

# Supporting Information for

## **Site-Selective Protein Immobilization on Polymeric Supports through N-Terminal Imidazolidinone Formation**

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## **Methods and Materials**

**General Methods and Instrumentation.** Unless otherwise noted, all reagents were obtained from commercial sources and used without any further purification. Amino PEGA resins (Novabiochem®, loading 0.3 – 0.5 mmol/g) were purchased from MiliporeSigma. Compound **3** and **8** were synthesized by following literature procedures.<sup>1</sup> Pro-sfGFP<sup>2</sup> and uteroglobin C69S<sup>3</sup> were expressed according to the literature procedures. Streptavidin (recombinant from *E. coli*) was purchased from MyBioSource (San Diego, CA). Ribonuclease A (from bovine pancreas),  $\alpha$ -chymotrypsin (from bovine pancreas),  $\beta$ -lactoglobulin (from bovine milk), thioredoxin (recombinant from *E. coli*), triose phosphate isomerase (from rabbit muscle), and cytochrome c (from bovine heart) were obtained from Sigma Aldrich (St. Louis, MO). OC-GFP was made via an oxidative coupling (OC) reaction at the N-terminus of pro-sfGFP according to the literature procedure.<sup>4</sup> Water (dd-H<sub>2</sub>O) used for biological procedures was deionized using a Barnstead NANOpure purification system (Waltham, MA, USA). All organic reactions were carried out under nitrogen with standard Schlenk techniques. Dry THF for cross-coupling reactions was degassed with nitrogen for 30 min. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica gel 60-f254 plates and visualized by ultraviolet (UV) irradiation at 254 nm and/or 365 nm. Purifications by flash silica gel chromatography were performed using EM silica gel 60 (230–400 mesh).

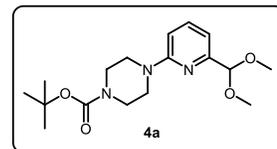
**NMR.** <sup>1</sup>H, <sup>13</sup>C, and <sup>19</sup>F spectra were measured with Bruker AV-300 (300 MHz, 75 MHz), AVB-400 (400 MHz, 100 MHz), or Bruker AV-600 (600 MHz, 151 MHz) spectrometer. <sup>1</sup>H NMR chemical shifts are reported as  $\delta$  in units of parts per million (ppm) relative to residual CHCl<sub>3</sub> ( $\delta$  7.26, singlet) or DMSO-d<sub>6</sub> ( $\delta$  2.50, pentet). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet or unresolved), or br (broad). Coupling constants are reported as a *J* value in Hertz (Hz). The number of protons (*n*) for a given resonance is indicated as *n*H and is based on spectral integration values. <sup>13</sup>C NMR chemical shifts are reported as  $\delta$  in units of parts per million (ppm) relative to CDCl<sub>3</sub> ( $\delta$  77.16, triplet) or DMSO-d<sub>6</sub> ( $\delta$  39.52, septet). <sup>19</sup>F NMR chemical shifts are reported as  $\delta$  in units of parts per million (ppm) relative to trifluoroacetic acid and ( $\delta$  -76.50 ppm vs. CFCl<sub>3</sub>).

**Mass Spectrometry.** High-resolution mass spectra (HRMS) for small molecule characterization were obtained at the UC Berkeley QB3/Chemistry Mass Spectrometry Facility using electrospray ionization (ESI). Proteins and protein conjugates were analyzed on an Agilent 6224 Time-of-Flight (TOF) mass spectrometer with a dual electrospray source (ESI) connected in-line with an Agilent 1200 series HPLC (Agilent Technologies, USA). Chromatography was performed using a Proswift RP-4H (Thermo Scientific, USA) column with a H<sub>2</sub>O/MeCN gradient mobile phase containing 0.1% formic acid. Mass spectra of proteins and protein conjugates were deconvoluted with MassHunter Qualitative Analysis Suite B.05 (Agilent Technologies, USA).

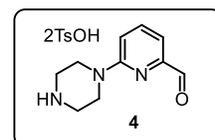
**Microscopy.** Resin modification with GFP and PEGA-2PCA-streptavidin binding with biotin-4-fluorescein were evaluated by fluorescence microscopy (EVOS Fluorescence Imaging System) with the excitation at 470 nm and the emission channel at 510 nm.

## **Experimental Procedures**

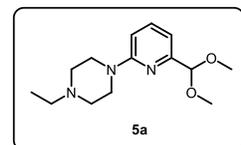
**tert-Butyl 4-(6-(dimethoxymethyl)pyridin-2-yl)piperazine-1-carboxylate (4a).** Following the general procedure for Pd-catalyzed cross-coupling with *tert*-butyl piperazine-1-carboxylate (1.2 mmol, 1.2 equiv, 224 mg) as a cross-coupling partner, the crude material was purified by column chromatography (hexane:ethyl acetate = 3:1 with 5% triethylamine), resulting in 99% yield (333 mg). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.54 (t, *J* = 7.9 Hz, 1H), 6.89 (d, *J* = 7.4 Hz, 1H), 6.61 (d, *J* = 8.5 Hz, 1H), 5.21 (s, 1H), 3.55 (s, 8H), 3.41 (s, 6H), 1.48 (s, 9H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 158.82, 155.50, 154.94, 138.20, 110.72, 106.96, 104.72, 80.00, 54.17, 53.94, 45.21, 28.55. HRMS (ESI) calculated for C<sub>17</sub>H<sub>28</sub>N<sub>3</sub>O<sub>4</sub> ([M+H]<sup>+</sup>) 338.2074; found, 338.2068.



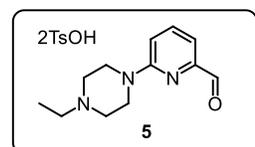
**6-(Piperazin-1-yl)picolinaldehyde (4).** To the reaction vessel containing **4a** (0.2 mmol, 68 mg) and *p*-toluenesulfonic acid monohydrate (0.42 mmol, 2.1 equiv, 80 mg), THF:H<sub>2</sub>O (1:1, 0.5 mL) was added and the mixture was stirred at 60 °C for 4 h. Upon cooling to room temperature, a small amount of methanol was added, followed by ethyl ether to induce precipitation. A viscous liquid was obtained as the product in quantitative yield (with two equivalents of tosylate salt). <sup>1</sup>H NMR (400 MHz, DMSO) δ 9.83 (s, 1H), 8.75 (s, 1H), 7.84 (t, *J* = 7.6 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 4H), 7.31 (d, *J* = 7.1 Hz, 1H), 7.26 (d, *J* = 8.5 Hz, 1H), 7.11 (d, *J* = 7.9 Hz, 4H), 3.81 (m, 4H), 3.22 (m, 4H), 2.29 (s, 6H). <sup>13</sup>C NMR (151 MHz, DMSO) δ 193.55, 158.20, 150.43, 145.54, 139.02, 137.72, 128.08, 125.48, 112.70, 112.64, 48.58, 42.50, 41.54, 20.76. HRMS (ESI) calculated for C<sub>10</sub>H<sub>14</sub>N<sub>3</sub>O ([M+H]<sup>+</sup>) 192.1131; found, 192.1130.



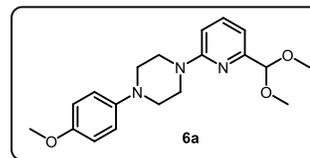
**1-(6-(dimethoxymethyl)pyridin-2-yl)-4-ethylpiperazine (5a).** Following the general procedure for Pd-catalyzed cross-coupling with 1-ethylpiperazine (1.2 mmol, 1.2 equiv, 137 mg) as a cross-coupling partner, the crude material was purified by column chromatography (hexane:ethyl acetate:MeOH:triethylamine = 3:1:0.2:0.2), resulting in 97% yield (257 mg). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.51 (t, *J* = 7.9 Hz, 1H), 6.87 (d, *J* = 7.3 Hz, 1H), 6.60 (d, *J* = 8.4 Hz, 1H), 5.18 (s, 1H), 3.63 (br, 4H), 3.41 (s, 6H), 2.61 (br, 4H), 2.54 (br, 2H), 1.17 (t, *J* = 6.9 Hz, 3H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 159.07, 155.51, 138.01, 110.40, 106.70, 104.85, 53.91, 52.71, 52.57, 45.16, 11.97. HRMS (ESI) calculated for C<sub>14</sub>H<sub>24</sub>N<sub>3</sub>O<sub>2</sub> ([M+H]<sup>+</sup>) 266.1863; found, 266.1859.



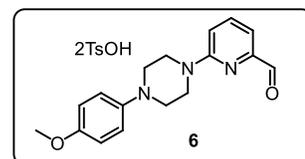
**6-(4-ethylpiperazin-1-yl)picolinaldehyde (5).** To a reaction vessel containing **5a** (0.2 mmol, 53 mg) and *p*-toluenesulfonic acid monohydrate (0.42 mmol, 2.1 equiv, 80 mg), THF:H<sub>2</sub>O (1:1, 0.5 mL) was added. The solution was stirred at 60 °C for 4 h. Upon cooling to room temperature, a small amount of methanol was added, followed by ethyl ether to induce precipitation. A viscous liquid was obtained as the product in quantitative yield (with two equivalents of tosylate salt). <sup>1</sup>H NMR (600 MHz, DMSO) δ 9.83 (s, 1H), 7.84 (t, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 8.1 Hz, 4H), 7.32 (d, *J* = 7.1 Hz, 1H), 7.29 (d, *J* = 8.5 Hz, 1H), 7.13 (d, *J* = 7.8 Hz, 4H), 4.52 (d, *J* = 14.3 Hz, 2H), 3.60 (d, *J* = 11.8 Hz, 2H), 3.25 – 3.17 (m, 4H), 3.11 – 3.02 (m, 2H), 2.28 (s, 6H), 1.25 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (151 MHz, DMSO) δ 193.57, 158.05, 150.45, 145.20, 139.12, 138.01, 128.20, 125.51, 112.90, 112.83, 50.91, 50.04, 41.78, 20.80, 8.99. HRMS (ESI) calculated for C<sub>12</sub>H<sub>18</sub>N<sub>3</sub>O ([M+H]<sup>+</sup>) 220.1444; found, 220.1444.



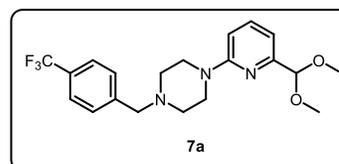
**1-(6-(dimethoxymethyl)pyridin-2-yl)-4-(4-methoxyphenyl)piperazine (6a).** Following the general procedure for Pd-catalyzed cross-coupling with 1-(4-methoxyphenyl)piperazine (1.2 mmol, 1.2 equiv, 231 mg) as a cross-coupling partner, the crude material was purified by column chromatography (hexane:ethyl acetate = 3:1 with 5% triethylamine), resulting in 95% yield (326 mg). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 7.54 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.04 – 6.92 (m, 2H), 6.89 (d, *J* = 7.3 Hz, 1H), 6.88 – 6.85 (m, 2H), 6.65 (d, *J* = 8.4 Hz, 1H), 5.20 (s, 1H), 3.78 (s, 3H), 3.74 (br, 4H), 3.42 (s, 6H), 3.19 (br, 4H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 159.02, 155.58, 138.12, 118.96, 118.87, 118.79, 114.68, 110.68, 106.89, 104.81, 55.73, 53.92, 51.02, 45.43. HRMS (ESI) calculated for C<sub>19</sub>H<sub>26</sub>N<sub>3</sub>O<sub>3</sub> ([M+H]<sup>+</sup>) 344.1969; found, 344.1963.



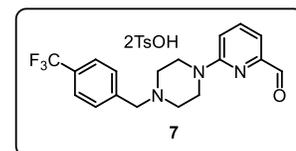
**6-(4-(4-methoxyphenyl)piperazin-1-yl)picolinaldehyde (6).** To the reaction vessel containing **6a** (0.2 mmol, 69 mg) and *p*-toluenesulfonic acid monohydrate (0.42 mmol, 2.1 equiv, 80 mg), THF:H<sub>2</sub>O (1:1, 0.5 mL) was added. The solution was stirred at 60 °C for 4 h. Upon cooling to room temperature, the mixture was precipitated from methanol/diethyl ether, producing pale yellow solid in quantitative yield (as two equivalent of tosylate salt). <sup>1</sup>H NMR (600 MHz, DMSO) δ 9.85 (s, 1H), 7.86 (t, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 8.7 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 4H), 7.33 (d, *J* = 7.7 Hz, 2H), 7.12 (d, *J* = 7.8 Hz, 4H), 7.09 (d, *J* = 9.1 Hz, 2H), 3.91 (br, 4H), 3.79 (s, 3H), 3.65 (br, 4H), 2.28 (s, 6H). <sup>13</sup>C NMR (151 MHz, DMSO) δ 193.61, 158.12, 150.45, 145.43, 139.07, 137.82, 128.12, 125.50, 122.07, 115.04, 112.69, 112.63, 55.62, 53.70, 42.65, 20.78. HRMS (ESI) calculated for C<sub>17</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub> ([M+H]<sup>+</sup>) 298.1550; found, 298.1549.



**1-(6-(dimethoxymethyl)pyridin-2-yl)-4-(4-(trifluoromethyl)benzyl)piperazine (7a).** Following the general procedure for Pd-catalyzed cross-coupling with 1-(4-(trifluoromethyl)benzyl)piperazine (1.2 mmol, 1.2 equiv, 293 mg) as a cross-coupling partner, the crude material was purified by column chromatography (hexane:ethyl acetate:MeOH:triethylamine = 3:1:0.1:0.2), resulting in 99% yield (390 mg). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 7.59 (d, *J* = 8.0 Hz, 2H), 7.54 – 7.48 (m, 3H), 6.86 (d, *J* = 7.3 Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 1H), 5.17 (s, 1H), 3.63 (s, 2H), 3.59 (br, 4H), 3.40 (s, 6H), 2.58 (br, 4H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 159.03, 155.56, 138.07, 129.48, 125.45, 125.42, 110.52, 106.77, 104.84, 62.54, 53.93, 52.99, 45.20. <sup>19</sup>F NMR (565 MHz, CDCl<sub>3</sub>) δ -63.36 (m). HRMS (ESI) calculated for C<sub>20</sub>H<sub>25</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub> ([M+H]<sup>+</sup>) 396.1893; found, 396.1885.



**6-(4-(4-(trifluoromethyl)benzyl)piperazin-1-yl)picolinaldehyde (7).** To the reaction vessel containing **7a** (0.2 mmol, 79 mg) and *p*-toluenesulfonic acid monohydrate (0.42 mmol, 2.1 equiv, 80 mg), THF:H<sub>2</sub>O (1:1, 0.5 mL) was added. The solution was stirred at 60 °C for 4 h. Upon cooling to room temperature, the mixture was precipitated from methanol/diethyl ether, producing pale yellow solid in quantitative yield (with two equivalents of tosylate salt). <sup>1</sup>H NMR (600 MHz, DMSO) δ 9.82 (s, 1H), 7.88 (d, *J* = 8.1 Hz, 2H), 7.85 (t, *J* = 7.8 Hz, 1H), 7.77 (d, *J* = 8.1 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 4H), 7.32 (d, *J* = 7.1 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 7.8 Hz, 4H), 4.52 (br, 4H), 3.47 (br, 2H), 3.27 – 3.08 (m, 4H), 2.28 (s, 6H). <sup>13</sup>C NMR (151 MHz, DMSO) δ 193.49, 157.92, 150.47, 145.46, 139.14, 137.78, 132.29, 128.10, 125.80, 125.77, 125.49, 112.91, 112.79, 58.09, 50.47, 41.64, 20.78. <sup>19</sup>F NMR (565 MHz, DMSO) δ -62.77 (s). HRMS (ESI) calculated for C<sub>18</sub>H<sub>19</sub>F<sub>3</sub>N<sub>3</sub>O ([M+H]<sup>+</sup>) 350.1475; found, 350.1473.



## Supporting Information References

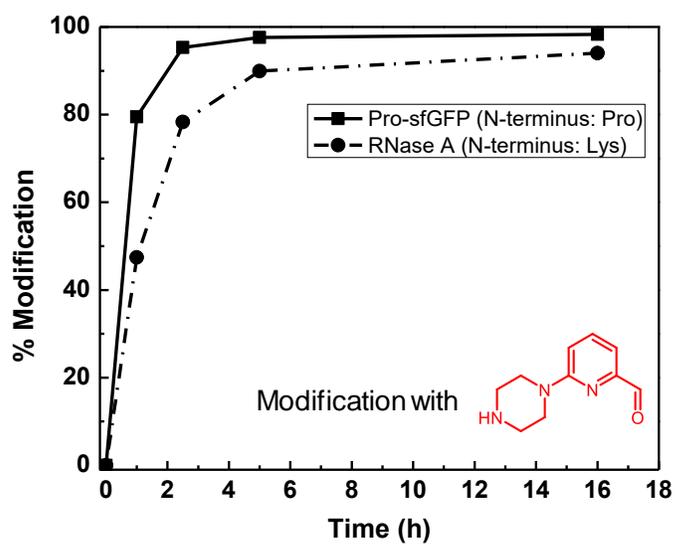
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Protein	MW	$\epsilon_{280}$ (cm <sup>-1</sup> M <sup>-1</sup> )	N-term
Pro-sfGFP	27.6 kDa	19.0 x 10 <sup>3</sup>	Pro-His
Streptavidin	13.3 kDa (tetramer: 53kDa)	41.9 x 10 <sup>3</sup> (1 chain)	Ala-Glu
RNase A	13.7 kDa	8.9 x 10 <sup>3</sup>	Lys-Glu
$\alpha$ -Chymotrypsin	25.7 kDa	50.6 x 10 <sup>3</sup>	Cys-Gly
$\beta$ -Lactoglobulin	18.4 kDa (A chain) 18.3 kDa (B chain)	18.7 x 10 <sup>3</sup> (A chain) 17.2 x 10 <sup>3</sup> (B chain)	Ala-Tyr (A) Leu-Ile (B)
Thioredoxin	11.7 kDa	14.1 x 10 <sup>3</sup>	Ser-Asp
Uteroglobin C69S	9.1 kDa (dimer: 18k)	1.5 x 10 <sup>3</sup> (1 chain)	Met-Glu
Triose Phosphate Isomerase	26.5 kDa (dimer: 53k)	33.7 x 10 <sup>3</sup> (1 chain)	Ala-Pro
Cytochrome c	12.3 kDa	106 x 10 <sup>3</sup> ( $\epsilon_{410}$ )	Ac-Gly-Asp

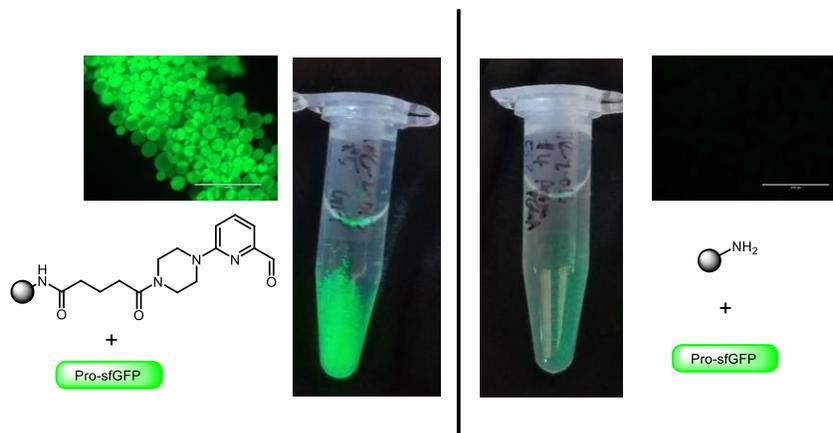
*Supporting Information Table S1.* Tabulated data for the proteins used in this work.

Protein	Initial	Protein Solution			PEGA-NH <sub>2</sub> + Protein			PEGA-2PCA + Protein			Loading nmol/mL
	0 h	20 h	46 h	72 h	20 h	46 h	72 h	20 h	46 h	72 h	
Pro-GFP	45.3	46.8	49.5	47.4	45.3	48.4	46.8	20.5	13.2	11.1	358
Streptavidin	48.4	49.9	51.8	52.0	49.4	49.4	49.6	15.0	2.9	1.0	487
RNase A	52.3	50.7	51.4	51.3	50.9	51.7	50.6	37.8	34.2	31.4	192
$\alpha$ -Chymotrypsin	49.8	51.2	52.4	52.2	49.4	50.0	49.0	18.2	12.3	10.9	381
$\beta$ -Lactoglobulin	48.3	51.7	54.7	50.0	47.1	46.5	45.9	31.4	22.7	17.4	285
Thioredoxin	49.6	53.2	54.6	55.3	49.6	48.2	47.5	39.7	38.3	36.9	106
Uteroglobin C69S	52.1	48.6	49.3	52.1	50	43.6	45.7	25	17.1	13.6	321
Triose Phosphate Isomerase	49.3	51.0	54.3	49.9	50.1	49.6	48.4	49.3	48.4	47.2	12
Cytochrome c	55.9	50.0	51.3	49.4	46.0	46.9	46.2	47.5	48.0	46.1	1

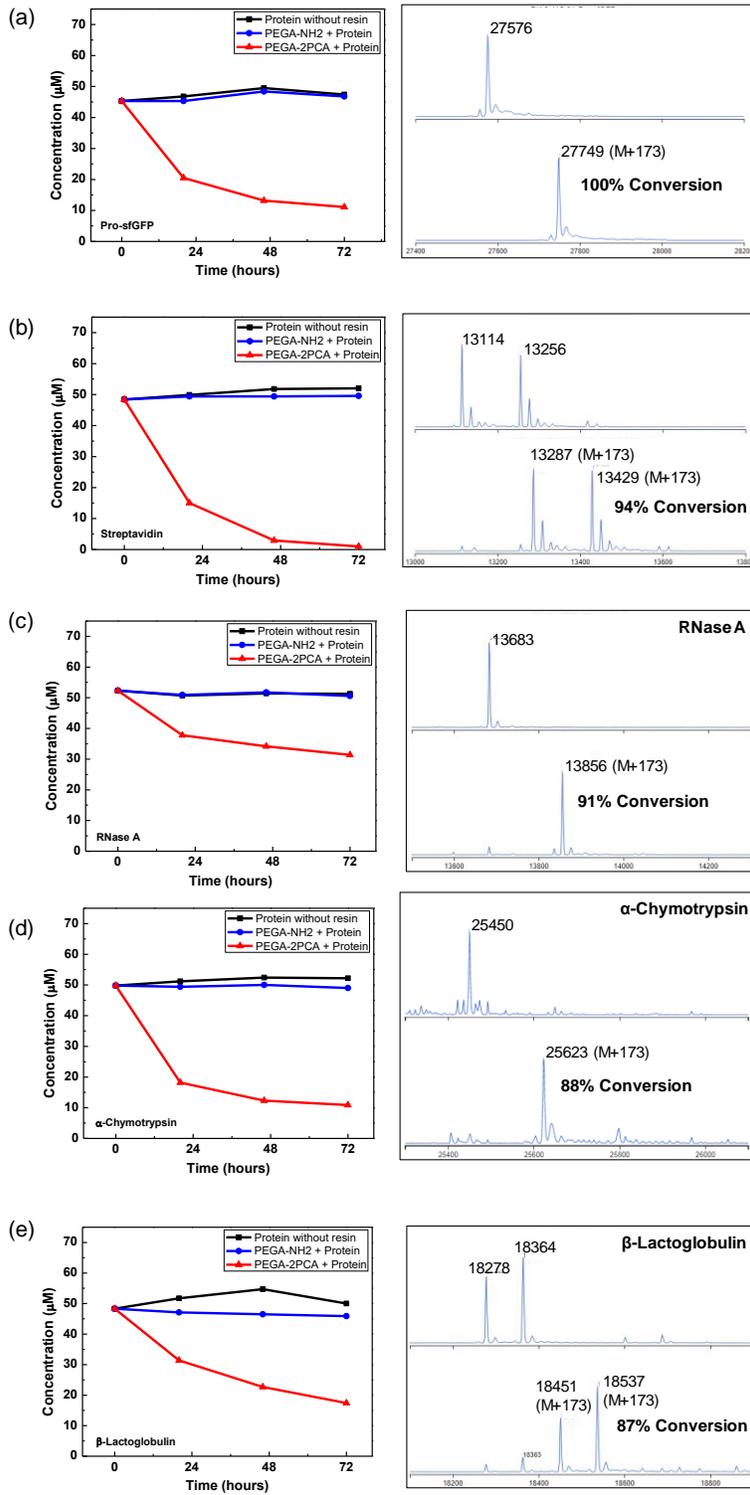
*Supporting Information Table S2.* Protein supernatant concentration changes during immobilization reactions. Protein concentrations (unit:  $\mu\text{M}$ ) were calculated from the absorption of the supernatant (280 nm) divided by extinction coefficient ( $\epsilon_{280}$ ). Note that cytochrome c concentration was obtained from the absorption at 410 nm divided by extinction coefficient ( $\epsilon_{410}$ ; reference: *PNAS* **2004**, 101, 5940). After 72 h, the concentrations of the supernatants were obtained. The concentration differences between PEGA-NH<sub>2</sub> + protein and PEGA-2PCA + protein, multiplied by the reaction volume (100  $\mu\text{L}$ ) was assumed to be the amount of proteins immobilized per 10  $\mu\text{L}$  of resin. For example, for pro-sfGFP, the loading =  $(46.84 - 11.05) \mu\text{M} * 100 \mu\text{L} * (10 \mu\text{L resin})^{-1} = 358 \text{ nmol/mL resin}$ .

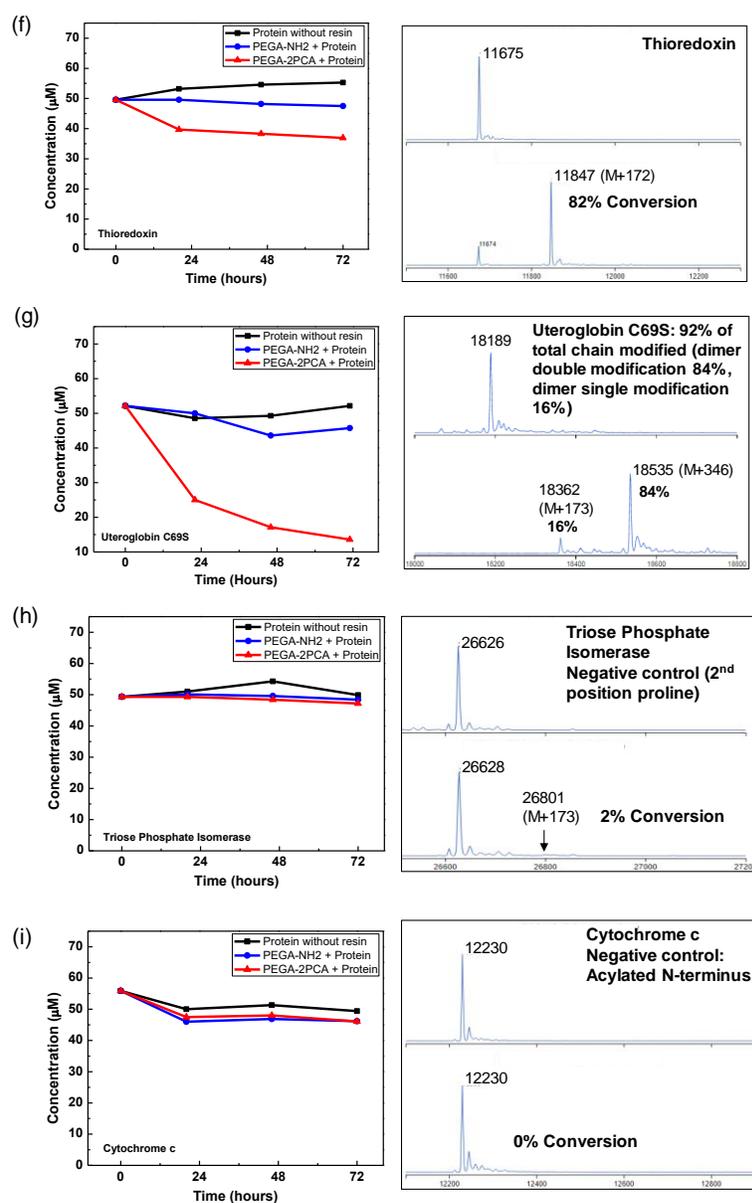


*Supporting Information Figure S1.* Kinetics of pro-sfGFP and RNase A modification in solution. The percent modification of pro-sfGFP (solid line) and RNase A (dash-dotted line) by compound **4** was monitored over time by calculating the percentage of modified proteins based on peak integration for the ESI-TOF-MS spectrum at different time points.

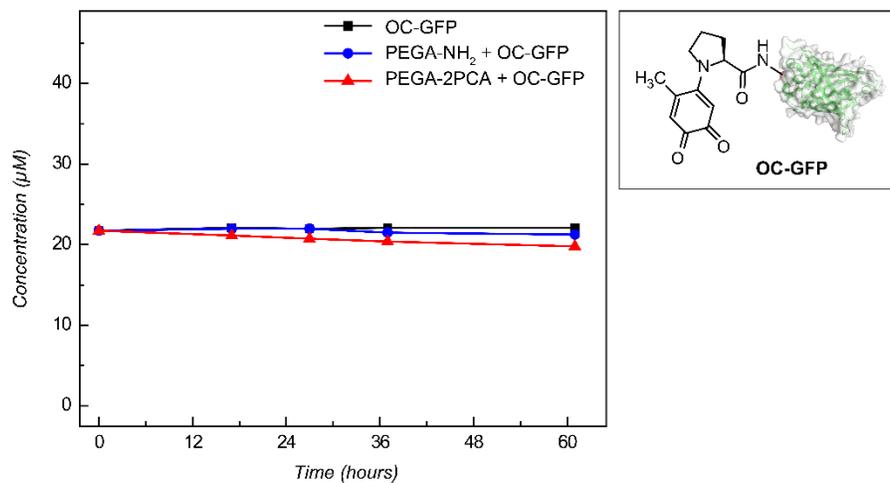


*Supporting Information Figure S2.* A control experiment for resin modification with pro-sfGFP. Both resins were charged with a pro-sfGFP solution ( $35 \mu\text{M}$  in pH 7.5 buffer) and incubated for 27 h. Pictures were taken after the resins were washed repeatedly with water. Left: Images of pro-sfGFP immobilization on the PEGA-2PCA resin; the beads were observed to be highly fluorescent. Right: Images of pro-sfGFP immobilization on PEGA-NH<sub>2</sub> resin; the beads were not fluorescent. The fluorescent microscopy image scale bar represents 1 mm.



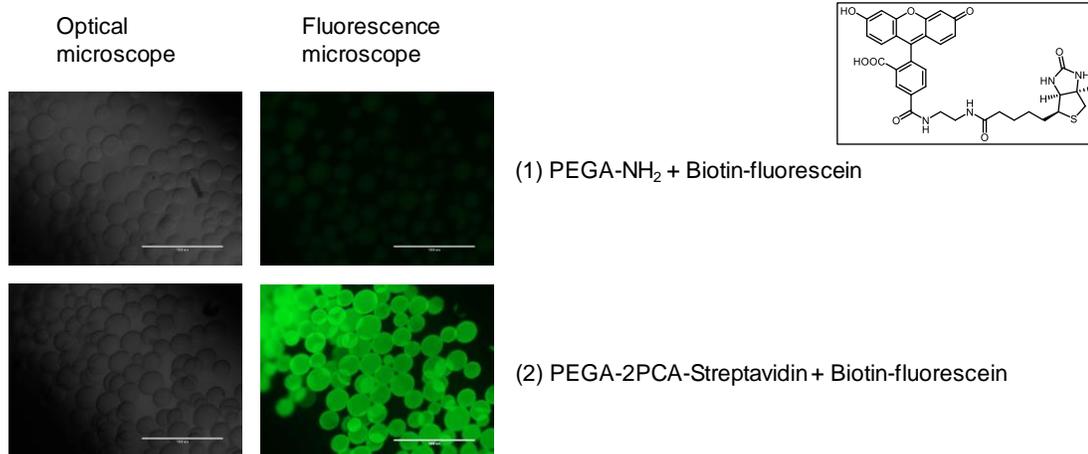


*Supporting Information Figure S3 (a)-(i).* Protein scope for immobilization. The left column shows the supernatant concentrations of protein samples without resin (black), proteins exposed to PEGA-NH<sub>2</sub> (blue), and proteins exposed to PEGA-2PCA (red). Each sample was monitored for 3 d. The right column shows ESI-TOF-MS data of protein modification reactions in solution without resin to confirm the reactivity of the substrates with compound **4** (top traces: before modification, and bottom traces: after modification). The solution reactions contained protein (50 µM) and **4** (5 mM) in 50 mM sodium phosphate buffer (pH 7.5), and were incubated at room temperature for 3 d. Following this, each sample was subjected to ultrafiltration against a molecular weight cut-off (MWCO) filter.

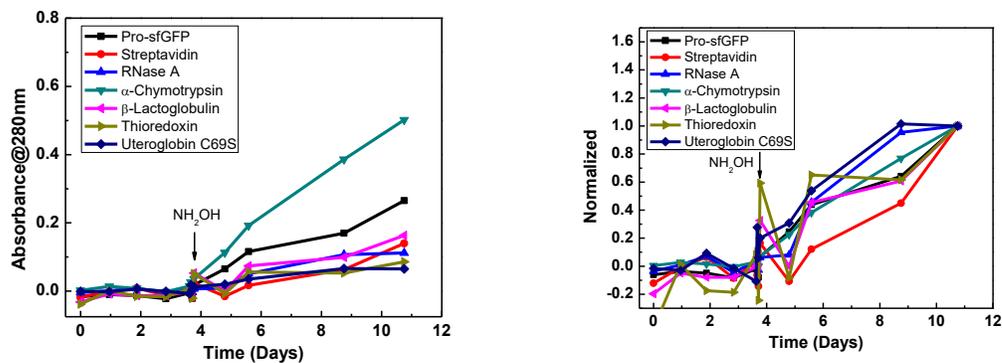


*Supporting Information Figure S4.* A negative control experiment with N-terminally blocked pro-sfGFP (OC-GFP). The constant supernatant protein concentration (measured from the absorption at 488 nm) in the presence of PEGA-2PCA (red) over a period of 2 days indicates that no immobilization occurred. During the measurement, OC-GFP was stable without resin (black) and in presence of unmodified resin (blue).

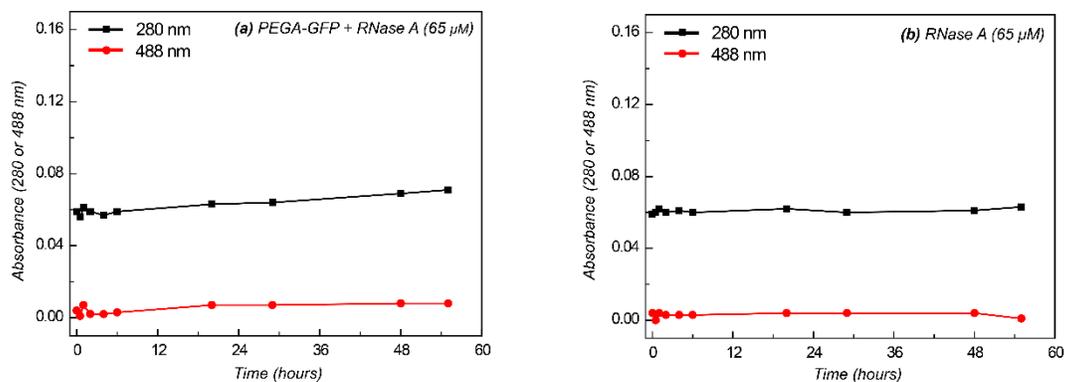




*Supporting Information Figure S6.* Characterization of PEGA-2PCA-streptavidin with biotin-fluorescein (structure shown in inset) with images at the top (PEGA-NH<sub>2</sub> + biotin-fluorescein, negative control), bottom (PEGA-2PCA-streptavidin + biotin-fluorescein, positive control), left column from optical microscope, and right column from fluorescence microscope. Biotin-fluorescein incubated with the PEGA-2PCA-streptavidin sample showed strong fluorescence, whereas the PEGA-NH<sub>2</sub> sample did not display fluorescence. Scale bar: 1 mm. Experimental procedure: the PEGA-2PCA-streptavidin was washed with water was added into a microcentrifuge tube. Biotin-4-fluorescein (a 500 μM solution dissolved in 10% DMSO in 10 mM sodium phosphate buffer) was added into each resin (PEGA-NH<sub>2</sub> and PEGA-2PCA-streptavidin) and placed in a rocking shaker for 19 h. The mixture was thoroughly washed with 10 mM sodium phosphate buffer (pH 7.5) and PBS (20 mM NaPhos and 300 mM NaCl, pH 7.5). The fluorescence microscope image was taken in 1:1 ethanol:10 mM sodium phosphate buffer, pH 7.5.

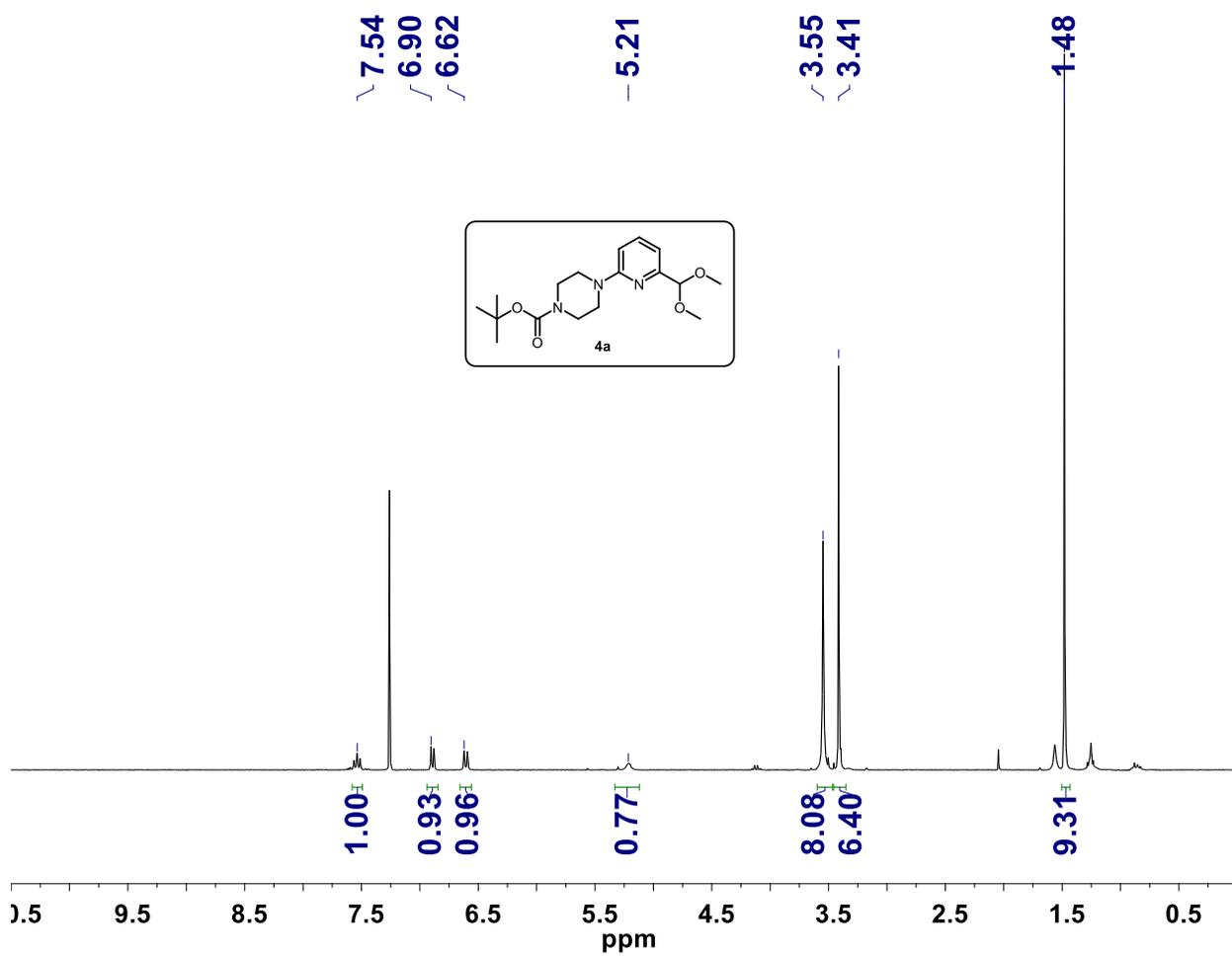


*Supporting Information Figure S7.* Monitoring the release of immobilized proteins from PEGA-2PCA resins. The constant absorbance measurements from 0-4 d indicated that no protein was leached from the resin during this time period. To induce reversibility, hydroxylamine was added to each reaction sample at day 4. Subsequent increase of protein was observed by increases in the supernatant absorbance values measured at 280 nm. The left graph shows the raw data, and the right graph represents the same measurements after normalization to the final data point values.

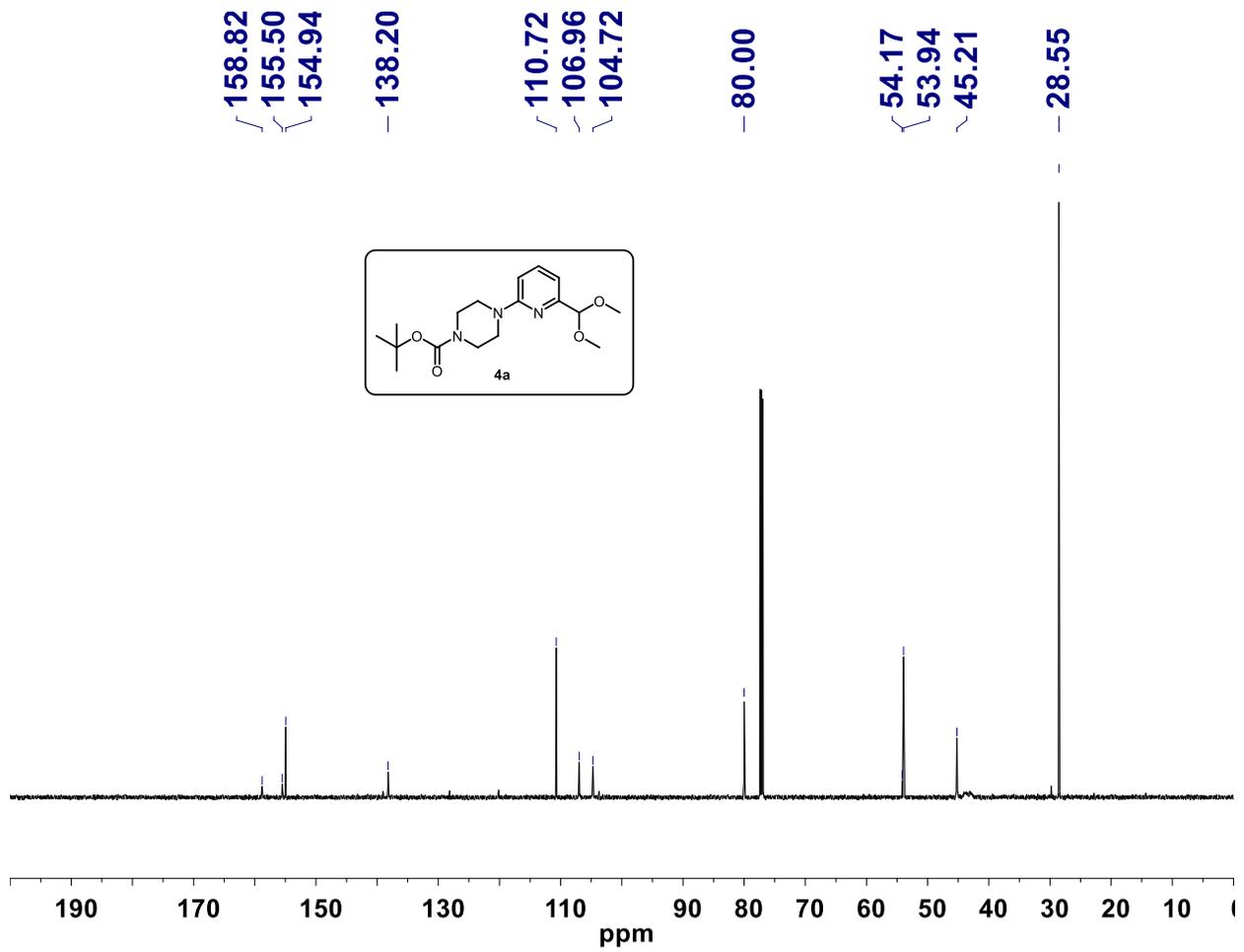


*Supporting Information Figure S8.* Monitoring the stability of PEGA-GFP in the presence of the second protein (RNase A). (a) The resin-bound protein (PEGA-GFP) was incubated with 65  $\mu$ M RNase A (100  $\mu$ L), while monitoring the absorption at 280 nm and 488 nm. The absorbance at 488 nm was close to zero over 2 d, indicating that resin-bound GFP did not leach from the resin. (b) As a control experiment, the absorption of the same concentration of RNase A in the absence of PEGA-GFP was measured.

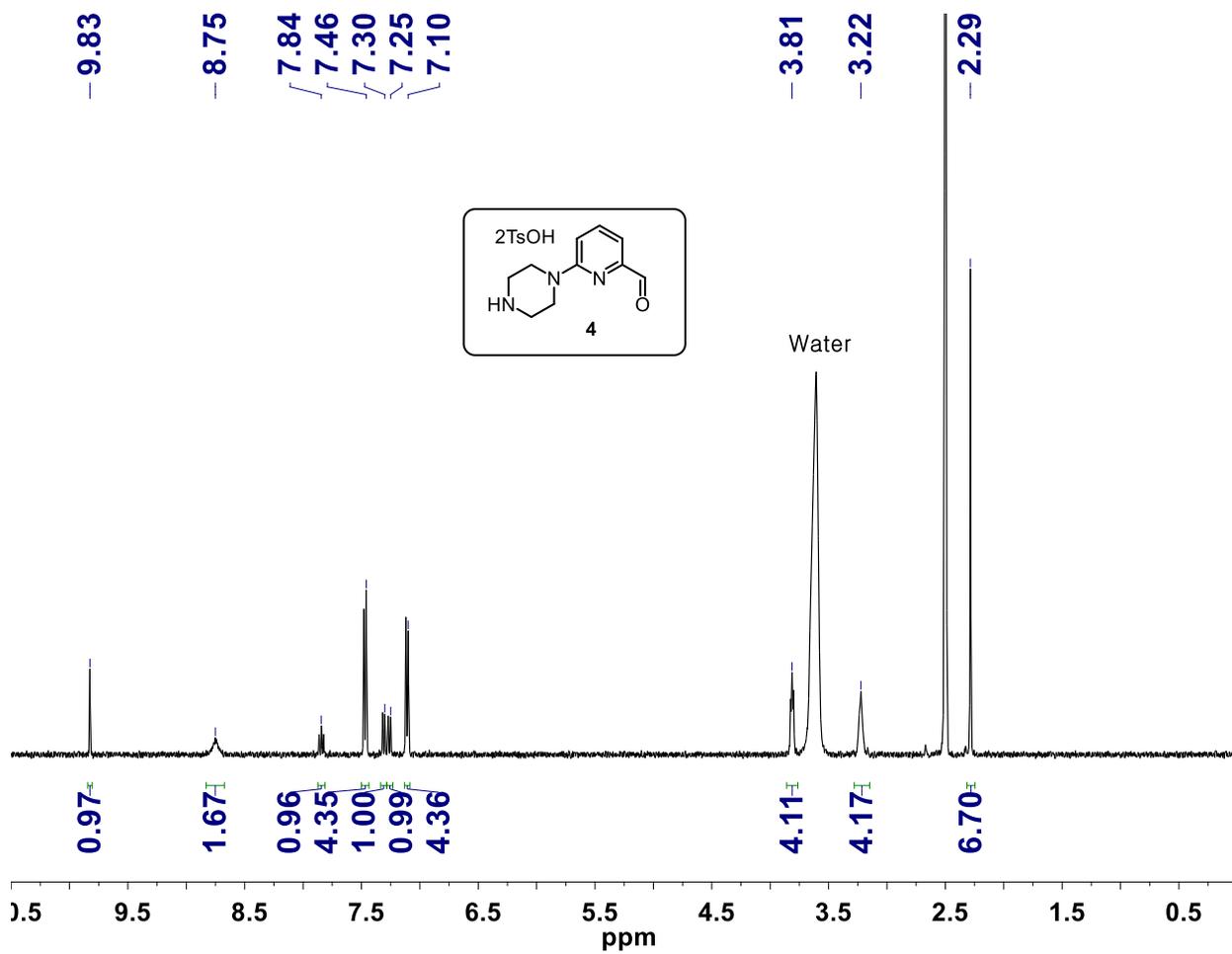
Supporting Information Figure S9. NMR Spectra



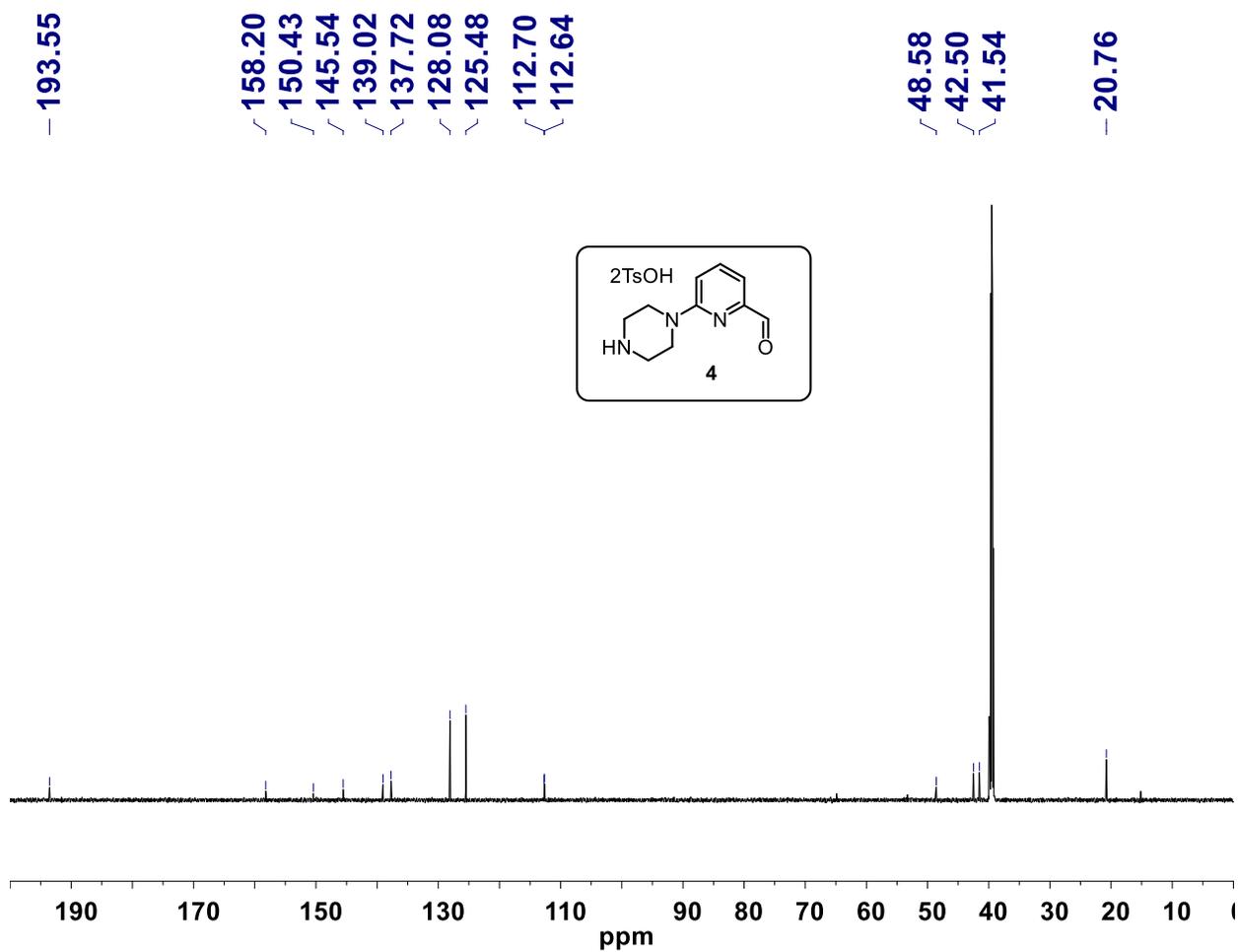
<sup>1</sup>H NMR spectrum of **4a** in CDCl<sub>3</sub>.



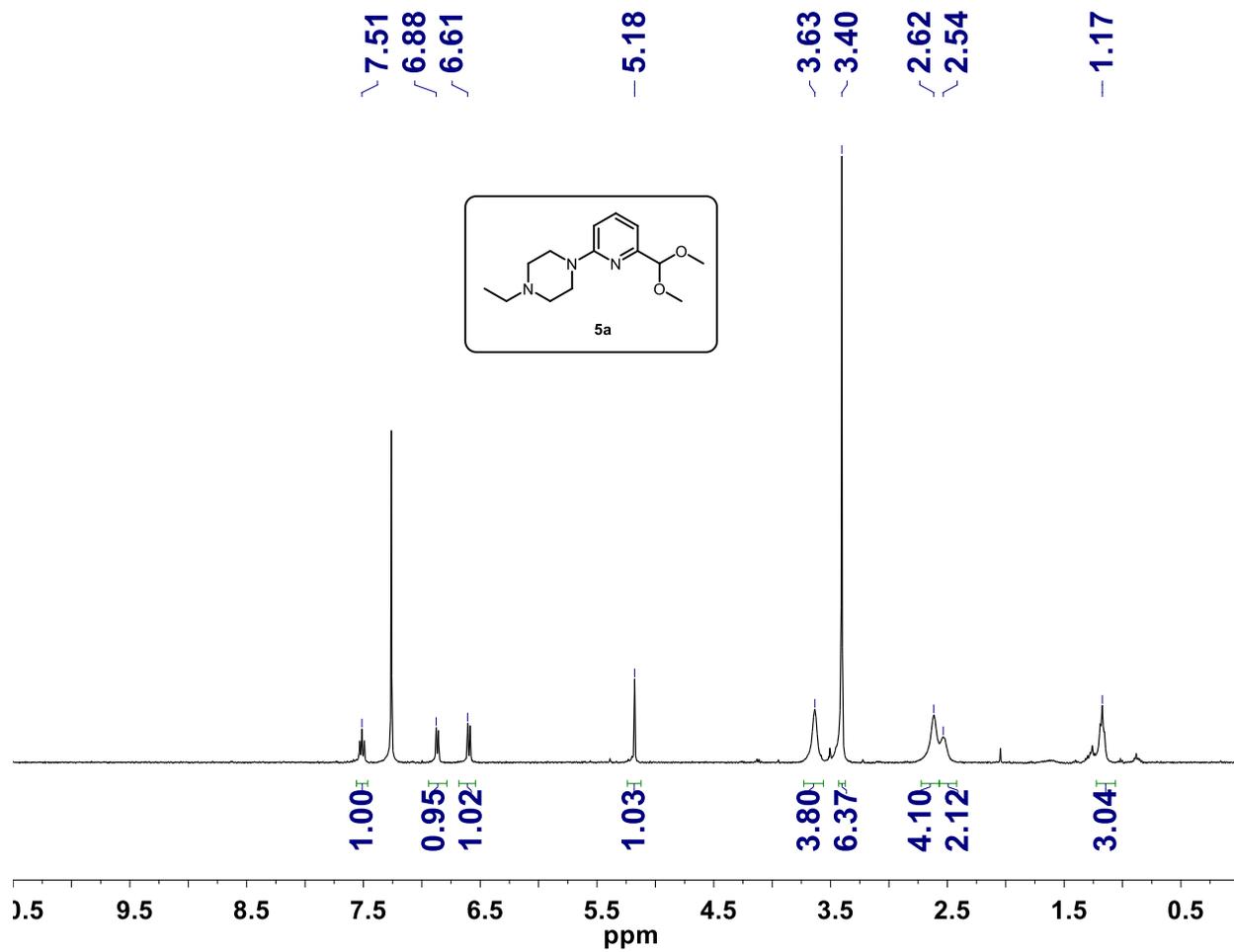
<sup>13</sup>C NMR spectrum of **4a** in CDCl<sub>3</sub>.



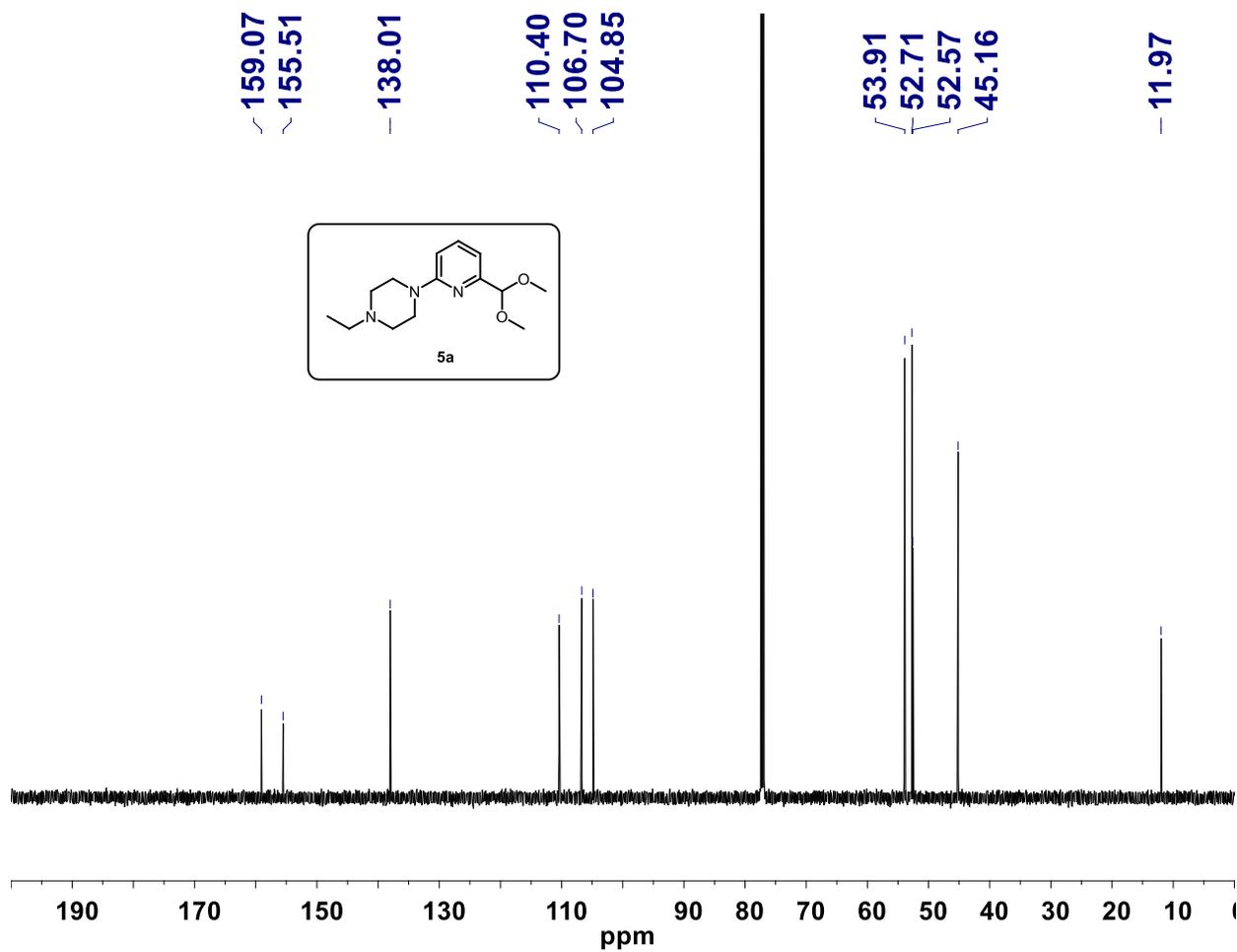
$^1\text{H}$  NMR spectrum of **4** (with two tosylate salts) in  $\text{DMSO-d}_6$ .



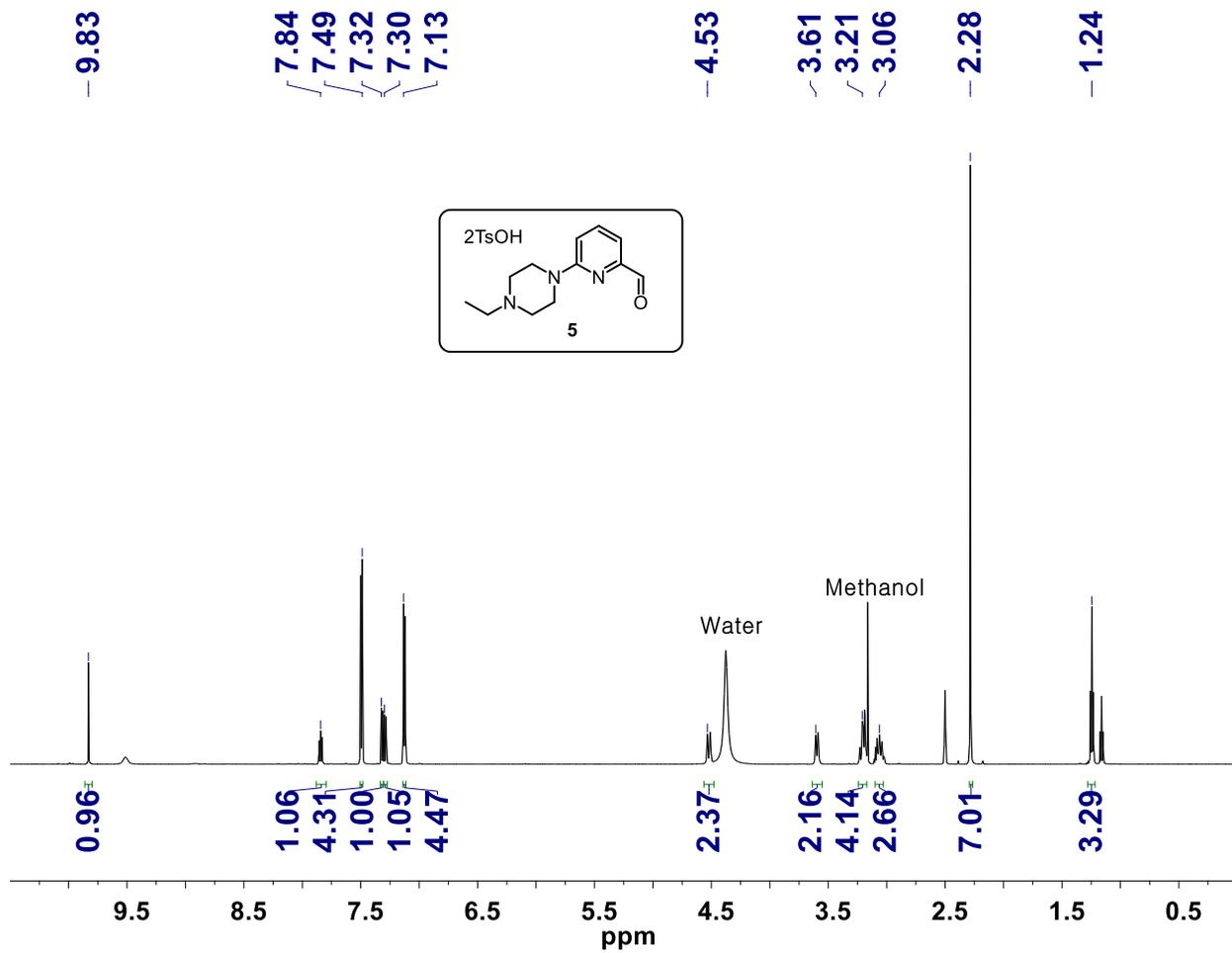
$^{13}\text{C}$  NMR spectrum of **4** (with two tosylate salts) in  $\text{DMSO-d}_6$ .



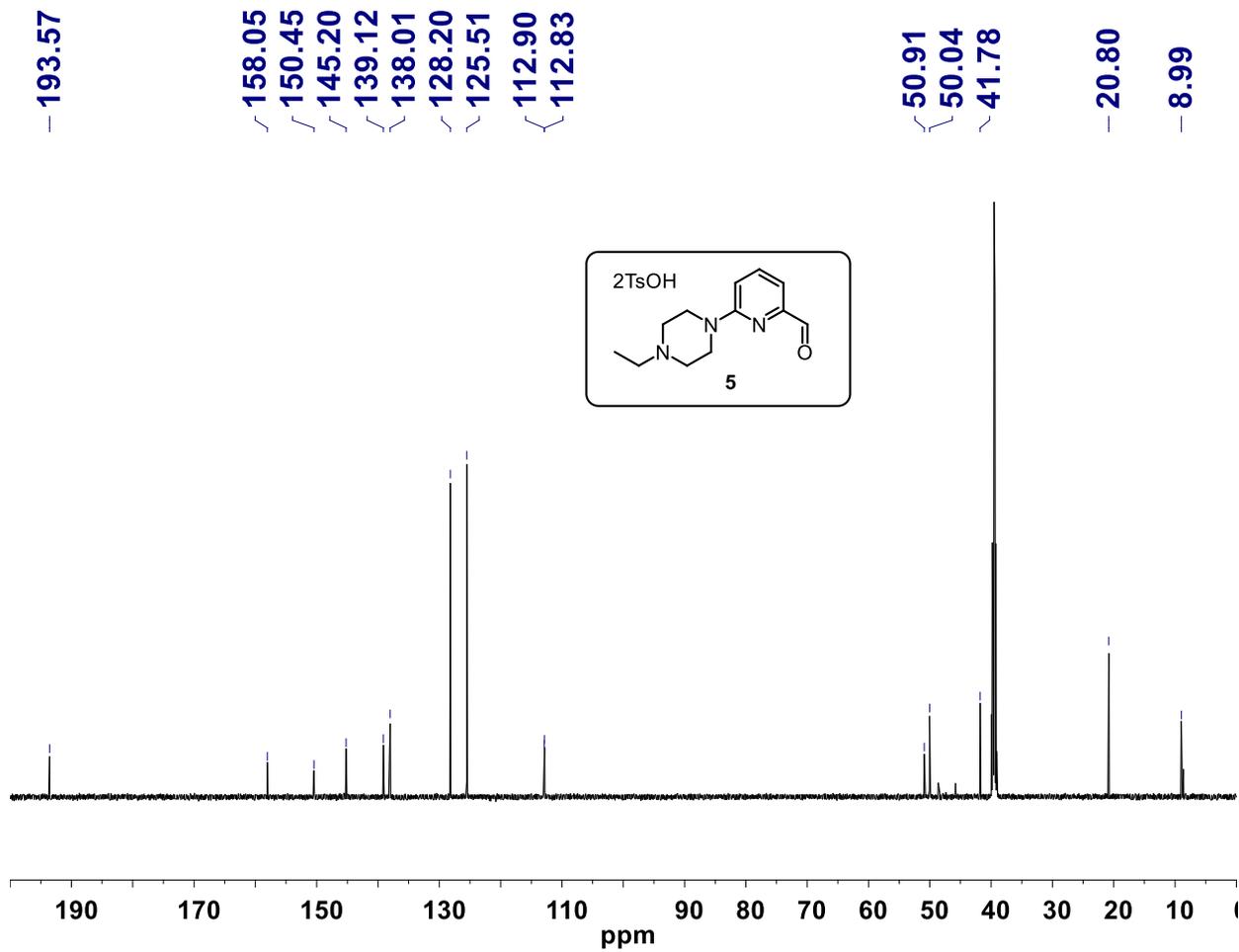
$^1\text{H}$  NMR spectrum of **5a** in  $\text{CDCl}_3$ .



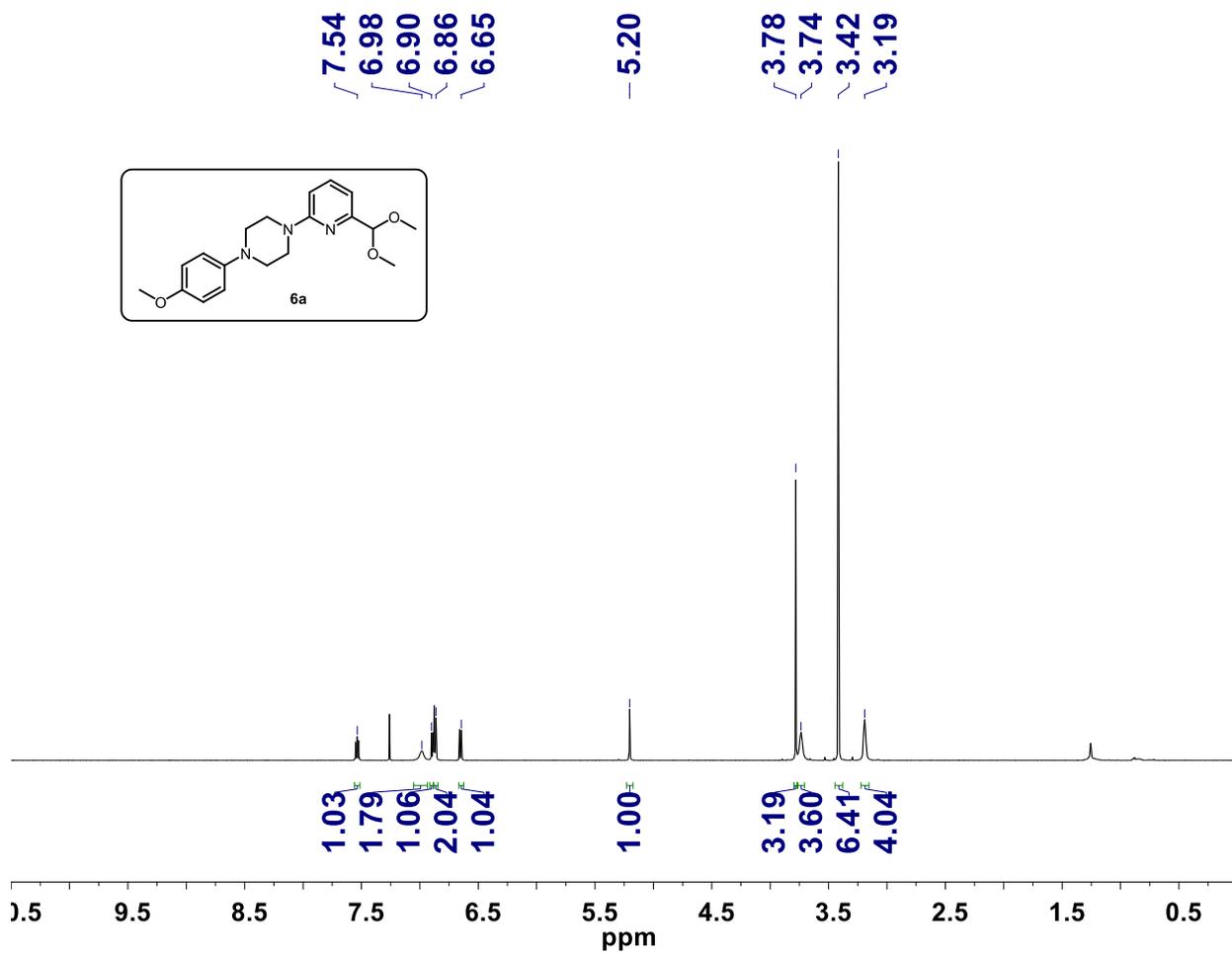
$^{13}\text{C}$  NMR spectrum of **5a** in  $\text{CDCl}_3$ .



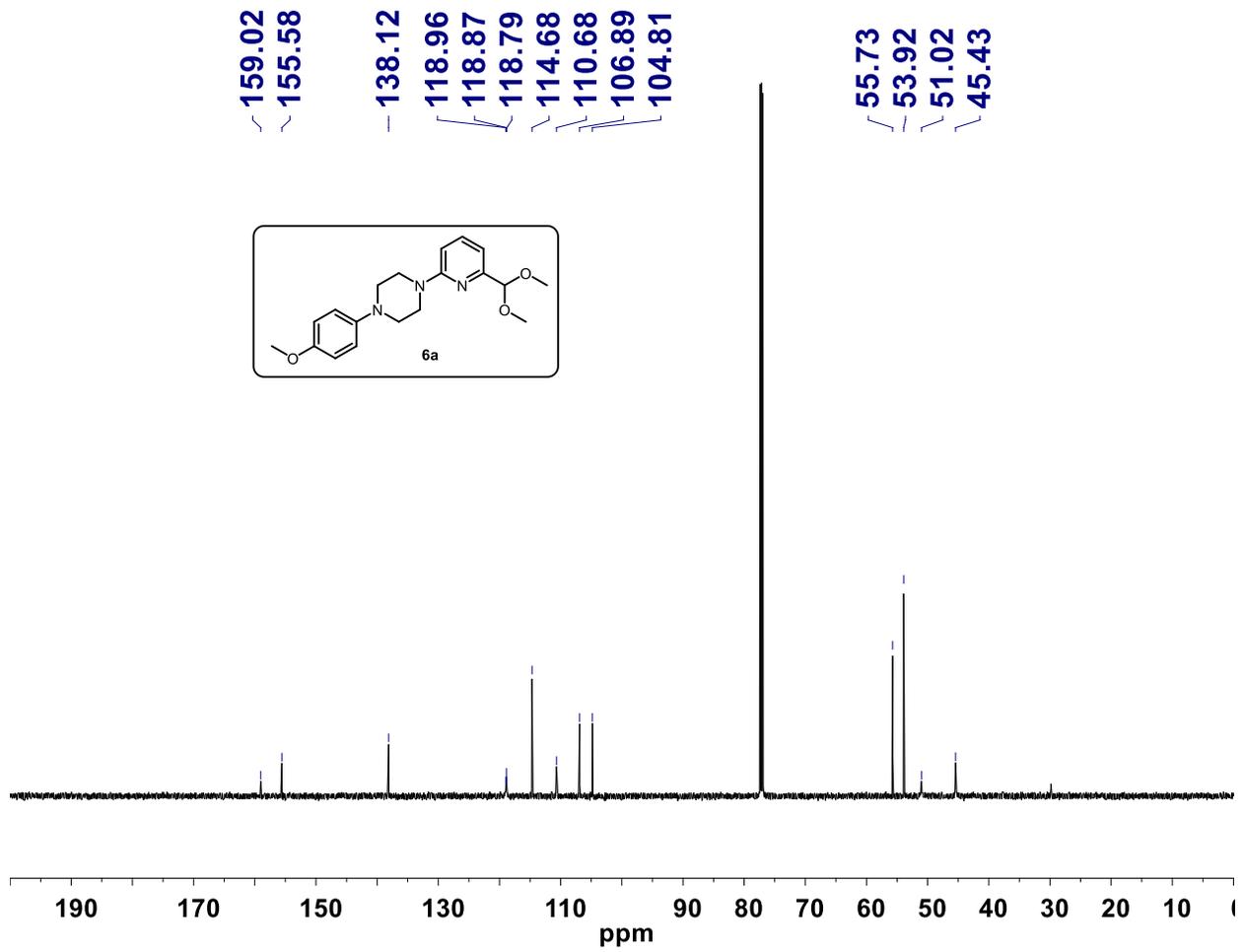
<sup>1</sup>H NMR spectrum of **5** (with two tosylate salts) in DMSO-d<sub>6</sub>.

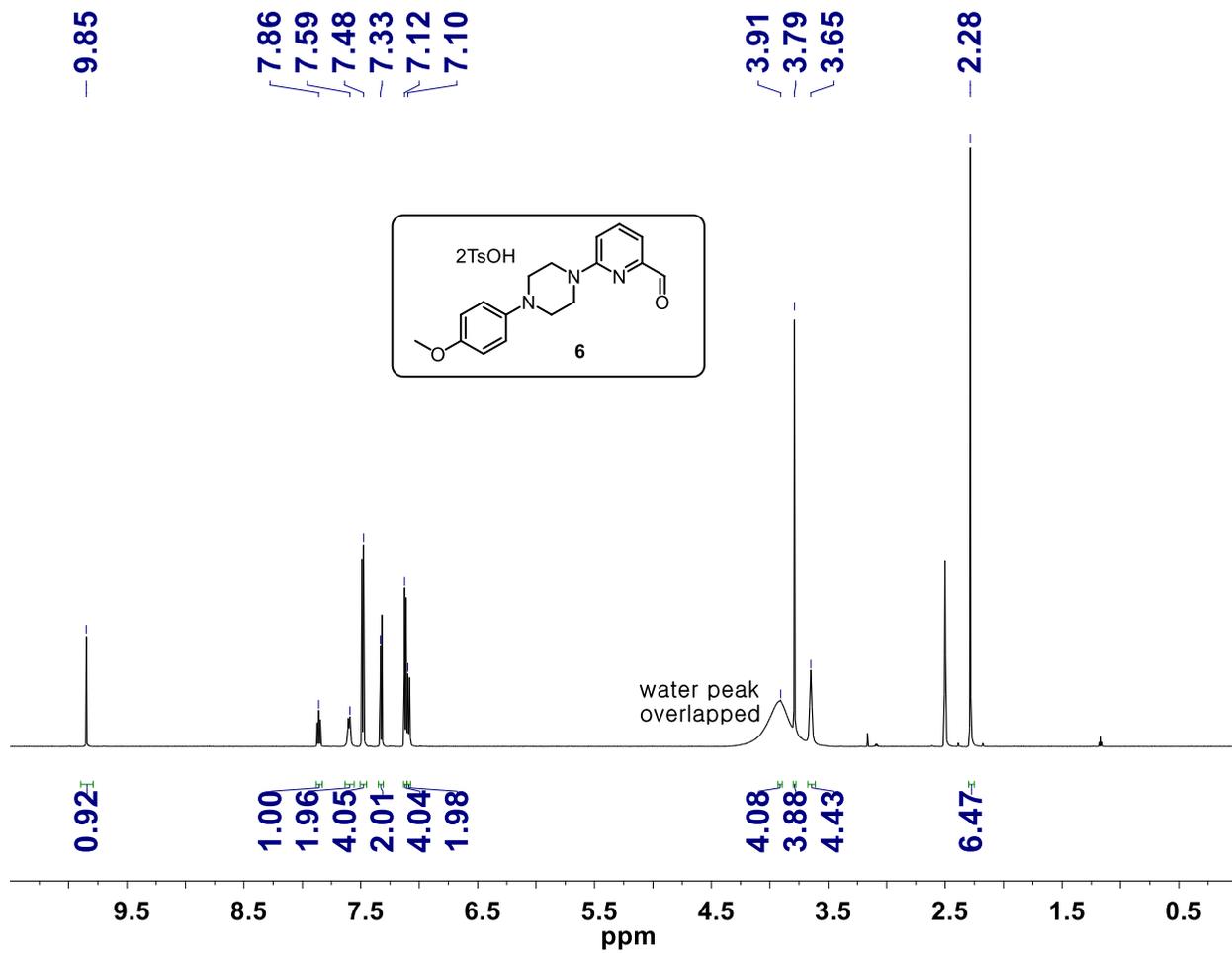


$^{13}\text{C}$  NMR spectrum of **5** (with two tosylate salts) in  $\text{DMSO-d}_6$ .

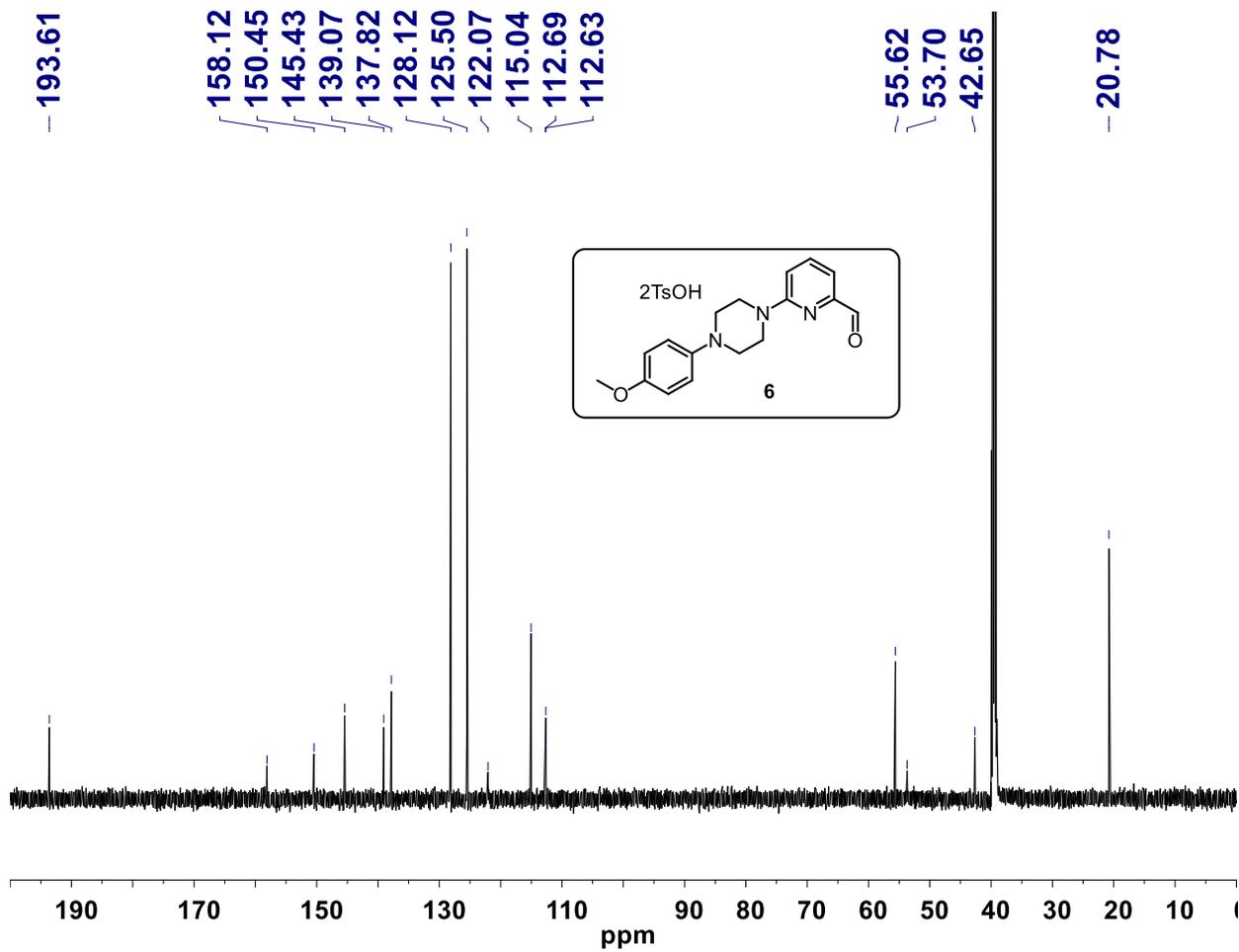


<sup>1</sup>H NMR spectrum of **6a** in CDCl<sub>3</sub>.

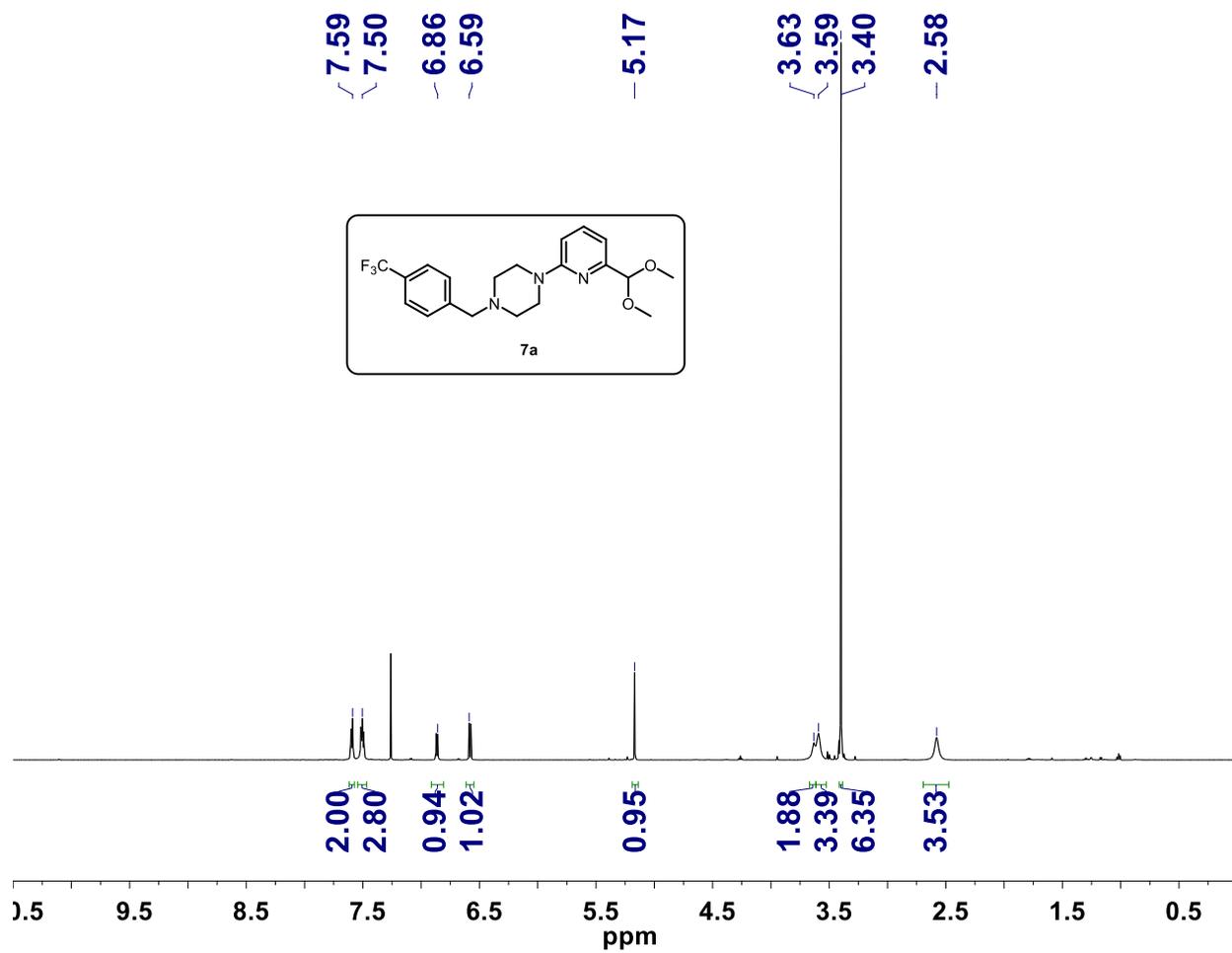




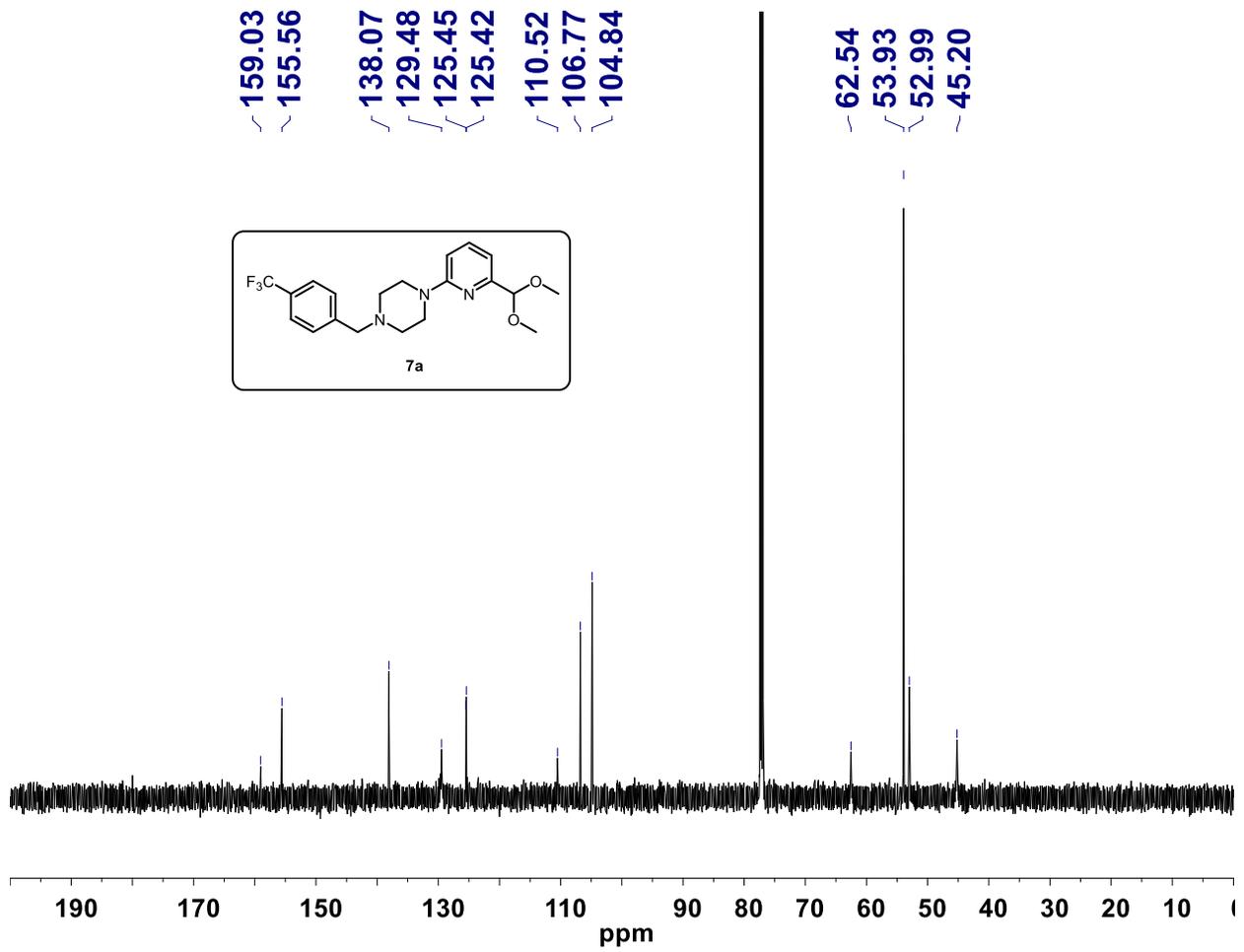
<sup>1</sup>H NMR spectrum of **6** (with two tosylate salts) in DMSO-d<sub>6</sub>.



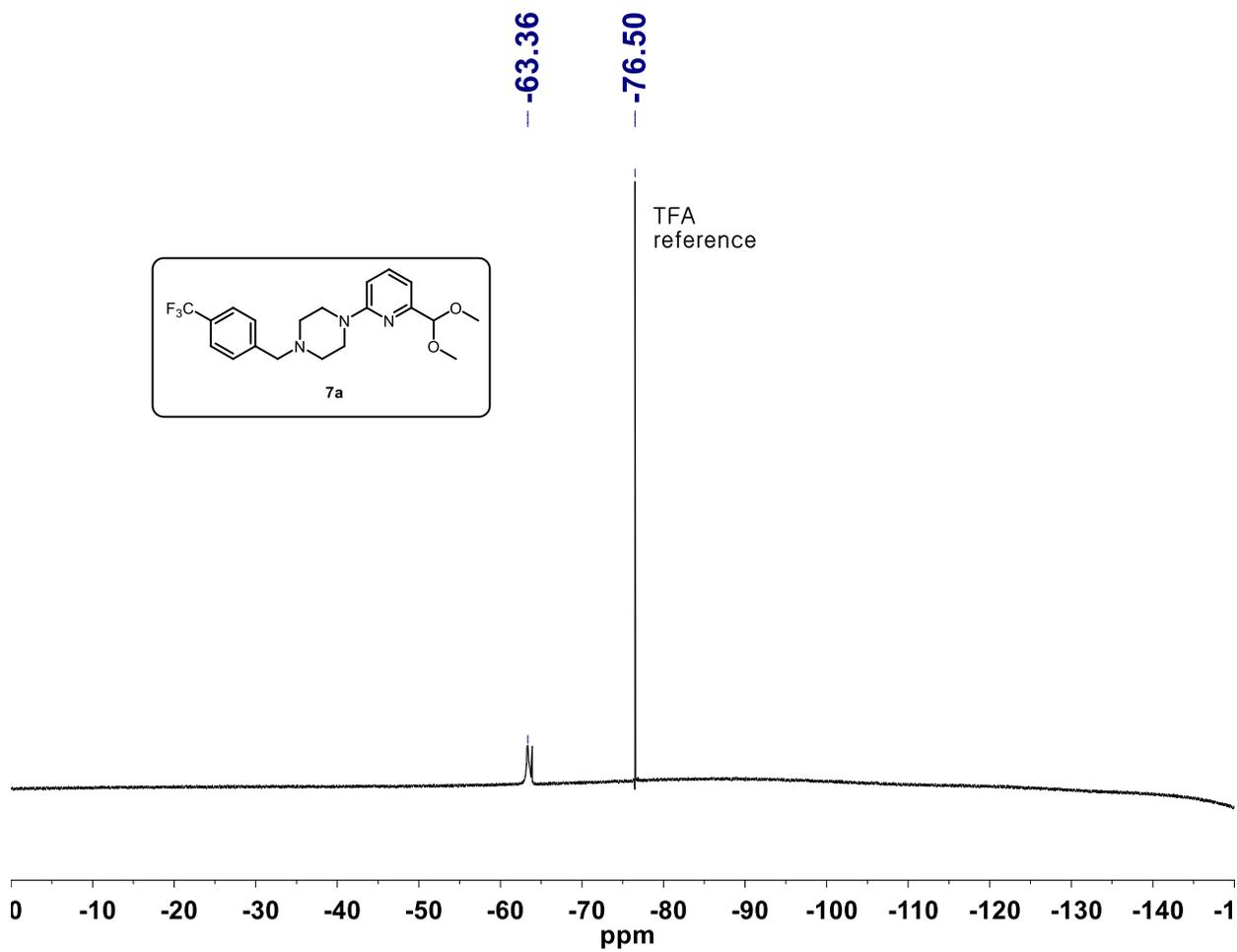
$^{13}\text{C}$  NMR spectrum of **6** (with two tosylate salts) in  $\text{DMSO-d}_6$ .



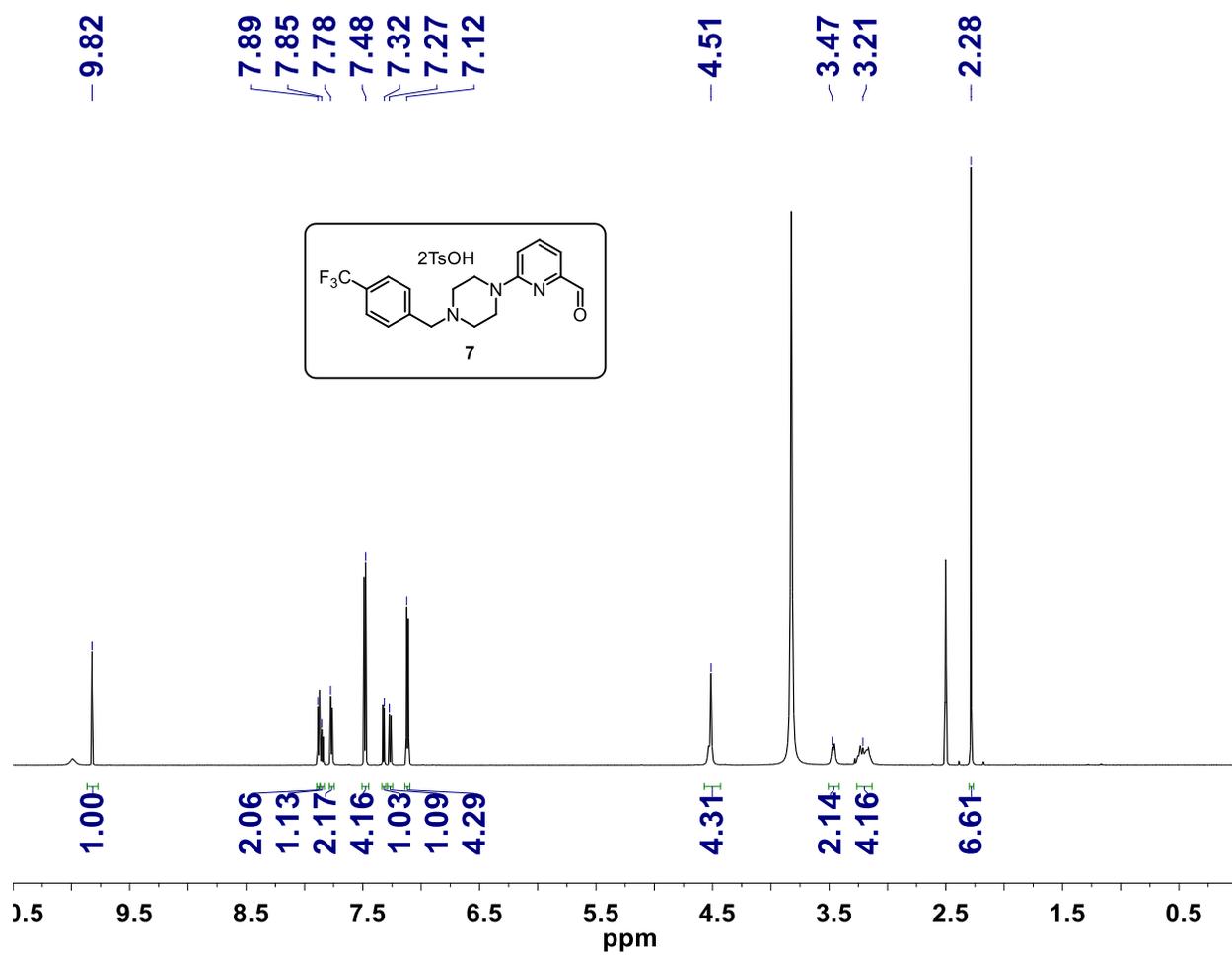
<sup>1</sup>H NMR spectrum of **7a** in CDCl<sub>3</sub>.



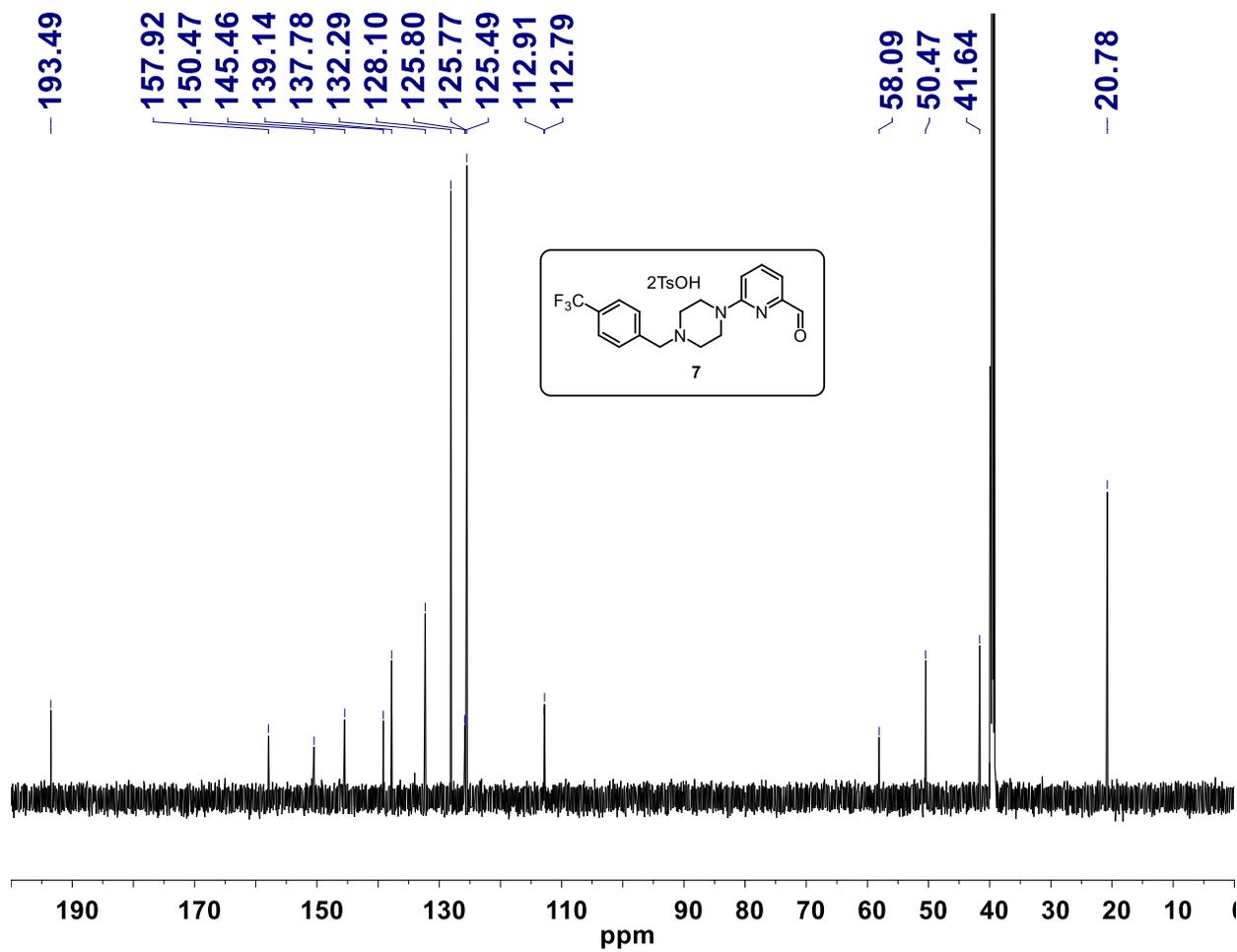
<sup>13</sup>C NMR spectrum of **7a** in CDCl<sub>3</sub>.



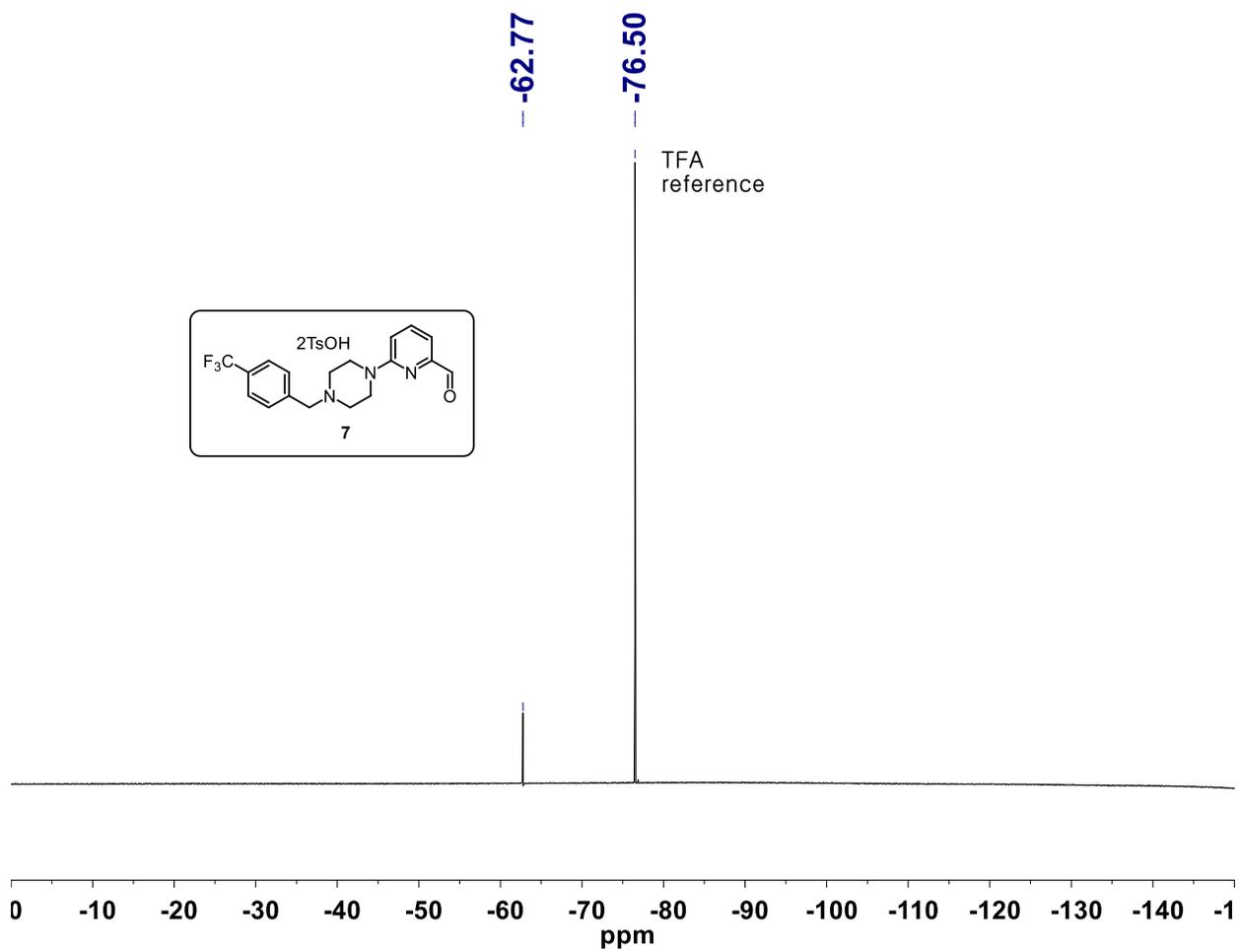
$^{19}\text{F}$  NMR spectrum of **7a** in  $\text{CDCl}_3$ .



<sup>1</sup>H NMR spectrum of **7** (with two tosylate salts) in DMSO-d<sub>6</sub>.



<sup>13</sup>C NMR spectrum of 7 (with two tosylate salts) in DMSO-d<sub>6</sub>.



$^{19}\text{F}$  NMR spectrum of **7** (with two tosylate salts) in  $\text{DMSO-d}_6$ .