

A Concise Route to MK-4482 (EIDD-2801) from Cytidine

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Contents

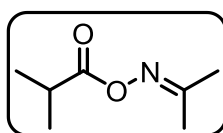
General Experimental Section:	2
Synthesis of Oxime Ester:.....	2
Enzymatic Acylation of Cytidine:	4
Transamination of 5'- <i>O</i> -isobutyrylcytidine:	8
Transamination of cytidine:	10
Enzymatic acylation of <i>N</i> -hydroxycytidine 3:	12
Synthesis of Triazole 6 from Uridine:	14
Transamination of Triazole 6:	16

General Experimental Section:

Instrumentation: For all compounds, ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance III spectrometer (300, 500, 600 MHz). Chemical shifts were measured relative to the residual solvent resonance for ^1H and ^{13}C NMR ($\text{CDCl}_3 = 7.26$ ppm for ^1H and 77.2 ppm for ^{13}C , $\text{DMSO}-d_6 = 2.50$ ppm for ^1H and 39.2 ppm for ^{13}C , $\text{CD}_3\text{OD} = 3.31$ ppm for ^1H and 49.0 ppm for ^{13}C). Coupling constants J are reported in hertz (Hz). The following abbreviations were used to designate signal multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; dd, doublet of doublet; ddd, doublet of doublet of doublet; dt, double of triplet; ddt, doublet of doublet of triplet; m, multiplet; br, broad. HRMS was recorded on an Agilent Technologies 6545 QTOF LC/MS. LRMS was recorded on an Agilent Technologies InfinityLab LC/MSD. Reactions were monitored by HPLC using the methods indicated. Glassware was oven-dried at 120 °C, assembled while hot, and cooled to ambient temperature under an inert atmosphere. Unless noted otherwise, reactions involving air sensitive reagents and/or requiring anhydrous conditions were performed under a nitrogen or argon atmosphere. Purity was assessed as noted by either quantitative NMR (qNMR) with benzyl benzoate as reference standard or by HPLC using purified intermediates and EIDD-2801 for system calibration.

Reagents and solvents. Reagents and solvents were purchased from Aldrich Chemical Company, Fisher Scientific, Alfa Aesar, Acros Organics, Oakwood, or TCI. Liquid reagents were purified by distillation when necessary. Unless otherwise noted, solid reagents were used without further purification. Methylene chloride (DCM), dioxane, and dimethylformamide (DMF) taken from a solid-sorbant Solvent Dispensing System purchased from Pure Process Technologies or distilled as described in the literature.

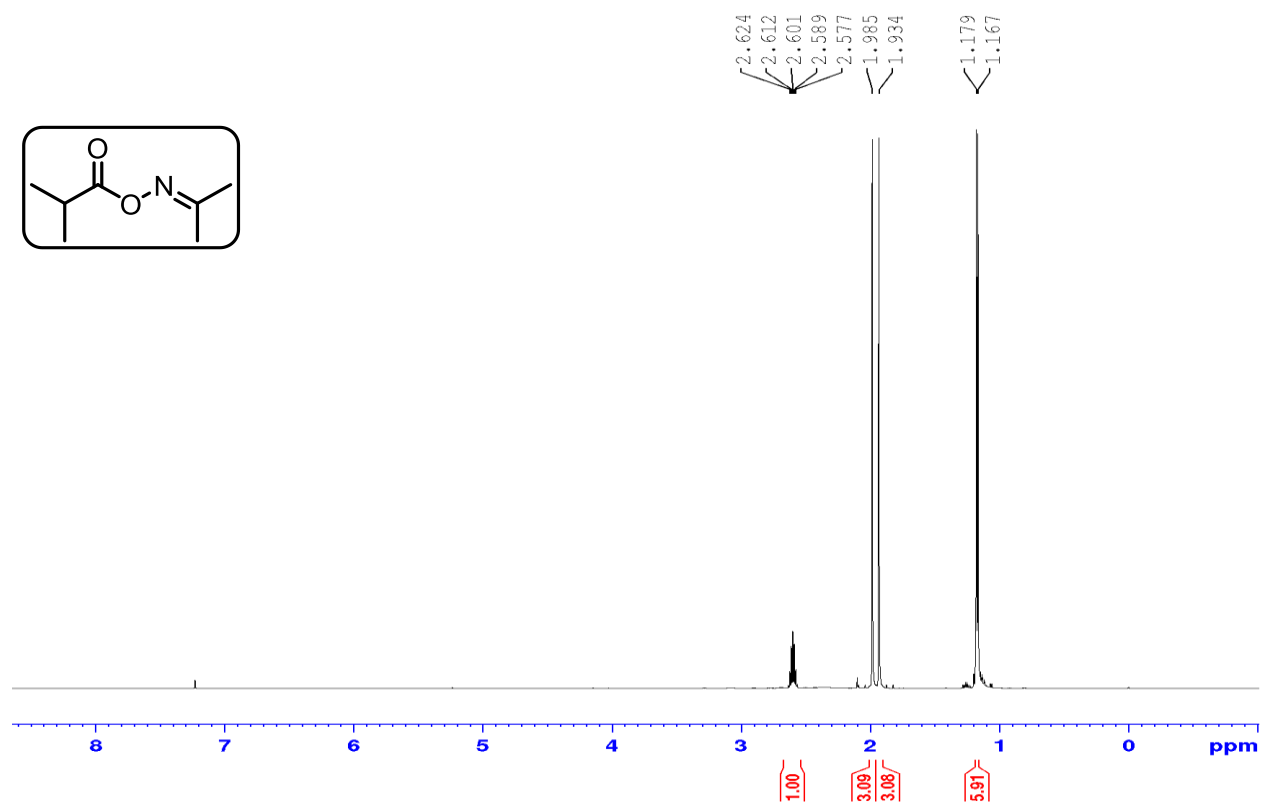
Synthesis of Oxime Ester:



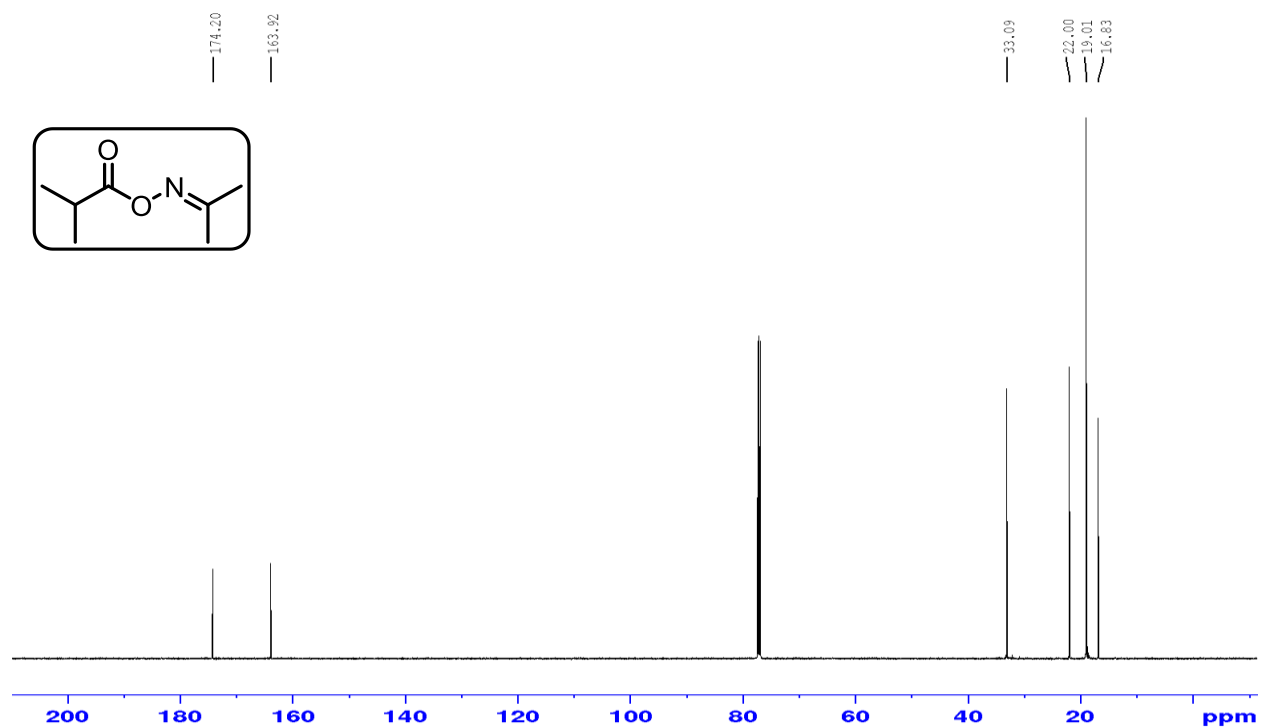
To a precooled solution of oxime (15.0 g, 205.21 mmol, 1.0 eq) in dichloromethane (750 mL) isobutyryl chloride (23.65 mL, 225.7 mmol, 1.1 eq) was added under nitrogen atmosphere at 0 °C, and then Et_3N (31.46 mL, 225.7, 1.1 eq) was added drop wise (~ 20 min) at 0 °C, and then this reaction mass was stirred at room temperature for 20h. Then reaction mass was washed with H_2O (2 X 150 mL), 5% solution of NaHCO_3 (2 X 100 mL), H_2O (1 X 150 mL), 1N aq. HCl (2 X 100 mL), H_2O (1 X 150 mL), and brine solution (1 X 50 mL), then organic layer was dried over anhydrous Na_2SO_4 , evaporated under vacuum in rotavapor to give desired oxime ester product as light-yellow oil (28.0 g, 95% yield).

^1H NMR (600 MHz, CDCl_3): δ 1.17 (d, $J = 7.15$ Hz, 6H), 1.93 (s, 3 H), 1.98 (s, 3 H), 2.60 (hept, $J = 7.0$ Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3): δ 16.8, 19.0 (2C), 22.0, 33.0, 163.9, 174.2 ppm.

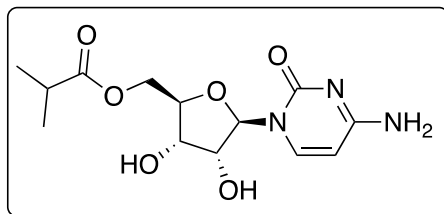
¹H NMR Spectra for oxime ester (600 MHz, CDCl₃):



¹³C NMR Spectra for oxime ester (151 MHz, CDCl₃):



Enzymatic Acylation of Cytidine:

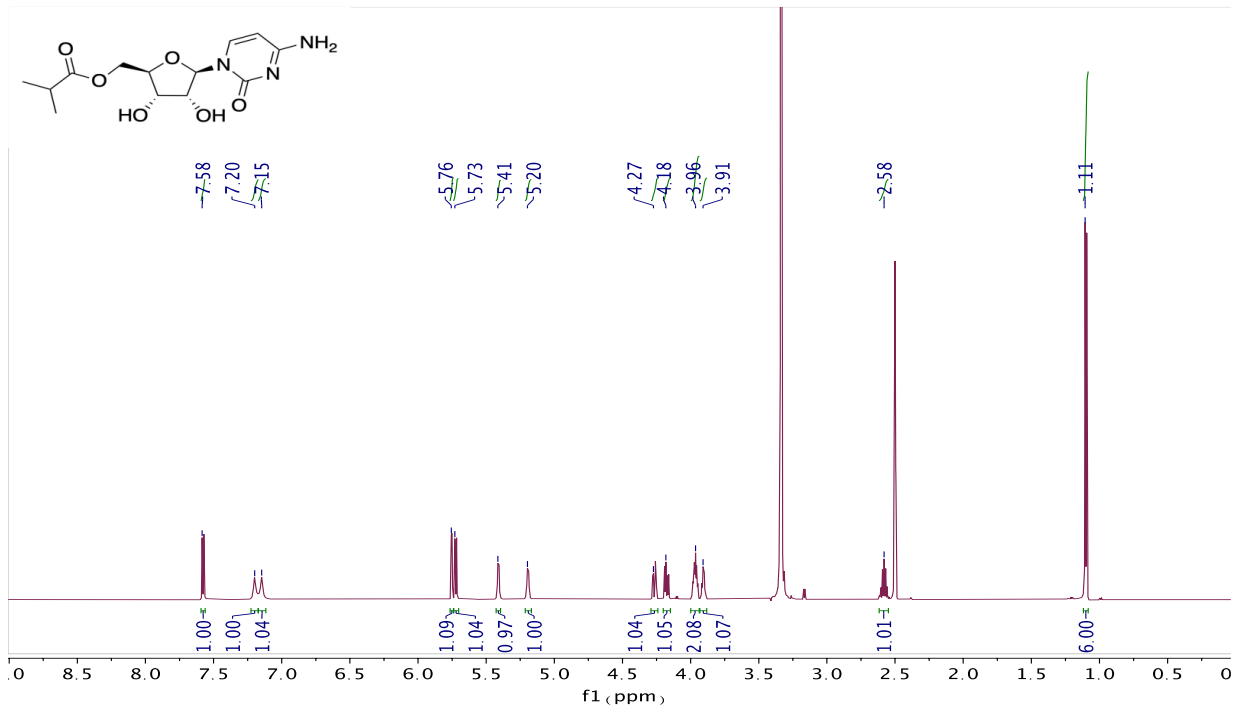


To an oven-dried 1 L 3-neck RBF was added cytidine **2** (5.0 g, 20.6 mmol), CALB (10.0 g, 200 wt %), 1,4-dioxane (500 mL), and crude acetone oxime *O*-isobutyryl ester (16.35 g, 102.8 mmol, assumed 90% purity). The reaction mixture was heated to 60 °C with an oil bath and stirred using a Heidolph RZR 2020 mechanical stirrer for 43 hours. The mixture was then filtered to remove enzymes and the solvent was removed by rotary evaporation, yielding the crude product as a yellow oil. The crude product was purified by column chromatography (2-20% gradient of MeOH in dichloromethane). The purified product **4** was obtained as a white crystalline solid (5.02 g, 98% purity, 78% yield). Characterization data matches with reported literature values.¹

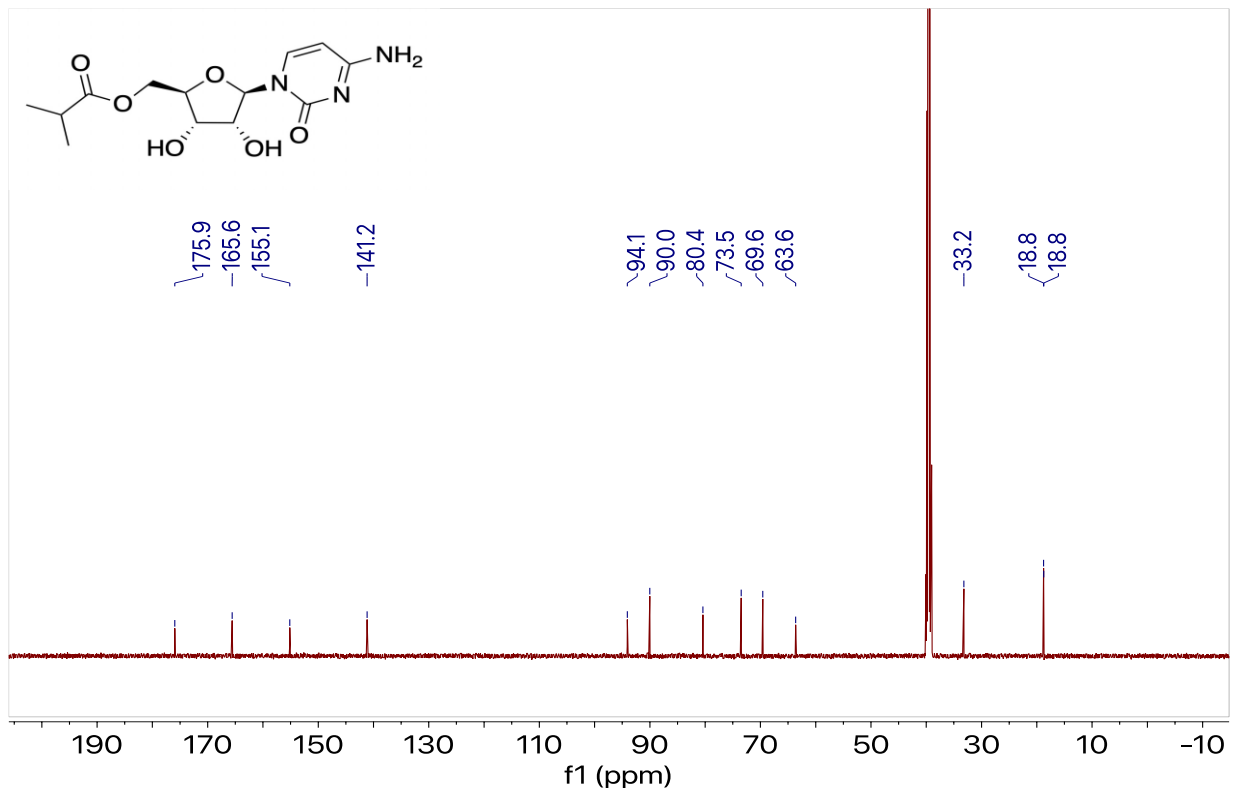
¹H NMR (600 MHz, DMSO-*d*₆): δ 1.11 (d, *J* = 7.0 Hz, 6H), 2.58 (h, *J* = 7.0 Hz, 1H), 3.91 (m, 1H), 3.94 – 4.01 (m, 2H), 4.18 (dd, *J* = 12.2, 5.4 Hz, 1H), 4.27 (dd, *J* = 12.2, 3.1 Hz, 1H), 5.20 (d, *J* = 6.0 Hz, 1H), 5.41 (d, *J* = 5.3 Hz, 1H), 5.73 (d, *J* = 7.4 Hz, 1H), 5.76 (d, *J* = 3.7 Hz, 1H), 7.15 (br s, 1H), 7.20 (br s, 1H), 7.58 (d, *J* = 7.4 Hz, 1H); **¹³C NMR (126 MHz, DMSO-*d*₆):** δ 18.8, 18.8, 33.2, 63.6, 69.6, 73.5, 80.4, 90.0, 94.1, 141.2, 155.1, 165.6, 176.0 ppm; **LRMS:** 314.1 [M+H]⁺; **HRMS (ESI):** calcd. for C₁₃H₁₉N₃O₆ [M+H]⁺ 314.1347, found 3.14.1352; **Purity:** 98% (assessed by qNMR).

¹ Nowak, I.; Jones, C. T.; Robins, M. J. *J. Org. Chem.* **2006**, *71* (8), 3077–3081.

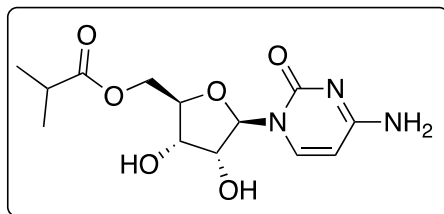
¹H NMR Spectra for 5'-O-Isobutyrylcytidine 4 (600 MHz, DMSO-d₆):



¹³C NMR Spectra for 5'-O-Isobutyrylcytidine 4 (151 MHz, DMSO-d₆):



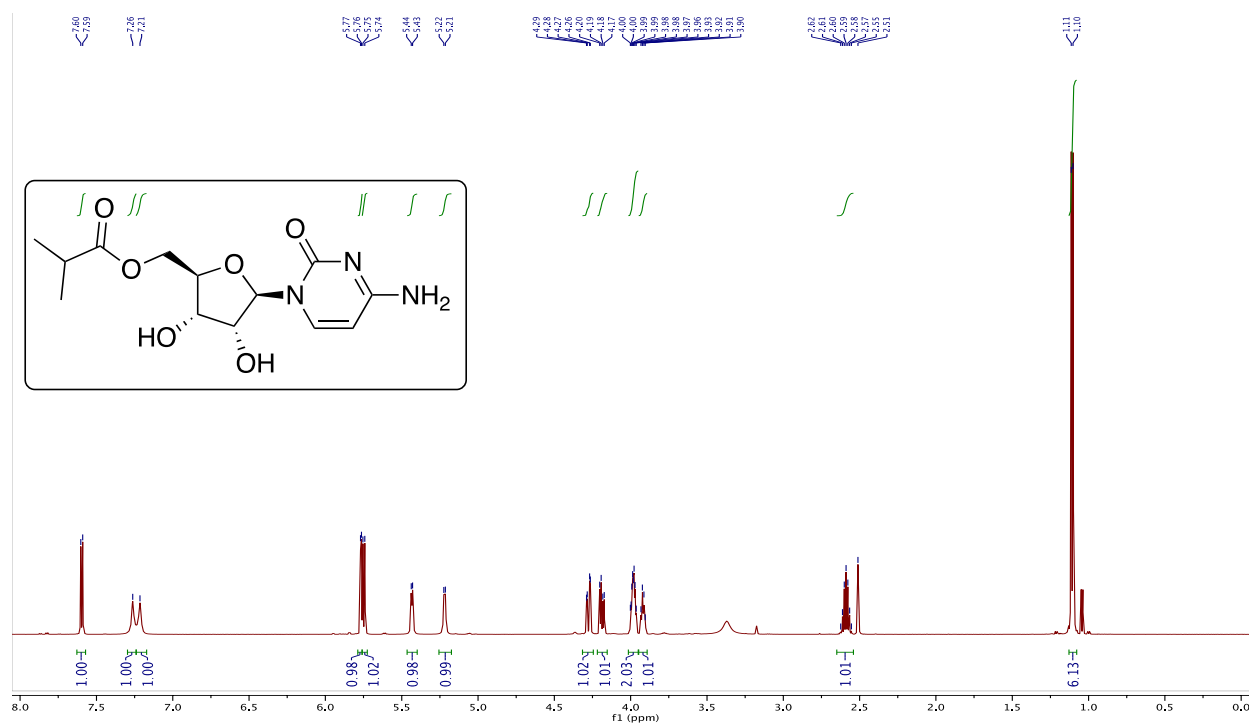
Chemical Acylation of Cytidine:



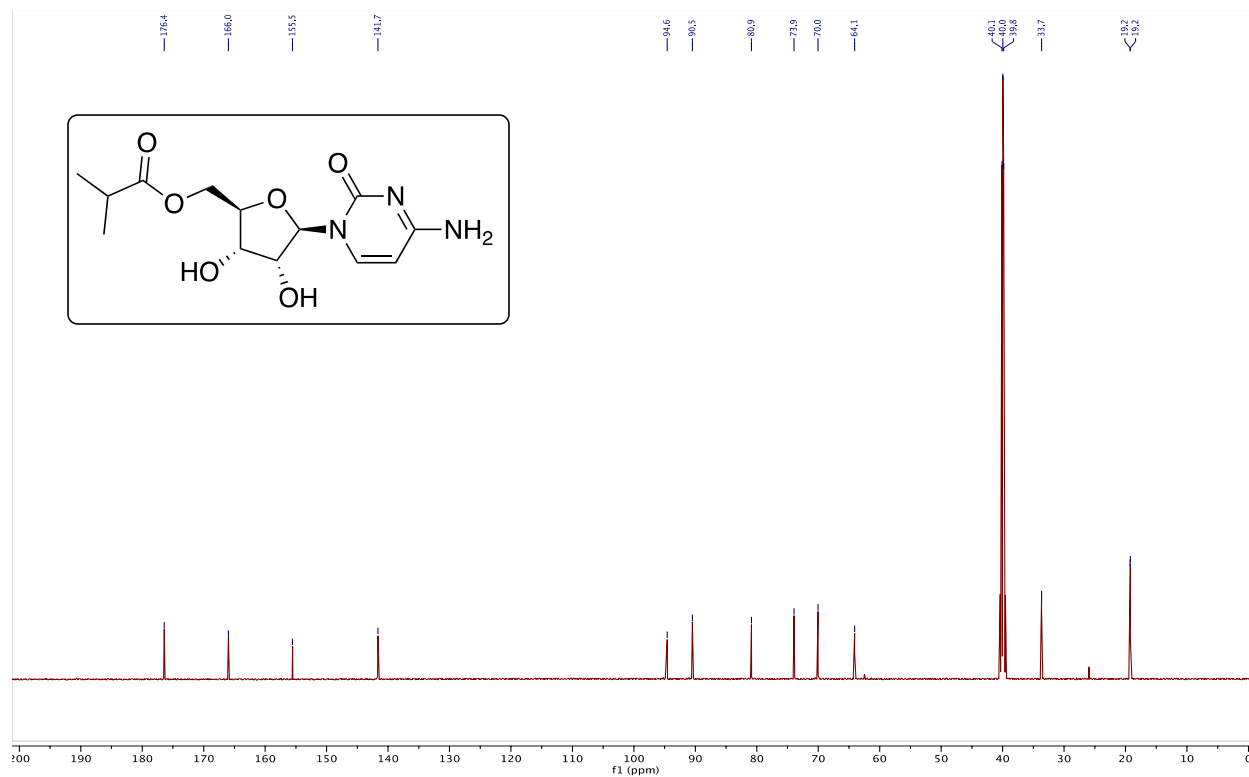
To a suspended solution of cytidine **2** (2.0 g, 8.22 mmol, 1.0 eq) in DMPU (4 mL), 4M HCl solution in 1,4-dioxane (3.08 mL, 12.33 mmol, 1.5 eq) was added and stirred at 20 °C for 15 minutes. The reaction mixture was cooled to 0 °C and isobutyryl chloride (1.03 mL, 9.87 mmol, 1.2 eq) was added at once. The reaction was stirred for 16h at same temperature. The reaction was monitored by HPLC, the unreacted cytidine (10%) and required product (75%) was observed. The reaction mixture was diluted with MTBE (40 mL) and stirred vigorously at 0 °C for 15 minutes. The supernatant liquid was removed (decant) and sticky residue was dissolved with MeOH (20 mL). The pH of reaction mixture was adjusted to 9 to 10 by addition sodium methoxide in methanol. The solvent was removed under reduced pressure and residue was subjected to column chromatography using MeOH/dichloromethane (0% to 20%) solvent combination. The white solid 5'-O-isobutyrylcytidine **4** (2.0 g, 94% purity, 76% yield) was obtained.

¹H NMR (600 MHz, DMSO-*d*₆): δ 1.11 (d, *J* = 7.0 Hz, 6H), 2.59 (hept, *J* = 7.0 Hz, 1H), 3.92 (m, 1H), 3.99 (ddd, *J* = 9.4, 6.3, 3.5 Hz, 2H), 4.19 (dd, *J* = 12.2, 5.4 Hz, 1H), 4.28 (dd, *J* = 12.2, 3.1 Hz, 1H), 5.22 (d, *J* = 6.0 Hz, 1H), 5.43 (d, *J* = 5.2 Hz, 1H), 5.75 (d, *J* = 7.4 Hz, 1H), 5.77 (d, *J* = 3.8 Hz, 1H), 7.21 (br s, 1H), 7.26 (br s, 1H), 7.60 (d, *J* = 7.5 Hz, 1H); **¹³C NMR (151 MHz, DMSO-*d*₆):** δ 19.2, 19.3, 33.7, 39.8, 40.0, 40.1, 64.1, 70.0, 73.9, 80.9, 90.5, 94.6, 141.7, 155.6, 166.0, 176.4 ppm; **Purity:** 94% (assessed by qNMR).

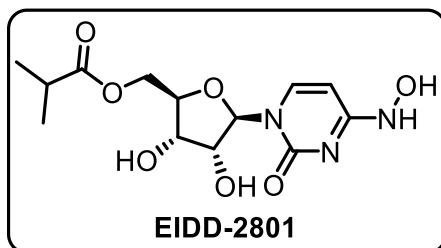
¹H NMR Spectra for 5'-O-Isobutyrylcytidine 4 (600 MHz, DMSO-d₆):



¹³C NMR Spectra for 5'-O-Isobutyrylcytidine 4 (151 MHz, DMSO-d₆):



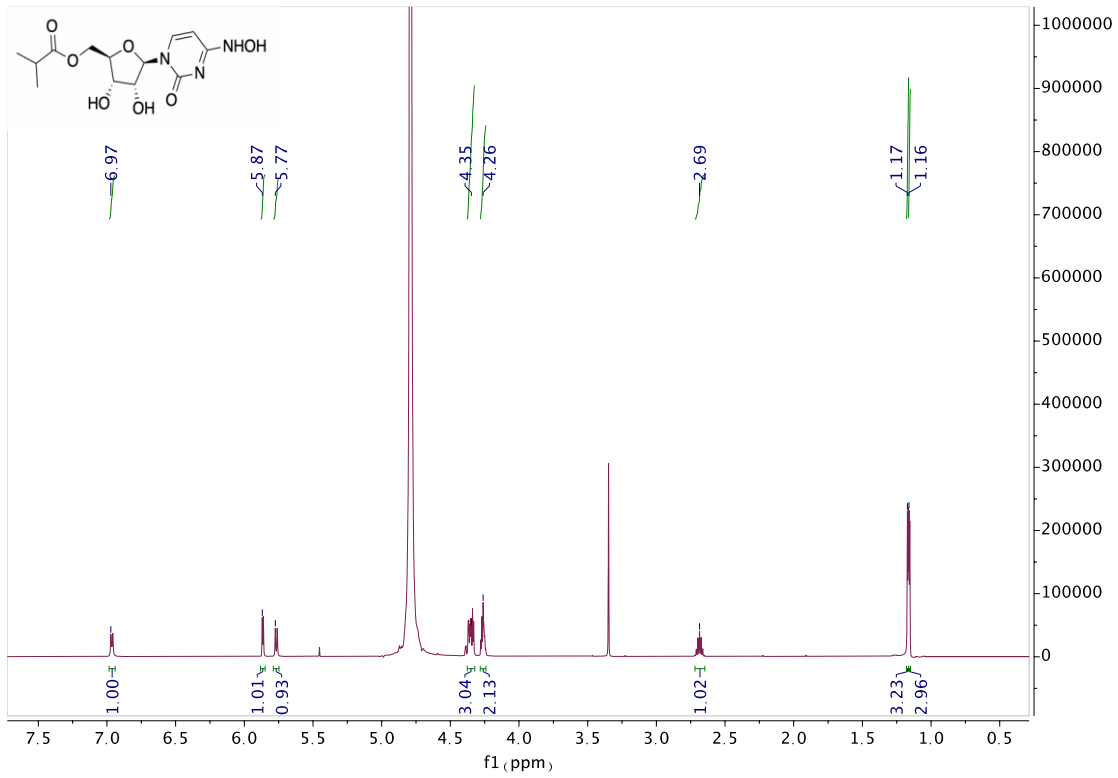
Transamination of 5'-*O*-isobutyrylcytidine:



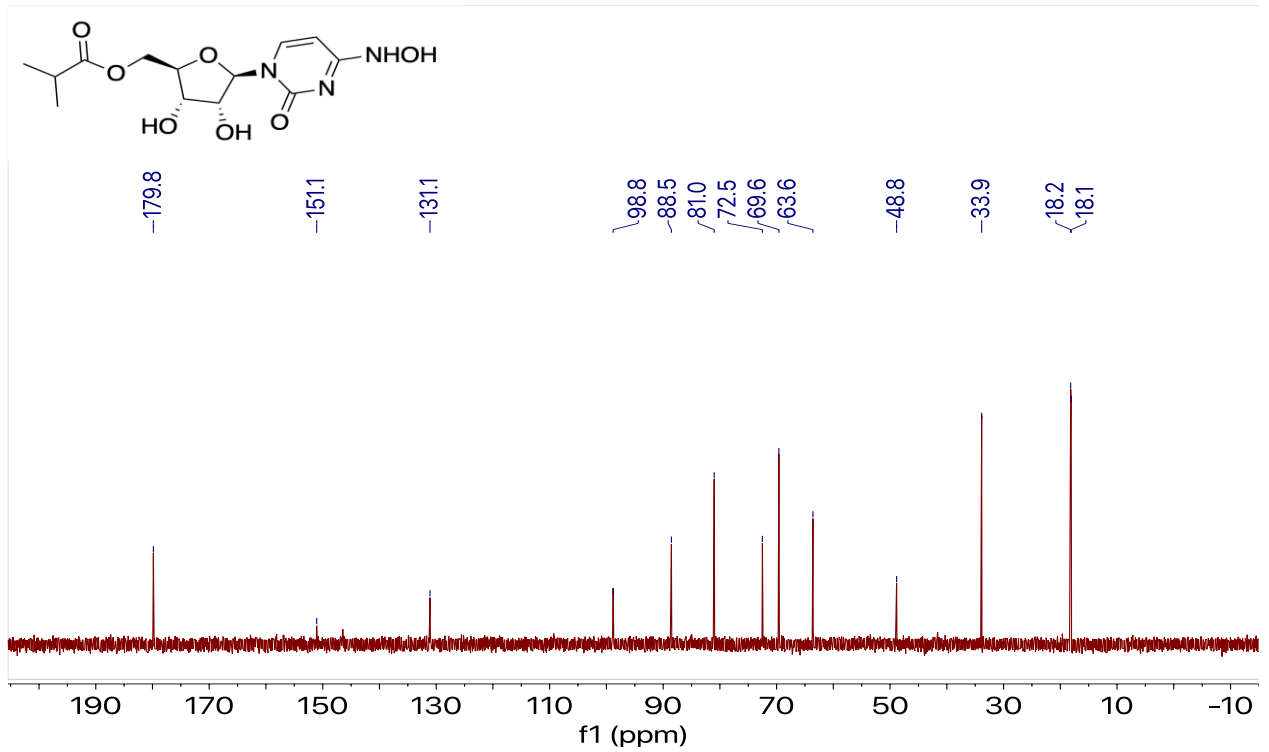
To a solution of 5'-*O*-isobutyrylcytidine **4** (1.0 g, 90% purity, 2.87 mmol, 1.0 eq) in 2-propanol (15 ml), hydroxylamine sulphate (2.12 g, 12.93 mmol, 4.5 eq.) was added and reaction was stirred for 20 h at 78 °C. Upon completion, the reaction was cooled to room temperature. The organic layer (upper layer) was separated from biphasic reaction mixture. The aqueous layer was washed with 2-propanol (2 X 5 mL). The combined organic layer was concentrated using rotary evaporation and the crude was purified by column chromatography with a gradient of 2-15% methanol in dichloromethane to yield EIDD-2801 (**1**) as a white solid (963 mg, 94% purity, 96% yield).

¹H NMR (600 MHz, D₂O) δ 6.98 (d, *J* = 8.3 Hz, 1H), 5.87 (d, *J* = 5.0 Hz, 1H), 5.78 (d, *J* = 8.2 Hz, 1H), 4.39 – 4.33 (m, 3H), 4.28 (dd, *J* = 6.6, 3.4 Hz, 2H), 2.69 (hept, *J* = 7.0 Hz, 1H), 1.17 (d, *J* = 3.7 Hz, 3H), 1.16 (d, *J* = 3.7 Hz, 3H). **¹³C NMR** (126 MHz, D₂O) δ 18.1, 18.2, 33.9, 48.8, 63.6, 69.6, 72.5, 81.0, 88.5, 98.8, 131.1, 151.1, 179.8 ppm; **LRMS**: 330.1 [M+H]⁺; **HRMS (ESI)**: calcd. for C₁₃H₁₉N₃O₇ [M+H]⁺ 330.1296, found 330.1302; **Purity**: 94% (assessed by qNMR).

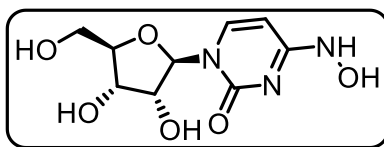
¹H NMR Spectra for EIDD-2801 (400 MHz, D₂O):



¹³C NMR Spectra for EIDD-2801 (126 MHz, D₂O):



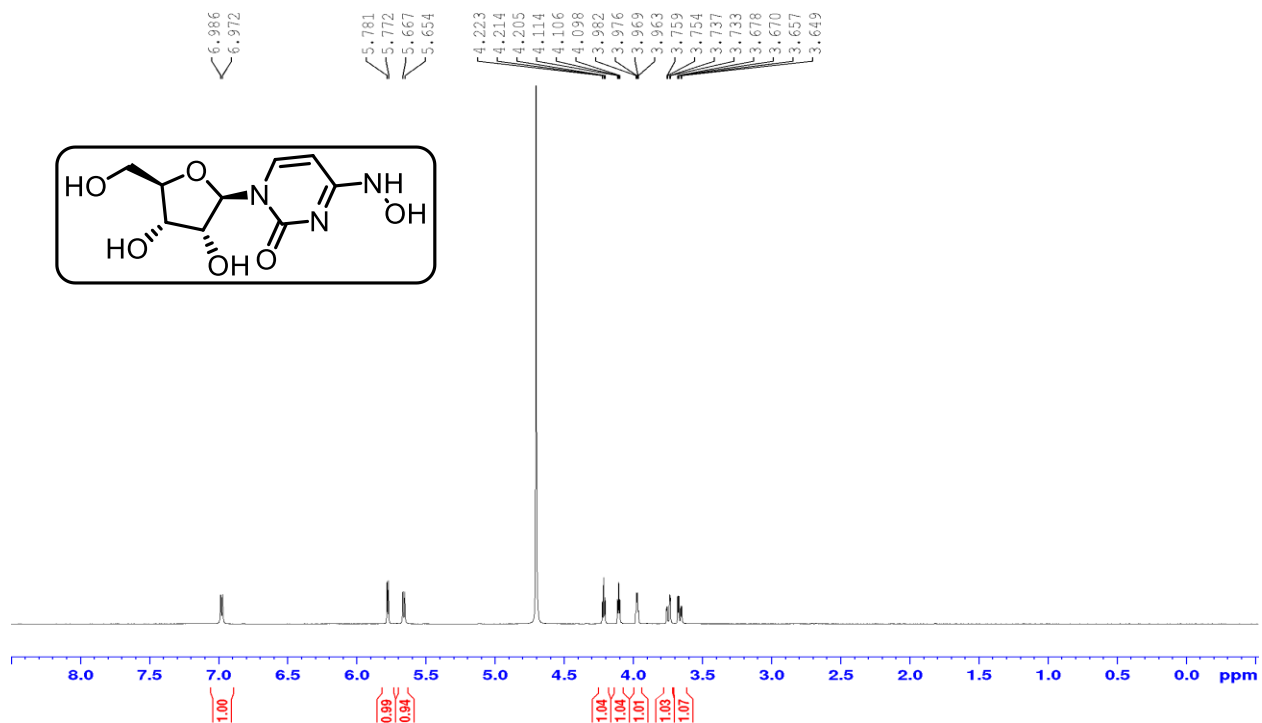
Transamination of cytidine:



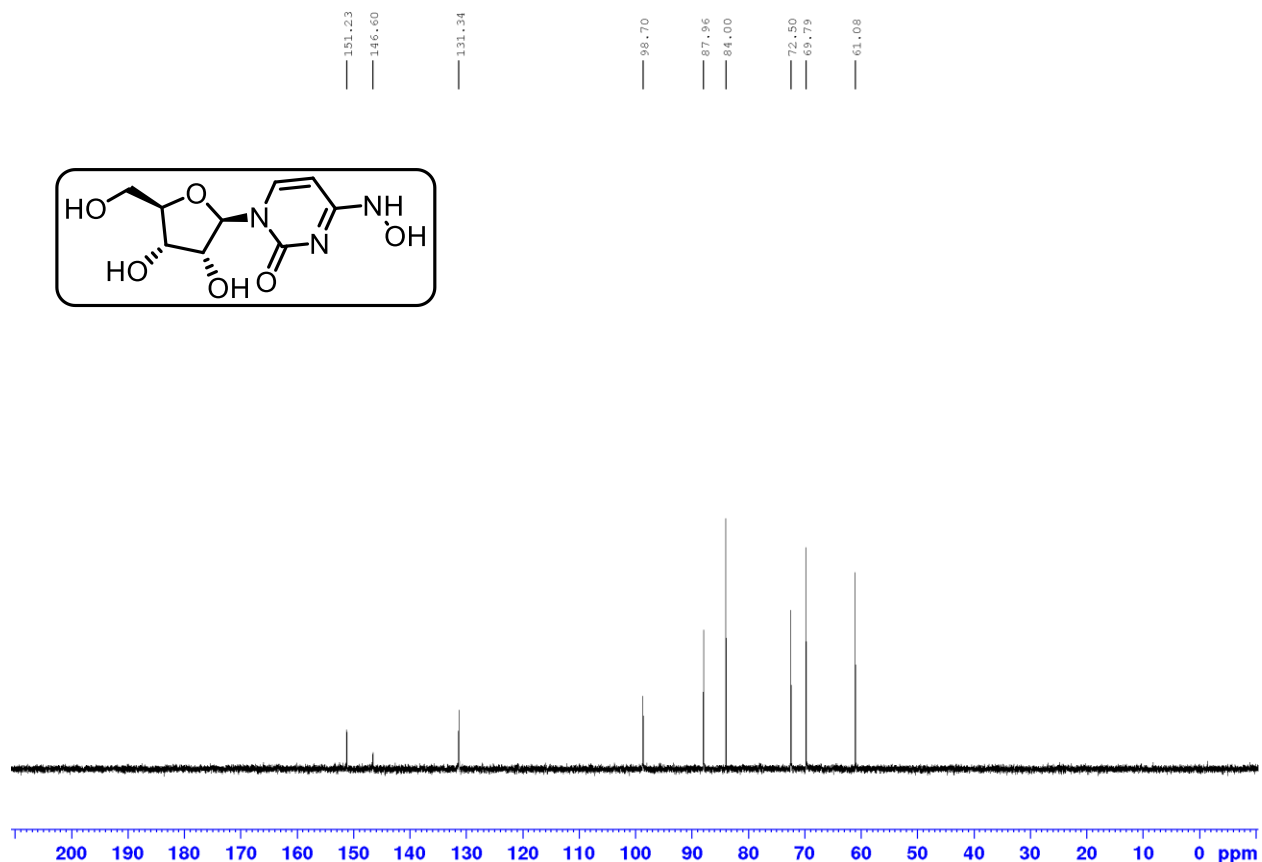
A mixture of cytidine **2** (10.0 g, 41.12 mmol, 1.0 eq) and $\text{NH}_2\text{OH}\cdot\text{AcOH}$ (11.5 g, 123.35 mmol, 3.0 eq) in H_2O (205 mL) was stirred for 48h at 40 °C. Reaction was monitored by HPLC and after completion of reaction, water was evaporated in rotavapor to give thick syrup, which was then suspended in 45 mL of water and placed in refrigerator for crystallization for 24h. The solid thus crystallized are filtered, washed with cold H_2O (~ 5.0 mL), dried under vacuum to yield the desired *N*-hydroxycytidine **3** as white solid (5.30 g, 98% pure, 50% yield).

^1H NMR (600 MHz, D_2O): δ 3.64 (dd, $J = 12.56, 4.49$ Hz, 1H), 3.75 (dd, $J = 12.65, 2.93$ Hz, 1H), 3.97 (q, $J = 3.85$ Hz, 1H), 4.11 (t, $J = 4.95$ Hz, 1H), 4.21 (t, $J = 5.59$ Hz, 1H), 5.66 (d, $J = 8.25$ Hz, 1H), 5.78 (d, $J = 5.69$ Hz, 1H), 6.98 (d, $J = 8.25$ Hz, 1H); **^{13}C NMR (151 MHz, D_2O):** δ 61.0, 69.7, 72.5, 84.0, 87.9, 98.7, 131.3, 146.6, 151.2 ppm. **MS:** 260 ($\text{M}+\text{H}$)⁺; **Purity:** 98% (assessed by HPLC).

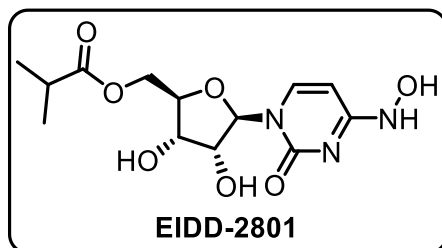
¹H NMR Spectra for compound 3 (600 MHz, DMSO-*d*₆):



¹³C NMR Spectra for compound 3 (151 MHz, DMSO-*d*₆):



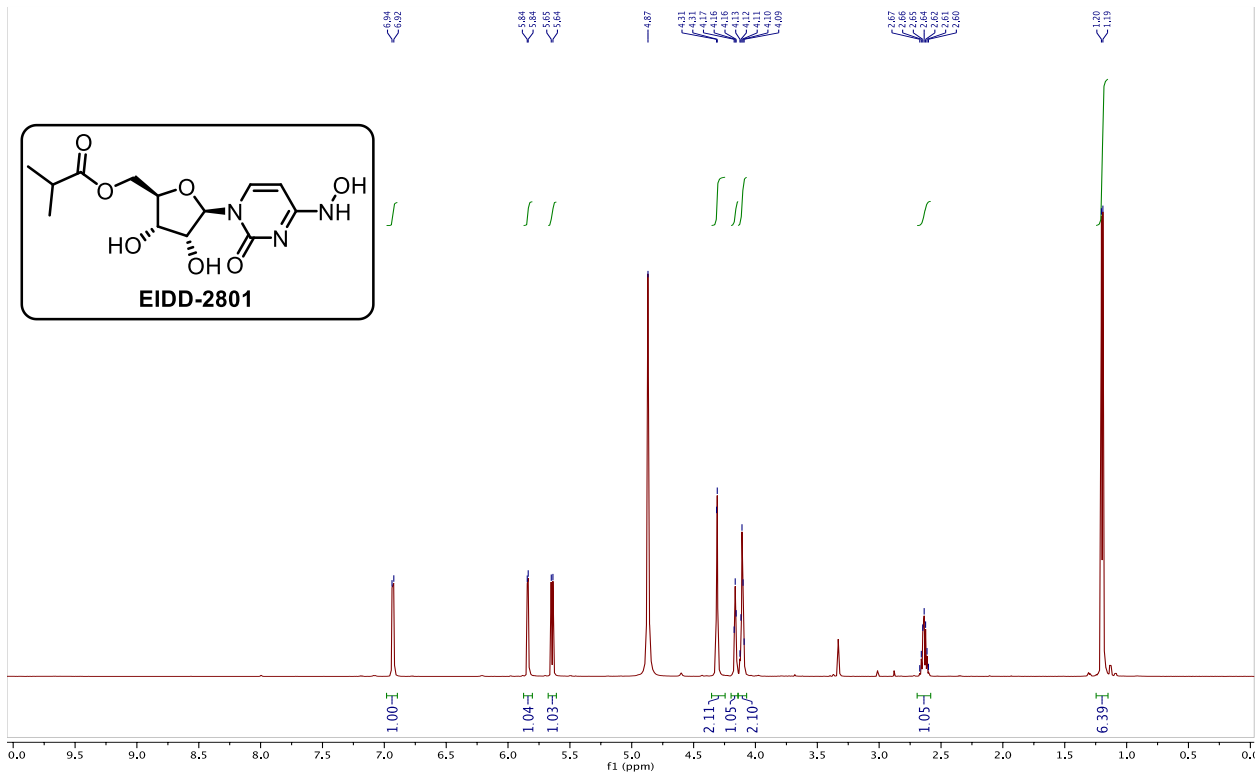
Enzymatic acylation of *N*-hydroxycytidine 3:



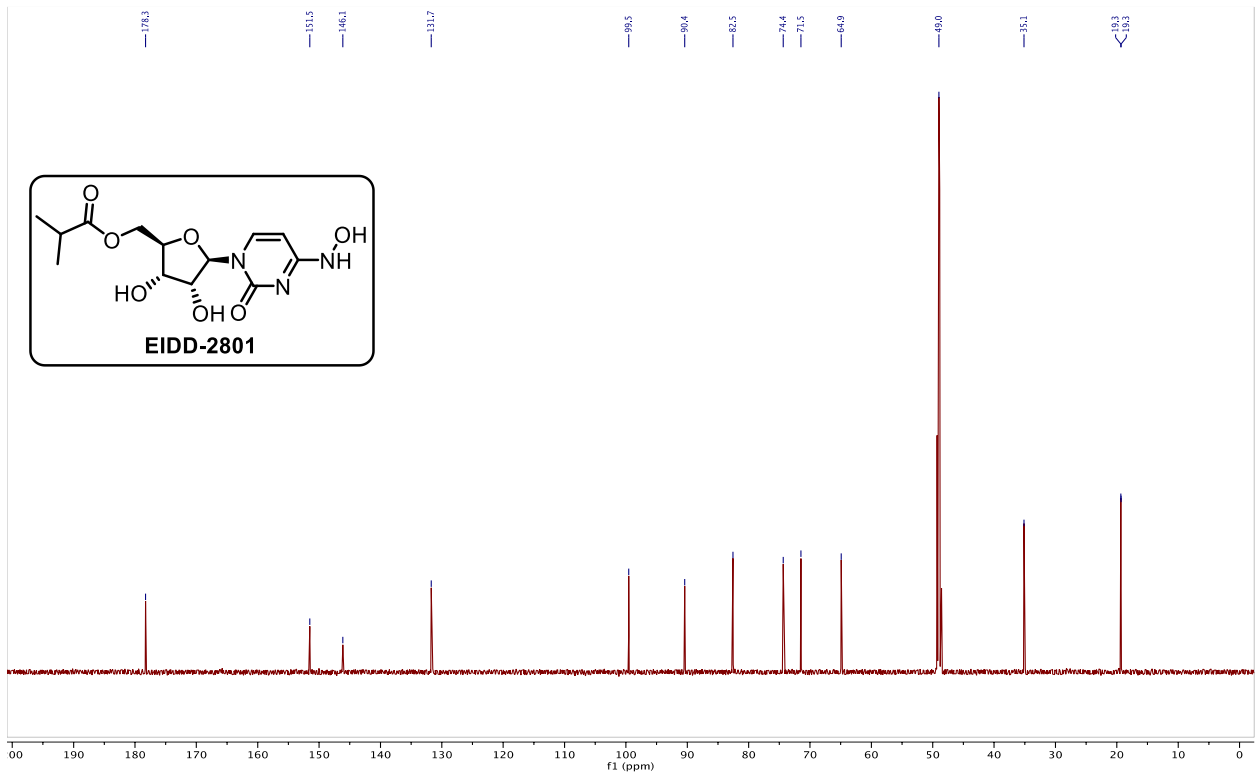
To a 100 mL three-neck round-bottom flask was added *N*-hydroxycytidine **3** (1.00 g, 3.86 mmol, 1.0 eq), oxime ester (1.66 g, 11.57 mmol, 3.0 eq) and 1,4-dioxane (50 mL). Then, Lipase acrylic resin $\geq 5,000$ U/g, recombinant, expressed in *Aspergillus niger* (synonym: Novozyme 435, purchased from Sigma Aldrich, 2.00 g) was added and reaction mass was stirred at 50 RPM at 40 °C. After 2h, Celite (2.00 g) was added and the mixture was filtered. The filter cake was washed with 1,4-dioxane (10 mL). The filtrate was concentrated and purified by column chromatography (Gradient, 0% to 20% MeOH in dichloromethane) giving **EIDD-2801 (1)** as a white solid (0.940 g, 98% pure, 74% yield).

^1H NMR (600 MHz, MeOH- d_4): δ 6.93 (d, J = 8.2 Hz, 1H), 5.84 (d, J = 4.8 Hz, 1H), 5.64 (d, J = 8.2 Hz, 1H), 4.31 (d, J = 3.6 Hz, 2H), 4.16 (t, J = 4.9 Hz, 1H), 4.11 (p, J = 4.9 Hz, 2H), 2.64 (p, J = 7.0 Hz, 1H), 1.20 (d, J = 7.0 Hz, 6H); **^{13}C NMR (151 MHz, MeOH- d_4):** δ 178.3, 151.5, 146.1, 131.7, 99.6, 90.4, 82.6, 74.4, 71.5, 64.9, 35.1, 19.4, 19.3 ppm; **HRMS (ESI):** calcd. for $\text{C}_{13}\text{H}_{19}\text{N}_3\text{O}_7$ 329.1223, found 329.1235; **Purity:** 98% (assessed by HPLC).

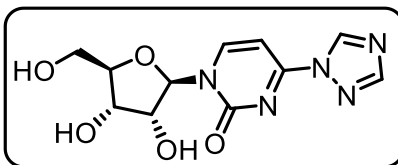
¹H NMR Spectra for EIDD-2801 (600 MHz, MeOH-d₄):



¹³C NMR Spectra for EIDD-2801 (151 MHz, MeOH-d₄):



Synthesis of Triazole 6 from Uridine:



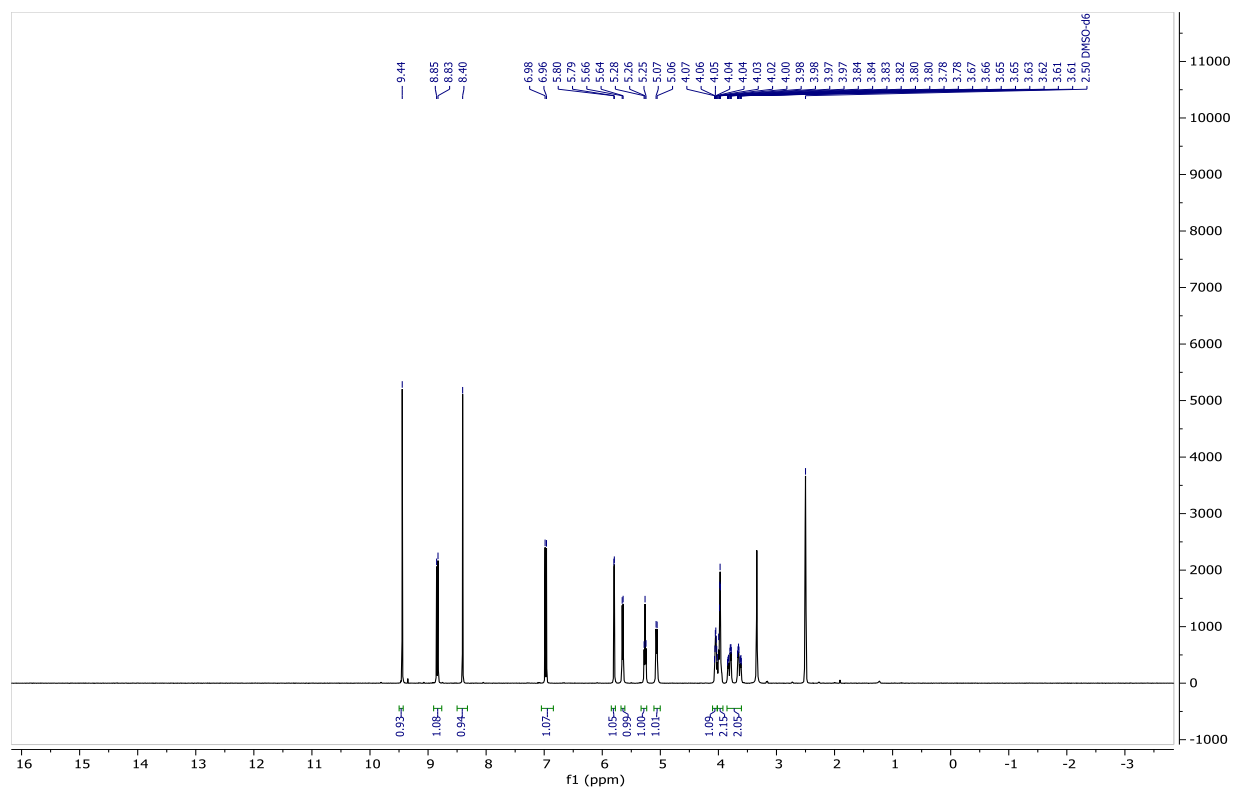
The compound was synthesized after a modified procedure by *Reese et al.*² To a stirred suspension of uridine (2.460 g, 10.00 mmol, 1.0 eq) in acetonitrile (100 mL) triethylamine (21 mL, 150.60 mmol, 15.0 eq) and TMSCl (6.4 mL, 50.40 mmol, 5.0 eq) were added at 0 °C. The cooling bath was removed, and the suspension was stirred for 1 h at room temperature. After that, the suspension was again cooled to 0 °C, POCl₃ (2.9 mL, 20.00 mmol, 2.0 eq) was added dropwise, the reaction mixture was stirred for 15 min and 1,2,4-triazole (6.90 g, 100.00 mmol, 10.0 eq) was added. The reaction was stirred for 14 h at rt and then poured into triethylammonium phosphate buffer (0.5 M, pH 7.0, 300 mL). The aqueous phase was extracted three times with DCM (each 50 mL), the combined organic layers were dried over sodium sulfate and the volatiles removed in vacuo. The residue was dissolved in MeOH:AcOH (4:1) and overnight, a slightly orange precipitate was formed. The precipitate was washed with cold methanol:EtOAc (9:1) and with diethyl ether and was dried in vacuo to provide triazole **6** as a colorless crystals (2.53 g, 86% yield).

R_f = 0.42 (C₁₈-silica; MeCN:H₂O, 1:20); **Melting point**: 195.2–187.8 °C (MeOH); **¹H NMR (300 MHz, DMSO-*d*₆)**: δ 9.44 (s, 1H, 5H), 8.84 (d, *J* = 7.2 Hz, 1H), 8.40 (s, 1H, 3H), 6.97 (d, *J* = 7.2 Hz, 1H), 5.80 (d, *J* = 2.2 Hz, 1H), 5.65 (d, *J* = 4.8 Hz, 1H), 5.26 (t, *J* = 5.0 Hz, 1H), 5.06 (d, *J* = 5.4 Hz, 1H), 4.09–4.01 (m, 1H), 4.01–3.93 (m, 2H), 3.81 (ddd, *J* = 12.2, 5.0, 2.0 Hz, 1H), 3.64 (ddd, *J* = 12.2, 5.0, 2.0 Hz, 1H); **¹³C NMR, (75 MHz, DMSO-*d*₆)**: δ 158.6, 154.1, 153.8, 148.4, 143.7, 93.7, 91.2, 84.2, 74.6, 68.1, 59.4 ppm; **IR (ATR)**: $\bar{\nu}$ [cm⁻¹] = 3321, 3125, 2951, 2920, 1656, 1516, 1546, 1100, 928, 788. **MS (ESI)**: *m/z* (%) = 296.1 (100) [M+H]⁺.

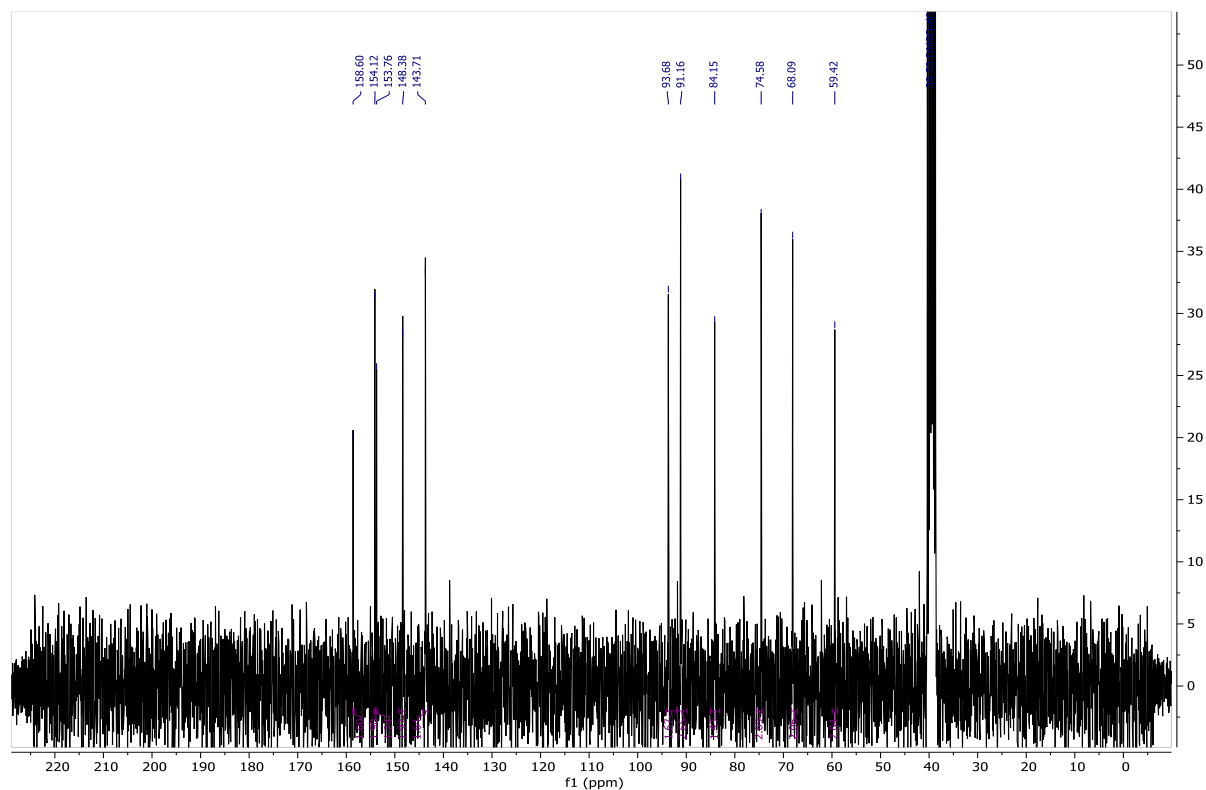
The analytical data are consistent with those reported in the literature.²

² Miah, A.; Reese, C. B.; Song, Q. Convenient Intermediates for the Preparation of C-4 Modified Derivatives of Pyrimidine Nucleosides. *Nucleosides Nucleotides Nucleic Acids* 1997, 16 (1–2), 53–65.

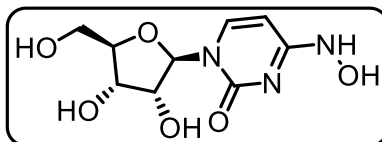
¹H NMR Spectrum for Compound 6 (300 MHz, DMSO-d₆):



¹³C NMR Spectrum for Compound 6 (75 MHz, DMSO-d₆):



Transamination of Triazole 6:

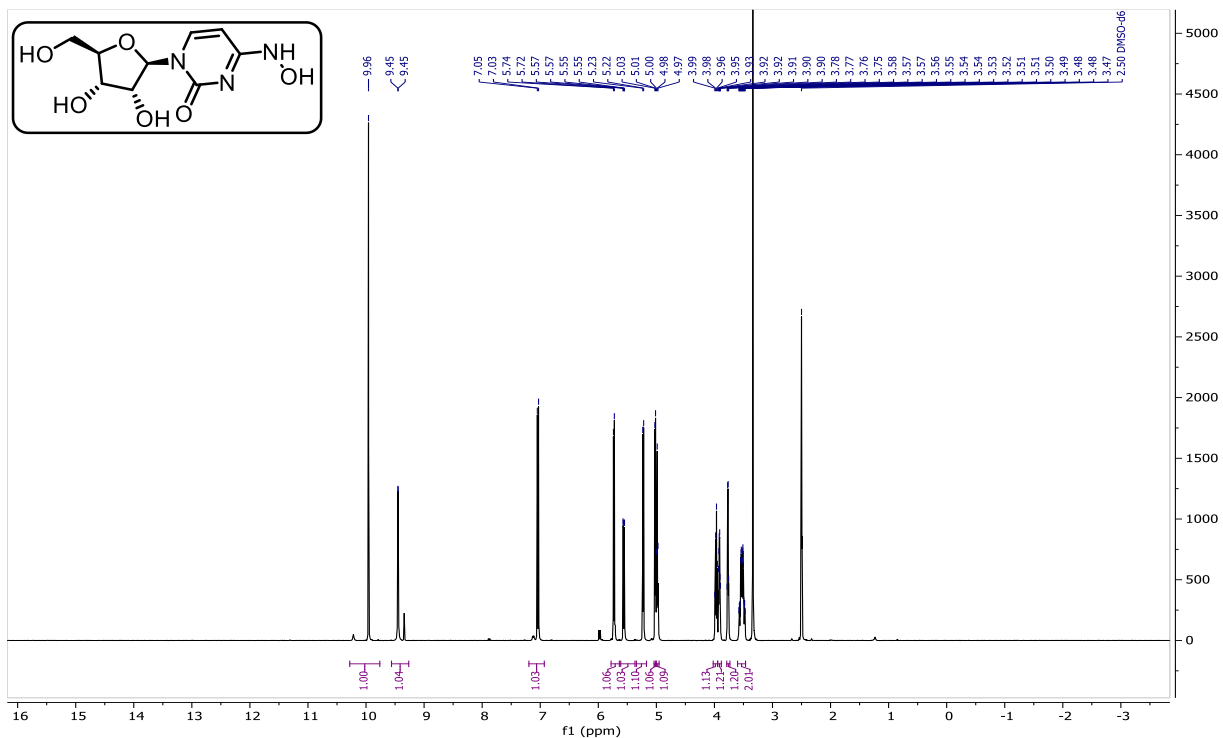


To a stirred suspension of triazole (**6**) (1.0 g, 3.39 mmol, 1.0 eq) in ethanol, hydroxylamine hydrochloride (329 mg, 4.75 mmol, 1.4 eq) and sodium acetate (361 mg, 4.41 mmol, 1.3 eq) were added. The suspension was heated to 65 °C for 14 h. The volatiles were removed in vacuo and the resulting residue was recrystallized from water at 0 °C. The resulting colorless crystals were dried in vacuo overnight to give triol **3** as colorless crystals (664 mg, 76% yield).

R_f = 0.78 (C_{18} -Silica; MeCN:H₂O, 1:20); **Melting point**: 173.6–175.2 °C; **¹H NMR (300 MHz, DMSO-*d*₆)**: δ 9.96 (s, 1H), 9.46 (d, J = 2.0 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 5.73 (d, J = 6.2 Hz, 1H), 5.56 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 5.23 (d, J = 6.0 Hz, 1H), 5.03 (d, J = 4.6 Hz, 1H), 4.99 (t, J = 5.3 Hz, 1H), 3.97 (app q, J = 5.9 Hz, 1H), 3.93–3.89 (m, 1H), 3.77 (app q, J = 3.4 Hz, 1H), 3.61–3.45 (m, 2H); **¹³C NMR, (75 MHz, DMSO-*d*₆)**: δ 149.7, 143.5, 130.2, 98.5, 86.8, 84.6, 72.5, 70.4, 61.4 ppm; **IR (ATR)**: $\bar{\nu}$ [cm⁻¹] = 3524, 3305, 3123, 2948, 2902, 1666, 1606, 1622, 1262, 1003, 778. **MS (ESI)**: m/z (%) = 260.0 (100) [M+H]⁺. The analytical data are consistent with those reported in the literature.³

³ Painter, G. R.; Bluemling, G. R.; Natchus, M. G.; Guthrie, D. N4-Hydroxycytidine and Derivatives and Anti-Viral Uses Related Thereto. WO2019113462A1, June 13, 2019

¹H NMR Spectrum for Compound 3 (300 MHz, DMSO-*d*₆):



¹³C NMR Spectrum for Compound 3 (75 MHz, DMSO-*d*₆):

