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Synthesis and cytotoxicity of novel cholesterol–cobalt bis(dicarbollide) conjugates

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Experimental

General. Oxonium derivatives of cobalt bis(dicarbollide) **1a** [S1,S2], **1b** [S3] and 3β-(2-hydroxyethoxy)cholest-5-ene **2** [S4] were synthesized according to the published procedures. Sodium hydride (60% dispersion in mineral oil) and cesium fluoride were purchased from Sigma-Aldrich and used without further purification. Tetrahydrofuran was distilled via a standard technique. Cholesterol, acetone, MeCN and CH_2Cl_2 were commercial reagents of analytical grade. The reaction course was monitored by thin layer chromatography (Merck F_{245} silica gel on aluminum plates) and visualized using 0.5% $PdCl_2$ in 1% HCl in aq. MeOH (1:10). The NMR spectra at 400.1 MHz (^{1}H), 128.4 MHz (^{11}B), and 100.0 MHz (^{13}C) were measured in $(CD_3)_2SO$ on a Bruker Avance 400 spectrometer. The residual signals of the NMR solvent relative to Me_4Si were used as the internal references for the ^{1}H and ^{13}C NMR spectra. The ^{11}B NMR spectra were referenced using $BF_3{\cdot}Et_2O$ as an external standard. The IR spectra were recorded on a SHIMADZU IR Prestige-21 spectrometer. The high-resolution mass spectra (HRMS) were measured on a Bruker micrOTOF II instrument using electrospray ionization (ESI). The measurements were carried out in a negative ion mode (interface capillary voltage 3200 V); mass range from m/z 50 to m/z 3000; external or internal calibration was performed with ESI Tuning Mix, Agilent. A syringe injection was used for solutions in $MeCN/H_2O$ (1:1) (flow rate 3 μl min^{-1}). Nitrogen was applied as a dry gas; the interface temperature was set at 180 ^oC. The electron ionization mass spectra were obtained with a Kratos MS 890 instrument operating in the mass range of 50-800.

Cisplatin is the commercial antitumor preparation (Cisplatin Teva, PHARMACHEMIE B.V., the Netherlands) was used as the positive control against of tumor cells. Aqueous 1% DMSO solution (Amresco, USA) was used to prepare solutions of the test compounds and was used as the control solution.

Cell culture. Cell cultures were obtained at the Institute of Cytology and Genetics of the Russian Academy of Sciences (Siberian Branch) (ICG SB RAS). All cell experiments were performed under sterile conditions.

The MTT test was used to determine the cytotoxic concentration and CC_{50} (the concentration causing 50 % cell death) of compounds **3a,b**. U-87 MG cells and non-transformed human embryo fibroblast cells FECh-15, were used for the MTT assay. Cells were seeded in 96-well plates at 2×10^4 cells/well and cultured in DMEM/F12 medium (1:1) (Biolot) with 10% fetal bovine serum (Invitrogen). Cell counting was performed using an automated Countess cell counter (Invitrogen). The plates were incubated in a CO_2 incubator with 5% CO_2. A day later, compound solutions were added to different wells in a volume of 1/10 of the total volume of the medium in the well and titrated. The control cells were incubated without drugs. Each experimental point was repeated 4-5 times. Three days later medium was removed from each well, and serum-free medium containing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Dia-m) at a concentration of 5 mg ml^{-1} was added. The plates were incubated with MTT for 4 hours, then the formed crystals of formazan DMSO (Biolot) were dissolved. The results were accumulated using a Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fisher Scientific Inc.) at 595 nm wavelengths. Data presented as percentage survival of experimental wells relative to control.

Statistical analysis. Statistical processing of the data obtained was carried out using the software STATISTICA 11.0 (StatSoft).

General procedure for the synthesis of cholesterol-cobalt bis(dicarbollide) conjugates 3a,b. Sodium hydride (60% dispersion in mineral oil, 2.0 eq.) was added to a solution of 3β-(2-hydroxyethoxy)cholest-5-ene **2** (1.2 eq.) in THF (30 ml) under an argon atmosphere. The resulting mixture was stirred for 1 h. Then, the corresponding oxonium derivative of cobalt bis(dicarbollide) **1a** or **1b** (1.0 eq.) was added. The reaction mixture was refluxed for 8 h. After cooling to room temperature, methanol (3-4 drops) was added to quench the excess of sodium hydride. The solvent was evaporated *in vacuo*. The residue was dissolved in acetone (5 ml) and treated with an excess of aqueous solution of CsCl and stored in a fridge for a few hours. The precipitate formed was recovered by filtration and purified by column chromatography on silica with a mixture of dichloromethane and acetonitrile (3/1 v/v) as eluent. The major fraction was collected and vacuum dried to give the target products as an orange foam.

Synthesis of cesium 8-[2-(2-(2-({(3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl}oxy)ethoxy)ethoxy)ethoxy]eucosahydro-1,1′,2,2′-tetracarba-3-*commo*-cobalt-*closo*-tri-cosaborate

(8-[3β-Chol-(O(CH$_2$)$_2$)$_3$O]-3,3'-Co(1,2-C$_2$B$_9$H$_{10}$)(1',2'-C$_2$B$_9$H$_{11}$))Cs (3a)

Compound **3a** was prepared according to the general procedure from 1,4-dioxane derivative of cobalt bis(dicarbollide) **1a** (0.20 g, 0.61 mmol), 3β-(2-hydroxyethoxy)cholest-5-ene **2** (0.25 g, 0.61 mmol), and sodium hydride (60% dispersion in mineral oil, 0.04 g). Yield: 0.30 g (65%). ^{1}H NMR (acetone-d_6, ppm): 5.37 (1H, s, Cst*H*(6)), 4.25 (4H, br. s, C*H*$_{carb}$), 3.61 (6H, d, *J* = 9.2 Hz, 3*OC*H*$_2$), 3.54 (2H, dd, *J* = 9.9, 5.1, Hz, OC*H*$_2$), 3.25 – 3.08 (1H, m, Cst*H*(3)), 2.86 (4H, s, 2*OC*H*$_2$), 2.39-1.06 (34H, br. m,), 1.03 (3H, s, Cst*H*$_3$(19)), 0.97 (3H, d, *J* = 6.4 Hz, Cst*H*$_3$(21)), 0.89 (3H, s, Cst*H*$_3$(26)), 0.88 (3H, s, Cst*H*$_3$(27)), 0.74 (3H, s, Cst*H*$_3$(18)). ^{11}B NMR (acetone-d_6, ppm): 22.9 (1B, s), 4.0 (1B, d, *J* = 128 Hz), 0.4 (1B, d, *J* = 143 Hz), -2.4 (1B, d, *J* = 143 Hz), -4.3 (2B, d, *J* = 55 Hz), -7.4 (2B, d, *J* = 134 Hz), -8.1 (4B, d, *J* = 151 Hz), -17.2 (2B, d, *J* = 151 Hz), -20.4 (2B, d, *J* = 155 Hz), -21.7 (1B, d, *J* = 146 Hz), -28.4 (1B, d, *J* = 146 Hz**)**. ^{13}C NMR (acetone-d_6, ppm): 140.7 (*C*st(5)), 120.9 (*C*st(6)), 78.9 (*C*st(3)), 71.7 (O*C*H$_2$), 70.6 (O*C*H$_2$), 70.0 (O*C*H$_2$), 69.4 (O*C*H$_2$), 68.3 (O*C*H$_2$), 67.0 (O*C*H$_2$), 61.5 (*C*st(14)), 56.7 (*C*st(17)), 56.2 (*C*H$_{carb}$), 54.0 (*C*st(9)), 50.3 (*C*H$_{carb}$), 42.2 (*C*st(4)), 39.8 (*C*st(12)), 39.4 (*C*st(13)), 39.1 (*C*st(24)), 37.1 (*C*st(1)), 36.7 (*C*st(10)), 36.1 (*C*st(22)), 35.7 (*C*st(20)), 31.9(*C*st(8)), 31.8 (*C*st(2)), 27.8 (*C*st(7)), 24.1 (*C*st(16)), 23.6 (*C*st(25)), 22.2 (*C*st(15)), 22.0 (*C*st(23)), 21.0 (*C*st(26), *C*st(27)), 19.0 (*C*st(11)), 18.9 (*C*st(19)), 18.3 (*C*st(21)), 11.3 (*C*st(18)). ESI-MS *m*/*z* for C$_{38}$H$_{81}$B$_{18}$CoO$_3$: calcd. 878.6960, found 878.6915 [M]$^-$. IR (solid, *v*, cm^{-1}): 2480 (BH).

Synthesis of cesium 8-[(5-(2-({(3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl}oxy)ethoxy)pentyl)oxy]-eucosahydro-1,1′,2,2′-tetracarba-3-*commo*-cobalt-*closo*-tri-cosaborate

(8-[3β-Chol-(O(CH$_2$)$_2$)$_2$(CH$_2$)$_3$O]-3,3'-Co(1,2-C$_2$B$_9$H$_{10}$)(1',2'-C$_2$B$_9$H$_{11}$))Cs (3b)

Compound **3b** was prepared according to the general procedure from tetrahydropyran derivative of cobalt bis(dicarbollide) **1b** (0.20 g, 0.61 mmol), 3β-(2-hydroxyethoxy)cholest-5-ene **2** (0.25 g, 0.61 mmol), and sodium hydride (60% dispersion in mineral oil, 0.04 g). Yield: 0.28 g (56%). ^{1}H NMR (acetone-d_6, ppm): 5.36 (1H, s, Cst*H*(6)), 4.30 (2H, s, C*H*$_{carb}$), 4.24 (2H, s, C*H*$_{carb}$), 3.66 – 3.57 (2H, m, OC*H*$_2$), 3.54 – 3.47 (2H, m, OC*H*$_2$), 3.43 (4H, dd, *J* = 8.3, 4.6 Hz, OC*H*$_2$C*H*$_2$O), 3.24 – 3.16 (1H, m, Cst*H* (3)), 2.92 (4H, s, 2*C*H*$_2$), 2.89 (2H, s, C*H*$_2$), 2.40-1.06 (34H br.m.,), 1.03 (3H, s, Cst*H*$_3$(19)), 0.97 (3H, d, *J* = 6.5 Hz, Cst*H*$_3$(21)), 0.89 (3H, s,

CstH_3(26)), 0.88 (3H, s, CstH_3(27)), 0.74 (3H, s, CstH_3(18)). ^{11}B NMR (acetone-d_6, ppm): 22.9 (1B, s), 3.6 (1B, d, J = 142 Hz), 0.3 (1B, d, J = 166 Hz), -2.5 (1B, d, J = 156 Hz), -4.2 (1B, d, J = 140 Hz), -7.5 (3B, d, J = 132 Hz), -8.3 (4B, d, J = 118 Hz), -17.4 (2B, d, J = 132 Hz), -20.4 (2B, d, J = 154 Hz), -22.1 (1B, d, J = 128 Hz), -28.6 (1B, d, J = 155 Hz). ^{13}C NMR (acetone-d_6, ppm): 141.0 (*C*st(5)), 121.2 (*C*st(6)), 79.0 (*C*st(3)), 70.90 (O*C*H$_2$), 70.4 (O*C*H$_2$), 68.7 (O*C*H$_2$), 67.1 (O*C*H$_2$), 56.7 (*C*st (14)), 56.1 (*C*H$_{carb}$), 54.6 (*C*st(17)), 50.3 (*C*H$_{carb}$), 46.3 (*C*st(9)), 42.2 (*C*st(4)), 39.8 (*C*st(12)), 39.4 (*C*st(13)), 39.2 (*C*st(24)), 37.2 (*C*st(1)), 36.7 (*C*st(10)), 36.1 (*C*st(22)), 35.7 (*C*H$_2$), 31.9 (*C*st(20)), 31.8 (*C*st(8)), 29.7 (*C*st(2)), 28.39 (*C*H$_2$), 28.1 (*C*H$_2$), 27.80 (*C*st(7)), 24.1 (*C*st(16)), 23.6 (*C*st(25)), 22.7 (*C*st(15)), 22.2 *C*st(23)), 22.0 (*C*st(26), *C*st(27)), 20.9 (*C*st(11)), 18.9 (*C*st(19)), 18.3 (*C*st(21)), 11.4 (*C*st(18)). ESI-MS *m*/*z* for $C_{37}H_{78}B_{18}CoO_4$: calcd. 840.7046, found 840.7035 $[M]^-$. IR (solid, v, cm^{-1}): 2563 (BH).

References

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S3. J. Llop, C. Masalles, C. Viñas, F. Teixidor, R. Sillanpää and R. Kivekäs, *Dalton Trans.*, 2003, 556.

S4. M. Berg, S. Nozinovic, M. Engeser and A. Lützen, *Eur. J. Org. Chem.*, 2015, 5966.

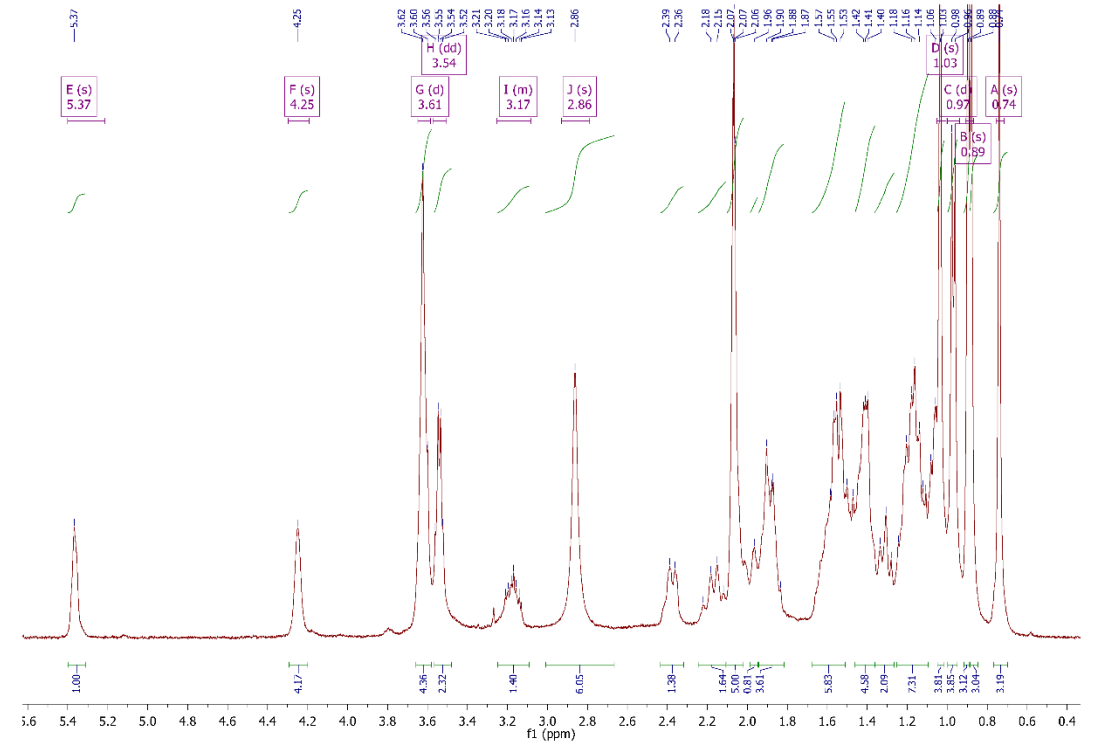

Figure S1. ^{1}H NMR spectrum of compound **3a** (acetone-d_6)

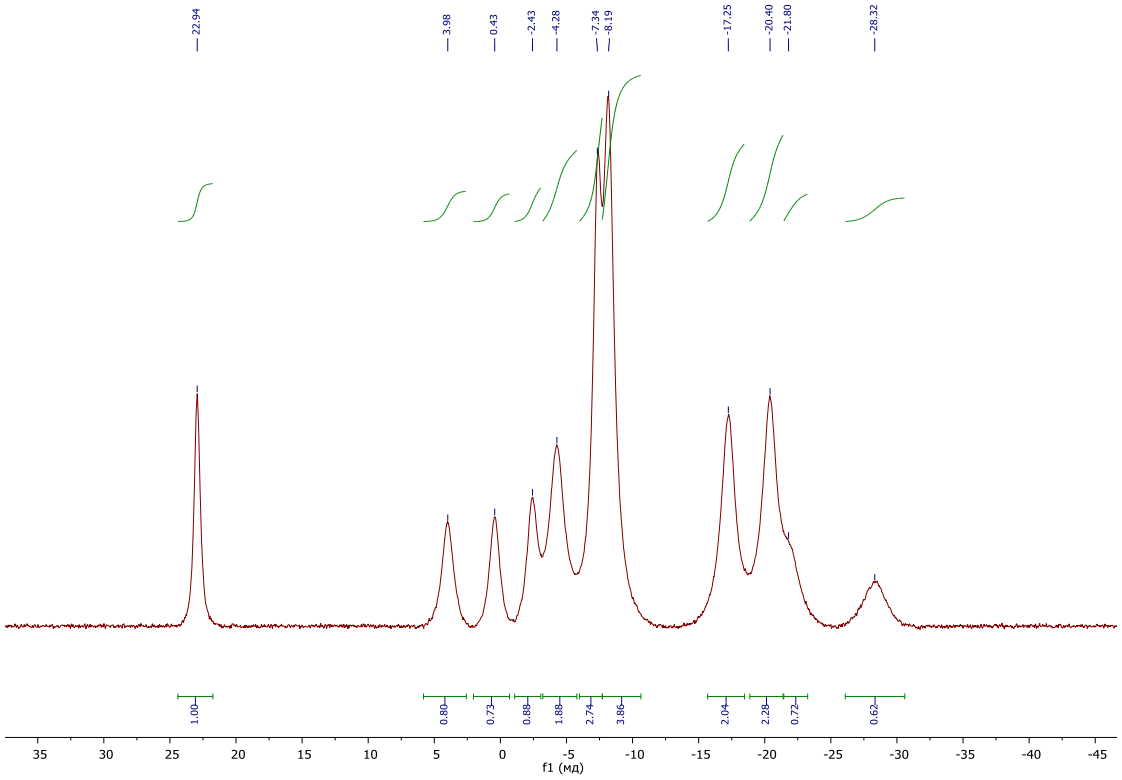

Figure S2. ^{11}B NMR spectrum of compound **3a** (acetone-d_6)

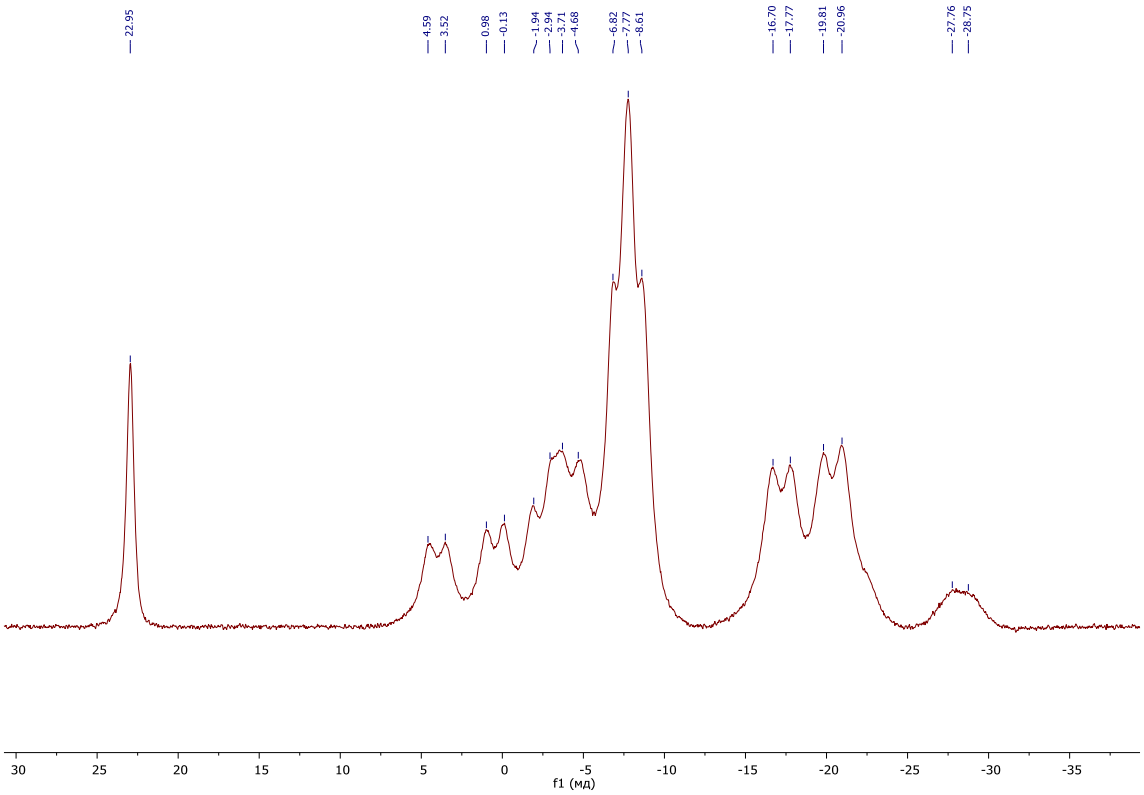

Figure S3. $^{11}B\{^{1}H\}$ NMR spectrum of compound **3a** (acetone-d_6)

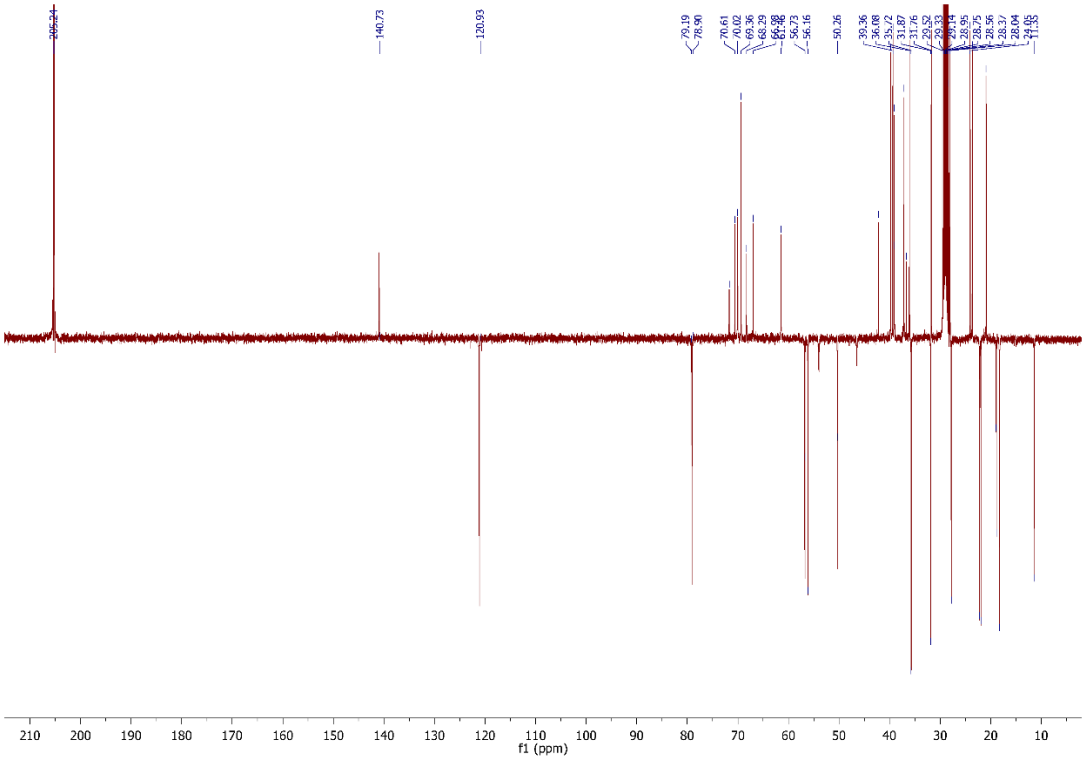

Figure S4. ^{13}C NMR spectrum of compound **3a** (acetone-d_6)

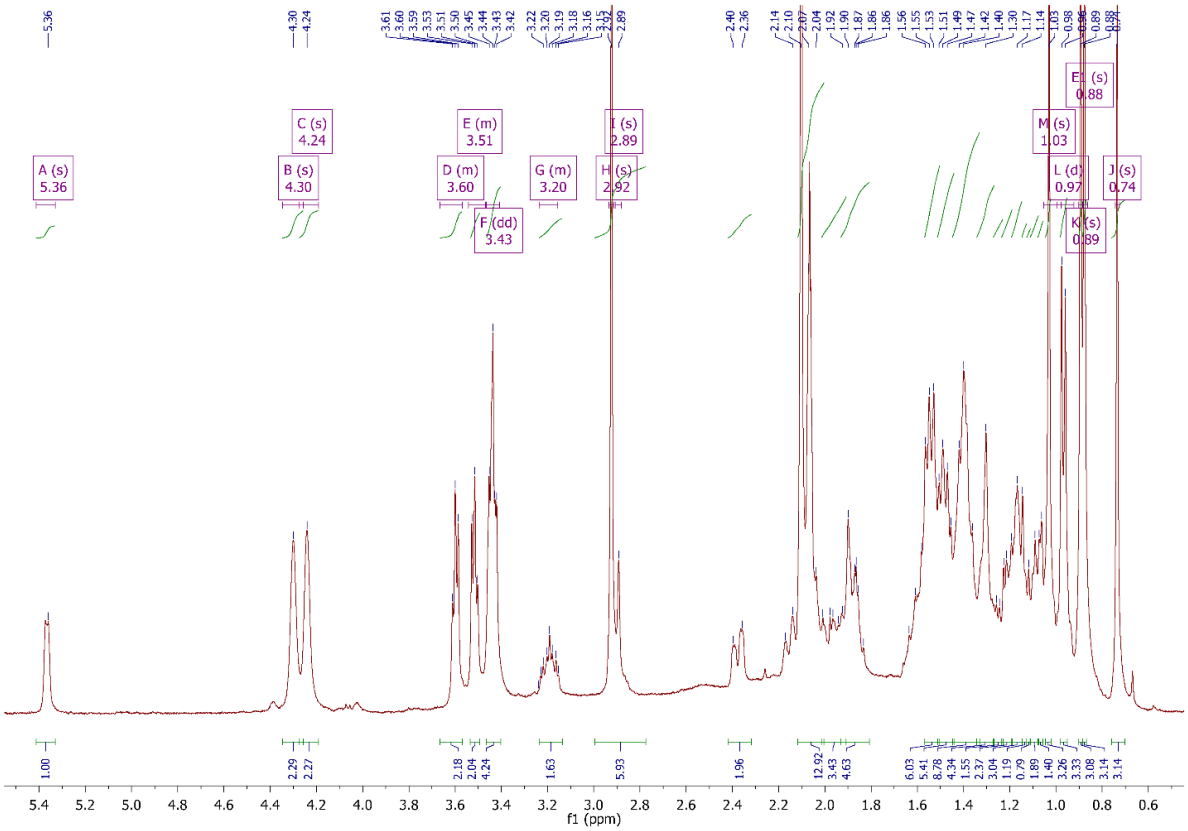

Figure S5. ^{1}H NMR spectrum of compound **3b** (acetone-d$_6$)

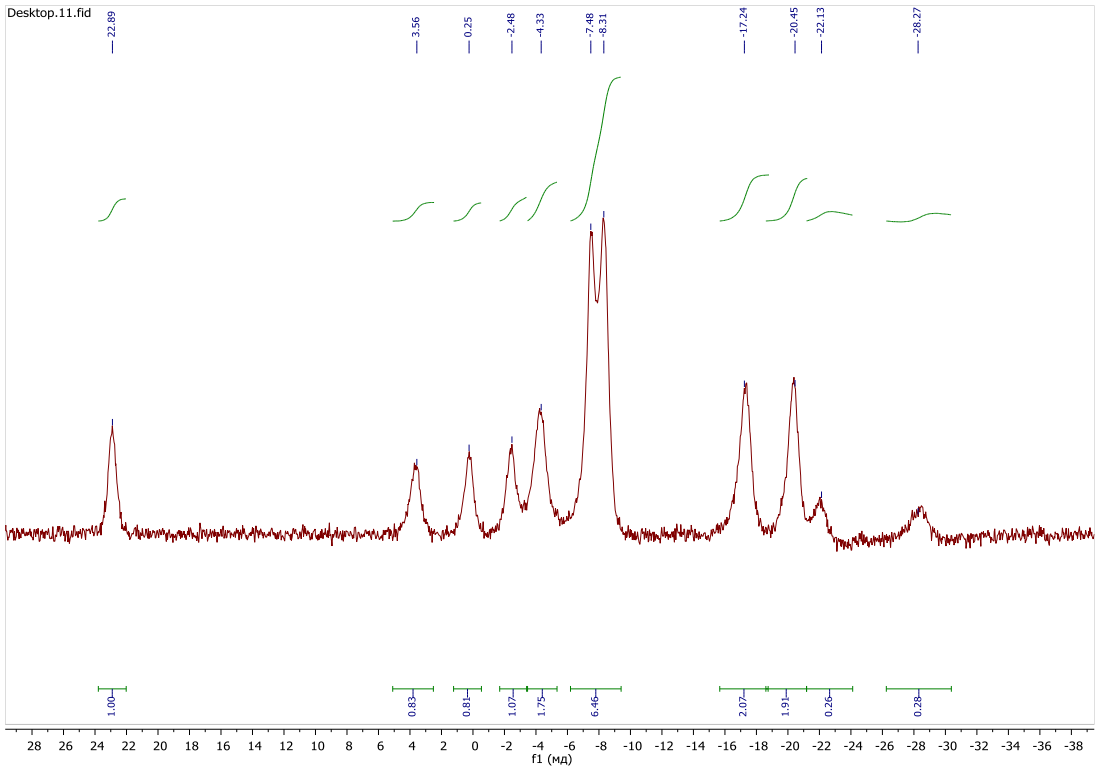

Figure S6. ^{11}B{^{1}H} NMR spectrum of compound 3**b** (acetone-d$_6$)

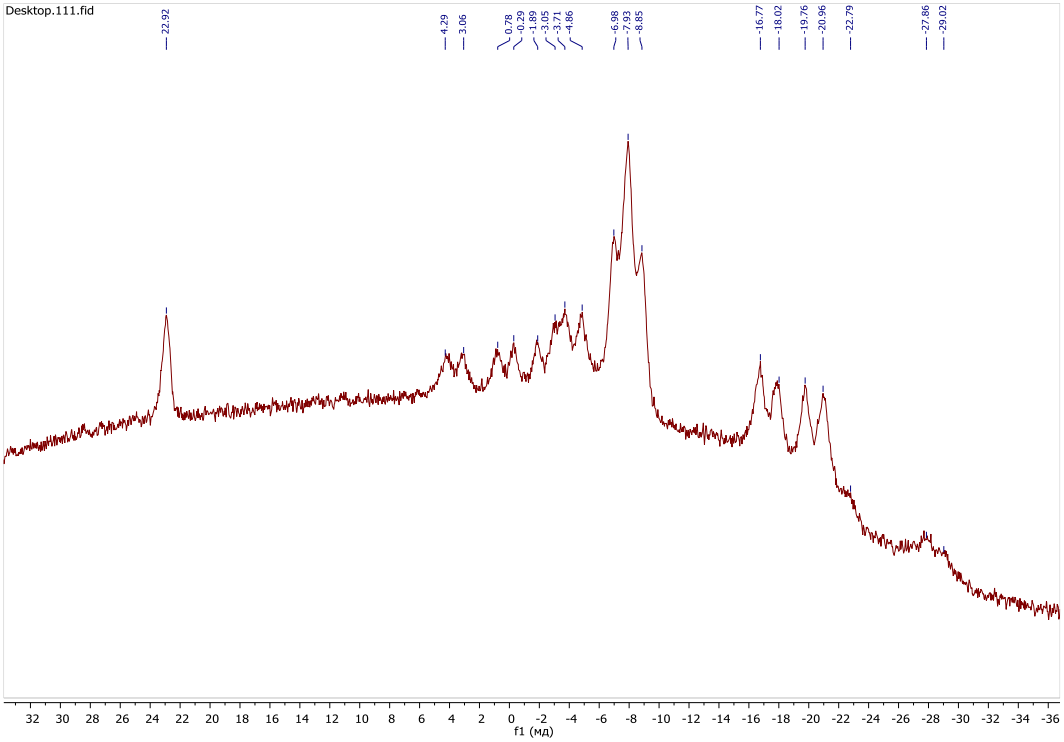

Figure S7. ^{11}B NMR spectrum of compound **3b** (acetone-d_6)

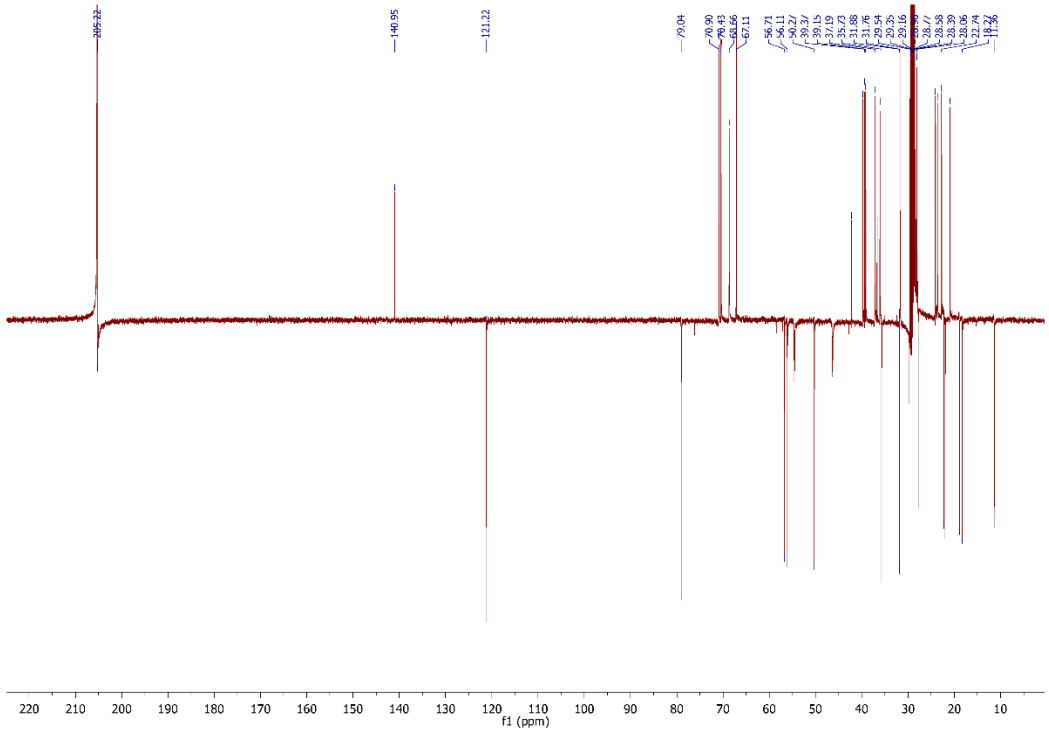

Figure S8. ^{13}C NMR spectrum of compound **3b** (acetone-d_6)

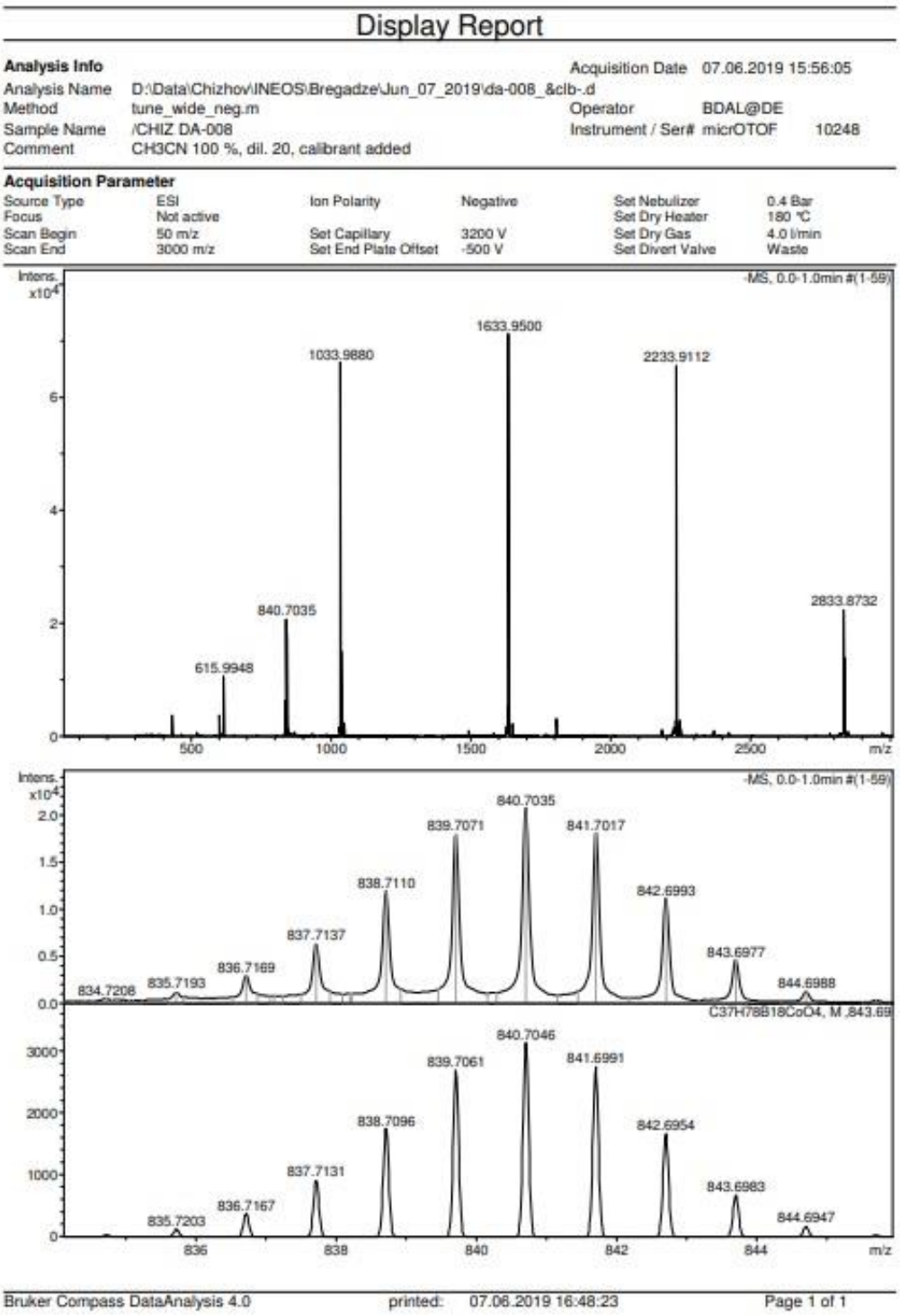

Figure S9. ESI-HRMS spectrum of compound **3a**

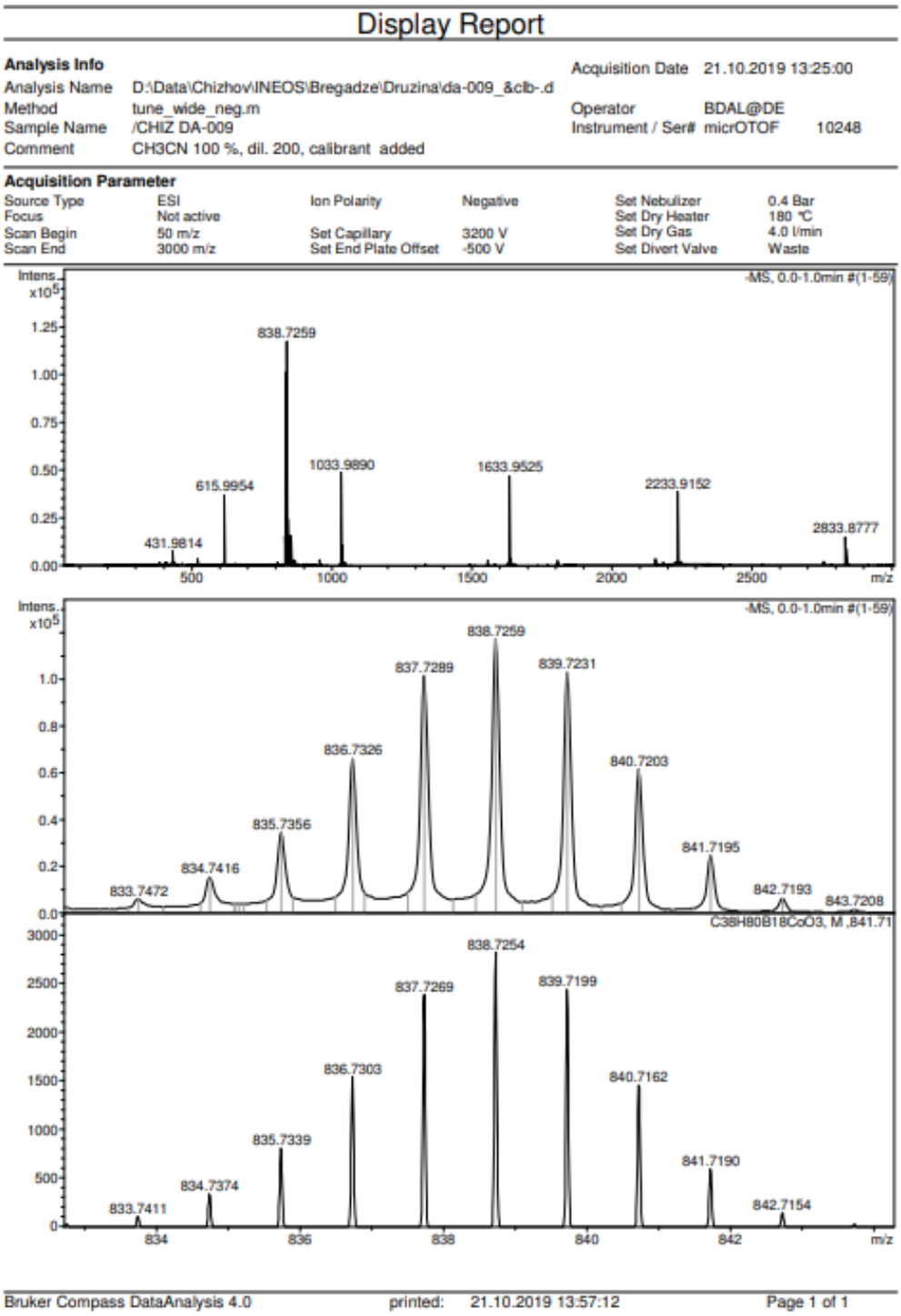

Figure S10. ESI-HRMS spectrum of compound **3b**