Amplified vibrational circular dichroism

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In this chapter we employ an auxiliary manifold of low-lying electronically excited states provided by a paramagnetic transition-metal ion to induce an amplification of VCD in amino acids and peptides in aqueous solutions. We find that the VCD of amino acids undergoes extraordinary signal enhancements. The mode-selectivity of the VCD amplification observed for peptides binding to metal ions indicates that the configuration of the binding pocket can be derived from the VCD-enhanced intensity of specific oscillators.
7.1 INTRODUCTION

Chirality plays an essential role in the functionality of biomolecular systems. A key step in understanding the functionality of such systems relies on the determination of their stereochemistry, conformation and structural heterogeneity. Vibrational circular dichroism (VCD)[52], the differential absorption of left- and right-handed circularly-polarised infrared light, has become one of the most powerful spectroscopic methods to determine the absolute configuration and conformational distribution of chiral molecules in solution.[2, 53, 55, 137, 138, 139, 140, 79, 141, 29] As yet however, the inherent small signal intensities have seriously impeded extensive application of this spectroscopic technique. In particular, VCD spectra of important systems such as amino acids, peptides and proteins, are difficult to obtain under biologically relevant conditions. Although in recent years impressive progress has been made in VCD instrumentation and analysis [142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152], one is commonly forced to work with highly concentrated samples to reach acceptable signal intensities, but this is often not possible due to low solubility and aggregation. One further issue that should be addressed in order to investigate large biologically active systems such as proteins and enzymes, is spectral congestion. The functionality of biomolecules is generally associated with a spatially restricted region, but in the VCD all parts of the molecule contribute with comparable amplitude, and the contribution of the functional part is difficult to observe. Spectroscopic studies of biomolecular functionality thus ideally would be able to zoom in on such active sites, but as yet this has been hard to realize.[153] In the present study we employ a paramagnetic transition-metal auxiliary to address both the issue of increasing the sensitivity of VCD as well as its application for probing local structure.

The peak intensities in a VCD spectrum are proportional to the rotational strength given by the imaginary part of the inner product of the electronic and magnetic transition-dipole moment vectors. Within a vibronic coupling approach the electronic contribution to the total magnetic transition dipole moment can be expressed as a sum of contributions from all electronically excited states, with weights that are
inversely proportional to their excitation energies\[^{14}\]. Theory thus leads one to expect that in systems with low-lying electronic states an enhancement of VCD signal intensities might occur as compared to analogous systems in which such low-lying electronic states are absent. Such enhanced VCD signals have indeed been observed, starting with studies on the CH-stretching region of (-)-sparteine transition-metal complexes.\[^{68}\] It is, however, only in the studies of Nafie et al. \[^{69}\] on the same system that a full explanation in terms of vibronic coupling was provided, and that the pertaining theoretical expressions for the VCD intensities were developed\[^{25}\]. In these particular transition-metal complexes the low-lying excited states are intrinsically already present. Conceptually, one should also be able to enhance VCD signal intensities by modulating the energies of the electronically excited-state manifold in such a way that a manifold is created with low-lying electronically excited states. We recently confirmed the validity of such an approach in a study in which electrochemical reduction was used to "create" the required low-lying electronically excited states, leading to an order-of-magnitude amplification of VCD signals\[^{40}\].

Since the initial studies on (-)-sparteine transition-metal complexes, a number of other studies have been reported in which analogous intensity enhancements in open-shell transition metal complexes were observed\[^{154, 155, 156, 69, 70, 157, 158}\]. However, practically all of these studies concerned complexes with rigid, non-biologically active ligands, and dissolved under non-physiologically relevant conditions. Here, we employ the manifold of low-lying electronically excited states provided by transition metal ions to induce enhanced VCD intensities in flexible biomolecular systems in aqueous solutions. We perform VCD studies on amino acids to determine to what extent vibrational circular dichroism can be enhanced in such systems. We show that amplification factors of more than two orders of magnitude can be obtained, bringing vibrational differential absorption on an equal footing with electronic differential absorption. Subsequently, we investigate how structural parameters influence this amplification. To this purpose we perform VCD studies on di- and tripeptides and show that VCD enhancement is strongly dependent on the distance of the oscillator from the amplifying center. This
spatial sensitivity of the VCD amplification thus provides excellent opportunities for its use as a structural tool for bio-inorganic systems.

7.2 VCD OF AMINO ACIDS NEARBY COBALT IONS

In Figure 31a and 31b we show infrared absorption and VCD spectra of monomeric L-proline (black dashed lines). The IR spectrum shows a number of readily identifiable bands, the strongest one occurring at 1600 cm\(^{-1}\), which is assigned to the carboxylate stretching mode. The VCD spectrum, on the other hand, shows bands with extremely small intensities that are hardly discernible from the noise of the measurement, typically \(\Delta A \approx 5 \times 10^{-6}\) OD. In order to enhance these differential absorptions we have altered our system in such way that L-proline is actively bound to a paramagnetic metal ion, thereby creating a model of a binding pocket where proline and water molecules can assume multiple configurations around the metal. To this purpose the hexacoordinated octahedral \(\text{Co}^{II}(\text{Pro})_2(\text{D}_2\text{O})_2\) complex was synthesized by adding \(\text{Co}^{2+}\) ions to a solution of L-proline in a molar ratio of 2:1 (see section 7.7.1). The IR and VCD spectra of this complex are shown in Figures 31a and 31b as blue solid lines. The IR spectrum confirms that L-proline is now incorporated into the \(\text{Co}^{II}(\text{Pro})_2(\text{D}_2\text{O})_2\) complex, since a splitting is observed for the carboxylate stretching mode as a consequence of exciton coupling between the two proline moieties.

Figures 31a and 31b show that complexation has a minor effect on the intensities of the bands in the IR spectrum, but leads to spectacular enhancements – by more than one order of magnitude – of the intensities of VCD bands. To confirm that this enhancement is indeed due to the presence of low-lying electronic states provided by the paramagnetic, high-spin \(\text{Co}^{2+}\) ion, we compare the VCD spectra of the proline-Co complex with Co in two distinct redox states: \(\text{Co}^{II}\) having low-lying electronically excited states, and diamagnetic \(\text{Co}^{III}\) having only high electronic states well-separated from ligand vibrational modes. The diamagnetic \(\text{Co}^{III}\)-proline complex was obtained by oxidation of the \(\text{Co}^{II}\)-proline complex following a previously reported procedure, which leaves the coordina-
Figure 31: IR absorption and VCD spectra of the amino acids L-proline (a,b), L-valine (c,d) and L-alanine (e,f) in D$_2$O (black dashed lines) and of the complexes formed by complexation with cobalt: Co$^{II}$(Pro)$_2$(D$_2$O)$_2$ (a,b), Co$^{II}$(Val)$_2$(D$_2$O)$_2$ (c,d) and Co$^{II}$(Ala)$_2$(D$_2$O)$_2$ (e,f) (solid filled lines). The samples were prepared in D$_2$O (c $\approx$ 25 mM). The VCD spectra of the amino acids and of the complexes were averaged with 4320 scans (1 hour) at a resolution of 4 cm$^{-1}$. The VCD spectra of the amino acids (dashed lines in b,d and f) have been scaled for better comparison with the enhanced VCD spectra of the complexes.

In Figure 32 we compare the VCD spectra of these Co$^{II}$$-$ and Co$^{III}$$-$proline complexes. The spectra clearly show that upon oxidation, the VCD signals of complexed L-proline reduce to the same magnitude as for uncomplexed L-proline. This observation is a direct proof that the VCD amplification is due to vibronic coupling to low-lying electronically excited states. The absence of unpaired electrons in the d-orbitals of the diamagnetic complex removes the low-lying states from the system, and the VCD signals are consequently no longer amplified. We have found similar amplification effects for other amino acids. Typical examples (L-alanine and L-valine) are shown in Figures 31c–31f. It is worth to notice that the VCD bands of L-valine are enhanced by more than two orders of magnitude by complexation to Co$^{2+}$ ions. The intensity enhancement of a vibrational transition is usually characterized by its anisotropy ratio $g = \Delta \varepsilon / \varepsilon$, which generally is in the range of $10^{-4} - 10^{-5}$. Here, we find for the complexed amino acids
ratios that range from $1 \times 10^{-3}$ up to $6.5 \times 10^{-3}$, and that thus promptly qualify as enhanced VCD. We notice in particular that the latter value is one of the largest molecular vibrational anisotropy ratios reported so far.

The Co$^{II}$(amino acid)$_2$(D$_2$O)$_2$ complexes can in principle adopt various binding configurations. To determine the binding configuration(s) that are actually present in our experiments, we have performed Density Functional Theory (DFT) calculations and simulated the experimental IR and VCD spectra of each of the complexes. For each of the complexes we find four isomers that differ in the arrangement of the amino acid pairs with respect to the cobalt ion (trans-trans, trans-cis, cis-trans, and cis-cis). Optimized molecular structures and associated energies are given in section 7.5. For the proline and the valine complexes we find that the trans-cis isomer is the one of lowest energy, in agreement with previous calculations on the proline complex [159]. Moreover, the relative energies of the other isomers are such that under our experimental conditions one does not expect them to be present in significant amounts. For the alanine complex, on the other hand, the trans-cis and cis-trans isomers are of similar energies, and one may expect that the experimental spectra contain contributions from both. Comparison of the predicted IR and
VCD spectra of the various isomers with the experimentally observed spectra confirms these conclusions.

In Figure 33 we show such a comparison for the trans-cis isomer of the proline and valine complexes as well as for the trans-cis and cis-trans isomers of the alanine complex, while in section 7.5 the predicted spectra for other isomers are reported. The VCD spectra have been calculated using the magnetic field perturbation formalism in which the influence of low-lying electronic states is not explicitly taken into account. One may thus anticipate that compared to the usual results obtained for other molecules - including transition metal complexes without any low-lying electronic states - the calculations will not achieve yet a similar satisfactory degree of agreement as is indeed observed[69, 160]. Nevertheless, for the proline and valine complexes comparison of the experimental spectrum with the predicted spectra for the four isomers favors an assignment to the trans-cis isomer. For the alanine complex the assignment to a single isomer is much less clear-cut. In fact, from the structure observed in the 1300 – 1450 cm\(^{-1}\) region one would tend to conclude that the experimental spectrum has contributions from more than one isomer. This observation is in agreement with the calculations that predict similar energies for the trans-cis and the cis-trans-isomers.

The experimental VCD spectra of the monomeric and complexed species (Figure 31) show that complexation leads to enhancements of the spectrum by a factor of roughly 8, 10, and 50 for alanine, proline, and valine, respectively [161]. Importantly, when we compare the absolute intensities of the bands in the experimental and predicted VCD spectra — as opposed to the comparison of the relative intensities that we have done so far — we find that the experimental spectra are enhanced with respect to the predicted spectra by similar factors (Fig. 33, grey lines). This observation thus fully supports the idea that vibronic coupling with low-lying electronic states is at the basis of both enhancements.

Figures 31 and 33 demonstrate that the three amino acid complexes display rather different enhancements. In order to rationalize this behavior we have performed TD-DFT calculations of the excitation energies and magnetic transition-dipole moments to the lower electronically excited states (see section 7.6). Interestingly, we find that the excitation en-
Figure 33: Experimental and calculated IR and VCD spectra of Co\textsuperscript{II}(Pro)\textsubscript{2}(D\textsubscript{2}O)\textsubscript{2} (top panels), Co\textsuperscript{II}(Val)\textsubscript{2}(D\textsubscript{2}O)\textsubscript{2} (center panels) and Co\textsuperscript{II}(Ala)\textsubscript{2}(D\textsubscript{2}O)\textsubscript{2} (lower panels). The calculated spectra (in black), corresponding to the isomer(s) energy minima for each of the complexes have been scaled (in grey) to better show the VCD peaks with weak intensities. DFT optimised structures of the trans-cis (and cis-trans for the alanine complex) isomers are displayed next to the spectra.

Energy of the lowest excited state in the valine complex is markedly lower than the excitation energy in the other two amino acid complexes. For the valine complex one therefore expects larger vibronic couplings, and
thus larger enhancements of VCD bands, as is indeed observed in the experiments. As yet, it would appear that there is no simple explanation for the differences in excitation energy. It would, however, be a worthwhile subject for further study because it might ultimately lead to amino acid identification using VCD enhancements as a probe.

7.3 ENHANCED VCD AS A LOCAL PROBE IN BIOLOGICAL SYSTEMS

As nearly one third of all biomolecules contain transition-metal ions, it is interesting to explore the applicability of the observed VCD enhancement to larger biomolecular systems [162]. The conformational details of metal-binding pockets of peptides and proteins in solution are often unknown due to the lack of suitable local probes that can assess site-specific geometry. We can locally probe the binding geometry of such systems using the intensity and shape of the amplified VCD signals. Since these signals are highly sensitive to molecular conformation, they can highlight important structural features of the binding sites that may not be detectable with other techniques. As a first step in this direction, we show in the following that one can retrieve the coordination geometries of larger molecular systems (di- and tripeptides) on the basis of the intensity and shape of the enhanced VCD spectral signatures.

7.3.1 Deriving the coordination geometry from the amplified VCD signals

Figure 34 displays IR (a) and VCD (b) spectra of bare Val-Val (black line) and Val-Val complexed to Co$^{2+}$ (green line). Figure 34a shows that upon complexation the amide I (mainly C=O stretch) band at 1650 cm$^{-1}$ disappears while a new band comes up at 1610 cm$^{-1}$. This red shift can be explained by the deprotonation of the amide nitrogen and the subsequent coordination of this nitrogen atom to the metal. [163] From the IR spectrum one can conclude that the carboxylate group (1580 cm$^{-1}$) does not participate in the binding since no shift is observed for its C=O-stretch vibration. The binding thus occur through the deprotonated amide nitrogen, the basic N-terminus and two water molecules forming a distorted
tetrahedral (high-spin, see experimental details in section 7.7) configuration (Figure 34, top right).

Comparison of the signal intensities of the salient bands in the VCD spectra of the bare and complexed dipeptide (Figure 34b) readily leads to the conclusion that in the complex VCD bands are significantly amplified. Interestingly, however, we find that this amplification is strongly mode dependent. This holds in particular for the amide I mode, which in the complex is enhanced by at least an order of magnitude more than the other modes. The VCD spectra thus give evidence for selective amplification of vibrational modes, and thereby demonstrate the potential of the method to zoom in on local details within a much larger, complex molecular system.

To investigate the spatial range of the ion-induced enhancement, we performed VCD measurements on a tripeptide (Val-Val-Val). In this case, two amide groups of the backbone can coordinate to the metal ion. In Figure 34c–d we show IR and VCD spectra of the unbound tripeptide (black lines) and the Co\(^{2+}\) bound tripeptide (red lines), respectively. In the amide I frequency range (1650 cm\(^{-1}\)) two amide I modes labeled 1 and 2 can be observed. Mode 1 is at a higher frequency and is assigned to the amide group closer to the N terminus of the tripeptide\[164\]. From the IR spectra it can be concluded that the two amide moieties do not participate equally in the binding. Upon coordination, the amide I band 1 changes into band 1\(^{\prime}\), and is thus red-shifted by approximately 40 cm\(^{-1}\), while amide I band 2\(^{\prime}\) is only shifted by 3 cm\(^{-1}\) from band 2. We thus conclude that the amide moiety 1\(^{\prime}\) is strongly bound to Co\(^{2+}\) (as in the dipeptide), while the non-deprotonated amide functionality 2\(^{\prime}\) is only weakly bound. Previous studies\[165\] on Cu\(^{II}\)-tripeptides have shown that for pH values between 7 and 9 these systems adopt a configuration in which one of the amide groups has a deprotonated nitrogen that is strongly bound to the metal, while the other non-deprotonated amide group is weakly bound through the amide oxygen (see Figure 34, lower right). For the Co\(^{II}\)-tripeptide considered here, detailed information on the conformation of the binding pocket can be obtained from the analysis of the VCD spectra, and in particular from the intensities of the two amide I bands. The two red-shifted amide I bands 1\(^{\prime}\) and 2\(^{\prime}\) give rise
Figure 34: FTIR (a) and VCD (b) spectra of L-Val-Val (black lines) and Co\textsuperscript{II}(L-Val-Val)(D\textsubscript{2}O)\textsubscript{2} (green lines) and FTIR (c) and VCD (d) of L-Val-Val-Val (black lines) and Co\textsuperscript{II}(L-Val-Val-Val)(D\textsubscript{2}O) (red lines). The numbered IR bands in panels a and c correspond to the VCD bands in panel b and d, respectively. The numbered moieties in the molecular structures of Co\textsuperscript{2+} bound valine dipeptide (high-spin) and Co\textsuperscript{2+} bound valine tripeptide (high-spin) correspond to the numbered peaks in the VCD spectra.

to a negative and a positive band, respectively, in the VCD spectra. It is striking to observe that the intensity amplification of these two bands is markedly different, with a much larger enhancement for 1' than for 2' (Figure 34d). This observation confirms that 1 has a much stronger interaction with the metal ion, and thus corresponds to the C=O-stretch vibration of the anionic amido moiety bound directly to Co\textsuperscript{2+} via its
nitrogen atom, while 2 corresponds to the C=O-stretch vibration of the weaker bound neutral amide group, in agreement with the frequency shifts of these bands and with conclusions drawn previously for the Cu$^{\text{II}}$–tripeptides.[165]

7.4 FINAL REMARKS

In summary, the present study has demonstrated that the signal intensities in a VCD spectrum can be enhanced up to two orders of magnitude provided there is an electronic manifold with low-lying electronic states that can be coupled to the molecule of interest. We have shown that VCD spectra of amino acids and peptides in water — which in the past have been notoriously difficult to obtain — become readily accessible with unprecedented signal-to-noise ratios by coupling the amino acid or peptide to an open-shell transition metal ion. Our studies on di- and tri-peptides demonstrate unambiguously that the enhancement of the VCD signal intensities is strongly localized, paving the way for its use as a probe of local structure in larger biomolecules. Many biomolecular systems have open-shell transition metal ions as part of their structure. Due to their low-lying electronically-excited states, these ions will amplify the VCD signals of surrounding functional groups. The same strategy can be employed to study biomolecules containing closed-shell metals such as zinc-containing biological complexes, simply by substituting Zn(II) for Co(II). VCD can therefore be used as a highly sensitive and site-specific structural probe to determine conformational details of binding pockets in biological systems.

7.5 CONFIGURATION ANALYSIS OF THE AMINO ACIDS BINDING POCKETS

Density Functional Theory (DFT) calculations were carried out with gaussian 09 [115]. Ground-state geometry optimizations and harmonic vibrational frequencies were computed using the B3LYP hybrid functional, which includes the Becke three-parameter exchange [105] and
the Lee, Yang and Parr correlation functionals [107]. The LANL2DZ effective core potential (ECP) was used for Co, whereas the 6-31+G(d) basis set was used for all other elements. Solvent effects were simulated with a polarizable continuum model (PCM). Excitation energies and electronic magnetic transition-dipole moments were calculated using time-dependent DFT.

In Fig 35, 36, 37 we show the optimized molecular structures of the Co$^{2+}$-bound amino acids valine, proline and alanine, respectively, and the corresponding IR and VCD spectra. The geometries of the complexes and associated VCD spectra were calculated for neutral, octahedral (high-spin) configurations with three unpaired electrons (quartets). The complexes can adopt four different isomers regarding the coordination of the amino acid pairs to the Co$^{2+}$ ions: cis-trans (ct), trans-cis (tc), cis-cis (cc) and trans-trans (tt).
Figure 35: Observed and calculated infrared absorption and VCD spectra for the four conformations (tc, ct, cc, tt) of Co\textsuperscript{11}(L-valinate)\textsubscript{2}(D\textsubscript{2}O)\textsubscript{2}. The energy of each isomer is given next to the respective spectrum.
Figure 36: Observed and calculated infrared absorption and VCD spectra for the four conformations (tc, ct, cc, tt) of Co^{11}(L-prolinate)$_2$(D$_2$O)$_2$. The energy of each isomer is given next to the respective spectrum.
Figure 37: Observed and calculated infrared absorption and VCD spectra for the four conformations (tc, ct, cc, tt) of Co$^{11}$L-alaninate$_2$(D$_2$O)$_2$. The energy of each isomer is given next to the respective spectrum.
7.6 TIME-DEPENDENT DFT STUDY OF THE BINDING POCKETS

Within a vibronic coupling approach, it can be shown that when a molecule is in an electronic state $|\psi_0\rangle$, the electronic part of the magnetic transition dipole moment of a transition between the $\nu = 0$ and $\nu = 1$ levels of a vibrational mode is given to first order by: [14]

$$ \langle \Psi_f | \bar{\mu}^e_{\text{mag}} | \Psi_i \rangle = \langle \chi_{\nu=0} | \sum_{\kappa \neq 0} \frac{\langle \psi_0 | \bar{\mu}^e_{\text{mag}} | \psi_\kappa \rangle}{W_\kappa - W_0} (\langle \psi_\kappa | T_{\text{nucl}} | \psi_0 \rangle - \langle \psi_0 | T_{\text{nucl}} | \psi_\kappa \rangle) | \chi_{\nu=1} \rangle \tag{61} $$

where $|\chi_{\nu=0}\rangle$ and $|\chi_{\nu=1}\rangle$ are the nuclear wave functions of the $\nu = 0$ and $\nu = 1$ states in the electronic ground state $|\psi_0\rangle$, and $T_{\text{nucl}}$ is the nuclear kinetic energy operator. $\bar{\mu}^e_{\text{mag}}$ is the electronic contribution to the magnetic transition dipole moment, $|\psi_0\rangle$ and $|\psi_\kappa\rangle$ are the Born-Oppenheimer electronic wave functions for the ground state and the $\kappa^{th}$ electronically excited state, with energies $W_0$ and $W_\kappa$, respectively. Fig 38 thus shows the excitation energies ($W_\kappa - W_0$, top panel) and the coefficients of the sum-over-states expansion (Eq. 61) for the electronic contribution to the magnetic transition-dipole moments between ground and the $\kappa^{th}$ electronically excited state ($\langle \psi_0 | \bar{\mu}^e_{\text{mag}} | \psi_\kappa \rangle / (W_\kappa - W_0)$, lower panel) for the lowest energy conformer of the valine, proline and alanine Cobalt complexes.

In the derivation of Eq. 61 it has been assumed that vibronic energies (that is, electronic plus vibrational energy) can be replaced by electronic excitation energies. For the systems studied in the present work, in which the lower electronically excited states have energies comparable to vibrational energies, this clearly is not the case. Eq 61 thus allows us to assess qualitatively the role of the various electronic excited states, but a correct description would require an extension of the theory including correction terms that account for such level of vibronic detail as has been derived by Nafie [25]. Our experimental results demonstrate the necessity to implement such correction terms to obtain agreement between calculated and measured enhanced VCD for systems with low-lying electronically excited states.
Figure 38: Excitation energies (top panel) and coefficients of the sum-over-states expansion for the electronic contribution to the magnetic transition-dipole moments between ground and the \( K^{th} \) electronically excited state (lower panel) for the lowest energy conformer of the valine, proline and alanine Cobalt complexes.

### 7.7 SYNTHESIS AND CHARACTERIZATION OF THE COMPLEXES

#### 7.7.1 \( \text{Co}^{II}(\text{L-prolinate})_2(\text{H}_2\text{O})_2 \)

Complex \([\text{Co}^{II}(\text{L-prolinate})_2(\text{H}_2\text{O})_2]\) was synthesized according to a slightly modified procedure reported by Guillon. \[159\] 229 mg anhydrous \( \text{CoCl}_2 \) (1.76 mmol) was dissolved in 3 mL of deoxygenated water under argon. In a separate schlenk tube, 406 mg L-proline (3.53 mmol) and 198 mg KOH (3.53 mmol) were dissolved in 5 mL deoxygenated water under argon. These solutions were mixed under argon to give a red solution, which was stirred for 1 h. Partial evaporation of water using a cold-trap under vacuum caused the precipitation of a red-purple microcrystalline solid. This was washed with small amounts of cold, deoxygenated water under argon. The solid was dried under vacuum. Yield 282 mg (50%). RT magnetic susceptibility measurement: \( \mu_{\text{eff}} = 3.9 \) BM (high spin cobalt(II)).
7.7 SYNTHESIS AND CHARACTERIZATION OF THE COMPLEXES

7.7.2 $\text{Co}^{\text{II}}(\text{L-alaninate})_2(\text{H}_2\text{O})_2$

Complex $[\text{Co}^{\text{II}}(\text{L-alaninate})_2(\text{H}_2\text{O})_2]$ was synthesized in a similar manner as described for $[\text{Co}^{\text{II}}(\text{L-prolinate})_2(\text{H}_2\text{O})_2]$: 228 mg anhydrous CoCl$_2$ (1.75 mmol) was dissolved in 2 mL of deoxygenated water under argon. In a separate schlenk tube, 312 mg L-alanine (3.51 mmol) and 197 mg KOH (3.53 mmol) were dissolved in 5 mL deoxygenated water under argon. These solutions were mixed under argon to give a red solution, which was stirred for 1 h. Partial evaporation of water using a cold-trap under vacuum caused the precipitation of a blue-purple powder. This was washed with small amounts of cold, deoxygenated water under argon. The solid was dried under vacuum. Yield 0.44 g (90%). RT magnetic susceptibility measurement: $\mu_{\text{eff}}$ = 3.5 BM (high spin cobalt(II)).

7.7.3 $\text{Co}^{\text{II}}(\text{L-valinate})_2(\text{H}_2\text{O})_2$

Complex $[\text{Co}^{\text{II}}(\text{L-valinate})_2(\text{H}_2\text{O})_2]$ was synthesized in a similar manner as described for $[\text{Co}^{\text{II}}(\text{L-prolinate})_2(\text{H}_2\text{O})_2]$: 114 mg anhydrous CoCl$_2$ (0.88 mmol) was dissolved in 2 mL of deoxygenated water under argon. In a separate schlenk tube, 205 mg L-valine (1.75 mmol) and 99 mg KOH (1.75 mmol) were dissolved in 2 mL deoxygenated water under argon. These solutions were mixed under argon to give a red solution, which was stirred for 1 h. Partial evaporation of water using a cold-trap under vacuum caused the precipitation of a purple powder. This solid was washed with small amounts of cold, deoxygenated water under argon. Yield 144 mg (50%). The solid was dried under vacuum. RT magnetic susceptibility measurement: $\mu_{\text{eff}}$ = 4.1 BM (high spin cobalt(II)).

7.7.4 $\text{Co}^{\text{II}}(\text{L-valinate-valinate})_2(\text{H}_2\text{O})_2$

Complex $[\text{Co}^{\text{II}}(\text{L-valinate-valinate})_2(\text{H}_2\text{O})_2]$ was synthesized in a similar manner as described for $[\text{Co}^{\text{II}}(\text{L-prolinate})_2(\text{H}_2\text{O})_2]$: 90.05 mg anhydrous CoCl$_2$ (0.69 mmol) was dissolved in 2 mL of deoxygenated water under argon. In a separate schlenk tube, 150 mg L-valine (0.69 mmol)
and 27.78 mg NaOH (0.69 mmol) were dissolved in 2 ml deoxygenated water under argon. These solutions were mixed under argon, which was stirred for 2 h. Blue/green precipitation occurred. This solid was washed with small amounts of cold, deoxygenated water under argon. Yield 95.6 mg (30%). The solid was dried under vacuum. RT magnetic susceptibility measurement: $\mu_{\text{eff}} = 3.9$ BM (high spin cobalt(II)).

7.7.5 Magnetic Susceptibility Measurements

Room temperature magnetic susceptibility measurements were performed on packed solid samples with a Sherwood Scientific MK 1 Magnetic Susceptibility Balance.

7.7.6 Sample Preparation and Methods

All samples were prepared in deuterium oxide (D$_2$O) with concentrations ranging from 5 to 40 mM. The solutions were prepared under inert conditions and inserted in sealed infrared cells with 3 mm thick CaF$_2$ windows separated by a 50 µm teflon spacer. Fourier-transform infrared (FTIR) and VCD spectra (with spectral resolution of 2 and 4 cm$^{-1}$, respectively) were obtained with a Bruker Vertex 70 spectrometer in combination with a PMA 50 module.