Supplementary Information

Androgen receptor targeted conjugates for bimodal photodynamic therapy of prostate cancer in vitro

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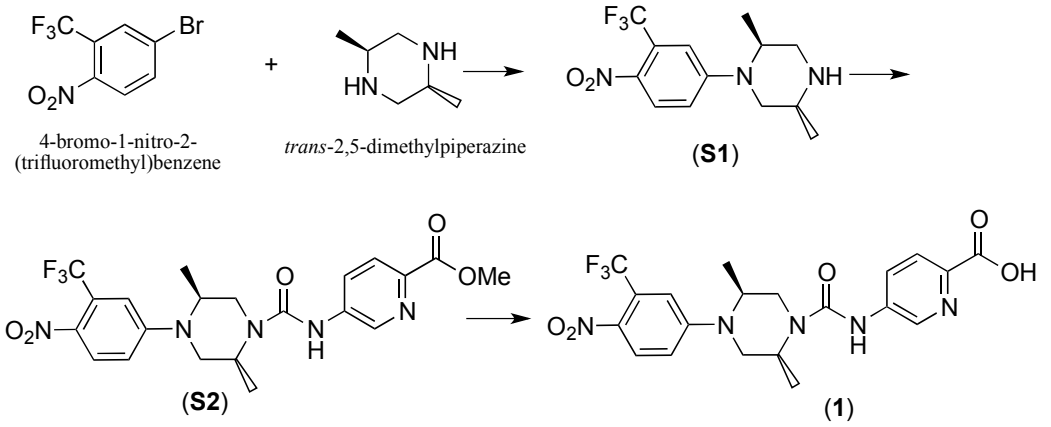
Synthesis of acid 1.

A slightly modified literature procedure was followed for the synthesis of acid (**1**).[1] The synthetic pathway is reported in **Scheme S1**.



Scheme S1. Synthesis of acid (**1**)

Synthesis of (**S1**): 4-Bromo-1-nitro-2-(trifluoromethyl) benzene (1 eq.) was reacted with racemic *trans*-2,5-dimethylpiperazine (4 eq.) in DMF (0.7 mL/mmol) and stirred at 80°C for 20 h. The reaction mixture was than purged into distilled water and extracted with EtAOc. The reaction crude was purified by silica gel flash chromatography (CH_3Cl/MeOH = 20/1) to afford **S1** as pale yellow solid in 53% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 9.2 Hz, 1H, ArH), 7.13 (d, *J* = 2.8 Hz, 1H, ArH), 6.92 (dd, *J* = 9.2, 2.8 Hz, 1H, ArH), 3.81 (dt, *J* = 6.6, 4.4 Hz, 1H, CH_2), 3.44 – 3.30 (m, 3H, 1H CH_2, 2 H CH), 3.17 (dd, *J* = 12.3, 4.4 Hz, 1H, CH_2), 2.73 (dd, *J* = 12.9, 4.4 Hz, 1H, CH_2), 1.62 (bs, 1H, NH), 1.22 (t, *J* = 6.5 Hz, 6H). ^{13}C NMR (100 MHz, CDCl3) δ 153.77, 148.79, 128.45, 123.67, 116.02, 113.47, 113.40, 50.55, 50.05, 48.13, 46.54, 18.65, 14.78. HRMS (m/z) calcd for $C_{21}H_{22}F_3N_5O_5$ $[M]^+$, 482.1573; found, 482.1829.

Synthesis of (**S2**): **S1** (1 eq.) was than reacted with methyl 4-isocyanatobenzoate (1.5 eq.) in anhydrous toluene (2 mL7mmol). The reaction mixture was stirred at room temperature for 10 min and than concentrated under reduced pressure. The crude material was purified by silica gel flash column chromatography (CH_3Cl/MeOH = 20/1) to afford **S2** as pale yellow solid in 84% yield. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.14 (s, 1H), 8.79 (d, *J* = 2.6 Hz, 1H, ArH), 8.15 – 8.04 (m, 2H, ArH), 7.97 (d, *J* = 8.6 Hz, 1H, ArH), 7.26 – 7.23 (m, 2H, ArH), 4.55 – 4.49 (m, 1H), 4.43 – 4.36 (m, 1H), 3.91 (d, *J* = 13.7 Hz, 1H), 3.82 (s, 3H, OCH_3), 3.81 – 3.74 (m, 1H), 3.48 – 3.43 (m, 2H), 1.19 (d, *J* = 6.5 Hz, 3H, CH_3), 1.12 (d, *J* = 6.4 Hz, 3H,

(1) Kinoyama, I.; Taniguchi, N.; Toyoshima, A.; Nozawa, E.; Kamikubo, T.; Imamura, M.; Matsuhisa, A.; Samizu, K.; Kawanimani, E.; Niimi, T.; Hamada, N.; Koutoku, H.; Furutani, T.; Kudoh, M.; Okada, M.; Ohta, M.; Tsukamoto, S. *J. Med. Chem.* **2006**, *49*, 716.

CH_3). ^{13}C NMR (101 MHz, DMSO) δ 165.63, 155.27, 153.66, 141.30, 141.16, 140.72, 135.74, 129.64, 126.26, 126.02, 115.78, 111.99, 79.86, 52.68, 49.52, 47.13, 45.63, 43.45, 16.20, 13.92. . HRMS (m/z) calcd for $C_{13}H_{16}F_3N_3O_2$ $[M]^+$, 304.1195; found, 304.1245.

Synthesis of activated Pba (4)

N-hydroxysuccinimide (1 equiv) and DCC (1 equiv) were subsequently added to a solution of P*ba* (1 eq.) in anhydrous 1,4-dioxane (30 mL/mmol) under nitrogen atmosphere. The reaction was kept in the dark and stirred at room temperature for 5 h. After that, the reaction mixture was concentrated under reduced pressure and absorbed on silica. The silica gel flash column chromatography (CH_2Cl_2/acetone = 10/1) afforded (**4**) as a dark-purple solid in 40% yield.

^1H NMR (400 MHz, $CDCl_3$) δ 9.53 (s, 1H), 9.38 (s, 1H), 8.63 (s, 1H), 7.96 (dd, J = 17.8, 11.6 Hz, 1H), 6.30 – 6.16 (m, 3H), 4.57 – 4.47 (m, 1H), 4.36 – 4.29 (m, 1H), 3.89 (s, 3H), 3.81 (s, 2H), 3.68 (d, *J* = 12.8 Hz, 2H), 3.40 (s, 3H), 3.20 (s, 3H), 2.79 (s, 4H) 2.55 – 2.32 (m, 2H), 1.84 (d, *J* = 7.3 Hz, 3H), 1.68 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.36, 171.97, 169.43, 168.91, 168.06, 145.12, 142.19, 138.00, 136.63, 136.37, 136.03, 132.11, 128.99, 122.97, 104.51, 97.59, 93.55, 64.62, 52.88, 50.81, 49.94, 29.31, 27.96, 25.53, 25.04, 19.43, 17.32, 12.15, 12.08, 11.18.

Supplementary figures

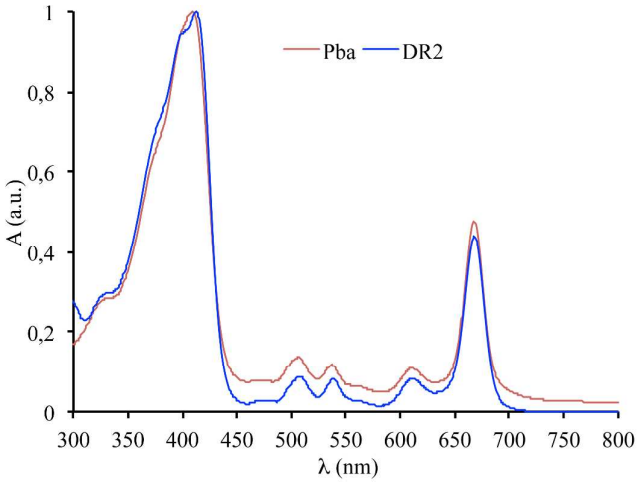


Figure S1. Absorption spectra of **DR2** and Pb*a* acquired in CH_2Cl_2.

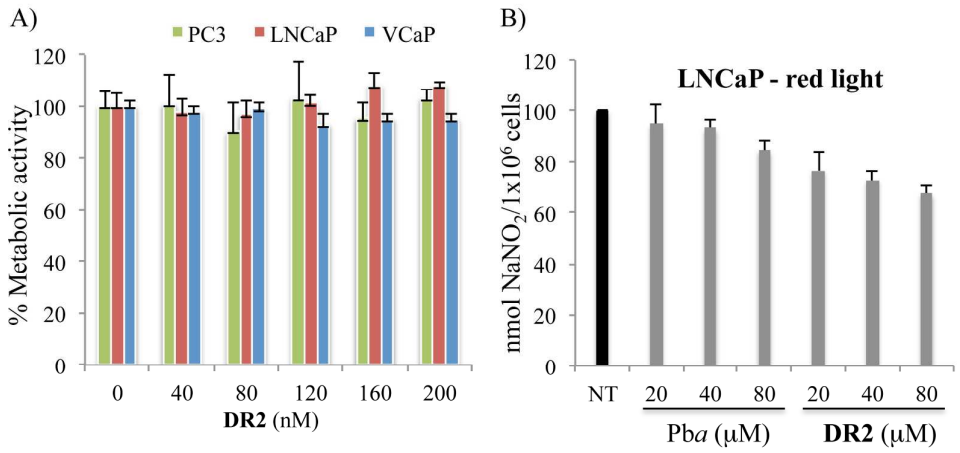
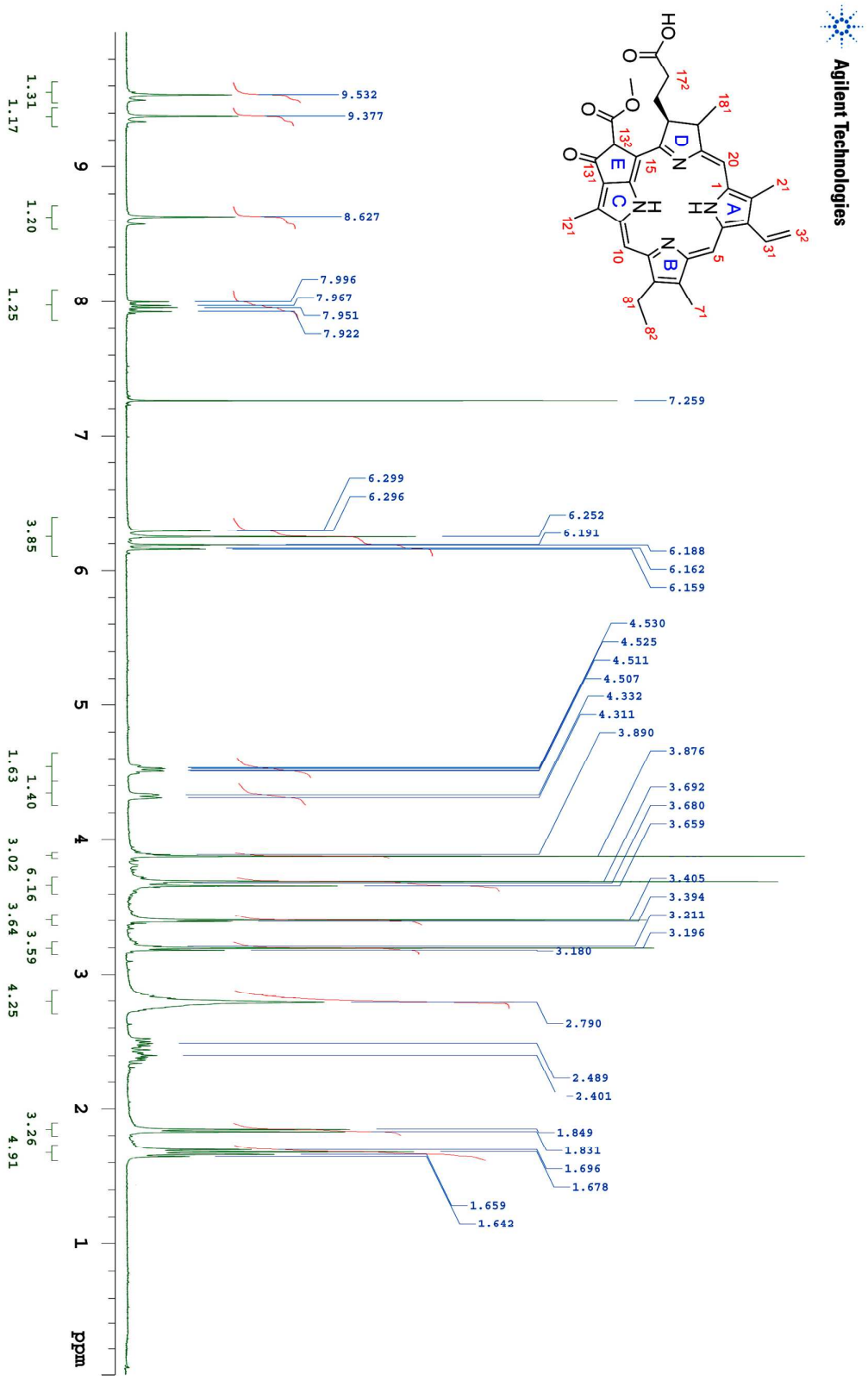
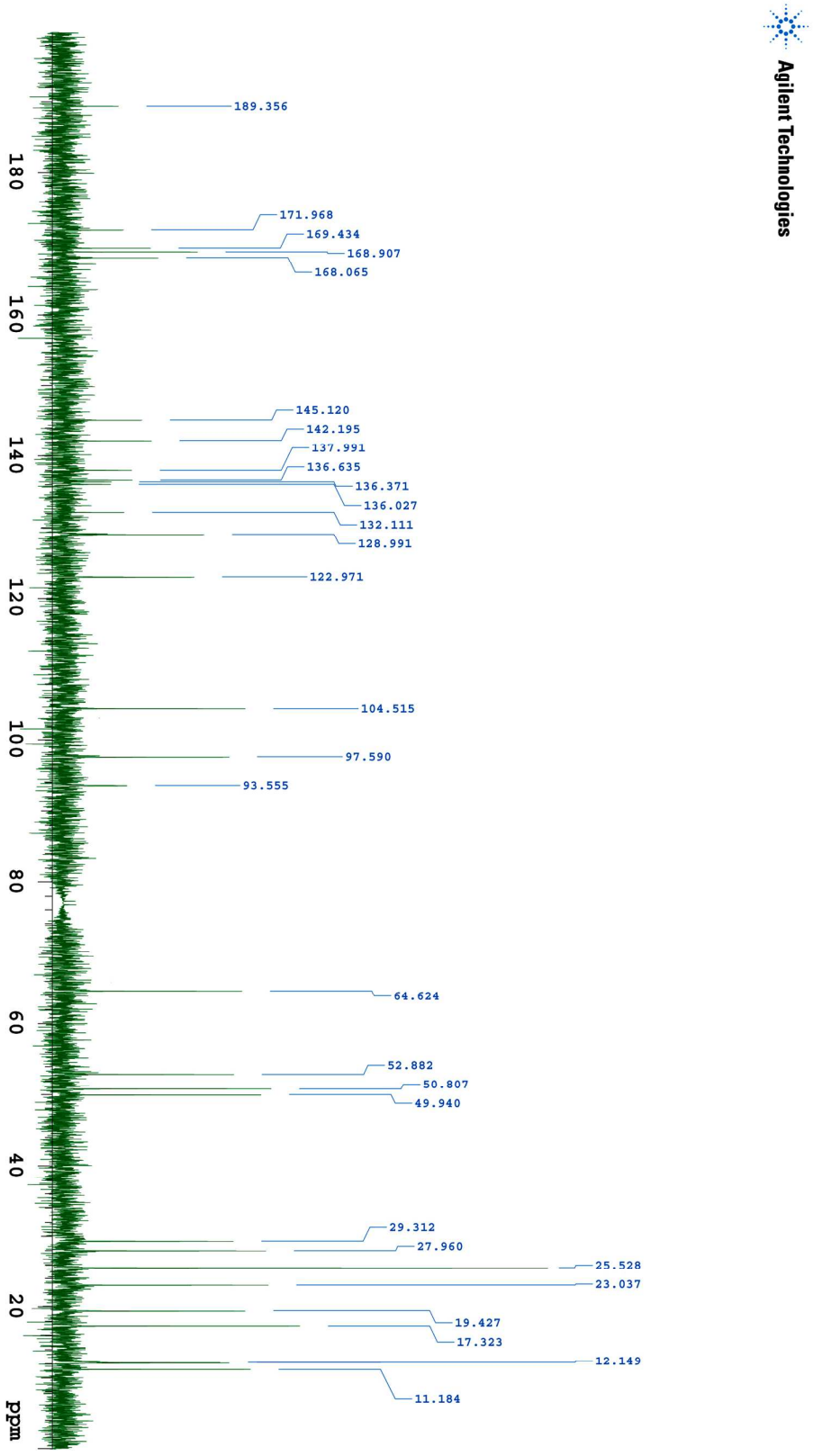


Figure S2. A) Toxicity in the dark of conjugate **DR2**. B) Photo-toxicity on LNCaP cells upon irradiation with white light equipped with a red filter after 6 h incubation time at increasing concentrations of the compounds. Data represent mean values ± SD of three independent experiments.

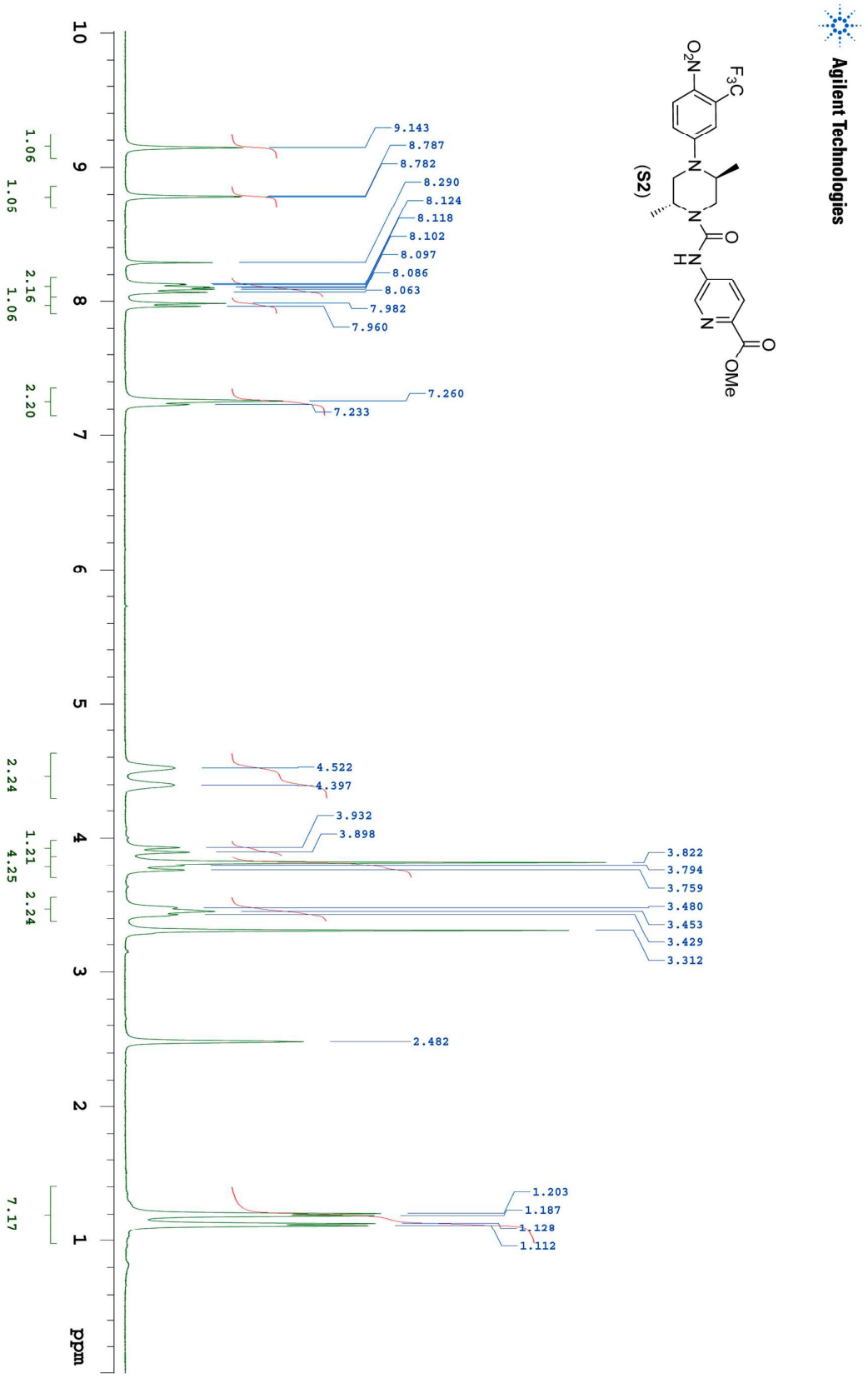
NMR Spectra



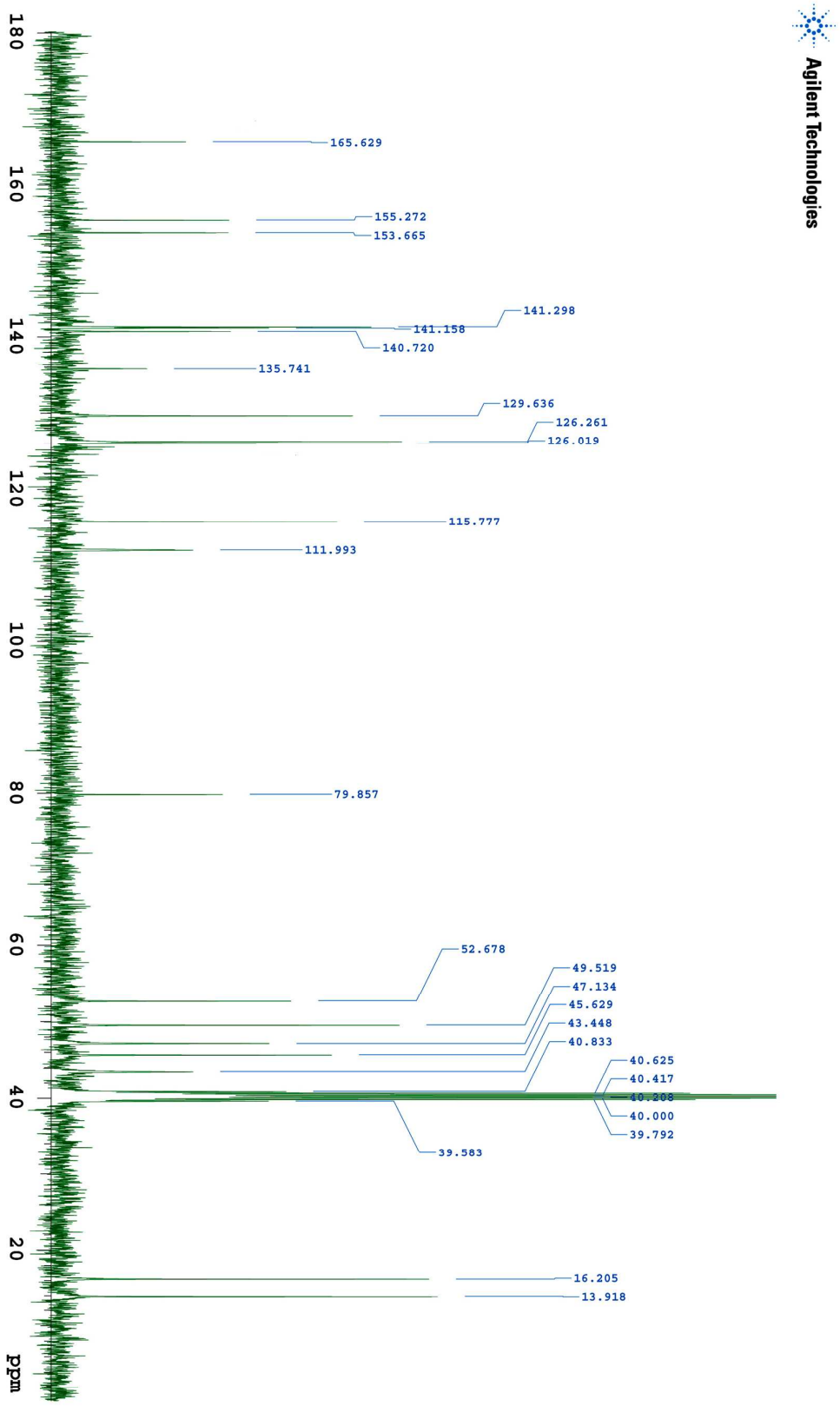
Compound (**4**): ^{1}HNMR ($CDCl_3$, 400 MHz)



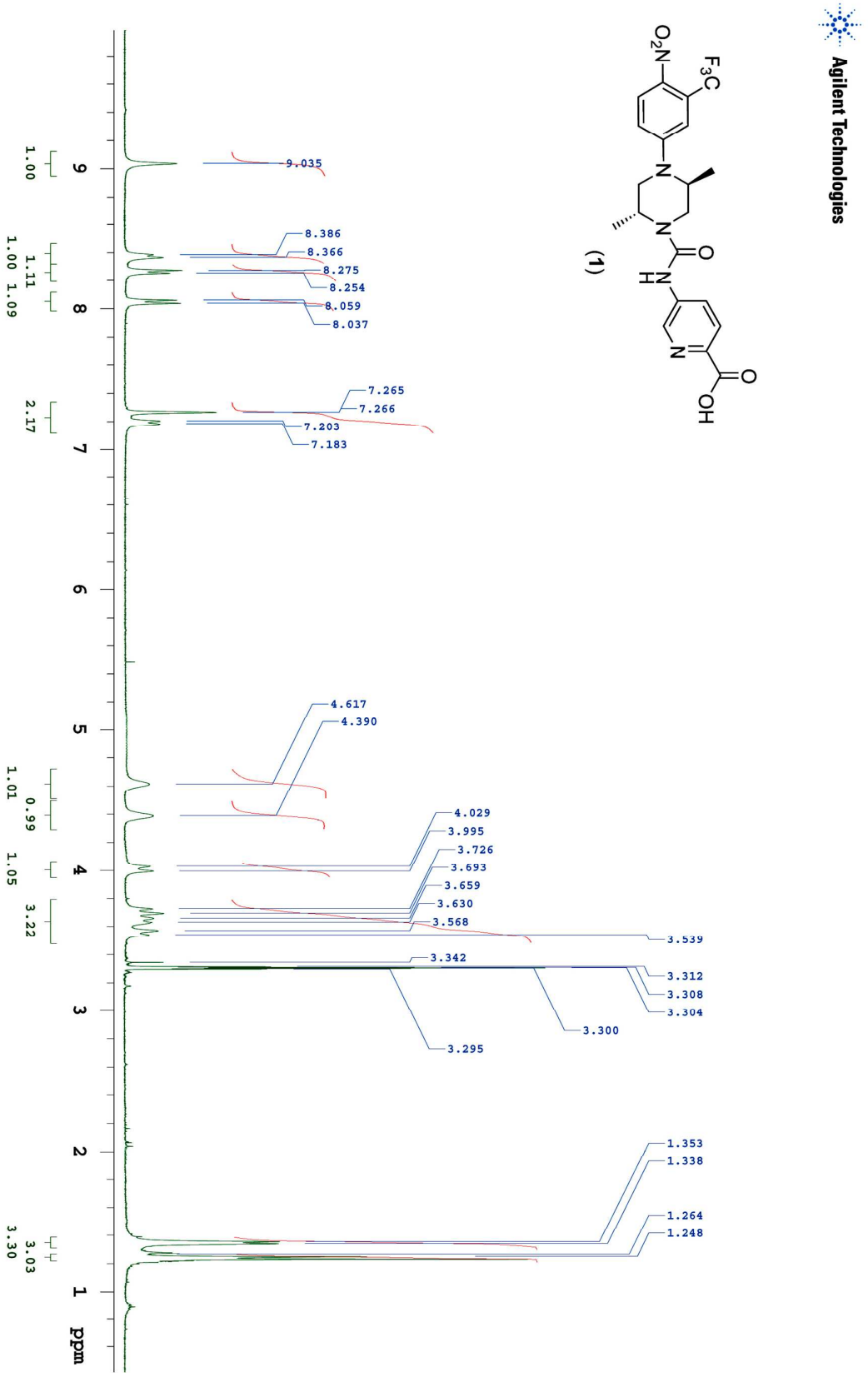
Compound (**4**): ^{13}CNMR ($CDCl_3$, 101 MHz) acquired with solvent (residual $CHCl_3$) pre-saturation sequence.



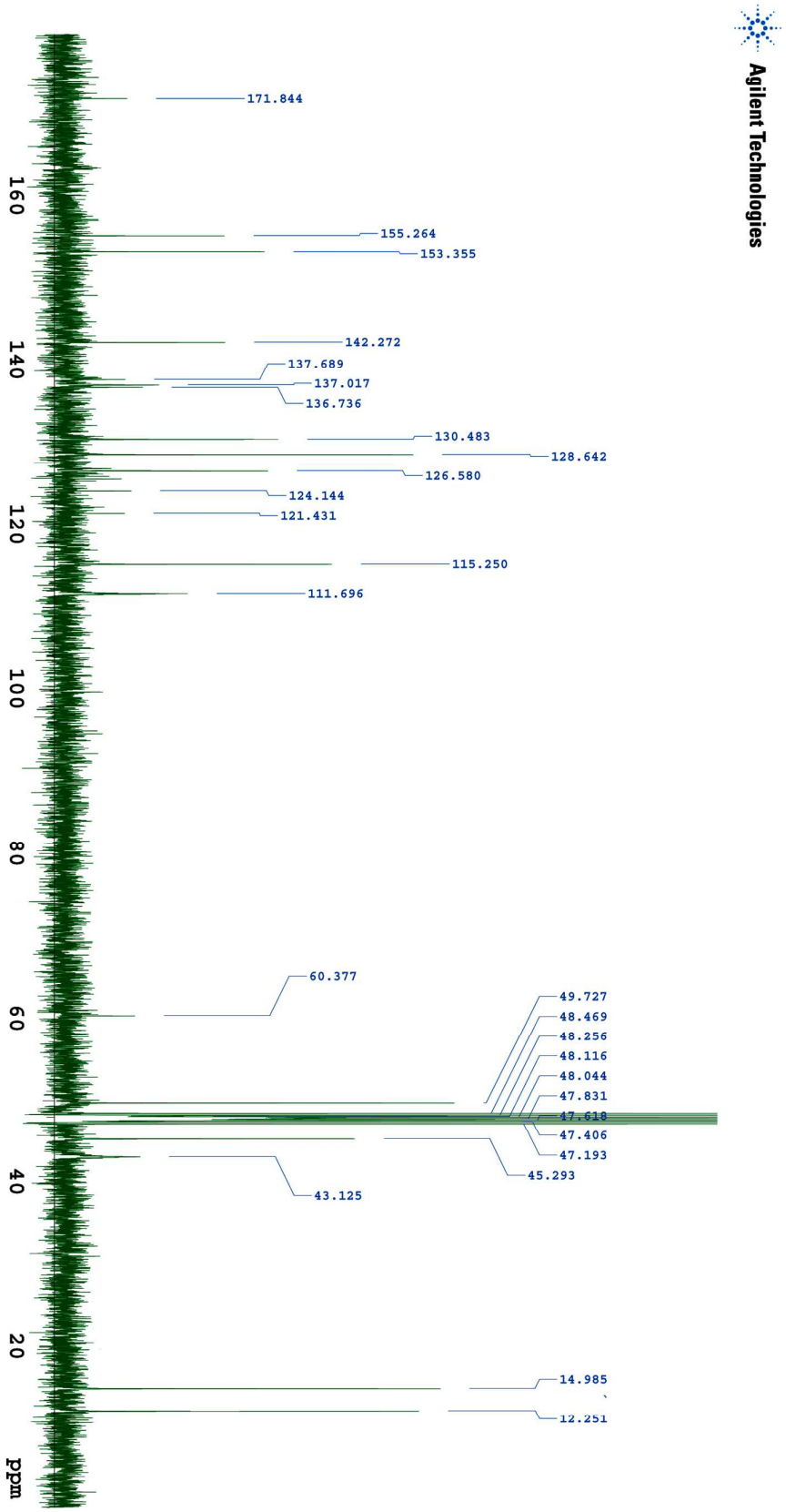
Compound (**S2**): ^{1}HNMR (DMSO, 400 MHz)



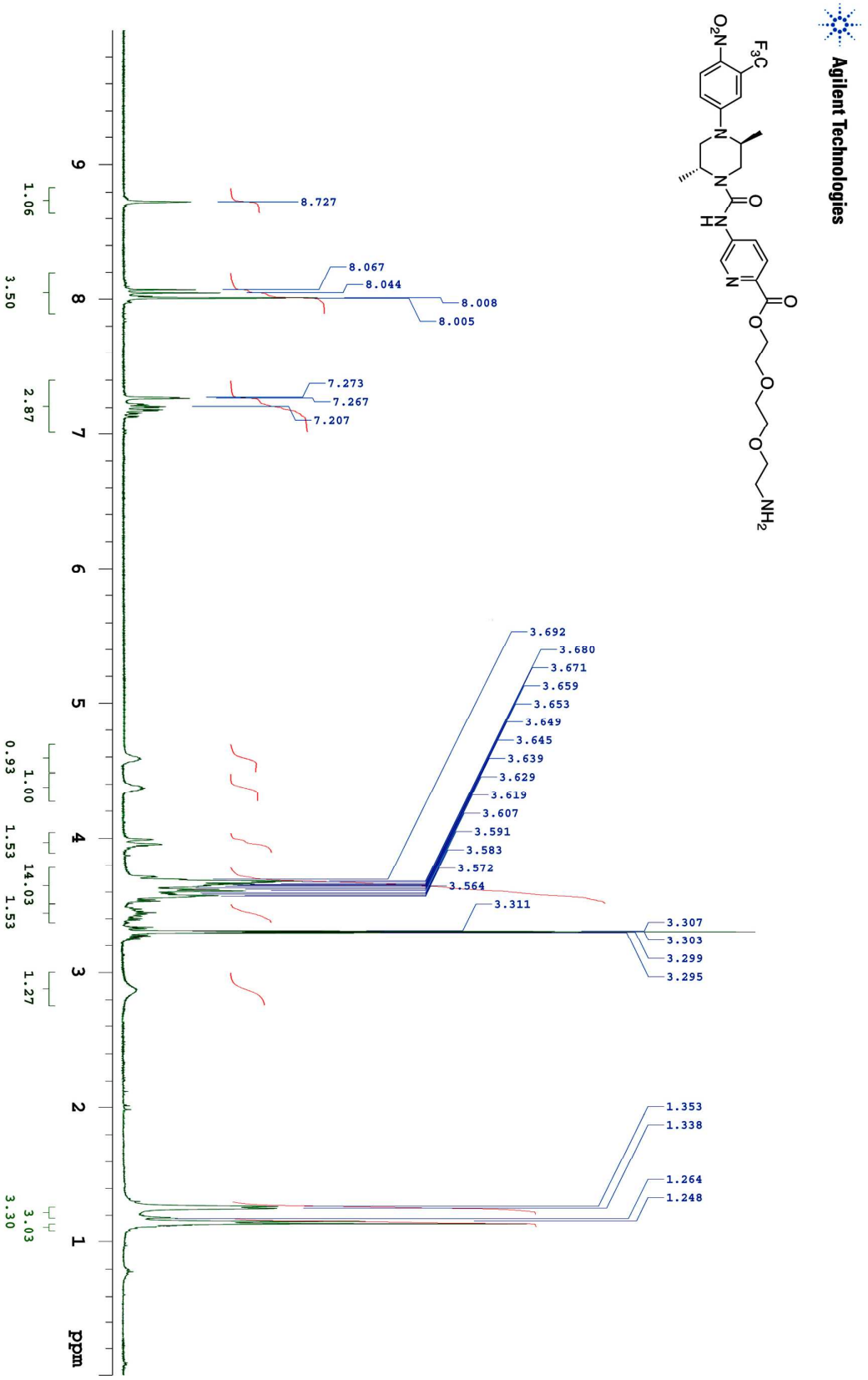
Compound (**S2**): ^{13}CNMR (DMSO, 101 MHz).



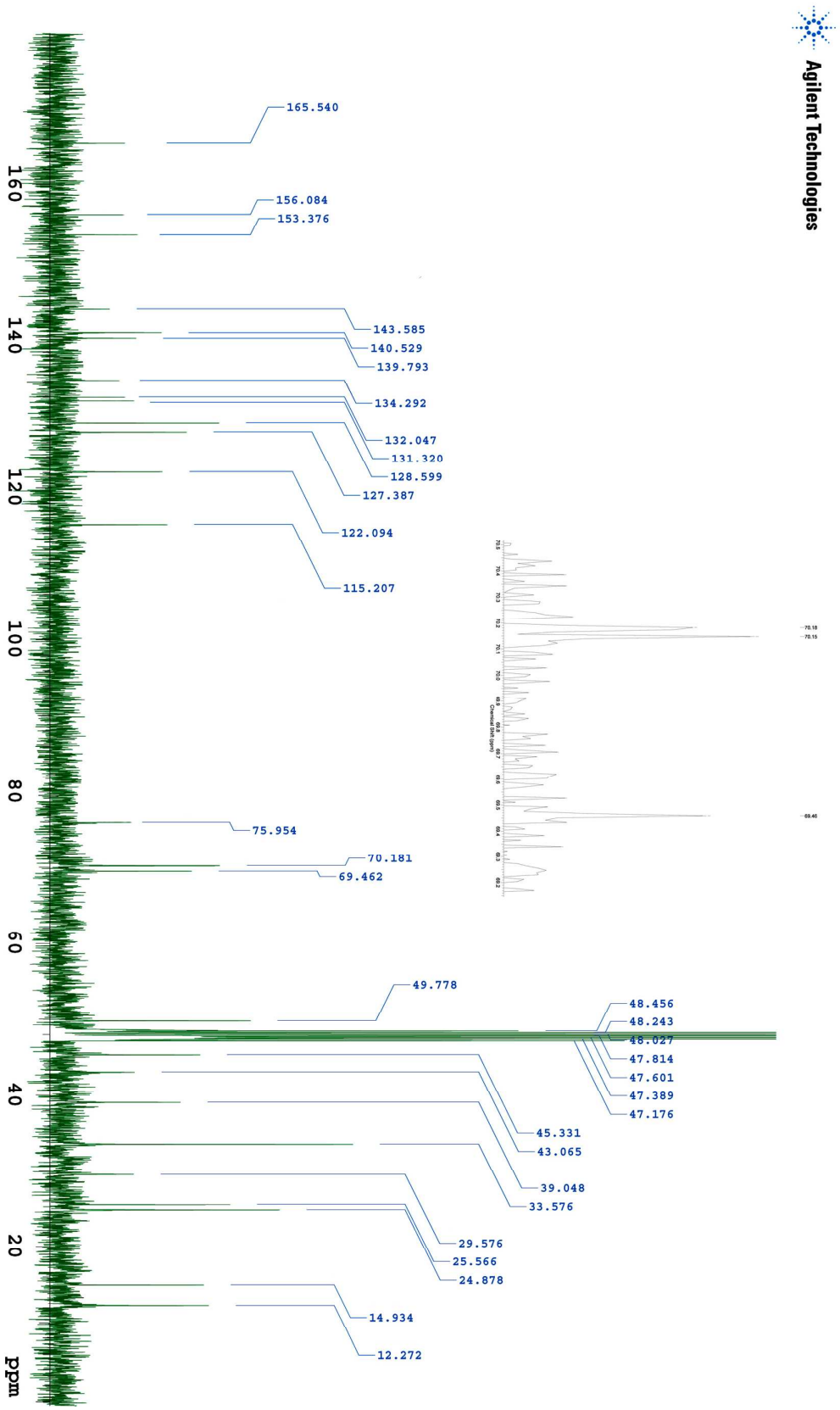
Acid (**1**): ^{1}HNMR (CD_3OD, 400 MHz)



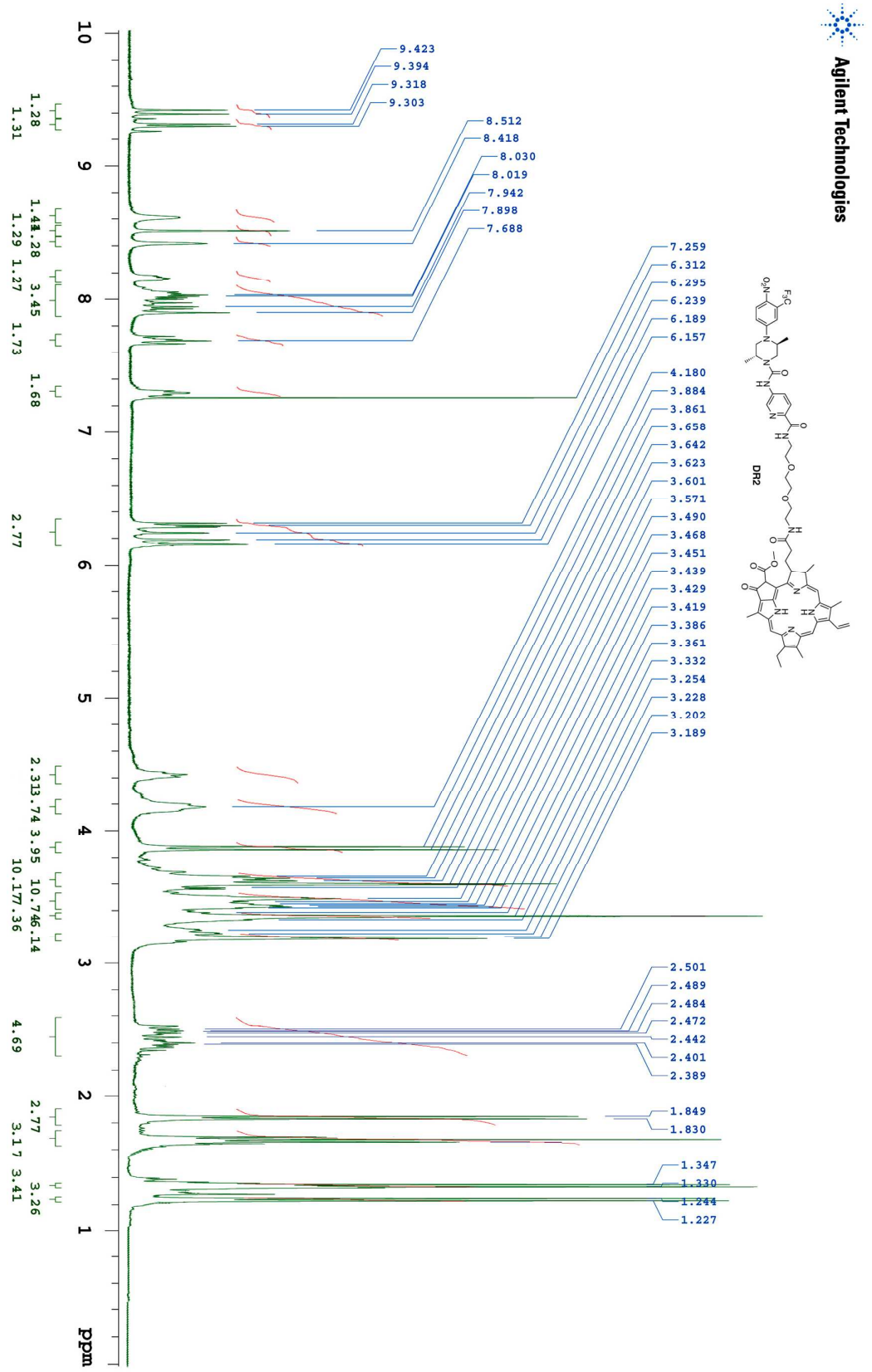
Acid (**1**): ^{13}CNMR (CD_3OD, 101 MHz).



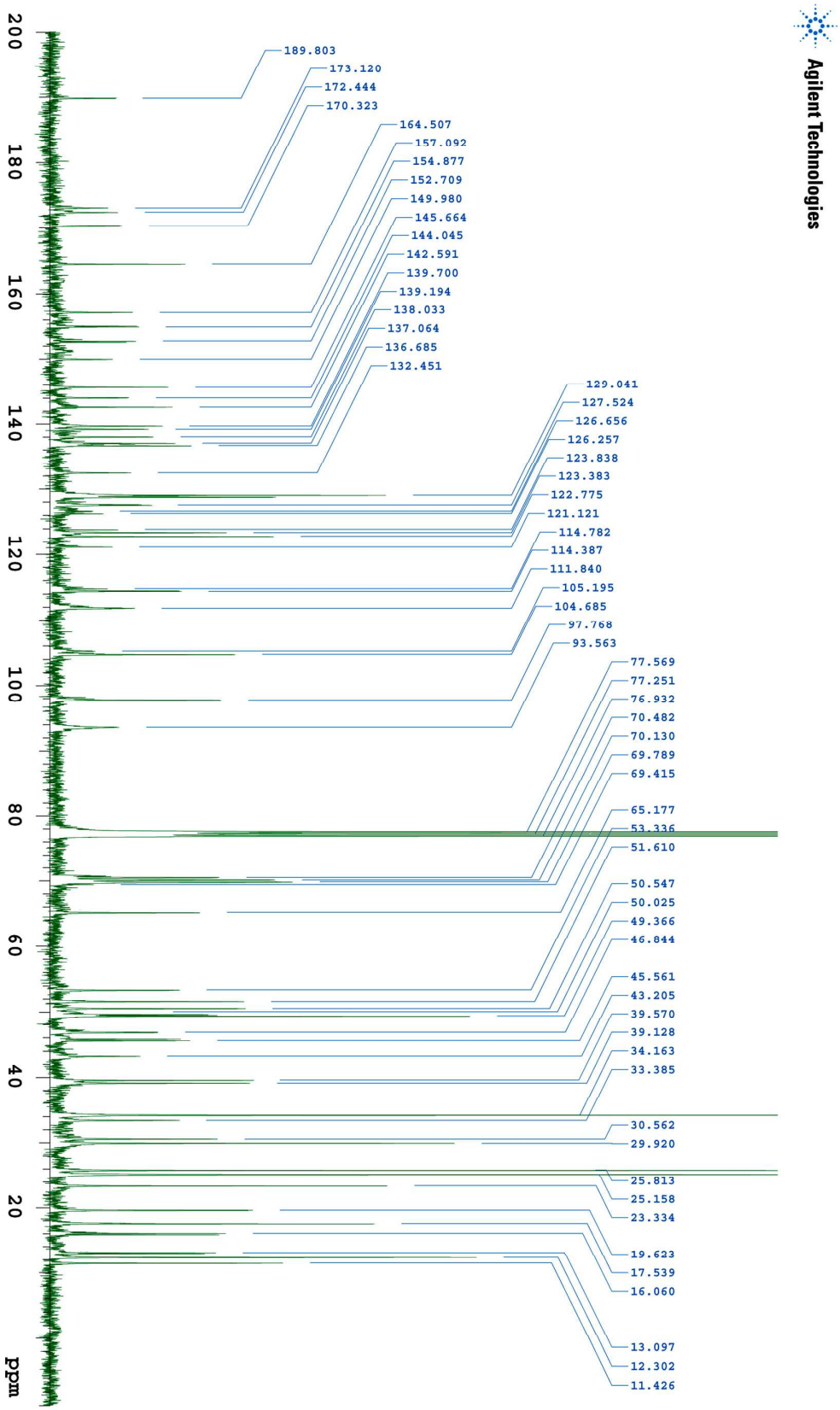
Compound (**3**): ^{1}HNMR (CD_3OD, 400 MHz).



Compound (**3**): ^{13}CNMR (CD_3OD, 101 MHz).



Compound (**DR2**): ^{1}HNMR ($CDCl_3$, 400 MHz).



Compound (**DR2**): ^{13}CNMR ($CDCl_3$, 101 MHz).