Supporting information

A two-step synthesis of 7,8-dichloro-riboflavin with high yield

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Experimental Section.

Synthesis of the dichloro-riboflavin using Boc protection (Scheme 1).

(1a) : Dichloro-1,2phenylenediamine (3.00g, 16.9mmol) was dissolved in equimolar proportion with sodium bicarbonate and di-tert-butyl dicarbonate in 75mL dioxane plus 75mL water. The reaction was carried out for 24 hours at room temperature and the reaction mixture was diluted in 150mL water. Then **1a** was extracted twice with 50mL dichloromethane. The organic phase was first washed with 50mL saturated sodium bicarbonate solution, then with 100mL brine. The organic phase was dried using magnesium sulphate and evaporated under vacuum. Solution (9mL of cyclohexan and 3mL of ethyl acetate) was added to the brown solid and filtrated to give the pure product. Yield: 2.74g (58%).

Mass spectrometry analysis (276.6g mol^{-1}). ^{1}H-NMR (400 MHz, DMSO D6): δ = 8.47 (s, 1H), 7.53 (s, 1H), 6.87 (s, 1H), 5.33 (s, 2H), 1.46 (s, 9H). ^{13}C-NMR (100 MHz, DMSO D6): δ = 153.2, 140.8, 125.7, 123.9, 116.3, 115.5, 79.5, 66.4, 28.1.

(**1b**) : **1a** (1.10g, 4mmoles), D-ribose (3.58g, 24mmoles) and sodium cyanoborohydride (0.50g, 16mmoles) were dissolved in 100mL dried methanol. The reaction was performed at 65°C, for 48 hours. Lower amounts of D-ribose and sodium cyanoborohydride led to lower yield of **1b**. After evaporation under vacuum, 30mL concentrated aqueous HCl was used to react with the remaining sodium cyanoborohydride. The solution was neutralized with saturated sodium bicarbonate solution. **1b** was extracted twice with 50mL ethyl acetate. The organic phase was washed with brine, dried using magnesium sulphate, then the solvent was removed by evaporation under vacuum. Purification was made with preparative chromatography (silica gel) using AcOEt. Yield: 0.46g (28%). Mass spectrometry analysis (410.8g mol^{-1}). ^{1}H-NMR (400 MHz, DMSO D6) : δ = 8.59 (s, 1H),7.42 (s, 1H), 6.76 (s, 1H), 5.32 (t, *J*=4.81Hz, 1H), 4.81 (d, *J*=5.50Hz, 1H), 4.77 (d, *J*=5.50Hz, 1H), 4.68 (d, *J*=5.04Hz, 1H), 4.42 (t, *J*=5.50Hz, 1H), 3.78 (m, 1H), 3.57 (m, 2H), 3.43 (m, 2H), 3.27 (m, 1H), 3.02 (m, 1H), 1.45 (s, 9H). ^{13}C-NMR (100 MHz, DMSO D6): δ = 153.6, 142.2, 127.1, 125.2, 124.1, 115.7, 111.2, 79.5, 73.2, 72.8, 69.9, 63.3, 45.6, 28.1.

(2) : 1b (1.10g, 2.7mmoles) was dissolved in 53mL of 4M HCl in dioxane. The reaction was performed at room temperature for 5 hours, under constant stirring. The solvent was removed by evaporation under vacuum and the product was dissolved in water (100mL) and washed three times using 20mL ether. Aqueous solution containing **2** was freeze, filtrate and dried without further purification. Yield: m = 0.67g (80%). Mass spectrometry (ESI) 310.9 g mol^{-1}. ^{1}H-NMR (400 MHz, EtOD D6) : δ = 6.75 (s, 1H), 6.60 (s, 1H), 5.42 (s, 2H), 3.95 (m, 1H), 3.74 (m, 2H),

3.64(m, 2H), 3.51 (b, 5H), 3.34 (m, 1H), 3.14 (m, 1H). ^{13}C-NMR (100 MHz, EtOD D6): δ = 137.2, 135.4, 121.0, 119.6, 115.9, 111.8, 73.1, 70.9, 63.3, 53.5, 46.4.

(**3**) : Alloxan (0.31g, 2.2 mmoles) and boric acid (0.58g, 9.3 mmoles) were dissolved in boiling pure acetic acid (25mL) for 1 hour, then the solution was cooled to room temperature. After adding 0.42g of **2**, the reaction was performed at 80°C for 3 hours and, then was left at room temperature overnight. Water (50mL) was added before evaporation under vacuum. About 80mL was evaporated and extraction was made twice by 50mL of cyclohexan and twice by 50mL by ethyle acetat. The aqueous phase was cooled and a precipited appear. Filtration gave the good product. Yield : m = 0.43g (77%). HRMS (ESI) 439.1 g mol^{-1}. ^{1}H-NMR (400 MHz, DMSO D6) : δ = 11.48 (b, 1H), 8.36 (s, 1H), 8.30 (s, 1H), 5.06 (s, 1H), 4.73 (m, 2H), 4.41 (s, 1H), 4.13 (s, 1H), 3.56 (s, 2H), 2.43 (s, 4H). ^{13}C-NMR (100 MHz, DMSO D6) : δ = 159.8, 155.8, 151.5, 140.2, 137.2, 134.5, 134.4, 132.2, 128.5, 120.1, 73.9, 73.1, 69.1, 63.8, 48.2.

Direct synthesis of dichloro-riboflavin (Scheme 2).

D-ribose (2.20g, 15moles), sodium cyanoborohydride (2.00g, 32mmoles) and **1** (1.77g, 10mmoles) were dissolved in dried methanol (100mL). The reaction was performed at 65°C, for 48 hours. Lower amounts of D-ribose and sodium cyanoborohydride led to lower yield of **2**. After evaporation under vacuum, 30mL concentrated aqueous HCl was used to react with the remaining sodium cyanoborohydride. The solution was neutralized with saturated sodium bicarbonate solution. **2** was extracted twice with 50mL ethyl acetate. The organic phase was dried using magnesium sulphate, then the solvent was removed by evaporation under vacuum. The crude product was partially dissolved in dichloromethane

and filtered on a n°5 frit. Unsoluble product was partially dissolved again with warm dichloromethane, cooled to room temperature and filtered. Yield : m = 1.01g (33%). RMN for compound **2** as described above.

Alloxan (0.32g, 2.2 mmoles) and boric acid (0.80g, 12mmoles) were dissolved in boiling pure acetic acid (25mL) for 1 hour and then cooled to 50°C. After adding 0.40g of **2**, the reaction was performed at 80°C for 3 hours and then left at room temperature overnight. Extraction and purification of **3** was performed as described above. Yield : m = 0.41g (77%). RMN for compound **3** as described above.

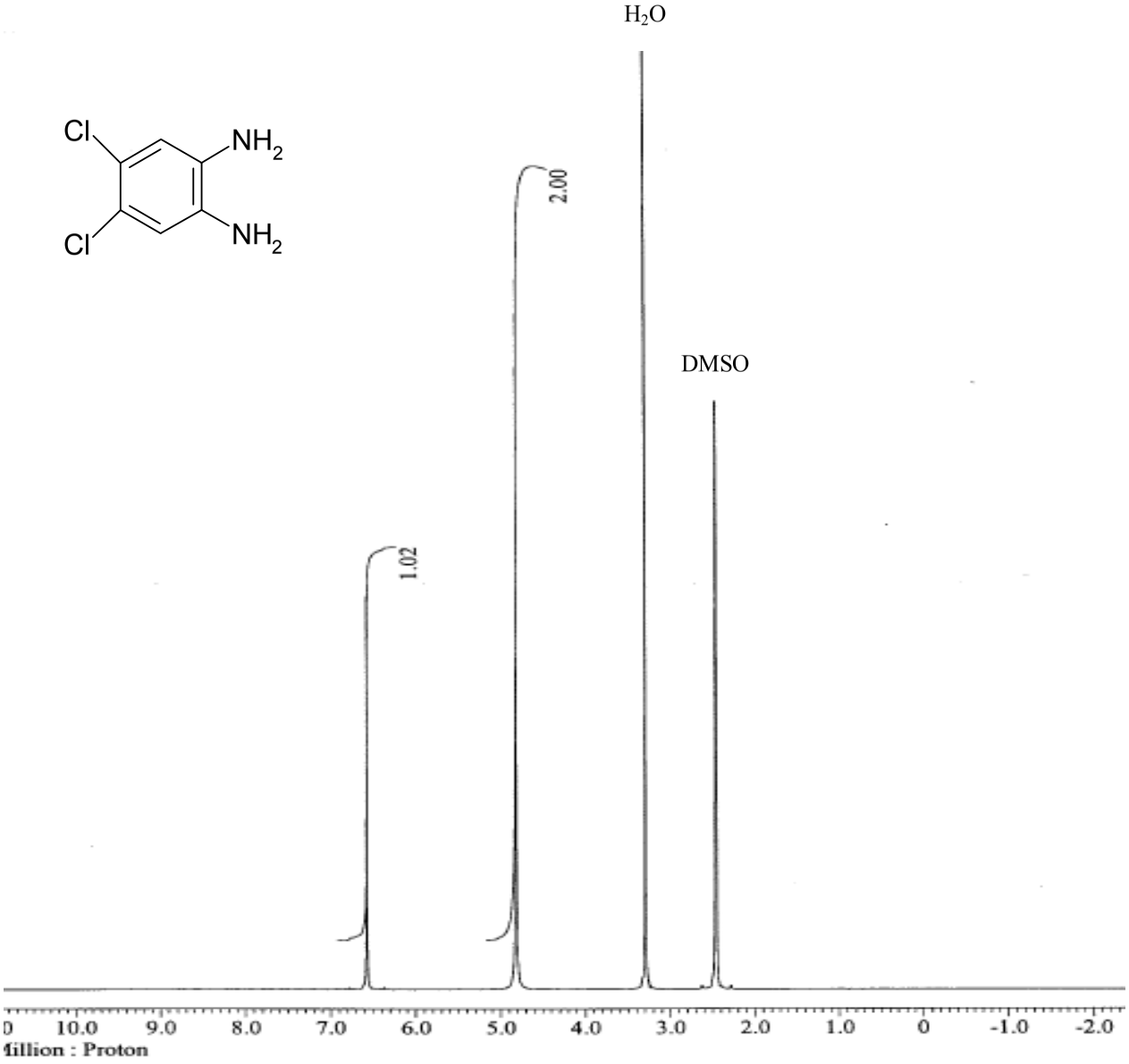
Spectroelectrochemical experiments

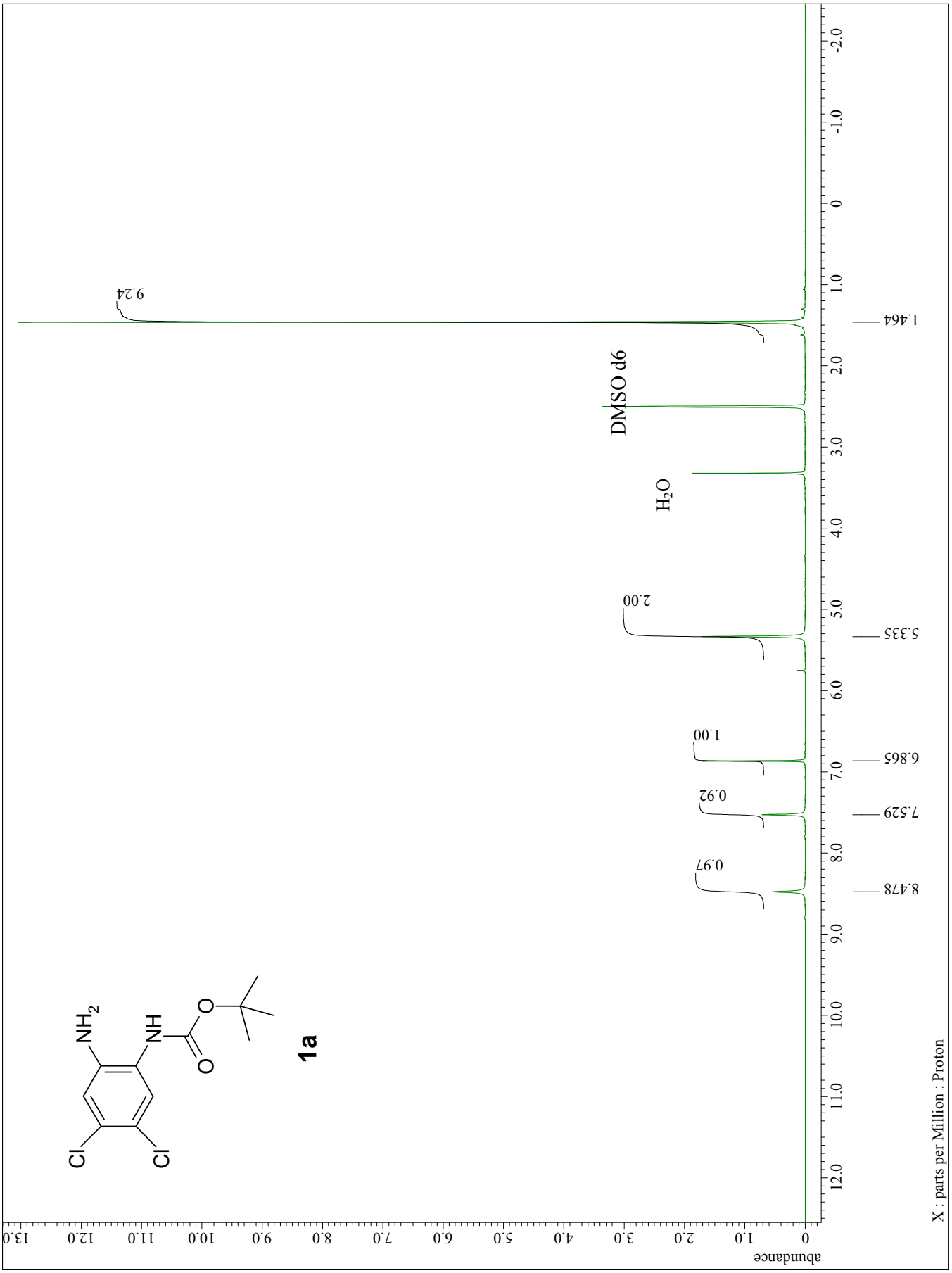
A thin layer quartz glass spectroelectrochemical cell with a light pass length of 1 mm (ALS) was used as an electrolysis cell. A Pt mesh (80 mesh, 6 mm × 7 mm) was used as the working electrode. A Pt wire and An Ag|AgCl|sat.KCl (ALS) were used as the auxiliary electrode and the reference electrode, respectively. These electrodes were fixed with a Teflon cap on the top of the cell. Electrolyte solution was 0.067 M phosphate buffer of pH 7.0 containing 0.67 M KCl, and the total volume was 300 µL. The concentration of **3** in the electrolyte solution was 0.33 mg mL^{-1}. The dissolved oxygen gas in electrolyte solution was removed with Ar gas for 5 min prior to the spectroelectrochemical measurement to minimize oxygen level. A blanket of Ar gas was maintained over the electrolyte solution during the entire electrochemical measurements. Argon gas was maintained over the electrolyte solution during electrolysis. Electrolysis was carried out on a potentiostat (CHI 660) thermostated at 15 ˚C. Spectrum change of **3** at each potential (-0.60, -0.50, -0.45, -0.40, -0.35, -0.30, -0.25, -0.20 and -0.15 V) was simultaneously monitored on a CARY 100 spectrophotometer. The potential dependence of the spectral change was analyzed at 450 nm according to following equation.

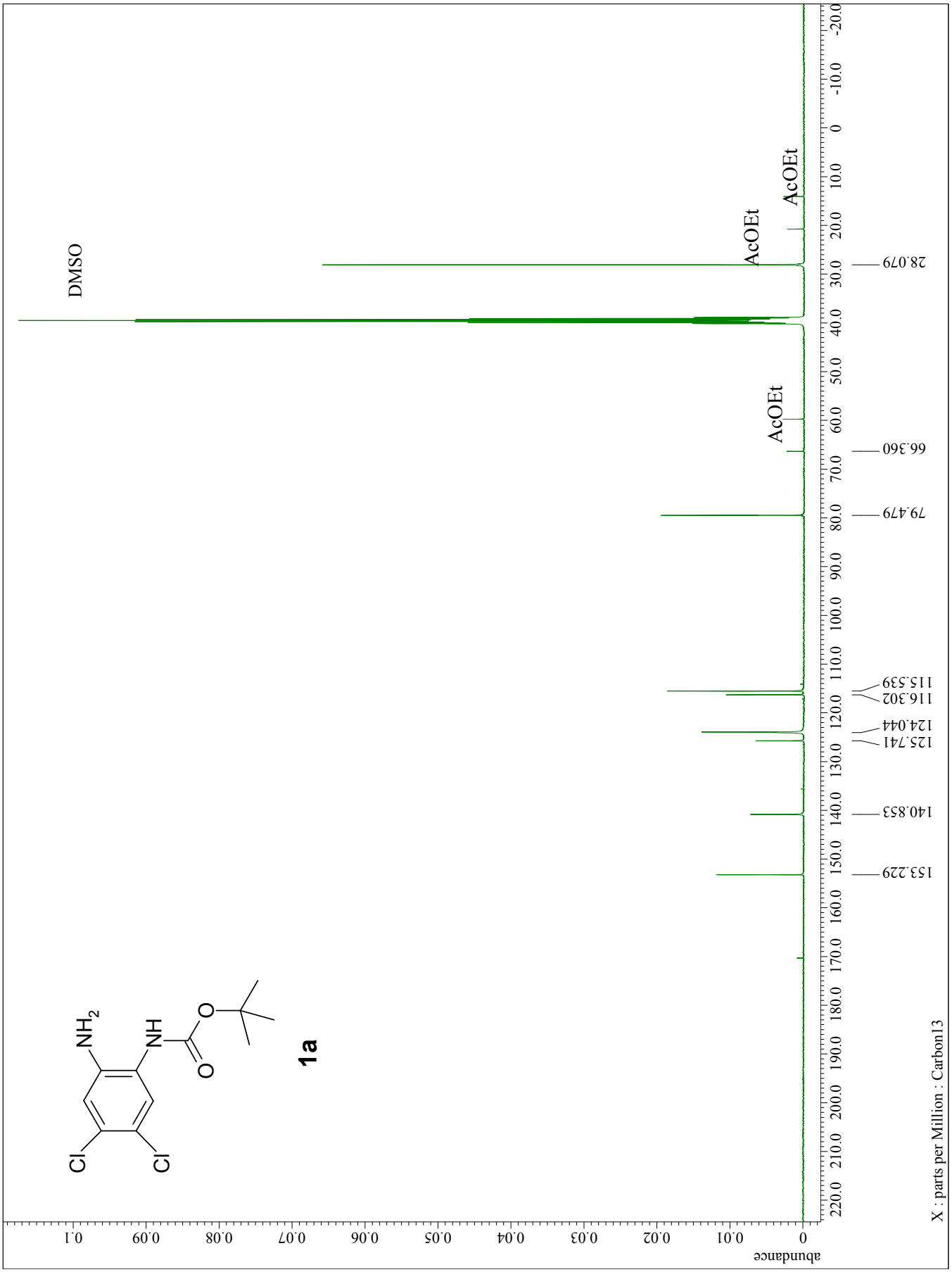
$$E = E^{\circ'} + \frac{RT}{nF}\ln\left(\frac{A_R - A(E)}{A(E) - A_O}\right) \qquad \text{equation (1)}$$

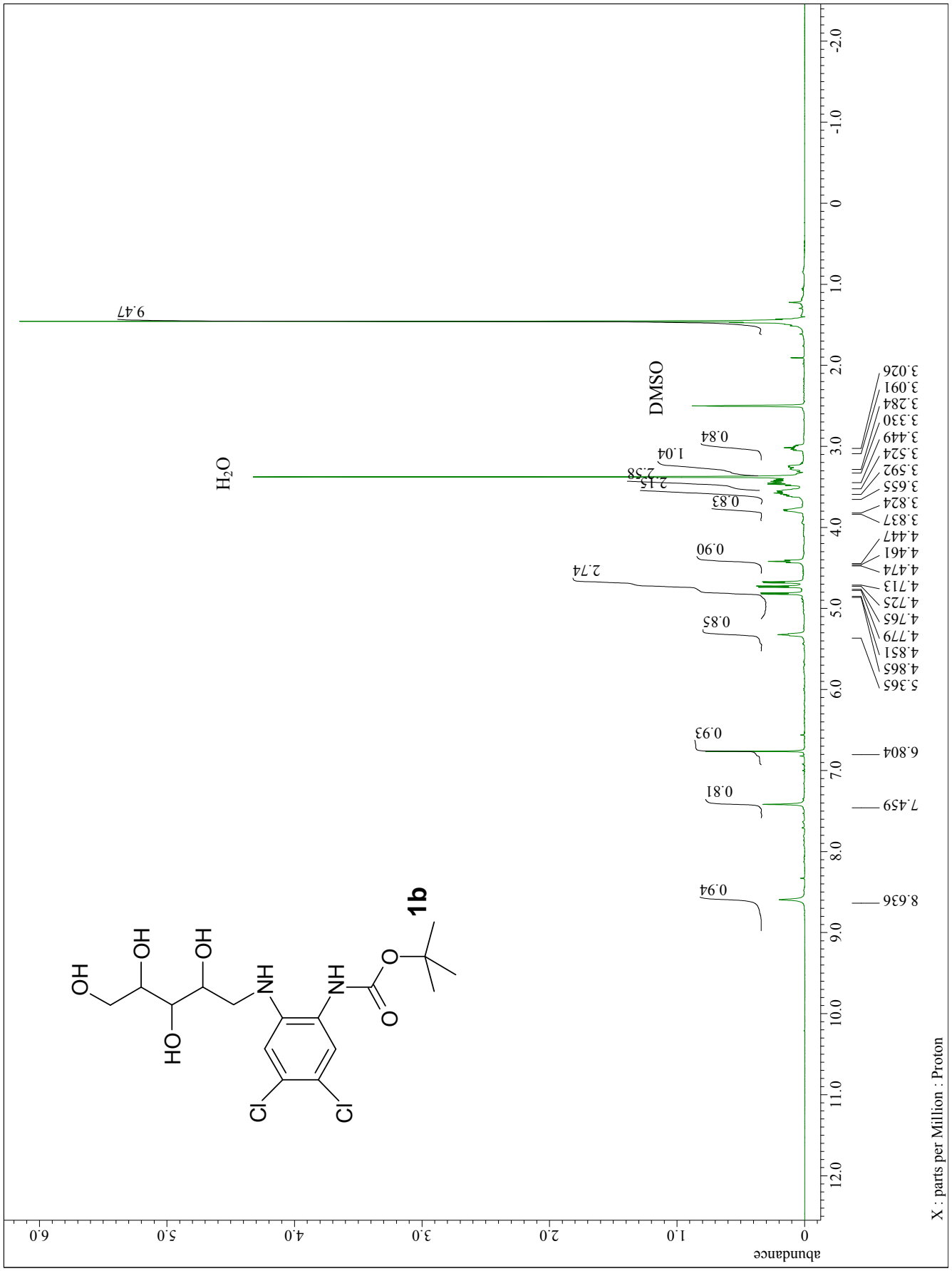
where n, E, E^{o}' are the number of electrons, electrode potential, and the redox potential of **3**, respectively. A_R and A_O are the absorbance at E = –0.6 V and –0.15 V, where **3** would be fully reduced and oxidized, respectively.

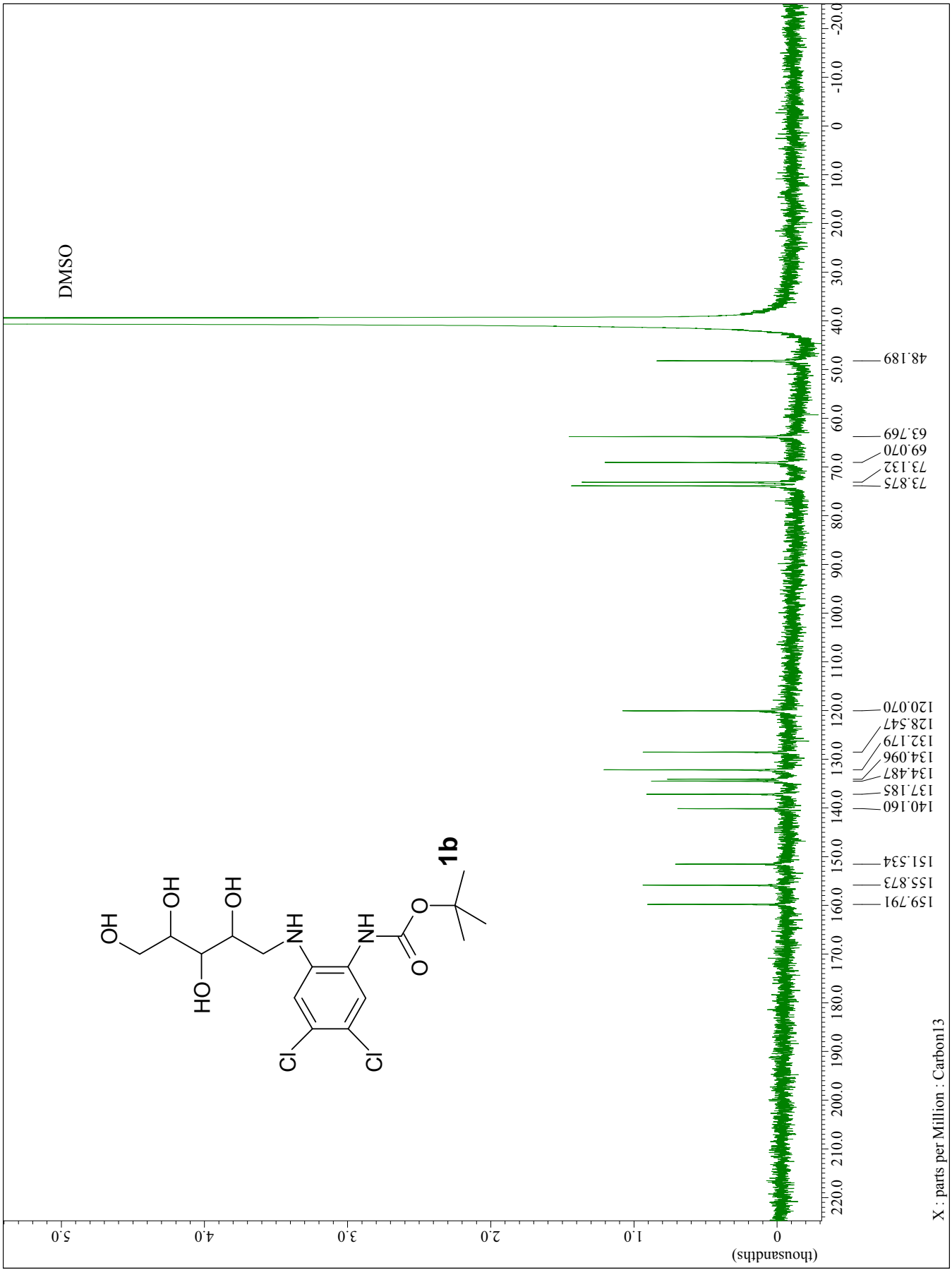
Proton NMR spectra of 1

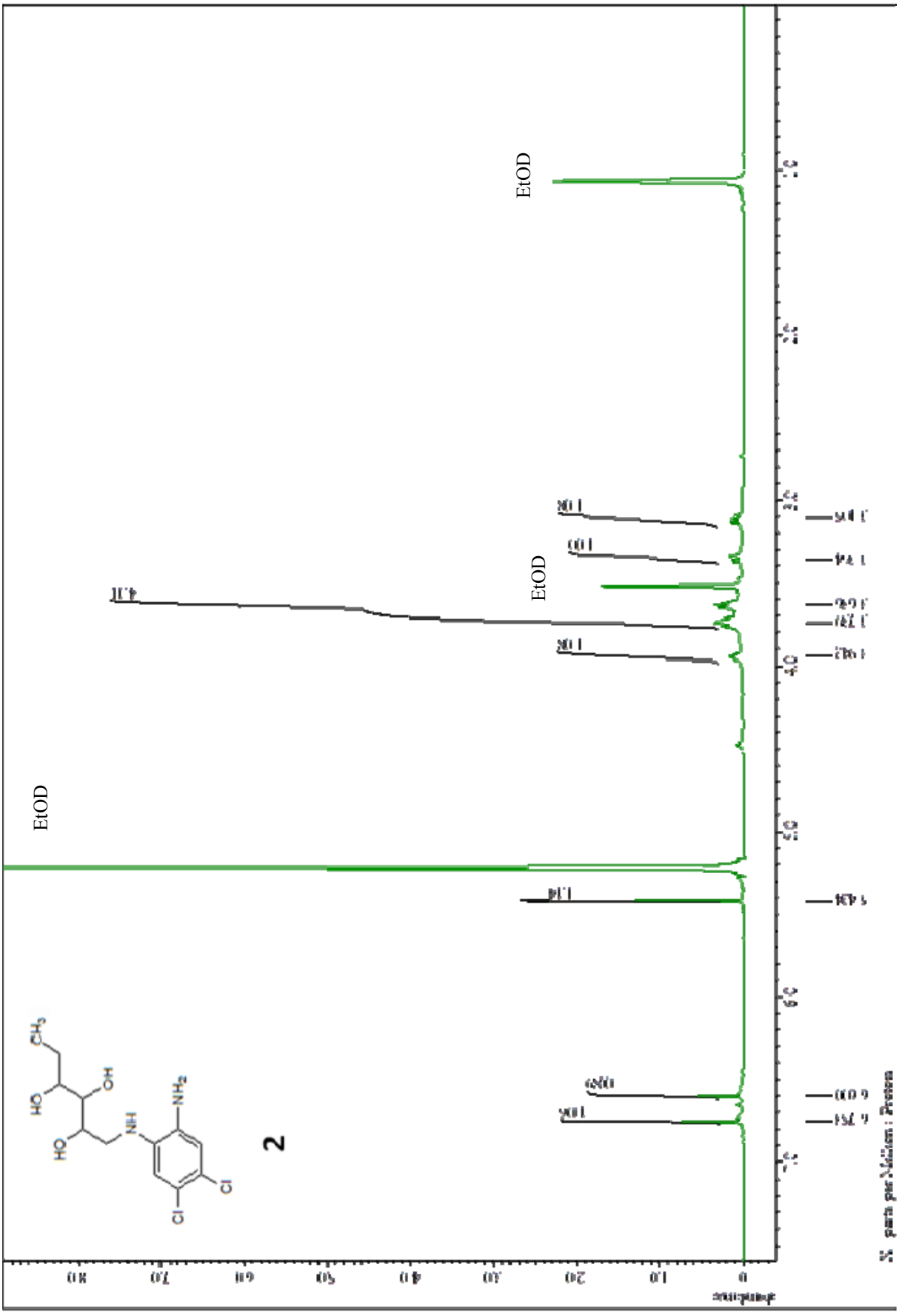








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EtOD
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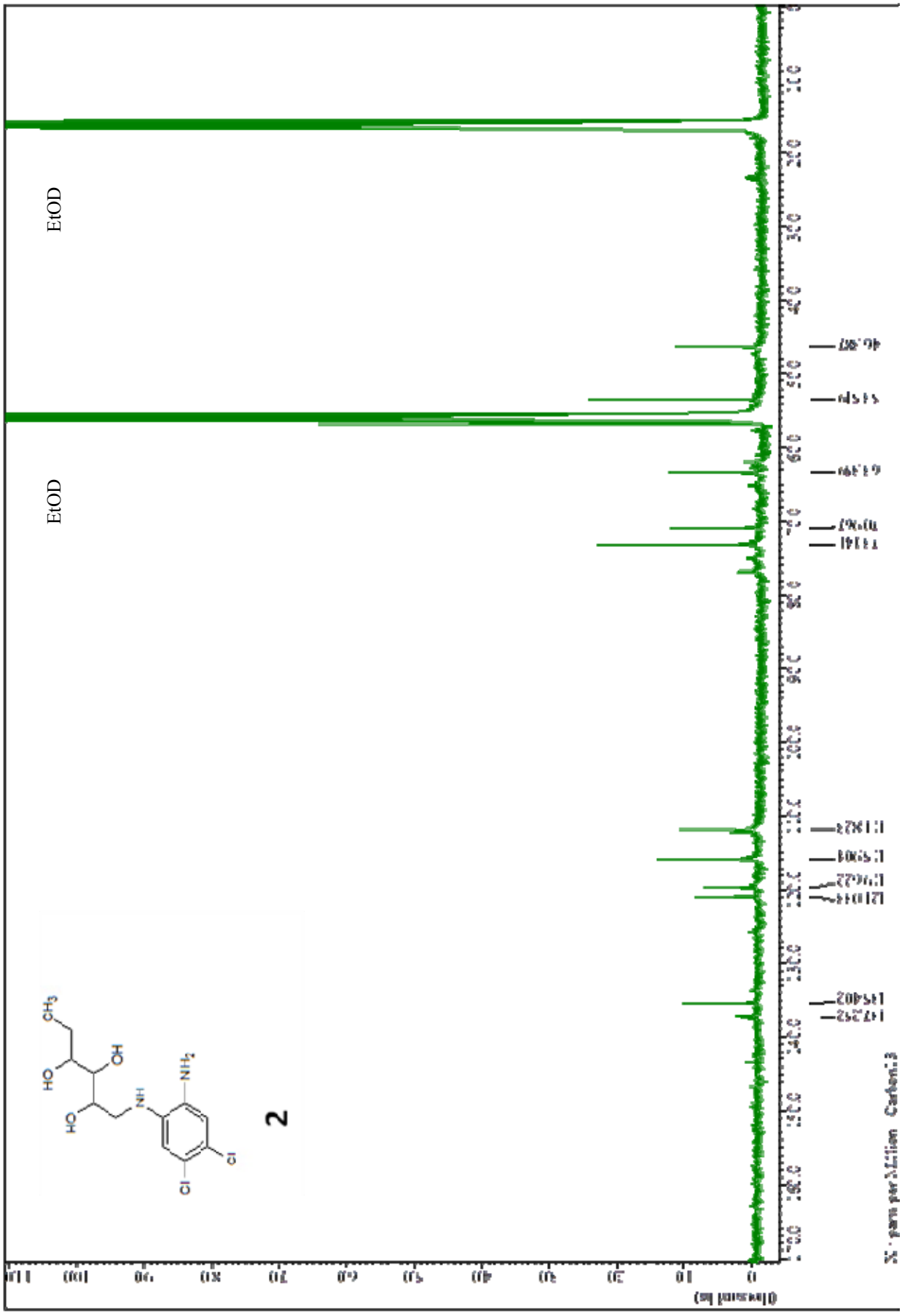
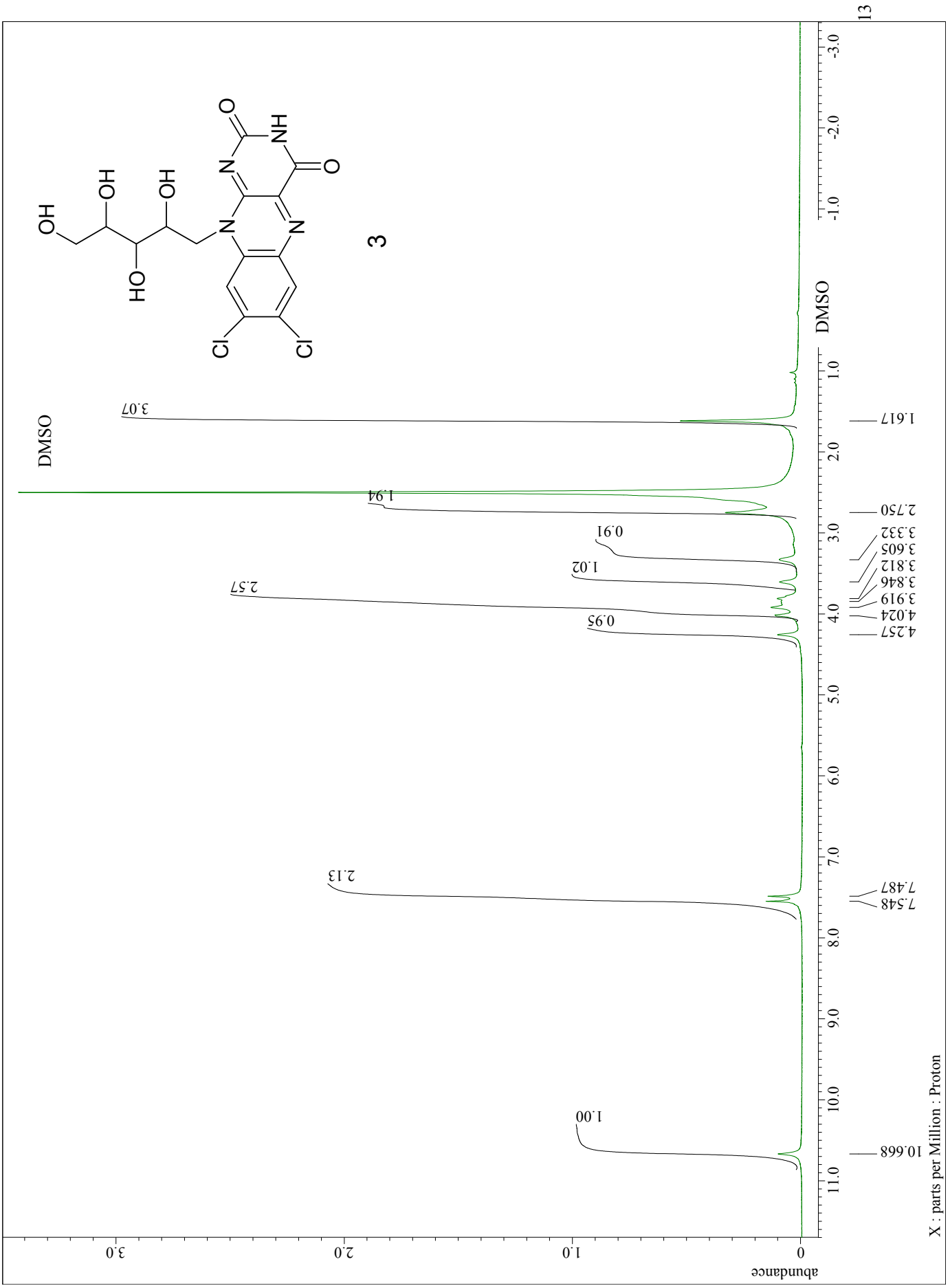
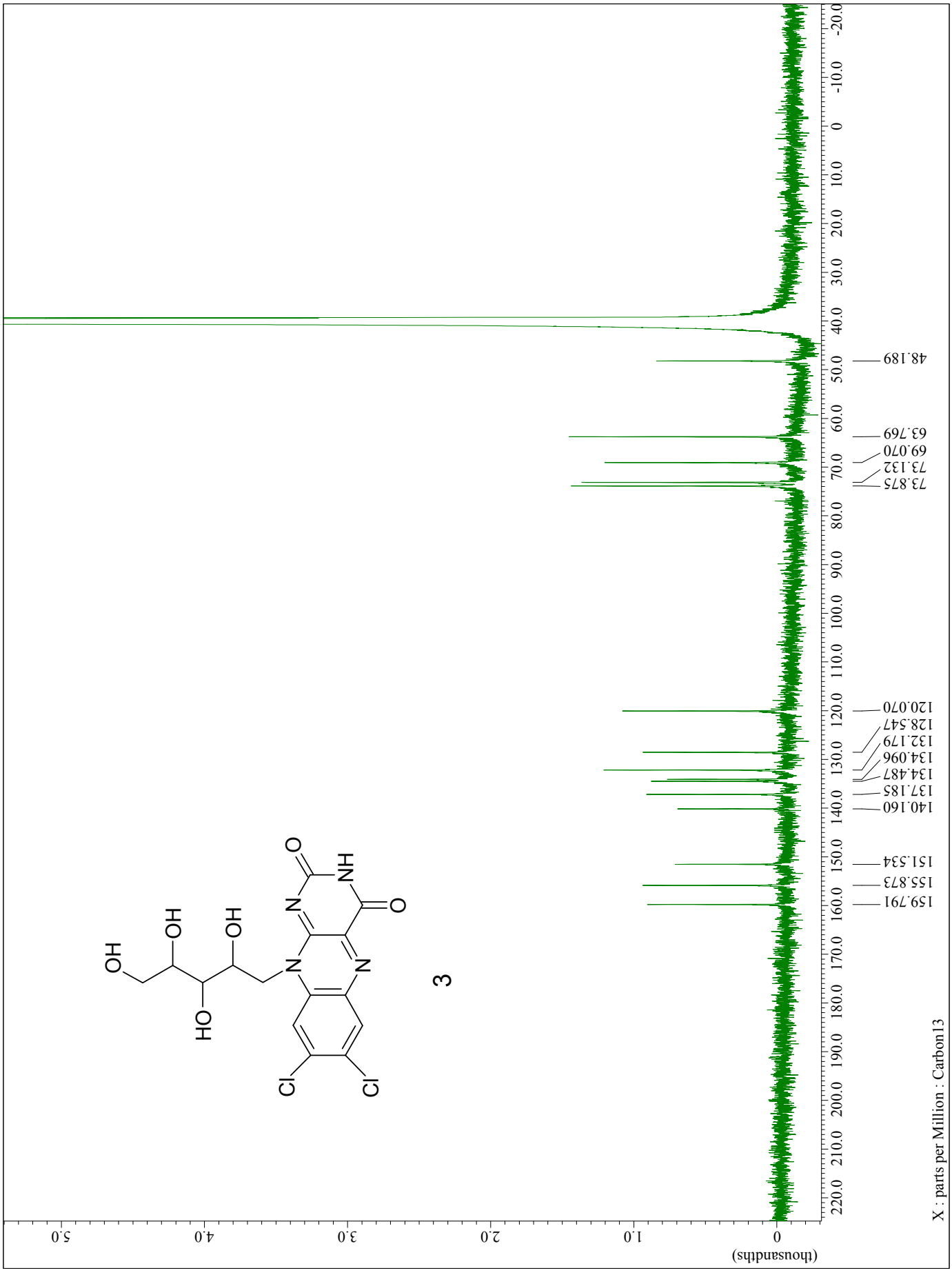
2



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63.769
48.189
3
OH
OH
OH
HO
N
N
N
N
O
NH
O
Cl
Cl