Supporting Information

{(1*R*,2*R*,4*R*)-4-Methyl-1,2-cyclohexanediamine}oxalatoplatinum(II) – a novel enantiomerically pure oxaliplatin derivative showing improved anticancer activity in vivo

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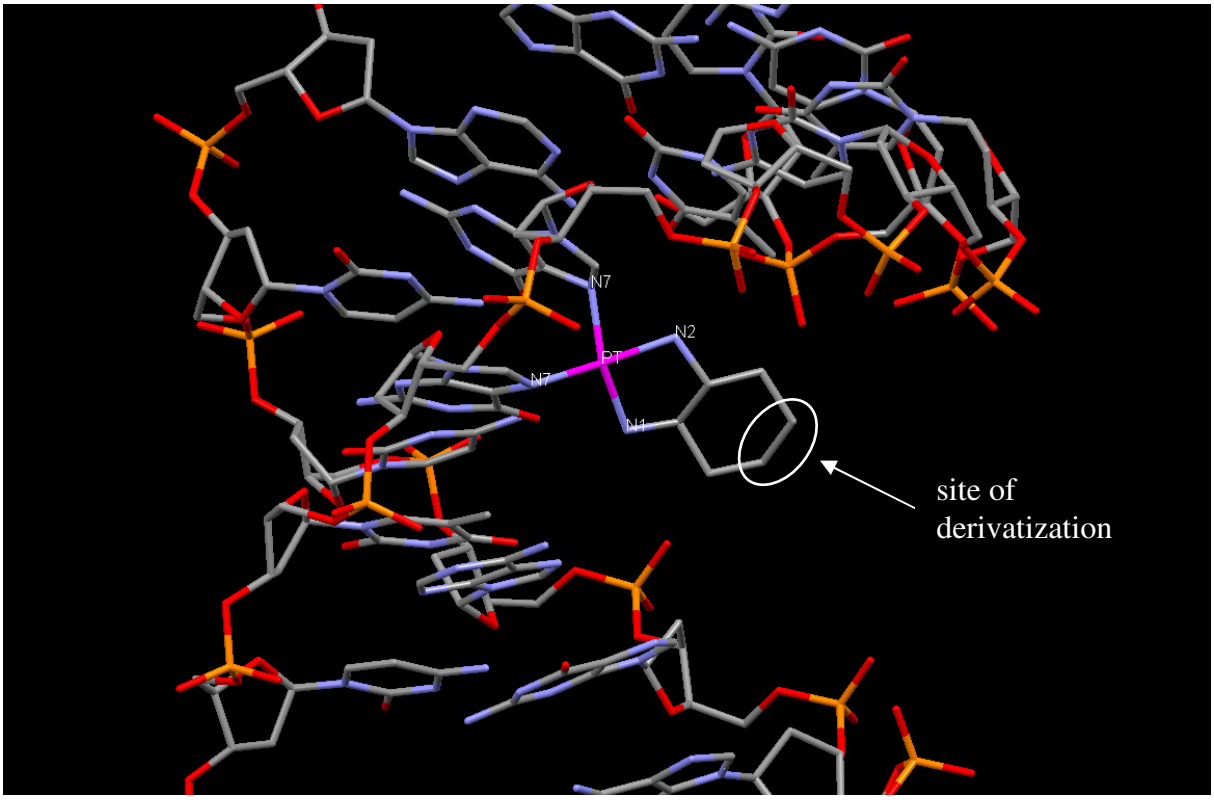
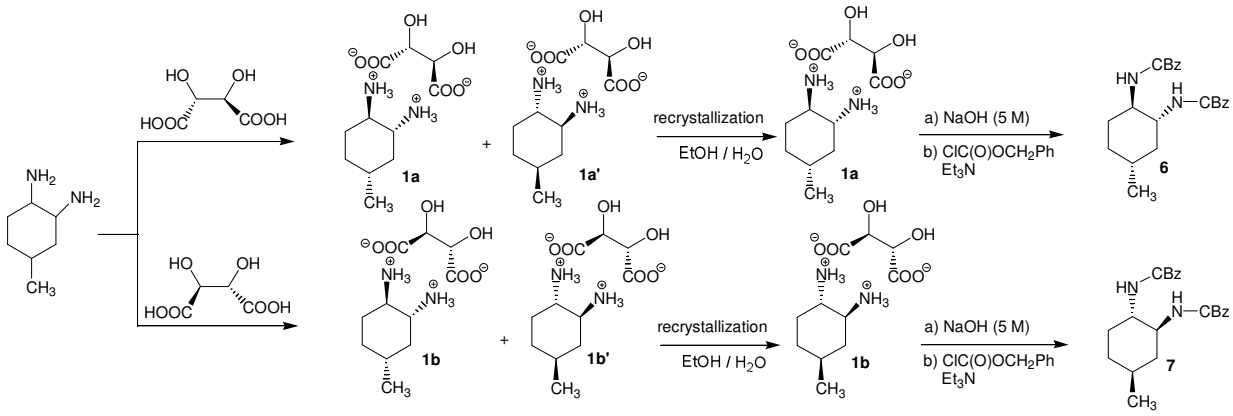


Figure S1. Crystal structure of an oxaliplatin 1,2-d(GpG) intrastrand cross-link in a DNA dodecamer duplex.
Protein Data Bank, PDB ID: 1IHH, Spingler, B., Whittington, D.A., Lippard, S.J. 2001, 2.4 Angstrom Crystal Structure of an Oxaliplatin 1,2-d(GpG) Intrastrand Cross-Link in a DNA Dodecamer Duplex, Inorg. Chem., 40, 5596.



Scheme S1. Separation of the racemic mixture of (1*R*,2*R*,4*R*)- and (1*S*,2*S*,4*S*)-4-methyl-1,2-cyclohexanediamine via recrystallization with the L- or D-tartrate and conversion of the diaminium tartrates into the UV-active CBz analogs used for chiral HPLC analysis.

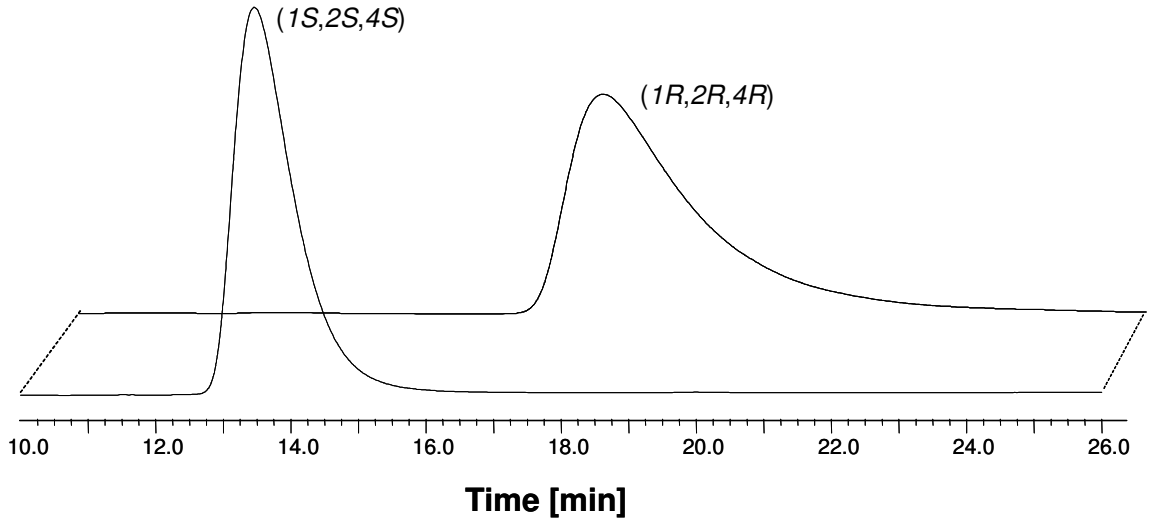
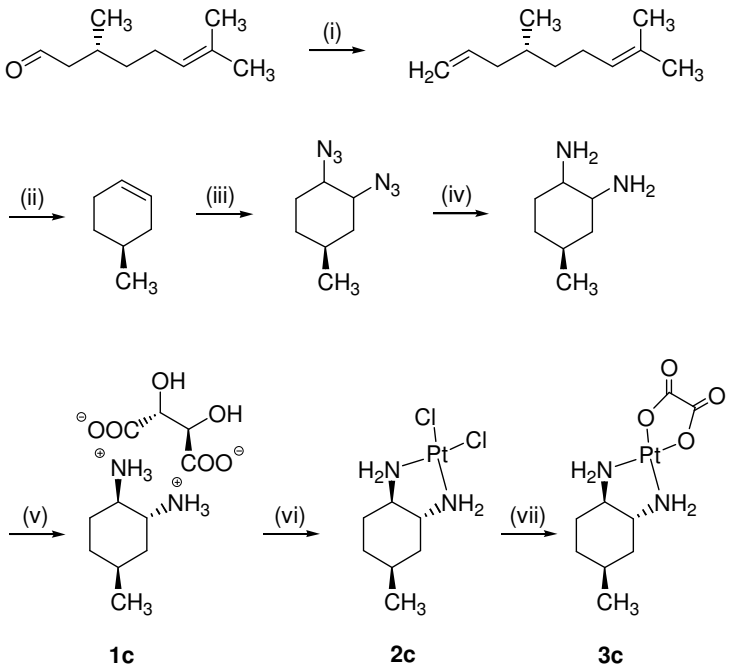


Figure S2. Confirmation of enantiomeric purity of (1*R*,2*R*,4*R*)- and (1*S*,2*S*,4*S*)-4-methyl-1,2-cyclohexanediamine after transformation into the CBz-derivatives by chiral HPLC.



Scheme S2. Synthesis of {(1*R*,2*R*,4*S*)-4-methyl-1,2-cyclohexanediamine}oxalato-platinum(II), **3c**. (i) $Me(PPh_3)_3Br$/BuLi, (ii) trans-$WOCl_2(OAr)_2$ (Ar = 2,6-dibromophenyl)/ tetraethyllead, (iii) $NaN_3/Mn(OAc)_3$, (iv) $Pd/CaCO_3$, H_2 (10 bar), (v) L-tartaric acid, (vi) K_2PtCl_4/NaOH and (vii) $AgNO_3$, basic ion exchanger, oxalic acid.

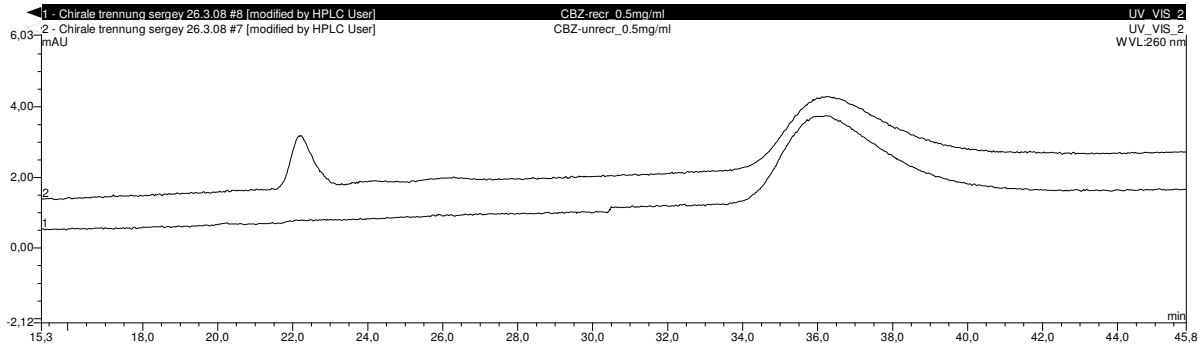


Figure S3. Confirmation of diastereomeric purity of (1*R*,2*R*,4*S*)-4-methyl-1,2-cyclohexanediamine after transformation into the CBz-derivatives by chiral HPLC (upper curve, mixture of (1*R*,2*R*,4*S*)- and (1*S*,2*S*,4*S*)-isomers; lower curve, pure (1*R*,2*R*,4*S*)-isomer.

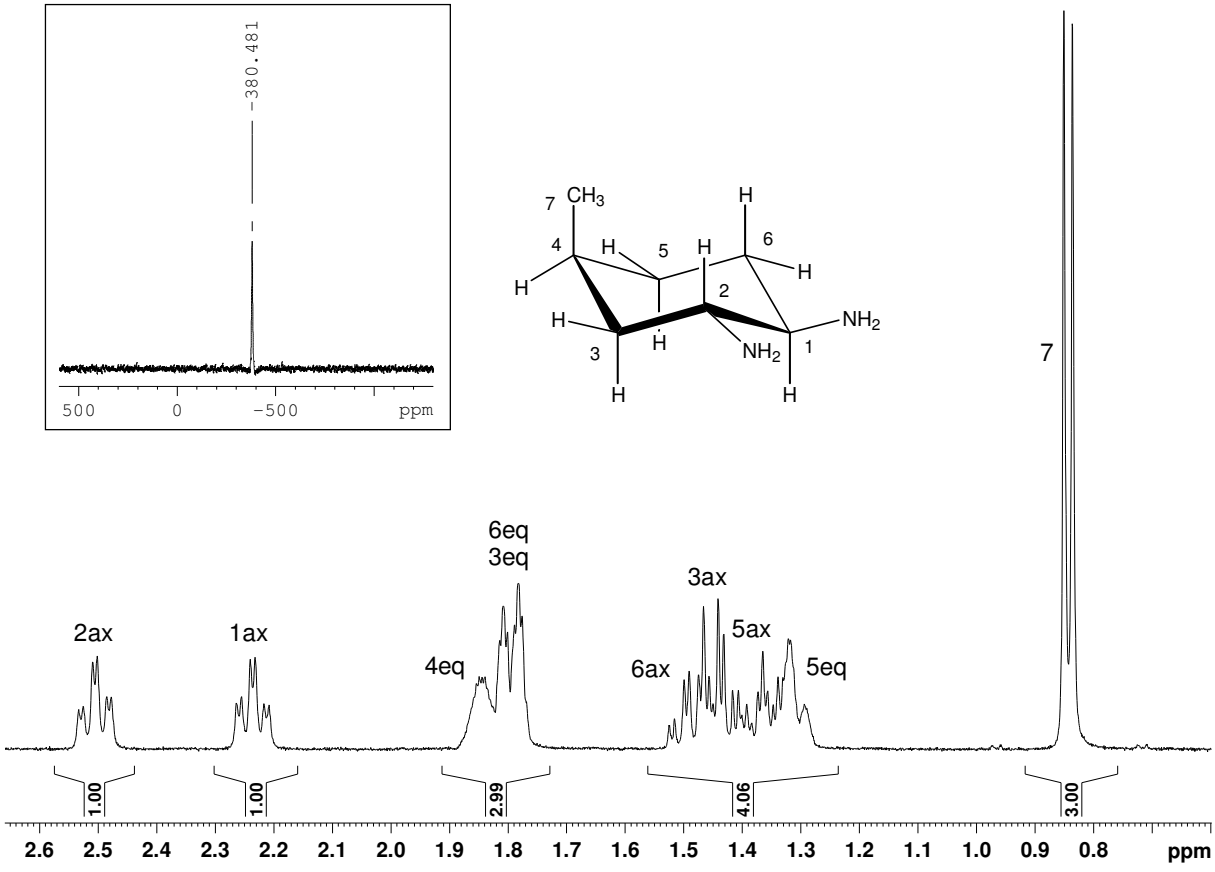


Figure S4. ^{1}H- and ^{195}Pt (small insert) NMR spectrum of {(1*R*,2*R*,4*S*)-4-methyl-1,2-cyclohexanediamine}oxalatoplatinum(II) **3c** with NMR numbering scheme and signal assignment, the oxalatoplatinum(II) fragment has been omitted for clarity.

Synthesis of (*S*)-4-Methylcyclohexene[1]

(*S*)-4,8-Dimethyl-l,7-nonadiene

A suspension of methyltriphenylphosphonium bromide (22.9 g, 64.1 mmol) in absolute tetrahydrofuran (400 mL) was filled into a three-necked flask under inert conditions and cooled to -20°C. Butyllithium (1.60 M in hexane; 38.0 mL, 60.8 mmol) was added via syringe. The orange mixture was stirred for 30 min. at room temperature and then cooled to -78°C. Thereafter, (*S*)-3,7-dimethyl-6-octenal ((*S*)-citronellal; 11.0 mL, 60.7 mmol) was added slowly, keeping the temperature low. The mixture was stirred for 1 h at -78°C and was then allowed to warm up slowly to room temperature. Acetone (10 mL) was added and the reaction mixture was stirred for 30 min to quench the excess of reagent. Eventually, the mixture was added to hexane (800 mL) and filtered. From the filtrate the solvent was removed with a rotary evaporator at reduced pressure, the solid formed was filtered off and washed with hexane. Again, hexane was removed using a rotary evaporator and the raw product was distilled under vacuum to afford the diene as a colorless liquid. Yield: 6.56 g (71%). ^{1}H NMR in $CDCl_3$: δ = 0.90 (d, ^{3}J=6.5 Hz, 3H), 1.12-1.21 (m, 1H), 1.33-1.41 (m, 1H), 1.48-1.57 (m, 1H), 1.62 (s, 3H), 1.70 (s, 3H), 1.87-2.13 (m, 4H), 4.97-5.04 (m, 2H), 5.12 (t, 1H), 5.74-5.86 (m, 1H).

***trans*-Dichlorobis(2,6-dibromphenoxy)oxotungsten(VI)**

A mixture of tungsten(VI) oxychloride (1.71 g, 5.00 mmol) and 2,6-dibromphenol (2.52 g, 10.0 mmol) in absolute toluene (25 mL) was heated at reflux for 1 h under argon. After the mixture was cooled down to room temperature, the solvent was removed under vacuum. The residue was stirred with absolute dichloromethane (100 mL) under argon for 10 min. The

[1] Nugent, W. A.; Feldman, J.; Calabrese, J. C. Practical Catalyst for Cyclic Metathesis. Synthesis of Functional and/or Enantiopure Cycloalkenes. *J. Am. Chem. Soc.* **1995**, *117*, 8992–8998.

solution was filtered inertly and filled into a sidearm round flask. Thereafter the solvent was removed in vacuum and the resulting amorphous, dark-red solid was stored under argon. The flask was directly used for the next reaction step.

(*S*)-4-Methylcyclohexene

trans-Dichlorobis(2,6-dibromphenoxy)oxotungsten(VI) was dissolved in dry trichlorobenzene (180 mL) under argon in a Schlenk flask, and (*S*)-4,8-dimethyl-l,7-nonadiene (31.5 g, 228 mmol), as well as tetraethyllead (50% solution in xylenes, 10g, 15 mmol) were added. The dark red mixture was heated up to 90°C, whereby a color change from deep-red to dark-blue, as well as a gas evolvement were observed. The mixture was kept at 90° for 2 h. After cooling down to room temperature, the solution was stirred shortly with silica (10 g) and filtered; a colorless liquid was obtained. Distillation at 97-120 °C produced a crude product containing about 10% of dissolved xylenes, which did not with subsequent transformations of the product. Yield: 19,5 g with about 10% xylenes (about 17,5 g of the product, 89 %). ^{1}H NMR in $CDCl_3$: δ = 0.97 (d, ^{3}J=6.5 Hz, 3H), 1.17-1.30 (m, 1H), 1.59-1.74 (m, 3H), 2.00-2.14 (m, 3H), 5.64-5.71 (m, 2H).

(*1R*,*2R*,*4R*)-4-Methyl-1,2-cyclohexanediaminium L-tartrate 1a

A mixture of 4-methyl-*trans*-(±)-1,2-cyclohexanediazide (2.1 g, 14.0 mmol) and palladium on $CaCO_3$ (Lindlar's catalyst, 5% Pd, 420 mg) in dry ethanol (30 mL) was stirred for 24 h under hydrogen atmosphere (3 bar). The catalyst was filtered off, the volume of the solvent was reduced to 2/3 and 4-methyl-*trans*-1,2-cyclohexanediaminium L-tartrate **1a/1a'** was precipitated by addition of L-tartaric acid until a pH of 7 was reached. The diaminium salt was filtered off, washed with diethyl ether (2 × 50 mL), and dried in vacuum. Yield 1.23 g (35%). A suspension of 4-methyl-1,2-*trans*-cyclohexanediaminium L-tartrate **1a/1a'** (3.0 g) in absolute ethanol (50 mL) was refluxed for 15 min, then water was added dropwise to obtain a clear solution. The solution was allowed to cool down to room temperature and was kept in the refrigerator for 12 h at 4 °C. The enantiomer **1a** was filtered off and dried in vacuum. Yield: 0.54 g (36%), m.p. 258–260 °C, $[\alpha]_{20}^{D}$ = +12 (c 0.5, acetone). Elemental analysis, found: C, 47.28; H, 8.04; N, 10.34. Calcd for $C_{11}H_{22}N_2O_6$: C, 47.47; H, 7.97; N, 10.07. MS (ESI^+) *m/z* 129 $[M+H]^+$. ^1H NMR in D_2O: δ = 0.85 (d, $^3J_{H,H}$ = 6.5 Hz, 3H, H-7), 0.99 (m, 1H, H-5), 1.15 (m, 1H, H-3), 1.41–1.58 (m, 2H, H-4, H-6), 1.71 (m, 1H, H-5`), 1.98–2.10 (m, 2H, H-6`, H-3`), 3.20–3.38 (m, 2H, H-1, H-2) 4.24 (s, 2H, CHOH). ^{13}C NMR in D_2O: δ = 20.7 (C-7), 29.5 (C-6), 30.2 (C-4), 31.5 (C-5), 37.6(C-3), 52.4 (C-1), 52.5 (C-2), 74.2 (CHOH), 178.9 (COO).

(*1S*,*2S*,*4S*)-4-Methyl-1,2-cyclohexanediaminium D-tartrate 1b

Following the same procedure as described for **1a**, compound **1b** was synthesized. Yield: 0.86 g (57%), m.p. 257–259 °C, $[\alpha]_{20}^{D}$ = -12 (c 0.5, acetone). Elemental analysis, found: C, 47.51; H, 7.79; N, 9.84. Calcd for $C_{11}H_{22}N_2O_6$: C, 47.47; H, 7.97; N, 10.07. MS (ESI^+) *m/z* 129 $[M+H]^+$. ^1H NMR in D_2O: δ = 0.85 (d, $^3J_{H,H}$ = 6.5 Hz, 3H, H-7), 0.98 (m, 1H, H-5), 1.14 (m, 1H, H-3), 1.43–1.53 (m, 2H, H-4, H-6), 1.71 (m, 1H, H-5`), 2.00–2.07 (m, 2H, H-6`, H-3`), 3.20–3.35 (m, 2H, H-1, H-2) 4.23 (s, 2H, CHOH). ^{13}C NMR in D_2O: δ = 20.7 (C-7),

29.6 (C-6), 30.3 (C-4), 31.5 (C-5), 37.7 (C-3), 52.4 (C-1), 52.5 (C-2), 74.2 (CHOH), 178.9 (COO).

(*1R,2R,4S*)-4-Methyl-1,2-cyclohexanediaminium L-tartrate 1c

(*S*)-4-Methylcyclohexene (10 g, 0.105 mol) and trifluoroacetic acid (120 mL) were added to a suspension of manganese acetate dihydrate (83.5 g, 0.31 mol) and sodium azide (34 g, 0.52 mol) in acetonitrile (1200 mL) under argon at -20°C. The reaction mixture was stirred for 3 h at -20°C and for 12 h at room temperature. Thereafter, $Na_2S_2O_5$ (10%, 300 mL) was added till the solution became clear. The intermediate diazide was extracted with petroleum ether (4 × 150 mL), washed with saturated Na_2CO_3 solution (2 × 100 mL) and brine (2 × 100 mL) and dried over Na_2SO_4. The solvent was removed in vacuum and the resulting oil was sissolved in 90 mL of ethanol. One third of the intermediate (30 mL of solution) was mixed with Lindlar catalyst (Pd on $CaCO_3$, 5% Pd, 1.6 g) and filled into an autoclave, which was then evacuated and pressurized with 10 bar of hydrogen. The suspension was stirred for 24 h at room temperature. The catalyst was filtered off and the product was precipitated by addition of a 20% stoichiometric excess of L-tartaric acid (6.3 g, 0.042 mol) in ethanol. The salt was filtered off, washed with diethyl ether (2 × 30 mL) and dried in vacuum. The product was recrystallyzed twice from a ethanol/water (1/1) mixture. The other two parts of the diazide intermediate were treated in the same way. Yield: 8.77 g (30%), elemental analysis, found: C, 47.49; H, 8.13; N, 10.34.Calcd for $C_{11}H_{22}N_2O_6$: C, 47.47; H, 7.97; N, 10.07. ^{1}H NMR in D_2O: δ = 0.90 (d, $^3J_{H,H}$ = 7.5 Hz, 3H, H-7), 1.36-1.46 (m, 1H, H-5), 1.58 (m, 1H, H-5`), 1.65-1.81 (m, 3H, H-3, H-6, H-3`), 1.84-2.10 (m, 2H, H-6`,H-4), 3.36 (m, 1H, H-1), 3.54 (m, 1H, H-2), 4.24 (s, 2H, CHOH). ^{13}C NMR in D_2O: δ = 17.5 (C-7), 23.7 (C-6), 25.4 (C-4), 27.4 (C-5), 33.5 (C-3), 48.3 (C-2), 50.9 (C-1), 73.8 (CHOH), 178.5 (COO).

(*SP*-4-3)-Dichlorido[(*1R*,*2R*,*4R*)-4-methyl-1,2-cyclohexanediamine-κ²*N*,*N*`]platinum(II) 2a

A solution of (*1R*,*2R*,*4R*)-4-methyl-1,2-cyclohexanediaminium L-tartrate (0.3 g, 1.1 mmol) in water (10 mL) was mixed with a solution of K_2PtCl_4 (0.45 g, 1.1 mmol) in water (15 mL). The pH of the reaction mixture was kept at ca. 7 over 24 h by addition of NaOH solution (0.25 M). The yellow product was filtered off, washed with HCl (2M, 2 × 5 mL) and water (3 ×10 mL), and dried in vacuum over P_2O_5. Yield: 0.33 g (78%), m.p. > 400 °C (decomp.). Elemental analysis, found: C, 21.41; H, 3.93; N, 6.89. Calcd for $C_7H_{16}N_2PtCl_2$: C, 21.33; H, 4.09; N, 7.11. MS (ESI⁺) *m/z* 417 $[M+Na]^+$, $[\alpha]_{20}^{D}$ = +100 (c = 0.5, DMSO). ^{1}H NMR in DMF-d_7: δ = 0.84-0.97 (m, 1H, H-5ax; d, $^3J_{H,H}$ = 6.5 Hz, 3H, H-7), 1.22 (q, $^3J_{H,H}$ = $^2J_{H,H}$ = 12 Hz, 1H, H-3ax), 1.41 (m, 1H, H-4ax), 1.48-1.60 (m+m, 2H, H-6ax, H-5eq), 1.99-2.11 (m+m, 2H, H-3eq, H-6eq), 2.46-2.66 (m+m, 2H, H-1ax, H-2ax), 5.01 (m, 2H, N^1-H/N^2-H), 5.58 (m, 2H, N^1-H/N^2-H). ^{13}C NMR in DMF-d_7: δ =20.6 (C-7), 31.0 (C-6), 31.5 (C-4), 33.0 (C-5), 40.2 (C-3), 63.2 (C-2), 63.4 (C-1). ^{15}N NMR in DMF-d_7: δ = –21.3. ^{195}Pt NMR in DMF-d_7: δ = –644.

(*SP*-4-3)-Dichlorido[(*1S*,*2S*,*4S*)-4-methyl-1,2-cyclohexanediamine-κ²*N*,*N*`]platinum(II) 2b

Following the same procedure as described for **1b**, complex **2b** was obtained from (*1S*,*2S*,*4S*)-4-methyl-1,2-cyclohexanediaminium D-tartrate (0.3 g, 1.1 mmol). Yield: 0.35 g (83%), m.p. > 400 °C (decomp.). Elemental analysis, found: C, 21.23; H, 3.87; N, 6.89. Calcd for $C_7H_{16}N_2PtCl_2$: C, 21.33; H, 4.09; N, 7.11. MS (ESI⁺) *m/z* 417 $[M+Na]^+$, $[\alpha]_{20}^{D}$ = -100 (c = 0.5, DMSO). ^{1}H NMR in DMF-d_7: δ = 0.84-0.97 (m, 1H, H-5ax; d, $^3J_{H,H}$ = 6.5 Hz, 3H, H-7), 1.22 (q, $^3J_{H,H}$ = $^2J_{H,H}$ = 12 Hz, 1H, H-3ax), 1.41 (m, 1H, H-4ax), 1.48-1.60 (m+m, 2H, H-6ax, H-5eq), 1.99-2.11 (m+m, 2H, H-3eq, H-6eq), 2.46-2.66 (m+m, 2H, H-1ax, H-2ax), 5.00 (m, 2H, N^1-H/N^2-H), 5.58 (m, 2H, N^1-H/N^2-H). ^{13}C NMR in DMF-d_7: δ =20.6 (C-7), 31.0 (C-6),

31.5 (C-4), 33.0 (C-5), 40.2 (C-3), 63.2 (C-2), 63.4 (C-1). ^{15}N NMR in DMF-d_7: δ = –21.4. ^{195}Pt NMR in DMF-d_7: δ = –644.

(*SP*-4-3)-Dichlorido[(*1R,2R,4S*)-4-methyl-1,2-cyclohexanediamine-κ²*N*,*N*`]platinum(II) 2c

Potassium tetrachloroplatinate (8.255 g, 0.02 mol) was dissolved in distilled water (50 mL) and filtered through a glass fiber filter. A solution of (*4S*)-methyl-(*1R,2R*)-cyclohexane-diaminium L-tartrate (5.566 g, 0.02 mol) in 50 mL of distilled water was added. The mixture was stirred for 24 h, whereby the pH of the reaction mixture was kept at 7.0 via addition of 0.25M NaOH. After the supernatant had become almost colorless and the pH was rising rapidly upon further addition of the base, the yellow precipitate was filtered off via a G4 glass sinter filter, washed twice with 2M HCl, then three times with distilled water and dried in vacuum over P_2O_5. Yield: 7.33 g (93%), elemental analysis, found: C, 21.17; H, 3.85; N, 7,15. Calcd for $C_7H_{16}N_2PtCl_2$: C, 21.33; H, 4.09; N, 7.11. ^{1}H NMR in DMF-d_7: δ = 0.96 (d, $^3J_{H,H}$ = 7.5 Hz, 3H, H-7), 1.37 (m, 1H, H-5eq), 1.45 (m, 1H, H-5ax), 1.69 (m, 1H, H-3ax), 1.76 (m, 1H, H-6ax), 1.85-1.97 (m+m+m, 3H, H-3eq, H-4eq, H-6eq), 2.48 (m, 1H, H-1ax), 2.74 (m, 1H, H-2ax), 4.97 (m, 2H, N^1-H/N^2-H), 5.47 (m, 1H, N^2-H), 5.60 (m, 1H, N^1-H). ^{13}C NMR in DMF-d_7: δ =17.2 (C-7), 26.7 (C-6), 27.6 (C-4), 30.0 (C-5), 37.5 (C-3), 58.7 (C-2), 63.9 (C-1). ^{15}N NMR in DMF-d_7: δ = –20.8 (N^2), –19.9 (N^1). ^{195}Pt NMR in DMF-d_7: δ = –657.

(*1R*,*2R*,*4R*)-*N*,*N*´-Bis(benzyloxycarbonyl)-4-methyl-1,2-cyclohexanedicarbamate 6

(*1R*,*2R*,*4R*)-4-Methyl-1,2-cyclohexanediamine was recovered from the respective diaminium tartrate **1a** (0.5 g) by reaction with a NaOH solution (5 M, 25 mL) and extraction with dichloromethane (3 × 20 mL). Triethylamine (0.68 mL, 4.87 mmol) was added to the combined CH_2Cl_2 fractions and the reaction mixture was treated with benzyloxycarbonylchloride (0.57 mL, 4.05 mmol) under vigorous stirring at 0–5 °C. Then it was allowed to warm up to room temperature and stirred for another 3 h (TLC control, hexane : ethyl acetate = 3 : 1). The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with brine (2 × 15 mL), and dried over Na_2SO_4. The solvent was removed to give a slightly yellow crude product which was purified by recrystallization from MeOH. Yield: 0.2 g (30%), m.p. 178–179 °C, $[\alpha]_{20}^{D}$ = -24 (c = 0.5, acetone). Elemental analysis, found: C, 69.45; H, 7.18; N, 7.36. Calcd for $C_{23}H_{28}N_2O_4$: C, 69.66; H, 7.12; N, 7.07. MS (ESI^+) *m/z* 419 $[M+Na]^+$. ^1H NMR in $CDCl_3$: δ = 0.94 (d, $^3J_{H,H}$ = 6.5 Hz, 3H, H-7), 1.08–0.93 (m, 2H, H-3, H-5), 1.32 (m, 1H, H-6), 1.54 (m, 1H, H-4), 1.72 (m, 1H, H-5´), 2.09–2.05 (m, 2H, H-3´, H-6`), 3.46-3.36 (m, 2H, H-2, H-1), 5.12–5.04 (m, 6H, OCH_2, NH), 7.34 (s, 10H, H-ar). ^{13}C NMR in $CDCl_3$: δ = 22.0 (C-7), 31.9 (C-4), 32.6 (C-5), 33.7 (C-6), 41.6 (C-3), 55.5 (C-2), 55.7 (C-1), 67.0 (OCH_2), 128.3 (C-ar), 128.4 (C-ar), 128.9 (C-ar), 137.0 (Cq-ar), 157.2 (C=O).

(*1S*,*2S*,*4S*)-*N*,*N*´-Bis(benzyloxycarbonyl)-4-methyl-1,2-cyclohexanedicarbamate 7

Following the same procedure as described for **6**, compound **7** was obtained from **1b** (0.1 g,). Yield: 0.76 mg (53%), m.p. 179–180 °C, $[\alpha]_{20}^{D}$ = +24 (c 0.5, acetone). Elemental analysis, found: C, 69.46; H, 7.26; N, 7.08. Calcd for $C_{23}H_{28}N_2O_4$: C, 69.66; H, 7.12; N, 7.07. MS (ESI^+) *m/z* 419 $[M+Na]^+$. ^1H NMR in $CDCl_3$: δ = 0.94 (d, $^3J_{H,H}$ = 6.5 Hz, 3H, H-7), 1.08–0.93 (m, 2H, H-3, H-5), 1.32 (m, 1H, H-6), 1.54 (m, 1H, H-4), 1.72 (m, 1H, H-5´), 2.09–2.05 (m, 2H, H-3´, H-6`), 3.46-3.36 (m, 2H, H-2, H-1), 5.12–5.04 (m, 6H, OCH_2, NH), 7.34 (s,

10H, H-ar). ^{13}C NMR in $CDCl_3$: δ = 22.1 (C-7), 31.9 (C-4), 32.6 (C-5), 33.6 (C-6), 41.5 (C-3), 55.5 (C-2), 55.7 (C-1), 67.0 (OCH_2), 128.3 (C-ar), 128.4 (C-ar), 128.9 (C-ar), 137.0 (Cq-ar), 157.2 (C=O).