Supporting information

STOP strategy to inhibit *P. falciparum* and *S. aureus* growth: molecular mechanism studies on purposely designed hybrids

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Table S1. MIC values obtained for the PTZ-quinoline hybrid compounds measured for the reference microbial strains

	MIC (μM)		
Compound	***S. aureus* ATCC 25923**	***E. coli* ATCC 25923**	***C. albicans* ATCC 10231**
1	>100	>100	>100
2	>100	>100	>100
3	>100	>100	>100
4a	12.5	>100	>100
4b	6.25-12.5	>100	>100
5a	12.5	>100	>100
5b	6.25-12.5	>100	>100
6a	>100	>100	>100
6b	50	>100	>100
7	>100	>100	>100
8	>100	>100	>100
GEN*	6.6	1.03	n.d
AMP*	4.2	67.3	n.d.
FLC*	n.d.^	n.d.	0.82

*GEN: Gentamicin; AMP: Ampicillin; FLC: Fluconazole.
^n.d. not detemined

Table S2. Antibiotic-resistance profile of the clinical isolates of *S. aureus*

Clinical isolate	**Antibiotic-resistance profile**
MRSA 1[§]	GEN^{S}, LVX^{R}, **OX^{R}**, **P^{R}**, TE^{S}, TEC^{S}, SXT^{S}, VA^{S}
MRSA 2[§]	GEN^{S}, LVX^{R}, **OX^{R}**, **P^{R}**, TE^{S}, TEC^{S}, SXT^{S}, VA^{S}
MRSA 3[§]	**CM^{R}, E^{R},** GEN^{S}, **LVX^{R}**, **OX^{R}**, **P^{R}**, TEC^{S}, TE^{S}, SXT^{S}, VA^{S}
MRSA 4[§]	CM^{S}, E^{S}, GEN^{S}, **LVX^{R}**, **OX^{R}**, **P^{R}**, TE^{S}, TEC^{S}, SXT^{S}, VA^{S}
MRSA 5[§]	CM^{S}, E^{S}, GEN^{S}, **LVX^{R}**, **OX^{R}**, **P^{R}**, TE^{S}, TEC^{S}, SXT^{S}, VA^{S}

CM = Clindamicyn; E = Erythromycin; GEN = Gentamicin; P = Penicillin; LVX = Levofloxacin; OX = Oxacillin; TE = Tetracycline; TEC = Teicoplanin; SXT = Trimethoprim/Sulfamethoxazole; VA = Vancomycin
R = Resistant; S = Susceptible; I = Intermediate, as defined following the EUCAST guidelines
[§]*Staphylococcus* species resistant to oxacillin were declared, by convention, methicillin-resistant.

Table S3. IC_{50} ranges (µM) obtained for MRSA and reference strains

S. aureus	**4a**	**4b**	**5a**	**5b**
ATCC 25923	7.34 – 7.64	3.97 – 5.75	5.41 – 8.05	3.31 – 6.75
MRSA (n = 5)	7.75 – 8.98	4.42 – 6.48	6.40 – 9.63	6.18 – 10.34

Table S4. Molecular docking scores of compounds 1-8 within the evaluated binding sites of *S. aureus* NDH-2 protein.

Compound	Docking score (kcal/mol) FAD binding site	Docking score (kcal/mol) NAD^+/NADH binding site	Docking score (kcal/mol) Q binding site
1	-8.159	-8.153	-8.537
2	-8.353	-8.007	-7.837
3	-8.551	-8.120	-7.211
4a	-8.757	-7.158	-9.794
4b	-8.281	-7.306	-8.129
5a	-9.059	-7.583	-9.575
5b	-9.297	-7.763	-8.628
6a	-9.198	-8.174	-9.732
6b	-9.856	-8.312	-11.174
7	-9.718	-8.011	-8.325
8	-9.895	-8.070	-9.590
NAD^+*	-	-6.420	-
HQNO*	-	-	-6.170
FAD*	-17.036	-	-

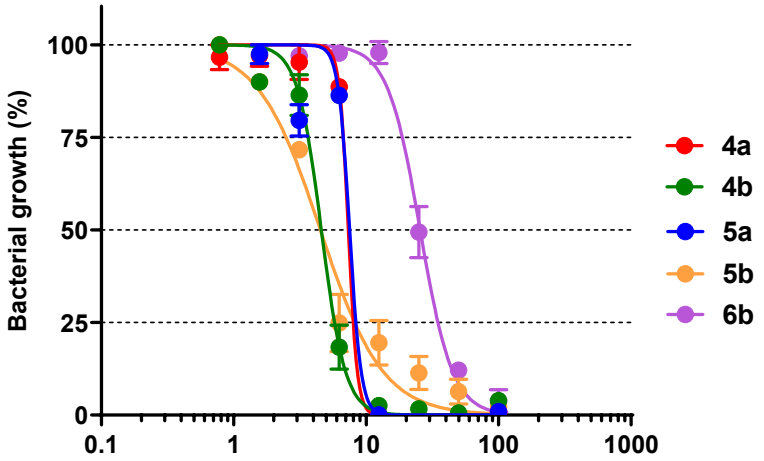
*NAD^+, HQNO and FAD are reported as reference scoring compounds.

Table S5. Molecular docking scores of compounds 1-8 within the evaluated binding sites of *P. falciparum* NDH-2 protein.

Compound	Docking score (kcal/mol) FAD binding site	Docking score (kcal/mol) NAD^+/NADH binding site	Docking score (kcal/mol) Q binding site
1	-6.446	-7.721	-7.983
2	-6.937	-6.455	-7.931
3	-6.662	-8.374	-8.014
4a	-7.150	-8.150	-8.206
4b	-7.061	-8.274	-10.208
5a	-7.223	-8.136	-8.911
5b	-7.435	-8.505	-9.374
6a	-7.604	-8.438	-7.987
6b	-7.882	-8.600	-7.566
7	-7.970	-7.274	-8.747
8	-7.867	-8.446	-9.120
NAD^+*	-	-8.879	-
HQNO*	-	-	-7.799
FAD*	-14.378	-	-

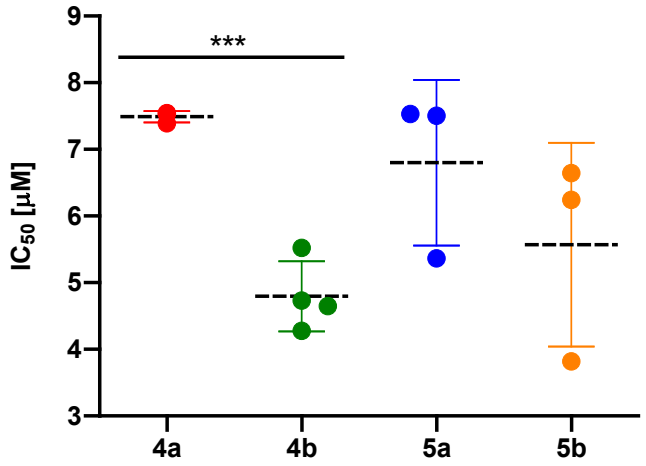
*NAD^+, HQNO and FAD are reported as reference scoring compounds.

Figure S1. Dose-response curves of the active PTZ-quinoline hybrid compounds against *S. aureus* ATCC 25923



Symboles represent mean values with standard deviations, and lines define the curves obtained from nonlinear regression analysis (GraphPad Prism version 9.4.1). Percentage values are relative to the positive control (bacteria grown in regular medium).

Figure S2. IC_{50} values measured for *S. aureus* ATCC 25923



Symboles represent IC_{50} values obtained in different experiments against *S. aureus*, and the dotted lines define the mean values for the PTZ-quinoline hybrid compounds. A statistically difference is measured comparing the triazole derivatives **4a** and **4b** (***$p<0.0001$ unpaired t test).

Figure S3. Hemolytic activity, expressed in percentage values, for the compounds measured on hRBCs.

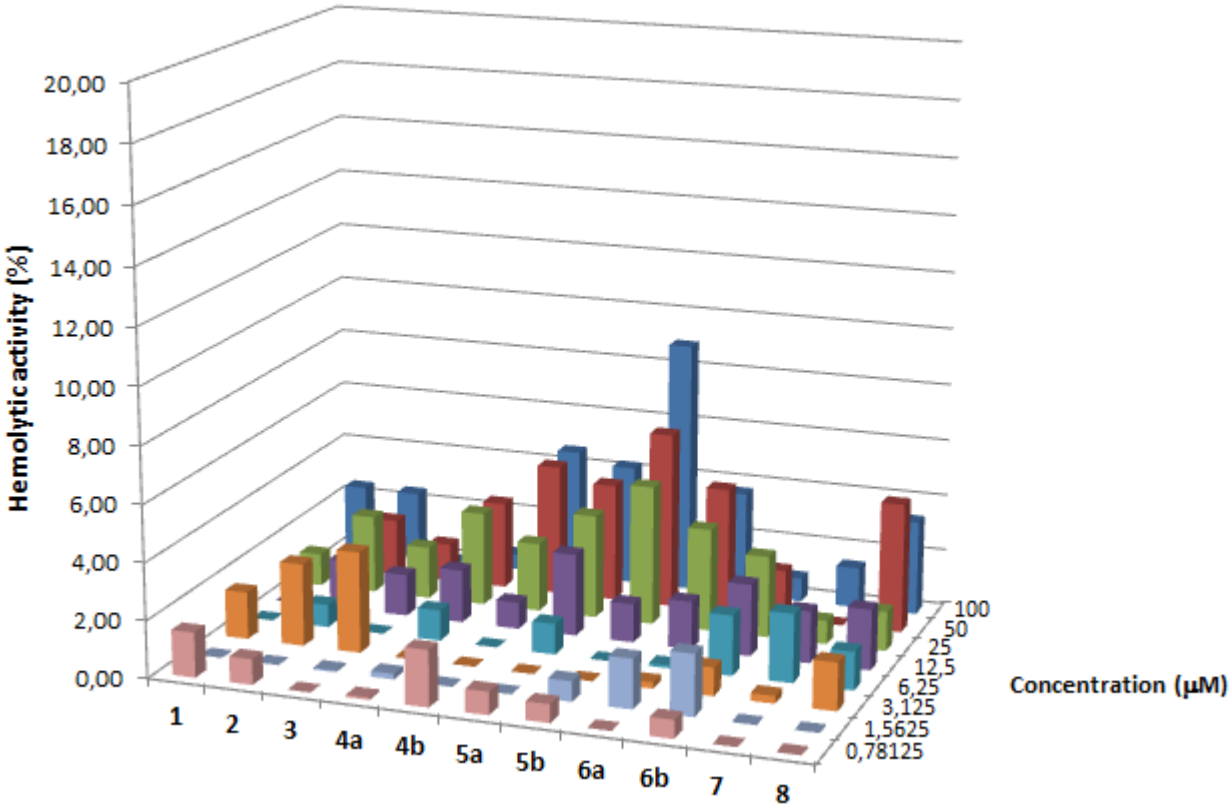
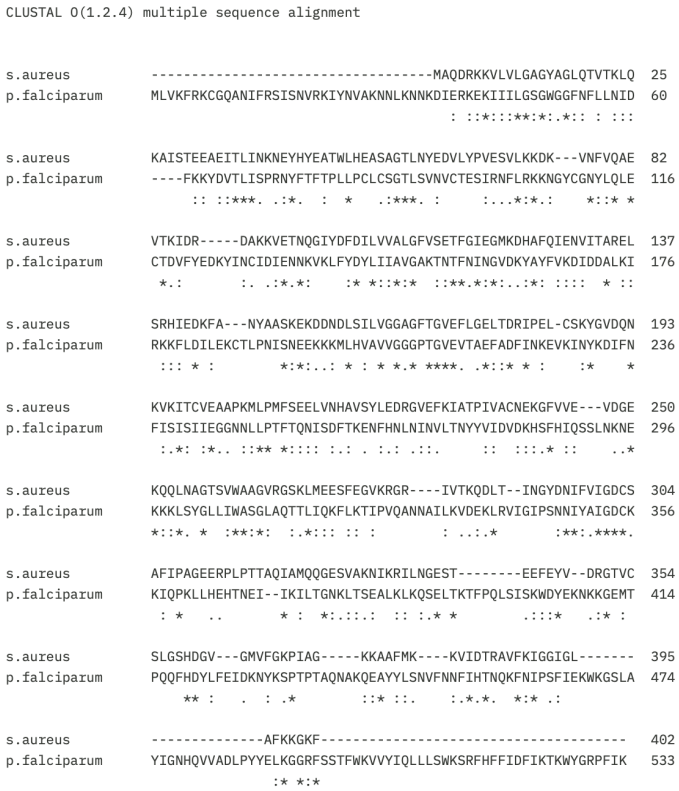


Figure S4. Sequence alignment between *S. aureus* and *P. falciparum* NDH-2 protein isoforms.



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CLUSTAL O(1.2.4) multiple sequence alignment


s.aureus          ----------------------------------MAQDRKKVLVLGAGYAGLQTVTKLQ  25
p.falciparum      MLVKFRKCGQANIFRSISNVRKIYNVAKNNLKNNKDIERKEKIIILGSGWGGFNFLLNID  60
                                                     : ::*:::**:*:.*:: : :::

s.aureus          KAISTEEAEITLINKNEYHYEATWLHEASAGTLNYEDVLYPVESVLKKDK---VNFVQAE  82
p.falciparum      ----FKKYDVTLISPRNYFTFTPLLPCLCSGTLSVNVCTESIRNFLRKKNGYCGNYLQLE  116
                       :: ::***. .:*.  :  *   .:***. :     :...*:*.:    *::* *

s.aureus          VTKIDR-----DAKKVETNQGIYDFDILVVALGFVSETFGIEGMKDHAFQIENVITAREL  137
p.falciparum      CTDVFYEDKYINCIDIENNKVKLFYDYLIIAVGAKTNTFNINGVDKYAYFVKDIDDALKI  176
                   *.:       :. .:*.*:    :* *::*:*  ::**.*:*:..:*: ::::  * ::

s.aureus          SRHIEDKFA---NYAASKEKDDNDLSILVGGAGFTGVEFLGELTDRIPEL-CSKYGVDQN  193
p.falciparum      RKKFLDILEKCTLPNISNEEKKKMLHVAVVGGGPTGVEVTAEFADFINKEVKINYKDIFN  236
                   ::: * :        *:*:..: * : * *.* ****. .*::* * :    :*    *

s.aureus          KVKITCVEAAPKMLPMFSEELVNHAVSYLEDRGVEFKIATPIVACNEKGFVVE---VDGE  250
p.falciparum      FISISIIEGGNNLLPTFTQNISDFTKENFHNLNINVLTNYYVIDVDKHSFHIQSSLNKNE  296
                   :.*: :*.. ::** *:::: :.: . :.: .::.     ::  :::.* ::    ..*

s.aureus          KQQLNAGTSVWAAGVRGSKLMEESFEGVKRGR----IVTKQDLT--INGYDNIFVIGDCS  304
p.falciparum      KKKLSYGLLIWASGLAQTTLIQKFLKTIPVQANNAILKVDEKLRVIGIPSNNIYAIGDCK  356
                  *::*. *  :**:*:  :.*::: :: :        : ..:.*       :**:.****.

s.aureus          AFIPAGEERPLPTTAQIAMQQGESVAKNIKRILNGEST--------EEFEYV--DRGTVC  354
p.falciparum      KIQPKLLHEHTNEI--IKILTGNKLTSEALKLKQSELTKTFPQLSISKWDYEKNKKGEMT  414
                   : *   ..       * :  *:.::.:  :: :.* *        .:::*   .:* :

s.aureus          SLGSHDGV---GMVFGKPIAG-----KKAAFMK----KVIDTRAVFKIGGIGL-------  395
p.falciparum      PQQFHDYLFEIDKNYKSPTPTAQNAKQEAYYLSNVFNNFIHTNQKFNIPSFIEKWKGSLA  474
                      ** :   .  : .*        ::* ::.    :.*.*.  *:* .:

s.aureus          --------------AFKKGKF-----------------------------------   402
p.falciparum      YIGNHQVVADLPYYELKGGRFSSTFWKVVYIQLLLSWKSRFHFFIDFIKTKWYGRPFIK   533
                                :* *:*
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The assignment of each output alignment position follows the general scheme: * (residue identity), : (residue semi-conservation) and . (residue conservation). The alignment was carried out using ClustalO (v1.2.4) [54].

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

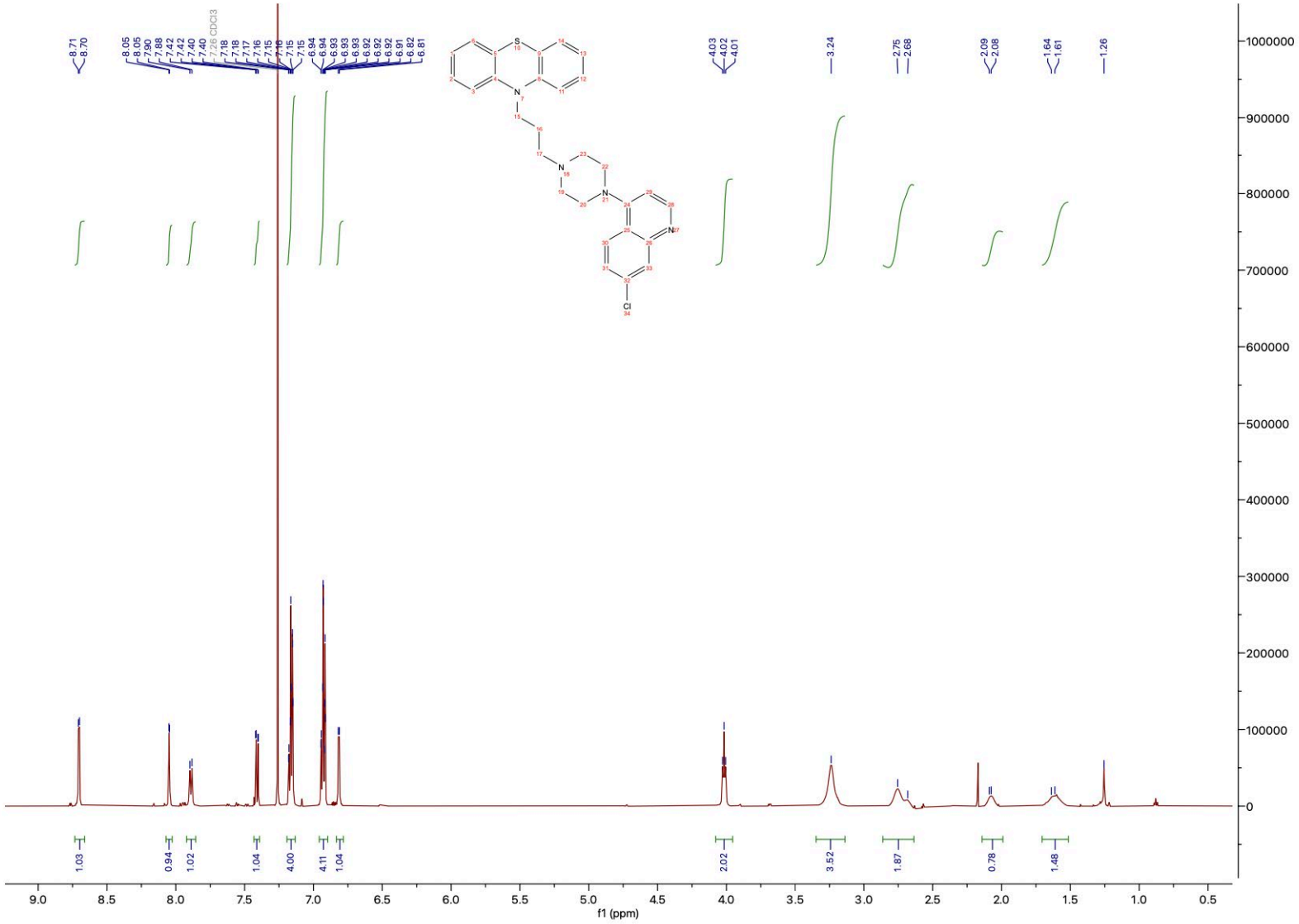

Figure S5: ^{1}H-NMR Spectra of compound **1**

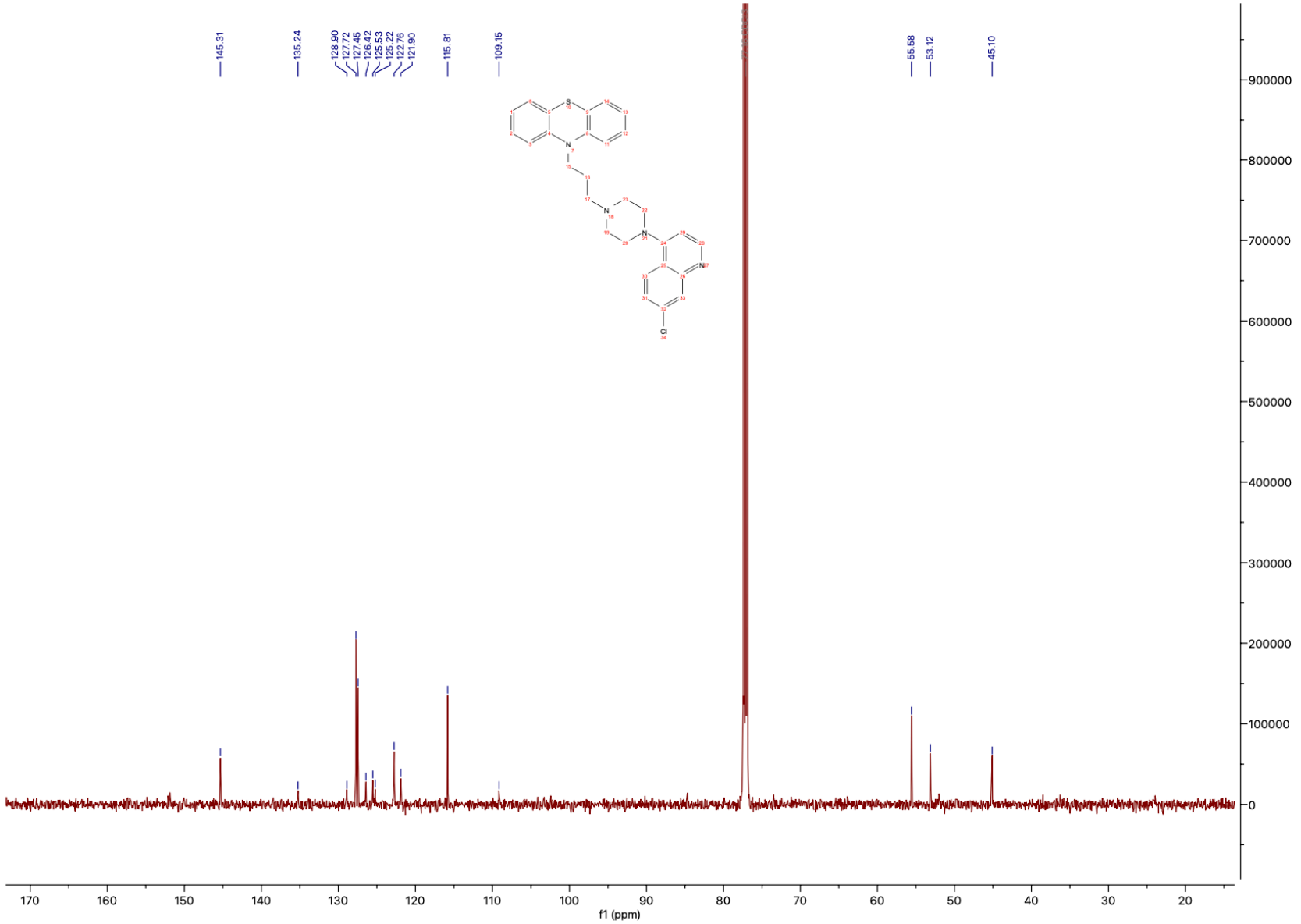

Figure S6: ^{13}C-NMR Spectra of compound **1**

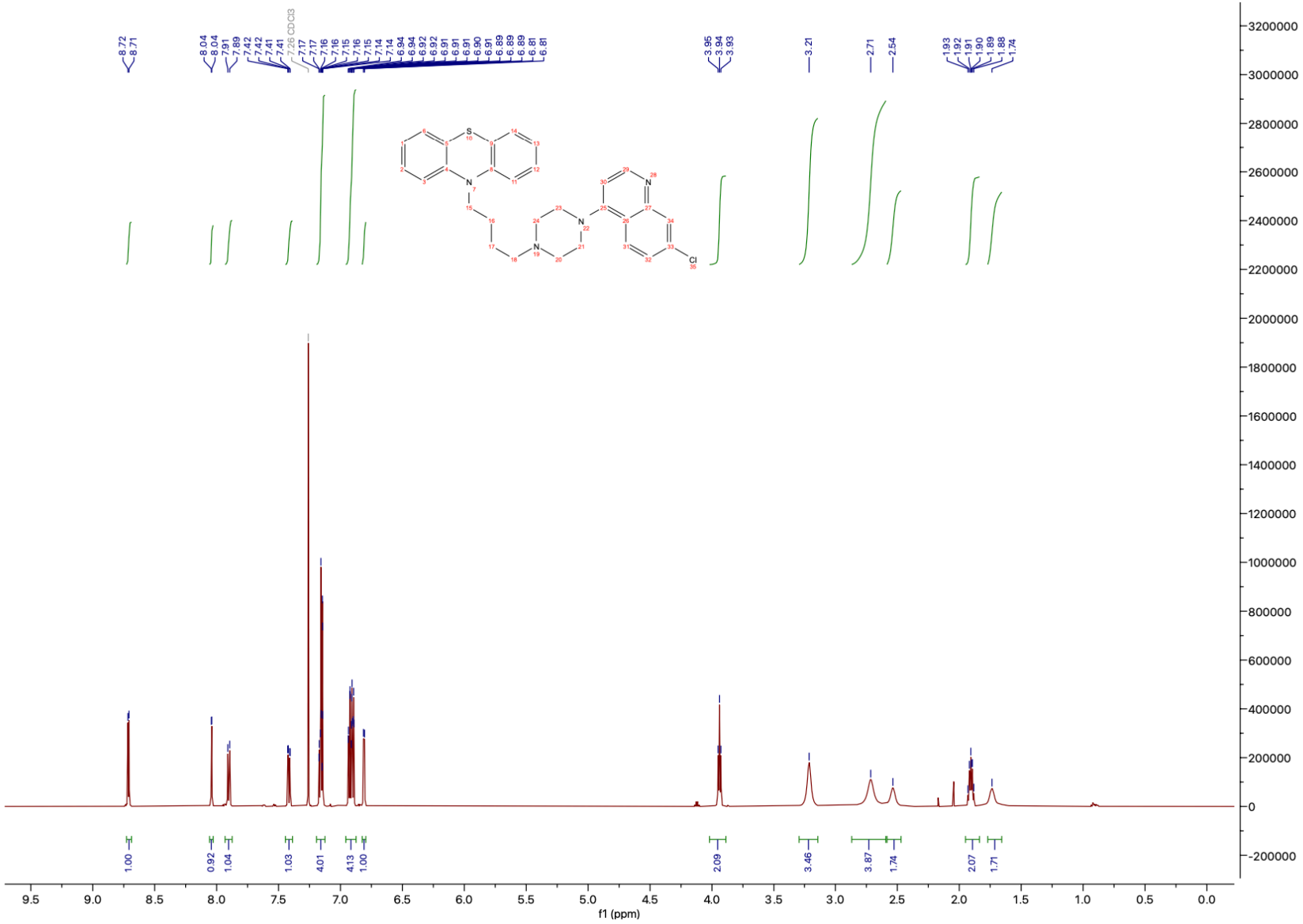

Figure S7: ^{1}H-NMR Spectra of compound **2**

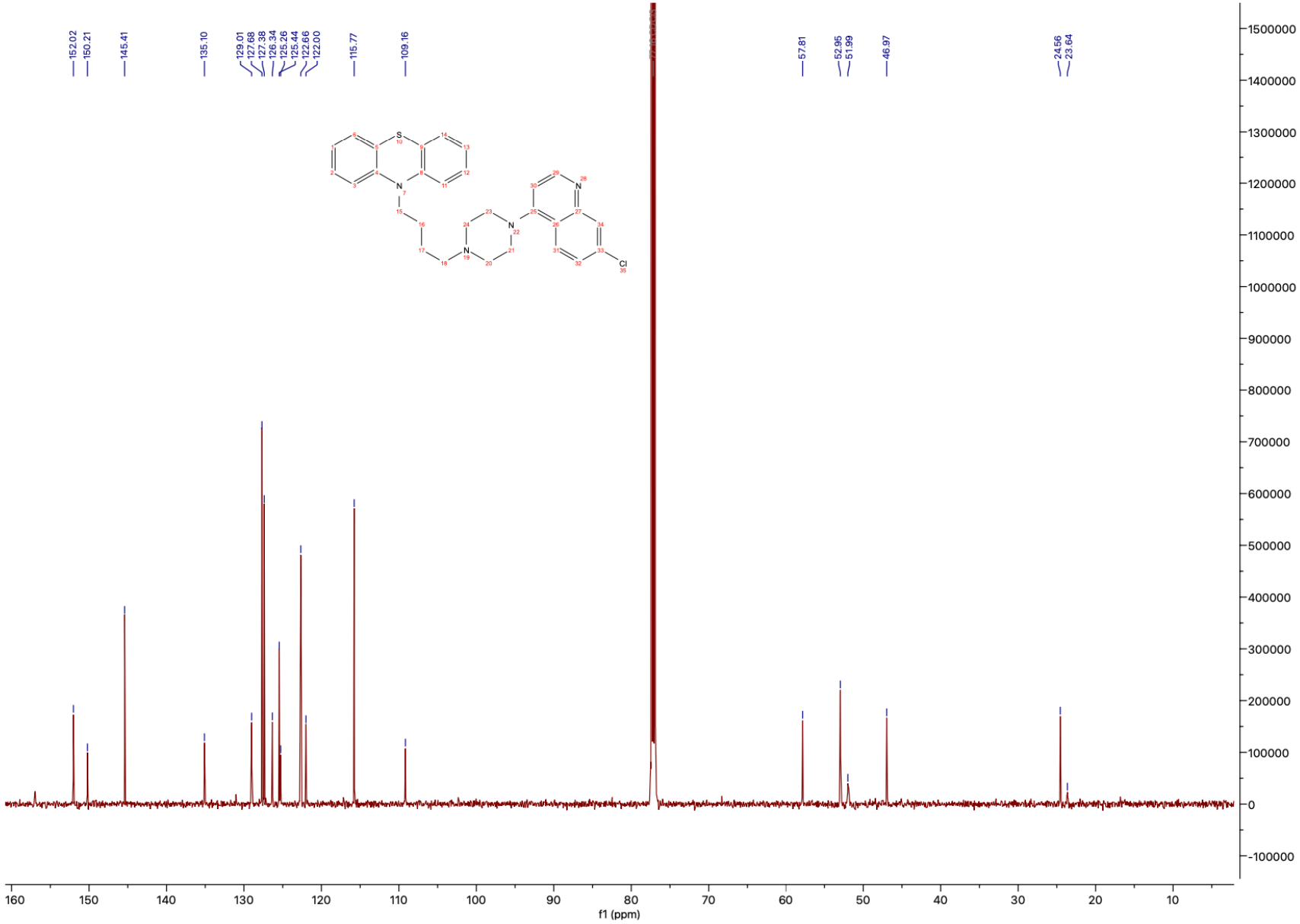

Figure S8: ^{13}C-NMR Spectra of compound **2**

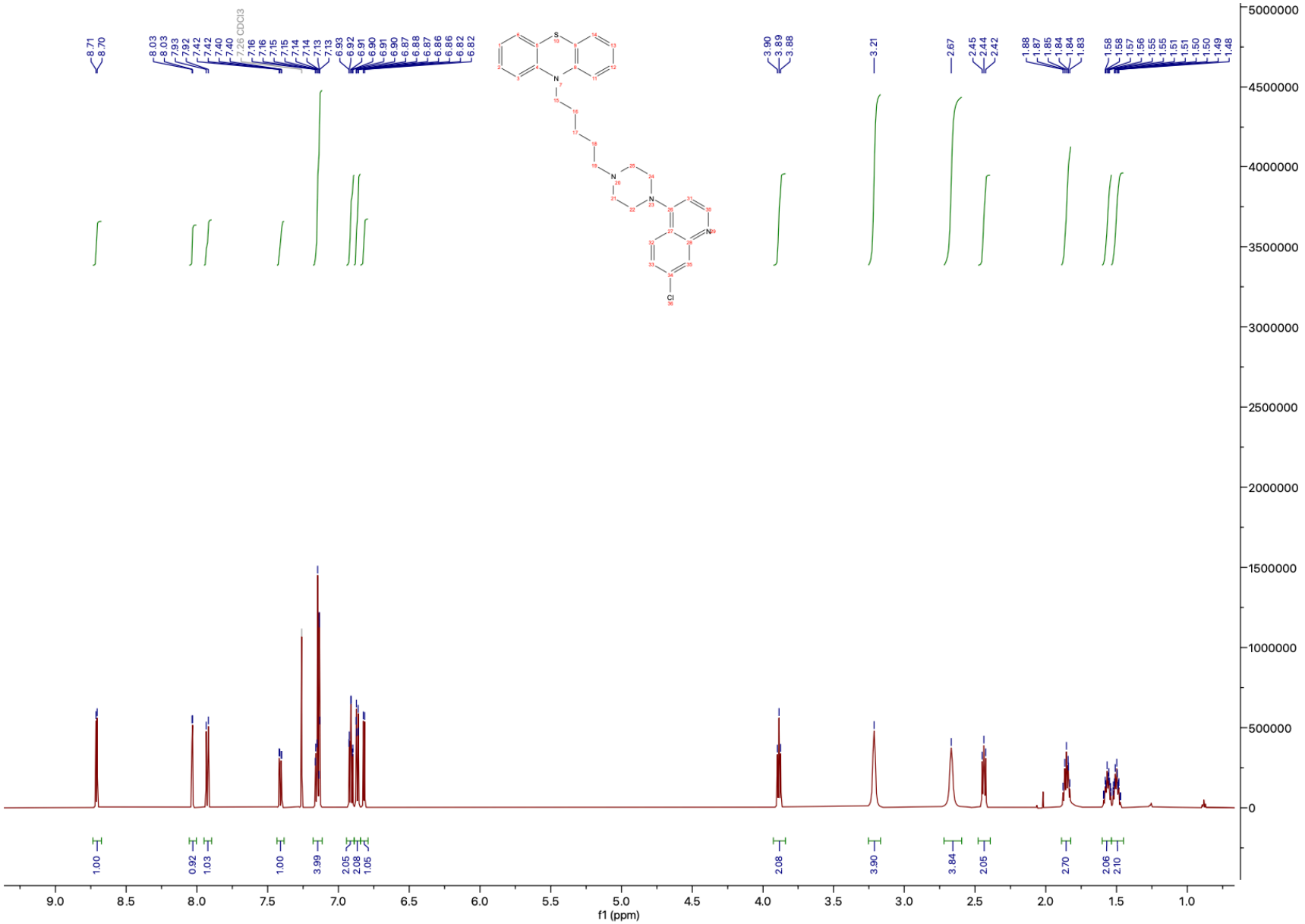

Figure S9: ^{1}H-NMR Spectra of compound **3**

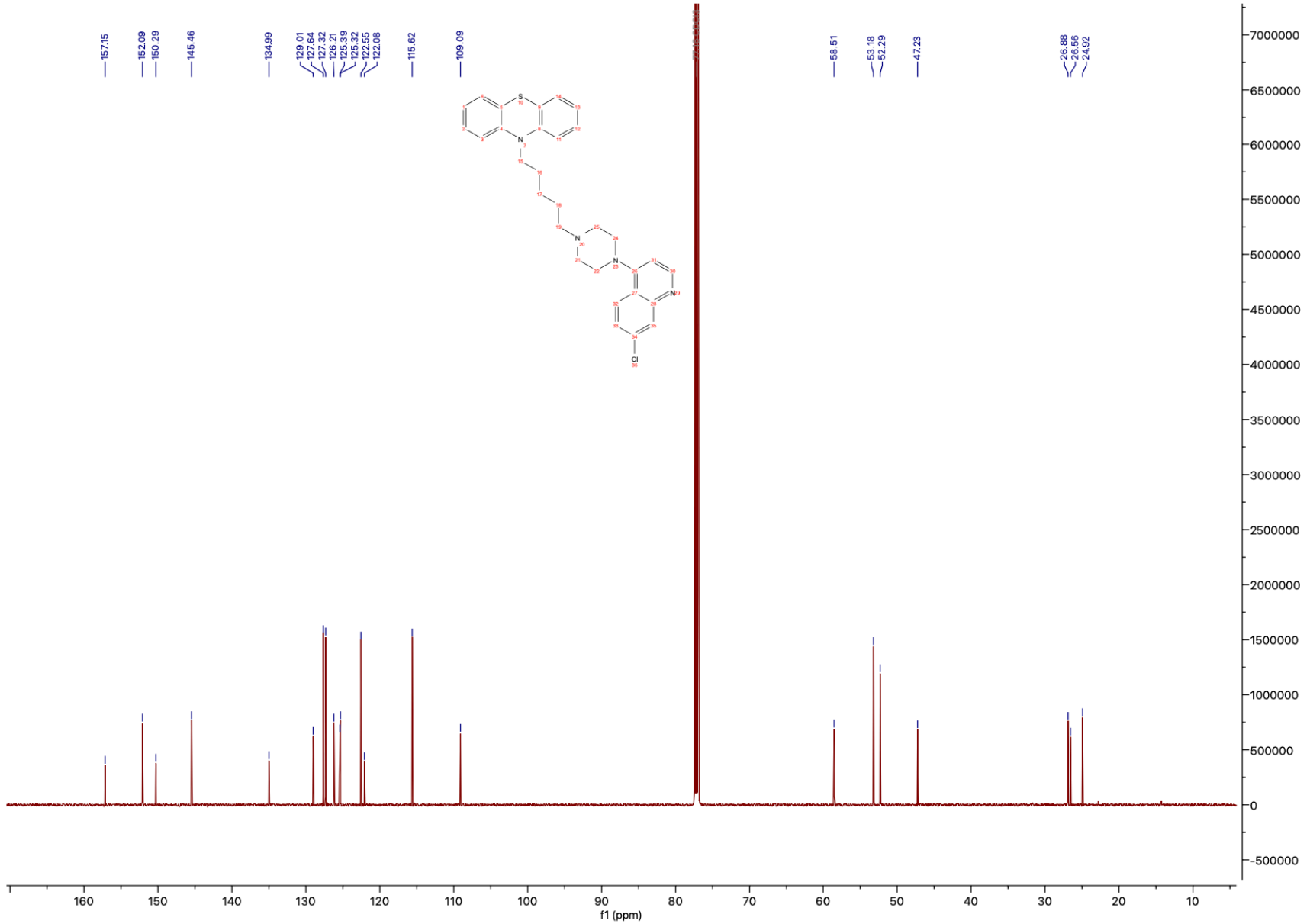

Figure S10: ^{13}C-NMR Spectra of compound **3**

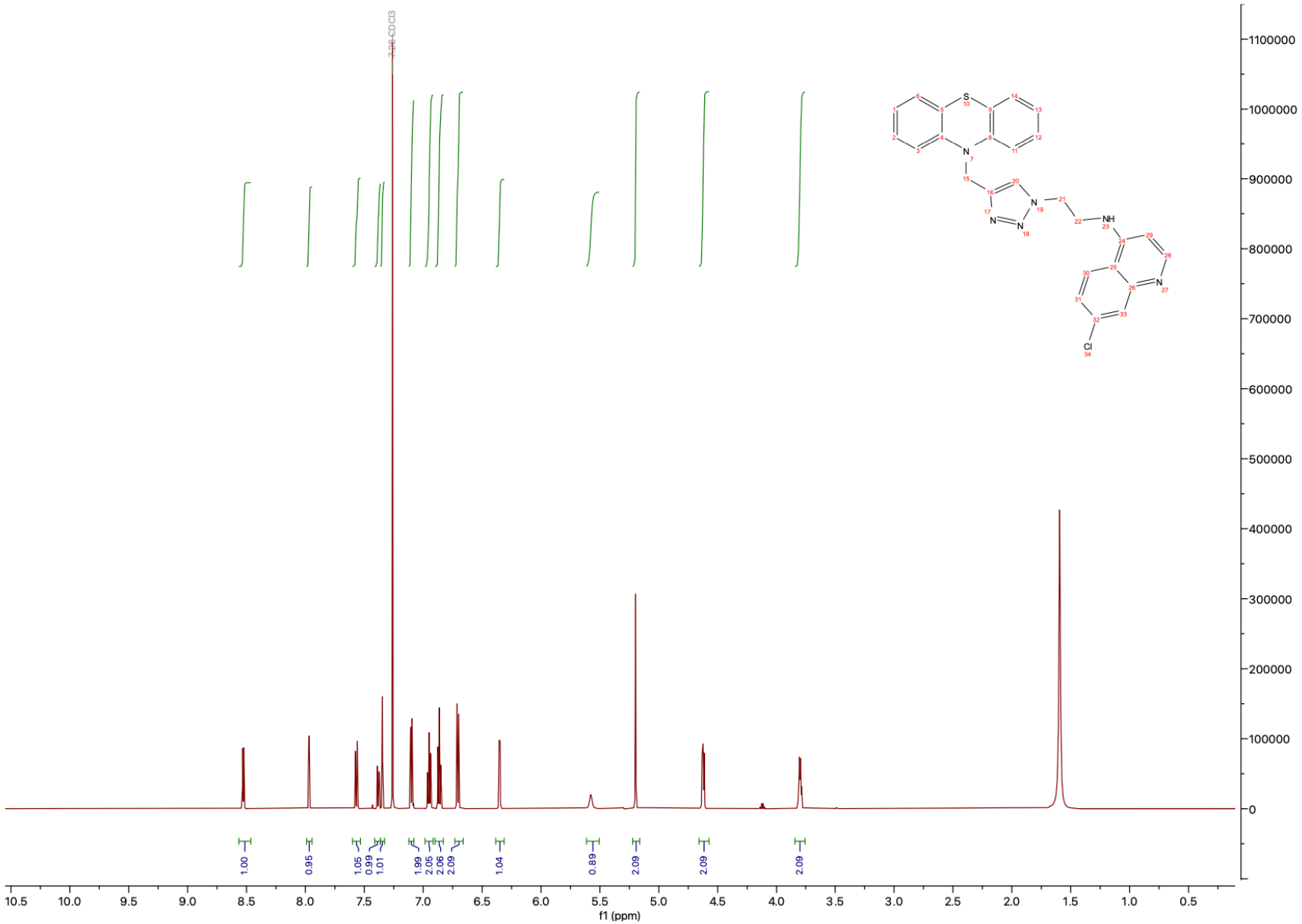

Figure S11: ^{1}H-NMR Spectra of compound **4a**

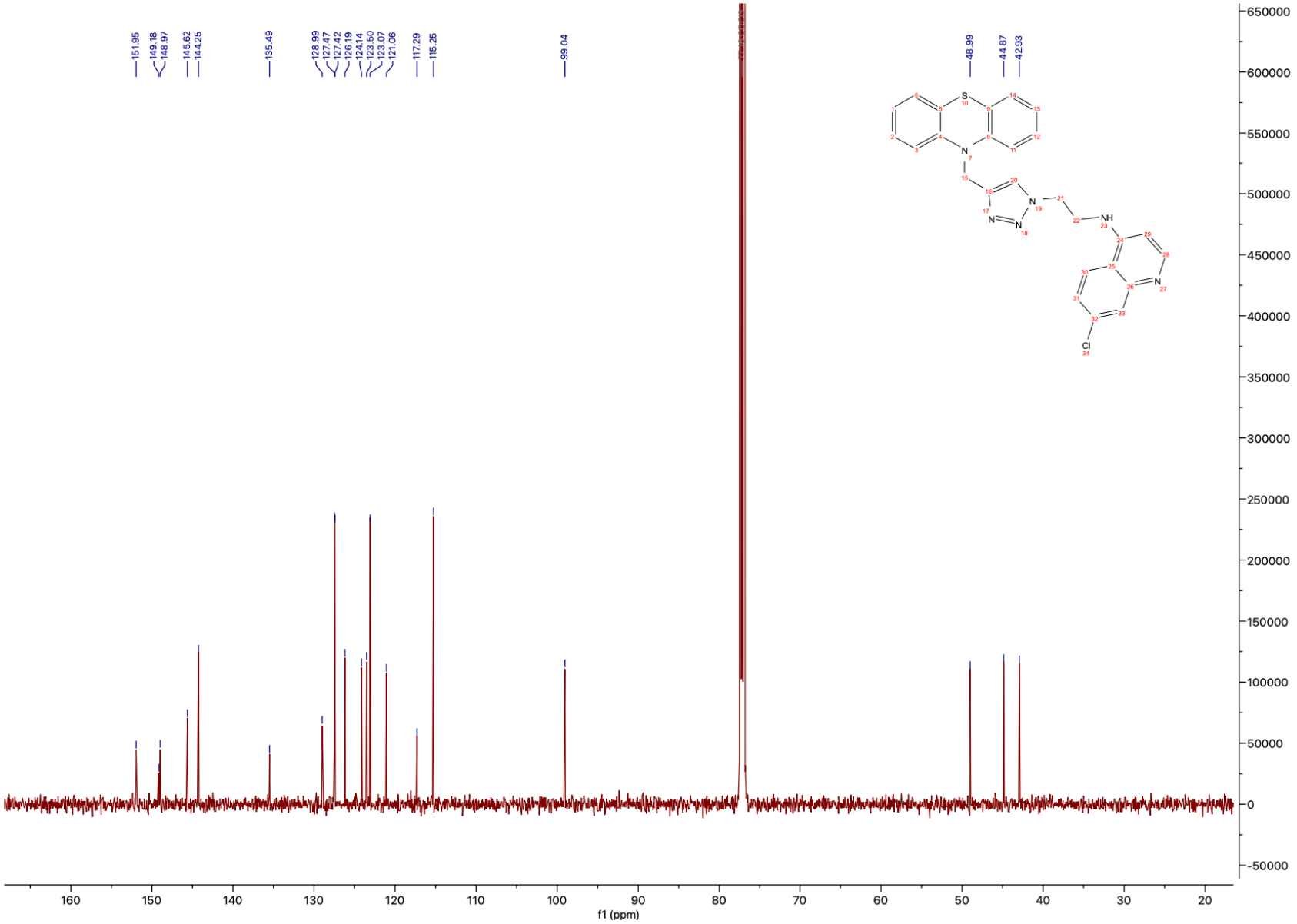

Figure S12: ^{13}C-NMR Spectra of compound **4a**

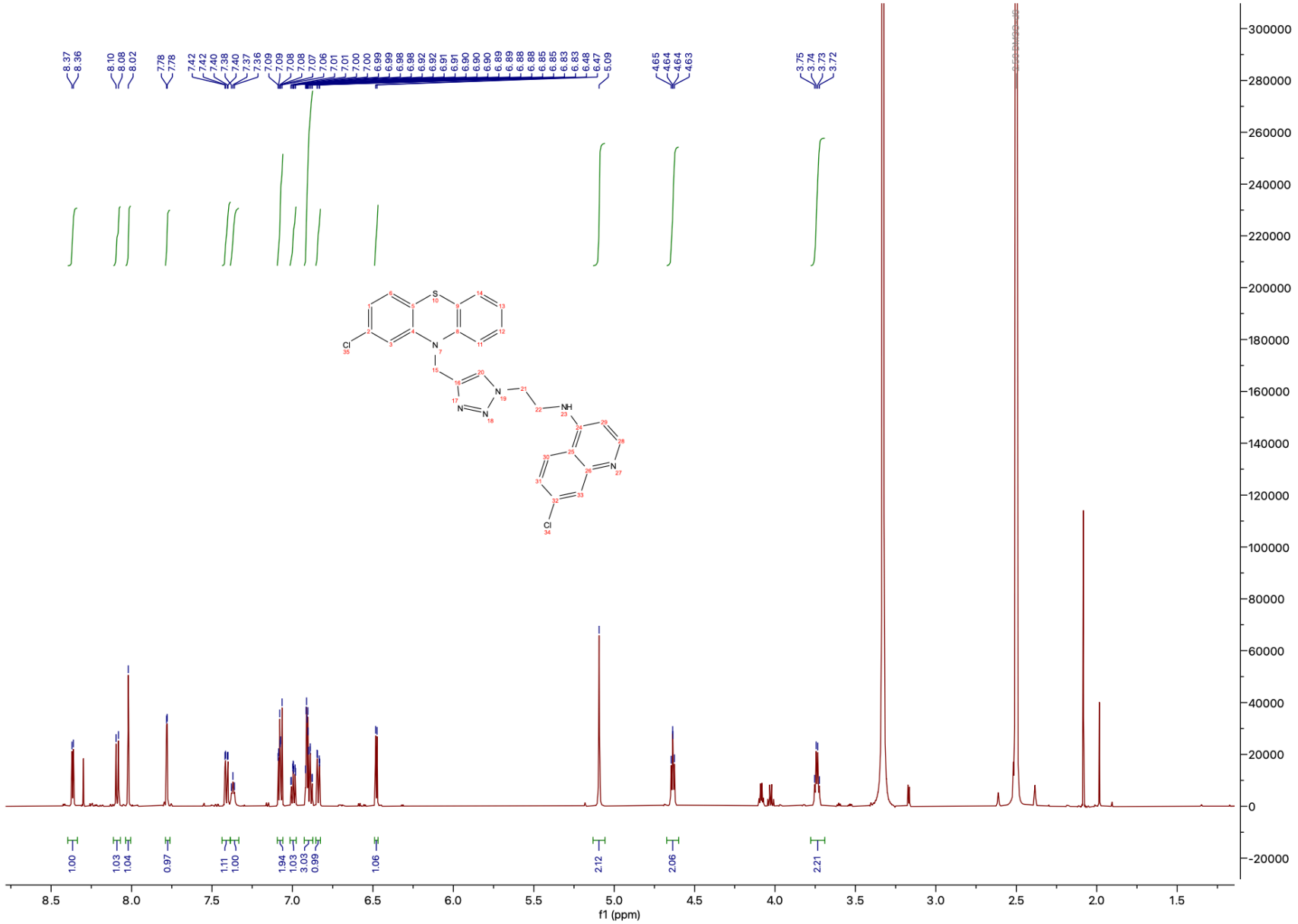

Figure S13: ^{1}H-NMR Spectra of compound **4b**

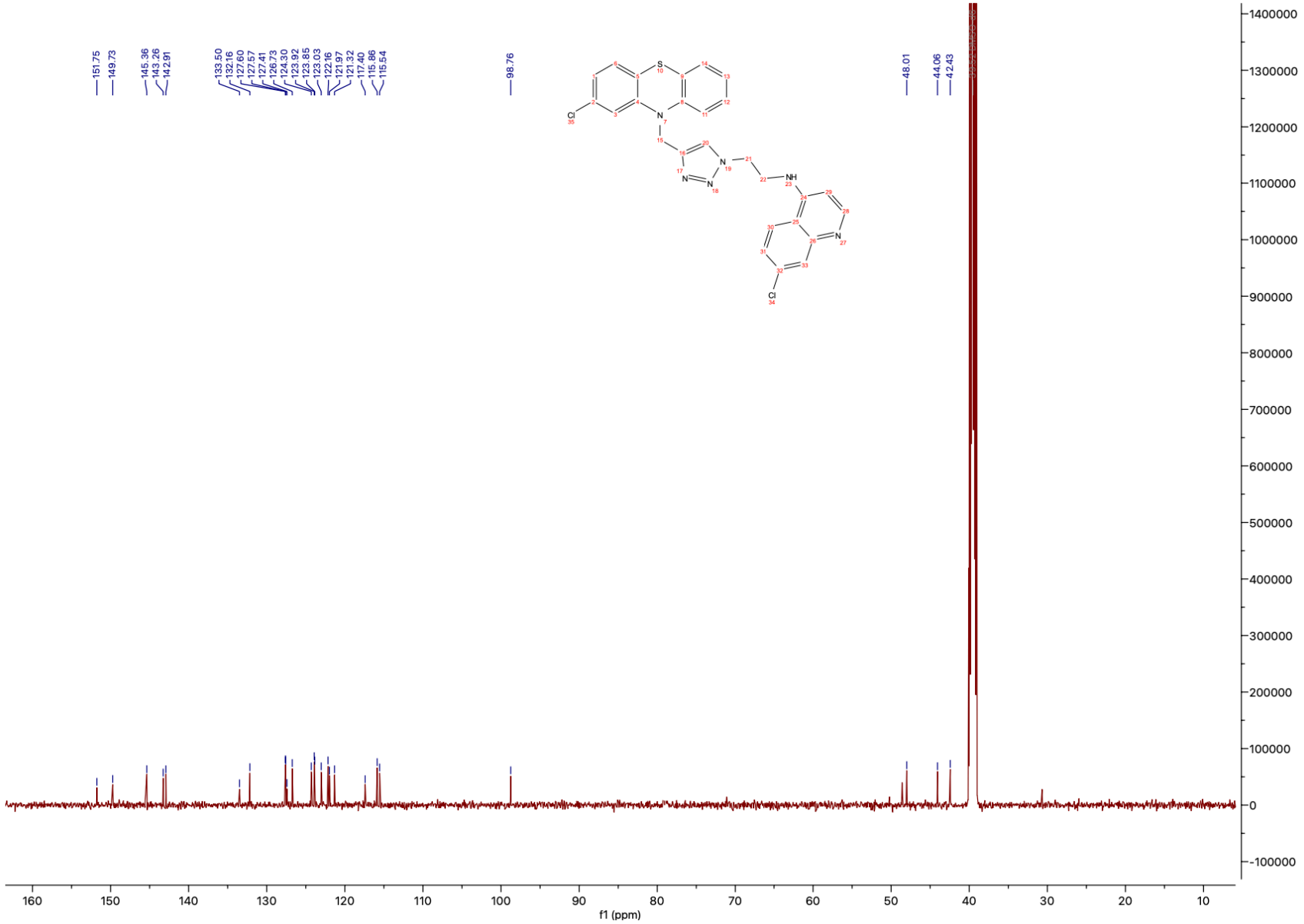

Figure S14: ^{13}C-NMR Spectra of compound **4b**

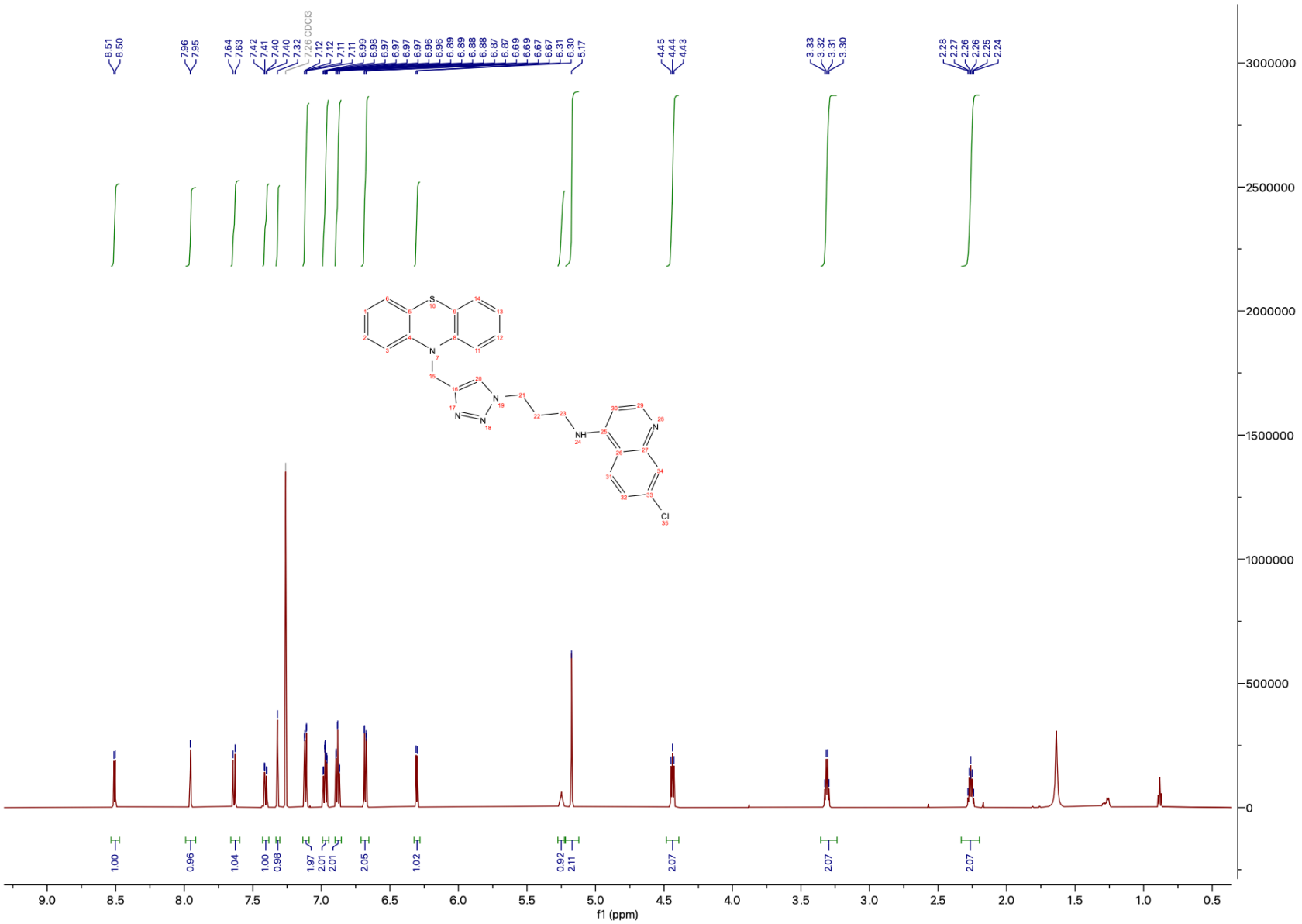

Figure S15: ^{1}H-NMR Spectra of compound **5a**

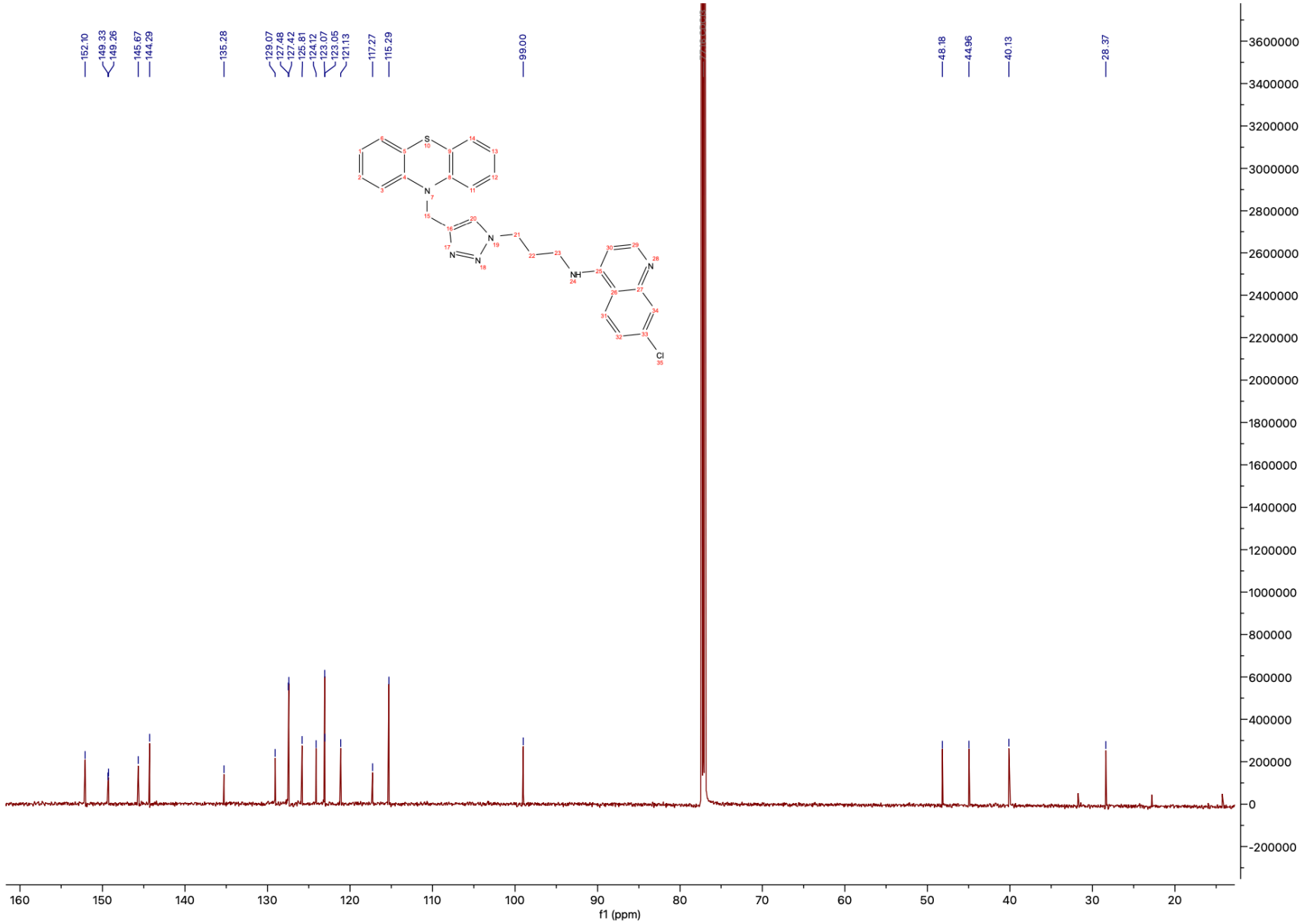

Figure S16: ^{13}C-NMR Spectra of compound **5a**

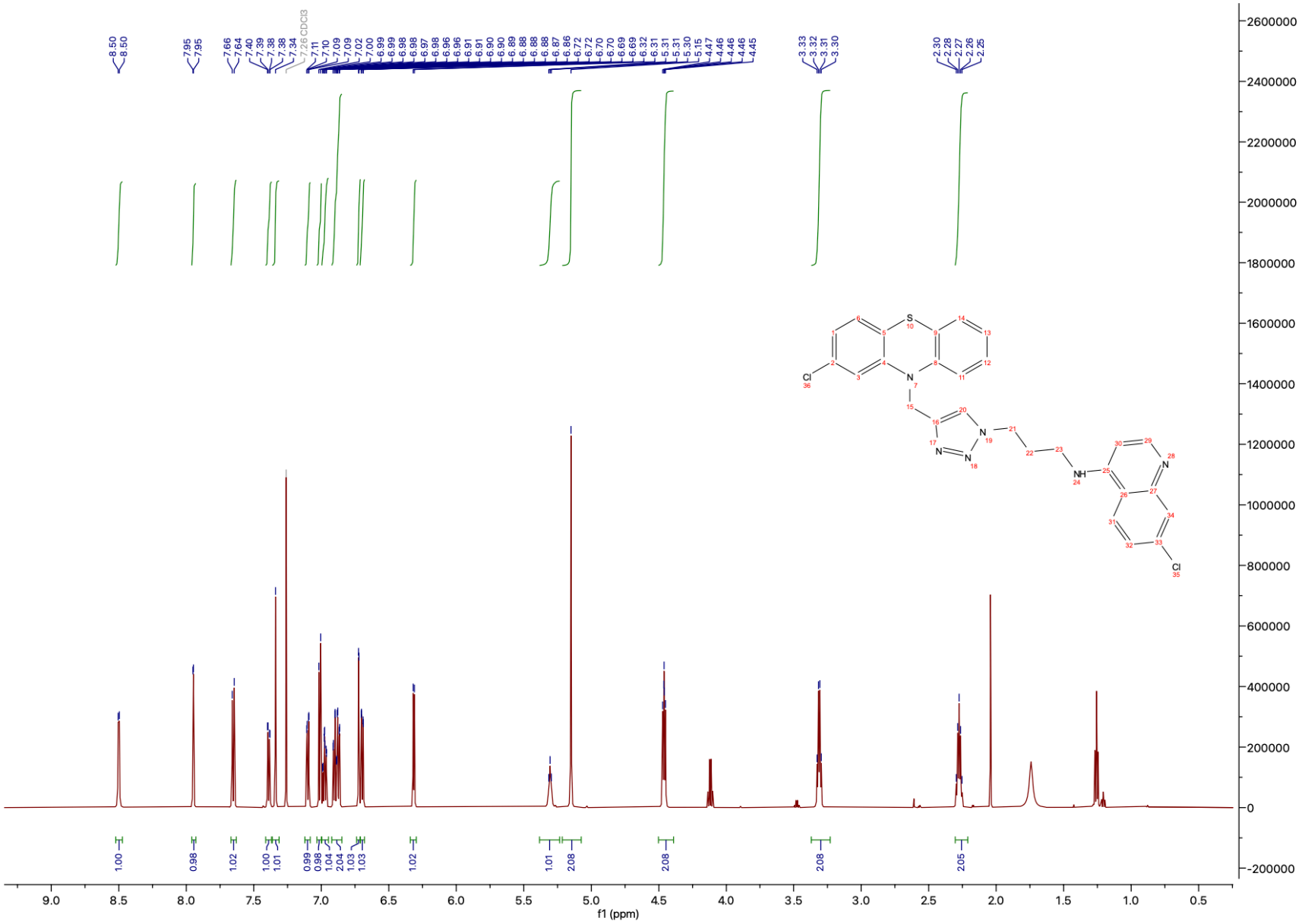

Figure S17: ^{1}H-NMR Spectra of compound **5b**

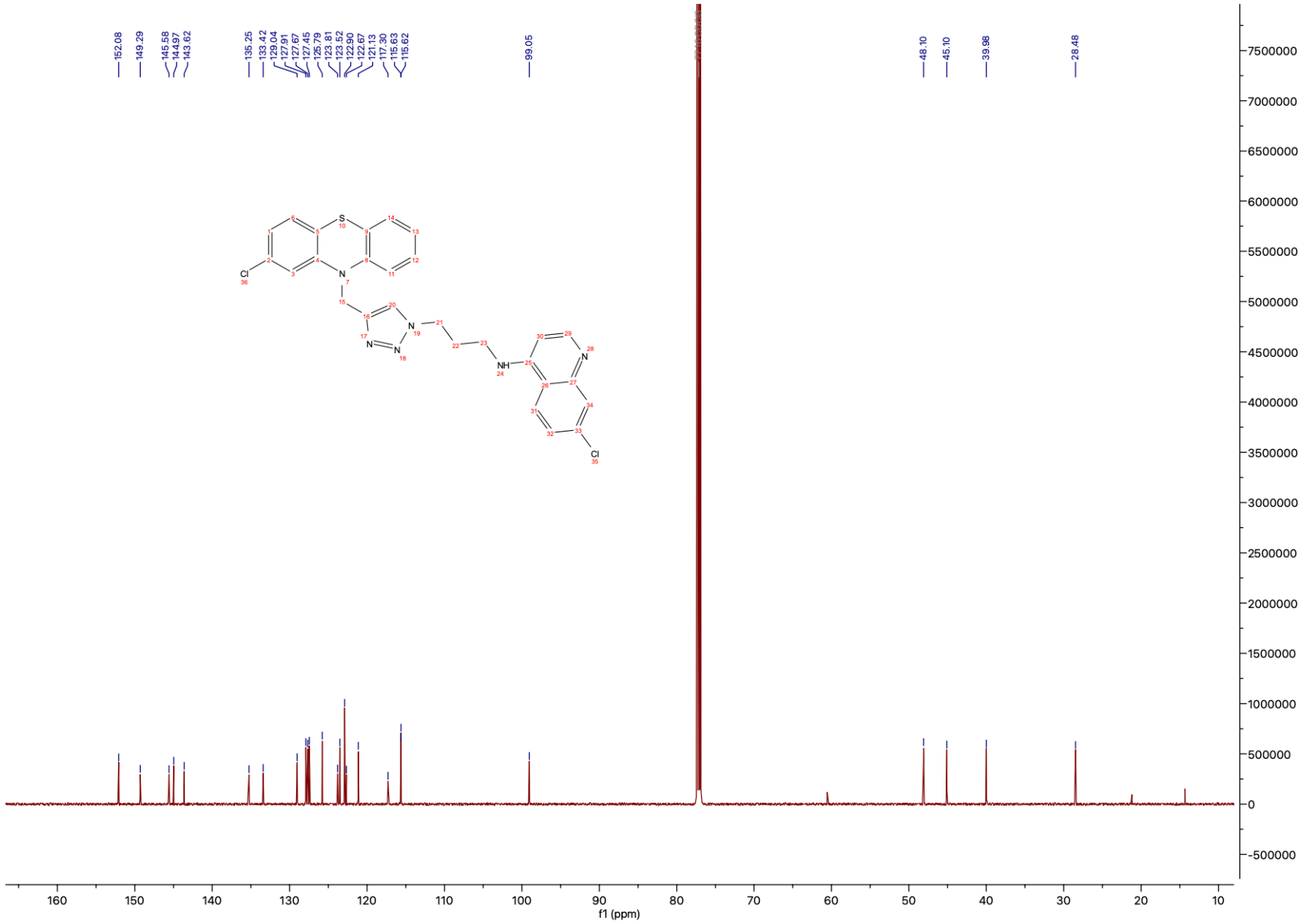

Figure S18: ^{13}C-NMR Spectra of compound **5b**

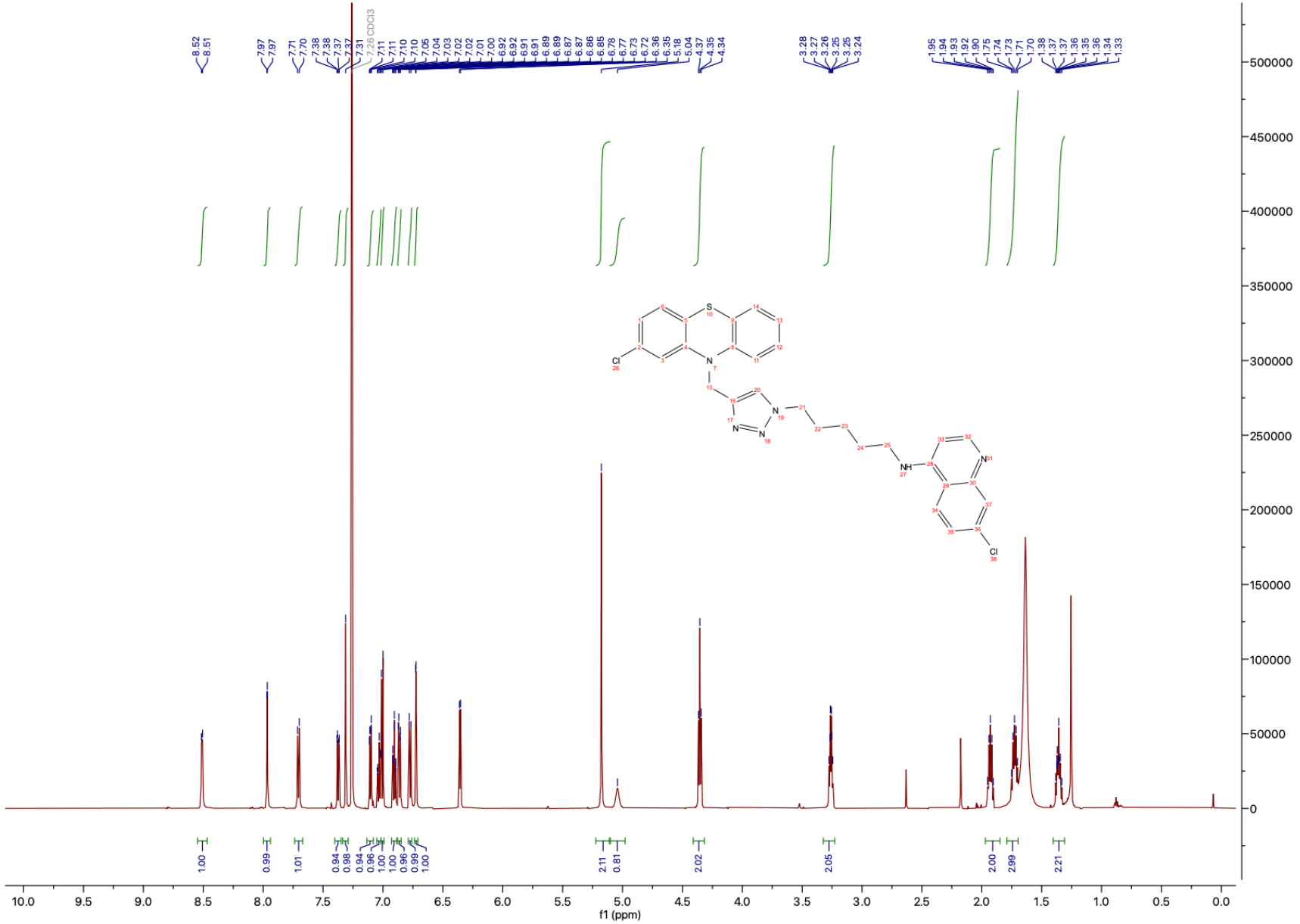

Figure S19: ^{1}H-NMR Spectra of compound **6b**

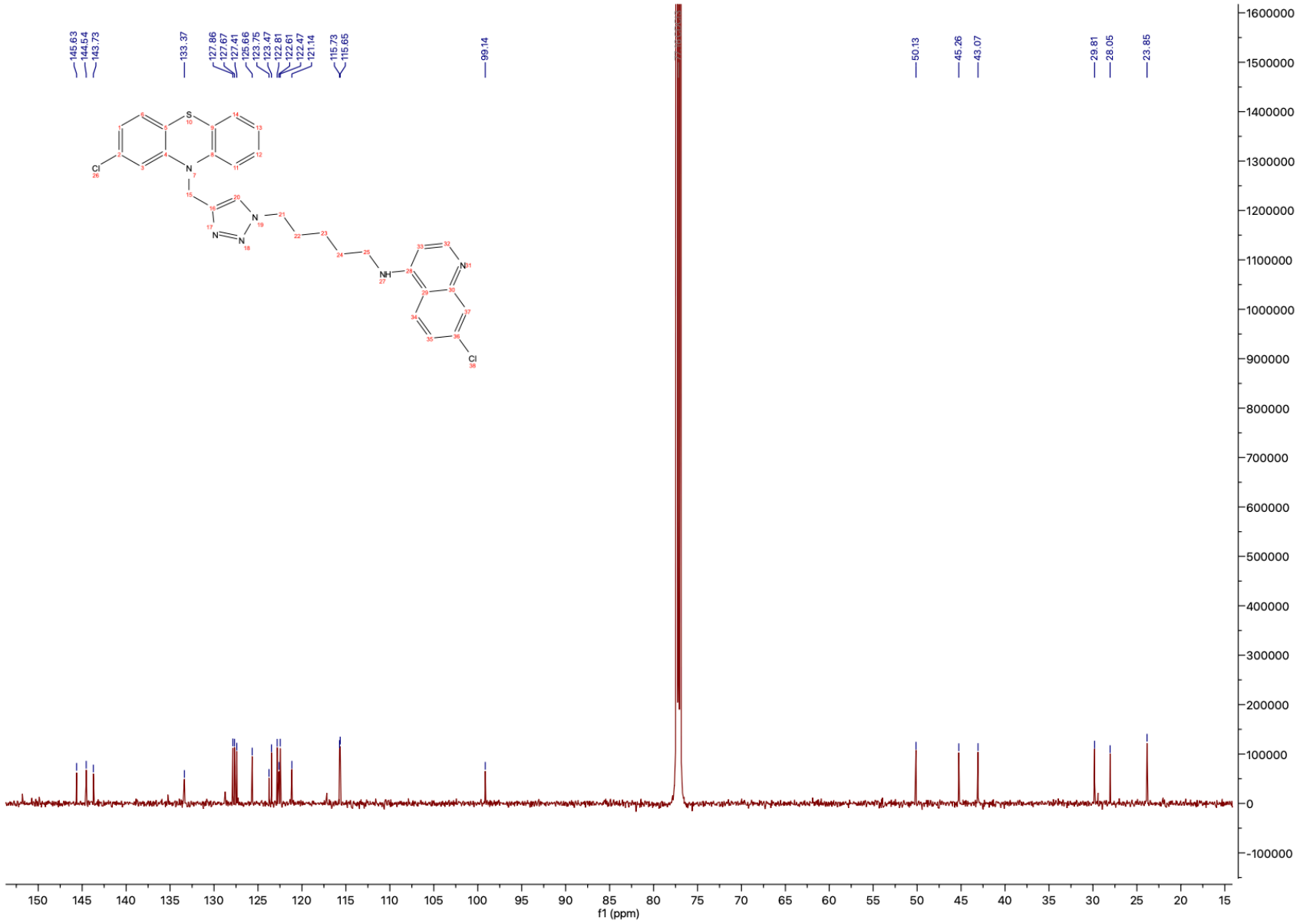

Figure S20: ^{13}C-NMR Spectra of compound **6b**

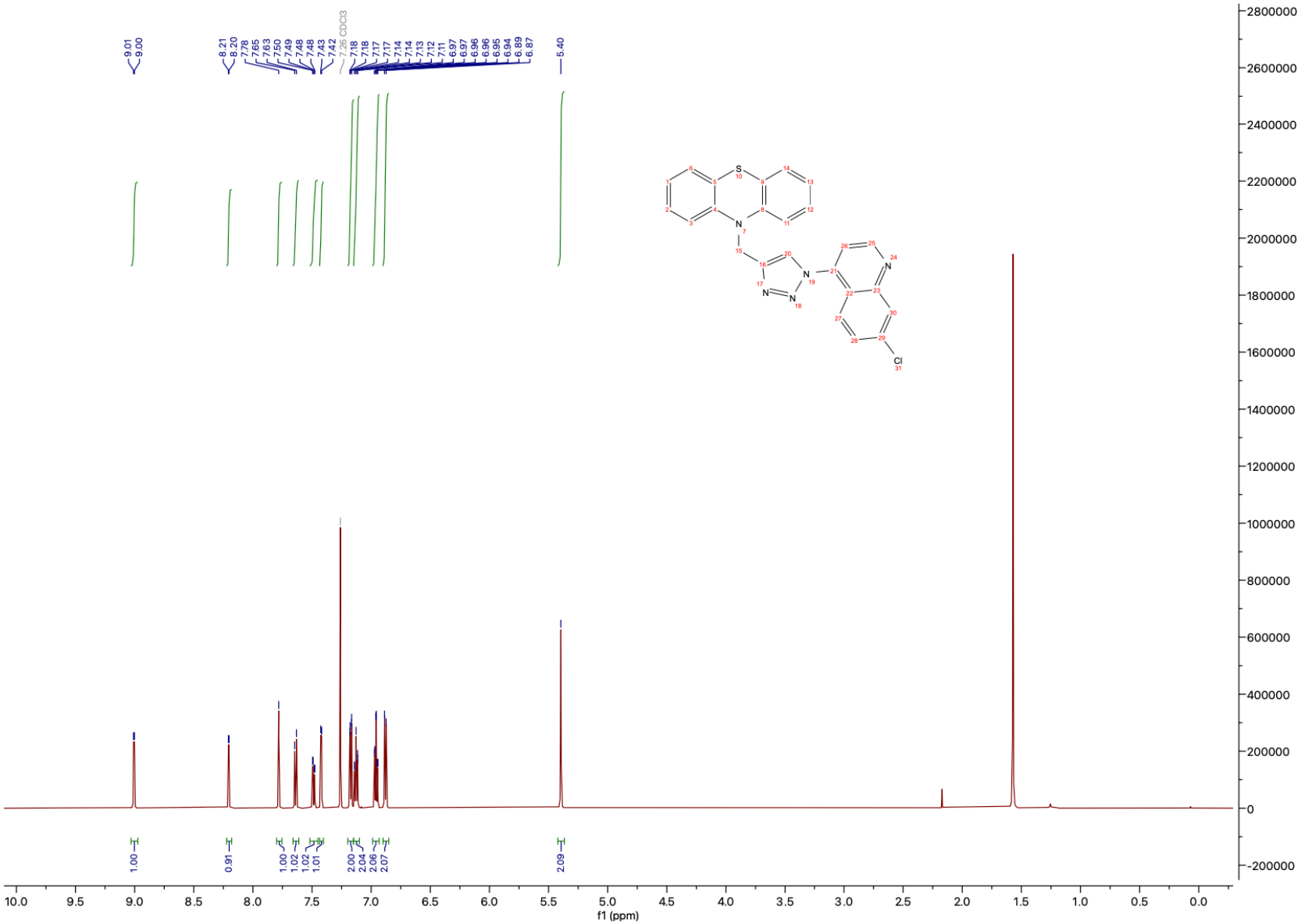

Figure S21: ^{13}C-NMR Spectra of compound **7**

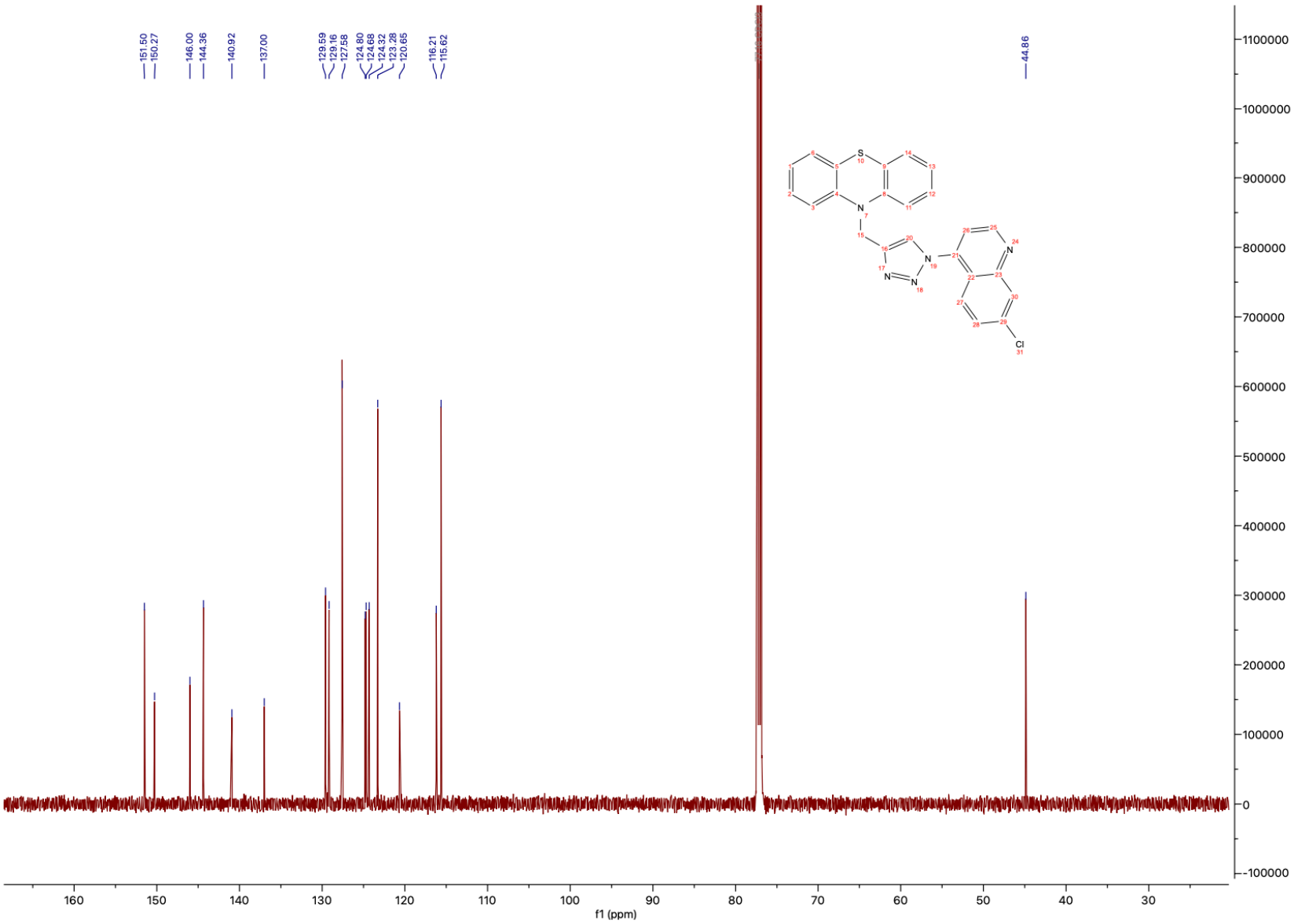

Figure S22: ^{13}C-NMR Spectra of compound **7**

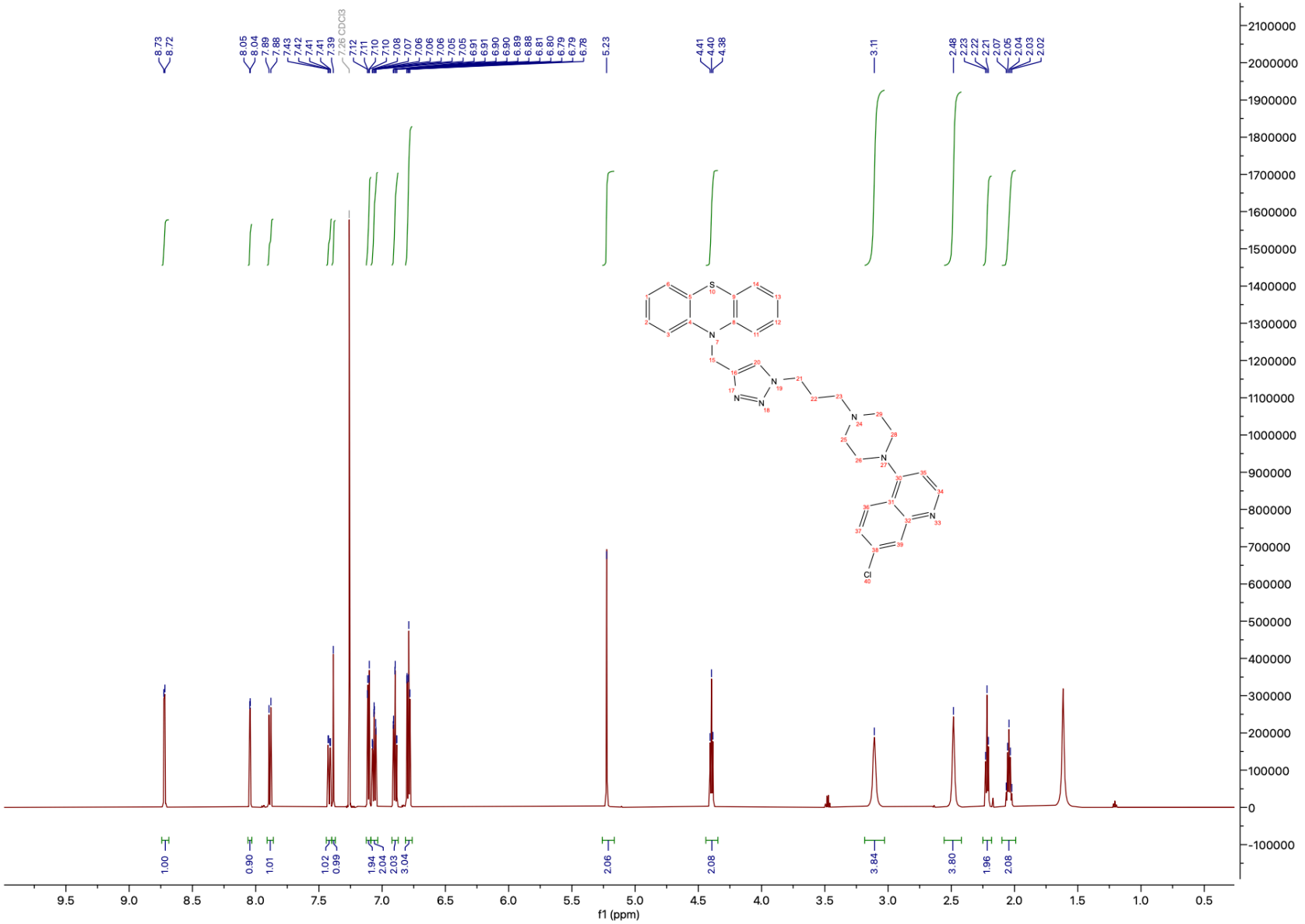

Figure S23: ^{1}H-NMR Spectra of compound **8**

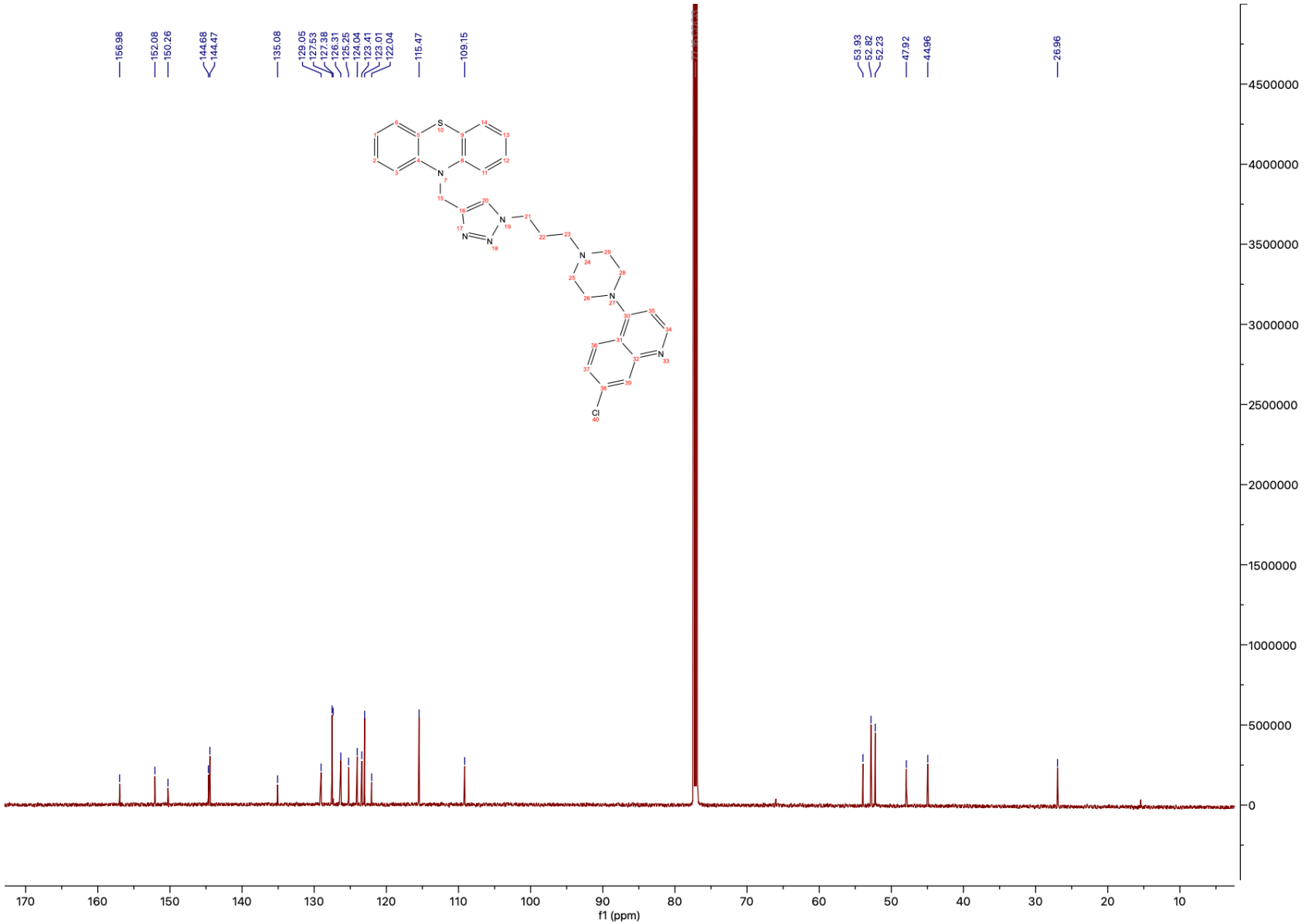

Figure S24: ^{13}C-NMR Spectra of compound **8**