

## **New orthogonally trifunctionalized morpholine nucleosides**

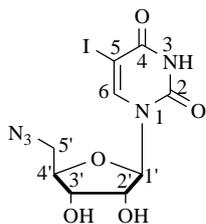
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### **Experimental**

#### **General**

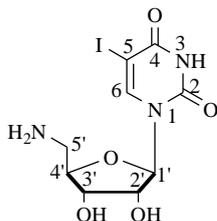
We used dimethyl oxalate, iodine monochloride, bromotrichloromethane and sodium periodate (Acros Organics, USA); sodium azide and glycine (Serva, Germany); uridine (ChemGenes Corporation, USA). Methyl *N*-(6-hydroxy-6-oxohexyl)oxamate [T. V. Abramova, S. S. Belov, Y. V. Tarasenko and V. N. Silnikov, *Beilstein J. Org. Chem.*, 2014, **10**, 1151] and 5-iodouridine **1a** [M. J. Robins, P. J. Barr and J. Giziewicz, *Can. J. Chem.*, 1982, **60**, 554] were synthesized as described in the literature. All other reagents and solvents were purchased from Sigma-Aldrich (USA) and Reachem (Russia). Organic solvents were dried and purified by standard procedures. NMR spectra were acquired on Bruker AM-400 and AV-300 instruments (Bruker, Germany) in appropriate deuterated solvents at 30 °C. Chemical shifts ( $\delta$ ) are reported in ppm relative to the tetramethylsilane (TMS) signal. Coupling constants *J* are reported in Hertz. MALDI TOF and ESI mass spectra were registered on Autoflex III mass spectrometer (Bruker Daltonics, Germany) using 2,5-dihydroxybenzoic acid as a matrix (MALDI TOF), or on Agilent ESI MSD XCT Ion Trap (Agilent Technologies, USA) in positive or negative mode at The Joint Center for genomic, proteomic and metabolomics studies (IChBFM SB RAN, Russia). Monitoring of a reaction progress was performed on a Milichrom A02 chromatograph system equipped with the MultiChrom program package (Econova, Russia) on a ProntoSIL 125 C18 column (2 × 75 mm) in a gradient of buffer B (0.1 M Et<sub>3</sub>N–AcOH, pH 7.0, 80% MeCN) in buffer A (0.1 M Et<sub>3</sub>N–AcOH, pH 7.0, water) with an elution rate of 0.2 ml min<sup>-1</sup> and UV detection at 250, 260, 280, and 300 nm. TLC was carried out on Kieselgel 60 F<sub>254</sub> plates (Merck, Germany) in the proper solvent systems (see below) and visualized by UV irradiation, ninhydrin (amine groups) or cysteine/aqueous sulfuric acid (nucleoside). Preparative silica gel column chromatography, reverse phase chromatography (RPC), and cation exchange chromatography were performed using silica gel 60 (40–63  $\mu$ m/230–400 mesh) (Macherey-Nagel, Germany), Porasil C 18 (55–105  $\mu$ m, 125 Å) (Waters, USA), and Servacel P23 (Serva, Germany) or Dowex 50Wx2 (Aldrich, USA), respectively. The compositions of all liquid mixtures are indicated as (v/v) percent. All evaporations were performed under reduced pressure.

### 5'-Azido-5'-deoxy-5-iodouridine **1b**



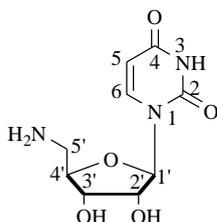
Triphenylphosphine (2.62 g, 10 mmol), sodium azide (3.25 g, 50 mmol), and bromotrichloromethane (1.0 ml, 10 mmol) were added to a solution of 5-iodouridine **1a** (1.57 g, 4.25 mmol) in DMF (50 ml). The reaction mixture was stirred for 48 h. The solvent was removed under reduced pressure and the residue was suspended in EtOH (70 ml). The precipitate formed in EtOH was filtered off, the filtrate was evaporated, the residue was suspended in water and the target product was purified by RPC in a linear gradient of EtOH in water (0→20%). Yield: 1.10 g (66 %).  $R_f$  0.64 (CH<sub>2</sub>Cl<sub>2</sub>:EtOH, 9:1); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 7.79 (1H, s, H6), 5.74 (1H, d, *J* 5.0, H1'), 4.00 (1H, app. t, *J* 4.9, H3'), 3.87-3.81 (2H, m, H5',H5''), 3.59-3.57 (1H, m, H4'), 3.56-3.53 (1H, m, H2'); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 168.19, 156.62, 143.50, 89.36, 81.71, 73.76, 73.00, 73.39, 51.89; MS ESI (*m/z*): [M-H]<sup>-</sup> calcd for C<sub>9</sub>H<sub>9</sub>IN<sub>5</sub>O<sub>5</sub> 393.96; found 394.000; [M+I]<sup>-</sup> calcd for C<sub>9</sub>H<sub>10</sub>I<sub>2</sub>N<sub>5</sub>O<sub>5</sub> 521.88; found 521.894.

### 5'-Amino-5'-deoxy-5-iodouridine **1c**



Triphenylphosphine (0.65 g, 2.5 mmol) was added to a solution of nucleoside **1b** (0.79 g, 2 mmol) in pyridine (2 ml) After 3 h of stirring, conc. aq. NH<sub>3</sub> (1 ml) was added, and the suspension was stirred overnight. The reaction mixture was then evaporated, the residue was suspended in 0.1% aq. TFA and filtered. The filtrate (total volume 200 ml) was passed through a column packed with Servacel P23 in NH<sub>4</sub><sup>+</sup> form. Elution was performed with a linear gradient of NH<sub>4</sub>HCO<sub>3</sub> (0→0.2 M) in 20% aq. EtOH. Fractions containing the product were combined and evaporated. The residue was evaporated several times from aq. EtOH to remove traces of NH<sub>4</sub>HCO<sub>3</sub>. Yield: 0.20 g (25%).  $R_f$  0.62 (Pr<sup>i</sup>OH:H<sub>2</sub>O:conc. aq. NH<sub>3</sub>, 6:3:1); <sup>1</sup>H NMR (D<sub>2</sub>O): 7.96 (1H, s, H6), 5.70 (1H, d, *J* 4.1, H1'), 4.48-4.44 (1H, m, H3'), 4.14-4.08 (2H, m, H2',H4'), 3.38-3.18 (2H, m, H5',H5''); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 171.80, 158.16, 145.37, 90.87, 82.36, 72.87, 70.95, 70.48, 70.39, 47.79; MS ESI (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>9</sub>H<sub>13</sub>IN<sub>3</sub>O<sub>5</sub> 369.99; found 369.996;

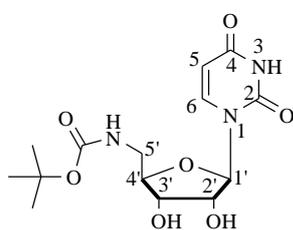
### 5'-Amino-5'-deoxyuridine **2b**



Triphenylphosphine (5.76 g, 22 mmol), sodium azide (6.50 g, 100 mmol), and bromotrichloromethane (2.2 ml, 22 mmol) were added to a solution of uridine (2.44 g, 10 mmol) in DMF (70 ml). After 30 h, bromotrichloromethane (0.55 ml, 5.5 mmol) and triphenylphosphine (1.44 g, 5.5 mmol) were added. The mixture was stirred for 48 h. The solvent was

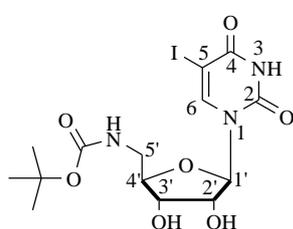
removed under reduced pressure and the residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (75 ml) followed by extraction with  $\text{H}_2\text{O}$  (3x75 ml). The aqueous extracts were evaporated, and EtOH (50 ml) was added to the oily residue. The precipitate formed in EtOH was filtered off. The filtrate was evaporated and the crude product **2a** was further used without additional purification. The residue after evaporation of the filtrate was dissolved in pyridine (10 ml), and triphenylphosphine (2.62 g, 10 mmol) was added. The mixture was stirred overnight and then conc. aq.  $\text{NH}_3$  (10 ml) was added. After 72 h the solvent was evaporated, the residue was twice co-evaporated with water and dissolved in water (100 ml) containing 0.1 % TFA. The precipitate was filtered off and washed with 20 % EtOH in water (30 ml). The combined filtrates (total volume 130 ml) were passed through a column with Dowex 50Wx2 in  $\text{H}^+$  form (30 ml), and then the column was washed by 20 % EtOH (40 ml). The product was eluted in a linear gradient of aq.  $\text{NH}_3$  in water (0→1 M). Fractions containing the product were combined and evaporated *in vacuo*. Yield: 1.95 g (80.1 %).  $R_f$  0.33 ( $\text{Pr}^i\text{OH}:\text{H}_2\text{O}:\text{conc. aq. NH}_3$ , 7:2:1);  $^1\text{H NMR}$  ( $\text{D}_2\text{O}$ ): 7.65 (1H, d,  $J$  7.9, H6), 5.90 (1H, d,  $J$  7.9, H5), 5.85 (1H, d,  $J$  4.5, H1'), 4.46 (1H, app. t, H3'), 4.21 (1H, app. t, H2'), 4.18-4.11 (1H, m, H4'), 3.24-3.04 (2H, m, H5',H5'').

#### 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxyuridine **2c**



Di-*tert*-butyl pyrocarbonate (2.54 g, 11.7 mmol) was added to a solution of compound **2b** (9.0 mmol) in a mixture of 1 M NaOH (20 ml) and  $\text{Pr}^i\text{OH}$  (9 ml). The mixture was stirred for 2 h at 50 °C then 1 M NaOH (10 ml) was added. The mixture was stirred overnight at 5 °C and then was washed with diethyl ether (70 ml). The aqueous layer was evaporated. The product was purified by RPC in a linear gradient of EtOH in water (0→20 %). The appropriate fractions were evaporated to give compound **2c**. Yield: 2.58 g (84%);  $R_f$  0.77 ( $\text{Pr}^i\text{OH}:\text{H}_2\text{O}:\text{conc. aq. NH}_3$ , 7:2:1);  $^1\text{H NMR}$  ( $\text{DMSO-}d_6$ ): 7.66 (1H, d,  $J$  8.1, H6), 7.02 (H, bt,  $J$  5.6, NH) 5.73 (1H, d,  $J$  6.2, H1'), 5.61 (1H, d,  $J$  7.9, H5), 4.10-3.96 (1H, m, H3'), 3.90-3.81 (1H, m, H2'), 3.80-3.73 (1H, m, H4'), 3.27-3.03 (2H, m, H5',H5''), 1.37 (9H, s,  $\text{CH}_3\text{-Boc}$ );  $^{13}\text{C NMR}$  ( $\text{DMSO-}d_6$ ): 173.04, 157.23, 156.05, 150,75, 139.90, 102.74, 88.90, 82.19, 77.82, 73.30, 71.14, 42.57,28.47; MS ESI ( $m/z$ ):  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{14}\text{H}_{21}\text{N}_3\text{NaO}_7$  366.13; found 366.196;  $[\text{2M}+\text{Na}]^+$  calcd for  $\text{C}_{28}\text{H}_{42}\text{N}_6\text{NaO}_{14}$  709.27; found 709.391.

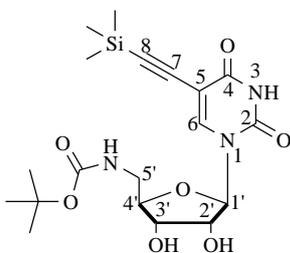
#### 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-iodouridine **3**



Iodine chloride (0.79 ml, 15.7 mmol) was added to a solution of compound **2c** (2.28 g, 6.6 mmol) in MeOH (30 ml). The mixture was

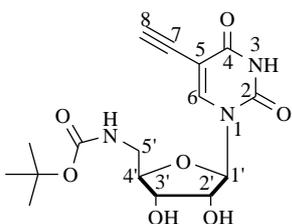
stirred for 1.5 h at 22°C, then conc. aq. NH<sub>3</sub> (30 ml) was added. In 20 min, 0.5 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (30 ml) was added. The mixture was evaporated, and EtOH (120 ml) was added to the oily residue, and the precipitate formed was filtered off. The filtrate was evaporated, and the product was purified by RPC in a linear gradient of EtOH in water (0→30 %). The appropriate fractions were evaporated to give compound **3**. Yield: 2.23 g (72%). R<sub>f</sub> 0.47 (Pr<sup>i</sup>OH:H<sub>2</sub>O:conc. aq. NH<sub>3</sub>, 7:2:1); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 11.75 (1H, s, NH-Ura), 8.08 (1H, s, H6), 7.03 (1H, bt, *J* 5.6, NH-Boc), 5.68 (1H, d, *J* 6.2, H1'), 5.38 (1H, d, *J* 5.5, OH), 5.13 (1H, d, *J* 4.7, OH), 4.18-4.07 (1H, m, H3'), 3.91-3.80 (1H, m, H2'), 3.79-3.71 (1H, m, H4'), 3.28-3.05 (2H, m, H5',H5''), 1.38 (9H, s, CH<sub>3</sub>-Boc); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 160.72, 156.07, 150.75, 145.54, 88.24, 83.24, 78.09, 72.35, 70.78, 70.42, 42.44, 28.50. MS ESI (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>21</sub>IN<sub>3</sub>O<sub>7</sub> 470.04; found 470.090; [M+Na]<sup>+</sup> calcd for C<sub>14</sub>H<sub>20</sub>IN<sub>3</sub>NaO<sub>7</sub> 492.02; found 492.000.

**5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-(2-trimethylsilylethynyl)uridine**



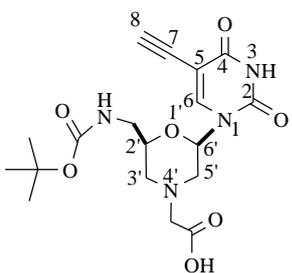
Tetrakis(triphenylphosphine)palladium(0) (0.60 g, 0.52 mmol), CuI (0.20 g, 1.03 mmol), diisopropylethylamine (1.77 ml, 10.4 mmol) were added to a solution of nucleoside **3** (2.43 g, 5.2 mmol) in anhydrous DMF (20 ml). The mixture was stirred for 1 h at 22°C in Ar atmosphere. Then 5 % disodium salt of EDTA/H<sub>2</sub>O (16 ml) was added, and the mixture was concentrated *in vacuo*. Diethyl ether (140 ml) was added to the residue and the precipitate was filtered off. The filtrate was evaporated and the residue obtained was purified on a silica gel using a gradient of MeOH in CH<sub>2</sub>Cl<sub>2</sub> (0→8%) as an eluent. The appropriate fractions were evaporated to give title compound. Yield: 1.71 g (74.7 %), R<sub>f</sub> 0.45 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH, 9:1); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 11.72 (1H, s, NH-Ura), 8.04 (1H, s, H6), 7.03 (1H, bt, *J* 5.7, NH-Boc), 5.71 (1H, d, *J* 6.6, H1'), 5.39 (1H, d, *J* 5.7, OH), 5.14 (1H, d, *J* 5.1, OH), 4.19-4.07 (1H, m, H3'), 3.90-3.81 (1H, m, H2'), 3.80-3.71 (1H, m, H4'), 3.28-3.08 (2H, m, H5',H5''), 1.38 (9H, s, CH<sub>3</sub>-Boc), 0.19 (9H, s, Si-CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 161.32, 155.82, 149.76, 145.15, 98.83, 97.53, 87.94, 83.25, 77.79, 72.17, 70.51, 70.42, 41.88, 28.23, -0.05; MS ESI (*m/z*): [M-H]<sup>-</sup> calcd for C<sub>19</sub>H<sub>28</sub>N<sub>3</sub>O<sub>7</sub>Si 438.17; found 438.295; [M+Cl]<sup>-</sup> calcd for C<sub>19</sub>H<sub>29</sub>ClN<sub>3</sub>O<sub>7</sub>Si 474.15; found 474.194.

#### 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-ethynyluridine **4**



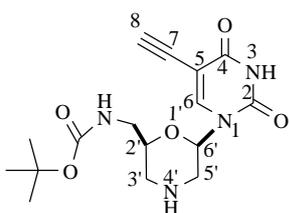
The above Boc,TMS-diprotected 5'-amino-5'-deoxy-5-ethynyluridine (1.24 g, 2.82 mmol) was dissolved in methanol (10 ml), and conc. aq. NH<sub>3</sub> (10 ml) was added. The mixture was stirred for 3 h at 22°C and then overnight at 5 °C, then was concentrated *in vacuo*. The residue was co-evaporated three times with MeCN to give compound **4** as a beige foam. Yield: 1.07 g (97%), R<sub>f</sub> 0.42 (CH<sub>2</sub>Cl<sub>2</sub>: MeOH, 9:1); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 11.72 (1H, s, NH-Ura), 8.04 (1H, s, H6), 7.03 (1H, bt, *J* 5.9, NH-Boc), 5.70 (1H, d, *J* 5.9, H1'), 5.40 (1H, d, *J* 5.6, OH), 5.13 (1H, d, *J* 4.4, OH), 4.14 (1H, s, H8), 4.13-4.05 (1H, m, H3'), 3.91-3.81 (1H, m, H2'), 3.80-3.72 (1H, m, H4'), 3.29-3.06 (2H, m, H5',H5''), 1.37 (9H, s, CH<sub>3</sub>-Boc); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 161.54, 155.84, 149.78, 145.15, 98.19, 88.00, 84.12, 83.00, 77.85, 76.09, 72.30, 70.47, 42.07, 28.23; MS ESI (*m/z*): [M+Na]<sup>+</sup> calcd for C<sub>16</sub>H<sub>21</sub>N<sub>3</sub>NaO<sub>7</sub> 390.13; found 390.190; [2M+Na]<sup>+</sup> calcd for C<sub>32</sub>H<sub>42</sub>N<sub>6</sub>NaO<sub>14</sub> 757.27; found 757.290.

#### {2'-[*N*-(*tert*-Butyloxycarbonyl)aminomethyl]-6'--(5-ethynyluracil-1-yl)morpholin-4'-yl}acetic acid **5**



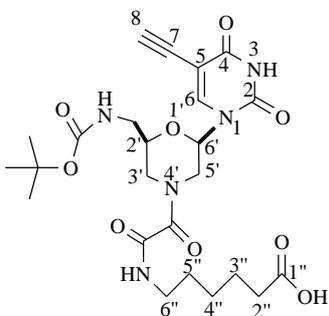
Sodium periodate (0.543 g, 2.54 mmol) in H<sub>2</sub>O (5 ml) was added to compound **4** (0.850 g, 2.32 mmol) in EtOH (40 ml). After 20 min stirring, solution of glycine (0.237 g, 3.16 mmol) in water (2 ml) adjusted by Et<sub>3</sub>N (0.38 ml) to pH 9.0–9.5 was added. The mixture was stirred for 1 h while pH was maintained at 9.0-9.5 (total Et<sub>3</sub>N was 1.15 ml). The precipitate was removed by filtration. Sodium cyanoborohydride (0.204 g, 3.25 mmol) was then added, and the mixture was stirred for 2.5 h. The solution was acidified with TFA (1.52 ml) to pH 5, stirred for 1 h and evaporated. The residue was purified on a silica gel using a gradient of MeOH in CH<sub>2</sub>Cl<sub>2</sub> (0→35 %) as an eluent. Some fractions were subjected to additional chromatography. The appropriate fractions were evaporated, the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and the product was precipitated with a ten-fold volume of diethyl ether/petroleum ether (1:1 v/v) to give compound **5**. Yield: 0.343 g (39%). R<sub>f</sub> 0.48 (CH<sub>2</sub>Cl<sub>2</sub>: MeOH:Et<sub>3</sub>N, 4:1:0.2); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 8.06 (1H, s, H6), 7.03 (1H, bt, *J* 5.6, NH-Boc), 5.58-5.49 (1H, m, H6'), 4.15 (1H, s, H8), 3.77-3.67 (1H, m, H2'), 3.30-2.97 (4H, m, NCH<sub>2</sub>C(O), 2'-NHCH<sub>2</sub>), 2.95-2.82 (1H, m, H3'), 2.79-2.70 (1H, m, H5'), 2.35 (1H, app. t, H3'), 2.18 (1H, app. t, H5'), 1.37 (9H, s, CH<sub>3</sub>-Boc); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 171.66, 161.48, 155.76, 148.96, 144.70, 97.87, 84.01, 79.52, 77.85, 76.11, 75.12, 58.35, 54.45, 52.87, 28.24; MS ESI (*m/z*): [M-H]<sup>-</sup> calcd for C<sub>18</sub>H<sub>23</sub>N<sub>4</sub>O<sub>7</sub> 407.16; found 407.200; [M+C<sub>2</sub>F<sub>3</sub>O<sub>2</sub>]<sup>-</sup> calcd for C<sub>20</sub>H<sub>24</sub>F<sub>3</sub>N<sub>4</sub>O<sub>9</sub> 521.15; found 521.194.

## 2'-[N-(*tert*-Butyloxycarbonyl)aminomethyl]-6'-(5-ethynyluracil-1-yl)morpholine **6**



Sodium periodate (0.058 g, 0.27 mmol) in H<sub>2</sub>O (0.5 ml) was added to compound **4** (0.095 g, 0.26 mmol) in EtOH (5 ml). After 20 min stirring, (NH<sub>4</sub>)<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·4H<sub>2</sub>O (0.082 g, 0.31 mmol) was added. The mixture was adjusted to pH 9.0-9.5 and stirred for 1 h maintaining pH in the same range with Et<sub>3</sub>N (total volume 0.2 ml). The precipitate was removed by filtration. Sodium cyanoborohydride (0.022 g, 0.338 mmol) was added to the filtrate, and the mixture was stirred for 2.5 h. The solution was acidified with TFA (0.2 ml) to pH 5, stirred for 1 h and evaporated. The residue was purified by RPC using a linear gradient of EtOH in water (0→50 %) as an eluent. The appropriate fractions were evaporated to give compound **6**. Yield: 0.035 g (40%). R<sub>f</sub> 0.57 (Pr<sup>i</sup>OH:H<sub>2</sub>O, 4:1); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 8.07 (1H, s, H<sub>6</sub>), 6.94 (1H, bt, NH-Boc), 5.47 (1H, d, *J* 5.5, H<sub>6'</sub>), 4.12 (1H, s, H<sub>8</sub>), 3.69-3.62 (1H, m, H<sub>2'</sub>), 3.08-2.99 (2H, m, 2'-NHCH<sub>2</sub>), 2.84 (1H, app. d, H<sub>3'</sub>), 2.69 (1H, app. d, H<sub>5'</sub>), 2.63-2.54 (1H, app. t, H<sub>3'</sub>), 2.43-2.34 (1H, app. t, H<sub>5'</sub>), 1.38 (9H, s, CH<sub>3</sub>-Boc); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 161.71, 155.99, 145.05, 97.93, 84.13, 80.58, 78.03, 77.39, 76.48, 48.30, 46.67, 42.56, 28.49, MS ESI (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>18</sub>H<sub>23</sub>N<sub>4</sub>O<sub>5</sub> 351.17; found 351.300; [M+Na]<sup>+</sup> calcd for C<sub>16</sub>H<sub>22</sub>N<sub>4</sub>NaO<sub>5</sub> 373.15; found 373.200.

## 9-{2'-[N-(*tert*-butyloxycarbonyl)aminomethyl]-6'-(5-ethynyluracil-1-yl)morpholin-4'-yl}-8,9-dioxo-7-azanonanoic acid **7**

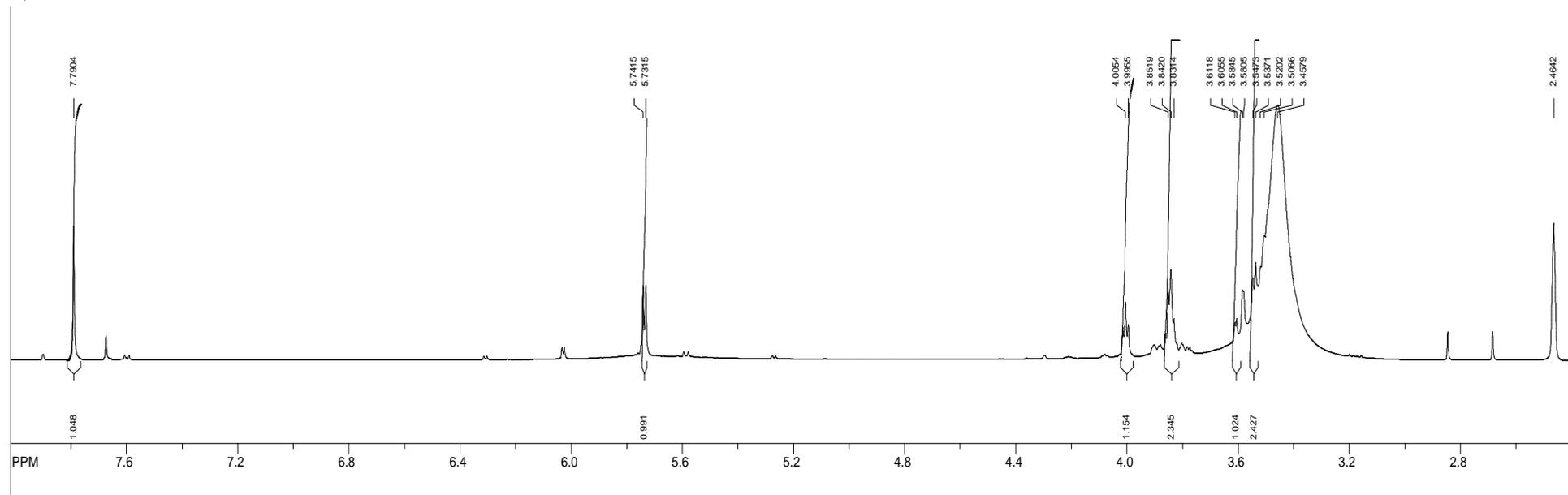


Boc-protected morpholino nucleoside **6** (0.022 g, 0.06 mmol), methyl *N*-(6-hydroxy-6-oxohexyl)oxamate (0.14 g, 0.6 mmol) and Et<sub>3</sub>N (0.1 ml, 0.7 mmol) in pyridine (1 ml) were stirred for 24 h at 50 °C. The mixture was evaporated several times to remove traces of pyridine and the target product was purified by RPC in a linear gradient of EtOH in water (0→75 %) as an eluent. The appropriate fractions were evaporated to give compound **7**. Yield: 0.024 g (70%). R<sub>f</sub> 0.67 (Pr<sup>i</sup>OH:H<sub>2</sub>O, 4:1); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, almost all signals are doubled as in Ref.<sup>1</sup>): 8.90-8.80 (1H, m, C(O)C(O)NH), 7.74, 7.72 (2x0.5H, 2s H<sub>6</sub>), 7.51, 7.14 (2x0.5H, 2bt, NH-Boc), 5.69, 5.62 (2x0.5H, 2dd, *J* 10.2, 2.4, H<sub>6'</sub>), 4.24-4.11 (1H, m, H<sub>2'</sub>), 3.78, 3.76 (2x0.5H, 2s, CH), 3.72-3.60 (2H, m, 2'-NHCH<sub>2</sub>), 3.30-3.23 (0.5H, m, H<sub>3'</sub>), 3.19-2.96 (4.5H, m, H<sub>3'</sub>, H<sub>6''</sub>, H<sub>5'</sub>), 2.92-2.86 (0.5H, m, H<sub>3'</sub>), 2.65-2.55 (0.5H, m, H<sub>5'</sub>), 1.94-1.81 (2H, m, H<sub>2''</sub>), 1.50-1.39 (3H, m, H<sub>3''</sub>, H<sub>4''</sub>, H<sub>5''</sub>), 1.38, 1.37 (2x4.5H, 2m, CH<sub>3</sub>-Boc), 1.29-1.13 (3H, m, H<sub>3''</sub>, H<sub>4''</sub>, H<sub>5''</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, almost all signals are doubled): 177.88, 177.41, 170.76, 170.53, 163.85, 163.56, 163.06, 162.92, 156.79, 155.76, 155.20, 154.99, 142.38, 97.98, 80.90, 78.89, 78.44, 77.95, 77.70, 75.03, 74.34, 71.44, 48.04, 47.39, 43.18, 42.07, 41.64, 38.27, 38.11, 37.90, 28.71, 28.60,

28.19, 26.87, 26.69, 25.99; MS ESI ( $m/z$ ):  $[M-H]^-$  calcd for  $C_{24}H_{32}N_5O_9$  534.22; found 534.393;  
 $[2M-H]^-$  calcd for  $C_{48}H_{65}N_{10}O_{18}$  1069.45; found 1069.585.

# 5'-Azido-5'-deoxy-5-iodouridine 1b

SpinWorks 2.5: N3IrU; DMSO-D6

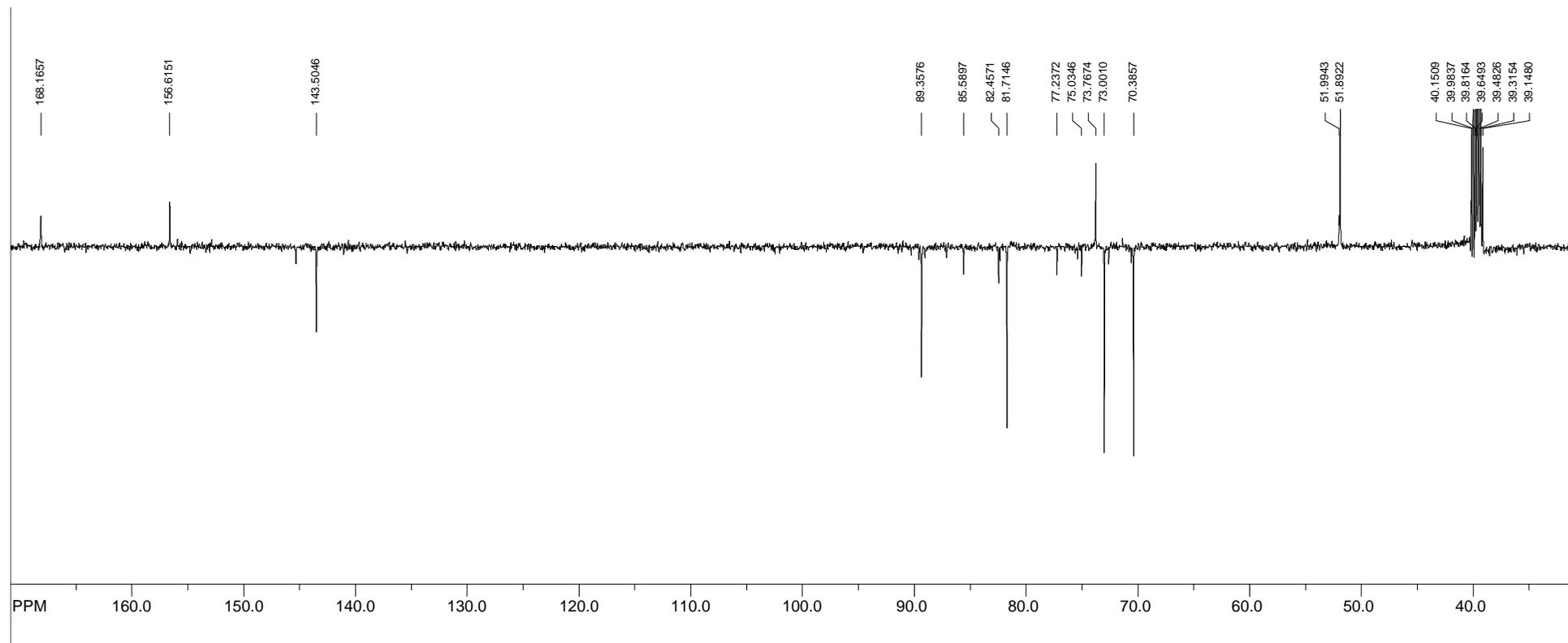


file: D:\0a00yia\_ar60iarou0airj\SIEMorGly 2016\2016 anibh-n3iru\1fid\_ expt <zg30>  
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time domain size: 65536 points  
width: 10964.91 Hz = 21.923916 ppm = 0.167311 Hz/pt  
number of scans: 40

freq. of 0 ppm: 500.130023 MHz  
processed size: 65536 complex points  
LB: 0.000 CB: 0.0000

# 5'-Azido-5'-deoxy-5-iodouridine 1b

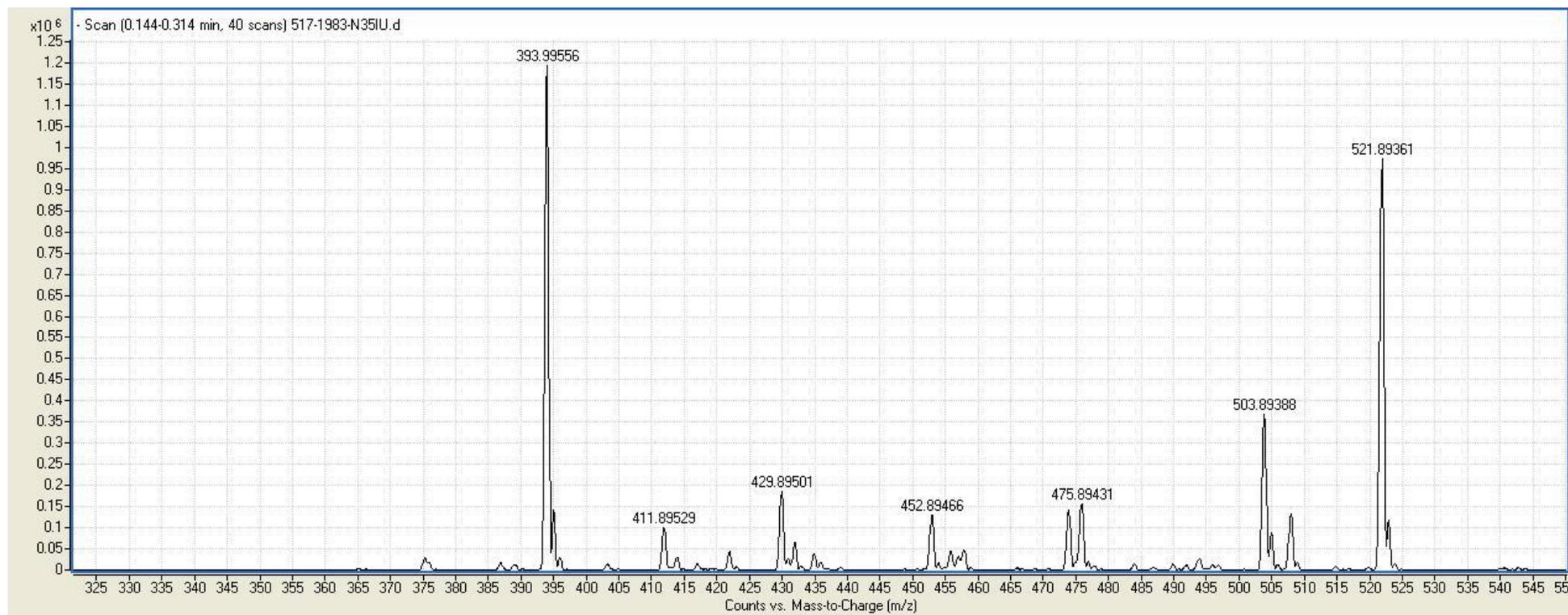
SpinWorks 2.5: N3lrU; DMSO-D6



file: D:\Ooüüüü\_äiëöiäiöü\Öäüü\äiö\MorGly 2016\2016 ä\nih-n3iru\1361fid expt: <jmod>  
transmitter freq.: 125.772075 MHz  
time domain size: 65536 points  
width: 30303.03 Hz = 240.936076 ppm = 0.462388 Hz/pt  
number of scans: 416

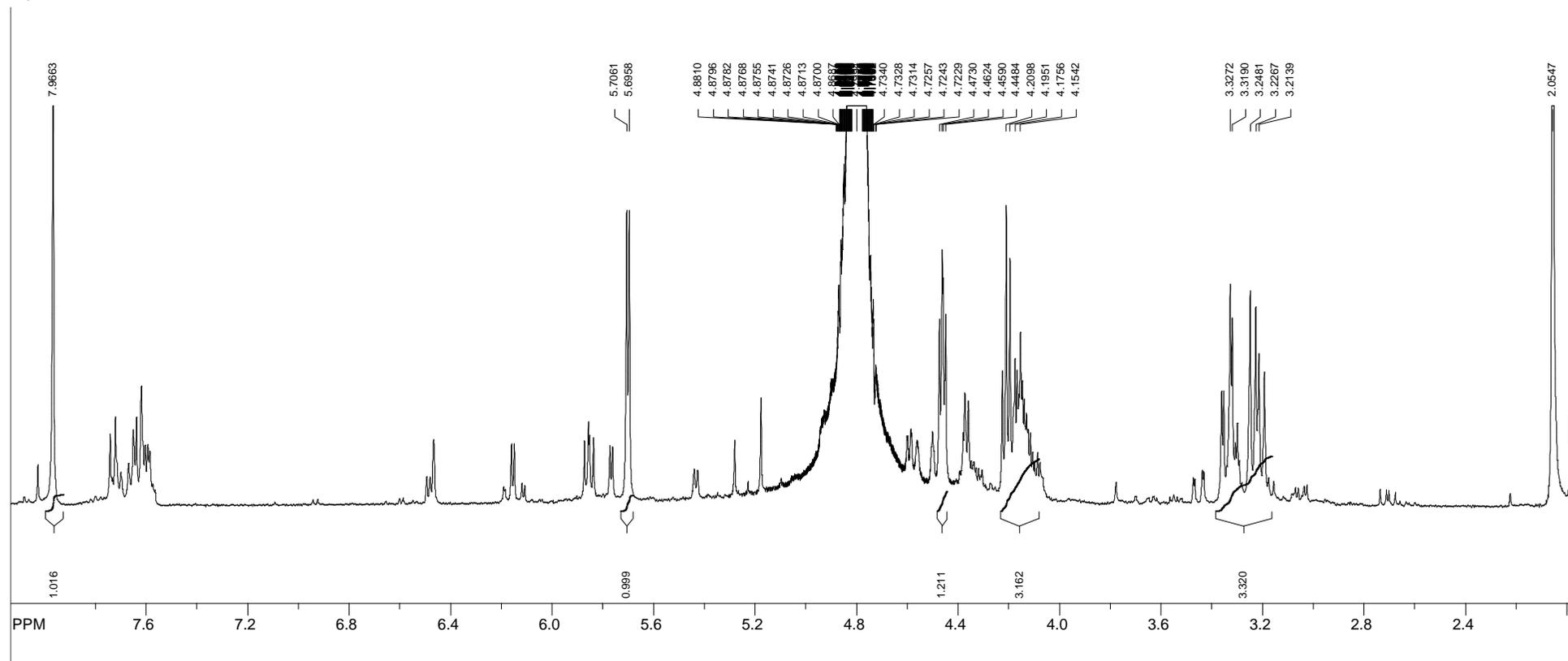
freq. of 0 ppm: 125.757816 MHz  
processed size: 131072 complex points  
LB: 0.000 GB: 0.0000

### 5'-Azido-5'-deoxy-5-iodouridine 1b



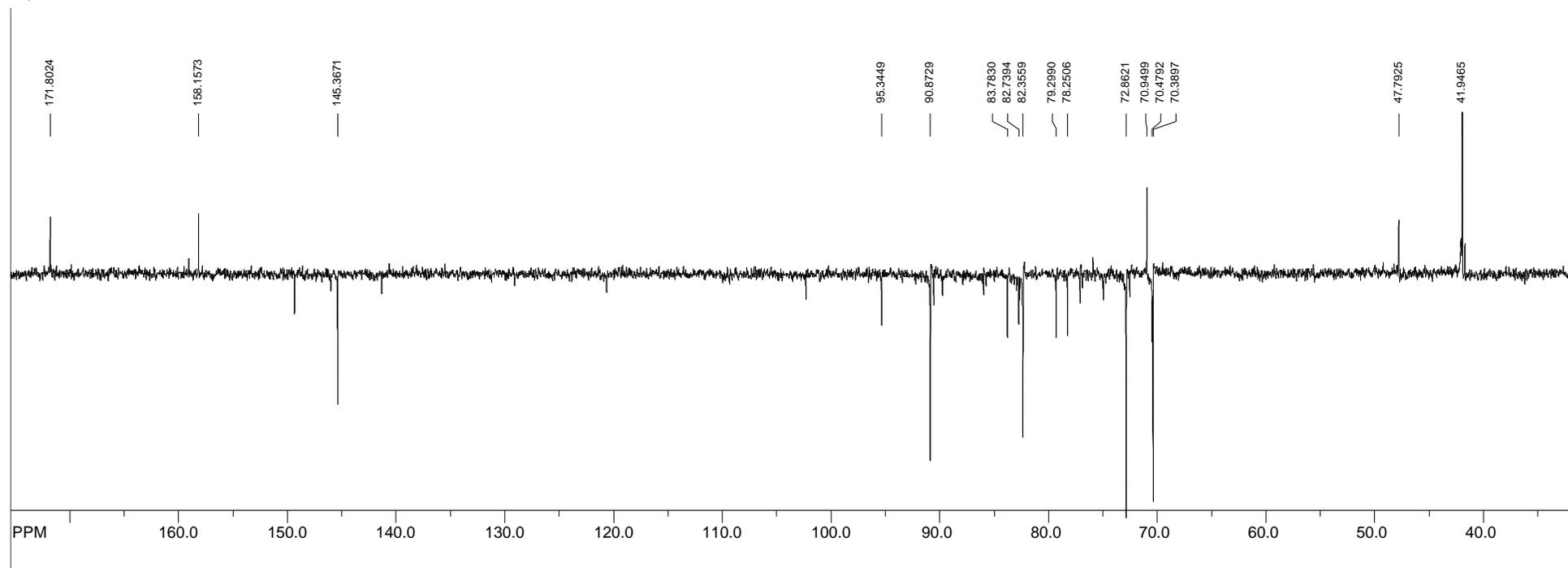
# 5'-Amino-5'-deoxy-5-iodouridine 1c

SpinWorks 2.5: F 2728; D2O



# 5'-Amino-5'-deoxy-5-iodouridine 1c

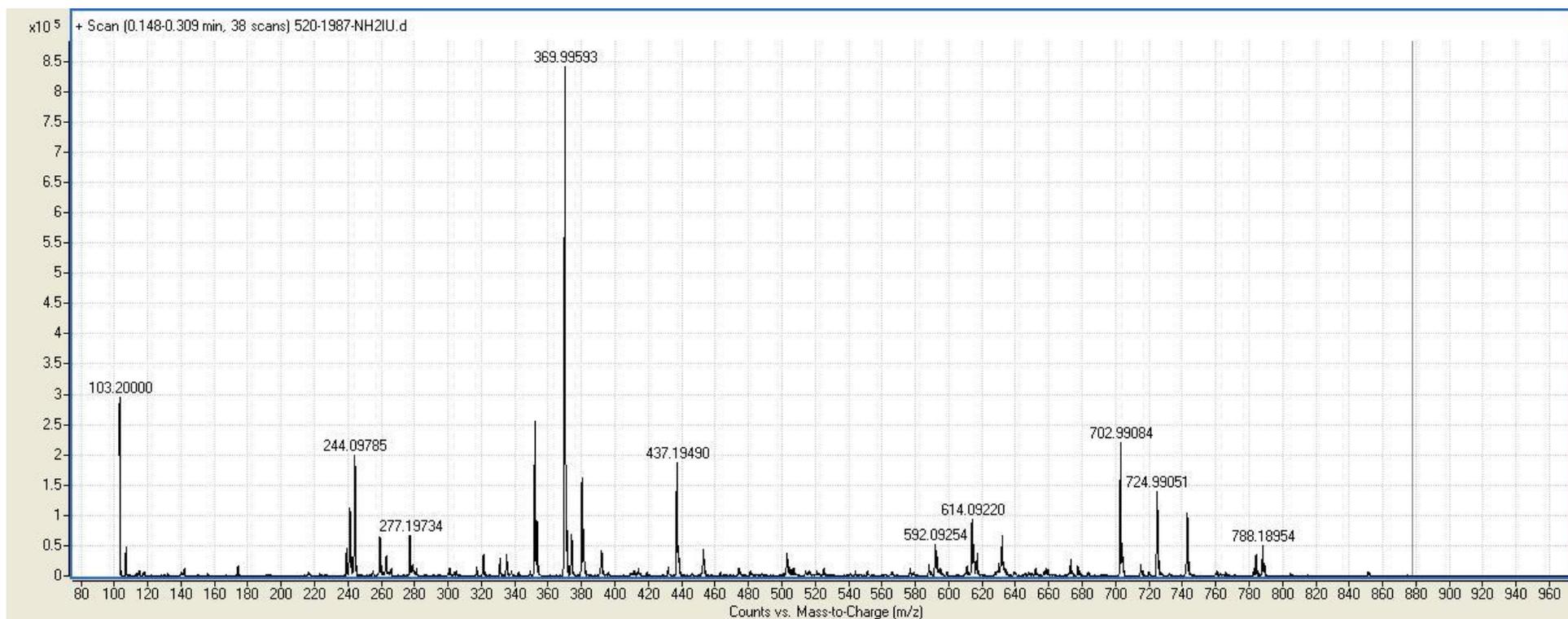
SpinWorks 2.5: NH2IU; D2O



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transmitter freq.: 125.772075 MHz  
time domain size: 65536 points  
width: 30303.03 Hz = 240.936076 ppm = 0.462388 Hz/pt  
number of scans: 432

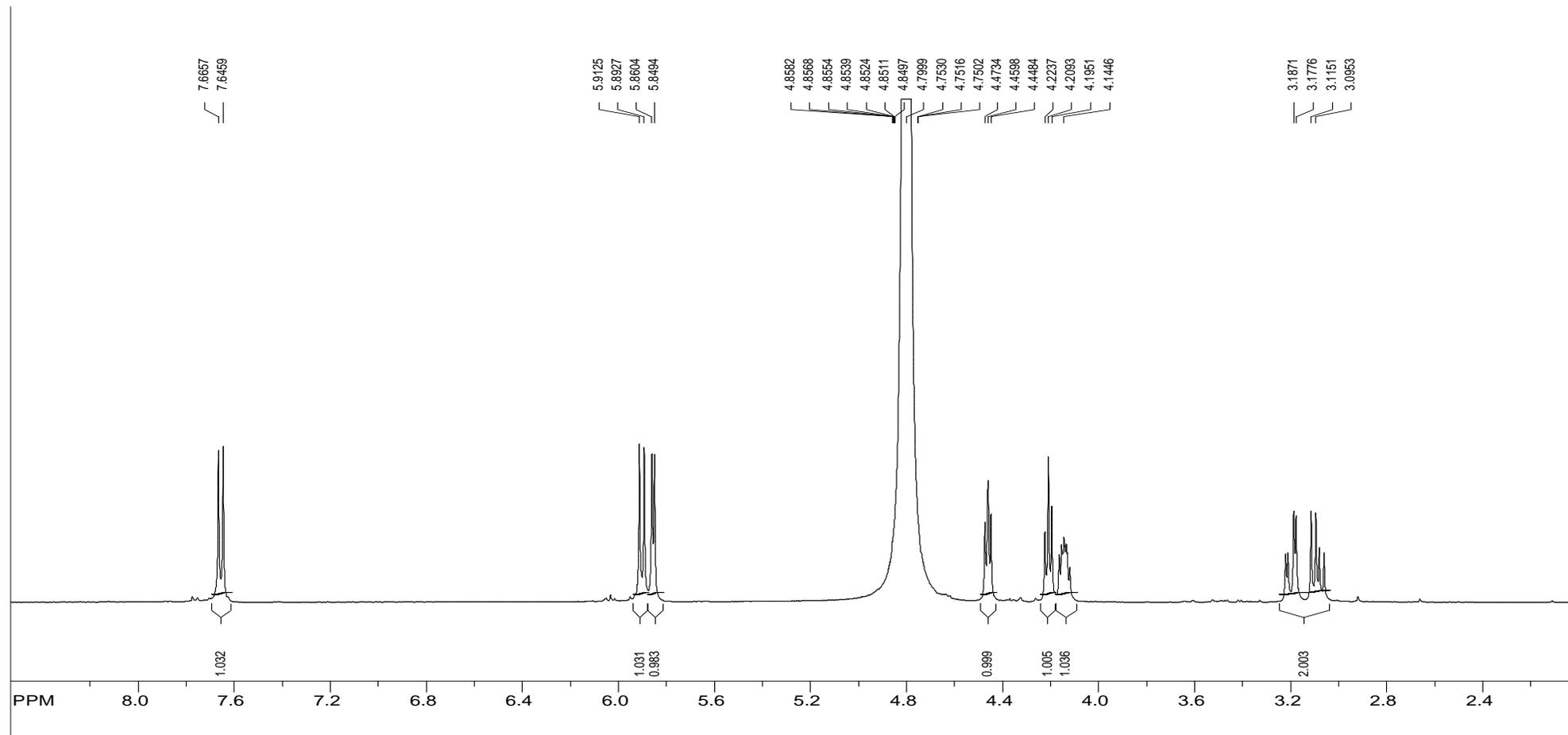
freq. of 0 ppm: 125.757816 MHz  
processed size: 131072 complex points  
LB: 0.000 GB: 0.0000

### 5'-Amino-5'-deoxy-5-iodouridine 1c



# 5'-Amino-5'-deoxyuridine 2b

SpinWorks 2.5: A - 567; D2O

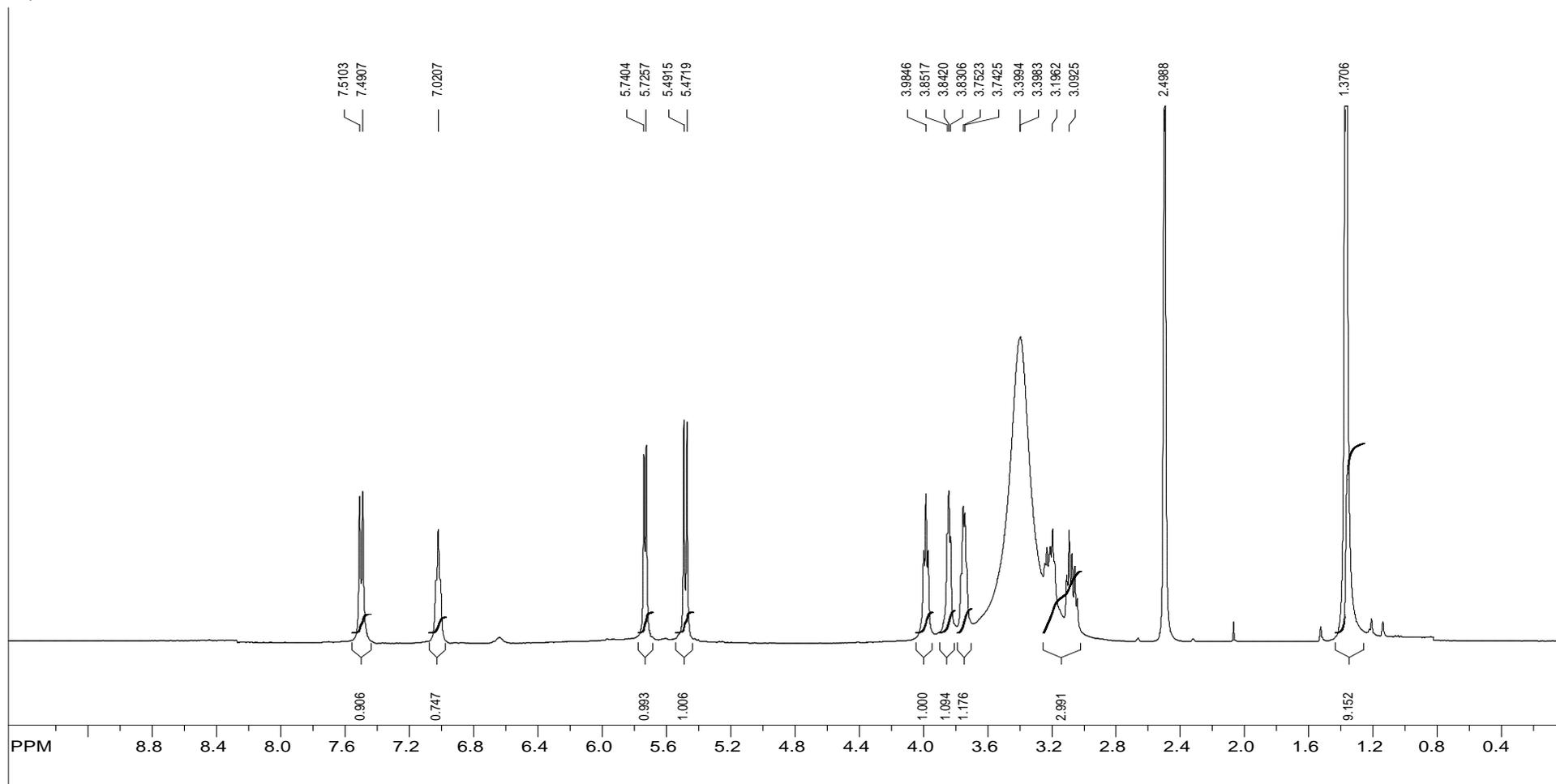


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time domain size: 32000 points  
width: 8802.82 Hz = 21.999672 ppm = 0.275088 Hz/pt  
number of scans: 64

freq. of 0 ppm: 400.129961 MHz  
processed size: 65536 complex points  
LB: 0.000 GB: 0.0000

# 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxyuridine 2c

SpinWorks 2.5: A - 570; DMSO - d6

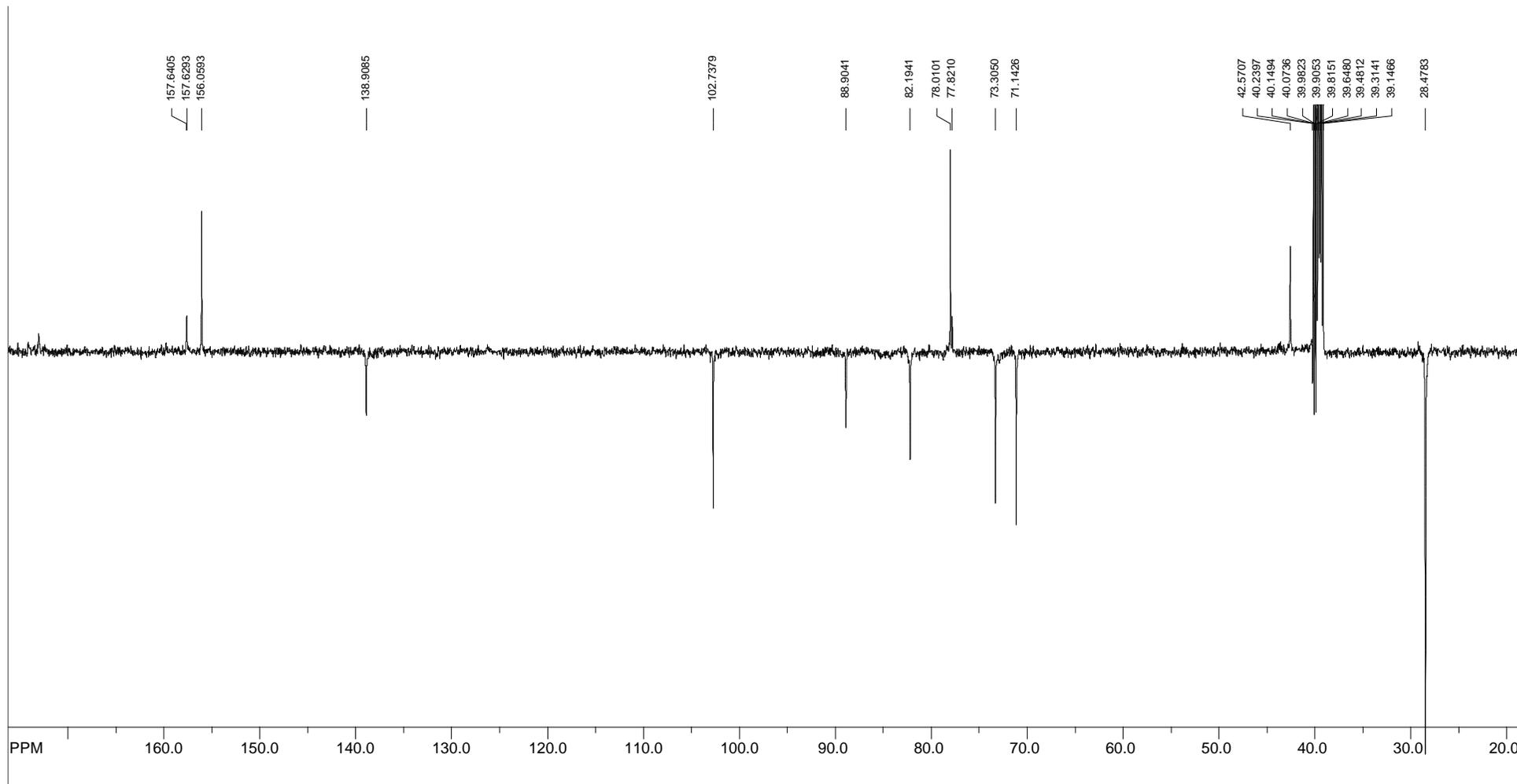


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time domain size: 32000 points  
width: 8802.82 Hz = 21.999672 ppm = 0.275088 Hz/pt  
number of scans: 32

freq. of 0 ppm: 400.130004 MHz  
processed size: 65536 complex points  
LB: 0.000 GB: 0.0000

# 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxyuridine 2c

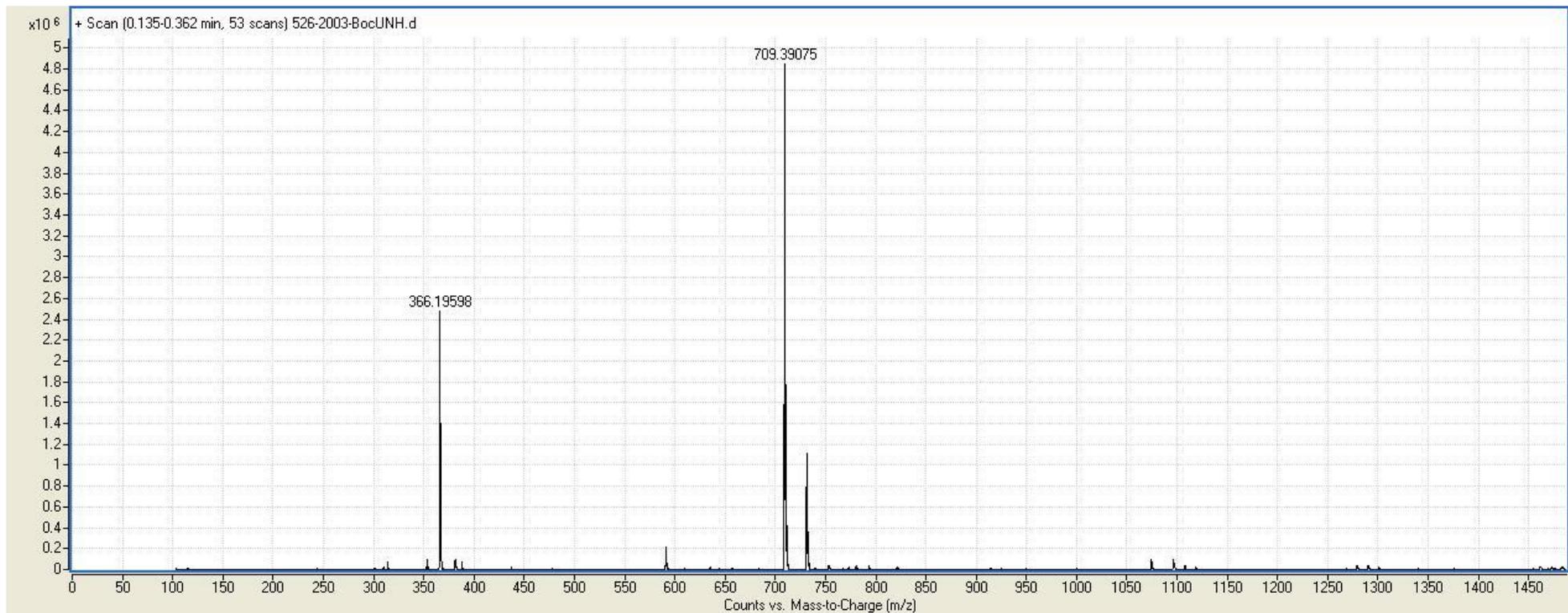
SpinWorks 2.5: BNHU; DMSO-d6



file: D:\Oaouyia\_aieoiarou\Oair\dl\MorGly 2016\2016 alnibh-bnhu\137\fid exp: <jmod>  
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time domain size: 65536 points  
width: 30303.03 Hz = 240.936076 ppm = 0.462388 Hz/pt  
number of scans: 2272

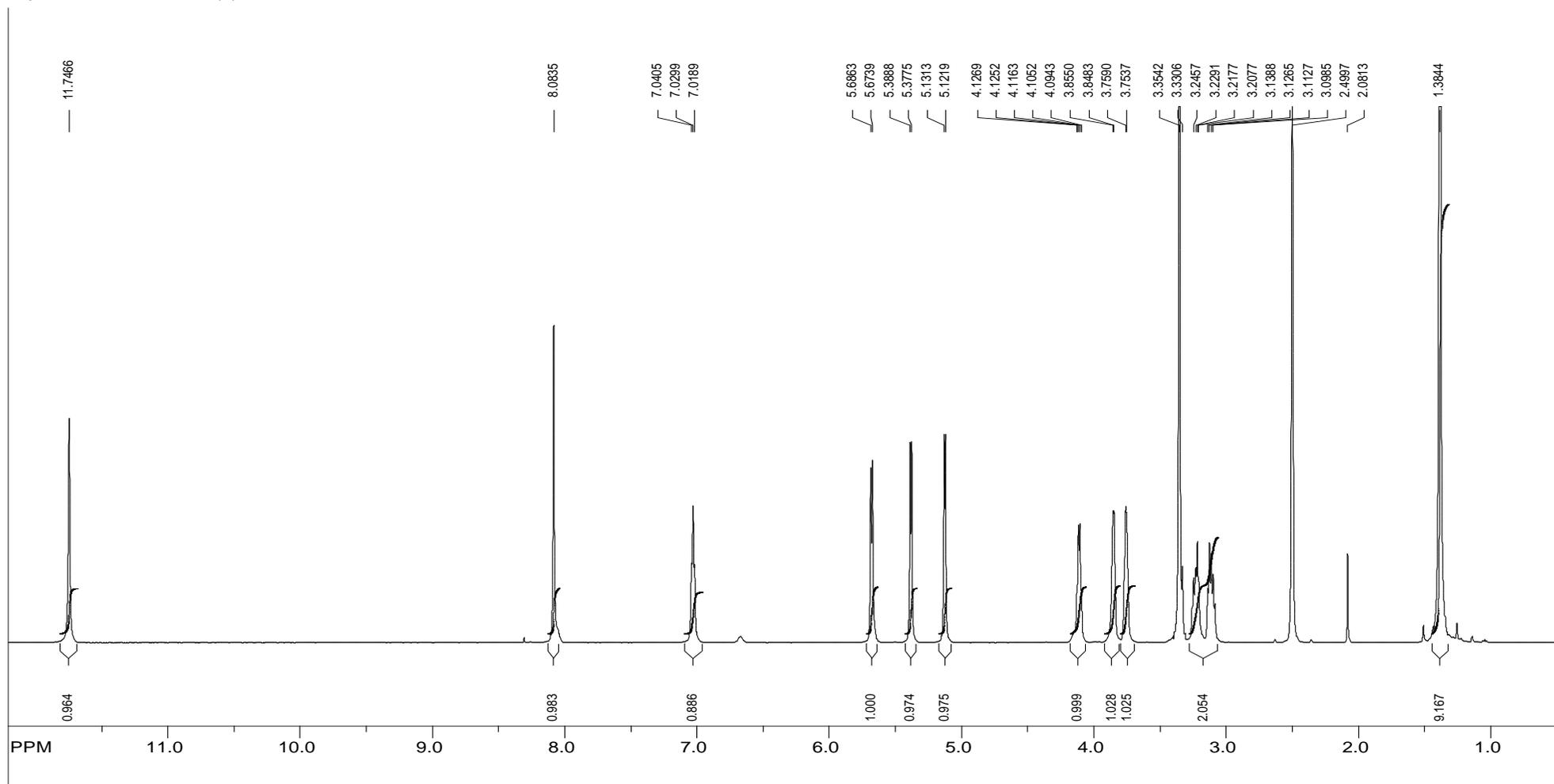
freq. of 0 ppm: 125.757816 MHz  
processed size: 131072 complex points  
LB: 0.000 GB: 0.0000

**5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxyuridine 2c**



# 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-iodouridine 3

SpinWorks 2.5: A-573(2); DMSO-d6

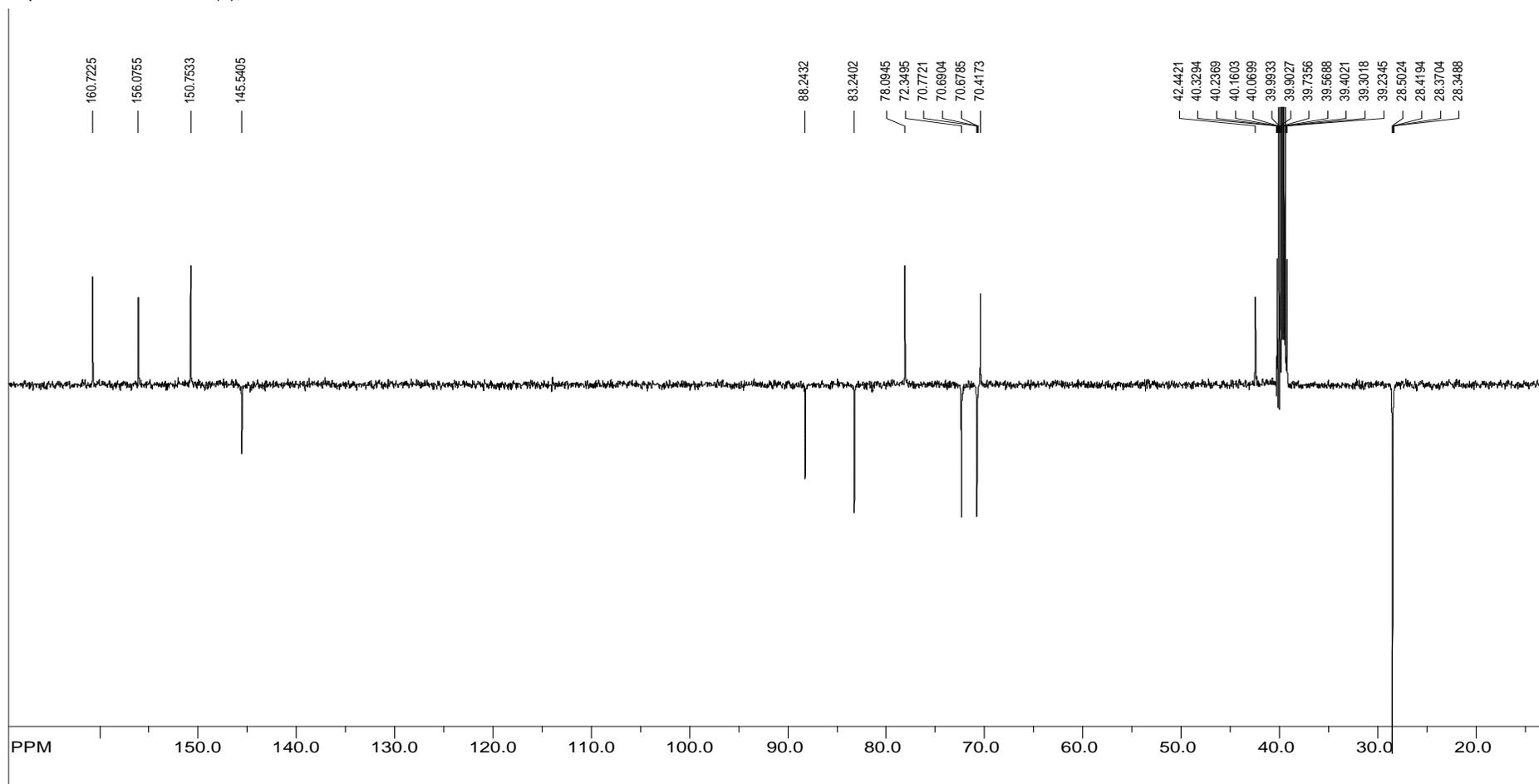


file: D:\Lyuda\analysis\NMR\nibh-a-573-2\nibh-a-573-2\1fid exp: <zg30>  
transmitter freq.: 500.134751 MHz  
time domain size: 65536 points  
width: 10964.91 Hz = 21.923916 ppm = 0.167311 Hz/pt  
number of scans: 16

freq. of 0 ppm: 500.130006 MHz  
processed size: 65536 complex points  
LB: 0.000 GB: 0.0000

# 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-iodouridine 3

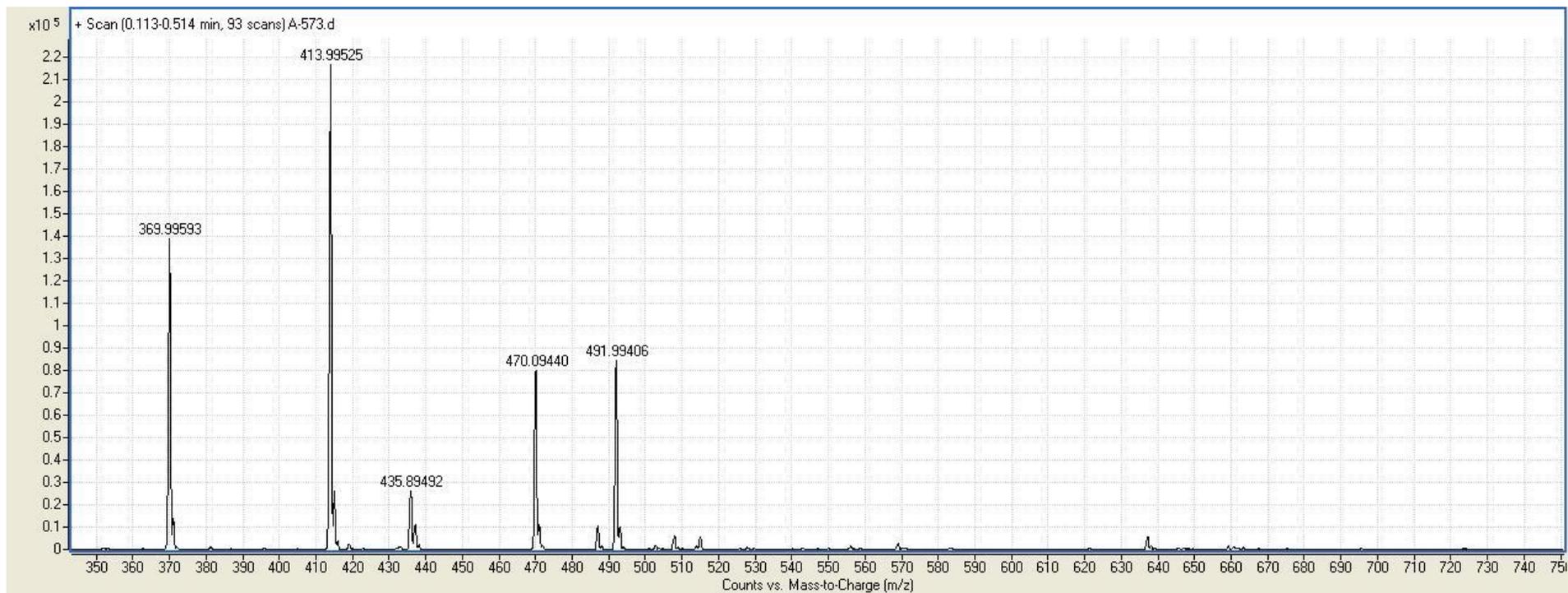
SpinWorks 2.5: A-573(2); DMSO-d6



file: D:\Lyuda\analysis\NMR\nibh-a-573-2\nibh-a-573-2\137fid exp: <jmod>  
transmitter freq.: 125.772075 MHz  
time domain size: 65536 points  
width: 30303.03 Hz = 240.936076 ppm = 0.462388 Hz/pt  
number of scans: 480

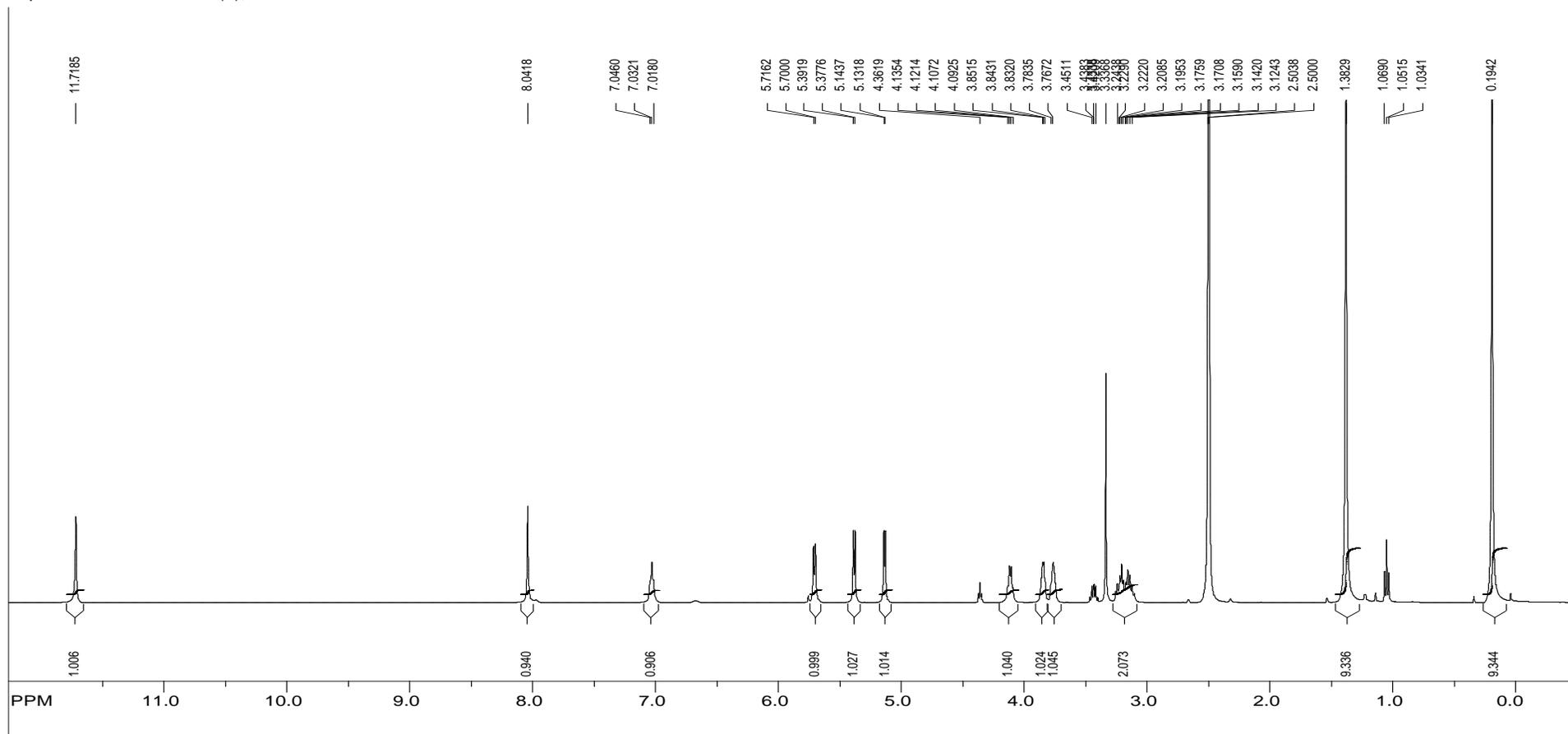
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processed size: 131072 complex points  
LB: 0.000 GB: 0.0000

**5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-iodouridine 3**



# 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-(2-trimethylsilylethynyl)uridine

SpinWorks 2.5: A-584(2); DMSO-d6

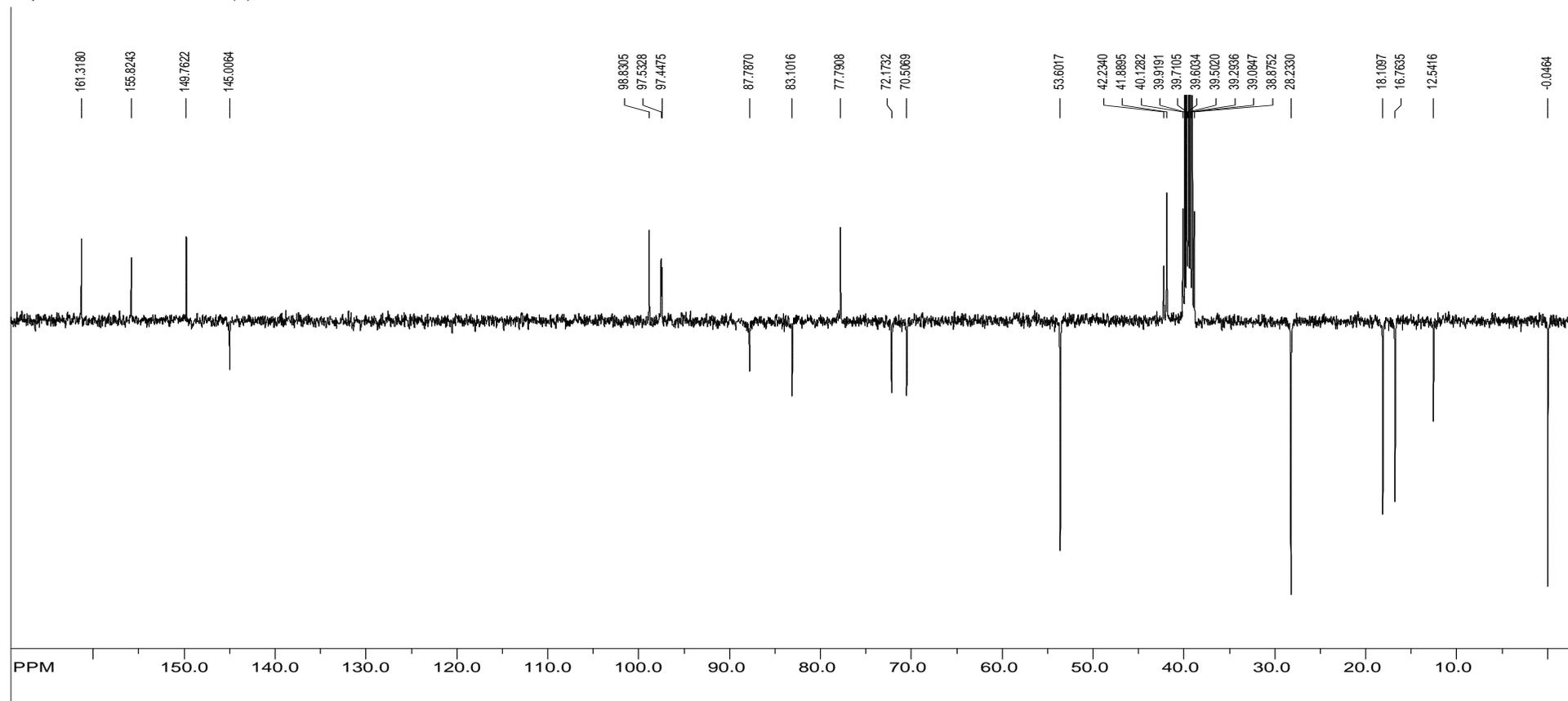


file: D:\Lyuda\analysis\NMR\nibh-a-584-2\nibh-a-584-2\1\fid exp: <zg30>  
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 time domain size: 32000 points  
 width: 8802.82 Hz = 21.999672 ppm = 0.275088 Hz/pt  
 number of scans: 88

freq. of 0 ppm: 400.130003 MHz  
 processed size: 65536 complex points  
 LB: 0.000 GB: 0.0000

# 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-(2-trimethylsilylethynyl)uridine

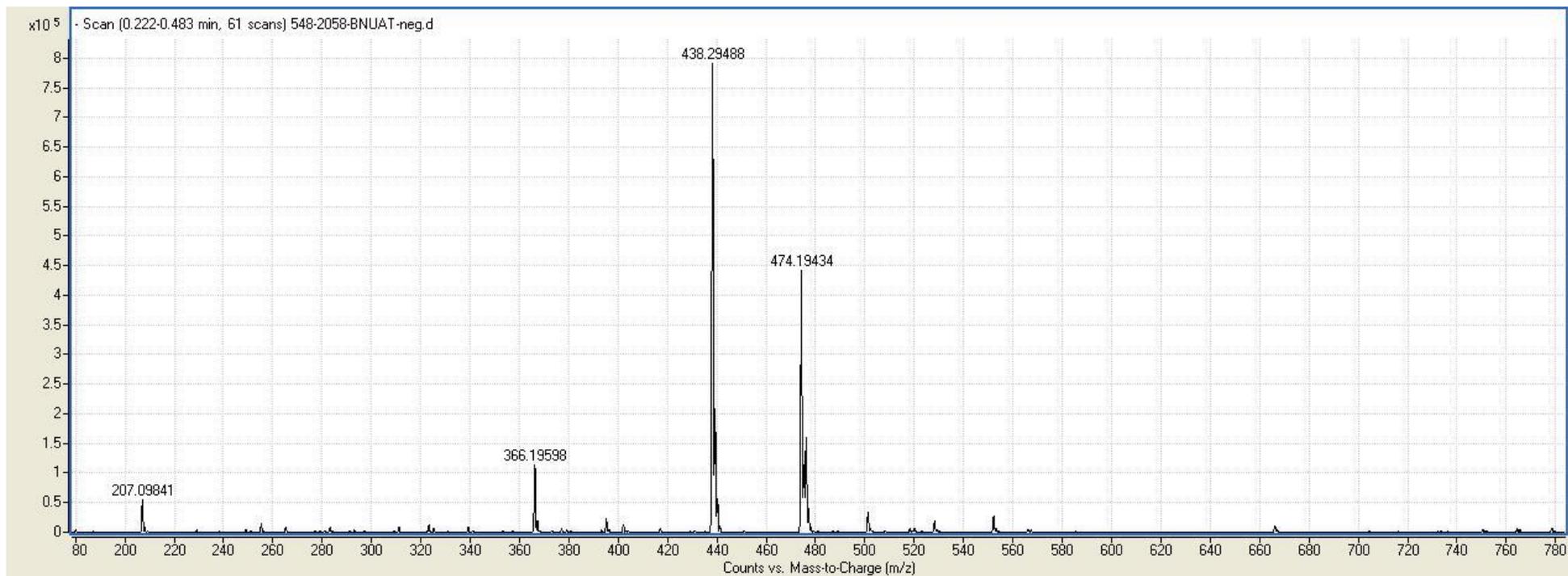
SpinWorks 2.5: A - 582(2); DMSO - d6



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transmitter freq.: 100.624339 MHz  
time domain size: 65536 points  
width: 24038.46 Hz = 238.893111 ppm = 0.366798 Hz/pt  
number of scans: 304

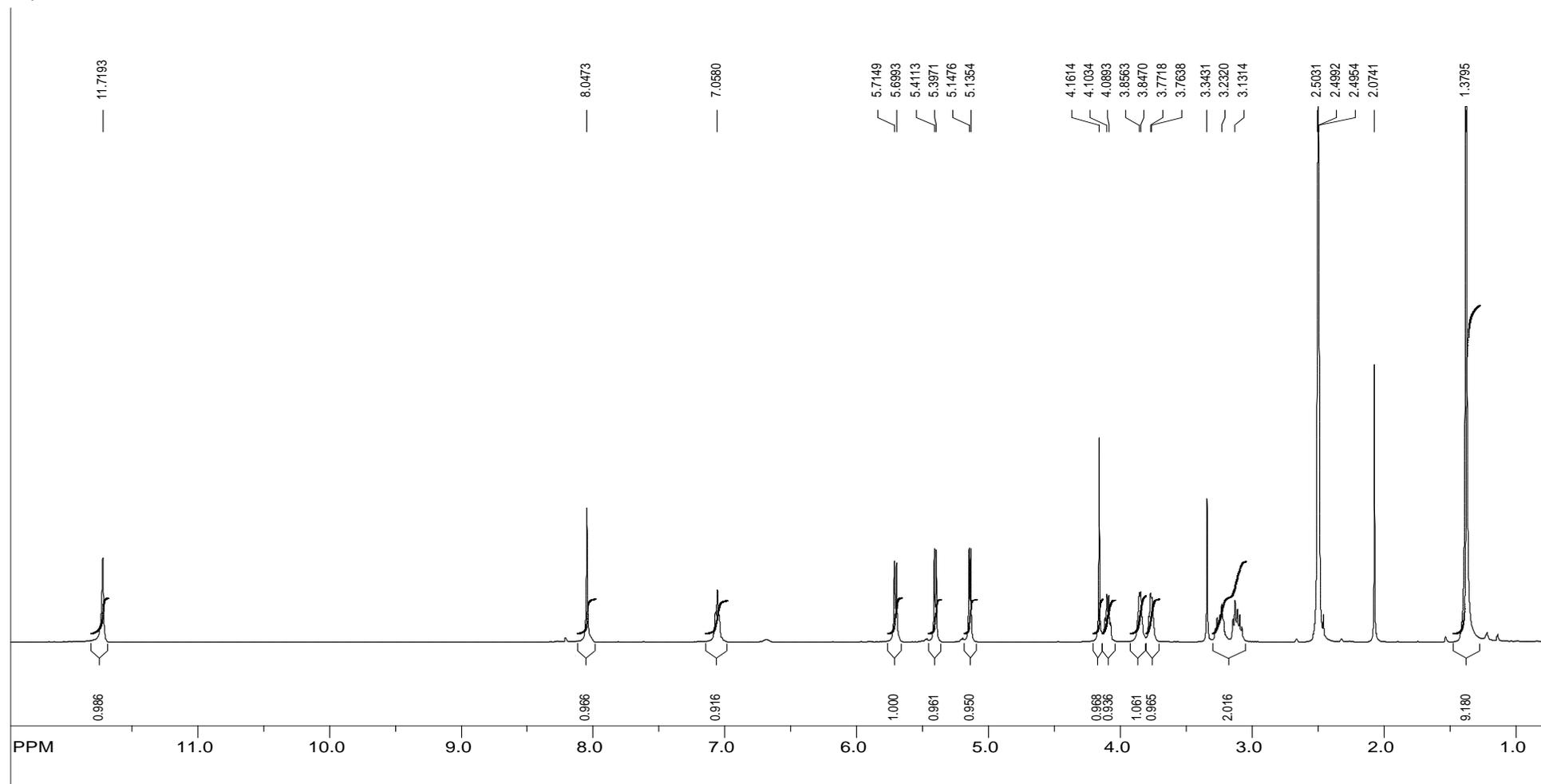
freq. of 0 ppm: 100.612815 MHz  
processed size: 131072 complex points  
LB: 0.000 GB: 0.0000

**5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-(2-trimethylsilylethynyl)uridine**



# 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-ethynyluridine 4

SpinWorks 2.5: A - 586; DMSO-D6

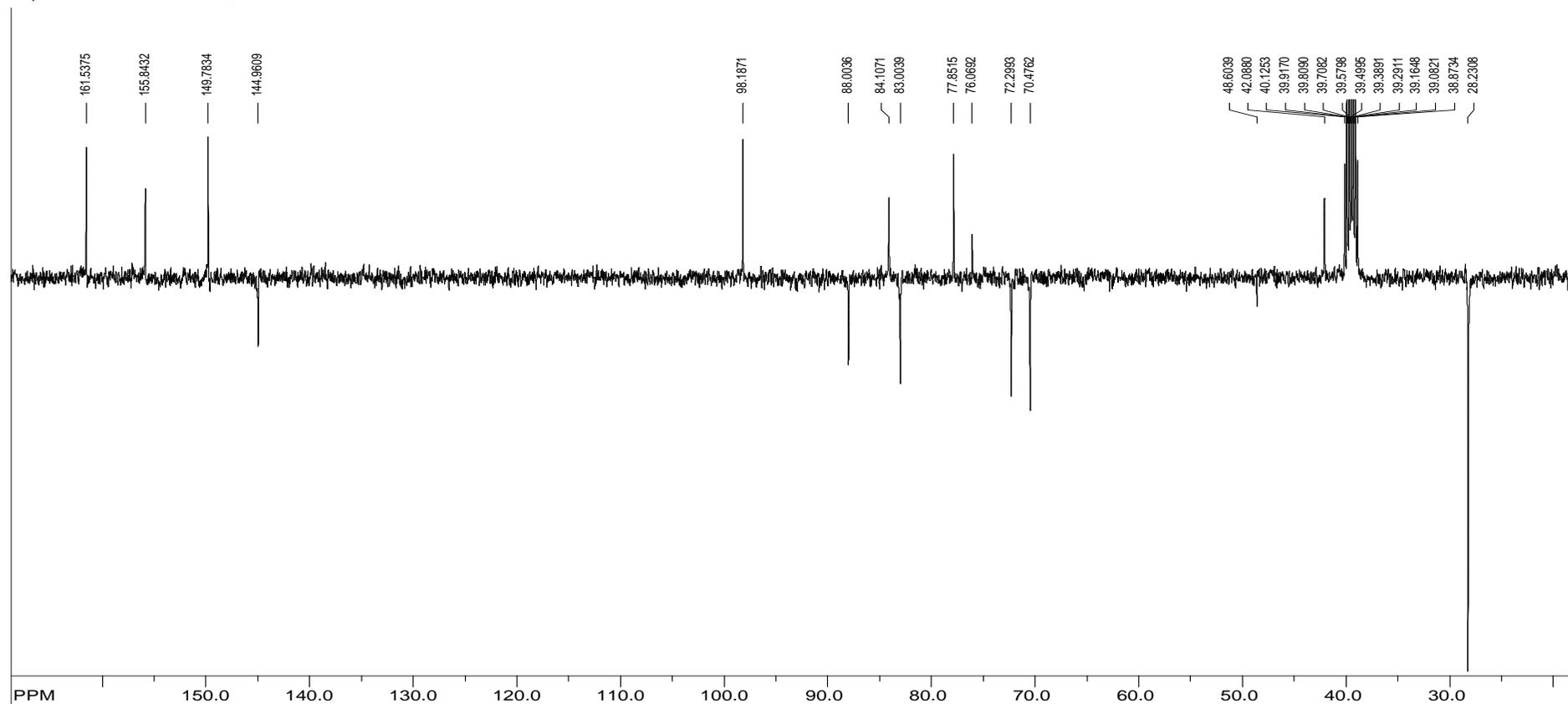


file: D:\Lyuda\analysis\NMR\nibh-a-586\nibh-a-586\1\fid exp: <zg30>  
transmitter freq.: 400.134001 MHz  
time domain size: 32000 points  
width: 8802.82 Hz = 21.999672 ppm = 0.275088 Hz/pt  
number of scans: 48

freq. of 0 ppm: 400.130003 MHz  
processed size: 65536 complex points  
LB: 0.000 GB: 0.0000

# 5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-ethynyluridine 4

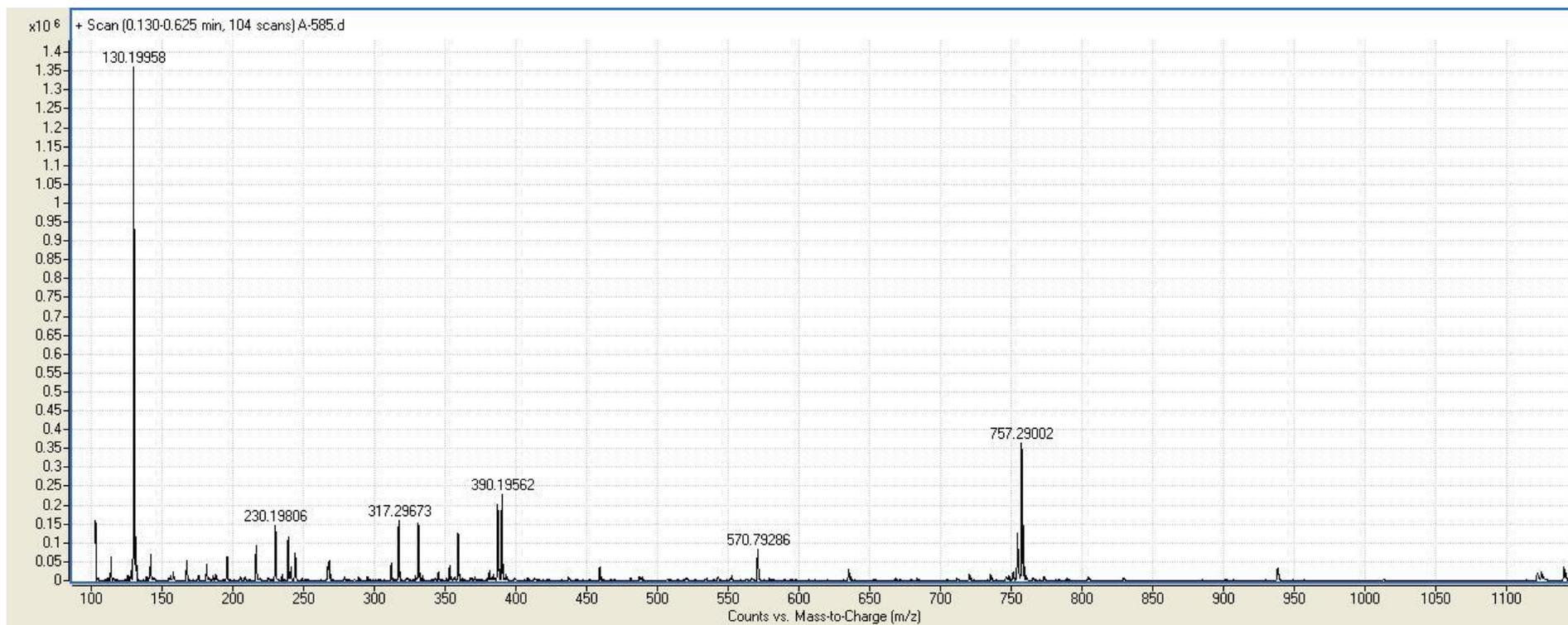
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time domain size: 65536 points  
width: 24038.46 Hz = 238.893111 ppm = 0.366798 Hz/pt  
number of scans: 176

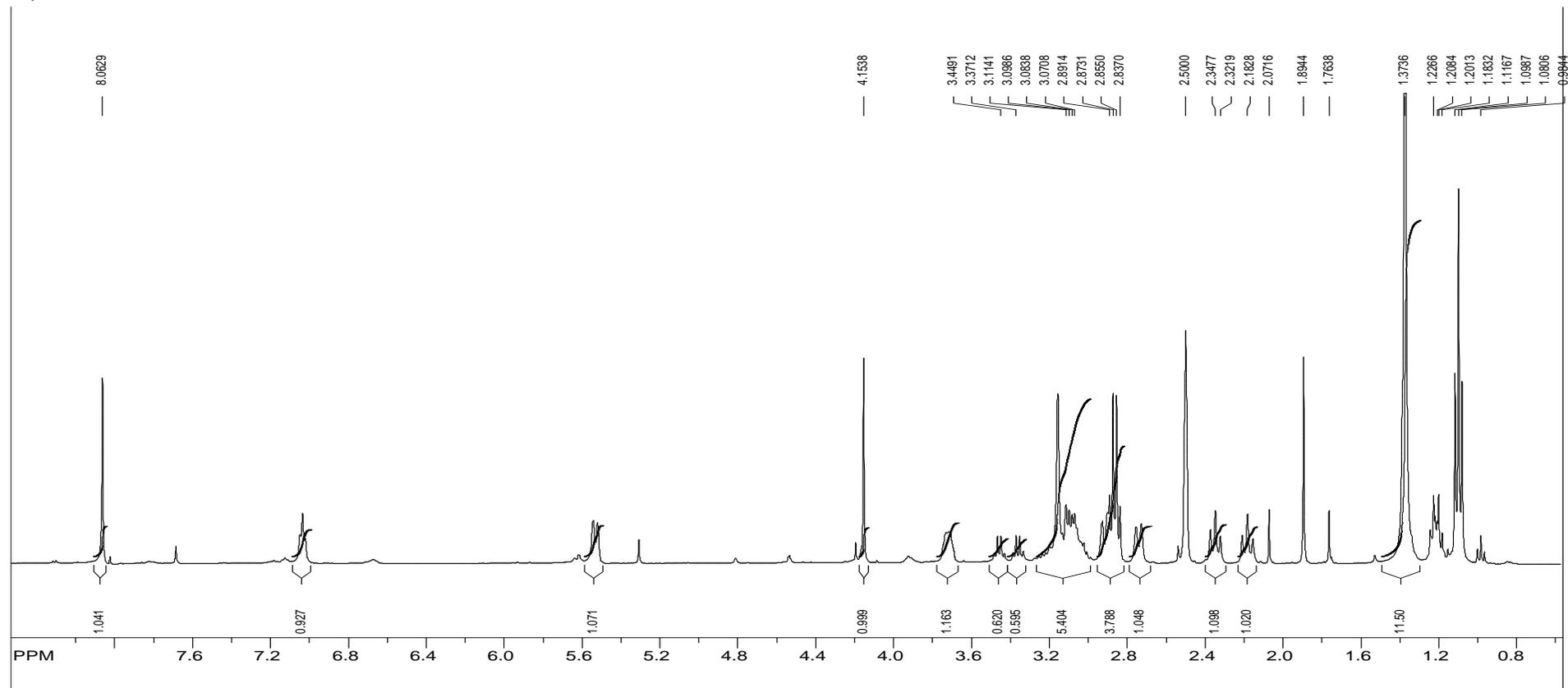
freq. of 0 ppm: 100.612815 MHz  
processed size: 131072 complex points  
LB: 0.000 GB: 0.0000

**5'-(*N*-*tert*-Butyloxycarbonyl)amino-5'-deoxy-5-ethynyluridine 4**



# {2'-[N-(*tert*-Butyloxycarbonyl)aminomethyl]-6'-(5-ethynyluracil-1-yl)morpholin-4'-yl}acetic acid 5

SpinWorks 2.5: A - 589; DMSO - d6

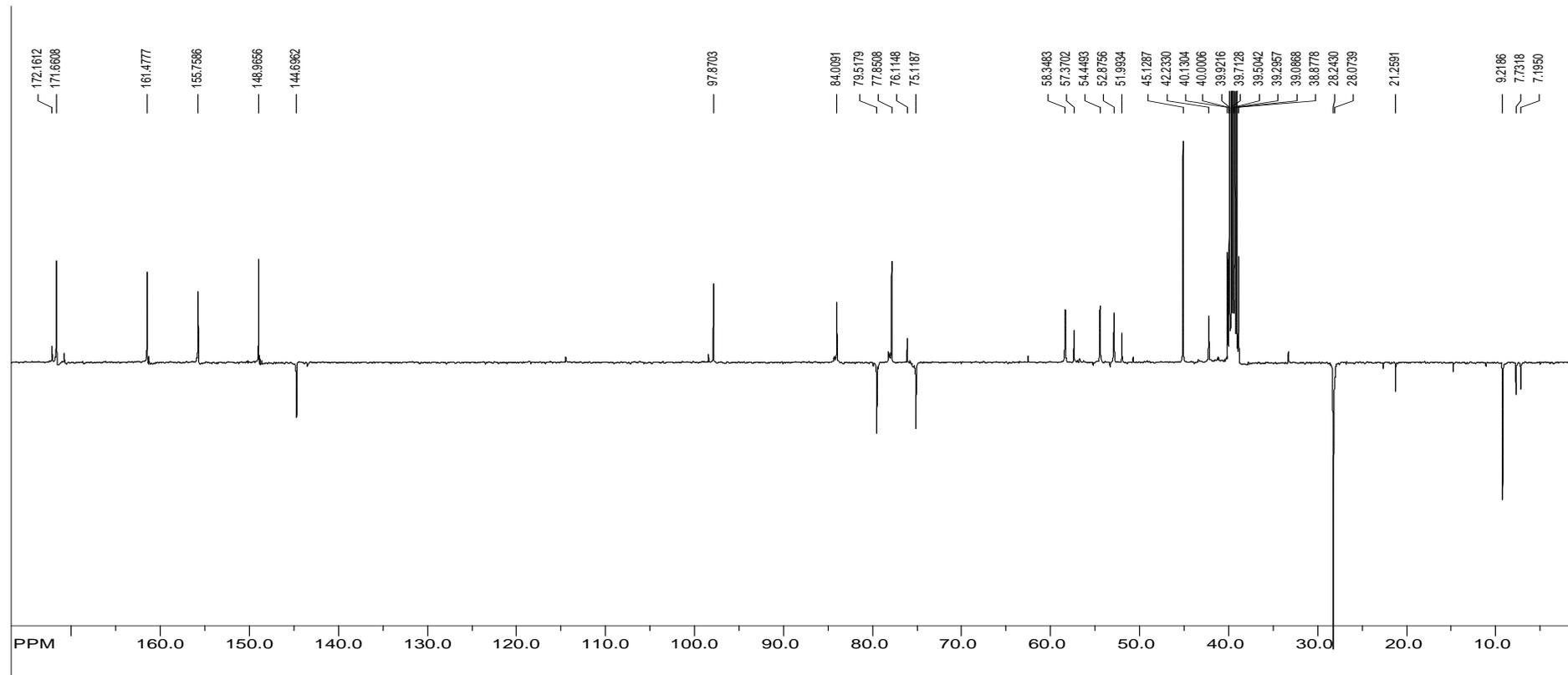


file: D:\Lyuda\analysis\NMR\nibh-a-589\1\fid expt: <zg30>  
transmitter freq.: 400.134001 MHz  
time domain size: 32000 points  
width: 8802.82 Hz = 21.999672 ppm = 0.275088 Hz/pt  
number of scans: 104

freq. of 0 ppm: 400.130003 MHz  
processed size: 65536 complex points  
LB: 0.000 GB: 0.0000

# {2'-[N-(*tert*-Butyloxycarbonyl)aminomethyl]-6'-(5-ethynyluracil-1-yl)morpholin-4'-yl}acetic acid 5

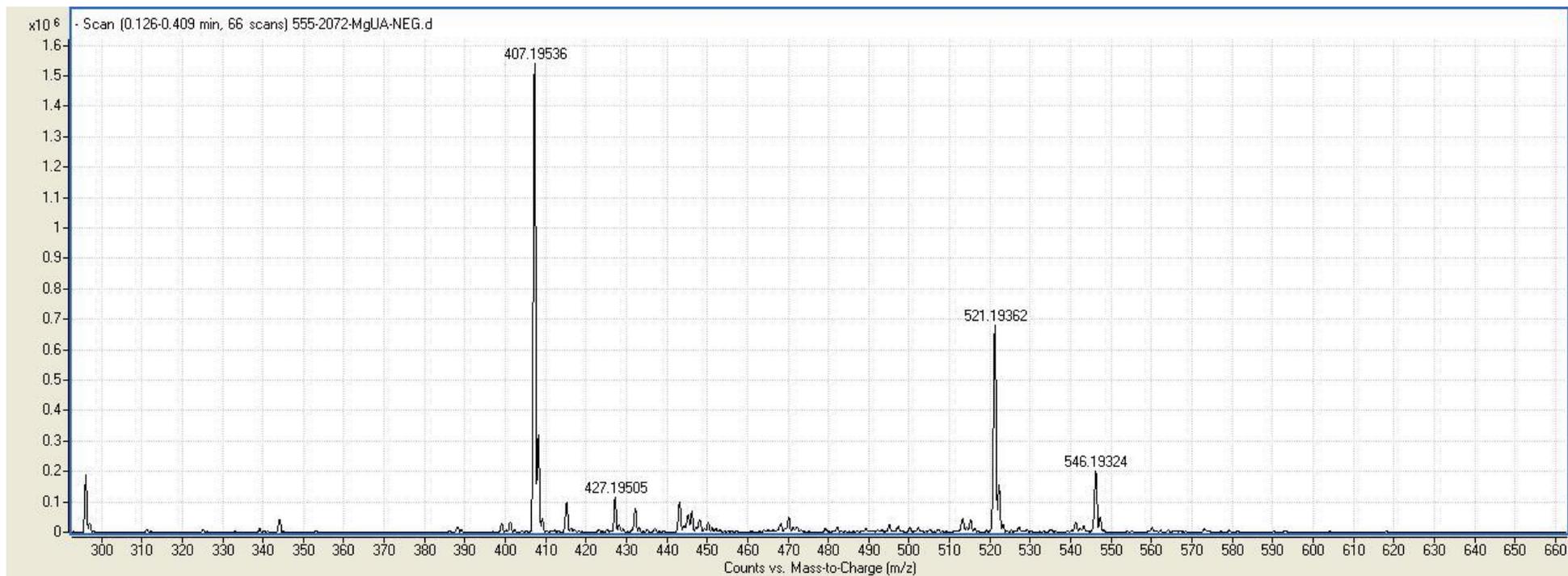
SpinWorks 2.5: A - 589; DMSO - d6



file: D:\Lyuda\analysis\NMR\nibh-a-589\137\fid expt: <jmod>  
transmitter freq.: 100.624339 MHz  
time domain size: 65536 points  
width: 24038.46 Hz = 238.893111 ppm = 0.366798 Hz/pt  
number of scans: 8016

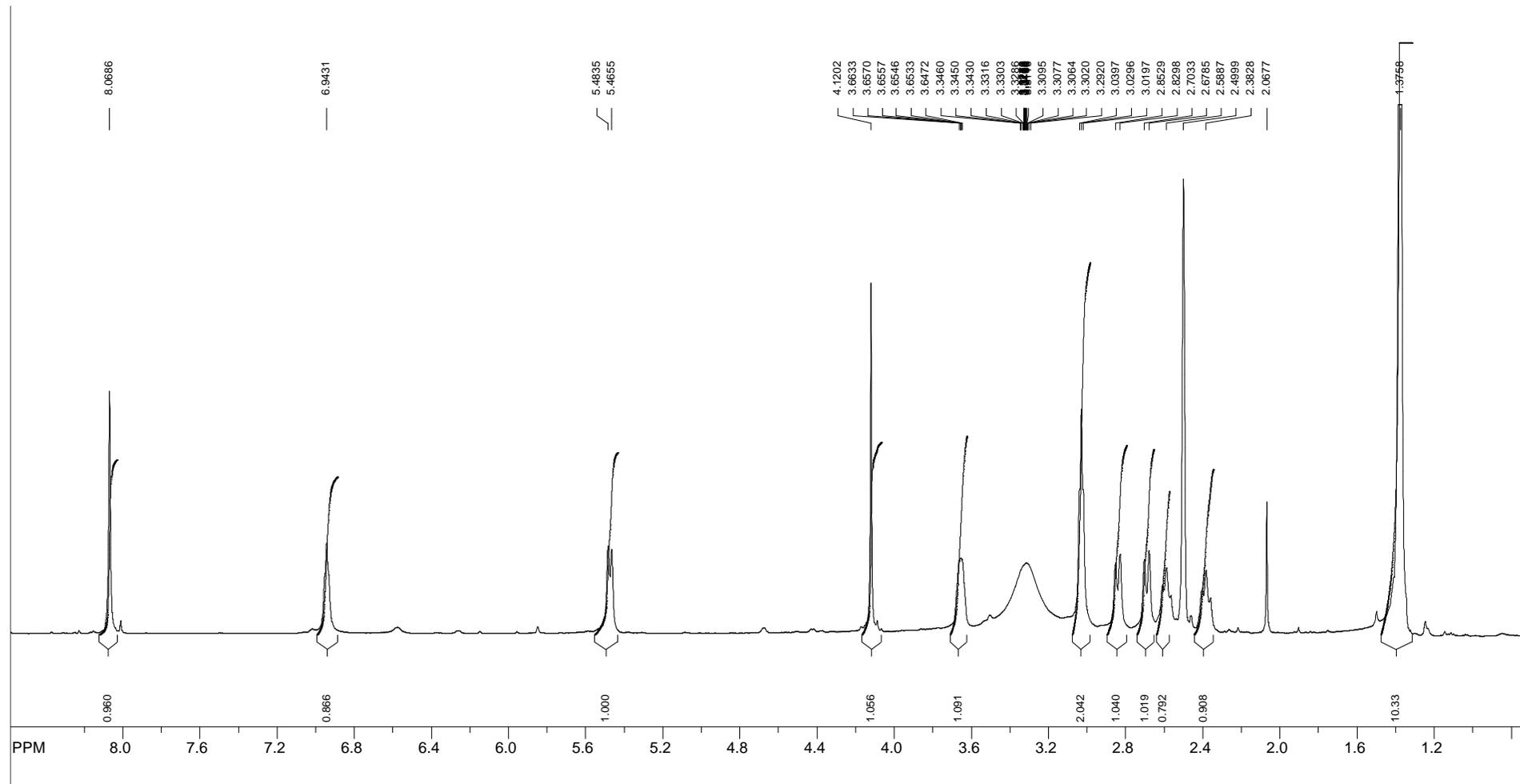
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processed size: 131072 complex points  
LB: 0.000 GB: 0.0000

**{2'-[N-(*tert*-Butyloxycarbonyl)aminomethyl]-6'-(5-ethynyluracil-1-yl)morpholin-4'-yl}acetic acid 5**



# 2'-[N-(*tert*-Butyloxycarbonyl)aminomethyl]-6'-(5-ethynyluracil-1-yl)morpholine 6

SpinWorks 2.5: BMUA; DMSO - d6

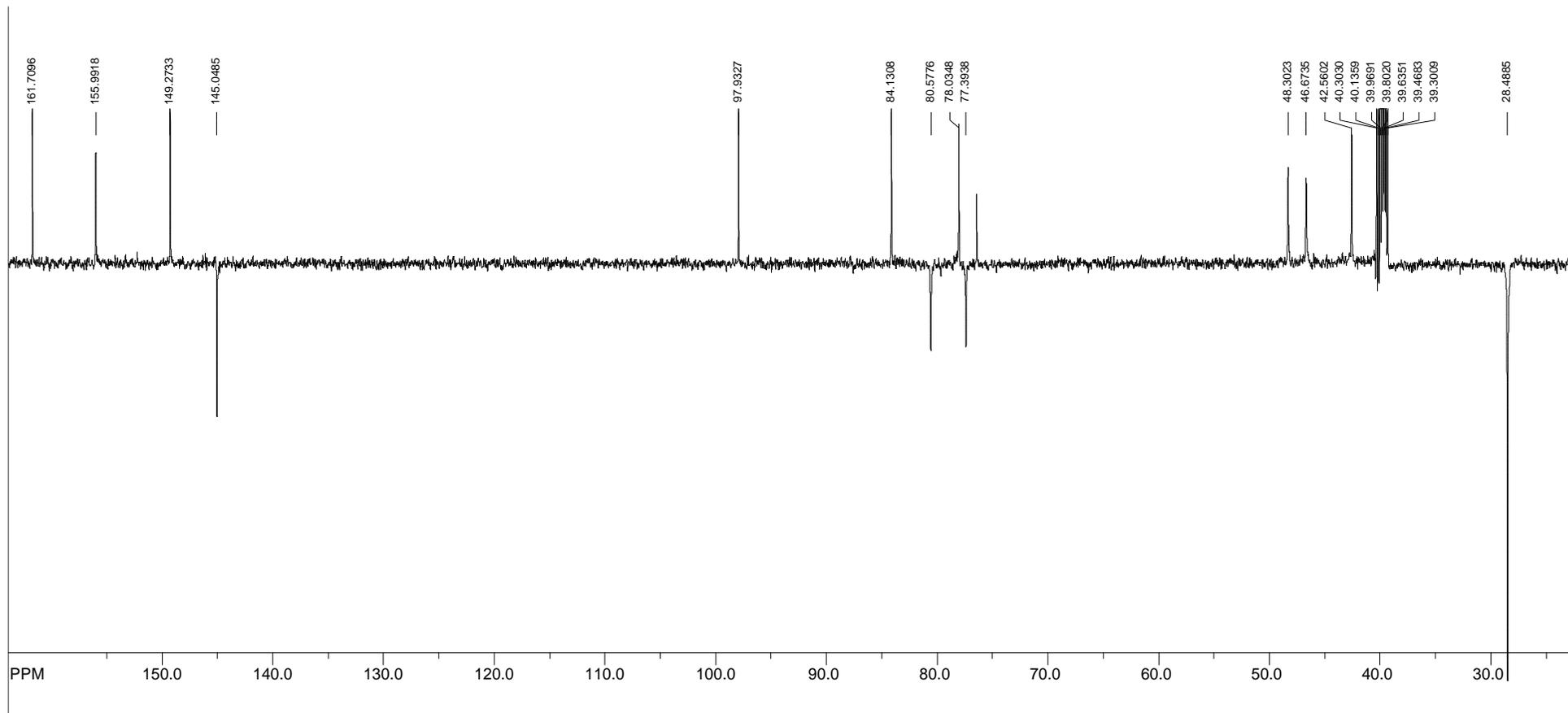


file: D:\0a0uy\ia\_aie0iaio\0aiy\5\5\5\MorGly 2016\2016 a\inibh-bmua\1\fid exp: <zg30>  
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 time domain size: 65536 points  
 width: 10964.91 Hz = 21.923916 ppm = 0.167311 Hz/pt  
 number of scans: 16

freq. of 0 ppm: 500.130006 MHz  
 processed size: 65536 complex points  
 LB: 0.000 GB: 0.0000

# 2'-[N-(*tert*-Butyloxycarbonyl)aminomethyl]-6'-(5-ethynyluracil-1-yl)morpholine 6

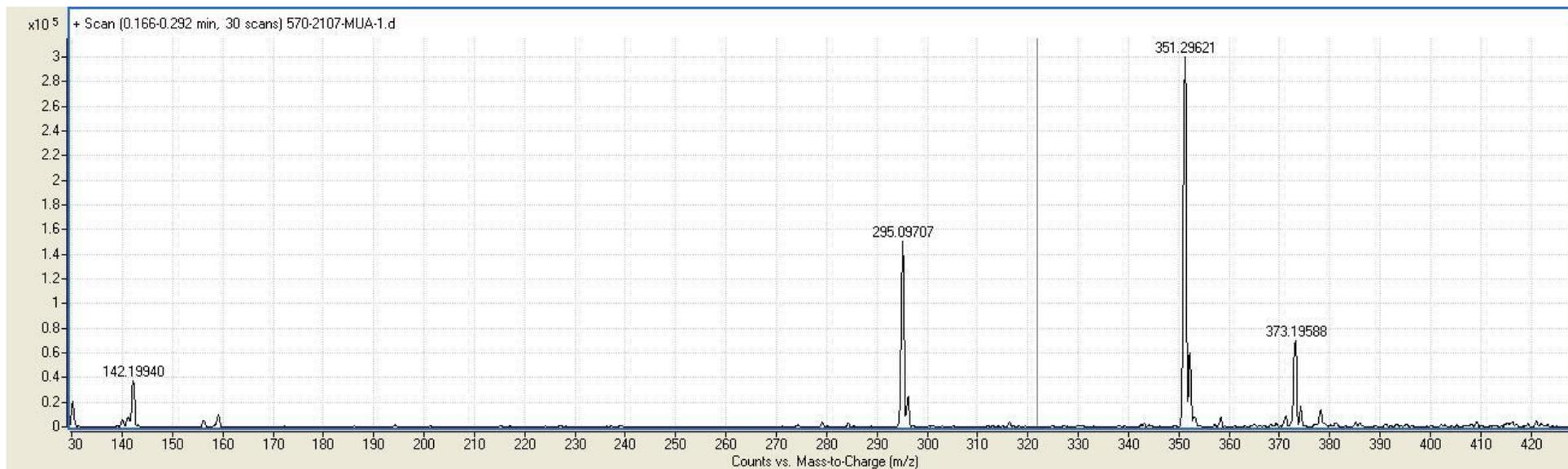
SpinWorks 2.5: BMUA; DMSO - d6



file: D:\00000000\_00000000\_00000000\MorGly 2016\2016 01\137\fid exp1 <jmod>  
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time domain size: 65536 points  
width: 30303.03 Hz = 240.936076 ppm = 0.462388 Hz/pt  
number of scans: 720

freq. of 0 ppm: 125.757816 MHz  
processed size: 131072 complex points  
LB: 0.000 GB: 0.0000

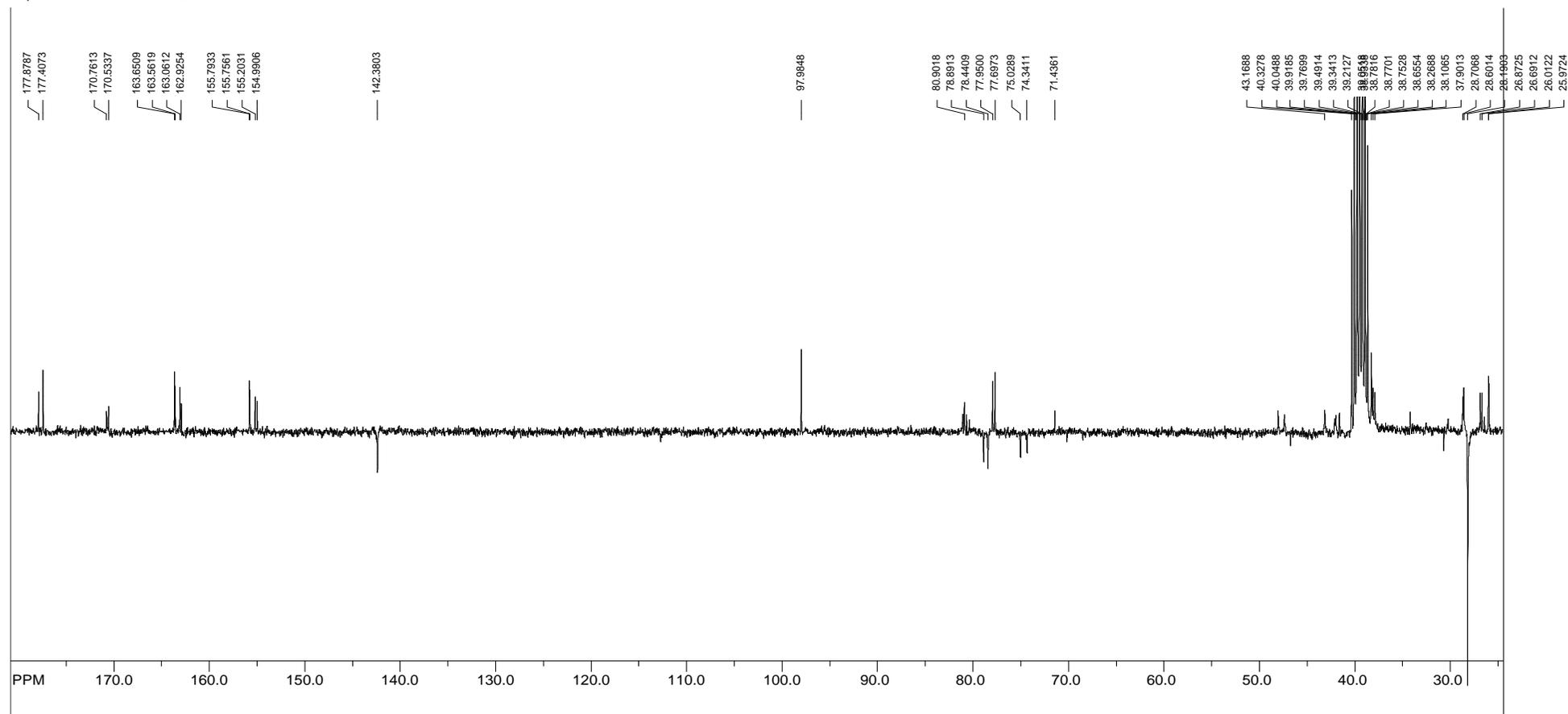
**2'-[N-(tert-Butyloxycarbonyl)aminomethyl]-6'-(5-ethynyluracil-1-yl)morpholine 6**





# 9-{2'-[N-(*tert*-butyloxycarbonyl)aminomethyl]-6'-(5-ethynyluracil-1-yl)morpholin-4'-yl]-8,9-dioxo-7-azanonanoic acid 7

SpinWorks 2.5: A - 645; DMSO - d6



file: D:\0a0uyia\_aieoiarou\0aiy\SD\MorGly 2016\2016 al\nibh-a-645\137\fid exp: <jmod>  
 transmitter freq.: 75.476050 MHz  
 time domain size: 32768 points  
 width: 18115.94 Hz = 240.022390 ppm = 0.552855 Hz/pt  
 number of scans: 8256

freq. of 0 ppm: 75.467785 MHz  
 processed size: 65536 complex points  
 LB: 0.000 GB: 0.0000

9-{2'-[N-(*tert*-butyloxycarbonyl)aminomethyl]-6'-(5-ethynyluracil-1-yl)morpholin-4'-yl]-8,9-dioxo-7-azanonanoic acid 7

