Synthesis of Pure Enantiomers of Titanium(IV) Complexes with Chiral Diaminobis(phenolato) Ligands and Their Biological Reactivity

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Racemic and enantiomerically pure titanium(IV) complexes with ortho-brominated or para-nitrated chiral diaminobis(phenolato) ligands were prepared with NH and NMe cyclohexyldiamino bridges through ligand to metal chiral induction. The hydrolytic behavior of the complexes was evaluated, identifying the N-methylated complex as the most stable. A representative NH complex hydrolyzed to first give a dimeric structure in solution as deduced by NMR diffusion measurements, followed by formation of clusters with higher nuclearity, as was supported by X-ray characterization of a tetranuclear cluster obtained in trace amounts following 30 days in water solutions. The cytotoxicity of the enantiomerically pure and racemic complexes was measured on HT-29 human colon cancer cell line based on the MTT assay; all stereochemical configurations of the N-methylated complex were inactive, whereas for the NH complexes, the racemic mixtures were mostly inactive but the pure enantiomers exhibited similarly high cytotoxicity, supporting a polynuclear active species. Analysis of the two enantiomers of the most active brominated complex for their cytotoxicity on human ovarian A2780, cisplatin resistant A2780cp and multi-drug-resistant A2780adr cell lines as well as for their apoptosis induction on the A2780 line revealed similar reactivity, supporting a similar mechanism for the two enantiomers.

Titanium(IV) compounds were the first non-platinum based metallodrugs entering clinical trials for treatment of cancer1–7. Two classes of compounds based on cyclopentadienide and diketonato ligands exhibited a wide range of activity and mild toxicity in vivo8–21, but their rapid hydrolysis under biological environment to form multiple unidentified products hampered further development14,22,23. Later “salan” type diaminobis(phenolato) Ti(IV) compounds (Fig. 1) showed: (a) markedly improved hydrolytic stability eventually giving defined polynuclear hydrolysis products, and (b) a wide range of activity both in vitro and in vivo, with no sign of toxicity to treated animals13,24–34. Wide structure-activity relationship studies pointed to a negative effect of steric bulk and a positive effect of ortho-halogenation on hydrolytic stability and cytotoxicity.

The salan type Ti(IV) complexes are chiral, exhibiting C2 or C1 symmetry. Therefore, when considered for medicinal applications, the evaluation of the biological activity of the pure enantiomers as well as their racemic mixture is essential35,36. In previous studies, employing chiral trans-cyclohexyldiamine- and bipyrrrolidine-based ligands induced ligand-to-metal chiral induction affording enantiomerically pure compounds that were analyzed for cytotoxicity37–40. In general, the enantiomerically pure forms of cyclohexyl-based NH complexes gave higher cytotoxicity relative to that of the racemic mixture37; however, for related active N-Me complexes, the racemic mixture was generally more active than the pure enantiomers38. In contrast, for the bipyrrrolidin-based complexes, the racemate was inactive whereas its enantiomerically pure isomers had similar biological activity39. Following studies suggested that the hydrolysis products participate as the active specie inside the cell37–44, providing an explanation to the different reactivity of racemates relative to that of the enantiomerically pure forms; whereas pure enantiomers gave homochiral dimeric clusters, the racemic mixtures produced heterochiral diastereomeric dimers37–40. This paper presents structure-activity relationship studies of differently substituted cyclohexyl-based complexes, analyzed as enantiomerically pure and as racemic. Preliminary mechanistic studies suggest a similar mode of action for the two active enantiomers.

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Results and Discussion
Synthesis and Characterization. Three sets of salan cyclohexyldiaminobis(phenolato) Ti(IV) complexes were synthesized according to a published procedure; each was produced as two separate enantiomers (Δ or Λ at metal) through ligand-to-metal chiral induction from optically pure trans-1,2-diaminocyclohexane (R,R or S,S at ligand, respectively)45–47, and as a racemic mixture (Δ + Λ at metal) starting from the racemic form of the starting material (Fig. 2). The compounds differed in the aromatic substitution and the diamino bridge. Ortho-bromination was selected for enhancing hydrolytic stability as reported previously26,32–34,40, and para-nitration was selected for improving solubility26,44.

Ligands L 1–3H 2 were synthesized according to a published procedures 37–39,48; ligand L 1,2H 2 were obatined by a condensation reaction between the substituted benzaldehyde and trans-diaminocyclohexane, and L 3H 2 was obtained by methylation of L 1H 2 with formaldehyde. The ligands were characterized by NMR and optical rotation. The corresponding Ti(IV) complexes were produced by reacting L 1–3H 2 with Ti(OiPr) 4 in THF, at room temperature, for 12–72 hours37–39. 1H NMR confirmed that the desired complex had been obtained quantitatively in >95% optical purity.

Single crystals suitable for X-ray crystallography were obtained at −30 °C for three compounds: rac-L 1Ti(OiPr) 2, crystallized from diethyl ether, and rac-L 2Ti(OiPr) 2 and S,S,Λ-L 3Ti(OiPr) 2 crystallized from a mixture of hexane and dichlormethane, the latter confirmed the aforementioned chiral induction. The structures are presented in Fig. 3 (Supplementary Table S1). All structures featured a C 2 symmetry, similarly to previously reported related Ti(IV) salan compounds37,40. The Ti(IV) center exhibited an octahedral geometry, whereby the phenolato oxygen atoms were in a trans configuration and the isopropoxo groups were in a cis orientation.

Hydrolysis. The comparative hydrolytic stability was assessed using 1H NMR as previously described25,26, adding 10% D 2O (>1000 equivalents) to THF-d 8 solutions of the compounds and monitoring the signals corresponding to the iso-propoxo labile ligands overtime. All experiments were conducted on the S,S,Λ- stereoisomers. The t 1/2 values are presented in Table 1.

In agreement with previous studies32,37,38,40, the most hydrolytically stable compound was the N-methylated complex L′Ti(OiPr) 2. Comparing the stability of the two NH compounds, similar stability, or even slightly higher stability for the nitrated compound implied that the ortho-bromination or the electron withdrawal by the nitro groups had a smaller effect on stability relative to that of the secondary amine, whereby added stability was provided by the cyclohexyl group relative to that of the ethylenediamino-based counterparts previously described26,32,37–40. Inspecting the 1H NMR of the product of the hydrolysis of S,S,Λ-L 3Ti(OiPr) 2, six aromatic signals evinced that a product of high symmetry was obtained, presumably dimeric37,40. The more complex corresponding spectrum of the hydrolysis product of the racemic complex implied that a mixture of homo- and hetero-chiral clusters had been obtained (Supplementary Figs. S1 and S2)37,40.
The anti-proliferative activity of the compounds was evaluated on human colon HT-29 cancer cells by the MTT (methylthiazolyldiphenyl-tetrazolium) assay as previously described. The results are illustrated in Fig. 5 and a summary of the relative IC_{50} values is provided in Table 1.

To gain more structural information on the hydrolysis product of L^1-Ti(OiPr)_2, the compound (S,S,A-stereoisomer) was reacted with water (>10,000 equivalents) for 30 days and the product was re-dissolved in diethyl ether and allowed to crystallize. Single crystals suitable for X-ray crystallography were obtained in trace amounts, and the structure is depicted in Fig. 4 (Supplementary Table S2). The structure (R \_S space group) features a tetrameric species of the type Ti_4(\mu-O)_8(S,S-L^1)_4 with four titanium centers bridged by oxo atoms, each metalo center binding a salan ligand with the phenolato donors having shifted to a cis orientation. The bond lengths and angles were generally similar to those of known related compounds. As this structure should yield twelve aromatic signals in the 1H NMR due to its C_2 symmetry, it is evident that the tetrameric complex is not the main product obtained in the hydrolysis reaction described above.

To shed more light on the possible hydrolysis products obtained in solution, diffusion NMR measurements were applied on S,S,A-L^3-Ti(OiPr)_2 upon addition of water using diffusion order spectroscopy (DOSY), whereby the diffusion coefficient (D) derived is proportional to the compound size (Table 2, Supplementary Fig. S3). Within 24 hours from water addition, the dominant species was a dinuclear compound, in agreement with previous studies on related salan diaminocyclohexyl-based complexes and in correlation with the aforementioned 1H NMR spectrum of the hydrolysis reaction. Prolonged incubation in water solutions gave indication of further decomposition to give products of higher nuclearity in trace amounts. It is plausible that the dimer forming decomposition to give products of higher nuclearity in trace amounts. It is plausible that the dimer forming

Cytotoxicity. The anti-proliferative activity of the compounds was evaluated on human colon HT-29 cancer cells by the MTT (methylthiazolyldiphenyl-tetrazolium) assay as previously described. The results are illustrated in Fig. 5 and a summary of the relative IC_{50} values is provided in Table 1.

Inspecting the reactivity of L^{1,2,3}-Ti(OiPr)_2, both with NH donors, no biological activity was detected for rac-L^3-Ti(OiPr)_2, whereas for each, the enantiomerically pure R,\_R,\_\Delta- and S,S,A-L^3-Ti(OiPr)_2 had similarly high cytotoxicity with no significant difference, in accordance with previous observations. Although the nitration was presumed to increase solubility as is also manifested by increased activity relative to other para-substituted derivatives, the maximal inhibition obtained by L^3-Ti(OiPr)_2 is slightly lower. Inspecting the reactivity of L^{1,3}-Ti(OiPr)_2, both with o-Br, it is evident that the N-methylation reduced the activity also for the enantiomerically pure forms. This observation may be explained by the added steric influence of the N-Methylated cyclohexyl ring together with the steric aromatic substitutions, overall creating sufficient bulk to abolish the activity. Similar effect was obtained for complexes with non chiral ligands as previously described.
The most active L$_1$Ti(O$_i$Pr)$_2$ was selected for further studies, aiming to investigate possible differences between the two enantiomers. Thus, cell viability studies were performed for the two enantiomers based on the MTT assay on human ovarian A2780 and its resistance lines: cisplatin-resistant human ovarian A2780cp and multi-drug-resistant (MDR) human ovarian A2780adr. The results are illustrated in Fig. 6 and a summary of the relative IC$_{50}$ values is provided in Table 3. Marked activity was obtained for all lines tested; importantly, similar activity was recorded for the two enantiomers on all lines, implying that there are no stereospecific interactions that are essential for overpassing drug resistance.

The induction of apoptosis vs. necrosis by the two enantiomers of L$_1$Ti(O$_i$Pr)$_2$ was investigated in vitro by double staining A2780 cells with annexin V-FITC and propidium iodide using flow cytometry. The cells were exposed to 8 $\mu$M (2xIC$_{50}$) of racemic (R,R,$\Delta$)-, R,R,$\Delta$- or S,S,$\Lambda$-L$_1$Ti(O$i$Pr)$_2$ isomers for 24 hours. The distribution of the populations between early and late apoptosis and necrosis is depicted in Fig. 7. In correlation with the cytotoxicity behavior on all tested cell lines, similar responses were observed for both enantiomers, showing similar induction of apoptosis within 24 hours. The racemic mixture had a negligible effect on the A2780 cells as anticipated by its inactivity, providing a distribution highly resembling that obtained in the control experiment (Supplementary Fig. S4).

Conclusions
Herein we presented new chiral derivatives of anticancer salan Ti(IV) complexes based on the chiral cyclohexyl moiety. The most stable derivative of the examined compounds, with ortho-bromination on the aromatic rings and a tertiary amino bridge, exhibited no biological activity for all isomers. This may be explained by the additive effect of the steric groups, evincing that more stable is not always more active. Inspecting the hydrolytic behavior of the least stable NH complex, it is evident that polymeric complexes are obtained in water. Interestingly, the first product appears to be a dimer, where the cluster nuclearity increases with time to yield a...
tetranuclear species, that even after 30 days in water does not decompose significantly to give titanium dioxide. The pure enantiomers of this complex showed a marked cytotoxicity on all lines tested; nevertheless, different reactivity of the racemic mixture supports the notion that the polynuclear hydrolysis products are the cellular active species37,41–44.

Comparing the behavior of the two active enantiomers in various aspects reveal no apparent difference, implying a similar mechanism 35,36,55. Both are similarly active on all lines tested, and similarly induce apoptosis. It is thus evident that the Ti(IV) complexes perform through a different mechanism than that of cisplatin, Adriamycin, or related drugs, providing them a potential advantage in the clinic. Additionally, similar reactivity of the two enantiomers may imply that tedious separation of enantiomers of chiral anticancer Ti(IV) complexes may be unnecessary, which is certainly another advantage. Nevertheless, identifying the biological target, if not necessarily chiral, and the mode of operation of these complexes remains enigmatic, and certainly merit additional mechanistic studies, currently underway in our laboratory.

Methods
Ligands L1–3H2 and bis(isopropoxo) titanium(IV) complexes L1–3Ti(OiPr)2 were synthesized according to published procedures37,38,48. All bis(isopropoxo) complexes were obtained in quantitative yields. Paraformaldehyde (97%), NaBH4 (97%), 1,2-trans-cyclohexanedianime (99%), and all substituted phenol and salicylaldehyde...
compounds (>96%) were purchased from Aldrich Chemical Company Inc., Fluka Riedel-DeHaen, Strem Chemicals Inc. or Alfa Aesar. Titanium tetra(isopropoxide) (97%) was purchased from Sigma Aldrich Chemical Company (Merck group). 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 resolution 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 and were calculated as the average of five measurements.

Cytotoxicity was measured on human colon HT-29 cancer cells (purchased from ATCC Inc.), human ovarian carcinoma A2780, human ovarian cisplatin-resistant carcinoma A2780cp and human ovarian adriamycin-resistant A2780adr carcinoma cell lines (purchased from ECACC Inc.) using the MTT assay as previously described\(^{34}\). Each measurement was repeated at least 3 x 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. Kinetic hydrolysis studies by NMR were performed at RT as previously described\(^{35}\), using ca. 3.5 mM of the complex solution in THF-d\(_8\) and adding >1000 equiv. of D\(_2\)O to give a final solution of 1:9 D\(_2\)O/THF-d\(_8\). The \(\mathrm{t}_{1/2}\) value is based on a pseudo first order fit for each compound. The results were verified by including p-dinitrobenzene (Sigma Aldrich Chemical Company Inc.) as an internal standard.

Apoptosis was measured using MEBCYTO apoptosis kit (annexin V-FITC kit, MBL). Cells were cultured in 6-well plates at density of 100,000 cells per well and allowed to attach overnight. The next day, the complex was added at 2xIC\(_{50}\) (8\(\mu\)M) concentration and was incubated for 24 h. All procedures were conducted according to the manufacturer’s instructions. The samples were analyzed by flow cytometry (Becton-Dickinson Excillum Bar FV-1000 Apoptosis Kit).

**Rac-L-H\(_2\)**

3-Bromo-2-hydroxy-benzaldehyde (0.90 g, 4.5 mmol) and trans-1,2-cyclohexanediamine (0.3 mL, 2.2 mmol) in methanol (40 mL) were heated for reflux for 2 hours. The reaction was cooled to 0 °C and ca. 15 equivalents of NaBH\(_4\) (1.2 g, 33 mmol) were added. The precipitate was filtered and washed with cold methanol to give the desired product in 73% yield. ESI-HRMS (C\(_{20}\)H\(_{24}\)N\(_4\)O\(_6\)\+[Na\(_2\)])\(^{+}\) m/z Calc.: 485.026 [M\(^{+}\)] Found: 485.02690. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 7.42\) (d, \(J = 8.1\) Hz, 1H; Ar), 7.01 (d, \(J = 7.6\) Hz, 1H; Ar), 6.71 (t, \(J = 7.6\) Hz, 1H; Ar), 4.07 (d, \(J = 13.6\) Hz, 1H; CH\(_2\)), 3.92 (d, \(J = 13.6\) Hz, 1H; CH\(_2\)), 2.54 (m, 1H; cy), 2.13 (m, 1H; cy), 1.74 (m, 1H; cy), 1.28 (m, 2H; cy) ppm. \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta = 154.6, 132.1, 127.2, 124.5, 120.0, 110.8, 59.7, 49.6, 30.7, 24.0\) ppm.

**R,R,\(\Delta\),S,S-L-H\(_2\)** (68%) and S,S,\(\Delta\),L-H\(_2\) (69%) were prepared similarly to rac-L-H\(_2\) from the optically pure trans-1,2-cyclohexanediamine. ESI-HRMS (C\(_{20}\)H\(_{24}\)Br\(_2\)N\(_2\)O\(_2\)+H\(^{+}\)) m/z Calc.: 485.026 [M\(^{+}\)]; R,R,\(\Delta\),L-H\(_2\) found 485.02475, S,S,\(\Delta\),L-H\(_2\) found 485.03051. Optical rotation for R,R,\(\Delta\),L-H\(_2\): [\(\alpha\)]\(_D\)\(^{36}\) = −35 ± 1°, for S,S,\(\Delta\),L-H\(_2\): [\(\alpha\)]\(_D\)\(^{36}\) = 28 ± 2° (for both: c = 3 mg/mL CHCl\(_3\)).

**Rac-L-H\(_2\)**

5-Nitro-2-hydroxy-benzaldehyde (0.80 g, 4.8 mmol) and trans-1,2-cyclohexanediamine (0.3 mL, 2.4 mmol) in methanol (40 mL) were heated for reflux for 2 hours. The reaction was cooled to 0 °C and ca. 15 equivalents of NaBH\(_4\) (1.4 g, 36 mmol) were added. The precipitate was filtered and washed with cold methanol to give the desired product in 40% yield. ESI-HRMS (C\(_{20}\)H\(_{24}\)N\(_4\)O\(_6\)+H\(^{+}\)) m/z Calc.: 417.177 [M\(^{+}\)] Found: 417.17564. \(^1\)H NMR (400 MHz, DMSO): \(\delta = 8.09\) (s, 1H; Ar), 7.90 (dd, \(J = 4.6\) Hz, 1H; Ar), 6.46 (s, 1H; Ar), 3.92 (d, \(J = 13.1\) Hz, 1H; CH\(_2\)), 3.77 (d, \(J = 13.2\) Hz, 1H; CH\(_2\)), 2.66 (m, 1H; cy), 2.07 (m, 1H; cy), 1.70 (m, 1H; cy), 1.23 (m, 2H; cy) ppm. \(^{13}\)C NMR (125 MHz, DMSO): \(\delta = 132.2, 128.2, 127.0, 126.2, 125.6, 118.7, 58.9, 46.0, 29.5, 24.7\) ppm.

**R,R,\(\Delta\),S,S-L-H\(_2\)** (56%) and S,S,\(\Delta\),L-H\(_2\) (66%) were prepared similarly to rac-L-H\(_2\) from optically pure trans-1,2-cyclohexanediamine. ESI-HRMS (C\(_{20}\)H\(_{24}\)N\(_4\)O\(_6\)+H\(^{+}\)) m/z Calc.: 417.177 [M\(^{+}\)]; R,R,\(\Delta\),L-H\(_2\) found 417.17668, S,S,\(\Delta\),L-H\(_2\) found 417.17773. Optical rotation for R,R,\(\Delta\),L-H\(_2\): [\(\alpha\)]\(_D\)\(^{24}\) = −19 ± 1°, for S,S,\(\Delta\),L-H\(_2\): [\(\alpha\)]\(_D\)\(^{30}\) = 29 ± 1° (for both: c = 3 mg/mL DMSO).

![Figure 7. Effect of L\(^2\)Ti(OiPr)\(_2\) (8 \(\mu\)M) ((a) control; (b) RR isomer; (c) SS isomer) on apoptosis (annexin V)/necrosis (propidium iodide) in human ovarian A2780 cells after 24 h of exposure using flow cytometry. Q1-necrotic (dead) cells; Q2- late apoptotic cells; Q3- viable cells; Q4- early apoptotic cells.](image-url)
**Rac-LH$_2$.** Rac-L$_2$H$_2$ (0.3 g, 0.6 mmol) was dissolved in a mixture of acetonitrile and acetic acid (10 ml) and formaldehyde (15 ml, 0.41 mol) was added. The reaction was stirred at room temperature for ca. 2 hours to give a solid precipitate, and then cooled to 0°C. Consequently, ca. 30 equivalents of NaBH$_4$ (0.7 g, 18 mmol) were added and the reaction was allowed to stand at room temperature overnight. NaBH$_4$ solution was added until pH 10 was reached. The precipitate was filtered and washed with water giving the desired product in 33% yield. ESI-HRMS ($C_3H_5Br_2N_2O_4Ti + H^+$) m/z Calc.: 513.058 [M$^+$] Found: 513.06056. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.43 (d, $J = 7.7$ Hz, 1H; Ar), 6.95 (d, $J = 7.4$ Hz, 1H; Ar), 6.67 (t, $J = 7.4$ Hz, 1H; Ar), 3.86 (s, $J = 13.7$ Hz, 1H; CH$_2$), 3.63 (d, $J = 12.9$ Hz, 1H; CH$_2$), 2.70 (m, 1H; cy), 2.24 (s, 3H; CH$_3$), 2.01 (m, 1H; cy), 1.82 (m, 1H; cy), 1.21 (m, 2H; cy) ppm. $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 154.7, 132.5, 128.2, 123.9, 119.9, 110.9, 61.8, 56.5, 35.8, 25.2, 22.9 ppm.

**R,R-Δ.** (31%) and S,S-Δ-LH$_2$ (43%) were prepared similarly to rac-L$_2$H$_2$, from optically pure L$_2$H$_2$, ESI-HRMS ($C_3H_5Br_2N_2O_4Ti + H^+$) m/z Calc.: 513.058 [M$^+$]; R,R-Δ-LH$_2$ found 513.06862, S,S-Δ-LH$_2$ found 513.07273. Optical rotation for R,R,Δ-LH$_2$; [α]$_D$$^23$ = 29 ± 2°, for S,S,Δ-LH$_2$; [α]$_D$$^29$ = −26 ± 1° (for both: c = 3 mg/mL CHCl$_3$).

**Rac-L$_2$Ti(OIPr)$_2$.** Ti(OIPr)$_2$ (0.044 g, 0.15 mmol) was reacted with rac-L$_2$H$_2$ (0.075 g, 0.15 mmol) in dry THF at room temperature for 2 hours. After evaporation, the crude product was obtained in a >95% purity in quantitative yield. ESI-HRMS ($C_2H_5Br_2N_2O_4Ti + H^+$) m/z Calc.: 649.058 [M$^+$] Found: 649.05792. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.45 (d, $J = 7.8$ Hz, 1H; Ar), 6.89 (d, $J = 7.4$ Hz, 1H; Ar), 6.52 (t, $J = 7.6$ Hz, 1H; Ar), 5.09 (sept, $J = 6.1$ Hz, 1H; CH), 4.87 (d, $J = 14.2$ Hz, 1H; CH$_2$), 3.89 (d, $J = 14.2$ Hz, 1H; CH$_2$), 2.22 (m, 1H; cy), 1.86 (m, 1H; cy), 1.23 (m, 6H; CH$_3$), 0.99 (m, 1H; cy), 0.85 (m, 1H; cy) ppm. $^{13}$C NMR (125 MHz, THF-$d_8$); $\delta$ = 158.7, 131.4, 128.3, 124.3, 117.2, 77.0, 62.7, 57.8, 48.9, 28.4, 25.4, 25.3 ppm.

**Crystal data for Rac-L$_2$Ti(OIPr)$_2$.** $C_6H_5Br_2N_2O_4Ti$, Mr = 648.29, monoclinic, a = 13.052(1) Å, b = 15.173(1) Å, $\alpha$ = 13.742(1) Å, $\beta$ = 93.513(2)$^\circ$, $V = 2716.3(4)$ Å$^3$, $T = 173(1)$ K, space group $P2_1/n$, $Z = 4$, $\mu$ (Mo-$K\alpha$) = 3.291 mm$^{-1}$, 31165 reflections measured, 6490 unique (Rint = 0.0482). R($F^2$) = 0.0429, R$_{wp}$ for $|F| > 2\sigma(F)$ = 0.0861.

**Rac-L$_2$Ti(OIPr)$_2$.** Ti(OIPr)$_2$ (0.058 g, 0.20 mmol) was reacted with rac-$L$$_2$H$_2$ (0.085 g, 0.20 mmol) in dry THF at room temperature overnight. After evaporation, the crude product was obtained in a >95% purity in quantitative yield. ESI-HRMS ($C_2H_5Br_2N_2O_4Ti + H^+$) m/z Calc.: 581.209 [M$^+$] Found: 581.20880. $^1$H NMR (400 MHz, DMSO): $\delta$ = 8.08 (d, $J = 2.1$ Hz, 1H; Ar), 8.00 (dd, $J = 7.2$ Hz, 1H; Ar), 6.64 (d, $J = 7.2$ Hz, 1H; Ar), 4.79 (sept, $J = 4.8$ Hz, 1H; CH), 4.40 (d, $J = 13.2$ Hz, 1H; CH$_2$), 4.02 (d, $J = 10.6$ Hz, 1H; CH$_2$), 2.14 (m, 1H; cy), 2.03 (m, 1H; cy), 1.55 (m, 1H; cy), 1.14 (m, 6H; CH$_3$), 1.04 (m, 1H; cy), 0.81 (m, 1H; cy) ppm. $^{13}$C NMR (125 MHz, DMSO): $\delta$ = 169.5, 137.5, 126.3, 125.4, 124.0, 118.5, 77.6, 67.5, 62.4, 58.5, 25.6, 25.0, 24.5 ppm.

**Crystal data for Rac-L$_2$Ti(OIPr)$_2$.** $C_6H_5Br_2N_2O_4Ti$, Mr = 648.29, monoclinic, a = 16.718(4) Å, b = 10.090(2) Å, c = 18.406(4) Å, $\alpha$ = 110.739(3)$^\circ$, $V = 2903.7(11)$ Å$^3$, $T = 173(1)$ K, space group $P2_1/n$, $Z = 4$, $\mu$ (Mo-$K\alpha$) = 3.082 mm$^{-1}$, 29963 reflections measured, 6286 unique (Rint = 0.0664). R($F^2$) = 0.0825, R$_{wp}$ for $|F| > 2\sigma(F)$ = 0.1623.

**Crystal data for Rac-L$_2$Ti(OIPr)$_2$.** $C_6H_5Br_2N_2O_4Ti$, Mr = 676.34, monoclinic, a = 14.4108(9) Å, c = 14.1029(6) Å, $\beta$ = 96.098(1)$^\circ$, $V = 1464.2(2)$ Å$^3$, $T = 173(1)$ K, space group $P2_1$, $Z = 2$, $\mu$ (Mo-$K\alpha$) = 3.057 mm$^{-1}$, 16766 reflections measured, 6789 unique (Rint = 0.0231). R($F^2$) = 0.0259, R$_{wp}$ for $|F| > 2\sigma(F)$ = 0.0554.

The hydrolysis product Ti$_4$(μ-O)$_4$(S,S-L)$_4$ was obtained by dissolving ca. 20 mg of S,S-L$_2$Ti(OIPr)$_2$ in THF (6 ml) and adding >10,000 equivalents of H$_2$O. The reaction was mixed for 30 days. The product was crystallized from diethyl ether. The structure contains disordered water molecules, for which H atoms were not detected.
Crystal data for Ti₄(μ-O)₄(S,S-L)₄: C₄₀H₆₈Br₄N₄O₁₇₃Ti₄, Mr = 2269.80, rhombohedral, a = 26.4920 (9) Å, c = 70.771 (3) Å, V = 43015(5) Å³, T = 173(1) K, space group R 3 c, Z = 18, μ (Mo-Kα) = 3.730 mm⁻¹, 159243 reflections measured, 11525 unique (Rint = 0.1489). R(F)₂ for |I| > 2σ(I) = 0.0660, Rw for |I| > 2σ(I) = 0.1270. CCDC 1817336–1817339 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via https://www.ccdc.cam.ac.uk/structures/.

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M.M. and E.Y.T. conceived the experiments, M.M. executed the experiments and analyzed the results. All authors contributed to the writing and review of the paper.

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