

Synthesis of new porphyrin/4-quinolone conjugates and evaluation of their efficiency in the photoinactivation of *Staphylococcus aureus*

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Supporting Information

Index

Structural characterization:.....	3
¹ H NMR spectrum of derivative 4a (CDCl ₃ , 300 MHz).	6
¹³ C NMR spectrum of derivative 4a (CDCl ₃ , 75 MHz).	7
Expansion of the ¹³ C NMR spectrum of derivative 4a (CDCl ₃ , 75 MHz).....	8
¹ H NMR spectrum of derivative 4b (CDCl ₃ , 300 MHz).	8
Expansion of the ¹ H NMR spectrum of derivative 4b (CDCl ₃ , 300 MHz).	9
Expansion of the ¹ H NMR spectrum of derivative 4b (CDCl ₃ , 300 MHz).	9
¹³ C NMR spectrum of derivative 4b (CDCl ₃ , 125 MHz).	10
Expansion of the ¹³ C NMR spectrum of derivative 4b (CDCl ₃ , 125 MHz).	10
¹ H NMR spectrum of derivative 4c (CDCl ₃ , 300 MHz).....	11
Expansion of the ¹ H NMR spectrum of derivative 4c (CDCl ₃ , 300 MHz).....	11
Expansion of the ¹ H NMR spectrum of derivative 4c (CDCl ₃ , 300 MHz).....	12
¹³ C NMR spectrum of derivative 4c (CDCl ₃ , 125 MHz).....	12
Expansion of the ¹³ C NMR spectrum of derivative 4c (CDCl ₃ , 125 MHz).....	13
Expansion of the ¹³ C NMR spectrum of derivative 4c (CDCl ₃ , 125 MHz).....	13

¹ H NMR spectrum of derivative 6a (CDCl ₃ , 300 MHz).	14
Expansion of the ¹ H NMR spectrum of derivative 6a (CDCl ₃ , 300 MHz).....	14
¹³ C NMR spectrum of derivative 6a (CDCl ₃ , 125 MHz).	15
Expansion of the ¹³ C NMR spectrum of derivative 6a (CDCl ₃ , 125 MHz).....	15
¹ H NMR spectrum of derivative 6b (CDCl ₃ , 300 MHz).	16
Expansion of the ¹ H NMR spectrum of derivative 6b (CDCl ₃ , 300 MHz).	16
¹³ C NMR spectrum of derivative 6b (CDCl ₃ , 125 MHz).	17
Expansion of the ¹³ C NMR spectrum of derivative 6b (CDCl ₃ , 125 MHz).	17
¹ H NMR spectrum of derivative 6c (CDCl ₃ /CD ₃ OD, 300 MHz).	18
Expansion of the ¹ H NMR spectrum of derivative 6c (CDCl ₃ /CD ₃ OD, 300 MHz).	18
Expansion of the ¹ H NMR spectrum of derivative 6c (CDCl ₃ /CD ₃ OD, 300 MHz).	19
¹³ C NMR spectrum of derivative 6c (CDCl ₃ /CD ₃ OD, 125 MHz).	19
Expansion of the ¹³ C NMR spectrum of derivative 6c (CDCl ₃ /CD ₃ OD, 125 MHz).	20
Expansion of the ¹³ C NMR spectrum of derivative 6c (CDCl ₃ /CD ₃ OD, 125 MHz).	20
¹ H NMR spectrum of derivative 8a (CDCl ₃ , 300 MHz).	21
Expansion of the ¹ H NMR spectrum of derivative 8a (CDCl ₃ , 300 MHz).....	21
¹³ C NMR spectrum of derivative 8a (CDCl ₃ , 75 MHz).	22
Expansion of the ¹³ C NMR spectrum of derivative 8a (CDCl ₃ , 75 MHz).....	22
¹ H NMR spectrum of derivative 8b (CDCl ₃ , 500 MHz).	23
Expansion of the ¹ H NMR spectrum of derivative 8b (CDCl ₃ , 500 MHz).	23
¹³ C NMR spectrum of derivative 8b (CDCl ₃ , 75 MHz).	24
Expansion of the ¹³ C NMR spectrum of derivative 8b (CDCl ₃ , 75 MHz).	24
UV-Vis spectra of derivatives 4a , 6a and 8a in DMF:H ₂ O (9:1)	25

Structural characterization:

The structures of new derivatives **4a-c**, **6a-c** and **8a-b** were confirmed and assigned according to their ^1H and ^{13}C NMR spectra and their molecular formulae were confirmed by HRMS. 2D NMR spectra (COSY, HSQC and HMBC) were also used in order to unequivocally identify the proton and carbon resonances.

The HRMS-ESI⁺ of the porphyrin/quinolone conjugates **4a** and **4b** show protonated molecular ions $[\text{M}+\text{H}]^+$ at m/z 929.27363 and 971.32075 confirming the success of the Buchwald-Hartwig coupling between porphyrin **1** and the bromo-quinolones **2a** and **2b**, respectively. The ^1H NMR spectra of these conjugates show similar patterns considering the resonances of the porphyrin and quinolone protons.

In the ^1H NMR spectrum of conjugate **4a**, it was possible to identify three AB systems at 8.70 and 8.68 ppm, 8.66 and 8.63 ppm and 8.59 and 8.55 ppm with the same coupling constant ($J = 4.7$ Hz) that are related with the resonances of six β -pyrrolic protons. The three singlets at 8.43, 8.35 and 6.48 ppm were assigned to the resonances of H-2', H-3 and NH , respectively. The *meso*-phenyl protons appeared as three sets of multiplets at 8.02-7.94 ppm, due to the *ortho* protons of 5,10,15-Ph; at 7.90-7.80 ppm, due to the *ortho* protons of 20-Ph and at 7.74-7.60 ppm due to the *meta* and *para* protons of 5,10,15,20-Ph. The protons of quinolone core were unequivocally assigned according with the signal multiplicity and the correlations observed in the COSY spectrum. The resonance of quinolone H-5' appeared as a doublet at 7.74 ppm ($J = 2.6$ Hz) as confirmed by the correlation with the double doublet at 7.55 ppm ($J = 9.0$ and 2.6 Hz), assigned to the resonance of H-7'. The correlation observed between the resonance of H-7' and the doublet at 7.32 ppm ($J = 9.0$ Hz) allowed the assignment of this signal to H-8' proton.

A careful analysis of the HMBC spectrum of **4a** allowed the unequivocal assignment of the carbonyl carbon resonances at 166.3 and 173.9 ppm. The resonance of H-2' correlates with both carbonyl signals but the resonance of protons H-5' only correlates with the signal at 173.9 ppm allowing its assignment to the C-4' resonance; the other signal at 166.3 ppm was assigned to the ester

carbonyl group. The correlation observed between the quartet at 4.43 ppm ($J = 7.0$ Hz) and the signal at 166.3 ppm confirms the assignment of this quartet to the methylene protons resonance of the ester group and therefore the triplet at 1.45 ppm ($J = 7.1$ Hz) is assigned to the CH_3 of the same group. The protons resonance of the ethyl group attached to $N-1$ were identified as the quartet at 4.21 ppm ($J = 7.1$ Hz) due to CH_2 protons and the triplet at 1.53 ppm ($J = 7.1$ Hz) due to the CH_3 protons.

Considering the 1H NMR of conjugate **4b**, the main difference when compared with the spectrum of **4a** relies on the resonance of the aliphatic pentyl group attached to $N-1$. COSY spectrum allowed to identify unequivocally the correlation between the multiplet at 1.95-1.80 ppm due to the resonance of H-2'' with the triplet at 4.12 ppm ($J = 7.0$ Hz) assigned to the two H-1'' protons. Additionally, the correlation of the multiplet at 1.95-1.80 ppm with the one at 1.42-1.32 ppm allowed to assign the resonances due to the four remaining methylene protons (H-3'' and H-4''); the correlation of this last multiplet with the triplet at 0.93 ppm ($J = 7.0$ Hz) allowed to identify the H-5'' protons.

In the case of the porphyrin/ribonucleoside conjugate **4c** the expected m/z value at 1345.36388 ($[M+H]^+$) observed in the HRMS-ESI⁺ spectrum confirmed its molecular formulae. The 1H NMR spectrum of this compound show a more complex pattern due to the presence of the protected ribose unit, although the resonances due to the protons of the porphyrinic core and quinolone unit show similar features to the ones observed for conjugates **4a** and **4b**. In this case, the 2D NMR spectra were fundamental for the unequivocal assignment of all sugar protons and of their protecting groups. The multiplets at 8.02-7.95 ppm and at 7.95-7.85 ppm assigned to the resonances of the *ortho* protons of 5,10,15-Ph and 20-Ph, respectively, also include the resonances of two *ortho* OBz protons each. The remaining two *ortho* OBz protons appear as a multiplet at 8.77-8.05 ppm. The most prominent difference observed for the quinolone proton resonances was related to H-2' which appeared as a singlet at lower field than in conjugates **4a** and **4b** (8.92 ppm *versus* 8.43 ppm); this fact can be justified by the electron-withdrawing character of the ribofuranosyl group attached to $N-1'$. The resonance of quinolone protons H-5', H-7' and H-8' maintained the same chemical shift pattern observed for derivative **4a**. The doublet at 7.77 ppm ($J = 2.5$ Hz) was assigned to H-5' resonance which correlates with the multiplet at 7.60-7.34 ppm

due to the resonances of H-7' and H-8' along with the nine *meta* and *para* OBz protons. The amino bridge proton (NH) was also identified as the singlet at 6.41 ppm. The COSY spectrum analysis was essential for the unequivocal assignments of the ribose moiety protons (Figure 2). The low field doublet in the aliphatic region at 6.48 ppm ($J = 4.6$ Hz) was attributed to the H-1''. The correlation between this signal with the triplet at 6.01 ppm ($J = 4.6$ Hz) allowed its assignment to H-2''. The H-3'' resonance was identified as the triplet at 5.90 ppm ($J = 4.6$ Hz) due to the correlations observed with H-2'' and with the multiplet at 4.96-4.82 ppm, assigned to the resonances of H-4'' and H-5''.

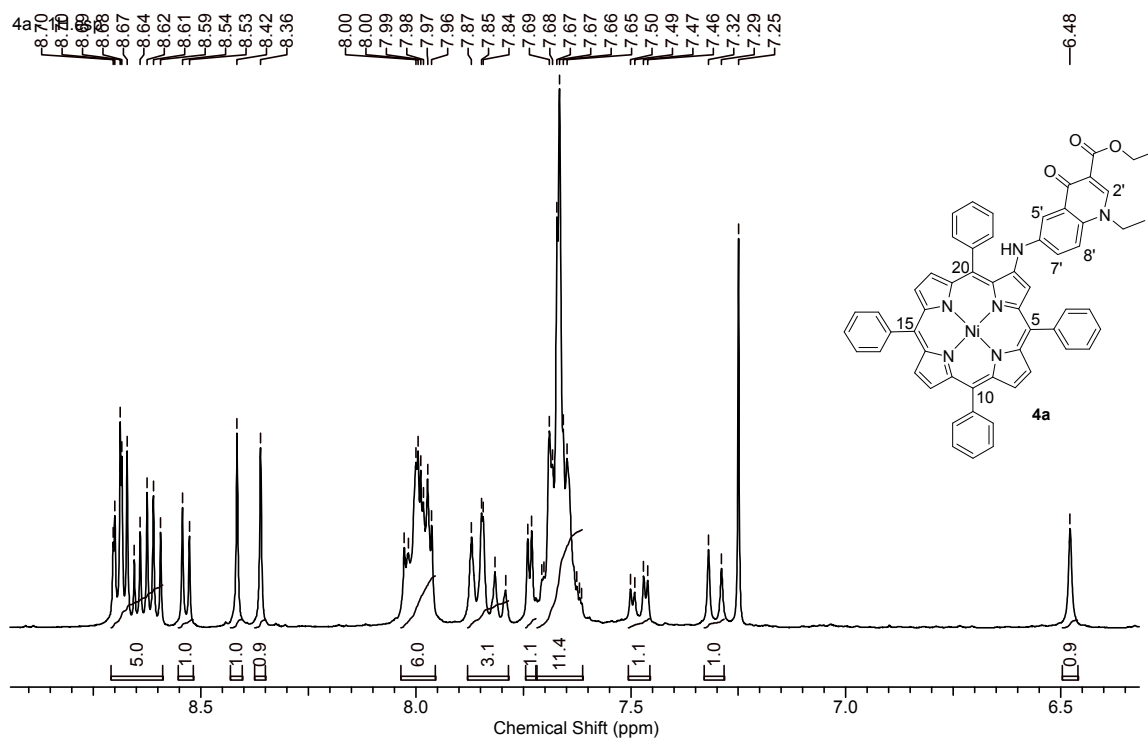
Based on heteronuclear (1H - ^{13}C) HSQC spectra it was possible to assign the signals at 90.3, 74.4 and 70.8 to the sugar C-1'', C-2'' and C-3'' resonances respectively. The resonances of C-4'' and C-5'' were attributed to the signals at 80.8 and 63.5 ppm, although their differentiation was not possible. The HMBC spectrum allowed the unequivocal identification of carbonyl C-4' resonance at 173.8 ppm and of CO_2Et at 165.0 ppm. The signals at 166.1, 165.1 and 164.7 ppm were assigned to the carbonyl resonances of the three benzoyl protecting groups.

An important feature of the 1H NMR spectra of conjugates **6a** and **6b** is the presence of a signal at around -2.60 ppm due to the resonances of the inner NH protons, which confirms the success of the demetallation step. In the case of porphyrin/quinolone **6c** it is possible to note also the absence of the signals due to the resonances of the benzoyl groups confirming the presence of deprotected hydroxyl groups. The HRMS-ESI⁺ of the porphyrin/quinolone conjugates **6a-c** show the expected $[M+H]^+$ molecular ions at the m/z values 873.35446, 915.40094 and 977.36557 respectively.

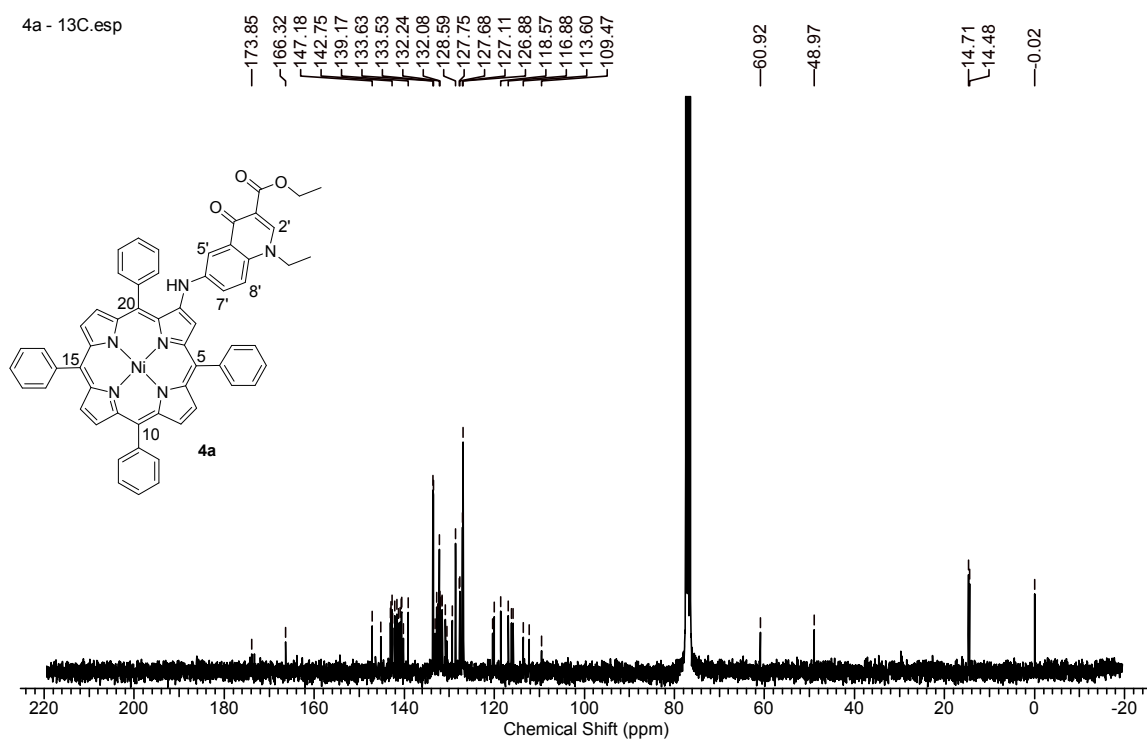
The molecular formulae of the intracyclized *N*-(6-quinolonil)quinolino[2,3,4-*af*]porphyrins **8a** and **8b** were also unambiguously confirmed by HRMS-ESI⁺ showing the expected $[M+H]^+$ molecular ions at m/z values 871.33852 and 913.38539 respectively.

The main difference between the 1H NMR spectra of conjugates **8a** and **8b** is related with the protons' signals of the aliphatic groups attached to *N*-1 being the pattern similar to the one of the corresponding non-cyclized precursors.

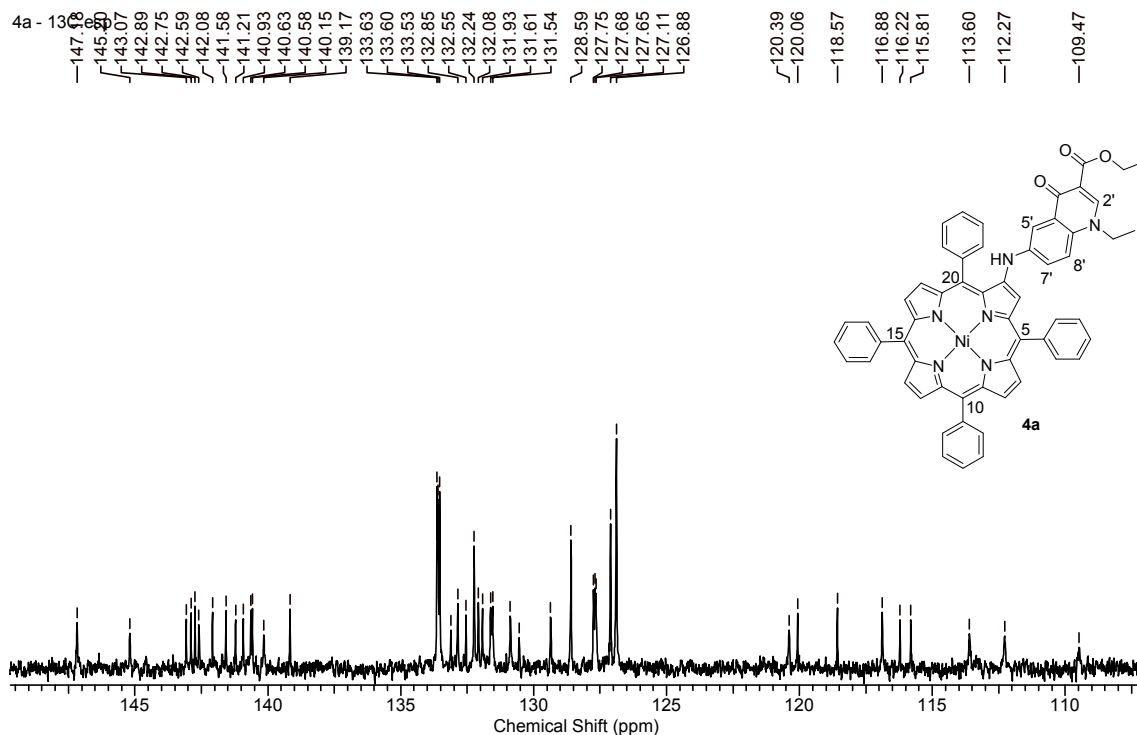
Taking into account the spectrum of derivative **8a**, the presence of two doublets at 9.69 ppm ($J = 4.7$ Hz) and 9.66 ppm ($J = 8.2$ Hz), due to H-18 and H-



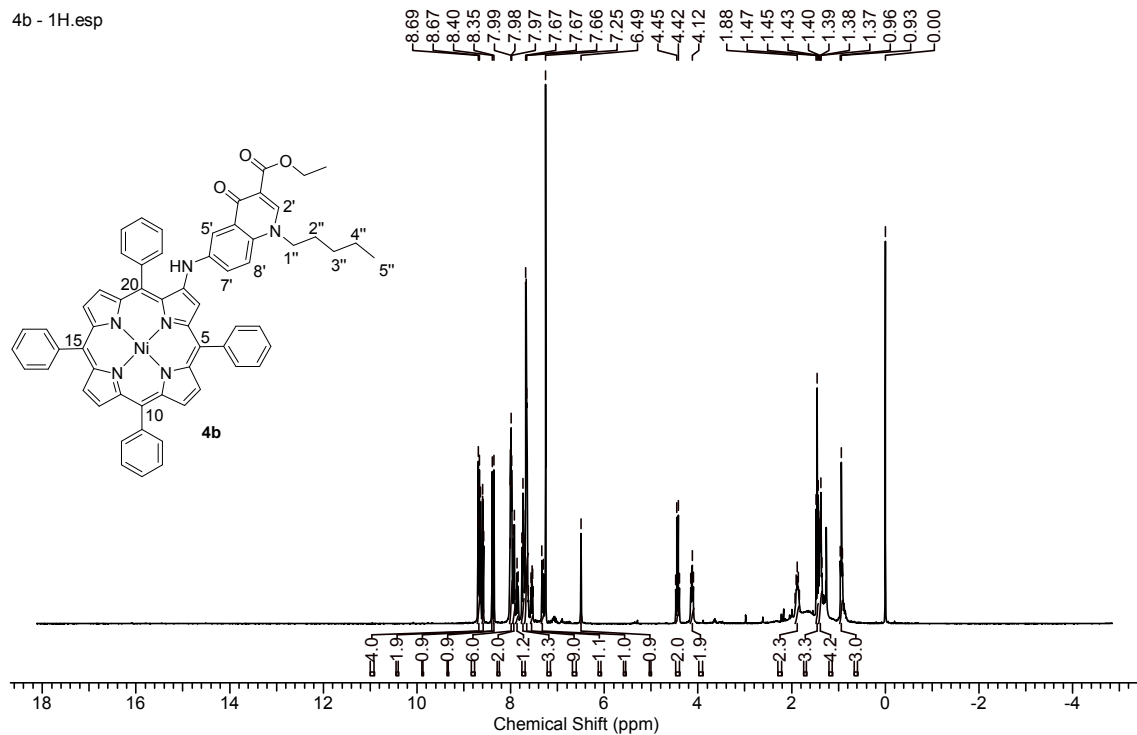
Expansion of the ^1H NMR spectrum of derivative 4a (CDCl_3 , 300 MHz).



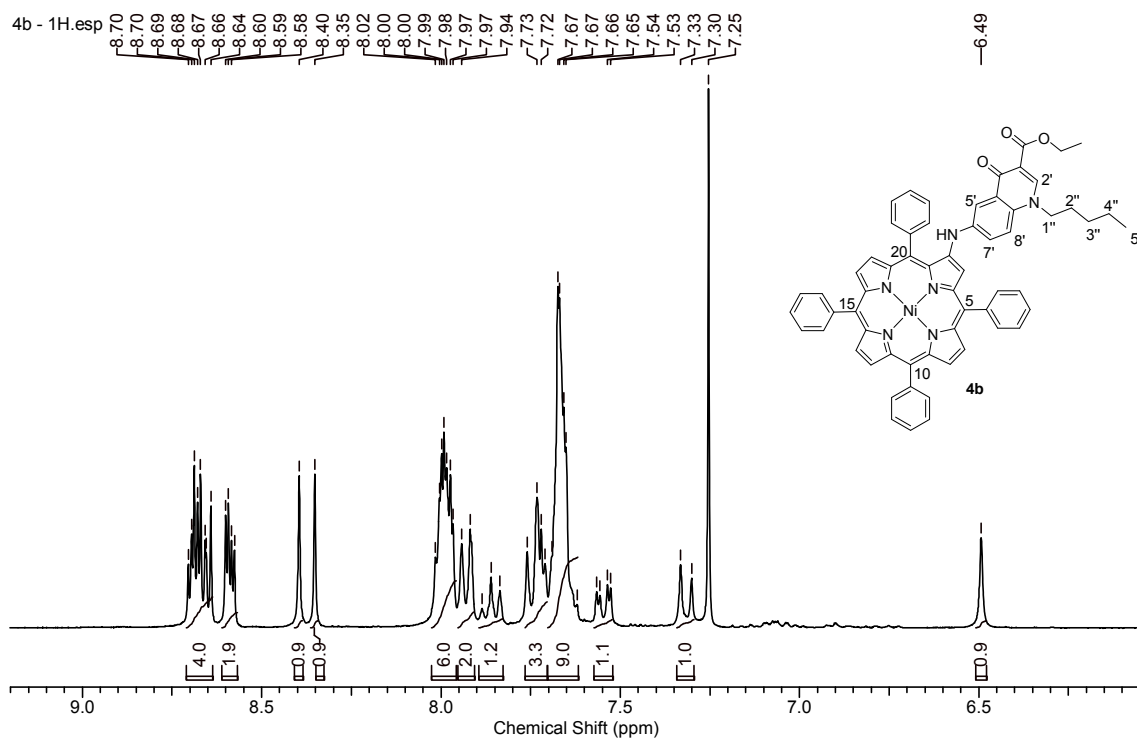
^{13}C NMR spectrum of derivative 4a (CDCl_3 , 75 MHz).



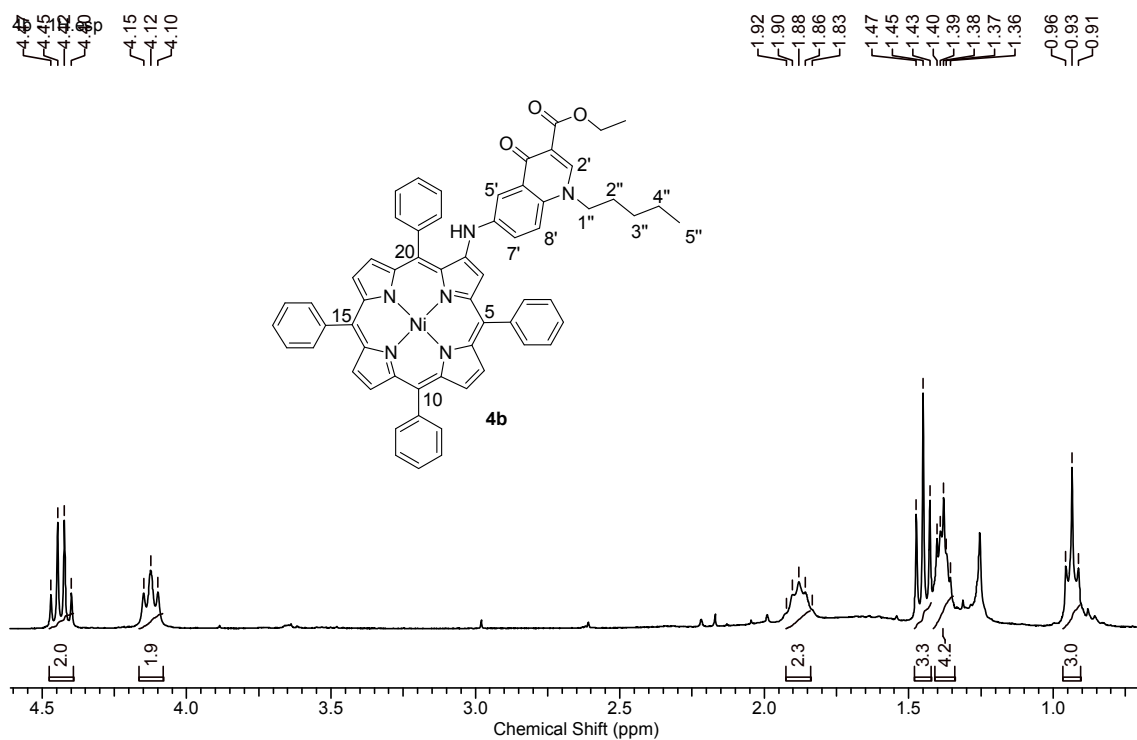
Expansion of the ^{13}C NMR spectrum of derivative 4a (CDCl_3 , 75 MHz).



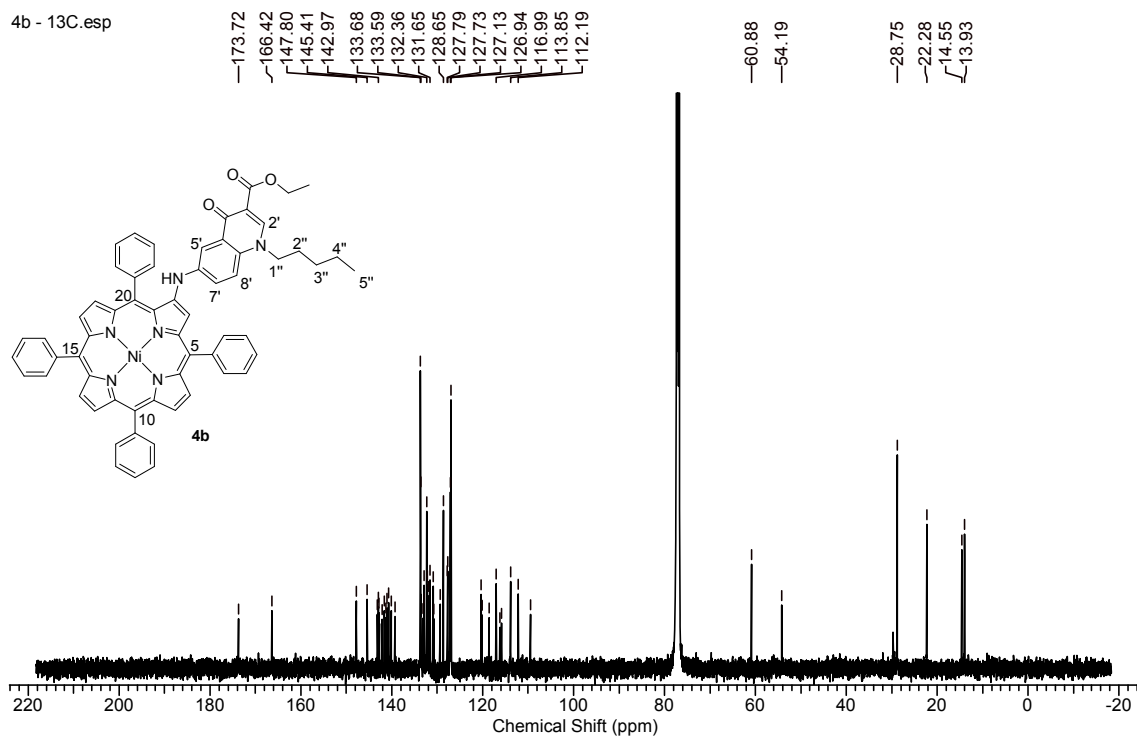
^1H NMR spectrum of derivative 4b (CDCl_3 , 300 MHz).



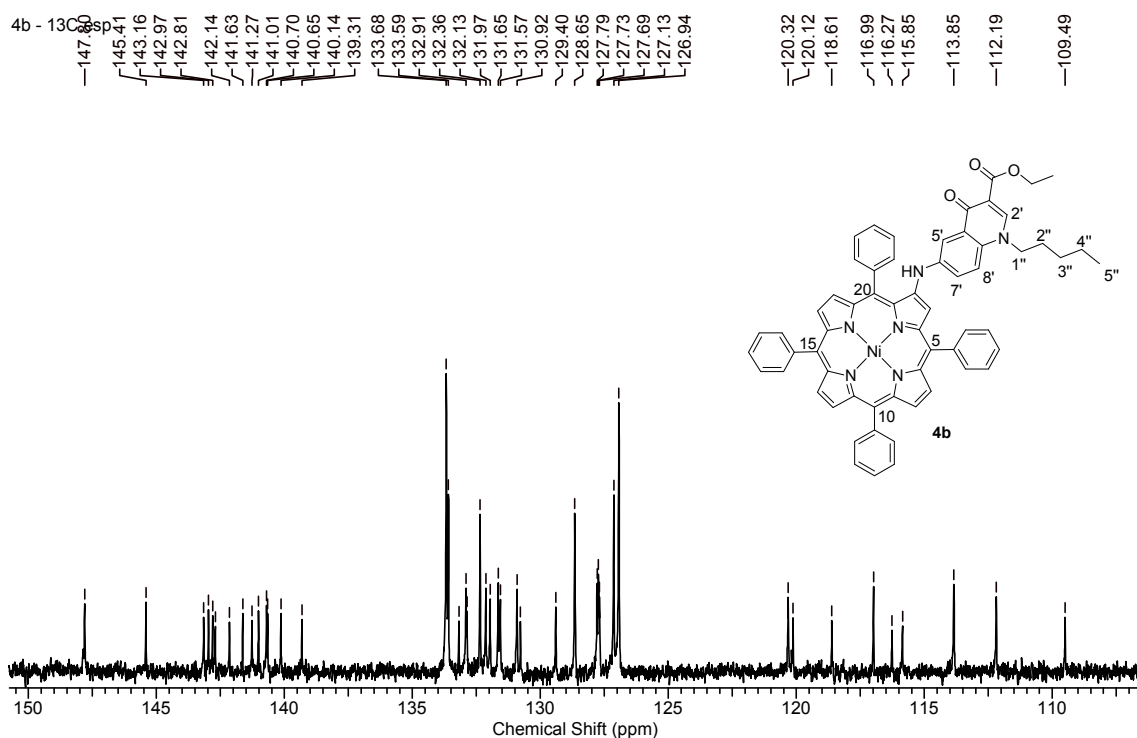
Expansion of the ^1H NMR spectrum of derivative 4b (CDCl_3 , 300 MHz).



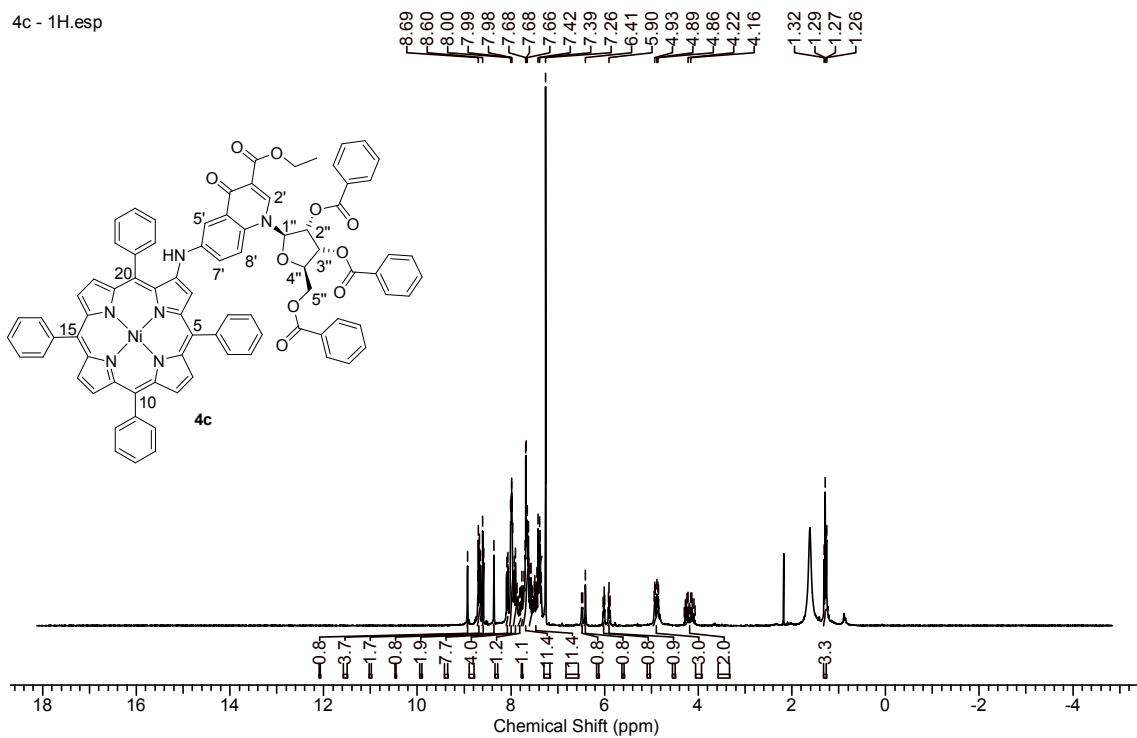
Expansion of the ^1H NMR spectrum of derivative 4b (CDCl_3 , 300 MHz).



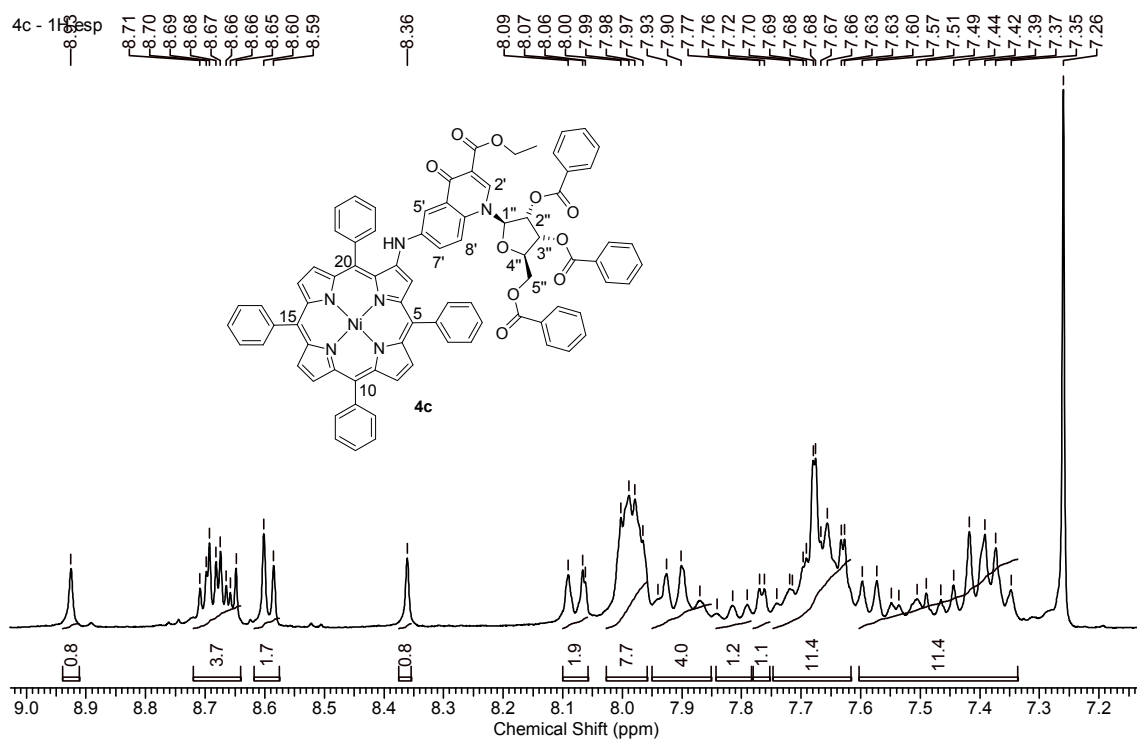
^{13}C NMR spectrum of derivative 4b (CDCl_3 , 125 MHz).



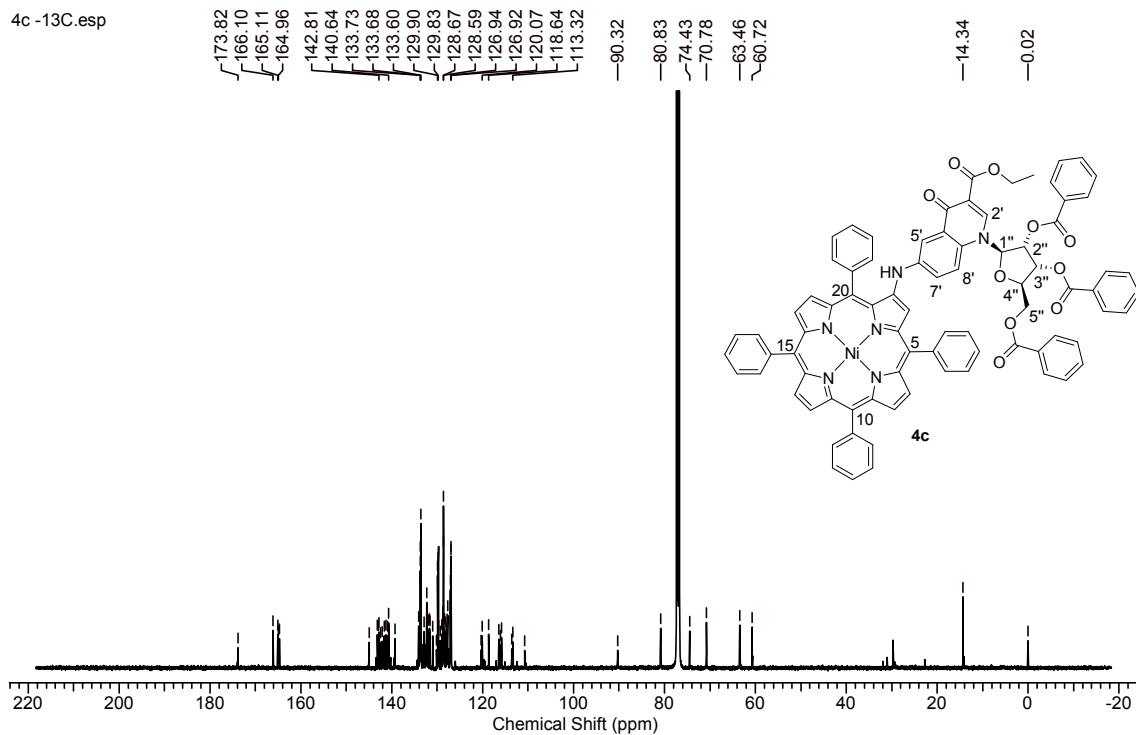
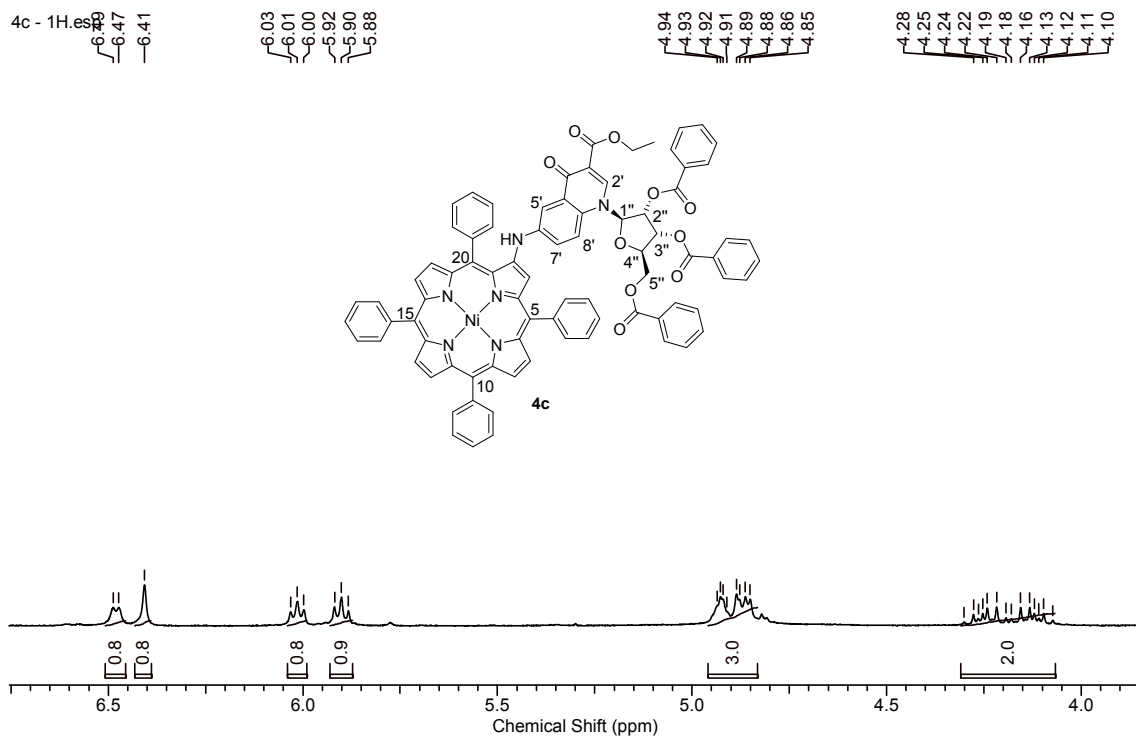
Expansion of the ^{13}C NMR spectrum of derivative 4b (CDCl_3 , 125 MHz).

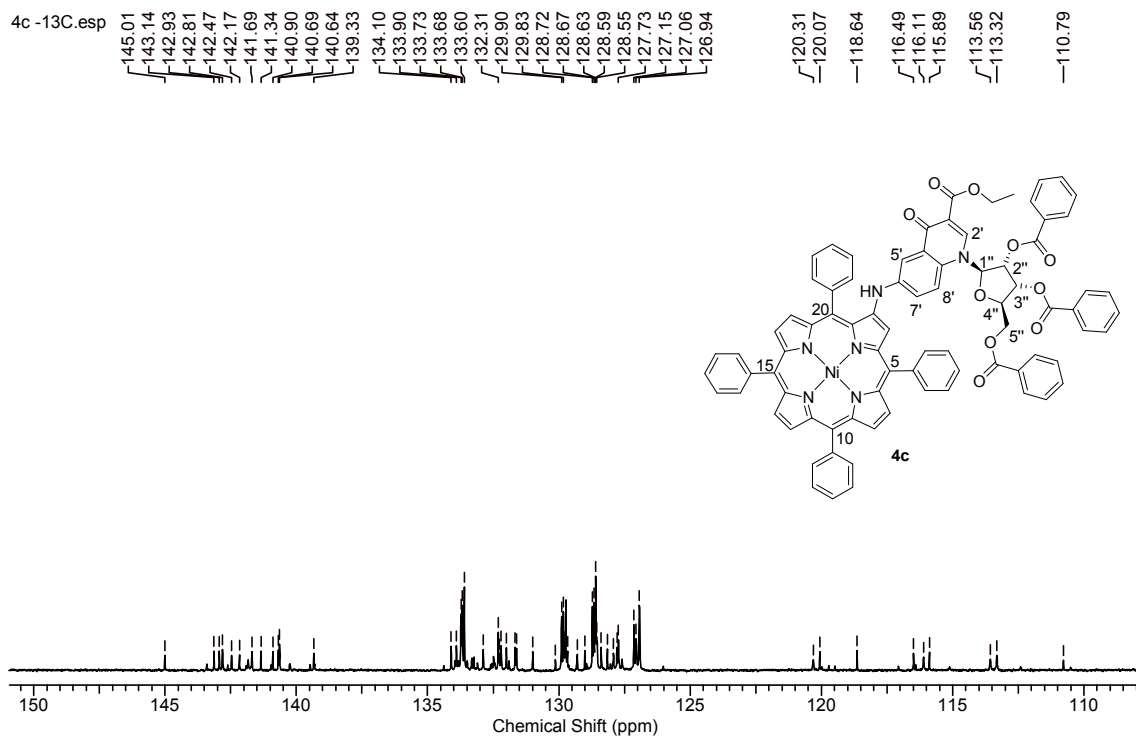


^1H NMR spectrum of derivative 4c (CDCl_3 , 300 MHz).

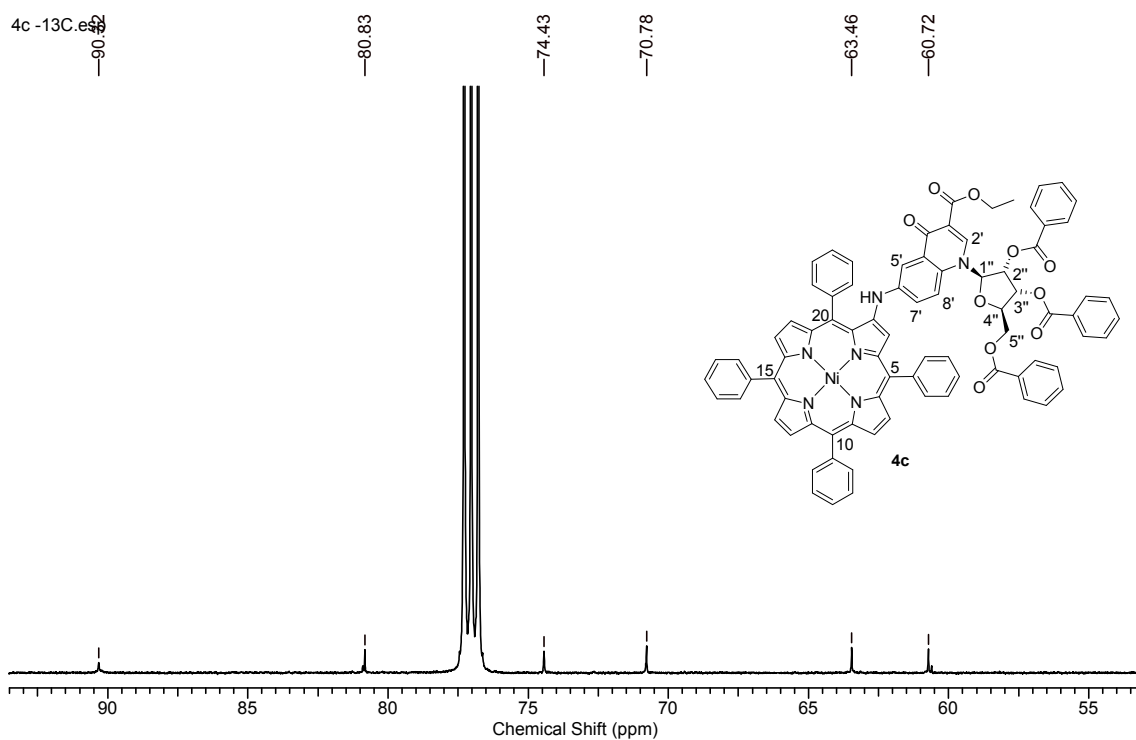


Expansion of the ^1H NMR spectrum of derivative 4c (CDCl_3 , 300 MHz).

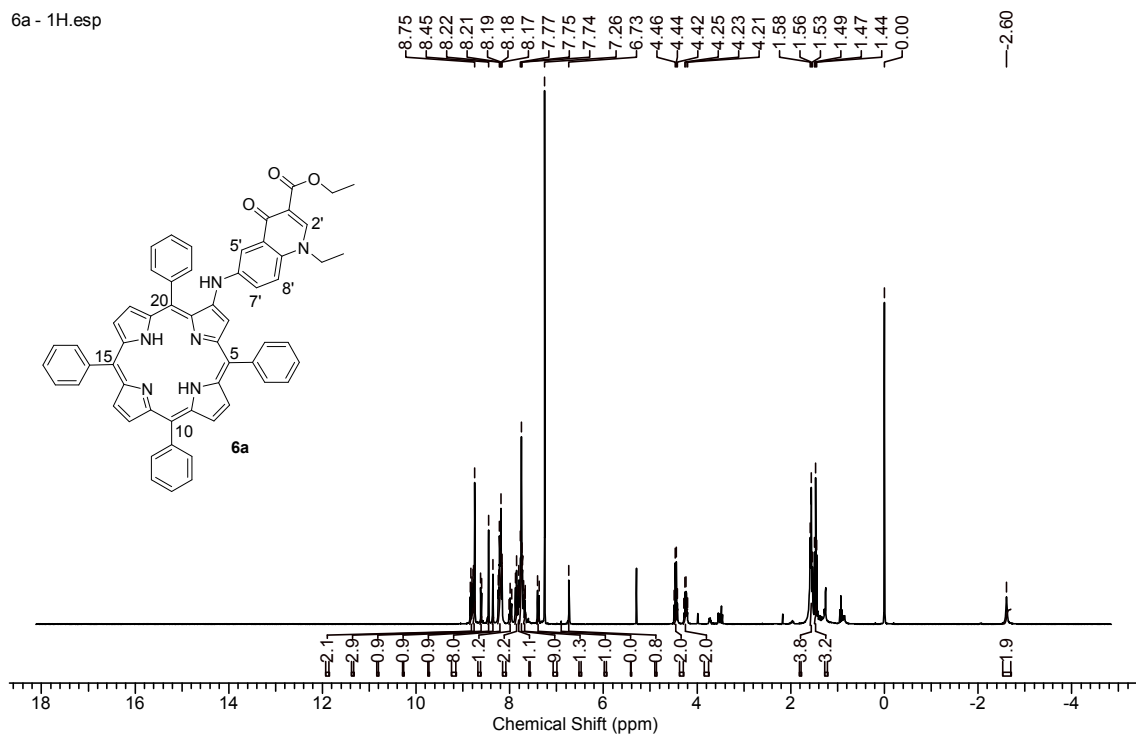




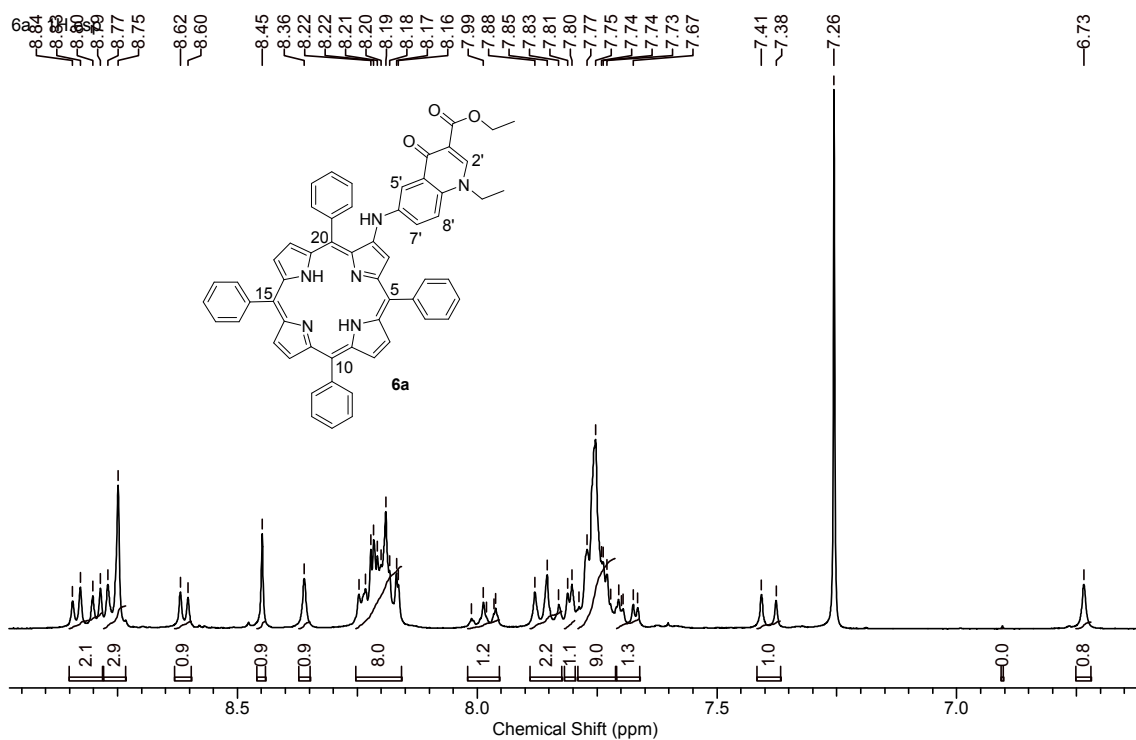
Expansion of the ^{13}C NMR spectrum of derivative 4c (CDCl_3 , 125 MHz).



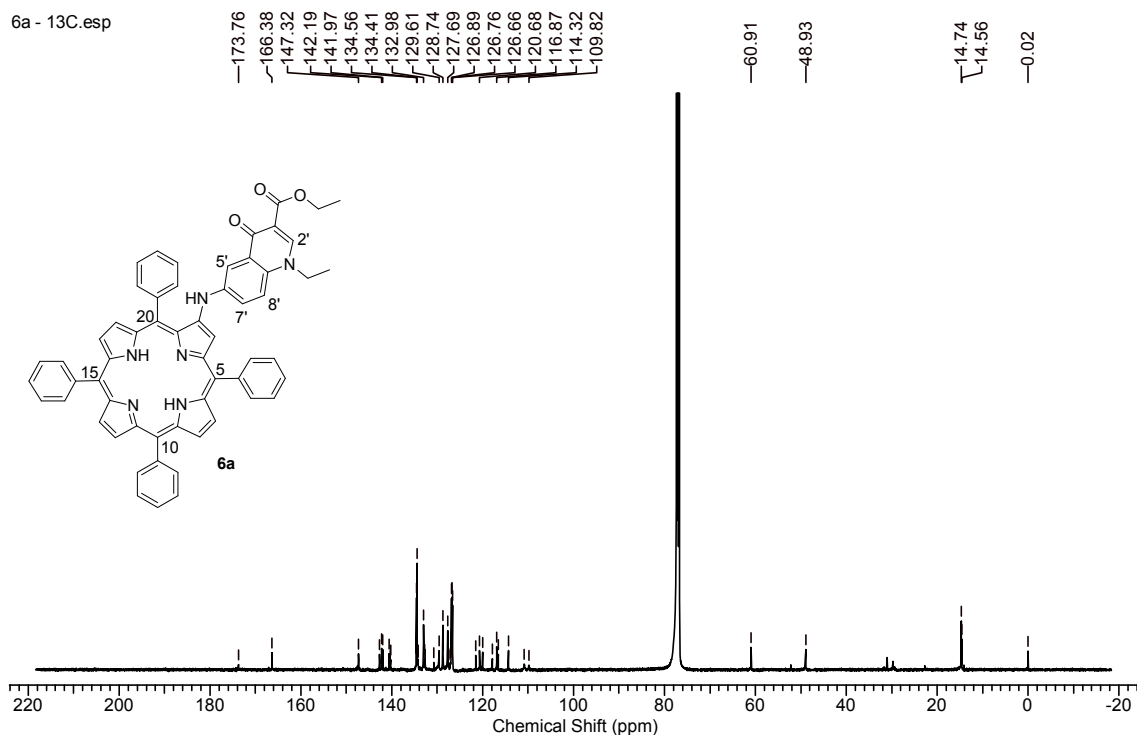
Expansion of the ^{13}C NMR spectrum of derivative 4c (CDCl_3 , 125 MHz).



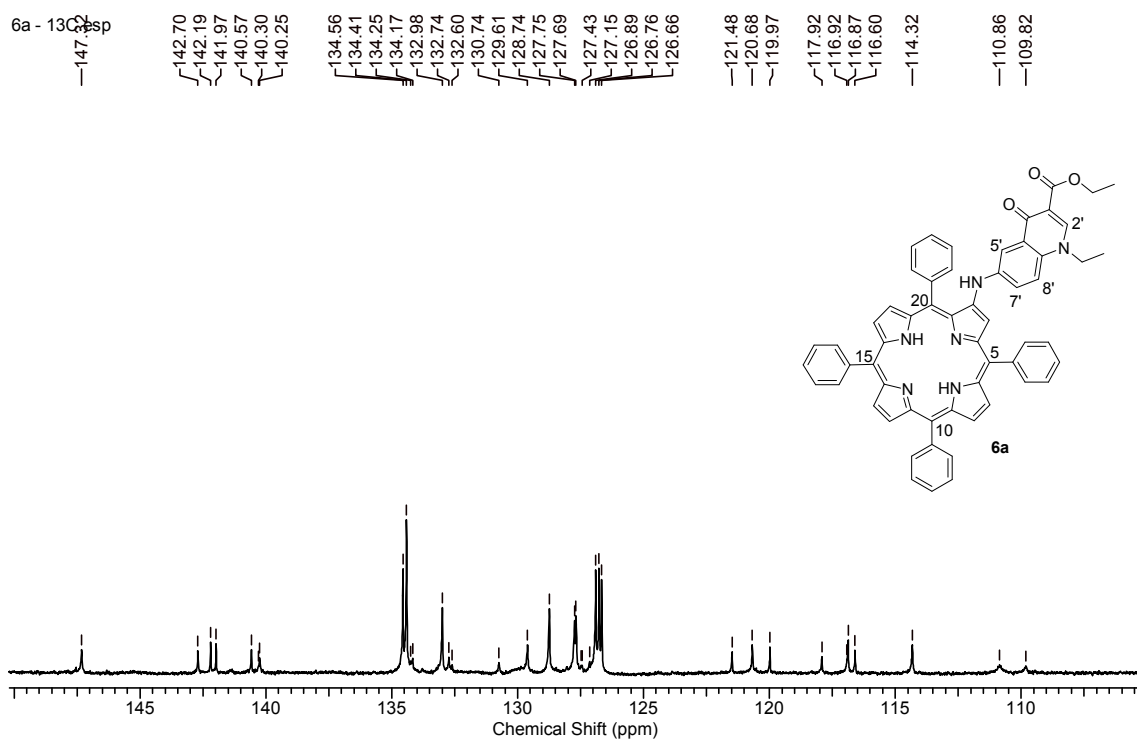
¹H NMR spectrum of derivative 6a (CDCl₃, 300 MHz).



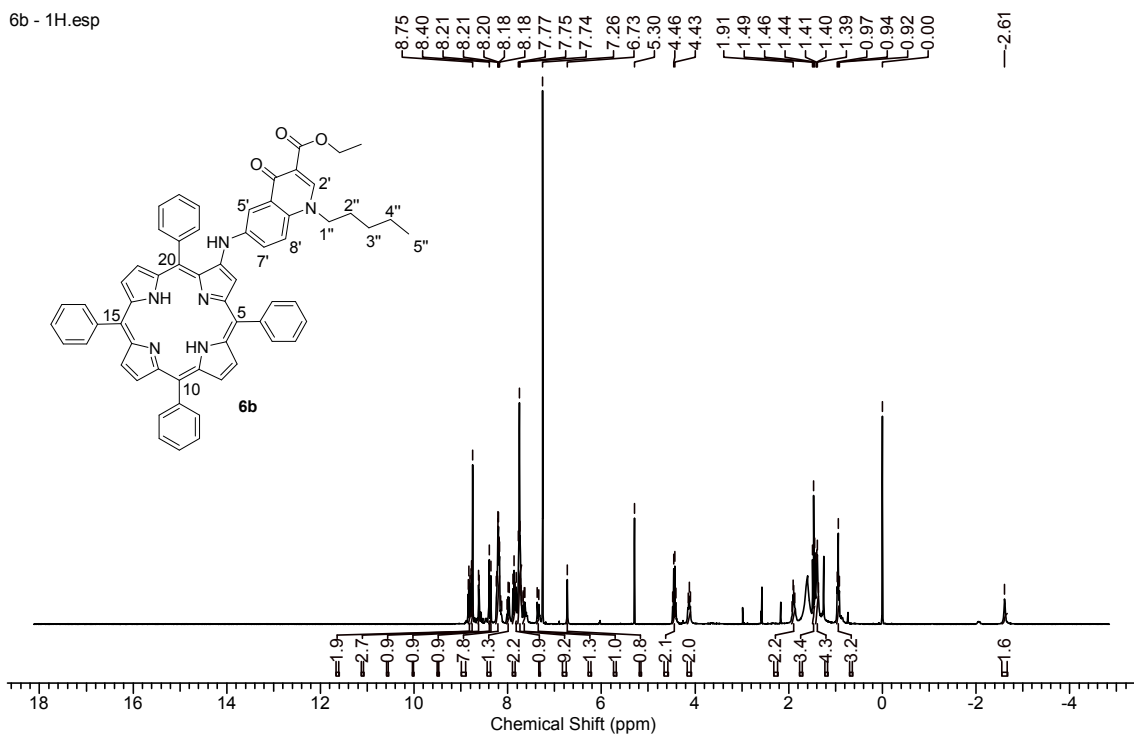
Expansion of the ¹H NMR spectrum of derivative 6a (CDCl₃, 300 MHz).



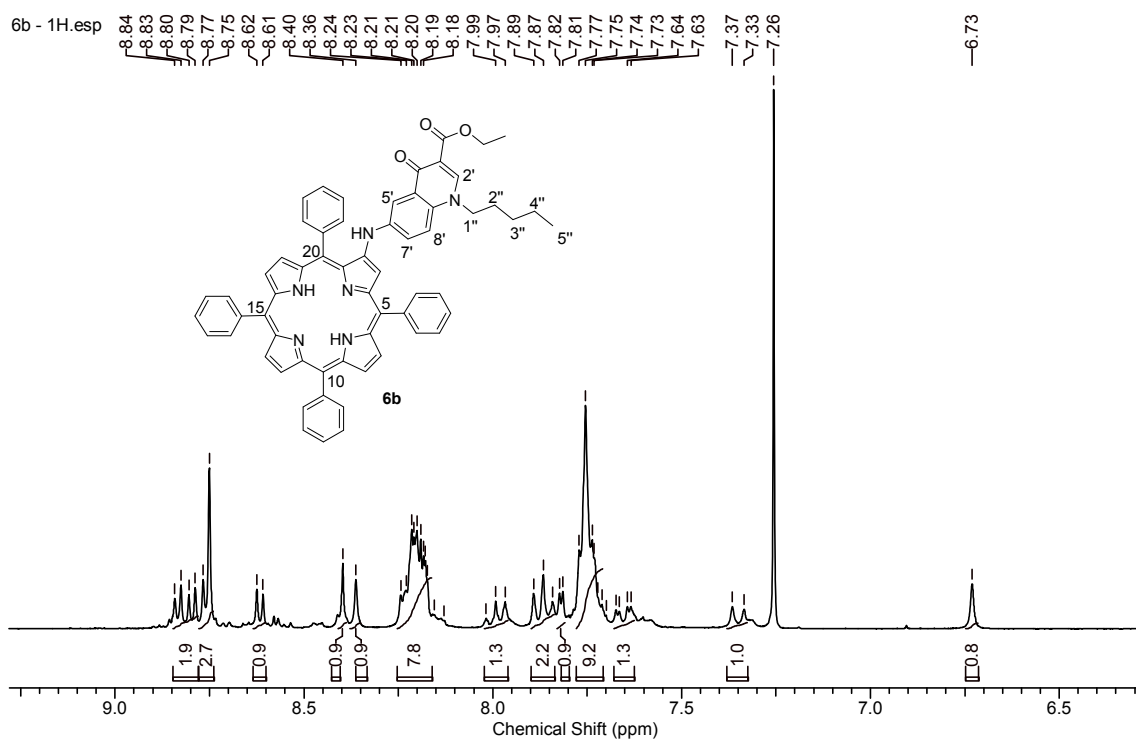
^{13}C NMR spectrum of derivative 6a (CDCl_3 , 125 MHz).



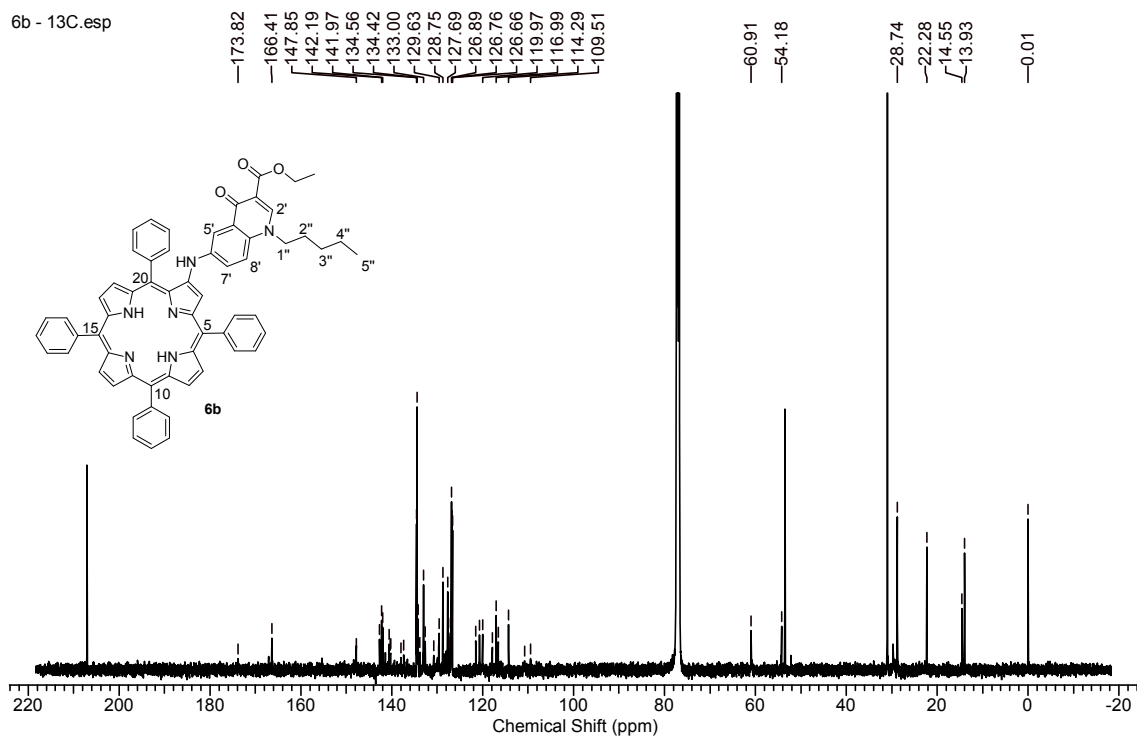
Expansion of the ^{13}C NMR spectrum of derivative 6a (CDCl_3 , 125 MHz).



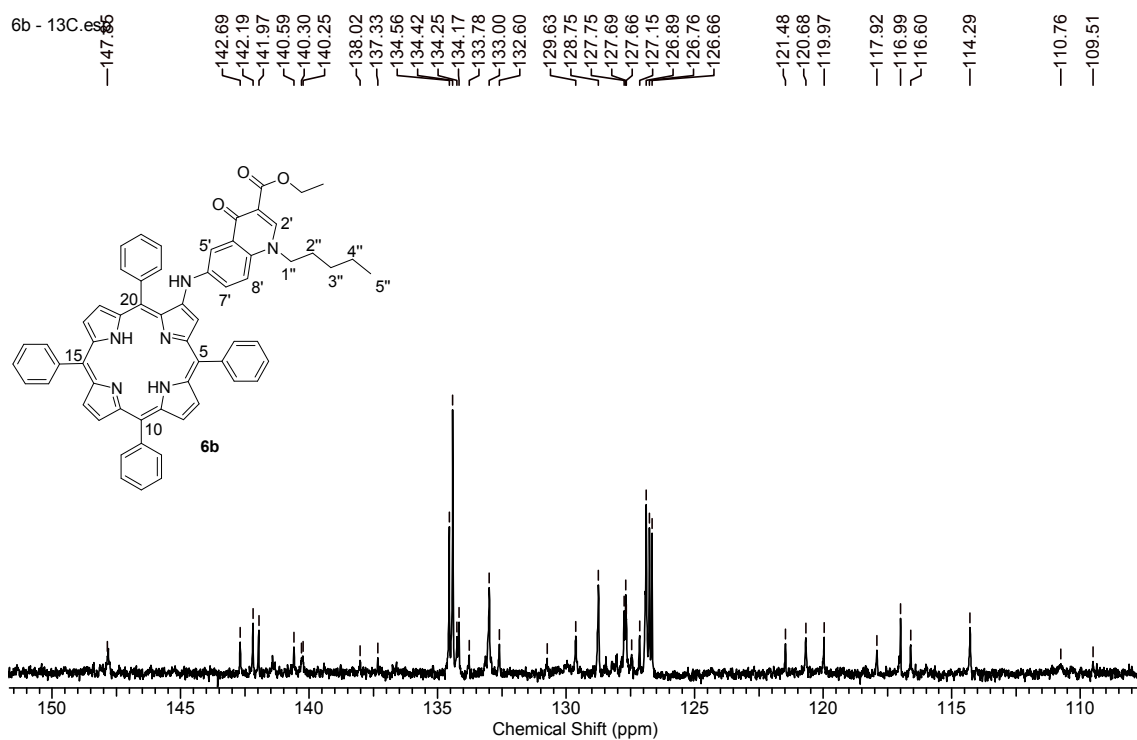
¹H NMR spectrum of derivative 6b (CDCl₃, 300 MHz).



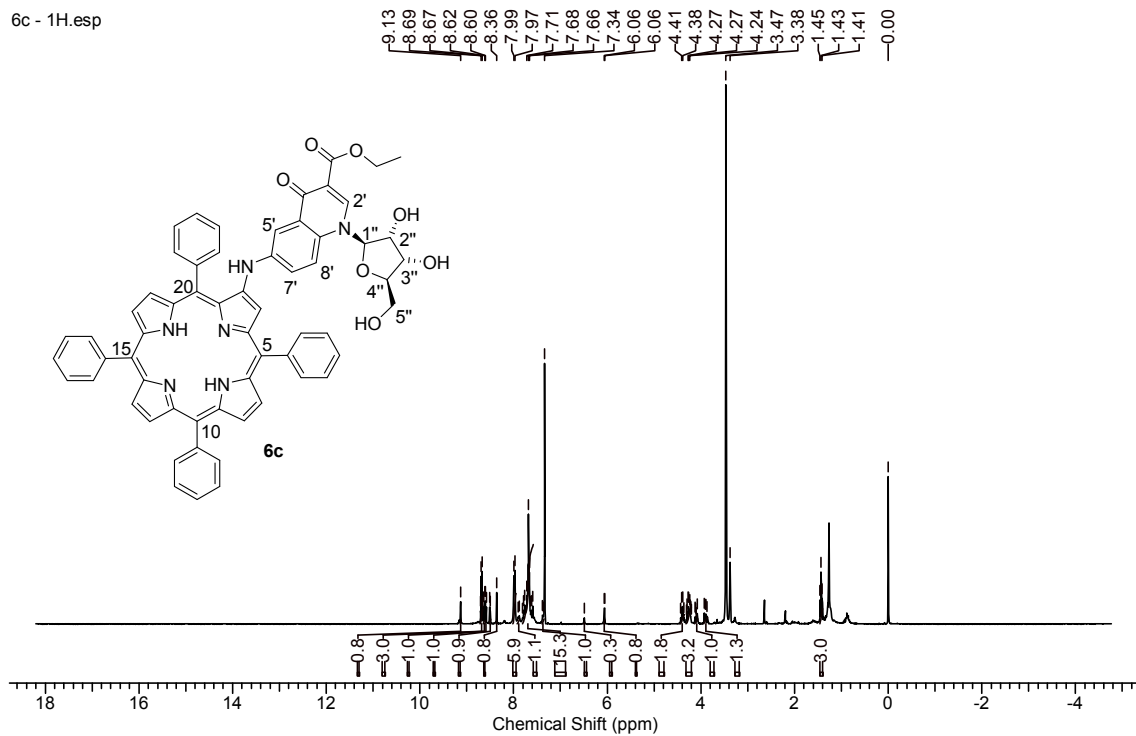
Expansion of the ¹H NMR spectrum of derivative 6b (CDCl₃, 300 MHz).



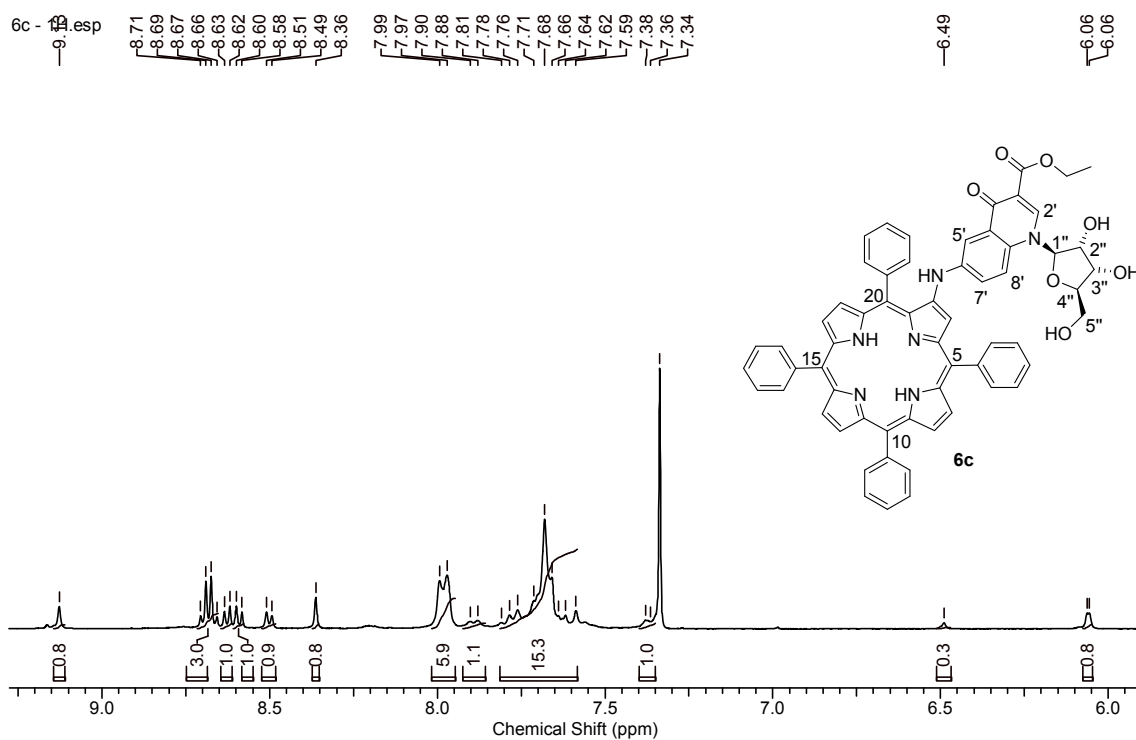
^{13}C NMR spectrum of derivative 6b (CDCl_3 , 125 MHz).



Expansion of the ^{13}C NMR spectrum of derivative 6b (CDCl_3 , 125 MHz).



^1H NMR spectrum of derivative 6c ($\text{CDCl}_3/\text{CD}_3\text{OD}$, 300 MHz).

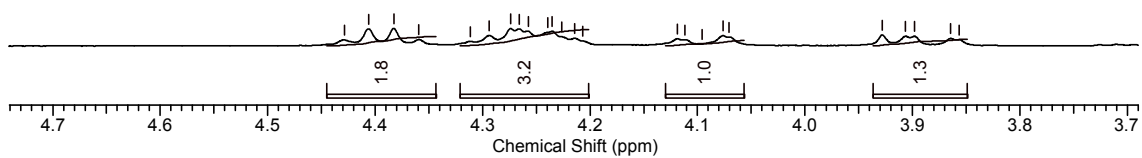
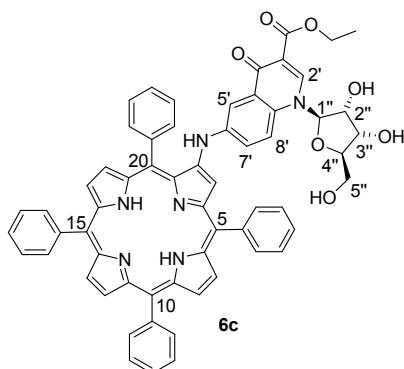


Expansion of the ^1H NMR spectrum of derivative 6c ($\text{CDCl}_3/\text{CD}_3\text{OD}$, 300 MHz).

6c - 1H.esp

4.43
4.41
4.38
4.36
4.31
4.29
4.27
4.26
4.24
4.24
4.23
4.21
4.21
4.12
4.11
4.10
4.08
4.07

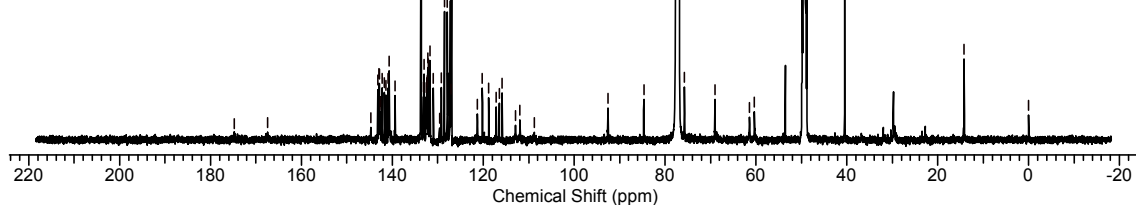
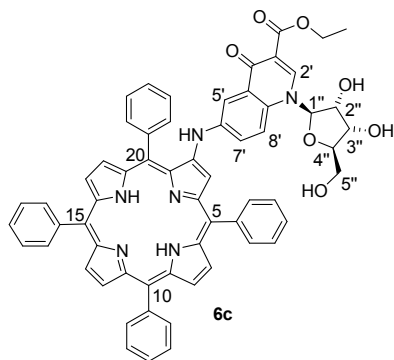
3.93
3.91
3.90
3.86
3.86



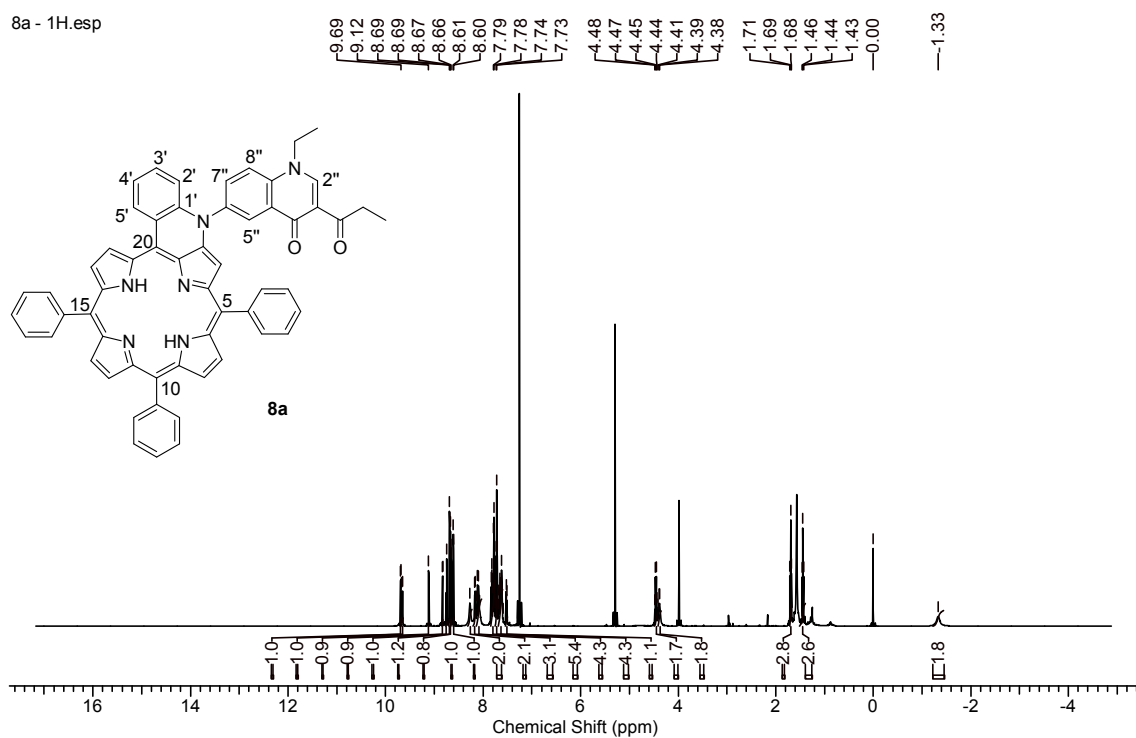
Expansion of the ^1H NMR spectrum of derivative 6c ($\text{CDCl}_3/\text{CD}_3\text{OD}$, 300 MHz).

6c - 13C.esp

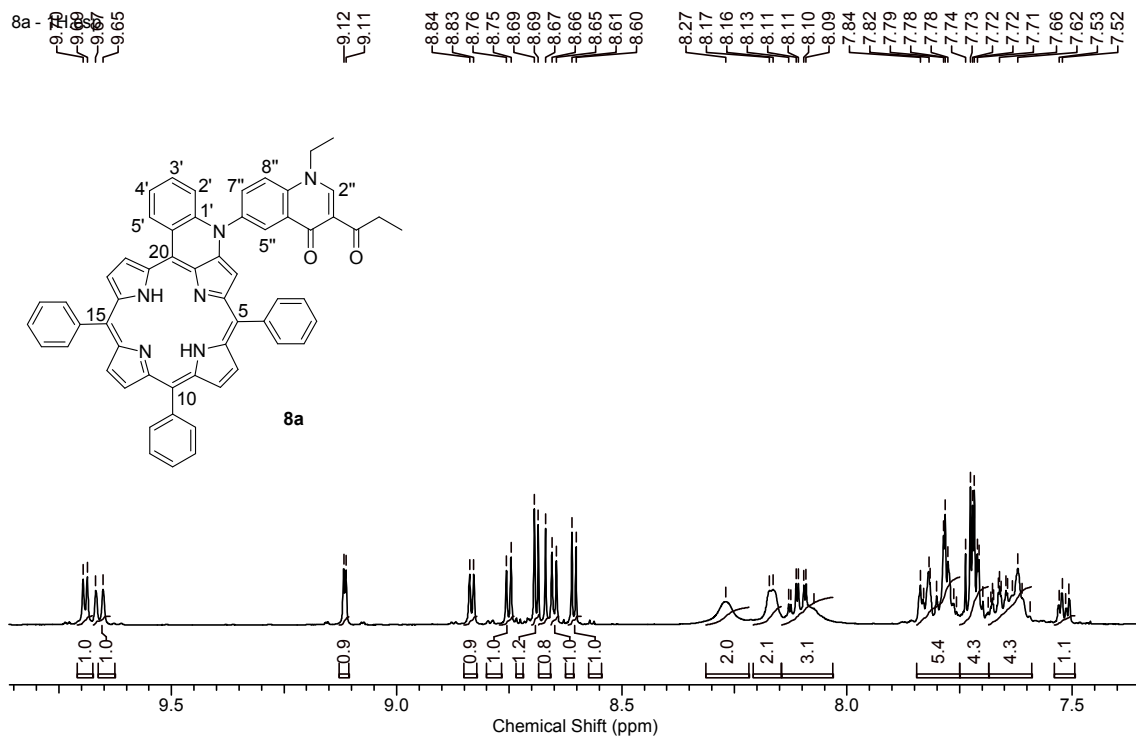
174.81
167.44
143.00
140.70
133.61
132.95
132.23
132.05
131.74
128.53
127.88
127.26
126.99
120.18
115.81
111.91
108.76
92.55
84.68
75.76
69.00
61.44
60.35
14.22
0.00



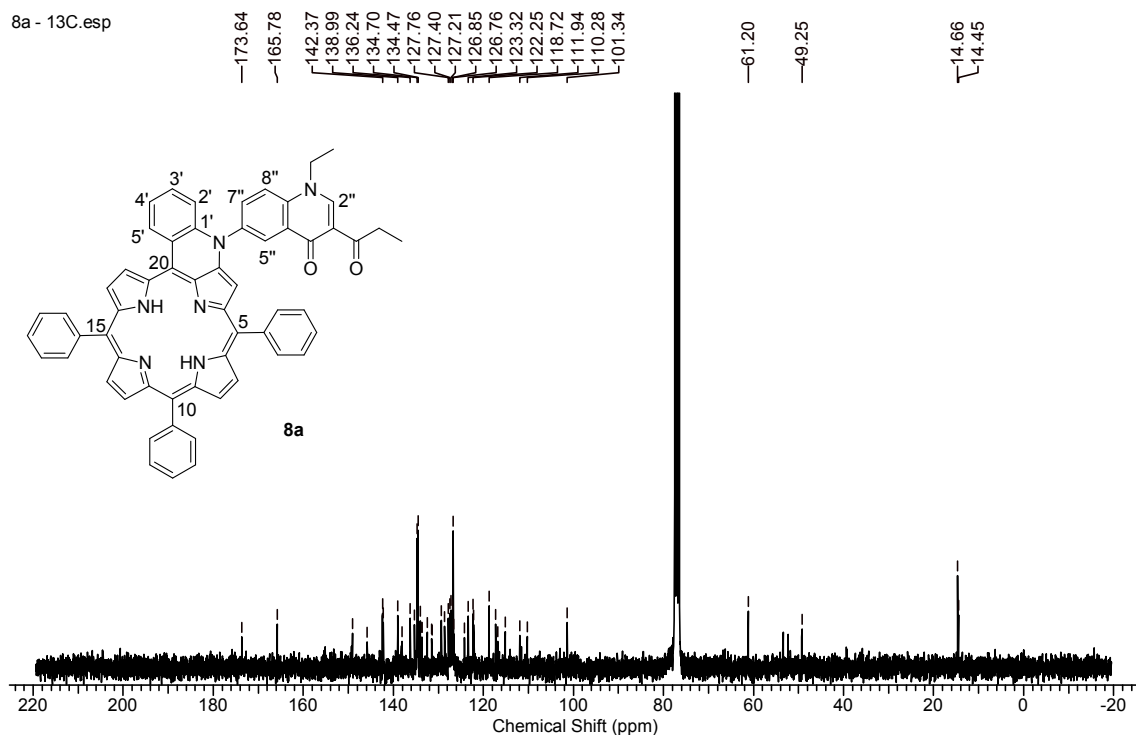
^{13}C NMR spectrum of derivative 6c ($\text{CDCl}_3/\text{CD}_3\text{OD}$, 125 MHz).



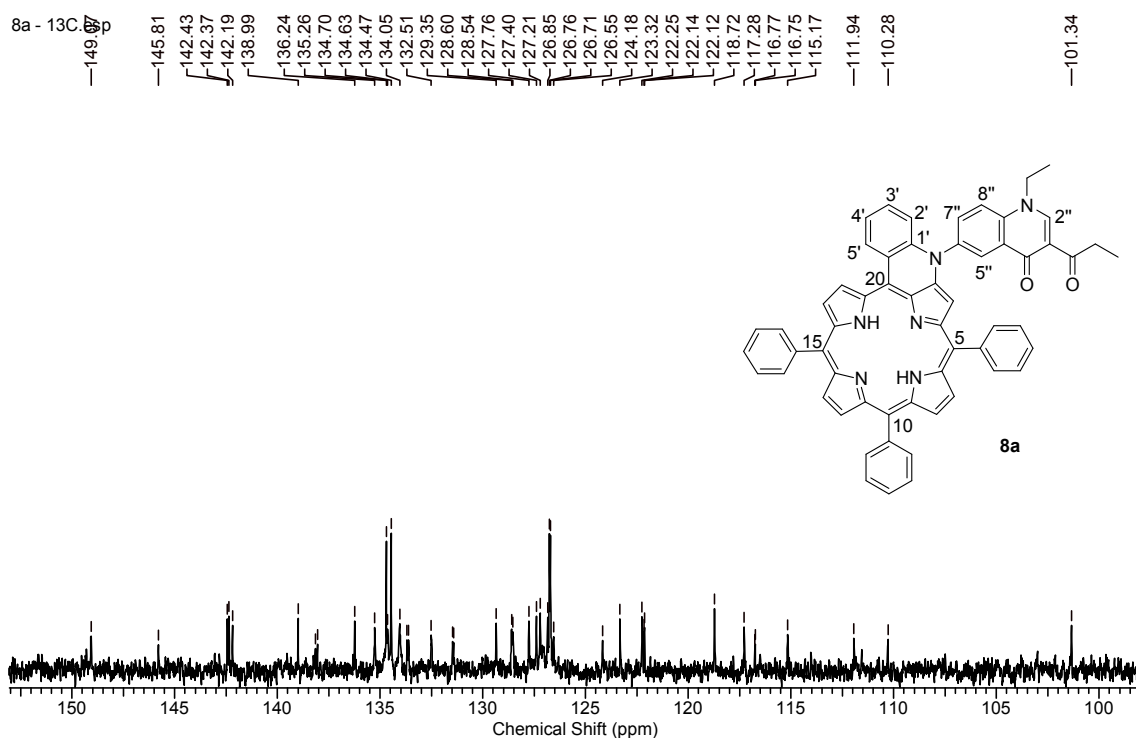
^1H NMR spectrum of derivative 8a (CDCl_3 , 300 MHz).



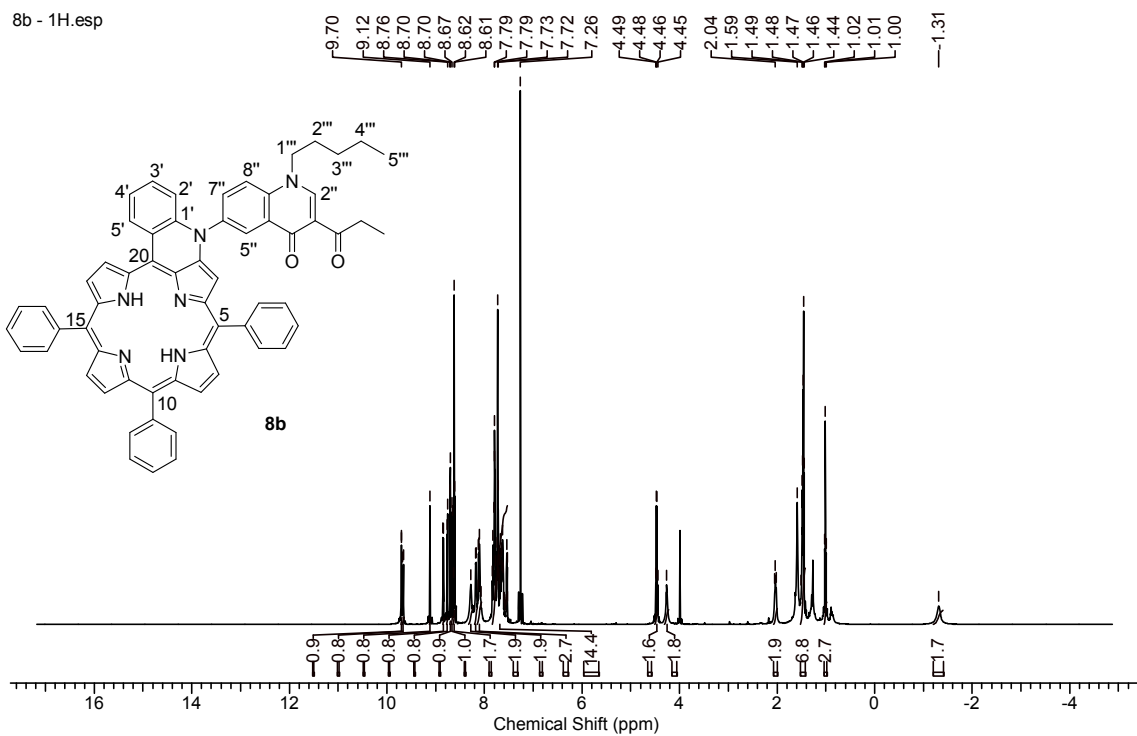
Expansion of the ^1H NMR spectrum of derivative 8a (CDCl_3 , 300 MHz).



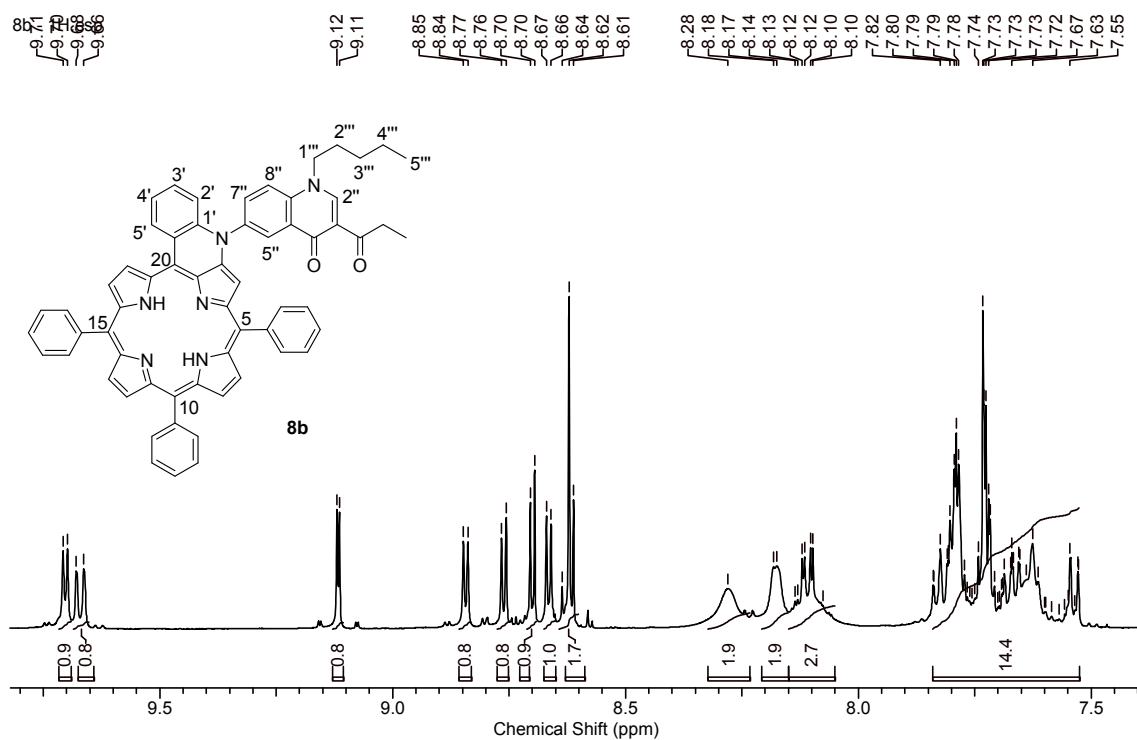
^{13}C NMR spectrum of derivative 8a (CDCl_3 , 75 MHz).



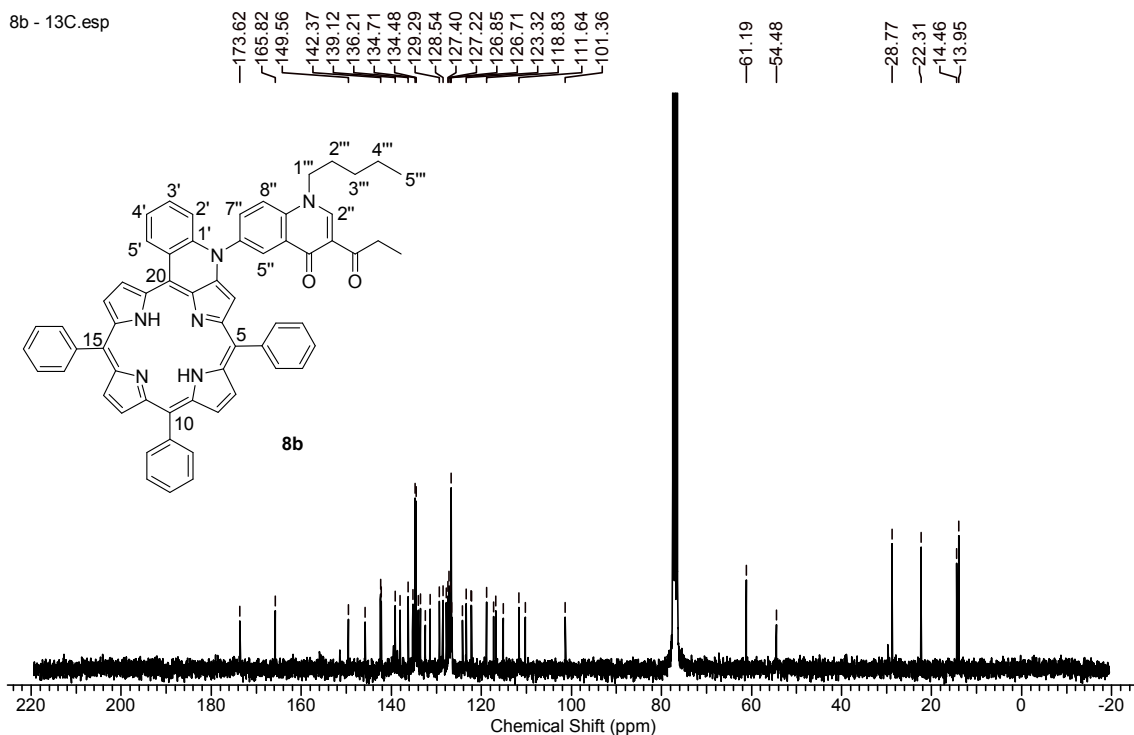
Expansion of the ^{13}C NMR spectrum of derivative 8a (CDCl_3 , 75 MHz).



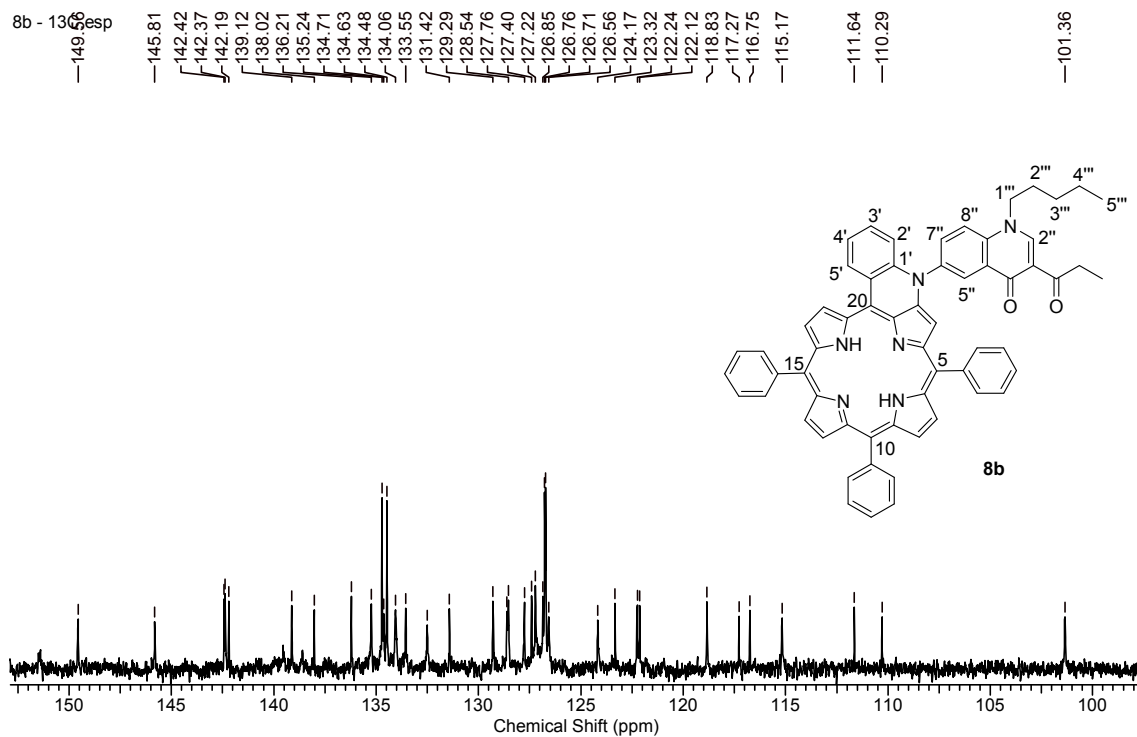
¹H NMR spectrum of derivative 8b (CDCl₃, 500 MHz).



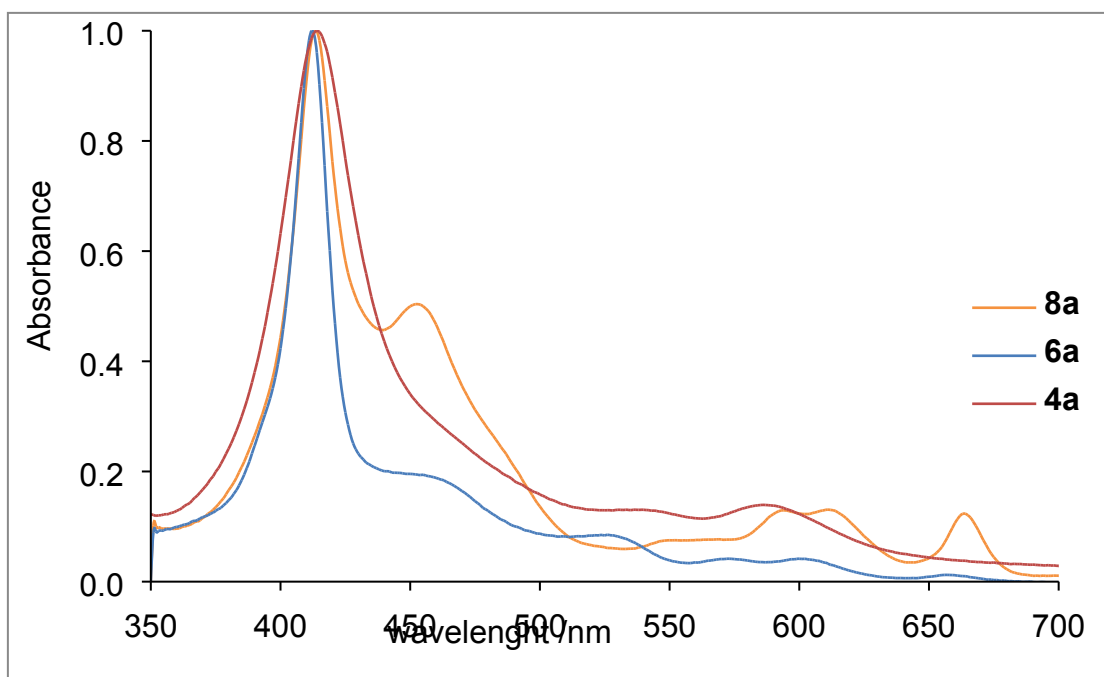
Expansion of the ¹H NMR spectrum of derivative 8b (CDCl₃, 500 MHz).



^{13}C NMR spectrum of derivative 8b (CDCl_3 , 75 MHz).



Expansion of the ^{13}C NMR spectrum of derivative 8b (CDCl_3 , 75 MHz).



Normalized UV-Vis spectra of derivatives 4a, 6a and 8a in DMF:H₂O (9:1)