Orientation- and Temperature-Dependent Rotational Behavior of Imidazole Ligands (L) in β-$[Ru(azpy)_2(L)_2](PF_6)_2$ Complexes

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The synthesis and characterization of the *cis* bifunctional coordinated ruthenium(II) complexes β-$[Ru(azpy)_2(MeIm)_2](PF_6)_2$ (β-MeIm) and β-$[Ru(azpy)_2(MeBim)_2](PF_6)_2$ (β-MeBim) (azpy = 2-phenylazopyridine, MeIm = 1-methylimidazole and MeBim = 1-methylbenzimidazole) is reported. In β-MeIm the two MeIm ligands can both freely rotate around the Ru–N axes on the NMR timescale. In β-MeBim the two MeBim ligands appear restricted in their rotation around the Ru–N axes, which becomes slow on the NMR timescale at low temperatures. In contrast to the analogous complexes α-$[Ru(azpy)_2(MeBim)_2](PF_6)_2$ and *cis*-$[Ru(bpy)_2(MeBim)_2](PF_6)_2$, only one atropisomer is observed for the two MeBim ligands in β-MeBim. The orientation of the MeBim ligands appears to correspond to an HT isomer which is similar to the orientation of the MeBim ligands in the most abundant atropisomer found in the related α-$[Ru(azpy)_2(MeBim)_2](PF_6)_2$. A stacking interaction between the phenyl ring of one azpy and one MeBim ligand is likely to stabilize the observed atropisomer of β-MeBim, and is such that the rotation of the phenyl ring of one of the two azpy ligands is restricted. At very low temperatures this rotation, or flipping of the phenyl ring between two identical positions, is in the slow-exchange range on the NMR timescale.

Introduction

Dichlorobis(azpy)ruthenium(II) isomers [azpy = 2-(phenylazo)pyridine] have gained interest because of the very different cytotoxicity they exhibit against a series of tumor cell lines, α-$[Ru(azpy)_2(Cl)_2]$ being very cytotoxic and β-$[Ru(azpy)_2(Cl)_2]$ only moderately so.[1] In a similar manner to platinum antitumor compounds, ruthenium complexes are thought to exhibit their cytotoxic activity through their coordination to biological target molecules like proteins or DNA.[2] Therefore, the differences in cytotoxicity found for the isomeric $[Ru(azpy)_2Cl_2]$ complexes might be very useful in the search for a structure-activity relationship (SAR) of antitumor-active ruthenium complexes. From steric aspects derived from the crystal structures[3,4] of α- and β-$[Ru(azpy)_2(Cl)_2]$ and *cis*-$[Ru(bpy)_2(Cl)_2]$, it was suggested that the potential *cis* binding sites in these three complexes are least accessible in the β isomer.[1,5] All three *cis*-$[Ru(LL')_2]$ moieties (LL′ = bpy or azpy) bind only monofunctionally to guanine derivatives.[5–7] Due to the absence of a C_2 axis, the β isomer has actually two different binding sites (see Figure 1), which both appear to be accessible for the binding of an oxopurine ligand; however, the bifunctional coordination of non-tethered purines appears impossible for this isomer.

Previously,[5,8,9] it has been described that the study of the coordination of ruthenium complexes to the lopsided heterocyclic ligand 1-methylbenzimidazole, MeBim, can provide information about the (steric) possibilities of a complex to bind in a *cis* bis(configuration) to the purines of DNA. In the present paper the binding of the lopsided imidazoles (L) 1-methylimidazole (MeIm) and MeBim (see Figure 2) to β-$[Ru(azpy)_2(NO_3)_2]$ is described and their ro-

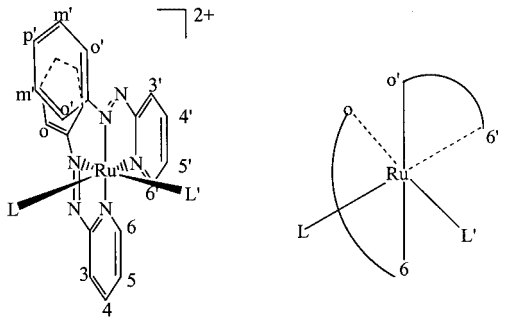

Figure 1. Structural (left) and schematic (right) representation of β-$[Ru(azpy)_2(L)_2](PF_6)_2$ and the used proton numbering scheme

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tational behavior discussed. The results are compared with those obtained for the analogous bpy complexes *cis*-[Ru(bpy)$_2$(L)$_2$](PF$_6$)$_2$,[8,9] and the isomeric α complexes α-[Ru(azpy)$_2$(L)$_2$](PF$_6$)$_2$.[5,10]

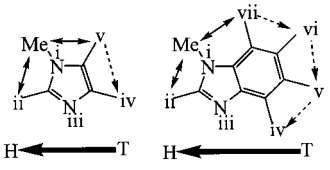

Figure 2. Structural representation, proton numbering scheme, NOE (solid arrows) and COSY (dashed arrows) connectivities of methylimidazole (MeIm, left) and methylbenzimidazole (MeBim, right); the bold arrows (bottom) represent the head-tail axis of the imidazole ligands, as will be used in the schematic drawings in Figures 4 and 9

Results and Discussion

The α- and β-isomers are generally accepted to be the most stable isomers of the bis(azpy)ruthenium(II) complexes.[11−13] However, in solution these two complexes can isomerize, as in fact is observed in the reaction of the isomeric complexes α- and β-[Ru(azpy)$_2$(NO$_3$)$_2$] with guanine derivatives under refluxing conditions in water.[5−7] In the synthesis of bis(adducts) of the type α-[Ru(azpy)$_2$(L)$_2$](PF$_6$)$_2$ no evidence for isomerisation has been found;[5,10] in the present synthesis of the β-isomer bis(adducts), no isomerized compounds were observed either. Apparently, once fully coordinated with nitrogen ligands the α- and β-bis(azpy)ruthenium complexes are stable to isomerisation, even under refluxing conditions in acetone/water.

Similar to the ^{1}H NMR spectra of the analogous complexes α-[Ru(azpy)$_2$(MeIm)$_2$](PF$_6$)$_2$ [5,10] and *cis*-[Ru(bpy)$_2$(MeIm)$_2$](PF$_6$)$_2$,[8,9] the β-MeIm complex shows a ^{1}H NMR spectrum with sharp signals, suggesting that the MeIm ligands are rotating rapidly on the NMR timescale (Figure 3). Also analogous to the bpy and α-azpy systems, the β-MeBim complex shows mainly broad peaks at room temperature, suggesting that the MeBim ligands are rotating at an intermediate speed on the NMR timescale. The possible

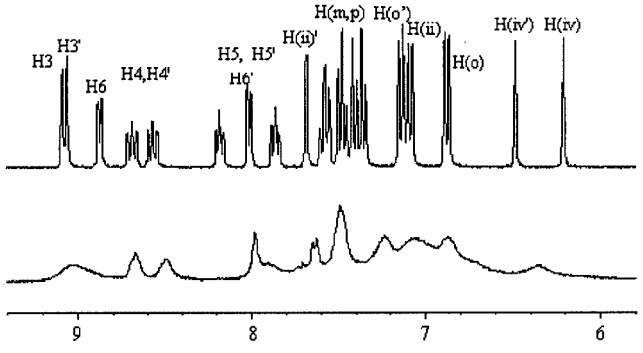

Figure 3. ^{1}H NMR spectra (δ scale in ppm) of the complexes β-MeIm (top) and β-MeBim (lower spectrum) in [D$_6$]acetone, at 25 °C; assignments are indicated in the figure

presence of rotameric structures in the β-MeBim complex requires a short introduction, which is given below.

For two *cis*-coordinated lopsided ligands on the ruthenium ion, the corresponding atoms can be on the same side (head-to-head, HH), or on opposite sides (head-to-tail, HT) of the ligand−metal−ligand plane.[14] As a lopsided ligand prefers not to orient in the same plane as another ligand,[15] four staggered orientations are possible. In a *cis* bifunctional octahedrally coordinated complex 16 possible atropisomers can be drawn.[5] It can be reasonably assumed that the bicyclic ligands will prefer an orientation with the longitudinal axis parallel to each other, and not orthogonal, therefore only the eight atropisomers depicted in Figure 4 have to be taken into account when identifying the atropisomers. In the complex *cis*-[Ru(bpy)$_2$(L)$_2$](PF$_6$)$_2$] the orientation of the imidazole ligands above the bpy ring system is not observed, diminishing the number of possible atropisomers.[8,9] The other four atropisomers, R5−R8, are not observed, the steric hindrance of the bpy rings most likely preventing the orientation of an MeBim unit above its ring system. It appears, however, that such an orientation cannot be excluded in α-[Ru(azpy)$_2$(L)$_2$](PF$_6$)$_2$ complexes.[5,10] The identification of the observed rotamers for bis(azpy)ruthenium complexes therefore requires a detailed investigation and consideration of all the eight atropisomers R1−R8. Most convenient for the identification of atropisomers in the α-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ system[5,10] is the fact that the C_2 symmetry in the α-[Ru(azpy)$_2$] moiety reduces the number of different rotamers from eight to six. Furthermore, four of the HT rotamers have C_2 symmetry, resulting in the two azpy and the two MeBim ligands being indistinguishable by ^{1}H NMR spectroscopy, which proves the presence of the HT isomers already from a 1D ^{1}H NMR spectrum. Due to the lack of C_2 symmetry in the β-[Ru(azpy)$_2$] moiety, all the eight atropisomers are different from each other, and each of them is expected to give different proton resonances for both azpy and both imidazole ligands.

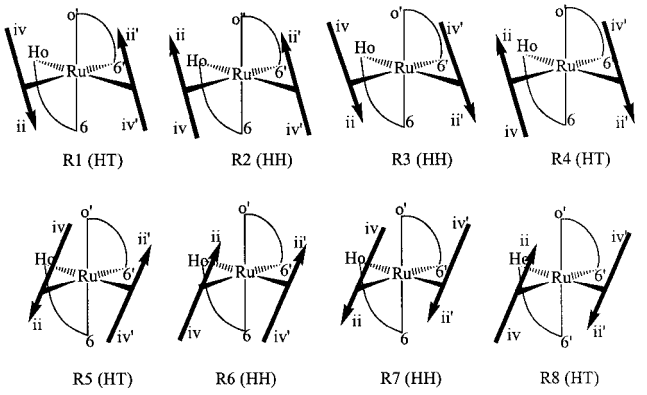

Figure 4. Schematic representation of eight theoretically possible rotameric structures of β-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$

β-[Ru(azpy)$_2$(MeIm)$_2$](PF$_6$)$_2$ (β-MeIm)

The ^{1}H NMR spectrum of β-MeIm at room temperature is shown in Figure 3. In the aromatic region, 20 resonances

are found, several of which overlap with other signals. With the use of COSY and NOESY data the complete assignment of all resonances has been done (Figure 3 and Table 1). One doublet and two triplet peaks with double intensity in the high-field region can immediately be attributed to the azpy *ortho* [H(*o*)], and *meta* [H(*m*)] protons of the phenyl ring, respectively, after which a COSY spectrum reveals the triplet at δ = 7.55 ppm to be attributed to H(*p*). The MeIm peaks were assigned starting from an NOESY spectrum in which the Me(i) resonances show interaction with the parent H(ii) as well as the H(v) proton resonances; the latter also shows a cross peak with H(iv). From a COSY spectrum, the four signals of the pyridine part of the azpy ligands could be correlated; on the basis of the ^3J coupling, the doublets at δ = 8.8 and 7.9 ppm were assigned to the H6 and H6′ resonances (≈ 6 Hz), whilst the doublet at δ = 9.0 ppm (≈ 9 Hz) was assigned to the two overlapping H3 and H3′ resonances. The peak assignment was further confirmed by the (weak) NOE cross-peaks observed between the H6 and the H6′ resonances, and the H(*o*) and H(*o*′) signals, as would be expected for the β-isomer.[5,7,16]

Table 1. Selection of ^{1}H NMR spectroscopic data (chemical shifts) of β-[Ru(azpy)$_2$(MeIm)$_2$](PF$_6$)$_2$, β-MeIm, and β-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$, β-MeBim, in [D$_6$]acetone at room temp. and at −95 °C, respectively

	H3	H6	H3′	H6′	H(ii)	H(iv)	H(ii′)	H(iv′)
β-MeIm	9.08	8.87	9.08	8.02	7.08	6.21	7.69	6.49
β-MeBim	9.35	7.82	9.35	8.87	8.02	5.3	7.76	6.35

On the basis of only the inter-azpy NOE interactions, it is impossible to attribute one of the two sets of pyridine- or one of the two sets of phenyl-ring proton resonances to a specific azpy ligand. Whereas the interligand NOE peaks of H6 with H(*o*) in a (non-C_2-symmetric) bis(adduct) of the α-isomer allow to distinguish between the azpy moieties, this is not the case for the β-isomer.[5,16] As the H6′ is oriented just above the pyridine ring of the other azpy ligand (Figure 5) a relatively strong shielding of this proton resonance is expected with respect to the H6 proton, and therefore the high-field doublet with a ^3J coupling of 6 Hz has been attributed to the H6′ proton, whilst the low-field doublet with a coupling of 6 Hz is assigned to H6. The

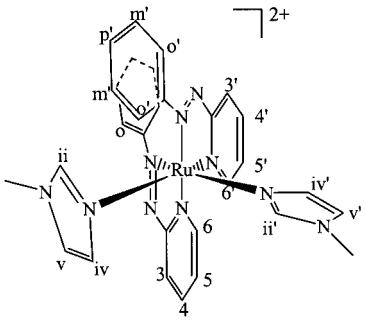

Figure 5. Structural representation and proton numbering scheme of β-[Ru(azpy)$_2$(MeIm)$_2$](PF$_6$)$_2$ (β-MeIm)

resonances of the two phenyl ring systems have been assigned on the basis of the interligand azpy-MeIm NOE interactions (vide infra).

The ^{1}H NMR spectrum of β-MeIm (Figure 3) shows two sets of azpy and two sets of imidazole signals. In theory, such a spectrum can either represent a complex in which both the MeIm ligands are rotating rapidly on the NMR timescale, or it can represent one of the eight atropisomers depicted in Figure 4. As the MeBim ligands in this complex show rotational behavior at room temp. (vide infra) it is reasonable to assume the smaller MeIm ligands do so, too. Upon lowering the temperature to −95 °C, the ^{1}H NMR spectrum of β-MeIm does not change significantly, indicating that at lower temperatures the MeIm ligands are still rotating rapidly on the NMR timescale. In the study with the *cis*-[Ru(bpy)$_2$(L)$_2$]$^{2+}$ [8,9] and α-[Ru(azpy)$_2$(L)$_2$]$^{2+}$ complexes[5,10] it is pointed out that the didentate heterocycles are spectator ligands, with the protons closest to the coordination sites functioning as probe protons that can be used to monitor the orientation and/or the rotation of the monodentate imidazole ligands L. The present results indicate that the H6/H6′ and the H(*o*)/H(*o*′) protons of the azpy ligand can also function as probe protons to monitor the orientation and rotational behavior of the *cis* bis(coordinated) ligands L. The NOESY spectrum of β-MeIm (Figure 6) shows that the azpy H6 proton interacts with the H(ii) and H(iv) protons of both MeIm ligands. These NOE peaks indicate that the MeIm ligands are rotating around their Ru−N axes on the NMR timescale, and this is further confirmed by the cross peaks between the two MeIm ligands: H(ii)-H(ii′), H(ii)−H(iv′), H(iv)−H(ii′) and H(ii)−H(iv′). The 2D ^{1}H NMR spectroscopic data clearly confirm, therefore, that the MeIm ligands are indeed rotating (fast) around their Ru−N(iii) axes.

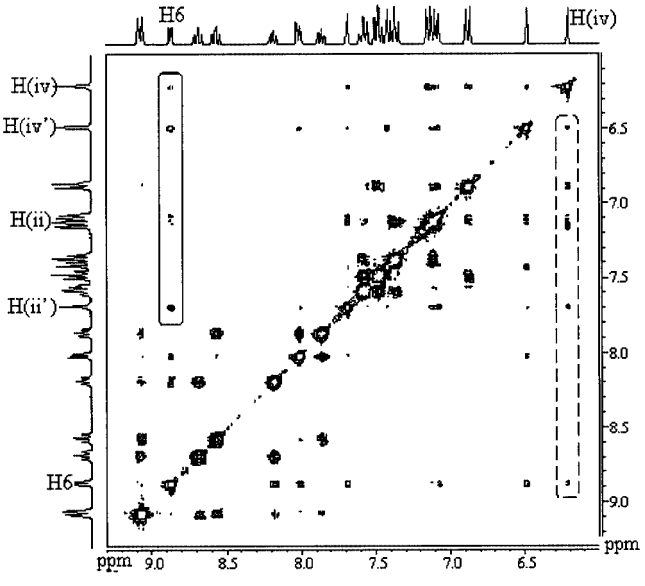

Figure 6. NOESY spectrum of β-[Ru(azpy)$_2$(MeIm)$_2$](PF$_6$)$_2$, in [D$_6$]acetone at 25 °C; the highlighted areas indicate the NOE cross peaks of the H6 (solid rectangle) and H(iv) (dashed rectangle) resonances

β-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ (β-MeBim)

Variable-Temperature ^{1}H NMR Behavior

At room temperature broad peaks are observed in the ^{1}H NMR spectrum of β-MeBim (Figure 3). These are characteristic of a complex with different rotamers that are exchanging with each other on the NMR timescale. In the ^{1}H NMR spectrum of β-MeBim at higher temperatures, no (complete) sharpening of the signals is observed. On lowering the temperature, on the other hand, a sharp set of signals is observed (Figure 7). All the azpy protons resonances can be assigned from the COSY and NOESY spectra in a similar way to β-MeIm. The identification of the rotamers was made from the 2D NOE data and by evaluating the (de)shielding effects of the ligands on the other proton resonances; the rotational aspects of the ligands at different temperatures were determined from the ROESY data.

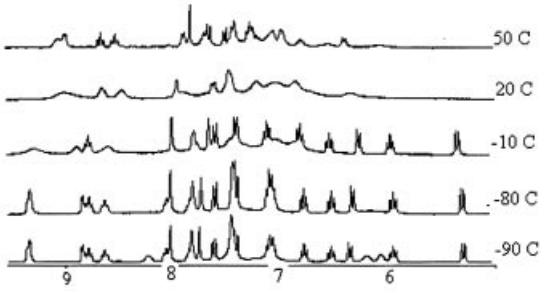

Figure 7. ^{1}H NMR spectra (δ scale in ppm) of β-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ at different temperatures; assignment of resonances in the low temperature spectrum is given in Table 1

Analogous to the discussion of the MeIm complex, for β-MeBim a theoretical total of 24 resonance signals in the aromatic region for the two azpy ligands and the two MeBim ligands could indicate the presence in solution of only one symmetric atropisomer, in slow exchange on the NMR timescale, or on the other hand it could indicate the average of a rotational system which is in fast exchange on the NMR timescale. For β-MeBim the ^{1}H NMR spectrum at room temperature shows broad peaks, suggesting that the presence of different atropisomers could possibly be observed at lower temperatures. The results obtained with the α-[Ru(azpy)$_2$(MeBim)$_2$]$^{2+}$ complex explicitly demonstrate[5,10] that one should take great care in interpreting the ^{1}H NMR spectroscopic data of rotational complexes, as a set of signals can indicate a system which has no rotating ligands on the NMR timescale, but can also indicate a system with fast moving ligands, which can freeze out into different atropisomers upon lowering the temperature. Furthermore, the sets of signals belonging to these "atropisomers" in slow exchange can sometimes represent the average signal of more possible atropisomers.[5,10] For β-MeBim, the spectrum at −95 °C is shown to represent one specific atropisomer (see next section), but the VT ^{1}H NMR spectroscopic data show one particular feature that should be treated in more detail. This regards the rotational behavior of the phenyl ring of one of the two azpy ligands, which, due to interaction with the lopsided ligands, becomes slow on the NMR timescale in the low-temperature region.

Orientations of the Two MeBim Ligands in β-MeBim

The orientation of the MeBim ligands in β-MeBim as observed at low temperatures was determined from the NOE data. As pointed out for the MeIm complex β-MeIm, the H(*o*) and H6 protons of the azpy ligands are found close to the imidazole ligands, and NOE interactions are most useful for determining the orientation of the MeBim ligands. Unfortunately, the H(*o*) resonances are very broad at the recording temperature and no NOE cross-peaks are observed. The most important NOE cross peaks observed for β-MeBim (Figure 8) are the H6−H(iv′) and the weak cross peaks between the H(ii) and H(iv′) resonances and H(iv) and H(ii′) resonances. These three cross peaks point to a conformer in which the imidazole H(ii) protons of both the benzimidazole ligands are oriented above the aza binding of their *fac*-coordinated azpy ligands (R5 in Figure 4; see Note added in proof). The six-membered ring of the MeBim ligand [H(iv)] is oriented in a face-to-face manner with the pyridine of the *mer*-coordinated azpy ligand, whilst the other MeBim ligand [H(iv′)] has its six-membered ring face-to-face with the phenyl ring of the *mer*-oriented azpy ligand. This HT atropisomer is different from the two HT atropisomers found for the *cis*-[Ru(bpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ system[8,9] in which none of the rotamers has the lopsided part of a MeBim ligand oriented above the didentate ligand. Interestingly, this rotamer is very similar to the most abundant (HT) atropisomer observed in the related α complex.[5,10]

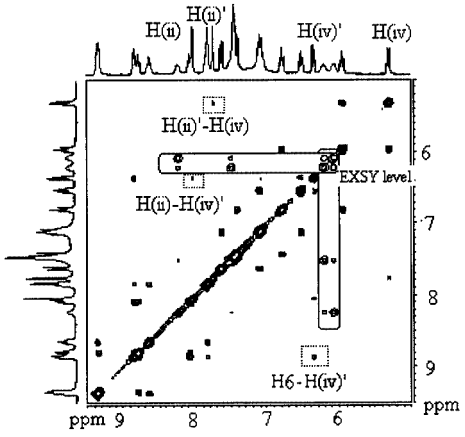

Figure 8. ROESY spectrum of β-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ (β-MeBim) in [D$_6$]acetone at −95 °C; NOE cross peaks are highlighted in the dotted areas (see Note added in proof)

It is most likely that the bulky, rotating phenyl rings of the two azpy ligands prevent the MeBim ligand from orienting in the direction of the two aromatic rings of the two azpy ligands (R1−R4).The orientation of the MeBim′ li-

gand with the six-membered ring above the azpy ligand is also not expected due to severe steric clashes (R7, R8). For the same reason atropisomer R6 is likely to be sterically disfavored. The remaining atropisomer, R5, has the MeBim ligands oriented in such a way that the imidazole ring protons are oriented close to the aza bond of the azpy ligands. Besides the steric effects preferring this orientation, an electronic effect is possibly also stabilizing the orientations of the MeBim ligands. Marzilli et al.[17] have pointed out that the orientation of imidazole ligands can partly be determined by the electrostatic interaction between the relatively positive NCHN δ^+ site of the imidazole ring with partly negative ligands like chloride ions. In β-MeBim there might be two such interactions between the imidazole ring of both MeBim ligands and the aza bond of the respectively *fac*-oriented azpy ligands.

In summary, taking into account that, for symmetry reasons the two HH rotamers in α-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ each represent two (identical) atropisomers, seven out of the eight possible orientations of the two MeBim ligands shown in Figure 4 are observed.[5,10] The isomeric complex β-MeBim, however, shows only one stable atropisomer, i.e. the HT isomer R5 (Figure 4).

From the discussion of the possible orientation of the non-tethered MeBim ligands in β-MeBim, it is concluded that only one stable atropisomer is to be expected. For the investigation of atropisomers in *cis*-[Ru(bpy)$_2$(L)$_2$](PF$_6$)$_2$ complexes, the mdbz ligand [bis(1-methyl-2-benzimidazolyl)-ethane] has been used to prepare the complex *cis*-[Ru(bpy)$_2$(mdbz)](PF$_6$)$_2$. In this mdbz complex, the two MeBim moieties are oriented as in the R1 atropisomer (A in the previous paper)[9] but are tethered, and therefore rotation of the imidazole ligands around the Ru−N axes cannot be expected and is indeed not observed. On reaction of β-[Ru(azpy)$_2$(NO$_3$)$_2$] with mdbz, however, no β-[Ru(azpy)$_2$(mdbz)](PF$_6$)$_2$ could be obtained at all. From a space-filling model of β-[Ru(azpy)$_2$(mdbz)](PF$_6$)$_2$, and from Figure 4, it is clear that the didentate ligand mdbz cannot coordinate in a bifunctional mode to the β-bis(azpy) isomer in such a way as to adopt the HT atropisomer R5, as the imidazole moieties in the R5 orientation are too distant. On the other hand, to adopt the HT orientation from atropisomer R1, one phenyl ring of the mdbz should be coordinated wedged in between the pyridine rings of the two azpy ligands. The "tail" part of the mdbz in β-[Ru(azpy)$_2$(mdbz)](PF$_6$)$_2$ should be wedged in between the phenyl rings of the two azpy ligands. In particular, the phenyl rings of the two azpy ligands are prevent the second benzimidazole moiety of the mdbz from coordinating to the ruthenium ion. (See the Supporting Information where the hypothetical structure of β-[Ru(azpy)$_2$(mdbz)](PF$_6$)$_2$ is drawn schematically.) This indicates that the (main) HT conformation in which the benzimidazole ligands coordinate to the *cis*-[Ru(bpy)$_2$] is not possible for the β-[Ru(azpy)$_2$] complex, and confirms the mdbz to be a good model ligand for an HT interaction in which the imidazole sites of the MeBim ligands are pointing towards each other.

(De)Shielding Effects

Because of the different (de)shielding effects caused by the different orientations of the MeBim ligands, the probe protons shift characteristically, as is nicely illustrated for the three main atropisomers found[5,10] in α-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ and the three atropisomers observed[8,9] in *cis*-[Ru(bpy)$_2$(MeBim)$_2$](PF$_6$)$_2$. In β-MeBim only one atropisomer is observed, and therefore the relative shifts of the probe protons in different atropisomers cannot be discussed. However, a few remarkable proton resonances do require further discussion.

The most upfield-shifted aromatic proton signal appears to be the H(iv) resonance at δ = 5.30 ppm. In the free ligand the H(iv) proton resonance is observed at δ = 7.6 ppm, and in the *cis*-[Ru(bpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ complex at δ = 7.1−7.2 ppm for the atropisomer R2/R3 and R4 (atropisomers B and C, respectively, as mentioned in the corresponding previous publications).[8,9] In the α-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ complex the most upfield-shifted H(iv) resonance signals are at δ = 6.1 and 6.2 ppm,[5,10] and belong to the least abundant atropisomer R6/R7. The exceptional upfield shift of H(iv) in β-MeBim can be nicely explained by the strong shielding effect of the proximate phenyl ring of the azpy ligand (see Figure 9).

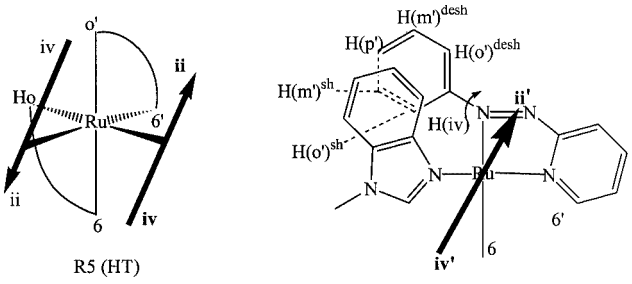

Figure 9. Schematic representation of the atropisomer R5 of β-MeBim (left) and projection along the MeBim′−Ru axis (right); the overlap and consequential mutual shielding effects of the six-membered rings of the azpy phenyl group and the MeBim ligand is visualized

It is most likely that some kind of stacking interaction is present, with H(iv) oriented just above the slowly flipping phenyl ring, which most of the time is oriented parallel and face-to-face with the MeBim six-membered ring. Consecutively, the two phenyl ring H(*o*) protons, once having become inequivalent due to the slow rotation of the ring around the C−N axis, are, in turn, expected to shift characteristically due to the shielding effect of the six-membered ring of the MeBim ligand. In fact, one resonance [δ = 6.1 ppm, H(*o*)^{sh}] is shifted strongly upfield whilst the other is deshielded [δ = 8.3 ppm, H(*o*)^{desh}] and shifted downfield. The H(*m*′) protons show a similar effect {δ = 6.3 [H(*m*′)^{sh}] and 7.6 ppm [H(*m*′)^{desh}]}, albeit with a frequency difference, $\Delta\nu$, less extreme than observed for the H(*o*) resonances (660 and 390 Hz, respectively).

From Figure 9 the mutual shielding effect of the azpy phenyl ring and the MeBim six-membered ring is evident. The H(*o*′)^{sh} signal is assigned to the proton which is oriented just above the shielding cone of the MeBim six-mem-

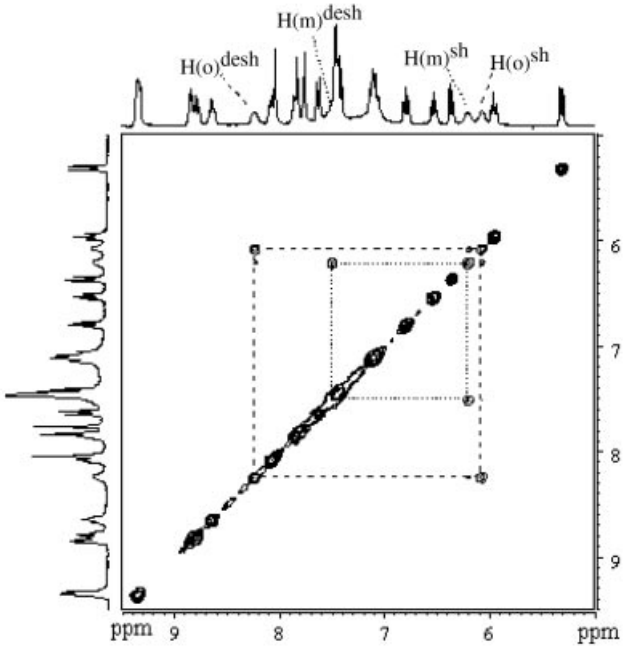

Figure 10. EXSY level of the ROESY spectrum of β-MeBim in [D$_6$]acetone at −95 °C; the H(*o′*) and H(*m′*) exchange peaks are indicated with dashed and dotted lines, respectively

bered ring, whilst the H(*o′*)^{desh} signal is attributed to the other H(*o′*) proton. NOE cross peaks between H(*o′*) and H(iv) would unambiguously confirm this orientation; however, none are observed. At −95 °C the phenyl ring is still flipping as can be concluded from the cross peaks observed in the EXSY level of a ROESY spectrum (Figure 10). The H(*o′*) resonance is too broad to detect any NOE interaction. However, all assignments and conclusions agree well with the results of the analogous 1,2-dimethylimidazole complex, in which the phenyl ring is rotating even more slowly on the NMR timescale than in the analogous MeBim complex, and the signals of the phenyl ring protons are sharper.[18]

Exchange Mechanism

The kinetics of exchanging systems can be studied by 1D and/or 2D NMR spectroscopic techniques. At higher temperatures a broadening of signals is observed for β-MeBim which might be due to rotational behavior of the MeBim ligands. The broadening of the peaks is not the result of interchanging rotamers present at low temperatures. The energy input upon increasing the temperature might allow the interconversion of the atropisomers R5 into (one of the) other atropisomers. In the highly symmetric system *cis*-[Ru(bpy)$_2$(4Pic)$_2$](PF$_6$)$_2$, in which the four rotamers present are all identical,[19] the thermodynamic parameters of the rotation of a heterocyclic six-ring ligand, 4-picoline, could be determined. The phenyl ring rotation in complex β-MeBim is observed to be relatively independent from the other rotational processes in the temperature range between −95 °C and −50 °C. However, close to the temperatures where the coalescence of the H(*o′*)^{desh} and H(*o′*)^{sh}, and the H(*m′*)^{desh} and H(*m′*)^{sh} signals are expected, the other signals of β-MeBim are also broadening, indicating that another rotational process is occurring. Besides the fact that the coalescence temperature of the phenyl ring protons is difficult to observe because of overlap with other signals, the occurrence of other dynamic processes in the same temperature region severely complicates the calculation of the thermodynamic parameters.

Conclusion

The synthesis and characterization of the bifunctional coordinated ruthenium(II) complexes β-[Ru(azpy)$_2$(L)$_2$](PF$_6$)$_2$ provides detailed information of the steric properties of the β-[Ru(azpy)$_2$] moiety. Both MeIm ligands in β-MeIm can rotate freely on the NMR timescale at all recording temperatures. The MeBim ligands in β-MeBim, in contrast to MeBim in the analogous complexes α-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ and *cis*-[Ru(bpy)$_2$(MeBim)$_2$](PF$_6$)$_2$, do not orient in different ways on the ruthenium ion at low recording temperatures, and only one atropisomer is observed. By using NMR techniques, including COSY and NOESY, the complete structural characterization of the conformation has been carried out, and the MeBim ligands appear to be oriented in an HT orientation similar to that found in the isomeric complex α-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$. An interesting observation is that the steric hindrance of the MeBim ligands is such that the rotation of the phenyl rings of one of the two azpy ligands is hindered. At very low temperatures the rotation is just at slow exchange on the NMR timescale, causing all five phenyl-ring protons to become inequivalent. This stacking interaction probably stabilizes the observed atropisomer.

The results for α-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ and β-[Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ might indicate that the cytotoxic effect of the α-[Ru(azpy)$_2$Cl$_2$] complex is not due to a (static) interaction with the DNA bases in an HT configuration, which is observed in the main atropisomer of all three complexes. The antitumor drug cisplatin coordinates in a *cis* bis(mode) to two neighboring guanine units in DNA, with the guanine units oriented in an HH conformation.[20] In the complex β-MeBim, neither of the two MeBim ligands appears to rotate around its Ru−N(iii) axis on the NMR timescale. The 1D and 2D VT ^{1}H NMR studies on complexes of the type *cis*-[Ru(Xpy)$_2$(L)$_2$]$^{2+}$ (with Xpy being a didentate ligand like bpy or azpy, and L an imidazole ligand) confirm clearly that complexes of the type *cis*-[Ru(Xpy)$_2$(L)$_2$]$^{2+}$ are sterically borderline cases in which bifunctional coordination of lopsided heterocycles depends on relatively small differences in the ligands or in their mutual orientations. The 1D and 2D VT ^{1}H NMR study presented here is an elaborate, but informative, method for determining such small differences in the steric properties of complexes of the type *cis*-[Ru(Xpy)$_2$]$^{2+}$. Whether the coordination and/or fluxional properties of heterocyclic ligands to *cis*-[Ru(Xpy)$_2$]$^{2+}$-type ruthenium complexes is indeed crucial for the biological activity of the complexes is a sub-

ject that should be investigated further with other *cis*-[Ru(Xpy)$_2$]$^{2+}$ complexes, as well as with in vitro DNA binding studies.

The current data prove that coordination of heterocyclic bicyclic ligands to bpy and azpy complexes differs significantly. The β-[Ru(azpy)$_2$]$^{2+}$ moiety is sterically less versatile than the *cis*-[Ru(bpy)$_2$]$^{2+}$ and α-[Ru(azpy)$_2$]$^{2+}$ moieties. Therefore, the β isomer, which is biologically not as active as the α isomer, shows a relatively reduced affinity regarding coordination to the heterocyclic ligands. Besides the orientation of DNA bases to platinum complexes, also the (ligand) rotational properties are accepted to be important factors influencing the antitumor properties.[20] If these differences in orientational and rotational behavior can indeed explain the differences in biological activity of *cis*-dichlororuthenium complexes, then a systematic, extensive investigation of a series of complexes of the type *cis*-[Ru(Xpy)$_2$] is of paramount importance.

Experimental Section

Materials and Physical Measurements: 1-Methylimidazole (MeIm, Aldrich) and 1-methylbenzimidazole (MeBim, Sigma) were used without further purification. Hydrated $RuCl_3$ was used as received from Johnson Matthey; azpy and β-[Ru(azpy)$_2$(Cl)$_2$]·$CHCl_3$ were prepared according to literature procedures.[11] For the purification of β-Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ neutral aluminum oxide (Alumina Woelm N Super I) was used. The complex β-[Ru(azpy)$_2$-(NO$_3$)$_2$]·$CHCl_3$ was prepared as described elsewhere.[5,7] NMR measurements were performed as described before[8,9] at 300.13 MHz with a Bruker 300 MHz DPX spectrometer, equipped with a Bruker B-VT1000 variable-temperature unit. Concentrations were in the 0.01 M region or below.

Syntheses

β-Ru(azpy)$_2$(MeIm)$_2$](PF$_6$)$_2$ (β-MeIm): β-[Ru(azpy)$_2$(NO$_3$)$_2$]·$CHCl_3$ (0.15 g, 0.21 mmol) was dissolved in a mixture of 10 mL of water and 4 mL of acetone and stirred at room temperature for 24 h. After addition of an excess of 1-methylimidazole (0.78 g, 9 mmol), the solution was refluxed for 3 h, and after addition of NH_4PF_6, β-MeIm was isolated by filtration. Recrystallization from ethanol/water, and from acetone/diethyl ether resulted in a purple microcrystalline material. Yield 0.10 g (54%). $C_{30}H_{30}F_{12}N_{10}P_2Ru$ (921.63): calcd. C 39.10, H 3.28, N 15.2; found C 38.5, H 3.18, N 14.7.

β-Ru(azpy)$_2$(MeBim)$_2$](PF$_6$)$_2$ (β-MeBim): β-[Ru(azpy)$_2$(NO$_3$)$_2$]·$CHCl_3$ (0.15 g, 0.21 mmol) was dissolved in a mixture of 10 mL of water and 4 mL of acetone and stirred at room temperature for 24 h. An excess of 1-methylbenzimidazole (1.19 g., 9 mmol) was added and the solute was refluxed for 3 h. The solid isolated after addition of NH_4PF_6 was recrystallized from ethanol/water and further purified by column chromatography with acetone/methanol (99:1) as eluent. Yield 0.060 g. (42%).^{1}H NMR spectroscopic data indicate the presence of a co-crystallized acetone molecule. $C_{38}H_{34}F_{12}N_{10}P_2Ru$·$(CH_3)_2CO$ (1079.83): calcd. C 45.6, H 3.73, N 12.9; found C 45.8, H 3.79, N 12.6.

Supporting Information: Schematic representation of the α and β isomers of Ru(azpy)$_2$L$_2$ species is given in Figure S1 (see also footnote on the first page of this article). A representation of the mdbz ligand and a hypothetical orientation of the mdbz ligand on the β-[Ru(azpy)$_2$] is given in Figures S2 and S3. The steric hindrance of the two phenyl rings of the two azpy ligands prevents the coordination of one of the MeBim moieties to the ruthenium ion.

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Note added in proof (December 16, 2002): The assignment to this atropisomer is further confirmed by the observation of (very) weak NOE cross peaks from both H(ii) and H(ii)′ with the (overlapping) H3 and H3′ proton resonances (not visible in the present Figure 8).

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