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Molecular design and synthesis of new heterobivalent compounds based on chlorambucil and colchicine

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Molecular modeling

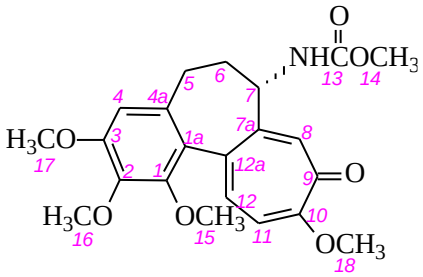
Molecular docking study was carried out using 3D model of colchicine binding site in α,β-tubulin (PDB ID 4O2B). The compounds used in protein structure determination and all water molecules were excluded from the model, other molecules and ions at the interface of α- and β-subunits of the protein were maintained. Assignment of atomic charges of protein amino acids was made by standard Kollman method using AutoDock Tools 1.5.6. Three-dimensional structures of the compounds were submitted to a conformational MMFF Amber ff14SB optimization using Gasteiger charges in USCF Chimera 1.13.1 program [1]. Molecular docking was carried out with AutoDock Vina 1.1.2 software [2] (grid box 23.25Å×25.5Å ×24.0Å, grid center size x=17.51 Å, y=69.503 Å, z=43.258 Å, exhaustiveness = 16). Complexes with the best values of scoring functions were selected and visualized using CLC Drug Discovery Workbench (Limited mode, Version 4).

Chemistry

Chlorambucil, colchicine, N,N'-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP), N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) and other initial reagents were commercially available (Sigma–Aldrich). N-deacetylcolchicine (**3**) was obtained in three steps from colchicine according to three-step procedure described in ref. [3]. Pimelic acid polyanhydride was synthesized from corresponding acid using standard procedure [4]. 3-(N-(*tert*-butoxycarbonyl)amino)adamantanol was obtained as described in ref. [5].

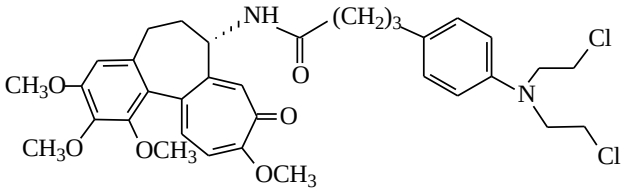
All solvents for extraction and chromatography were technical grade and were purified by standard procedures prior to use. Column chromatography purification was performed using silica gel Macherey-Nagel (0.063–0.2 mm). Thin layer chromatography (TLC) on TLC silica gel plates ALUGRAM Xtra G/UV254 was used for reactions monitoring and additional purification of compound **4**. ^{1}H and ^{13}C NMR spectra were recorded on spectrometer Agilent 400-MR (400.0 MHz for ^{1}H; 100.6 MHz for ^{13}C) at room temperature; chemical shifts were measured with reference to the solvent ($CDCl_3$, δ_H=7.27 ppm, δ_C=77.0 ppm). Chemical shifts (δ) are given in

ppm, multiplicities are indicated by s (singlet), d (doublet), t (triplet), m (multiplet), br (broad), ddd (double-double-doublet), spin-spin coupling constants (*J*) are reported in Hz. Signals of atoms in colchicine fragment in NMR spectra were assigned according to the data in ref. [6] using the following numbering of atoms:



Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass-spectra were acquired on a Bruker Autoflex II mass spectrometer (accelerating voltage of 20 kV). Electrospray mass spectra (ESI-MS) were recorded on an Agilent 6538 UHD mass spectrometer in positive atom mode.

N-{4-[4-(bis(2-chloroethyl)amino)phenyl]butanoyl}-N-deacetylcolchicine (**4**). To a solution of chlorambucil (0.051 g, 0.167 mmol), in CH_2Cl_2 (5 ml) was added N-deacetylcolchicine (**3**) (0.050 g, 0.140 mmol) and EEDQ (0.050 g, 0.202 mmol). The mixture was stirred for 24 h at room temperature then the solvent was removed under reduced pressure. The residue was first purified by column chromatography (ethyl acetate/petroleum ether (40–70ºC) 1:1, then 1% methanol in CH_2Cl_2) and then by TLC (1% methanol in CH_2Cl_2) to give 0.035 g of compound **4** (yield 39%) as yellowish waxy solid.

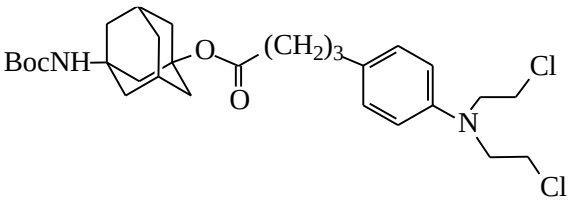


^{1}H NMR (CDCl$_3$, δ, *J*/Hz): 1.82 – 1.90 (m, 3H,), 2.21 – 2.29 (m, 3H), 2.39 (m, 1H, H^{5colch}), 2.49 – 2.55 (m, 3H), 3.59 – 3.63 (m, 4H, N(CH$_2$C<u>H</u>$_2$Cl)$_2$), 3.66 (s, 3H, OCH$_3$), 3.68 – 3.72 (m, 4H, N(C<u>H</u>$_2$CH$_2$Cl)$_2$), 3.91 (s, 3H, OCH$_3$), 3.95 (s, 3H, OCH$_3$), 4.01 (s, 3H, OCH$_3$), 4.67 (ddd, *J* = 6.8, 6.8, 12.1 Hz, 1H, H^{7colch}), 6.54 (s, 1H, H ^{4colch}), 6.62 (d, *J* = 8.8 Hz, 2H, H$^{3'Ar}$), 6.87 (d, 1H, H^{11colch}, *J* = 11.0 Hz,), 7.04 (d, *J* = 8.6 Hz, 2H, H$^{2'Ar}$), 7.33 (d, 1H, *J* =10.8 Hz, H^{12colch}), 7.49 (s, 1H, H^{8colch}).

^{13}C NMR: (CDCl$_3$, δ): 26.86; 29.88; 34.00; 35.50; 37.00; 40.43 (CH$_2$Cl); 52.05 (C^{7colch}); 53.69 (CH$_2$N); 56.08 (OCH$_3$); 56.39 (OCH$_3$); 61.37 (OCH$_3$); 61.59 (OCH$_3$); 107.32 (C^{4colch}); 112.34 (C$^{3'Ar}$); 112.74 (C^{11colch}); 125.53 (C$^{1a\ colch}$); 129.67 (C$^{2'Ar}$); 130.60 (C^{8colch}); 130.85; 134.13 (C$^{4a\ colch}$); 135.56 (C^{12colch}); 136.69 (C$^{12a\ colch}$); 141.65 (C$^{2\ colch}$); 144.00; 151.19 (C^{1colch}); 151.80 (C$^{7a\ colch}$); 153.51 (C^{3colch}); 163.97 (C^{10colch}); 172.34 (C(O)NH); 179.05 (C^{9colch}).

MS (MALDI-TOF), *m/z*: 642 $[M]^+$, 644 $[M+2]^+$; Calculated for $C_{34}H_{40}Cl_2N_2O_6$ MW=643.6

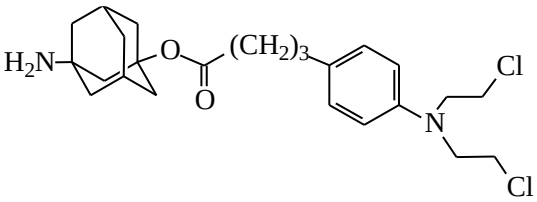
3-((*Tert*-butoxycarbonyl)amino)adamantan-1-yl 4-(4-(bis(2-chloroethyl)amino)phenyl)butanoate (**6a**). To a solution of 3-(N-(*tert*-butoxycarbonyl)amino)adamantanol (0.2 g, 0.749 mmol) in CH_2Cl_2 (15 ml) was added chlorambucil (0.15 g, 0.493 mmol), DCC (0.165 g, 0.800 mmol) and catalytic amount of DMAP (0.005 g). The mixture was stirred for 12 h at room temperature, then ~10 μl of acetic acid was added and in 15 min the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (10 ml) and kept at 4°C for 2–3 h. The sediment was filtered off and the filtrate was washed with cold ethyl acetate (2×10 ml), 0.1 N HCl, water (10 ml), brine (10 ml), dried over Na_2SO_4 and evaporated. Chromatographic purification [petroleum ether (40–70°C), then 1% ethyl acetate in petroleum ether (40–70°C)] gave 0.180 g **6a** (yield 66%) as yellowish oily liquid.



^{1}H NMR ($CDCl_3$, δ, *J*/Hz): 1.43 (s, 9H, ^{t}Bu), 1.58 (m, 2H, H^{Ad}), 1.81 – 1.88 (m, 4H, $ArCH_2CH_2$+H^{Ad}), 1.92 – 1.95 (m, 2H, H^{Ad}), 2.01 – 2.04 (m, 2H, H^{Ad}), 2.09 – 2.12 (m, 2H, H^{Ad}), 2.22 (t, *J* = 7.5 Hz, 2H, CH_2CO_2Ad), 2.27 – 2.29 (m, 4H, H^{Ad}), 2.54 (t, *J* = 7.5 Hz, 2H, $ArCH_2$), 3.60 – 3.72 (m, 8H, $N(CH_2CH_2Cl)_2$), 4.45 (br s, 1H, NH), 6.63 (d, *J* = 8.6 Hz, 2H, $H^{3',5'Ar}$), 7.07 (d, *J* = 8.6 Hz, 2H, $H^{2',6'Ar}$).

^{13}C NMR: ($CDCl_3$, δ): 27.35, 28.84 ($(CH_3)_3$), 30.95, 34.34, 35.31, 35.40, 40.50, 40.91 (CH_2Cl), 41.11, 45.82, 53.24 (C^{Ad}-N), 53.99 (CH_2N), 77.62 ($C(CH_3)_3$), 80.56 (C^{Ad}-O), 112.48 ($C^{3',5'Ar}$), 130.10 ($C^{2',6'Ar}$), 131.18 ($C^{1'Ar}$), 144.63 ($C^{4'Ar}$), 154.45 (CO^tBu), 173.07 (C=O)

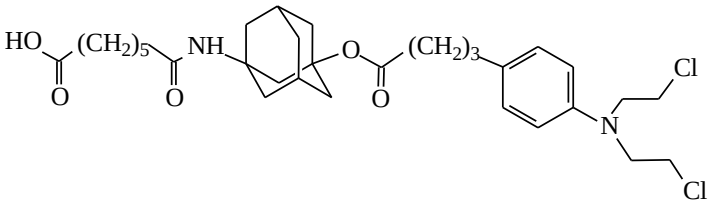
3-(4-(4-(Bis(2-chloroethyl)amino)phenyl)butanoyloxy)-1-adamantanylamine (**6b**). Protected amine **6a** (0.180 g, 0.325 mmol) was dissolved in CH_2Cl_2 (15 ml) and treated with TFA (0.1 ml, 1.307 mmol) for 24 h at room temperature. The solvent was evaporated under reduced pressure at 60°C for 3 h to yield 0.147 g **6b** (yield 100%) as beige waxy solid used in the next step without additional purification.



^{1}H NMR ($CDCl_3$, δ, *J*/Hz): 1.59 (m, 2H, H^{Ad}), 1.81 – 1.88 (m, 6H, $ArCH_2CH_2$+H^{Ad}), 1.99 – 2.02 (m, 2H, H^{Ad}), 2.14 – 2.17 (m, 2H, H^{Ad}), 2.23 – 2.27 (m, 4H, CH_2CO_2Ad+H^{Ad}), 2.35 (m, 2H, H^{Ad}), 2.54 (t, *J* = 7.5 Hz, 2H, $ArCH_2$), 3.60 – 3.72 (m, 8H, $N(CH_2CH_2Cl)_2$), 6.62 (d, *J* = 8.6 Hz, 2H, $H^{3',5'Ar}$), 7.06 (d, *J* = 8.6 Hz, 2H, $H^{2',6'Ar}$), 7.64 (br s, 2H, NH_2).

^{13}C NMR: ($CDCl_3$, δ): 26.85, 30.13, 33.88, 33.98, 34.77, 39.17, 39.34, 40.56 (CH_2Cl), 44.20, 53.62 (CH_2N), 54.35 (C^{Ad}-N), 79.11 (C^{Ad}-O), 112.17 ($C^{3',5'Ar}$), 129.68 ($C^{2',6'Ar}$), 130.59 ($C^{1'Ar}$), 144.33 ($C^{4'Ar}$), 172.93 (C=O).

7-[3-((4-(4-(Bis(2-chloroethyl)amino)phenyl)butanoyl)oxy)-adamant-1-ylamino]-7-oxoheptanoic acid (7). To a solution of 3-(4-(4-(bis(2-chloroethyl)amino)phenyl)butanoyloxy)-1-adamantanylamine (**6b**) (0.120 g, 0.265 mmol) in CH_2Cl_2 (5 ml) was added pimelic acid polyanhydride (0.060 g, 0.422 mmol) and DMAP (0.04 g, 0.328 mmol). The mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with aqueous acetone (ca. 10% of water, 10 ml) and stirred at room temperature for 2 h, then concentrated under reduced pressure, taken up in CH_2Cl_2 (20 ml), dried over Na_2SO_4, filtered and evaporated. Chromatographic purification [petroleum ether (40–70°C), then 25% ethyl acetate in petroleum ether (40–70°C)] gave 0.110 g **6a** (yield 70%) as colorless oily liquid.



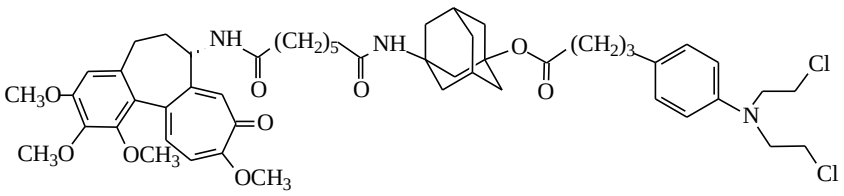
^{1}H NMR ($CDCl_3$, δ, *J*/Hz): 1.32 – 1.40 (m, 2H, $C^{pim}H_2$), 1,57 – 1.60 (m, 2H, H^{Ad}), 1,61 – 1.69 (m, 4H, $C^{pim}H_2$), 1,80 – 1.88 (m, 2H, $ArCH_2CH_2$), 1.90 – 1.93 (m, 2H, H^{Ad}), 1.98 – 2.01 (m, 2H, H^{Ad}), 2.03 – 2.10 (m, 4H), 2.10 (t, *J* = 7.5 Hz, 2H, CH_2CONH), 2.23 (t, *J* = 7.5 Hz, 2H, CH_2CO_2Ad), 2.26 – 2.30 (m, 2H, H^{Ad}), 2.36 (t, *J* = 7.4 Hz, 2H, CH_2CONH), 2.37 – 2.39 (m, 2H, H), 2.54 (t, *J* = 7.5 Hz, 2H, $ArCH_2$), 3.61 – 3.73 (m, 8H, $N(CH_2CH_2Cl)_2$), 5.23 (br s, 1H, NH), 6.63 (d, *J* = 8.6 Hz, 2H, $H^{3'Ar}$), 7.06 (d, *J* = 8.6 Hz, 2H, $H^{2'Ar}$).

^{13}C NMR: ($CDCl_3$, δ): 24.33, 25.37, 26.93, 28.42, 30.55, 33.85, 33.93, 34.92, 37.17, 40.16, 40.26, 40.55 (CH_2Cl), 44.97, 51.58, 53.61 (CH_2N), 54.23 ($C^{Ad}N$), 80.16 (C^{Ad}-O), 112.21 ($C^{3'Ar}$), 129.70 ($C^{2'Ar}$), 130.80 ($C^{1'Ar}$), 144.23 ($C^{4'Ar}$), 172.96 (C=O), 174.22 (C=O), 178.93 (CO_2H).

MS (ESI, m/z (Int%)): 595.5 $[M+H]^+$ (100), 597.3 $[M+H+2]^+$ (75), 599.3 $[M+H+4]^+$ (18). Calculated for $C_{31}H_{44}Cl_2N_2O_5$ MW =595.6

Found, %: C 62.48, H 7.40, N 4.65. $C_{31}H_{44}Cl_2N_2O_5$ requires, %: C 62.51; H, 7.45; N, 4.70.

N-{7-[3-((4-(4-(bis(2-chloroethyl)amino)phenyl)butanoyl)oxy)-adamant-1-ylamino]-7-oxoheptanoyl}-N-deacetylcolchicine (**8**). Prepared from **7** (0.100 g, 0.168 mmol), EEDQ (0.060 g, 0.243 mmol) and N-deacetylcolchicine (0.065 g, 0.182 mmol) similar to compound **4**.



Chromatographic purification (ethyl acetate/petroleum ether 1:5, then CH_2Cl_2/acetone 10:1–5:1) gave (**5**) as yellowish oil (0.071 g, yield 45%).

^{1}H NMR ($CDCl_3$, δ, *J*/Hz): 1.31 – 1.40 (m, 2H, $C^{pim}H_2$), 1.45 – 1.55 (m, 2H), 1,57 – 1.60 (m, 2H), 1,66 – 1.90 (m, 11H), 1.96 – 2.01 (m, 2H, H^{Ad}), 2.01 – 2.08 (m, 1H), 2.14 – 2.34 (m, 10H), 2.39 – 2.47 (m, 1H, H^{5colch}), 2.53 (t, *J* = 7.5 Hz, 2H, $ArCH_2$), 2.54 (m, 1H, H^{5colch}), 3.60 – 3.72 (m, 8H, $N(CH_2CH_2Cl)_2$), 3.66 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.57 (ddd, *J* = 6.6, 6.6,11.8 Hz, 1H, H^{7colch}), 6.54 (s, 1H, H^{4colch}), 6.62 (d, *J* = 8.6 Hz, 2H, $H^{3'Ar}$), 6.86 (d, *J* = 10.8 Hz, 1H, $H^{11colch}$), 7.04 (d, *J* = 8.6 Hz, 2H, $H^{2'Ar}$). 7.07 (br s, 1H, NH), 7.35 (d, *J* = 10.8 Hz, 1H, $H^{12colch}$), 7.41 (br s., 1H, NH), 7.47 (s, 1H, H^{8colch}).

^{13}C NMR: ($CDCl_3$, δ): 25.65, 25.79, 26.79, 28.66, 29.83, 30.34, 30.47, 33.83, 34.79, 35.19, 36.29, 36.60, 37.15, 39.58, 40.01, 40.47 (CH_2Cl), 44.38, 52.95 (C^{7colch}), 53.52 (CH_2N), 54.22 ($C^{Ad}N$), 56.06 (OCH_3), 56.33 (OCH_3), 61.35 (OCH_3), 61.59 (OCH_3), 80.74 (C^{Ad}-O), 107.28 (C^{4colch}), 112.09 ($C^{3'Ar}$), 112.62 ($C^{11colch}$), 125.71 ($C^{1a\ colch}$), 129.58 ($C^{2'Ar}$), 130.42, 130.53 (C^{8colch}), 134.10 ($C^{4a\ colch}$), 135.53 ($C^{12colch}$), 136.56 ($C^{12a\ colch}$), 141.65 ($C^{2\ colch}$), 144.27, 150.60 (C^{1colch}), 152.11 ($C^{7a\ colch}$), 153.42 (C^{3colch}), 164.13 ($C^{10colch}$), 173.04 (C=O), 173.29 (C=O), 173.89 (C=O), 179.04 (C^{9colch}).

MS (ESI, m/z, Int%): 934.8 $[M+H]^+$ (100), 937.0 $[M+H+2]^+$ (62), 938.8 $[M+H+4]^+$ (19). Calculated for $C_{51}H_{65}Cl_2N_3O_9$ MW = 934.98

MS (MALDI-TOF, m/z): 934 $[M]^+$, 957 $[M+Na]^+$, 973 $[M+K]^+$.

Found: C, 65.47; H, 7.00; N, 4.43. $C_{51}H_{65}Cl_2N_3O_9$ requires: C, 65.51; H, 7.01; N, 4.49.

Biological testing

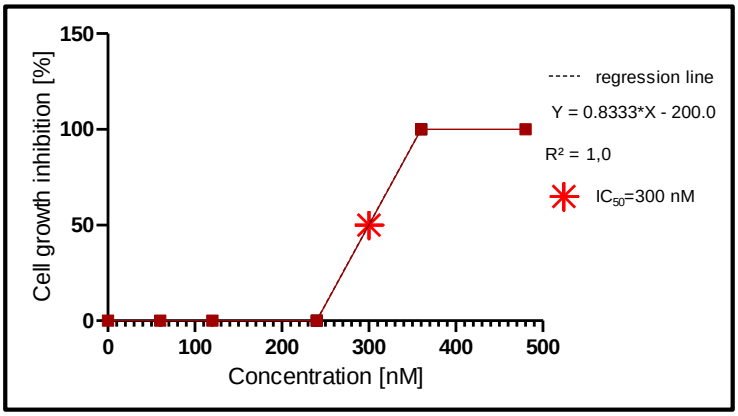
Cell lines. A549 human lung epithelial carcinoma cells (CCL-185™) were cultured under a 5% CO_2 humidified atmosphere with Dulbecco´s Modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C. A549_EGFP cells and immortalized lung fibroblasts VA13_Katushka were cultured in DMEM/F-12 containing 10% fetal bovine serum, penicillin (50 U/ml), and streptomycin (0.05 mg/ml) at 37°C under 5% CO_2.

MTT Cytotoxicity Assay. 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl-2*H*-tetrazoliumbromide (MTT, Roth GmbH, Karlsruhe, Germany) was used in a standard quantitative colorimetric assay to measure the cytotoxicity, viability and metabolic activity [7]. The A549 cells were seeded in 96-well plates (4000–6000 cells per well). Stock solutions of tested compounds (20 mM) were prepared in DMSO (0.5% served as a negative control). Cells were treated for 24 h with each selected concentration. Optical density was measured at 550 nm with 690 nm reference compounds at 1–600 nM (8 wells for filter using a plate reader SpectraMax iD3 (Molecular

devices, San Jose, USA). Experiments were repeated 3–6 times and EC_{50} values were determined by sigmoid curve fitting using Excel-based software.

Screening of selectivity of cytotoxic action of compound 4 was based on evaluation of viability of modified cell lines A549_EGFP and VA13_Katushka by determination of fluorescence of the expressed fluorescent protein as described in details in [8]. In brief, the cells were seeded in 384-well plate (400 A549_EGFP cells per well, 800 VA13_Katushka cells per well) in 40 µl of F-12 medium and in 20 h the attached cells were treated with tested compound. Stock solution of compound **4** (300 µM) was prepared in DMSO, 40 µl of the centrifuged (2000 g/2 min) solution of the tested compound in F-12 medium (DMSO content did not exceed 2%) in concentrations 3µM, 0.6 µM, 0.015 µM (and without compound **4** for negative control) were added to each well. The cells were treated for 72 h with each selected concentration of **4**. Fluorescent images were obtained on TYPHOON FLA950 (GE Healthcare) scanner at a resolution of 10 µm (excitation wavelength 473 nm and emission filter 520–540 nm for GFP, excitation wavelength 636 nm and emission filter 665 nm for Katushka). Data were processed using ImageJ programme as described in [8] and for evaluation of cell viability the cell fluorescence intensity was normalized to the wells without test compound.

***Proliferation assay for compound 8*.** The 6-well plates were seeded with a 2 ml solution containing 80 000 A549 cells per well. After a settling period of 24 h, 10 µl of substances to final concentrations 60 nM, 120 nM, 240 nM 360 nM and 480 nM of compound **8** were added (0.5% DMSO was used as a control). The next day the wells were washed twice with PBS, the cells were detached from substrate by trypsinization and resuspended in 1 ml medium. The number of all cells in suspentions was counted directly by phase-contract microscopy using hemocytometer.



The data of typical cell growth experiment in the presence of compound 8.

In order to calculate IC_{50}, a regression line was set from the point where the values started increased to the intersection of the control line (number of cell before incubation with

compounds). The regression line provided a formula, which could be implemented to determine the IC_{50}.

Immunofluorescence staining of cellular microtubules. Lung carcinoma A549 cells were cultured in 12-well plates on 11 mm glass coverslips (~20000 cells per coverslip). Cells were incubated with tested compounds at 2 μM (and 10 μM for compound **8**) for 24 h (0.5 % DMSO served as a negative control). The cells were fixed and stained as described in [9]. Fixed cells were labeled with mouse monoclonal antibody against α-tubulin (Sigma, St. Louis, USA) at a dilution of 1:300, followed by incubation of Alexa Fluor488 labeled goat anti-mouse IgG (Invitrogen, Germany) at a dilution of 1:300. Images of all samples were acquired with a Nikon Diaphot 300 inverted microscope (Nikon GmbH, Düsseldorf, Germany) equipped with a cooled charge-couple device camera system (SenSys; Photometrics, Munich, Germany).

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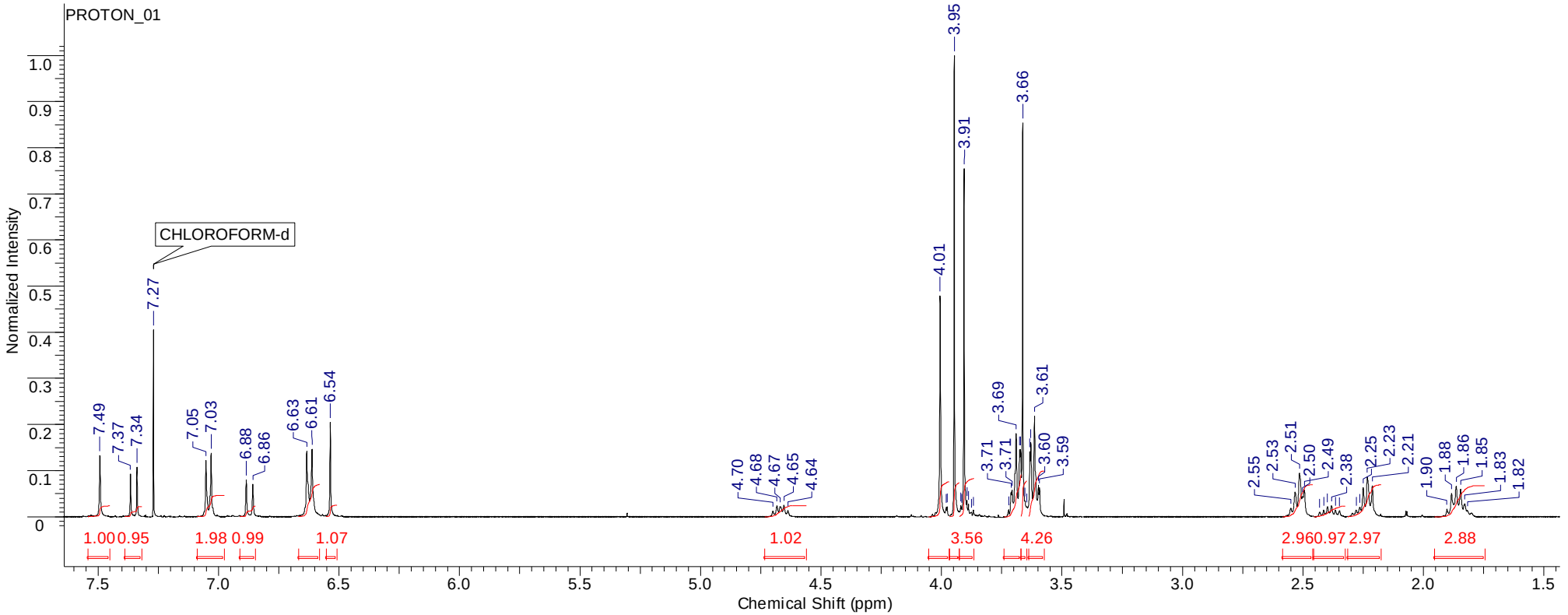
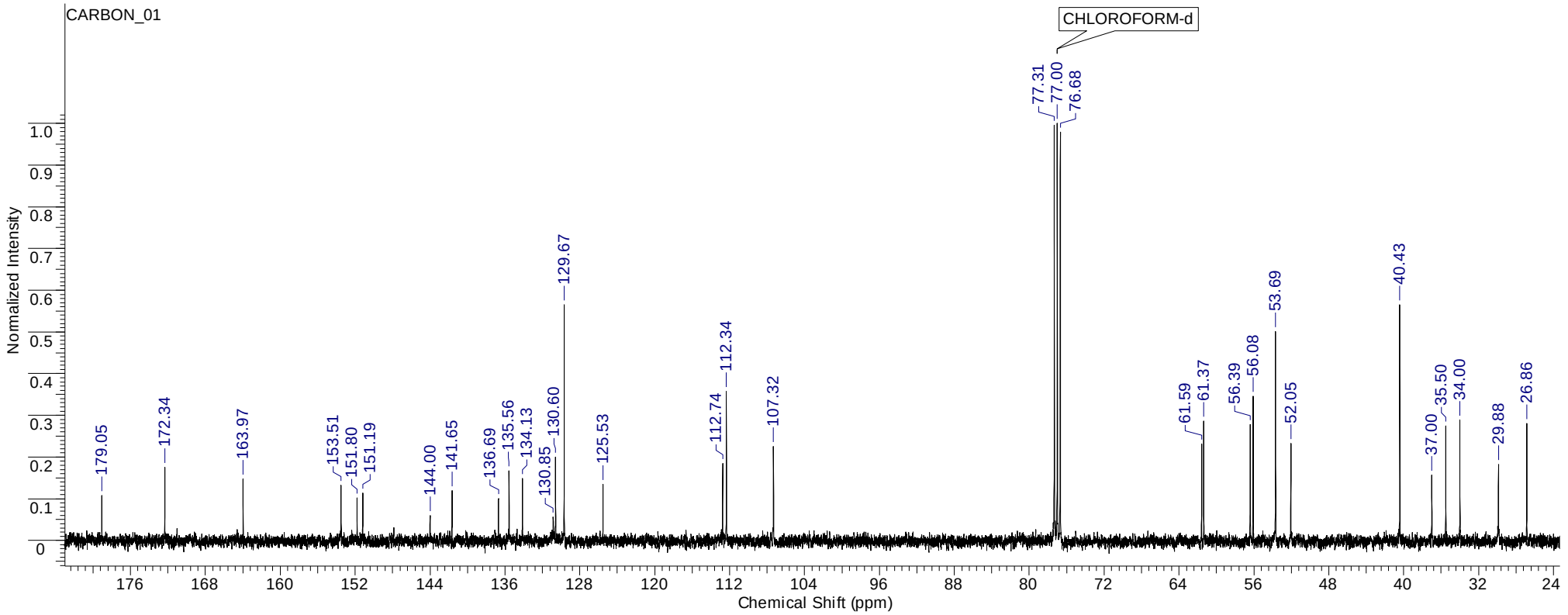
1. O. Trott and A. J. Olson, *J. Comput. Chem*., 2010, **31**, 455.
2. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin, *J. Comput. Chem*., 2004, **25**, 1605.
3. J. D. Bagnato, A. L. Eilers, R. A. Horton and C. B. Grissom, *J. Org. Chem*., 2004, **69**, 8987.
4. J. W. Hill and W. H. Carothers, *J. Am. Chem. Soc*., 1933, **55**, 5023.
5. O.N. Zefirova, E.V. Nurieva, B. Wobith, V. V. Gogol, N. A. Zefirov, A. V. Ogonkov, D. V. Shishov, N. S. Zefirov and S. A. Kuznetsov, *Mol. Diversity*, 2017, **21**, 547.
6. D. Meksuriyen, L.-J. Lin, G. A. Cordell, S. Mukhopadhyay and S. K. Banerjee, *J. Nat. Prod*. 1988, **51**, 88.
7. D. Gerlier and N. Thomasset, *J. Immunol. Methods*, 1986, **94**, 57.
8. M. A. Kalinina, D. A. Skvortsov, M. P. Rubtsova, E. S. Komarova and O. A. Dontsova, *Mol. Imaging Biol.* 2018, **20**, 368.
9. A. Al-Haddad, M. A. Shonn, B. Redlich, A. Blocker, J. K. Burkhardt, H. Yu, J. A. Hammer 3rd, D, G. Weiss, W. Steffen, G. Griffiths and S. A. Kuznetsov, *Mol. Biol. Cell*, 2001, **12**, 2742.

^{1}H and ^{13}C NMR spectra of the compounds

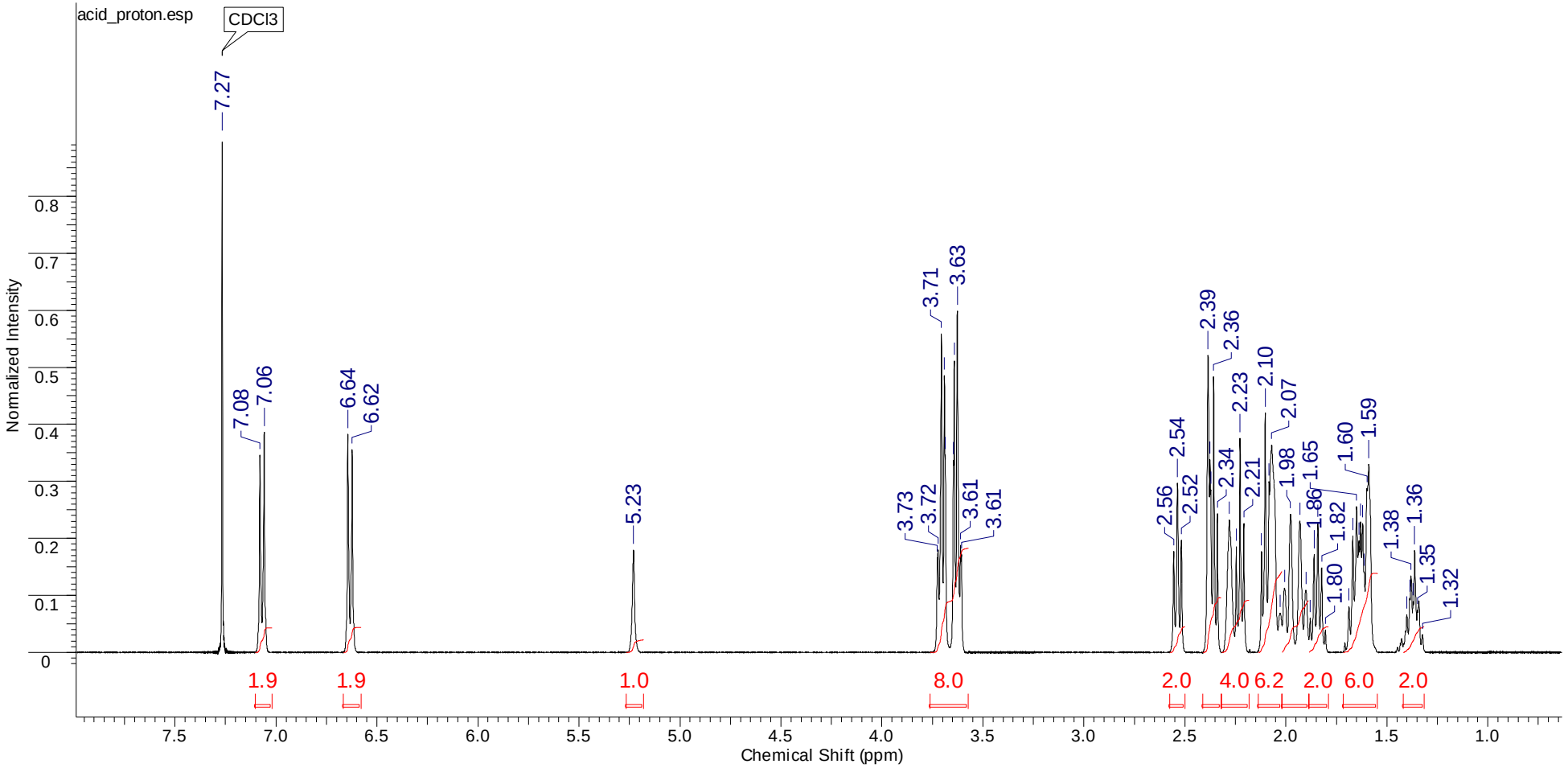
*^{1}H NMR spectrum of N-{4-[4-(bis(2-chloroethyl)amino)phenyl]butanoyl}-N-deacetylcolchicine (**4**)*



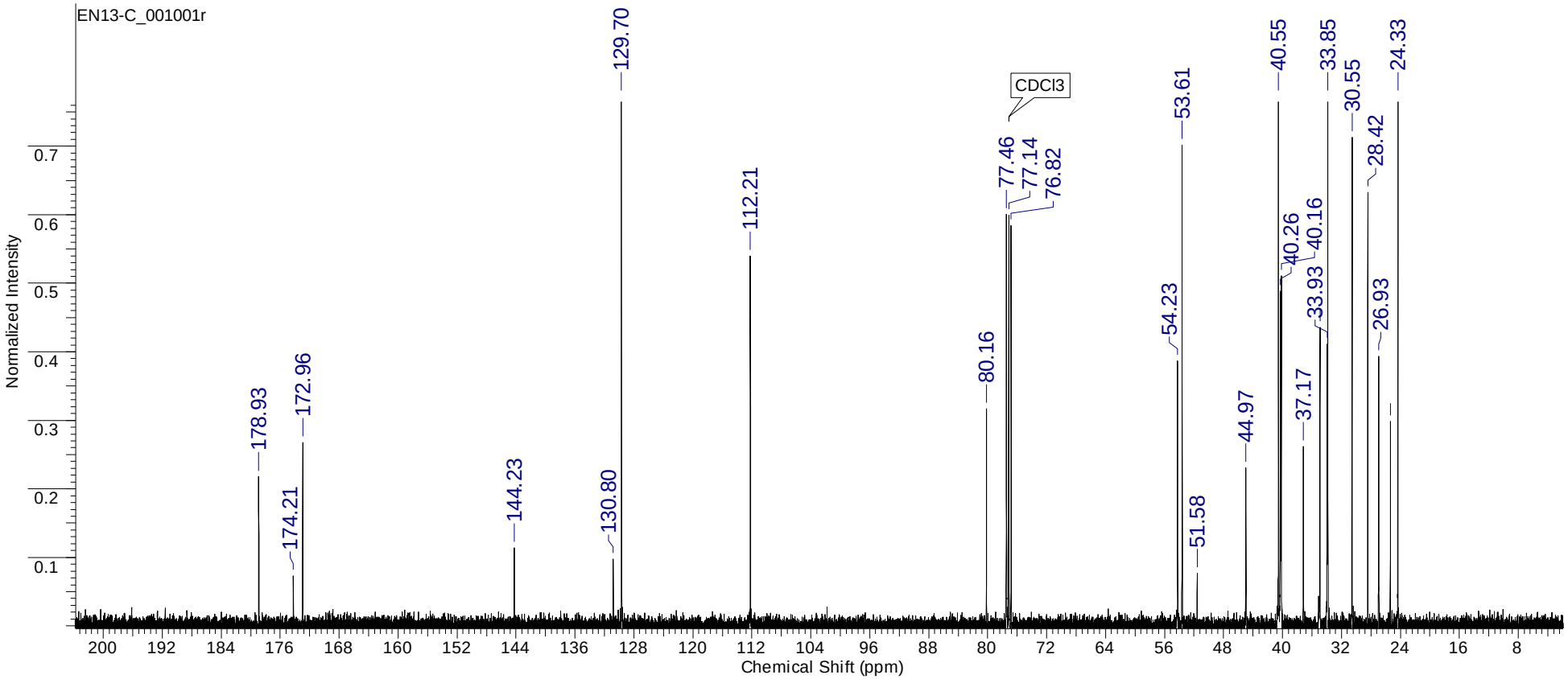
*^{13}C NMR spectrum of N-{4-[4-(bis(2-chloroethyl)amino)phenyl]butanoyl}-N-deacetylcolchicine (**4**)*



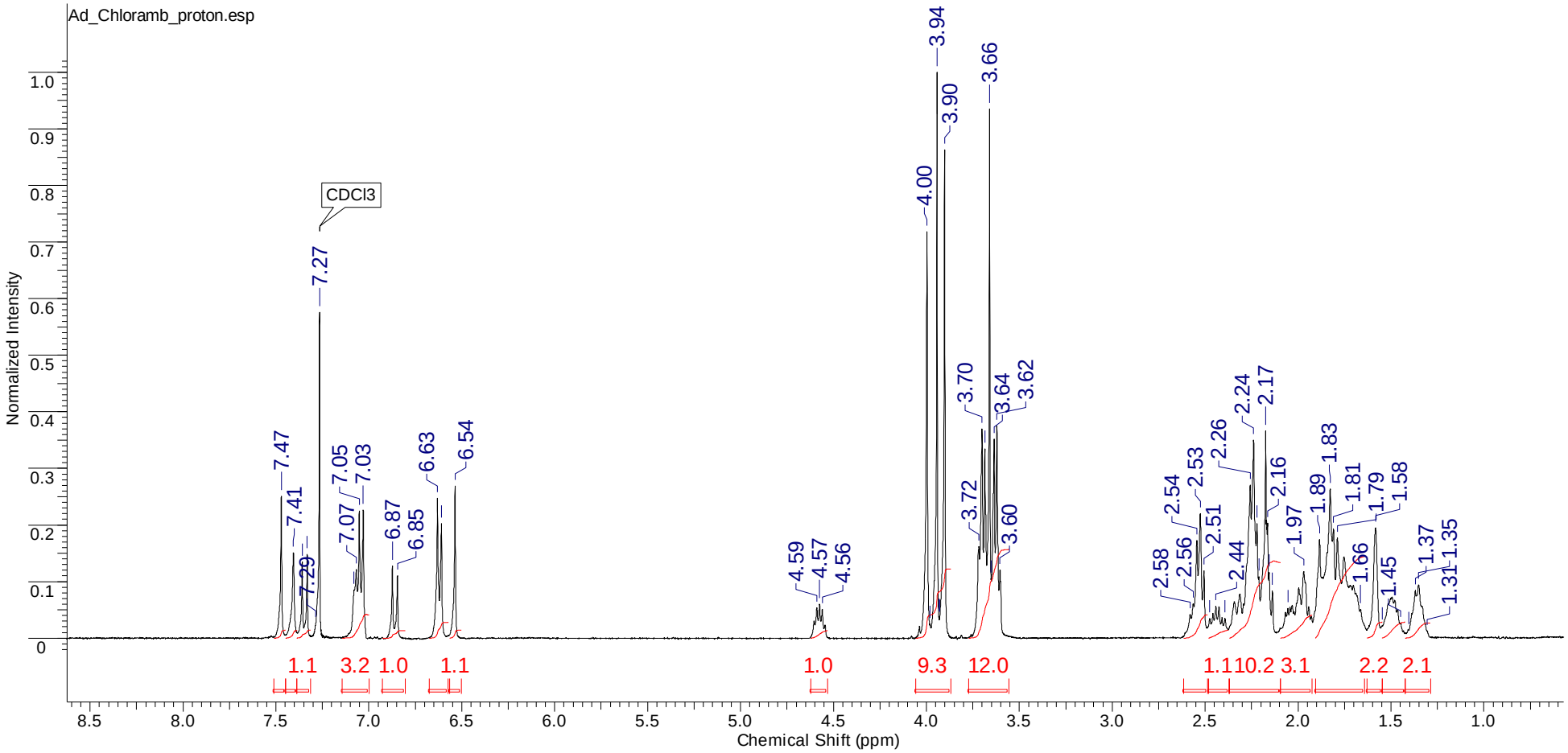
^{1}H NMR spectrum of 7-[3-((4-(4-(bis(2-chloroethyl)amino)phenyl)butanoyl)oxy)-adamant-1-ylamino]-7-oxoheptanoic acid (7)



^{13}C NMR spectrum of 7-[3-((4-(4-(bis(2-chloroethyl)amino)phenyl)butanoyl)oxy)-adamant-1-ylamino]-7-oxoheptanoic acid (7)



*^{1}H NMR spectrum of N-{7-[3-((4-(4-(bis(2-chloroethyl)amino)phenyl)butanoyl)oxy)-adamant-1-ylamino]-7-oxoheptanoyl}-N-deacetylcolchicine (**8**)*



^{13}C NMR spectrum of N-{7-[3-((4-(4-(bis(2-chloroethyl)amino)phenyl)butanoyl)oxy)-adamant-1-ylamino]-7-oxoheptanoyl}-N-deacetylcolchicine ***(8)***