Supplementary Material

Synthesis and evaluation of benzochalcogenazole-benzimidazole derivatives as potential DNA-binding radioprotectors

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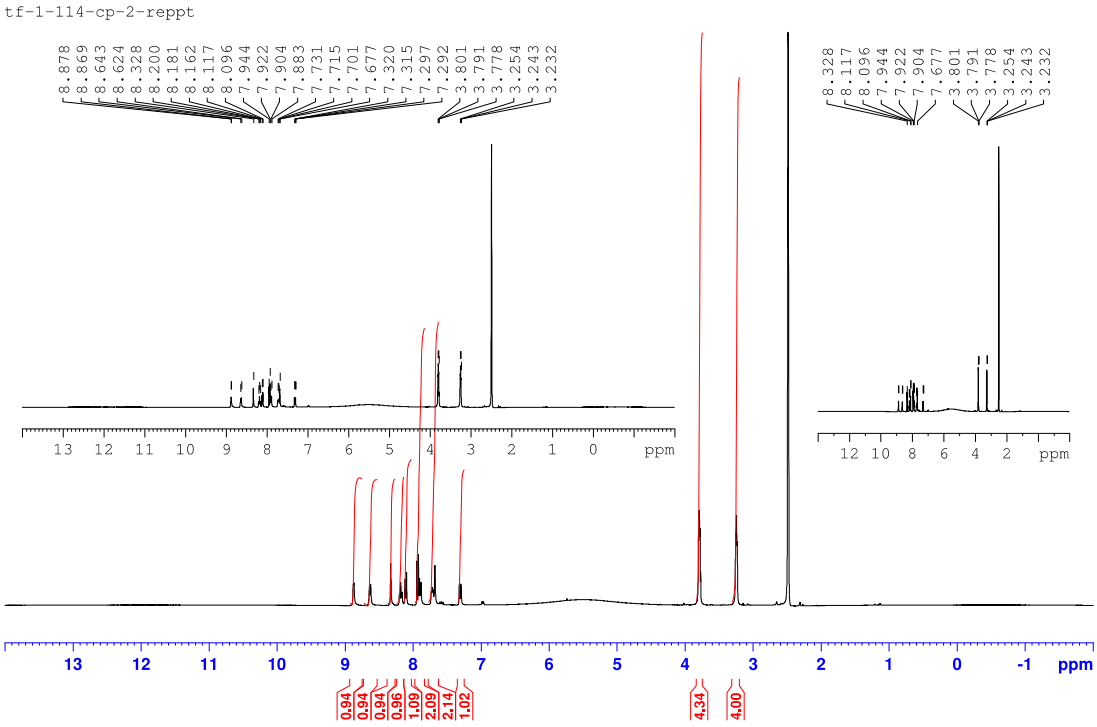

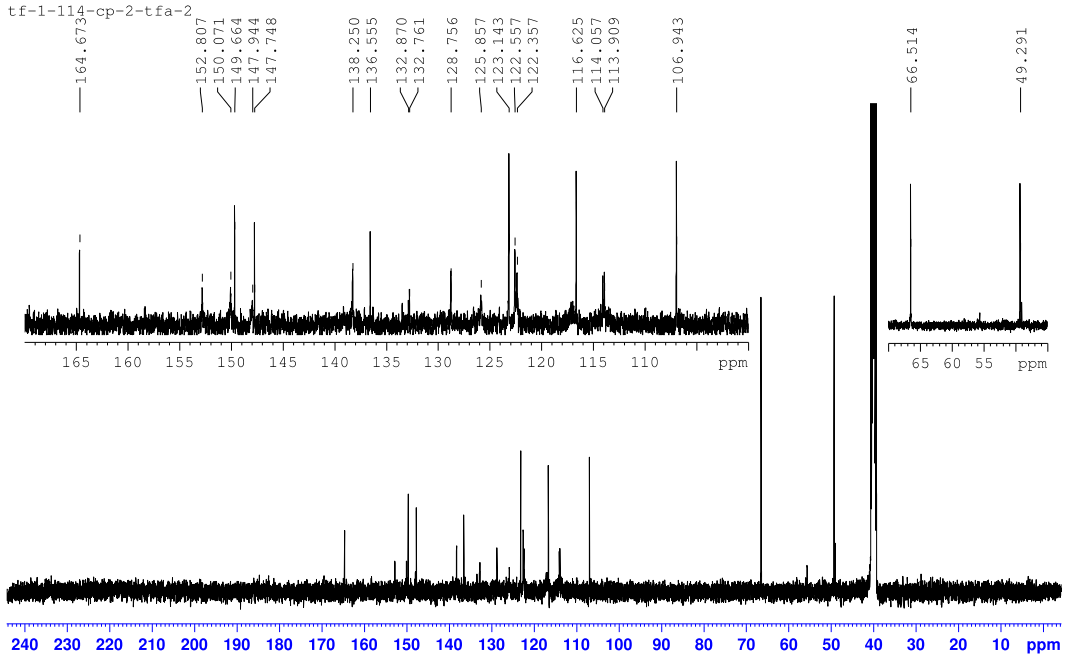

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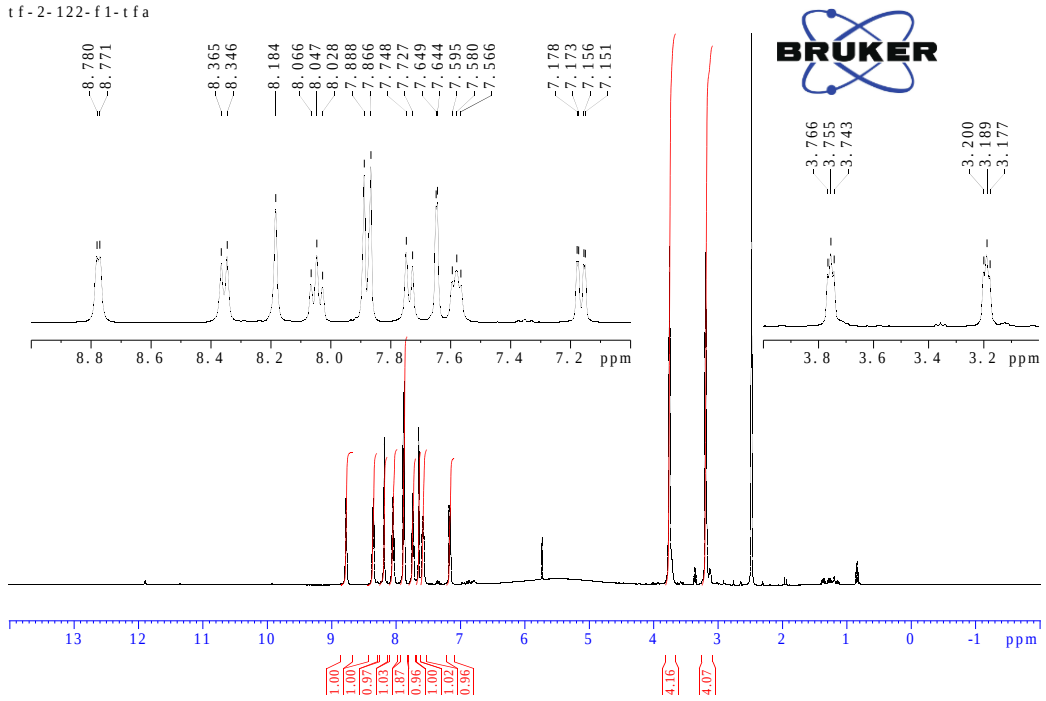

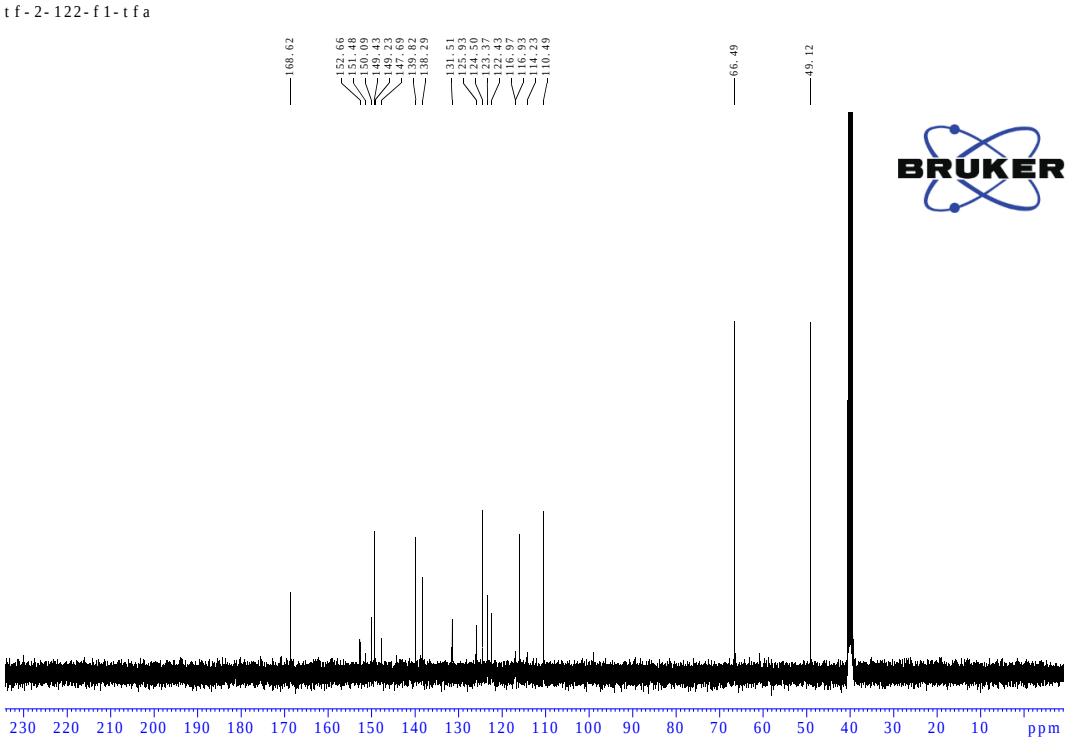

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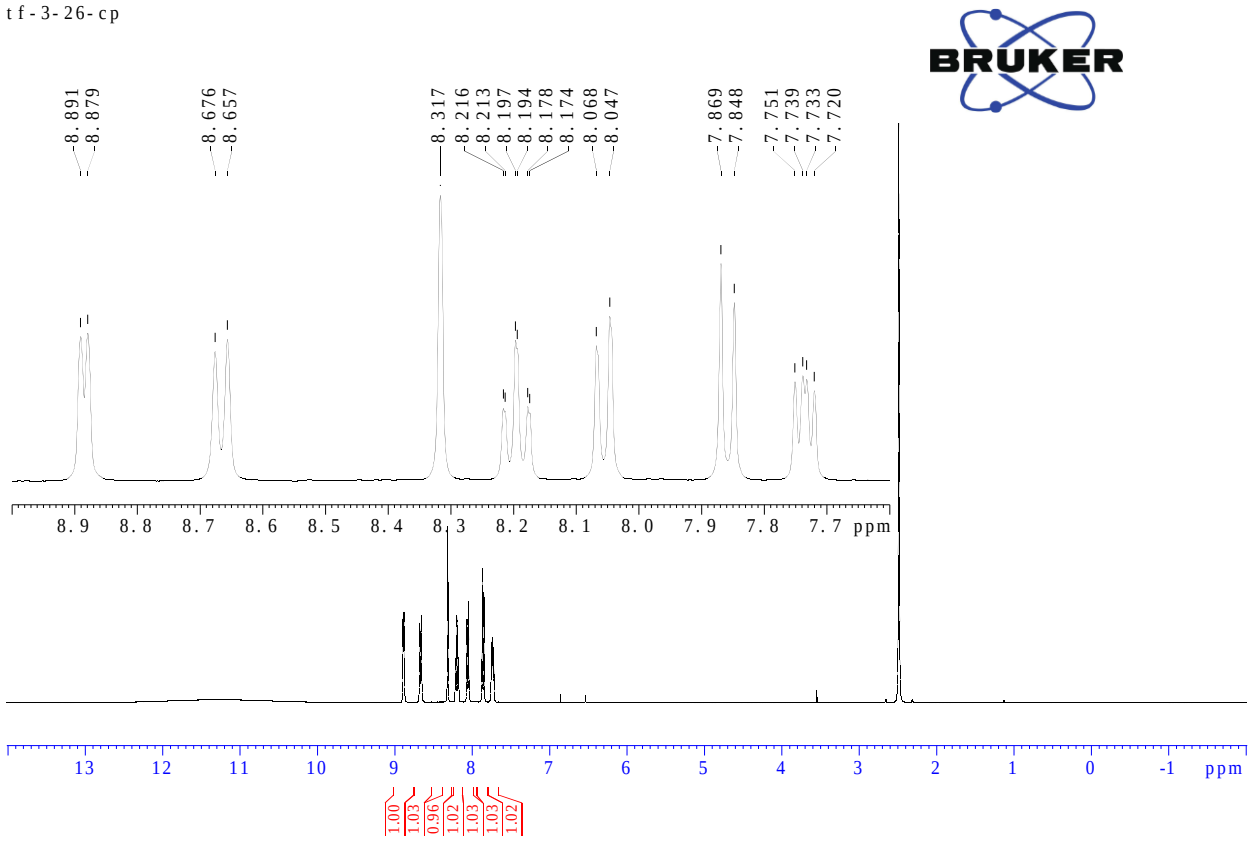

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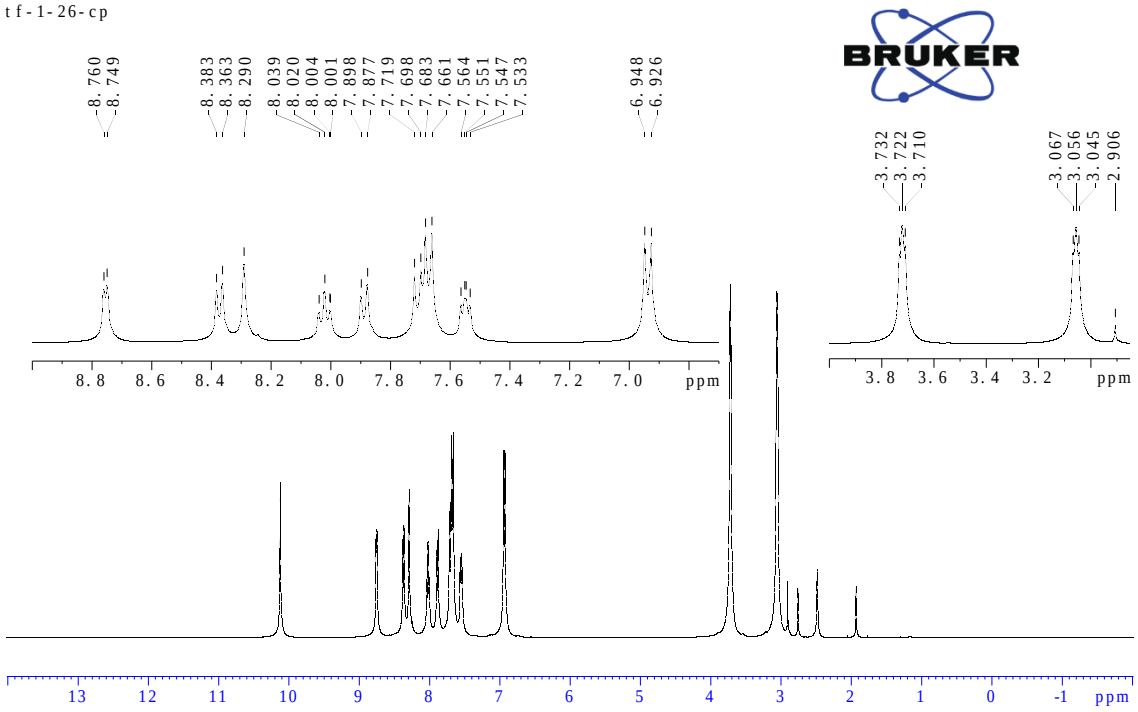

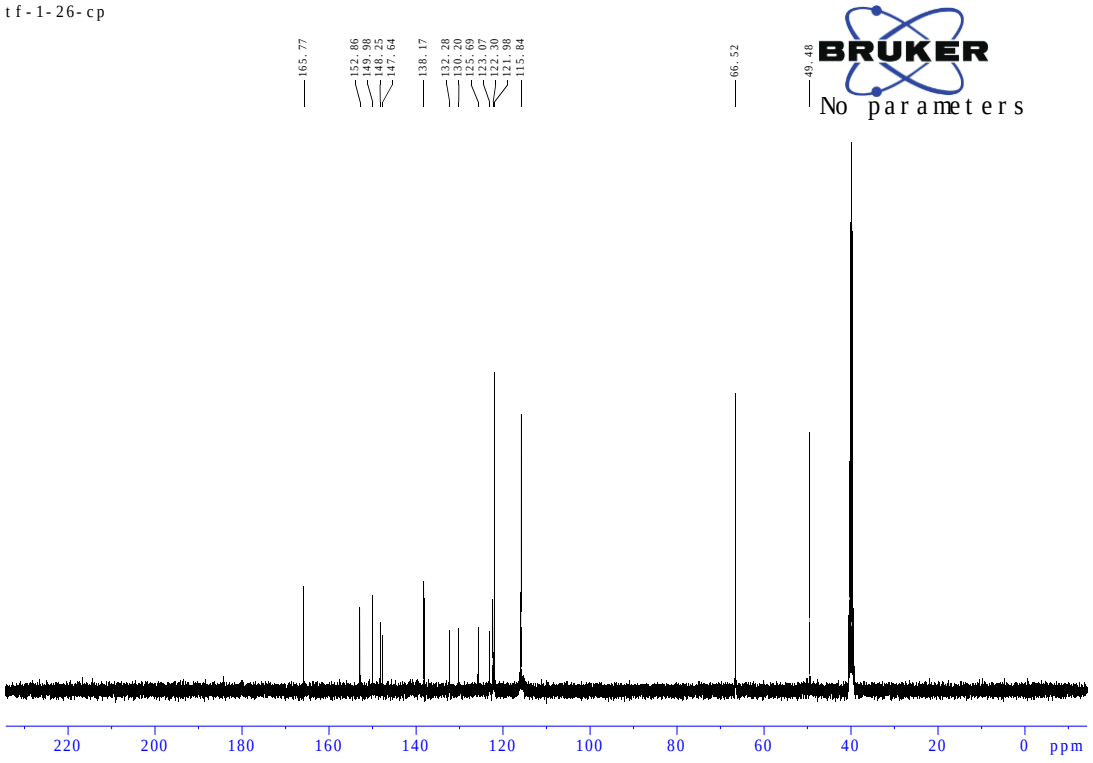

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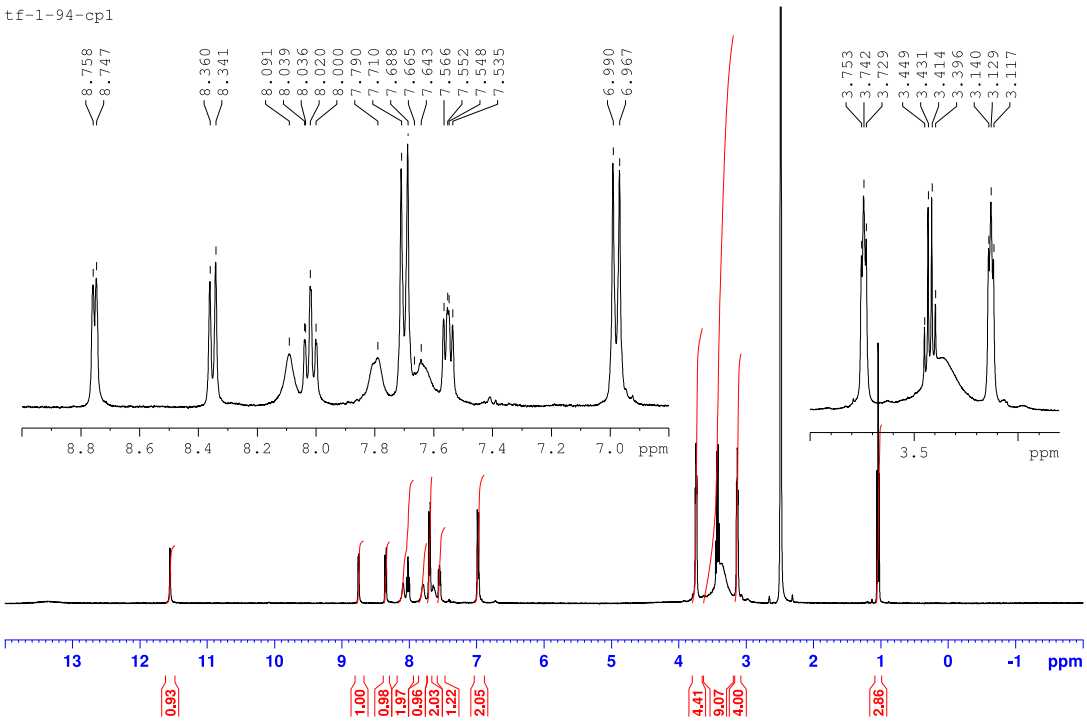
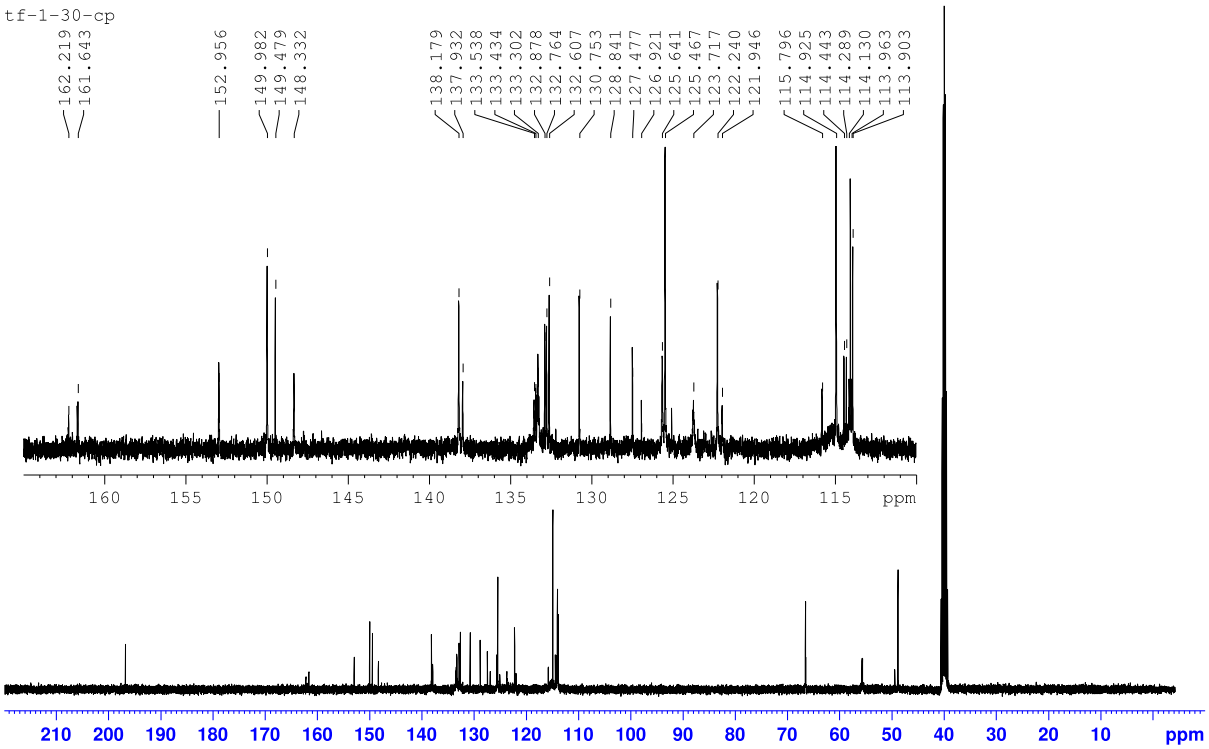


Signals at 3.42 (q) and 1.05 (t) attributed to residual ethanol



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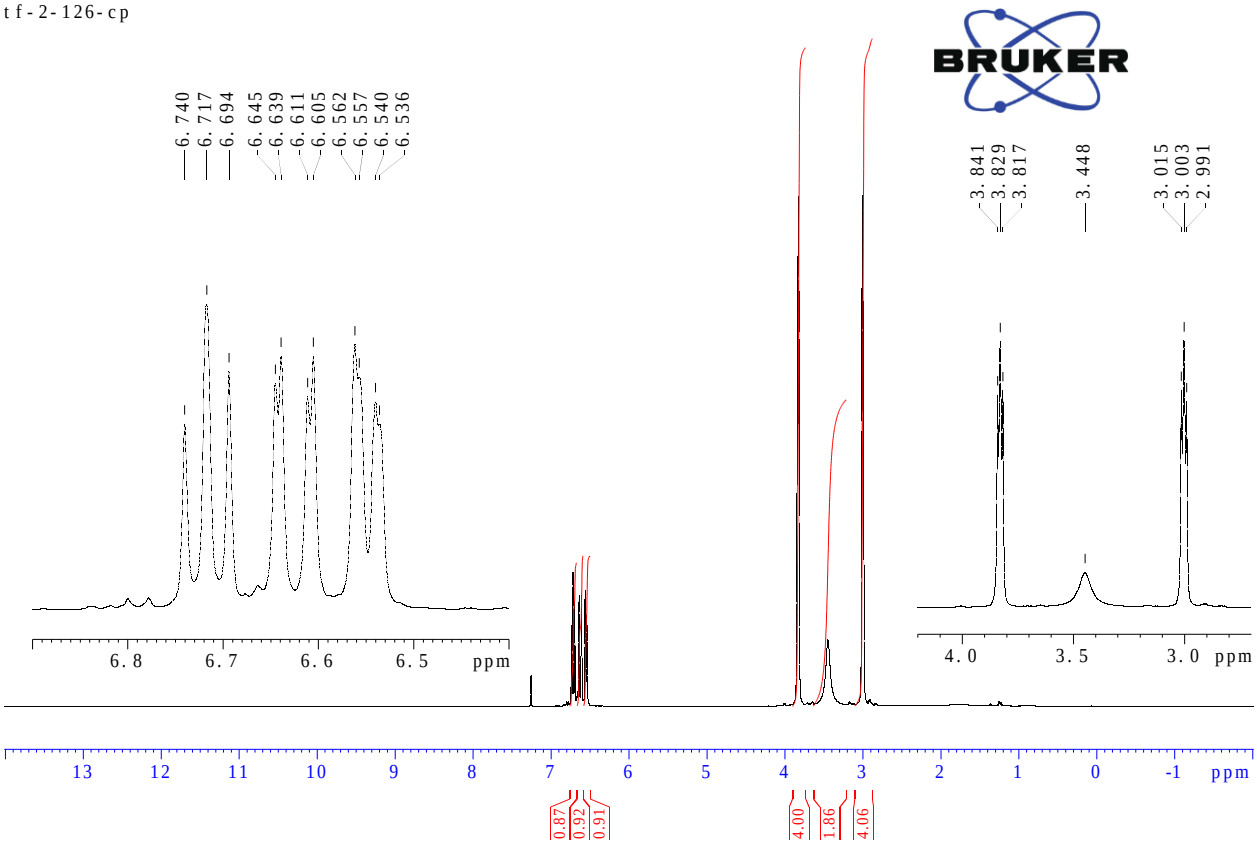

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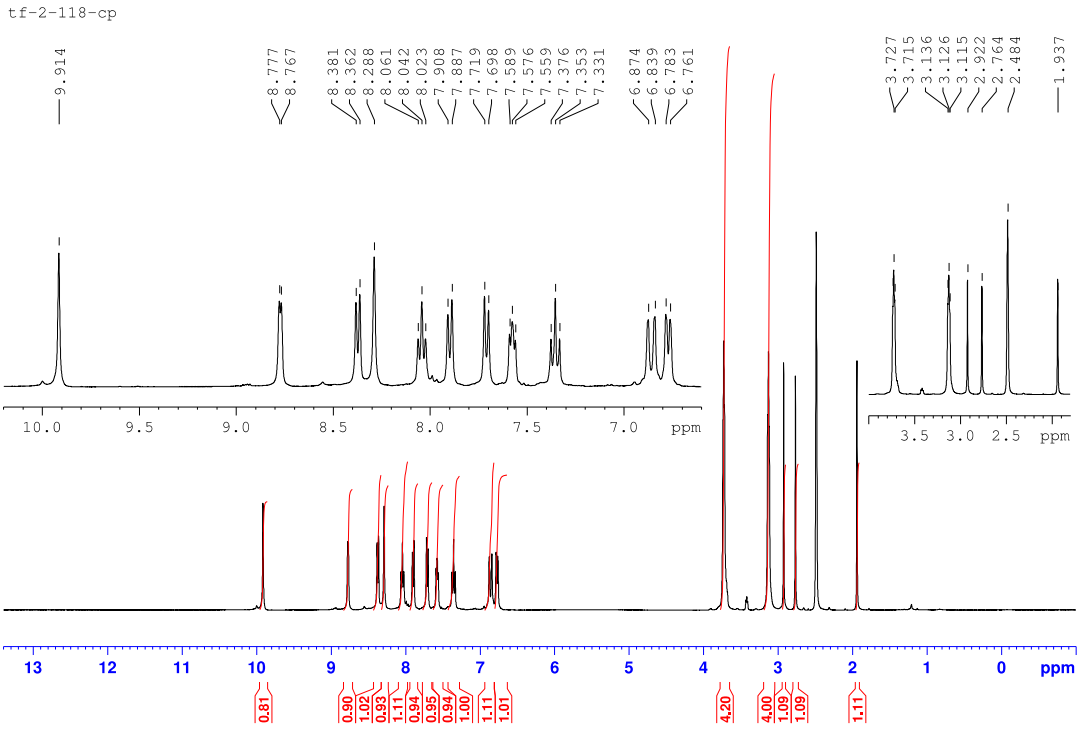
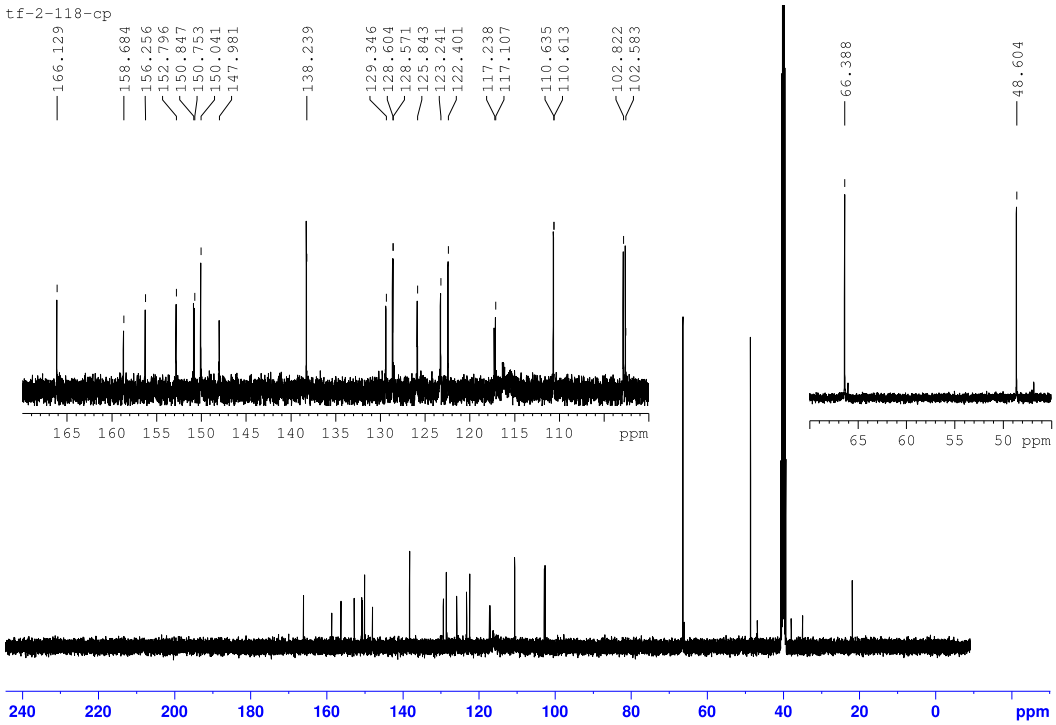


2.922 (s), 2.764 (s), 1.937 (s) attributed to N,N-dimethylacetamide impurity (0.3 eq)



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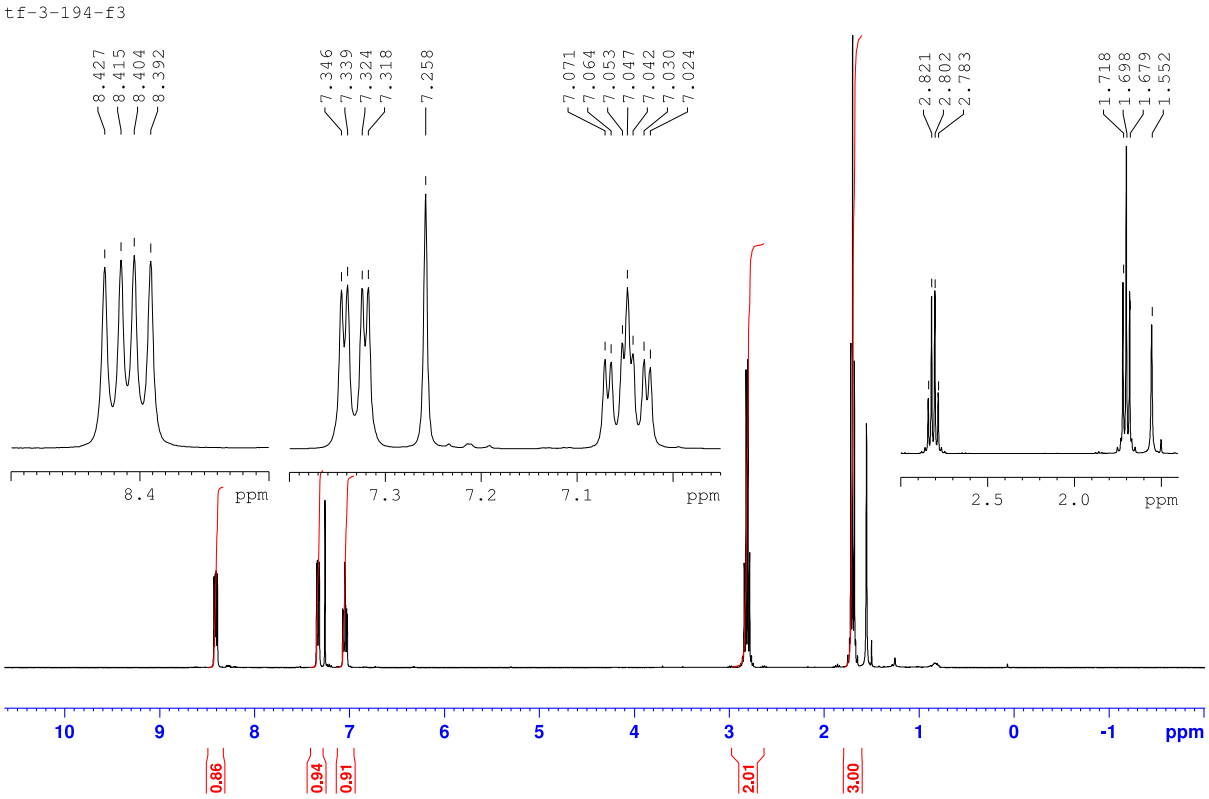

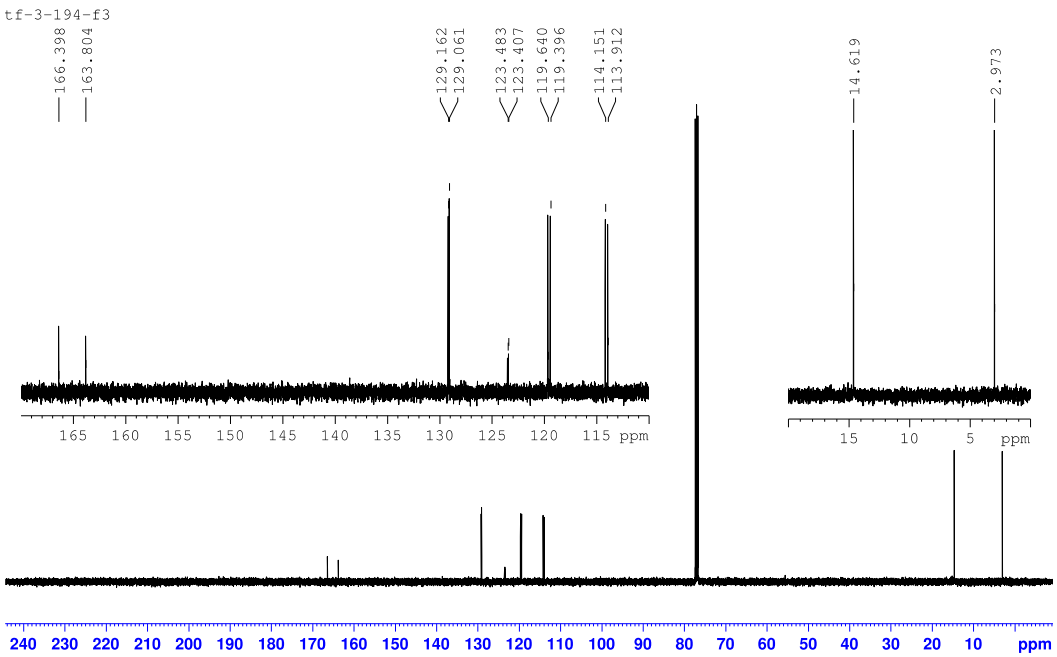

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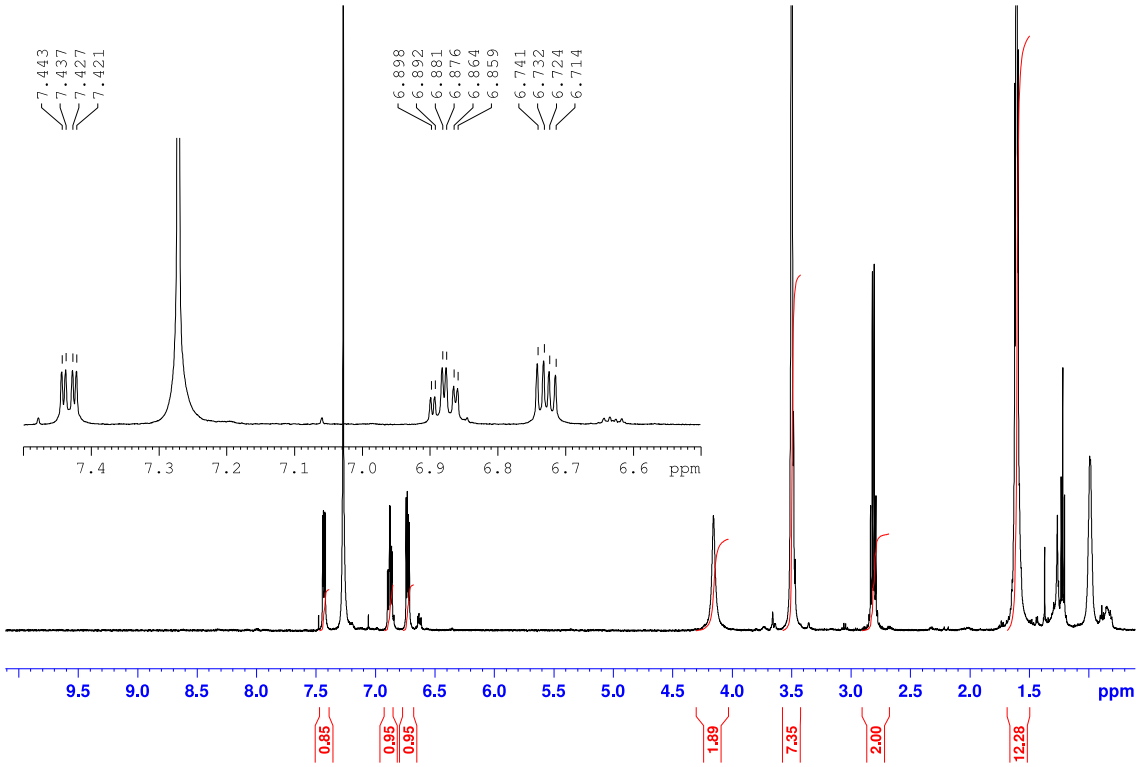

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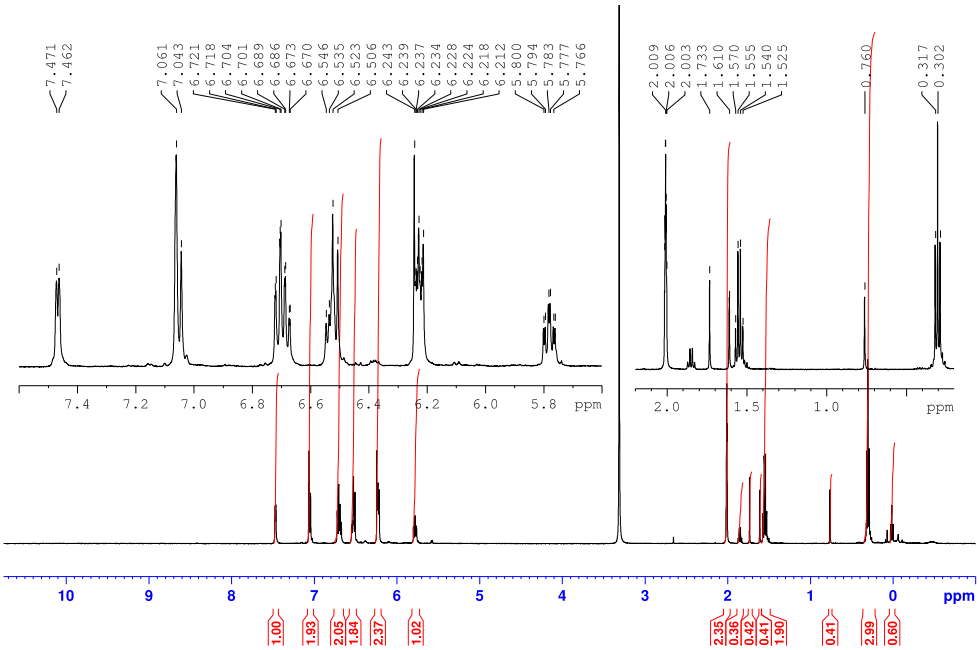

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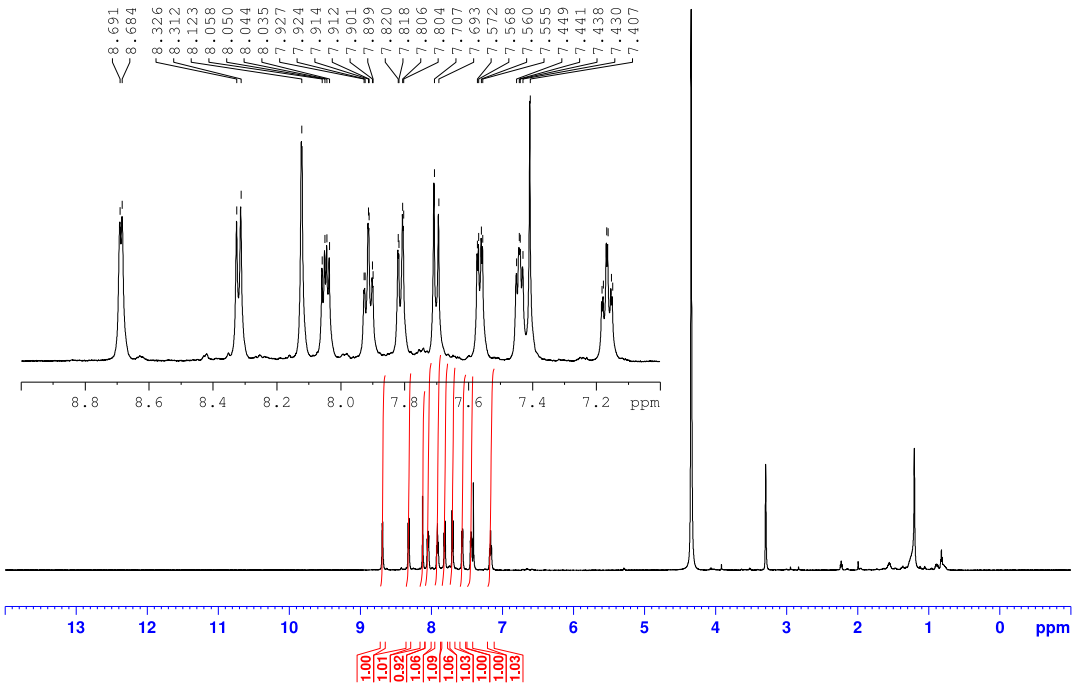

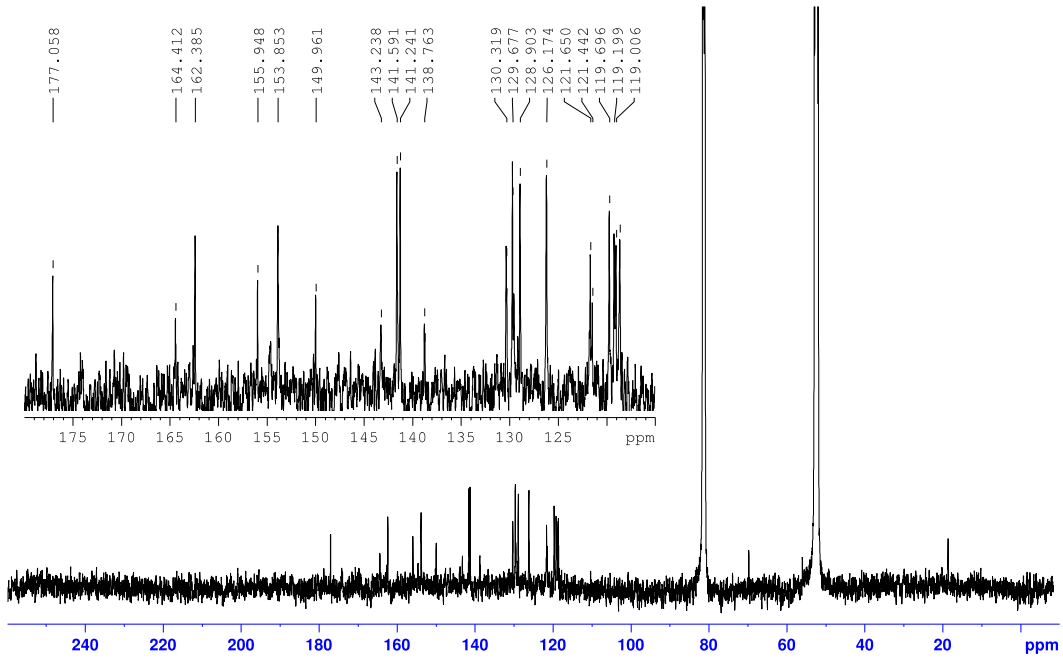

Ligand-DNA binding Analysis

1 Theoretical approach

The purpose of the analysis described in the following section is to calculate the ligand-DNA binding parameters, namely the binding dissociation constant K_d (measured in M – molar) and the frequency of binding sites N (measured in 1/bp – per base pair of DNA), from the changes in the absorbance spectrum of the ligand upon binding to DNA.

1.1 General formalism

The basic equilibrium equation for binding of species *A* to species *B* is described by the equation:

$$K_d = \frac{[A][B]}{[AB]} \qquad \text{S1}$$

where [*A*] is the concentration of free species *A*, [*B*] is the concentration of free species *B*, [*AB*] is the concentration of *AB* complexes and K_d is the binding dissociation constant. Application of the equation S1 to the case of ligand-DNA binding produces the following equation:

$$K_d = \frac{(Nx - \alpha h)(1-\alpha)h}{\alpha h} \qquad \text{S2}$$

where *x* is the DNA concentration, *h* is the total ligand concentration, □ is the fraction of the bound ligand and *N* is the frequency of binding sites.

In the equation S2, (*Nx*- αh) is the concentration of the free (not occupied) binding sites (species *A* in equation S1), (1- α)*h* is the concentration of free ligand (species *B* in equation S1), and αh is the concentration of complexes (equals to the concentration of bound ligand). Equation S2 is easily converted to the standard quadratic equation relative to unknown variable α:

$$h\alpha^2 - (K_d + Nx + h)\alpha + Nx = 0\,. \qquad \text{S3}$$

The positive solution of this equation relative to □ produces the following expression:

$$\alpha = \frac{K_d + Nx + h - \sqrt{(K_d + Nx + h)^2 - 4Nxh}}{2h}\,. \qquad \text{S4}$$

Expression S4 can be regarded as a basis for non-linear regression analysis of the experimental data for the measured fraction of the bound ligand α assuming K_d and *N* as free parameters to be estimated as a result of the regression, and *x* and *h* as experimentally measurable variables.

1.2 Data Analysis Method

Experimental data for evaluation of DNA-ligand binding parameters consists of a range of absorbance spectra obtained following titration of the ligand solution with DNA. Two variables are under control: the DNA concentration (denoted as *x*) and the total ligand concentration (denoted as *h*). In a general case, both variables may change for each titration point. Assuming that there are *n* titration points, we can denote the absorbance spectrum for each titration point x_i and h_i as $\mathbf{Y_i}$, (*i* = 0, 1, …, *n*). We consider spectrum $\mathbf{Y_i}$ as a vector (or an array) each element of which is the absorbance at a particular wavelength. The DNA concentration at the first titration point (*i* = 0) x_0 = 0, so $\mathbf{Y_0}$ is the spectrum of free ligand. Unfortunately, it is very difficult to achieve conditions providing 100% binding of the ligand, so at the titration point with the highest DNA concentration x_n the measured spectrum $\mathbf{Y_n}$ is not the spectrum of bound ligand. Therefore we denote the spectrum of bound ligand which is usually unknown as $\mathbf{Y_b}$. Any spectrum **Y** of a ligand-DNA solution at particular values of *x* and *h* can be then represented as a linear combination of the $\mathbf{Y_0}$ and $\mathbf{Y_b}$ spectra by the following expression:

$$\mathbf{Y} = (1-\alpha)\mathbf{Y_0} + \alpha\mathbf{Y_b} \qquad \text{S5}$$

Since absorbance of the ligand is proportional to the ligand concentration *h*, we assume that in expression S5 all spectra are normalised to the same ligand concentration. When applied to the spectra $\mathbf{Y_i}$, normalisation is

done to the concentration at the first point (measured spectrum multiplied by h_0/h_i). To compensate the contribution of DNA absorbance, we perform the titration using the dual beam mode and add DNA to both the reference and sample cuvette. Since the spectrum $\mathbf{Y_b}$ in expression S5 is not available, direct calculation of from experimental data using this expression is not possible. However it is easy to demonstrate that any spectrum **Y** can be represented by a linear combination of any two fixed spectra from the $\mathbf{Y_i}$ range. An obvious choice of these two spectra is $\mathbf{Y_0}$ and $\mathbf{Y_n}$, since one of them is the spectrum of the first species (free ligand) and the second one has the largest contribution of the second species (bound ligand). Then:

$$\mathbf{Y} = \beta_0 \mathbf{Y_0} + \beta_1 \mathbf{Y_n}, \qquad \text{S6}$$

where β_0 and β_1 are coefficients.

To establish the relationship between coefficients and and , we can substitute for $\mathbf{Y_n}$ in expression S6 using the following expression:

$$\mathbf{Y_n} = (1-\alpha_n)\mathbf{Y_0} + \alpha_n \mathbf{Y_b}. \qquad \text{S7}$$

This substitution transforms expression S6 into:

$$\mathbf{Y} = (\beta_0 + \beta_1(1-\alpha_n))\mathbf{Y_0} + \beta_1 \alpha_n \mathbf{Y_b}, \qquad \text{S8}$$

Comparison of expressions S5 and S8 allows deriving following expressions for β_0 and β_1:

$$\begin{aligned} \beta_0 &= 1 - \frac{\alpha}{\alpha_n} \\ \beta_1 &= \frac{\alpha}{\alpha_n} \end{aligned}. \qquad \text{S9}$$

Considering that $\beta_1 = 1 - \beta_0$, (as follows from expressions S9), and provided that both spectra in expression S6 $\mathbf{Y_0}$ and $\mathbf{Y_n}$, are available from the titration experiment, we applied the non-linear regression analysis for each experimental spectrum $\mathbf{Y_i}$ according to expression S6 using $\mathbf{Y_0}$ and $\mathbf{Y_n}$, as independent variables to obtain the best fit values of coefficient β_1 for each titration point (β_1^i).

A substitution in expression S9 for α from expression S4 produces the following expression for β_1:

$$\beta_1 = \frac{h_n\left(K_d + Nx + h - \sqrt{(K_d + Nx + h)^2 - 4Nxh}\right)}{h\left(K_d + Nx_n + h_n - \sqrt{(K_d + Nx_n + h_n)^2 - 4Nx_n h_n}\right)}. \qquad \text{S10}$$

We used expression S10 for the non-linear regression analysis of values β_1^i obtained at the previous step, considering *h* and *x* as independent variables and K_d and *N* as fitting parameters values of which are obtained as a result of this analysis. The spectrum of bound ligand $\mathbf{Y_b}$ can be calculated from expression S7 as follows:

$$\mathbf{Y_b} = \frac{\mathbf{Y_n} - (1-\alpha_n)\mathbf{Y_0}}{\alpha_n}. \qquad \text{S11}$$

2 Experimental

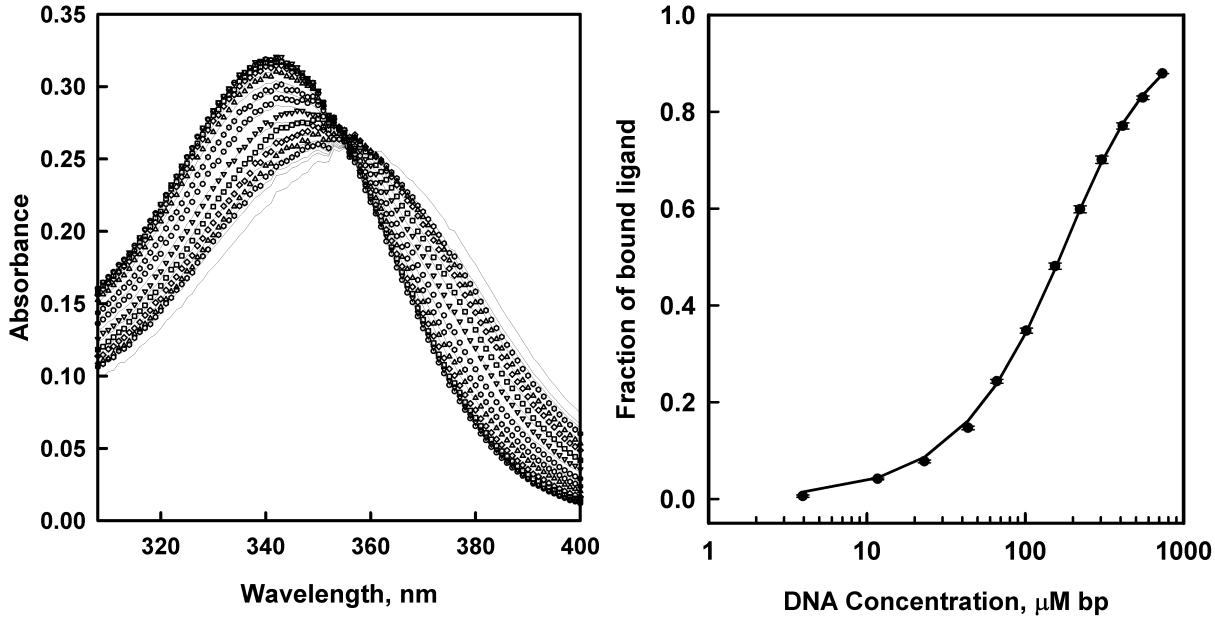

Figure S1. M2PB DNA binding analysis.

Left panel - experimental absorbance spectra of M2PB following titration with DLB16A DNA (symbols) and the results of regression analysis (lines). The range of DNA concentrations is from 0 to 730 μM bp. The range of ligand concentration is from 8.1 to 5.7 μM.
Right panel - non-linear regression analysis of fractions of bound ligand α. Values of DNA binding parameters are: K_d = 3.92 ± 0.30 μM, N = 0.0451 ± 0.002 1/bp.