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Supporting Information

Structure-Based Design and Synthesis of New Estrane-Pyridine Derivatives as Cytochrome P450 (CYP) 1B1 Inhibitors

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1. Docking methodology

Docking studies were performed using the 3D-structure of CYP1B1 (PDB ID: 3PM0) and GOLD-5.4 software.[1] The chemical structure of estrone (E1), 17β-estradiol (E2) and the co-crystallized inhibitor α-naphthoflavone (ANF) were retrieved from the ZINC database,[2] whereas the estrane-pyridine derivatives were built from the systematic modifications of E1 and E2. All ligands were energy-minimized by the semi-empirical PM6 method using Gaussian 09 software.[3] Docking simulations were carried out within a 10 Å radius of the co-crystallized molecule using the following parameters: 100 GA runs per molecule and 125,000 operations. ChemPLP fitness score was chosen as scoring function whereas GOLD score was selected as restore function within the goldscore_p450_csd parameter file. The dockings were ranked according to the value of the GOLD score and ChemPLP fitness score function; only the best ranked solution for each ligand was included in further analysis.

2. Experimental procedures for the synthesis, characterization, and NMR spectra of compounds 1, 2a-b, 3a-b, 4a-b, 6a-b, 7a-b, 9, 10a-b, 11a-b, and 12a-b

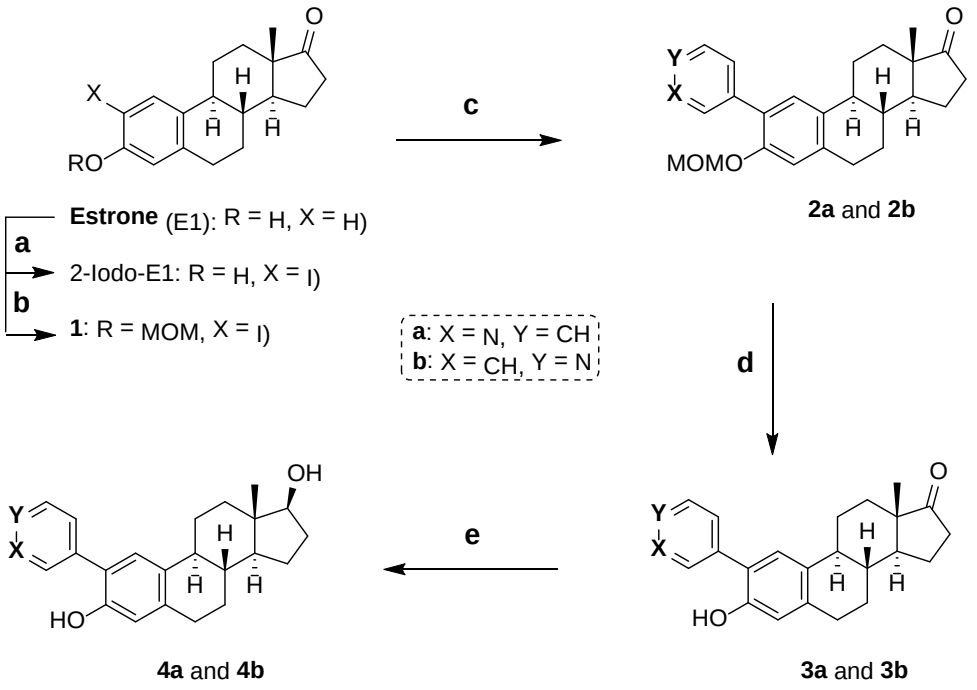
General

Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Dry dichloromethane (DCM) and dimethylformamide (DMF) were purchased from Sigma-Aldrich while acetonitrile (ACN), ethyl acetate (EtOAc), hexanes and methanol (MeOH) were obtained from Fisher Scientific (Montréal, Qc, Canada) and used as received. Reactions using microwave irradiations were performed with a Biotage Initiator (Charlotte, NC, USA). Thin-layer chromatography (TLC) and flash-column chromatography were performed on 0.20 mm silica gel 60 F254 plates (E. Merck; Darmstadt, Germany) and with 230–400 mesh ASTM silica gel 60 (Silicycle, Québec, Qc, Canada), respectively. The purity of final compounds to be tested was determined with a Shimadzu HPLC apparatus (Kyoto, Japan) using a Shimadzu SPD-M20 photodiode array detector, an Alltima HP C18 column (250 mm x 4.6 mm, 5 μm), and a solvent gradient of MeOH:water to MeOH (100%). The wavelength of the UV detector was selected between 190 and 205 nm. For some compounds, a preparative HPLC purification was performed on the same Shimadzu apparatus with a Phenomenex C18 reversed-phase column (250 mm x 21.2 mm, 4 μm) and a solvent gradient of MeOH (70%):water (30%) to MeOH (100%) over a 60 min run. Infrared (IR) spectra were recorded on a MB 3000 ABB FTIR spectrometer (Québec, QC, Canada), and only the significant bands are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^{1}H and 100.6 MHz for ^{13}C on a Bruker Avance 400 digital spectrometer (Billerica, MA, USA). The chemical shifts (δ) were expressed in ppm and referenced to chloroform (7.26 and 77.0 ppm), dimethylsulfoxide (2.49 and 39.5 ppm) or methanol (3.31 ppm and 49.0 ppm) for ^{1}H and ^{13}C NMR, respectively. Low-resolution mass spectra (LRMS) were recorded on a Shimadzu apparatus (Kyoto, Japan) equipped with an atmospheric pressure chemical ionization (APCI) source. High-resolution mass spectra (HRMS) were provided by Pierre Audet at the Chemistry Department of Université Laval (Québec, Qc, Canada).

The chemical reactions described below were generally complete, but yields decreased due to the drying conditions of the organic phase and conditions for chromatography. First, it should be emphasized that the use of sodium sulfate instead of magnesium sulfate is precognized to dry the organic phase after the extraction because magnesium can form complexes with pyridine, leading to a loss of product during the filtration. Second, it is difficult to purify compounds bearing a pyridine on a silica gel stationary normal phase. So, the use of triethylamine (TEA) is necessary to purify this kind of compound and the silica gel

must be packaged with this organic base to avoid the protonation of the aromatic nitrogen of the pyridine nucleus leading to the formation of a salt. The optimal eluent system to purify these compounds is not the same, depending on the structure of the molecules. Indeed, we noticed a trend between the eluent system used and the position of the pyridine moiety on the steroid backbone. Concerning the compounds with a pyridine at position 2 or 4, the use of an elution system of DCM, MeOH, and TEA is preconized, because these compounds are slightly soluble in EtOAc. In fact, the hydroxy function at position 2 can form intermolecular hydrogen bonds with pyridine cores. It is better to begin the column chromatography with just DCM and TEA, because MeOH hinders separation. Nevertheless, this elution system rarely allows the purification of all the material; a part being indeed mixed with small impurities, thus leading to a loss of yield. An elution system of hexanes, EtOAc and TEA is recommended for the compounds with the pyridine in C3. Indeed, the absence of the 3-OH facilitates the purification of these compounds. Note that an addition of MeOH is neccessary to elute the totality of these products, because an important part remains hung in the column.

Copies of ^{1}H NMR and ^{13} NMR spectra of compounds **1**, **2a-b**, **3a-b**, **4a-b**, **6a-b**, **7a-b**, **9**, **10a-b**, **11a-b**, and **12a-b** are reported at the end of this section.



*Synthesis of 2-iodo-3-methoxymethyloxy-estra-1,3,5(10)-trien-17-one (**1**)*

To a solution of 2-iodo-estrone[4] (100 mg, 0.25 mmol) and Cs_2CO_3 (6 eq.) in ACN (15 mL) was added chloromethyl methyl ether (4 eq.). The mixture was then stirred and heated to reflux for 2 h. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1) as eluent to give compound **1** as a white solid (82 mg, 74%). IR (KBr) *v*: 1736 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.90 (s, 3H, CH_3-18), 1.35-2.38 (m,

residual CH and CH_2), 2.50 (dd, J_1 = 8.4 Hz and J_2 = 18.9 Hz, 1H, CH-16β), 2.86 (m, 2H, CH_2-6), 3.51 (s, 3H, OCH_3), 5.20 (s, 2H, OCH_2O), 6.80 (s, 1H, CH-4), 7.65 (s, 1H, CH-1); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.5, 25.8, 26.3, 29.4, 31.4, 35.8, 38.0, 43.7, 47.9, 50.3, 56.4, 83.9, 94.9, 115.1, 135.7, 136.3, 138.2, 153.9, 220.6; LRMS for $C_{20}H_{26}IO_3$ $[M + H]^+$: calc 441.1, found 441.0.

*Synthesis of 2-(3-pyridinyl)-3-methoxymethyloxy-estra-1,3,5(10)-trien-17-one (**2a**)*

To a solution of compound **1** (80 mg, 0.18 mmol) in DMF (1.5 mL) were added 3-pyridine boronic acid (5 eq.), potassium phosphate tribasic (K_3PO_4) (5 eq.) and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (Pd(dppf)Cl_2) (0.1 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 2 h. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM/MeOH (97:3) as eluent to give compound **2a** as a yellow-white amorphous solid (49 mg, 69%). IR (KBr) *v*: 1736 (C=O); ^1H NMR ($CDCl_3$) δ: 0.92 (s, 3H, CH_3-18), 1.42-2.45 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.5 Hz and J_2 = 18.8 Hz, 1H, CH-16β), 2.96 (m, 2H, CH_2-6), 3.39 (s, 3H, OCH_3), 5.12 (s, 2H, OCH_2O), 6.99 (s, 1H, CH-4), 7.24 (s, 1H, CH-1), 7.32 (dd, J_1 = 4.9 Hz and J_2 = 7.8 Hz, 1H, CH of Py), 7.81 (td, J_1 = 1.9 Hz and J_2 = 7.9 Hz, 1H, CH of Py), 8.54 (dd, J_1 = 1.4 Hz and J_2 = 4.8 Hz, 1H, CH of Py), 8.75 (d, J = 1.7 Hz, 1H, CH of Py); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 25.9, 26.5, 29.6, 31.5, 35.8, 38.2, 43.9, 48.0, 50.4, 56.2, 94.9, 115.5, 122.9, 125.6, 127.8, 132.2, 133.8, 136.7, 138.2, 147.8, 150.3, 152.2, 220.4 (very weak); LRMS for $C_{25}H_{30}NO_3$ $[M + H]^+$: calc 392.2, found 392.2.

*Synthesis of 2-(3-pyridinyl)-3-hydroxy-estra-1,3,5(10)-trien-17-one (**3a**)*

Compound **2a** (65 mg, 0.17 mmol) was dissolved in 10 mL of a solution of 10 % aqueous hydrochloric acid in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM (100%), DCM/MeOH (99:1) and DCM/MeOH (95:5) as eluent to give compound **3a** as a light brown solid (29 mg, 50%). IR (KBr) *v*: 3433 (OH), 1728 (C=O); ^1H NMR (DMSO-d_6) δ: 0.82 (s, 3H, CH_3-18), 1.20-2.50 (m, residual CH and CH_2), 2.80 (m, 2H, CH_2-6), 6.66 (s, 1H, CH-4), 7.15 (s, 1H, CH-1), 7.38 (dd, J_1 = 4.8 Hz and J_2 = 7.8 Hz, 1H, CH of Py), 7.89 (td, J_1 = 1.9 Hz and J_2 = 7.9 Hz, 1H, CH of Py), 8.44 (dd, J_1 = 1.5 Hz and J_2 = 4.8 Hz, 1H, CH of Py), 8.69 (d, J = 1.9 Hz, 1H, CH of Py), 9.43 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ: 14.0, 21.6, 25.9, 26.6, 29.2, 31.8, 35.9, 38.3, 43.8, 47.8, 50.1, 116.3, 122.3, 123.5, 127.5, 131.4, 135.0, 136.6, 137.9, 147.6, 150.0, 152.7, 220.2; HRMS for $C_{23}H_{26}NO_2$ $[M + H]^+$: calc 348.1958, found 348.1952; HPLC purity: 94.2%.

*Synthesis of 2-(3-pyridinyl)-estra-1,3,5(10)-trien-3,17β-diol (**4a**)*

To a solution of compound **3a** (16 mg, 0.05 mmol) in MeOH/DCM 9:1 (5 mL) was added under argon atmosphere and at 0 °C sodium borohydride ($NaBH_4$) (12 eq.). The mixture was then stirred at 0 °C under argon for 2.5 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with DCM. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM/MeOH (99:1) to DCM/MeOH (95:5) as eluent to give compound **4a** as a light yellow solid (10 mg, 62%). IR (KBr) *v*: 3425 (OH); ^1H NMR ($CD_3OD/CDCl_3$, 1:3) δ: 0.73 (s, 3H, CH_3-18), 1.10-2.31 (m, residual CH and CH_2), 2.80 (m, 2H, CH_2-6), 3.64 (t, J = 8.6 Hz, 1H, CH-17α), 6.63 (s, 1H, CH-4), 7.14 (s, 1H,

CH-1), 7.34 (dd, J_1 = 5.0 Hz and J_2 = 7.7 Hz, 1H, CH of Py), 7.94 (td, J_1 = 1.8 Hz and J_2 = 8.0 Hz, 1H, CH of Py), 8.36 (d, J = 3.5 Hz, 1H, CH of Py), 8.68 (s, 1H, CH of Py); ^{13}C NMR ($CD_3OD/CDCl_3$, 1:1) δ: 11.5, 23.6, 27.0, 27.8, 30.0, 30.2, 37.3, 39.6, 43.8, 44.5, 50.6, 81.9, 116.6, 122.5, 124.1, 127.9, 132.9, 136.4, 138.2, 139.2, 146.8, 149.7, 152.6; HRMS for $C_{23}H_{28}NO_2$ $[M + H]^+$: calc 350.2115, found 350.2103; HPLC purity: 99.7%.

*Synthesis of 2-(4-pyridinyl-3-methoxymethyloxy-estra-1,3,5(10)-trien-17-one (**2b**)*

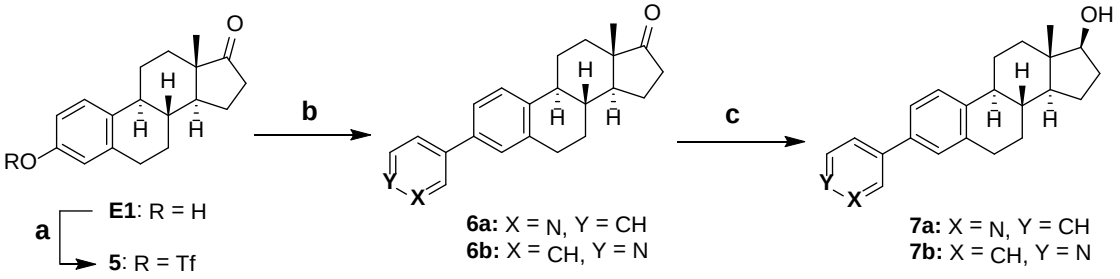
To a solution of compound **1** (180 mg, 0.41 mmol) in DMF (2.5 mL) were added 4-pyridine boronic acid (5 eq.), K_3PO_4 (5 eq.) and $Pd(dppf)Cl_2$ (0.1 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 3 h. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM + 1% TEA as eluent to give compound **2b** as a yellow-white solid (127 mg, 79%). IR (KBr) *v*: 1736 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, 3H, CH_3-18), 1.41-2.46 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.5 Hz and J_2 = 18.7 Hz, 1H, CH-16β), 2.96 (m, 2H, CH_2-6), 3.40 (s, 3H, OCH_3), 5.13 (s, 2H, OCH_2O), 6.98 (s, 1H, CH-4), 7.26 (s, 1H, CH-1), 7.44 (d, J = 6.0 Hz, 2H, 2 x CH of Py), 8.60 (d, J = 5.9 Hz, 2H, 2 x CH of Py); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 25.9, 26.4, 29.6, 31.5, 35.8, 38.2, 43.9, 47.9, 50.4, 56.2, 94.9, 115.7, 124.3 (2C), 126.3, 127.5, 133.9, 138.9, 146.6, 149.4 (2C), 152.2, 220.6 (weak); LRMS for $C_{25}H_{30}NO_3$ $[M + H]^+$: calc 392.2, found 392.5.

*Synthesis of 2-(4-pyridinyl)-3-hydroxy-estra-1,3,5(10)-trien-17-one (**3b**)*

Compound **2b** (16 mg, 0.04 mmol) was dissolved in 5 mL of a solution of 10 % aqueous hydrochloric acid in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3) + 1% TEA to EtOAc + 1% TEA as eluent to give compound **3b** as a light yellow solid (8 mg, 56%). IR (KBr) *v*: 1736 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.93 (s, 3H, CH_3-18), 1.25-2.45 (m, residual CH and CH_2), 2.52 (dd, J_1 = 8.5 Hz and J_2 = 18.8 Hz, 1H, CH-16β), 2.92 (m, 2H, CH_2-6), 6.71 (s, 1H, CH-4), 7.22 (s, 1H, CH-1), 7.47 (d, J = 6.0 Hz, 2H, 2 x CH of Py), 8.65 (d, J = 6.0 Hz, 2H, 2 x CH of Py); ^{13}C NMR ($CD_3OD/CDCl_3$, 1:1) δ: 14.2, 22.1, 26.5, 27.0, 29.8, 32.1, 36.4, 39.0, 44.5, 48.8, 51.0, 116.8, 123.2, 125.1 (2C), 127.7, 132.2, 139.8, 148.5, 148.9 (2C), 153.1, 223.4; HRMS for $C_{23}H_{26}NO_2$ $[M + H]^+$: calc 348.1958, found 348.1963; HPLC purity: 95.5%.

*Synthesis of 2-(4-pyridinyl)-estra-1,3,5(10)-trien-3,17β-diol (**4b**)*

To a solution of compound **3b** (45 mg, 0.13 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (12 eq.). The mixture was then stirred at 0 °C under argon for 2.5 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with DCM. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM + 1% TEA as eluent to give compound **4b** as a yellow-white solid (29 mg, 64%). IR (KBr) *v*: 3155 (OH); ^{1}H NMR (CD_3OD) δ: 0.79 (s, 3H, CH_3-18), 1.15-2.40 (m, residual CH and CH_2), 2.82 (m, 2H, CH_2-6), 3.66 (t, J = 8.5 Hz, 1H, CH-17α), 6.63 (s, 1H, CH-4), 7.26 (s, 1H, CH-1), 7.66 (d, J = 6.3 Hz, 2H, 2 x CH of Py), 8.47 (d, J = 6.2 Hz, 2H, 2 x CH of Py); ^{13}C NMR (DMSO-d_6) δ: 11.3, 22.8, 26.0, 26.8, 28.9, 29.9, 36.5, 38.6, 42.8, 43.4, 49.5, 80.0, 116.0, 122.0, 123.7 (2C), 126.8, 131.5, 138.5, 146.3, 149.2 (2C), 152.4; HRMS for $C_{23}H_{28}NO_2$ $[M + H]^+$: calc 350.2115, found 350.2100; HPLC purity: 98.5%.



*Synthesis of 3-(3-pyridinyl)-estra-1,3,5(10)-trien-17-one (**6a**)*

To a solution compound **5**[5] (150 mg, 0.37 mmol) in DMF (2.5 mL) were added 3-pyridine boronic acid (5 eq.), K_3PO_4 (5 eq.) and Pd(dppf)Cl_2 (0.1 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 3 h. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM + 1% TEA and then with hexanes/EtOAc (8:2) + 1% TEA to hexanes/EtOAc (7:3) + 1% TEA as eluent to give compound **6a** as a white solid (66 mg, 54%). IR (KBr) *v*: 1728 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.93 (s, 3H, CH_3-18), 1.45-2.50 (m, residual CH and CH_2), 2.52 (dd, J_1 = 9.0 Hz and J_2 = 19.1 Hz, 1H, CH-16β), 3.02 (m, 2H, CH_2-6), 7.33-7.43 (m, 4H, CH-1, CH-2, CH-4 and CH of Py), 7.86 (td, J_1 = 2.0 Hz and J_2 = 8.0 Hz, 1H, CH of Py), 8.57 (dd, J_1 = 1.4 Hz and J_2 = 4.7 Hz, 1H, CH of Py), 8.83 (d, J = 2.0 Hz, 1H, CH of Py); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 25.7, 26.4, 29.5, 31.6, 35.8, 38.1, 44.3, 48.0, 50.5, 123.5, 124.5, 126.2, 127.7, 134.1, 135.4, 136.4, 137.3, 139.9, 148.2, 148.3, 220.7 (very weak); HRMS for $C_{23}H_{26}NO$ $[M + H]^+$: calc 332.2009, found 332.2000; HPLC purity: 99.4%.

*Synthesis of 3-(3-pyridinyl)-estra-1,3,5(10)-trien-3,17β-diol (**7a**)*

To a solution of compound **6a** (54 mg, 0.16 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (12 eq.). The mixture was then stirred at 0 °C under argon for 2.5 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with DCM. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) + 1% TEA to hexanes/EtOAc (7:3) + 3% MeOH + 1% TEA as eluent to give compound **7a** as a yellow-white solid (50 mg, 94%). IR (KBr) *v*: 3202 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.80 (s, 3H, CH_3-18), 1.19-2.42 (m, residual CH and CH_2), 2.96 (m, 2H, CH_2-6), 3.76 (t, J = 8.5 Hz, 1H, CH-17α), 7.31-7.43 (m, 4H, CH-1, CH-2, CH-4 and CH of Py), 7.86 (dd, J_1 = 1.8 Hz and J_2 = 7.9 Hz, 1H, CH of Py), 8.56 (d, J = 3.6 Hz, 1H, CH of Py), 8.83 (s, 1H, CH of Py); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.1, 27.1, 29.6, 30.6, 36.7, 38.6, 43.2, 44.3, 50.1, 81.8, 123.5, 124.4, 126.2, 127.7, 134.2, 135.1, 136.6, 137.6, 140.5, 148.1, 148.2; HRMS for $C_{23}H_{28}NO$ $[M + H]^+$: calc 334.2165, found 334.2162; HPLC purity: 99.5%.

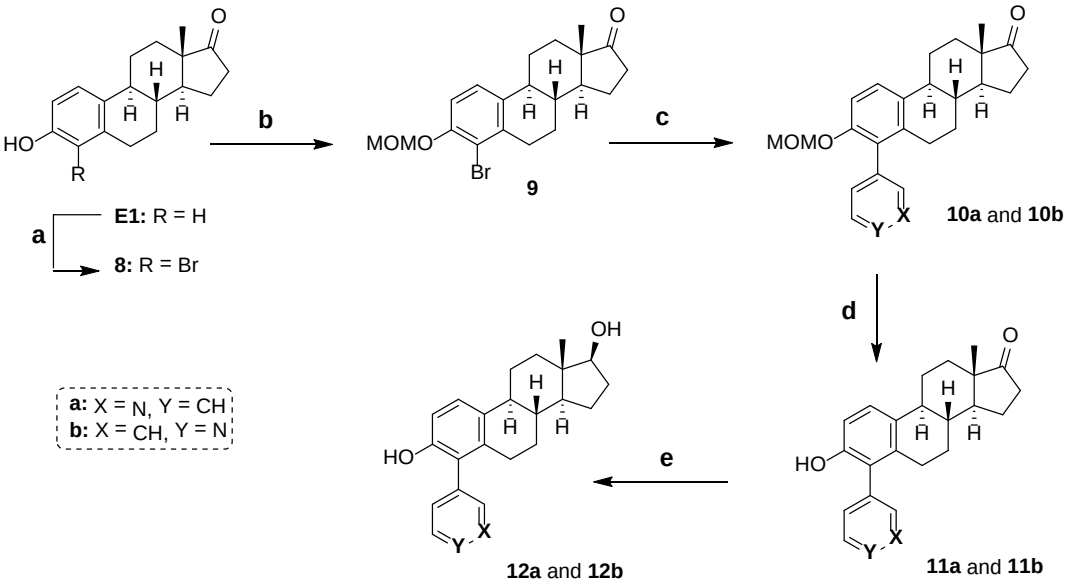
*Synthesis of 3-(4-pyridinyl)-estra-1,3,5(10)-trien-17-one (**6b**)*

To a solution of compound **5**[5] (150 mg, 0.37 mmol) in DMF (2.5 mL) were added 4-pyridine boronic acid (5 eq.), K_3PO_4 (5 eq.) and Pd(dppf)Cl_2 (0.1 eq.). The mixture was then stirred and heated at 120 °C for 4 h. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was

purified by flash chromatography with hexanes/EtOAc (8:2) + 1% TEA to hexanes/EtOAc (7:3) + 1% TEA as eluent to give compound **6b** as a yellow-white solid (73 mg, 60%). IR (KBr) *v*: 1728 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.93 (s, 3H, CH_3-18), 1.45-2.50 (m, residual CH and CH_2), 2.53 (dd, J_1 = 8.5 Hz and J_2 = 18.6 Hz, 1H, CH-16β), 3.02 (m, 2H, CH_2-6), 7.39 (s, 1H, CH-4), 7.43 (m, 2H, CH-1 and CH-2), 7.49 (d, J = 6.2 Hz, 2H, 2 x CH of Py), 8.63 (d, J = 6.2 Hz, 2H, 2 x CH of Py); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 25.7, 26.4, 29.5, 31.5, 35.8, 38.0, 44.4, 47.9, 50.5, 121.4 (2C), 124.3, 126.2, 127.5, 135.6, 137.4, 141.0, 148.1, 150.2 (2C), 220.7; HRMS for $C_{23}H_{26}NO$ $[M + H]^+$: calc 332.2009, found 332.1997; HPLC purity: 97.2%.

*Synthesis of 3-(4-pyridinyl)-estra-1,3,5(10)-trien-3,17β-diol (**7b**)*

To a solution of compound **6b** (55 mg, 0.17 mmol) in MeOH/DCM (9:1) (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (12 eq.). The mixture was then stirred at 0 °C under argon for 2.5 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with DCM. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) + 1% TEA to hexanes/EtOAc (7:3) + 1% TEA as eluent to give compound **7b** as a white solid (50 mg, 90%). IR (KBr) *v*: 3256 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.80 (s, 3H, CH_3-18), 1.20-2.43 (m, residual CH and CH_2), 2.96 (m, 2H, CH_2-6), 3.76 (dd, J_1 = 8.4 Hz and J_2 = 13.8 Hz, 1H, CH-17α), 7.37 (s, 1H, CH-4), 7.43 (s, 2H, CH-1 and CH-2), 7.49 (d, J = 6.2 Hz, 2H, 2 x CH of Py), 8.63 (d, J = 6.1 Hz, 2H, 2 x CH of Py); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.1, 27.1, 29.6, 30.5, 36.7, 38.5, 43.2, 44.4, 50.1, 81.7, 121.4 (2C), 124.1, 126.2, 127.5, 135.3, 137.6, 141.7, 148.2, 150.1 (2C); HRMS for $C_{23}H_{28}NO$ $[M + H]^+$: calc 334.2165, found 334.2155; HPLC purity: 99.0%.



*Synthesis of 3-methoxymethyloxy-4-bromo-estra-1,3,5(10)-trien-17-one (**9**)*

To a solution of compound **8**[6] (100 mg, 0.29 mmol) and Cs_2CO_3 (6 eq.) in ACN (15 mL) was added chloromethyl methyl ether (4 eq.). The mixture was then stirred and heated to reflux for 3 h. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1) as eluent to give compound **9** as a white solid (91 mg, 80%). IR (KBr) *v*: 1736 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.90 (s, 3H, CH_3-18), 1.38-2.42 (m,

residual CH and CH_2), 2.51 (dd, J_1 = 8.4 Hz and J_2 = 18.2 Hz, 1H, CH-16β), 2.75 (m, 1H, CH of CH_2-6), 3.05 (dd, J_1 = 5.9 Hz and J_2 = 18.0 Hz, 1H, CH of CH_2-6), 3.52 (s, 3H, OCH_3), 5.24 (s, 2H, OCH_2O), 6.98 (d, J = 8.6 Hz, 1H, CH-2), 7.21 (d, J = 8.6 Hz, 1H, CH-1); ^{13}C NMR ($CDCl_3$) δ: 13.7, 21.5, 26.0, 26.7, 31.1, 31.4, 35.8, 37.4, 44.2, 47.8, 50.3, 56.3, 95.1, 113.2, 116.1, 124.8, 135.4, 137.5, 151.7, 220.7 (weak); LRMS for $C_{20}H_{26}{}^{79}BrO_3$ $[M + H]^+$: calc 393.1, found 393.0.

*Synthesis of 3-methoxymethyloxy-4-(3-pyridinyl)-estra-1,3,5(10)-trien-17-one (**10a**)*

Compound **9** (350 mg, 0.89 mmol), 3-pyridine boronic acid (2 eq.) and tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) (0.05 eq.) were dissolved under argon atmosphere in toluene (3.5 mL) and ethanol (1.5 mL). An aqueous solution of K_2CO_3 (2M) was then added, and the resulting mixture was stirred under argon and heated at 90 °C overnight. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) + 1% TEA to hexanes/EtOAc (6:4) + 1% TEA as eluent to give compound **10a** as a white solid (250 mg, 72%). IR (KBr) *v*: 1736 (C=O); ^1H NMR ($CDCl_3$) δ: 0.92 (s, 3H, CH_3-18), 1.24-2.55 (m, residual CH and CH_2), 3.28 (s, 3H, OCH_3), 5.01 (s, 2H, OCH_2O), 7.05 (d, J = 8.7 Hz, 1H, CH-2), 7.33 (d, J = 8.3 Hz, 1H, CH-1), 7.36 (m, 1H, CH of Py), 7.55 (m, 1H, CH of Py), 8.47 (d, J = 15.0 Hz, 1H, CH of Py), 8.58 (s, 1H, CH of Py); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.5, 26.1, 26.5, 28.7, 31.6, 35.8, 37.7, 44.3, 47.9, 50.4, 55.9, 94.5, 112.3, 123.2, 126.2, 127.8, 133.6, 133.9, 136.3, 137.4, 148.0, 150.7, 152.6, 220.6 (weak); LRMS for $C_{25}H_{30}NO_3$ $[M + H]^+$: calc 392.2, found 392.2.

*Synthesis of 3-hydroxy-4-(3-pyridinyl-estra-1,3,5(10)-trien-17-one (**11a**)*

Compound **10a** (69 mg, 0.18 mmol) was dissolved in 10 mL of a solution of 10% aqueous hydrochloric acid in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) + 1% TEA to EtOAc + 1% TEA as eluent to give compound **11a** as a yellow-white solid (44 mg, 72%). IR (KBr) *v*: 3456 (OH), 1736 (C=O); ^1H NMR ($CDCl_3$) δ: 0.92 (s, 3H, CH_3-18), 1.24-2.56 (m, residual CH and CH_2), 5.20 (s, 1H, OH), 6.85 (d, J = 8.6 Hz, 1H, CH-2), 7.28 (d, J = 8.6 Hz, 1H, CH-1), 7.42 (m, 1H, CH of Py), 7.63 (m, 1H, CH of Py), 8.51 (d, J = 13.1 Hz, 1H, CH of Py), 8.61 (s, 1H, CH of Py); ^{13}C NMR ($CD_3OD/CDCl_3$, 1:1) δ: 14.2, 22.0, 26.7, 27.1, 29.3, 32.1, 36.4, 38.4, 44.9, 48.8, 51.0, 113.6, 124.4, 125.0, 126.9, 131.9, 135.3, 136.3, 139.5, 147.5, 150.6, 153.1, 223.5; HRMS for $C_{23}H_{26}NO_2$ $[M + H]^+$: calc 348.1958, found 348.1965; HPLC purity: 97.3%.

*Synthesis of 4-(4-pyridinyl-estra-1,3,5(10)-trien-3,17β-diol (**12a**)*

To a solution of compound **11a** (103 mg, 0.3 mmol) in MeOH/DCM (9:1) (20 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (12 eq.). The mixture was then stirred under argon at 0 °C for 2.5 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with DCM. The organic phase was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM + 1% TEA to DCM/MeOH (97:3) + 1% TEA as eluent to give compound **12a** as a white solid (84 mg, 81%). IR (KBr) *v*: 3394 (OH); ^1H NMR ($CDCl_3$) δ: 0.79 (s, 3H, CH_3-18), 1.21-2.52 (m, residual CH and CH_2), 3.73 (t, J = 8.4 Hz, 1H, CH-17α), 6.84 (d, J = 8.6 Hz, 1H, CH-2), 7.28 (d, J = 8.7 Hz, 1H, CH-1), 7.41 (m, 1H, CH of Py), 7.62 (m, 1H, CH of Py), 8.51 (d, J = 10.4 Hz, 1H, CH of Py), 8.61 (s, 1H, CH of Py); ^{13}C NMR

($CD_3OD/CDCl_3$, 1:1) δ: 11.4, 23.5, 27.2, 27.8, 29.5, 30.2, 37.3, 38.9, 43.7, 44.9, 50.6, 81.9, 113.5, 124.4, 124.8, 126.9, 132.7, 135.4, 136.5, 139.6, 147.3, 150.5, 152.8; HRMS for $C_{23}H_{28}NO_2$ $[M + H]^+$: calc 350.2115, found 350.2109; HPLC purity: 99.2%.

*Synthesis of 3-methoxymethyloxy-4-(4-pyridinyl)-estra-1,3,5(10)-trien-17-one (**10b**)*

Compound **9** (350 mg, 0.89 mmol), 4-pyridine boronic acid (3 eq.) and tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) (0.1 eq.) were dissolved under argon atmosphere in toluene (4.2 mL) and ethanol (1.8 mL). An aqueous solution of K_2CO_3 (2M) was then added and the resulting mixture was stirred under argon and heated at 90 °C overnight. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM + 1% TEA as eluent to give compound **10b** as a light yellow solid (200 mg, 57%). IR (KBr) *v*: 1736 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, 3H, CH_3-18), 1.24-2.53 (m, residual CH and CH_2), 3.28 (s, 3H, OCH_3), 5.01 (s, 2H, OCH_2O), 7.04 (d, J = 8.8 Hz, 1H, CH-2), 7.16 (dd, J_1 = 5.0 Hz and J_2 = 12.0 Hz , 2H, 2 x CH of Py), 7.32 (d, J = 8.7 Hz, 1H, CH-1), 8.65 (t, J = 5.4 Hz, 2H, 2 x CH of Py); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.5, 26.0, 26.4, 28.4, 31.5, 35.8, 37.6, 44.3, 47.9, 50.4, 55.9, 94.5, 112.4, 125.2, 126.3 (2C), 128.8, 133.9, 135.3, 146.6, 149.6 (2C), 151.9, 220.8 (very weak); LRMS for $C_{25}H_{30}NO_3$ $[M + H]^+$: calc 392.2, found 392.2.

*Synthesis of 3-hydroxy-4-(4-pyridinyl)-estra-1,3,5(10)-trien-17-one (**11b**)*

Compound **10b** (60 mg, 0.15 mmol) was dissolved in 10 mL of a solution of 10% aqueous hydrochloric acid in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM + 1% TEA to DCM/MeOH (97:3) + 1% TEA as eluent to give compound **11b** as a yellow-white solid (46 mg, 88%). IR (KBr) *v*: 3425 (OH), 1736 (C=O); ^{1}H NMR ($CD_3OD/CDCl_3$, 1:1) δ: 0.91 (s, 3H, CH_3-18), 1.22-2.54 (m, residual CH and CH_2), 6.76 (d, J = 8.6 Hz, 1H, CH-2), 7.19 (d, J = 8.6 Hz, 1H, CH-1), 7.25 (s, 2H, 2 x CH of Py), 8.51 (s, 2H, 2 x CH of Py); ^{13}C NMR ($CD_3OD/CDCl_3$, 1:1) δ: 14.2, 22.0, 26.7, 27.0, 29.0, 32.1, 36.4, 38.4, 44.9, 48.7, 50.9, 113.7 (2C), 126.2, 126.8, 126.9, 131.9, 135.4, 148.8, 149.2 (2C), 152.3, 223.4; HRMS for $C_{23}H_{26}NO_2$ $[M + H]^+$: calc 348.1958, found 348.1954; HPLC purity: 85.7%.

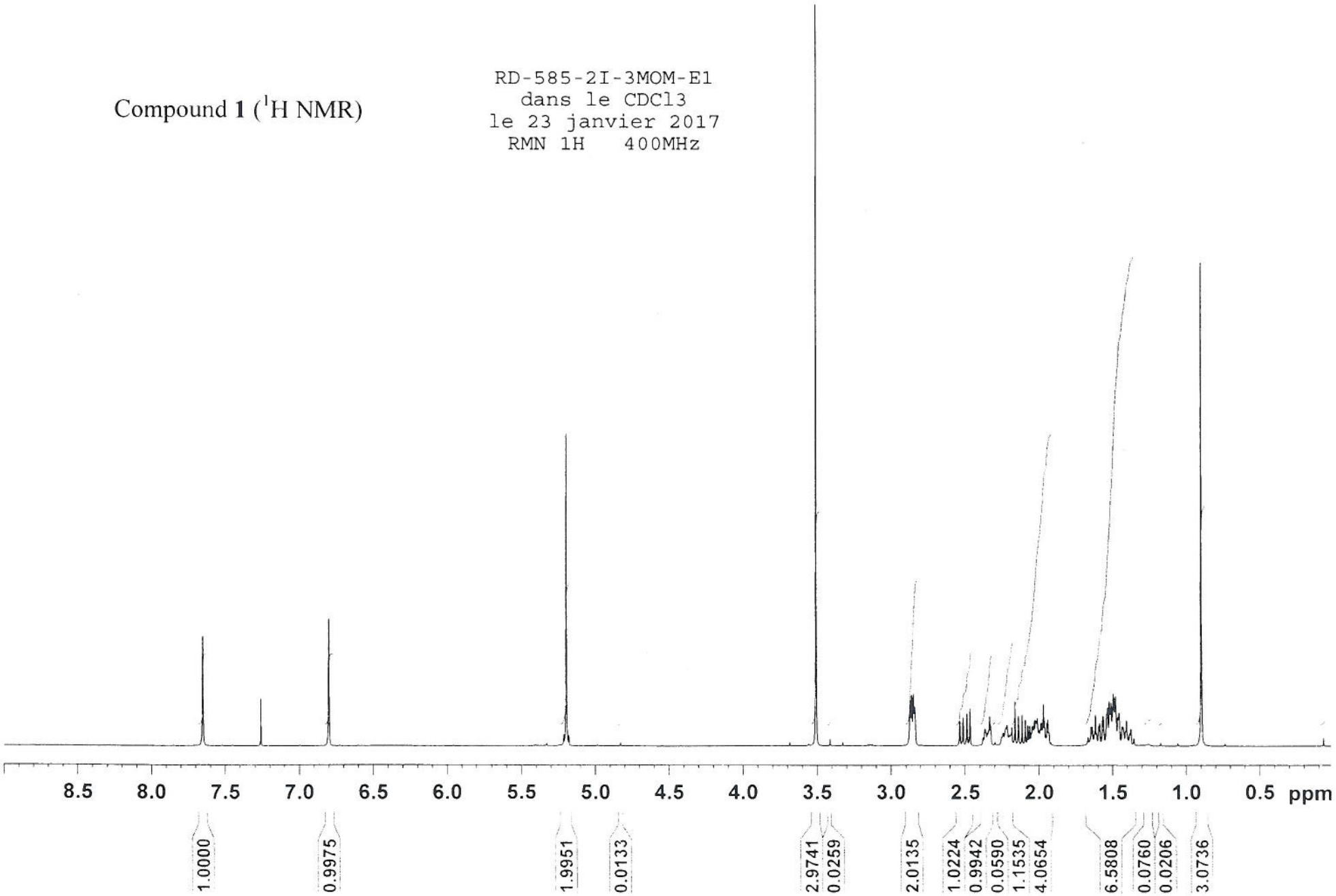
*Synthesis of 4-(4-pyridinyl)-estra-1,3,5(10)-trien-3,17β-diol (**12b**)*

To a solution of compound **11a** (115 mg, 0.33 mmol) in MeOH/DCM (9:1) (20 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (12 eq.). The mixture was then stirred under argon at 0 °C for 2.5 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with DCM. The organic phase was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM + 1% TEA, DCM/MeOH (99:1) + 1% TEA and DCM/MeOH (97:3) + 1% TEA as eluent to give compound **12b** as a white solid (53 mg, 46%). IR (KBr) *v*: 3526 (OH); ^{1}H NMR (CD_3OD) δ: 0.75 (s, 3H, CH_3-18), 1.12-2.49 (m, residual CH and CH_2), 3.64 (t, J = 8.6 Hz, 1H, CH-17α), 6.74 (d, J = 8.5 Hz, 1H, CH-2), 7.19 (d, J = 8.5 Hz, 1H, CH-1), 7.25 (d, J = 4.3 Hz, 2H, 2 x CH of Py), 7.54 (s, 1H, OH), 8.51 (d, J = 5.4 Hz, 2H, 2 x CH of Py); ^{13}C NMR (CD_3OD) δ: 11.4, 23.5, 27.2, 27.8, 29.1, 30.2, 37.3, 38.9, 43.7, 44.9, 50.6, 81.8, 113.5 (2C), 126.2, 126.8, 127.0, 132.7, 135.5, 149.0, 149.2 (2C), 152.1; HRMS for $C_{23}H_{28}NO_2$ $[M + H]^+$: calc 350.2115, found 350.2118; HPLC purity: 94.9%.

Copies of ^{1}H NMR and ^{13}C NMR spectra of compounds

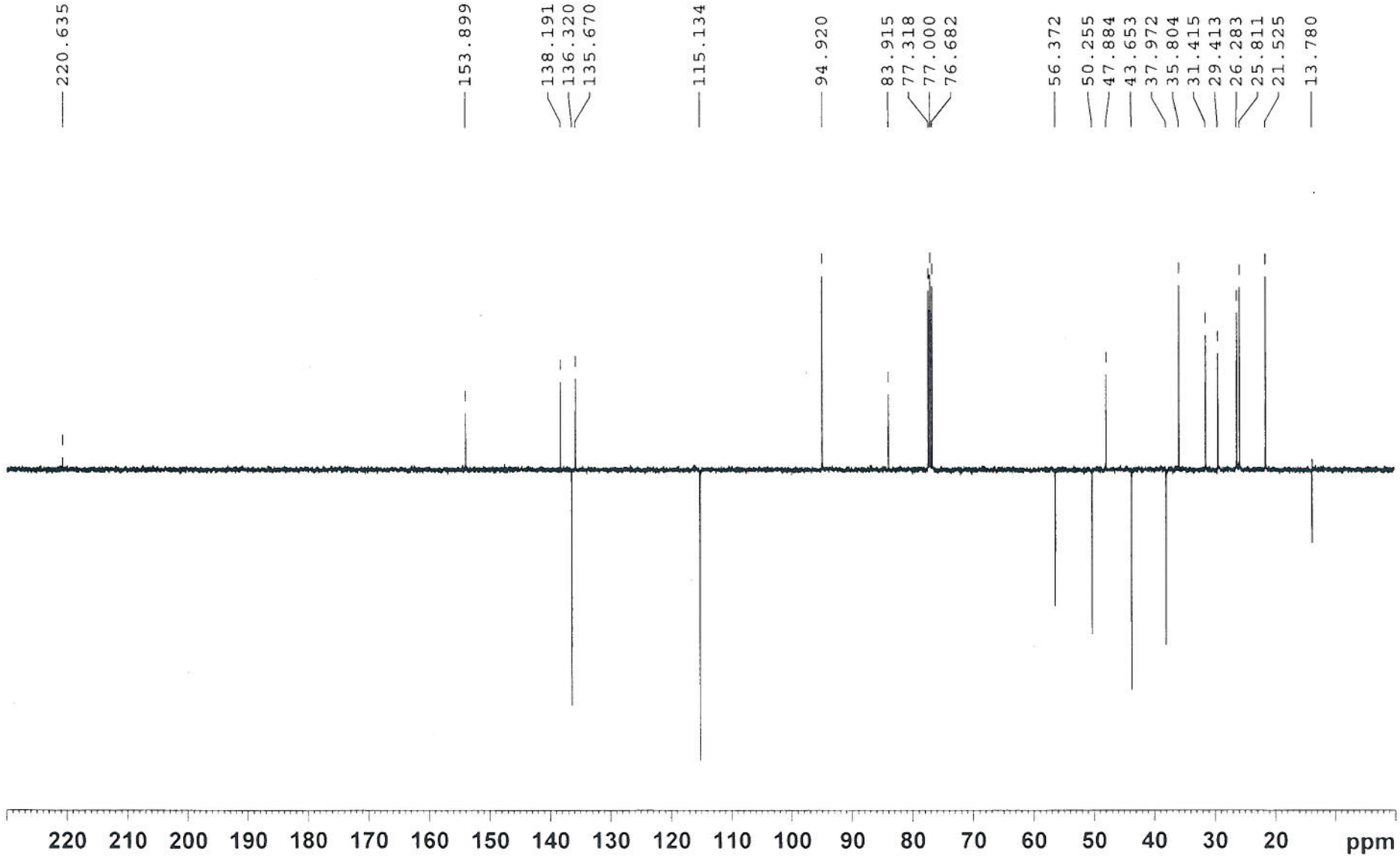
(compounds **1**, **2a-b**, **3a-b**, **4a-b**, **6a-b**, **7a-b**, **9**, **10a-b**, **11a-b**, and **12a-b**)

Compound **1** (^{1}H NMR)

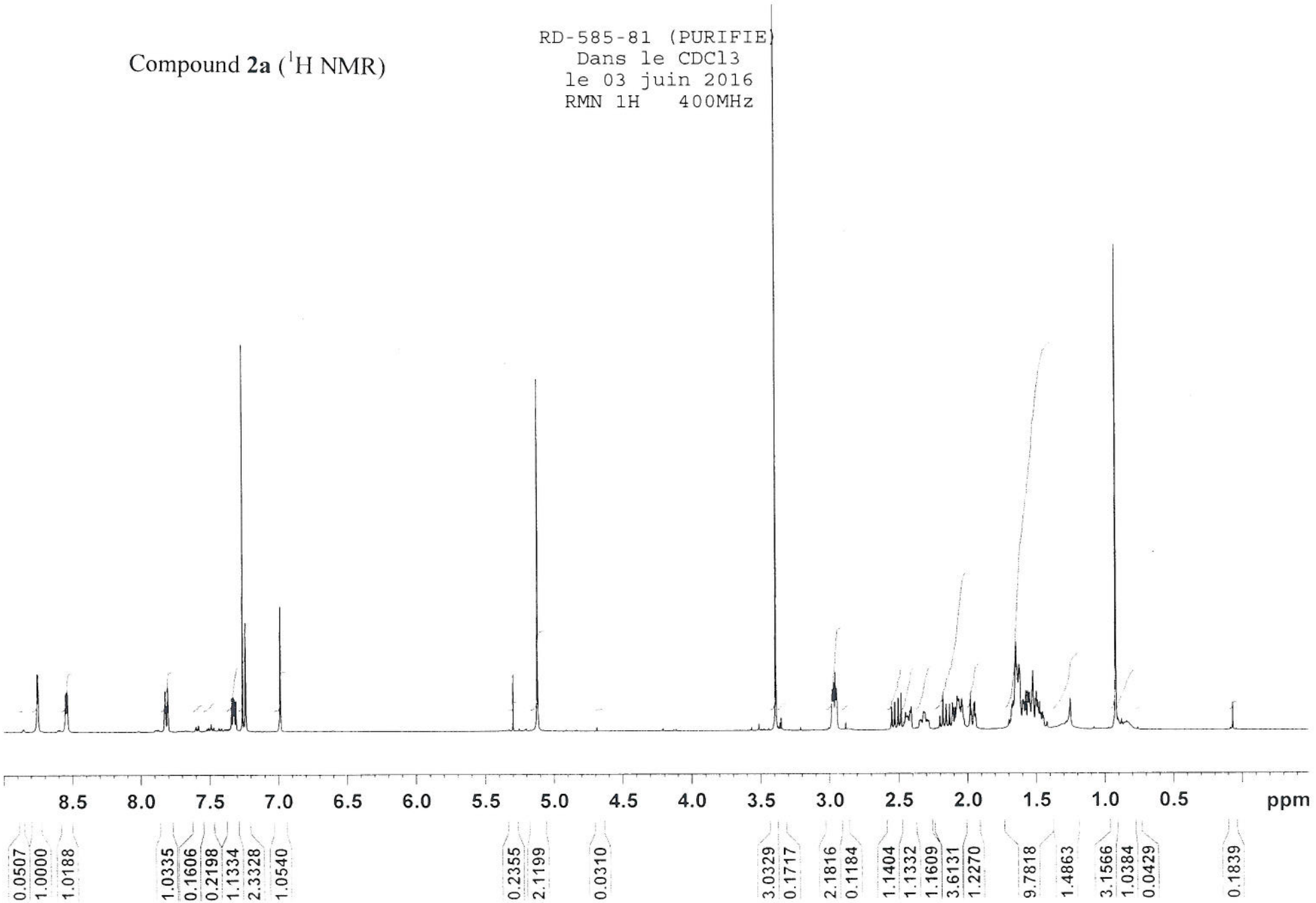


Compound **1** (^{13}C NMR)

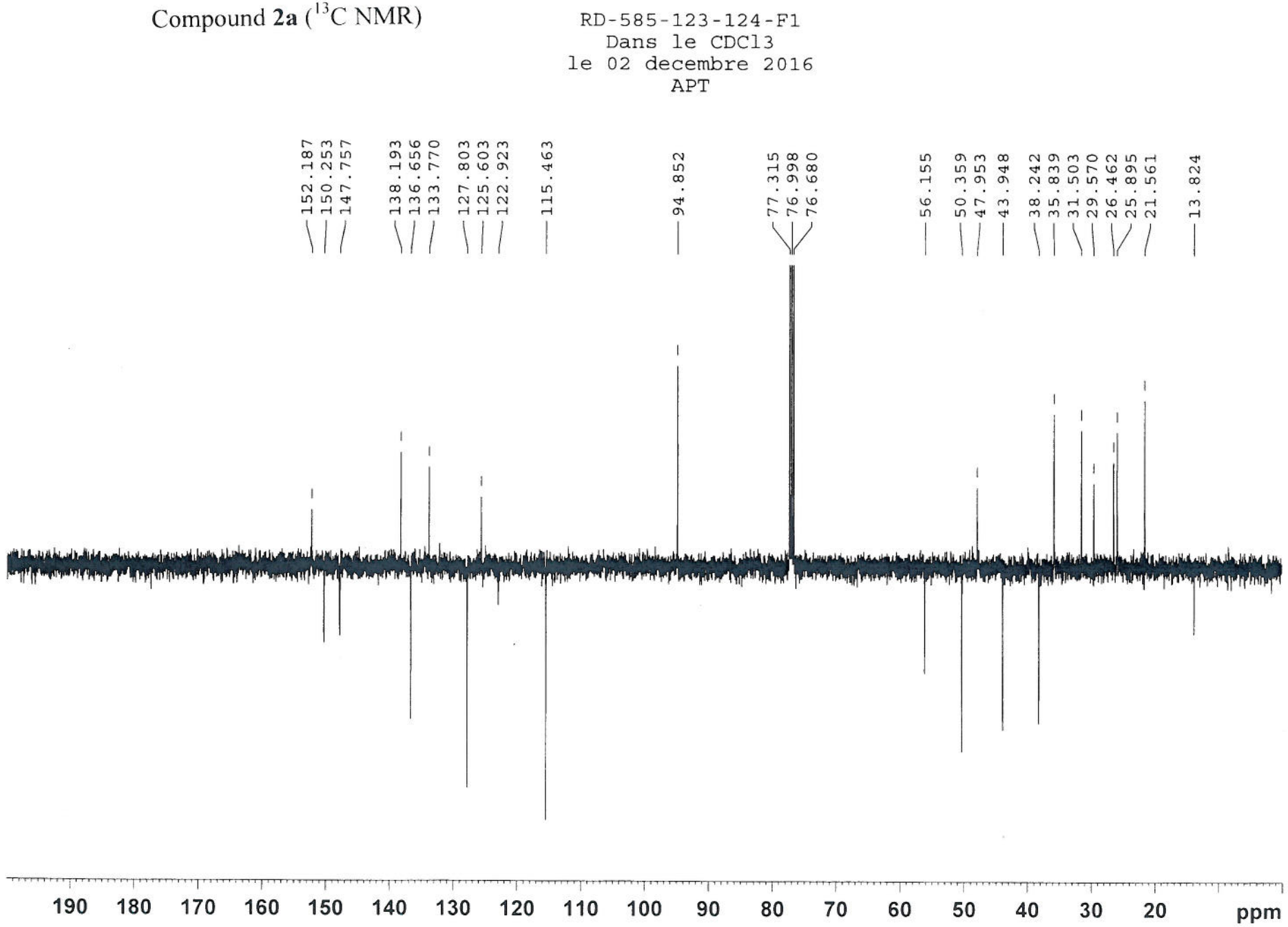
RD-585-2I-3MOM-E1
dans le CDCl3
le 23 janvier 2017
APT



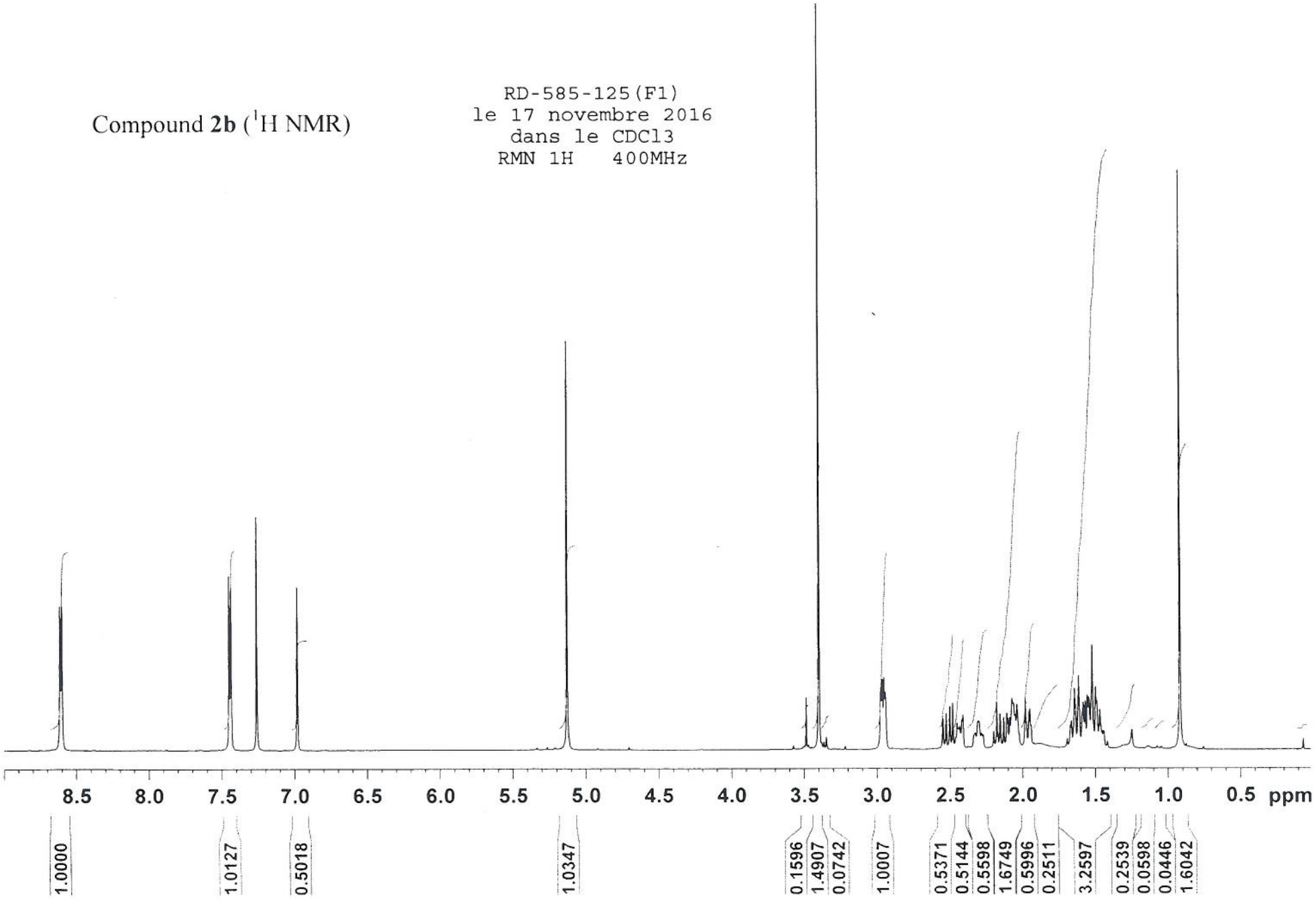
Compound **2a** (^{1}H NMR)



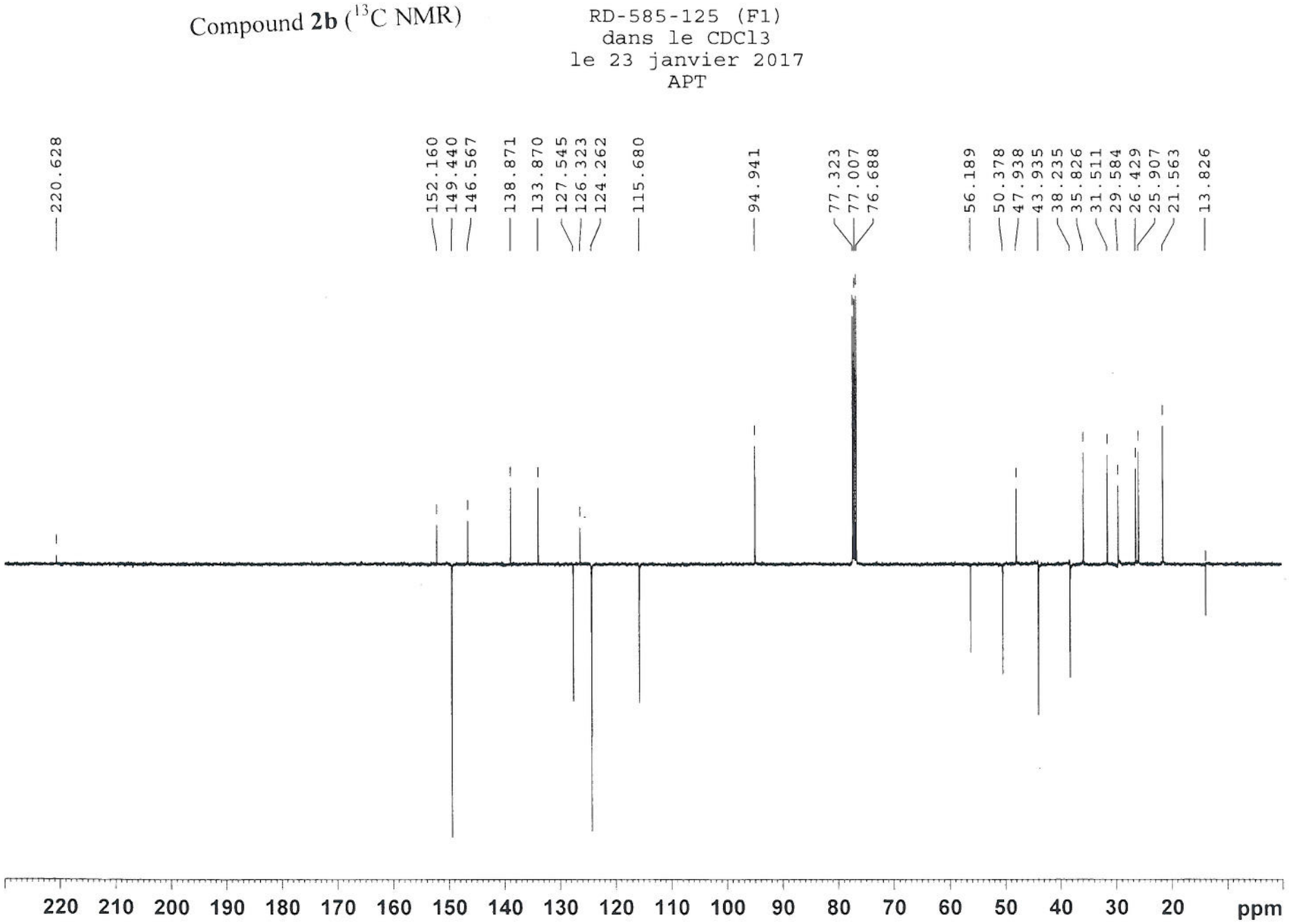
Compound **2a** (^{13}C NMR)



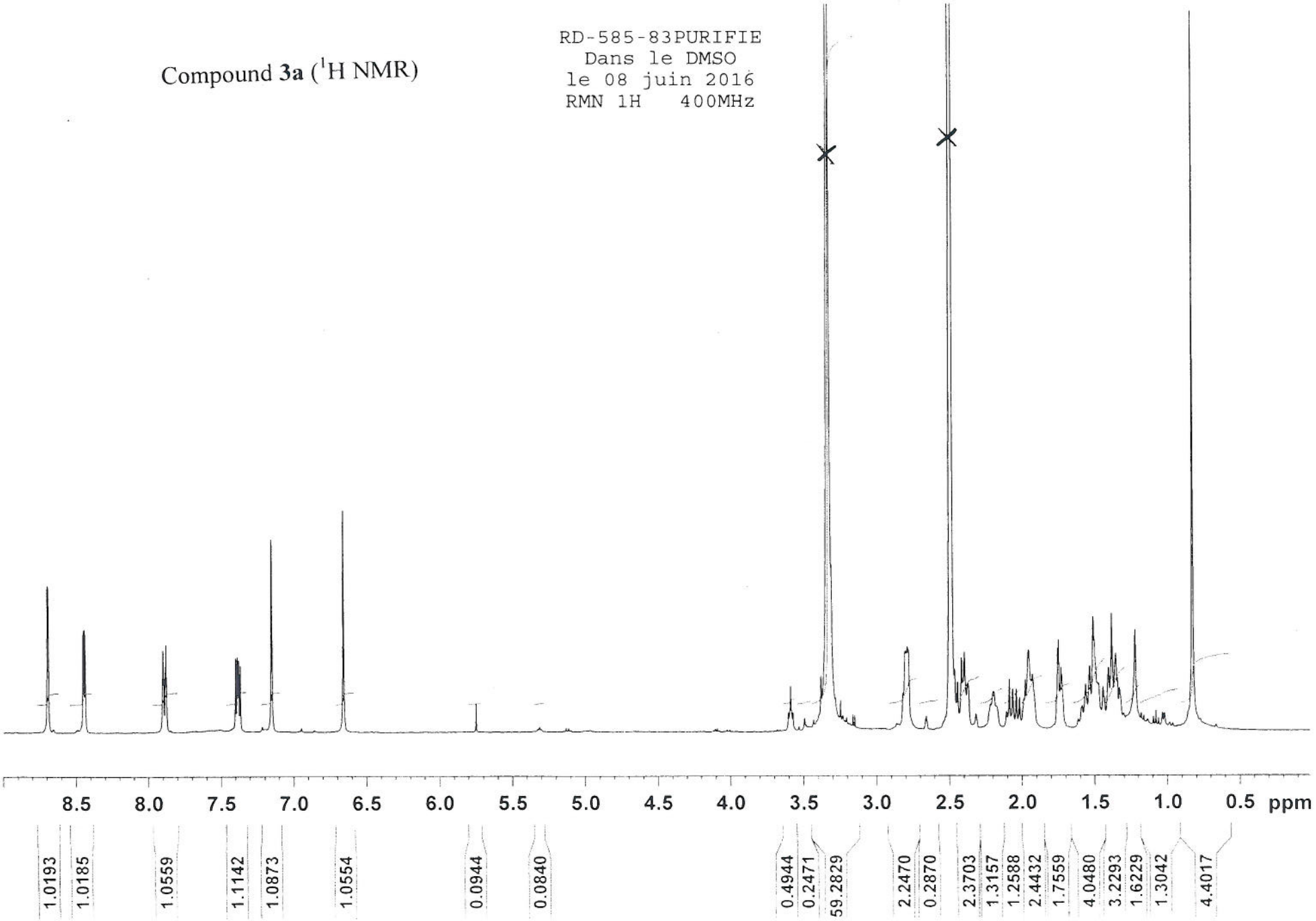
Compound **2b** (^{1}H NMR)



Compound **2b** (^{13}C NMR)



Compound **3a** (^{1}H NMR)



Compound **3a** (^{13}C NMR)

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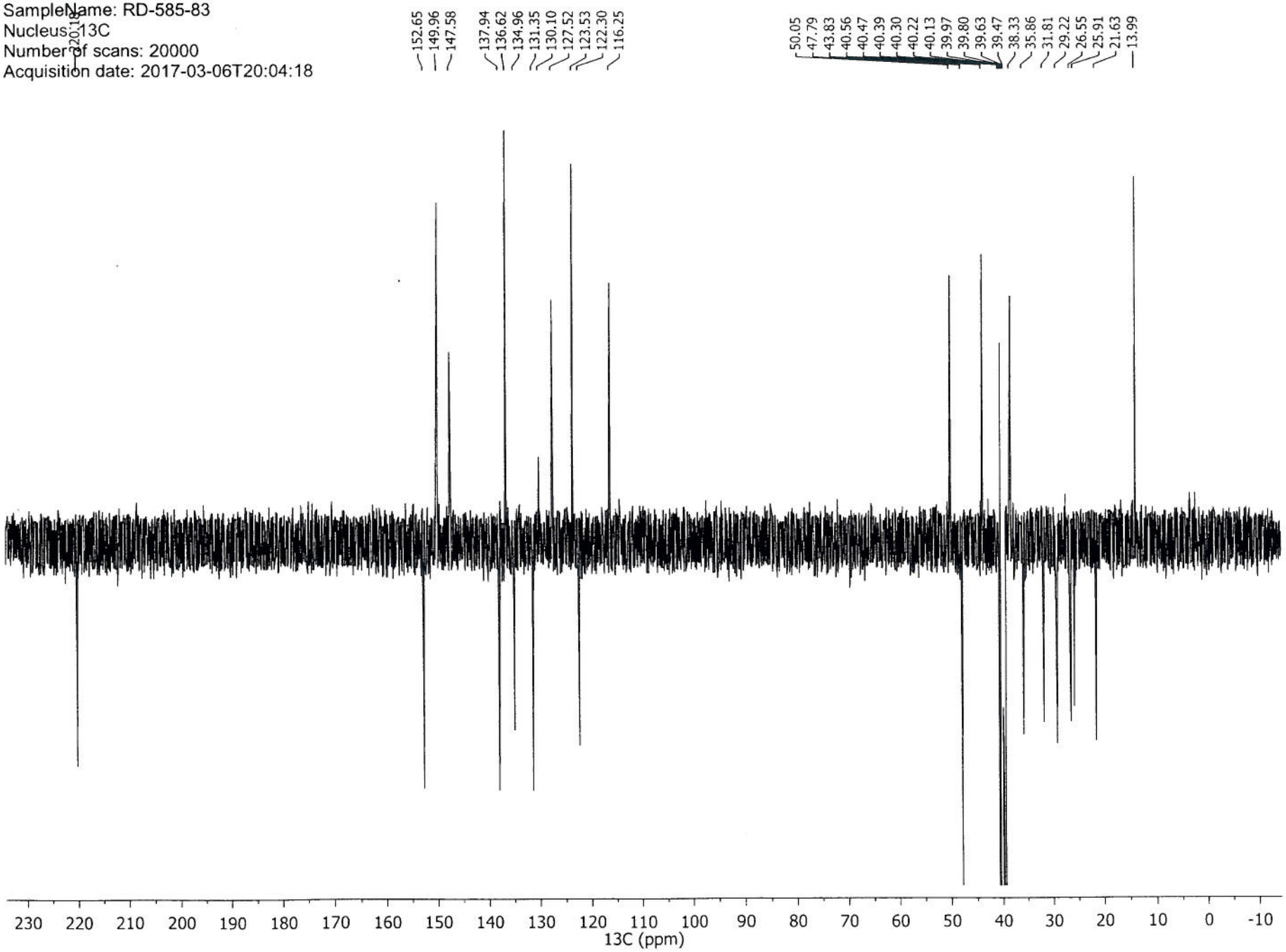
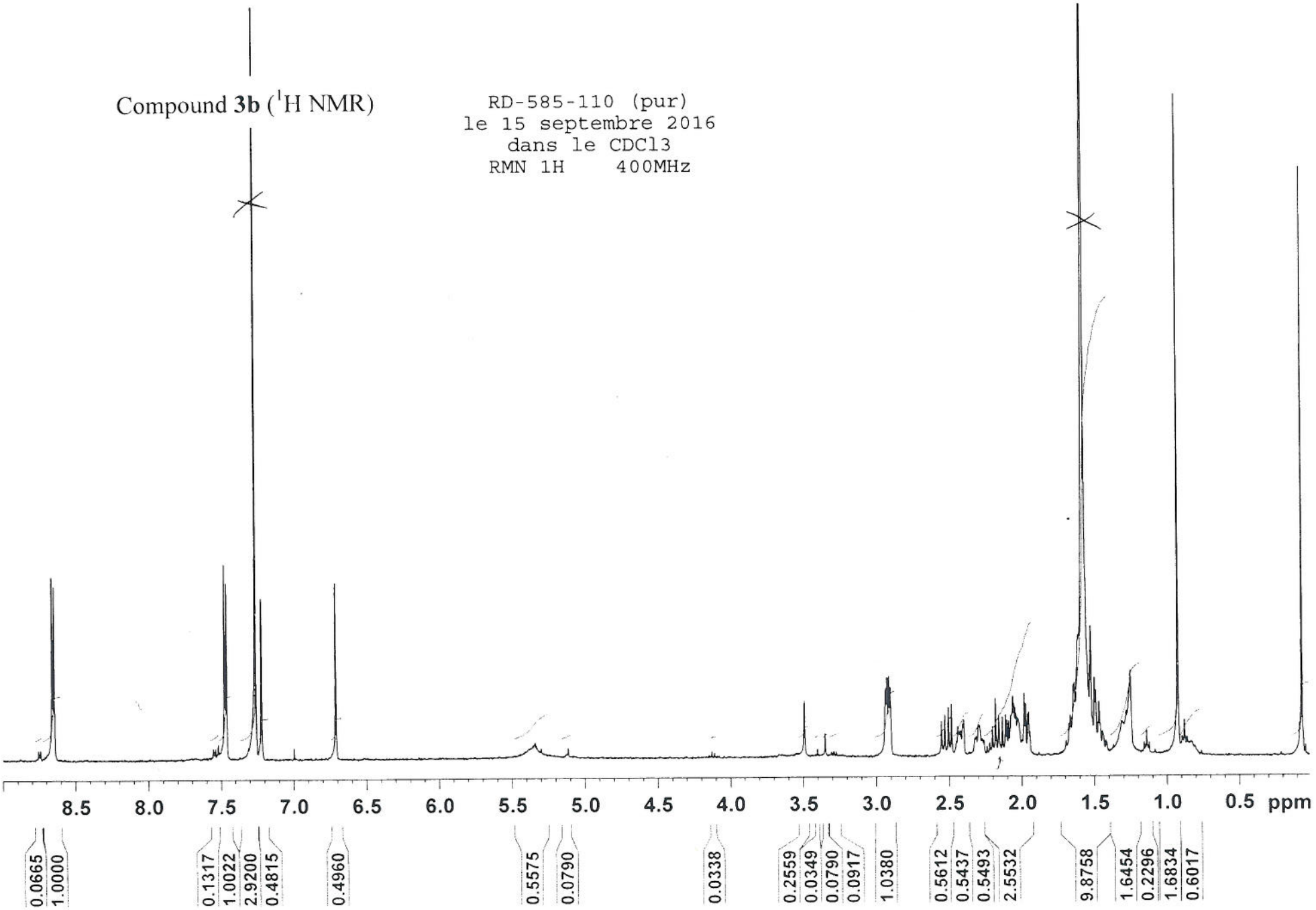
Nucleus: 13C
Number of scans: 20000
Acquisition date: 2017-03-06T20:04:18



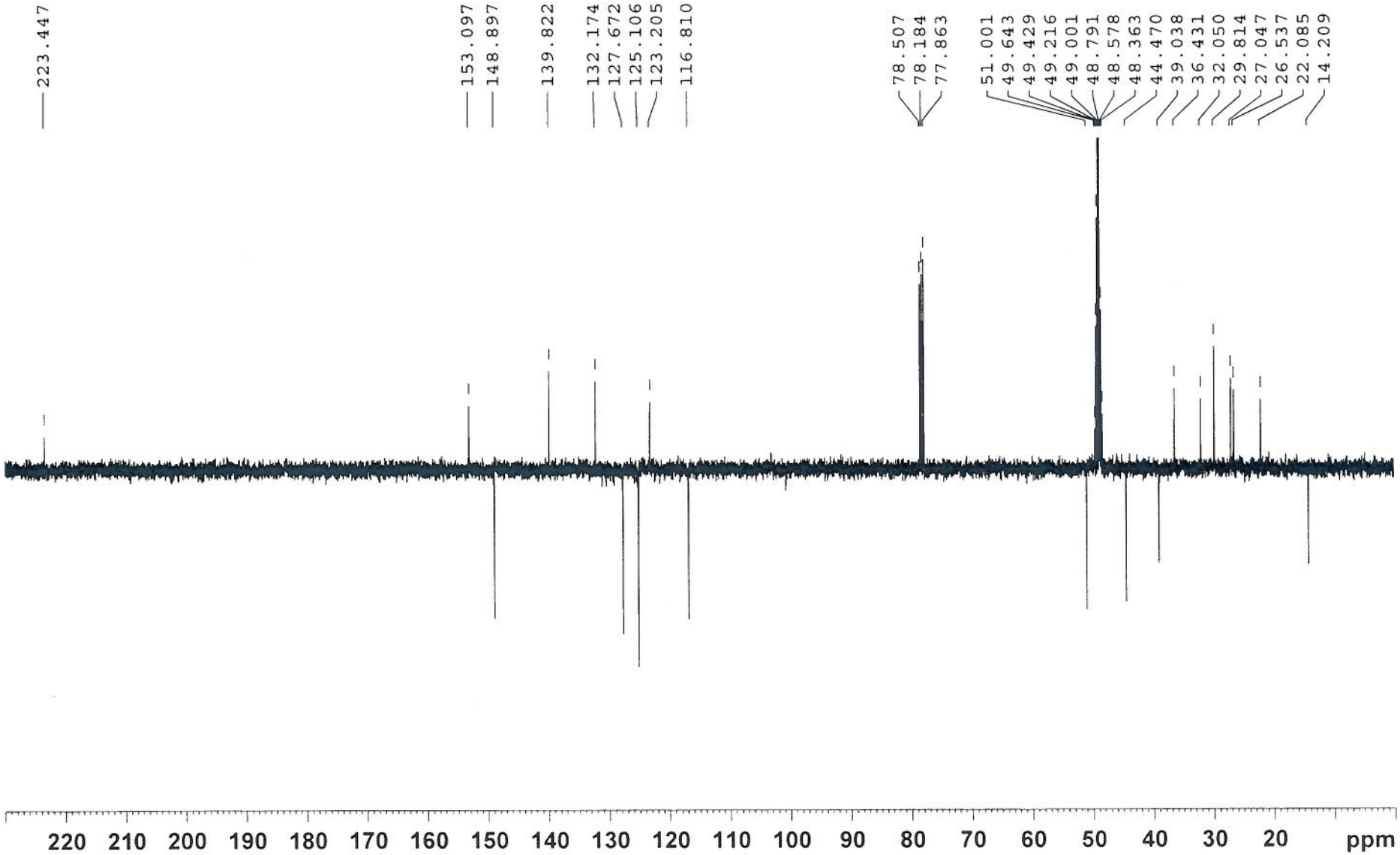
Compound **3b** (^{1}H NMR)

RD-585-110 (pur)
le 15 septembre 2016
dans le CDCl3
RMN 1H 400MHz

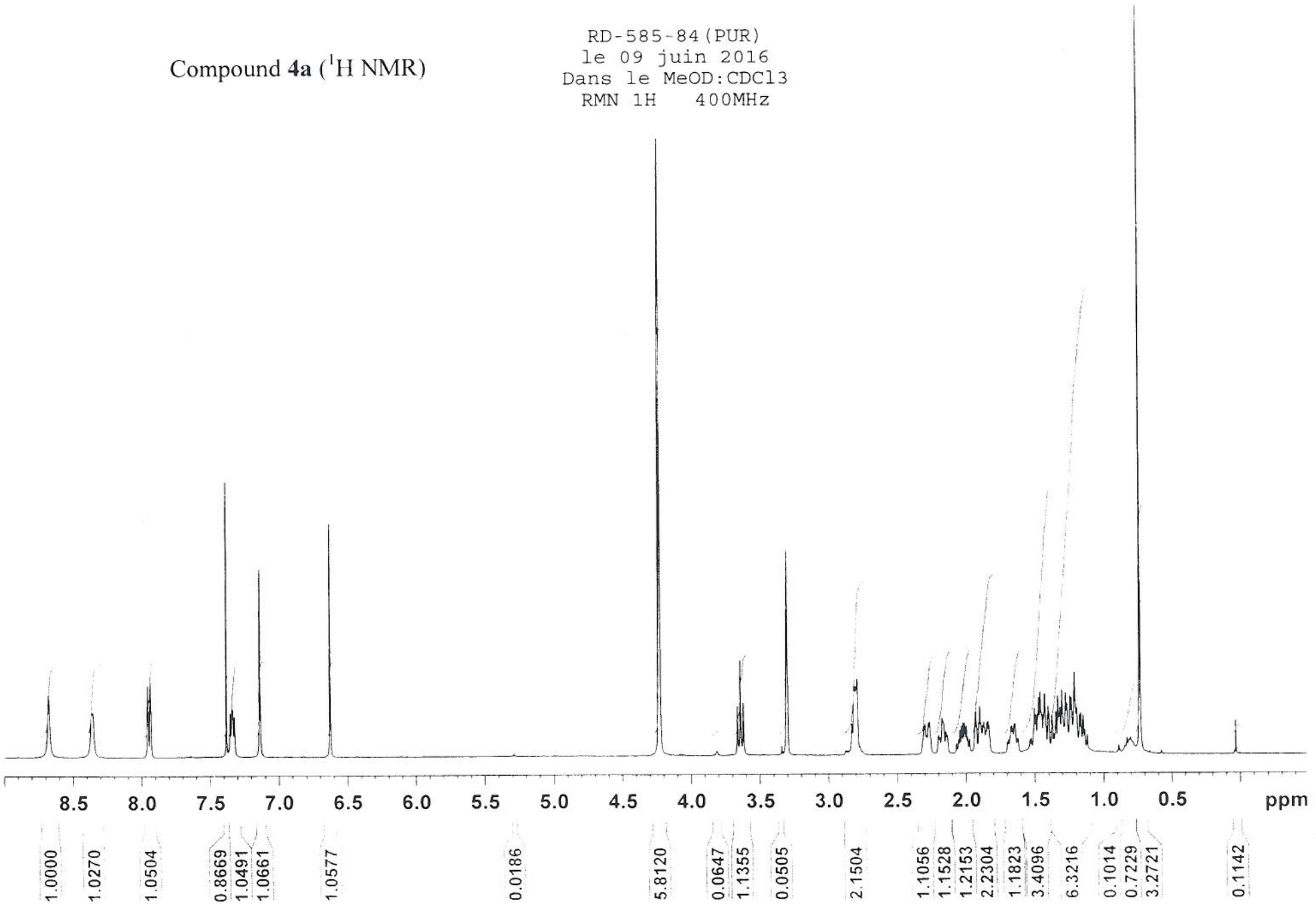


Compound **3b** (^{13}C NMR)

RD-585-110
le 18 novembre 2016
dans le MeOD:CDCl3 1:1
APT

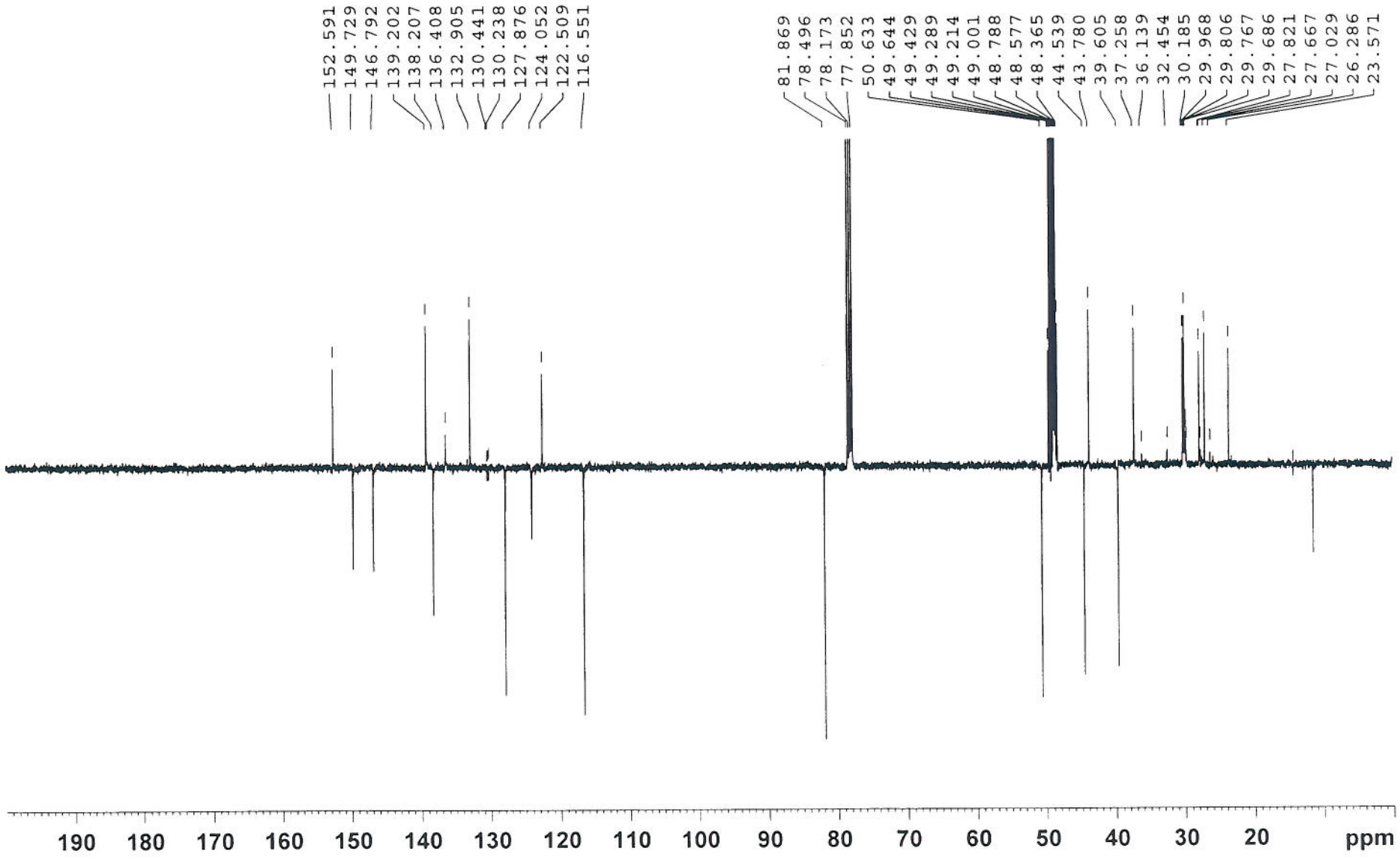


Compound **4a** (^{1}H NMR)

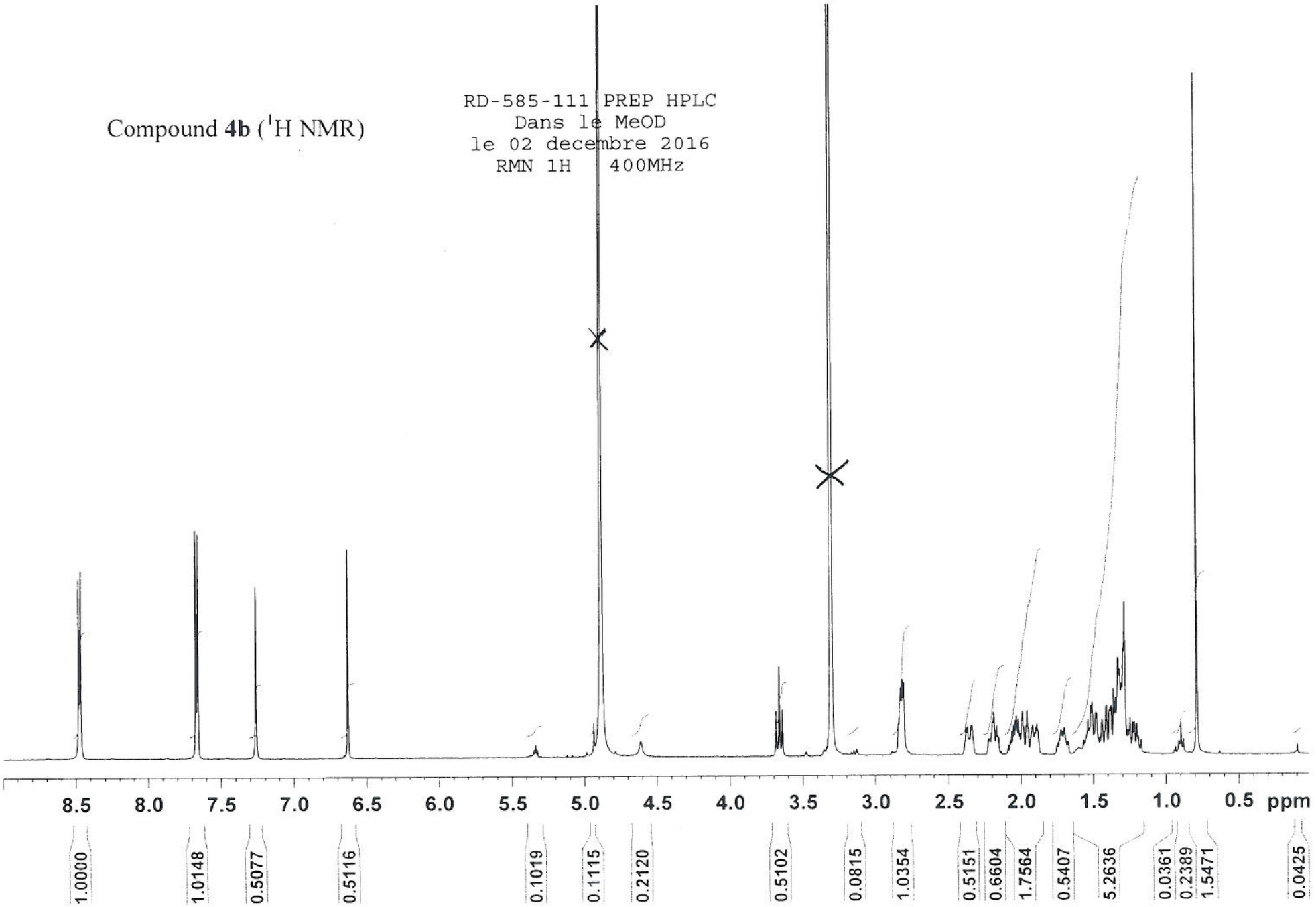


Compound **4a** (^{13}C NMR)

RD-585-84
le 18 novembre 2016
dans le MeOD:CDCl3 1:1
APT

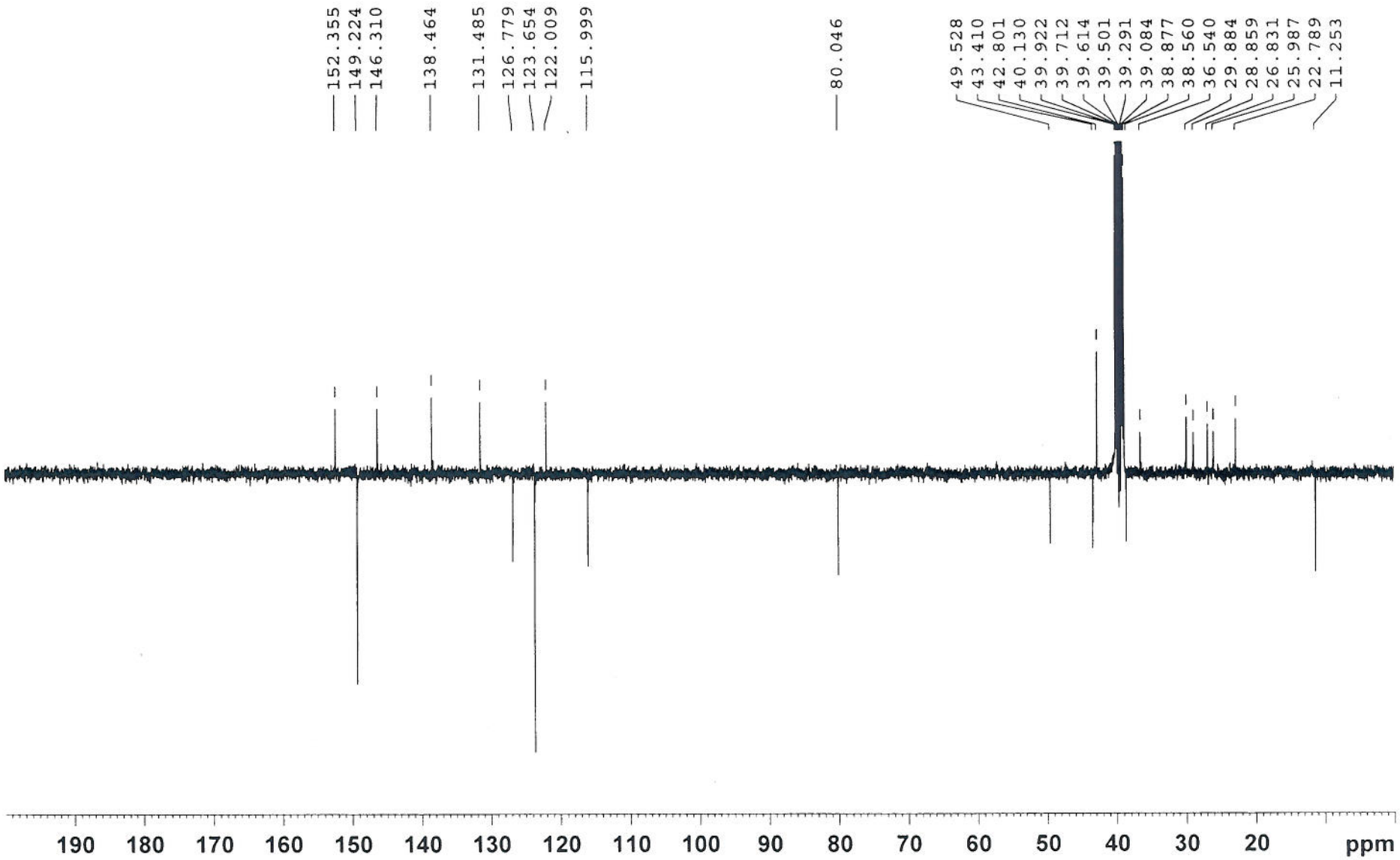


Compound **4b** (^{1}H NMR)

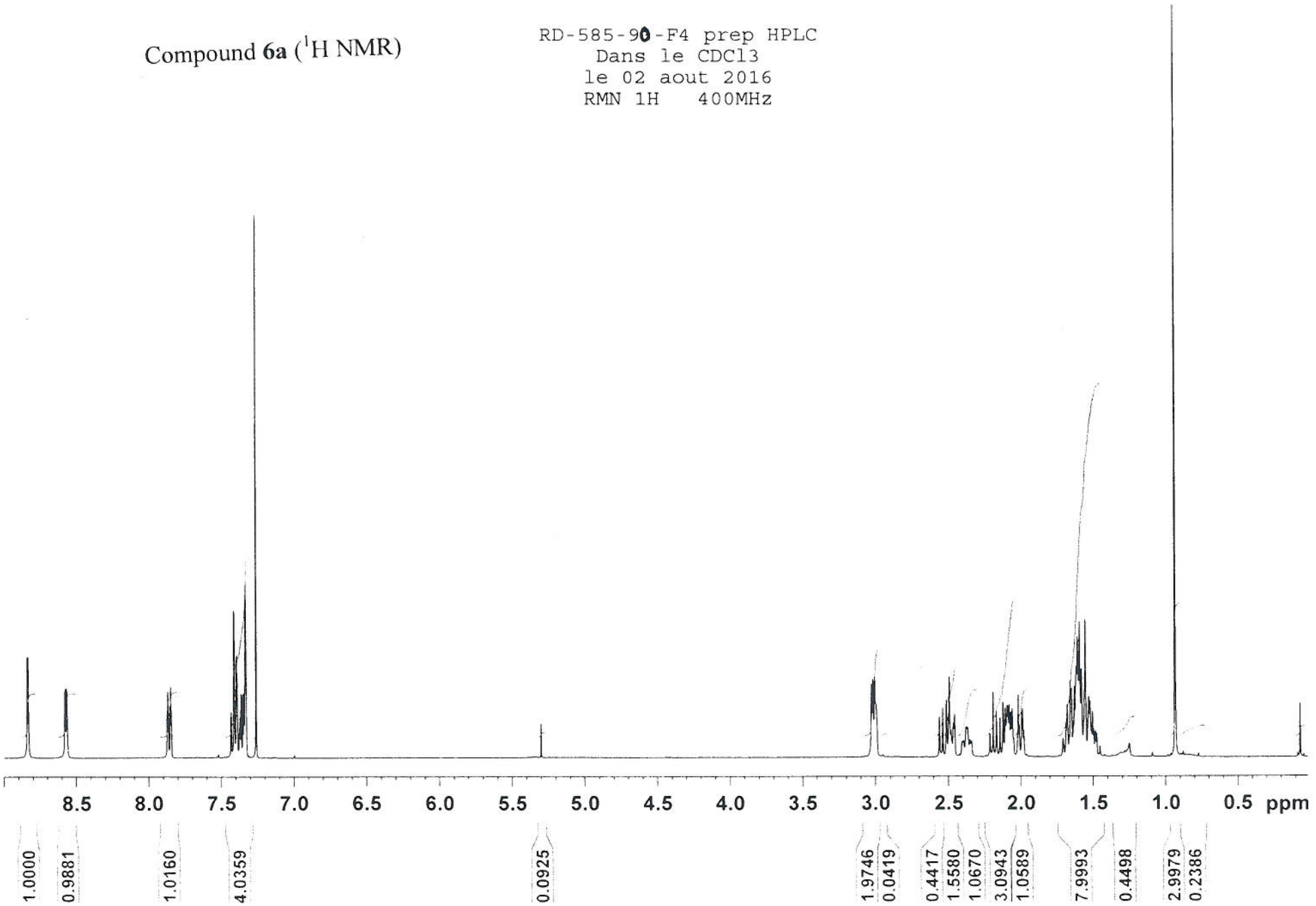


Compound **4b** (^{13}C NMR)

RD-585-111 (PREP HPLC)
dans le DMSO
le 24 janvier 2017
APT

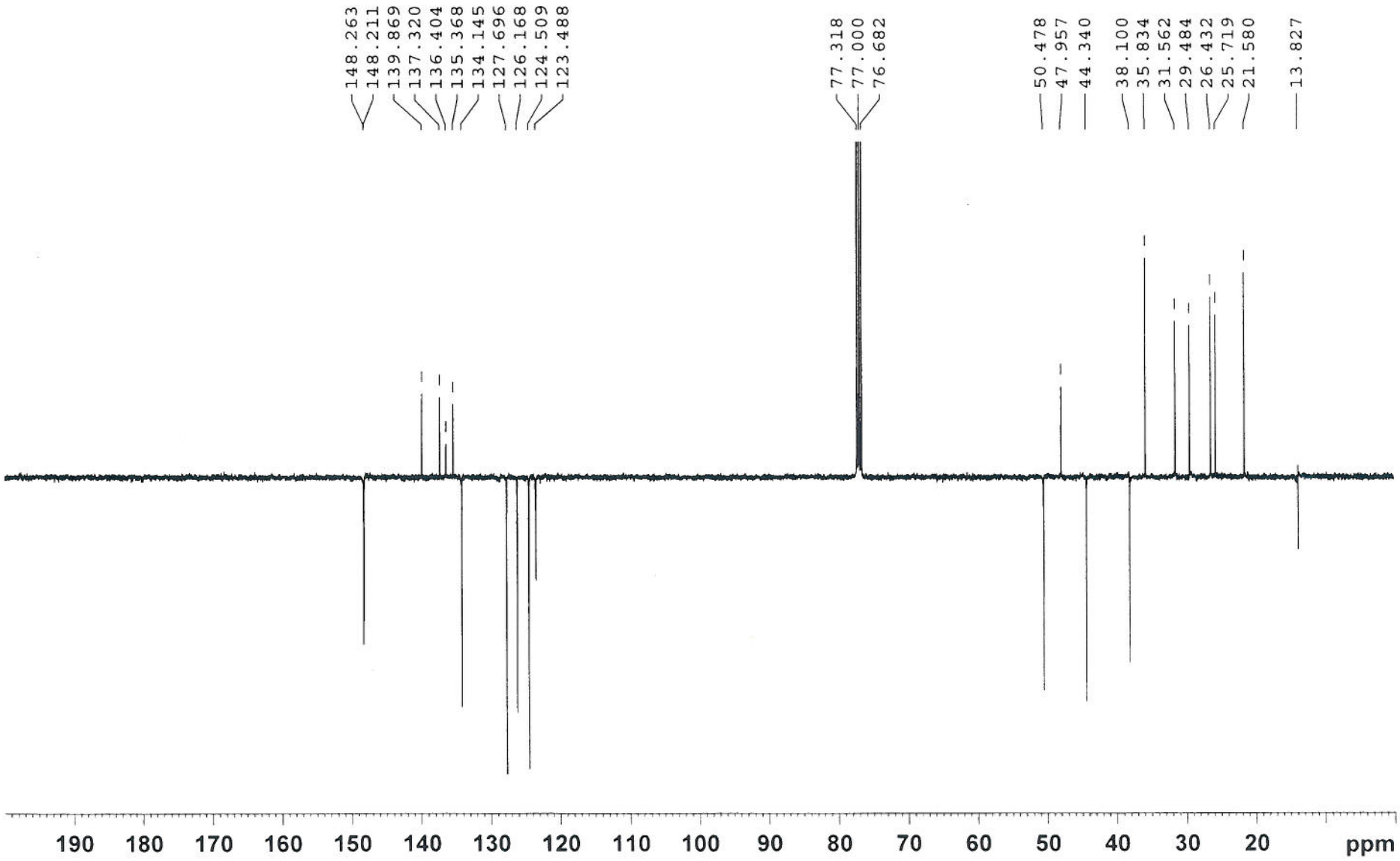


Compound **6a** (^{1}H NMR)

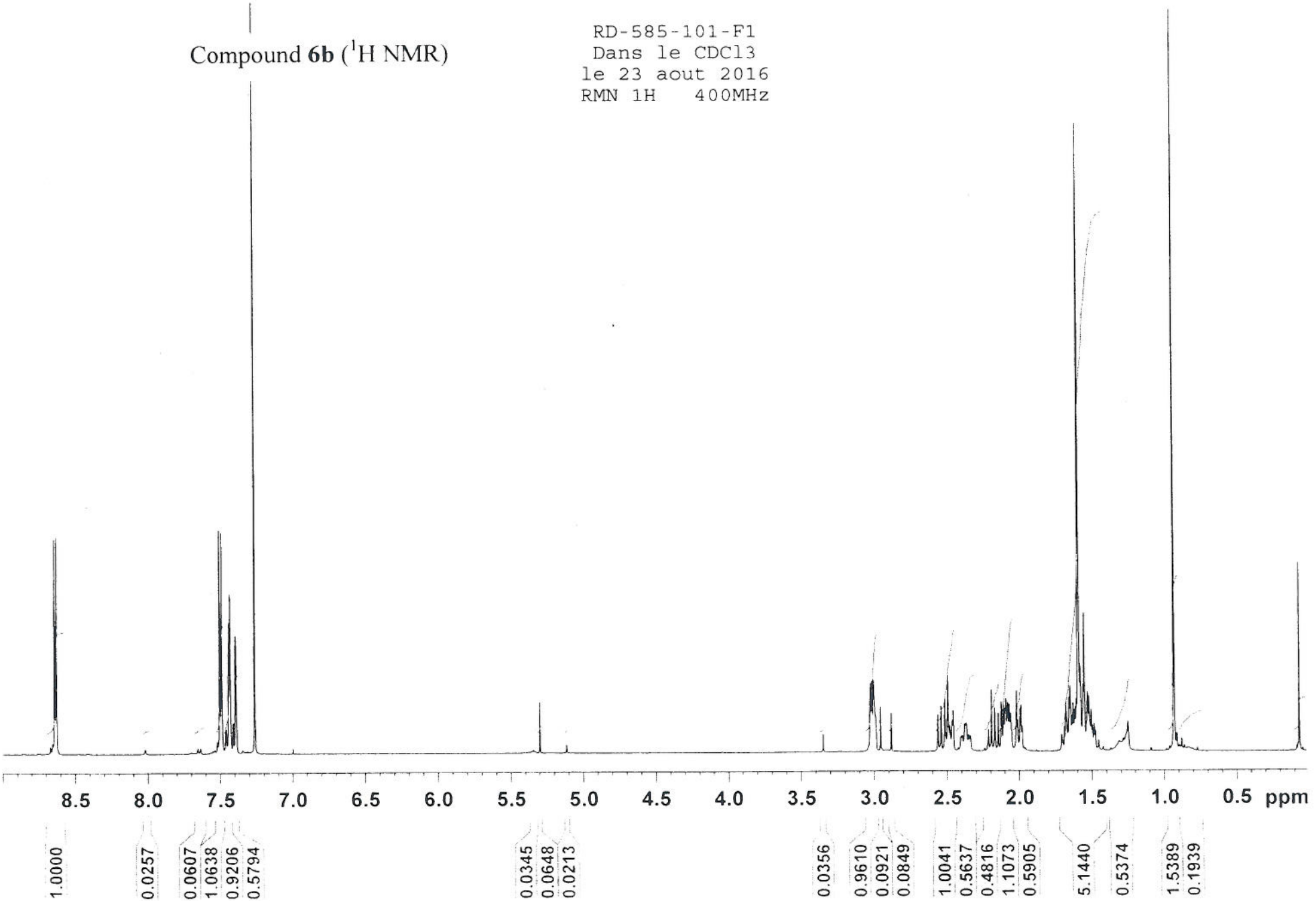


Compound **6a** (^{13}C NMR)

RD-585-90-F4
le 09 novembre 2016
dans le CDCl3
APT

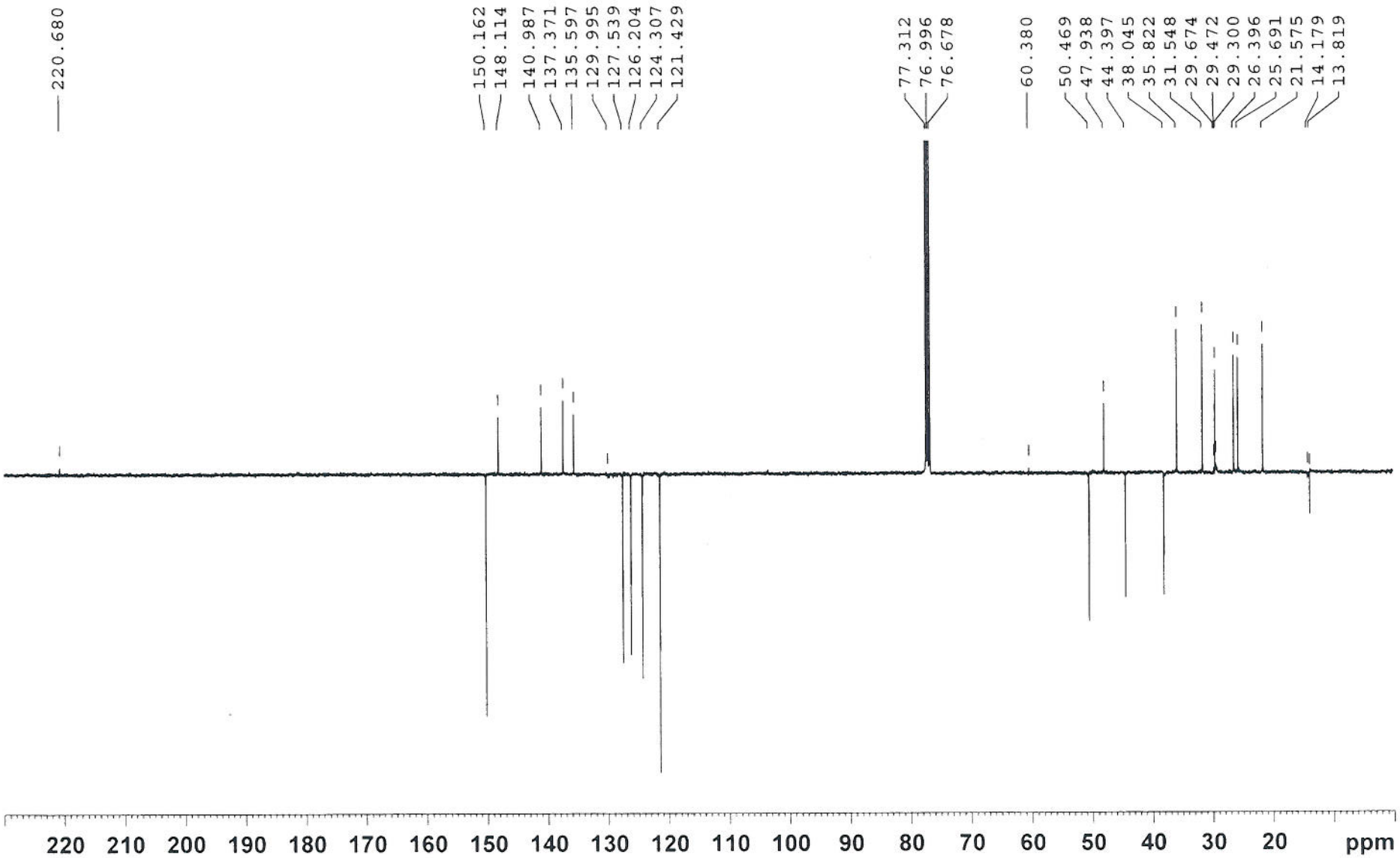


Compound **6b** (^{1}H NMR)



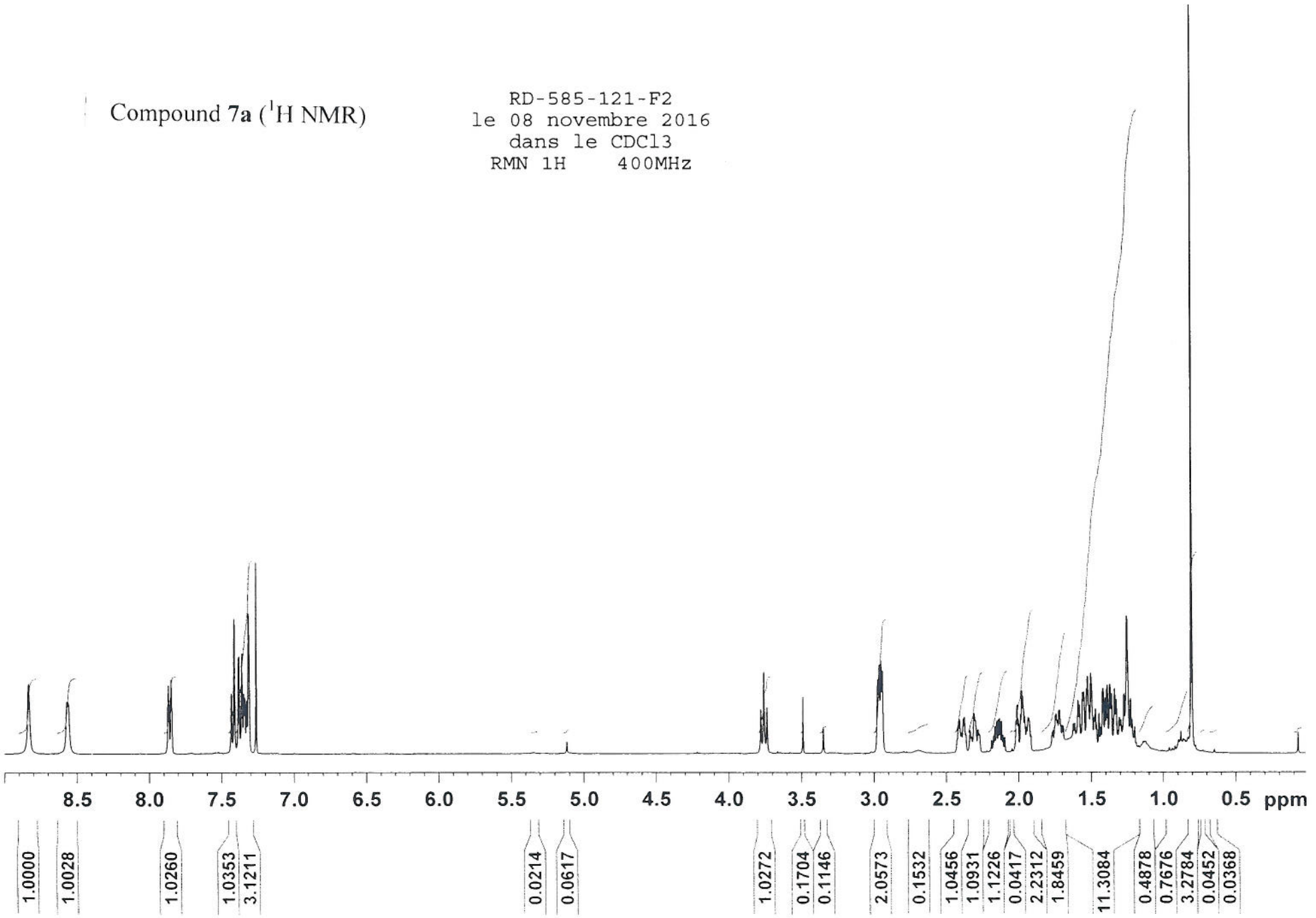
Compound **6b** (^{13}C NMR)

RD-585-101 (F1)
dans le CDCl3
le 20 janvier 2017
APT



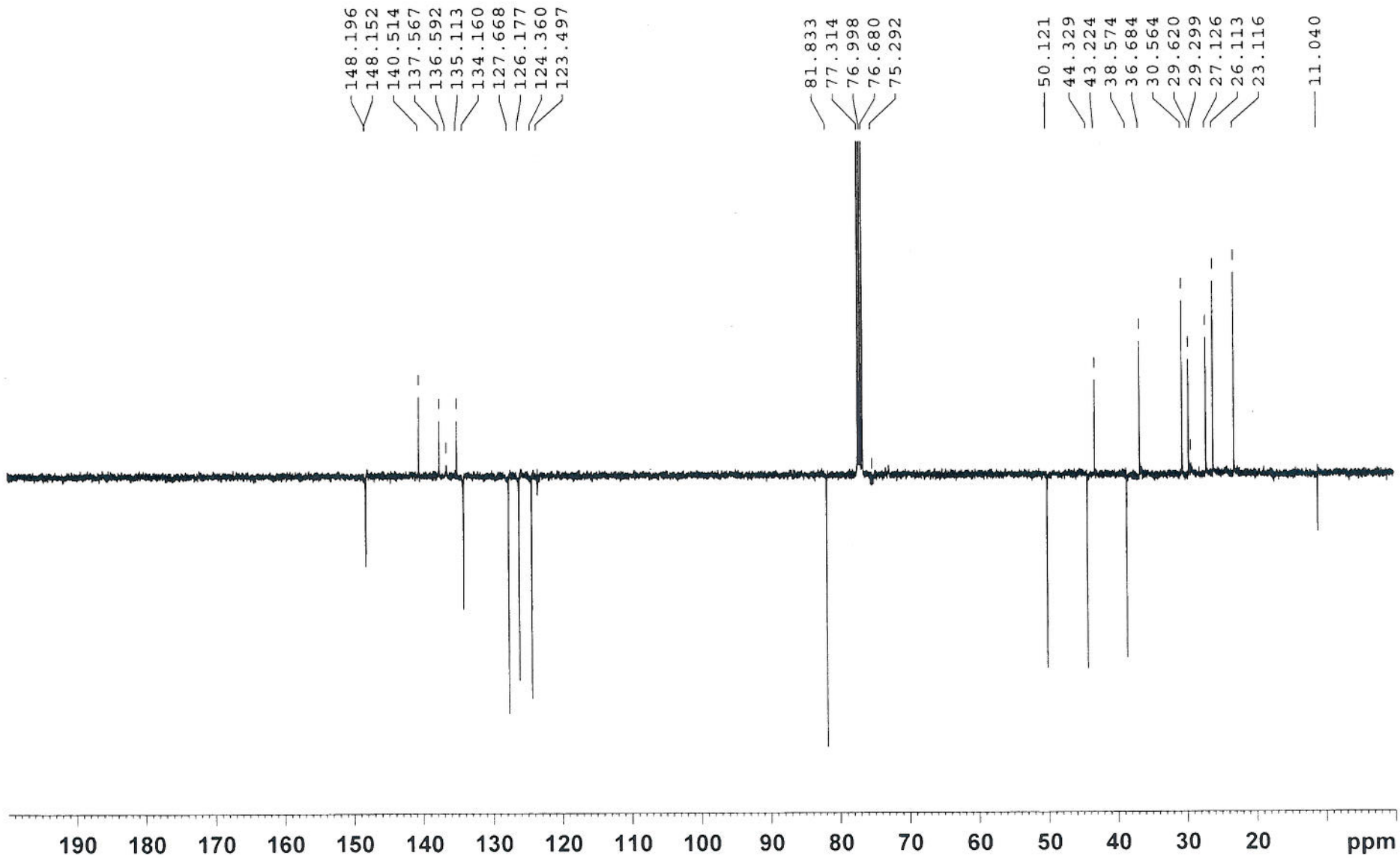
Compound **7a** (^{1}H NMR)

RD-585-121-F2
le 08 novembre 2016
dans le CDCl3
RMN 1H 400MHz



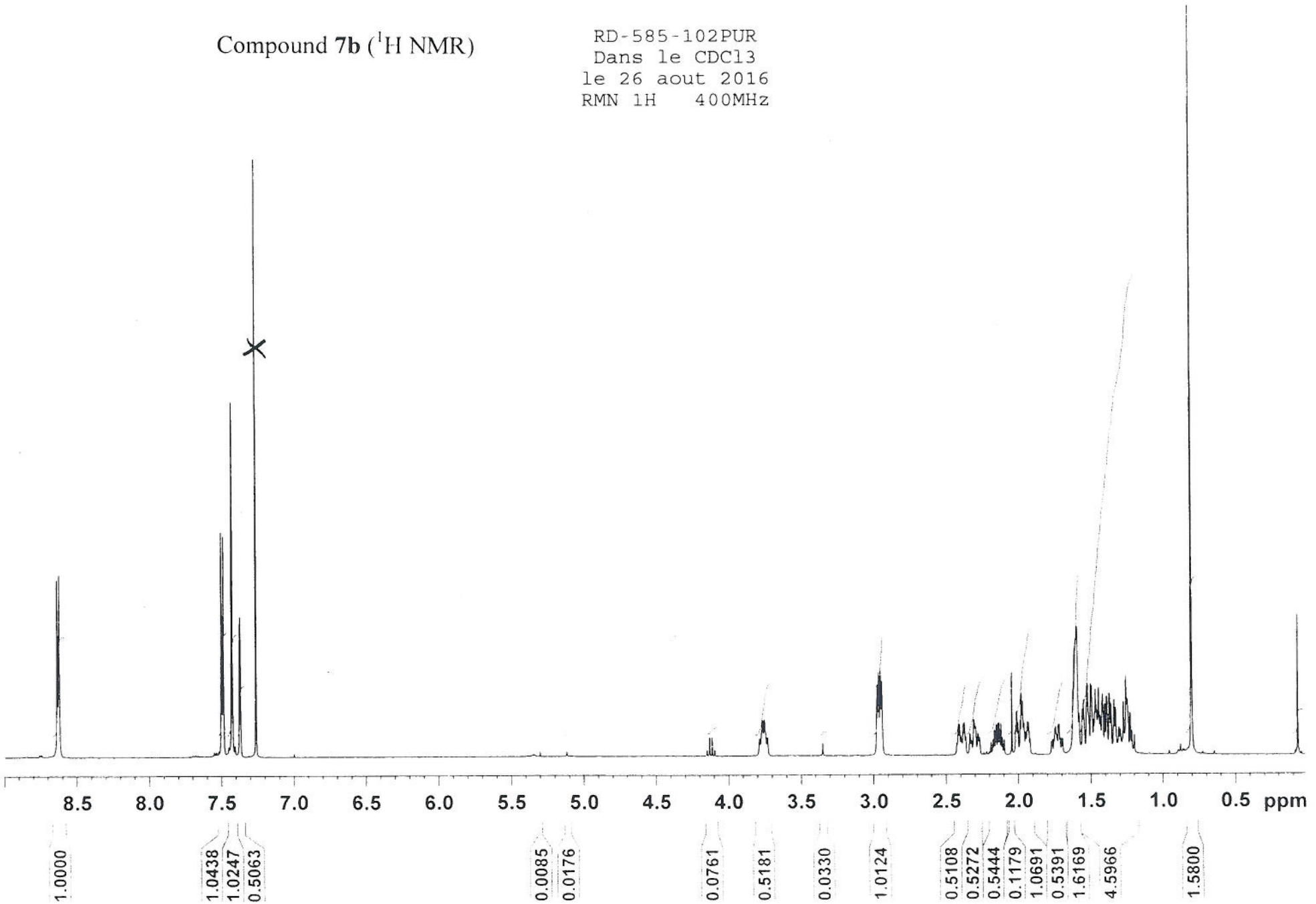
Compound **7a** (^{13}C NMR)

RD-585-91-F1-LAVAGE
le 23 novembre 2016
dans le CDCl3
APT



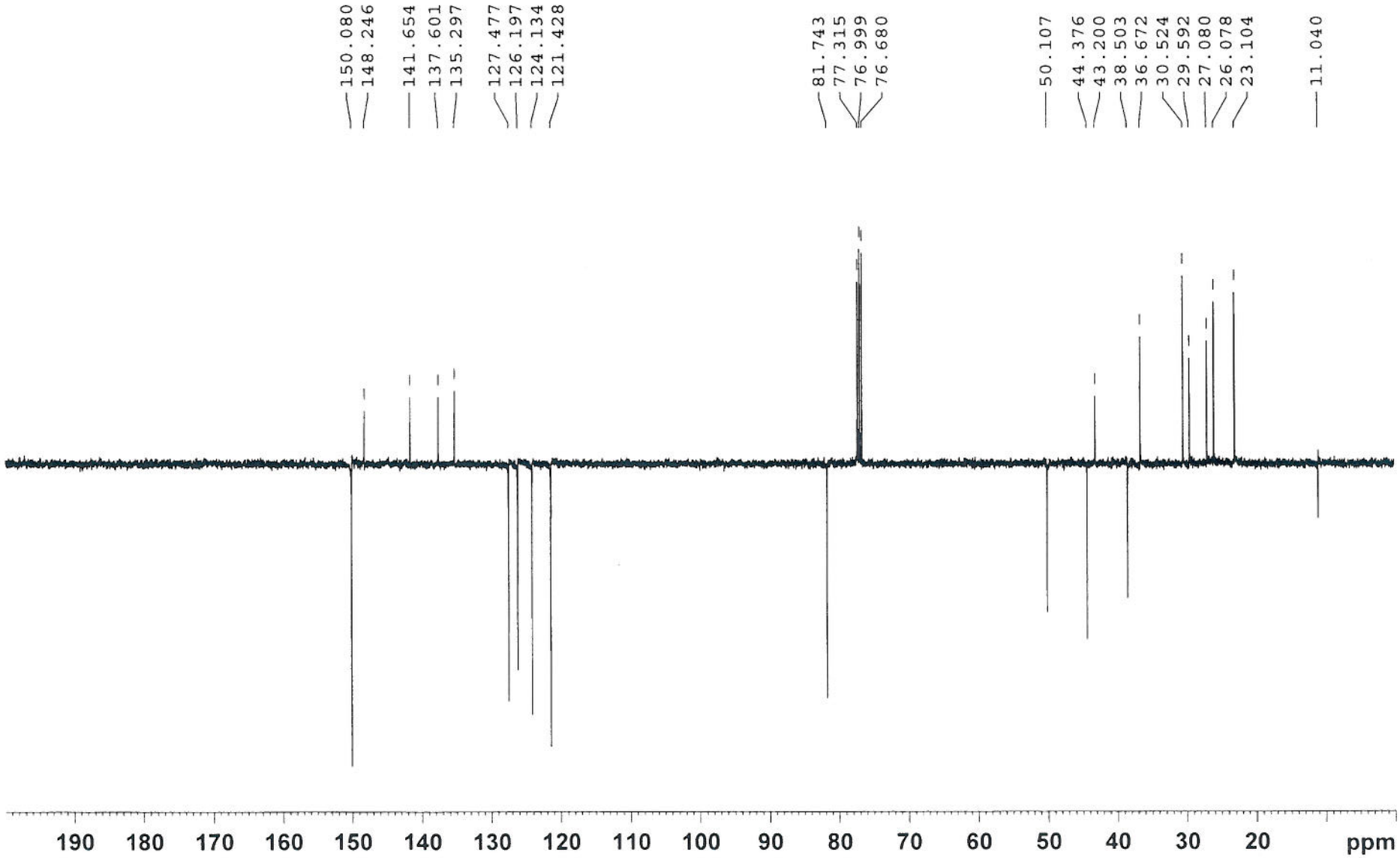
Compound **7b** (^{1}H NMR)

RD-585-102PUR
Dans le CDCl3
le 26 aout 2016
RMN 1H 400MHz

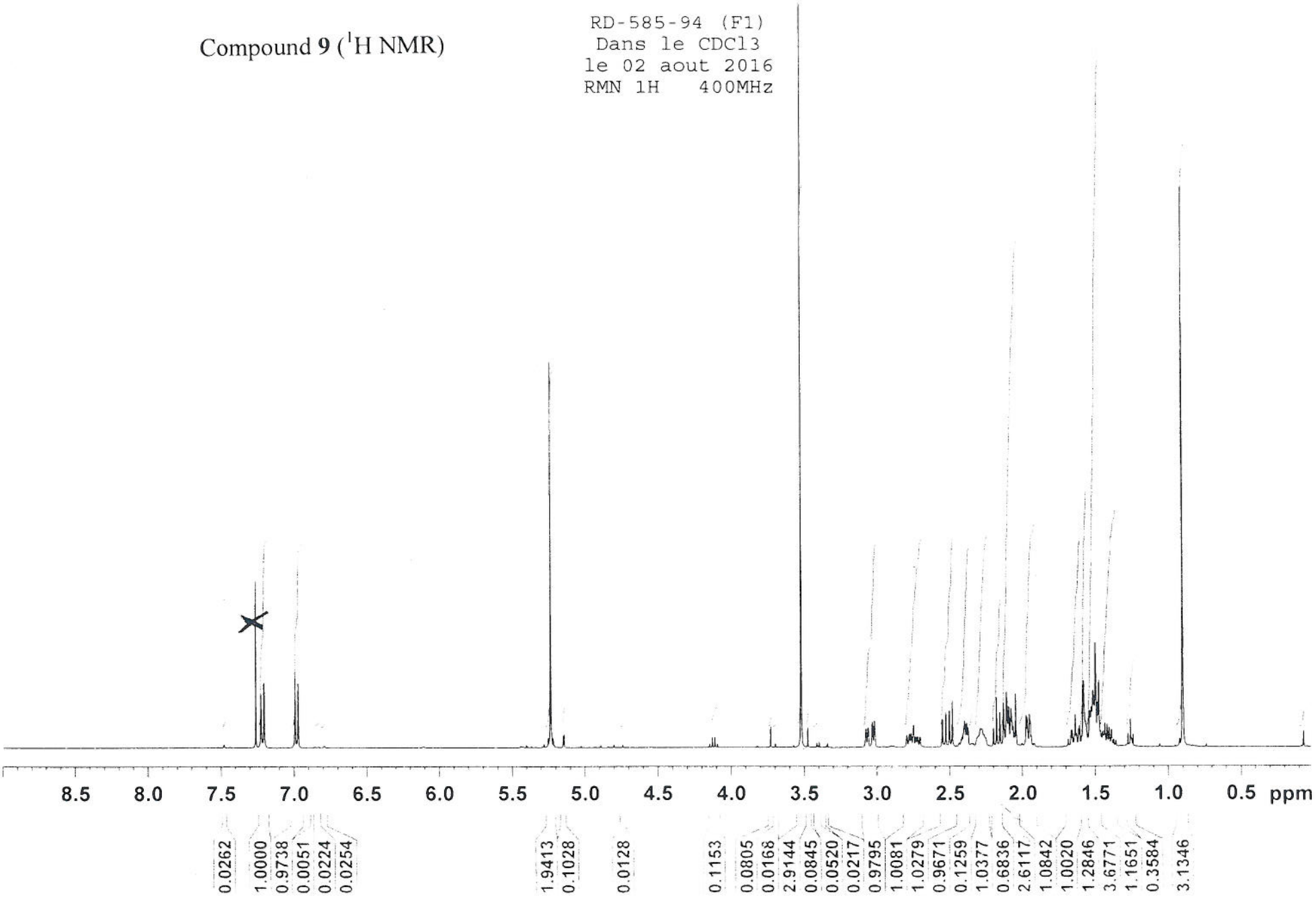


Compound **7b** (^{13}C NMR)

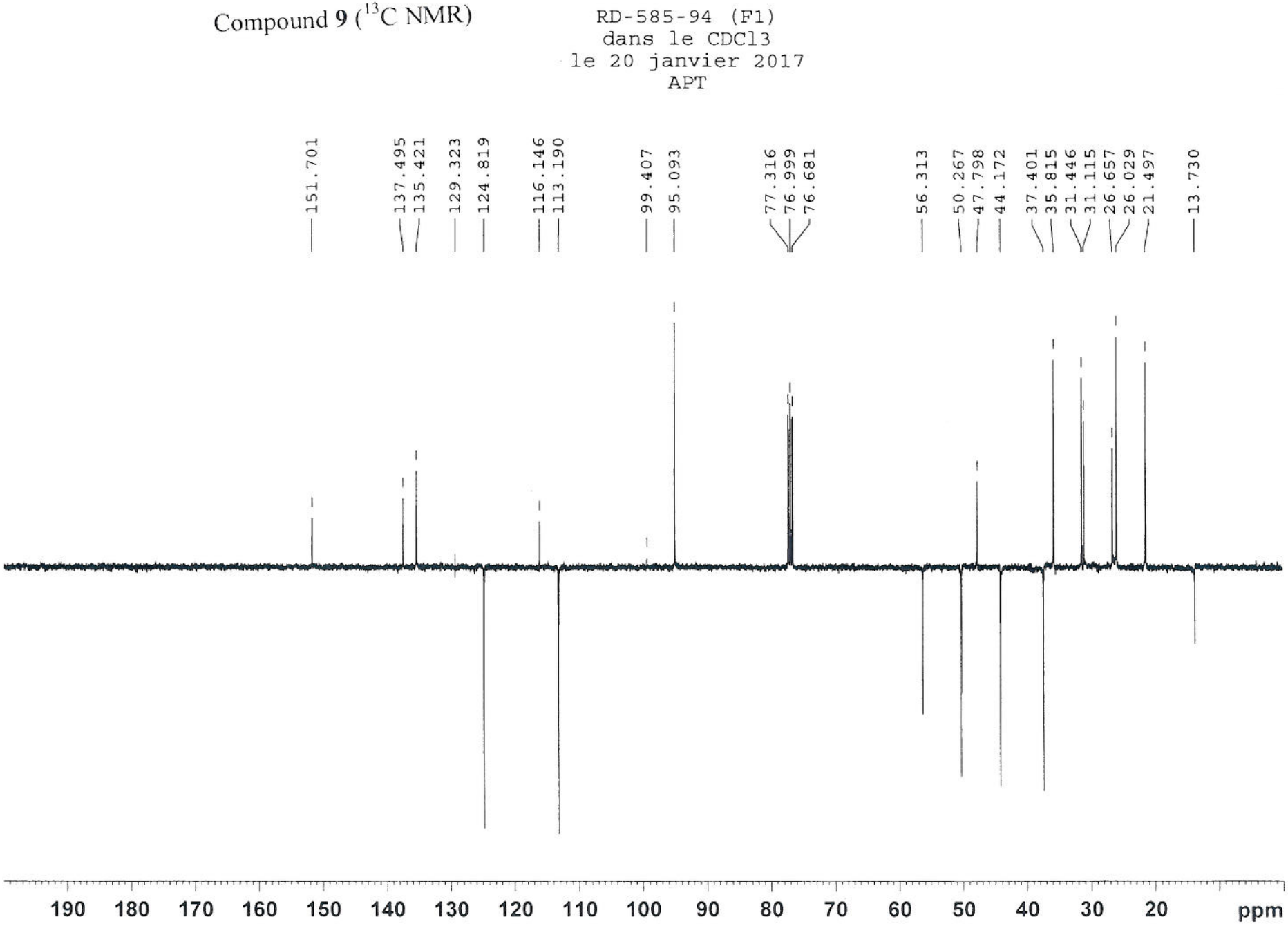
RD-585-102
le 08 novembre 2016
dans le CDCl3
APT



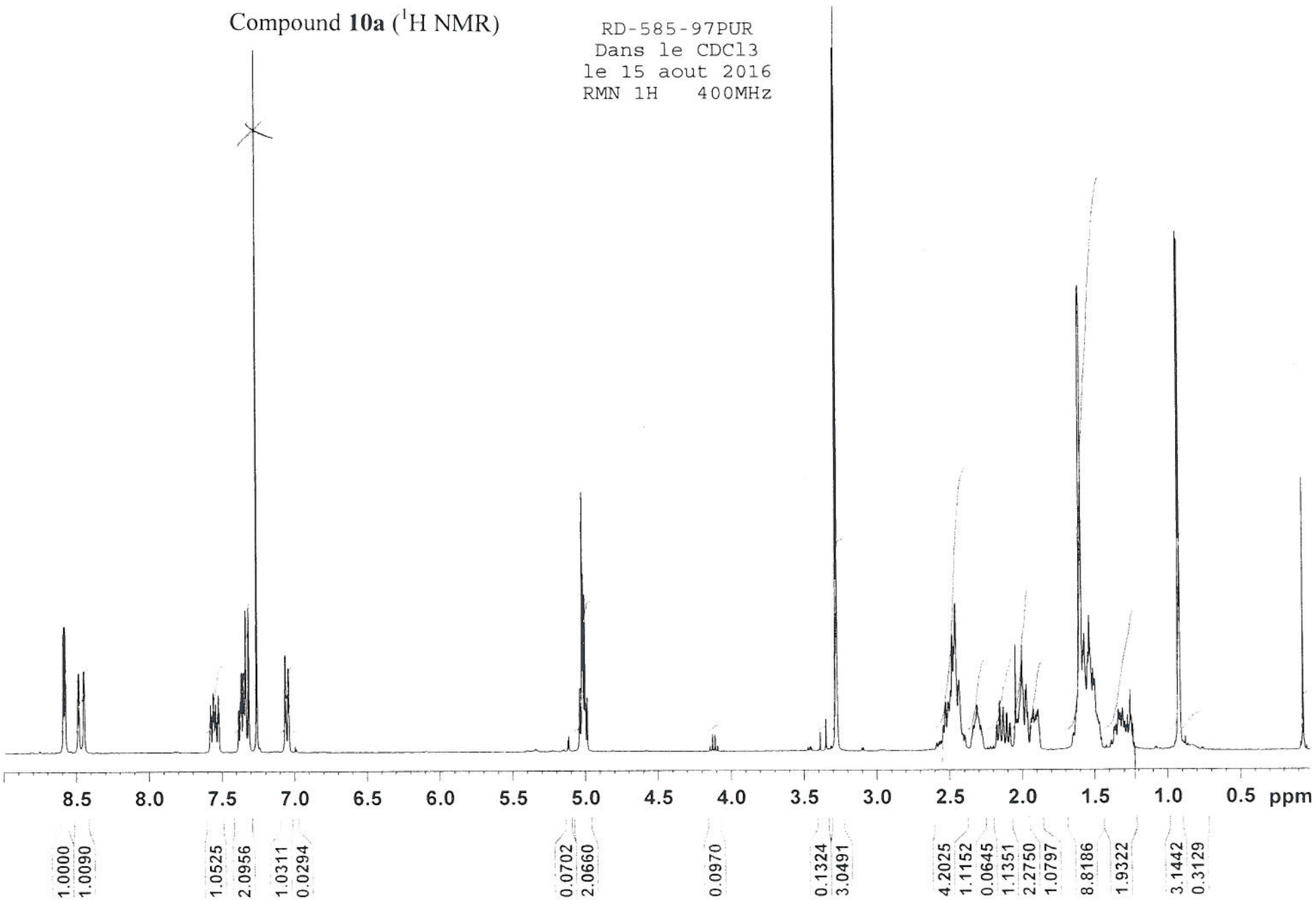
Compound **9** (^{1}H NMR)



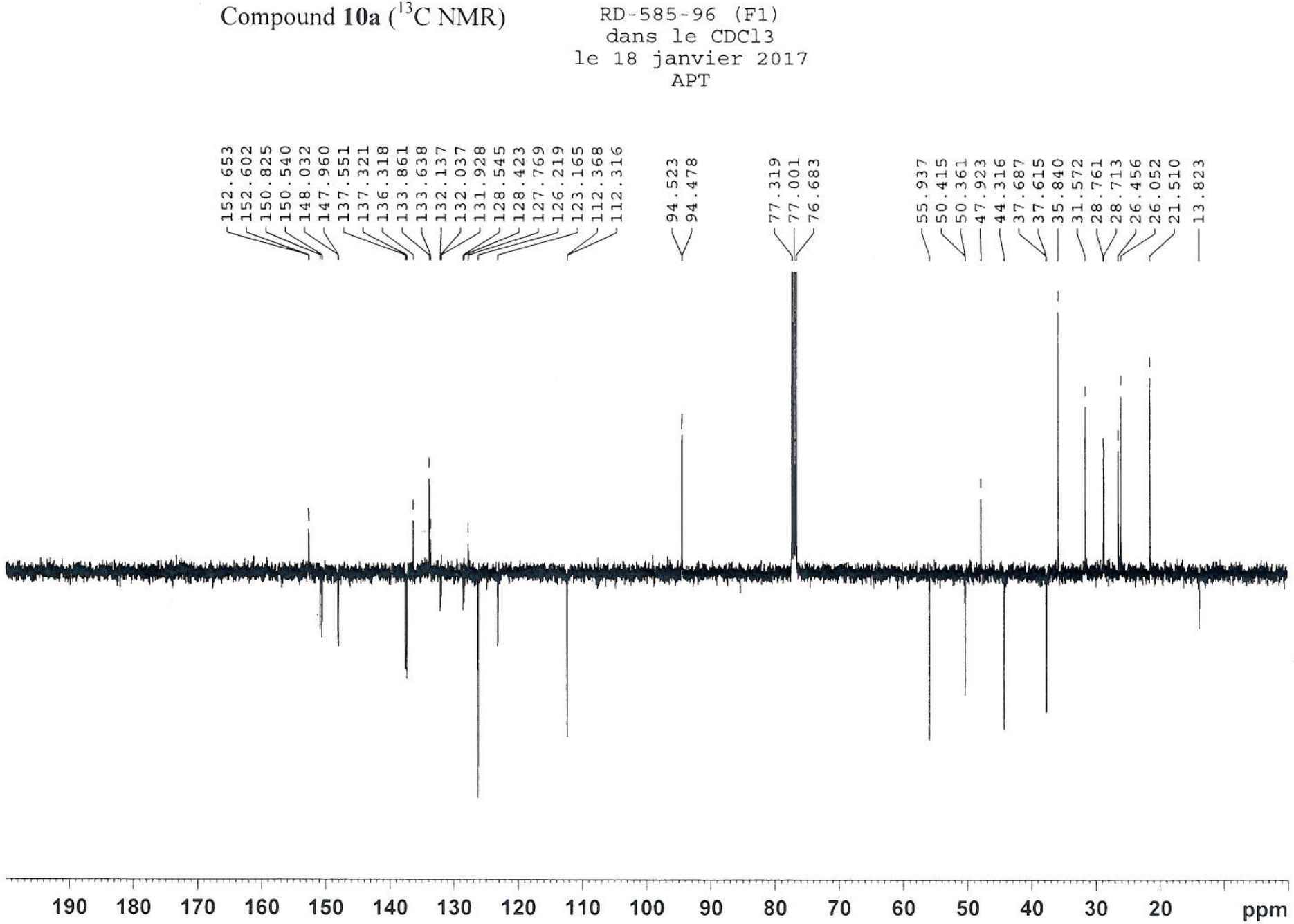
Compound **9** (^{13}C NMR)



Compound **10a** (^{1}H NMR)

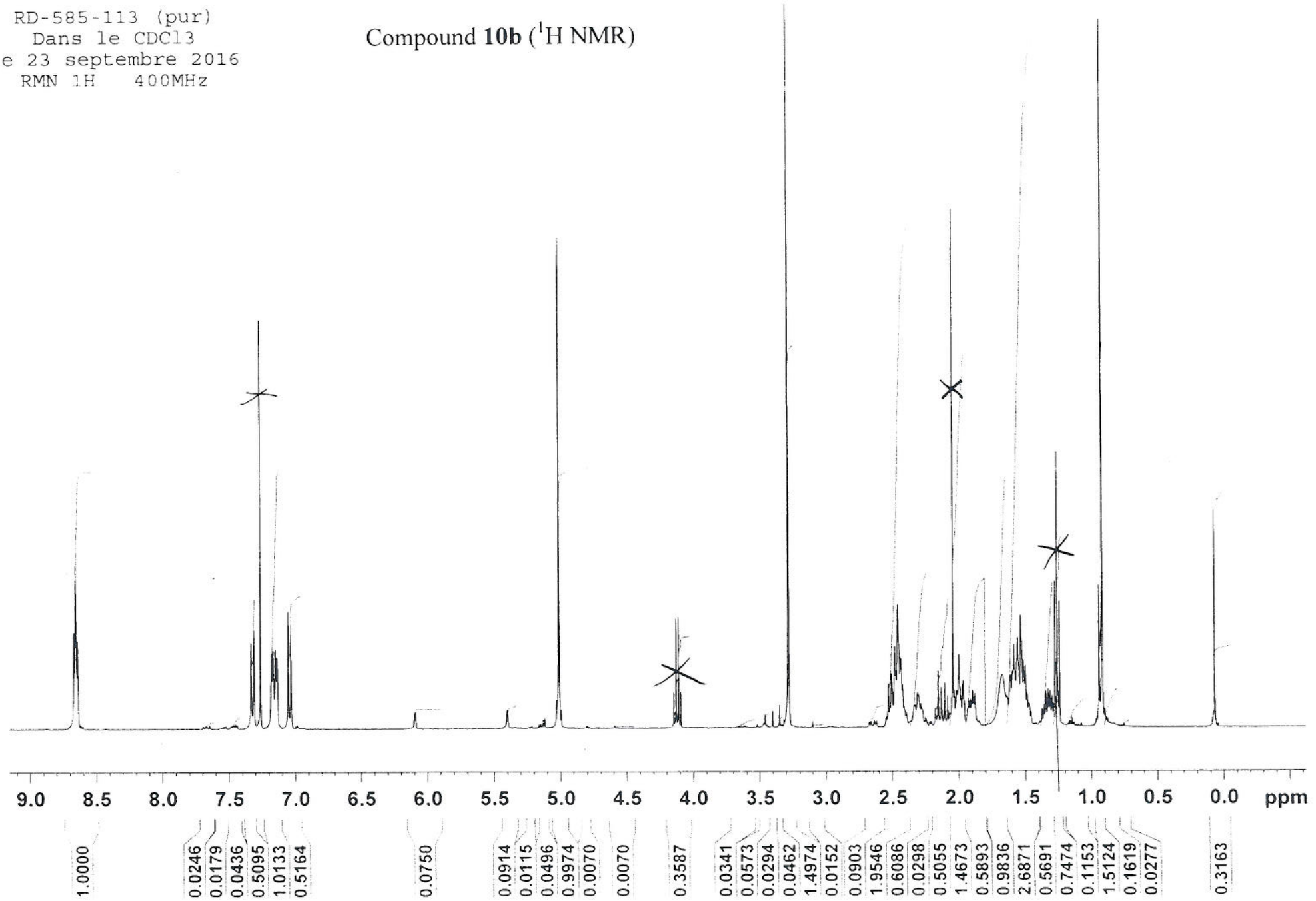


Compound **10a** (^{13}C NMR)

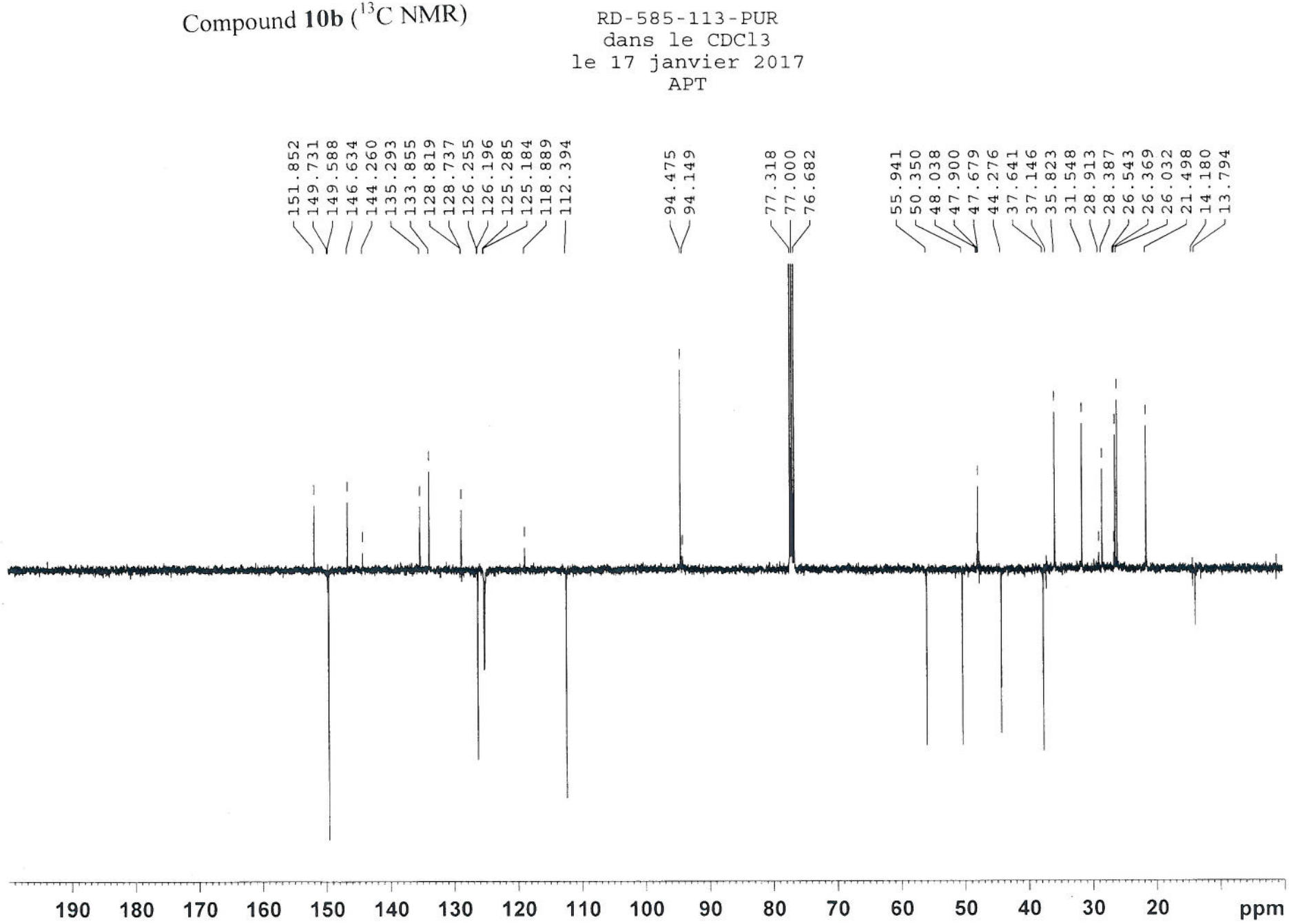


RD-585-113 (pur)
Dans le CDCl3
le 23 septembre 2016
RMN 1H 400MHz

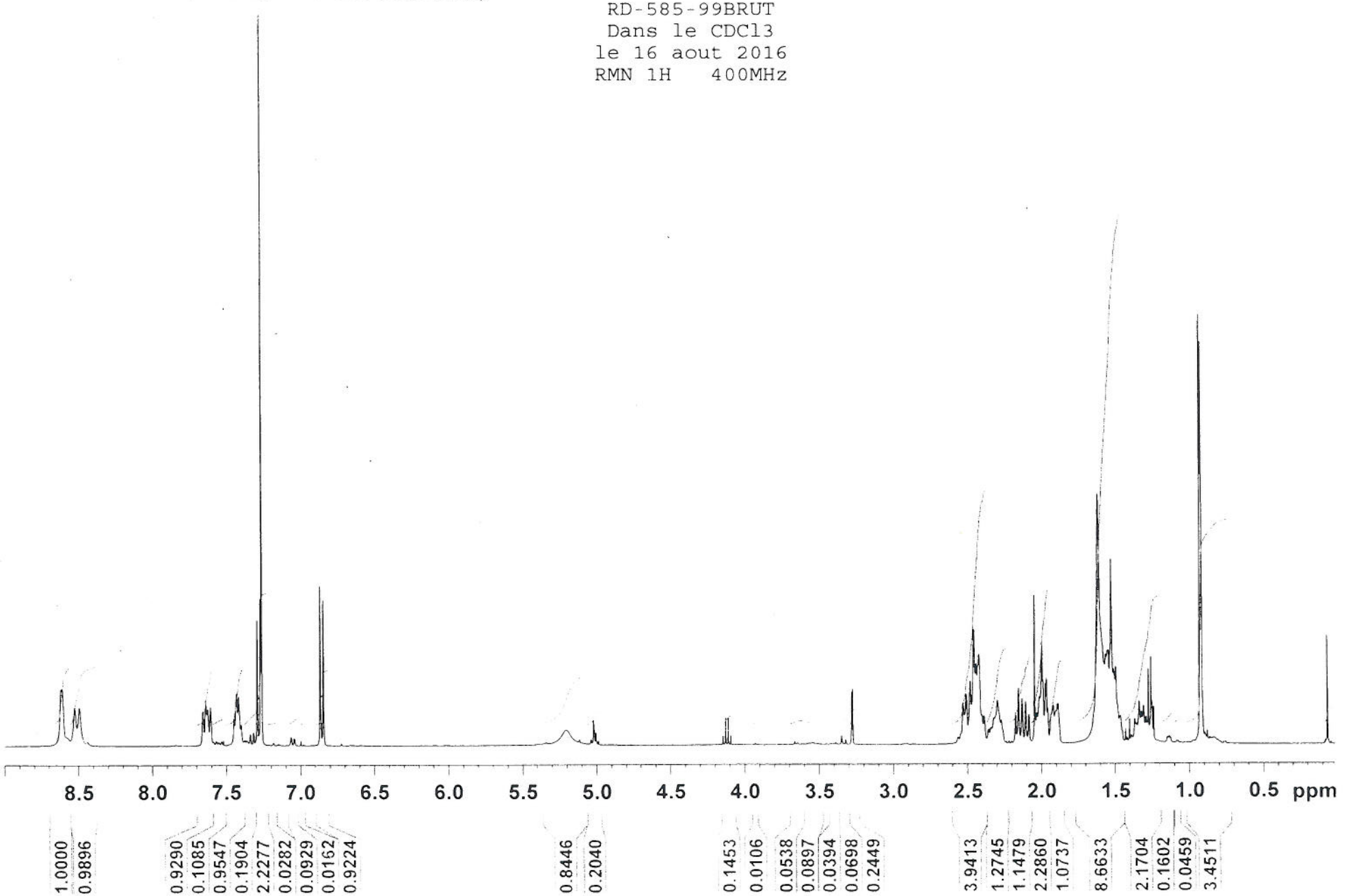
Compound **10b** (^{1}H NMR)



Compound **10b** (^{13}C NMR)

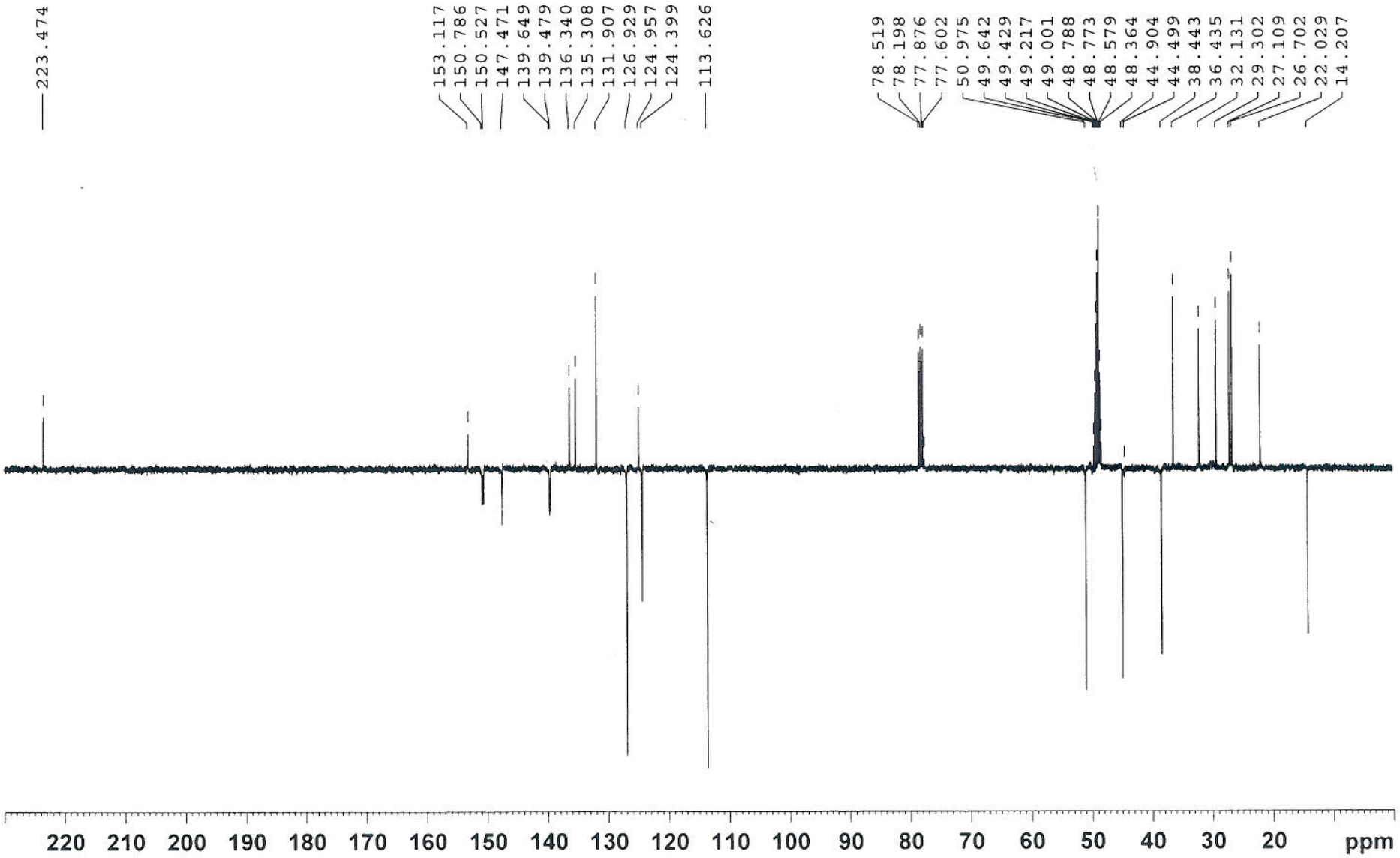


Compound **11a** (^{1}H NMR)



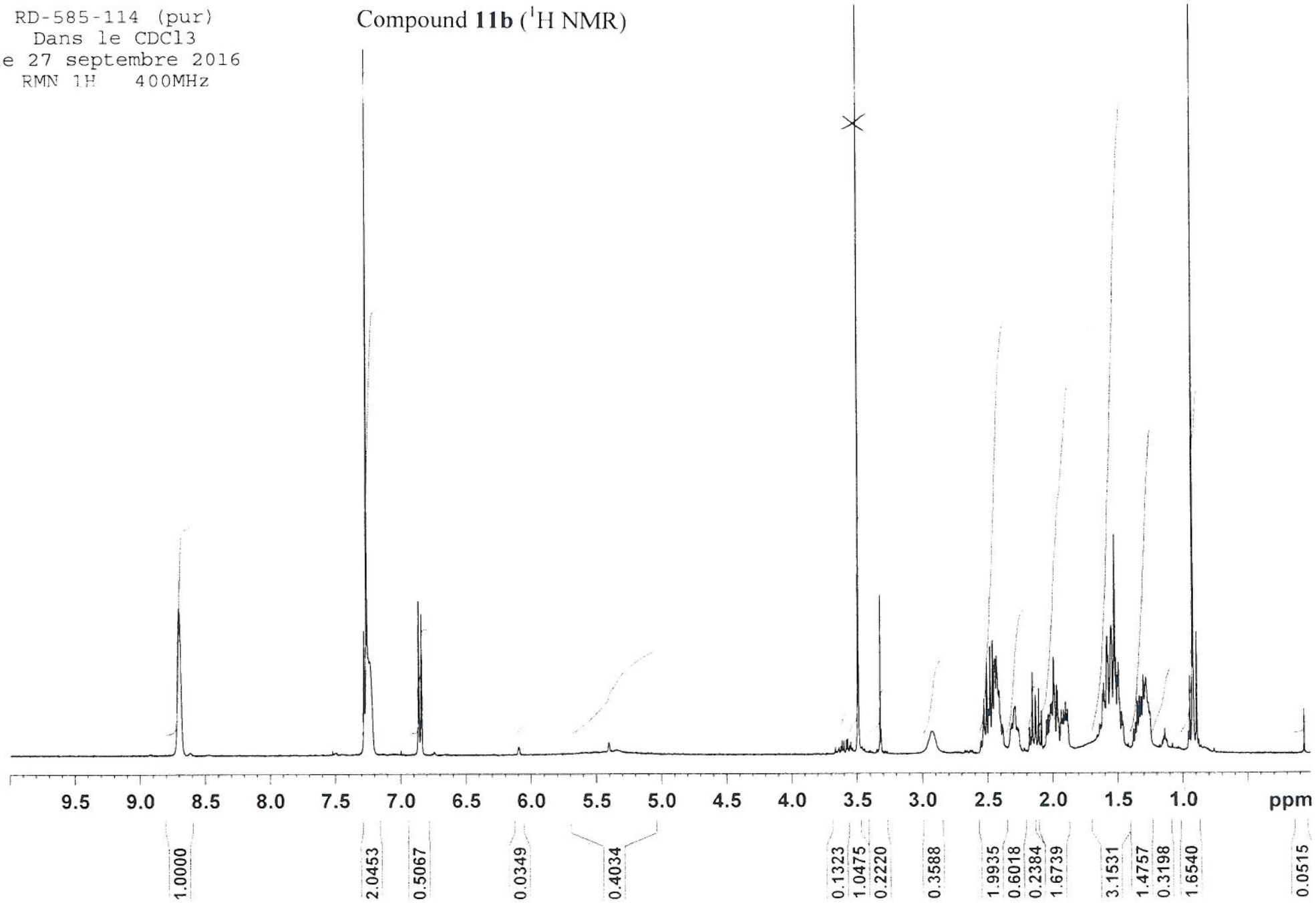
Compound **11a** (^{13}C NMR)

RD-585-99
le 17 novembre 2016
dans le MeOD:CDCl3 1:1
APT



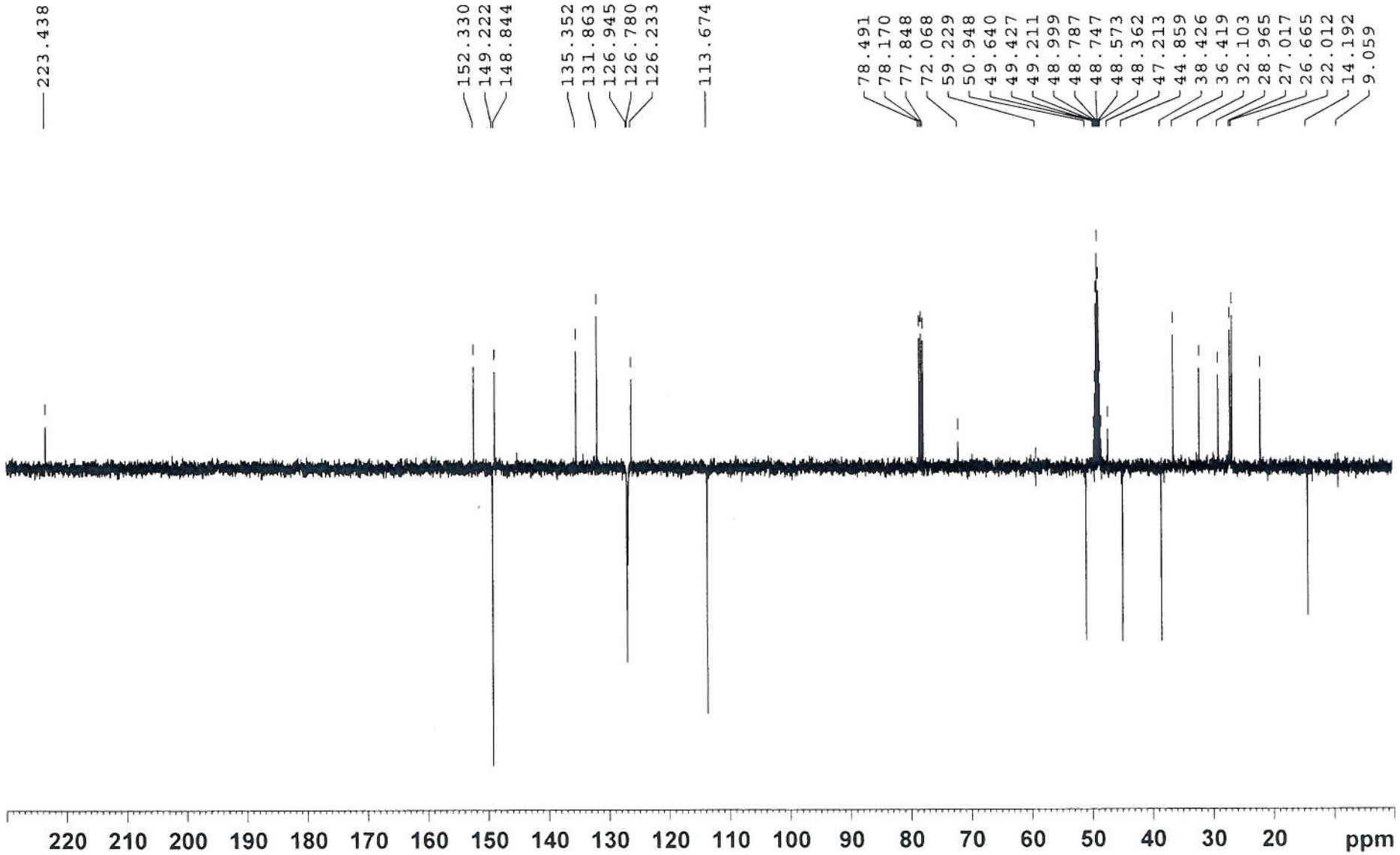
RD-585-114 (pur)
Dans le CDCl3
le 27 septembre 2016
RMN 1H 400MHz

Compound **11b** (^{1}H NMR)

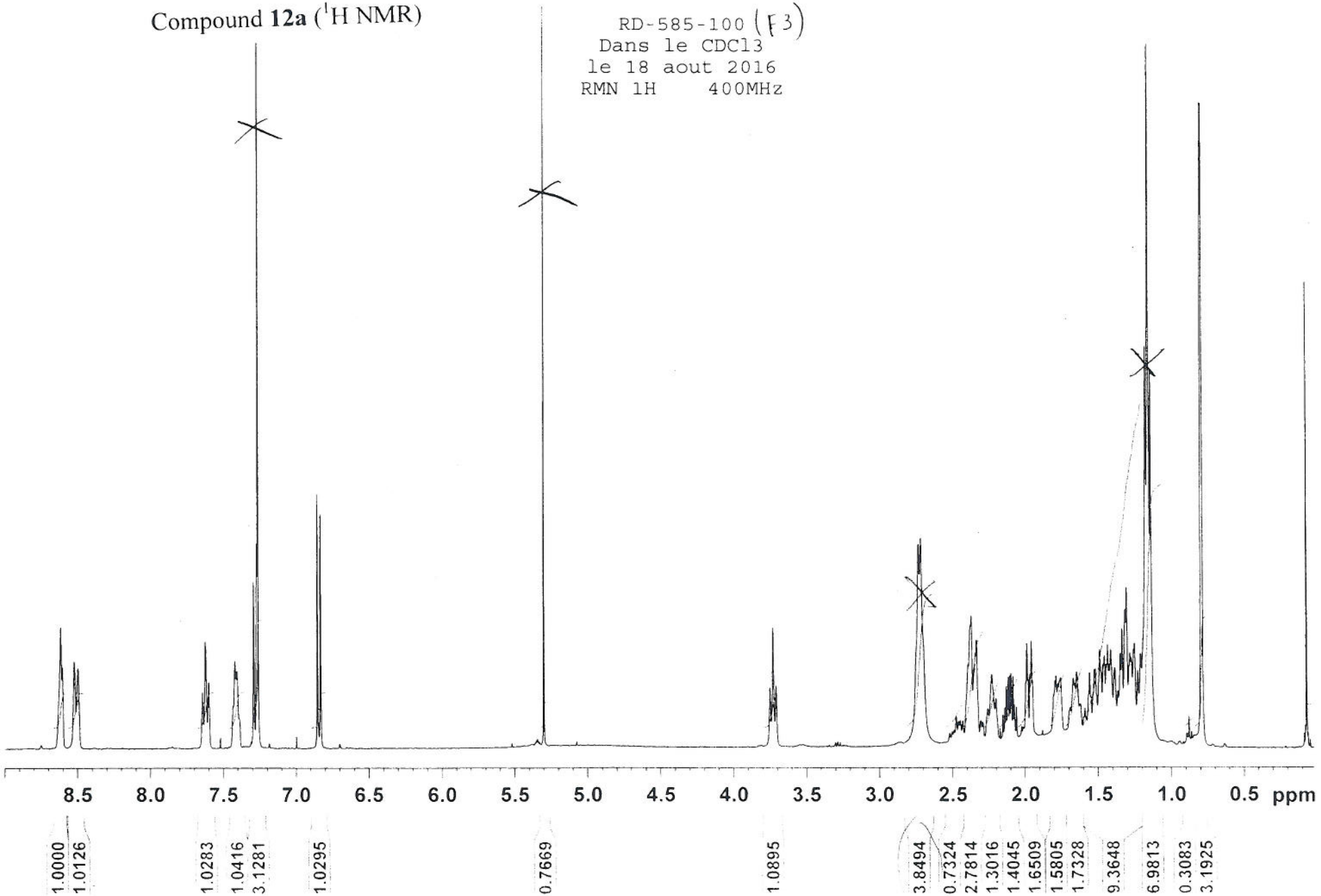


Compound **11b** (^{13}C NMR)

RD-585-114
le 18 novembre 2016
dans le MeOD:CDCl3 1:1
APT

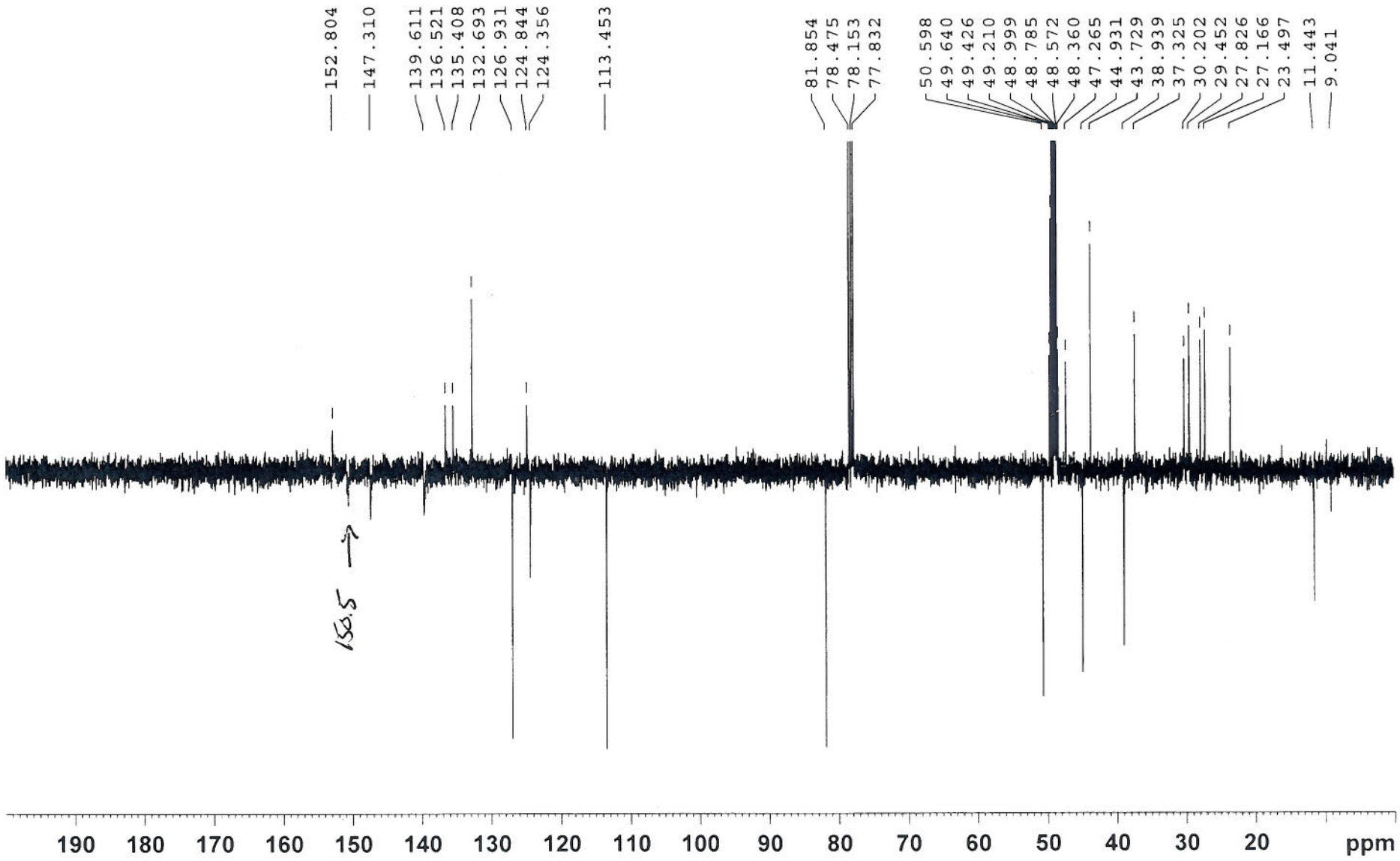


Compound **12a** (^{1}H NMR)

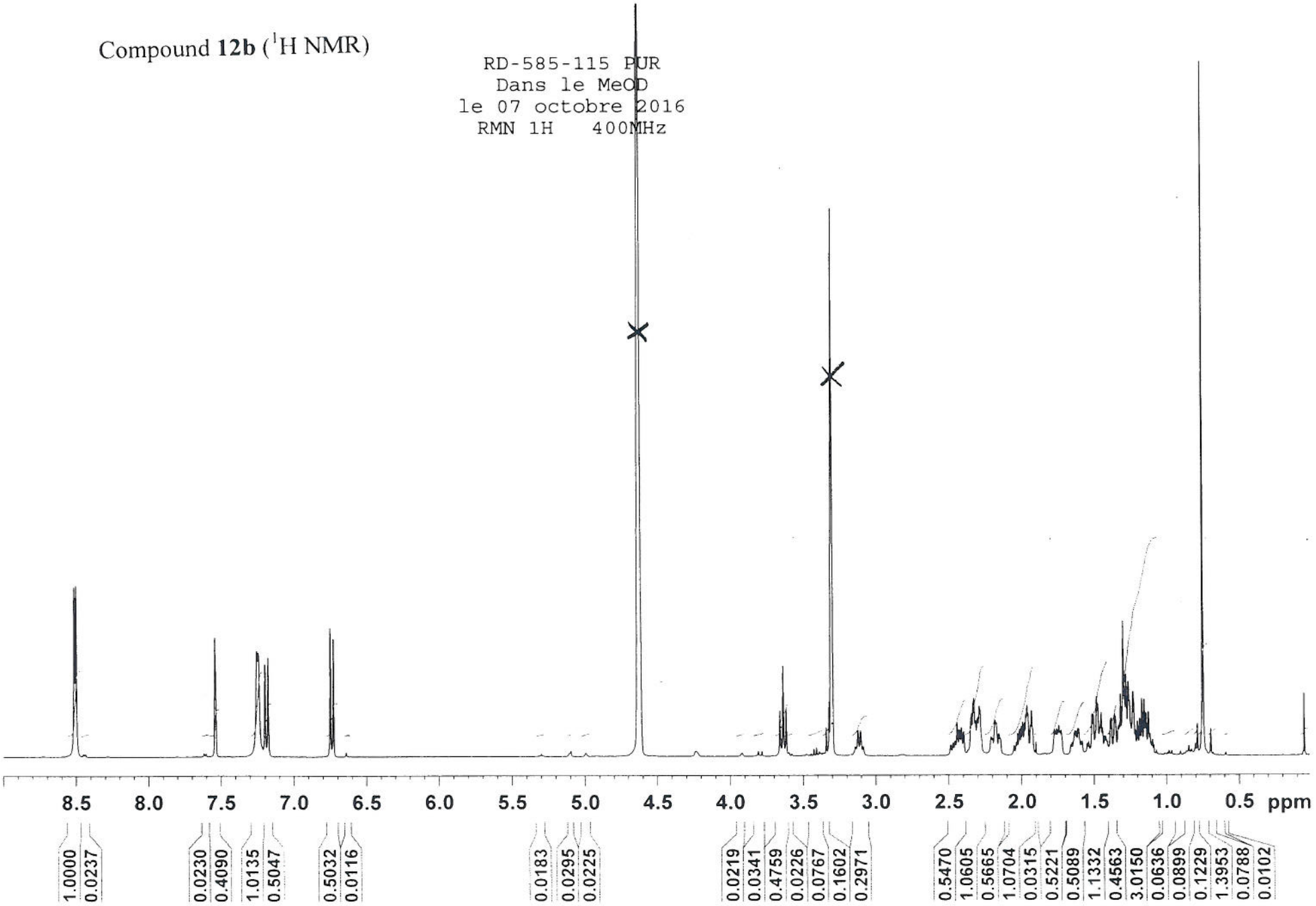


Compound **12a** (^{13}C NMR)

RD-585-100F3
Dans le MeOD;CDCl3 1:1
le 01 decembre 2016
APT

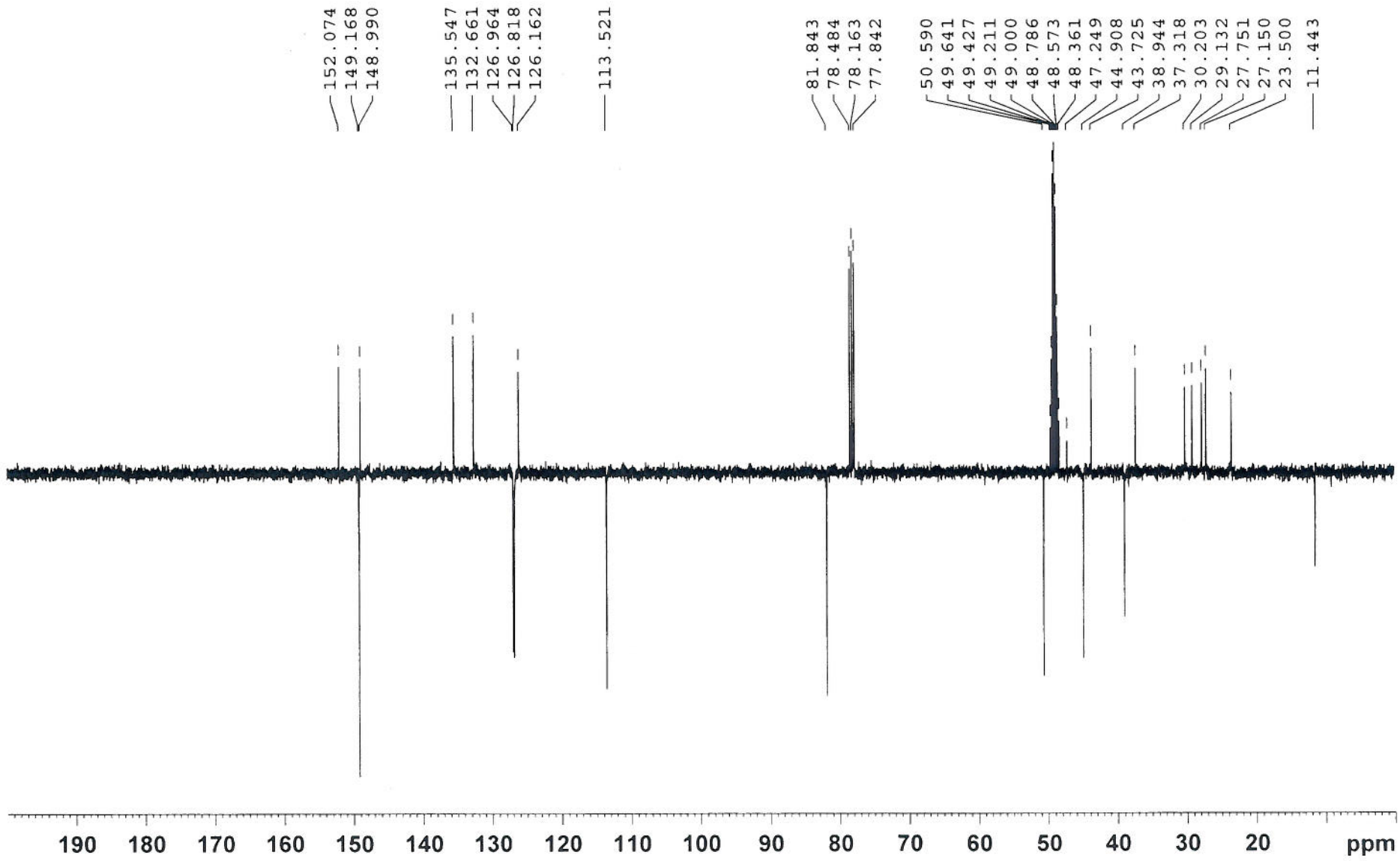


Compound **12b** (^{1}H NMR)



Compound **12b** (^{13}C NMR)

RD-585-115
le 11 novembre 2016
dans le MeOD
APT



3. Description of the enzymatic assay (EROD assay)

The inhibitory activity of tested compounds against CYP1B1 was determined using the ethoxyresorufin-*O*-deethylase (EROD) assay according to the manufacturer's instructions (Corning, Woburn, MA, USA; BD Bioscience, Mississauga, ON, Canada). Briefly, 7-ethyl-*O*-resorufin (4 μM) was used as an enzyme substrate and a NADPH regenerating system containing 1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 3.3 mM $MgCl_2$ (solution A) and 0.5 U/mL glucose-6-phosphate dehydrogenase (solution B) was used instead of NADPH (1.67 mM) in a final volume of 90 μL in tris-acetate buffer pH 7.4 by well. Each compound was dissolved in DMSO and added (5 μL) to the incubation mixture to obtain the final concentration needed. The DMSO concentration in the well was adjusted to 0.1%. Recombinant human CYP1B1 equipped with P450 reductase (Supersomes; BD Bioscience) was used as an enzyme source and the enzymatic reaction in triplicate was initiated by adding 5 μL of CYP1B1 (0.7 pmol) dissolved in tris-acetate buffer. The plate was incubated for 45 min at 37 °C and fluorescence derived from the formation of resorufin was recorded (96-well microplate reader INFINITE 500 PRO series; Tecan, Männedorf, Switzerland) with excitation and emission filters at 535 and 590 nm, respectively. For the screening, the percentage of inhibition was calculated at one concentration (0.3 μM), whereas several concentrations (0.01, 0.05, 0.1, 0.5, 1.0, 3.0, and 5.0 μM) were used for the IC_{50} value determined using GraphPad Prism 6 software.

4. Plasmatic concentration assay (4a injected in rats)

This *in vivo* experiment was approved by our Institutional Animal Care and Use Committee *(Comités de protection des animaux de l'Université Laval, Québec, Qc, Canada)* and carried out according to the guidelines of the Canadian Council on Animal Care. During the acclimatization and study periods, the animals were housed under a controlled environment at 22 ± 3 °C, with 50 ± 20% relative humidity and light set at 12 h/day (light on at 07:15). Sterile rodent food (Rodent diet #T.2018.15, Harlan Teklad, Madison, WI) and water were provided ad libitum. During this experiment, rats were housed individually and were fasted for 8 h before the inhibitor injection.

For the experiment, three male rats weighing approximately 340 g received a single subcutaneous injection (0.5 mL) of compound **4a** (2.0 mg/kg) in propylene glycol:DMSO (92:8). The compound was firstly dissolved in DMSO and thereafter we added propylene glycol to obtain a final concentration of 8 % DMSO. After 2, 4, 6, 12, and 24 h post-dose, a blood sample was collected in the jugular vein (0.4 mL) and added into a Microvette potassium-EDTA (ethylenediamine tetraacetic acid)-coated tube (Sarstedt, Aktiegesellchaft & Co, Germany), and centrifuged at 3200 rpm for 10 min at 4 °C. The plasma concentrations of **4a** and its oxidized metabolite **3a** were determined by LC-MS/MS using a procedure developed at the CHUQ (CHUL) - Research Center (Québec, QC, Canada).[7,8]

Briefly, for extraction from plasma, 100 μL of sample is transferred to individual tubes, and 600 μL of ammonium acetate (1 mM) is added. A methanolic solution (50 μL) containing a steroidal internal standard is then added to each tube. Samples are transferred on Strata-X SPE columns (Phenomenex, Torrance, CA, USA), which has been conditioned with 2 mL of MeOH and 2 mL of water. Each column is washed with 2 mL of MeOH:water (10:90, v/v). The aminosteroid is then eluted with 5 mL of MeOH containing 5 mM ammonium acetate. MeOH is evaporated at 45 °C under inert atmosphere and the residue dissolved in 100 μL of MeOH:water (85:15, v/v). For the aminosteroid analysis, the HPLC system uses a 75 mm x 4.6 mm, 3 μm reversed-phase Luna Phenyl-Hexyl column (Phenomenex, Torrance, CA, USA) at a flow rate of 0.8 mL/min. The inhibitor is detected

using an API 3000 mass spectrometer, equipped with TurboIonSpray (Applied Biosystems, Canada). ESI in positive ion mode was used.

5. Figure A: E1, E2, and ANF scaffolds and docking results

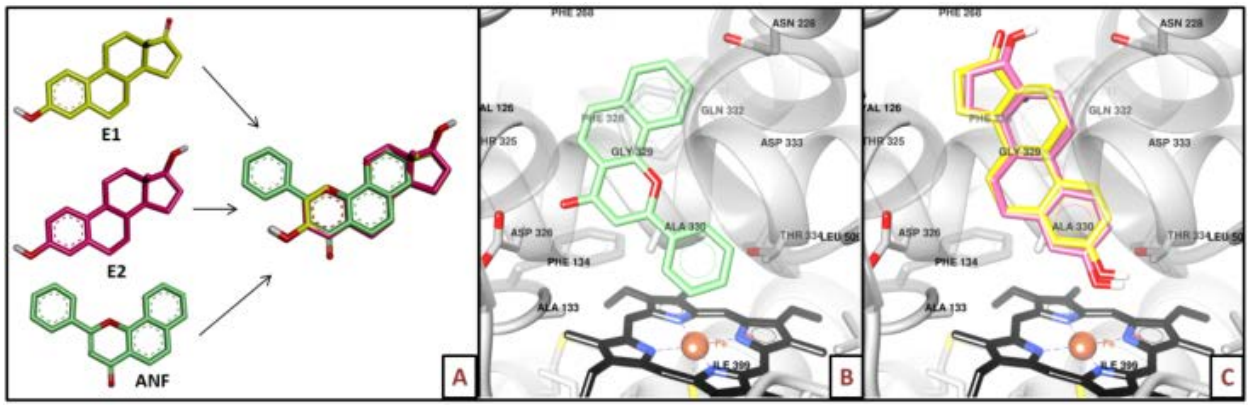


Figure A. Steroidal E1 and E2 scaffolds compared to the flavonoid scaffold of ANF (A), co-crystal structure of ANF in CYP1B1 (**B**) and the overlay of docked E1 and E2 in CYP1B1 (E1: pink and E2: yellow) (**C**).

6. Figure B: Inhibition curves for 3a, 3b, 4a, 4b, and ANF (IC_{50} determination)

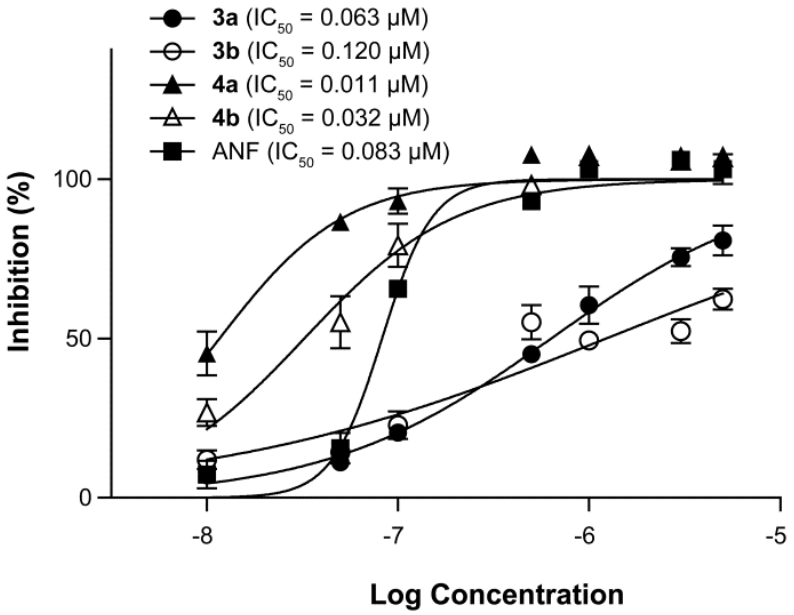


Figure B. Inhibition of CYP1B1 activity (EROD assay) by steroid derivatives **3a** (2-(pyridin-3-yl)-E1), **3b** (2-(pyridin-4-yl)-E1), **4a** (2-(pyridin-3-yl)-E2), and **4b** (2-(pyridin-4-yl)-E2) as well as non-steroidal known inhibitor ANF (α-naphthoflavone). IC_{50} value was determined from the inhibition curve of each compound tested at several concentrations (0.01, 0.05, 0.1, 0.5, 1.0, 3.0, and 5.0 µM).

7. References

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