Electronic Supporting Information (ESI)

Racemic vs. Enantiopure Inert Ti(IV) Complex of a Single Diaminotetrakis(phenolato) Ligand in Anticancer Activity Toward Human Drug-Sensitive and -Resistant Cancer Cell Lines

Maya Miller[a] and Edit Y. Tshuva[a]*

[a]*Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 9190401, Israel. Email: edit.tshuva@mail.huji.ac.il*

Experimental section

Ligands *R,R*-, *S,S*-, and rac-LH_4 and the corresponding titanium(IV) complexes *R,R,Δ-, S,S,Λ-,* and *rac*-LTi were synthesized according to a published procedures.[1–3] $NaBH_4$ (97%), 2,2'-dihydroxybenzophenone (98%), titanium tetra(isopropoxide) (97%) and 1,2-*trans*-cyclohexanediamine (99%), were purchased from Aldrich Chemical Company Inc. or Alfa Aesar. All solvents were dried over aluminum column on an MBraun drying system SPS-800. All experiments requiring dry atmosphere were performed in an M. Braun or LC-technologies dry-box or under nitrogen atmosphere using Schlenck line technique. NMR spectroscopic data were recorded with an AMX-400 MHz or AMX-500 MHz Bruker spectrometer. X-ray diffraction data were obtained with Bruker Smart Apex diffractometer. High resultion electrospray ionisation mass spectrometry were performed in the microanalytical laboratory in our institute. Specific optical rotation measurements were performed by Autopol I Automatic Polarimeter from Rudolph Research analytical and were calculated as the average of five measurements. Circular dichroism (CD) spectra of the complexes and the ligands were recorded on JASCO J-810 Spectrophotometer (JASCO, Japan) using supplied SpectraManager software. The temperature was kept constant at 20 ˚C using a temperature controlled water bath. Samples were prepared fresh from stock before each measurement. A range of 2-7 mg of the tested compound/ligand was dissolved in 200/400 µL in DMSO and used as stock solution. Consequently dilutions provided concentrations ranging from 0.65 to 1.45 mM and were placed in a 1mm quartz cell. The spectra were recorded in wavelength rang of λ = 240 to 500 nm, with 3 accumulations for

each measurement and a data pitch of 0.1 nm. Background CD spectrum of the solvent was recorded and subtracted form each spectrum.

Cytotoxicity was measured on human colon HT-29 (purchased from ATCC Inc.), human ovarian carcinoma A2780, human ovarian cisplatin-resistant carcinoma A2780cp, and human ovarian adriamycin-resistant carcinoma A2780adr (purchased from ECACC Inc.) cancer cell lines using the MTT assay as previously described.[4] Cells (0.6×10^6) in medium (containing 10% fetal bovine serum, 1% L-glutamine, 1% penicillin/streptomycin antibiotics and 88% Roswell Park Memorial Institute (RPMI) 1640 medium, purchased from Biological Industries Inc.) were seeded in 96 well plates (Thermo Scientific NUNC™) and were allowed to attach overnight. Following 1 day, the cells were treated with the tested compound or combination of different ratios of the pure enantiomers at 10 different concentrations and the cells were incubated for 3 days at 37 ˚C in 5% CO_2 atmosphere. Consequently, MTT (0.1 mg in 20 µL) was added to the cells for additional incubation of 3 hours. The medium was then removed, 200 µL of isopropanol was added, and the absorbance was measured at 550 nm by Bio-Tek EL-800 microplate reader or by a Spark 10M multimode microplate reader spectrophotometer (Tecan Group Ltd. Mannedorf, Switzerland). Each measurement was repeated at least 3×3 times, namely, three repeats per plate, all repeated three times on different days (9 repeats altogether). Relative IC_{50} values with standard error of means were determined by a nonlinear regression of a variable slope (four parameters) model by Graph Pad Prism5.0 program.

Hydrolysis studies by NMR were performed at room temperature as previously described,[5,6] using ca. 3.6 mM of the complex solution in DMSO and adding >10000 equiv. of H_2O to give a final solution of 9:1 H_2O / DMSO. Following 17 days, the volatiles were removed by reduced pressure, the remaining mixture lyophilized, and a final spectrum was recorded in DMSO-d_6. An additional hydrolysis study was performed in DMSO, adding >1000 equiv. of D_2O to give a final solution of 9:1 DMSO / D_2O, monitored over time by NMR, and calibrated to dinitrobenzene internal standard (Sigma Aldrich ltd.). The results are provided below.

***Rac*-LH_4:** The ligand was synthesized based on a published procedure[1] in 87% isolated yield. ESI-HRMS $(C_{32}H_{34}N_2O_4 + H)^+$ m/z Calc.: 511.26023 $[M^+]$ Found: 511.26026. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.11-6.99 (m, 4H), 6.82-6.75 (m, 4H), 6.75-6.68 (m, 4H), 6.68-6.60 (m, 4H), 5.35 (s, 2H), 2.48-2.38 (m, 2H), 2.07-1.94 (m, 2H), 1.61-1.48 (m, 2H), 1.22-1.09 (m, 4H) ppm. ^{13}C NMR

(125 MHz, DMSO-d_6): δ = 157.1, 156.3, 129.0, 128.6, 128.4, 128.3, 128.2, 127.2, 119.3, 118.9, 116.1, 115.9, 57.5, 55.9, 25.0, 23.4 ppm.

***R,R*-** (47%) and ***S,S* -LH$_4$** (68%) were prepared similarly to *rac*-LH_4 from the optically pure *trans*-1,2-cyclohexanediamine. ESI-HRMS ($C_{32}H_{34}N_2O_4$ + H)$^+$ m/z Calc.: 511.26023 [M$^+$]; *R,R,Δ*-LH_4 found 511.26458, *S,S,Λ*-LH_4 found 511.25829. Optical rotation for *R,R,Δ*-LH_4: $[\alpha]_D^{25}$=-64±2°, for *S,S,Λ*-LH_4: $[\alpha]_D^{24}$=68±1° (*c* = 1.6 and 1.4 mg/mL in DMSO, respectivly).

***Rac*-LTi:** Ti(O*i*Pr)$_4$ (0.097 g, 3.4 µmol) was reacted with *rac*-LH_4 (0.018 g, 3.4 µmol) in dry THF at room temperature overnight. Spontaneous yellow precipitation was isolated by decantation and obtained in a >95% purity (isolated yield: 88%). ESI-HRMS ($C_{32}H_{30}N_2O_4Ti$ + H)$^+$ m/z Calc.: 555.17688 [M$^+$] Found: 555.17303. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.45 (d, *J*=6.0 Hz, 2H), 7.22 (d, *J*=6.0 Hz, 2H), 7.10 (t, *J*=6.1 Hz, 2H), 6.96 (t, *J*=6.1 Hz, 2H), 6.76 (t, *J*=5.9 Hz, 2H), 6.58 (t, *J*=5.8 Hz, 2H), 6.52 (d, *J*=6.3 Hz, 2H), 6.33 (d, *J*=6.4 Hz, 2H), 5.32 (s, 2H), 2.78-2.69 (m, 2H), 2.36-2.25 (m, 2H), 1.71-1.61 (m, 2H), 1.51-1.38 (m, 2H), 0.95-0.83 (m, 2H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 161.4, 160.5, 133.5, 130.1, 129.8, 128.7, 120.2, 118.8, 117.7, 115.8, 62.3, 61.2, 28.0, 27.6, 25.8, 24.7 ppm.

***R,R,Δ*-** (72%) and ***S,S,Λ*-LTi** (61%) were prepared similarly to *rac*-LTi from optically pure LH_4. ESI-HRMS ($C_{32}H_{30}N_2O_4Ti$ + H)$^+$ m/z Calc.: 555. 17688 [M$^+$]; *R,R,Δ*-LTi found 555.17328, *S,S,Λ*-LTi found 555.17231. Optical rotation for *R,R,Δ*-LTi: $[\alpha]_D^{27}$=103±4°, for *S,S,Λ*-LTi: $[\alpha]_D^{25}$=-93±1° (*c* = 0.7 mg/mL DMSO).

Crystal data for *S,S,Λ*-LTi: $C_{32}H_{30}N_2O_4Ti$, M_r = 554.48, tetragonal, *a* = b = 15.934(2) Å, *c* = 12.007(2) Å, *V* = 3048.2(6) Å^3, *T* = 293(1) K, space group $P4_12_12$, *Z* = 4, μ(Mo-K$_\alpha$) =0.317 mm^{-1}, 35268 reflections measured, 3667 unique (Rint = 0.0396). $R(F^2_o)$ for [I>2σ(I)] = 0.0710, R_w for [I>2σ(I)] = 0.1658.

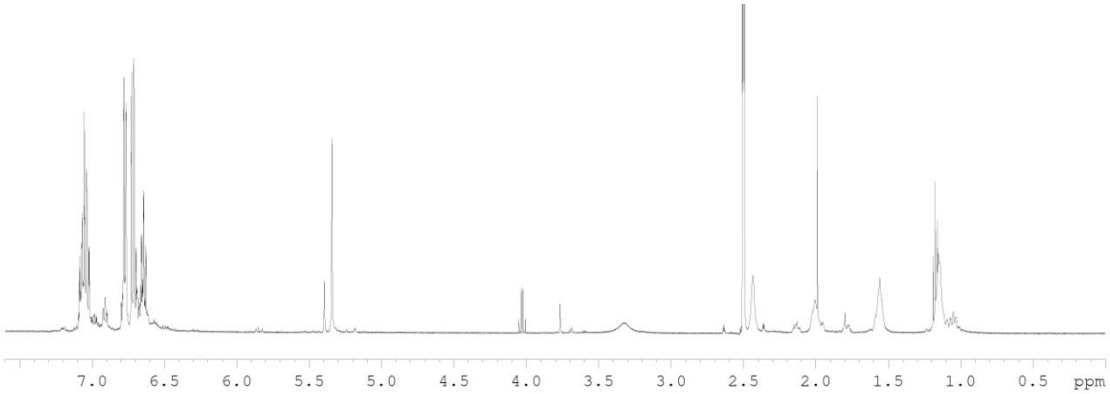

Figure S1: ^{1}H NMR spectra of *S,S*-LH_4 in DMSO-d_6

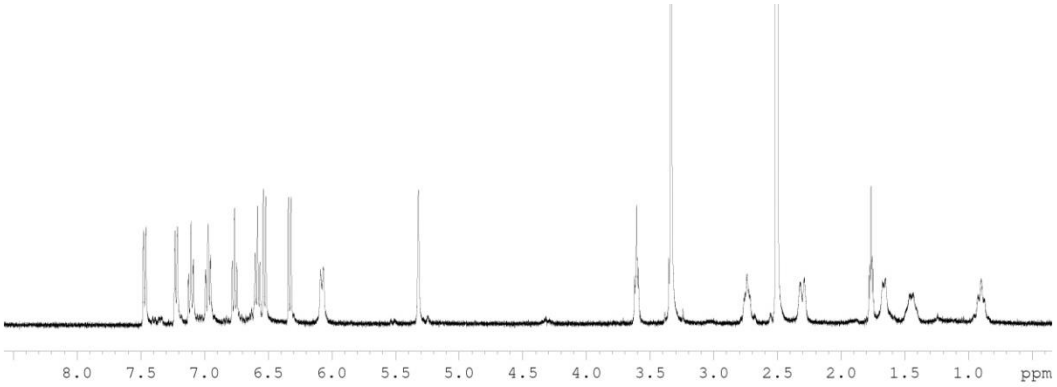

Figure S2: ^{1}H NMR spectra of *S,S,Λ*-LTi in DMSO-d_6

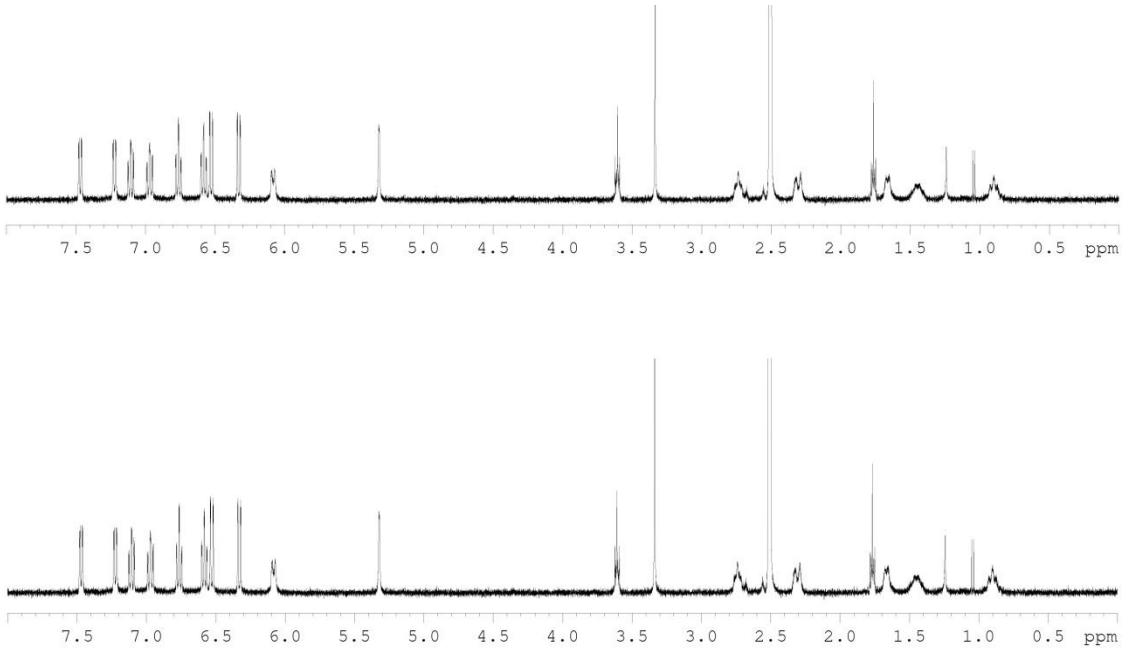

Figure S3: ^{1}H NMR spectrum for *S,S,Λ*-LTi in DMSO-d_6, before (top) and 17 days after (bottom), the addition of 90% H_2O (spectrum of the latter taken in neat DMSO-d_6 following evaporation)

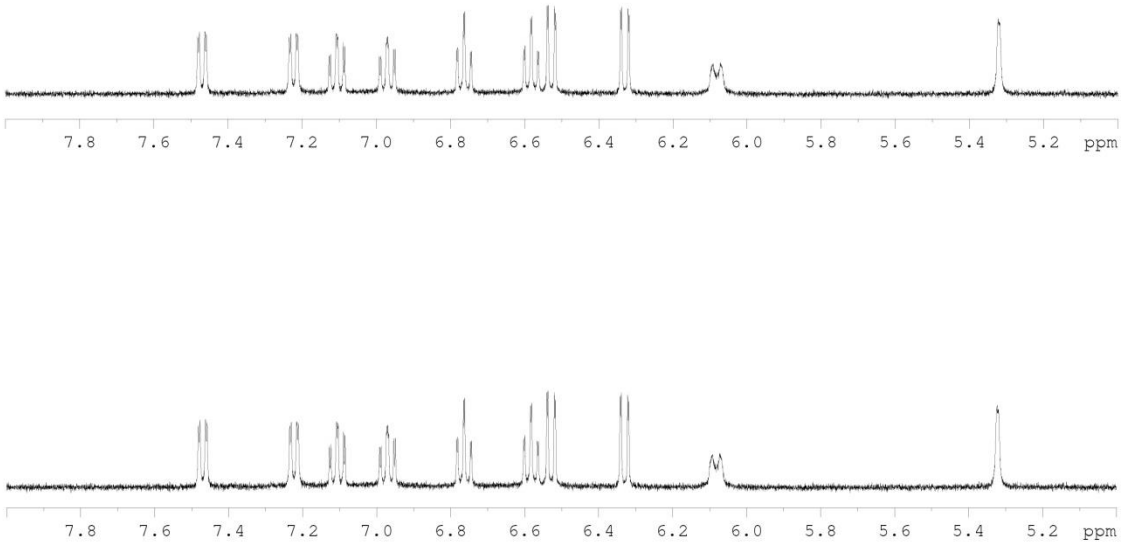

Figure S4: ^{1}H NMR spectrum for aromatic region of *S,S,Λ*-LTi in DMSO-d_6, before (top) and 17 days after (bottom), the addition of 90% H_2O (spectrum of the latter taken in neat DMSO-d_6 following evaporation)

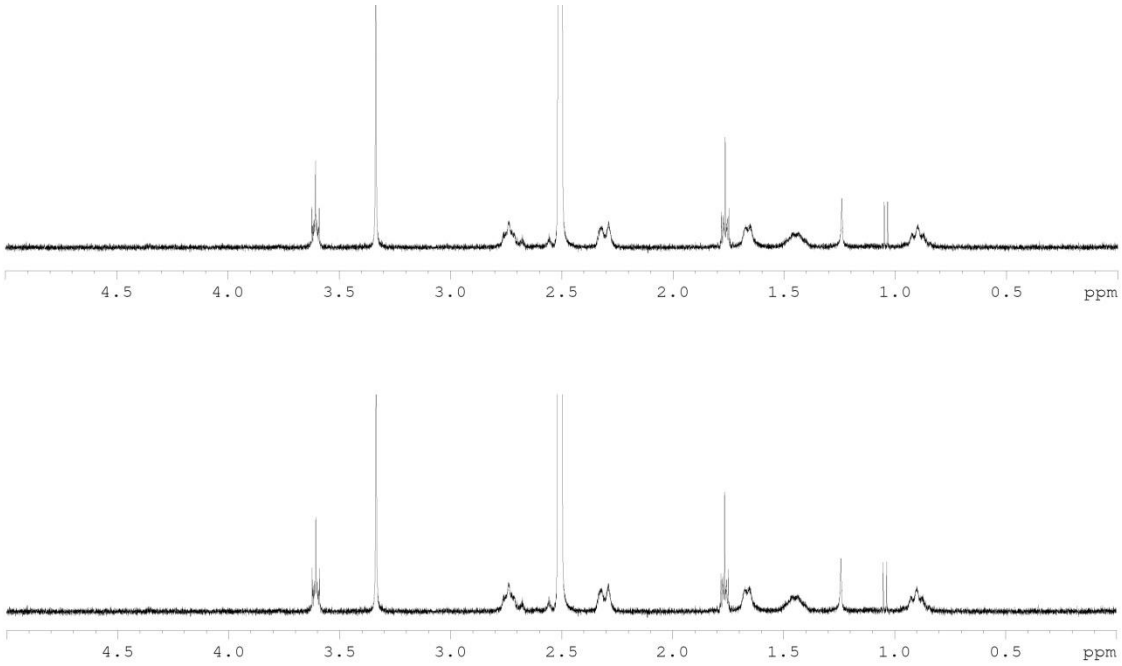

Figure S5: ^{1}H NMR spectrum for aliphatic region of *S,S,Λ*-LTi in DMSO-d_6, before (top) and 17 days after (bottom), the addition of 90% H_2O (spectrum of the latter taken in neat DMSO-d_6 following evaporation)

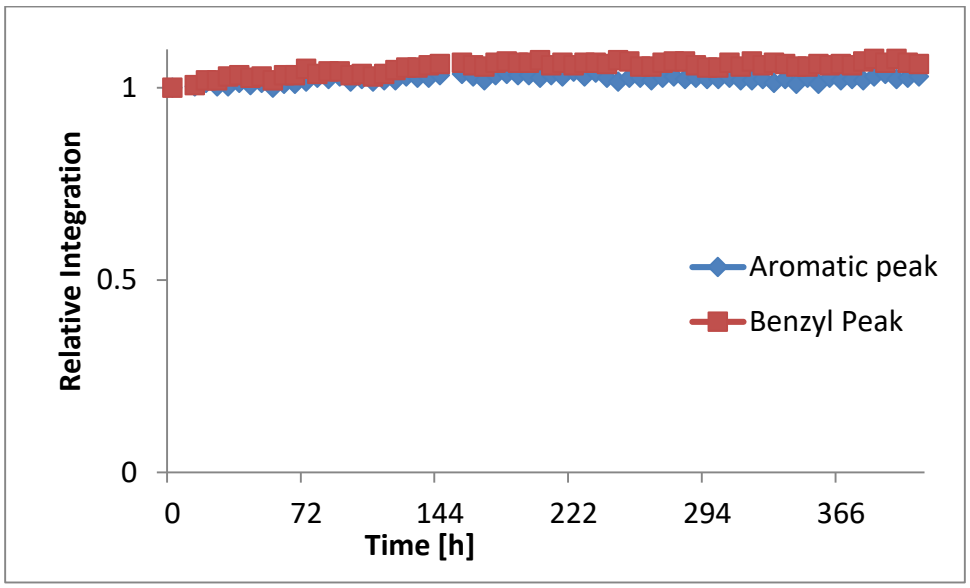

Figure S6: A plot of the relative integration (vs an internal standard) of selected signals in the ^{1}H NMR spectrum of *S,S,Λ*-LTi versus time following the addition of 10% D_2O to a solution of the complex in DMSO-d_6 at RT.

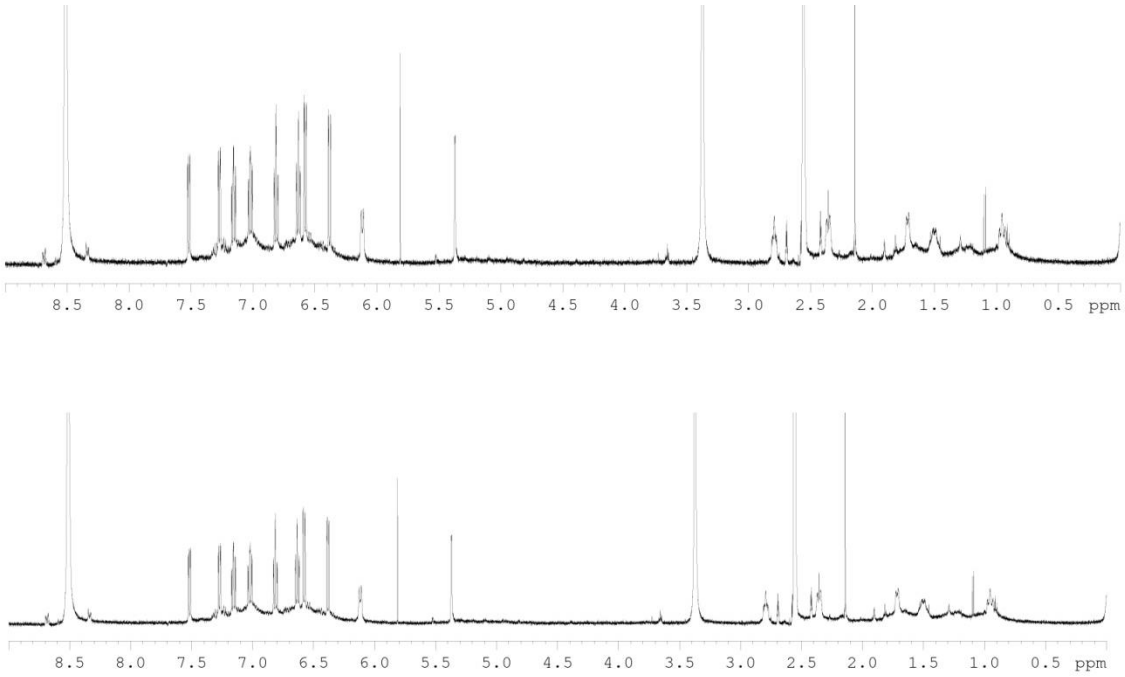

Figure S7: ^{1}H NMR spectrum for *S,S,Λ*-LTi in DMSO-d_6, immediatly after (top) and 17 days following (bottom), the addition of 10% D_2O (specta taken in 90% DMSO-d_6; 10% D_2O)

Table S1: Relative IC_{50} [μM] values for complexes LTi toward human A2780 and HT-29 cancer cell lines

	IC_{50} [μM] Racemic	*R,R,Δ*	*S,S,Λ*
A2780	78.8±19.1	*	*
HT-29	77.9±13.3	71.7±14.5	59.4±23.9

*Could not be derived

Table S2: Relative IC_{50} [μM] values for stereochemical isomers of LTi toward human A2780cp and A2780adr cancer cell lines

	IC_{50} [μM] Racemic	*R,R,Δ*	*S,S,Λ*	*Δ: Λ* (3:1)	*Δ: Λ* (1:3)
A2780cp	14.6±5.0	*	*	*	1.3±0.3
A2780adr	13.1±1.8	*	*	*	21.6±14.8

* Could not be derived

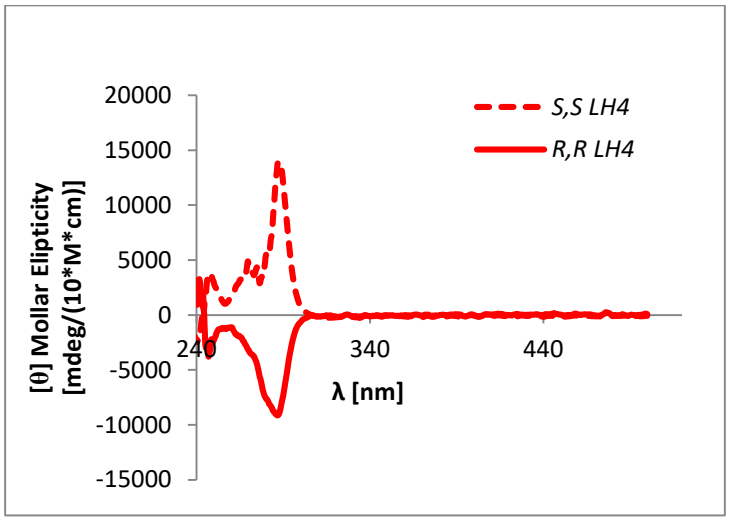

Figure S8: CD spectra of *R,R*- and *S,S*- LH_4

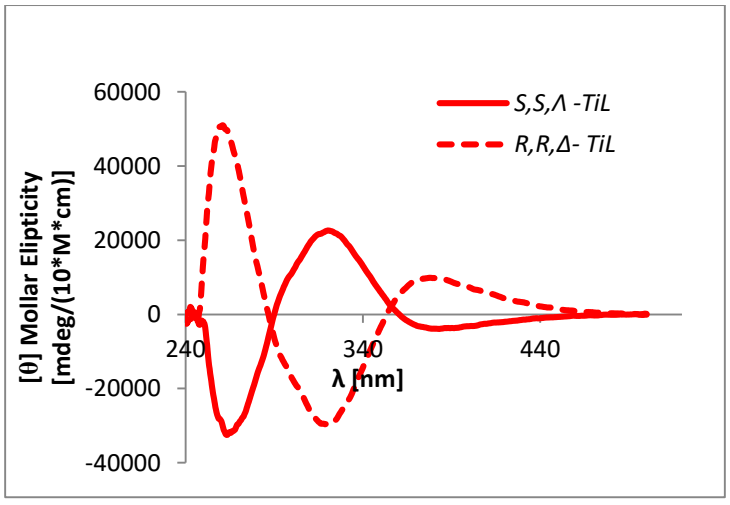

Figure S9: CD spectra of *R,R,Δ*- and *S,S,Λ*- TiL

References:

1 M. S. Seo and H. Kim, *J. Am. Chem. Soc.*, 2015, **137**, 14190–14195.

2 C. M. Manna, G. Armony and E. Y. Tshuva, *Inorg. Chem.*, 2011, **50**, 10284–10291.

3 M. Miller and E. Y. Tshuva, *Eur. J. Inorg. Chem.*, 2014, 1485–1491.

4 N. Ganot, S. Meker, L. Reytman, A. Tzubery and E. Y. Tshuva, *J. Vis. Exp.*, 2013, e50767.

5 D. Peri, S. Meker, M. Shavit and E. Y. Tshuva, *Chem.Eur. J.*, 2009, **15**, 2403–2415.

6 D. Peri, S. Meker, C. M. Manna and E. Y. Tshuva, *Inorg. Chem.*, 2011, 1030–1038.