SUPPORTING INFORMATION

Spectroscopic and Computational Study of the Protonation Equilibria of Amino-Substituted benzo[*b*]thieno[2,3-*b*]pyrido[1,2-*a*]benzimidazoles as Novel pH-Sensing Materi-als

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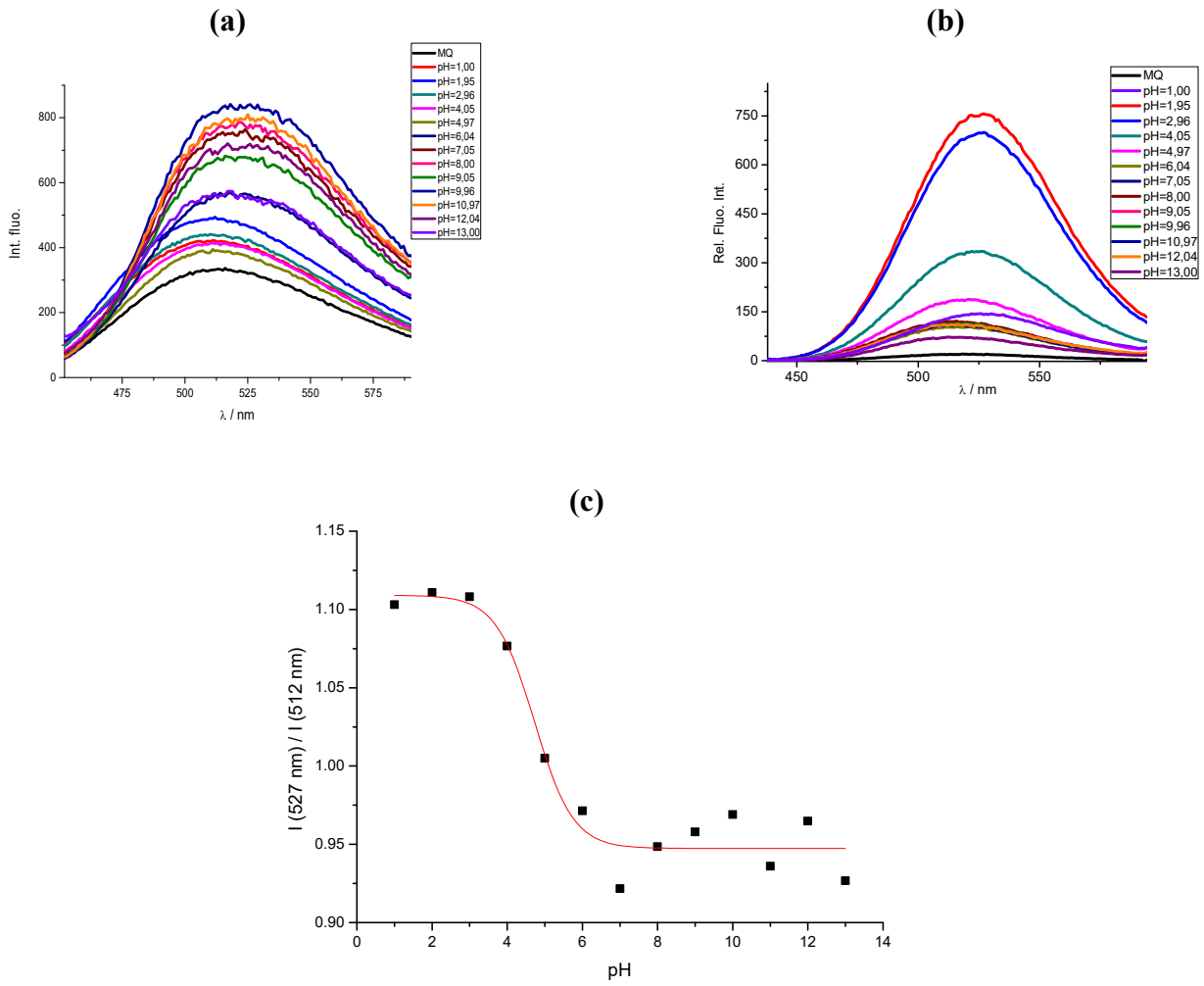
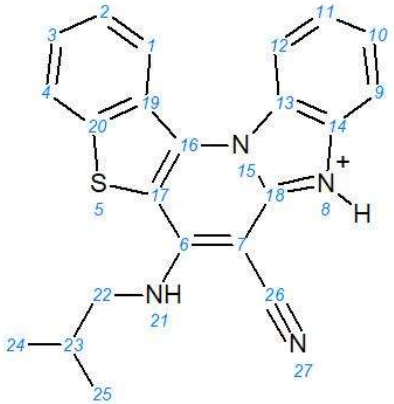


Figure S1. Emission spectra of **5** (a) and **6** (b) at different pH values in buffer (λ_{exc} = 310nm for **5**, λ_{exc} = 308 nm for **6**). (c) Ratiometric fluorescence pH titration curve for **6** from which its pK_a value was obtained.



Shift1 (ppm)	H's	Type	J (Hz)	Atom1	Multiplet1
0.981	6.000	d	6.6	24, 25	M11
2.132	1.000	spt	6.9	23	M10
3.664	2.000	d	7.3	22<'>, 22<''>	M09
7.565	1.000	ddd	8.5, 7.2, 1.0	11	M08
7.681	1.000	t	7.7	10	M07
7.723	1.000	ddd	8.0, 7.2, 1.0	2	M06
7.787	1.000	ddd	8.0, 7.2, 1.0	3	M05
7.816	1.000	d	8.1	9	M04
8.328	1.000	d	8.1	4	M03
8.365	1.000	d	8.4	12	M02
8.624	1.000	d	8.4	1	M01

Solvent	DMSO-d6
Pulse Sequence	zg30
Temperature (degree C)	25.000
Acquisition Time (sec)	1.3631
Number of Transients	32
Points Count	32768
Sweep Width (Hz)	12018.86
Spectrum Offset (Hz)	5399.9355
Original Points Count	16384
Nucleus	1H
Frequency (MHz)	600.1300

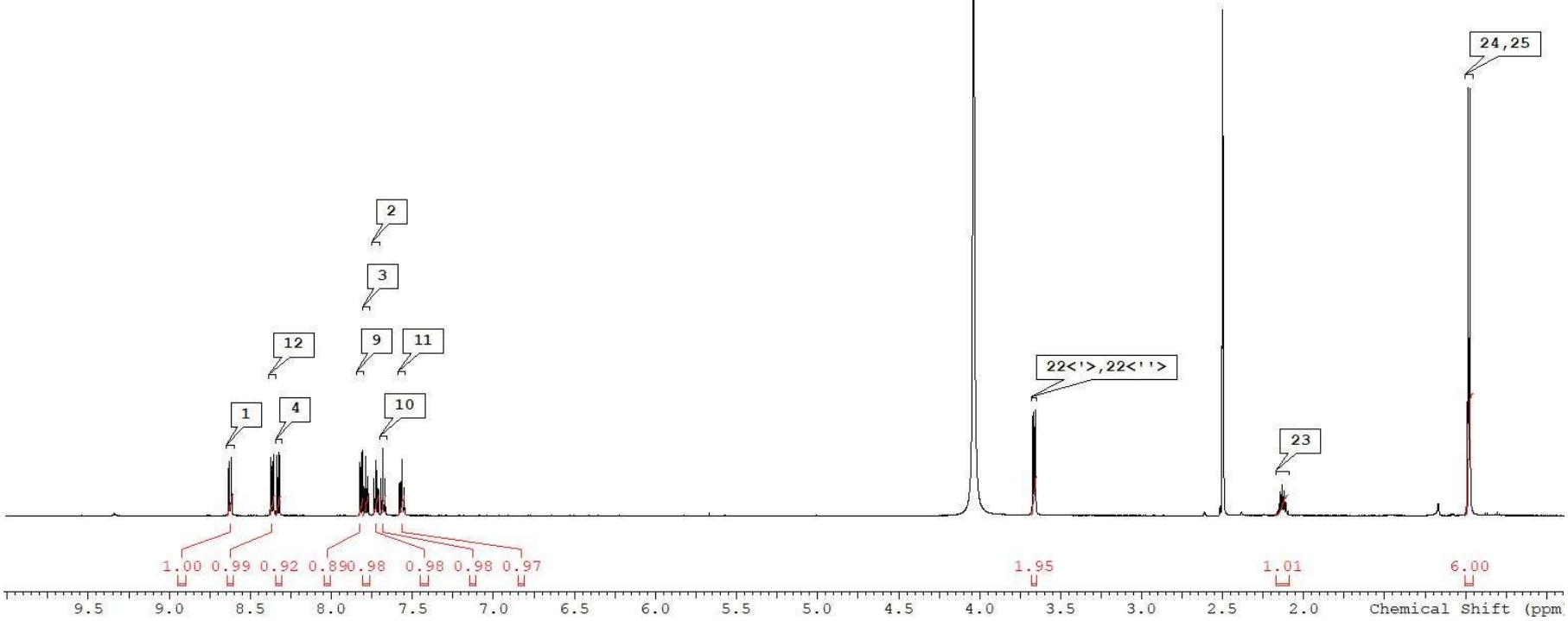
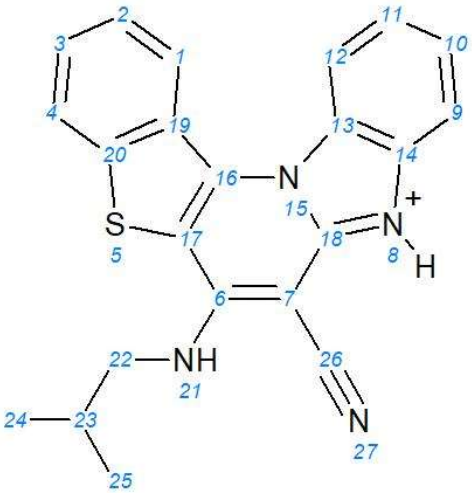


Figure S2. ^{1}H spectrum of **5** in 90% DMSO-d_6 / 10% D_2O at 25 °C, pH 2.4



Atom	Exp. Shift (ppm)	Atom	Exp. Shift (ppm)
24	19.67	3	129.95
25	19.67	14	132.40
23	28.99	16	133.23
22	51.17	20	139.70
7	68.55	18	148.52
9	113.90	6	150.71
17	115.09		
12	115.97		
26	120.70		
11	123.80		
4	124.68		
1	125.10		
2	126.27		
10	127.78		
19	128.45		
13	128.47		

Solvent	**DMSO-d6**
Pulse Sequence	**deptqgpsp.2**
Temperature (degree C)	**25.000**
Acquisition Time (sec)	**0.5999**
Number of Transients	**8192**
Points Count	**32768**
Sweep Width (Hz)	**39368.88**
Spectrum Offset (Hz)	**15083.2031**
Original Points Count	**23619**
Nucleus	**13C**
Frequency (MHz)	**150.9029**

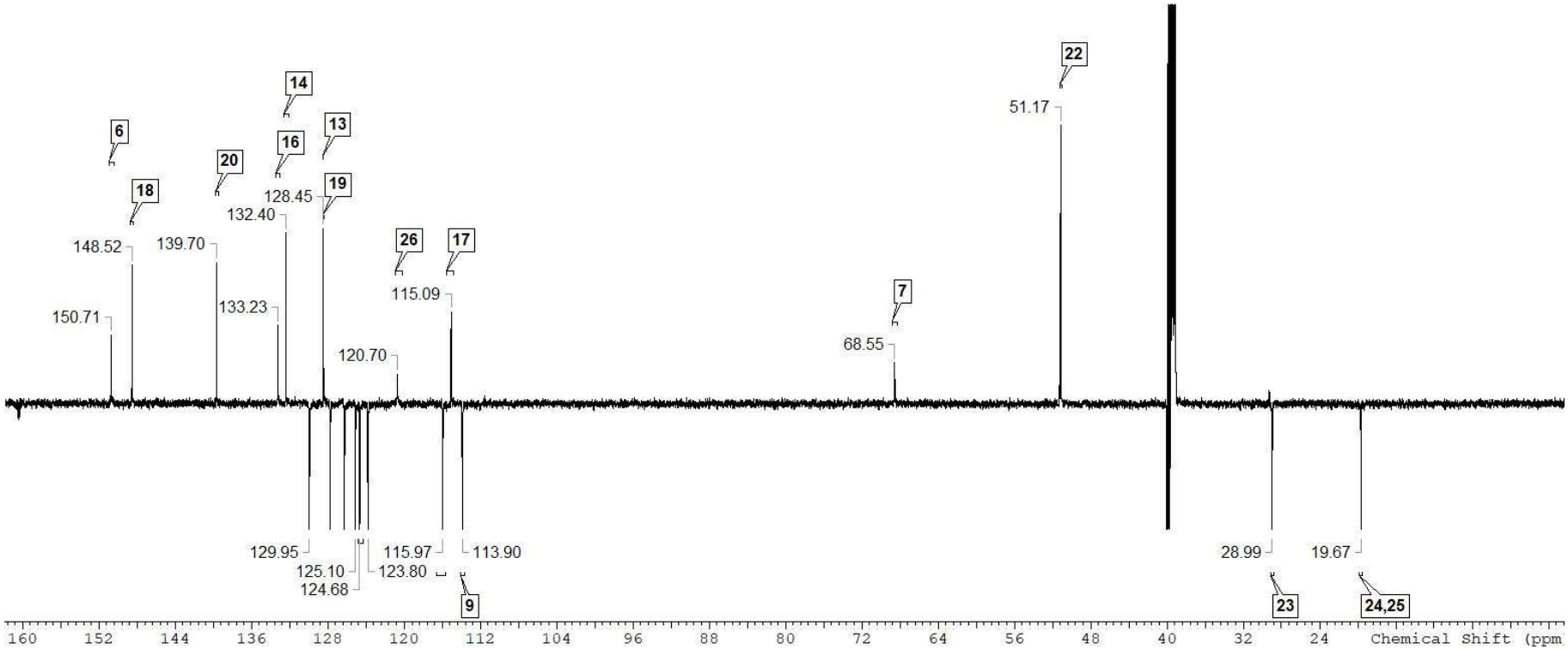


Figure S3. ^{13}C spectrum of **5** in 90% DMSO-d_6 / 10% D_2O at 25 °C, pH 2.4

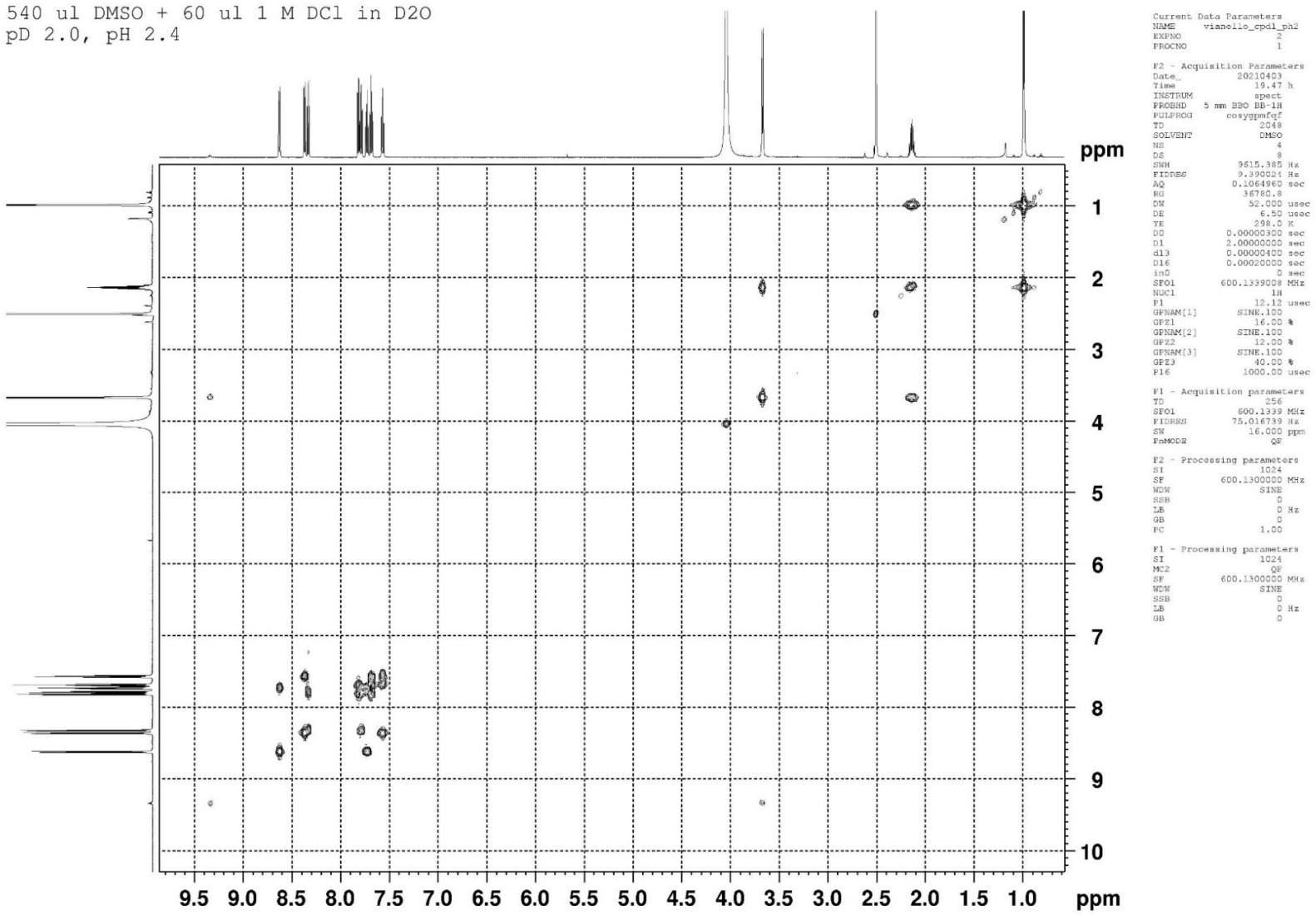


Figure S4. COSY spectrum **5** in 90% DMSO-d_6 / 10% D_2O at 25 °C, pH 2.4

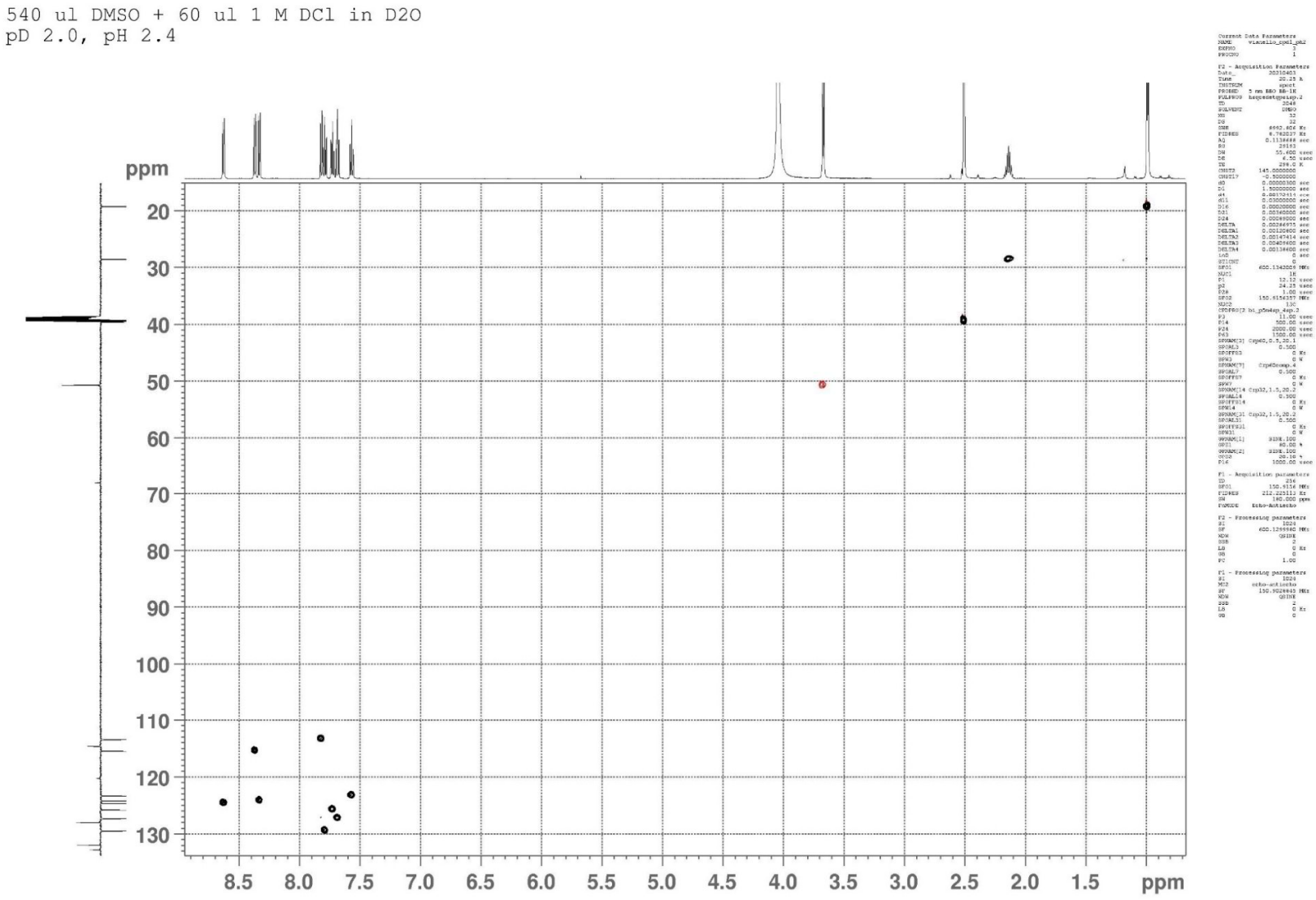
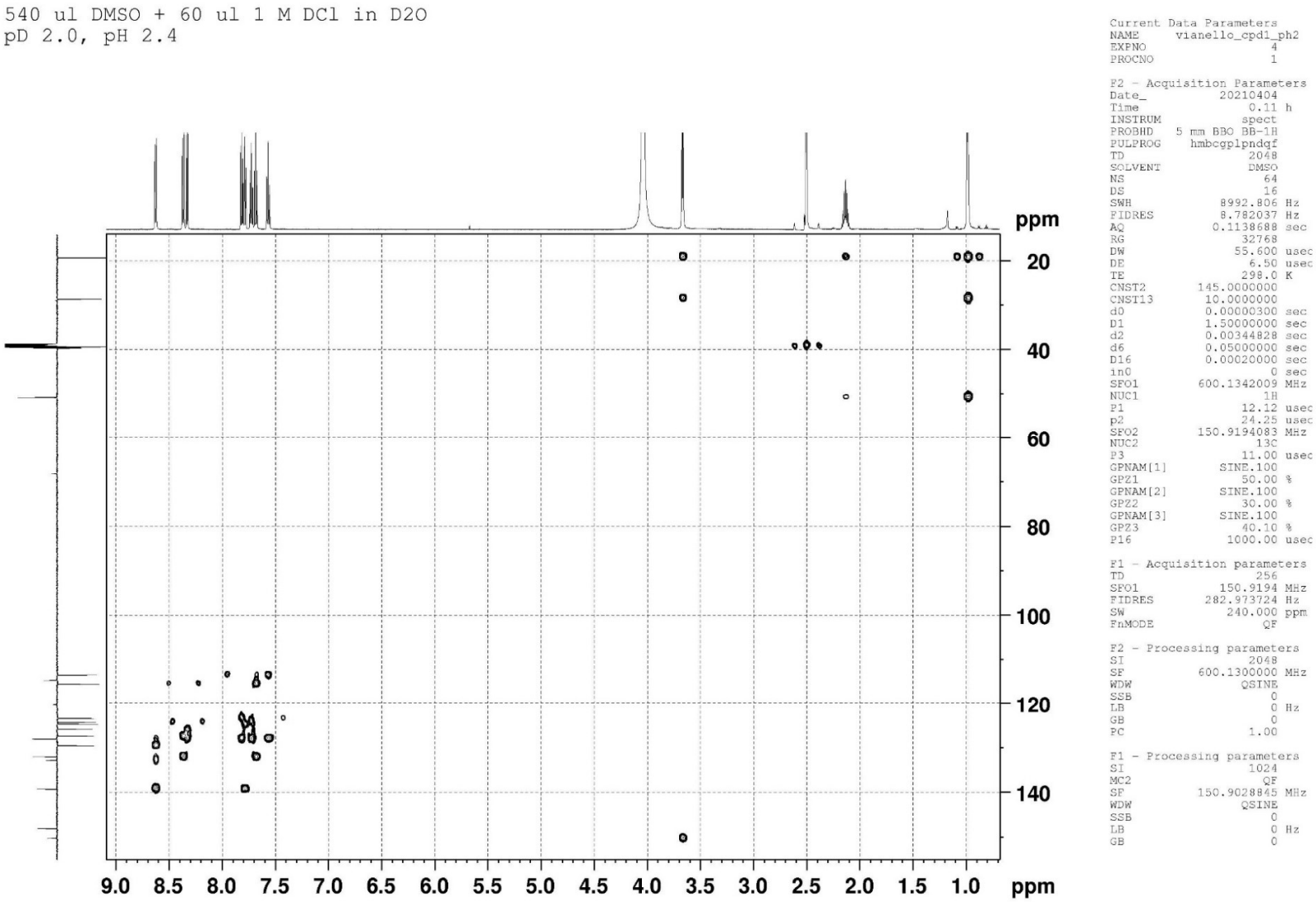


Figure S5. ^{1}H-^{13}C HSQC spectrum of **5** in 90% DMSO-d_6 / 10% D_2O at 25 °C, pH 2.4

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PROCNO                    1

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TD                   2048
SOLVENT              DMSO
NS                     64
DS                     16
SWH              8992.806 Hz
FIDRES           8.782037 Hz
AQ             0.1138688 sec
RG                 32768
DW                55.600 usec
DE                  6.50 usec
TE                298.0 K
CNST2        145.0000000
CNST13        10.0000000
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D1            1.50000000 sec
d2            0.00344828 sec
d6            0.05000000 sec
D16           0.00020000 sec
in0                    0 sec
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NUC1                  1H
P1                 12.12 usec
p2                 24.25 usec
SFO2         150.9194083 MHz
NUC2                 13C
P3                 11.00 usec
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GPZ1               50.00 %
GPNAM[2]        SINE.100
GPZ2               30.00 %
GPNAM[3]        SINE.100
GPZ3               40.10 %
P16              1000.00 usec

F1 - Acquisition parameters
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SFO1            150.9194 MHz
FIDRES        282.973724 Hz
SW               240.000 ppm
FnMODE                 QF

F2 - Processing parameters
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SF           600.1300000 MHz
WDW                 QSINE
SSB                     0
LB                      0 Hz
GB                      0
PC                   1.00

F1 - Processing parameters
SI                   1024
MC2                    QF
SF           150.9028845 MHz
WDW                 QSINE
SSB                     0
LB                      0 Hz
GB                      0
```

Figure S6. ^{1}H-^{13}C HMBC spectrum of **5** in 90% DMSO-d_6 / 10% D_2O at 25 °C, pH 2.4

Shift1 (ppm)	H's	Type	J (Hz)	Atom1	Multiplet1
0.965	6.000	d	6.6	24, 25	M11
2.119	1.000	spt	7.1	23	M10
3.617	2.000	d	7.3	22<'>, 22<">	M09
7.320	1.000	ddd	8.2, 7.2, 0.9	11	M08
7.457	1.000	td	8.2, 7.1, 0.9	10	M07
7.698	1.000	m	-	2	M06
7.719	1.000	m	-	3	M05
7.770	1.000	d	8.1	9	M04
8.259	1.000	dd	7.0, 2.2	4	M03
8.301	1.000	d	8.4	12	M02
8.757	1.000	dd	8.0, 1.8	1	M01

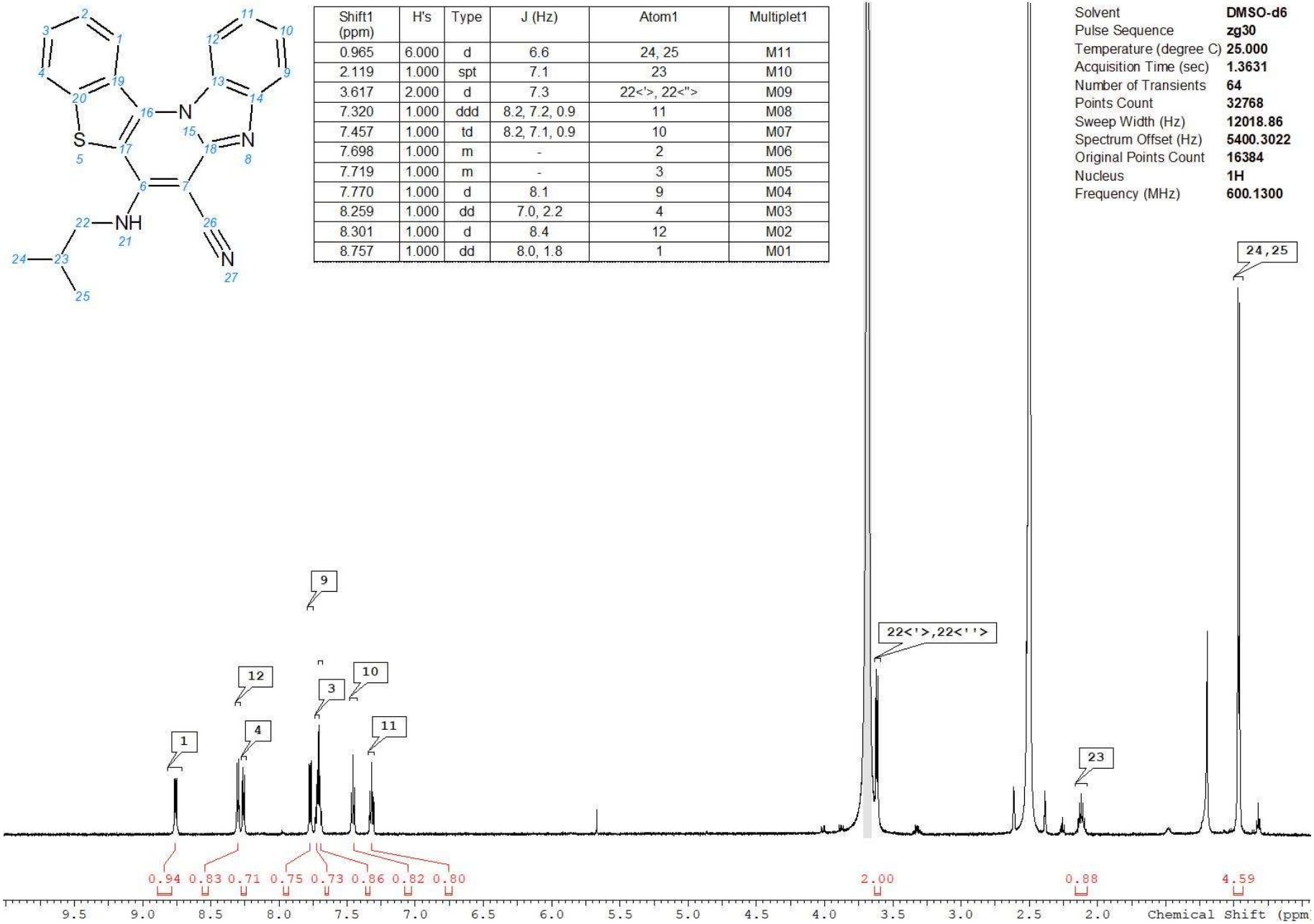
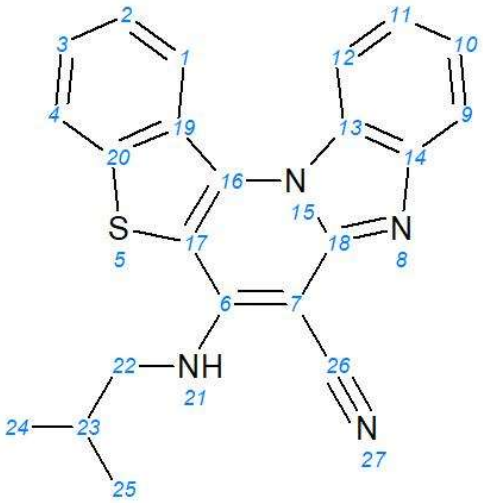


Figure S7. ^{1}H spectrum of **5** in 90% DMSO-d_6 / 10% D_2O at 25 °C, pH 7.5



Atom	Exp. Shift (ppm)	Atom	Exp. Shift (ppm)
24	19.71	13	130.41
25	19.71	16	133.50
23	28.73	20	138.87
22	50.94	14	144.86
7	71.75	6	148.67
12	114.51	18	151.53
17	117.65		
26	117.89		
9	118.80		
11	120.22		
4	124.47		
1	124.63		
10	124.95		
2	125.72		
3	128.75		
19	129.23		

Solvent	**DMSO-d6**
Pulse Sequence	**deptqgpsp.2**
Temperature (degree C)	**25.000**
Acquisition Time (sec)	**0.5999**
Number of Transients	**22300**
Points Count	**32768**
Sweep Width (Hz)	**39368.88**
Spectrum Offset (Hz)	**15082.0010**
Original Points Count	**23619**
Nucleus	**13C**
Frequency (MHz)	**150.9029**

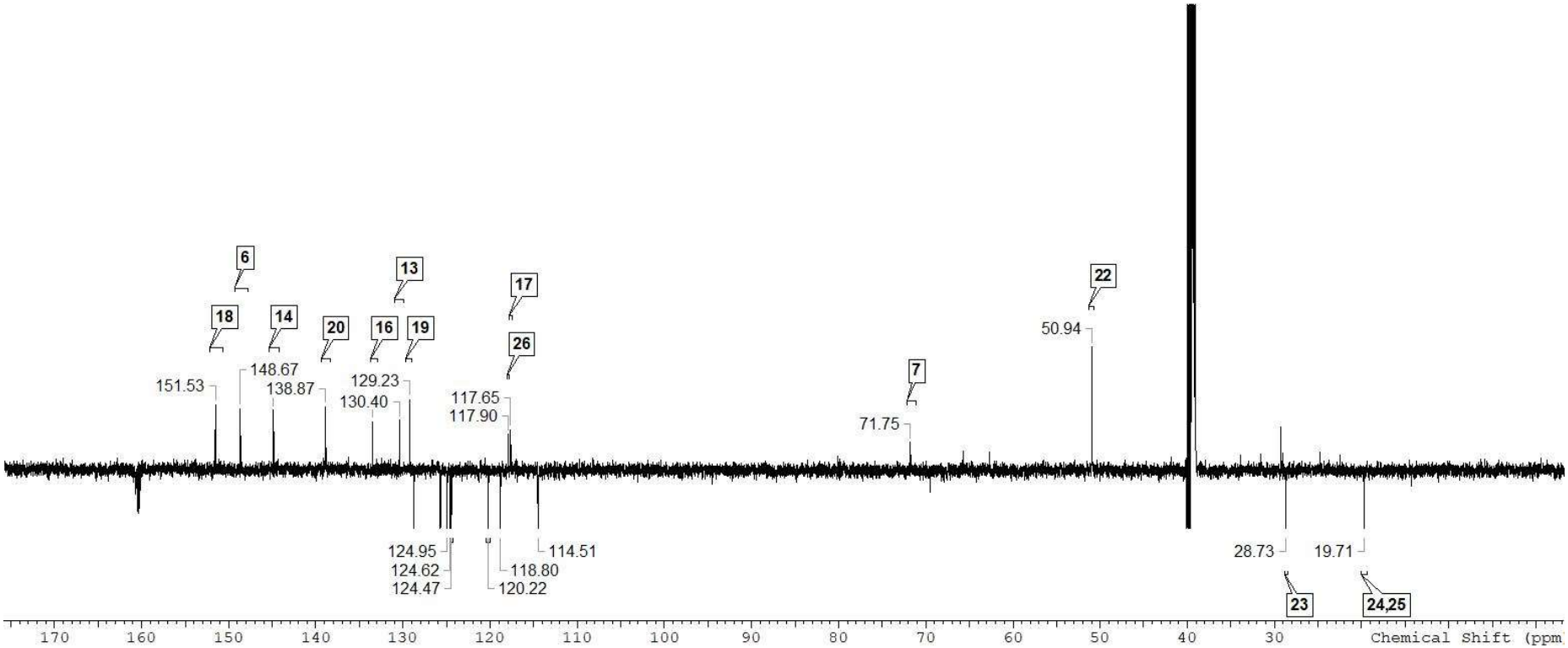


Figure S8. ^{13}C spectrum of **5** in 90% DMSO-d_6 / 10% D_2O at 25 °C, pH 7.5

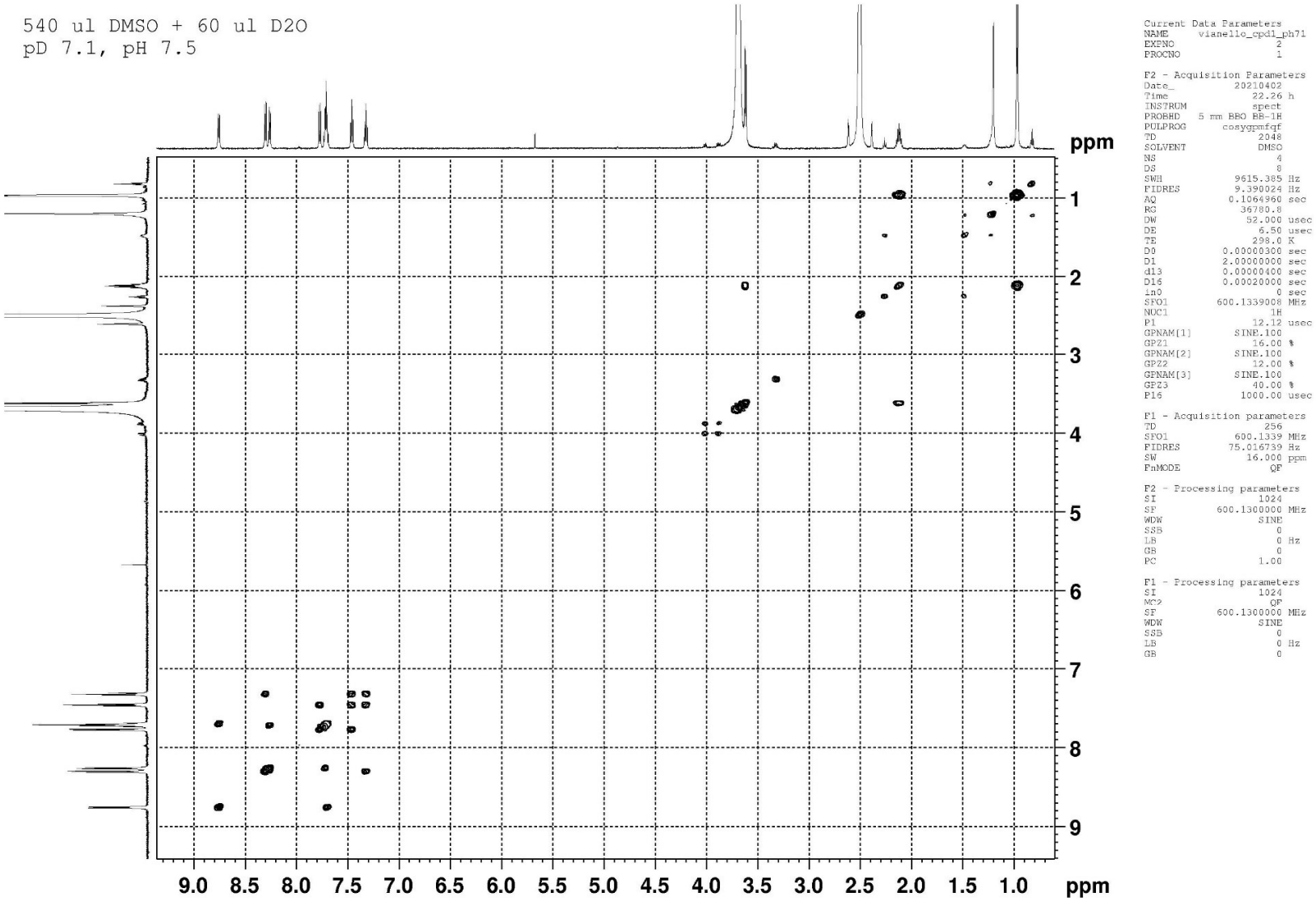


Figure S9. COSY spectrum of **5** in 90% DMSO-d_6 / 10% D_2O at 25 °C, pH 7.5

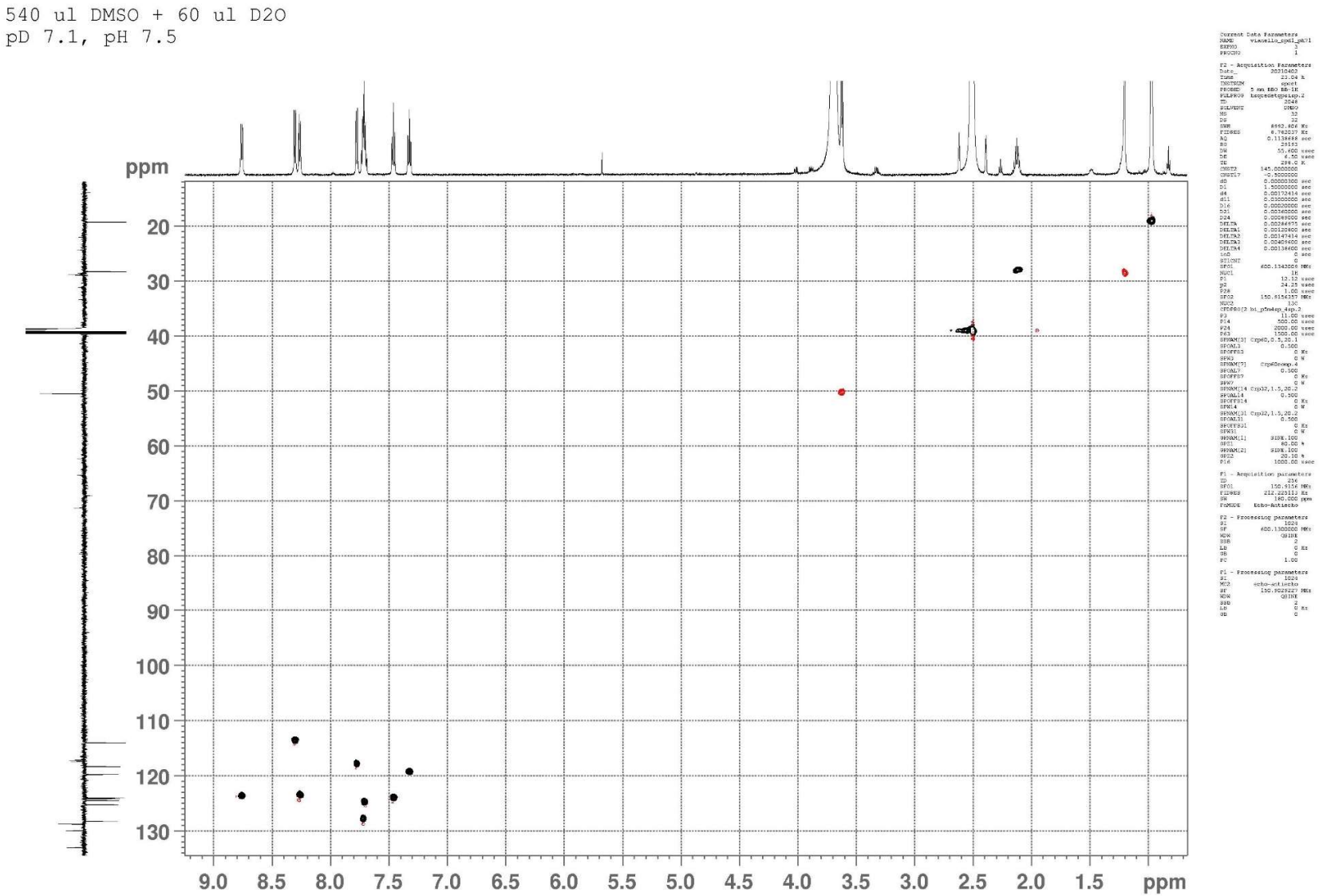
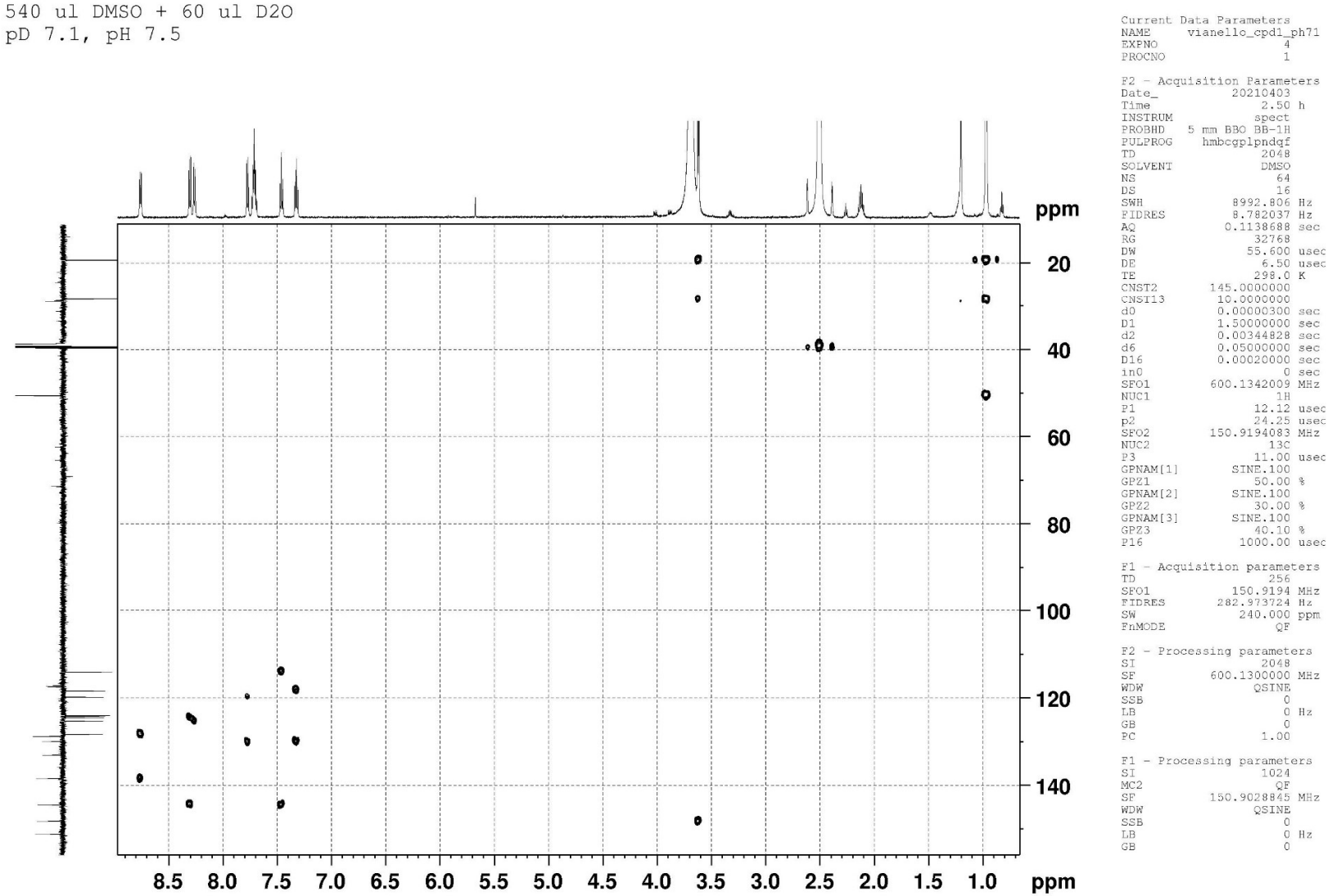


Figure S10. ^{1}H-^{13}C HSQC spectrum of **5** in 90% DMSO-d_6 / 10% D_2O at 25 °C, pH 7.5

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PROCNO                     1

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PULPROG     hmbcgplpndqf
TD                  2048
SOLVENT             DMSO
NS                    64
DS                    16
SWH             8992.806 Hz
FIDRES          8.782037 Hz
AQ            0.1138688 sec
RG                32768
DW               55.600 usec
DE                 6.50 usec
TE                298.0 K
CNST2        145.0000000
CNST13        10.0000000
d0            0.00000300 sec
D1            1.50000000 sec
d2            0.00344828 sec
d6            0.05000000 sec
D16           0.00020000 sec
in0                    0 sec
SFO1         600.1342009 MHz
NUC1                  1H
P1                12.12 usec
p2                24.25 usec
SFO2         150.9194083 MHz
NUC2                 13C
P3                11.00 usec
GPNAM[1]        SINE.100
GPZ1              50.00 %
GPNAM[2]        SINE.100
GPZ2              30.00 %
GPNAM[3]        SINE.100
GPZ3              40.10 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                   256
SFO1            150.9194 MHz
FIDRES        282.973724 Hz
SW               240.000 ppm
FnMODE                QF

F2 - Processing parameters
SI                   2048
SF           600.1300000 MHz
WDW                 QSINE
SSB                     0
LB                      0 Hz
GB                      0
PC                   1.00

F1 - Processing parameters
SI                   1024
MC2                    QF
SF           150.9028845 MHz
WDW                 QSINE
SSB                     0
LB                      0 Hz
GB                      0
```

Figure S11. ^{1}H-^{13}C HMBC spectrum of **5** in 90% DMSO-d_6 / 10% D_2O at 25 °C, pH 7.5

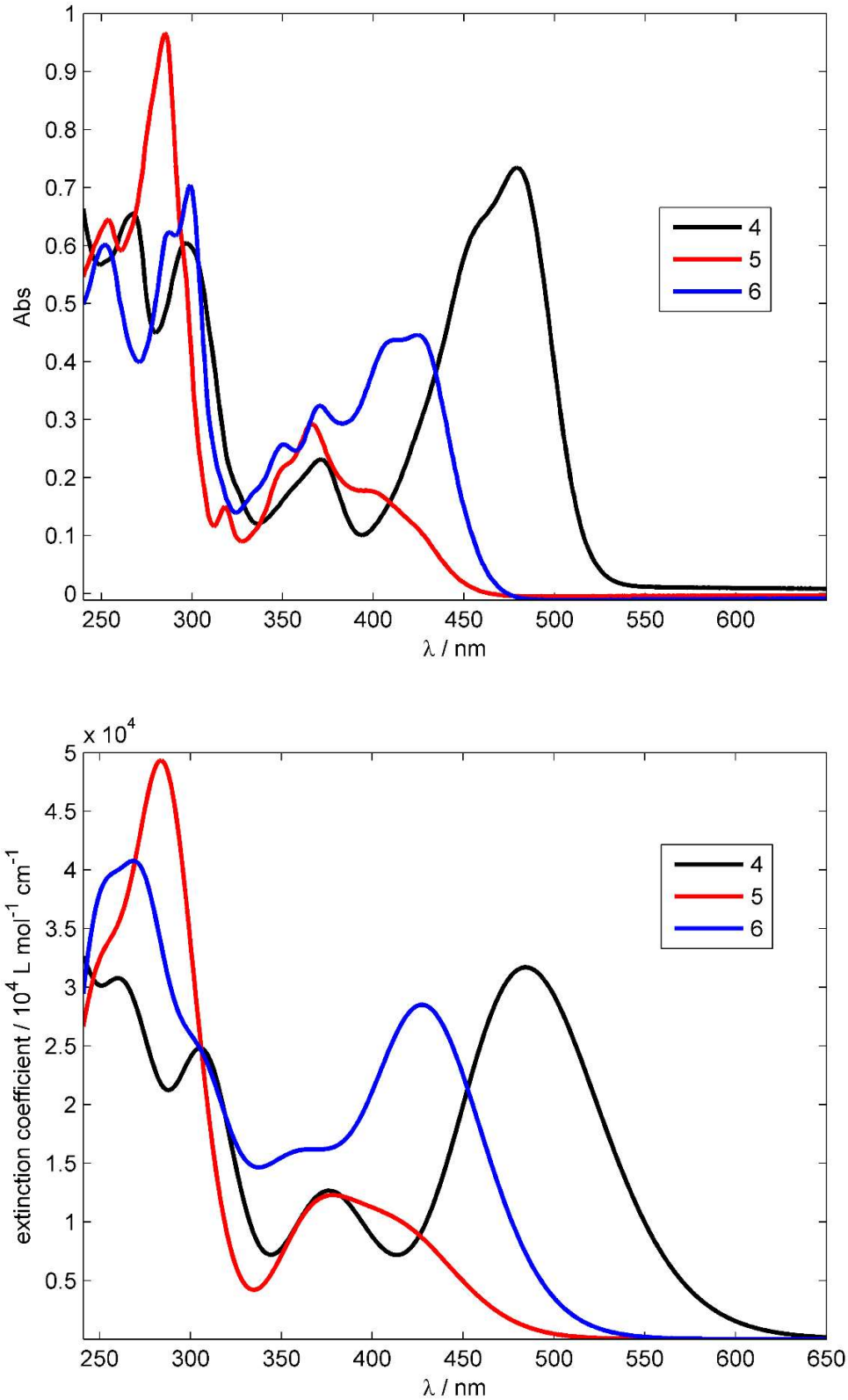


Figure S12. Comparison of experimental (top) and caluclated (bottom) absorption spectra of systems **4**–**6**.

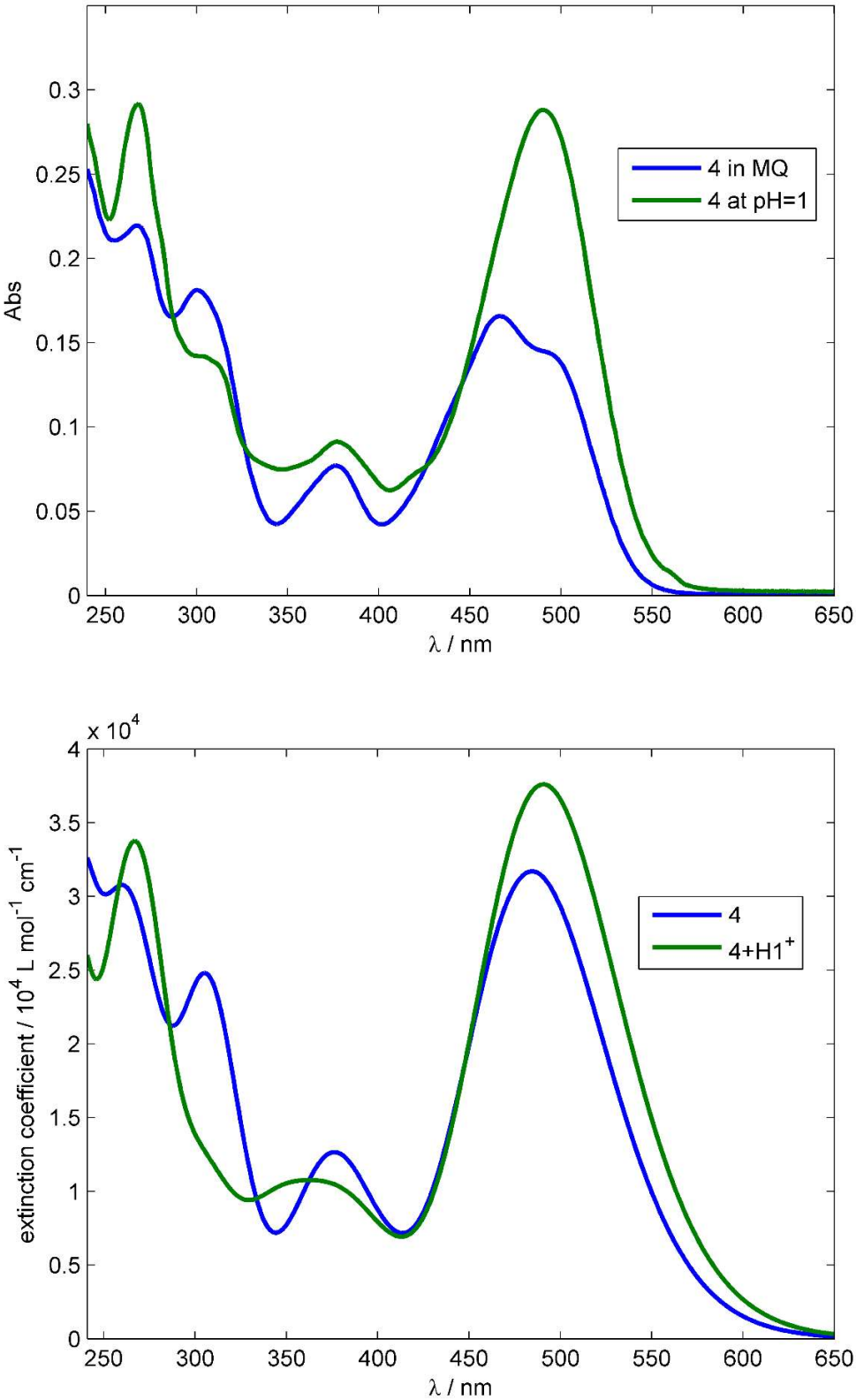


Figure S13. Comparison of the experimental absorption spectra of **4** in pure and acidified MQ (top) and those caluclated for different protonation forms of **4** (bottom).

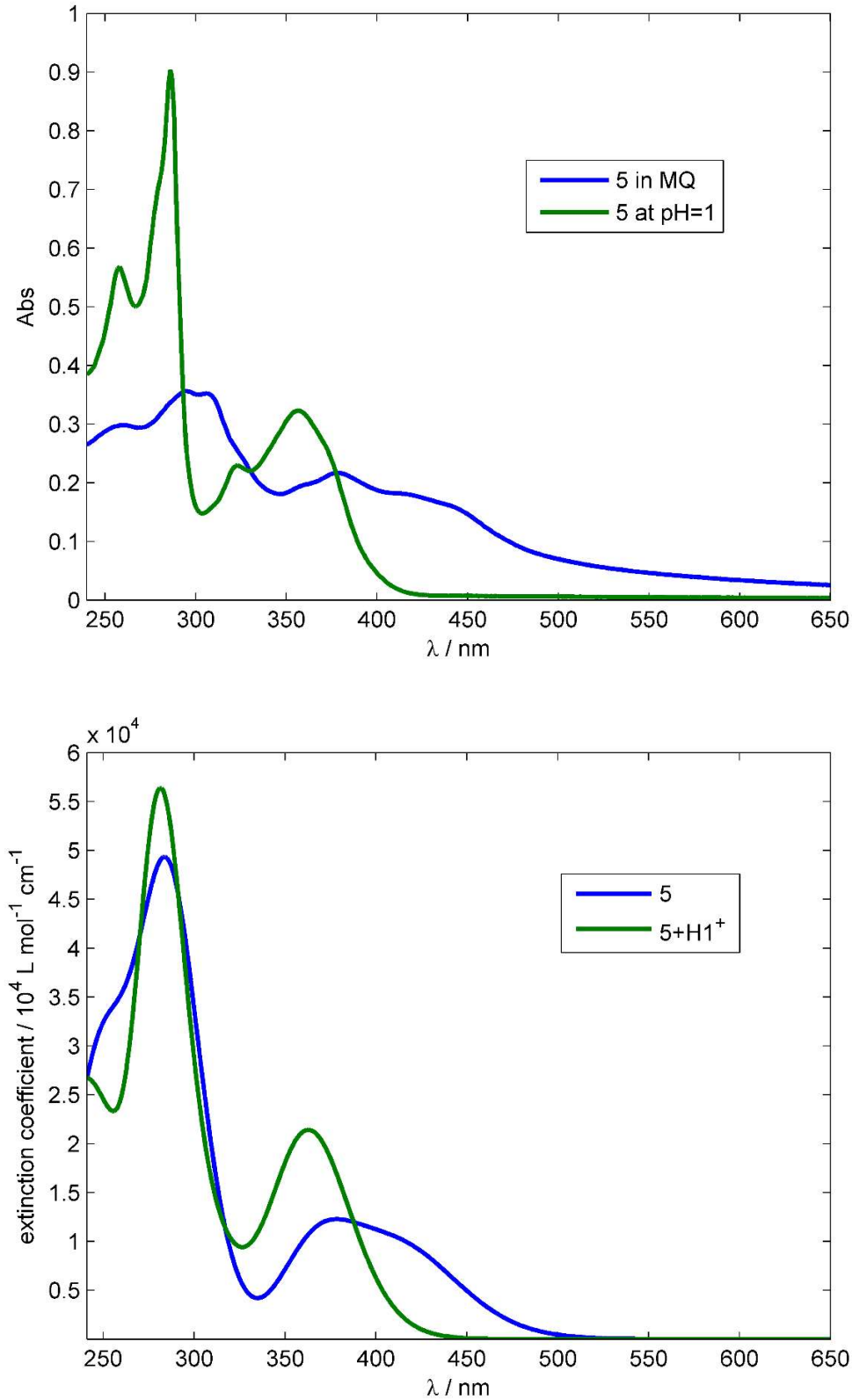


Figure S14. Comparison of the experimental absorption spectra of **5** in pure and acidified MQ (top) and those caluclated for different protonation forms of **5** (bottom).

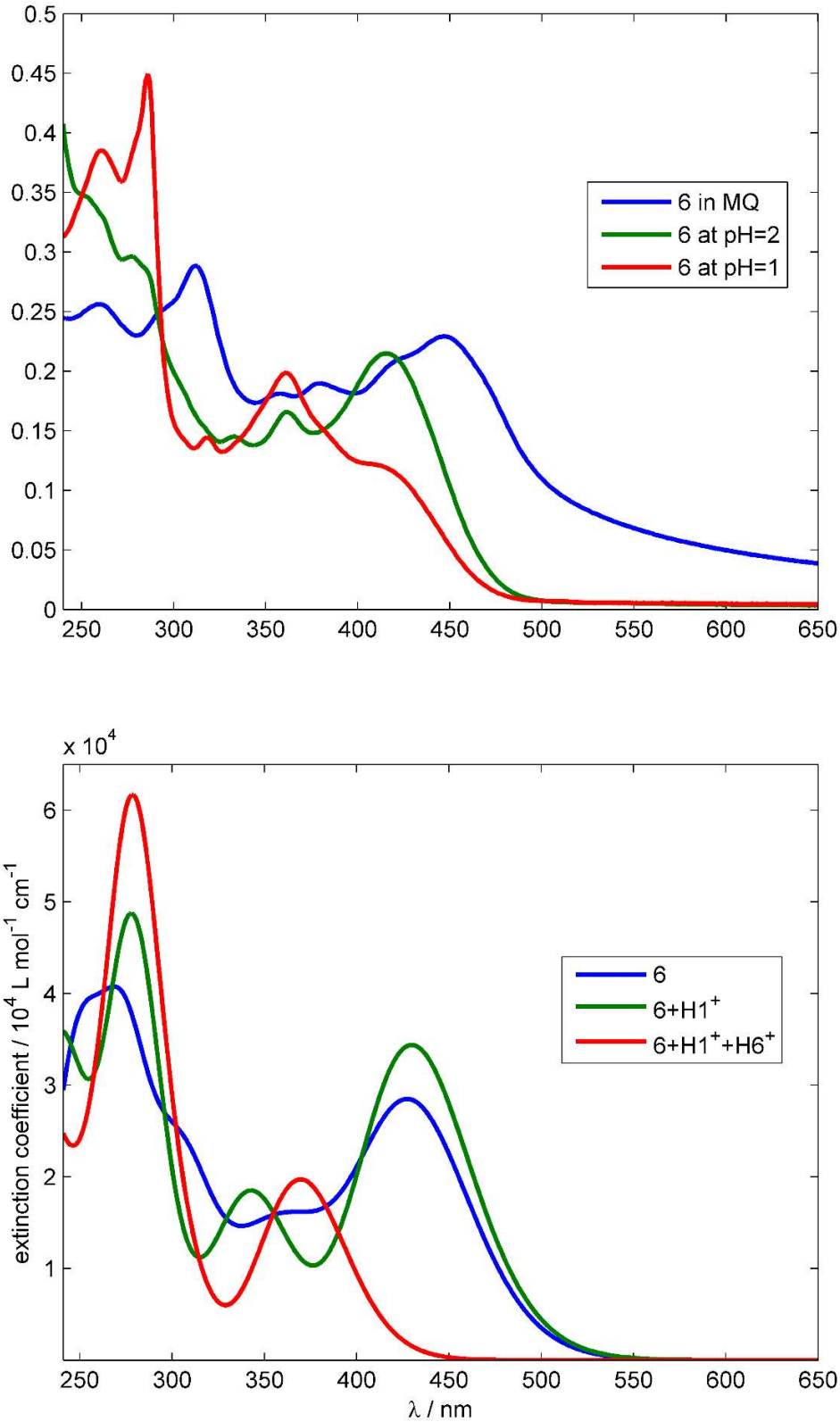


Figure S15. Comparison of the experimental absorption spectra of **6** in pure and acidified MQ (top) and those caluclated for different protonation forms of **6** (bottom).

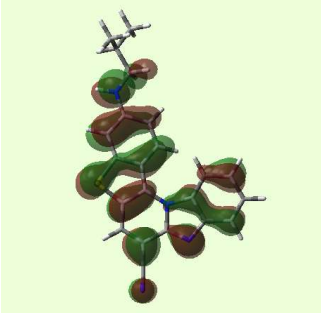
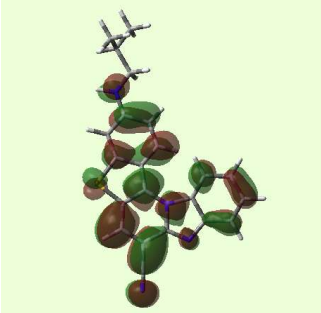
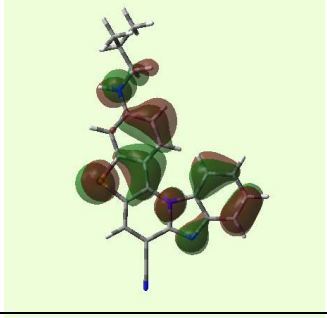
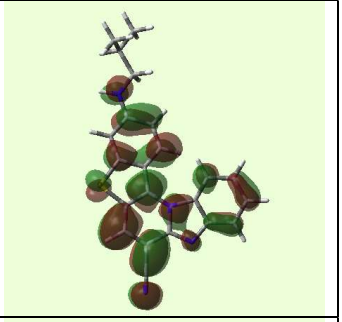
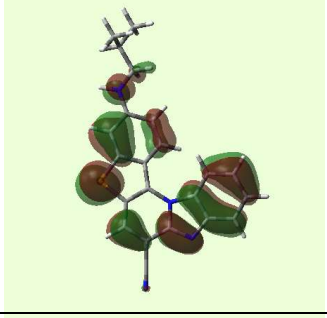
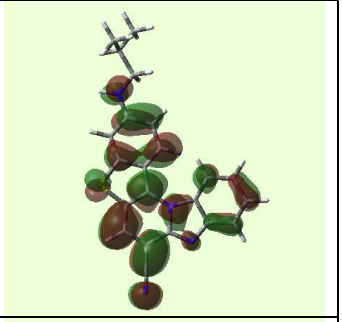
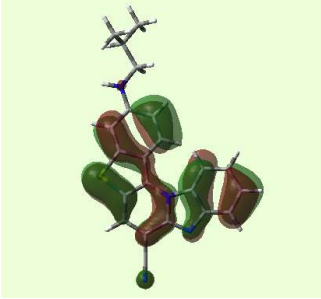
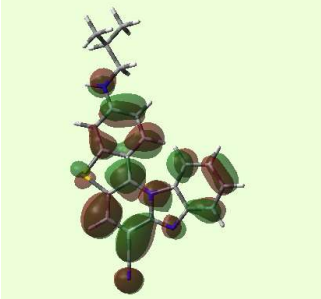
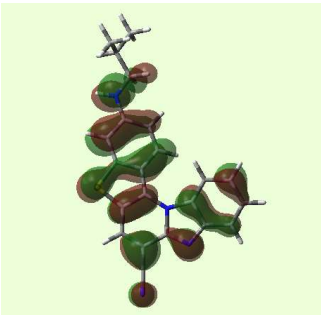
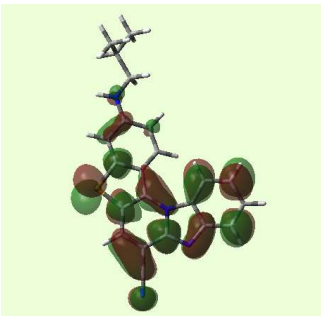
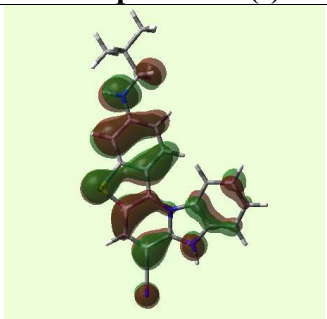
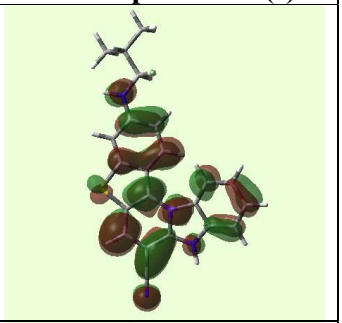
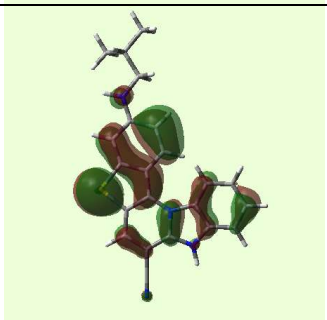
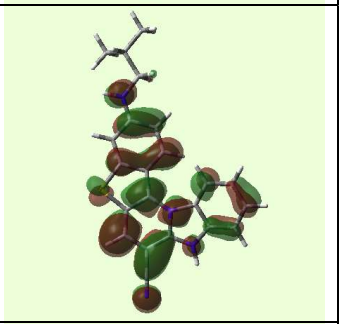
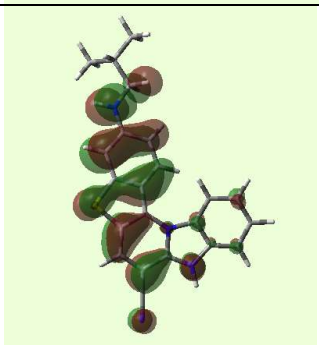
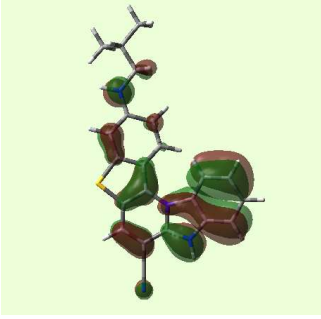
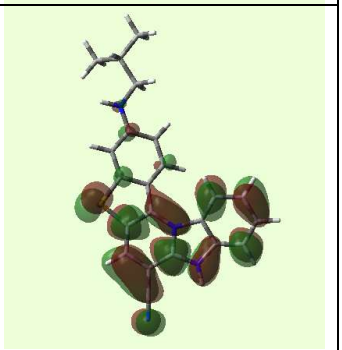
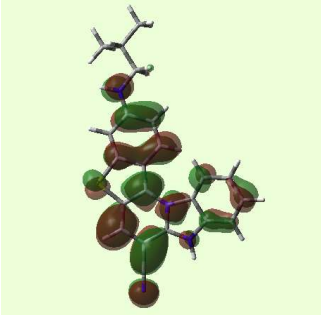
Transition	Occupied NTO(s)	Unoccupied NTO(s)
1 λ= 484.54 nm f=0.5533 100%		
2 λ= 378.66 nm f=0.1618 98%		
3 λ= 370.48 nm f=0.0601 94%		
4 λ= 322.68 nm f=0.0379 43%		
    56%		

Figure S16. Natural transition orbitals for the first four excited states of **4**. Transitions are described by their ordinal numbers, absorption wavelengths (λ), oscillator strengths (f), and contributions of the natural transition orbital pairs shown in the table rows.

Transition	Occupied NTO(s)	Unoccupied NTO(s)
1 λ= 490.78 nm f=0.6562 99%		
2 λ= 385.46 nm f=0.1422 97%		
3 λ= 349.57 nm f=0.0141 37% 62%	 	 

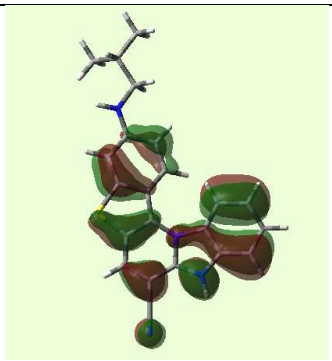
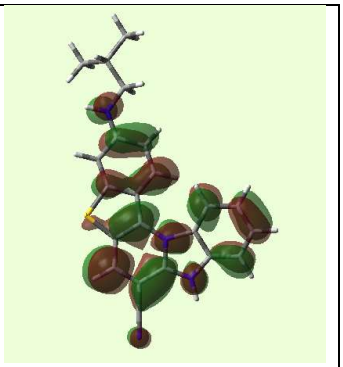
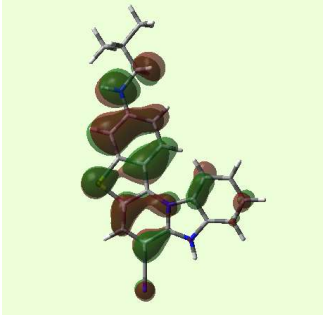
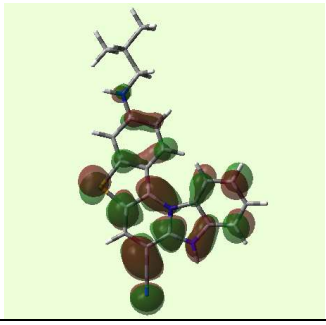
4 λ= 345.78 nm f=0.1271 39% 60%		
		

Figure S17. Natural transition orbitals for the first four excited states of **4-H$^+$(1)**. Transitions are described by their ordinal numbers, absorption wavelengths (λ), oscillator strengths (f), and contributions of the natural transition orbital pairs shown in the table rows.

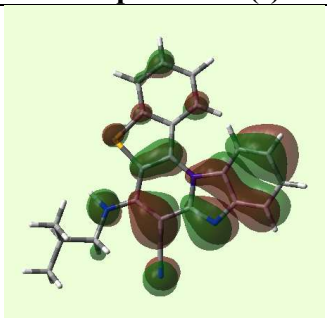
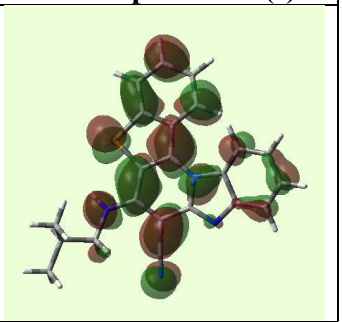
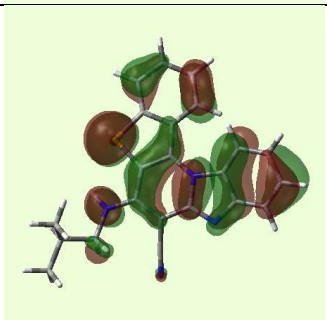
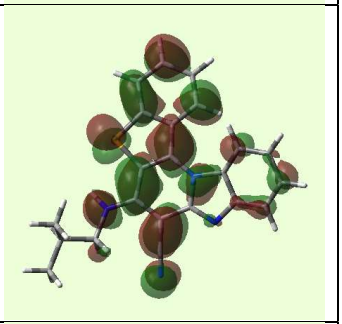
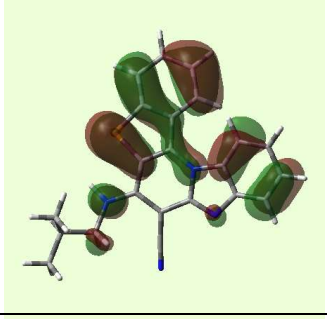
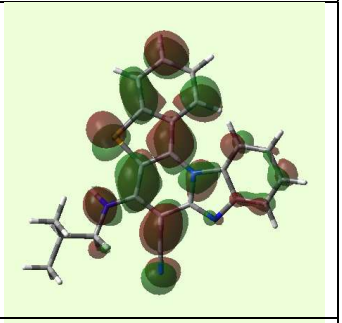
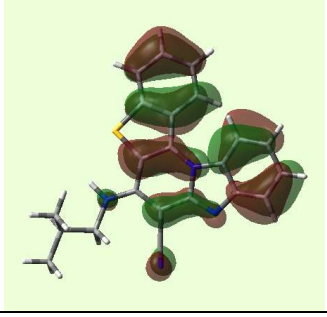
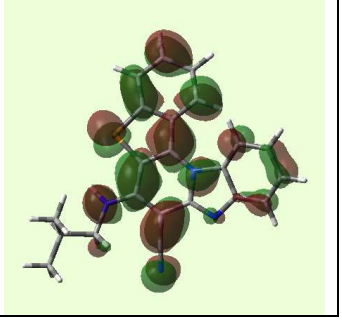
Transition	Occupied NTO(s)	Unoccupied NTO(s)
1 λ= 418.85 nm f=0.1423 99%		
2 λ= 370.03 nm f=0.1860 96%		
3 λ= 310.34 nm f=0.0226 91%		
4 λ= 300.81 nm f=0.1993 81%		

Figure S18. Natural transition orbitals for the first four excited states of **5**. Transitions are described by their ordinal numbers, absorption wavelengths (λ), oscillator strengths (f), and contributions of the natural transition orbital pairs shown in the table rows.

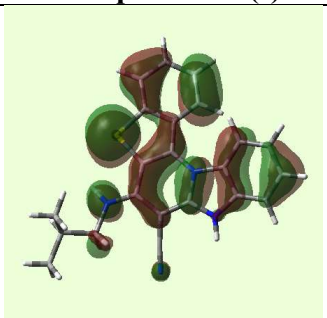
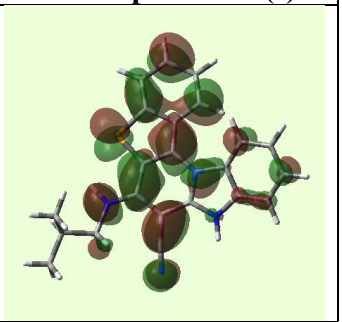
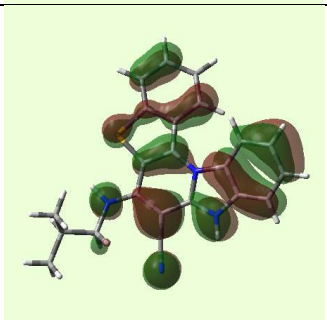
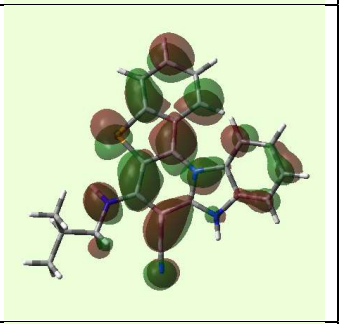
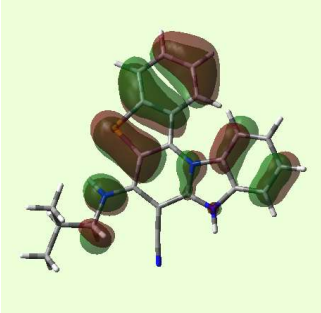
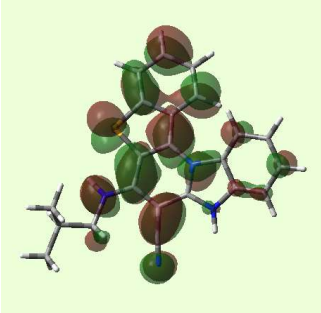
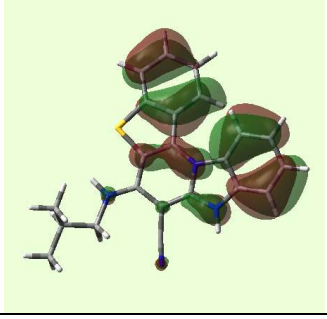
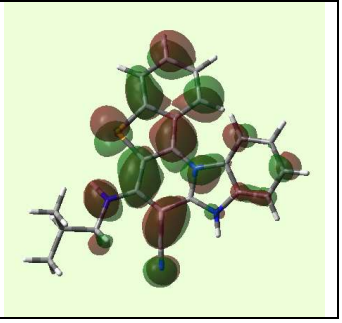
Transition	Occupied NTO(s)	Unoccupied NTO(s)
1 λ= 363.69 nm f=0.1773 96%		
2 λ= 363.13 nm f=0.1930 99%		
3 λ= 315.66 nm f=0.0957 89%		
4 λ= 299.89 nm f=0.0835 97%		

Figure S19. Natural transition orbitals for the first four excited states of **5-H$^+$(1)**. Transitions are described by their ordinal numbers, absorption wavelengths (λ), oscillator strengths (f), and contributions of the natural transition orbital pairs shown in the table rows.

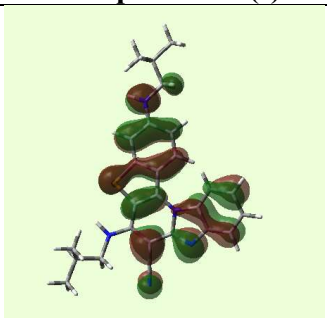
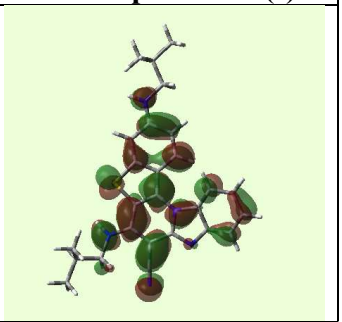
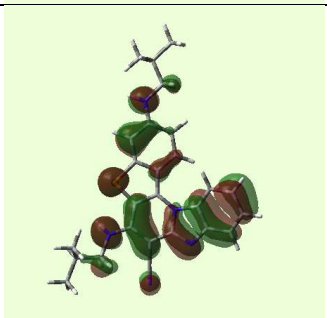
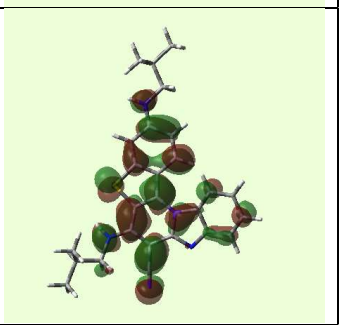
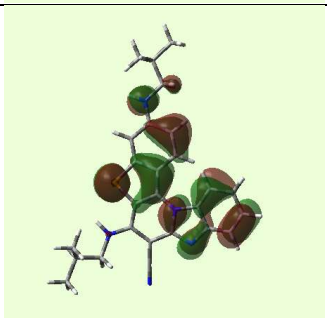
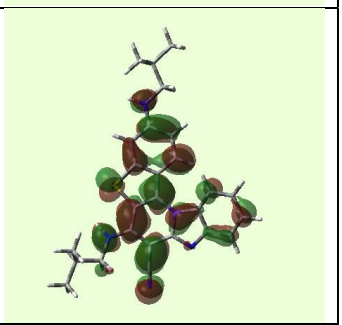
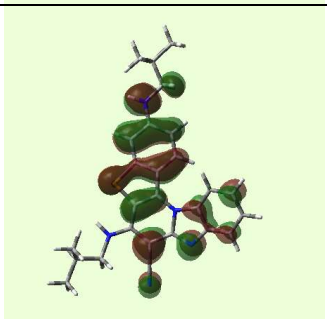
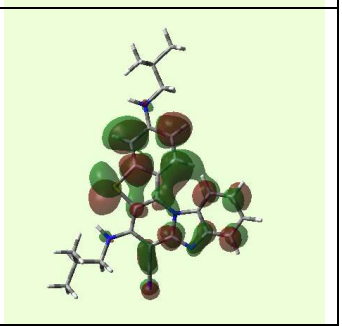
Transition	Occupied NTO(s)	Unoccupied NTO(s)
1 λ= 429.74 nm f=0.4816 99%		
2 λ= 371.78 nm f=0.1824 95%		
3 λ= 343.96 nm f=0.1514 91%		
4 λ= 312.06 nm f=0.0265 87%		

Figure S20. Natural transition orbitals for the first four excited states of **6**. Transitions are described by their ordinal numbers, absorption wavelengths (λ), oscillator strengths (f), and contributions of the natural transition orbital pairs shown in the table rows.

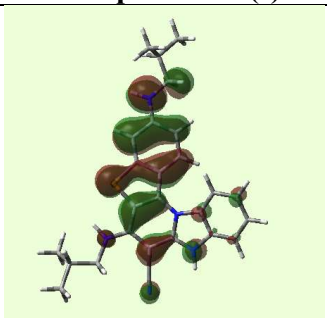
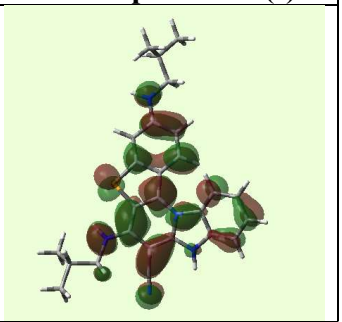
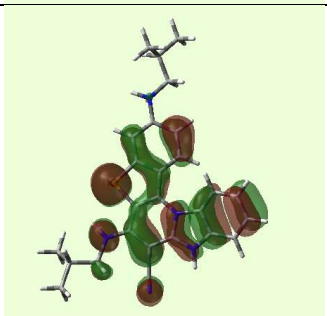
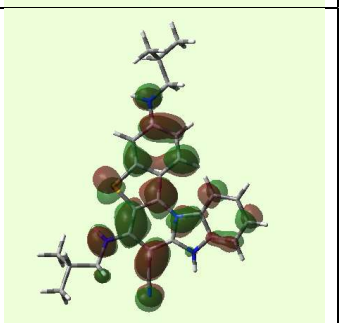
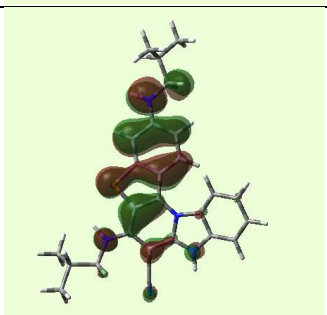
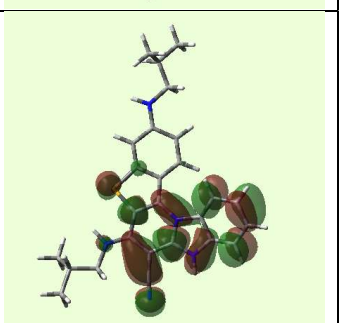
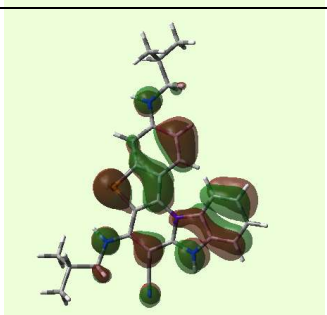
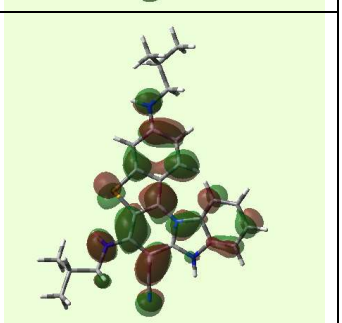
Transition	Occupied NTO(s)	Unoccupied NTO(s)
1 λ= 429.94 nm f=0.5997 99%		
2 λ= 350.41 nm f=0.1715 86%		
3 λ= 338.05 nm f=0.1324 88%		
4 λ= 328.91 nm f=0.0442 94%		

Figure S21. Natural transition orbitals for the first four excited states of **6-H$^+$(1)**. Transitions are described by their ordinal numbers, absorption wavelengths (λ), oscillator strengths (f), and contributions of the natural transition orbital pairs shown in the table rows.

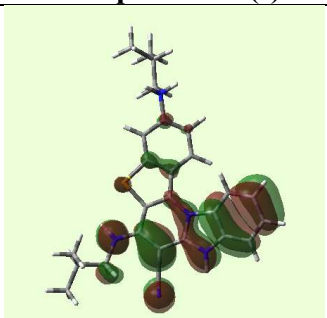
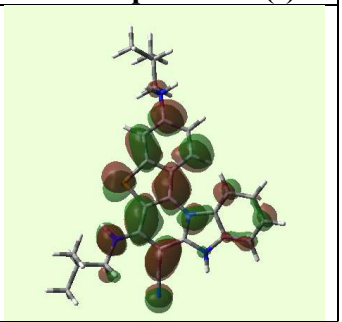
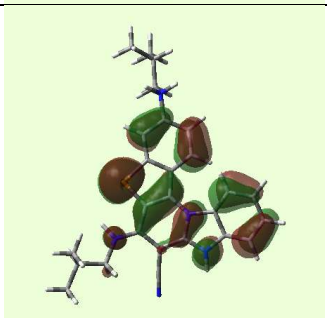
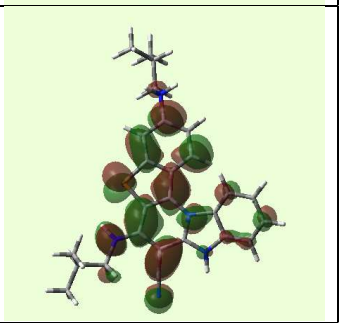
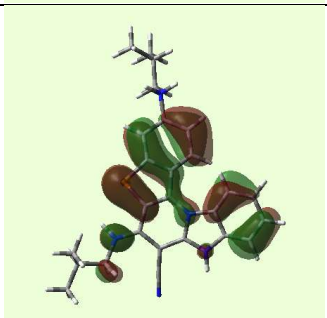
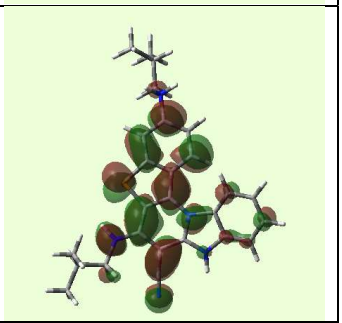
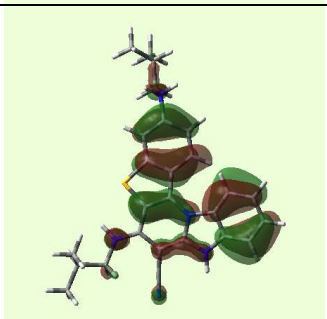
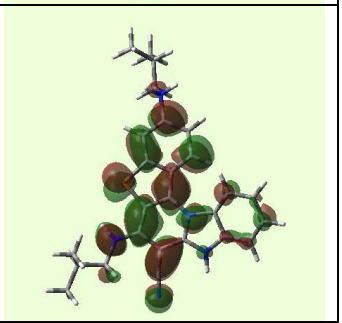
Transition	Occupied NTO(s)	Unoccupied NTO(s)
1 λ= 374.51 nm f=0.2365 99%		
2 λ= 360.26 nm f=0.1247 95%		
3 λ= 310.47 nm f=0.0403 91%		
4 λ= 299.96 nm f=0.1519 87%		

Figure S22. Natural transition orbitals for the first four excited states of **6-H^{++}(1,6)**. Transitions are described by their ordinal numbers, absorption wavelengths (λ), oscillator strengths (f), and contributions of the natural transition orbital pairs shown in the table rows.

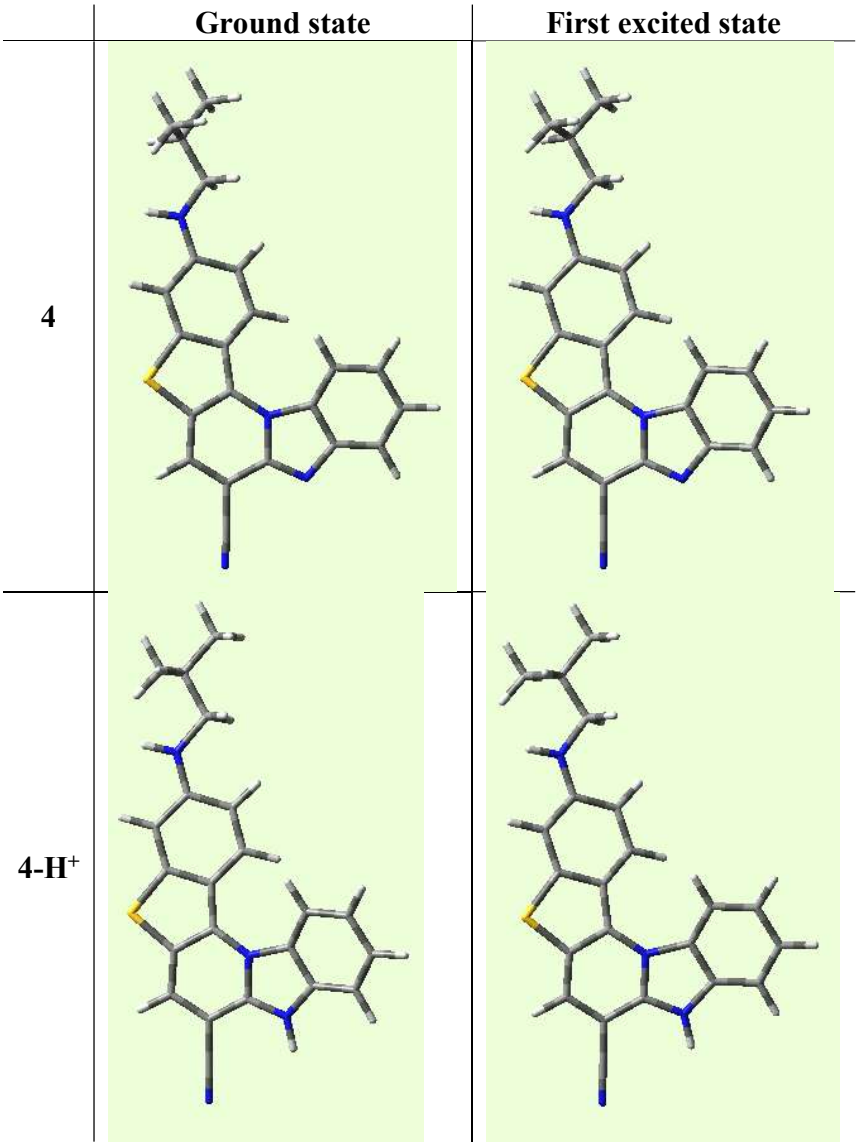


Figure S23. Optimized geometries of the neutral and protonated **4** in the ground and in the first excited state

	Ground state	First excited state
5		
5-H$^+$		

Figure S24. Optimized geometries of the neutral and protonated **5** in the ground and in the first excited state

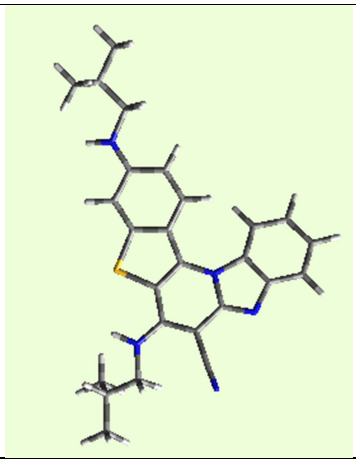
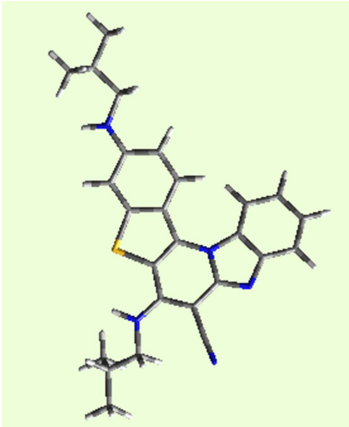
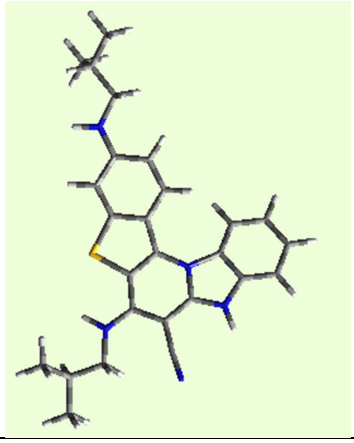
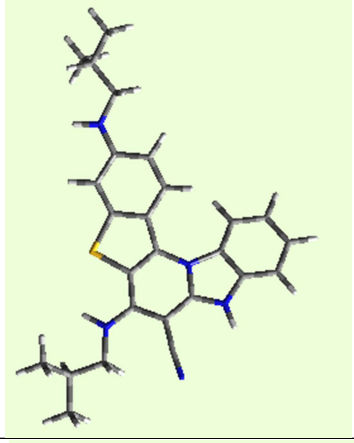
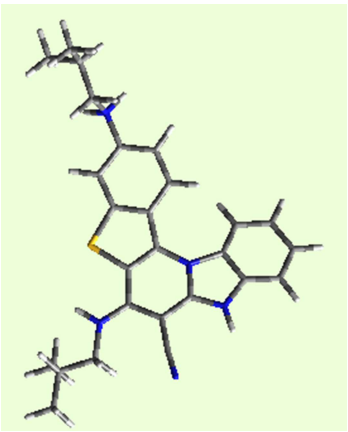
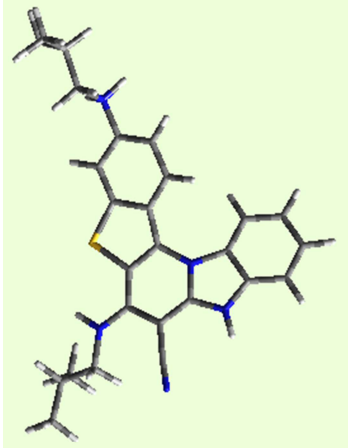
	Ground state	First excited state
6		
6-H^{+}		
6-H^{++}		

Figure S25. Optimized geometries of the neutral, protonated and diprotonated **6** in the ground and in the first excited state