

# Supplementary data

## Dual-targeted anti-CMV/anti-HIV heterodimers

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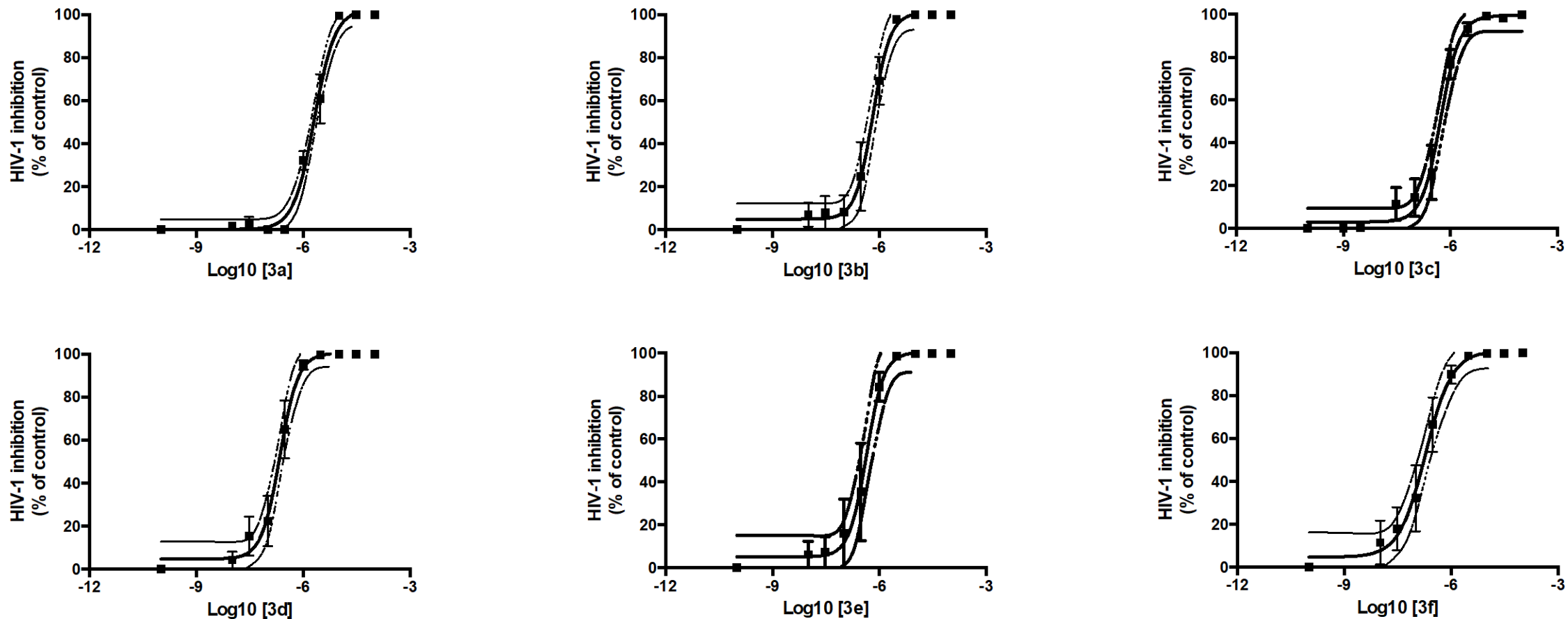


Figure S1. Anti-HIV activity of heterodimers in MT-4 cell cultures

$10^6$  MT-4 cells were inoculated with 100  $\mu$ L of HIV-1 viral stock of X4<sub>LAI.04</sub> at a p24<sub>gag</sub> concentration of 50 ng/mL for 1 h 30 min at 37°C. After incubation, excess of HIV was washed off with PBS and  $10^5$  infected cells were transferred to 24-well plates, mixed with 1 mL of medium containing each of the 6 test compounds at concentrations ranging from 100 pM to 100  $\mu$ M (in triplicate). After 3 days of culture at 37°C, the anti-HIV activity was evaluated from the suppression of viral replication compared with untreated MT-4 cell cultures. EC<sub>50</sub>s or effective concentrations that inhibited HIV-1 replication by 50% and the 95% confidence intervals were estimated by fitting the data points to a sigmoidal dose-response curve, using Prism software (Table 1).

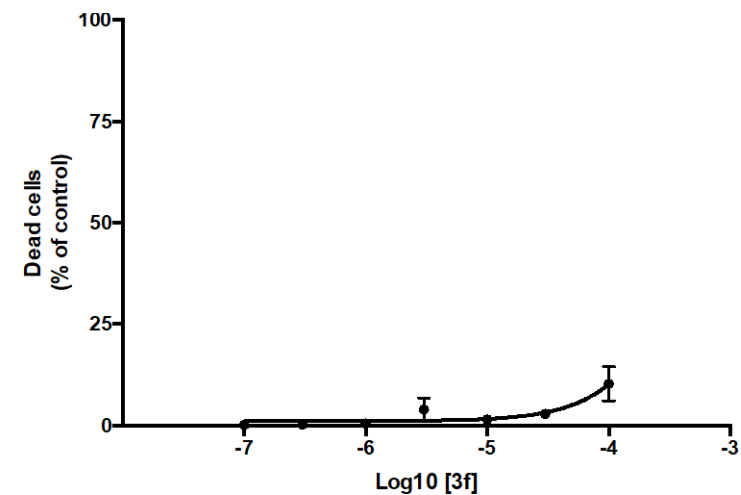
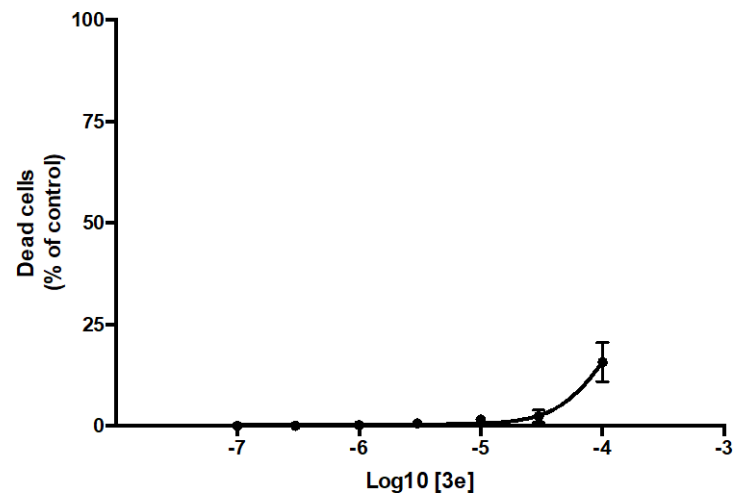
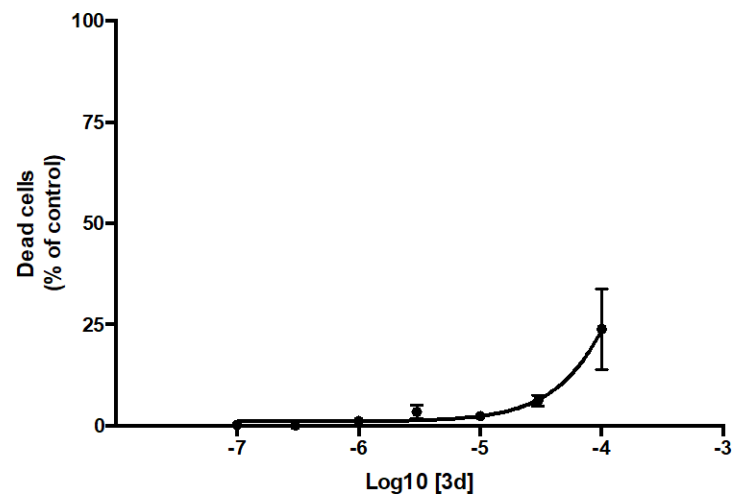
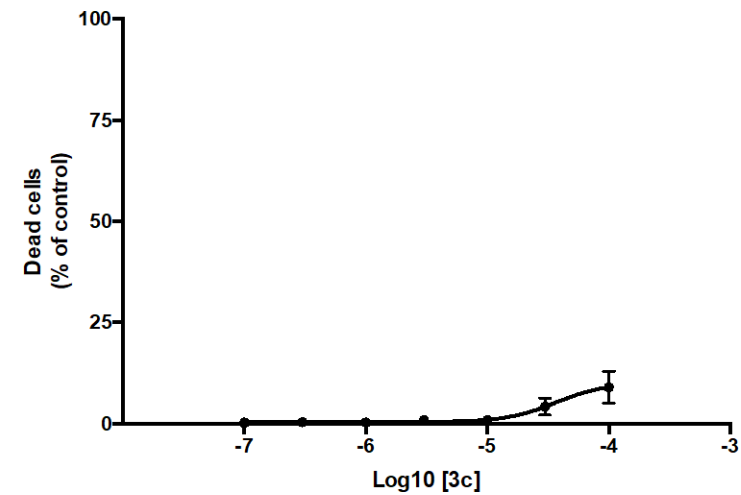
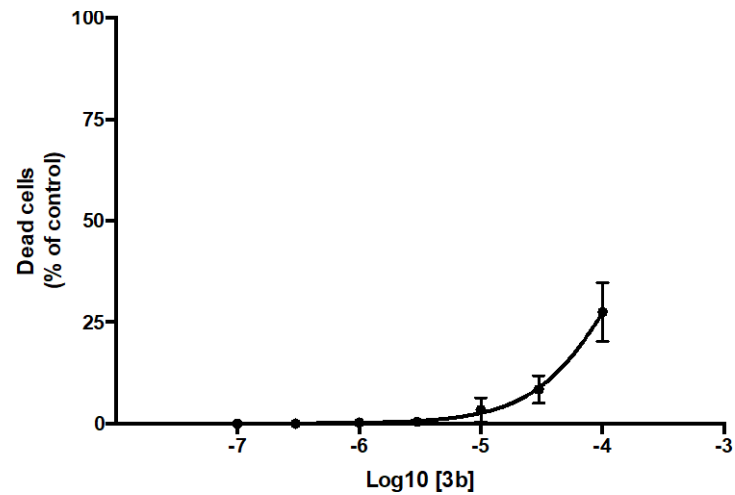
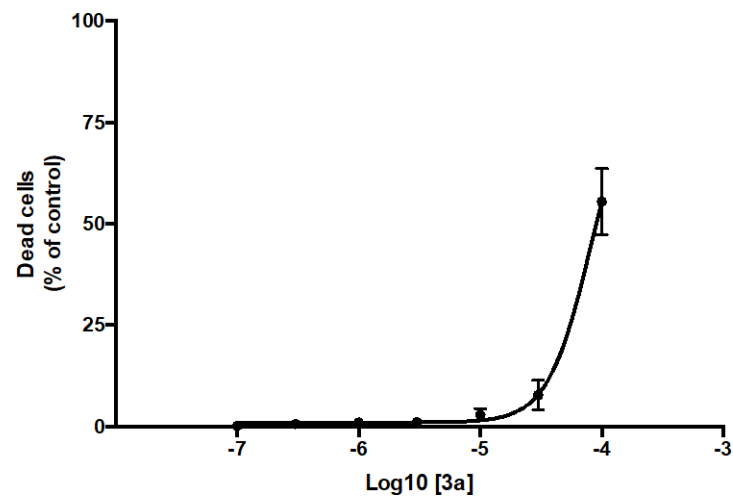
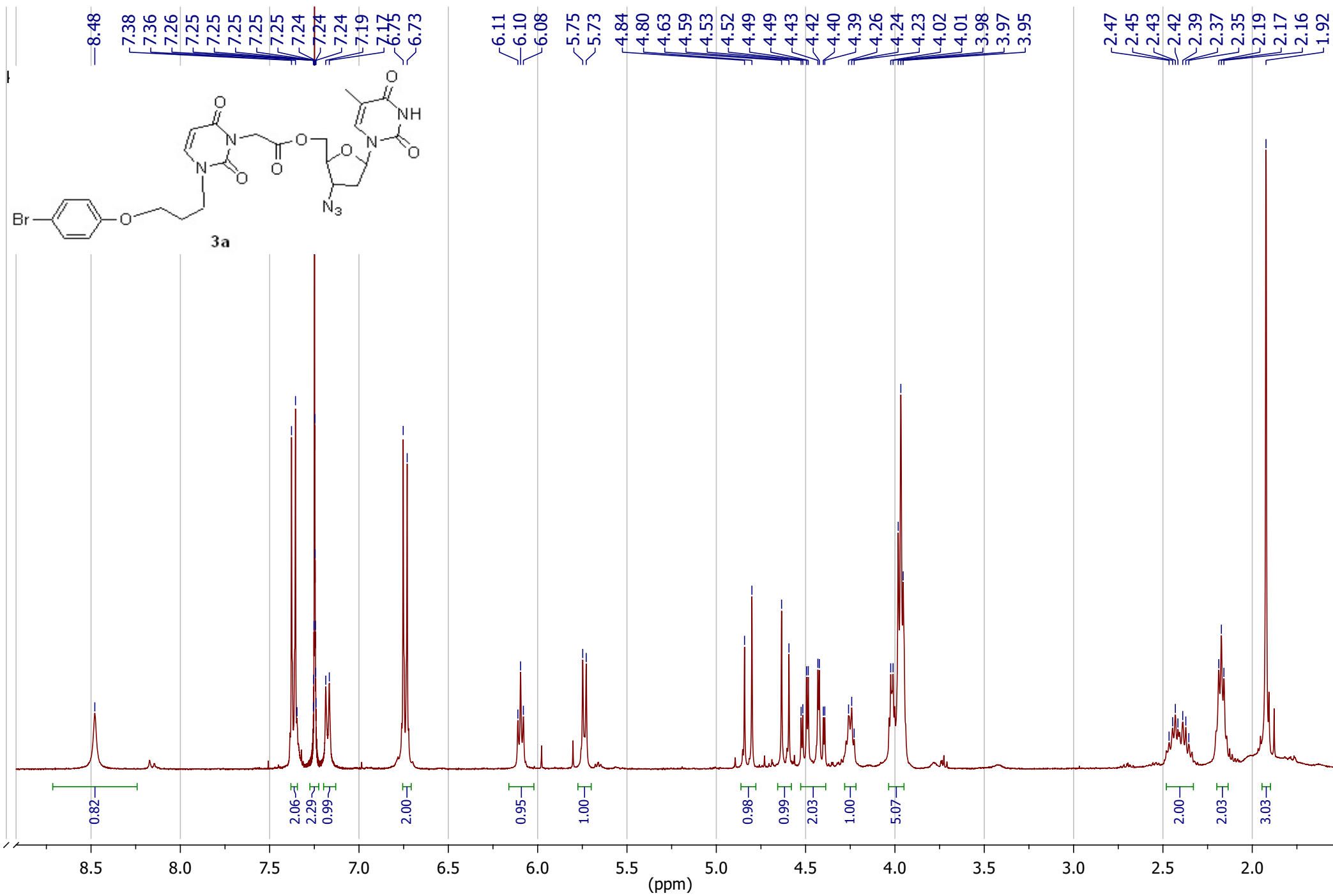
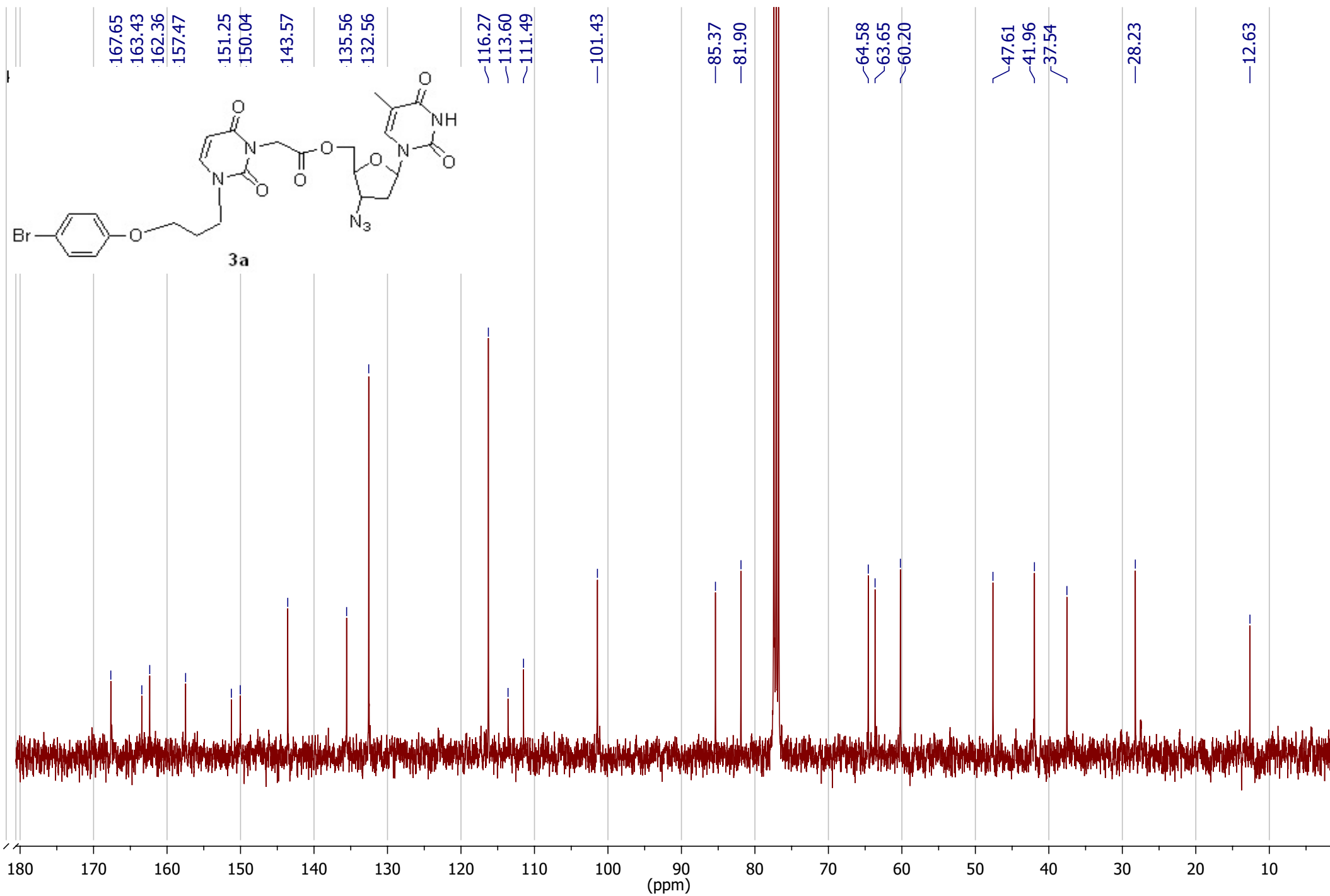


Figure S2. Low cytotoxicity of heterodimers in MT-4 cell cultures

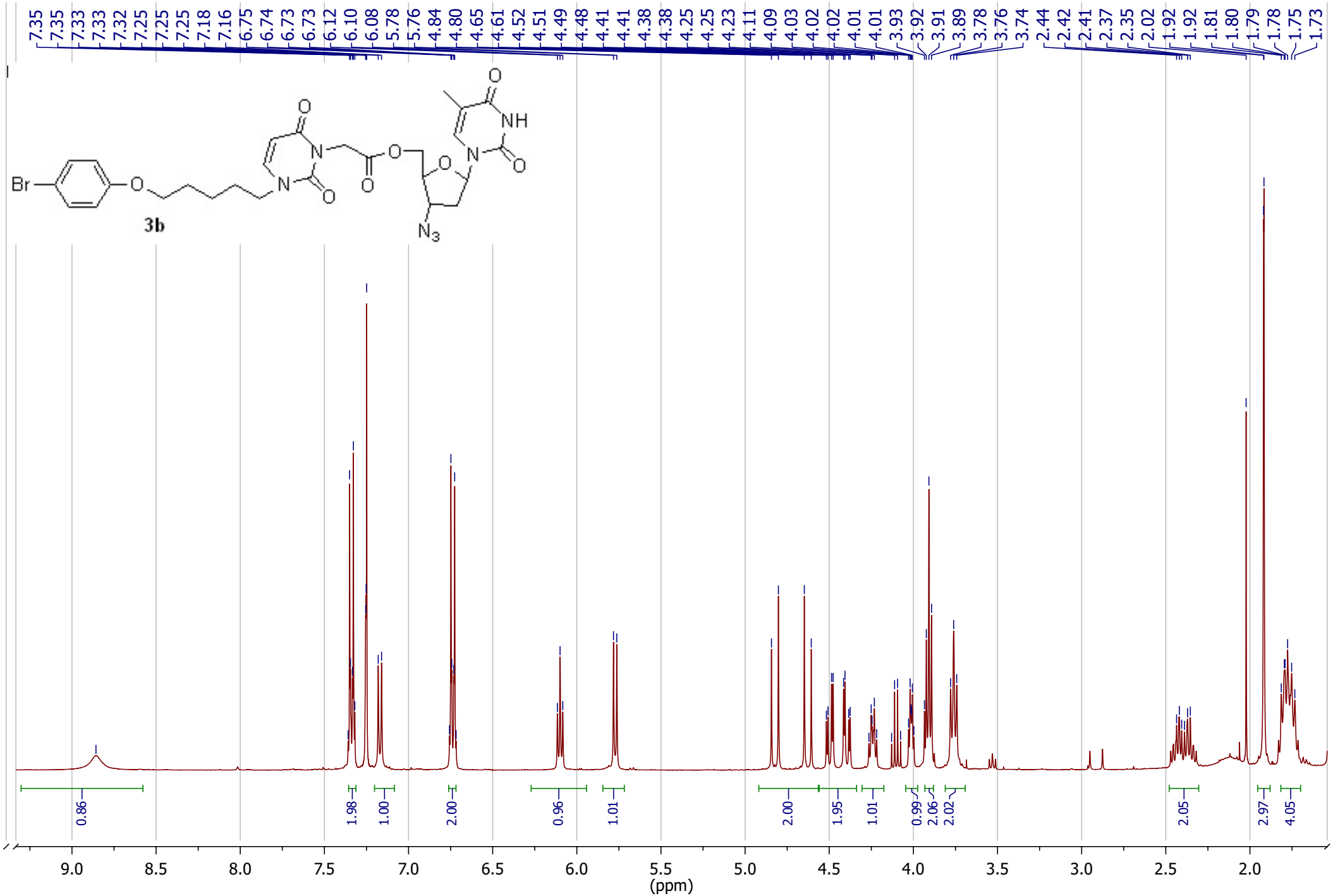
$10^5$  cells MT-4 cells were incubated at 37°C with increasing concentrations of heterodimers and cultured for 3 days. Cells were counted using a Nucleocounter® and cell death (expressed as percent of control) was estimated from five independent experiments, each of them testing seven different concentrations (ranging from 100 nM to 100  $\mu$ M).



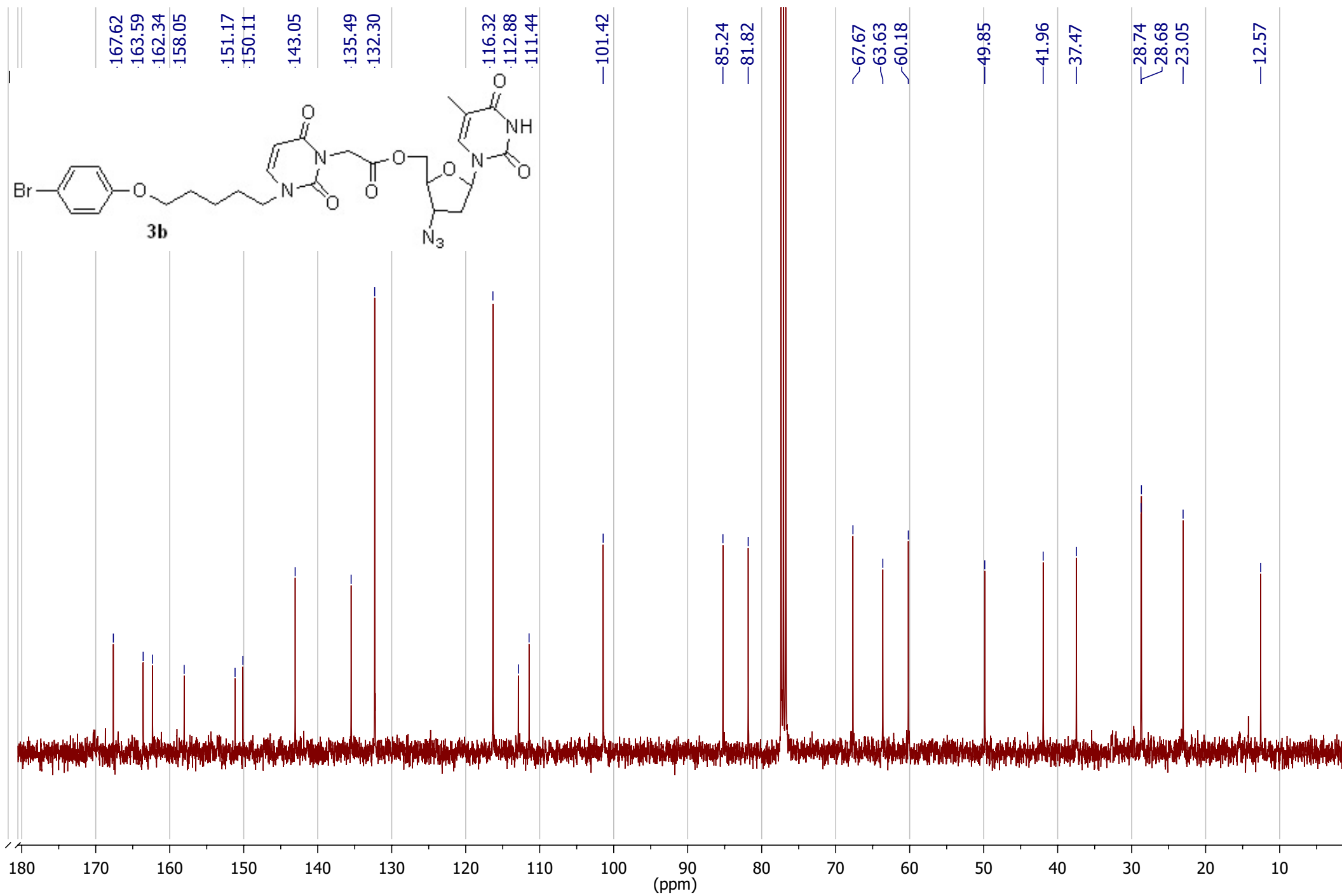
S5  $^1\text{H NMR}$  spectrum of **3a** in  $\text{CDCl}_3$



S6  $^{13}\text{C}$  NMR spectrum of **3a** in  $\text{CDCl}_3$

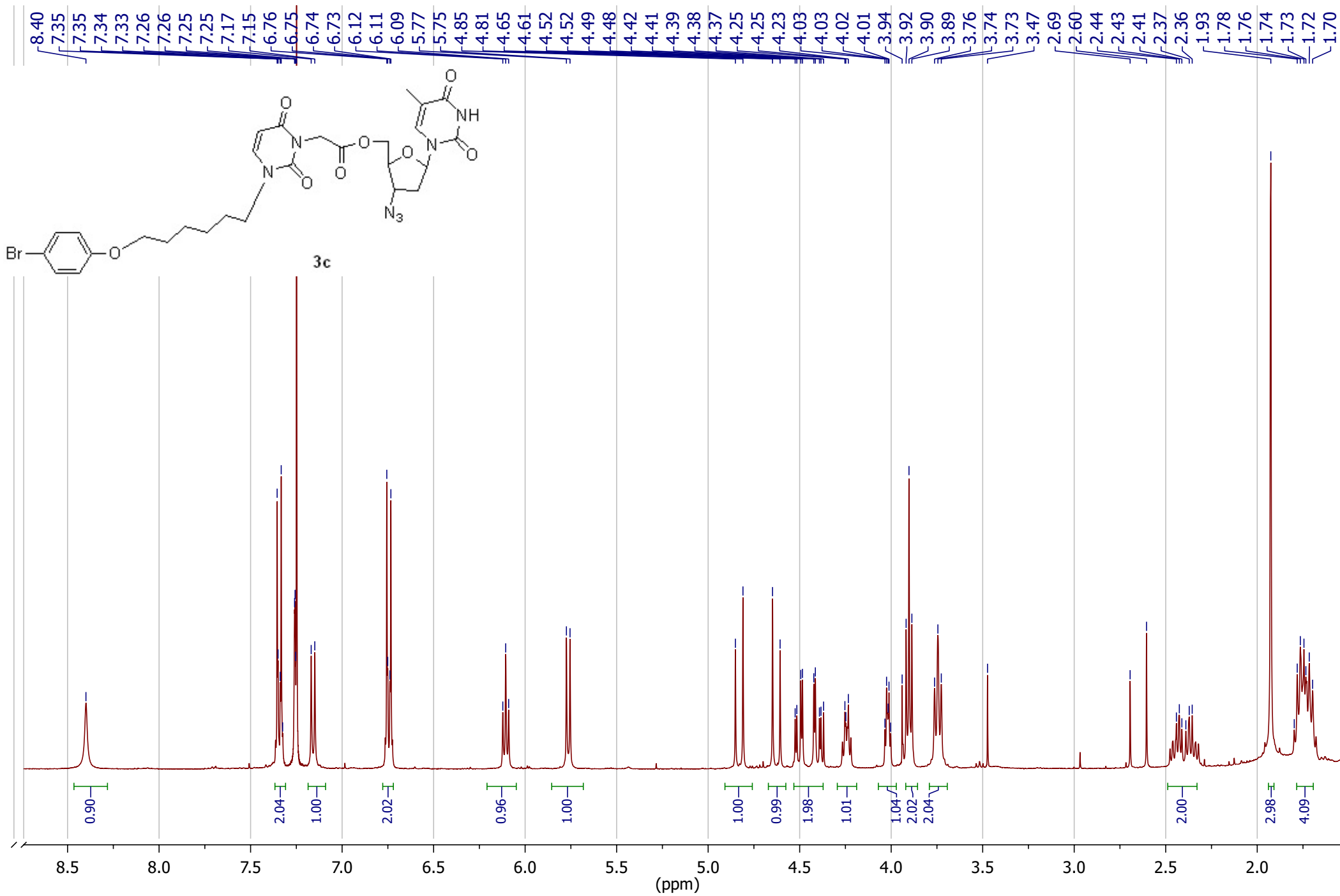


S7  $^1\text{H NMR}$  spectrum of **3b** in  $\text{CDCl}_3$

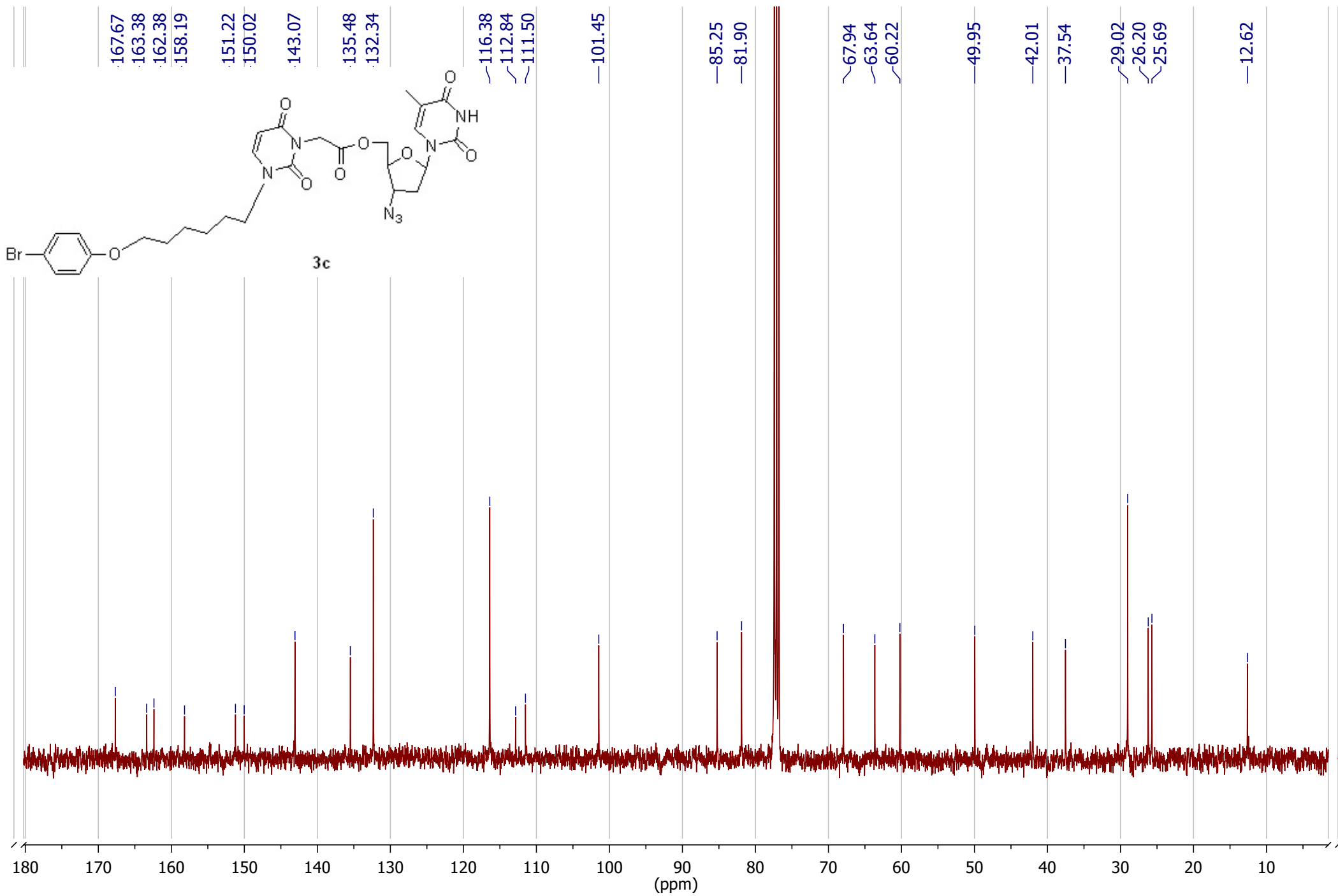


S8 <sup>13</sup>C NMR spectrum of **3b** in CDCl<sub>3</sub>

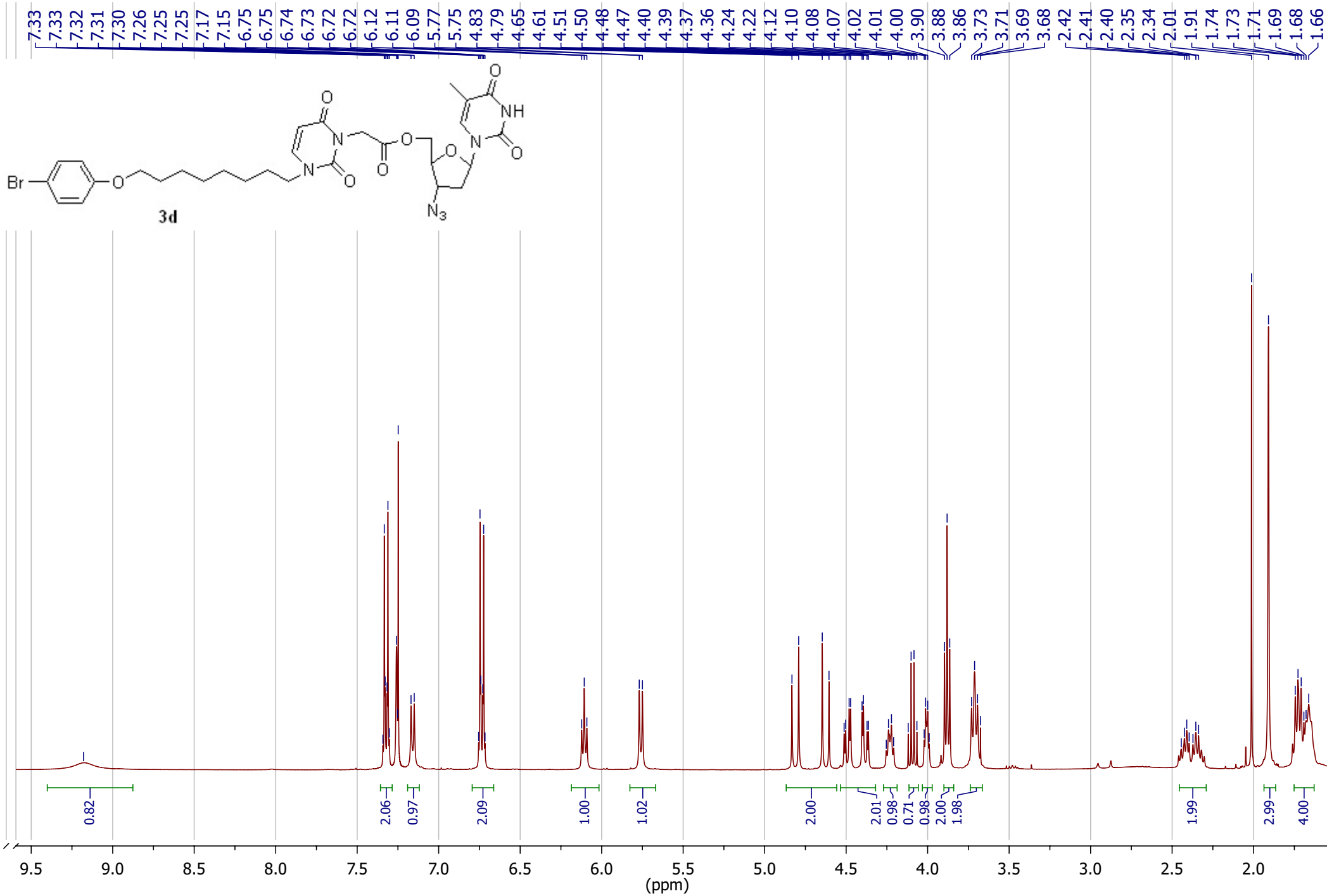




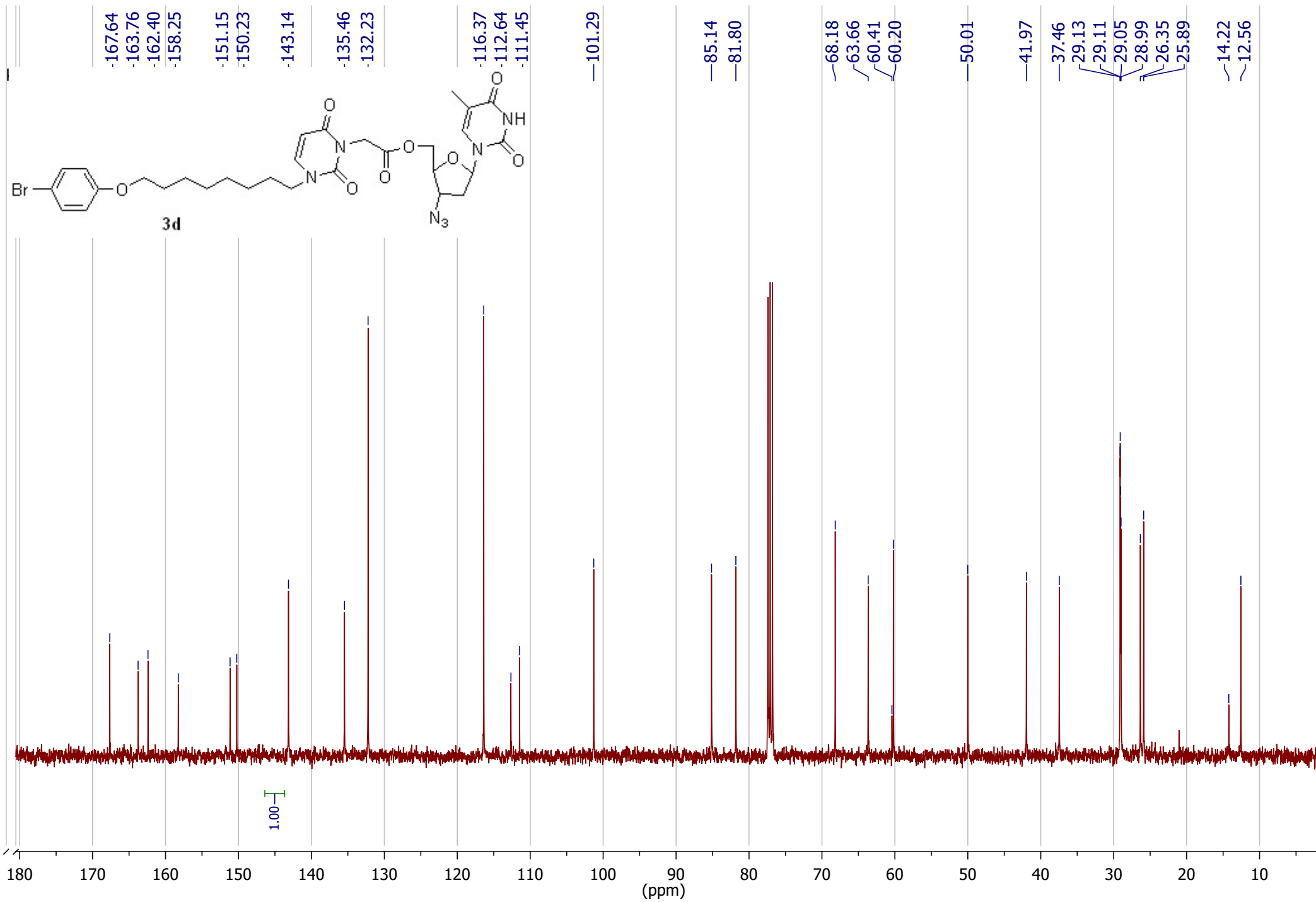
S9 <sup>1</sup>H NMR spectrum of **3c** in CDCl<sub>3</sub>

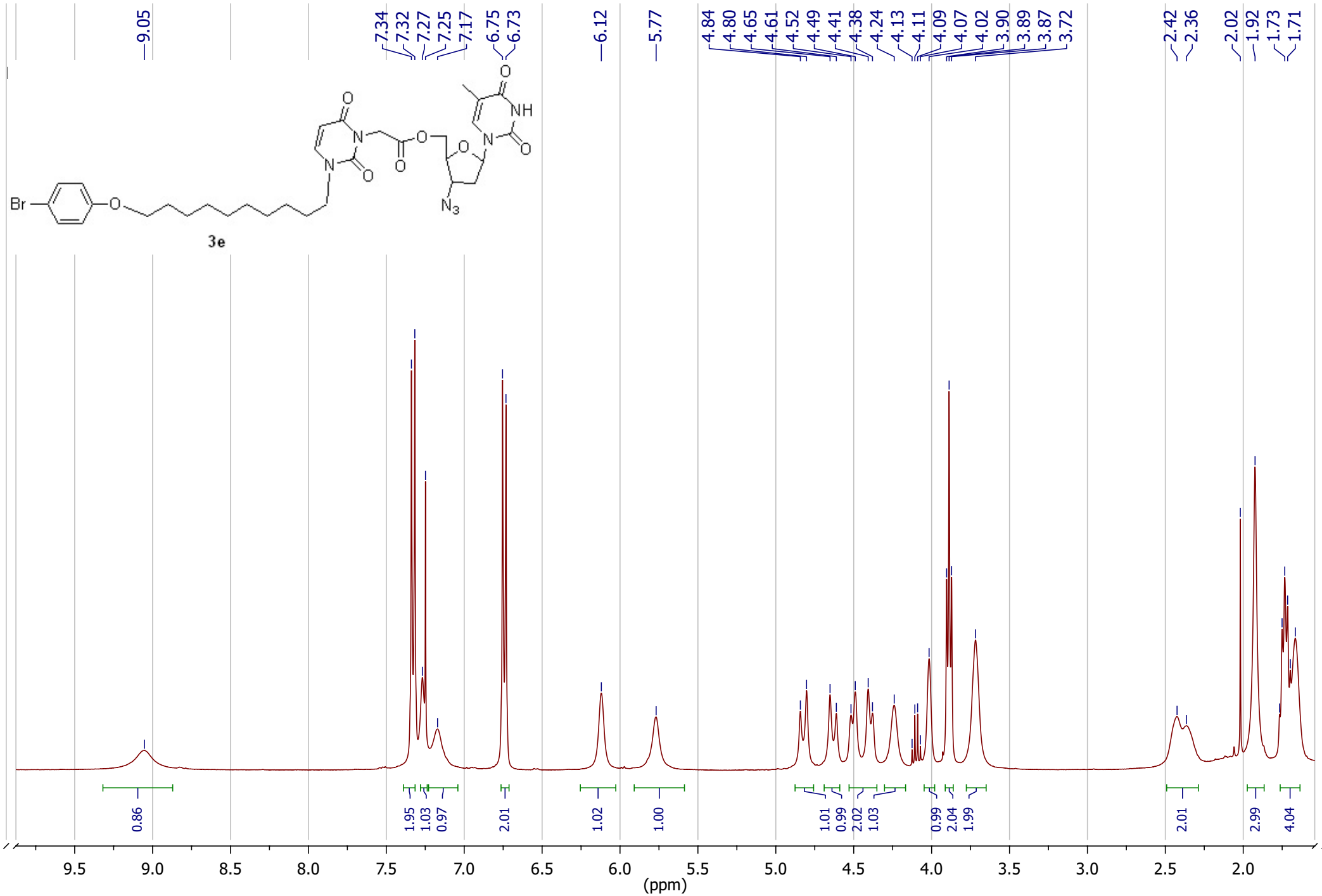


S10  $^{13}\text{C}$  NMR spectrum of **3c** in  $\text{CDCl}_3$

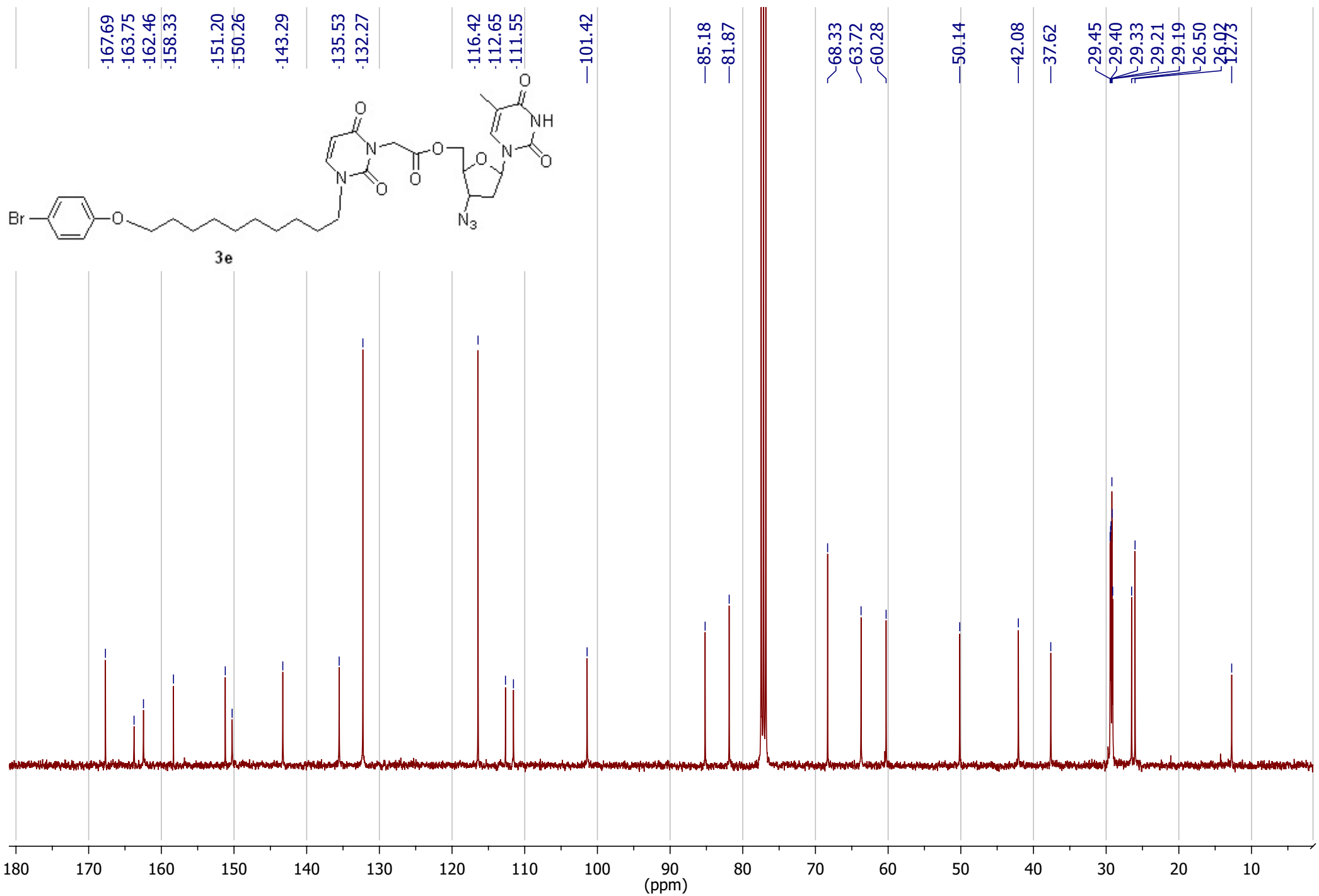


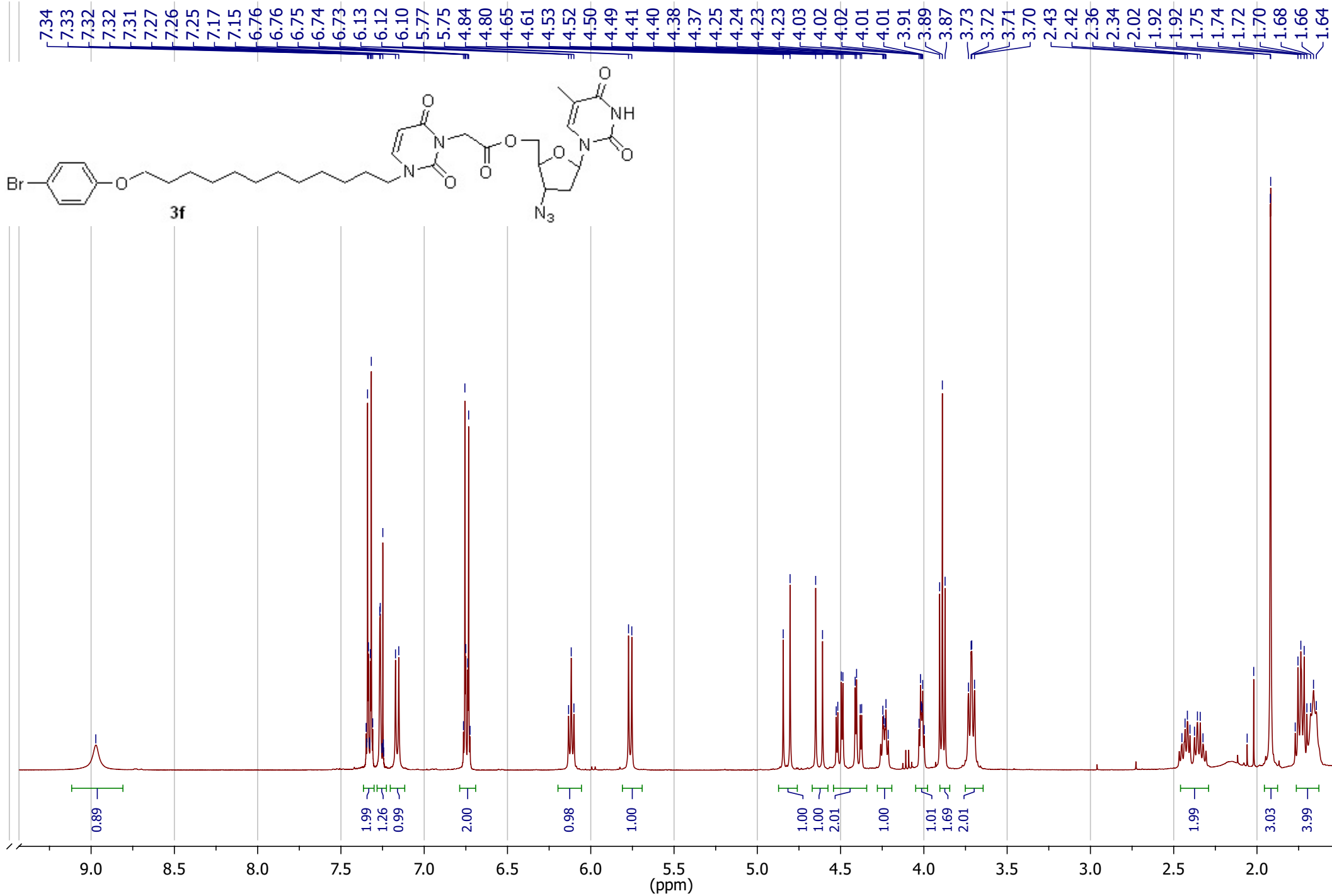
S11 <sup>1</sup>H NMR spectrum of **3d** in CDCl<sub>3</sub>



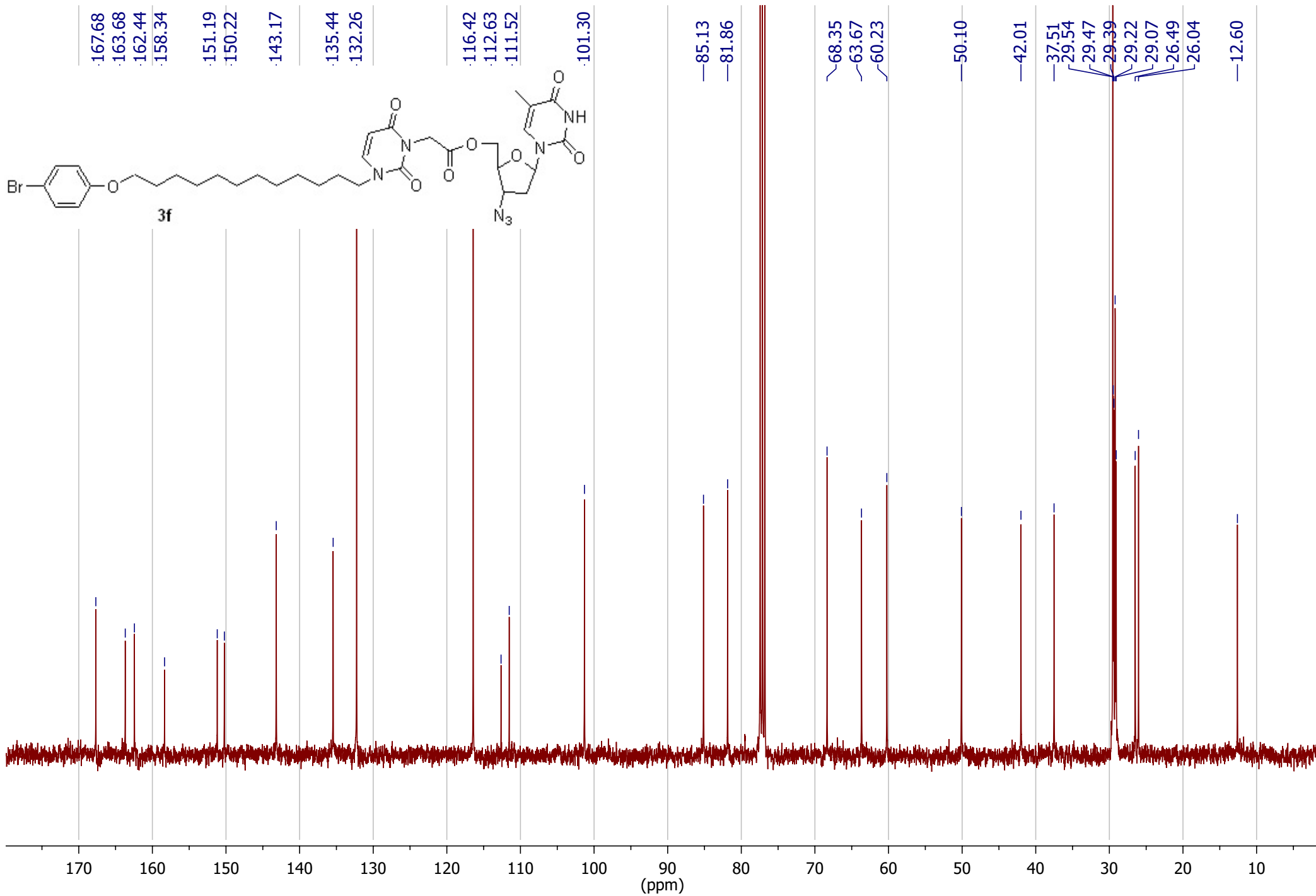


S13 <sup>1</sup>H NMR spectrum of **3e** in CDCl<sub>3</sub>



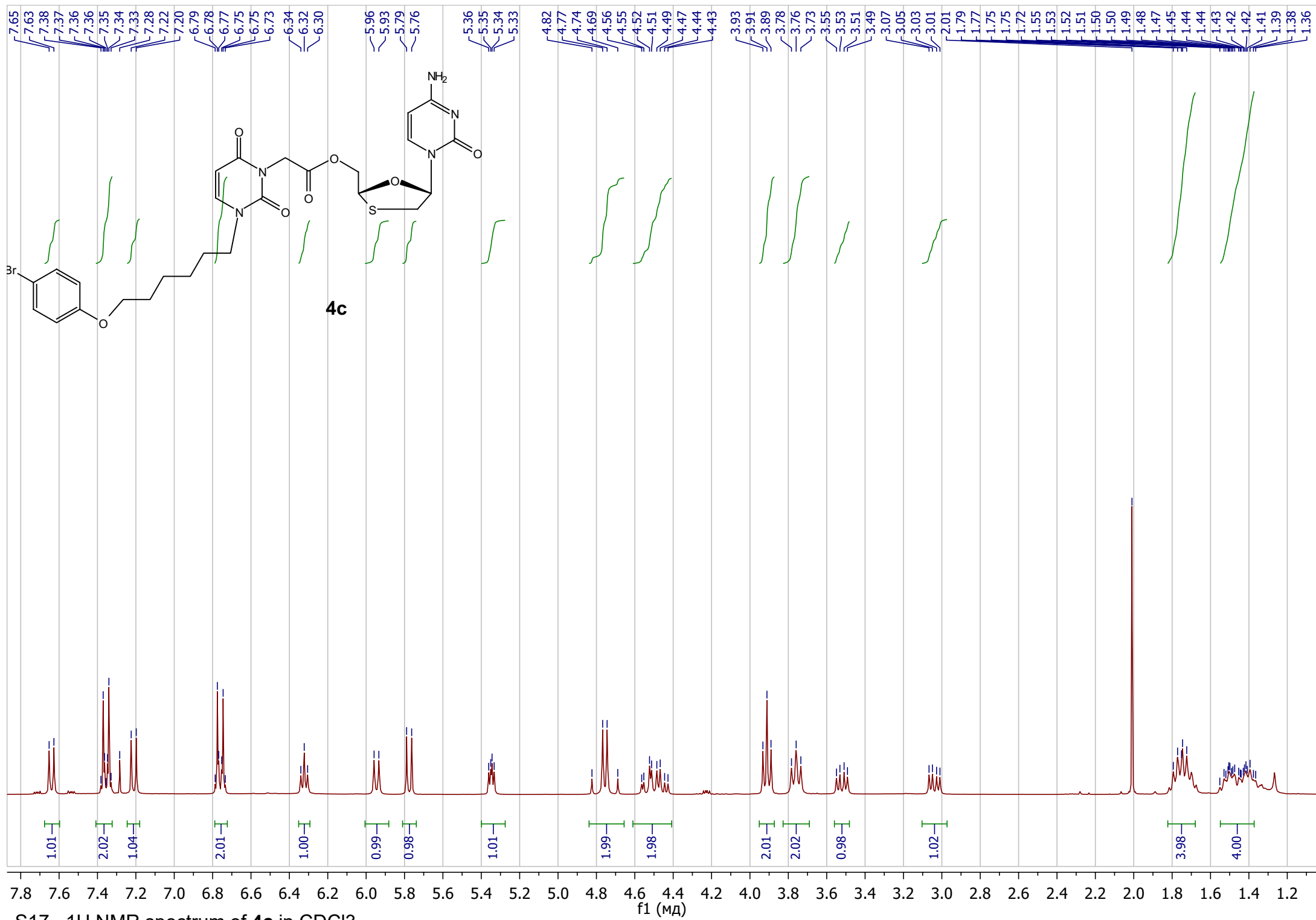


S15 <sup>1</sup>H NMR spectrum of **3f** in CDCl<sub>3</sub>

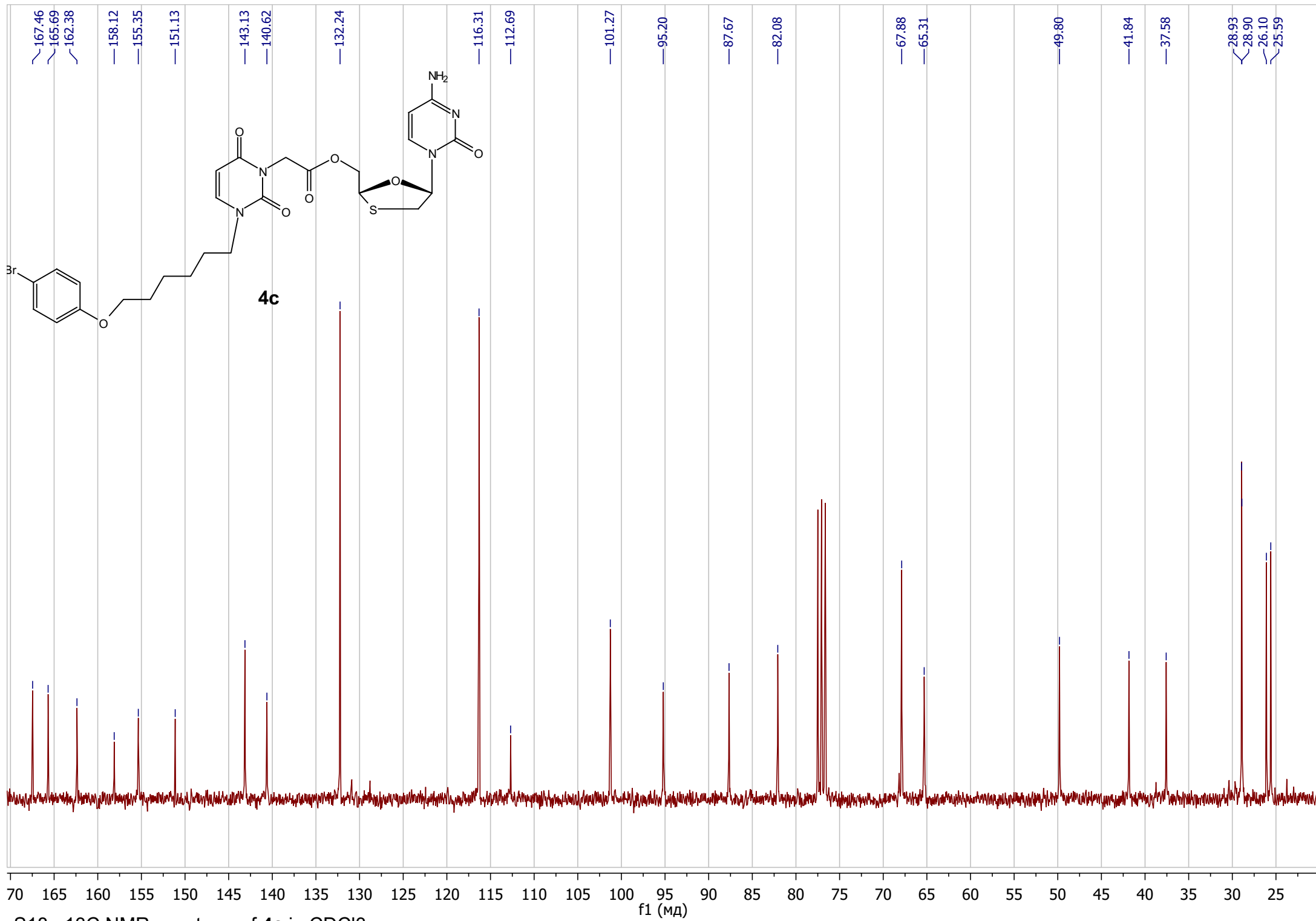


S16  $^{13}\text{C}$  NMR spectrum of **3f** in  $\text{CDCl}_3$





S17 1H NMR spectrum of **4c** in CDCl<sub>3</sub>



## 1. Description-of-PASS-prediction-for-antiviral-compounds

The coordinates of structures **2c** were saved in SDF format. PASS software was used to predict the spectra of biological activity for this structure. PASS uses the naïve Bayes approach [29755970]. A chemical structure is represented as a set of the so-called multilevel neighborhoods of atoms (MNA descriptors). MNA descriptors allow taking into account each atom of the molecule along with its nearest neighbors. In the last version of PASS, MNA-descriptors of the second level (MNA-2) are used. Estimated values  $P_a$  and  $P_i$  are calculated based on the frequencies of MNA-descriptors in the sets of active and inactive compounds of the training set.  $P_a$  and  $P_i$  reflect the probabilities for the chemical compound to be either active ( $P_a$ ) or inactive ( $P_i$ ) in certain biological activities. A compound can be active in case  $P_a$  exceeds  $P_i$ . During training process MNA descriptors along with the data on compounds, they are associated with and experiments activity spectra for these compounds are stored in SAR (structure-activity relationship) knowledge base.

To predict antiviral activity of the compound **2c** and AZT we used PASS (v.2010) specially developed for predicting antiviral properties. SAR base included 266697 compounds with information about 5825 activities. There are data on over 10000 compounds with overall antiviral properties, over 6600 compounds active against human immunodeficiency virus HIV. Also this specifically compiled SAR base have information about chemical compounds which selectively inhibit herpesviruses, including cytomegalovirus (CMV). It also includes information about compounds active against respiratory syncytial virus (RSV), adenovirus and some others.

According to the results of PASS prediction, the compound **2c** must have antiviral activity. In particular, it should be active against herpesviruses, including specific activity against CMV. It can also be active against RSV, adenoviruses, rhinovirus. In other words, it may have a wide spectrum of antiviral activity. According to the PASS results, AZT must have antiviral activity against HIV, which corresponds to the experimental results. The full predicted spectra on biological activity is provided in \*.CSV format along with SDF file containing 2D coordinates of the structure.

## PASS for compound 2c

PASS - C:\Users\fiola\Desktop\4Nastya\_Khandazhinskaya\_2a-f.mol

File Base Predict View Options Help

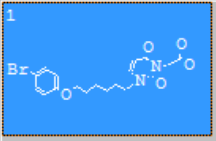
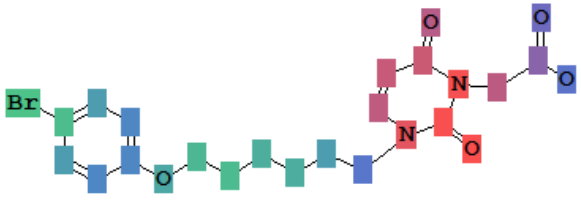
Pa > Pi

Z:\exchanger\!! PASS SAR\2010 04 12\PASS2010.SAR

C:\Users\fiola\Desktop\4Nastya\_Khandazhinskaya\_2a-f.mol

GRAPH | TEXT | MNA

1

Antiviral (CMV)

Chart	General	Effects	Mechanisms	Toxicity	Metabolism	Genes	Transporters
0.373	0.022	Intermittent claudication treatment					
0.450	0.131	Antiviral (Rhinovirus)					
0.324	0.015	Uric acid excretion stimulant					
0.410	0.104	Vasodilator, cerebral					
0.291	0.027	Antieczematous atopic					
0.325	0.080	Antiinfective					
0.267	0.039	Antidiarrheal					
0.217	0.013	Antiviral (RSV)					
0.280	0.081	Cell adhesion molecule inhibitor					
0.251	0.068	Platelet aggregation inhibitor					
0.223	0.039	Antiseptic					
0.267	0.084	Antidiabetic					
0.255	0.075	Ophthalmic drug					
0.206	0.029	Chelator					
0.298	0.122	Antithrombotic					
0.273	0.100	Antitoxic					
0.256	0.087	Muscle relaxant					
0.343	0.176	Cytoprotectant					
0.319	0.154	Antianginal					
0.265	0.112	Antiviral (Herpes)					
0.191	0.040	Platelet antagonist					
0.293	0.149	Radioisensitizer					
0.169	0.026	Renal failure treatment					
0.197	0.072	Urticaria treatment					
0.212	0.090	Antiviral (CMV)					
0.204	0.084	Diabetic nephropathy treatment					
0.229	0.113	Prostate disorders treatment					
0.170	0.057	Restenosis treatment					

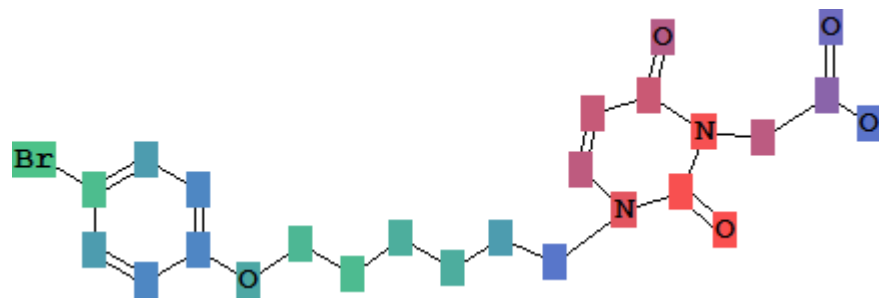
46 Substructure Descriptors; 0 new.

Drug-Likeness: 0.640

387 of 4130 Possible Activities  
 72 of 501 Possible Pharmacological Effects  
 301 of 3295 Possible Molecular Mechanisms  
 2 of 57 Possible Side Effects and Toxicity  
 6 of 199 Possible Metabolism-Related Actions  
 3 of 29 Possible Gene Expression Regulation  
 3 of 49 Possible Transporters-Related Actions

1/1 | 0.212 0.090 Antiviral (CMV)

# Prediction of the toxicity for compound 2c by PASS



2 of 57 Possible Side Effects and Toxicity at Pa > Pi

0.341	0.183	Cardiotoxic
0.324	0.280	Emetic

# Search results for compound 2c in ChEMBL

← → ↻ ebi.ac.uk/chembl/g/#similarity\_search\_results/O%3DC(O)Cn1c(%3DO)ccn(CCCCCOc2ccc(Br)cc2)c1%3DO/95

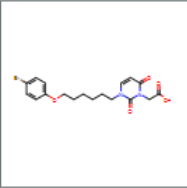
EMBL-EBI Services Research Training About us

**ChEMBL** Search in ChEMBL  
Examples: Imatinib erbB2 brain MDCK c1ccccc1N Draw a Structure | Enter a Sequence

UniChem ChEMBL-NTD SureChEMBL Downloads Web Services More Share

EBI > Databases > Chemical Biology > ChEMBL Database > Similarity Search Results

## Similarity Search Results



**Threshold:** 95%  
**Query:** O=C(O)Cn1c(=O)ccn(CCCCCOc2ccc(Br)cc2)c1=O






**Status:** Results Ready These results will expire on 2020-03-10T11:58:49.420557+00:00. [Learn More.](#) [Edit Search](#)

[Show Full Query](#)

No records were found.

Search results with similarity cut-off threshold > 95% - no similar structures found

# Search results for compound 2c in ChEMBL

<input type="checkbox"/>	Molecule ChEMBL ID	Compound Key	Standard Type	Standard Relation	Standard Value	Standard Units	pChEMBL Value	Comment	Assay ChEMBL ID	Assay Description	BAO Label	Assay Organism	Target ChEMBL ID	Target Name	Target Organism	Target Type	Document ChEMBL ID	Source Description	Cell ChEMBL ID
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<input type="checkbox"/>	 CHEMBL3623860	7g	Inhibition	No Data	No Data	No Data	No Data	Not Active	CHEMBL3626155	Antiviral activity against Human coxsackievirus B4	organism-based format	Human coxsackievirus B4	CHEMBL612320	Human coxsackievirus B4	Human coxsackievirus B4	ORGANISM	CHEMBL3621200	Scientific Literature	No Data
<input type="checkbox"/>	 CHEMBL3623865	7i	Inhibition	No Data	No Data	No Data	No Data	Not Active	CHEMBL3626150	Antiviral activity against HSV1 KOS	organism-based format	Human herpesvirus 1 strain KOS	CHEMBL2367034	Human herpesvirus 1 strain KOS	Human herpesvirus 1 strain KOS	ORGANISM	CHEMBL3621200	Scientific Literature	No Data
<input type="checkbox"/>	 CHEMBL3623857	7d	CC50	=	4900	nM	No Data	No Data	CHEMBL3626041	Cytotoxicity against human cytomegalovirus infected HEL cells assessed as cell growth inhibition incubated for 3 days by coulter counter method	cell-based format	Homo sapiens	CHEMBL613888	HEL	Homo sapiens	CELL-LINE	CHEMBL3621200	Scientific Literature	CHEMBL3308090
<input type="checkbox"/>	 CHEMBL4074053	33	Ratio EC50	No Data	No Data	No Data	No Data	Not Determined	CHEMBL4036856	Ratio of EC50 for agonist activity at CB1 receptor (unknown origin) expressed in CHO cells to EC50 for agonist activity at CB2 receptor (unknown origin) expressed in CHO cells	cell-based format	Homo sapiens	CHEMBL612545	Unchecked	No Data	UNCHECKED	CHEMBL4033762	Scientific Literature	No Data