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## Supplementary information

# Practical and concise synthesis of nucleoside analogs

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# Practical and concise synthesis of nucleoside analogs

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# SUPPORTING INFORMATION

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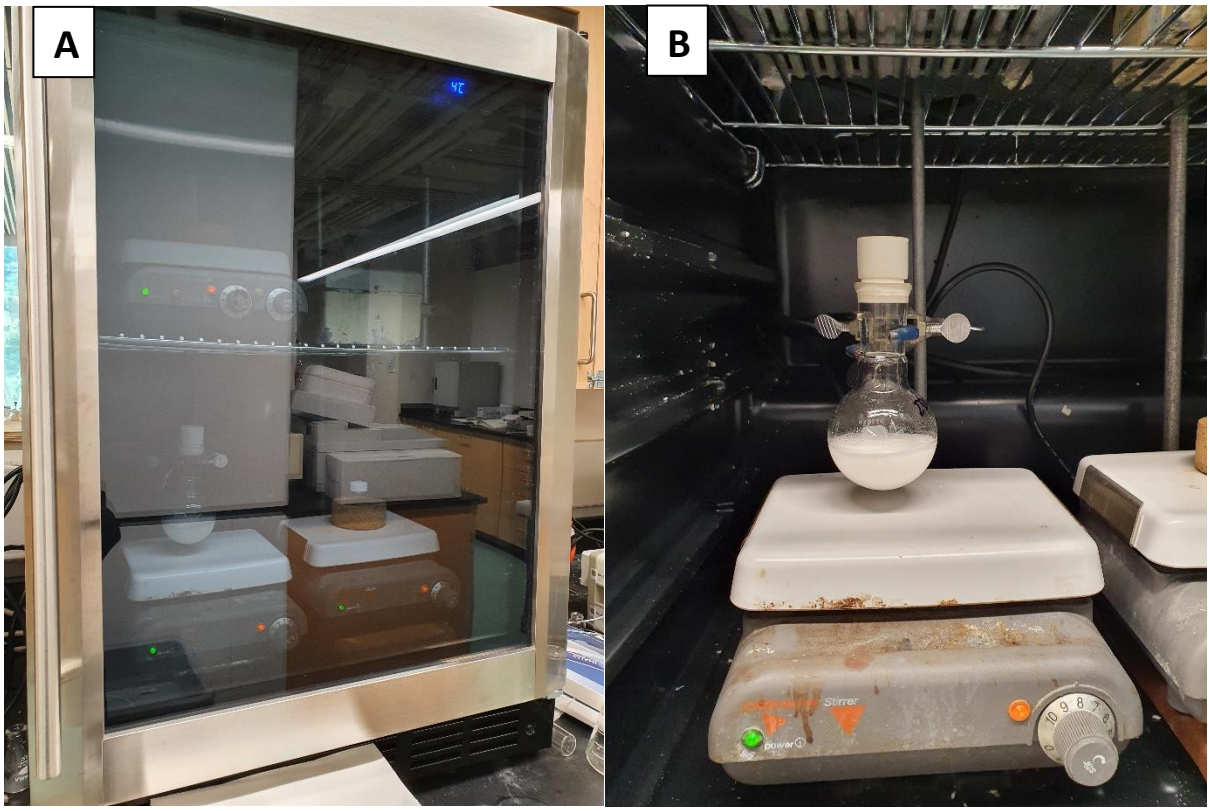
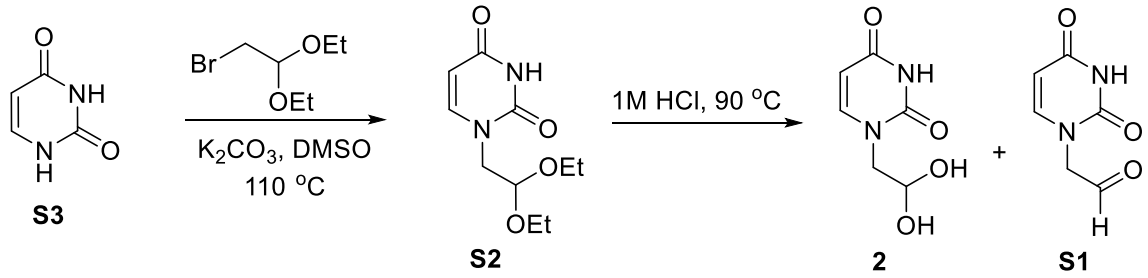



**Figure S1** | (a) Dedicated 4 °C fridge employed for the synthesis of fluorohydrin **4a/b** on gram-scale (steps 4-5); (b) α-Fluorination reaction mixture at 4 °C (step 5)

**Procedure S1 | Research-scale synthesis of aldehyde S1 and hydrate 2**




**Synthesis of acetal S2** TIMING Reaction ~20 hours, purification ~5-24 hours

1. Weigh uracil (**S3**) (10.0 g, 89.2 mmol) into a 500 mL round-bottomed flask
2. Weigh potassium carbonate (12.3 g, 89.2 mmol) and add this to the flask, along with a Teflon coated magnetic stir bar and dimethylsulfoxide (178 mL)
3. Begin stirring the mixture at room temperature, then add 2-bromoacetaldehyde diethyl acetal (14.8 mL, 98.1 mmol) in a steady stream
4. Fit a reflux condenser to the flask and a rubber septum to the top of the condenser. Attach a nitrogen filled balloon fitted with a needle attachment, to the top of the condenser.

**CRITICAL STEP** 2-bromoacetaldehyde diethyl acetal has a boiling point of ~175 °C and will partially evaporate if a reflux condenser is not used, leading to lower yields of **S2**

5. Heat the mixture to 110 °C in an oil bath for 16 hours **CAUTION** Prolonged heating of DMSO can cause its degradation. The decomposition process is autocatalytic and can led to a runaway reaction and explosion hazard, use a blast shield
6. Cool the mixture to room temperature and check reaction progress by TLC. To do this, take a small aliquot of the reaction mixture (~0.1 mL) and dilute two-fold with ethyl acetate in a 1 dram vial. Add ~0.1 mL of water to the vial and gently agitate the mixture. Run a TLC of the mini workup spotted next to uracil (**S3**) to determine completion of the reaction (eluting with 9:1 vol/vol dichloromethane/methanol; retention factor ($R_f$) uracil (**S3**) = 0.13, acetal **S2** = 0.38, dialkylated uracil side product = 0.74). Visualise the TLC plate under ultraviolet (UV) irradiation at 254 nm and develop the TLC plate with *p*-anisaldehyde stain (**S2** is UV active and will stain light grey-brown). If the reaction is incomplete, continue stirring until only trace starting material is observed by TLC **TROUBLESHOOTING**
7. Once cooled to room temperature, dilute the reaction mixture with ethyl acetate (500 mL) and transfer the mixture to a 1 L separatory funnel, rinsing the round bottomed-flask with ethyl acetate (50 mL)
8. Add brine (300 mL), stopper the funnel and mix the layers by gentle shaking, pausing to vent the pressure every few seconds. Clamp the separatory funnel upright on a retort stand, remove the stopper and separate the bottom aqueous layer into a 1 L beaker
9. Repeat step 8 two more times (combining the aqueous layers)
10. Drain out the organic layer into a clean 600 mL conical flask, rinsing the separatory funnel with ethyl acetate (50 mL)
11. Add sufficient anhydrous sodium sulfate to the organic solution to absorb residual water, swirling the flask as you do so
12. Carefully decant the solution into a 1 L round bottomed flask, washing the conical flask and sodium sulfate with ethyl acetate (50 mL)
13. Remove the ethyl acetate on a rotary evaporator to provide a crude yellow oil
14. Add ~3 mg of **S2** seed crystals to the crude yellow oil and allow to sit undisturbed at 0 °C for at least 3 hours. **PAUSE POINT** The crude material can be left to crystalize in the fridge overnight if desired **TROUBLESHOOTING**
15. Using a Pasteur pipette, carefully remove as much of the residual yellow oil as possible
16. Add a Teflon coated stirrer bar to the round bottomed flask and dissolve the colourless **S2** crystals in a minimum amount of hot toluene (~100 mL)
17. Cool 400 mL of hexanes on ice, then slowly pour this to the stirred toluene solution until **S2** crashes out as a white precipitate
18. Stop the stirring and allow to sit undisturbed at 0 °C for at least 1 hour
19. Set up a Büchner funnel (12.5cm diameter) fitted with a piece of filter paper (11cm diameter) and a rubber ring, on the top of a 1 L Büchner flask. Use a rubber hose to connect the Büchner flask to a water aspirator or a Piab low vacuum system. Moisten the filter paper with 20 mL of hexanes
20. Swirl the contents of the round-bottomed flask, turn on the low vacuum system, and slowly pour the mixture into the Büchner funnel
21. Wash the **S2** powder collected in the funnel with cold hexanes (80 mL), and air dry for ~1 minute
22. Disconnect the vacuum and transfer the colourless fine powder into a pre-weighed 250 mL round-bottomed flask, then dry under high vacuum for at least 1 hour
23. Weigh the flask and record the yield of **S2**
24. Confirm the identity of the product using HRMS, $^{1}$H NMR and $^{13}$C NMR

**Synthesis of aldehyde S1 and hydrate 2** TIMING Reaction ~3 hours, purification ~24 hours

25. Weigh acetal **S2** (9.09 g, 39.8 mmol) into a 250 mL round-bottomed flask
26. Add a Teflon coated magnetic stir bar and 1M aqueous hydrochloric acid (80 mL), and begin stirring the mixture
27. Fit a reflux condenser to the flask and a rubber septum to the top of the condenser. Add a nitrogen filled balloon fitted with a needle attachment to the top of the condenser
28. Heat the mixture to 90 °C for 3 hours in a sand or oil bath
29. Cool the mixture to room temperature and check reaction by TLC. To do this, take a small aliquot of the reaction mixture (~0.1 mL) and dilute two-fold with ethyl acetate in a 1 dram vial and gently agitate the mixture. Run a TLC of the mini workup spotted next to acetal **S2** to determine completion of the reaction (eluting with 9:1 v/v dichloromethane/methanol; $R_f$ **S2** = 0.38, aldehyde **S1** and hydrate **2** = 0.28). Visualise the TLC plate under UV irradiation at 254 nm and develop the TLC plate with permanganate stain (**2** and **S1** will both stain yellow on a purple background, both are UV active). If the reaction is incomplete, continue stirring at 90 °C until **S2** is completely consumed as observed by TLC
30. Once complete, cool the mixture to room temperature, then transfer the round-bottomed flask to a freezer and leave it overnight **PAUSE POINT** The mixture can be left in the freezer for up to a week if desired
31. Remove the flask from the freezer. In most cases, hydrate **2** has already precipitated from the solution, in which case proceed directly to step 32. Otherwise, thaw the solution a little with agitation and hydrate **1** should precipitate from the solution **TROUBLESHOOTING**
32. Ensure the contents of the round-bottomed flask have completely thawed (1-2 hours). Set up a Büchner funnel and flask as for step 19 above. Swirl the thawed contents of the round-bottomed flask, turn on the low vacuum system, and slowly pour the mixture into the Büchner funnel
33. Wash the aldehyde hydrate **2** powder collected in the Büchner funnel with cold water (50 mL), then hexanes (50 mL) and air dry for ~1 minute
34. Transfer the colourless fine powder (**2**) into a pre-weighed 250 mL round-bottomed flask, then dry under high vacuum for at least 5 hours. Weigh the flask and record the yield of **2** **CRITICAL STEP** Ensure **2** is transferred only as a solid, and is not dissolved in any alcoholic solvents or reformation of an acetal will occur
35. While **2** is drying under high vacuum, transfer the filtrate containing aldehyde **S1** to a pre-weighed 500 mL round-bottomed flask, rinsing the Büchner flask with acetonitrile (50 mL) and freeze the solution as for step 45 (B) (iv) (Procedure 1, main text)
36. Lyophilize the filtrate to obtain aldehyde **S1** (often as a mixture with hydrate **2**) as a white powder. Weigh the flask and record the yield **CRITICAL STEP** Lyophilisation is critical to removing the HCl from **S1**. Complete removal of HCl is imperative before commencing the subsequent α-FAR reaction
37. Confirm the purity and identity of both **S1** and **2** using HRMS, $^{1}$H NMR and $^{13}$C NMR **TROUBLESHOOTING**

**Procedure S2 | Process scale synthesis of aldehyde S1 and hydrate 2**

**Synthesis of acetal S2** TIMING Reaction ~24 hours, purification ~24 hours

38. Fit the central 45 size joint of a 4-neck (24, 24, 24 and 45 joint sizes) 3000 mL round-bottom flask with an overhead stirrer holding a glass stir rod terminated with a 10 cm stirring paddle
39. Fit the left-most 24 size joint with a rubber septum housing a JKEM thermoprobe
40. Fit the right-most 24 size joint with a water-cooled reflux condenser topped with a 24 sized glass $N_2$ inlet adaptor

41. Charge the vessel with uracil (**S3**) (50.0 g, 446 mmol) and potassium carbonate (61.7 g, 446 mmol) via a powder funnel fitted in the remaining open 24 size joint
42. Add DMSO (890 mL) to the vessel
43. With stirring (350 rpm), add 2-bromoacetaldehyde diethyl acetal (73.8 mL, 491 mmol) in a steady stream via the remaining joint opening
44. Seal the remaining 24 sized joint with a glass stopper lined with a Teflon sleeve
45. Using a large aluminum block, heat the resulting suspension such that the internal temperature reaches 90 °C. **CAUTION** Prolonged heating of DMSO can cause its degradation. The decomposition process is autocatalytic and can led to a runaway reaction and explosion hazard, use a blast shield
46. After 24 hours, check the reaction progress by TLC (see Step 6 for details). If the reaction is incomplete, continue stirring until only trace starting material is observed by TLC **TROUBLESHOOTING**
47. Cool the dark mixture to room temperature, and transfer it to a 5 L extractor vessel containing a drain valve
48. Fit this vessel with an overhead stirrer holding a glass stir rod terminated with a 10 cm stirring paddle
49. Add ethyl acetate (2500 mL) and brine solution (1000 mL) and stir the biphasic mixture at 450 rpm for 5 minutes
50. Allow the layers to separate and collect the organic layer between two 2000 mL Erlenmeyer flasks
51. Extract the aqueous layer again with ethyl acetate (1000 mL) as for Step 49
52. Wash the combined organic layers with brine (3 × 1000 mL) and 10% aq. LiCl (3 × 1000 mL). **CRITICAL STEP** Each extraction utilized stirring at 450 rpm for 5 minutes before the phases were allowed to separate
53. Add sufficient anhydrous sodium sulfate to the organic solution to absorb residual water, swirling the flask as you do so
54. Using a 600 mL fritted funnel, filter the solution to remove the drying agent, and remove all volatiles on the rotary evaporator to afford a colored solid
55. Suspend the residue in methyl *tert*-butyl ether or diethyl ether (200 mL) and stir for 30 minutes at 750 rpm
56. Collect the colorless precipitate by vacuum filtration using a 600 mL fritted funnel and dry under high vacuum (batch 1)
57. Concentrate the filtrate on the rotary evaporator and take the resulting residue up in 3:7 (vol/vol) diethyl ether/hexanes (200 mL) and stir the suspension (750 rpm) overnight at 4 °C in a cold room
58. Collect the colorless precipitate by vacuum filtration using a 600 mL fritted funnel and dry it to a constant weight under high vacuum (batch 2)
59. Combine batches 1 and 2 into a pre-weighed round-bottomed flask
60. Weigh the flask and record the yield of acetal **S2**
61. Confirm the identity of the product using HRMS, $^{1}$H NMR and $^{13}$C NMR

**Synthesis of aldehyde S3 and hydrate 1 TIMING** Reaction ~4 hours, purification ~24 hours

62. Fit the left-most joint of a 3-neck 2000 mL round-bottom flask (all 24 joint sizes) containing a large magnetic stirrer-bar fitted with rubber septa housing a JKEM thermoprobe
63. Fit the central joint with a water-cooled reflux condenser topped with a 24 sized glass $N_2$ inlet adaptor

64. Charge the vessel with acetal **S2** (51.0 g, 223 mmol) and 1M aqueous hydrochloric acid (453 mL) via a powder funnel fitted in the remaining open 24 size joint
65. Seal this 24 sized joint with a glass stopper lined with a Teflon sleeve
66. Using a large aluminum block, heat the resulting suspension such that the internal temperature reaches 90 °C **TROUBLESHOOTING**
67. After 3 hours, check the reaction by LCMS (Method info: Agilent Zorbax Eclipse C18 RRHD 1.8um, 2.1 x 50 mm, 40 °C, 1.0 ml/min; 15-99% B in 2.0 min then hold 0.5 min; 0.75 min post time, 1 µL inj volume, 210 and 254 nm. A = Water + $NH_4HCO_2$ @ pH 3.5; B = CAN. Starting material elutes at 0.457 minutes and the product elutes at 0.130 minutes.) If the reaction is incomplete, continue stirring at 90 °C until **S2** is completely consumed
68. Once complete, cool the mixture to room temperature with stirring
69. Transfer the reaction vessel to the cold room (4 °C) and continue stirring for approximately 18 hours
70. Collect the resulting colorless precipitate via vacuum filtration using a 600 mL fritted funnel
71. Wash the cake with decanted ice water (2 × 100 mL), acetonitrile (2 × 100 mL), and diethyl ether (2 × 100 mL)
72. Collect the solids into a pre-weighed round-bottomed flask and dry to a constant weight under high-vacuum
73. Weigh the flask and record the yield of aldehyde **S1**/hydrate **2**
74. Confirm the identity of the product using HRMS, $^{1}H$ NMR and $^{13}C$ NMR **TROUBLESHOOTING**

**Table S1 | Troubleshooting for synthesis of aldehyde 1 and hydrate S3 (procedures S1 and S2)**

| Step | Problem | Possible reason | Solution |
|---|---|---|---|
| 6 and 46 | Incomplete reaction after 24 hours at 110 °C | Dialkylation of uracil starting material is unavoidable. Thus with 1.1 equivalents of alkylating agent the reaction will always leave some uracil unreacted | Do not add more 2-bromoacetaldehyde diethyl acetal as while this drives the reaction to completion, it also yield more dialkylated uracil. Best yields of **S2** have been obtained when some uracil has been left unreacted. If unsure, leave for ~5 hours, and if the reaction progress appears unchanged by TLC then proceed to the next step |
| 14 | No **S2** seed crystal on hand | Reaction being conducted for the first time | Leave crude oil in the fridge overnight, **S2** often crystalizes out on its own. If this fails, proceed directly to step 16 |
| 31 | Hydrate **2** doesn't precipitate from the solution | Uncertain | If on hand, add ~3 mg of seed crystals of **2**, and leave in the freezer for at least 5 hours |
| 37 | The $^{1}$H NMR of the lyophilised aldehyde **S1** shows hydrate **2** is present | Incomplete crystallization of hydrate **2** from the reaction mixture | The mixture of **S1** and **2** may be used in the α-FAR without consequence |
| 65 | The reaction mixture is heterogenous | Acetal **S2** is insoluble in aqueous media | The mixture became homogeneous at approximately 50 °C |
| 74 | NMR indicates aldehyde **S1** and hydrate **2** are present as a mixture | Aldehyde is suspected to form from **S1** in dry solvents | The mixture of **S1** and **2** may be used in the α-FAR without consequence |

## Anticipated results:

**Diethyl Acetal S2.** Fine white powder. Typically obtained in 45-55% yield.

$^{1}$H NMR (500 MHz, $CDCl_3$) δ 8.51 (br s, 1H), 7.26 (d, *J* = 7.9 Hz, 1H), 5.66 (dd, *J* = 7.9, 2.3 Hz, 1H), 4.62 (t, *J* = 5.3 Hz, 1H), 3.79 (d, *J* = 5.3 Hz, 2H), 3.76 (dq, *J* = 9.4, 7.0 Hz, 2H), 3.54 (dq, *J* = 9.4, 7.0 Hz, 2H), 1.20 (t, *J* = 7.0 Hz, 6H)

$^{13}$C NMR (150 MHz, $CDCl_3$) δ 164.1 (C), 151.3 (C), 146.2 (CH), 101.7 (CH), 100.3 (CH), 64.5 (2 × $CH_2$), 51.1 ($CH_2$), 15.4 (2 × $CH_3$)

IR (neat, $cm^{-1}$) 2978, 1705, 1673, 1352, 1252, 1115, 1053, 1026, 897, 822, 767

HRMS (EI+) calcd for $C_{10}H_{17}N_2O_4$ $[M+H]^+$ 229.1183; found: 229.1164.

**Aldehyde hydrate 2.** Colourless amorphous solid. Typically obtained in 60-75% yield (research scale).

$^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 11.22 (br s, 1H), 7.49 (d, *J* = 7.8 Hz, 1H), 6.13 (d, *J* = 6.2 Hz, 2H), 5.50 (dd, *J* = 7.8, 2.3 Hz, 1H), 4.91 (pent, *J* = 5.9 Hz, 1H), 3.36 (d, *J* = 5.5 Hz, 2H)

$^{13}$C NMR (150 MHz, DMSO-$d_6$) δ 163.8 (C), 151.1 (C), 147.0 (CH), 100.1 (CH), 87.1 (CH), 53.5 ($CH_2$);

IR (neat, $cm^{-1}$) 3352, 1652, 1435, 1244, 1075, 1036, 883, 762

HRMS (EI+) calcd for $C_6H_9N_2O_4$ $[M+H]^+$ 173.0557; found: 173.0560.

**Aldehyde S1.** Colourless amorphous solid. Typically obtained in 20-30% yield, as a ~1:1 mixture with aldehyde hydrate **2** (research scale). On process scale, a ~1:1 mixture of **S1** and **2** was obtained in 91% yield.

$^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 11.38 (br s, 1H), 9.54 (s, 1H), 7.51 (d, *J* = 7.9 Hz, 1H), 5.61 (dd, *J* = 7.9, 2.3 Hz, 1H), 4.63 (s, 2H)

$^{13}$C NMR (150 MHz, DMSO-$d_6$) δ 197.4 (CH), 163.8 (C), 151.0 (C), 145.9 (CH), 101.0 (CH), 56.5 ($CH_2$)

IR (neat, $cm^{-1}$) 2817, 1661, 1075, 1036, 885, 808, 722

HRMS (EI+) calcd for $C_6H_7N_2O_3$ $[M+H]^+$ 155.0451; found: 155.0427.

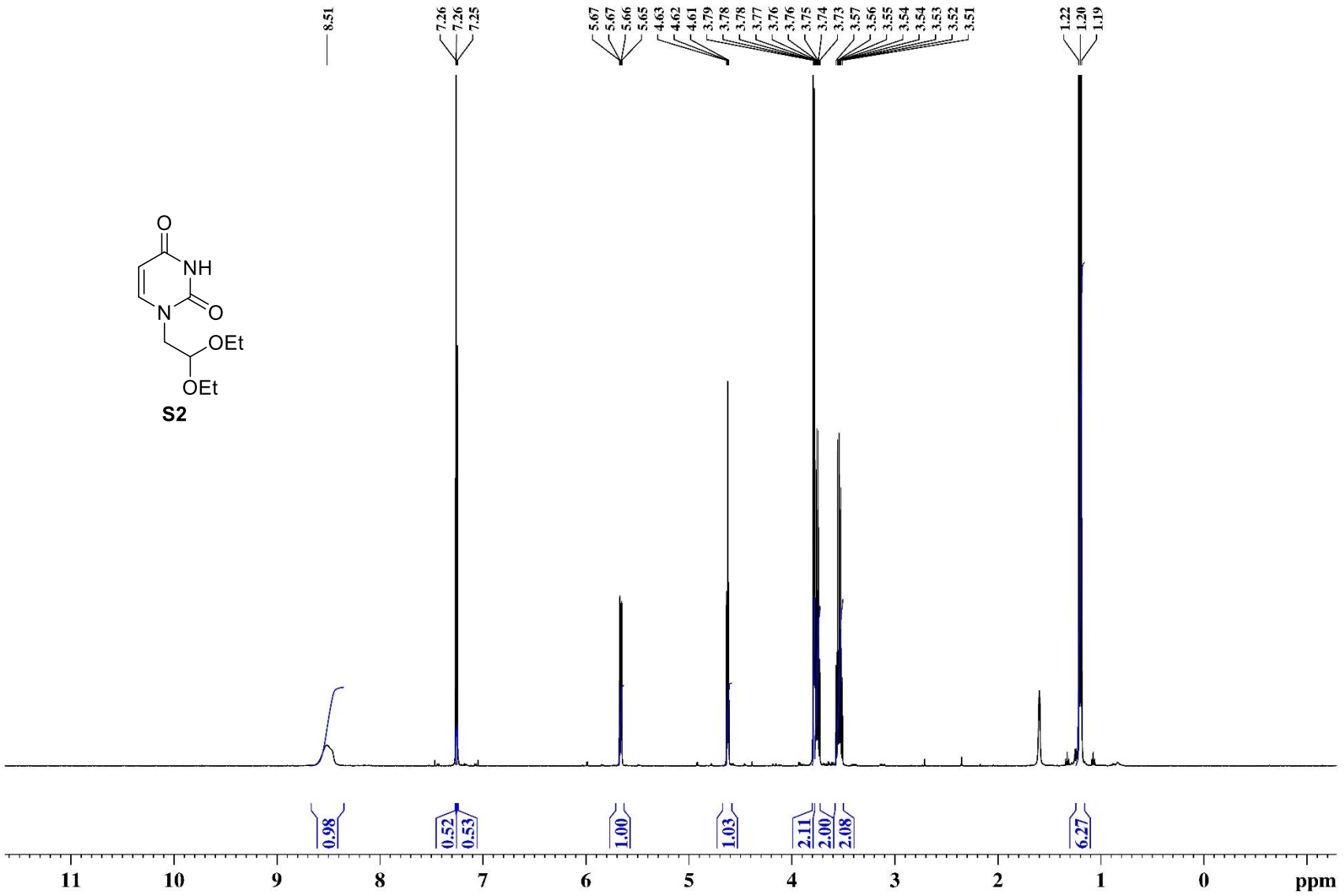



**Figure S2.** $^{1}$H NMR spectrum of diethyl acetal **S2** ($CDCl_3$, 500 MHz)

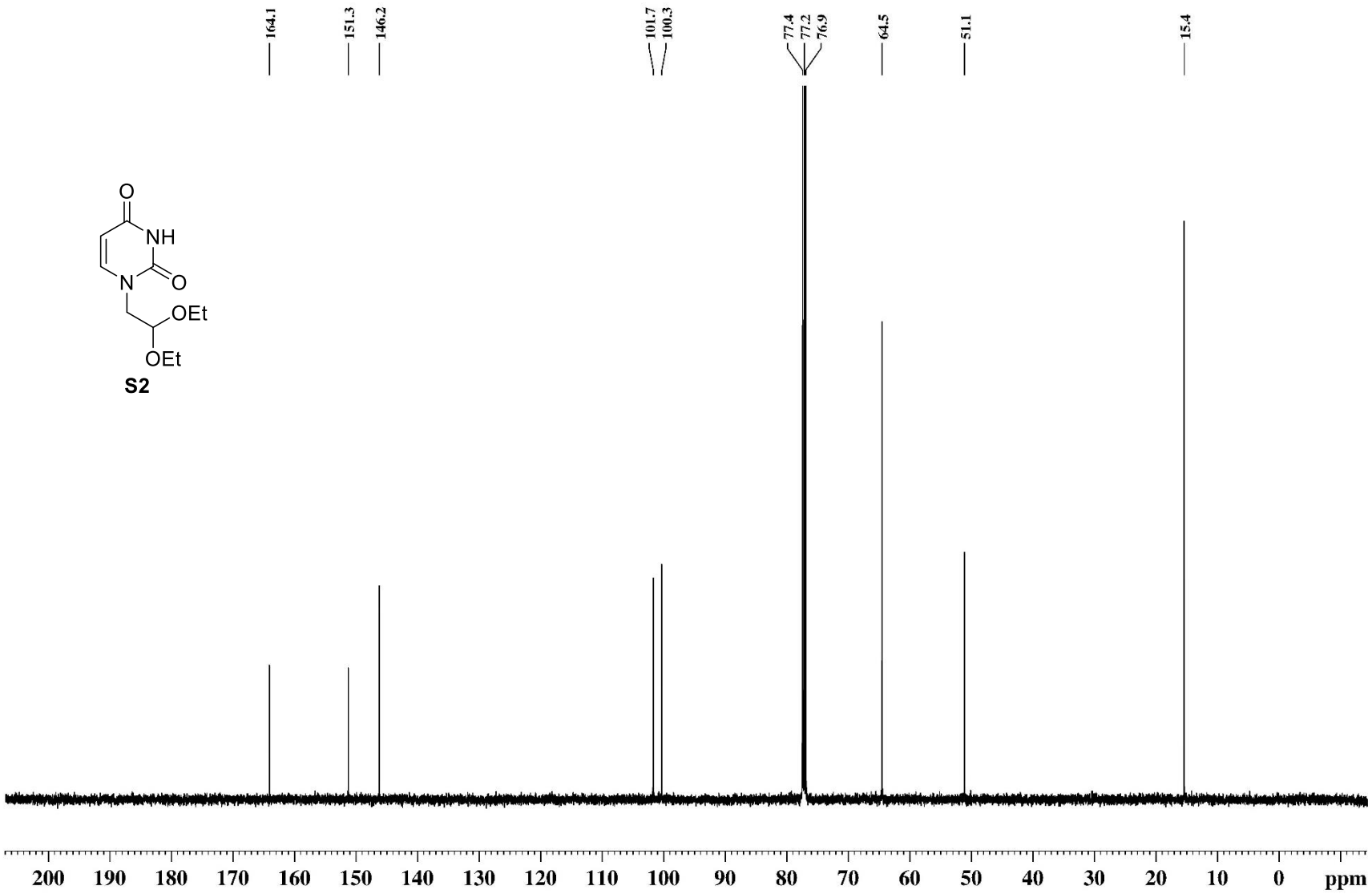



**Figure S3.** $^{13}C$ NMR spectrum of diethyl acetal **S2** ($CDCl_3$, 125 MHz)

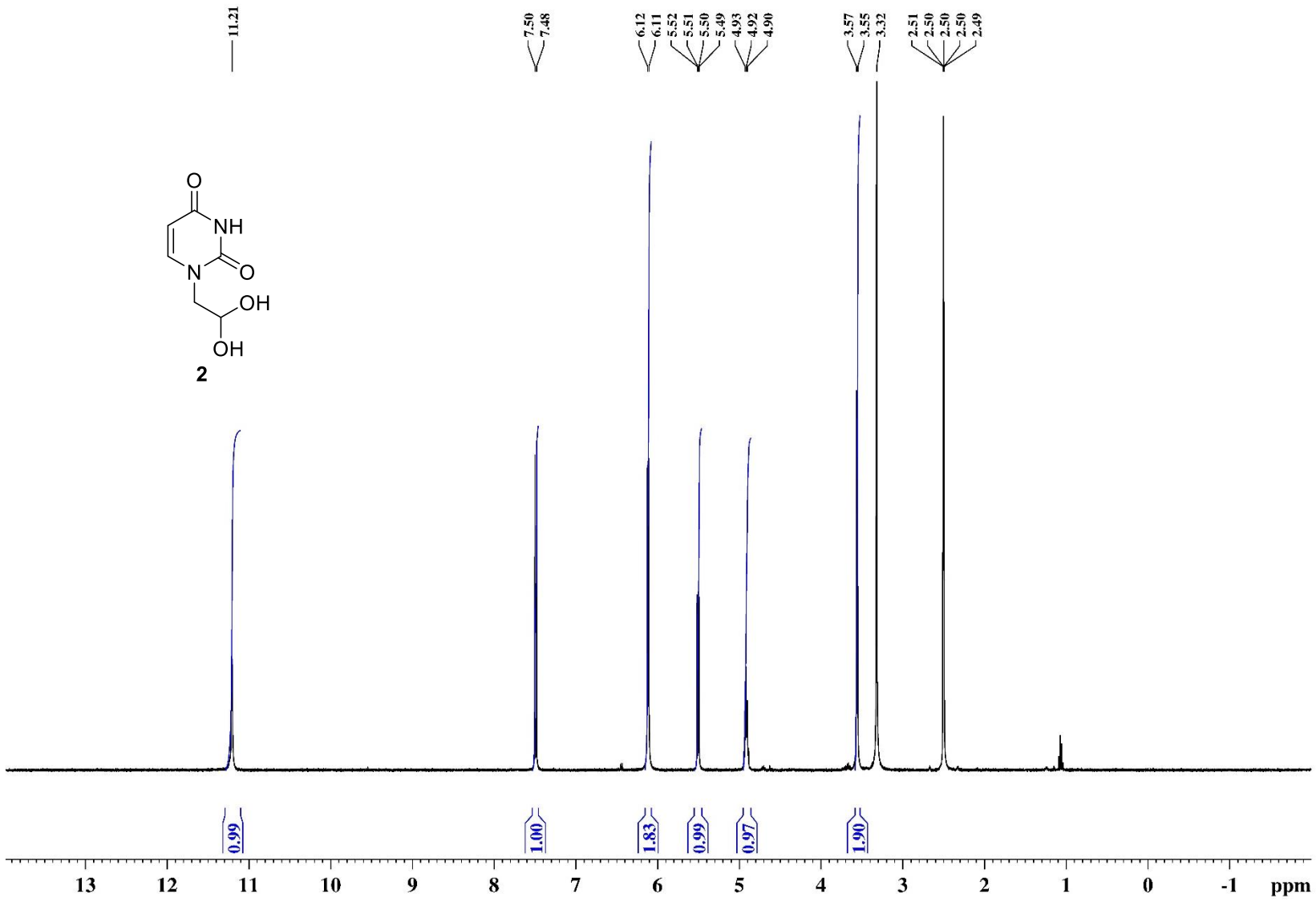



**Figure S4.** $^{1}$H NMR spectrum of aldehyde hydrate **2** (DMSO-$d_6$, 500 MHz)

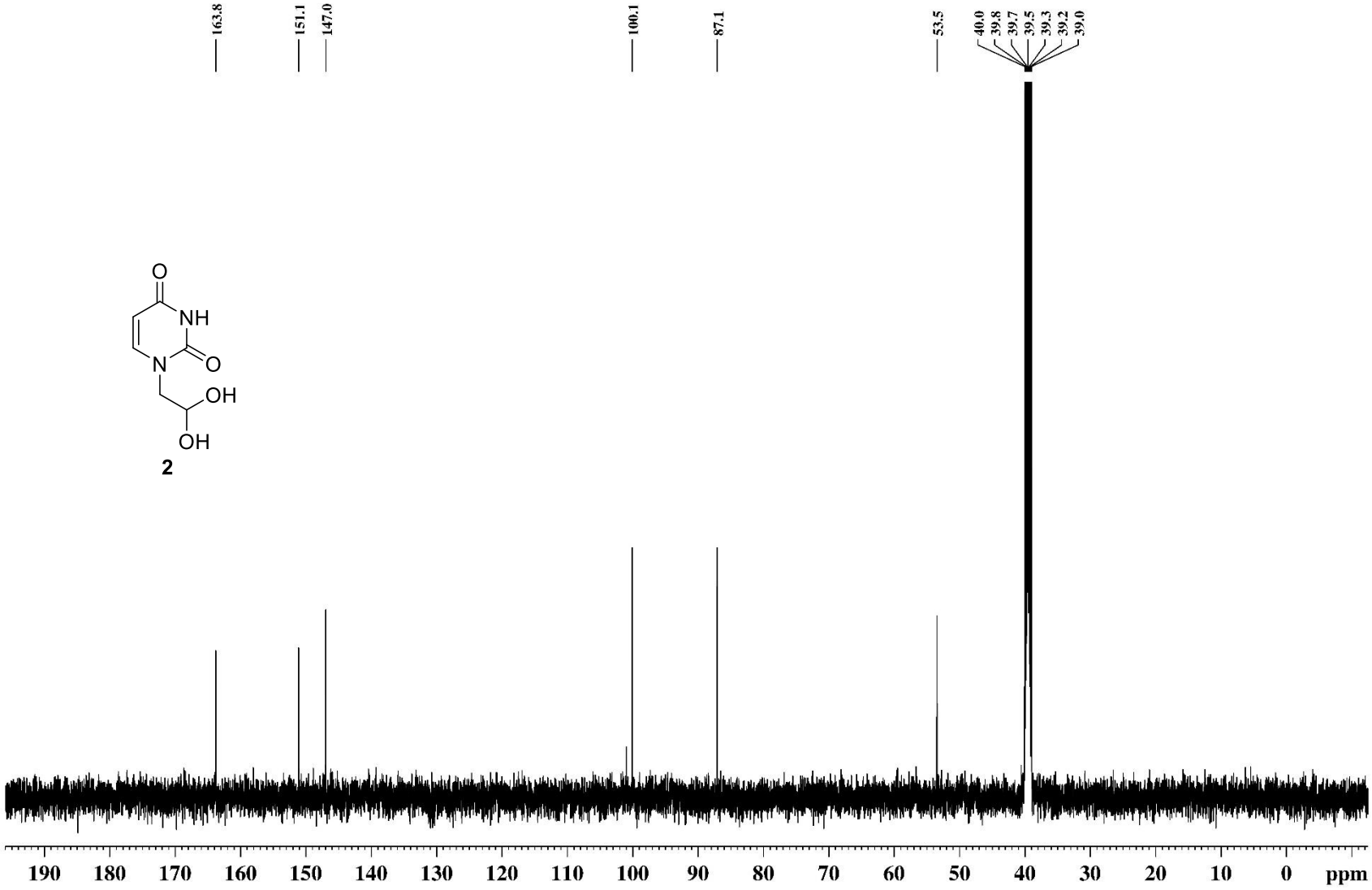



**Figure S5.** $^{13}C$ NMR spectrum of aldehyde hydrate **2** (DMSO-$d_6$, 125 MHz)

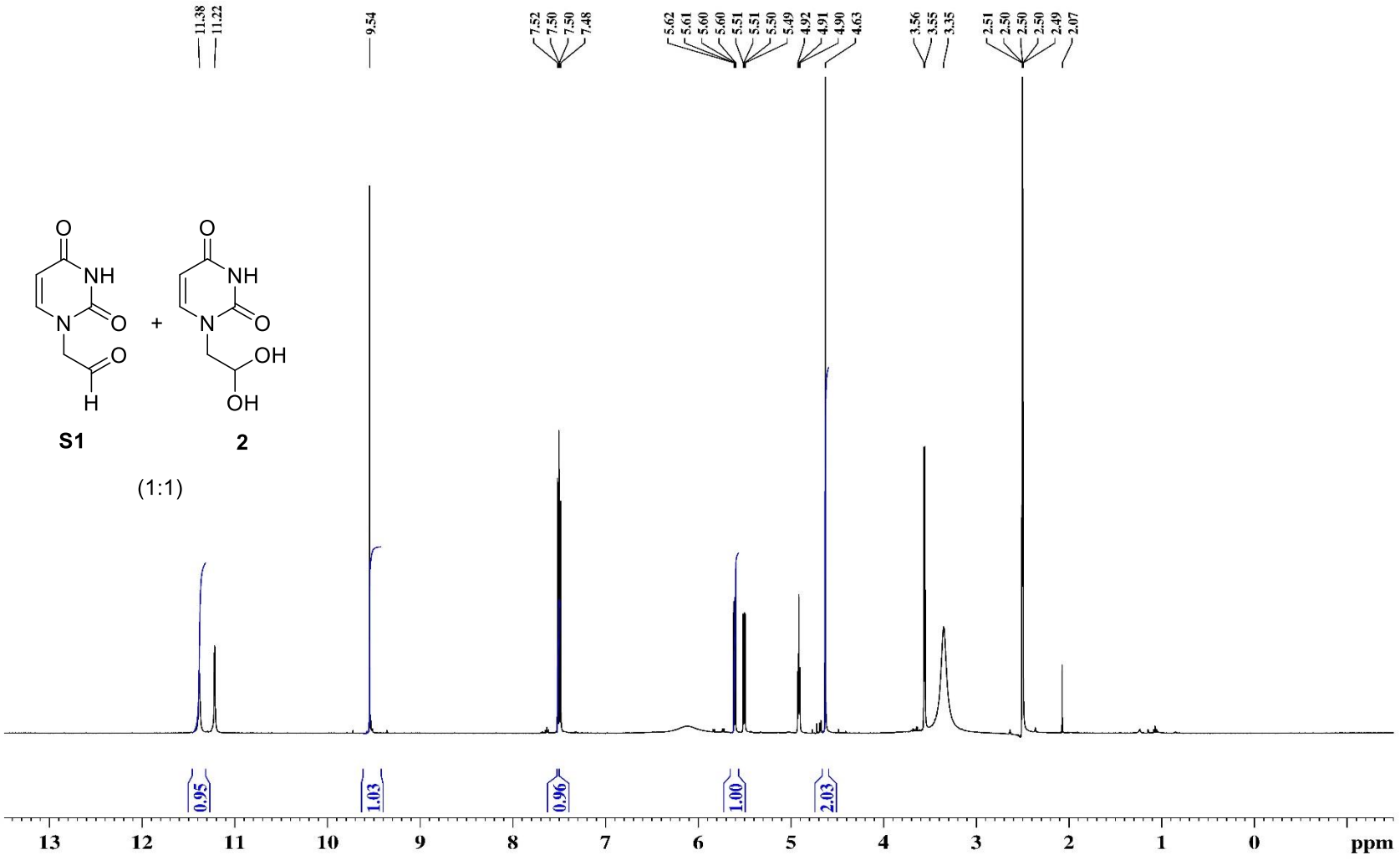



**Figure S6.** $^{1}$H NMR spectrum of aldehyde hydrate **2** and aldehyde **S1** (1:1) (DMSO-$d_6$, 500 MHz)

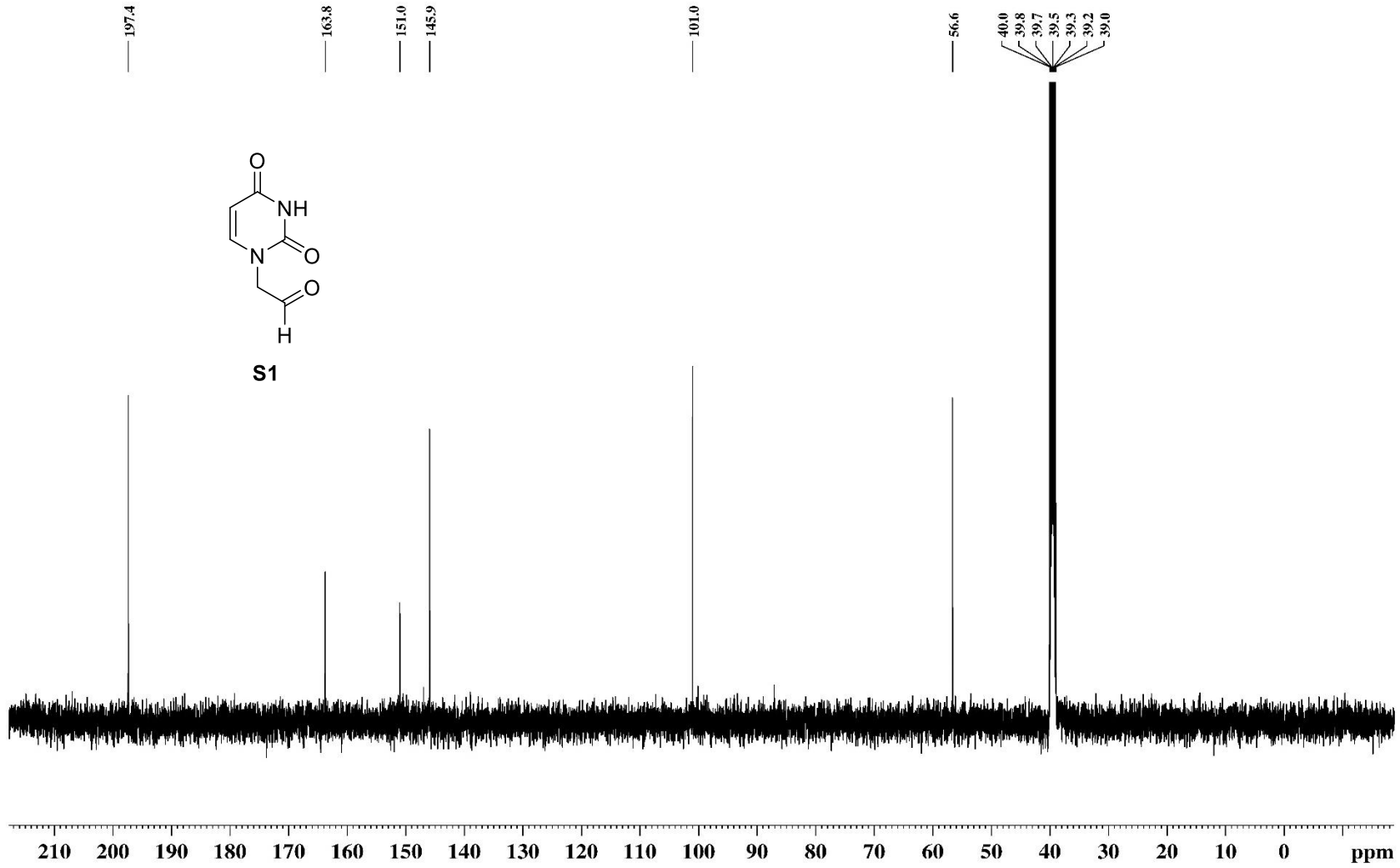



**Figure S7.** $^{13}C$ NMR spectrum of aldehyde **S1** (DMSO-$d_6$, 125 MHz)