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Synthesis and Investigation of Biological Activity of Phosphorylated Amines and Amides

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Abstract—A series of phosphoryl-substituted amines and amides was synthesized and their inhibition activity towards acetylcholinesterase, butyrylcholinesterase, and carboxylesterase was investigated.

Keywords: phosphorylated amines, phosphorylated amides, acetylcholinesterase, butyrylcholinesterase, carboxylesterase, serine esterase inhibitors

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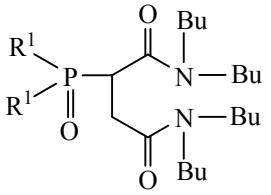
Phosphine oxides with amino group contain two types of coordination centers in the same molecule; the properties of these centers significantly depend on the nature of the substituents at the heteroatoms. Dialkylamino-substituted phosphine oxides are the convenient model for revealing main regularities of influence of the functional groups structure on biological activity. Among polyfunctional amino-containing phosphine oxides new highly reactive blockers of glutamate-induced capture of $^{45}Ca^{2+}$ ions were found [1] that allows considering them as the promising compounds for further investigation as alternative neuroprotectors [2].

Phosphorylated dialkyldiamides of succinic acid exhibit similar properties [3], whereas phosphorylated succinic acid, its esters, and esteramides as well as investigated phosphorylated acetamides do not possess physiological activity under similar testing.

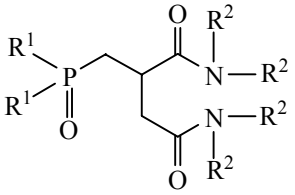
Therefore more detailed investigation of phosphorylated amines and amides as model compounds for studying the general regularities of their structure impact on the other types of biological activity, particularly on the ability of the compounds to inhibit serine esterases, is of apparent interest.

It is known that inhibition of serine esterases can lead to various pharmacological effects. In connection with it the search for and construction of new inhibitors of the enzymes is an urgent task. Particularly inhibitors of acetylcholinesterase (EC 3.1.1.7) promote the improvement of cognitive functions at dementias of different origin [4]. Butyrylcholinesterase inhibitors (EC 3.1.1.8) also promote improvement of cognitive functions that is especially important for the progressive, severe stages of Alzheimer's disease, when the activity of acetylcholinesterase decreases and butyrylcholinesterase replaces it in acetylcholine hydrolysis. Herewith as distinct from acetylcholinesterase inhibitors, butyrylcholinesterase inhibitors do not possess such dangerous side-effect as an acute cholinergic toxicity and other undesirable effects of acetylcholinesterase inhibitors [5, 6]. Carboxylesterase (EC 3.1.1.1) and butyrylcholinesterase can hydrolyze many therapeutically important drugs containing the ester groups; therefore the selective inhibitors of the enzymes are valuable for the metabolism modulation and pharmacokinetics of these medicines [7]. The earlier developed approach to the determination of the esterase profile of the compounds based on the comparative estimation of the inhibition activity towards several enzymes-esterases is widely applied [8–10]. This approach allows obtaining more clear comprehension of biological activity of compounds and therefore promotes the estimation of therapeutic potential and possible side-effects of the investigated compounds.

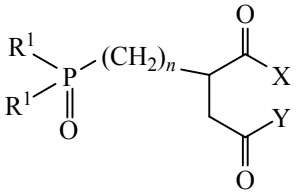
Scheme 1.



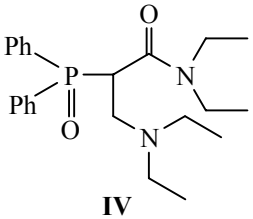
Ia–If



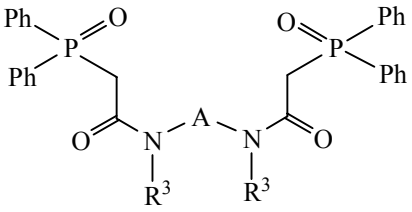
IIa–IIc



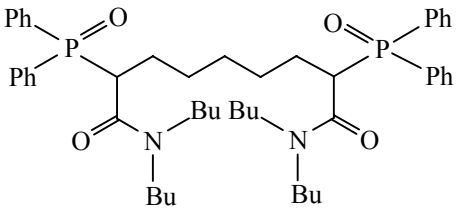
IIIa–IIIe



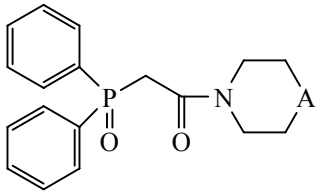
IV



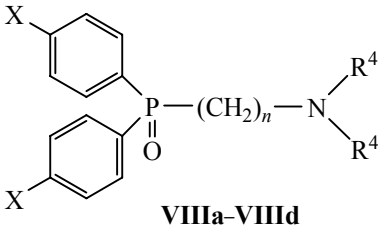
Va–Ve



VI



VIIa–VIIc



VIIIa–VIIId

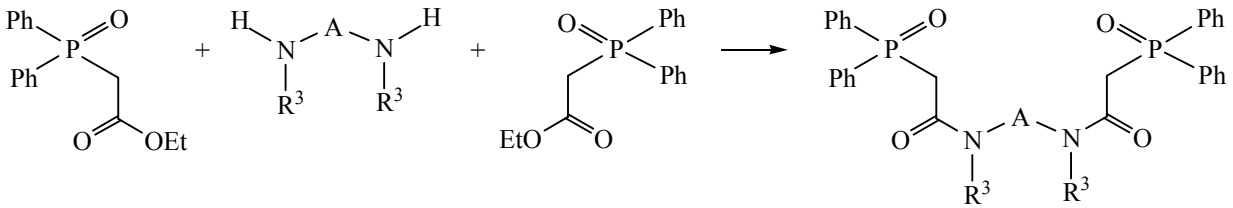
I, R^1 = Et (**a**), Bu (**b**), Ph (**c**), *o*-$CH_3C_6H_4$ (**d**), *p*-ClC_6H_4 (**e**), *p*-$CH_3OC_6H_4$ (**f**); **II**, R^2 = Et, R^1 = Ph (**a**), R^2 = Bu, R^1 = *p*-$CH_3OC_6H_4$ (**b**), 2,5-$(CH_3)_2C_6H_3$ (**c**); **III**, *n* = 0, X = Y = OH, R^1 = *i*-Pr (**a**), *p*-$CH_3C_6H_4$ (**b**); X = Y = OEt, R^1 = Ph (**c**); X = NBu_2, Y = OEt, R^1 = Ph (**d**); *n* = 1, X = Y = OH, R^1 = *p*-$CH_3OC_6H_4$ (**e**); **V**, R^3 = Pr, A = $(CH_2)_2$ (**a**); R^3 = Oct, A = $(CH_2)_4$ (**b**); R^3 = Oct, A = $(CH_2)_6$ (**c**); R^3 = Me, A = $(CH_2)_2N(Me)(CH_2)_2N(Me)(CH_2)_2$ (**d**); R^3 = cyclo-Hex, A = C(O) (**e**); **VII**, A = CH_2 (**a**), O (**b**), NH (**c**); **VIII**, *n* = 1, X = H , R^4,R^4 = $(CH_2)_5$ (**a**); *n* = 1, X = Me, R^4, R^4 = $(CH_2)_5$ (**b**); R^4 = cyclo-Hex (**c**); *n* = 3, X = Br, R^4 = Bu (**d**).

For this goal in the present work a number of the model compounds was synthesized; the compounds were phosphorylated dialkyldiamides of succinic acid and phosphoryl-containing amines distinguished by the nature of the substituents at the nitrogen atom and the distance between the functional groups. The experiments *in vitro* allowed the investigation of the impact of the compounds' structure on their inhibition activity and selectivity towards a number of pharmacologically significant serine esterases like acetylcholinesterase, butyrylcholinesterase, and carboxylesterase.

Monophosphorylated dialkyldiamides of succinic acid **I** and **II** were investigated as the main objects compared to their oxygen-containing **III** and nitrogen-containing **IV** analogs. Besides that we have chosen for the primary screening also diphosphorylated diamides where an alkylene bridge binds two symmetric parts of the molecule through the amide **V** or methylene **VI** fragments. For comparison, phosphorylated monoamides **VII**, where the nitrogen atom was involved into aliphatic ring, and amines **VIII**, which differed by the character of the substituents in the amino group and spatial distance between the heteroatoms, were also investigated (Scheme 1).

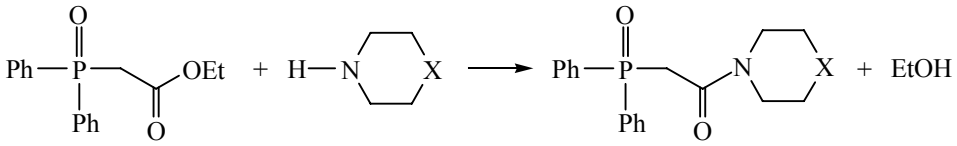
Bisphosphorylated diamides **Va–Ve** were prepared by fusion of ethyl diphenylphosphono acetate

Scheme 2.



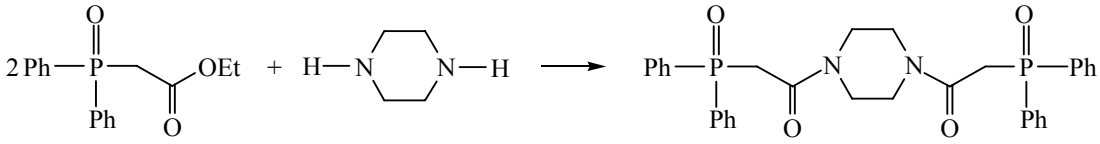
Va–Ve

Scheme 3.



VIIa–VIIc

Scheme 4.



IX

with secondary amines in an evacuated ampule (Scheme 2).

The presence of the diphenylphosphinoyl moiety in the α-position with respect to the ethoxycarbonyl group allowed the direct amidation of the ester with the formation of the products in high yields. Only compound **Ve** was prepared in a low yield since under the reaction conditions the starting dicyclohexylurea underwent a partial decomposition.

Similarly heating the equimolar amounts of ethyl diphenylphosphono acetate with secondary cyclic amines like morpholine, piperidine, and piperazine with distillation of the released alcohol provided monoamides **VIIa–VIIc** (Scheme 3).

The reaction of ethyl diphenylphosphono acetate with piperazine at molar ratio of the starting reagents 2 : 1 afforded bisamide **IX**; however its activity was not investigated due to low solubility (Scheme 4).

For compounds **I–VIII** the inhibition activity towards acetylcholinesterase, butyrylcholinesterase, and carboxylesterase was measured (see the table). Among diamides **I** and **II** a significant inhibition activity towards butyrylcholinesterase was found only for compound **If** (IC_{50} = 5.54 × 10^{-5}) possessing the most electron-donor substituents at the phosphorus atom. Herewith the introduction of the methylene bridge between phosphoryl and amide parts of the molecule reduced the inhibition activity towards butyrylcholinesterase.

Monoamides **VIIa–VIIc** occurred to be similar by their activity to diarylphosphinoyl-substituted diamides **I**, **II**. The closest oxygen-containing **III** and nitrogen-containing **IV** analogs practically did not possess meaningful inhibition activity and therefore were considered to be unpromising for further investigations. The most interesting results were obtained in a series of diphosphorylated diamides **Va–Ve** and for compound **VI**. Thus, compounds **Vb**, **Vc**, and **VI** were found to possess micromolar inhibition activity towards butyrylcholinesterase. For the rest of compounds of the group containing a short bridge between nitrogen atoms like **Va** or the bridges modified with heteroatoms **Vd** and **Ve** any meaningful inhibition activity was not found.

It should be noted that compound **VIIIc** with cycloalkyl substituents at the amino group exhibited an inhibition activity towards butyrylcholinesterase (IC_{50} = 4.55 × 10^{-5}).

The compounds which expressed meaningful inhibition activity are of various chemical classes that allows their further modification with the goal of preparation of compounds with high inhibition activity and selectivity towards butyrylcholinesterase.

EXPERIMENTAL

Commercially available preparations of acetylcholinesterase of human erythrocytes, butyrylcholinesterase of horse serum, and carboxylesterase of pig liver by Sigma (USA) were used for the investigation of inhibition activity of the compounds regarding acetylcholinesterase, butyrylcholinesterase, and carboxylesterase. An activity of acetylcholinesterase and butyrylcholinesterase was determined according to the Ellman method (λ = 412 nm) [11] with the use of acetylthiocholine (1 mM) and butyrylthiocholine (1 mM) by Sigma (USA) as a substrate, respectively. The activity was determined under the following conditions: 100 mM phosphate buffer, pH = 7.5, 25°C. Activity of carboxylesterase was determined spectrophotometrically [12] by release of 4-nitrophenol (λ = 405 nm), substrate was 4-nitrophenyl acetate (1 mM) by Sigma (USA); the conditions were 100 mM phosphate buffer, pH = 8.0, 25°C. The measurements were performed on a microplate spectrophotometer BioRad Benchmark Plus (France). The compounds were dissolved in DMSO (Sigma, USA); the incubation mixture contained 2% of the solvent.

An estimation of inhibition activity of the compounds was done by measuring the degree of the enzymes inhibition at concentration of the compound 20 μM. To do this a sample of the appropriate enzyme was incubated in a mixture with the investigated compound for 10 min followed by determination of the residual activity of the enzyme. Each experiment was repeated three times. For the most active compounds the values IC_{50} were determined that is the concentration of an inhibitor required to reduce an enzyme activity by 50%. To determine IC_{50} of acetylcholinesterase, butyrylcholinesterase, and carboxylesterase inhibition, a sample of the corresponding enzyme was incubated in a mixture with the investigated compound (concentration DMSO was 2 vol %) for 10 min followed by determination of the residual enzyme activity. A range of concentrations of the investigated compound was (1×10^{-11})–(1×10^{-4}) M. Each experiment was repeated three times.

^{1}H and ^{31}P NMR spectra were registered on a Bruker CXP-200 instrument (Germany) with Me_4Si as

Inhibition activity of compounds **I–VIII** towards acetylcholinesterase, butyrylcholinesterase, and carboxylesterase[a]

Comp. no.	Acetylcholinesterase	Butyrylcholinesterase	Carboxylesterase
Ia	1.5±0.5	15.1±2.3	0
Ib	0	13.9±2.8	0
Ic	7.8±1.6	23.0±2.5	17.3±1.9
Id	7.6±1.5	15.1±2.3	19.6±2.2
Ie	7.7±1.4	24.2±2.7	0
If	0	32.1±2.9 [$(5.54\pm0.61) \times 10^{-5}$]	9.3±1.9
IIa	6.4±1.3	20.0±2.4	3.0±0.9
IIb	6.5±1.4	14.1±2.5	0
IIc	4.2±1.2	22.0±2.0	2.3±0.7
IIIa	0	0	7.9±1.7
IIIb	2.5±0.8	3.5±1.0	2.0±0.6
IIIc	0	0	6.2±1.4
IIId	2.4±0.7	2.4±0.6	6.7±1.5
IIIe	0	0	2.2±0.6
IV	2.9±0.9	6.8±1.4	5.8±1.8
Va	2.5±0.8	18.0±1.9	16.7±2.2
Vb	18.1±2.2	59.2±6.5 [$(8.23\pm0.74) \times 10^{-6}$]	22.3±2.5
Vc	2.6±0.8	57.7±5.2 [$(1.46\pm0.07) \times 10^{-5}$]	23.6±2.6
Vd	0	8.3±1.6	3.9±1.2
Ve	3.4±1.0	7.6±1.9	3.1±0.9
VI	0	35.1±3.5 [$(7.84\pm0.86) \times 10^{-5}$]	2.8±0.8
VIIa	6.0±1.6	18.8±2.1	0
VIIb	0	13.9±2.5	0
VIIc	1.5±0.5	18.8±2.3	0
VIIIa	0	1.8±0.5	7.5±1.7
VIIIb	0	1.9±0.6	11.0±2.2
VIIIc	5.6±1.7	48.3±4.4 [$(4.55\pm0.50) \times 10^{-5}$]	15.2±2.7
VIIId	17.7±2.3	11.3±2.2	16.0±2.4

[a] Inhibition activity was determined as a degree of inhibition (%) of the enzyme activity with the investigated compound in 20 μM concentration; the values of IC_{50}, M are given in brackets.

reference. Melting points were determined on a Boetius PHMK instrument (Germany). Before the biological investigations to be performed the compounds were additionally purified by recrystallization or chromatographically on a column filled with silica gel L 100/160 eluting with chloroform, a mixture hexane–isopropanol (10 : 1), and dried in a vacuum.

Compounds **I-II** [1], **III** [13], **IV** [14], **VI** [15], and **VIII** [3] were prepared by the known procedures.

4,7-Bis(diphenylphosphinoylacetyl)-4,7-diazadecane (Va). A mixture of $Ph_2P(O)CH_2COOEt$ (2.88 g, 0.01 mol) and *N*,*N*'-dipropylethylenediamine (0.72 g, 0.005 mol) was heated for 2 h at 170°C in an evacuated ampule. After cooling, 20 mL of chloroform was added. The organic layer was separated, washed with water (3 × 7 mL), dried over Na_2SO_4, and concentrated. Yield 2.43 g (78%), mp 209–211°C (toluene). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.85 m (6H, $2CH_3$), 1.48 m (4H, CCH_2CN), 3.00 s (4H, NCH_2CH_2N), 3.24 m (4H, CH_2N), 3.50 d (4H, $2CH_2P$, $^2J_{HP}$ 16.0 Hz), 7.44 m (12H, Ph), 7.82 m (8H, Ph). ^{31}P NMR spectrum ($CDCl_3$): δ_P 29.35 ppm.

9,14-Bis(diphenylphosphinoylacetyl)-9,14-diazadocosane (Vb) was prepared similarly from $Ph_2P(O)\cdot CH_2COOEt$ (1.44 g, 0.005 mol) and *N*,*N*'-dioctylbutylenediamine (0.79 g, 0.0025 mol); the reaction conditions: 190–200°C, 5 h. Yield 1.83 g (92%), mp 118–119.5°C (benzene–pentane, 1 : 2). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.86 t (6H, CH_3, *J* 6.5 Hz), 1.18 m (24H, CH_2), 1.48 m (4H, CH_2N), 3.15 m (8H, $N(CH_2)_4N$), 3.54 d and 3.58 d (4H, CH_2P, $^2J_{HP}$ 16.0 Hz), 7.46 m (12H, Ph), 7.86 m (8H, Ph). ^{31}P NMR spectrum ($CDCl_3$), δ_P, ppm: 29.55, 29.76.

9,16-Bis(diphenylphosphinoylacetyl)-9,16-diazatetracosane (Vc) was prepared similarly from $Ph_2P(O)\cdot CH_2COOEt$ (0.86 g, 0.003 mol) and *N*,*N*'-dioctylhexylenediamine (0.51 g, 0.0015 mol); the reaction conditions: 190–200°C, 5 h. Yield 1.09 g (88%), mp 125–126°C (benzene–pentane, 1 : 2). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.85 t (6H, CH_3, *J* 6.5 Hz), 1.00–1.50 m (32H, CH_2), 3.12 m (4H, CH_2NCH_2), 3.32 m (4H, CH_2NCH_2), 3.40 d (4H, CH_2P, $^2J_{HP}$ 16.0 Hz), 7.42 m (12H, Ph), 7.82 m (8H, Ph). ^{31}P NMR spectrum ($CDCl_3$): δ_P 29.80 ppm.

5,8-Dimethyl-2,11-bis(diphenylphosphinoylacetyl)-2,5,8,11-tetraazadodecane (Vd) was prepared similarly from $Ph_2P(O)CH_2COOEt$ (2.88 g, 0.01 mol) and 5,8-dimethyl-2,5,8,11-tetraazadodecane (1.01 g, 0.005 mol); the reaction conditions: 100°C, 8 h. Yield 2.08 g (61%). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 2.20 m (6H, CH_3), 2.40 m [8H, $CH_2N(Me)CH_2$], 2.85 s and 3.10 s (6H, CH_3N), 3.30 m and 3.50 m (4H, CH_2NCO), 3.56 d and 3.58 d (4H, CH_2P, $^2J_{HP}$ 20.0 Hz), 7.46 m (12H, Ph), 7.85 m (8H, Ph). ^{31}P NMR spectrum ($CDCl_3$), δ_P, ppm: 29.46, 29.72.

***N*,*N*'-Dicyclohexyl-*N*,*N*'-bis(diphenylphosphinoylacetyl)urea (Ve)** was prepared similarly from $Ph_2P(O)\cdot CH_2COOEt$ (1.44 g, 0.005 mol) and dicyclohexylurea (0.56 g, 0.0025 mol); the reaction conditions: 200 °C, 1 h. Yield 0.71 g (40%), mp 169–170°C (benzene). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.20 m (12H, CH_2), 1.60 m (10H, CH_2CHNCH_2), 3.28 d (4H, CH_2P, $^2J_{HP}$ 12.5 Hz), 3.70 m (2H, CHN), 7.46 m (12H, Ph), 7.72 m (8H, Ph). ^{31}P NMR spectrum ($CDCl_3$): δ_P 30.81 ppm.

***N*-(2-Diphenylphosphinoylacetyl)morpholine (VIIa)**. A mixture of $Ph_2P(O)CH_2COOEt$ (1.44 g, 0.005 mol) and morpholine (0.44 g, 0.005 mol) was heated at 170°C in the Claisen flask till the alcohol distilling completed (about 5 h). After cooling, the residue was dissolved in 15 mL of chloroform. The organic layer was washed with water, dried over Na_2SO_4, and evaporated. Yield 1.35 g (82%), mp 177–178.5°C (toluene). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 3.52 s (4H, CH_2OCH_2), 3.56 d (2H, CH_2P, $^2J_{HP}$ 17.0 Hz), 3.62 s (4H, CH_2NCH_2), 7.50 m (6H, Ph), 7.82 m (4H, Ph). ^{31}P NMR spectrum (DMSO): δ_P 28.68 ppm.

***N*-(2-Diphenylphosphinoylacetyl)piperidine (VIIb)** was prepared similarly from $Ph_2P(O)CH_2COOEt$ (1.44 g, 0.005 mol) and piperidine (0.43 g, 0.005 mol); the reaction conditions: 200°C, 3 h. Yield 1.40 g (85%), mp 162–163°C (benzene). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.40 m (2H, CH_2CCN), 1.50 m (4H, CH_2CNCCH_2), 3.38 m (4H, CH_2NCH_2), 3.60 d (2H, CH_2P, $^2J_{HP}$ 16.0 Hz), 7.46 m (6H, Ph), 7.72 m (4H, Ph). ^{31}P NMR spectrum ($CDCl_3$): δ_P 28.23 ppm.

***N*-(2-Diphenylphosphinoylacetyl)piperazine (VIIc)** was prepared similarly from $Ph_2P(O)CH_2COOEt$ (2.88 g, 0.01 mol) and piperazine (0.86 g, 0.01 mol); the reaction conditions: 170°C, 4 h. Yield 2.00 g (61%). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.80 br.s (1H, NH), 2.78 t and 2.89 t [4H, $CH_2N(H)CH_2$, $^2J_{HH}$ 4.7 Hz], 3.50 m [4H, $CH_2N(CO)CH_2$], 3.58 d (2H, CH_2P, $^2J_{HP}$ 17.0 Hz), 7.48 m (6H, Ph), 7.84 m (4H, Ph). ^{31}P NMR spectrum ($CDCl_3$): δ_P 28.71 ppm.

***N*,*N*'-Bis(2-diphenylphosphinoylacetyl)piperazine (IX)** was prepared similarly from $Ph_2P(O)CH_2COOEt$ (2.88 g, 0.01 mol) and piperazine (0.43 g, 0.005 mol);

the reaction conditions: 190°C, 2 h. Yield 2.20 g (77%), mp 251–252°C (ethanol). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 3.40–3.80 m (12H, CH_2P, CH_2NCH_2), 7.50 m (6H, Ph), 7.80 m (4H, Ph). ^{31}P NMR spectrum ($CDCl_3$), δ_P, ppm: 28.66, 28.75.

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