Electronic Supplementary Material (ESI) for Dalton Transactions.

Formylation of a metathesis-derived *ansa*[4]-ferrocene: a simple route to anticancer organometallics

Maria Mazur, Michał Mrozowicz, Włodzimierz Buchowicz,* Mariola Koszytkowska-Stawińska, Radosław Kamiński, Zbigniew Ochal, Patrycja Wińska, Maria Bretner

SUPPORTING INFORMATION

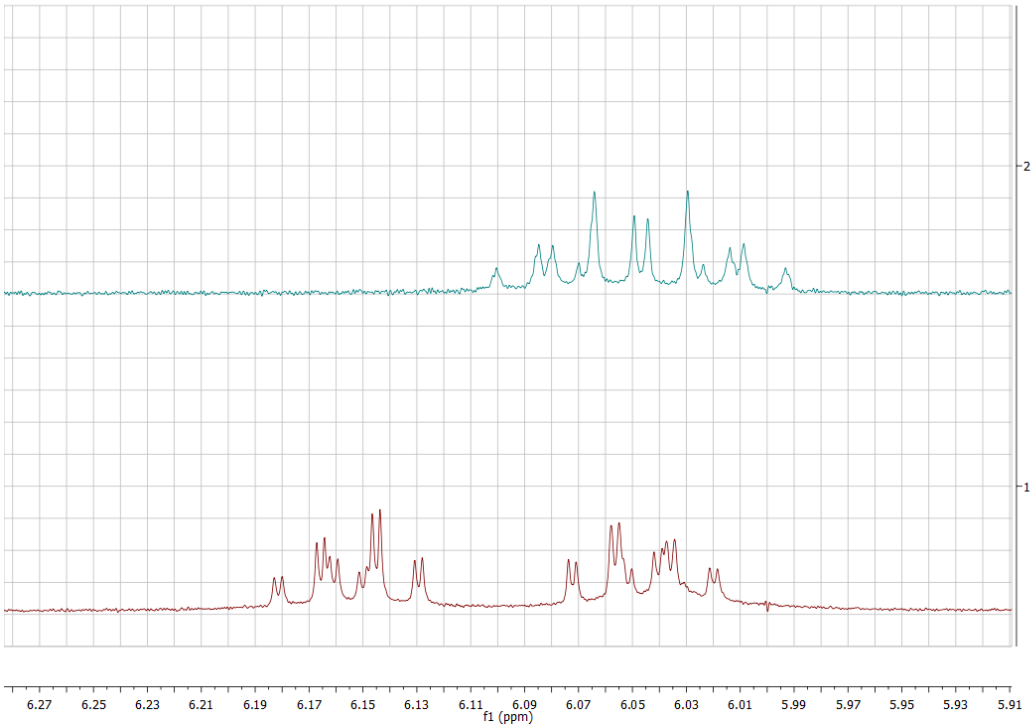


Figure S1. The olefinic range of the ^{1}H NMR spectra of aldehydes **(±)-3** (top) and **(±)-4** (bottom).

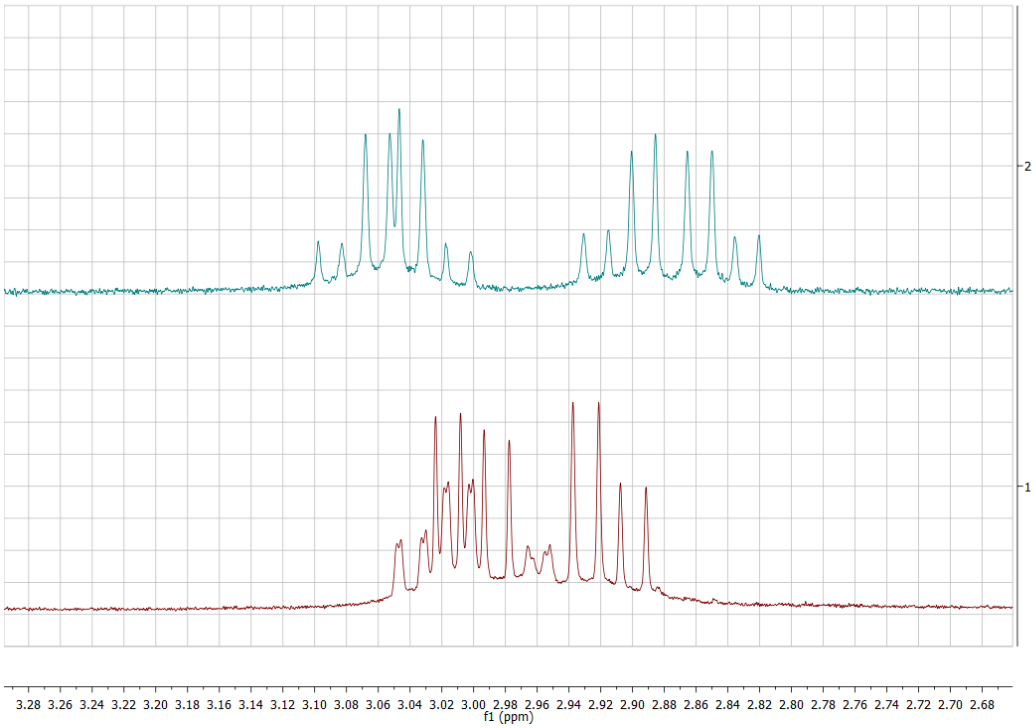


Figure S2. The allylic range of the ^{1}H NMR spectra of aldehydes **(±)-3** (top) and **(±)-4** (bottom).

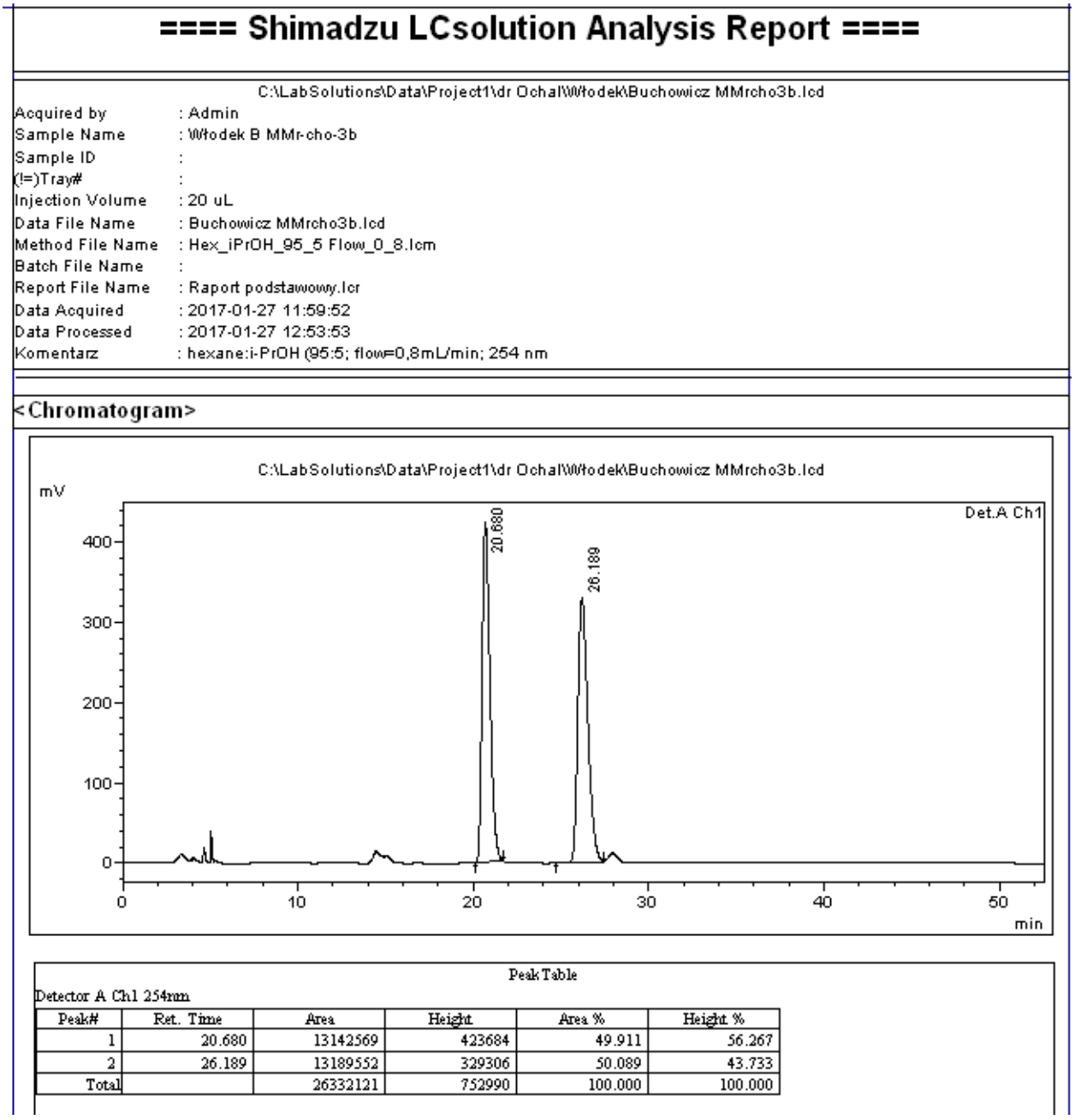


Figure S3. The chiral HPLC trace of compound **(±)-3**. Conditions: Chiralcel OD-H chiral column (4.6 mm × 250 mm, from Daicel Chemical Ind., Ltd.), equipped with a precolumn (4 mm × 10 mm, 5 μm) [n-hexane/2-PrOH (95:5, v/v); f = 0.8 mL/min; λ = 254 nm, 30°C].

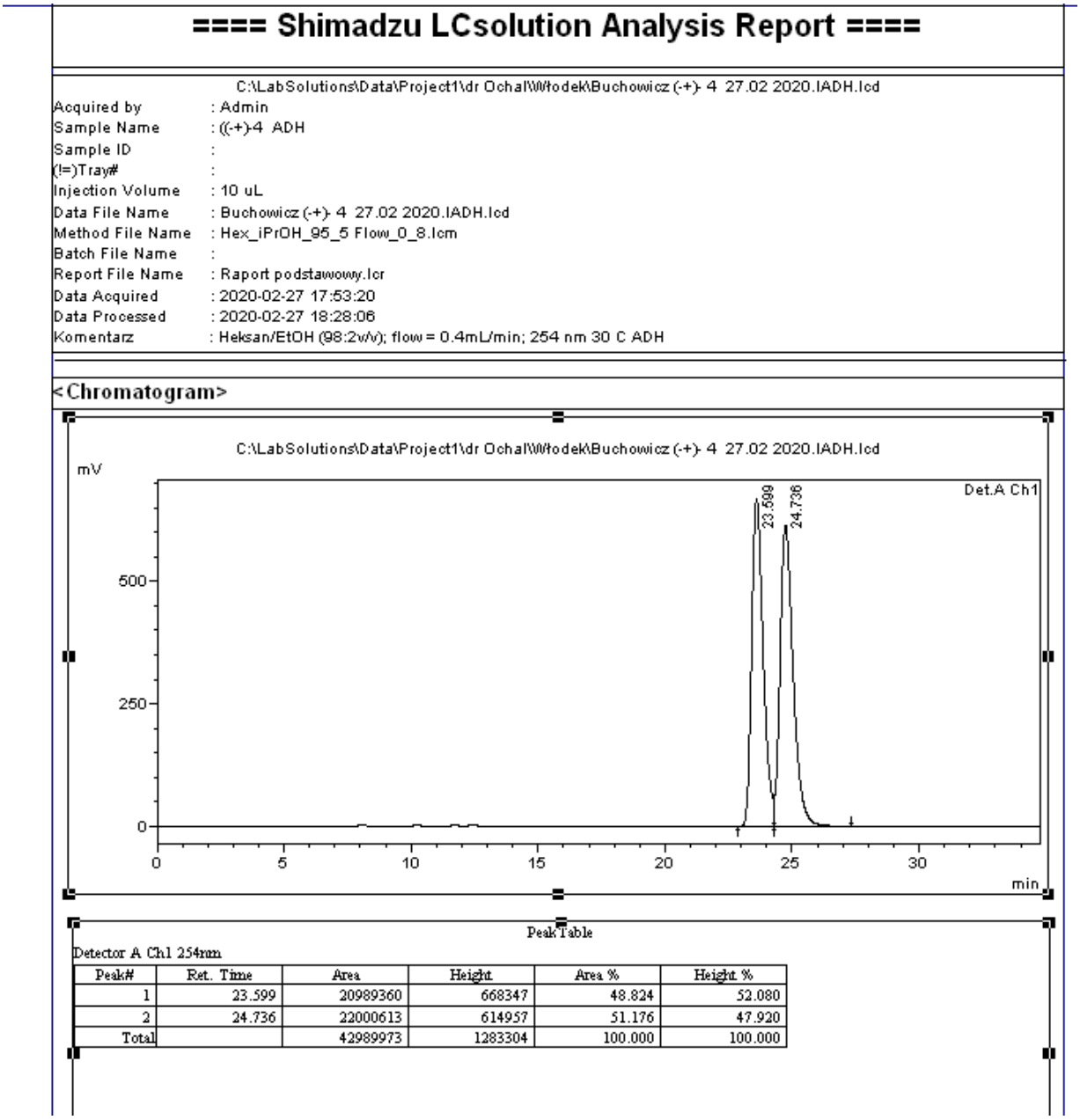


Figure S4. The chiral HPLC trace of compound **(±)-4**. Conditions: Chiralcel AD-H chiral column (4.6 mm × 250 mm, from Daicel Chemical Ind., Ltd.), equipped with a precolumn (4 mm × 10 mm, 5 μm) [n-hexane/EtOH (98:2, v/v); f = 0.4 mL/min; λ = 254 nm, 30°C].

Resolution of **(±)-3** into enantiomers

Under an inert atmosphere, a catalytic amount of *p*-toluenesulfonic acid monohydrate (4.0 mg, 0.021 mmol) was added to a solution of **(±)-3** (55.0 mg, 0.24 mmol) in trimethyl orthoformate (3.50 mL). The resulting solution was stirred overnight at 80-90 °C. Then anhydrous potassium carbonate was added with stirring while the solution was cooled to room temperature. Diethyl ether (10 mL) was added and the mixture was filtered through Celite and concentrated *in vacuo.* The crude dimethylacetal **(±)-5** was used directly in the next step without further purification.

In an another Schlenk flask, molecular sieves 4 Å were added to an emulsion of (S)-(-)-1,2,4-butanetriol (0.10 mL, 0.119 g, 1.12 mmol) in chloroform (2.0 mL), then *p*-toluenesulfonic acid monohydrate (4.0 mg, 0.021 mmol) was added. The crude dimethylacetal **(±)-5** was dissolved in chloroform (1.0 mL) and added to the flask. The resulting mixture was stirred for 2 h at 60 °C. Anhydrous potassium carbonate was added followed by diethyl ether (10 mL). The mixture was filtered and concentrated *in vacuo* to yield brown oil, which was purified by column chromatography on SiO_2 (hexane:ethyl acetate 2.5:1). A yellow band was collected that afforded after the solvents' removal acetals **6** (45.0 mg, 0.12 mmol, 50% yield, 1:1 mixture of diastereoisomers) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.01 (m, 2H, CH=), 5.332 (1H, O-CH-O), 5.326 (1H, O-CH-O), 4.28 (m, 1H, C_5H_3), 4.17 (m, 2H, C_5H_4) 4.14 (m, 1H, C_5H_3), 4.10 (m, 1H, C_5H_3), 4.03 (m, 2H, C_5H_4), 3.87 (m, CH-O), 3.64 (m, 2H CH_2-OH), 2.93 (m, 2H, CH_2CH=), 2.36 (m, 1H, CH_2CH=), 2.00 (m, 1H, CH_2CH=), 1.83 (m, 1H, CH_2), 1.39 (m, 1H, CH_2) ppm. MS (EI, 70 eV) *m/z* (rel int, %) (^{56}Fe): 354 (M^+, 100), 282 (14), 266 (32), 238 (32), 121 (14). HRMS (EI): *m/z* Found for 354.0920 (Calc for $C_{19}H_{22}{}^{56}FeO_3$ 354.0918). Anal. Found: C, 65.68; H, 6.68. Calc. for $C_{19}H_{22}FeO_3 \times 0.2C_6H_{14}$: C, 65.35; H, 6.68%.

Acetal **6** (1:1 mixture of diastereoisomers) was dissolved in hexanes and placed in a fridge at +4 °C for 1 day. Yellow crystals were formed that were collected, dried under vacuum and analyzed by

^{1}H NMR. The ratio of the two diastereoisomers was established by integration of the diagnostic O-CH-O signals at δ 5.332 and 5.326 ppm. The isolated crystals were re-dissolved in hexanes. After two crystallizations ratio of two diastereoisomers was 1:3 but the repetition of this operation did not significantly increase this ratio. Maximum enantiomeric excess obtained by this method (after hydrolysis) was 55% (Figure S5).

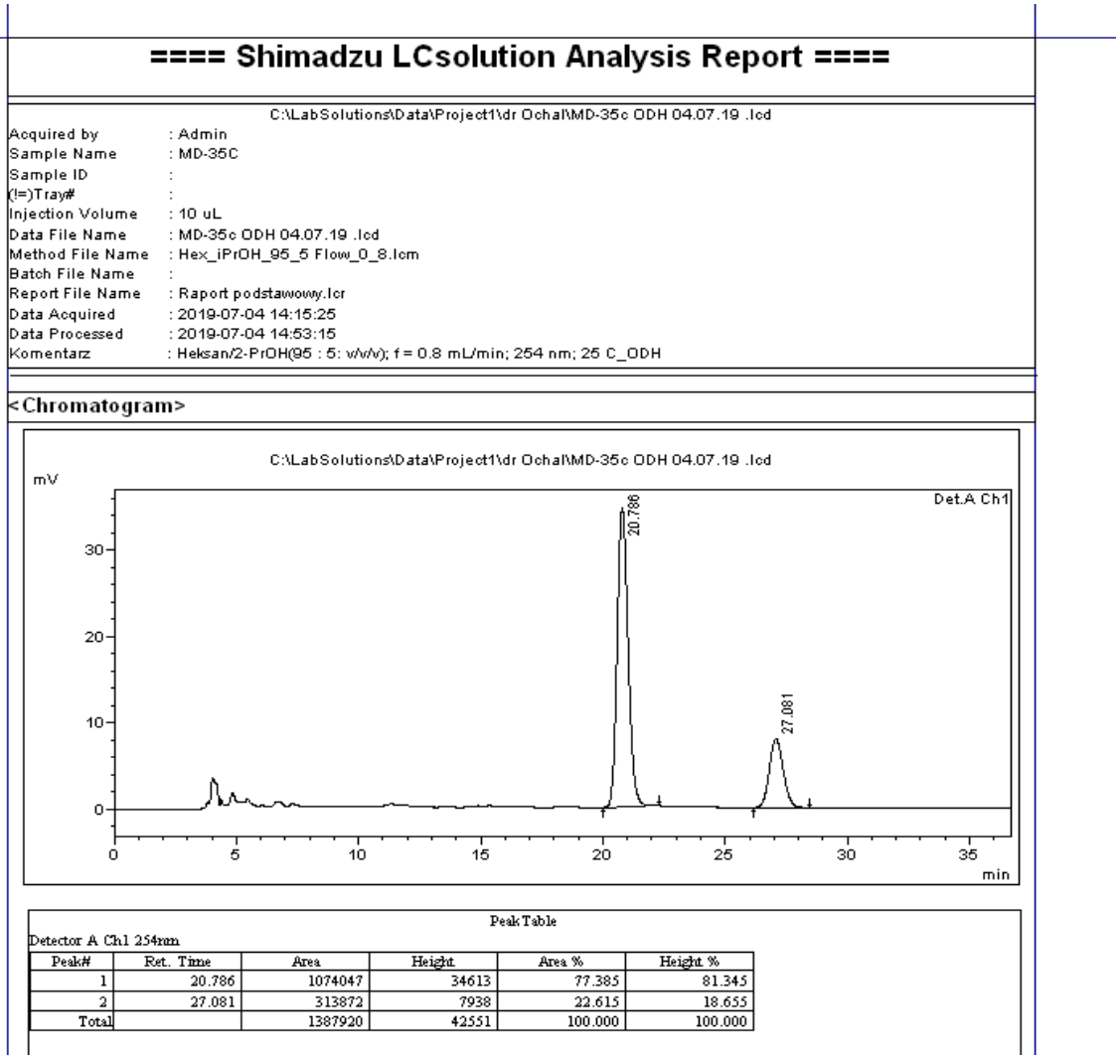


Figure S5. The chiral HPLC trace of enantiomerically enriched compound **(-)-3** (55% ee).

Another sample of acetal **6** (1:1 mixture of diastereoisomers) was crystalized from hexanes/2-propanol mixture (10:1, +4 °C, 3 days) giving the second diastereoisomer of **6** with 80% excess

(determined by ^{1}H NMR). This sample of **6** (20 mg, 0.05 mmol) was hydrolyzed by heating with *p*-toluenesulfonic acid for 1h in dichloromethane to yield **(+)-3** (8.0 mg, 0.03 mmol, 60%): $[\alpha]_D^{20}$ = 167.5 (c = 0.08 in $CHCl_3$), 76% ee (Figure S6). The configuration of the major enantiomer in the enriched batch was determined via crystallization (from hexanes at +4 °C) and crystals structure determination. All randomly chosen crystals displayed the (S_p) configuration (note that the space group is chiral).

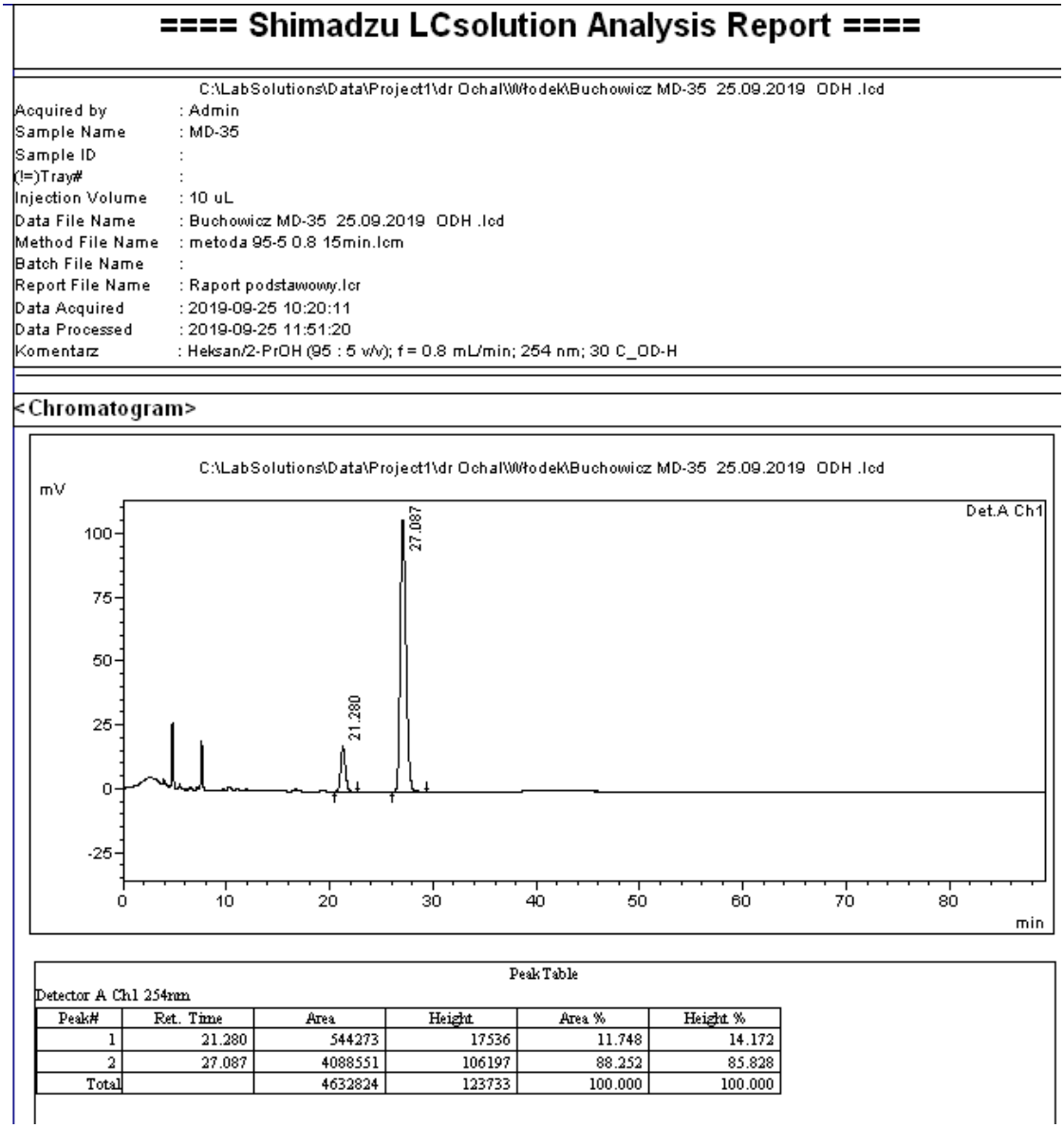
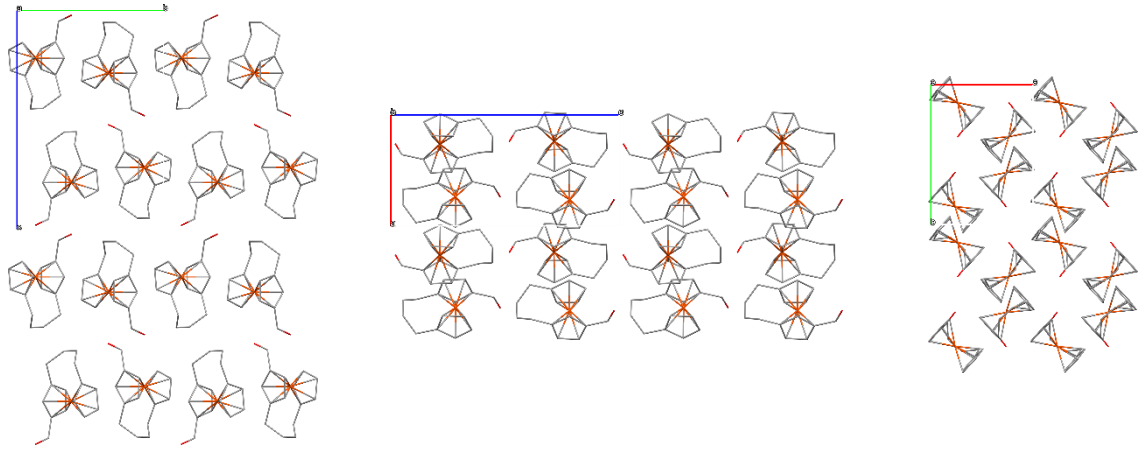


Figure S6. The chiral HPLC trace of enantiomerically enriched compound **(+)-3** (76% ee).

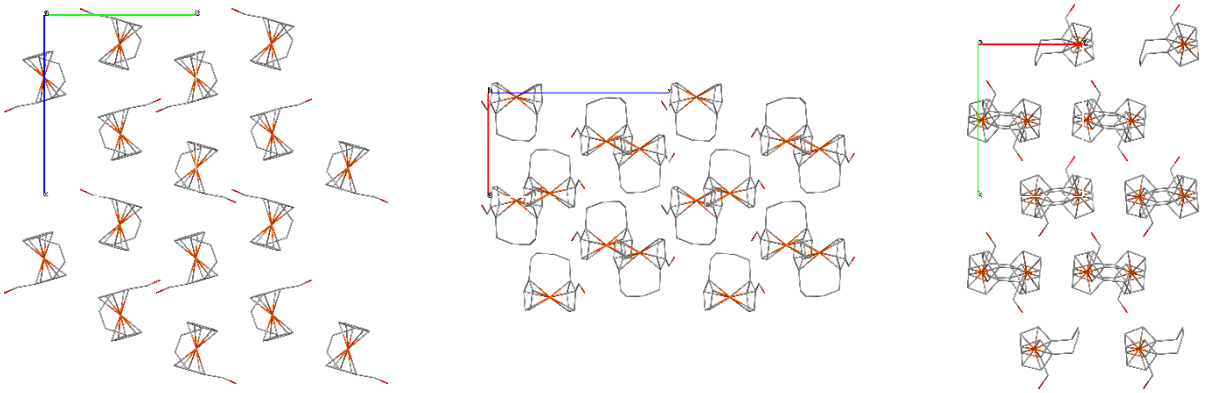
Table S1. Selected X-ray data collection, processing and refinement parameters for all presented crystal structures.

Structure	**(R_p)-3**	**(R_p)-4**
Moiety formula	$C_{15}H_{14}FeO$	$C_{15}H_{14}FeO$
Moiety formula mass, M_r / a.u.	266.1	266.1
Crystal system	orthorhombic	orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
a / Å	7.4133(5)	7.6075(4)
b / Å	10.1403(8)	11.1031(7)
c / Å	15.2345(8)	13.2087(6)
α / °	90	90
β / °	90	90
γ / °	90	90
V / Å^3	1145.22(13)	1115.70(10)
Z	4	4
T / K	100	100
F_{000}	552	552
d_{calc} / g·cm^{-3}	1.544	1.584
θ range	5.24° − 75.94°	5.20° − 76.48°
Absorption coefficient, μ / mm^{-1}	10.360	10.621
Crystal color & shape	orange, cut crystal	orange, cut crystal
Crystal size / mm^3	0.03×0.15×0.16	0.10×0.12×0.17
No. of reflections collected / unique	3160 / 1251	9171 / 2287
R_{int}	8.71%	7.04%
No. of reflections with $I > 3\sigma(I)$	992	1988
No. of parameters / restraints / constraints	142 / 0 / 68	154 / 0 / 56
Flack parameter	0.13(2)	0.039(7)
$R[F]$ ($I > 3\sigma(I)$)	5.70%	4.33%
$R[F]$ (all data)	7.08%	4.72%
$\varrho_{res}^{min/max}$ / e·Å^{-3}	−0.38 / +0.75	−0.40 / +1.01
CCDC code	1935549	1935550



(*a*) (*b*) (*c*)

Figure S7. Packing of molecules of compound **3**; views along *X* (*a*), *Y* (*b*) and *Z* (*c*) axes.



(*a*) (*b*) (*c*)

Figure S8. Packing of molecules of compound **4**; views along *X* (*a*), *Y* (*b*) and *Z* (*c*) axes.

Biological evaluation

MTT-based assay

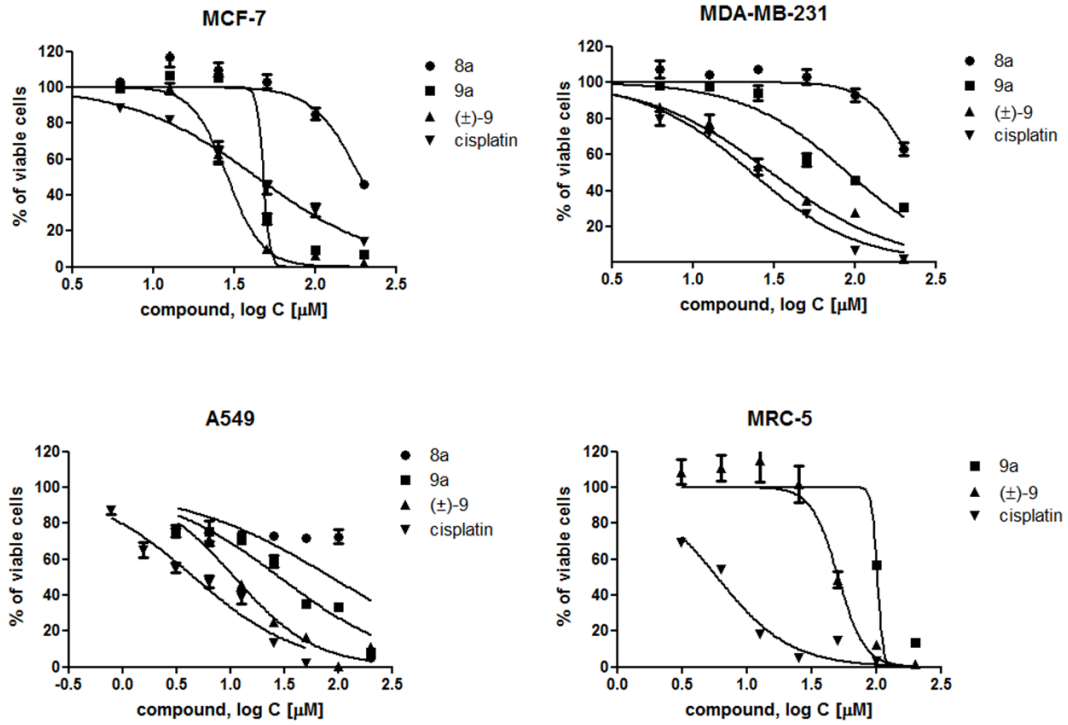


Figure S9. Plots generated by GraphPad Prism after fitting the MTT data obtained for MCF-7, MDA-MB-231, A549 and MRC-5 to sigmoidal dose response equation Y = 100/(1+10^((LogEC50-X)*HillSlope))); the concentration (EC_{50}) that causes a response half way between the maximal (Top) response and the maximally inhibited (Bottom) response was calculated.

Senescence studies

Detection of pH-dependent β-galactosidase activity was used as a senescence marker in MCF-7 cell line. Etoposide, a well-known senescence inducing agent was used as a positive control.

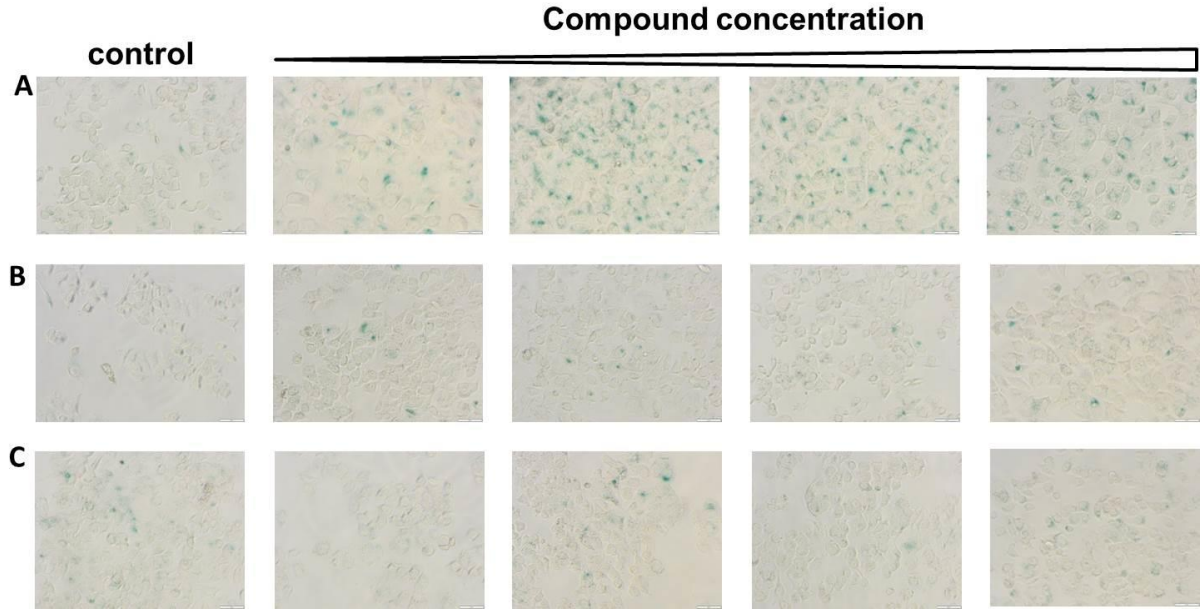


Figure S10. The β-galactosidase activity in MCF-7 cells after treatment (48 h) by etoposide (A) and compounds **(±)-9** (B) and **9a** (C).