Electronic Supporting Information

Article

Heterostilbene Carbamates with Selective and Remarkably Butyrylcholinesterase Inhibition: Computational Study and Physico-Chemical Properties

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1. Dose-response curves for the inhibition of BChE by carbamates 2 – 8, 10 – 14 and 17 – 19

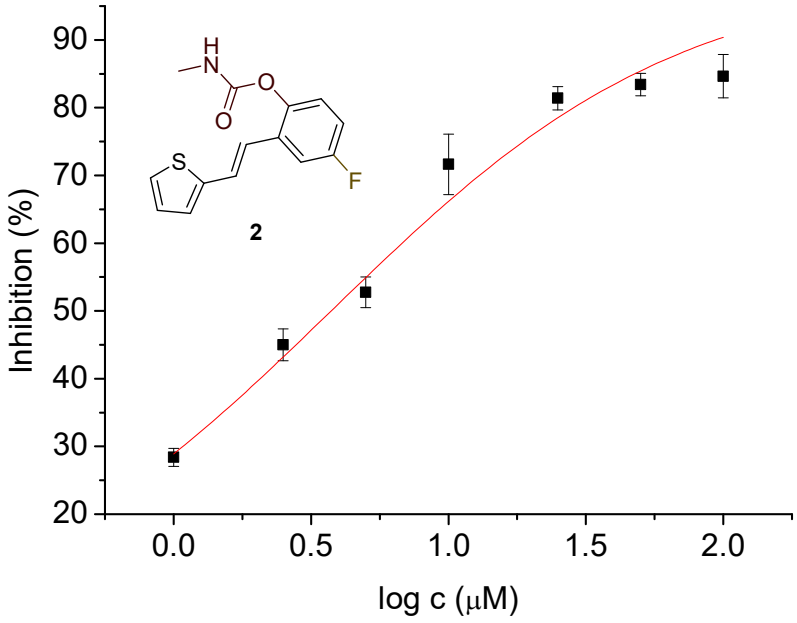


Figure S1. Dose-response curve for the inhibition of BChE by **2**.

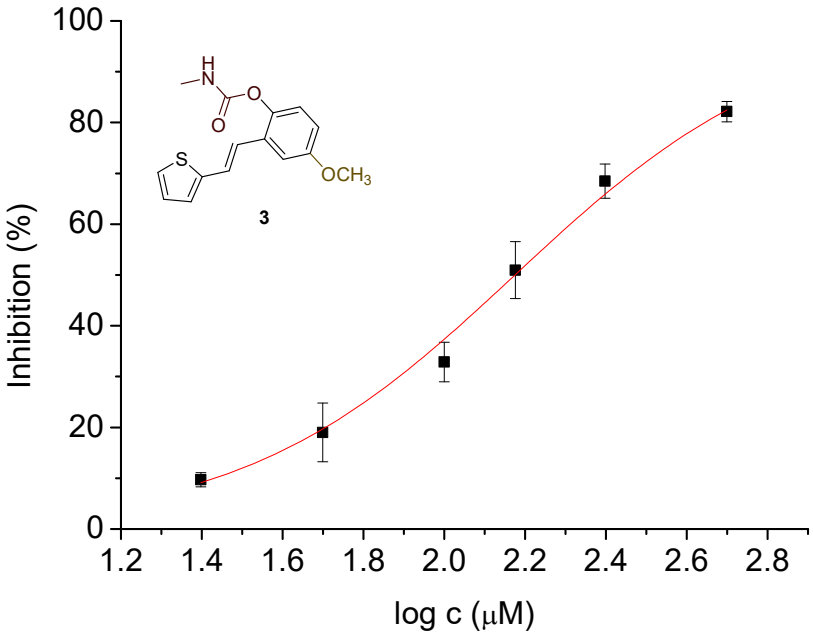


Figure S2. Dose-response curve for the inhibition of BChE by **3**.

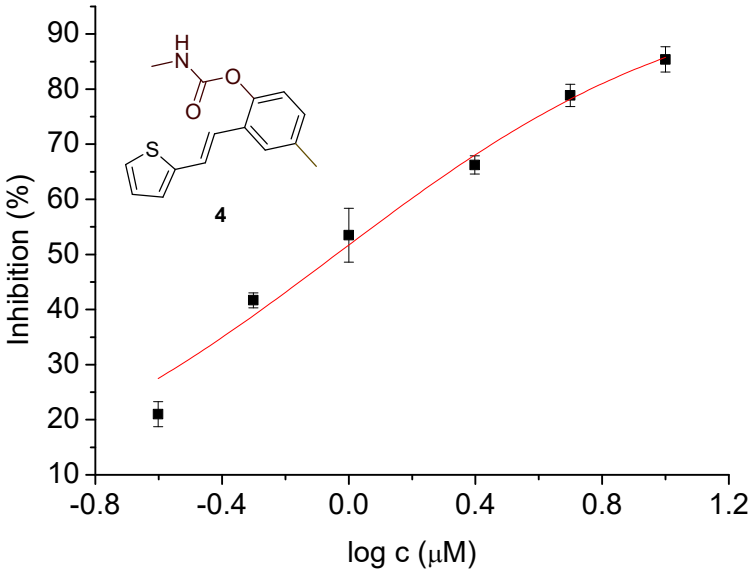


Figure S3. Dose-response curve for the inhibition of BChE by **4**.

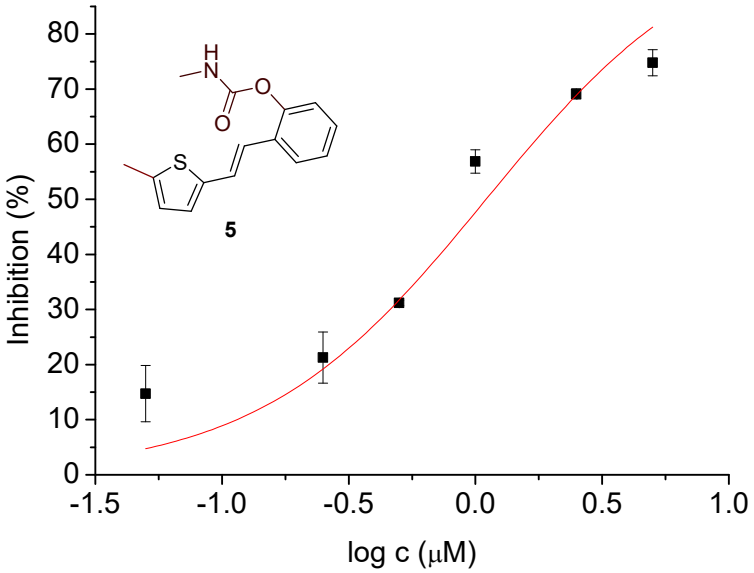


Figure S4. Dose-response curve for the inhibition of BChE by **5**.

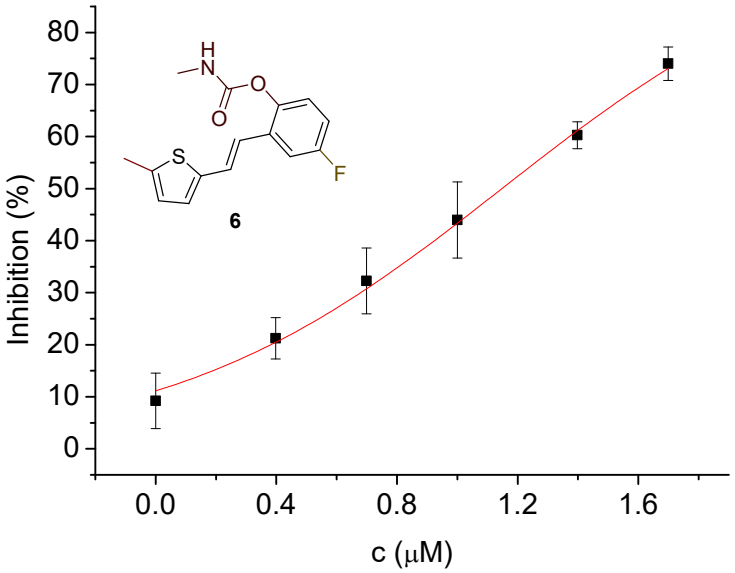


Figure S5. Dose-response curve for the inhibition of BChE by **6**.

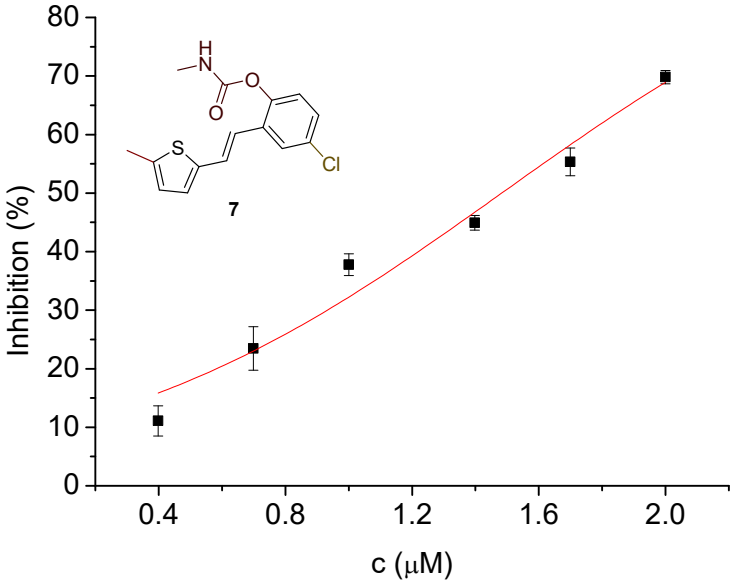


Figure S6. Dose-response curve for the inhibition of BChE by **7**.

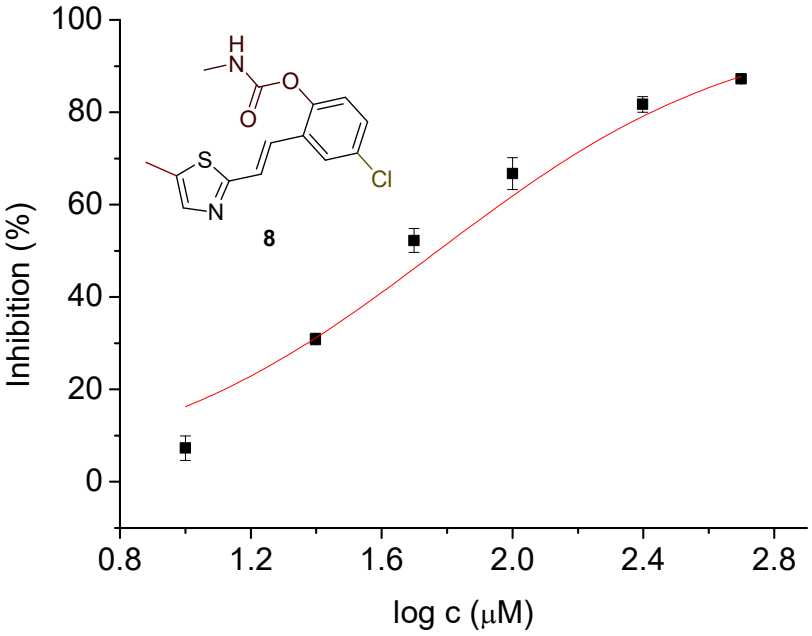


Figure S7. Dose-response curve for the inhibition of BChE by **8**.

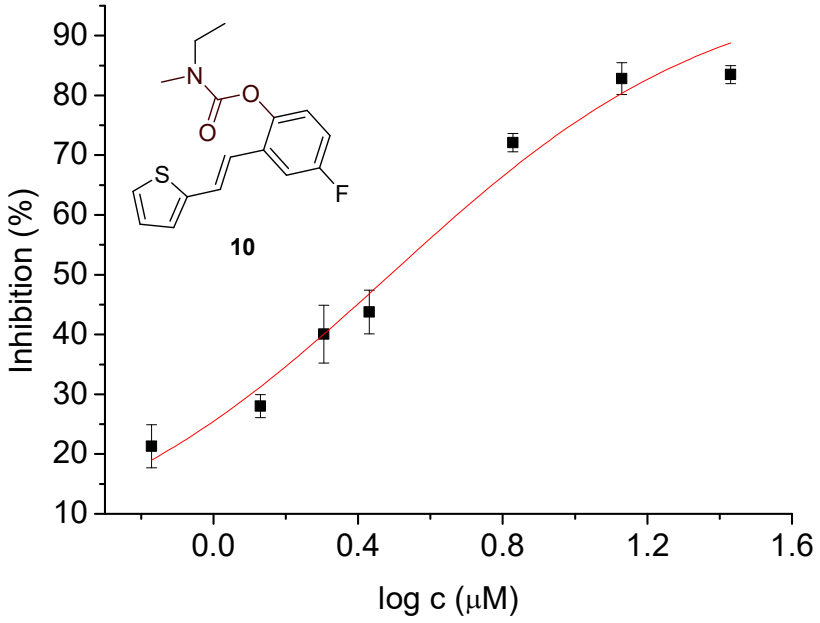


Figure S8. Dose-response curve for the inhibition of BChE by **10**.

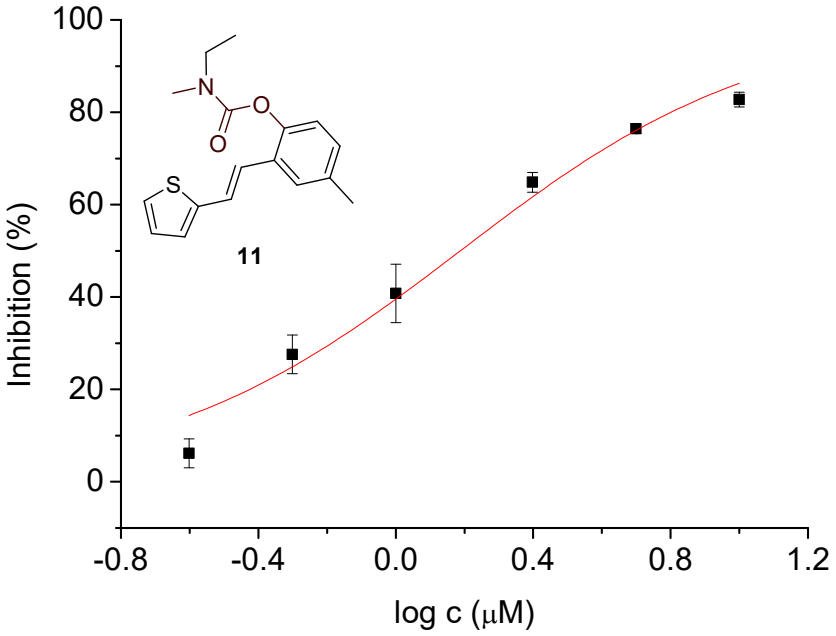


Figure S9. Dose-response curve for the inhibition of BChE by **11**.

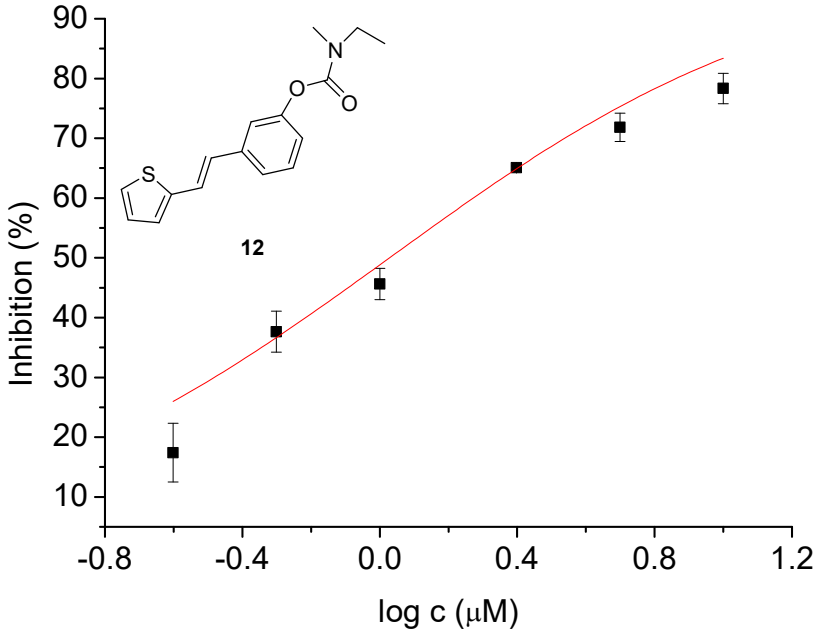


Figure S10. Dose-response curve for the inhibition of BChE by **12**.

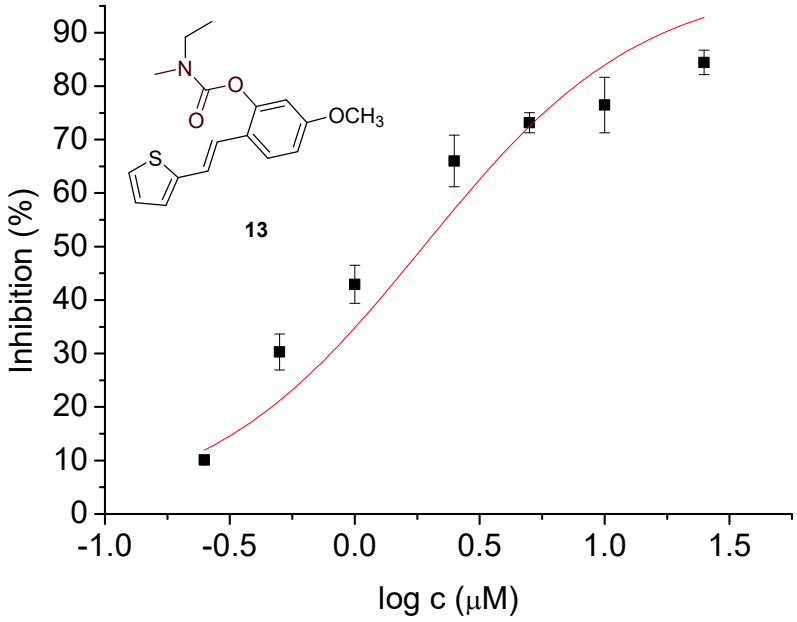


Figure S11. Dose-response curve for the inhibition of BChE by **13**.

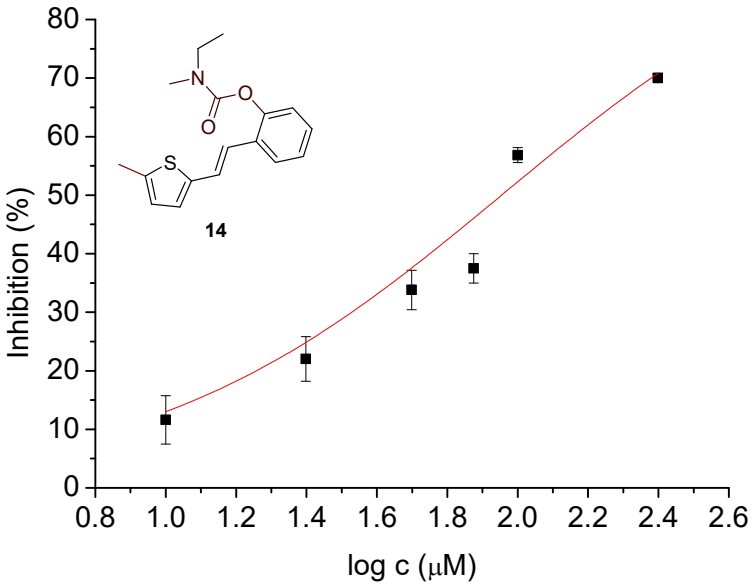


Figure S12. Dose-response curve for the inhibition of BChE by **14**.

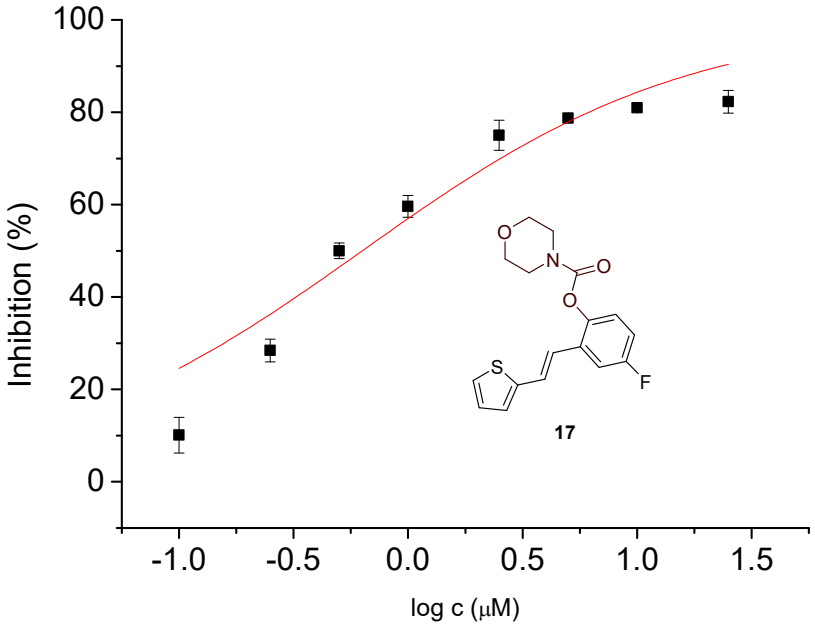


Figure S13. Dose-response curve for the inhibition of BChE by **17**.

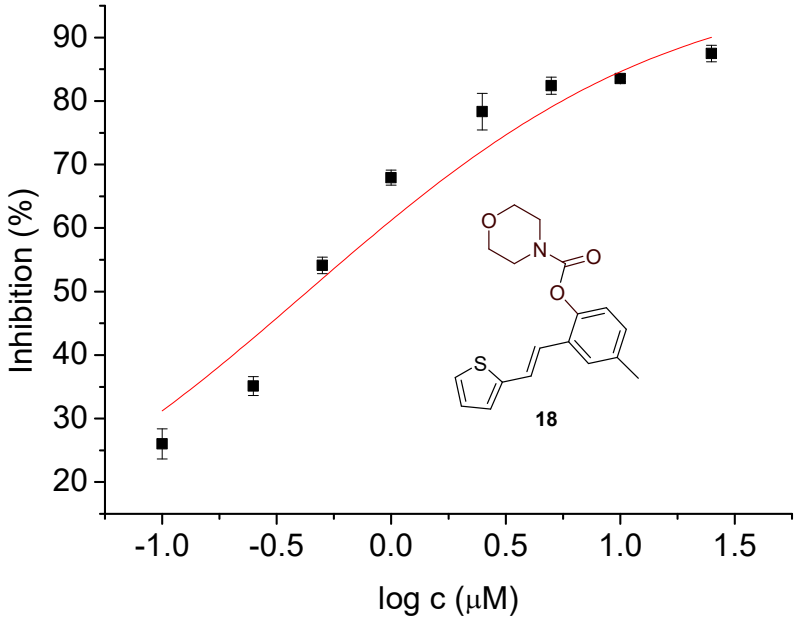


Figure S14. Dose-response curve for the inhibition of BChE by **18**.

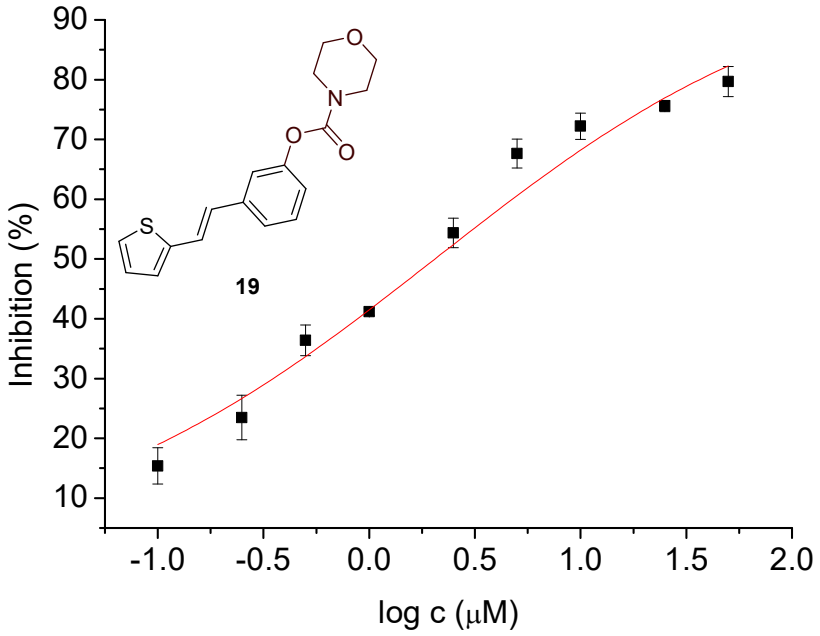
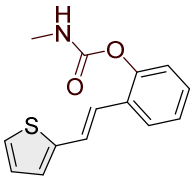
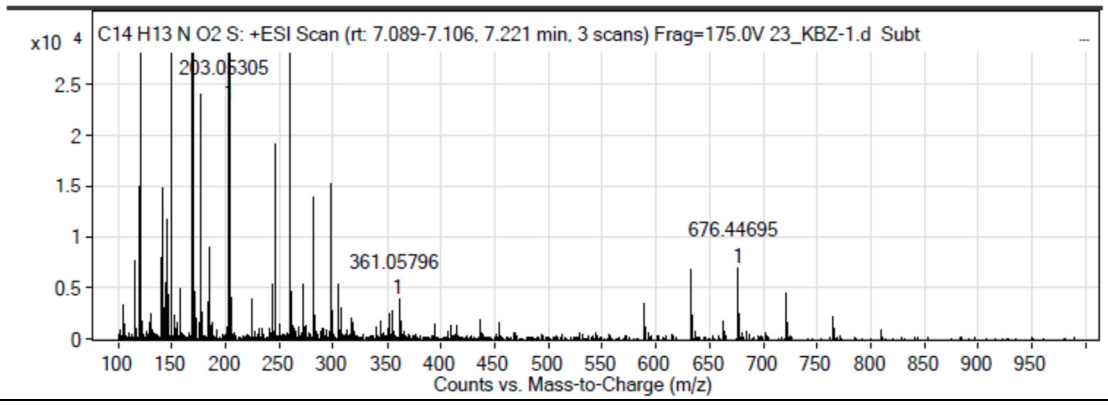


Figure S15. Dose-response curve for the inhibition of BChE by **19**.

2. Mass spectra and HRMS analyses of carbamates 1 – 19

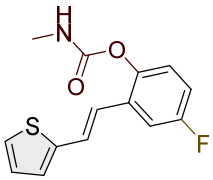


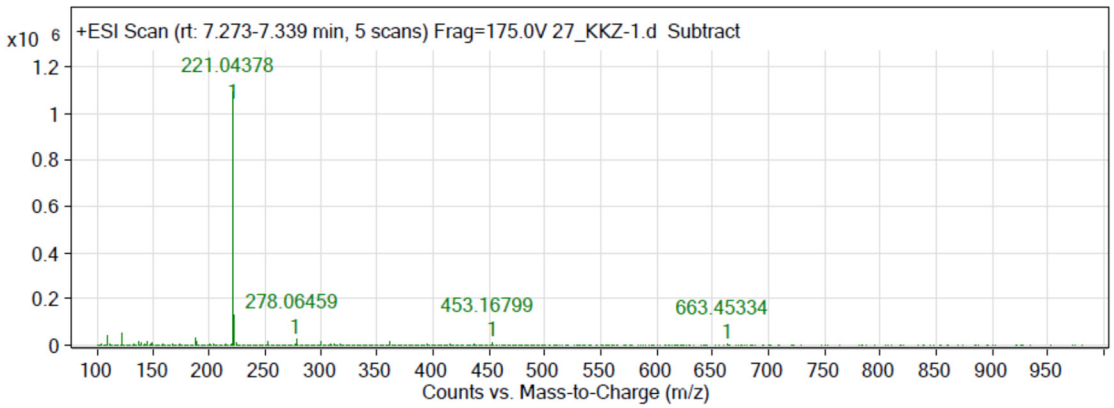


Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C14 H13 N O2 S	True	259.06662	259.0667	0.32	C14 H14 N O2 S	99.01

Figure S16. Mass spectra and HRMS analysis of carbamate **1**.

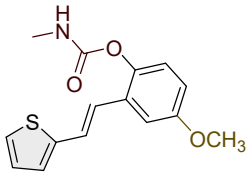




Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C14 H12 F N O2 S	True	277.05727	277.05728	0.04	C14 H13 F N O2 S	98.79

Figure S17. Mass spectra and HRMS analysis of carbamate **2**.



Peak List

z	z	Abund	Formula	Ion
149.02346		37591.32		
233.06391	1	251074.22		
234.06681	1	33451.94		
279.09362	1	31940.43		
290.08461	1	40905.91	C15 H15 N O3 S	(M+H)+
293.07568	1	32330.35		
312.06647	1	29945.77		
350.25446	1	53062.36		
720.47394	1	39330.24		
764.50024	1	41317.5		

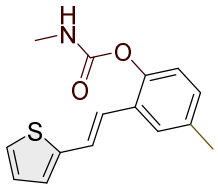
Formula Calculator Element Limits

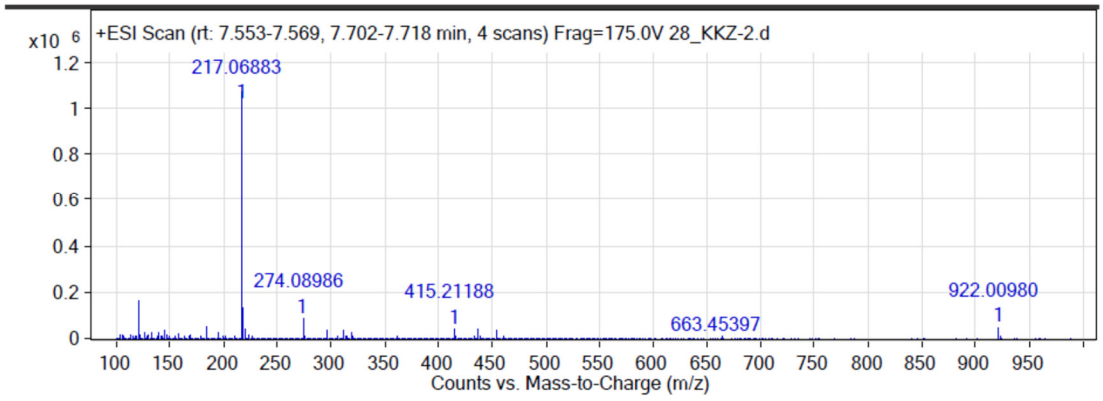
Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	30
S	0	5
Cl	0	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C15 H15 N O3 S	True	289.07733	289.07726	-0.21	C15 H16 N O3 S	99.27

Figure S18. Table with masses and HRMS analysis of carbamate **3**.

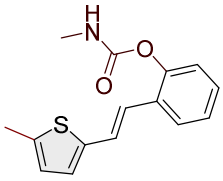


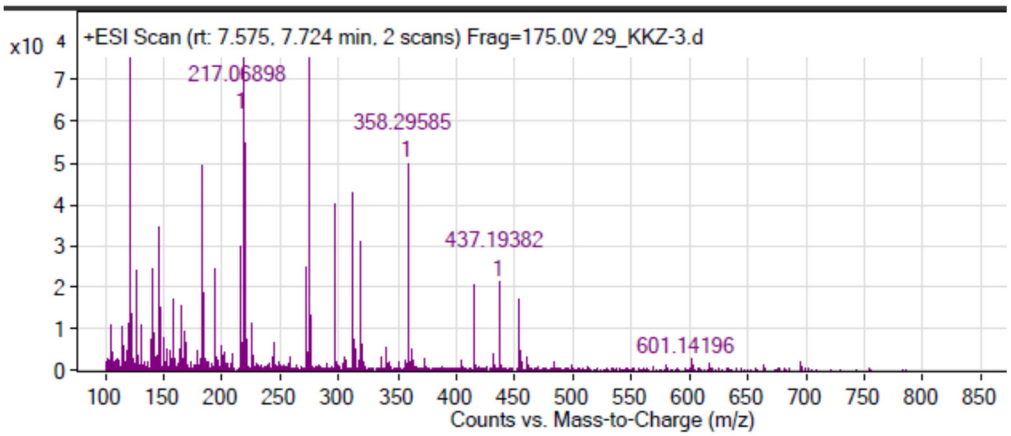


Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C15 H15 N O2 S	True	273.08261	273.08235	-0.95	C15 H16 N O2 S	98.46

Figure S19. Mass spectra and HRMS analysis of carbamate **4**.

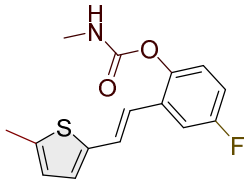


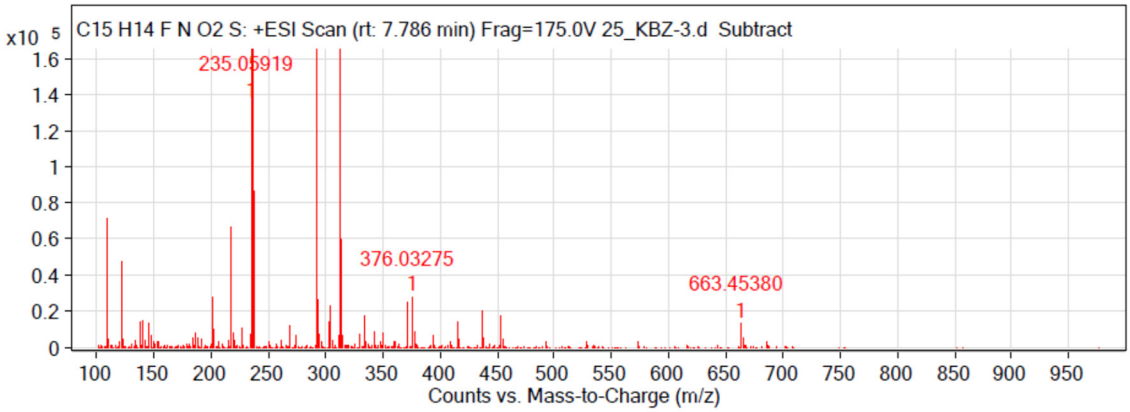


Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C15 H15 N O2 S	False	273.0825	273.08235	-0.57	C15 H16 N O2 S	99.26

Figure S20. Mass spectra and HRMS analysis of carbamate **5**.

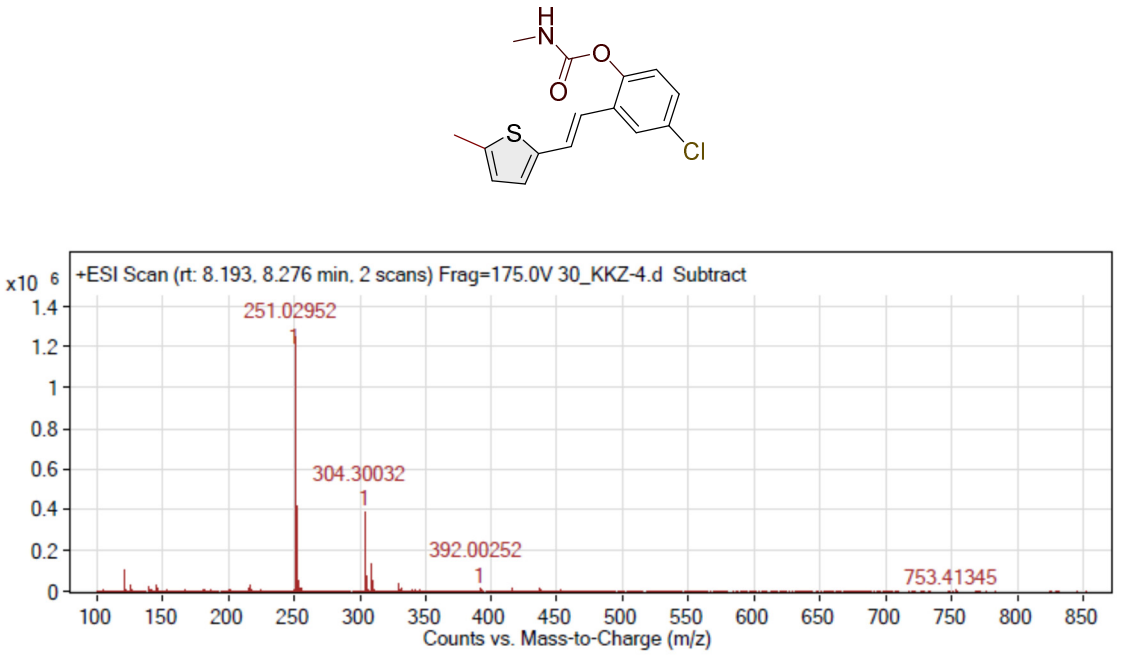




Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C15 H14 F N O2 S	True	291.07308	291.07293	-0.53	C15 H15 F N O2 S	98.4

Figure S21. Mass spectra and HRMS analysis of carbamate **6**.



Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C15 H14 Cl N O2 S	True	307.04329	307.04338	0.28	C15 H15 Cl N O2 S	99.2

Figure S22. Mass spectra and HRMS analysis of carbamate **7**.

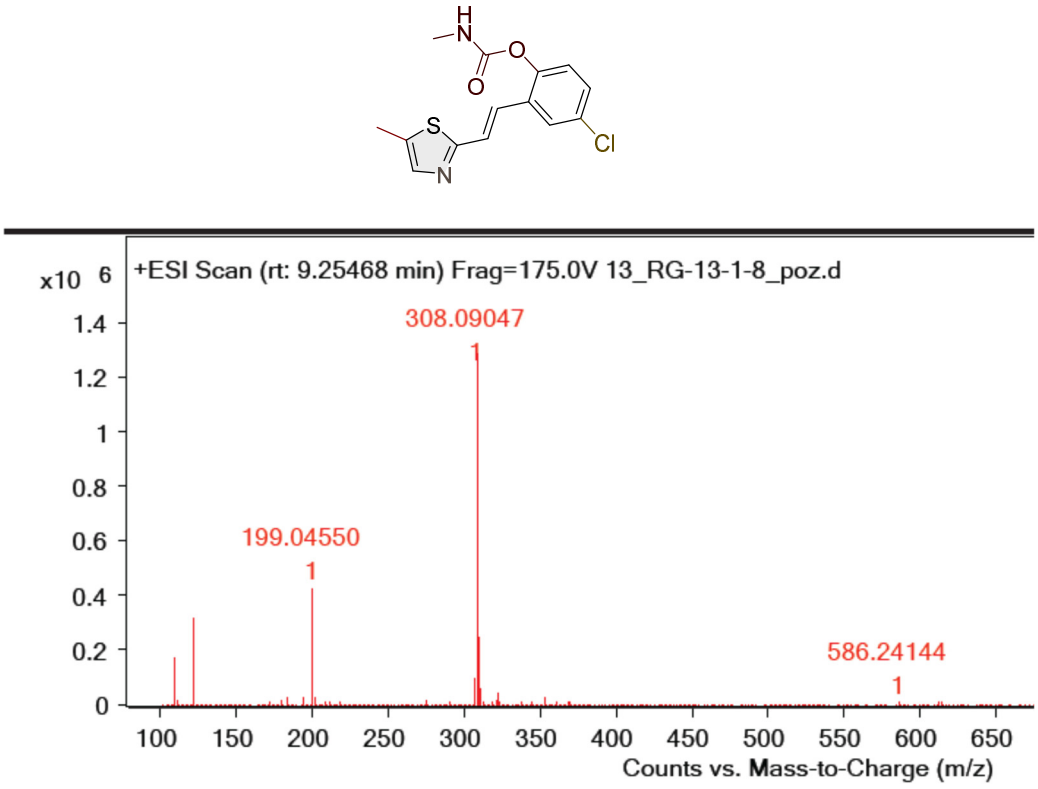
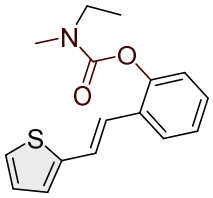
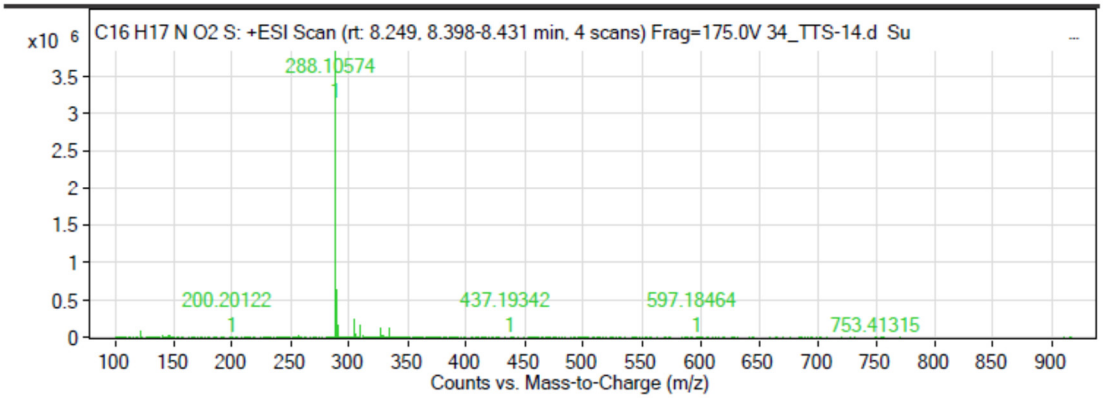


Figure S23. Mass spectra of carbamate **8**.

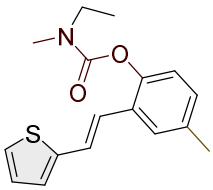


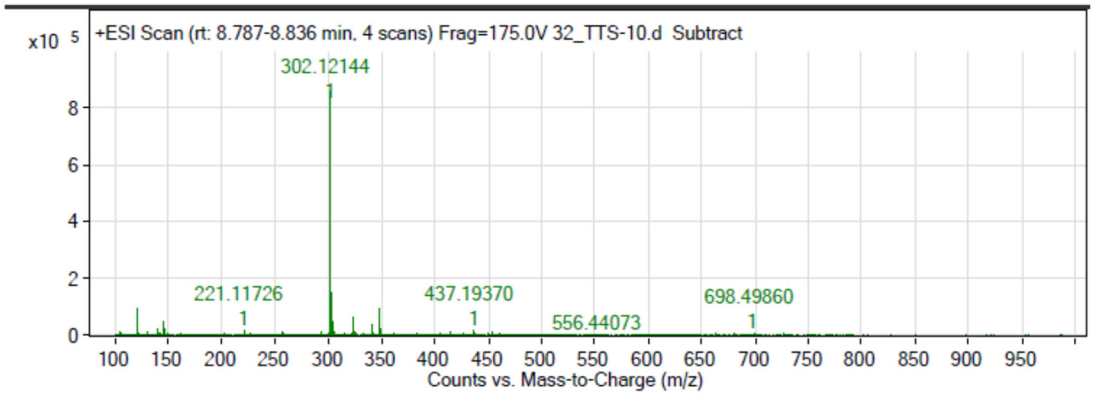


Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C16 H17 N O2 S	True	287.09853	287.098	-1.84	C16 H18 N O2 S	96.51

Figure S24. Mass spectra and HRMS analysis of carbamate **9**.

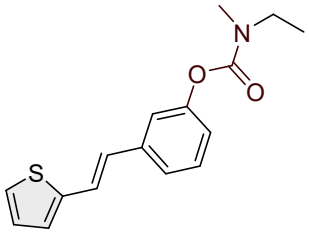


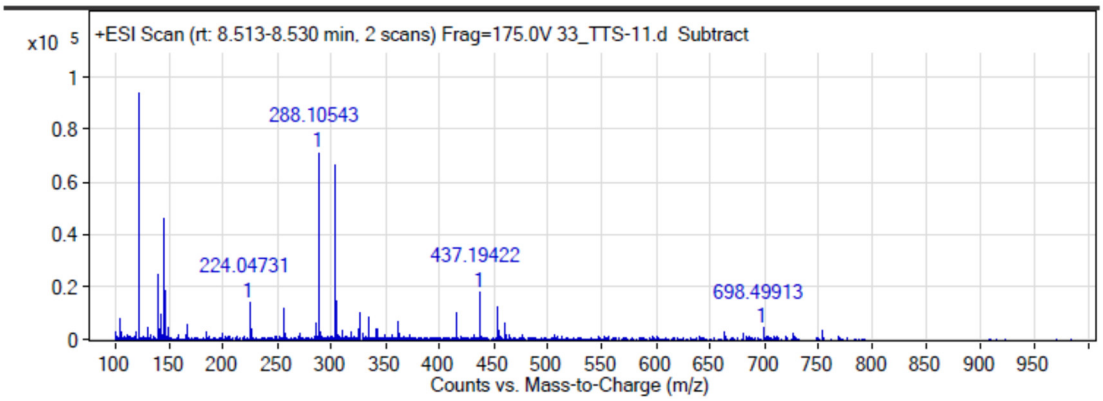


Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C17 H19 N O2 S	False	301.11414	301.11365	-1.64	C17 H20 N O2 S	96.49

Figure S25. Mass spectra and HRMS analysis of carbamate **11**.

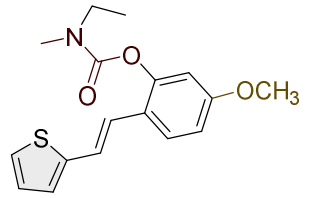




Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C16 H17 N O2 S	True	287.09817	287.098	-0.6	C16 H18 N O2 S	98.11

Figure S26. Mass spectra and HRMS analysis of carbamate **12**.



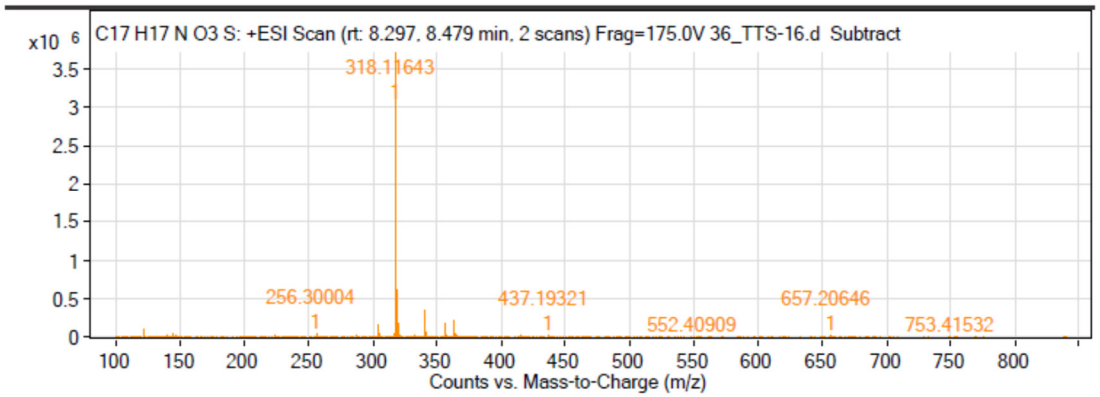
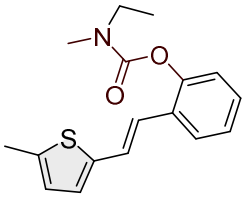


Figure S27. Mass spectra of carbamate **13**.



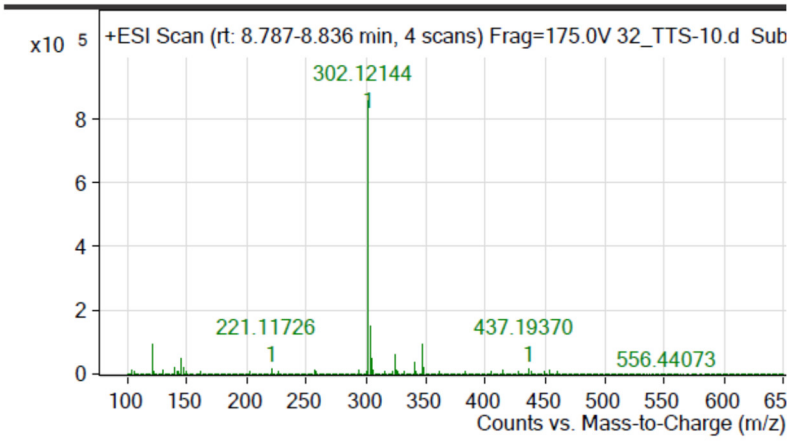
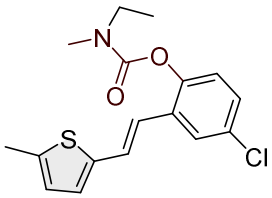
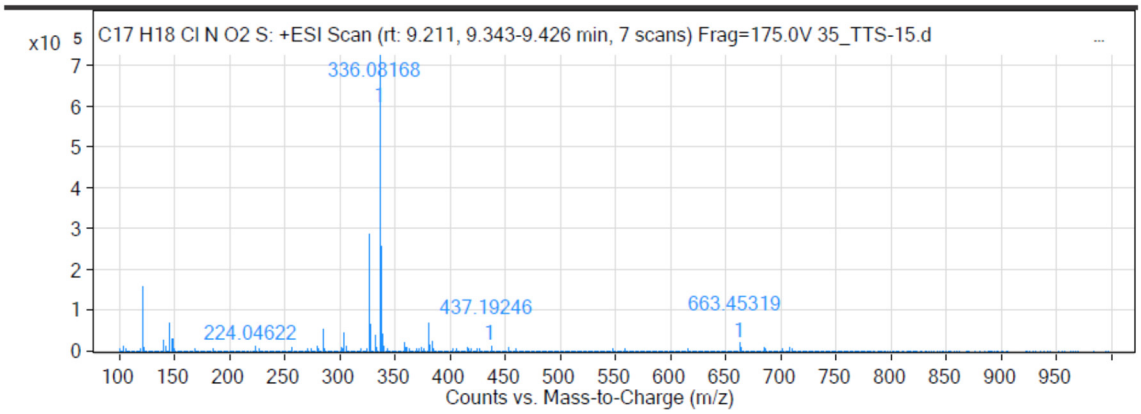


Figure S28. Mass spectra of carbamate **14**.

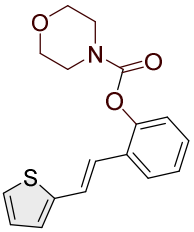


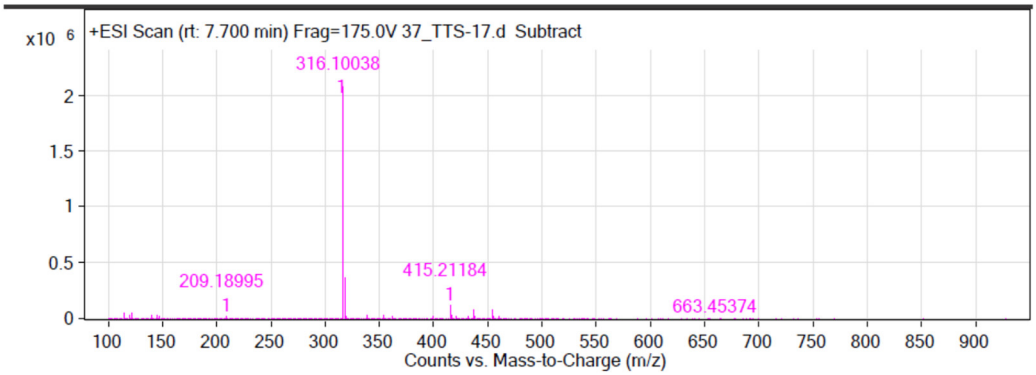


Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C17 H18 Cl N O2 S	True	335.07437	335.07468	0.9	C17 H19 Cl N O2 S	98.32

Figure S29. Mass spectra and HRMS analysis of carbamate **15**.

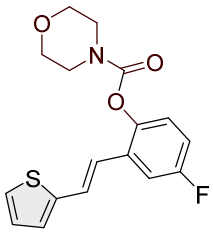


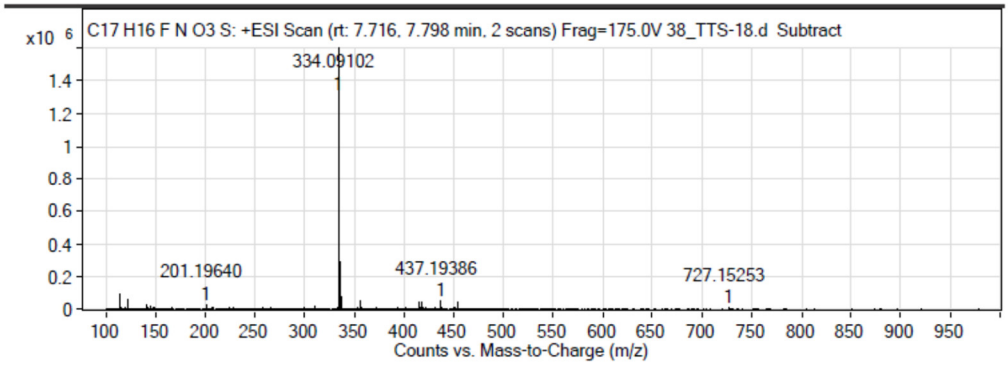


Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C17 H17 N O3 S	True	315.0932	315.09291	-0.91	C17 H18 N O3 S	96.27

Figure S30. Mass spectra and HRMS analysis of carbamate **16**.

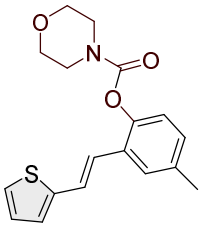


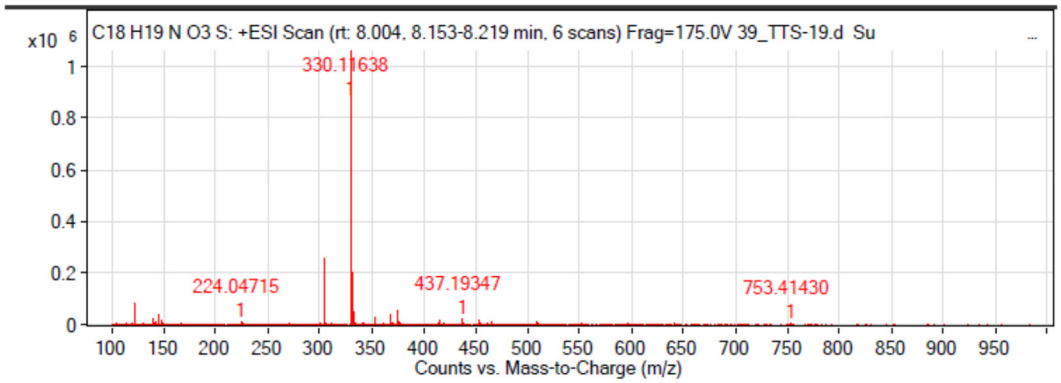


Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C17 H16 F N O3 S	True	333.08379	333.08349	-0.89	C17 H17 F N O3 S	97.71

Figure S31. Mass spectra and HRMS analysis of carbamate **17**.

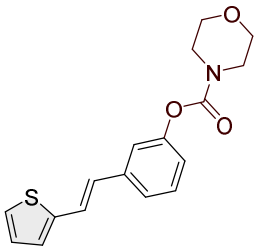


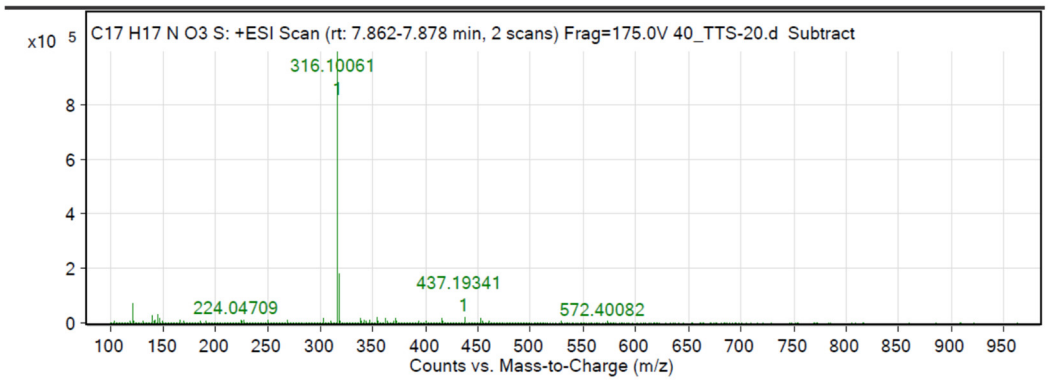


Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C18 H19 N O3 S	True	329.10913	329.10856	-1.72	C18 H20 N O3 S	96.7

Figure S32. Mass spectra and HRMS analysis of carbamate **18**.





Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C17 H17 N O3 S	True	315.09343	315.09291	-1.63	C17 H18 N O3 S	96.77

Figure S33. Mass spectra and HRMS analysis of carbamate **19**.

3. ^{1}H and ^{13}C NMR spectra of carbamates 1 – 19

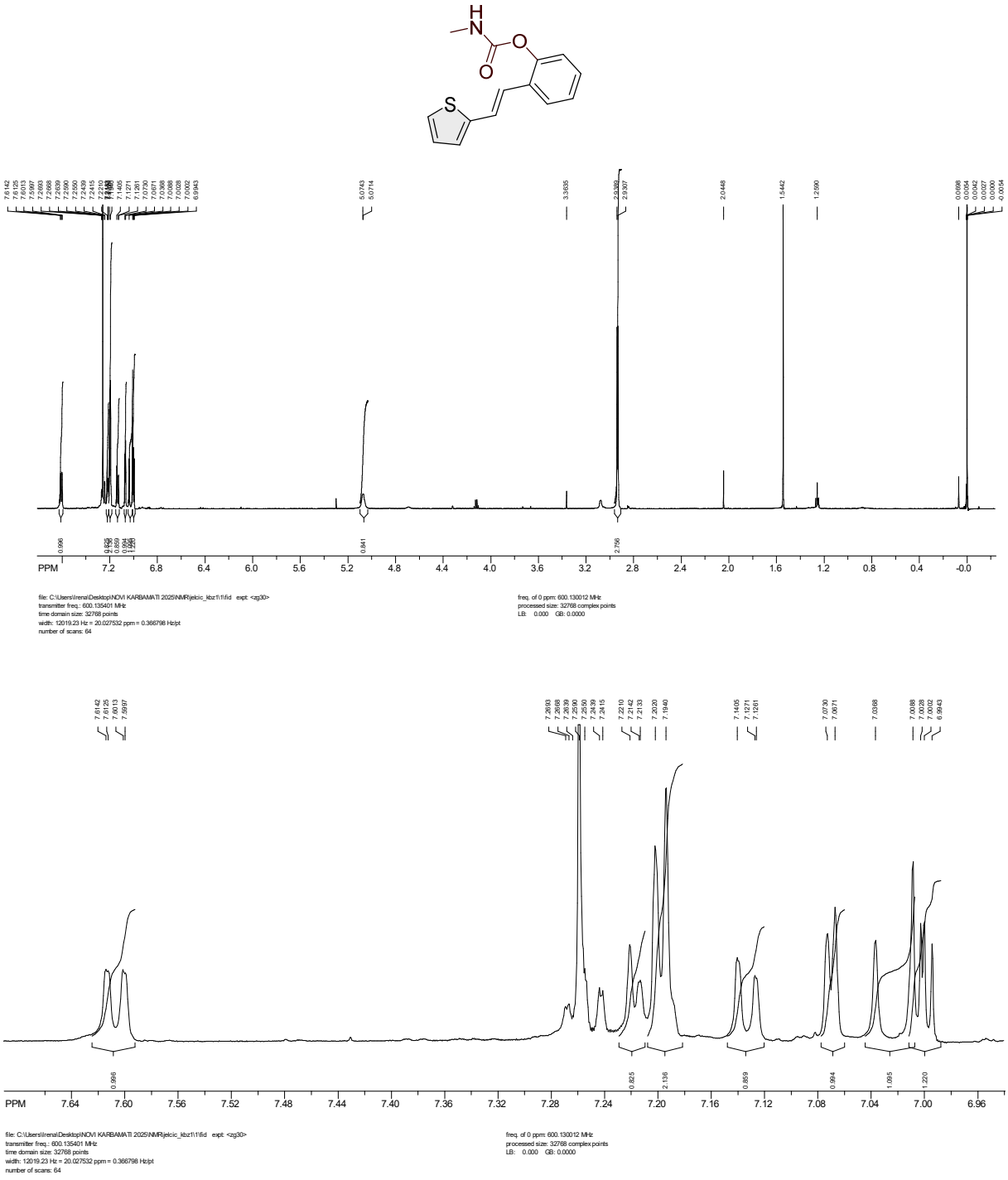


Figure S34. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **1**.

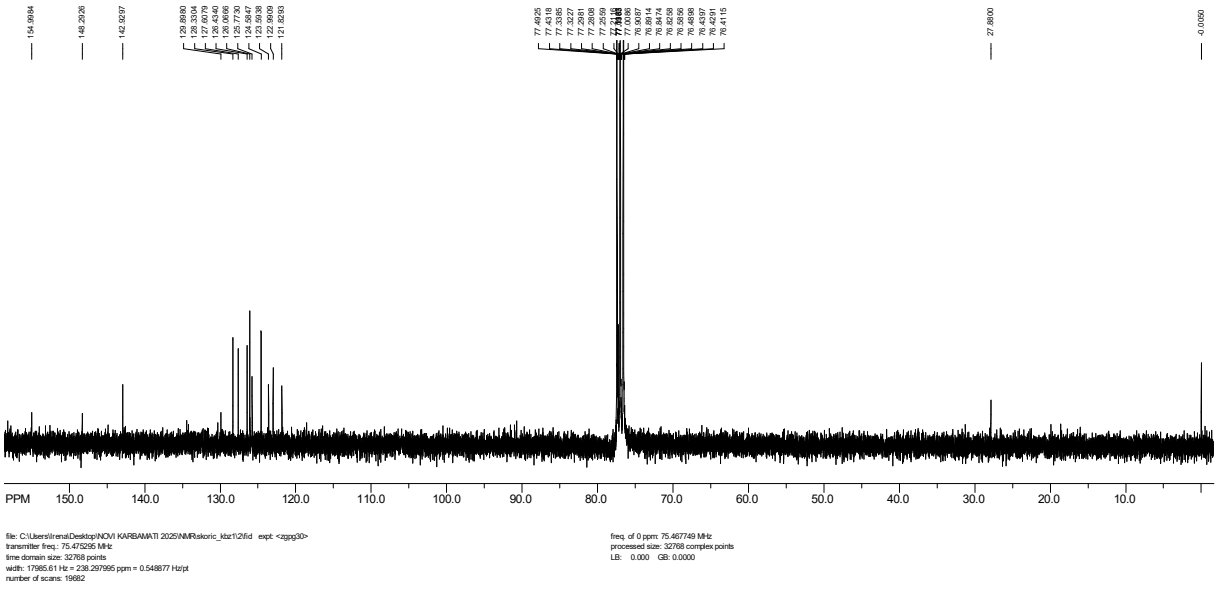


Figure S35. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **1**.

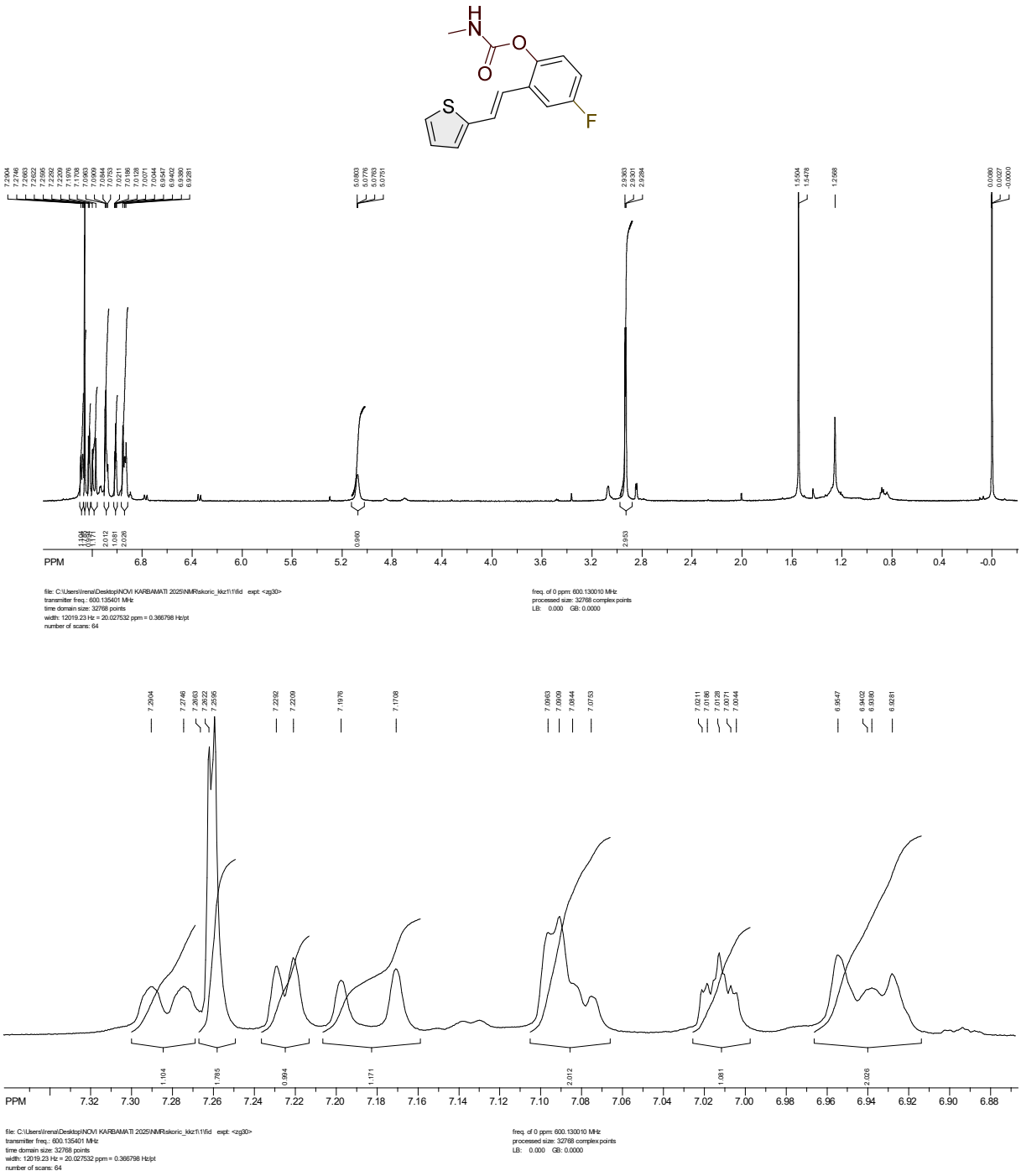


Figure S36. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **2**.

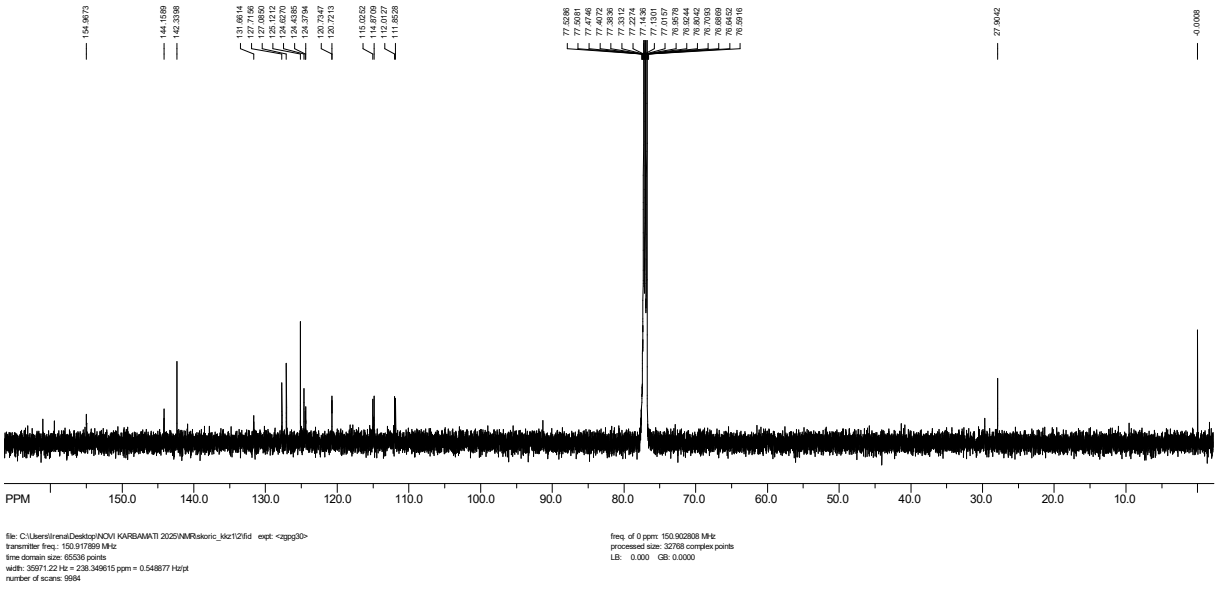


Figure S37. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **2**.

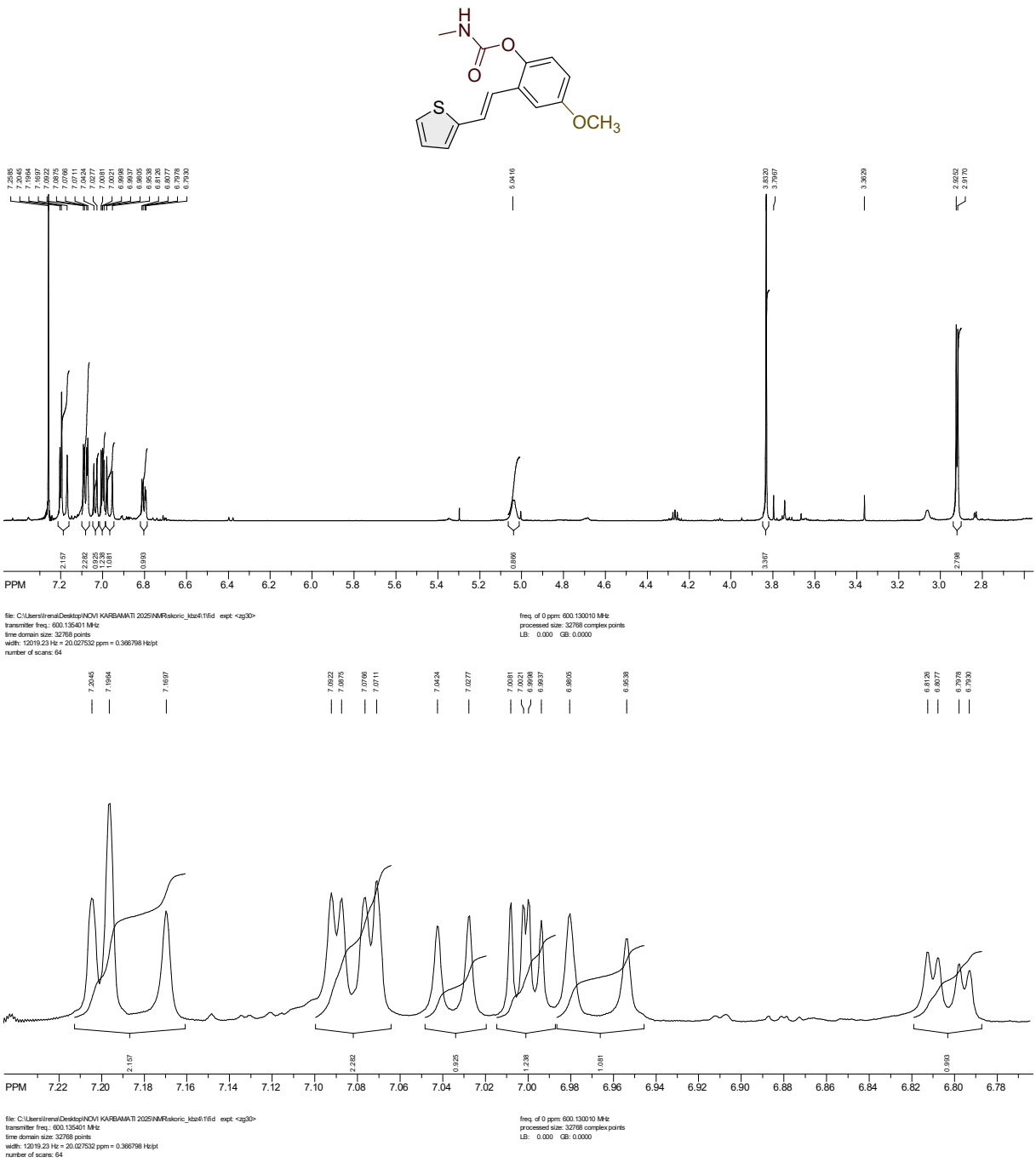


Figure S38. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **3**.

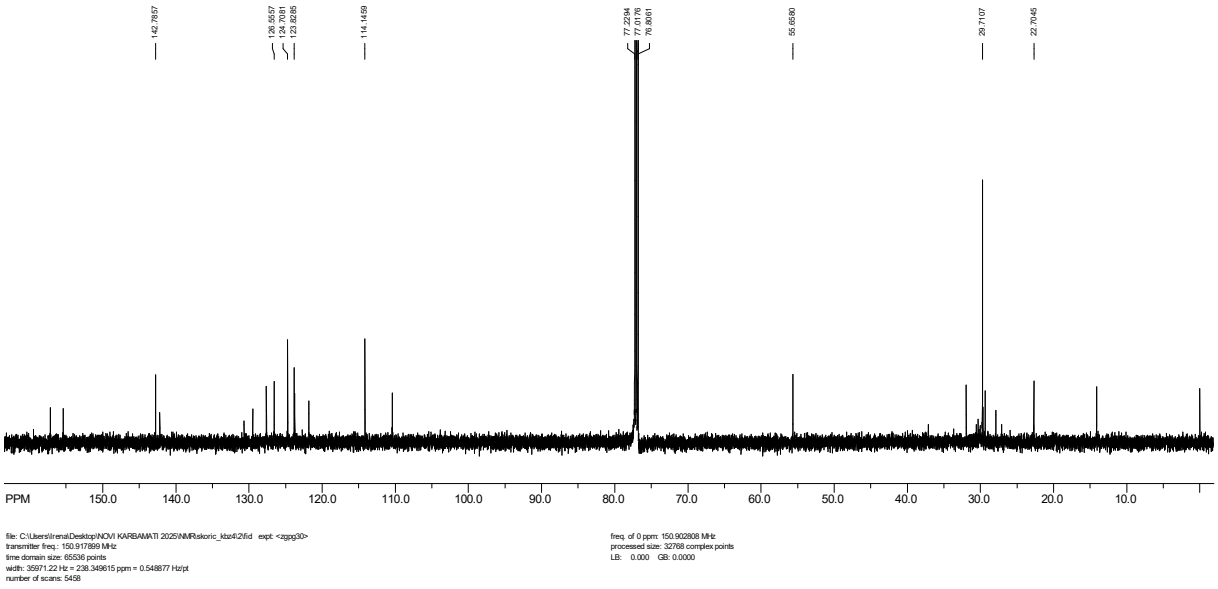


Figure S39. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **3**.

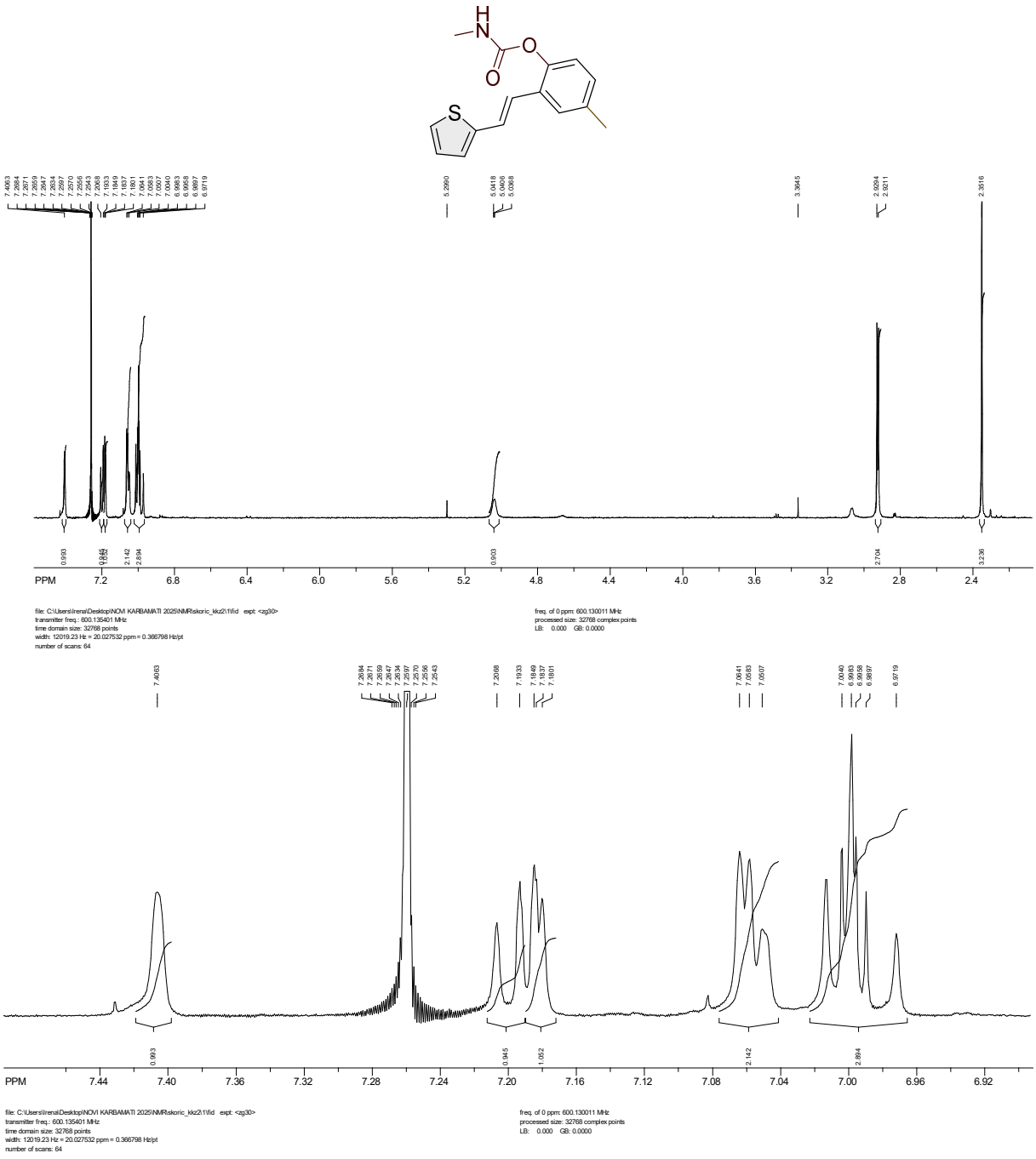


Figure S40. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **4**.

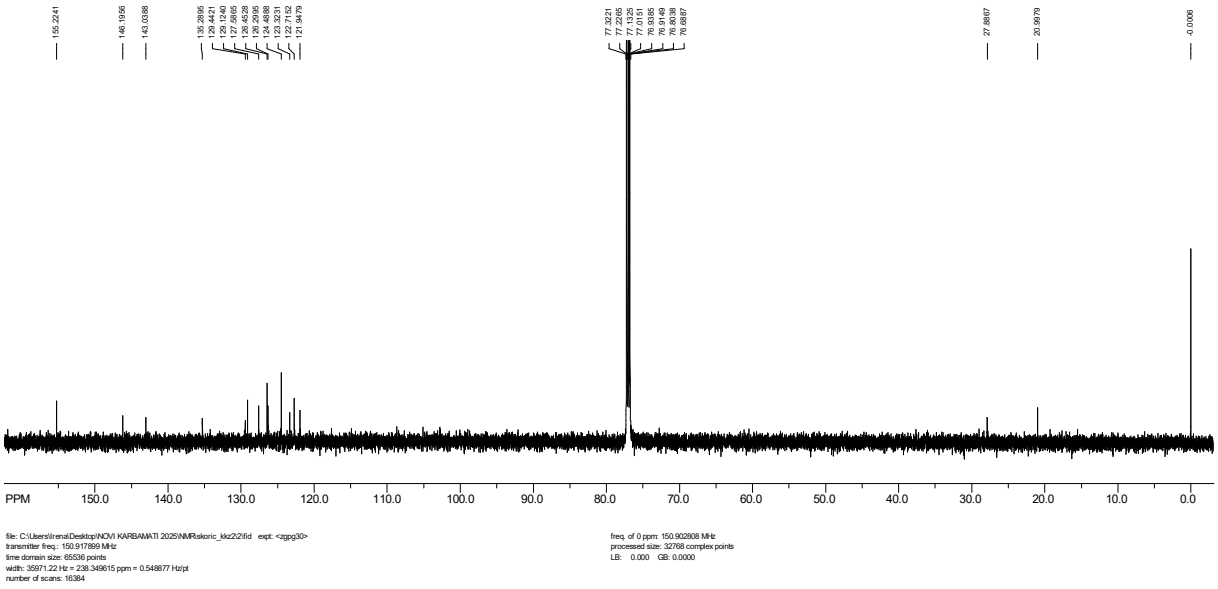


Figure S41. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **4**.

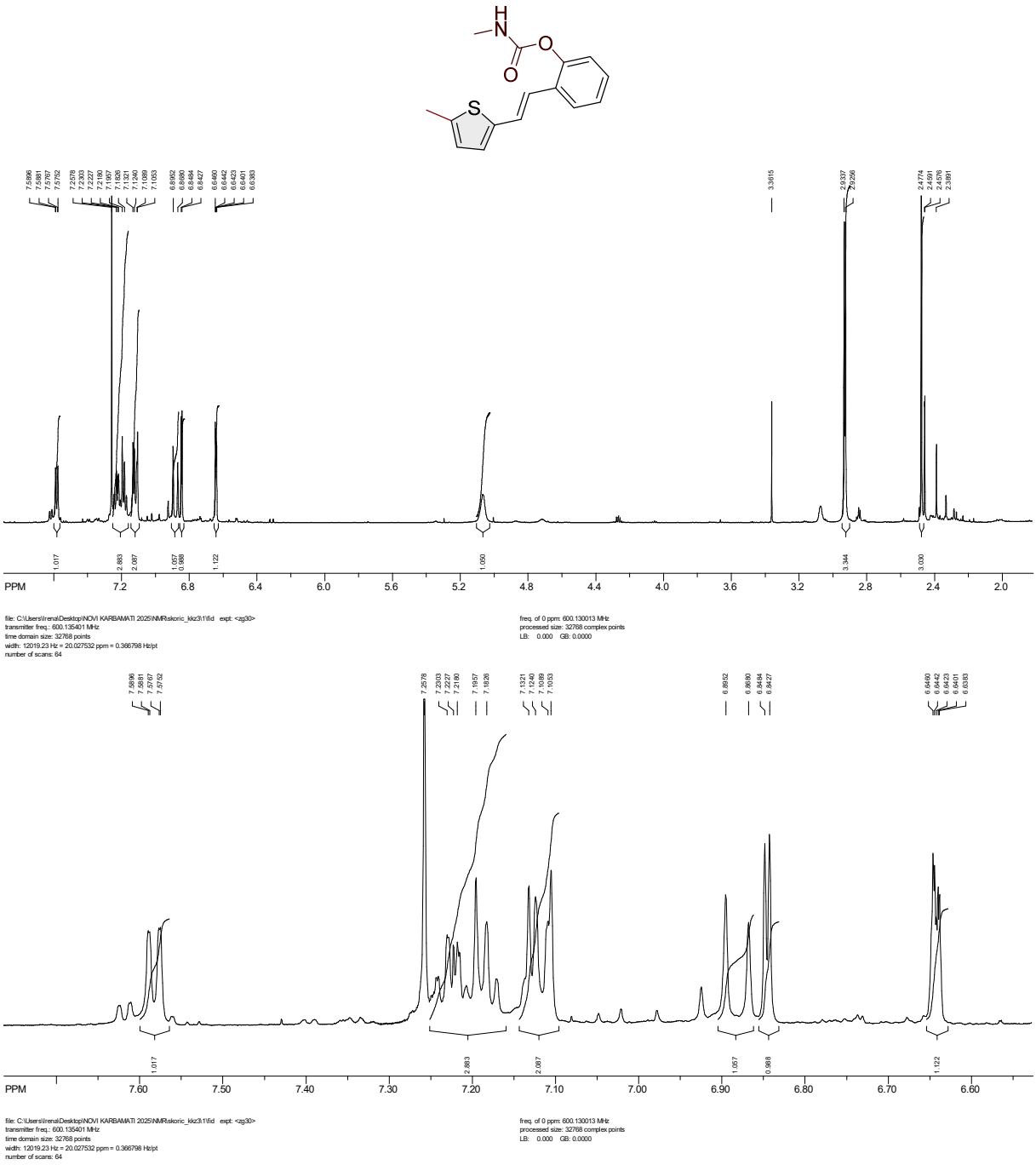


Figure S42. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **5**.

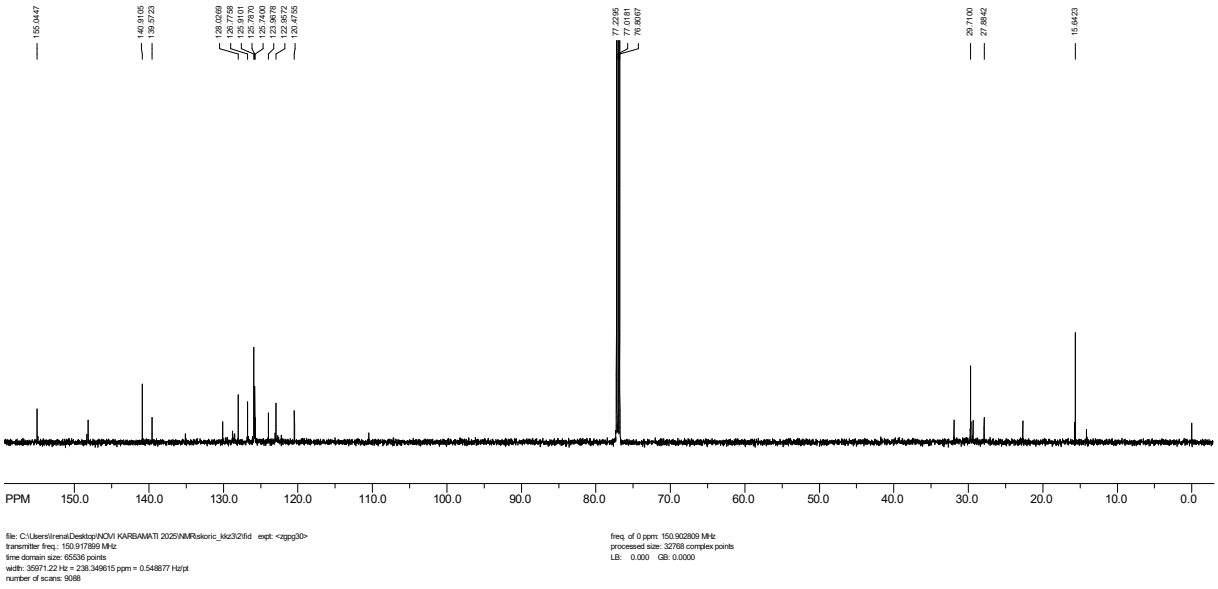


Figure S43. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **5**.

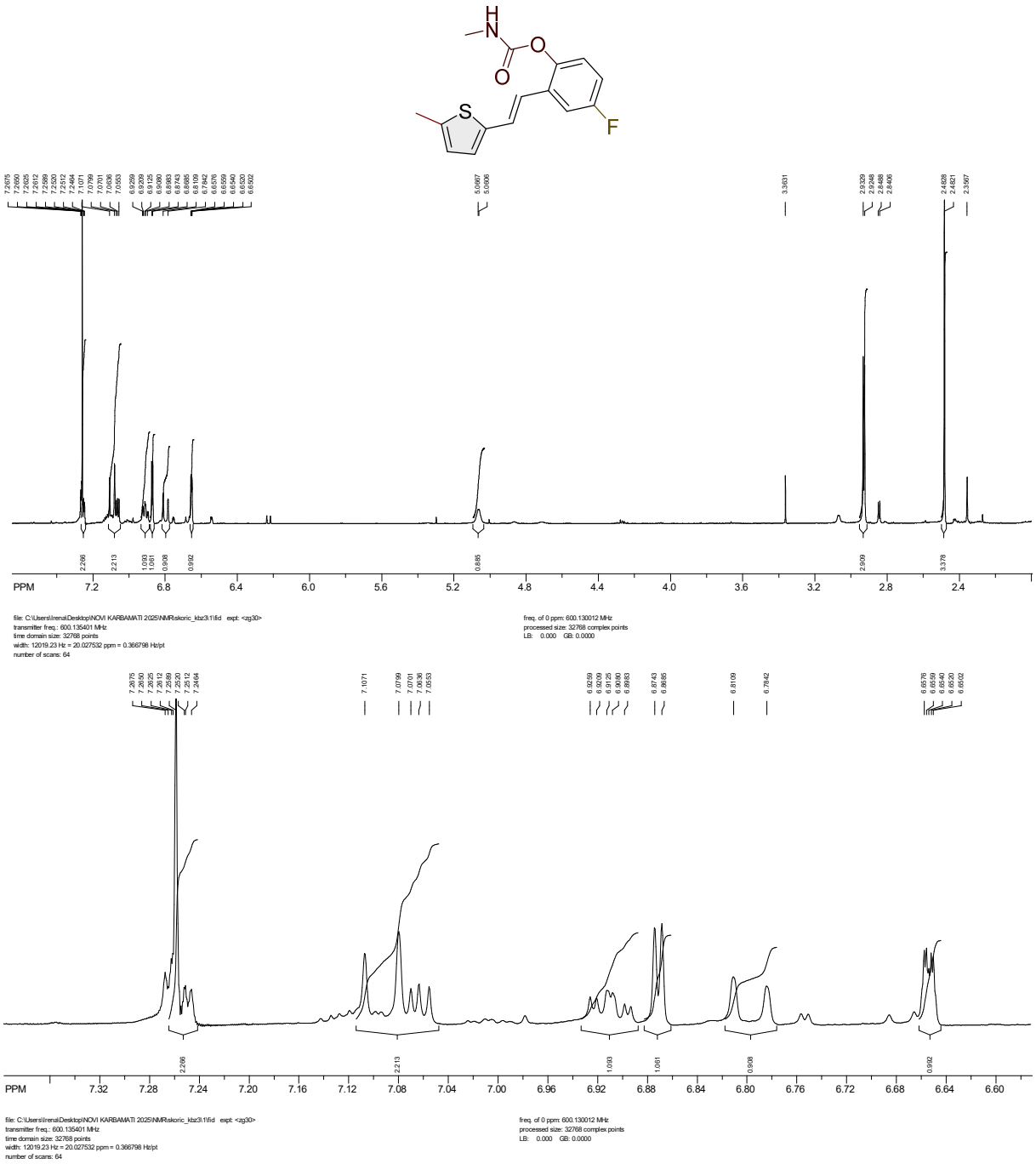


Figure S44. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **6**.

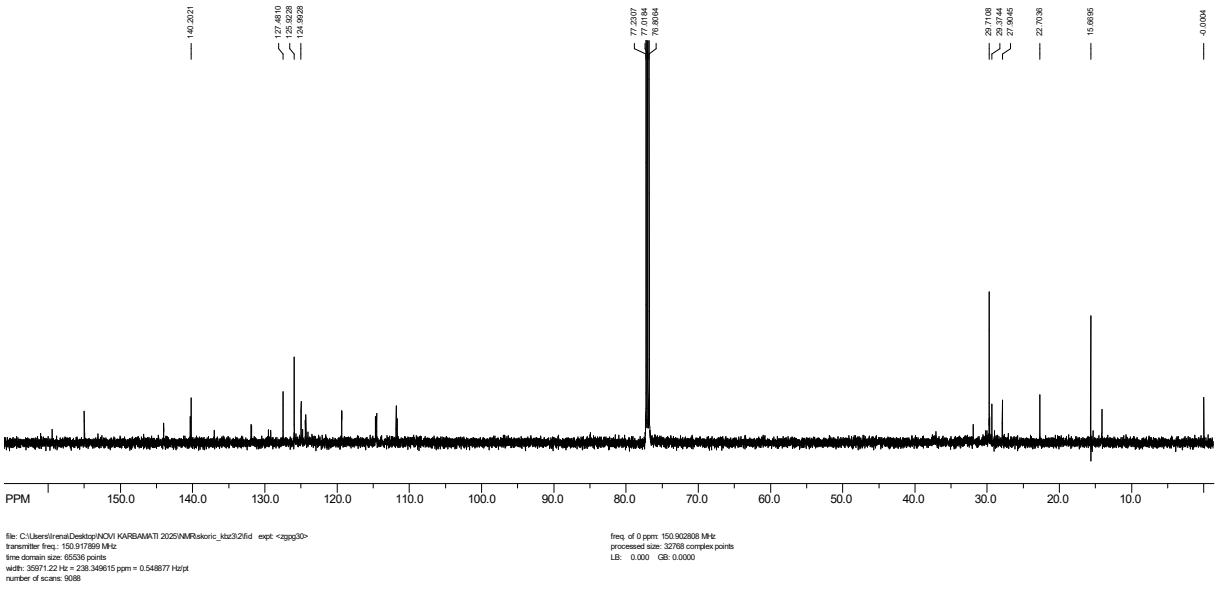


Figure S45. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **6**.

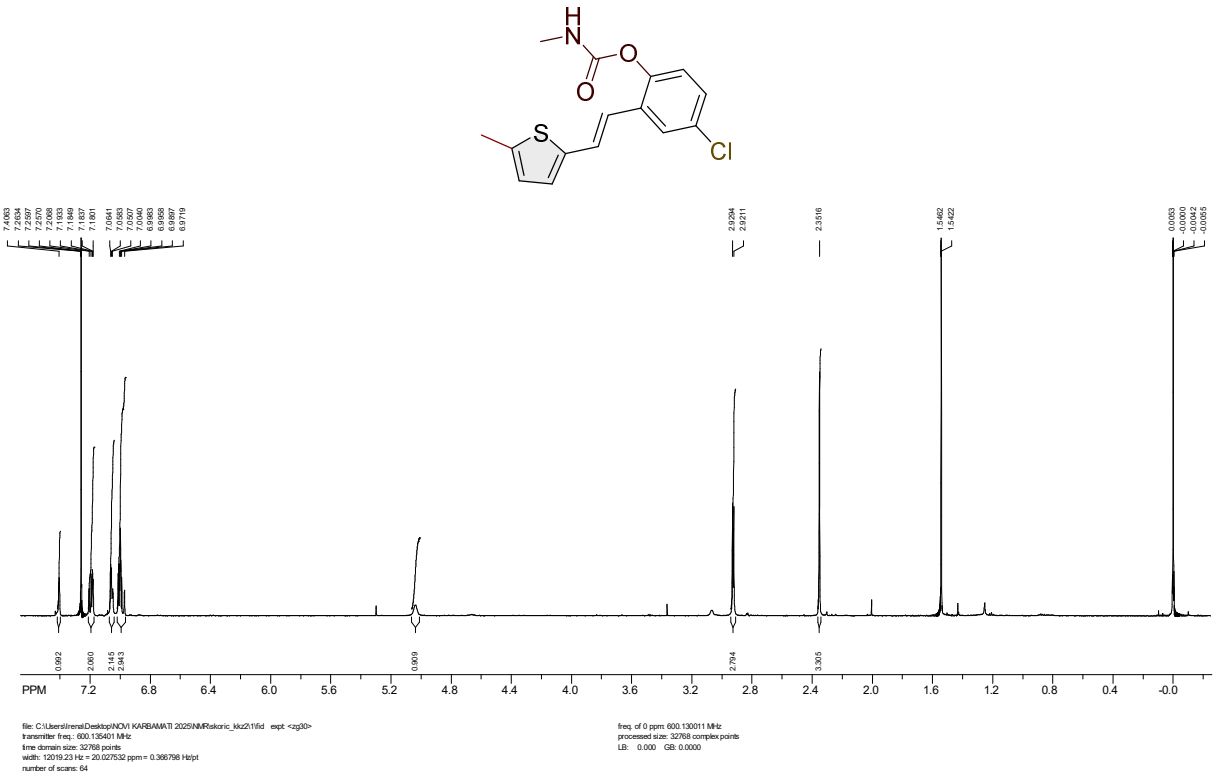


Figure S46. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **7**.

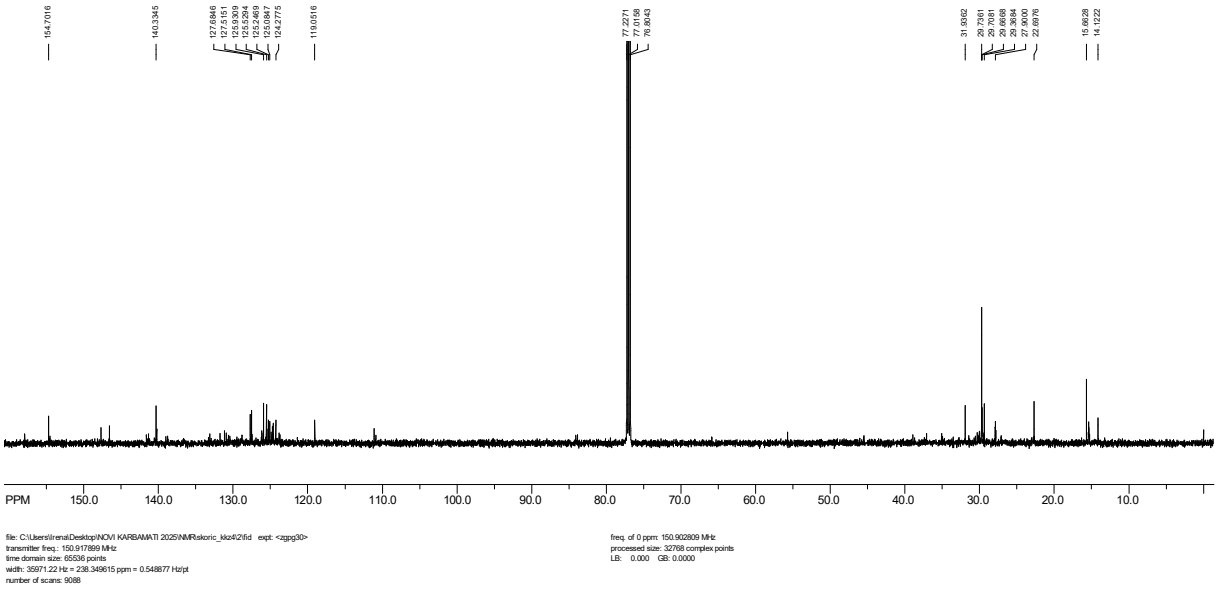


Figure S47. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **7**.

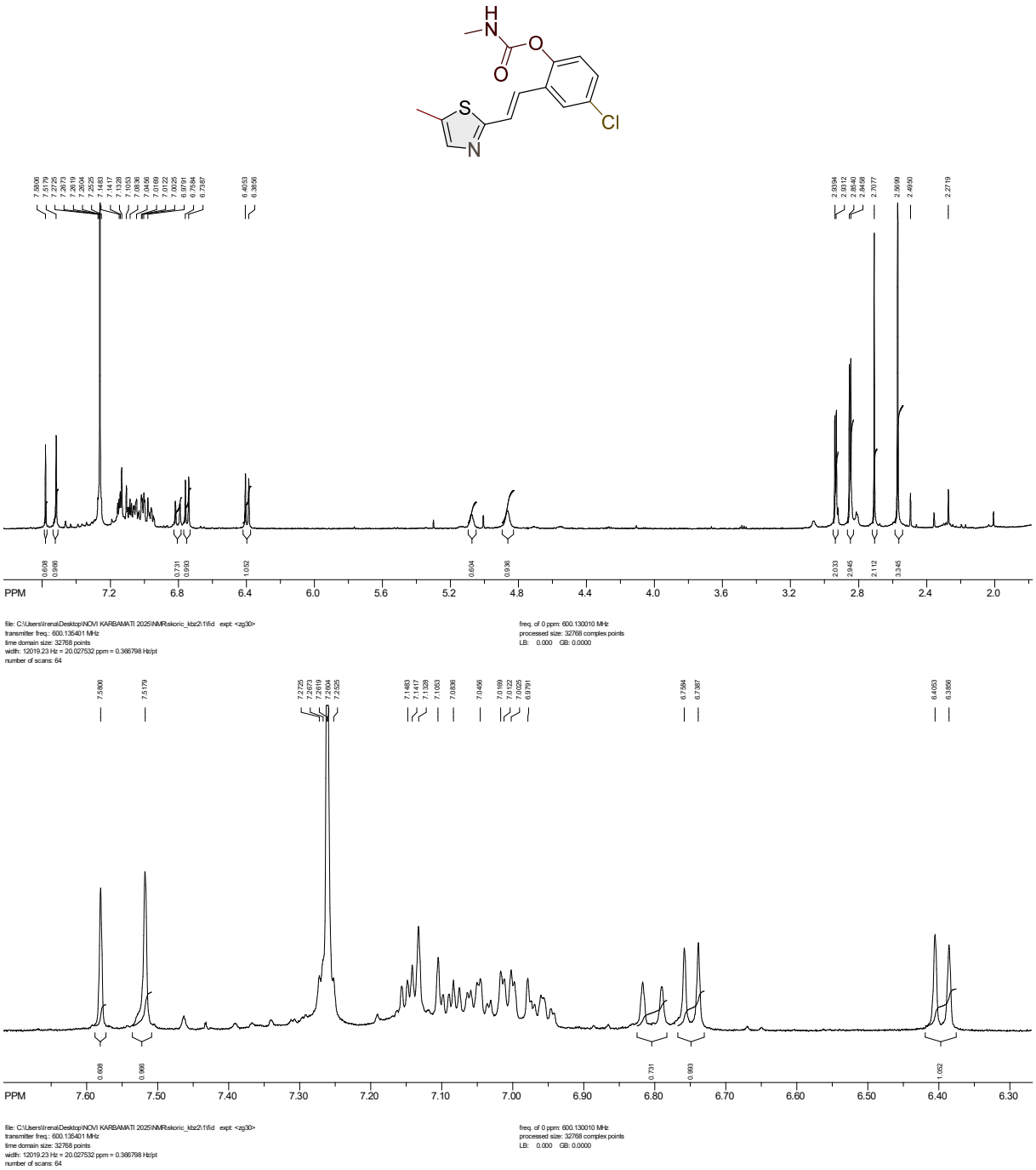


Figure S48. ^{1}H NMR spectrum ($CDCl_3$) of the mixture of (*E*)- and (*Z*)-isomer of carbamate **8**.

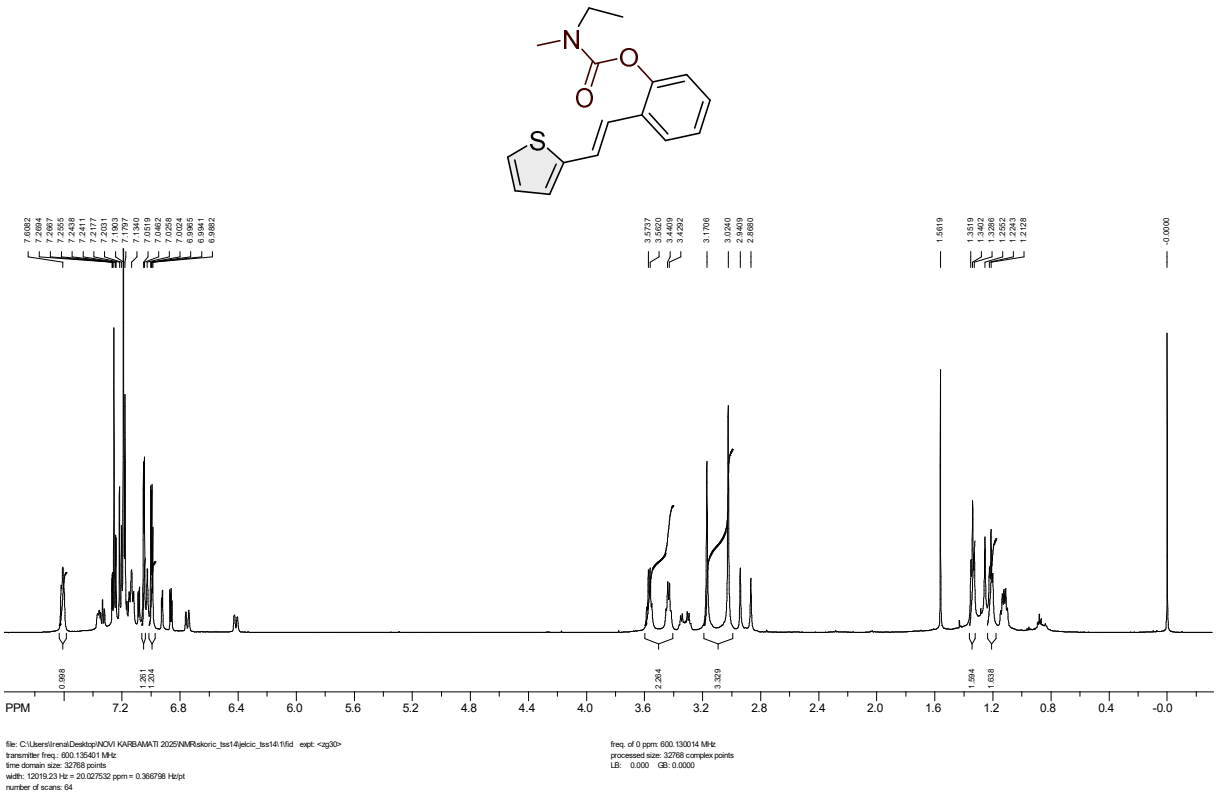


Figure S49. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **9** (with small amount of (*Z*)-isomer).

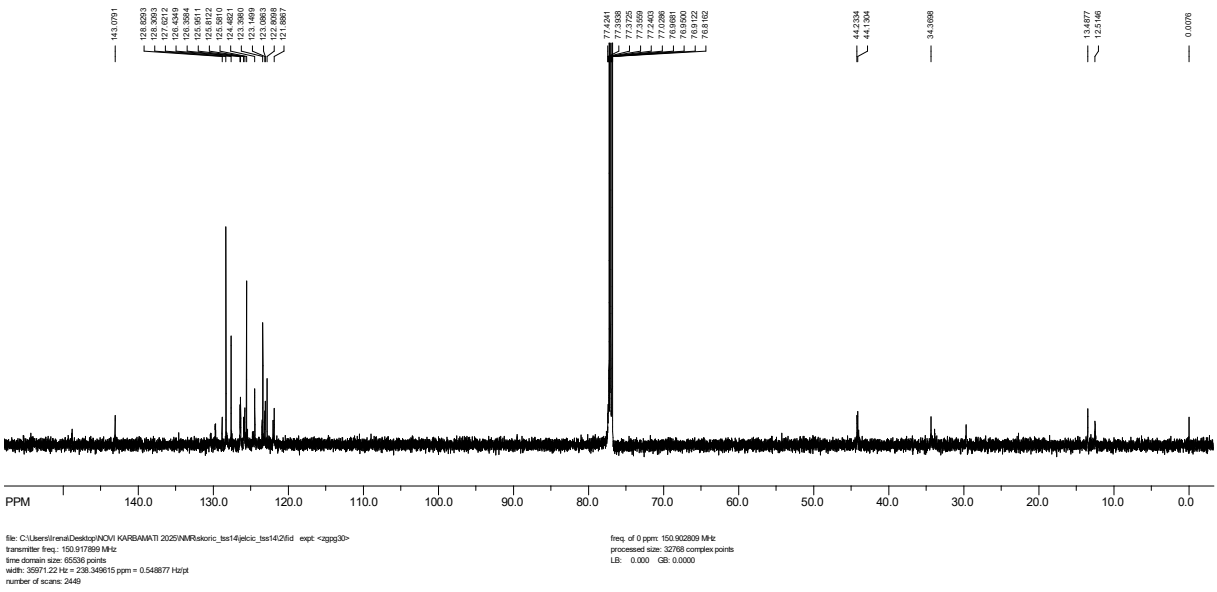


Figure S50. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **9**.

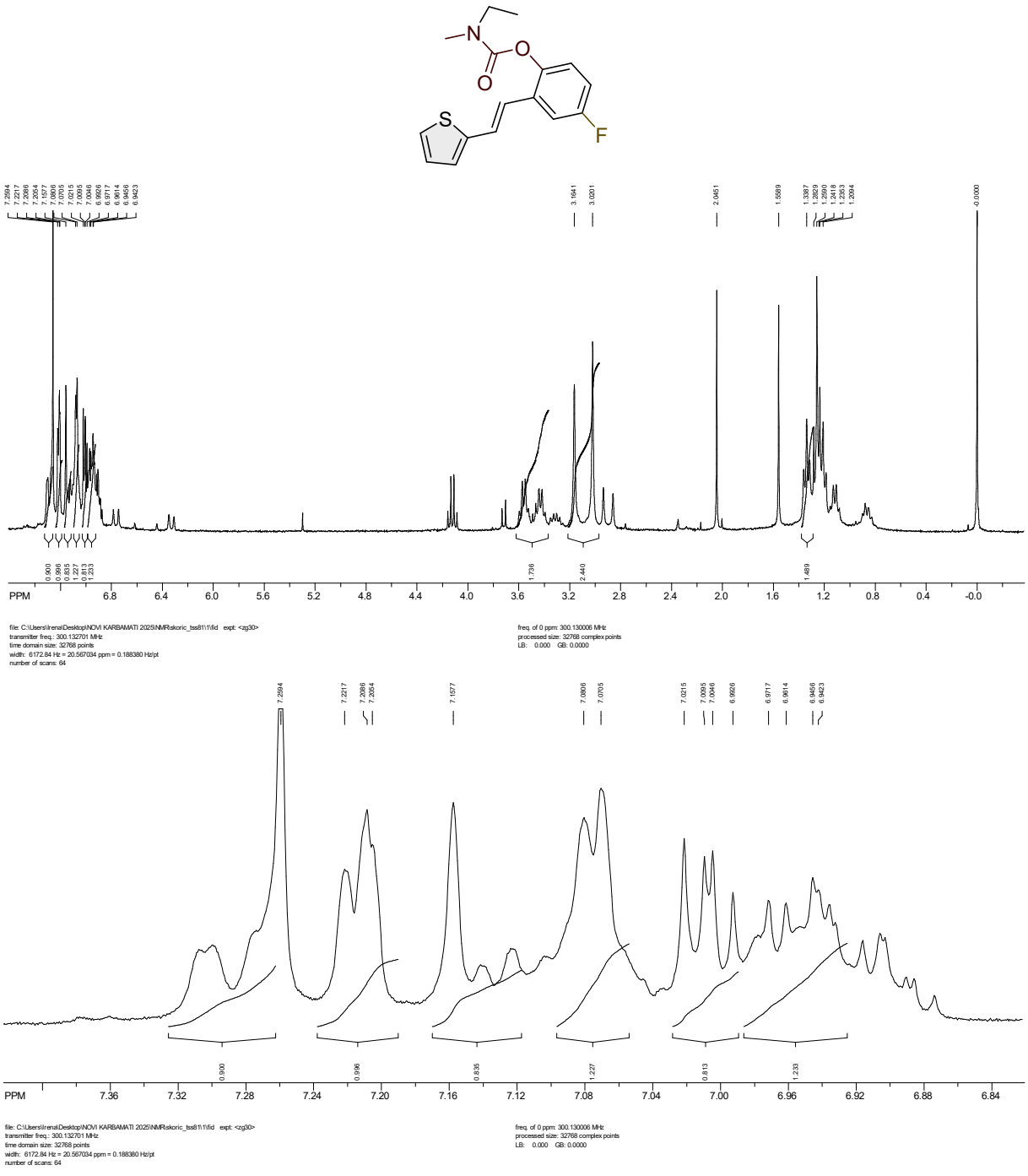


Figure S51. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **10**.

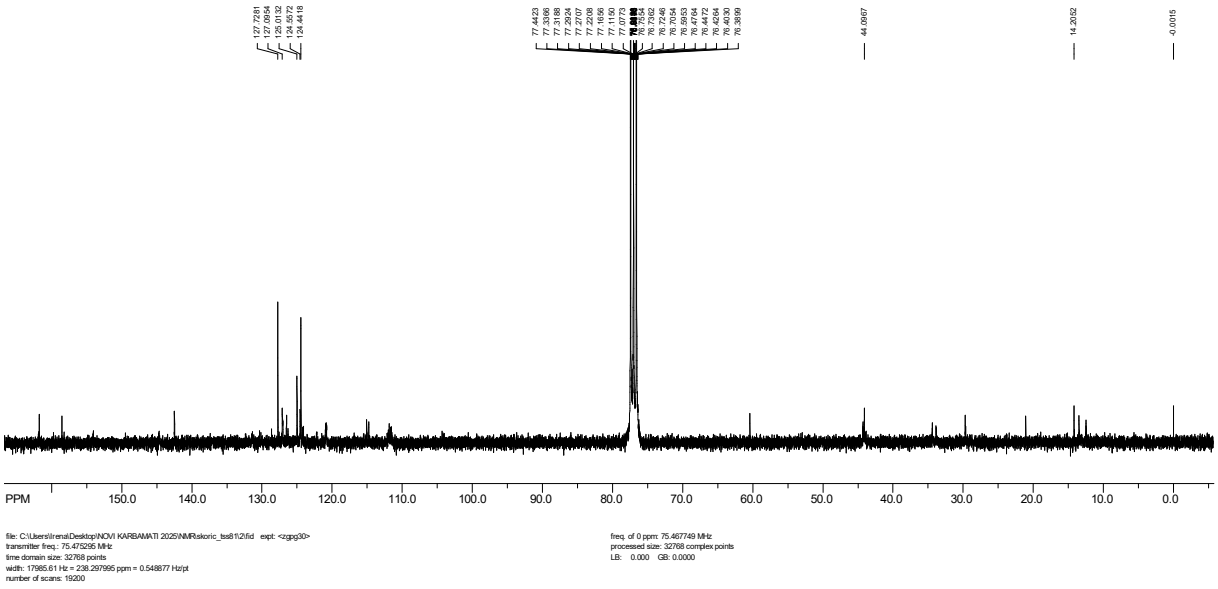


Figure S52. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **10**.

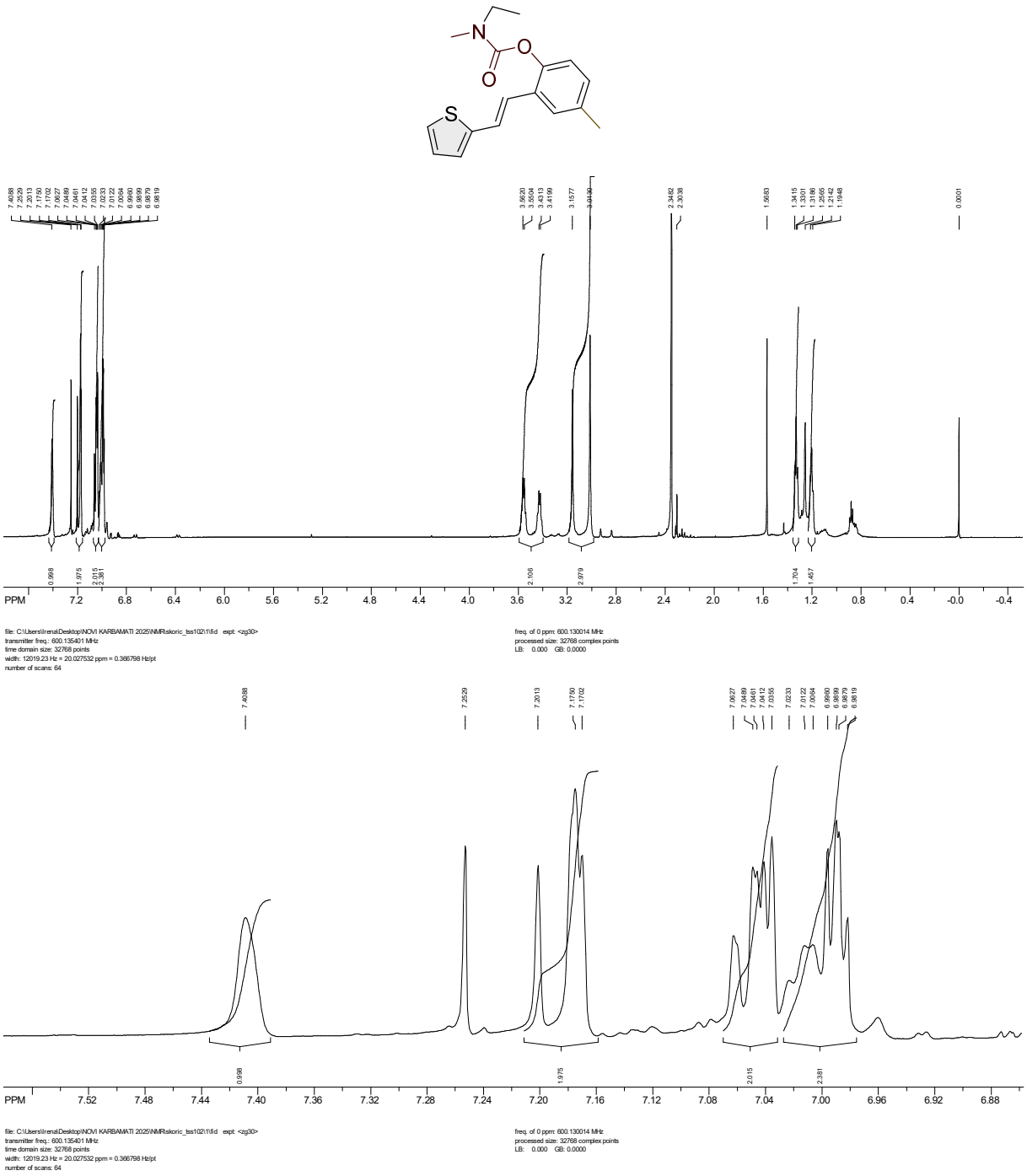


Figure S53. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **11**.

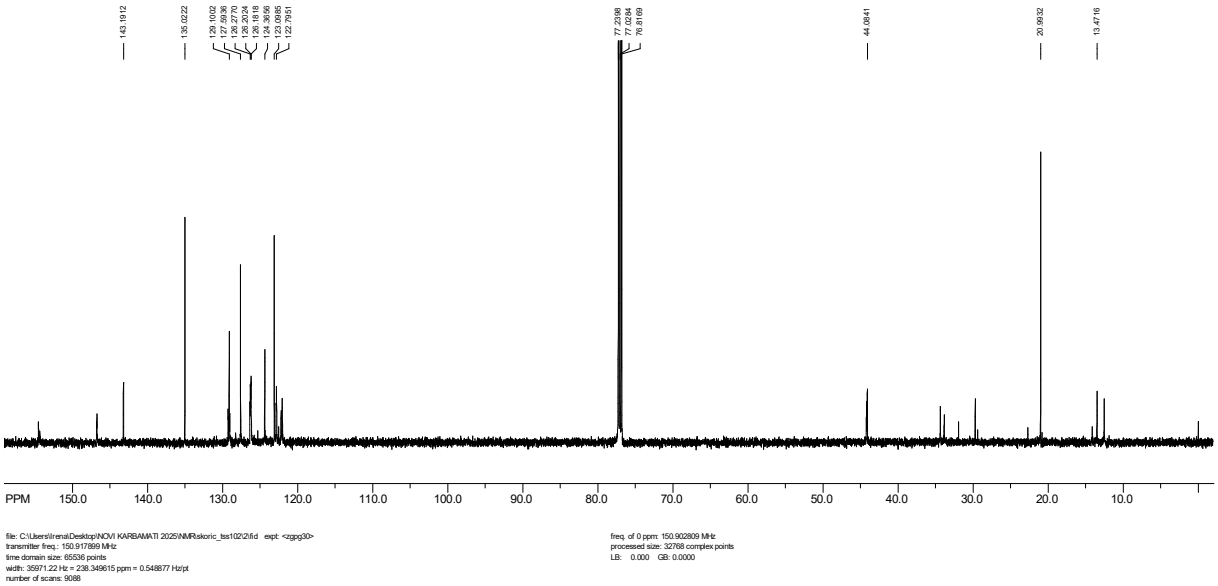


Figure S54. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **11**.

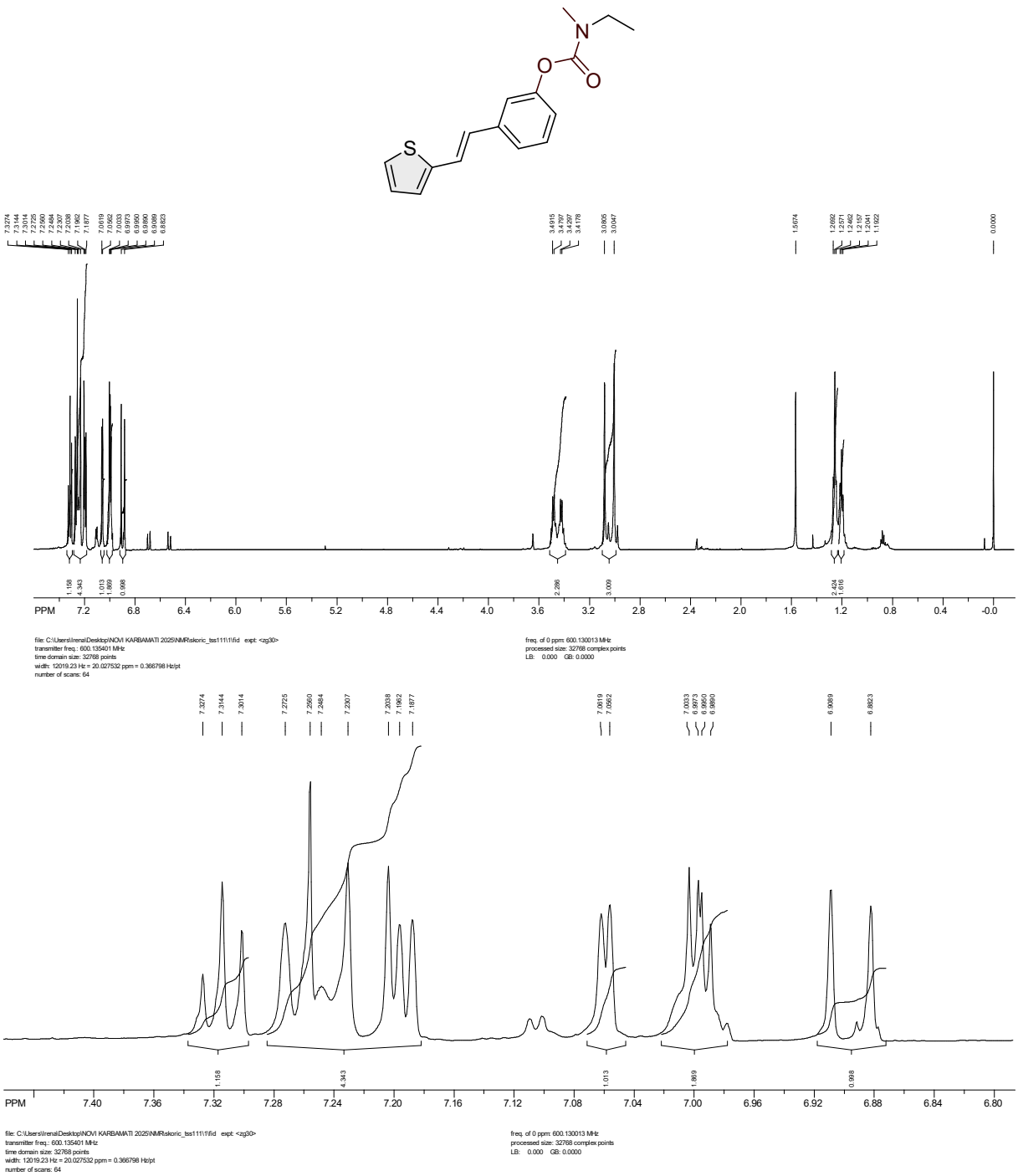


Figure S55. ^{1}H NMR spectrum ($CDCl_3$) of carbamate 1**2**.

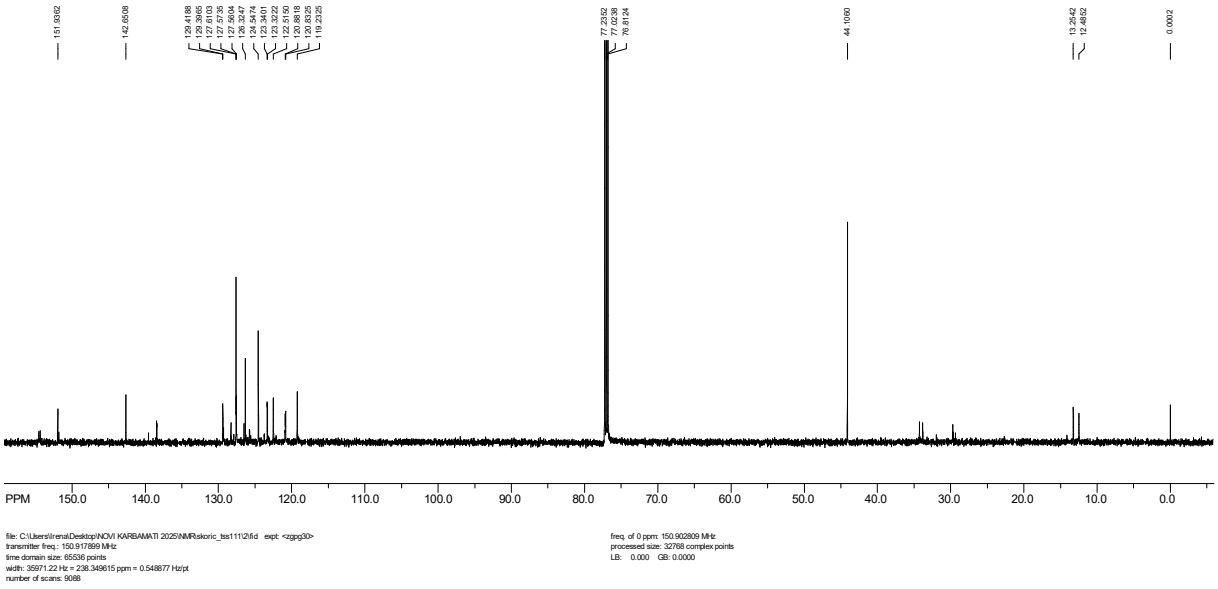


Figure S56. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **12**.

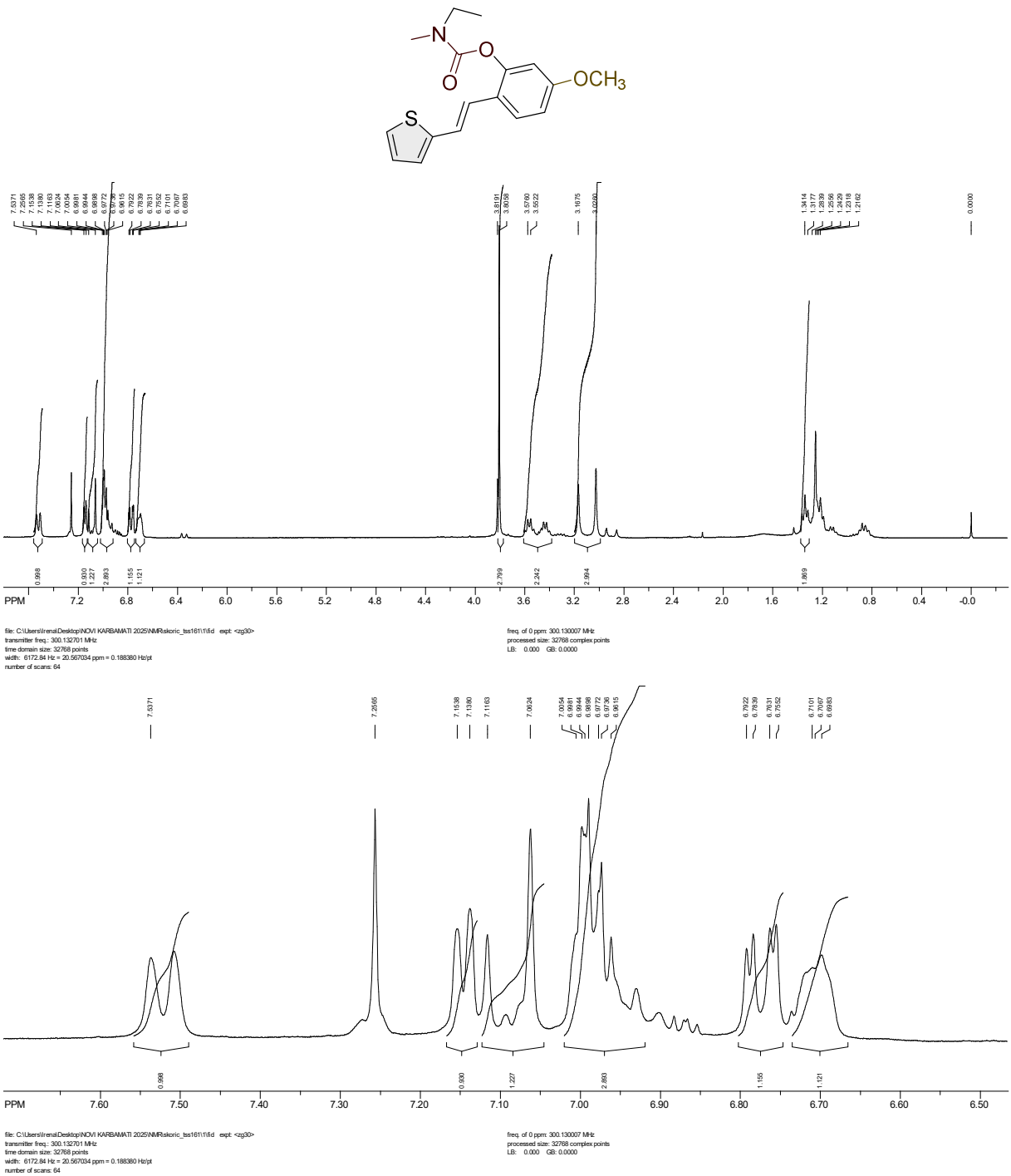


Figure S57. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **13**.

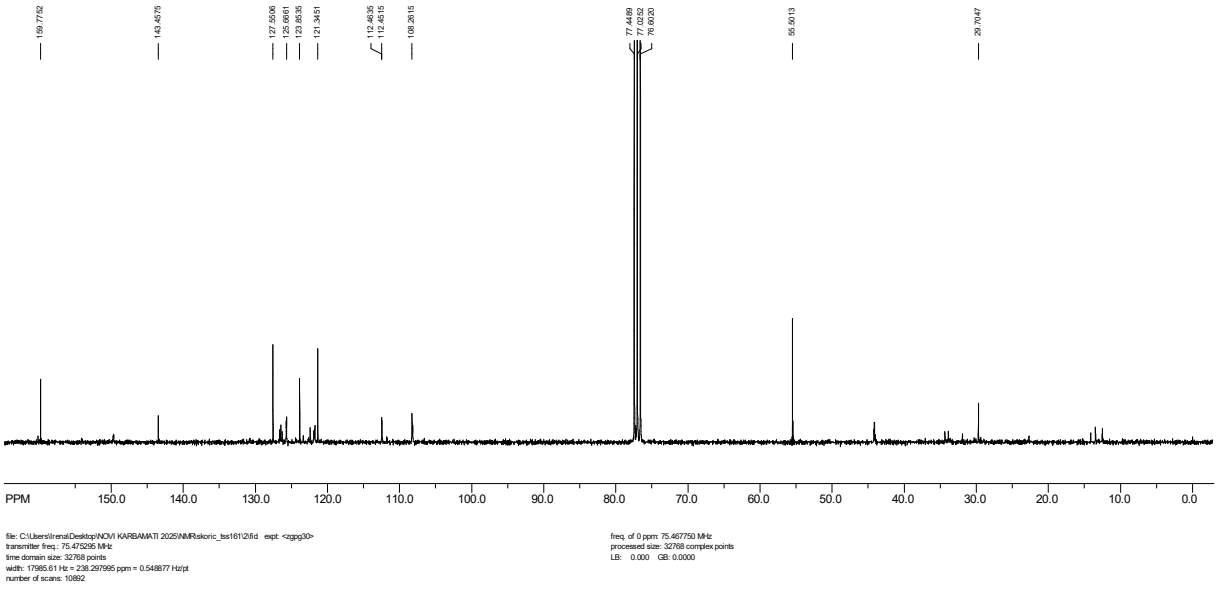


Figure S58. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **13**.

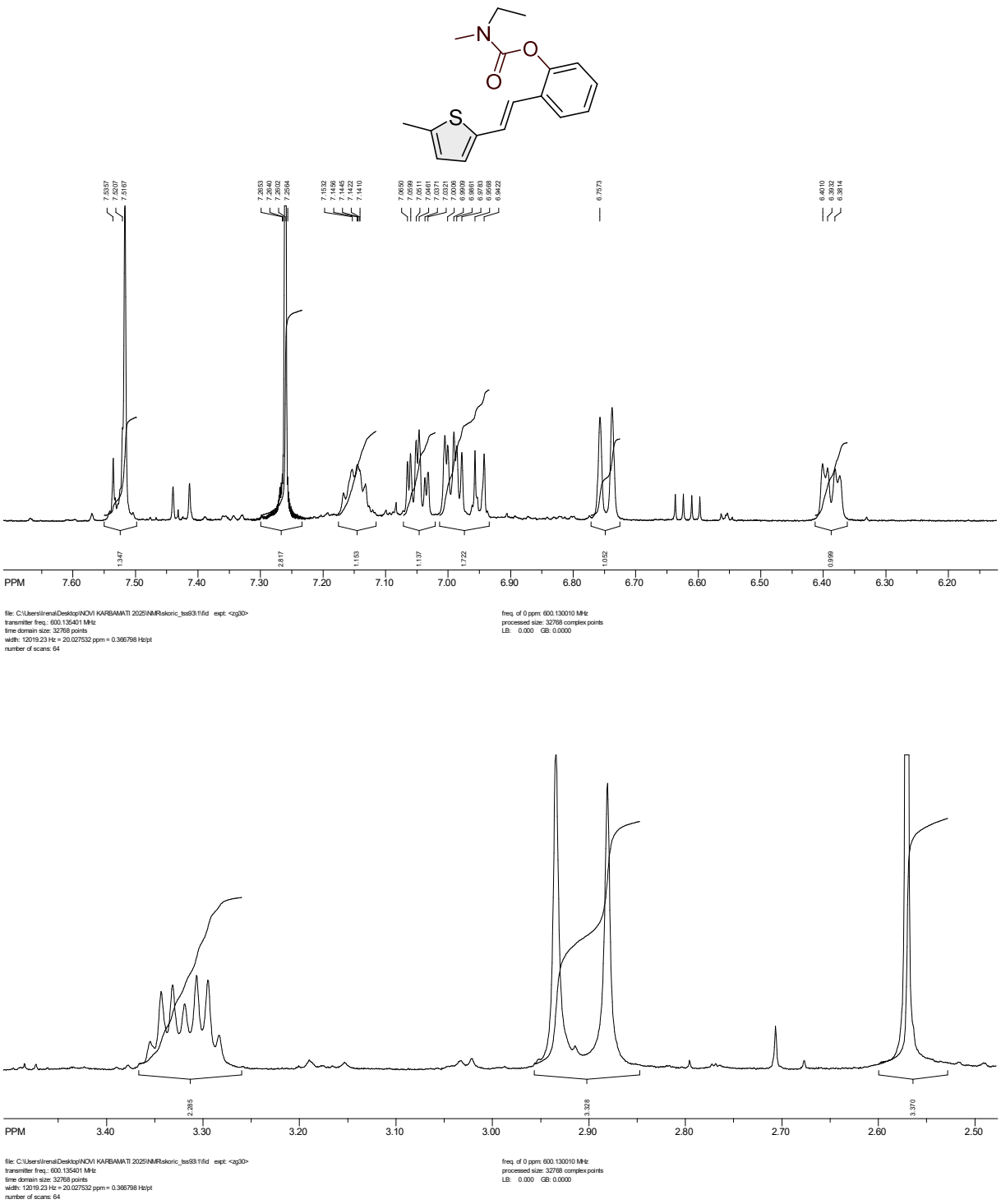


Figure S59. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **14**.

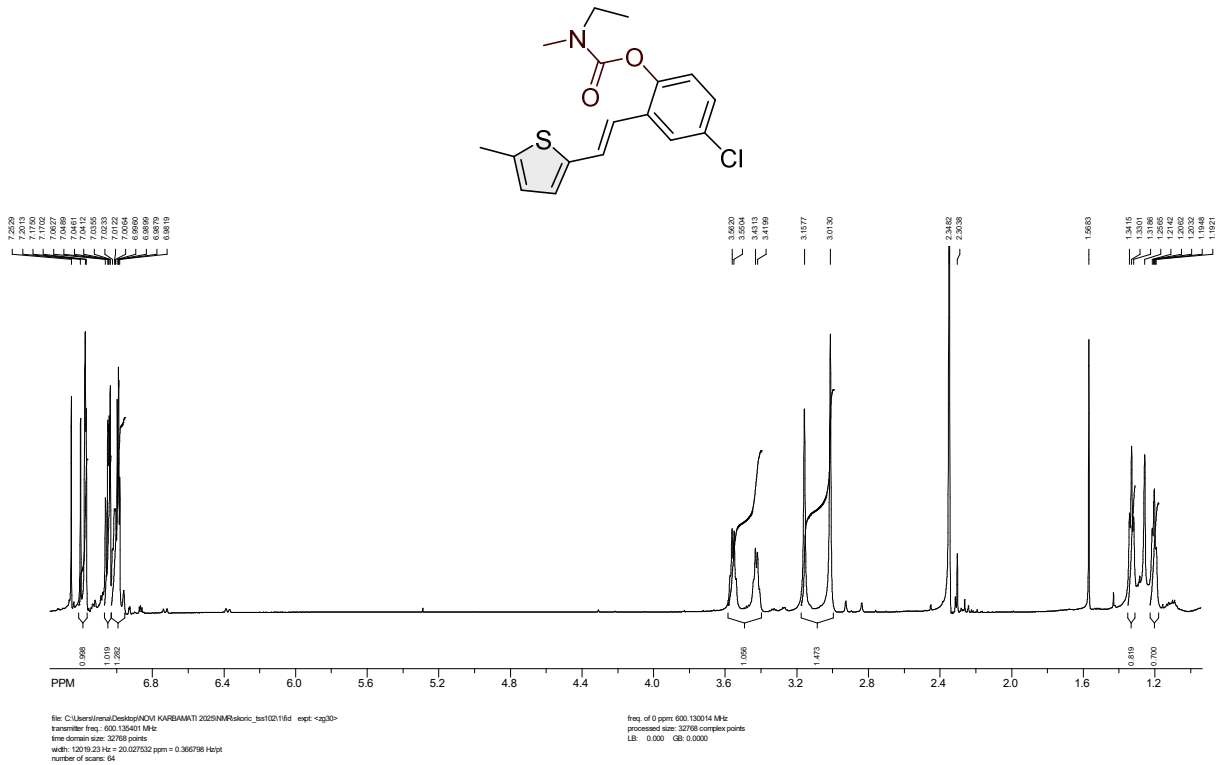


Figure S60. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **15**.

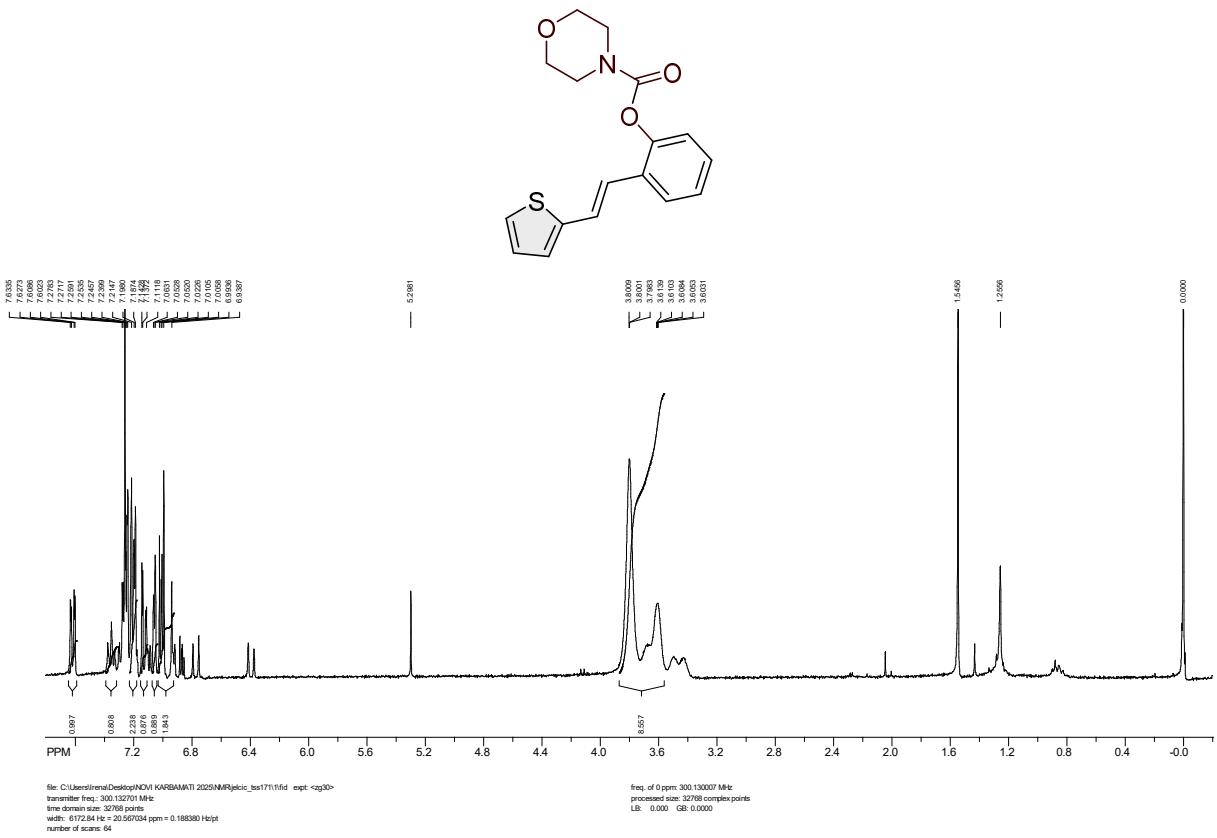


Figure S61. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **16** (with a small proportion of (*Z*)-isomer).

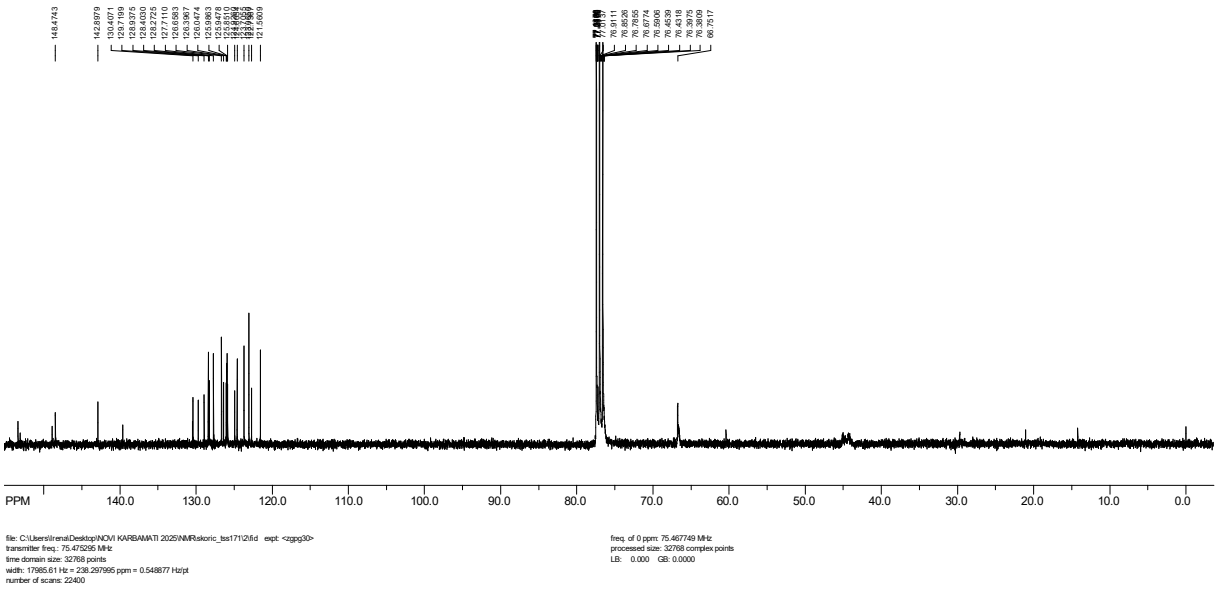


Figure S62. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **16**.

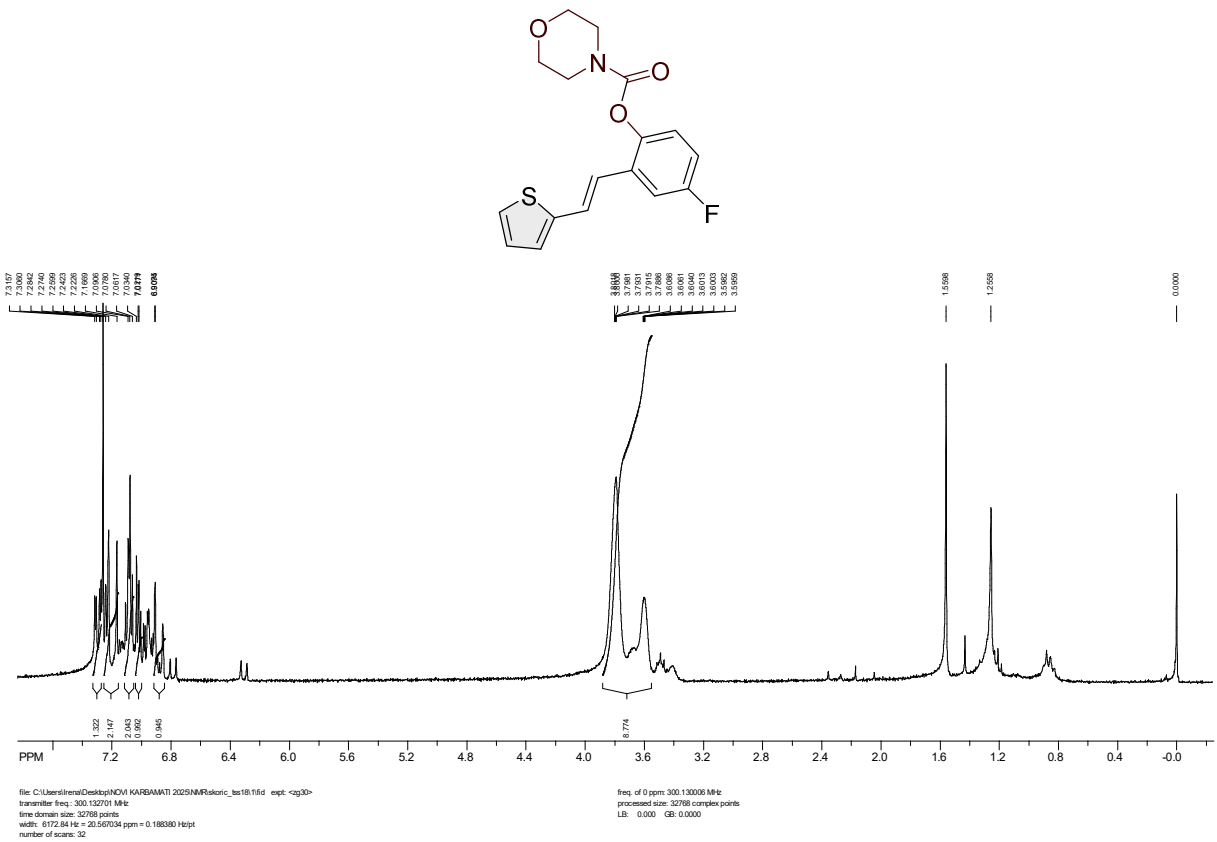


Figure S63. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **17** (with a small proportion of (*Z*)-isomer).

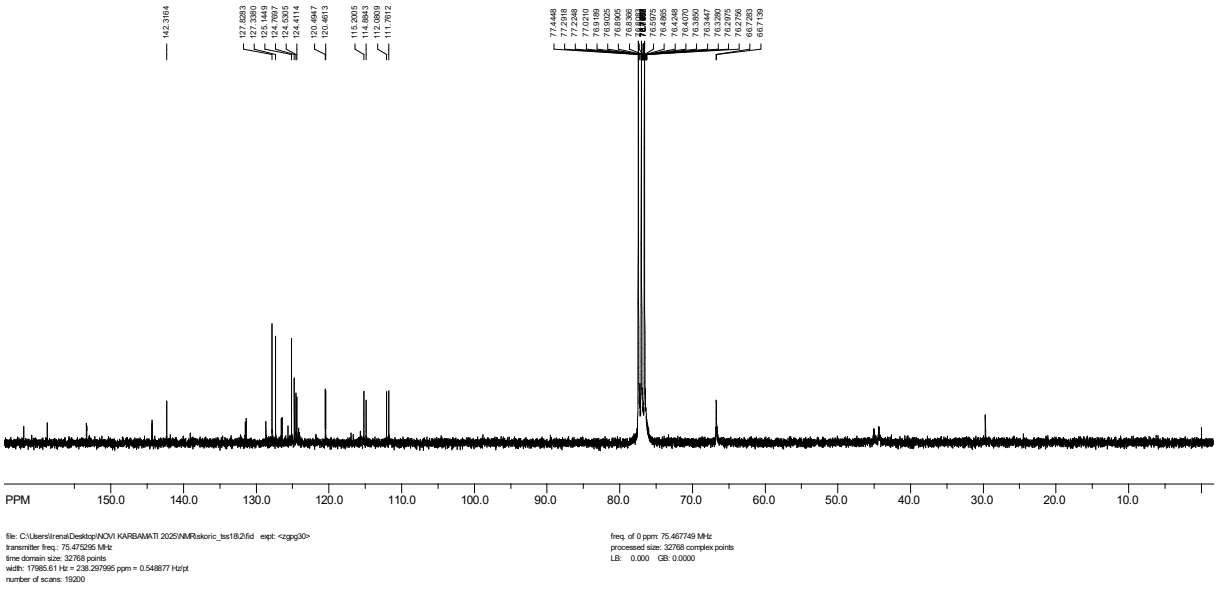


Figure S64. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **17**.

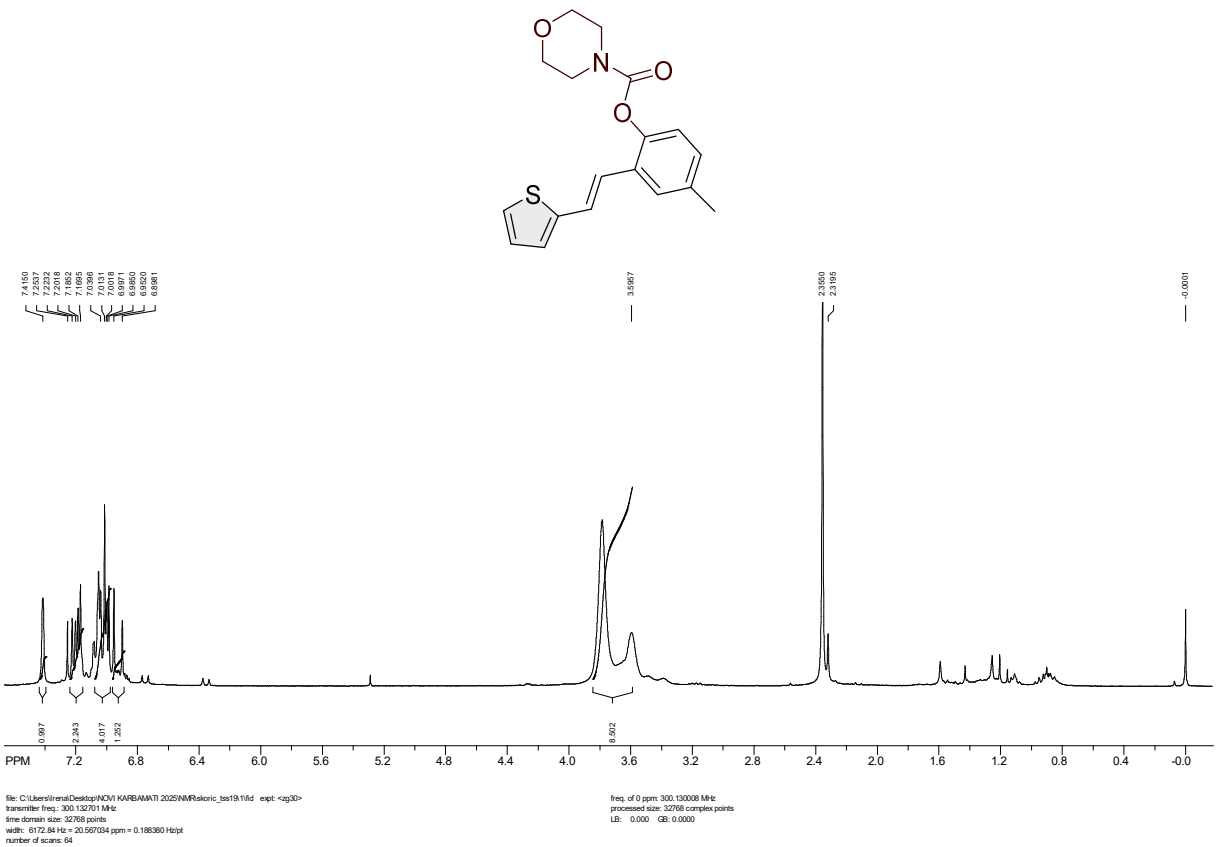


Figure S65. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **18**.

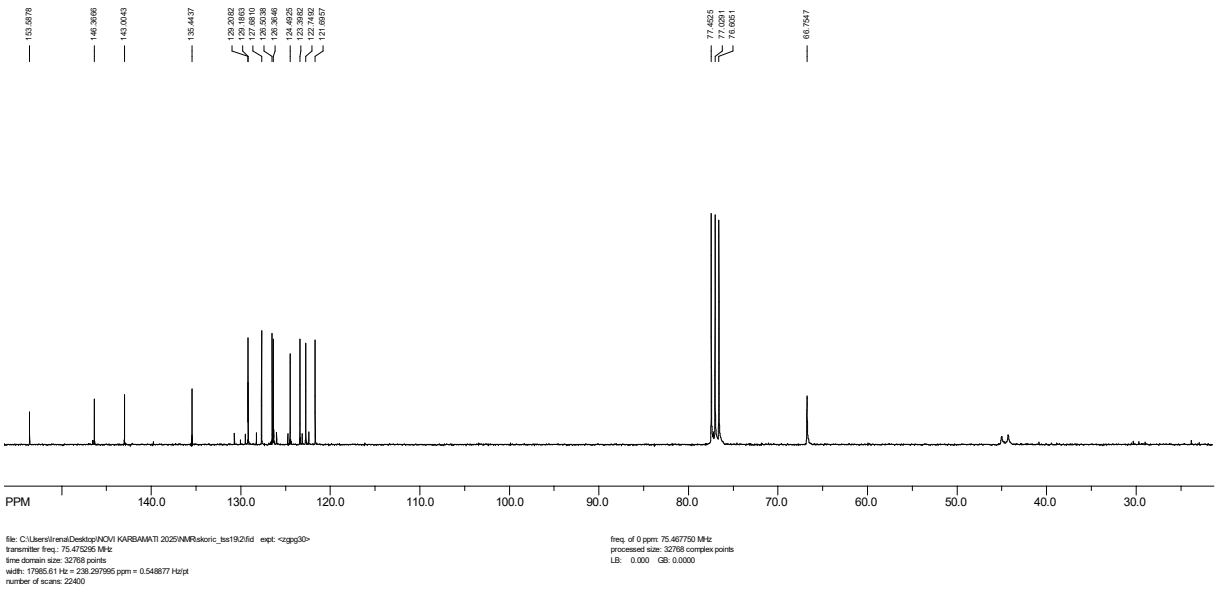


Figure S66. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **18**.

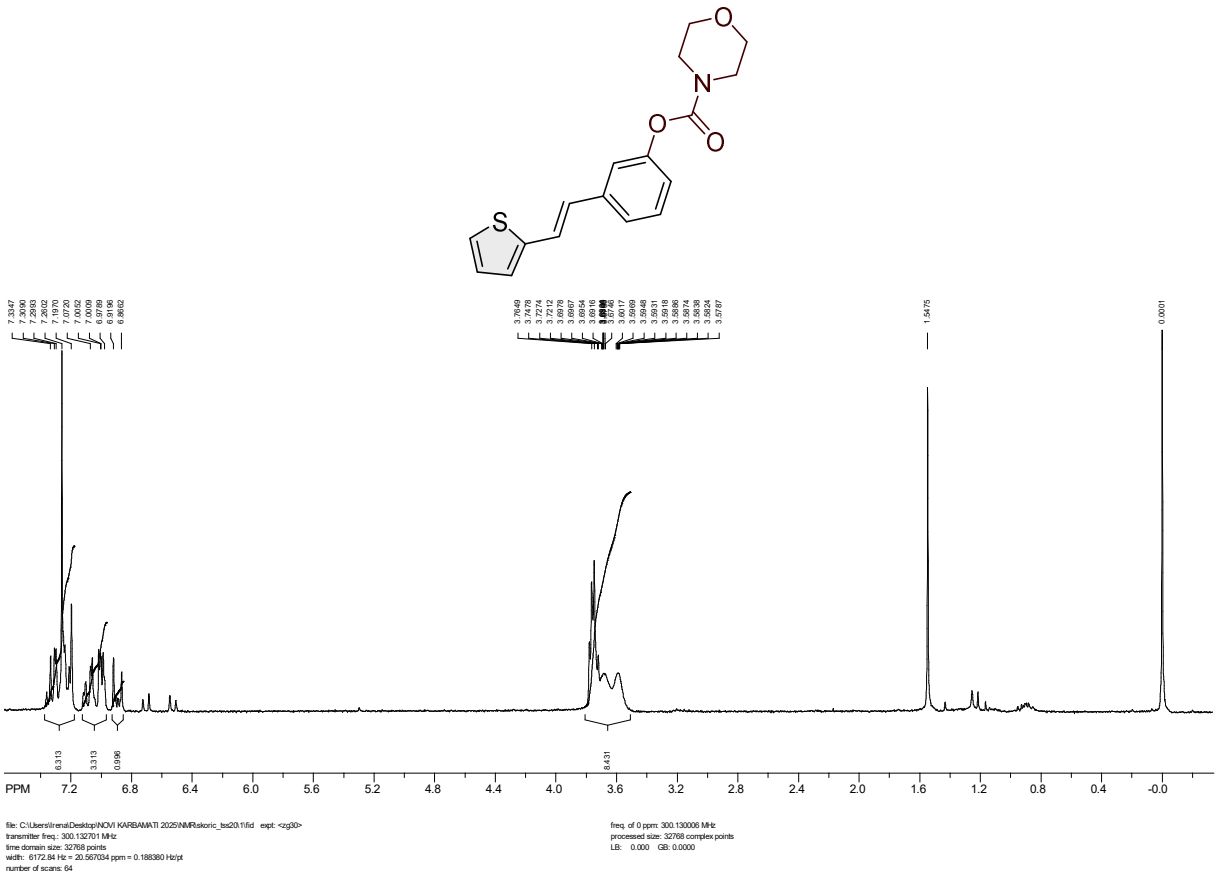


Figure S67. ^{1}H NMR spectrum ($CDCl_3$) of carbamate **19** (with a small proportion of (*Z*)-isomer).

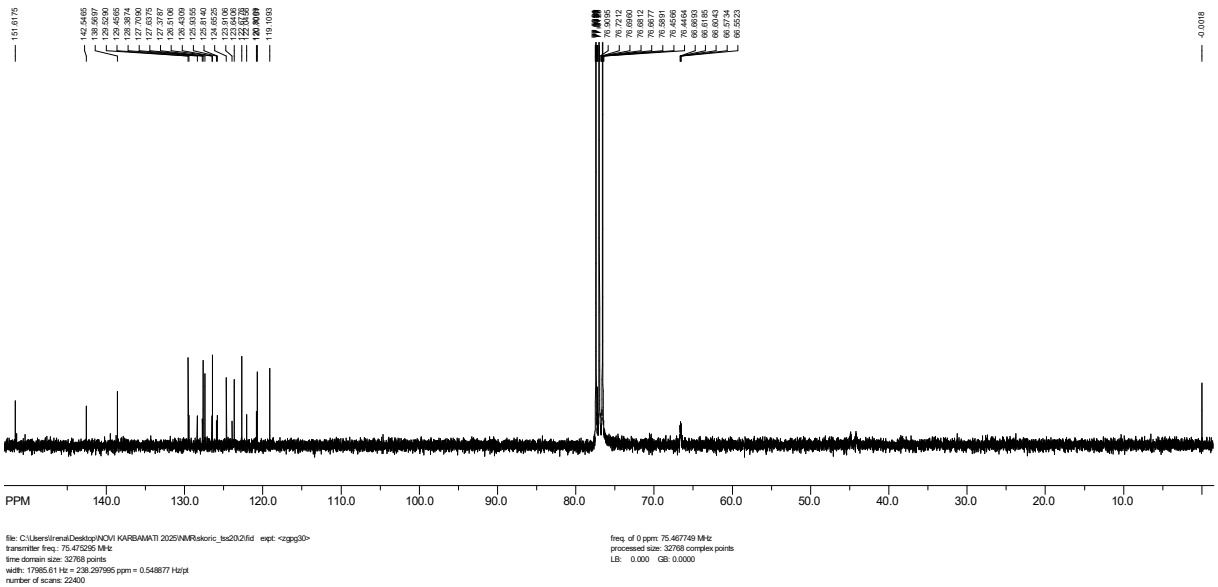


Figure S68. ^{13}C NMR spectrum ($CDCl_3$) of carbamate **19**.

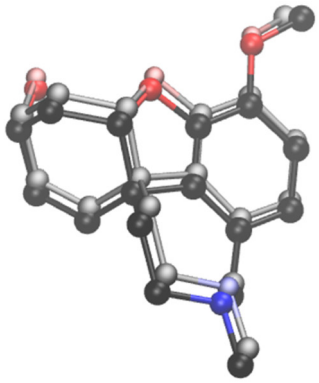


Figure S69. The docking protocol was validated as a cross-docking of galantamine into the active site of AChE, using the crystal structure of the AChE-galantamine complex (PDB ID: 1QTI) as a reference. Heavy atom RMSD between predicted pose and experimental reference is 0.304 A. Heavy atoms of the crystal structure are presented in black (C), red (O), and blue (N), while pale colors depict heavy atoms of the docked ligand.

Cartesian coordinates of ligands docked into the active site of BChE

Compound **1**, ligand pose in the most stable complex

C	-22.017	-7.574	41.381
H	-21.936	-6.529	41.726
C	-20.957	-8.285	40.872
S	-21.460	-9.889	40.428
C	-23.078	-9.585	40.937
H	-23.893	-10.326	40.879
C	-23.232	-8.318	41.420
H	-24.187	-7.916	41.797
C	-19.595	-7.807	40.709
H	-19.443	-6.714	40.705
C	-18.503	-8.577	40.563
H	-18.643	-9.671	40.588
C	-17.139	-8.071	40.373
C	-16.812	-6.711	40.484
H	-17.606	-5.982	40.717
C	-15.509	-6.264	40.307
H	-15.275	-5.191	40.406
C	-14.495	-7.175	40.004
H	-13.460	-6.821	39.863
C	-14.789	-8.528	39.880
H	-13.992	-9.253	39.642
C	-16.099	-8.957	40.059
O	-16.421	-10.306	40.026
C	-17.001	-10.829	38.898
O	-18.088	-10.517	38.471
N	-16.159	-11.734	38.348
H	-15.316	-11.956	38.855
C	-16.563	-12.527	37.203
H	-15.869	-13.273	36.750
H	-16.897	-11.830	36.399
H	-17.514	-13.050	37.461

Compound **1**, the pose of the ligand in the most populated conformational cluster

```
C  -21.815  -7.771  41.436
H  -21.339  -6.873  41.864
C  -21.132  -8.708  40.699
S  -22.205  -9.977  40.187
C  -23.549  -9.237  40.972
H  -24.570  -9.654  40.972
C  -23.199  -8.073  41.594
H  -23.906  -7.439  42.155
C  -19.717  -8.685  40.371
H  -19.362  -9.417  39.626
C  -18.802  -7.852  40.894
H  -19.152  -7.102  41.623
C  -17.372  -7.865  40.568
C  -16.635  -6.692  40.342
H  -17.143  -5.715  40.403
C  -15.280  -6.737  40.043
H  -14.721  -5.801  39.875
C  -14.625  -7.967  39.955
H  -13.548  -8.003  39.719
C  -15.330  -9.147  40.165
H  -14.819 -10.122  40.097
C  -16.686  -9.083  40.462
O  -17.408 -10.230  40.761
C  -17.420 -10.698  42.050
O  -16.437 -10.870  42.732
N  -18.700 -10.923  42.426
H  -19.418 -10.813  41.727
C  -19.003 -11.529  43.708
H  -20.058 -11.714  44.018
H  -18.509 -10.924  44.503
H  -18.447 -12.493  43.777
```

Compound **16**, ligand pose in the most stable complex

```
C  -18.786  -8.376  41.153
H  -19.607  -7.734  40.792
C  -19.048  -9.442  42.005
C  -18.034 -10.291  42.475
C  -16.721 -10.011  42.068
H  -15.896 -10.648  42.428
C  -16.440  -8.948  41.221
H  -15.400  -8.749  40.911
C  -17.475  -8.130  40.762
H  -17.251  -7.286  40.087
O  -20.342  -9.791  42.365
C  -21.096 -10.573  41.535
N  -21.958  -9.806  40.813
C  -22.732 -10.451  39.757
H  -22.816 -11.553  39.905
H  -22.191 -10.465  38.782
C  -21.866  -8.353  40.701
H  -21.310  -7.895  41.552
```

```
H  -21.184  -8.032  39.880
C  -24.092  -9.774  39.656
H  -24.724 -10.238  38.863
H  -24.732 -10.004  40.540
O  -23.953  -8.379  39.462
C  -23.271  -7.782  40.548
H  -23.853  -7.869  41.495
H  -23.254  -6.671  40.459
O  -21.011 -11.780  41.476
C  -18.371 -11.413  43.359
H  -19.360 -11.884  43.231
C  -17.565 -11.910  44.312
H  -16.571 -11.445  44.424
C  -17.891 -13.008  45.208
C  -18.681 -14.093  44.916
H  -19.160 -14.247  43.935
C  -18.820 -15.000  46.007
H  -19.415 -15.927  45.962
C  -18.133 -14.591  47.113
H  -18.110 -15.146  48.066
S  -17.305 -13.103  46.842
```

Compound **16**, the pose of the ligand in the most populated conformational cluster

```
C   -18.304 -13.244  44.538
H   -17.391 -12.844  45.010
C   -19.108 -12.426  43.752
C   -20.269 -12.900  43.123
C   -20.612 -14.243  43.333
H   -21.526 -14.647  42.866
C   -19.824 -15.074  44.118
H   -20.111 -16.129  44.266
C   -18.668 -14.574  44.720
H   -18.042 -15.236  45.342
O   -18.757 -11.115  43.464
C   -17.564 -10.845  42.852
N   -17.660  -9.712  42.105
C   -16.441  -9.177  41.507
H   -15.903  -8.480  42.191
H   -15.642  -9.948  41.410
C   -18.760  -8.756  42.202
H   -18.593  -7.995  42.999
H   -19.687  -9.215  42.617
C   -16.786  -8.529  40.173
H   -17.066  -9.289  39.407
H   -15.882  -8.110  39.673
O   -17.796  -7.550  40.330
C   -18.986  -8.125  40.833
H   -19.820  -7.385  40.856
H   -19.424  -8.855  40.113
O   -16.575 -11.538  42.954
C   -21.070 -11.995  42.290
H   -21.072 -12.173  41.201
C   -21.800 -10.967  42.752
H   -21.805 -10.803  43.843
C   -22.584 -10.051  41.939
C   -22.200  -8.802  41.515
```

```
H   -21.220  -8.355  41.754
C   -23.194  -8.140  40.735
H   -23.068  -7.131  40.308
C   -24.318  -8.897  40.578
H   -25.207  -8.571  40.012
S   -24.184 -10.423  41.369
```

Galantamine

```
C  -22.383 -10.415  42.154 H  -21.530 -10.579  42.833
C  -23.576 -10.869  42.537
H  -23.638 -11.416  43.493
C  -24.849 -10.682  41.744
H  -25.645 -10.363  42.457
C  -24.690  -9.573  40.705
H  -25.558  -9.544  40.006
H  -24.804  -8.564  41.166
C  -23.380  -9.692  39.954
H  -23.306  -8.856  39.220
C  -22.117  -9.689  40.849
O  -23.347 -10.952  39.238
C  -21.518 -12.259  38.280
C  -20.140 -12.473  38.318
H  -19.688 -13.227  37.651
C  -19.324 -11.745  39.190
H  -18.237 -11.933  39.185
C  -18.946 -10.067  41.044
H  -17.881 -10.351  40.873
H  -19.095 -10.475  42.071
N  -19.082  -8.608  41.057
C  -20.251  -8.147  41.804
H  -20.314  -8.670  42.787
H  -20.099  -7.099  42.153
C  -21.576  -8.270  41.050
H  -22.350  -7.640  41.546
H  -21.496  -7.755  40.064
C  -22.024 -11.286  39.144
C  -21.215 -10.575  40.011
C  -19.839 -10.789  40.065
C  -17.877  -8.022  41.634
H  -17.980  -6.912  41.644
H  -16.951  -8.357  41.112
H  -17.646  -8.435  42.643
O  -25.267 -11.903  41.157
H  -25.086 -11.820  40.206
O  -22.409 -12.901  37.485
C  -21.920 -13.406  36.262
H  -22.653 -13.934  35.608
H  -21.045 -14.070  36.455
H  -21.431 -12.585  35.688
```

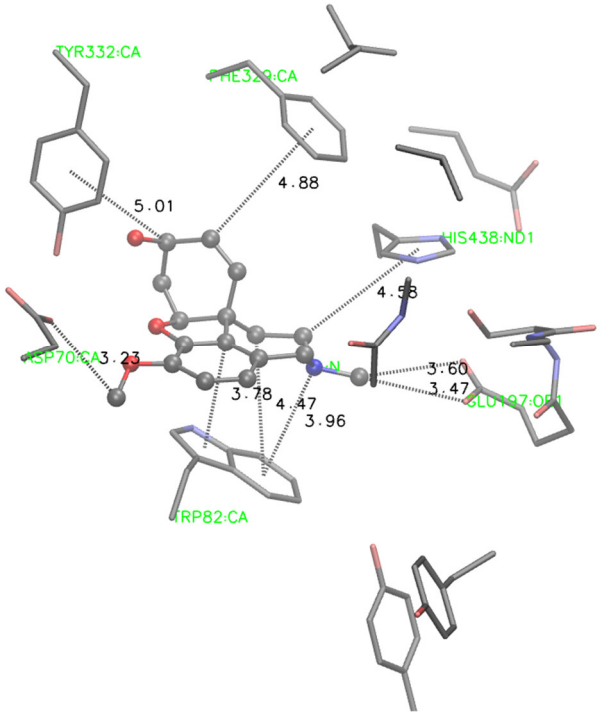


Figure S70. Galantamine docked in the active site of BChE. The ligand is involved in hydrophobic interactions with the choline-binding site (residue Trp82), and an electrostatic interaction between the tertiary amine of galantamine and Glu197. The bicyclic ring system reaches toward the peripheral site (Asp70 and Tyr332), stabilizing the pose via long-range electrostatic and hydrophobic contacts.

Table S1. Free energies of binding, ΔG_{bind}, obtained by molecular docking of ligands **1**, **16,** and reference ligand galantamine into the active site of BChE (7AIY.pdb), along with the number of conformational clusters and distribution of conformations.

Ligand	ΔG_{bind}/kcal mol^{-1}		Number of distinctive conformational clusters	Distribution of conformations within clusters with $n > 1$ (n = cluster population)
	lowest	highest		
1	−4.96	−4.55	5	3, 11, 9
16	−5.44	−5.38	6	6, 7, 5, 5
Galantamine	−5.20	−5.17	3	23

Table S2. Root-mean-square displacement (RMSD), RMS fluctuations of alpha carbons of the protein backbone, and radius of gyration for complexes of BChE and **1** and **16**, derived by molecular dynamics simulation of 40 ns.

Enzyme-ligand complex	RMSD/Å		RMSF/Å		Rg/Å	
	average	min, max	average	min, max	average	min, max
BChE-**1**(I)	2.02	0.78, 2.37	0.76	0.33, 2.62	23.14	22.86, 23.33
BChE-**1**(II)	2.05	0.80, 2.45	0.84	0.37, 4.65	23.04	22.86, 23.27
BChE-**16**(I)	1.93	0.87, 2.23	0.78	0.35, 3.89	22.99	22.78, 23.23
BChE-**16**(II)	2.17	0.89, 2.59	0.87	0.39, 2.65	23.11	22.81, 23.34