Inorganica Chimica Acta

LETTER

Phosphine analogues of 'cisplatin'. Pyrimidyl nucleobase platinum(II) complexes stabilized by trimethylphosphine: synthesis and characterization

Guendalina Trovó and Bruno Longato*

Centro di Studio sulla Stabilitá e Reattivitá dei Composti di Coordinazione, C.N.R., c/o Dipartimento di Chimica Inorganica, Metallorganica ed Analitica, via Marzolo 1, 35131 Padua (Italy)

(Received November 21, 1991; revised December 24, 1991)

In the course of our studies on the properties of heteropolymetallic phosphino complexes we have shown that *cis*-(dppf)$PtCl_2$ (dppf = 1,1'-bis(diphenylphosphino)ferrocene) is the precursor of species which are very reactive towards thymidines [1]. The same precursor turned out to be moderately cytostatic in tests with Eagle's KB cell line [2]. These results prompted us to undertake a detailed investigation on the reactivity of nucleic acids constituents with *cis*-$\{L_2Pt^{II}\}$ units, in which the donor atoms are phosphorus of tertiary phosphines [3]. The aim of these studies was the evaluation of the effect of: (i) the higher *trans* labilizing power of the phosphine compared to the amine, (ii) the higher thermodynamic stability of the Pt–P bonds on the coordination ability of nucleobases in platinum(II) complexes.

We have recently shown that the water soluble complex *cis*-$[(PMe_3)_2Pt(\mu\text{-}OH)]_2^{2+}$ deprotonates the exocyclic amino group of 1-methylcytosine (1-MeCy) to give the dinuclear complex *cis*-$[(PMe_3)_2Pt(\mu\text{-}1\text{-}MeCy(-H))]_2^{2+}$, in which the anionic ligands bridge two metal centers in a N^3,N^4 head-to-tail fashion [4]. Remarkably, this coordination mode had been previously observed in the amino analogue *cis*-$[(NH_3)_2Pt(\mu\text{-}1\text{-}MeCy(-H))]_2^{2+}$ [5]. Interestingly enough, unlike the amino complex, the phosphino derivative slowly converts into a mononuclear species which will be described elsewhere.

In this paper we report on the reactivity of the aquo complex *cis*-$[(PMe_3)_2Pt(H_2O)_2]^{2+}$ with 1-methylsubstituted pyrimidyl nucleobases cytosine, thymine (1-MeTy) and uracil (1-MeU). The synthesis and characterization of the new complexes *cis*-$[(PMe_3)_2Pt(1\text{-}MeCy)_2]^{2+}$, *cis*-$(PMe_3)_2Pt(1\text{-}MeTy(-H))_2$ and *cis*-$(PMe_3)_2Pt(1\text{-}MeU(-H))_2$ is described. Evidences for slow rotation, on the 400 MHz NMR time scale, about the Pt–N(3) bond of the thyminate ligands are presented. This finding appears to be the first example of a platinum–nucleobase complex in which the barrier to rotation is large enough to allow the detection of stable stereoisomers.

Experimental

The nucleobases 1-methylthymine, 1-methylcytosine and 1-methyluracil were obtained from Sigma. The complex *cis*-$(PMe_3)_2Pt(NO_3)_2$ was prepared as previously described [4]. The NMR spectra were obtained with a JEOL FX 90 Q and a Bruker AM400 spectrometer at 27 °C. Chemical shifts are given on the δ scale and referenced as follows: internal tetramethylsilane in DMSO-d_6 and the sodium salt of the 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid in D_2O, for the proton spectra; external H_3PO_4 (85% wt./wt.) for the phosphorus spectra. The IR spectra were obtained with a Perkin-Elmer 580 B.

Preparation of cis-[(PMe$_3$)$_2$Pt(1-MeCy)$_2$](NO$_3$)$_2$

A water solution of $AgNO_3$ (160 mg; 0.945 mmol) was added to a suspension of *cis*-$(PMe_3)_2PtCl_2$ (197 mg; 0.473 mmol) in 7 ml of H_2O and stirred at room temperature for 4 h. The precipitate of AgCl was separated by filtration and 1-MeCy (118 mg; 0.945 mmol) was added to the resulting solution. After 1 h the solvent was evaporated under vacuum and the glassy solid so formed was purified by dissolution in CH_3OH (3 ml) and left to concentrate at room temperature to *c.* 1 ml. The colorless microcrystalline product obtained was recovered by filtration and dried under vacuum (289 mg), yield 85%. *Anal.* Calc. for $C_{16}H_{32}N_8O_8P_2Pt$: C, 26.63; H, 4.47; N, 15.53. Found: C, 26.55; H, 4.43; N, 15.62%. IR: $\nu(NH_2)$ 3478, 3344 (strong); 3401 and 3253 (weak); ν(CO) 1655, 1614 (very strong); $\nu(NO_3^-)$ 979.8, 951.9 cm^{-1}. ^1H NMR (D_2O): (cytosine resonances) 7.73 (doublet (d), *J*(HH) 7.3 Hz, H(6)), 6.13 (doublet of doublets (dd), *J*(HH) 7.3 Hz, ^5J(PH) 0.7 Hz, ^4J(PH) *c.* 10 Hz, H(5)), 3.47 (singlet (s), CH_3); 1.69 (d, *J*(PH) 11.2 Hz, *J*(PtH) 36.4 Hz, PCH_3). ^1H NMR (DMSO-d_6): 8.81 (broad singlet, NH); 7.88 (d, *J*(HH) 7.1 Hz, H(6)); 7.71 (br, s, NH); 5.98 (d, *J*(HH) 7.1 Hz, *J*(PtH) *c.* 11 Hz, H(5); 3.31 (s, NCH_3), 1.59 (d, *J*(PH) 11 Hz, *J*(PtH) 35 Hz, PCH_3). $^{31}P\{^1H\}$ NMR in DMSO-d_6: δ −27.0 (s, *J*(PtP) 3269 Hz); in D_2O: δ −28.3 (s, *J*(PtP) 3251 Hz).

*Author to whom correspondence should be addressed.

0020-1693/92/$5.00

© 1992 – Elsevier Sequoia. All rights reserved

Preparation of cis-$(PMe_3)_2Pt(1\text{-}MeTy(-H))_2 \cdot H_2O$ and cis-$(PMe_3)_2Pt(1\text{-}MeU(-H))_2$

A suspension of *cis*-$(PMe_3)_2PtCl_2$ (315 mg; 0.755 mmol) and Ag_2O (175 mg; 0.755 mmol) in 10 ml of H_2O was stirred at room temperature for 20 min. 1-MeTy (212 mg, 1.51 mmol) was then added and the resulting mixture was stirred for 3 h, in the dark. AgCl was separated by filtration; the filtrate was rotary evaporated to give a white solid which was dried under high vacuum for 24 h. The crude product was purified by dissolution in ethanol and precipitation with diethyl ether. *Anal.* Calc. for $C_{18}H_{34}N_4O_5P_2Pt$: C, 33.60; H, 5.32; N, 8.70. Found: C, 33.61; H, 5.30; N, 8.59%. ^1H NMR (400 MHz, in D_2O at 27 °C): (thyminate resonances) 7.218 (s, H_6); 3.267 (s, NCH_3); 3.241 (s, NCH_3); 1.758 (s, CH_3); 1.730 (s, CH_3); (phosphine resonances) 1.644 (d, J(PH) 10.5 Hz, J(PtH) 34 Hz, PCH_3). ^1H NMR (at 90 MHz, in DMSO-d_6 at 27 °C): (thyminate resonances) 7.19 (quartet, J(HH) 0.86 Hz, H(6)); 3.10 (s, NCH_3); 1.63 (d, CH_3); (phosphine resonances) 1.54 (d, J(PH) 10.8 Hz, J(PtH) 33.7 Hz, PCH_3). ^{13}C NMR (DMSO-d_6): (thyminate resonances) 170.57 (C_4); 156.13 (C_2); 140.23 (C_6); 107.91 (C_5); 35.86 (NCH_3); 13.26 (CH_3); (phosphine resonances) 14.2 (complex multiplet). $^{31}P\{^1H\}$ NMR (162 MHz, at 27 °C): in D_2O δ −33.17 (s, J(PtP) 3358 Hz); in DMSO-d_6 δ −31.44 (s, J(PtP) 3318 Hz).

cis-$(PMe_3)_2Pt(1\text{-}MeU(-H))_2$ was prepared by the same procedure. *Anal.* Calc. for $C_{16}H_{28}N_4O_4P_2Pt$: C, 32.16; H, 4.72; N, 9.38. Found: C, 32.18; H, 4.96; N, 9.33%. ^1H NMR in D_2O at 27 °C: δ 7.383 (d, J(HH) 7.4 Hz, 1H, C(6)H), 5.58 (d, J(HH) *c.* 7 Hz, J(PtH) *c.* 10 Hz, 1H, C(5)H), 3.281 (s, 3H, NCH_3), 1.629 (d, J(PH) 11 Hz, J(PtH) 36 Hz, 9H, PCH_3). $^{31}P\{^1H\}$ NMR (162 MHz, at 27 °C): in D_2O δ −32.097 (s, J(PtP) 3347 Hz). IR: ν(CO) 1650 and 1575 (broad) cm^{-1}.

Results and discussion

Reactivity of cis-$(PMe_3)_2Pt(NO_3)_2$ with 1-methylcytosine

Addition of two equivalents of nucleobase to a water solution of *cis*-$(PMe_3)_2Pt(NO_3)_2$ leads to the immediate formation of the adduct *cis*-$[(PMe_3)_2Pt(1\text{-}MeCy)_2](NO_3)_2$. The reaction, followed by ^{31}P NMR spectroscopy, is evidenced by the quantitative replacement of the singlet due to the aquo complex (δ −25.25 ppm [4]) by a new one at higher field (δ −28.24), with a smaller ^{195}Pt–^{31}P coupling constant (J(PtP) 3251 Hz). The complex has been isolated as a microcrystalline solid and characterized by elemental analysis, IR and multinuclear NMR spectroscopies. In the ^1H NMR spectrum the H(5) and H(6) resonances of 1-MeCy are shifted downfield from the corresponding resonances of the uncoordinated base (0.26 ppm in D_2O and 0.35 ppm in DMSO-d_6). The H(5) proton appears coupled, in addition to H(6), to the platinum (^4J(PtH) = *c.* 10 Hz) and to the phosphorus of the *trans* ligand (^5J(PH) = *c.* 0.7 Hz). Moreover, the resonance of the NH_2 protons, in DMSO-d_6, occurs as two well separated singlets, both shifted downfield relative to the broad singlet typical of the free base. The coordination of the nucleobase to the metal center, therefore, results in the hindered rotation of the NH_2 group, a circumstance noted in the related amino derivative *cis*-$[(NH_3)_2Pt(1\text{-}MeCy)_2]^{2+}$ [6].

The intermediate *cis*-$[(PMe_3)_2Pt(H_2O)(1\text{-}MeCy)]^{2+}$ cannot be obtained free from *cis*-$[(PMe_3)_2Pt(1\text{-}MeCy)_2]^{2+}$ when just one equivalent of 1-MeCy is added to *cis*-$(PMe_3)_2Pt(NO_3)_2$, thus indicating a higher formation constant of the bis-adduct. The ^{31}P NMR spectrum of the monocytosine adduct in D_2O is characterized by an AB multiplet flanked by ^{195}Pt satellites, centered at δ −23.6 (J(PtP) 3251 Hz) and −29.0 (J(PtP) 3710 Hz), respectively, with J(PP) 24.4 Hz. The latter resonance, with the stronger Pt–P coupling effect, is attributable to the phosphine *trans* to the unsubstituted solvent molecule. The formation of a mononuclear solvent complex is in line with the monodentate behaviour of the neutral 1-methylcytosine ligand which is seen to use exclusively its N(3) donor atom in all its platinum complexes [7].

Similar results are obtained in the interaction of 1-MeCy with *cis*-$(PMe_3)_2Pt(NO_3)_2$ in DMSO. The $^{31}P\{^1H\}$ NMR parameters obtained in DMSO-d_6 (δ −21.9 (J(PtP) 3369 Hz), −28.7 (J(PtP) 3606 Hz), J(PP) 24.4 Hz) suggest the involvement of a solvent molecule as fourth ligand in *cis*-$[(PMe_3)_2Pt(S)(1\text{-}MeCy)]^{2+}$ (S = DMSO). Moreover, the remarkable increase of J(PtP) of the low field doublet attributable to the phosphorus *trans* to the nucleobase ligand, i.e. the *cis*-influence of S, can be rationalized by considering the bifunctional nature exhibited by the DMSO ligand toward the platinum(II) electrophiles [8].

Interaction of cis-$(PMe_3)_2Pt(NO_3)_2$ with 1-methylthymine and 1-methyluracil

Unlike the bis(phosphino)complex *cis*-$\{(dppf)Pt\}^{2+}$ (where dppf is 1,2-bis(diphenylphosphino)ferrocene), which exhibits rapid and extensive interaction with neutral 1-methylthymine in DMSO solution [1], the same base proves to be unreactive towards the cationic moiety *cis*-$\{(PMe_3)_2Pt\}^{2+}$ in water. In fact, the ^{31}P NMR spectrum of *cis*-$(PMe_3)_2Pt(NO_3)_2$ (0.1 M in D_2O) appears unchanged upon addition of 2 equiv. of 1-MeTy (saturated solution at 30 °C). As expected, however, addition of 2 equiv. of NaOH leads to the rapid dissolution of the nucleobase and the concomitant appearance of a singlet at −33.17 ppm flanked by ^{195}Pt satellites (J(PtP) = 3358 Hz) attributable to the dithy-

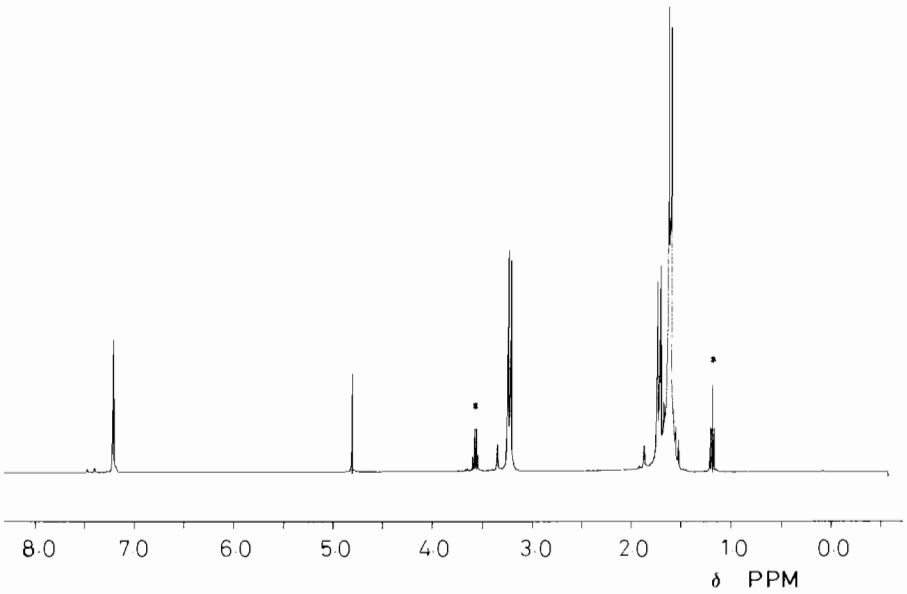

Fig. 1. 400 MHz ^{1}H NMR spectrum of *cis*-$(PMe_3)_2Pt(1\text{-}MeTy(-H))_2$ (*c.* 0.1 M in D_2O) at 298 K. The resonances indicated by * are due to entrapped crystallization solvent.

minate complex *cis*-$(PMe_3)_2Pt(1\text{-}MeTy(-H))_2$ containing the N(3)-deprotonated N(3)-bonded nucleobase. Since the fractional crystallization of the reaction mixture does not allow the separation of the by-product $NaNO_3$, pure *cis*-$(PMe_3)_2Pt(1\text{-}MeTy(-H))_2$, as well as *cis*-$(PMe_3)_2Pt(1\text{-}MeU(-H))_2$, are conveniently prepared by reacting *cis*-$(PMe_3)_2PtCl_2$ with Ag_2O and a following addition of the stoichiometric amount of the pertinent nucleobase. The solid obtained after AgCl filtration and solvent removal contains one mole of H_2O per mole of complex as indicated by its elemental analysis and as qualitatively confirmed by the IR spectrum. Crystallization of the crude product from EtOH/Et_2O affords colorless crystals which rapidly lose part of the entrapped solvent when removed from the mother liquor.

The interesting feature of the ^{1}H NMR spectrum of *cis*-$(PMe_3)_2Pt(1\text{-}MeTy(-H))_2$, obtained at 400 MHz in D_2O at 298 K, is the splitting of both the C(5)CH_3 and NCH_3 thyminate resonances into two singlets, in 1:1 intensity ratio (Fig. 1) which merge to single resonances at δ 3.21 and 1.74, respectively, at 333 K. This finding suggests that the barrier to rotation about the Pt–N(3) bond of the thyminato ligands at room temperature is large enough to allow the detection of the stereoisomers deriving from the two possible arrangements of the exocyclic groups on the pyrimidyl rings with respect to the metal coordination plane. By contrast, the uracil derivative *cis*-$(PMe_3)_2Pt(1\text{-}MeU(-H))_2$ exhibits a single set of resonances of the coordinated nucleobases. At 400 MHz, the ^{1}H NMR spectrum shows a sharp singlet at 3.281 ppm and a doublet at 7.383 ppm (J(H(5)H(6)) 7.4 Hz) for the NCH_3 and C(6)H protons, respectively, while C(5)H shows some broadening due to unresolved coupling of the phosphorous of the *trans* ligand. At 90 MHz the latter proton appears coupled with ^{195}Pt with ^4J(PtH) *c.* 10 Hz and the same occurs for the phosphine methyl protons which are seen as an apparent doublet (J(PH) 11 Hz) with ^3J(PtH) 36 Hz indicating that no dissociative mechanism of the ligands is involved.

The restricted rotation about the platinum–nitrogen bond of the pyrimidyl ring observed in *cis*-$(PMe_3)_2Pt(1\text{-}MeTy(-H))_2$ has a precedent in the bis(guanosine)-platinum(II) complex containing the relatively bulky *N,N,N',N'*-tetramethylethylenediamine ligand [9].

As noted in related bis(phosphino) derivatives [1] and in the analogous amino complexes [10], the binding of the deprotonated nucleobase to the metal centre determines a shift at higher field of all the proton resonances.

In the case of *cis*-$(PMe_3)_2Pt(1\text{-}MeTy(-H))_2$, this shift is evidenced by the weak resonances shown in Fig. 1. Some of these signals are attributable to free 1-MeTy formed by partial hydrolysis of the complex, thus suggesting that the stability of *cis*-$(PMe_3)_2Pt(1\text{-}MeTy(-H))_2$ and *cis*-$(PMe_3)_2Pt(1\text{-}MeU(-H))_2$ is appreciably lower than that exhibited by their amino analogs, under comparable conditions [10].

Acknowledgements

Parke-Davis is gratefully acknowledged for a research grant to G.T. We thank the Progetto Finalizzato Chimica Fine II, CNR, for partial financial support.

References

1 B. Longato, G. Pilloni, G. M. Bonora and B. Corain, *J. Chem. Soc., Chem. Commun.*, (1986) 1478.

2 V. Scarcia, A. Furlani, B. Longato, B. Corain and G. Pilloni, *Inorg. Chim. Acta, 153* (1988) 67.

3 (a) B. Longato, B. Corain, G. M. Bonora and G. Pilloni, *Inorg. Chim. Acta, 137* (1987) 75; (b) B. Longato, B. Corain, G. M. Bonora and G. Pilloni, in M. Nicolini (ed.), *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*, Martinus Nijhoff, Boston, MA, 1988, p. 705; (c) G. Bandoli, G. Trovó, A. Dolmella and B. Longato, *Inorg. Chem., 31* (1992) 45.

4 G. Trovó, G. Bandoli, U. Casellato, B. Corain, M. Nicolini and B. Longato, *Inorg. Chem., 29* (1990) 4616.

5 R. Faggiani, B. Lippert, C. J. L. Lock and R. A. Speranzini, *J. Am. Chem. Soc., 103* (1981) 1111.

6 (a) J. D. Orbell, L. G. Marzilli and T. J. Kistenmacher, *J. Am. Chem. Soc., 103* (1981) 5126; (b) R. Faggiani, B. Lippert and C. J. L. Lock, *Inorg. Chem., 21* (1982) 3210.

7 B. Lippert, *Prog. Inorg. Chem., 37* (1989) 1.

8 A. Davies, F. R. Hartley and S. G. Murray, *J. Chem. Soc., Dalton Trans.*, (1979) 1705.

9 R. E. Cramer and P. L. Dahlstrom, *J. Am. Chem. Soc., 101* (1979) 3679.

10 B. Lippert, *Gazz. Chim. Ital., 118* (1989) 153, and refs. therein.