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Antitumor effect of Pt(II) amine phosphonate complexes on sarcoma Sa-180 in mice. Crystal structure of *cis*-dichlorobis(diethyl-4-pyridylmethylphosphonate-κN)platinum(II) hydrate, *cis*-$[PtCl_2(4\text{-pmpe})_2] \cdot H_2O$

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The cisplatin analogoues platinum(II) complexes of the general formula *cis*-$[PtL_2Cl_2]$, where L is monodentate diethyl 2-, 3- or 4-pyridylmethylphosphonate (2-, 3- or 4-pmpe) ligand, have been synthesized and characterized by means of IR and NMR (^1H, ^{31}P, ^{195}Pt) spectroscopy. The crystal and molecular structure of *cis*-$[Pt(4\text{-pmpe})_2Cl_2] \cdot H_2O$ (**A3**) shows a square planar geometry of PtL_2Cl_2, with two organic molecules and two chloride leaving ligands in a *cis* configuration. The antitumor activity of the platinum(II) complexes was examined against Sarcoma Sa-180 in mice. The obtained results indicate a marked anticancer effect of platinum phosphonate complexes, and moderate nephrotoxicity evaluated in the BUN and creatinine levels in comparison with cisplatin (CDDP).

1. Introduction

Cisplatin (CDDP, *cis*-$[Pt(NH_3)_2Cl_2]$) is one of the most frequently used antitumor agents since its significant activity against a number of solid carcinomas. Although cisplatin exhibits a significant therapeutic effect, the utility of the drug is limited because of severe adverse effects and resistance of tumors (Lippert 1999; Barnes and Lippard 2004). Nephrotoxicity, the most serious side effect, is manifested clinically by elevated blood urea nitrogen (BUN) and serum creatinine level. Although the renal function may return to normal, significant pathologic damage appears to persist after cisplatin treatment (Arany and Safirstein 2003).

In carboplatin and oxaliplatin, the second-generation platinum complexes in clinical use, the chloride leaving ligands are replaced by a dicarboxylate chelating ligand. These compounds are less chemically reactive than cisplatin, and due to this fact have reduced toxicity, primarily nephrotoxicity, but nausea and vomiting do frequently occur (O'Dwyer et al. 2000).

Since cisplatin and their clinically used analogues are not ideal drugs, the search for new platinum complexes that possess improved therapeutic properties is continuing. A great number of platinum heterocyclic amine complexes have been synthesized and tested against various tumor systems. Platinum(II) complexes of the formula *cis*-$[Pt(NH_3)_2(Am)Cl]^+$, where Am = pyridine or 4-methylpyridine, have demonstrated antitumor activity against Sarcoma 180 ascites (S180a) in mice (Hollis et al. 1989). The sterically hindered complex AMD473 (*cis*-amminedichloro(2-methylpyridine)platinum(II)) was selected in search for compounds with activity against cisplatin-resistant cell lines (Holford et al. 1998) and has recently entered phase III clinical studies in patients with ovarian cancer.

Diverse ligands containing phosphonic acids and their derivatives are of interest because of their broad spectrum of biological properties (Sánchez-Moreno et al. 2004). Several platinum complexes linked to phosphonato carboxylate ligands (Hollis et al. 1990) and to amino phosphonic acids (Bloemink et al. 1999) have been reported as a new class of antitumor agents.

In our search for effective antineoplastic compounds we reported on *cis*-platinum(II) complexes linked to ligands containing both pyridine ring and phosphonate group, i.e. *cis*-$[Pt(2\text{-pmpe})_2Cl_2]$ (**A1**), *cis*-$[Pt(3\text{-pmpe})_2Cl_2]$ (**A2**) and *cis*-$[Pt(4\text{-pmpe})_2Cl_2]$ (**A3**).

These compounds, structurally related to cispatin, belong to the classic type of platinum complexes containing two leaving chloride ligands and two monodentate, uncharged diethyl 2- or 3- or 4-pyridylmethylphosphonates[1] (2- or 3- or 4-pmpe) as amine ligands. The pyridine ring of a covalently attached platinum atom could possibly intercalate into a neighboring interbase pair site on the DNA.

In the cytotoxicity assay (MTT test) against mouse leukemia L1210 cell lines all the complexes showed moderate activity (data not published).

In this study, we report chemical and biological data on the Pt(II) amino phosphonate complexes **A1, A2** and **A3,** that demonstrate anticancer activity against a murine tumor system. In addition, the compounds display reduced or comparable renal toxicity relative to cisplatin. The results obtained show that the *cis*-Pt(II) complexes of pyridylmethylphosphonate ligands could be considered as a new class of potential anticancer agents.

2. Investigations, results and discussion

The *cis*-Pt(II) complexes with pyridylmethylphosphonate ligands were prepared in one step by reaction of an appropriate pmpe ligand with K_2PtCl_4.

The complexes were characterized by IR and NMR (^1H, ^{31}P, ^{195}Pt) spectroscopy. The IR spectra display a very intensive band at 1250 cm^{-1} characteristic for ν(P=O) and broad, intensive bands in the region 1050–1020 cm^{-1}, corresponding to ν(P−O−C) (Ochocki et al. 1997). In the far IR region ν(Pt−N) and ν(Pt−Cl) bands are detected (Kettle 1996). As would be expected for *cis* configurated complexes **A1**, **A2** and **A3**, IR spectra show split (or doublet) ν(Pt−Cl) band at about 333 cm^{-1}. The weak, not sharp band, assigned to the asymmetric Pt−N stretch, appears at 498–475 cm^{-1}. In the ^1H NMR spectra the signal assigned to the CH_2−P methylene group appears as a doublet with vicinal J = 22 Hz, characteristic for $^{31}P-^1H$ coupling (Ochocki et al. 1997). For complex **A1** can be observed characteristic downfield shift of this methylene protons signal (δ = 5.85), comparing to **A2** and **A3** (δ = 3.23 and 3.13 ppm, respectively). It may be explained by the lowering in electron density of the pyridine ring due to coordination site and seems to be a general trend in transition-metal complexes containing different ligands, coordinated via heterocyclic N atom (Ochocki et al. 1992, 1998).

The Figure shows the molecule of complex **A3** with the atom-numbering scheme. Two 4-pyridylmethylphosphonate ligands (4-pmpe) are coordinated to the Pt(II) atom, *via* the pyridyl *N* atoms [Pt1−N1 = 2.013(6) Å, Pt1−N2 = 1.998(6) Å]. Two *cis*-chloro ligands, at Pt−Cl distances of 2.298(2) and 2.302(2) Å, complete the distorted square-planar Pt coordination environment. All the metal-ligand distances are slightly shorter than literature values of Pt−N and Pt−Cl, 2.05(5) and 2.32(4) Å (Orpen et al. 1989), but they are comparable with values observed for isomeric platinum(II) complex *cis*-[$PtCl_2$(2-pmpe)$_2$] · 0.5 H_2O (**A1**) [Pt−N = 2.035(5) and 2.038(5) Å; Pt−Cl = 2.2851(15) and 2.2896(16) Å] (Chęcińska et al. 2003). The *cis* angles, N1−Pt1−N2 = 87.6(2) and Cl1−Pt1−Cl2 = 92.14(8)°, deviate by up to 3° from the ideal value of 90°.

Both pyridyl rings are essentially planar, with the maximum deviation of 0.021(7) Å for C14 atom. Dihedral angle between planes of these rings is 74.5(2)°. In the presented complex, *cis*-[$PtCl_2$(4-pmpe)$_2$] · H_2O, the mean planes of the two pyridyl rings have similar orientation with respect to the Pt1 coordination plane defined by atoms: Pt1, Cl1, Cl2, N1 and N2. The corresponding dihedral angles are 56.8(2) and 60.2(2)°, respectively. Whereas, in isomeric complex **A1**, presented earlier (Chęcińska et al. 2003), the different orientations of the pyridyl rings in regard to the $PtCl_2N_2$ plane were observed, with the respective dihedral angles between them: 70.5(2) and 85.9(2)°.

In the presented complex, the conformations of two methylphosphonate fragments differ significantly from each other with respect to pyridyl rings. The corresponding torsion angles describing these differences are: C2−C3−C6−P1 = 84.8(8)° and C11−C12−C15−P2 = 107.6(8)°; C3−C6−P1−O1 = −48.0(7)° and C12−C15−P2−O5 = 63.9(6)°.

In both phosphonate groups, a deformation from the ideal tetrahedral shape is observed, especially in the angles O1−P1−O3 = 116.2(3)° and O5−P2−O4 = 114.1(4)° as well as C6−P1−O3 = 99.9(3)° and C15−P2−O6 = 99.5(3)°.

Due to the omission of the hydrogen of water molecule from the model, it was not possible to fully analyse the hydrogen-bonding interactions. However, the relatively short O7…O5 $\left[1-x,\ y-{}^1/_2,\ {}^1/_2-z\right]$ contact of 2.86(1) Å could possibly be considered as classic O−H…O hydrogen bond. The arrangement of the water and [$PtCl_2$(4-pmpe)$_2$] molecules in the lattice is determined mainly by intermolecular C−H…O interactions. The weak interac-

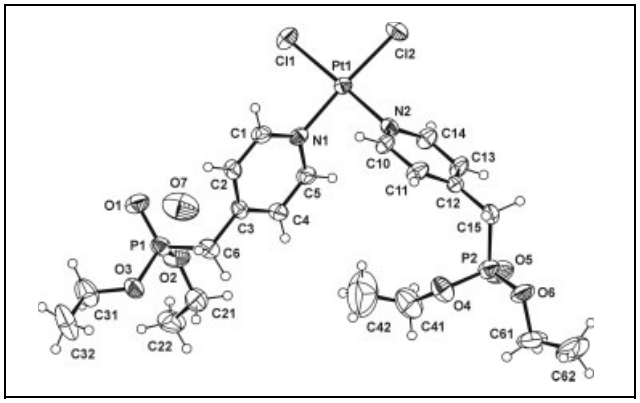

Fig.: The molecular structure of **A3** with the atom-numbering scheme. Displacement ellipsoids are drawn at 40% probability level and H atoms are shown as small spheres of arbitrary radii. H atoms of the water molecule are omitted

Table 1: The hydrogen-bonding geometries for A3

	D−H [Å]	H…A [Å]	D…A [Å]	D…HA [°]
C4−H4…O5^{i}	0.93	2.44	3.348(9)	165
C6−H6A…O4^{ii}	0.97	2.43	3.368(9)	163
C6−H6B…O7^{iii}	0.97	2.58	3.547(9)	175
C32−H32B…Cg^{iii}	0.97	2.82	3.57(1)	135

Symmetry codes: (i) 1 − x, 1 − y, 1 − z; (ii) −x, 1 − y, 1 − z; (iii) x, $^1/_2$ − y, $^1/_2$ + z; Cg is the centroid of the pyridyl ring (N1, C1, C2, C3, C4, C5)

Table 2: Antitumor screening results for investigated Pt(II) amine phosphonate complexes against Sarcoma Sa-180 in mice

Compound	LD_{50} 7 × i.p. (mg/kg)	Dose 7 × i.p. (mg/kg)	MTD (%)	Average tumor weight (mg)[a]	% T/C in tumor weight	Inhibition of tumor weight (%)	Antitumor effect (NCI)	ED_{50} 7 × i.p. (mg/kg)[c]	TI[d]
Control	–	–	–	509.5 ± 40.9	100	–	–	–	–
A1	35.7	28.6	100	67.0 ± 8.2[b]	13.1	86.9	+	14.8 ± 3.1	2.4
		14.3	50	262.0 ± 18.1[b]	51.4	48.6	+		
A2	17.1	11.5	100	107.2 ± 12.6[b]	21.0	79.0	+	5.7 ± 1.6	3.0
		5.8	50	258.6 ± 25.4[b]	50.7	49.3	+		
A3	22.9	18.3	100	202.2 ± 29.1[b]	39.7	60.3	+	14.8 ± 2.8	1.6
		9.2	50	338.4 ± 51.0	66.4	33.6	–		
Cisplatin	1.1	0.94	100	199.4 ± 42.0[b]	39.1	60.9	+	0.4 ± 0.06	1.7
		0.47	50	340.6 ± 40.0	66.9	33.1	–		

[a] values represent the means ± SE, n = 6; [b] mean is significantly different from control group; $p < 0.05$; [c] values represent ED_{50} ± SE; [d] therapeutic index $TI = \frac{LD_{50}}{ED_{50}}$

Table 3: Serum levels of BUN and creatinine in mice on the 8th day after a single i.p. administration of Pt(II) amine phosphonate complexes

Compound	Dose (mg/kg)	BUN (mg/100 ml)[a]	Creatinine (mg/100 ml)[a]
Control	–	25.1 ± 1.1	0.27 ± 0.01
A1	200.0	38.2 ± 2.8[b]	0.40 ± 0.02[b]
A2	80.5	30.1 ± 2.6[b]	0.32 ± 0.03[b]
A3	128.0	40.7 ± 1.3[b]	0.38 ± 0.01[b]
Cisplatin	6.0[c]	32.5 ± 2.4[c]	0.40 ± 0.03[c]

[a] values represent the means ± SE, n = 5
[b] mean is significantly different from control group; $p < 0.05$
[c] Kim et al. 1994

tion C−H...π is also observed. The hydrogen-bonding geometries are presented in Table 1.

The results on antitumor activity against Sarcoma Sa-180 in mice of *cis*-Pt(II) pyridylmethylphosphonate complexes compared with cisplatin are displayed in Table 2. The performed investigations have revealed that the complexes **A1** and **A2** inhibit the development of solid tumor after their administration at a total dose equal to 100% and 50% MTD. Complex **A3** demonstrates antineoplastic activity after its administration at a total dose equal to 100% MTD. LD_{50} and ED_{50} values for cisplatin against Sarcoma Sa-180 in mice are 6.6 mg/kg and 4 mg/kg, respectively, and therapeutic index ($TI = LD_{50}/ED_{50}$) for cisplatin is 1.7 (Kadłubowski 1971). The same results were reported by Iba et al. (1993). Complexes **A1** and **A2** have higher TI (2.4 and 3.0, respectively) than cisplatin and complex **A3** has TI = 1.6, which is similar to that of cisplatin.

On the basis of blood urea nitrogen (BUN) and serum creatinine levels in treated mice, the nephrotoxicity profile of the complexes was determined. Compounds **A1** and **A3** caused a significant increase of BUN and creatinine levels in mouse blood serum in comparison with the group of healthy controls (Table 3). Both the BUN and serum creatinine parameter increased in a non significant manner after the administration of complex **A2**, what indicates the lowest nephrotoxicity of this compound.

The results presented above show that the platinum(II) complexes of pyridylmethylphosphonate isomers exhibit *in vivo* antineoplastic activity against Sarcoma Sa-180 and could be useful in structure-activity relationship studies of antitumor-active platinum complexes, as among the compounds there are differences in activity, nephrotoxicity and therapeutic index values.

3. Experimental

3.1. Synthesis and spectroscopy

All reagents used in synthesis were obtained from Aldrich and used without further purification. Cisplatin was prepared by the Dhara (1970) method. Melting points were determined on a Boethius apparatus and were uncorrected. IR spectra were obtained on an ALT Mattson Infinity Series FTIR using CsI disks. ^1H and ^{31}P NMR spectra were recorded on a Varian 300 (300 MHz). Chemical shifts were reported in respect to TMS and H_3PO_4 as standard. ^{195}Pt NMR spectra were recorded on a MSL-500 Bruker, using K_2PtCl_4 as an external standard ($\delta = -1631$ ppm vs. K_2PtCl_6, $\delta = 0$). Results of elemental analyses (C, H, N) were in an acceptable error range (less than 0.4%).

The platinum amine phosphonate complexes **A1** and **A2** were prepared by a modified method described previously (Kostka and Ochocki 1996) for complex **A3**. To the solution of the appropriate pmpe ligand (0.46 g, 2 mmol) in methanol (2 ml) the water solution (10 ml) of K_2PtCl_4 (0.42 g, 1 mmol) was added dropwise. The reaction mixture was stirred at ambient temperature for 5 h. The pale yellow precipitate was collected by filtration, washed with water and diethyl ether and recrystallized from ethanol.

3.1.1. cis-[Pt(2-pmpe)$_2$Cl$_2$] (**A1**)

Yield 0.36 g (49%); m.p. 142–145 °C; IR (CsI): ν (cm^{-1}) 1250, 1050, 1027, 475, 335; ^1H NMR ($CDCl_3$): δ (ppm) 1.43 (t, 12 H, J = 7.0 Hz, CH_3), 4.30 (dq, 8 H, J = 7.0 Hz, $POCH_2$), 5.85 (d, 4 H, $J_{P-C-H} = 22.0$ Hz, CH_2P), 7.30–7.43 (m, 4 H, Ar−H C3, C5), 7.70–7.91 (m, 2 H, Ar−H C4), 9.63 (d, 2 H, Ar−H C6); ^{31}P NMR ($CDCl_3$): δ (ppm) 23.43; ^{195}Pt NMR ($CDCl_3$): δ (ppm) −1947.
$C_{20}H_{32}N_2O_6P_2Cl_2Pt$ (724)

3.1.2. cis-[Pt(3-pmpe)$_2$Cl$_2$] (**A2**)

Yield 0.29 g (40%); m.p. 177–179 °C; IR (CsI): ν (cm^{-1}) 1265, 1053, 1026, 477, 332; ^1H NMR ($CDCl_3$): δ (ppm): 1.26 (t, 12 H, J = 6.0 Hz, CH_3), 3.23 (d, 4 H, $J_{P-C-H} = 22.0$ Hz, CH_2P), 4.10 (dq, 8 H, J = 6.0 Hz, $POCH_2$), 7.10–7.41 (m, 2 H, Ar−H C5), 7.76–7.91 (m, 2 H, Ar−H C4), 8.89–9.13 (m, 4 H, Ar−H C2, C6); ^{31}P NMR ($CDCl_3$): δ (ppm): 23.77; ^{195}Pt NMR ($CDCl_3$): δ (ppm) −1993.
$C_{20}H_{32}N_2O_6P_2Cl_2Pt$ (724)

3.1.3. cis-[Pt(4-pmpe)$_2$Cl$_2$] (**A3**) (Kostka and Ochocki 1996)

Yield 0.45 g (62%); m.p. 183–185 °C; IR (CsI): ν (cm^{-1}) 1242, 1050, 1022, 498, 333; ^1H NMR ($CDCl_3$): δ (ppm) 1.27 (t, 12 H, J = 6.9 Hz, CH_3), 3.13 (d, 4 H, $J_{P-C-H} = 22.6$ Hz, CH_2P), 4.07 (dq, 8 H, J = 6.9 Hz, $POCH_2$), 7.26–7.29 (m, 4 H, Ar−H C3, C5), 8.76 (d, 4 H, Ar−H C2, C6); ^{31}P NMR ($CDCl_3$): δ (ppm) 22.68; ^{195}Pt NMR ($CDCl_3$): δ (ppm) −1944.
$C_{20}H_{32}N_2O_6P_2Cl_2Pt$ (724)

3.2. Crystal structure determination

Crystals suitable for X-ray measurement were obtained by slow evaporation of solvent (ethanol). The light yellow single crystal was used for measurement on an AFC5S Rigaku diffractometer (MSC 1989). X-ray intensities were collected using graphite monochromatized MoK_α radiation and ω scan. After each group of 150 reflections three standard intensities were monitored and insignificant (1.15%) intensity fluctuation was observed. All data were corrected for Lorentz and polarization effects (Rigaku/MSC 2002). Absorption correction (Meulenaer and Tompa 1965) was also applied.

The structure was solved by the Patterson method (SHELXS97) (Sheldrick 1997) and refined on F^2 by full-matrix least-squares technique (SHELXL97) (Sheldrick 1997). All non-hydrogen atoms were refined anisotropically. During the refinement the displacement ellipsoids for the methyl atoms C42 and C62 suggest positional disorder. Although quite a number of disorder models were explored, no reasonable disorder was found.

Table 4: Crystal and refinement data for A3

Empirical formula	$C_{20}H_{32}Cl_2N_2O_6Pt$, H_2O
Formula weight	742.42
Crystal colour	Light yellow
Crystal size [mm]	0.5 × 0.1 × 0.05
Crystal system	Monoclinic
Space group	$P2_1/c$
a [Å]	9.458(4)
b [Å]	24.744(2)
c [Å]	15.091(4)
β [°]	127.10(3)
V [Å^3]	2816.8(18)
Z	4
Density [g cm^{-3}]	1.751
Absorption coefficient [mm^{-1}]	5.324
F(000)	1464
Diffractometer	AFC5S Rigaku
Radiation [Å]	MoK_α (0.7107)
Temperature [K]	293(2)
2θ range [°]	2.70–27.50
Index ranges, hkl	$-12 \le h \le 0$ $0 \le k \le 32$ $-15 \le l \le 19$
No. of reflections collected	6849
No. of reflections independent	6473
No. of reflections observed	2728
No. of parameters	308
Extinction coefficient	0.00020(3)
Goodness of fit	0.818
R [$I \ge 2\sigma(I)$]	0.0317
wR [$I \ge 2\sigma(I)$]	0.0524
R (all data)	0.1551
wR (all data)	0.0620
Largest diff. Peak [eÅ^{-3}]	0.720
Largest diff. Hole [eÅ^{-3}]	−1.037

All H-atoms bound to C atoms were included in the refinement, at calculated positions, in the riding-model approximation, with C−H distances of 0.93 Å (aromatic), 0.97 Å (CH_2) and 0.98 Å (CH_3). The isotropic displacement parameters were set at 1.2–1.5 *Ueq* of the carrier atom. The H-atoms of the water molecule were not included because their positions could not be determined precisely.
The low ratio of observed to unique reflections (42%) is the result of the poor quality crystals obtained.
The molecular geometry was calculated by PLATON (Spek 2001).
The selected crystal data and details of the structure refinement are given in Table 4. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication. The CCDC reference number is 263598. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ,UK (Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

3.3. Animals

The study was carried out on male Swiss strain mice, weighing 18–22 g, fed with standard diet and receiving water *ad libitum*, purchased from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław. The experiments were carried out in strict accordance with the Polish governmental regulations concerning experiments on animals (Dz. U. 97. 111, 724) and rules followed the Medical University of Łódź.

3.4. Acute and subacute toxicity test

Approximate LD_{50} as an indicator of acute toxicity of compounds as well as the maximum tolerated dose (MTD) were determined according to Deichmann and Le Blanck (1943). The investigated compounds were administered i.p. into mice in aqueous suspension with 1% methylcellulose added. After a single and multiple i.p. administration of the compounds at various doses, the observations were carried out for a period of 14 days. The LD_{50} values (1 × i.p.) for compounds **A1**, **A2**, and **A3** were approximately 250, 120, 160 mg/kg of body weight, respectively, whereas the MTD were 200, 80.5, 128 mg/kg 1 × i.p., respectively, and 28.6, 11.5, 18.3 mg/kg 7 × i.p., respectively (Table 1).

3.5. Antitumor assay

Antineoplastic activity was assessed on Sarcoma Sa-180 in mice according to Goldin et al. (1961). Sarcoma Sa-180 was purchased from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław. The solid tumor was implanted subcutaneously from the passage in donor mice to the experimental animals. The day of implantation was regarded as the "0" day. The mice with implanted tumors were divided into 7 groups, six mice were used per group. The animals which were not treated with any compound constituted the control group. The animals from the experimental groups received the compounds i.p. during 7 subsequent days, with the total dose equal to 100% MTD or 50% MTD, starting 24 h after the implantation. On the 8th day after sarcoma implantation the tumor was prepared and weighed. The inhibition of tumor mass increase in treated mice as compared with non-treated ones was regarded as the criterion of antineoplastic activity. The inhibition of tumor mass increase by at least 42% is the evidence of antineoplastic effect of the administered dose (Goldin et al. 1961). Values ED_{50} of the compounds were determined according to the Kadłubowski (1971) method.

3.6. Nephrotoxicity assay

The assessment of nephrotoxicity of the investigated compounds was carried out by the determination of blood urea nitrogen (BUN) and creatinine levels in blood serum of mice on the 8th day after a single i.p. administration of the dose equal to 100% MTD, according to Pawelski (1977).

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[1] The IUPAC name for diethyl 2- or 3- or 4-pyridylmethylphosphonate is diethyl pyridin-2- or 3- or 4-ylmethylphosphonate.

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