Synthesis and the crystal structures of *N*-(2-nitroxyethyl)isonicotinamide and its complexes with $PdCl_2$ and $PtCl_2$ as potential antitumor medicines

B. S. Fedorov, N. I. Golovina, M. A. Fadeev, G. V. Strukov, V. V. Kedrov, G. V. Shilov, G. N. Boiko, and L. O. Atovmyan*

Institute of Problems of Chemical Physics, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation.
Fax: +7 (096) 515 3588. E-mail: boris@icp.ac.ru

Previously unknown *N*-(2-nitroxyethyl)isonicotinamide was synthesized by the reaction of isonicotinoyl chloride with 2-nitroxyethylamine and was used as a ligand in the reactions with $PdCl_2$ and $PtCl_2$ to prepare new complexes, *viz.*, *trans*-bis[(2-nitroxyethyl)isonicotinamide-*N*]dichloropalladium(II) and *cis*-bis[(2-nitroxyethyl)isonicotinamide-*N*]dichloroplatinum(II), respectively. The structures of the ligand and the resulting complexes were established by X-ray diffraction analysis.

Key words: 2-nitroxyethylamine, isonicotinoyl chloride, *N*-(2-nitroxyethyl)isonicotinamide, reactions with palladium(II) and platinum(II) chlorides, *trans*-bis[(2-nitroxyethyl)isonicotinamide-*N*]dichloropalladium(II), *cis*-bis[(2-nitroxyethyl)isonicotinamide-*N*]dichloroplatinum(II), crystal structure, X-ray diffraction analysis.

Recently, a new approach to the synthesis of antitumor medicines exhibiting combined action has been developed.* Previously, we have reported selected concepts of this approach.[1] The basic idea resides in the fact that the molecule of an antitumor medicine should contain three functionally important fragments:

1) the transition-metal atom, which is able to form a covalent bond with DNA of tumor cells;

2) a variable metabolically active ligand bearing physiologically active groups;

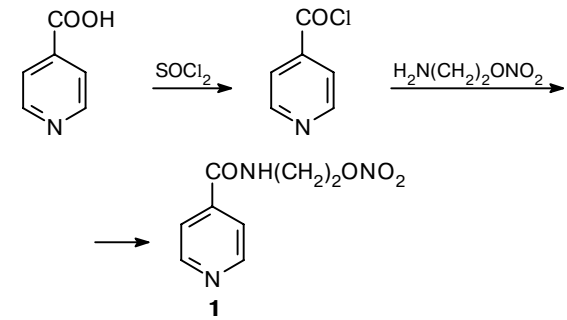
3) a group that ensures generation of nitrogen monoxide activating a system of cyclic nucleotides.

The known structure—activity relationship (SAR-1) should also be taken into account. According to this rule, *cis*-complexes exhibit higher antitumor activity than *trans*-complexes.[2,3]

Previously, we have used the above-considered approach for preparing Pd^{II} complexes with different derivatives of pyridinecarboxylic acids containing nitroxyethyl groups.[1,4,5]

It is known that 2-nitroxyethyl nicotinate and *N*-(2-nitroxyethyl)nicotinamide form *trans*-complexes with Pd^{II} and Pt^{II},[1,6] which is attributable to steric hindrances to the formation of the corresponding *cis*-complexes. In the present study, we synthesized potential antitumor medicines, *viz.*, the Pd^{II} and Pt^{II} complexes with *N*-(2-nitroxyethyl)isonicotinamide, and investigated their structures. These complexes were expected to have *cis* structures.

Previously unknown *N*-(2-nitroxyethyl)isonicotinamide (**1**) was prepared by the reaction of isonicotinoyl chloride with 2-nitroxyethylamine.



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The overall view of molecule **1** is shown in Fig. 1. The bond lengths and bond angles in the pyridine fragment are typical of this type of molecules. The plane of the pyridine ring forms angles of 22.9° and 91° with the planes of the C(1)C(6)O(1)N(2) fragment and the nitrate C(8)O(2)N(3)O(3)O(4) moiety, respectively. The angle between the C(1)C(6)O(1)N(2) and C(6)N(2)C(7) planes is 3.9°.

trans-Bis[(2-nitroxyethyl)isonicotinamide-*N*]dichloropalladium(II) (**2**) was synthesized by the reaction of an

* The approach has been developed at the Department of Kinetics of Chemical and Biological Processes of the Institute of Problems of Chemical Physics of the Russian Academy of Sciences.

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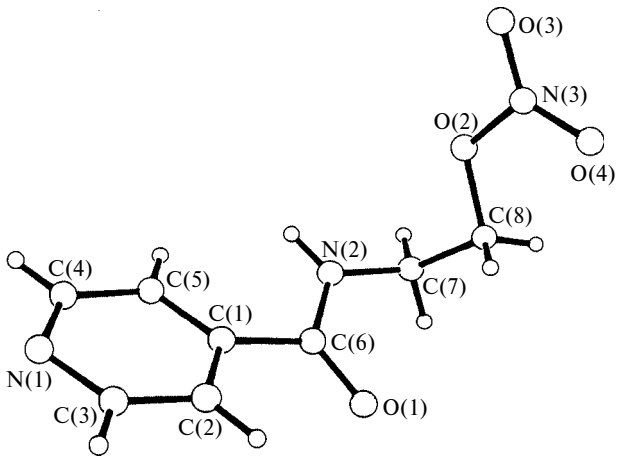
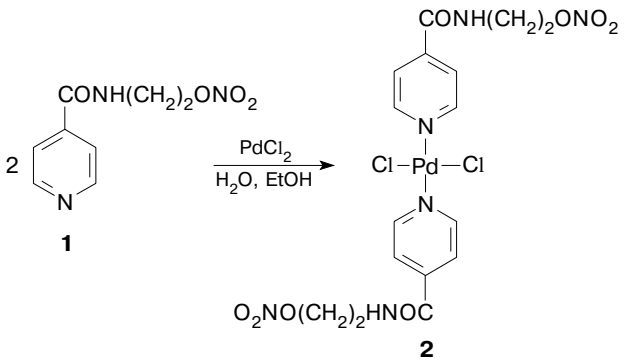


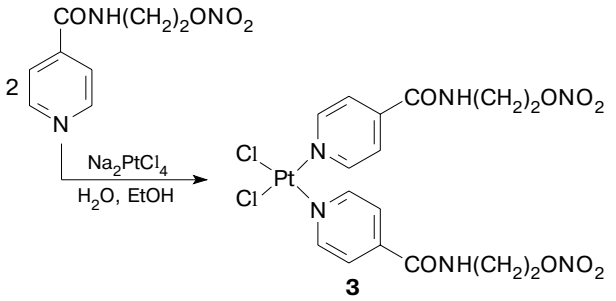
Fig. 1. Molecular structure of *N*-(2-nitroxyethyl)isonicotinamide (**1**).

aqueous solution of $PdCl_2$ with an aqueous-ethanolic solution of compound **1** in 96% yield.



In the crystal, the planar molecule of complex **2** is located in a general position (Fig. 2). The plane passing through the coordination unit forms angles of 87.4° and 87.3° with the planes of the pyridine rings. The Pd(1)—N(1) and Pd(1)—N(2) bond lengths in complex **2** are 1.991(3) and 2.03(3) Å, respectively. Probably, the *trans* arrangement of the pyridine ligands is energetically favorable due to d_{xy}—π^*-orbital interactions between the Pd^{2+} ion and the pyridine rings. The Pd(1)—Cl(1) and Pd(1)—Cl(2) bond lengths are 2.27(3) and 2.35(3) Å, respectively.

Platinum(II) chloride, unlike $PdCl_2$, forms the *cis*-complex with amide **1**, *viz.*, *cis*-bis[(2-nitroxyethyl)isonicotinamide-*N*]dichloroplatinum(II) (**3**). Complex **3** was prepared by mixing an aqueous solution of Na_2PtCl_4 with an aqueous-alcoholic solution of amide **1** in 73% yield.



The structure of complex **3** is shown in Fig. 3. The plane passing through the coordination unit forms angles of 147.9° and 59.2° with the planes of the N(1)C(1)C(2)C(3)C(4)C(5) and N(2)C(9)C(10)C(11)C(12)C(13) pyridine rings, respectively. The angle between the pyridine rings is 112.1°. The first two angles differ substantially from 0 and 90°, *i.e.*, the mutual arrangement of the coordination unit and the pyridine rings does not require efficient d_{xz}- and d_{yz}—π^*-orbital interactions between the Pt^{2+} ion and the pyridine rings. The specific rotation of the pyridine rings relative to the plane of the coordination unit may be a consequence of steric hindrances.

Apparently, the structures of the resulting complexes are determined not only by the position of the nitroxyethylamide substituent in the pyridine ring, but also by the ability of the d_{xy} orbitals of the Pd^{2+} and Pt^{2+} ions to be involved in π-conjugation with the ligands.

According to the results of assays of the acute toxicity performed at the Laboratory of Experimental Tumor Chemotherapy of the Institute of Problems of Chemical Physics of the Russian Academy of Sciences, the complexes under study are weakly toxic (LD_{50} ranged from 170 to 750 mg/kg). Presently, these complexes are being tested for antitumor activity. Even the first results of screening of complex **2** for antitumor activity demonstrated that it exhibits moderate antimetastatic activity with respect to experimental melanoma B-16. The index of inhibition of metastases, which takes into account the

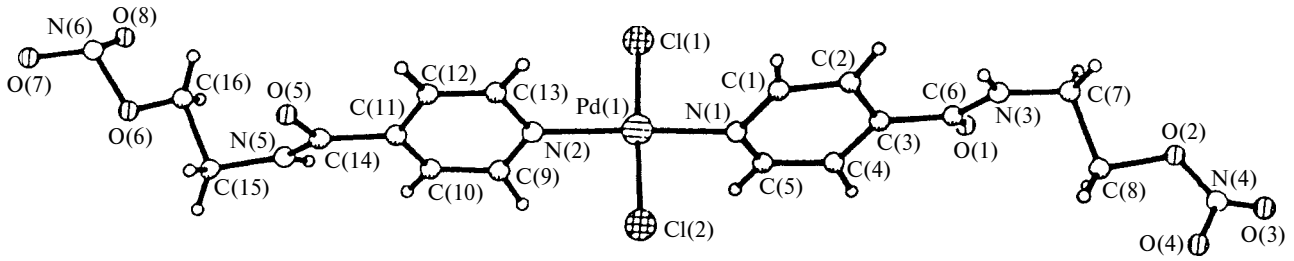


Fig. 2. Molecular structure of *trans*-bis[(2-nitroxyethyl)isonicotinamide-*N*]-dichloropalladium(II) (**2**).

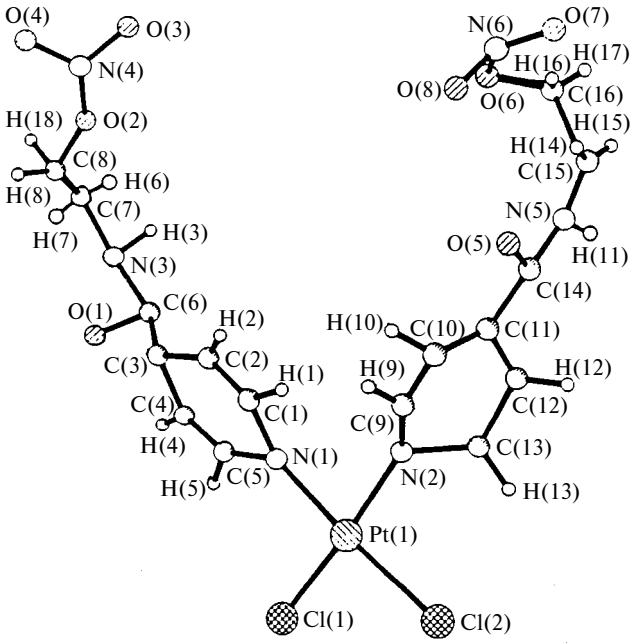
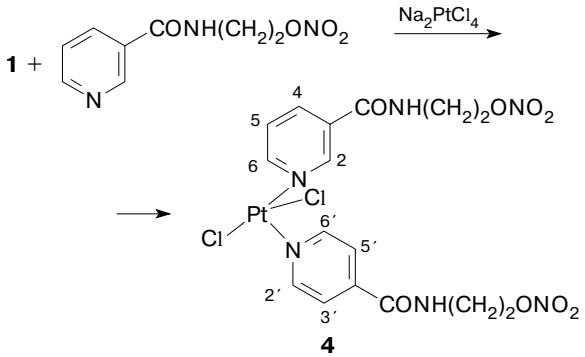


Fig. 3. Molecular structure of *cis*-bis[(2-nitroxyethyl)isonicotinamide-*N*]dichloroplatinum(II) (**3**).

frequency and intensity of metastazing, was 60—70% depending on the dose and mode of introduction.* Apparently, this complex exhibits relatively low antitumor activity due to its *trans* structure, which agrees with the SAR-1 rule.

Taking into account the results of this study, there is good reason to hope that the approach developed will be useful in the synthesis of this series of antitumor medicines. It should be noted that the Pt^{II} complexes with the isomeric *N*-(2-nitroxyethyl)nicotinamide and *N*-(2-nitroxyethyl)isonicotinamide ligands have different structures (*trans* and *cis* isomers), which also differ substantially in toxicity. In this connection, complexes containing isomeric ligands at the same transition-metal atom are of obvious interest. We synthesized complex of platinum(II) chloride with *N*-(2-nitroxyethyl)isonicotinamide and *N*-(2-nitroxyethyl)nicotinamide (**4**) analogously to complexes **2** and **3**.



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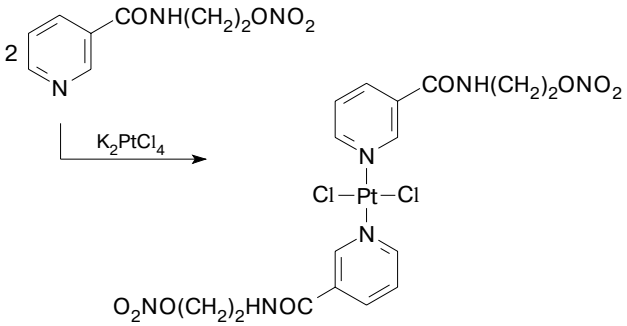
* N. P. Konovalova, private communication.

The NMR spectrum confirmed the presence of two isomeric ligands in complex **4**. A comparison of the IR spectrum of complex **4** with those of complexes **3** and **5** (the complex of $PtCl_2$ with nicorandil) disclosed the presence of a single band at 331 cm^{-1}, which is also typical of *trans*-complex **5**, and the absence of a doublet of absorption bands of Pt—Cl at 347 and 337 cm^{-1}, which is characteristic of *cis*-complex **3**. This is indicative of the *trans*-structure of complex **4**.



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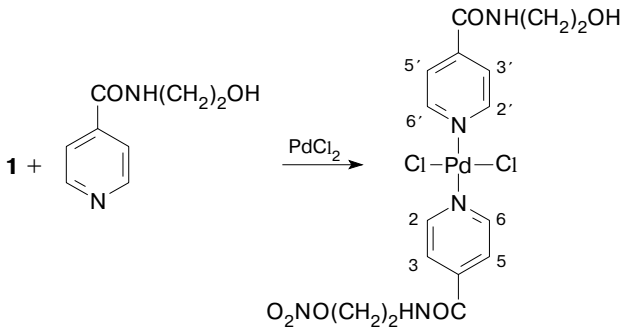
We also synthesized a Pd^{II} complex with two different ligands one of which contains the nitroexyethyl group and another ligand contains the hydrophilic (OH) group instead of the nitrate group (complex **6**).



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Complex **6** would be expected to have the geometry analogous to that of complex **2** formed by $PdCl_2$ and two molecules of amide **1**. It is of interest to estimate the effect of such combination of the functional groups on the solubility of complex **6** in water, its total toxicity, and antitumor activity.

Experimental

The IR spectra were recorded on a Specord M-80 spectrophotometer in KBr pellets. The ^1H NMR spectra were measured on a Bruker AC-200 P spectrometer.

***N*-(2-Nitroxyethyl)isonicotinamide (1).** A mixture of isonicotinic acid (analytical grade, 3.7 g, 3 mmol) and freshly distilled $SOCl_2$ was heated to boiling after which heating was terminated. The crystalline residue was evacuated first using a water-aspirator pump and then using an oil pump at 30—40 °C. Dichloroethane (80 mL), which has been dried over P_2O_5, was

added to the pale-yellow precipitate of isonicotinoyl chloride hydrochloride that formed. Then 2-nitroxyethylamine nitrate[7] (5.07 g, 3 mmol) and a solution of freshly distilled triethylamine (6 mL) in dichloroethane (12 mL) were successively added with stirring at 5—10 °C. The reaction mixture was stirred at ~20 °C for 4—6 h and then water (40 mL) was added. The reaction mixture was cooled to 0—5 °C. The precipitate that formed was filtered off, washed with water, and dried in air. Compound **1** was obtained in a yield of 4.7 g (74%) with respect to isonicotinic acid, m.p. 110—111 °C (from dichloroethane). Found (%): C, 45.38; H, 4.21; N, 19.87. $C_8H_9N_3O_4$. Calculated (%): C, 45.50; H, 4.29; N, 19.89. IR (KBr), ν/cm^{-1}: 759 (NO_2); 865 (O—NO_2); 1012 (C—O); 1268 (C—N, amide); 1274 and 1638 (ONO_2); 703, 738, 1420, and 1591 (C—H and C—C, Py). ^{1}H NMR (DMSO-d_6), δ: 9.02 (br.t, 1 H, NH, J = 6.1 Hz); 8.36 (br.d, 2 H, H(2), H(6), J = 6.4 Hz); 7.74 (br.d, 2 H, H(3), H(5), J = 6.4 Hz); 4.67 (t, 2 H, CH_2O, J = 5.1 Hz); 3.63 (dt, 2 H, CH_2N, J = 5.6 and 5.1 Hz). Crystals suitable for X-ray diffraction study were prepared by slow crystallization from dichloroethane.

***trans*-Bis[(2-nitroxyethyl)isonicotinamide-*N*]-dichloropalladium(II) (2).** Concentrated HCl (~0.4 mL) was added to a suspension of *N*-(2-nitroxyethyl)isonicotinamide (**1**) (2.39 g, 11.3 mmol) in water (120 mL) to pH 2—3 at 20—30 °C. Then a solution of $PdCl_2$ (0.6 g, 5.65 mmol) in water (40 mL) was added with stirring at the same temperature. The reaction mixture was neutralized with an aqueous solution of sodium carbonate to pH 6.0—6.5 and stirred for 12 h. The precipitate that formed was filtered off, washed with water and alcohol, and dried in air. Compound **2** was obtained in a yield of 3.3 g (97%), m.p. >210 °C. Found (%): C, 31.92; H, 3.01; N, 14.10; Cl, 11.63; Pd, 17.53. $C_{16}H_{18}Cl_2N_6O_8Pd$. Calculated (%): C, 32.05; H, 3.03; N, 14.01; Cl, 11.82; Pd, 17.74. IR (KBr), ν/cm^{-1}: 761 (NO_2); 866 (O—NO_2); 1011 (C—O); 1266 (C—N, amide); 1280 and 1614 (ONO_2); 704, 740, 1398, 1492, and 1589 (C—H and C—C, Py). ^{1}H NMR (DMSO-d_6), δ: 9.21 (br.t, 1 H, NH, J = 6.1 Hz); 8.93 (br.d, 2 H, H(2), H(6), J = 6.4 Hz); 7.87 (br.d, 2 H, H(3), H(5), J = 6.4 Hz); 4.66 (t, 2 H, CH_2O, J = 5.1 Hz); 3.63 (td, 2 H, CH_2N, J = 5.6 and 5.1 Hz). Crystals suitable for X-ray diffraction study were prepared by crystallization from CH_3NO_2.

***cis*-Bis[(2-nitroxyethyl)isonicotinamide-*N*]dichloroplatinum(II) (3).** A solution of *N*-(2-nitroxyethyl)isonicotinamide (**1**) (2.1 g, 10 mmol) in a mixture of alcohol (80 mL) and water (30 mL) was added with stirring to a solution of Na_2PtCl_4 (1.915 g, 5 mmol) in water (75 mL) at 20—30 °C. The reaction mixture was stirred for 14 h. The precipitate that formed was filtered off, washed with water and alcohol, and dried in air. Compound **3** was obtained in a yield of 2.52 g (73.7%), m.p. >214 °C. Found (%): C, 27.63; H, 2.54; N, 12.08; Cl, 10.11; Pt, 28.04. $C_{16}H_{18}Cl_2N_6O_8Pt$. Calculated (%): C, 27.90; H, 2.61; N, 12.21; Cl, 10.32; Pt, 28.35. IR (KBr), ν/cm^{-1}: 337, 347 (Pt—Cl), 761 (NO_2); 867 (O—NO_2); 1010 (C—O); 1266 (C—N, amide); 1280 and 1614 (ONO_2); 704, 740, 1398, 1492, and 1589 (C—H and C—C, Py). ^{1}H NMR (DMSO-d_6), δ: 9.13 (br.t, 1 H, NH, J = 6.1 Hz); 8.94 (br.d, 2 H, H(2) and H(6), J = 6.4 Hz); 7.78 (br.d, 2 H, H(3) and H(5), J = 6.4 Hz); 4.63 (t, 2 H, CH_2O, J = 5.1 Hz); 3.61 (td, 2 H, CH_2N, J = 5.1 and 5.6 Hz). Crystals suitable for X-ray diffraction study were prepared by crystallization from a 1 : 1 $(CH_3)_2CO$—CH_3CN mixture.

***trans*-[(2-Nitroxyethyl)isonicotinamide-*N*′][(2-nitroxyethyl)nicotinamide-*N*]dichloroplatinum(II) (4).** A solution of Na_2PtCl_4 (1.915 g, 5 mmol) in water (60 mL) was added with stirring to a solution of *N*-(2-nitroxyethyl)isonicotinamide (**1**) (1.05 g, 5 mmol) and *N*-(2-nitroxyethyl)nicotinamide[8] (nicorandil) (1.05 g, 5 mmol) in 60% aqueous alcohol (80 mL) at 20—30 °C. The reaction mixture was stirred for 11 h. The yellow precipitate that formed was filtered off, washed with water and alcohol, and dried in air. Compound **4** was obtained in a yield of 2.94 g (86%), m.p. >200 °C. Found (%): C, 27.78; H, 2.36; N, 12.02; Cl, 9.98; Pt, 28.17. $C_{16}H_{18}Cl_2N_6O_8Pt$. Calculated (%): C, 27.90; H, 2.61; N, 12.21; Cl, 10.32; Pt, 28.35. IR (KBr), ν/cm^{-1}: 331 (Pt—Cl), 761 (NO_2); 867 (O—NO_2); 1010 (C—O); 1266 (C—N, amide); 1280 and 1614 (ONO_2); 704, 740, 1398, 1492, and 1589 (C—H and C—C, Py). ^{1}H NMR (DMSO-d_6), δ: 9.24 (br.m, 2 H, H(2), NH); 9.15 (br.d, 1 H, H(6), J = 4.8 Hz); 8.95 (br.t, 1 H, NH′, J = 6.1 Hz); 8.94 (br.d, 2 H, H(6′), H(2′), J = 6.7 Hz); 8.37 (br.d, 1 H, H(4), J = 7.9 Hz); 7.78 (br.d, 2 H, H(3′), H(5′), J = 6.4 Hz); 7.64 (br.dd, 1 H, H(5), J = 4.8 and 8.0 Hz); 4.64 (br.m, 4 H, CH_2O, $CH_2'O$); 3.63 (br.m, 4 H, CH_2N, $CH_2'N$).

***trans*-[(2-Nitroxyethyl)isonicotinamide-*N*′][(2-hydroxyethyl)isonicotinamide-*N*]dichloropalladium(II) (6).** An aqueous solution of $PdCl_2$ (40 mg mL^{-1}, 22.5 mL, 5.08 mmol) was added with stirring to a solution of *N*-(2-nitroxyethyl)isonicotinamide (**1**) (1.045 g, 4.95 mmol) and *N*-(2-hydroxyethyl)isonicotinamide (0.822 g, 4.95 mmol) in a mixture of alcohol (30 mL) and water (10 mL) at 20—30 °C. Then concentrated HCl was added to pH 2—2.5 and the reaction mixture was stirred for 2 h. The yellow precipitate that formed was filtered off, washed with water and alcohol, and dried in air. Compound **6** was obtained in a yield of 2.58 g (94%), m.p. >200 °C. Found (%): C, 34.51; H, 3.23; N, 12.57; Cl, 12.39; Pd, 18.87. $C_{16}H_{19}Cl_2N_5O_6Pd$. Calculated (%): C, 34.64; H, 3.45; N, 12.64; Cl, 12.78; Pd 19.18. IR (KBr), ν/cm^{-1}: 332 (Pd—Cl), 761 (NO_2); 867 (O—NO_2); 1010 (C—O); 1266 (C—N, amide); 1280 and 1614 (ONO_2); 704, 740, 1398, 1492, and 1589 (C—H and C—C, Py). ^{1}H NMR (DMSO-d_6), δ: 9.20 (br.t, 1 H, NH, J = 6.1 Hz); 8.92 (br.m, 5 H, H(2) and H(6), H(2′) and H(6′), NH′); 7.86 (br.m, 4 H, H(3) and H(5), H(3′) and H(5′)); 4.79 (t, 1 H, OH, J = 3.1 Hz); 4.66 (t, 2 H, CH_2O, J = 5.1 Hz); 3.64 (dt, 2 H, CH_2N, J = 5.1 and 5.6 Hz); 3.51 (dt, 2 H, $CH_2'N$, J = 5.1 and 5.6 Hz); 3.35 (dt, 2 H, $CH_2'O$, J = 3.1 and 5.1 Hz).

X-ray diffraction study of compounds 1, 2, and 3. Crystals of compound **1** are monoclinic, mol. weight 211.18, a = 9.155(1) Å, b = 11.454(1) Å, c = 9.902(1) Å, β = 111.09(9)°, V = 968.85(9) Å^3, d = 1.447(5) g cm^{-3}, λ = 0.7107 Å, μ = 0.110 mm^{-1}, space group $P2_1/c$, Z = 4; 1931 reflections with $I > 2\sigma(I)$ were measured in the region $\sin(\theta/\lambda) \leq 0.63$.

Crystals of complex **2** are triclinic, mol. weight 599.98, a = 14.890(15) Å, b = 7.968(7) Å, c = 4.844(6) Å, α = 81.51 (9)°, β = 84.22(9)°, γ = 82.93(7)°, V = 562.20(8) Å^3, d = 1.771(7) g cm^{-3}, λ = 1.5418 Å, μ = 2.330 mm^{-1}, space group $P1$, Z = 1; 1936 reflections with $I > 2\sigma(I)$ were measured in the region $\sin(\theta/\lambda) \leq 0.63$.

Crystals of complex **3** are orthorhombic, mol. weight 688.52, a = 26.692(8) Å, b = 9.801(2) Å, c = 10.057(2) Å, V = 2631.10(10) Å^3, d = 1.737(8) g cm^{-3}, λ = 0.7107 Å, μ = 9.358 mm^{-1}, space group $Pc2_1n$, Z = 4; 1450 reflections with $I > 2\sigma(I)$ were measured in the region $\sin(\theta/\lambda) \leq 0.59$.

The intensities of the observed independent reflections (1931 (**1**), 1936 (**2**), and 1450 (**3**)) were measured on a four-circle KM-4 diffractometer (KUMA-Diffraction, Poland) using the ω/2θ scanning technique. The structures of **1**, **2**, and **3** were solved by the direct method with the use of the SHELX-97 program package.

An attempt to solve the structure of complex **2** in the space group $P\bar{1}$ failed. Hence, we solved and refined the structure of complex **2** in the space group $P1$. It should be noted that the central portion of complex **2** has a local center of symmetry.

However, this symmetry is substantially distorted due to the presence of the terminal nitrate groups of the ligands. Thus, the plane passing though the coordination unit forms angles of 14.8° and 6.5° with the planes of the C(8)O(2)N(4)O(3)O(4) and C(16)O(6)N(6)O(7)O(8) nitrate groups, respectively. The angles between the planes of the pyridine rings and the nitrate groups in the ligands are 102.0° and 89.4°.

The atomic coordinates for the structures of **1**, **2**, and **3** were refined by the full-matrix least-squares method (**1**: 1077 reflections with $F_0 > 4\sigma(F_0)$, $R = 0.052$; **2**: 1741 reflections with $F_0 > 4\sigma(F_0)$, $R = 0.065$; **3**: 751 reflections with $F_0 > 4\sigma(F_0)$, $R = 0.12$). For the structures of complexes **2** and **3**, the absorption corrections were applied using the DIFABS program. The nonhydrogen and hydrogen atoms were refined anisotropically and isotropically, respectively. For the structure of **3**, the hydrogen atoms were not refined.

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