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5-Amino-1,2,4-triazole-3-carboxamide homologues and their biological potential

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Table of Contents

1. Synthetic Section
1.1. Materials and Methods

All the chemical reagents were obtained from commercial suppliers (Acros organics, USA and Macklin, China) and used without further purification. All the solvents were distilled before use, and some of them were absolutized according to known methods[S1]. PTX-AF-A-UV silica gel plates (Sorbfil, Russia) were used for thin-layer chromatography. The substances visualization on thin-layer chromatograms were carried out using UV irradiation at 254 nm and iodine followed by phosphomolybdic acid or ninhydrin. Column chromatography was carried out on silica gel Kiselgel 60 0.040-0.063 mm (Merck, Germany).

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a DPX-300 spectrometer (Bruker, Germany) at 25 °C. ^{1}H and ^{13}C NMR spectra were recorded at an operating frequency of 300 and 75 MHz respectively. Chemical shifts are present in ppm relative to the solvent $CDCl_3$ (7.26 ppm), DMSO-d_6 (2.50 ppm) for ^{1}H NMR and $CDCl_3$ (77.0 ppm), DMSO-d_6 (39.5 ppm) for ^{13}C NMR. The determination of solvent peaks was carried out in accordance with the literature [S2]. Designations in ^{1}H spectra: s – singlet, br.s – broad singlet, d – doublet, t – triplet, q – quartet, m – multiplet. The data are presented as follows: chemical shifts, multiplicity, J-coupling constants (Hz) and the relative value of integration.

High-resolution mass spectra (HRMS) were recorded on the Agilent 6224 instrument using the electron spray ionization (ESI) method. HPLC-MS measurements were performed in the Agilent InfinityLab LC/MSD iQ chromatographic system consisting of: Agilent 1260 Infinity II SFC Control Module, Agilent 1260 Infinity High Performance Degasser, Agilent 1260 Infinity II SFC Quaternary Pump, Agilent 1260 Infinity II Multisampler, Agilent 1260 Infinity II Multicolumn Thermostat, and Agilent Single Quadrupole LC/MSD iQ mass Spectrometric detector (m/z range 2-1450). Separation was performed on an Agilent Poroshell 300SB-C18 chromatographic column, 2.1 x 75 mm, 5 um.

Separation conditions:

A: MeCN;

B: 0.1% trifluoroacetic acid in H_2O.

The elution mode was gradient:

0 min – 10% A and 90% B;

10 min – 95% A and 5% B;

10.01 min – 10% A and 90% B;

14 min – 10% A and 90% B.

The volume of the injected sample is 5 μl.

The flow rate of the mobile phase is 0.5 ml/min.

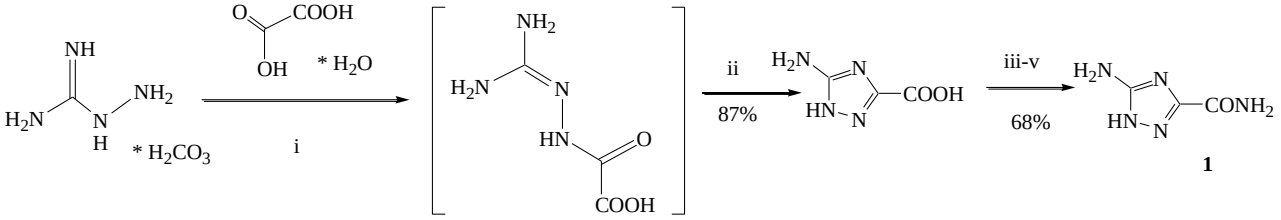
The temperature of the speakers is 30 °C.

ESI ionization ±3.5 kV, spray gas – nitrogen, 350 °C.

The solvents were removed on a Heidolph LABOROTA 4000 rotary vacuum evaporator with a water jet pump. Melting temperatures are determined on the Cole-Parmer MP-200D-120 device in an open capillary.

1.2. Synthetic procedures

1.2.1. 5-Amino-1,2,4-triazole-3-carboxamide synthesis



Scheme S1 *Reagents and conditions:* i, H_2O, reflux; ii, KOH, H_2O, reflux; iii, $SOCl_2$, MeOH (anhydrous); iv, NH_3, MeOH; v, HCl, dioxane (anhydrous), room temperature.

5-Amino-1,2,4-triazole-3-carboxylic acid hemihydrate

Oxalic acid dihydrate (38.7 g, 307 mmol) was dissolved in 204 ml of water at heating, then 30.7 g (226 mmol) aminoguanidine bicarbonate was added in small portions with stirring to the solution. The reaction mixture was refluxed for 6 hours. After cooling the reaction mixture to room temperature, the precipitate that formed was filtered and washed with hot water. The resulting precipitate was suspended at 270 ml of aqueous solution of potassium hydroxide (1.7 M) and refluxed for 2 hours. The conversion of the intermediate amidrazone was controlled using TLC, and after its full conversion the pH value of the reaction medium was adjusted to 1 using hydrochloric acid (36% w/w). The precipitate was filtered, washed with 20 ml of water and dried in a vacuum desiccator over sodium hydroxide for about 12 hours. The yield was 27.2 g (94%) as white crystals. R_f 0.45 (ammonia water and acetonitrile in a volume ratio of 1:1), m.p. = 180-181 °C, m.p (lit.) = 182–183 °C [S3].

^{13}C NMR (DMSO-d_6: 30% DCl in D_2O (9:1)) δ: 142.82; 152.32; 157.63. Found (%): C, 26.38; H, 3.74; N, 40.43. Calc. for $C_3H_4N_4O_2 \cdot 0.5\ H_2O$ (%): C, 26.28; H, 3.68; N, 40.87.

5-Amino-1,2,4-triazole-3-carboxamide (1)

5-Amino-1,2,4-triazole-3-carboxamide was prepared by a modification of a literature procedure[S4]. To a suspension of 10.0 g (78.0 mmol) of the above 5-amino-1,2,4-triazole-3-carboxylic acid in 70.0 ml of ethanol, 12.0 ml (164 mmol) of thionyl chloride was added dropwise on an ice bath. After the addition was complete the reaction mass was stirred for 2.5 hours at the reflux. The reaction mixture was filtered, the volatile components from filtrate were evaporated at reduced pressure. Both precipitate and residue was used without further purification as intermediate ethyl 5-amino-1,2,4-triazole-3-carboxylate hydrochloride, R_f 0.40 (5% methanol in chloroform + 0.1% TEA). To 1.60 g of the intermediate 3.00 ml of 14.6 M aqueous ammonia solution was added. The reaction mixture was refluxed and 0.50 ml of 14.6 M aqueous ammonia solution was added every 3 hours. Conversion of the intermediate product was monitored using TLC (5% methanol in chloroform + 0.1% TEA). After its complete conversion, the reaction mixture was acidified with a solution of HCl in dioxane (3.42 M) to pH 4-5. The precipitate was filtered and washed with 10.0 ml of water. The precipitate was dried in a vacuum desiccator over NaOH. The yield was 0.94 g (68%) as white crystals.

^{1}H NMR (DMSO-d_6) δ: 6.07 (s, 2H, -$CONH_2$); 7.35 (s, 2H, -NH_2); 12.39 (br.s, 1H, Tr). ^{13}C NMR (DMSO-d_6+DCl) δ: 148.31; 154.46; 158.76. HRMS (ESI), *m/z*: 128.0577 $[M+H]^+$, (calc. for $C_3H_6N_5O$, m/z: 128.0572), *m/z*: 126.0420 $[M-H]^+$, (calc. for $C_3H_4N_5O$, m/z: 126.0416). Found (%): C, 28.55; H, 3.77; N, 55.04. Calc. for $C_3H_5N_5O$ (%): C, 28.35; H, 3.96; N, 55.10.

1.2.2. 5-(ω-Aminoalkyl)-1,2,4-triazole-3-carboxamide homologues synthesis

N-Boc-amino acids methyl esters

General procedure

To a suspension of amino acid **2** in anhydrous methanol (20 ml per 2.5 g) 2 eq. thionyl chloride was added drop by drop on an ice bath. After the addition was complete the reaction mixture was stirred at room temperature for 2.5 hours. The conversion of the amino acid was monitored by TLC (20% methanol in chloroform + 0.5% TEA). The volatile components were evaporated. The residue was suspended in 20 ml of methanol, 2 eq. of TEA and then a solution of 0.98 eq. di-*tert*-butyl dicarbonate in methanol were added. The reaction mass was stirred at room temperature, until the full conversion of di-*tert*-butyl dicarbonate. The precipitated were filtered out, filtrate was evaporated. The residue was dissolved in 20 ml of ethyl acetate, washed with water (3 x 10 ml) and 10% hydrochloric acid (10 ml, pH 5). After that, the solvent was evaporated at reduced pressure. The R_f values of the products were given in a chloroform system

Methyl tert-butoxycarbonylaminoacetate

From 2.50 g (33.3 mmol) of glycine, 4.84 ml (66.6 mmol) of thionyl chloride, 9.01 ml (66.0 mmol) of TEA, 6.97 g (31.9 mmol) of di-*tert*-butyl dicarbonate in 48 hours, 2.89 g (84%) of product was obtained as colorless oil.

*R*f 0.65. ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 9H, $(-CH_3)_3$); 3.72 (s, 3H, $-CH_3$); 3.88 (m, 2H, -NH-$\underline{CH_2}$-); 5.02 (br.s, 1H, -$\underline{NH}$-CH_2-). ^{13}C NMR ($CDCl_3$) δ: 28.24; 42.22; 52.19; 79.97; 155.66; 170.82.

Methyl 3-tert-butoxycarbonylaminopropanoate

From 2.50 g (28.0 mmol) of *β*-alanine, 4.07 ml (56.0 mmol) of thionyl chloride, 7.70 ml (56.0 mmol) of TEA, 5.9 g (27.0 mmol) of di-*tert*-butyl dicarbonate in 10 hours, 4.29 g (76%) of product was obtained as colorless oil.

*R*f 0.68. ^{1}H NMR ($CDCl_3$) δ: 1.40 (s, 9H, $(-CH_3)_3$); 2.50 (t, 2H, J=6.03, -$\underline{CH_2}$-CH_2-); 3.36 (m, 2H, -NH-$\underline{CH_2}$-CH_2-); 3.67 (s, 3H, $-CH_3$); 5.02 (br.s, 1H, -$\underline{NH}$-CH_2-). ^{13}C NMR ($CDCl_3$) δ: 28.28; 34.35; 35.97; 51.66; 79.25; 155.70; 172.86.

Methyl 4-tert-butoxycarbonylaminobutanoate

From 2.50 g (24.0 mmol) of *γ*-aminobutyric acid, 3.52 ml (48.0 mmol) of thionyl chloride, 6.60 ml (50 mmol) of TEA, 5.00 g (23.1 mmol) of di-*tert*-butyl dicarbonate in 12 hours, 4.11 g (80%) of product was obtained as colorless oil.

*R*f 0.70. ^{1}H NMR ($CDCl_3$) δ: 1.37 (s, 9H, $(-CH_3)_3$); 1.64-1.75 (m, 2H, -CH_2-$\underline{CH_2}$-CH_2-); 2.30 (t, 2H, J=7.37, -$(CH_2)_2$-$\underline{CH_2}$-); 3.08 (t, 2H, J=7.09, -$\underline{CH_2}$-$(CH_2)_2$-); 3.61 (s, 3H, $-CH_3$); 4.83 (br.s, 1H, -NH-). ^{13}C NMR ($CDCl_3$) δ: 25.08; 28.17; 31.07; 39.67; 51.37; 78.82; 155.84; 173.53.

Methyl 6-tert-butoxycarbonylaminohexanoate

From 2.50 g (19.0 mmol) of 6-aminohexanoic acid, 2.76 ml (38.0 mmol) of thionyl chloride, 5.19 ml (37.0 mmol) of TEA, 3.95 g (18.0 mmol) di-*tert*-butyl dicarbonate in 8 hours, 4.16 g (90%) of product was obtained as colorless oil.

*R*f 0.75. ^{1}H NMR ($CDCl_3$) δ: 1.22-1.56 (m, 4H, -CH_2-$\underline{CH_2}$-$\underline{CH_2}$-$(CH_2)_2$-); 1.40 (s, 9H, $(-CH_3)_3$); 1.60 (m, 2H, -$(CH_2)_3$-$\underline{CH_2}$-CH_2-); 2.27 (t, 2H, J=7.45, -$(CH_2)_4$-$\underline{CH_2}$-); 3.06 (m, 2H, -NH-$\underline{CH_2}$-$(CH_2)_4$-); 3.63 (s, 3H, $-CH_3$); 4.55 (br.s, 1H, -$\underline{NH}$-CH_2-). ^{13}C NMR ($CDCl_3$) δ: 24.48; 26.20; 28.32; 29.67; 33.83; 40.27; 51.41; 78.94; 155.90; 173.96.

N-Boc-amino acid hydrazides (3)

General procedure

Hydrazine hydrate (3 equiv.) was added to a solution methyl ester of the above *tert*-butyloxycarbonyl amino acid in methanol. The reaction mass was stirred at room temperature, until the full conversion of the methyl ester. The volatile components were evaporated at reduced pressure. The residue was suspended in 10 ml of toluene and re-evaporated twice.

tert-Butoxycarbonylaminoethanoic acid hydrazide (3a)

From 1.54 g (7.61 mmol) Me-ester, 1.11 ml (23.0 mmol) of hydrazine hydrate in 1.5 ml of methanol after 3 hours, 1.40 g (98%) of product **3a** was obtained as colorless oil.

R_f 0.50 (10% methanol in chloroform). ^{1}H NMR (DMSO- d_6) δ: 1.36 (s, 9H, (-CH_3)$_3$); 3.46 (d, 2H, J= 6.13, -NH-$\underline{CH_2}$-); 4.19 (br.s, 2H, -NH-$\underline{NH_2}$); 6.92 (t, 1H, J=6.05, -$\underline{NH}$-CH_2-); 8.94 (s, 1H, -$\underline{NH}$-NH_2). ^{13}C NMR (DMSO- d_6) δ: 28.22; 41.88; 77.97; 155.74; 168.79.

3-tert-Butoxycarbonylaminopropanoic acid hydrazide (3b)

From 4.10 g (20.2 mmol) Me-ester, 2.94 ml (60.5 mmol) hydrazine hydrate in 3.5 ml of methanol after 1 hours 4.10 g (100%) of product **3b** was obtained as colorless oil.

R_f 0.40 (3% methanol in chloroform). ^{1}H NMR (DMSO-d_6) δ: 1.35 (s, 9H, (-CH_3)$_3$); 2.14 (t, 2H, J=7.38, -NH-CH_2-$\underline{CH_2}$-); 3.06-3.13 (m, 2H, -NH-$\underline{CH_2}$-CH_2-); 4.14 (s, 2H, -NH-$\underline{NH_2}$); 6.73 (t, 1H, J=5.73, -NH-); 8.97 (s, 1H, -$\underline{NH}$-NH_2). ^{13}C NMR (DMSO- d_6) δ: 28.22; 33.94; 36.72; 77.59; 155.43; 169.75.

4-tert-Butoxycarbonylaminobutanoic acid hydrazide (3c)

From 4.09 g (18.8 mmol) Me-ester, 2.75 ml (56.5 mmol) of hydrazine hydrate in 3.5 ml of methanol after 2 hours 4.00 g (98%) of product **3c** was obtained as colorless oil.

R_f 0.55 (3% methanol in chloroform). ^{1}H NMR (DMSO-d_6) δ: 1.36 (s, 9H, (-CH_3)$_3$); 1.55-1.63 (m, 2H, -CH_2-$\underline{CH_2}$-CH_2-); 1.99 (t, 2H, J=7.54, -(CH_2)$_2$-$\underline{CH_2}$-); 2.89 (t, 2H, J=6.62, -$\underline{CH_2}$-(CH_2)$_2$-); 4.10 (s, 2H, -NH-$\underline{NH_2}$); 6.68 (br.s, 1H, -NH-); 8.84 (s, 1H, -$\underline{NH}$-NH_2). ^{13}C NMR (DMSO-d_6) δ: 25.82; 28.28; 30.98; 40.05; 77.47; 155.60; 171.34.

6-tert-Butoxycarbonylaminohexanoic acid hydrazide (3d)

From 2.50 g (10.0 mmol) Me-ester, 1.49 ml (30 mmol) of hydrazine hydrate in 2.5 ml of methanol after 3 hours 2.32 g (95%) of product **3d** was obtained as colorless oil.

R_f 0.25 (2% methanol in chloroform). ^{1}H NMR (DMSO-d_6) δ: 1.12-1.22 (m, 2H, -(CH_2)$_2$-$\underline{CH_2}$-(CH_2)$_2$-); 1.28-1.33 (m, 2H, -CH_2-$\underline{CH_2}$-(CH_2)$_3$-); 1.35 (s, 9H, (-CH_3)$_3$); 1.40-1.50 (m, 2H, -(CH_2)$_3$-$\underline{CH_2}$-CH_2-); 1.95-2.00 (m, 2H, -(CH_2)$_4$-$\underline{CH_2}$-); 2.82-2.89 (m, 2H, -NH-$\underline{CH_2}$-(CH_2)$_4$-); 4.12 (s, 2H, -NH-$\underline{NH_2}$); 6.75 (t, 1H, J=5.46, -NH-); 8.90 (s, 1H, -$\underline{NH}$-NH_2). ^{13}C NMR (DMSO- d_6) δ: 24.99; 26.00; 28.28; 29.27; 33.40; 39.77; 77.31; 155.57; 171.59.

Ethyl β-*N*-acyloxalamidrazones (4)

General procedure

Two solutions of *N*-Boc-amino acid hydrazide **3** and 1.1 eq. ethyl 2-thiooxamate each in 1.5 ml of ethanol were combined. The reaction mass was stirred at room temperature until the full conversion of hydrazide **3** was controlled by TLC (4% methanol in chloroform). Then the precipitate was filtered and washed on a filter with 5 ml of diethyl ether and dried on a filter. The R_f values of the products were given in a system of 10% methanol in chloroform.

Ethyl β-N-(tert-butoxycarbonylaminoacetyl)oxalamidrazone (4a)

From 1.41 g (7.40 mmol) of **3a**, 1.08 g (8.1 mmol) ethyl ether of thiooxamic acid in 96 hours 1.26 g (59%) of product **4a** was obtained as yellow crystals.

R_f 0.70. M.p. 133-135 °C. ^{1}H NMR (DMSO-d_6) δ: 1.24 (t, 3H, J=7.31, -CH_2-$\underline{CH_3}$); 1.37 (s, 9H, (-CH_3)$_3$); 3.55-3.59 and 3.88-3.94 (m, 2H, -NH-$\underline{CH_2}$-); 4.17-4.25 (m, 2H, -$\underline{CH_2}$-CH_3); 6.45-6.48 (m, 2H, -NH_2); 6.72-6.76 and 6.98-7.03 (m, 1H, -$\underline{NH}$-CH_2-); 9.86 (d, 1H, J= 66.65, -NH-N=). ^{13}C NMR (DMSO-d_6) δ: 13.94; 28.19; 41.27; 61.53; 77.85; 136.34; 155.81; 161.58; 170.70. HRMS (ESI), *m/z*: 289.1516 $[M+H]^+$, (calc. for $C_{11}H_{21}N_4O_5$, *m/z*: 289.1512), *m/z*: 287.1358 $[M-H]^+$, (calc. for $C_{11}H_{19}N_4O_5$, *m/z*: 287.1355).

Ethyl β-N-(3-(tert-butoxycarbonylamino)propanoyl)oxalamidrazone (4b)

From 2.10 g (10.3 mmol) of **3b**, 1.51 g (11.3 mmol) ethyl ether of thiooxamic acid in 56 hours 2.8 g (93%) of product **4b** was obtained as yellow crystals.

R_f 0.60. M.p. 180-181 °C. ^{1}H NMR (DMSO-d_6) δ: 1.21-1.26 (m, 3H, -CH_2-$\underline{CH_3}$); 1.36 (s, 9H, (-CH_3)$_3$); 2.31 (t, 1H, J = 7.15, -NH-$\underline{CH}$H-CH_2-); 2.64 (t, 1H, J = 7.06, NH-CH$\underline{H}$-CH_2-); 3.11-3.19 (m, 2H, -$\underline{CH_2}$-CH_2-); 4.20 (q, 2H, J = 7.81, -$\underline{CH_2}$-CH_3); 6.43 (d, 2H, J = 13.58, -NH_2); 6.70-6.83 (m, 1H, -$\underline{NH}$-CH_2-CH_2-); 9.78 (d, 1H, J = 43.08, -NH-N=). ^{13}C NMR (DMSO-d_6) δ: 14.00; 28.25; 32.38; 34.65; 35.73; 36.67; 61.56; 77.64; 136.03; 139.03; 155.54; 161.75; 162.27; 166.50; 172.83. HRMS (ESI), *m/z*: 303.1670 $[M+H]^+$, (calc. for $C_{12}H_{23}N_4O_5$, *m/z*: 303.1668), *m/z*: 301.1515 $[M-H]^+$, (calc. for $C_{12}H_{21}N_4O_5$, *m/z*: 301.1512).

Ethyl β-N-(4-(tert-butoxycarbonylamino)butanoyl)oxalamidrazone (4c)

From 1.49 g (6.87 mmol) of **3c**, 1.00 g (7.55 mmol) ethyl ether of thiooxamic acid in 48 hours 2.07 g (87%) of product **4c** was obtained as yellow crystals.

R_f 0.50. M.p. 178-180 °C. ^{1}H NMR (DMSO-d_6) δ: 1.21-1.26 (m, 3H, -CH_2-$\underline{CH_3}$); 1.36 (s, 9H, (-CH_3)$_3$); 1.57-1.66 (m, 2H, -CH_2-$\underline{CH_2}$-CH_2-); 2.13 (t, 2H, J=7.35, -$(CH_2)_2$-$\underline{CH_2}$-); 2.88-2.95 (m, 2H, -$\underline{CH_2}$-$(CH_2)_2$-); 4.20 (q, 2H, J=6.93, -$\underline{CH_2}$-CH_3); 6.42 (d, 2H, J=10.52, -NH_2); 6.75-6.84 (m, 1H, -NH); 9.73 (d, 1H, J=39.85, -NH-N=). ^{13}C NMR (DMSO-d_6) δ: 14.03; 24.39; 25.72; 28.29; 28.94; 31.65; 39.78; 61.53; 61.60; 77. 43; 77.52; 135.83; 138.83; 155.63; 161, 81; 162.33; 168.08; 174.20. HRMS (ESI), *m/z*: 317.1827 $[M+H]^+$, (calc. for $C_{13}H_{25}N_4O_5$, *m/z*: 317.1825), *m/z*: 315.1671 $[M-H]^+$, (calc. for $C_{13}H_{23}N_4O_5$, *m/z*: 315.1668).

Ethyl β-N-(6-(tert-butoxycarbonylamino)hexanoyl)oxalamidrazone (4d)

From 2.32 g (9.45 mmol) of **3d**, 1.38 g (10.4 mmol) ethyl ether of thiooxamic acid in 48 hours 2.84 g (87%) of product **4d** was obtained as yellow crystals.

R_f 0.75. M.p. 172-173 °C. ^{1}H NMR (DMSO-d_6) δ: 1.19-1.27 (m, 5H, -$(CH_2)_2$-$\underline{CH_2}$-$(CH_2)_2$- and -CH_2-$\underline{CH_3}$); 1.35 (m, 11H, (-CH_3)$_3$ and -CH_2-$\underline{CH_2}$-$(CH_2)_3$-); 1.46-1.56 (m, 2H, -$\underline{CH_2}$-CH_2-$(CH_2)_3$-); 2.13 (t, 2H, J = 7.29, -$(CH_2)_4$-$\underline{CH_2}$-); 2.88 (q, 2H, J = 6.45, -$\underline{CH_2}$-$(CH_2)_4$-); 4.16-4.24 (m, 2H, -$\underline{CH_2}$-CH_3); 6.39 (d, 2H, J = 17.94, -NH_2); 6.73-6.74 (m, 1H, -NH-); 9.70 (d, 1H, J = 38.02, -NH-N=). ^{13}C NMR (DMSO-d_6) δ: 14.06; 23.82; 25.00; 26.04; 28.31; 29.37; 34.13; 39.78; 61.59; 77.35; 135.71; 138.68; 155.62; 161.84; 162.33; 168.37; 174.50. HRMS (ESI), *m/z*: 345.2142 $[M+H]^+$, (calc. for $C_{15}H_{29}N_4O_5$, *m/z*: 345.2138), *m/z*: 343.1985 $[M-H]^+$, (calc. for $C_{15}H_{27}N_4O_5$, *m/z*: 343.1981).

5-Substituted ethyl 1,2,4-triazole-3-carboxylates (5)

General procedure

Amidrazone **4** was refluxed in *o*-xylene (20 ml of *o*-xylene per 1 g of amidrazone) until complete conversion of amidrazone, control using TLC (10% methanol in chloroform). O-xylene was evaporated. The reaction product **5** was isolated by column chromatography on silica gel in a toluene/acetone solvent system (with an acetone gradient from 0 to 25%).

Ethyl 5-[N-(tert-butoxycarbonyl)aminomethyl]-1,2,4-triazole-3-carboxylate (5a)

From 1.96 g (6.80 mmol) **4a** in 20 ml of *o*-xylene in 7 hours was obtained 1.14 g (62%) of product **5a** as white crystals.

R_f 0.45 (5% methanol in chloroform). M.p. 152-156 °C. ^{1}H NMR (DMSO-d_6) δ: 1.35-1.40 (m, 12H, -C(-CH_3)$_3$ and -O-CH_2-$\underline{CH_3}$); 4.44 (q, 2H, J=7.14, -O-$\underline{CH_2}$-CH_3), 4.56 (br.s, 2H, -NH-$\underline{CH_2}$-); 6.00 (br.s, 1H, -$\underline{NH}$-CH_2-). ^{13}C NMR (DMSO-d_6) δ: 14.61; 27.96; 36.39; 61.29; 78.24; 154.35; 156.09; 156.64; 160.25. HRMS (ESI), *m/z*: 271.1409 $[M+H]^+$, (calc. for $C_{11}H_{19}N_4O_4$, *m/z*: 271.1406), *m/z*: 269.1254 $[M-H]^+$, (calc. for $C_{11}H_{17}N_4O_4$, *m/z*: 269.1249).

Ethyl 5-[N-(tert-butoxycarbonyl)aminoethyl]-1,2,4-triazole-3-carboxylate (5b)

From 2.60 g (8.61 mmol) **4b** in 25 ml of *o*-xylene in 24 hours was obtained 1.40 g (57%) of product **5b** as white crystals.

R_f 0.45 (4% methanol in chloroform). M.p. 121-122 °C. ^{1}H NMR ($CDCl_3$) δ: 1.36-1.41 (m, 12H, -CH_2-$\underline{CH_3}$ and (-CH_3)$_3$); 3.13-3.17 (m, 2H, -NH-$\underline{CH_2}$-CH_2-); 3.58-3.60 (m, 2H, -NH-CH_2-$\underline{CH_2}$-); 4.45 (q, 2H, J=7.13, -$\underline{CH_2}$-CH_3); 5.41 (s, 1H, -NH-). ^{13}C NMR ($CDCl_3$) δ: 14.22; 27.81; 28.29; 38.27; 62.05; 80.27; 153.76; 154.42; 156.81; 160.20. HRMS (ESI), *m/z*: 285.1568 $[M+H]^+$, (calc. for $C_{12}H_{21}N_4O_4$, *m/z*: 285.1563), *m/z*: 283.1410 $[M-H]^+$, (calc. for $C_{12}H_{19}N_4O_4$, *m/z*: 283.1406).

Ethyl 5-[N-(tert-butoxycarbonyl)aminopropyl]-1,2,4-triazole-3-carboxylate (5c)

and

ethyl 5-[3-(tert-butoxycarbonyl)aminopropyl]-1,3,4-oxadiazole-2-carboxylate (5′c)

From 2.85 g (9.00 mmol) **4c** in 30 ml of *o*-xylene in 12 hours an a crude mixture was obtained. By column chromatography 1.65 g (yield 61%) of product **5c** as white crystals and ethyl 5-[3-(*tert*-butoxycarbonyl)aminopropyl]-1,3,4-oxadiazole-2-carboxylate **5′c** 0.21 g (yield 8%) as colorless oil were isolated.

For 5c: R_f 0.50 (2% methanol in chloroform). M.p. 137-138 °C. ^{1}H NMR ($CDCl_3$) δ: 1.37-1.41 (m, 12H, -CH_2-$\underline{CH_3}$ and (-CH_3)$_3$); 1.84-1.92 (m, 2H, -CH_2-$\underline{CH_2}$-CH_2-); 2.91-2.95 (t, 2H, J= 6.14, -(CH_2)$_2$-$\underline{CH_2}$-); 3.16 (q, 2H, J=6.44, -$\underline{CH_2}$-(CH_2)$_2$-); 4.44 (q, 2H, J=6.96, -$\underline{CH_2}$-CH_3); 4.98-5.01 (m, 1H, -NH-). ^{13}C NMR ($CDCl_3$) δ: 14.20; 22.91; 28.27; 28.46; 38.69; 61.83; 80.25; 154.48; 157.37; 158.00; 160.36. HRMS (ESI), *m/z*: 299.1725 $[M+H]^+$, (calc. for $C_{13}H_{23}N_4O_4$, *m/z*: 299.1719), *m/z*: 297.1565 $[M-H]^+$, (calc. for $C_{13}H_{21}N_4O_4$, *m/z*: 297.1562).

For 5′c: R_f 0.45 (15% acetone in toluene). ^{1}H NMR ($CDCl_3$) δ: 1.38-1.43 (m, 12H, (-CH_3)$_3$ и -CH_2-$\underline{CH_3}$); 1.96-2.05 (m, 2H, -CH_2-$\underline{CH_2}$-CH_2-); 2.93-2.98 (m, 2H, -(CH_2)$_2$-$\underline{CH_2}$-); 3.16-3.24 (m, 2H, -$\underline{CH_2}$-(CH_2)$_2$-); 4.43-4.50 (m, 2H, -$\underline{CH_2}$-CH_3); 4.75 (br.s, 1H, -NH-). ^{13}C NMR ($CDCl_3$) δ,: 13.96; 22.83; 26.65; 28.26; 39.40; 63.39; 79.35; 154.24; 156.86; 158.61;168.74. HRMS (ESI), *m/z*: 300.1561 $[M+H]^+$, (calc. for $C_{13}H_{22}N_3O_5$, *m/z*: 300.1559).

Ethyl 5-[N-(tert-butoxycarbonyl)aminopentyl]-1,2,4-triazole-3-carboxylate (5d)

From 2.64 g (7.67 mmol) **4d** in 25 ml of *o*-xylene in 16 hours was obtained 1.63 g (65%) of product **5d** as white crystals.

R_f 0.70 (2% methanol in chloroform). M.p. 140-141 °C. ^{1}H NMR ($CDCl_3$) δ: 1.27-1.48 (m, 16H, -NH-$\underline{CH_2}$-$(CH_2)_4$- and -NH-$(CH_2)_2$-$\underline{CH_2}$-$(CH_2)_2$-, and -CH_2-$\underline{CH_3}$, and (-$\underline{CH_3}$)$_3$); 1.73-1.83 (m, 2H, -NH-$(CH_2)_3$-$\underline{CH_2}$-CH_2-); 2.88 (t, 2H, J = 7.48, -NH-$(CH_2)_4$-$\underline{CH_2}$-); 3.02-3.09 (m, 2H, -NH-$\underline{CH_2}$-$(CH_2)_4$-); 4.41 (q, 2H, J = 7.12, -$\underline{CH_2}$-CH_3); 4.74-4.78 (m, 1H, -$\underline{NH}$-$(CH_2)_5$-). ^{13}C NMR ($CDCl_3$) δ: 14.20; 26.01; 26.30, 27.23, 28.37; 29.53; 40.16; 61.93; 79.50; 154.07; 156.46; 159.15; 160.33. HRMS (ESI), *m/z*: 327.2035 $[M+H]^+$, (calc. for $C_{15}H_{27}N_4O_4$, *m/z*: 327.2032), *m/z*: 325.1880 $[M-H]^+$, (calc. for $C_{15}H_{25}N_4O_4$, m/z: 325.1876).

5-Substituted 1,2,4-triazole-3-carboxamide (6)

General procedure

An ammonia solution in methanol (12 M) was added to 5-substituted ethyl 1,2,4-triazole-3-carboxylates **5** (10 ml per 1g of **5**) and the mixture was stirred at the reflux until the full conversion of ester **5** was completely converted (TLC control). 0.5 ml of ammonia solution in methanol (12 M) was added every two hours. After the full conversion of ester **5** the volatile components were evaporated at reduced pressure. The product **6** was isolated by column chromatography on silica gel in a methanol-chloroform solvent system.

5-[N-(tert-Butoxycarbonyl)aminomethyl]-1,2,4-triazole-3-carboxamide (6a)

From 1.14 g (4.20 mmol) of ethyl 5-[*N*-(*tert*-butoxycarbonyl)aminomethyl]-1,2,4-triazole-3-carboxylate **5a** in 24 hours 0.42 g (42%) of the product **6a** was isolated by column chromatography on silica gel, eluent: methanol-chloroform (methanol gradient from 0 to 15%), as white crystals.

R_f 0.50 (10% methanol in chloroform). ^{1}H NMR (DMSO-d_6) δ: 1.38 (s, 9H, (-CH_3)$_3$); 4.23 (br.s, 2H, -NH-$\underline{CH_2}$-); 7.29 (s, 1H; -NH-); 7.60 and 7.82 (2 br.s, 2H, -$CONH_2$); 14.36 (br.s, 1H, triazole). ^{13}C NMR (DMSO-d_6) δ: 28.46; 36.96; 78.77; 153.87; 155.95; 158.14; 160.07. HRMS (ESI), *m/z*: 242.1255 $[M+H]^+$, (calc. for $C_9H_{16}N_5O_3$, *m/z*: 242.1253), *m/z*: 240.1100 $[M-H]^+$, (calc. for $C_9H_{14}N_5O_3$, *m/z*: 240.1097).

5-[N-(tert-Butoxycarbonyl)aminoethyl]-1,2,4-triazole-3-carboxamide (6b)

From 0.50 g (1.76 mmol) ethyl 5-[*N*-(*tert*-butoxycarbonyl)aminoethyl]-1,2,4-triazole-3-carboxylate **5b** in 42 hours 0.32 g (71%) of the product **6b** was isolated by column chromatography on silica gel, eluent: methanol-chloroform (methanol gradient from 0 to 10%), as white crystals.

R_f 0.40 (5% methanol in chloroform). ^{1}H NMR (DMSO-d_6) δ: 1.35 (s, 9H, (-CH_3)$_3$); 2.78-2.83 (m, 2H, -NH-CH_2-$\underline{CH_2}$-); 3.23-3.30 (m, 2H, -NH-$\underline{CH_2}$-CH_2-); 6.88 (s, 1H; -NH-); 7.55 and 7.79 (2 br.s, 2H, -$CONH_2$); 14.15 (br.s, 1H, triazole). ^{13}C NMR (DMSO-d_6) δ: 26.92; 28.24; 38.29; 77.98; 152.90; 155.62; 156.75; 159.30. HRMS (ESI), *m/z*: 256.1414 $[M+H]^+$, (calc. for $C_{10}H_{18}N_5O_3$, *m/z*: 256.1409), *m/z*: 254.1256 $[M-H]^+$, (calc. for $C_{10}H_{16}N_5O_3$, *m/z*: 254.1253).

5-[N-(tert-Butoxycarbonyl)aminopropyl]-1,2,4-triazole-3-carboxamide (6c)

From 0.88 g (2.90 mol) ethyl 5-[N-(*tert*-butoxycarbonyl)aminopropyl]-1,2,4-triazole-3-carboxylate **5c** in 36 hours 0.56 g (71%) of the product **6c** was isolated by column chromatography on silica gel, eluent: methanol-chloroform (methanol gradient from 0 to 10%), as white crystals.

R_f 0.45 (7% methanol in chloroform). ^{1}H NMR (DMSO-d_6) δ: 1.36 (s, 9H, (-CH_3)$_3$); 1.71-1.81 (m, 2H, -CH_2-$\underline{CH_2}$-CH_2-); 2.67 (br.s, 2H, -$(CH_2)_2$-$\underline{CH_2}$-); 2.93-2.99 (m, 2H, -$\underline{CH_2}$-$(CH_2)_2$-); 6.83 (s, 1H; -NH-); 7.39-7.97 (m, 2H, -$CONH_2$); 13.96-14.44 (m, 1H, triazole). ^{13}C NMR (DMSO-d_6) δ: 23.13;

27.51; 28.32; 40.06; 77.78; 152.73; 155.76; 158.12; 159.17. HRMS (ESI), *m/z*: 270.1569 $[M+H]^+$, (calc. for $C_{11}H_{20}N_5O_3$, *m/z*: 270.1566), *m/z*: 268.1413 $[M-H]^+$, (calc. for $C_{11}H_{18}N_5O_3$, *m/z*: 268.1409).

5-[N-(tert-Butoxycarbonyl)aminopentyl]-1,2,4-triazole-3-carboxamide (6d)

From 0.50 g (1.53 mmol) ethyl 5-[*N*-(*tert*-butoxycarbonyl)aminopentyl]-1,2,4-triazole-3-carboxylate **5d** in 36 hours 0.37 g (82%) of the product **6d** was isolated by column chromatography on silica gel, eluent: methanol-chloroform (methanol gradient from 0 to 7%), as white crystals.

R_f 0.40 (3% methanol in chloroform in the system). ^{1}H NMR (DMSO-d_6) δ: 1.20-1.38 (m, 4H, -NH-$(CH_2)_2$-$\underline{CH_2}$-$(CH_2)_2$- and -NH-CH_2-$\underline{CH_2}$-$(CH_2)_3$-); 1.35 (s, 9H, $(-CH_3)_3$); 1.63-1.70 (m, 2H, -NH-$(CH_2)_3$-$\underline{CH_2}$-CH_2-); 2.61-2.72 (m, 2H, -NH-$(CH_2)_4$-$\underline{CH_2}$-); 2.85-2.92 (m, 2H, -NH-$\underline{CH_2}$-$(CH_2)_4$-); 6.71 (s, 1H, -NH-); 7.39-7.99 (m, 2H, $-CONH_2$); 13.93-14.45 (m, 1H, triazole). ^{13}C NMR (DMSO-d_6) δ: 25.52; 25.78; 26.80; 28.38; 29.17; 39.78; 77.56; 152.98; 155.75; 158.41; 159.36. HRMS (ESI), *m/z*: 298.1884 $[M+H]^+$, (calc. for $C_{13}H_{24}N_5O_3$, *m/z*: 298.1879), *m/z*: 296.1731 $[M-H]^+$, (calc. for $C_{13}H_{22}N_5O_3$, *m/z*: 296.1726).

tert-Butoxycarbonyl protective group removal (compounds (7))

General procedure

HCl in 1,4-dioxane (3.42 M) was added to carboxamide **6**. The reaction mass was stirred at room temperature under anhydrous conditions. The conversion of compounds **6** was controlled by TLC. The precipitate was filtered and washed with 10 ml of anhydrous diethyl ether. The crystals were dried in a desiccator over NaOH for 24 hours.

5-Aminomethyl-1,2,4-triazole-3-carboxamide dihydrochloride (7a)

From 0.38 g (1.57 mmol) 5-[*N*-(*tert*-butoxycarbonyl)aminomethyl]-1,2,4-triazole-3-carboxamide **6a** in 6 ml HCl in 1.4-dioxane in 36 hours. The reaction was controlled in a system of 5% methanol in chloroform. The yield of **7a** was 0.27 g (80%) as white crystals.

^{1}H NMR (DMSO-d_6) δ: 4.08-4.14 (m, 2H, $-CH_2-$); 7.88 and 8.00 (2 br.s, 2H, $-CONH_2$); 8.79 (s, 3H, $-NH_3^+$); 10.10 (br.s, 2H, $=NH_2^+$). ^{13}C NMR (DMSO-d_6) δ: 35.72; 152.56; 155.81; 159.10. HRMS (ESI), *m/z*: 142.0733 $[M+H]^+$, (calc. for $C_4H_8N_5O$, *m/z*: 142.0729), *m/z*: 140.0577 $[M-H]^+$, (calc. for $C_4H_6N_5O$, *m/z*: 140.0572).

5-(2-Aminoethyl)-1,2,4-triazole-3-carboxamide dihydrochloride (7b)

From 0.30 g (1.18mmol) 5-[*N*-(*tert*-butoxycarbonyl)aminoethyl]-1,2,4-triazole-3-carboxamide **6b** in 4 ml HCl in 1.4-dioxane in 1 hour. The reaction was controlled in a system of 4% methanol in chloroform. The yield of **7b** was 0.24 g (89%) as white crystals.

^{1}H NMR (DMSO-d_6) δ: 3.09-3.19 (m, 4H, $-CH_2-CH_2-$); 7.71 and 8.08 (2 br.s, 2H, $-CONH_2$); 8.37 (s, 3H, $-NH_3^+$); 9.83 (br.s, 2H, $=NH_2^+$). 7.62-8.18 (m, 2H, $-CH_2-\underline{NH_2}$); 8.33 (s, 2H, $-C(O)NH_2$). ^{13}C NMR (DMSO-d_6) δ: 24.37; 37.23; 153.72; 156.61; 160.03. HRMS (ESI), *m/z*: 156.0890 $[M+H]^+$, (calc. for $C_5H_{10}N_5O$, *m/z*: 156.0885), *m/z*: 154.0736 $[M-H]^+$, (calc. for $C_5H_8N_5O$, *m/z*: 154.0729).

5-(3-Aminopropyl)-1,2,4-triazole-3-carboxamide dihydrochloride (7c)

From 0.50 g (1.86 mmol) 5-[*N*-(*tert*-butyloxycarbonyl)aminopropyl]-1,2,4-triazole-3-carboxamide **6c**, 4 ml HCl in 1.4-dioxane, 6 hours. The reaction was controlled in a system of 2% methanol in chloroform. The yield of **7c** was 0.44 g (97%) as white crystals.

^{1}H NMR (DMSO-d_6) δ: 1.95-2.05 (m, 2H, $-CH_2-\underline{CH_2}-CH_2-$); 2.81-2.87 (m, 4H, $-\underline{CH_2}-(CH_2)_2-$ and $-(CH_2)_2-\underline{CH_2}-$); 7.72 and 7.98 (2 br.s, 2H, $-CONH_2$); 8.21 (s, 3H, $-NH_3^+$); 11.18 (s, 2H, $-NH_2^+$). ^{13}C NMR (DMSO-d_6) δ: 22.94; 24.95; 30.94; 152.76; 157.65; 159.21. HRMS (ESI), *m/z*: 170.1044 $[M+H]^+$, (calc. for $C_6H_{12}N_5O$, *m/z*: 170.1042), *m/z*: 168.0889 $[M-H]^+$, (calc. for $C_5H_{10}N_5O$, *m/z*: 168.0885).

5-(5-Aminopentyl)-1,2,4-triazole-3-carboxamide dihydrochloride (7d)

From 0.35 g (1.18 mmol) 5-[*N*-(*tert*-butoxycarbonyl)aminopentyl]-1,2,4-triazole-3-carboxamide **6d** in 4 ml HCl in 1.4-dioxane in 8 hours. The reaction was controlled in a system of 2% methanol in chloroform. The yield of **7d** was 0.24 g (89%) as white crystals.

^{1}H NMR (DMSO-d_6) δ: 1.28-1.38 (m, 2H, NH_2-$(CH_2)_2$-$\underline{CH_2}$-$(CH_2)_2$-); 1.54-1.62 (m, 2H, -NH-CH_2-$\underline{CH_2}$-$(CH_2)_3$-); 1.67-1.77 (m, 2H, -NH-$(CH_2)_3$-$\underline{CH_2}$-CH_2-); 2.68-2.82 (m, 4H, -NH-$\underline{CH_2}$-$(CH_2)_4$- and -NH-$(CH_2)_4$-$\underline{CH_2}$-); 7.78-8.12 (m, 5H, -$\underline{H_3N^+}$-$(CH_2)_5$- and -CO$\underline{NH_2}$); 8.58 (br.s, 2H, =NH_2^+). ^{13}C NMR (DMSO-d_6) δ: 25.04; 25.10; 25.37; 26.64; 38.78; 152.38; 158.07, 159.07. HRMS (ESI), *m/z*: 198.1360 $[M+H]^+$, (calc. for $C_8H_{16}N_5O$, *m/z*: 198.1355), *m/z*: 196.1202 $[M-H]^+$, (calc. for $C_8H_{14}N_5O$, *m/z*: 196.1198).

1.3. Biological Section

1.3.1. Antiviral effect

1.3.1.1. Virus

The H3N2 influenza A virus strain Aichi/2/68 was obtained from the State Virus Collection of the National Research Center for Epidemiology and Microbiology named after Honorary Academician N. F. Gamaleya of the Ministry of Health of the Russian Federation.

1.3.1.2. Cell culture

The Madin-Darby Canine Kidney (MDCK) cell line was used in the work. The cell line was obtained from the State Collection of Cell Cultures of the Federal State Budgetary Institution "National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya" of the Ministry of Health of the Russian Federation (a division of the D.I. Ivanovsky Institute of Virology).

1.3.1.3. Medium

Cultivation medium: Igla-MEM (PanEco, Moscow) + 10% TFP (HyClone, Thermo Scientific, USA); A confluent monolayer of the transplantable cell line was used in the work, grown in a culture medium with the addition of 0.3 mg/ml L-glutamine and antibiotics - 100 ME/ml penicillin and 100 ME/ml streptomycin. The virus-containing medium (medium for working with the virus) was a culture medium with the addition of trypsin to its final concentration in the medium of 0.001 mg/ml.

1.3.1.4. Cytotoxicity of synthesized compounds

MTT-test

For the MTT test, a commercial reagent kit was used to control proliferation and determine cytotoxicity of cells with thiazolyl (MTT), Art. G4101-200T (Service bio). The study was performed according to the manufacturer's instructions. The absorption was measured at 490 nm.

LDG-test

A commercial lactate dehydrogenase cytotoxicity assay kit, G1610-100T, (Servicebio) was used for the LDH test. The study was performed according to the manufacturer's instructions, determining the total intracellular LDH. The absorption was measured at 490 nm.

1.3.1.5. RGA

The hemagglutination reaction (HGA) was performed using a conventional method[S5] in round-bottomed microplates for immunological reactions (Medpolymer, Russia). 50 µl of virus-containing medium from the corresponding well of the cell culture tablet was added to the wells of the microplate for immunological reactions, after which an equal amount of 1% suspension of human erythrocytes was added to the wells. The reaction was taken into account after 40-60 minutes of incubation at +4 °C.

1.3.1.6. Antiviral activity

A cell culture monolayer ($2*10^4$ cells/well) grown in plastic 96-well plates (Corning, USA) was incubated at 37°C, 5% CO_2 for 24 hours, then solutions of the tested substances in the culture medium and the virus in virus-containing medium were added to the wells (multiplicity of infection 0.1 lg $TCID_{50}$). After that the plate was incubated in a thermostat at 37°C, 5% CO_2 for 24 hours and samples were taken from the wells to determine the infectious titer of the virus, which was established using RGA[S6].

Table S1 Compounds antiviral activity and CC_{50} for the compounds **1** and **7a-d**.

Compound	CC_{50} by MTT assay, mM	CC_{50} by LDG assay, mM	Suppression of viral replication
Rib	8.8 ± 1.5	13.4 ± 0.3	virus is not detected
1	17.9 ± 3.1	-	4 times
7a	10.7 ± 2.7	25.0 ± 0.6	4 times
7b	18.0 ± 1.2	25.3 ± 0.4	4 times
7c	19.0 ± 3.8	25.6 ± 0.9	4 times
7d	18.8 ± 5.3	26.9 ± 1.2	virus is not detected

«-» - not determined due to poor solubility

1.3.2. Anticancer activity

1.3.2.1. Cell Cultures

Acute lymphoblastic leukemia CCRF-SB and chronic myeloid leukemia K562 cell lines were obtained from the Bioresource collection of cell lines of N.N. Blokhin National Medical Research Center of Oncology. Cells were cultured in RPMI-1640 media ("Paneco", Russia) supplemented with a 10% fetal bovine serum ("Biowest", France), 2 mM L-glutamine, 5 ME/mL penicillin and 5 μg/mL streptomycin ("Paneco", Russia) at 37 °C and 5% CO_2.

1.3.2.2. MTT Assay

Cells were seeded in 96-well plates (25 000 cells/well for CCRF-SB or 20 000 cell/well for K562) and treated with various concentrations (1.56 mM – 50 mM) of Rib or tested compounds for 24 h. Cell viability was determined using the MTT assay. Cells were incubated at 37 °C for 3 h with a 0.25 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, "Paneco", Russia) in phosphate-saline buffer (PBS, pH 7.4). The supernatant was discarded, and the crystals of formazan was dissolved in DMSO. The absorbance values were measured at 570 nm on a Microplate Photometer Multiskan FC ("Thermo Fisher Scientific", USA). The percentage of viable cells was calculated as a percentage of solvent-treated control.

Table S2 The studied compounds CC_{50}, with an exposure time of 24 h.

Compound [*,**]	CCRF-SB CC_{50}, mM	K562 CC_{50}, mM
7a	43 ± 8	15 ± 4
7b	26 ± 4	15 ± 2
7c	20 ± 1	6 ± 2
7d	20 ± 1	14 ± 1

[*] for compound **1** CC_{50} not determined due to poor solubility.

[**] for the comparison drug **Rib** no effect was observed on the CCRF-SB cell line, on the K562 line, CC_{50} was 0.27 ± 0.01

1.3.2.3. Cell Cycle

Cells were cultured in 24-well plates (30 000 cells/well) and treated with a 10 nM **Cyt** (positive control), 0.5 mM (for CCRF-SB) or 5 mM (for K562) Rib or tested compounds for 72 h. Then, cells were washed with PBS and fixed in 70% ice-cold ethanol for 2 h. Cells were then washed twice with ice-cold PBS, then suspended in a 500 μL propidium iodide (PI) solution (50 μg/mL PI, 1% Triton X-100 and 100 μg/mL RNase A in PBS). The cell cycle distribution of cells in samples were analyzed using a FACSCalibur Flow Cytometer ("BD Biosciences", San Jose, CA, USA).

All data were calculated as the mean ± standard error of mean (S.E.M.). The data were analyzed using GraphPad v8.2.1 software. The treatment effects in each experiment were compared by one-way ANOVA test. Differences between groups were considered significant at $p<0.05$. All *in vitro* experiments in this section were repeated three times in 2 technical replications.

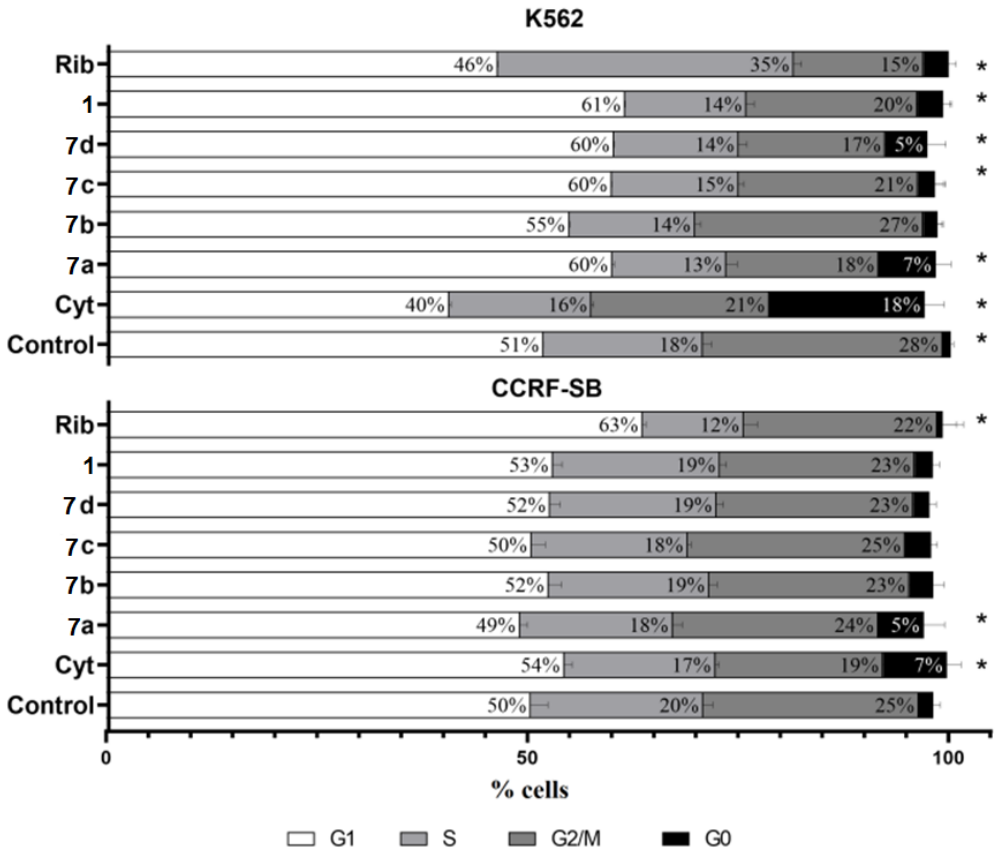


Figure S1 Distribution of K562 and CCRF-SB cells by cell cycle phases. Cells were cultured with **Rib**, **1**, **7a-d** or **Cyt** for 72 h. Cells were fixed with ethanol and then stained with PI and analyzed by flow cytometry. All data are expressed as percent of non-treated control. Significant differences were analyzed by the one-way ANOVA test. *- significant differences from the control ($p<0.05$).

1.3.3. Antimicrobial assays

1.3.3.1. Microorganisms cultures

The following microorganisms from the collection of cultures of the Gause Institute of New Antibiotics were used as test cultures: *Staphylococcus aureus* INA 00985, *Micrococcus luteus* ATCC 9341, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 14053, *Mycobacterium smegmatis* ATCC 607.

1.3.3.2. Agar well diffusion method

Antimicrobial activity was determined with standard agar wells method by measuring the diameter of the inhibition zones[S7]. The cultures grown at 35 °C on the following media: Mueller-Hinton agar (*Staphylococcus aureus* INA 00985, *Micrococcus luteus* ATCC 9341, *Pseudomonas aeruginosa* ATCC 27853), Sabouraud agar (*Candida albicans* ATCC 14053) for 24 h before assay setting. Preparation of inoculum: the cells of the bacterial were suspended in sterile saline to a turbidity of 0.5 McFarland by shaking on a vortex mixer by 10-15 sec and applied to petri dishes with Mueller-Hinton agar (Mueller-Hinton agar with 2% glucose for *Candida albicans)*. Dishes were incubated at 35°C. Growth inhibition zones size were measured after 24 h of incubation.

Table S3 Zone of microorganism growth inhibition by synthesized compounds **1**, **7a-d** at a concentration of 50 mM.

Compound	Zone of growth inhibition, mm			
	Bacteria			Fungi
	M. luteus ATCC 9341	*S. aureus* INA 00985	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 14053
Rib	0	0	25.0	30.0
1	23.0	18.0	15.0	0
7a	0	0	0	0
7b	14.5	14.0	11.0	0
7c	18.5	15.0	11.0	0
7d	17.5	15.0	11.0	0

1.3.3.3. MIC values determination

Minimum inhibitory concentrations of the compounds were determined according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Gram-positive (*Staphylococcus aureus* INA 00985, *Micrococcus luteus* ATCC 9341) and Gram-negative (*Pseudomonas aeruginosa* ATCC 27853) strains were used. The test cultures were grown on Mueller-Hinton agar (HiMedia, India) for 20 h at 35 °C. The preparation of the bacterial suspension is described in 1.3.1. The suspension, with a turbidity of 0.5 McFarland units, were applied to 96-well microplates filled with fixed volumes of dilutions of the test substances. The tested substances were dissolved in a sterile medium and sterilized by filtration through membrane filters with a pore size of 0.22 microns. The positive control was ciprofloxacin, and the negative control was culture without additives. The microplates were incubated for 24 h at 35 °C, and minimum inhibitory concentrations (MICs) of the test substances were determined by microplate photometer Hipo (Biosan, Latvia) at the wavelength 620 nm. The experiment was triplicated.

Table S4 MIC for compounds **1** and **7b-d** determined by serial dilution method.

Compound	MIC, mM		
	M. luteus ATCC 9341	*S. aureus* INA 00985	*P. aeruginosa* ATCC 27853
1	-	-	12.5
7b	-	-	12.5
7c	6.25	12.5	12.5
7d	6.25	6.25	12.5

«-» - not determined due to poor solubility

1.3.3.4. Antimicobacterial effect

A 48 h culture of *M. smegmatis ATCC 607* grown on peptone agar medium (Fizlabpribor, Moscow, Russia) at 37 °C was aseptically suspended in PBS buffer with glass beads. The suspension with a turbidity of 0.5 McFarland units was added to a sterile medium up to 10%. The tested substances were dissolved in a sterile medium and sterilized by filtration through membrane filters with a pore size of 0.22 microns. Cultivation was carried out in a 96-well plate with the test substances at concentrations of 100 and 10 mM. The positive control was isoniazid at 300, 30, and 3 μM, and the negative control was the culture without additives. The experiment was triplicated. Cultivation was carried out at 37 °C and 300 rpm for 48 h. Percent inhibition was determined by optical density at the wavelength 620 nm.

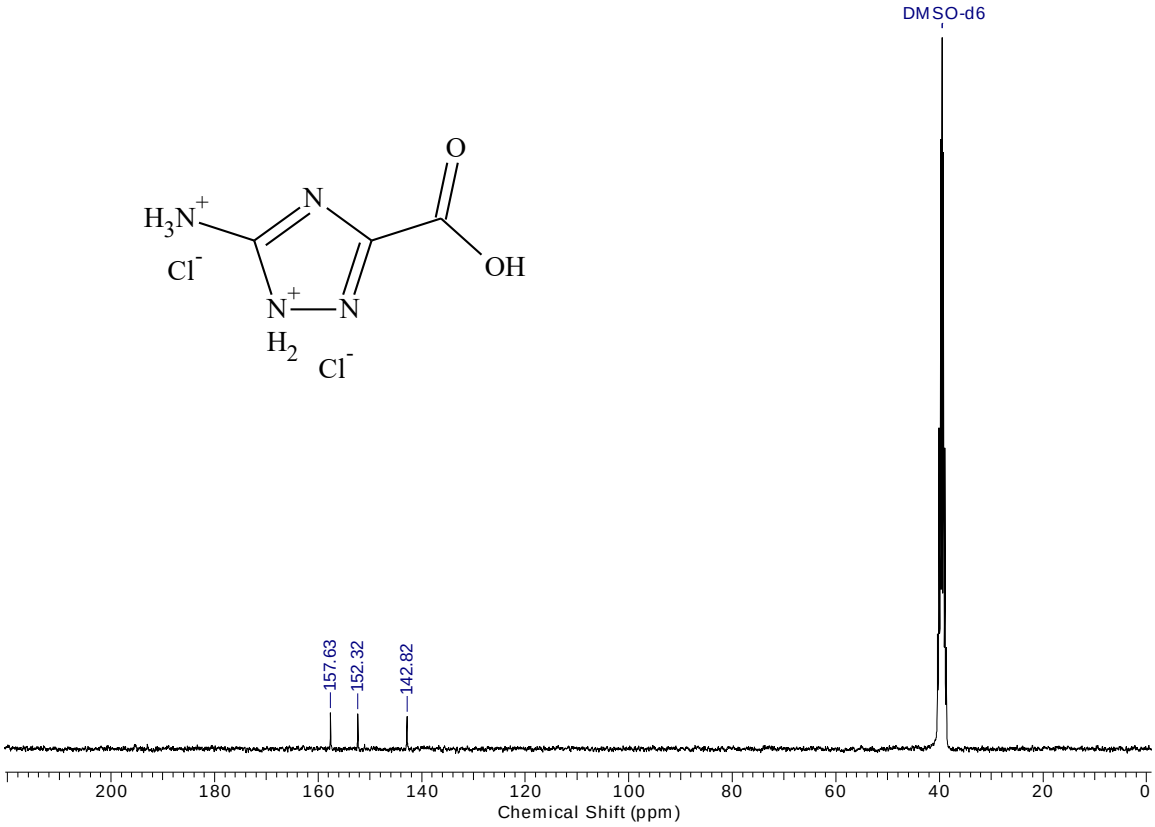
Table S5 *M. smegmatis* ATCC 607 growth inhibition by synthesized compounds.

Compound*	Proportion of inhibition growth *M. smegmatis* (48 hours)	
	C=10 mM	C=100 mM
1	0.06 ± 0.02	0.10 ± 0.02
7a	0.20 ± 0.03	0.72 ± 0.04
7b	0.10 ± 0.02	0.74 ± 0.02
7c	0.51 ± 0.04	0.77 ± 0.03
7d	0.17 ± 0.04	0.75 ± 0.05

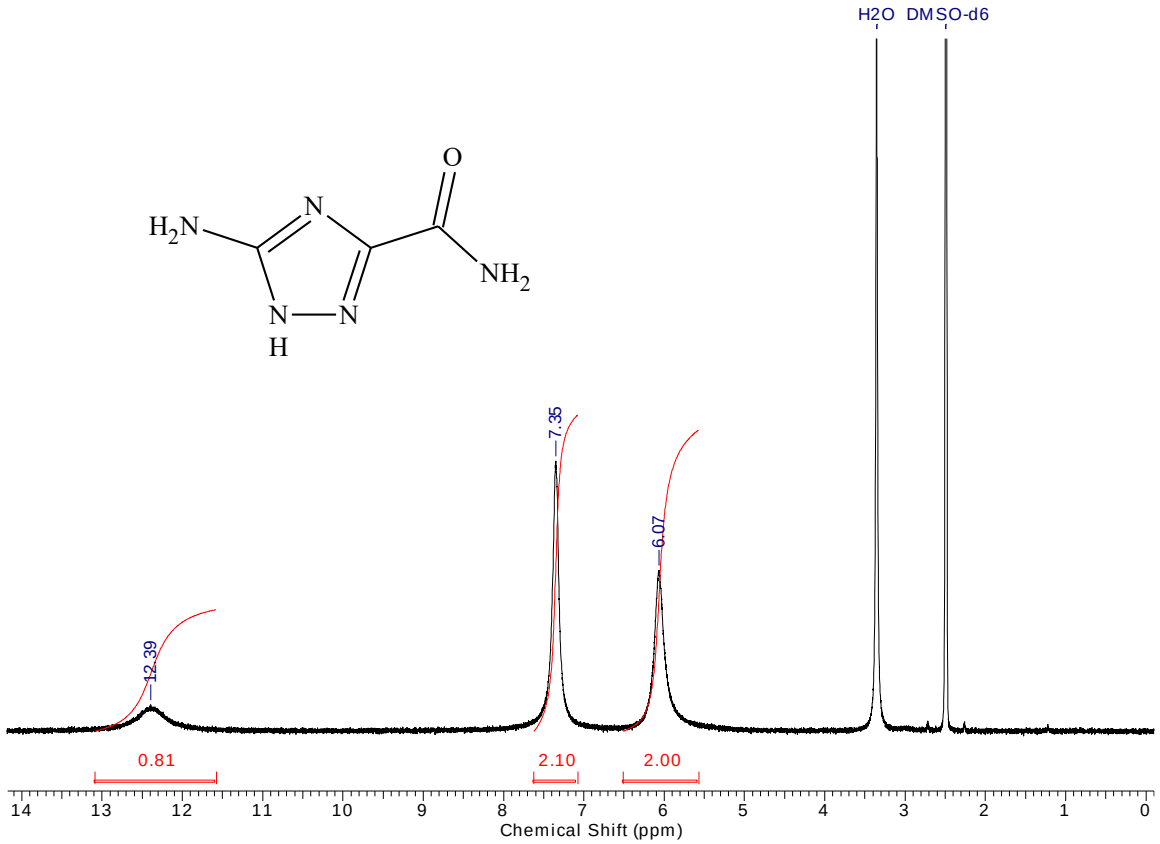
* for the comparison drug isoniazid at a concentration of 30 μM – 0.80 ± 0.04 (48 h)

1.4. NMR Spectra

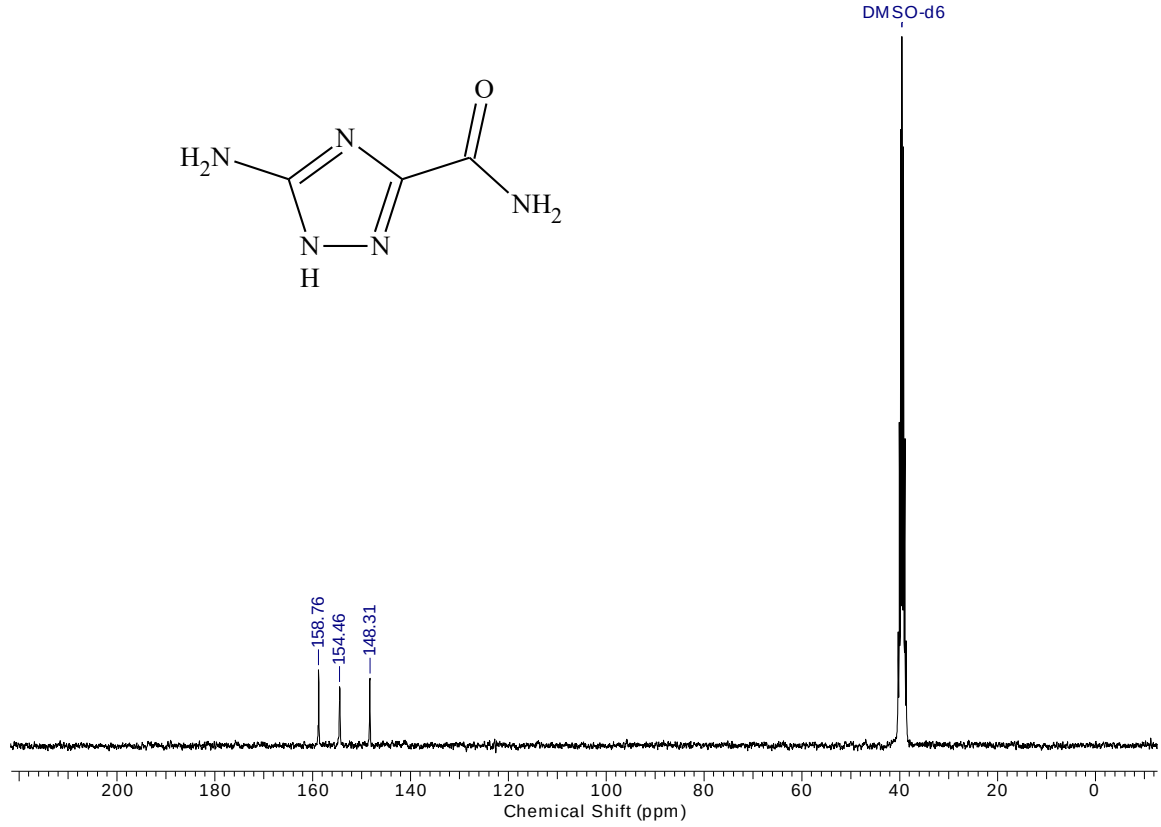
^{13}C NMR spectrum of 5-amino-1,2,4-triazole-3-carboxylic acid



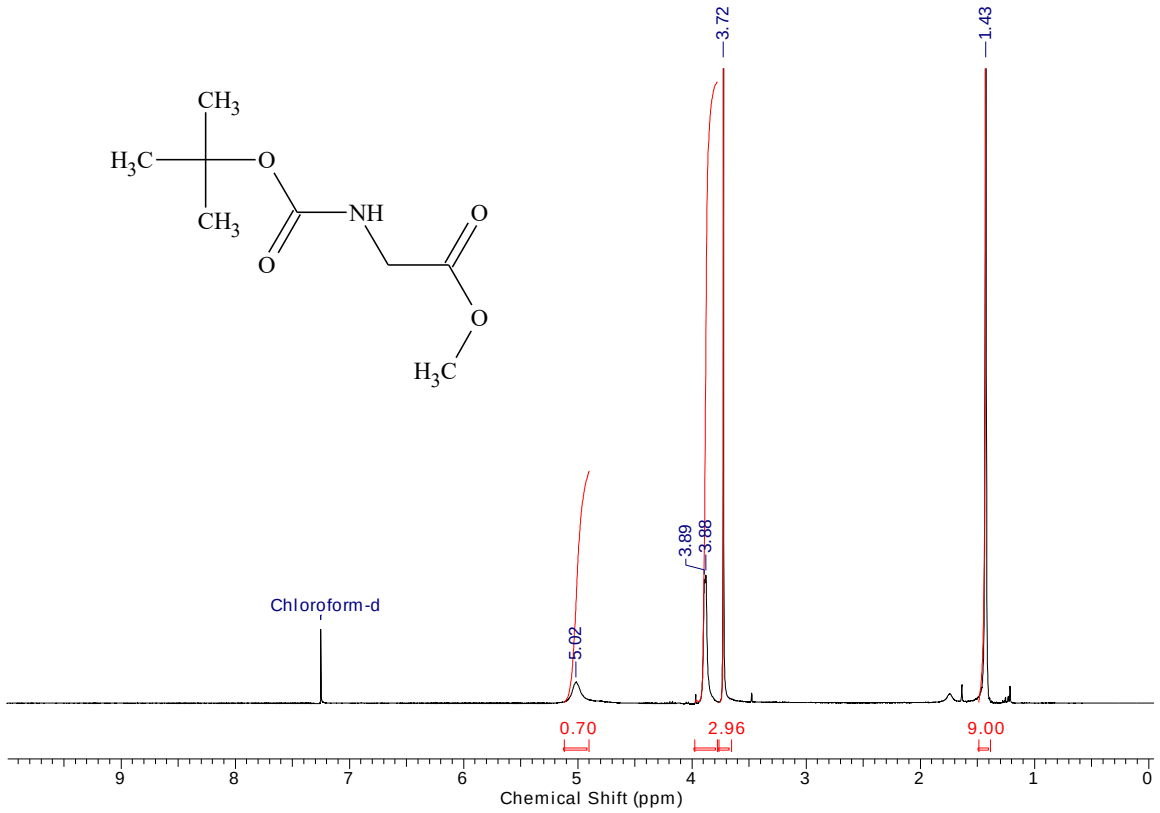
*^{1}H NMR spectrum of **1***



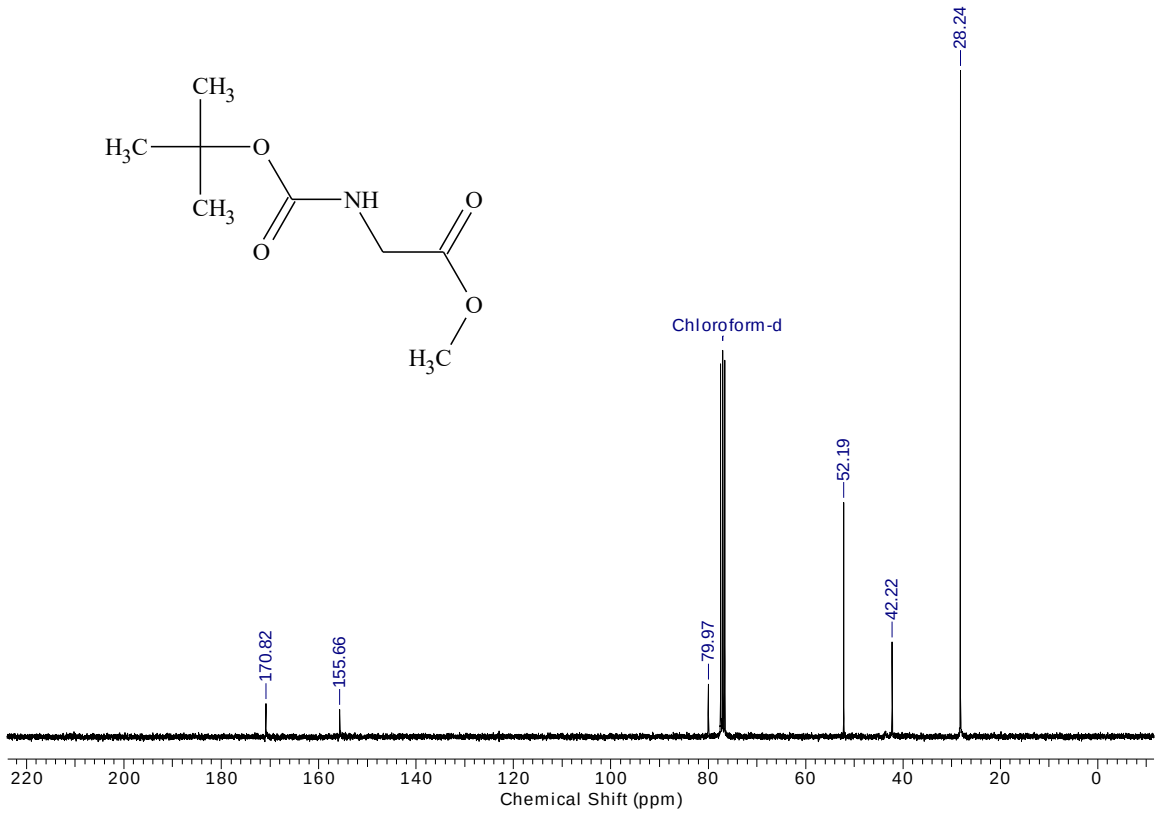
*^{13}C NMR spectrum of **1***



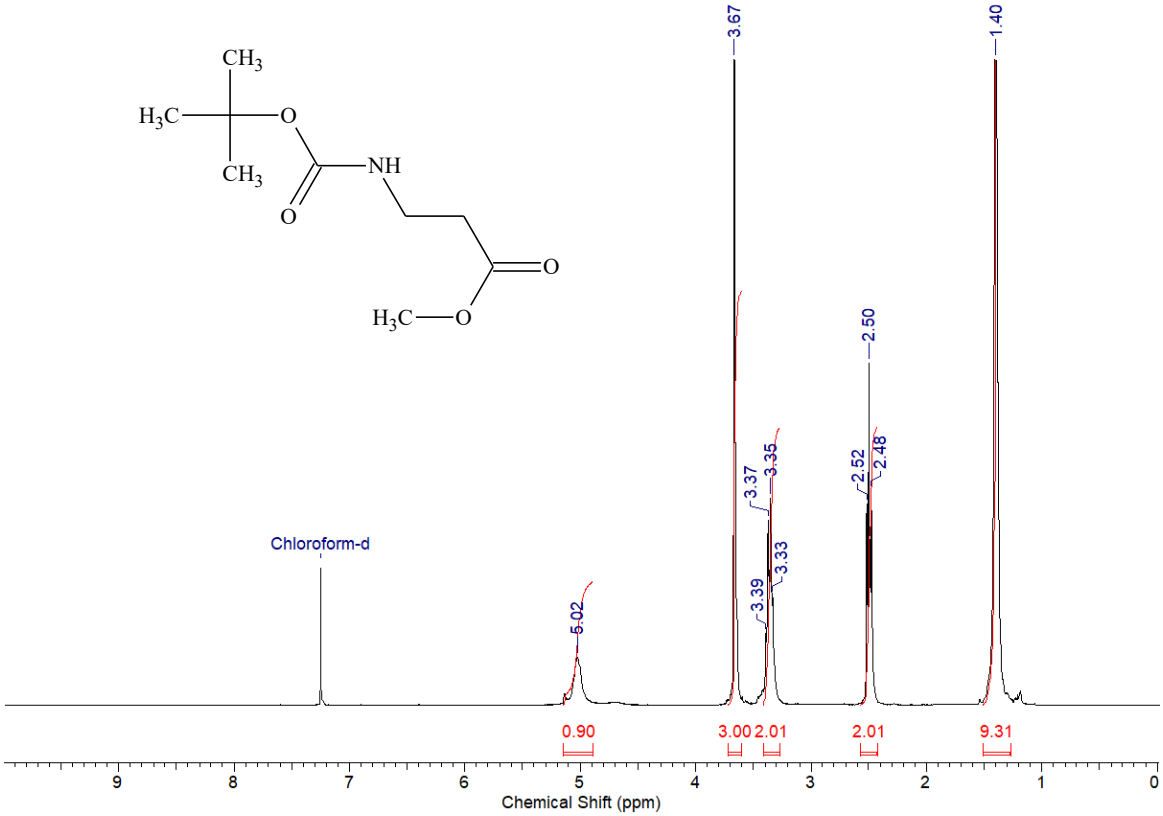
^{1}H NMR spectrum of N-Boc-glycine methyl ester



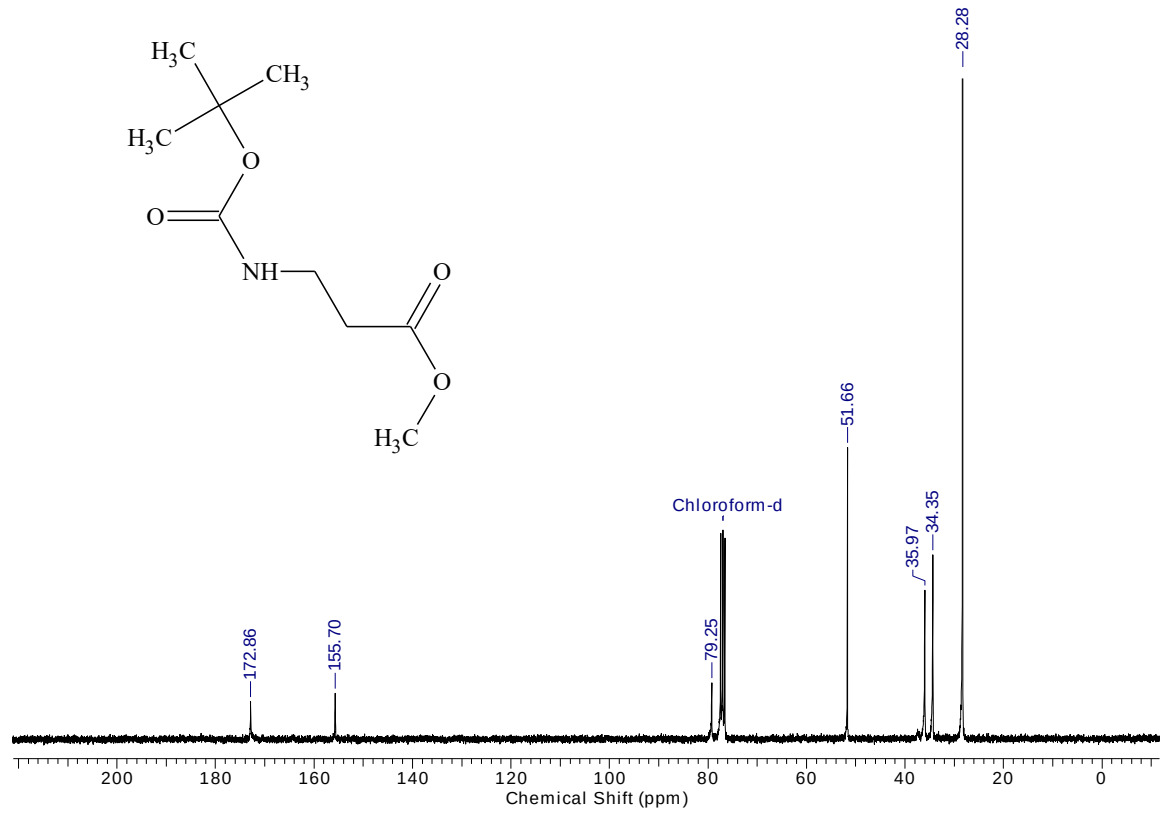
^{13}C NMR spectrum of N-Boc-glycine methyl ester



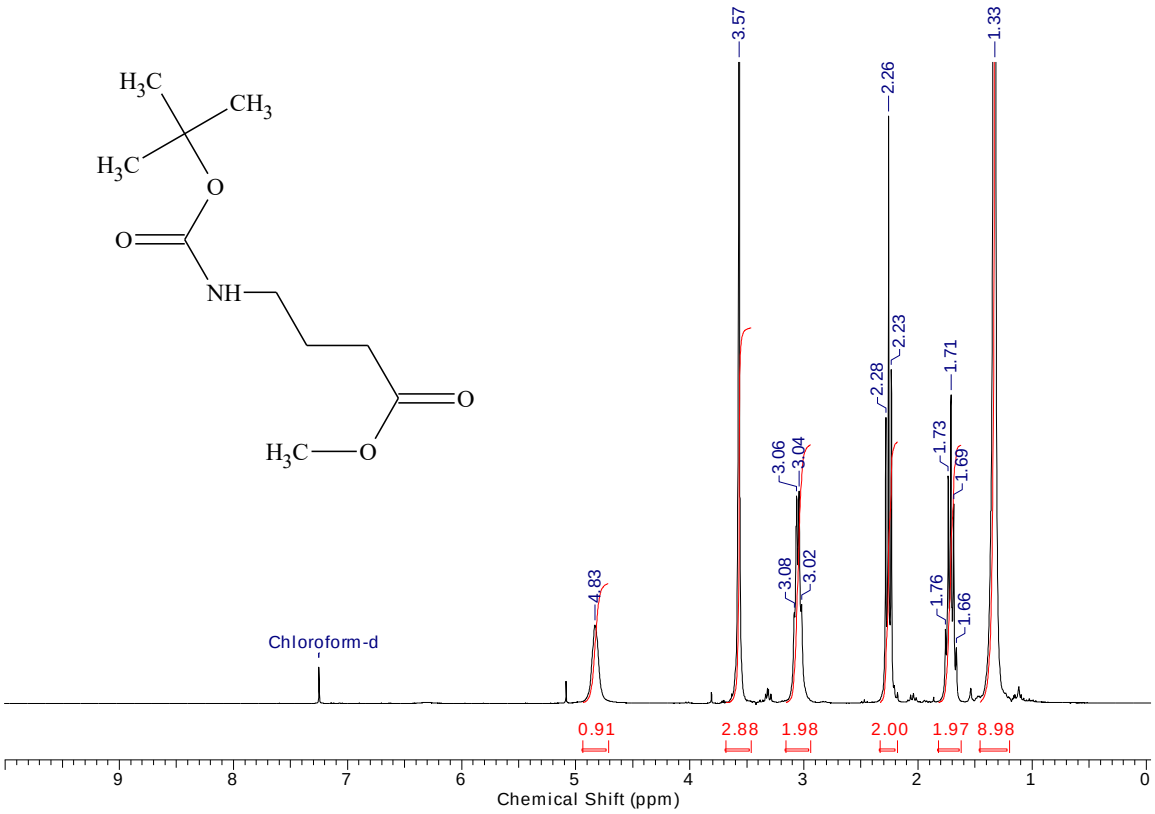
^{1}H NMR spectrum of N-Boc-β-alanine methyl ester



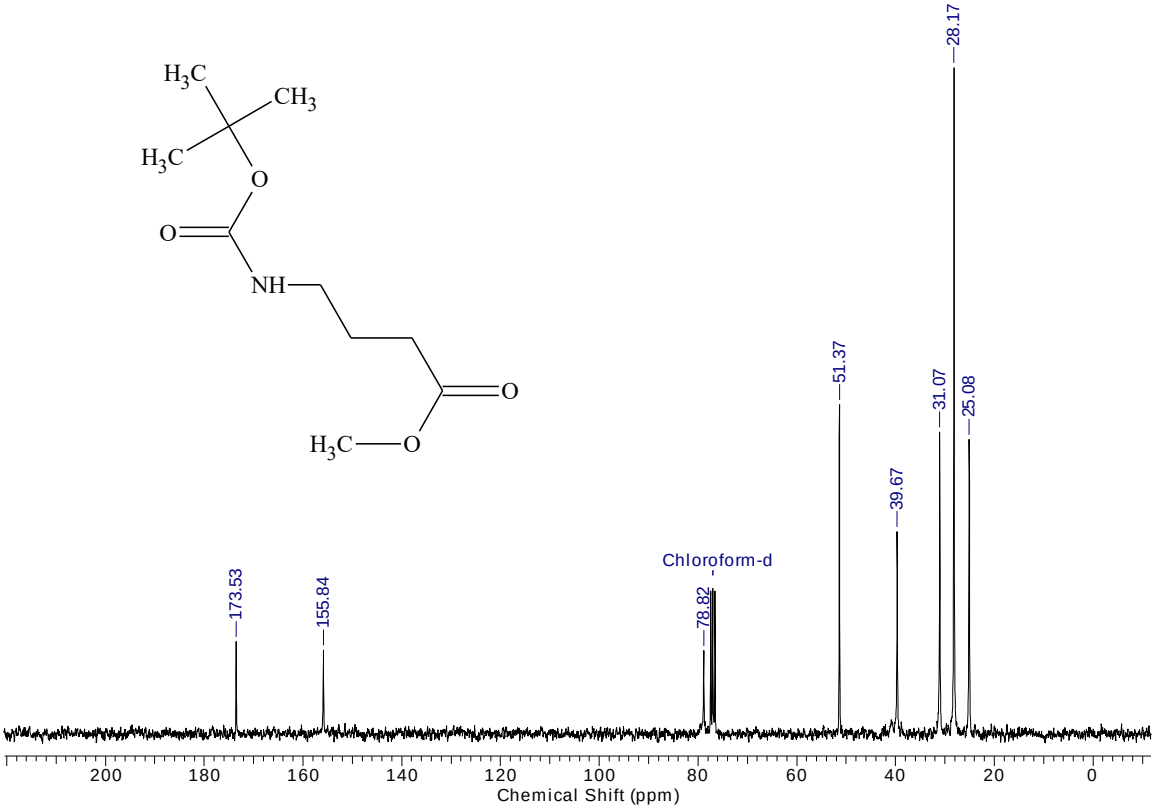
^{13}C NMR spectrum of N-Boc-β-alanine methyl ester



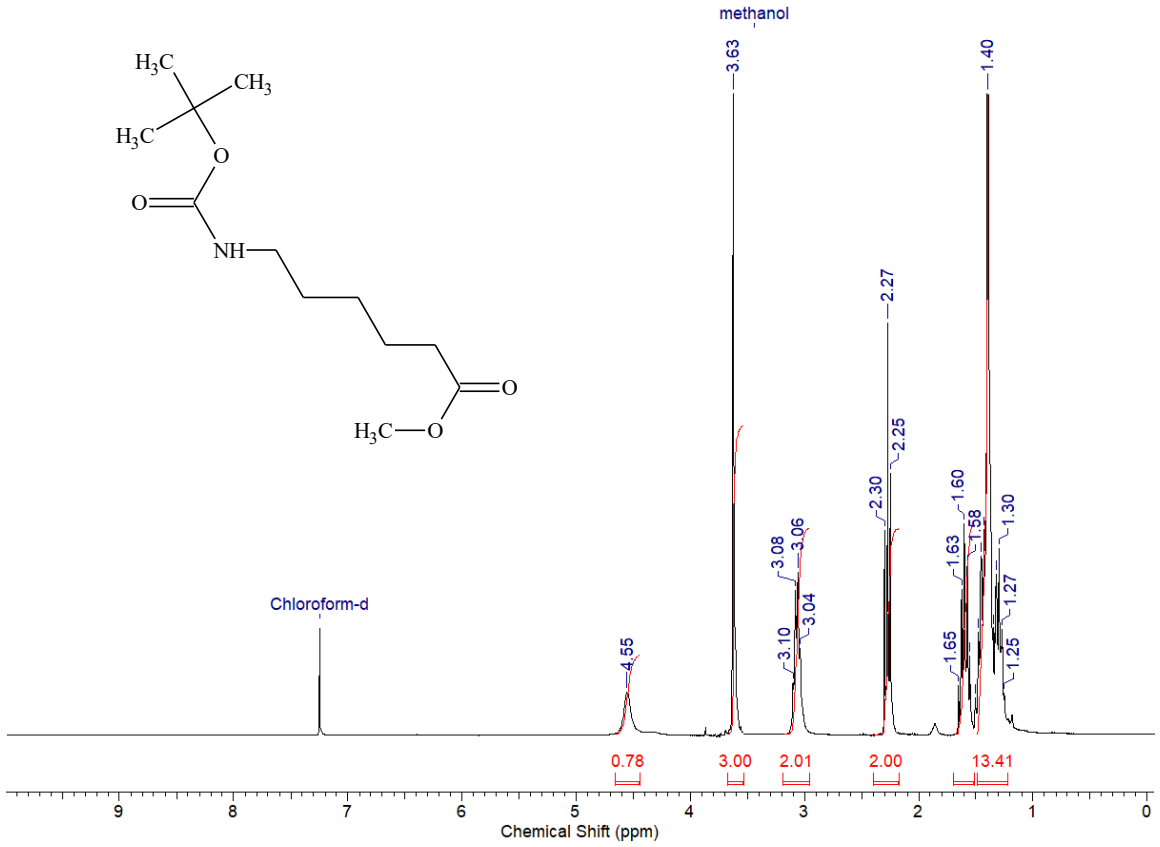
^{1}H NMR spectrum of N-Boc-4-aminobutyric acid methyl ester



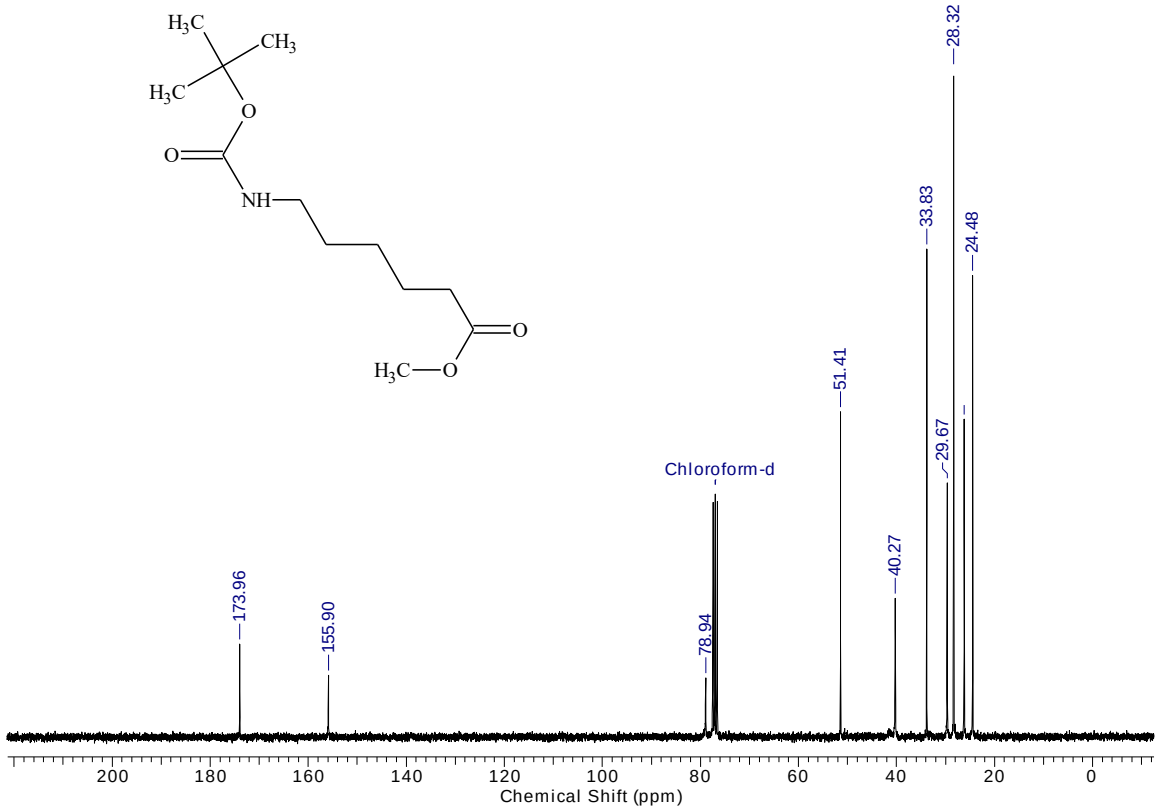
^{13}C NMR spectrum of N-Boc-4-aminobutyric acid methyl ester



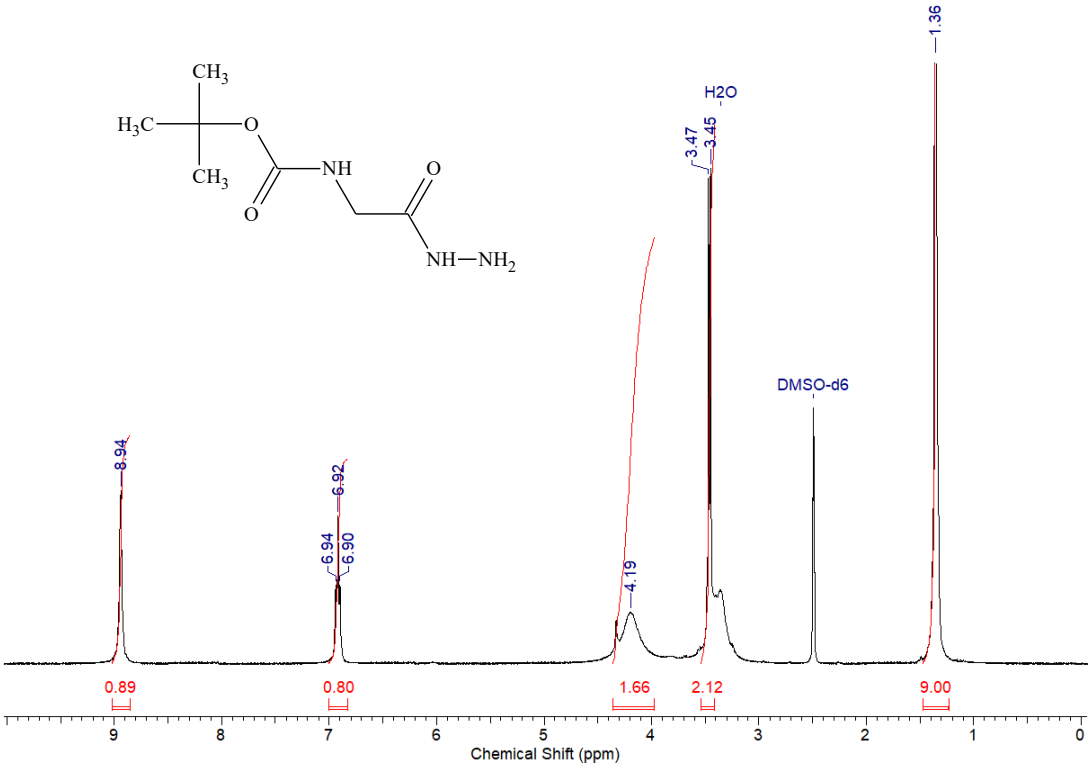
^{1}H NMR spectrum of N-Boc-6-aminohexanoic acid methyl ester



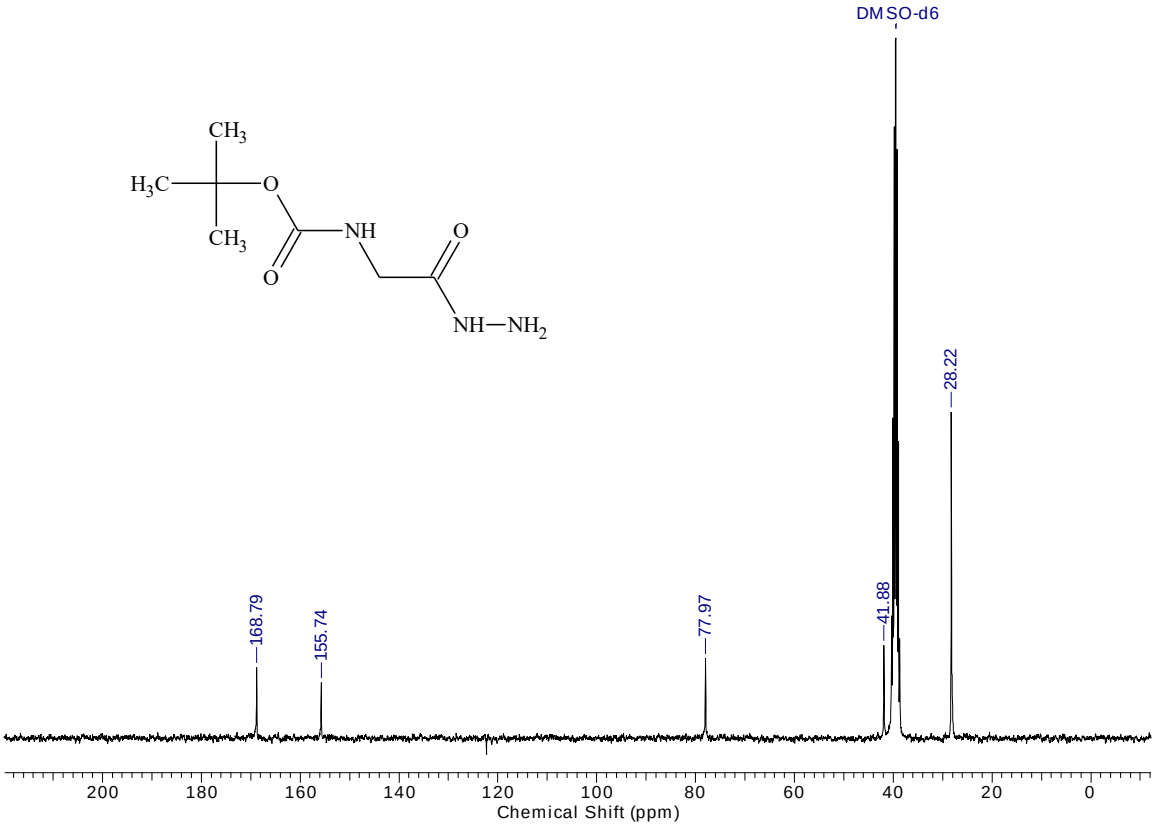
^{13}C NMR spectrum of N-Boc-6-aminohexanoic acid methyl ester



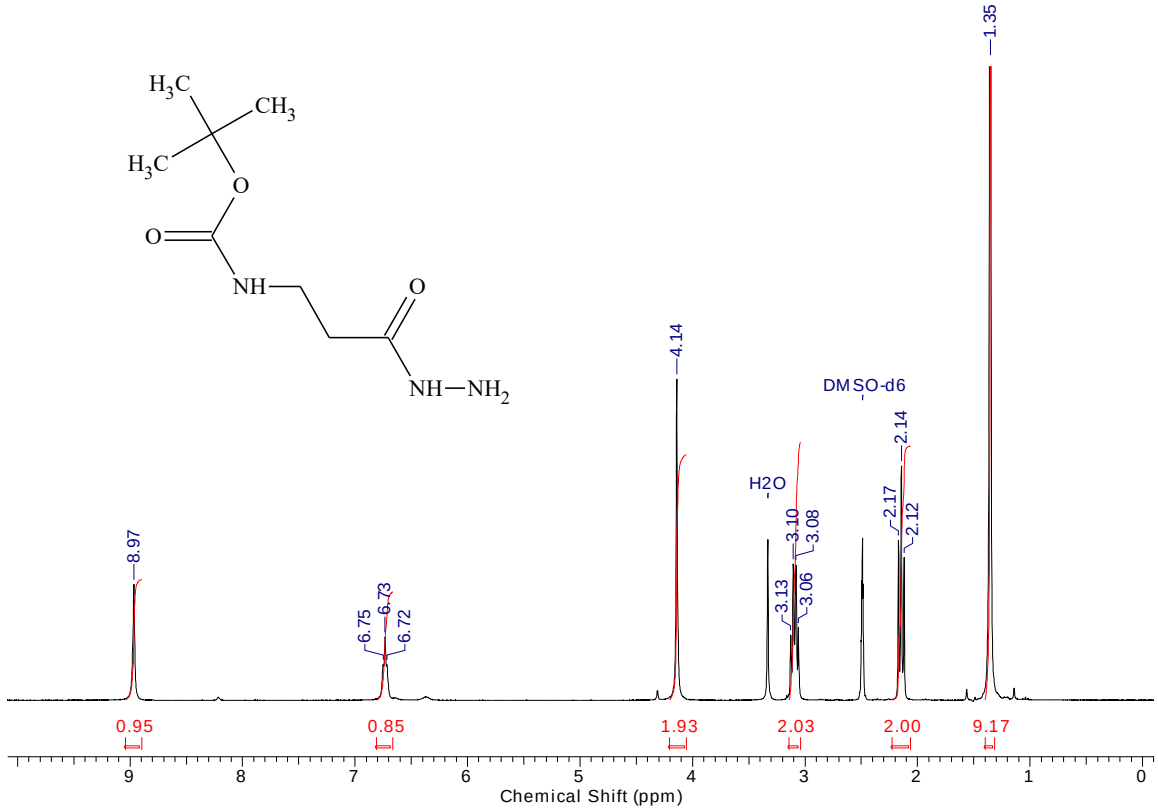
*^{1}H NMR spectrum of **3a***



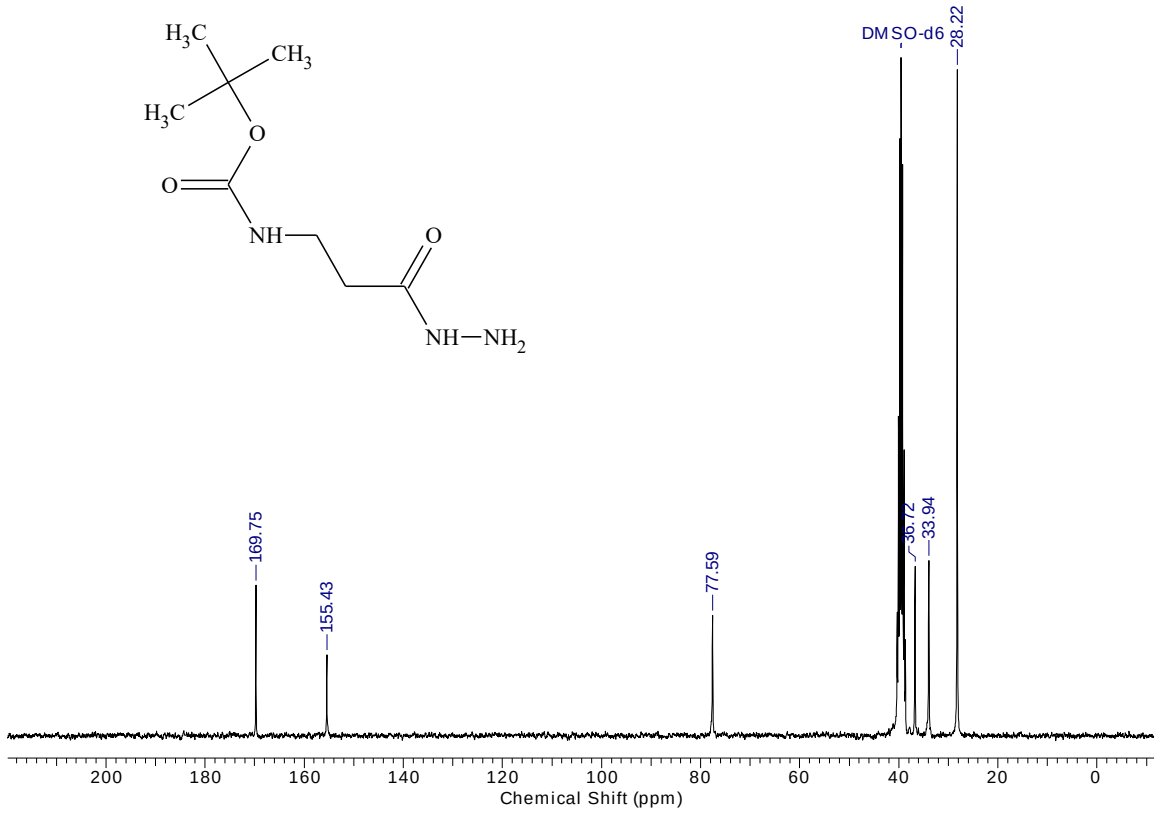
*^{13}C NMR spectrum of **3a***



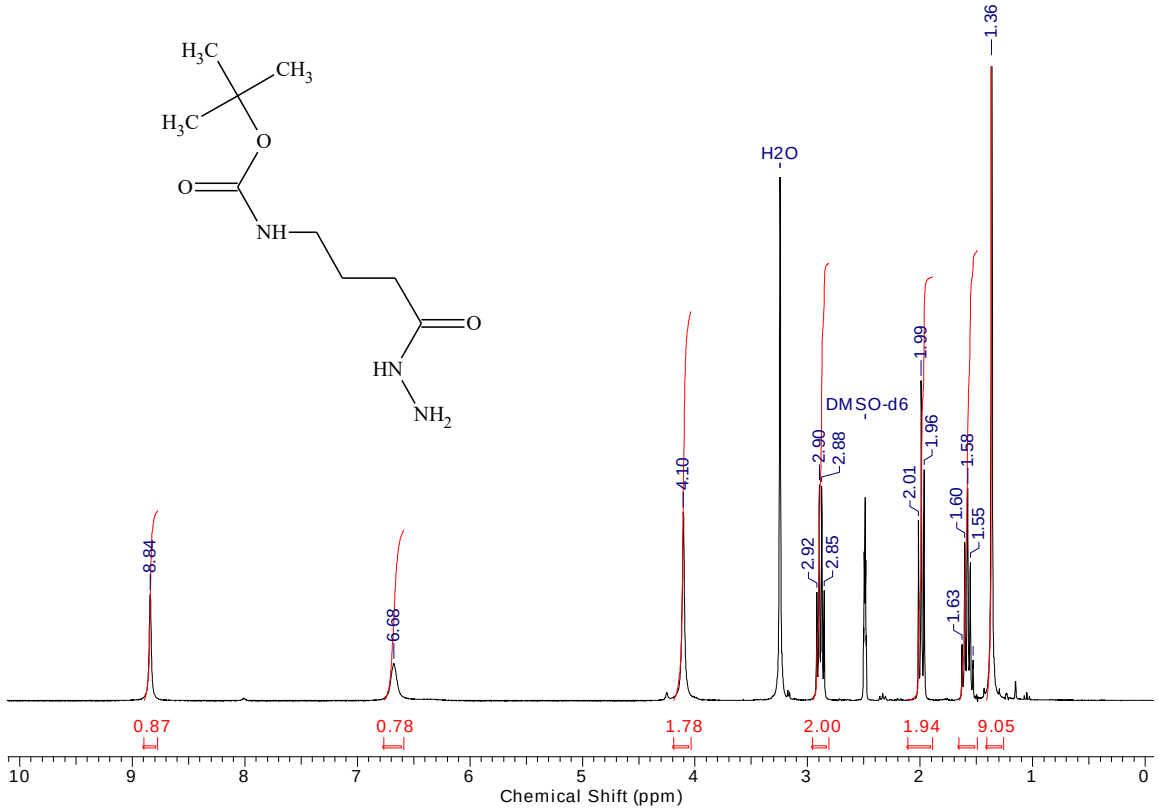
*^{1}H NMR spectrum of **3b***



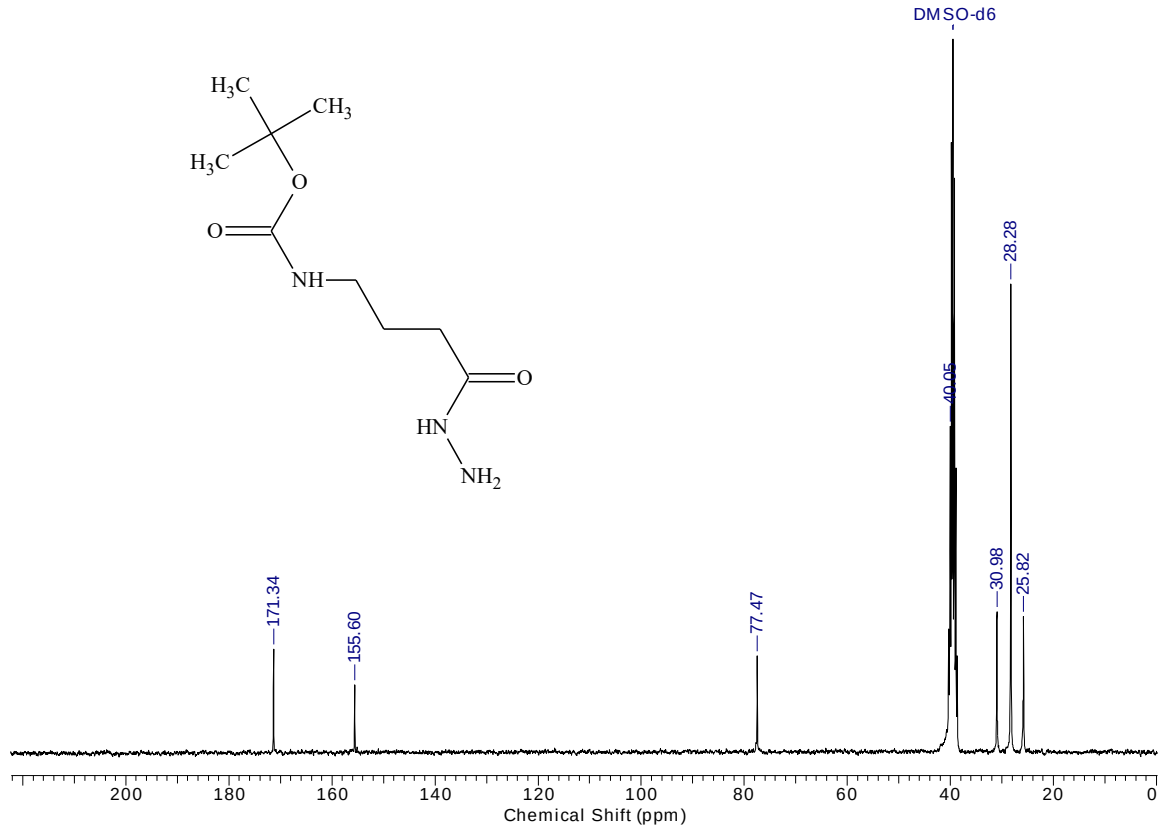
*^{13}C NMR spectrum of **3b***



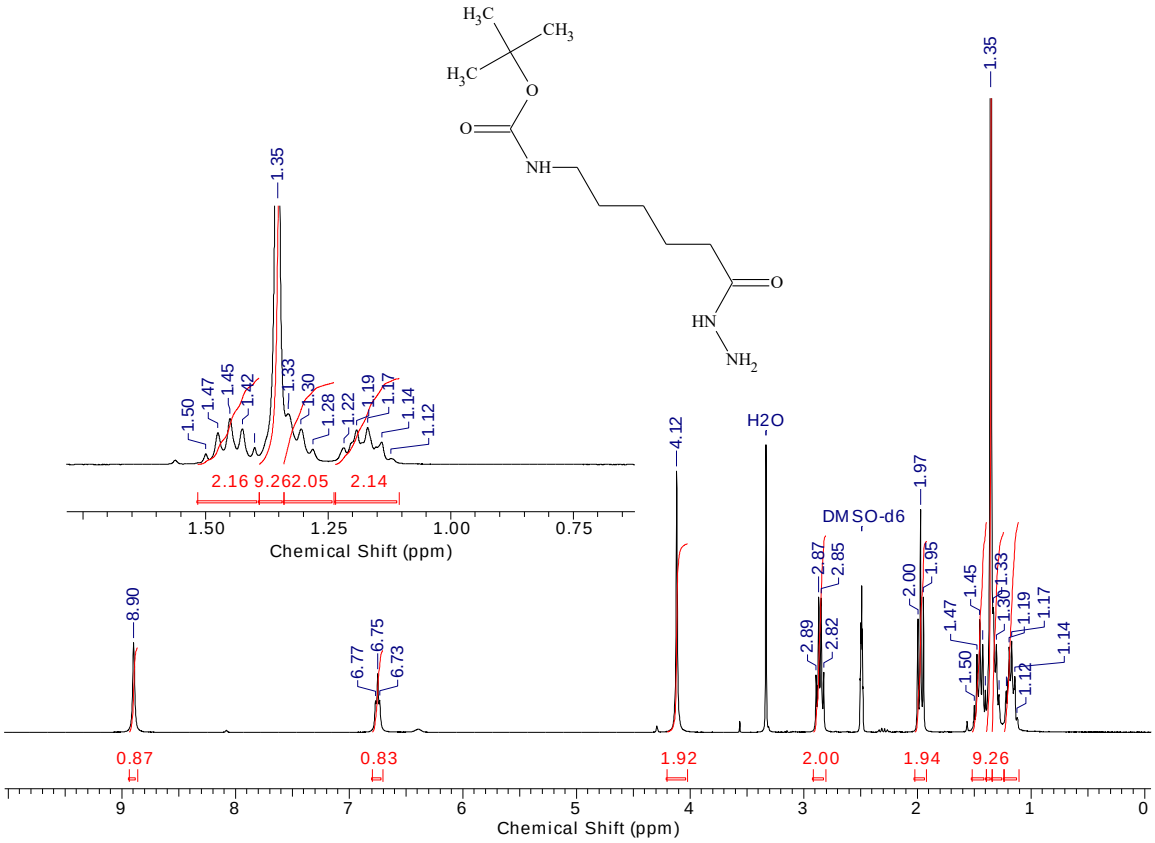
*^{1}H NMR spectrum of **3c***



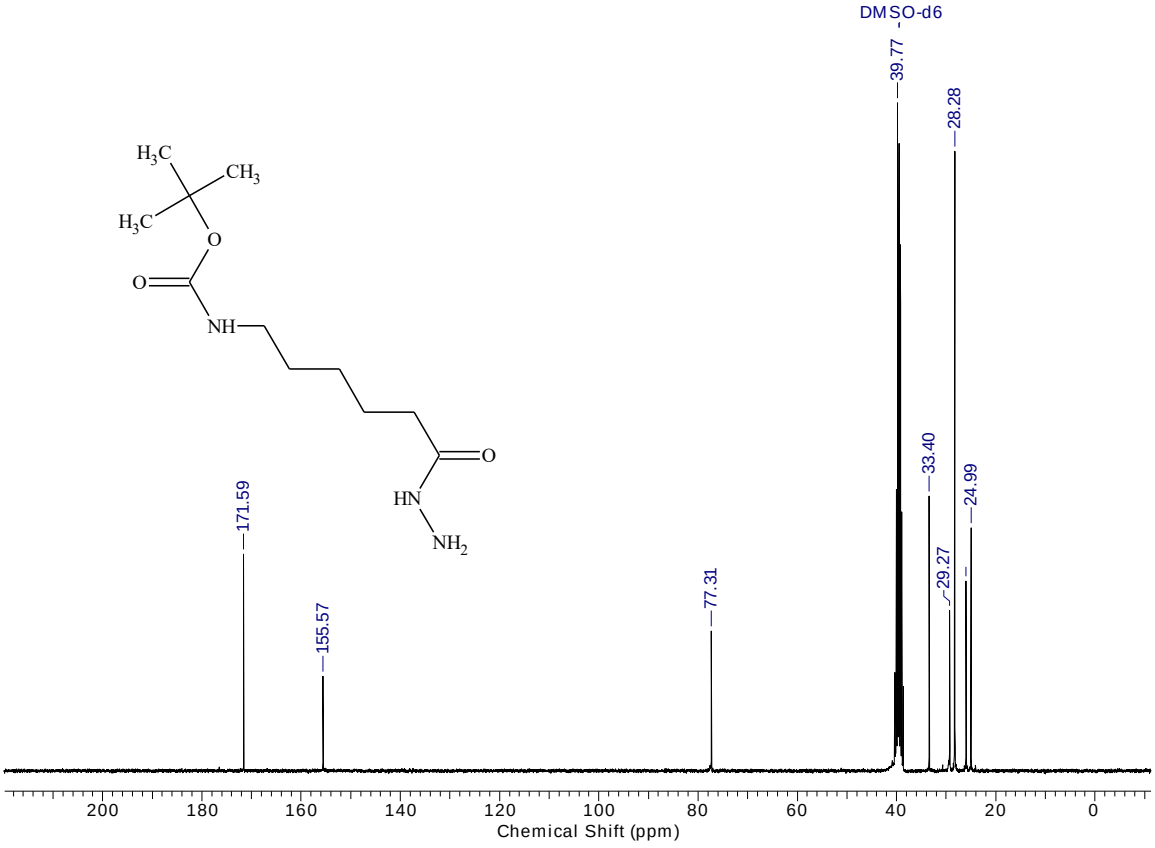
*^{13}C NMR spectrum of **3c***



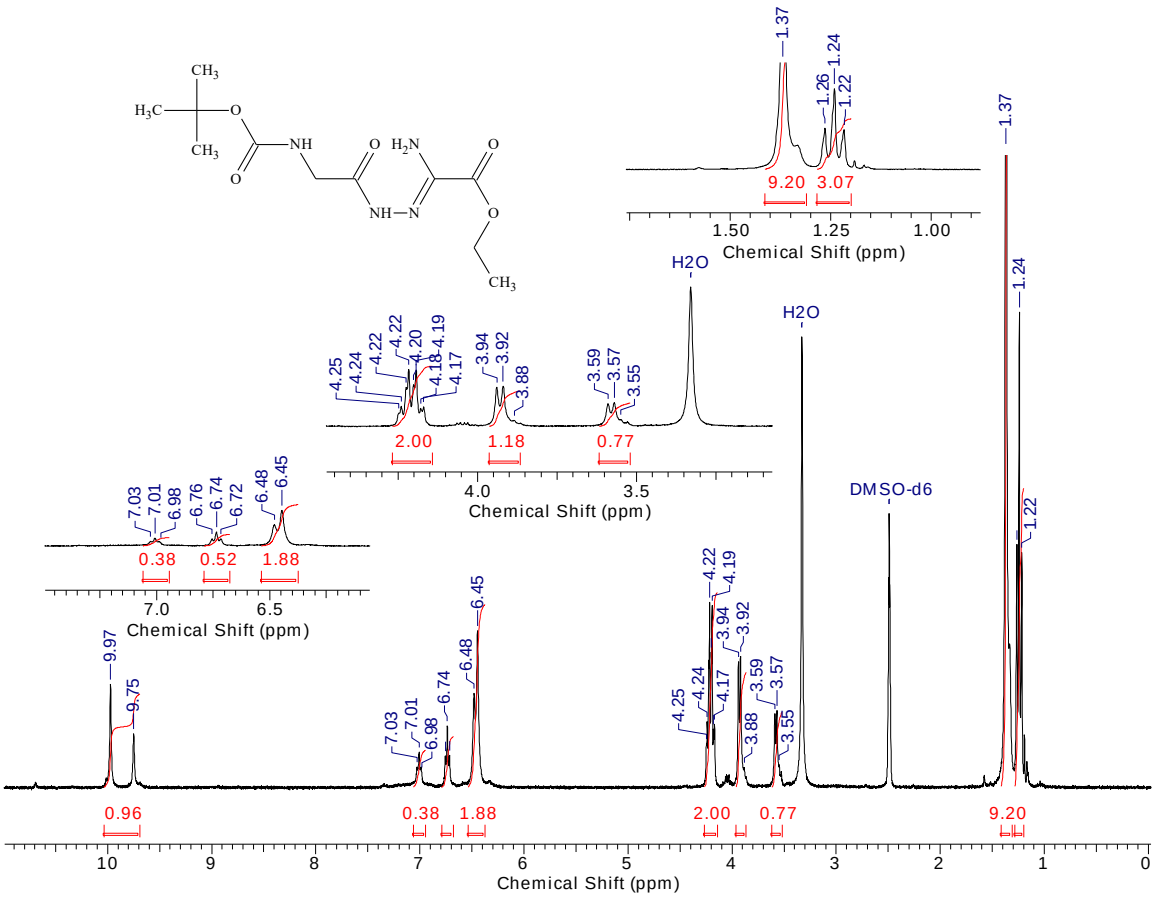
*[1]H NMR spectrum of **3d***



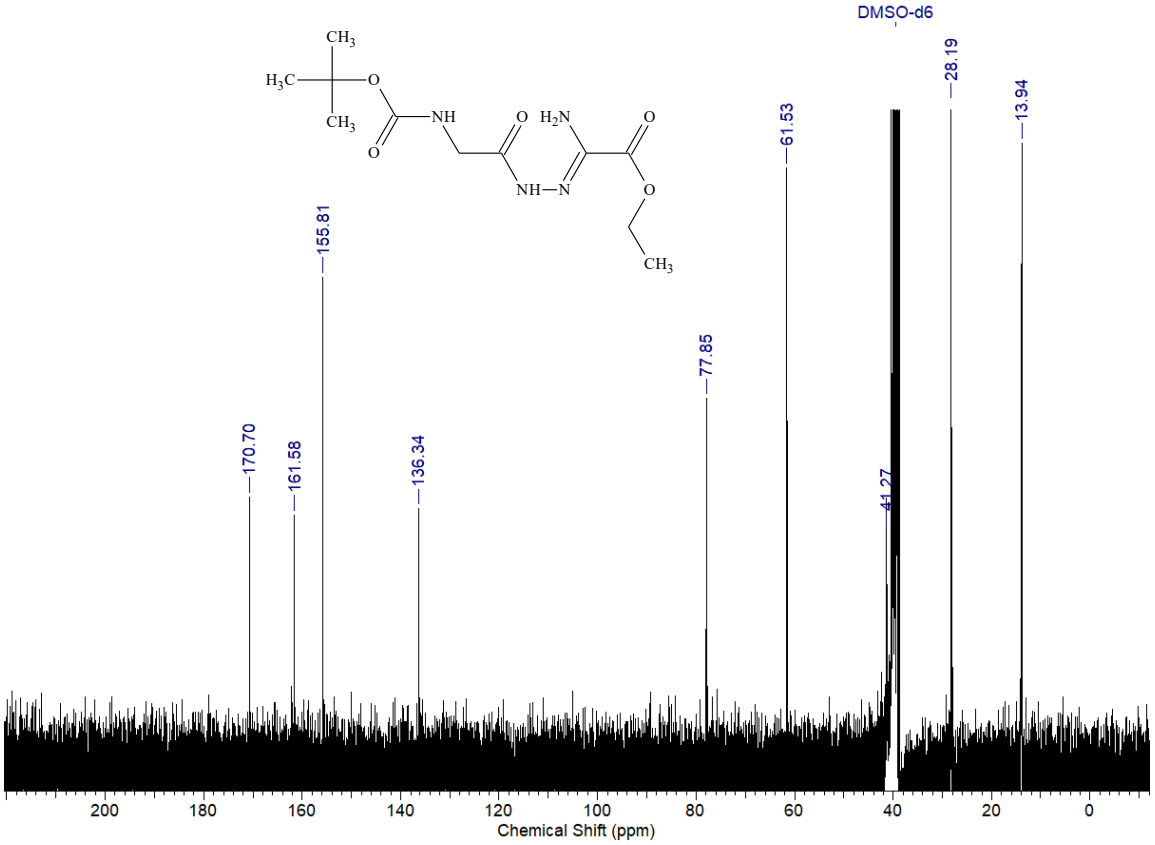
*[13]C NMR spectrum of **3d***



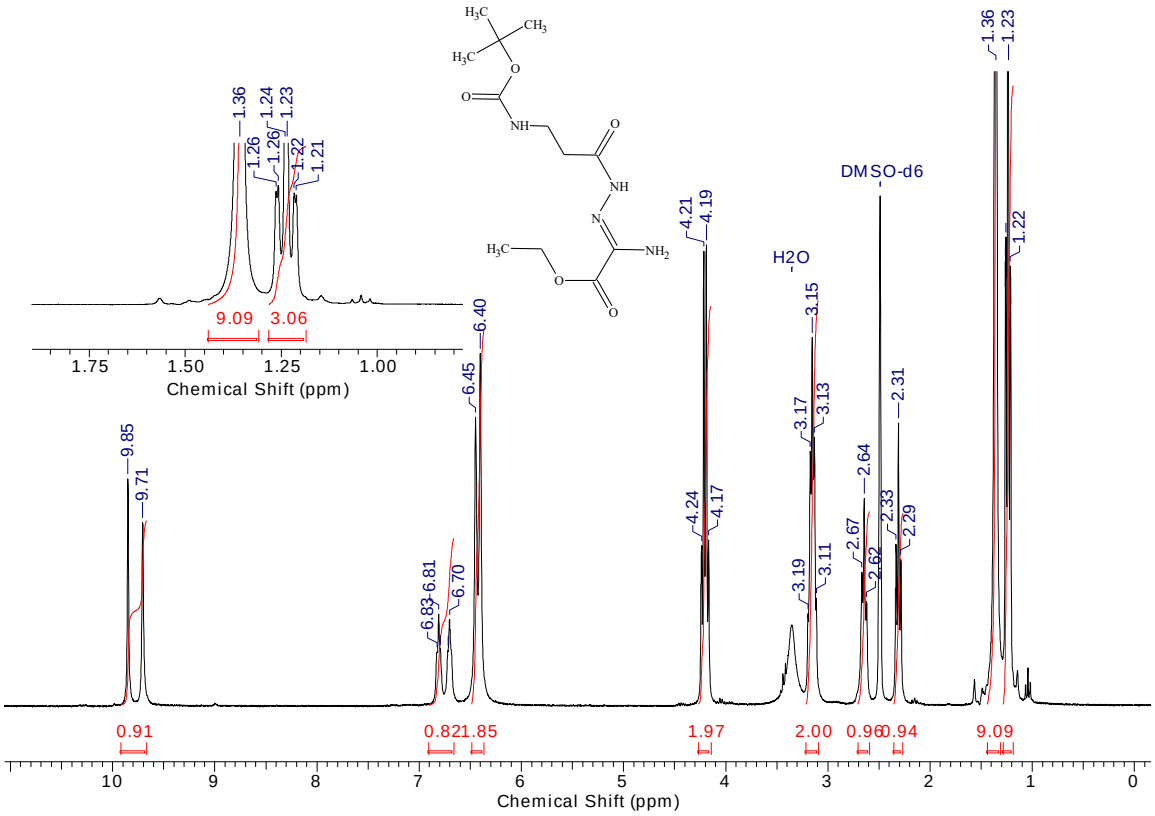
*^{1}H NMR spectrum of **4a***



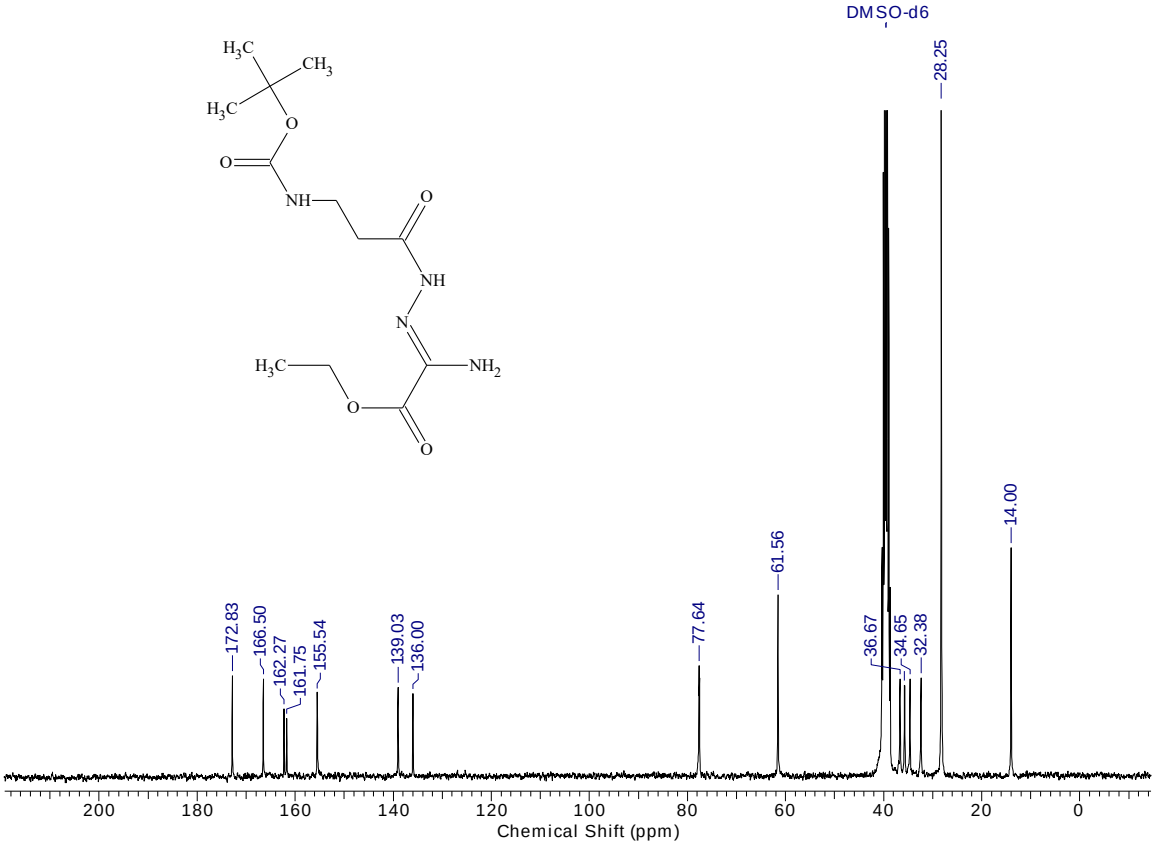
*^{13}C NMR spectrum of **4a***



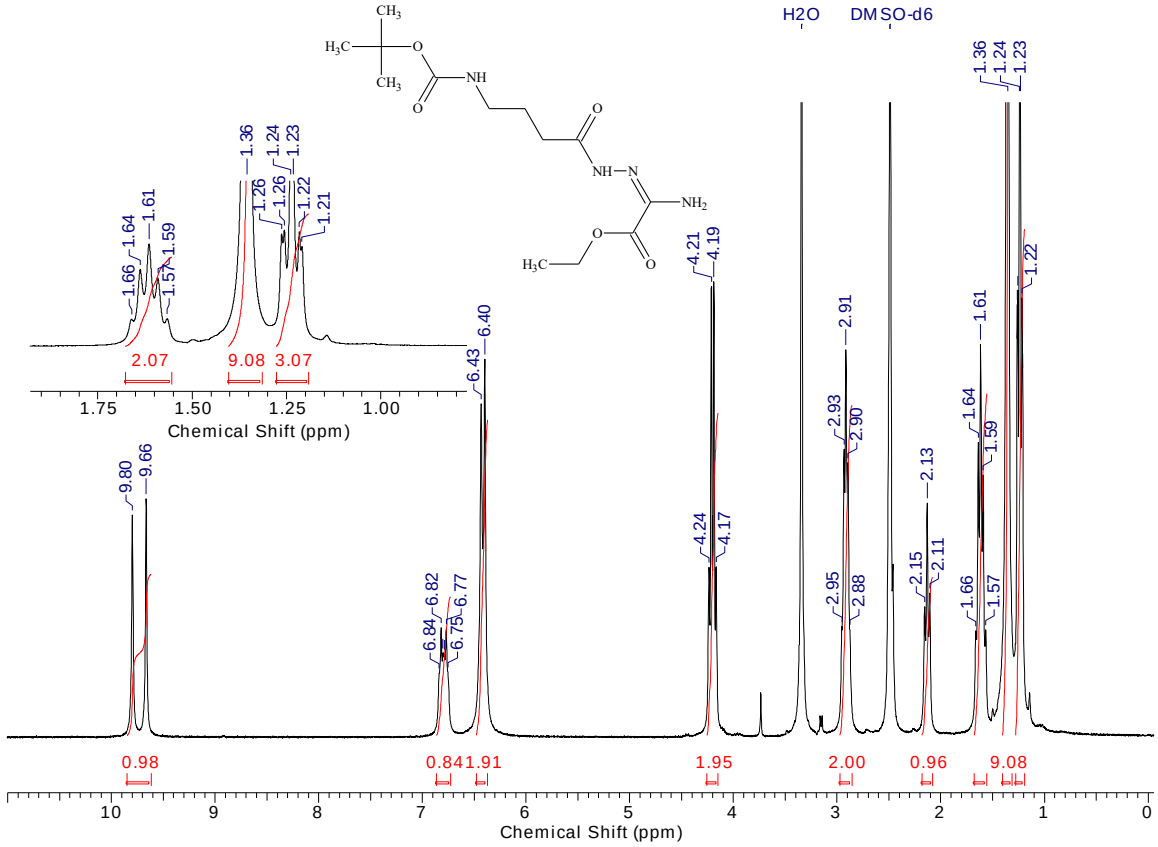
*^{1}H NMR spectrum of **4b***



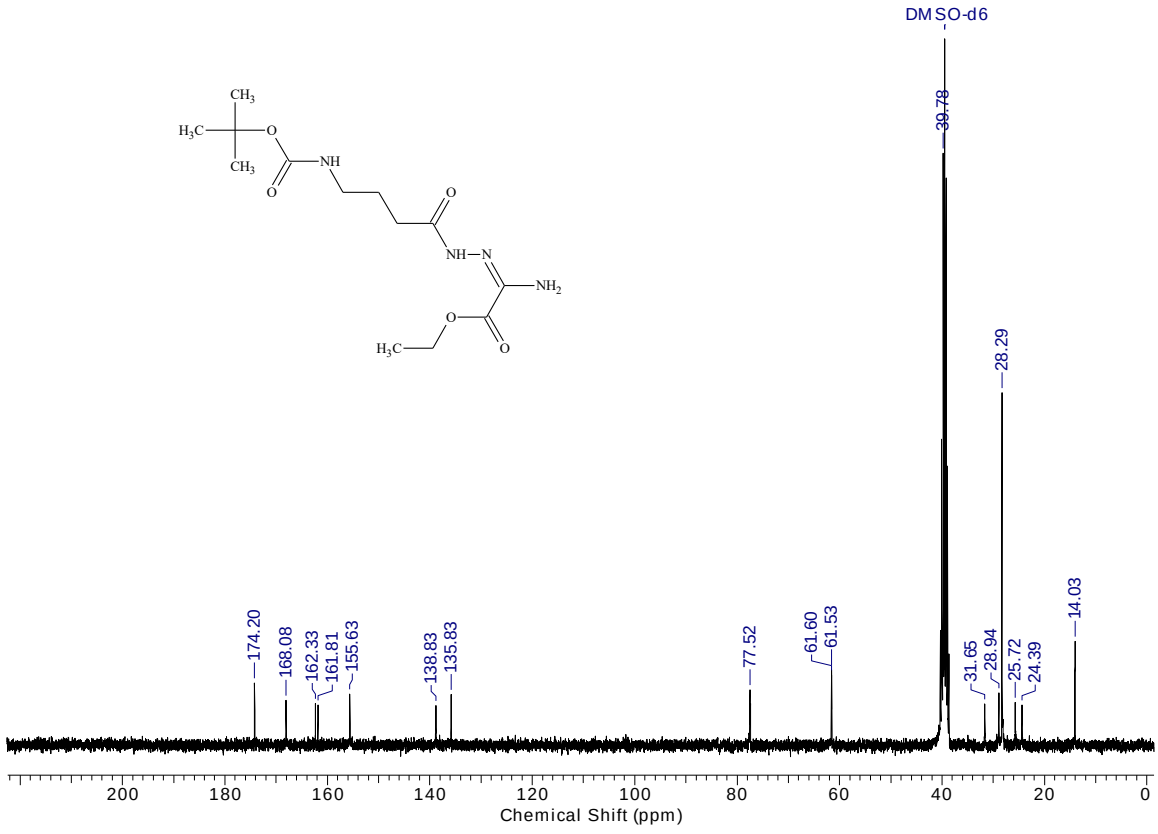
*^{13}C NMR spectrum of **4b***



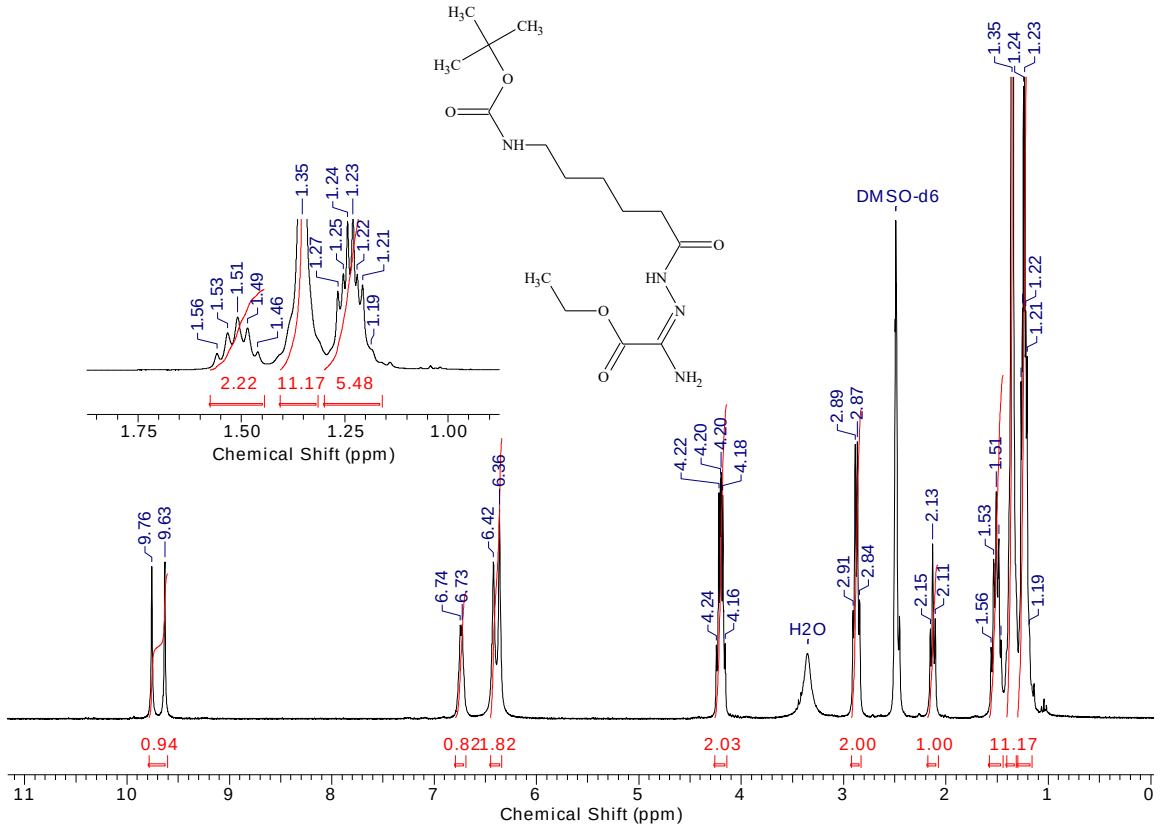
*^{1}H NMR spectrum of **4c***



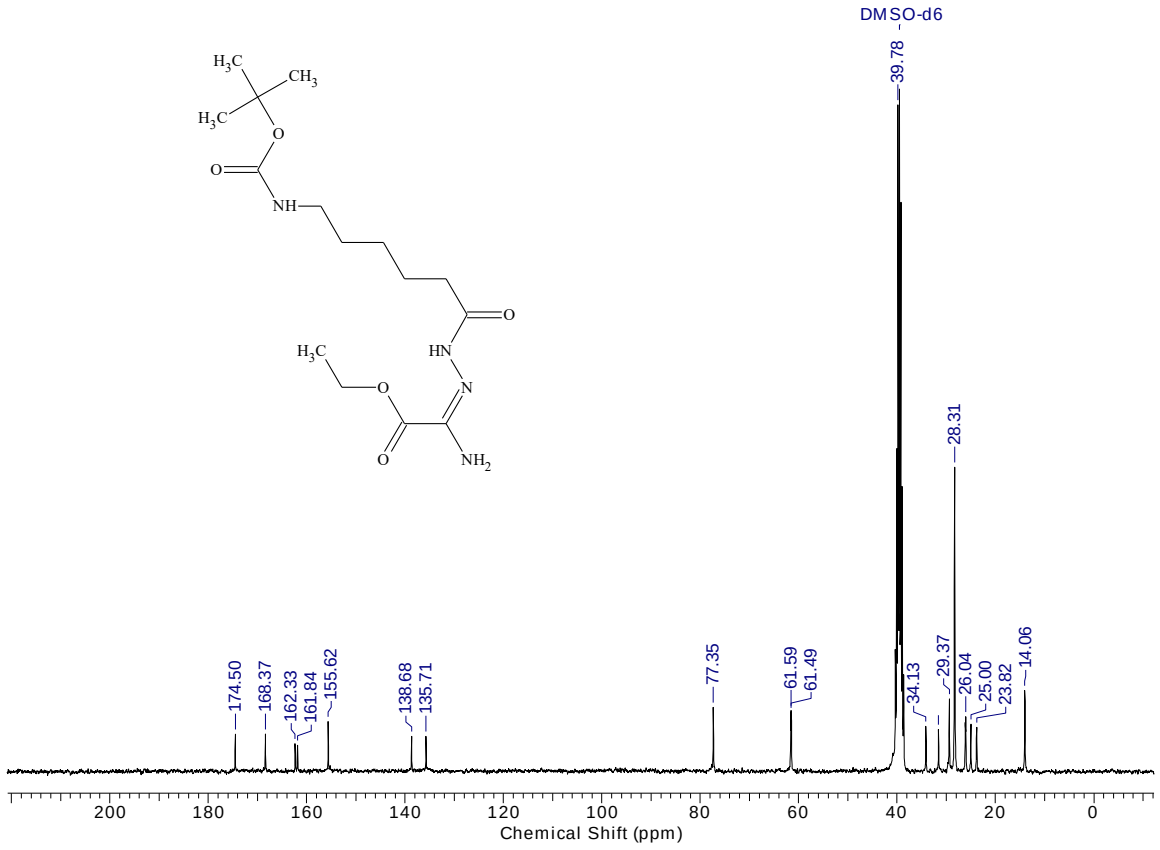
*^{13}C NMR spectrum of **4c***



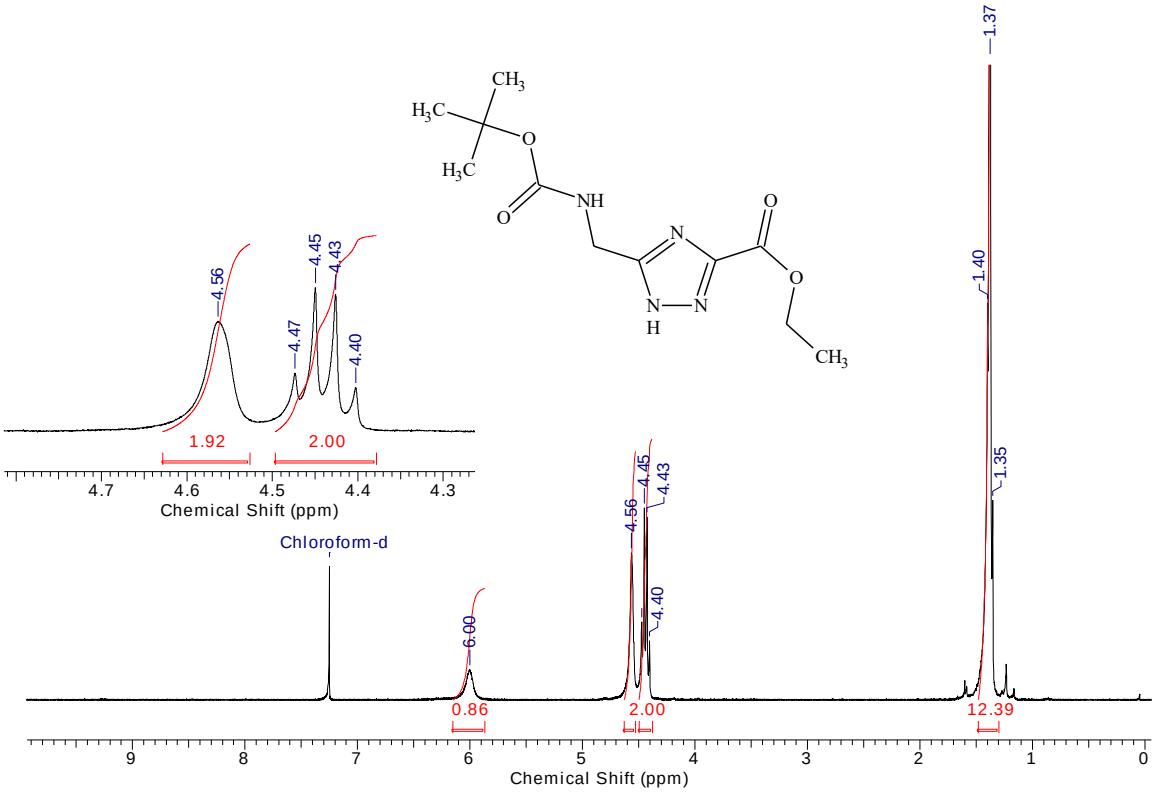
*^{1}H NMR spectrum of **4d***



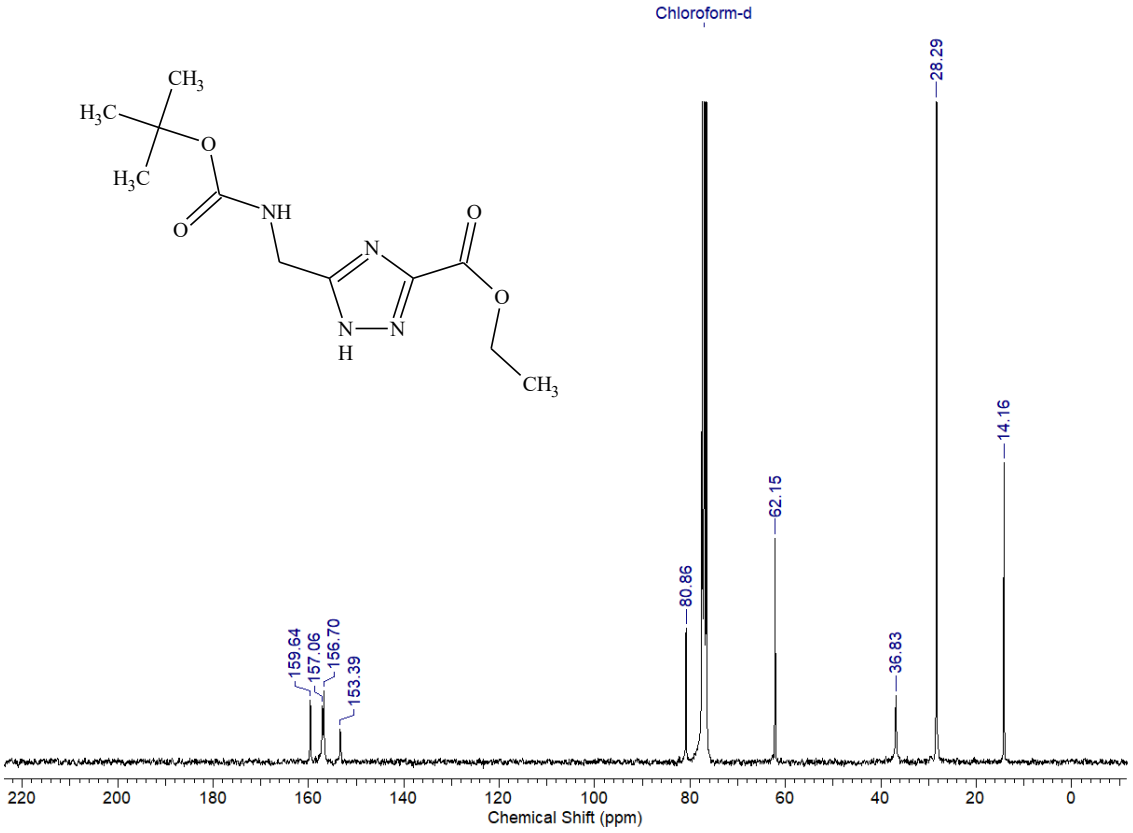
*^{13}C NMR spectrum of **4d***



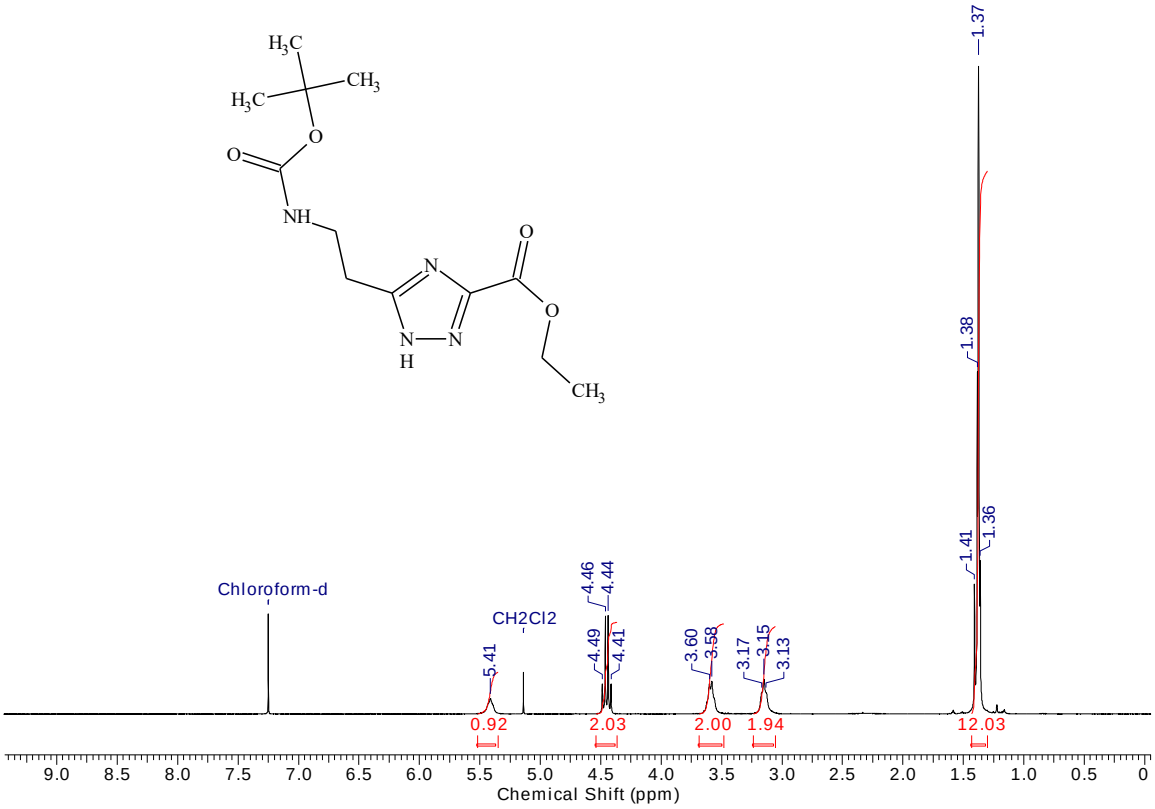
*^{1}H NMR spectrum of **5a***



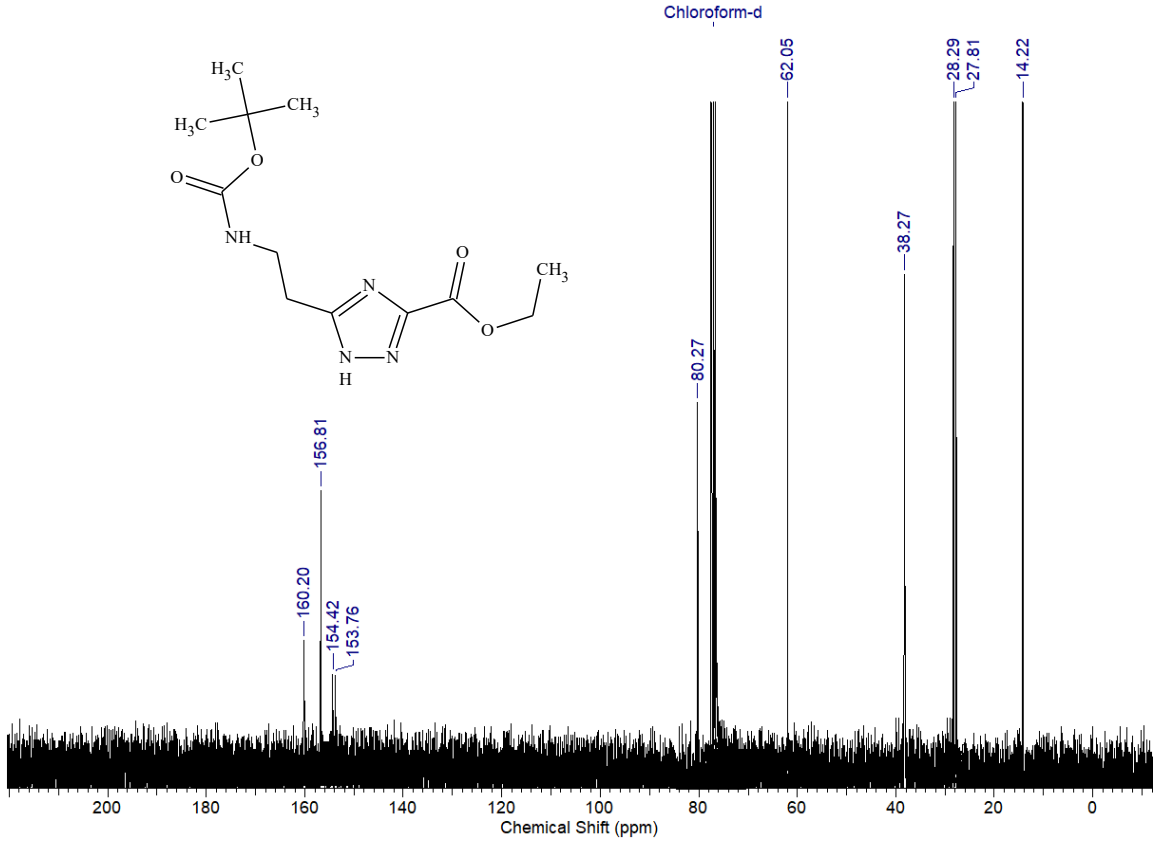
*^{13}C NMR spectrum of **5a***



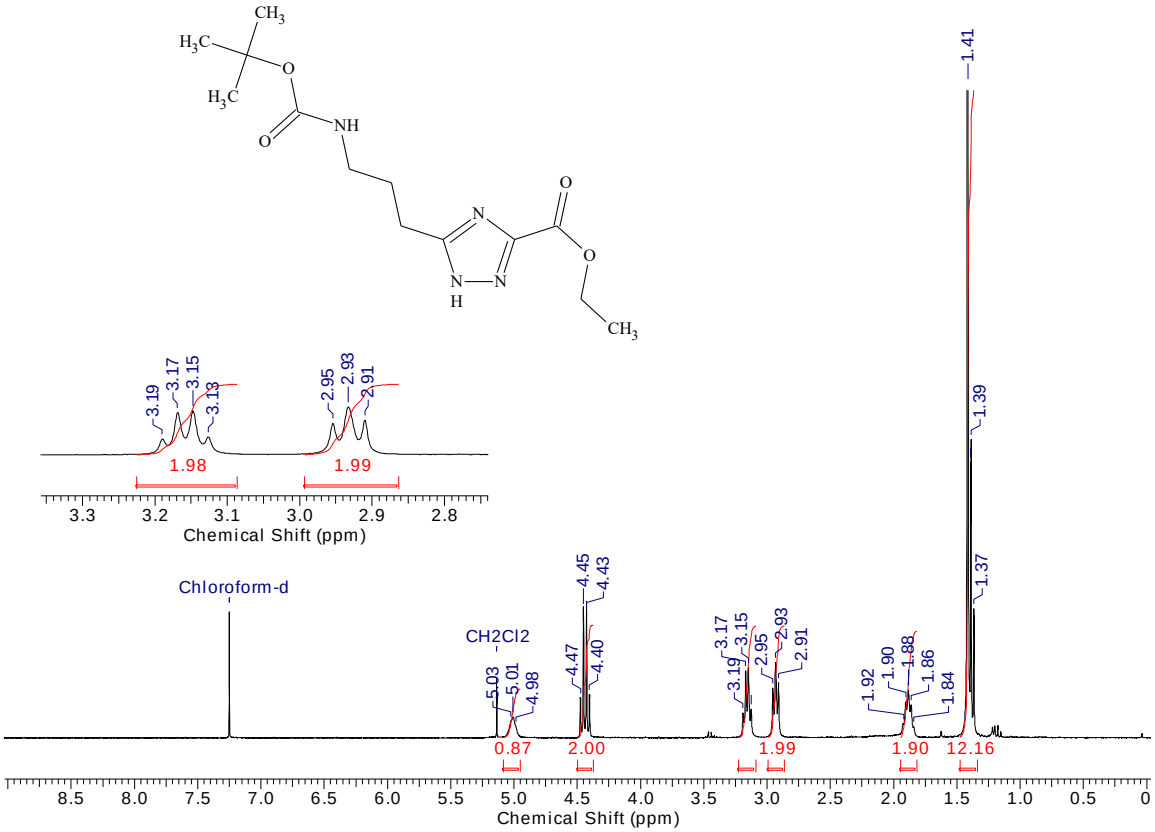
*^{1}H NMR spectrum of **5b***



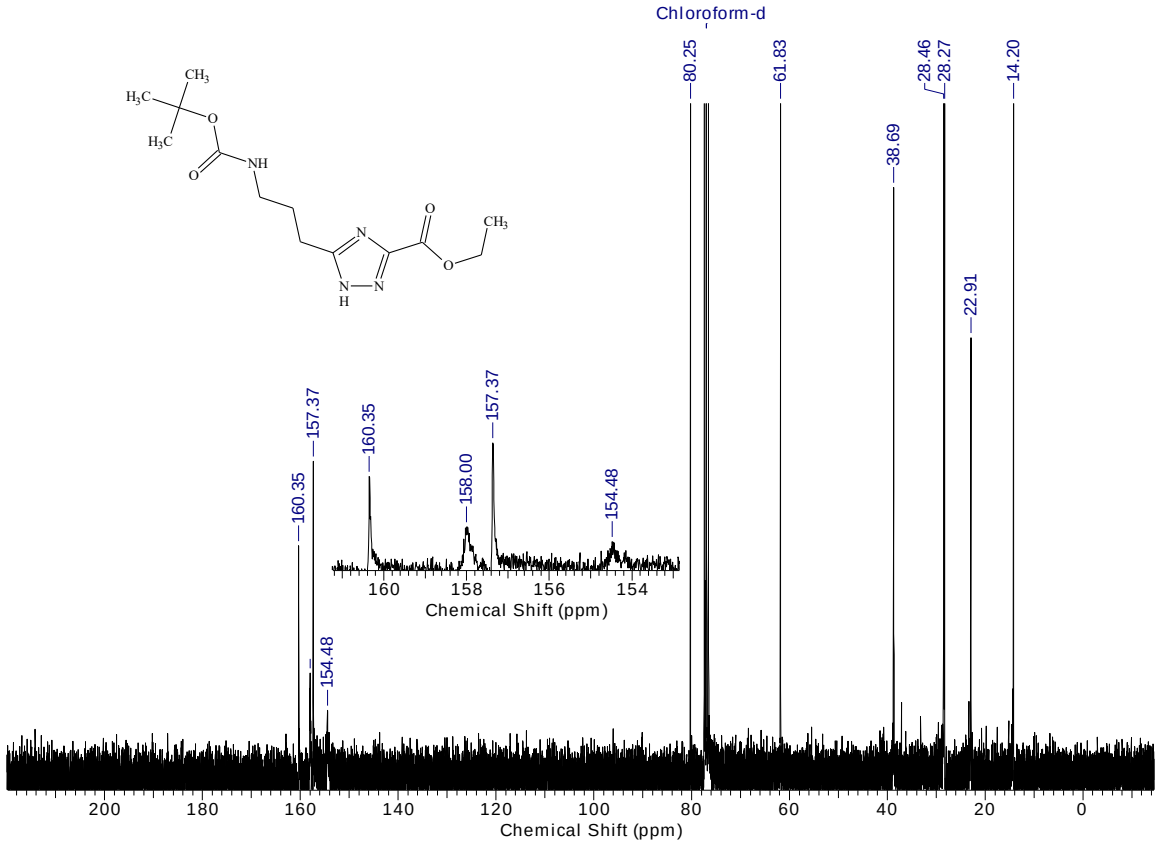
*^{13}C NMR spectrum of **5b***



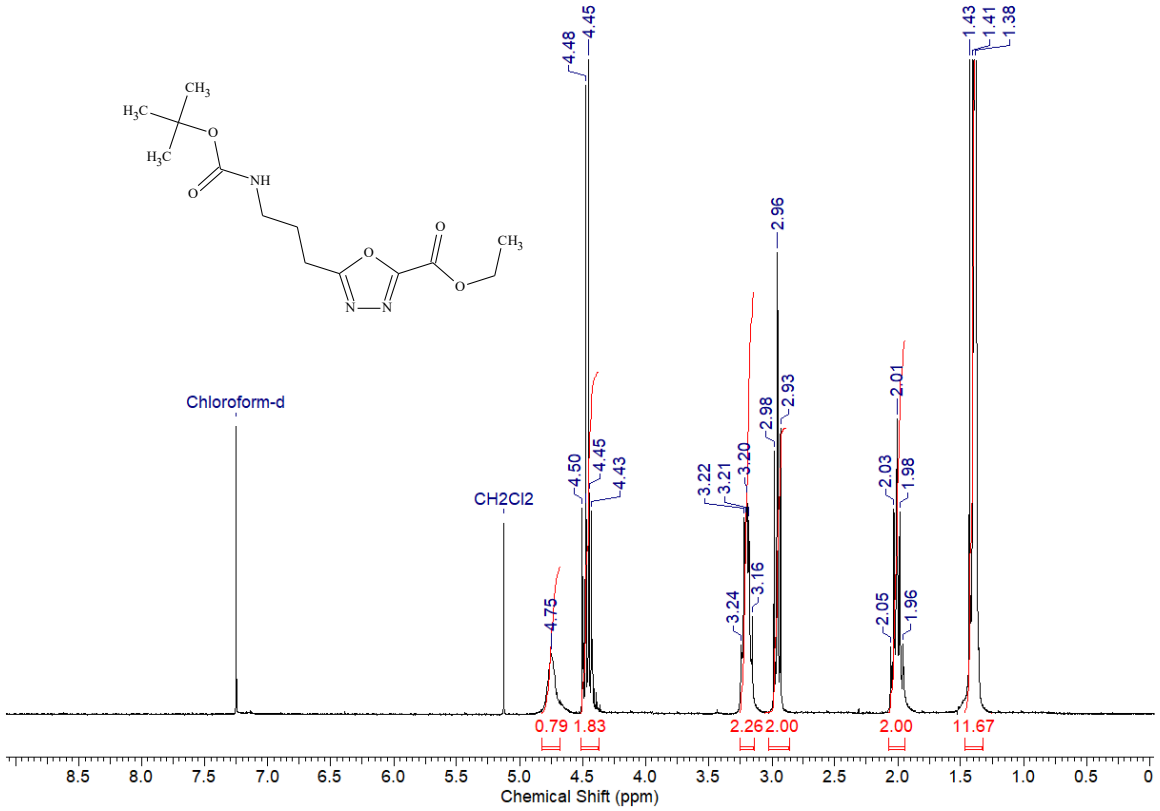
*^{1}H NMR spectrum of **5c***



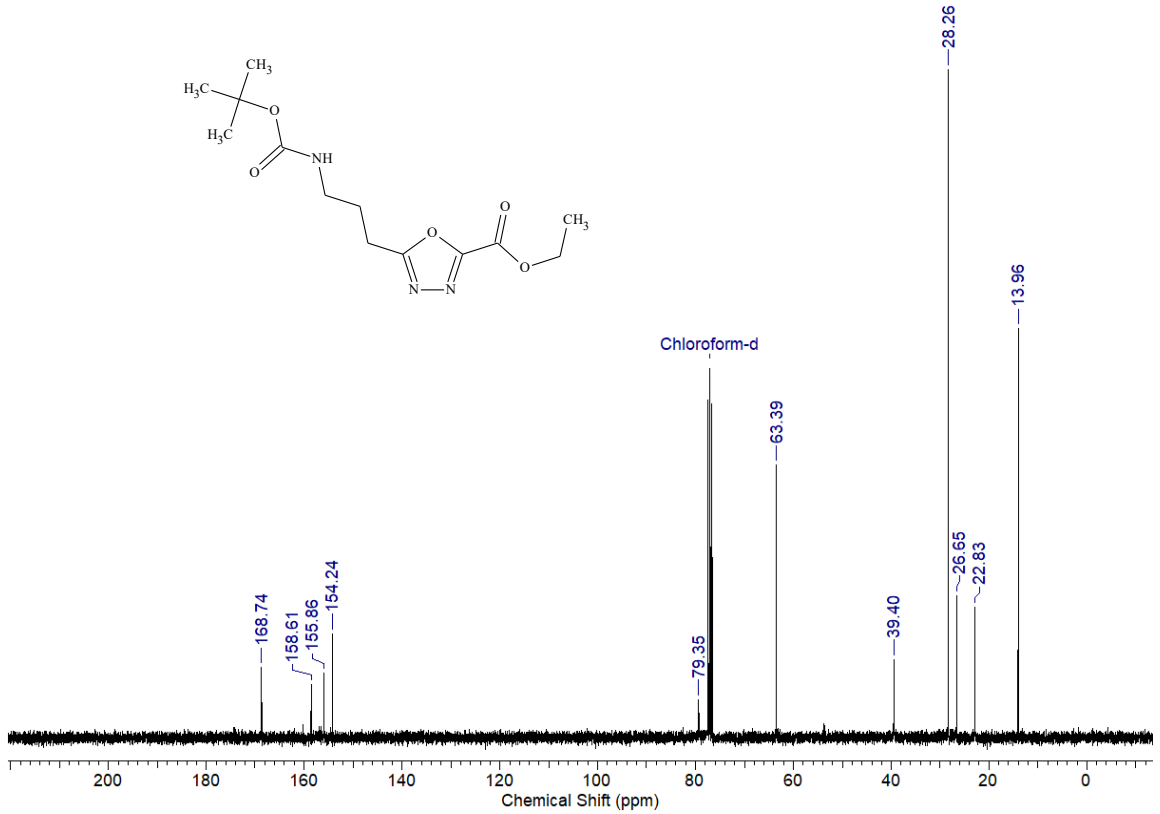
*^{13}C NMR spectrum of **5c***



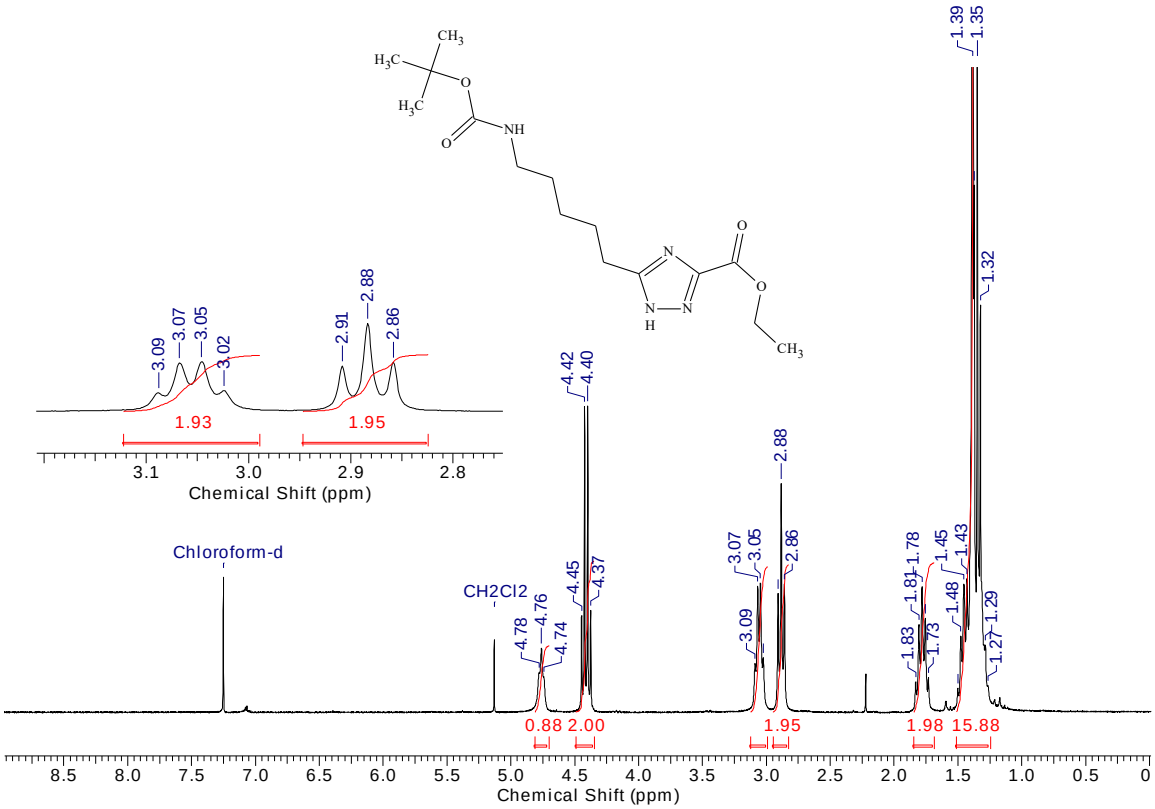
*^{1}H NMR spectrum of **5'c***



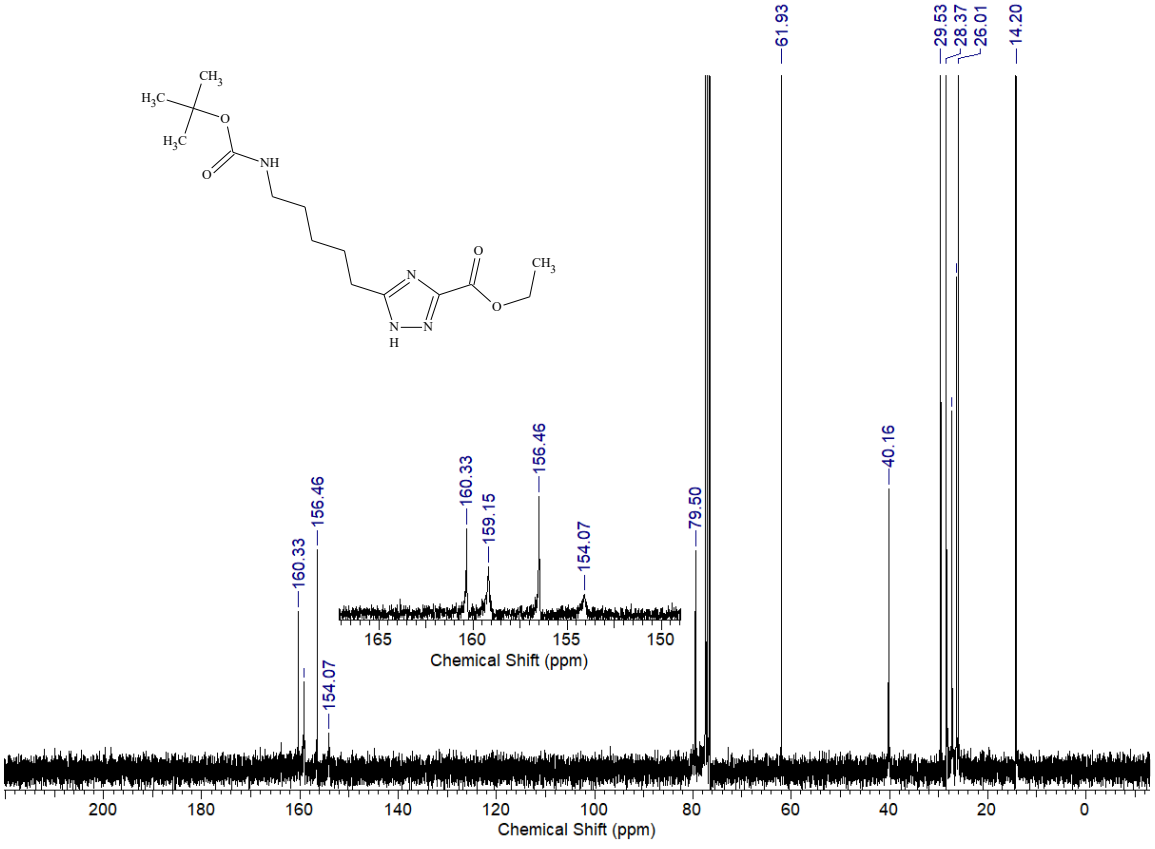
*^{13}C NMR spectrum of **5'c***



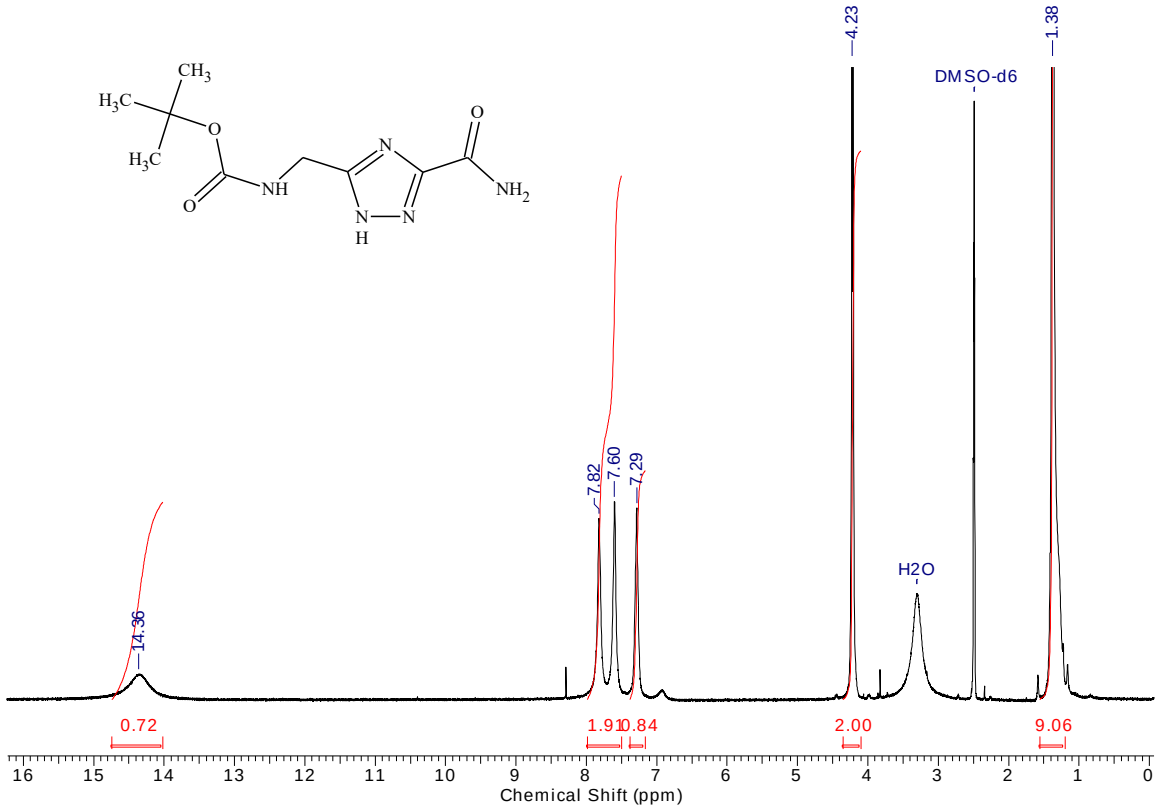
*^{1}H NMR spectrum of **5d***



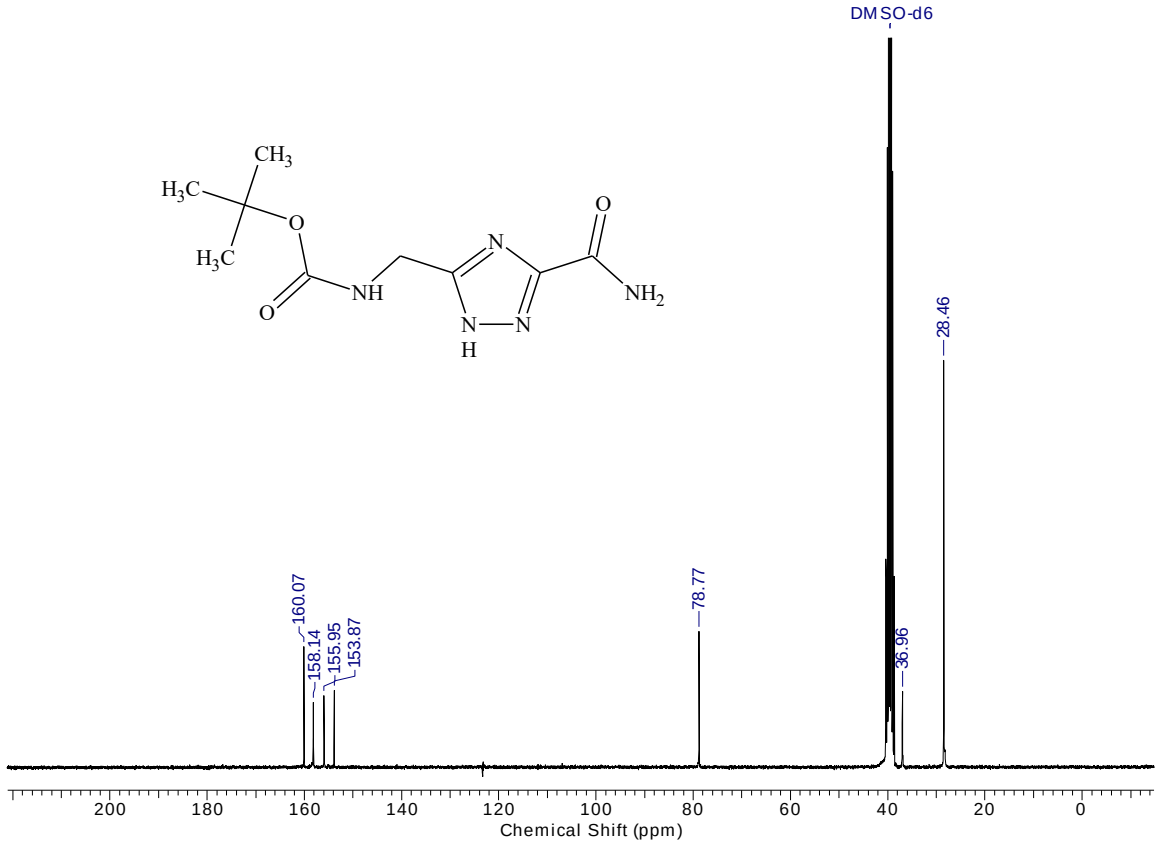
*^{13}C NMR spectrum of **5d***



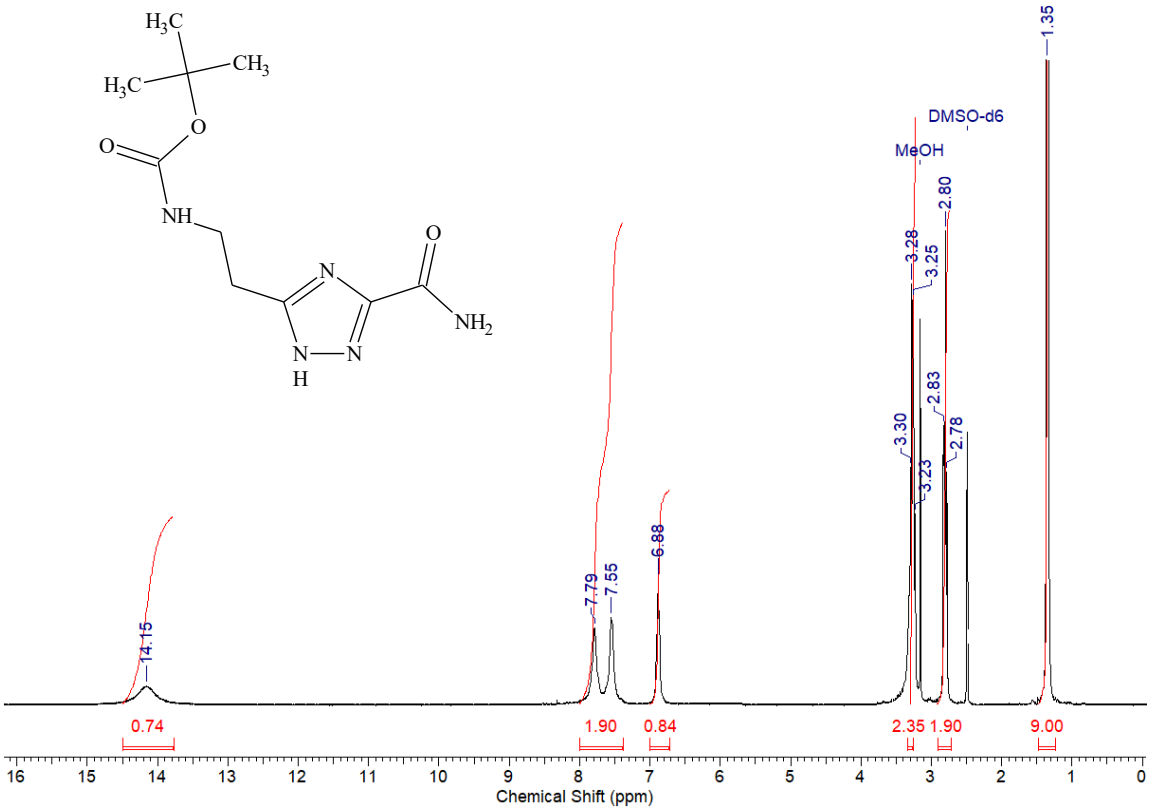
*^{1}H NMR spectrum of **6a***



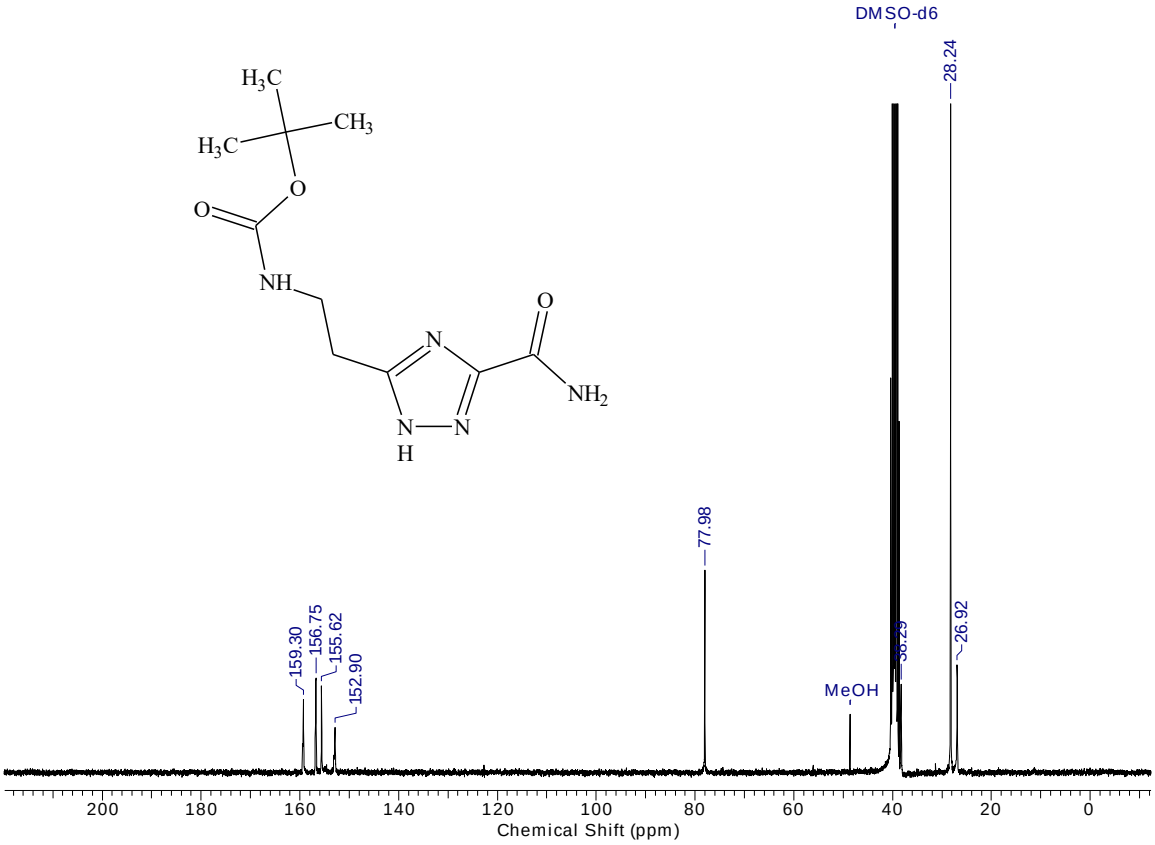
*^{13}C NMR spectrum of **6a***



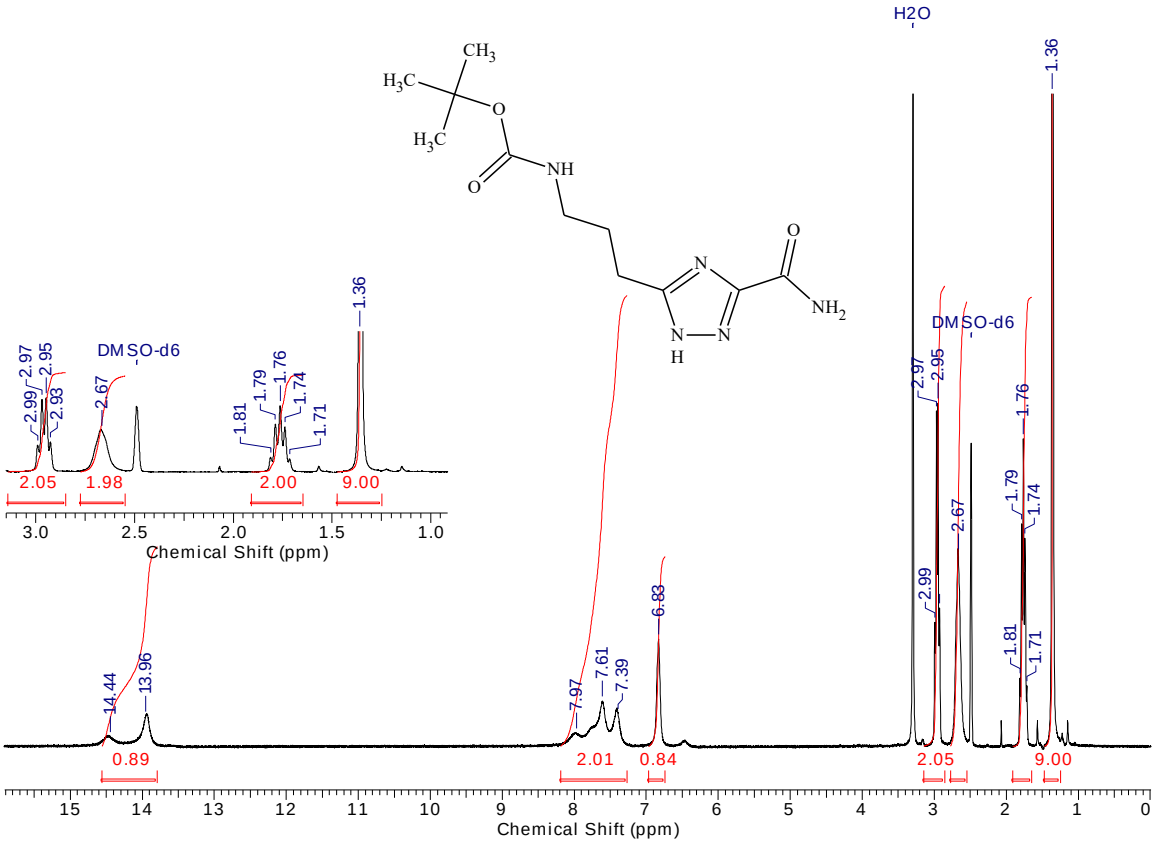
*^{1}H NMR spectrum of **6b***



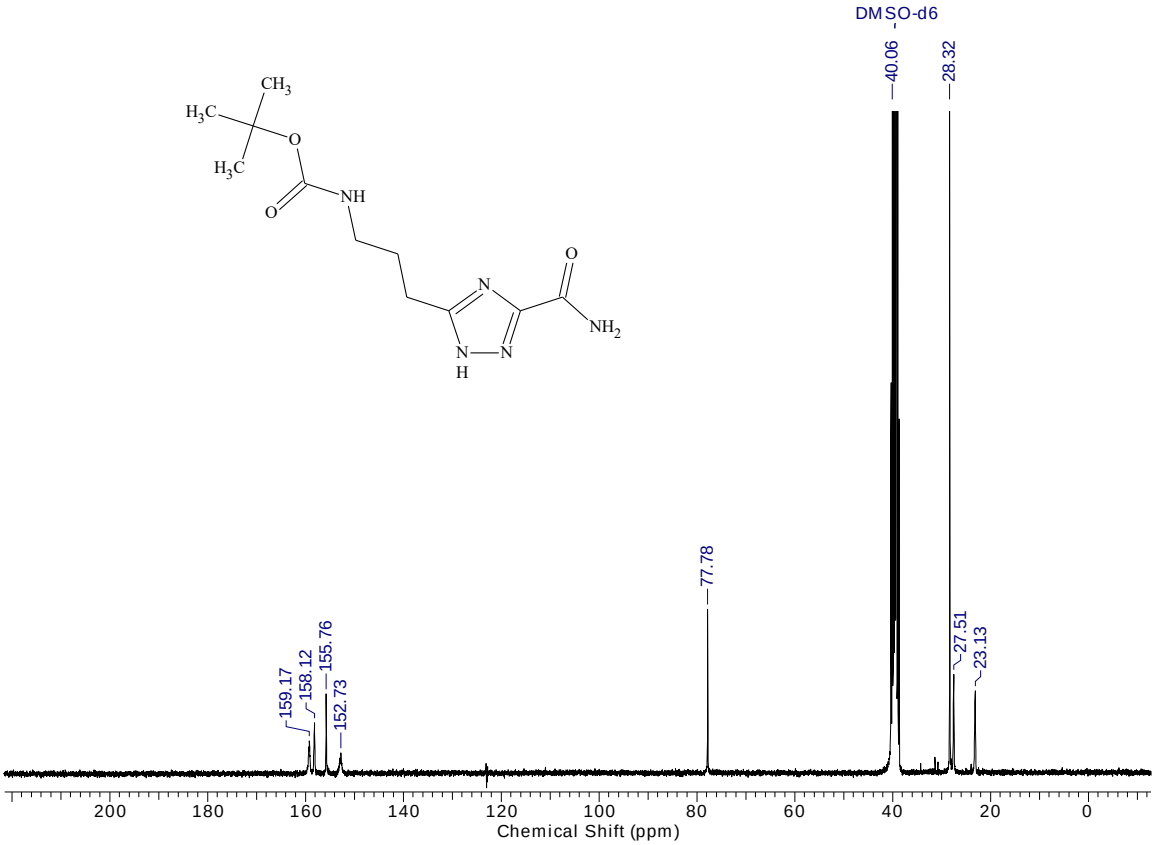
*^{13}C NMR spectrum of **6b***



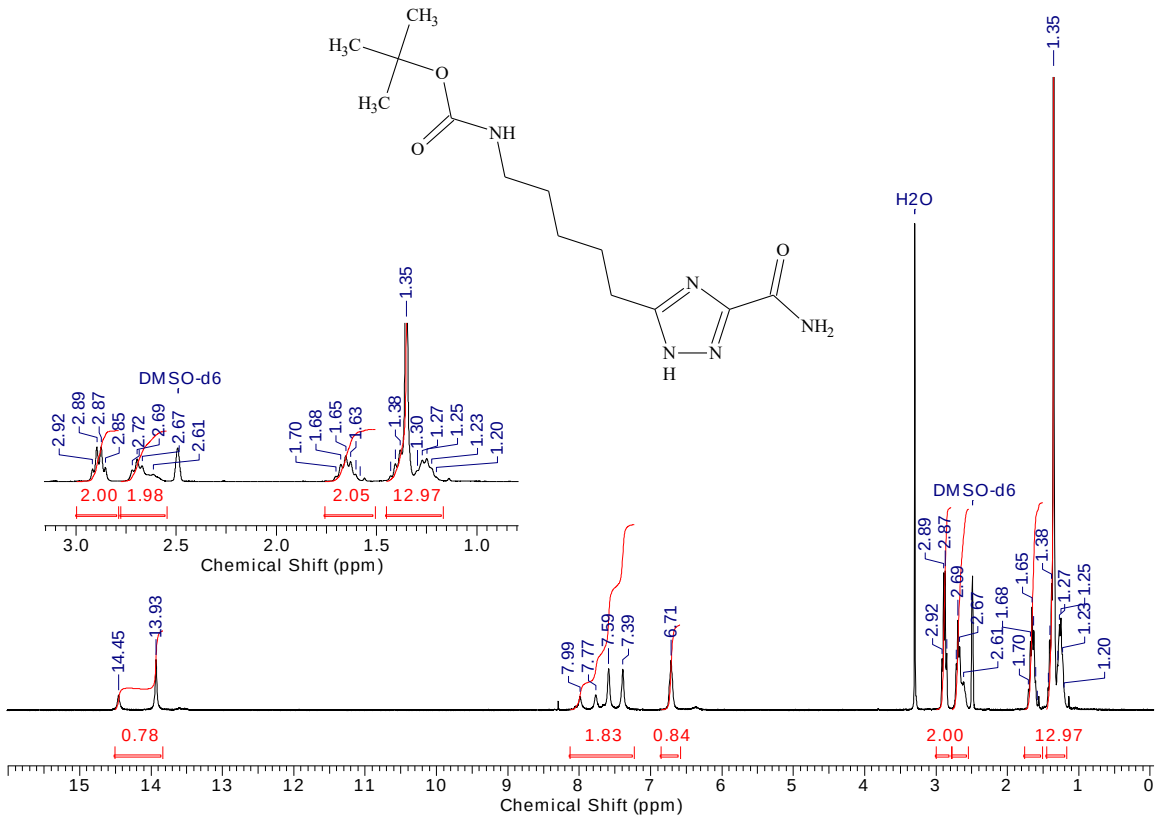
*^{1}H NMR spectrum of **6c***



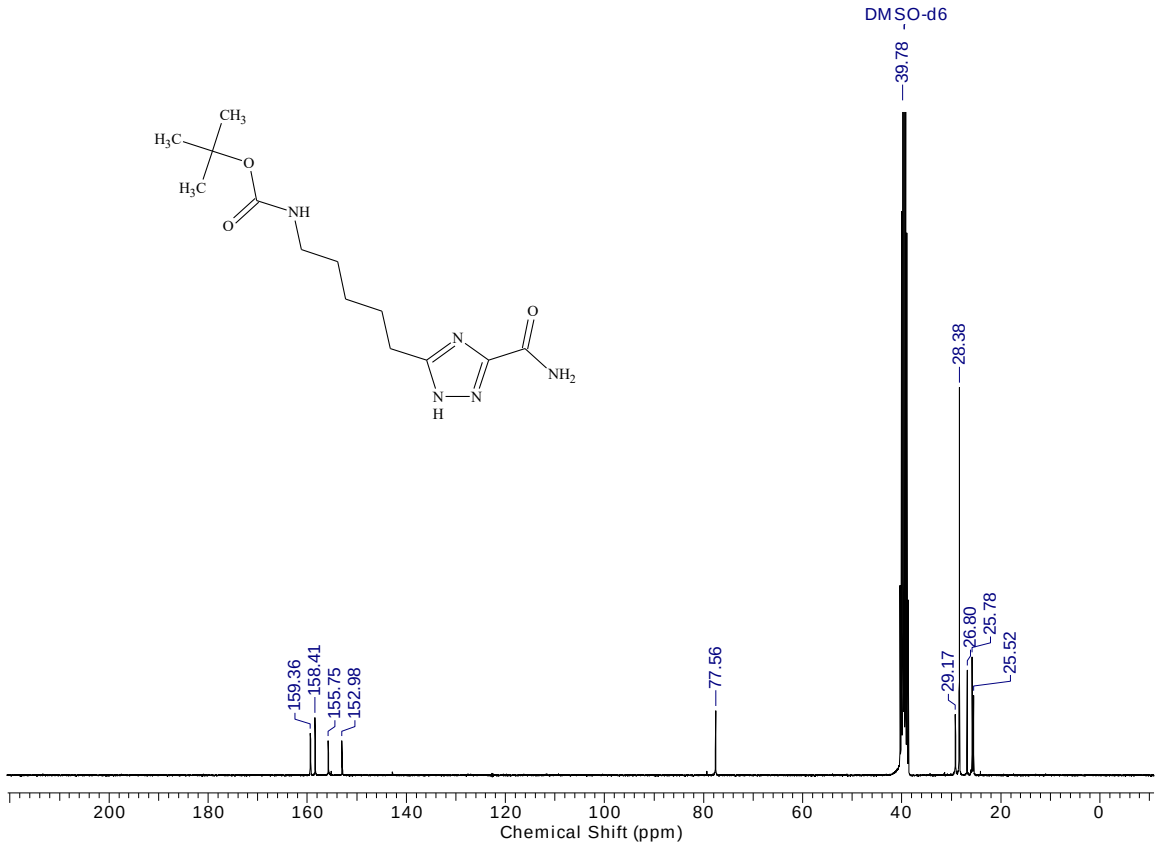
*^{13}C NMR spectrum of **6c***



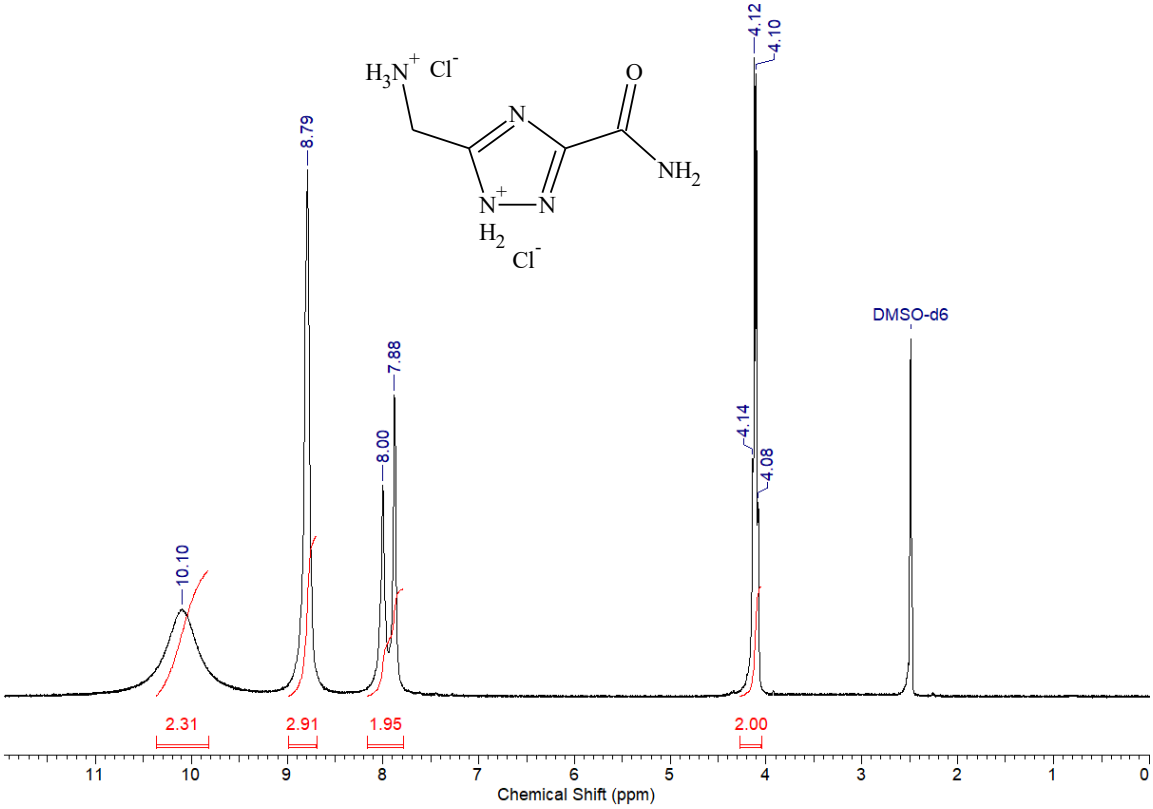
^{1}H NMR spectrum of ***6d***



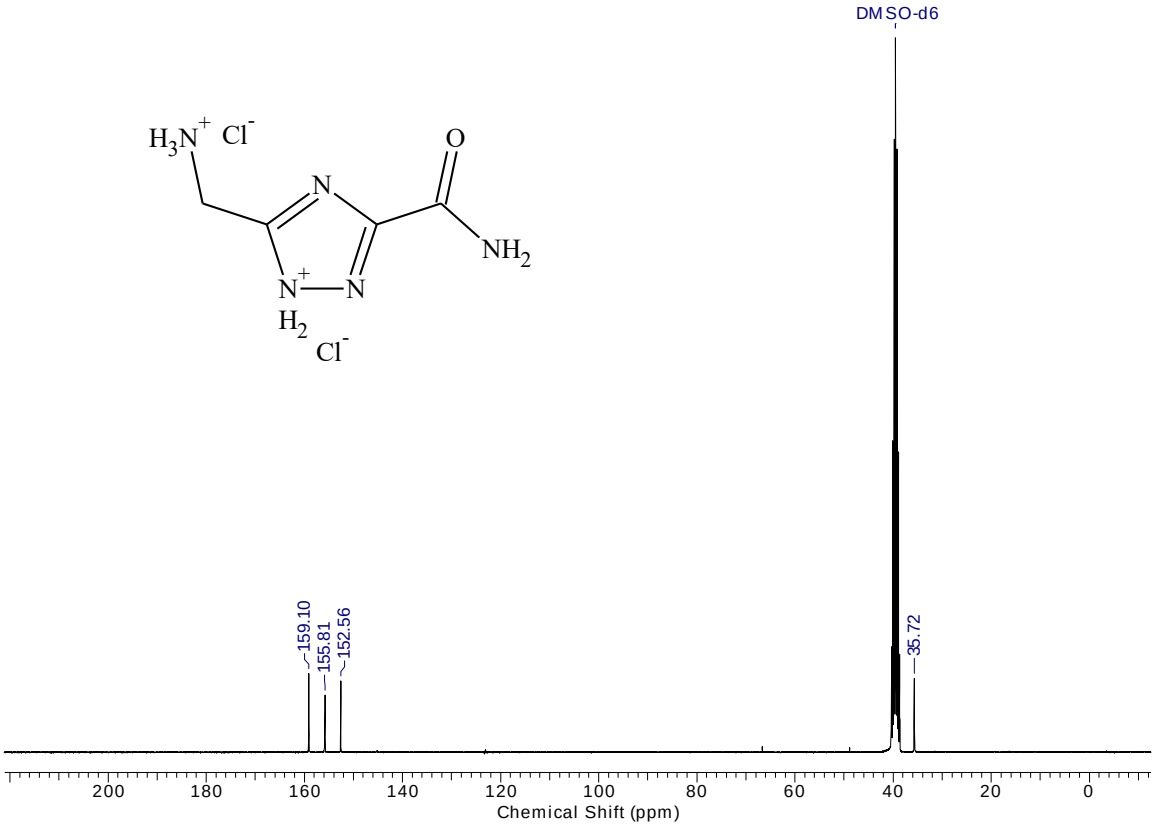
^{13}C NMR spectrum of ***6d***



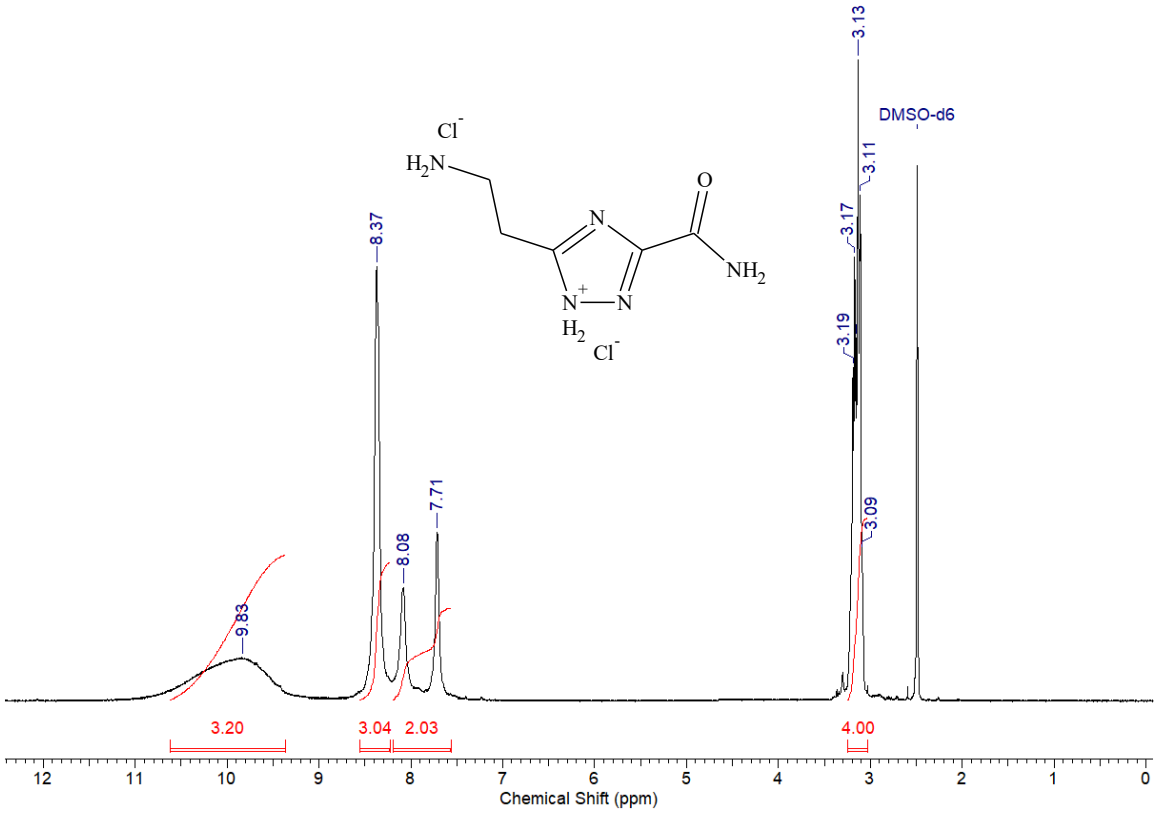
*^{1}H NMR spectrum of **7a***



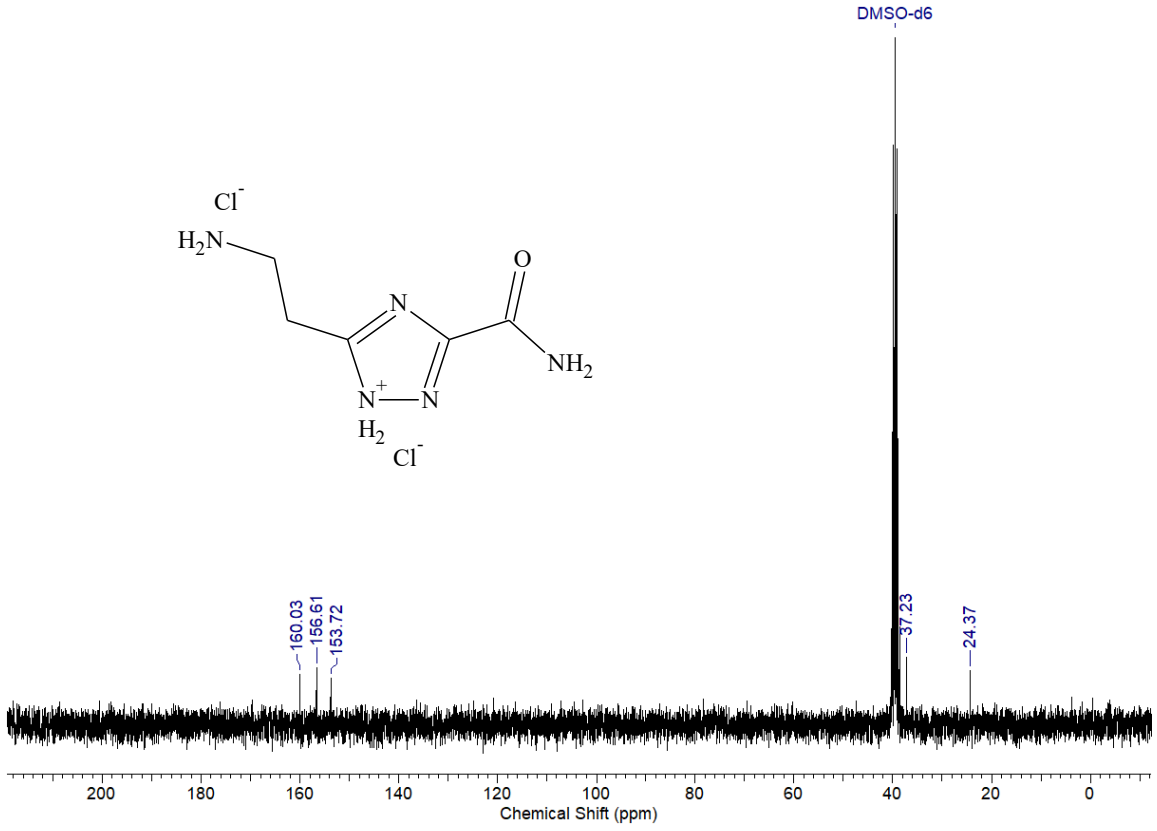
*^{13}C NMR spectrum of **7a***



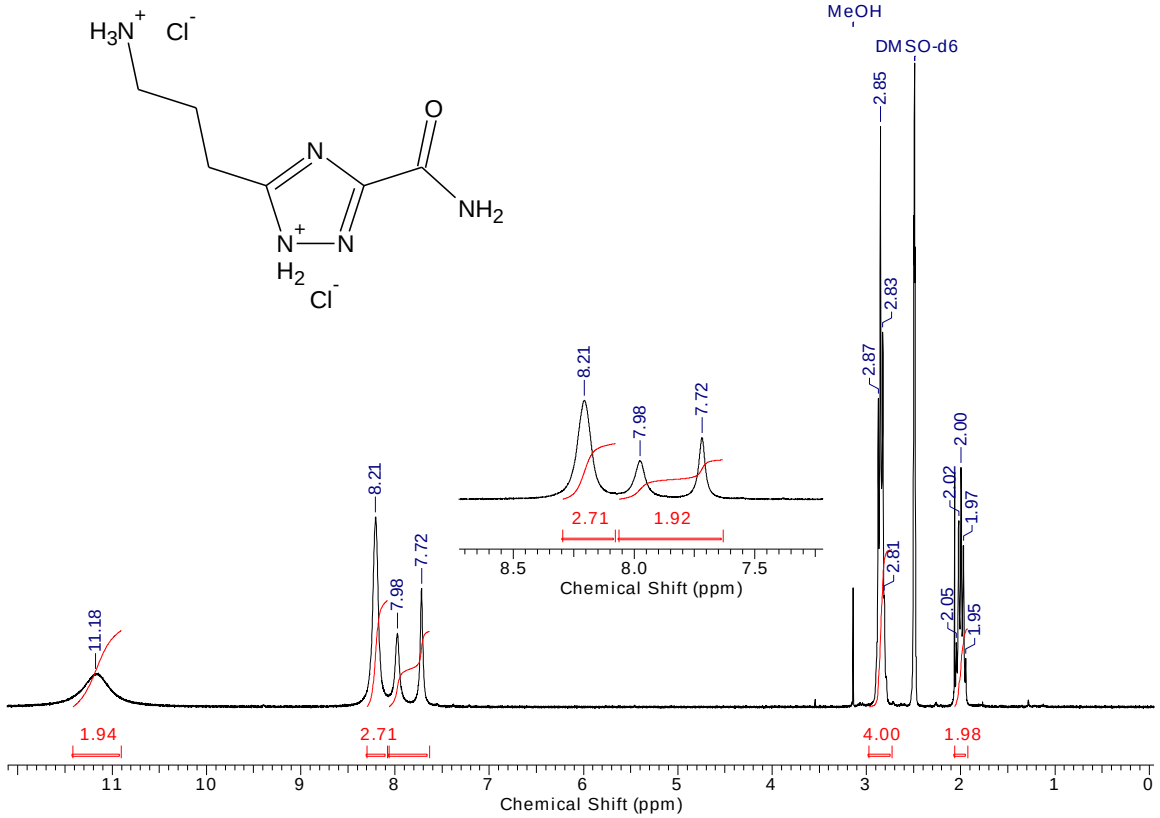
*^{1}H NMR spectrum of **7b***



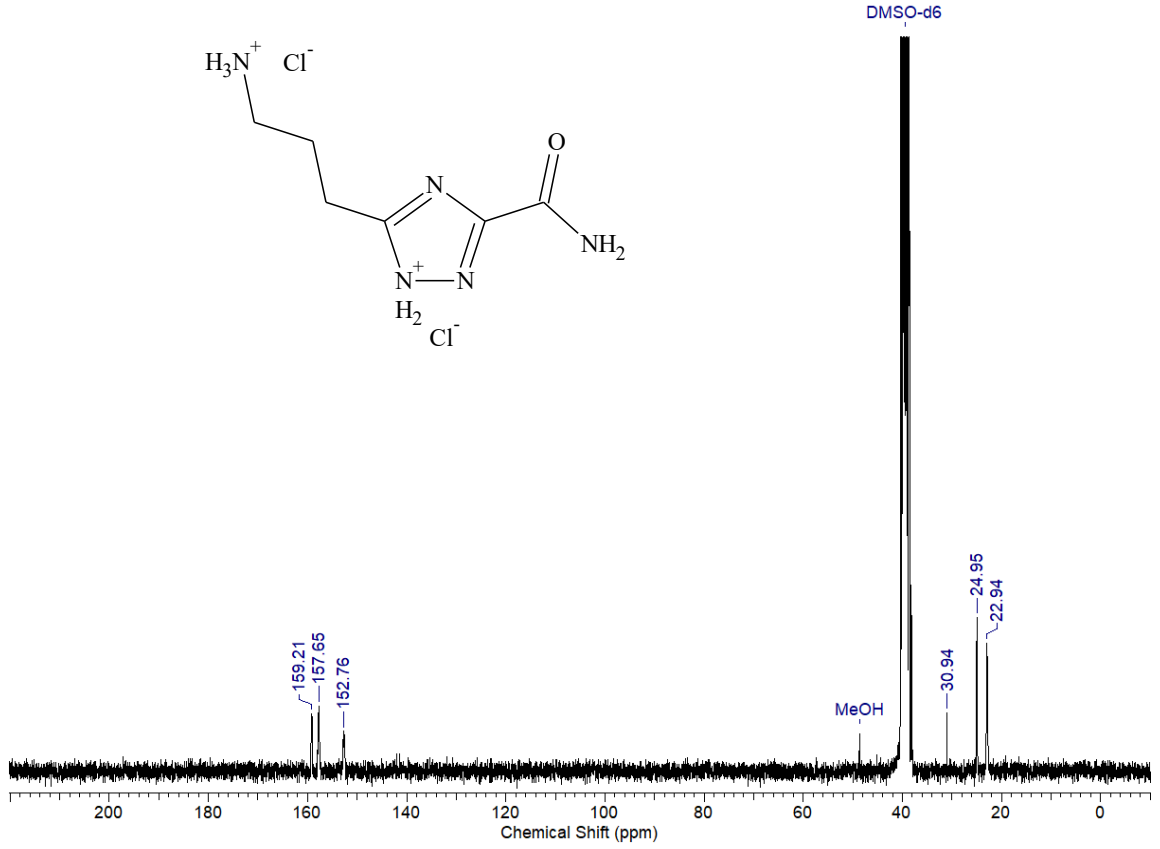
*^{13}C NMR spectrum of **7b***



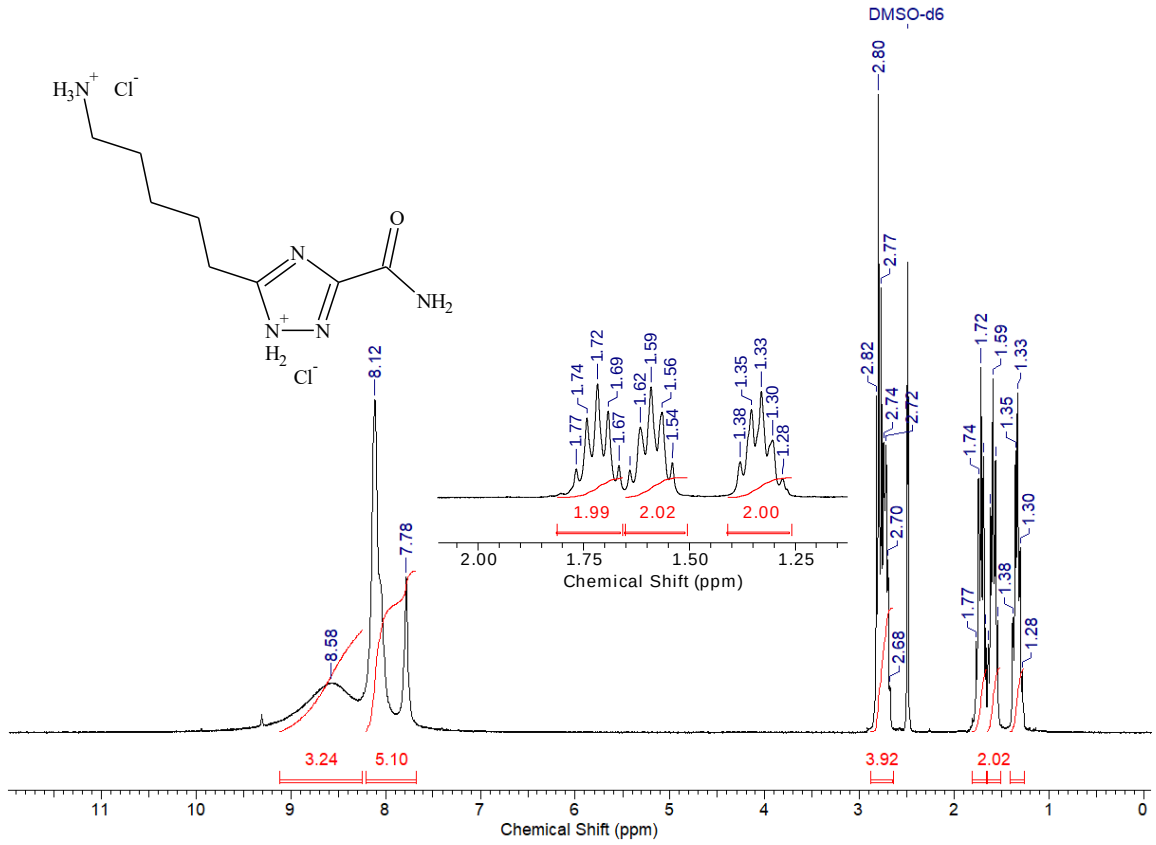
*^{1}H NMR spectrum of **7c***



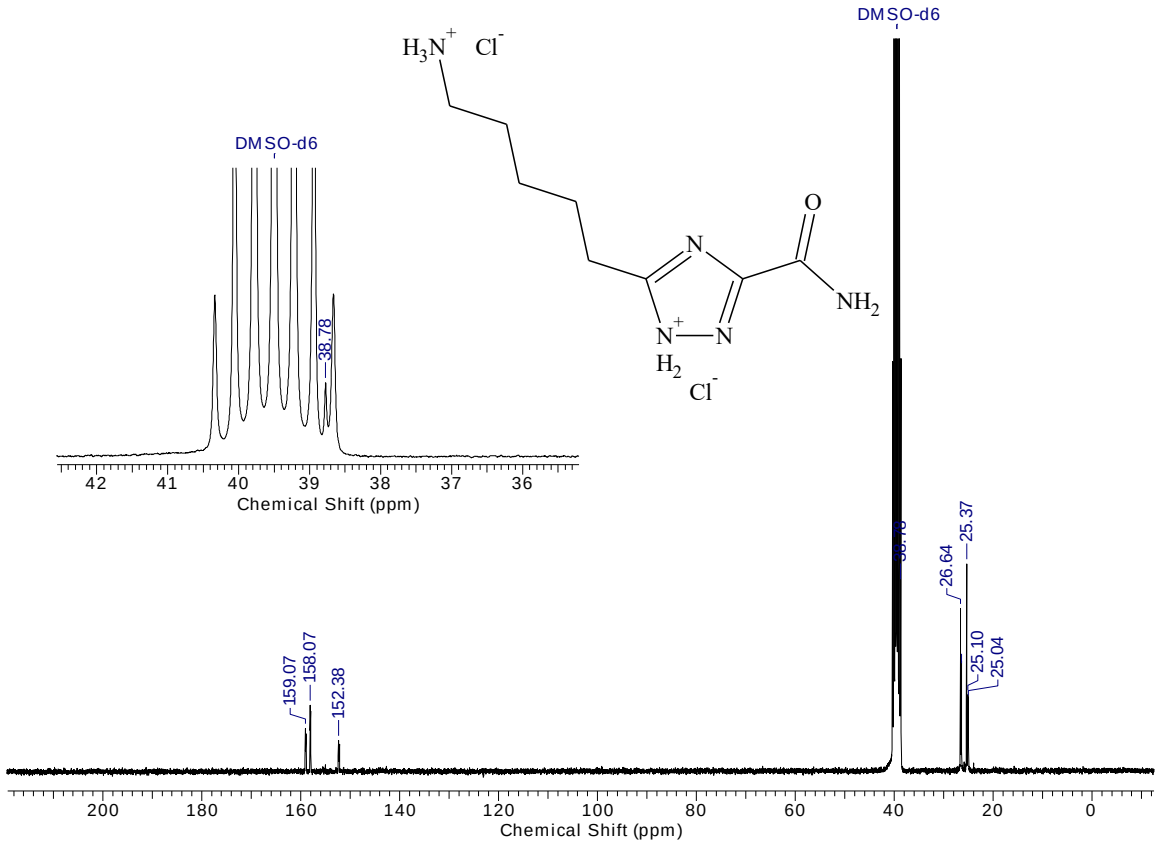
*^{13}C NMR spectrum of **7c***



*^{1}H NMR spectrum of **7d***



*^{13}C NMR spectrum of **7d***



1.5. References

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S2. H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512, https://doi.org/10.1021/jo971176v.

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