Supplementary Material

NOVEL FLEXIMER PYRAZOLE-CONTAINING ADENOSINE ANALOGUES: CHEMICAL AND HIGLY-EFFICIENT BIOTECHNOLOGICAL SYNTHESIS

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Table S1 Experimental data for the enzymatic synthesis of *β*-D-ribonucleosides (**11**-**13**)[a] and *β*-D-2'-deoxyribosides(**14**-**16**)

Comp.	Accep-tor	Donor	Volume, mL 10 mM KH_2PO_4	**Substrates**				Enzymes				Conversion after 24 h (HPLC data), %	Final conversion (HPLC data), %	Yield (%), (mg) purity
				Acceptor		Donor		PNP,		UP,				
				g (mmol)	C, mM	g (mmol)	C, mM	e.u.(A)[b]	e.u./mL	e.u. (B)[b]	e.u./mL			
11	**8**	Urd	**156**	0.05 (0.312)	2	0.152 (0.62)	4	**167** (535)	**1.07**	**73** (118)	**0.47**	46	74	30 (29) **99.7**
12	**9**		**62**	0.020 (0.125)	2.02	0.061 (0.25)	4	**18.6** (149)	**0.3**	**49.6** (198)	**0.8**	69	96	44 (16) **91.2**
13	**10**		**62**	0.020 (0.125)	2.02	0.061 (0.25)	4	**18.6** (149)	**0.3**	**49.6** (198)	**0.8**	44	99	55 (20) **95.8**
14	**8**	dUrd	**156**	0.05 (0.312)	2	0.142 (0.62)	4	**167** (535)	**1.07**	**73** (198)	**0.47**	81	99	51 (22) **98.3**
15	**9**		**62**	0.020 (0.125)	2.02	0.057 (0.25)	4	**18.6** (149)	**0.3**	**49.6** (198)	**0.8**	95	98	52 (18) **95.2**
16	**10**		**62**	0.020 (0.125)	2.02	0.057 (0.25)	4	**18.6** (149)	**0.3**	**49.6** (198)	**0.8**	89	100	64 (22) **95.1**

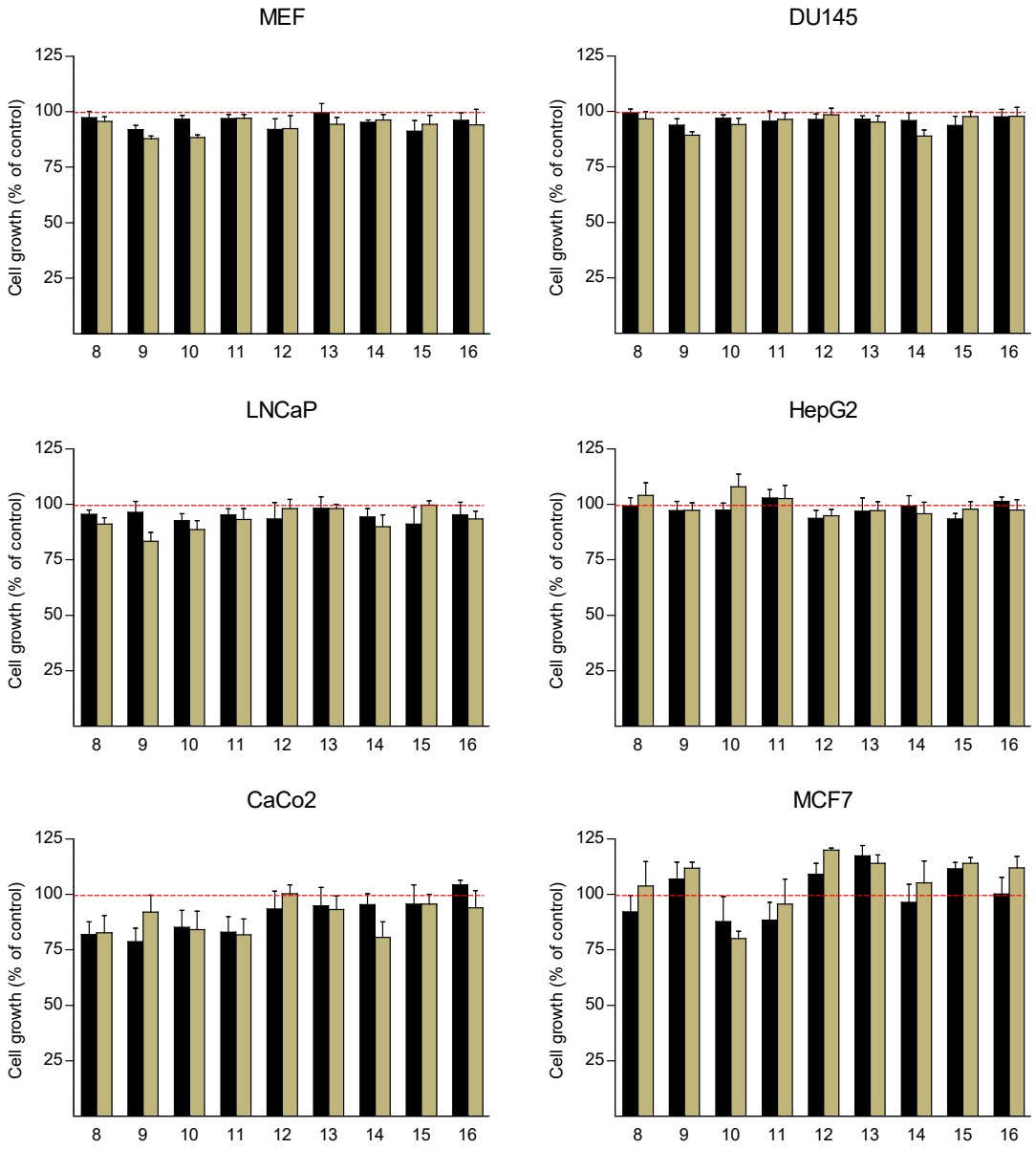
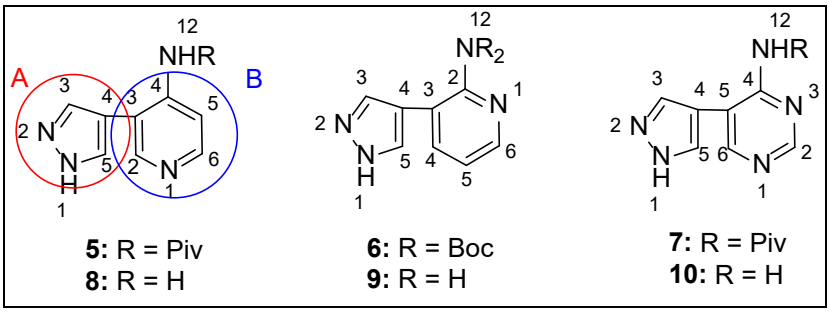
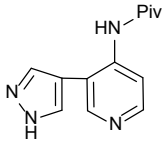


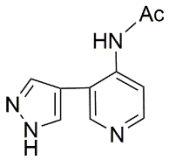
Figure S1. Effect of flexible bases **8-10** and nucleoside analogues **11-16** on the growth of five different human cancer cell lines and non-cancer mouse embryonic fibroblasts (MEF). The cells were treated for 3 days in medium supplemented with 10 µM (black bars) or 100 µM (green bars) compounds and analyzed for resazurin proliferative index. The data are means ± S.D., n=6.

CHARACTERIZATION DATA OF PRODUCTS

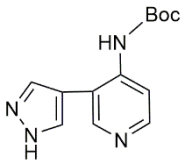




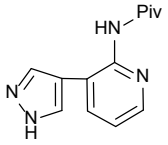
4-(4-Pivaloylaminopyridin-3-yl)-1*H*-pyrazole (5). Compound **5** was synthesized from 3-bromo-4-pivaloylaminopyridine **2** (1.3 g, 3.3mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxoboran-2-yl)-1*H*-pyrazole **1** (850 mg, 3.3mmol). After purification on a silica gel column eluting with chloroform:methanol (95:5), **5** was obtained as a white powder (550 mg, 69%). ^{1}H NMR (300.1 MHz, DMSO-*d6*) δ: 13.19 (1H, s, NH-A), 8.76 (1H, s, H-2B), 8.59 (1H, s, H-5A), 8.38 (1H, d *J* = 5.4 Hz, H-6B), 8.45-8.47 (1H, s, H-3A), 7.84 (1H, s, NH-B), 7.76 (1H, d *J* = 5.5 Hz, H-5B), 1.20 (9H, s, Piv). ^{13}C NMR (75.5 MHz, DMSO-*d6*) δ: 176.5 (C(O)), 150.0 (C-2B), 147.9 (C-6B), 141.9 (C-4B), 137.9 (C-3A), 127.6 (C-5A), 122.0 (C-3B), 118.0 (C-5B), 114.0 (C-4A), 38.6 (C-$(CH_3)_3$), 26.7 (3C, $(CH_3)_3$).



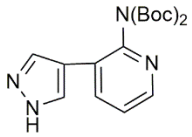
4-(4-Acetylaminopyridin-3-yl)-1*H*-pyrazole. Was synthesized and purified in a similar manner as compounds **5** from 3-bromo-4-acetylaminopyridine (540 mg; 2.5 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxoboran-2-yl)-1*H*-pyrazole **1** (645 mg, 2.5mmol) with 30% yield. ^{1}H NMR (300.1 MHz, *DMSO-d6*) δ: 13.13 (1H, br s, NH-A), 9.33 (1H, s, H-2B), 8.58 (1H, s, H-5A), 8.33 (1H, d *J* = 5.5 Hz, H-6B), 8.14 (1H, br s, H-3A), 7.84 (1H, br s, NH-B), 7.75 (1H, d *J* = 5.5 Hz, H-5B), 1.20 (3H, s, Ac). ^{13}C NMR (75.5 MHz, DMSO-d6) δ: 169.2 (C(O)), 150.0 (C-2B), 147.6 (C-6B), 141.5 (C-4B), 137.6 (C-3A), 127.6 (C-5A), 121.2 (C-3B), 118.0 (C-5B), 114.1 (C-4A), 23.7 (C, CH_3).



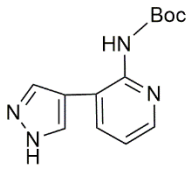
4-(4-*tert*-Butyloxycarbonylamino)pyridine-3-yl)-*1H*-pyrazole. Was synthesized and purified in a similar manner as compounds **5** from 3-bromo-4-*tert*-butyloxycarbonylaminopyridine (740 mg; 2.7mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxoboran-2-yl)-1*H*-pyrazole **1** (690mg, 2.7mmol) with 33% yield.^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.43 (1H, d, *J* = 5.8 Hz, H-6B), 8.36 (1H, s, H-2B), 8.15 (1H, d, *J* = 5.8 Hz, H-5B), 7.75 (2H, s, H-3A, H-5A), 6.88 (1H, s, NH-B), 1.49 (9H, s, Boc). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 151.9 (C(O)), 150.4 (C-2B), 149.7 (C-6B), 143.6 (C-4B), 133.6 (C-3A), 116.8 (C-5A), 114.3 (C-3B), 112.2 (C-5B), 82.0 (C-4A), 29.7 (C-$(CH_3)_3$), 28.1 (3C, $(CH_3)_3$).



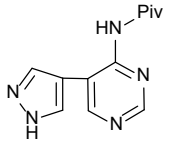
4-(2-Pivaloylaminopyridin-3-yl)-1*H*-pyrazole (6). Compound was synthesized starting from 3-bromo-2-pivaloylaminopyridine **3** (590 mg, 2.3 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxoboran-2-yl)-1*H*-pyrazole **1** (450 mg, 2.3 mmol). After purification on a silica gel column using chloroform: methanol (95:5) system for elution, the yield of compound **6** as white powder was 72% (400 mg). ^{1}H NMR (300.1 MHz, CD_3OD) δ: 8.38 (1H, dd, *J* = 4.8, 1.8 Hz, H-5A), 7.99 (1H, dd, *J* = 7.7, 1.8 Hz, H-6B), 7.86 (2H, s, H-3A, H-4B), 7.41 (1H, dd, *J* = 7.7, 4.8 Hz, H-5B), 1.27 (9H, s, Piv). ^{13}C NMR (75.5 MHz, CD_3OD) δ: 178.7 (C(O)), 147.4 (C-2B), 145.8 (C-4B), 138.2 (C-5B, C-6B), 126.9 (C-3B), 122.5 (C-3A, C-5A), 116.6 (C-4A), 38.2 (C-$(CH_3)_3$), 25.7 (3C, $(CH_3)_3$).



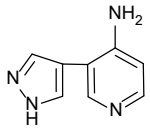
4-(2-(bis(*tert*-Butyloxycarbonyl)amino)pyridin-3-yl)-1*H*-pyrazole. Was synthesized starting from 3-bromo-2-di-tert-butyloxycarbonylaminopyridine (860 mg, 2.3 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxoboran-2-yl)-1*H*-pyrazole **1** (446 mg; 2.3 mmol). After purification on a silica gel column using chloroform: methanol (95:5) system for elution, the yield of compound as white powder was 56% (460mg). ^{1}H NMR (300.1 MHz, $CDCl_3$) δ: 8.45 (1H, dd, *J* = 4.7, 1.8 Hz, H-5A), 7.9 (1H, dd, *J* = 7.7, 1.9 Hz, H-6B), 7.82 (2H, s, H-3A, H-4B), 7.33 (1H, ddd, *J* = 7.7, 4.7, 0.7 Hz, H-5B), 1.29 (18H, s, Boc_2). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 150.8 (2×C(O)), 148.8 (C-2B), 146.9 (C-4B), 137.0 (C-6B), 132.6 (C-5B), 126.9 (C-3B), 123.5 (C-3A, C-5A), 117.0 (C-4A), 83.1 (2×C, $C(CH_3)_3$), 27.7 (6C, 2x$(CH_3)_3$).



4-(2-*tert*-Butyloxycarbonylamino)pyridine-3-yl)-*1H*-pyrazole. The title compound was obtained together with compound **6** and the same purification procedure gave the product as white powder with 27% (160 mg) yield. ^{1}H NMR (300.1 MHz, $CDCl_3$) δ: 8.47 (1H, dd, *J* = 4.7, 1.9 Hz, H-5A), 7.84 (2H, s, H-3A, H-4B), 7.66 (1H, dd, *J* = 7.6, 1.9 Hz, H-6B), 7.11 (1H, dd, *J* = 7.6, 4.9 Hz, H-5B), 1.47 (s, 9H, Boc). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 152.0 (C(O)), 148.5 (C-2B), 146.7 (C-4B, C-6B), 132.9 (C-5B), 120.2 (C-3B), 119.8 (C-3A, C-5A), 116.7 (C-4A), 81.2 (C-$(CH_3)_3$), 28.2 (3C, $(CH_3)_3$).

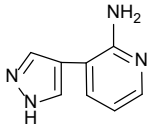


4-(4-Pivaloylaminopyrimidin-5-yl)-1*H*-pyrazole (7). Was synthesized starting from 4-pivaloylamino-5-bromopyrimidine **4** (550 mg, 2.8 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxoboran-2-yl)-1*H*-pyrazole **1** (722 mg; 2.8 mmol). Was purified on a silica gel column using chloroform: methanol (98:2) to (95:5) system for elution and obtained as white powder with 74% (515mg) yield. ^{1}H NMR (300.1 MHz, DMSO-*d6*) δ: 13.09 (1H, s, NH-A), 9.79 (1H, s, NH-B), 8.94 (1H, s, H-2B), 8.90 (1H, s, H-6B), 8.09 (1H, s, H-5A), 7.82 (1H, s, H-3A), 1.20 (9H, s, Piv). ^{13}C NMR (75.5 MHz, DMSO-*d6*) δ: 176.9 (C(O)), 157.4 (C-2B), 156.1 (C-6B), 155.5 (C-4B), 137.9 (C-3A), 127.8 (C-5A), 123.7 (C-5B), 114.2(C-4A), 40.9 (C-$(CH_3)_3$), 27.31 (3C, $(CH_3)_3$).

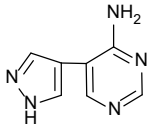


4-(4-Aminopyridin-3-yl)-1*H*-pyrazol (8). Was obtained from **5** (500 mg, 2 mmol) by reflux in methanol with K_2CO_3 (360 mg, 2.6 mmol) during 12 h. Purification on a silica gel column using chloroform: methanol (8:2) system for elution gave the product **8** as white powder with 84% (275 mg) yield. ^{1}H NMR (300.1 MHz, DMSO-*d6*) δ: 8.14 (1H, s, H-2B), 7.94 (1H, d, *J* = 5.6 Hz, H-6B), 7.89

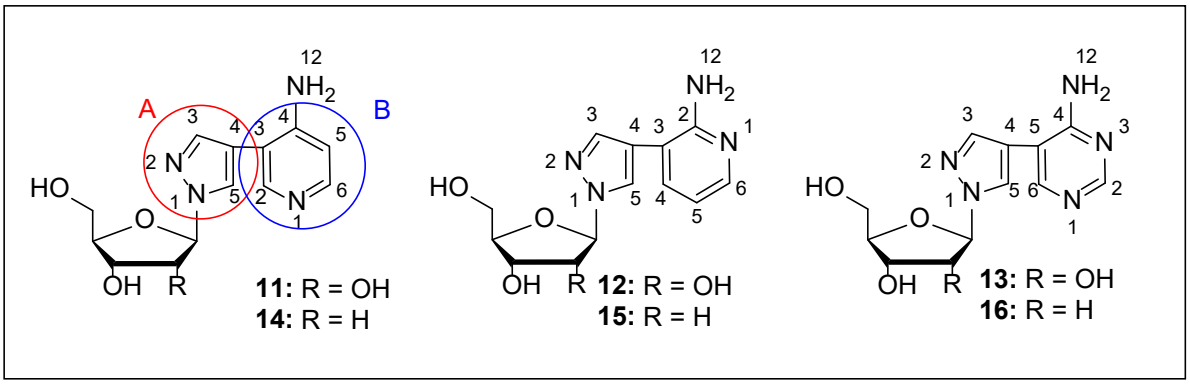
(2H, s, H-3A, H-5A), 6.68 (1H, d, *J* = 5.7 Hz, H-5B), 5.93 (2H, s, NH_2). ^{13}C NMR (75.5 MHz, DMSO-*d6*) δ: 151.9 (C-4B), 148.4 (C-2B), 147.0 (C-6B), 132.6 (C-3A, C-5A), 115.1 (C-3B), 114.0 (C-4A), 109.88 (C-5B). HRMS *m/z*: calculated for $C_8H_8N_4$ $[M+H]^+$ 161.0822; found $[M+H]^+$ 161.0822; UV: λ_{max} 285 (ε 6700).

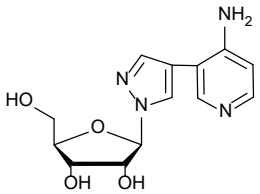


4-(2-Aminopyridin-3-yl)-1*H*-pyrazole (9). Was obtained from **6** (350 mg, 1.4 mmol) by reflux in methanol with K_2CO_3 (250 mg, 1.8 mmol) during 15 h. The solvent was removed *in vacuo* and the residue was purified on a silica gel column using chloroform: methanol (8:2) system for elution to give the product **9** as pale-yellow powder with 71% (160 mg) yield. ^{1}H NMR (300.1 MHz, DMSO-*d6*) δ 13.01 (1H, s, NH), 8.01 (1H, br s, H-5A), 7.88 (1H, dd, *J* = 4.9, 1.8 Hz, H-6B), 7.80 (1H, br s, H-3A), 7.50 (1H, dd, *J* = 7.4, 1.8 Hz, H-4B), 6.62 (1H, dd, *J* = 7.4, 4.9 Hz, H-5B), 5.56 (2H, s, NH_2). ^{13}C NMR (75.5 MHz, DMSO-*d6*) δ: 156.8 (C-6B), 146.21 (C-2B), 138.0 (C-3A), 136.5 (C-4B), 127.0 (C-5A), 117.5 (C-3B), 113.6 (C-5B), 113.1 (C-4A). HRMS *m/z*: calculated for $C_8H_8N_4$ $[M+H]^+$ 161.0822; found $[M+H]^+$ 161.0833. λ_{max} 310 (ε 4800).



4-(4-Aminopyrimidin-5-yl)-1*H*-pyrazole (10). Was synthesized starting from **7** (275mg, 1.1mmol) by reflux in methanol with K_2CO_3 (193 mg, 1.4 mmol) during 12 h. Purification on a silica gel column using chloroform: methanol (9:1) to (8:2) system for elution gave product **10** as white powder with 69% (120 mg) yield. ^{1}H NMR (300 MHz, DMSO-*d6*) δ: 13.11 (1H, s, NH), 8.30 (1H, s, H-2B), 8.18 (1H, s, H5A), 8.07 (1H, s, H-6B), 7.80 (1H, s, H-3A), 6.58 (2H, s, NH_2). ^{13}C NMR (75.5 MHz, DMSO-*d6*) δ: 160.8 (C-4B), 156.7 (C-2B), 153.7 (C-6B), 137.9 (C-3A), 127.4 (C-5A), 113.7 (C-5B), 110.6 (C-4A). HRMS *m/z*: calculated for $C_7H_7N_5$ $[M+H]^+$ 162.0774 found $[M+H]^+$ 162.0774; UV: λ_{max} 289 (ε 8000).



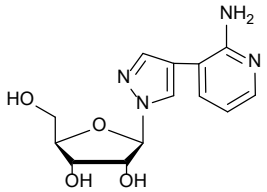


1-(β-*D*-Ribofuranosyl)-4-(4-aminopyridin-3-yl)pyrazole (11).

The reaction mixture (31.2 mL in total) contained 60 mM uridine (457 mg), 20 mM **8** (100 mg), 30 mM potassium phosphate buffer pH 7.0, 50 μg of *E. coli* cells overexpressing recombinant UP (dry weight, 54 units of activity), 65 mg of *E. coli* cells overexpressing recombinant PNP (dry weight, 15730 units of activity). The reaction mixture was stirred 24 h at 57 °C until the

yield of the product in reaction mixture reached 90% according to RP HPLC, buffer for the elution contained 1% acetonitrile. The reaction mixture was applied on 45 mL of Dowex-1x8 resin (HO^-). The resin was thoroughly washed with water (400 mL). Fleximer **11** was eluted with 60% ethanol. The eluent was evaporated to dryness *in vacuo* to give 80 mg of 95% pure (RP HPLC) **11**. The residue was dissolved in water (100 mL) and applied onto the column (2.7 x 180 mm) of reverse phase Chromatorex C18 (100Å, 20-45 µm; Fuji Silysia Chemical Ltd). The reverse phase was washed with 10% acetonitrile (250 mL) and the target product was eluted with 20% acetonitrile. The solution was evaporated to dryness *in vacuo* and the residue was thoroughly dried in high *vacuo* to give 51 mg (28%) of **11** as white powder.

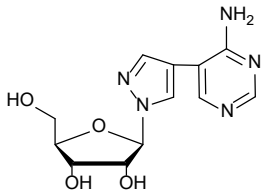
^{1}H NMR (600.2 MHz, D_20, TSP) δ: 8.21, (1 H, s, H-5A), 8.06 (1 H, s, H-2B), 8.00 (1 H, d, *J* = 7.0, H-6B), 7.89 (1 H, s, H-3A), 6.97 (1 H, d, *J* = 7.0, H-5B), 5.91 (1 H, d, *J* = 5.2, H-1'), 4.68 (1 H, t, *J* = 5.2, H-2'), 4.39 (1 H, t, *J* = 4.8, H-3'), 4.23-4.18 (1 H, m, H-4'), 3.85 (1 H, dd, *J* = 12.6, 3.4, Ha-5'), 3.75 (1 H, dd, *J* = 12.6, 5.2, Hb-5'). ^{13}C NMR (150.9 MHz, D_20, TSP) δ: 159.3 (C-4B), 141.7 (C-2B), 139.7 C-6B), 139.2 (C-3A), 131.7 (C-5A), 114.5 (C-3B), 114.1 (C-4A), 110.3 (C-5B), 93.5 (C-1'), 85.8 (C-4'), 75.0 (C-2'), 71.1 (C-3'), 62.3 (C-5'). ^{15}N NMR (71 MHz, DMSO-d_6) δ: 303.97 (N2), 279.58 (N1), 222.72 (N1), 67.79 (6-NH_2). λ_{max} 273 (ε 5500). HRMS *m/z*: calculated for $C_{13}H_{16}O_4N_4$ $[M+H]^+$ 293.1244; found $[M+H]^+$ 293.1197.



1-(β-*D*-Ribofuranosyl)-4-(2-aminopyridin-3-yl)pyrazole (12).

The reaction mixture (13.5 mL in total) contained 60 mM Uridine (198 mg), 20 mM **9** (43.3 mg), 30 mM potassium phosphate buffer pH 7.0, 13.5 µg of *E. coli* cells overexpressing recombinant UP (dry weight, 14.7 units of activity), 13.5 mg of *E. coli* cells overexpressing recombinant PNP (dry weight, 3267 units of activity). The reaction mixture was stirred 7 h at 57 ^{o}C until the yield of the product in reaction mixture reached 95.4% according to RP HPLC, buffer for the elution contained 10% acetonitrile. The reaction mixture was applied on 25 mL of Dowex-1x8 resin (HO^-). The resin was thoroughly washed with water (300 mL). Fleximer **12** was eluted with 60% ethanol. The eluent was evaporated to dryness *in vacuo* and the residue was thoroughly dried in high *vacuo* to give 36 mg (45.6%) of **12** as white powder.

^{1}H NMR (600.2 MHz, D_20, TSP) δ: 8.13, (1 H, s, H-5A), 7.95 (1 H, dd, *J* = 5.1, 1.5, H-6B), 7.89 (1 H, s, H-3A), 7.60 (1 H, dd, *J* = 7.4, 1.7, 1H, H-4B), 6.89 (1 H, dd, *J* =7.4, 5.2, H-5B), 5.90 (1 H, d, *J* = 5.2, H-1'), 4.67 (1 H, t, *J* = 5.2, H-2'), 4.41 (1 H, dd, *J* = 4.8, 5.0, H-3'), 4.24-4.18 (1 H, m, H-4'), 3.87 (1 H, dd, *J* = 12.6, 3.5, Ha-5'), 3,77 (1 H, dd, *J* = 12.6, 5.2, Hb-5'). ^{13}C NMR (150.9 MHz, D_20, TSP) δ: 156.7 (C-6B), 146.7 (C-2B), 141.4 (C-3A), 139.7 (C-4B), 130.4 (C-5A), 119.2 (C-3B), 116.1 (C-5B), 114.6 (C-4A), 93.4 (C-1'), 85.6 (C-4'), 74.9 (C-2'), 71.1 (C-3'), 62.3 (C-5'). ^{15}N NMR (71 MHz, DMSO-d_6) δ 303.54 (N2), 270,63 (N1), 222.20 (N1), 76.22 (6-NH_2). λ_{max} 305 (ε 5800). HRMS *m/z*: calculated for $C_{13}H_{16}O_4N_4$ $[M+H]^+$ 293.1244; found $[M+H]^+$ 293.1241.

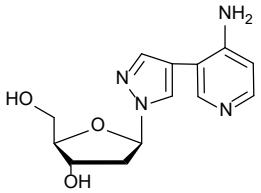


1-β-*D*-Ribofuranosyl-4-(4-aminopyrimidin-5-yl)pyrazole (13).

The reaction mixture (60 mL in total) contained 60 mM Uridine (908 mg), 20 mM **10** (210 mg), 30 mM potassium phosphate buffer pH 7.0, 60 µg of *E. coli* cells overexpressing recombinant UP (dry weight, 65.4 units of activity), 62 mg of *E. coli* cells

overexpressing recombinant PNP (dry weight, 14520 units of activity). The reaction mixture was stirred 10 h at 57 °C until the yield of the product in reaction mixture reached 98% according to RP HPLC, buffer for the elution contained 5% acetonitrile. The reaction mixture was applied on 80 mL of Dowex-1x8 resin (HO^-). The resin was thoroughly washed with water (1200 mL). Fleximer **13** was eluted with 60% ethanol. The eluent was evaporated *in vacuo* to dryness and the residue was thoroughly dried in high *vacuo* to give 205 mg (53.8%) of **13** as white powder.

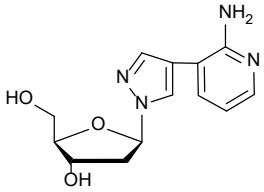
^{1}H NMR (600.2 MHz, $D_2$0, TSP) δ: 8.35, (1 H, s, 1H, H-2B), 8.17 (1 H, s, H-5A), 8.13 (1 H, s, H-6B), 7.89 (1 H, s, H-3A), 5.91 (1 H, d, *J* = 5.2, H-1'), 4.68 (1 H, t, *J* = 5.2, H-2'), 4.41 (1 H, dd, *J* = 4.8, 5.0, H-3'), 4.24-4.19 (1 H, m, H-4'), 3.87 (1 H, dd, *J* = 12.6, 3.5, Ha-5'), 3.77(1 H, dd, *J* = 12.6, 5.2, Hb-5'). ^{13}C NMR (150.9 MHz, $D_2$0, TSP) δ: 162.1 (C-4B), 157.1 (C-2B), 154.2 (C-6B), 141.3 (C-3A), 130.9 (C-5A), 115.5 (C-5B), 111.3 (C-4A), 93.4 (C-1'), 85.7 (C-4'), 74.9 (C-2'), 71.1 (C-3'), 62.3 (C-5'). ^{15}N NMR (71 MHz, DMSO-d_6) δ: 304.05 (N2), 261.38 (N3), 249.97 (N1), 223.24 (N1), 83.27 (6-NH_2). λ_{max} 285 (ε 5200). HRMS *m/z*: calculated for $C_{12}H_{15}O_4N_5$ $[M+H]^+$ 294.1197; found $[M+H]^+$ 294.1198.



1-(β-*D*-2'-Deoxyribofuranosyl)-4-(4-aminopyridin-3-yl)pyrazole (14).

The reaction mixture (28.1 mL in total) contained 60 mM Thymidine (408 mg), 20 mM **8** (90 mg), 30 mM potassium phosphate buffer pH 7.0, 77 μg of *E. coli* cells overexpressing recombinant TP (dry weight, 152 units of activity), 56 mg of *E. coli* cells overexpressing recombinant PNP (dry weight, 13552 units of activity). The reaction mixture was stirred 60 h at 47 °C until the yield of the product in reaction mixture reached 93% according to RP HPLC, buffer for the elution contained 3% acetonitrile. The reaction mixture was applied on 70 mL of Dowex-1x8 resin (HO^-). The resin was thoroughly washed with water (600 mL). Fleximer **14** was eluted with 60% ethanol. The eluent was evaporated to dryness *in vacuo* and the residue was thoroughly dried in high *vacuo* to give 73 mg (45.3%) of **14** as light-yellow viscous oil.

^{1}H NMR (600.2 MHz, $D_2$0, TSP) δ: 8.12, (1 H, s, H-5A), 8.10 (1 H, s, H-2B), 8.05 (1 H, d, *J* = 5.4, H-6B), 7.85 (1 H, s, H-3A), 6.83 (1 H, d, *J* = 5.7, H-5B), 6.31 (1 H, t, *J* = 6.5, H-1'), 4.63-4.59 (1 H, m, H-3'), 4.14-4.09 (1 H, m, H-4'), 3.79 (1 H, dd, *J* = 12.3, 4.0, Ha-5'), 3.69 (1 H, dd, *J* = 12.3, 5.9, Hb-5'), 2.88-2.81 (1 H, m, Ha-2'), 2.57-2.50 (1 H, m, Hb-2'). ^{13}C NMR (150.9 MHz, $D_2$0, TSP) δ: 153.6 (C-4B), 148.7 (C-2B), 147.8 (C-6B), 141.1 (C-3A), 130.3 (C-5A), 116.7 (C-3B), 114.8 (C-4A), 111.1 (C-5B), 90.1 (C-1'), 87.8 (C-4'), 72.0 (C-3'), 62.6 (C-5'), 39.5 (C-2'). ^{15}N NMR (71 MHz, DMSO-d_6) δ: 302.52 (N2), 273.49 (N1), 226.21 (N1), 68.88 (6-NH_2). λ_{max} 272 (ε 7000). HRMS *m/z*: calculated for $C_{13}H_{16}O_3N_4$ $[M+H]^+$ 277.1295; found $[M+H]^+$ 277.1294.

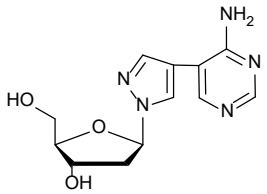


1-(β-*D*-2'-Deoxyribofuranosyl)-4-(2-aminopyridin-3-yl)pyrazole (15).

The reaction mixture (19 mL in total) contained 60 mM Thymidine (276 mg), 20 mM **9** (59 mg), 30 mM potassium phosphate buffer pH 7.0, 31.3 μg of *E. coli* cells overexpressing recombinant TP (dry weight, 61.9 units of activity), 19 mg of *E. coli* cells overexpressing recombinant PNP (dry weight, 4598 units of activity). The reaction mixture was stirred 8 h at 47 °C until the yield of the product in reaction mixture reached 93% according to RP HPLC, buffer for the elution contained 10 % acetonitrile. The

reaction mixture was applied on 25 mL of Dowex-1x8 resin (HO^-). The resin was thoroughly washed with water (300 mL). Fleximer **15** was eluted with 60% ethanol. The eluent was evaporated to dryness *in vacuo* and the residue was thoroughly dried in high *vacuo* to give 43 mg (45.3%) of **15** as light-yellow viscous oil.

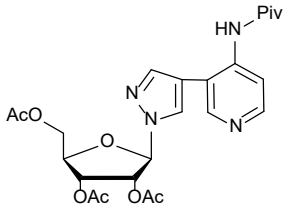
^{1}H NMR (600.2 MHz, D_20, TSP) δ: 8.12, (1 H, s, H-5A), 7.96 (1 H, dd, *J* = 5.2, 1.6, H-6B), 7.88 (1 H, s, H-3A), 7.62 (1 H, dd, *J* = 7.5, 1.7, H-4B), 6.89 (1 H, dd, *J* = 7.4, 5.2, H-5B), 6.30 (1 H, t, *J* = 6.5, H-1'), 4.63-4.59 (1 H, m, H-3'), 4.13-4.10 (1 H, m, H-4'), 3.79 (1 H, dd, *J* = 12.3, 4.0, Ha-5'), 3.69 (1 H, dd, *J* = 12.3, 5.8, Hb-5'), 2.87-2.81 (1 H, m, Ha-2'), 2.56-2.50 (1 H, m, Hb-2'). ^{13}C NMR (150.9 MHz, D_20, TSP) δ: 156.7 (C-2B), 146.8 (C-6B), 140.9 (C-3A), 139.6 (C-4B), 130.0 (C-5A), 119.1 (C-3B), 116.2 (C-5B), 114.7 (C-4A), 90.1 (C-1'), 87.8 (C-4'), 71.9 (C-3'), 62.6 (C-5'), 39.5 (C-2'). ^{15}N NMR (71 MHz, DMSO-d_6) δ: 302.96 (N2), 271,19 (N1), 225.96 (N1), 73.253 (6-NH_2). λ_{max} 309 (ε 5000). HRMS *m/z*: calculated for $C_{13}H_{16}O_3N_4$ $[M+H]^+$ 277.1295; found $[M+H]^+$ 277.1287.



1-(β-*D*-2'-Deoxyribofuranosyl)-4-(4-aminopyrimidin-5-yl)pyrazole (16).

The reaction mixture (15.2 mL in total) contained 60 mM Thymidine (220.7 mg), 20 mM **10** (48.6 mg), 30 mM potassium phosphate buffer pH 7.0, 25 µg of *E. coli* cells overexpressing recombinant TP (dry weight, 49.5 units of activity), 15.2 mg of PNP-MC (dry weight, 3678 units of activity). The reaction mixture was stirred 12 h at 47 °C until the yield of the product in reaction mixture reached 94% according to RP HPLC, buffer for the elution contained 5 % acetonitrile. The reaction mixture was applied on 25 mL of Dowex-1x8 resin (HO^-). The resin was thoroughly washed with water (300 mL). Fleximer **16** was eluted with 60% ethanol. The eluent was evaporated to dryness *in vacuo* and the residue was thoroughly dried in high vacuo to give 43 mg (51.4%) of **16** as light-yellow viscous oil.

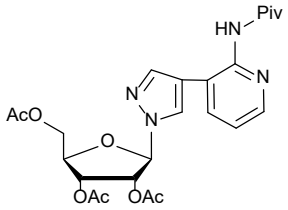
^{1}H NMR (600.2 MHz, D_20, TSP) δ: 8.35 (1 H, s, H-2B), 8.14 (2 H, s, H-5A, H-6B), 7.87 (1 H, s, H-3A), 6.30 (1 H, t, *J* = 6.5, H-1'), 4.63-4.59 (1 H, m, 1H, H-3'), 4.14-4.09 (1 H, m, H-4'), 3.79 (1 H, dd, *J* = 12.3, 4.0, Ha-5'), 3.69 (1 H, dd, *J* = 12.3, 5.9, Hb-5'), 2.87-2.81 (1 H, m, 1H, Ha-2'), 2.57-2.51 (1 H, m, Hb-2'). ^{13}C NMR (150.9 MHz, D_20, TSP) δ: 162.0 (C-4B), 157.0 (C-2B), 154.1 (C-6B), 140.9 (C-3A), 130.4 (C-5A), 115.4 (C-5B), 111.4 (C-4A), 90.2 (C-1'), 87.8 (C-4'), 71.9 (C-3'), 62.6 (C-5'), 39.5 (C-2'). ^{15}N NMR (71 MHz, DMSO-d_6) δ: 303.33 (N2), 261,31 (N3), 249,45 (N1), 226.82 (N1), 82.53 (6-NH_2). λ_{max} 285 (ε 5300). HRMS *m/z*: calculated for $C_{12}H_{15}O_3N_5$ $[M+H]^+$ 278.1248; found $[M+H]^+$ 278.1247.



1-(β-*D*-(2',3',5'-Triacetylribofuranosyl))-4-(4-pivaloylaminopyridin-3-yl)pyrazole (17).

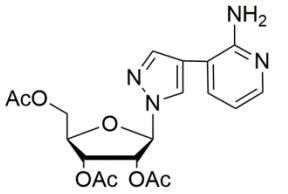
Was synthesized starting from 4-(4-pivaloylaminopyridin-3-yl)-1*H*-pyrazole **5**. After purification on a silica gel column using 5% of methanol in chloroform for elution and additional purification by PLC in ethyl acetate the yield of compound **17** as white powder was 51 % (125 mg). ^{1}H NMR (300.1 MHz, $CDCl_3$), δ: 8.46, (1H, d, *J* = 5.9 Hz, H-6B), 8.42-8.34 (2H, m, H-2B, H-5B), 7.79 (1H, s, NH), 7.74 (1H, s, H-3A), 7.71 (1H, s, H-5A), 5.95 (1H, d, *J* = 3.1 Hz, H-1'), 5.80 (1H, dd, *J* = 5.2, 3.1 Hz, H-2'), 5.66 (1H, t, *J* = 5.7 Hz, H-3'), 4.48-4.37 (2H, m, H-5'), 4.26-4.17 (1H, m, H-4'), 2.12 (3H, s, Ac), 2.11 (3H, s, Ac), 2.06 (3H, s, Ac), 1.19 (9H, s, Piv). ^{13}C NMR (75.5

MHz, $CDCl_3$), δ: 177.2 (C(O)Piv), 170.5 (C(O)Ac), 169.6 (C(O)Ac), 169.5 (C(O)Ac), 149.8 (C-2B), 149.7 (C-6B), 143.4 (C-4B), 140.5 (C-3A), 129.0 (C-5A), 117.2 (C-3B), 115.4 (C-4A), 113.9 (C-5B), 91.7 (C-1'), 80.3 (C-4'), 74.5 (C-2'), 71.1 (C-3'), 63.5 (C-5'), 29.8 (C-$C(CH_3)_3$), 27.4 ($(CH_3)_3$), 20.8 (C-Ac), 20.6 (2C, 2×Ac).

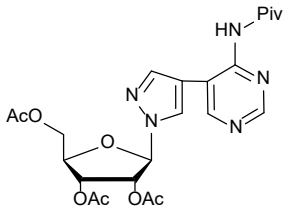


1-(β-*D*-(2',3',5'-Triacetylribofuranosyl))-4-(2-pivaloylaminopyridin-3-yl)pyrazole (18).

Was synthesized starting from 4-(2-pivaloylaminopyridin-3-yl)-1*H*-pyrazole **6**. After purification on a silica gel column using 5% of methanol in chloroform for elution and additional purification by PLC in ethyl acetate the yield of compound **18** as white powder was 57 % (144 mg). ^{1}H NMR (300.1 MHz, $CDCl_3$), δ: 8.39 (1H, dd, *J* = 4.7, 1.5 Hz, H-6B), 7.94 (1H, br s, NH), 7.75 (1H, s, H-5A), 7.71 (1H, s, H-3A), 7.68 (1H, dd, *J* = 7.6, 1.8 Hz, H-4B), 7.19 (1H, dd, *J* = 7.6, 4.8 Hz, H-5B), 5.90 (1H, d, *J* = 3.2 Hz, H-1'), 5.81 (1H, dd, *J* = 5.2, 3.2 Hz, H-2'), 5.69 (1H, t, *J* = 5.4 Hz, H-3'), 4.47-4.37 (2H, m, H-5'), 4.20 (1H, dd, *J* = 6.3, 6.3 Hz, H-4'), 2.12 (3H, s, Ac), 2.11 (3H, s, Ac), 2.07 (3H, s, Ac), 1.23 (9H, s, Piv). ^{13}C NMR (176 MHz, $CDCl_3$) δ: 176.2 (C(O)Piv), 170.0 (C(O)Ac), 169.0 (C(O)Ac), 168.8 (C(O)Ac), 147.9 (C-6B), 146.5 (C-2B), 139.6 (C-3A), 138.2 (C-4B), 127.8 (C-5A), 122.9 (C-3B), 120.9 (C-5B), 118.5 (C-4A), 90.9 (C-1'), 79.6 (C-4'), 73.8 (C-2'), 70.6 (C-3'), 63.0 (C-5'), 29.1 (C-$(CH_3)_3$), 26.8 (3C, $(CH_3)_3$), 20.2 (C-Ac), 20.0 (C-Ac), 19.9 (C-Ac).

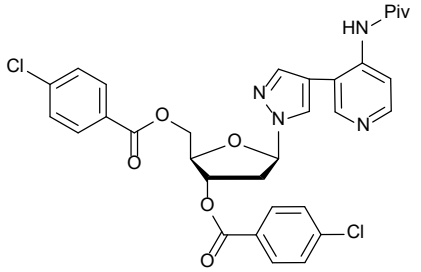


1-(β-*D*-(2',3',5'-Triacetylribofuranosyl))-4-(2-aminopyrimidin-5-yl)pyrazole. Was synthesized starting from 4-(2-(bis(*tert*-butyloxycarbonyl)amino)pyridin-3-yl)-1*H*-pyrazole. After purification on a silica gel column using 5% of methanol in chloroform for elution and additional purification by PLC in ethyl acetate the yield of compound **18** as white powder was 27 % (56 mg). ^{1}H NMR (300.1 MHz, $CDCl_3$), δ: 7.85 (1H, s, H-5A), 7.81 (1H, dd, *J* = 5.7, 1.7 Hz, H-6B), 7.64 (1H, s, H-3A), 7.53 (1H, dd, *J* = 7.4, 1.7 Hz, H-4B), 6.76 (1H, dd, *J* = 7.4, 5.8 Hz, H-5B), 6.34 – 6.26 (1H, m, H-1'), 6.15 (2H, brs, NH_2), 5.60 (1H, t, *J* = 5.6 Hz, H-2'), 5.36 (1H, dd, *J* = 5.9, 5.2 Hz, H-3'), 4.67-4.63 (1H, m, H-4'), 4.37-4.32 (1H, m, Hb-5'), 4.20-4.15 (1H, m, Ha-5'), 2.08 (3H, s, Ac), 2.03 (3H, s, Ac), 1.85 (3H, s, Ac). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 173.5 (C(O)Ac), 173.2 (C(O)Ac), 172.9 (C(O)Ac), 154.1 (C-6B), 146.6 (C-2B), 139.6 (C-3A), 138.3 (C-4B), 127.2 (C-5A), 113.2 (C-3B), 95.6 (C-5B), 93.7 (C-4A), 98.0 (C-1'), 79.8 (C-4'), 70.3 (C-2'), 70.2 (C-3'), 62.6 (C-5'), 20.3 (C-Ac), 20.0 (C-Ac), 19.7 (C-Ac).



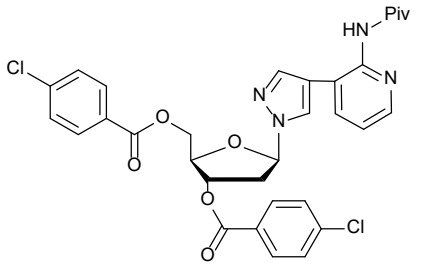
1-(β-*D*-(2',3',5'-Triacetylribofuranosyl))-4-(4-pivaloylaminopyrimidin-5-yl)pyrazole (19). Was synthesized starting from 4-(4-pivaloylaminopyrimidin-5-yl)-1*H*-pyrazole **7**. After purification on a silica gel column using 5% of methanol in chloroform for elution and additional purification by PLC in ethyl acetate the yield of compound **19** as white powder was 68 % (171 mg). ^{1}H NMR (300.1 MHz, CD_3OD), δ: 8.91, (1H, s, H-2B), 8.80 (1H, s, H-5A), 8.13 (1H, s, H-6B), 7.91 (1H, s, 6-NHPiv), 7.88 (1H, s, H-3A),

6.09 (1H, d, *J* = 3.5 Hz, H-1'), 5.82 (1H, dd, *J* = 5.3, 3.5 Hz, H-2'), 5.73-5.66 (1H, m, H-3'), 4.48-4.37 (2H, m, H-5'), 4.26-4.18 (1H, m, H-4'), 2.12 (3H, s, Ac), 2.13 (3H, s, Ac), 2.07 (3H, s, Ac), 1.26 (9H, s, Piv). ^{13}C NMR (75.5 MHz, CD_3OD), δ: 179.4 (C(O)Piv), 172.3 (C(O)Ac), 171.4 (C(O)Ac), 171.1 (C(O)Ac), 158.6 (C-2B), 157.5 (C-6B), 157.3 (C-4B), 141.1 (C-3A), 130.7 (C-5A), 123.3 (C-5B), 116.8 (C-4A), 92.5 (C-1'), 81.4 (C-4'), 75.5 (C-2'), 72.5 (C-3'), 64.5 (C-5'), 40.7 ($\underline{C}$-$C(CH_3)_3$), 27.4 ($(CH_3)_3$), 20.7 (C-Ac), 20.41 (C-Ac), 20.35 (C-Ac).



1-(β-*D*-(3',5'-di-(4-Chlorobenzoyl)-2'-deoxyribofuranosyl))-4-(4-pivaloylaminopyridin-3-yl)pyrazole (20). Was synthesized starting from 4-(4-pivaloylaminopyridin-3-yl)-1*H*-pyrazole **5**. After purification on a silica gel column using 5% of methanol in chloroform for elution and additional purification by PLC in ethyl acetate the yield of **β-20** as inseparable anomeric mixture with 13% of **α** anomer was 19% (60.5 mg). ^{1}H NMR (300.1 MHz, $CDCl_3$), δ: 8.47, (1H, d, *J* =5.7 Hz, H-6B), 8.43-8.34 (2H, m, H-2B, H-5B), 8.00-7.88 (3H, m, 2H-Ph, H-3A), 7.82 (1H, s, NH), 7.79-7.66 (3H, m, H-5A, 2H-Ph), 7.46-7.28 (4H, m, 4H-Ph), 6.30 (1H, dd, *J* = 6.8, 2.2 Hz, H-1'), 5.61 (1H, ddd, *J* = 7.3, 2.9, 2.7 Hz, H-3'), 4.82-4.75 (1H, m, H-4'), 4.67-4.53 (2H, m, 2H-5'), 3.17 (1H, ddd, *J* = 14.9, 4.8, 2.4 Hz, H-2'a), 3.06-2.93 (1H, m, H-2'b), 1.16 (9H, 2s, Piv). ^{13}C NMR (75.5 MHz, $CDCl_3$), δ: 177.0 (C(O)Piv)), 165.3 (C(O)), 165.1 (C(O)), 149.71 (C-2B), 149.65 (C-6B), 143.2 (C-4B), 140.5 and 140.1 (2C, 2xC4(Ph)), 139.2(C-3A), 131.2, 130.0 and 129.0x2 (8C, 2xC2, 2xC3, 2xC5 and 2xC6(Ph)), 128.9 (2C, 2xC1(Ph)), 127.4 (C-5A), 117.5 (C-3B), 115.2 (C-4A), 113.9 (C-5B), 90.7 (C-1'), 83.6 (C-4'), 74.9 (C-3'), 70.5 (C-5'), 64.2 (C-2'), 29.8 ($\underline{C}$-$C(CH_3)_3$), 27.4 ($(CH_3)_3$).

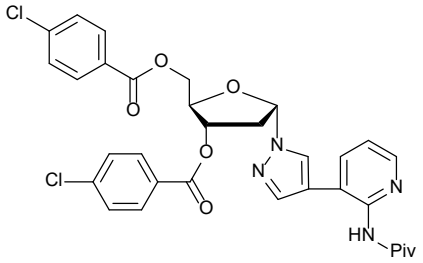
1-(β-*D*-(3',5'-Di-(4-chlorobenzoyl)-2'-deoxyribofuranosyl))-4-(2-pivaloylaminopyrimidin-3-yl)pyrazole (β-21) and 1-(α-*D*-(3',5'-Di-(4-chlorobenzoyl)-2'-deoxyribofuranosyl))-4-(2-pivaloylaminopyrimidin-3-yl)pyrazole (α-21). Were synthesized starting from 4-(**2-pivaloylaminopyrimidin-5-yl**)-1*H*-pyrazole **6**. After purification on a silica gel column using 5% of methanol in chloroform for elution the yield of anomeric mixture **β-21** and **α-21** (3:2) was 91 % (290 mg). Additional purification by PLC in ethyl acetate gave individual isomers.



1-(β-*D*-(3',5'-Di-(4-chlorobenzoyl)-2'-deoxyribofuranosyl))-4-(2-pivaloylaminopyrimidin-5-yl)pyrazole (β-21).

^{1}H NMR (300.1 MHz, $CDCl_3$), δ: 8.37 (1H, dd, *J* = 4.8, 1.6 Hz, H-6B), 8.11-7.95 (3H, m, NH, 2H-Ph), 7.92 (1H, s, H-5A), 7.83-7.75 (2H, m, 2H-Ph), 7.72 (1H, s, H-3A), 7.63 (1H, dd, *J* = 7.7, 1.8 Hz, H-4B), 7.46-7.38 (2H, m, 2H-Ph), 7.33-7.26 (2H, m, 2H-Ph), 7.17 (1H, dd, *J* = 7.7, 4.8 Hz, H-5B), 6.24 (1H, dd, *J* = 6.8, 2.3 Hz, H-1'), 5.59 (1H, ddd, *J* = 7.4, 3.1, 2.9 Hz, H-3'), 4.82-4.73 (1H, m, H-4'), 4.64-4.53 (2H, m, H-5'), 3.14 (1H, ddd, *J* = 14.8, 5.1, 2.6 Hz, Hb-2'), 3.03-2.89 (1H, m, Ha-2'), 1.23 (9H, s, Piv). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 176.8 (C(O)Piv)), 165.3 (C(O)), 165.1 (C(O)), 148.3 (C-6B), 146.7 (C-2B), 140.1 (C-3A), 138.9 x2 (2xC4 (Ph)), 138.3 (C-4B),

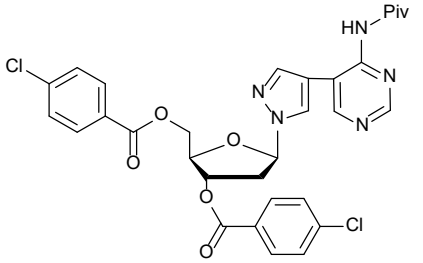
131.2x2, 131.1x2, 129.0x2 and 128.9x2 (2xC2, 2xC3, 2xC5 and 2xC6 (Ph)), 128.0 (C-5A), 124.0 (C-3B), 121.5 (C-5B), 118.9 (C-4A), 90.3 (C-1'), 83.2 (C-4'), 74.9 (C-3'), 64.3 (C-5'), 37.8 (C-2'), 29.7 (C-$C(CH_3)_3$), 27.4 ($(CH_3)_3$).



1-(α-*D*-(3',5'-Di-(4-chlorobenzoyl)-2'-deoxyribofuranosyl))-4-(2-pivaloylaminopyrimidin-5-yl)pyrazole (α-21).

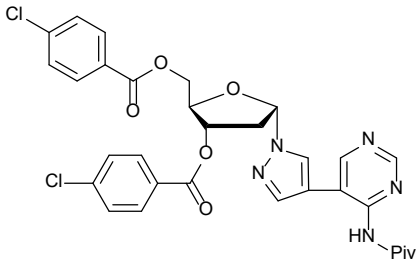
^{1}H NMR (300.1 MHz, $CDCl_3$), δ: 8.49 (1H, br s, NH), 8.37 (1H, dd, *J* = 5.1, 1.7 Hz, H-6B), 8.02-7.93 (4H, m, 4H-Ph), 7.80 (1H, s, H-5A), 7.75 (1H, dd, *J* = 7.7, 1.7 Hz, H-4B), 7.68 (1H, s, H-3A), 7.47-7.43 (2H, m, 2H-Ph), 7.39-7.32 (2H, m, 2H-Ph), 7.30-7.23 (1H, m, H-5B), 6.23 (1H, t, *J* = 6.0 Hz, H-1'), 5.88-5.78 (1H, m, H-3'), 4.66-4.44 (3H, m, H-3' and H-5'), 3.32 (1H, ddd, *J* = 14.2, 6.7, 5.5 Hz, Hb-2'), 2.76-2.65 (1H, m, Ha-2'), 1.25 (9H, s, Piv). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 177.0 (C(O)Piv)), 165.5 (C(O)), 165.2 (C(O)), 148.2 (C-6B), 144.4 (C-2B), 140.4 (C-3A), 139.9 (C-4B), 139.3 x2 (2xC4 (Ph)), 131.6x2, 131.3x2, 129.1x2 and 128.9x2 (2xC2, 2xC3, 2xC5 and 2xC6 (Ph)), 128.2 (C-5A), 127.9 (2xC1 (Ph)), 125.1 (C-3B), 121.7 (C-5B), 118.7 (C-4A), 89.9 (C-1'), 82.9 (C-4'), 75.7 (C-3'), 64.6 (C-5'), 37.3 (C-2'), 29.8 (C-$C(CH_3)_3$), 27.4 ($(CH_3)_3$).

1-(β-*D*-(3',5'-Di-(4-chlorobenzoyl)-2'-deoxyribofuranosyl))-4-(4-pivaloylaminopyrimidin-5-yl)pyrazole (β-22) and 1-(α-*D*-(3',5'-Di-(4-chlorobenzoyl)-2'-deoxyribofuranosyl))-4-(4-pivaloylaminopyrimidin-5-yl)pyrazole (α-22). Were synthesized starting from 4-(4-pivaloylaminopyrimidin-5-yl)-1*H*-pyrazole **7**. After purification on a silica gel column using 5% of methanol in chloroform for elution the yield of anomeric mixture **β-22** and **α-22** (3:1) was 63 % (201 mg). Additional purification by PLC in ethyl acetate gave individual isomers.



1-(β-*D*-(3',5'-Di-(4-chlorobenzoyl)-2'-deoxyribofuranosyl))-4-(4-pivaloylaminopyrimidin-5-yl)pyrazole (β-22).

^{1}H NMR (300.1 MHz, $CDCl_3$), δ: 8.93 (1H, s, H-2B), 8.53 (1H, s, H-5A), 8.03-7.88 (4H, m, 2H-Ph, NH, H-6B), 7.81-7.74 (2H, m, 2H-Ph), 7.71 (1H, d, *J* = 0.8 Hz, H-3A), 7.44-7.38 (2H, m, 4H-Ph), 7.36-7.28 (2H, m, 4H-Ph), 6.26 (1H, dd, *J* = 6.8, 2.3 Hz, H-1'), 5.59-5.49 (1H, ddd, *J* = 7.3, 2.9, 2.8 Hz, H-3'), 4.81-4.73 (1H, m, H-4'), 4.65-4.54 (2H, m, H-5'), 3.17 (1H, ddd, *J* = 14.9, 5.0, 2.5 Hz, Hb-2'), 3.04-2.90 (1H, m, Ha-2'), 1.19 (9H, s, Piv). ^{13}C NMR (75.5 MHz, $CDCl_3$), δ: 175.6 (C(O)Piv)), 165.3 (C(O)), 165.2 (C(O)), 157.1 (C-2B), 155.3 (C-6B), 140.3 (C-4B), 140.1 (C-3A), 138.6x2 (2xC4 (Ph)), 131.13x2, 131.07x2, 129.02x2 and 128.97x2 (2xC2, 2xC3, 2xC5 and 2xC6 (Ph)), 127.6 (2xC1, (Ph)), 126.9 (C-5A), 117.8 (C-5B), 114.9 (C-4A), 90.5 (C-1'), 83.5 (C-4'), 74.9 (C-3'), 64.2 (C-5'), 37.8 (C-2'), 29.8 (C-$C(CH_3)_3$), 27.3 ($(CH_3)_3$).



1-(α-*D*-(3',5'-Di-(4-chlorobenzoyl)-2'-deoxyribofuranosyl))-4-(4-pivaloylaminopyrimidin-5-yl)pyrazole (α-22)

^{1}H NMR (300.1 MHz, $CDCl_3$) δ 8.97 (1H, s, H-2B), 8.53 (1H, s, H-5A), 8.03-7.91 (4H, m, 2H-Ph), 7.80 (1H, s, H-6B), 7.69 (1H, s, H-3A), 7.49-7.42 (2H, m, 4H-Ph), 7.40-7.33 (m, 2H, 4H-Ph), 6.27 (1H, dd, *J* = 6.6, 5.2 Hz, H-1'), 5.89-5.79 (1H, m, H-3'), 4.67-4.44 (3H, m, H-4' and 2xH-5'), 3.35 (1H, ddd, *J* = 14.2, 6.4, 5.7 Hz, Hb-2'), 2.81-2.69 (1H, m, Ha-2'), 1.23 (s, 9H, Piv). ^{13}C NMR (75.5 MHz, $CDCl_3$), δ: 165.4 (C(O)), 165.2 (C(O)), 157.1 (C(O)Piv)), 156.9 (C-2B), 155.7 (C-6B), 140.4 (C-4B), 139.99 (C-3A), 139.2x2 (2xC4 (Ph)), 131.3x4, 129.1x2 and 128.4x2 (2xC2, 2xC3, 2xC5 and 2xC6 (Ph)), 128.1 (C-5A), 127.8 (2xC1, (Ph)), 117.4 (C-5B), 115.1 (C-4A), 90.1 (C-1'), 83.1 (C-4'), 75.7 (C-3'), 64.5 (C-5'), 40.5 (C-2'), 29.8 (C-$C(CH_3)_3$), 27.3 ($(CH_3)_3$).

Nucleoside phosphorylase activity

Uridine Phosphorylase (UP)

The reaction mixture (1 mL in total), containing 100 mM of Uridine, 50 mM of Potassium phosphate buffer (pH 7.0) and about 0.5 µg of recombinant cells overexpressing the UP (calculated on dry cell mass) was incubated 5 min. at 60 °C the cell debris was spined down. An aliquot of 10 µl of reaction mixture was added to 400 µl of triethylammonium acetate buffer (pH7.0), heated to 100 °C. After 1 min the cell debris was spined down and the supernatant was analyzed with HPLC on the Waters Breeze system using Gemini C18, 5 µm, 150 x 4.6 mm column and isocratic elution with 5% acetonitrile in 0.1 M Triethylammonium acetate, pH 7.0, flow rate – 1 mL/min, injection volume – 5 µl. The HPLC column was calibrated with Uracil and Uridine. The UP activity was 1090 µmol/min x mg dry cells

Purine nucleoside phosphorylase (PNP)

Procedure was similar to the above for UP, but the reaction mixture contained Inosine instead of Uridine and 1 µg recombinant cells, overexpressing the PNP (calculated on dry cell mass). The HPLC column was calibrated with Hypoxantine and Inosine. HPLC analysis was as above; the PNP activity was 242 µmol/min x mg dry cells.

Thymidine phosphorylase (TP)

The procedure was similar to the determination of UP, but the reaction mixture contained Thymidine, instead of Uridine and the incubation was at 50 °C. The HPLC column was calibrated with Thymine and Thymidine. HPLC analysis was as above, the PNP activity was 1978 µmol/min x mg dry cells.

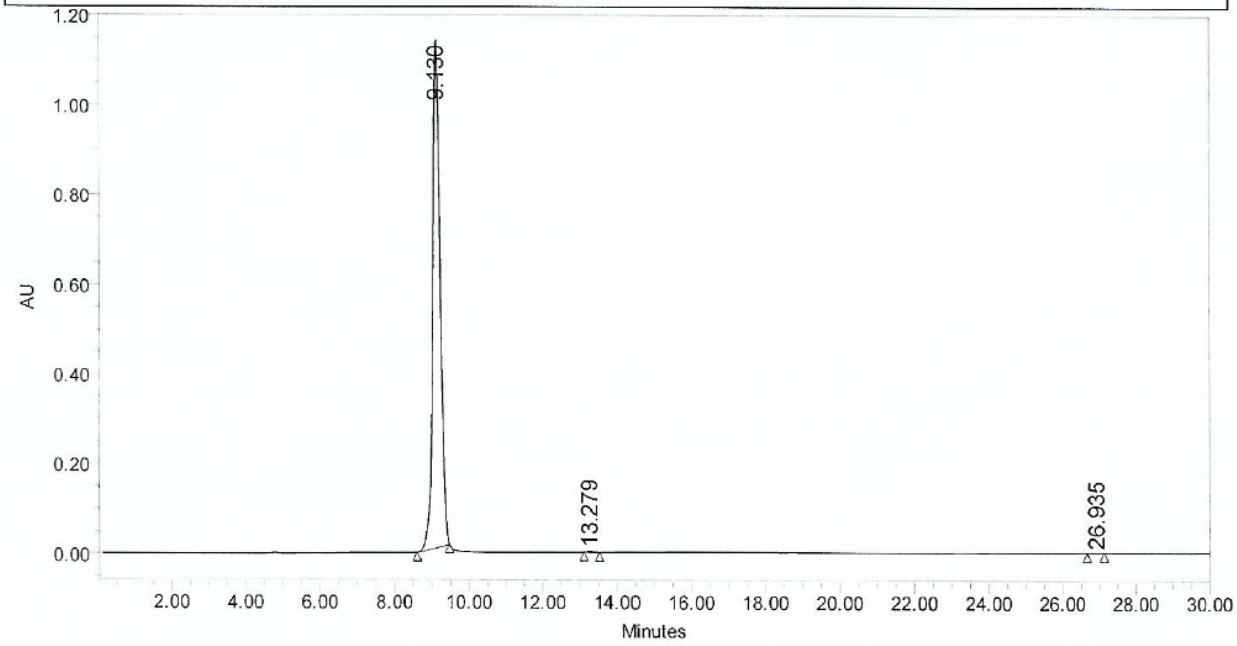
HPLC DATA

1-β-D-Ribofuranosyl-4-(4-aminopyridin-3-yl)pyrazole **(11)**

Compound	Fleximer LN-98-R		Lot # LN98R0121
Column	Gemini C18,110Å	5µm	4.6 x 150 mm
Column Temperature	30 °C		
Solvent A	0.1 M TEAA		
Solvent B	80% CH_3CN		
Pump program	0% - 15% B, 30 min;		Flow = 1 ml/min
Detector	λ = 260 nm and		
Comments			

SAMPLE INFORMATION

Sample Name:	LN=98-R	Acquired By:	System
Sample Type:	Unknow n	Date Acquired:	1/5/2021 3:24:39 PM
Vial:	1	Acq. Method:	Fleximers
Injection #:	2	Date Processed:	1/5/2021 4:01:10 PM
Injection Volume:	5.00 ul	Channel Name:	2487Channel 1
Run Time:	30.00 Minutes	Channel Desc.:	260
		Sample Set Name:	



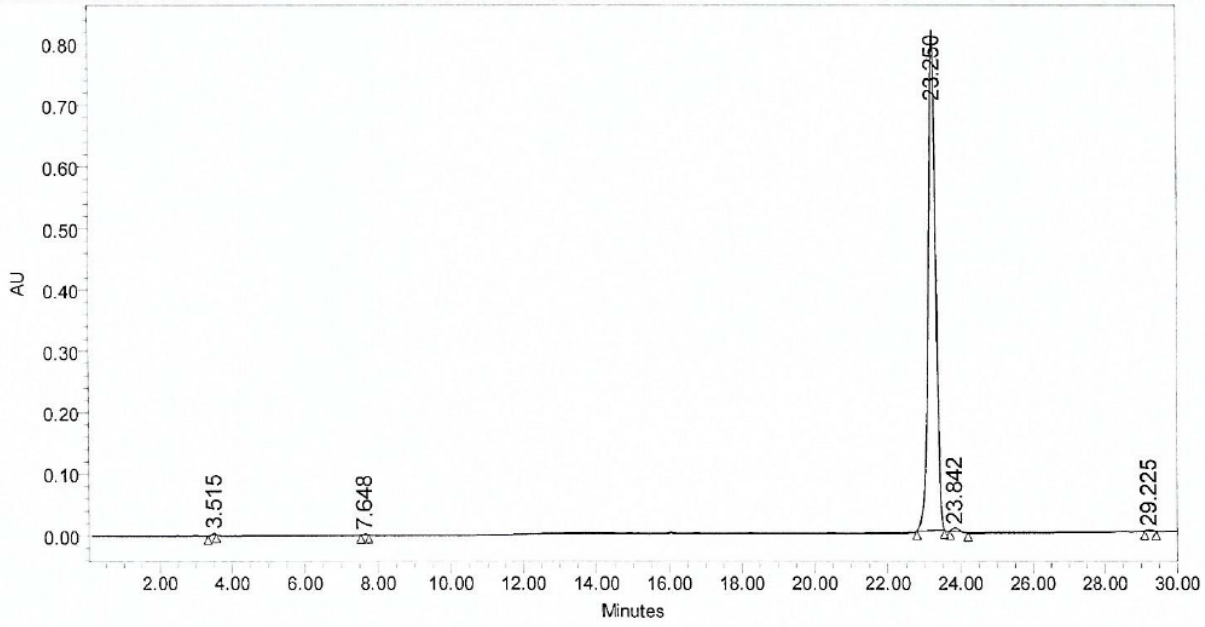
	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	9.130	15343525	99.80	1131312	99.76
2	13.279	29865	0.19	2678	0.24
3	26.935	1622	0.01	94	0.01

1-β-*D*-Ribofuranosyl-4-(2-aminopyridin-3-yl)pyrazole **(12)**

Compound	Fleximer LN-75-R		Lot # LN75R1020
Column	Gemini C18,110Å	5µm	4.6 x 150 mm
Column Temperature	30 °C		
Solvent A	0.1 M TEAA		
Solvent B	80% CH_3CN		
Pump program	0% - 20% B, 30 min;		Flow = 1 ml/min
Detector	λ = 260 nm and		
Comments			

SAMPLE INFORMATION

Sample Name:	LN-75-R	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	10/7/2020 5:21:50 PM
Vial:	1	Acq. Method:	Fleximers2
Injection #:	1	Date Processed:	10/7/2020 6:33:57 PM
Injection Volume:	5.00 ul	Channel Name:	2487Channel 1
Run Time:	30.00 Minutes	Channel Desc.:	260
		Sample Set Name:	



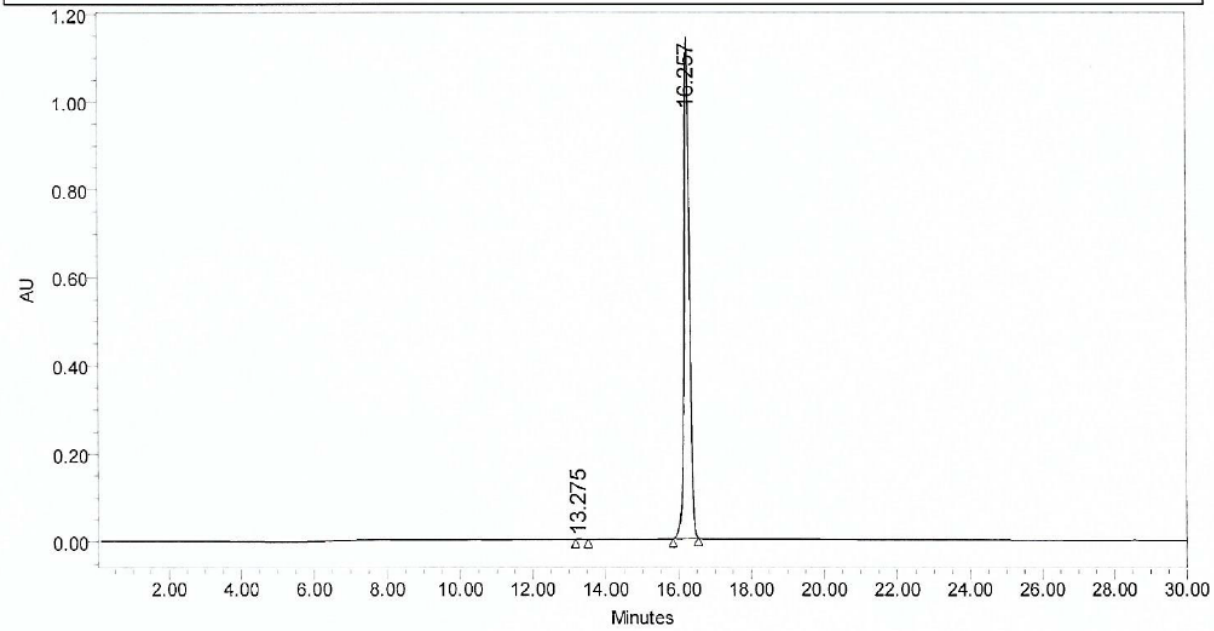
	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	3.515	13287	0.12	2372	0.29
2	7.648	10843	0.09	1376	0.17
3	23.250	11392956	99.02	813907	98.62
4	23.842	67543	0.59	5707	0.69
5	29.225	20691	0.18	1906	0.23

1-β-*D*-Ribofuranosyl-4-(4-aminopyrimidin-5-yl)pyrazole **(13)**

Compound	Fleximer LN-74-R		Lot # LN74R0820
Column	Gemini C18,110Å	5µm	4.6 x 150 mm
Column Temperature	30 °C		
Solvent A	0.1 M TEAA		
Solvent B	80% CH_3CN		
Pump program	0% - 15% B, 30 min;		Flow = 1 ml/min
Detector	λ = 260 nm and		
Comments			

SAMPLE INFORMATION

Sample Name:	LN74R0820	Acquired By:	System
Sample Type:	Unknow n	Date Acquired:	8/13/2020 2:58:07 PM
Vial:	1	Acq. Method:	Flexomer LN74R
Injection #:	1	Date Processed:	8/13/2020 3:37:04 PM
Injection Volume:	5.00 ul	Channel Name:	2487Channel 1
Run Time:	30.00 Minutes	Channel Desc.:	260
		Sample Set Name:	



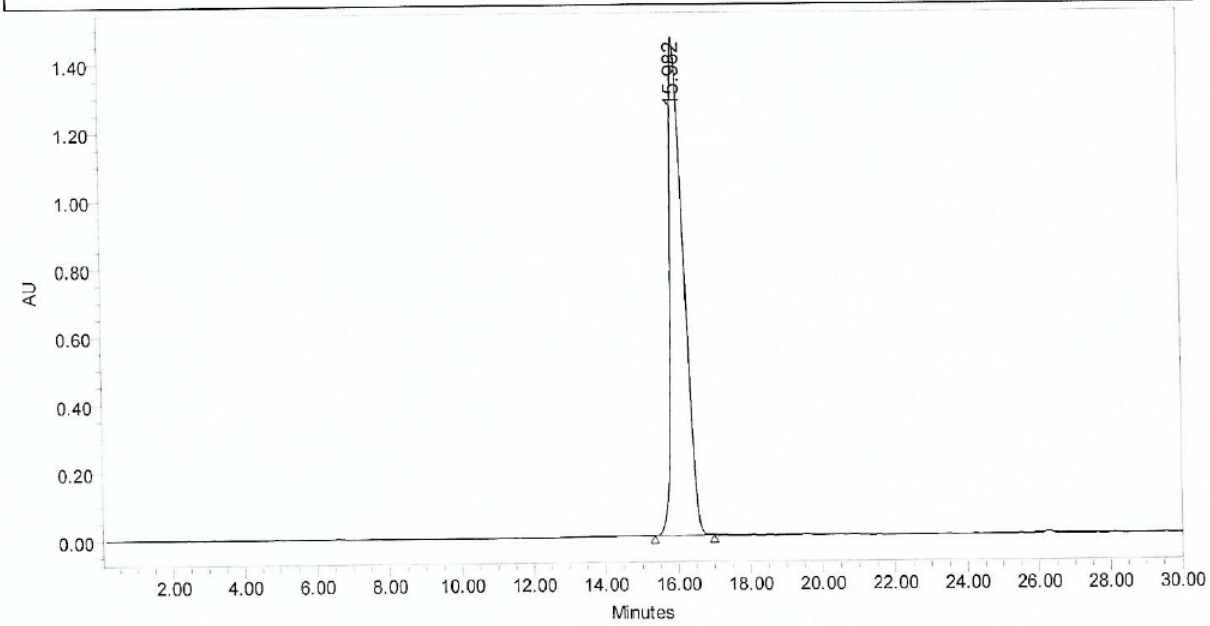
	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	13.275	9346	0.08	1225	0.11
2	16.257	11653483	99.92	1145205	99.89

1-β-D-2'-Deoxyribofuranosyl-4-(4-aminopyridin-3-yl)pyrazole **(14)**

Compound	Fleximer LN-98-D		Lot # LN98D1220
Column	Gemini C18,110Å	5µm	4.6 x 150 mm
Column Temperature	30 °C		
Solvent A	0.1 M TEAA		
Solvent B	80% CH_3CN		
Pump program	0% - 15% B, 30 min;		Flow = 1 ml/min
Detector	λ = 260 nm		
Comments			

SAMPLE INFORMATION

Sample Name:	LN(=98-D	Acquired By:	System
Sample Type:	Unknow n	Date Acquired:	1/5/2021 2:35:36 PM
Vial:	1	Acq. Method:	Fleximers
Injection #:	1	Date Processed:	1/5/2021 3:08:35 PM
Injection Volume:	5.00 ul	Channel Name:	2487Channel 1
Run Time:	30.00 Minutes	Channel Desc.:	260
		Sample Set Name:	



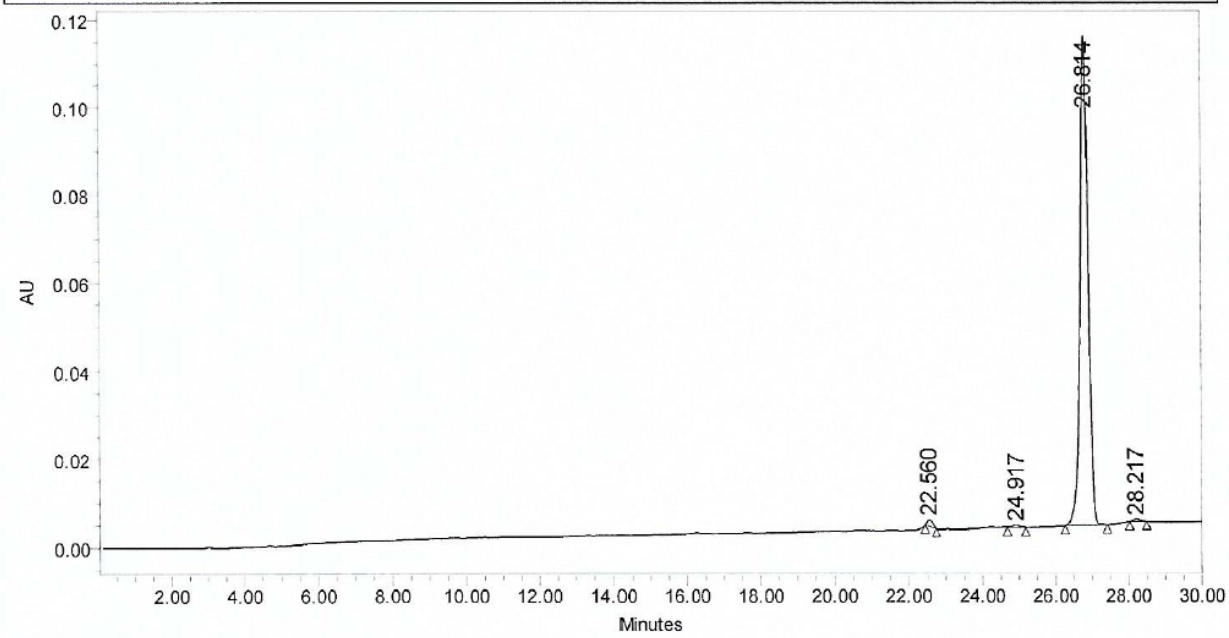
	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	15.982	38303591	100.00	1465470	100.00

1-β-D-2'-Deoxyribofuranosyl-4-(2-aminopyridin-3-yl)pyrazole **(15)**

Compound	Fleximer LN-75-D		Lot # LN75D1020
Column	Gemini C18,110Å	5µm	4.6 x 150 mm
Column Temperature	30 °C		
Solvent A	0.1 M TEAA		
Solvent B	80% CH_3CN		
Pump program	0% - 20% B, 30 min;		Flow = 1 ml/min
Detector	λ = 260 nm and		
Comments			

SAMPLE INFORMATION

Sample Name:	LN-75-D	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	10/7/2020 5:59:57 PM
Vial:	1	Acq. Method:	Fleximers2
Injection #:	1	Date Processed:	10/7/2020 6:37:54 PM
Injection Volume:	5.00 ul	Channel Name:	2487Channel 1
Run Time:	30.00 Minutes	Channel Desc.:	260
		Sample Set Name:	



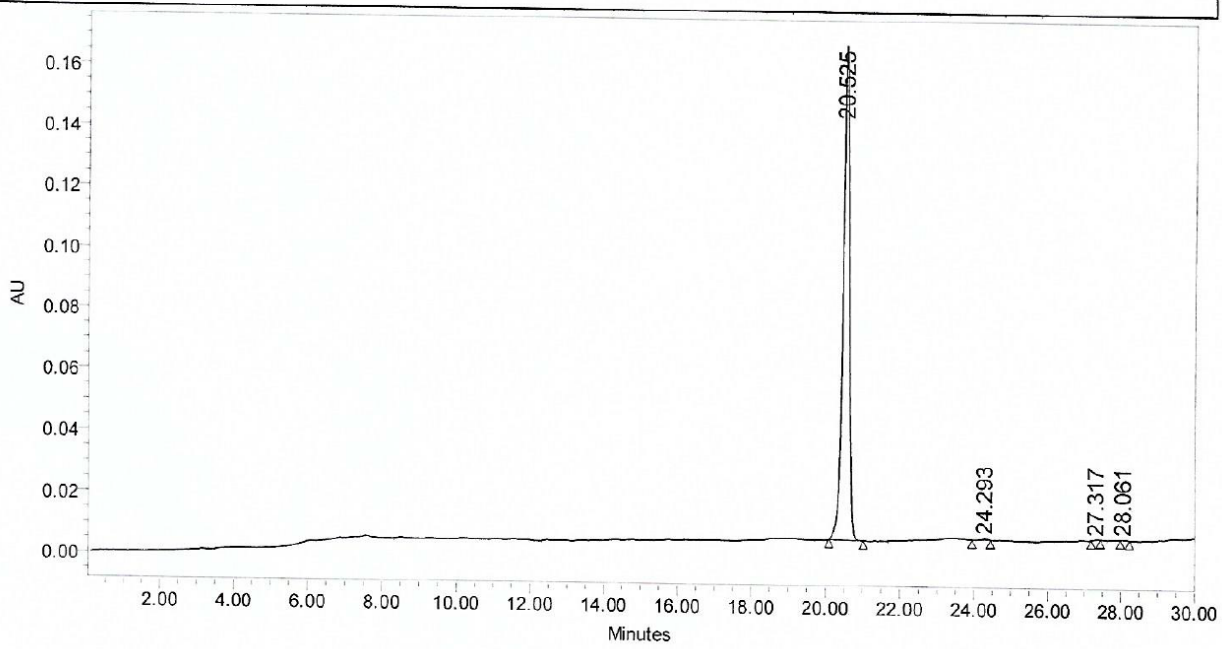
	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	22.560	14619	0.85	1472	1.29
2	24.917	7112	0.42	571	0.50
3	26.814	1681546	98.26	111189	97.73
4	28.217	8031	0.47	539	0.47

1-β-*D*-2'-Deoxyribofuranosyl-4-(4-aminopyrimidin-5-yl)pyrazole **(16)**

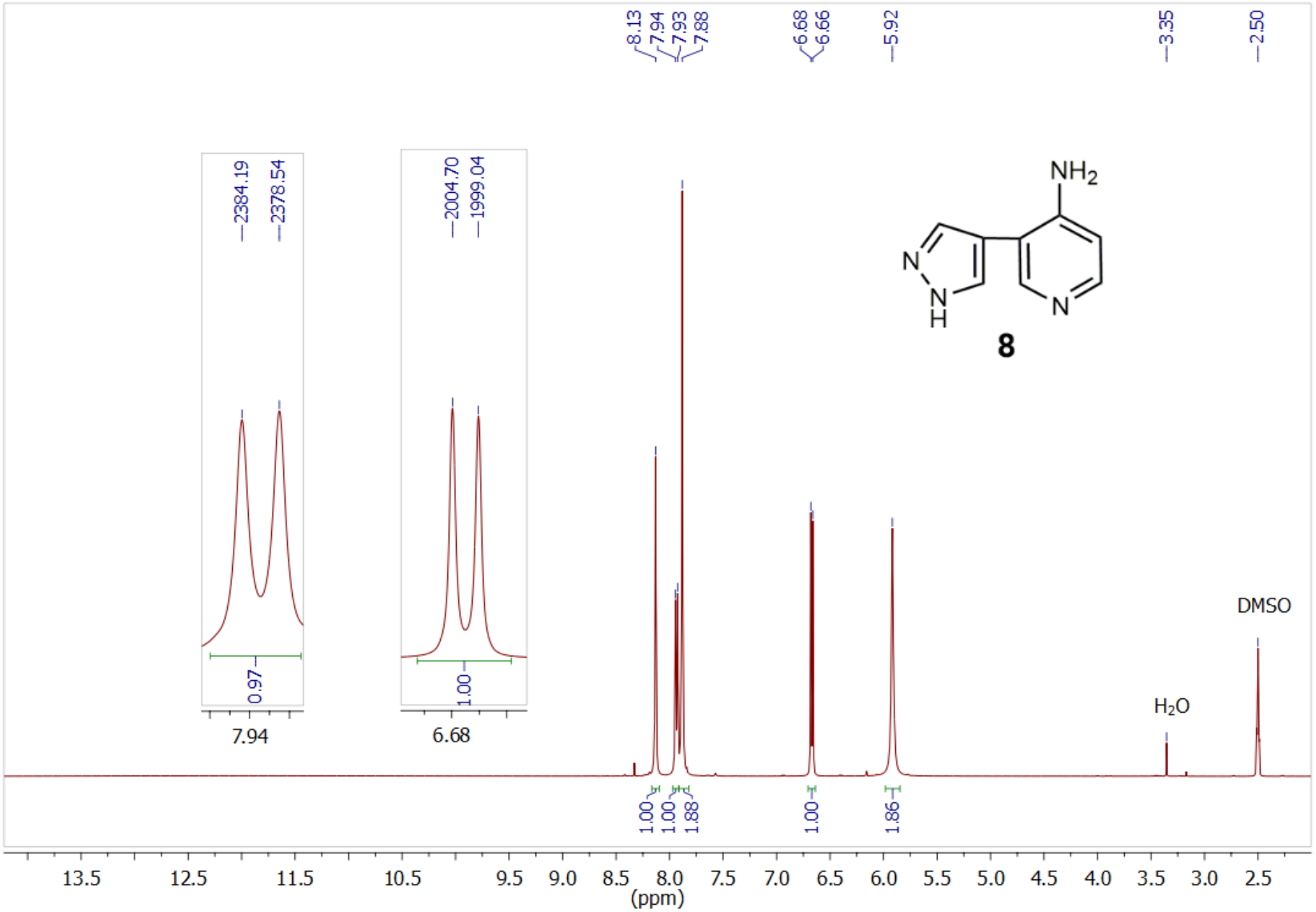
Compound	Fleximer LN-74-D		Lot # LN74D0920
Column	Gemini C18,110Å	5µm	4.6 x 150 mm
Column Temperature	30 °C		
Solvent A	0.1 M TEAA		
Solvent B	80% CH_3CN		
Pump program	0% - 15% B, 30 min;		Flow = 1 ml/min
Detector	λ = 260 nm and		
Comments			

SAMPLE INFORMATION

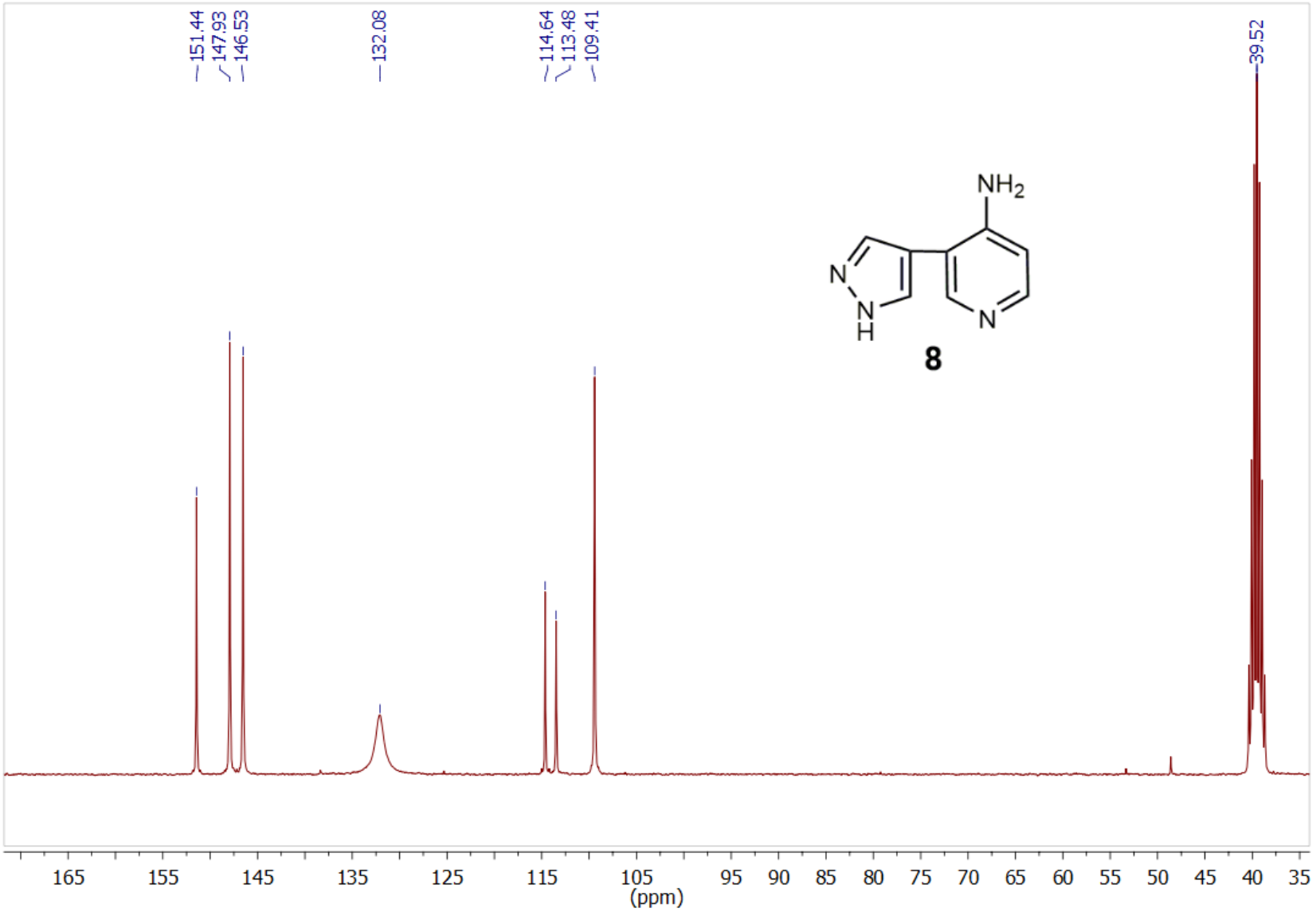
Sample Name:	LN74D	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	9/24/2020 5:01:37 PM
Vial:	1	Acq. Method:	Fleximers
Injection #:	1	Date Processed:	9/24/2020 5:32:50 PM
Injection Volume:	5.00 ul	Channel Name:	2487Channel 1
Run Time:	30.00 Minutes	Channel Desc.:	260
		Sample Set Name:	



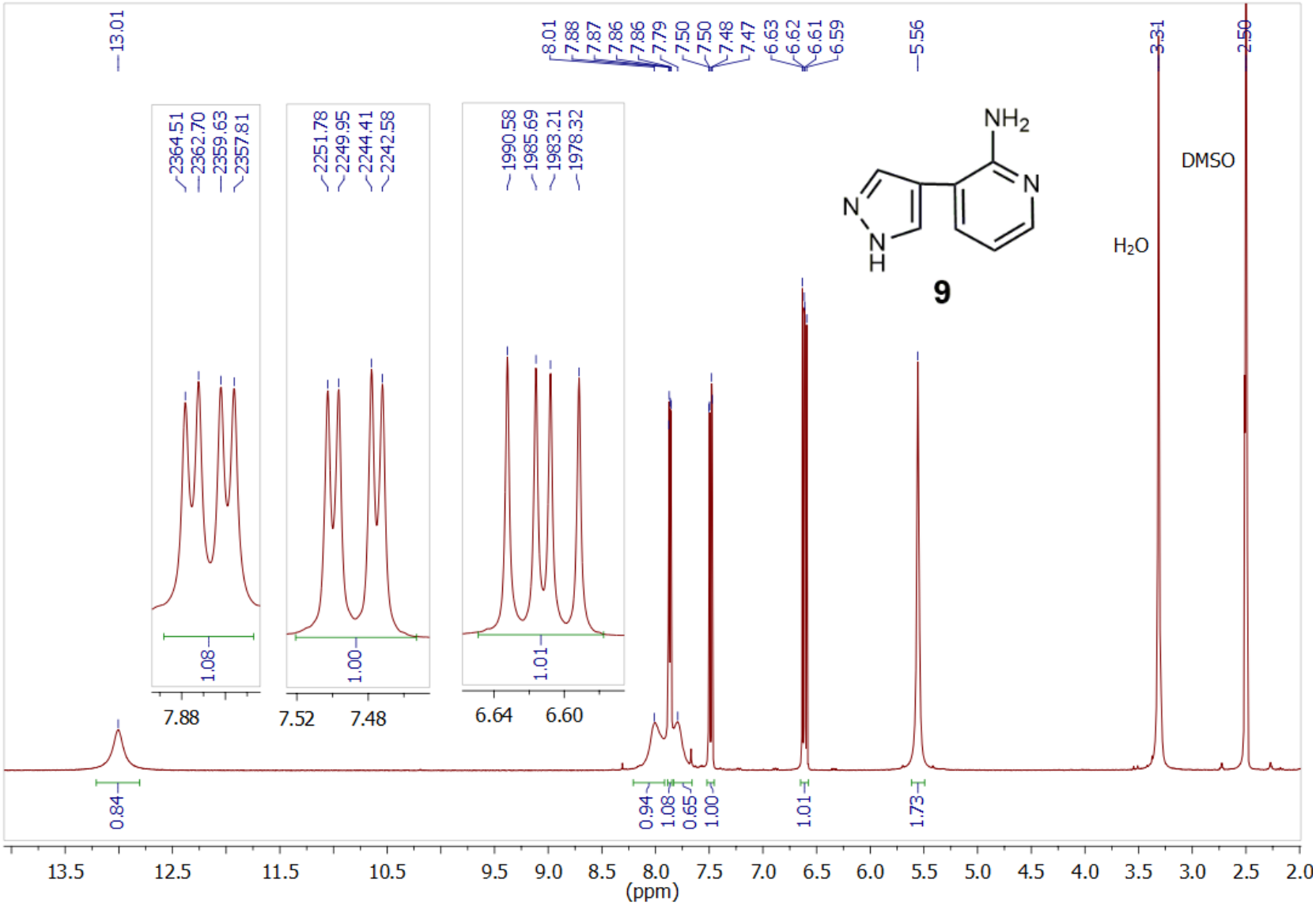
	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	20.525	1898363	99.56	162016	99.50
2	24.293	6085	0.32	572	0.35
3	27.317	980	0.05	120	0.07
4	28.061	1329	0.07	119	0.07



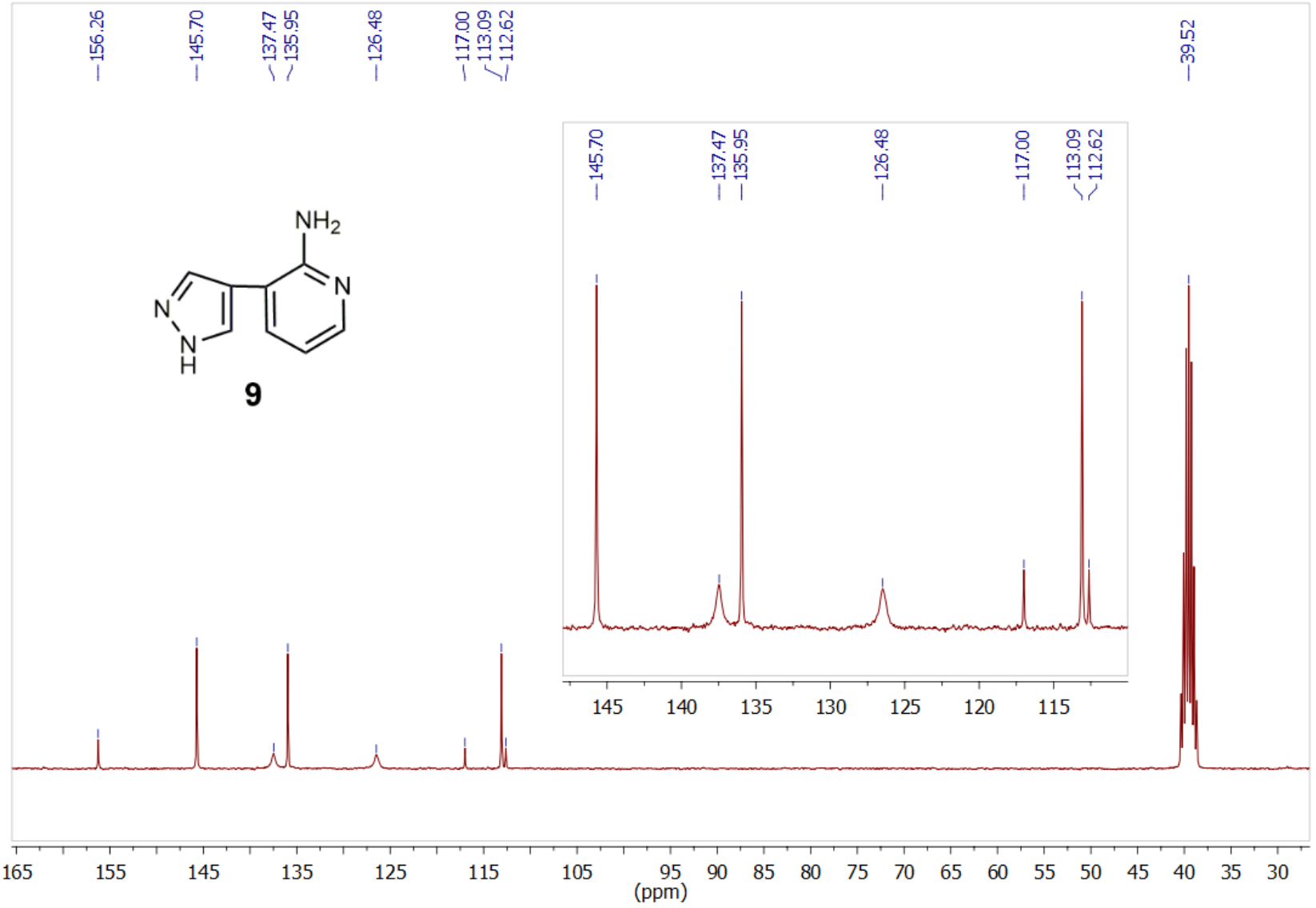
^{1}H NMR spectrum (300.1 MHz) of **8** in DMSO-d^{6}



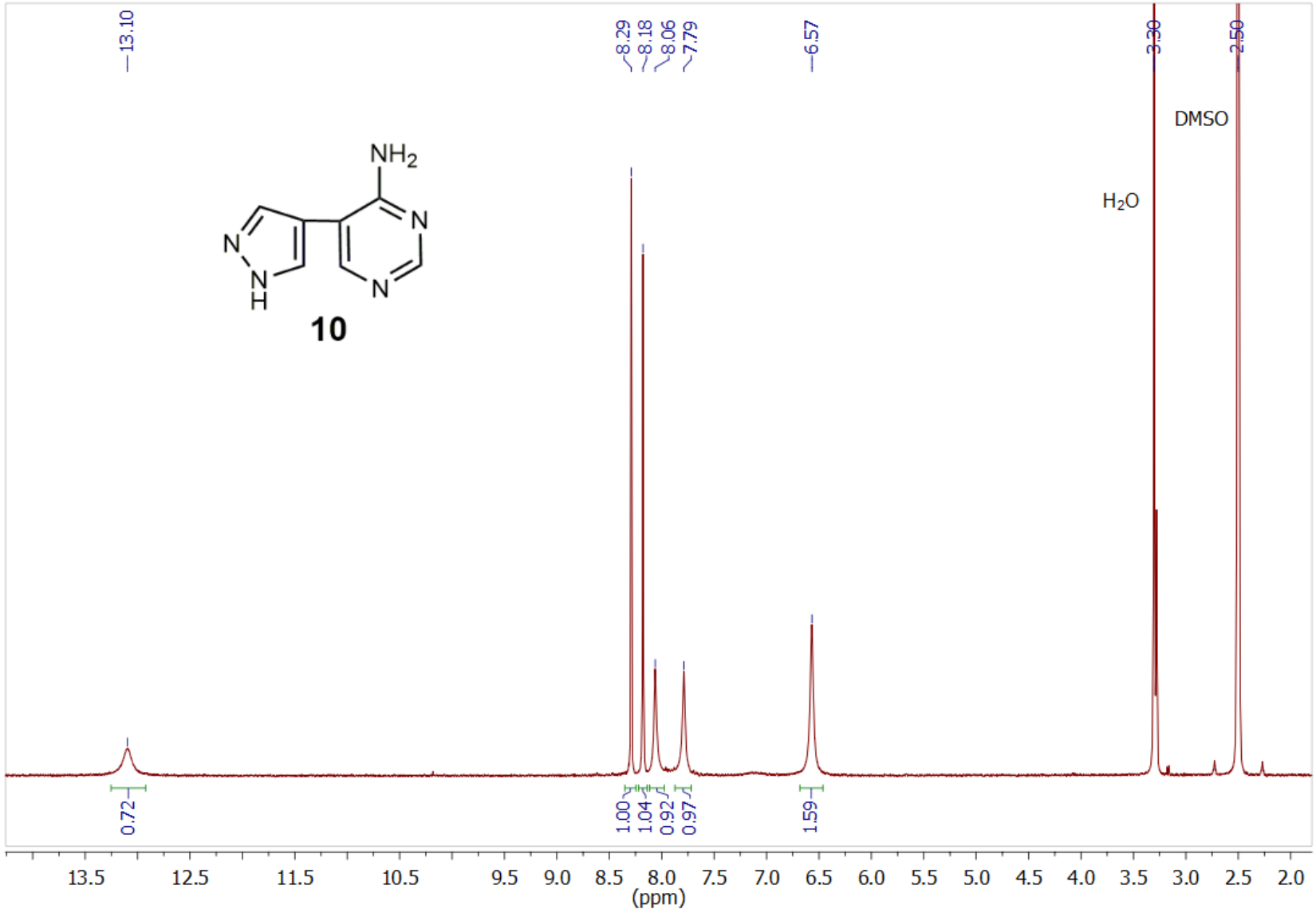
^{13}C NMR spectrum (75.5 MHz) of **8** in DMSO-d^6



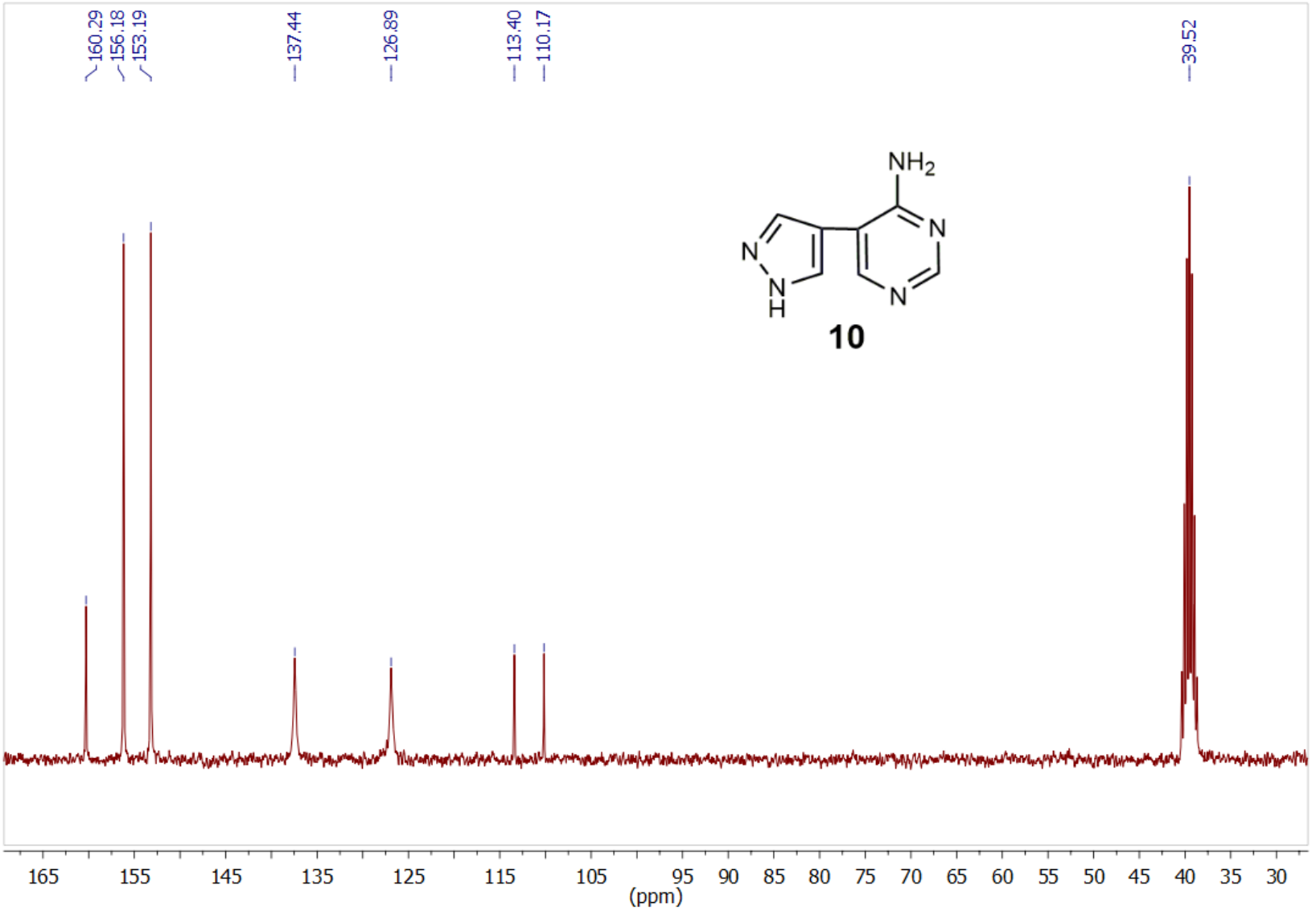
[1]H NMR spectrum (300.1 MHz) of **9** in DMSO-d^6



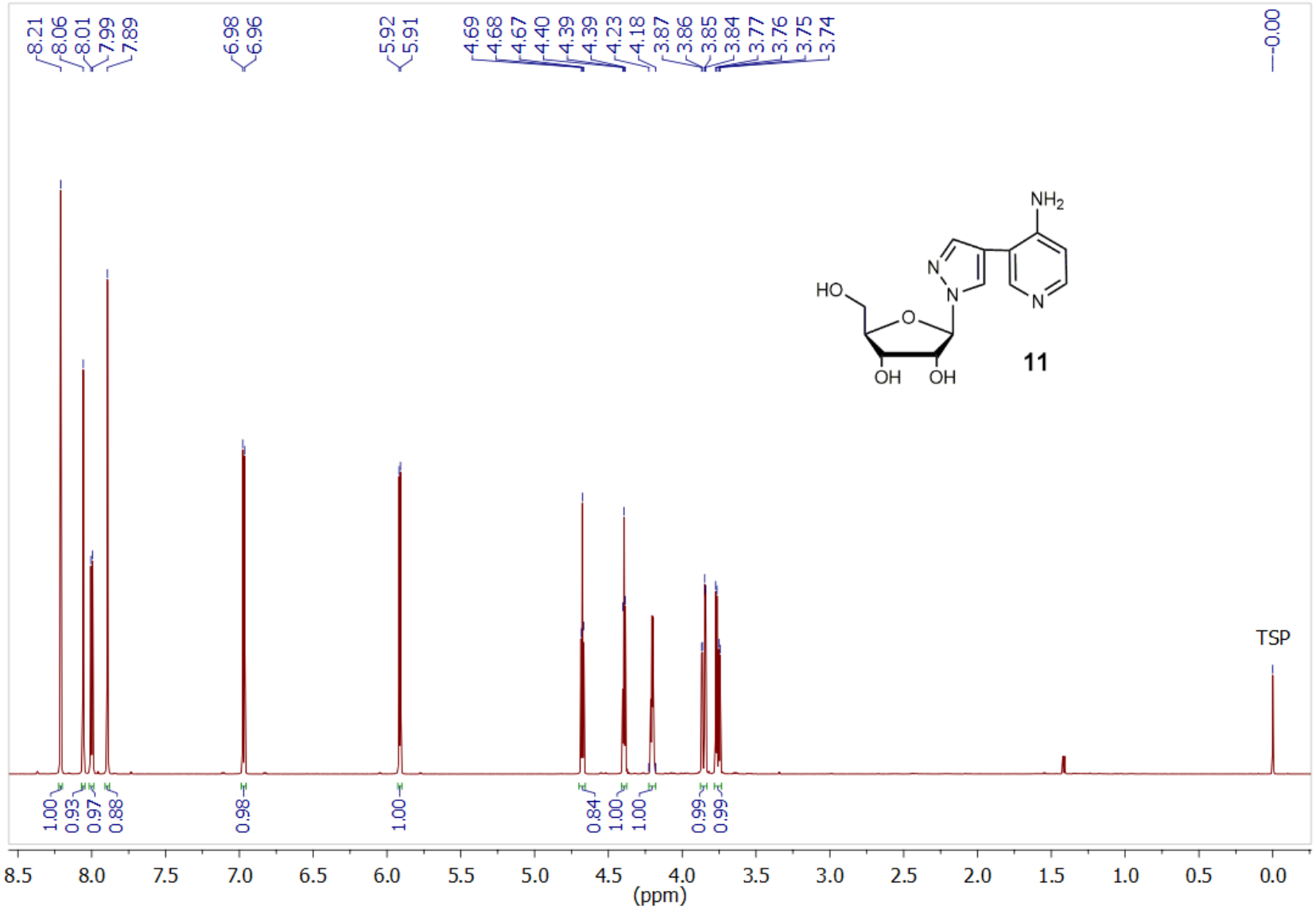
^{13}C NMR spectrum (75.5 MHz) of **9** in DMSO-d^6



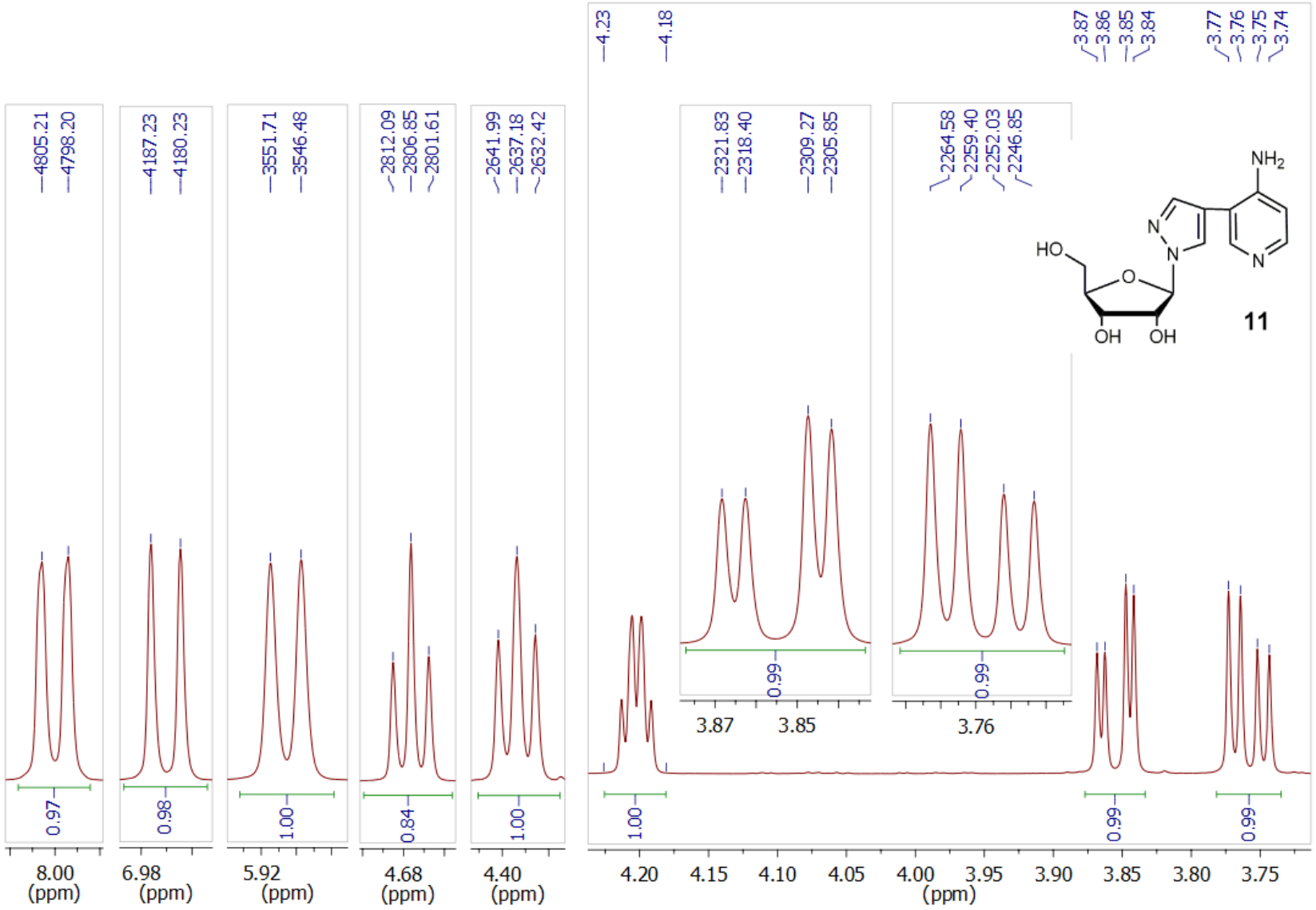
^{1}H NMR spectrum (300.1 MHz) of **10** in DMSO-d^{6}



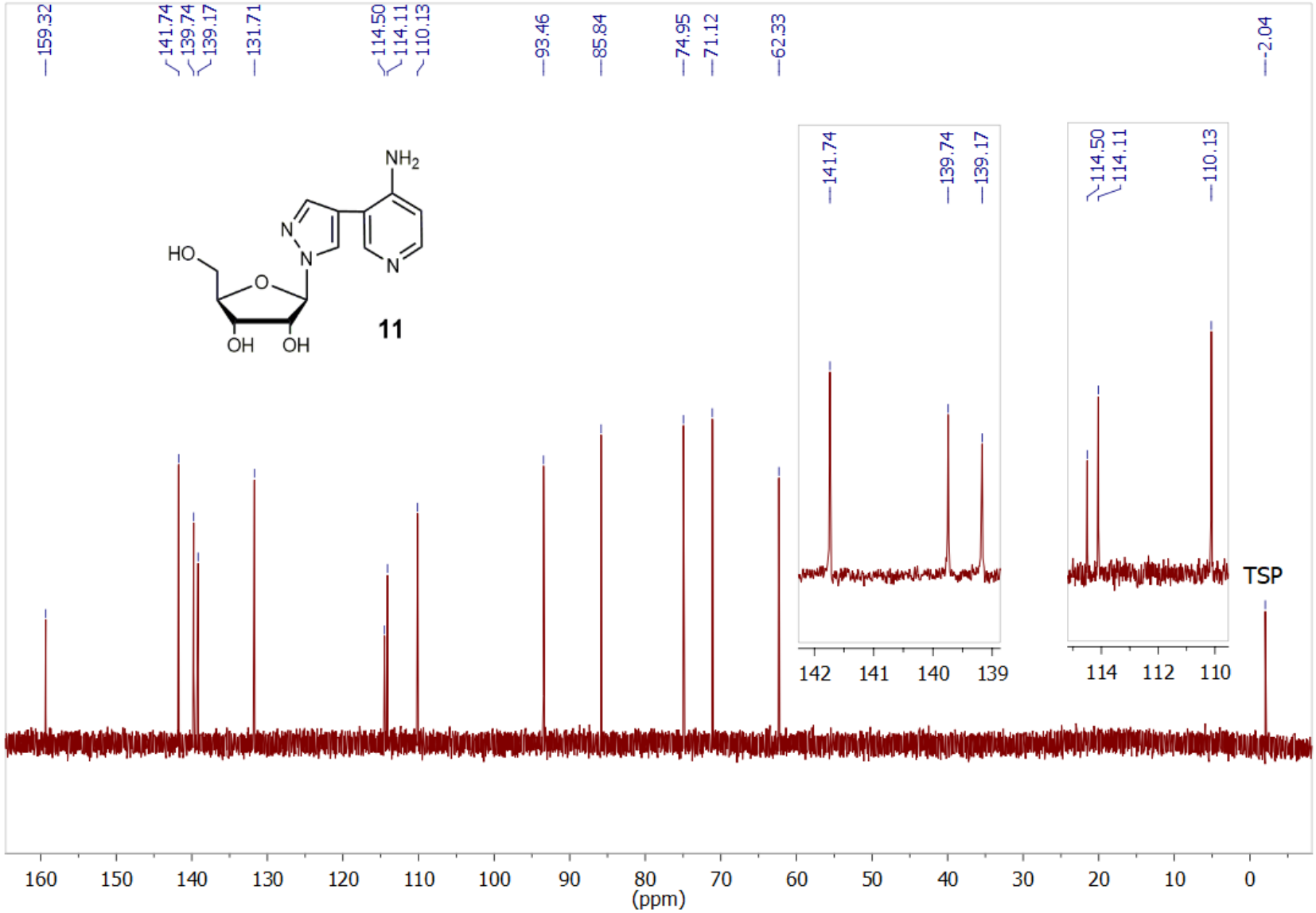
^{13}C NMR spectrum (75.5 MHz) of **10** in DMSO-d^6



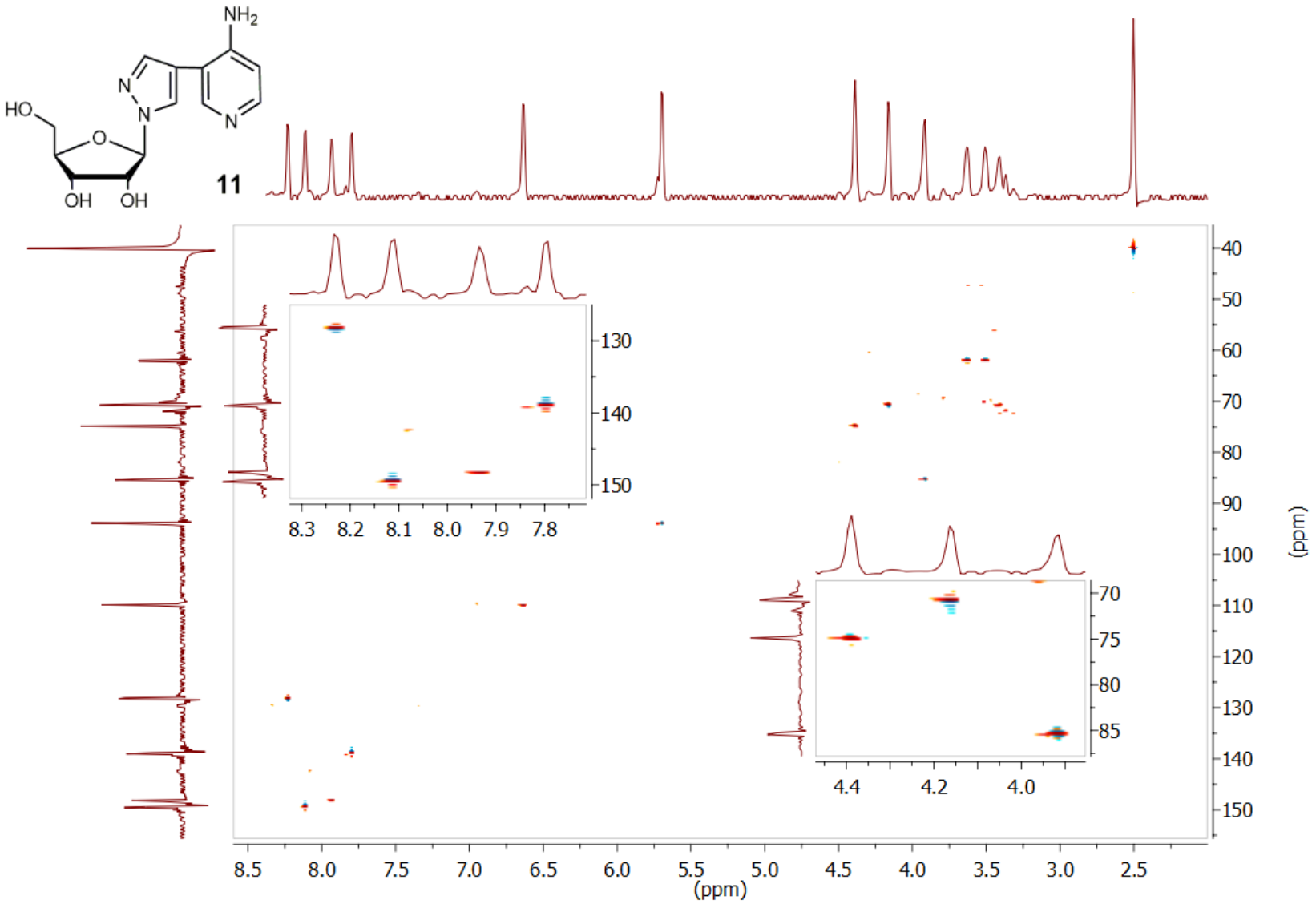
^{1}H NMR spectrum (600.2 MHz) of **11** in D_2O



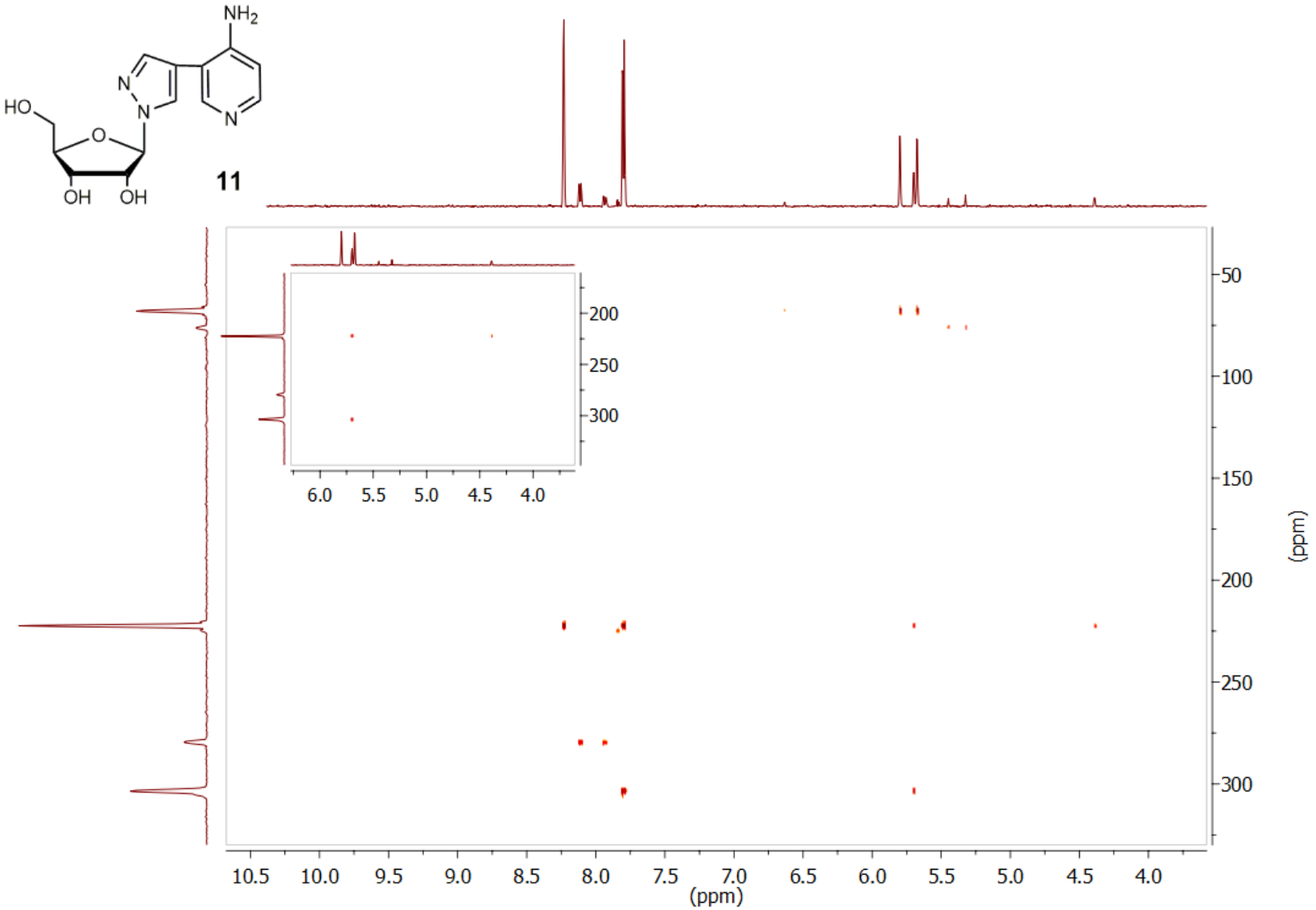
Detailed ^{1}H NMR spectrum (600.2 MHz) of **11** in D_2O



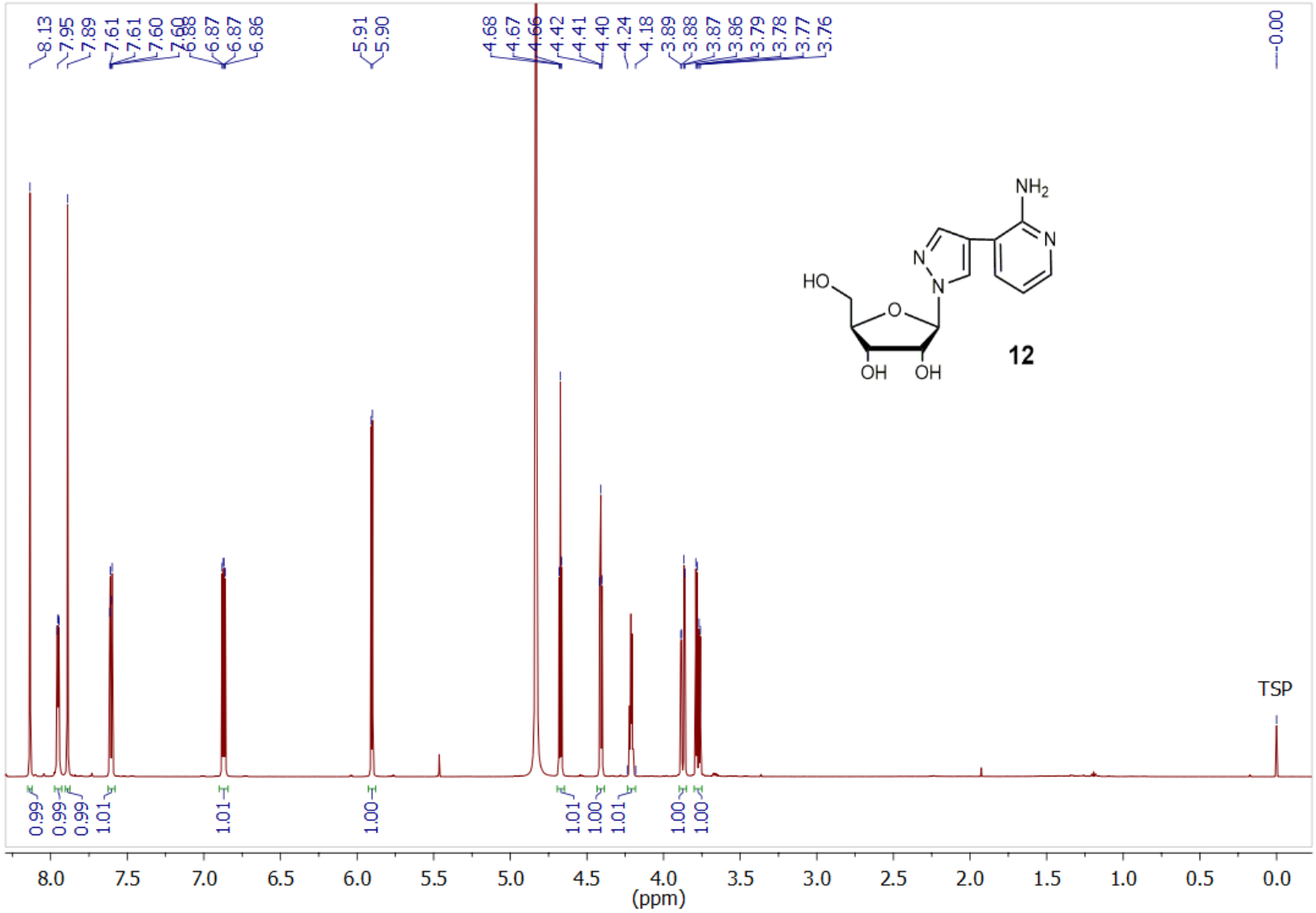
^{13}C NMR spectrum (150.9 MHz) of **11** in D_2O



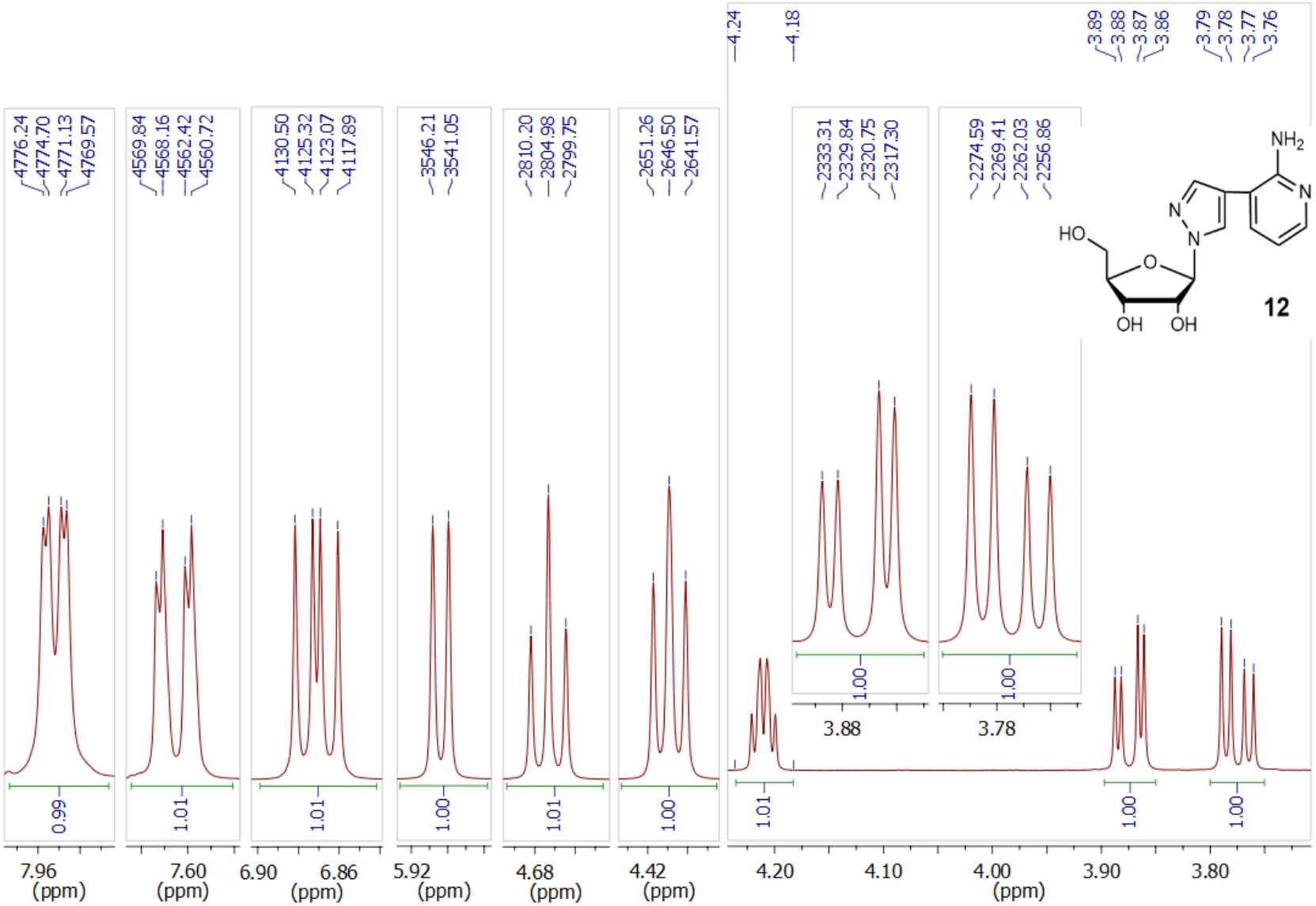
^{1}H-^{13}C HSQC (700.2 MHz) spectrum of **11** in DMSO-*d6*



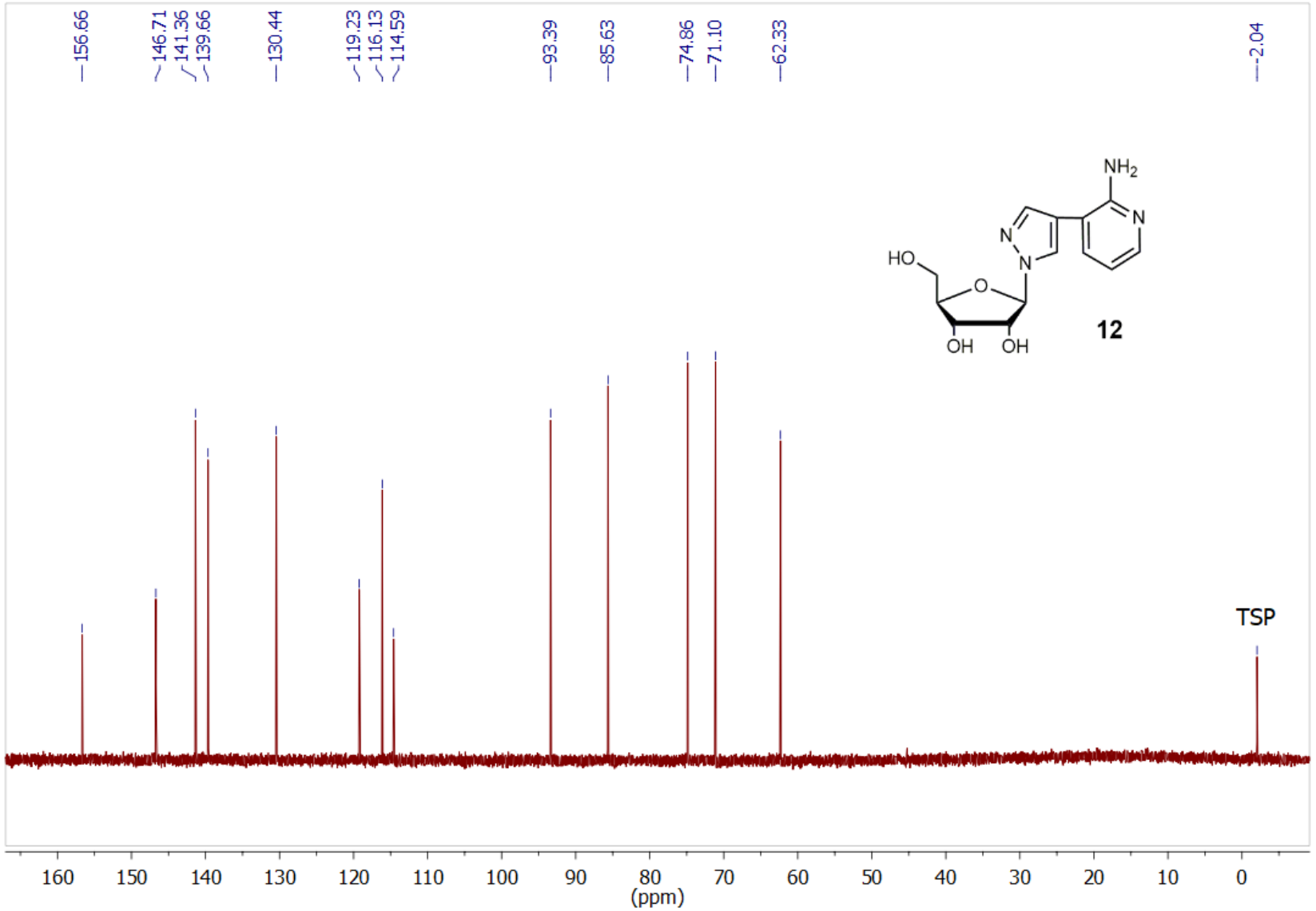
Superimposed ^{1}H-^{15}N spectra (700.2 MHz) of **11** in DMSO-*d6*



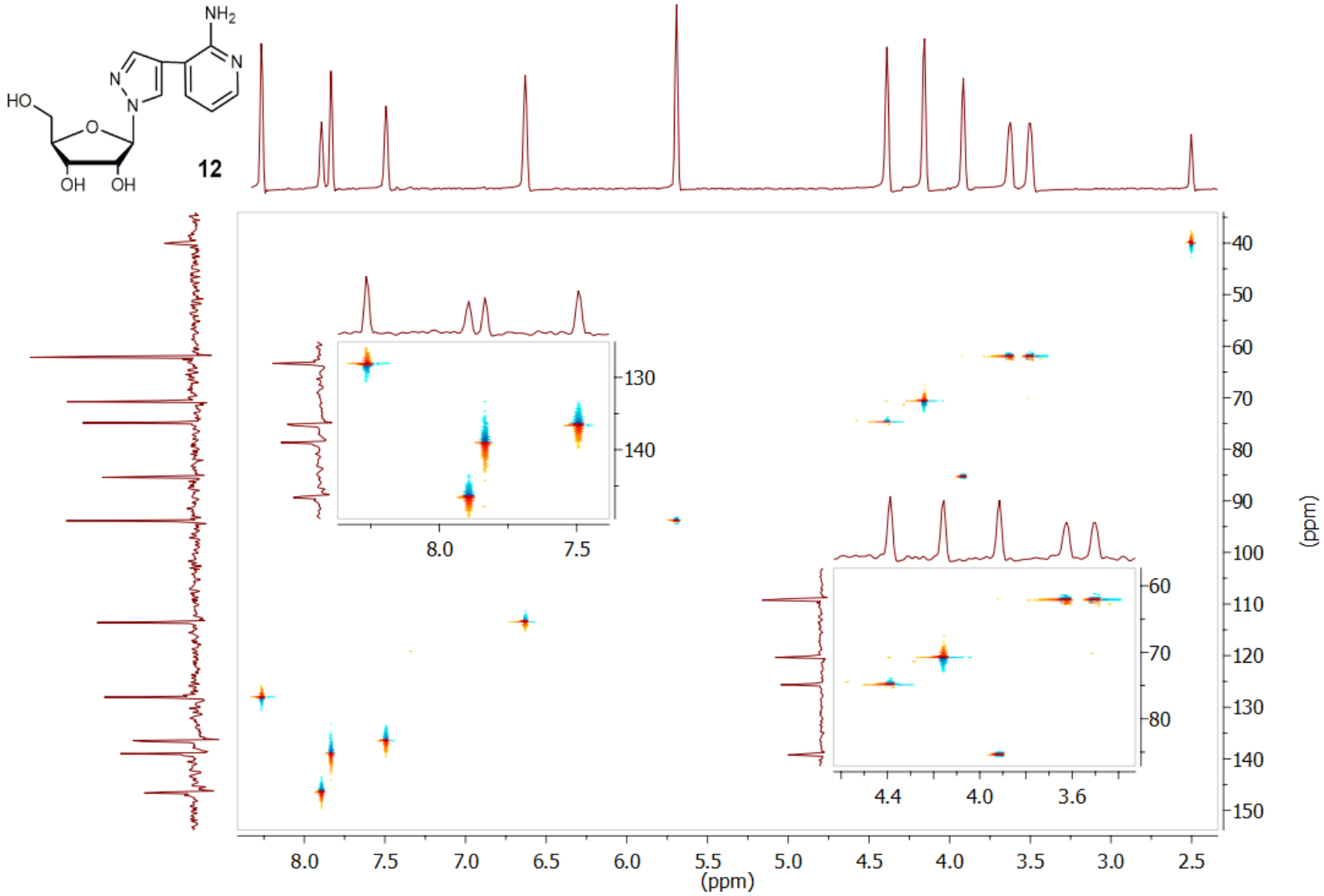
^{1}H NMR spectrum (600.2 MHz) of **12** in D_2O



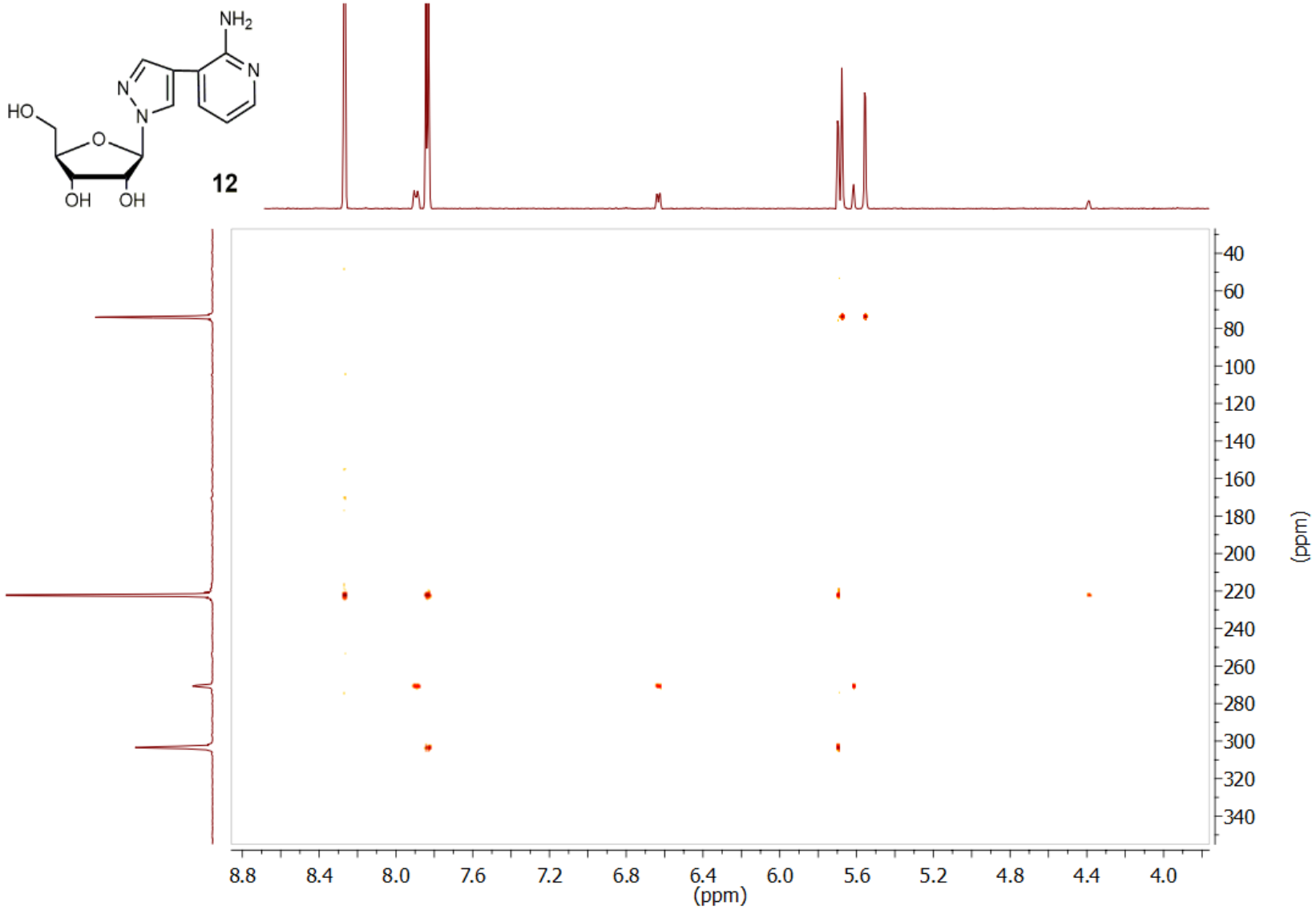
Detailed ^{1}H NMR spectrum (600.2 MHz) of **12** in D_2O



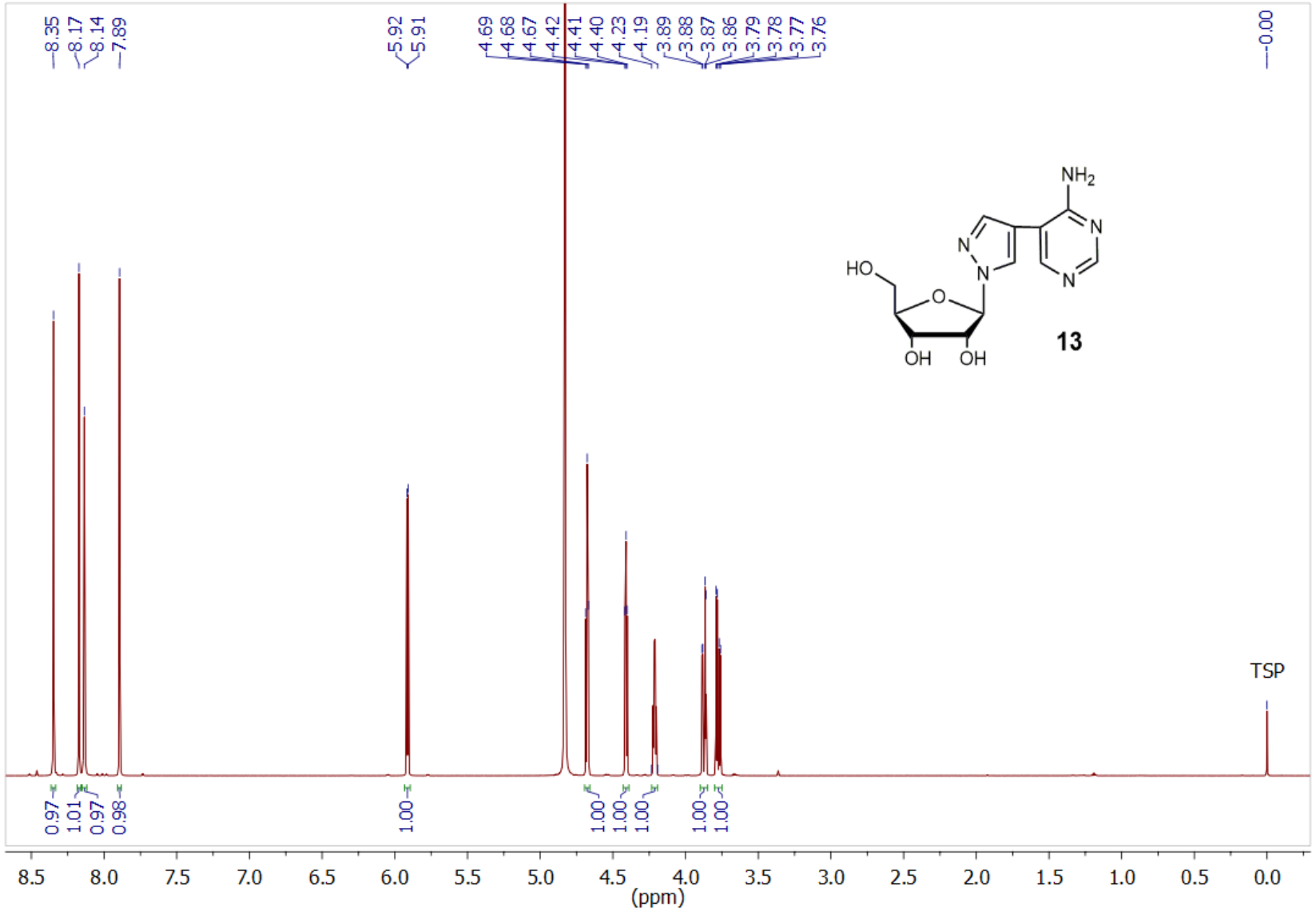
^{13}C NMR spectrum (150.9 MHz) of **12** in D_2O



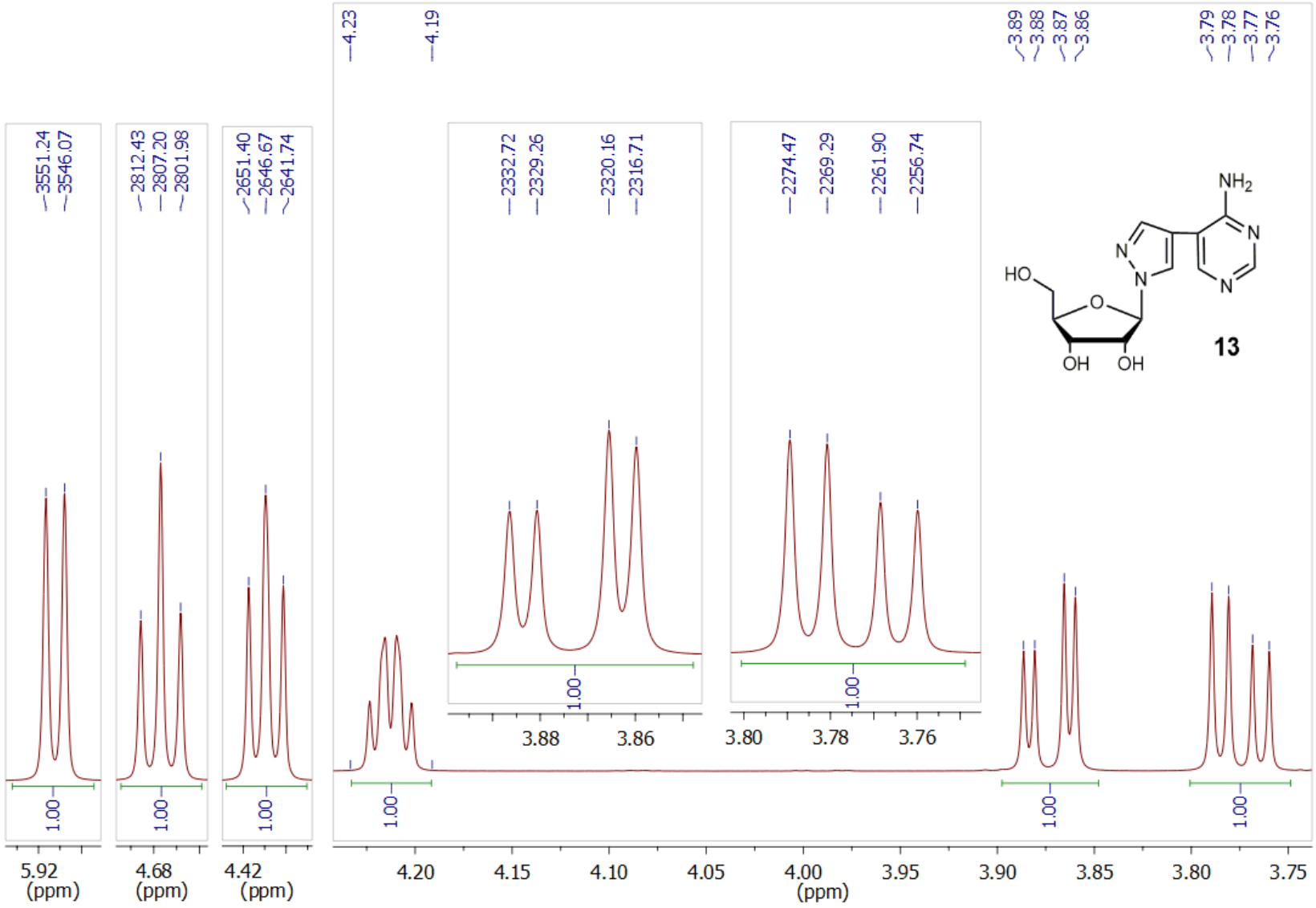
^{1}H-^{13}C HSQC spectrum (700.2 MHz) of **12** in DMSO-*d6*



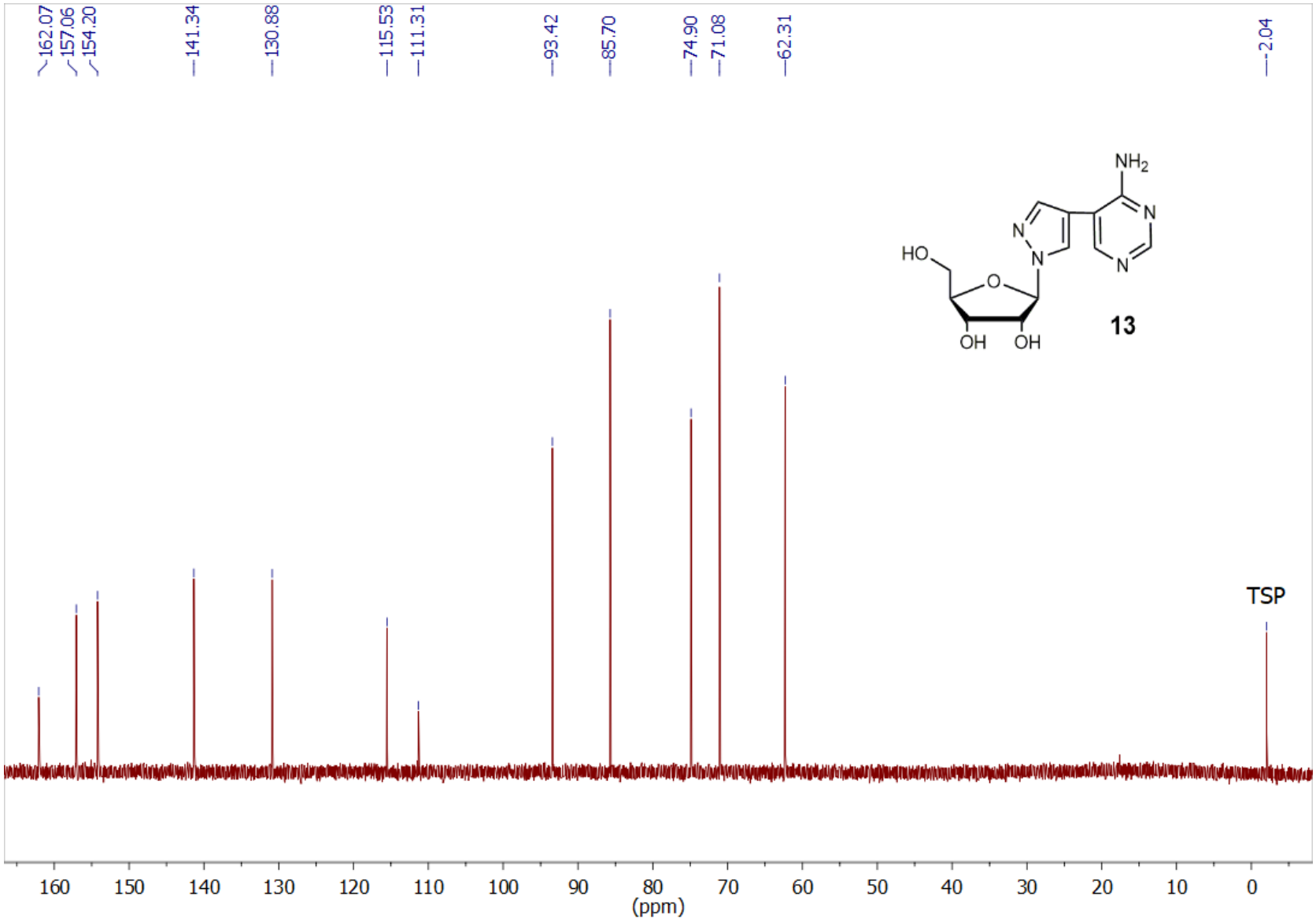
Superimposed ^{1}H-^{15}N spectra (700.2 MHz) of **12** in DMSO-*d6*



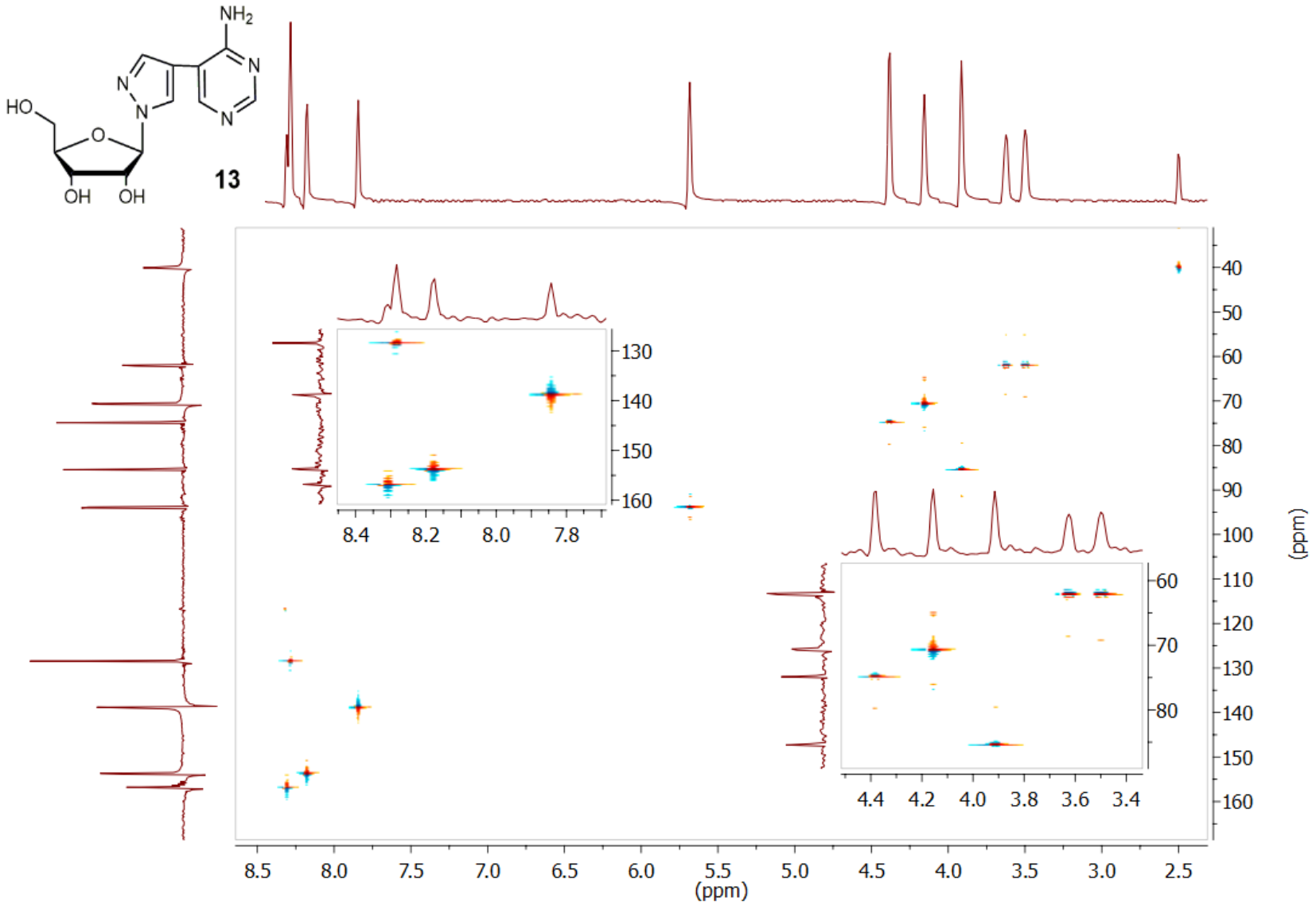
^{1}H NMR spectrum (600.2 MHz) of **13** in D_2O



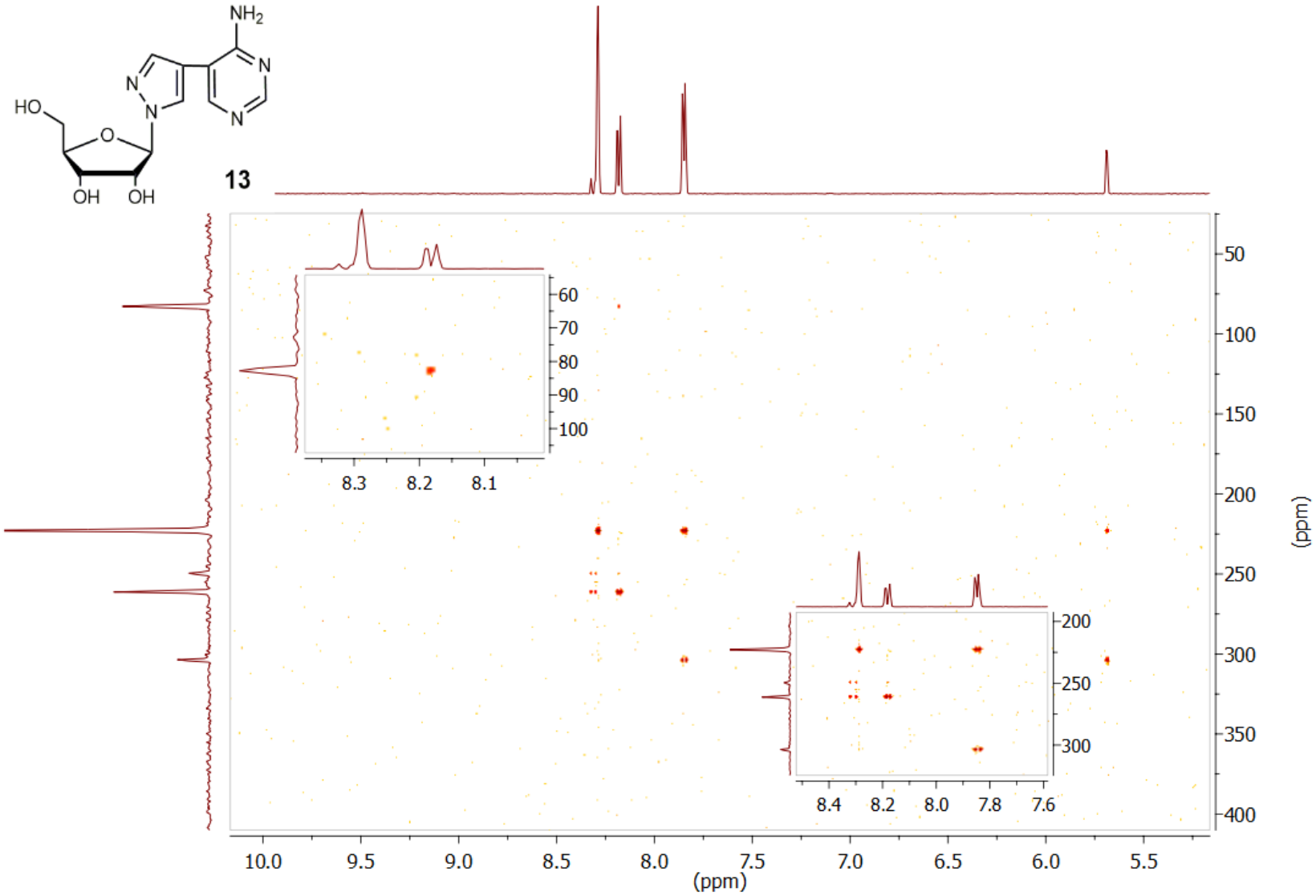
Detailed ^{1}H NMR spectrum (600.2 MHz) of **13** in D_2O



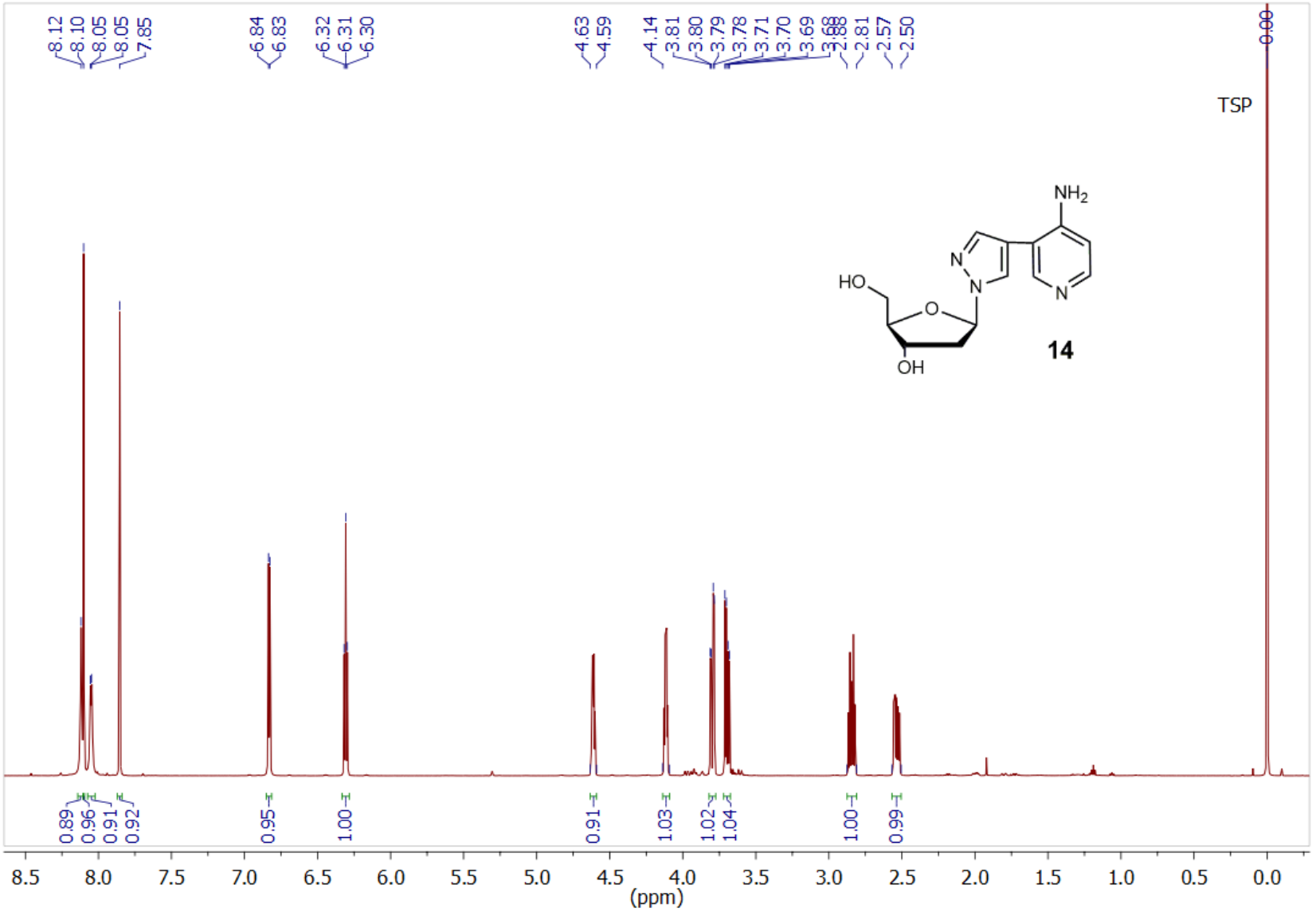
^{13}C NMR spectrum (150.9 MHz) of **13** in D_2O



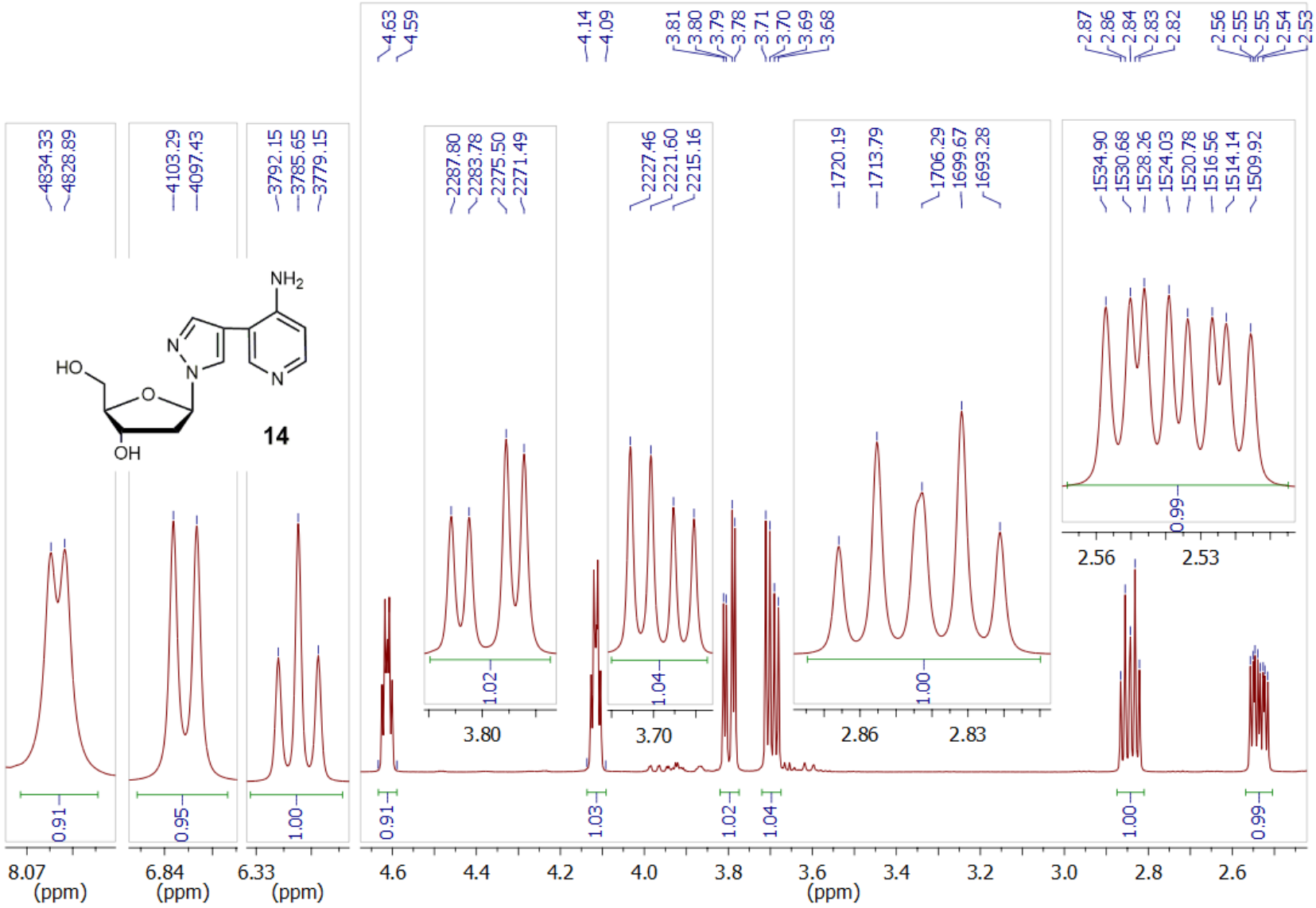
^{1}H-^{13}C HSQC spectrum (700.2 MHz) of **13** in DMSO-*d6*



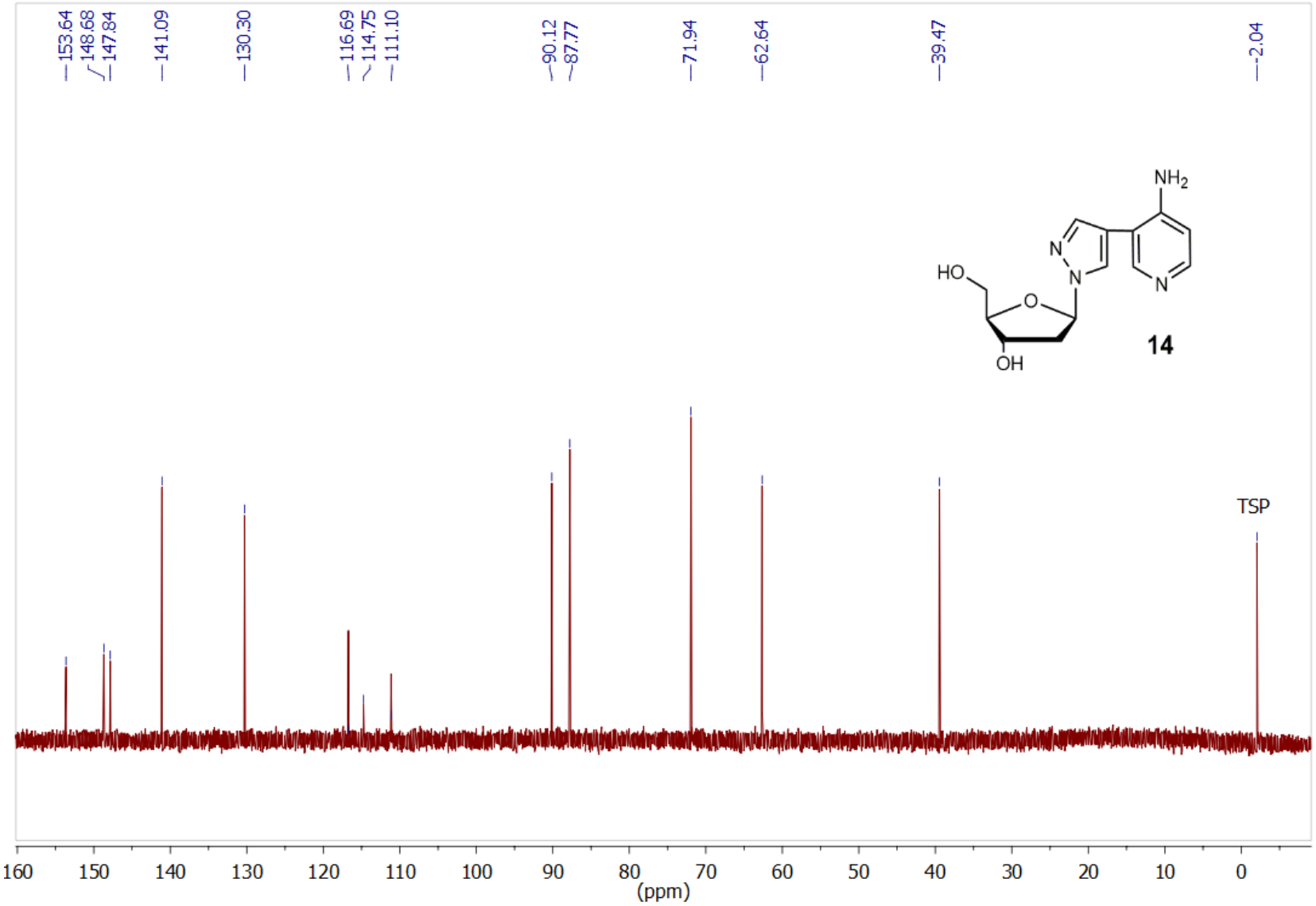
Superimposed ^{1}H-^{15}N spectra (700.2 MHz) of **13** in DMSO-*d6*



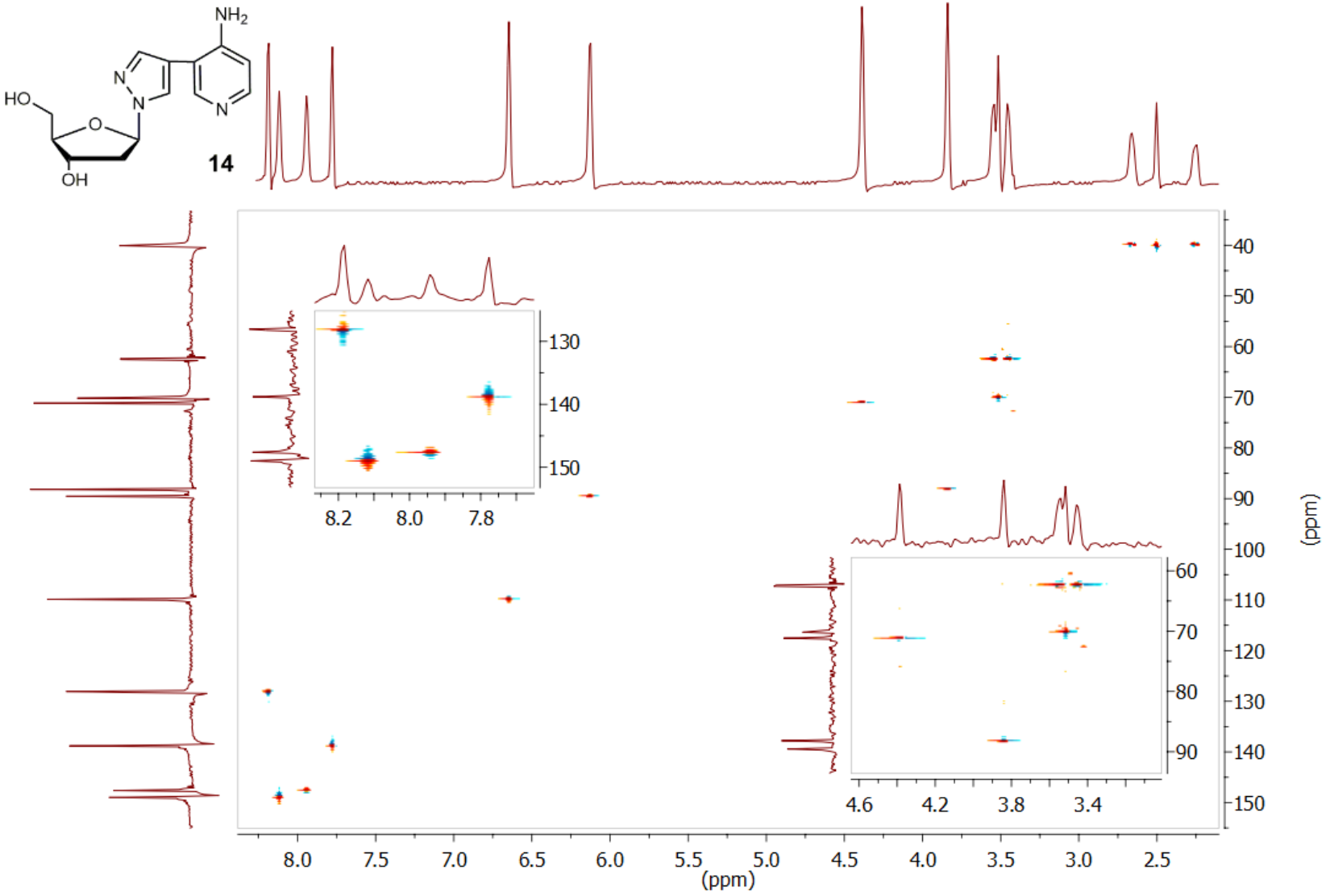
^{1}H NMR spectrum (600.2 MHz) of **14** in D_2O



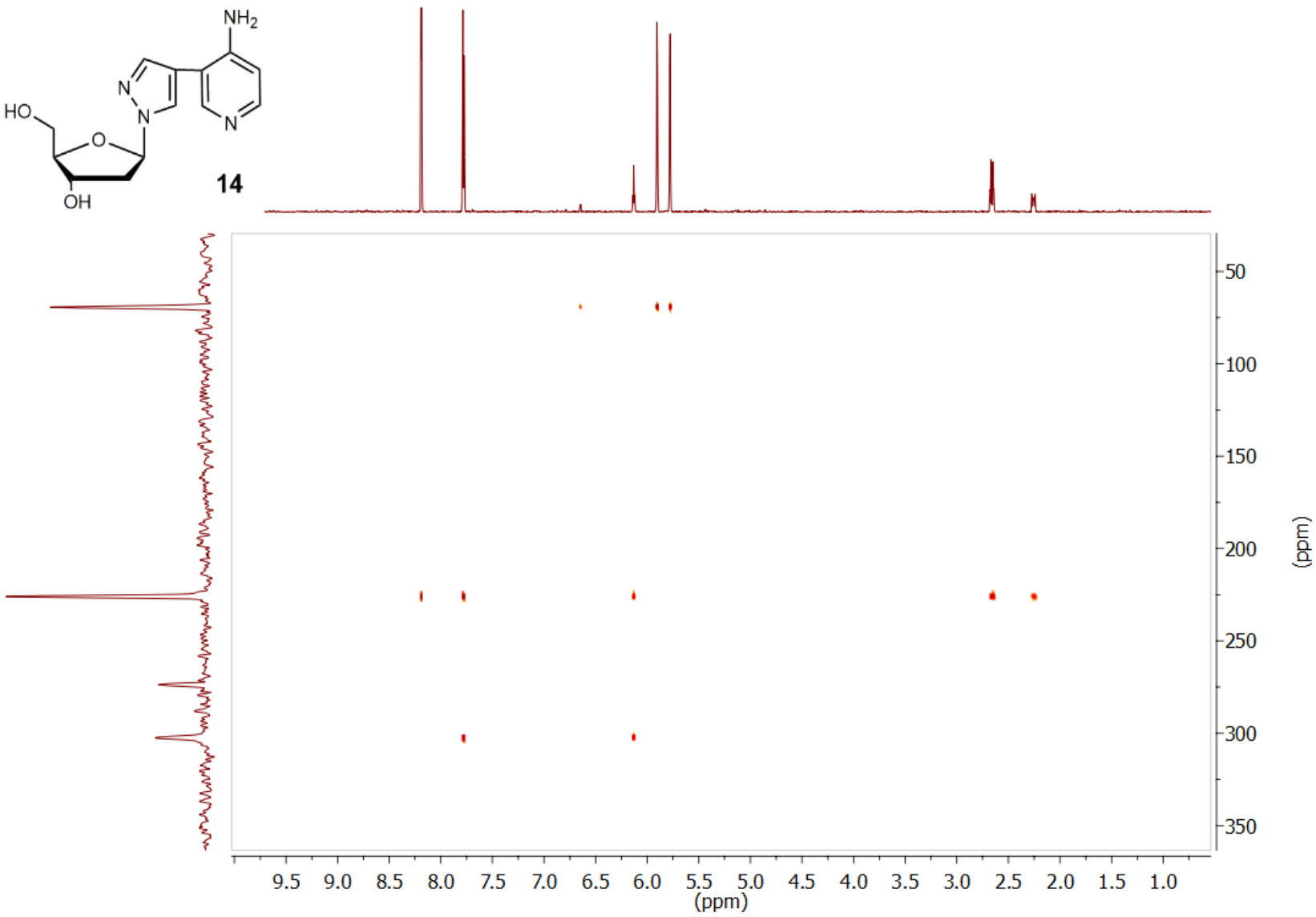
Detailed ^{1}H NMR spectrum (600.2 MHz) of **14** in D_2O



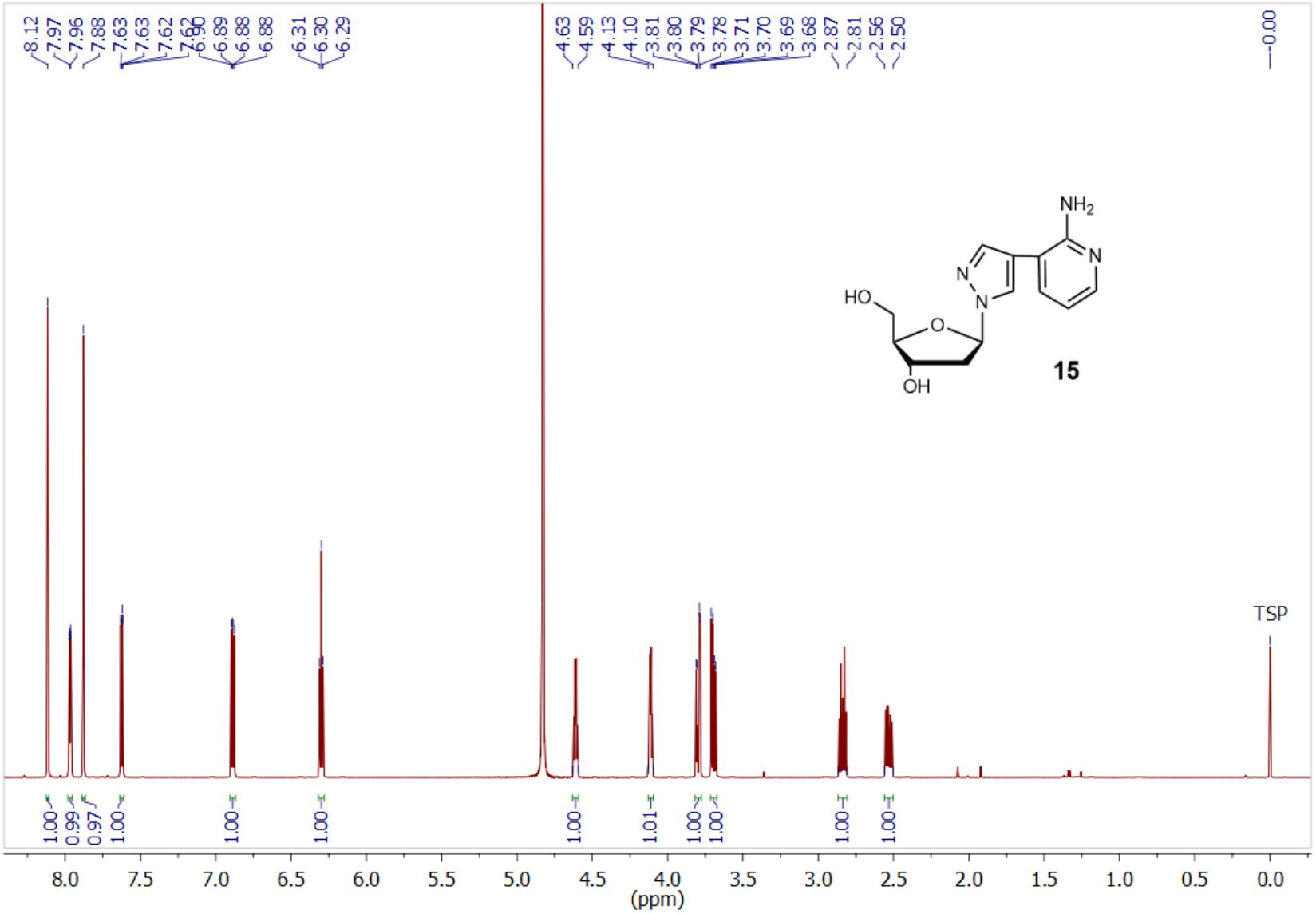
^{13}C NMR spectrum (150.9 MHz) of **14** in D_2O



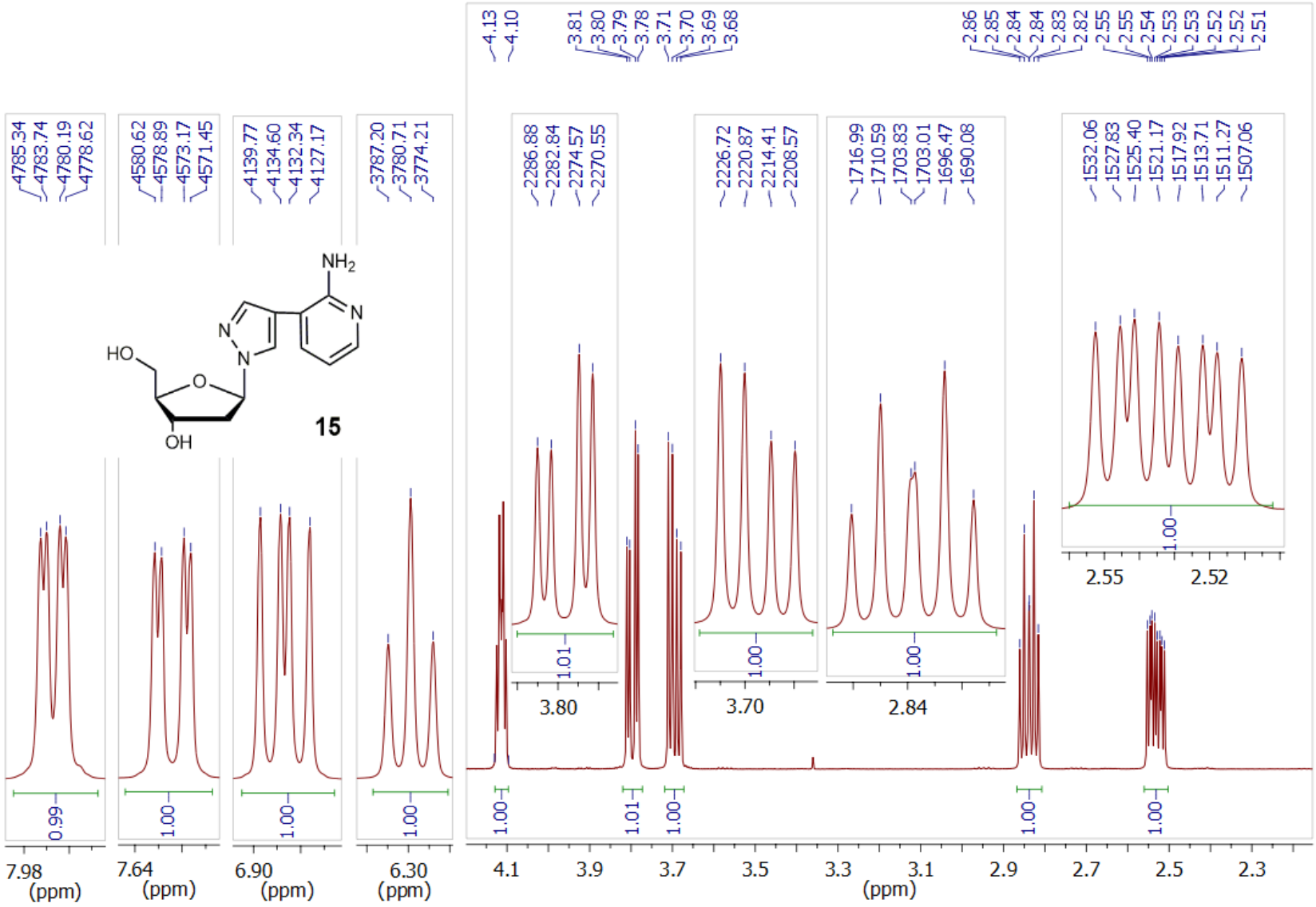
^{1}H-^{13}C HSQC spectrum (700.2 MHz) of **14** in DMSO-*d6*



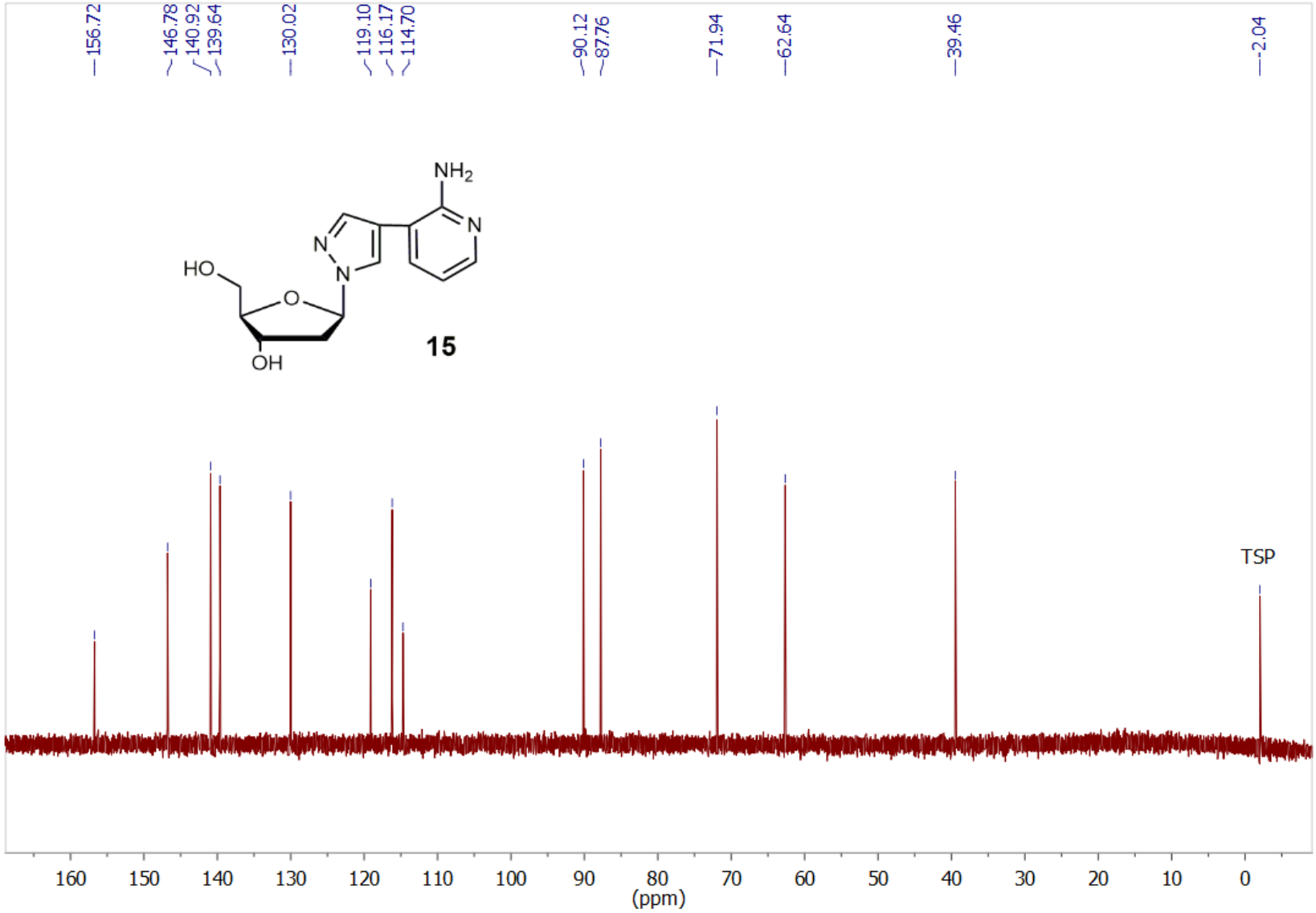
Superimposed ^{1}H-^{15}N spectra (700.2 MHz) of **14** in DMSO-*d6*



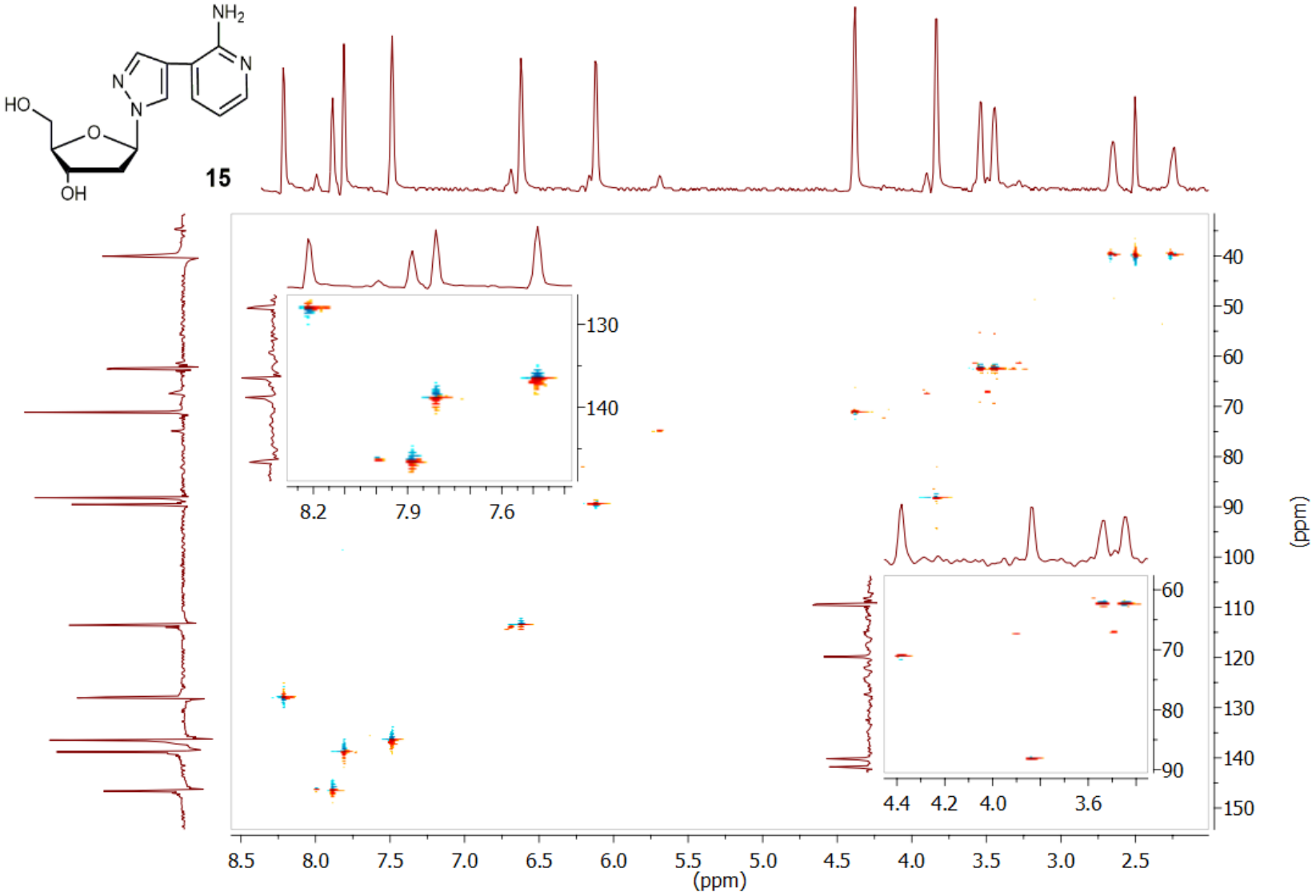
^{1}H NMR spectrum (600.2 MHz) of **15** in D_2O



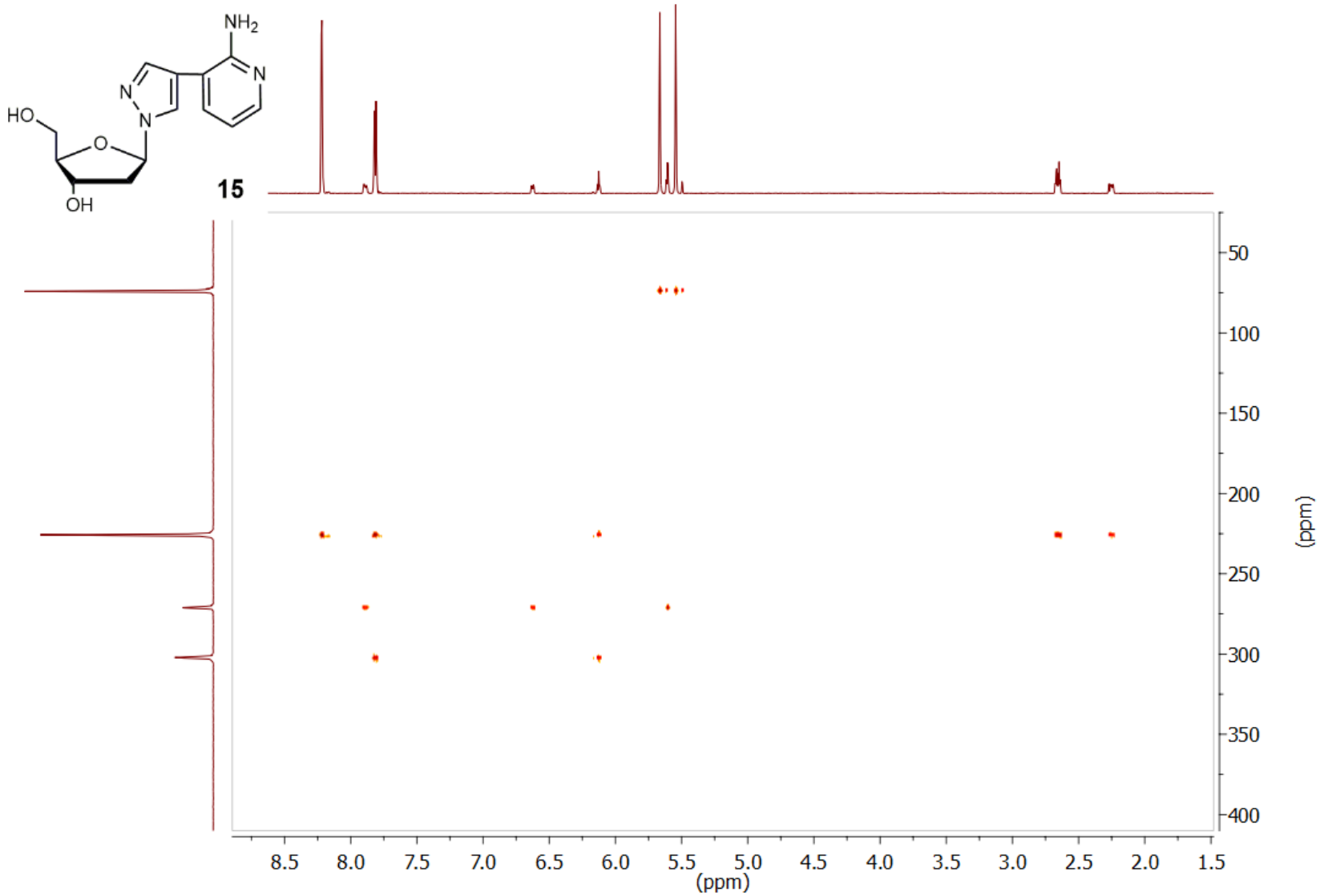
Detailed ^{1}H NMR spectrum (600.2 MHz) of **15** in D_2O



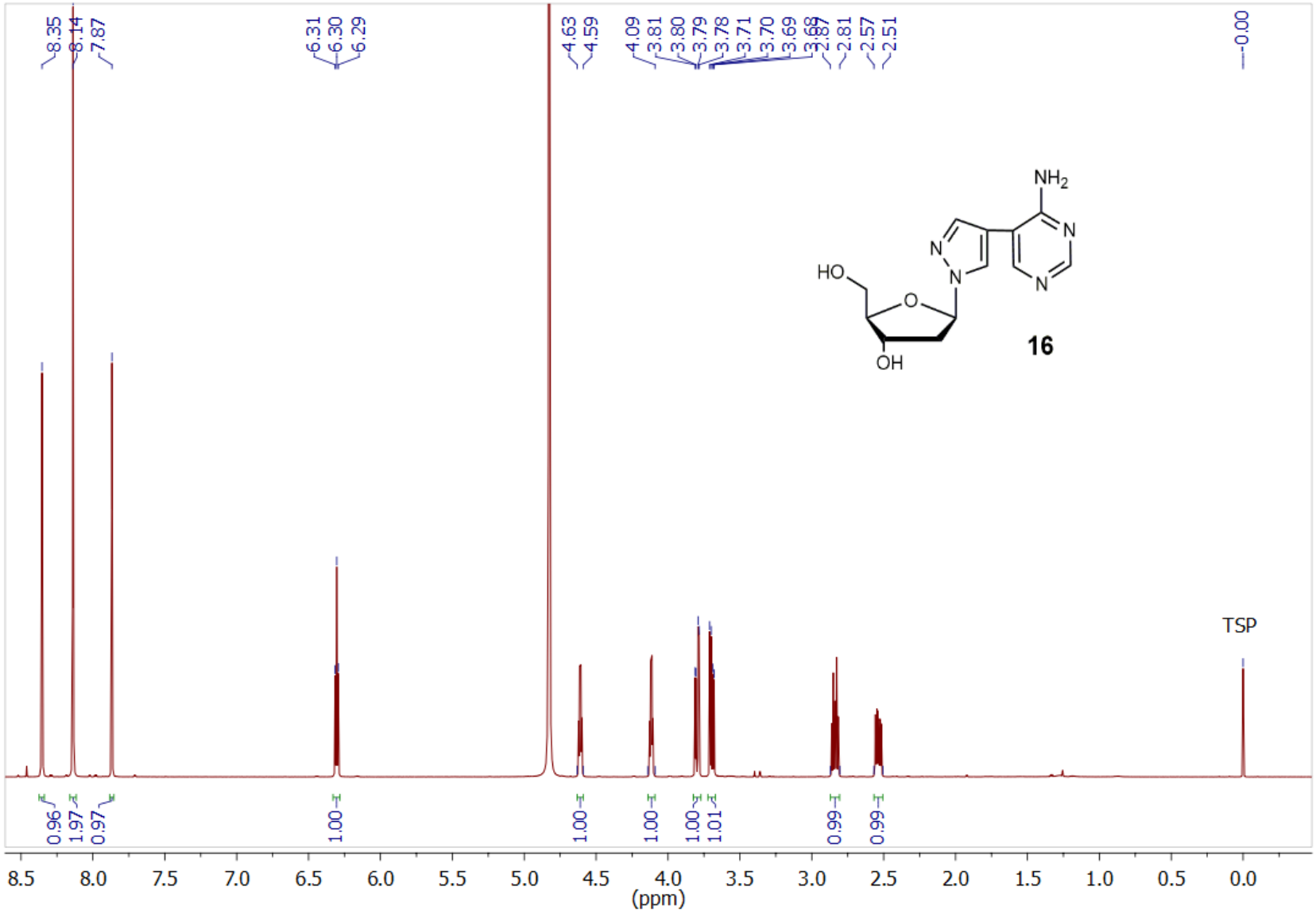
^{13}C NMR spectrum (150.9 MHz) of **15** in D_2O



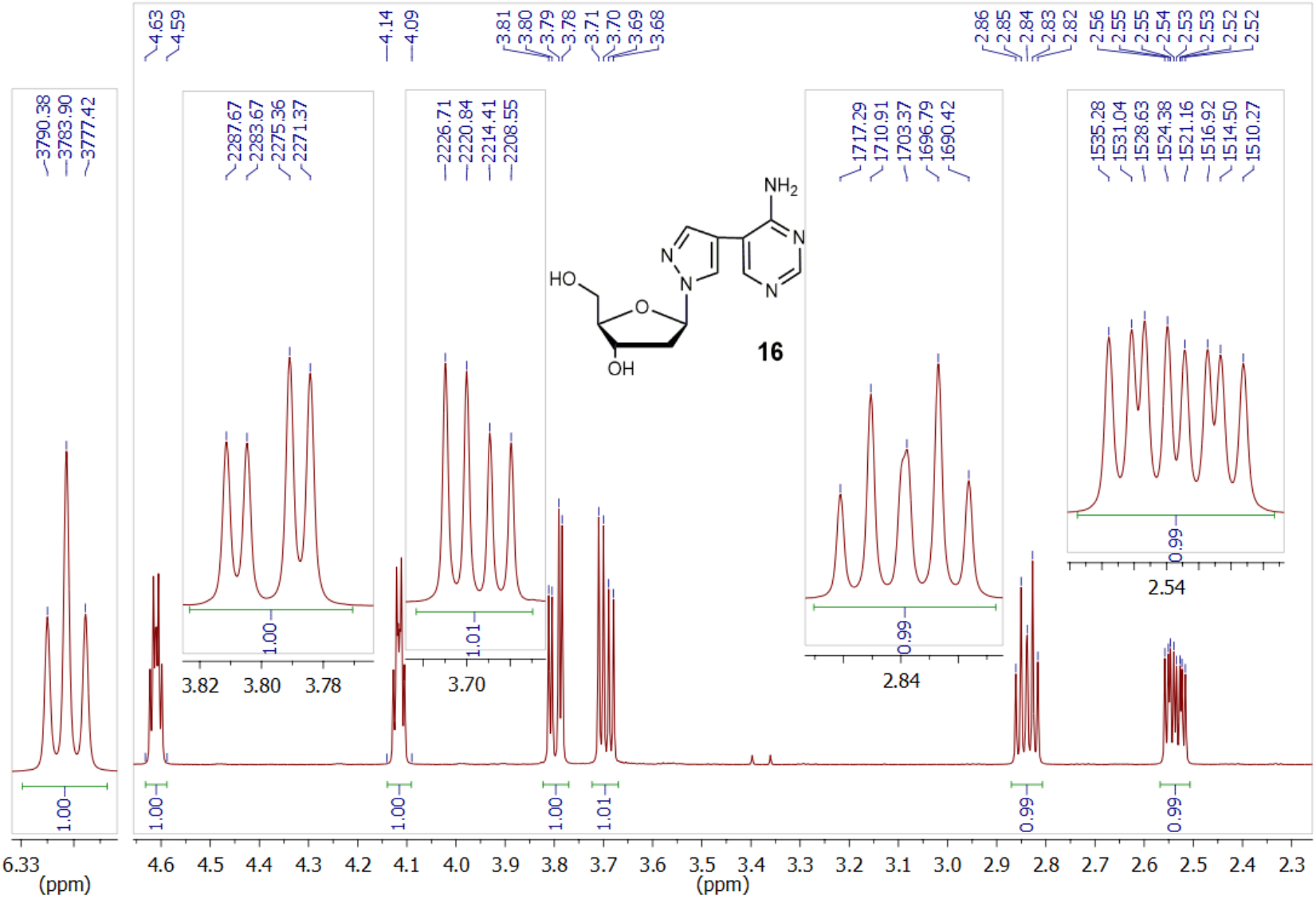
^{1}H-^{13}C HSQC spectrum (700.2 MHz) of **15** in DMSO-*d6*



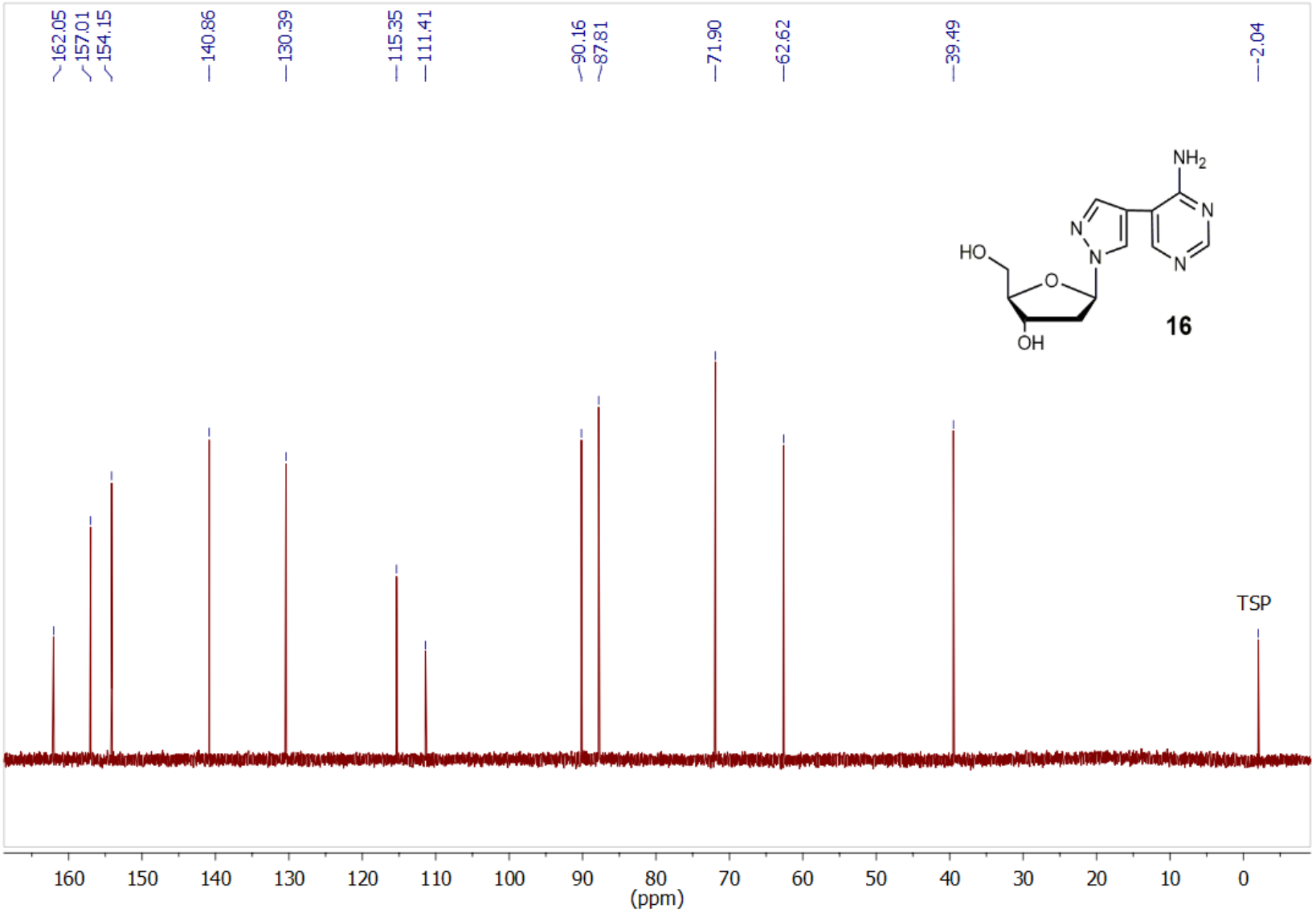
Superimposed ^{1}H-^{15}N spectra (700.2 MHz) of **15** in DMSO-*d6*



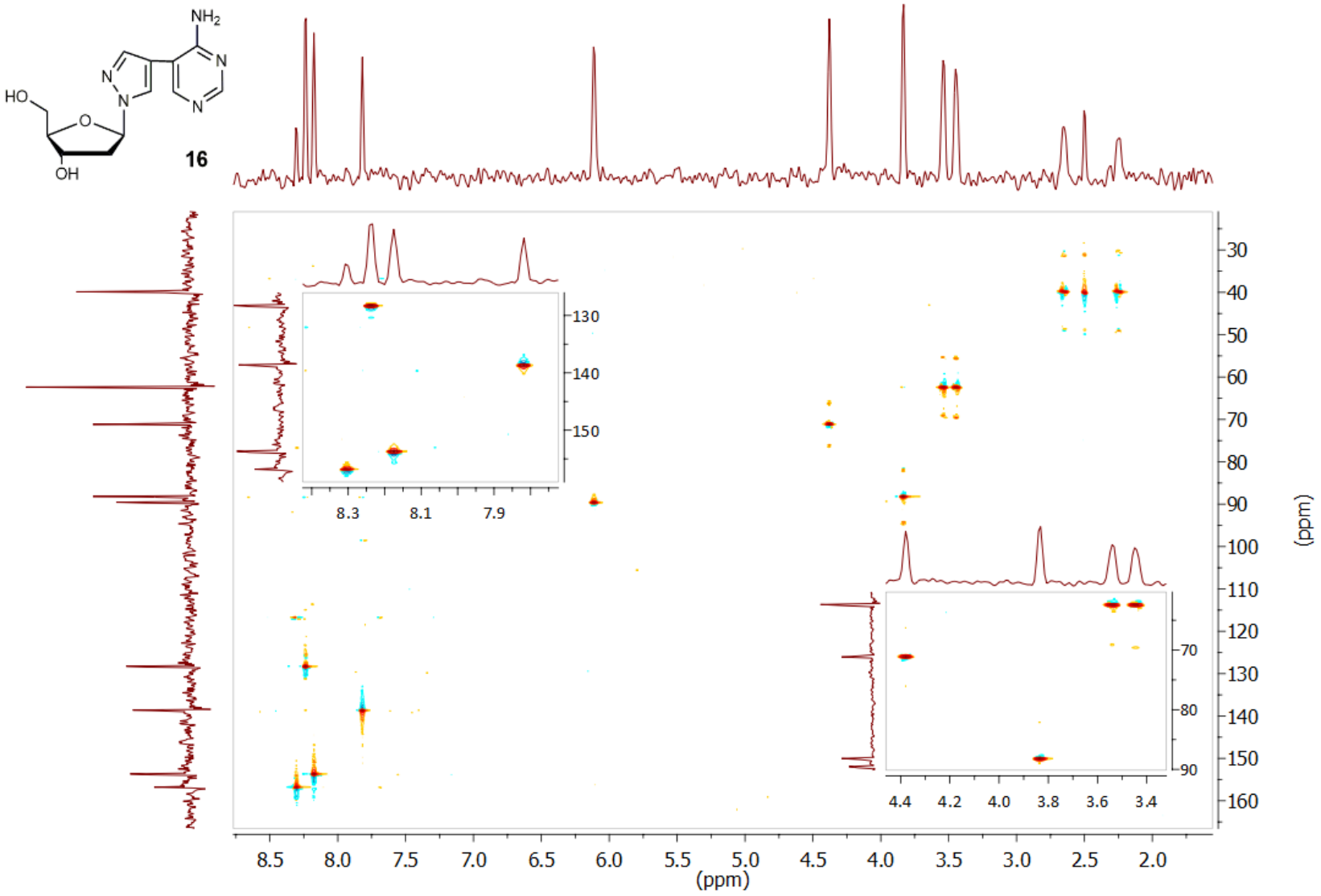
^{1}H NMR spectrum (600.2 MHz) of **16** in D_2O



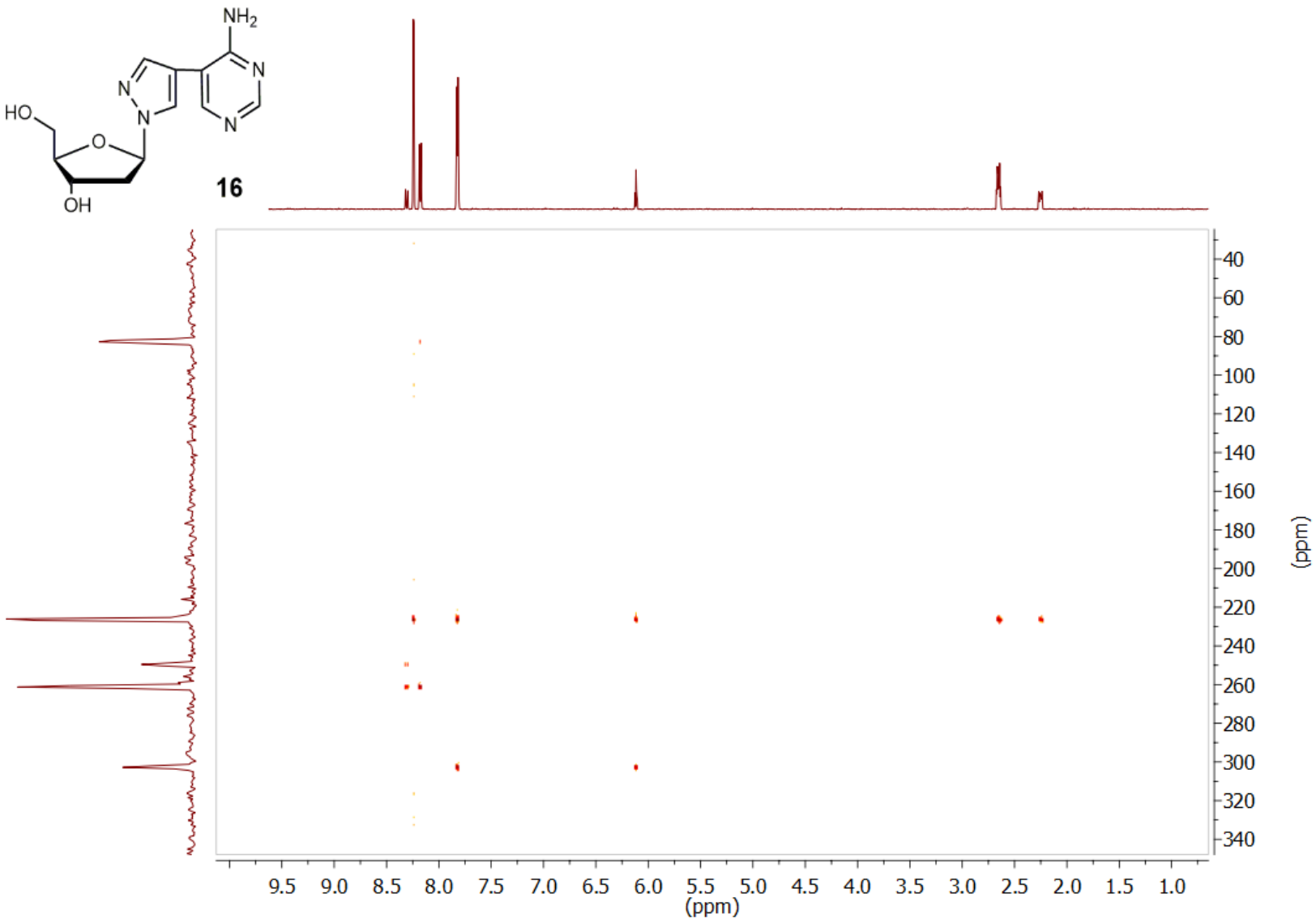
Detailed ^{1}H NMR spectrum (600.2 MHz) of **16** in D_2O



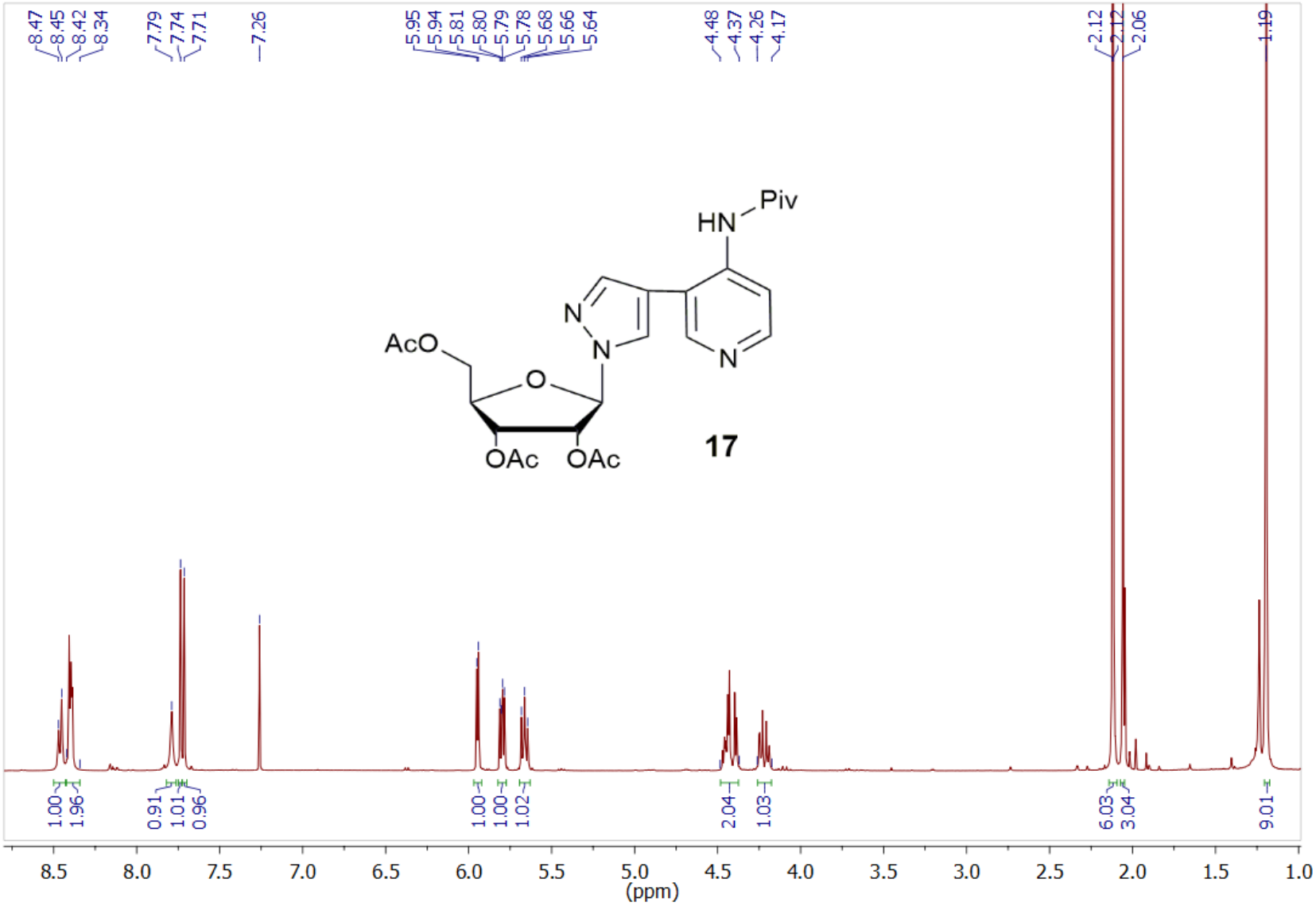
^{13}C NMR spectrum (150.9 MHz) of **16** in D_2O



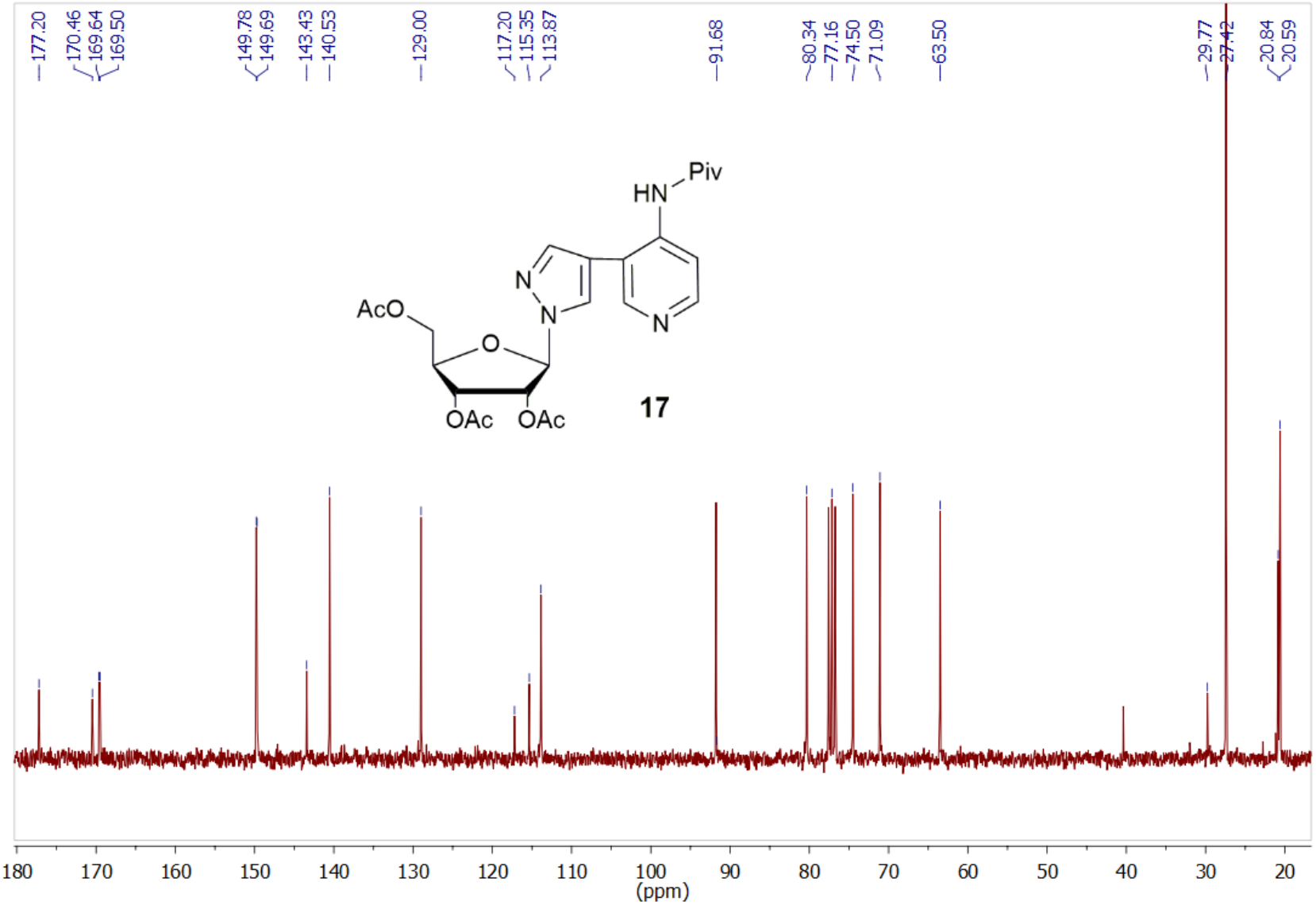
^{1}H-^{13}C HSQC spectrum (700.2 MHz) of **16** in DMSO-*d6*



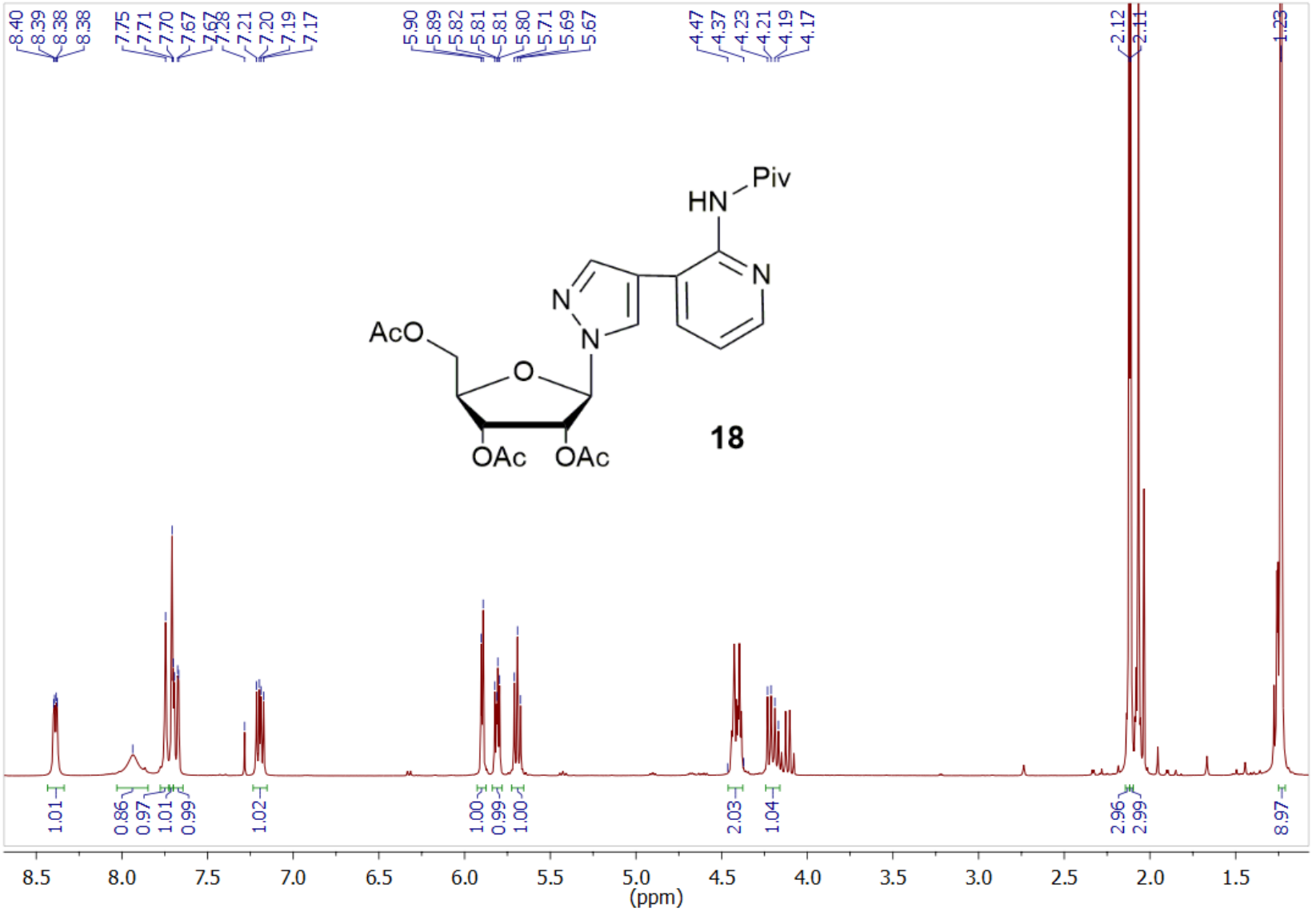
Superimposed ^{1}H-^{15}N spectra (700.2 MHz) of **16** in DMSO-*d6*



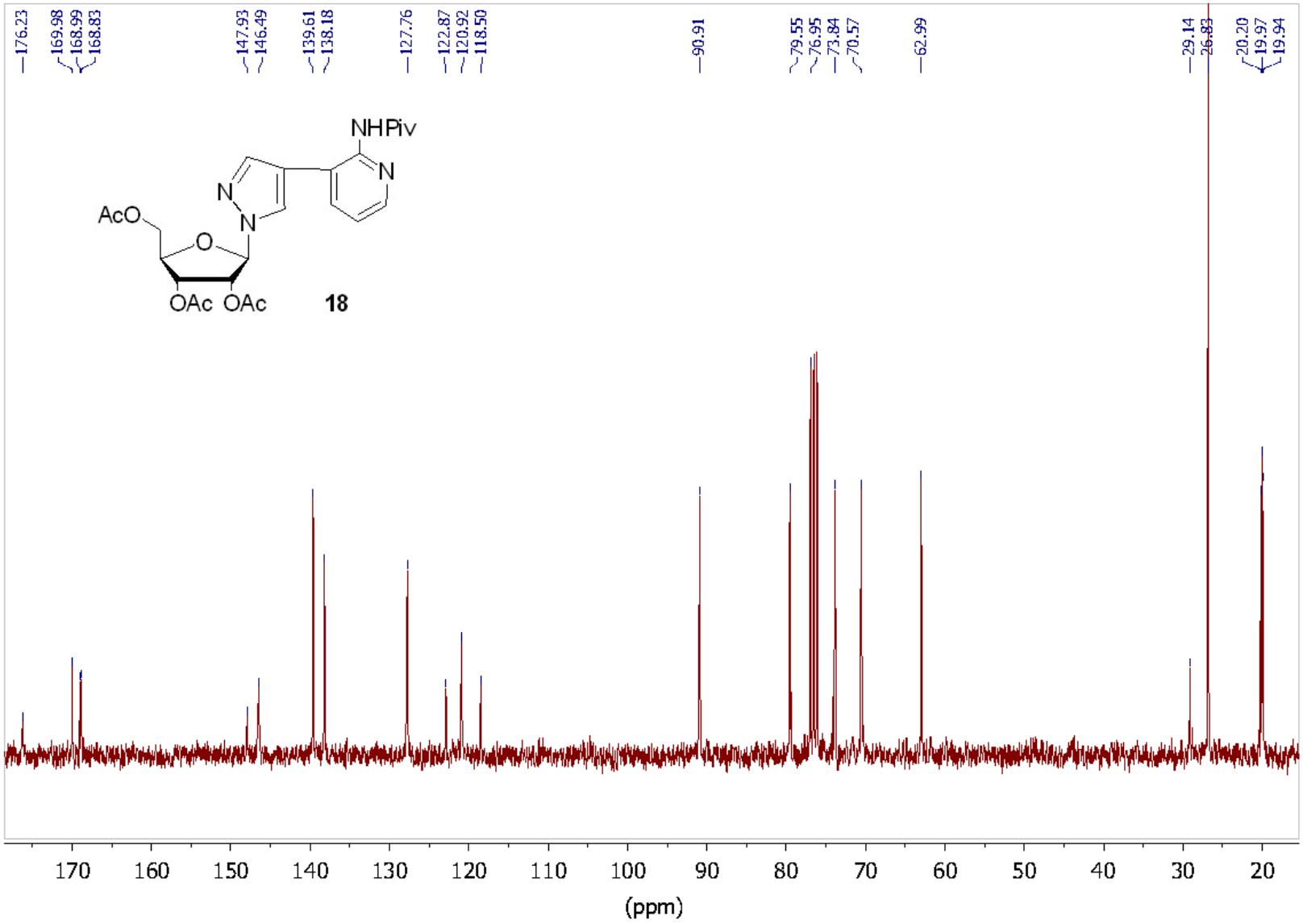
^{1}H NMR spectrum (300.1 MHz) of **17** in $CDCl_3$



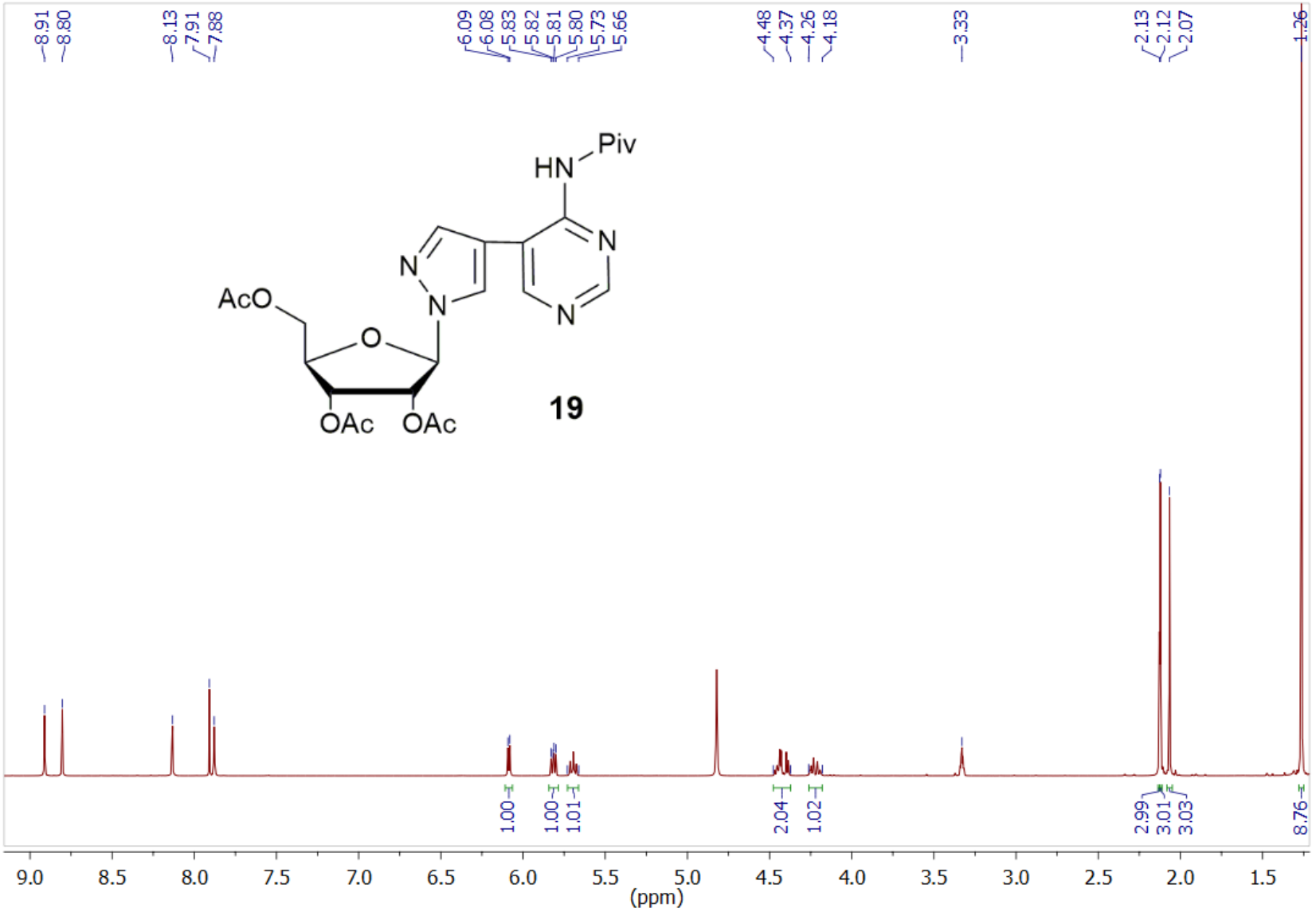
^{13}C NMR spectrum (75.5 MHz) of **17** in DMSO-d^6



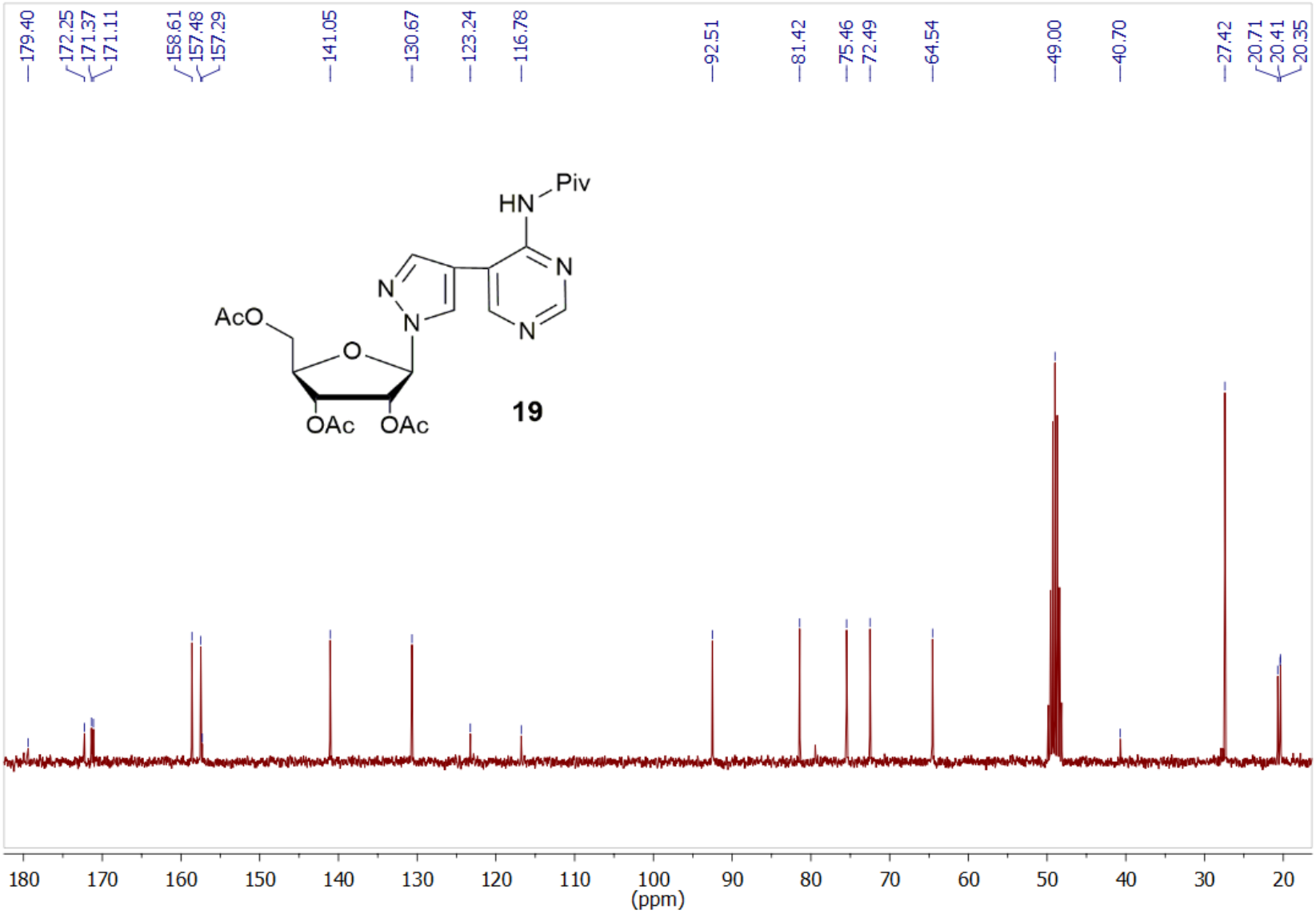
^{1}H NMR spectrum (300.1 MHz) of **18** in $CDCl_3$



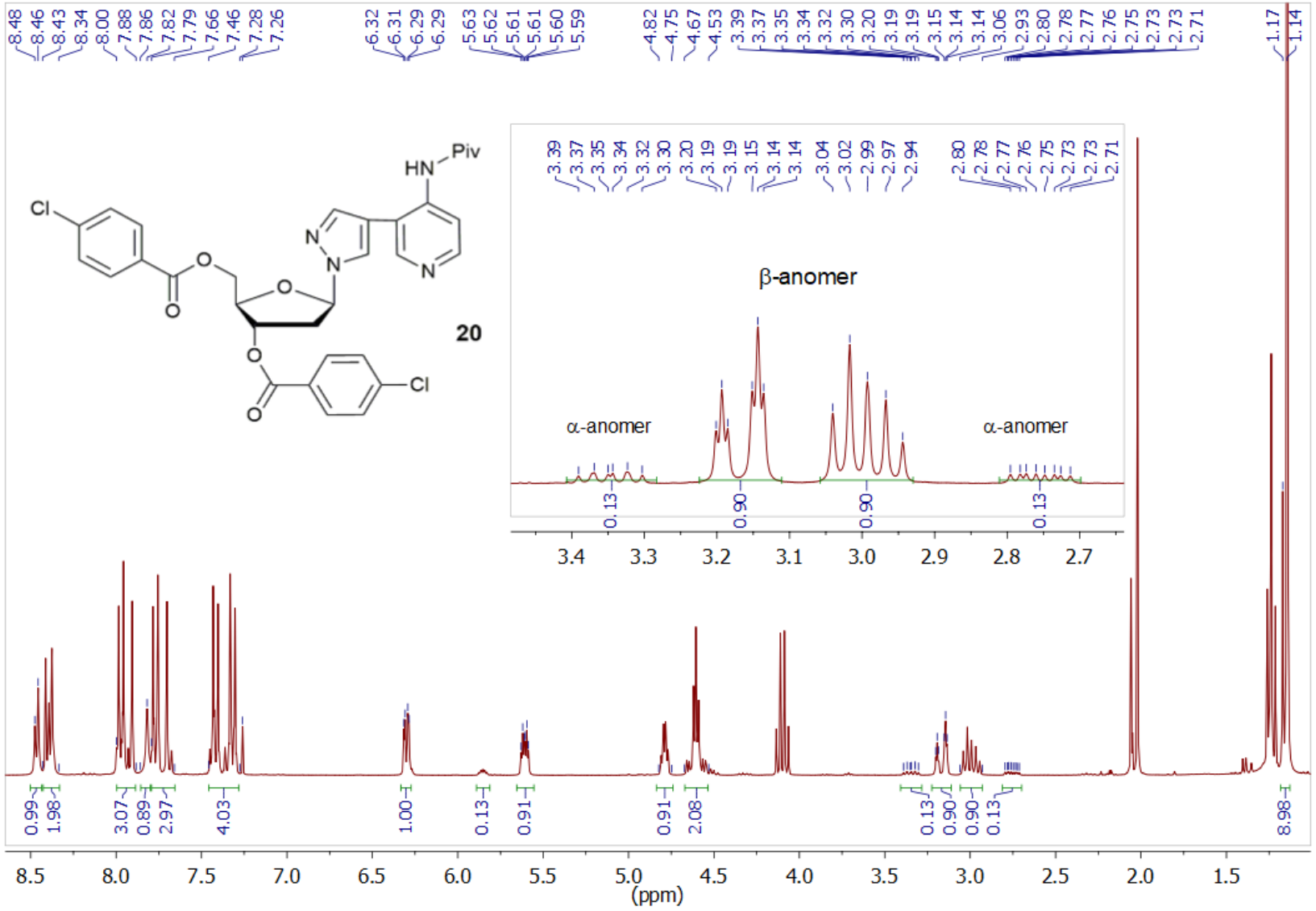
^{13}C NMR spectrum (75.5 MHz) of **18** in CD_3Cl_3



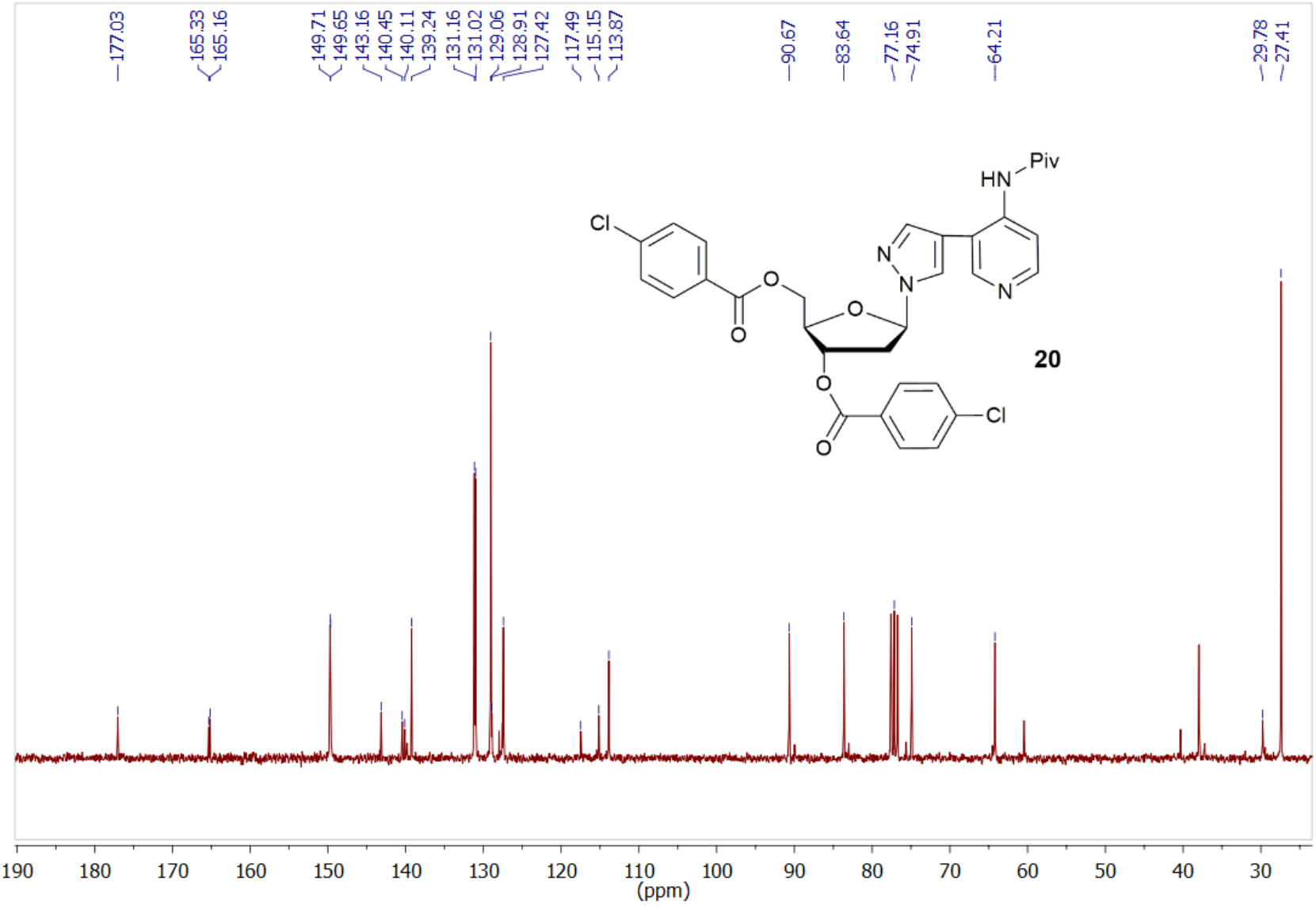
[1]H NMR spectrum (300.1 MHz) of **19** in CD_3OD



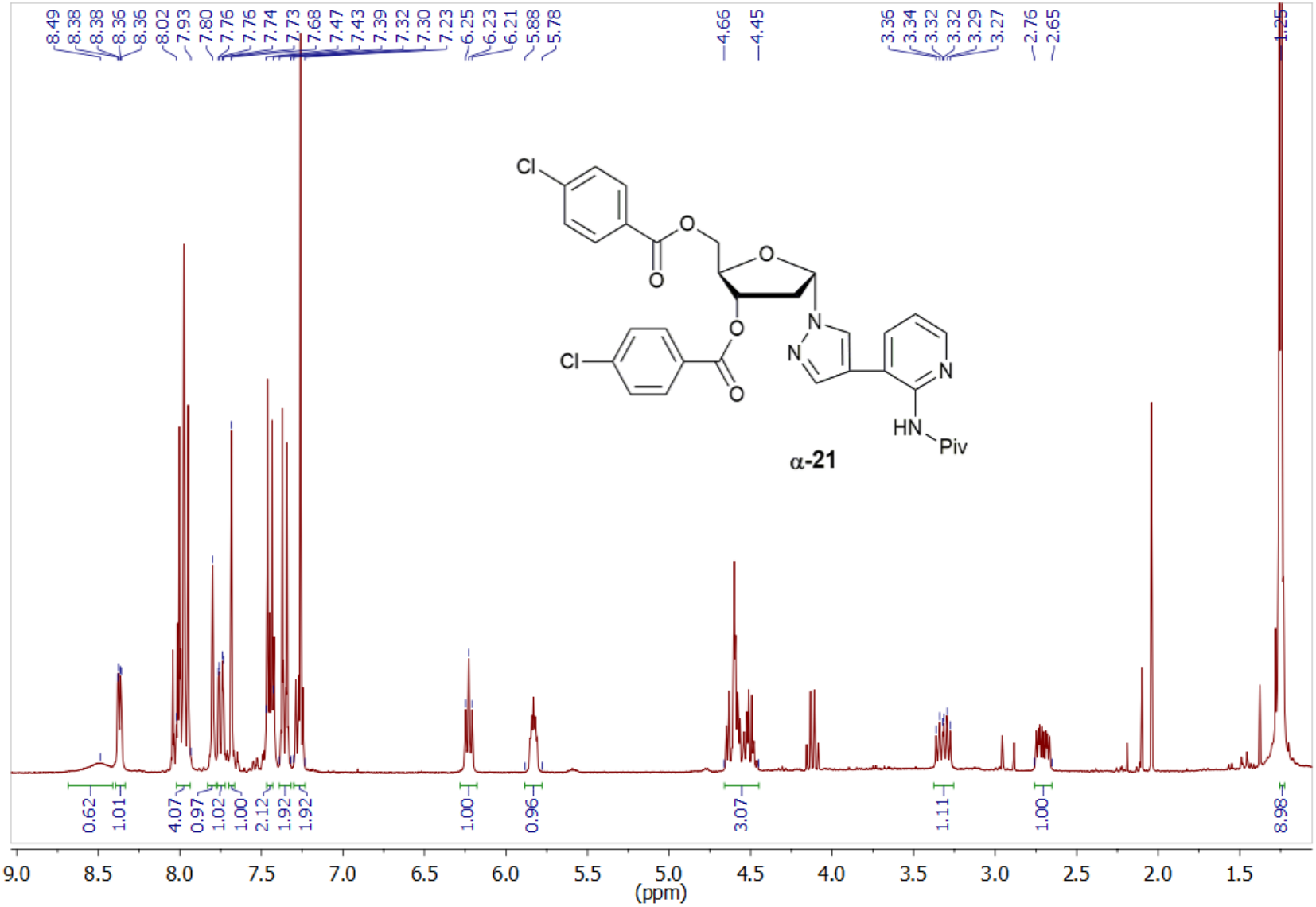
^{13}C NMR spectrum (75.5 MHz) of **19** in CD_3OD



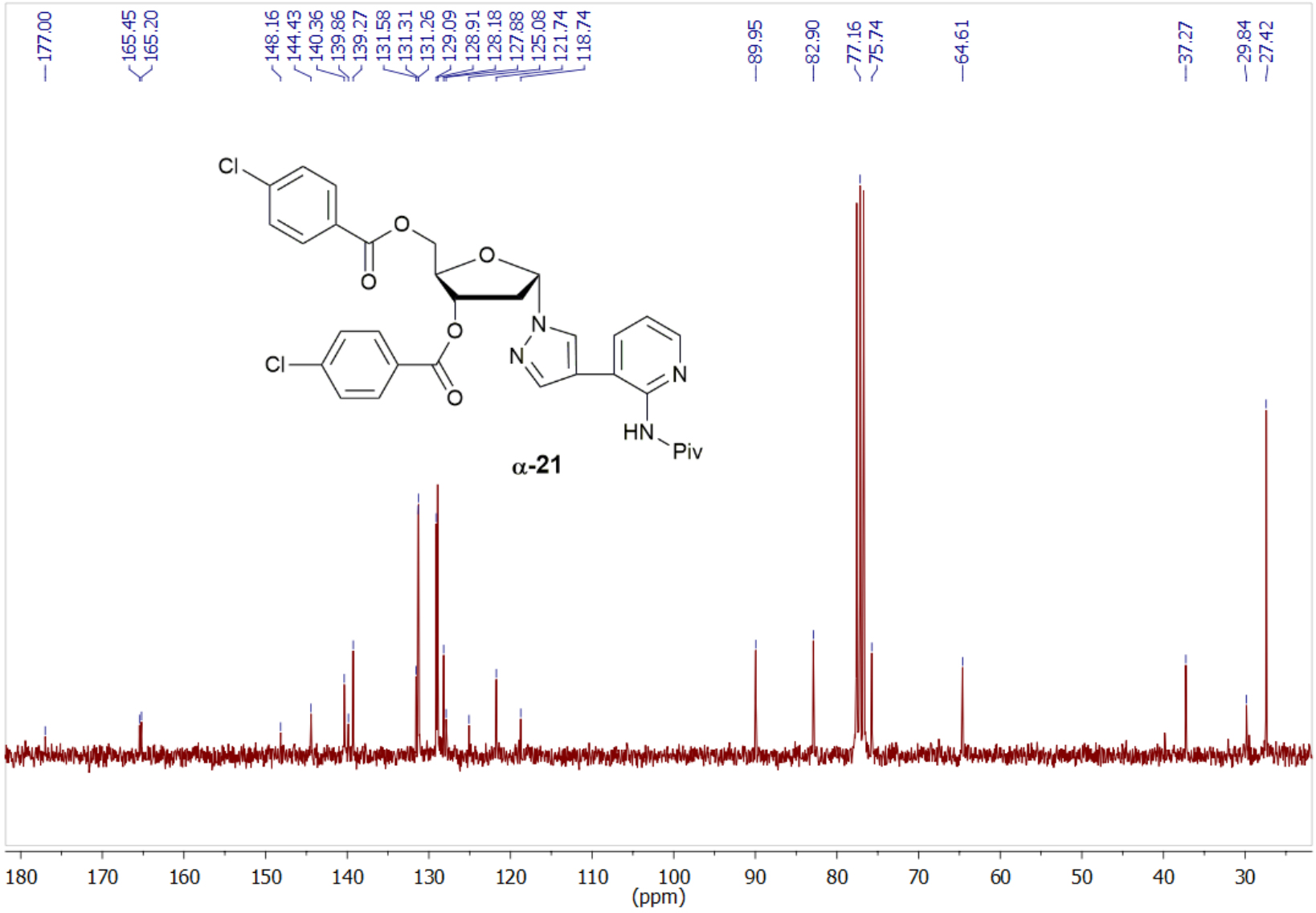
^{1}H NMR spectrum (300.1 MHz) of **20** in $CDCl_3$



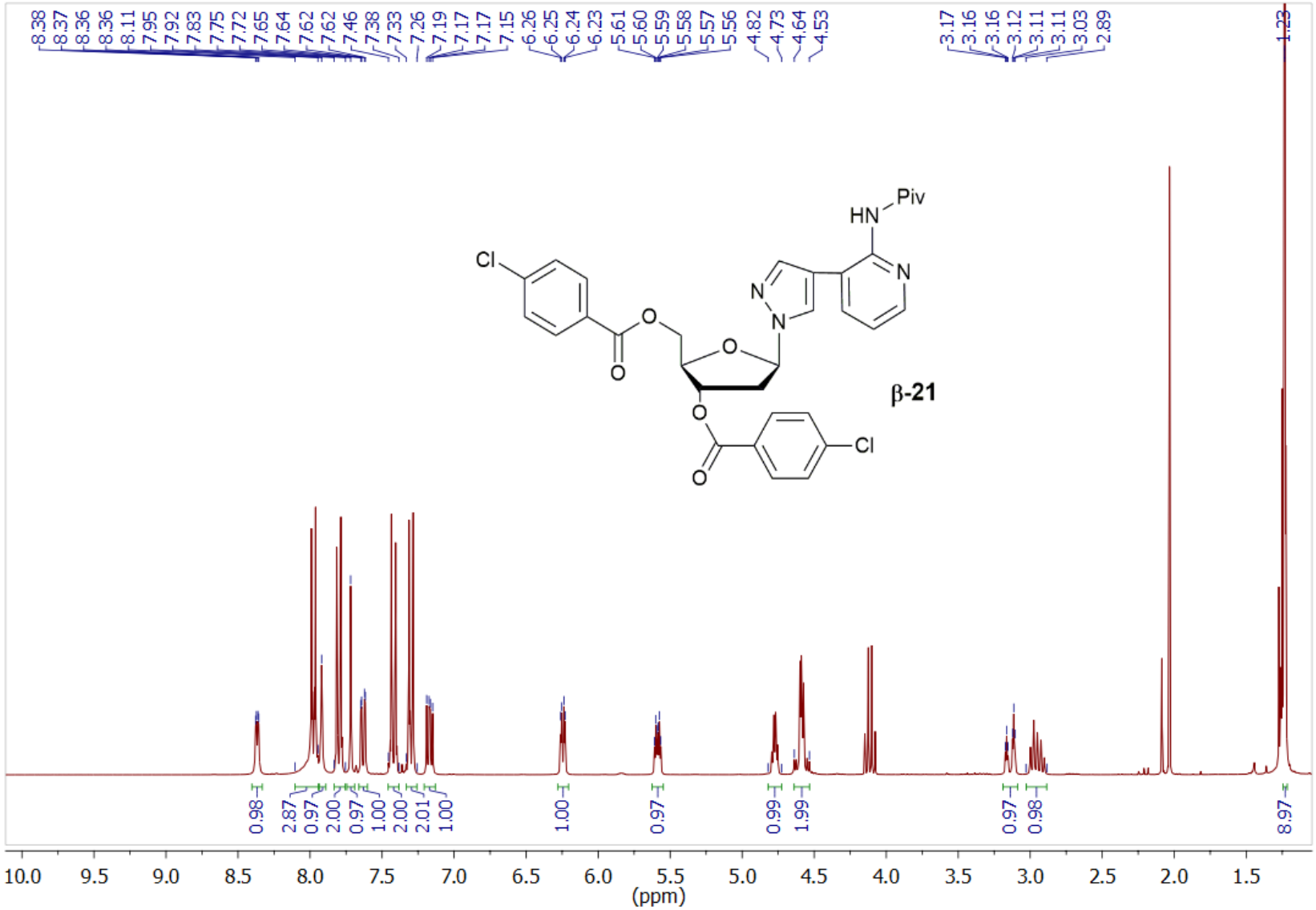
^{13}C NMR spectrum (75.5 MHz) of **20** in $CDCl_3$



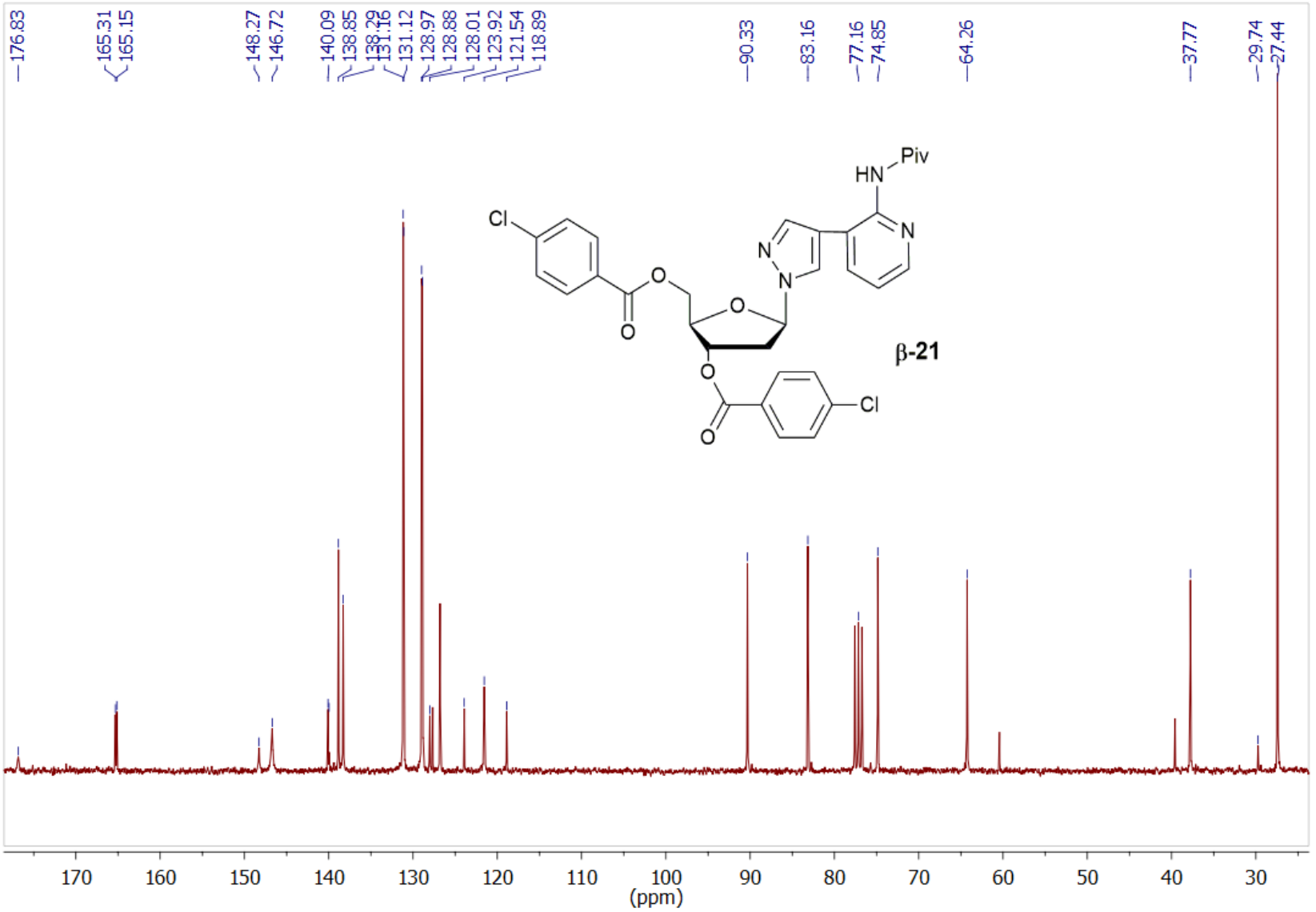
^{1}H NMR spectrum (300.1 MHz) of **21 α-anomer** in $CDCl_3$



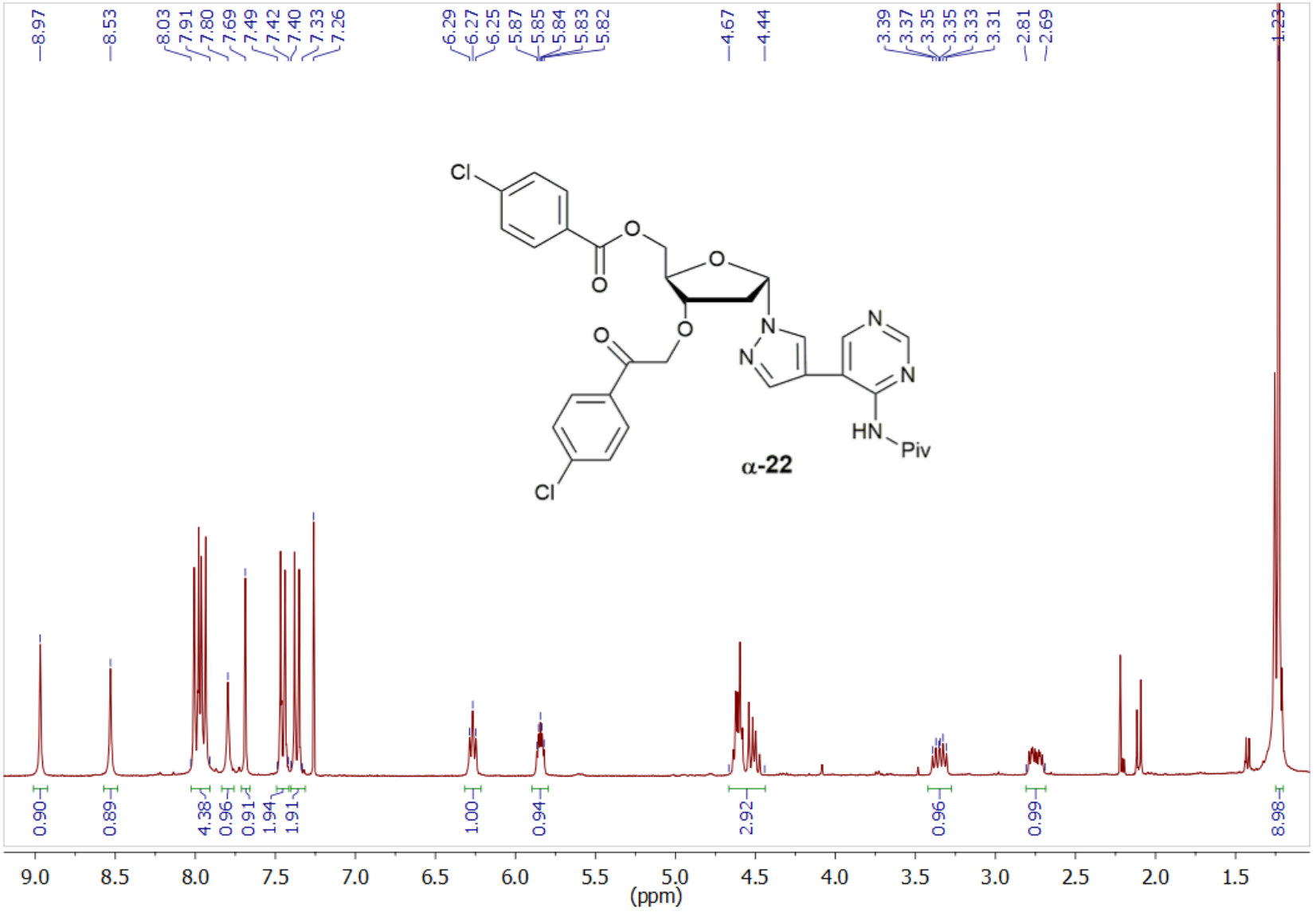
^{13}C NMR spectrum (75.5 MHz) of **21 α-anomer** in $CDCl_3$



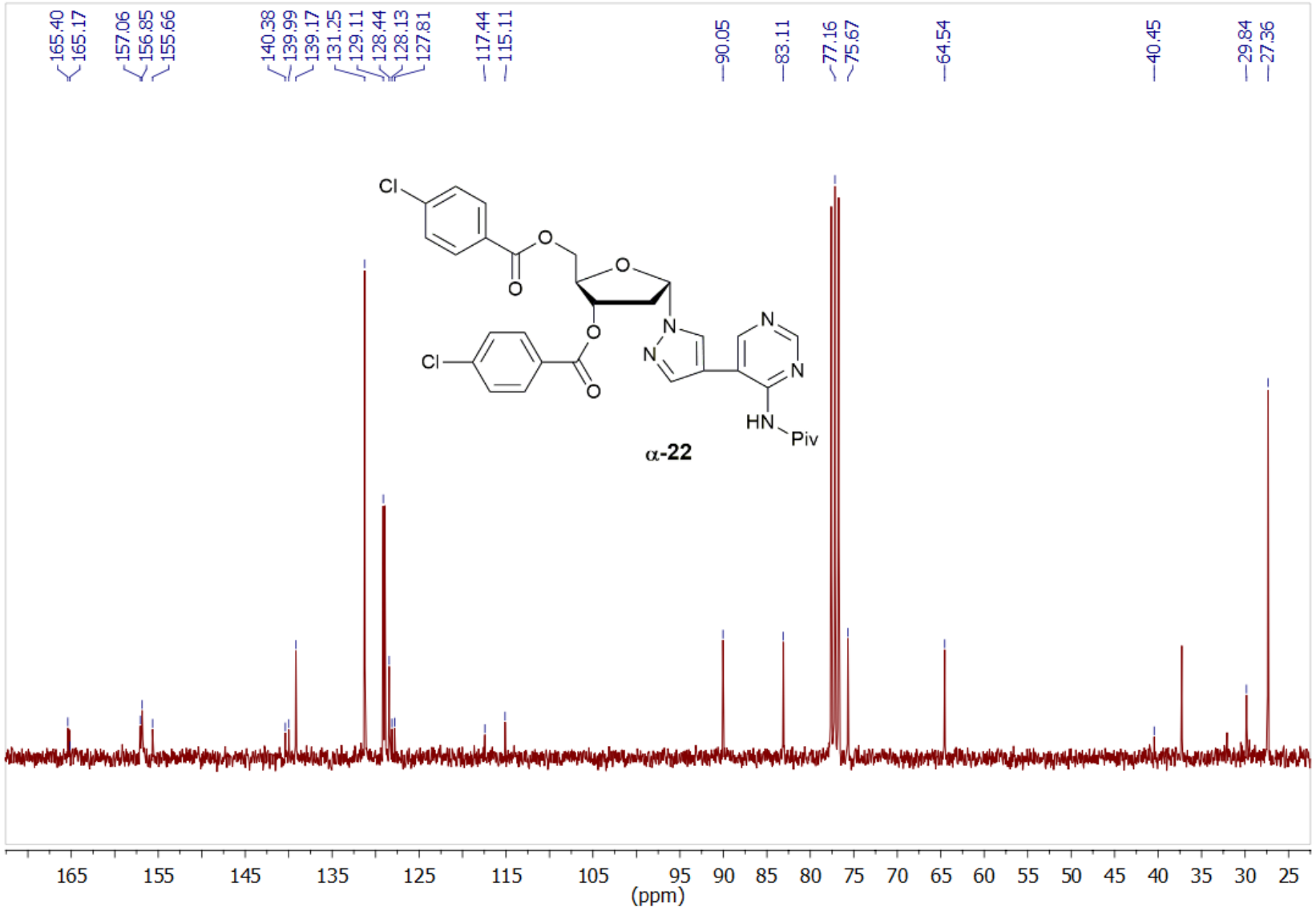
^{1}H NMR spectrum (300.1 MHz) of **21 β-anomer** in $CDCl_3$



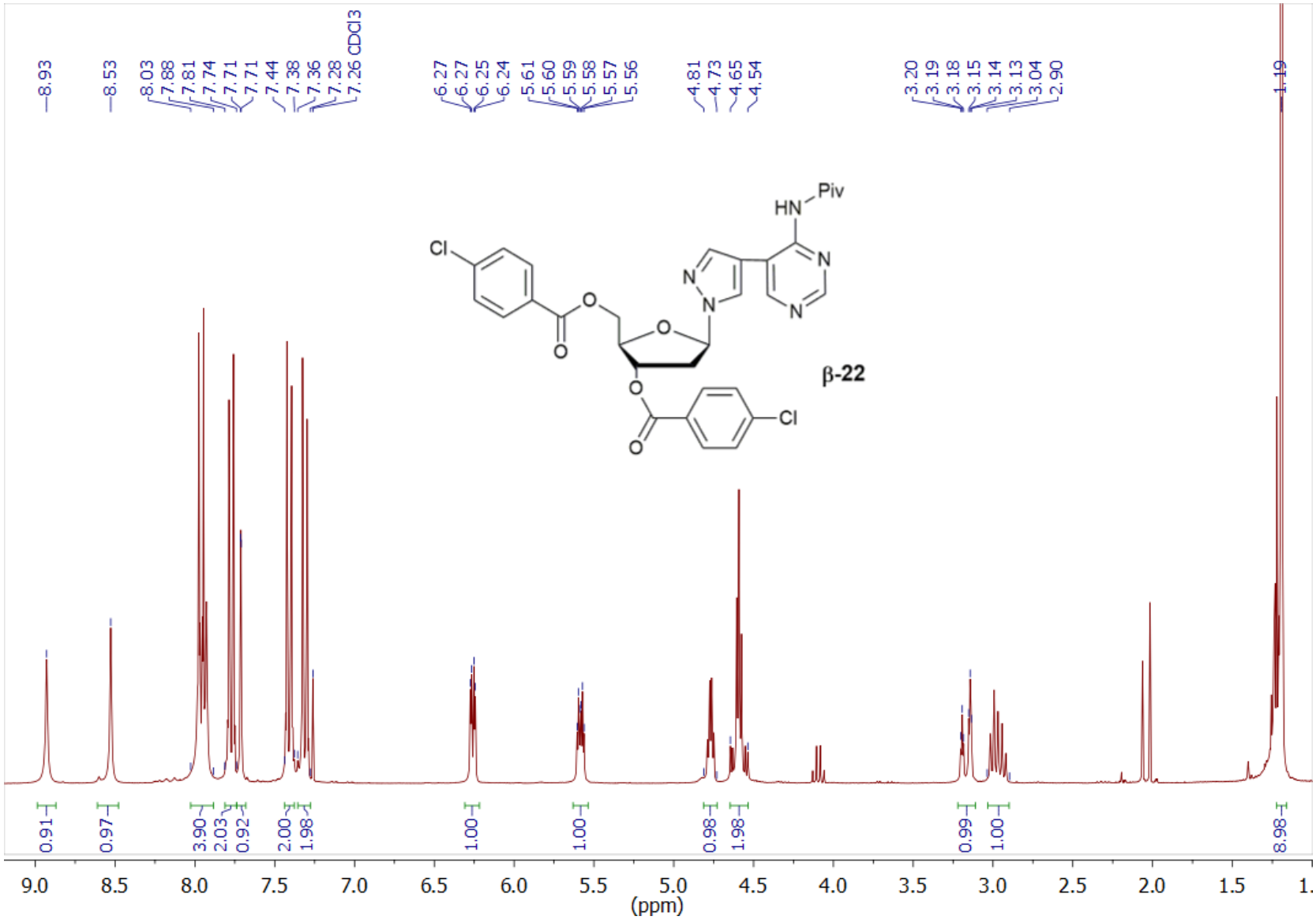
^{13}C NMR spectrum (75.5 MHz) of **21 β-anomer** in $CDCl_3$



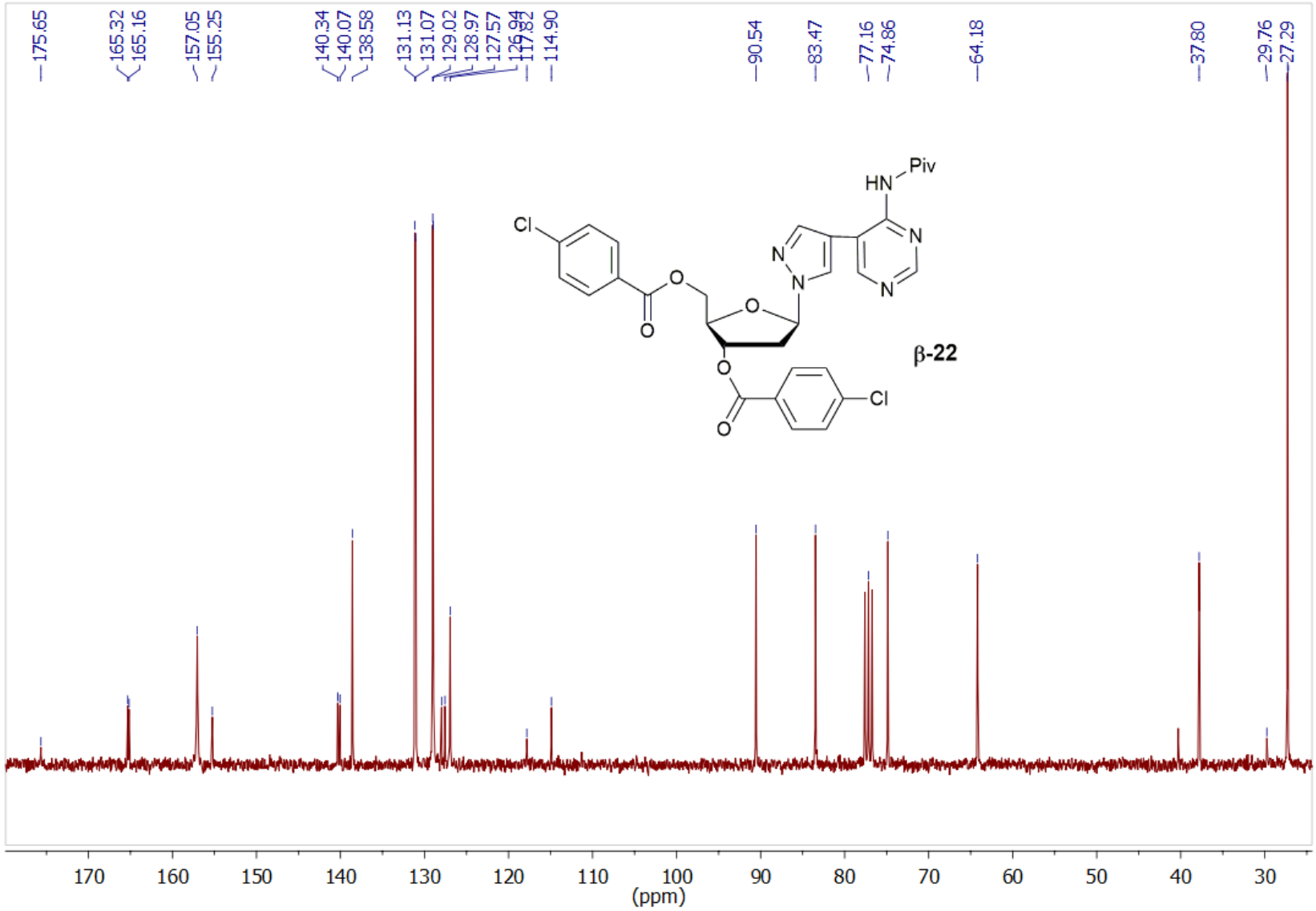
^{1}H NMR spectrum (300.1 MHz) of **22 α-anomer** in $CDCl_3$



^{13}C NMR spectrum (75.5 MHz) of **22 α-anomer** in $CDCl_3$



^{1}H NMR spectrum (300.1 MHz) of **22 β-anomer** in $CDCl_3$



^{13}C NMR spectrum (75.5 MHz) of **22 β-anomer** in $CDCl_3$