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Novel 5-alkyl(aryl)-substituted ribavirine analogues: synthesis and antiviral evaluation

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1. General experimental information.

All reactions were carried out in glassware with magnetic stirring. Anhydrous methanol and ethanol were prepared by standard methods. Other solvents and commercial reagents were used without additional purification. Visualization on TLC (analytical thin layer chromatography) was achieved by the use of UV light (254 nm) and treatment with phosphomolybdic acid followed by heating. TLC was performed on Imid Ltd Sorbfil plates. Kieselgel F254 (Merck) silica gel was used as a sorbent for column chromatography. Unless otherwise noted, yields refer to chromatographically and spectroscopically pure compounds. High-resolution mass spectra (HRMS) were recorded on Agilent 6224 instrument using electron spray ionization (ESI). Proton and carbon magnetic resonance spectra (^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz) were recorded on a Bruker DPX-300 spectrometer with solvent resonance as the internal standard (^{1}H NMR: $CDCl_3$ at 7.25 ppm, DMSO-d_6 at 2.49 ppm; acetone-d_6 at 2.04 ppm; ^{13}C NMR: $CDCl_3$ at 77.00 ppm, DMSO-d_6 at 39.50 ppm). NMR data are represented as follows: chemical shift (δ ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad), coupling constant in Hertz (Hz). Melting points were reported uncorrected. Nucleoside **6a** was synthesized from compound **5a** and guanosine according the reported procedure [1] with 62% yield. ^{1}H NMR spectrum and purity of **6a** were adequate.

2. General procedure for 5-alkyl/aryl-1,2,4-triazole-3-carboxamides (5) preparation.

Ethyl 5-alkyl/aryl-1,2,4-triazole-3-carboxylates **4a-h** were refluxed with 50-fold excess of 10M methanol ammonia solution with stirring for 72 h. Every 12 hours a 5-fold excess of 10M methanol ammonia solution was added. Then the solution was evaporated on a vacuum rotary evaporator and 5-alkyl/aryl-1,2,4-triazole-3-carboxamides **5a-h** were purified by recrystallization from water. Compounds **5a,e,f,g,h** have melting points above 250 °C.

5-Methyl-1,2,4-triazole-3-carboxamide **5a**

From 300 mg (1.93 mmol) of ethyl 5-methyl-1,2,4-triazole-3-carboxylate (**4a**) 230 mg (95%) of product (**5a**) as a white solid was obtained. ^{1}H NMR (DMSO-d_6) δ: 2,34 (s, 3H, CH_3); 7,49; 7,74 (2H, 2bs, -$CONH_2$); 8.51 (1H, c, 1-NH). ^{13}C NMR (DMSO-d_6) δ: 12.5; 155.4; 157.9; 163.2. HRMS (ESI): m/z calculated for $[C_4H_6N_4O+H]^+$ 127.0614, m/z found 127.0618.

5-Ethyl-1,2,4-triazole-3-carboxamide **5b**

From 300 mg (1.77 mmol) of ethyl 5-ethyl-1,2,4-triazole-3-carboxylate (**4b**) 226 mg (91%) of product **5b** as a white solid was obtained. ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J=7.64 Hz, -$CH_2\underline{CH_3}$); 2.92 (2H, q, J=7.64 Hz, -$\underline{CH_2}CH_3$); 7.52, 7.83 (2H, 2bs, -$CONH_2$); 9.70 (1H, c, 1-NH). ^{13}C NMR (DMSO-d_6) δ: 11.3; 16.1; 153.1; 162.2; 165.9. HRMS (ESI): m/z calculated for $[C_5H_8N_4O+H]^+$ 141.0771, m/z found 141.0772. Mp 196-201°C.

5-Propyl-1,2,4-triazole-3-carboxamide **5c**

From 400 mg (2.19 mmol) of ethyl 5-propyl-1,2,4-triazole-3-carboxylate (**4c**) 313 mg (93%) of product **5c** as a white solid was obtained. ^{1}H NMR (DMSO-d_6) δ: 0.94 (3H, t, J=7.31 Hz, -CH_3); 1.79 (2H, m, J=7.53 Hz, -CH_2); 2.89 (2H, t, J=7.53 Hz, -CH_2); 7.48, 7.79 (2H, 2bs, $CONH_2$), 9.74 (1H, c, 1-NH). ^{13}C NMR (DMSO-d_6) δ: 13.3; 21.5; 28.6; 150.4; 159.2; 165.5. HRMS (ESI): m/z calculated for $[C_6H_{10}N_4O+H]^+$ 155.0927, m/z found 155.0924. Mp 192-194°C (sublimation at 138-141°C).

5-Isopropyl-1,2,4-triazole-3-carboxamide **5d**

From 400 mg (2.19 mmol) of ethyl 5-isopropyl-1,2,4-triazole-3-carboxylate (**4d**) 280 mg (83%) of product **5d** as a white solid was obtained. ^{1}H NMR (DMSO-d_6) δ: 1.36 (3H, s, -CH_3); 1.38 (3H, s, -CH_3); 3.31 (1H, m, -CH); 7.52, 7.83 (2H, 2bs, $CONH_2$), 9.78 (1H, c, 1-NH). ^{13}C NMR (DMSO-d_6) δ: 23.0; 31.0; 151.8; 163.4; 166.2. HRMS (ESI): m/z calculated for $[C_6H_{10}N_4O+H]^+$ 155.0927, m/z found 155.0931. Mp 185-188°C (sublimation at 135-140°C).

5-Cyclopropyl-1,2,4-triazole-3-carboxamide **5e**

From 300 mg (1.66 mmol) of ethyl 5-cyclopropyl-1,2,4-triazole-3-carboxylate (**4e**) 220 mg (87%) of product **5e** as a white solid was obtained. ^{1}H NMR (DMSO-d_6) δ: 1.05-1.18 (4H, m, -CH_2-CH_2-); 2.11-2.14 (1H, m, -CH); 7.48, 7.79 (2H, 2bs, $CONH_2$); 9.81 (1H, c, 1-NH). ^{13}C NMR (DMSO-d_6) δ: 9.3; 11.0; 150.6; 160.2; 168.6. HRMS (ESI): m/z calculated for $[C_6H_8N_4O+H]^+$ 153.0771, m/z found 153.0766. Sublimation at 141-143°C.

5-Isobutyl-1,2,4-triazole-3-carboxamide **5f**

From 1 g (5.07 mmol) of ethyl 5-isobutyl-1,2,4-triazole-3-carboxylate (**4f**) 760 mg (90%) of product **5f** as a white solid was obtained. ^{1}H NMR (DMSO-d_6) δ: 0.85,0.89 (6H, 2s, -CH($\underline{CH_3}$)$_2$); 1.88-2.07 (3H, m, -$\underline{CH_2}$ $\underline{CH}$(CH_3)$_2$); 7.38, 7.77 (2H, 2bs, $CONH_2$); 9.61 (1H, c, 1-NH). ^{13}C NMR (DMSO-d_6) δ: 21.6; 27.4; 33.0; 150.2; 159.4; 165.6. HRMS (ESI): m/z calculated for $[C_7H_{12}N_4O+H]^+$ 169.1084, m/z found 169.1092.

5-tert-*Butyl-1,2,4-triazole-3-carboxamide* **5g**

From 300 mg (1.52 mmol) of ethyl 5-*tert*-butyl-1,2,4-triazole-3-carboxylate (**4g**) 200 mg (79%) of product **5g** as a white solid was obtained. ^{1}H NMR (DMSO-d_6) δ: 1.36 (9H, s, -C($\underline{CH_3}$)$_3$); 7.35, 7.67 (2H, 2bs, $CONH_2$); 9.68 (1H, c, 1-NH). ^{13}C NMR (DMSO-d_6) δ: 27.8; 31.3; 154.4; 165.2; 167.9. HRMS (ESI): m/z calculated for $[C_7H_{12}N_4O+H]^+$ 169.1084, m/z found 169.1090. Sublimation at 180-185°C.

5-Phenyl-1,2,4-triazole-3-carboxamide **5h**

From 500 mg (2.30 mmol) of ethyl 5-phenyl-1,2,4-triazole-3-carboxylate (**4h**) 380 mg (88%) of product **5h** as a white solid was obtained. ^{1}H NMR (DMSO-d_6) δ: 7.43-7.36 (3H, m, -Ph); 7.58, 7.74 (2H, 2bs, $CONH_2$); 8.01-8.09 (2H, m, -Ph); 9.89 (1H, c, 1-NH). ^{13}C NMR (DMSO-d_6) δ: 127.3; 127.9; 128.81; 129.43; 155.2; 158.9; 160.8. HRMS (ESI): m/z calculated for $[C_9H_8N_4O+H]^+$ 189.0771, m/z found 189.0778. Sublimation at 190-195°C.

3. General procedure for ethyl 5-alkyl/aryl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylates (7) preparation.

An equimolar mixture of ethyl 5-alkyl/aryl-1,2,4-triazole-3-carboxylate, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and 3% mol of bis(*p*-nitrophenyl)phosphate (BNPP) was dissolved in a minimum volume of ethanol, then the solvent was removed on a rotary evaporator under vacuum and the oily residue was heated on an oil bath under vacuum at 155-160 °C for 45 min. The desired product was isolated as pale yellow oil by column chromatography on silica gel using 1:19 acetone-toluene as eluent.

*Ethyl 5-ethyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate* **7b**

From 1 g (5.9 mmol) of ethyl 5-ethyl-1,2,4-triazole-3-carboxylate (**5b**), 1.88 g (5.9 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and BNPP (0.064 g) 1.35 g (53.5%) of product **7b** was obtained. ^{1}H NMR ($CDCl_3$) δ: 1.27 (3H, t, J= 7.8 Hz, -CH_2*CH_3*); 1.29 (3H, t, J= 7.2 Hz, -OCH_2*CH_3*); 2.00-2.02 (9H, 3s, 3 *CH_3*COO); 2.79 (2H, q, J=7.6 Hz, -*CH_2*CH_3); 4.02-4.38 (5H, m, $C_{5'}H_2$, $C_{4'}H$, -O*CH_2*CH_3); 5.59 (1H, t, J=5.3 Hz, $C_{3'}H$); 5.73-5.76 (1H, m, $C_{2'}H$); 5.86 (1H, d, J=3.8 Hz, $C_{1'}H$).

*Ethyl 5-propyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate* **7c**

From 1 g (5.5 mmol) of ethyl 5-propyl-1,2,4-triazole-3-carboxylate (**5c**), 1.74 g (5.5 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and BNPP (0.06 g) 1.40 g (58%) of product **7c** was obtained. ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, t, J= 7.4 Hz, -CH_2CH_2*CH_3*); 1.38 (3H, t, J= 7.1 Hz, -OCH_2*CH_3*); 1.74-1.87 (2H, m, -CH_2*CH_2*CH_3); 2.09-2.12 (9H, m, *CH_3*COO); 2.78-2.83 (2H, m, -*CH_2*CH_2CH_3); 4.11-4.47 (5H, m, $C_{5'}H_2$, $C_{4'}H$, -O*CH_2*CH_3); 5.68 (1H, t, J=5,3 Hz, $C_{3'}H$); 5.83-5.86 (1H, m, $C_{2'}H$); 5.92 (1H, d, J=3.7 Hz, $C_{1'}H$).

*Ethyl 5-isopropyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate* **7d**

From 1 g (5.5 mmol) of ethyl 5-isopropyl-1,2,4-triazole-3-carboxylate (**5d**), 1.74 g (5.5 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and BNPP (0.06 g) 1.25 g (51%) of product **7d** was obtained. ^{1}H NMR ($CDCl_3$) δ: 1.37-1.42 (9H, m, $2CH_3$-iPr, -OCH_2*CH_3*); 2.11-2.13 (9H, 3s, *CH_3*COO); 3.16-3.19 (1H, m, CH i-Pr); 4.12-4.49 (5H, m, $C_{5'}H_2$, $C_{4'}H$, -O*CH_2*CH_3); 5.71 (1H, t, J=5.3 Hz, $C_{3'}H$); 5.86-5.89 (1H, m, $C_{2'}H$); 5.98 (1H, d, J=3.6 Hz, $C_{1'}H$).

*Ethyl 5-cyclopropyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate* **7e**

From 1.5 g (6.6 mmol) of ethyl 5-cyclopropyl-1,2,4-triazole-3-carboxylate (**5e**), 2.11 g (6.6 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and BNPP (0.07 g) 1.5 g (52%) of product **7e** was obtained. ^{1}H NMR ($CDCl_3$) δ: 1.11-1.28 (4H, m, -CH_2CH_2- c-Pr); 1.38 (3H, t, J=7.1 Hz, -CH_2*CH_3*);

1.91-2.00 (1H, m, -CH- c-Pr); 2.09-2.12 (9H, 3s, CH_3COO); 4.12 - 4.49 (5H, m, $C_{5'}H_2$, $C_{4'}H$, -O$\underline{CH_2}CH_3$); 5.70 (1H, t, J=5.3 Hz, $C_{3'}H$); 5.85-5.88 (1H, m, $C_{2'}H$); 6.13 (1H, d, J=3.6 Hz, $C_{1'}H$).

*Ethyl 5-isobutyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate* **7f**

From 1 g (5.1 mmol) of ethyl 5-isobutyl-1,2,4-triazole-3-carboxylate (**5f**), 1.61 g (5.1 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and BNPP (0.06 g) 1.1 g (43%) of product **7f** was obtained. ^{1}H NMR ($CDCl_3$) δ: 0.95 (6H, t, J=7.4 Hz, $2CH_3$ i-Bu); 1.38 (3H, t, J=7.1 Hz, -$OCH_2\underline{CH_3}$); 2.08-2.11 (9H, 3s, $3CH_3COO$); 2.14-2.23 (1H, m, CH i-Bu); 2.71 (2H, d, J=7.3 Hz, CH_2 i-Bu) 4.11 - 4.46 (5H, m, $C_{5'}H_2$, $C_{4'}H$, -O$\underline{CH_2}CH_3$); 5.69 (1H, t, J=5.2 Hz, $C_{3'}H$); 5.85 (1H, t, J=5.2 Hz, $C_{2'}H$); 5.91 (1H, d, J=3.7 Hz, $C_{1'}H$).

*Ethyl 5-*tert*-butyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate* **7g**

From 0.5 g (2.5 mmol) of ethyl 5-*tert*-butyl-1,2,4-triazole-3-carboxylate (**5g**), 0.8 g (2.5 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and BNPP (0.03 g) 0.4 g of crude product **7g** was obtained. Crude product was used in the next step without further purification.

*Ethyl 5-phenyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate* **7h**

From 1 g (4.6 mmol) of ethyl 5-phenyl-1,2,4-triazole-3-carboxylate (**5h**), 1.47 g (4.6 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and BNPP (0.06 g) 0.6 g (28%) of product **7h** was obtained. ^{1}H NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.1 Hz, -$OCH_2\underline{CH_3}$); 1.99-2.09 (9H, 3s, 3 CH_3COO); 4.13-4.49 (5H, m, $C_{5'}H_2$, $C_{4'}H$, -O$\underline{CH_2}CH_3$); 5.76 (1H, t, J=5.2 Hz, $C_{3'}H$); 5.93-6.00 (2H, m, $C_{2'}H$, $C_{1'}H$); 7.45-7.52, 7.72-7.76 (5H, 2m, Ph).

4. General procedure for 5-alkyl/aryl-1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamides (6) preparation.

Ethyl 5-alkyl/aryl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylates (**7**) were treated with 50-fold excess of 10M methanol ammonia solution with stirring for 48 h. Then the solution was evaporated on a vacuum rotary evaporator, 5-alkyl/aryl-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamides (**6**) were isolated by flash chromatography on silica gel eluting with ethyl acetate−methanol 9:1 followed by recrystallization from ethanol.

5-Ethyl-1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide **6b**

From 1.1 g (3.5 mmol) of ethyl 5-ethyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**7b**) and 15 ml of 10M methanol ammonia solution a crude product was obtained. After purification 0.4 g (57%) of compound **6b** was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.24 (3H, t, J=7.4 Hz, -$CH_2\underline{CH_3}$); 2.83 (2H, q, J=7.4 Hz, -$\underline{CH_2}CH_3$); 3.37-3.58 (2H, m, $C_{5'}H_2$); 3.92 (1H, q, J=4.6 Hz, $C_{4'}H$); 4.18 (1H, q, J=5.1 Hz, $C_{3'}H$); 4.48 (1H, q, J=4.9 Hz, $C_{2'}H$); 4.75 (1H, t, J=5.8 Hz, $C_{5'}OH$); 5.18 (1H, d, J=5.5 Hz, $C_{3'}OH$); 5.45 (1H, t, J=6.1 Hz, $C_{2'}OH$); 5.73 (1H, d, J=4.4 Hz, $C_{1'}H$); 7.55, 7.69 (2H, 2bs, NH_2). ^{13}C NMR (D_2O) δ: 11.9; 19.5; 62.2; 71.0; 74.9; 85.8; 89.8; 155.5; 162.2; 163.5. HRMS (ESI): m/z calculated for $[C_{10}H_{16}N_4O_5+H]^+$ 273.1193, m/z found 273.1191. Mp 203-205°C.

5-Propyl-1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide **6c**

From 1.4 g (3.5 mmol) of ethyl 5-propyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**7c**) and 15 ml of 10M methanol ammonia solution a crude product was obtained. After purification 0.85 g (88%) of compound **6c** was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ: 0.94 (3H, t, J=7.4 Hz, -$CH_2CH_2\underline{CH_3}$); 1.69 (2H, sextet, J=7.6 Hz, -$CH_2\underline{CH_2}CH_3$); 2.80 (3H, t, J=7.4 Hz, -$\underline{CH_2}CH_2CH_3$); 3.38-3.58 (2H, m, $C_{5'}H_2$); 3.92 (1H, q, J=5.6 Hz, $C_{4'}H$); 4.18 (1H, q, J=5.0 Hz, $C_{3'}H$); 4.49 (1H, q, J=5.6 Hz, $C_{2'}H$); 4.75 (1H, t, J=5.6 Hz, $C_{5'}OH$); 5.18 (1H, d, J=5.5 Hz, $C_{3'}OH$); 5.45 (1H, d, J=5.9 Hz, $C_{2'}OH$); 5.74 (1H, d, J=4.4 Hz, $C_{1'}H$); 7.56, 7.70 (2H, 2bs, NH_2). ^{13}C NMR (DMSO-d_6) δ: 14.0; 22.0; 28.1; 63.6; 72.4; 76.2; 87.7; 91.2; 157.0; 159.2; 161.4. HRMS (ESI): m/z calculated for $[C_{11}H_{18}N_4O_5+H]^+$ 287.1350, m/z found 287.1386. Mp 168-170°C.

5-Isopropyl-1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide **6d**

From 1.5 g (3.4 mmol) of ethyl 5-isopropyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**7d**) and 15 ml of 10M methanol ammonia solution a crude product was obtained. After purification 0.5 g (51%) of compound **6d** was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.24-1.27 (6H, 2d, J=6.8 Hz, $2CH_3$ i-Pr); 3.23-3.27 (1H, m, CH i-Pr); 3.37-3.55 (2H,

m, $C_{5'}H_2$); 3.92 (1H, q, J=4.8 Hz, $C_{4'}H$); 4.17 (1H, q, J=4.4 Hz, $C_{3'}H$); 4.49 (1H, q, J=4.9 Hz, $C_{2'}H$); 4.71-4.75 (1H, m, $C_{5'}OH$); 5.16 (1H, d, J=5.1 Hz, $C_{3'}OH$); 5.44 (1H, d, J=5.9 Hz, $C_{2'}OH$); 5.75 (1H, d, J=4.6 Hz, $C_{1'}H$); 7.55, 7.64 (2H, 2bs, NH_2). ^{13}C NMR (DMSO-d_6) δ: 21.4; 24.8; 62.1; 70.6; 73.9; 85.9; 88.9; 155.6; 160.6; 162.8. HRMS (ESI): m/z calculated for $[C_{11}H_{18}N_4O_5+H]^+$ 287.1350, m/z found 287.1349. Mp 165-168°C.

5-Cyclopropyl-1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide **6e**

From 1.5 g (3.5 mmol) of ethyl 5-cyclopropyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**7e**) and 15 ml of 10M methanol ammonia solution a crude product was obtained. After purification 0.75 g (75%) of compound **6e** was obtained as a white solid. ^1H NMR (DMSO-d_6) δ: 0.96-1.09 (4H, m, CH_2 c-Pr); 2.19-2.28 (1H, m, CH c-Pr); 3.38-3.58 (2H, m, $C_{5'}H_2$); 3.92 (1H, q, J=4.9 Hz, $C_{4'}H$); 4.18 (1H, q, J=5 Hz, $C_{3'}H$); 4.50 (1H, q, J=5 Hz, $C_{2'}H$); 4.76 (1H, t, J=5.6 Hz, $C_{5'}OH$); 5.16 (1H, d, J=5.6 Hz, OH); 5.47 (1H, t, J=5.7 Hz, OH); 5.92 (1H, d, J=4.2 Hz, $C_{1'}H$); 7.52, 7.60 (2H, 2bs, NH_2). ^{13}C NMR (DMSO-d_6) δ: 5.83; 8.66; 8.73; 62.10; 70.55; 73.79; 85.74; 89.18; 155.47; 159.78; 160.53. HRMS (ESI): m/z calculated for $[C_{11}H_{16}N_4O_5+H]^+$ 285.1193, m/z found 285.1194. Mp 216-218°C.

5-Isobutyl-1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide **6f**

From 1 g (2.2 mmol) of ethyl 5-isobutyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**7f**) and 10 ml of 10M methanol ammonia solution a crude product was obtained. After purification 0.35 g (53%) of compound **6f** was obtained as a white solid. ^1H NMR (DMSO-d_6) δ: 0.93 (6H, 2t, J=6.4 Hz, $2CH_3$ i-Bu); 2.01-2.08 (1H, m, CH i-Bu); 2.71 (1H, d, J=7.2 Hz, CH_2 i-Bu); 3.37-3.55 (2H, m, $C_{5'}H_2$); 3.92 (1H, q, J=4.8 Hz, $C_{4'}H$); 4.17 (1H, q, J=4.4 Hz, $C_{3'}H$); 4.49 (1H, q, J=4.9 Hz, $C_{2'}H$); 4.73 (1H, t, J=5.6 Hz, $C_{5'}OH$); 5.16 (1H, d, J=5.1 Hz, $C_{3'}OH$); 5.44 (1H, d, J=5.9 Hz, $C_{2'}OH$); 5.75 (1H, d, J=4.6 Hz, $C_{1'}H$); 7.56, 7.69 (2H, 2bs, NH_2). ^{13}C NMR (DMSO-d_6) δ: 22.2; 27.6; 33.6; 62.1; 70.6; 74.1; 85.9; 89.2; 155.8; 157.4; 160.6. HRMS (ESI): m/z calculated for $[C_{12}H_{20}N_4O_5+H]^+$ 301.1506, m/z found 301.1511. Mp 162-165°C.

*5-*tert*-Butyl-1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide* **6g**

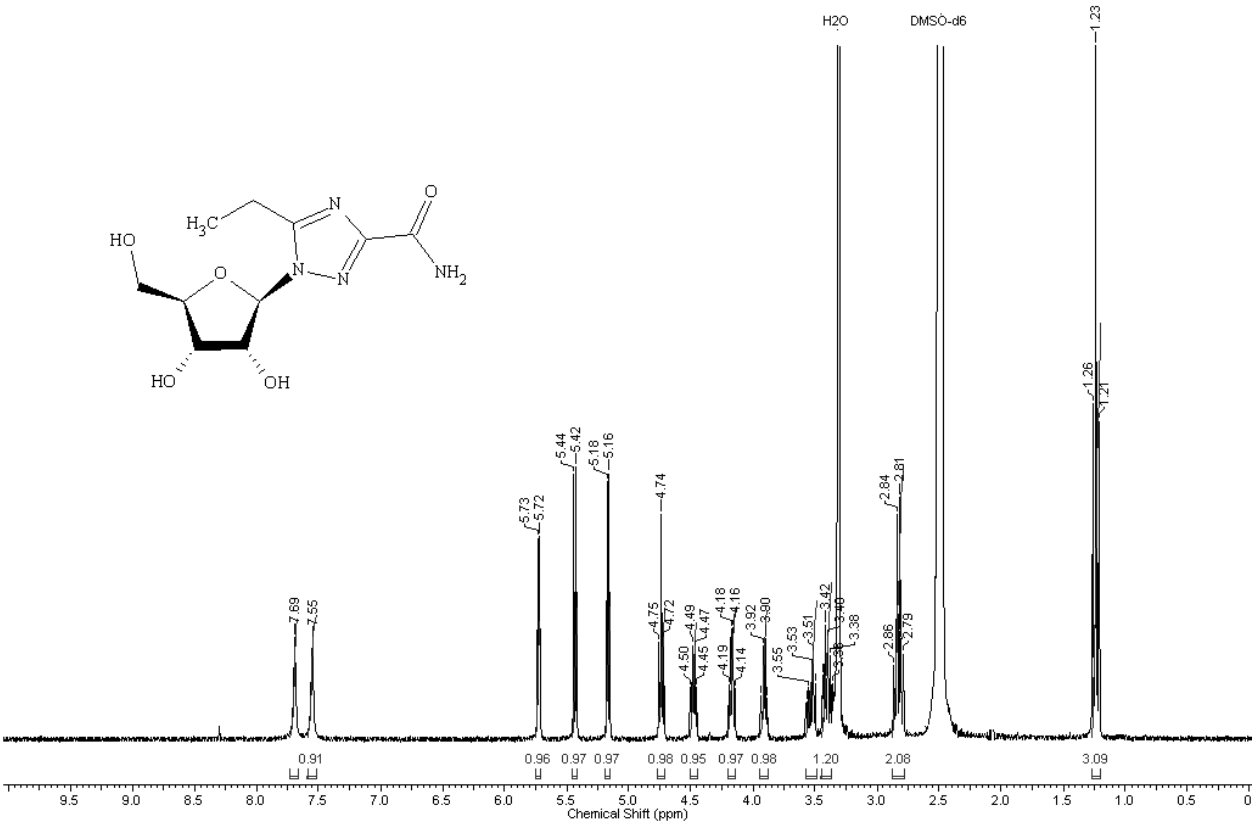
From 0.6 g of crude compound (**7g**) and 10 ml of 10M methanol ammonia solution a crude product was obtained. After purification 0.15 g (21%) of compound **6g** was obtained as a yellow pale oil. ^1H NMR (DMSO-d_6) δ: 1.30 (9H, s, $3CH_3$ t-Bu); 3.39-3.63 (2H, m, $C_{5'}H_2$); 3.86-3.92 (1H, m, $C_{4'}H$); 4.22-4.34 (2H, m, $C_{3'}H$, $C_{2'}H$); 4.71 (1H, t, J=6.0 Hz, $C_{5'}OH$); 5.08 (1H, d, J=6.0 Hz, $C_{3'}OH$); 5.40 (1H, d, J=5.3 Hz, $C_{2'}OH$); 6.68 (1H, d, J=2.9 Hz, $C_{1'}H$); 7.21, 7.72 (2H, 2bs, NH_2). ^{13}C NMR

(DMSO-d_6) δ: 29.2; 32.5; 62.4; 70.9; 74.5; 85.4; 90.6; 147.7; 158.8; 169.6. HRMS (ESI): m/z calculated for $[C_{12}H_{20}N_4O_5+H]^+$ 301.1506, m/z found 301.1512.

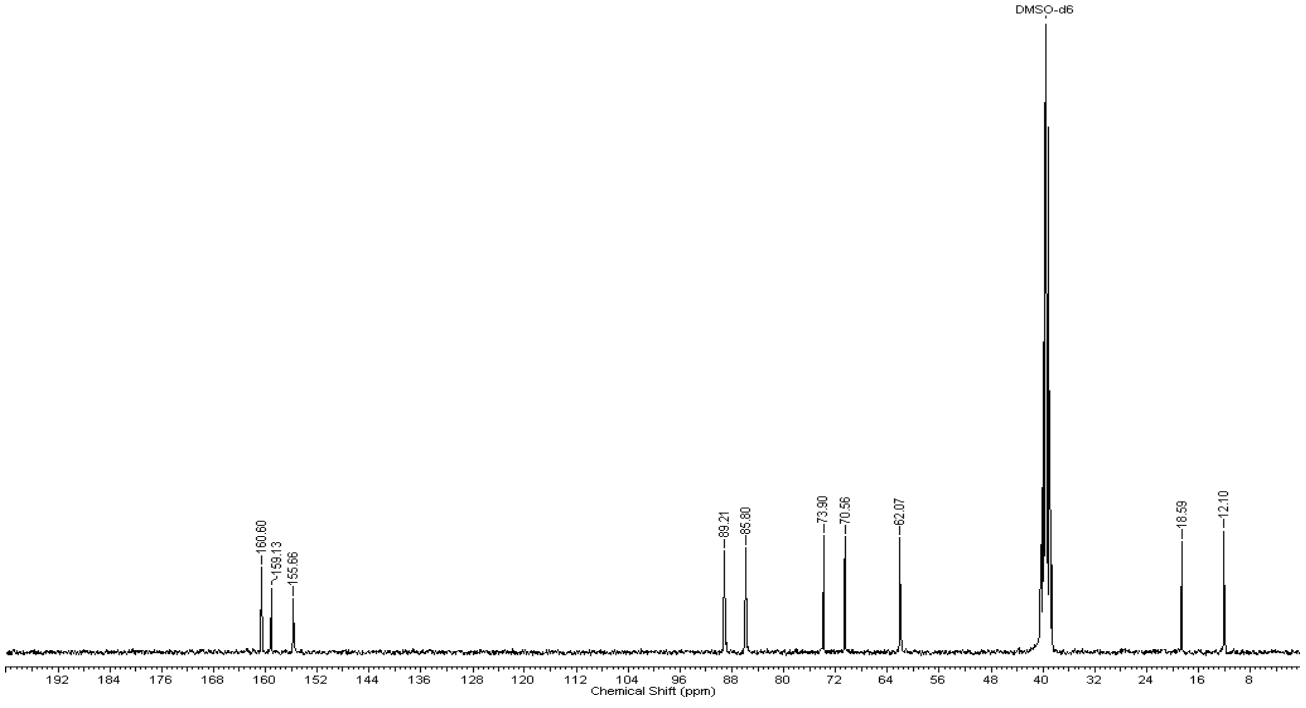
5-Phenyl-1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide **6h**

From 0.6 g (1.2 mmol) of ethyl 5-phenyl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**7h**) and 10 ml of 10M methanol ammonia solution a crude product was obtained. After purification 0.32 g (83%) of compound **6h** was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ: 3.63-3.84 (2H, m, $C_{5'}H_2$); 4.08 (1H, q, J=5.3 Hz, $C_{4'}H$); 4.15 (1H, t, J=4.3 Hz, $C_{5'}OH$); 4.43 (1H, q, J=5.3 Hz, $C_{3'}H$); 4.56 (1H, d, J=4.7 Hz, $C_{3'}OH$); 4.75 (1H, d, J=5.5 Hz, $C_{2'}H$); 4.88 (1H, d, J=4.2 Hz, $C_{2'}OH$); 5.86 (1H, d, J=4.0 Hz, $C_{1'}H$); 6.89,7.52 (2H, 2bs, NH_2); 7.61-7.65, 7.82-7.86 (5H, 2m, Ph). ^{13}C NMR (DMSO-d_6) δ: 62.6; 71.5; 75.2; 86.4; 91.2; 126.9; 129.8; 131.9; 156.3; 158.3; 162.4. HRMS (ESI): m/z calculated for $[C_{14}H_{16}N_4O_5+H]^+$ 321.1193, m/z found 321.1199. Mp 201-204°C.

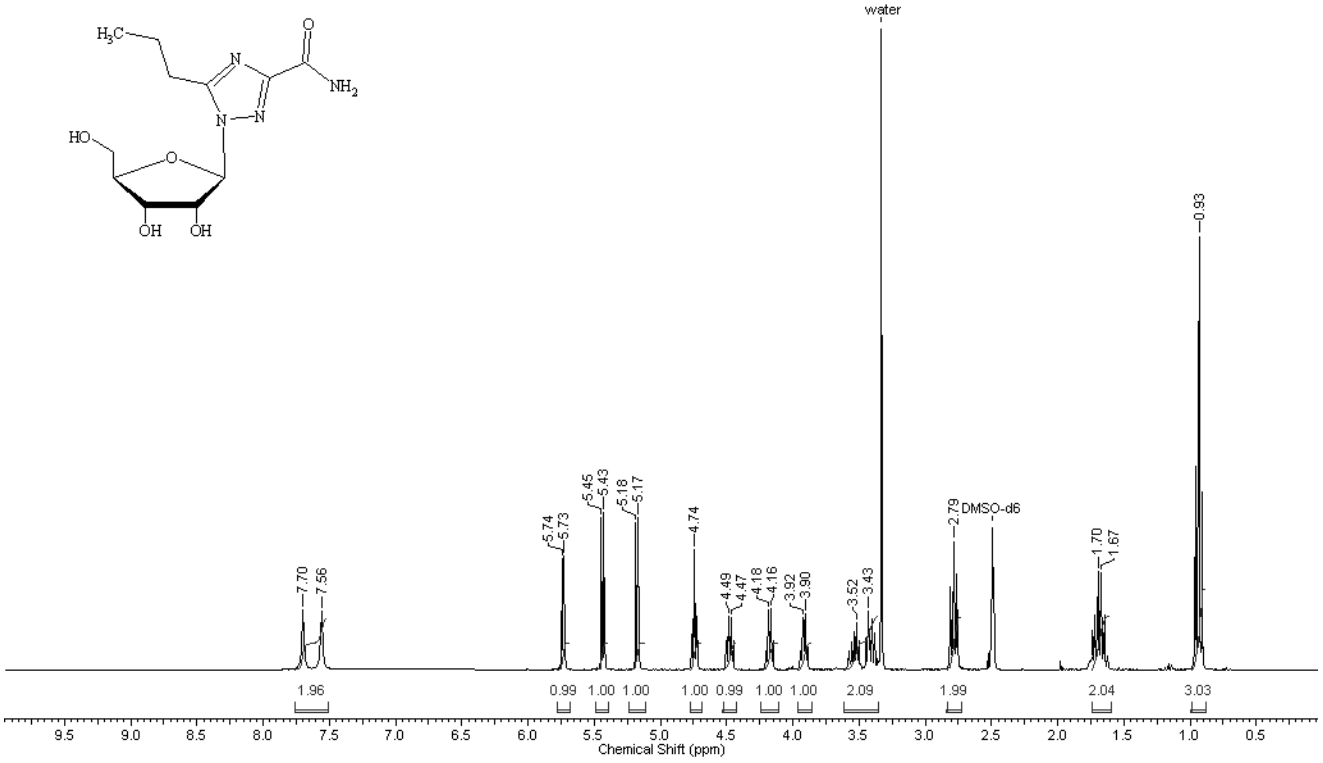
5. NMR spectra of new compounds.



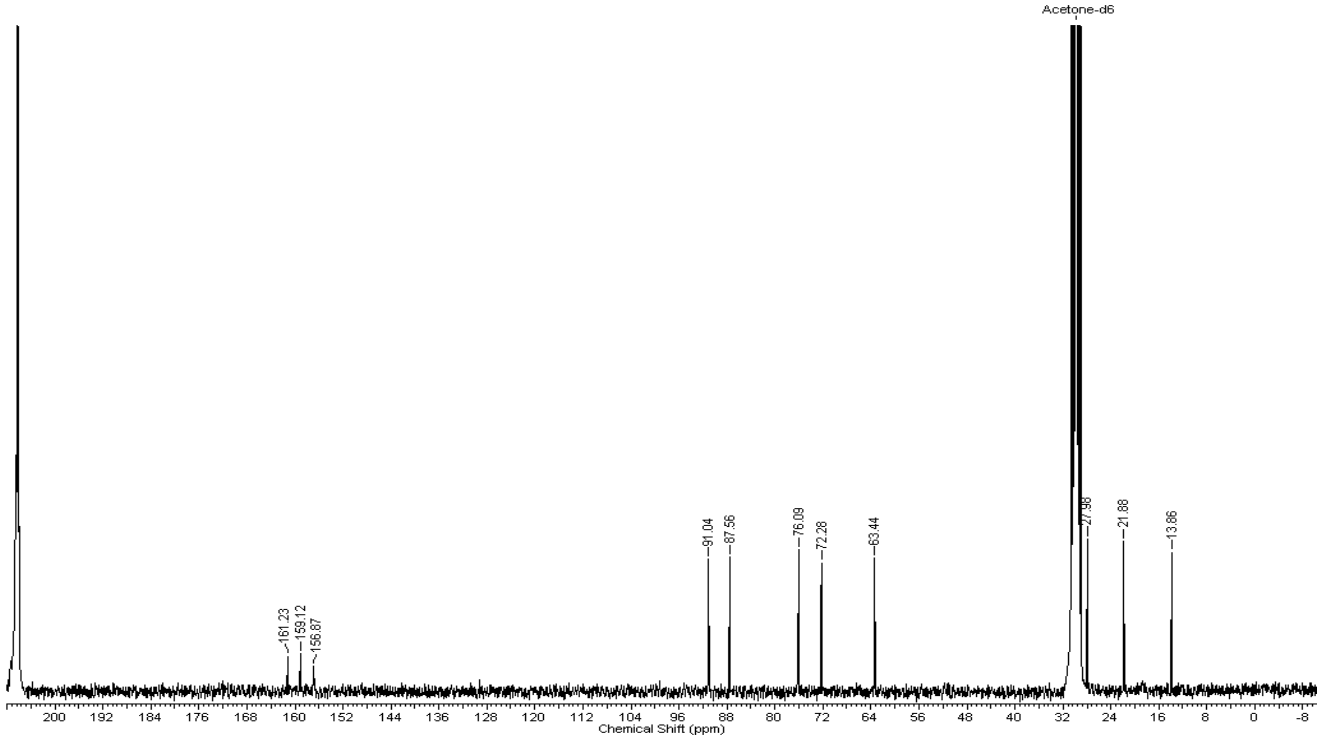
^{1}H NMR spectrum (300 MHz, DMSO-d_6) of **6b**



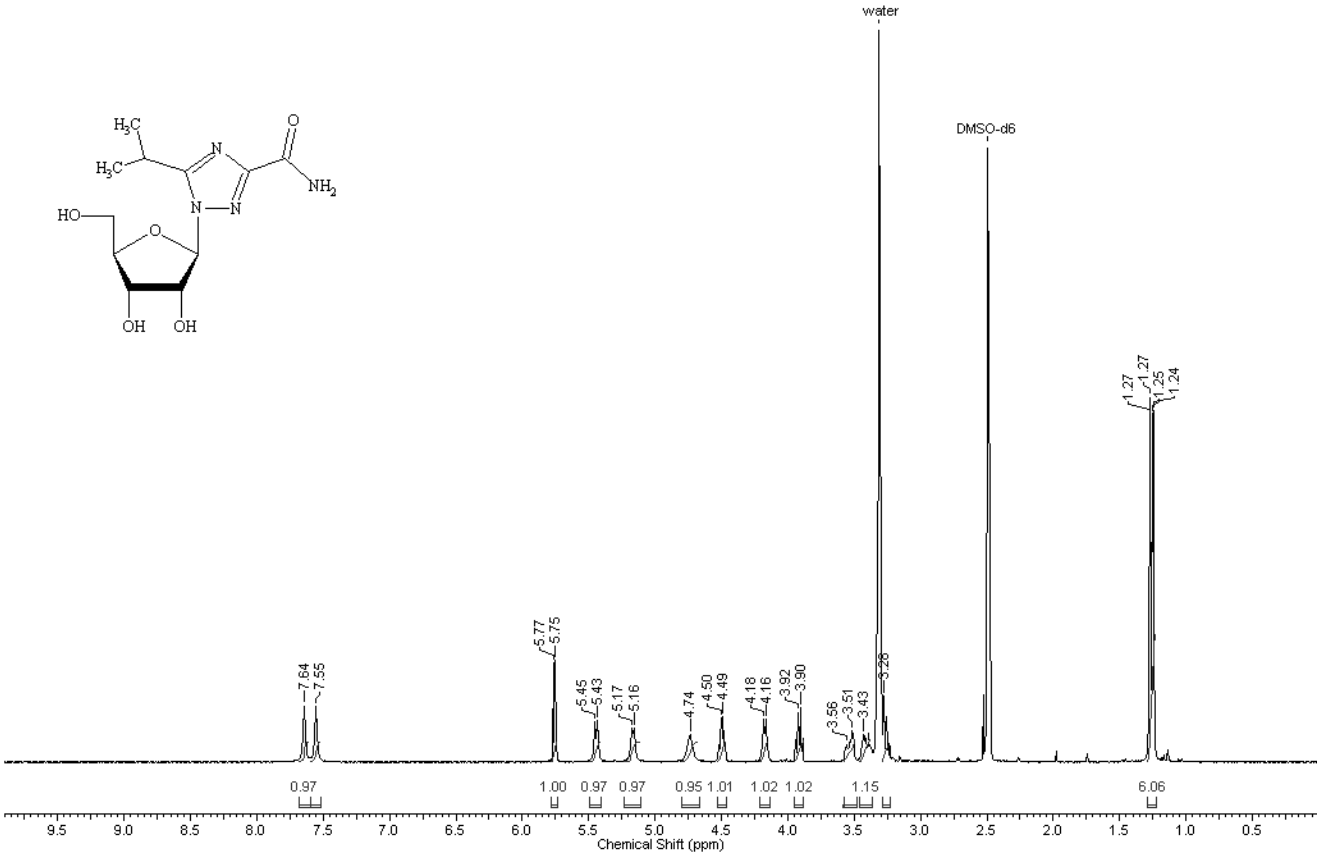
^{13}C NMR spectrum (75 MHz, DMSO-d_6) of **6b**



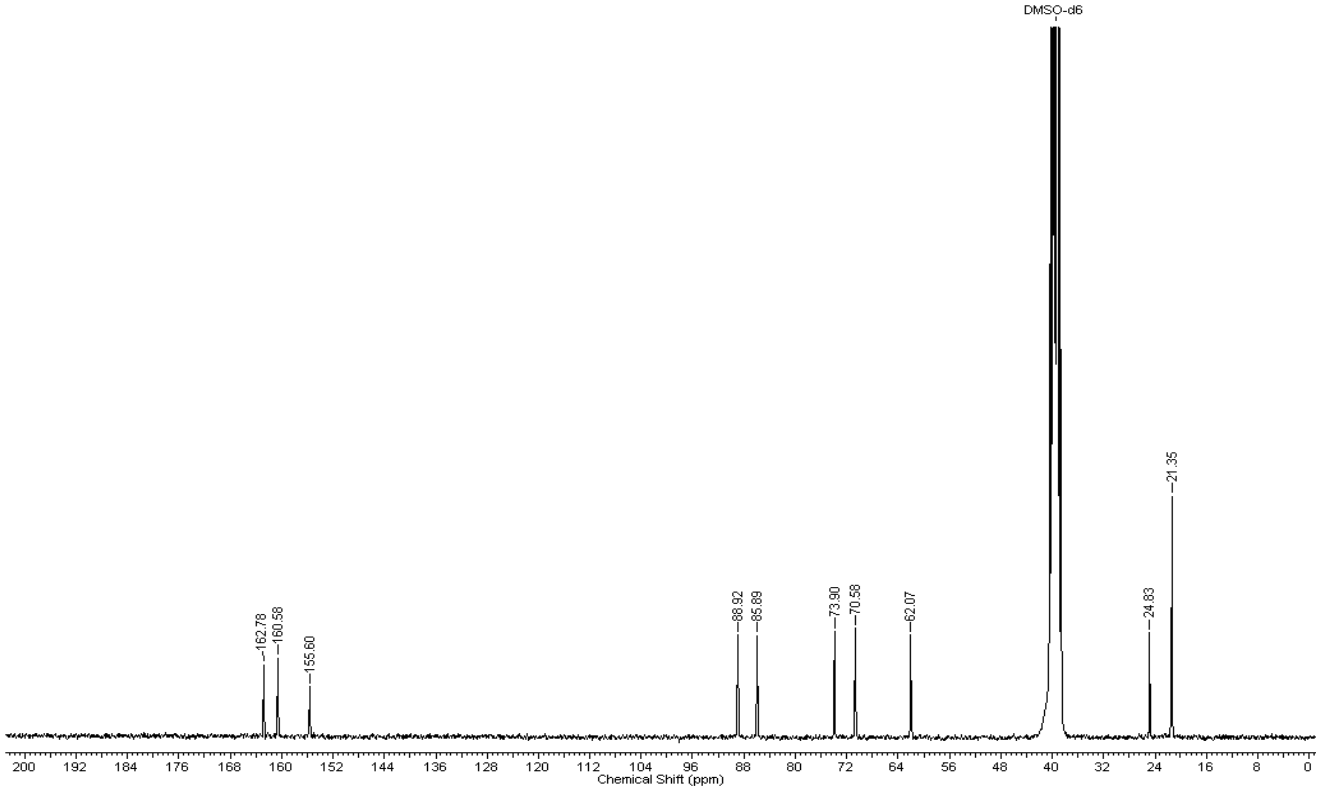
^{1}H NMR spectrum (300 MHz, DMSO-d_6) of **6c**



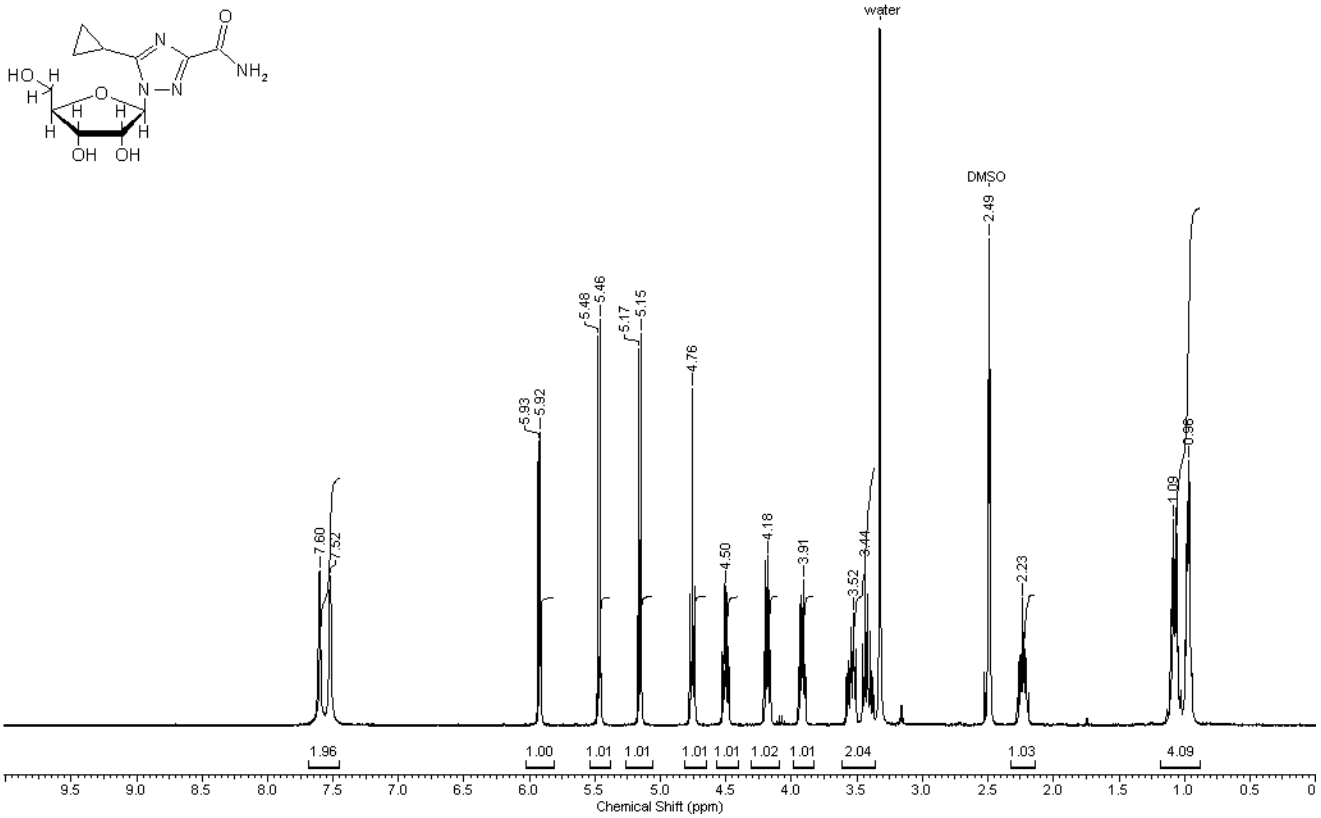
^{13}C NMR spectrum (75 MHz, acetone-d_6) of **6c**



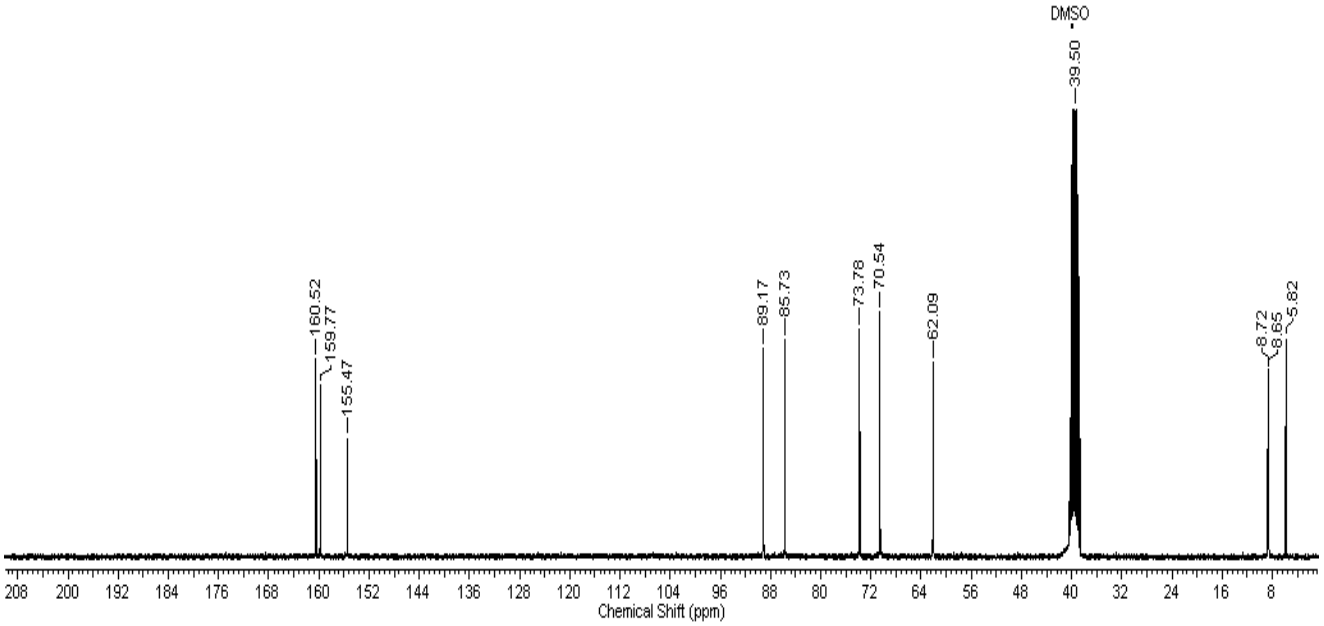
^{1}H NMR spectrum (300 MHz, DMSO-d_6) of **6d**



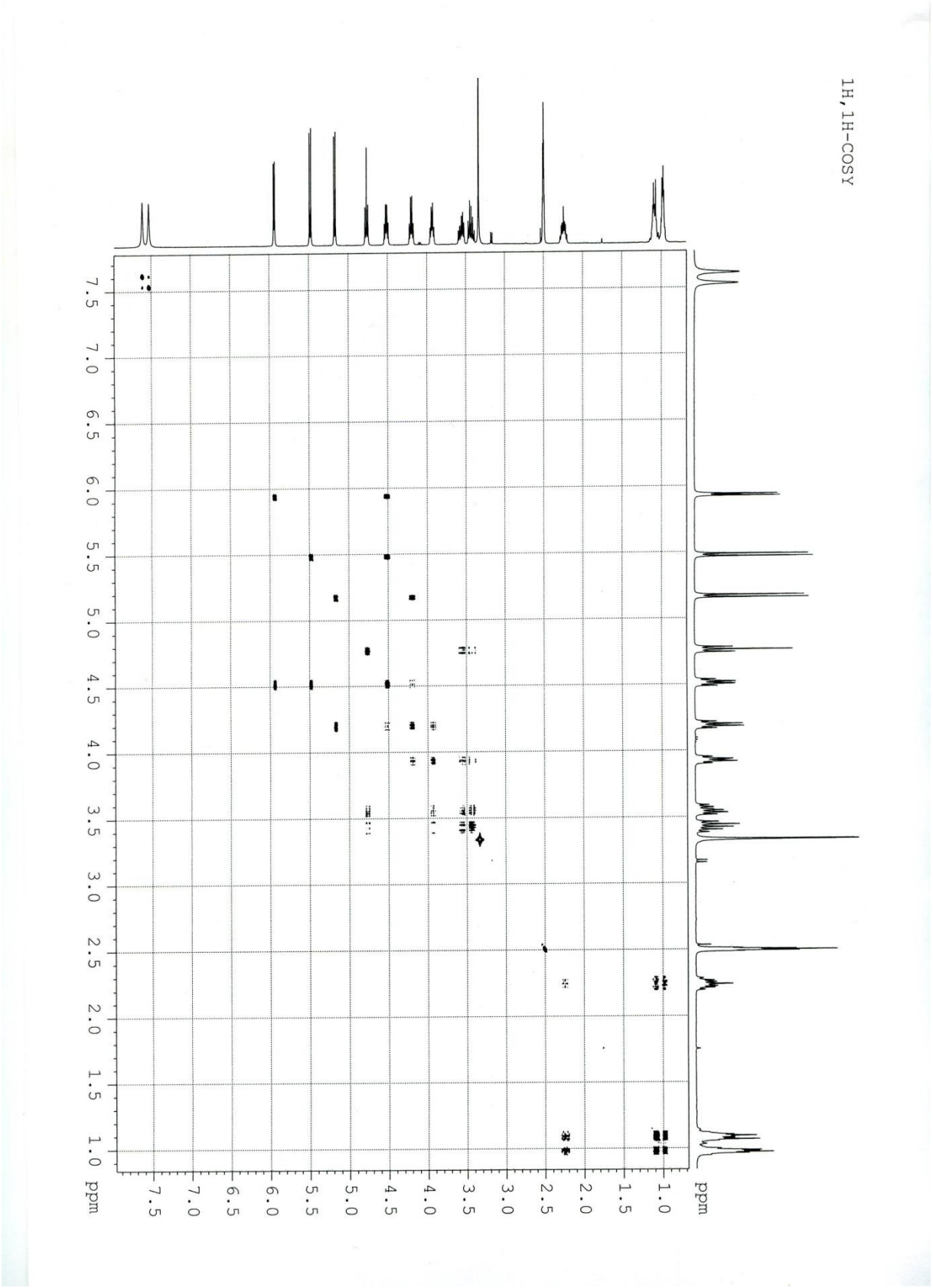
^{13}C NMR spectrum (75 MHz, DMSO-d_6) of **6d**



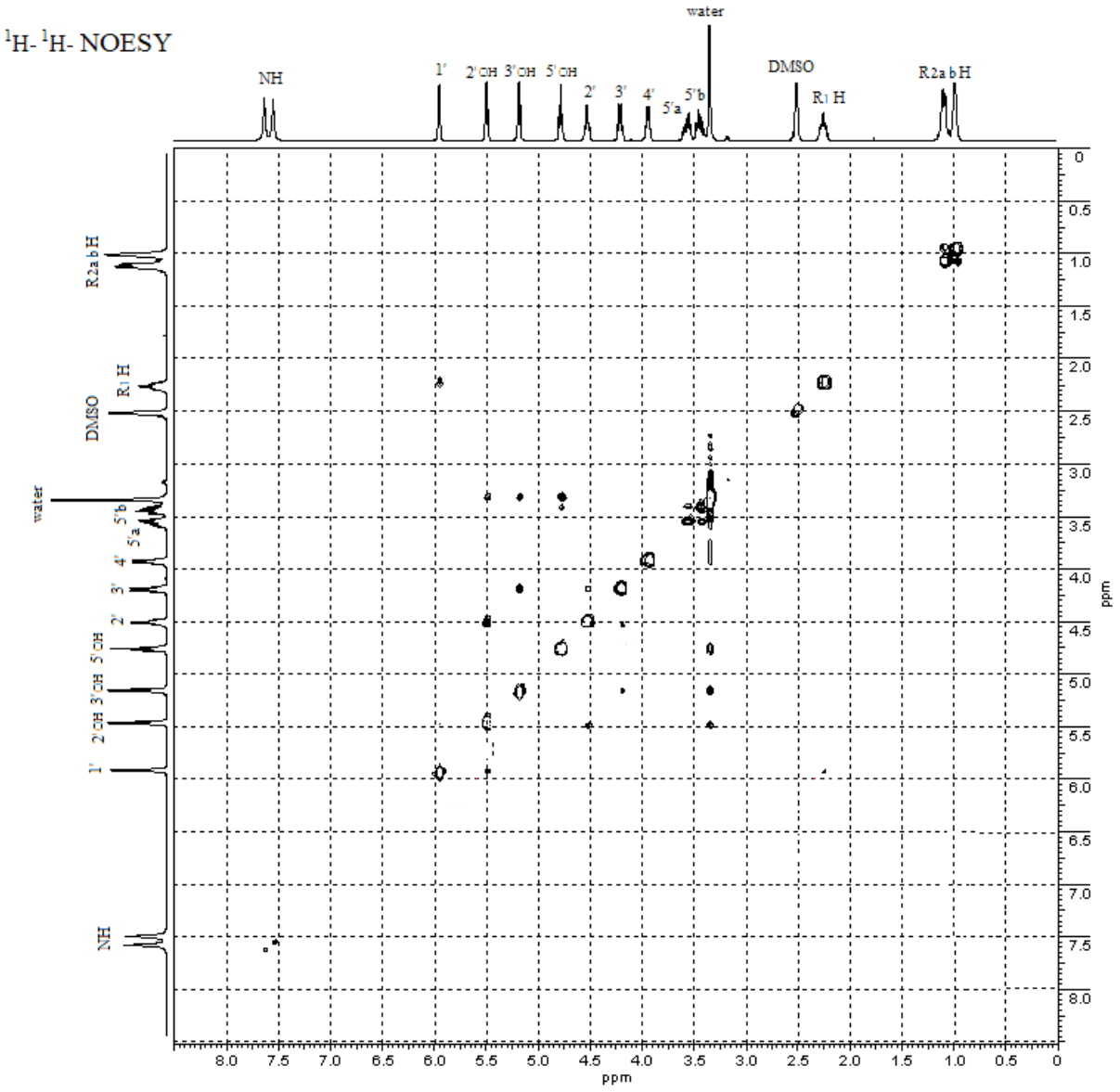
^{1}H NMR spectrum (300 MHz, DMSO-d_6) of **6e**



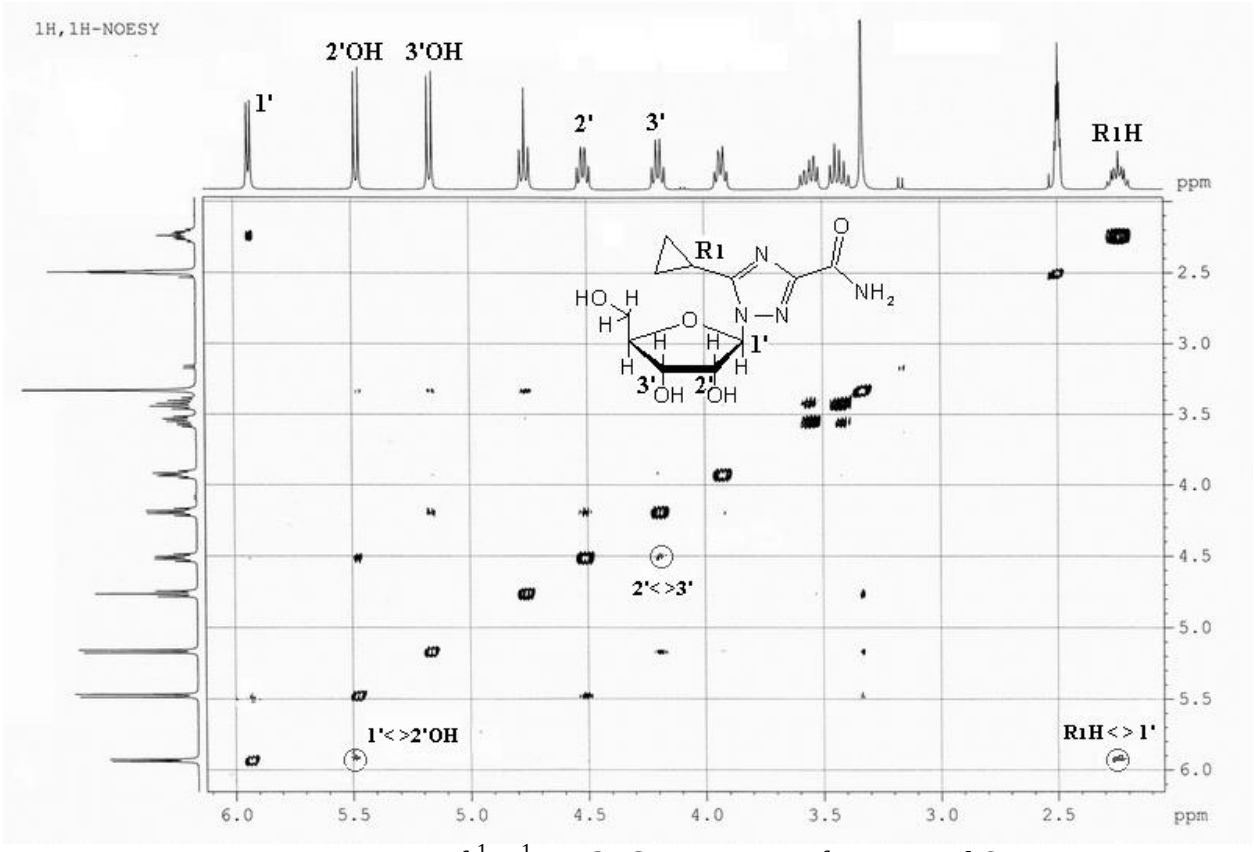
^{13}C NMR spectrum (75 MHz, DMSO-d_6) of **6e**



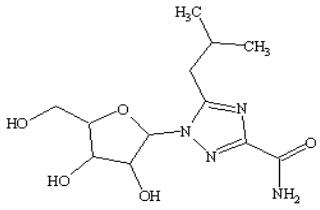
^{1}H-^{1}H COSY spectrum (300 MHz, DMSO-d_6) of **6e**

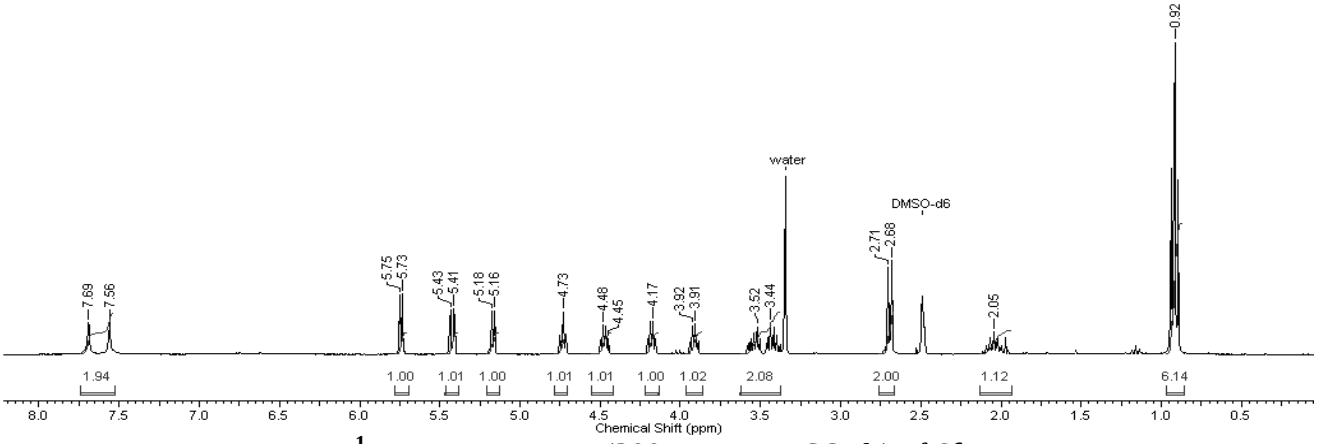


^{1}H-^{1}H NOESY spectrum (300 MHz, DMSO-d_6) of **6e**

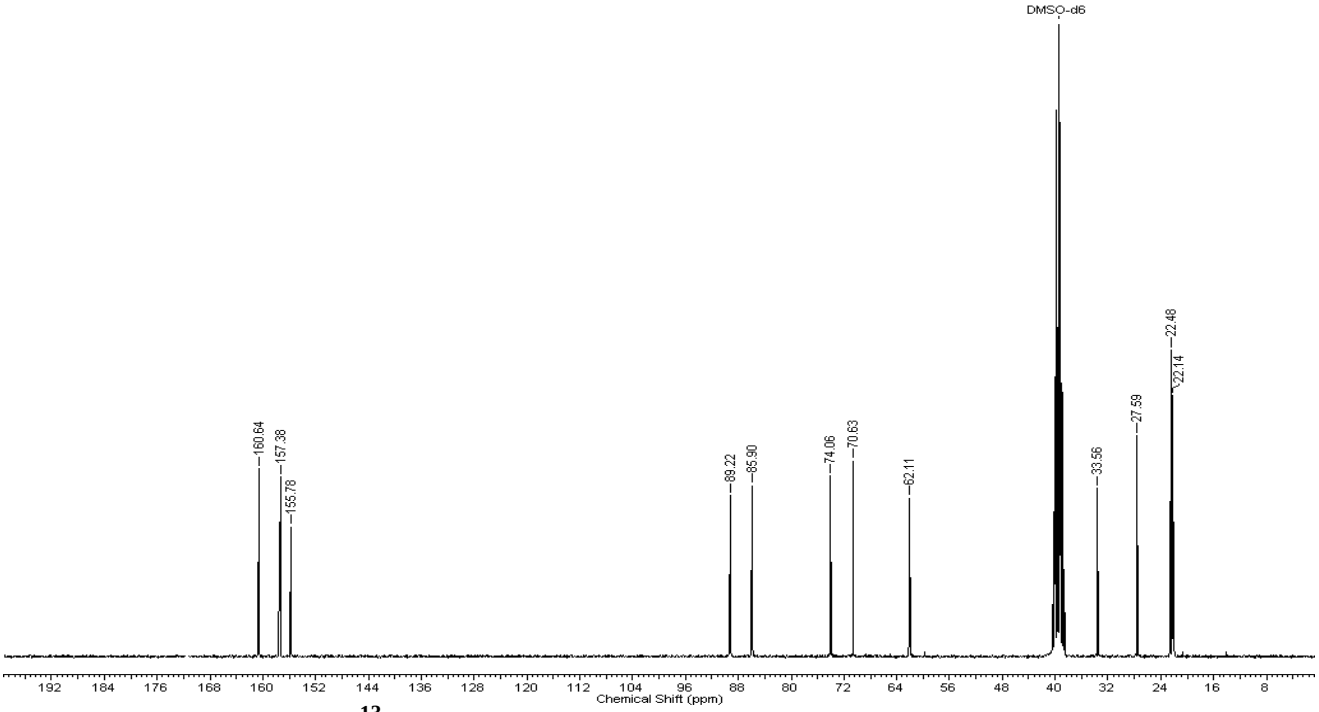


Fragment of ^{1}H-^{1}H NOESY spectrum of compound **6e**

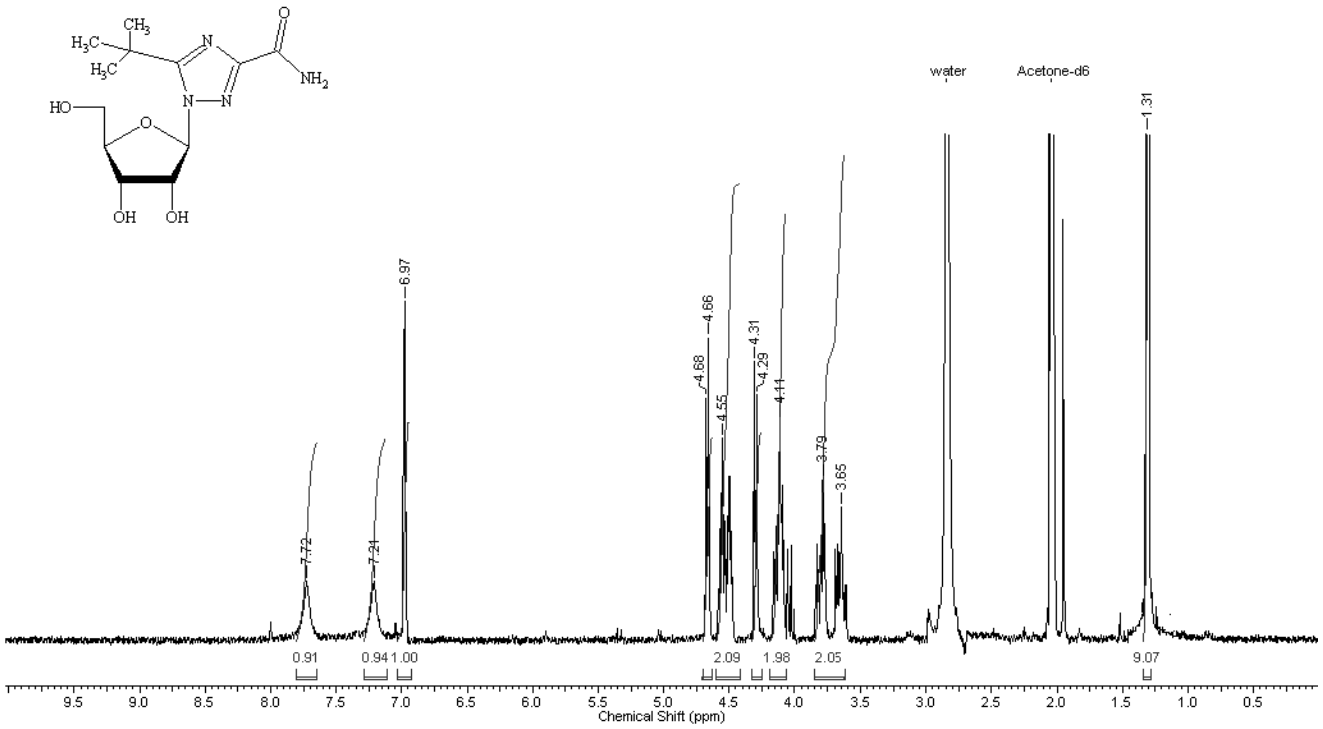




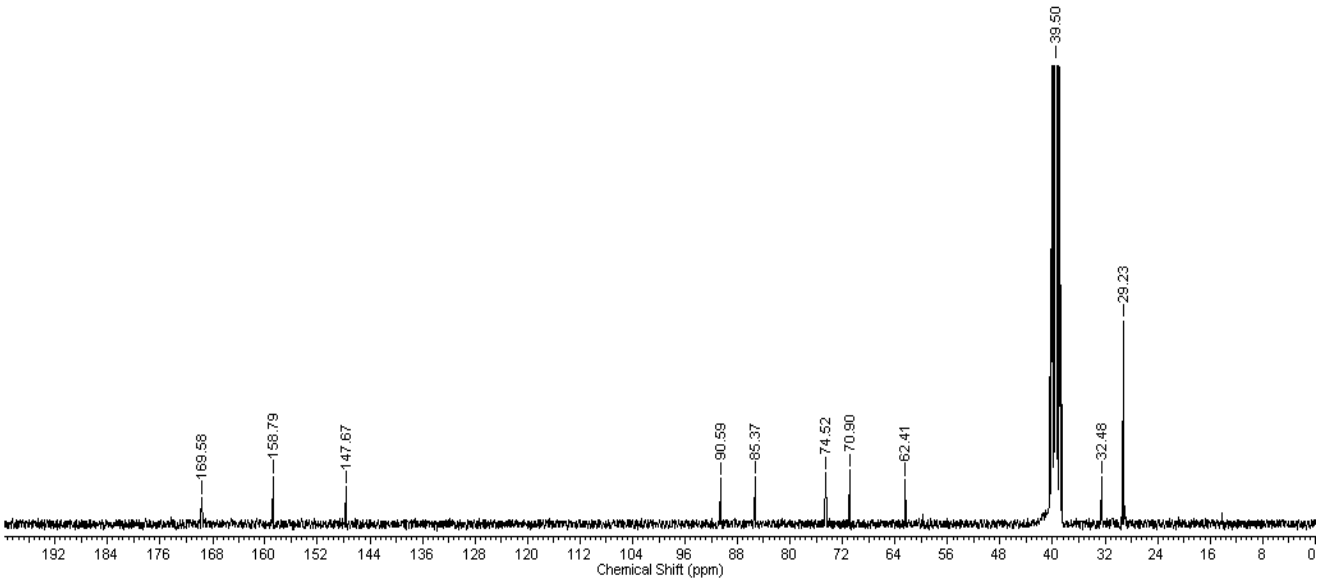
[1]H NMR spectrum (300 MHz, DMSO-d_6) of **6f**



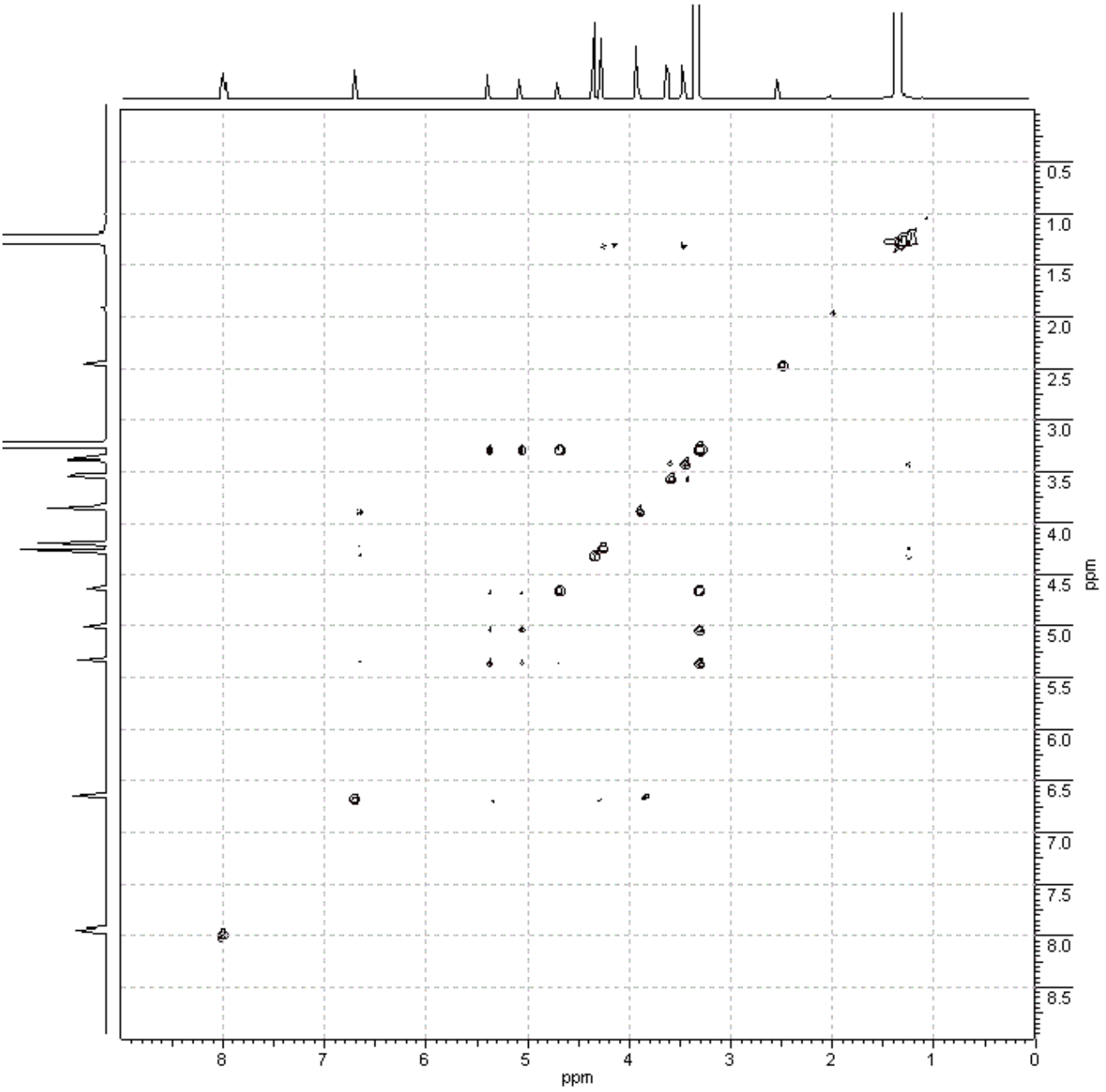
[13]C NMR spectrum (75 MHz, DMSO-d_6) of **6f**



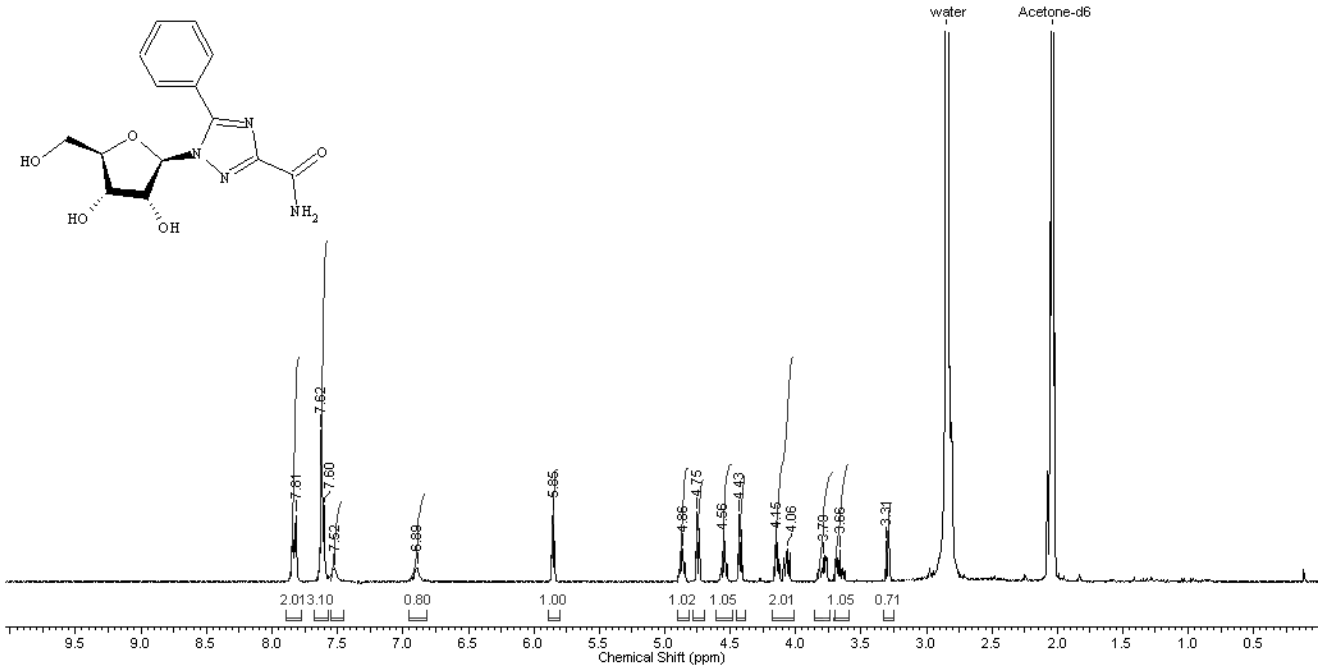
^{1}H NMR spectrum (300 MHz, acetone-d_6) of **6g**



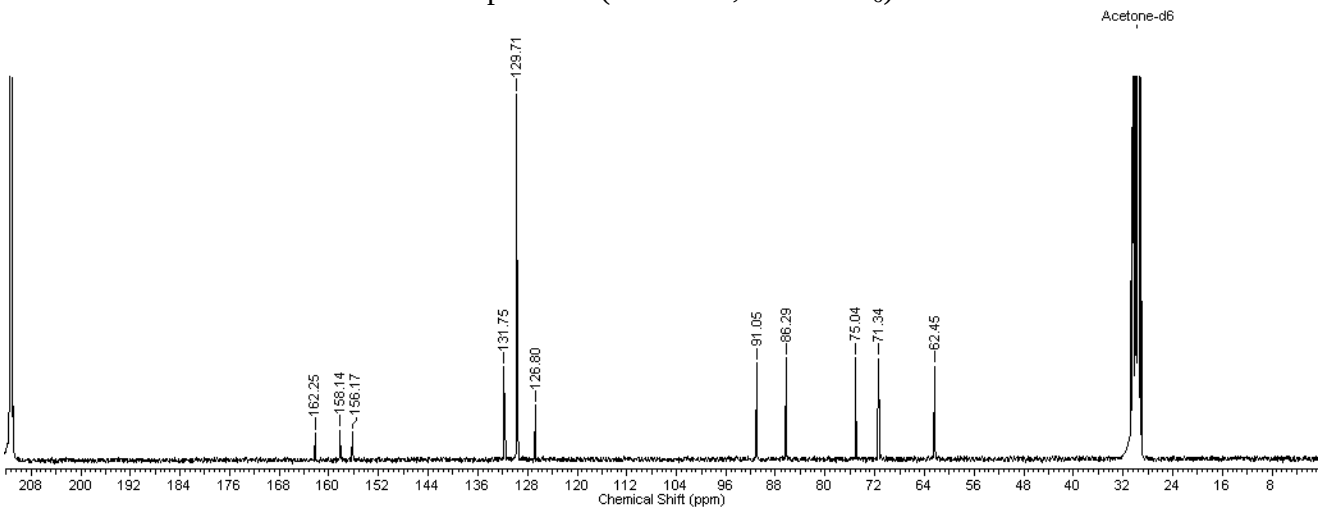
^{13}C NMR spectrum (75 MHz, DMSO-d_6) of **6g**



^{1}H-^{1}H NOESY spectrum (400 MHz, DMSO-d_6) of **6g**



^{1}H NMR spectrum (300 MHz, acetone-d_6) of **6h**



^{13}C NMR spectrum (75 MHz, acetone-d_6) of **6h**

6. Antiviral evaluation.

Anti-HCV activity of the obtained compounds **6a-h** was investigated in a stable Huh7 cell line harbouring the luciferase-encoding full-length HCV replicon (isolate Con1). Cytotoxicity of the ribavirin analogues was accessed in uninfected Huh7 cells by a standard MTT test.

Anti-HSV activity of the obtained compounds (7c-h) was investigated in a VeroE6 cell (kidney cells from green monkeys) line harbouring Herpes simplex virus type 1, strain L2. The VeroE6 cell was grown in medium "Igla" with 5% fetal calf serum and lincomycin hydrochloride (600 U/ml). The VeroE6 cell monolayer culture grown at 37°C under 5% CO_2 for 24 h were used. The evaluation of compounds against herpes virus activity in vitro was performed according to the CPE inhibition assay [2,3] after 48-hour infection. Cytotoxicity of the ribavirin analogues was accessed in uninfected VeroE6 cell by visual inspection of the cell monolayer after 72 h of cultivation in the medium of uninfected cells with the test compound.

Anti-influenza activity of the obtained compounds **6c-h** was investigated in a MDCK cell line harbouring influenza virus A H1N1/USSR/77. The MDCK cell was grown in medium "Igla" with 5% fetal calf serum. The MDCK cell monolayer culture grown at 37°C under 5% CO_2 for 24-hour were used. The evaluation of compounds against influenza virus activity in vitro was performed by visual inspection of the cell monolayer after 72 h of cultivation in the "Igla" medium with 0.2% bovine serum albumin (bovine albumin fraction V 7.5%, Gibco) and 2 mg/ml trypsin-TRNC (tolylsulfonylphenilalanylchloromethylketone-trypsine, Sigma) of infected cells. Cytotoxicity of the ribavirin analogues was accessed in uninfected MDCK cell by visual inspection of the cell monolayer after 72 h of cultivation in the medium of uninfected cells with the test compound.

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2. E. DeClercq, J. Descamps, G. Verheist, R.T. Walker, A.S. Jones, P.F. Torrence and D. Shugar *J. Infect. Dis*., 1980, **141**, 563.

3. I. I. Fedorov, E.M. Kazmina, G.V. Gurskaya, M.V. Jasko, V.E. Zavodnic, J. Balzarini, E. De Clercq, A. Faraj, J.-P. Sommadossi, J.-L. Imbach and G. Gosselin, *J. Med. Chem*., 1997, **40**, 486.