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Synthesis of Asymmetrical Diaminobis(alkoxo)-bisphenol Compounds and their C1-Symmetrical Mono-Ligated Titanium(IV) Complexes as Highly Stable Highly Active Antitumor Compounds

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Experimental Data

The syntheses of ligands and complexes were inspired by previously published syntheses of diaminobis(phenolato) salan compounds.[1] The syntheses of $L^{2,2}H_4$ and $L^{2,2}Ti$ were based on a Mannich condensation as published previously.[2] 3,5-dibromo-2-hydroxybenzaldehyde (98%), 3,5-dichloro-2-hydroxybenzaldehyde (98%), 3,5-dibromo-2-hydroxybenzyl bromide (98%), and triethylamine (99%) were purchased from Tokyo Chemical Industry Co., Ltd. 3,5-difluoro-2-hydroxybenzaldehyde (98%) was purchased from Apollo Scientific Ltd. N,N'-Bis(2-hydroxyethyl)ethylenediamine(97%), 2,4-dichlorophenol (99%), $NaBH_4$ (98%), and Ti(O*i*Pr)$_4$ (97%) were purchased from Sigma Aldrich Chemical Company Inc. Formaldehyde (37% in H_2O) and 2-hydroxy-5-nitrobenzyl bromide were purchased from J&K Scientific Ltd. The syntheses of the complexes were conducted under inert conditions in LC-Technologies Dry-box, employing solvents that were dried over aluminum column on an M. Braun drying system SPS-800. NMR spectroscopic data were recorded using AMX-500 MHz Bruker spectrometer. *J* values are given in Hz. High resolution electrospray ionization mass spectrometry were performed using ESI Agilent LC-MSMS 6520 instrument. Elemental analyses were performed in the microanalytical laboratory in our institute. X-ray diffraction data were obtained with an XtaLAB Synergy, Mo Single source HyPix diffractometer, while keeping the crystals at 150 K during data collection. Using Olex2, the structure was solved with the ShelXT structure solution program using Intrinsic Phasing and refined with the ShelXL refinement package using Least Squares minimisation.

Hydrolytic stability studies were conducted as previously detailed,[2] by recording the ^{1}H NMR spectra of D_2O:DMSO-d_6 (1:9) solutions of the Ti(IV) complexes for 72 h, using 1,4-dinitrobenzene (98%, Sigma Aldrich Chemical Company Inc.) as an internal standard. The $t_{1/2}$ value of $L^{1,4}Ti$ was calculated based on a pseudo-first order fit.

Cytotoxicity was measured on human colon HT-29 cancer cells (purchased from ATCC Inc.), human ovarian A2780 cancer cells, and human ovarian cisplatin-resistant A2780cp cancer cells (purchased from ECACC Inc.) using the MTT assay as previously published.[3] Cells (0.6×10^6) in medium (containing 88% RPMI-1640, 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin; all purchased from Biological Industries Inc.) were seeded in 96-well plate in medium and allowed to attach for one day. The cells were subsequently administered with reagent tested at 10 different concentrations. 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 incubated for additional 3 h. Thereafter, the MTT solution was removed, and 200 µL of isopropanol was added. The absorbance at 550 nm was measured by Spark 10 M Multimode Microplate Reader spectrophotometer (Tecan Group Ltd.). Each measurement was repeated a least 3 × 3 times, namely, three repeats per plate, all repeated at least 3 times on different days. Relative IC_{50} values were determined by a nonlinear regression of a variable slope (four parameters) model by GraphPad Prism 5.04 softeware, and reported as mean ± SD. Despite the high stability of the complexes under biological conditions, cytotoxicity plots of free ligands are provided below, showing markedly lower (if any) activity.

2,4-difluoro-6-(((2-hydroxyethyl)(2-((2-hydroxyethyl)amino)ethyl)amino)methyl)phenol. To a stirred solution of 3,5-difluoro-2-hydroxybenzaldehyde (1.01 gr, 6.39 mmol) in methanol (30 mL) was added a solution of N,N'-Bis(2-hydroxyethyl)ethylenediamine (0.95 gr, 6.39 mmol) in methanol (30 mL). The yellow solution was stirred for 2 h and $NaBH_4$ (0.48 gr., 12.78 mol) was added slowly. After stirring overnight at rt, the volatiles were evaporated. The precipitate was dissolved in distilled water (25 mL) and the solution was neutralized employing HCl solution. Following evaporation, the residue was dissolved in methanol

(20 mL) and the precipitate was filtered. The filtrate was evaporated and dried overnight resulting in a colorless precipitate (0.76 gr, 41%). HRMS ($C_{13}H_{20}F_2N_2O_3$ + Na)$^+$ *m/z* Calc.: 313.1334. Found: 313.1353. ^{1}H NMR (500 MHz, MeOD): δ = 6.83–6.78 (m, 1H, Ar), 6.75–6.72 (m, 1H, Ar), 3.74 (s, 2H, CH_2), 3.71 (t, *J* = 5.4 Hz, 2H, CH_2), 3.62 (t, *J* = 5.6 Hz, 2H, CH_2), 2.95 (t, *J* = 6.2 Hz, 2H, CH_2), 2.84 (t, *J* = 5.4 Hz, 2H, CH_2), 2.80 (t, *J* = 6.2 Hz, 2H, CH_2), 2.69 (t, *J* = 5.6 Hz, 2H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 152.8 (dd, *J* = 235, 12 Hz), 150.6 (dd, *J* = 242, 13 Hz), 142.6 (d, *J* = 14 Hz), 127.8 (dd, *J* = 8, 4 Hz), 111.1 (dd, *J* = 22, 3 Hz), 102.8 (dd, *J* = 27, 23 Hz), 59.0, 58.4, 55.4, 54.2, 51.6, 50.7, 45.8 ppm. ^{19}F NMR (470 MHz, DMSO-d_6): δ = −125.44 (t, *J* = 8.4 Hz, 1F), −133.53 (d, *J* = 10.8 Hz, 1F) ppm

2,4-dichloro-6-(((2-hydroxyethyl)(2-((2-hydroxyethyl)amino)ethyl)amino)methyl)phenol. The compound was synthesized similarly by reacting 3,5-dichloro-2-hydroxybenzaldehyde (2.76 gr, 14.45 mmol) with N,N′-Bis(2-hydroxyethyl)ethylenediamine (2.14 gr, 14.45 mmol) and $NaBH_4$ (1.09 gr, 28.9 mol) yielding a colorless precipitate (2.18 gr, 47%). HRMS ($C_{13}H_{20}Cl_2N_2O_3$ + Na)$^+$ *m/z* Calc.: 345.0743. Found: 345.0757. ^{1}H NMR (500 MHz, MeOD): δ = 7.16 (d, *J* = 2.8 Hz, 1H, Ar), 6.94 (d, *J* = 2.8 Hz, 1H, Ar), 3.78–3.76 (m, 2H, CH_2), 3.63 (s, 2H, CH_2), 3.58 (t, *J* = 5.7 Hz, 2H, CH_2), 3.04 (t, *J* = 6.2 Hz, 2H, CH_2), 2.94–2.92 (m, 2H, CH_2), 2.84 (t, *J* = 6.2 Hz, 2H, CH_2), 2.68 (t, *J* = 5.7 Hz, 2H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 159.4, 128.4, 128.2, 127.0, 121.8, 113.6, 58.5, 58.4, 55.2, 53.8, 50.6, 50.2, 45.3 ppm.

2,4-dibromo-6-(((2-hydroxyethyl)(2-((2-hydroxyethyl)amino)ethyl)amino)methyl)phenol. The compound was synthesized similarly by reacting 3,5-dibromo-2-hydroxybenzaldehyde (1.94 gr, 6.93 mmol) with N,N′-Bis(2-hydroxyethyl)ethylenediamine (1.03 gr, 6.93 mmol) and $NaBH_4$ (0.52 gr, 13.86 mol) yielding orange precipitate (1.74 gr, 61%). HRMS ($C_{13}H_{20}Br_2N_2O_3$ + H)$^+$ *m/z* Calc.: 412.9894. Found: 412.9910. ^{1}H NMR (500 MHz, MeOD): δ = 7.49 (d, *J* = 2.5 Hz, 1H, Ar), 7.18 (d, *J* = 2.5 Hz, 1H, Ar), 3.80–3.78 (m, 2H, CH_2), 3.70 (s, 2H, CH_2), 3.62 (t, *J* = 5.5 Hz, 2H, CH_2), 3.12 (t, *J* = 6.3 Hz, 2H, CH_2), 3.01–2.99 (m, 2H, CH_2), 2.86 (t, *J* = 6.3 Hz, 2H, CH_2), 2.71 (t, *J* = 5.5 Hz, 2H, CH_2) ppm. ^{13}C NMR (125 MHz, MeOD): δ = 148.8, 125.5, 123.7, 119.9, 104.7, 99.1, 50.8, 49.6, 47.2, 47.0, 41.7, 41.5, 36.9 ppm.

$L^{1,4}H_4$. 2-hydroxy-5-nitrobenzyl bromide (0.48 gr, 2.07 mmol) in THF (25 mL) was added to a stirred solution of 2,4-difluoro-6-(((2-hydroxyethyl)(2-((2-hydroxyethyl)amino)ethyl)amino)methyl)phenol (0.60 gr, 2.07 mmol) in THF (25 mL) followed by an addition of triethylamine (0.42 gr, 4.14 mmol). The solution was refluxed for 2 h and stirred at rt overnight. The formed precipitate was filtered off and the volatiles were removed under vacuum. The oily product was dissolved in distilled water (25 mL) and the solution was neutralized by HCl solution, followed by extraction with dichloromethane (20 mL). The organic phase was dried with $MgSO_4$, filtrated, and the solvent was removed under vacuum, yielding a yellow precipitate (0.35 gr, 38%). HRMS ($C_{20}H_{25}F_2N_3O_6$ + H)$^+$ *m/z* Calc.: 442.1784. Found: 442.1768. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.05 (d, *J* = 2.9 Hz, 1H, Ar), 7.99 (dd, *J* = 9.0, 2.9 Hz, 1H, Ar), 7.06–7.00 (m, 1H, Ar), 6.85–6.83 (m, 1H, Ar), 6.78 (d, *J* = 9.0 Hz, 1H, Ar), 3.82 (s, 2H, CH_2), 3.74 (s, 2H, CH_2), 3.52 (t, *J* = 5.9 Hz, 2H, CH_2), 3.50 (t, *J* = 5.8 Hz, 2H, CH_2), 2.77–2.68 (m, 4H, CH_2CH_2), 2.61 (t, *J* = 5.9 Hz, 2H, CH_2), 2.55 (t, *J* = 5.8 Hz, 2H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 165.3, 153.8 (dd, *J* = 236, 12 Hz), 150.2 (dd, *J* = 243, 12 Hz), 140.9 (dd, *J* = 13, 2 Hz), 138.1, 127.2 (dd, *J* = 8, 4 Hz), 125.3, 124.8, 124.1, 116.0, 110.8 (dd, *J* = 23, 3 Hz), 102.9 (dd, *J* = 27, 23 Hz), 58.2, 58.0, 55.5, 55.4, 54.7, 54.5, 50.4, 50.3 ppm. ^{19}F NMR (470 MHz, DMSO-d_6): δ = −123.18 (t, *J* = 8.9 Hz, 1F), −133.20 (d, *J* = 11.06 Hz, 1F) ppm.

$L^{2,4}H_4$. The compound was synthesized similarly by reacting 2-hydroxy-5-nitrobenzyl bromide (0.72 gr, 3.12 mmol) with 2,4-dichloro-6-(((2-hydroxyethyl)(2-((2-hydroxyethyl)amino)ethyl)amino)methyl)phenol (1.01 gr, 3.12 mmol) and triethylamine (0.63 gr, 6.24 mmol), yielding a yellow precipitate (0.80 gr, 54%).

HRMS ($C_{20}H_{25}Cl_2N_3O_6$ + Na)$^+$ *m/z* Calc.: 496.1013. Found: 496.1033. Anal. calcd (%) for $C_{20}H_{25}Cl_2N_3O_6$: C, 50.64; H, 5.31; N, 8.86. Found: C, 50.49; H, 5.31; N, 8.83. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.07 (d, *J* = 2.9 Hz, 1H, Ar), 8.00 (dd, *J* = 9.0, 2.9 Hz, 1H, Ar), 7.31 (d, *J* = 2.6, 1H, Ar), 7.07 (d, *J* = 2.6, 1H, Ar), 6.82 (d, *J* = 9.0 Hz, 1H, Ar), 3.81 (s, 2H, CH_2), 3.79 (s, 2H, CH_2), 3.54–3.50 (m, 4H, CH_2), 2.72–2.68 (m, 4H, CH_2CH_2), 2.60–2.57 (m, 4H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 161.1, 152.5 ,127.6, 127.2, 126.1, 125.3, 124.7, 124.5, 121.8, 120.4, 115.7, 58.2, 57.8, 56.0, 55.6, 55.2, 54.1, 50.1, 50.0 ppm.

$L^{3,4}H_4$. The compound was synthesized similarly by reacting 2-hydroxy-5-nitrobenzyl bromide (0.87 gr, 3.76 mmol) with 2,4-dibromo-6-(((2-hydroxyethyl)(2-((2-hydroxyethyl)amino)ethyl)amino)methyl)phenol (1.55 gr, 3.76 mmol) and triethylamine (0.76 gr, 7.52 mmol), yielding a yellow precipitate (1.52 gr, 72%). HRMS ($C_{20}H_{25}Br_2N_3O_6$ + H)$^+$ *m/z* Calc.: 564.0164. Found: 564.0157. Anal. calcd (%) for $C_{20}H_{25}Br_2N_3O_6$: C, 42.65; H, 4.47; N, 7.46. Found: C, 42.85; H, 4.56; N, 7.15. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.07 (d, *J* = 2.9 Hz, 1H, Ar), 8.01 (dd, *J* = 9.0, 2.9 Hz, 1H, Ar), 7.53 (d, *J* = 2.4, 1H, Ar), 7.21 (d, *J* = 2.4, 1H, Ar), 6.83 (d, *J* = 9.0 Hz, 1H, Ar), 3.82 (s, 2H, CH_2), 3.78 (s, 2H, CH_2), 3.54-–3.50 (m, 4H, CH_2), 2.71 (s, 4H, CH_2CH_2), 2.58 (t, *J* = 5.7 Hz, 4H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 164.0, 154.1 ,138.9, 132.9, 130.6, 126.3, 125.3, 124.7, 124.6, 115.7, 110.4, 109.3, 58.2, 57.8, 56.2, 55.6, 55.1, 54.1, 50.1, 50.0 ppm.

$L^{2,3}H_4$. The compound was synthesized similarly by reacting 3,5-dibromo-2-hydroxybenzyl bromide (1.01 gr, 2.94 mmol) with 2,4-dichloro-6-(((2-hydroxyethyl)(2-((2-hydroxyethyl)amino)ethyl)amino)methyl) phenol (0.95 gr, 2.94 mmol) and triethylamine (0.59 gr, 5.88 mmol), yielding an orange precipitate (1.24 gr, 72%). HRMS ($C_{20}H_{24}Br_2Cl_2N_2O_4$ + H)$^+$ *m/z* Calc.: 586.9531. Found: 586.9551. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.57 (d, *J* = 2.4 Hz, 1H, Ar), 7.35 (d, *J* = 2.6 Hz, 1H, Ar), 7.23 (d, *J* = 2.4, 1H, Ar), 7.09 (d, *J* = 2.6, 1H, Ar), 3.81 (s, 2H, CH_2), 3.80 (s, 2H, CH_2), 3.53–3.51 (m, 4H, CH_2), 2.69 (s, 4H, CH_2CH_2), 2.57 (t, *J* = 5.6 Hz, 4H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 153.99, 152.4, 133.0, 130.5, 127.6, 127.2, 126.2, 126.0, 122.0, 120.4, 110.3, 109.5, 57.8, 57.7, 56.2, 56.0, 55.3, 55.2, 50.0, 49.4 ppm.

$L^{2,2}H_4$. The compound was synthesized by refluxing 2,4-dichlorophenol (1.66 gr, 10.18 mmol), N,N'-Bis(2-hydroxyethyl)ethylenediamine (0.75 gr, 5.09 mmol) and formaldehyde (0.61 gr, 20.36 mmol) in methanol overnight. The solution was allowed to cool and the solvent was removed under vacuum. The oily residue was dissolved in methanol and kept at 4 °C overnight. The resulting precipitate was collected by vacuum filtration (0.56 gr, 22%). HRMS ($C_{20}H_{24}Cl_4N_2O_4$ + H)$^+$ *m/z* Calc.: 499.0535. Found: 499.0528. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.34 (d, *J* = 2.2 Hz, 2H, Ar), 7.09 (d, *J* = 2.2 Hz, 2H, Ar), 3.80 (s, 4H, CH_2), 3.52 (t, *J* = 5.4 Hz, 4H, CH_2), 2.68 (s, 4H, CH_2CH_2), 2.57 (t, *J* = 5.4 Hz, 4H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 152.4, 127.6, 127.2, 126.1, 121.9, 120.4, 57.8, 56.1, 55.3, 49.6 ppm.

$L^{1,4}$Ti. Ti(O*i*Pr)$_4$ (0.075 gr, 0.26 mmol) in dry THF (2 mL) was added to a stirred solution of $L^{1,4}H_4$ (0.115 gr, 0.26 mmol) in THF (4 mL) under inert conditions in a glovebox. After stirring overnight at rt, the resulted precipitate was washed twice with THF and the pale-yellow product was isolated by decantation (0.092 gr, 74%). HRMS ($C_{20}H_{21}F_2N_3O_6Ti$ + H)$^+$ *m/z* Calc.: 486.0953. Found: 486.0970. Anal. calcd (%) for $C_{20}H_{21}F_2N_3O_6Ti$: C, 49.50; H, 4.36; N, 8.66. Found: C, 49.53; H, 4.44; N, 8.32. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.17 (d, *J* = 2.9 Hz, 1H, Ar), 8.09 (dd, *J* = 9.0, 2.9 Hz, 1H, Ar), 7.18–7.14 (m, 1H, Ar), 6.95–6.93 (m, 1H, Ar), 6.82 (d, *J* = 9.0 Hz, 1H, Ar), 4.57–4.48 (m, 2H, CH_2), 4.40 (d, *J* = 14.0 Hz, 1H, CH_2), 4.35 (d, *J* = 14.3 Hz, 1H, CH_2), 4.13 (d, *J* = 14.0 Hz, 1H, CH_2), 4.01 (d, *J* = 14.3 Hz, 1H, CH_2), 3.94–3.90 (m, 1H, CH_2), 3.87–3.83 (m, 1H, CH_2), 3.65–3.57 (m, 2H, CH_2), 3.30–3.20 (m, 4H, CH_2), 3.05–2.98 (m, 2H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 167.3, 153.6 (dd, *J* = 238, 12 Hz), 149.2 (dd, *J* = 244, 13 Hz), 145.2 (dd, *J* = 13, 3 Hz), 138.7, 127.7 (dd, *J* = 9, 3 Hz), 126.2, 126.0, 125.6, 117.1, 111.2 (dd, *J* = 23, 2 Hz), 103.7 (dd, *J* = 26, 23 Hz),

69.5, 69.3, 62.5, 62.2, 60.9, 60.3, 60.1, 60.0 ppm. ^{19}F NMR (470 MHz, DMSO-d_6): δ = −122.73 (t, J = 8.9 Hz, 1F), −129.39 (d, J = 10.81 Hz, 1F) ppm.

$L^{2,4}$Ti. The compound was synthesized similarly by reacting Ti(O*i*Pr)$_4$ (0.078 gr, 0.27 mmol) with $L^{2,4}H_4$ (0.128 gr, 0.27 mmol) to give the pale-yellow product (0.113 gr, 81%). HRMS ($C_{20}H_{21}Cl_2N_3O_6Ti$ + H)$^+$ *m/z* Calc.: 519.0383. Found: 519.0407. Anal. calcd (%) for $C_{20}H_{21}Cl_2N_3O_6Ti$: C, 46.36; H, 4.08; N, 8.11. Found: C, 46.12; H, 4.14; N, 7.87. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.18 (s, 1H, Ar), 8.09 (d, J = 9.0 Hz, 1H, Ar), 7.46 (s, 1H, Ar), 7.26 (s, 1H, Ar), 6.81 (d, J = 9.0 Hz, 1H, Ar), 4.55–4.48 (m, 2H, CH_2), 4.39 (d, J = 14.0 Hz, 1H, CH_2), 4.36 (d, J = 14.1 Hz, 1H, CH_2), 4.15 (d, J = 14.0 Hz, 1H, CH_2), 4.02 (d, J = 14.1 Hz, 1H, CH_2), 3.92–3.84 (m, 2H, CH_2), 3.68–3.55 (m, 2H, CH_2), 3.28–3.22 (m, 4H, CH_2), 3.05–3.01 (m, 2H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 167.2, 155.2, 138.8, 128.3, 128.2, 128.0, 126.1, 125.9, 125.6, 121.8, 121.0, 117.1, 69.6, 69.5, 62.6, 62.2, 60.8, 60.5, 60.1, 60.0 ppm.

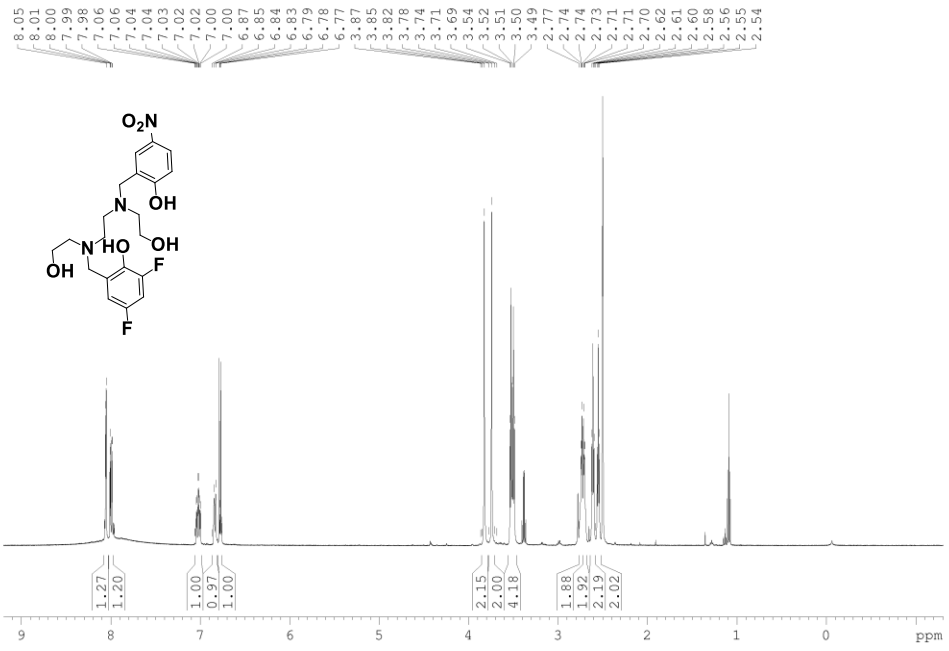
$L^{3,4}$Ti. The compound was synthesized similarly by reacting Ti(O*i*Pr)$_4$ (0.066 gr, 0.23 mmol) with $L^{3,4}H_4$ (0.130 gr, 0.23 mmol) to give a pale-yellow product (0.115 gr, 83%). Single crystals suitable for X-ray crystallography were grown from dichloromethane solution at −30 °C. HRMS ($C_{20}H_{21}Br_2N_3O_6Ti$ + H)$^+$ *m/z* Calc.: 607.9333. Found: 607.9311. Anal. calcd (%) for $C_{20}H_{21}Br_2N_3O_6Ti$: C, 39.57; H, 3.49; N, 6.92. Found: C, 39.50; H, 3.55; N, 6.56. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.18 (d, J = 2.9 Hz, 1H, Ar), 8.09 (d, J = 9.0, 2.9 Hz, 1H, Ar), 7.67 (d, J = 2.4 Hz, 1H, Ar), 7.41 (d, J = 2.4 Hz, 1H, Ar), 6.80 (d, J = 9.0 Hz, 1H, Ar), 4.55–4.48 (m, 2H, CH_2), 4.39 (d, J = 14.0 Hz, 1H, CH_2), 4.37 (d, J = 14.2 Hz, 1H, CH_2), 4.15 (d, J = 14.0 Hz, 1H, CH_2), 4.02 (d, J = 14.2 Hz, 1H, CH_2), 3.90–3.84 (m, 2H, CH_2), 3.65–3.56 (m, 2H, CH_2), 3.30–3.22 (m, 4H, CH_2), 3.05–3.00 (m, 2H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 167.1, 156.6, 138.7, 133.7, 131.7, 128.3, 126.1, 125.9, 125.6, 117.1, 111.4, 109.4, 69.5, 69.4, 62.6, 62.2, 60.8, 60.5, 60.1, 60.0 ppm.

Crystal data for $L^{3,4}$Ti (CCDC 2053342). $C_{21}H_{23}Br_2Cl_2N_3O_6Ti$ (M =692.04 g/mol), monoclinic, space group $P2_1/c$ (no. 14), a = 10.0658(2) Å, b = 24.2061(4) Å, c = 10.8597(2) Å, β = 107.976(2)°, V = 2516.84(8) Å^3, Z = 4, T = 149.99(10) K, μ(Mo Kα) = 3.771 mm^{-1}, *Dcalc* = 1.826 g/cm^3, 46034 reflections measured (4.254° ≤ 2Θ ≤ 64.422°), 7875 unique (R_{int} = 0.0368, R_{sigma} = 0.0354) which were used in all calculations. The final R_1 was 0.0481 (I > 2σ(I)) and wR_2 was 0.0892 (all data).

$L^{2,3}$Ti. The compound was synthesized similarly by reacting Ti(O*i*Pr)$_4$ (0.071 gr, 0.25 mmol) with $L^{2,3}H_4$ (0.147 gr, 0.25 mmol) to give the yellow product (0.140 gr, 88%). HRMS ($C_{20}H_{20}Br_2Cl_2N_2O_4Ti$ + H)$^+$ *m/z* Calc.: 630.8701. Found: 630.8717. Anal. calcd (%) for $C_{20}H_{20}Br_2Cl_2N_2O_4Ti$: C, 38.07; H, 3.19; N, 4.44. Found: C, 38.04; H, 3.24; N, 4.13. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.67 (d, J = 2.4 Hz, 1H, Ar), 7.45 (d, J = 2.6 Hz, 1H, Ar), 7.40 (d, J = 2.4 Hz, 1H, Ar), 7.25 (d, J = 2.6 Hz, 1H, Ar), 4.51–4.45 (m, 2H, CH_2), 4.38 (d, J = 14.0 Hz, 1H, CH_2), 4.36 (d, J = 14.0 Hz, 1H, CH_2), 3.99 (d, J = 14.1 Hz, 2H, CH_2), 3.89–3.84 (m, 2H, CH_2), 3.61–3.53 (m, 2H, CH_2), 3.25–3.19 (m, 4H, CH_2), 3.01–2.97 (m, 2H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 156.70, 155.32, 133.69, 131.66, 128.37, 128.31, 128.21, 128.03, 121.62, 120.92, 111.40, 109.29, 69.31, 69.26, 62.55, 62.53, 60.61, 60.59, 60.56, 59.90 ppm.

$L^{2,2}$Ti. The compound was synthesized similarly by reacting Ti(O*i*Pr)$_4$ (0.091 gr, 0.32 mmol) with $L^{2,2}H_4$ (0.159 gr, 0.32 mmol) to give the pale-yellow product (0.145 gr, 84%). HRMS ($C_{20}H_{20}Cl_4N_2O_4Ti$ + H)$^+$ *m/z* Calc.: 542.9705. Found: 542.9681. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.45 (d, J = 2.1 Hz, 2H, Ar), 7.25 (d, J = 2.1 Hz, 2H, Ar), 4.51–4.46 (m, 2H, CH_2), 4.37 (d, J = 14.0 Hz, 2H, CH_2), 3.99 (d, J = 14.0 Hz, 2H, CH_2), 3.89–3.85 (m, 2H, CH_2), 3.61–3.53 (m, 2H, CH_2), 3.25–3.19 (m, 4H, CH_2), 3.02–2.98 (m, 2H, CH_2) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 155.3, 128.3, 128.2, 128.0, 121.6, 120.9, 69.3, 62.5, 60.6, 59.9 ppm.

NMR spectra


8.05
8.01
8.00
7.99
7.98
7.06
7.06
7.04
7.04
7.03
7.02
7.02
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7.00
6.87
6.85
6.84
6.83
6.79
6.78
6.77
3.87
3.85
3.82
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2.74
2.73
2.71
2.71
2.70
2.62
2.61
2.60
2.58
2.56
2.55
2.54
O2N
OH
N
HO
OH
N
OH
F
F
1.27
1.20
1.00
0.97
1.00
2.15
2.00
4.18
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1.92
2.19
2.02
9
8
7
6
5
4
3
2
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0
ppm

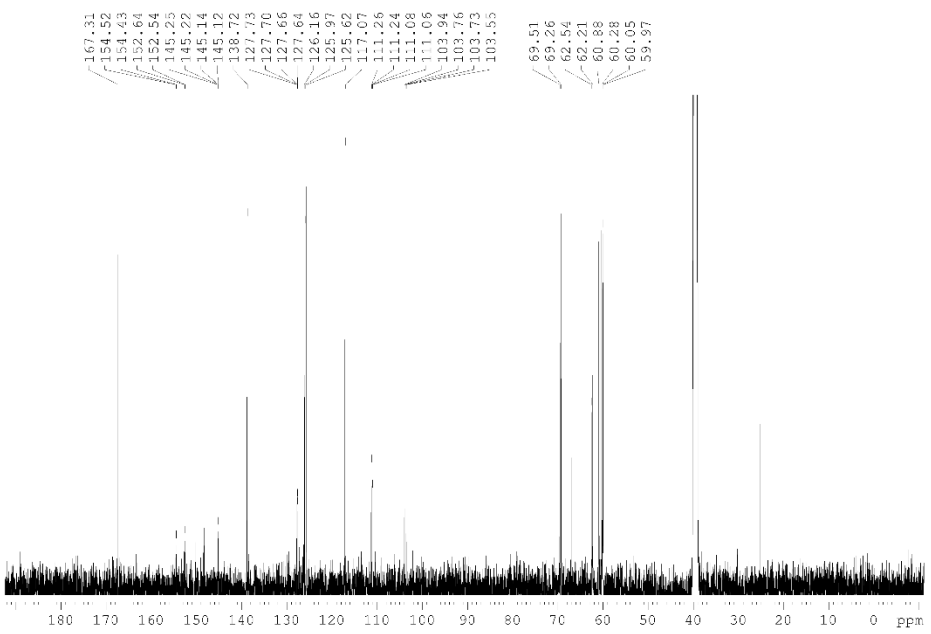
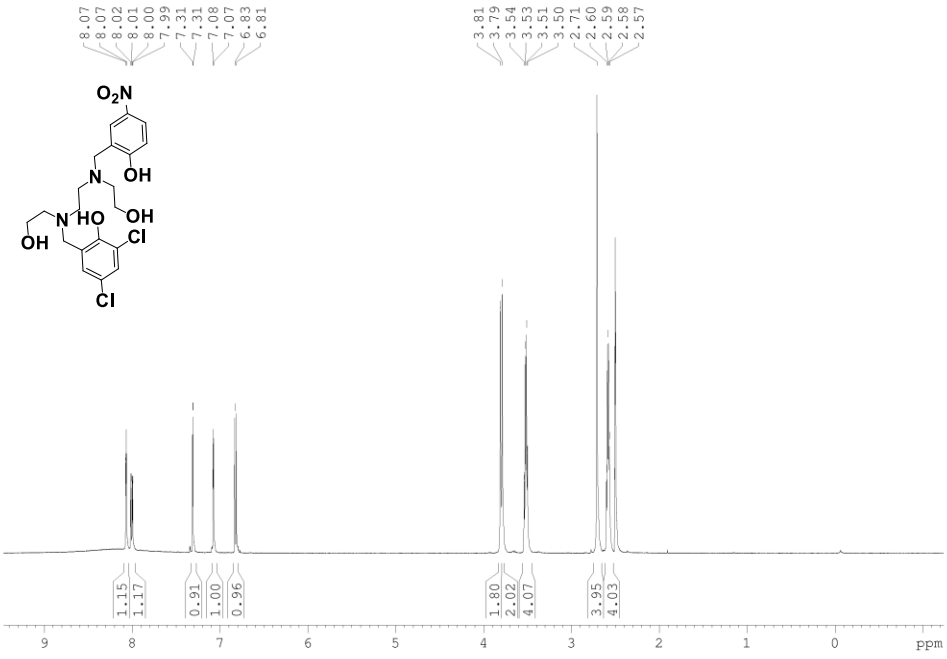


Figure S1: ^{1}H (500 MHz, top) and ^{13}C (125 MHz, bottom) NMR spectra of $L^{1,4}H_4$ in DMSO-d_6



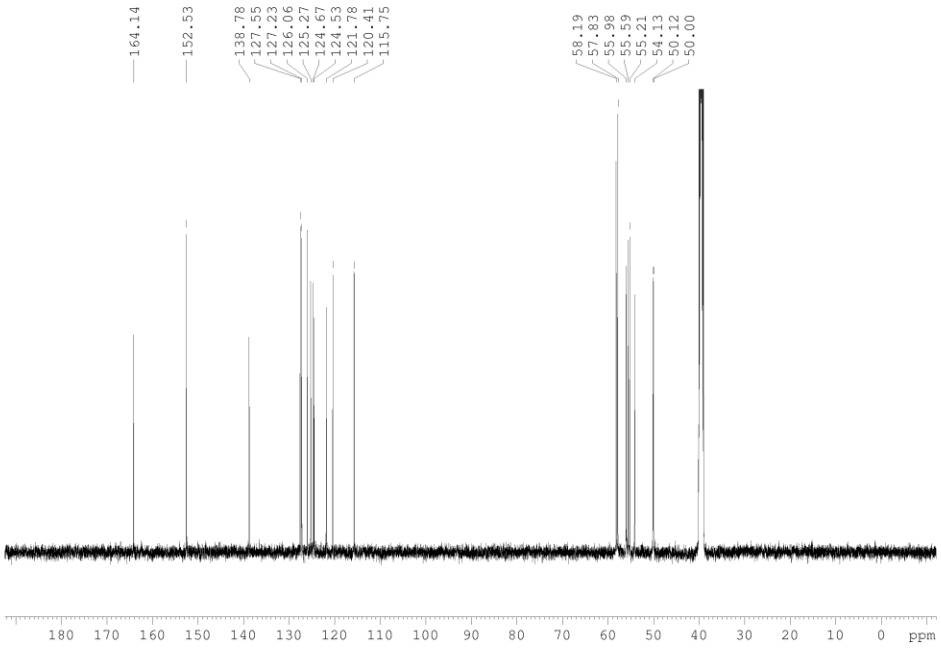
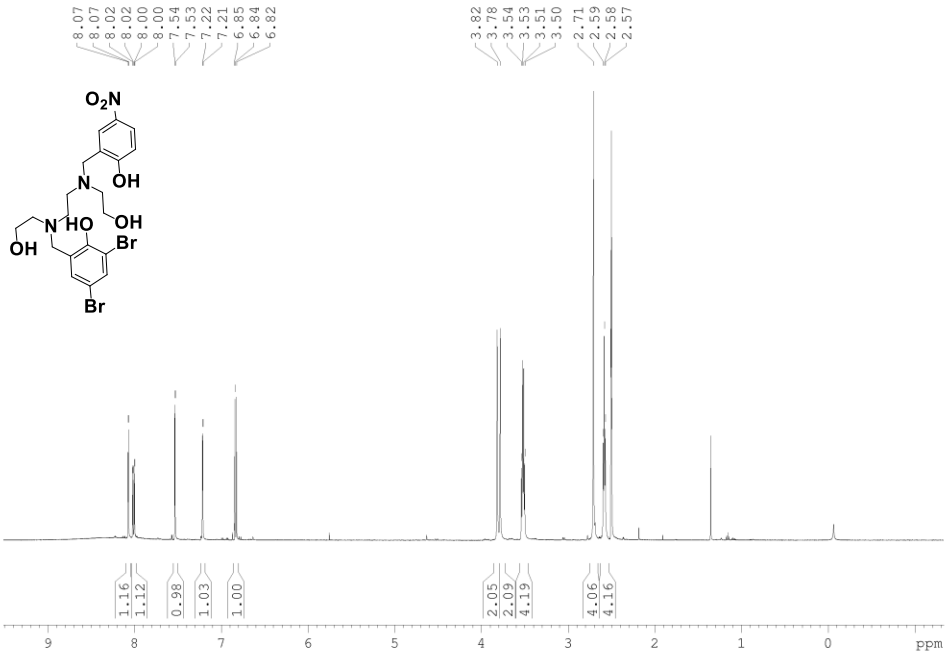


Figure S2: ^{1}H (500 MHz, top) and ^{13}C (125 MHz, bottom) NMR spectra of $L^{2,4}H_4$ in DMSO-d_6



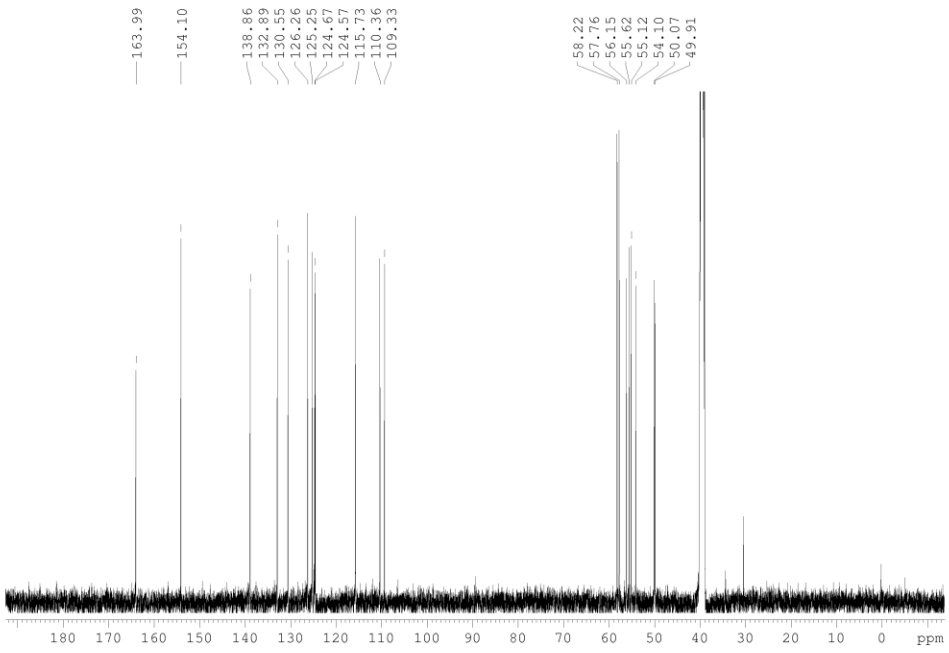
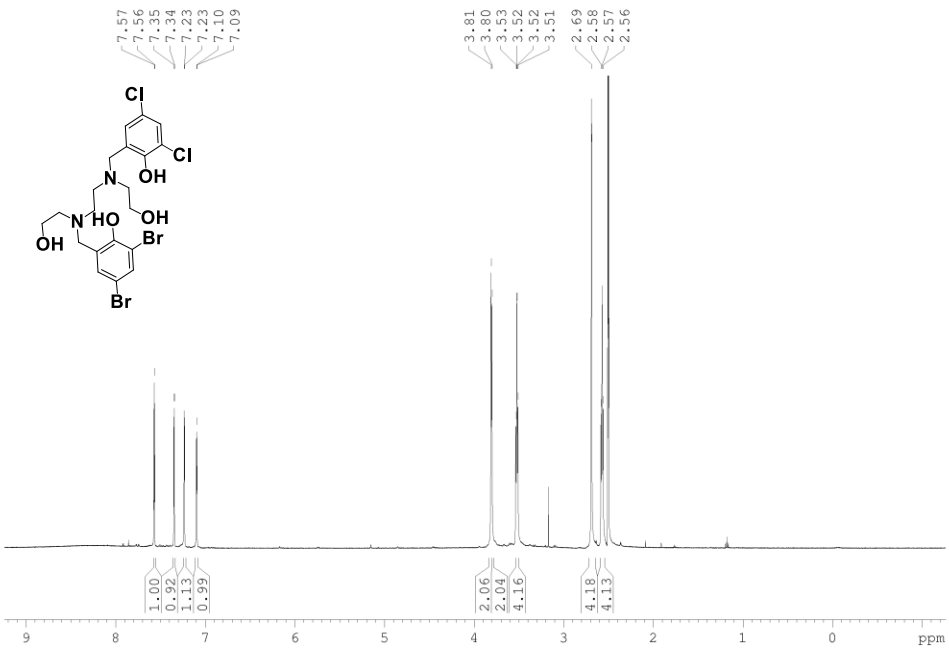


Figure S3: ^{1}H (500 MHz, top) and ^{13}C (125 MHz, bottom) NMR spectra of $L^{3,4}H_4$ in DMSO-d_6



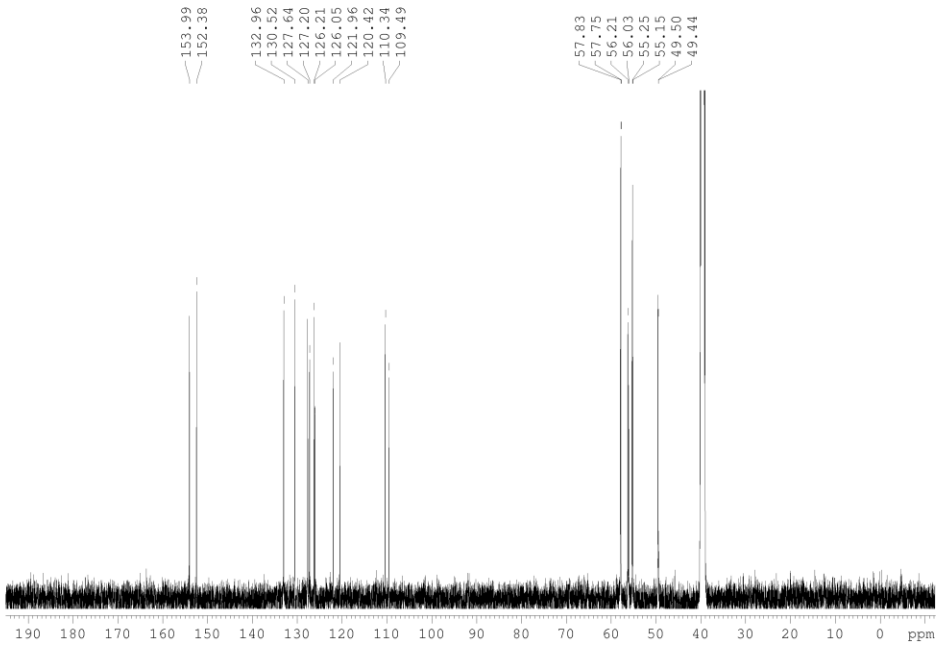
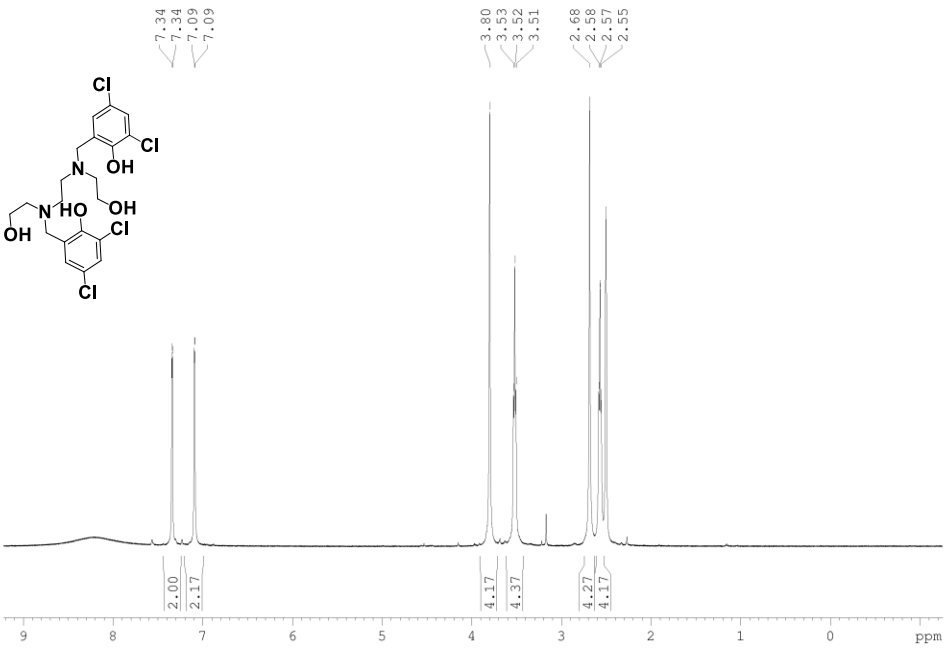


Figure S4: ^{1}H (500 MHz, top) and ^{13}C (125 MHz, bottom) NMR spectra of $L^{2,3}H_4$ in DMSO-d_6



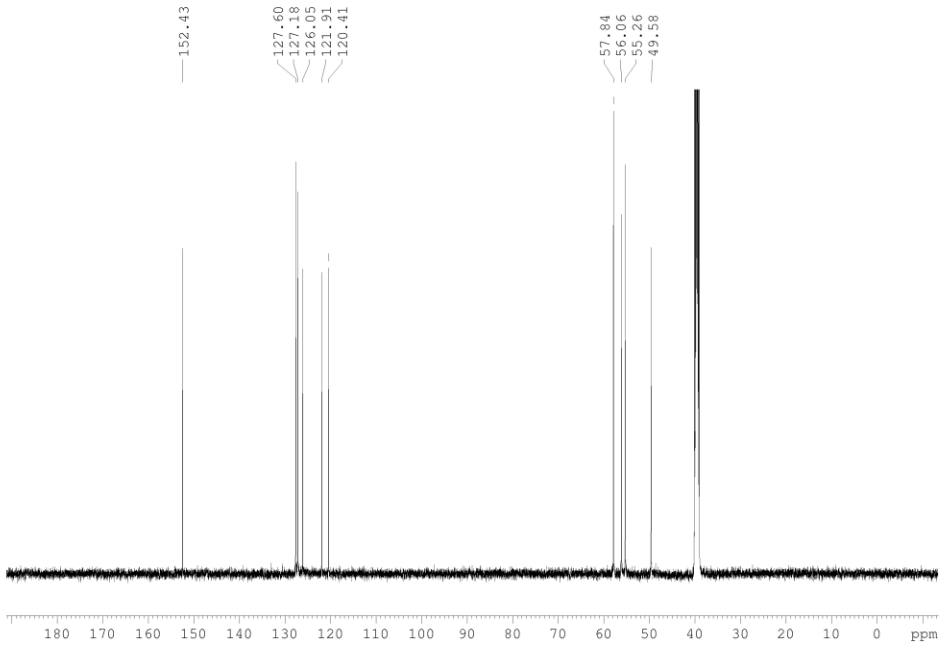
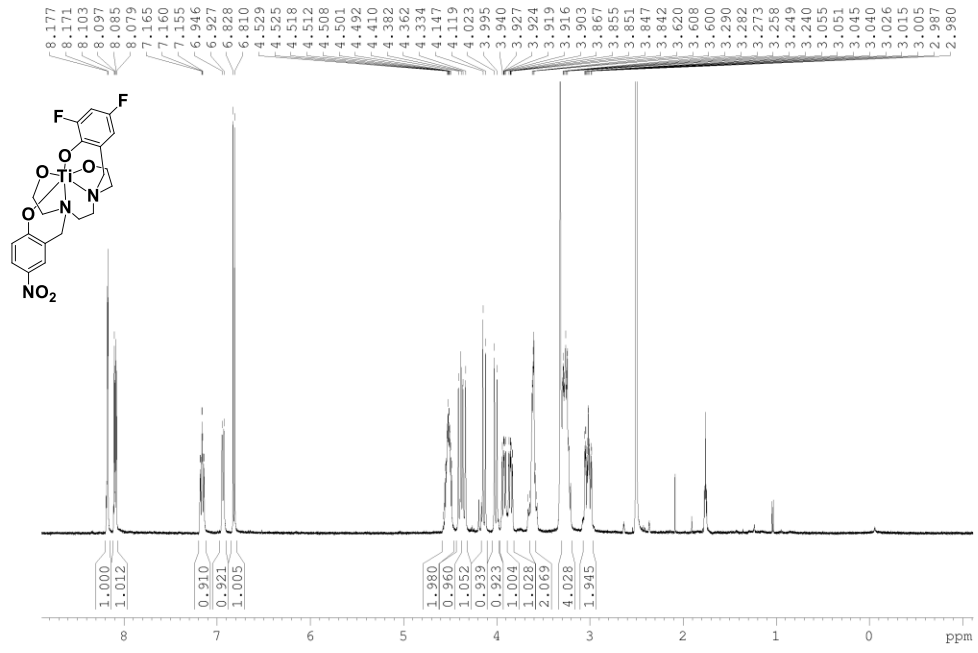


Figure S5: ^{1}H (500 MHz, top) and ^{13}C (125 MHz, bottom) NMR spectra of $L^{2,2}H_4$ in DMSO-d_6



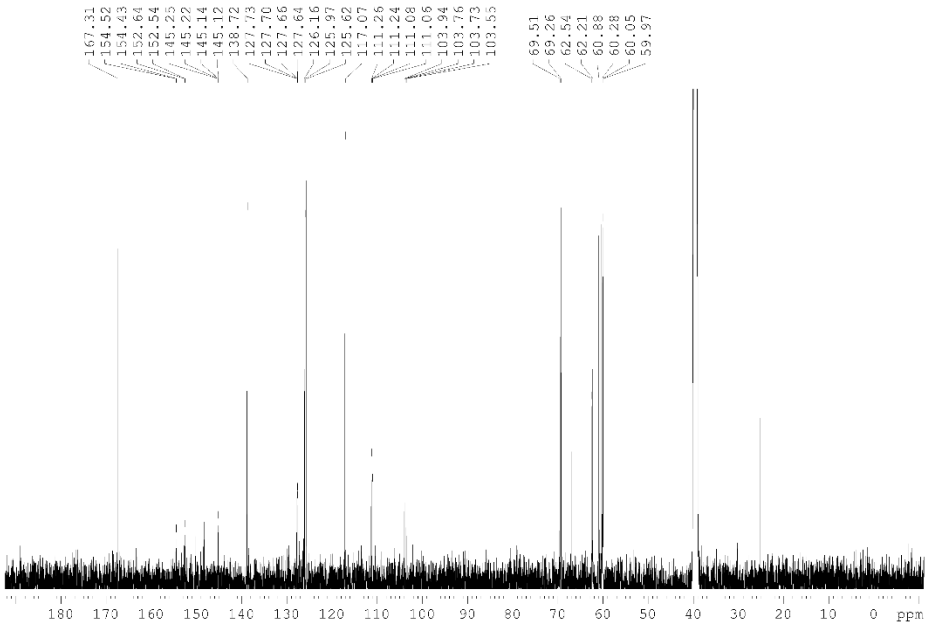
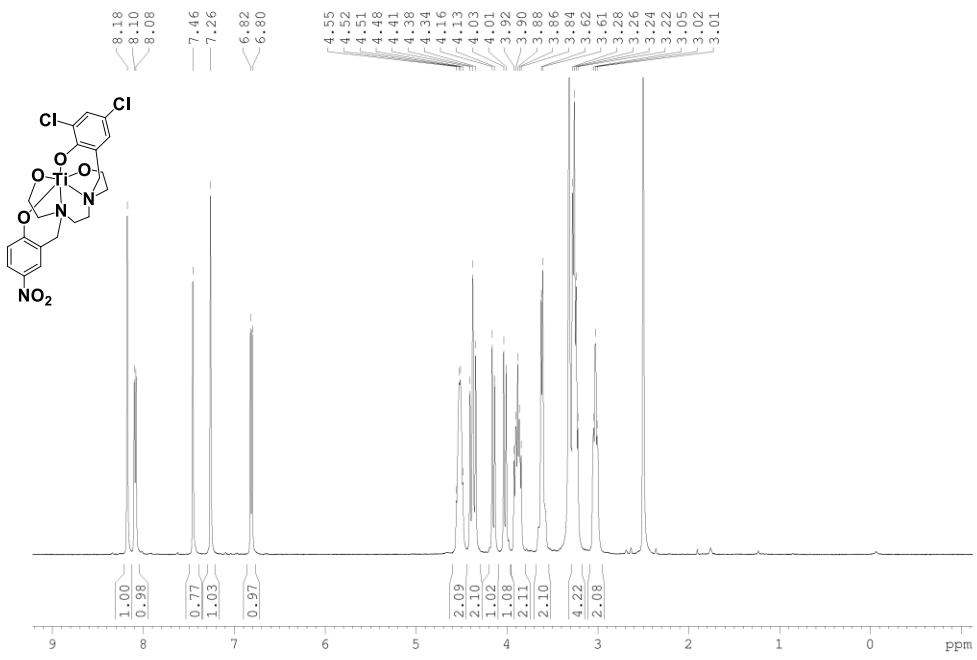


Figure S6: ^{1}H (500 MHz, top) and ^{13}C (125 MHz, bottom) NMR spectra of $L^{1,4}Ti$ in DMSO-d_6



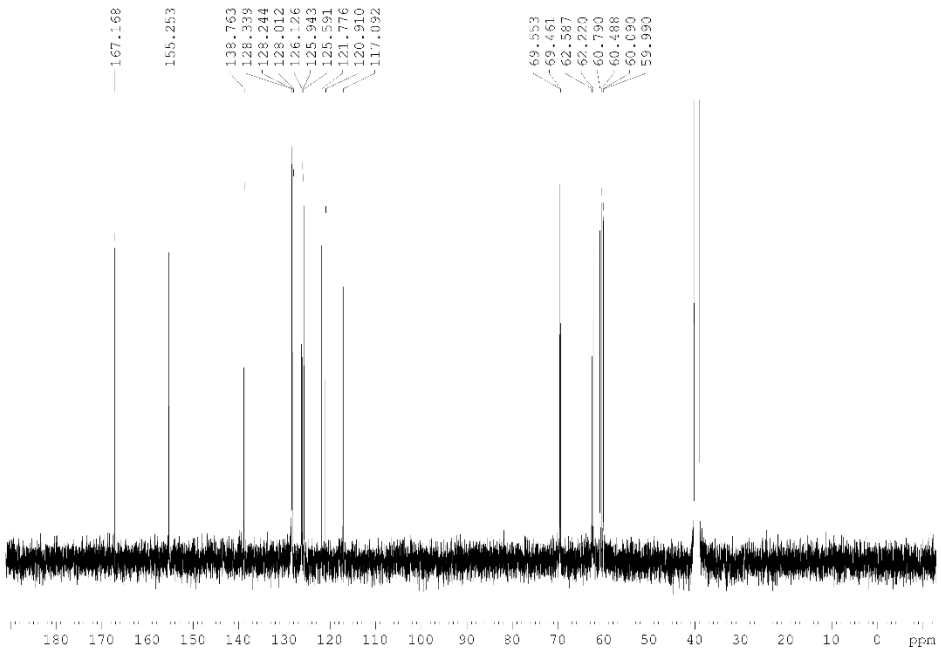
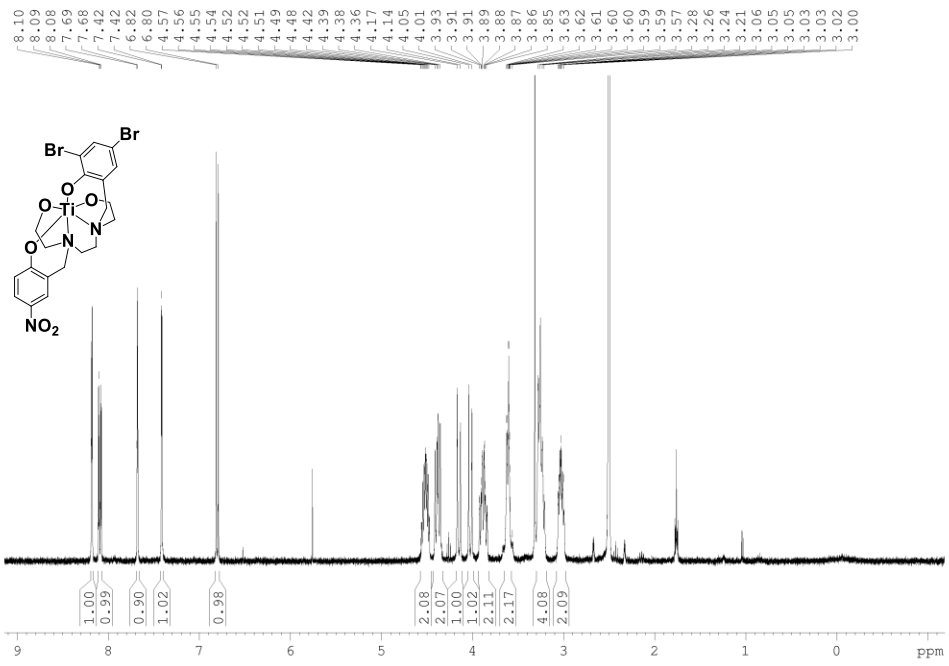


Figure S7: ^{1}H (500 MHz, top) and ^{13}C (125 MHz, bottom) NMR spectra of $L^{2,4}$Ti in DMSO-d_6



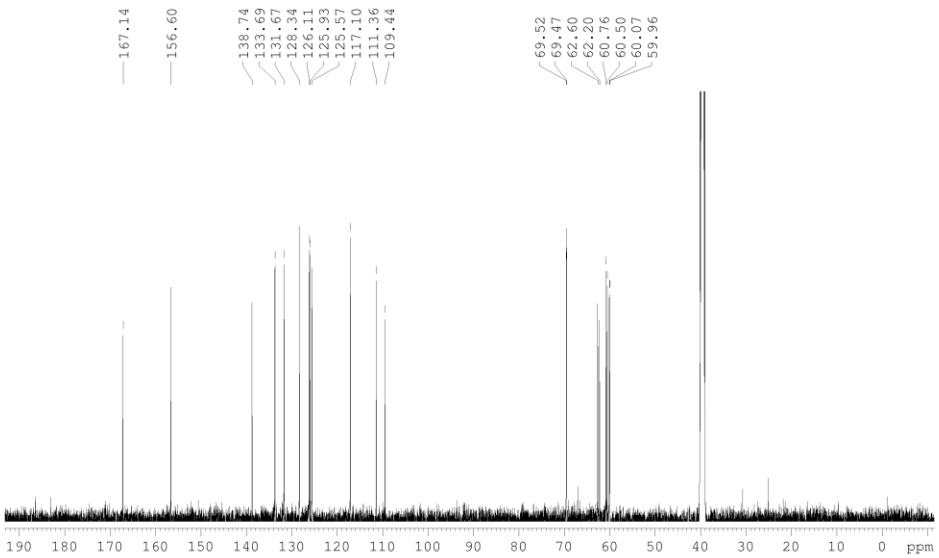
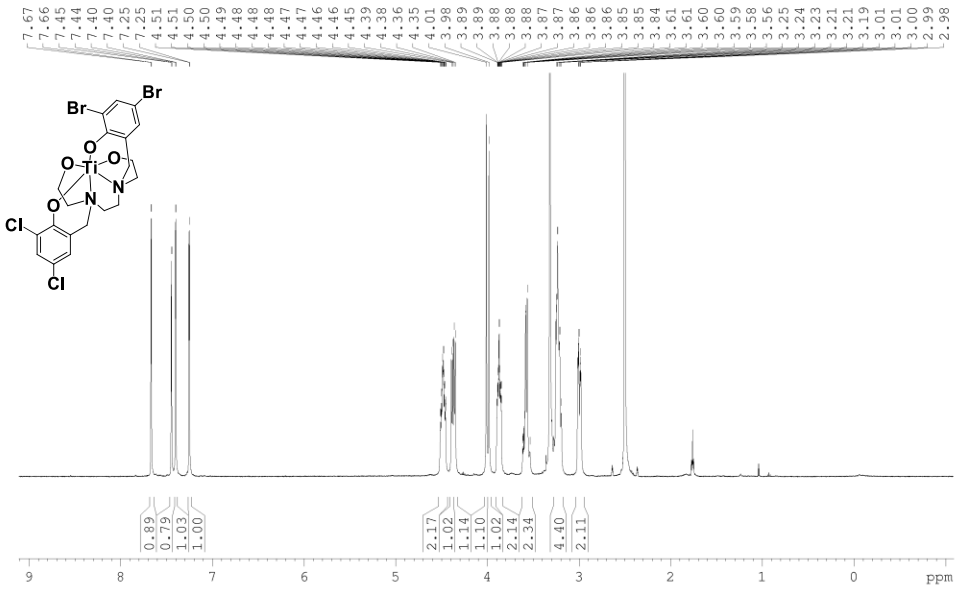


Figure S8: ^{1}H (500 MHz, top) and ^{13}C (125 MHz, bottom) NMR spectra of $L^{3,4}$Ti in DMSO-d_6



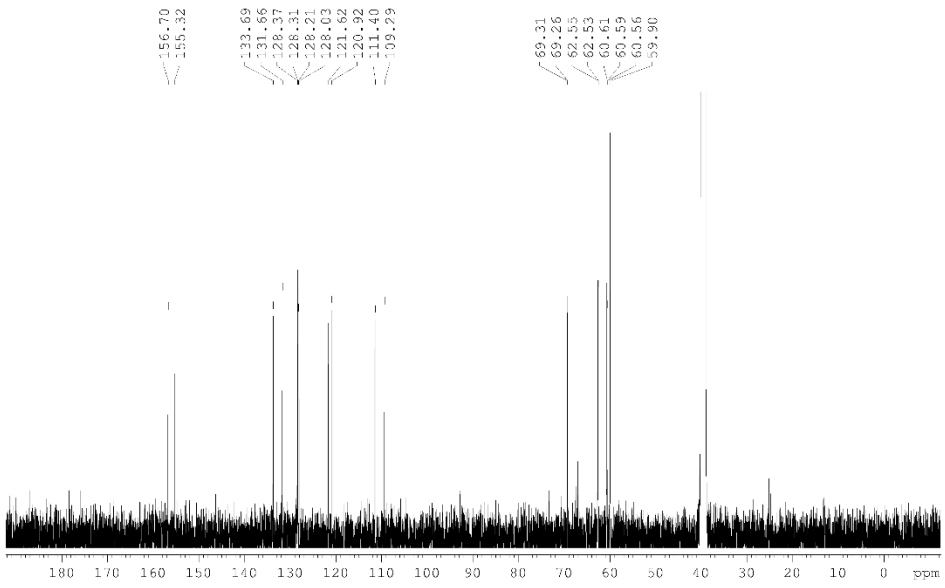
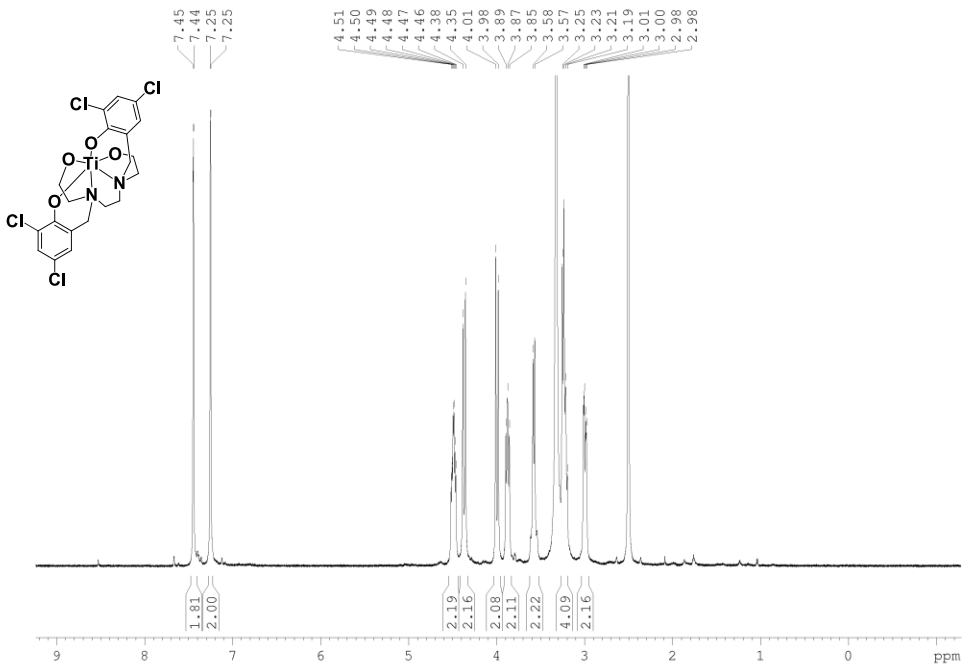


Figure S9: ^{1}H (500 MHz, top) and ^{13}C (125 MHz, bottom) NMR spectra of $L^{2,3}Ti$ in DMSO-d_6



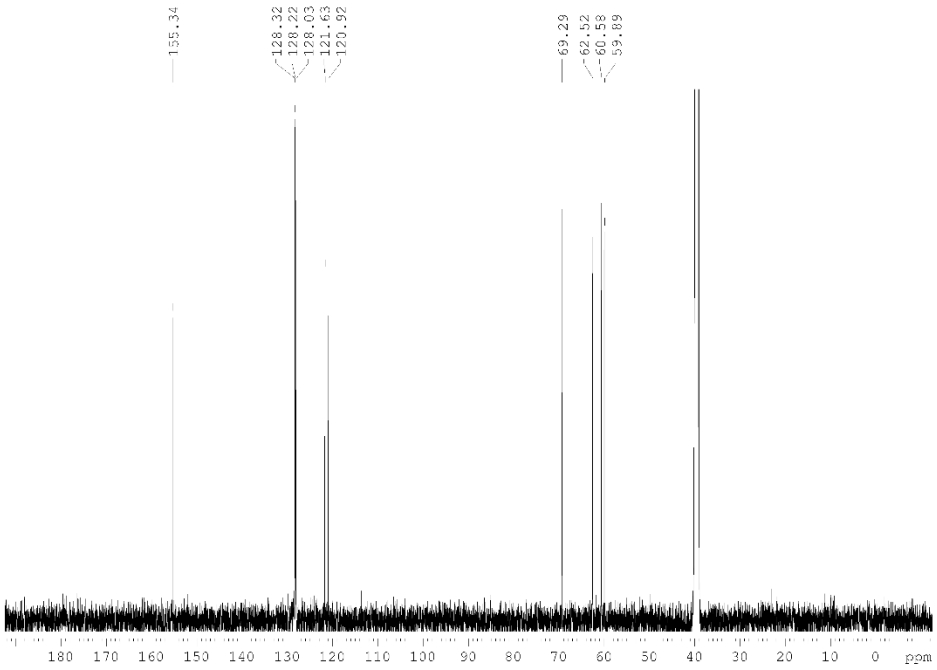


Figure S10: ^{1}H (500 MHz, top) and ^{13}C (125 MHz, bottom) NMR spectra of $L^{2,2}$Ti in DMSO-d_6

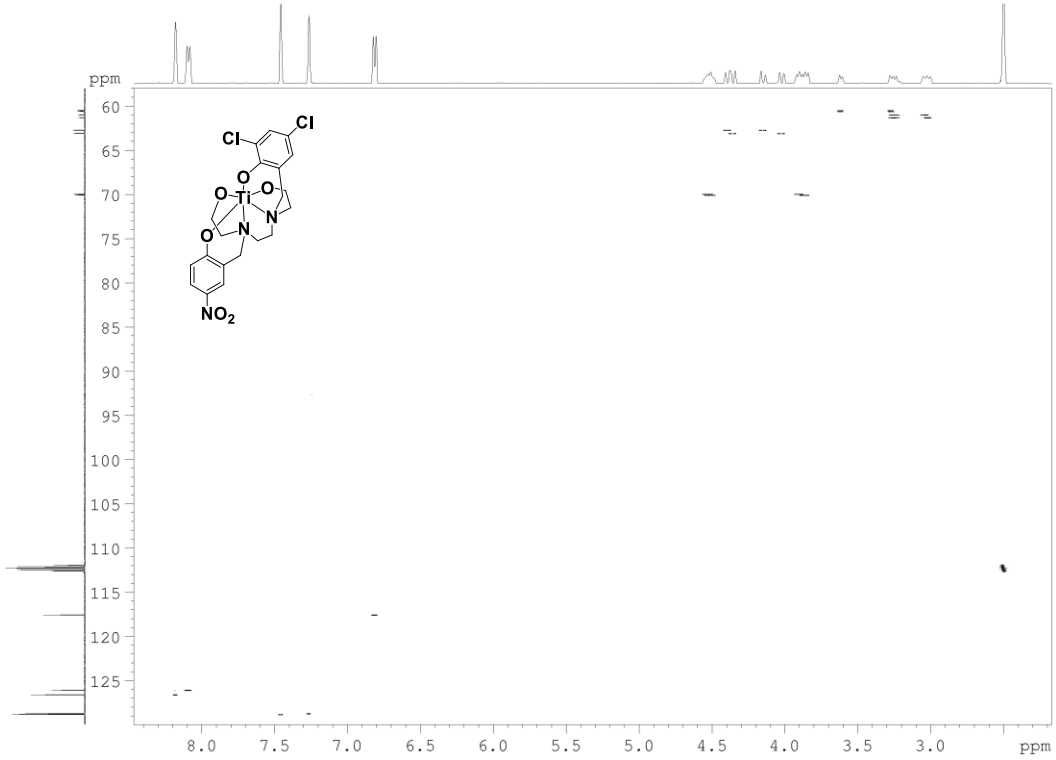


Figure S11: HSQC (500 MHz) NMR spectrum of $L^{2,4}Ti$ in DMSO-d_6

Hydrolysis of LTi

Table S1: Relative amount of hydrolysis products complex (%) of LTi after 3 days in D_2O:DMSO-d_6 (1:9) solution

Complex	Relative amount of hydrolysis products (%)[a]
$L^{1,4}Ti$	>50[b]
$L^{2,4}Ti$	22
$L^{3,4}Ti$	25
$L^{2,3}Ti$	19
$L^{2,2}Ti$	17

[a] relative to internal standard; [b] $t_{1/2} = 25\ h$

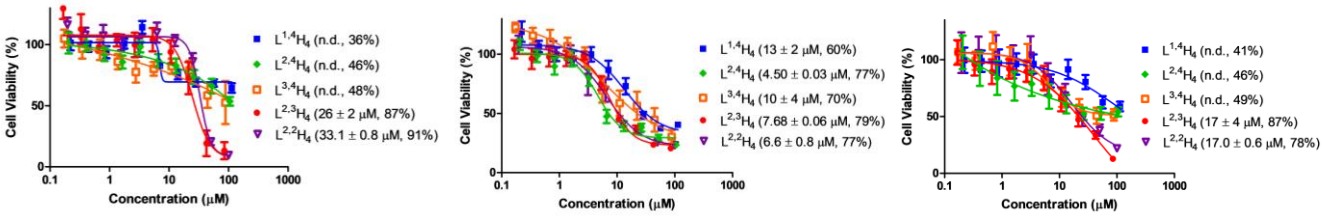


Figure S12: Dependence of HT-29 (left), A2780 (middle), and A2780cp (right) cell viability based on the MTT assay following a three day incubation period with administered concentration of LH_4; in parenthesis: IC_{50} values (μM), maximal cell growth inhibition (MI) (%).

References

1 H. Glasner and E. Y. Tshuva, *Inorg. Chem.*, 2014, **53**, 3170–3176.

2 S. Meker, O. Braitbard, M. D. Hall, J. Hochman and E. Y. Tshuva, *Chem. Eur. J.*, 2016, **22**, 9986–9995.

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