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New Insights on the Active Species and Mechanism of Cytotoxicity of Salan-Ti(IV) Complexes: A Stereochemical Study

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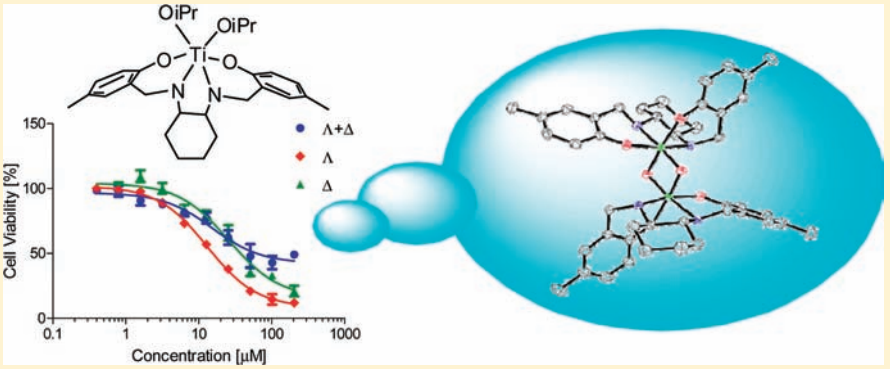
Supporting Information

ABSTRACT:



Following the discovery of cisplatin, much effort has been devoted to the exploration of transition metal complexes as cytotoxic agents. We have recently introduced the highly efficient C_2-symmetrical salan-Ti(IV) family of complexes, demonstrating high cytotoxicity toward colon and ovarian cells and enhanced hydrolytic stability in mixed organic/water solutions. The effect of stereochemistry is hereby reported, by comparing the cytotoxic activity and hydrolysis of pure enantiomers and their racemic mixture for four complexes of this family with different aromatic substitutions: *para*-Me, *para*-Cl, *ortho*-Cl, and *ortho*-OMe. These complexes include the *trans*-diaminocyclohexyl bridge, which enables ligand-to-metal chiral induction to give solely the Δ isomer when starting from the *R,R* ligand and vice versa. Different activity is obtained for the different stereochemical forms (Δ, Λ, and rac) in two of the four complexes, where for the other two either all forms are inactive or all are highly active. Additionally, where not all are of similar activity, the racemic mixture is the least active of the three. We therefore conclude that the salan ligand is essential for the fruitful biological interaction, which probably involves a chiral cellular target. The activity of the racemate differing from that expected from a simple mixture of enantiomers operating separately may be explained by the involvement of a polynuclear active species, where different metal centers might be of different configurations. This is particularly supported by the different polynuclear products of hydrolysis obtained from an optically pure complex and from the racemic one, as analyzed crystallographically. The former is an all-*R,R* chiral C_1-symmetrical homodimer, while the latter is an achiral *R,R*-*S,S* C_i-symmetrical heterodimer obtained through chiral recognition.

INTRODUCTION

Cisplatin marked a new era in the field of chemotherapy.[1] Nevertheless, its disadvantages of high toxicity and limited activity range led to wide research of other compounds, including transition metal complexes that are based on different metal centers.[2] The Ti(IV) complexes titanocene dichloride (Cp_2TiCl_2, Scheme 1a) and budotitane ($(bzac)_2Ti(OEt)_2$, Scheme 1b), and their derivatives based on Cp or diketonato type ligands, demonstrated promising activity with lower toxicity compared to cisplatin.[3] However, rapid hydrolysis in biologically relevant solutions is the main drawback of the titanium complexes which impedes their utility.[3d,f,4] We recently introduced a new family of Ti(IV) complexes based on salan ligands (Scheme 1c), which exhibits enhanced hydrolytic stability and cytotoxicity toward colon and ovarian cells compared to Cp_2TiCl_2 and $(bzac)_2Ti(OiPr)_2$.[5] Upon slow hydrolysis of the labile isopropoxo groups (within up to weeks in 10% D_2O solutions), salan-bound polynuclear complexes are formed, which are stable for days but lack any cytotoxicity. It was also observed that complexes of large steric bulk that precludes formation of the cluster do not exhibit cytotoxicity as well, which led us to propose that the cluster formation in the cell plays a meaningful role in activity.

The salan complexes possess a C_2-symmetry, rendering chirality (Figure 1). The effect of stereochemistry on biological activity is of great importance especially for medicinal applications, as many of the biological targets in the cells are chiral. And yet, all chiral cytotoxic Ti(IV) complexes reported prior to our

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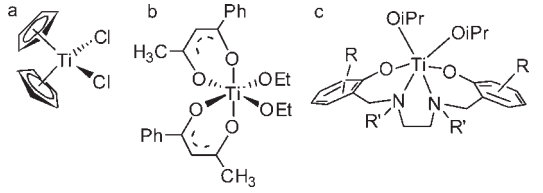
Scheme 1



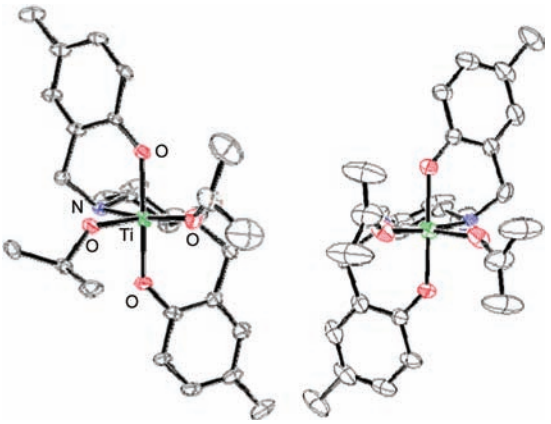
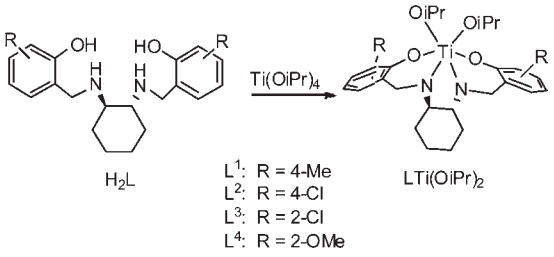


Figure 1. ORTEP drawings of one molecule of Δ,R,R-$L^1Ti(OiPr)_2$ found in the asymmetric unit of rac-$L^1Ti(OiPr)_2$ (left) and Λ,S,S-$L^1Ti(OiPr)_2$ (right).[7]

Scheme 2. Preparation of Complexes of Chiral Salan Ligands



studies, including budotitane and *ansa*-metallocenes,[6] were measured as racemic only. Thus, in our recent communication we reported our preliminary studies on the effect of stereochemistry on cytotoxicity, with the aim of not only identifying the most active isomer for future medicinal utility, but in particular for elucidating mechanistic aspects relating to the importance of the ligand and the nature of the cellular target.[7] We synthesized pure enantiomers by chiral induction from an optically pure chiral ligand and found that for $L^1Ti(OiPr)_2$, (Scheme 2, Figure 1) the Λ isomer is markedly more active than the Δ. Herein, we elaborate on these investigations to include a series of four complexes, each prepared as rac, Δ, and Λ, and evaluate the effect of stereochemistry on cytotoxicity and hydrolysis while analyzing mechanistic implications.

RESULTS AND DISCUSSION

Synthesis and Characterization. The salan ligands employed are based on the chiral *trans*-1,2-diaminocyclohexyl moiety, which are known to enable chiral induction on the metal center and provide optically pure Ti(IV) complexes.[8] Thus, when applying the racemic diamine, a single pair of enantiomers, namely R,R,Δ and S,S,Λ is obtained, while starting from the optically pure diamine yields a single diastereomer.[8]

The synthesis of the ligands is based on a two-step procedure involving condensation of salicylaldehyde derivatives with *trans*-1,2-diaminocyclohexane followed by reduction with $NaBH_4$.[7,9] ^1H NMR confirmed that the desired ligand was obtained with two doublets of an AB system representing the methylene bridge. Rac-$L^{1-4}H_2$ were obtained from rac-1,2-diaminocyclohexane, while S,S-$L^{1-4}H_2$ and R,R-$L^{1-4}H_2$ were prepared similarly, starting from the corresponding commercially available optically pure diamine.

The Ti(IV) complexes were obtained as previously described,[5a,b] by dissolving the ligands in THF followed by addition of titanium tetra(isopropoxide) and stirring the solution for 2 h. The complexes rac-, Δ,R,R-, and Λ,S,S-$L^{1-4}Ti(OiPr)_2$ were obtained in a quantitative yield (Scheme 2). The ligands $L^{1-4}H_2$ gave complete induction; namely, by starting the complexation with an optically pure ligand, only a single diastereomer of the complex was identified by ^1H NMR. The complexes feature a C_2-symmetry as evident by ^1H NMR, with a single type of an aromatic ring and two doublets of the methylene protons, and as also presented in the ORTEP diagram of one of the molecules found in the asymmetric unit of rac-$L^1Ti(OiPr)_2$ and of Λ,S,S-$L^1Ti(OiPr)_2$ (Figure 1).[7]

Cytotoxicity. The cytotoxicity of complexes rac-, Δ,R,R-, and Λ,S,S-$L^{1-4}Ti(OiPr)_2$ was measured toward two representative cell lines, HT-29 colon and OVCAR-1 ovarian cells based on the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay as previously described.[5a] In general, similar results were obtained for both cell types. The results for colon cells are presented in Figure 2, and those for ovarian cells are given in Figures S1−S4. The relative IC_{50} and maximal cell growth inhibition values are summarized in Table 1.

The complexes exhibit variable cytotoxic activities with relative IC_{50} values ranging from 2 to 44 μM, depending on the substituent on the aromatic ring and the stereochemical configuration. Even more pronounced is the effect of these parameters on the maximal cell growth inhibition, which changes quite significantly for different compounds and different configurations, varying between 55% and 100%. For two complexes of the series, $L^{2,4}Ti(OiPr)_2$, the activity for all three stereochemical configurations, namely, rac, Δ, and Λ, is similar; either no activity ($L^2Ti(OiPr)_2$) or similarly high activity ($L^4Ti(OiPr)_2$) is obtained for all. However, whereas for $L^3Ti(OiPr)_2$ the two pure enantiomers are of a similar activity that is noticeably higher than that of the racemate, for $L^1Ti(OiPr)_2$ the activity of the two pure enantiomers is also different. We may thus generalize and say that if not all stereochemical combinations are of similar activity, at least one of the pure enantiomers is the most active. These observations suggest that the biological target is chiral, and that the salan ligand is meaningful and is involved in the interaction with this target, rather than the active species being a "stripped" aquated or protein-bound ion following early dissociation of all ligands.[10] The lower activity of the racemic mixtures than that expected of a mixture of enantiomers operating in an unrelated fashion may suggest the involvement of a polynuclear active species, where several metal centers may be of different stereochemical configurations. Thus, different

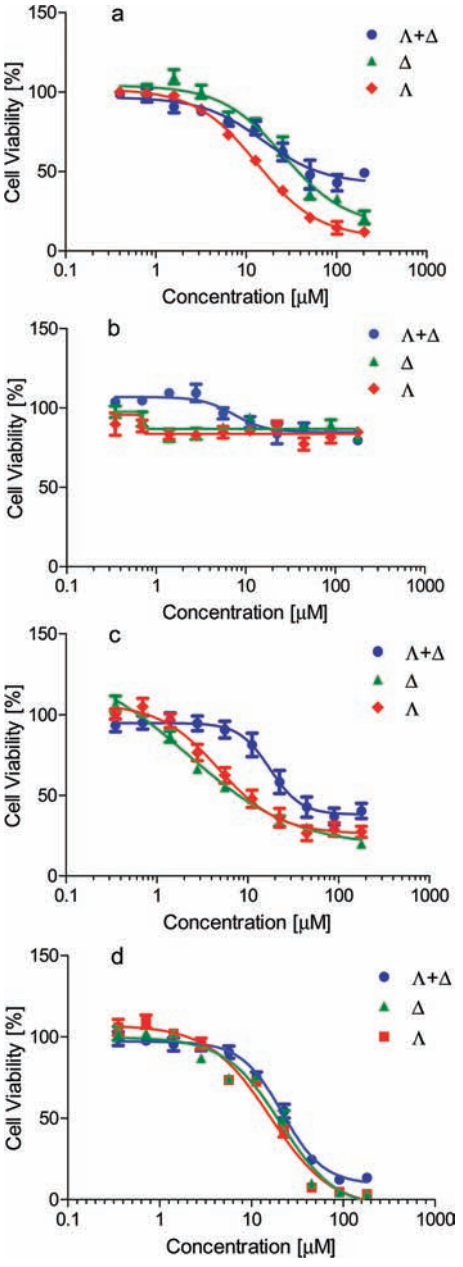


Figure 2. Dependence of HT-29 cell viability after 3 days incubation period on administered concentration of rac-, Δ,*R*,*R*-, and Λ,*S*,*S*-$L^{1-4}Ti(OiPr)_2$ based on 3 × 4 independent repetitions: (a) $L^1Ti(OiPr)_2$, (b) $L^2Ti(OiPr)_2$, (c) $L^3Ti(OiPr)_2$, (d) $L^4Ti(OiPr)_2$.

enantiomers or diastereomers of the active species should certainly demonstrate different activity toward a chiral target.

Hydrolysis. The hydrolytic stability of the four racemic complexes at 5 mM concentration was measured by ^1H NMR at RT in 10% D_2O (>1000 D_2O equivalents relative to Ti) solutions as previously described.[5a,b] The $t_{1/2}$ of hydrolysis of the labile isopropoxo groups for complexes rac-$L^{1-4}Ti(OiPr)_2$ is similar, between 1 (for rac-$L^{1,2}Ti(OiPr)_2$) and 4 (for rac-$L^{3,4}Ti(OiPr)_2$) hours. The somewhat enhanced stability of the latter may relate to their *ortho*-steric bulk, which may inhibit cluster formation as observed in our previous studies.[5a,b] Additional measurements conducted with enantiomerically pure complexes revealed similar hydrolysis rates to those of their racemic mixtures.

Table 1. Relative IC_{50} and Maximal Inhibition Values for the Complexes rac-, Λ-, and Δ-$L^{1-4}Ti(OiPr)_2$, and Known Compounds towards HT-29 and OVCAR-1 Cell Lines Based on 3 × 4 Independent Repetitions

	HT-29		OVCAR-1	
compd	IC_{50} [μM]	maximal inhibition (%)	IC_{50} [μM]	maximal inhibition (%)
rac-$L^1Ti(OiPr)_2$	15 ± 2	58	20 ± 2	55
Λ,*S*,*S*-$L^1Ti(OiPr)_2$	13 ± 1	92	13 ± 1	89
Δ,*R*,*R*-$L^1Ti(OiPr)_2$	26 ± 2	84	26 ± 2	74
rac-$L^2Ti(OiPr)_2$	inactive	0	inactive	0
Λ,*S*,*S*-$L^2Ti(OiPr)_2$	inactive	0	inactive	0
Δ,*R*,*R*-$L^2Ti(OiPr)_2$	inactive	0	inactive	0
rac-$L^3Ti(OiPr)_2$	17.5 ± 0.9	62	21.3 ± 0.5	62
Λ,*S*,*S*-$L^3Ti(OiPr)_2$	4.8 ± 2.2	74	5.2 ± 0.4	70
Δ,*R*,*R*-$L^3Ti(OiPr)_2$	2.1 ± 1.0	82	3.4 ± 0.2	75
rac-$L^4Ti(OiPr)_2$	21.7 ± 0.5	91	20.2 ± 0.4	92
Λ,*S*,*S*-$L^4Ti(OiPr)_2$	16.6 ± 0.6	100	17.7 ± 0.6	100
Δ,*R*,*R*-$L^4Ti(OiPr)_2$	20.8 ± 0.6	100	23.7 ± 0.7	100
Cp_2TiCl_2	609 ± 4	90	701 ± 4	90
$(bzac)_2Ti(OiPr)_2$	15.2 ± 0.3	90	14.9 ± 0.4	90
cisplatin	11.1 ± 0.4	88	8.6 ± 0.2	90

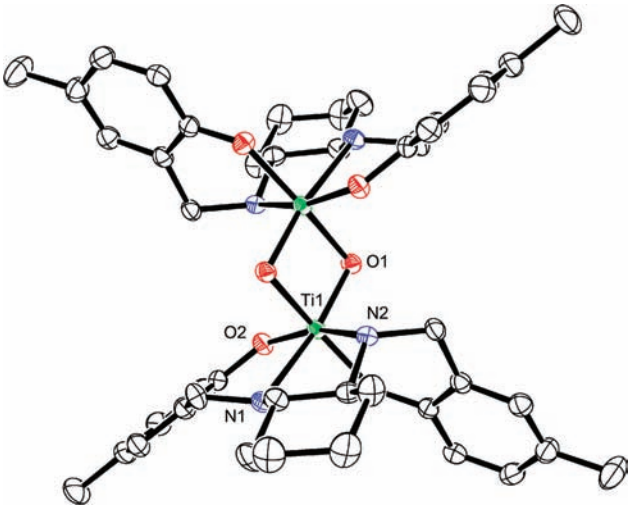


Figure 3. ORTEP presentation of $Ti_2(\mu\text{-}O)_2$(*R*,*R*-L^1)(*S*,*S*-L^1), product of hydrolysis of rac-$L^1Ti(OiPr)_2$, at 50% probability ellipsoids; H atoms and solvent were omitted for clarity.

In order to shed light on the structure of the hydrolysis product of this series of complexes, $L^1Ti(OiPr)_2$ was reacted with 1000 water equivalents in THF for three days, and the product was allowed to crystallize from methylene chloride and hexane. The ORTEP drawings of the products of rac-$L^1Ti(OiPr)_2$ and Δ,*R*,*R*-$L^1Ti(OiPr)_2$ are given in Figures 3 and 4, respectively, with a list of bond lengths and angles summarized in Tables 2 and 3, respectively. The aromatic region of the ^1H NMR of these crystals is provided in Figure S5, featuring twice as many signals for the product of the optically pure compound.

The X-ray structures of both hydrolysis products feature dinuclear species[8b] which are generally similar, with similar bond

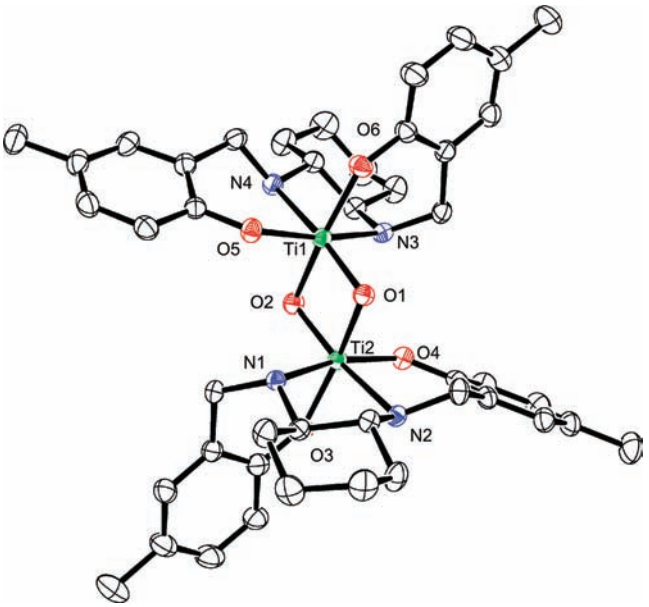


Figure 4. ORTEP presentation of $Ti_2(\mu\text{-}O)_2(R,R\text{-}L^1)_2$, product of hydrolysis of $\Delta,R,R\text{-}L^1Ti(OiPr)_2$, at 50% probability ellipsoids; H atoms and solvent were omitted for clarity.

Table 2. Selected Bond Lengths (Å) and Angles (deg) for Complex $Ti_2(\mu\text{-}O)_2(R,R\text{-}L^1)(S,S\text{-}L^1)$

atoms	value	atoms	value
		Lengths	
N(1)−Ti(1)	2.283(3)	N(2)−Ti(1)	2.219(3)
O(1)−Ti(1)	1.784(2)	O(1)−Ti(1)#1	1.951(2)
O(2)−Ti(1)	1.879(2)	O(4)−Ti(1)	1.918(2)
		Ti···Ti	2.793
		Angles	
Ti(1)−O(1)−Ti(1)#1	96.7(1)	O(1)−Ti(1)−N(2)	97.3(1)
O(1)−Ti(1)−O(1)#1	83.3(1)	O(4)−Ti(1)−N(2)	81.5(1)
O(1)−Ti(1)−O(4)	102.0(1)	N(2)−Ti(1)−N(1)	76.3(1)
O(2)−Ti(1)−O(4)	97.2(1)		

lengths and angles around the metal center, which are also similar to those of the mononuclear precursors.[7] The two Ti(IV) centers with a distance of 2.8 Å are bridged by two oxo atoms coming from the water molecules replacing the labile isopropoxo ligands, and each Ti(IV) center is bound to the salan ligand. The configuration of the phenolato groups changes from *trans* in the staring isopropoxo complex to *cis* in the dimer,[8b,11] as occurred for two out of the three metal centers of the trinuclear products obtained previously for other complexes of this family.[5a,b] A close look at both structures, however, reveals that the symmetry of the two clusters is different, due to different configurations. Whereas the optically pure compound formed the chiral C_1-symmetrical homodimer $Ti_2(\mu\text{-}O)_2(R,R\text{-}L^1)_2$, the racemic complex formed the achiral C_i symmetrical heterodimer $Ti_2(\mu\text{-}O)_2$-$(R,R\text{-}L^1)(S,S\text{-}L^1)$ with the crystallographic inversion center as the main product,[8b] as also corroborated by superposition of its ^1H NMR (Figure S5) with that of the crude hydrolysis products. This indicates that a chiral recognition occurred in the dimer formation when starting from the racemic precursor.

Table 3. Selected Bond Lengths (Å) and Angles (deg) for Complex $Ti_2(\mu\text{-}O)_2(R,R\text{-}L^1)_2$

atoms	value	atoms	value
Lengths			
N(1)−Ti(2)	2.2883(16)	N(4)−Ti(1)	2.2021(15)
N(2)−Ti(2)	2.2281(16)	O(1)−Ti(1)	1.7827(12)
N(3)−Ti(1)	2.2780(15)	O(1)−Ti(2)	1.9579(13)
		Ti(1)···Ti(2)	2.805
Angles			
Ti(1)−O(1)−Ti(2)	102.06(6)	O(5)−Ti(1)−N(4)	162.13(5)
O(1)−Ti(1)−O(2)	82.69(5)	N(4)−Ti(1)−N(3)	76.88(5)
O(1)−Ti(1)−N(4)	95.41(6)	O(4)−Ti(2)−O(1)	164.11(6)

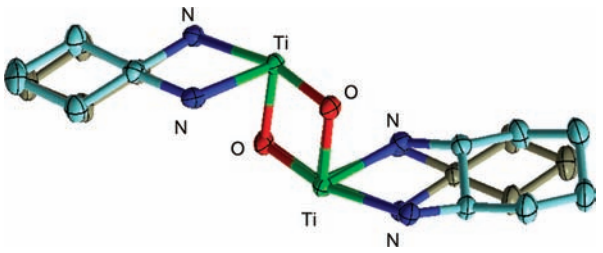


Figure 5. Superposition of X-ray structures of $Ti_2(\mu\text{-}O)_2(R,R\text{-}L^1)(S,S\text{-}L^1)$ (light blue) and $Ti_2(\mu\text{-}O)_2(R,R\text{-}L^1)_2$ (gray), presenting only the dimetal core and the diaminocyclohexane moiety; one cyclohexyl ring is of an identical configuration in both structures (left) while the other is in an opposite one (right).

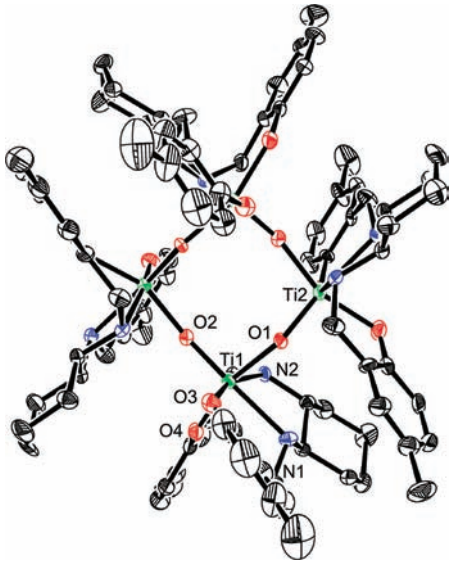


Figure 6. ORTEP presentation of $Ti_4(\mu\text{-}O)_4(R,R\text{-}L^1)_4$ at 50% probability ellipsoids; H atoms and solvent were omitted for clarity.

A superposition of the metal cores of the two structures is depicted in Figure 5, where the opposite configuration of one diaminocyclohexyl ring may be observed.

These results make the dinuclear hydrolysis products good candidates for the cellular active species in the cytotoxicity mechanism, where indeed we would expect different activity

Table 4. Selected Bond Lengths (Å) and Angles (deg) for Complex $Ti_4(\mu\text{-}O)_4(R,R\text{-}L^1)_4$

atoms	value	atoms	value
		Lengths	
N(1)−Ti(1)	2.274(5)	O(1)−Ti(1)	1.755(4)
N(2)−Ti(1)	2.189(5)	O(3)−Ti(1)	1.895(5)
		Ti(1)···Ti(2)	3.546
		Angles	
Ti(1)−O(1)−Ti(2)	152.9(3)	O(3)−Ti(1)−O(4)	96.5(2)
O(1)−Ti(1)−O(2)	102.56(18)	O(3)−Ti(1)−N(1)	85.03(19)
O(2)−Ti(1)−O(4)	157.22(18)	O(3)−Ti(2)−N(2)	163.0(2)

with a chiral target. Thus, the cytotoxicity of both dimeric hydrolysis products $Ti_2(\mu\text{-}O)_2(R,R\text{-}L^1)_2$ and $Ti_2(\mu\text{-}O)_2(R,R\text{-}L^1)$-$(S,S\text{-}L^1)$ was tested on HT-29 colon and OVCAR-1 ovarian cells, and no activity was detected, similarly to the observation with trinuclear products of hydrolysis obtained for other salan complexes as analyzed previously.[5b] This does not rule out the possible participation of these dimers as the active species inside the cell, as their lack of cytotoxicity may relate to their inability to penetrate the cell membrane due to increased size, while where formed inside the cell following penetration of a relatively stable bis(isopropoxo) precursor, might be of fruitful biological interactions.

Interestingly, additional crystals of a different hydrolysis product of $\Delta,R,R\text{-}L^1Ti(OiPr)_2$ were obtained in trace amount (<5%) from methylene chloride (Figure 6, Table 4). A tetrameric C_2-symmetrical $(Ti_4(\mu\text{-}O)_4(R,R\text{-}L^1)_4$ complex with a crystallographic inversion center was obtained, with four Ti(IV) centers bridged by four oxo atoms, and each metal atom is bound to the salan ligand with *cis* orientation of the phenolato oxygen atoms as in the dimers.[12] Bond lengths and angles around the metal center are also similar to those obtained for the dimeric structures. This indicates that other clusters with different nuclearities are possible, although in this case they were obtained in small amounts only.

CONCLUSIONS

Herein we report the first analysis of the effect of stereochemistry on cytotoxicity and hydrolysis of chiral salan Ti(IV) complexes, while pointing to the possible relationship between these parameters. Four salan Ti(IV) complexes of different aromatic substitutions were analyzed as enantiomerically pure and as racemic. It appears that, on the line of activity, the effect of stereochemistry is mostly pronounced in the intermediate cases. The complexes of mediocre activity clearly show that the racemic mixture is less active than at least one of the pure enantiomers that may be of either similar or different activity. Assuming a generally similar mechanism of all complexes of this family, this corroborates the participation of the ligand in fruitful interaction with the presumably chiral target, although inactivation of a particular enantiomer by a different chiral competitor cannot be ruled out. This also implies that the active species in the cell is polynuclear, and that, for this series of complexes, homochiral polynuclear complexes are more active than mixed-configured ones that should form with preference. This was corroborated by the crystal structures of the dimeric products of hydrolysis of racemic and an enantiomerically pure complex, which revealed that, despite the similar rates of cluster formation, indeed chiral recognition occurs and mainly an achiral heterodinuclear complex formed from a racemic precursor, which is a diastereomer of the product obtained from the optically pure precursor. The inactivity of the two dimers raises the possibility that the dimers may lack cell penetration ability, but if formed inside the cells, might manifest their activity. This emphasizes the advantage of our precursor bis(isopropoxo) complexes in being of relatively high hydrolytic stability, which also means that their cell penetration should occur relatively rapidly. Nevertheless, the involvement of other active intermediate polynuclear species formed prior to the particular clusters characterized herein, that may form in the outer-cellular environment and are able to penetrate the cell membrane, cannot be ruled out.

In the edges of activity, the *ortho*-methoxylated complex showed the highest activity when considering not only the relative IC_{50} values, but the maximal inhibition as well, for all stereochemical combinations, namely for the two enantiomers Δ and Λ and for the racemate. In contrast, the *para*-chlorinated complex demonstrated negligible activity in all stereochemical forms. The different activity of differently substituted complexes is surely a result of different features of the ligand, which may relate either to steric and/or electronic influences, or to indirect effects such as solubility.[5a,b] Nevertheless, different chiral recognition in the cluster formation upon hydrolysis should also be considered, as it is certainly possible that, for differently substituted complexes, hydrolysis of the racemic complexes may yield the homodimeric chiral structures as the main products, thus leading to similar activity for all stereochemical combinations of the precursor bis(isopropoxo) complexes.

In summary, it is clear that stereochemistry plays a major role in determining cytotoxicity and should be considered in the future design of active complexes. Additional studies in this respect are underway.

EXPERIMENTAL SECTION

L^1H_2 and rac-, Δ,R,R-, and $\Lambda,S,S\text{-}L^1Ti(OiPr)_2$ were prepared as previously described.[7,9] All other ligands and Ti(IV) complexes were prepared similarly. The configuration assignment of complexes was made under the assumption that all *R,R* ligands of this class give Δ at metal and vice versa, based on the observations of representative complexes by us and others.[8] All bis(isopropoxo) complexes were obtained in a quantitative yield and may be further washed with diethyl ether. $NaBH_4$ (97%), 1,2-*trans*-cyclohexanediamine (99%), and all substituted salicylaldehyde compounds (>96%) were purchased from Aldrich Chemical Co. Inc. or Fluka Riedel-deHaën. Titanium tetra(isopropoxide) (97%) was purchased from Aldrich Chemical Co., Inc. Cp_2TiCl_2 (98%) for reference measurements was purchased from Arapahoe Chemicals Inc. $(bzac)_2Ti(OiPr)_2$ for reference measurements was synthesized based on a published procedure.[3f] All solvents were distilled from K or K/benzophenone under nitrogen or dried over aluminum column on an MBrawn drying system SPS-800. All experiments requiring dry atmosphere were performed in an M. Braun drybox or under nitrogen atmosphere using Schlenck line techniques. NMR data were recorded using AMX-400 MHz or AMX-500 MHz Bruker spectrometer. X-ray diffraction data were obtained with a Bruker SMART APEX CCD 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 analysis was conducted in the microanalytical laboratory of 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.

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.[5a] Cells (1.0×10^6) in medium (containing 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 a 96-well plate and allowed to attach for 24 h. The cells were consequently treated with the reagent tested at 10 different concentrations. Solution of reagent was prepared by dissolving 4 mg of the reagent in 200 μL of THF and diluting 10 μL of this solution with 90 μL of medium. From the resulting solution, 10 μL was added to each well already containing 200 μL of the above solution of cells in the medium to give final concentration of up to 100 mg/L. After a standard of 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 h. The MTT solution was then removed, and the cells were dissolved in 200 μL of isopropanol. The absorbance at 550 nm was measured by a Bio-Tek EL-800 microplate reader spectrophotometer. Each measurement was repeated at least 3 × 4 times, namely, three repeats per plate, all repeated four times on different days (12 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 Prism 5.0 program. Kinetic hydrolysis studies by NMR were performed at RT as previously described,[5a,b] using 5 mM of the complex solution in THF-d_8 and adding >1000 equiv of D_2O to give a final solution of 1:9 D_2O/THF-d_8. The $t_{1/2}$ value is based on a pseudo-first-order fit for each compound. The results were verified by including *p*-dinitrobenzene as an internal standard.

rac-L^2H_2 was synthesized in analogy to rac-L^1H_2[7] from rac-1,2-diaminocyclohexane and 5-chlorosalicylaldehyde (1.0 g, 67% yield). (Found: C, 60.75; H, 6.07, N, 6.94%. Calcd for $C_{20}H_{24}Cl_2N_2O_2$: C, 60.76; H, 6.12; N, 7.09%.) δ_H(500 MHz; $CDCl_3$) 7.00 (2 H, dd, *J* 8.6, 2.3 Hz, Ar), 6.88 (2 H, s, Ar), 6.97 (2 H, d, *J* 8.6 Hz, Ar) 3.94 (2 H, d, *J* 14.2 Hz, CH2), 3.81 (2 H, d, *J* 14.6 Hz, CH2), 2.34 (2 H, m, cy), 2.08 (2 H, m, cy), 1.66 (2 H, m, cy), 1.16 (4 H, m, cy); δ_C(125 MHz; $CDCl_3$) 156.6, 128.6, 128.0, 124.1, 123.7, 117.7, 59.7, 49.2, 30.5, 24.0.

For *R,R*-L^2H_2 (1.1 g, 73%): $[\alpha]_D^{25} = 4.5 \pm 0.3°$ (*c* 0.13% in $CHCl_3$). (Found: C, 60.89; H, 5.84, N, 6.98%. Calcd for $C_{20}H_{24}Cl_2N_2O_2$: C, 60.76; H, 6.12; N, 7.09%.) δ_H(500 MHz; $CDCl_3$) 7.01 (2 H, dd, *J* 8.6, 2.3 Hz, Ar), 6.88 (2 H, s, Ar), 6.97 (2 H, d, *J* 8.6 Hz, Ar), 3.94 (2 H, d, *J* 14.2 Hz, CH2), 3.81 (2 H, d, *J* 14.6 Hz, CH2), 2.34 (2 H, m, cy), 2.08 (2 H, m, cy), 1.66 (2 H, m, cy), 1.16 (4 H, m, cy); δ_C(125 MHz; $CDCl_3$) 156.6, 128.6, 128.0, 124.1, 123.7, 117.7, 59.7, 49.2, 30.5, 24.0. For *S,S*-L^2H_2 (1.4 g, 85%): $[\alpha]_D^{25} = -4.7 \pm 0.5°$, (*c* = 0.25% in $CHCl_3$). (Found: C, 60.79; H, 5.78, N, 6.58%. Calcd for $C_{20}H_{24}Cl_2N_2O_2$: C, 60.76; H, 6.12; N, 7.09%.) δ_H(500 MHz; $CDCl_3$) 7.01 (2 H, dd, *J* 8.6, 2.3 Hz, Ar), 6.88 (2 H, s, Ar), 6.97 (2 H, d, *J* 8.6 Hz, Ar) 3.94 (2 H, d, *J* 14.2 Hz, CH2), 3.81 (2 H, d, *J* 14.6 Hz, CH2), 2.34 (2 H, m, cy), 2.08 (2 H, m, cy), 1.66 (2 H, m, cy), 1.16 (4 H, m, cy); δ_C(125 MHz; $CDCl_3$) 156.6, 128.6, 128.0, 124.1, 123.7, 117.7, 59.7, 49.2, 30.5, 24.0. These compounds were synthesized in analogy to rac-L^2H_2 from *R,R*-1,2-diaminocyclohexane and *S,S*-1,2-diaminocyclohexane, respectively.

rac-L^3H_2 was synthesized in analogy to rac-L^1H_2[7] from rac-1,2-diaminocyclohexane and 3-chlorosalicylaldehyde (1.0 g, 67% yield). (Found: C, 60.70; H, 6.04, N, 6.98%. Calcd for $C_{20}H_{24}Cl_2N_2O_2$: C, 60.76; H, 6.12; N, 7.09%.) δ_H(500 MHz; $CDCl_3$) 7.18 (2 H, dd, *J* 5.2, 2.1 Hz, Ar), 6.83 (2 H, d, *J* 8.0 Hz, Ar), 6.65 (2 H, m, Ar), 3.94 (2 H, d, *J* 14.2 Hz, CH2), 3.82 (2 H, d, *J* 14.6 Hz, CH2), 2.36 (2 H, m, cy), 2.05 (2 H, s, cy), 1.66 (2 H, m, cy), 1.16 (4 H, m, cy); δ_C(125 MHz; $CDCl_3$) 153.4, 129.1, 126.7, 124.4, 121.1, 119.4, 59.9, 49.5, 30.3, 24.0.

For *R,R*-L^3H_2 (1.1 g, 73%): $[\alpha]_D^{24} = -130 \pm 8°$ (*c* 0.33% in $CHCl_3$). (Found: C, 61.03; H, 6.12, N, 7.11%. Calcd for $C_{20}H_{24}Cl_2N_2O_2$: C, 60.76; H, 6.12; N, 7.09%.) δ_H(500 MHz; $CDCl_3$) 7.18 (2 H, d, *J* 2.0, 5.0 Hz, Ar), 6.83 (2 H, d, *J* 8.0 Hz, Ar), 6.65 (2 H, m, Ar), 3.94 (2 H, d, *J* 14.2 Hz, CH2), 3.82 (2 H, d, *J* 14.6 Hz, CH2), 2.36 (2 H, m, cy), 2.05 (2 H, s, cy), 1.66 (2 H, m, cy), 1.16 (4 H, m, cy); δ_C(125 MHz; $CDCl_3$) 153.4, 129.1, 126.7, 124.4, 121.1, 119.4, 59.9, 49.5, 30.3, 24.0. For *S,S*-L^3H_2 (1.3 g, 87%): $[\alpha]_D^{26} = 128 \pm 1°$ (*c* 0.36% in $CHCl_3$). (Found: C, 60.93; H, 6.12, N, 7.01%. Calcd for $C_{20}H_{24}Cl_2N_2O_2$: C, 60.76; H, 6.12; N, 7.09%.) δ_H(500 MHz; $CDCl_3$) 7.18 (2 H, d, *J* 2.0, 5.4 Hz, Ar), 6.83 (2 H, d, *J* 8.0 Hz, Ar), 6.65 (2 H, m, Ar), 3.94 (2 H, d, *J* 14.2 Hz, CH2), 3.82 (2 H, d, *J* 14.6 Hz, CH2), 2.36 (2 H, m, cy), 2.05 (2 H, s, cy), 1.66 (2 H, m, cy), 1.16 (4 H, m, cy); δ_C(125 MHz; $CDCl_3$) 153.4, 129.1, 126.7, 124.4, 121.1, 119.4, 59.9, 49.5, 30.3, 24.0. These compounds were synthesized in analogy to rac-L^3H_2 from *R,R*-1,2-diaminocyclohexane and *S,S*-1,2-diaminocyclohexane, respectively.

rac-L^4H_2 was synthesized in analogy to rac-L^1H_2[7] from rac-1,2-diaminocyclohexane and 2-hydroxy-3-methoxybenzaldehyde (1.0 g, 68% yield). (Found: C, 68.15; H, 7.69, N, 7.17%. Calcd for $C_{22}H_{30}N_2O_4$: C, 68.37; H, 7.82; N, 7.25%.) δ_H(500 MHz; $CDCl_3$) 6.71 (2 H, dd, *J* 8.3, 2.2 Hz, Ar), 6.65 (2 H, t, *J* 8.0 Hz, Ar), 6.65 (2 H, d, *J* 7.4 Hz, Ar), 3.94 (2 H, d, *J* 13.7 Hz, CH2), 3.82 (2 H, d, *J* 13.7 Hz, CH2), 3.79 (6 H, s, CH_3) 2.36 (2 H, m, cy), 2.05 (2 H, s, cy), 1.66 (2 H, m, cy), 1.14 (4 H, m, cy); δ_C(125 MHz; $CDCl_3$) 147.8, 146.7, 124.1, 120.6, 118.8, 110.7, 60.5, 59.9, 49.1, 30.3, 24.0.

For *R,R*-L^4H_2 (1.1 g, 75%): $[\alpha]_D^{27} = -100 \pm 2°$ (*c* 0.35% in $CHCl_3$). (Found: C, 68.34; H, 7.75, N, 7.20%. Calcd for $C_{22}H_{30}N_2O_4$: C, 68.37; H, 7.82; N, 7.25%.) δ_H(500 MHz; $CDCl_3$) 6.71 (2 H, dd, *J* 8.0, 2.0 Hz, Ar), 6.65 (2 H, t, *J* 8.0 Hz, Ar), 6.65 (2 H, d, *J* 7.3 Hz, Hz, Ar), 3.94 (2 H, d, *J* 13.7 Hz, CH2), 3.82 (2 H, d, *J* 13.6 Hz, CH2), 3.79 (6 H, s, CH_3) 2.36 (2 H, m, cy), 2.05 (2 H, s, cy), 1.66 (2 H, m, cy), 1.14 (4 H, m, cy); δ_C(125 MHz; $CDCl_3$) 147.8, 146.7, 124.1, 120.6, 118.8, 110.7, 60.5, 59.9, 49.1, 30.3, 24.0. For *S,S*-L^4H_2[13] (1.3 g, 82%): $[\alpha]_D^{26} = 98.3 \pm 0.3°$, (*c* 0.35 in $CHCl_3$). (Found: C, 68.24; H, 7.91, N, 7.14%. Calcd for $C_{22}H_{30}N_2O_4$: C, 68.37; H, 7.82; N, 7.25%). δ_H(500 MHz; $CDCl_3$) 6.71 (2 H, dd, *J* 8.1, 2.0 Hz, Ar), 6.65 (2 H, t, *J* 8.0 Hz, Ar), 6.65 (2 H, d, *J* 7.2 Hz, Ar), 3.94 (2 H, d, *J* 13.8 Hz, CH2), 3.82 (2 H, d, *J* 13.6 Hz, CH2), 3.79 (6 H, s, CH_3), 2.36 (2 H, m, cy), 2.05 (2 H, s, cy), 1.66 (2 H, m, cy), 1.14 (4 H, m, cy); δ_C(125 MHz; $CDCl_3$) 147.8, 146.7, 124.1, 120.6, 118.8, 110.7, 60.5, 59.9, 49.1, 30.3, 24.0. These compounds were synthesized in analogy to rac-L^4H_2 from *R,R*-1,2-diaminocyclohexane and *S,S*-1,2-diaminocyclohexane, respectively.

rac-$L^2Ti(OiPr)_2$ was synthesized similarly to rac-$L^1Ti(OiPr)_2$[7] (0.02 g, >95%, >95% induction). (Found: C, 55.77; H, 6.27, N, 4.73%. Calcd for $C_{26}H_{36}Cl_2N_2O_4Ti$: C, 55.83; H, 6.49; N, 5.01%.) δ_H(500 MHz; $CDCl_3$) 6.98 (2 H, d, *J* 7.2 Hz, Ar), 6.83 (2 H, s, Ar), 6.52 (2 H, d, *J* 8.7 Hz, Ar), 4.71 (2 H, sept, *J* 6.0 Hz, CH), 4.65 (2 H, d, *J* 14.3 Hz, CH2), 3.72 (2 H, d, 14.3 Hz, CH2), 2.25 (2 H, m, cy), 2.16 (2 H, m, cy), 1.62 (2 H, m, cy), 1.20 (6 H, d, *J* 6.2 Hz, CH3), 1.0 (6 H, d, *J* 6.1 Hz, CH3), 0.97(2 H, m, cy), 0.76 (2 H, m, cy); δ_C (125 MHz; $CDCl_3$) 161.0, 129.6, 128.5, 123.2, 121.5, 119.2, 77.8, 64.4, 58.0, 48.8, 29.8, 25.7, 24.4.

For Δ,*R,R*-$L^2Ti(OiPr)_2$ (0.02 g, >95%, >95% induction): $[\alpha]_D^{27} = -110 \pm 5°$ (*c* 0.14% in $CHCl_3$). (Found: C, 55.72; H, 6.22, N, 4.69%. Calcd for $C_{26}H_{36}Cl_2N_2O_4Ti$: C, 55.83; H, 6.49; N, 5.01%.) δ_H(500 MHz; $CDCl_3$) 6.98 (2 H, d, *J* 7.2 Hz, Ar), 6.83 (2 H, s, Ar), 6.52 (2 H, d, *J* 8.7 Hz, Ar), 4.71 (2 H, sept, *J* 6.2 Hz, CH), 4.65 (2 H, d, *J* 14.3 Hz, CH2), 3.72 (2 H, d, 14.3 Hz, CH2), 2.25 (2 H, m, cy), 2.16 (2 H, m, cy), 1.62 (2 H, m, cy), 1.20 (6 H, d, *J* 6.1 Hz, CH3), 1.0 (6 H, d, *J* 6.1 Hz, CH3), 0.97(2 H, m, cy), 0.76 (2 H, m, cy); δ_C (125 MHz; CDCl3) 161.0, 129.6, 128.5, 123.2, 121.5, 119.2, 77.8, 64.4, 58.0, 48.8, 29.8, 25.7, 24.4. For Λ,*S,S*-$L^2Ti(OiPr)_2$ (0.03 g, >95%, >95% induction): $[\alpha]_D^{27} = 111 \pm 3°$, (*c* 0.15% in $CHCl_3$). (Found: C, 55.61; H, 6.20, N, 4.64%. Calcd for $C_{26}H_{36}Cl_2N_2O_4Ti$: C, 55.83; H, 6.49; N, 5.01%.) δ_H(500 MHz; $CDCl_3$) 6.98 (2 H, d, *J* 7.2 Hz, Ar), 6.83 (2 H, s, Ar), 6.52 (2 H, d, *J* 8.7 Hz, Ar), 4.71 (2 H, sept, *J* 6.0 Hz, CH), 4.65 (2 H, d, *J* 14.3 Hz, CH2), 3.72 (2 H, d, 14.3 Hz, CH2), 2.25 (2 H, m, cy), 2.16 (2 H, m, cy), 1.62 (2 H, m, cy), 1.20 (6 H, d, *J* 6.0 Hz, CH3), 1.0 (6 H, d, *J* 6.0 Hz, CH3), 0.97(2 H, m, cy), 0.76 (2 H, m, cy); δ_C (125 MHz; $CDCl_3$)

161.0, 129.6, 128.5, 123.2, 121.5, 119.2, 77.8, 64.4, 58.0, 48.8, 29.8, 25.7, 24.4 These compounds were synthesized in analogy to rac-L^{2}Ti(OiPr)$_2$ from *R*,*R*-L^{2}H$_2$ and *S*,*S*-L^{2}H$_2$, respectively.

rac-L^{3}Ti(OiPr)$_2$ was synthesized similarly to rac-L^{1}Ti(OiPr)$_2$[7] (0.02 g, >95%, >95% induction). (Found: C, 55.45; H, 6.13, N, 5.02%. Calcd for $C_{26}H_{36}Cl_2N_2O_4Ti$: C, 55.83; H, 6.49; N, 5.01%.) δ_H(500 MHz; $CDCl_3$) 7.19 (2 H, d, *J* 7.0 Hz, Ar), 6.78 (2 H, d, *J* 7.6 Hz Ar), 6.52 (2 H, t, *J* 7.6 Hz, Ar), 4.97 (2 H, sept, *J* 6.2 Hz, CH), 4.78 (2 H, d, *J* 14.0 Hz, CH2), 3.84 (2 H, d, 14.0 Hz, CH2), 2.21 (2 H, m, cy), 2.13 (2 H, m, cy), 1.59 (2 H, m, cy), 1.20 (6 H, d, *J* 6.0 Hz, CH3), 1.0 (6 H, d, *J* 6.0 Hz, CH3), 0.92 (2 H, m, cy), 0.76 (2 H, m, cy); δ_C (125 MHz; $CDCl_3$) 159.9, 129.0, 127.4, 123.4, 122.3, 117.0, 79.4, 67.8, 57.8, 49.3, 29.8, 25.7, 24.4.

For Δ,*R*,*R*-L^{3}Ti(OiPr)$_2$ (0.02 g, >95%, >95% induction): $[\alpha]_D^{24}$ = −188 ± 2° (*c* 0.15% in $CHCl_3$). (Found: C, 56.03; H, 6.23, N, 5.13%. Calcd for $C_{26}H_{34}Cl_2N_2O_4Ti$: C, 55.83; H, 6.49; N, 5.01%). δ_H(500 MHz; $CDCl_3$) 7.19 (2 H, d, *J* 7.0 Hz, Ar), 6.78 (2 H, d, *J* 7.6 Hz Ar), 6.52 (2 H, t, *J* 7.6 Hz, Ar), 4.97 (2 H, sept, *J* 6.2 Hz, CH), 4.78 (2 H, d, *J* 14.0 Hz, CH2), 3.84 (2 H, d, 14.0 Hz, CH2), 2.21 (2 H, m, cy), 2.13 (2 H, m, cy), 1.59 (2 H, m, cy), 1.20 (6 H, d, *J* 6.0 Hz, CH3), 1.0 (6 H, d, *J* 6.0 Hz, CH3), 0.92 (2 H, m, cy), 0.76 (2 H, m, cy); δ_C (125 MHz; $CDCl_3$) 159.9, 129.0, 127.4, 123.4, 122.3, 117.0, 79.4, 67.8, 57.8, 49.3, 29.8, 25.7, 24.4. For Λ,*S*,*S*-L^{3}Ti(OiPr)$_2$ (0.02 g, >95%, >95% induction): $[\alpha]_D^{26}$ = 195 ± 3° (*c* 0.13% in $CHCl_3$). (Found: C, 56.05; H, 6.35, N, 5.14%. Calcd for $C_{26}H_{34}Cl_2N_2O_4Ti$: C, 55.83; H, 6.49; N, 5.01%.) δ_H(500 MHz; $CDCl_3$) 7.19 (2 H, d, *J* 7.0 Hz, Ar), 6.78 (2 H, d, *J* 7.6 Hz Ar), 6.52 (2 H, t, *J* 7.6 Hz, Ar), 4.97 (2 H, sept, *J* 6.2 Hz, CH), 4.78 (2 H, d, *J* 14.0 Hz, CH2), 3.84 (2 H, d, *J* 14.0 Hz, CH2), 2.21 (2 H, m, cy), 2.13 (2 H, m, cy), 1.59 (2 H, m, cy), 1.20 (6 H, d, *J* 6.2 Hz, CH3), 1.0 (6 H, d, *J* 6.0 Hz, CH3), 0.92 (2 H, m, cy), 0.76 (2 H, m, cy); δ_C (125 MHz; $CDCl_3$) 159.9, 129.0, 127.4, 123.4, 122.3, 117.0, 79.4, 67.8, 57.8, 49.3, 29.8, 25.7, 24.4. These compounds were synthesized in analogy to rac-L^{4a}Ti(OiPr)$_2$ from *R*,*R*-L^{3}H$_2$ and *S*,*S*-L^{3}H$_2$, respectively.

rac-L^{4}Ti(OiPr)$_2$ was synthesized similarly to rac-L^{1}Ti(OiPr)$_2$[7] (0.02 g, >95%, >95% induction). (Found: C, 60.87; H, 7.70, N, 4.80%. Calcd for $C_{28}H_{42}N_2O_6Ti$: C, 61.09; H, 7.69; N, 5.09%.) δ_H(500 MHz; $CDCl_3$) 6.73 (2 H, t, *J* 5.0 Hz, Ar), 6.52 (4 H, d, *J* 4.0 Hz Ar), 4.78 (2 H, sept, *J* 6.1 Hz, CH), 4.71 (2 H, d, *J* 14.0 Hz, CH2), 3.82 (2 H, d, 14.1 Hz, CH2), 3.80 (6 H, s, CH_3), 2.35 (2 H, m, cy), 2.16 (2 H, m, cy), 1.59 (2 H, m, cy), 1.20 (6 H, d, *J* 6.3 Hz, CH3), 1.0 (6 H, d, *J* 6.5 Hz, CH3), 0.92 (2 H, m, cy), 0.76 (2 H, m, cy); δ_C (125 MHz; $CDCl_3$) 153.5, 148.6, 122.6, 121.8, 116.2, 113.1, 77.5, 57.9, 57.0, 49.0, 29.8, 25.8, 25.7, 24.4.

For Δ,*R*,*R*-L^{4}Ti(OiPr)$_2$ (0.02 g, >95%, >95% induction): $[\alpha]_D^{27}$ = −235 ± 8° (*c* 0.15% in $CHCl_3$). (Found: C, 60.83; H, 7.65, N, 5.16%. Calcd for $C_{28}H_{42}N_2O_6Ti$: C, 61.09; H, 7.69; N, 5.09%.) δ_H(500 MHz; $CDCl_3$) 6.73 (2 H, t, *J* 5.1 Hz, Ar), 6.52 (4 H, d, *J* 4.2 Hz Ar), 4.78 (2 H, sept, *J* 6.1 Hz, CH), 4.71 (2 H, d, *J* 14.0 Hz, CH2), 3.82 (2 H, d, 14.2 Hz, CH2), 3.80 (6.0 H, s, CH_3), 2.35 (2 H, m, cy), 2.16 (2 H, m, cy), 1.59 (2 H, m, cy), 1.20 (6 H, d, *J* 6 Hz, CH3), 1.0 (6 H, d, *J* 6.4 Hz, CH3), 0.92 (2 H, m, cy), 0.76 (2 H, m, cy); δ_C (125 MHz; $CDCl_3$) 153.5, 148.6, 122.6, 121.8, 116.2, 113.1, 77.5, 57.9, 57.0, 49.0, 29.8, 25.8, 25.7, 24.4. For Λ,*S*,*S*-L^{4}Ti(OiPr)$_2$ (0.02 g, >95%, >95% induction): $[\alpha]_D^{27}$ = 248 ± 10° (*c* 0.16% in $CHCl_3$). (Found: C, 60.67; H, 7.48, N, 5.11%. Calcd for $C_{28}H_{42}N_2O_6Ti$: C, 61.09; H, 7.69; N, 5.09%.) δ_H(500 MHz; $CDCl_3$) 6.73 (2 H, t, *J* 5.0 Hz, Ar), 6.52 (4 H, d, *J* 4.3 Hz Ar), 4.78 (2 H, sept, *J* 6.1 Hz, CH), 4.71 (2 H, d, *J* 14.5 Hz, CH2), 3.82 (2 H, d, 14.5 Hz, CH2), 3.80 (6 H, s, CH_3), 2.35 (2 H, m, cy), 2.16 (2 H, m, cy), 1.59 (2 H, m, cy), 1.20 (6 H, d, *J* 6.0 Hz, CH3), 1.0 (6 H, d, *J* 6.3 Hz, CH3), 0.92 (2 H, m, cy), 0.76 (2 H, m, cy); δ_C (125 MHz; $CDCl_3$) 153.5, 148.6, 122.6, 121.8, 116.2, 113.1, 77.5, 57.9, 57.0, 49.0, 29.8, 25.8, 25.7, 24.4. These compounds were synthesized in analogy to rac-L^{5a}Ti(OiPr)$_2$ from *R*,*R*-L^{4}H$_2$ and *S*,*S*-L^{4}H$_2$, respectively.

Ti$_2$(μ-O)$_2$(*R*,*R*-L^1)(*S*,*S*-L^1) was obtained from mixing rac-L^{1}Ti(OiPr)$_2$ with 1000 equiv of water and crystallized from methylene chloride and hexane (0.01 g, 30%). (Found: C, 63.47; H, 6.57, N, 6.93%. Calcd for $C_{44}H_{56}N_4O_4Ti_2$: C, 63.47; H, 6.78; N, 6.73%.) δ_H(400 MHz; $CDCl_3$) 6.95 (2 H, dd, *J* 10.1, 2.2 Hz), 6.79 (2 H, dd, *J* 10.4, 2.7 Hz), 6.71 (2 H, s), 6.70 (2 H, d, *J* 10.2), 6.60 (2 H, d, *J* 2.2), 6.46 (2 H, d, *J* 10.2), 4.42 (4 H, m), 3.78 (2 H, t, *J* 1.2), 3.64 (2 H, dd, *J* 14.3, 0.8 Hz), 3.39 (2 H, d, *J* 10.7), 2.80 (2 H, dq, *J* 14.4, 4.6 Hz), 2.24 (4 H, m), 2.17 (6 H, s), 2.10 (6 H, s), 2.24 (2 H, dq, *J* 14.0, 4.8 Hz), 1.68 (4 H, t, *J* 14.8 Hz), 1.34 (2 H, t, *J* 14.3 Hz), 1.13 (4 H, m), 1.00 (2 H, m), 0.68 (2 H, dq, *J* 15.4, 4.1 Hz); δ_C (100 MHz; $CDCl_3$) 160.8, 159.2, 129.9, 129.8, 129.8, 129.4, 127.3, 126.6, 124.9, 122.9, 117.5, 116.7, 59.9, 58.5, 49.2, 48.1, 30.3, 29.9, 24.5, 24.5, 20.5, 20.4.

Crystal data for Ti$_2$(μ-O)$_2$(*R*,*R*-L^1)(*S*,*S*-L^1) follow: $C_{44}H_{56}N_4O_6Ti_2 \cdot CH_2Cl_2$, *M* = 917.65, triclinic, *a* = 10.531(1) Å, *b* = 12.598(2) Å, *c* = 12.731(2) Å, α = 115.766(2)°, β = 100.287(2)°, γ = 106.986(2)°, *V* = 1360.0(3) Å^3, *T* = 173(1) K, space group $P\bar{1}$, *Z* = 1, μ(Mo Kα) = 0.736 mm^{-1}, 14 907 reflections measured, 5841 unique (R_{int} = 0.0302), $R(F^{o2})$ for $[I > 2\sigma(I)]$ = 0.0728, w*R* for $[I > 2\sigma(I)]$ = 0.1842.

Ti$_2$(μ-O)$_2$(*R*,*R*-L^1)$_2$ was obtained from mixing Δ,*R*,*R*-L^{1}Ti(OiPr)$_2$ with 1000 equiv of water and further crystallized from methylene chloride and hexane (0.01 g, 30%). (Found: C, 63.55; H, 6.67, N, 6.82%. Calcd for $C_{44}H_{56}N_4O_4Ti_2$: C, 63.47; H, 6.78; N, 6.73%.) δ_H(400 MHz; $CDCl_3$) 7.13 (1 H, d, *J* 5.5 Hz), 6.85 (1 H, d, *J* 8.2 Hz), 6.78 (1 H, d, *J* 8.6 Hz), 6.71 (1 H, s), 6.64 (1 H, d, *J* 10.1 Hz), 6.55 (2 H, m), 6.42 (1 H, s), 6.35 (1 H, d, *J* 8.1 Hz), 6.19 (1 H, d, *J* 7.9 Hz), 6.09 (1 H, d, *J* 7.8 Hz), 6.04 (1 H, s), 4.31 (2 H, m), 3.88 (2 H, m), 3.60 (4 H, m), 3.07 (1 H, d, *J* 11.4 Hz), 2.87 (1 H, d, *J* 11.0 Hz), 2.20 (3 H, s), 2.16 (3 H, s), 2.13 (3 H, s), 2.11 (3 H, s), 2.25−0.38 (22 H); δ_C (100 MHz; $CDCl_3$) 161.6, 160.7, 160.2, 159.6, 130.9, 130.2, 130.2, 129.8, 129.4, 129.1, 128.9, 127.9, 126.9, 126.7, 126.6, 126.3, 124.7, 124.5, 124.2, 122.0, 117.6, 116.6, 116.4, 115.7, 60.7, 60.1, 60.1, 59.8, 49.8, 48.7, 47.8, 47.4, 30.7, 30.5, 29.4, 28.4, 25.4, 24.7, 24.6, 21.2, 20.7, 20.6, 20.5, 20.4.

Crystal data for Ti$_2$(μ-O)$_2$(*R*,*R*-L^1)$_2$ follow: $C_{44}H_{56}N_4O_6Ti_2 \cdot 2\,CH_2Cl_2$, *M* = 917.65, monoclinic, *a* = 8.4605(7) Å, *b* = 14.937(1) Å, *c* = 17.662(2) Å, β = 96.117(1)°, *V* = 2219.2(3) Å^3, *T* = 173(1) K, space group $P_2\bar{1}$, *Z* = 2, μ(Mo Kα) = 0.532 mm^{-1}, 25 290 reflections measured, 10 287 unique (R_{int} = 0.0188), $R(F^{o2})$ for $[I > 2\sigma(I)]$ = 0.0320, w*R* for $[I > 2\sigma(I)]$ = 0.0856.

Crystal data for Ti$_4$(μ-O)$_4$(*R*,*R*-L^1)$_4$ follow: $C_{88}H_{112}N_8O_{12}Ti_4 \cdot CH_2Cl_2$, *M* = 1750.38, monoclinic, *a* = 22.568(3) Å, *b* = 13.3559(15) Å, *c* = 31.153(4) Å, β = 100.928(2)°, *V* = 9219.4(18) Å^3, *T* = 173(1) K, space group *C*2, *Z* = 4, μ(Mo Kα) = 0.453 mm^{-1}, 50 725 reflections measured, 19 798 unique (R_{int} = 0.0623), $R(F^{o2})$ for $[I > 2\sigma(I)]$ = 0.0896, w*R* for $[I > 2\sigma(I)]$ = 0.2196.

■ ASSOCIATED CONTENT

Supporting Information. Cytotoxic activity for complexes rac-, Δ,*R*,*R*-, and Λ,*S*,*S*-L$^{1-4}$Ti(OiPr)$_2$ toward OVCAR-1 cancer cell lines (Figures S1−S4), ^{1}H NMR (aromatic region) of Ti$_2$(μ-O)$_2$(*R*,*R*-L^1)(*S*,*S*-L^1) and Ti$_2$(μ-O)$_2$(*R*,*R*-L^1)$_2$ (Figure S5), and CD spectra of Δ,*R*,*R*- and Λ,*S*,*S*-L$^{1-4}$Ti(OiPr)$_2$ (Figure S6). Crystal data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) (a) Abu-Surrah, A. S.; Kettunen, M. *Curr. Med. Chem.* **2006**, *13*, 1337. (b) Barnes, K. R.; Lippard, S. J. *Met. Ions Biol. Syst.* **2004**, *42*, 143. (c) Cepeda, V.; Fuertes, M. A.; Castilla, J.; Alonso, C.; Quevedo, C.; Perez, J. M. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 3.

(2) (a) Bruijnincx, P. C. A.; Sadler, P. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 197. (b) Desoize, B. *Anticancer Res.* **2004**, *24*, 1529. (c) Galanski, M.; Arion, V. B.; Jakupec, M. A.; Keppler, B. K. *Curr. Pharm. Des.* **2003**, *9*, 2078. (d) Jakupec, M. A.; Galanski, M.; Arion, V. B.; Hartinger, C. G.; Keppler, B. K. *Dalton Trans.* **2008**, 183. (e) Ott, I.; Gust, R. *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 117. (f) van Rijt, S. H.; Sadler, P. J. *Drug Discovery Today* **2009**, *14*, 1089. (g) Xu, G.; Cui, Y. B.; Cui, K.; Gou, S. H. *Prog. Chem.* **2006**, *18*, 107.

(3) (a) Abeysinghe, P. M.; Harding, M. M. *Dalton Trans.* **2007**, 3474. (b) Caruso, F.; Rossi, M. *Mini-Rev. Med. Chem.* **2004**, *4*, 49. (c) Caruso, F.; Rossi, M.; Pettinari, C. *Expert Opin. Ther. Pat.* **2001**, *11*, 969. (d) Christodoulou, C. V.; Eliopoulos, A. G.; Young, L. S.; Hodgkins, L.; Ferry, D. R.; Kerr, D. J. *Br. J. Cancer* **1998**, *77*, 2088. (e) Kelter, G.; Sweeney, N. J.; Strohfeldt, K.; Fiebig, H.-H.; Tacke, M. *Anti-Cancer Drugs* **2005**, *16*, 1091. (f) Keppler, B. K.; Friesen, C.; Moritz, H. G.; Vongerichten, H.; Vogel, E. *Struct. Bonding (Berlin)* **1991**, *78*, 97. (g) Köpf-Maier, P.; Köpf, H. *Chem. Rev.* **1987**, *87*, 1137. (h) Köpf-Maier, P.; Köpf, H. *Struct. Bonding (Berlin)* **1988**, *70*, 103. (i) Meléndez, E. *Crit. Rev. Oncol. Hematol.* **2002**, *42*, 309. (j) Strohfeldt, K.; Tacke, M. *Chem. Soc. Rev.* **2008**, *37*, 1174.

(4) (a) Caruso, F.; Massa, L.; Gindulyte, A.; Pettinari, C.; Marchetti, F.; Pettinari, R.; Ricciutelli, M.; Costamagna, J.; Canales, J. C.; Tanski, J.; Rossi, M. *Eur. J. Inorg. Chem.* **2003**, 3221. (b) Toney, J. H.; Marks, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 947.

(5) (a) Peri, D.; Meker, S.; Manna, C. M.; Tshuva, E. Y. *Inorg. Chem.* **2011**, *50*, 1030. (b) Peri, D.; Meker, S.; Shavit, M.; Tshuva, E. Y. *Chem.—Eur. J.* **2009**, *15*, 2403. (c) Shavit, M.; Peri, D.; Manna, C. M.; Alexander, J. S.; Tshuva, E. Y. *J. Am. Chem. Soc.* **2007**, *129*, 12098. (d) Tshuva, E. Y.; Ashenhurst, J. A. *Eur. J. Inorg. Chem.* **2009**, 2203. (e) Tshuva, E. Y.; Peri, D. *Coord. Chem. Rev.* **2009**, *253*, 2098.

(6) (a) Gomez-Ruiz, S.; Kaluderovic, G. N.; Polo-Ceron, D.; Prashar, S.; Fajardo, M.; Zizak, Z.; Juranic, Z. D.; Sabo, T. J. *Inorg. Chem. Commun.* **2007**, *10*, 748. (b) Gomez-Ruiz, S.; Kaluderovic, G. N.; Prashar, S.; Polo-Ceron, D.; Fajardo, M.; Zizak, Z.; Sabo, T. J.; Juranic, Z. D. *J. Inorg. Biochem.* **2008**, *102*, 1558.

(7) Manna, C. M.; Tshuva, E. Y. *Dalton Trans.* **2010**, *39*, 1182.

(8) (a) Yeori, A.; Groysman, S.; Goldberg, I.; Kol, M. *Inorg. Chem.* **2005**, *44*, 4466. (b) Kondo, S.; Saruhashi, K.; Seki, K.; Matsubara, K.; Miyaji, K.; Kubo, T.; Matsumoto, K.; Katsuki, T. *Angew. Chem., Int. Ed.* **2008**, *47*, 10195.

(9) Gao, J.; Liu, Y. G.; Liu, R.; Zingaro, R. A. *ChemMedChem* **2008**, *3*, 954.

(10) (a) Guo, M.; Sun, H.; McArdle, H. J.; Gambling, L.; Sadler, P. J. *Biochemistry* **2000**, *39*, 10023. (b) Tinoco, A. D.; Incarvito, C. D.; Valentine, A. M. *J. Am. Chem. Soc.* **2007**, *129*, 3444.

(11) Groysman, S.; Sergeeva, E.; Goldberg, I.; Kol, M. *Eur. J. Inorg. Chem.* **2005**, *2005*, 2480.

(12) Adão, P.; Avecilla, F.; Bonchio, M.; Carraro, M.; Costa Pessoa, J.; Correia, I. *Eur. J. Inorg. Chem.* **2010**, *2010*, 5568.

(13) Adão, P.; Costa Pessoa, J. o.; Henriques, R. T.; Kuznetsov, M. L.; Avecilla, F.; Maurya, M. R.; Kumar, U.; Correia, I. *Inorg. Chem.* **2009**, *48*, 3542.