Supporting Information

Cobalt bis(dicarbollide) is a DNA-neutral pharmacophore

Krzysztof Fink*[,†,‡], Jakub Cebula[†,‡], Zdeněk Tošner[§], Mateusz Psurski[‖], Mariusz Uchman[‡], and Tomasz M. Goszczyński[†]

[†] Laboratory of Biomedical Chemistry, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 12 Rudolf Weigl St., 53-114 Wrocław, Poland

[‡] Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Hlavova 2030, 128 40 Prague 2, Czechia

[§] Department of Chemistry, Faculty of Science, Charles University, Hlavova 2030, 128 40 Prague 2, Czechia

[‖] Laboratory of Experimental Anticancer Therapy, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 12 Rudolf Weigl St., 53-114 Wrocław, Poland

Contents

1. Materials and equipment

Cs[3-cobalt bis(1,2-dicarbollide)] was purchased from Katchem (Prague, Czech Republic). The cesium salt of Cs[3-cobalt bis(1,2-dicarbollide)] was converted to sodium salt (Na[COSAN]) and potassium salt (K[COSAN]) according to the published procedure[1] using a cationic exchange resin (Amberlite IR120, Acros Organics, US). Calf thymus DNA (ctDNA) and oligonucleotide (5'-CGCGAATTCGCG-3') were purchased from Merck Life Science Sp.z.o.o. (Poznań, Poland). Dialysis tubes (MWCO 3500 Da) were from Serva Electrophoresis GmbH (Heidelberg, Germany), and sodium dihydrogen phosphate dihydrate was purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland). Rapid Equilibrium Dialysis (RED) plates were purchased from VWR International Sp. z o.o. (Poland). All other chemicals were purchased from Merck Life Science and used without further purification. All solutions were prepared in Milli-Q (MQ) water (18.2 M cm^{-1}) from a Direct-Q3 UV system (Millipore, US).

Absorption and circular dichroism (CD) spectra were recorded on a J-1500 spectropolarimeter (Jasco, Japan) equipped with a thermostated cell holder and PML-534 FDCD detector at 25 °C. For DNA melting temperature studies, absorption spectra were also recorded in the temperature range of 25 to 90 °C. Measurements were performed in rectangular quartz cuvettes with 0.1 cm and 1 cm optical path lengths for measurements in the range of 220 – 320 nm and 380 – 600 nm, respectively.

Linear dichroism spectra were recorded on a J-1500 spectropolarimeter (Jasco, Japan) equipped with a CFC-573 couette flow cell unit at ambient temperature. Samples were oriented with a flow couette cell with an inner rotating cylinder and an experimental path length of 0.05 cm at a spinning speed of 2800–2900 RPM.

Differential scanning calorimetry (DSC) experiments were carried out in a Nano DSC (TA, Waters LLC, New Castle, USA) with a nominal cell volume of 0.3 mL. The sample solutions were previously degassed in a degassing station to minimize the formation of bubbles upon heating (TA, Waters LLC, New Castle, USA).

Viscosity was measured on an m-VROC® viscometer (RheoSense, Inc., USA) equipped with a Thermo Cube Liquid–Liquid (Solid State Cooling Systems, USA) at 25.00 ± 0.05 °C. The flow rate was 500 µl/min, the measurement time was 10.0 s, and the waiting time was 3.0 s.

Isothermal titration calorimetry (ITC) measurements were conducted on a Nano ITC Low Volume calorimeter (TA Instruments, USA). The calorimeter consists of a sample cell and a reference cell (24K gold). The sample cell is connected to a 50 µl syringe with a flattened, twisted paddle at the tip, which ensures continuous mixing of the solutions. The syringe stirring rate was 350 RPM. The reference cell was filled with Milli-Q water. The sample cell (170 µl) was filled with buffer or ctDNA solution.

Equilibrium dialysis experiments were conducted using Rapid Equilibrium Dialysis (RED) plate with a molecular-weight cutoff (MWCO) = 8 kDa. Each plate contained 48 pairs of side-by-side chambers (buffer and sample chamber) separated by a dialysis membrane. The concentration of COSAN in each chamber was determined by HPLC. HPLC analyses were carried out using the Ultimate 3000 RS HPLC system (Dionex, Sunnyvale, CA) equipped with a DAD detector.

DOSY spectra were acquired on a Bruker Avance III 600 MHz spectrometer equipped with a cryogenically cooled TCI probe using the dstebpgp3s pulse program with the diffusion time set to 0.1 s, gradient length 1250 µs, and 32 linearly spaced gradient amplitude values. Spectra were integrated and fitted to the Stejskal-Tanner formula using Topsin 3.4 spectrometer software. The doped 10% H_2O/90% D_2O standard sample was used to calibrate the evaluation procedure to yield a diffusion constant of 1.9×10^{-9} m^2s^{-1}. The samples were equilibrated for at least 15 min, and the temperature was controlled within ±0.1 degrees.

2. Methods

2.1. Sample preparation

Calf thymus DNA (ctDNA) solution preparation: ctDNA was dissolved in MQ water with gentle shaking and kept overnight at 4 °C to dissolve to a clear solution. Next, the solution was dialyzed in a dialysis tube with MWCO 3500 Da against 20 mM phosphate buffer with 150 mM NaCl and 1 mM EDTA, pH 7.4. The concentrations of ctDNA were calculated from the absorbance at 260 nm using ε_{260nm} = 6600 $M^{-1}cm^{-1}$ per base (pb). [2] The A_{260nm}/A_{280nm} was >1.8, indicating that ctDNA samples were sufficiently free from proteins. [3,4] The stock solution was stored at 4 °C.

Oligonucleotide (oligoDNA) solution preparation: Oligonucleotide 5'-CGCGAATTCGCG-3' (oligoDNA) was dissolved in 10 mM phosphate buffer with 100 mM NaCl and 0.1 mM EDTA, pH 7.0. The concentration of oligoDNA was calculated from the absorbance at 260 nm using ε_{260nm} = 110,700 $M^{-1}cm^{-1}$ per DNA single strand. The stock solution was stored at –20 °C.

KCOSAN stock preparation: The KCOSAN stock solution was prepared in MQ water.

NaCOSAN stock preparation: The NaCOSAN stock solution was prepared in 20 mM phosphate buffer with 150 mM NaCl and 1 mM EDTA, pH 7.4. The concentration of NaCOSAN was determined by UV–Vis spectroscopy using ε_{282nm} = 30,866 $M^{-1}cm^{-1}$.

2.2. Determination of molar extinction coefficient for COSAN

To determine the molar extinction coefficient for COSAN, we used potassium salt of COSAN (KCOSAN), which is water-free. [1] Thus, the mass concentration of KCOSAN can be directly recalculated to its molar concentration. Absorption spectra were recorded from 220 nm to 320 nm at increasing concentrations of KCOSAN (from 0.001 to 0.03 mM). Additionally, absorption spectra were recorded from 380 nm to 600 nm at increasing concentrations of KCOSAN (from 0.1 to 2.5 mM). All samples were in MQ water. The spectra were recorded with a data pitch of 0.2 nm, bandwidth of 1 nm, and data integration time of 1 sec at 50 nm min^{-1} (Fig. S1A and C). The spectra were corrected by the water blank. The absorption intensities at the absorption maxima (λ_{max}) for each wavelength range – λ_{max} = 282 nm and 448 nm for 220 to 320 nm and 380 to 600 nm, respectively – were plotted against the concentration of KCOSAN (Fig. S1B and D). The molar extinction coefficients (ε_{282nm} and ε_{448nm}) were determined from the slopes of the lines after linear regression analysis.

2.3. UV–Vis and circular dichroism measurements

Absorption and CD spectra were recorded from 220 nm to 320 nm at a constant ctDNA concentration of 0.4 mM (per base, pb) and varying NaCOSAN concentrations (from 0 to 0.4 mM). Additionally, absorption spectra were recorded from 380 nm to 550 nm at a constant NaCOSAN concentration of 1.5 mM and varying ctDNA concentrations (from 0 to 2.25 mM (pb)). All samples were in 20 mM phosphate buffer with 150 mM NaCl and 1 mM EDTA, pH 7.4. Twelve spectra (recorded with a data pitch of 1 nm, bandwidth of 1 nm, and data integration time of 1 sec at 50 nm min^{-1}) were averaged for each sample. Each spectrum was corrected by the corresponding concentration of NaCOSAN (for measurements in the 220 – 320 nm range) or by the buffer blank (for measurements in the 380 – 600 nm range).

2.4. UV–Vis and linear dichroism measurements

Absorption and LD spectra were recorded from 220 nm to 320 nm at a constant ctDNA concentration of 1.0 mM (pb) and varying NaCOSAN concentrations (from 0 to 0.7 mM). All samples were in 20 mM phosphate buffer with 150 mM NaCl and 1 mM EDTA, pH 7.4. Four spectra (recorded with a data pitch of 0.2 nm, bandwidth of 1 nm, and data integration time of 1 sec at 100 nm min^{-1}) were averaged for each sample. LD spectra were recorded at a couette cell spinning speed of 2800–2900 RPM and corrected by the measurement of the same sample at a spinning speed of 0 RPM. Absorption spectra were recorded at a couette cell spinning speed of 2800–2900 RPM and corrected by the buffer blank.

2.5. Viscosity

Viscosity measurements were conducted for buffer (20 mM phosphate buffer with 150 mM NaCl and 1 mM EDTA, pH 7.4) alone in triplicate and at a constant ctDNA concentration of 0.24 mM (pb) and increasing concentrations of NaCOSAN (from 0 to 0.12 mM) in four replicates for each COSAN concentration (Table S2). Intrinsic viscosity was calculated by subtracting values of measured viscosity for samples with the average of three replicates of measured viscosity for the buffer (Table S3). The values of relative intrinsic viscosity $(\eta/\eta_0)^{1/3}$, where η_0 is the intrinsic viscosity of ctDNA in the absence of NaCOSAN and η is the intrinsic viscosity of ctDNA in the presence of NaCOSAN (Table S4), were plotted against [NaCOSAN]/[ctDNA(pb)].

2.6. Differential scanning calorimetry (DSC)

A scan rate of 1 °C min^{-1} was used from 20 to 120 °C at 3 atm. pressure was chosen for the experiments. Raw data were corrected by subtraction of a buffer–buffer baseline to eliminate instrumental effects (Fig. S5). The excess heat capacity function (μJ/s) was converted to molar heat capacity (kJ/molꞏK). The DSC scans of molar heat capacity versus temperature were analyzed using NanoAnalyze software. A polynomial baseline was constructed and subtracted from the data. Five Gaussian models were used to deconvolute and fit the data.

2.7. Melting temperature

Melting temperatures T_m for free oligoDNA (0.05 mM (pb)) and oligoDNA in the presence of NaCOSAN (0.01 mM) were measured by following the changes in absorption at 260 nm as a function of temperature. All experiments were conducted in 10 mM phosphate buffer with 100 mM NaCl and 0.1 mM EDTA, pH 7.0. The absorption intensities at 260 nm were normalized using the equation:

$$f(ss) = (A-A_0)/(A_f-A_0) \quad (1)$$

where A is the absorption at 260 nm at temperatures between 25 and 90 °C, A_0 is the absorption at 260 nm at 25 °C and A_f is the absorption at 260 nm at 90 °C. The normalized values were plotted against

individual temperatures, and nonlinear fit analysis using GraphPad Prism 7.0 software was conducted to calculate DNA melting temperatures (T_m).

2.8. Isothermal titration calorimetry

NaCOSAN at a concentration of 5.0 mM was titrated to either ctDNA (1.0 mM (pb)) or a buffer. All measurements were conducted at 298.15 K. Twenty-five 2 µl injections were conducted into the cell with 300 s intervals between each injection. All solutions were degassed before use. ITC data were imported into NanoAnalyze software. The titration baseline was corrected, and the mean enthalpy per injection was calculated using the Blank (constant) model in the software.

2.9. Equilibrium dialysis

The solutions of 0.05 mM NaCOSAN containing different concentrations of ctDNA (0, 0.01, 0.05, 0.2, and 1.0 mM (pb)) were placed in sample chambers of the RED plate and were dialyzed against buffer (20 mM phosphate buffer with 150 mM NaCl and 1 mM EDTA, pH 7.4) placed in buffer chambers. The plate was covered with a gas-permeable membrane and agitated at 350 RPM for 24 hours at RT. Fifty-microliter aliquots were removed from both chambers. A solution of 0.05 mM NaCOSAN and 0.05 mM human serum albumin (HSA) served as a positive control since COSAN has a high affinity for HSA. [5] Equilibrium was achieved within 24 h. The total concentration of COSAN (C_t) and concentration of COSAN in the buffer chamber (C_b) and sample chamber (C_s) were determined from HPLC analysis (Fig. S7–S18). Samples were diluted 10 times with 0.1% TFA and were analyzed by HPLC for COSAN levels. A Vanquish C18+ (1.5 µm, 2.1 x 50 mm) column was used for sample analysis. The mobile phases consisted of 0.1% trifluoroacetic acid (TFA) in water or acetonitrile. The flow rate was 0.4 ml/min with gradient elution from 5 to 95% B in 10 minutes.

The recovery was calculated from the equation:

$$\%Recovery = \frac{C_b \times V_b + C_s \times V_s}{C_t \times V_t} \quad (2)$$

where V_b, V_s, and V_t are the volumes of samples in the buffer chamber and sample chamber after dialysis and the volume added to the sample chamber before dialysis, respectively. [6] The calculated values of recovery were from 24 to 32% for chambers with free COSAN and with COSAN and ctDNA and from 65 to 70% for the chamber with COSAN and HSA. The fraction of bound COSAN was determined from the equation:

$$\%Bound = \left(1 - \frac{C_b}{C_s}\right) \times 100\% \quad (3)$$

Experiments were performed in three replicates.

2.10. NMR

DOSY spectra were acquired on a Bruker Avance III 600 MHz spectrometer equipped with a cryogenically cooled TCI probe using the dstebpgp3s pulse program with the diffusion time set to 0.1 s, gradient length 1250 µs, and 32 linearly spaced gradient amplitude values. Spectra were integrated and fitted to the Stejskal-Tanner formula using Topsin 3.4 spectrometer software. The doped 10% H_2O/90% D_2O standard sample was used to calibrate the evaluation procedure to yield a diffusion constant of 1.9×10^{-9} m^2s^{-1}. The samples were equilibrated for at least 15 min, and the temperature was controlled within ±0.1 degrees.

2.11. Statistical analysis

ANOVA tests were performed to compare three or more groups, a nonlinear fit was performed to determine oligoDNA melting temperature, and linear regressions were performed to calculate the COSAN extinction coefficient using GraphPad Prism 7.0 (GraphPad Software). For the statistical analyses, $p < 0.05$ was considered significant.

2.12. Cell culture lines and culture conditions

A549 (human lung carcinoma) and PC-3 (human prostate adenocarcinoma) cells were purchased from the European Collection of Authenticated Cell Cultures (ECACC; Porton Down, UK). UM-UC-3 (human urinary bladder transitional cell carcinoma), MCF 10A (nontumorigenic human mammary gland cells), MV 4 11 (human biphenotypic leukemia) and Balb/3T3 (nontumorigenic murine fibroblasts) cells were purchased from the American Type Culture Collection (ATCC; Rockville, USA). All cell lines were maintained at the Hirszfeld Institute of Immunology and Experimental Therapy (HIIET), Wrocław, Poland. The Balb/3T3 and UM-UC-3 cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM; Life Technologies, Scotland) supplemented with 10% (v/v) fetal bovine serum (FBS; GE Healthcare HyClone, Logan, USA) and 2 mM L-glutamine (Sigma–Aldrich, Poznań, Poland). The A549 cell line was cultured in OptiMEM (HIIET, PAS, Wroclaw, Poland) supplemented with 5% (v/v) FBS (GE Healthcare HyClone, Logan, USA) and 2 mM L-glutamine. The MV-4-11-cell line was cultured in RPMI 1640 (Thermo Fisher Scientific) supplemented with 10% (v/v) FBS, 2 mM L-Gln and 1 mM sodium pyruvate (Sigma–Aldrich, Poznań, Poland). The PC-3 cell line was cultured in RPMI 1640 (Thermo Fisher Scientific) supplemented with 10% (v/v) FBS (GE Healthcare HyClone, Logan, USA) and 2 mM L-Gln (Sigma–Aldrich, Poznań, Poland). The MCF 10A cell line was cultured in Ham's F12 medium with glutamine (Corning) supplemented with 5% (v/v) FBS, 5% (v/v) horse serum, 10 µg/mL insulin, 0.05 µg/mL cholera toxin, 0.5 µg/mL hydrocortisone, and 20 ng/mL hEGF (all from Sigma–Aldrich). All culture media were supplemented with antibiotics – 100 µg/mL streptomycin (Polfa Tarchomin, Warsaw, Poland) and 100 U/mL penicillin (Sigma–Aldrich, Poznań, Poland). The cells were grown at 37 °C in a humid atmosphere saturated with 5% CO_2.

2.13. Antiproliferative activity assessment by sulforhodamine B assay

The cells were seeded on 384-well plates (Greiner Bio One, Kremsmünster, Austria) at 1×10^3 cells/well density for the A-549, PC-3, and UM-UC-3 cell lines and 2×10^3 cells/well for the MCF-10A and Balb/3T3 cell lines. After overnight incubation, compounds were applied at various concentrations (ranging from 50 µM to 0.01 nM). After 72 h of incubation, the sulforhodamine B (SRB) assay based on Skehan et al. [7] was carried out with slight modifications. In brief, 50 µL of medium was replaced with 30 µL/well of 25% (w/v) trichloroacetic acid (Avantor Performance Materials, Gliwice, Poland). After 1 h of incubation at room temperature, the plates were washed three times with tap water, and 20 µL of a 0.1% (w/v) solution of sulforhodamine B (Sigma–Aldrich, Poznan, Poland) in 1% (v/v) acetic acid (Avantor Performance Materials, Gliwice, Poland) was added to each well. After 30 min of incubation at room temperature, unbound dye was washed out with 1% (v/v) acetic acid. Bound dye was solubilized with 70 µL of 10 mM unbuffered TRIS (Avantor Performance Materials, Gliwice, Poland) solution. The procedure was performed using a BioTek EL-406 washing station (BioTek Instruments, USA). Absorbance was read using a Biotek Hybrid H4 reader (BioTek Instruments, USA) at 540 nm wavelength. Crude absorbance data were used to calculate proliferation inhibition using the following formula:

$$\%Inh = \left[\left(\frac{A_p - A_m}{A_k - A_m}\right) \times 100\right] - 100 \quad (4)$$

where:
Am - absorbance for cell-free wells,
Ak - absorbance for vehicle-treated, control wells,
Ap - absorbance for compound-treated wells;
The %Inh was next used for IC_{50} calculations performed in GraphPad Prism 7.05 (GraphPad Software, Inc.) utilizing the '[Inhibitor] vs. response – Variable slope (four parameters)' model.

2.14. Antiproliferative activity assessment by MTT assay

MV-4-11 cells were seeded on 384-well plates (Greiner Bio-One, Kremsmünster, Austria) at a density of 1×10^3 cells/well. After overnight incubation, compounds were applied at various concentrations (ranging from 100 µM to 0.03 nM). After 72 h of incubation, the MTT assay based on Mosmann, T. [8] was carried out with slight modifications. In brief, 20 µL of MTT (Sigma–Aldrich, Poznań, Poland) solution in PBS (5 mg/mL) was added to each well, and after 4 h of incubation, the plates were gently centrifuged (rt, 5 min, 300×g). The culture medium was gently removed using a BioTek EL-406 washing station, and the remaining formazan was solubilized in DMSO (Avantor Performance Materials, Gliwice, Poland; 75 µL/well). After 1 h of incubation, the absorbance was read using a Biotek Hybrid H4 reader (BioTek Instruments, USA) at a wavelength of 570 nm. Crude absorbance data were used to calculate proliferation inhibition using the formula (4).

2.15. Hemolytic Assay

The hemolytic activity of the metallacarborane derivatives was evaluated as the amount of hemoglobin released by the disruption of mouse red blood cells (mRBCs) using a modified method described by Oddo et al. [9]. Erythrocytes were collected by centrifugation at 1500 × g for 15 min, washed two times with PBS (10 mM, pH 7.4) and suspended to a final concentration of 5% v/v. Then, an equal volume of erythrocyte suspension and peptide solution with various concentrations was mixed in 96-well plates and incubated for 1 h at 37 °C. After centrifugation at 1000×g for 5 min, the absorbance of the supernatant was measured at 490 nm to monitor the release of hemoglobin using a microplate reader (Biotek SynergyH4 Hybrid Reader). Untreated erythrocytes and erythrocytes treated with 1% Triton X-100 were employed as negative and positive controls, respectively. The hemolysis percentage was calculated from the following equation:

$$Hemolysis\ rate\ (\%) = \frac{OD_{490nm\ (compounds)} - OD_{490nm\ (negative\ control)}}{OD_{490nm\ \ (positive\ control)} - OD_{490nm\ (negative\ control)}} \quad (5)$$

Half maximal effective concentrations (EC_{50}) were defined as the compound concentrations causing 50% hemolysis. The experiments were performed in triplicate.

3. Results

3.1. Determination of molar extinction coefficient for COSAN

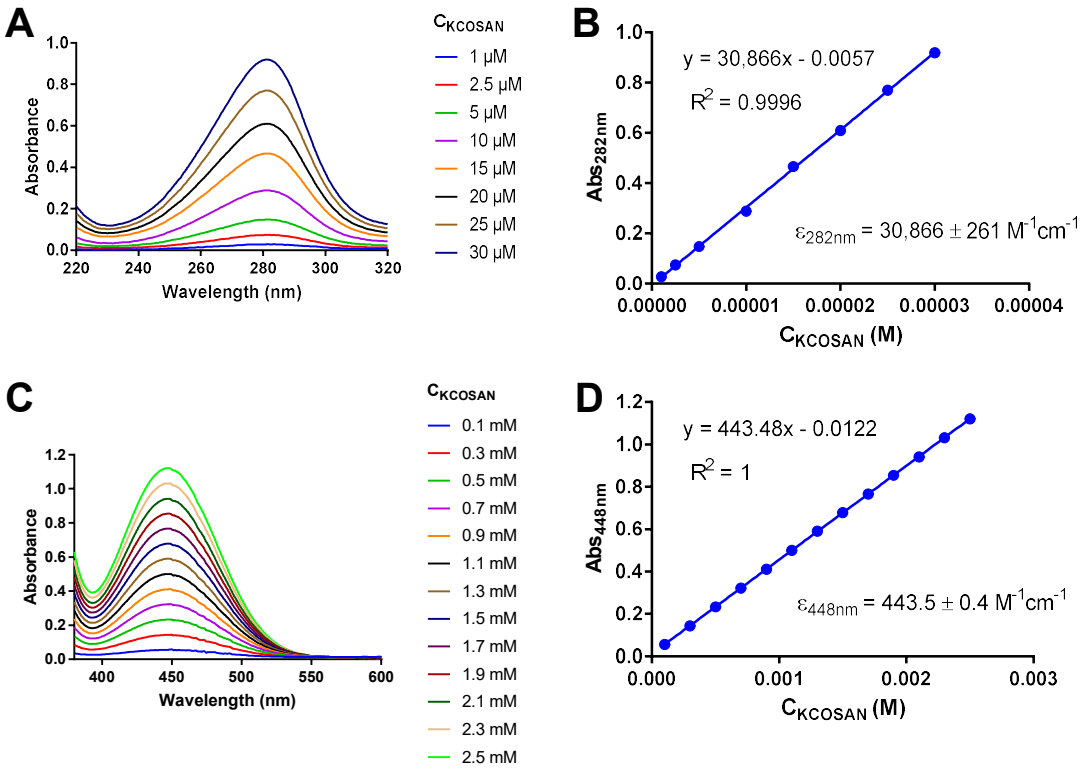


Figure S1. (**A**) Absorption spectra of KCOSAN (1 to 30 µM). (**B**) Plot of the absorbance at 282 nm versus KCOSAN concentration. (**C**) Absorption spectra of KCOSAN (0.1 to 2.5 mM). (**D**) Plot of the absorbance at 448 nm versus KCOSAN concentration. The absorption spectra were measured in MQ water.

3.2. Circular dichroism (CD) measurements using a double-couvette system

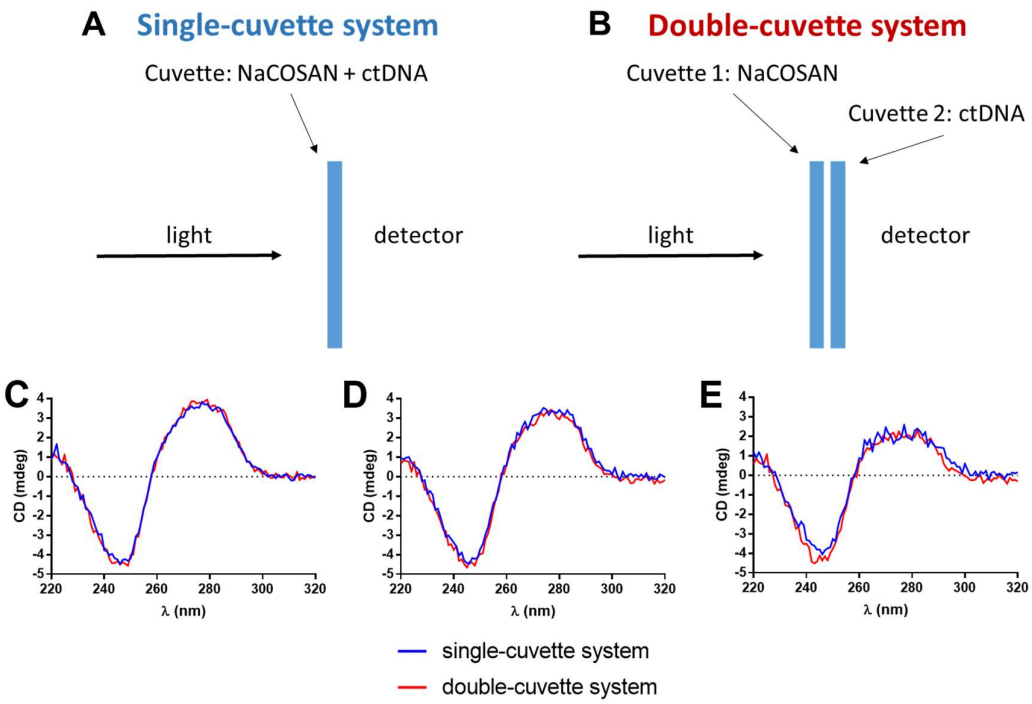


Figure S2. (**A**) Single-cuvette system (SCS), in which a single cuvette contained a solution of ctDNA with different COSAN concentrations. (**B**) Double-cuvette system (DCS), in which a cuvette closer to the light source (cuvette 1) contained a solution with different concentrations of COSAN and a cuvette further from the light source (cuvette 2) contained a solution of ctDNA. Circular dichroism (CD) spectra of (**C**) SCS: ctDNA (0.4 mM (pb)), DSC: cuvette 1 contained the buffer and cuvette 2 contained solution of ctDNA (0.4 mM (pb)); (**D**) SCS: ctDNA in presence of NaCOSAN (0.2 mM), DCS: cuvette 1 contained solution of NaCOSAN (0.2 mM) and cuvette 2 contained solution of ctDNA (0.4 mM (pb)) and (**E**) SCS: ctDNA in presence of NaCOSAN (0.4 mM), DCS: cuvette 1 contained solution of NaCOSAN (0.4 mM) and cuvette 2 contained solution of ctDNA (0.4 mM (pb)). All spectra were measured in 20 mM phosphate buffer with 150 mM NaCl and 1 mM EDTA, pH 7.4.

3.3. Absorption and circular dichroism (CD) spectra of ctDNA in the presence of COSAN after seven days of incubation

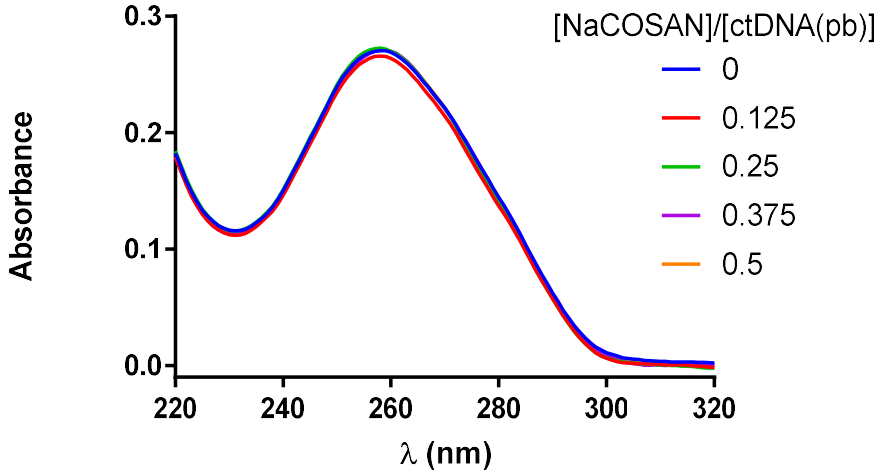


Figure S3. Absorption spectra of (**A**) ctDNA (0.4 mM (pb)) in the presence of increasing concentrations of NaCOSAN (0 to 0.4 mM) for samples incubated for seven days at room temperature. All spectra were measured in 20 mM phosphate buffer with 150 mM NaCl and 1 mM EDTA, pH 7.4.

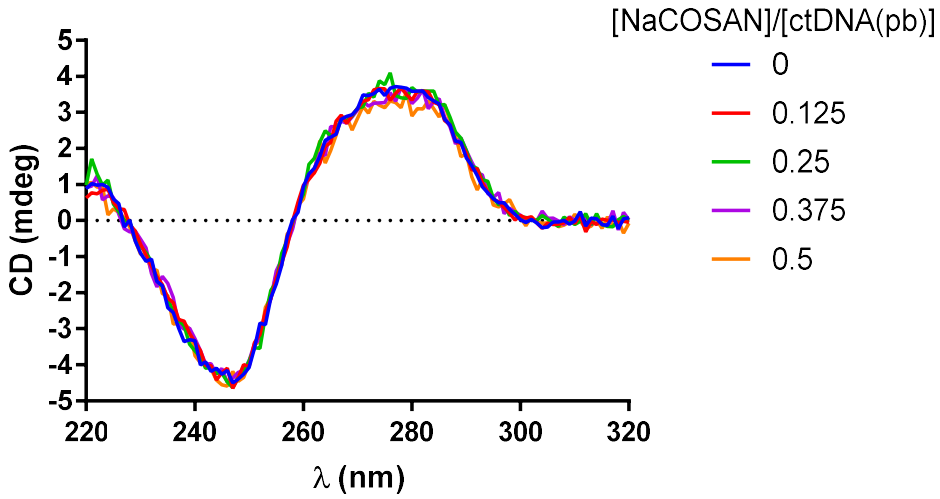


Figure S4. Circular dichroism (CD) spectra of (**A**) ctDNA (0.4 mM (pb)) in the presence of increasing concentrations of NaCOSAN (0 to 0.4 mM) for samples incubated for seven days at room temperature. All spectra were measured in 20 mM phosphate buffer with 150 mM NaCl and 1 mM EDTA, pH 7.4.

3.4. Differential scanning calorimetry (DSC) measurements of ctDNA in the presence of COSAN

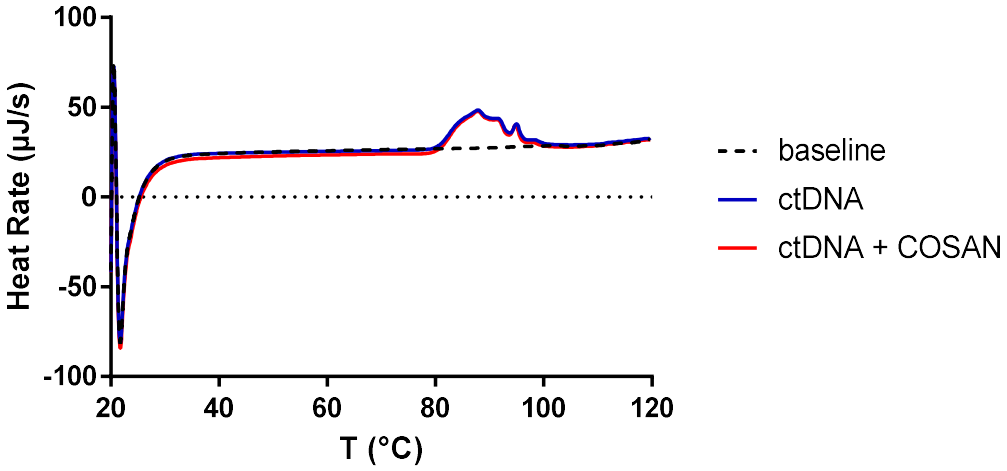


Figure S5. DSC thermograms before baseline subtraction of free ctDNA (3.0 mM (pb)) and ctDNA with NaCOSAN (0.4 mM); temperature range 20 to 120 °C, heating rate 1 °C/min, pressure 3 atm.

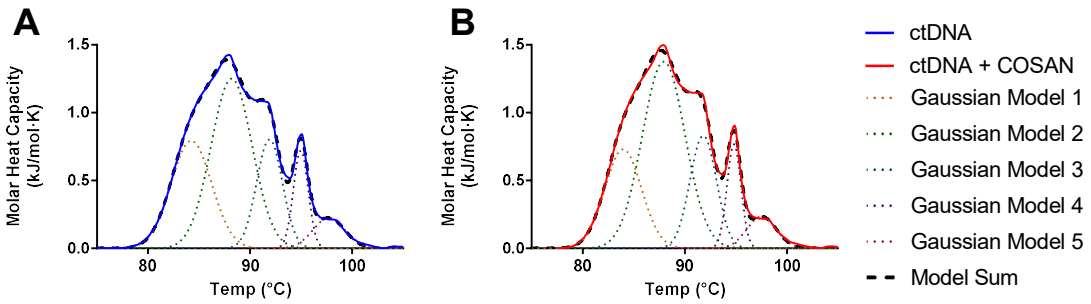


Figure S6. DSC thermograms with melting curves of free ctDNA (3.0 mM (pb)) and ctDNA with NaCOSAN (0.4 mM) with DSC thermograms after deconvolution and fitting using five Gaussian models.

Table S1. Parameters calculated from deconvolution and fitting using five Gaussian models of DSC thermograms of free ctDNA (3.0 mM (p)) and ctDNA with NaCOSAN (0.4 mM).

Gaussian Model No.	Parameters	ctDNA	ctDNA + COSAN
1	Amplitude	0.7934 ± 0.0627	0.7299 ± 0.0427
	FWHM (°C)	4.66 ± 0.248	4.244 ± 0.104
	T (°C)	84.25 ± 0.222	83.94 ± 0.11
	Area	3.936	3.298
2	Amplitude	1.2502 ± 0.056	1.3763 ± 0.0203
	FWHM (°C)	4.732 ± 0.225	4.92 ± 0.162
	T (°C)	88.02 ± 0.149	87.91 ± 0.075
	Area	6.297	7.208
3	Amplitude	0.8028 ± 0.0342	0.829 ± 0.0269
	FWHM (°C)	2.967 ± 0.125	3.033 ± 0.074
	T (°C)	91.91 ± 0.054	91.76 ± 0.034
	Area	2.535	2.677
4	Amplitude	0.7207 ± 0.0257	0.7665 ± 0.0168
	FWHM (°C)	1.711 ± 0.066	1.614 ± 0.044
	T (°C)	94.97 ± 0.034	94.77 ± 0.019
	Area	1.312	1.316
5	Amplitude	0.2253 ± 0.0113	0.2355 ± 0.0085
	FWHM (°C)	3.663 ± 0.213	3.393 ± 0.12
	T (°C)	97.73 ± 0.187	97.49 ± 0.084
	Area	0.878	0.851

(Confidence Level = 95%)

3.5. Viscosity measurements of ctDNA in the presence of COSAN

Table S2. Values of viscosity measured for the buffer, free ctDNA (0.24 mM (pb)), and ctDNA (0.24 mM (pb)) in the presence of increasing concentrations of NaCOSAN (0 to 0.12 mM). The average measured viscosity was calculated for the buffer and was further used for calculations of intrinsic viscosities (Table S3). All viscosities were measured in 20 mM phosphate buffer with 150 mM NaCl and 1 mM EDTA, pH 7.4.

Sample	Measured viscosity (mPa*s)				Measured viscosity (mPa*s)	
					Average	St. dev.
buffer	0.927	0.926	0.922		0.925	0.002
ctDNA	0.972	0.973	0.981	0.977		
ctDNA+COSAN (20 µM)	0.975	0.977	0.976	0.976		
ctDNA+COSAN (40 µM)	0.975	0.979	0.975	0.976		
ctDNA+COSAN (60 µM)	0.976	0.975	0.971	0.976		
ctDNA+COSAN (80 µM)	0.975	0.971	0.973	0.972		
ctDNA+COSAN (100 µM)	0.972	0.967	0.973	0.969		
ctDNA+COSAN (120 µM)	0.977	0.975	0.971	0.975		

Table S3. Values of intrinsic viscosity for free ctDNA (0.24 mM (pb)) and ctDNA (0.24 mM (pb)) in the presence of increasing concentrations of NaCOSAN (0 to 0.12 mM). Average intrinsic viscosity was calculated for free ctDNA and was further used for calculations of values of $(\eta/\eta_0)^{1/3}$ (Table S4).

Sample	Intrinsic viscosity (mPa*s)				Intrinsic viscosity (mPa*s)	
					Average	St. dev.
ctDNA	0.047	0.048	0.056	0.052	0.051	0.004
ctDNA+COSAN (20 µM)	0.050	0.052	0.051	0.051	n.a.	
ctDNA+COSAN (40 µM)	0.050	0.054	0.050	0.051		
ctDNA+COSAN (60 µM)	0.051	0.050	0.046	0.051		
ctDNA+COSAN (80 µM)	0.050	0.046	0.048	0.047		
ctDNA+COSAN (100 µM)	0.047	0.042	0.048	0.044		
ctDNA+COSAN (120 µM)	0.052	0.050	0.046	0.050		

Table S4. Values of $(\eta/\eta_0)^{1/3}$ calculated for free ctDNA (0.24 mM (pb)) and ctDNA (0.24 mM (pb)) in the presence of increasing concentrations of NaCOSAN (0 to 0.12 mM).

Sample	$(\eta/\eta_0)^{1/3}$				$(\eta/\eta_0)^{1/3}$	
					Average	St. dev.
ctDNA	0.975	0.982	1.033	1.008	1.000	0.023
ctDNA+COSAN (20 µM)	0.995	1.008	1.002	1.002	1.002	0.005
ctDNA+COSAN (40 µM)	0.995	1.021	0.995	1.002	1.003	0.011
ctDNA+COSAN (60 µM)	1.002	0.995	0.968	1.002	0.992	0.014
ctDNA+COSAN (80 µM)	0.995	0.968	0.982	0.975	0.980	0.010
ctDNA+COSAN (100 µM)	0.975	0.939	0.982	0.954	0.963	0.017
ctDNA+COSAN (120 µM)	1.008	0.995	0.968	0.995	0.992	0.015

3.6. Equilibrium dialysis of COSAN in the presence of ctDNA

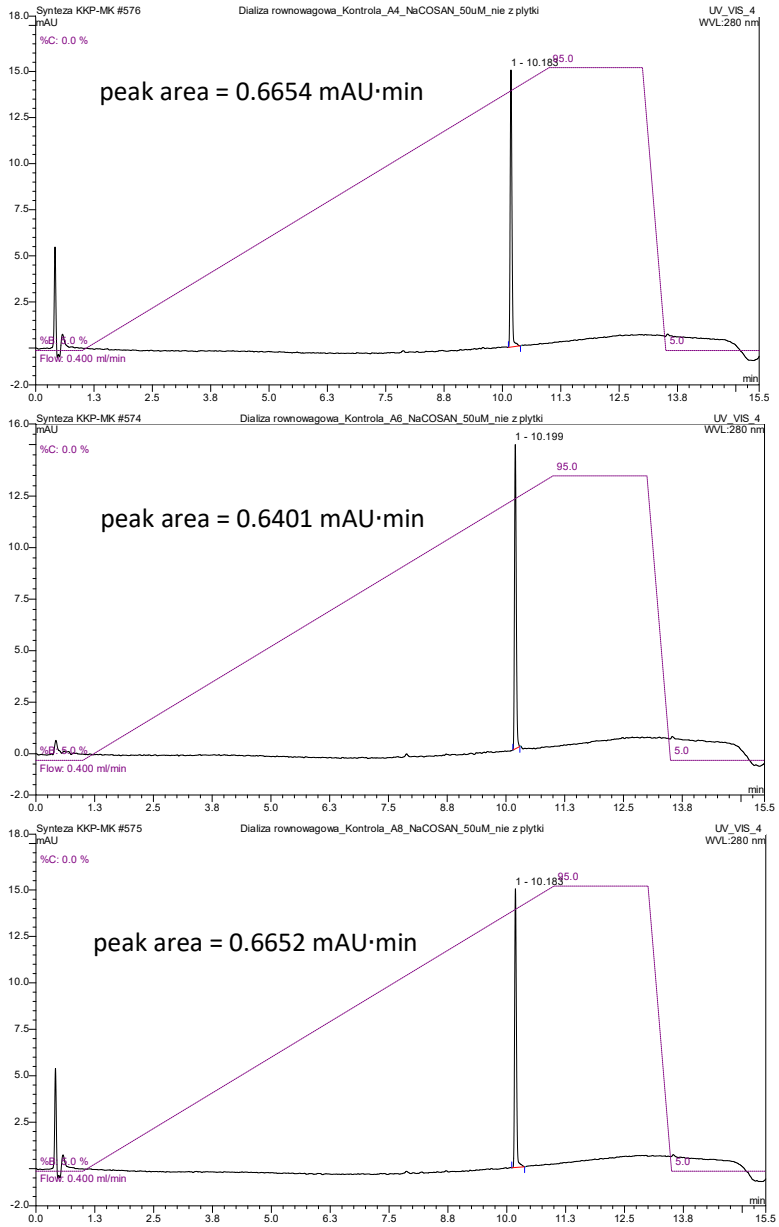


Figure S7. Chromatograms of samples of 50 µM NaCOSAN before equilibrium dialysis in RED plates ($V_{Inj.}$ = 0.2 µl).

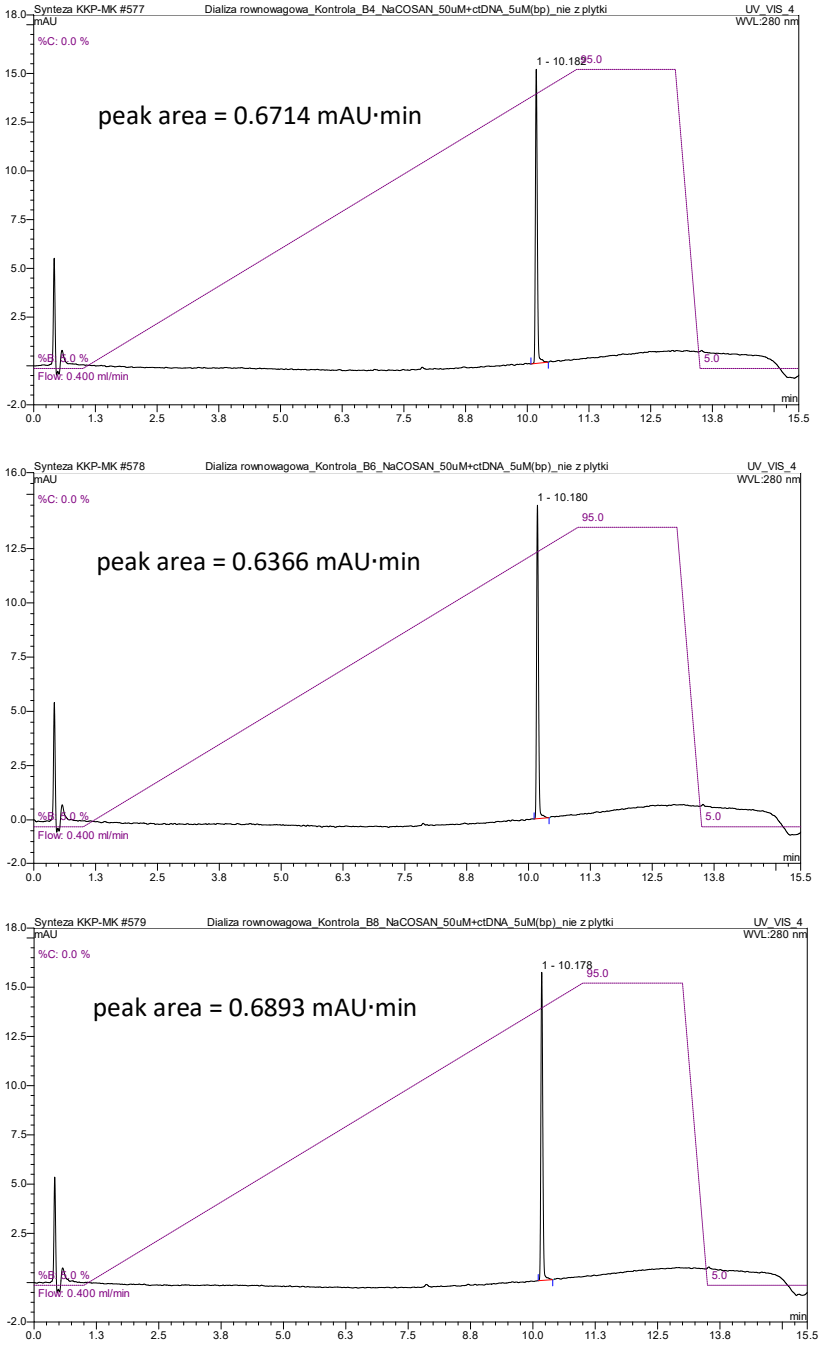


Figure S8. Chromatograms of samples of 50 µM NaCOSAN and 10 µM (pb) ctDNA before equilibrium dialysis in RED plates ($V_{Inj.}$ = 0.2 µl).

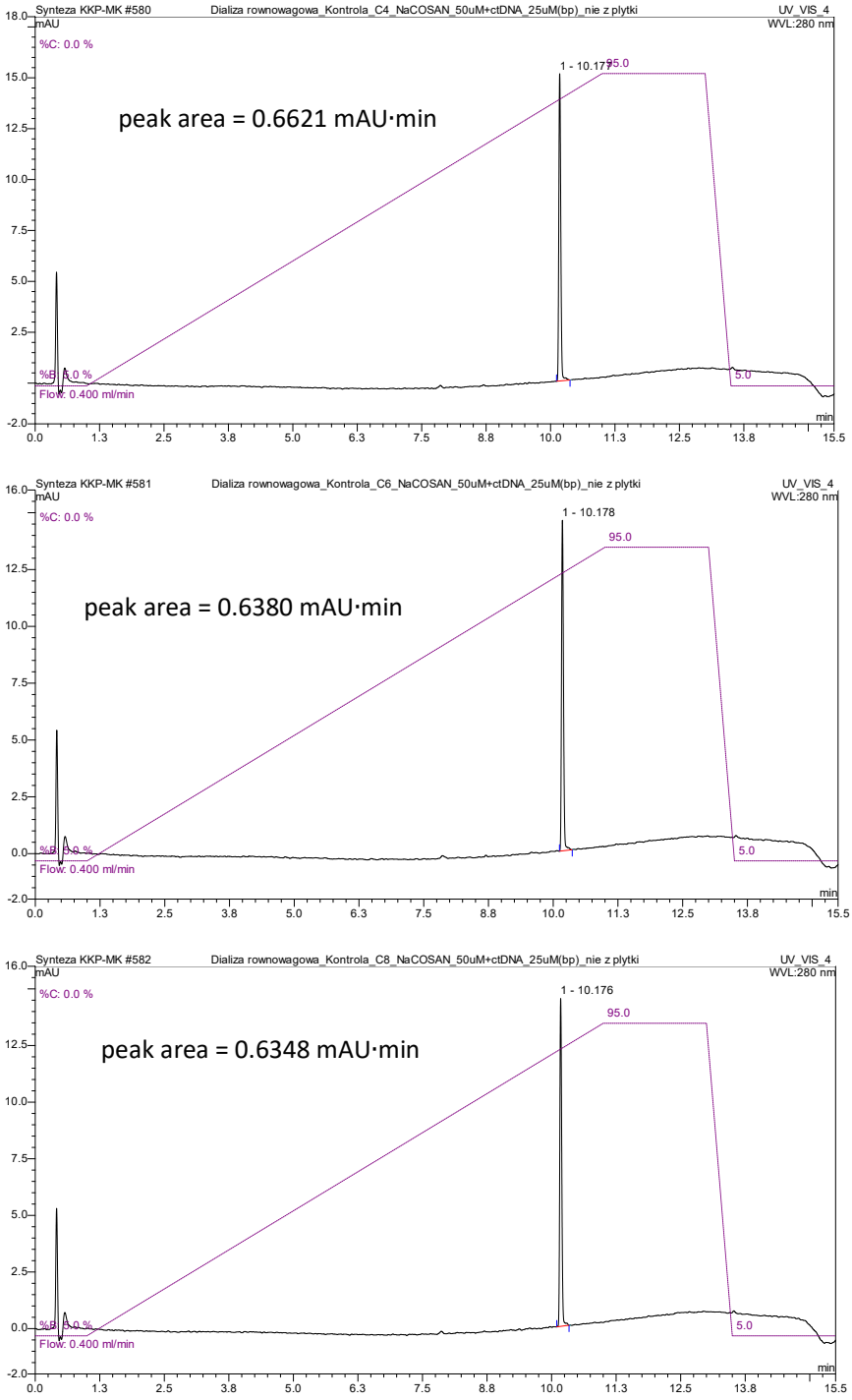


Figure S9. Chromatograms of samples of 50 µM NaCOSAN and 50 µM (pb) ctDNA before equilibrium dialysis in RED plates ($V_{Inj.}$ = 0.2 µl).

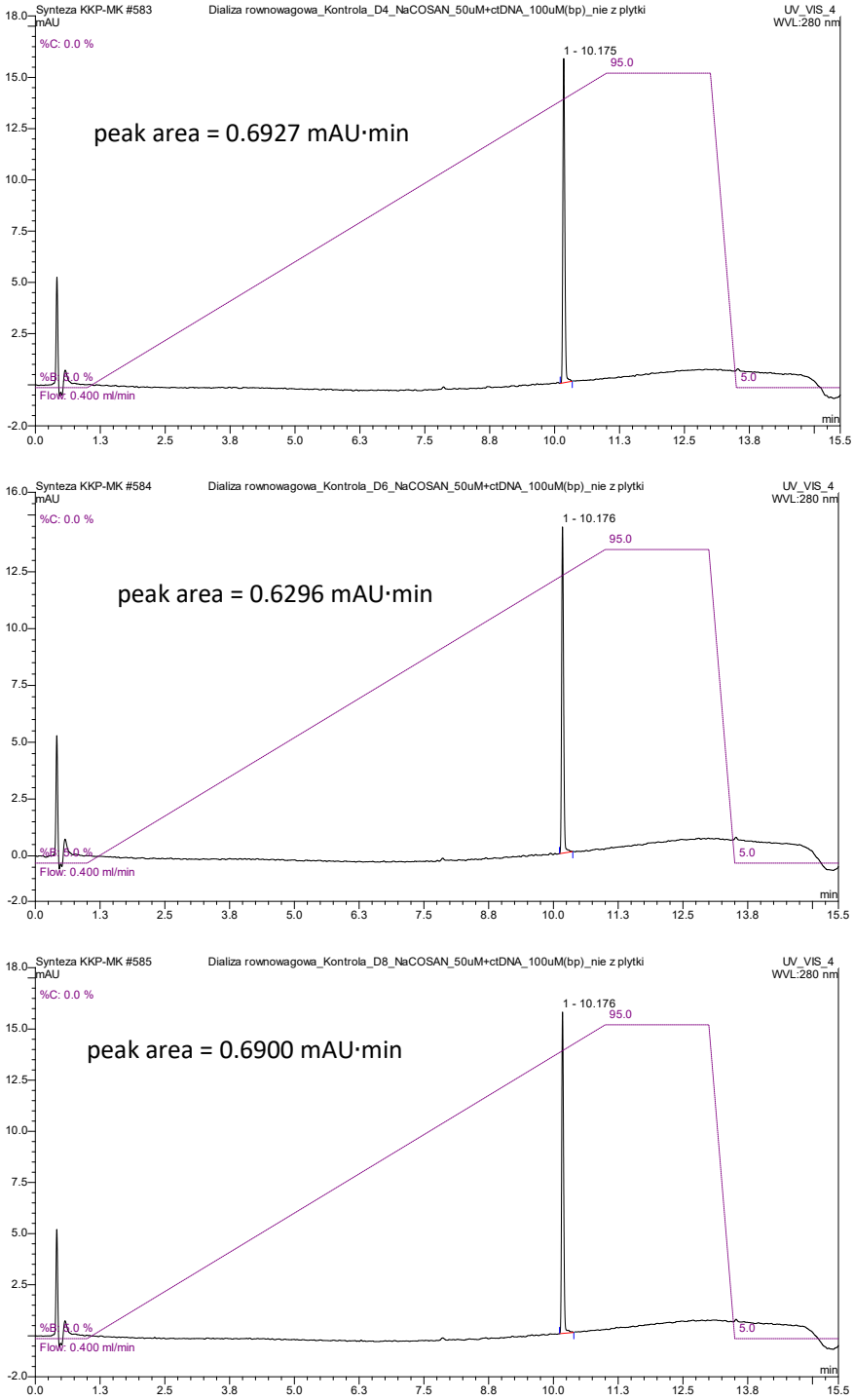


Figure S10. Chromatograms of samples of 50 µM NaCOSAN and 200 µM (pb) ctDNA before equilibrium dialysis in RED plates ($V_{Inj.}$ = 0.2 µl).

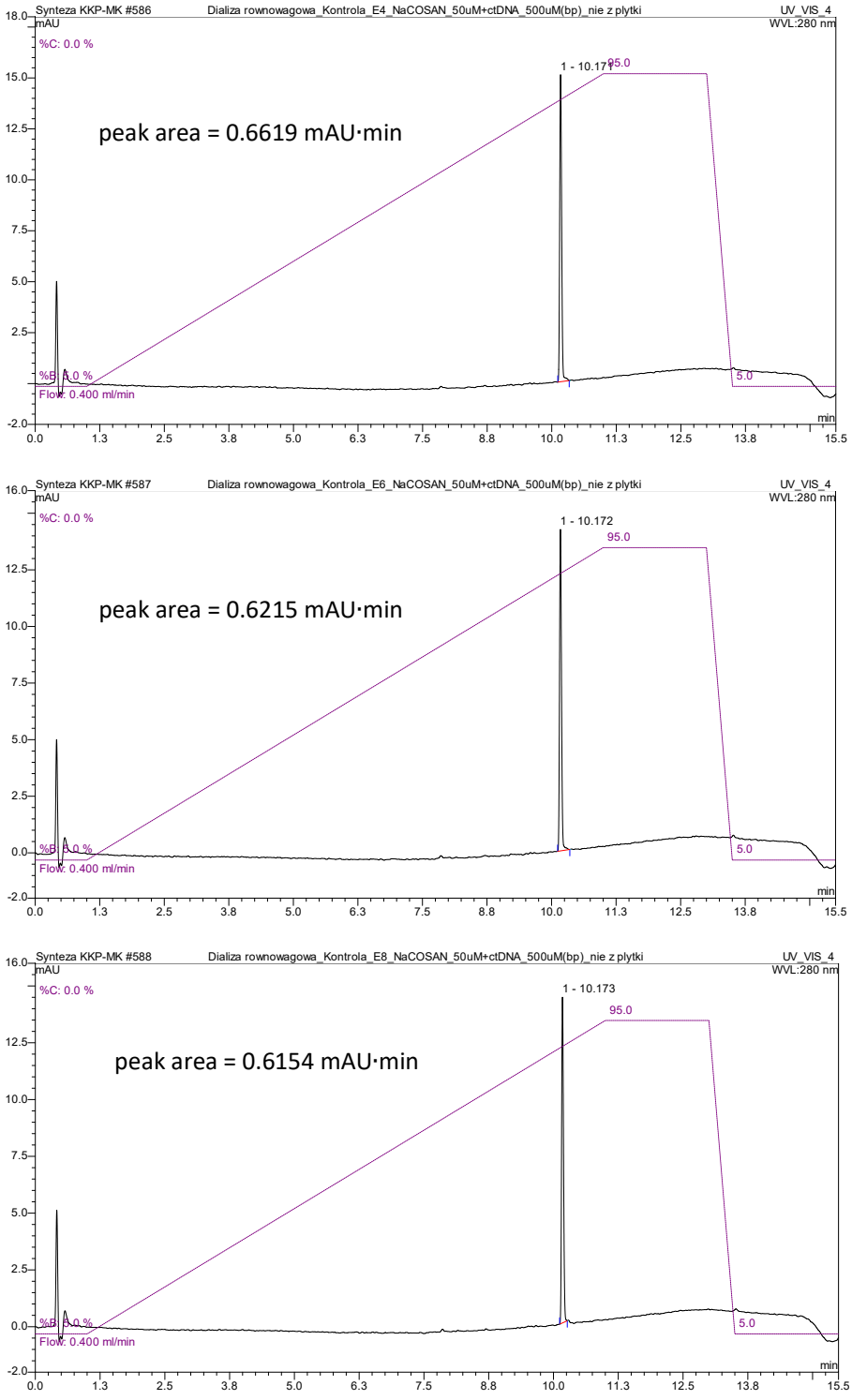


Figure S11. Chromatograms of samples of 50 µM NaCOSAN and 1000 µM (pb) ctDNA before equilibrium dialysis in RED plates ($V_{Inj.}$ = 0.2 µl).

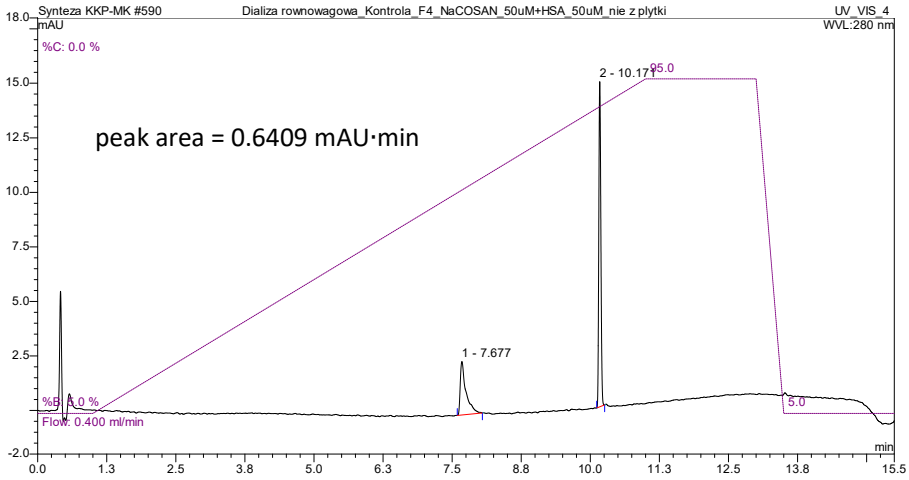


Figure S12. A chromatogram of the sample of 50 µM NaCOSAN and 50 µM human serum albumin (HSA) before equilibrium dialysis in RED plates ($V_{Inj.}$ = 0.2 µl).

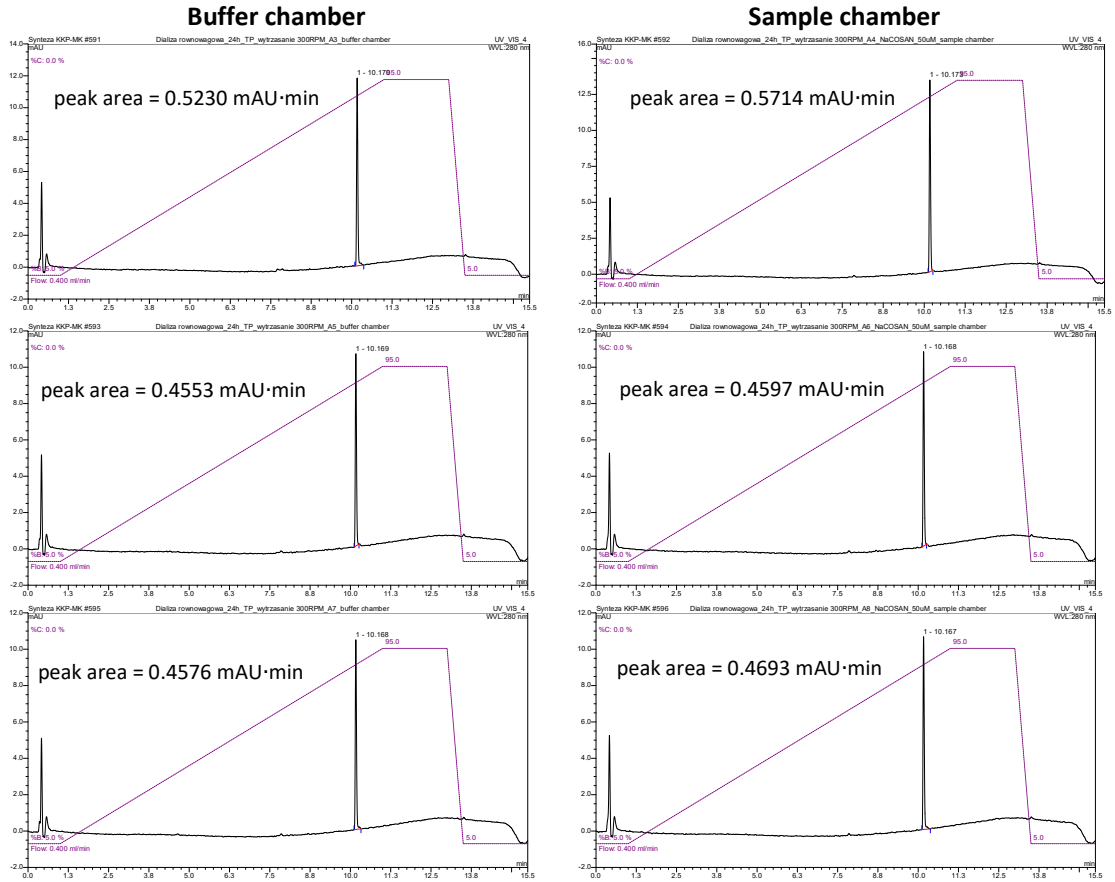


Figure S13. Chromatograms of samples of 50 µM NaCOSAN after equilibrium dialysis in RED plates ($V_{Inj.}$ = 1.0 µl).

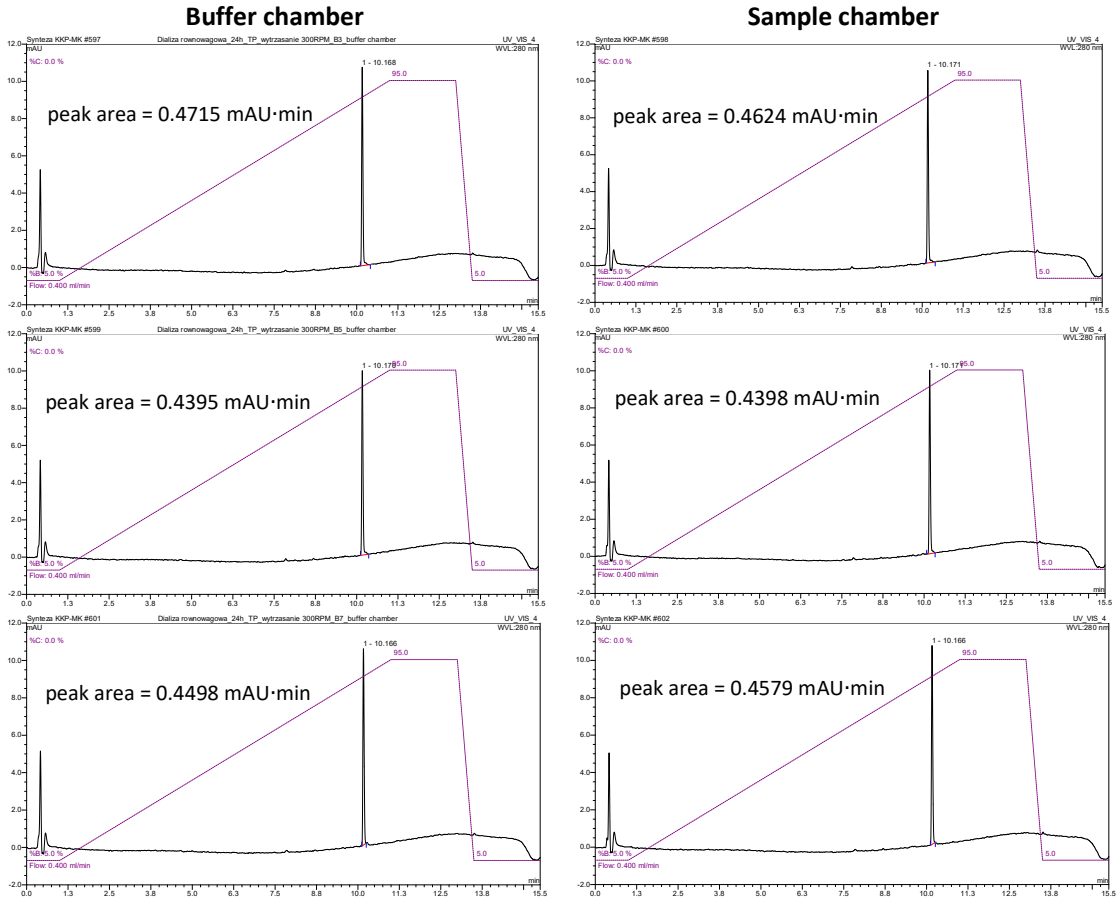


Figure S14. Chromatograms of samples of 50 µM NaCOSAN and 10 µM (pb) ctDNA after equilibrium dialysis in RED plates ($V_{Inj.}$ = 1.0 µl).

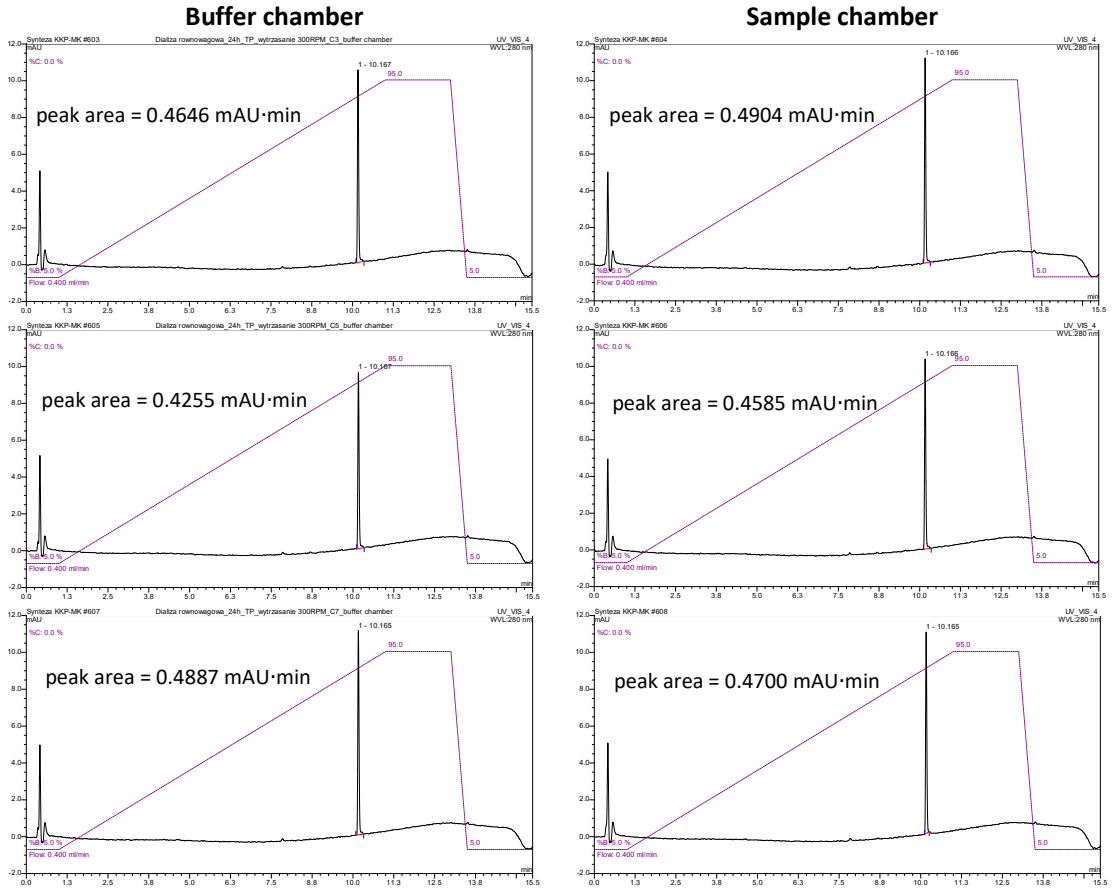


Figure S15. Chromatograms of samples of 50 µM NaCOSAN and 50 µM (pb) ctDNA after equilibrium dialysis in RED plates ($V_{Inj.}$ = 1.0 µl).

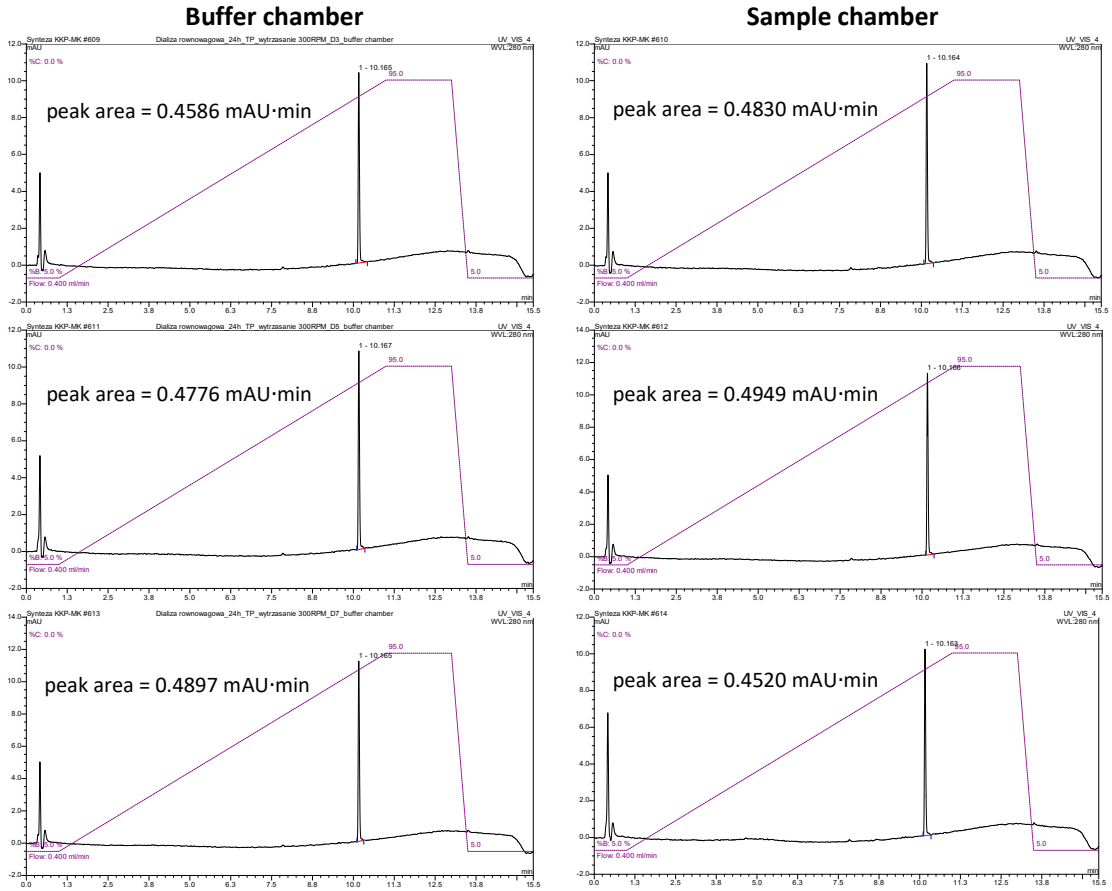


Figure S16. Chromatograms of samples of 50 µM NaCOSAN and 200 µM (pb) ctDNA after equilibrium dialysis in RED plates ($V_{Inj.}$ = 1.0 µl).

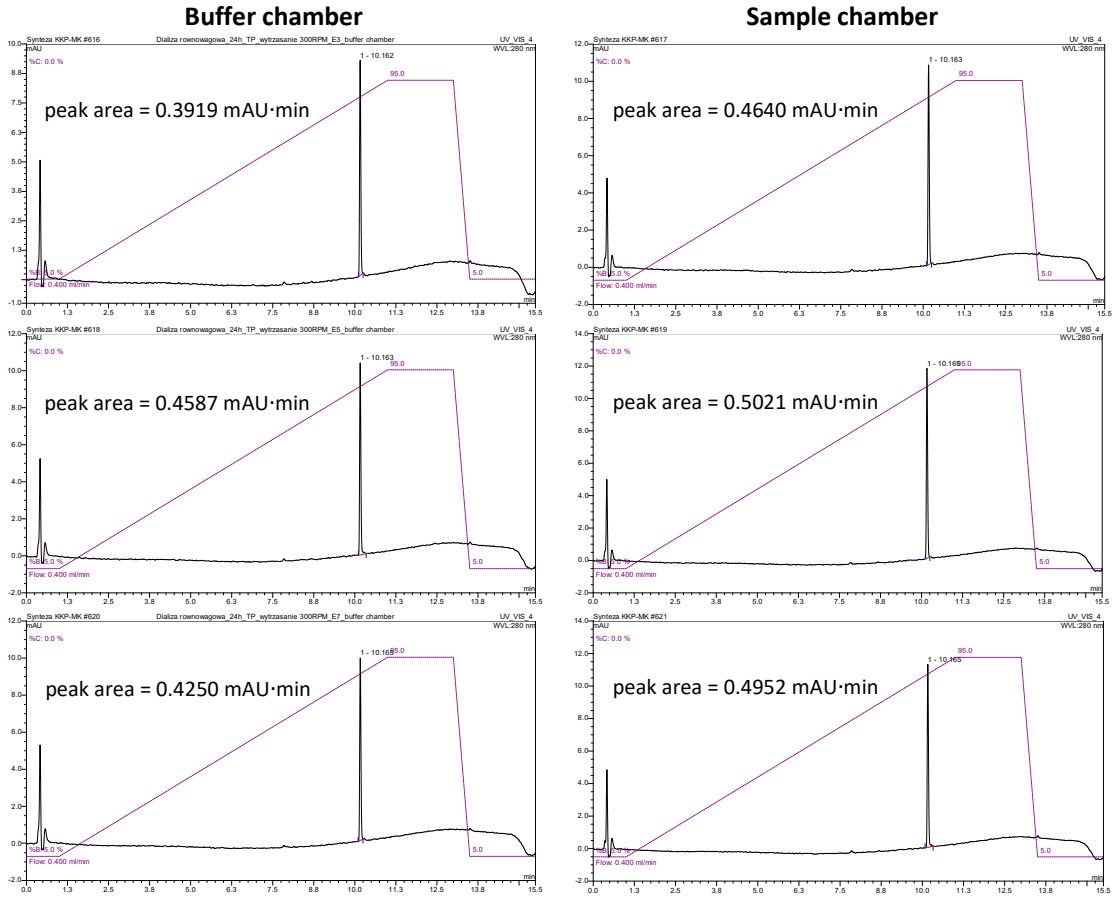


Figure S17. Chromatograms of samples of 50 µM NaCOSAN and 1000 µM (pb) ctDNA after equilibrium dialysis in RED plates ($V_{Inj.}$ = 1.0 µl).

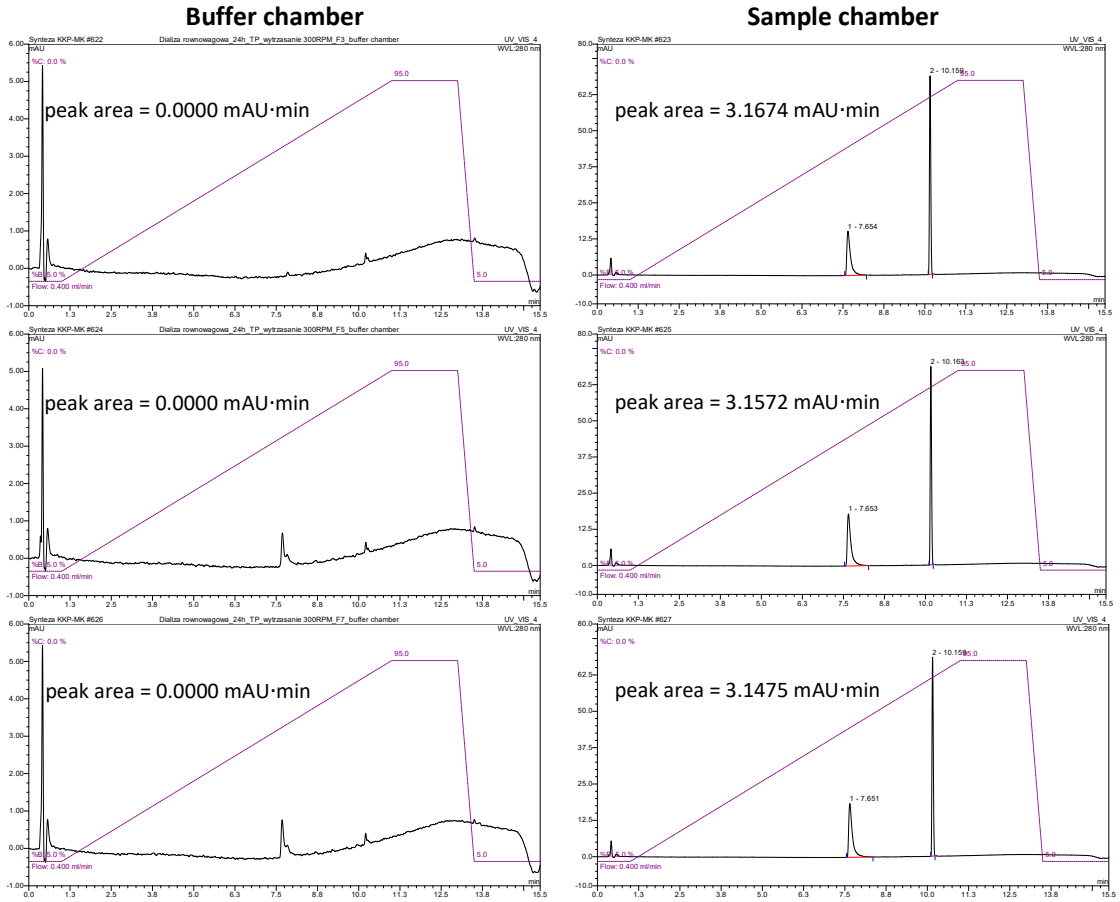
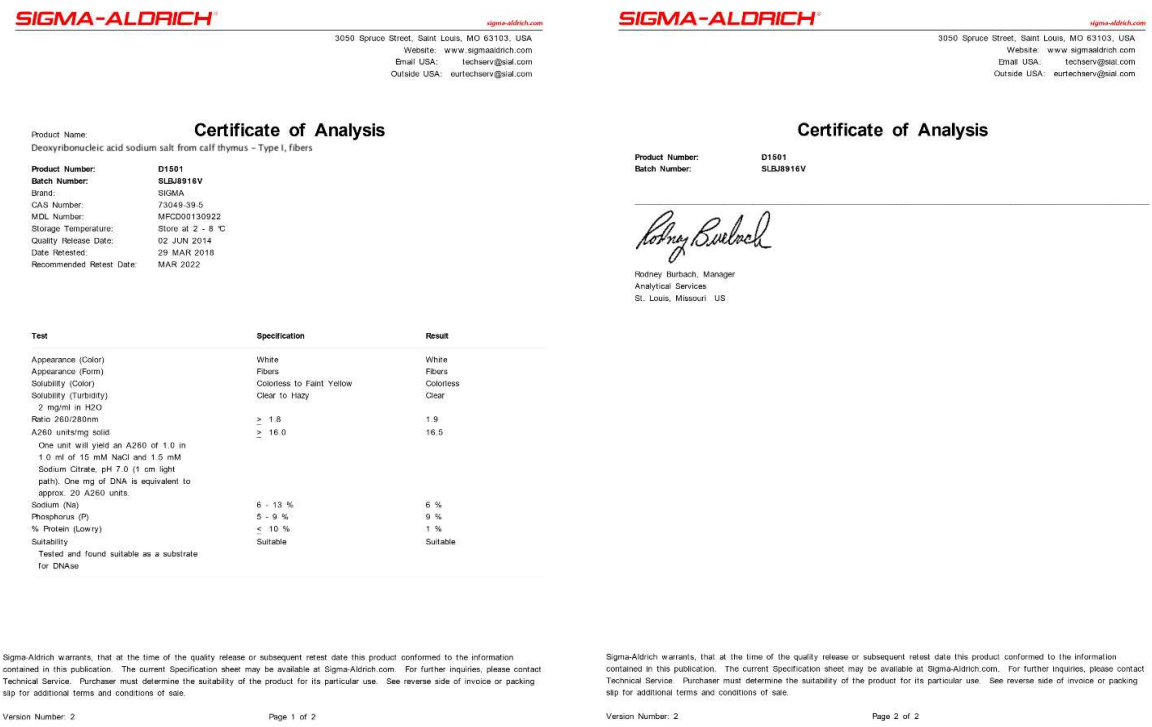


Figure S18. Chromatograms of samples of 50 µM NaCOSAN and 50 µM human serum albumin (HSA) after equilibrium dialysis in RED plates ($V_{Inj.}$ = 1.0 µl). The peak at a retention time of 7.56 comes from HSA.

3.7. Certificate of Analysis of ctDNA



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Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Deoxyribonucleic acid sodium salt from calf thymus - Type I, fibers

Product Number:	**D1501**
Batch Number:	**SLBJ8916V**
Brand:	SIGMA
CAS Number:	73049-39-5
MDL Number:	MFCD00130922
Storage Temperature:	Store at 2 - 8 °C
Quality Release Date:	02 JUN 2014
Date Retested:	29 MAR 2018
Recommended Retest Date:	MAR 2022

Test	Specification	Result
Appearance (Color)	White	White
Appearance (Form)	Fibers	Fibers
Solubility (Color)	Colorless to Faint Yellow	Colorless
Solubility (Turbidity) 2 mg/ml in H2O	Clear to Hazy	Clear
Ratio 260/280nm	≥ 1.8	1.9
A260 units/mg solid One unit will yield an A260 of 1.0 in 1.0 ml of 15 mM NaCl and 1.5 mM Sodium Citrate, pH 7.0 (1 cm light path). One mg of DNA is equivalent to approx. 20 A260 units.	≥ 16.0	16.5
Sodium (Na)	6 - 13 %	6 %
Phosphorus (P)	5 - 9 %	9 %
% Protein (Lowry)	< 10 %	1 %
Suitability Tested and found suitable as a substrate for DNAse	Suitable	Suitable

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Version Number: 2 Page 1 of 2

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St. Louis, Missouri US

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Version Number: 2 Page 2 of 2

Figure S19. Certificate of analysis for calf thymus DNA (ctDNA).

3.8. NMR measurements of COSAN or ethidium bromide (EB) in the presence of ctDNA

All samples were prepared in D_2O containing 150 mM NaCl, 20 mM phosphate, and 1 mM Na_2EDTA (disodium ethylenediaminetetraacetic), pH 7.4, with the addition of 1 mM DSS (sodium trimethylsilylpropanesulfonate). Chemical shifts (δ) were expressed in parts per million (ppm), while multiplicity was reported as follows: s = singlet, d = dublet, t = triplet, q = quartet, m = multiplet (complex pattern), br = broad. For clarity, the solvent peak (4.79 ppm, HDO) as well as the set of broad multiplets of B-H protons *ca.* 1-4 ppm has been omitted in the ^{1}H NMR description. In all spectra below, integrations are normalized to the signal of the trimethylsilyl protons of DSS (0.03-0.04 ppm), as they do not overlap with the BH signals of COSAN.

3.8.1. 0.6 mM ethidium bromide (EB) sample

EB ^{1}H NMR (600 MHz, D_2O) δ 1.47 (m, CH_3, 3H), 4.66 (q, J = 7.2 Hz, CH_2, 2H), 6.61 (d, J = 2.4 Hz, ArH, 1H), 7.43 (dd, J = 8.4 Hz, 1.2 Hz, ArH, 1H), 7.49 (d, J = 1.8 Hz, ArH, 1H), 7.54 (d, J = 7.2 Hz, ArH, 2H), 7.60-7.58 (dd, J = 9 Hz, J = 2.4 Hz, ArH, 1H), 7.85-7.75 (m, Ar-H, 3H), 8.47-8.46 (d, Ar-H, 1H), 8.55-8.53 (d, Ar-H, 1H). NH_2 signals not observed.

DSS ^{1}H NMR (600 MHz, D_2O) δ 0.66 (t, J = 9.6 Hz, CH_2, 2H), 1.79 (m, CH_2, 2H), 0.03 (s, 3xCH_3, 9H), 2.94 (t, J = 7.8 Hz, CH_2, 2H).

EDTA ^{1}H NMR (600 MHz, D_2O) δ 3.19 (s, CH_2, 4H) 3.61 (s, CH_2, 8H).

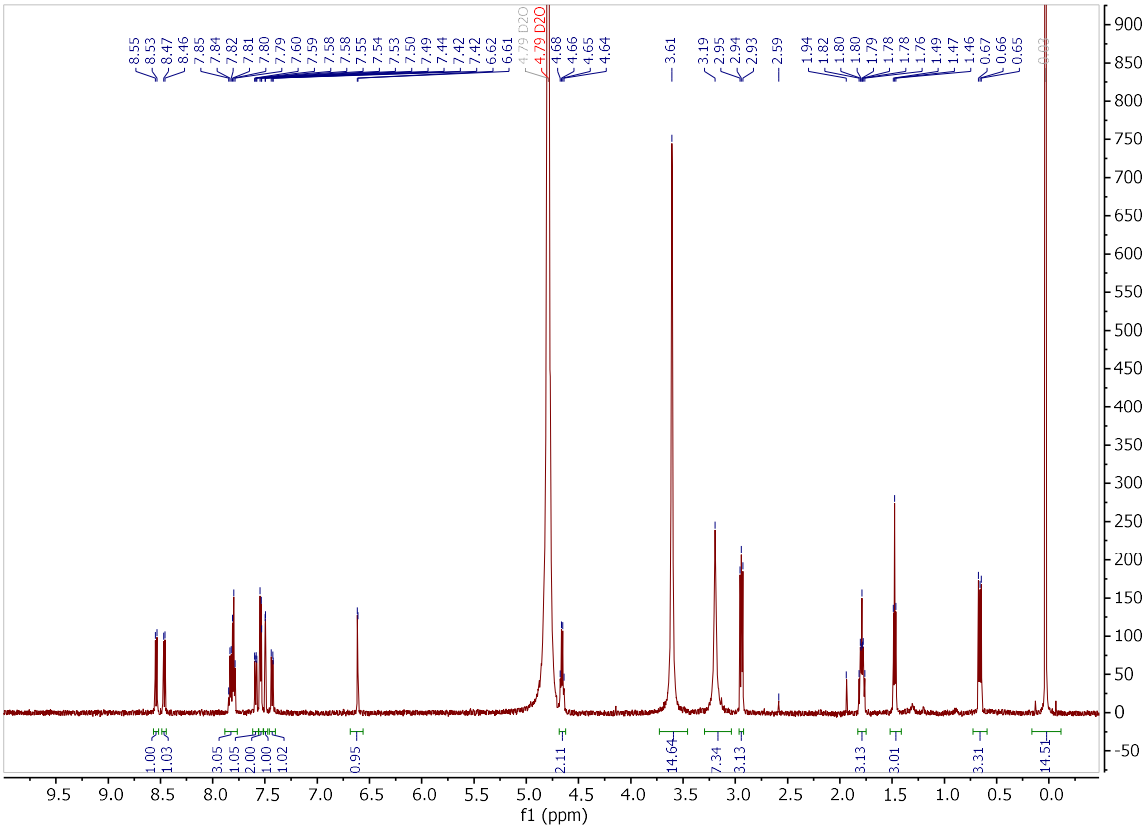

Figure S20. ^{1}H NMR spectrum of 0.6 mM EB.

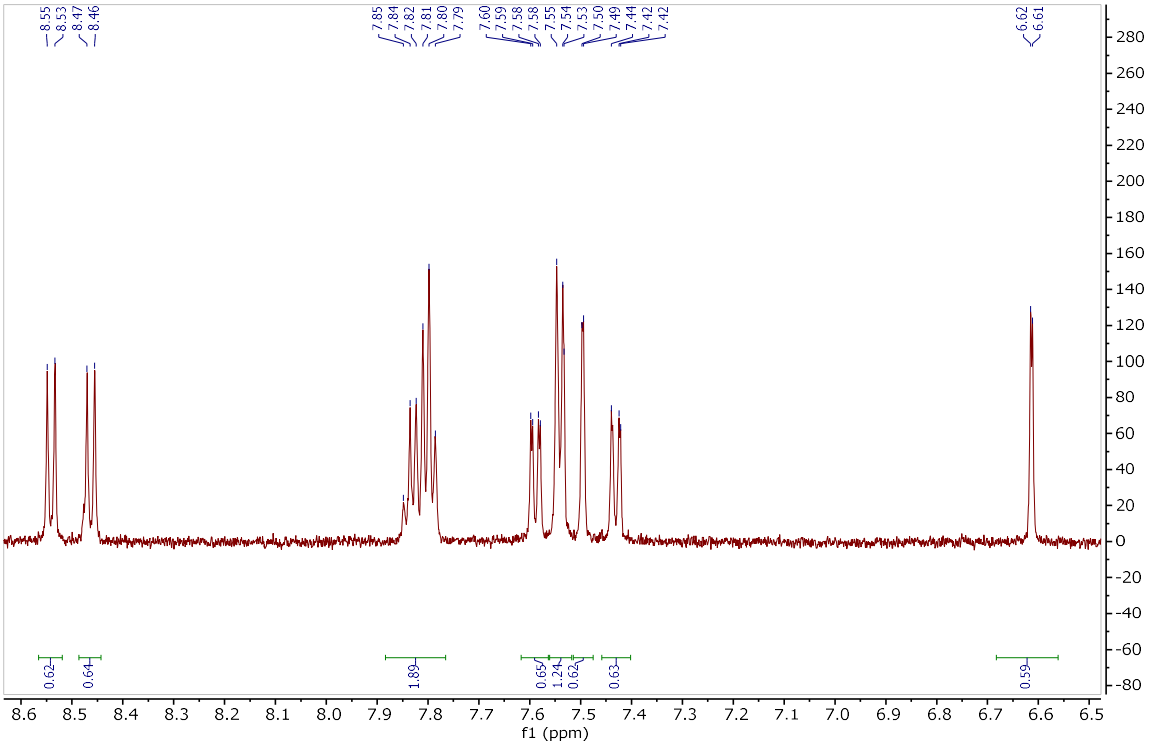

Figure S21. Expanded ^{1}H NMR spectrum of 0.6 mM EB.

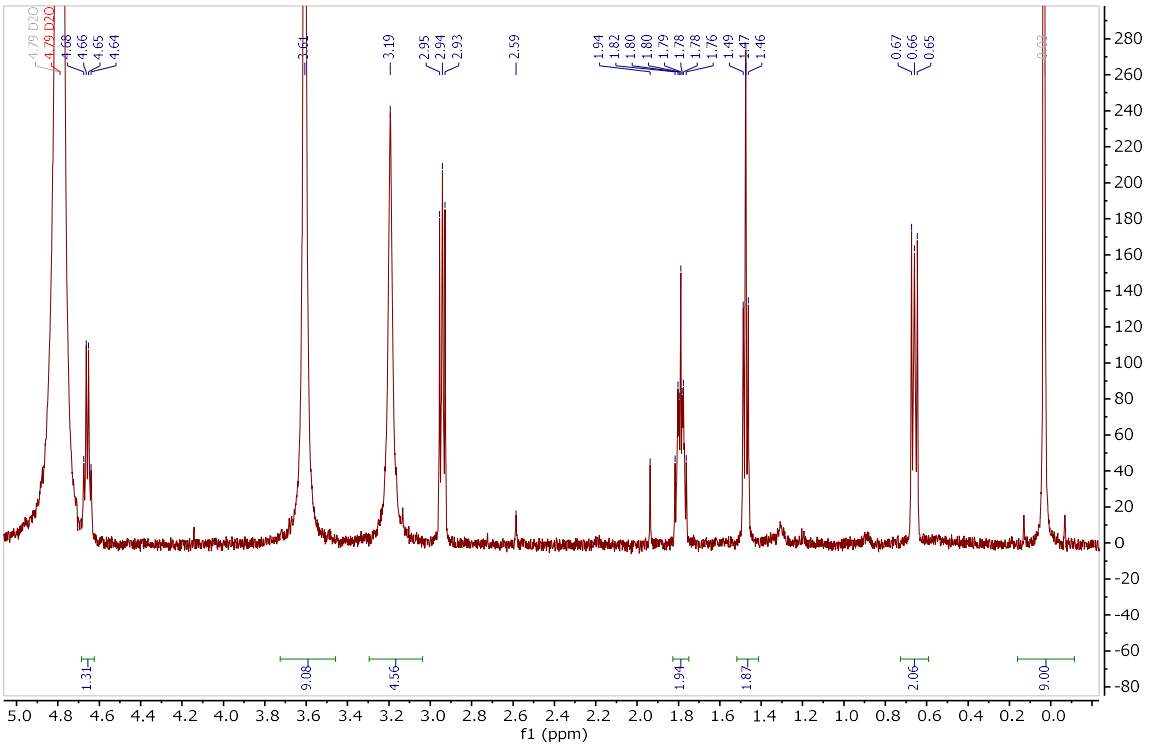

Figure S22. Expanded ^{1}H NMR spectrum of 0.6 mM EB.

3.8.2. 0.6 mM EB in the presence of 2 mM ctDNA (pb)

EB ^{1}H NMR (600 MHz, D_2O) δ 1.29 (s, CH_3, 3H)

DSS ^{1}H NMR (600 MHz, D_2O) δ 0.03 (s, 3xCH_3, 9H), 0.66 (t, J = 9.0 Hz, CH_2, 2H), 1.79 (m, CH_2, 2H), 2.94 (t, J = 7.8 Hz, CH_2, 2H)

EDTA ^{1}H NMR (600 MHz, D_2O) δ 3.18 (s, CH_2, 4H), 3.60 (s, CH_2, 8H)

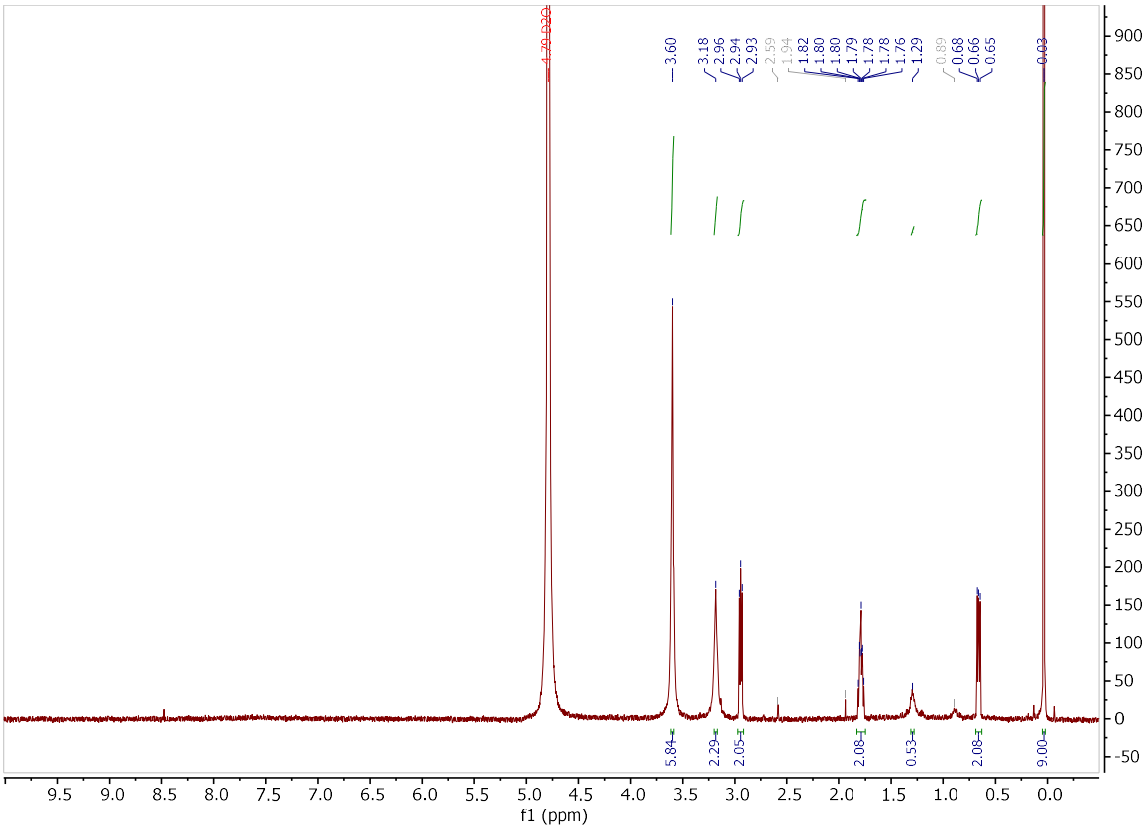

Figure S23. ^{1}H NMR spectrum of 0.6 mM EB in the presence of 2 mM ctDNA (pb).

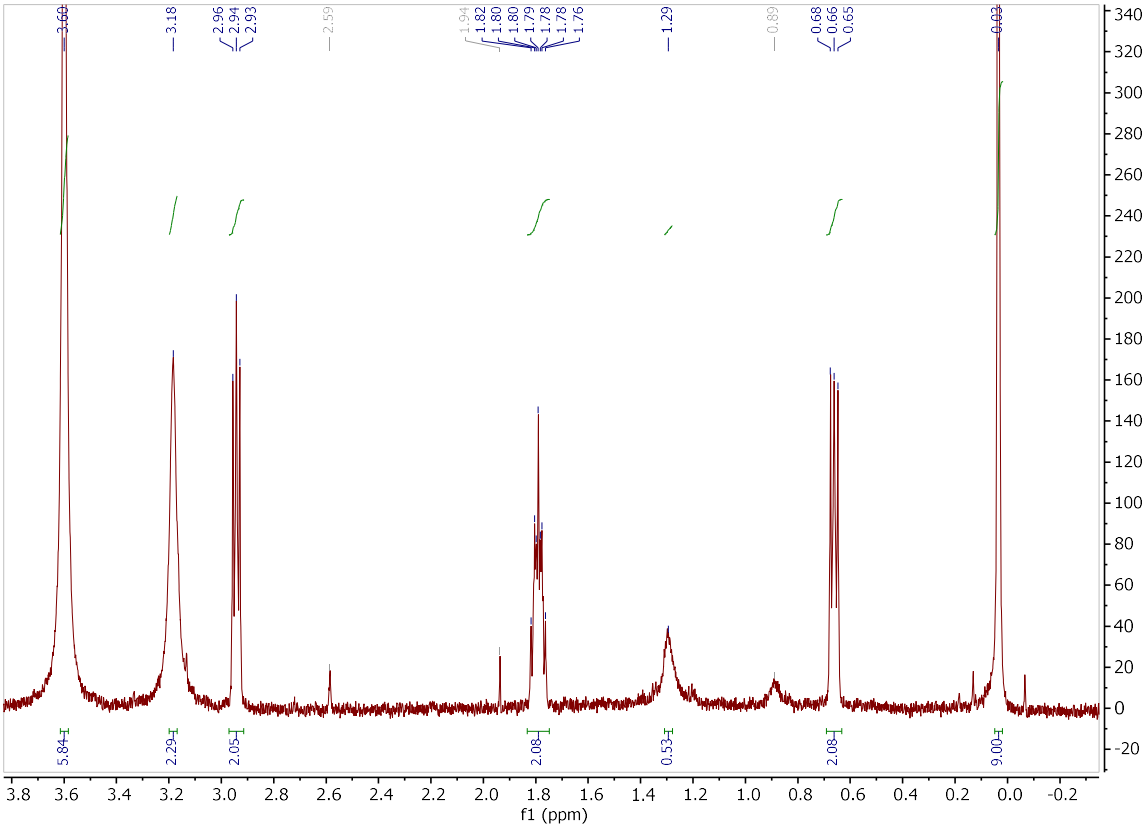

Figure S24. Expanded ^{1}H NMR spectrum of 0.6 mM ethidium bromide (EB) in the presence of 2 mM (pb) ctDNA

3.8.3. 0.2 mM NaCOSAN

COSAN ^{1}H NMR (600 MHz, D_2O) δ 4.08 (s, 4xBCH, 4H)

DSS ^{1}H NMR (600 MHz, D_2O) δ 0.03 (s, 3xCH_3, 9H), 0.66 (t, J = 9.0 Hz, CH_2, 2H), 1.79 (m, CH_2, 2H), 2.94 (t, J = 7.8 Hz, CH_2, 2H)

EDTA ^{1}H NMR (600 MHz, D_2O) δ 3.15 (s, 2xCH_2, 4H), 3.57 (s, 4xCH_2, 8H)

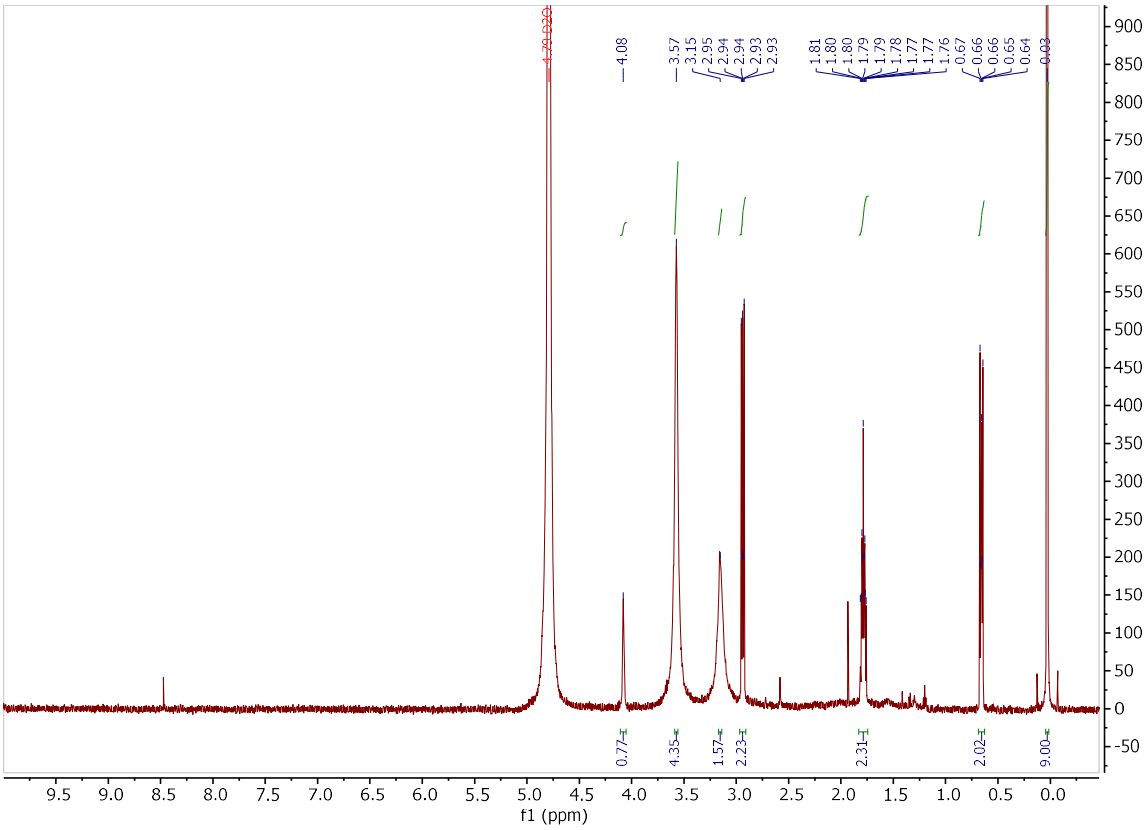

Figure S25. ^{1}H NMR spectrum of 0.2 mM NaCOSAN.

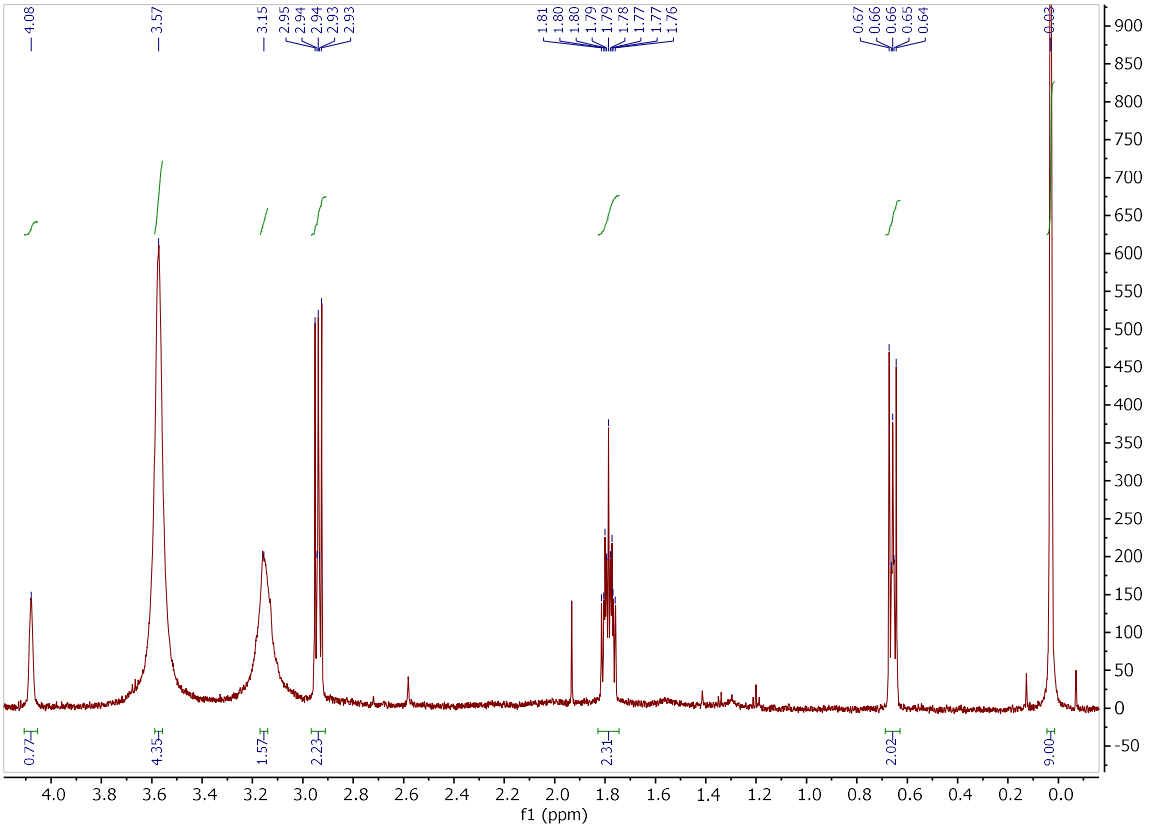

Figure S26. Expanded ^{1}H NMR spectrum of 0.2 mM NaCOSAN

3.8.4. 0.2 mM NaCOSAN in the presence of 2 mM ctDNA (pb)

COSAN ^{1}H NMR (600 MHz, D_2O) δ 4.08 (s, 4xBCH, 4H)

DSS ^{1}H NMR (600 MHz, D_2O) δ 0.03 (s, 3xCH_3, 9H), 0.66 (t, J = 9.0 Hz, CH_2, 2H), 1.79 (m, CH_2, 2H), 2.94 (t, J = 7.8 Hz, CH_2, 2H)

EDTA ^{1}H NMR (600 MHz, D_2O) δ 3.16 (s, 2xCH_2, 4H), 3.58 (s, 4xCH_2, 8H)

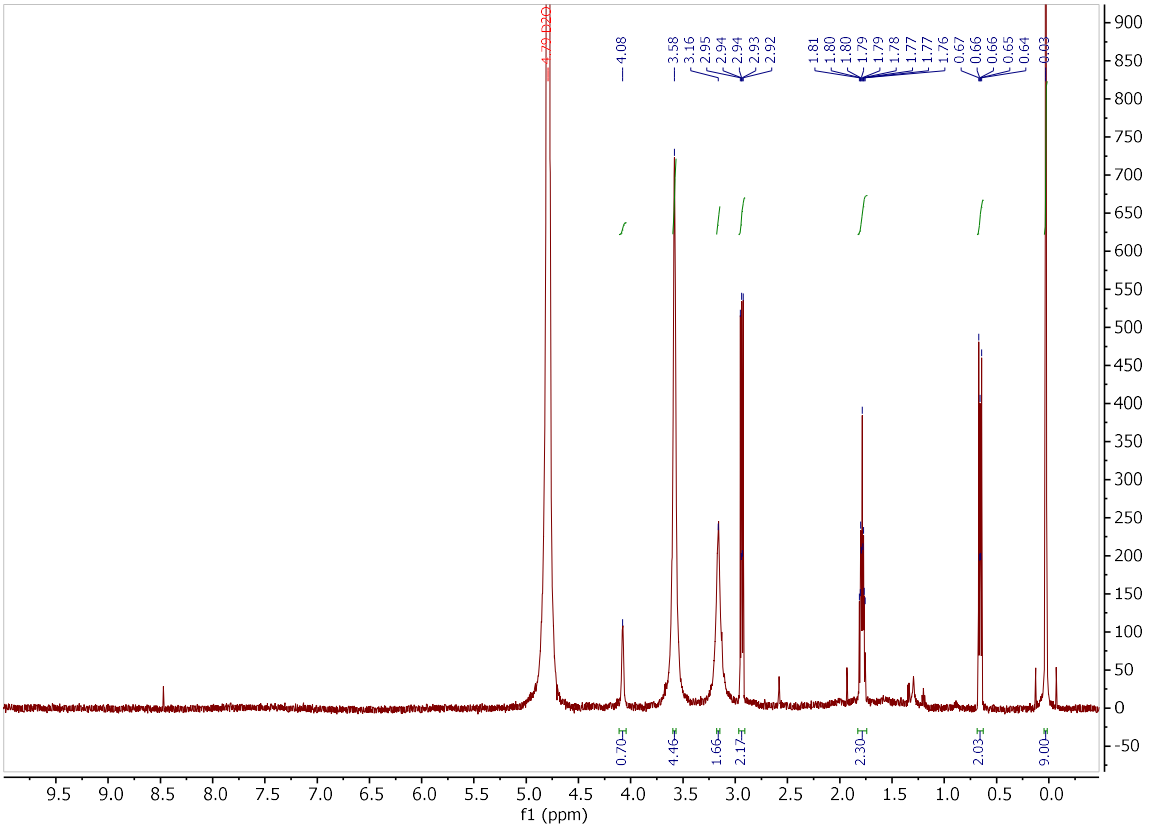

Figure S27. ^{1}H NMR spectrum of 0.2 mM NaCOSAN in the presence of 2 mM ctDNA (pb).

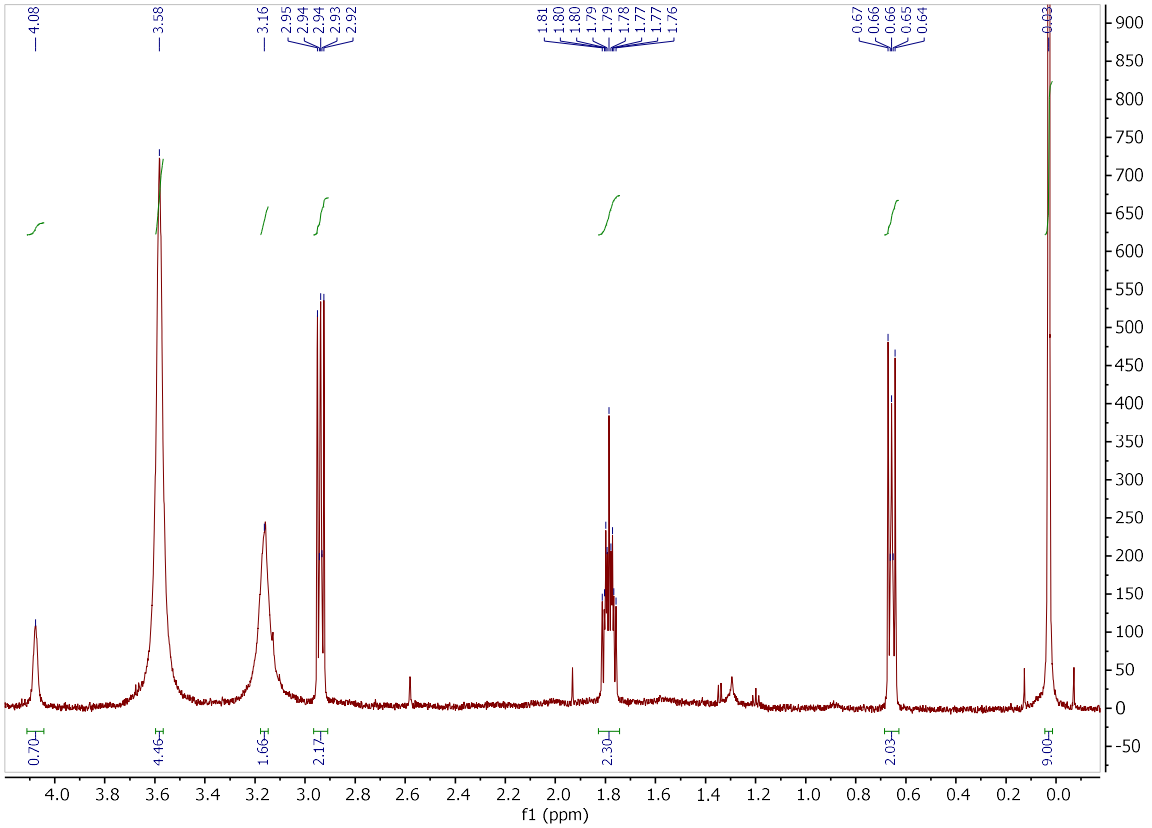

Figure S28. Expanded ^{1}H NMR spectrum of 0.2 mM NaCOSAN in the presence of 2 mM ctDNA (pb).

3.8.5. 0.6 mM NaCOSAN

COSAN ^{1}H NMR (600 MHz, D_2O) δ 4.08 (s, 4xBCH, 4H)

DSS ^{1}H NMR (600 MHz, D_2O) δ 0.03 (s, 3xCH_3, 9H), 0.66 (t, J = 9.0 Hz, CH_2, 2H), 1.79 (m, CH_2, 2H), 2.94 (t, J = 7.8 Hz, CH_2, 2H)

EDTA ^{1}H NMR (600 MHz, D_2O) δ 3.17 (s, 2xCH_2, 4H), 3.59 (s, 4xCH_2, 8H)

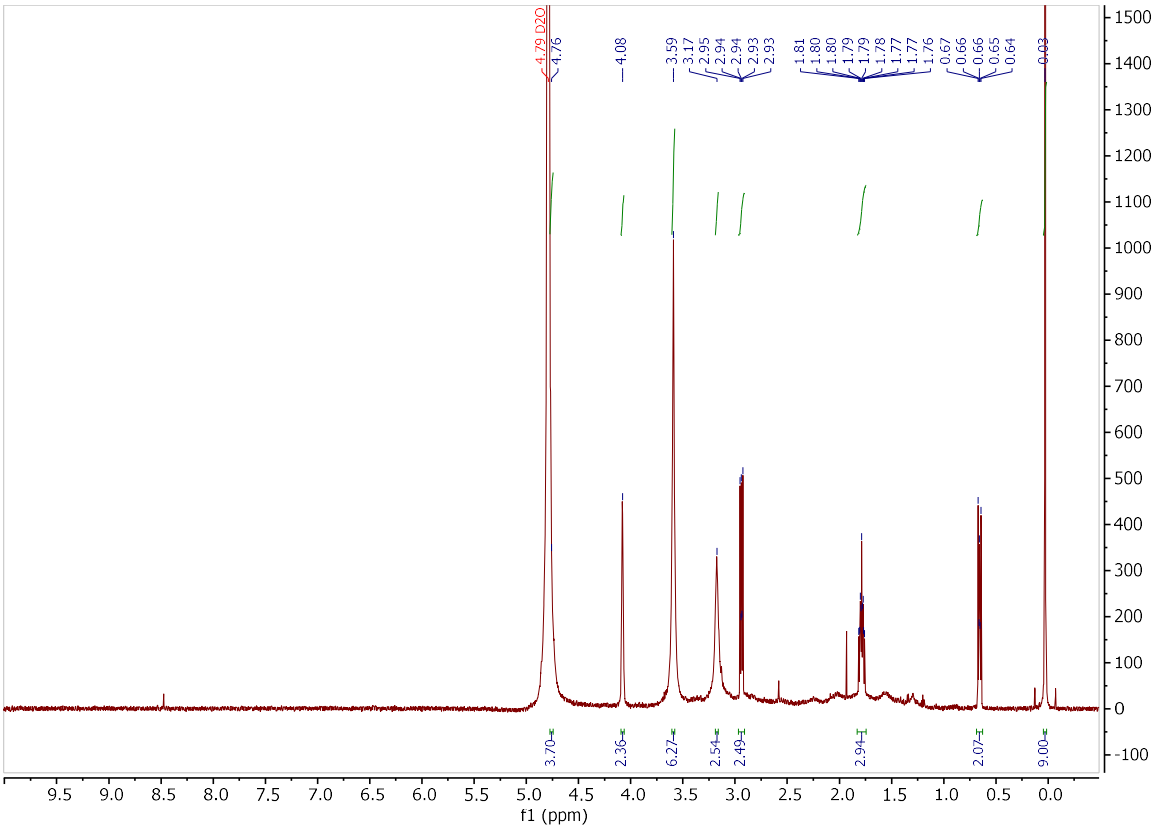

Figure S29. ^{1}H NMR spectrum of 0.6 mM NaCOSAN.

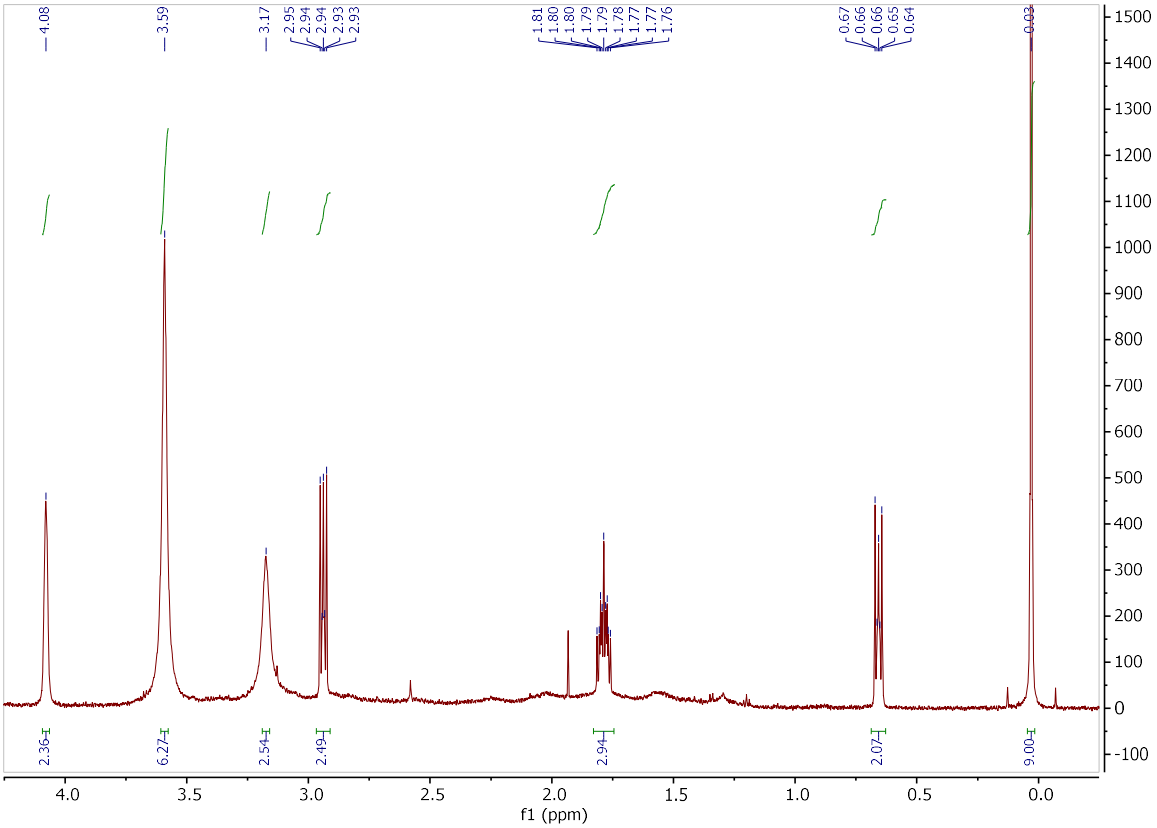

Figure S30. Expanded ^{1}H NMR spectrum of 0.6 mM NaCOSAN.

3.8.6. 0.6 mM NaCOSAN in the presence of 2 mM ctDNA (pb)

COSAN ^{1}H NMR (600 MHz, D_2O) δ 4.08 (s, 4xBCH, 4H)

DSS ^{1}H NMR (600 MHz, D_2O) δ 0.03 (s, 3xCH_3, 9H), 0.66 (t, J = 9.0 Hz, CH_2, 2H), 1.79 (m, CH_2, 2H), 2.94 (t, J = 7.8 Hz, CH_2, 2H)

EDTA ^{1}H NMR (600 MHz, D_2O) δ 3.17 (s, 2xCH_2, 4H), 3.59 (s, 4xCH_2, 8H)

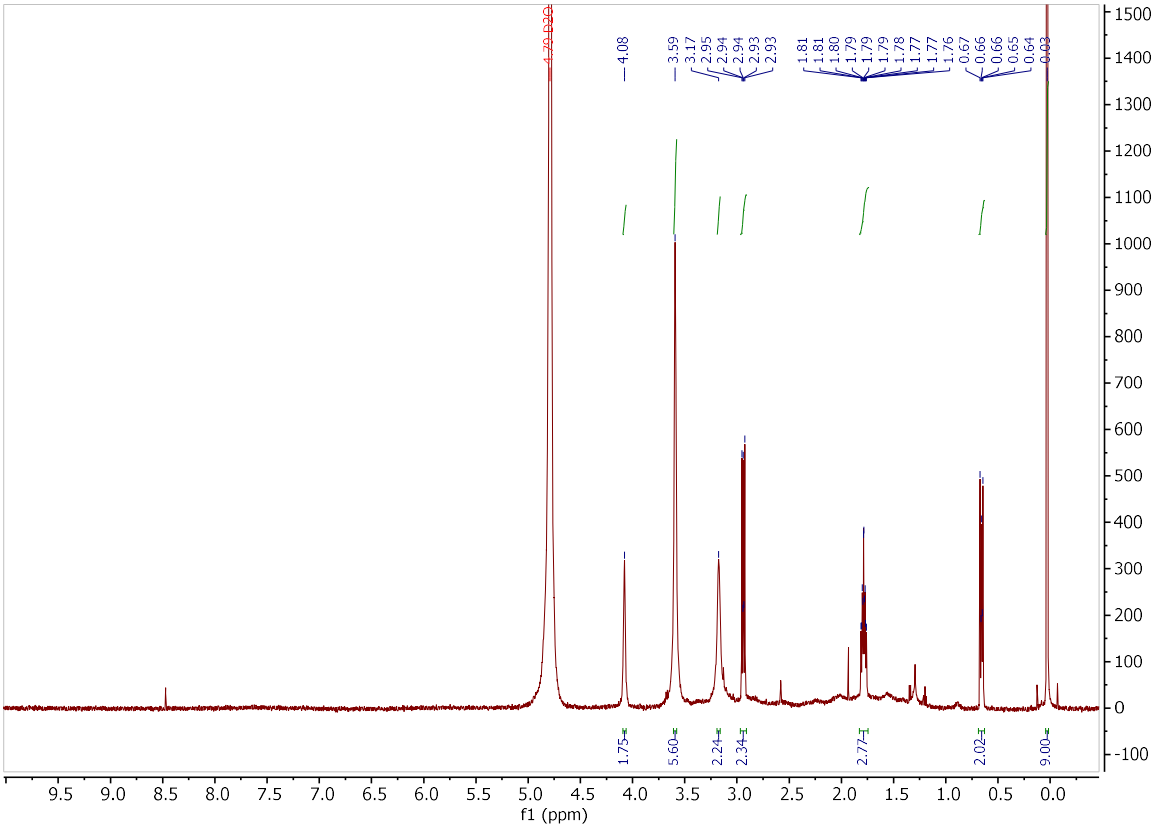

Figure S31. ^{1}H NMR spectrum of 0.6 mM NaCOSAN in the presence of 2 mM ctDNA (pb).

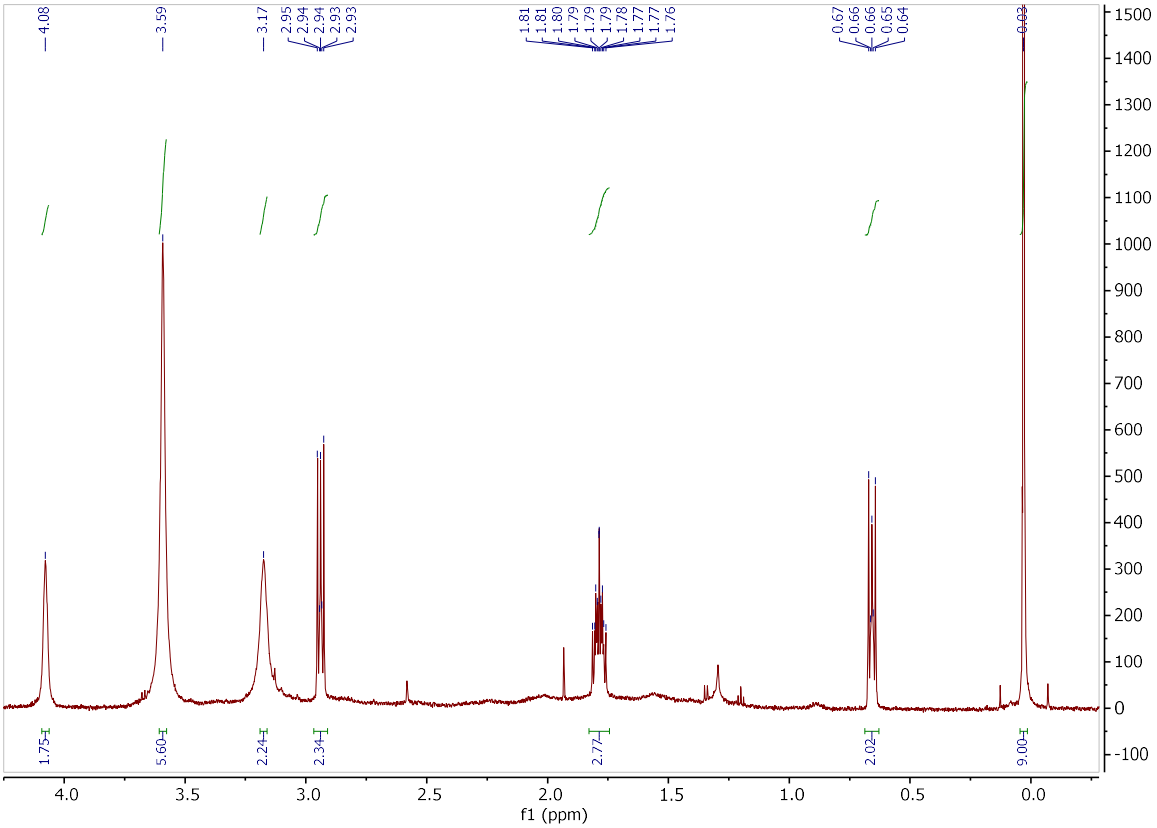

Figure S32. Expanded ^{1}H NMR spectrum of 0.6 mM NaCOSAN in the presence of 2 mM ctDNA (pb)

3.8.7. 2 mM NaCOSAN

COSAN ^{1}H NMR (600 MHz, D_2O) δ 4.08 (s, 4xBCH, 4H)

DSS ^{1}H NMR (600 MHz, D_2O) δ 0.03 (s, 3xCH_3, 9H), 0.66 (t, J = 9.0 Hz, CH_2, 2H), 1.79 (m, CH_2, 2H), 2.94 (t, J = 7.8 Hz, CH_2, 2H)

EDTA ^{1}H NMR (600 MHz, D_2O) δ 3.20 (s, 2xCH_2, 4H), 3.61 (s, 4xCH_2, 8H)

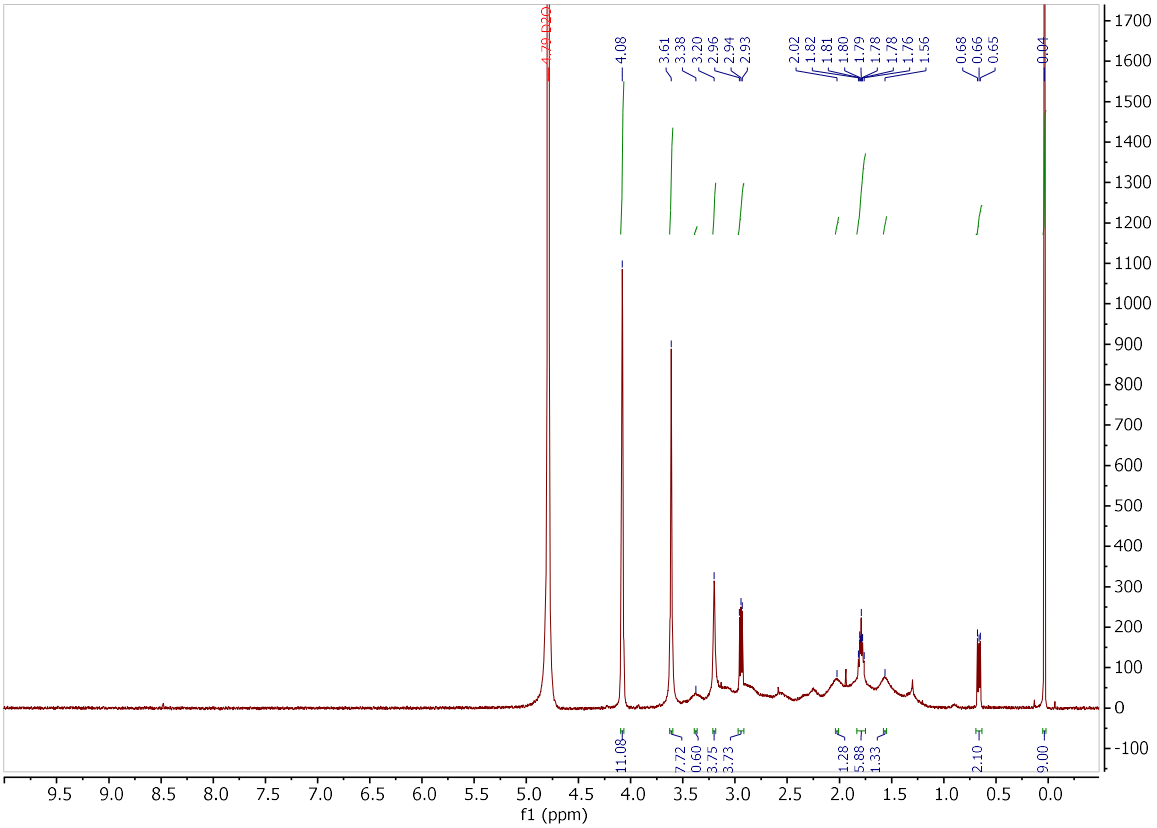

Figure S33. ^{1}H NMR spectrum of 2 mM NaCOSAN.

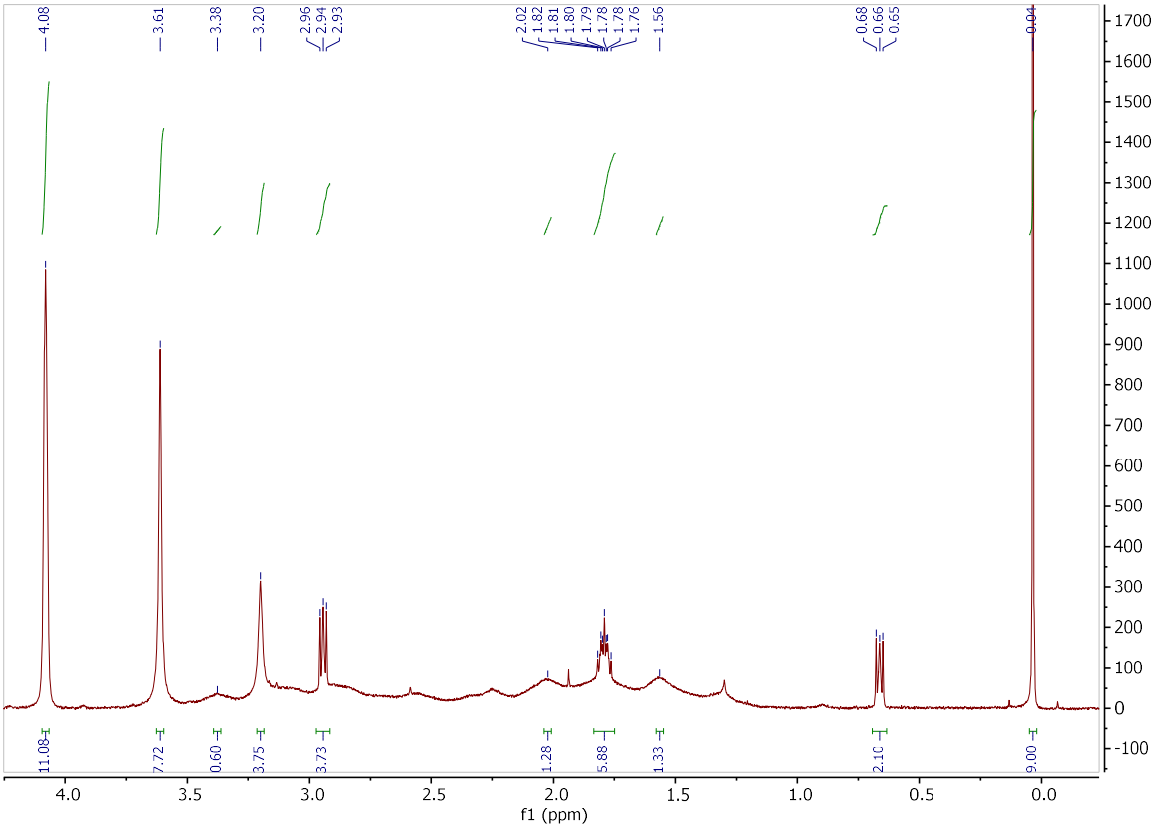

Figure S34. Expanded ^{1}H NMR spectrum of 2 mM NaCOSAN.

3.8.8. 2 mM NaCOSAN in the presence of 2 mM ctDNA (pb)

COSAN ^{1}H NMR (600 MHz, D_2O) δ 4.08 (s, 4xBCH, 4H)

DSS ^{1}H NMR (600 MHz, D_2O) δ 0.03 (s, 3xCH_3, 9H), 0.66 (t, J = 9.0 Hz, CH_2, 2H), 1.79 (m, CH_2, 2H), 2.94 (t, J = 7.8 Hz, CH_2, 2H)

EDTA ^{1}H NMR (600 MHz, D_2O) δ 3.20 (s, 2xCH_2, 4H), 3.61 (s, 4xCH_2, 8H)

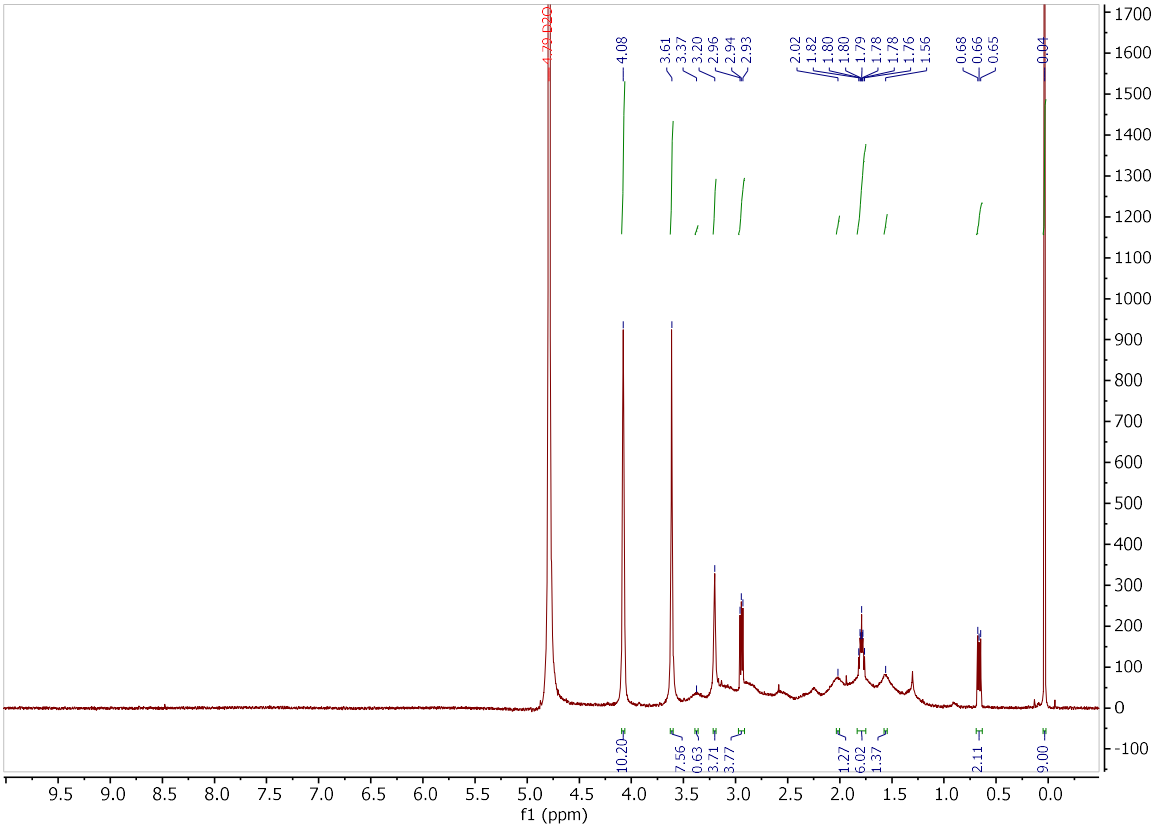

Figure S35. ^{1}H NMR spectrum of 2 mM NaCOSAN in the presence of 2 mM ctDNA (pb).

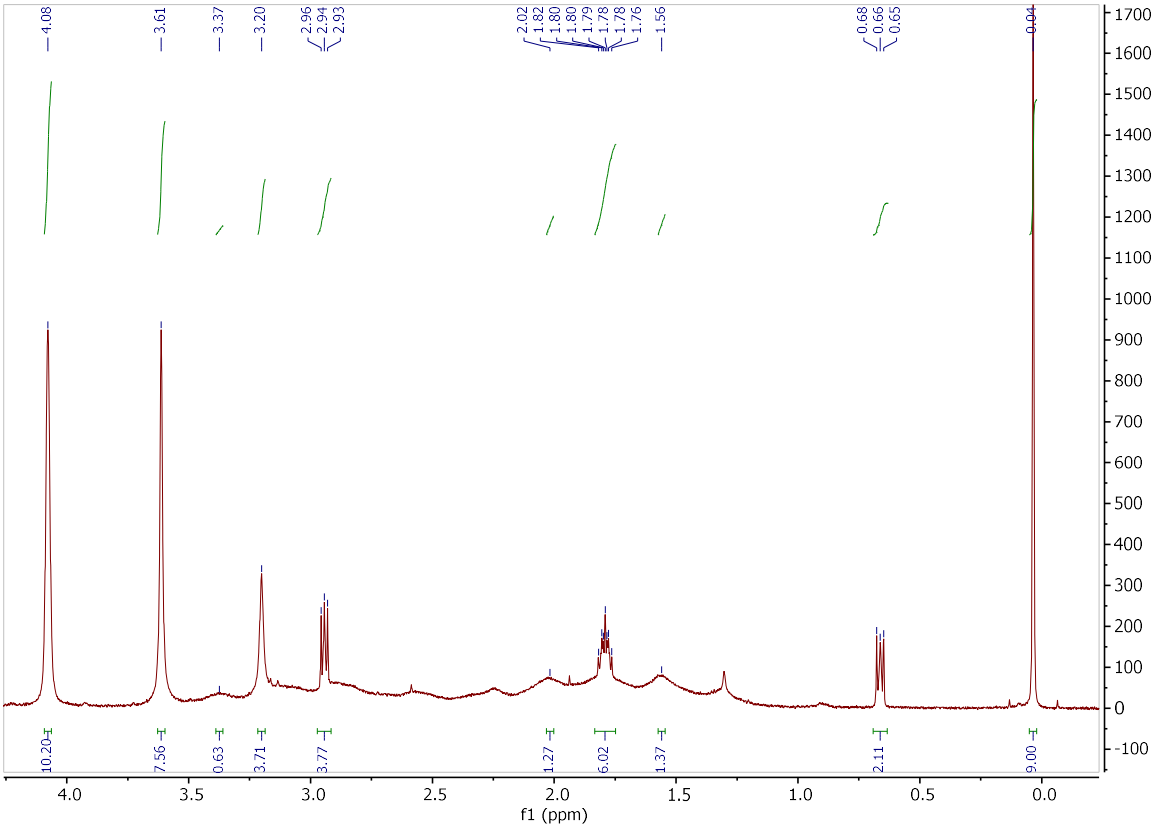

Figure S36. Expanded ^{1}H NMR spectrum of 2 mM NaCOSAN in the presence of 2 mM ctDNA (pb).

3.9. Hemolytic assay

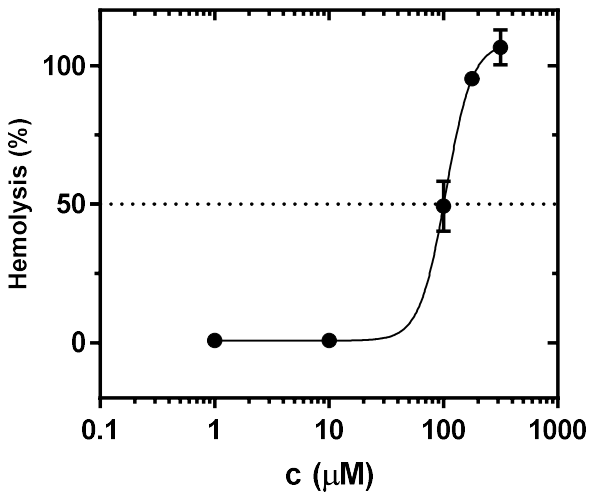

Figure S37. Hemolytic activity of COSAN.

3.10. Cytotoxicity assays

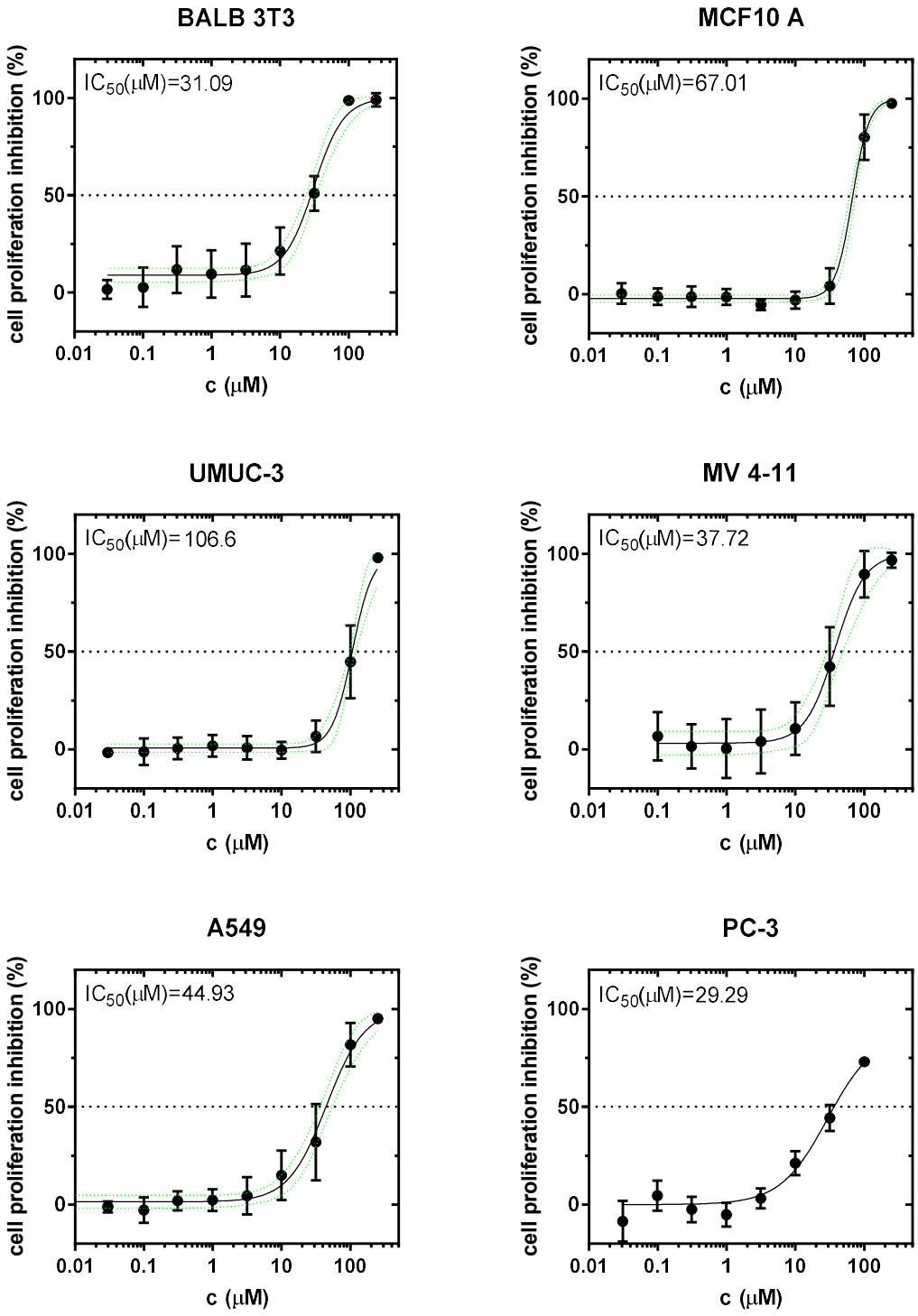

Figure S38. Antiproliferative activity of COSAN after 72 h of treatment in six different cell lines. Balb/3T3 (nontumorigenic murine fibroblasts), MCF 10A (nontumorigenic human mammary gland cells), A549 (human lung carcinoma), MV 4 11 (human biphenotypic leukemia), UM-UC-3 (human urinary bladder transitional cell carcinoma), and PC-3 (human prostate adenocarcinoma).

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