Markedly Different Cytotoxicity of the Two Enantiomers of C_2-Symmetrical Ti(IV) Phenolato Complexes; Mechanistic Implications

Cesar M. Manna and Edit Y. Tshuva*

The Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel. Fax: +972 2 6584282; Tel: +972 2 6586084; Email: tshuva@chem.ch.huji.ac.il

Supporting Information

Experimental Section

The ligand synthesis is a modified known procedure.[1] ±-*Trans*-1,2-diaminocyclohexane (98%), was purchased from Across Organic, Inc. and used without further purification. *S,S*-1,2-diaminocyclohexane (98%; >99% *ee*), *R,R*-1,2-diaminocyclohexane (98%; >99% *ee*), 2-hydroxy-5-methylbenzaldehyde (98%), $NaBH_4$ (98%), and titanium tetra(isopropoxide) (97%) were purchased from Aldrich Chemical Company, Inc and used without further purification. All solvents used for procedures requiring an inert atmosphere were distilled from potassium or potassium/benzophenone under nitrogen. All experiments requiring dry atmosphere were performed in a M. Braun dry-box or under nitrogen atmosphere using

Schlenck line techniques. NMR data were recorded using AMX-400 or -500 MHz Bruker spectrometer. $CDCl_3$ (99.8%) was purchased from Cambridge Isotope Laboratories Inc. and used without further purification. Optical rotation was measured on a Jasco P-1010 polarimeter at 589 nm with d = 0.2 dm. X-ray diffraction data were obtained with Bruker Smart Apex diffractometer, running the SMART software package. After collection, the raw data frames were integrated by the SAINT software package. The structures were solved and refined using the SHELXTL software package. Elemental analyses were performed in the microanalytical laboratory in our institute. Cytotoxicity was measured on HT-29 colon and OVCAR-1 ovarian cells obtained from ATCC Inc. using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay as previously described:[2,3] cells ($1.2x10^6$) in medium (contains: 1% penicillin/streptomycin antibiotics; 1% L-glutamine; 10% fetal bovine serum (FBS), all purchased from Biological Industries Inc., and 88% medium RPMI-1640, purchased from Sigma Inc.) were seeded into 55 wells in a 96-well plate and allowed to attach for 24 hours. The cells were consequently treated with the reagent tested at different concentrations. Solution of reagent was prepared by dissolving the reagent in 10 μL of THF and diluting with 90 μL of medium to give final concentrations of up to 200 mg/L. From the resulting solution, 10 μL was added to each well already containing 200 μL of the aforementioned solution of cells in the medium. After 3 days incubation at 37°C in 5% CO_2 atmosphere, MTT (0.1 mg in 20 μL) was added, and the cells were incubated for additional 3-4 hours. The MTT solution was then removed, and the cells were dissolved in 200 μL 2-propanol. The absorbance at 550 nm was measured for 200 μL of the aforementioned solution by a Bio-Tek EL-800 microplate reader spectrophotometer. Each measurement was repeated at least 3×5 times. Control experiments with THF only (10 μL solvent in 210 μL total volume as described above) verified no influence on the cells in the above partial volumes.

Rac-H$_2$L^5 was synthesized similarly to published procedures[1,4] by adding dropwise *trans*-1,2-diaminocyclohexane (0.42 g, 3.7 mmol) to a stirred solution of 2-hydroxy-5-methylbenzaldehyde (1 g, 7.34 mmol) in toluene (10 mL). The reaction mixture was stirred and refluxed for 2 hours, after which the reaction was cooled and the solvent evaporated. The yellow solid obtained, corresponding to the salen ligand, was dissolved in methanol, and 5 equiv of NaBH$_4$ (0.69 g, 18.5 mmol) were added portion-wise with stirring. The reaction mixture was stirred for additional 2 hours at RT, during which a color change to colorless was observed. The reaction mixture was poured into water (100 mL), and the product was isolated by vacuum filtration (0.8 g, 60% yield) (Found: C, 74.7; H, 8.7, N, 8.1. Calc. for $C_{22}H_{30}N_2O_2$: C, 74.6; H, 8.6; N, 8.0%). δ_H(400 MHz; CDCl$_3$) 6.96 (2 H, dd, *J* 8.2, 1.8 Hz, Ar), 6.78 (2 H, d, *J* 1.8 Hz, Ar), 6.72 (2 H, d, *J* 8.2 Hz, Ar), 4.00 (2 H, d, *J* 13.9 Hz, CH$_2$), 3.89 (2 H, d, *J* 13.8 Hz, CH$_2$), 2.45 (2 H, m, cy), 2.24 (6 H, s, CH$_3$), 2.16 (2 H, m, cy), 1.70 (2 H, m, cy), 1.24 (m, 4H, cy). δ_C(400 MHz; CDCl$_3$) 155.5, 129.2, 128.8, 128.2, 122.5, 116.1, 59.8, 49.8, 30.5, 24.1, 20.4.

***R,R*-H$_2$L^5** [1] (0.7 g, 54%. $[\alpha]_D^{27}$= -16°, (*c* 15.6 mM in CHCl$_3$). Found: C, 74.6; H, 8.6, N, 8.0. Calc. for $C_{22}H_{30}N_2O_2$: C, 74.6; H, 8.6; N, 8.0%). δ_H(400 MHz; CDCl$_3$) 6.96 (2 H, d, *J* 8.0 Hz, Ar), 6.78 (2 H, s, Ar), 6.72 (2 H, d, *J* 8.0 Hz, Ar), 4.00 (2 H, d, *J* 13.8 Hz, CH$_2$), 3.90 (2 H, d, *J* 13.8 Hz, CH$_2$), 2.45 (2 H, m, cy), 2.23 (6 H, s, CH$_3$), 2.16 (2 H, m, cy), 1.69 (2 H, m, cy), 1.24 (4 H, m, cy). δ_C(400 MHz; CDCl$_3$) 155.5, 129.2, 128.8, 128.2, 122.5, 116.1, 59.8, 49.8, 30.5, 24.1, 20.4.) and ***S,S*-H$_2$L^5** [1] (0.8 g, 61%. $[\alpha]_D^{26}$= 13° (*c* 15.1 mM in CHCl$_3$).). δ_H(400 MHz; CDCl$_3$) 6.96 (2 H, d, *J* 8.1 Hz, Ar), 6.79 (2 H, s, Ar), 6.73 (2 H, d, *J* 8.1 Hz, Ar), 4.00 (2 H, d, *J* 13.8 Hz, CH$_2$), 3.89 (2 H, d, *J* 13.8 Hz, CH$_2$), 2.45 (2 H, m, cy), 2.23 (6 H, s, CH$_3$), 2.16 (2 H, m, cy), 1.71 (2 H, m, cy), 1.24 (4 H, m, cy). δ_C(400 MHz; CDCl$_3$) 155.5, 129.2, 128.8, 128.2, 122.5, 116.1, 59.8, 49.8, 30.5, 24.1, 20.4.) were synthesized in a direct analogy to **rac-H_2L^5** from *R*,*R*-1,2-diaminocyclohexane and *S*,*S*-1,2-diaminocyclohexane, respectively.

Rac-L^5Ti(OiPr)$_2$ was synthesized similarly to $L^1Ti(OiPr)_2$[5] by reacting $Ti(OiPr)_4$ (0.3 g, 1.0 mmol) with *rac*-H_2L^5 (0.8 g, 1.0 mmol) in THF at RT under nitrogen atmosphere to give the light yellow product in a quantitative yield, which may be recrystallized from diethylether at RT (0.3 g, 60%). (Found: C, 65.0; H, 8.6, N, 5.4. Calc. for $C_{28}H_{42}N_2O_2Ti$: C, 64.9; H, 8.2; N, 5.4%). δ_H(400 MHz; $CDCl_3$) 6.92 (2 H, d, *J* 8 Hz, Ar), 6.71 (2 H, s, Ar), 6.57 (2 H, d, 8 Hz, Ar), 4.80 (2 H, sept, *J* 6 Hz, CH), 4.73 (2 H, d, *J* 14 Hz, CH_2), 3.83 (2 H, d, 14 Hz, CH_2), 2.35 (2 H, m, cy), 2.22 (6 H, s, CH_3), 1.60 (2 H, m, cy), 1.20 (14 H, m, cy, CH_3), 1.00 (2 H, m, cy), 0.80 (2 H, m, cy). δ_C (500 MHz; $CDCl_3$) 160.4, 129.6, 129.1, 126.1, 121.8, 117.7, 57.9, 49.3, 30.0, 26.1, 26.0, 24.5, 20.6.

Δ,R,R*-L^5Ti(OiPr)$_2$** (>95%. 0.25 g, 52% following recrystallization. $[\alpha]_D^{27}$= -113°, (*c* 5.4 mM in $CHCl_3$). Found: C, 64.6; H, 8.2, N, 5.5. Calc. for $C_{28}H_{42}N_2O_2Ti$: C, 64.9; H, 8.2; N, 5.4%. δ_H(500 MHz; $CDCl_3$) 6.92 (2 H, d, *J* 8 Hz, Ar), 6.72 (2 H, s, Ar), 6.58 (2 H, d, *J* 8 Hz, Ar), 4.83 (2 H, sept, *J* 6 Hz, CH), 4.75 (2 H, d, *J* 14 Hz, CH_2), 3.85 (2 H, d, *J* 14 Hz, CH_2), 2.35 (2 H, m, cy), 2.22 (6 H, s, CH_3), 1.6 (2 H, m, cy), 1.2 (14 H, m, cy, CH_3), 1 (2 H, m, cy), 0.8 (2 H, m, cy). δ_C(500 MHz; $CDCl_3$) 160.4, 129.6, 129.1, 126.1, 121.8, 117.7, 57.9, 49.3, 30.0, 26.1, 26.0, 24.5, 20.6.) and ***Λ,S,S*-L^5Ti(OiPr)$_2$** (>95%. 0.25 g, 52% following recrystallization $[\alpha]_D^{28}$= 114°, (*c* 3.6 mM in $CHCl_3$). Found: C, 64.9; H, 8.3, N, 5.4. Calc. for $C_{28}H_{42}N_2O_2Ti$: C, 64.9; H, 8.2; N, 5.4%. δ_H(500 MHz; $CDCl_3$) 6.92 (2 H, d, *J* 8 Hz, Ar), 6.72 (2 H, s, Ar), 6.58 (2 H, d, *J* 8 Hz, Ar), 4.83 (2 H, sept, *J* 6 Hz, CH), 4.75 (2 H, d, *J* 14 Hz, CH_2), 3.85 (2 H, d, *J* 14 Hz, CH_2), 2.35 (2 H, m, cy), 2.22 (6 H, s, CH_3), 1.63 (2 H, m, cy), 1.2 (14 H, m, cy, CH_3), 1.02 (2 H, m, cy), 0.8 (2 H, m, cy). δ_C (500 MHz; $CDCl_3$) 160.4, 129.6, 129.1, 126.1, 121.8, 117.7, 57.9, 49.3, 30.0, 26.1, 26.0 24.5, 20.6.) were synthesized in a direct analogy to *rac*-$L^5Ti(OiPr)_2$ from ***R**,*****R*****-**$\mathbf{H_2L^5}$ and ***S*****,*****S*****-**$\mathbf{H_2L^5}$, respectively.

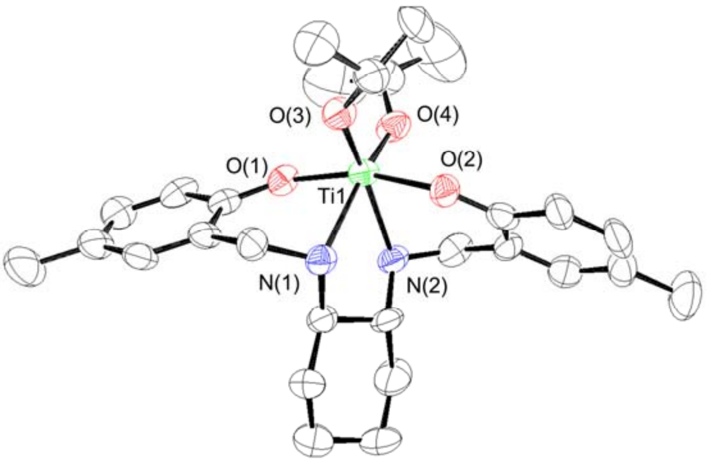

Figure 1. ORTEP drawing of one molecule of *rac*-L^{5}Ti(OiPr)$_2$ out of three similar ones found in the asymmetric unit at 50% probability ellipsoids; H atoms and solvent were omitted

Table 1: Selected bond length (Å) and angles (°) for one molecule of Δ,*R*,*R*-L^{5}Ti(OiPr)$_2$ found in the asymmetric unit of *rac*-L^{5}Ti(OiPr)$_2$

O(1)–Ti(1)	1.912(4)	O(4)–Ti(1)	1.808(4)
O(2)–Ti(1)	1.910(4)	N(1)–Ti(1)	2.284(5)
O(3)–Ti(1)	1.810(4)	N(2)–Ti(1)	2.259(5)
O(4)–Ti(1)–O(3)	105.3(2)	O(1)–Ti(1)–N(1)	80.31(18)
O(4)–Ti(1)–O(2)	98.4(2)	O(2)–Ti(1)–N(1)	86.08(18)
O(3)–Ti(1)–O(2)	91.37(18)	O(3)–Ti(1)–N(1)	91.81(19)
O(4)–Ti(1)–O(1)	92.4(2)	O(4)–Ti(1)–N(1)	162.17(19)
O(3)–Ti(1)–O(1)	96.97(18)	O(1)–Ti(1)–N(2)	88.50(18)
O(2)–Ti(1)–O(1)	164.22(19)	O(2)–Ti(1)–N(2)	80.19(18)
N(1)–Ti(1)–N(2)'	74.72(19)	O(3)–Ti(1)–N(2)	164.5(2)
		O(4)–Ti(1)–N(2)	89.0(2)

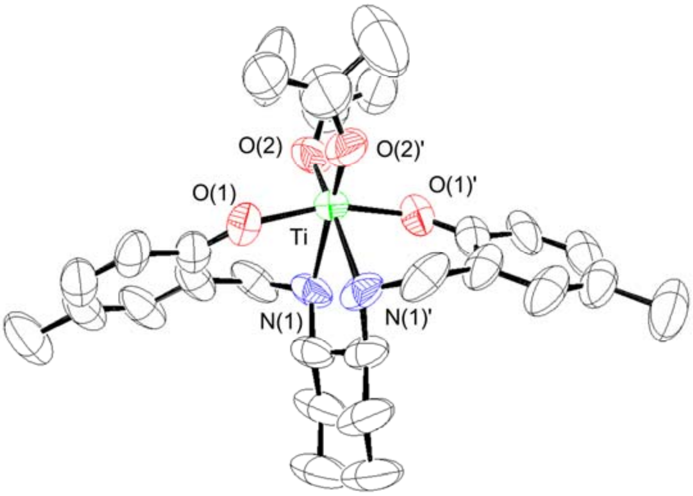

Figure 2. ORTEP drawing of *Λ*,*S*,*S*-L^{5}Ti(OiPr)$_2$ at 50% probability ellipsoids; H atoms were omitted

Table 2: Selected bond length (Å) and angles (°) for *Λ*,*S*,*S*-L^{5}Ti(OiPr)$_2$

O(1)–Ti	1.922(3)	N(1)–Ti	2.253(4)
O(2)–Ti	1.811(3)		
O(2)–Ti–O(2)'	103.9(3)	O(2)–Ti–N(1)'	164.90(18)
O(2)–Ti–O(1)	92.76(16)	O(2)–Ti–N(1)	90.53(16)
O(2)–Ti–O(1)'	98.23(15)	O(1)–Ti–N(1)	80.71(14)
O(1)–Ti–O(1)'	162.1(2)	O(1)–Ti–N(1)'	85.17(14)
N(1)–Ti–N(1)'	75.4(2)		

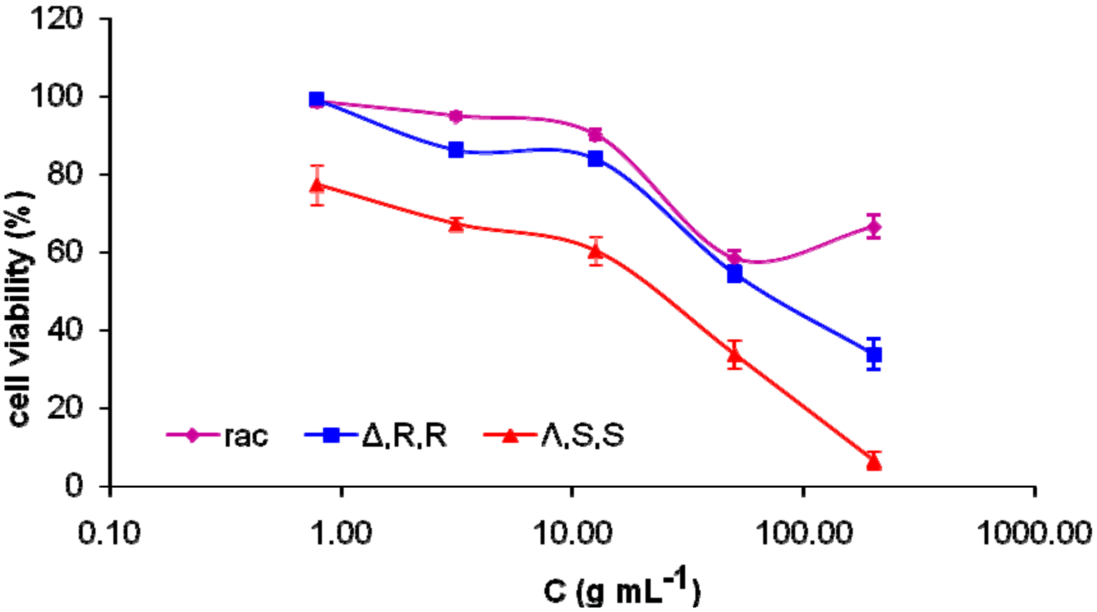

Figure 3. Dependence of OVCAR-1 cell viability based on the MTT assay following a 3d incubation period on concentration of *rac*-$L^5Ti(OiPr)_2$, *Δ*,*R*,*R*-$L^5Ti(OiPr)_2$, and *Λ*,*S*,*S*-$L^5Ti(OiPr)_2$ presented on a logarithmic scale

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