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Supporting Information

Pyrimidine Carboxylic Acids Linked through 1, 4, 5- Trisubstituted 1, 2, 3-Triazoles: Synthesis and RNase A Inhibition Studies

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Experimental Section for the Synthesis of Compounds

General methods

All reagents and fine chemicals were purchased from commercial suppliers and were used without further purification. Column chromatographic separations were done using silica gel (230-400 mesh). Solvents were dried and distilled following standard procedures. TLC was carried out on precoated plates (Merck silica gel 60, f_{254}) and the spots were visualized with UV light or by charring the plates dipped in 5% H_2SO_4-MeOH solution or 5% H_2SO_4/vanillin/EtOH solution. ^1H and ^{13}C NMR for the compounds were recorded at 200/400/600 and 50/100/150 MHz respectively using a Bruker NMR instrument unless stated otherwise. Chemical shifts are reported in parts per million (ppm, δ scale). DEPT experiments have been carried out to identify the methylene carbons. Melting points were determined in open-end capillary tubes. Bovine pancreatic RNase A, RNA (*Torulautilis*) and 2′, 3′-cCMPwere purchased from commercial suppliers. UV-Vis measurements were made using a UV-Vis spectrophotometer (Shimadzu 2450). Concentrations of the solutions were estimated spectrophotometrically using the following data: $\varepsilon_{278.5}$ = 9800 $M^{-1}cm^{-1}$ (RNase A)[55] andε_{268} = 8500 $M^{-1}cm^{-1}$ (2′, 3′-cCMP)[17]. Mass spectroscopy data were obtained from Xevo G2QT mass spectrometer.

General procedure for the triazolylation reaction

To a well-stirred solution of the azide (1 mmol) in CCl_4 (5 ml) was added DMAD (1.5 mmol). The resulting mixture was allowed to reflux for 24h. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography under reduced pressure (EtOAc-pet ether) to obtain pure product.

General procedure for ester hydrolysis

To a well stirred solution of the compound (1 mmol) in methanol (5 ml) at 0 °C, was added 1 (N) NaOH solution (2 ml) dropwise. The resulting solution was allowed to warm back to room temperature and stirred for 2h. After evaporation of methanol under reduced pressure, the resulting residue was dissolved in water (10 ml) and neutralized with acidic amberlyte. The resulting mixture was filtered, and the filtrate was evaporated under reduced pressure to obtain pure product.

Compound 4

Compound **4** (0.42 g, 75%) was synthesized from compound **3** (0.6 g, 1.46 mmol) following the general procedure of ester hydrolysis. White solid. ^{1}H NMR (600 MHz, DMSO-*d*$_6$):δ 1.80 (s, 3H), 2.51-2.63 (m, 1H), 2.69-2.73 (m, 1H), 3.69-3.77 (m, 2H), 4.27-4.28 (m, 1H), 6.07-6.08 (m, 1H), 6.53-6.55 (m, 1H), 7.94 (s, 1H).

(ref: Häbic, D.; Barth, W.; Rösner, M. *Heterocycles* **1989**, *29*, 2083)

Compound 6

Compound **6** (0.50 g, 87%) was obtained from compound **5** (0.42 g, 1.10 mmol) using the general procedure of triazolylation. [Eluent: 35-45% EtOAc in pet ether]. Colourless gum. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.04 (s, 3H), 0.08 (s, 3H), 0.84 (s, 9H), 1.81 (s, 3H), 2.00-2.28 (m, 2H), 3.83 (s, 3H), 3.90 (s, 3H), 4.02-4.09 (m, 1H), 4.27-4.36 (m, 1H), 4.76-5.01 (m, 2H), 6.07 (t, *J* = 6.4 Hz, 1H), 6.60 (s, 1H), 9.80 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ -4.7, -4.6, 12.3, 17.9, 25.6, 39.4 (CH_2), 49.9 (CH_2), 52.8, 53.4, 71.5, 83.7, 85.4, 111.5, 131.7, 135.7, 139.5, 150.4, 159.1, 160.2, 164.0. HRMS (ESI^+): m/z calcd for $C_{22}H_{34}N_5O_8Si(M+H)^+$: 524.2177; found: 524.2155.

Compound 7

To a well-stirred solution of compound **6**(0.43 g, 0.82 mmol) in methanol (10 ml), was added CH_3COCl (cat). The resulting solution was allowed to stir for 2h at room temperature. The solvent was evaporated under reduced pressure and the resulting residue was purified by column chromatography over silica gel to obtain **7**(0.28 g, 83%). [Eluent: 4% MeOH in DCM]. ^{1}H NMR (200 MHz, DMSO-*d*$_6$): δ 1.74 (s, 1H), 2.05-2.35 (m, 2H), 3.80 (s, 3H), 3.84 (s, 3H), 3.95-4.02 (m, 1H), 4.17-4.25 (m, 1H), 4.79-4.98 (m, 2H), 5.56 (d, *J* = 4.8 Hz, 1H), 6.09 (t, *J* = 6.2 Hz, 1H), 7.13 (s, 1H). 11.29 (s, 1H).

Compound 8

Compound **8** (0.17 g, 78%) was obtained from compound **7**(0.23 g, 0.56 mmol) applying general procedure of ester hydrolysis. White solid. M.P: 196-198 °C. ^{1}H NMR (200 MHz, DMSO-*d*$_6$): δ 1.82 (s, 3H), 2.02-2.19 (m, 2H), 4.22-4.30 (m, 2H), 4.95-5.18 (m, 2H), 6.15 (t, *J* = 7.4 Hz, 1H), 7.55 (s, 1H), 11.30 (s, 1H). ^{13}C NMR (50 MHz, DMSO-*d*$_6$): δ 12.0, 38.0 (CH_2), 51.3 (CH_2), 71.1, 84.3 (2xC), 109.7, 132.9, 136.1, 140.6, 150.4, 159.2, 161.2, 163.7. HRMS (ESI^+): m/z calcd for $C_{14}H_{16}N_5O_8(M+H)^+$: 382.0999; found:382.0887.

Compound 10

Compound **9** (0.35 g, 1.13 mmol) was converted to **10** (0.41 g, 81%) using general procedure of triazolylation. [Eluent: 50-60% EtOAc in pet ether]. Yellow solid. M.P: 115-117 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.33 (s, 3H), 1.52 (s, 3H), 3.86 (s, 3H), 3.95 (s, 3H), 4.45-4.53 (m, 1H), 4.88-5.14 (m, 4H), 5.39 (s, 1H), 5.71-5.75 (m, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 9.13 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 25.3, 27.1, 51.5 (CH_2), 52.8, 53.5, 81.8, 84.5, 87.3, 96.9, 102.8, 114.7, 131.3, 139.7, 144.0, 150.4, 159.1, 160.5, 163.9. HRMS (ESI^+): m/z calcd for $C_{18}H_{22}N_5O_9(M+H)^+$: 452.1418; found: 452.1411.

Compound 11

1:1 TFA:DCM mixture (2 ml) was added to compound **10**(0.35 g, 0.77 mmol), and stirred at room temperature for one hour. The reaction mixture was then evaporated under reduced pressure, and purified by column chromatography over silica gel to obtain compound **11** (0.24g, 74%).[Eluent: 5-10% MeOH in DCM]. Yellow gum. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.82 (s, 3H), 3.85 (s, 3H), 3.94-4.18 (m, 3H), 4.79-5.00 (m, 2H), 5.38 (d, *J* = 6.0 Hz , 1H), 5.5 (d, *J* = 5.4 Hz, 1H), 5.57-5.62 (m, 2H), 7.48 (d, *J* = 8.2 Hz, 1H), 11.34 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 51.0 (CH2), 52.5, 53.4, 70.1, 72.0, 81.0, 90.6, 101.9, 131.5, 138.6, 141.9, 150.4, 158.7, 160.0, 163.1. HRMS (ESI^+): m/z calcd for $C_{15}H_{18}N_5O_9(M+H)^+$: 412.1105; found: 412.1085.

Compound 12

Compound **12** (0.11 g, 64%) was generated from compound **11**(0.18 g, 0.44 mmol) following general procedure of ester hydrolysis. White solid. M.P: 118-120 °C.^{1}H NMR (600 MHz, DMSO-d_6): δ 3.17 (s, 1H), 4.01-4.02 (t, *J* = 4.8 Hz, 1H), 4.15-4.16 (t, *J* =5.4 Hz, 1H), 4.19-4.21 (m, 1H), 5.62-5.63 (d, *J* = 8.4 Hz, 1H), 5.72-5.73 (d, *J* = 5.4 Hz, 1H), 7.61-7.63 (m, 2H), 11.34 (s, 1H).^{13}C NMR (50 MHz, DMSO-d_6): δ 51.3 (CH_2), 70.7, 72.2, 81.8, 88.6, 102.0, 132.5, 140.3, 141.2, 150.7, 159.3, 161.4, 163.1. HRMS (ESI^+): m/z calcd for $C_{13}H_{14}N_5O_9(M+H)^+$: 384.0792; found: 384.0796.

Compound 14

Compund**13** (0.40 g, 0.78 mmol) was transformed to compound **14** (0.29 g, 56%) using the general procedure of triazolylation over 48 h. [Eluent: 60-70% EtOAc in pet ether]. Colourless gum. ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.45-3.47 (m, 1H), 3.61-3.63 (m, 1H), 3.87 (s, 1H), 3.97 (s, 3H), 4.74-4.77 (m, 1H), 5.04 (t, *J* = 6.0 Hz, 1H), 5.41 (d, *J* = 7.8 Hz, 1H), 5.70-5.72(m, 1H),

6.36 (d, *J* = 5.4 Hz, 1H), 7.23-7.41 (m, 15H), 7.96 (d, *J* = 8.4 Hz, 1H), 10.23 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): 52.9, 53.7, 61.6 (CH_2), 64.4, 76.1, 79.0, 85.3, 87.7, 101.4, 127.4, 128.0, 128.1, 128.6, 128.7, 131.2, 139.9, 142.0, 143.1, 150.8, 158.4, 160.2, 164.6. HRMS (ESI^+): m/z calcd for $C_{34}H_{31}N_5O_9(M+H)^+$: 654.2200; found: 654.2207.

Compound 15

1:1 TFA:DCM mixture (2 ml) was added to compound **14**(0.23 g, 0.35 mmol), and stirred at room temperature for one hour. The reaction mixture was then evaporated under reduced pressure, and purified by column chromatography over silica gel to obtain compound **15** (0.11 g, 77%).[Eluent: 5-10% MeOH in DCM]. Yellow gum. ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.58-3.60 (m, 1H), 3.71-3.73 (m, 1H), 3.89 (s, 3H), 3.95 (s, 3H), 4.36-4.38 (m, 1H), 4.83 (q, *J* = 5.4 Hz, 1H), 5.25 (s, 1H), 5.28-5.30 (m, 1H), 5.65-5.66 (m, 1H), 6.27 (d, *J* = 5.4 Hz, 1H), 6.31 (d, *J* = 5.4 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 11.36 (d, *J* = 1.8 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 53.2, 54.3, 60.3 (CH_2), 66.1, 75.0, 81.2, 85.1, 101.0, 131.9, 139.3, 142.6, 150.9, 158.8, 160.5, 163.7. HRMS (ESI^+): m/z calcd for $C_{15}H_{18}N_5O_9(M+H)^+$: 412.1105; found: 412.1118.

Compound 16

Compound **15** (0.09 g, 0.22 mmol) was converted to compound **16** (0.05 g, 62%) following the general procedure of ester hydrolysis. White solid. M.P: > 200 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.59-3.62 (m, 1H), 3.69-3.72 (m, 1H), 4.29-4.32 (m, 1H), 4.73 (t, *J* = 4.2 Hz, 1H), 5.63 (d, *J* = 7.8 Hz, 1H), 5.79-5.81 (m, 1H), 6.34 (d, *J* = 4.8 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 11.33 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 61.0 (CH_2), 66.2, 75.0, 82.5, 85.7, 100.7, 133.4, 140.8, 142.8, 150.9, 159.7, 161.6, 163.8. HRMS (ESI^+): m/z calcd for $C_{13}H_{14}N_5O_9(M+H)^+$: 384.0792; found: 384.0786.

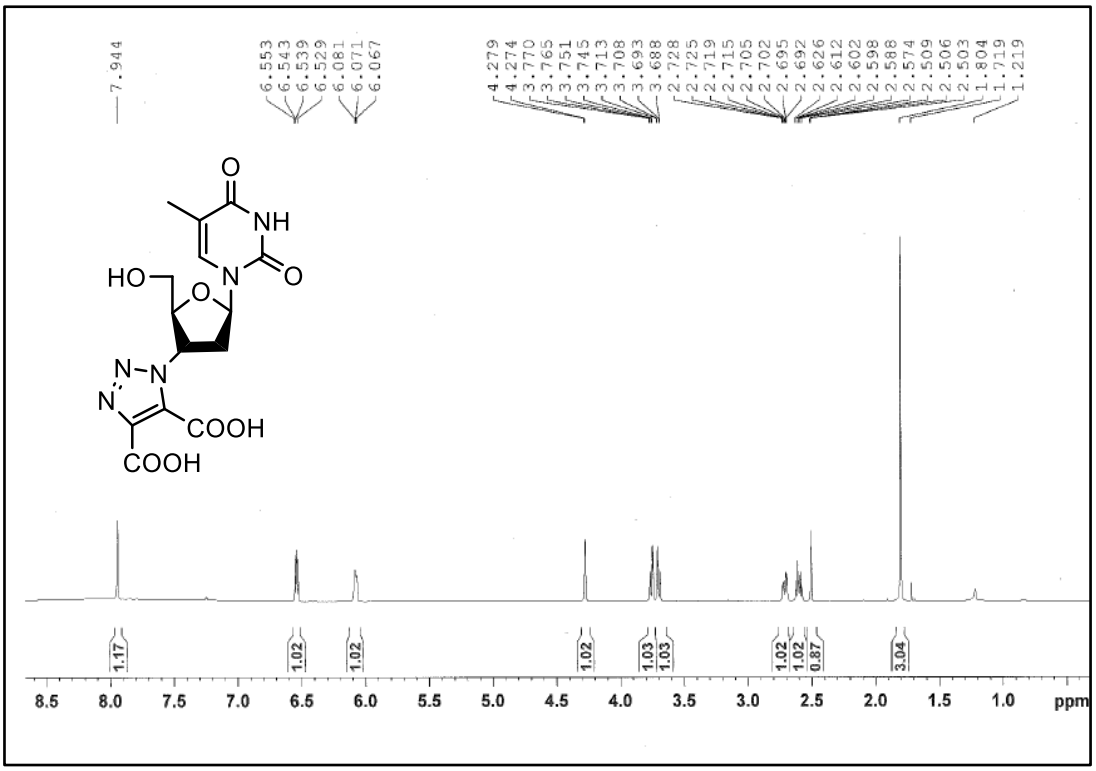
Compound 18

Compound **17** (0.32 g, 1.09 mmol) was converted to compound **18** (0.49 g, 77%) following the general procedure of triazolylation, using 3.0 equivalents of DMAD. [Eluent: 70-75% EtOAc in pet ether]. Yellow gum. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.87 (s, 3H) 2.93-3.03 (m, 2H), 3.85 (s, 3H), 3.91 (s, 3H), 3.94 (s, 3H), 4.02 (s, 3H), 4.89-4.97 (m, 2H), 5.09-5.20 (m, 1H), 5.63-5.74 (m, 1H), 6.13 (m, 1H), 6.90 (s, 1H), 9.46 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 12.3, 36.7 (CH_2), 50.0 (CH_2), 52.8, 52.9, 53.6, 54.2, 60.1, 81.8, 88.7, 111.6, 131.0, 131.7, 137.6,

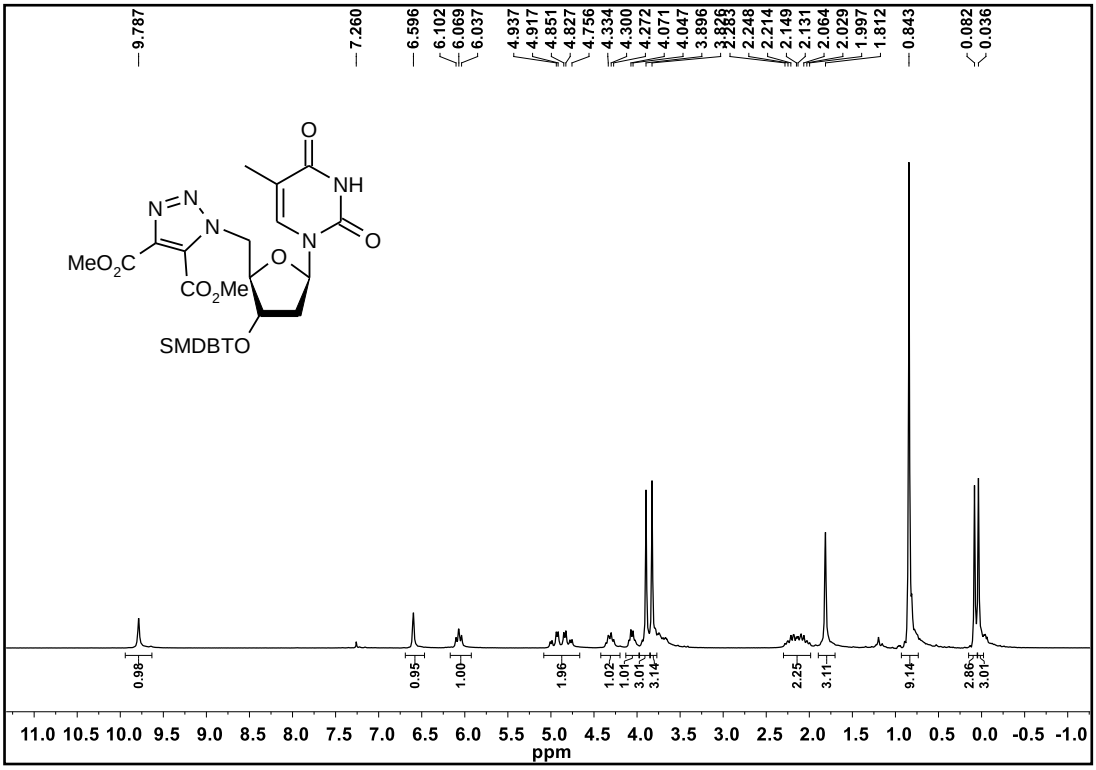
139.8, 140.1, 150.2, 158.8, 159.0, 160.2, 160.3, 163.9. HRMS (ESI^+): m/z calcd for $C_{22}H_{25}N_8O_{11}(M+H)^+$: 577.1643; found: 577.1652.

Compound 19

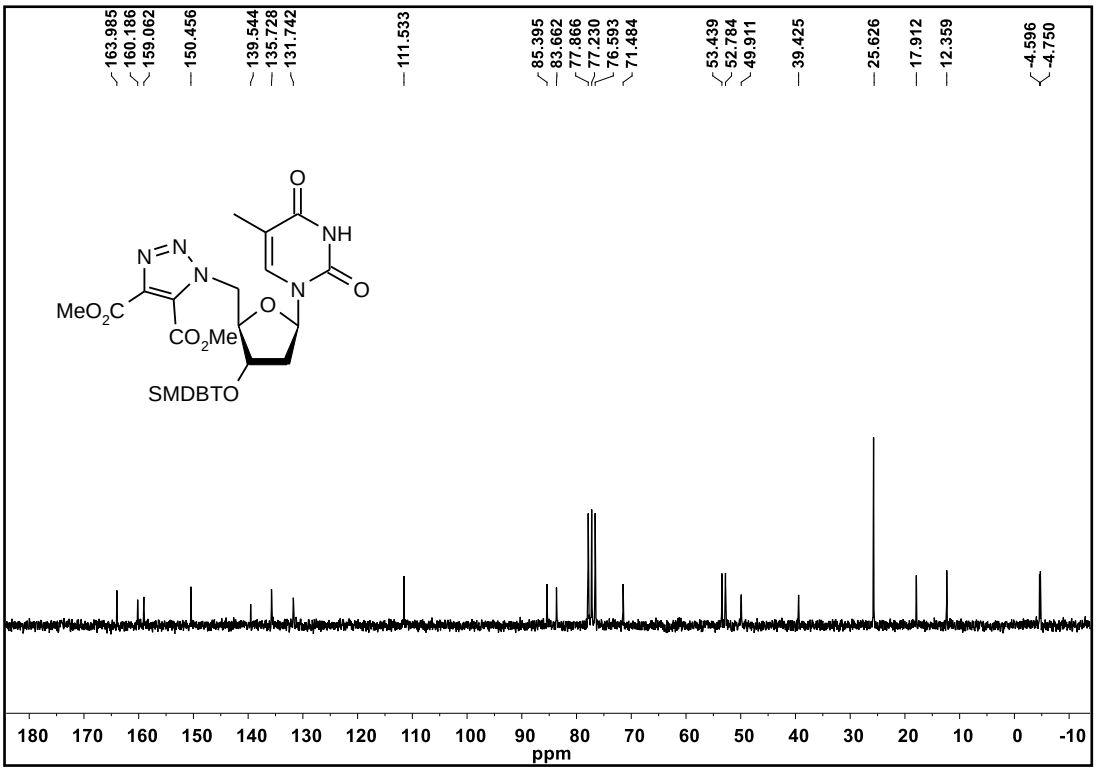
Compund**19** (61%) was obtained from compound **18** (0.25 g, 0.43 mmol) following the general procedure of ester hydrolysis. White solid. M.P: 196-198 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 1.83 (s, 3H), 2.76-2.80 (m, 2H), 4.76-4.78 (m, 1H), 5.15-5.18 (m, 1H), 5.25-5.31 (m, 1H), 6.31 (bs, 2H), 6.45 (t, J = 6.8 Hz, 1H), 7.61 (s, 1H), 11.35 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.1, 36.1 (CH_2), 51.7 (CH_2), 60.5, 82.1, 85.1, 110.0, 132.5, 132.6, 136.3, 140.3, 140.7, 150.4, 159.2, 161.3, 161.4, 163.7 (2xC). HRMS (ESI^+): m/z calcd for $C_{18}H_{16}N_8O_{11}Na(M+Na)^+$: 543.0836; found: 543.0765.



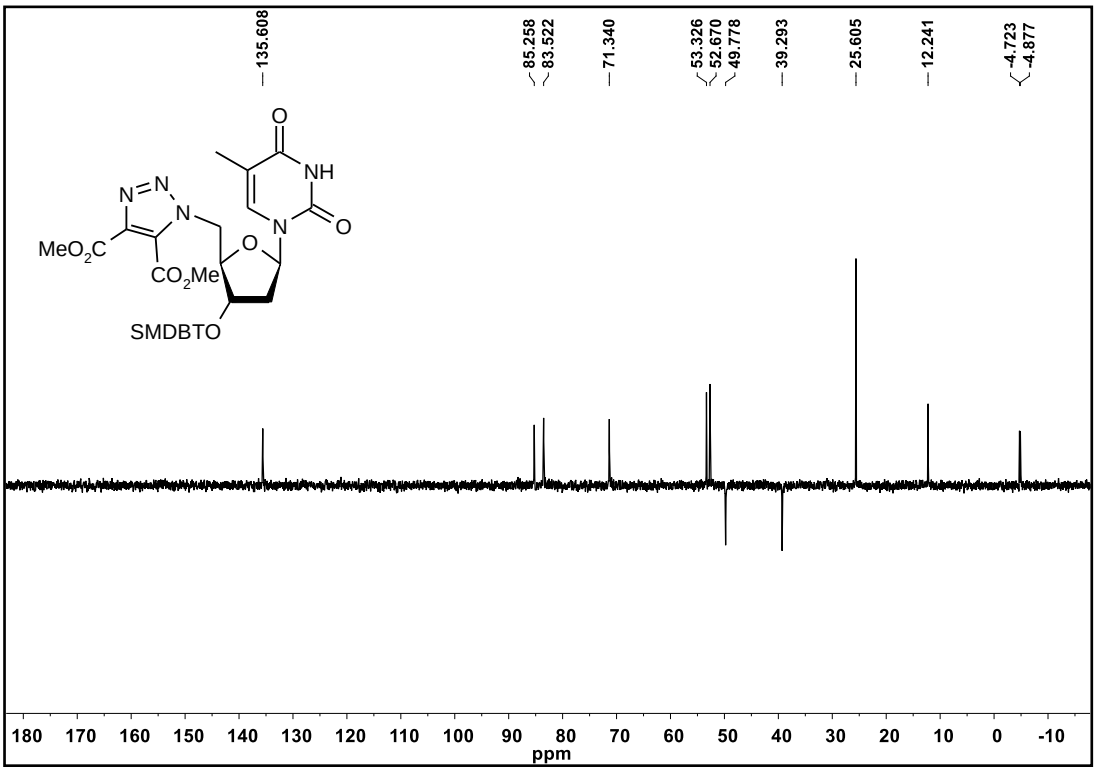
^{1}H NMR of compound **4**



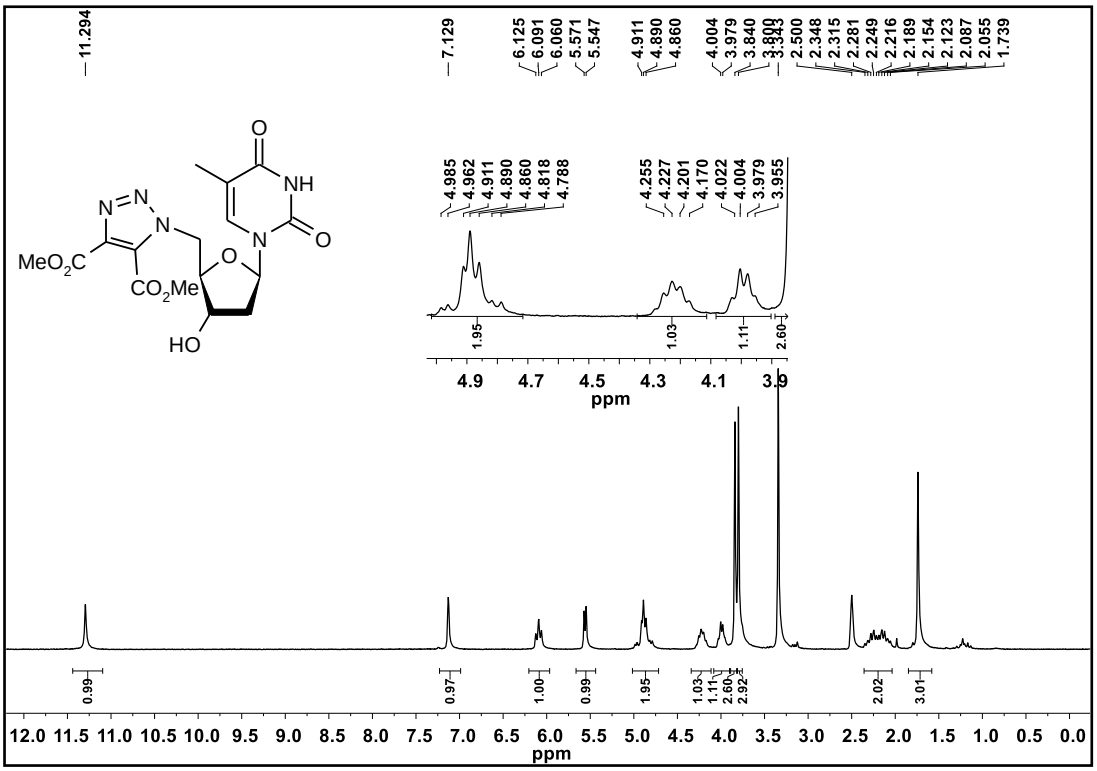
^{1}H NMR of compound **6**



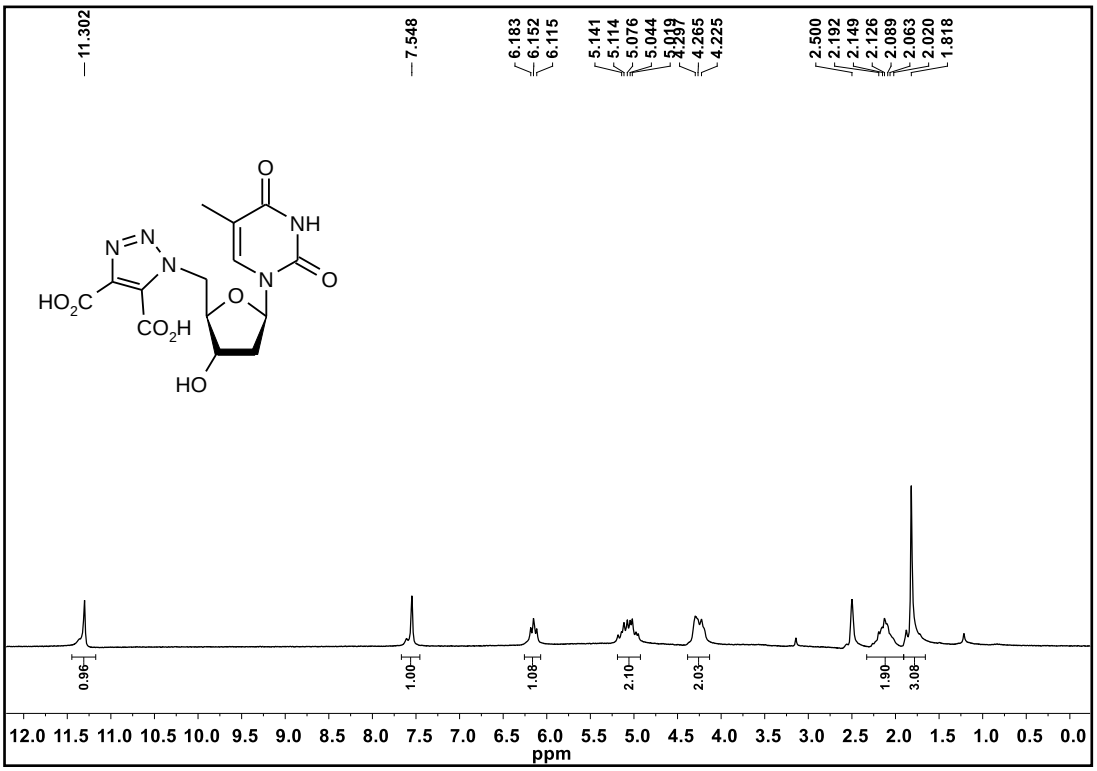
^{13}C NMR of compound **6**



DEPT 135 NMR of compound **6**



^{1}H NMR of compound **7**



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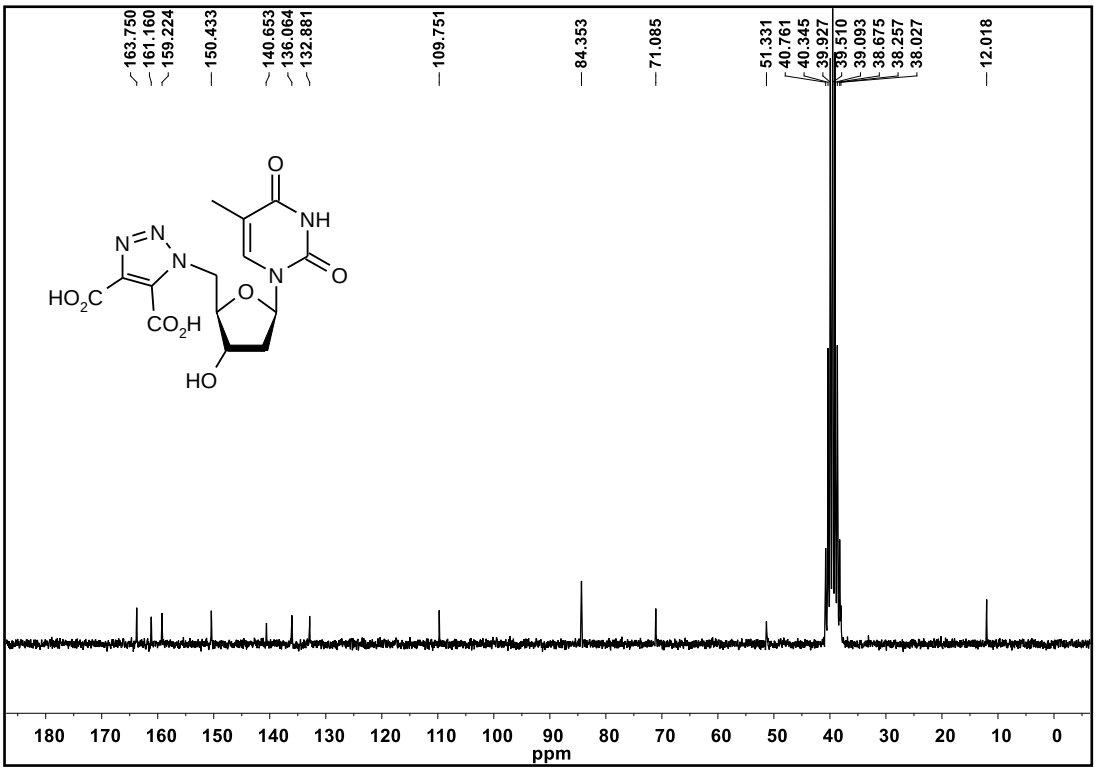

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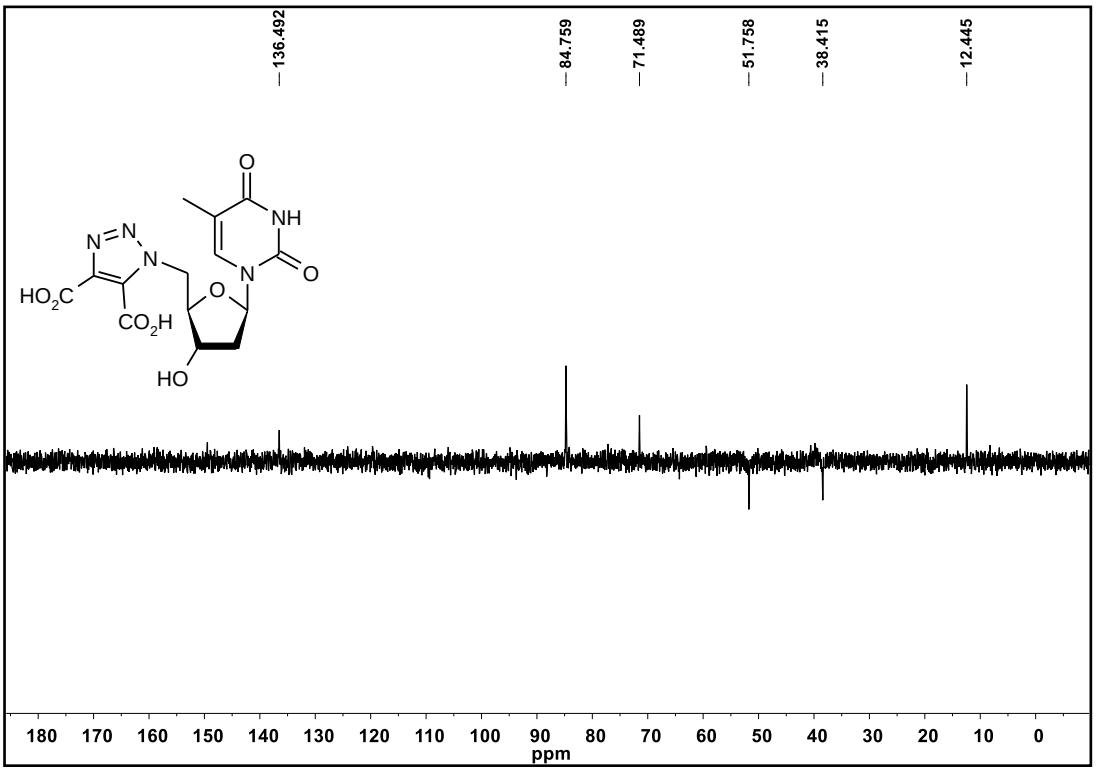

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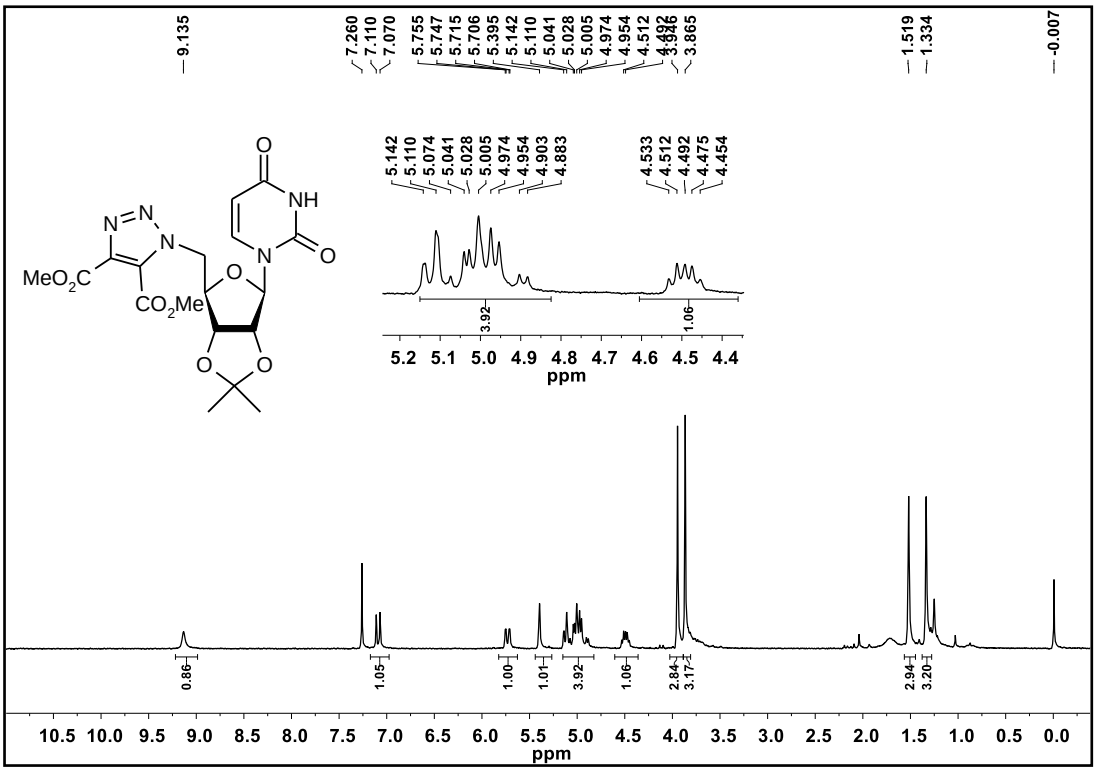
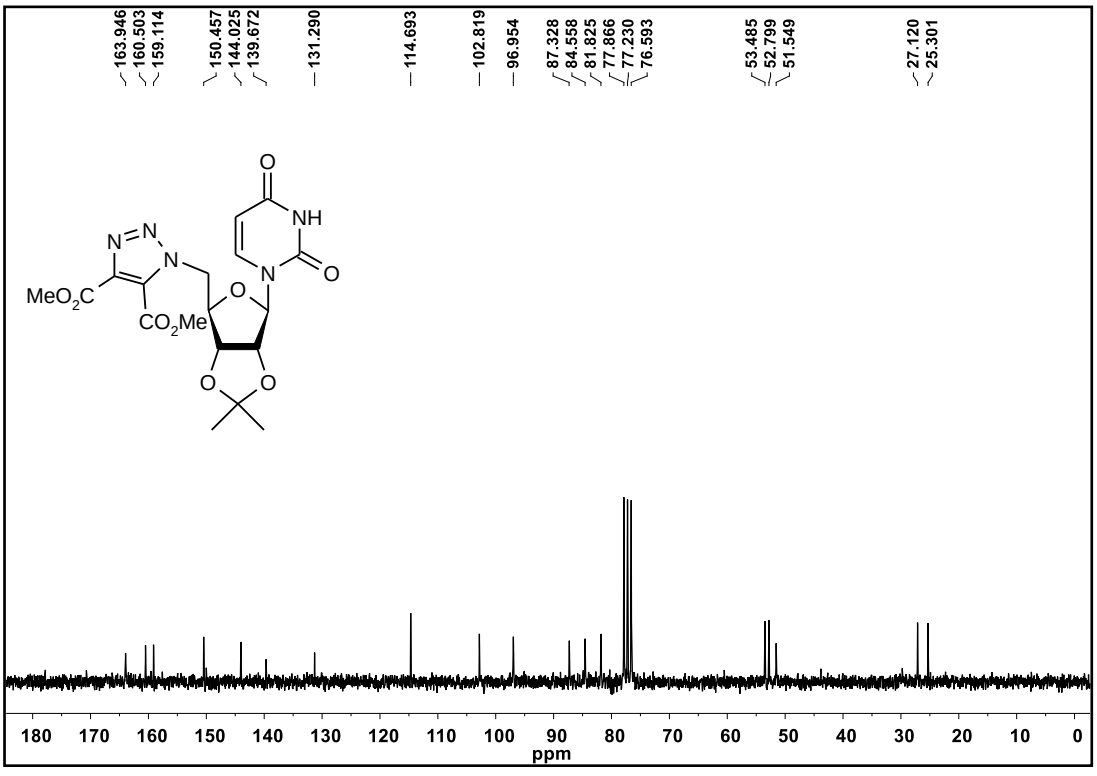


^{1}H NMR of compound **10**



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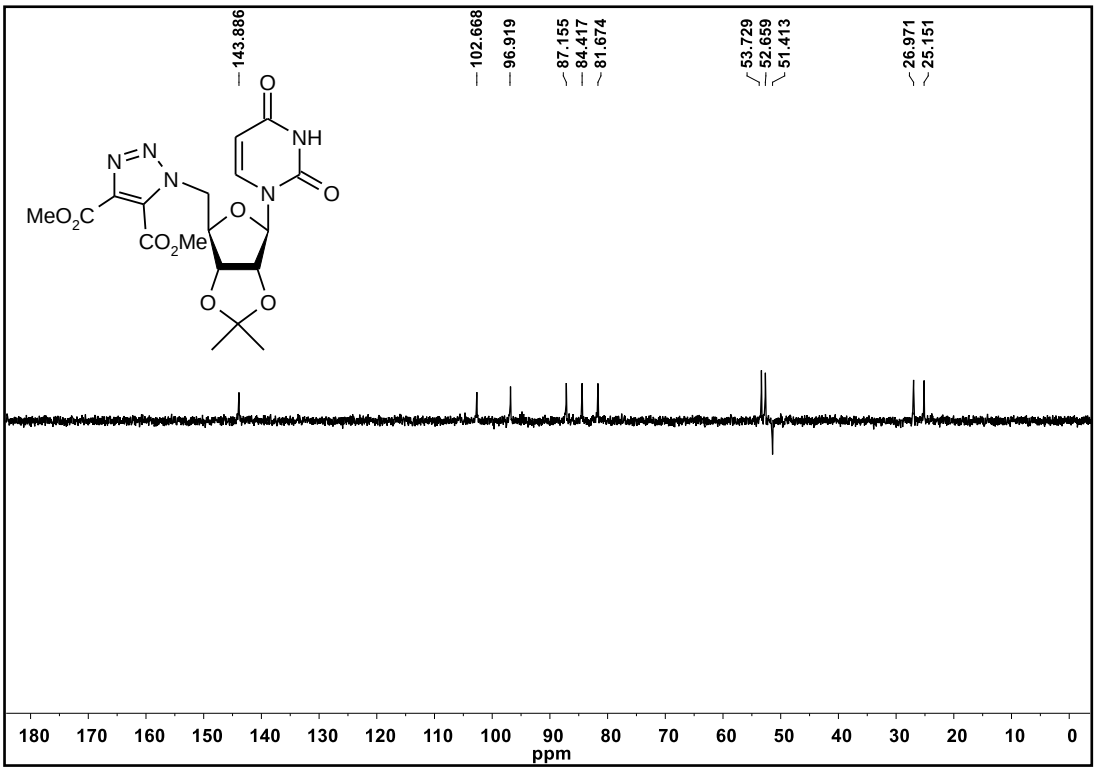
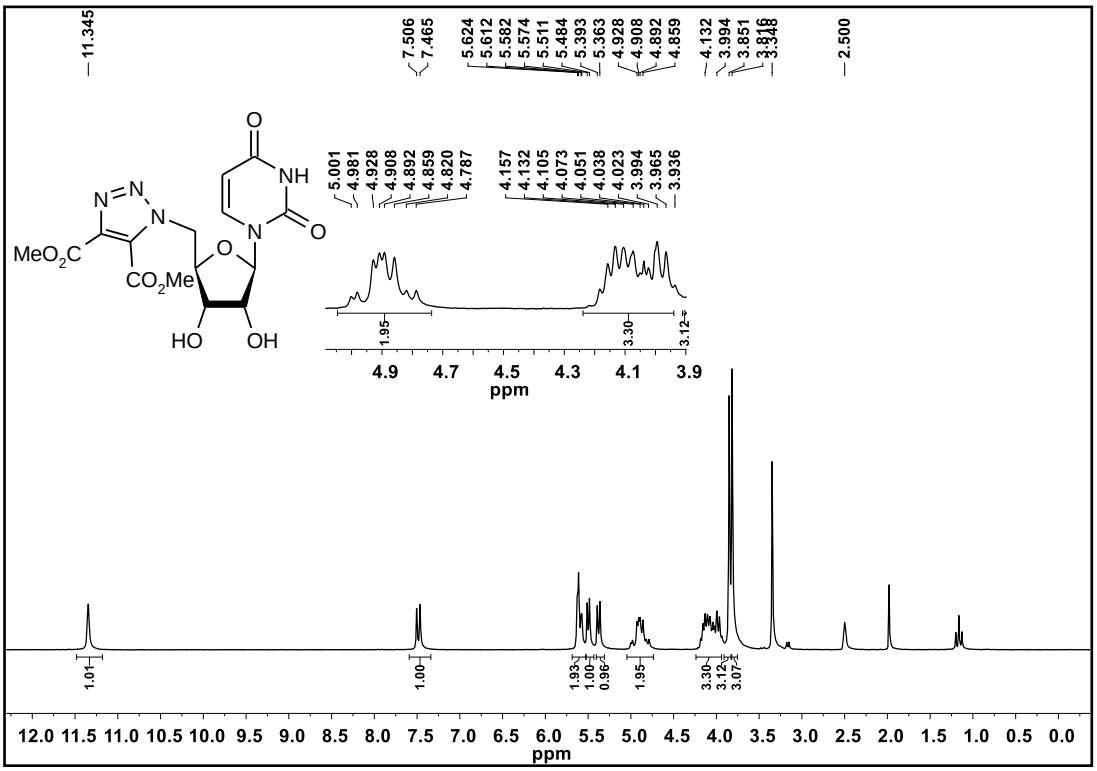
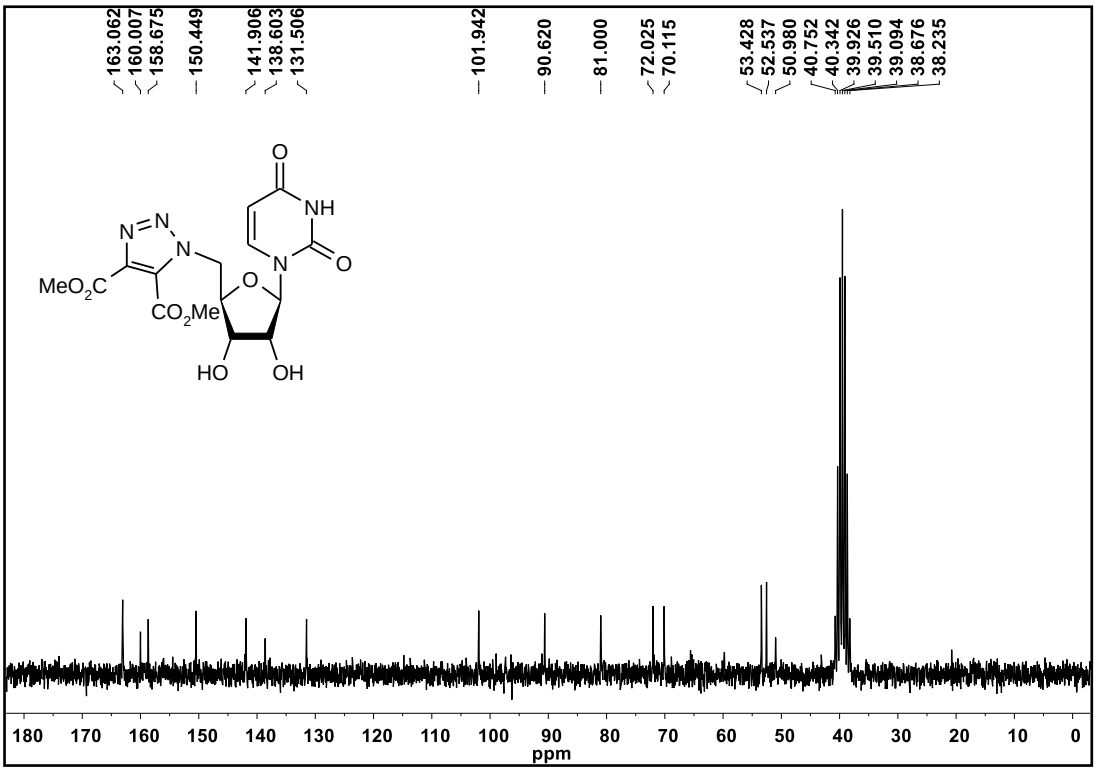


DEPT 135 NMR of compound **10**



^{1}H NMR of compound **11**



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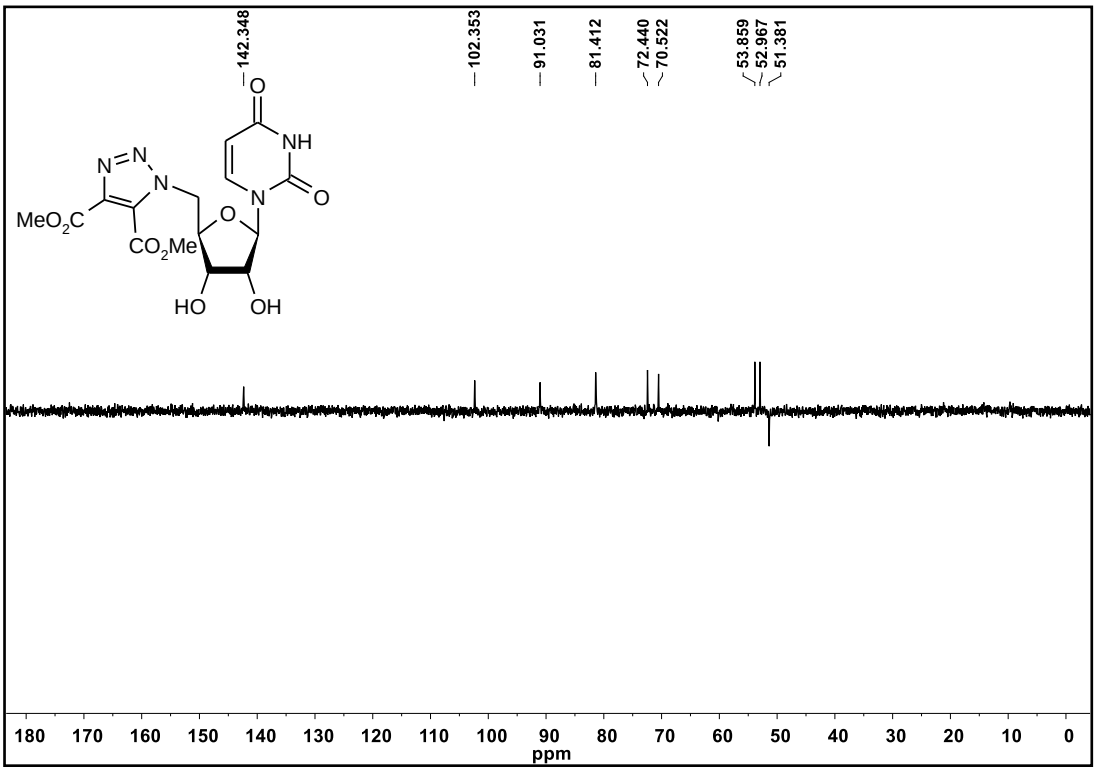

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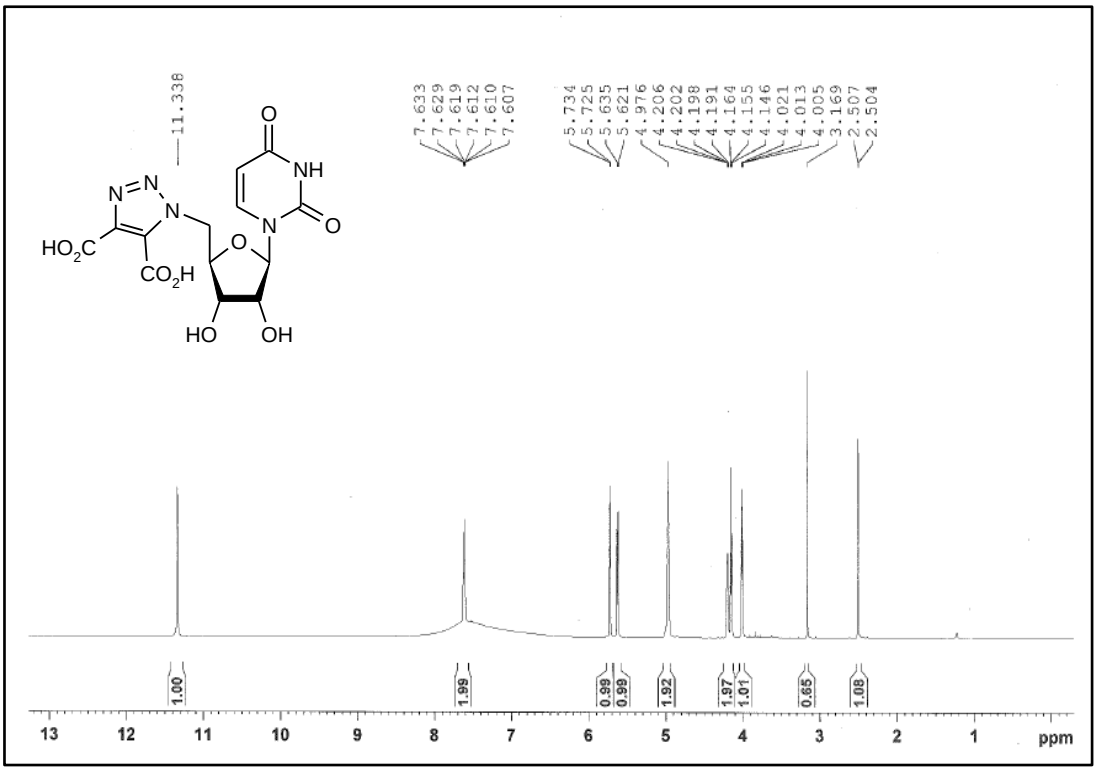

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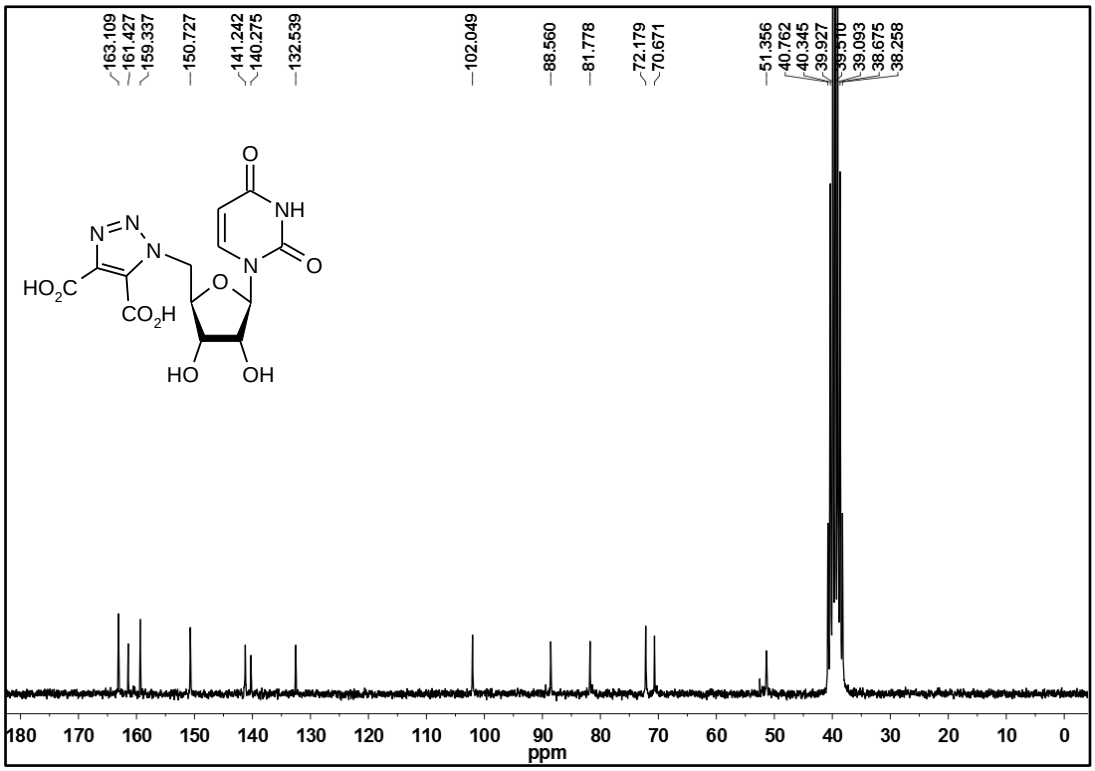
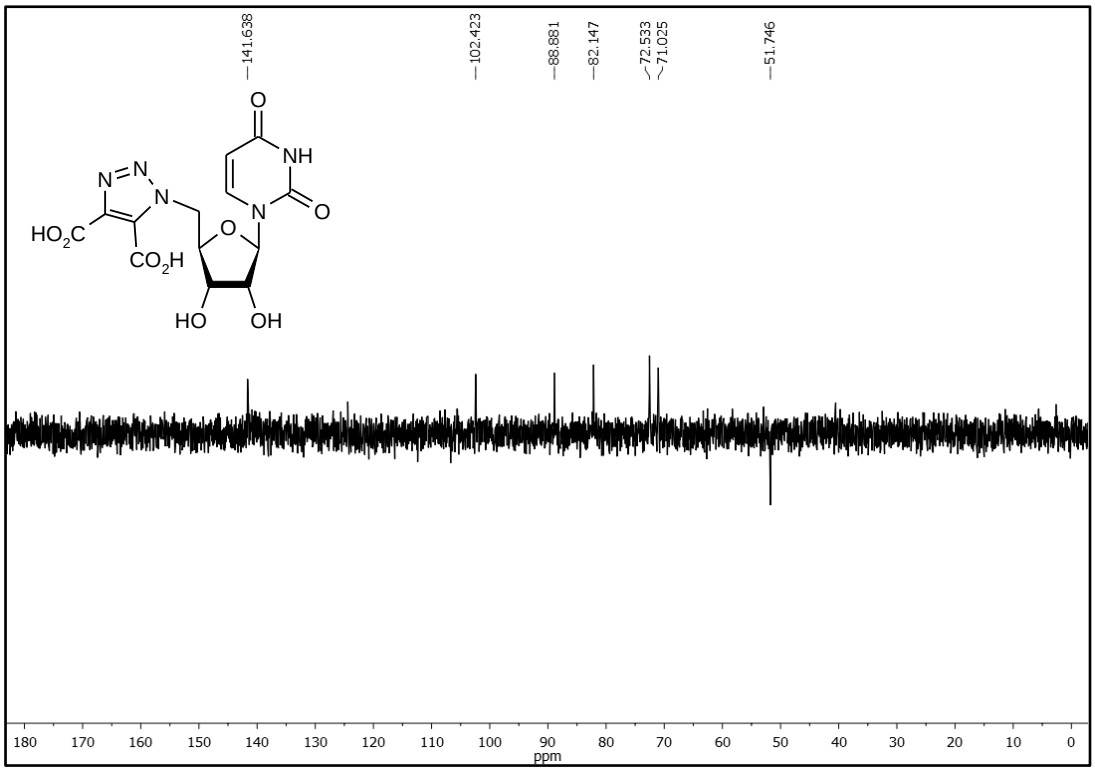
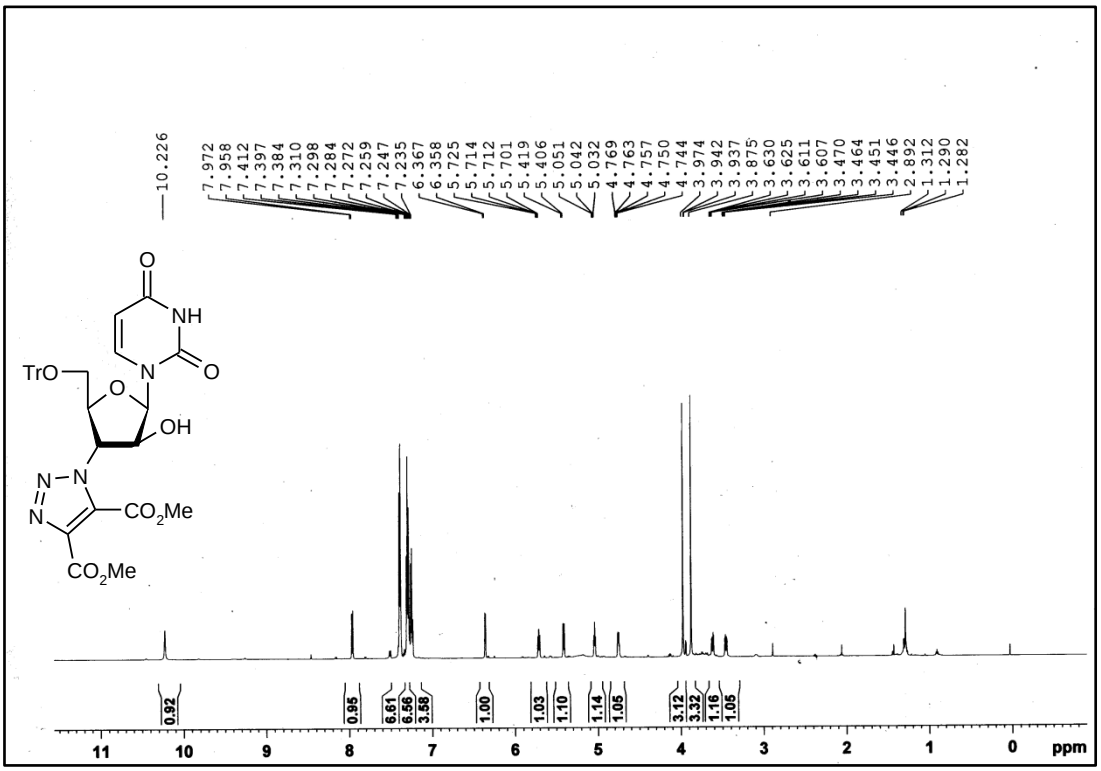


^{13}C NMR of compound **12**



DEPT 135 NMR of compound **12**



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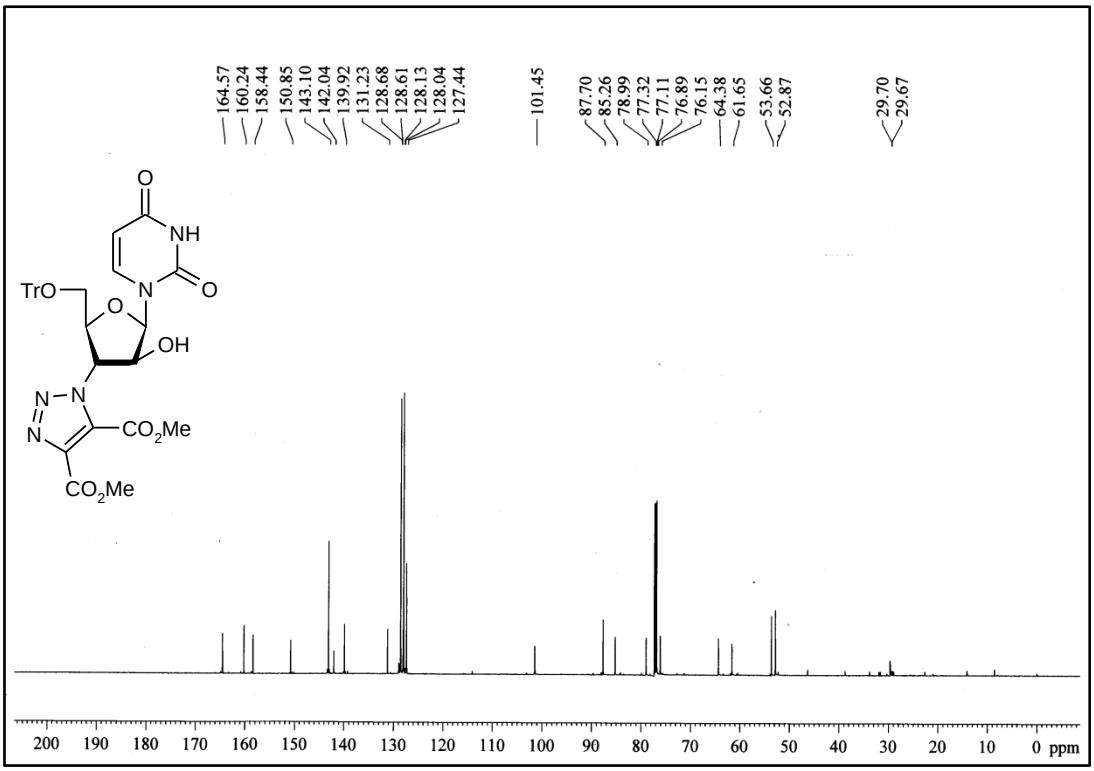

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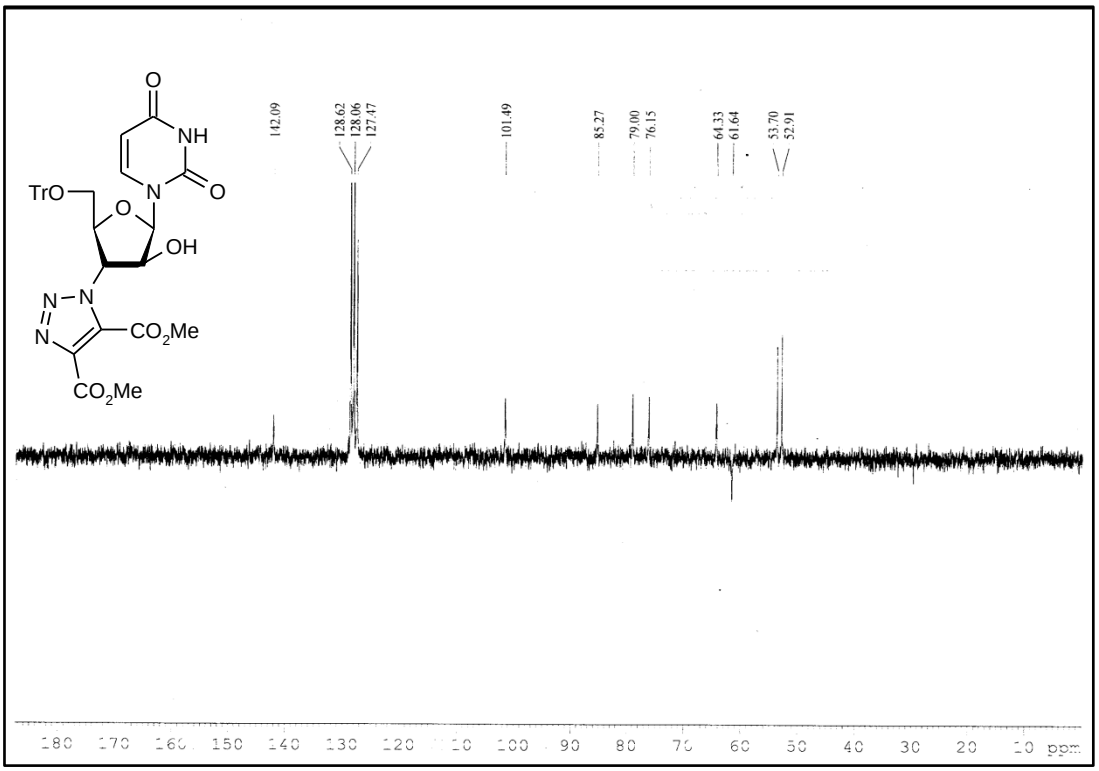
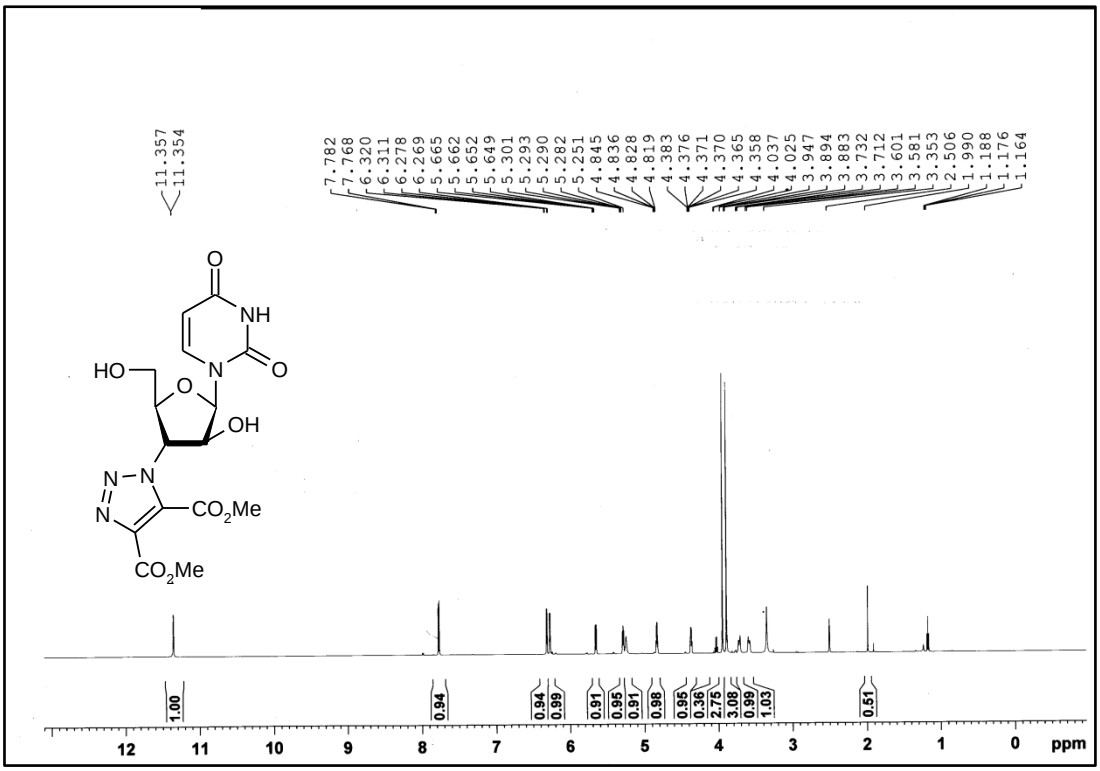


DEPT 135 NMR of compound **14**



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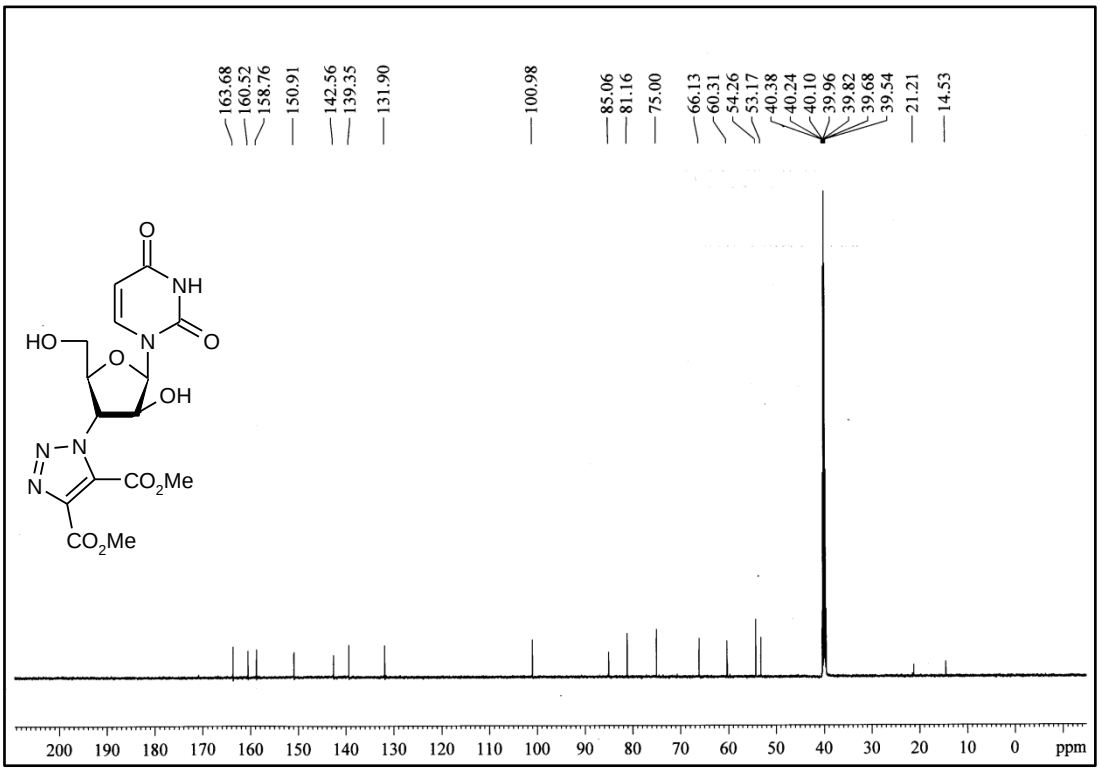

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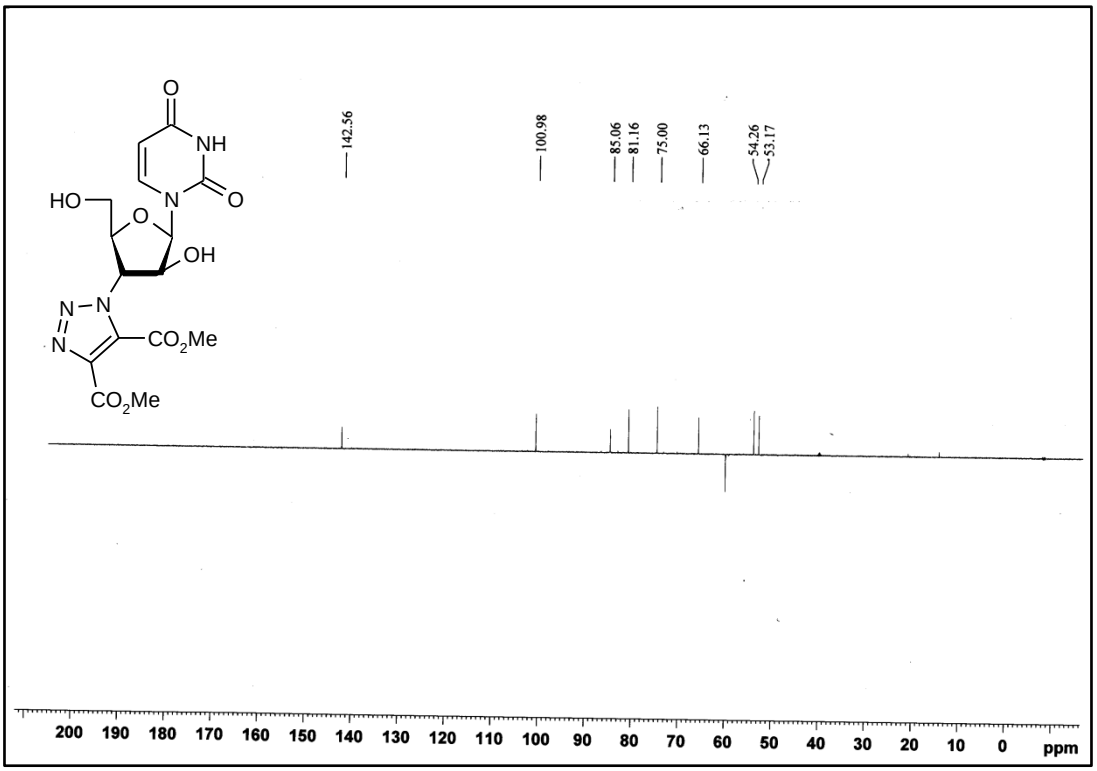
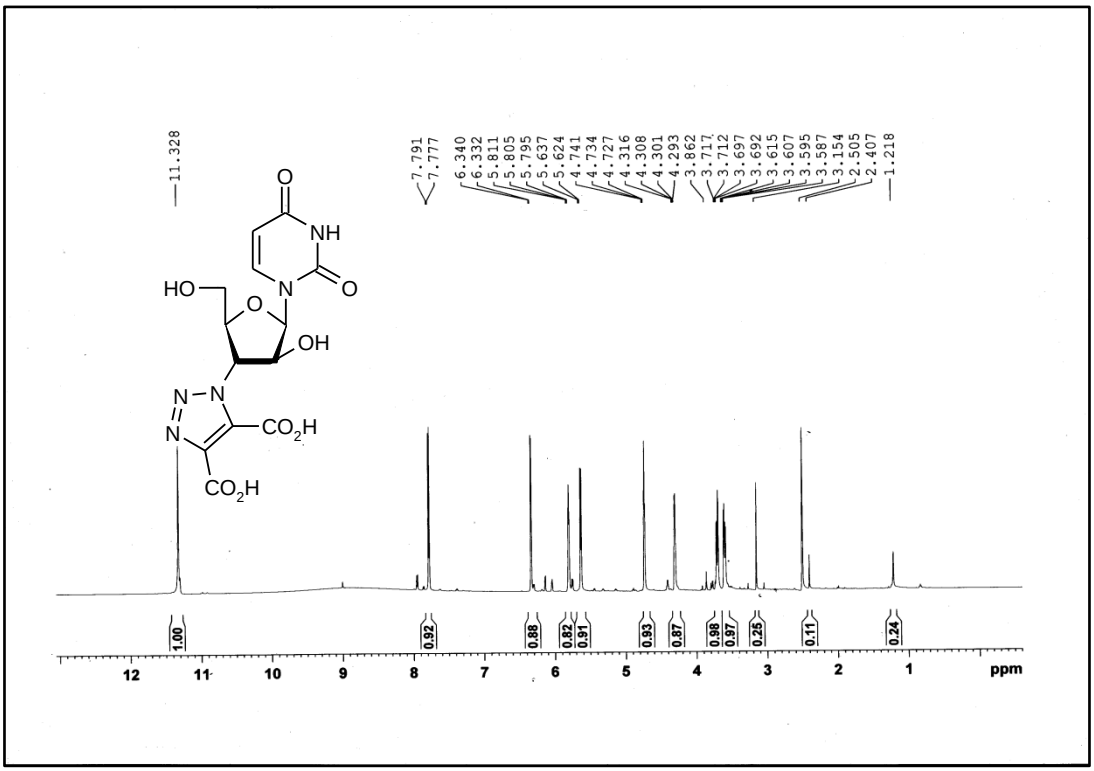
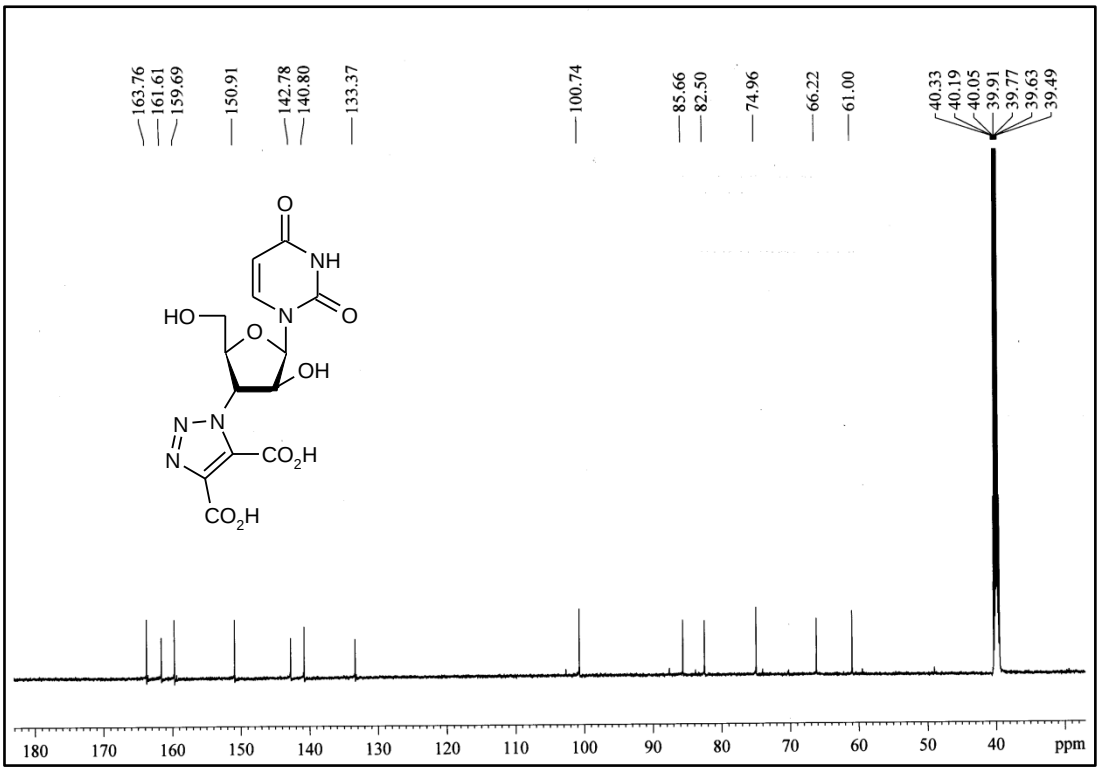


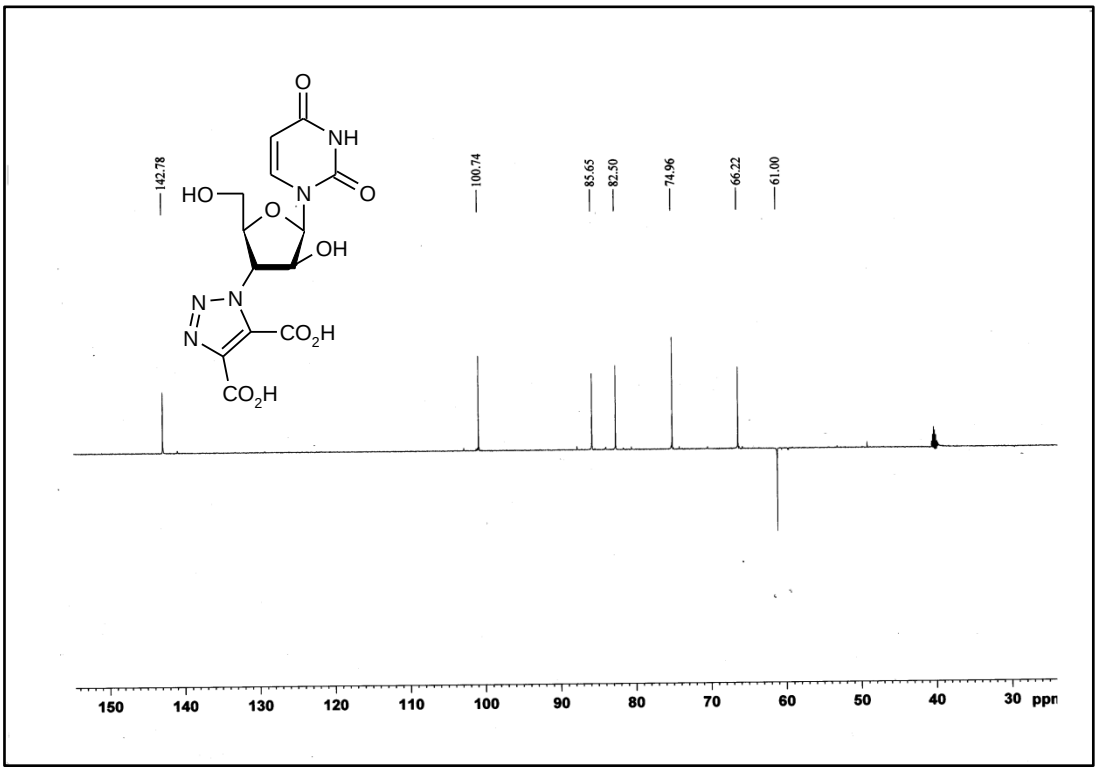
DEPT 135 NMR of compound **15**



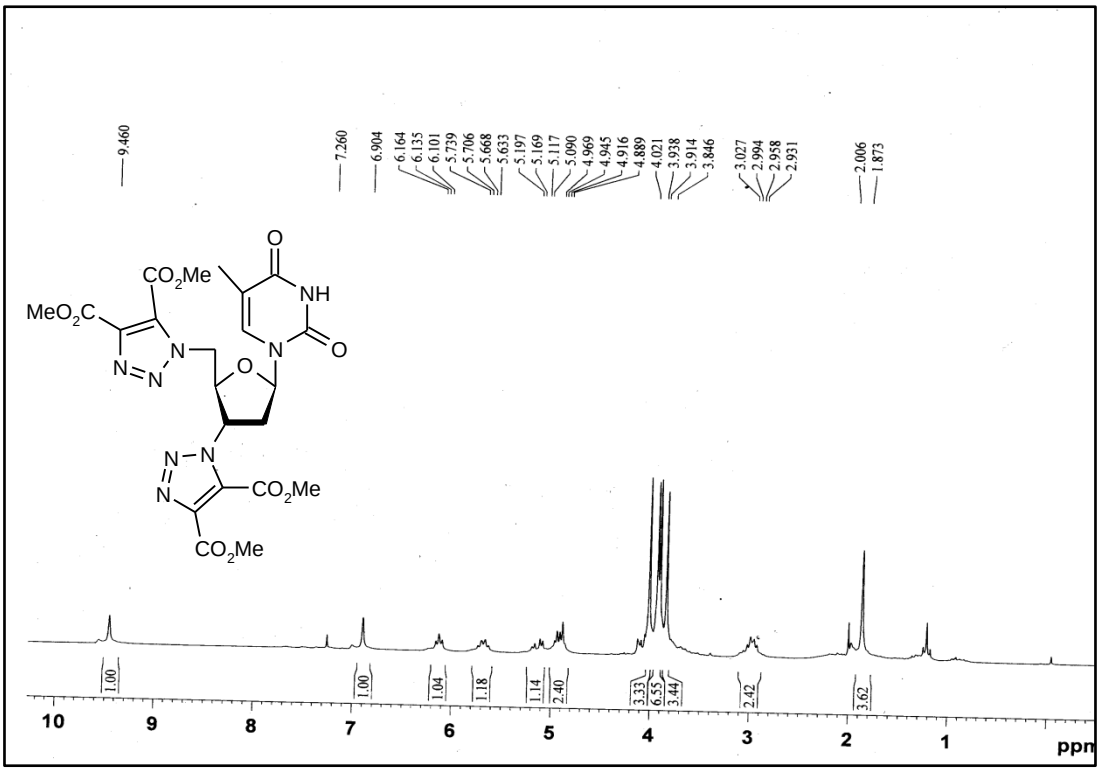
^{1}H NMR of compound **16**



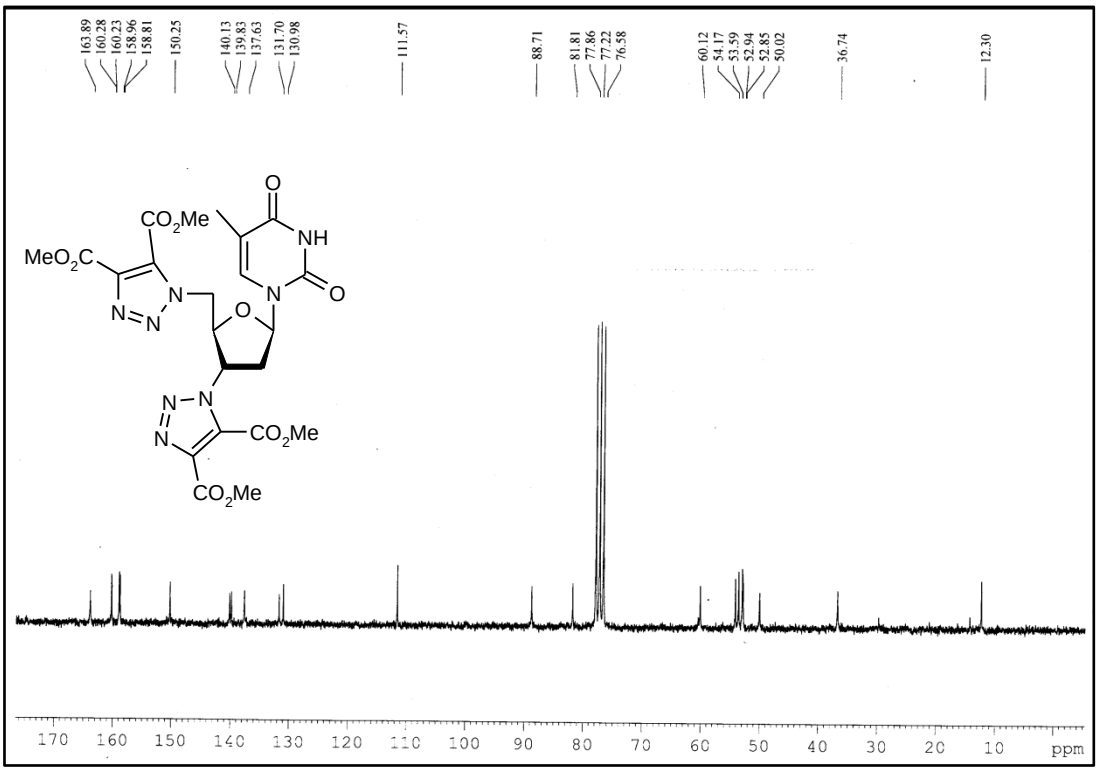
^{13}C NMR of compound **16**



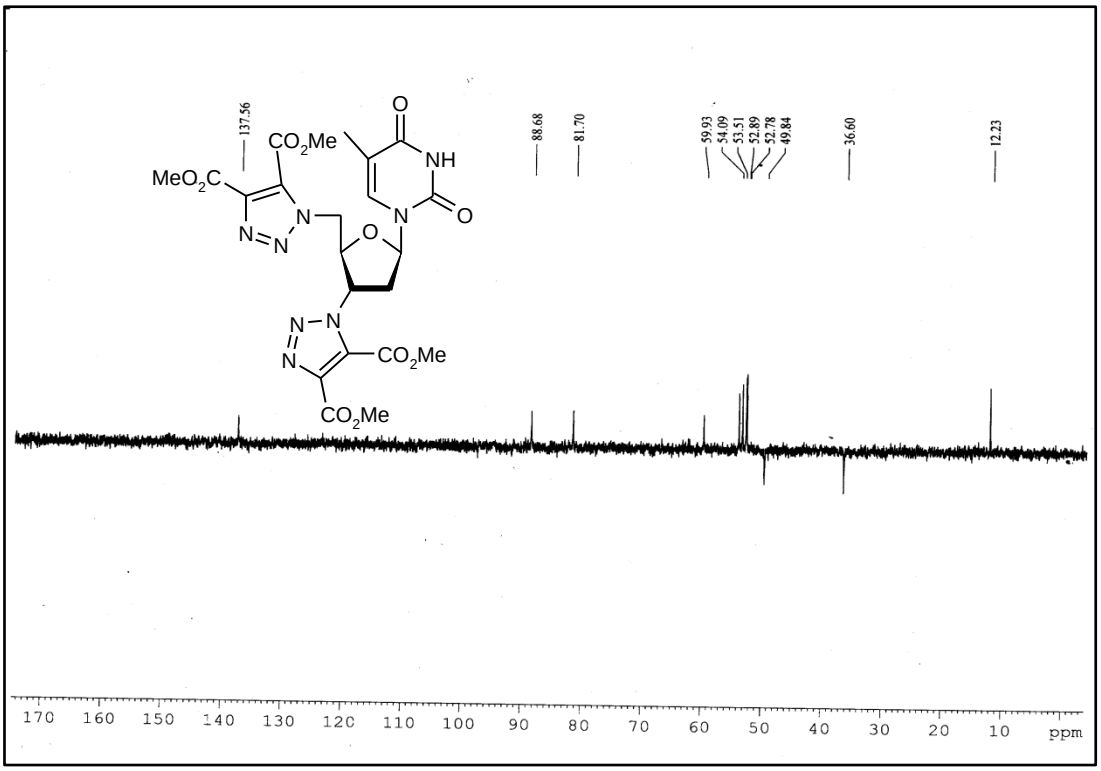
DEPT 135 NMR of compound **16**



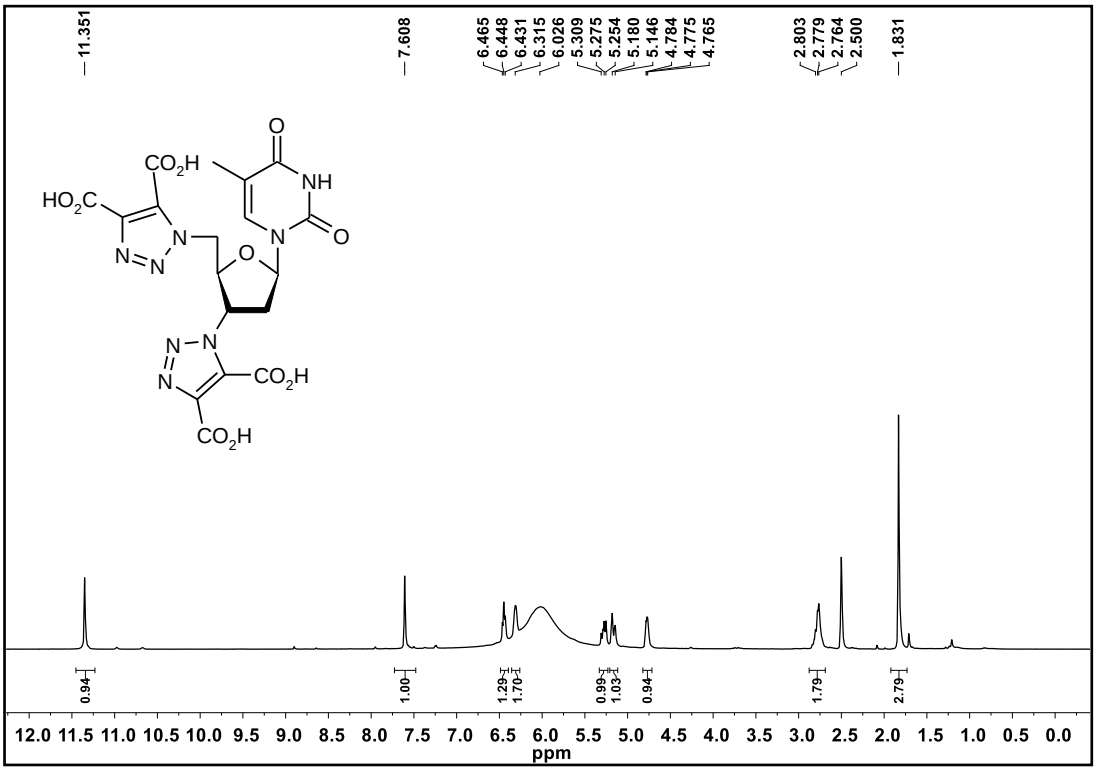
^{1}H NMR of compound **18**



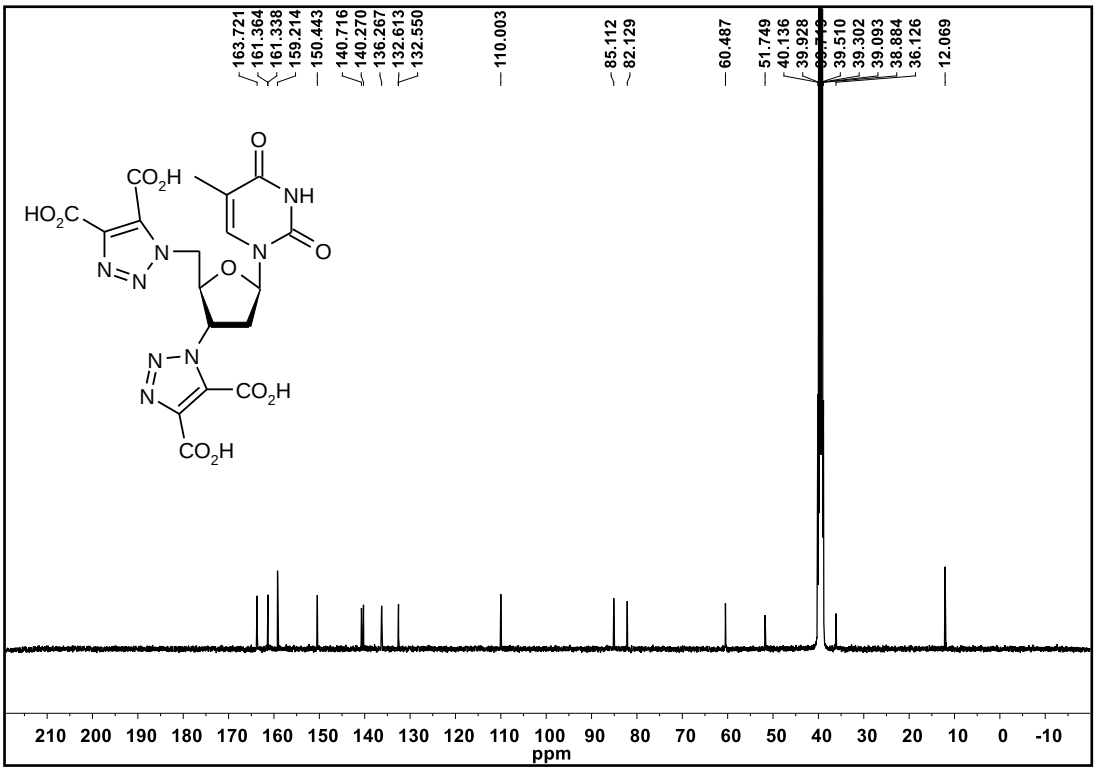
^{13}C NMR of compound **18**



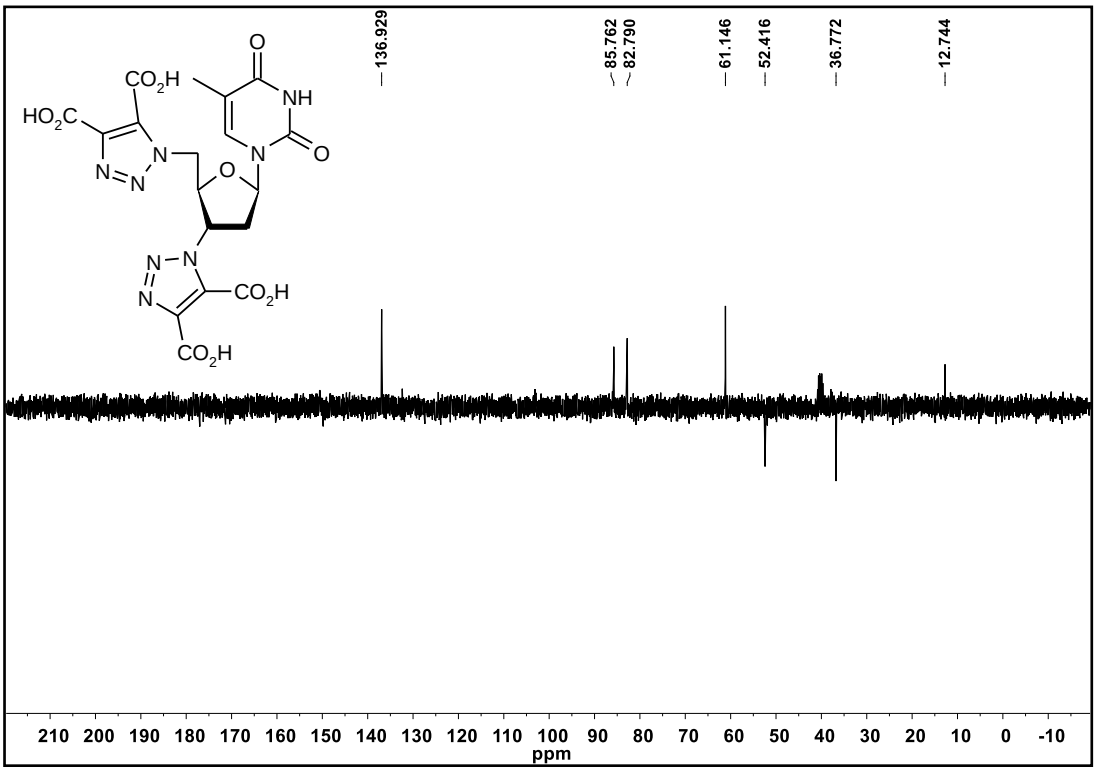
DEPT 135 NMR of compound **18**



^{1}H NMR of compound **19**



^{13}C NMR of compound **19**



DEPT 135 NMR of compound **19**

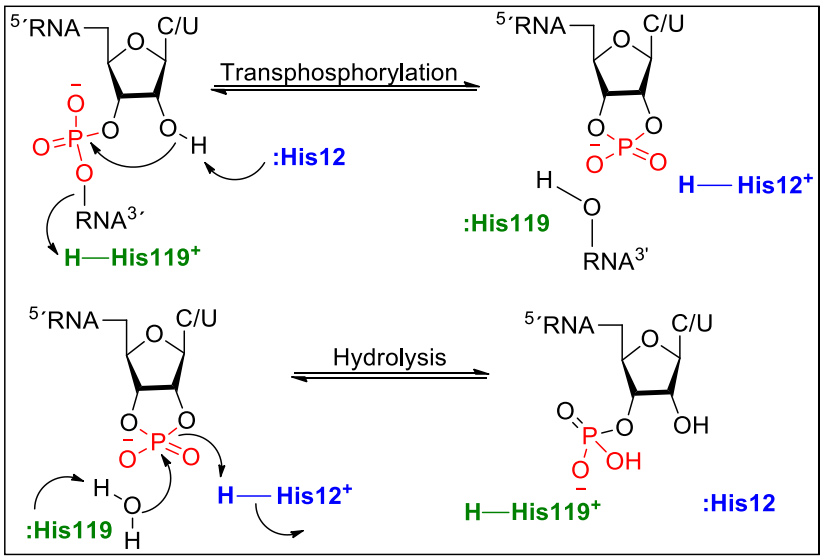


Figure S1: Mechanism for the transphosphorylation and hydrolysis reactions catalyzed by enzyme RNase A.

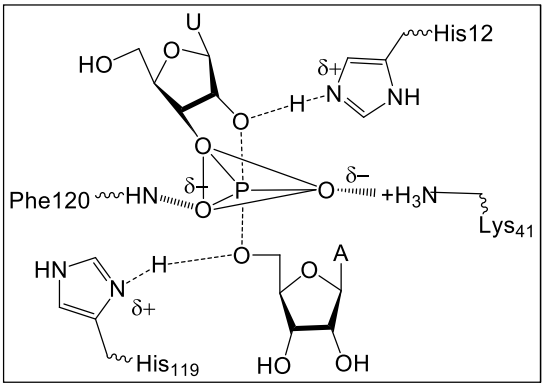


Figure S2: Transition state during transphosphorylation of UpA by RNase A

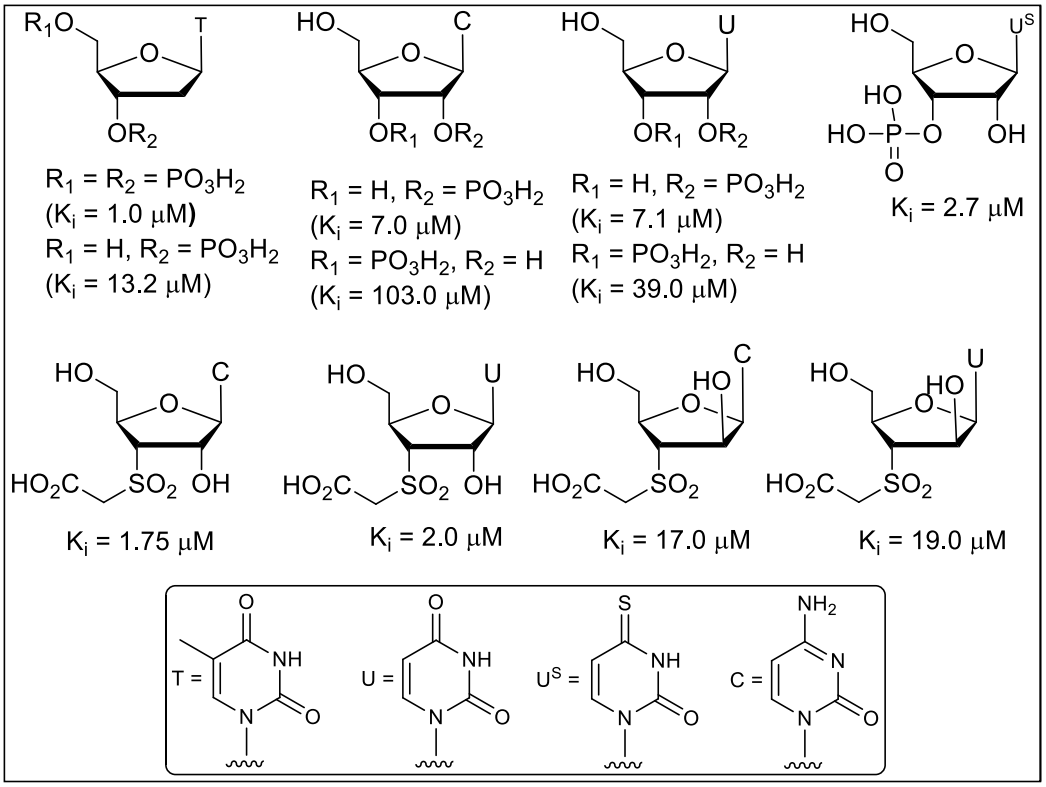


Figure S3: Reported nucleoside based Ribonuclease A inhibitors

Reference:

Sulfonic nucleic acids (SNAs): a new class of substrate mimics for ribonuclease A inhibition

Dhrubajyoti Datta, Swagata Dasgupta, Tanmaya Pathak

Org. Biomol. Chem. **2019**, *30*, 7215-7221 (doi: 10.1039/c9ob01250h)

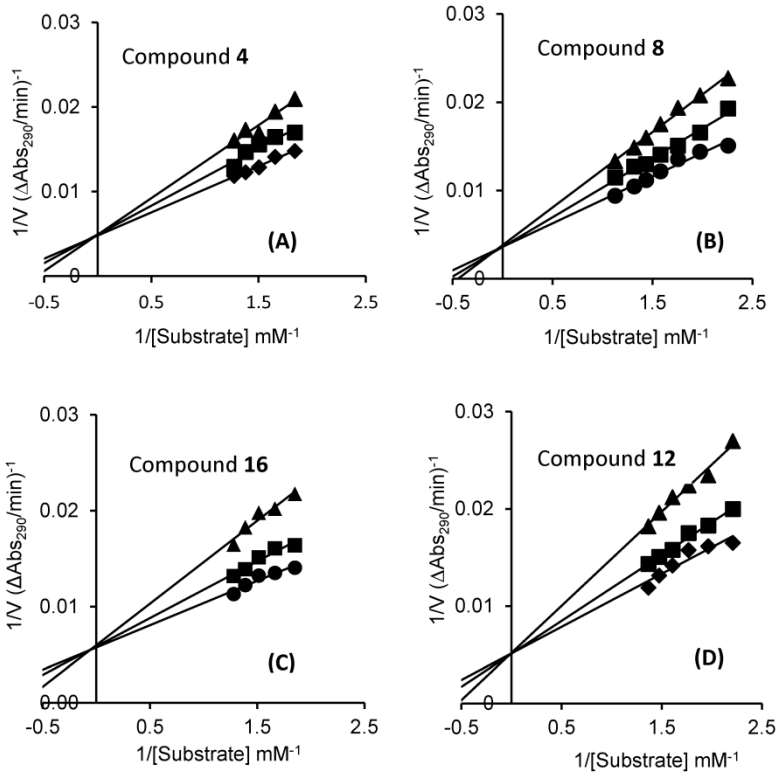


Figure S4: Lineweaver-Burk plot for inhibition of RNase A by **(A) 4** of 0.075 (▲), 0.025 (■), 0 (●) mM. **(B) 8** of 0.06 (▲), 0.02 (■), 0 (●) mM. (**C**) **16** of 0.05 (▲), 0.02 (■), 0 (●) mM. **(D) 12** of 0.03 (▲), 0.01 (■), 0 (●) mM. 2′,3′-cCMP concentrations of 0.89-0.45 mM and RNase A concentration of 9.8-10.1 μM.

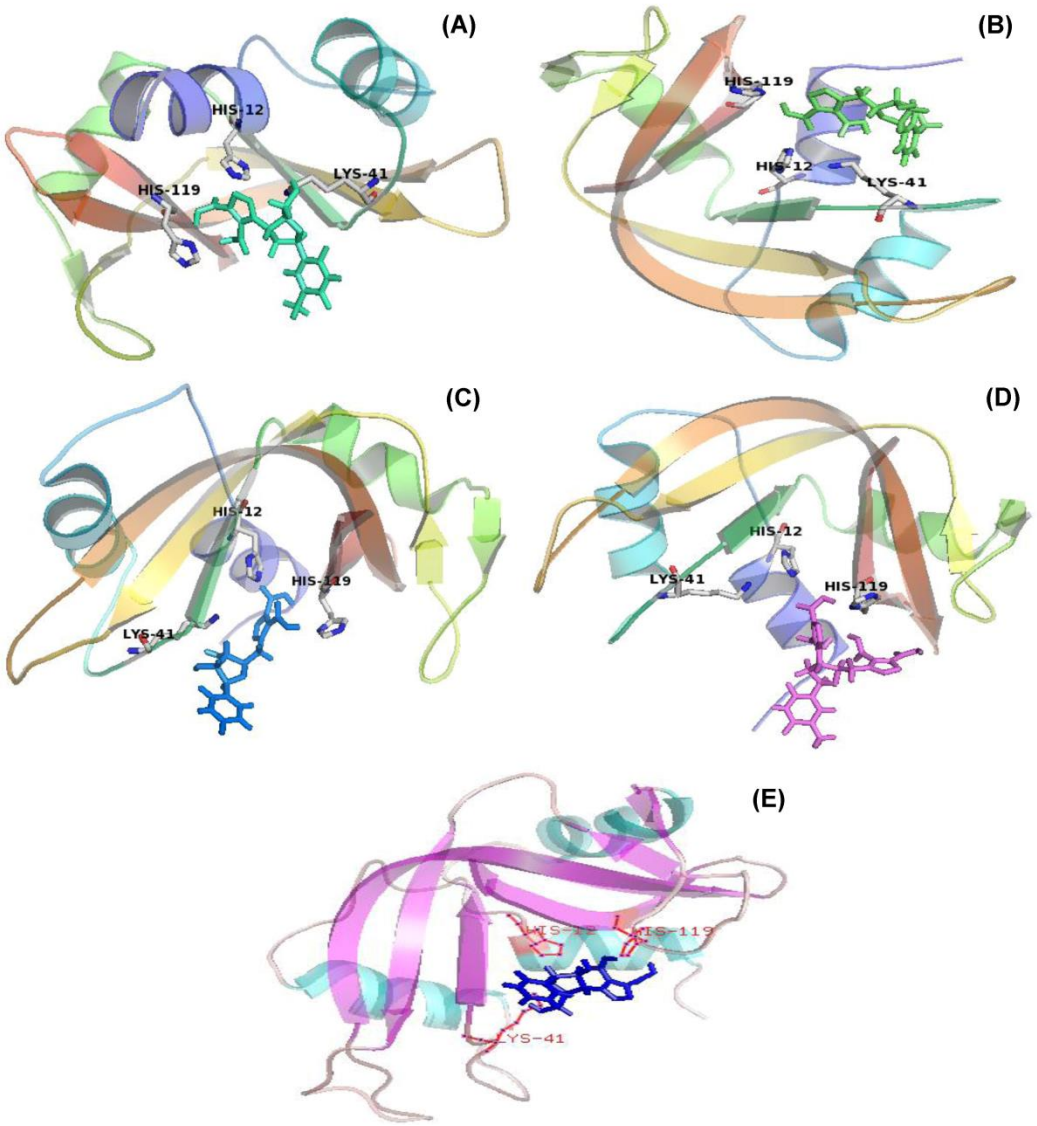


Figure S5: Docked poses of **(A) 4** (cyan), **(B) 8** (green), **(C) 12** (light blue), **(D) 19** (magenta), and **(E) 16** (dark blue) with **RNase A** (1FS3)

Table S1: Distances of polar contacts between inhibitors and amino acid residues of RNase A (1FS3)

1FS3	4	8	12	19	16
Arg39 NH1	2.71 Å [O of C2 C=O of nucleobase]		2.45 Å [O of C2 C=O of nucleobase]		
Arg39 NH2	2.94 Å [5' OH]	2.5 Å [O of C2 C=O of nucleobase]	3.2 Å [O of C2 C=O of nucleobase] 2.7 Å [2' OH]		
His119 Nδ1	2.0 Å [OH of acid of C5 of triazole ring]	2.0 Å [OH of acid of C4 of triazole ring]	1.7 Å [OH of acid of C5 of triazole ring]	1.86 Å [C=O of acid of C5 of 3'-triazole ring]	2.03 Å [OH of acid of C5 of triazole ring]
His12 Nε2	2.9 Å [C=O of acid of C5 of triazole ring]	2.9 Å [C=O of acid of C4 of triazole ring]	3.0 Å [C=O of acid of C4 of triazole ring] 2.58 Å [C=O of acid of C5 of triazole ring]	3.0 Å [C=O of acid of C5 of 3'-triazole ring]	2.9 Å [C=O of acid of C5 of triazole ring]
Cys26 CA	3.2 Å [N2 of triazole ring] 3.3 Å [N3 of triazole ring]				
Gln11 Nε2	2.6 Å [N3 of triazole ring]	2.6 Å [C=O of acid of C5 of triazole ring]		3.4 Å [C=O of acid of C4 of triazole ring] 2.75 Å [C=O of acid of C5 of 3'-triazole ring]	2.9 Å [C=O of acid of C5 of triazole ring]
Gln11 CD		2.0 Å [OH of acid of C5 of triazole ring]			
Phe120 N		1.9 Å [OH of acid of C5 of triazole ring]	3.0 Å [C=O of acid of C4 of triazole ring]	2.65 Å [OH of acid of C4 of 3'-triazole ring]	2.76 Å [OH of acid of C5 of triazole ring]
Lys41 Nζ		2.9 Å [C=O of acid of C4 of triazole ring]			
Thr45 CA		2.9 Å [O of C4 C=O of nucleobase]			
Lys7 Nζ			3.0 Å [3' OH] 2.2 Å [N2 of triazole ring] 2.4 Å [N3 of triazole ring]	3.0 Å [O of C2 C=O of nucleobase] 2.74 Å [C=O of acid of C5 of 3'-triazole ring]	2.9 Å [C=O of acid of C4 of triazole ring]
Val118 O				1.96 Å [OH of acid of C5 of 3'-triazole ring] 2.0 Å [OH of acid of C5 of 5'-triazole ring]	2.12 Å [OH of acid of C4 of triazole ring]
Gln69 Nε2				3.2 Å [N3 of 5'-triazole ring]	
Gln69 Oε1				2.35 Å [OH of acid of C5 of 5'-triazole ring]	
Cys 58					2.9 Å [C=O of acid of C4 of triazole ring]
Val 43 O					OH of 5'-CH_2OH