterase-catalyzed hydrolysis of acetylcholine than the corresponding ammonium and phosphonium compounds. In this connection, it was of interest to prepare phosphonium bis-zwitterions based on 2-cyanoacrylates and study them as inhibitors of choline esterases.

Target compounds **3** were prepared by the reactions of tripropyl- and hexaethyltriamidophosphines with bis-2-cyanoacrylates (**2**), derivatives of 1,8- and 1,10-diols.[4] The reactions were carried out similarly to the preparation of phosphonium monobetaines (**1**)[2] (Scheme 1).

Scheme 1



	a	**b**	**c**
R	Et_2N	Pr	Pr
n	10	8	10

All compounds synthesized are viscous oils. During these reactions, in the ^{31}P NMR spectra, a signal characteristic of bis-zwitterions of **3** appears and increases to a finite value in parallel with decrease in the signal of R_3P down to zero. No other signals were observed in

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the ^{31}P NMR spectrum. This suggested that the yields of compound **3** are quantitative according to the ^{31}P NMR data. The ^{1}H NMR spectra of compounds **3a—c** and mono-zwitterions **1** contain a characteristic doublet of protons of the methylene group in the region of 3.3—3.4 ppm with the constant J_{H-P} = 7 Hz. The IR spectra of compound **3** contain two intense absorption bands at 1610 and 2150 cm^{-1} characteristic of pentad anions in mono-zwitterions and salts of cyanoacetic ester.

Taking into account that the anticholine esterase activity of bisammonium compounds with the common formula $H_3N^+(CH_2)_nN^+H_3$ increases as n increases, and the highest effect is achieved at n = 10 (see Ref. 5), we chose bis-zwitterions **3a,c** for studying anticholine esterase activity, which was compared with that for mono-zwitterions **1a—d** (R^1 = Et_2N (**a**, **b**), Pr (**c**, **d**); R^2 = Me (**a**, **c**), Et (**b**, **d**)). It should not be expected that compounds **3** possess the highest anticholine esterase activity. We have no grounds yet to assume that the same regularities as for bisammonium salts are valid for bis-P-zwitterions **3**, because compounds **3** contain in the chain cationic centers and additional anionic charges as well.

Similarly to mono-zwitterions, bis-zwitterions are reversible inhibitors of choline esterases from various biological sources. For example, when the incubation time of an enzyme with an inhibitor increases from 0 to 10 min, the activity of the enzyme remains almost unchanged, which indicates the absence of irreversible inhibition.

The I_{50} values that characterize the ability of compounds **3a,c** to inhibit choline esterase hydrolysis of acetylcholine are presented in Table 1. For comparison, I_{50} determined during inhibition of choline esterases by known mono-zwitterions **1a—d** [1] are also presented in Table 1.

It follows from the data in Table 1 that compounds **3a,c** possess a weak inhibition effect on choline esterases from various sources. These compounds manifest a stronger inhibition effect than compound **1** (I_{50} for compound **3** are lower than those for compound **1**). It is of interest that the choline esterases studied substantially differ in sensitivity from each of the tested compounds in the series of **3**. This is also inherent in each of the compounds in the series of **1**, but the character of these differences is not the same. For example, compounds **3a,c** and **1a,b** more strongly inhibit butyrylcholine esterases than acetylcholine esterases, whereas compounds **1c,d**, by contrast, more strongly inhibit acetylcholine esterases. Enzymes of CEH and CENS exhibit the lowest sensitivity toward all compounds under study.

Table 1. Inhibition effect of mono- and bis-zwitterions based on bis-2-cyanoacrylates on different choline esterases

Enzyme*	$I_{50} \cdot 10^4$/mol L^{-1}					
	1a	**1b**	**1c**	**1d**	**3a**	**3c**
ACEM	21.0	11.0	9.7	4.2	2.5	2.2
ACEB	21.0	12.0	9.9	4.8	2.4	2.2
BuCEH	3.6	2.5	12.0	6.2	1.5	1.0
BuCEF	9.2	6.0	16.0	12.0	1.3	1.3
BuCEP	10.0	6.6	15.0	12.0	1.6	1.4
CEH	22.0	15.0	29.0	28.0	10.0	8.0
CENS	21.0	12.0	25.0	23.0	6.0	5.0

* See Experimental.

It is noteworthy that the nature of substituent R in compounds **3a,c** has almost no effect on their anticholine esterase properties. This distinguishes compounds **3** from compounds **1**, for which the anticholine esterase effect noticeably depends on their structure.

Thus, the results of measuring the inhibition effect of compounds of series **3a,c** and **1a,b** argue additionally in favor of the differences in the structure of the active centers of both acetylcholine esterases and butyrylcholine esterases and indicate the specific features of the structure of the active centers of choline esterases from the blood serum of hen and the cerebral tissue of squid, which is manifested as their especially low sensitivity toward the inhibition effect of cation-containing compounds.

Experimental

Bis-2-cyanoacrylates synthesized by the known procedure[4] were used. ^{1}H and ^{31}P NMR spectra were obtained on a Bruker WP 200 SY instrument (^{1}H 200.13, ^{31}P 81.01 MHz) and presented relative to $SiMe_4$ and 85% H_3PO_4 (external standards), respectively.

IR spectra were recorded on a Zeiss UR-20 instrument in Nujol. All reactions were carried out in an atmosphere of dry argon.

Synthesis of bis-zwitterions 3. Bis-2-cyanoacrylate **1** (0.01 mol) in anhydrous benzene (5 mL) was slowly added dropwise with stirring to a solution of an organophosphorus nucleophile (0.02 mol) in anhydrous benzene (10 mL). After 2—3 h, the solvent was evaporated by 2/3, *n*-pentane was added to the residue, the mixture was stirred, and the upper layer was decanted. The procedure was multiply repeated. The oils obtained were dried *in vacuo*. When stored over P_2O_5, they are stable for several months. According to the ^{31}P NMR data, the yields are quantitative.

1,10-Bis[2-hexaethyltriamidophosphonio-1-(cyano)ethanidocarbonyloxy]decane (3a). Viscous orange oil. Found (%): C, 61.19; H, 9.95; P, 7.05. $C_{42}H_{84}N_8O_4P_2$. Calculated (%): C, 60.99; H, 10.24; P, 7.49. IR, ν/cm^{-1}: 1610 (C=O), 2150 (CN). ^{31}P NMR (C_6H_6), δ: 58.01. ^{1}H NMR (C_6D_6), δ: 0.97 (t, 18 H, CH_3CH_2N); 1.40 (m, 8 H, $(CH_2)_4CH_2O$); 2.98 (m, 12 H, CH_2N); 3.55 (d, 2 H, CH_2P, J_{H-P} = 7.2 Hz); 4.61 (m, 2 H, CH_2O).

1,8-Bis[2-tri-*n*-propylphosphonio-1-(cyano)ethanidocarbonyloxy]octane (3b). IR, ν/cm^{-1}: 1610 (C=O), 2152 (CN). ^{31}P NMR (C_6H_6), δ: 31.9. ^{1}H NMR (C_6D_6), δ: 0.95 (t, 9 H, $CH_3(CH_2)_2P$, J = 7.0 Hz); 1.23 (m, 6 H, CH_2CH_2P); 1.45 (m, 6 H, $(CH_2)_3CH_2O$); 1.64 (m, 6 H, CH_2CH_2P); 3.24 (d, 2 H, CH_2P, J_{H-P} = 6.8 Hz); 4.10 (m, 2 H, CH_2O).

1,10-Bis[2-tri-*n*-propylphosphonio-1-(cyano)ethanidocarbonyloxy]decane (3c). Colorless oil. Found (%): C, 65.93;

H, 10.12; P, 10.05. $C_{36}H_{66}N_2O_4P_2$. Calculated (%): C, 66.23; H, 10.19; P, 9.49. IR, ν/cm^{-1}: 1612 (C=O), 2149 (CN). ^{31}P NMR (C_6D_6), δ: 32.0. ^1H NMR (C_6D_6), δ: 0.98 (t, 9 H, $CH_3(CH_2)_2P$, J = 7.0 Hz); 1.19 (m, 6 H, CH_2CH_2P); 1.52 (m, 8 H, $(CH_2)_4CH_2O$); 1.70 (m, 6 H, CH_2CH_2P); 3.12 (d, 2 H, CH_2P, J_{H-P} = 6.9 Hz); 4.01 (m, 2 H, CH_2O).

In the study of the anticholine esterase effect of compound **3**, choline esterases from different biological sources were used as enzymes[1]: acetylcholine esterase (KF 3.1.1.7) from erythrocytes of blood of man (ACEM) and bull (ACEB); butyrylcholine esterases (KF 3.1.1.8) from the blood serum of horse (BuCEH), fish (BuCEF), and pigeon (BuCEP); choline esterase (KF 3.1.1.8) from the blood serum of hen (CEH) and the cerebral tissue of New Zealand squid (CENS).

Catalytic activity of enzymes in the absence and presence of an inhibitor was determined by the photometric Ellman method[6] at 25 °C and pH 7.5 using acetylcholine (2 mmol L^{-1}) as a substrate. Efficiency of the inhibition effect of a compound was estimated from its concentration (I_{50}) that results in a 50% decrease in the hydrolysis rate of the substrate. Conditions of determination of the catalytic activity and I_{50} were similar to those in Ref. 1.

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Synthesis of 5-fluoro-2,4,6-tris(perfluoroalkyl)pyrimidines

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5-Fluoro-2,4,6-tris(perfluoroalkyl)pyrimidines were synthesized by reactions of polyfluorinated iminoenamines with perfluorocarboxylic acid anhydrides and perfluorobutanoyl chloride.

Key words: polyfluorinated iminoenamines; condensation with perfluorocarboxylic acid anhydrides and perfluorobutanoyl chloride; perfluoropyrimidines.

Pyrimidines constitute an abundant class of natural bases and are widespread among drugs possessing a pronounced antiviral,[1] gastric antisecretory,[2] diuretic,[3] antimalarial,[4] and anti-HIV-1-activity.[5,6] It is also known that the presence of fluorine atoms in a molecule increases the physiological activity of compounds as compared with their nonfluorinated analogs.[7] Therefore, interest in the synthesis of new fluorine-containing pyrimidines, which is the subject of this work, seems to be quite natural.

It is known that reactions of polyfluorinated imidoylamidines with perfluorocarboxylic acid anhy-

Table 1. Characteristics of compounds **1a—c**

Compound	Yield (%)	B.p./°C (*p*/Torr)	Found / Calculated (%) C	F	N	Molecular formula
1a	71.3	111—112	28.0 / 27.8	61.75 / 62.90	9.04 / 9.27	$C_7F_{10}N_2$
1b	73.0	44 (50)	27.27 / 27.27	64.94 / 64.77	8.00 / 7.95	$C_8F_{12}N_2$
1c	88.0	50—51 (24)	27.07 / 26.87	66.19 / 66.17	6.93 / 6.97	$C_9F_{14}N_2$

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