

NH $\cdots\pi$ Interactions Stabilize the Most-Hindered Rotamer of the (S)-Tyrosinato Side Group in Bis[(S)-tyrosinato](diamine)cobalt(III) Complexes: An NMR Spectroscopic and DFT Study^[‡]

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Dedicated to the memory of Professor Milenko B. Čelap^[‡‡]

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Conformational analysis based on an analysis of the vicinal α - and β -proton coupling constants of (S)-tyrosinato ligands in diastereomers of a bis[(S)-tyrosinato](1,3-diaminopropane)-cobalt(III) complex is used to calculate the mol fractions of the three most stable rotamers (*t*, *g*, *h*) of the (S)-tyrosinato ligand's side groups in D₂O solution. The results of this conformational analysis indicate a population increase in the sterically least favorable rotamer *h* in diastereomers of C₁-molecular symmetry (complexes **5** and **6**). The TOCSY spectrum of complex **6** in aqueous solution shows an exceptionally small chemical shift of one NH₂ proton from the coordinated diamine, which is explained by an interligand NH $\cdots\pi$ interac-

tion. These findings demonstrate the persistence of this weak, noncovalent interaction in water solution. DFT calculations for complex **6**, whose diamine ring is in a chair conformation, indicate that the complex with an *h* conformation in both its (S)-tyrosinato ligand side-residues, which yields the most frequent NH $\cdots\pi$ interactions, represents an energy minimum. The fact that coordinated 1,3-diaminopropane in the examined complex **6** is in a chair conformation in aqueous solution is proved by the NMR analysis.

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Introduction

The side-residues of aromatic amino acids participate in many in vivo molecular recognitions, such as antibody–antigen,^[1–3] enzyme–substrate,^[4–7] protein–nucleic acid bases^[8,9] etc. Molecular recognition is brought about by various forms of noncovalent interactions in which the side-residues of aromatic amino acids take part, such as hydrogen bonding (Tyr, Trp), stacking interactions, hydrophobic interactions, cation– π interactions, and various forms of XH $\cdots\pi$ interactions (X = C, N, O). During the past decade the importance of NH $\cdots\pi$ interactions in molecular recognition in biochemical systems and in maintaining the native protein structure has emerged,^[10–14] although such systems are very complex to study because of the multiple weak

interactions which act in synergy. This gives rise to a need for simple model systems suitable for studying these interactions.

Although NH $\cdots\pi$ interactions in proteins have been known since 1984,^[15] in complexes of transition metals they have been discovered only recently^[16] as, because of their low interaction energy,^[17,18] they are difficult to detect in solution. One of the best ways to identify the presence of NH $\cdots\pi$ interactions in aqueous solution is the anomalous chemical shift of the NH protons involved in the interaction.^[19–25]

We recently reported that complexes of cobalt(III) with (S)-tyrosinato and (S)-phenylalaninato ligands can serve as simple model systems for studying NH $\cdots\pi$ and $\pi\cdots\pi$ interactions.^[26,27] In our previous paper,^[27] conformational analysis based on an analysis of the vicinal α - and β -proton coupling constants was used to calculate the mol fractions of the three most stable rotamers of (S)-tyrosinato and (S)-phenylalaninato side-groups in diastereomers of [Co{(S)-tyr}₂en]⁺ and [Co{(S)-phe}₂en]⁺ in D₂O solution. The results of this analysis indicated a population increase in the sterically least favorable rotamer *h* in C₁-molecular symmetry diastereomers, which for complex **5 tyr** is proved to be due to the intra- and interligand NH $\cdots\pi$ interactions.^[27]

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In this paper, on the basis of conformational analysis, our aim was to obtain the data on the rotamer population of the (*S*)-tyrosinato ligand side-groups in diastereomers of the previously synthesized $[\text{Co}\{(\textit{S})\text{-tyr}\}_2\text{tn}]^+$ complex^[28] and to establish whether a population increase of rotamer *h* in C_1 -molecular symmetry diastereomers of the complex exist, and if so, whether that is due to NH $\cdots\pi$ interactions as well. We also used ab initio DFT molecular orbital calculations in our study.

Results and Discussion

Rotamer Distribution – NMR Study

The complex bis[(*S*)-tyrosinato](1,3-diaminopropane)cobalt(III) chloride was synthesized by treatment of the alkali metal salt of (*S*)-tyrosine with bis(1,3-diaminopropane)carbonato)cobalt(III) and then separated into its diastereomers (Figure 1) on an optically active Sephadex QAE column.^[28]

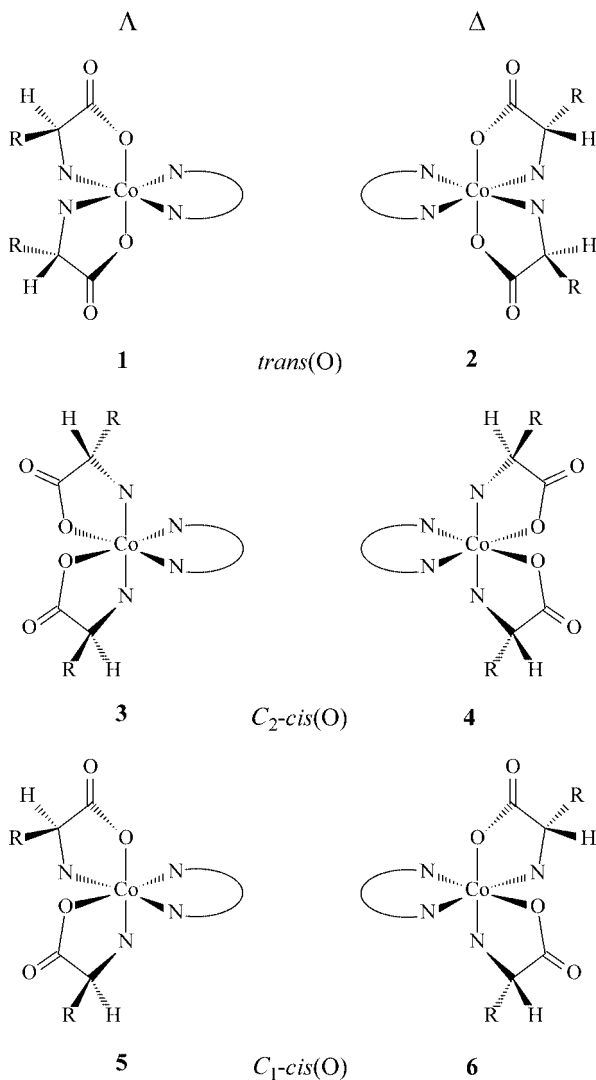


Figure 1. Theoretically possible diastereomers of $[\text{Co}\{(\textit{S})\text{-tyr}\}_2\text{tn}]$ ($\text{R} = \text{CH}_2\text{C}_6\text{H}_4\text{OH}$).

According to symmetry rules, the diastereomers can be divided into two groups: those having a C_2 -molecular axis and higher degree of molecular symmetry [*trans*(O)- and *trans*(N) isomers, complexes **1–4**] and those having no symmetry elements [C_1 -*cis*(O) isomers, complexes **5** and **6**].

Newman projections for the three most stable rotamers of the (*S*)-tyrosinato ligand side-group in the complex are shown in Figure 2. The mol fractions of the *t*, *g*, and *h* rotamers of the (*S*)-tyrosinato ligand side-groups in D_2O solution were calculated on the basis of the vicinal coupling constants of the α - and β -protons (J_{AX} and J_{BX})^[29–31] as in our previous paper^[27] (Table 1).

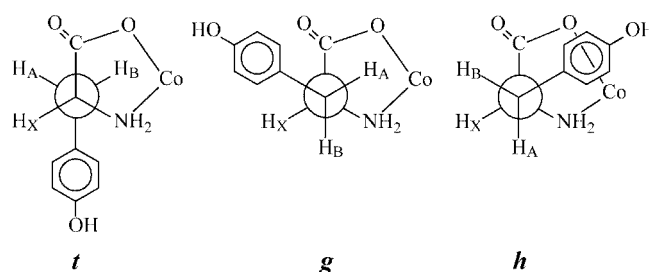


Figure 2. Newman projections of the three staggered rotamers of the (*S*)-tyrosinato side group in the cobalt(III) complex (the β carbon is in front and the α carbon is in the rear).

Table 1. Coupling constants and calculated rotamer mol fractions^[31] of the (*S*)-tyrosinato side-groups in noncoordinated (*S*)-tyrosine, and in diastereomers of $[\text{Co}\{(\textit{S})\text{-tyr}\}_2\text{tn}]^+$ (bold), and $[\text{Co}\{(\textit{S})\text{-tyr}\}_2\text{en}]^+$ ^[27] (*) in D_2O solution.

	J_{AX}	J_{BX}	<i>t</i>	<i>g</i>	<i>h</i>
(<i>S</i>)-tyrosine	5.4	7.4	45	24	31
Λ - C_2 - <i>cis</i> (O) (3)	4.7	8.1	53	16	31
(3 *)	4.3	10.3	77	12	11
Δ - C_2 - <i>cis</i> (O) (4)	4.6	9.0	63	15	22
(4 *) ^[a]	5.0	9.4	67	19	14
Λ - C_1 - <i>cis</i> (O) (5)					
tyr I	5.3	8.3	55	22	23
tyr I*	5.3	8.5	57	23	20
tyr II	5.7	5.7	<i>t</i> + <i>g</i> = 54		46
tyr II*	5.6	5.6	<i>t</i> + <i>g</i> = 51		49
Δ - C_1 - <i>cis</i> (O) (6)					
tyr I	5.2	6.2	32	21	47
tyr I*	4.9	7.2	43	18	39
tyr II	4.6	8.6	59	15	26
tyr II*	4.3	7.8	50	11	39

[a] These results have not been published to date.

With regard to the differences in symmetry, in diastereomers of C_2 -molecular symmetry the two (*S*)-tyrosinato ligands have the same chemical environment in solution, and hence the α - and β -protons of both (*S*)-tyrosinato ligands show the same chemical shift as well as the same value for J_{AX} and J_{BX} . Therefore, the side-residues of the (*S*)-tyrosinato ligands have the same mol fractions of rotamers in solution (Table 1, complexes **3** and **4**). In the unsymmetrical complexes, the side-residues of the (*S*)-tyrosin-

ato ligands have different chemical environments and hence different mol fractions of rotamers *t*, *g*, and *h* in solution (Table 1, complexes **5** and **6**). As is well known, the α -proton of an aminocarboxylate ligand whose NH_2 group is *trans* to an NH_2 group resonates at lower field (in this paper **tyr I**) than the α -proton of an aminocarboxylate ligand whose NH_2 group is *trans* to oxygen (in this paper **tyr II**).^[32,33]

The population analysis of the side-group rotamers of the (*S*)-tyrosinato ligands in diastereomers of $[\text{Co}\{(\text{S})\text{-tyr}\}_2\text{tn}]^+$ in D_2O solution indicates that the (*S*)-tyrosinato coordination increases the population of the sterically most favorable rotamer *t* relative to the population of this rotamer in the noncoordinated amino acid (Table 1). On the other hand, rotamer *g* is the least favorable rotamer energy-wise (from the MO calculations) because the phenyl group is closest to the coordinated carboxylic group. Therefore, rotamer *g* is the least abundant rotamer. These results are analogous to the results obtained from an examination of the rotamer population of the side-group of (*S*)-phenylalaninato and (*S*)-tyrosinato ligands in diastereomers of $[\text{Co}\{(\text{S})\text{-tyr}\}_2\text{en}]^+$ and $[\text{Co}\{(\text{S})\text{-phe}\}_2\text{en}]^+$ in D_2O solution.^[27] At the same time, it has been noticed^[27] that in diastereomers of these complexes with C_1 -molecular symmetry, the population of rotamer *h* increases due to intra- and interligand $\text{NH}\cdots\pi$ interactions.

In this context, in this paper we intended to examine whether, in the diastereomers of C_1 -molecular symmetry of $[\text{Co}\{(\text{S})\text{-tyr}\}_2\text{tn}]^+$, the population of rotamer *h* also increases and whether this increase is caused by $\text{NH}\cdots\pi$ interactions. In order to allow this comparison, the results of the mol fraction abundance of the side-group rotamers of (*S*)-tyrosinato ligands in diastereomers of $[\text{Co}\{(\text{S})\text{-tyr}\}_2\text{tn}]^+$ and $[\text{Co}\{(\text{S})\text{-tyr}\}_2\text{en}]^+$ ^[27] in D_2O solution are given in Table 1. A considerable increase can be seen in the population of rotamer *h* in one residue of the (*S*)-tyrosinato ligand in complexes of C_1 -molecular symmetry (**5 tyr II** and **6 tyr I**) relative to the population of this rotamer in noncoordinated (*S*)-tyrosine (mol fractions of rotamer *h* are 46 and 47% respectively). As the first result is in agreement with the result obtained in an analogous diastereomer with 1,2-diaminoethane, the population increase of rotamer *h* in the side-residue **tyr I** in complex **6** was investigated more closely.

If $\text{NH}\cdots\pi$ interactions lead to a population increase of rotamer *h* in complex **6** as well, then the NMR spectrum should show a small chemical shift of the NH proton that takes part in this interaction. A molecular model of the complex with the side-residues of the (*S*)-tyrosinato ligands in conformation *h* indicates the possibility of three different $\text{NH}\cdots\pi$ interactions in the molecule (Figure 3, a), namely an $\text{NH}\cdots\pi$ interaction within (*S*)-tyrosinato itself, an $\text{NH}\cdots\pi$ interaction between two coordinated (*S*)-tyrosinato ligands (one is a donor and the other an acceptor of the hydrogen atom), and an $\text{NH}\cdots\pi$ interaction between a partially positively charged hydrogen atom from the coordinated 1,3-diaminopropane and the aromatic ring of the (*S*)-tyrosinato ligand.

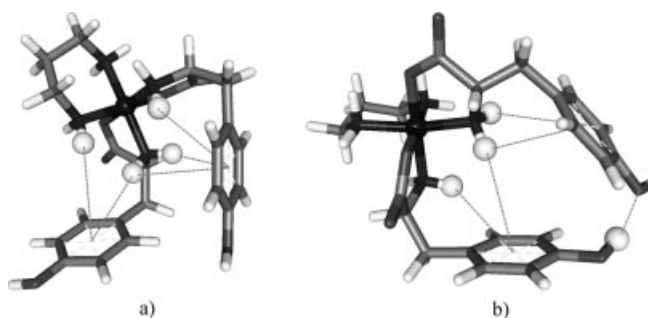


Figure 3. a) Calculated geometry of the Δ - C_1 -*cis*(O)- $[\text{Co}\{(\text{S})\text{-tyr}\}_2\text{tn}]^+$ complex (**6**) [both (*S*)-tyrosinato ligand side-groups adopt an *h* conformation and the diamine adopts a chair **A** conformation]. All possible $\text{NH}\cdots\pi$ interactions in the molecule are shown. b) Calculated geometry of complex **6** (**tyr I** adopts a *t* and **tyr II** an *h* conformation; the diamine adopts an **A_{1w}** conformation). Intramolecular $\text{NH}\cdots\pi$ interactions and H-bonds in the molecule are shown.

Since the mol fraction of rotamer *h* in the solution is small for the side-residue of **tyr II** (26%), the assumption of an $\text{NH}\cdots\pi$ interaction between two coordinated (*S*)-tyrosinato ligands can be rejected. In order to assign the NH protons, a TOCSY spectrum of complex **6** in H_2O solution was recorded (Figure 4). A small chemical shift of one NH proton at $\delta = 3.7$ ppm (overlaps the signal of the α -proton), originating from coordinated 1,3-diaminopropane, can be seen in the spectrum. The chemical shift of this proton is indicative of strong shielding effects associated with the ring current of the aromatic ring.^[23] In the 1,3-diaminopropane ligand, the difference in chemical shift between NH proton involved in the $\text{NH}\cdots\pi$ interaction and its geminal partner is -1.3 ppm. Thus we can conclude that the population increase of rotamer *h* of the side-residue **tyr I** in complex **6** is the result of an interligand $\text{NH}\cdots\pi$ interaction, i.e., an $\text{NH}\cdots\pi$ interaction between the partially positively charged hydrogen atom of the 1,3-diaminopropane ligand and the aromatic ring in the (*S*)-tyrosinato ligand.

A significant increase of the population of rotamer *h* is not found in analogous diastereomers of the (*S*)-phenylalanine complex. Williams and co-authors^[23,34] have recently reported the existence of $\text{NH}\cdots\pi$ interactions in complexes of Co^{III} with (*S*)- and (*R*)-tryptophan and its derivatives. Our results, and those published by Williams,^[23,34] are in accordance with the observation of Steiner and Koellner,^[14] who noted that Trp and Tyr are the aromatic amino acids most frequently involved in $\text{NH}\cdots\pi$ interactions in proteins. The order efficacy of an aromatic acceptor for hydrogen bonding is $\text{Trp} \gg \text{Tyr} > \text{Phe} \gg \text{His}$.

DFT Study

DFT calculations were carried out for different conformations of the side-residues of (*S*)-tyrosinato ligands in complex **6** and for two different diamine chair conformations **A** and **B** because this isomer has no symmetry elements (Table 2). In the case of the diamine chair conformation in which the axial protons of the terminal methylene

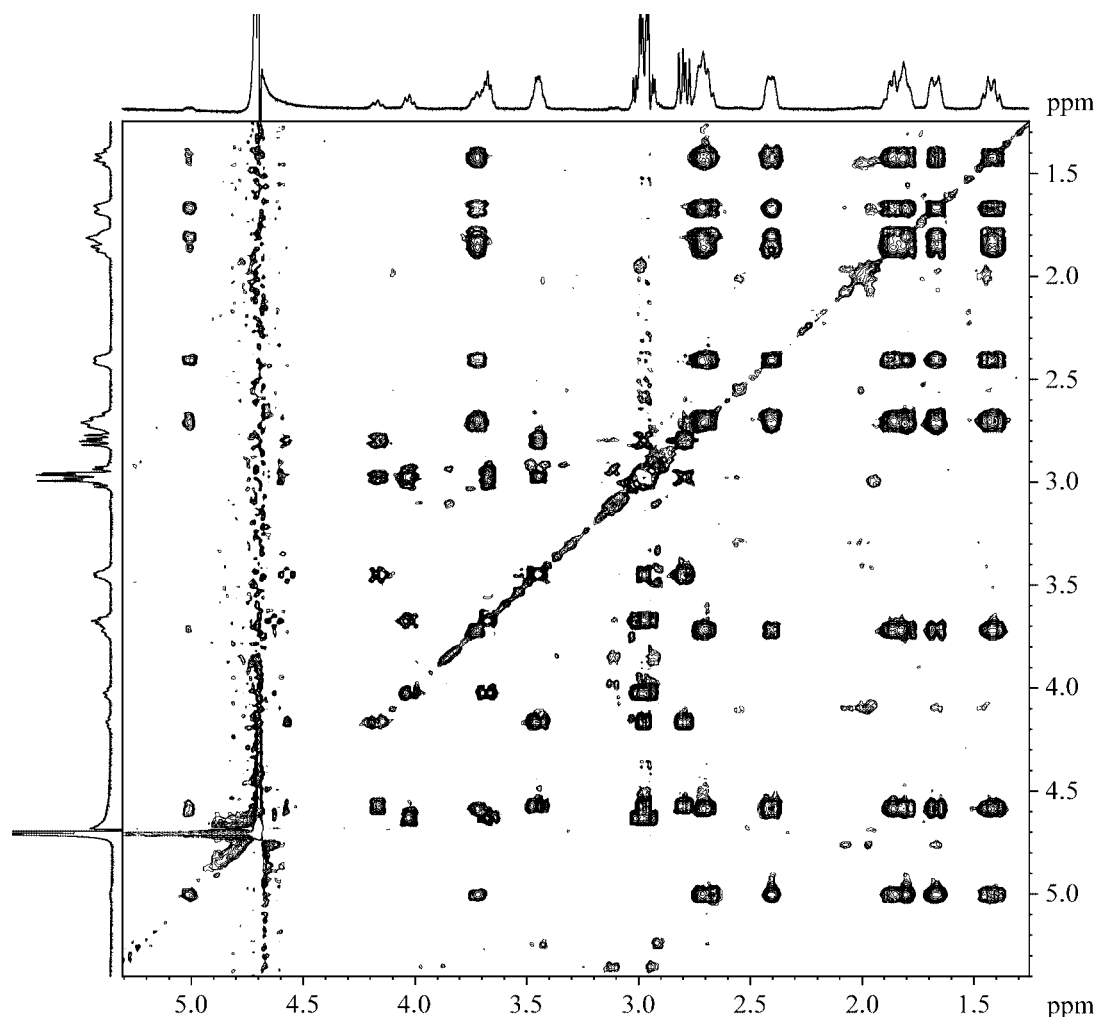


Figure 4. Partial TOCSY (500 MHz; mixing time 60 ms) spectrum of complex **6** in water solution.

groups are pointing toward the coordinated carboxylic group (conformation **A**, Table 2), the complex with two side-residues in conformation **h** represents an energy minimum. In this **h** conformation, the greatest number of NH $\cdots\pi$ interactions exist in the molecule (Figure 3, a), and it is followed, energy-wise, by the complexes in which one side-residue assumes an **h** and the second a **t** conformation. The least stable complex is that with both side-residues in conformation **g** (Figure 2). Here, the aromatic ring and the exocyclic oxygen of the coordinated carboxylic group are at the shortest distance, hence the repulsive forces are the greatest. The complexes in which only one side-residue takes conformation **g** are less stable energy-wise.

In the case of the diamine chair conformation, in which the axial protons of the terminal methylene groups point towards the α - and NH $_2$ protons of the coordinated (*S*)-tyrosinato ligand (conformation **B**, Table 2), the same order of stability of the conformers was obtained, but it is obvious that all complexes with the chair conformation **B** of the diamine are less stable than the corresponding complexes with conformation **A**. This is not surprising because in the chair conformation large steric hindrances are present between the axial protons of the terminal methylene groups

Table 2. The relative energies of conformers^[a] of complex **6**. The diamine adopts a chair (**A** and **B**) or twisted chair (**A_{tw}** and **B_{tw}**) conformation.

tyr I, tyr II	$E_{\text{rel}}^{[b]}$			
	Chair A	Chair B	A_{tw}	B_{tw}
h, g	33.811	45.837	47.297	49.706
h, h	0.000	12.041	14.080	15.418
h, t	22.204	32.146	35.832	38.605
t, g	42.126	56.878	57.415	66.742
t, h	10.302	20.978	9.549	9.718
t, t	30.766	44.833	49.982	57.887

[a] All energies [kJ mol $^{-1}$] are calculated relative to the energy of complex **6** (data obtained by a B3LYP/LANL2DZ//B3LYP/6-31G* calculation using an Onsager model) with chair conformation **A** and **h** conformations of the (*S*)-tyrosinato ligand side-chains, which represents an energy minimum. [b] In kJ mol $^{-1}$.

of the diamine and the α - and NH $_2$ protons of the coordinated (*S*)-tyrosinato ligand.

DFT calculations were also carried out for the case where the diamine adopts a twisted chair conformation (**A_{tw}** and **B_{tw}**, Table 2). The most stable geometry of the complex is obtained when **tyr I** adopts a **t** and **tyr II** an **h** conformation. The molecular model of the complex (Fig-

ure 3, b) indicates the possibility of forming an intramolecular H-bond only for these conformations of the (S)-tyrosinato side-chains.

A full optimization was performed for the most stable conformers of complex **6** (Table 3). The Boltzmann distribution indicates that complex **6** with a chair **A** conformation of the diamine and with both (S)-tyrosinato ligands side-chains in the **h** conformation is present at about 90%.

Table 3. Relative energies of the fully optimized^[a] conformers of complex **6** and Boltzman distribution of the conformers.

tyr I, tyr II	Diamine	$E_{\text{rel}}^{[b]}$		Population ^[c]	
		Chair	Chair _{tw}	Chair	Chair _{tw}
h, h	B	12.754	15.304	0.531	0.191
h, h	A	0.000	14.261	88.369	0.290
h, t	B	32.125	37.831	0.000	0.000
h, t	A	21.496	35.227	0.016	0.000
t, h	B	19.594	8.595	0.034	2.817
t, h	A	7.218	8.568	4.892	2.847

[a] Obtained by a B3LYP/LANL2DZ calculation using an Onsager solvation model. [b] In kJ mol⁻¹. [c] Percentage at 298 K.

The results obtained on the basis of the NMR spectroscopic data for the rotamer population in complex **6** indicate that **tyr I** in D₂O solution prefers an **h** conformation and **tyr II** a **t** conformation. On the other hand, the DFT calculations have shown that both (S)-tyrosinato side-chains prefer an **h** conformation (this complex is energetically the most stable). Because of this discrepancy we included in our DFT calculations two water molecules that are bonded to the hydroxyl groups of the (S)-tyrosinato side-chains by hydrogen bonds. The calculation (Table 4) was performed for conformation **A** of the diamine, which in all cases investigated was more stable than conformation **B** (Table 2). One significant difference can be seen in comparison with the data in Table 2, namely that the complex in which **tyr I** adopts an **h** and **tyr II** a **t** conformation, which is in accordance with experimental results, is now more stable than the complex with the opposite conformation of the side-chains. As mentioned above, the only combination of (S)-tyrosinato side-chain rotamers that can form intramolecular H-bonds is when **tyr I** adopts a **t** and **tyr II** an **h** conformation (Figure 3, b). Incorporation of two water molecules disrupts the intramolecular H-bonds by forming solute-solvent H-bonds, and these intermolecular

Table 4. Relative energies of the fully optimized^[a] conformers of complex **6** (diamine adopts a more stable **A** conformation).

tyr I, tyr II	$E_{\text{rel}}^{[b]}$
h, h	0.000
h, t	21.310
t, h	23.914
g, h	35.290
t, t	37.468
h, g	41.366
g, g	62.894
t, g	67.219
g, t	70.064

[a] Data obtained by a B3LYP/LANL2DZ calculation using an Onsager model and two water molecules. [b] In kJ mol⁻¹.

solute-solvent interactions could enhance or compete with the forces responsible for the stabilization of a particular rotamer in solution.^[23,24] Complex solute-solvent interactions and bulk entropy effects cannot be modeled at the MO level, and this could be the reason for the slight difference between the theoretical and experimental results.

Conformation of the 1,3-Diaminopropane Chelate Ring in Solution

The data obtained from the analysis of the vicinal coupling constants of the methylene protons indicate that the 1,3-diaminopropane in complex **6** in D₂O (and H₂O) solution has a chair conformation. Thus, according to the Karplus equation,^[35] which describes the dependence of vicinal coupling constant on the dihedral angle of the coupling protons, for the chair conformation the following values of the vicinal coupling constants are expected: ³J_{aa} (10–16 Hz) and ³J_{ae} and ³J_{ee} (3–5 Hz).^[36] By extracting the data from the NMR spectrum for complex **6** (at 500 MHz) in D₂O solution, the following data were obtained: the methylene protons bonded to the NH₂ group positioned *trans* to the nitrogen atom, resonate at δ = 2.7 (³J = 10.6 and 2.7 Hz) and 2.4 ppm (³J = 5.9 and 2.9 Hz), the methylene group protons bonded to the NH₂ group positioned *trans* to the oxygen atom resonate at δ = 1.9 (³J = 10.3 and 2.7 Hz) and = 1.8 ppm (³J = 5.8 and 3.0 Hz), while the protons of the central methylene group resonate at δ = 1.4 ppm. The existence of one vicinal coupling constant greater than 10 Hz indicates that the coordinated 1,3-diaminopropane adopts a chair conformation in D₂O (and H₂O) solution. It is worth mentioning that the relative chemical shifts of the axial and equatorial protons in terminal (bearing NH₂) methylene groups are opposite to the chemical shifts of these protons in a saturated cyclohexane ring, i.e. the axial protons resonate at higher δ values. The molecular model of complex **6** indicates that in the case of a chair conformation of the diamine ring the equatorial protons are found in the region of diamagnetic shielding of the aromatic ring in the tyrosinato ligand, which could explain the anomalous chemical shifts of the axial and equatorial protons of the terminal methylene groups.

Conclusions

The results of a conformational analysis indicate a population increase of the sterically least favorable **h** rotamer of the (S)-tyrosinato ligand side-group in diastereomers of C₁-molecular symmetry of [Co{(S)-tyr}₂(diamine)]⁺-type complexes (diamine = 1,2-diaminoethane or 1,3-diaminopropane) in D₂O (and H₂O) solution that is caused by attractive intra- and/or interligand NH \cdots π interactions.

DFT calculations for complex **6**, where the diamine adopts a chair conformation (as supported by NMR spectroscopic data), indicate that the complex with an **h** conformation of both the (S)-tyrosinato side-residues, which yields

the most efficient NH $\cdots\pi$ interactions, represents an energy minimum.

The results obtained on the basis of ^1H NMR spectroscopy indicate that intramolecular NH $\cdots\pi$ interactions persist in aqueous solution, regardless of the energy-wise favored interactions with water molecules. This is in agreement with the results obtained by the investigation of amino–aromatic interactions in peptides.^[21] Our results indicate the significance of noncovalent NH $\cdots\pi$ interactions involving (S)-tyrosinato side-chains and highlight the suitability of these simple cobalt(III) complexes to serve as models for studying these relatively poorly understood attractive forces.

Experimental Section

Synthesis: Diastereomers of the bis[(S)-tyrosinato](1,3-diaminopropane)cobalt(III) complex were synthesized by a previously described procedure.^[28]

NMR Spectroscopy: ^1H NMR spectra were recorded on a GEMINI 2000 spectrometer at 200 MHz in D_2O solution (complexes 3–6). The TOCSY spectrum of complex 6 in H_2O solution was recorded at 500 MHz on a Bruker ABX-500 spectrometer (mixing time 60 ms).

Computational Details: The optimized geometries and energies of conformers of $\Delta\text{-C}_1\text{-cis}(\text{O})\text{-}[\text{Co}\{(\text{S})\text{-tyr}\}_2\text{tn}]^+$ (6) were obtained using the density functional theory (DFT) with the Becke three-parameter exchange functional (B3),^[37] and the Lee–Yang–Parr (LYP) correlation functional.^[38] The DFT method was used as it gives good results for all transition metal complexes of the first row.^[39,40] These B3LYP calculations were carried out with the GAUSSIAN 98 program.^[41] The geometries of all conformers of the complex were fully optimized using 6-31G* basis sets, and for these optimized geometries the energy was calculated with LANL2DZ basis set in the presence of a solvent (H_2O), using an Onsager model.^[42–47] Full optimization for the most stable conformers was performed with the LANL2DZ basis set in the presence of a solvent, using an Onsager model and with explicit inclusion of two water molecules.

Acknowledgments

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