Supplementary Information (SI) for Chemical Communications.

Supporting Information

A simple strategy for measuring the genuine Resonance Raman Optical Activity (RROA) of nonpolar molecules

Joanna Mazurkiewicz[a,b], Aleksandra Orlef[a], Tomasz Misiaszek[c], Tomasz K. Olszewski[c] and Agnieszka Kaczor*[a]

[a] *Faculty of Chemistry, Jagiellonian University, 2 Gronostajowa Str., 30-387 Krakow, Poland.*

[b] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, 11 Lojasiewicza Str., 30-348 Krakow, Poland.*

[c] *Faculty of Chemistry, Wroclaw University of Science and Technology, Wybrzeże Wyspiańskiego 27 Str., 50-370, Wroclaw, Poland.*

**Corresponding author e-mail: agnieszka.kaczor@uj.edu.pl*

Table of Contents:

Experimental:

Preparation of R,R and *S,S* enantiomers of Ni-complex

The *R,R* and *S,S* enantiomers of Ni-complex were synthesized according to the known procedure.[1]

Preparation of solutions

Preparation of micelles

Polymer micelles were formed from Pluronic F-127 (Merck) by dissolving the copolymer in ultrapure water in a concentration higher than CMC (critical micelle concentration), which is 0.7 w/v % at 25°C.[2] To completely dissolve Pluronic F-127 in water, the system was stirred for 30 min and then left for 1 h to stabilize. The final concentration of Pluronic F-127 in water was 0.0020 M.

Encapsulation:

R,R or *S,S* enantiomers of bis(pyrrol 2-ylmethyleneamine)-cyclohexane nickel^{II} were dissolved in tetrahydrofuran (THF, Thermo Scientific, 99,9%, stabilized with BHT) to obtain the concentration of 0.0200 M. The prepared THF solutions of the complexes were then mixed with the formed polymer micelles in a volume ratio of 6:94, and left for 12 hours to stabilize. The final concentration of complexes was equal to 0.0012 M. In the final systems THF constitutes 6% of the volume of the entire solution.

ECD measurements:

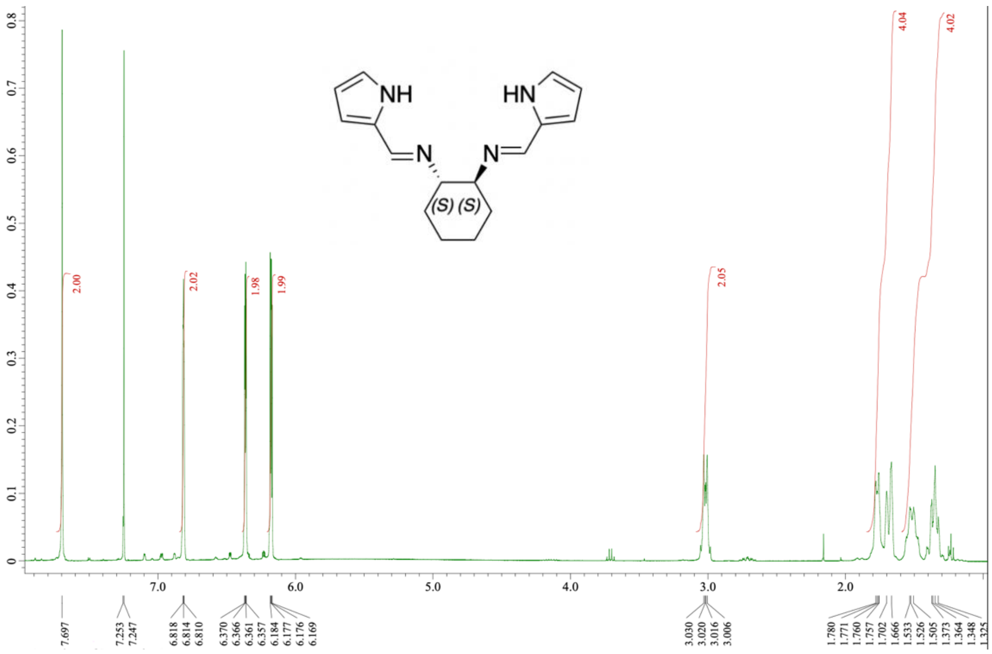
Electronic absorption and electronic circular dichroism (ECD) spectra were registered using the Jasco J-1500 spectrometer (with JASCO 1.52.00 software) in two ranges: 500-600 nm and 300-700 nm. The measurements were made for both enantiomers of the complex at the concentration of 0.0030 M for the 500-600 nm range and 0.0003 M for the 300-700 nm range. Analogous measurement ranges were also used for the measurements of both enantiomers of the complex encapsulated in micelles. For the 500-600 nm range the optical pathlength of 1 mm, the scan speed of 50 nm/min and 0.5 nm data pitch were used. For the 300-700 nm range, 1 mm, 100 nm/min, and 0.5 nm were applied. 5 individual scans were accumulated and averaged for all electronic absorption and ECD spectra. The solvent was subtracted from each recorded spectrum in the dedicated JASCO program (Jasco 1.52.00 version).

'ECD-Raman/RROA', RROA and *DOC* measurements:

Resonance Raman Optical Activity (RROA) or 'ECD-Raman/RROA', resonance Raman and degree of circularity (*DOC*) spectra were measured using the BioTools Inc. ChiralRAMAN-2x spectrometer equipped with a CCD camera and 532 nm excitation wavelength (with ROA Display and Control 4.2.0.0 software). The measurements were registered for both enantiomers of the complex at a concentration of 0.0200 M, with 1 h acquisition time, 10 mW laser power and an illumination period of 3.087 s. The same parameters was used for the measurement of Raman spectra od THF. The spectra of both enantiomers of the complex encapsulated in micelles (final complex concentration of 0.0012M) were registered using 4 days acquisition time, 20 mW laser power and the illumination period of 3.087 respectively. The same parameters was used for the measurement of Raman spectra od micelles with the addition of THF (trace amount, same as for the complex encapsulated in micelles). All spectra were registered in the 500–2500 cm^{-1} range. Averaging of the obtained spectra was performed using the OPUS 7.2 program. *DOC* measurements, which were the result of averaging 3 individual measurements, were performed analogously, but using then accumulation time of 30 s for each spectra.

Figures and tables:

A



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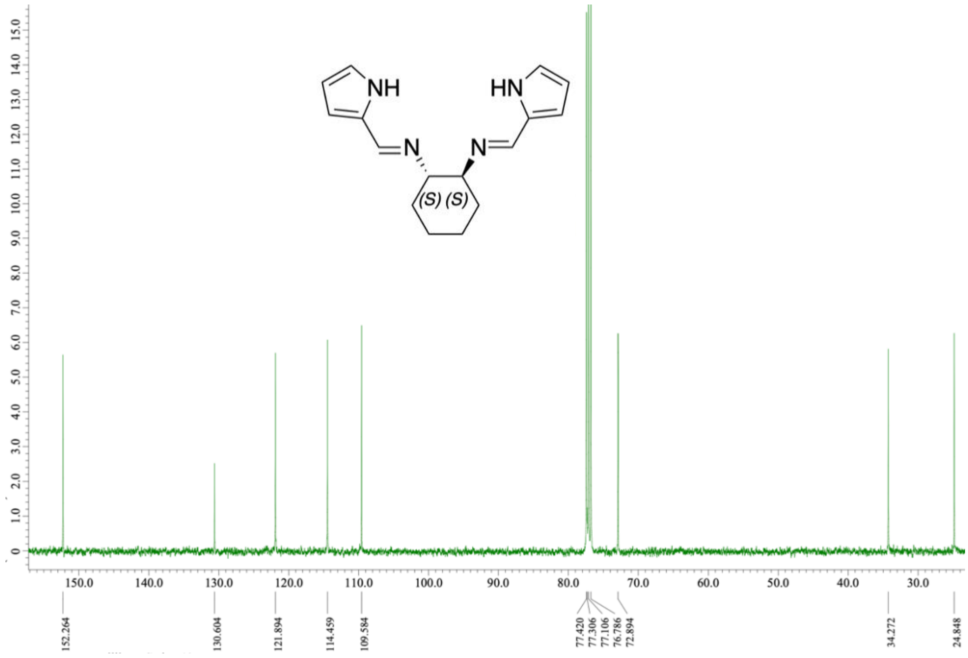

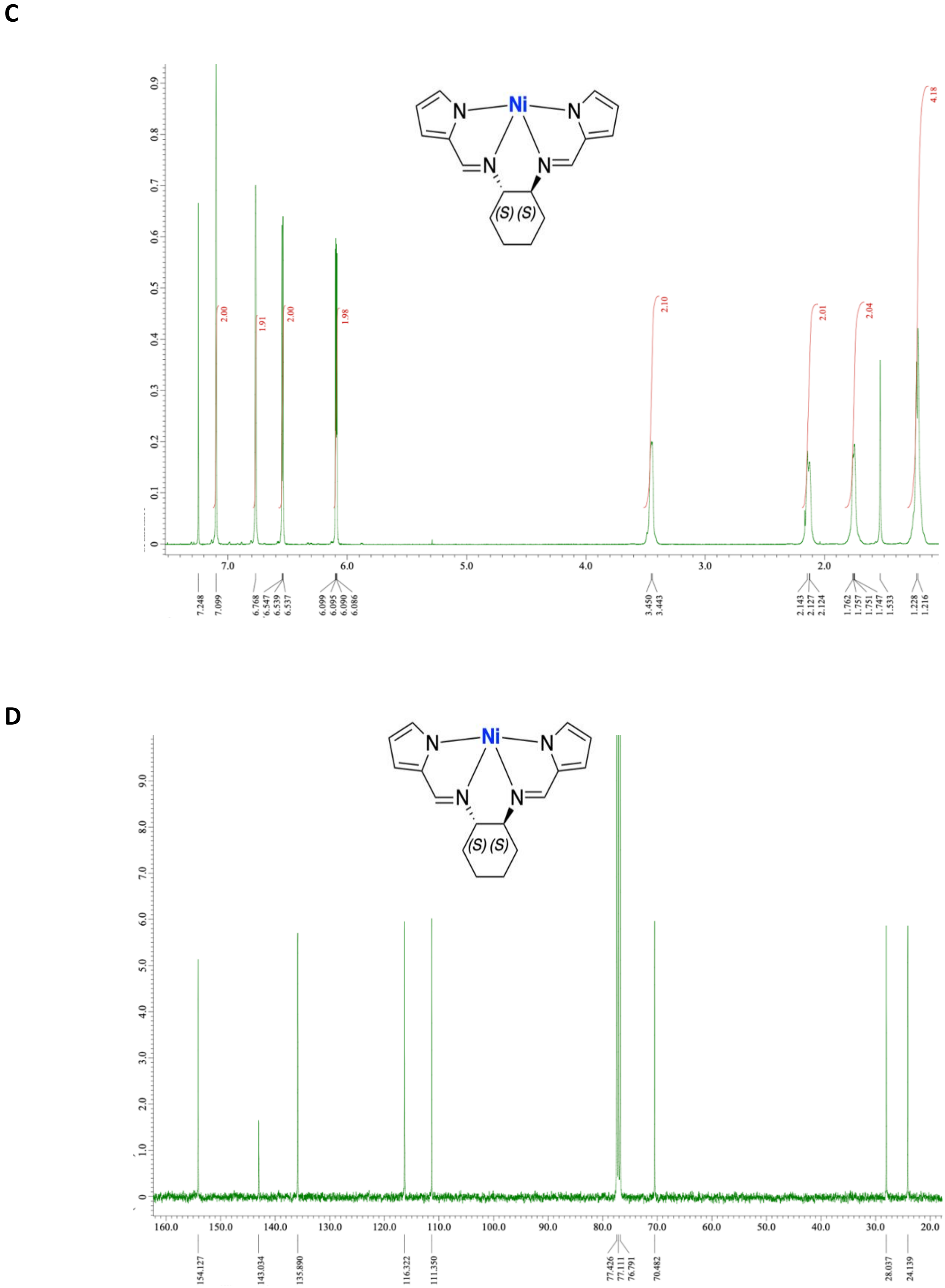

Fig. S1. ^{1}H NMR and ^{13}C NMR spectra (measured in $CDCl_3$, on Jeol 400yh NMR instrument, 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) of the *S,S* enantiomer of the ligand (^{1}H NMR, **A**; ^{13}C NMR, **B**) and *S,S* enantiomer of Ni-complex (^{1}H NMR, **C**; ^{13}C NMR, **D**). The ^{1}H and ^{13}C NMR spectra of the *R,R* enantiomer of the ligand and *R,R* enantiomer Ni-complex were identical.

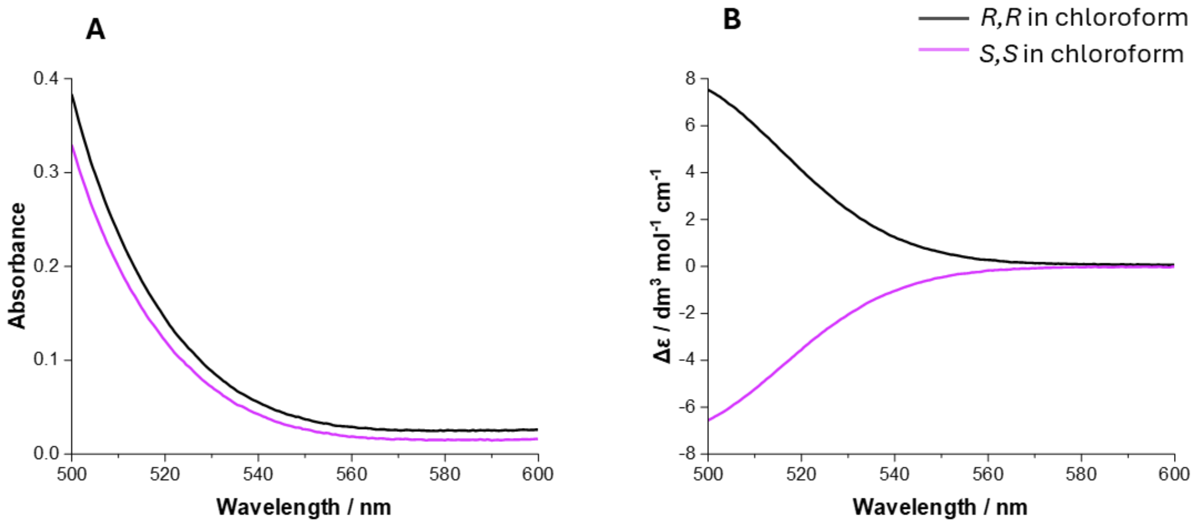

Fig. S2. Electronic absorption (A) and ECD (B) spectra of *R,R* and *S,S* enantiomers of Ni-complex in chloroform. The concentration of Ni-complex was equal to 0.0030 M. This experiment was performed to compare the spectra with those available in the literature.[3]

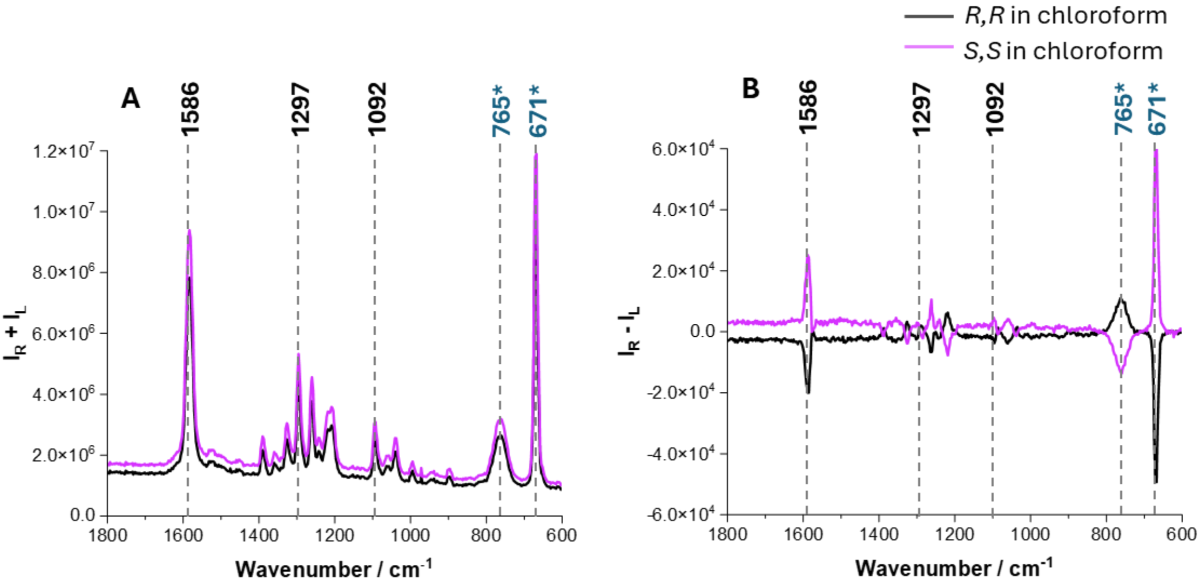

Fig. S3. Resonance Raman (A) and 'ECD-Raman/RROA' (B) spectra of *R,R* and *S,S* enantiomers of Ni-complex in chloroform. The concentration of Ni-complex equal to 0.0400 M. This experiment was performed to compare the spectra with those available in the literature.[3] Asterix (*) and blue color mark the most intense bands from the solvent.

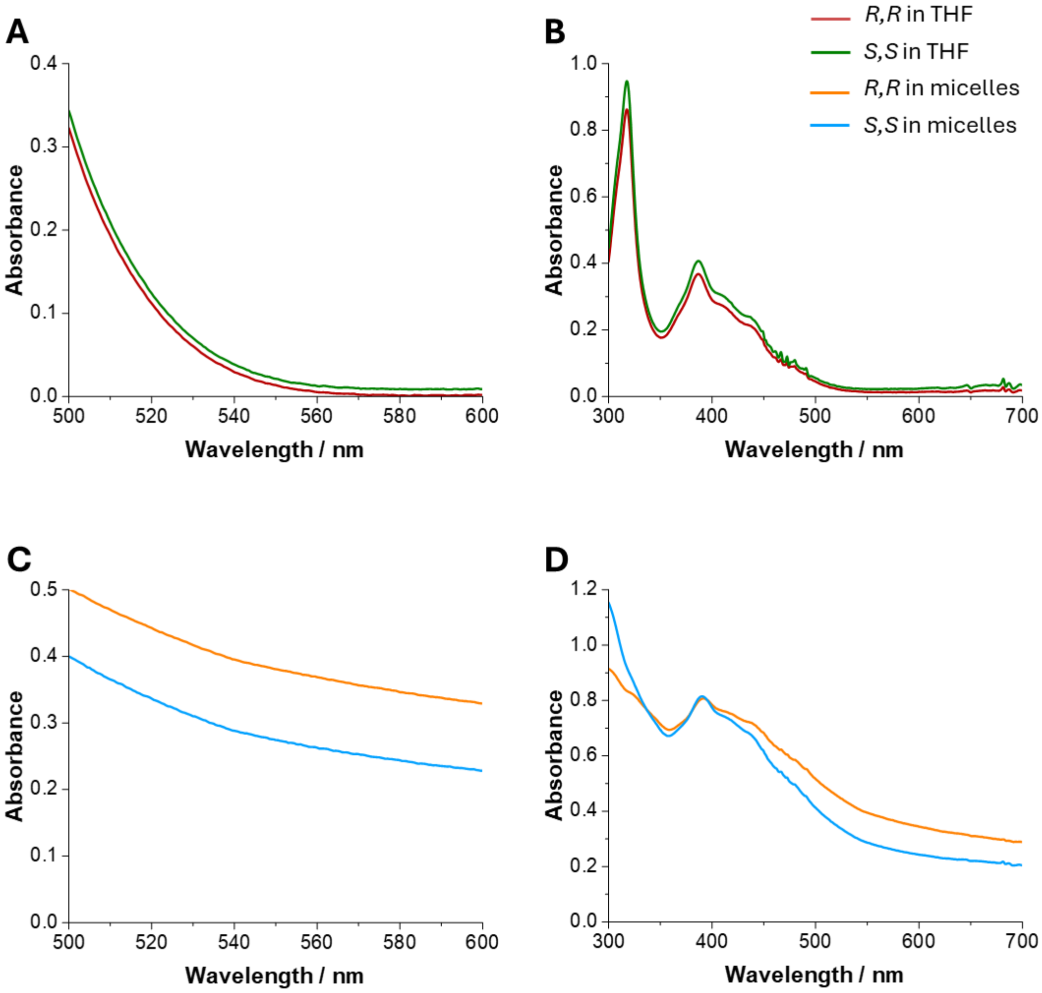

Fig. S4. Electronic absorption spectra of *R*,*R* and *S*,*S* enantiomers of Ni-complex in THF and after encapsulation in micelles. The concentration of Ni-complex in THF was equal to 0.003 M for 500-600 nm spectral range (**A**) and 0.0003 M for 300-700 nm range (**B**), respectively. The concentration of Ni-complex in micelles was equal to 0.0012 M for both 500-600 nm (**C**) and 300-700 nm range (**D**).

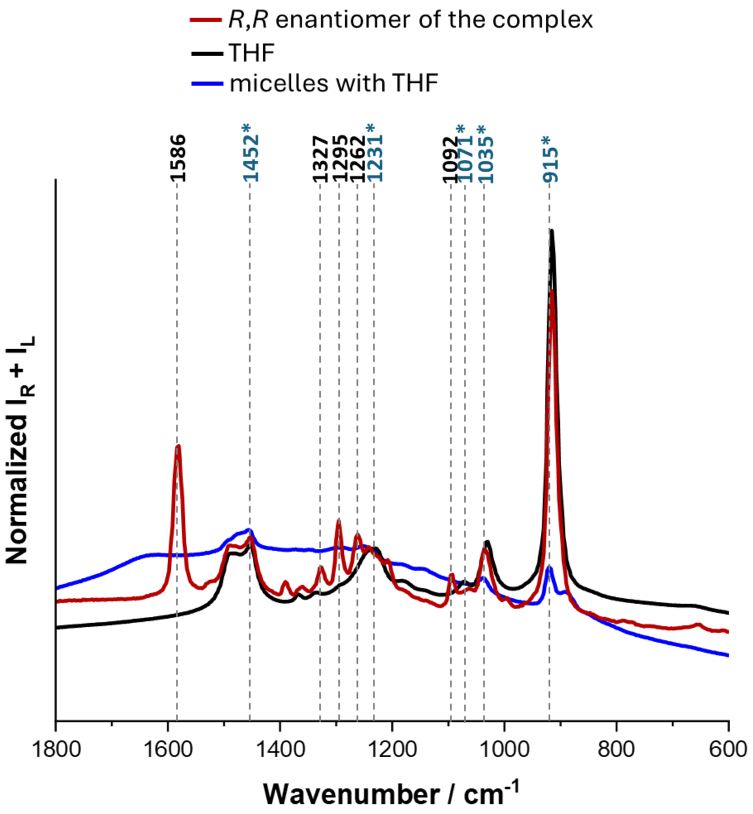

Fig. S5. The comparison of normalized Raman spectra of *R,R* enantiomer of Ni-complex in THF, pure THF and micelles with the addition of THF (trace amount). The system of micelles with the addition of THF is prepared and measured in the same conditions as *R,R* enantiomer of Ni-complex encapsulated in micelles (with trace amount of THF). Asterix (*) and blue color mark bands from THF. From the above figure, it can be seen that the bands with the highest intensity visible in the Raman spectrum of system containing micelles and THF come from THF.

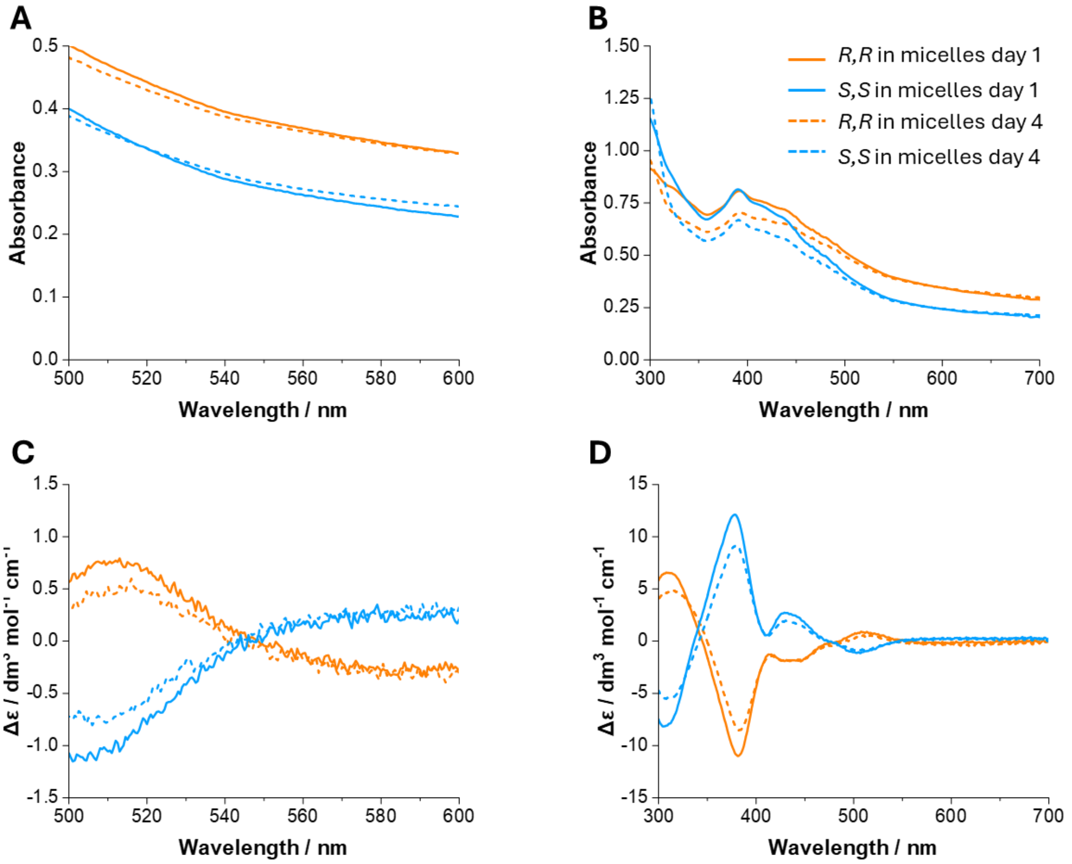

Fig. S6. Electronic absorption (A,B) and ECD (C,D) spectra showing the stability of *R,R* and *S,S* enantiomers of Ni-complex encapsulated in micelles by comparing the spectra of freshly prepared systems (1 day, solid line) and after 4 days (dashed line).

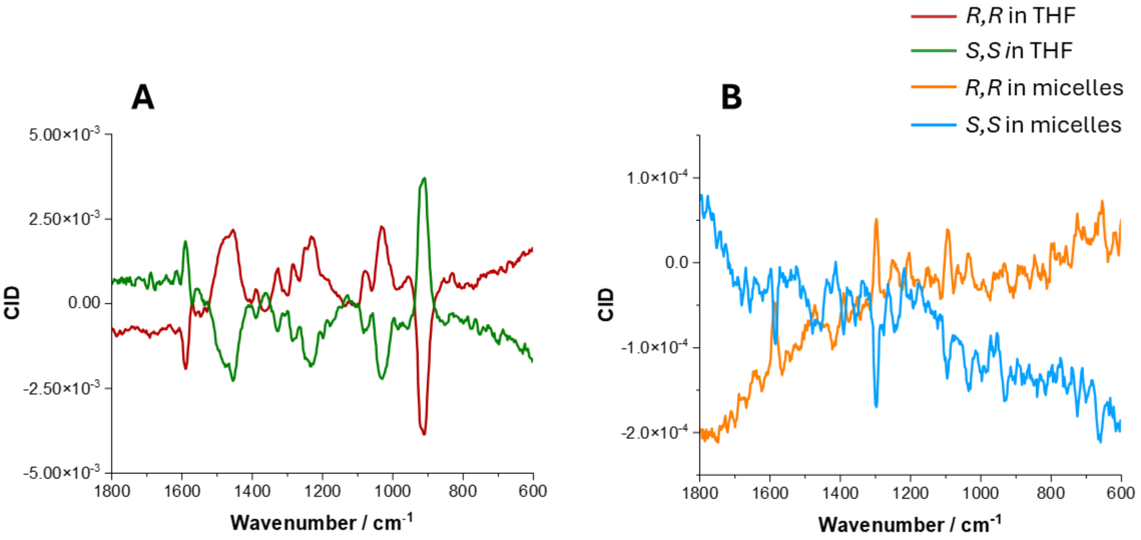

Fig. S7. CID spectra of *R,R* and *S,S* enantiomers of Ni-complex dissolved in THF (A) and after encapsulation in micelles (B). CID values were obtained by division of 'ECD-Raman/RROA' spectra by the respective Raman spectra.

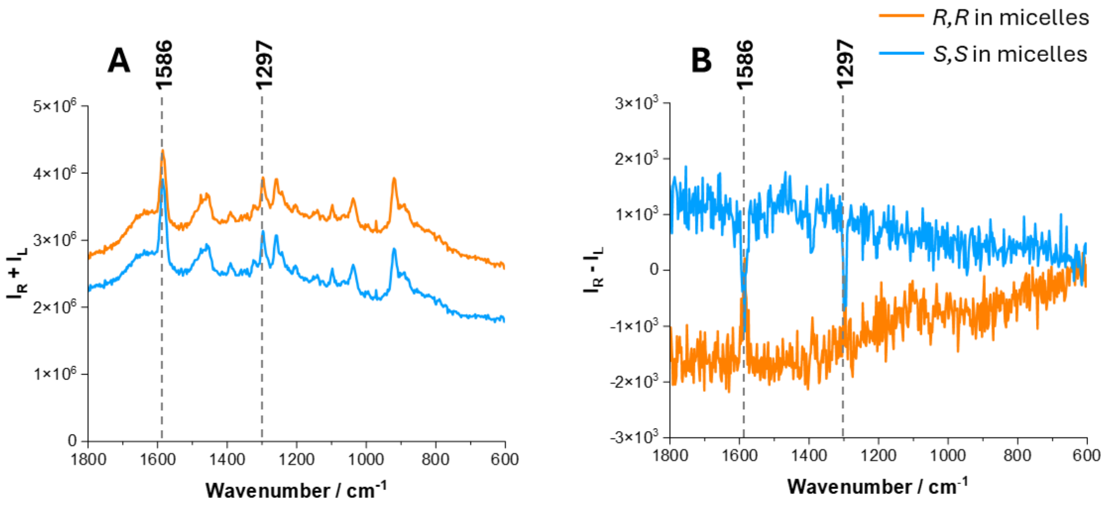

Fig. S8. Resonance Raman (A) and RROA (B) spectra of *R,R* and *S,S* enantiomers of Ni-complex encapsulated in micelles. The concentration of the micelles and Ni-complex were equal to 0.0010 M and 0.00058 M respectively. Spectra were collected for 24 hours.

Notes on the ECD-Raman formula

Reading carefully the previous works on the ECD-Raman effect, we have noticed that the relative intensities of the bands in the experimental 'ECD-Raman/RROA' spectra are not perfectly reproduced by the proposed ECD-Raman formula.[3-4] The analysis of *DOC*-dependent and independent terms of the equation (presented in the main text, **Fig. 3**) made us start pondering on signs of both terms in the ECD-Raman formula. In the case of the proposed formula[4], for the Ni-complex in THF, the first term sign is opposite to the sign of the second term (**Fig. 3**, main text).

But if the first term is negative (-$\Delta\varepsilon'$ instead of $\Delta\varepsilon'$, as below), the final equation will be:

$$I_R - I_L = \left(\frac{ln10}{4} c\,L\right)(-\Delta\varepsilon')(I_R + I_L) + \left(\frac{ln10}{4} c\,L\,\Delta\varepsilon\,DOC\right)\,(I_R + I_L)$$

where $\Delta\varepsilon$ and $\Delta\varepsilon'$ denote ECD intensity (in L·mol^{-1}·cm^{-1}) for the excitation and scattering, respectively, *L* is the optical path length (in cm), *c* is the concentration of the solute (in mol·L^{-1}), *DOC* is the degree of circularity of either the solvent or the solute and I_R+I_L is the Raman intensity at the given wavenumber.

For such case (**Fig. S9C**) the agreement of the 'ECD-Raman/RROA' spectra with the calculated ECD-Raman spectra is nearly perfect. Additionally, in this case the sign of the solute band is correct.

As the ECD-Raman formula is empirical, we conclude that it is possible that the factors are a bit different than proposed and that the $\Delta\varepsilon'$ requires the minus sign. However, we do not present it here as a fact, just as a hypothesis, which definitely needs to be verified for other examples.

$I_R - I_L = \left(\frac{ln10}{4} cL\Delta\varepsilon'\right)(I_R + I_L) + \left(\frac{ln10}{4} cL\Delta\varepsilon DOC\right)(I_R + I_L)$

$I_R - I_L = \left(\frac{ln10}{4} cL\right)(-\Delta\varepsilon')(I_R + I_L) + \left(\frac{ln10}{4} cL\Delta\varepsilon DOC\right)(I_R + I_L)$

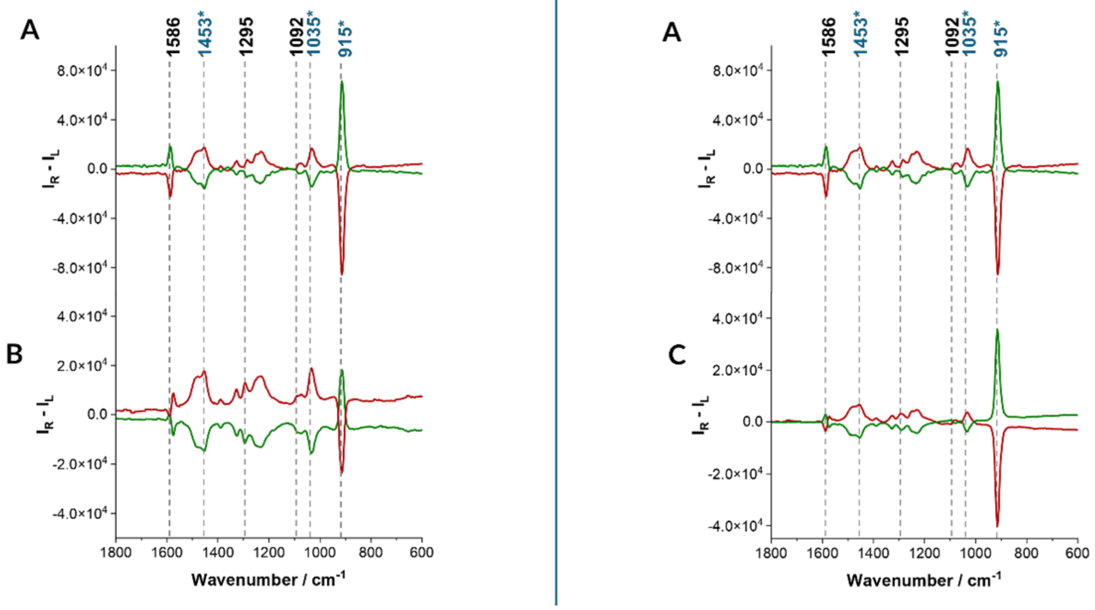

Fig. S9. The experimental 'ECD-Raman/RROA' spectra of *R*,*R* and *S*,*S* enantiomers of Ni-complex dissolved in THF (A) compared with the ECD-Raman spectra calculated employing currently used formula (B) and the formula with the negative $\Delta\varepsilon'$ value (C).

Table 1. CID values for signals at 1586, 1297, and 1092 cm^{-1} for *R*,*R* and *S*,*S* enantiomers of Ni-complex encapsulated in micelles. CID values are ratios of integral intensities of the respective RROA to Raman bands.

R*,*R			
Band wavenumber / cm^{-1}	1586	1297	1092
CID values	$2.84\cdot10^{-4}$	$8.12\cdot10^{-4}$	$7.38\cdot10^{-4}$
S*,*S			
Band wavenumber / cm^{-1}	1586	1297	1092
CID values	$-2.60\cdot10^{-4}$	$-1.09\cdot10^{-3}$	$-6.03\cdot10^{-4}$

References:

1. X. F. Shan, L. Z. Wu, X. Y Liu, L. P. Zhang, and C. H. Tung, *Eur. J. Inorg. Chem.*, **2007**, 21, 3315.
2. A. M. Bodratti and P. Alexandridis, *J. Funct. Biomater.*, **2018**, 9, 11.
3. T. Wu, G. Li, J. Kapitán, J. Kessler, Y. Xu and P. Bouř, *Angew. Chem. Int. Ed.*, **2020**, 59, 21895.
4. G. Li, M. Alshalalfeh, Y. Yang, J. R. Cheeseman, P. Bouř and Y. Xu, *Angew. Chem. Int. Ed.*, **2021**, 60, 22004.