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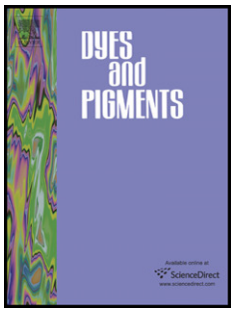

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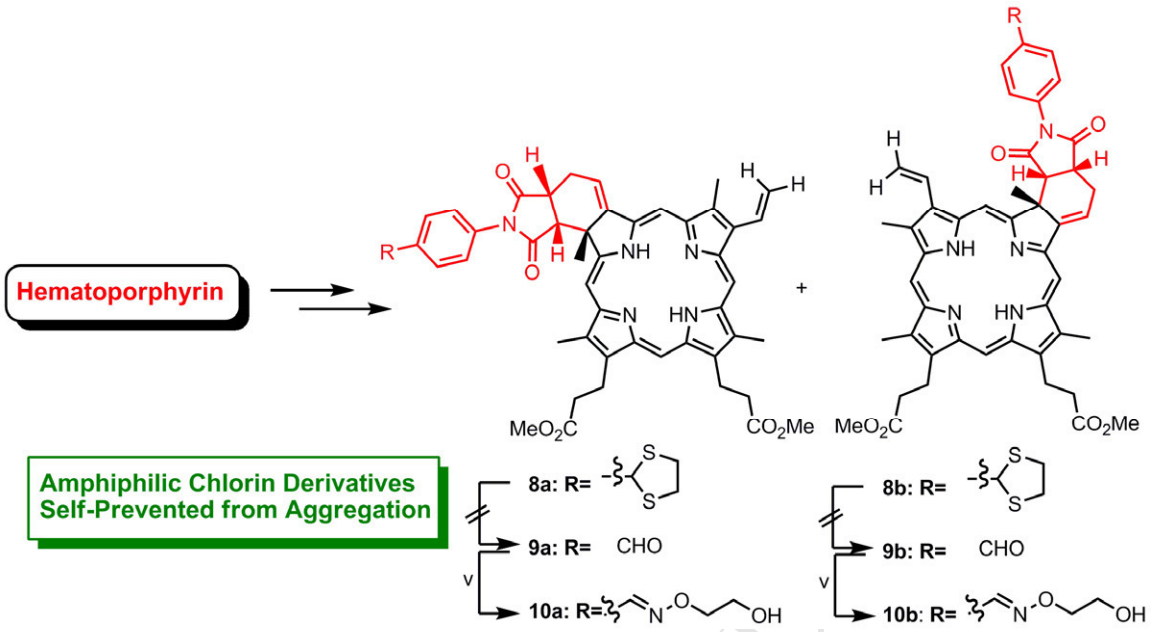

Synthesis of Functionalized Chlorins Sterically-Prevented from Self-Aggregation

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Abstract

The synthesis of six new regioisomeric chlorin derivatives sterically-prevented from self-aggregation is described. The compounds were prepared by the Diels-Alder reaction between protoporphyrin IX dimethyl ester, and *N*-[*p*-(1,3-dithiolan)phenyl]maleimide and *N*-(p-formylphenyl)maleimide. The protopophyrin IX dimethyl ester was synthesized in 2 steps from natural hematoporphyrin using a modified procedure from literature, and the substituted maleimides were conveniently synthesized, aiming at producing formyl-chlorins for subsequent functionalization with amphiphilic groups. The Diels-Alder reactions were systematically studied in order to establish optimized conditions for the cycloadditions.

The regioisomers were fully characterized and the aggregation studies were performed by NMR, UV-Vis spectroscopy, and also HRMS (ESI-TOF and MALDI-TOF). Preliminary evaluations on the photosensitizing activities and amphiphilicity were carried out indicating that these new compounds are potential candidates, to be studied in more advanced tests of Photodynamic Therapy (PDT). This work represents on advance on our previous study, with respect to these new structures, their photophysical properties and amphiphilicity.

Keywords: Protoporphyrin IX, chlorin derivatives, Diels-Alder, non-aggregation, photosensitizers, PDT and amphiphilicity.

Highlights:

-Synthesis of six chlorins sterically self-prevented from aggregation is described

-A vinyl porphyrin and *p*-substituted maleimides were used as building blocks.

-Amphiphilic chlorins were obtained after functionalizations

-These new chlorins have proven to be good candidates for PDT studies.

Introduction

Protoporphyrin IX and chlorophylls can be considered as the dyes of life since animal and vegetal existence on earth depends on the activities of these molecules.[1] For animals, protoporphyrin IX is essential in cellular breathing, and for plants the chlorophylls are essential to transfer light energy through the photosynthetic process. Therefore, there is no surprise that these biosynthetically-related pigments can act as photosensitizers, and some modified structures present improved ability to transfer light energy.[2] It is well known that when light excites a photosensitizer this may provide a type-II redox reaction in cells through singlet oxygen formation, which is an essential component of photodynamic therapy (PDT).[3]

In this context, the chemistry of protopophyrin IX has attracted attention since the discovery of the photosensitizing ability of this compound, and many papers have been published recently.[2,4,5,6] In general, protoporphyrin IX exhibits an exceptional combination of advantages, such as optimal photophysical properties, low systemic toxicity, and a great affinity for hyperproliferating tissues.[2] In addition, several of the successful commercial photosensitizers, such as Visudyne® and Lemuteporfin® are an excellent example of chlorin derivatives synthesized from protoporphyrin IX, and present remarkable photodynamic activities.[2]

However, a general limitation for the use of chlorin derivatives in PDT is their strong tendency for aggregation in solution and their low chemical stability. Stability of chlorin derivatives has been achieved by using other methodologies,

highlighting those based on C-C bond formation at the β-position by using cycloaddition reactions.[4,5,6]

In conclusion, methodologies to produce stable and non-aggregating photosensitizers are the aim of our research, since the aggregation phenomenon has also been decisive for low singlet oxygen generation and for decreased ability to act as a photosensitizer in PDT treatments.

In previous work we have demonstrated that chlorin derivatives, which were synthesized through the Diels-Alder reaction between protoporphyrin IX dimethyl ester and maleimides, presented a special “L-shape” structure and were sterically prevented from self-aggregation.[6] We now describe the synthesis and preliminary evaluation of some functionalized chlorins prepared from different substituted maleimides, which were designed to produce non-aggregating and amphiphilic photosensitizers.

1. Materials and Methods

1.1. Reagents and measurements

All reagents were of analytical grade and were purchased from Aldrich® or some national suppliers. If necessary, solvents and reagents were only used after purification according to standard procedures.[7] Protoporphyrin IX was supplied by Frontier (Texas, USA). Ultrasound irradiation was employed for deoxygenation of the toluene used in the Diels-Alder reactions. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400.13 MHz and 100.13 MHz, respectively, using $CDCl_3$ as the solvent and TMS as the

internal reference. Unequivocal ^{1}H and ^{13}C assignments were carried out by using 2D *g*COSY ($^{1}H/^{1}H$), gHSQC ($^{1}H/^{13}C$), gHMBC ($^{1}H/^{13}C$) and NOESY spectra (mixing time of 800 ms). The chemical shifts are expressed in δ (ppm) and the coupling constants (J) are given in Hertz (Hz). HRMS were obtained using the ESI-TOF (Max Impact Bruker) and MALDI-TOF (AutoFlex Speed Bruker, laser 355 nm, 50 Hz, spectrometer, α-cyano-4-hydroxycinnamic acid as matrix, and Flex analysis as processing software). The UV-Vis absorption spectra were recorded on a Perkin Elmer Lambda 25 spectrophotometer.

Column chromatography was carried out using silica gel 200-400 mesh from Aldrich, and the preparative thin layer chromatography (TLC) was conducted on Aluminum sheets (1 mm thick) – Merck TLC Silica gel 60 F_{254}.

1.2. *Synthesis of 2-(4-nitrophenyl)-1,3-dithiolane* (**2**):

To a solution of 4-nitrobenzaldehyde (**1**) (5.10 g, 34.0 mmol) in anhydrous dichloromethane (150 mL), 1,2-ethanedithiol (14.8 mL, 17.6 mmol) and $BF_3.OEt_2$ (0.8 mL) were added, maintaining the stirring at room temperature for 6 h. After that, the reaction was washed with 5% NaOH (100 mL), water (100 mL) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was distilled off under reduced pressure. Compound **2** was purified by flash chromatography in silica gel using hexane:ethyl acetate (8:2) as eluent. Yield: 6.90 g; 30.4 mmol; 89%. **mp**: 76-78^{o}C. **^{1}H NMR (**$CDCl_3$, 400 MHz**)** *δ* (ppm)***:*** 3.36-3.56 (m, 4H); 5.65 (s, 1H); 7.67 (d, 2H, *J* = 8.6 Hz); 8.16 (d, 2H, *J* = 8.6 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) *δ* (ppm)***:*** 148.6 (1C); 147.4 (1C); 128.8 (2CH); 123.7 (2CH); 54.9 (1CH); 40.5 ($2CH_2$). **IR** (ν_{max}., KBr, cm^{-1}): 3115,

3099, 3078, 2920, 2853,1108, 1605, 1593, 1517, 1349, 866, 727 , 715. **GC-MS** m/z: 227, 199, 182, 166, 152, 121, 77.

1.3. *Synthesis of 4-(1,3-dithiolan-2-yl)aniline* (**3**):

To a solution of compound **2** (5.08 g, 22.0 mmol) in 95% ethanol, $SnCl_2.2H_2O$ (25.3 g, 0.11 mol) was added. The reaction was heated at 70^oC for 3h. The reaction mixture was then poured onto crushed ice (100 g) and the pH was adjusted with aqueous $NaHCO_3$ 5% to about 8. The mixture was extracted with ethyl acetate (300 mL) and the organic layer was washed with brine (100 mL) and dried over Na_2CO_3. The solvent was distilled off under reduced pressure and compound **3** was obtained without further purification. Yield: 4.30 g; 21.7 mmol; 93%. **mp**: 56-58^oC.**^{1}H NMR** ($CDCl_3$, 400MHz) δ (ppm): 3.28-3.36 (m, 2H); 3.45-3.52 (m, 2H); 3.69 (br.s, 2H); 5.61 (s, 1H); 6.61 (d, 2H, *J* = 8.4 Hz); 7.31 (d, 2H, *J* = 8.4 Hz). **^{13}C NMR** ($CDCl_3$, 100MHz) δ (ppm)**:** 146.3 (1C); 129.1 (1C); 129.0 (2CH); 114.9 (2CH); 56.4 (1CH); 40.1 ($2CH_2$). **IR** (ν_{max}., KBr, cm^{-1}): 3423, 3383, 3308, 3032, 3007, 2916, 1624, 1607, 1513, 1279, 1174, 750, 685, 525.

1.4. *Synthesis of 1-[4-(1,3-dithiolan-2-yl)phenyl]-1H-pyrrole-2,5-dione* (**4**):

To a solution of compound **3** (3.73 g, 18.9 mmol) in CH_2Cl_2 (20 mL) at 0^oC, maleic anhydride (1.85 g, 18.9 mmol) dissolved in CH_2Cl_2 (20 mL) was slowly added. After 1h, the solid formed was filtered and washed with small portions of

cooled CH_2Cl_2. The solid amide-acid was dried under vacuum and used without further purification. Yield: 4.98 g; 16.9 mmol; 84%. **mp**: 181-183^oC. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 3.30-3.55 (m, 4H); 5.72 (s, 1H); 6.31 (d, 1H, J = 12.1Hz); 6.47 (d,1H, J = 12.1 Hz); 7.46-7.48 (m, 2H); 7.56-7.58 (m, 2H); 10.43 (s,1H); 12.97 (s,1H). **^{13}C NMR** (DMSO-d_6, 100 MHz) δ (ppm): 39.7 ($2CH_2$); 54.7 (1CH); 119.4 (1CH); 128.4 (1CH); 130.5 (2CH); 131.5 (2CH); 136.0 (1C); 138.1 (1C); 163.2 (1C); 166.9 (1C). **IR** (ν_{max}., KBr, cm^{-1}): 3092; 1920; 1857; 1794; 1698; 1524; 1504; 1323; 852.

To the amide-acid obtained previously (2.50 g, 8.46 mmol), anhydrous sodium acetate (1.21 g, 8.46 mmol) and freshly purified acetic anhydride (100 mL) were added at room temperature. After 1h stirring under a N_2 atmosphere, the reaction was heated at 90^{o}C for 1h. Cold water (200 mL) was added and the reaction was stirred up to room temperature (~ 30 min). The reaction was extracted with ethyl acetate (2 x 100 mL) and the organic layer washed with saturated $NaHCO_3$ solution (2 x 100 mL), brine (100 mL) and dried over anhydrous Na_2SO_4. Compound **4** was obtained without further purification, after solvent evaporation under reduced pressure. Yield**:** 2.14 g; 7.75 mmol; 92%. **mp**: 168-170^{o}C. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 3.34-3.40 (m, 2H); 3.47-3.54 (m, 2H); 5.65 (s, 1H); 6.85 (s, 2H); 7.31 (d, 2H, J = 8.5 Hz); 7.63 (d, 2H, J = 8.5 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 169.4 (2C); 140.3 (1C);134.2 (2CH); 130.7 (1C); 128.8 (2CH); 125.9 (2CH); 55.6(1CH); 40.2 ($2CH_2$). **IR** (ν_{max}., KBr, cm^{-1}): 3097, 2920,1513, 1400, 1711, 1151, 832, 710. **HRMS (ESI-TOF)**: *m/z* calculated for $[M+H]^+$ 278.0304; found 278.0301.

1.5. *Synthesis of 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzaldehyde* (**5**):

To a solution of compound **4** (2.00 g, 7.21 mmol) in acetone (80 mL) at 0°C, NBS (5.13 g, 28.8 mmol) dissolved in acetone:water 9:1 (80 mL) was added, and the stirring was maintained for 1h. Water (100 mL) was added and the reaction was extracted with CH_2Cl_2 (3 x 100 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent distilled off under reduced pressure. Compound **5** was purified by flash chromatography in silica gel using toluene:ethyl acetate (9:1) as eluent. Yield**:** 1.26 g; 6.26 mmol; 87%. **mp**: 129-131°C. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 10.04 (s, 1H); 6.91 (s, 2H); 7.63 (d, 2H, *J* = 8.6 Hz); 7.99 (d, 2H, *J* = 8.6 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 125.7 (2CH); 130.4 (2CH); 134.5 (2CH); 135.0 (C); 136.6 (C); 168.8 (2C); 191.1 (1CH). **IR** (ν_{max}., KBr, cm^{-1}): 2854, 1776, 1718, 1701, 1603, 1395, 837, 827. **HRMS (ESI-TOF)**: *m/z* calculated for $[M+H]^+$ 202.0499; found 202.0504.

*1.6. Synthesis of protoporphyrin IX dimethyl ester (**7**):*

To a solution of 2.00 g of hematophorphyrin (**6**) (3.34 mmol) in chlorobenzene (1L), dried *p*-toluenesulfonic acid (5.0 g, 30 mmol) was added and the reaction was heated at 140°C under a N_2 atmosphere for 3h. After that, the chlorobenzene was distilled off under reduced pressure, and the reaction was treated with a 5% methanolic solution of H_2SO_4 (670 mL) for 12h at room temperature and with protection from light. The reaction was then poured onto 800 g of crushed ice and neutralized with concentrated NH_4OH solution to a pH of about 9. The reaction was extracted with CH_2Cl_2 (3 x 100 mL) and the

organic layer was dried over Na_2SO_4. Compound **7** was obtained after solvent evaporation and crystallization from $CHCl_3$:MeOH (1:3). Yield: 1.40 g, 2.40 mmol, 72%. **^{1}H NMR** ($CDCl_3$, 400MHz) δ ppm*:* –3.69 (s, 2H); 3.28 (t, 4H, *J* = 7.5 Hz); 3.62 (s, 3H); 3.63 (s, 3H); 3.66 (s, 6H); 3.70 (s, 3H); 3.71 (s, 3H); 4.40 (t, 4H, *J* = 7.5 Hz); 6.17 (d, 2H, *J* = 11.6 Hz); 6.37 (d, 2H, *J* = 17.7 Hz), 8.27 (dd, 2H, *J* = 17.7 Hz, *J* = 11.6 Hz); 10.03 (s, 3H); 10.08 (s, 1H); 10.16 (s, 3H); 10.21 (s, 3H).

*1.7. General procedure for the Diels-Alder reactions between **7** and* maleimides **4** and **5***.*

To a solution of protoporphyrin dimethyl ester (**7**) (60 mg, 0.1 mmol) in degassed and dry toluene (5 mL), maleimides **4** or **5** were added in different molar proportions (Tables 1 and 2). The reactions were performed in glass pressure tubes (Ace pressure tubes from Aldrich®) at different temperatures and reaction times. The reactions were monitored by TLC and UV-Vis spectroscopy in order to verify the end of the starting material. The reaction products were purified by flash column chromatography in silica gel using toluene:ethyl acetate 8.5:1.5 as eluent, and the chlorin derivatives were crystallized from CH_2Cl_2 and hexanes. Compounds **8a-b** and **9a-b** were obtained in different yields as described in Tables 1 and 2.

2^1,2^2[*N*,*N*-dicarbonyl-*N*-4-(1,3-dithiolan-2-yl)phenyl]-13,17-bis[2-(methoxycarbonyl)ethyl]-2,7,12,18-tetramethyl-8-vinyl-2,2^1,2^2,2^3-

tetrahydrobenzo[*b*]porphyrin (**8a**)**: ^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm)*:* -2.40 and -2.48 (br.s, 2H, H-22 e H-24); 2.08 (s, 3H, CH_3-H2^{5}); 3.05-3.11 and 3.14-3.22 (m, 6H e 2H, H-13^{2}, H-17^{2}, H2^{15}, H2^{16}); 3.40 (s, 3H, H-12^{1}); 3.45-3.47 (m, 2H, H-2$^{3\alpha}$ e 2$^{3\beta}$); 3.49 (s, 3H, H-18^{1}); 3.56 (s, 3H, H-7^{1}); 3.65 and 3.68 (s, 6H; H-13^{4} and H-17^{4}); 3.90-3.94 (m, 1H, H-2^{2}); 4.18 (t, 2H, *J* = 7.8 Hz, H-13^{1}); 4.31 (t, 2H, *J* = 6.6 Hz, H-17^{1}), 4.65 (d, 1H, *J* = 8.6 Hz, H-2^{1}); 6.15 (dd, 1H, *J* = 1.3 Hz, *J* = 11.6 Hz, H-8$^{2\alpha}$); 6.35 (dd, H, *J* = 1.3 Hz and *J* = 17.8 Hz, H-8$^{2\beta}$); 6.64 (d, 2H, *J* = 8.6 Hz, H-2^{9} and H-2^{13}); 7.10 (d, 2H, *J* = 8.6 Hz, H-2^{10} and H-2^{12}); 7.41 (t, 1H, *J* = 5.3 Hz, H-2^{4}); 8.19 (dd, 1H, *J* = 17.8 Hz and 11.6 Hz, H-8^{1}); 9.09 (s, 1H, H-20); 9.32 (s, 1H, H-5); 9.67 (s, 1H, H-15), 9.85 (s, 1H, H-10)**. ^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm)*:* 11.4 (C-18^{1}); 11.6 (C-12^{1}); 12.3 (C-7^{1}); 21.5 (C-17^{1}); 21.9 (C-13^{1}); 25.6 (C-2^{3}); 26.5 (C-2^{5}); 36.6 (C-17^{2}); 37.0 (C-13^{2}); 38.5 (C-2^{2}); 39,8 and 39,9 (C2^{15} and C2^{16}); 50.1 (C-2^{1}); 51.7 (C-13^{4}); 51.8 (C-17^{4}); 52.3 (C-2); 55.3 (C-2^{14}); 90.4 (C-5); 93.4 (C-20); 97.9(C-15); 99.8 (C-10); 115.6 (C-2^{4}); 121.4 (C-8^{2}); 125.9 (C-2^{9} and C-2^{13}); 128.2 (C-2^{10} and C-2^{12}); 129.2 (C-7); 129.8 (C-8^{1}); 130.8 (C-2^{8})**;** 130.9 (C-18); 132.6 (C-9); 133.7 (C-8); 133.9 (C-16); 136.2 (C-17**)**; 136.5 (C-6);138.3 (C-12 e C-19);139.6 (C-13); 140.5 (C-2^{11}); 149.5 (C-3)**;** 151.0 (C-14); 151.3 (C-11); 152.2 (C-4); 165.9 (C-1); 173,4 (C-17^{3}); 173.8 (C-13^{3});174.8 (C-2^{6}); 178.5 (C-2^{7}). **HRMS (MALDI-TOF)**: *m/z* calculated for $[M+H]^+$ 868.3197; found 868.3207. **UV-Vis** (CH_2Cl_2) $\lambda_{max\ nm}$ (log ε) 407 (5.26), 505 (4.08), 540 (4.10), 610 (3.59), 667 (4.58).

2^{1},2^{2}[*N*,*N*-dicarbonyl-*N*-4-(formylphenyl)]-13,17-bis[2-(methoxycarbonyl)ethyl]-2,7,12,18-tetramethyl-18-vinyl-2,2^{1},2^{2},2^{3}-tetrahydrobenzo[*b*]porphyrin (**9a**): **^{1}H**

NMR ($CDCl_3$, 400 MHz) δ (ppm)*:* -2.47 and -2.39 (br.s, 2H, H-22 e H-24); 2.07 (s, 3H, H-2^5); 3.15 (t, 2H, *J* = 7.8 Hz, H-13^2); 3.20 (t, 2H, *J* = 7.8 Hz, H-17^2); 3.40 (s, 3H, H-12^1); 3.44-3.47 (m, 2H, H-$2^{3\alpha}$ and $2^{3\beta}$); 3.50 (s, 3H, H-18^1); 3.55 (s, 3H, H-7^1); 3.65 and 3.68 (s, 6H; H-13^4 and H-17^4); 3.89-3.93 (m, 1H, H-2^2); 4.18 (t, 2H, *J* = 7.8 Hz, H-13^1); 4.31 (t, 2H, *J* = 7.6 Hz, H-17^1), 4.64 (d, 1H, *J* = 8.6 Hz, H-2^1); 6.15 (dd, 1H, *J* = 1.5 Hz, *J* = 11.5 Hz, H-$8^{2\alpha}$); 6.34 (dd, H, *J* = 1.5 Hz, *J* = 17.8 Hz, H-$8^{2\beta}$); 6.91-6.94 (m, 2H, H-2^9 and 2^{13});7.44-7.47 (m, 2H, H-2^{11} and H-2^{12}); 7.41 (t, 1H, *J* = 5.4 Hz, H-2^4); 8.17 (dd, 1H, *J* = 17.8 and 11.5 Hz, H-8^1); 9.07 (s, 1H, H-20); 9.33 (s, 1H, H-5); 9.69 (s, 1H, H-15), 9.86 (s, 1H, H-10); 9.64 (s, 1H, H-2^{14}). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ *(*ppm)*:* 11.4 (C-18^1); 11.6 (C-12^1); 12.3 (C-7^1); 21.5 (C-17^1); 21.9 (C-13^1); 25.6 (C-2^3); 26.4 (C-2^5); 36.6 (C-17^2); 37.0 (C-13^2); 38.5 (C-2^2); 50.2 (C-2^1); 51.7 (C-13^4); 51.8 (C-17^4); 52.3 (C-2); 90.4 (C-5); 93.2 (C-20); 98.1(C-15); 99.9 (C-10); 115.6 (C-2^4); 121.4 (C-8^2); 126.3 (C-2^9 and C-2^{13}); 129.7 (C-2^{10} and C-2^{12}); 129.2 (C-7); 129.8 (C-8^1); 130.9 (C-18); 132.6 (C-9); 133.8 (C-8); 133.9 (C-16); 135.2 (C-2^{11}); 136.3 (C-17**)**; 136.5 (C-6 and C-2^8);138.3 (C-19); 138.4 (C-12); 139.7 (C-13); 149.6 (C-3); 151.1 (C- 14); 151.5 (C-11); 152.0 (C-4); 165.7 (C-1); 173.4 (C-17^3); 173.8 (C-13^3); 174.3 (C-2^6); 178.1 (C-2^7); 190.8 (C-2^{14}). **HRMS (MALDI-TOF)**: *m/z* calculated for $[M+H]^+$ 792.3392; found 792.3420. **UV-Vis** (CH_2Cl_2) $\lambda_{max\ nm}$ (log ε) 407 (5.25), 505 (4.11), 540 (4.11), 610 (3.65), 667 (4.65).

2^1,2^2[*N*,*N*-dicarbonyl-*N*-4-(1,3-dithiolan-2-yl)phenyl]-8,12-bis[2-(methoxycarbonyl)ethyl]-2,7,13,17-tetramethyl-8-vinyl-2,2^1,2^2,2^3-tetrahydrobenzo[*b*]porphyrin (**8b**)**:** **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm):-2.44

(br.s, 2H, H-21 and H-23); 2.06 (s, 3H, H-2^5); 3.15-3.22 and 3.05–3.12 (m, 8H, H-12^2, H-8^2, H-2^{15}, H-2^{16}); 3.42 (s, 3H, H-13^1); 3.44-3.45 (m, 2H, H-$2^{3\alpha}$ and $2^{3\beta}$); 3.47 (s, 3H, H-7^1); 3.61 (s, 3H, H-17^1); 3.65 and 3.66 (s, 6H; H-12^4 and H-8^4); 3.89-3.93 (m, 1H, H-2^2); 4.18 (t, 2H, *J* = 7.7 Hz, H-12^1); 4.32 (t, 2H, *J* = 7.7 Hz, H-8^1), 4.63 (d, 1H, *J* = 8.6 Hz, H-2^1); 6.11 (dd, 1H, *J* = 1.4 Hz, *J* = 11.5 Hz, H-$18^{2\alpha}$); 6.33 (dd, H, *J* = 1.4 Hz, *J* = 17.8 Hz, H-$18^{2\beta}$); 6,62-6,64 (m, 2H, H-2^9 and 2^{13}); 7,08-7,10 (m, 2H, H-2^{10} and H-2^{12}); 7.41 (t, 1H, *J* = 5.3 Hz, H-2^4); 8.13 (dd, 1H, *J* = 17.8 Hz and 11.5 Hz, H-18^1); 9.25 (s, 1H, H-5); 9.27 (s, 1H, H-20), 9.68 (s, 1H, H-10), 9.75 (s, 1H, H-15). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 11.2 (C-7^1); 11.7 (C-13^1); 12.4 (C-17^1); 21.6 (C-8^1); 21.9 (C-12^1); 25.6 (C-2^3); 26.6 (C-2^5); 36.7 (C-8^2); 37.1 (C-12^2); 38.6 (C-2^2); 39.8 and 39.9 (C2^{15} and C2^{16}); 50.1 (C-2^1); 51.7 (C-12^4); 51.8 (C-8^4); 52.2 (C-2); 55.3 (C2^{14}); 89.9 (C-5); 94.1 (C-20); 98.4 (C-10); 99.4 (C-15); 116.0 (C-2^4); 120.9 (C-18^2); 125.8 (C-2^9 and C-2^{13}); 128.2 (C-2^{10} and C-2^{12}); 129.9 (C-18^1); 130,4 (C-7); 130,8 (C-2^8); 131.2 (C-18); 133.3 (C-9 and C-17); 133.8 (C-16); 135.9 (C-8); 136.8 (C-6); 137.3 (C-19); 138.0 (C-13); 139.7 (C-12); 140.4 (C-2^{11}); 149.6 (C-3); 150.6 (C-11); 151.4 (C-14); 152.8 (C-4); 165.6 (C-1); 173.4 (C-8^3); 173.8 (C-12^3); 174.7 (C-2^6); 178.5 (C-2^7). **HRMS (MALDI-TOF)**: *m/z* calculated for $[M+H]^+$ 868.3197; found 868.3192. **UV-Vis** (CH_2Cl_2) $\lambda_{max\ nm}$ (log ε) 407 (5.22), 505 (4.07), 539 (4.10), 574 (3.63), 667 (4.64).

2^1,2^2[*N*,*N*-dicarbonyl-*N*-4-(formylphenyl)]-8,12-bis[2-(methoxycarbonyl)ethyl]-2,7,13,17-tetramethyl-18-vinyl-2,2^1,2^2,2^3-tetrahydrobenzo[*b*]porphyrin (**9b**): **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): -2.43 (br.s, 2H, H-21 and H-23); 2.07 (s, 3H, H-2^5); 3.14-3.17 (m, 2H, H-12^2); 3.18-3.21 (m, 2H, H-8^2); 3.41 (s, 3H, H-13^1);

3.44-3.45 (m, 2H, H-$2^{3\alpha}$ and H-$2^{3\beta}$); 3.46 (s, 3H, H-7^{1}); 3.61 (s, 3H, H-17^{1}); 3.64 and 3.66 (s, 6H; H-12^{4} and H-8^{4}); 3.89-3.93 (m, 1H, H-2^{2}); 4.18 (t, 2H, *J* = 7.8 Hz, H-12^{1}); 4.31 (t, 2H, *J* = 7.7 Hz, H-8^{1}), 4.64 (d, 1H, *J* = 11.8 Hz, H-2^{1}); 6.11 (dd, 1H, *J* = 1.6 Hz, *J* = 11.6 Hz, H-$18^{2\alpha}$); 6.33 (dd, H, *J* = 1.6 Hz, *J* = 17.8 Hz, H-$18^{2\beta}$); 6.91-6.93 (m, 2H, H-2^{9} and H-2^{13}); 7.44-7.46 (m, 2H, H-2^{10} and H-2^{12}); 7.40 (t, 1H, *J* = 5.4 Hz, H-2^{4}); 8.11 (dd, 1H, *J* = 17.8 Hz and 11.6 Hz, H-18^{1}); 9.26 (s, 2H, H-5 and H-20), 9.70 (s, 1H, H-10), 9.75 (s, 1H, H-15); 9.64 (s, 1H, H-2^{14}). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 11.2 (C-7^{1}); 11.7 (C-13^{1}); 12.4 (C-17^{1}); 21.5 (C-8^{1}); 21.9 (C-12^{1}); 25.6 (C-2^{3}); 26.5 (C-2^{5}); 36.7 (C-8^{2}); 37.0 (C-12^{2}); 38.6 (C-2^{2}); 50.1 (C-2^{1}); 51.7 (C-12^{4}); 51.8 (C-8^{4}); 52.2 (C-2); 89,9 (C-5); 93.9 (C-20); 98.5 (C-10); 99.5 (C-15); 115.8 (C-2^{4}); 120.9 (C-18^{2}); 126.3 (C-2^{9} and C-2^{13}); 129.7 (C-2^{10} and C-2^{12}); 129.9 (C-18^{1}); 130.4 (C-7)**;** 131.2 (C-18); 133.4 (C-9); 133.4 (C-17); 133.8 (C-16); 135.2 (C-2^{11}); 136.0 (C-8); 136.5 (C-2^{8});136,8 (C-19); 137.3 (C-6); 138.1 (C-13); 139.8 (C-12); 149,7 (C-3); 150.7 (C-11); 151.5 (C-14); 152.7(C-4); 165.4 (C-1); 173.3 (C-8^{3}); 173.8 (C-12^{3}); 174.2 (C-2^{6}); 178.1 (C-2^{7}); 190.8 (C-2^{14}). **HRMS (MALDI-TOF)**: *m/z* calculated for $[M+H]^+$ 792.3392; found 792.3356. **UV-Vis** (CH_2Cl_2) $\lambda_{max\ nm}$ (log ε) 407 (5.25), 505 (4.09), 539 (4.10), 574 (3.62), 667 (4.65).

*2.8. General procedure for the reaction between **9a-b** and 2-(aminooxy)ethanol:*

To a solution of 10 mg (0.012 mmol) of compounds **9a** or **9b** in anhydrous and deoxygenated THF, 2-(aminooxy)ethanol (1.5 mg, 0.02 mmol) and PTSA (2 mg) were added. The reaction was performed in a glass pressure tube (Ace pressure tube from Aldrich®), purged with argon and stirred at room

temperature for 6 h. The consumption of starting material **9a-b** was monitored by TLC and UV-Vis spectroscopy. The solvent was removed under reduced pressure when no starting material was detected. The purification was carried out by flash column chromatography in silica gel using dichloromethane:methanol 99:1 as eluent, and the chlorin derivatives were crystallized from CH_2Cl_2/hexane.

2^1,2^2[*N*,*N*-dicarbonyl-*N*-4-(*O*-2-hydroxyethyloxime)phenyl]-13,17-bis[2-(methoxycarbonyl)ethyl]-2,7,12,18-tetramethyl-18-vinyl-2,2^1,2^2,2^3-tetrahydrobenzo[*b*]porphyrin (**10a**): Yield: 7.5 mg; 8.0 μmol; 70%. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): -2.47 e -2.39 (sl, 2H); 2.09 (s, 3H); 3.16 (t, 2H, *J* = 7.9 Hz); 3.21 (t, 2H, *J* = 8.1 Hz); 3.41 (s, 3H); 3.46-3.48 (m, 2H); 3.50 (s, 3H); 3.56 (s, 3H); 3.65 and 3.68 (2s, 3H and 3H); 3.73 (t, 2H, *J* = 4.4Hz) 3.93-3.97 (m, 1H); 4.09-4.07 (m, 2H); 4.18 (t, 2H, *J* = 7.8 Hz); 4.31 (t, 2H, *J* = 7.5 Hz), 4.68 (d, 1H, *J* = 8.7 Hz); 4.72 (br.s, 1H); 6.15 (dd, 1H, *J* = 1.3 Hz and 11.5 Hz, H-$8^{2\alpha}$); 6.35 (dd, H, *J* = 1.3 Hz and 17.9 Hz); 6.77-6.74 (m, 2H); 7.17-7.15 (m, 2H,); 7.42 (t, 1H, *J* = 5.3 Hz); 7.76 (s, 1H,); 8.19 (dd, 1H, *J* = 17.9 and =11.5Hz); 9.09 (s, 1H, H-20); 9.33 (s, 1H, H-5); 9.68 (s, 1H,), 9.86 (s, 1H,). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm)***:*** 11.4 ($1CH_3$); 11.6 ($1CH_3$); 12.3 ($1CH_3$); 21.5 ($1CH_2$); 21.9 ($1CH_2$); 25.6 ($1CH_2$); 26.4 ($1CH_3$); 36.6 ($1CH_2$); 37.0 ($1CH_2$); 38.5 (1CH); 50.1 (1CH); 51.7 ($1CH_3$); 51.8 ($1CH_3$); 52.3 (1C); 62.0 ($1CH_2$); 75.1 ($1CH_2$); 90.4 (1CH); 93.2 (1CH); 98.0 (1CH); 99.8 (1CH); 115.5 (1CH); 121.3 ($1CH_2$); 126.2 (2CH); 127.1 (2CH); 129.2 (1C); 129.8 (1CH); 130.9 (1C); 131.5 (1C); 132.57 (1C); 132.64 (1C); 133,8 (1C); 133.9 (1C); 136.3 (1C**)**; 136.5 (1C);138.3 (1C); 138.4 (1C); 139.7 (1C); 148.1 (1CH); 149,6 (1C); 151.1 (1C);

151.4 (1C); 152.1 (1C); 165.8 (1C); 173.4 (1C); 173.8 (1C); 174.6 (1C); 178.4 (1C). **HRMS (ESI-TOF)**: *m/z* calculated for [M^+] 850.3685; found 850.3684. UV-Vis (CH_2Cl_2) λmax (log ε) 407 (5.14), 503 (4.00), 540 (4.00), 610 (3.57), 668 (4.50).

2^1,2^2[*N*,*N*-dicarbonyl-*N*-4-(*O*-2-hydroxyethyloxime)]-8,12-bis[2-(methoxycarbonyl)ethyl]-2,7,13,17-tetramethyl-18-vinyl-2,2^1,2^2,2^3-tetrahydrobenzo[*b*]porphyrin (**10b**): Yield: 8.0 mg; 9.0 μmol; 74%. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): -2.43 (br.s, 2H); 2.07 (s, 3H); 3,15-3,18(m, 2H); 3,18-3,22 (m, 2H); 3.42 (s, 3H); 3.45-3.46 (m, 2H); 3.47 (s, 3H); 3.62 (s, 3H); 3.85 and 3.86 (2s, 3H and 3H); 3.72-3.74(m, 2H); 3,92-3,96 (m, 1H); 4.07-4.09 (m, 2H) 4.19 (t, 2H, *J* = 7.8 Hz); 4.32 (t, 2H, *J* = 7.8 Hz), 4.65 (d, 1H, *J* = 8.7 Hz); 6.11 (dd, 1H, *J* = 1.1 Hz and 11.6 Hz); 6.33 (dd, H, *J* = 1.1 Hz and 17.9 Hz); 6.74 (d, 2H, *J* = 8.7Hz); 7.15 (d, 2H, *J* = 8.7Hz); 7.42 (t, 1H, *J* = 5.3 Hz); 7.76 (s, 1H); 8.13 (dd, 1H, *J* = 11.6 and 17.9 Hz); 9.27 (s, 2H), 9.69 (s, 1H), 9.76 (s, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 11.2 ($1CH_3$); 11.7 ($1CH_3$); 12.4 ($1CH_3$); 21.5 ($1CH_2$); 21.9 ($1CH_2$); 25.6 ($1CH_2$); 26.5 ($1CH_3$); 36.7 ($1CH_2$); 37.1 ($1CH_2$); 38.6 (1CH); 50.1 (1CH); 51.7 ($1CH_3$); 51.8 ($1CH_3$); 52.2 (1C); 62.0 ($1CH_2$); 75.1 ($1CH_2$); 89,9 (1CH); 94.0 (1CH); 98.4 (1CH); 99.4 (1CH); 115.9 (1CH); 120.9 ($2CH_2$); 126.2 (2CH); 127.0 (2CH); 129.9 (1CH); 130.4 (1C)**;** 131.2 (1C); 131.4 (1C); 132.7 (1C); 133.35 (1C); 133.41 (1C); 133,8 (1C); 136.0 (1C); 136.9 (1C); 137.4 (1C); 138.0 (1C); 139.8 (1C); 148.1 (1CH); 149.7 (1C); 150.6 (1C); 151.4 (1C); 152.8 (1C); 165.6 (1C); 173.4 (1C); 173.8 (1C); 174.5 (1C); 178.4 (1C). **HRMS (ESI-TOF)**: *m/z* calculated for [M^+] 850.3685;

found 850.3692. UV-Vis (CH_2Cl_2) λmax (log ε) 407 (5.20), 502 (4.07), 539 (4.05), 611 (3.56), 668 (4.55) nm.

2.9. Aggregation studies

The self-aggregation on porphyrins and chlorins is a natural tendency due to the strong attractive interactions between π-systems of the polyaromatic compounds. As it is usual in any aggregation equilibrium, the amount of aggregates should increase with the total solution concentration of the photosensitizers, unless there are sterically-inhibiting functions on molecules as those present in **8a-b, 9a-b, 10a-b**. To evaluate this phenomenon, NMR and UV-Vis analyses give clear evidences about the presence or absence of the aggregates in solution.

Thus, the ^{1}H NMR spectra were acquired in different concentrations in order to evaluate the phenomenon. Chemical shift variations for each sample were the analysed parameters. The same was performed for the UV-Vis analyses at different concentrations and different solvents (CH_2Cl_2 and water). Changes in the λ_{max} from the bands in UV-Vis spectra were monitored.

2.10. Singlet oxygen measurements

The singlet oxygen quantum yield was determined using 1,3-diphenylisobenzofuran (DPBF) as singlet oxygen quencher.[8] A $1.01x10^{-4}$ $mol.L^{-1}$ solution of DPBF in ethanol was prepared. Solutions of the new chlorins (**8a-b, 9a-b, 10a-b**) were prepared in the same solvent, with an absorbance of

0.4 at the Soret band. The cuvette was filled with 1.5 mL of the chlorin solution and 1.5 mL of the DPBF solution. The absorbance was recorded and then the solution was irradiated for 3 second periods, using a red laser operating at 50 mW and 661 nm. Absorbance was recorded again and twelve more irradiations were made, each one followed by an absorbance measurement. Experiments were done in triplicate. The same was performed with standard methylene blue (MB). The singlet oxygen quantum yields were obtained from Equation (1).[9]

Equation (1)

In this equation, (Φ_{Δ}) represents the singlet oxygen quantum yield of the sample and (Φ_{Δ}^{Std}) is the standard singlet oxygen quantum yield. R and R^{Std} are the rate constants for DPBF consumption in the presence of the chlorins (**8a-b, 9a-b, 10a-b**) and MB, respectively. Since it is a first order reaction, these parameters were obtained from a plot of the natural logarithm (ln) of DPBF absorbance versus the irradiation time. I_{abs} and I_{abs}^{Std} are the intensity of absorbed light for the sample and the standard, respectively.

2.11. Photobleaching studies

A solution of the new chlorins (**8a-b, 9a-b, 10a-b**) in CH_2Cl_2 with an absorbance value around 1 was prepared, and irradiated for 1 min using 50 mW as laser potency. The absorbance spectra were recorded always after the irradiations (10 times), in order to observe the possible photobleaching by reduction of the photosensitizer concentration.[5]

2.12. Partition coefficients in n-octanol/water (log P)

The partition coefficients were determined at room temperature in *n*-octanol /water (log P_{OW}) according to the shake-flask method. Distilled water and n-butanol were mixed vigorously for 24 h at room temperature, to promote solvent saturation in both phases. The chlorins (**10a-b**) were dissolved in the organic phase (absorbance~0.5 at the Soret band). Then, 4 mL of each, water and stock chlorin solution were individually taken, and the vessel was vigorously stirred for 10 min to allow the partitioning between the aqueous and organic phase. The mixture was centrifuged at 3×10^3 g for 10 min, and the solvents were separated and kept for equilibration at the test temperature (25 ^{o}C) for 2 h before the analysis. The UV–Vis absorption spectra were measured for organic phases, before and after partitioning and the differences were measured at the Soret band. The value of log P_{OW} can be easily determined by Equation (2):

Equation (2)

Where A_W is the absorbance of water, given by difference between the stock solution before and after partitioning and A_O is the absorbance of n-octanol, given by stock solution after partitioning. Both absorbances were measured at the Soret band. V_W and V_B are the volumes of aqueous and n-octanol phases, respectively.[10]

2. Results and Discussion

First, we have performed the syntheses of the new dienophiles **4** and **5** (Scheme 1) which were designed to avoid aggregation in the Diels-Alder adducts with protopophyrin IX dimethyl ester (**7**). For this purpose, *p*-nitrobenzaldehyde (**1**) was protected with ethanedithiol under catalytic acid conditions (yield 89%).[11] Then, compound **2** was reduced with $SnCl_2.H_2O$ in CH_2Cl_2 yielding the amine **3** (93%).[11] Maleimide **4** was synthesized in two steps: the first using an addition of **3** to maleic anhydride (84%) [6]; after that, maleimide **4** was prepared with the mixed anhydride of Ac_2O/NaOAc (92%).[12] The imide **5** was obtained from the reaction between **4** and NBS/acetone-water (87%).[13]

Scheme 1

Aiming at the chlorin syntheses, we first prepared protoporphyrin IX dimethyl ester (**7**) starting from hematoporphyrin (**6**) through a modified literature procedure.[14] Hematoporphyrin (**6**) was dehydrated in chlorobenzene under reflux and acid catalysis (PTSA). The solvent was distilled off and the crude product was immediately esterified in methanol:H_2SO_4 (5%) with protection from light, yielding **7** in 72% (2 steps) after crystallization.

Scheme 2.

After the preparation of compounds **4**, **5** and **7,** we then performed a systematic study of the Diels-Alder reaction between **4** and **7**, as presented in Table 1.

Table 1

In our hands, the best condition was obtained by using toluene as solvent, catalytic amounts of BHT (10 mg) to avoid polymerizations and 20 equiv of maleimide **4**, maintaining the temperature at 125 ^{o}C (glass pressure tube) for 24h. In these conditions, the regioisomeric chlorins **8a** and **8b** were each obtained in 37% yield (74% overall yield) after purifications.

Cycloaddition studies were also performed by using maleimide **5** as presented in Table 2. In all entries, chlorins **9a** and **9b** were obtained in a shorter time and by using a reduced amount of equivalents of dienophile **5**. The best result was obtained by using 8 equiv of maleimide **5** for 14h, at 125^{o}C (glass pressure tube) in toluene. In these conditions, chlorins **9a** and **9b** were obtained in 36% and 35%, respectively (71% overall yield) after purification.

Table 2

Attempts to deprotect chlorins **8a** and **8b** were performed in order to have convergent syntheses of chlorins **9a** and **9b**, but there was no success. Thus, the desired functionalized chlorins **9a** and **9b** were only successfully obtained

from the reaction between **7** and **5.** The amphiphilic derivatives **10a** and **10b** were then prepared from **9a** or **9b** by the reaction with 2-(hydroxyamino)ethanol in THF under PTSA catalysis. The oxime derivatives **10a** and **10b** were obtained after purifications in 70% and 74% yield, respectively.

3. Characterizations using 1D and 2D NMR.

Compounds **8**-**10** were characterized using 1D (^{1}H, ^{13}C and DEPT 135) and 2D (*g*HMBC, *g*HSQC, *g*COSY and *g*NOESY) NMR analyses, MALDI-TOF or ESI-TOF HRMS and UV-Vis. Particularly, the 2D NOESY analyses were decisive to define the regiochemistry of the Diels–Alder reaction (reaction in ring A or B of porphyrin **7**) and the stereochemistry (*cis*-*endo*) of the products. For example, Figure 1 depicts the main NOE correlations for compounds **9a** and **9b** to exemplify the characterizations. First, we started from the principle that chlorins **9a** and **9b** presented a doublet in the ^{1}H NMRspectrum near to 4.65 ppm, and this signal corresponds to H-2^{1} in both cases (Figure 1). For both isolated products (higher or lower R_f) we observed a NOE correlation between H-2^{1} and one *meso*-hydrogen (H-20) in both regioisomers **9a** and **9b**. For the product with higher R_f, the meso-hydrogen H-20 presented a NOE correlation with one vinylic hydrogen (double doublet at 8.11 ppm, J = 17.8 Hz and J = 11.6 Hz) giving us the first evidence that it could be the isomer **9b** (Figure 1b). Looking at the structures of compounds **9a** and **9b**, only **9b** should present a NOE correlation between H-20 and the vinylic hydrogen H-18^{1}. In addition, H-18^{1} presents a very elucidative multiplicity (double doublet at 8.11 ppm, J = 17.8 Hz and J = 11.6 Hz) as expected for this hydrogen in the compound **9b**. Other

correlations were decisive to conclude about the regiochemistry and the stereochemistry of **9b** and can be seen in Figure 1b. Remarkably, the NOE correlation between H-2^1 (Figure 1b) and the easily distinguished methyl group H-2^5 (at 2.06 ppm) was decisive to assign the *cis*-*endo* structure of chlorin **9b**. Also, the NOE correlation between H-2^1 and H-2^2 gave us support to conclude about the *endo*-stereoselectivity.

Figure 1

The compound with lower Rf, proposed to be **9a**, was also deduced from the 2D NOESY spectrum. We observed NOE correlations between H-2^1 and H-20 and between H-2^1 and H-2^5. However, in this case we observed a NOE effect between the easily distinguished exocyclic vinylic hydrogen H-2^4 (triplet at 7.41 ppm and J = 5.4 Hz) and another meso-hydrogen, apparently H-5. Considering other NOE correlations, we have observed that the proposed H-5 presents a NOE correlation with the methyl group H-7^1, and these hydrogen atoms present a NOE correlation with a vinylic hydrogen, with an unambiguous multiplicity (double doublet at 6.34 ppm, J = 17.8 Hz and J = 1.5 Hz); this last hydrogen has to be assigned as H-$8^{2\beta}$, and all the previous correlations gave us a confirmation about the regiochemistry of this more polar isomer **9a**. Similarly, the *cis*-*endo* structure of chlorin **9a** was finally deduced from the NOE correlations between H-2^1 and H-2^5 and between H-2^1 and H2^2 (Figure 1a). As depicted in Figure 1a, other NOE correlations were decisive for the complete assignment of structure **9a**. The complete assignments of the ^{1}H and ^{13}C of

chlorins **9a** and **9b** are described in the experimental section, and all the 1D and 2D NMR analyses are included in the supporting information.

The chlorins **8a** and **8b** were completely elucidated by using 1D and 2D NMR as carried out for **9a** and **9b**. Since compounds **10a** and **10b** were synthesized from **9a** and **9b** respectively, only the ^{1}H and ^{13}C NMR and HRMS analyses were performed and were thus conclusive to assign the structures of compounds **10a** and **10b**.

4. Aggregation studies by ^{1}H NMR spectroscopy.

Aggregation is a very undesirable phenomenon presented by photosensitizers in solutions, and this behavior makes such photosensitizers less efficient because the singlet oxygen production is strongly affected. In order to evaluate the aggregation behavior of compounds **8**-**10** in solution, we decided to carry out a large number of ^{1}H NMR analyses at different concentrations. First, we prepared 8 solutions of porphyrin **7** in $CDCl_3$ and the ^{1}H NMR spectra were recorded with 64 scans. As observed in Figure 2 there was a remarkable variation in the chemical shift of meso-hydrogen starting from $0.2x10^{-2}$ mol.L^{-1} to $2.1x10^{-2}$ mol.L^{-1}. These results indicate that the porphyrin **7** presents a strong aggregation in solution, as described in our previous literature.[6] We have shown that the magnetic anisotropy is strongly affected by aggregation, resulting in a change of the chemical shift in ^{1}H NMR (around 0.1-0.5 ppm) in more concentrated solutions.

After that, solutions of compounds **8a**, **8b,** **9a**, **9b**, **10a** and **10b** were individually prepared in $CDCl_3$ and the ^1H NMR analyses were recorded (64 scans) using the same concentrations as for the compound **7** analysis. As shown in Figure 3 and Figures S1-S7 (Supporting information) all the new chlorin derivatives were completely prevented from aggregation due to the "L shape" of these potential photosensitizers. It is important to mention that all the chlorin concentrations used in these studies were larger than the concentrations used in common PDT treatments.

Figure 3

5. Aggregation studies by UV-Vis spectroscopy

Aggregation phenomena were also measured by UV-Vis spectroscopy since this technique is very sensitive, and the concentrations used here are in the same scale of PDT treatments. The measurements were performed in CH_2Cl_2 and water (containing 4% of DMSO) (Figures 4-5 and Figures S8-S13 - supporting information). Note that there is no shift at the maximum absorbance wavelengths. Also, there is a linear increase in the absorption with concentration confirming the very low tendency for aggregation of the new chlorins **8a**, **8b,** **9a**, **9b**, **10a** and **10b**. In addition, the solubilization of compounds **10a-b** in aqueous solution, attest their amphiphilic character, which may have good impact in the PDT efficiency

Figure 4

Figure 5

6. Singlet oxygen measurements *(Φ_Δ)*

In order to verify the ability of these new chlorins to produce singlet oxygen, we measured the singlet oxygen quantum yield as described in the literature.[15] For the quantifications, methylene blue (MB) was used as standard and 1,3-diphenylisobenzofuran (DPBF) as scavenger reagent. The measurements were performed in ethanol (Figures 6 and 7), detecting the consumption of DPBF after successive irradiations in the presence of MB or compounds **8a**, **8b**, **9a**, **9b**, **10a** and **10b**, respectively, giving the rate of singlet oxygen production (first order kinetics).

Figure 6

Figure 7

Comparing these rates and using the Equation (1) the Φ_Δ values for the series were **8a**, Φ_Δ= 0.41; **8b,** Φ_Δ= 0.38; **9a,** Φ_Δ= 0.35; **9b,** Φ_Δ= 0.39; **10a,** Φ_Δ= 0.37; **10b,** Φ_Δ=0.36, when compared with methylene blue (Φ_Δ= 0.52). These singlet oxygen quantum yields showed that the compounds are good candidates for PDT treatments.

8. Photobleaching studies

Photobleaching is the photodegradation of photosensitizers after irradiation with light where singlet oxygen reacts with the proper photosensitizer promoting its consumption. This is an important parameter to be determined because stability under light irradiation represents a fundamental characteristic for a good photosensitizer.[16] All the studies described here were performed in dichloromethane, by selective irradiation with a red laser at 661 nm and 50 mW. After 10 irradiation periods of 1 min. each, we obtained the curves shown in Figure 8 and Figures S14-S19 (supporting information). All the new chlorin derivatives showed an absorbance decrease around 0.1-0.2 as exemplified for chlorin **10a** in Figure 8. Only the chlorin **8a** presented a strong photobleaching effect with an absorbance decrease around 0.4.

Figure 8

9. Preliminary Amphiphilicity Measurements (Log P)

Another essential property of a dye for PDT treatments is its ability to incorporate into the cell. This is especially crucial in the case of hydrophobic compounds, in which passive partitioning into the cytoplasm membrane appears to be a pre-requisite for their cellular absorption. For such molecules, a partition coefficient (logP) between an organic solvent phase and water is one factor that is used to predict the ability of a molecule to diffuse into biomembranes. In quantitative terms, the hydrophilic character is associated

with poor distribution of chlorins in n-octanol, with log P <0, and lipophilic compounds present log P> 1.5. Amphiphilic substances have intermediate values of log P.[17]

The procedure employed for determining log P was the shake-flask method. We measured the log P for chlorins **10a** and **10b** obtaining the same value for both (log P= 1.3). Based on these results we can suggest that the designed compounds **10a** and **10b** can be considered amphiphilic with potential for future PDT treatments.[18]

7. Conclusions

In this paper we have described a study on the synthesis of six new chlorin derivatives, using protoporphyrin IX dimethyl ester and *p*-substituted phenylmaleimides as building blocks, thus obtaining the chlorin derivations in very good yields. All the compounds were fully characterized by ^{1}H and ^{13}C NMR (1D and 2D), HRMS and UV-Vis. Preliminary photophysical and photochemical evaluations were performed, showing that the new photosensitizers are self-prevented from aggregation in solution, and good candidates for PDT studies. This work was inspired by results that we have described previously, but here we have achieved advances in improving the structural features of more amphiphilic photosensitizers for PDT studies.

8. Appendix

Supporting information available for spectral data include: ^{1}H NMR and ^{13}C NMR (1D/2D), HRMS spectra, absorption and mass spectra of the synthesized compounds.

Acknowledgments

The authors wish to thank the following Brazilian agencies for the financial support and fellowships; FAPESP, CAPES, and CNPq. Thanks are also due to Edson R. Filho and Marília A. Trapp of this Department for the MALDI-HRMS measurements, and the Bruker laboratory in Brazil for ESI-TOF measurements.

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Highlights:

-Synthesis of six chlorins sterically self-prevented from aggregation is described

-A vinyl porphyrin and *p*-substituted maleimides were used as building blocks.

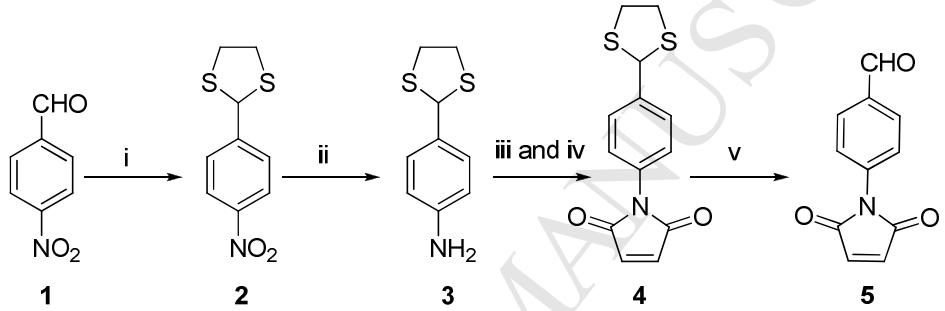
-Amphiphilic chlorins were obtained after functionalizations

-These new chlorins have proven to be good candidates for PDT studies.

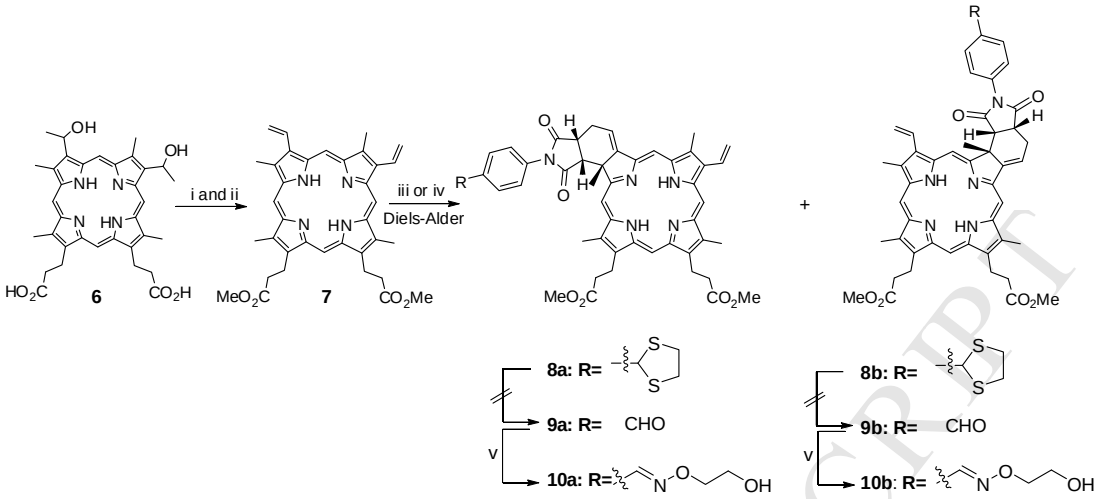
Figures, Schemes, Tables and Equations

$$\Phi_\Delta = \Phi_\Delta^{Std}\frac{R.I_{abs}^{std}}{R^{std}.I_{abs}}$$ Equation (1)

$$\log P_{OW} = \log\left(\frac{A_O}{A_w}\frac{V_w}{V_O}\right)$$ Equation (2)



Scheme 1: Synthesis of maleimides **4** and **5**. i) ethanedithiol/CH_2Cl_2, $BF_3.Et_2O$, rt.,6h, 80% yield; ii) $SnCl_2.2H_2O$/EtOH, 70^{o}C, 6h, 93% yield; iii) maleic anhydride/CH_2Cl_2, 0^{o}C, 1h, 84% yield; iv) Ac_2O/NaOAc, rt, to 90^{o}C, 92% yield; v) NBS, H_2O-acetone, 0^{o}C, 87% yield.



Scheme 2. Synthesis of new chlorins. i) C_6H_5Cl/PTSA, reflux, 2h and then ii) MeOH/H_2SO_4 5%, rt, overnight, 72% yield (2 steps); iii) solvent/conditions (Table 1) and maleimide **4**; iv) solvent/conditions (Table 2) and maleimide **5**; v) $NH_2OCH_2CH_2OH$, THF, PTSA, r.t.

Table 1: Conditions for the synthesis of chlorins **8a** and **8b**.

Temperature (°C)	Equiv. of maleimide 4	Time (h)	Solvent	BHT in catalytic amounts	Yield 8a:8b (%)
110-140	4	40	Toluene	no	14/14
120	10	66	Toluene	no	14/14
125	20	24	Toluene	no	29/30
125	20	24	Chlorobenzene	yes	28/24
125	20	24	Toluene	yes	35/32
125	20	24	Toluene	yes	37/37

Table 2: Conditions for the synthesis of chlorins **9a** and **9b**.

Equiv. of imide 5	Time (h)	Catalytic BHT	Yield 9a:9b (%)
6	19	no	21/21
8	14	no	36/35
10	10	no	34/35
10	10	yes	35/34

All reactions were performed in toluene at 125°C in a sealed tube

a) Main NOE effects in **9a**

b) Main NOE effects in **9b**

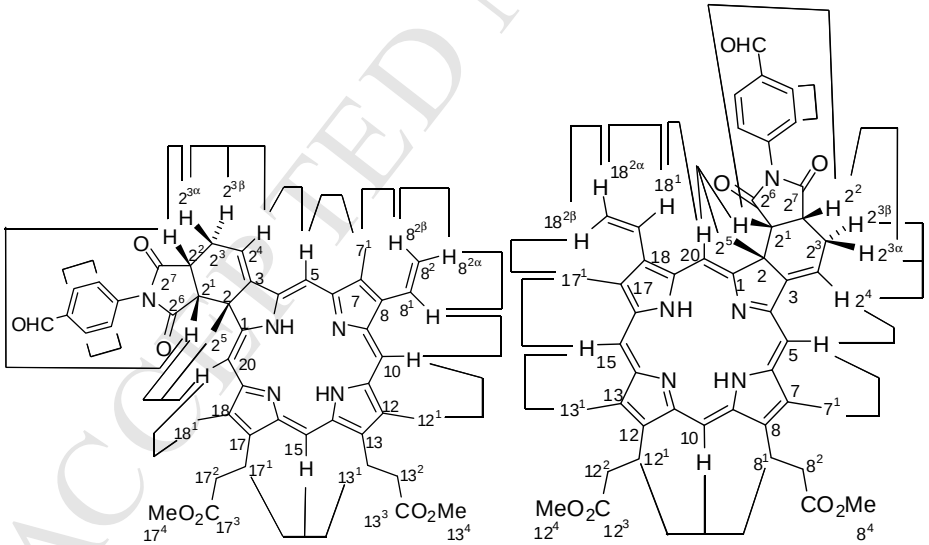


Figure 1.(a) Main NOE effects in compound **9a** (b) main NOE effects in compound **9b**.

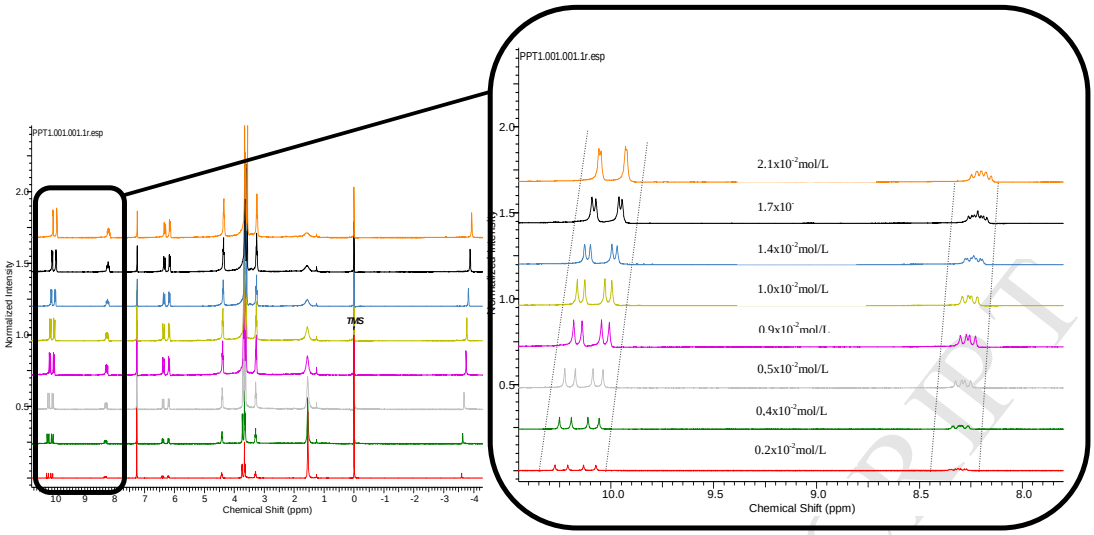


Figure 2. Changes of the ^{1}H chemical shifts of protoporphyrin IX dimethyl ester (**7**), in $CDCl_3$ at 25℃, as a function of the concentration.

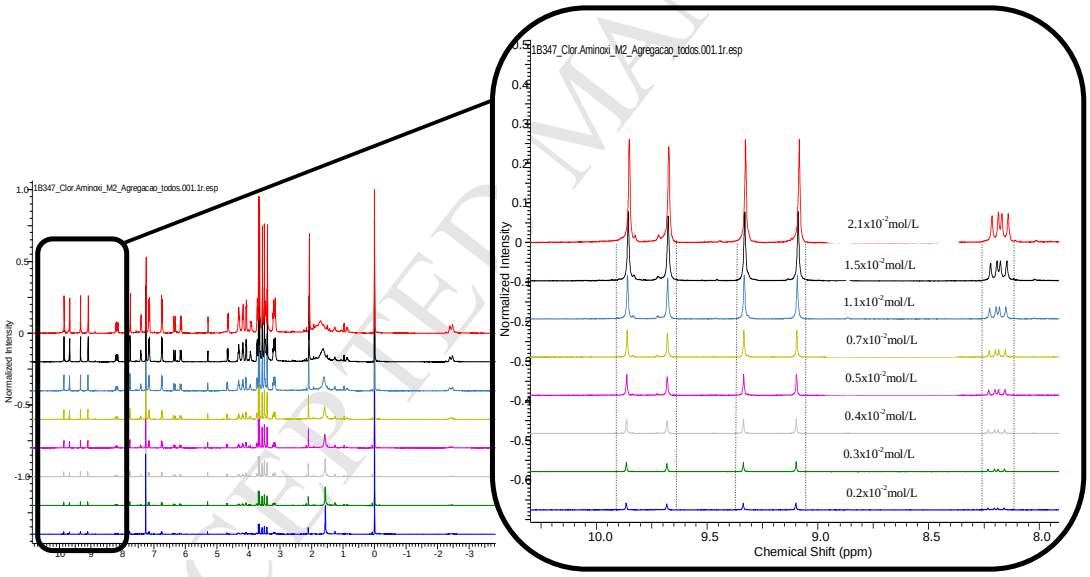


Figure 3. Example of the maintenance of the ^{1}H chemical shifts of compound **10a** in $CDCl_3$ at 25 ℃ as a function of the concentration.

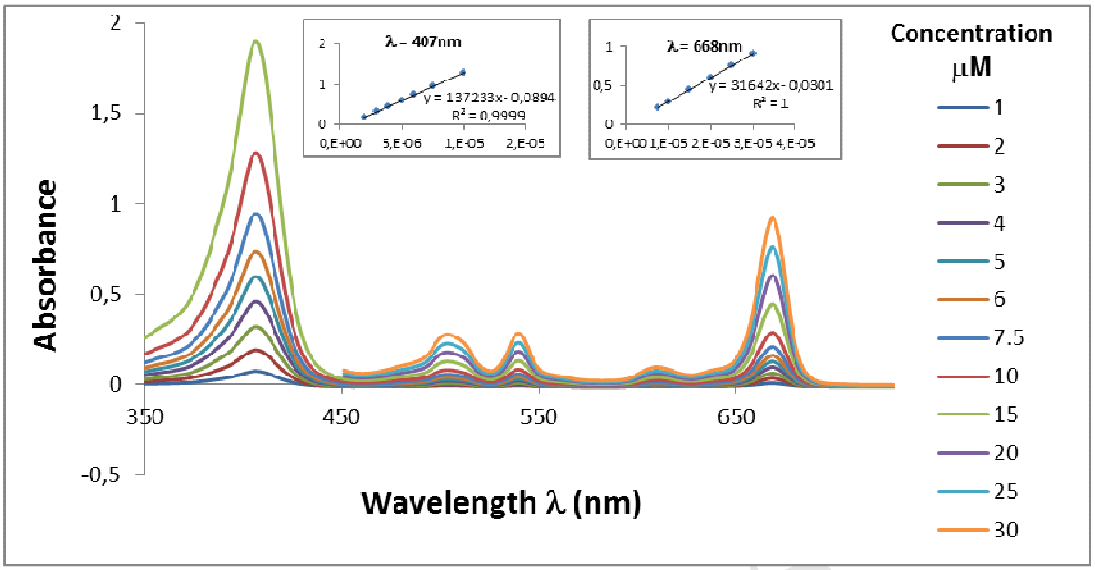


Figure 4. Example of aggregation study by UV-Vis analyses of compound **10a** in CH_2Cl_2.

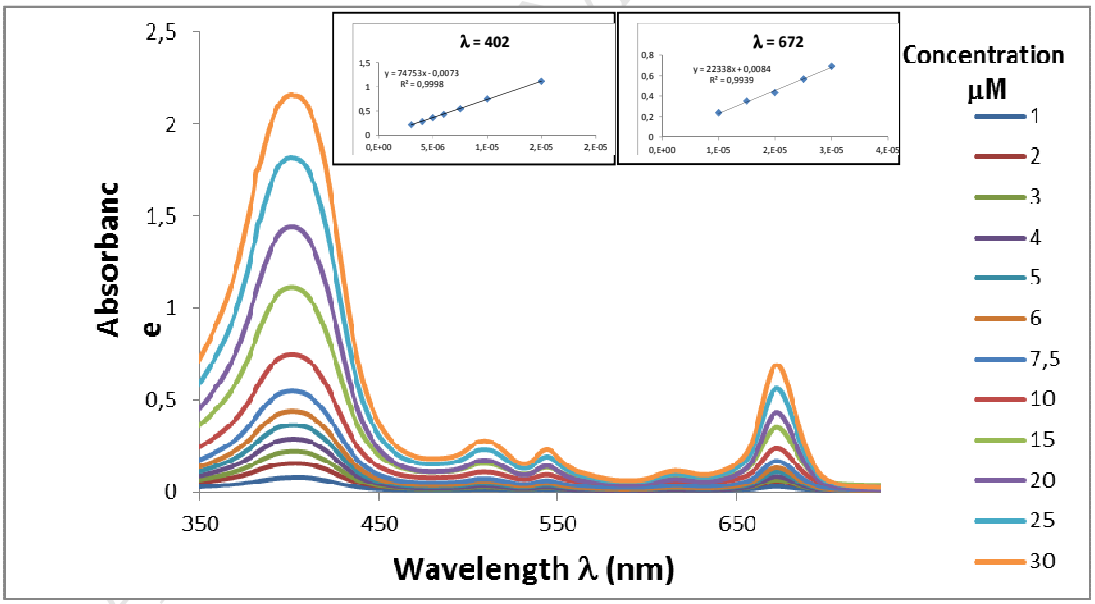


Figure 5. Example of aggregation study by UV-Vis analyses of compound **10a** in water containing 4% of DMSO.

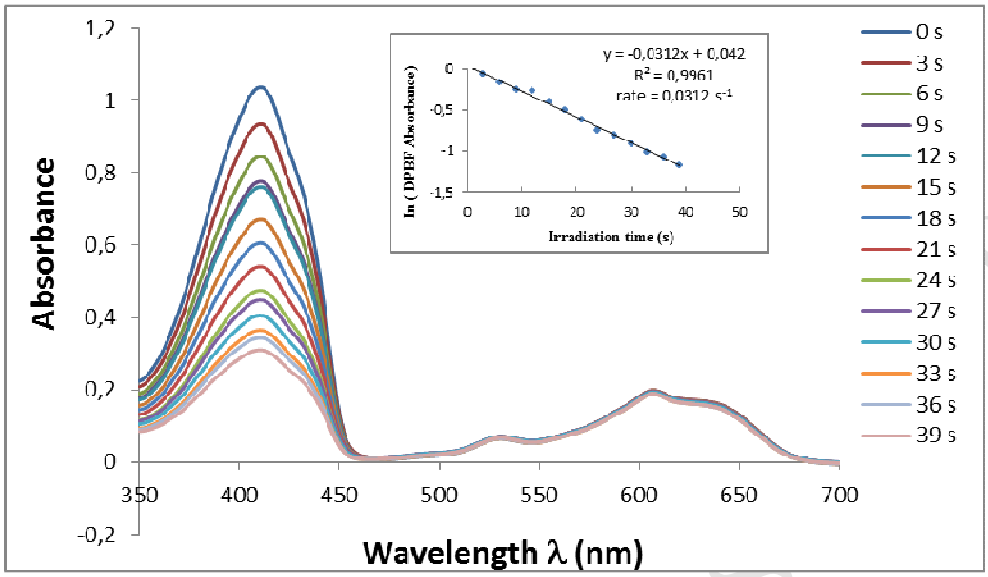


Figure 6. DPBF consumption in the presence of MB in ethanol. (inset: ln (DPBF absorbance) vs irradiation time).

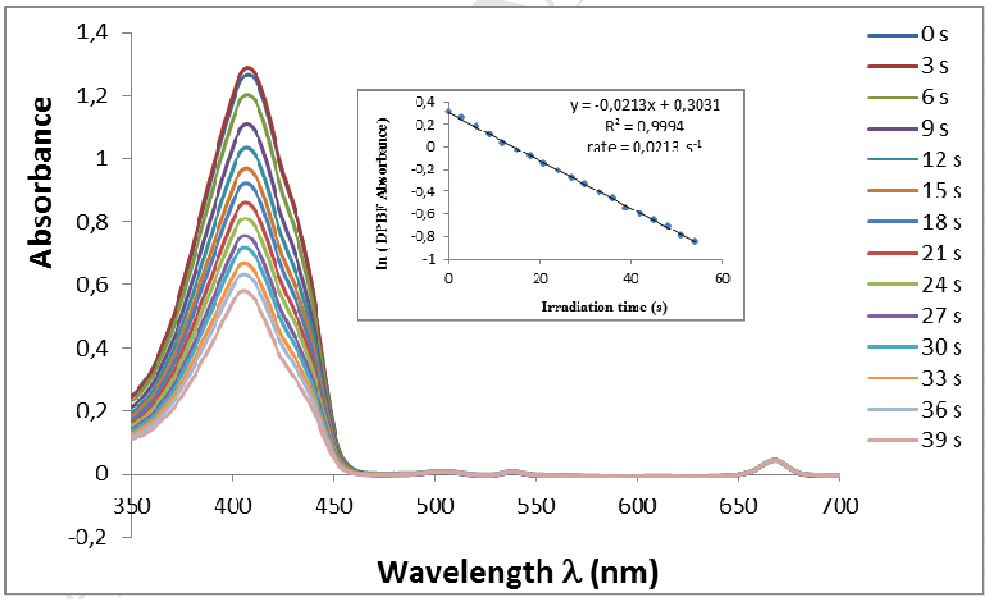


Figure 7. DPBF consumption in the presence of chlorin **10a** in ethanol. (inset: ln (DPBF absorbance) vs irradiation time).

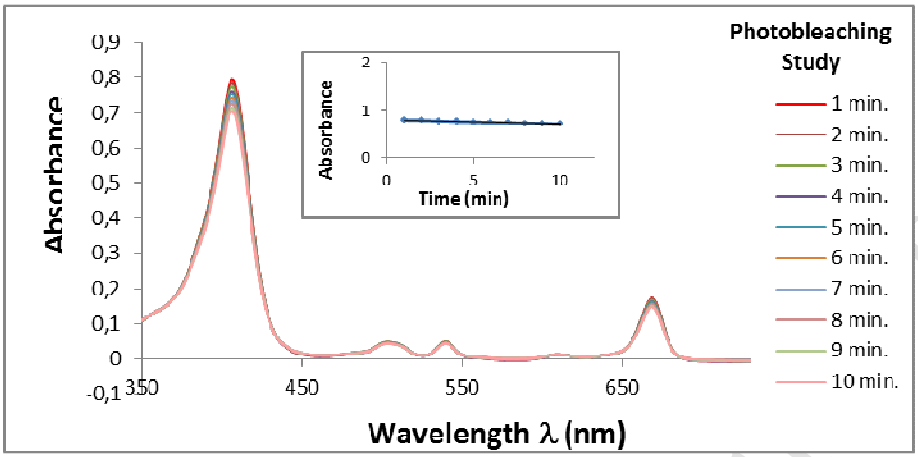


Figure 8. Photobleaching study for chlorin **10a**.

Synthesis of Functionalized ChlorinsSterically-Prevented from Self-Aggregation

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Supporting Information

Aggregation studies - ^{1}H NMR spectra

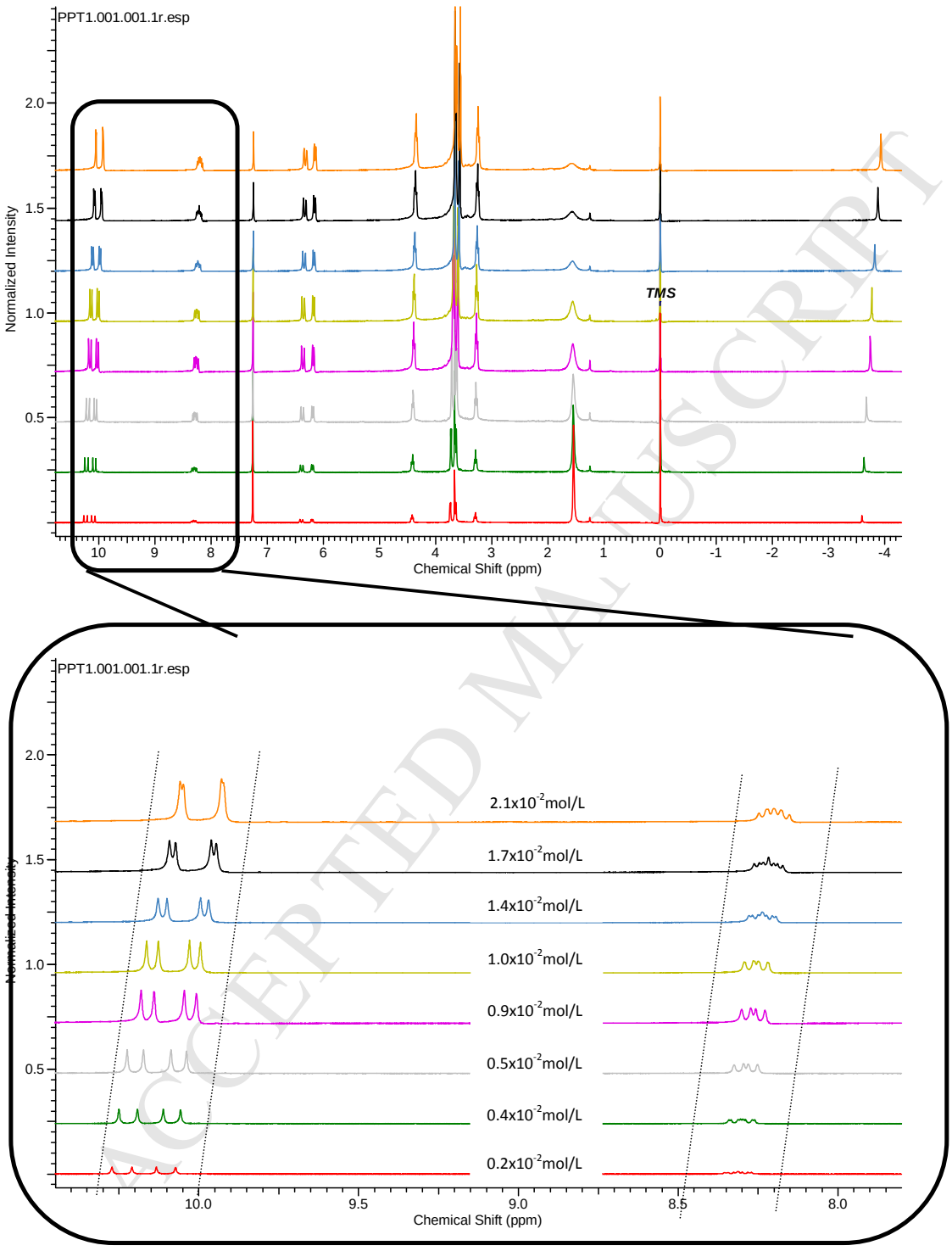


Figure S1. ^{1}H-NMR chemical shift changes for protopophyrin IX dimethyl ester (**7**), in $CDCl_3$ at 25℃, as a function of the concentration.

Aggregation studies - ^{1}H NMR spectra

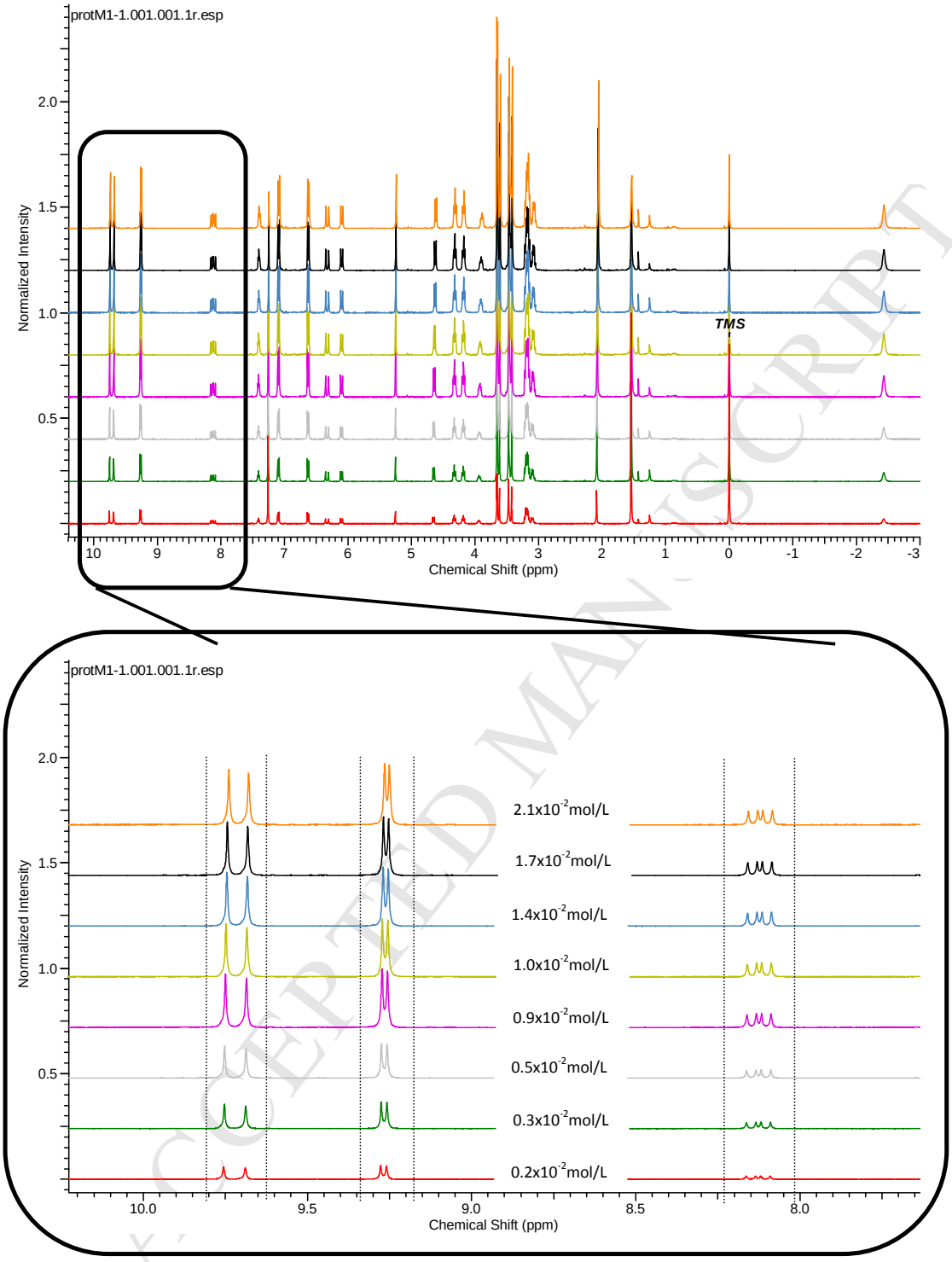


Figure S2. ^{1}H-NMR chemical shift changes for compound **8b**, in $CDCl_3$ at 25℃, as a function of the concentration.

Aggregation studies - ^{1}H NMR spectra

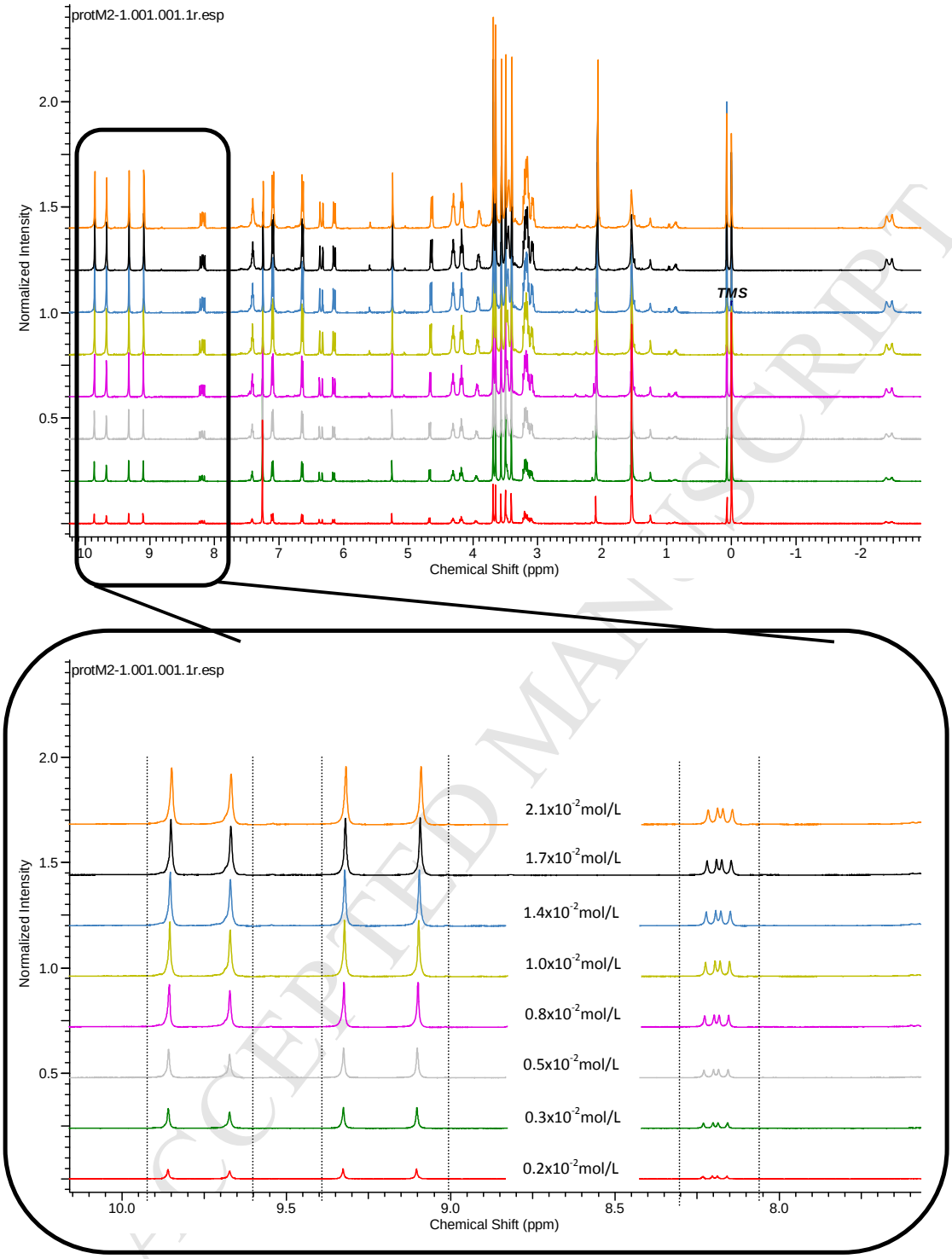


Figure S3. ^{1}H-NMR chemical shift changes for compound **8a**, in $CDCl_3$ at 25℃, as a function of the concentration.

Aggregation studies - ^{1}H NMR spectra

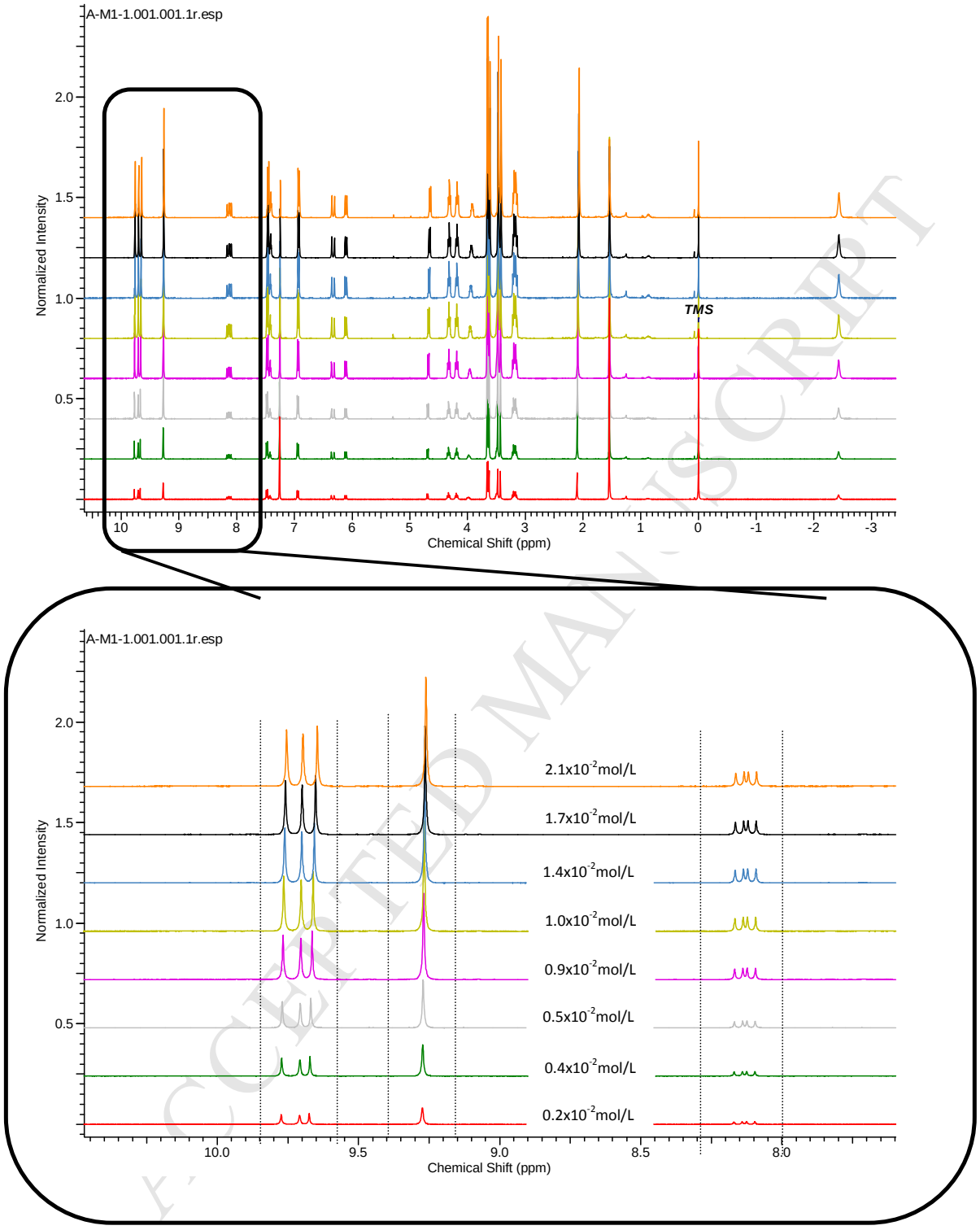


Figure S4. ^{1}H-NMR chemical shift changes for compound **9b,** in $CDCl_3$ at 25℃, as a function of the concentration.

Aggregation studies - ^{1}H NMR spectra

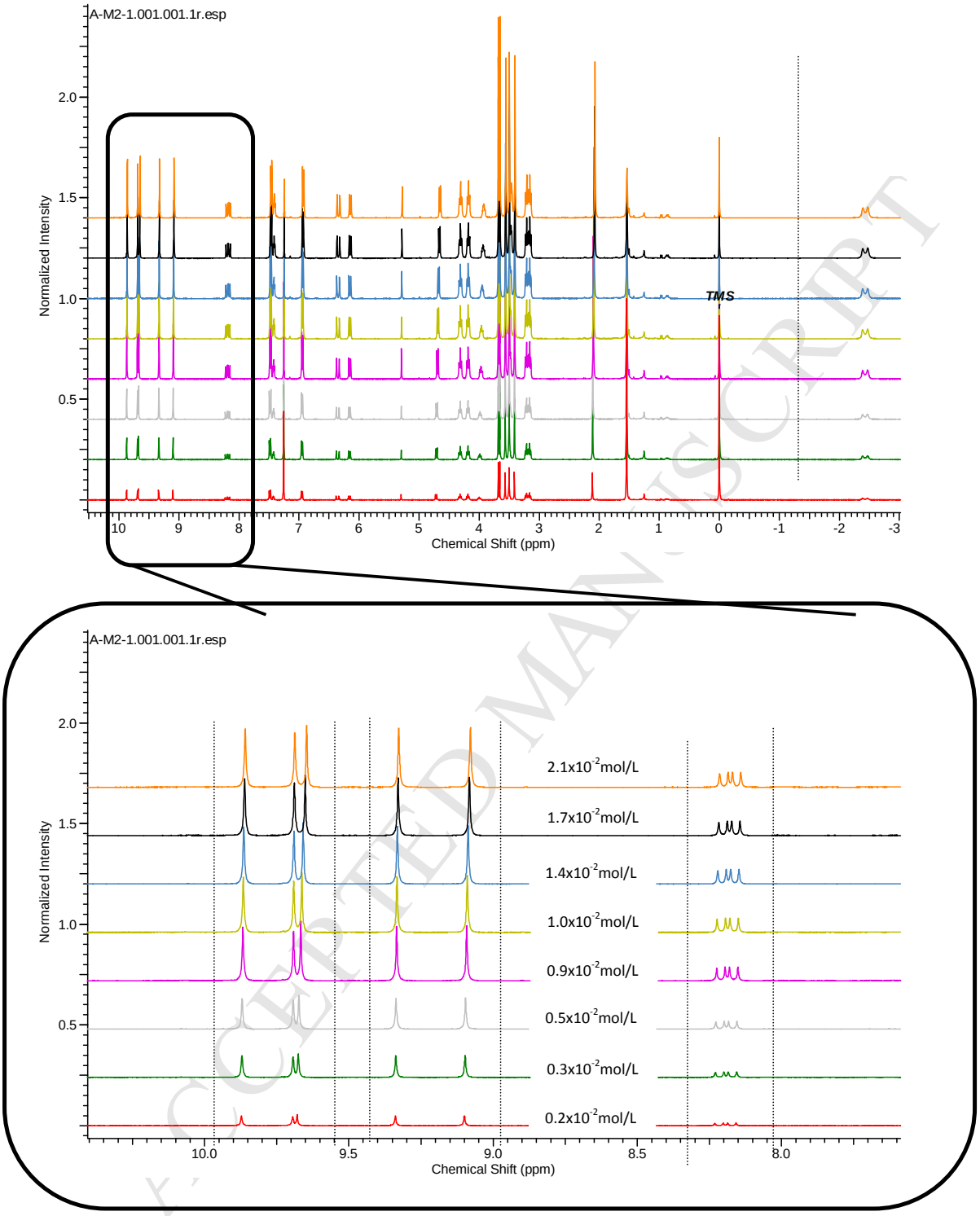


Figure S5. ^{1}H-NMR chemical shift changes for compound **9a**, in $CDCl_3$ at 25℃, as a function of the concentration.

Aggregation studies - ^{1}H NMR spectra

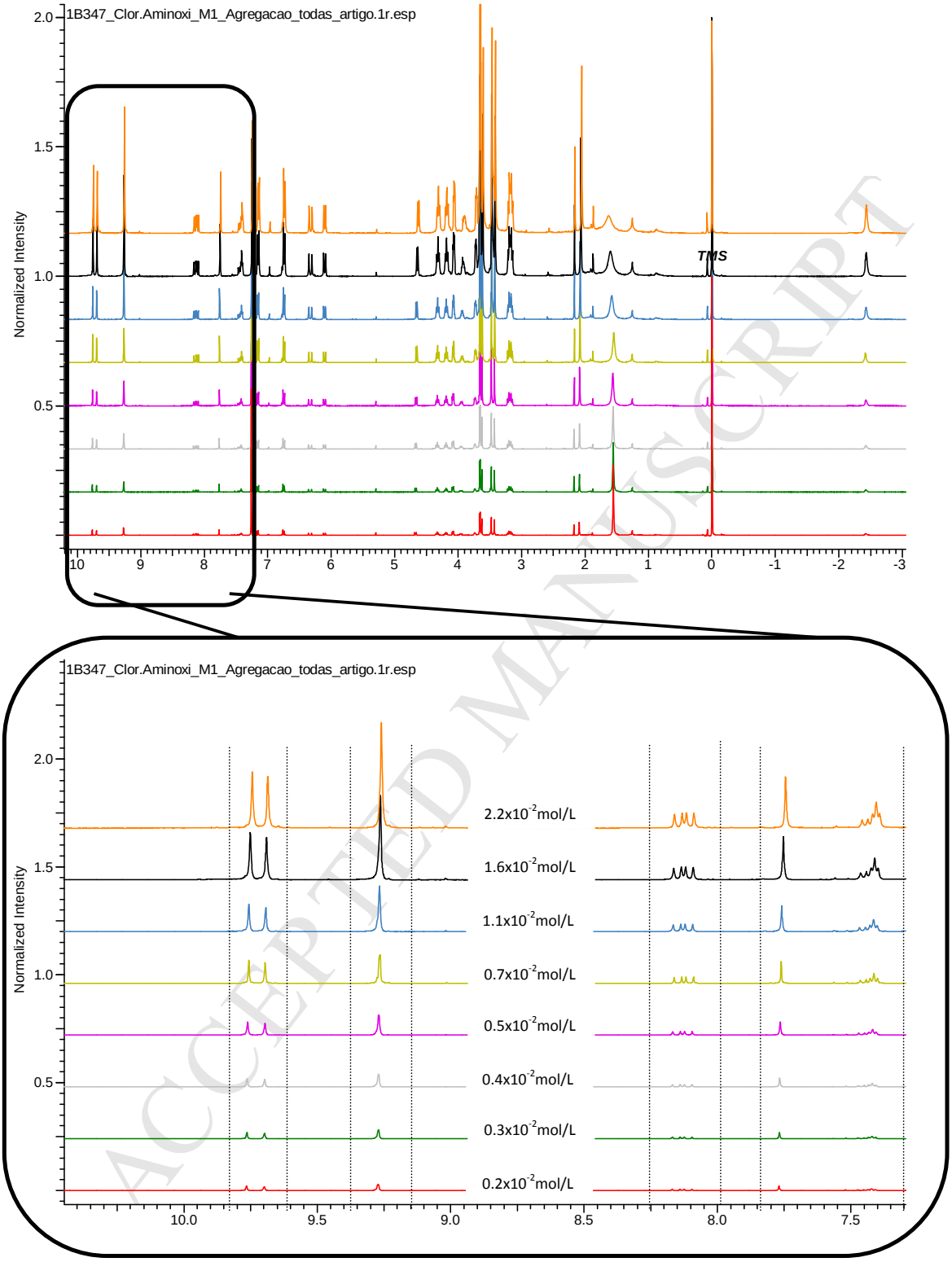


Figure S6. ^{1}H-NMR chemical shift changes for compound **10b**, in $CDCl_3$ at 25℃, as a function of the concentration.

Aggregation studies - ^{1}H NMR spectra

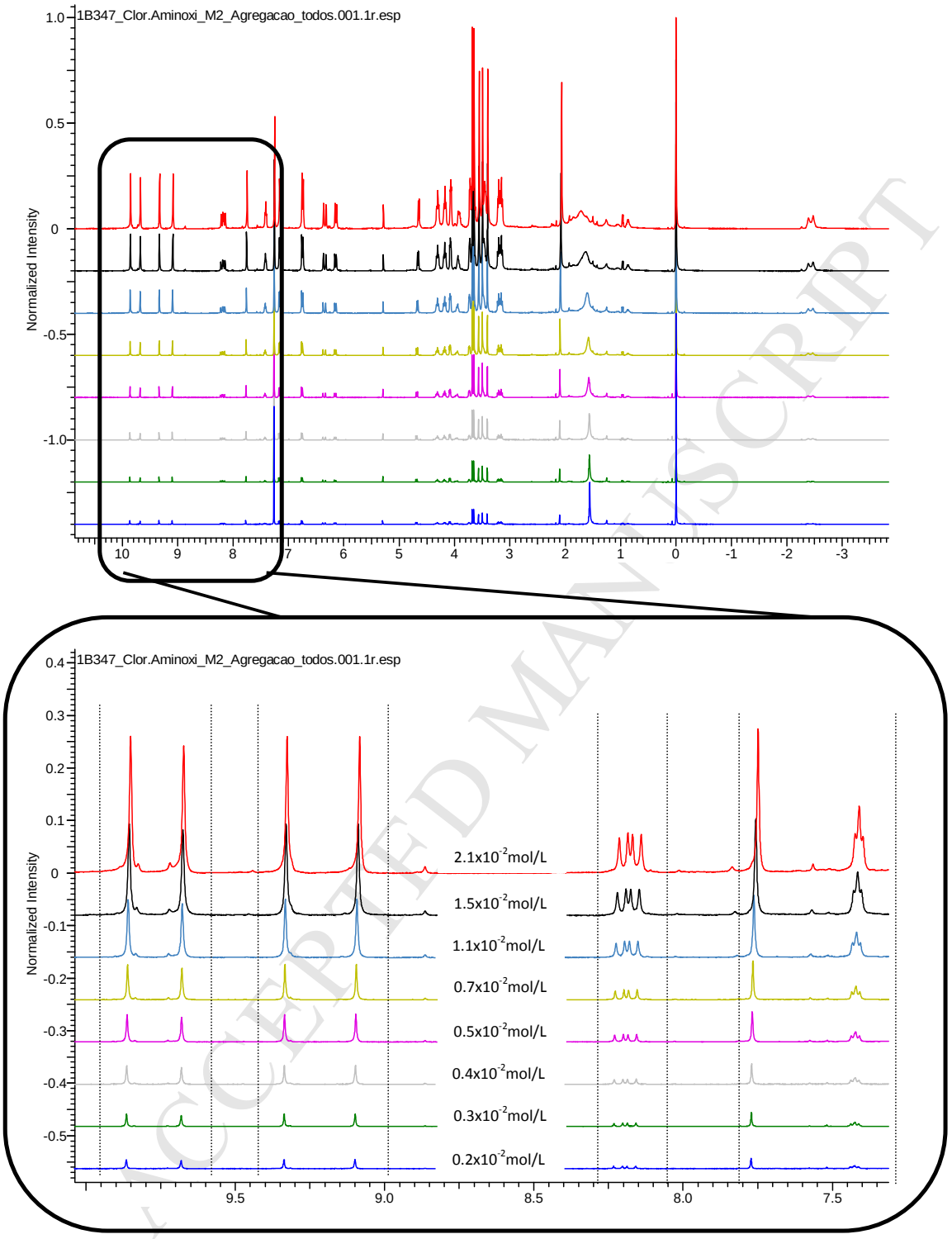


Figure S7. ^{1}H-NMR chemical shift changes for compound **10a**, in $CDCl_3$ at 25℃, as a function of the concentration.

Aggregation studies- UV-Vis Spectra

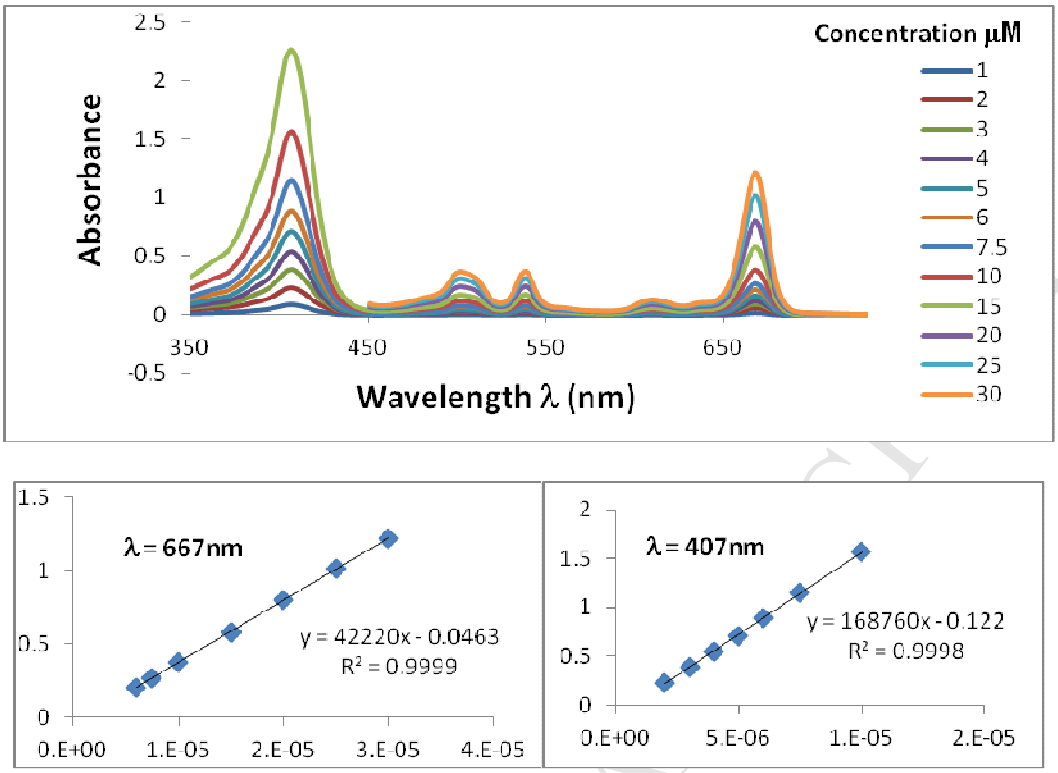


Figure S8. Aggregation studies by UV-Vis analyses of compound **8b** in CH_2Cl_2.

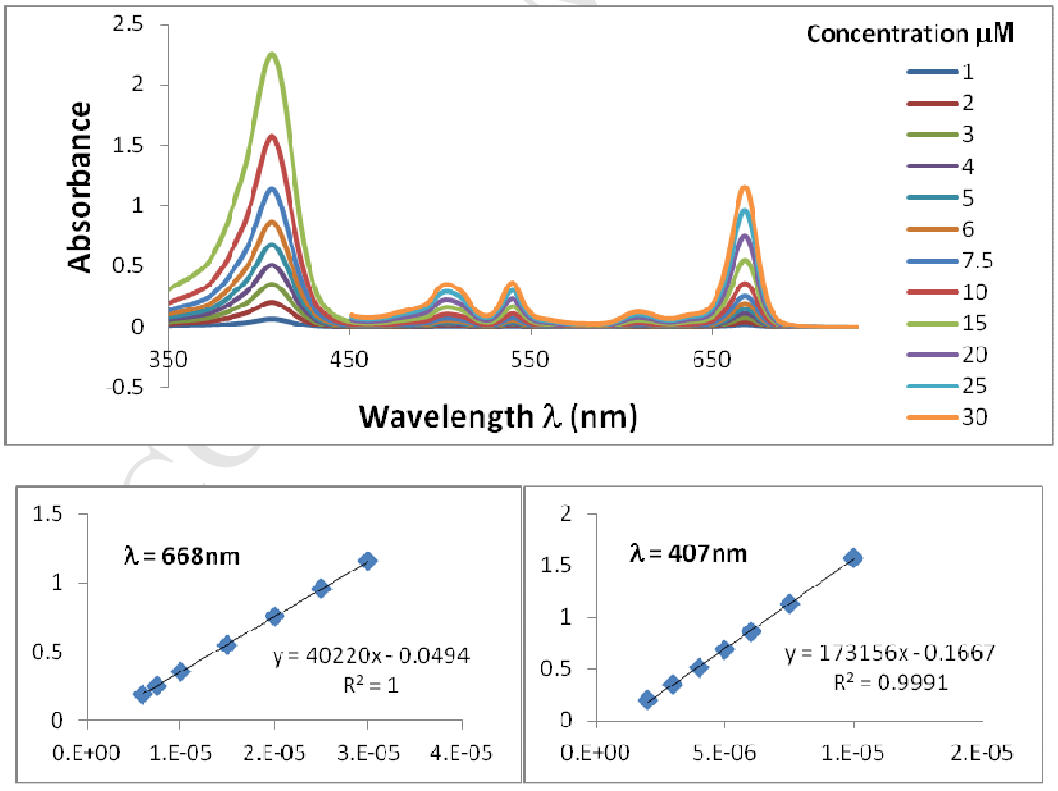


Figure S9. Aggregation studies by UV-Vis analyses of compound **8a** in CH_2Cl_2.

Aggregation studies- UV-Vis Spectra

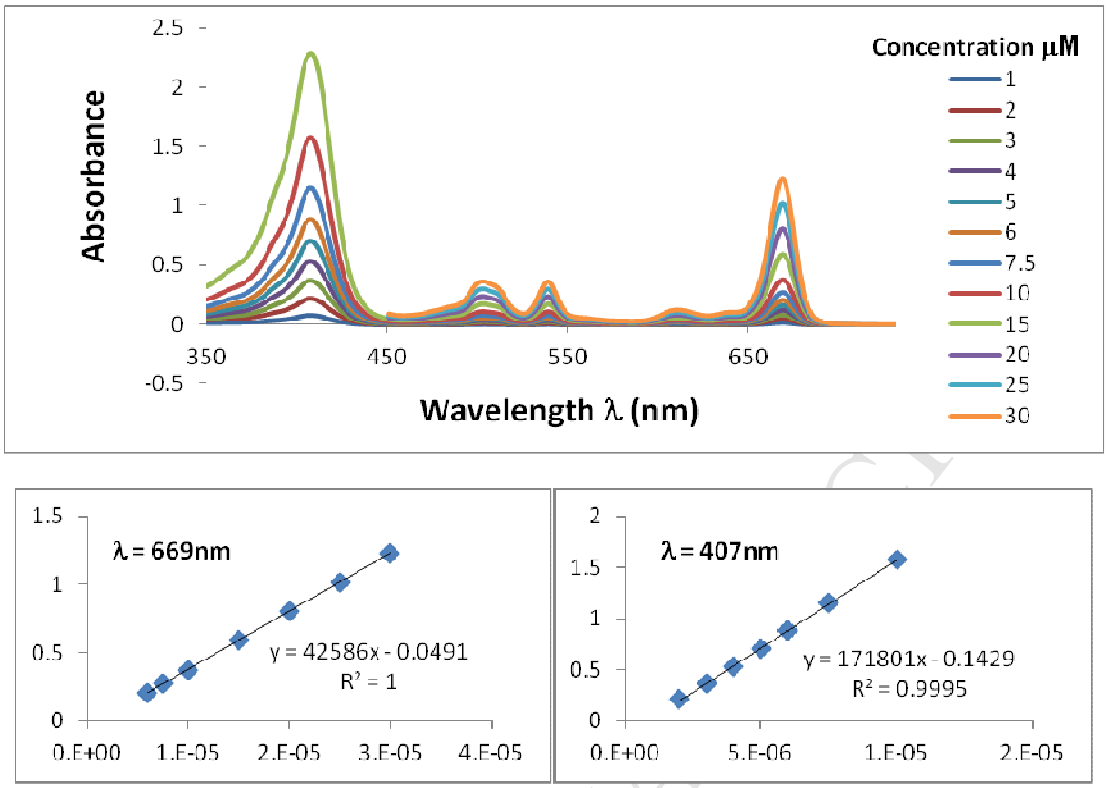


Figure S10. Aggregation studies by UV-Vis analyses of compound **9b** in CH_2Cl_2.

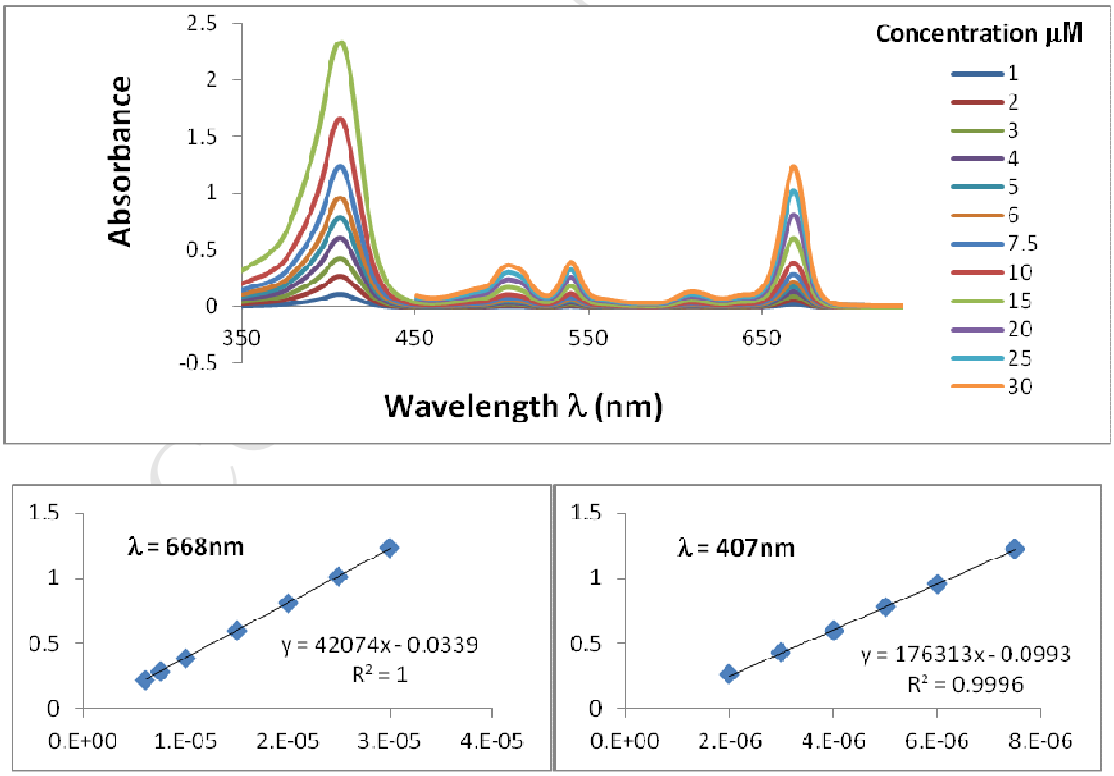


Figure S11. Aggregation studies by UV-Vis analyses of compound **9a** in CH_2Cl_2.

Aggregation studies- UV-Vis Spectra

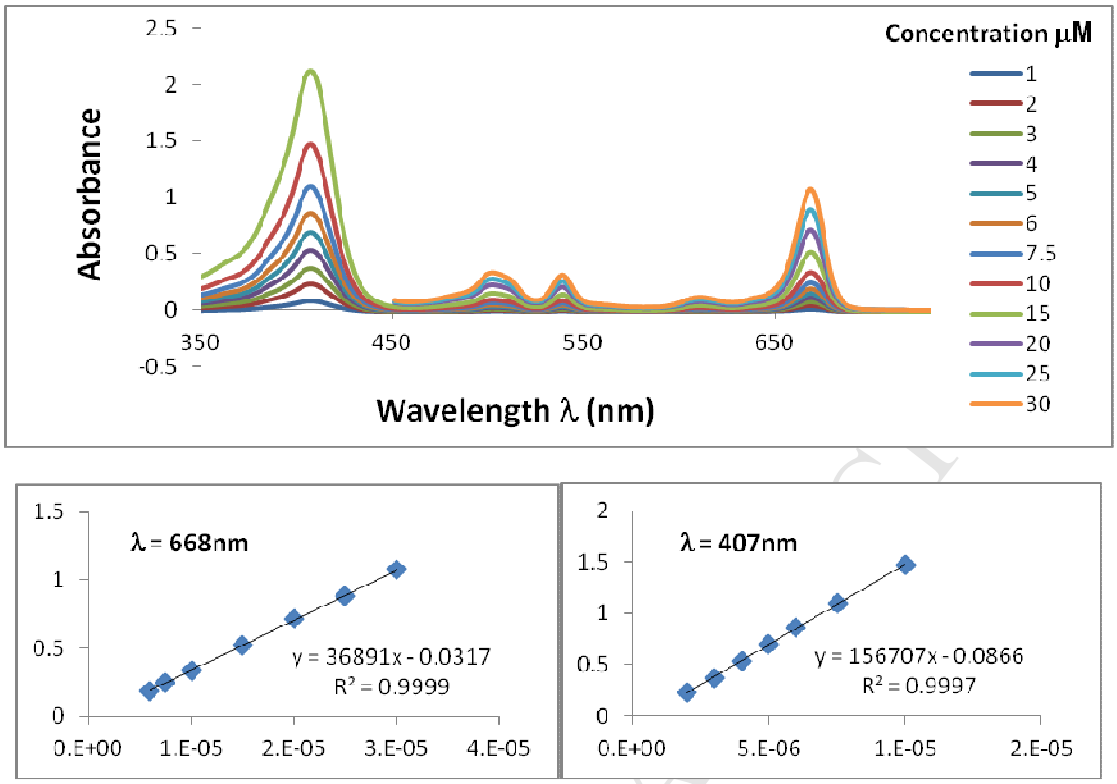


Figure S12. Aggregation studies by UV-Vis analyses of compound **10b** in CH_2Cl_2.

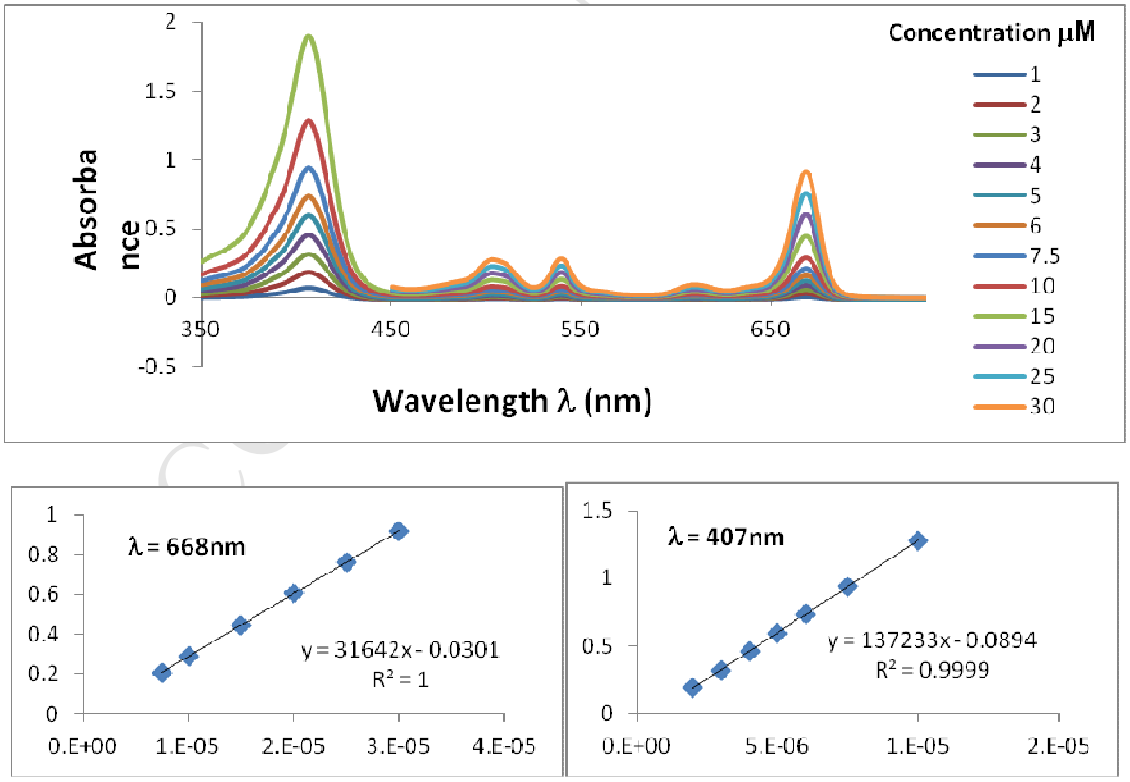


Figure S13. Aggregation studies by UV-Vis analyses of compound **10a** in CH_2Cl_2.

Aggregation ***studies- UV-Vis Spectra***

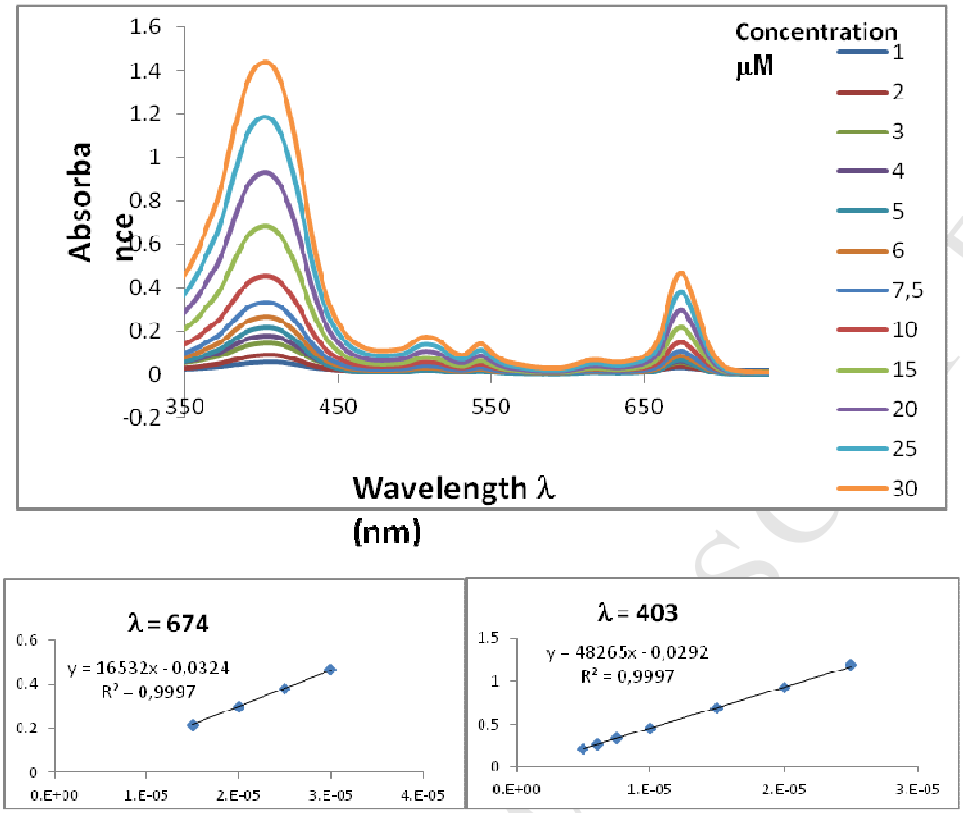
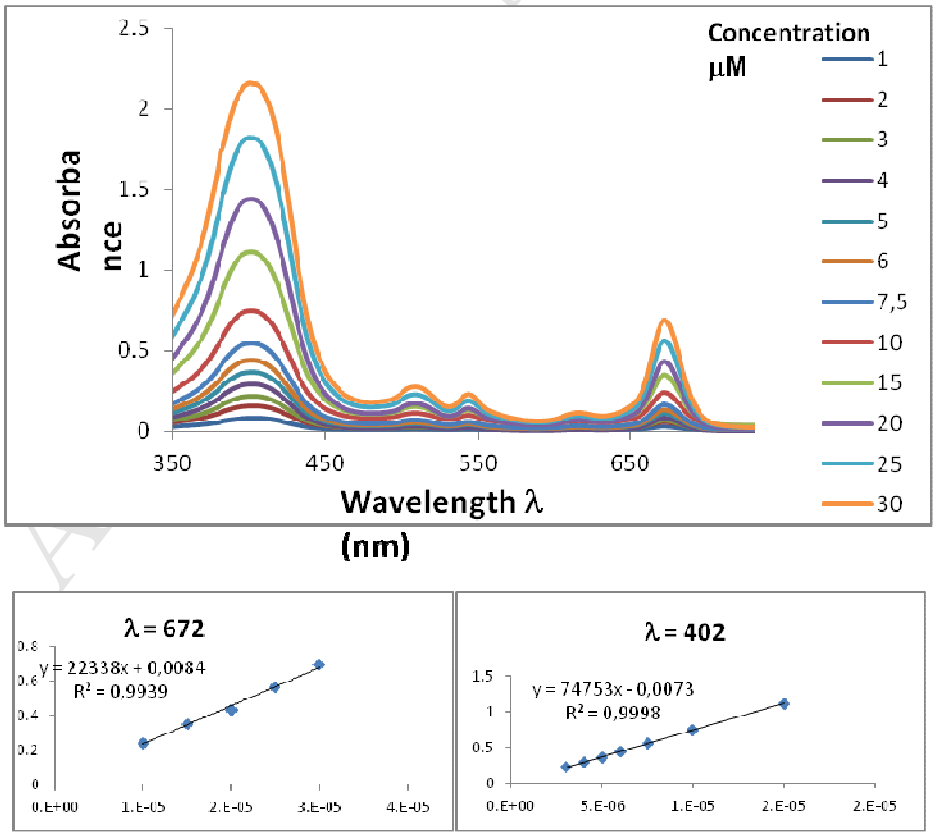


Figure S14a. Aggregation studies by UV-Vis analyses of compound **10b** in water with 4% of DMSO.



Aggregation ***studies- UV-Vis Spectra***

Figure S13a. Aggregation studies by UV-Vis analyses of compound **10a** in water with 4% of DMSO.

Photobleaching Study- UV-Vis Spectra

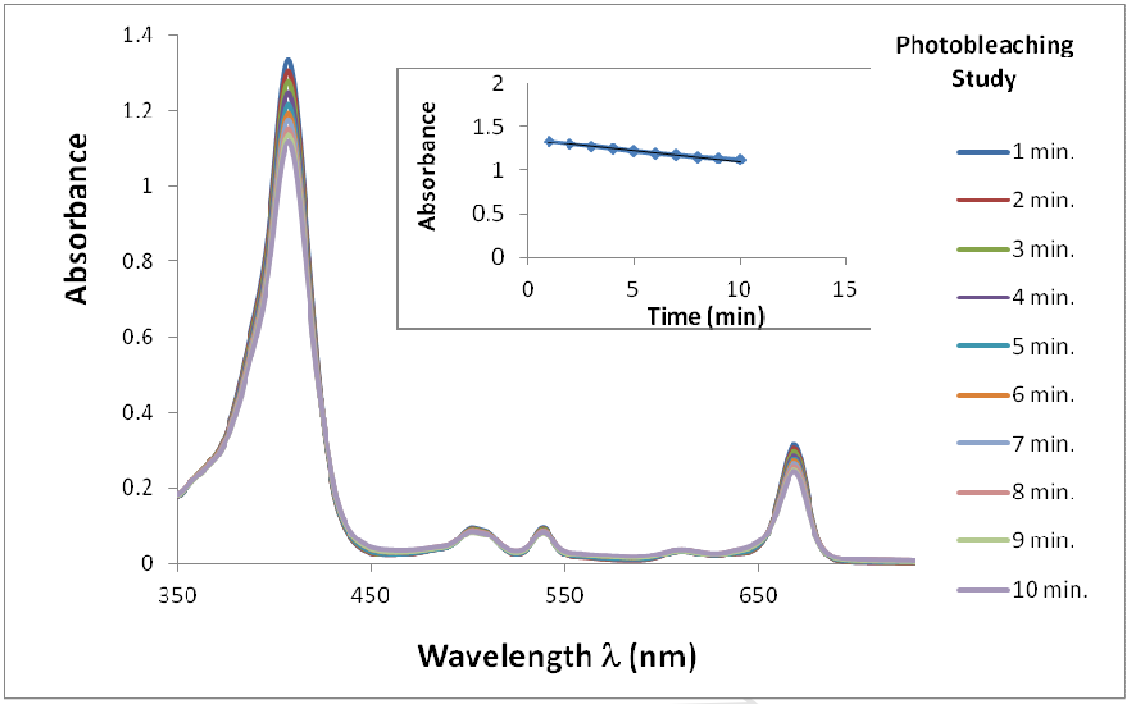


Figure S15. Photobleaching study by UV-Vis analyses of compound **8b** in CH_2Cl_2

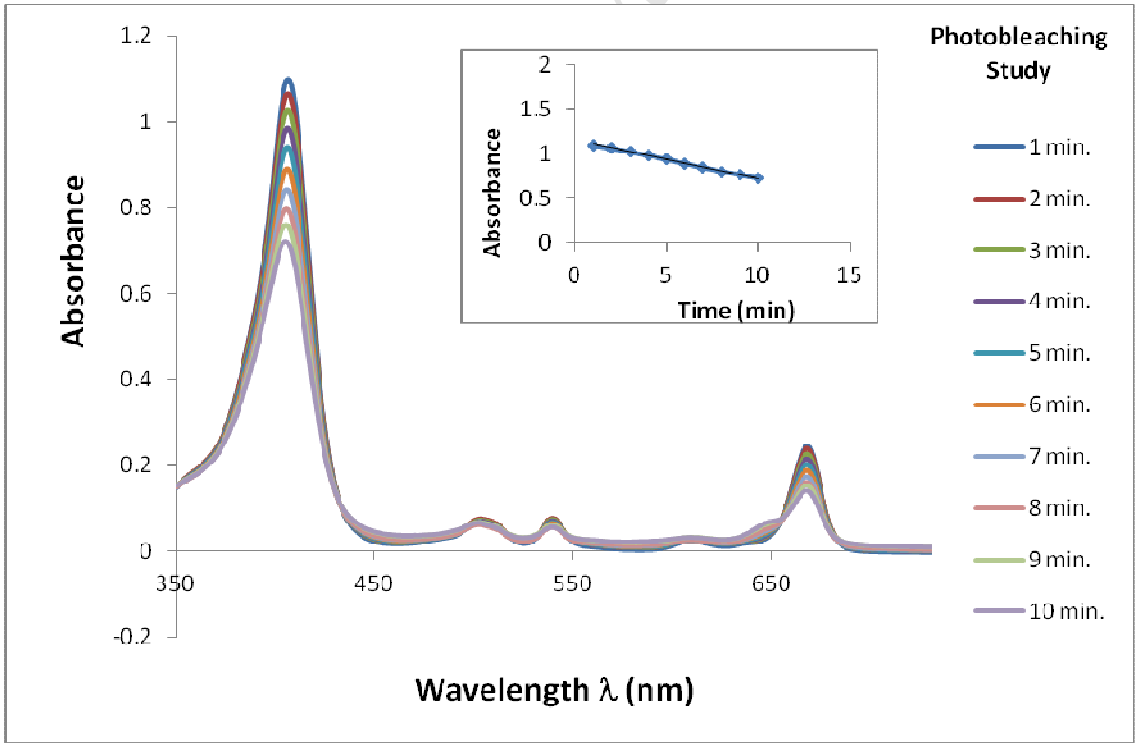


Figure S16. Photobleaching study by UV-Vis analyses of compound **8a** in CH_2Cl_2.

Photobleaching Study- UV-Vis Spectra

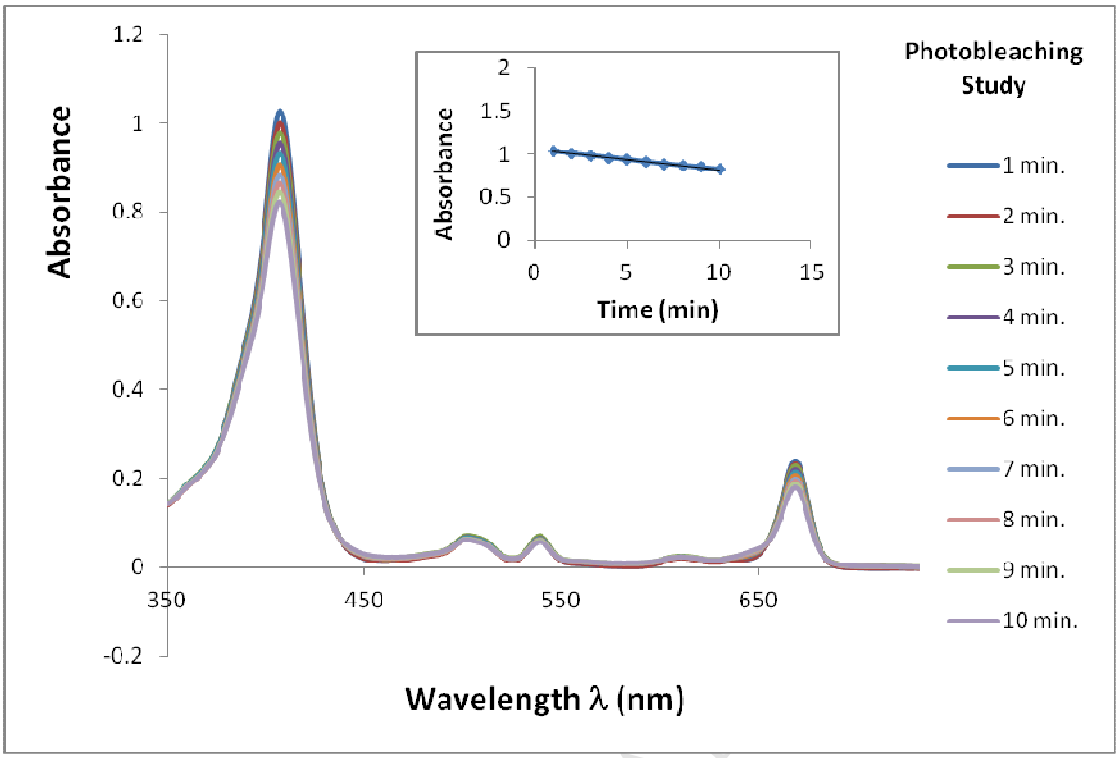


Figure S17. Photobleaching study by UV-Vis analyses of compound **9b** in CH_2Cl_2

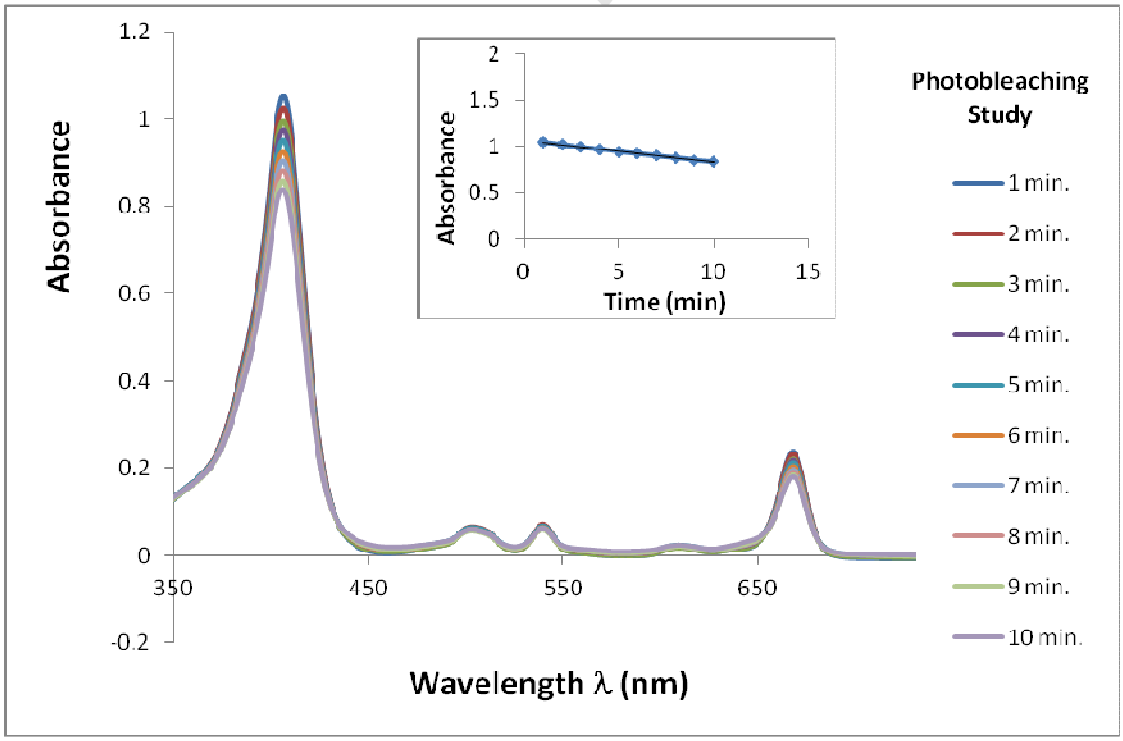


Figure S18. Photobleaching study by UV-Vis analyses of compound **9a** in CH_2Cl_2.

Photobleaching Study- UV-Vis Spectra

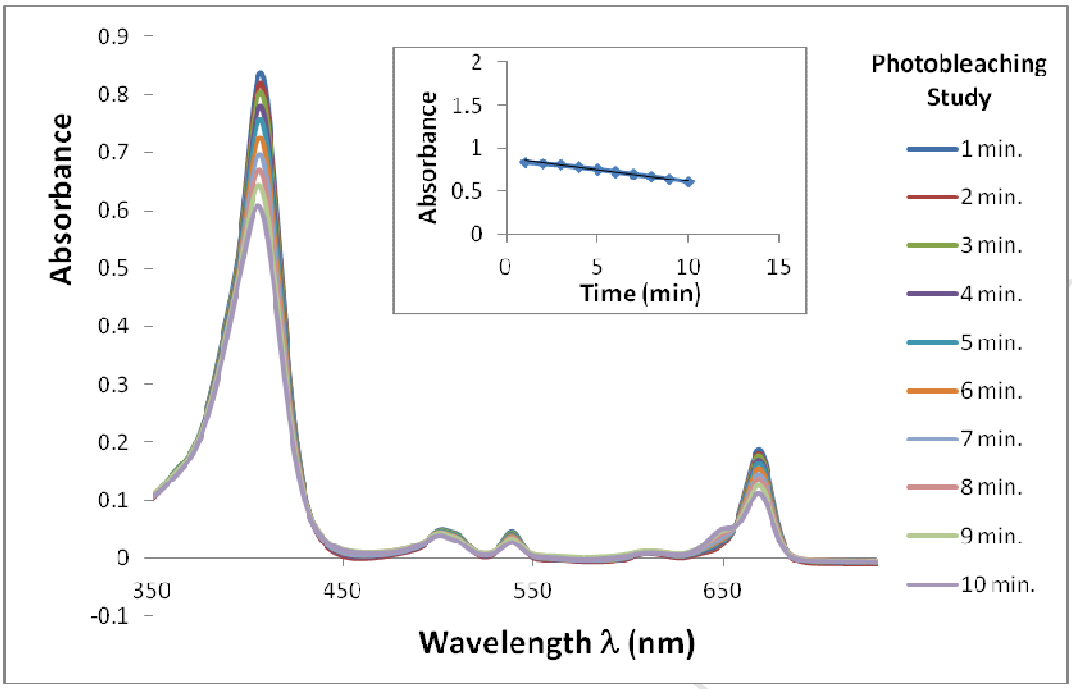


Figure S19.Photobleaching study by UV-Vis analyses of compound **10b** in CH_2Cl_2.

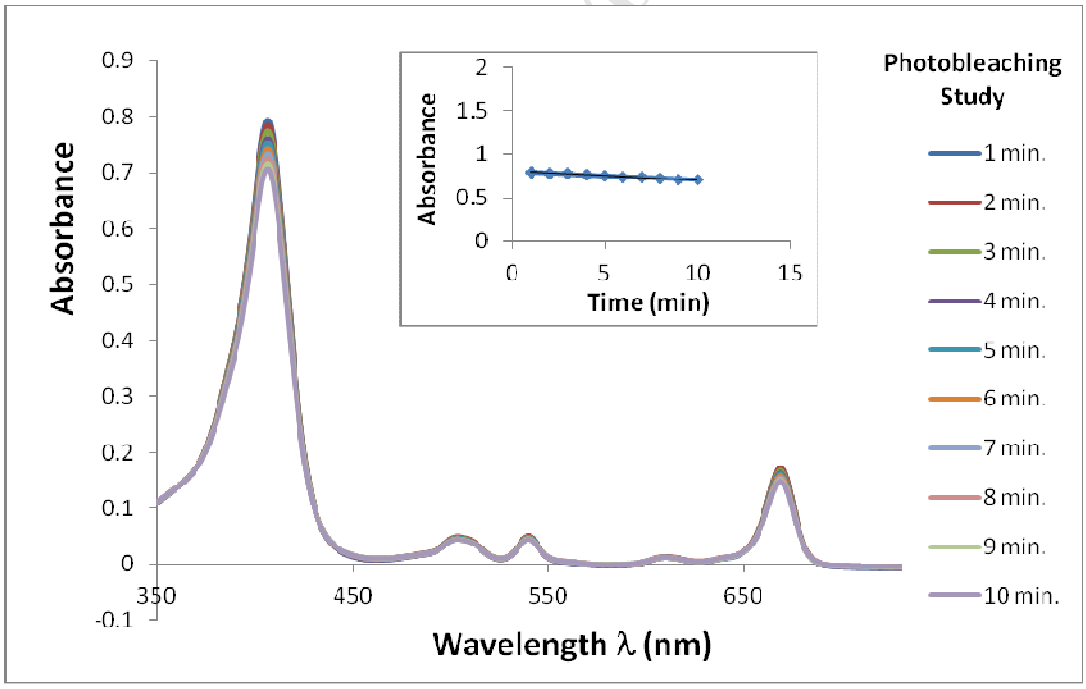


Figure S20.Photobleaching study by UV-Vis analyses of compound **10a** in CH_2Cl_2.

NMR Spectrum

Acquisition Time (sec)	4.8060	***File Name***	FBS71	***Date***	14 Sep 2011 14:57:20	***Date Stamp***	14 Sep 2011 14:57:20

Frequency (MHz)	400.15	***Nucleus***	1H	***Number of Transients***	128
Origin	spect	***Original Points Count***	32768	***Owner***	nmrsu
Points Count	65536	***Pulse Sequence***	zg30	***Receiver Gain***	114.00
SW(cyclical) (Hz)	6818.18	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	2993.4102
Spectrum Type	STANDARD	***Sweep Width (Hz)***	6818.08	***Temperature (degree C)***	26.800

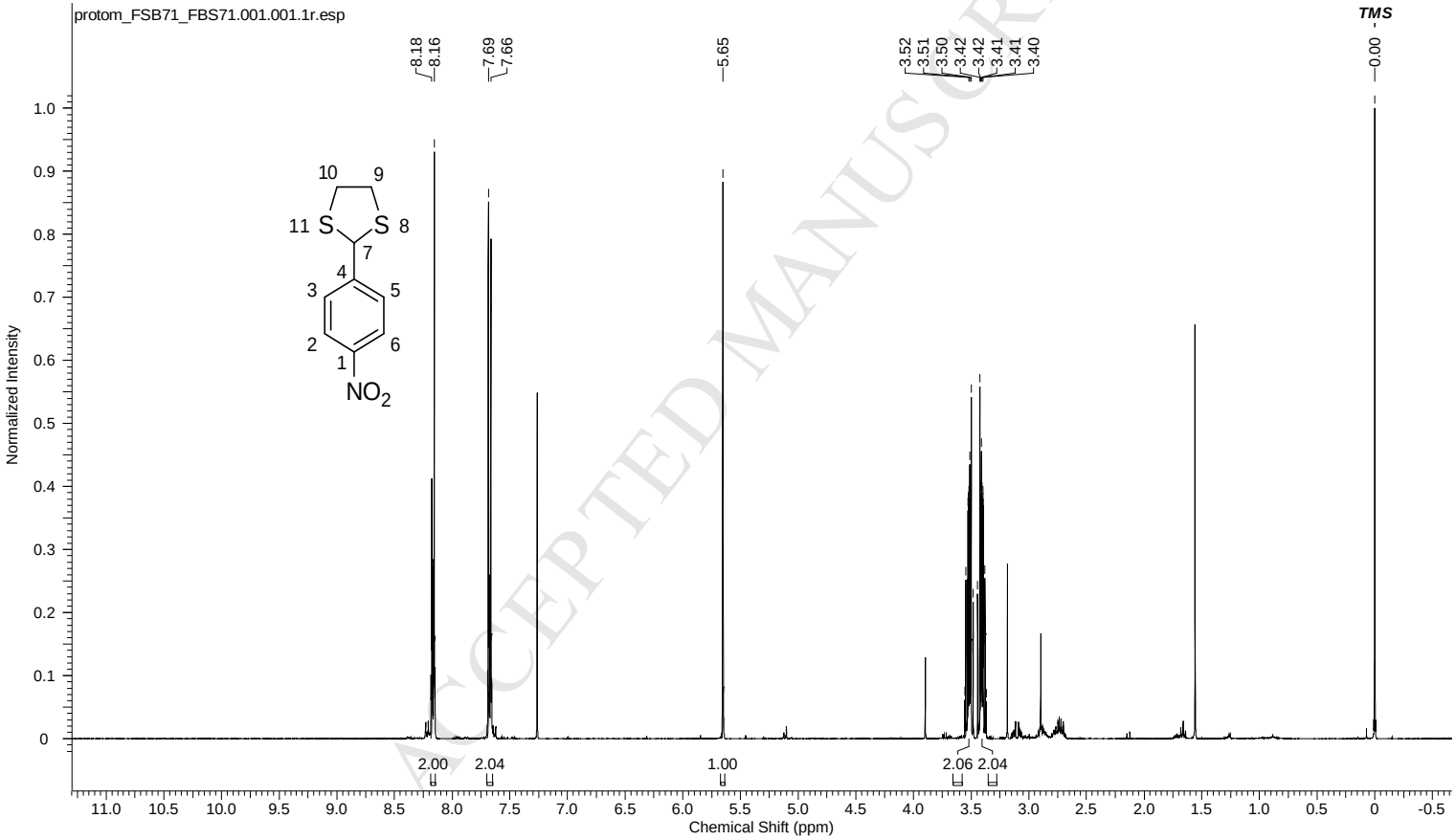


Figure S21. ^{1}H NMR (400 MHz) in $CDCl_3$ of compound **2**.

NMR Spectrum

		Acquisition Time (sec)	0.6641	***Date*** 14 Sep 2011 15:14:24	***File Name*** _FBS71
Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	1024
Origin	spect	***Original Points Count***	16384	***Owner***	nmrsu
Points Count	32768	***Pulse Sequence***	zgpg30	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	24671.05	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	10058.5996
Spectrum Type	STANDARD	***Sweep Width (Hz)***	24670.30	***Temperature (degree C)***	26.800

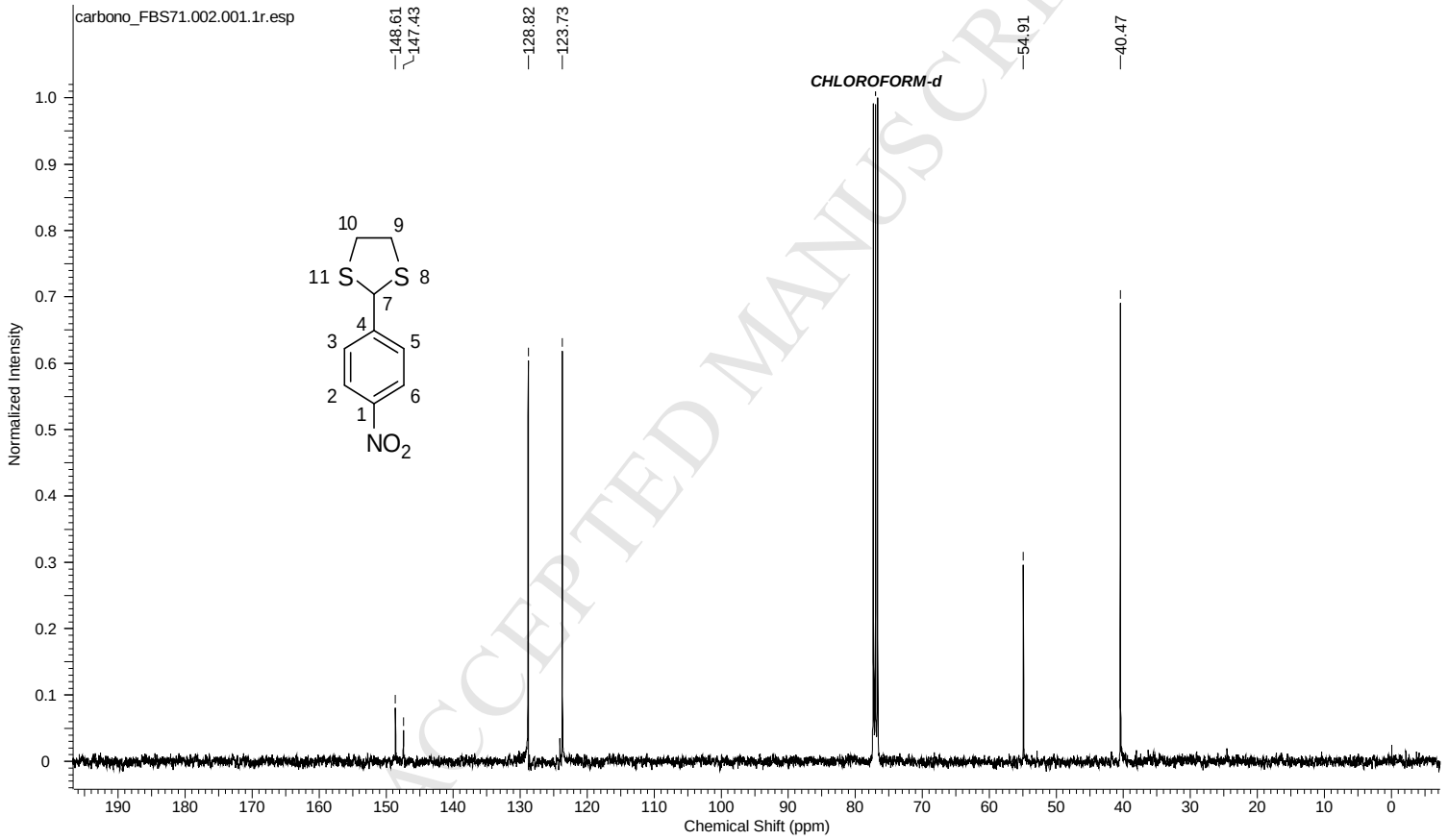


Figure S22. ^{13}C NMR (100 MHz) in $CDCl_3$ of compound **2**.

NMR Spectrum

Acquisition Time (sec)	0.8126	***Date***	14 Sep 2011 15:37:52	***File Name***	FBS71
Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	1024
Origin	spect	***Original Points Count***	16384	***Owner***	nmrsu
Points Count	32768	***Pulse Sequence***	deptsp135	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	20161.29	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	8048.4780
Spectrum Type	DEPT135	***Sweep Width (Hz)***	20160.68	***Temperature (degree C)***	26.800

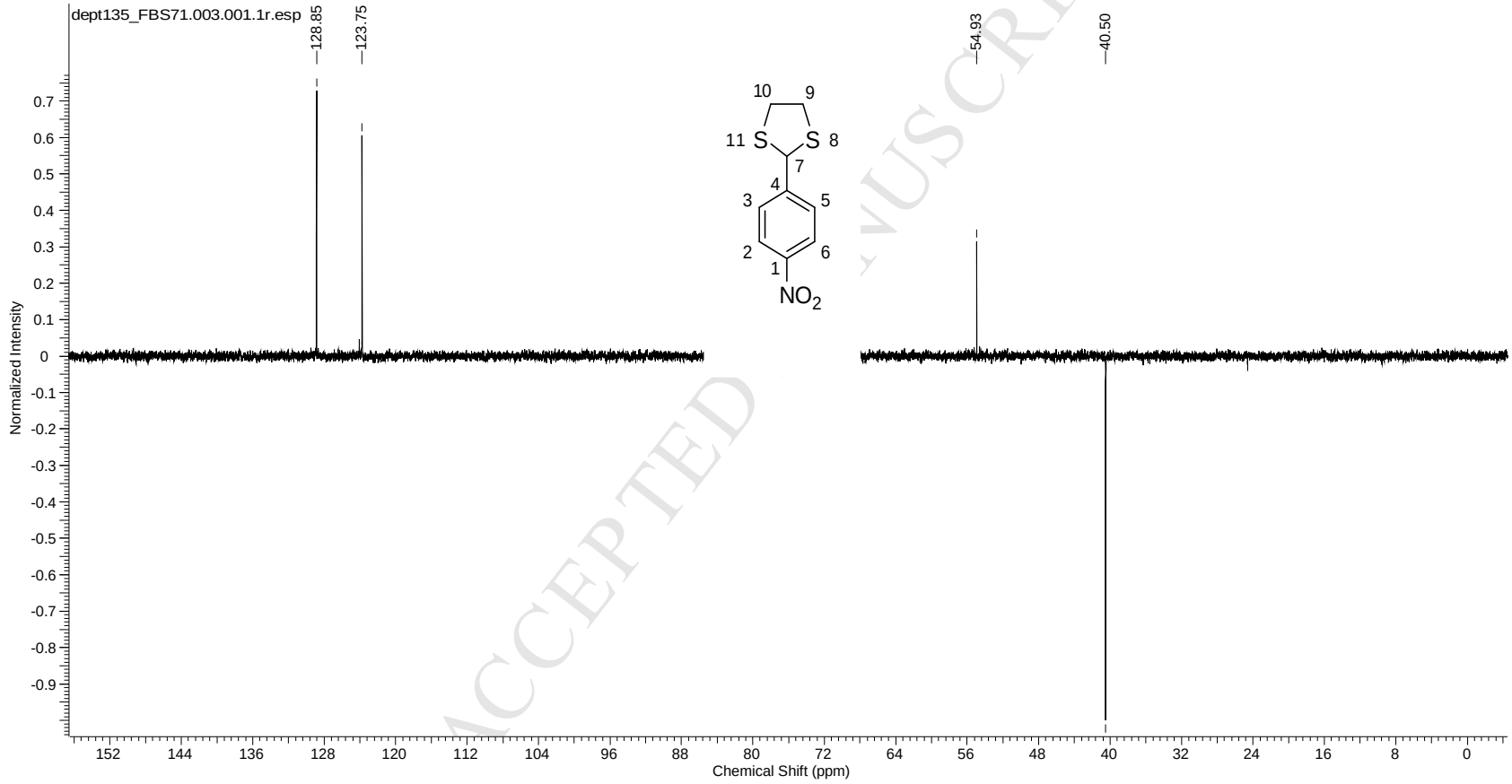


Figure S23. ^{13}C (DEPT-135) in $CDCl_3$ of compound **2**.

NMR Spectrum

Acquisition Time (sec)	4.8060	***File Name***	FBS26	***Date***	07 Feb 2011 11:07:12
Frequency (MHz)	400.15	***Nucleus***	1H	***Number of Transients***	16
Origin	spect	***Original Points Count***	32768	***Owner***	nmrsu
Points Count	65536	***Pulse Sequence***	zg30	***Receiver Gain***	114.00
SW(cyclical) (Hz)	6818.18	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	2991.2253
Spectrum Type	STANDARD	***Sweep Width (Hz)***	6818.08	***Temperature (degree C)***	24.800

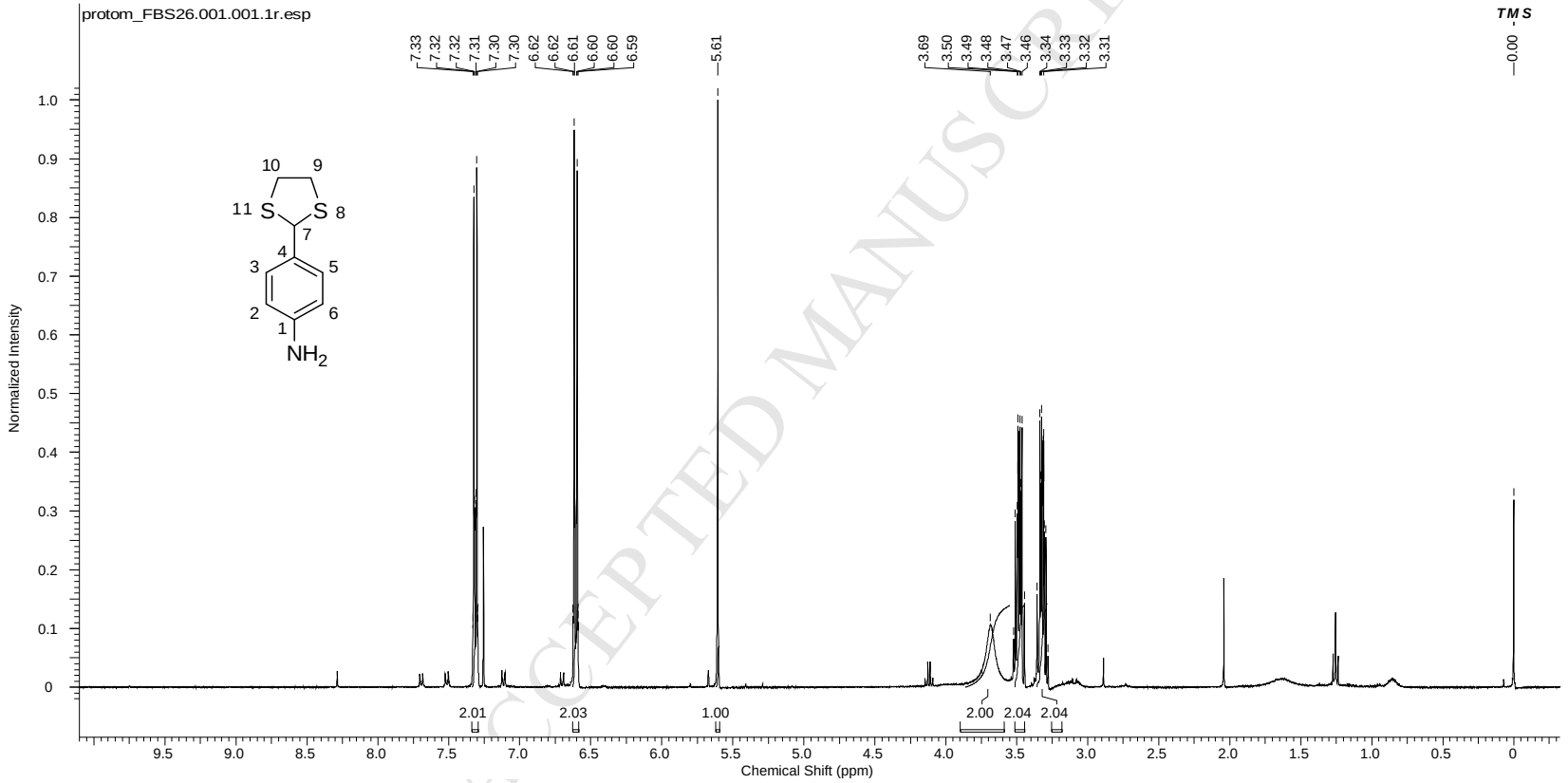


Figure S24. ^{1}H NMR (400 MHz) in $CDCl_3$ of compound **3**.

NMR Spectrum

Acquisition Time (sec)	0.6641	***File Name***	FBS72_	***Date***	07 Feb 2011 11:22:08
Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	1024
Origin	spect	***Original Points Count***	16384	***Owner***	nmrsu
Points Count	32768	***Pulse Sequence***	zgpg30	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	24671.05	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	10054.8350
Spectrum Type	STANDARD	***Sweep Width (Hz)***	24670.30	***Temperature (degree C)***	24.800

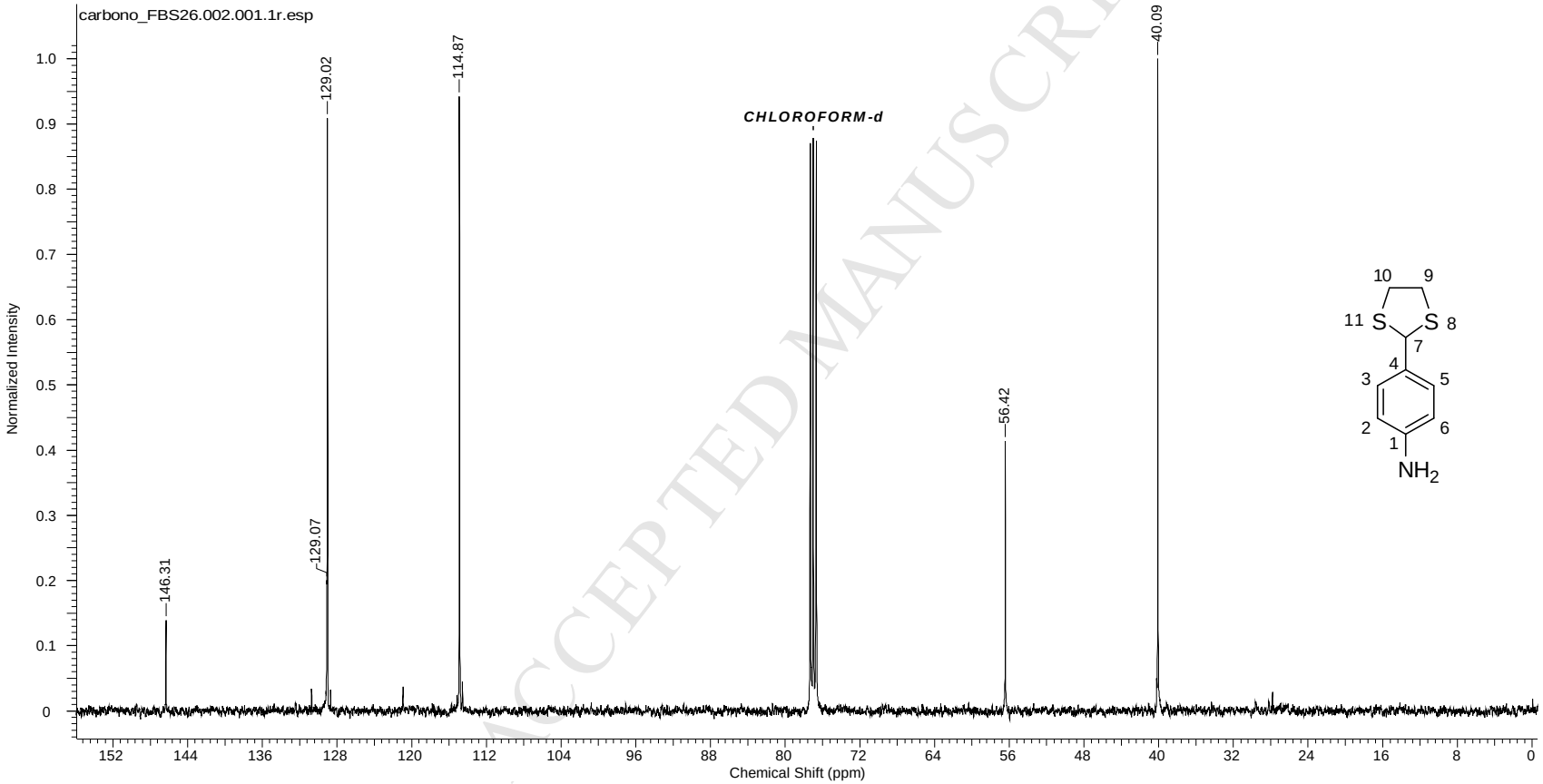


Figure S25. ^{13}C NMR (100 MHz) in $CDCl_3$ of compound**3**.

NMR Spectrum

Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	1024
File Name	FBS72	***Original Points Count***	16384	***Date***	07 Feb 2011 18:39:28
Points Count	32768	***Pulse Sequence***	deptsp135	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	20161.29	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	8048.4780
Spectrum Type	DEPT135	***Sweep Width (Hz)***	20160.68	***Temperature (degree C)***	24.800

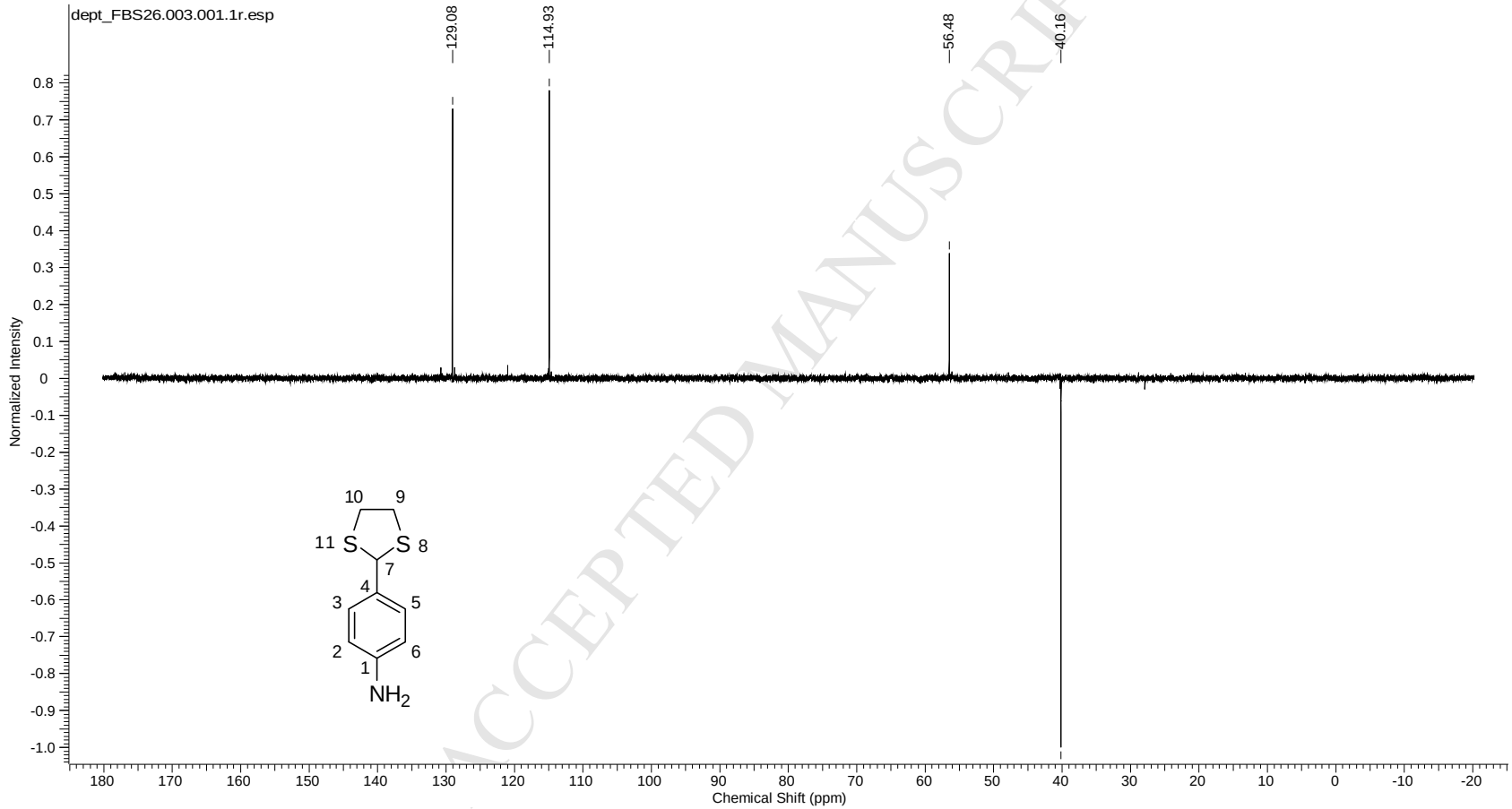


Figure S26. ^{13}C (DEPT-135) in $CDCl_3$ of compound **3**.

NMR Spectrum

Frequency (MHz)	400.15	***Nucleus***	1H	***Number of Transients***	16
File Name	FBS27	***Original Points Count***	32768	***Date***	14 Feb 2011 15:18:56
Points Count	65536	***Pulse Sequence***	zg30	***Receiver Gain***	114.00
SW(cyclical) (Hz)	6818.18	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	3001.1238
Spectrum Type	STANDARD	***Sweep Width (Hz)***	6818.08	***Temperature (degree C)***	23.800

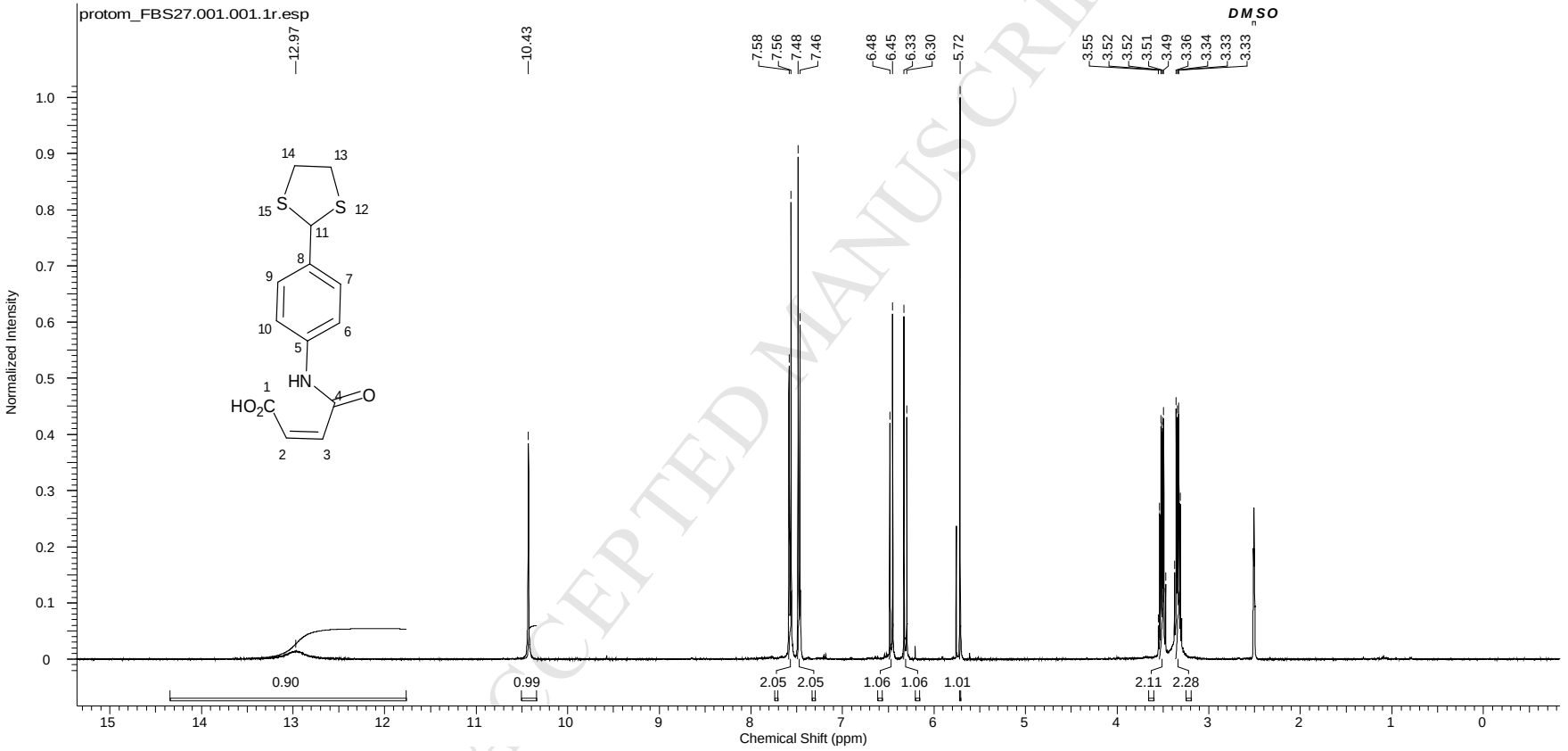


Figure S27. ^{1}H NMR (400 MHz) in DMSO-d6 of the intermediate for the preparation of compound **4**.

NMR Spectrum

File Name	_FBS73_Prot_S_Acido_			***Frequency (MHz)***	100.62	***Nucleus***	13C
Number of Transients	2048	***Temperature (degree C)***	23.600	***Original Points Count***	16384	***Date***	22 Jul 2012 23:52:48
Points Count	32768	***Pulse Sequence***	zgpg30	***Receiver Gain***	2050.00	***SW(cyclical) (Hz)***	24671.05
Solvent	DMSO-d6	***Spectrum Offset (Hz)***	10014.8047	***Spectrum Type***	STANDARD	***Sweep Width (Hz)***	24670.30

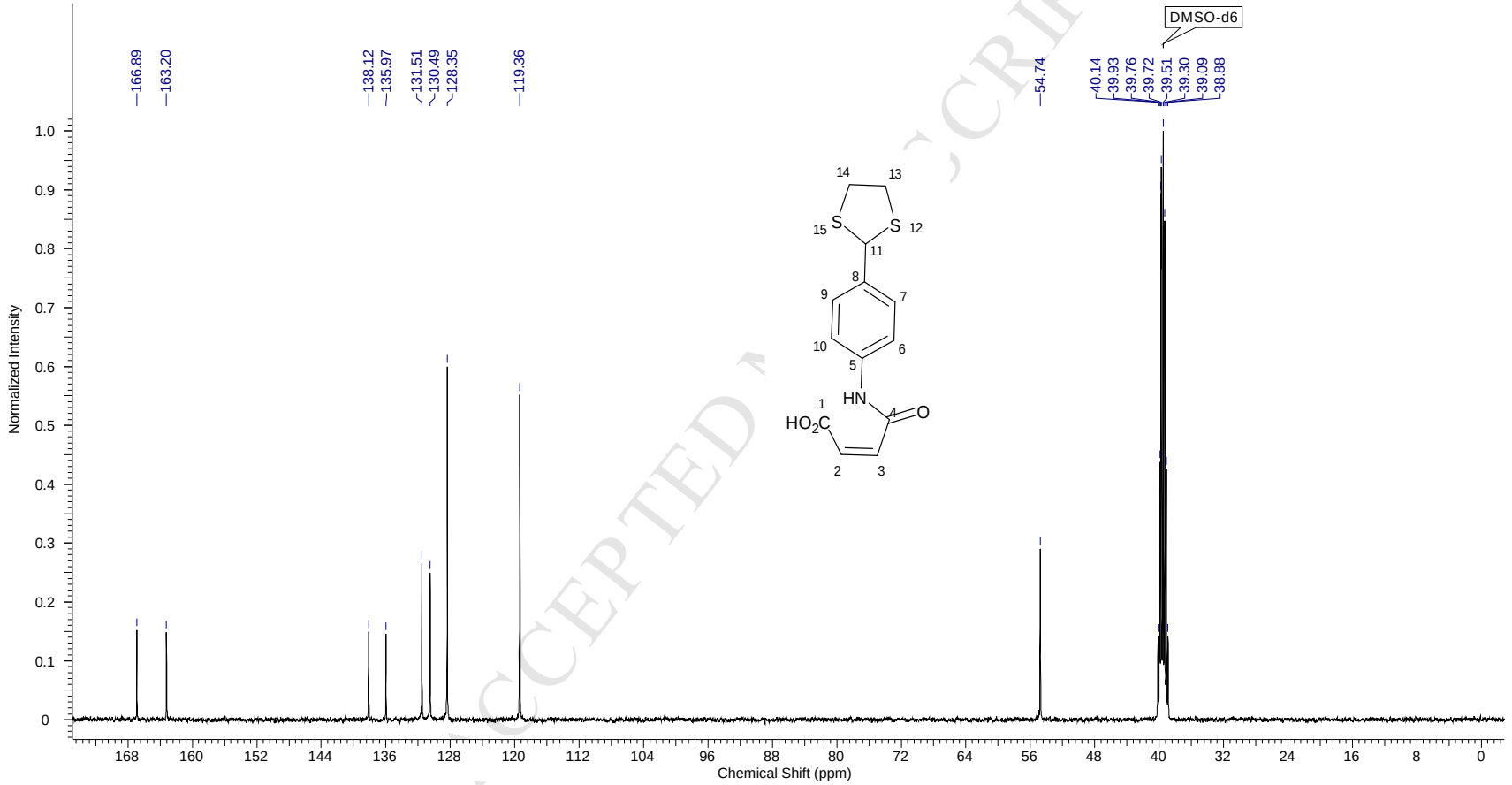


Figure S28. ^{13}C NMR (100 MHz) in DMSO-d6 of the intermediate for the preparation of compound **4**.

NMR Spectrum

Temperature (degree C)	23.200			***Date Stamp***	22 Jul 2012 23:22:56		
File Name	_FBS73_Prot_S_Acido_			***Frequency (MHz)***	100.62	***Nucleus***	13C
Number of Transients	1024	***Origin***	spect	***Original Points Count***	16384	***Date***	22 Jul 2012 23:22:56
Points Count	32768	***Pulse Sequence***	deptsp135	***Receiver Gain***	2050.00	***SW(cyclical) (Hz)***	20161.29
Solvent	DMSO-d6	***Spectrum Offset (Hz)***	7998.1680	***Spectrum Type***	DEPT135	***Sweep Width (Hz)***	20160.68

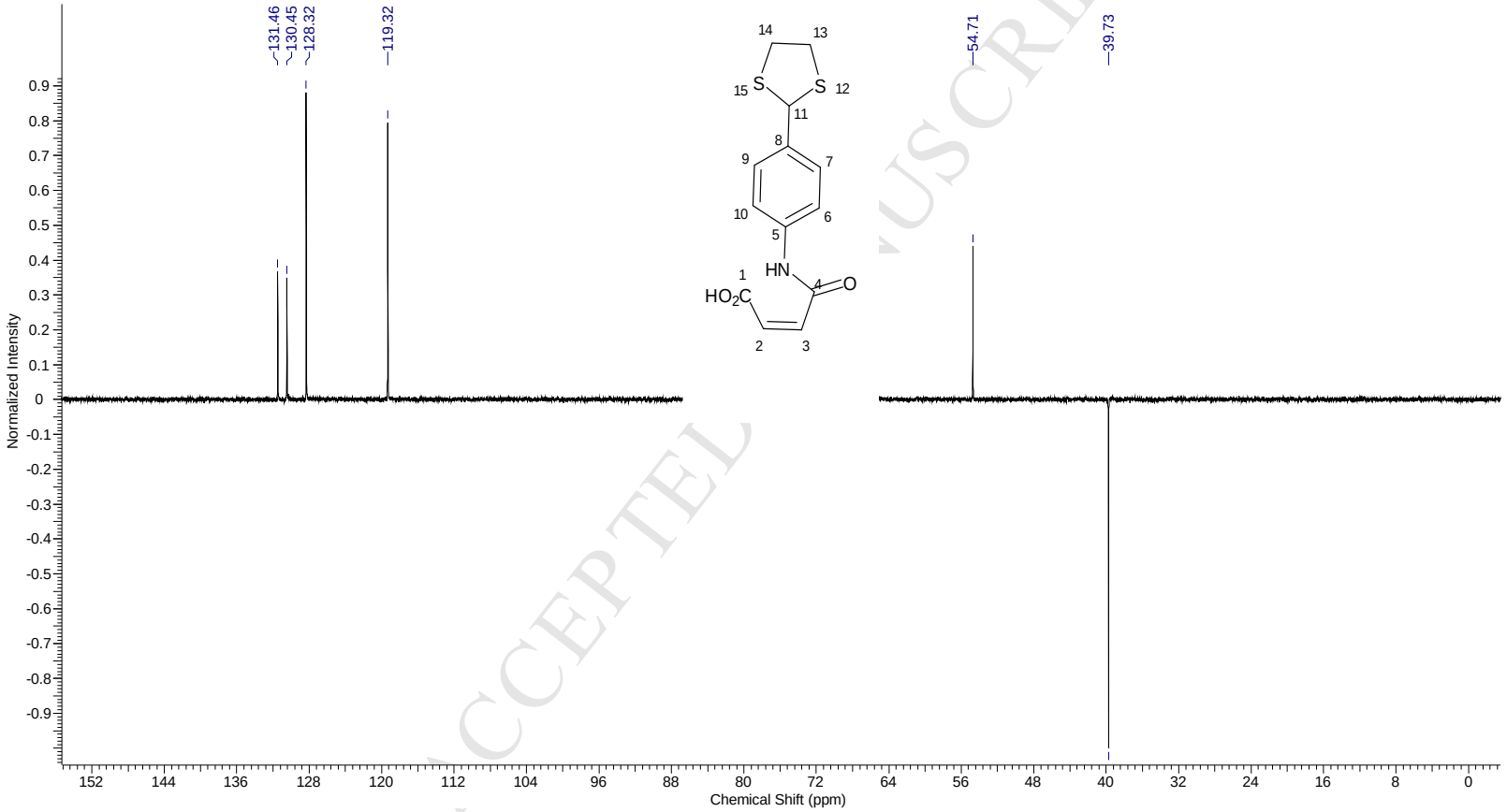


Figure S29.^{13}C (DEPT-135) in DMSO-d6 of the intermediate for the preparation of compound **4**.

NMR Spectrum

Frequency (MHz)	400.15	***Nucleus***	1H	***Number of Transients***	16
File Name	FBS37	***Original Points Count***	32768	***Date***	11 Apr 2011 17:39:28
Points Count	65536	***Pulse Sequence***	zg30	***Receiver Gain***	203.00
SW(cyclical) (Hz)	6818.18	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	2993.6184
Spectrum Type	STANDARD	***Sweep Width (Hz)***	6818.08	***Temperature (degree C)***	24.800

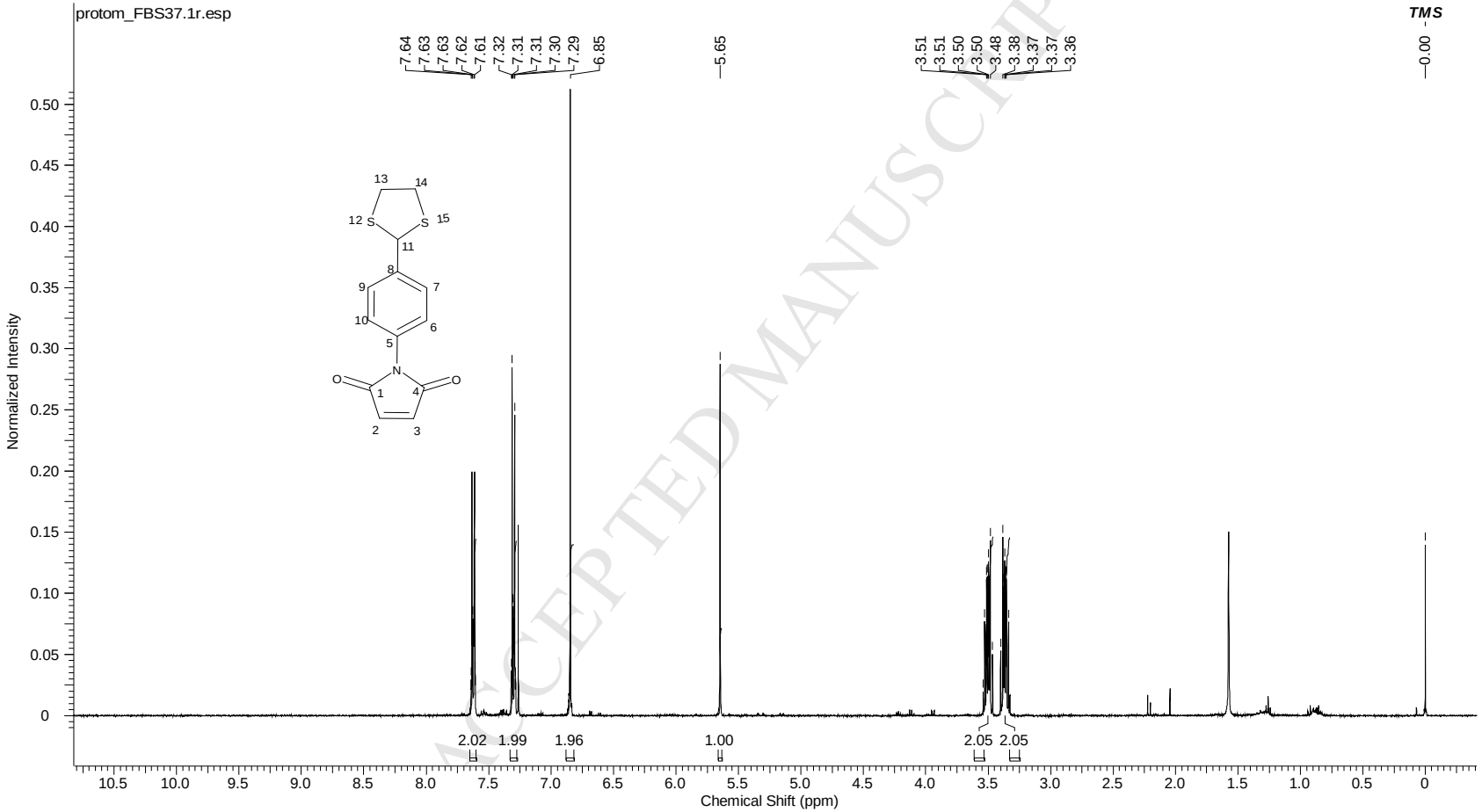


Figure S30. ^{1}H NMR (400 MHz) in $CDCl_3$ of compound **4**.

NMR Spectrum

Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	1024
File Name	FBS42	***Original Points Count***	16384	***Date***	21 Apr 2011 15:14:24
Points Count	32768	***Pulse Sequence***	zgpg30	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	24671.05	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	10058.5996
Spectrum Type	STANDARD	***Sweep Width (Hz)***	24670.30	***Temperature (degree C)***	24.900

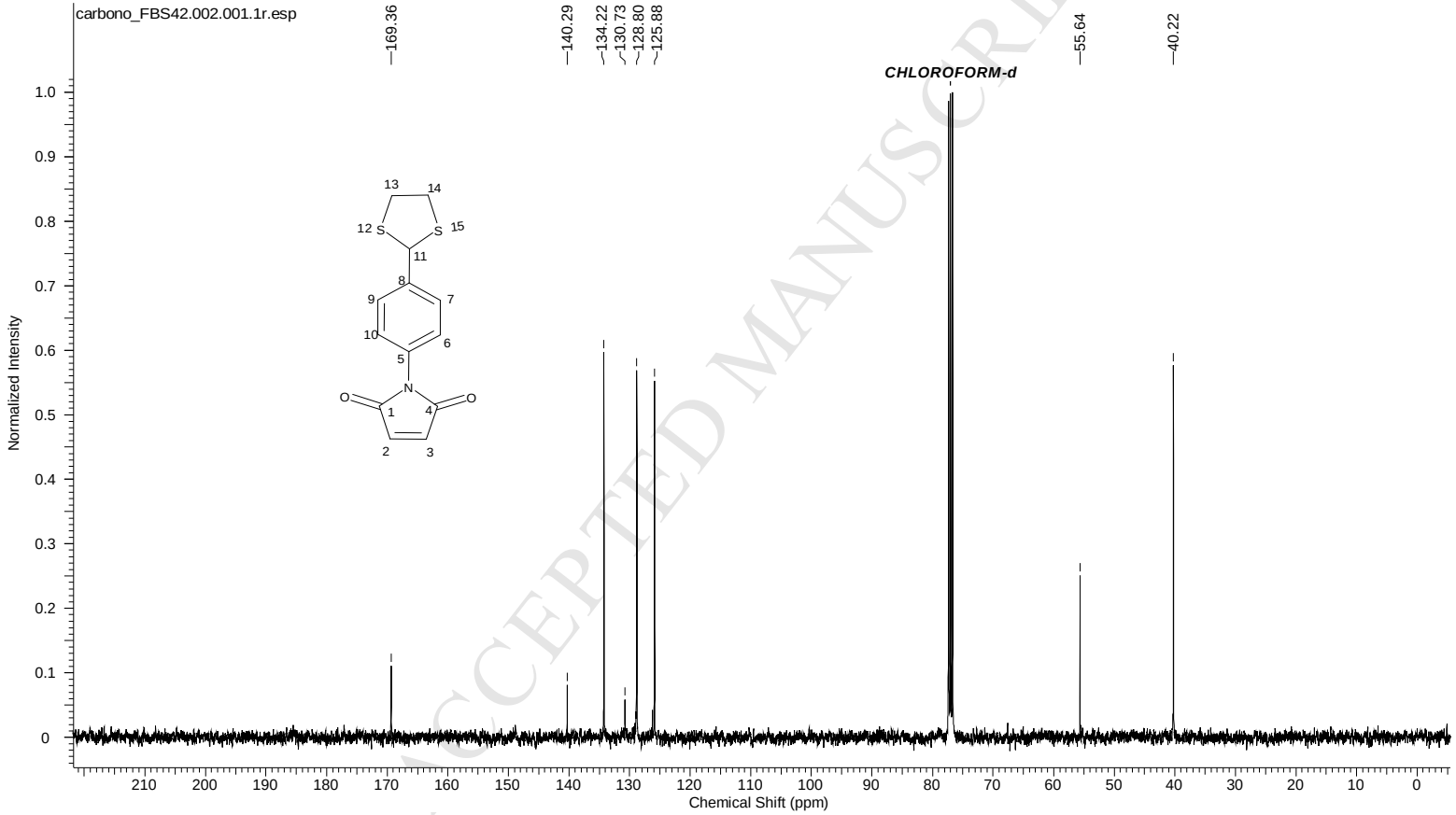


Figure S31.^{13}C NMR (100 MHz) in $CDCl_3$ of compound **4**.

NMR Spectrum

Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	1024
File Name	FBS37	***Original Points Count***	16384	***Date***	11 Apr 2011 18:02:56
Points Count	32768	***Pulse Sequence***	deptsp135	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	20161.29	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	8048.4780
Spectrum Type	DEPT135	***Sweep Width (Hz)***	20160.68	***Temperature (degree C)***	24.800

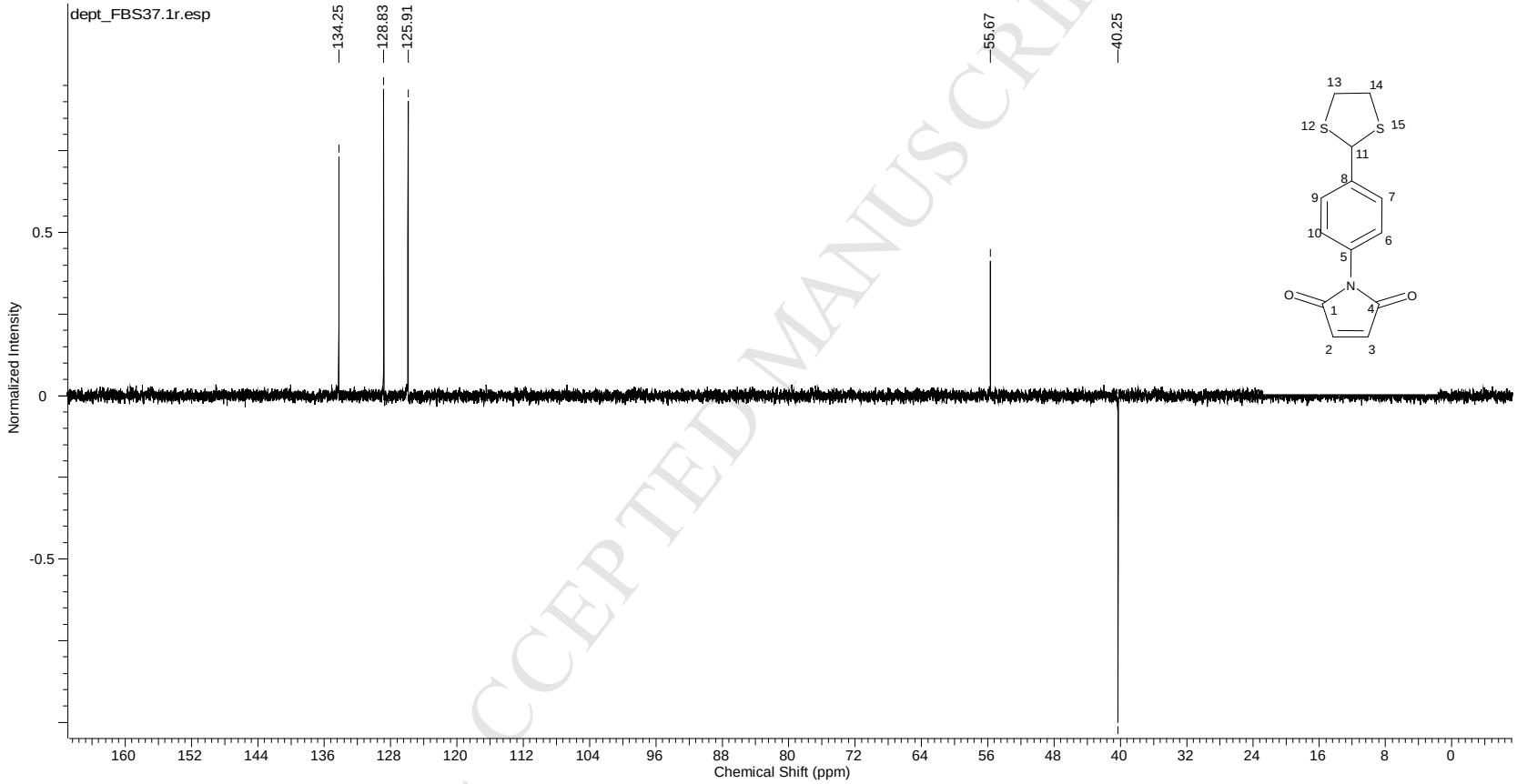


Figure S32. ^{13}C (DEPT-135) in $CDCl_3$ of compound **4**.

NMR Spectrum

File Name	E:\1B329_FBS60-imida_aldeido\1\pdata\1\1r			***Frequency (MHz)***	400.15	***Nucleus***	1H
Number of Transients	16	***Origin***	spect	***Original Points Count***	32768	***Owner***	nmrsu
Points Count	65536	***Pulse Sequence***	zg30	***Receiver Gain***	203.00	***SW(cyclical) (Hz)***	6818.18
Solvent	CHLOROFORM-d	***Spectrum Offset (Hz)***	2994.0344	***Spectrum Type***	STANDARD	***Sweep Width (Hz)***	6818.08
Temperature (degree C)	26.800						

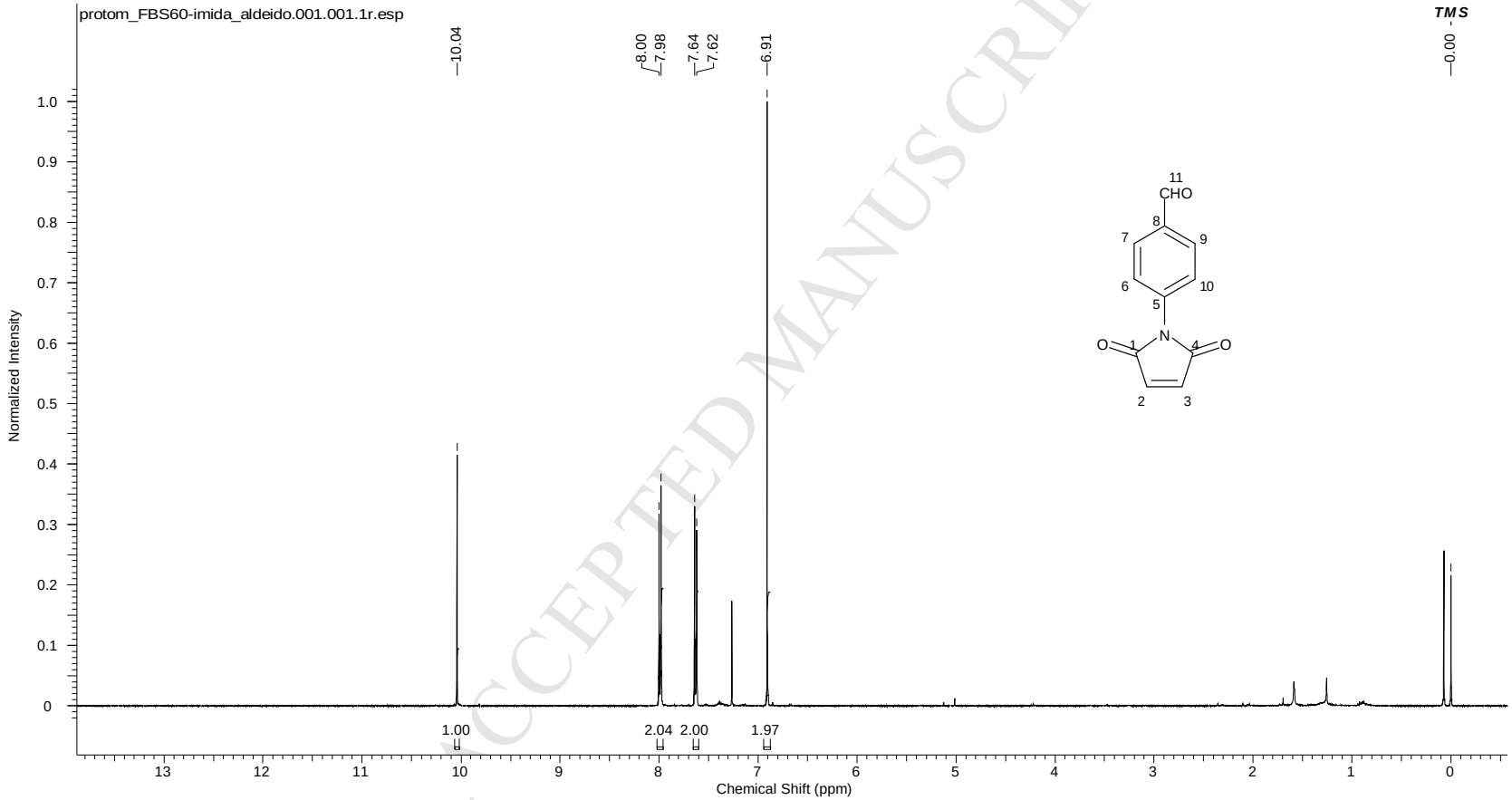


Figure S33.^{1}H NMR (400 MHz) in $CDCl_3$ of compound **5**.

NMR Spectrum

File Name	FBS81_Meleimida_Aldeido					***Frequency (MHz)***	100.62
Nucleus	13C	***Number of Transients***	2048	***Date***	23 Jul 2012 00:48:16	***Original Points Count***	16384
Owner	nmrsu	***Points Count***	32768	***Pulse Sequence***	zgpg30	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	24671.05	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	10057.0938	***Spectrum Type***	STANDARD
Sweep Width (Hz)	24670.30	***Temperature (degree C)***	23.600				

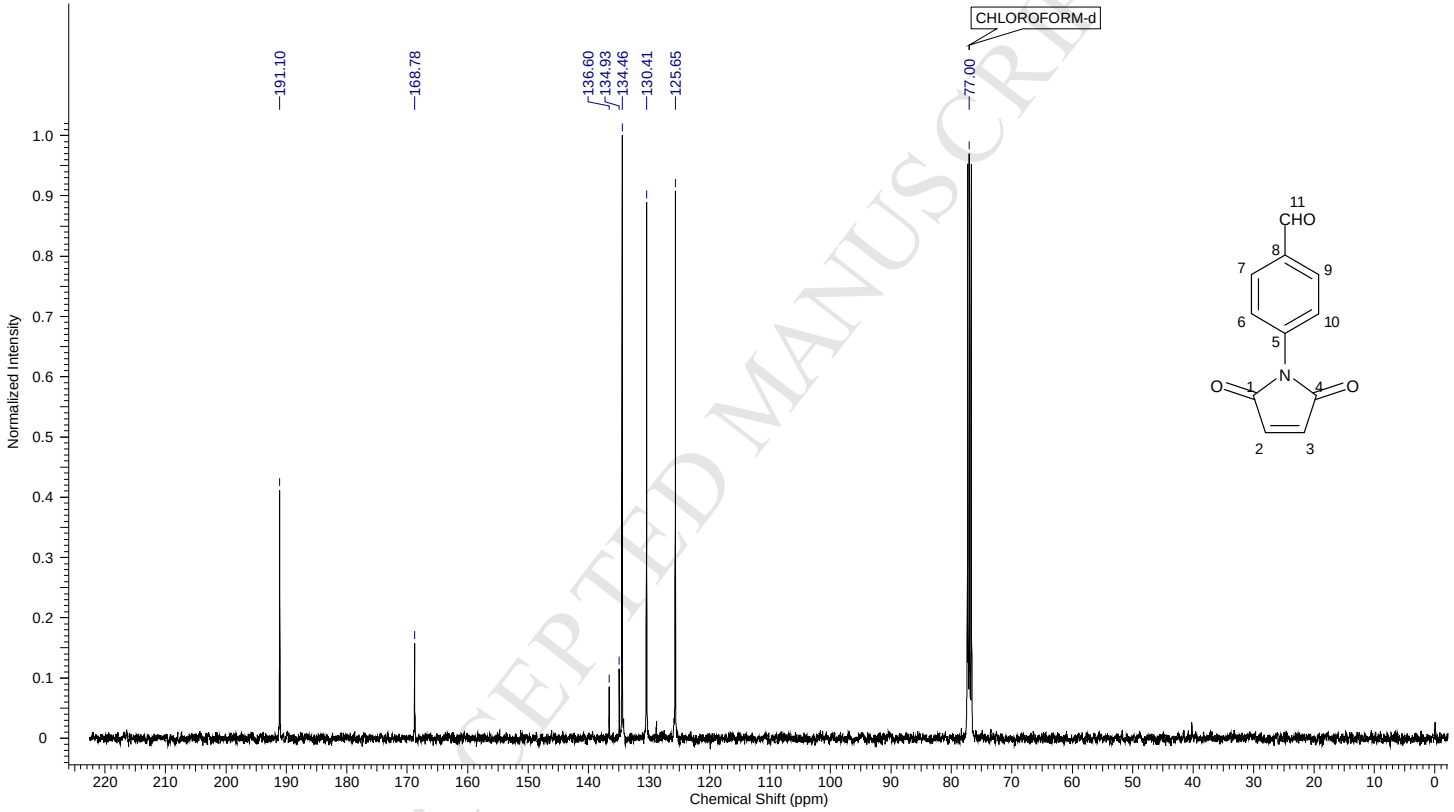


Figure S34.^{13}C NMR (100 MHz) in $CDCl_3$ of compound **5**.

NMR Spectrum

File Name	FBS81_Meleimida_Aldeido_			***Frequency (MHz)***	100.62	***Nucleus***	13C
Number of Transients	1024	***Origin***	spect	***Original Points Count***	16384	***Date***	23 Jul 2012 00:20:32
Points Count	32768	***Pulse Sequence***	deptsp135	***Receiver Gain***	2050.00	***SW(cyclical) (Hz)***	20161.29
Solvent	CHLOROFORM-d	***Spectrum Offset (Hz)***	8048.4780	***Spectrum Type***	DEPT135	***Sweep Width (Hz)***	20160.68
Temperature (degree C)	23.300						

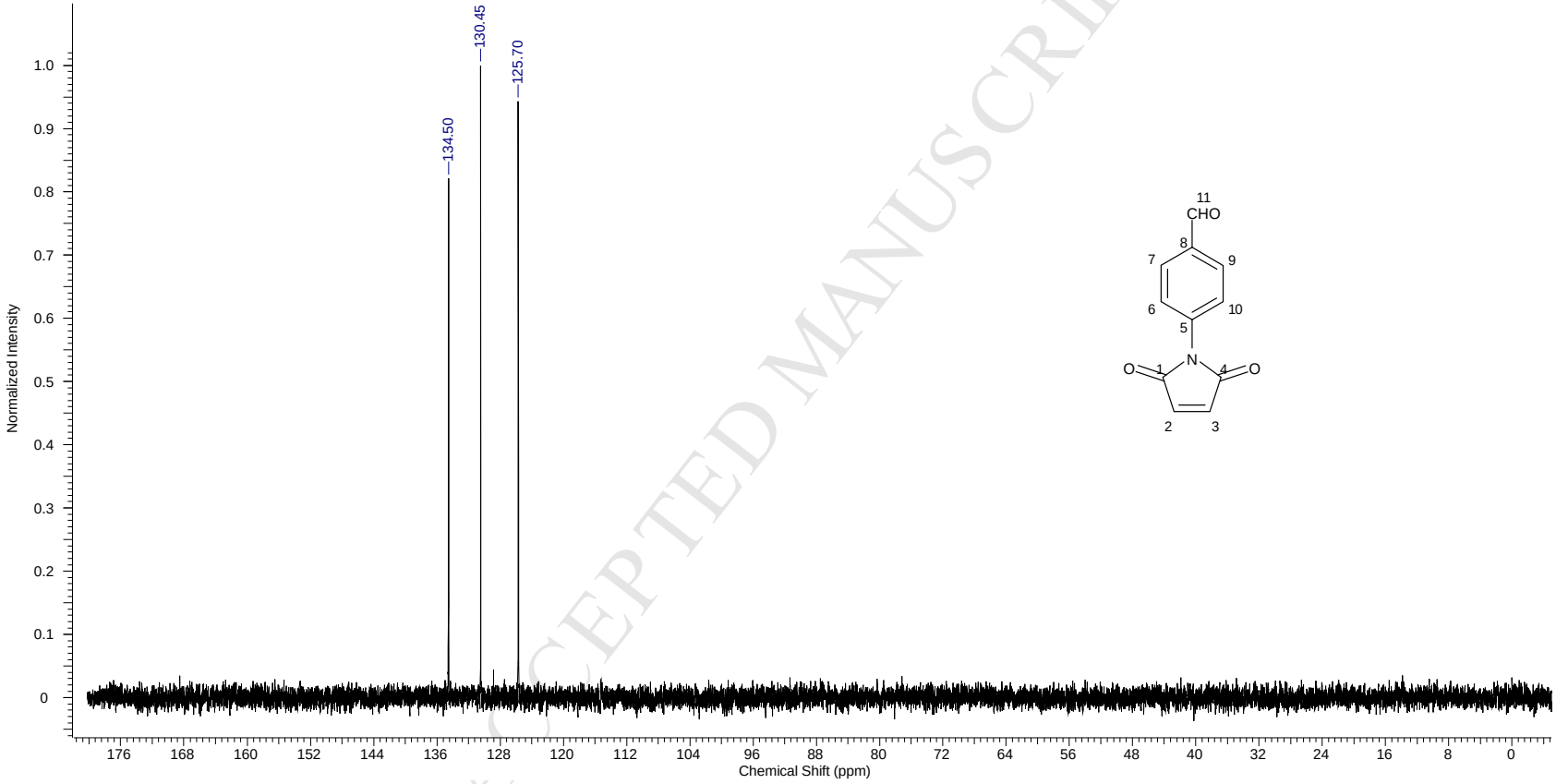


Figure S35.^{13}C (DEPT-135) in $CDCl_3$ of compound **5**.

NMR Spectrum

Acquisition Time (sec)	3.2768	***Comment***	FBS pg 04	***Date***	04 Oct 2010 16:37:36		
Nucleus	1H	***Number of Transients***	32	***Origin***	spect	***Frequency (MHz)***	400.15
Owner	RMN	***Points Count***	65536	***Pulse Sequence***	zg30	***Original Points Count***	32768
SW(cyclical) (Hz)	10000.00	***Solvent***	CHLOROFORM-d			***Receiver Gain***	144.00
Spectrum Type	STANDARD	***Sweep Width (Hz)***	9999.85	***Temperature (degree C)***	25.000	***Spectrum Offset (Hz)***	2460.0984

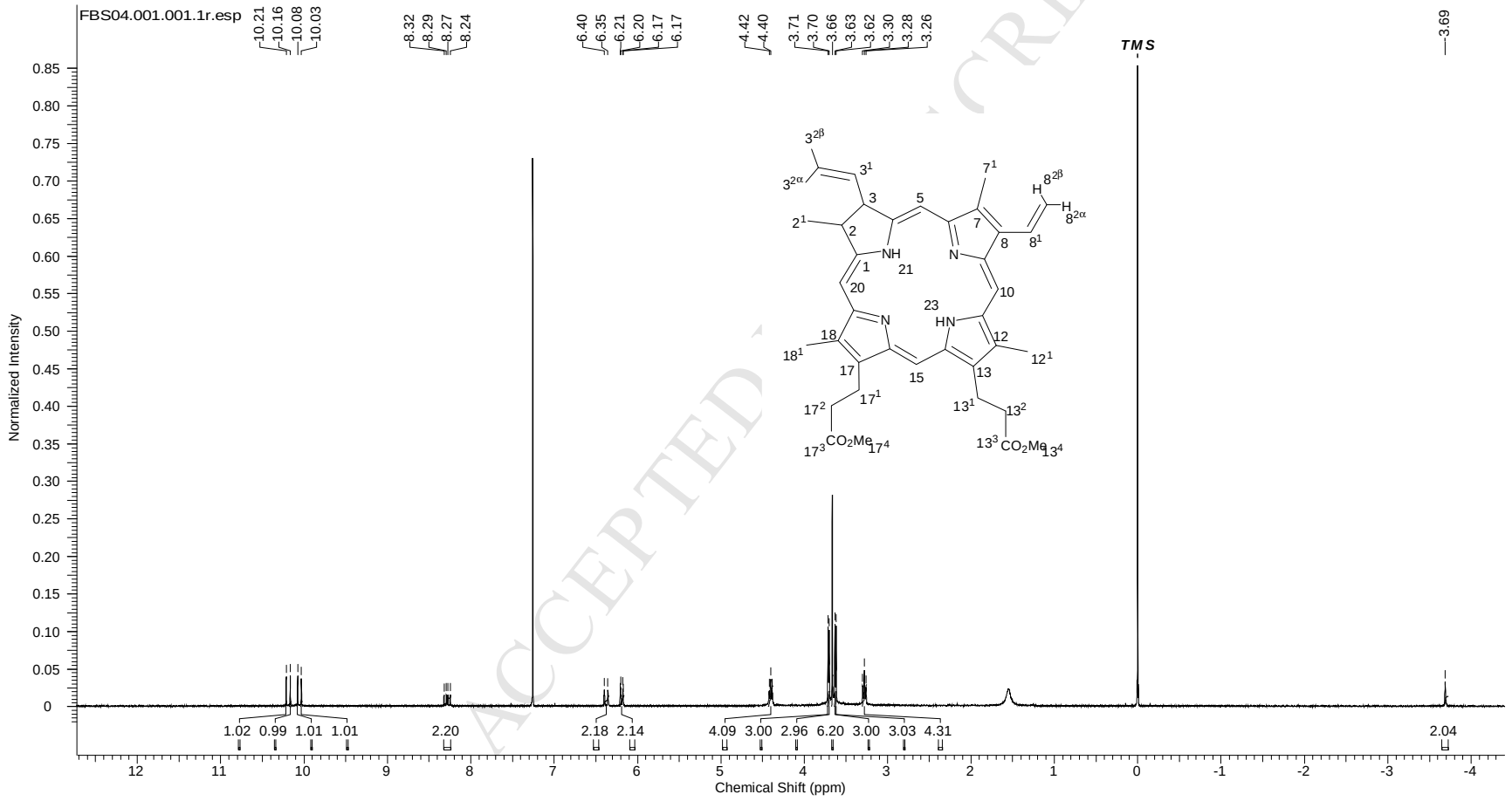


Figure S36.^{1}H NMR (400 MHz) in $CDCl_3$ of compound **7**.

NMR Spectrum

Frequency (MHz)	400.13	***Nucleus***	1H	***Number of Transients***	32	***File Name***	C. Protegida M2
Original Points Count	32768	***Date***	31 Jan 2012 19:27:12	***Points Count***	65536	***Pulse Sequence***	zg
Receiver Gain	114.00	***SW(cyclical) (Hz)***	8370.54	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	1931.2543
Spectrum Type	STANDARD	***Sweep Width (Hz)***	8370.41	***Temperature (degree C)***	24.993		

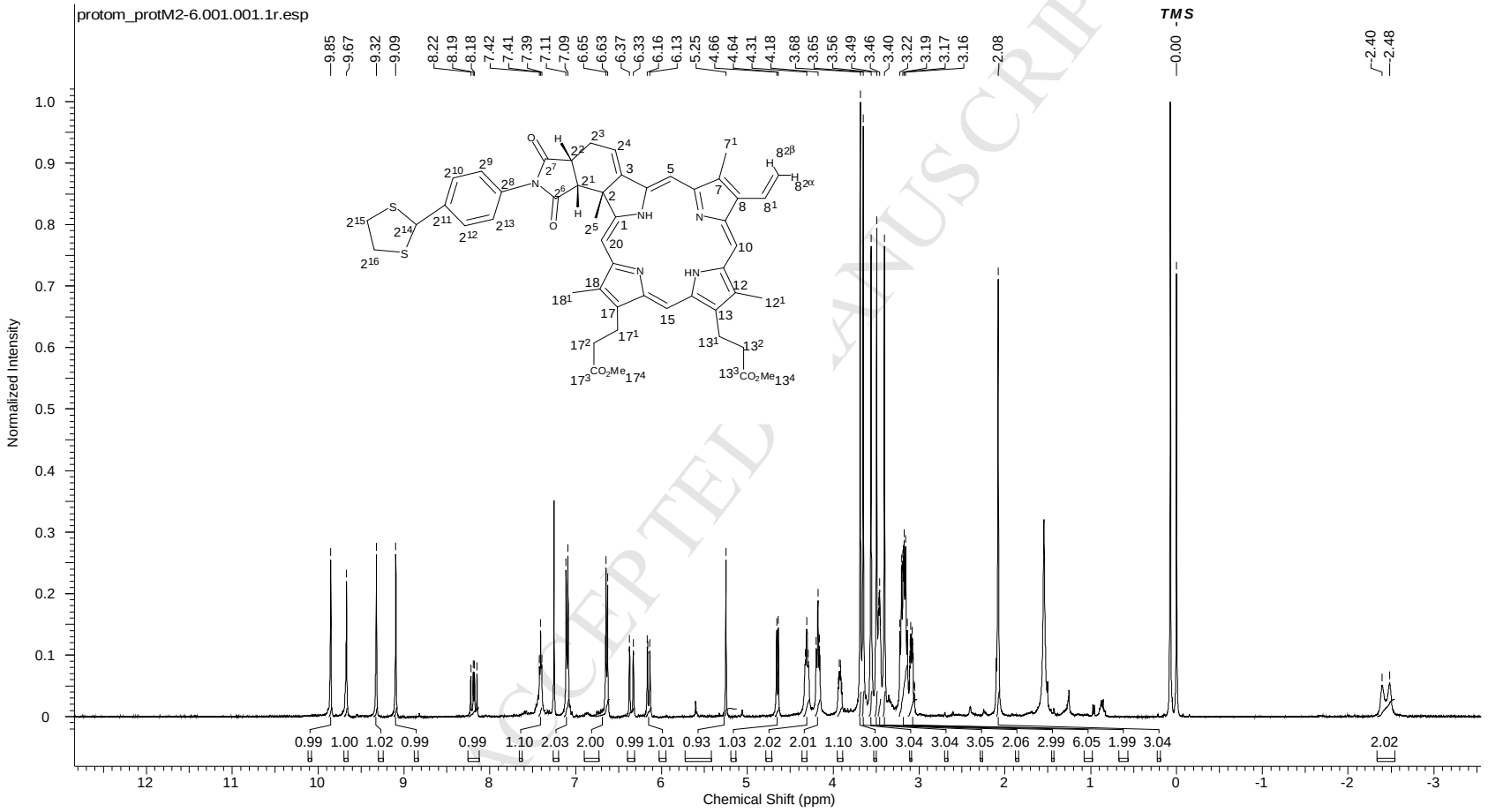


Figure S37.^{1}H NMR (400 MHz) in $CDCl_3$ of compound **8a**.

NMR Spectrum

Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	4096
Date	23 Jun 2012 11:00:32	***Original Points Count***	16384	***File Name***	C.Protegida M2
Points Count	32768	***Pulse Sequence***	zgpg30	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	24671.05	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	10057.8467
Spectrum Type	STANDARD	***Sweep Width (Hz)***	24670.30	***Temperature (degree C)***	24.400

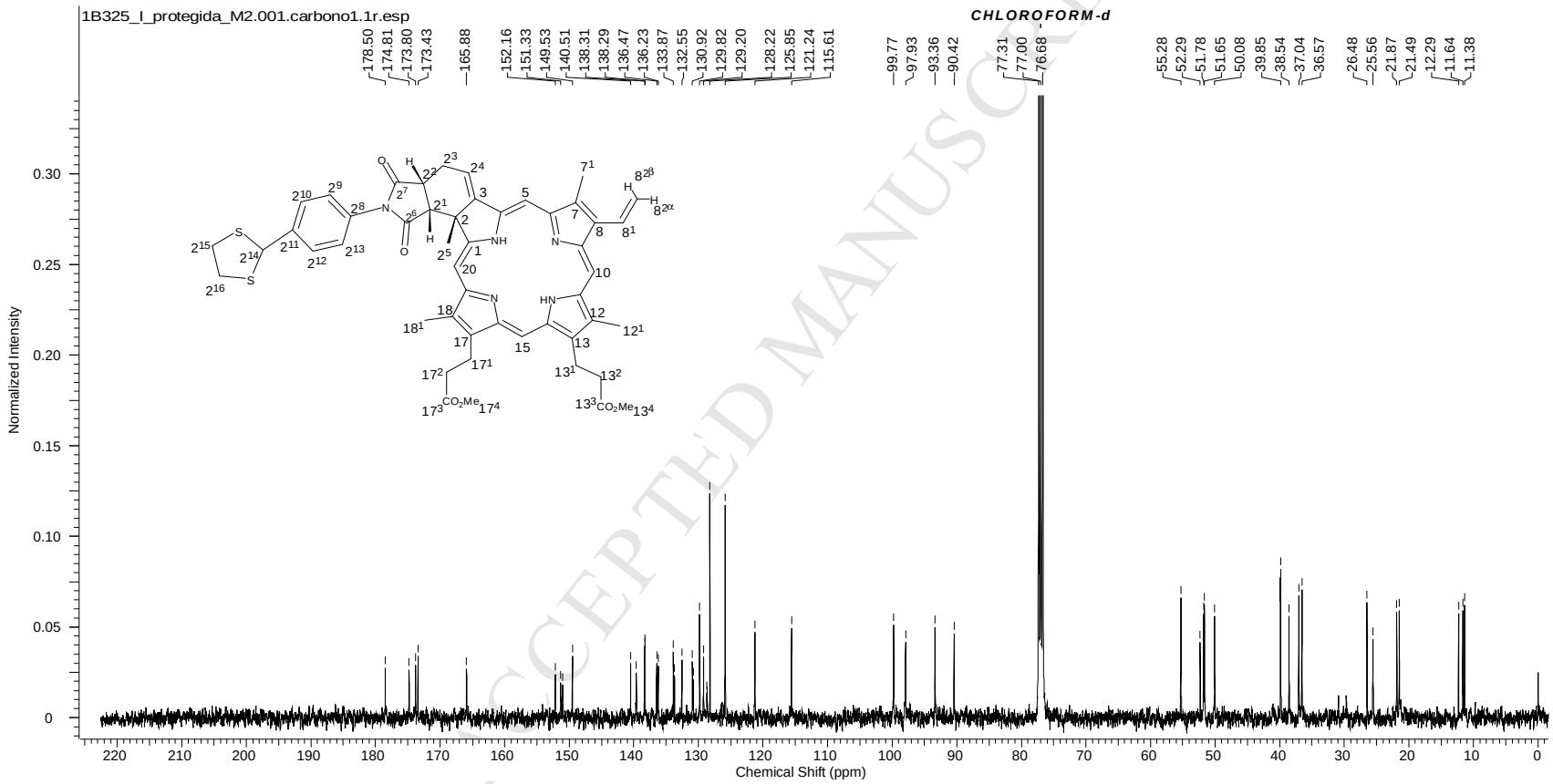


Figure S38.^{13}C NMR (100 MHz) in $CDCl_3$ of compound **8a**.

NMR Spectrum

Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	2048
Date	04 Jul 2011 16:05:36	***Original Points Count***	16384	***File Name***	
Points Count	32768	***Pulse Sequence***	deptsp135	***Receiver Gain***	724.00
SW(cyclical) (Hz)	20161.29	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	8048.4780
Spectrum Type	DEPT135	***Sweep Width (Hz)***	20160.68	***Temperature (degree C)***	26.800

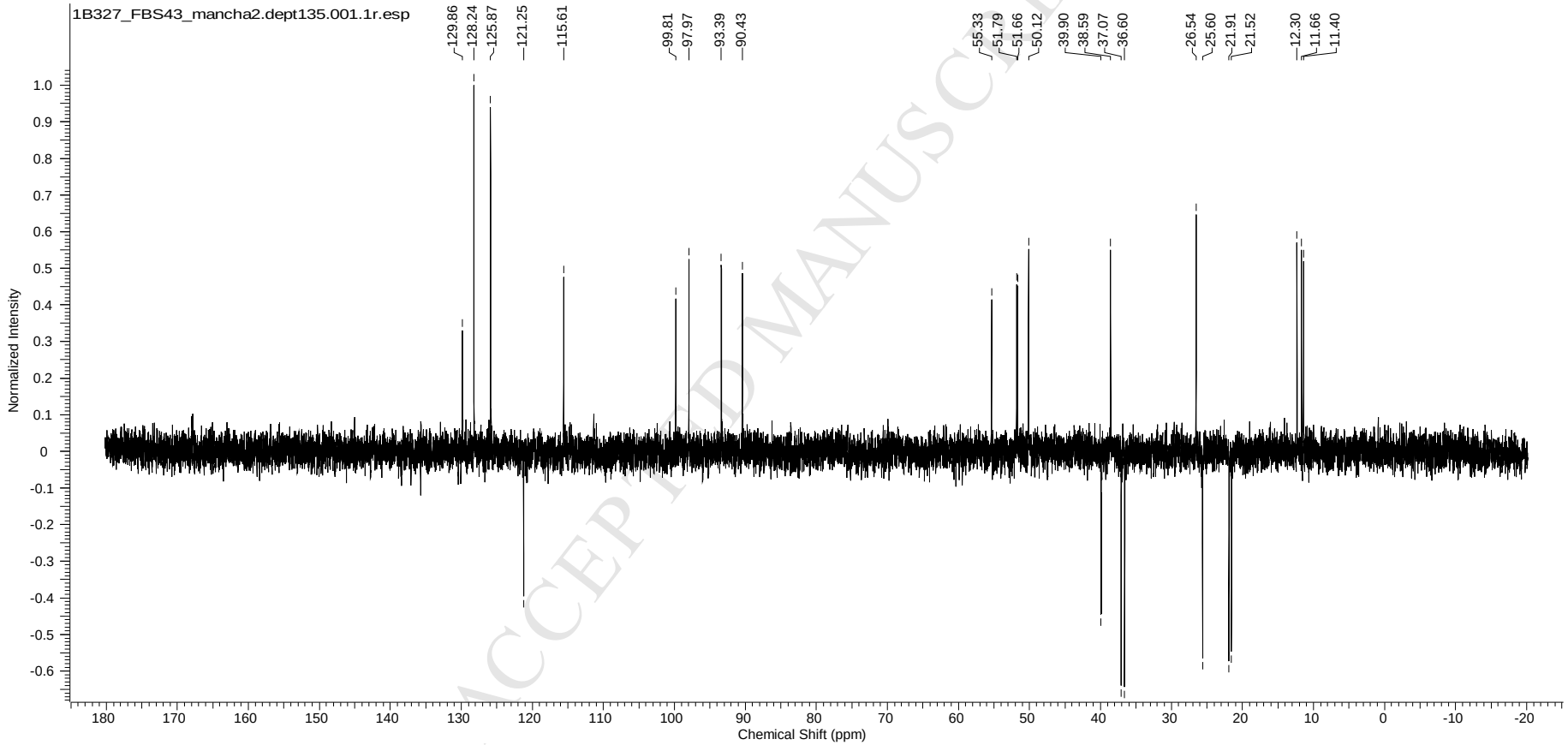
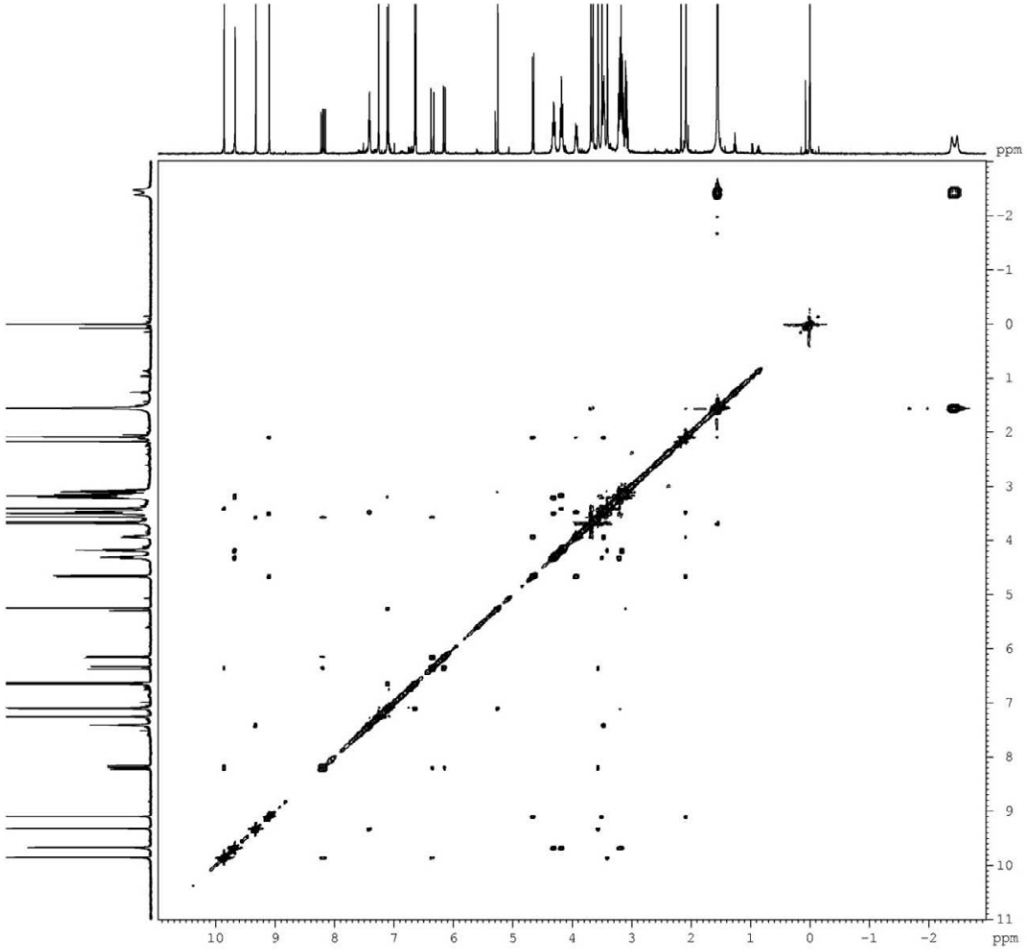


Figure S39.^{13}C (DEPT-135) in $CDCl_3$ of compound **8a**.

NMR Spectrum

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FIDRES          1.667627 Hz
AQ              0.2998772 sec
RG                   181
DW                73.200 use
DE                 6.50 use
TE                298.2 K
D0           0.00006284 sec
D1           1.00000000 sec
D8           0.80000001 sec
D11          0.03000000 sec
D12          0.00002000 sec
IN0          0.00014640 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                 0.14 use
P17             2500.00 use
PLW1         10.00000000 W
PLW10         0.98017001 W
SFO1        400.1317985 MHz

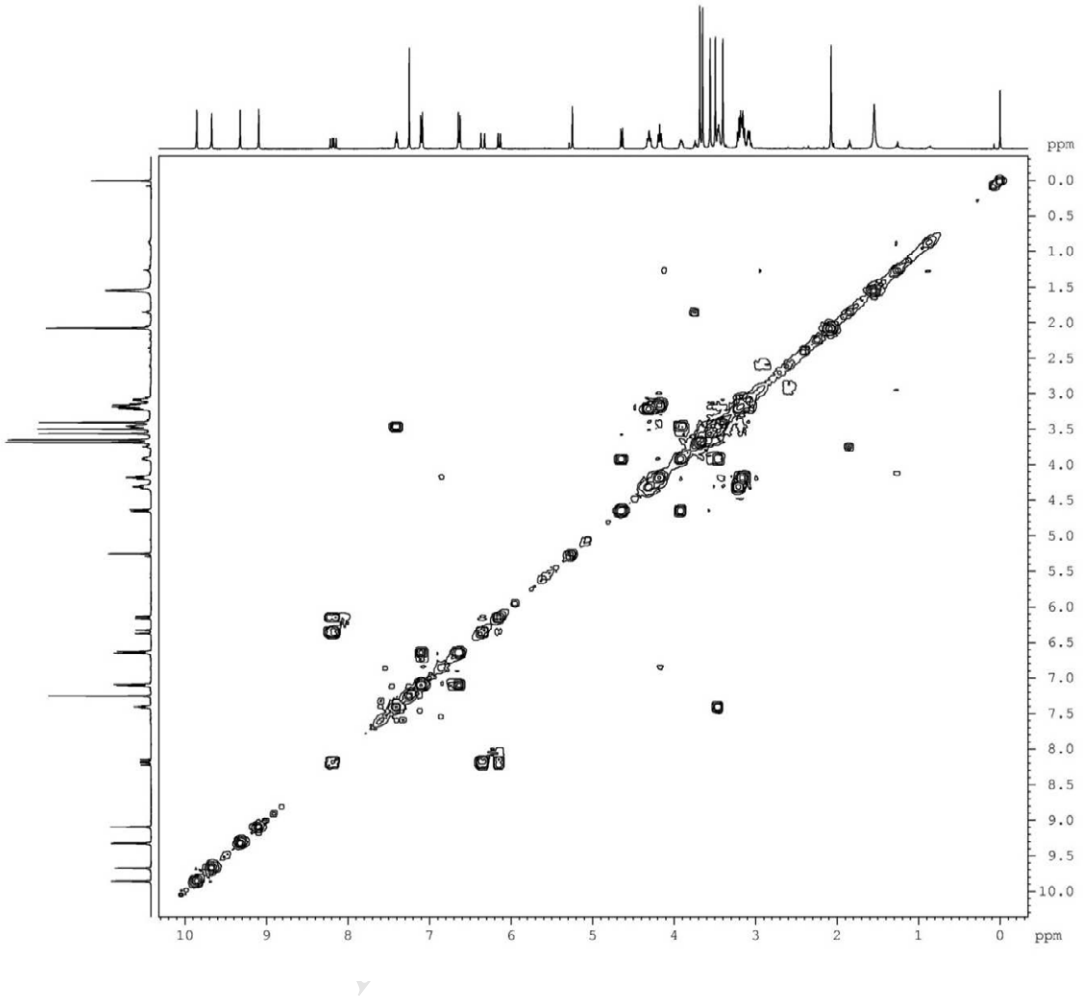
F1 - Acquisition parameters
TD                   256
SFO1        400.1318 MHz
FIDRES       26.682035 Hz
SW              17.071 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                  1024
SF          400.1300118 MHz
WDW                QSINE
SSB                    2
LB        0 Hz
GB        0
PC                  1.00

F1 - Processing parameters
SI                  1024
MC2          States-TPPI
SF          400.1300090 MHz
WDW                    A
SSB                    2
LB        0 Hz
GB        0
```

Figure S40. gNOESY (400 MHz) in $CDCl_3$ of compound **8a**.

NMR Spectrum




```
Current Data Parameters
NAME      1A823_fbs43-m2
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110610
Time                0.45
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG       cosygpppqf
TD                  4096
SOLVENT             CDCl3
NS                     8
DS                    16
SWH             8802.817 Hz
FIDRES          2.149125 Hz
AQ             0.2327028 sec
RG                   181
DW                56.800 usec
DE                10.00 usec
TE                299.8 K
D0           0.00000300 sec
D1           1.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
D13          0.00000400 sec
D16          0.00020000 sec
IN0          0.00011360 sec

-------- CHANNEL f1 --------
NUC1                   1H
P0                  8.76 usec
P1                  8.76 usec
P17              2500.00 usec
PLW1          18.00000000 W
PLW10          2.04329991 W
SFO1          400.1534013 MHz

====== GRADIENT CHANNEL =====
GPNAM1         SMSQ10.100
GPZ1                10.00 %
P16               1000.00 usec

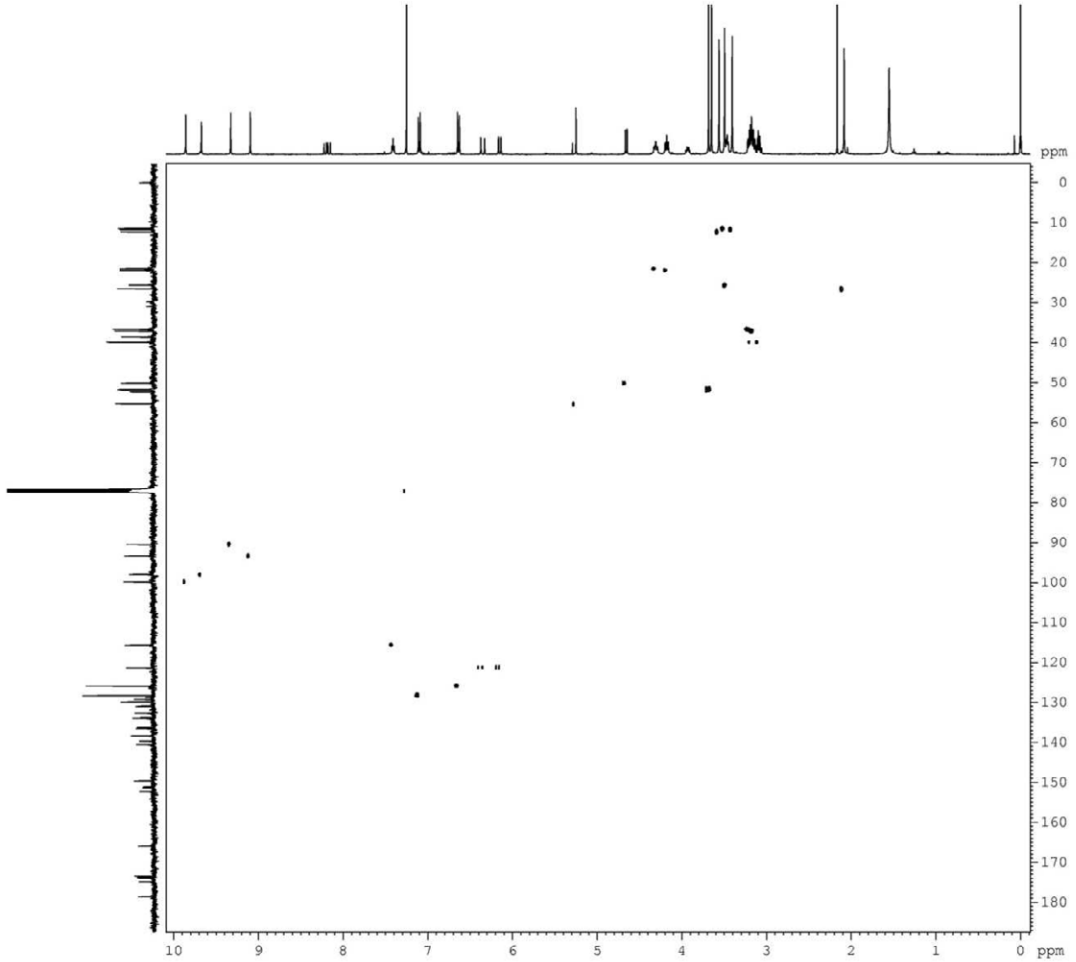
F1 - Acquisition parameters
TD                    256
SFO1           400.1534 MHz
FIDRES         34.385994 Hz
SW               21.999 ppm
FnMODE                QF

F2 - Processing parameters
SI                   1024
SF           400.1500095 MHz
WDW                 QSINE
SSB       0
LB        0 Hz
GB        0
PC                  1.40

F1 - Processing parameters
SI                   1024
MC2                    QF
SF           400.1500078 MHz
WDW
SSB       0
LB        0 Hz
GB        0
```

Figure S41. gCOSY (400 MHz) in $CDCl_3$ of compound **8a**.

NMR Spectrum




```
Current Data Parameters
NAME      Lu1B346_ImidaProtegidaM2
EXPNO                  200
PROCNO                   1

F2 - Acquisition Parameters
Date_               20111117
Time                   22.12
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG          hsqcetgp
TD                     4096
SOLVENT              CDCl3
NS                       24
DS                       16
SWH                4795.396 Hz
FIDRES             1.170751 Hz
AQ                 0.4271263 sec
RG                      2050
DW                  104.267 usec
DE                   10.00 usec
TE                   299.8 K
CNST2            145.00000000
D0                0.00000300 sec
D1                1.00000000 sec
D4                0.00172414 sec
D11               0.03000000 sec
D13               0.00000400 sec
D16               0.00020000 sec
IN0               0.00002310 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                     1H
P1                    8.76 usec
P2                   17.52 usec
P28                2000.00 usec
PLW1          18.00000000 W
SFO1          400.1522030 MHz

======== CHANNEL f2 ========
CPDPRG2                garp
NUC2                    13C
P3                    8.75 usec
P4                   17.50 usec
PCPD2                70.00 usec
PLW2          60.06999969 W
PLW12          0.93858999 W
SFO2          100.6278598 MHz

====== GRADIENT CHANNEL =====
GPNAM1          SMSQ10.100
GPNAM2          SMSQ10.100
GPZ1                 80.00 %
GPZ2                 20.10 %
P16                1000.00 usec

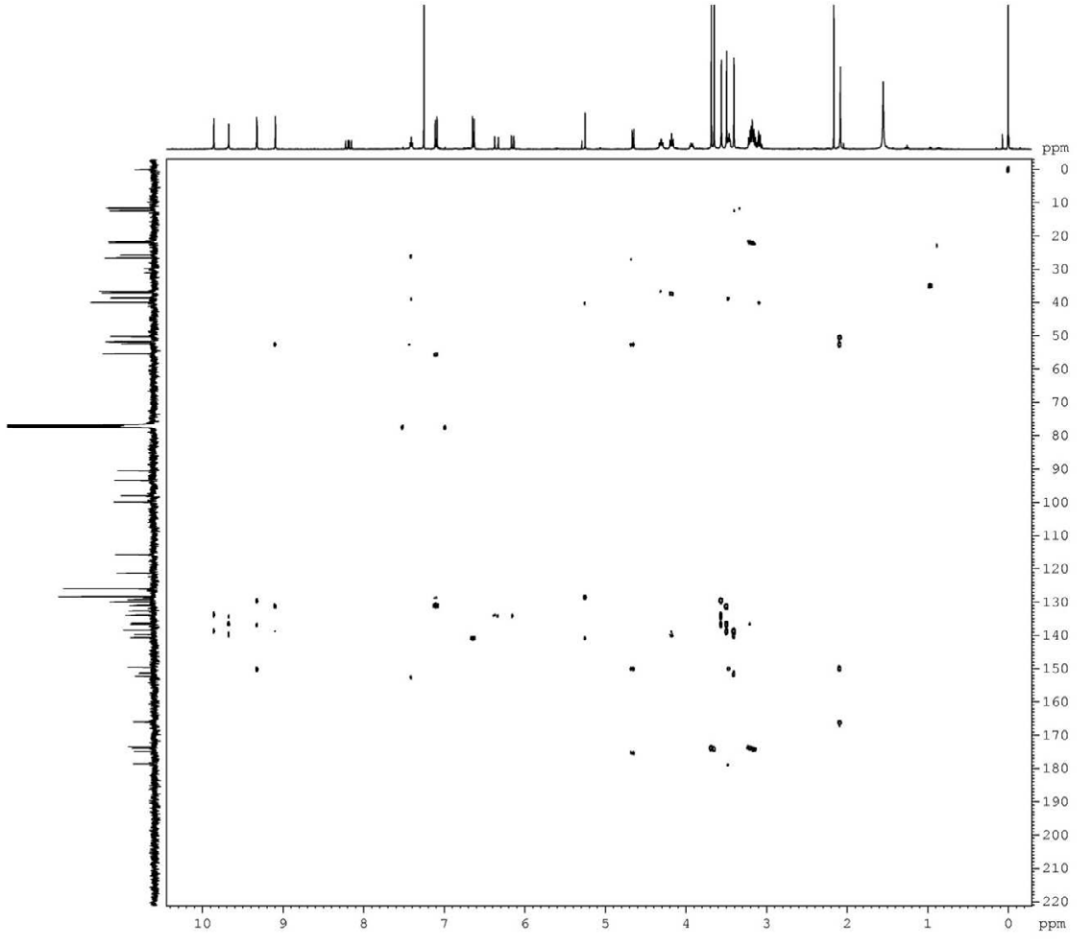
F1 - Acquisition parameters
TD                       256
SFO1          100.6279 MHz
FIDRES         84.509529 Hz
SW                 214.995 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                      1024
SF            400.1500000 MHz
WDW                   QSINE
SSB                      2
LB         0 Hz
GB         0
PC                     1.40

F1 - Processing parameters
SI                      1024
MC2         echo-antiecho
SF            100.6177980 MHz
WDW                      A
SSB                      2
LB         0 Hz
GB         0
```

Figure S42.gHSQC (400 MHz) in $CDCl_3$ of compound **8a**.

NMR Spectrum




```
Current Data Parameters
NAME      LuIB346_1midaProtegidaM2
EXPNO              300
PROCNO               1

F2 - Acquisition Parameters
Date_          20111118
Time               0.47
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG    hmbcgplpndqf
TD                 4096
SOLVENT           CDCl3
NS                   32
DS                   16
SWH             6818.182 Hz
FIDRES          1.664595 Hz
AQ              0.3004233 sec
RG                 2050
DW                73.333 usec
DE                10.00 usec
TE                299.7 K
CNST2         145.0000000
CNST13         10.0000000
D0             0.00000300 sec
D1             1.20000005 sec
D2             0.00344828 sec
D6             0.05000000 sec
D16            0.00020000 sec
IN0            0.00002030 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                 8.76 usec
P2                17.52 usec
PLW1         18.00000000 W
SFO1         400.1530011 MHz

======== CHANNEL f2 ========
NUC2                13C
P3                 8.75 usec
PLW2         60.06999969 W
SFO2         100.6289660 MHz

------ GRADIENT CHANNEL -----
GPNAM1        SMSQ10.100
GPNAM2        SMSQ10.100
GPNAM3        SMSQ10.100
GPZ1               50.00 %
GPZ2               30.00 %
GPZ3               40.10 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                  256
SFO1          100.6289 MHz
FIDRES         96.303993 Hz
SW             244.998 ppm
FnMODE               QF

F2 - Processing parameters
SI                 2048
SF          400.1500098 MHz
WDW                SINE
SSB       0
LB        0 Hz
GB        0
PC                 1.40

F1 - Processing parameters
SI                 1024
MC2                  QF
SF          100.6177684 MHz
WDW                 `v   `v
SSB       0
LB        0 Hz
GB        0
```

Figure S43. gHMBC (400 MHz) in $CDCl_3$ of compound **8a**.

NMR Spectrum

Frequency (MHz)	400.15	***Nucleus***	1H	***Number of Transients***	128
File Name	C.Protegida_M1	***Original Points Count***	32768	***Date***	14 Oct 2011 12:51:28
Points Count	65536	***Pulse Sequence***	zg30	***Receiver Gain***	181.00
SW(cyclical) (Hz)	10000.00	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	2985.9163
Spectrum Type	STANDARD	***Sweep Width (Hz)***	9999.85	***Temperature (degree C)***	23.200

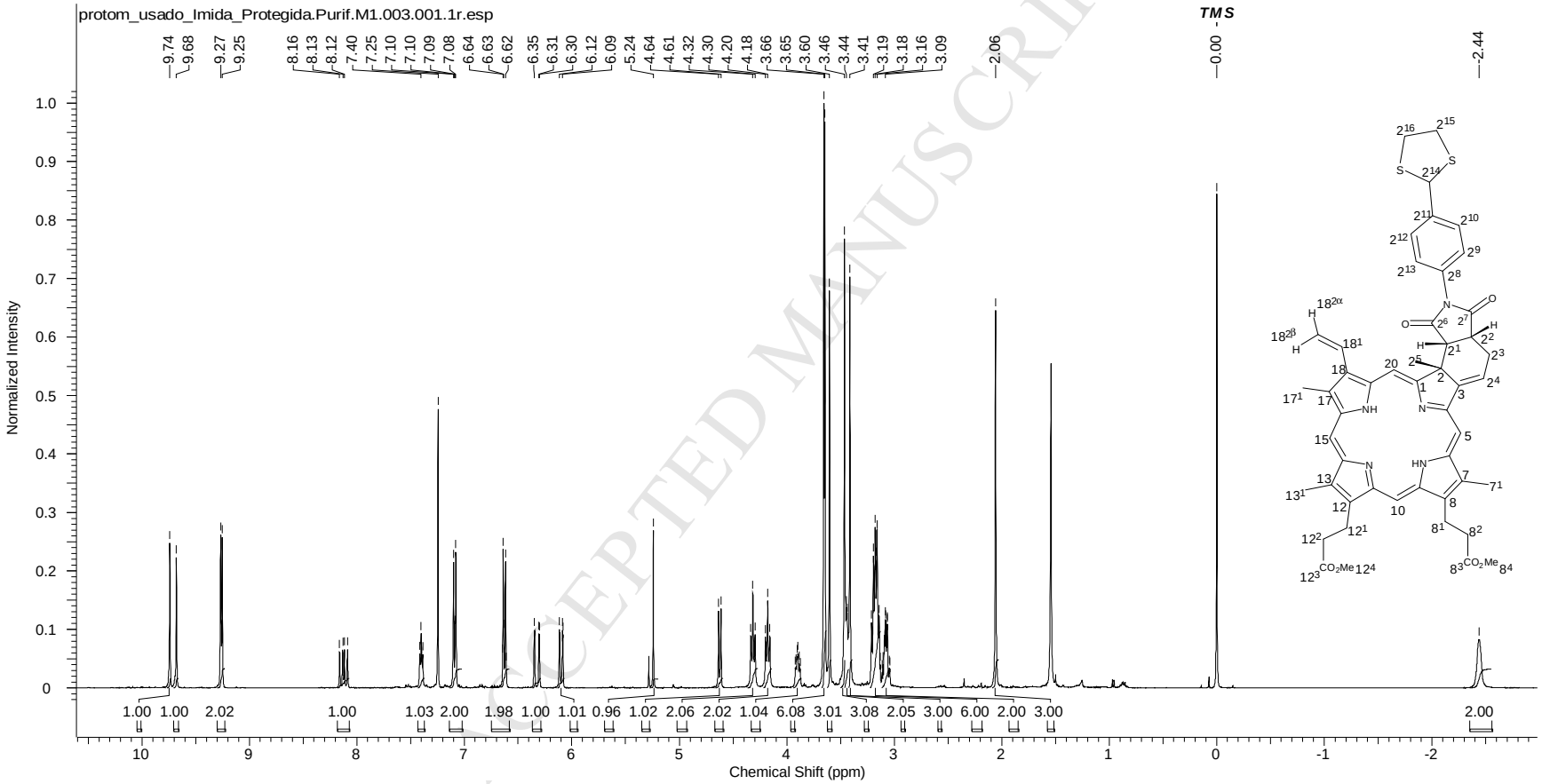


Figure S44. ^{1}H NMR (400 MHz) in $CDCl_3$ of compound **8b**.

NMR Spectrum

Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	4096
File Name	C.Protegida_M1	***Original Points Count***	16384	***Date***	17 Oct 2011 14:53:20
Points Count	32768	***Pulse Sequence***	zgpg30	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	24671.05	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	10057.8467
Spectrum Type	STANDARD	***Sweep Width (Hz)***	24670.30	***Temperature (degree C)***	24.200

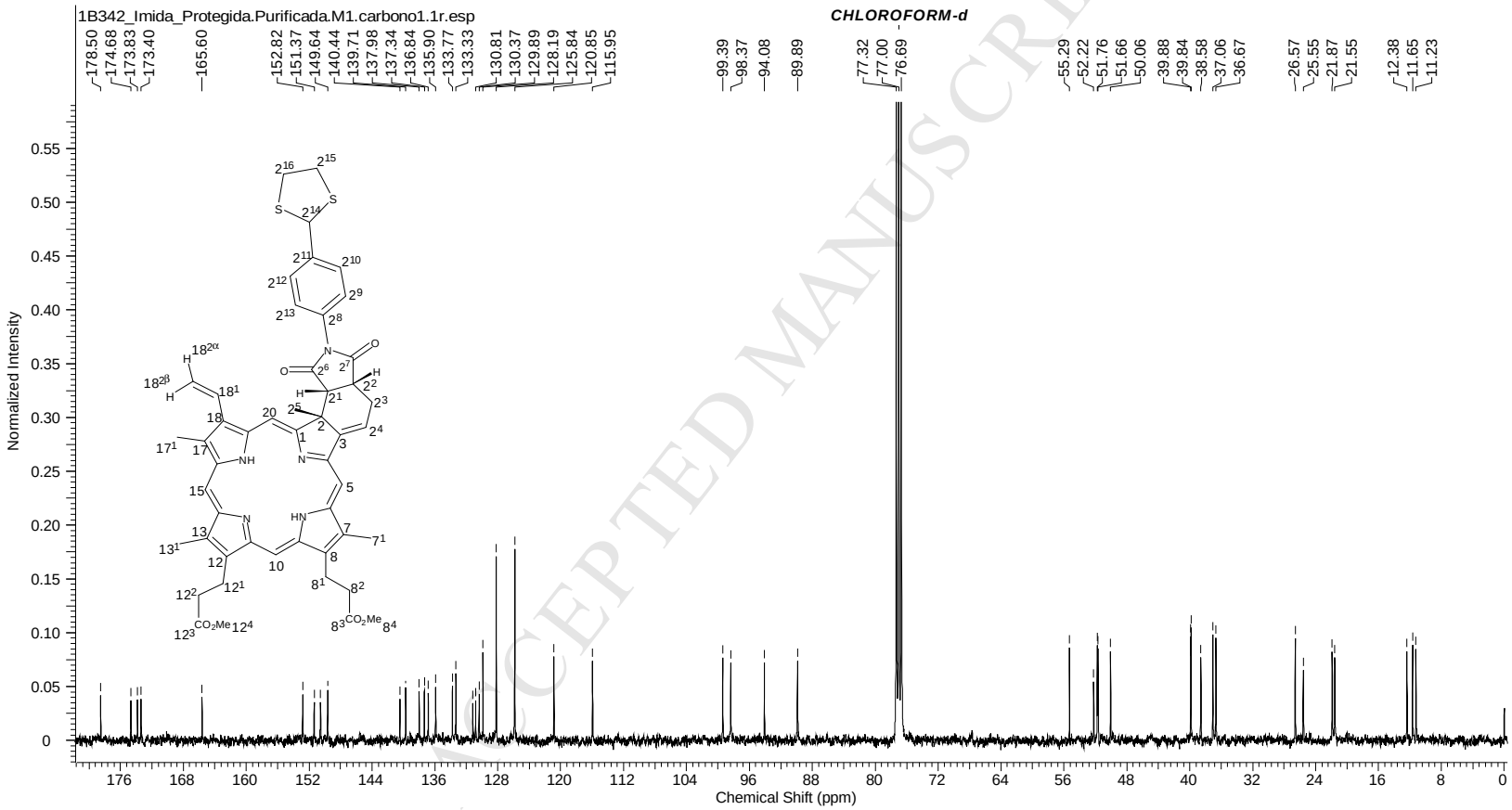


Figure S45. ^{13}C NMR (100 MHz) in $CDCl_3$ of compound **8b**.

NMR Spectrum

Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	2048
File Name	C.Protegida_M1	***Original Points Count***	16384	***Date***	29 Apr 2011 05:21:20
Points Count	32768	***Pulse Sequence***	deptsp135	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	20161.29	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	8048.4780
Spectrum Type	DEPT135	***Sweep Width (Hz)***	20160.68	***Temperature (degree C)***	26.800

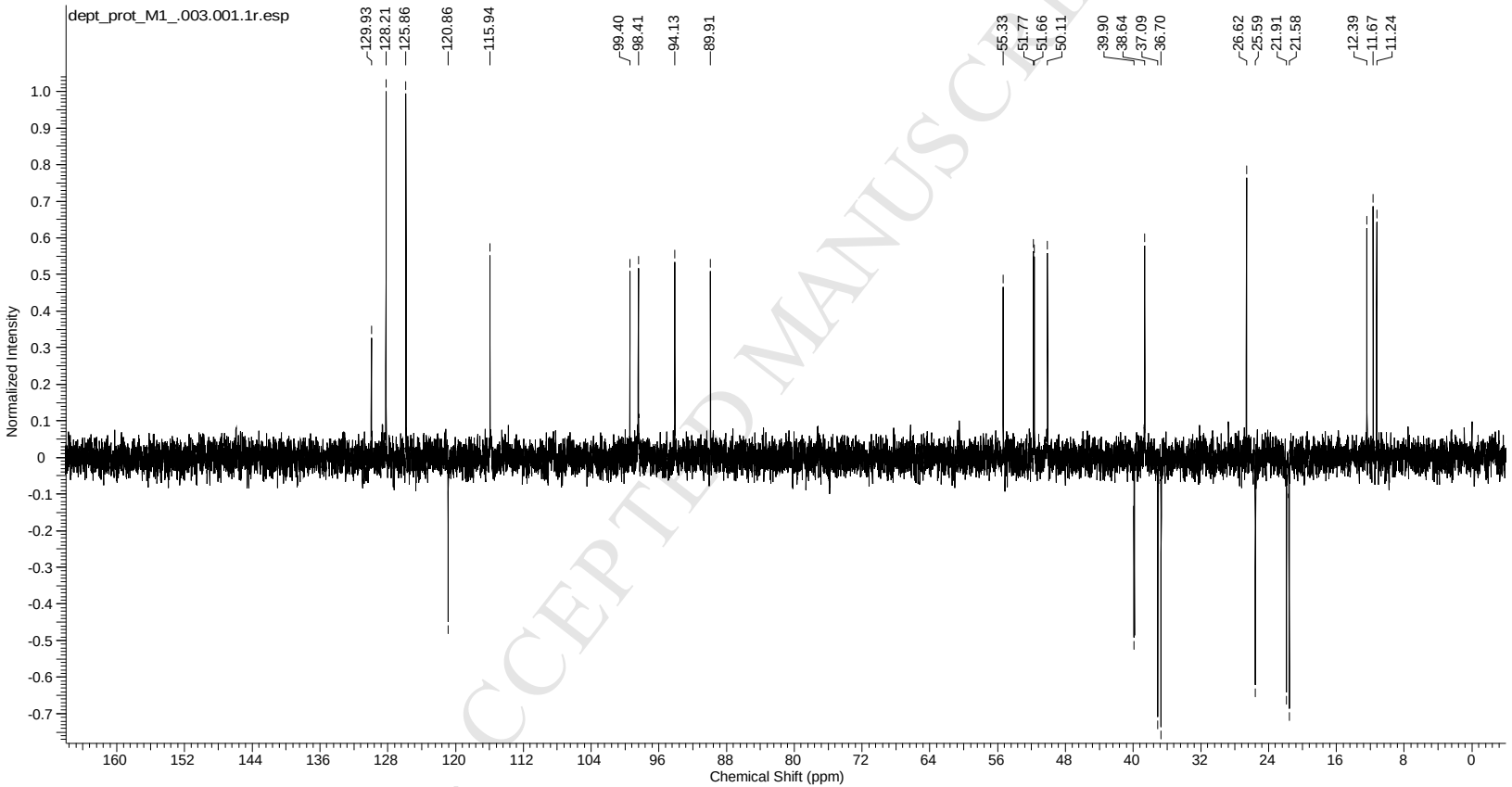
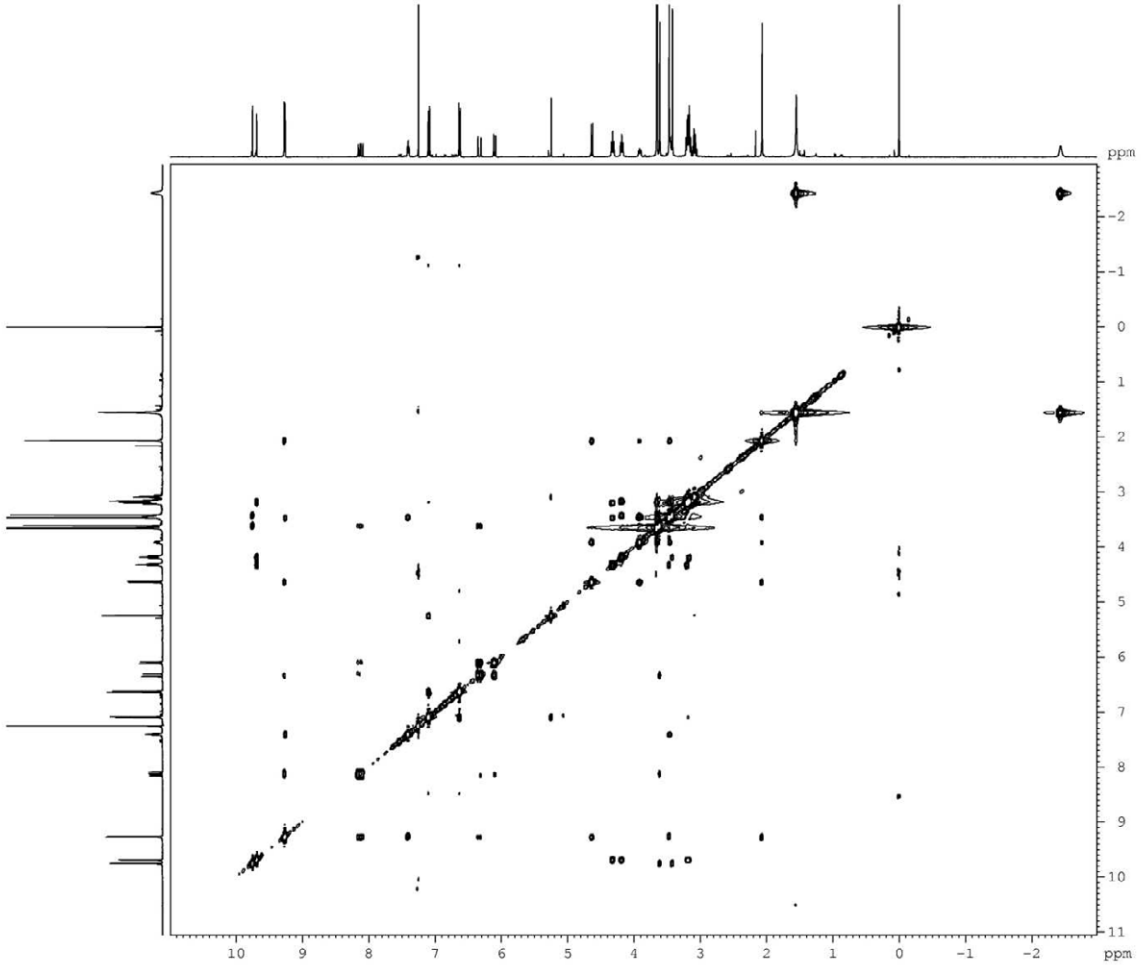


Figure S46.^{13}C (DEPT-135) in $CDCl_3$ of compound **8b**.

NMR Spectrum




```
Current Data Parameters
NAME      1B326_IProt-M1
EXPNO                400
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120626
Time              11.03
INSTRUM           spect
PROBHD    5 mm PABBI 1H/
PULPROG       noesyphpp
TD                4096
SOLVENT           CDCl3
NS                  40
DS                  16
SWH           6830.601 Hz
FIDRES        1.667627 Hz
AQ            0.2998772 sec
RG                181
DW             73.200 use
DE              6.50 use
TE             298.2 K
D0          0.00006284 sec
D1          1.00000000 sec
D8          0.80000001 sec
D11         0.03000000 sec
D12         0.00002000 sec
IN0         0.00014640 sec

======== CHANNEL f1 ========
NUC1               1H
P1               8.14 use
P17           2500.00 use
PLW1      10.00000000 W
PLW10      0.98017001 W
SFO1      400.1317985 MHz

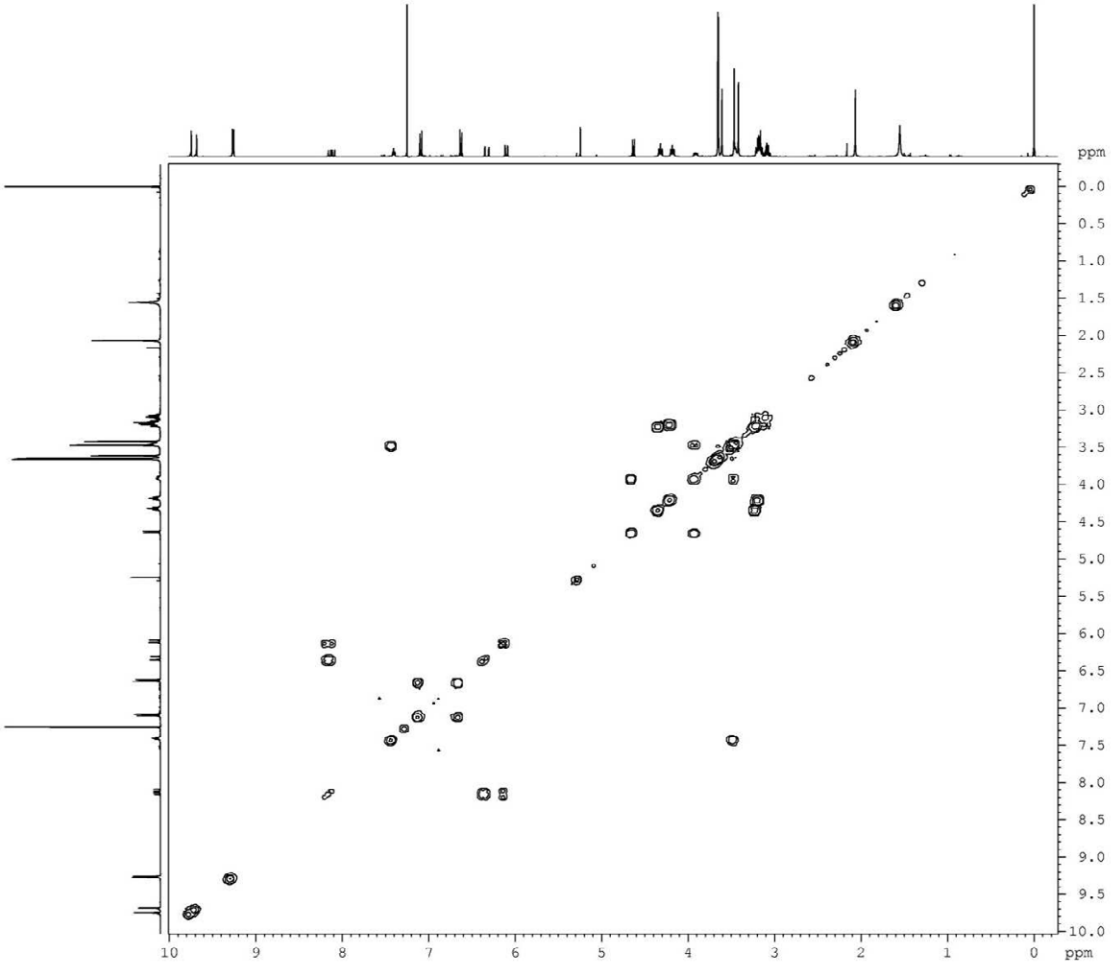
F1 - Acquisition parameters
TD                256
SFO1        400.1318 MHz
FIDRES       26.682035 Hz
SW             17.071 ppm
FnMODE      States-TPPI

F2 - Processing parameters
SI                1024
SF          400.1300101 MHz
WDW               QSINE
SSB                   2
LB        0 Hz
GB        0
PC                1.00

F1 - Processing parameters
SI                1024
MC2         States-TPPI
SF          400.1300101 MHz
WDW             x u   u
SSB                   2
LB        0 Hz
GB        0
```

Figure S47.gNOESY (400 MHz) in $CDCl_3$ of compound **8b**.

NMR Spectrum




```
Current Data Parameters
NAME      Lu1B342_Imida_protegida_Pur
EXPNO              100
PROCNO               1

F2 - Acquisition Parameters
Date_          20111018
Time              10.32
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG      cosygpppqf
TD                 4096
SOLVENT            CDCl3
NS                    8
DS                   16
SWH            8802.817 Hz
FIDRES         2.149125 Hz
AQ             0.2327028 sec
RG                  406
DW                56.800 usec
DE                10.00 usec
TE                296.2 K
D0             0.00000300 sec
D1             1.00000000 sec
D11            0.03000000 sec
D12            0.00002000 sec
D13            0.00000400 sec
D16            0.00020000 sec
IN0            0.00011360 sec

======== CHANNEL f1 ========
NUC1                 1H
P0                8.76 usec
P1                8.76 usec
P17            2500.00 usec
PLW1       18.00000000 W
PLW10       2.04329991 W
SFO1       400.1534013 MHz

====== GRADIENT CHANNEL =====
GPNAM1       SMSQ10.100
GPZ1              10.00 %
P16             1000.00 usec

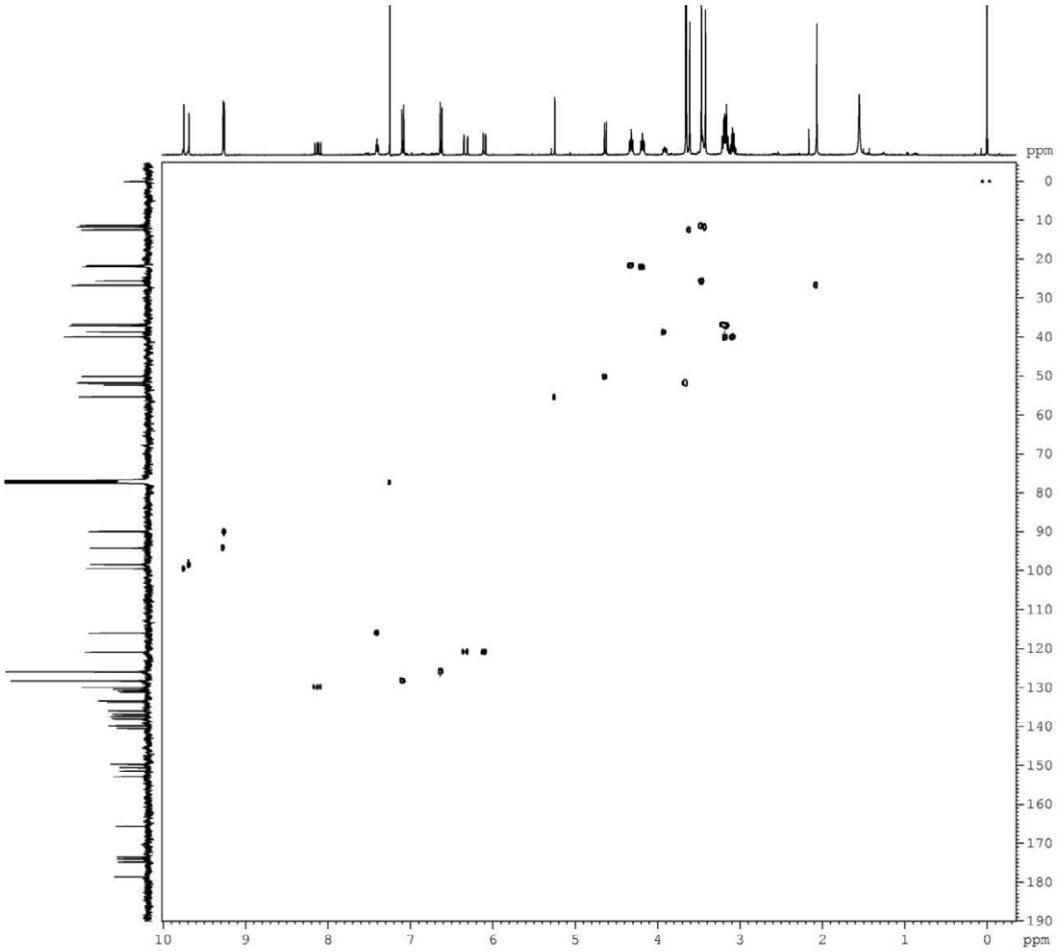
F1 - Acquisition parameters
TD                   256
SFO1           400.1534 MHz
FIDRES         34.385994 Hz
SW                21.999 ppm
FnMODE                QF

F2 - Processing parameters
SI                   1024
SF          400.1500000 MHz
WDW                 QSINE
SSB       0
LB        0 Hz
GB        0
PC                  1.40

F1 - Processing parameters
SI                   1024
MC2                   QF
SF          400.1500000 MHz
WDW
SSB       0
LB        0 Hz
GB        0
```

Figure S48. gCOSY (400 MHz) in $CDCl_3$ of compound **8b**.

NMR Spectrum




```
Current Data Parameters
NAME      18325_1.Protegida_M1
EXPNO                        1
PROCNO                       1

F2 - Acquisition Parameters
Date_              20120623
Time                 16.04
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG          hsqcetgp
TD                   4096
SOLVENT              CDCl3
NS                       8
DS                      16
SWH               4795.396 Hz
FIDRES            1.170751 Hz
AQ                0.4271263 sec
RG                    2050
DW                 104.267 usec
DE                   10.00 usec
TE                  296.9 K
CNST2            145.00000000
D0                0.00000300 sec
D1                1.00000000 sec
D4                0.00172414 sec
D11               0.03000000 sec
D13               0.00000400 sec
D16               0.00020000 sec
IN0               0.00002310 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                      1H
P1                      8.76 usec
P2                     17.52 usec
P28                  2000.00 usec
PLW1            18.00000000 W
SFO1           400.1522030 MHz

======== CHANNEL f2 ========
CPDPRG2                 garp
NUC2                      13C
P3                      8.75 usec
P4                     17.50 usec
PCPD2                  70.00 usec
PLW2            60.06999969 W
PLW12            0.93858999 W
SFO2           100.6278598 MHz

====== GRADIENT CHANNEL =====
GPNAM1          SMSQ10.100
GPNAM2          SMSQ10.100
GPZ1                 80.00 %
GPZ2                 20.10 %
P16                1000.00 usec

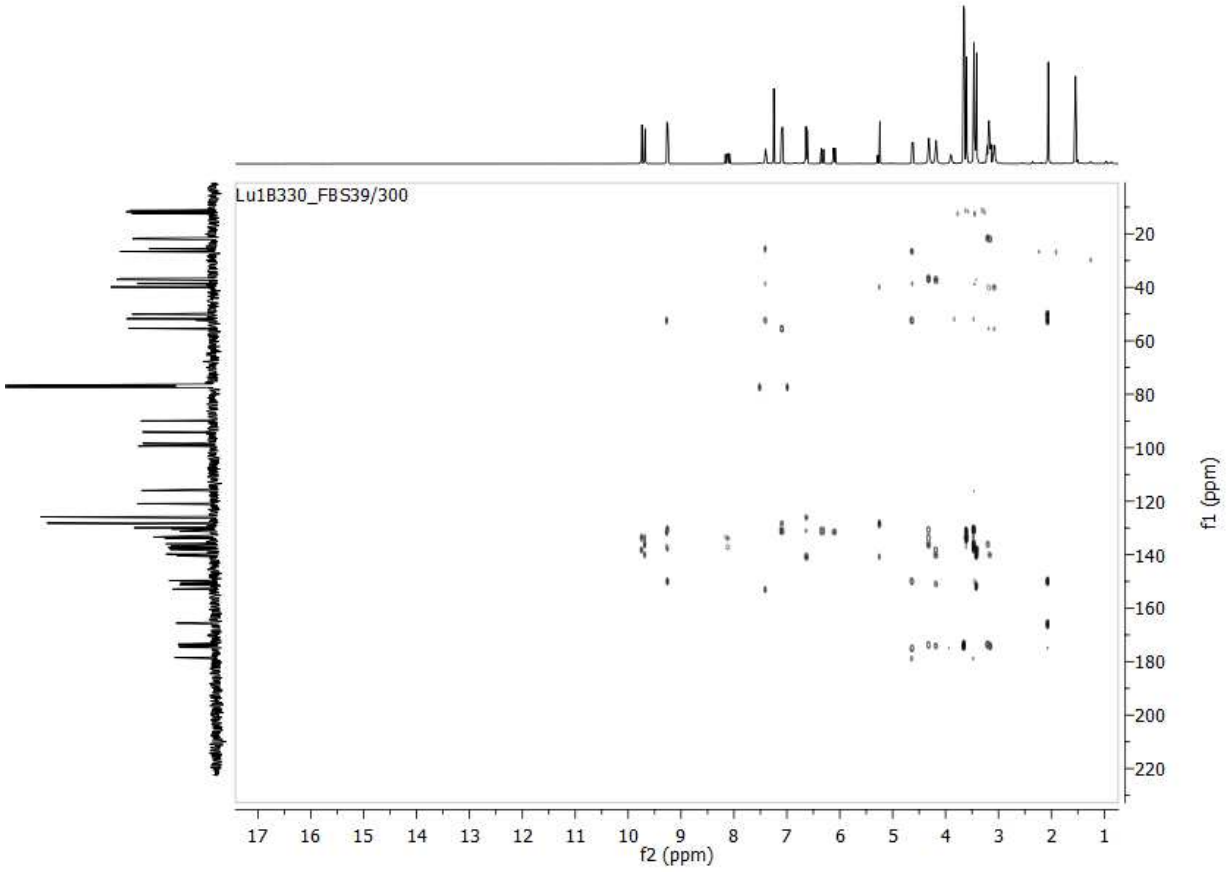
F1 - Acquisition parameters
TD                       256
SFO1              100.6279 MHz
FIDRES            84.509529 Hz
SW                  214.995 ppm
FnMODE      Echo-Antiecho

F2 - Processing parameters
SI                      1024
SF              400.1500090 MHz
WDW                    QSINE
SSB                        2
LB          0 Hz
GB          0
PC                      1.40

F1 - Processing parameters
SI                      1024
MC2          echo-antiecho
SF              100.6177915 MHz
WDW                  x ⊛   ⊛
SSB                        2
LB          0 Hz
GB          0
```

Figure S49.gHSQC (400 MHz) in $CDCl_3$ of compound **8b**.

NMR Spectrum



	Parameter	Value (f2, f1)
1	Data File Name	G:/RMNeditados mestre NOVA/ RMN da imda protegida/ Iprotegida M1 todas análises/ Lu1B330_FBS39/ 300/ ser
2	Title	Lu1B330_FBS39/ 300
3	Comment	
4	Origin	Bruker BioSpin GmbH
5	Owner	nmrsu
6	Site	
7	Spectrometer	spect
8	Author	
9	Solvent	CDCl3
10	Temperature	299.8
11	Pulse Sequence	hmbcgplpndqf
12	Experiment	2D-HMBC
13	Number of Scans	32
14	Receiver Gain	2050
15	Relaxation Delay	1.5000
16	Pulse Width	8.7600
17	Acquisition Time	0.2327
18	Acquisition Date	2011-07-29T03:41:37
19	Modification Date	2011-07-29T11:09:00
20	Spectrometer Frequency	(400.15, 100.63)
21	Spectral Width	(8802.8, 24653.8)
22	Lowest Frequency	(-1008.5, -1224.6)
23	Nucleus	(1H, 13C)
24	Acquired Size	(2048, 256)
25	Spectral Size	(2048, 1024)

Figure S50. gHMBC (400 MHz) in $CDCl_3$ of compound **8b**.

NMR Spectrum

Frequency (MHz)	400.13	***Nucleus***	1H	***Number of Transients***	8	***File Name***	C.Aldeido_M2
Original Points Count	32768	***Owner***	root	***Points Count***	32768	***Pulse Sequence***	zg
Receiver Gain	71.80	***SW(cyclical) (Hz)***	6578.95	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	1622.5515
Spectrum Type	STANDARD	***Sweep Width (Hz)***	6578.75	***Temperature (degree C)***	24.988		

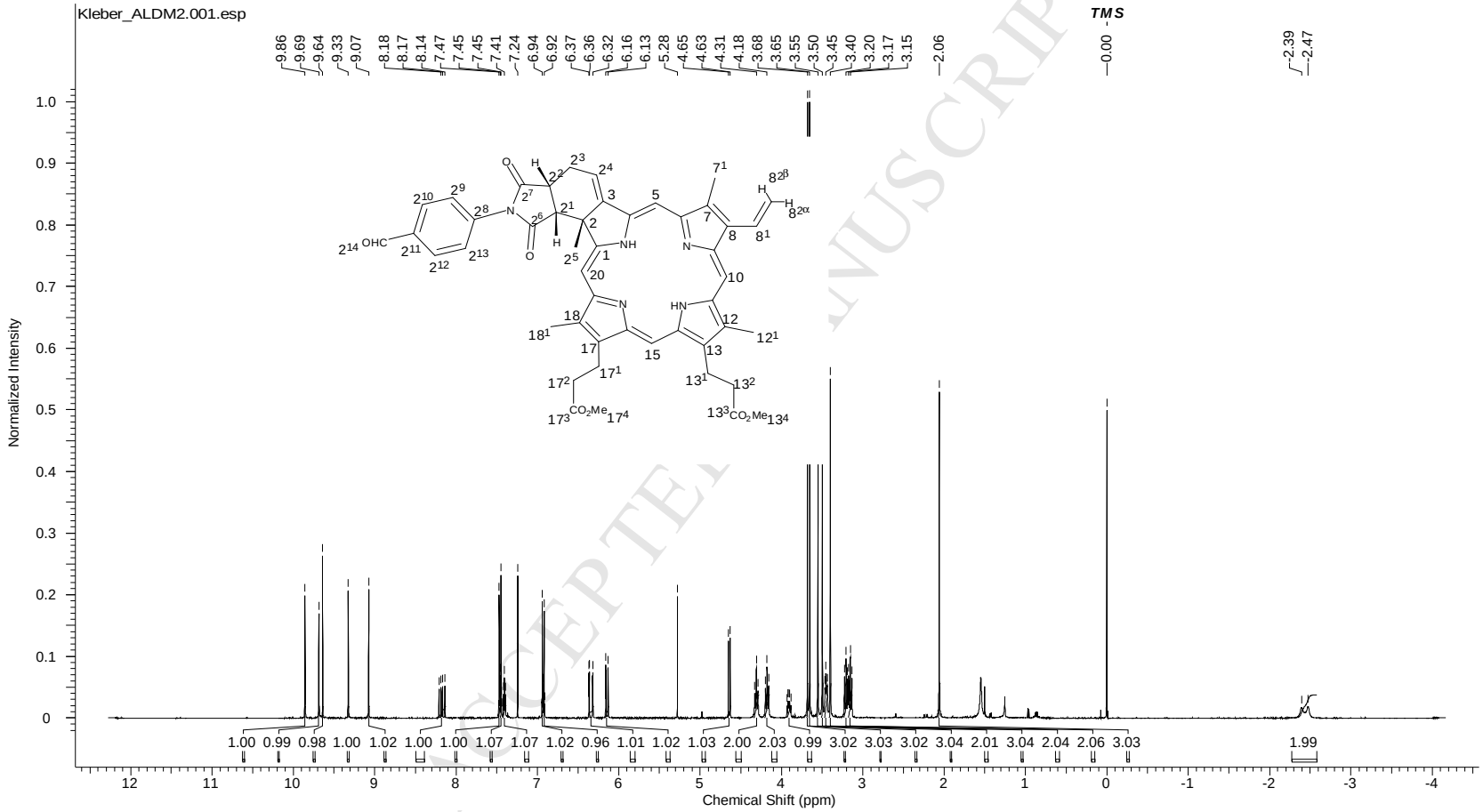


Figure S51. ^{1}H NMR (400 MHz) in $CDCl_3$ of compound **9a**.

NMR Spectrum

Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	7168	***File Name***	C.Aldeido_M2
Original Points Count	16384	***Owner***	root	***Points Count***	16384	***Pulse Sequence***	zgpg30
Receiver Gain	912.00	***SW(cyclical) (Hz)***	24038.46	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	10056.5830
Spectrum Type	STANDARD	***Sweep Width (Hz)***	24036.99	***Temperature (degree C)***	24.998		

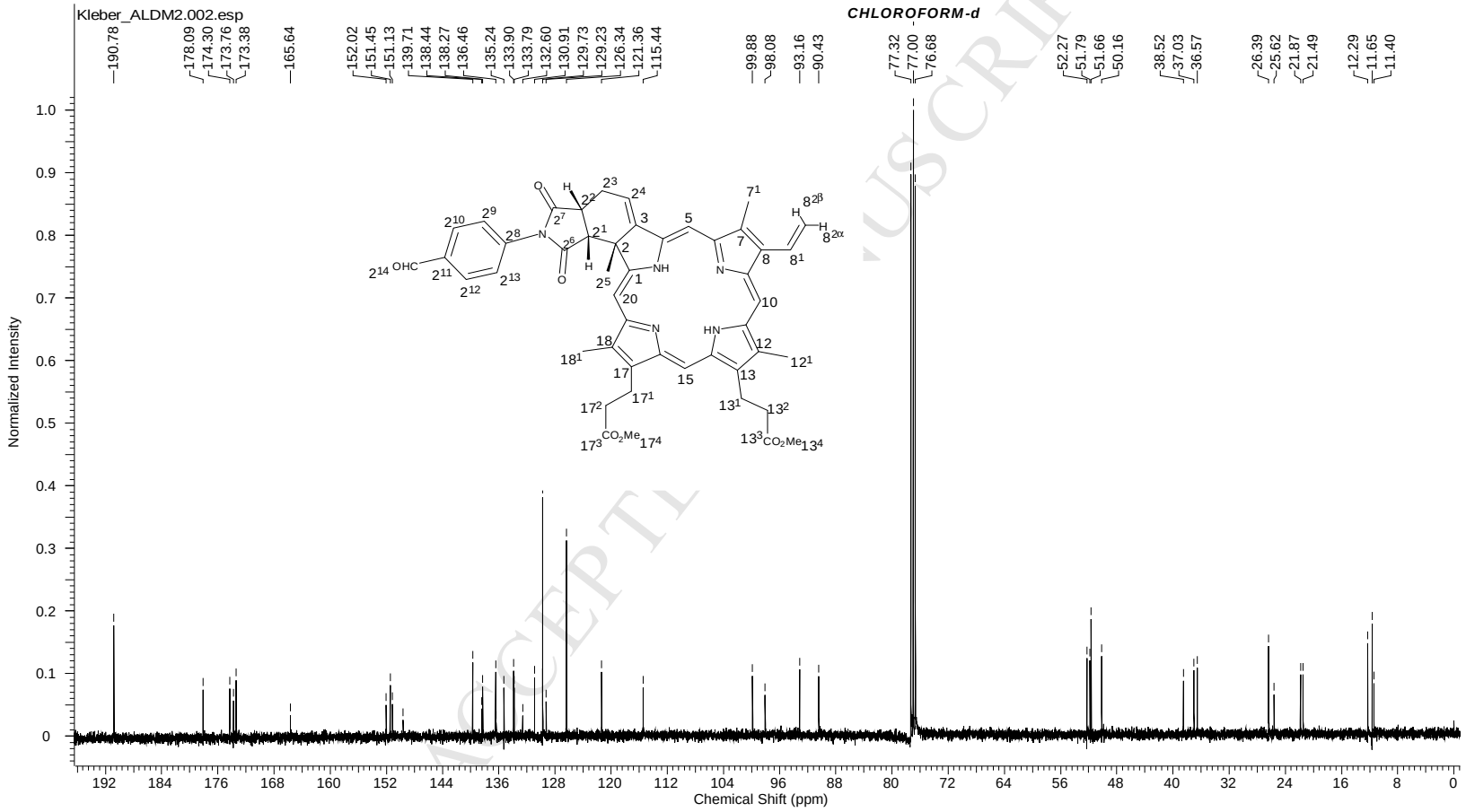


Figure S52. ^{13}C NMR (100 MHz) in $CDCl_3$ of compound **9a**.

NMR Spectrum

Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	2048	***File Name***	C.Aldeido_M2
Original Points Count	32768	***Owner***	root	***Points Count***	32768	***Pulse Sequence***	deptsp135
Receiver Gain	2050.00	***SW(cyclical) (Hz)***	23437.50	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	10403.8057
Spectrum Type	DEPT135	***Sweep Width (Hz)***	23436.79	***Temperature (degree C)***	24.995		

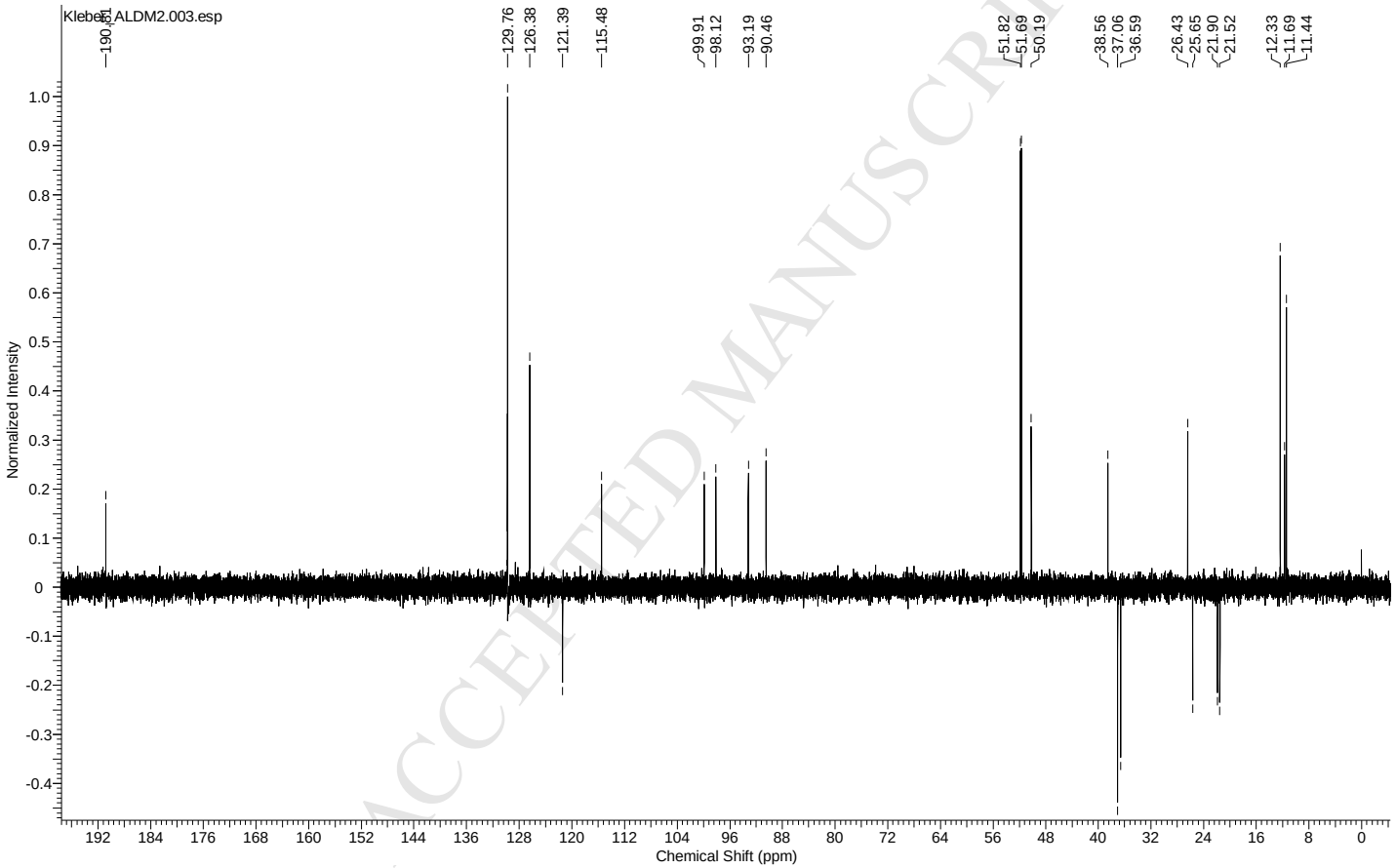
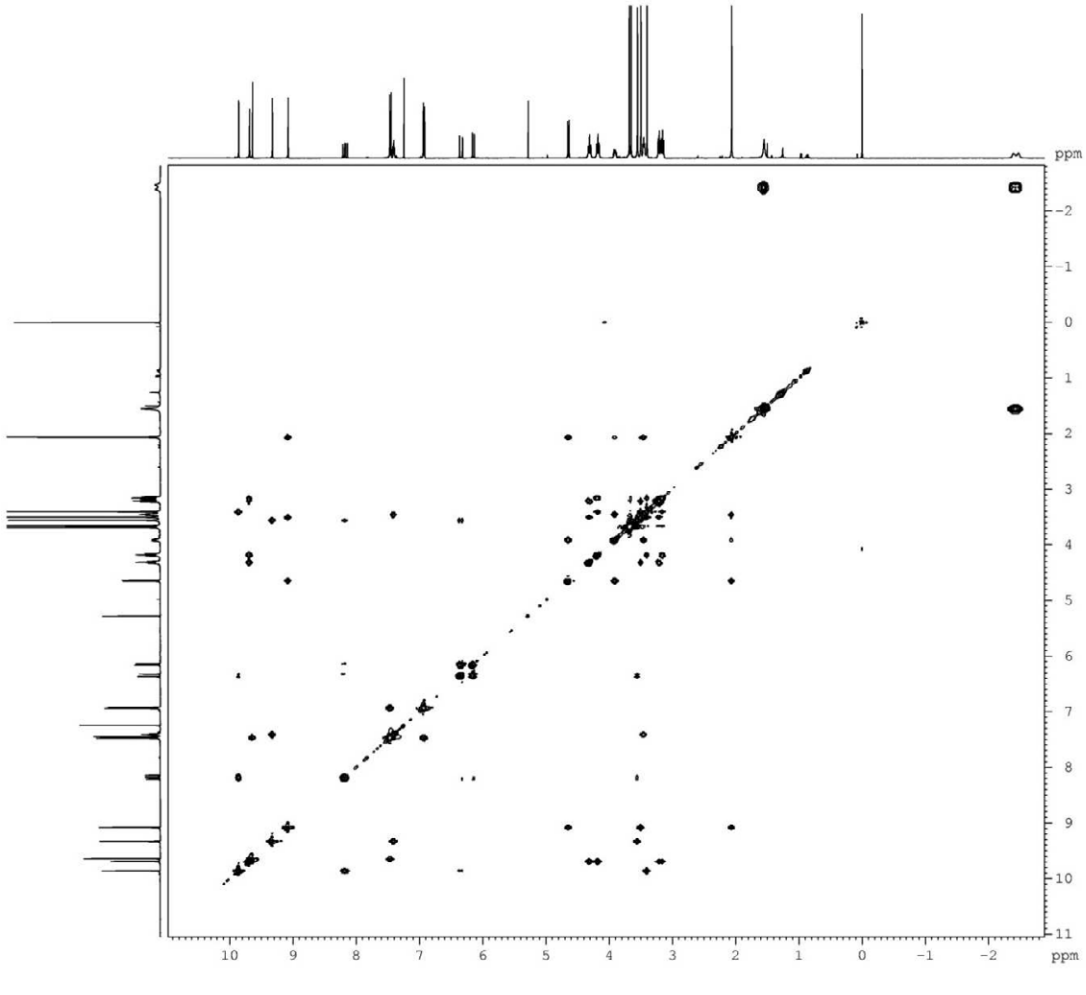


Figure S53. ^{13}C (DEPT-135) in $CDCl_3$ of compound **9a**.

NMR Spectrum




```
Current Data Parameters
NAME        Kleber_ALDM2
EXPNO                400
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120219
Time               13.56
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG        noesyphpp
TD                  4096
SOLVENT             CDCl3
NS                    32
DS                    16
SWH            6578.947 Hz
FIDRES         1.606188 Hz
AQ             0.3113460 sec
RG                    64
DW               76.000 use
DE                6.50 use
TE                298.0 K
D0           0.00006564 sec
D1           1.00000000 sec
D8           0.80000001 sec
D11          0.03000000 sec
D12          0.00002000 sec
IN0          0.00015200 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                  8.14 use
P17              2500.00 use
PLW1        10.00000000 W
PLW10        0.98017001 W
SFO1        400.1316395 MHz

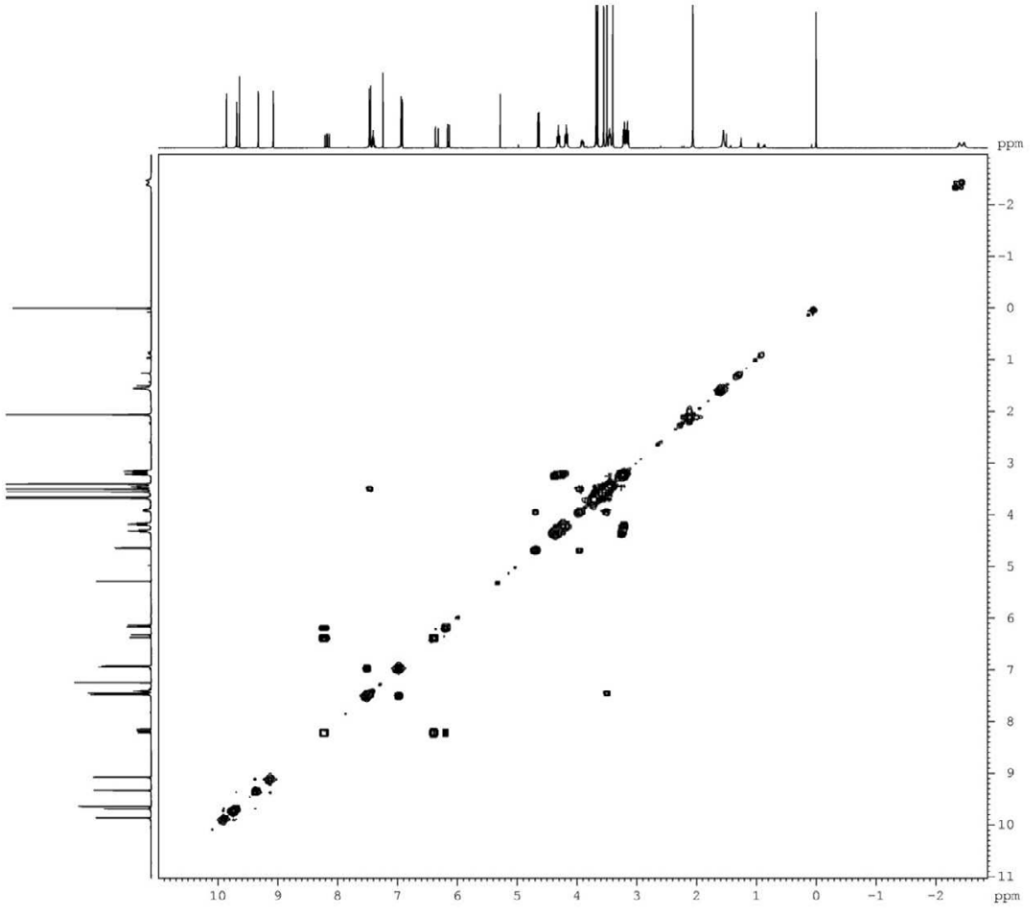
F1 - Acquisition parameters
TD                   256
SFO1          400.1316 MHz
FIDRES       25.699011 Hz
SW              16.442 ppm
FnMODE      States-TPPI

F2 - Processing parameters
SI                  1024
SF          400.1300144 MHz
WDW                 QSINE
SSB                     2
LB        0 Hz
GB        0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2         States-TPPI
SF          400.1300145 MHz
WDW                   A #
SSB                     2
LB        0 Hz
GB        0
```

Figure S54. gNOESY (400 MHz) in $CDCl_3$ of compound **9a**.

NMR Spectrum




```
Current Data Parameters
NAME        Kleber_ALDM2
EXPNO                100
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120219
Time               13.09
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG        cosygpqf
TD                 4096
SOLVENT            CDCl3
NS                    8
DS                   16
SWH             6578.947 Hz
FIDRES          1.606188 Hz
AQ              0.3113460 sec
RG                   64
DW                76.000 usec
DE                 6.50 usec
TE                297.9 K
D0             0.00000300 sec
D1             1.00000000 sec
D13            0.00000400 sec
D16            0.00020000 sec
IN0            0.00015200 sec

======== CHANNEL f1 ========
NUC1                  1H
P0                 8.14 usec
P1                 8.14 usec
PLW1          10.00000000 W
SFO1          400.1316395 MHz

====== GRADIENT CHANNEL =====
GPNAM1         SMSQ10.100
GPZ1               10.00 %
P16              1000.00 usec

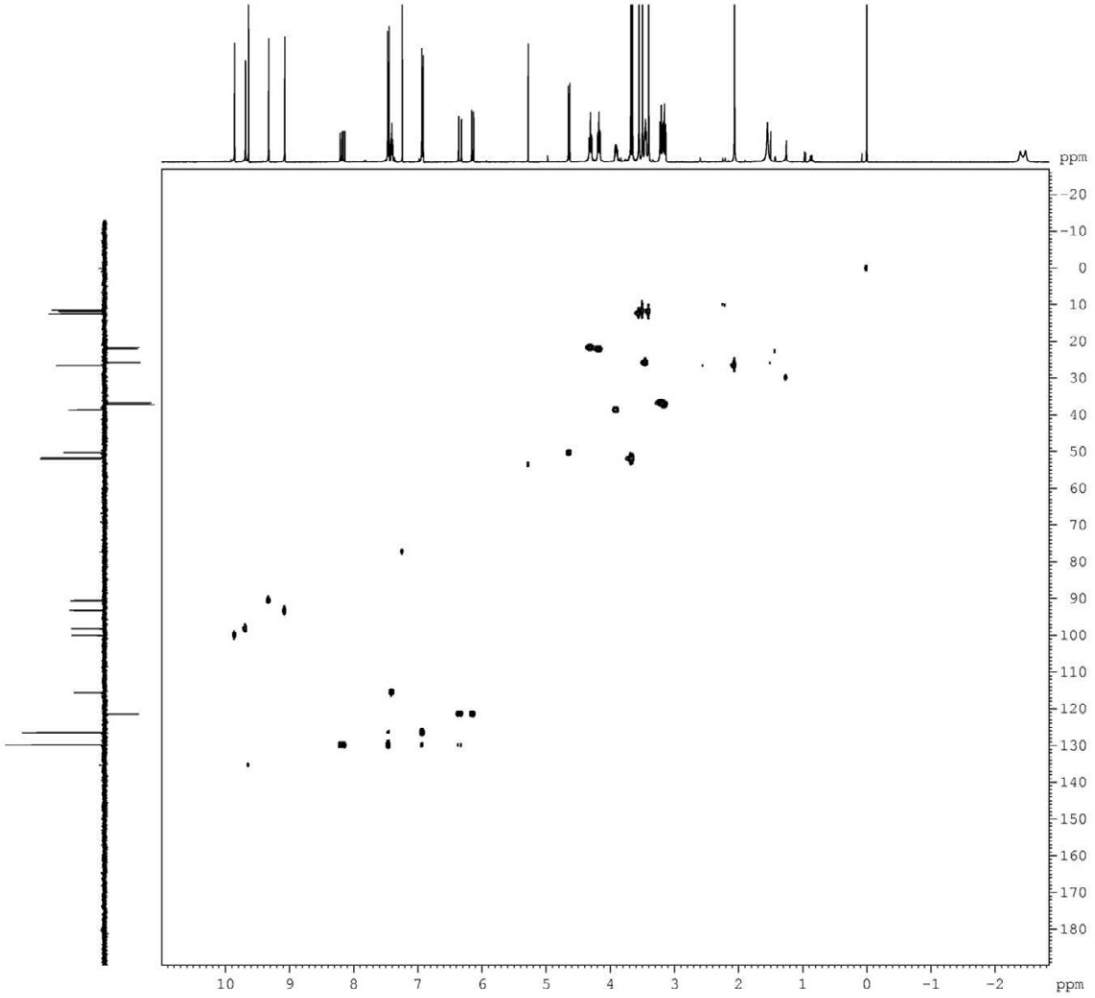
F1 - Acquisition parameters
TD                   256
SFO1          400.1316 MHz
FIDRES         25.699011 Hz
SW                16.442 ppm
FnMODE                QF

F2 - Processing parameters
SI                   1024
SF            400.1299971 MHz
WDW                  SINE
SSB       0
LB        0 Hz
GB        0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2                   QF
SF            400.1299981 MHz
WDW
SSB       0
LB        0 Hz
GB        0
```

Figure S55. gCOSY (400 MHz) in CDCl3 of compound **9a**.

NMR Spectrum




```
Current Data Parameters
NAME         Kleber_ALDM2
EXPNO                 200
PROCNO                  1

F2 - Acquisition Parameters
Date_           20120219
Time              18.51
INSTRUM             spect
PROBHD   5 mm PABBI 1H/
PULPROG          hsqcetgp
TD                  4096
SOLVENT             CDCl3
NS                    16
DS                    16
SWH              6578.947 Hz
FIDRES           1.606188 Hz
AQ               0.3113460 sec
RG                  2050
DW                 76.000 usec
DE                  6.50 usec
TE                298.9 K
CNST2          145.0000000
D0               0.00000300 sec
D1               1.00000000 sec
D4               0.00172414 sec
D11              0.03000000 sec
D16              0.00020000 sec
IN0              0.00002160 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                   1H
P1                   8.14 usec
P2                  16.28 usec
P28               1000.00 usec
PLW1          10.00000000 W
SFO1          400.1316395 MHz

======== CHANNEL f2 ========
CPDPRG2              garp
NUC2                  13C
P3                  15.00 usec
P4                  30.00 usec
PCPD2               80.00 usec
PLW2          80.00000000 W
PLW12          2.81250000 W
SFO2          100.6203150 MHz

====== GRADIENT CHANNEL =====
GPNAM1         SMSQ10.100
GPNAM2         SMSQ10.100
GPZ1                80.00 %
GPZ2                20.10 %
P16               1000.00 usec

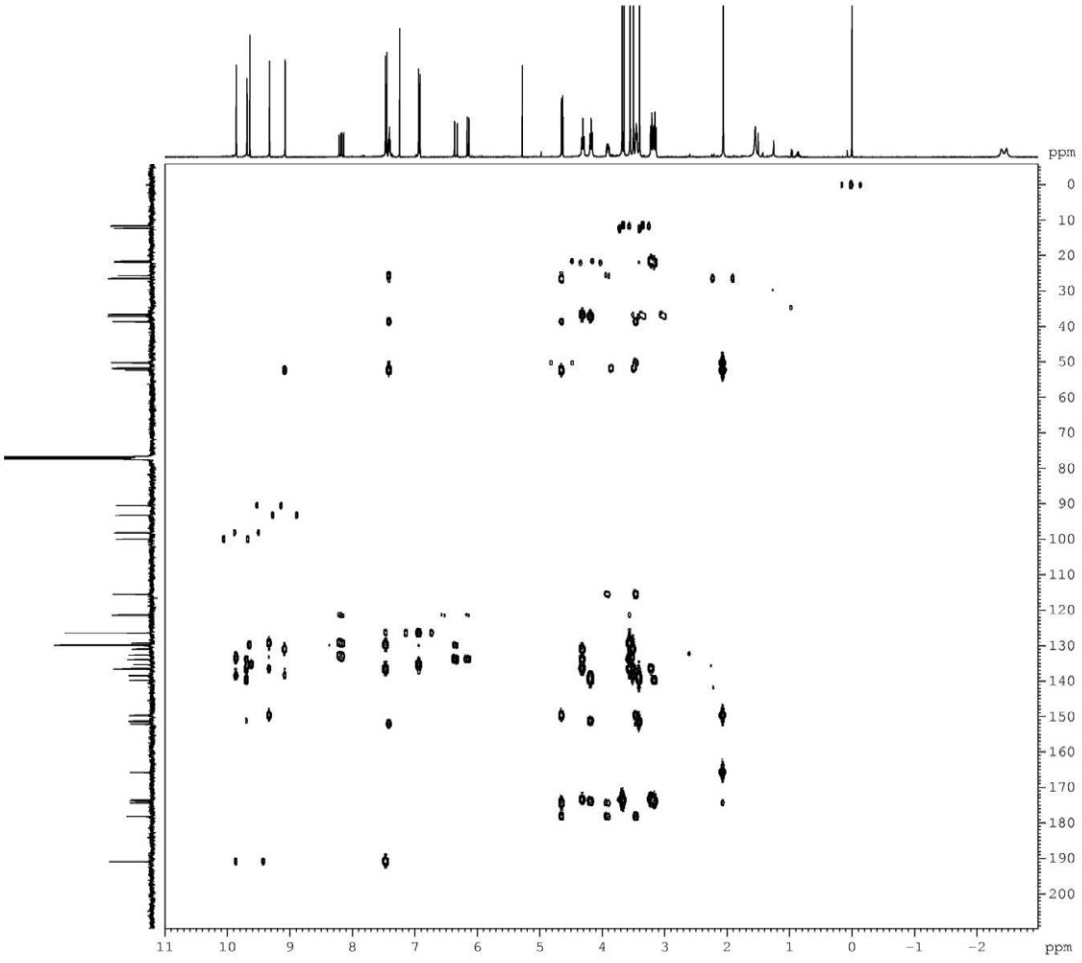
F1 - Acquisition parameters
TD                   256
SFO1            100.6203 MHz
FIDRES          90.401062 Hz
SW                230.000 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                  4096
SF            400.1300164 MHz
WDW                 QSINE
SSB                    2
LB         0 Hz
GB         0
PC                   1.40

F1 - Processing parameters
SI                  1024
MC2          echo-antiecho
SF            100.6127629 MHz
WDW                 x
SSB                    2
LB         0 Hz
GB         0
```

Figure S56. gHSQC (400 MHz) in $CDCl_3$ of compound **9a**.

NMR Spectrum




```
Current Data Parameters
NAME        Kleber_ALDM2
EXPNO                300
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120219
Time               20.25
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG    hmbcgplpndqf
TD                 4096
SOLVENT            CDCl3
NS                  128
DS                   16
SWH             6578.947 Hz
FIDRES          1.606188 Hz
AQ              0.3113460 sec
RG                 2050
DW                76.000 usec
DE                 6.50 usec
TE                297.9 K
CNST2         145.0000000
CNST13          8.0000000
D0            0.00000300 sec
D1            1.00000000 sec
D2            0.00344828 sec
D6            0.06250000 sec
D16           0.00020000 sec
IN0           0.00002080 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                 8.14 usec
P2                16.28 usec
PLW1         10.00000000 W
SFO1        400.1316395 MHz

======== CHANNEL f2 ========
NUC2                13C
P3                15.00 usec
PLW2         80.00000000 W
SFO2        100.6228293 MHz

====== GRADIENT CHANNEL =====
GPNAM1         SMSQ10.100
GPNAM2         SMSQ10.100
GPNAM3         SMSQ10.100
GPZ1                50.00 %
GPZ2                30.00 %
GPZ3                40.10 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                   256
SFO1            100.6228 MHz
FIDRES          93.900238 Hz
SW                238.897 ppm
FnMODE                QF

F2 - Processing parameters
SI                  1024
SF          400.1300139 MHz
WDW                 SINE
SSB       0
LB        0 Hz
GB        0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2                   QF
SF          100.6127592 MHz
WDW                   ~6
SSB       0
LB        0 Hz
GB        0
```

Figure S57. gHMBC (400 MHz) in $CDCl_3$ of compound **9a**.

NMR Spectrum

File Name	ALDM1					***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	8	***Origin***	spect	***Original Points Count***	32768
Owner	root	***Points Count***	32768	***Pulse Sequence***	zg	***Receiver Gain***	71.80
SW(cyclical) (Hz)	6578.95	***Solvent***	CHLOROFORM-d			***Spectrum Offset (Hz)***	1622.5515
Spectrum Type	STANDARD	***Sweep Width (Hz)***	6578.75	***Temperature (degree C)***	24.992		

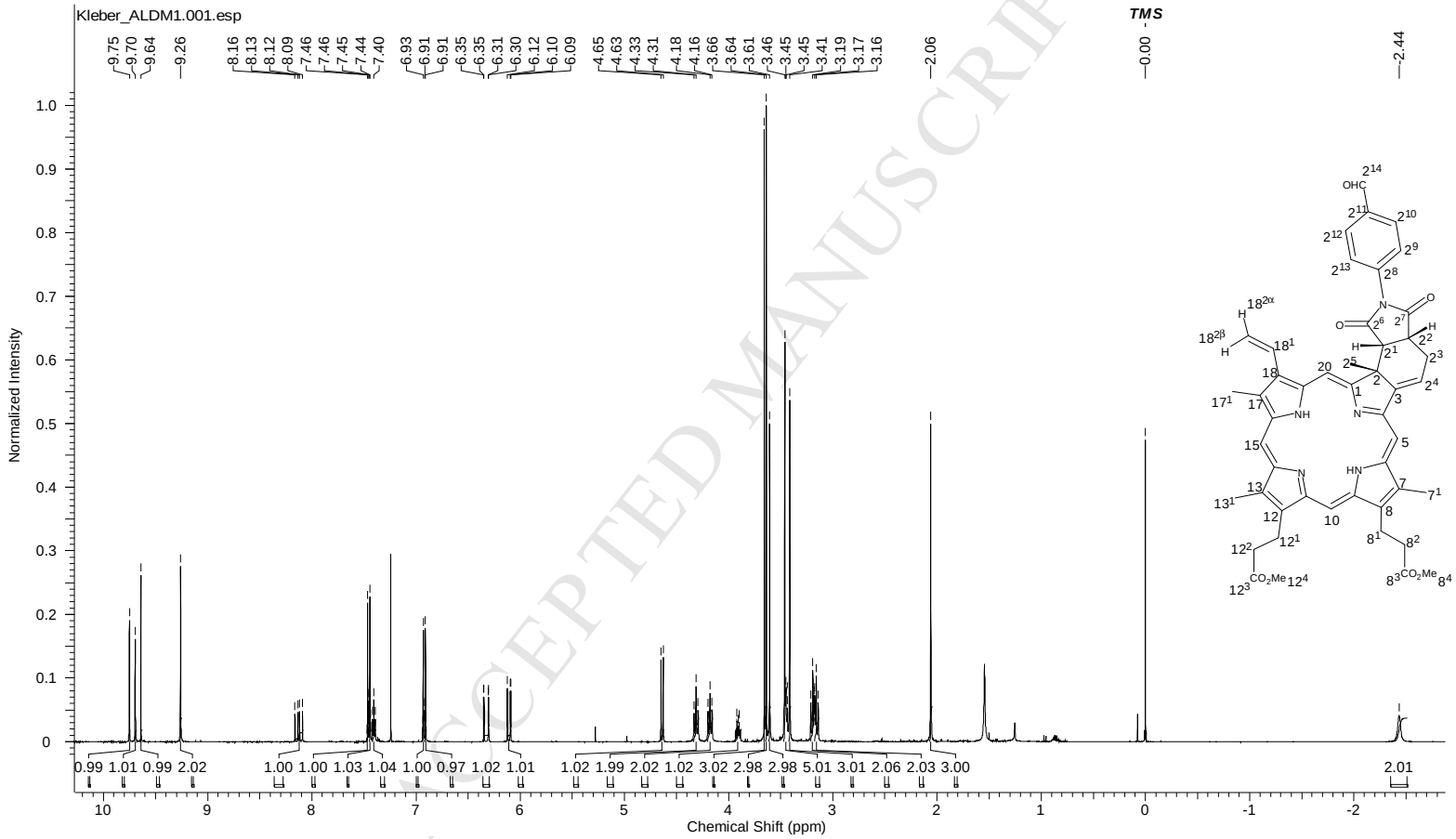


Figure S58. ^{1}H NMR (400 MHz) in $CDCl_3$ of compound **9b**.

NMR Spectrum

File Name	_ALDM1					***Frequency (MHz)***	100.62
Nucleus	13C	***Number of Transients***	8192	***Origin***	spect	***Original Points Count***	16384
Owner	root	***Points Count***	16384	***Pulse Sequence***	zgpg30	***Receiver Gain***	912.00
SW(cyclical) (Hz)	24038.46	***Solvent***	CHLOROFORM-d			***Spectrum Offset (Hz)***	10056.5830
Spectrum Type	STANDARD	***Sweep Width (Hz)***	24036.99	***Temperature (degree C)***	25.017		

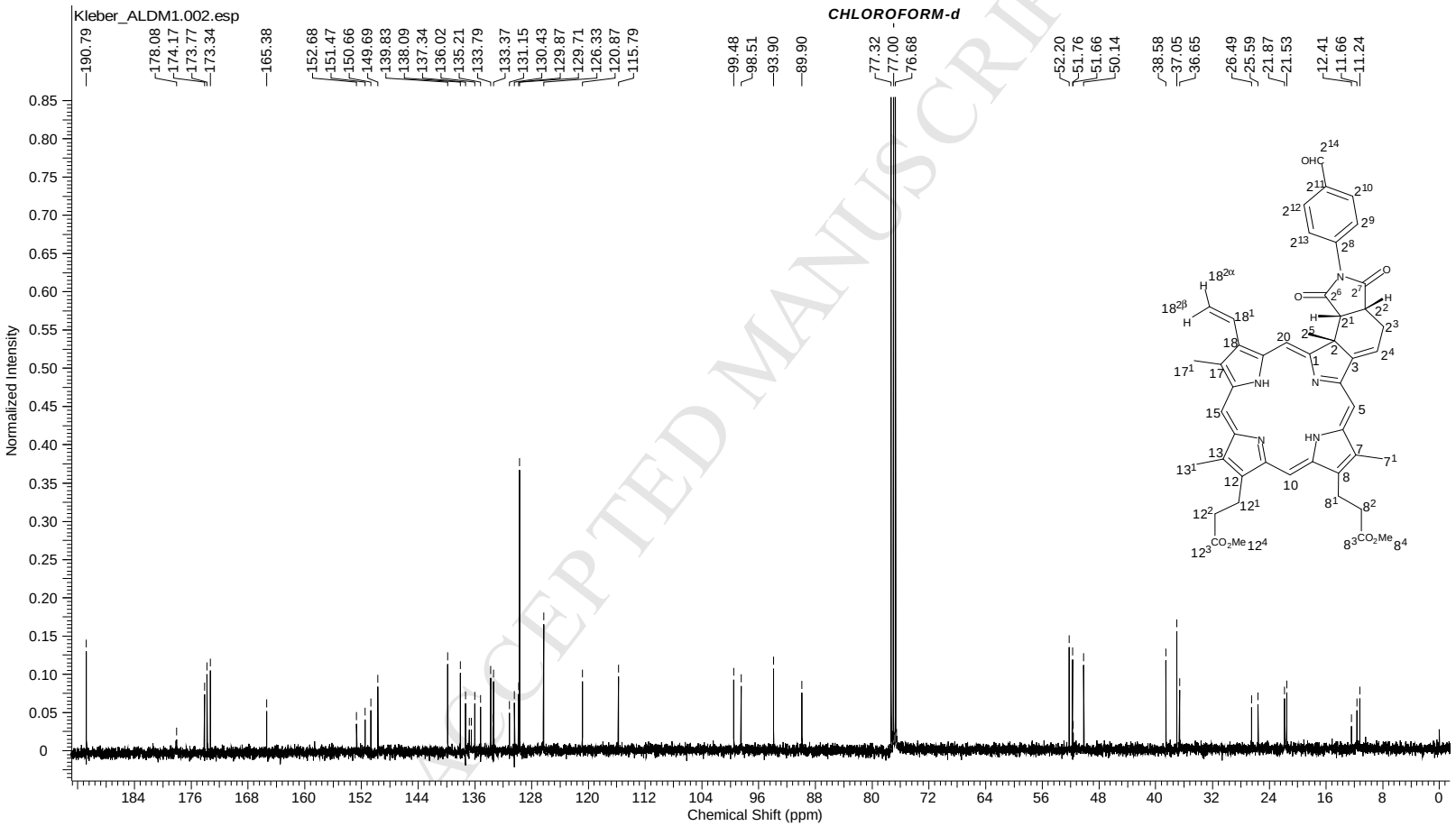


Figure S59. ^{13}C NMR (100 MHz) in $CDCl_3$ of compound **9b**.

NMR Spectrum

File Name	_ALDM1					***Frequency (MHz)***	100.62
Nucleus	13C	***Number of Transients***	2048	***Origin***	spect	***Original Points Count***	32768
Owner	root	***Points Count***	32768	***Pulse Sequence***	deptsp135	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	23437.50	***Solvent***	CHLOROFORM-d			***Spectrum Offset (Hz)***	10403.8057
Spectrum Type	DEPT135	***Sweep Width (Hz)***	23436.79	***Temperature (degree C)***	24.994		

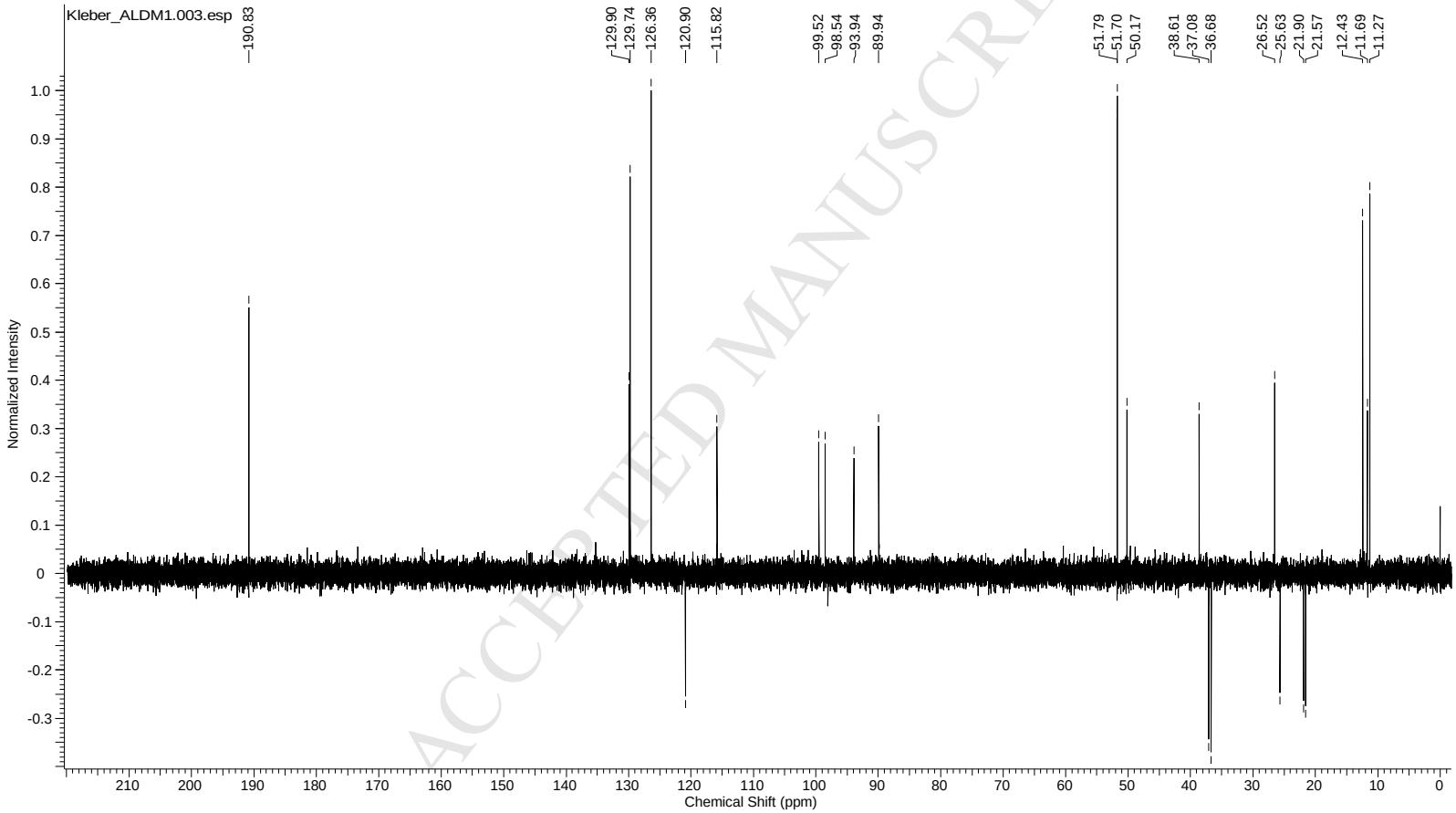


Figure S60. ^{13}C (DEPT-135) in $CDCl_3$ of compound **9b**.

NMR Spectrum

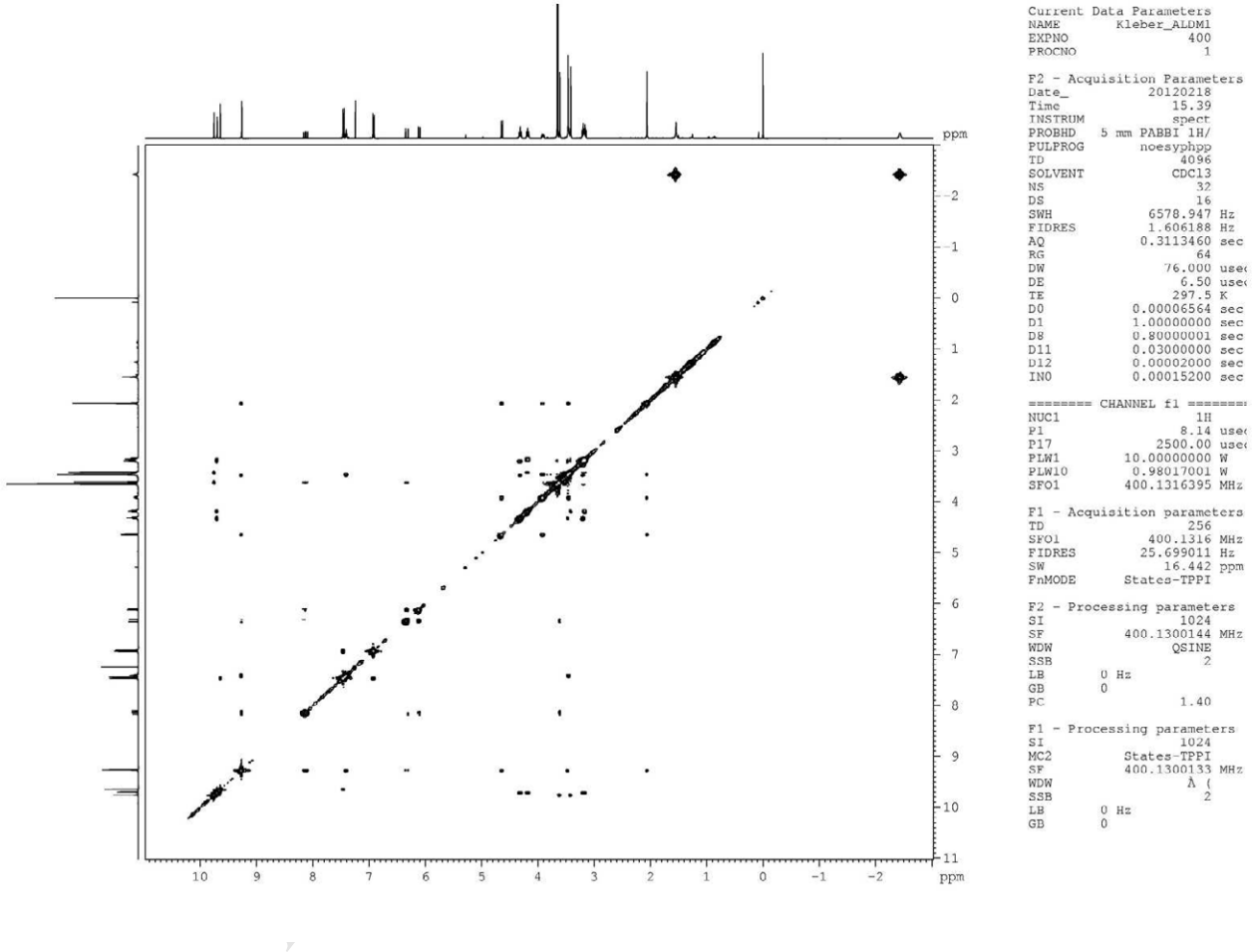
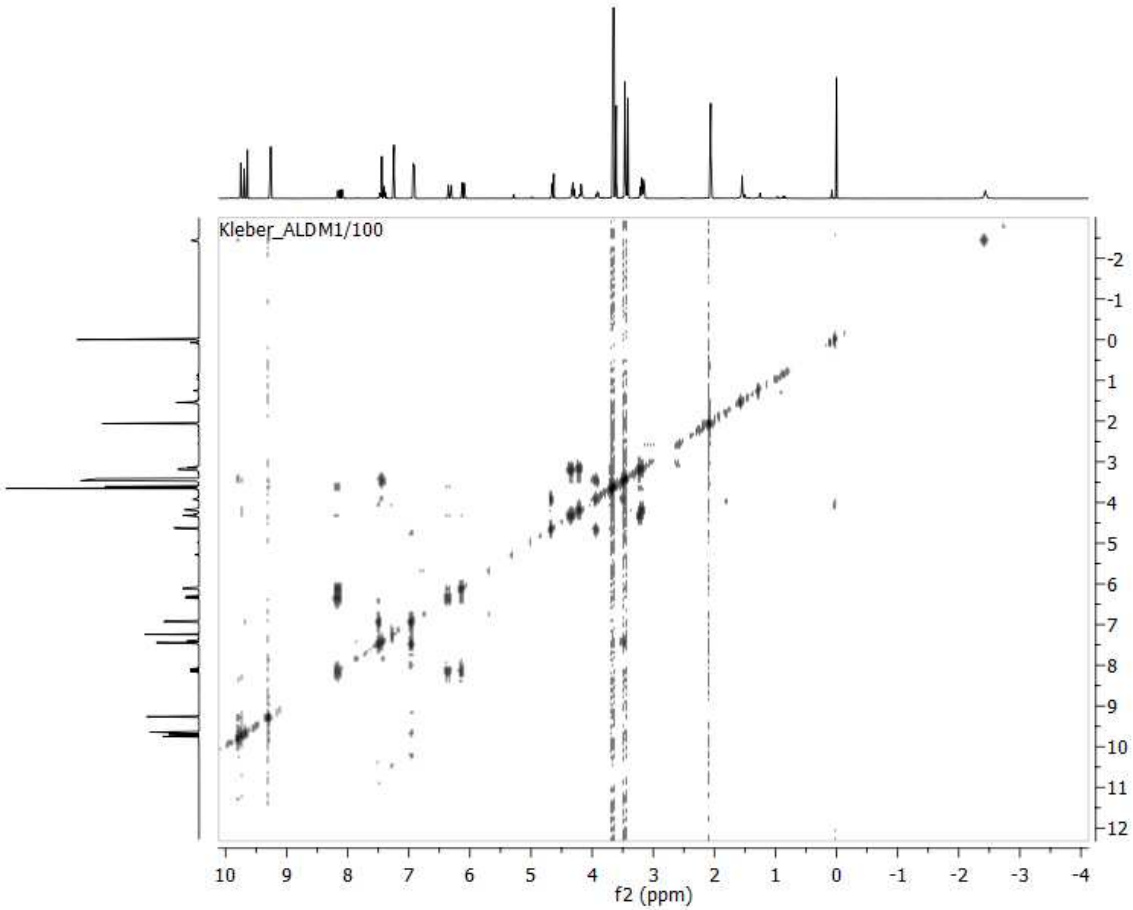


Figure S61.gNOESY (400 MHz) in $CDCl_3$ of compound **9b**.

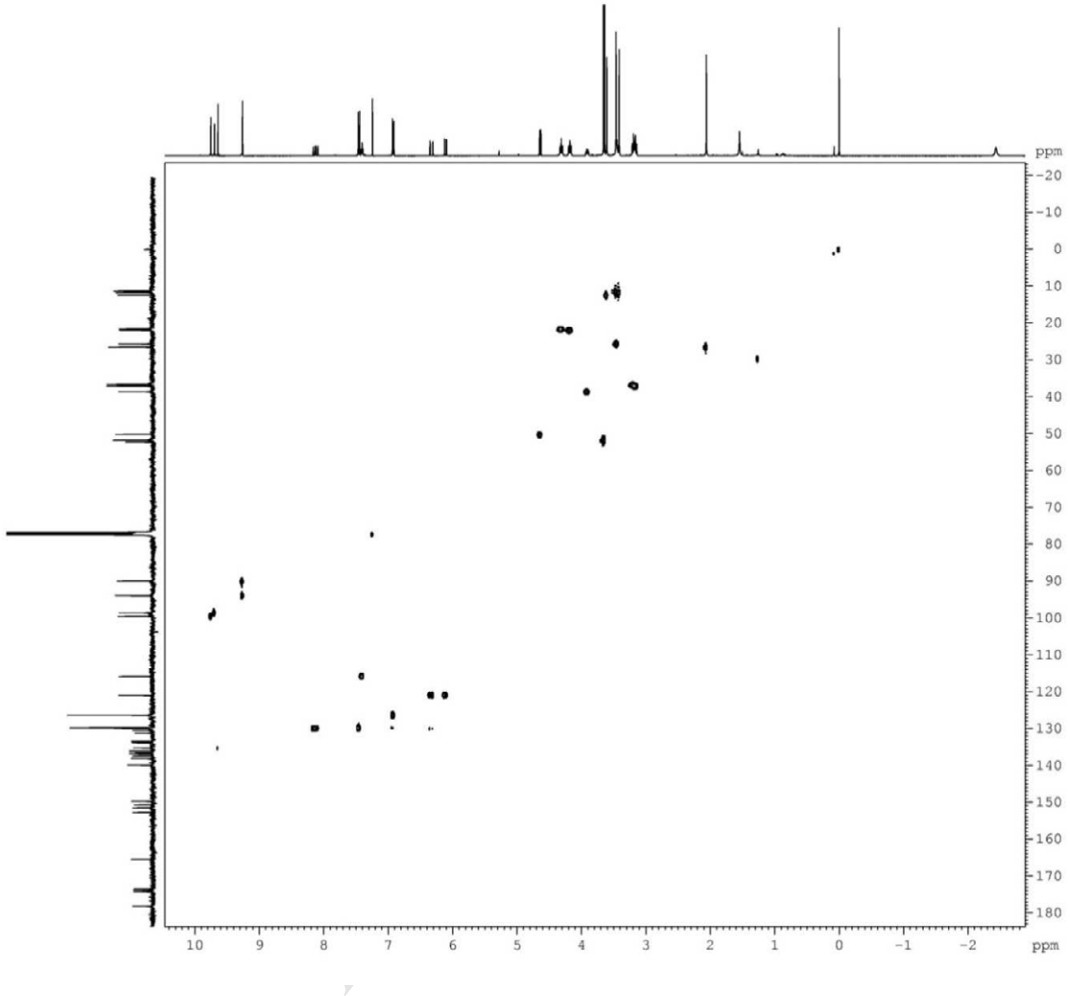
NMR Spectrum



	Parameter	Value (f2, f1)
1	Data File Name	G:/ RMNeditados mestre NOVA/ RMN imida aldeido/ Kleber_ALDM1/ 100/ ser
2	Title	Kleber_ALDM1/ 100
3	Comment	
4	Origin	Bruker BioSpin GmbH
5	Owner	root
6	Site	
7	Spectrometer	spect
8	Author	
9	Solvent	CDCl3
10	Temperature	297.9
11	Pulse Sequence	cosygpqf
12	Experiment	2D-COSY
13	Number of Scans	8
14	Receiver Gain	64
15	Relaxation Delay	1.0000
16	Pulse Width	8.1400
17	Acquisition Time	0.3113
18	Acquisition Date	2012-02-18T10:21:24
19	Modification Date	2012-02-18T15:06:54
20	Spectrometer Frequency	(400.13, 400.13)
21	Spectral Width	(6578.9, 6578.9)
22	Lowest Frequency	(-1650.0, -1650.0)
23	Nucleus	(1H, 1H)
24	Acquired Size	(2048, 256)
25	Spectral Size	(2048, 256)

Figure S62. gCOSY (400 MHz) in $CDCl_3$ of compound **9b**.

NMR Spectrum




```
Current Data Parameters
NAME        Kleber_ALDM1
EXPNO                200
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120218
Time               14.07
INSTRUM             spect
PROBHD   5 mm PABBI 1H/
PULPROG         hsqcetgp
TD                 4096
SOLVENT             CDCl3
NS                    16
DS                    16
SWH             6578.947 Hz
FIDRES          1.606188 Hz
AQ              0.3113460 sec
RG                  2050
DW                76.000 usec
DE                 6.50 usec
TE                298.9 K
CNST2         145.0000000
D0              0.00000300 sec
D1              1.00000000 sec
D4              0.00172414 sec
D11             0.03000000 sec
D16             0.00020000 sec
IN0             0.00002160 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                  1H
P1                  8.14 usec
P2                 16.28 usec
P28              1000.00 usec
PLW1          10.00000000 W
SFO1          400.1316395 MHz

======== CHANNEL f2 ========
CPDPRG2             garp
NUC2                13C
P3                 15.00 usec
P4                 30.00 usec
PCPD2              80.00 usec
PLW2          80.00000000 W
PLW12          2.81250000 W
SFO2          100.6203150 MHz

====== GRADIENT CHANNEL =====
GPNAM1         SMSQ10.100
GPNAM2         SMSQ10.100
GPZ1               80.00 %
GPZ2               20.10 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                   256
SFO1          100.6203 MHz
FIDRES         90.401062 Hz
SW               230.000 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                  4096
SF          400.1300165 MHz
WDW                 QSINE
SSB                    2
LB         0 Hz
GB         0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2        echo-antiecho
SF          100.6127573 MHz
WDW                   A (
SSB                    2
LB         0 Hz
GB         0
```

Figure S63. gHSQC (400 MHz) in $CDCl_3$ of compound **9b**.

NMR Spectrum

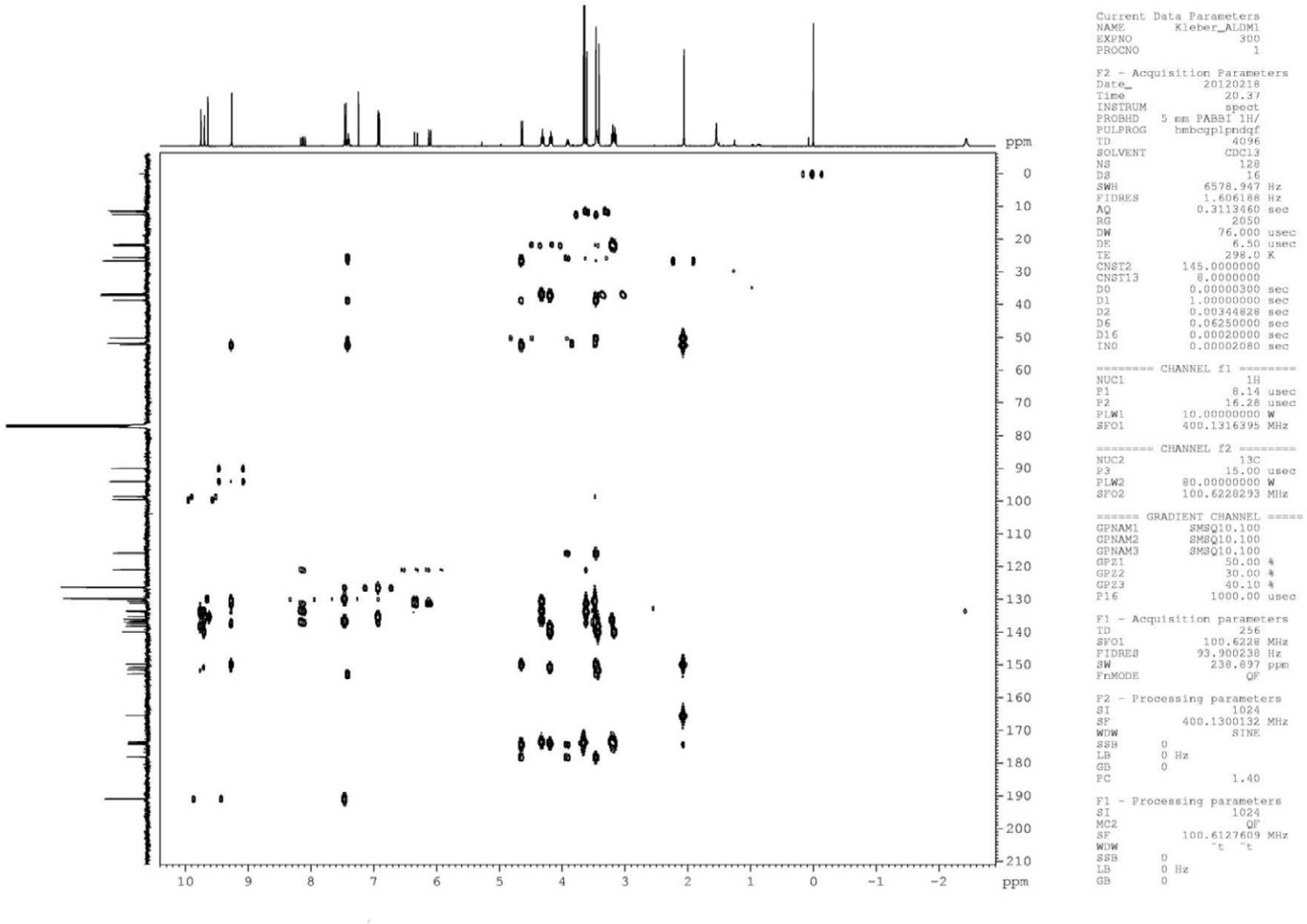


Figure S64. gHMBC (400 MHz) in $CDCl_3$ of compound **9b**.

NMR Spectrum

Date	21 Sep 2012 08:01:20			***Date Stamp***	21 Sep 2012 08:01:20		
File Name	F:\1B338_Clorina_desp_M2_OHCCONH2\1\pdata\1\1r			***Frequency (MHz)***	400.15	***Nucleus***	1H
Number of Transients	128	***Origin***	spect	***Original Points Count***	32768	***Owner***	nmrsu
Points Count	65536	***Pulse Sequence***	zg30	***Receiver Gain***	181.00	***SW(cyclical) (Hz)***	10000.00
Solvent	CHLOROFORM-d	***Spectrum Offset (Hz)***	2990.4939	***Spectrum Type***	STANDARD	***Sweep Width (Hz)***	9999.85

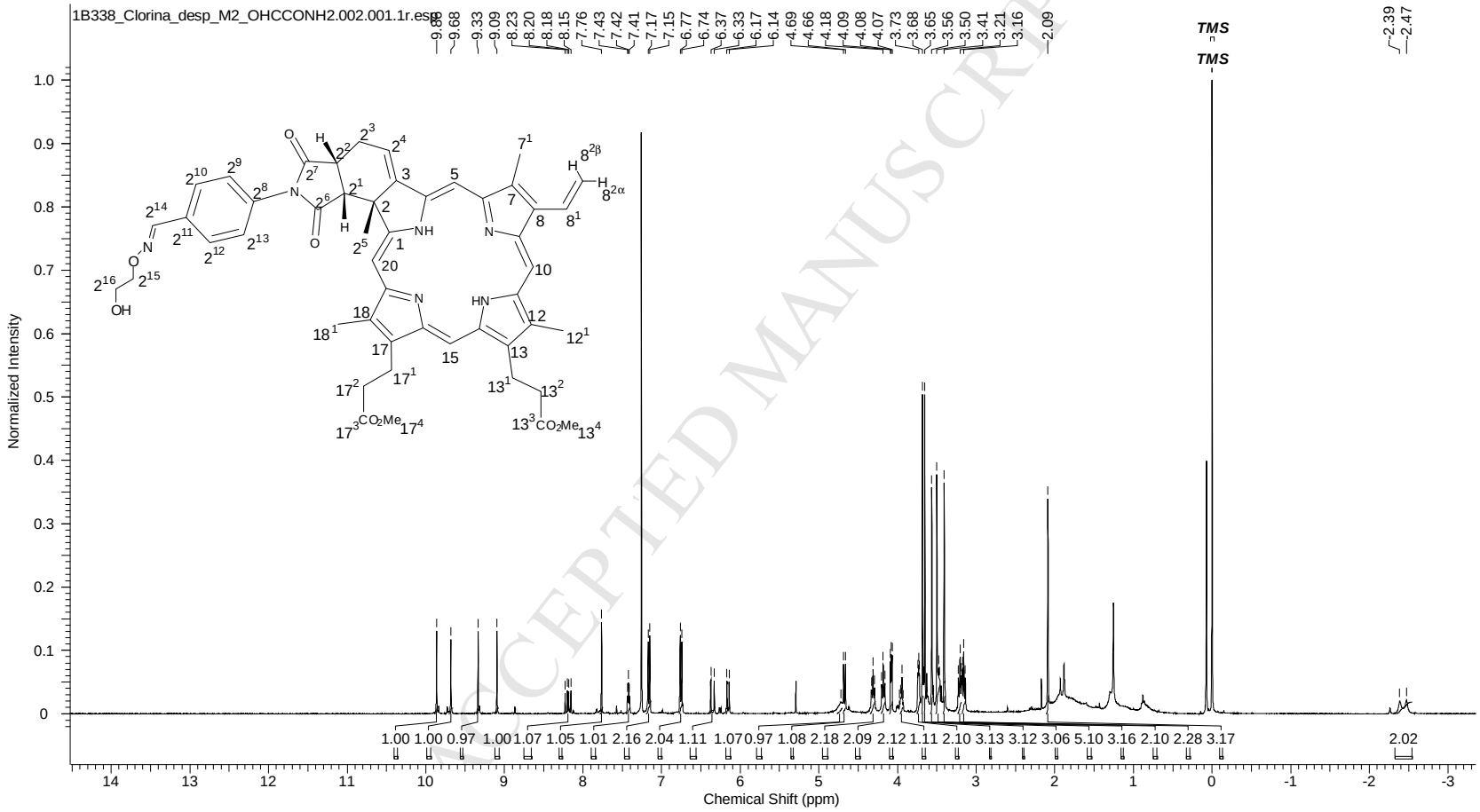


Figure S65. ^{1}H NMR (400 MHz) in $CDCl_3$ of compound **10a**.

NMR Spectrum

File Name	Aminoxi_M2_carb				
Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	4096
Origin	spect	***Original Points Count***	16384	***Temperature (degree C)***	25.600
Points Count	32768	***Pulse Sequence***	zgpg30	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	24671.05	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	10058.5996

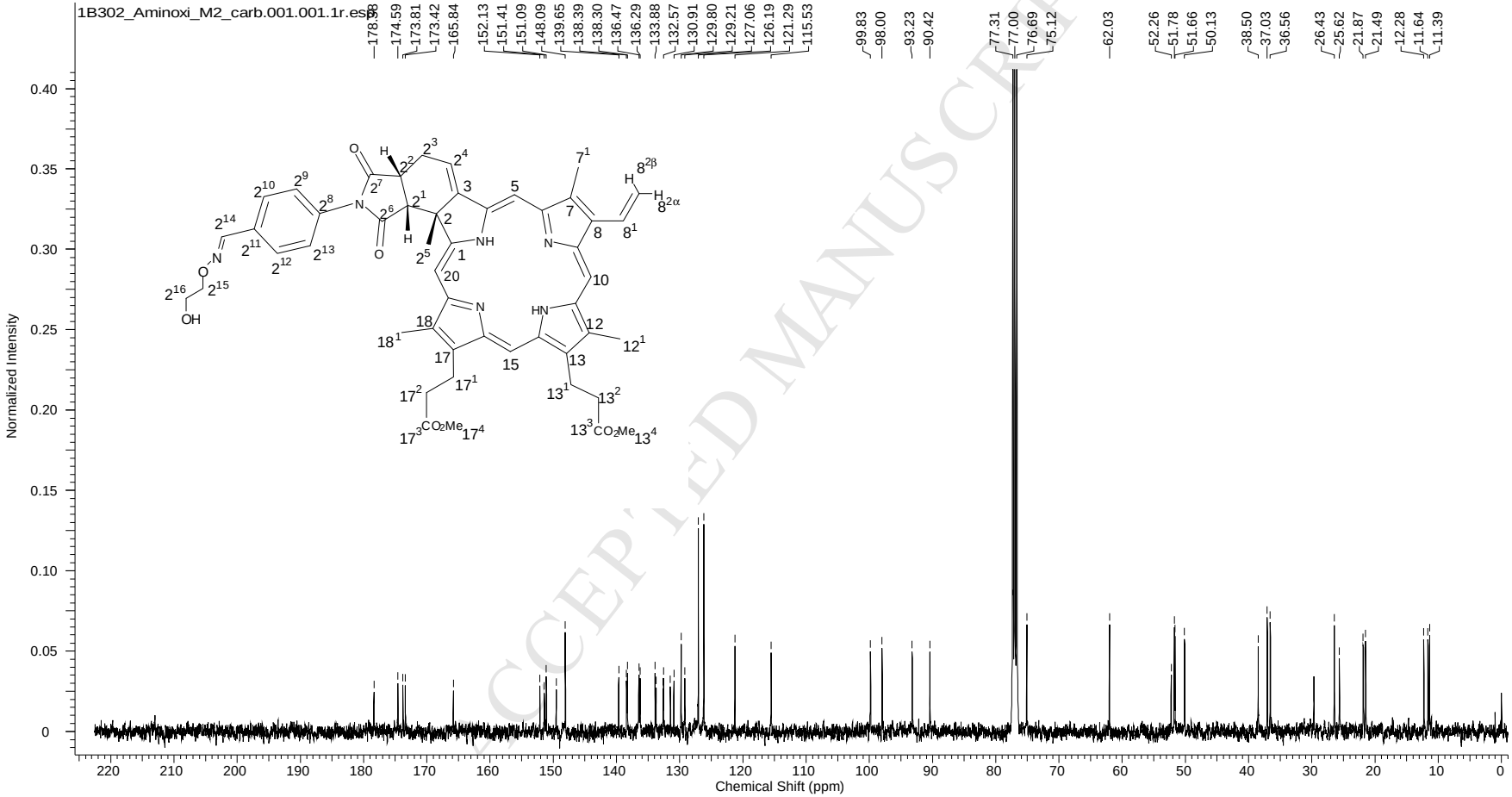


Figure S66. ^{13}C NMR (100 MHz) in $CDCl_3$ of compound **10a**.

NMR Spectrum

Date	26 Sep 2012 07:52:48			***Date Stamp***	26 Sep 2012 07:52:48		
File Name	F:\1B339_Clorina_despM1_OHCCONH2\1\pdata\1\1r			***Frequency (MHz)***	400.15	***Nucleus***	1H
Number of Transients	128	***Origin***	spect	***Original Points Count***	32768	***Owner***	nmrsu
Points Count	65536	***Pulse Sequence***	zg30	***Receiver Gain***	181.00	***SW(cyclical) (Hz)***	10000.00
Solvent	CHLOROFORM-d	***Spectrum Offset (Hz)***	2989.8835	***Spectrum Type***	STANDARD	***Sweep Width (Hz)***	9999.85

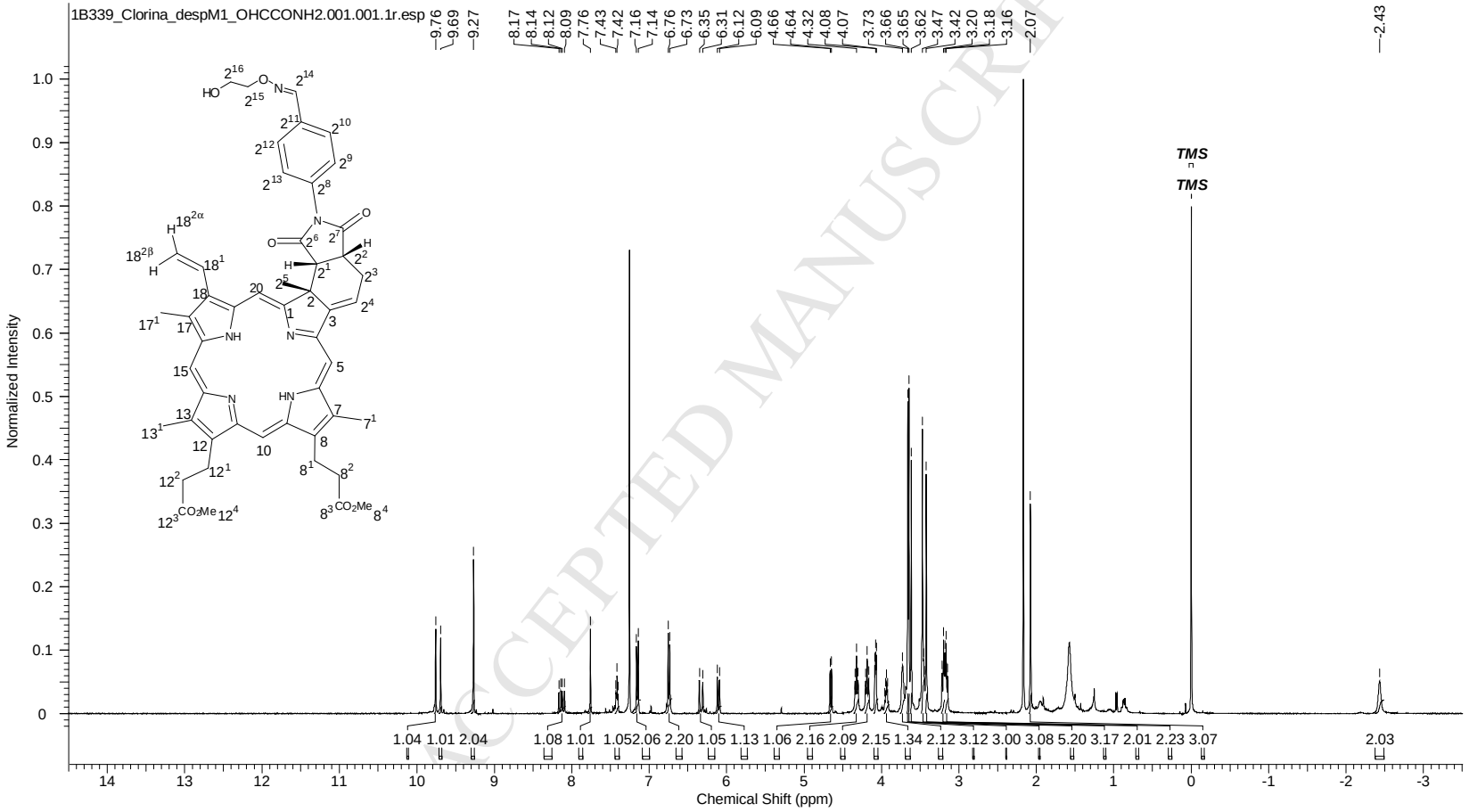


Figure S67. ^{1}H NMR (400 MHz) in $CDCl_3$ of compound **10b**.

NMR Spectrum

File Name	_Aminoxi_M1_carb				
Frequency (MHz)	100.62	***Nucleus***	13C	***Number of Transients***	4096
Origin	spect	***Original Points Count***	16384	***Temperature (degree C)***	25.700
Points Count	32768	***Pulse Sequence***	zgpg30	***Receiver Gain***	2050.00
SW(cyclical) (Hz)	24671.05	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	10058.5996

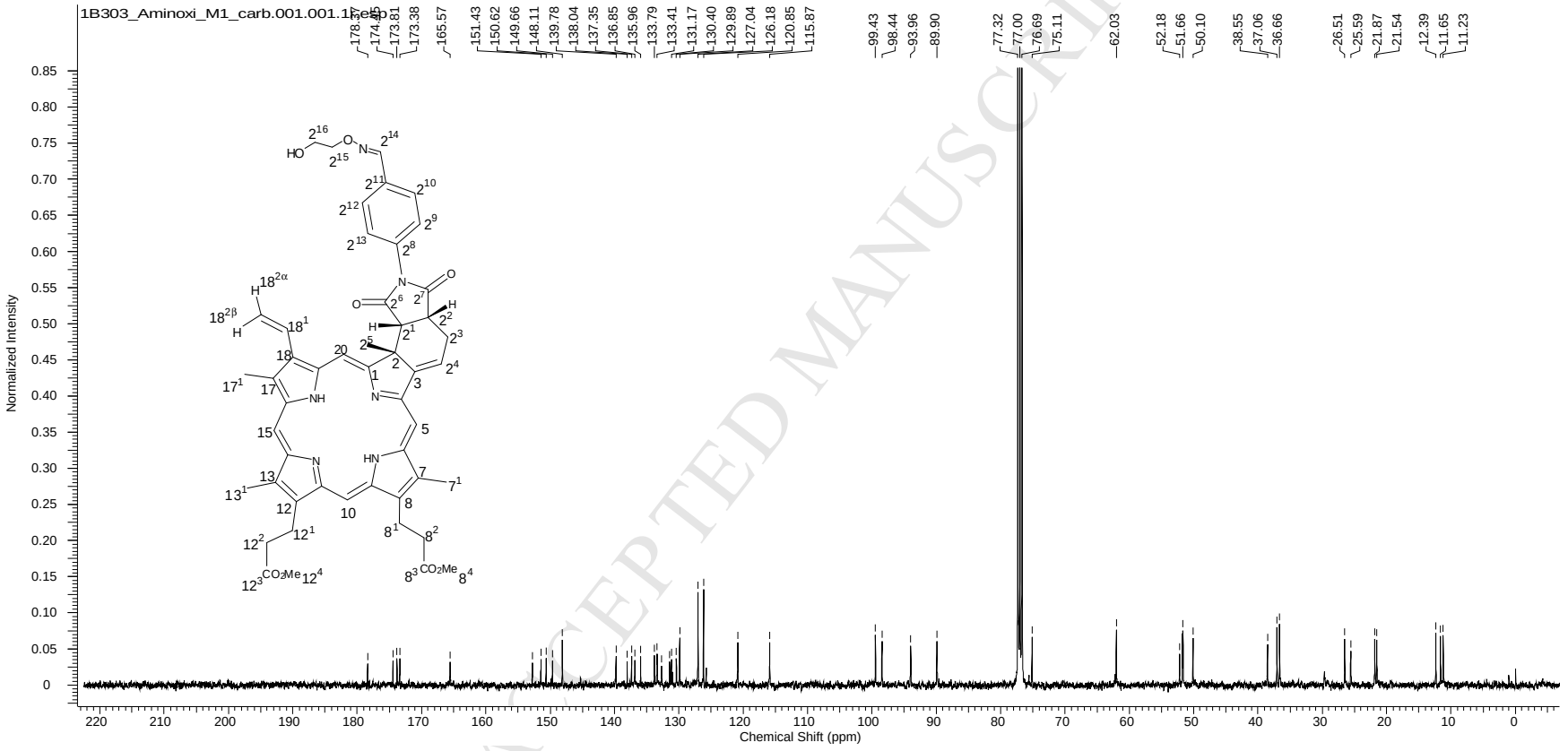


Figure S68. ^{13}C NMR (100 MHz) in $CDCl_3$ of compound **10b**.

Mass Spectrum

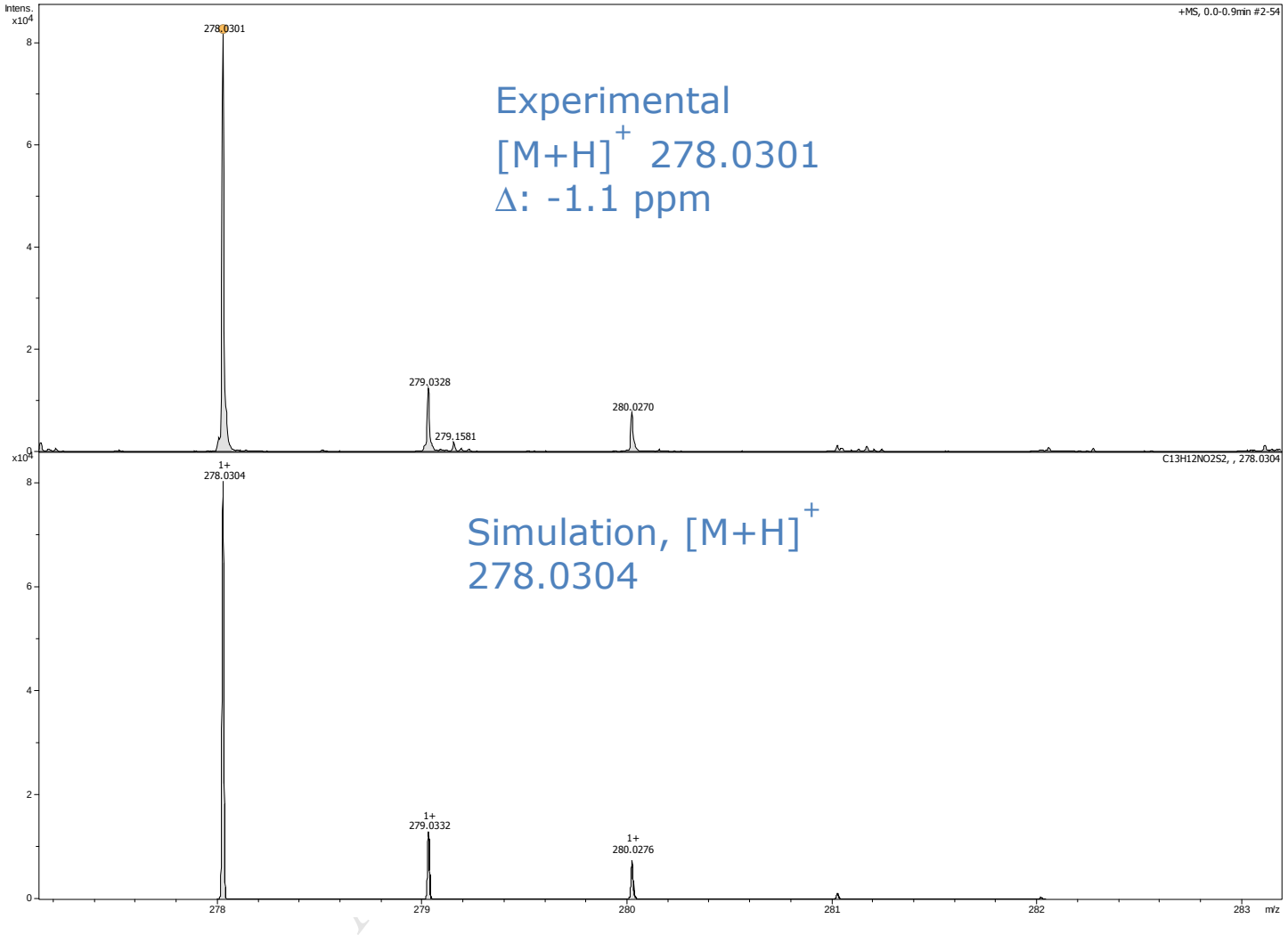


Figure S69. HRMS ESI-TOF of compound 4.

Mass Spectrum

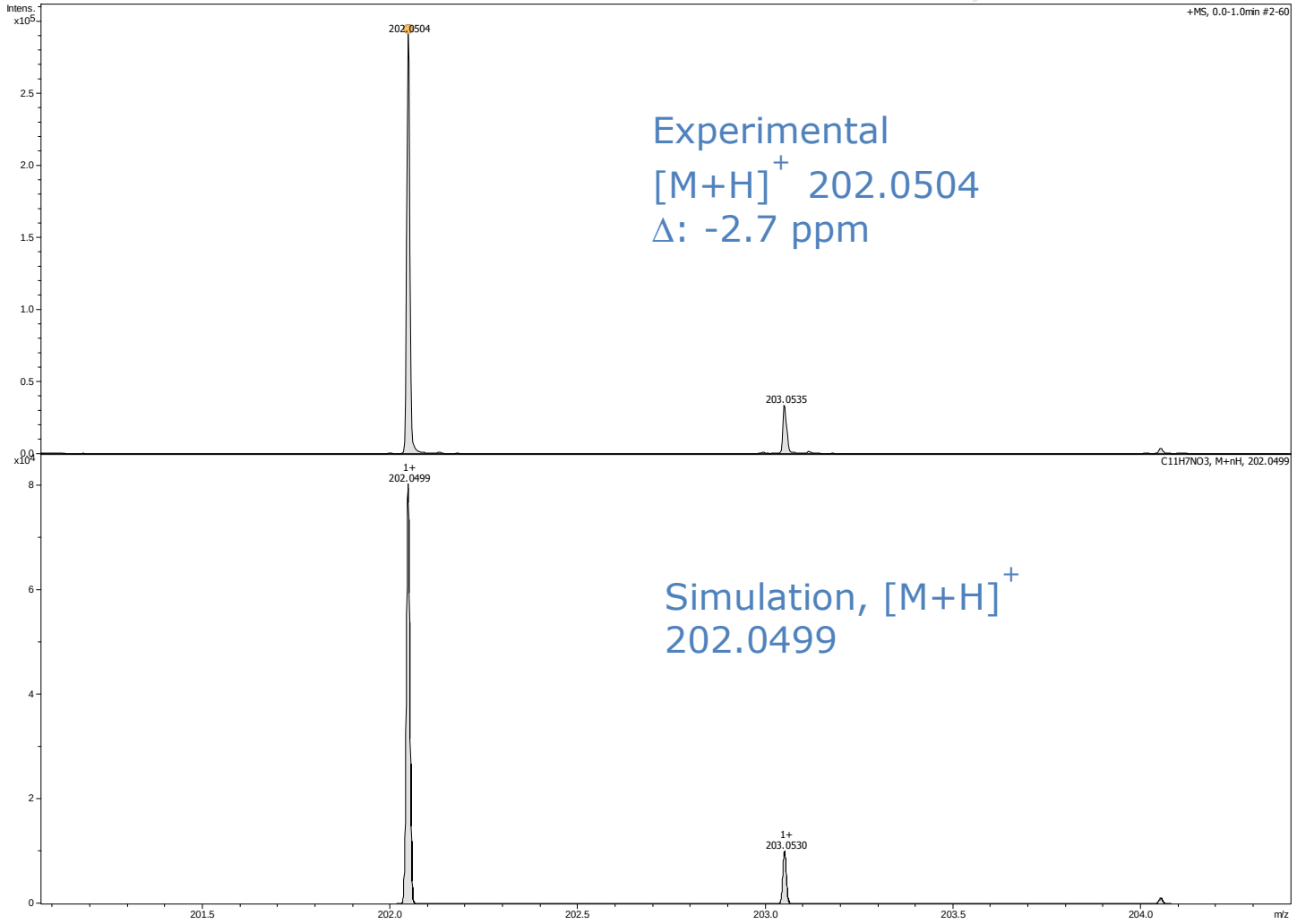


Figure S70. HRMS ESI-TOF of compound 5.

Mass Spectrum

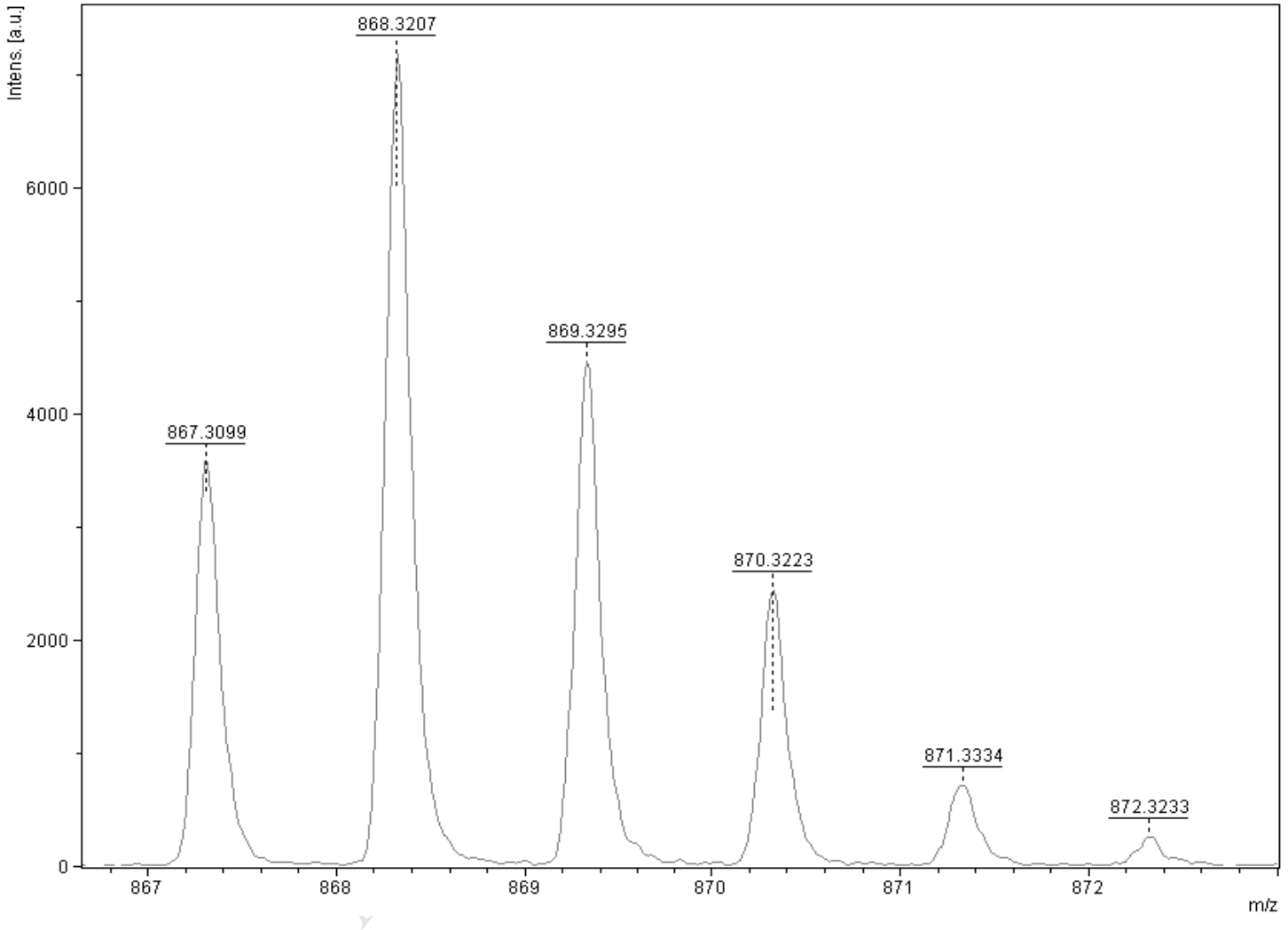
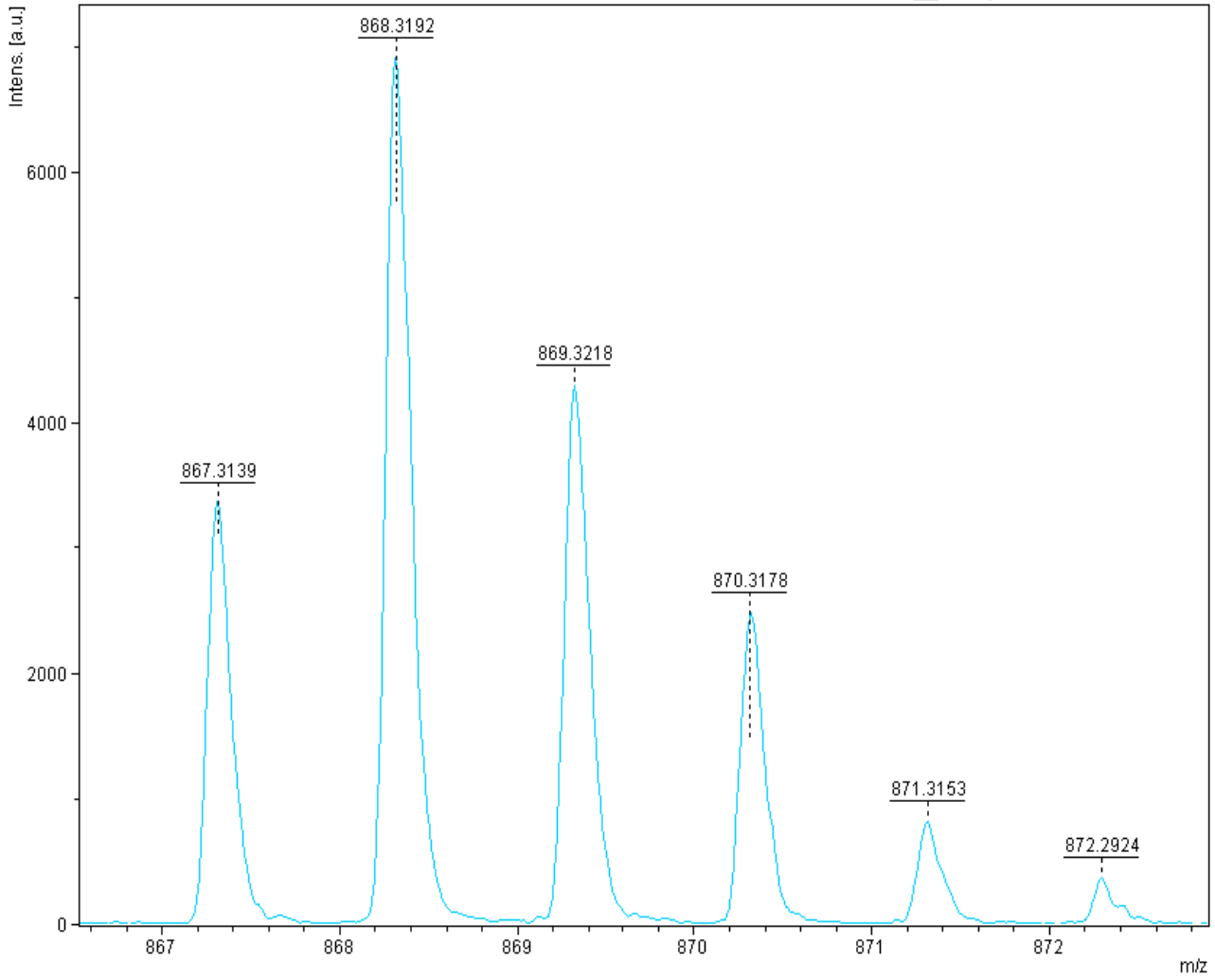


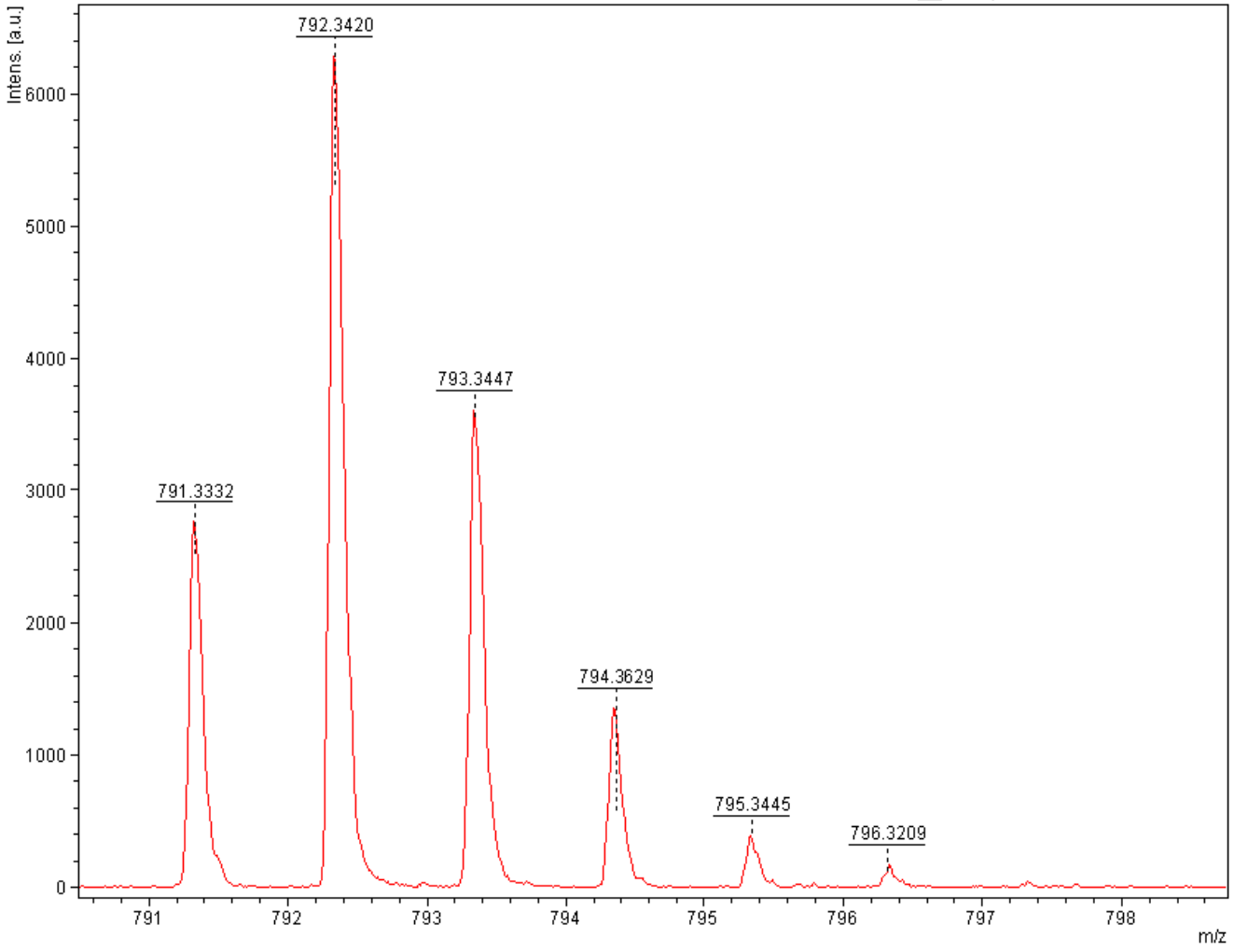
Figure S71. MALDI-HRMS of compound **8a**.

Mass Spectrum



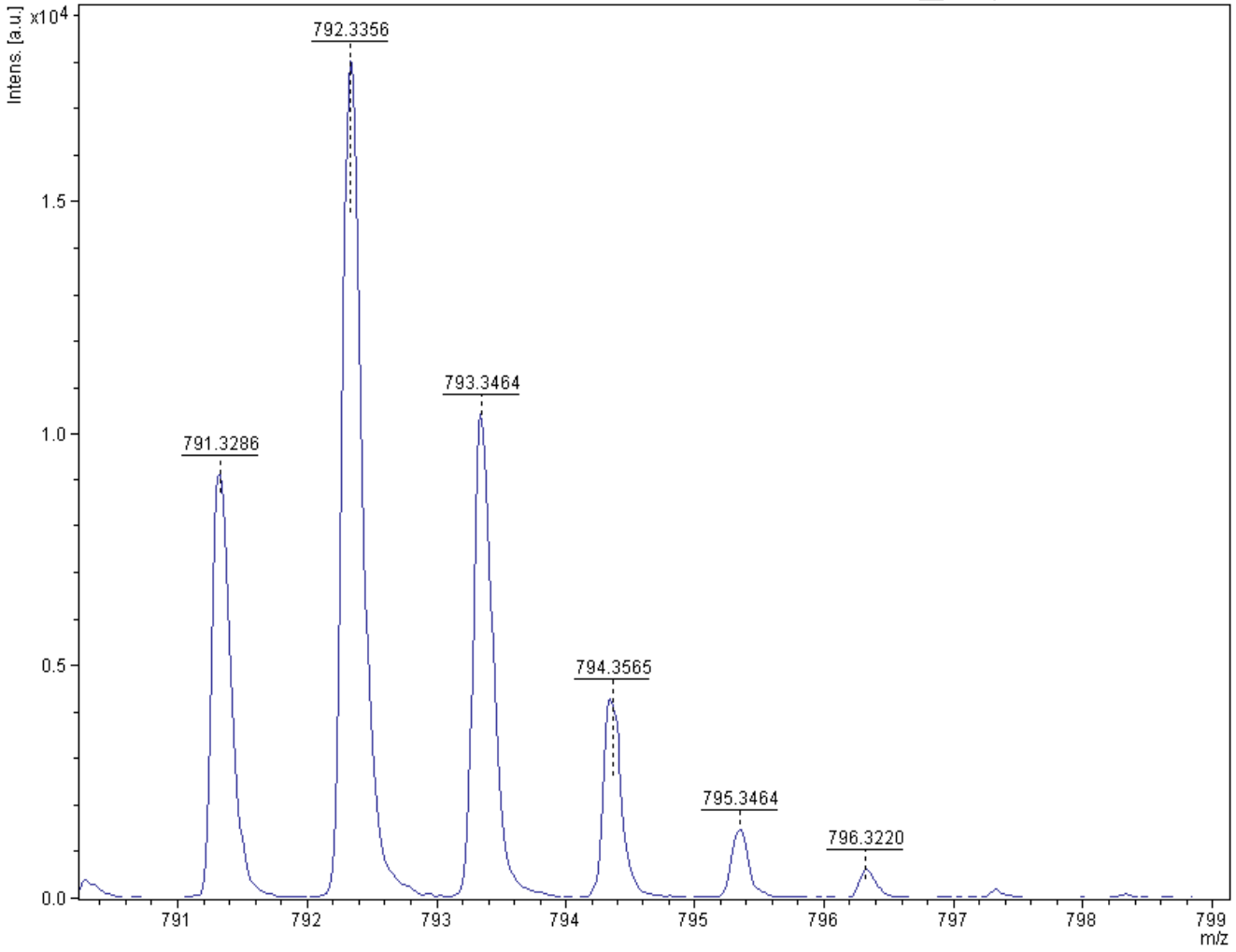
Mass Spectrum

Figure S72. MALDI-HRMS of compound **8b**.



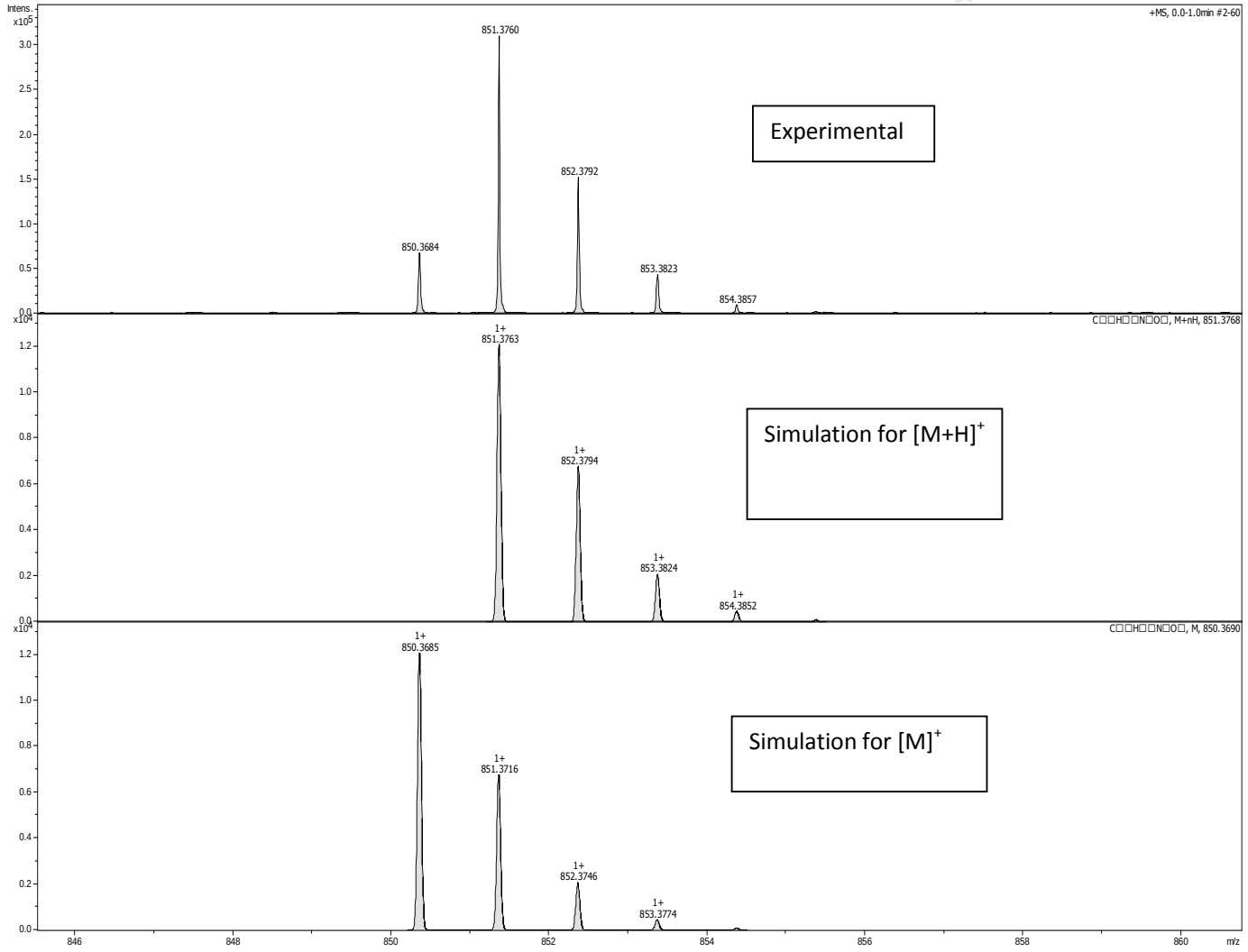
Mass Spectrum

Figure S73. MALDI-HRMS of compound **9a**.



Mass Spectrum

Figure S74. MALDI-HRMS of compound **9b**.



Mass Spectrum

Figure S75. ESI-TOF HRMS of compound **10a**.

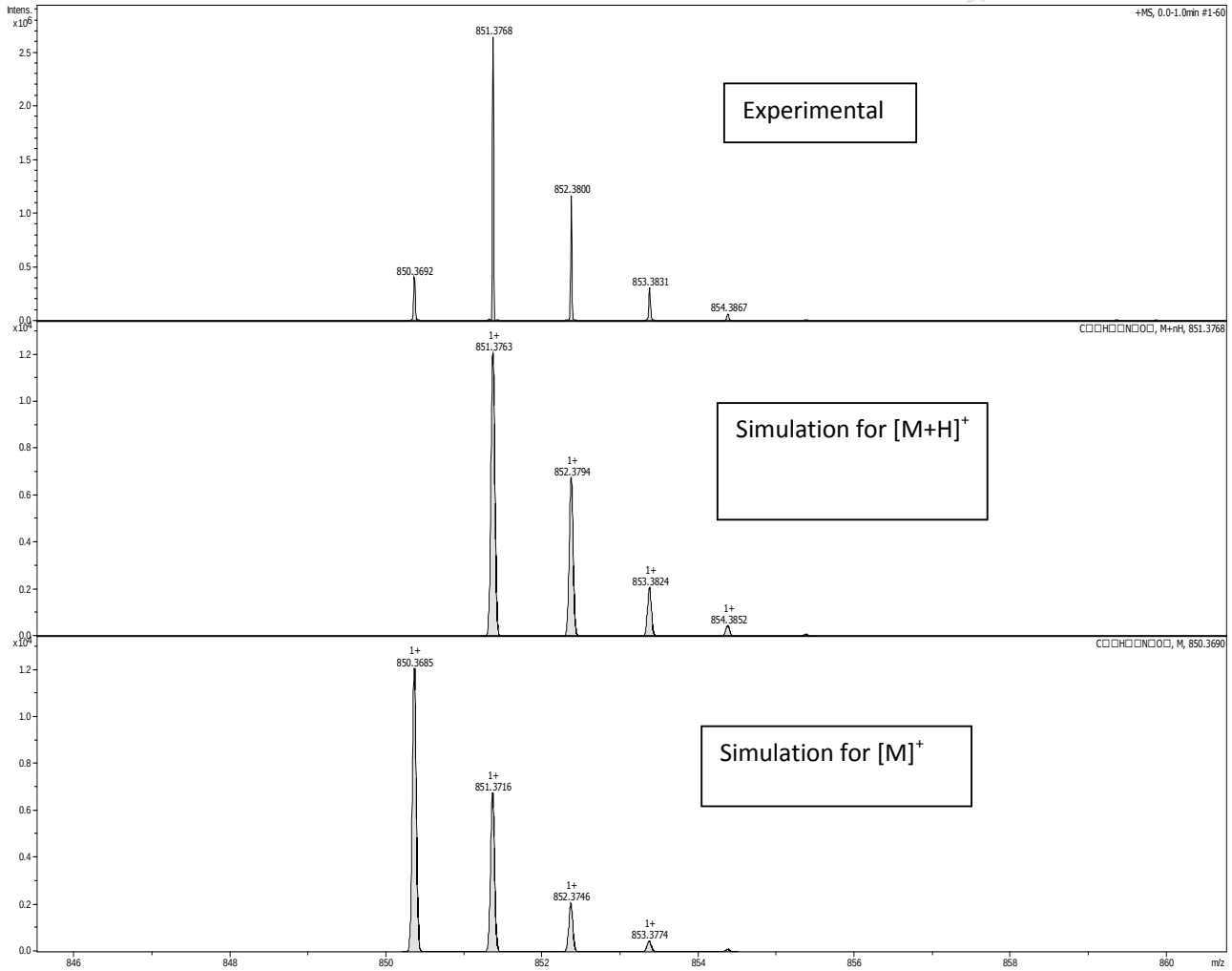


Figure S76. ESI-TOF HRMS of compound **10b**.

Scheme 1: Synthesis of maleimides **4** and **5**. i) ethanedithiol/CH_2Cl_2, $BF_3.Et_2O$, rt.,6h, 80% yield; ii) $SnCl_2.2H_2O$/EtOH, 70^oC, 6h, 93% yield; iii) maleic anhydride/CH_2Cl_2, 0^oC, 1h, 84% yield; iv) Ac_2O/NaOAc, rt, to 90^oC, 92% yield; v) NBS, H_2O-acetone, 0^oC, 87% yield.

Scheme 2. Synthesis of new chlorins. i) C_6H_5Cl/PTSA, reflux, 2h and then ii) MeOH/H_2SO_4 5%, rt, overnight, 72% yield (2 steps); iii) solvent/conditions (Table 1) and maleimide **4**; iv) solvent/conditions (Table 2) and maleimide **5**; v) $NH_2OCH_2CH_2OH$, THF, PTSA, r.t.

Table 1: Conditions for the synthesis of chlorins **8a** and **8b**.

Table 2: Conditions for the synthesis of chlorins **9a** and **9b**.

Figure 1.(a) Main NOE effects in compound **9a** (b) main NOE effects in compound **9b**.

a) Main NOE effects in **9a** b) Main NOE effects in **9b**

Figure 2. Changes of the ^1H chemical shifts of protoporphyrin IX dimethyl ester (**7**), in $CDCl_3$ at 25℃, as a function of the concentration.

Figure 3. Example of the maintenance of the ^1H chemical shifts of compound **10a** in $CDCl_3$ at 25 ℃ as a function of the concentration.

Figure 4. Example of aggregation study by UV-Vis analyses of compound **10a** in CH_2Cl_2.

Figure 5. Example of aggregation study by UV-Vis analyses of compound **10a** in water containing 4% of DMSO.

Figure 6. DPBF consumption in the presence of MB in ethanol. (inset: ln (DPBF absorbance) vs irradiation time).

Figure 7. DPBF consumption in the presence of chlorin **10a** in ethanol. (inset: ln (DPBF absorbance) vs irradiation time).

Figure 8. Photobleaching study for chlorin **10a**.