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Novel platinum-based anticancer drug: a complete vibrational study

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The introduction of cisplatin to oncology, in the 1970s, marked the onset of the search for novel and improved metal-based anticancer drugs. Polynuclear Pt^{II} and Pd^{II} complexes with linear alkylamines as bridging ligands are a class of potential antineoplastic agents that have shown promising cytotoxicity against low-prognosis human cancers, such as metastatic breast adenocarcinoma and osteosarcoma. The present study reports an analysis of [μ -(*N,N'*-bis(3-aminopropyl)butane-1,4-diamine- $\kappa^4N,N''N'''$)]bis[dichloridoplatinum(II)], [Pt₂Cl₄(C₁₀H₂₆N₄)], denoted Pt₂Spm (Spm is spermine), by vibrational spectroscopy coupled to theoretical calculations. Within the latter, the Density Functional Theory (DFT – mPW1PW/6-31G*) and Effective Core Potential (ECP – LANL2DZ) approaches were used, in order to ensure the most accurate representation of the molecule and achieve a maximum agreement with the experimental data. The solid-state geometry of Pt₂Spm corresponds to C_i symmetry, displaying 132 vibrational modes. A complete assignment of the experimental vibrational profile of the system was attained through the combined application of complementary Raman, FT-IR and Inelastic Neutron Scattering (INS) techniques. INS allowed an unequivocal identification of the CH₂ and NH₂ rocking modes, not clearly detected by the optical techniques, while Raman measurements led to a clear discrimination of the Pt–N stretching frequencies from the two distinct Pt–N moieties within the chelate. The metal-to-metal distances calculated for the molecule under study were found to allow the establishment of effective inter- and intrastrand crosslinks with DNA. These results will hopefully help to clarify the mode of action of the compound, at the molecular level, contributing to the development of improved cisplatin-like chemotherapeutic drugs having a higher efficacy and specificity coupled to lower acquired resistance and deleterious side effects.

1. Introduction

Anticancer platinum-based agents have been the object of intense research since the introduction of cisplatin to oncology in the 1970s (Rosenberg *et al.*, 1965; Rosenberg *et al.*, 1969; Wang & Lippard, 2005). Actually, deleterious side effects of cisplatin (*e.g.* nephrotoxicity) and the occurrence of acquired resistance urge for the development of new and improved antineoplastic drugs, displaying a significant antitumour activity coupled to a high selectivity (low toxicity) and negligible resistance. Polynuclear Pt^{II} and Pd^{II} complexes with polyamines as bridging ligands are one such group of compounds, their cytotoxicity being mediated by covalent binding to DNA (their main pharmacological target), through a nonconventional mechanism responsible for an enhanced antitumour effect (Kelland & Farrell, 2000; Marques, Girão *et al.*, 2002; Teixeira *et al.*, 2004; Chvalova *et al.*, 2006; Fiuza *et al.*, 2006; Soares *et al.*, 2007; Mitchell *et al.*, 2007; Hegmans *et al.*, 2008; Tummala *et al.*, 2010; Fiuza *et al.*, 2011; Muchova *et al.*, 2012; Komeda, 2011; Silva *et al.*, 2013; Batista de Carvalho *et al.*, 2016). These types of compounds yield specific DNA adducts *via* long-distance intra- and interstrand crosslinks (Marques *et al.*, 2015), triggering an increased DNA damage relative to conventional drugs (such as cisplatin or carboplatin). Upon cellular uptake of the Pt/Pd complexes, drug activation takes place *via* hydrolysis of the leaving ligands (*e.g.* chloride) and deleterious DNA conformational rearrangements are triggered by binding to the purine and pyrimidine bases. The detailed pharmacokinetic and pharmacodynamic profiles of these compounds, however, are still scarcely known, and are recognised to be strongly dependent on the structural preferences of the drug under physiological conditions. Accordingly, these preferences should be determined accurately in order to predict cytotoxicity, thus allowing for optimization of anticancer activity. In the last decade, the authors have studied these types of third-generation Pt^{II} and Pd^{II} complexes with alkyl polyamines, having achieved very promising results, particularly towards human metastatic breast cancer (Fiuza *et al.*, 2011).

Coordination with amine ligands other than the biogenic tetramine spermine [*i.e.* $\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2$, Spm] has been investigated previously by our group (Silva *et al.*, 2012). The aim of this research is to provide a more thorough understanding, at a molecular level, of the behaviour of these kinds of polynuclear chelates and therefore of their cytotoxicity profile *via* interaction with DNA. The use of vibrational spectroscopy is a reliable and appropriate approach for these types of studies, mainly through the combination of optical [Fourier transform IR (FT-IR) and Raman] and neutron-based (inelastic neutron scattering, INS) techniques, as they allow an accurate assessment of the conformational preferences of metal–polyamine coordination compounds, under specific conditions (such as temperature and pH). Since these types of systems are highly hydrogenated, INS is particularly useful, yielding complementary information to that obtained from Raman and FT-IR, since it allows observation of some low-frequency modes unavailable to the optical techniques. The neutron scattering cross-section of an atom (σ) is characteristic of that atom and independent of its chemical environment, the value for hydrogen (80 barns) far exceeding that of all other elements (*ca* 5 barns). Hence, the INS spectrum is dominated by the vibrational modes involving a significant hydrogen displacement (u_i), the intensity from a powdered sample at energy ν_i being represented by:

$$S_i^*(Q, \nu_k) = \frac{(Q^2 u_i^2) \sigma}{3} \exp\left(-\frac{Q^2 \alpha_i^2}{3}\right), \quad (1)$$

where Q ($\text{\AA}^{-1}$) is the momentum transferred from the neutron to the sample and α_i ($\text{\AA}$) is associated with a weighted sum of all the displacements of the atom. Therefore, experimental data provide the energies of the vibrational transitions (the eigenvalues, ν_i), as well as the atomic displacements (the eigenvectors, u_i). In addition, the spectral intensities can be quantitatively compared with those calculated theoretically, allowing us to relate molecular geometry (calculated results) with the experimental spectroscopic features, which provides a consistent conformational picture of the system under study. The present work focuses on a full vibrational spectroscopic analysis of the polynuclear Pt^{II} chelate with spermine, namely $[\mu, -N, N'$ -bis(3-amino-propyl)butane-1,4-diamine- $\kappa^4 N, N'', N''', N''''$]bis[dichloridoplatinum(II)]–bis[dichloridoplatinum(II)], Pt_2Spm (Fig. 1) – in the light of quantum mechanical calculations using the density functional theory (DFT) approach and effective core potentials (ECP) for representing the metal. A complete spectral assignment of the complex was achieved, since several vibrational spectroscopic techniques were available for this study. The Pt^{II} complex with 1,3-diaminopropane [$\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}_2$, Dap; Batista de Carvalho *et al.*, 2012] was taken as a suitable model of the larger Pt_2Spm chelate (which contains identical moieties to PtDap).

2. Experimental

2.1. Synthesis

The synthesis of $\text{Pt}_2(\text{Spm})\text{Cl}_4$ was carried out based on a published procedure (Codina *et al.*, 1999), optimized by the authors for the homologous compound Pd_2Spm (Teixeira *et al.*, 2004; Fiuza *et al.*, 2011, 2015). Briefly, K_2PtCl_4 (2 mmol) was dissolved in a minimal amount of water and an aqueous solution containing spermine (1 mmol) was added dropwise under continuous stirring. The reaction was allowed to proceed for 24 h, after which time the resulting yellow powder was filtered off and washed with acetone. Recrystallization of this product from water afforded yellow–orange needle-shaped crystals (yield 60%). Elemental analysis found for $\text{C}_{10}\text{H}_{26}\text{Cl}_4\text{N}_4\text{Pt}_2$ (%): C 16.2, H 3.5, N 7.6, Cl 19.1; calculated: C 16.4, H 3.6, N 7.5, Cl 19.3.

Figure 1

Structural representation of the Pt_2Spm complex, showing (a) the solid-state conformer and (b) the minimum-energy conformer. The atom numbering is included.

Table 1
Experimental (X-ray) and calculated (mPW1PW) structural parameters for Pt₂Spm.

Structural parameter	Exp ^a	LANL2DZ/6-31G*	♦ ^b
Bond lengths (pm)			
Pt—N ₁	201.3	206.9	5.6
Pt—N ₂	204.7	208.3	3.6
Pt—Cl ₁	231.0	233.3	2.3
Pt—Cl ₂	231.2	233.5	2.3
N ₁ —C ₁	150.3	147.6	-2.7
N ₂ —C ₃	150.6	148.1	-2.5
C ₁ —C ₂	150.4	152.4	2.0
C ₂ —C ₃	149.9	152.7	2.8
N ₂ —C ₄	148.8	148.4	-0.7
C ₄ —C ₅	152.4	152.0	-0.4
C ₅ —C ₆	152.7	152.6	-0.1
Pt...Pt	789	817	28
Bond angles (°)			
Cl ₁ —Pt—Cl ₂	201.3	95.90	2.2
N ₁ —Pt—N ₂	204.7	92.89	1.9
N ₁ —Pt—Cl ₁	231.0	85.19	-3.2
N ₂ —Pt—Cl ₂	231.2	85.97	-1.0
Pt—N ₁ —C ₁	150.3	114.2	0.0
Pt—N ₂ —C ₃	150.6	111.7	-0.7
Pt—N ₂ —C ₄	150.4	115.3	1.9
N ₁ —C ₁ —C ₂	149.9	112.3	1.9
N ₂ —C ₃ —C ₂	148.8	114.3	0.0
C ₁ —C ₂ —C ₃	152.4	115.9	0.1
C ₃ —N ₂ —C ₄	152.7	113.0	-0.1
N ₂ —C ₄ —C ₅	201.3	112.4	0.1
C ₄ —C ₅ —C ₆	204.7	111.1	-0.6
Torsion angles (°)			
N ₂ —Pt ₁ —N ₁ —C ₁	-53.6	-48.5	5.1
N ₁ —Pt ₁ —N ₂ —C ₃	49.7	47.4	-2.3
Pt ₁ —N ₁ —C ₁ —C ₂	65.1	60.4	-4.7
Pt ₁ —N ₂ —C ₃ —C ₂	-60.3	-60.8	-0.5
Cl ₁ —Pt ₁ —N ₁ —C ₁	128.5	130.7	2.2
N ₁ —Pt ₁ —N ₂ —C ₄	-80.2	-83.4	-3.2
Cl ₂ —Pt ₁ —N ₂ —C ₄	103.3	99.6	-3.7
Cl ₂ —Pt ₁ —N ₂ —C ₃	-126.9	-129.6	-2.7
N ₁ —C ₁ —C ₂ —C ₃	-65.7	-65.8	-0.1
C ₁ —C ₂ —C ₃ —N ₂	65.1	67.6	2.5
C ₄ —N ₂ —C ₃ —C ₂	69.7	71.2	1.5
C ₃ —N ₂ —C ₄ —C ₅	178.1	169.6	-8.5
Pt ₁ —N ₂ —C ₄ —C ₅	-52.5	-60.2	-7.7
N ₂ —C ₄ —C ₅ —C ₆	-179.9	-179.8	0.1
♦♦ ^b			2.1

Notes: (a) Odoko & Okabe (2006); (b) ♦ = calculated value - experimental value and ♦♦ = overall mean percent difference (Amado *et al.*, 2007).

2.2. Quantum mechanical calculations

Quantum mechanical calculations were performed for $\text{Pt}_2(\text{Spm})\text{Cl}_4$ on *GAUSSIAN* (Frisch *et al.*, 2004) – both for geometry optimization and calculation of the harmonic vibrational frequencies. The DFT approach was used, at a level that was previously shown by the authors to be the best choice for describing this type of Pt^{II} –amine complex, since it presents the optimal compromise between accuracy and computational demands (Fiuza *et al.*, 2008; Amado *et al.*, 2007). The mPW1PW method, which comprises a modified version of the exchange term of Perdew–Wang and the Perdew–Wang 91 correlation functional (Adamo & Barone, 1998; Perdew *et al.*, 1996) was applied, along with the split-valence basis set 6-31G* (Harihara & Pople, 1973), which was used for all atoms except the metal. Pt^{II} was represented by the relativistic Effective Core Potentials of Hay & Wadt (1985) (*GAUSSIAN* keyword LANL2DZ), $n = 5$ and $n = 6$ being considered as valence electron shells. Molecular geometries were fully optimized by the Berny algorithm, using redundant internal coordinates (Peng *et al.*, 1996), *viz.* bond lengths to within *ca* 0.1 pm and the bond angles to within *ca* 0.1°. The final r.m.s. gradients were always less than 3×10^{-4} Hartree Bohr⁻¹ or Hartree radian⁻¹. No geometrical constraints were imposed on the molecule. The harmonic vibrational wave-numbers, as well as the Raman activities and IR intensities, were obtained at the same level of theory as the geometry optimization procedure.

Simulation of the INS spectra (both the theoretical transition intensities and the wavenumbers) was carried out with the dedicated *aCLIMAX* program (Ramirez-Cuesta, 2004).

2.3. Vibrational spectroscopy

The FT–IR spectra were recorded using a Bruker Optics Vertex 70 spectrometer, purged with CO₂-free dry air.

The mid-IR absorbance spectrum (400–4000 cm⁻¹) of the solid complex was recorded from KBr disks (*ca* 0.5% *w/w*) using a Ge-on-KBr substrate beamsplitter and a liquid-nitrogen-cooled wide-band Mercury Cadmium Telluride (MCT) detector.

The far-IR attenuated total reflection spectrum (FIR–ATR, 50–600 cm⁻¹) was measured using pure powder on the Bruker Platinum ATR single-reflection diamond accessory, using a silicon solid-state beamsplitter and a deuterated L-alanine-doped triglycine sulfate (DLATGS) detector with a polyethylene window. The spectrum was corrected for the frequency dependence of the penetration depth of the electric field in ATR, using the standard *Opus* software option.

In both cases, spectra were the sum of 64 scans, at 2 cm⁻¹ resolution, and the 3-term Blackman–Harris apodization function was applied. Under these conditions, the accuracy in wavenumbers was well below 1 cm⁻¹.

The Raman spectra were acquired at room temperature using a Bruker RFS-100 FT–Raman spectrometer, equipped with an InGaAs detector, with near-IR excitation (in order to overcome fluorescence) provided by the 1064 nm line of an Nd:YAG laser (Coherent, model Compass-1064/500N), yielding *ca* 150 mW at the sample position. A 180° geometry was employed, and each spectrum was the average of three repeated measurements of 50 scans each, the resolution being set at 2 cm⁻¹.

The INS spectrum of the complex was obtained in the TOSCA spectrometer (Parker *et al.*, 2014; ISIS, <http://www.isis.stfc.ac.uk/instruments/tosca/>) at the ISIS Neutron and Muon Source of the STFC Rutherford Appleton Laboratory (United Kingdom). This is an indirect geometry time-of-flight high-resolution [$(\Delta E/E)$ *ca* 1.25%] broad-range spectrometer. A crystalline sample of the synthesized complex (*ca* 1 g) was wrapped in an aluminium foil sachet and placed in a 0.2 cm thick (4 x 4 cm) flat aluminium can. To reduce the impact of the Debye–Waller factor [the exponential term in equation (1)] on the observed spectral intensity, the sample was cooled to cryogenic temperatures (*ca* 10 K).

3. Results and discussion

Table 1 comprises the LANL2DZ/6-31G*-optimized geometry for the solid-state conformation of Pt_2Spm (Fig. 1*a*), as well as the X-ray crystal structure parameters determined by Odoko & Okabe (2006) (CSD refcode UCOBEU). The differences between the experimental and calculated values (♦ values), and the corresponding overall error (♦♦ value), calculated as described previously for cisplatin (Amado *et al.*, 2007), are also shown. Generally speaking, the DFT structural parameters currently obtained are in good agreement with the experimental ones; the overall mean percent difference was determined as 2.1%. However, when only the bond lengths and angles are considered, this value becomes 0.9%, which constitutes a significant improvement relative to the values previously obtained for the homologous chelate with palladium, with several basis sets (Fiuza *et al.*, 2015). The presently calculated distance between the two metal centres within the Pt_2Spm chelate is 817 pm, in rather good agreement with the experimental value (789 pm).

Nevertheless, the minimum-energy conformer obtained for Pt_2Spm (Fig. 1*b*), displaying a Pt···Pt distance of 430 pm differs significantly from the reported X-ray value (789 pm; Fig. 1*a*). This allows us to conclude that despite the very high energy difference between these two conformations, *i.e.* 41.2 kJ mol⁻¹, the intermolecular interactions between

Figure 2
FT-IR, INS and Raman spectra ($50\text{--}1700\text{ cm}^{-1}$) for Pt_2Spm , showing the experimental (solid line) and calculated (dashed line).

Figure 3
FT-IR and Raman spectra ($2700\text{--}3400\text{ cm}^{-1}$) for Pt_2Spm , showing the experimental (solid line) and calculated (dashed line).

Table 2

Experimental (Raman, FT-IR and INS) and calculated (LANL2DZ/6-31G*) vibrational wavenumbers (cm^{-1}) for Pt_2Spm (isolated molecule).

Experimental			Calculated ^a	Sym. species	Approximate description ^b
Raman	FT-IR	INS			
3273			3261	A_g	$\nu_{\text{as}}\text{NH}_2$
	3267		3261	A_u	$\nu_{\text{as}}\text{NH}_2$
3203			3166	A_g	$\nu_s\text{NH}_2$
			3164	A_g	νNH
	3197		3166	A_u	$\nu_s\text{NH}_2$
			3164	A_u	νNH
	3133				$2 \times \nu_s(\text{NH}_2)$ FR $\nu_s(\text{NH}_2)$
			3001	A_u	$\nu_{\text{as}}\text{CH}_2$ (ring)
			3001	A_g	$\nu_{\text{as}}\text{CH}_2$ (ring)
	2960		2992	A_u	$\nu_{\text{as}}\text{CH}_2$ (chain)
			2990	A_u	$\nu_{\text{as}}\text{CH}_2$ (ring)
			2990	A_g	$\nu_{\text{as}}\text{CH}_2$ (ring)
			2982	A_g	$\nu_{\text{as}}\text{CH}_2$ (chain)
2941		2940	2971	A_g	$\nu_{\text{as}}\text{CH}_2$ (ring)
	2935		2969	A_u	$\nu_{\text{as}}\text{CH}_2$ (ring)
			2974	A_u	$\nu_{\text{as}}\text{CH}_2$
			2963	A_g	$\nu_{\text{as}}\text{CH}_2$ (chain)
	2880		2931	A_u	$\nu_s\text{CH}_2$ (chain)
			2930	A_u	$\nu_s\text{CH}_2$ (ring)
2885			2930	A_g	$\nu_s\text{CH}_2$ (ring)
			2925	A_g	$\nu_s\text{CH}_2$ (ring)
	2867		2925	A_u	$\nu_s\text{CH}_2$ (ring)
			2921	A_g	$\nu_s\text{CH}_2$ (chain)
	2855		2913	A_u	$\nu_s\text{CH}_2$ (ring)
			2913	A_g	$\nu_s\text{CH}_2$ (ring)
			2903	A_g	$\nu_s\text{CH}_2$ (chain)
			2903	A_u	$\nu_s\text{CH}_2$ (chain)
1582	1584	1597	1580	A_u	δNH_2
			1580	A_g	δNH_2
			1463	A_u	δCH_2 (chain)
			1457	A_g	δCH_2 (chain)
	1458		1454	A_u	δCH_2 (ring)
			1454	A_g	δCH_2 (ring)
1447	1446	1450	1441	A_u	δCH_2
			1439	A_g	δCH_2 (ring) + $\beta\text{N}^2\text{H}$
	1439		1438	A_u	δCH_2 + $\beta\text{N}^2\text{H}$
			1436	A_g	δCH_2 + $\beta\text{N}^2\text{H}$
1433	1436		1436	A_u	δCH_2 (ring)
			1435	A_u	$\beta\text{N}^2\text{H}$ + δCH_2
			1435	A_g	$\beta\text{N}^2\text{H}$ + δCH_2
			1435	A_g	δCH_2
	1396		1380	A_u	ωCH_2 (ring)
1387		1390	1383	A_g	ωCH_2
			1376	A_g	ωCH_2
	1385		1369	A_u	ωCH_2
1364		1368	1367	A_g	ωCH_2 + $\delta\text{C}^4\text{N}^2\text{H}$
	1355		1354	A_u	ωCH_2 + $\delta\text{C}^4\text{N}^2\text{H}$
			1337	A_g	ωCH_2 (ring)
	1323	1325	1336	A_u	ωCH_2 (ring)
1321			1311	A_g	tCH_2 (ring) + ωCH_2 (chain)
			1301	A_u	tCH_2
1286		1291	1295	A_g	tCH_2 (ring) + ωCH_2 (chain)
	1283		1292	A_u	tCH_2 (chain)
			1285	A_g	tCH_2 (chain)
			1265	A_g	tCH_2
			1265	A_u	tCH_2
	1243	1253	1259	A_u	tCH_2 (ring)
1237			1258	A_g	tCH_2 (ring) + tNH_2
			1234	A_g	tCH_2 (chain)
	1223	1231	1223	A_u	ωCH_2 (chain)
1194		1188	1188	A_g	ωNH_2
	1184		1186	A_u	ωNH_2
			1190	A_u	tCH_2 (chain) + tNH_2
1166		1167	1168	A_g	tNH_2 + ρCH_2 (chain)
	1125	1132	1128	A_u	$\delta\text{PtN}^1\text{H}^1$
1125			1121	A_g	$\delta\text{PtN}^1\text{H}^1 + \delta\text{PtN}^2\text{H}$
1114			1107	A_g	$\nu_s\text{N}^2\text{C}^3\text{C}^2 + \omega\text{NH}_2$
	1092		1104	A_u	$\delta\text{PtN}^2\text{H} + \nu_s\text{N}^2\text{C}^3\text{C}^2$

Table 2 (continued)

Experimental			Calculated ^a	Sym. species	Approximate description ^b
Raman	FT-IR	INS			
1071	1065	1071	1068	A_u	$\delta\text{PtN}^2\text{H} + \text{tCH}_2$ (chain)
			1056	A_g	$\nu_s\text{N}^1\text{C}^3\text{C}^2 + \delta\text{PtN}^2\text{H}$
	1051	1050	1054	A_u	$\nu_s\text{N}^1\text{C}^3\text{C}^2 + \delta\text{PtN}^2\text{H}$
1048			1051	A_g	$\nu\text{N}^1\text{C}^1 + \nu\text{N}^2\text{C}^3 + \nu\text{C}^4\text{C}^5$
			1049	A_g	$\delta\text{PtN}^2\text{H}$
			1042	A_g	$\nu\text{C}^5\text{C}^6 + \nu\text{N}^2\text{C}^4$
			1034	A_u	$\delta\text{PtN}^1\text{H}^2$
1012			1023	A_g	$\delta\text{PtN}^1\text{H}^2$
		1004	1021	A_u	$\nu\text{C}^4\text{C}^5 + \text{tNH}_2$
958			964	A_g	$\nu\text{N}^2\text{C}^3 + \nu\text{C}^4\text{C}^5 + \text{tNH}_2$
	960		949	A_u	$\nu\text{N}^2\text{C}^3 + \text{tNH}_2$
	935	937	928	A_u	$\nu\text{N}^1\text{C}^1 + \nu\text{N}^2\text{C}^3 + \rho\text{CH}_2$
929			920	A_g	$\nu\text{N}^1\text{C}^1 + \nu\text{N}^2\text{C}^3 + \rho\text{CH}_2$
	920	917	912	A_u	$\rho\text{CH}_2 + \text{tCH}_2 \delta\text{PtNH}$
886		873	901	A_g	ρCH_2 (ring) + δPtNH
	880		892	A_u	$\rho\text{CH}_2 + \text{tCH}_2$
857			857	A_g	ρCH_2 (ring)
	866		853	A_u	ρCH_2 (ring)
	794	800	789	A_u	$\nu_s\text{C}^1\text{C}^2\text{C}^3$
810		818	787	A_g	$\nu_s\text{C}^1\text{C}^2\text{C}^3$
755		765	775	A_g	ρCH_2 (chain)
	742	748	756	A_u	ρNH_2
737			755	A_g	ρNH_2
			710	A_u	ρCH_2 (chain)
	604/566		595	A_u	$\delta\text{N}^2\text{C}^3\text{C}^2$
614/592		593	587	A_g	$\delta\text{N}^2\text{C}^3\text{C}^2$
531		526	530	A_g	$\delta\text{C}^3\text{N}^2\text{C}^4 + \delta\text{C}^4\text{C}^5\text{C}^6 + \nu\text{PtN}^2$
			509	A_u	$\delta\text{N}^1\text{C}^1\text{C}^2 + \nu\text{PtN}^1$
			508	A_g	$\delta\text{N}^1\text{C}^1\text{C}^2 + \nu\text{PtN}^1$
		464	496	A_u	$\nu_s\text{NPtN} + \delta\text{C}^3\text{N}^2\text{C}^4\text{C}^5$
471	467		482	A_u	$\nu_s\text{NPtN} + \delta\text{C}^1\text{C}^2\text{C}^3$
			464	A_g	$\nu_s\text{NPtN} + \delta\text{C}^1\text{C}^2\text{C}^3$
			437	A_u	$\delta\text{C}^1\text{C}^2\text{C}^3 + \delta\text{C}^4\text{C}^5\text{C}^6$
375	—		379	A_g	$\delta\text{C}^4\text{C}^5\text{C}^6 + \gamma$ ring
	—	365	360	A_u	γ' ring
—			354	A_g	γ ring
			341	A_u	$\nu_s\text{Cl} - \text{Pt} - \text{Cl}$
327	—		340	A_u	$\nu_s\text{Cl} - \text{Pt} - \text{Cl}$
			332	A_g	$\nu_{\text{as}}\text{Cl} - \text{Pt} - \text{Cl}$
			332	A_u	$\nu_{\text{as}}\text{Cl} - \text{Pt} - \text{Cl}$
	321		312	A_g	$\delta\text{N}^2\text{C}^4\text{C}^5$
	—	300	293	A_u	γ' ring
244	—	244	247	A_g	δNPtN
	240		231	A_u	δNPtN
	—		226	A_g	γ ring
	—		218	A_u	γ (N—Pt—N)
	—		191	A_u	γ (Pt—N ¹)
161	—		162	A_g	γ (Pt—N ¹)
	—		155	A_g	$\gamma\text{C} - \text{C} - \text{C}_{\text{chain}}$
	—		154	A_u	$\gamma\text{C} - \text{C} - \text{C}_{\text{chain}}$
	—		147	A_g	$\gamma\text{C} - \text{C} - \text{C}_{\text{chain}}$
	151	145	145	A_u	$\gamma\text{C} - \text{C} - \text{C}_{\text{chain}}$
	—		144	A_u	$\delta\text{Cl} - \text{Pt} - \text{Cl}$
	—		142	A_g	$\delta\text{Cl} - \text{Pt} - \text{Cl}$

Notes: (a) scaled according to: 0.9499 for the bands above 700 cm^{-1} (Merrick *et al.*, 2007), 0.920 for $\nu(\text{NH}_2)$, 0.933 for $\delta(\text{NH}_2)$ and 0.986 for $\omega(\text{NH}_2)$ (Batista de Carvalho *et al.*, 2012). (b) ν = stretching, δ/β = in-plane deformation, ρ = rocking, t = twisting and γ/γ' = in-phase and out-of-phase out-of-plane deformation; s, as, and a refer to symmetric, antisymmetric and asymmetric modes, respectively; FR = Fermi resonance. The atom numbering is according to Fig. 1.

neighbouring molecules in the crystal lattice play a decisive role on the solid-state conformational preferences of the chelate, the larger metal-to-metal distance being favoured in the condensed state. Moreover, the two calculated Pt...Pt values (817 and 430 pm) correspond to the longest and shortest possible distances between the metal centres in the molecule. Therefore, several stable conformations can be found between these limits, each of them with a different metal-metal distance (corresponding to distinct conformations of the diamine bridge ligand) (Fig. 1).

Considering that (i) >75% hydrated DNA (B-DNA native conformation) is the main target of this kind of metal-based anticancer agents, (ii) the distance between two neighbouring bases that are perpendicular to the B-DNA main axis is *ca* 340 pm (base packing arrangement) and (iii) the average B-DNA double helix minor and major groove widths are 600 and 1160 pm, respectively, one can conclude that the possible conformers of the dinuclear platinum(II) chelate under study span the needed range of distances in order to establish either inter- or intrastrand crosslinks through DNA bases (preferably at the N7 atom of purines) (Lopes *et al.*, 2012, 2013; Yue *et al.*, 2013).

The solid-state geometry of Pt₂Spm (Fig. 1) corresponds to *C_i* symmetry, giving rise to 132 vibrational modes – 66 Raman active (with *A_g* symmetry) and 66 IR active (with *A_u* symmetry). While all the modes are IR or Raman allowed, this does not mean that all the modes are observable, as an allowed mode may have near zero intensity. INS provides additional information, particularly in the low-energy region of Pt₂Spm.

The FT-IR, Raman and INS vibrational spectra measured for this compound are shown in Figs. 2 and 3, allowing access to all the vibrational modes of the molecule and a thorough assignment of its vibrational profile. Special attention was paid to the features that evidenced noteworthy changes upon complexation; the characteristic Cl–Pt–Cl and N–Pt–N deformation and stretching bands, in particular, allowed the metal coordination to be probed (Fig. 2). In fact, when comparing the spectrum of the free ligand (Spm; Marques, Batista de Carvalho *et al.*, 2002; Amorin da Costa *et al.*, 2003; Batista de Carvalho *et al.*, 2006) to the vibrational pattern for its dinuclear Pt^{II} complex, this complexation is clear, the bands from the amine moieties being the most affected.

Table 2 collects the experimental and calculated vibrational wavenumbers obtained for Pt₂Spm. When comparing with the smaller model complex PtDap, the N–Pt–N deformation is observed at 244 cm⁻¹ for the Spm complex and at *ca* 246 cm⁻¹ for PtDap (Batista de Carvalho *et al.*, 2012). Regarding the Pt–Cl stretching mode, it occurs in the Raman at 327 cm⁻¹ for Pt₂Spm and at 323 cm⁻¹ for cisplatin (for which it is also detected by INS at ~330 cm⁻¹; Batista de Carvalho *et al.*, 2011). The Pt–N stretching at 531 (Raman) and 526 cm⁻¹ (INS) was also identified for cisplatin (at 524/521 cm⁻¹) and PtDap (at 532/535 cm⁻¹) (Batista de Carvalho *et al.*, 2012). This band also has contributions from skeletal deformations (Table 2), being slightly blue shifted relative to the free ligand. In the complex, these modes are degenerate.

The CH₂ stretching modes of the intramolecular ring moiety within the Pt₂Spm chelate (Fig. 1), at 2885 and 2941 cm⁻¹ in the Raman spectrum, were blue shifted relative to free spermine (Amorin da Costa *et al.*, 2003), which is a clear proof of metal coordination. A similar behaviour had been observed previously for PtDap (Batista de Carvalho *et al.*, 2012).

The INS spectrum of the Pt^{II}–spermine chelate allowed an unequivocal identification of both CH₂ and NH₂ rocking modes (not clearly detected by the optical techniques), as well as of the torsion bands from the intramolecular ring structures formed upon complexation (Fig. 1). These have also been observed in PtDap, which also contains this moiety.

Previous conformational studies on biogenic polyamines carried out by the authors (Marques, Batista de Carvalho *et al.*, 2002; Batista de Carvalho *et al.*, 2006) demonstrated that INS is particularly suited for detecting low-frequency vibrational modes in these highly hydrogenated molecules, namely their characteristic transverse and longitudinal acoustic modes (TAMs and LAMs, respectively). In the Pt₂Spm chelate, however, since the two metal centres are bridged by the tetraamine ligand (Fig. 1), the low-frequency vibrations of the chain are not real TAM and LAM modes, as the polyamine is anchored by coordination to the two Pt^{II} ions and therefore loses flexibility.

Interestingly enough, the Raman data reported here allowed the discrimination of the distinct Pt–N stretching frequencies due to the different environments in the Pt–N² and Pt–N¹ moieties within the chelate (Fig. 1). Actually, these modes are nondegenerate, yielding more than the two bands assigned to the symmetric and antisymmetric stretching modes, which would be the only ones detected if the two metal–nitrogen bonds at each metal centre were symmetry-equivalent.

4. Conclusions

Overall, the analysis reported here for the dinuclear Pt^{II} complex, with a biogenic polyamine, which has been shown by the authors to act as a promising anticancer agent towards human metastatic breast cancer, allows the determination of its main conformational preferences. These are confirmed by the very good agreement between the experimental vibrational data and the calculated spectra for the conformer present in the solid.

Overall, the results presently attained clearly evidence that the drug–DNA adducts responsible for cytotoxicity strongly depend on the conformation of the metal complex, particularly on the metal-to-metal distance. Indeed, this structural parameter determines the effectiveness of the drug interaction with its target, and the consequent damage caused in the

native structure of the nucleic acid. Moreover, the flexibility of the bridging amine linkers within the Pt₂Spm chelate constitute an advantage for an effective interaction with DNA, both prompting a pre-association mechanism with the double helix and favouring a suitable adjustment between drug and target aiming at a maximum number of stable Pt–N7_{bases} covalent bonds.

For Pt₂Spm, the calculated Pt···Pt distances span a suitable range of values for establishing effective inter- and intrastrand crosslinks with DNA.

The full conformational characterization of these types of Pt^{II}–polyamine complexes will help to clarify their pharmacodynamics (interaction with their main pharmacological target, DNA), as well as their mechanism of action within a biological matrix (*e.g.* a cell, a tissue or even a living organism). This will hopefully expose the molecular basis of their cytotoxicity, thus contributing for a tailored design of new and more efficient cisplatin-like anticancer drugs.

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supporting information

Novel platinum-based anticancer drug: a complete vibrational study

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Computing details

Data collection: ?; cell refinement: ?; data reduction: ?; program(s) used to solve structure: ?; program(s) used to refine structure: ?; molecular graphics: ?; software used to prepare material for publication: ?.

(4a*R*,4b*R*,7*R*,10a*S*)-4b,7-Dihydroxy-7-isopropyl-1,1-dimethyl-9-oxododecahydrobenzo[*a*]azulene-4a(2*H*)-carboxylic acid

Crystal data

C₂₀H₃₂O₅

M_r = 352.45

Orthorhombic, *P*2₁2₁2₁

a = 6.4641 (2) Å

b = 11.6518 (4) Å

c = 25.4249 (9) Å

V = 1914.96 (11) Å³

Z = 4

F(000) = 768

D_x = 1.223 Mg m⁻³

Cu *Kα* radiation, λ = 1.54178 Å

Cell parameters from 3751 reflections

θ = 5.6–48.6°

μ = 0.70 mm⁻¹

T = 170 K

Block, colourless

0.28 × 0.15 × 0.12 mm

Data collection

Bruker APEXII CCD

diffractometer

φ and ω scan

Absorption correction: multi-scan

*T*_{min} = 0.722, *T*_{max} = 0.786

24714 measured reflections

3648 independent reflections

3630 reflections with *I* > 2σ(*I*)

*R*_{int} = 0.032

θ_{max} = 71.9°, θ_{min} = 4.2°

h = -7→7

k = -13→12

l = -29→30

Refinement

Refinement on *F*²

Least-squares matrix: full

R [*F*² > 2σ(*F*²)] = 0.028

wR (*F*²) = 0.074

S = 1.05

H atoms treated by a mixture of independent and constrained refinement

w = 1/[σ²(*F_o*)² + (0.0418*P*)² + 0.3285*P*]

where *P* = (*F_o*² + 2*F*²)/3
(Δ/σ)_{max} = 0.001

3648 reflections

242 parameters

0 restraints

Hydrogen site location: mixed

Δρ_{max} = 0.23 e Å⁻³

Δρ_{min} = -0.14 e Å⁻³

Absolute structure: Flack *x* determined using
1495 quotients [(*I*⁺)-(*I*⁻)]/[(*I*⁺)+(*I*⁻)]

Absolute structure parameter: 0.04 (3)

Special details

Geometry. All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ($\text{\AA}^2$)

	<i>x</i>	<i>y</i>	<i>z</i>	$U_{\text{iso}}^*/U_{\text{eq}}$
O2	0.94217 (16)	0.28421 (10)	0.28036 (4)	0.0226 (2)
H2	0.963 (4)	0.214 (2)	0.2739 (9)	0.042 (6)*
O3	0.30306 (18)	0.35567 (9)	0.31740 (5)	0.0273 (3)
H3	0.179 (4)	0.335 (2)	0.3103 (9)	0.045 (6)*
O5	0.55585 (18)	0.42514 (10)	0.16027 (5)	0.0284 (3)
H5	0.576 (4)	0.494 (2)	0.1522 (9)	0.048 (7)*
O4	0.31887 (18)	0.17124 (9)	0.33942 (5)	0.0302 (3)
O1	0.9565 (2)	0.57373 (10)	0.25697 (5)	0.0359 (3)
C8	0.8617 (2)	0.49022 (13)	0.24168 (6)	0.0226 (3)
C13	0.7537 (2)	0.36813 (13)	0.15861 (6)	0.0239 (3)
C20	0.4053 (2)	0.26256 (13)	0.33269 (6)	0.0222 (3)
C5	0.7133 (2)	0.38684 (13)	0.36756 (6)	0.0233 (3)
H5A	0.8674	0.3794	0.3681	0.028*
C10	0.6389 (2)	0.27869 (12)	0.33756 (6)	0.0207 (3)
C1	0.7326 (3)	0.17358 (13)	0.36456 (6)	0.0251 (3)
H1A	0.8853	0.1764	0.3617	0.030*
H1B	0.6839	0.1029	0.3468	0.030*
C14	0.9149 (2)	0.44040 (13)	0.18854 (6)	0.0241 (3)
H14A	1.0396	0.3921	0.1931	0.029*
H14B	0.9547	0.5051	0.1654	0.029*
C7	0.6936 (2)	0.43776 (12)	0.27520 (6)	0.0208 (3)
H7	0.5592	0.4508	0.2567	0.025*
C6	0.6729 (3)	0.48890 (13)	0.33102 (6)	0.0247 (3)
H6A	0.7760	0.5504	0.3368	0.030*
H6B	0.5326	0.5206	0.3367	0.030*
C9	0.7232 (2)	0.30567 (12)	0.28054 (6)	0.0195 (3)
C11	0.6197 (2)	0.23782 (13)	0.23618 (6)	0.0226 (3)
H11A	0.4744	0.2638	0.2331	0.027*
H11B	0.6171	0.1558	0.2463	0.027*
C12	0.7222 (3)	0.24793 (13)	0.18195 (6)	0.0250 (3)
H12A	0.6380	0.2031	0.1567	0.030*
H12B	0.8594	0.2105	0.1841	0.030*
C4	0.6520 (3)	0.39262 (15)	0.42607 (6)	0.0302 (4)
C2	0.6694 (3)	0.17070 (15)	0.42285 (7)	0.0319 (4)
H2A	0.5176	0.1608	0.4254	0.038*
H2B	0.7357	0.1041	0.4402	0.038*
C15	0.8242 (3)	0.36049 (15)	0.10016 (6)	0.0325 (4)
H15	0.8295	0.4411	0.0868	0.039*
C3	0.7326 (3)	0.28090 (16)	0.45154 (6)	0.0349 (4)

H3A	0.8855	0.2844	0.4531	0.042*
H3B	0.6808	0.2770	0.4881	0.042*
C18	0.4185 (3)	0.40647 (16)	0.43549 (7)	0.0373 (4)
H18A	0.3671	0.4721	0.4153	0.056*
H18B	0.3930	0.4195	0.4730	0.056*
H18C	0.3466	0.3367	0.4242	0.056*
C19	0.7637 (4)	0.49480 (18)	0.45147 (7)	0.0443 (5)
H19A	0.9117	0.4909	0.4433	0.066*
H19B	0.7445	0.4922	0.4897	0.066*
H19C	0.7064	0.5666	0.4377	0.066*
C17	0.6691 (4)	0.29680 (18)	0.06540 (7)	0.0468 (5)
H17A	0.6767	0.2143	0.0727	0.070*
H17B	0.7020	0.3107	0.0283	0.070*
H17C	0.5290	0.3246	0.0729	0.070*
C16	1.0403 (4)	0.3110 (2)	0.09255 (8)	0.0511 (6)
H16A	1.1410	0.3585	0.1114	0.077*
H16B	1.0744	0.3102	0.0550	0.077*
H16C	1.0445	0.2325	0.1063	0.077*

Atomic displacement parameters (Å²)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
O2	0.0176 (5)	0.0180 (6)	0.0320 (5)	0.0018 (4)	−0.0007 (4)	0.0001 (4)
O3	0.0180 (6)	0.0220 (6)	0.0420 (6)	0.0006 (4)	−0.0023 (5)	0.0025 (4)
O5	0.0272 (6)	0.0225 (6)	0.0354 (6)	0.0028 (5)	−0.0040 (5)	0.0033 (5)
O4	0.0235 (6)	0.0219 (6)	0.0453 (7)	−0.0047 (5)	−0.0011 (5)	0.0043 (5)
O1	0.0467 (7)	0.0299 (6)	0.0311 (6)	−0.0201 (6)	−0.0010 (5)	0.0007 (5)
C8	0.0219 (7)	0.0176 (7)	0.0284 (7)	−0.0004 (6)	−0.0055 (6)	0.0037 (6)
C13	0.0258 (7)	0.0194 (7)	0.0266 (7)	0.0009 (6)	−0.0009 (6)	0.0007 (6)
C20	0.0216 (7)	0.0188 (7)	0.0261 (7)	0.0002 (6)	0.0006 (6)	−0.0008 (6)
C5	0.0209 (7)	0.0216 (7)	0.0276 (8)	−0.0022 (6)	−0.0007 (6)	−0.0020 (6)
C10	0.0194 (7)	0.0166 (7)	0.0262 (7)	−0.0006 (5)	−0.0014 (6)	0.0012 (6)
C1	0.0236 (8)	0.0219 (8)	0.0298 (8)	0.0013 (6)	−0.0019 (6)	0.0038 (6)
C14	0.0241 (7)	0.0192 (7)	0.0290 (7)	−0.0013 (6)	0.0013 (6)	0.0025 (6)
C7	0.0193 (7)	0.0155 (7)	0.0275 (7)	0.0004 (6)	−0.0011 (6)	0.0000 (5)
C6	0.0267 (8)	0.0175 (7)	0.0298 (8)	−0.0018 (6)	0.0025 (6)	−0.0028 (6)
C9	0.0166 (7)	0.0156 (7)	0.0262 (7)	0.0003 (5)	−0.0009 (6)	0.0004 (5)
C11	0.0241 (7)	0.0167 (7)	0.0272 (7)	−0.0020 (6)	−0.0023 (6)	0.0004 (6)
C12	0.0318 (8)	0.0177 (7)	0.0254 (7)	−0.0007 (6)	−0.0031 (6)	−0.0004 (6)
C4	0.0359 (9)	0.0282 (8)	0.0265 (8)	−0.0031 (7)	0.0012 (7)	−0.0015 (6)
C2	0.0362 (9)	0.0284 (9)	0.0311 (8)	0.0010 (7)	−0.0016 (7)	0.0093 (6)
C15	0.0435 (10)	0.0259 (8)	0.0281 (8)	−0.0022 (8)	0.0025 (7)	0.0021 (6)
C3	0.0395 (10)	0.0385 (10)	0.0268 (8)	−0.0007 (8)	−0.0034 (7)	0.0033 (7)
C18	0.0434 (10)	0.0351 (10)	0.0334 (9)	0.0008 (8)	0.0121 (8)	−0.0010 (7)
C19	0.0610 (13)	0.0410 (10)	0.0309 (9)	−0.0094 (10)	0.0001 (9)	−0.0095 (8)
C17	0.0684 (14)	0.0457 (11)	0.0262 (8)	−0.0134 (10)	−0.0033 (9)	−0.0011 (8)
C16	0.0549 (13)	0.0580 (13)	0.0403 (11)	0.0074 (11)	0.0172 (10)	−0.0049 (10)

Geometric parameters (Å, °)

O2—H2	0.84 (3)	C9—C11	1.531 (2)
O2—C9	1.4377 (18)	C11—H11A	0.9900
O3—H3	0.86 (3)	C11—H11B	0.9900
O3—C20	1.3285 (19)	C11—C12	1.534 (2)
O5—H5	0.84 (3)	C12—H12A	0.9900
O5—C13	1.4417 (19)	C12—H12B	0.9900
O4—C20	1.2138 (19)	C4—C3	1.544 (2)
O1—C8	1.214 (2)	C4—C18	1.537 (3)
C8—C14	1.510 (2)	C4—C19	1.535 (3)
C8—C7	1.510 (2)	C2—H2A	0.9900
C13—C14	1.541 (2)	C2—H2B	0.9900
C13—C12	1.535 (2)	C2—C3	1.532 (3)
C13—C15	1.557 (2)	C15—H15	1.0000
C20—C10	1.527 (2)	C15—C17	1.529 (3)
C5—H5A	1.0000	C15—C16	1.523 (3)
C5—C10	1.549 (2)	C3—H3A	0.9900
C5—C6	1.531 (2)	C3—H3B	0.9900
C5—C4	1.541 (2)	C18—H18A	0.9800
C10—C1	1.529 (2)	C18—H18B	0.9800
C10—C9	1.580 (2)	C18—H18C	0.9800
C1—H1A	0.9900	C19—H19A	0.9800
C1—H1B	0.9900	C19—H19B	0.9800
C1—C2	1.538 (2)	C19—H19C	0.9800
C14—H14A	0.9900	C17—H17A	0.9800
C14—H14B	0.9900	C17—H17B	0.9800
C7—H7	1.0000	C17—H17C	0.9800
C7—C6	1.545 (2)	C16—H16A	0.9800
C7—C9	1.557 (2)	C16—H16B	0.9800
C6—H6A	0.9900	C16—H16C	0.9800
C6—H6B	0.9900		
C9—O2—H2	109.0 (17)	C9—C11—C12	115.71 (13)
C20—O3—H3	107.7 (16)	H11A—C11—H11B	107.4
C13—O5—H5	107.4 (18)	C12—C11—H11A	108.4
O1—C8—C14	118.68 (15)	C12—C11—H11B	108.4
O1—C8—C7	120.45 (14)	C13—C12—H12A	107.7
C14—C8—C7	120.88 (13)	C13—C12—H12B	107.7
O5—C13—C14	109.49 (12)	C11—C12—C13	118.36 (13)
O5—C13—C12	106.94 (13)	C11—C12—H12A	107.7
O5—C13—C15	108.30 (13)	C11—C12—H12B	107.7
C14—C13—C15	107.74 (13)	H12A—C12—H12B	107.1
C12—C13—C14	113.42 (13)	C5—C4—C3	106.34 (14)
C12—C13—C15	110.84 (13)	C18—C4—C5	114.05 (14)
O3—C20—C10	114.53 (13)	C18—C4—C3	110.75 (15)
O4—C20—O3	121.89 (14)	C19—C4—C5	108.61 (14)
O4—C20—C10	123.48 (14)	C19—C4—C3	108.59 (15)

C10—C5—H5A	104.2	C19—C4—C18	108.36 (16)
C6—C5—H5A	104.2	C1—C2—H2A	109.3
C6—C5—C10	106.26 (12)	C1—C2—H2B	109.3
C6—C5—C4	120.52 (13)	H2A—C2—H2B	107.9
C4—C5—H5A	104.2	C3—C2—C1	111.69 (14)
C4—C5—C10	115.53 (13)	C3—C2—H2A	109.3
C20—C10—C5	116.55 (12)	C3—C2—H2B	109.3
C20—C10—C1	109.24 (12)	C13—C15—H15	106.3
C20—C10—C9	106.91 (12)	C17—C15—C13	112.79 (15)
C5—C10—C9	100.54 (11)	C17—C15—H15	106.3
C1—C10—C5	107.90 (12)	C16—C15—C13	114.29 (15)
C1—C10—C9	115.77 (12)	C16—C15—H15	106.3
C10—C1—H1A	109.6	C16—C15—C17	110.15 (17)
C10—C1—H1B	109.6	C4—C3—H3A	108.6
C10—C1—C2	110.18 (13)	C4—C3—H3B	108.6
H1A—C1—H1B	108.1	C2—C3—C4	114.65 (14)
C2—C1—H1A	109.6	C2—C3—H3A	108.6
C2—C1—H1B	109.6	C2—C3—H3B	108.6
C8—C14—C13	119.87 (13)	H3A—C3—H3B	107.6
C8—C14—H14A	107.4	C4—C18—H18A	109.5
C8—C14—H14B	107.4	C4—C18—H18B	109.5
C13—C14—H14A	107.4	C4—C18—H18C	109.5
C13—C14—H14B	107.4	H18A—C18—H18B	109.5
H14A—C14—H14B	106.9	H18A—C18—H18C	109.5
C8—C7—H7	107.3	H18B—C18—H18C	109.5
C8—C7—C6	115.14 (13)	C4—C19—H19A	109.5
C8—C7—C9	111.16 (12)	C4—C19—H19B	109.5
C6—C7—H7	107.3	C4—C19—H19C	109.5
C6—C7—C9	108.17 (12)	H19A—C19—H19B	109.5
C9—C7—H7	107.3	H19A—C19—H19C	109.5
C5—C6—C7	104.06 (12)	H19B—C19—H19C	109.5
C5—C6—H6A	110.9	C15—C17—H17A	109.5
C5—C6—H6B	110.9	C15—C17—H17B	109.5
C7—C6—H6A	110.9	C15—C17—H17C	109.5
C7—C6—H6B	110.9	H17A—C17—H17B	109.5
H6A—C6—H6B	109.0	H17A—C17—H17C	109.5
O2—C9—C10	107.92 (12)	H17B—C17—H17C	109.5
O2—C9—C7	107.00 (12)	C15—C16—H16A	109.5
O2—C9—C11	109.75 (12)	C15—C16—H16B	109.5
C7—C9—C10	103.55 (11)	C15—C16—H16C	109.5
C11—C9—C10	115.00 (12)	H16A—C16—H16B	109.5
C11—C9—C7	113.13 (12)	H16A—C16—H16C	109.5
C9—C11—H11A	108.4	H16B—C16—H16C	109.5
C9—C11—H11B	108.4		
O2—C9—C11—C12	−48.56 (17)	C1—C10—C9—C7	−152.65 (13)
O3—C20—C10—C5	47.36 (18)	C1—C10—C9—C11	83.42 (16)
O3—C20—C10—C1	169.94 (12)	C1—C2—C3—C4	54.8 (2)

O3—C20—C10—C9	−64.09 (15)	C14—C8—C7—C6	173.36 (13)
O5—C13—C14—C8	45.65 (18)	C14—C8—C7—C9	49.92 (18)
O5—C13—C12—C11	−52.52 (18)	C14—C13—C12—C11	68.28 (18)
O5—C13—C15—C17	−58.06 (18)	C14—C13—C15—C17	−176.41 (15)
O5—C13—C15—C16	175.10 (15)	C14—C13—C15—C16	56.76 (19)
O4—C20—C10—C5	−136.33 (15)	C7—C8—C14—C13	23.1 (2)
O4—C20—C10—C1	−13.8 (2)	C7—C9—C11—C12	70.86 (17)
O4—C20—C10—C9	112.22 (16)	C6—C5—C10—C20	−73.26 (16)
O1—C8—C14—C13	−156.69 (15)	C6—C5—C10—C1	163.47 (13)
O1—C8—C7—C6	−6.8 (2)	C6—C5—C10—C9	41.81 (15)
O1—C8—C7—C9	−130.28 (15)	C6—C5—C4—C3	−175.00 (15)
C8—C7—C6—C5	−119.38 (14)	C6—C5—C4—C18	62.6 (2)
C8—C7—C9—O2	33.29 (16)	C6—C5—C4—C19	−58.3 (2)
C8—C7—C9—C10	147.15 (12)	C6—C7—C9—O2	−94.05 (14)
C8—C7—C9—C11	−87.70 (15)	C6—C7—C9—C10	19.82 (15)
C20—C10—C1—C2	−70.13 (17)	C6—C7—C9—C11	144.96 (13)
C20—C10—C9—O2	−161.41 (12)	C9—C10—C1—C2	169.16 (13)
C20—C10—C9—C7	85.39 (13)	C9—C7—C6—C5	5.64 (16)
C20—C10—C9—C11	−38.54 (16)	C9—C11—C12—C13	−55.38 (19)
C5—C10—C1—C2	57.48 (16)	C12—C13—C14—C8	−73.70 (18)
C5—C10—C9—O2	76.47 (13)	C12—C13—C15—C17	58.96 (19)
C5—C10—C9—C7	−36.74 (13)	C12—C13—C15—C16	−67.9 (2)
C5—C10—C9—C11	−160.67 (12)	C4—C5—C10—C20	63.25 (18)
C5—C4—C3—C2	−51.34 (19)	C4—C5—C10—C1	−60.02 (17)
C10—C5—C6—C7	−29.94 (16)	C4—C5—C10—C9	178.32 (13)
C10—C5—C4—C3	55.08 (18)	C4—C5—C6—C7	−163.82 (13)
C10—C5—C4—C18	−67.28 (19)	C15—C13—C14—C8	163.23 (13)
C10—C5—C4—C19	171.78 (14)	C15—C13—C12—C11	−170.37 (14)
C10—C1—C2—C3	−56.66 (19)	C18—C4—C3—C2	73.09 (19)
C10—C9—C11—C12	−170.44 (12)	C19—C4—C3—C2	−168.04 (16)
C1—C10—C9—O2	−39.44 (16)		

Hydrogen-bond geometry (Å, °)

<i>D</i> —H $\cdots$ <i>A</i>	<i>D</i> —H	H $\cdots$ <i>A</i>	<i>D</i> $\cdots$ <i>A</i>	<i>D</i> —H $\cdots$ <i>A</i>
O2—H2 $\cdots$ O1 ⁱ	0.84 (3)	1.89 (3)	2.7101 (17)	165 (2)
O3—H3 $\cdots$ O2 ⁱⁱ	0.86 (3)	1.81 (3)	2.6499 (16)	166 (2)
O5—H5 $\cdots$ O4 ⁱⁱⁱ	0.84 (3)	2.18 (3)	2.9797 (17)	158 (2)

Symmetry codes: (i) $-x+2, y-1/2, -z+1/2$; (ii) $x-1, y, z$; (iii) $-x+1, y+1/2, -z+1/2$.