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# Dichlorobis(2-phenylazopyridine)ruthenium(II) complexes: characterisation, spectroscopic and structural properties of four isomers †

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The didentate ligand 2-phenylazopyridine (azpy) can – in theory – give rise to five different isomeric complexes of the type $[Ru(azpy)_2Cl_2]$, of which three have been known since 1980. The molecular structures of the *cis*-dichlorobis(2-phenylazopyridine) ruthenium(II) complexes α-$[Ru(azpy)_2Cl_2]$ and β-$[Ru(azpy)_2Cl_2]$ (in which the coordinating pyridine nitrogen atoms are in mutually *trans* and *cis* positions, respectively, whilst the azo nitrogen atoms are in mutually *cis* positions) were unambiguously determined in the early 1980s. The third isomer, γ-$[Ru(azpy)_2Cl_2]$, has for two decades, erroneously, been assumed to be the all-*trans* isomer. In a recent communication we have proven that for this γ isomer the chloride ions are indeed in a *trans* geometry, but the pyridine nitrogen and azo nitrogen atoms of the two azpy ligands are in mutually *cis* geometries. In this paper the isolation of a fourth isomer is presented, the hitherto unknown δ-$[Ru(azpy)_2Cl_2]$. The isomeric structure of δ-$[Ru(azpy)_2Cl_2]$ has been determined by $^1$H-NMR spectroscopy and single-crystal X-ray diffraction analysis, and is the all-*trans* isomer. The bis(azpy)–ruthenium(II) isomers are of interest because of the pronounced cytotoxicity they exhibit against tumour cell lines and could be very useful in the search for structure–activity relationships of antitumour-active ruthenium complexes, as among the isomers there is a significant difference in activity. It is of paramount importance to have a good understanding of the structural and spectroscopic properties of these complexes, which in this paper are compared and discussed, with a particular emphasis on 1D and 2D $^1$H NMR spectroscopies.

## Introduction

Azpy (2-phenylazopyridine, Fig. 1),[1] is a didentate ligand that can coordinate to a metal ion through the lone electron pairs of the pyridinic and azo nitrogen atoms, forming a stable chelating 5-membered ring.[2] The pyridinic nitrogen of azpy is in its coordinating properties similar to the 2,2′-bipy nitrogen atoms as it is a weak σ-donor and intermediate π-acceptor. The azo nitrogen is also a weak σ-donor atom, but its π-accepting properties are much stronger than for a 2,2′-bipy nitrogen atom.[3–7] The first report of the use of azpy as a ligand in the synthesis of ruthenium complexes is from 1980, when Krause and Krause[3] published the synthesis of three isomers of the complex [Ru(azpy)$_2$Cl$_2$], namely α-[Ru(azpy)$_2$Cl$_2$], β-[Ru(azpy)$_2$Cl$_2$]·CHCl$_3$ and γ-[Ru(azpy)$_2$Cl$_2$], from now on referred to as **α-Cl**, **β-Cl** and **γ-Cl**, respectively. Also in the early 1980s, the group of Chakravorty started to study this kind of ruthenium complex.[4] As azpy is not a $C_2$-symmetric didentate ligand, in theory, five different isomers of [Ru(azpy)$_2$Cl$_2$] can exist, indicated as *ccc*, *cct*, *ctc*, *tcc*, and *ttt*, referring to the coordinating Cl, N(pyridine) and N(azo) atoms that can be in mutually *cis* (*c*) or *trans* (*t*) positions (Fig. 2). One of the three complexes reported in 1980, **β-Cl**, a blue–purple crystalline material, could be readily and unambiguously ascribed to the all-*cis* isomer *ccc* as this is the only isomer lacking $C_2$-symmetry, and therefore was readily recognised from a ($^{13}$C) NMR spectrum. The other four isomers all have a $C_2$ axis, due to which the two azpy ligands are magnetically equivalent and therefore indistinguishable by NMR spectroscopy. What appeared to be the most stable isomer, **α-Cl**, a blue crystalline compound, was identified as the *ctc* isomer on basis of IR data. The third complex described in 1980 by Krause and Krause (**γ-Cl**),[3] and later also by Chakravorty *et al.*,[4,8] a green felt-like microcrystalline powder with a purple lustre, was assigned to the all-*trans* isomer *ttt*, mainly on the basis of the number of the Ru–ligand vibrations in the IR spectra.

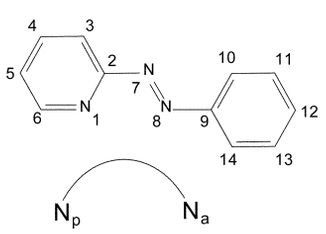


**Fig. 1** Structural (upper) and schematic (lower) representation of the didentate ligand azpy and the proton numbering scheme. For the NMR discussion, the phenyl ring *ortho* (10 and 14) and *meta* (11 and 13) protons are referred to as H(o) and H(m), respectively, the proton in *para* (12) as H(p). The $N_A$ and $N_P$ represent the azo and pyridine donor atoms, respectively.

The initial isomer assignment of the two *cis*-dichloro complexes **α-Cl** and **β-Cl** was confirmed by a crystallographic study in 1984,[9] and also by comparison of spectroscopic data of related [Ru(azpy)$_2$X$_2$] (X = NNN or NO$_2$) complexes whose structures were determined by single-crystal X-ray diffraction studies.[10] A single-crystal X-ray diffraction study of the third isomer, as previewed by Chakravorty *et al.*,[11] to the best of our knowledge has never been published by this group. Recently we have communicated a crystallographic study, unambiguously proving the geometry of the ligands in **γ-Cl** to be *tcc*, *i.e.* with the chlorides in a *trans* configuration, but with the pyridines as

† Electronic supplementary information (ESI) available: Pluton packing plots of the left-handed helices present in the crystals of **γ-Cl**; NOESY spectrum of **α-Cl** in acetone-$d_6$. See http://www.rsc.org/suppdata/dt/b3/b313182c/

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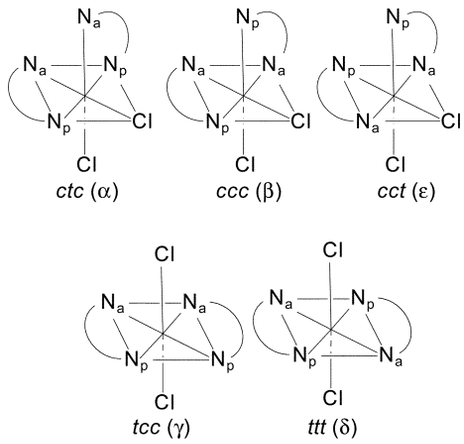


**Fig. 2** The five possible $[Ru(azpy)_2Cl_2]$ isomers, with a systematic three-letter code indicating the mutually *cis* (*c*) or *trans* (*t*) geometries of the chlorides (Cl), the pyridine ($N_p$) and the azo nitrogens ($N_A$), respectively; between brackets the Greek letter code is given. Throughout this paper mainly the Greek letters are used to indicate the isomers. Note, **γ-Cl** (the *tcc* isomer), for two decades has erroneously been thought to be the *ttt* isomer, see text). The arcs represent the azpy ligand (see Fig. 1). The *cis*-dichlorocomplexes, **α-Cl**, **β-Cl** and **ε-Cl**, exist in two enantiomeric forms, Δ and Λ; only the Λ form is shown in this figure.

well as the azo groups in mutually *cis* geometries.[12] So, for two decades the **γ-Cl** has erroneously been assumed to be the all-*trans* isomer. In this paper we now also present the crystal structure of a new isomer, which in fact has the all-*trans* configuration. As there is confusion about the structural characteristics of the ruthenium–azpy isomers, we prefer to identify them by the Greek letter codes as initiated by Krause, and nominating the here presented isomer as δ-$[Ru(azpy)_2Cl_2]$ (**δ-Cl**). Referring to the different isomers with a two-letter code as proposed in early papers,[11,13] is not sufficient for more than three isomers; actually it was already erroneous for identification of the first three known isomers, as the **γ-Cl** appears to be not the suggested (*t*)*tt*, but the (*t*)*cc* isomer, and therefore should be discriminated from the (*c*)*cc* isomer.

The recent discovery of the cytotoxicity of the $[Ru(azpy)_2Cl_2]$ complexes (and in particular that of the α isomer) against a series of tumour cell lines, has renewed the interest in these dichlororuthenium(II) complexes and is a promising lead in search for new antitumour compounds.[12] The synthesis of series of ruthenium–azpy complexes for antitumour studies,[12,14] as well as the investigation of their interaction with potential biological target molecules like DNA bases,[15–19] require a good understanding of the structural and spectroscopic properties of this type of complexes. The $[Ru(azpy)_2Cl_2]$ isomers constitute the class of complexes bearing two didentate azo ligands. From the five $[Ru(azpy)_2Cl_2]$ isomers, four have now been fully characterised. In this paper the synthesis and characterisation of these $[Ru(azpy)_2Cl_2]$ isomers are discussed, with a particular emphasis on the molecular structures, and 1D and 2D $^1$H NMR data, and put in perspective to their biological activity. A detailed discussion of ruthenium(II) complexes with azpy-related ligands like arylazopyridines (bearing substituents on the phenyl and/or pyridine ring), phenyl- and aryl-azoimidazoles, phenylazopyrimidines or phenylazoquinoline, is out of the scope of this paper and is not addressed in detail here, but only when useful in discussion; similarly, a discussion of azpy complexes with other metal ions is not elaborated.

## Experimental

### Materials and physical measurements

The ligand azpy[3] and the ruthenium(II) complexes α-$[Ru(azpy)_2Cl_2]$ (**α-Cl**), β-$[Ru(azpy)_2Cl_2]\cdot CHCl_3$ (**β-Cl**) and γ-$[Ru(azpy)_2Cl_2]$ (**γ-Cl**) were prepared from literature procedures.[3,4,20] UV-Vis absorption spectra were measured on a Perkin Elmer Lambda 900 Spectrometer. Elemental analyses were performed by the Microanalytical Laboratory, University College, Dublin, Ireland (C, H, N). $^1$H NMR spectra in deuterated acetone or deuterated chloroform were recorded on a Bruker DPX 300 spectrometer. Resonances were referenced to the central peak of partially deuterated solvent at $\delta$ 2.06 (for acetone-$d_6$) or $\delta$ 7.26 ppm (for chloroform-*d*). The figures of the molecular (XRD) structures have been prepared using PLATON.[21]

### Syntheses

**γ-$[Ru(azpy)_2Cl_2]$ (γ-Cl).** A crystalline sample of **γ-Cl** was prepared by slow diffusion of diethyl ether in a concentrated solution of **γ-Cl** in chloroform. The green crystalline material with a purply-golden lustre was isolated by filtration, and washed with diethyl ether. The appearance of the compound under a microscope at a tenfold enhancement is that of a "haystack" of needle-shaped crystals of *ca.* 1 cm and a diameter of approximately 0.05 mm. Details of the XRD-analysis of this complex have been described elsewhere.[12]

**δ-$[Ru(azpy)_2Cl_2]$ (δ-Cl).** A 1.5 g blue–green crude mixture of **γ-Cl** was prepared from *cis*-$[Ru(dmso)_4Cl_2]$ and azpy in refluxing acetone.[3] This mixture of isomers, containing *ca.* 20% **δ-Cl** as determined from $^1$H NMR data, was extensively washed with acetone (*ca.* 3 l), until $^1$H NMR (in $CDCl_3$) showed no more presence of **γ-Cl** in the residue. The resulting pure **δ-Cl** was obtained as a sea-green powder in a yield of 50 mg (*ca.* 5% with respect to the starting ruthenium(II)–dmso complex). Elemental analysis for the non-recrystallised material, probably containing some residual solvent (Found: C, 48.0; H, 3.46; N, 14.5. $RuC_{22}H_{18}N_6Cl_2$ requires C, 49.1; H, 3.37; N, 15.6; $[RuC_{22}H_{18}Cl_2N_6]\cdot H_2O\cdot 1/3(CH_3)_2CO$ requires C, 48.0; H, 3.82; N, 14.6%.). Recrystallisation from chloroform/benzene (3/1) by slow evaporation of the solvent yielded dark-green rod-like crystals suitable for X-ray diffraction analysis. These crystals were proven to be the same isomer as the sea-green powder by $^1$H NMR, with the spectrum of the latter showing an additional multiplet signal deriving from the co-crystallised benzene molecule.

**Crystal structure data of δ-$[Ru(azpy)_2Cl_2]\cdot C_6H_6$.** $C_{22}H_{18}Cl_2N_6Ru\cdot C_6H_6$, $C_{2h}$ isomer, $M_r$ = 616.50, monoclinic, space group $P2_1/c$ (no. 14) with $a$ = 9.175 (3), $b$ = 9.7409 (18), $c$ = 15.8783 (17) Å, $\beta$ = 109.957 (12)°, $V$ = 1333.9(5) Å$^3$, $Z$ = 2, $\rho_{calc}$ = 1.535 g cm$^{-3}$, $F(000)$ = 624, $\mu_{MoK\alpha}$ = 0.8 mm$^{-1}$. 8874 reflections were measured on an Nonius Kappa CCD diffractometer on rotating anode ($\lambda_{MoK\alpha}$ = 0.71073 Å, $T$ = 150 K, $\theta_{max}$ = 27.5°), 2962 of which were unique ($R_{int}$ = 0.0593). The structure was solved with direct methods (SHELXS86).[45] 305 parameters were refined with SHELXL-97.[46] $R_1$ = 0.0452, $wR_2$ = 0.0946, $S$ = 1.034. Hydrogen atoms were included on calculated positions, riding on their carrier atoms. Non-H atoms were refined with anisotropic displacement parameters, hydrogen atom displacement parameters were linked to the equivalent displacement parameter of their carrier atom.§

## Results and discussion

### Synthesis and characterisation

In the early papers describing the synthesis and characterisation of the three isomers **α-Cl**, **β-Cl** and **γ-Cl**, the presence of an impurity is mentioned which was eliminated from the other

§ CCDC reference number 222437. See http://www.rsc.org/suppdata/dt/b3/b313182c/ for crystallographic data in CIF or other electronic format.

isomers by column chromatography.[3–5,20] The reason why the synthesis of the crude mixture of $[Ru(azpy)_2Cl_2]$ isomers does not always yield the same ratio of isomers, might well be the presence of this "impurity" which has now been isolated and proven to be the all-*trans* isomer, **δ-Cl**, *vide infra*. The **δ-Cl** is isolated from a crude mixtures of isomers by extensive washing with acetone, in which this isomer appears much less soluble than the other three. The **γ-Cl** isomer was shown to be the kinetically most favoured isomer and is found as main product in the synthesis of the crude mixture of isomers starting from $RuCl_3 \cdot xH_2O$ in methanol as well as from *cis*-$[Ru(dmso)_4Cl_2]$ in acetone. In both cases we have observed **δ-Cl** as a by-product, albeit in higher quantities using the latter synthetic route (*ca.* 6% *versus ca.* 20%). The poor solubility of **δ-Cl** in acetone most likely explains the higher yield of this isomer in the latter route, and prompted us to investigate a direct synthesis of this isomer from $RuCl_3 \cdot xH_2O$ in acetone, and from *cis*-$[Ru(dmso)_4Cl_2]$ at lower temperatures; however, this did not achieve good results.

The **α-Cl** and **β-Cl** isomers can be obtained by isomerisation of crude mixtures of isomers in high-boiling solvents; the thermodynamically most stable isomer being the α-isomer.[3,4,20] However, isomerisation from the α to the β isomer has also been observed.[5,15,19] The low yield of the **δ-Cl** did not allow us to thoroughly investigate its isomerisation processes. Some preliminary data suggest, however, that the **δ-Cl** converts to **α-Cl** under basic conditions in ethanol/dichloromethane mixtures, whereas the **γ-Cl** under the same conditions isomerizes to **β-Cl**. During the writing of this paper, we became aware of a report regarding the 'missing' fifth isomer, ε-$[Ru(azpy)_2Cl_2]$ (**ε-Cl**). In 1990, Popov and co-workers published a short letter on thermodynamic and kinetic aspects of $[Ru(azpy)_2Cl_2]$ complexes, reporting the synthesis of an unknown isomer, attributed to the *cct* (*i.e.* **ε-Cl**) on the basis of spectrophotometric data, *vide infra*.[22] This isomer appears thermodynamically unstable and, at temperatures slightly higher than room temperature, isomerizes to **α-Cl**.

For obvious reasons, not all new compounds are directly characterised by single-crystal XRD analyses and one often has to rely on spectroscopic techniques. Initial conclusions drawn for the geometric configuration of the ligands in the γ isomer on basis of spectroscopic, and in particular IR, data, have unfortunately turned out to be wrong. Our doubts regarding the assignment of the **γ-Cl** to the all-*trans* isomer arouse when analysing in detail 1D proton NMR shifts of the different isomers **α-Cl**, **β-Cl**, and **γ-Cl**. 2D NMR experiments then underlined our doubts, and they were finally confirmed by a crystallographic study of the γ isomer.[12] Although in the first reports on ruthenium(II)–azpy complexes the use of NMR spectroscopy is mentioned,[3,4] no detailed discussions were given there. The characterisation of the **δ-Cl** as presented in this paper further underlines $^1$H NMR spectroscopy to be a most powerful and valuable tool for the characterisation of this kind of isomeric complexes. In the following section, a detailed discussion is given of the 1D and 2D proton NMR data of the four $[Ru(azpy)_2Cl_2]$ isomers, **α-Cl**, **β-Cl**, **γ-Cl** and **δ-Cl** in comparison with the XRD-derived structural data. For clarity reasons, these data are discussed in reverse order: first the structural data of the individual isomers are discussed, consecutively, the HH-NOESY data are presented, and finally the 1D proton NMR data are discussed, with a particular emphasis on shielding and deshielding effects.

### XRD data of α-Cl, β-Cl, γ-Cl, and δ-Cl

Single-crystal X-ray diffraction studies provide the most convincing evidence for the molecular structure and absolute geometry of a compound. The molecular structures of two $[Ru(azpy)_2Cl_2]$ isomers, **α-Cl** and **β-Cl**, were published in 1984,[9] and the structure of the third isomer, **γ-Cl**, was reported in 2000.[12] In Fig. 3 the molecular structures of **α-Cl** and **β-Cl** are

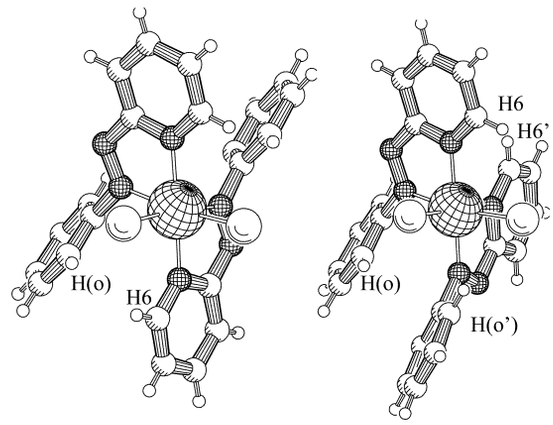


**Fig. 3** The molecular structures of **α-Cl** (left) and **β-Cl**.[10] The co-crystallised chloroform molecule in the structure of the latter is omitted for clarity.

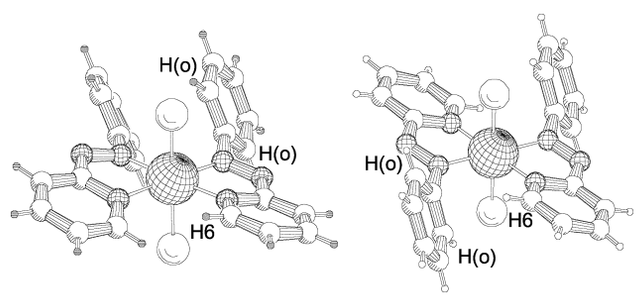


**Fig. 4** The molecular structures of **γ-Cl** (left),[12] and the all-*trans* isomer **δ-Cl** (the co-crystallised benzene molecule is omitted for clarity).

shown. In Fig. 4 the molecular structures of **γ-Cl** and the new isomer, **δ-Cl**, are presented. In Table 1 some relevant structural data of the four isomeric $[Ru(azpy)_2Cl_2]$ complexes are summarised. Characteristic for all four isomers is the relative shortness of the $Ru–N_{azo}$ bonds, reflecting a strong π-back-bonding interaction from the ruthenium(II) d orbitals with the π*-orbitals of the azo bond.[9,10,23–26] Conversely, the azo bond is dramatically longer in the ruthenium(II) complexes in comparison with the free ligand,[27] further evidence for significant electron back-bonding in the anti-bonding orbitals of the azo bond.[9,10,23–26] A more detailed discussion regarding the packing structure of the **γ-Cl** isomer is given in a separate paragraph, *vide infra*.

The molecular structure of **δ-Cl** (see Fig. 4) unambiguously proves the all-*trans* geometry for this isomer, *i.e.* the chloride ions as well as the pyridine and the azo nitrogen atoms are all in mutually *trans* geometries. The complex is located on a crystallographic inversion centre, enforcing all *trans* angles to be exactly 180°. The structural data indicate a considerable steric interaction between the phenyl ring of one azpy ligand and the pyridine ring of the other azpy ligand, as is illustrated by the occurence of an intramolecular C–H ··· π interaction with dimensions (C ··· Cg) = 3.501 Å and (C–H ··· Cg) = 145° (where Cg is the geometrical center of the phenyl ring). Similar to what is observed in the other three isomers, the relative shortness of the Ru–N(azo) bond with respect to the Ru–N(pyridine) bond, as well as the long N–N(azo) bond, indicates substantial π bonding to ruthenium through an azo nitrogen. A *trans* N(azo)–M–N(azo) arrangement is very rare, and has only been reported very recently, for the *mer*-tris(azpy)–ruthenium(II) complex.[7] In a *trans* N(azo)–Ru(II)–N(azo) complex the two azo bonds are thought to compete for the back-bonding of the Ru(II) (4d) $t_2$ electron density, resulting in relatively long Ru–N(azo) bond lengths. In fact, the significantly longer Ru–N(azo) distance in the **δ-Cl** with respect to the equivalent bond lengths in **α-Cl**, **β-Cl** and **γ-Cl** (Table 1) confirms the decrease in the Ru–N(azo) bond order for the

**Table 1** Selected geometrical parameters from **α-Cl** and **β-Cl**, and **γ-Cl**, and **δ-Cl**. Distances are reported in Å, angles in °, with standard uncertainties in parentheses. **β-Cl** has two independent molecules, **δ-Cl** is located on a crystallographic inversion centre and therefore has only one independent azpy ligand and Cl ion

| | Ru–$N_a$ | Ru–$N_p$ | Ru–Cl | $N_a$–$N_a$ | $N_a$–Ru–$N_p$ | Cl–Ru–Cl′ | $N_p$–Ru–$N_{p'}$ | $N_a$–Ru–$N_{a'}$ |
|---|---|---|---|---|---|---|---|---|
| **α-Cl** | 1.977(4)<br>1.984(4) | 2.045(4)<br>2.051(4) | 2.401(1)<br>2.397(1) | 1.279(7)<br>1.283(6) | 76.1(2)<br>76.6(2) | 89.52(6) | 174.5(2) | 93.5(2) |
| **β-Cl**<br>(mol1) | 1.960(9)<br>2.003(9) | 2.059(9)<br>2.020(9) | 2.401(4)<br>2.410(3) | 1.30(1)<br>1.29(1) | 76.2(4)<br>76.1(4) | 92.2(1) | 98.6(4) | 98.8(4) |
| **β-Cl**<br>(mol2) | 1.958(9)<br>1.96(1) | 2.069(9)<br>2.028(9) | 2.389(4)<br>2.379(4) | 1.31(1)<br>1.30(2) | 76.1(4)<br>77.5(4) | 91.1(2) | 101.9(4) | 103.0(4) |
| **γ-Cl** | 1.986(5)<br>1.988(5) | 2.116(6)<br>2.099(5) | 2.3768(15)<br>2.3683(16) | 1.302(8)<br>1.306(7) | 76.4(2)<br>75.80(19) | 170.5(7) | 103.8(2) | 104.1(2) |
| **δ-Cl** | 2.016(3) | 2.060(3) | 2.3843(10) | 1.282(4) | 75.30(11) | 180 | 180 | 180 |

δ isomer, which is furthermore also in correspondence with IR and UV-Vis data, *vide infra*. In addition, the N–N azo bond in the **δ-Cl** structure is relatively short, evidencing a reduced Ru–azpy π bonding with respect to the other isomers.

### Reconsidering γ-Cl, the *tcc* isomer

The *tcc* geometry was originally assumed to be the least favoured of all five possible $[Ru(azpy)_2Cl_2]$ isomers, because of the severe steric interaction of the two pyridine rings[3] or the aryl rings[4,8] of the two azpy ligands in an in-plane head-to-head orientation. Paradoxically, it appears that the proximity of the two phenyl rings actually stabilises this geometry, *i.e. via* significant stacking interactions of the two rings![12] The proximity of the two phenyl rings can be clearly seen from the molecular structure of **γ-Cl** in Fig. 4 and also from the space-filling plot in Fig. 9, *vide infra* (the geometric centres of the two phenyl rings are 3.493(8) Å apart, the angle between the ring planes is 16.9(2)°). Also the crystal structures of related (γ-isomeric) complexes with arylazoimidazole (Cg ··· Cg distance: 3.513(4) Å, plane angle: 11.1(5)°),[23] phenylazopyrimidine (Cg ··· Cg distance: 3.715(3) Å, plane angle: 22.2(2)°),[26] and phenylazoisoquinoline ligands (Cg ··· Cg distance: 3.712(2) Å, plane angle: 27.07(16)°)[28] show such an interaction.

In the solid state, the crystals of **γ-Cl** are found to have the relatively rare hexagonal space group $P6_5$. In Fig. 5 the interesting packing of the molecules in the crystal structure is shown. The molecules are stacked in helices along the six-fold screw axes running parallel to the *c* translation axis (see also ESI †). In the centre of each helix is a solvent-filled channel.[12] Two neigbouring helical columns of **γ-Cl** interact with each other through π ··· π stacking. One clear C–H ··· π interaction can be discerned with a C ··· Cg distance of 3.558 Å and a C–H ··· Cg angle of 160° (Cg is the geometrical centre of the accepting aromatic ring). The stabilising effect of the stacked phenyl rings, in combination with the easy formation of poorly soluble crystalline needles, might explain the first and main product in ruthenium–azpy complex syntheses to be the γ isomer. In fact, also with related ligand systems, like phenylazoimidazole[23,24,29] and phenylazopyrimidine ligands the main formed isomer appears to be the *tcc* isomer (γ).

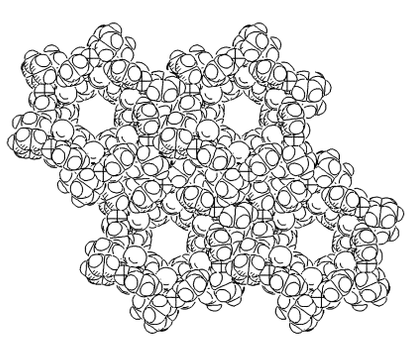

**Fig. 5** Crystal-packing plot of **γ-Cl** (view along the *c*-axis).

### HH-NOESY data of α-Cl, β-Cl, γ-Cl, and δ-Cl

The **α-Cl**, **γ-Cl** and **δ-Cl** isomers all three have a $C_2$ symmetry, causing the mutual protons of the azpy ligands in each isomer to show identical NMR signals. As a consequence, one should take care in interpreting the presence and/or absence of NOE cross-peaks in their 2D spectra. In the last column of Table 2 the (intra-molecular) inter-ligand NOE cross-peaks that are observed for the different isomers are indicated. Actually, in solution the phenyl ring of the azpy is rotating fast around its CN axis, explaining the single resonance signal for the two H(o) protons as well as the two H(m) protons. Although the phenyl ring is rotating, on average, the H(o) protons can be close enough to other protons to allow well-observable NOE cross-peaks. The averaged inter-proton H6–H(o) distances from solid-state XRD-data appear a useful indication to predict NOE in solution experiments.

From the molecular structure of **α-Cl** (see Fig. 3),[9] it is easily seen that the H6 proton of one azpy pyridine moiety is close to the phenyl ring of the other azpy ligand (H6–H(o) distances of 2.5 and 5.7 Å). The NOE cross-peak observed between the pyridine H6 and the phenyl ring H(o) proton resonances (of the other, magnetically indistinguishable, azpy ligand) in the NOESY spectrum (see ESI †) suggests that this feature is preserved also in solution. This H6–H(o) NOE gives valuable reference peaks in related compounds of the type α-$[Ru(azpy)_2X_2]$ (X being an anion like $NO_3^-$),[15,19] and α-$[Ru(azpy)_2Y]$ (Y being a dianion like oxalate, malonate or 1,1-cyclobutanedicarboxylate).[14] Also for the sometimes very complicated spectra of (atropisomers of) α-$[Ru(azpy)_2L_2]^{2+}$ complexes (L being a heterocyclic nitrogen ligand) the H6–H(o) NOE is an important reference point, in particular also when the $C_2$ symmetry is removed due to the orientation of the L ligands.[19,30]

In Fig. 6 the NOESY spectrum of **β-Cl** is shown. Due to the absence of $C_2$ symmetry in this isomer, the proton resonances of the two azpy ligands are not equivalent. From the molecular structure of **β-Cl** (see Fig. 3)[9] it can be seen that the mutual H6 protons and the H(o) protons are relatively close to one another (less than 3.5 Å for the H6 protons, and a shortest distance between two H(o) protons of 3.1 Å). In the NOESY spectrum indeed cross-peaks are observed between H6–H6′ and the H(o)–H(o′) resonances. These cross-peaks are most valuable for characterisation of related complexes like β-$[Ru(azpy)_2X_2]$ and β-$[Ru(azpy)_2L_2]^{2+}$ complexes.[19,31,32] The only other isomer in which the two H6/H6′ protons as well as the H(o)/H(o′) protons are close enough to allow NOE cross-peaks is the

**Table 2** $^1$H chemical shifts of **α-Cl**, **β-Cl**, **γ-Cl** and **δ-Cl** in $CDCl_3$ (see Fig. 8) and observed NOE cross-peaks

| | H6/6′ | H5/5′ | H4/4′ | H3/3′ | H(o)/(o′) | H(m)/(m′) | H(p)/(p′) | NOEs |
|---|---|---|---|---|---|---|---|---|
| **α-Cl** | 9.37 | 7.51 | 7.98 | 8.51 | 6.82 | 7.11 | 7.28 | H6–H(o) |
| **β-Cl** | 9.79<br>7.35 | 7.86<br>7.48 | 8.16<br>7.92 | 8.52<br>8.39 | 6.71<br>7.81 | 7.19<br>7.39 | 7.22<br>7.29 | H6–H6′<br>H(o)–H(o′) |
| **γ-Cl** | 8.92 | 7.82 | 8.19 | 8.59 | 7.54 | 6.98 | 7.18 | — |
| **δ-Cl** | 7.70 | 7.18 | 7.93 | 8.54 | 8.07 | 7.64 | 7.67 | H6–H(o) |

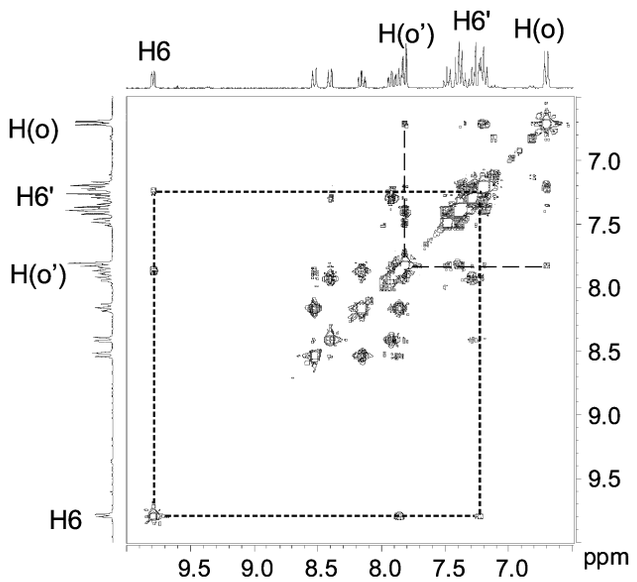


**Fig. 6** NOESY spectrum of **β-Cl** in acetone-$d_6$. The H6–H6′ and the H(o)–H(o′) NOE cross-peaks are indicated with dotted and dashed lines, respectively.

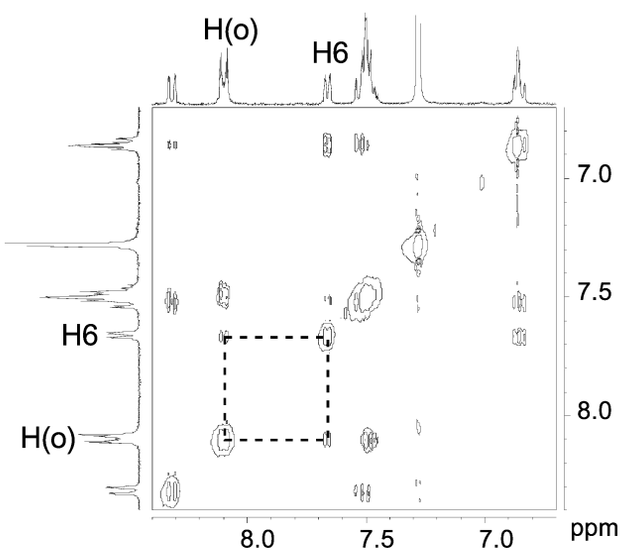


**Fig. 7** NOESY spectrum of **δ-Cl** recorded in $CDCl_3$/benzene-$d_6$. The dashed line indicates the NOE cross-peak between the H6 and H(o) protons.

γ isomer, but due to the $C_2$ symmetry in the latter, these cross-peaks are not off-diagonal and are not observed.

In Fig. 4 the molecular structure[12] of **γ-Cl**, is shown. The chloride ions are in a *trans* geometry, whilst the azpy ligands are in a head-to-head orientation. Important to notice in the NOESY spectrum of **γ-Cl** is the absence of a NOE cross-peak between the H6 and the H(o) resonances (see supporting information in ref. 12). In the all-*trans* structure, which the **γ-Cl** was assumed to have for twenty years,[3–5,20] a NOE interaction between these protons would be expected because of the proximity of the pyridine of one azpy and the phenyl ring of the other ligand (see discussion of the **δ-Cl** structure, in the next section). In the *tcc* configuration, which **γ-Cl** actually appears to be, in fact no NOE cross-peak between the H6 and H(o) proton resonances are to be expected.[12]

In Fig. 7 the NOESY spectrum of **δ-Cl** is shown. The NOESY experiment was performed in a chloroform-*d*/benzene-$d_6$ (3 : 1) solution in order to avoid overlap of the H6 proton resonance with other signals which occurs in pure chloroform-*d*. The NOE cross-peak between the H6 and the H(o) protons is clearly present, as expected for the *ttt* isomer. In the molecular structure of **δ-Cl** (see Fig. 4) the H6 is 3.61 and 2.98 Å distant from the two H(o) protons, which agrees well with the observed intense NOE cross-peak.

Regarding the presence or absence of NOE cross-peaks in the 2D NMR spectra of the bis(azpy)–ruthenium(II) isomers, a few remarks have to be made. The NOESY data presented above show characteristic inter-ligand cross-peaks, which perfectly agree with the XRD-derived structural data. However, these data alone are not sufficient to identify an isomer unambiguously. A strong H6–H(o) NOE peak is expected and observed for the *ttt* geometry (δ), as well as for the *ctc* isomer (α). A H6–H(o) NOE coupling is not observed for the other *trans*-Cl isomer *tcc* (γ), but a (weak) NOE can be expected to be observed also in the *cct* structure (ε).[33] Therefore, the presence of a H6–H(o) NOE cross-peak alone cannot discriminate between the **α-Cl**, **δ-Cl** and **ε-Cl**. The absence of inter-ligand NOE cross-peaks, on the other hand, is expected only for the **γ-Cl** and therefore is a unique characteristic for the *tcc* configuration.[34]

### $^1$H NMR data of α-Cl, β-Cl, γ-Cl and δ-Cl

In Fig. 8 the $^1$H NMR spectra of **α-Cl**, **β-Cl**, **γ-Cl** and **δ-Cl** in $CDCl_3$ are displayed and in Table 2 the $^1$H NMR data of these four isomers are listed. The H6 doublet of the azpy ligands in the spectra of the four isomers have been assigned on account of the $^3$J coupling (typical values of ~5 Hz for the H6 *versus* ~9 Hz for the H3 doublet) and the further assignments of the H4 and H5 triplets (double doublets) were made on the basis of HH COSY spectra. Regarding the phenyl ring proton resonances, the single peaks for the H(o) and H(m) protons in

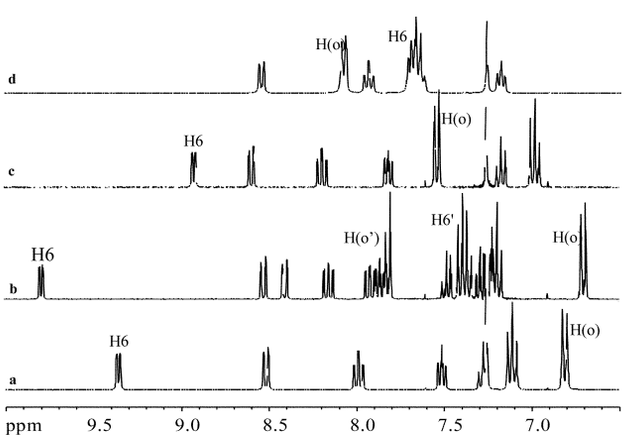


**Fig. 8** $^1$H NMR spectra of **α-Cl** (a), **β-Cl** (b), **γ-Cl** (c) and **δ-Cl** (d) in $CDCl_3$. Assignment of the peaks is given in Table 2.

the spectra indicate free rotation (on the NMR time-scale) of the phenyl rings for each isomer. This is even the case for the γ isomer, in which the phenyl rings are sterically the most encumbered of those of all of the isomers. In this context, an interesting observation is that even at low temperatures the $^1$H NMR spectrum of **γ-Cl** is similar to the RT spectrum, *i.e.* the H(o) and H(m) phenyl ring protons of the azpy ligands are double in intensity with respect to the other proton resonances. From Fig. 4 and Fig. 9 it is clear that the two H(o) protons of one azpy ligand are not in the same magnetic environment; one H(o) proton is located just above the shielding phenyl ring of the second azpy, while the other one is not. However, the double intensity of the H(o) signals indicates that the two H(o) protons of one azpy ligand are identical (on the NMR time scale), and therefore the phenyl rings are concluded to rotate rapidly around their C(9)–N(8) axis. From the space-filling plot in Fig. 9, it can be seen that a rotation of the phenyl rings around this CN axes by 360° is for steric reasons rather improbable, even taking into account that the Ru–N(azo) distances and the N–Ru–N angle might increase due to so-called "breathing" of the metal–ligand system. So it can be expected that the phenyl rings of both azpy ligands are rather involved in a parallel, synchronous and continuous flipping around their CN axes by about 90°.

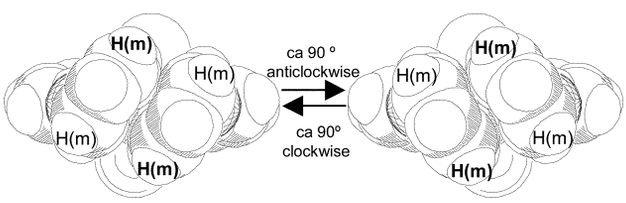


**Fig. 9** Space-filling plot of **γ-Cl** and representation of the synchronous rotation of the two phenyl rings. The two H(m) protons (and similarly the two H(o) protons) of one phenyl ring are in a magnetically different environment (**H(m)** indicating the shielded proton), but due to the fast flipping (on the NMR time scale) they yield only one, averaged, proton resonance.

The **β-Cl** isomer is readily distinguished based on the double number of NMR signals with respect to the other isomers **α-Cl**, **γ-Cl** and **δ-Cl** which all have a twofold symmetry axis and are expected to show only nine lines in the $^1$H NMR spectra. For the assignment of the NMR signals to the two, non-equivalent, azpy ligands in **β-Cl**, a discussion on the shielding and deshielding effect is necessary. In the NOESY spectra of **β-Cl** cross-peaks are observed between the H6 and H6′ resonances and between the H(o) and H(o′) resonances; no inter-ligand NOE cross-peaks are observed between one pyridine with the phenyl ring of the other azpy. Therefore, it is impossible to determine which set of pyridine signals and which set of the two phenyl groups belong to the same azpy ligand, on the basis of these 2D data. However, in the molecular structure of **β-Cl** (see Fig. 3) it is clearly seen that the H(o′) and the H6 protons of the **β-Cl** isomer are directed toward the chloride ions; it is therefore plausible that these signals are observed downfield with respect to the H(o) and H6′ resonances, respectively, and together with the COSY data all resonances are readily assigned.

A first discussion of (1D) $^1$H NMR data of ruthenium–azpy complexes dates from 1986,[13] when the diagnostic value of the H6 and H(o) proton resonances was recognised. The qualitative discussion regarding shielding/deshielding effects in this latter paper is, however, of limited value, as the authors, erroneously assumed their green isomer (**γ-Cl**) to be the all-*trans* isomer on basis of previous reports. Comparing all four isomers (Fig. 8), in fact, the most characteristic peaks in the ruthenium(azpy) complexes are the H6 and the H(o) protons, which are found in different magnetic environments due the shielding and deshielding effect of the proximate neighbouring ligands. Between the four isomers, the H(o) resonance differs up to 1.4 ppm, whilst the H6 resonance can differ up to even 2 ppm (actually, even 2.5 ppm between H6 and H6′of **β-Cl**). The H6 signals of both **α-Cl** (9.37 ppm) and **β-Cl** (9.79 ppm) appear at relatively low field, whereas the H(o) signals are shifted to relatively high field. This can readily be explained by the deshielding effect of the chloride ions on the H6 atoms,[14,15,19] and the shielding effect of the pyridine ring or the azo bond on the H(o) atoms. Conversely, the H6′ and H(o′) signals of the second azpy ligand in **β-Cl** are shifted significantly up-field and down-field, respectively, with respect to the equivalent signals of the first-mentioned azpy ligand. This is explained by shielding of the H6′ atom by the pyridine ring and deshielding of the H(o′) protons by a chloride ion. In **γ-Cl** the pyridine H6 protons (8.92 ppm) are oriented in the plane of the pyridine ring of the neighbouring azpy ligand, and a modest deshielding effect is expected. The H6 in the **δ-Cl** (7.70 ppm) on the other hand is oriented close above the shielding cone of the phenyl ring of the other azpy ligand, and a strong shielding is expected and indeed observed.

As discussed above, on basis of NOE cross-peaks alone not all the different isomers can be discriminated. The **β-Cl** shows unique H6–H6′ and H(o)–H(o′) cross-peaks, and the **γ-Cl** isomer can be identified on basis of the absence of H6–H(o) NOE cross-peaks; however, the α, δ and ε isomers are all expected to show H6–H(o) NOE interactions. Notwithstanding this, a discrimination between the $C_2$-symmetric isomers can be made on basis of shielding and deshielding effects. The H6 resonance in **α-Cl** is expected to be shifted strongly down-field, whilst in **δ-Cl** the H6 is strongly shielded. For the H(o) resonances the opposite is true, resulting in the difference between H6–H(o) resonances of +2.6 ppm for **α-Cl** and −0.5 ppm (the H6 is up-field with respect to the H(o) resonance) for the **δ-Cl**. The NMR resonances for **ε-Cl** are expected to be similar to the azpy′ proton resonances of **β-Cl**, so most characteristic shielding of the H6 and deshielding of the H(o), leading to the inversion of the H(6) and H(o) resonances.[35] The NMR spectra of **δ-Cl** and **ε-Cl** might therefore resemble one another, but the H(6) and H(o) resonances of the former should be at lower field, and furthermore show a much stronger NOE cross-peak than the analogous cross-peak of the latter. Finally, discrimination of **δ-Cl** and **ε-Cl** should further be readily confirmed by UV-Vis absorption data, *vide infra*. Summarising, by using 1D and 2D $^1$H NMR spectroscopy it is possible to determine the geometries of the $[Ru(azpy)_2Cl_2]$ isomers, combining the 2D NOESY data with rationalising the deshielding and shielding effects of the chloride and azpy ligands on the H(6) and H(o) protons in the 1D spectra.

### UV-Vis and IR spectroscopies of $[Ru(azpy)_2Cl_2]$ complexes

In the first reports on the bis(azpy)–ruthenium(II) isomers, UV-Vis and IR spectroscopies were the main spectroscopic tools used for the assignment of the **α-Cl** and **γ-Cl** complexes.[3,4] These two techniques provide indeed valuable information, but has unfortunately also led to the mis-assignment of the latter isomer. The IR vibrations of the three isomers **α-Cl**, **β-Cl** and **γ-Cl** have been elaborately discussed by Krause *et al.*,[3] and Chakravorty *et al.*,[4] although the former probably incorrectly located the Ru–N vibrations in the 300–400 cm$^{-1}$ region instead of the 200–300 cm$^{-1}$ region. The **γ-Cl** showing only one Ru–Cl vibration and one Ru–pyridine vibration was assigned to the $C_{2h}$ symmetry (all-*trans*), as the other *trans*-chloro isomer, *tcc* ($C_{2v}$ symmetry), was expected to show more ruthenium–ligand vibrations. We have carefully examined the ruthenium–ligand vibrations region (150–400 cm$^{-1}$) of **γ-Cl** and **δ-Cl**, in CsI pellets, as well as in polyethylene disks, but the spectra of these two isomers appear very similar and do not allow a straightforward discrimination of the two *trans*-Cl isomers on basis of IR spectra. At higher wavenumbers, in the finger-print region, the **δ-Cl** shows a strong absorption of the azo bond at 1347 cm$^{-1}$, which is at higher energy than observed for the other three isomers (1300–1325 cm$^{-1}$), indicating more double-bond

character and less π-bonding to the ruthenium, as is in correspondence with the XRD data, *vide supra*.

Solutions of the four bis(azpy)–ruthenium(II) isomers are all intensely coloured. In Fig. 10 the UV-Vis absorption spectra of the **α-Cl** (sky-blue), **β-Cl** (blue–purple), **γ-Cl** (grass-green), and **δ-Cl** (turquoise) in chloroform are shown. Non-coordinated azpy displays absorption bands at 440 nm ($\varepsilon \approx 0.7 \times 10^4$) and at ~320 nm ($\varepsilon \approx 1.9 \times 10^4$) which have been assigned to n ⟶ π* and π ⟶ π* transitions, respectively, centered primarily on the azo group.[4] In the dichlorobis(azpy)–ruthenium(II) complexes, intense absorption bands appear between 570 and 700 nm. High values for the molar extinction coefficients (~$10^4$) indicate these absorption bands to be of charge-transfer origin, thus assigned to $t_2$(Ru) ⟶ π*(L) transitions (MLCT).[3,4] The shift of the MLCT band of the δ complex to lower energy relative to the equivalent bands of the other isomers, might suggest a smaller Ru–N(azo) bond order, which is in correspondence with the relatively strong double-bond character of the NN bond as concluded from IR data.[5]

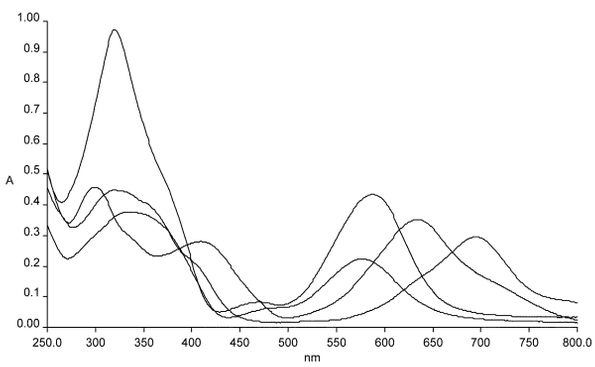


**Fig. 10** UV-Vis spectra of **α-Cl**, **β-Cl**, **γ-Cl** and **δ-Cl** in chloroform. $\lambda_{max}$ (nm) and molar extinction coefficient ($dm^3\ mol^{-1}\ cm^{-1}$) between brackets for **α-Cl**: 589 ($\varepsilon \approx 1.20 \times 10^4$), 469, 320; for **β-Cl**: 576 ($\varepsilon \approx 0.62 \times 10^4$), 480, 334; for **γ-Cl**: 633 ($\varepsilon \approx 0.97 \times 10^4$), 409, 300; for **δ-Cl**: 694 ($\varepsilon \approx 0.82 \times 10^4$), 405, 320.

### α-Cl, β-Cl, γ-Cl, δ-Cl and the missing isomer ε-Cl

Regarding the ε-[Ru(azpy)$_2$Cl$_2$], **ε-Cl**, in which the chloride ions and the pyridine ligands are both in *cis* positions and the azo nitrogens in *trans*, to date, no structural characterisation has been published. Popov and co-workers, in a short letter on thermodynamic and kinetic aspects of the presumed *cct* isomer, report also spectrophotometric data: ($CHCl_3$) $\lambda_{max}$ 540 nm ($\varepsilon \approx 1.0 \times 10^4$).[22] The UV-Vis absorption data of the above presented four isomers, are significantly different from that reported by Popov *et al.*,[22] indicating that Popo's ruthenium(II)–azpy compound indeed could be the missing, third, *cis* dichloro isomer, **ε-Cl**. Although the synthesis of the presumed **ε-Cl** has been published, it is probably not very informative to study its biological activity, as close above room temperature, and surely at physiological temperatures, the **ε-Cl** isomerizes to another (the very active α) isomer.

Some additional, structural, information regarding ruthenium(II)–bis(azpy) isomers with ε configuration is available. In the reaction of the α isomer with the potassium salt of dithiocarbonate the azpy ligands are thiolated and a geometrical reorganisation of the ligands occurs resulting in a complex in which the geometry of the two azpy ligands corresponds to the ε isomer.[36] Furthermore, in 1982 the synthesis of the tris(azpy)–ruthenium(II) complex *mer*-[Ru(azpy)$_3$]$^{2+}$ has been reported,[6] which contains three, non-equivalent, azpy ligands. Analysing the geometries of the three pairs of azpy ligands in *mer*-[Ru(azpy)$_3$]$^{2+}$, the azpy ligands are two-by-two in mutually α, β and ε-geometries. Recently, the molecular structure of this compound has been determined.[7,18] From the inter-ligand distances in these complexes a H6–H(o) NOE cross-peak is expected to be observed also for **ε-Cl**.

With ligands related to 2-phenylazopyridine (azpy), like bis(azopyridine), organometallic complexes have been reported in which the two azo–imine ligands are positioned in an ε-configuration.[37] Also, 2-(arylazo)imidazole ligands have been reported to form stable dichlororuthenium(II) complexes.[23] Even four isomers of the dichlorobis(2-(arylazo)imidazole)-ruthenium complexes have been reported, including the *cct* (ε) isomer,[24] however, this claim is based on spectroscopic analysis only. Surprisingly, for these arylazoimidazole complexes the NMR signals of mutual protons in the different isomers are relatively similar.

### Physicochemical and biological properties of isomeric [Ru(azpy)$_2$Cl$_2$] complexes

The difference in biological activity of the two isomeric *cis*-, and *trans*-dichlorobisammineplatinum(II) complexes has led to one of the main structure–activity relationships for platinum antitumour complexes, *i.e.* the requirement of two *cis*-coordinated leaving groups, *e.g.* chloride ions, which allow the platinum to coordinate in a specific way to two neighbouring guanine bases in DNA.[38] Many ruthenium complexes have shown antitumour, cytotoxic or antimetastatic activity,[39,40] but no clear structure–activity relationships (SARs) have been found, yet. The recent discovery of the different cytotoxicity of isomeric [Ru(azpy)$_2$Cl$_2$] complexes (azpy = 2-phenylazopyridine), and in particular that of the α isomer, is of great interest.[12] Particularly striking is the difference in cytotoxicity between the two *cis*-dichloro isomers, **α-Cl** and **β-Cl** and the structurally related *cis*-[Ru(bpy)$_2$Cl$_2$]. Unfortunately, the neutral dichlorobis(azpy)–ruthenium(II) complexes are rather insoluble in aqueous solution, and so it is difficult to investigate the hydrolysis of the chloride ions and the chemical reactivity of these complexes under physiological relevant conditions. Therefore, several series of water-soluble bis(azpy)–ruthenium(II) complexes have been prepared and are investigated for their biological activity in search for SARs.[12,14,15,18,19] Although the cytotoxicity data of these complexes are not as pronounced as that of the parent **α-Cl**, they do provide useful data to proceed with the *in vitro* and *in vivo* investigation of isomeric ruthenium–(azpy) complexes, as well as related compounds.

The difference in biological activity observed for isomeric ruthenium(II)–bis(azpy) complexes might indicate that specific structural aspects of the ruthenium complexes are crucial for activity, *e.g.* the coordination of the ruthenium to biologically relevant ligands like DNA bases. Ruthenium(II) complexes are generally octahedrally six-coordinated, in contrast to the square-planar coordination of platinum(II) complexes, and therefore the coordination sites are likely to be sterically more hindered than in related platinum complexes. In fact, for α- and β-ruthenium(II)–bis(azpy) isomers the accessibilities of the two potential coordination sites for biologically-relevant nitrogen bases show some interesting differences. Whereas the mono-functional binding of guanine derivatives,[15,19] and adenine derivatives,[16,17] to the α isomer is very similar to that to the β isomer,[19,32] and *cis*-[Ru(bpy)$_2$Cl$_2$],[19,41] the bifunctional co-ordination of the purine model base 1-methylbenzimidazole to the α isomer[19,30] shows significant differences with respect to the binding to the β isomer,[19,32] or to *cis*-[Ru(bpy)$_2$Cl$_2$].[42,43] These differences in orientation and rotational behavior of coordinated nitrogen heterocycles parallel the higher cytotoxicity found for the **α-Cl** and suggest the coordination to biological relevant ligands might play a role in causing the biological activity of antitumour-active ruthenium(II) complexes.

Besides the structural differences between the isomeric ruthenium–azpy complexes that might explain the differences in biological activity, other physicochemical properties of the isomers should not be excluded beforehand. For instance, the azo bond is a very strong π-acidic ligand with a significant effect on *trans*-coordinated ligands. The influence of this effect is

evident in the reaction of azpy and bpy complexes with the thiocyanate ligand, which can bind either *via* the sulfur or the nitrogen atom. Whereas in the related *cis*-$[Ru(bpy)_2(NCS)_2]$ complex only the N-bonded isomer is observed, for α-$[Ru(azpy)_2(NCS)_2]$ all three possible linkage isomers are observed; the *trans*-azo groups have better π-acceptor properties than pyridine and favour also the S-linkage isomer.[44] In the β isomer, one of the labile coordination sites is opposite to an azo bond, whilst the other one is opposite to a pyridine nitrogen. The β-bis(azpy)–ruthenium(II) isomer is therefore expected to be significantly different from the α-isomer in its kinetics and thermodynamics of coordination to (bio)ligands, another possible explanation for the different biological activities of these two isomeric complexes.

## Conclusion

The $[Ru(azpy)_2Cl_2]$ isomers are a unique and interesting set of coordination compounds, which moreover are most interesting because of the different biological activities of the isomers. For structure–activity relationship studies a good understanding of the structural and spectroscopic properties of the compounds under investigation is of paramount importance. Furthermore, a detailed analysis of the structural and spectroscopic data of the bis(azpy)–ruthenium(II) complexes appeared necessary, as, for over two decades, one of the three known isomers, **γ-Cl**, has erroneously been accepted to be the all-*trans* isomer. Papers from the last two decades that regard γ-$[Ru(azpy)_2Cl_2]$, *ttt*-$[Ru(azpy)_2Cl_2]$ or related complexes, should therefore be read with caution. We have unambiguously demonstrated the γ-$[Ru(azpy)_2Cl_2]$ not to be the all-*trans* structure, but the *tcc*-isomer. In this paper a new isomer, δ-$[Ru(azpy)_2Cl_2]$, is presented which has the all-*trans* geometry. From all five possible isomers of $[Ru(azpy)_2Cl_2]$ four have now been unambiguously identified and a short report[22] appears to reveal the fifth isomer. The analyses of structural and spectroscopic data clearly demonstrate that for the azpy complexes the isomeric form of the $[Ru(azpy)_2Cl_2]$ isomers can be determined using a combination of standard 1D and 2D $^1H$ NMR techniques and a discussion of (de)shielding effects.

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33 Although no NMR data of this isomer have been published, from XRD-structures of bis(azpy) complexes with a *cct* configuration, and *mer*-tris(azpy) complexes, *vide infra*, we can expect a H6–H(o) NOE cross-peak for this isomer.
34 It might seem questionable to draw conclusions on the basis of absence of NOE cross-peaks alone. However, if one measures a series of isomeric compounds, one can reasonably assume them to have approximately the same physical-chemical properties relevant for NMR measurements, *e.g.* correlation times. So, by using the same experimental conditions (solvent, concentration, temperature *etc.*) and the same parameter set (mixing time, delay time *etc.*) for recording NOESY spectra, if NOEs are then observed between some protons, the absence of NOEs between other protons is very likely to be of significant value, too.
35 No NMR data of the *cct* isomer are available, but we can expect the H6 resonance, relatively shielded by the aza bond, to fall at about 7.4 ppm. The H(o) resonance, on the other hand, is expected to be close to the resonance observed for the H(o′) in the all-*cis* isomer (compare the isomers in Fig. 3), *i.e.* ~7.8 ppm. Therefore, the H6 is expected to be up-field with respect to the H(o) resonance.
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