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A High-Yielding Synthesis of EIDD-2801 from Uridine

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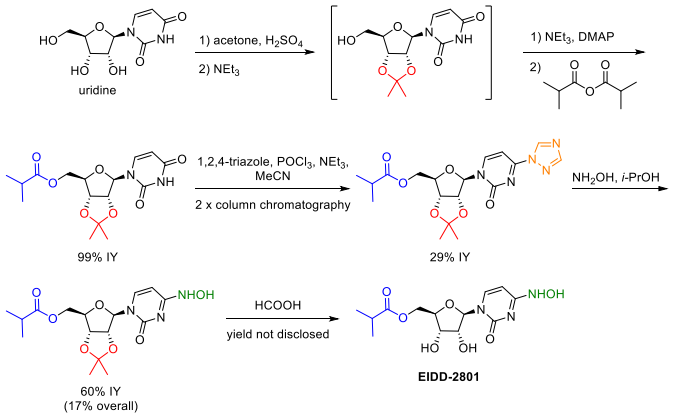
Supporting information for this article is available online.

Abstract: A simple reordering of the reaction sequence allowed the improved synthesis of EIDD-2801, an antiviral with promising activity against the SARS-CoV-2 virus, starting from uridine. Compared to the original route, the yield was enhanced from 17% to 61%, and fewer isolation/purification steps were needed. In addition, a continuous flow procedure for the final acetonide deprotection was developed, which proved to be favorable toward selectivity and reproducibility.

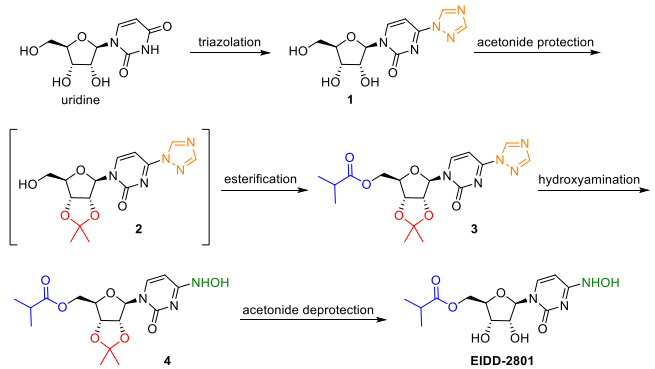
The current medical crisis caused by the SARS-CoV-2 virus, has impacted people's lives and economy worldwide, with presently more than 35 million confirmed cases.[1] In view of the severity of the COVID-19 pandemic, there is a continuous quest for new drugs for the treatment of this disease. EIDD-2801, a NHC (β-D-N^4-hydroxycytidine) prodrug, has shown potent activity against multiple CoVs in animal studies[2] and is currently in Phase II clinical trials on symptomatic patients with COVID-19.[3] This antiviral candidate is structurally similar to remdesivir, but blocks RNA polymerase differently, which possibly makes them complimentary.[4] Since EIDD-2801 can be taken orally in the form of a pill, the potential treatment against COVID-19 would be made more accessible compared to remdesivir that currently has to be administered intravenously.

EIDD-2801 was developed by Emory University researchers, and the disclosed route consists of a five-step synthesis starting from uridine (Scheme 1).[5] This was the only reported synthesis in the open literature until recently, when routes toward EIDD-2801 were reported that replaced uridine for cytidine.[6,7] In the original route, first an acetonide protection followed by selective esterification of the 5'-hydroxy group is performed in a one-pot fashion. Then, the molecule is activated by introduction of the 1,2,4-triazole moiety which is further displaced by hydroxylamine. After removal of the acetonide protecting group, EIDD-2801 is obtained via a complex crystallization procedure. As no isolated yield for the final deprotection step has been disclosed in the patent, an overall yield (17%) can only be stated for the synthesis of protected EIDD-2801. The main disadvantage of the reported route is the low yield (29%) in the triazole coupling step.



Scheme 1. Patented route toward EIDD-2801.

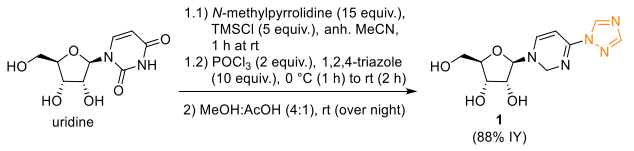
In 1997, Reese and co-workers reported the triazolation of uridine to proceed in 89% yield after a simple extractive work-up and crystallization.[8] Therefore, we anticipated, that a simple reordering of the steps in the original synthetic sequence would



Scheme 2. Proposed route toward EIDD-2801: reordering of steps for improved overall yield and isolation.

result in an overall higher yield and improved isolation procedures. Further, by starting from uridine the impurity profile should closely match that of the commercial process which might accelerate uptake of the modified process by minimizing regulatory changes. As illustrated in Scheme 2, we planned our synthesis toward EIDD-2801 starting with the triazolation according to Reese and co-workers. The other transformations (acetonide protection, esterification, hydroxyamination) should ideally proceed in a similar approach as in the patented route. Acetonide deprotection from **4** as final step and isolation of EIDD-2801 is envisaged as reported in the original patent.[5]

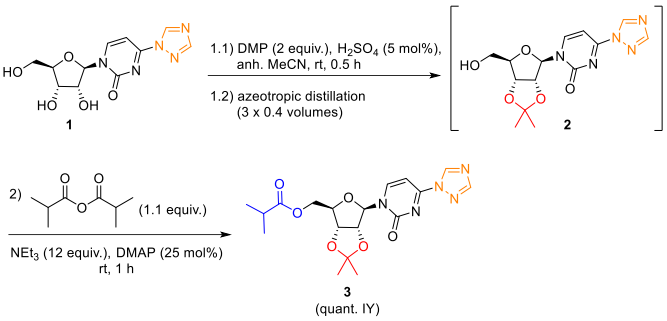
Following the original procedure of Reese and co-workers which involves a one-pot TMS protection of uridine and phosphorus oxychloride-promoted triazole coupling using Et_3N as base,[8] **1** was obtained in 74% yield. When changing the base to *N*-methylpyrrolidine the yield of **1** increased to 88%. Product isolation consisted of a simple filtration/washing step, as **1** precipitated during deprotection from the MeOH/AcOH mixture. Additionally, we attempted to reduce the amount of 1,2,4-triazole (10 equiv.), because of the associated health risks.[9] Unfortunately, less triazole also leads to lower isolated yields of **1** (8 equiv.: 78%, 6 equiv.: 58%). Compared to the original route,[5] we were able to increase the yield of the triazolated compound from 29% to 88% by introducing the triazole moiety as first step. Importantly, also column chromatographic work-up could be avoided.



Scheme 3. Step 1: Triazolation of uridine.

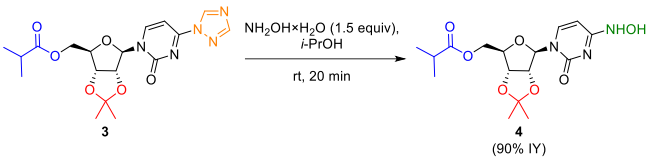
To selectively introduce the isobutyl ester at the 5'-hydroxy position, the other two hydroxy groups need to be protected first. When using the conditions for the acetonide protection of uridine described in the original patent (5 mol% H_2SO_4 in acetone),[5] no conversion of **1** to the desired acetonide **2** was observed. Higher concentrations (0.5 equiv.) of H_2SO_4 lead to hydrolysis of the triazole moiety. Addition of 2 equiv. of 2,2-dimethoxypropane (DMP) did not significantly improve the reaction. However, when exchanging acetone for MeCN as solvent in combination with the use of DMP, a 73% conversion to **2** was achieved. Further optimization revealed, that the amount of H_2SO_4 could be reduced to 5 mol% (Scheme 4).

Upon attempting the acetonide formation and esterification in a one-pot fashion similar to the patented route (see Scheme 1), we observed that 3 equiv. of isobutyric anhydride were needed in order to reach full conversion of **2**. The excess of anhydride was necessary because it was quenched by the 2 equiv. of MeOH that were released from DMP. Therefore, after stirring of **1** with DMP and H_2SO_4 in MeCN for 30 min at room temperature, a stepwise azeotropic distillation was performed to remove MeOH. Applying this protocol resulted in only 1.1 equiv. of isobutyric anhydride being necessary for full conversion of **2** (Scheme 4). A single distillation step of 1.2 volumes proved to be less efficient, as 1.3 equiv. of anhydride were required. Acetonide ester **3** was obtained in quantitative yield and ≥99% HPLC purity after extractive work-up in this one-pot procedure.



Scheme 4. Steps 2 and 3: One-pot acetonide protection and esterification. See the Supporting Information for more details.

Acetonide ester **3** can be readily converted to hydroxylamine **4**, by stirring in *i*-PrOH at room temperature with 1.5 equiv. of hydroxylamine (50 wt% in H_2O) for 20 min (Scheme 5). After extractive work-up, **4** was isolated in 90% yield and 91% purity (HPLC area% at 260 nm). The purity could be improved to 99% by washing with cold diethyl ether, but ca 50% of product was lost in this washing step. Therefore, the washing step was omitted, and the crude product after extraction was used for the acetonide deprotection step.



Scheme 5. Step 4: Hydroxylamine formation.

Removal of the acetonide protecting group of **4** leading to EIDD-2801 has been described to proceed in formic acid at room temperature overnight (see Scheme 1).[5] However, by performing the reaction exactly under the reported conditions only 47 area% of EIDD-2801 were observed (HPLC at 260 nm). Heating to 60 °C for 5 h or 100 °C for 30 min resulted in the increased formation of side products. An acid screening (see Table S2) revealed that H_2SO_4 in combination with *i*-PrOH as solvent furnished the highest conversion (80%) to EIDD-2801 after heating at 60 °C for 30 min. The main side reaction was found to be the ester hydrolysis yielding *N*-hydroxycytidine **5** (EIDD-1931, see also Figure 1), in particular when the reaction was conducted at higher temperatures.

Since the same solvent system (*i*-PrOH) was employed in the hydroxyamination and acetonide deprotection, we envisioned that these two steps could be combined to a one-pot procedure. A similar process has been recently demonstrated in an alternative route toward EIDD-2801.[7] During batch optimization studies (see Table S3) we experienced reproducibility issues that were mainly related to the exothermic nature upon H_2SO_4 addition in the deprotection step. Therefore, we decided to transfer the batch process to continuous flow, where better mixing and temperature control allows for superior control of exotherms, and in return would provide reproducible results.[10–12]

A simple flow set-up was therefore established using a heated reaction coil together with individual streams for introducing solutions of the substrate and the acid reagent, which were combined in a Y-shaped mixer (Figure 1A). Triazole **3** was converted to hydroxylamine **4** in batch under the same conditions as shown in Scheme 5. However, **4** was not isolated, but after 15

min of stirring, the reaction mixture was introduced directly into the flow reactor via a sample loop for the acetonide deprotection. The flow process was investigated in various solvents using HCOOH, H_2SO_4, CF_3COOH or TfOH as acid component (Tables S4–S7). During the deprotection optimization studies, ester hydrolysis and hydroxyl amine–hydroxyl exchange occurred as most prominent side reactions yielding compounds **5** and **6** (Figure 1B, see also the Supporting Information). The effects of reaction temperature, residence time and excess of acid were therefore carefully examined with the aim to minimize side product formation while simultaneously maximize conversion to EIDD-2801 (see Tables S4–S7 for details). Similarly as in batch, H_2SO_4 proved to be superior to the other tested acids (see Table S5). Because of precipitation issues in *i*-PrOH, MeOH was selected as solvent. The highest conversion to EIDD-2801 (79%) was obtained at only 5 min of residence time using 2.75 equiv. of H_2SO_4 at 100 °C, while **5** (11%) and **6** (8%) were at a minimum. Importantly, the flow process under optimum conditions not only permitted higher yields, but it also enabled a significant chemical intensification and a proper reproducibility as compared to our initial batch attempts. By employing these flow conditions for scale-out (16 min), pure EIDD-2801 was obtained in 69% isolated yield (307 mg) after chromatographic purification.

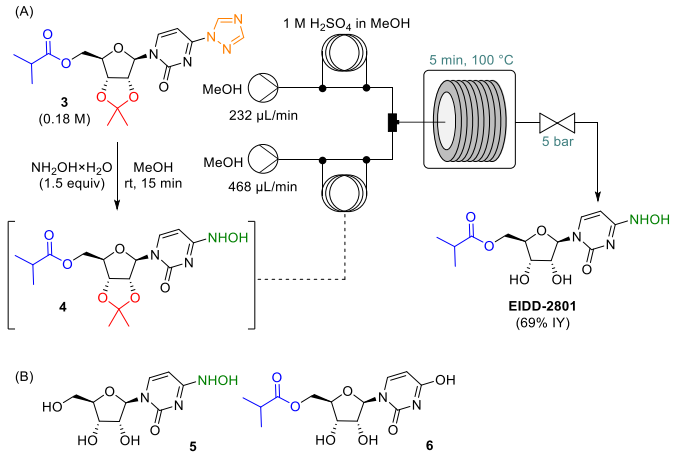
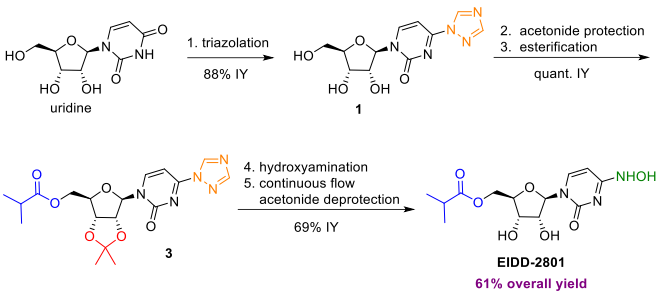


Figure 1. Steps 4 and 5: Telescoped batch hydroxyamination and continuous flow acetonide deprotection. (A) Schematic representation of the reaction set-up. (B) Structures of identified side products.

In conclusion, an improved protocol was developed to access EIDD-2801 from uridine as starting material (Scheme 6). By strategic reordering of synthetic steps and by employing a continuous flow process for the final acetonide deprotection, the overall yield was improved from 17% to 61%. Importantly, this strategy presents fewer and significantly simplified product isolation procedures, as two telescoped procedures (acetonide protection/esterification and hydroxyamination/acetonide deprotection) are included within the 5-step route.



Scheme 6. Improved protocol for the synthesis of EIDD-2801.

Acknowledgements

We thank the Bill and Melinda Gates Foundation for their longstanding support of our research.

Keywords: EIDD-2801 • COVID-19 • continuous flow • acetonide deprotection • triazolation

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Supporting Information

A High-Yielding Synthesis of EIDD-2801 from Uridine

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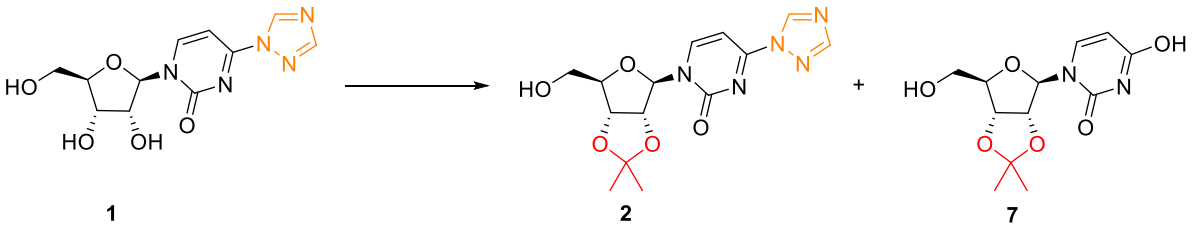
Contents

1. General Methods

All solvents and chemicals were obtained from standard commercial vendors (TCI, Sigma-Aldrich/Merck or VWR) and were used without any further purification, unless otherwise noted. **^{1}H NMR spectra** were recorded on a Bruker 300 MHz instrument. **^{13}C NMR spectra** were recorded on the same instrument at 75 MHz. Chemical shifts (δ) are expressed in ppm downfield from TMS as internal standard. The letters s, d, t, q, sept, dd and m are used to indicate singlet, doublet, triplet, quadruplet, septet, doublet of doublets and multiplet. **Analytical HPLC analysis** was carried out on a Shimadzu instrument using a C18 reversed-phase (RP) analytical column (150 mm × 4.6 mm, particle size 5 µm) at 37 °C using mobile phases A (H_2O/MeCN (90:10 v/v) + 0.1% TFA) and B (MeCN + 0.1% TFA) at a flow rate of 1.5 mL/min. The following gradient was applied: start at 3 % solvent B, increase to 5 % solvent B until 3 min, increase to 30 % solvent B until 7 min and finally increase to 100 % solvent B until 10 min. **LC-MS analysis** was carried out on a Shimadzu instrument using a C18 reversed-phase (RP) analytical column (150 mm × 4.6 mm, particle size 5 µm) using mobile phases A (H_2O/MeCN 90:10 (v/v) + 0.1% HCOOH) and B (MeCN + 0.1 % HCOOH) at a flow rate of 0.6 mL/min. The following gradient was applied: hold at 5% solvent B until 2 min, increase to 20% solvent B until 8 min, increase to 100% solvent B until 16 min and hold until 22 min at 100% solvent B. Low resolution mass spectra were obtained on a Shimadzu LCMS-QP2020 instrument using electrospray ionization (ESI) in positive or negative mode. **Melting points** were obtained on a Stuart melting point apparatus in open capillary tubes. **High-resolution mass spectrometry** was performed on an Agilent 6230 TOF mass spectrometer, after separation of the compounds with an Agilent 1260 Infinity Series HPLC-system. The injection volume was set to 0.5 µL and the flow rate to 0.3 mL/min of a mixture of 40% H_2O (0.1% 5M ammoniumformate) and 60% MeCN/H_2O (5:1 +0.1% 5 M ammoniumformate). The HRMS module comprises an electrospray ionization source (Dual AJS ESI) and uses nitrogen as the nebulizer (15 psig) and the drying gas (5 L/min). ESI experiments were performed using the positive ionization mode (Gas Temp. = 300 °C, Fragmentor = 150 V, Skimmer = 65 V, OCT 1 RF Vpp = 750 V, Vcap = 1400, Nozzle Voltage = 2000 V, Reference Masses = 121.050873 and 922.009798, Acquisition = 100-1,100 m/z, 1 spectra/s). Data was acquired with MassHunter Workstation Rev.B.05.01SP2. **Microwave irradiation** experiments were carried out in a Monowave 400 single-mode microwave reactor from Anton Paar or an Initiator+ single-mode microwave reactor from Biotage, respectively, using 10 mL Pyrex vials. The reaction temperature was controlled by an external infrared sensor. Reaction times refer to hold times at the temperature indicated. **Column chromatography** was carried out using a Biotage Isolera automated flash chromatography system.

2. Optimization Studies: One-pot Acetonide Protection and Esterification

Table S1. Optimization of Acetonide Formation.



Entry	Solvent	Reagent[a]	Time [h]	**1** [%][b]	**2** [%][b]	**7** [%][b]
1	acetone	H_2SO_4 (0.5)	24	1	3	96
2	acetone	H_2SO_4 (0.2) DMP (2)	4	78	12	10
3	MeCN	H_2SO_4 (0.2) DMP (2)	1	23	73	4

[a] Equivalents given in parenthesis.
[b] Area% determined by HPLC at 260 nm.

The hydrolysis product **7** was identified by LC-MS analysis (see Figure S1). The generation of **7** was additionally verified, since upon reaction with isobutyric anhydride, the corresponding ester **6** was obtained. The identity of **8** was confirmed by NMR analysis (see Figures S2 and S3).

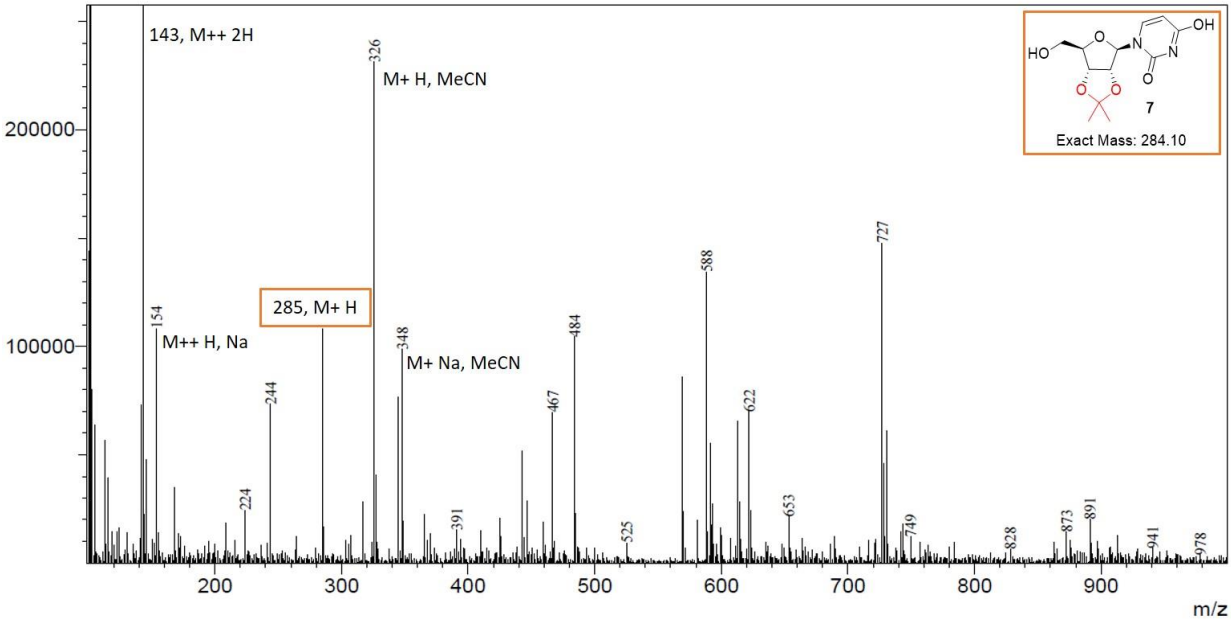


Figure S1. LC-MS of hydrolysis product **7.**

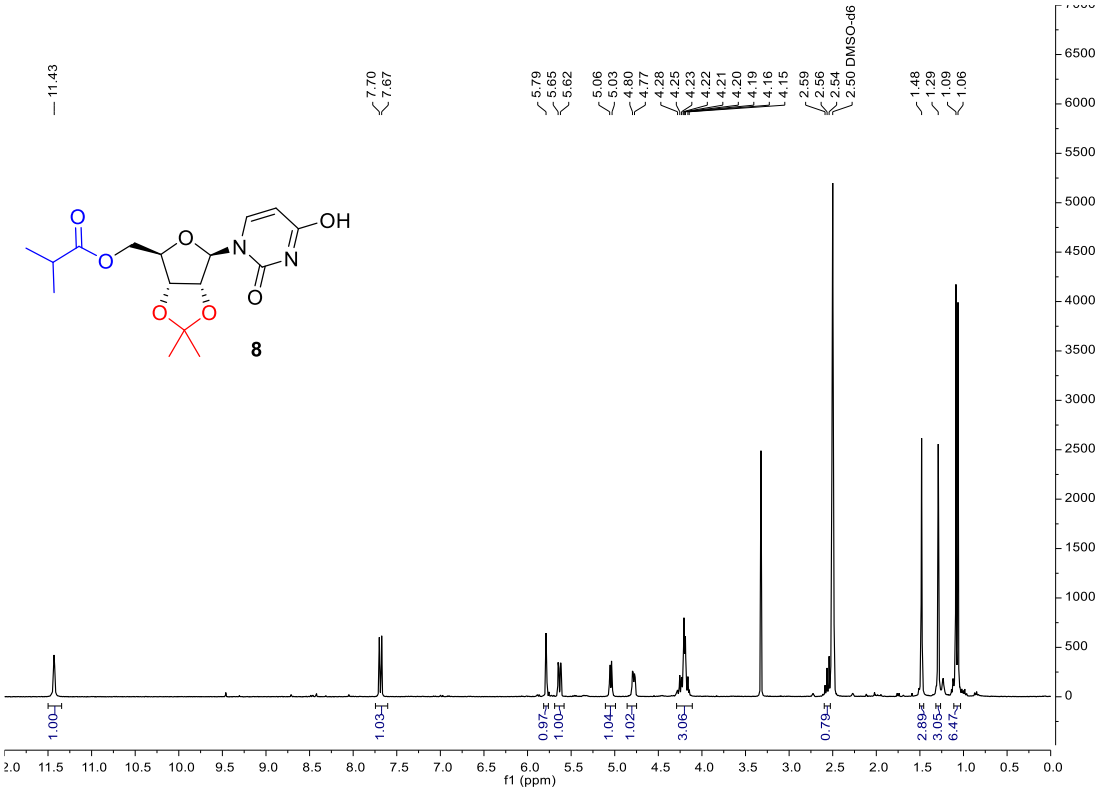


Figure S2. ^{1}H NMR (300 MHz, DMSO-d_6) of product **8.**

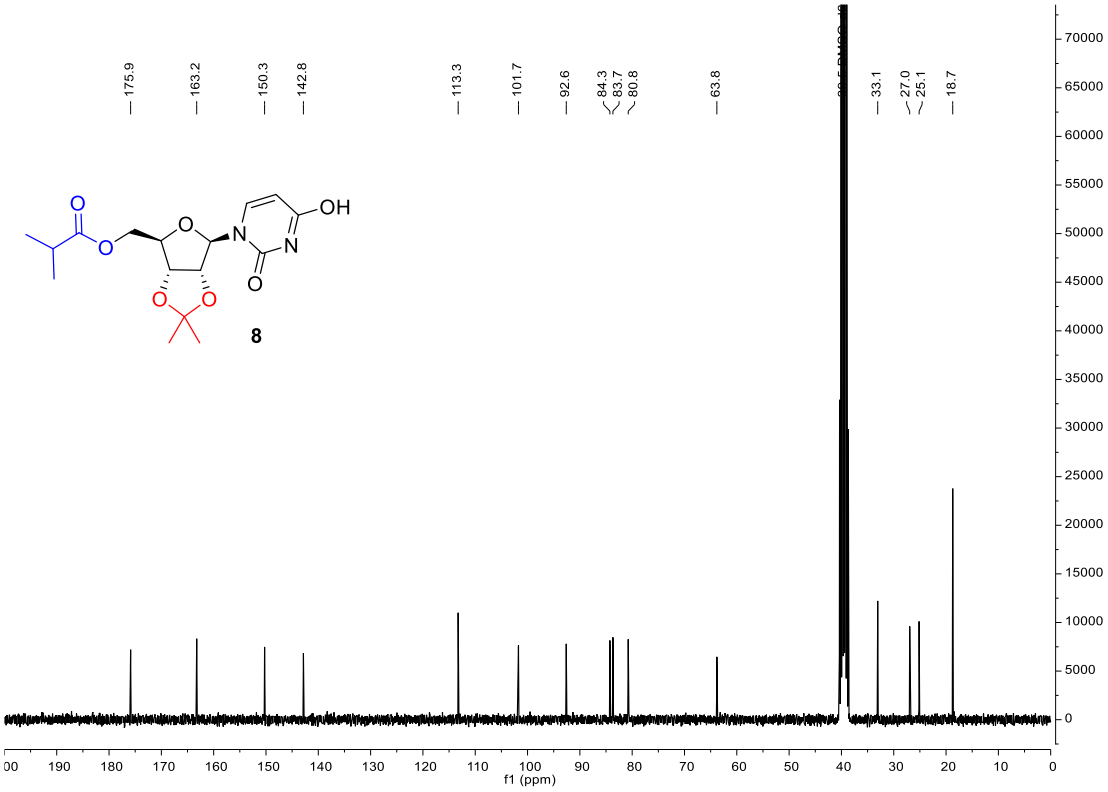
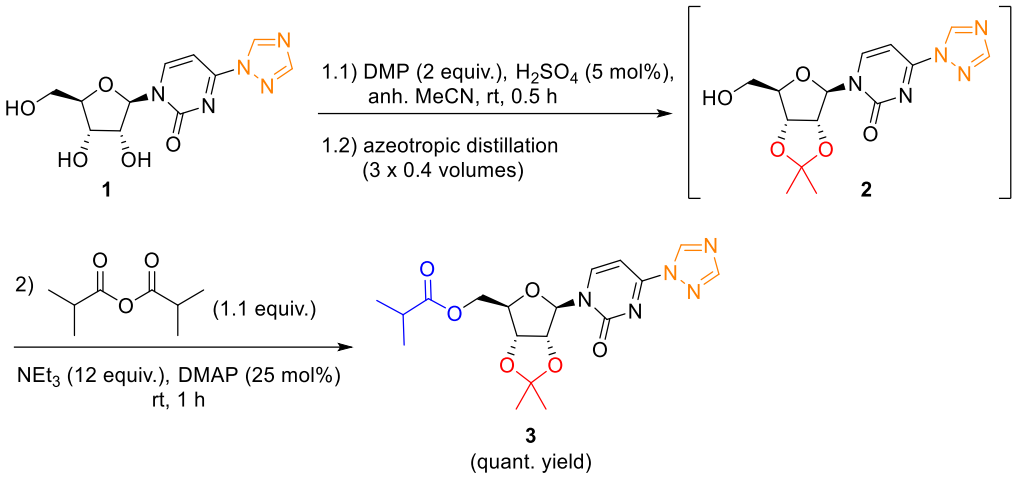


Figure S3. ^{13}C NMR (75 MHz, DMSO-d_6) of product **8.**



Scheme S1. One-pot acetonide protection and esterification.

In the protection step, the reaction mixture was stirred first for 30 min at rt, then the stepwise azeotropic distillation was performed. We chose this reaction regime out of precautionary reasons, because the bp of DMP (85 °C) is close to the bp of the azeotrope MeOH/MeCN (63 °C). Therefore, in order not to potentially distill DMP during the azeotropic distillation, initially stirring at rt was preferred. A 73% conversion to **2** was achieved after 30 min, and full conversion was accomplished during the azeotropic distillation (Figure S4). Reaction monitoring of the esterification step could be done visually, as with full conversion of **2**→**3** the suspension became a clear solution. The reaction time was dependent on the scale: On a 600 mg scale the reaction went to completion within 40 min (Figure S4), while on a 5 g scale the reaction time needed to be prolonged to 1 h (Scheme S1). In general, the reaction sequence proved to be remarkably clean, as no side products were detected. After extractive work-up, **3** was isolated in quantitative yield and a purity of ≥99%.

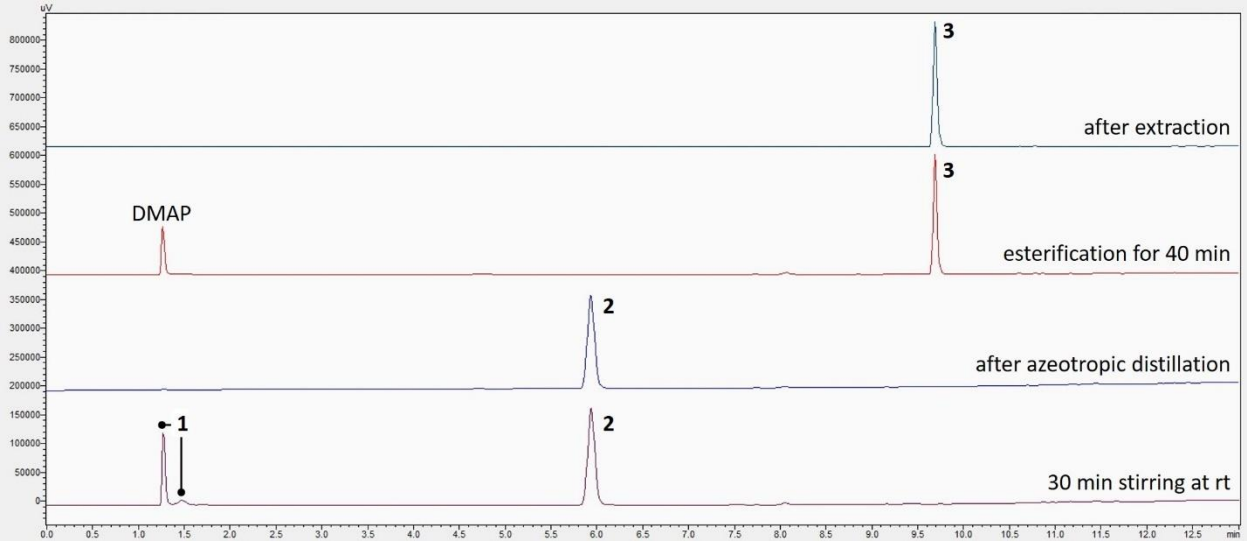
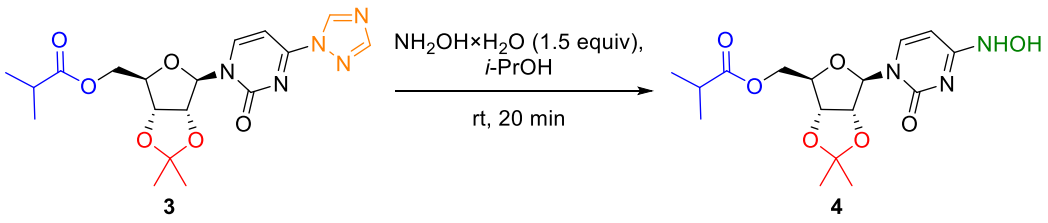


Figure S4. HPLC (260 nm) reaction monitoring of the one-pot acetonide protection and esterification on a 600 mg scale.

3. Hydroxylamine Formation



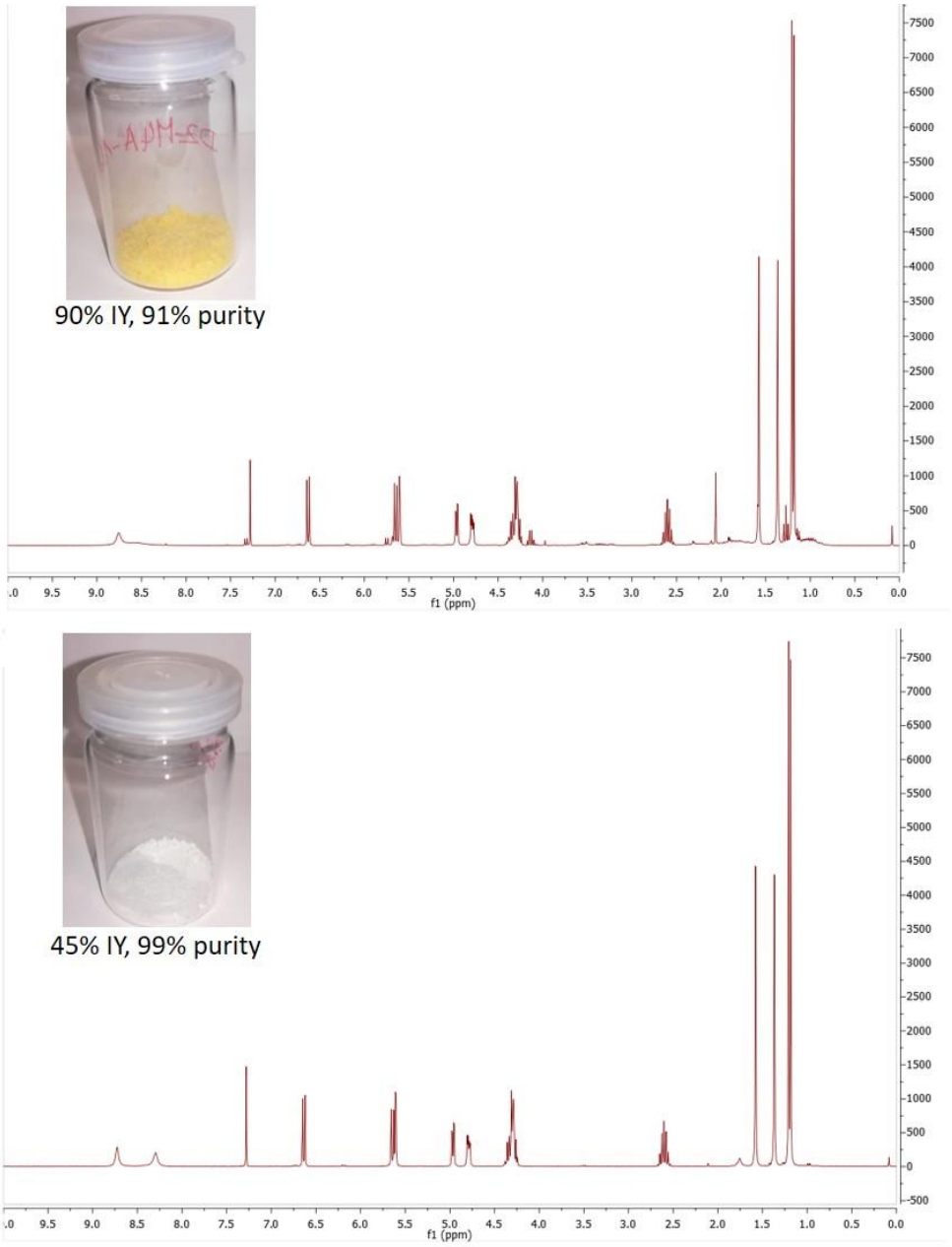
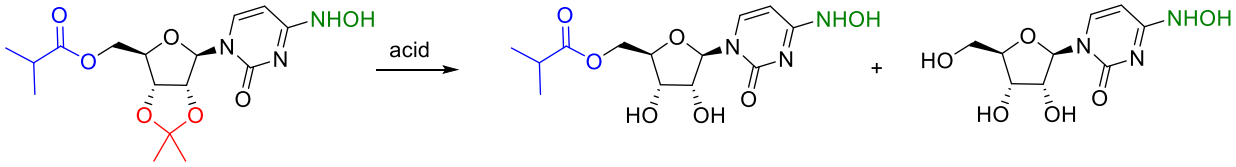


Figure S5. ^{1}H NMR comparison of isolated product **4** before (top) and after (bottom) the washing step with Et_2O.

4. Optimization Studies: Acetonide Deprotection in Batch

General procedure for the acid screening: A 1 mL HPLC vial was charged with **4** (50 mg, 0.135 mmol) and 1 mL of acid. The vial was crimped and stirred at rt or heated in an aluminum heating block at 60 °C.

Table S2. Acid Screening for the Acetonide Deprotection of **4**.



4 **EIDD-2801** **5**

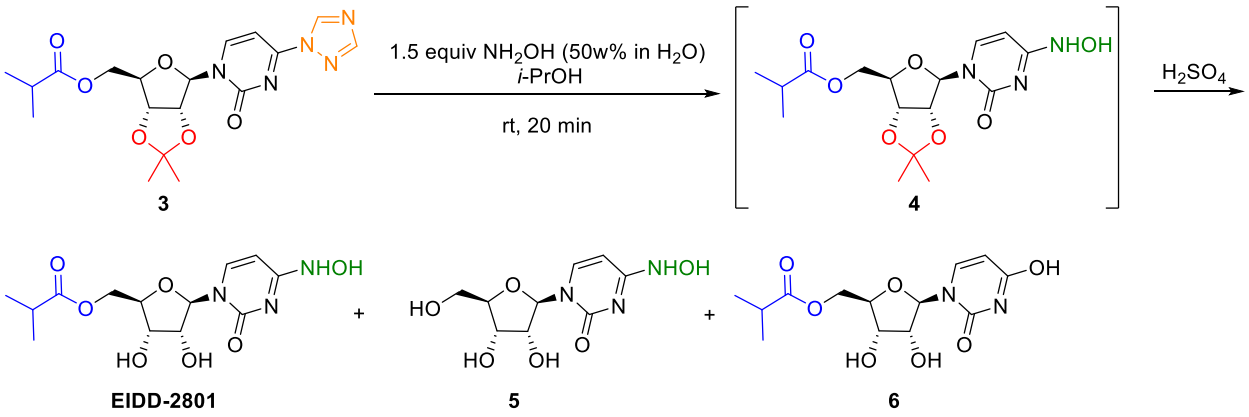
Entry	Acid	T [°C]	Time [h]	**4** [%][b]	**EIDD-2801** [%][b]	**5** [%][b]
1	HCOOH	rt	22	40	**47**	-
2	HCOOH	60	5	6	**33**	-
3	H_2SO_4 (*i*-PrOH)[a]	rt	7	28	**62**	5
4	H_2SO_4 (*i*-PrOH)[a]	60	0.5	10	**80**	8
5	H_2SO_4 (*i*-PrOH)[a]	60	1	8	**74**	15
6	H_2SO_4 (H_2O)[a]	rt	1	40	**52**	4
7	H_2SO_4 (H_2O)[a]	60	0.5	-	**43**	56
8	HCl_{conc} (*i*-PrOH)[a]	rt	7	55	**32**	6
9	HCl_{conc} (*i*-PrOH)[a]	60	0.5	-	**36**	64
10	HCl conc	rt	1	33	**59**	5

[a] Acids were 1 M in the respective solvent.
[b] Area% determined by HPLC at 260 nm. Except for entry 9, further unidentified impurities were detected.

General procedure for the one-pot hydroxyamination and acetonide deprotection: A microwave vial was charged with **3** (50 mg, 0.123 mmol). *i*-PrOH (617 µL) and hydroxylamine (50w% in water, 11.1 µL, 1.5 equiv.) were added and the reaction mixture was stirred at room temperature for 20 minutes to ensure full conversion to **4**. Next, conc H_2SO_4 was added dropwise under stirring, the microwave vial crimped and subjected to microwave heating.

As can be seen in Table 3 (entries 1 and 2), the preformation of hydroxylamine **4** is required in order to drive the reaction toward EIDD-2801 while concomitantly reduce the formation of side products **5** and **6**. Nevertheless, we experienced reproducibility issues in this optimization study, most likely because of the exotherm upon addition of H_2SO_4, which proved to be difficult to control in batch. Unfortunately, the reproducibility could not be improved by diluting the conc H_2SO_4 with *i*-ProH.

Table S3. Optimization for the One-pot Hydroxyamination and Acetonide Deprotection.



Entry	Equiv H_2SO_4	T [°C][a]	Time [h]	**4** [%][b]	**EIDD-2801** [%][b]	**5** [%][b]	**6** [%][b]
1[c]	5	60	0.5	-	**9**	29	62
2[c]	5	60	1	-	**7**	44	49
3	5	60	0.5	1	**71**	12	12
4	5	40	0.5	30	**53**	3	8
5	1.5	60	0.5	80	**7**	-	-
6	6	60	0.5	25	**57**	5	7
7	10	rt	1	16	**68**	3	7
8	10	rt	2	12	**75**	5	8
9	10	rt	4	6	**72**	10	8

[a] Close-vessel microwave heating for T ≥40 °C.
[b] Area% determined by HPLC at 260 nm. Except for entries 1, 2 and 8, further unidentified impurities were detected.
[c] No preformation of **4**.

5. Optimization Studies: Acetonide Deprotection in Continuous Flow

The flow set-up consisted of a 3.5 mL reaction coil (PFA, 1/16" OD, 0.80 mm ID) immersed in a heated oil bath, a Syrris Asia syringe pump module (P1 and P2) equipped with two injection valves, two sample loops (SL1 and SL2, 2 mL and 1.5 mL, respectively; PFA, 1/16" OD, 0.80 mm ID, each) and a Zaiput back pressure regulator which kept a constant pressure of 5 bar.

General procedure: For each experiment, a 2 mL solution containing 0.18 M of compound **3** (146 mg, 0.36 mmol) and 1.5 equiv. of NH_2OH (32.4 µL, 50 wt% in H_2O) in the corresponding solvent was stirred for 15 min at room temperature to ensure full conversion to **4**. This reaction mixture was then directly transferred to SL2. SL1 was filled with neat HCOOH or with a solution of H_2SO_4, CF_3COOH or TfOH prepared in the same solvent as the substrate solution. The liquid feeds were combined in a Y-mixer, and the resulting stream was directed through the heated reaction coil. In each run, approx. 0.5 mL sample of product mixture was collected which was next analyzed directly by HPLC at 260 nm.

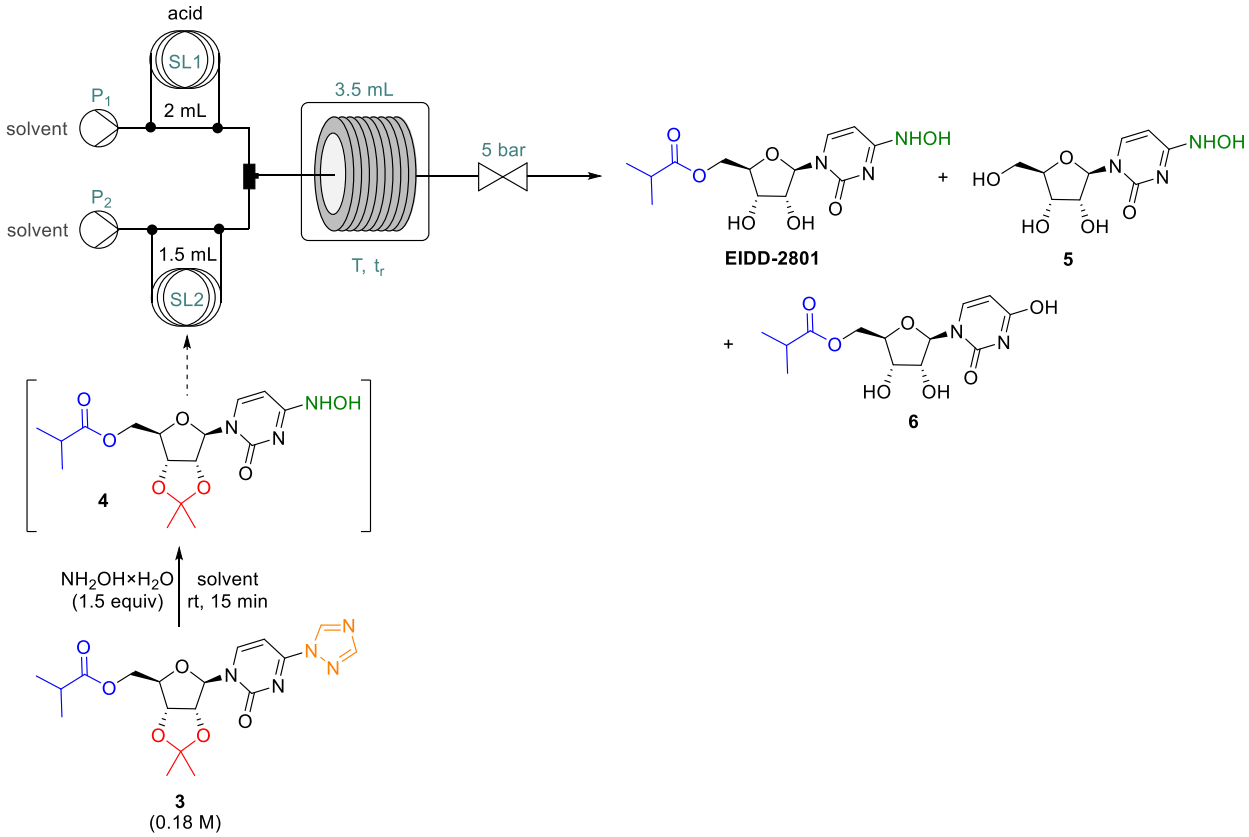


Figure S6. Flow set-up used for the optimization experiments.

Table S4. Optimization of the Flow Acetonide Deprotection using **HCOOH** as Acid Reagent.[a]

Entry	Solvent	Flow rate [µL/min]		Acid equiv	t_r [min]	T [°C]	**4** [%][b]	**EIDD-2801** [%][b]	**5** [%][b]	**6** [%][b]
		P1	P2							
1	*i*-PrOH	175	175	144	10	60	95.8	**4.2**	-	-
2	MeOH	175	175	144	10	60	94.0	**6.0**	-	-
3[c]	MeOH	106	69	222	20	100	11.3	**71.7**	1.4	6.4

[a] Neat HCOOH (≥98%) was employed which corresponds to approx. 26 M.
[b] Area% determined by HPLC at 260 nm.
[c] Further unidentified impurities.

Table S5. Optimization of the Flow Acetonide Deprotection using **H_2SO_4** as Acid Reagent.[a]

Entry	Solvent	Flow rate [µL/min]		Acid equiv	t_r [min]	T [°C]	**4** [%][b]	**EIDD-2801** [%][b]	**5** [%][b]	**6** [%][b]
		P1	P2							
1[c]	*i*-PrOH	175	175	5.55	10	60	56.3	**38.3**	1.0	4.5
2[c]	THF	175	175	5.55	10	60	-	**-**	-	-
3	MeOH	175	175	5.55	10	60	6.7	**73.5**	13.6	6.2
4	MeOH	58	117	2.75	20	60	24.6	**60.8**	9.5	5.1
5	MeOH	500	250	11.1	5	60	3.0	**72.9**	18.6	5.5
6	MeOH	232	468	2.75	5	80	38.2	**54.6**	3.4	3.9
7	MeOH	116	234	2.75	10	80	7.3	**73.7**	12.0	7.0
8	MeOH	350	350	5.55	5	100	-	**46.2**	47.6	6.2
9	MeOH	232	468	2.75	5	100	1.8	**79.1**	11.0	8.1
10	MeOH	116	234	2.75	10	100	-	**55.7**	36.2	8.1
11	MeOH	80	270	1.65	10	100	5.4	**70.2**	16.8	7.6
12	MeOH	342	534	3.55	4	100	-	**70.3**	19.7	10.0
13	MeOH	167	417	2.22	6	100	3.7	**75.9**	11.9	8.5
14	EtOH	232	468	2.75	5	100	3.7	**73.6**	7.0	15.7
15[d]	MeCN	232	468	2.75	5	100	9.4	**17.1**	2.1	62.6
16	MeOH/H_2O 8:1	232	468	2.75	5	100	2.3	**70.8**	10.3	16.5
17	MeOH/H_2O 4:1	232	468	2.75	5	100	1.8	**67.0**	10.5	20.8

[a] H_2SO_4 was employed as 1 M solution in MeOH.
[b] Area% determined by HPLC at 260 nm.
[c] Precipitation after mixing of streams.
[d] Further unidentified impurities.

Table S6. Optimization of the Flow Acetonide Deprotection using **CF_3COOH** as Acid Reagent.

Entry	Solvent	Flow rate [µL/min]		Acid equiv	t_r [min]	T [°C]	**4** [%][a]	**EIDD-2801** [%][a]	**5** [%][a]	**6** [%][a]
		P1	P2							
1[b]	MeOH	232	468	2.75	5	100	89.7	**10.3**	0	0
2[b]	MeOH	175	175	5.55	10	100	76.6	**23.4**	0	0
3[c]	MeOH	156	80	70	15	100	10.4	**61.9**	11.7	16.0

[a] Area% determined by HPLC at 260 nm.
[b] CF_3COOH was employed as 1 M solution in MeOH.
[c] CF_3COOH was employed as 6.5 M solution in MeOH.

Table S7. Optimization of the Flow Acetonide Deprotection using **TfOH** as Acid Reagent.[a]

Entry	Solvent	Flow rate [µL/min]		Acid equiv	t_r [min]	T [°C]	**4** [%][b]	**EIDD-2801** [%][b]	**5** [%][b]	**6** [%][b]
		P1	P2							
1	MeOH	116	234	2.75	10	80	6.7	**70.8**	14.5	8.0
2	MeOH	232	468	2.75	5	100	0.9	**73.7**	17.7	7.6

[a] TfOH was employed as 1 M solution in MeOH.
[b] Area% determined by HPLC at 260 nm.

6. Acetonide Deprotection: Analysis of Side Products

In the acetonide deprotection reactions, ester hydrolysis and the exchange of the hydroxyl amine moiety occurred as most prominent side reactions yielding compounds **5** and **6** as side products as detailed above. The identity of these substances was corroborated by means of LC-MS.

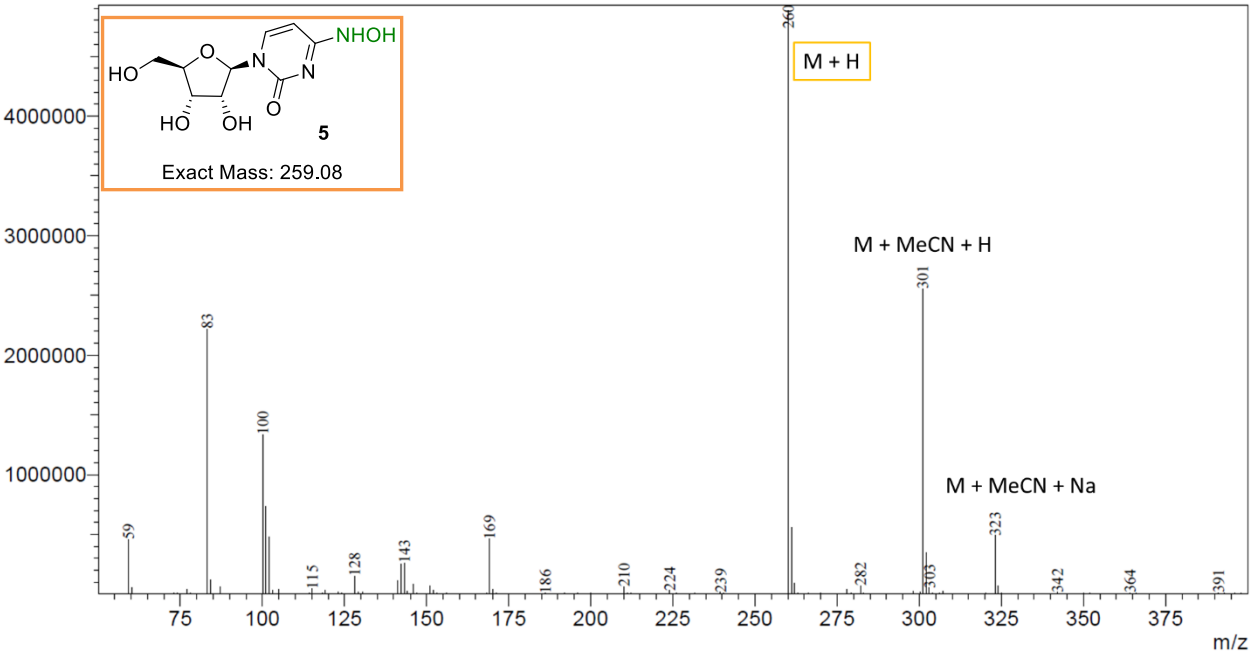


Figure S7. LC-MS of compound **5**.

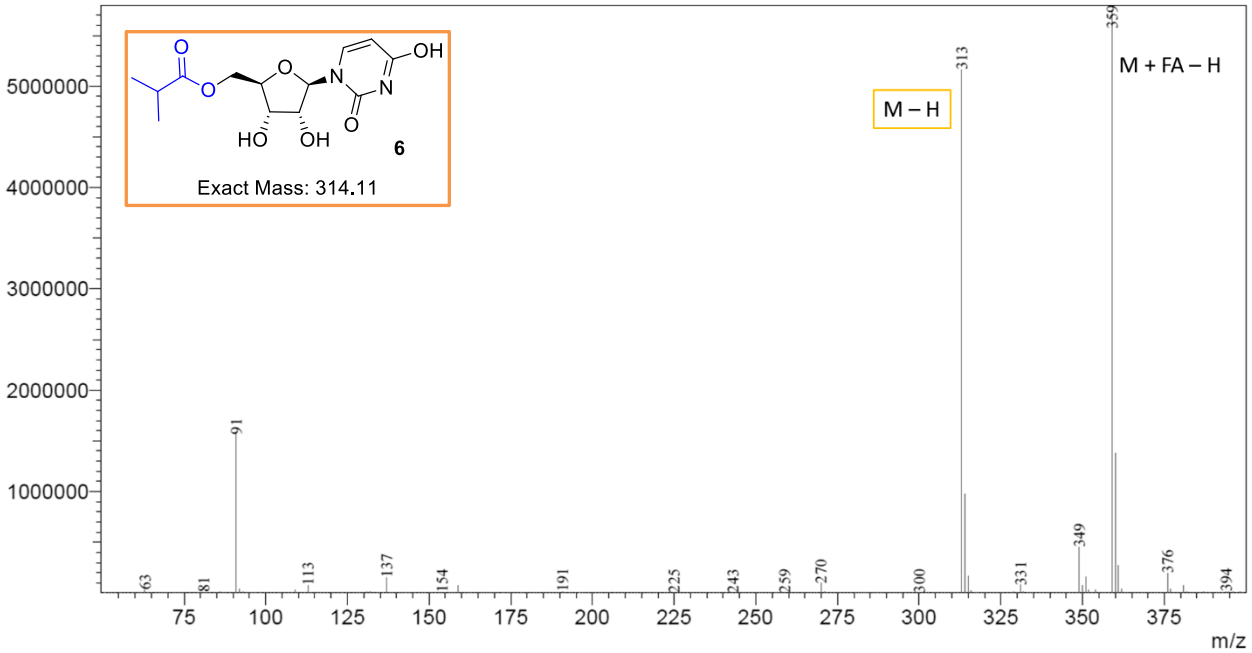
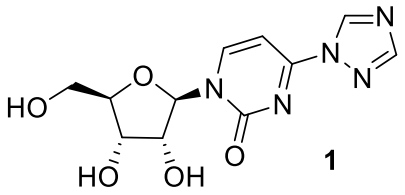


Figure S9. LC-MS of compound **6** (FA: formic acid).

7. Experimental Procedure

7.1. Synthesis of Triazolated Uridine 1



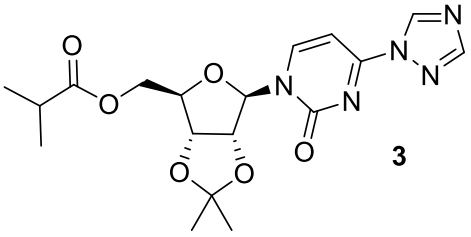
1

An oven dried 500 mL 2-necked round bottom flask was flushed with argon and charged with uridine (5.0 g, 20.5 mmol) and 164 mL of anhydrous MeCN (AcroSeal™, max. 0.001% H_2O). *N*-Methylpyrrolidine (31.9 mL, 307 mmol, 15 equiv) and TMSCl (13 mL, 102 mmol, 5 equiv) were added, and the reaction mixture was allowed to stir for 1 h at room temperature. The solution was cooled in an ice bath to 0 °C. $POCl_3$ (3.74 mL, 41.0 mmol, 2 equiv) was added. After stirring for 10 min, 1,2,4-triazole (14.1 g, 205 mmol, 10 equiv) was added, and stirring was continued at 0 °C for 1 h and at room temperature for another 2 h. The yellow solution was then poured onto 700 mL triethylammonium phosphate buffer (0.5 M, pH 7) and extracted with DCM (3 × 100 mL). The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. A mixture of MeOH:AcOH (4:1 v/v) was added to the residue and the reaction mixture was stirred at room temperature overnight. The precipitated product was collected by filtration, washed with diethyl ether and dried under reduced pressure. **1** was obtained as a white solid in 88% yield (5.34 g) and ≥99% purity.

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.45 (s, 1H), 8.84 (d, *J* = 7.2 Hz, 1H), 8.41 (s, 1H), 6.97 (d, *J* = 7.2 Hz, 1H), 5.80 (s, 1H), 5.65 (d, *J* = 4.6 Hz, 1H), 5.26 (t, *J* = 4.5 Hz, 1H), 5.06 (d, *J* = 5.2 Hz, 1H), 4.05 – 3.97 (m, 3H), 3.81 (dd, *J* = 11.6, 4.0 Hz, 1H), 3.64 (dd, *J* = 12.3, 3.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ158.6, 154.1, 153.8, 148.4, 143.7, 93.7, 91.2, 84.2, 74.6, 68.1, 59.4.

The NMR data is in agreement with previously published values.[1]

7.2. One-pot Synthesis of Acetonide Ester 3



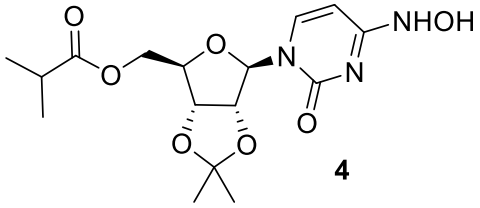
3

An oven dried 500 mL 3-necked round bottom flask equipped with a Dean-Stark apparatus was flushed with argon and charged with **1** (5.34 g, 18.1 mmol) and 121 mL of anhydrous MeCN. 2,2-Dimethoxypropane (4.46 mL, 36.2 mmol, 2 equiv) and 95% H_2SO_4 (53.4 µL, 0.91 mmol, 5 mol%) were added. The suspension was stirred at room temperature for 30 min. Then, 45 mL of anhydrous MeCN were added and 45 mL of solvent were distilled off ($T_{oilbath}$ = 95 °C). This azeotropic distillation was performed three times. After cooling to room temperature, Et_3N (30.3 mL, 217 mmol, 12 equiv), *N*,*N*-

dimethylaminopyridine (553 mg, 4.53 mmol, 25 mol%) and isobutyric anhydride (3.3 mL, 19.9 mmol, 1,1 equiv) were added. The reaction mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure. After addition of 270 mL ethyl acetate, the organic phase was washed with sat. $NaHCO_3$ (2 × 90 mL), water (90 mL) and brine (90 mL) and dried with Na_2SO_4. After removal of the solvent under reduce pressure, the product was obtained as a yellow solid in quantitative yield (7.43 g) and 101% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.12 (s, 1H), 8.05 (d, *J* = 7.3 Hz, 1H), 7.04 (d, *J* = 7.2 Hz, 1H), 5.82 (d, *J* = 1.4 Hz, 1H), 5.02 (dd, *J* = 6.3, 1.5 Hz, 1H), 4.82 (dd, *J* = 6.3, 3.8 Hz, 1H), 4.54 – 4.50 (m, 1H), 4.38 – 4.36 (m, 2H), 2.50 (sept, *J* = 7.0 Hz, 1H), 1.59 (s, 3H), 1.37 (s, 3H), 1.13 (d, *J* = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.5, 159.9, 154.3, 154.2, 147.8, 143.5, 114.6, 96.7, 94.8, 86.6, 85.5, 81.1, 64.1, 34.0, 27.2, 25.4, 19.1, 19.0. HRMS (ESI, positive mode): *m/z* $[M + H]^+$ Calcd for $[C_{18}H_{23}N_5O_6 + H]^+$: 406.1721, found: 406.1720.

7.3. Synthesis of Acetonide-protected Hydroxylamine 4



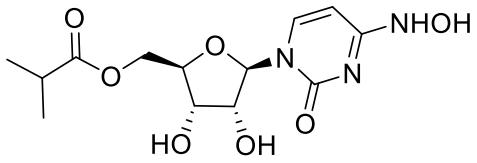
4

A 25 mL round bottom flask was charged with **3** (828 mg, 2.0 mmol) and 10 mL of *i*-PrOH. Hydroxylamine (50w% in H_2O, 185 µL, 3.0 mmol, 1.5 equiv) was added and stirred at rt for 20 min. Next, the solvent was removed under reduced pressure and the solid residue dissolved in 60 mL of EtOAc. The organic phase was washed with water (2 × 40 mL) and brine (1 × 40 mL) and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the product was obtained as a yellow solid in 90% yield (682 mg) and 91% purity. The product was then further purified by washing with cold diethyl ether to obtain a white solid (341 mg, 45% yield, 99% purity).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (brs, 1H), 8.27 (brs, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 5.64 – 5.58 (m, 2H), 4.95 (dd, *J* = 6.5, 2.1 Hz, 1H), 4.77 (dd, *J* = 6.4, 3.5 Hz, 1H), 4.40 – 4.18 (m, 3H), 2.58 (sept, *J* = 7.0 Hz, 1H), 1.56 (s, 3H), 1.35 (s, 3H), 1.18 (d, *J* = 7.0 Hz, 6H).^{13}C NMR (75 MHz, $CDCl_3$) δ 176.9, 149.4, 145.1, 132.9, 114.6, 98.9, 94.0, 84.6, 84.4, 81.2, 64.2, 34.0, 27.3, 25.5, 19.1, 19.1.

The NMR data is in agreement with previously published values.[2]

7.4. Telescoped Synthesis of EIDD-2801 in Continuous Flow



EIDD-2801

The flow set-up used was identical with the one described in Section 5 (Figure S6, see also Figure 1 in the main text). Sample loops of 5 mL (SL1) and 10 mL (SL2) were used (PFA, 1/16" OD, 0.80 mm ID, each).

Scale-out was performed under optimum flow conditions (see Table S5, entry 9) as follows:

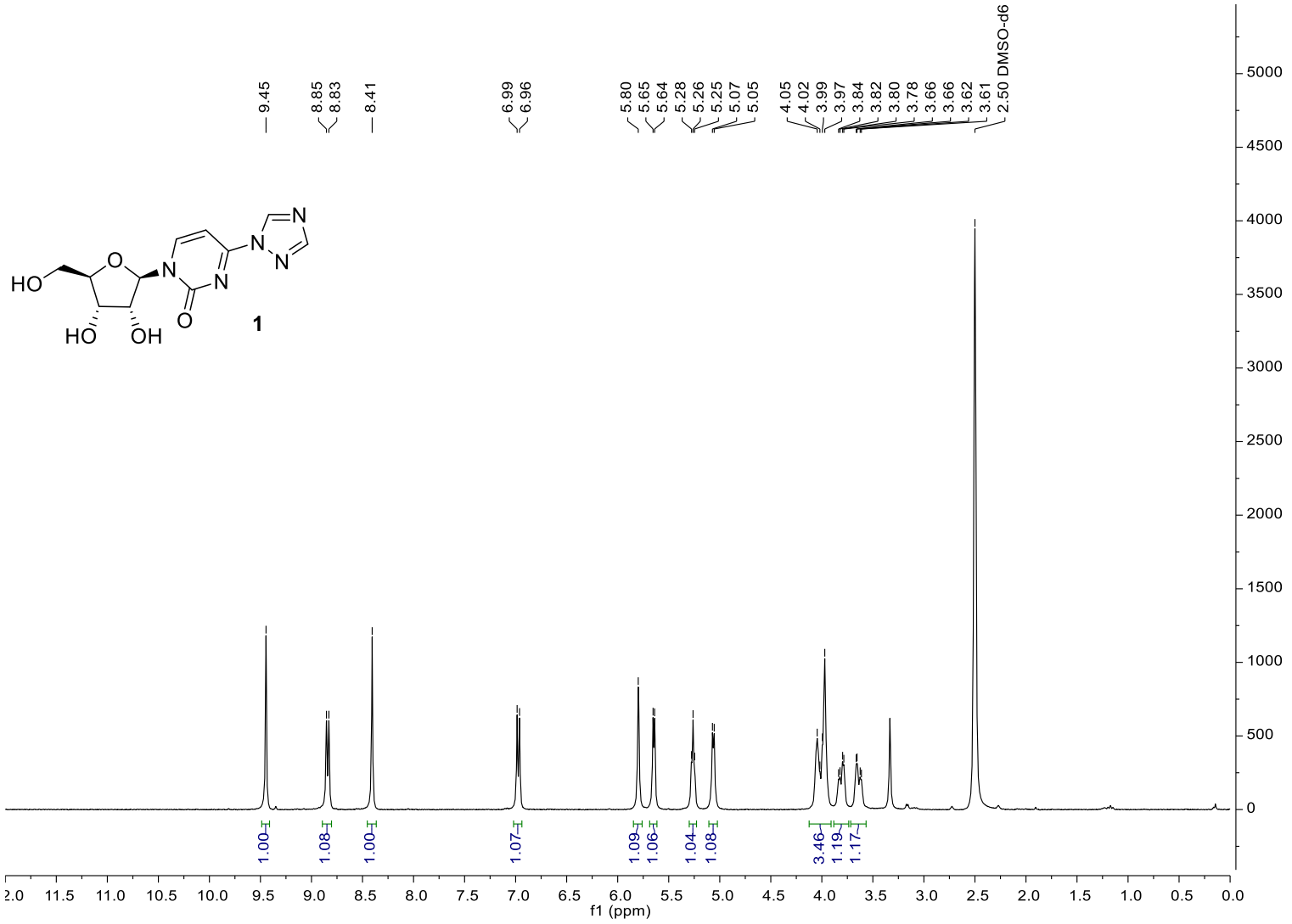
A 10.5 mL solution containing 0.18 M of compound **3** (766 mg, 1.89 mmol) and 1.5 equiv. of NH_2OH (170 µL, 50 wt% in H_2O) in MeOH was prepared and was stirred for 15 min at room temperature. This reaction mixture was then directly transferred to SL2. SL1was filled with 1 M H_2SO_4 solution in MeOH. P1 was set to 232 µL/min and P2 to 468 µL/min. The liquid feeds were combined in a Y-mixer, and the resulting stream was directed through a 3.5-mL reaction coil at 100 °C. The product mixture leaving the coil was collected for 16 min under steady state conditions. The collected mixture was neutralized (pH 7) with a 4 M aq. NaOH solution and was next purified by column chromatography using a 6–16% gradient of MeOH in CH_2Cl_2 as eluent. EIDD-2801 was isolated in 69% yield (307 mg) and ≥99% purity as a white solid.

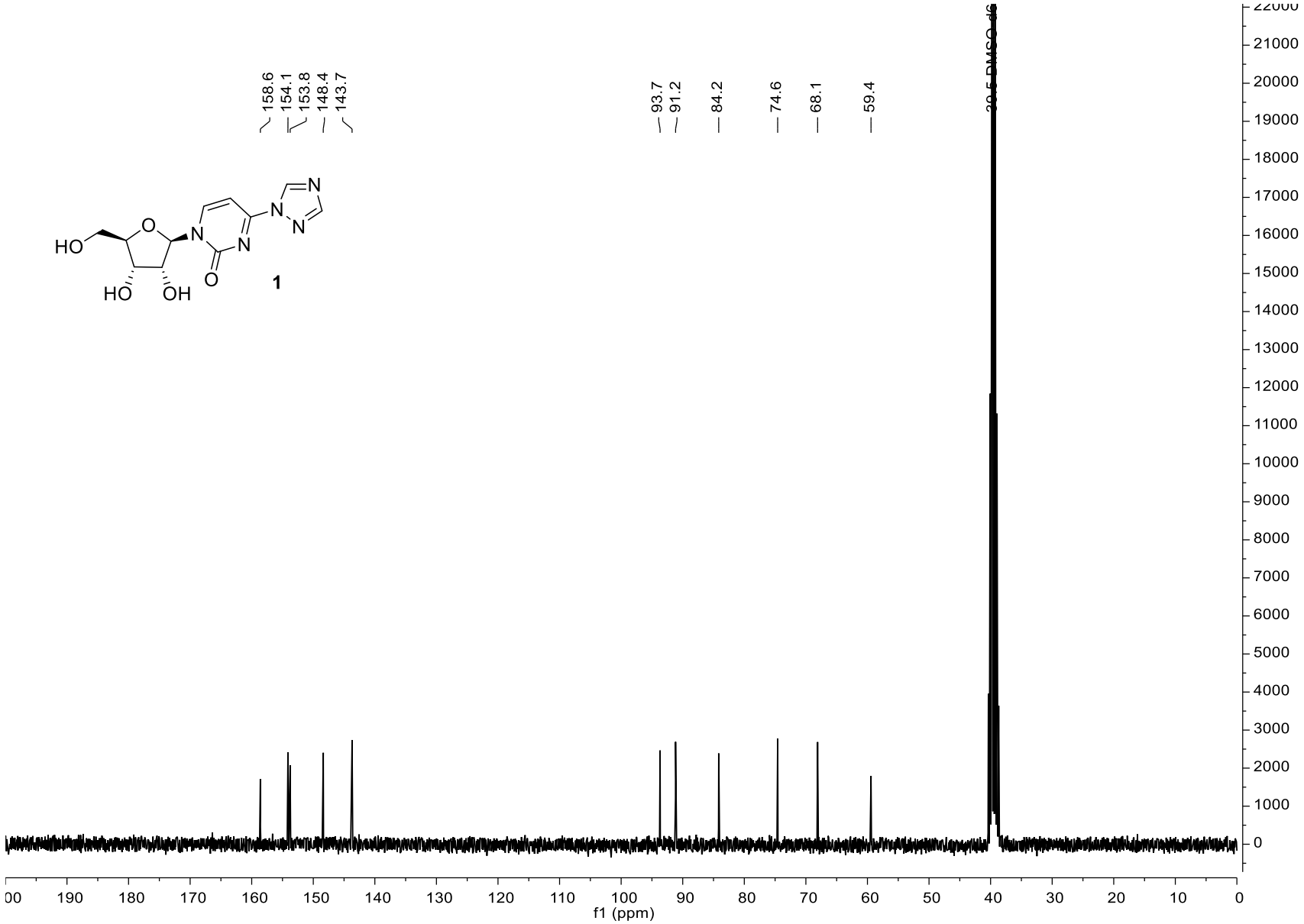
^{1}H-NMR (300 MHz, MeOH-d_4) δ 6.91 (d, *J*= 8.3 Hz, 1H), 5.82 (d, *J*= 4.8 Hz, 1H), 5.61 (d, *J*= 8.2 Hz, 1H), 4.29 (d, *J*= 3.6 Hz, 2H), 4.15-4.07 (m, 3H), 2.62 (sept, *J*= 7.0 Hz, 1H), 1.18 (d, *J*= 7.0 Hz 6H); ^{13}C-NMR (75 MHz, MeOH-d_4) δ 178.3, 151.5, 146.1, 131.7, 99.5, 90.4, 82.6, 74.4, 71.5, 64.9, 35.2, 19.4, 19.3. The NMR data is in agreement with previously published values.[2]

HRMS (ESI, positive mode): *m/z* $[M + H]^+$ Calcd for $[C_{13}H_{20}N_3O_7 + H]^+$: 330.1296, found: 330.1297.

8. NMR Spectra

^{1}H NMRs: 300 MHz, ^{13}C NMRs: 75 MHz




158.6
154.1
153.8
148.4
143.7
93.7
91.2
84.2
74.6
68.1
59.4
39.5 DMSO-d6
HO
HO
OH
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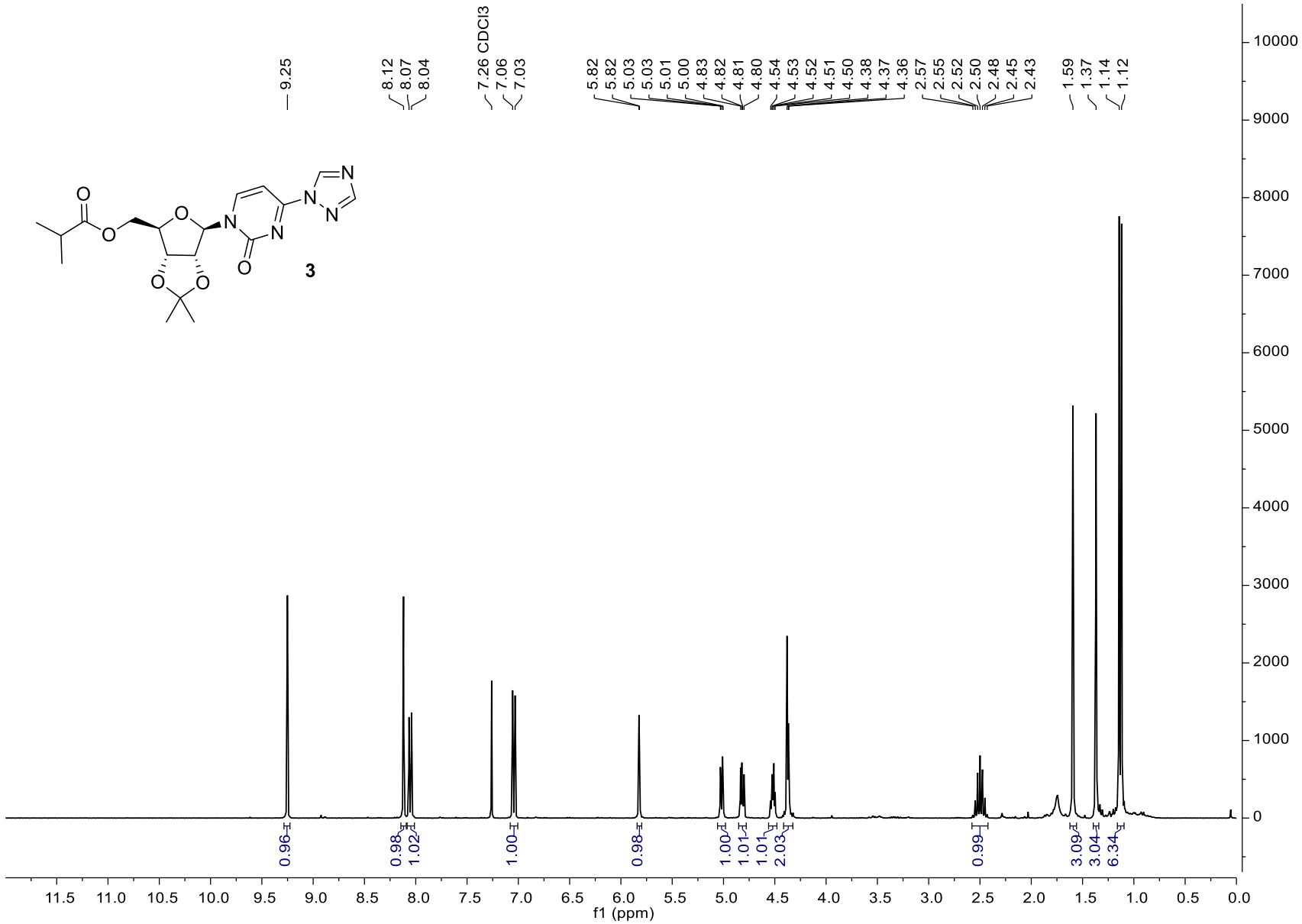
N
N
N
N
O
1
f1 (ppm)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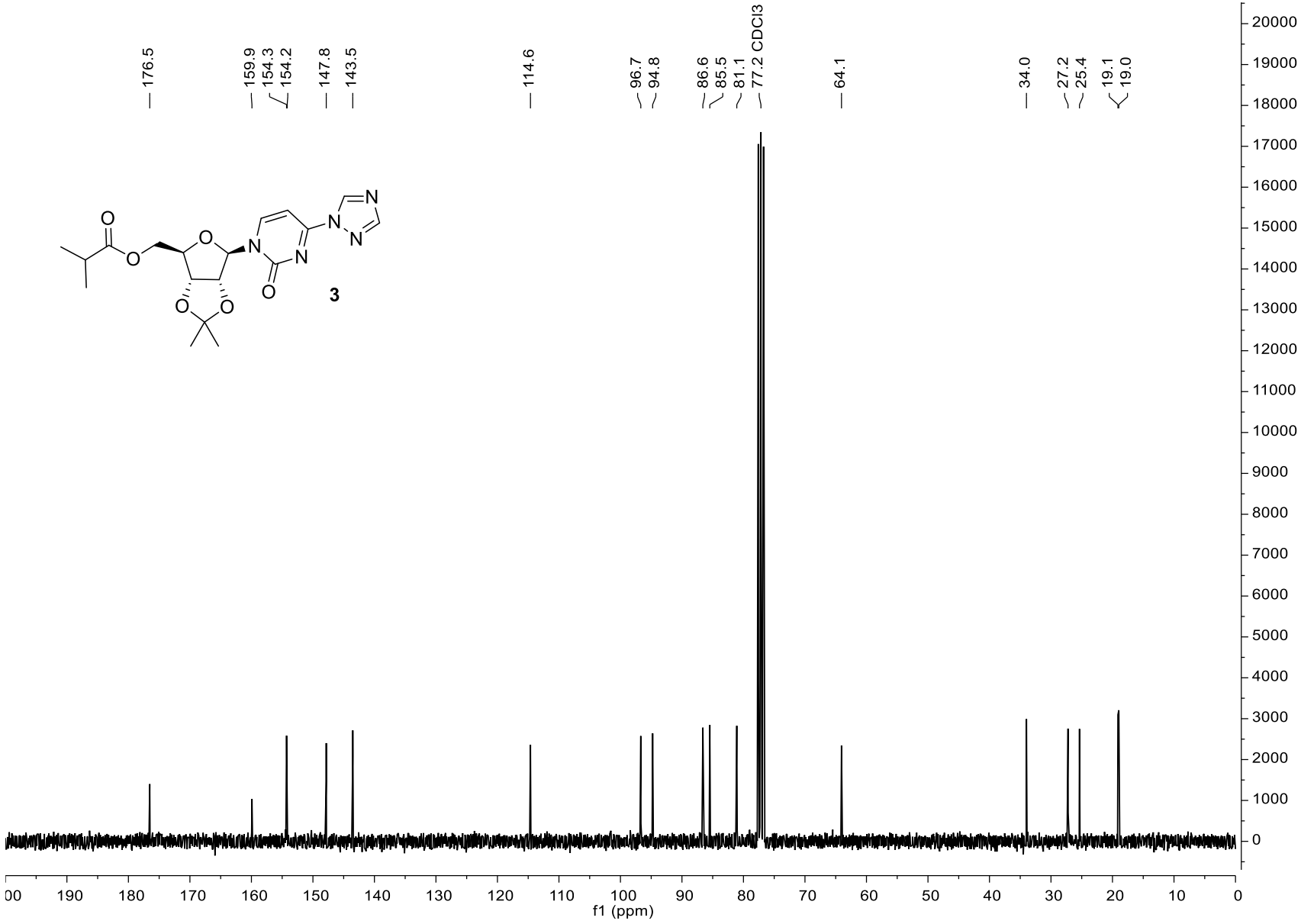
8.12
8.07
8.04
7.26 CDCl3
7.06
7.03
5.82
5.82
5.03
5.03
5.01
5.00
4.83
4.82
4.81
4.80
4.54
4.53
4.52
4.51
4.50
4.38
4.37
4.36
2.57
2.55
2.52
2.50
2.48
2.45
2.43
1.59
1.37
1.14
1.12
3
0.96
0.98
1.02
1.00
0.98
1.00
1.01
1.01
2.03
0.99
3.09
3.04
6.34
f1 (ppm)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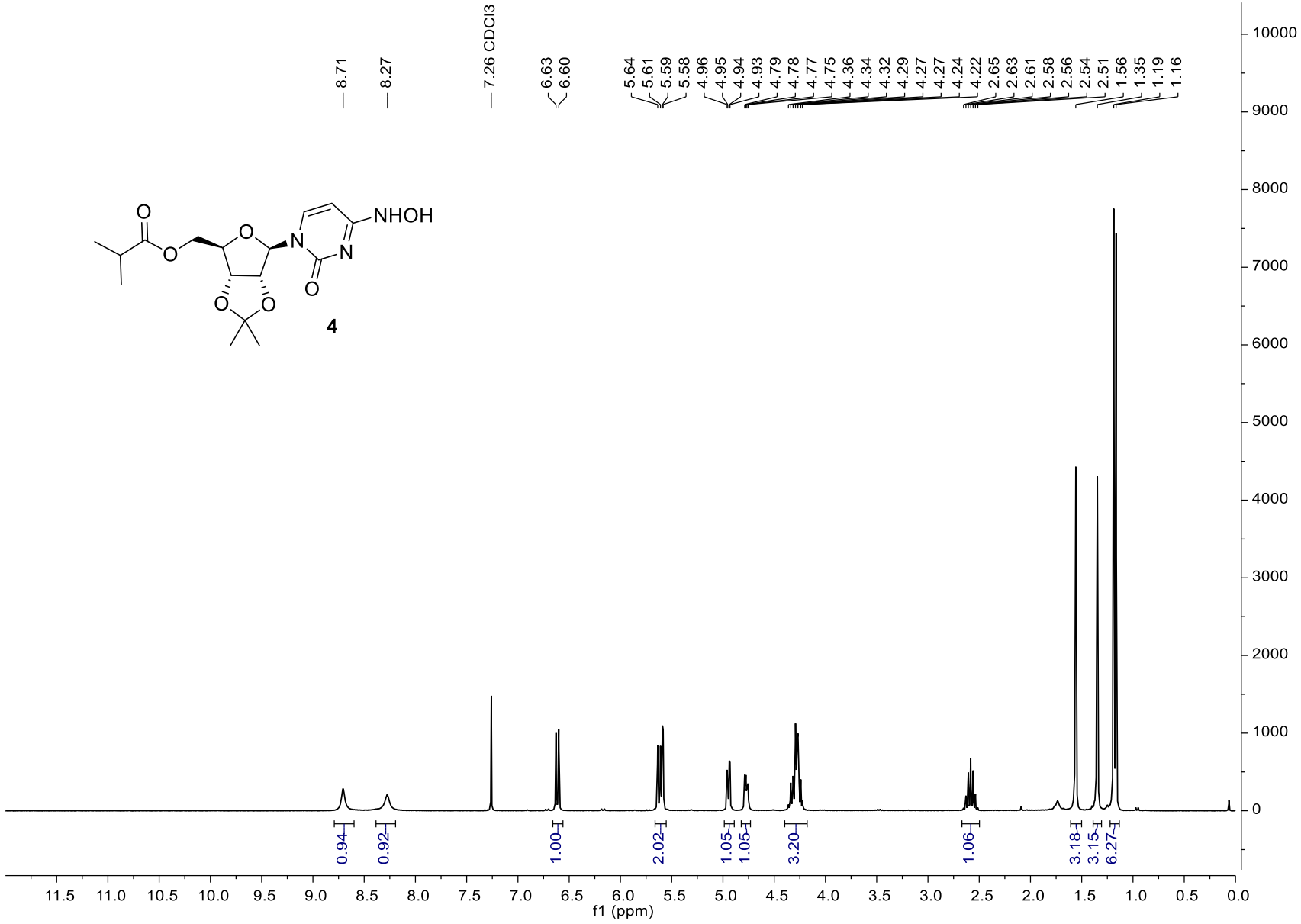
159.9
154.3
154.2
147.8
143.5
114.6
96.7
94.8
86.6
85.5
81.1
77.2 CDCl3
64.1
34.0
27.2
25.4
19.1
19.0
3
f1 (ppm)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5.59
5.58
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4.78
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4.27
4.27
4.24
4.22
2.65
2.63
2.61
2.58
2.56
2.54
2.51
1.56
1.35
1.19
1.16
NHOH
4
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0.92
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1.05
1.05
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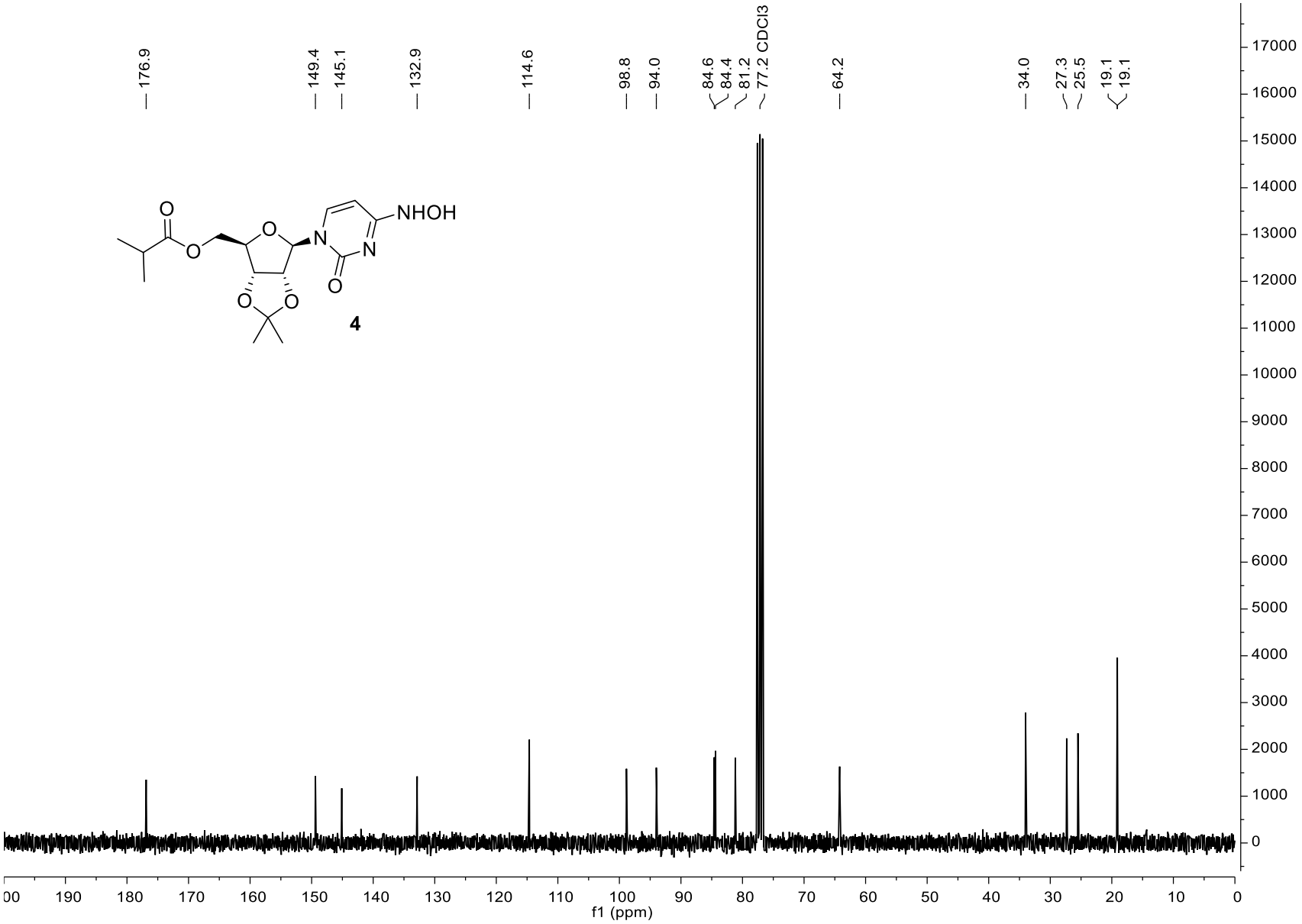
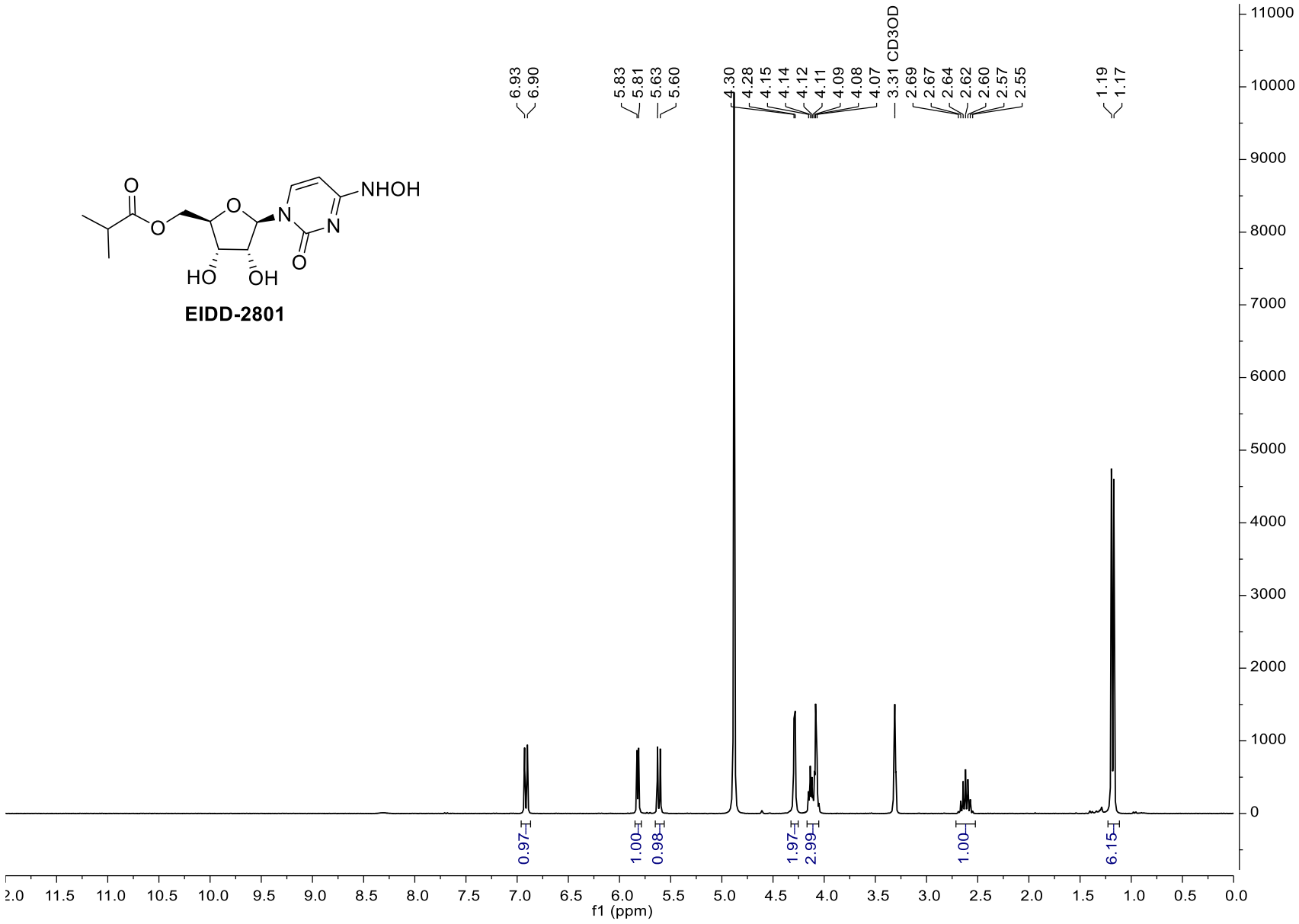
1.06
3.18
3.15
6.27
f1 (ppm)


176.9
149.4
145.1
132.9
114.6
98.8
94.0
84.6
84.4
81.2
77.2 CDCl3
64.2
34.0
27.3
25.5
19.1
19.1
NHOH
O
O
O
N
N
O
O
O
4
f1 (ppm)


EIDD-2801
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5.63
5.60
4.30
4.28
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4.14
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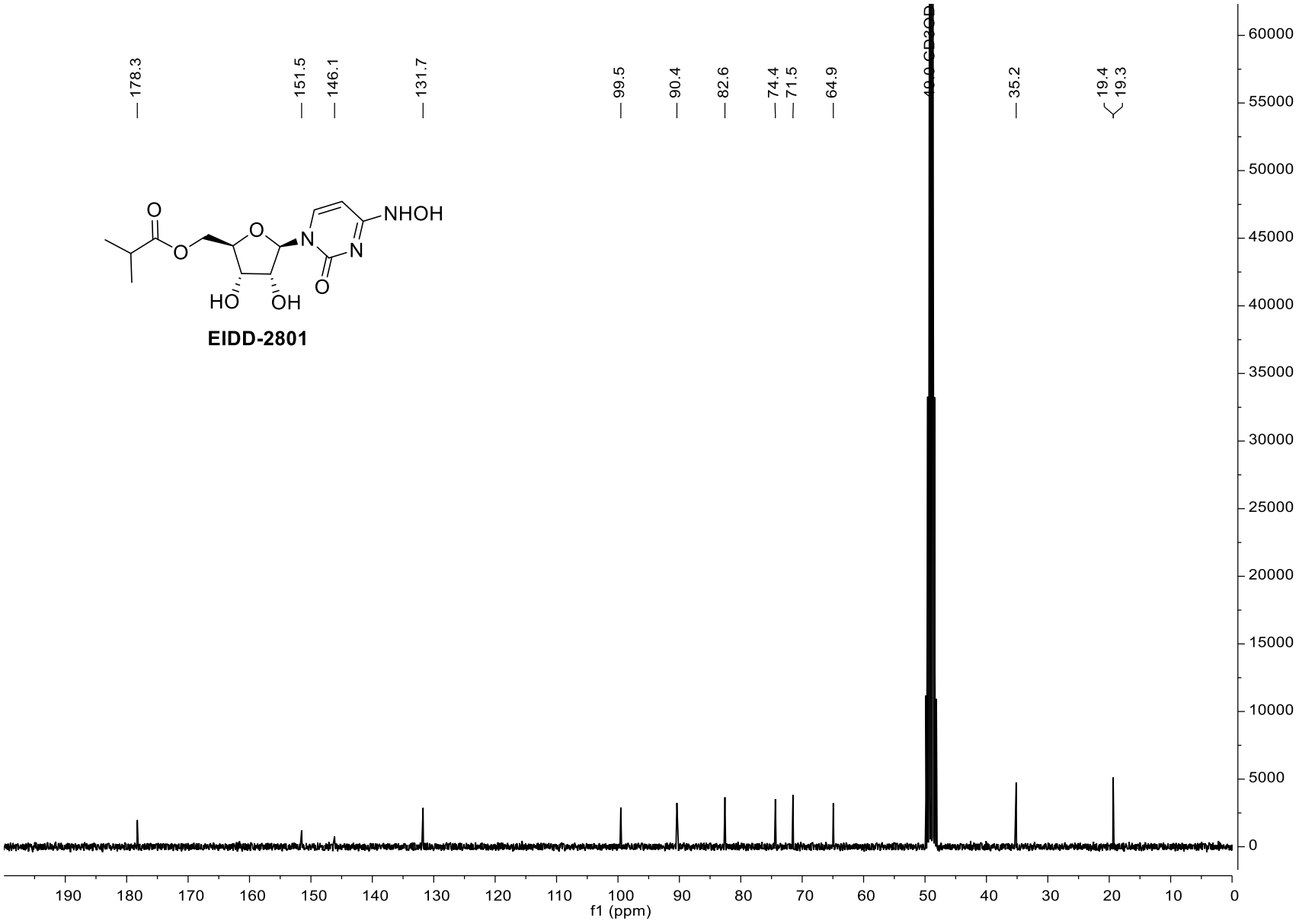
4.11
4.09
4.08
4.07
3.31 CD3OD
2.69
2.67
2.64
2.62
2.60
2.57
2.55
1.19
1.17
0.97
1.00
0.98
1.97
2.99
1.00
6.15
f1 (ppm)


178.3
151.5
146.1
131.7
99.5
90.4
82.6
74.4
71.5
64.9
49.0 CD3OD
35.2
19.4
19.3
NHOH
O
O
O
N
N
N
O
HO
OH
EIDD-2801
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

9. References

[1] A. Miah, C. B. Reese, Q. Song, *Nucleosides and Nucleotides* **1997**, *16*, 53–65.

[2] V. Gopalsamuthiram, C. Williams, J. Noble, T. F. Jamison, B. F. Gupton, D. R. Snead, *Synlett* **2020**, DOI 10.1055/a-1275-2848.

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