# SYNTHESIS AND ANTIVIRAL ACTIVITY OF PHOSPHORUS-CONTAINING DERIVATIVES OF DIAZA-18-CROWN-6

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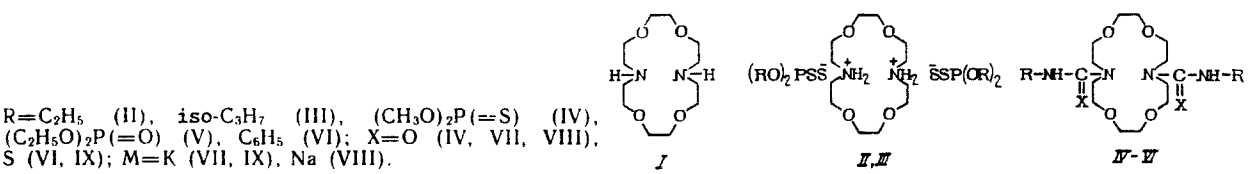
Crown ethers have a wide range of exploitable properties, and they are used in a variety of areas of science and technology [2, 4]. Although their biological activity is of great interest [1], the literature contains only a few reports of the biological properties of crown ethers and their derivatives.

The aim of the present work was to prepare a series of organophosphorus derivatives of diaza-18-crown-6, in which the phosphorus-containing fragment is linked with the backbone ring by different types of bonds, and to study their antiviral activities and toxicities.

Diaza-18-crown-6 (I), containing two reactive NH-groups, is easily modified by organophosphorus reagents. Thus, dialkyldithiophosphoric acids react to form crystalline salts containing two of the latter molecules per molecule of diaza-18-crown-6(compounds II and III).

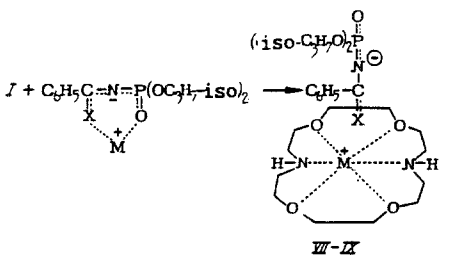
The macrocyclic diamine I reacts easily with the C=N bond of isocyanates and isothiocyanates, to form products in a ratio of 1:2 (compounds IV-VI).




The alkaline salts of N-phosphorylated amides and thioamides form stable complexes (VIII-IX) with diaza-18-crown-6, in which the phosphorus-containing fragment is bound to a metal ion, which is located within the cavity of the large ring by coordination bonds.

Compounds II-IX are crystalline solids, stable to prolonged storage, freely soluble (except for VI) in most organic solvents, though they are insoluble in water. The structures of II-IX were determined by infra-red- and NMR ($^1H$ and $^{31}P$)-spectroscopy, and the results were supported by elemental analysis. The properties of II-IX are shown in Table 1.

Complexes VII-IX have liquid crystal properties, as shown by the wide temperature range over which they melt.




## MATERIALS AND METHODS

### Chemical Methods

Infra-red spectra were taken using a UR-20 (GDR) spectrometer over the range 400-3700 $cm^{-1}$, and PMR spectra were taken on a "Tesla BS 467 A" (Czechoslovakia) instrument, at 60 MHz, using hexamethyldisiloxane as the internal standard, and $CCl_4$ and $(CD_3)$ CO as solvents. $^{31}P$-NMR spectra were taken on an RYa-2305 (USSR) apparatus, with a working frequency of

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TABLE 1. Physicochemical Characteristics of Diaza-18-crown-6 Derivatives

| Compound | Yield, % | mp, °C | Composition formula | $\delta_P$, ppm |
|---|---|---|---|---|
| II | 90,5 | 103—104 | $C_{20}H_{48}N_2O_8P_2S_4$ | 110 |
| III | 99,1 | 108—109 | $C_{24}H_{56}N_2O_8P_2S_4$ | 109 |
| IV | 74,1 | 132—135 | $C_{18}H_{38}N_4O_{10}P_2S_2$ | 65 |
| V | 86,4 | 97—99 | $C_{22}H_{46}N_4O_{10}P_2S_2$ | 4 |
| VI | 97,7 | 190—191 | $C_{26}H_{36}N_4O_4S_2$ | — |
| VII | 89,1 | 105—136 | $C_{25}H_{45}KN_3O_8P$ | 9 |
| VIII | 75,8 | 80—101 | $C_{25}H_{45}N_3NaO_8P$ | 8 |
| IX | 92,3 | 100—125 | $C_{25}X_{45}KN_3O_7P$ | 6 |

TABLE 2. Protective Effects of Diaza-18-crown-6 (I) and its Derivatives in experimental Influenza Infection in White Mice with A/Aichi 2/68 (H3N2)

| Compound | MID, mg/kg | Dose given, mg/kg | | Survival rate, % | Protection, % | Index of protection, % | Prolongation of life, % |
|---|---|---|---|---|---|---|---|
| | | qnty. per dose | qnty. per course | | | | |
| I | 300 | 30 | 150 | 24,1 | 0 | 0 | 0 |
| II | 1000 | 100 | 500 | 70,0 | 40,0 | 57,0 | 0 |
| III | 500 | 50 | 250 | 70,0 | 40,0 | 57,0 | 9 |
| IV | 1000 | 100 | 500 | 86,7 | 38,4 | 65,0 | 13,3 |
| V | 50 | — | — | — | — | — | — |
| VI | 5000 | 500 | 2500 | 83,3 | 31,6 | 65,5 | 14,0 |
| VII | 200 | 20 | 100 | 89,7 | 39,7 | 79,4 | 21,4 |
| VIII | 400 | 40 | 200 | 76,7 | 26,7 | 53,7 | 15,0 |
| IX | 500 | 50 | 250 | 80,0 | 30,0 | 60,0 | 11,3 |

8 MHz and 85% $H_3PO_4$ as the internal standard. Phase transition temperatures were determined by a thermooptical method, using a Boezius (GDR) apparatus with an RNMK 05 visual monitor. Elemental analysis results were in agreement with values calculated from structural analyses.

**Bis-dialkyldithiophosphate-1,10-diaza-dication-18-crown-6 Compounds (II, III).** Diethyl- or diisopropyl-diisophosphoric acid (0.02 moles) in 10-15 ml of anhydrous benzene was added with constant mixing to a solution of 0.01 moles of diaza-18-crown-6 in 30-40 ml benzene. The reaction was exothermic. Hexane (150-200 ml) was added to the reaction; the resulting crystals were filtered, washed with hexane, and dried.

**N,N'-Bis-dialkyloxy(thio)phosphorylamido(thio)carbonyl-1,10-diaza-18-crown-6 (VI, V).** A solution of 0.04 moles of dimethoxythiophosphorylisocyanate or diethoxyphosphorylisothiocyanate in 10 ml of benzene was added dropwise to a solution of 0.02 moles of diaza-18-crown-6 (I) in 40 ml of anhydrous benzene. The reaction mixture was agitated for 2 h, after which it was kept at room temperature for 24 h. The solvent was removed in vacuo (water-jet pump), and the residue was treated with hexane. The crystals thus formed were separated by filtration, and were purified by reprecipitation from benzene with hexane.

**N,N'-Bis-phenylaminothiocarbonyl-1,10-diaza-18-crown-6 (VI).** A solution of 0.01 moles of diaza-18-crown-6 in 15 ml of benzene was added dropwise with mixing to a solution of 0.02 moles of phenylisothiocyanate in 10 ml of anhydrous benzene. The reaction mixture produced heat. The mix was kept at room temperature for 24 h. Crystals of VI were formed, and were washed repeatedly with benzene and hexane.

**Complexes of the Alkaline Salts of N-Phosphorylated(Thio)amides with Diaza-18-crown-6 (VII-IX).** A solution of 0.1 mole of diaza-18-crown-6 in 100 ml of acetone was added to 0.1 moles of a solution of the alkaline salt of N-phosphorylated (thio)amide in 100 ml of anhydrous acetone. The mixture was heated with a reflux condenser for 15 min. The solvent was removed in vacuo (water-jet pump), and the residue was treated with hexane. The products obtained were purified by recrystallization from benzene.

**Biological Methods**

The antiviral activities of I-IX were studied *in vitro* and *in vivo*.

*In vitro* antiviral activity was evaluated in terms of the reduction of the replication of influenza A/Leningrad 34/72 (H3N2) virus in isolated fragments of chick embryo chorioallantoic membrane (CAM) [5].

The maximum concentration of compounds was half the minimal toxic dose for CAM cells. All compounds had low toxicity, in the range 250-2000 μg/ml. Cell cultures were infected with 1, 10, and 100 infective doses of allantoic virus. Virus was assayed in the culture fluid by hemagglutination of 1% chick erythrocytes in saline. Because of the water-insolubility of the compounds, samples were dissolved in dimethylsulfoxide and were diluted to the required concentration with sterile Hank's solution. An antiviral activity was studied for compounds II-V and VII-IX, since I and VI formed precipitates on addition to culture medium. The results showed that the compounds studied did not efficiently inhibit the reproduction of influenza A (H3N2) virus *in vitro*.

*In vivo* antiviral activity was measured by the ability to protect mice from death from experimental influenza. Experiments were carried out on mongrel mice of both sexes (16-18 g) obtained from the "White Moss" breeding station. Mice were infected with the mouse lung-adapted strain A/Aichi 2/68 (H3N2). Virus grown on CAM was used, with an $LD_{50}$ for white mice of $10^{-4.5}$ and a hemagglutinin titer of 1:2048. Mice were infected intranasally under light ether anesthesia with 0.03 ml of virus in Hank's solution. Mice received the compounds subcutaneously five times on a therapeutic-prophylactic regime, each dose was one tenth of the minimum toxic dose. Antiviral activity was determined using standard methods [3]; observations were continued for 14 days.

## RESULTS AND DISCUSSION

The toxicity of compounds I-IX was found to depend on the type of chemical bond joining the phosphorus-containing fragment and the large ring of the crown ether. Thus, complexes of N-phosphorylated (thio)amides with diaza-18-crown-6 VII-IX had a level of toxicity similar to that of the initial crown ether I. The minimum toxic dose (MTD) varied over the range 200-500 mg/kg (Table 2). The bis-dialkyldithiophosphate salts of diaza-18-crown-6 (compounds II and III) were less toxic than the initial crown ether I, with toxicity for mammals of 500-1000 mg/kg. The toxicity of compounds with side chains linked by covalent bonds appears to be determined primarily by toxicity of the substituent. Thus, compound IV was 3.3 times less toxic than the original compound I and, on the other hand, compound V was six times more toxic than I. The high toxicity of compound V (MTD 50 mg/kg) prevented *in vivo* studies of its antiviral activity. The anomalously low toxicity of bis-thiourea (compound VI, MTD 5000 mg/kg) is apparently due to its extremely low solubility in water and in organic solvents.

Diaza-18-crown-6 (I) did not protect mice from death resulting from experimental influenza, while all the derivatives did have protective activity (Table 2). Complexes VII and IX, and bis-(thio)ureas IV and VI had the highest levels of activity. The indices of protection with these compounds were in the range 60-79.4%. These compounds increased the survival time by 11.3-21.1%. Compounds II, III, and VIII were marginally less protective: they had indices of protection of 53.7-57%, and they increased survival time by 9-15%.

Our studies have thus demonstrated the potential for finding antiviral agents among derivatives of crown ethers. The most interesting compounds are those in which the crown ether ring contains a side chain with potential biological activity (for example, urea or thiourea derivatives). The lack of antiviral activity in the starting material, diaza-18-crown-6, and the appearance of these properties in a variety of its derivatives demonstrates that the phosphorus-containing fragments are responsible for their biological properties. In these compounds, the ring functions as a lipophilizing fragment, and also confers the ability to penetrate cell membranes easily, i.e., it promotes access of the antiviral fragment to the site of action. The latter, in particular, is indicated by the fact that neither alkyldithiophosphoric acids nor their salts with linear amines had antiviral activity, in which they differed from II and III.

The presence of high levels of antiviral activity against experimental influenza without any direct antiviral activity may demonstrate that diaza-18-crown-6 derivatives act on a variety of protective systems of the body, e.g., activating the antiviral immune response, or activating non-specific resistance; this will be the subject of our future studies.

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