nature protocols

PROTOCOL

https://doi.org/10.1038/s41596-022-00705-7

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Practical and concise synthesis of nucleoside analogs

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Nucleoside analogs are valuable commodities in the development of antisense oligonucleotides or as stand-alone antiviral and anticancer therapies. Syntheses of nucleoside analogs are typically challenged by a reliance on chiral pool starting materials and inefficient synthetic routes that are not readily amenable to diversification. The novel methodology described in this protocol addresses several longstanding challenges in nucleoside analog synthesis by enabling flexible and selective access to nucleoside analogs possessing variable nucleobase substitution, D- or L-configuration, selective protection of C3′/C5′ alcohols and C2′ or C4′ derivatizations. This protocol provides direct access to C3′/C5′ protected nucleoside analogs in three steps from simple, achiral starting materials and is described on both research (2.8 g) and process (30 g) scales for the synthesis of C3′/C5′-acetonide protected uridine. Using this protocol, proline catalyzes the fluorination of simple heteroaryl-substituted aldehyde starting materials, which are then directly engaged in a one-pot enantioselective aldol reaction with a dioxanone. Reduction, followed by intramolecular annulative fluoride displacement, forges the nucleoside analog. The three-step parent protocol can be completed in ~5 d by using simple mix-and-stir reaction procedures and standard column chromatographic purification techniques.

Introduction

Nucleoside analogs (NAs) possess a rich history spanning half a century as stand-alone treatments for cancer and viral infections[1]. Recently, the antiviral nucleoside analog molnupiravir was approved in the United Kingdom by the Medicines and Healthcare products Regulatory Agency for treatment of patients with mild to moderate COVID-19 with at least one risk factor for developing severe illness[2]. Additional regulatory applications for molnupiravir are currently under review in the European Union and United States among other countries[3]. As the first orally dosed antiviral approved for treatment of COVID-19, molnupiravir highlights the importance of this class of therapeutics. NAs are also indispensable commodities in the development of antisense oligonucleotides, an emerging area of clinical therapeutics that holds incredible promise in the field of personalized therapies because they can be rationally designed in a sequence-specific manner to match patient-specific mutations[4,5]. However, the full exploitation of NAs has been limited by their synthetic access.

Despite decades of research, contemporary syntheses of NAs are generally inefficient and reliant on chiral pool starting materials that are not readily amenable to diversification. For example, a process-scale (10 kg) synthesis of potent anti-HIV nucleoside analog 4′-ethynyl-2-fluoro-2′-deoxyadenosine by Merck & Co. requires 12 linear steps, an enzymatic desymmetrization and a poorly diastereoselective penultimate Vorbrüggen glycosylation[6]. Sofosbuvir is a nucleotide analog therapeutic used for the treatment of hepatitis C. A de novo synthesis of the nucleoside core, PSI-6130, of sofosbuvir required 10 linear steps from commercial materials, and analogously to that of 4′-ethynyl-2-fluoro-2′-deoxyadenosine, a penultimate glycosylation reaction displayed modest diastereoselectivity[7]. Gilead's synthesis of the broad-spectrum antiviral agent remdesivir requires chiral pool starting materials, eight steps and a final chiral HPLC separation of diastereomers[8].

Recently, we disclosed a straightforward synthesis of NAs from achiral starting materials in only two or three steps (Fig. 1)[9]. The process uses proline catalysis to fluorinate simple heteroaryl-substituted aldehyde starting materials, which are then directly engaged in a one-pot enantioselective aldol reaction with a dioxanone. Reduction (or organometallic addition) followed by intramolecular annulative fluoride displacement (AFD) then forges the NA. This novel methodology addresses

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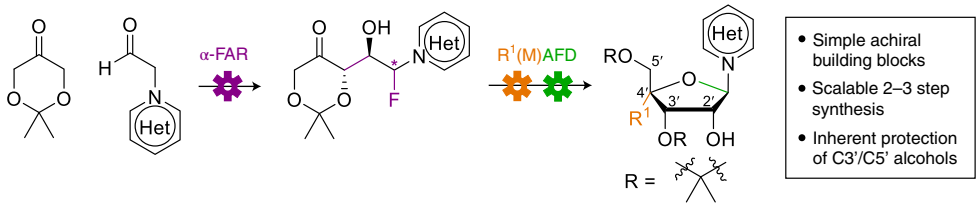

Fig. 1 | Simple three-step synthesis of nucleoside analogs. AFD, annulative fluoride displacement (green); Het, heteroaryl; R¹[M], reduction or organometallic addition (orange); α-FAR, α-fluorination aldol reaction (purple)[6].

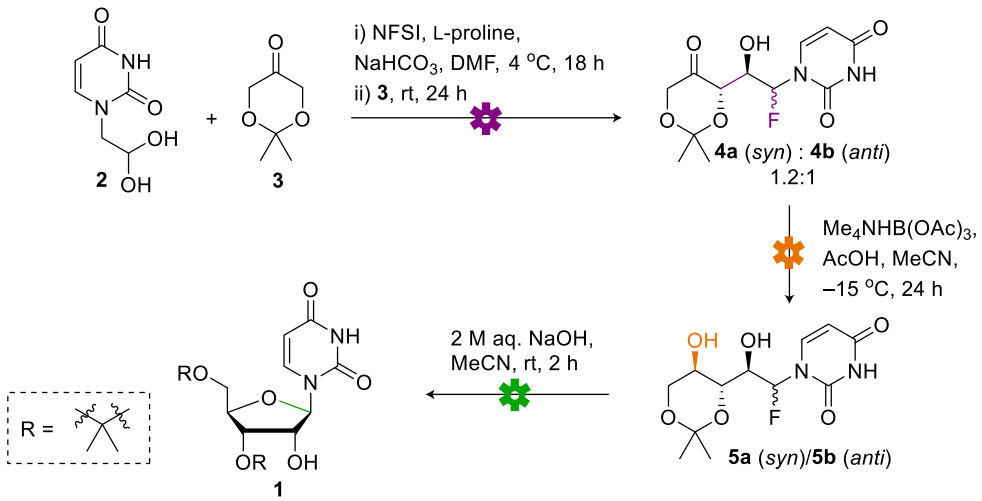

Fig. 2 | Parent protocol detailed herein. Synthesis of uridine **1** α-FAR (purple); reduction (orange); AFD (green). AcOH, acetic acid; aq., aqueous; DMF, *N*,*N*-dimethylformamide; MeCN, acetonitrile; NFSI, *N*-fluorobenzenesulfonimide; rt, room temperature.

several longstanding challenges in nucleoside analog synthesis;[10] direct access to C3′/C5′ protected and C4′ functionalized nucleoside analogs such as locked nucleic acid (LNA) monomers is enabled; in addition, the process provides flexibility in nucleobase substitution and D- or L-configuration. Moreover, the utility of the α-functionalization aldol reaction was further emphasized through the recent synthesis of a diverse portfolio of glycomimetics[11].

This technology is amenable to both research (2.8 g) and process (30 g) scales, and variations in the protocol for both scales will be detailed herein.

Experimental design

This protocol describes the synthesis of C3′/C5′-acetonide protected uridine **1** in three steps from aldehyde hydrate **2** (Fig. 2). The protocol is operationally simple, requiring achiral and commercially available materials, inexpensive proline catalysis and simple mix-and-stir procedures. All three reactions are performed under a N_2 atmosphere (standard practice within our laboratory); however, we have not found this to be critical to the reaction outcomes, because similar yields were obtained when the reactions were performed open to air. This protocol has two procedure sections: follow Procedure 1 to conduct the synthesis on a 2.8-g scale and Procedure 2 for the 30-g scale reaction.

The α-fluorination aldol reaction (α-FAR) is a one-pot procedure in which aldehyde hydrate **2** is fluorinated with *N*-fluorobenzenesulfonimide (NFSI) and L-proline catalyst at 4 °C before dioxanone **3** is added to generate fluorohydrin intermediate **4a/b**. Use of D-proline during the α-FAR reaction will ultimately afford the L-configured NA (*ent*-**1**). To ensure a stable 4 °C environment for the duration of the fluorination step (overnight, typically 18 h), we set up a dedicated refrigerator containing several stirrer plates for the research-scale procedure. A refrigerator possessing an internal power outlet and Dixell Prime temperature control panel was used for this purpose (Supplementary Fig. 1, Electronic Supporting Information (ESI)). However, if readers do not have a refrigerator with internal power outlets readily available, then we recommend feeding the power cable of a single stirrer

plate through the door of a laboratory refrigerator. The rubber seal of the door should hold closed with the power cable protruding in most cases; however, duct tape may be used to hold the refrigerator door closed if necessary. For the process-scale procedure, a cold room was used to maintain a stable temperature of 4 °C for the duration of the α-FAR reaction.

Aldehyde hydrate **2** was used as starting material for this protocol because it can be synthesized from uracil in two steps on 10–50-g scales. In addition, a simple crystallization allows isolation of aldehyde hydrate **2**. (Research- and process-scale synthesis procedures for making this starting material are provided; Supplementary Methods 1 and 2, respectively, and Supplementary Data.) However, readers may wish to use a different aldehyde (or mixture of aldehyde and aldehyde hydrate) depending on their desired product. The desired aldehyde or hydrate analog (or mixture of aldehyde and aldehyde hydrate) may be used interchangeably without any operational changes to the procedure.

Access to C3′/C5′ protected uridine **1** from fluorohydrin **4a/b** requires *syn*-reduction of the carbonyl by using tetramethylammonium triacetoxyborohydride to generate *syn*-diol **5a/b** at −15 °C (2.8-g scale) or −25 °C (30-g scale). *Syn*-diol **5a/b** is then treated with 2 M aqueous sodium hydroxide in acetonitrile to mediate the AFD and generate NA **1**. Readers can choose from two workup procedures to isolate NA **1** on the gram scale; the steps for these options are described in Step 45 of Procedure 1. Option A (Step 45A) involves neutralization and extraction of the reaction mixture and then removal of the solvent to provide a crude sample of **1**, which is wet-loaded onto a silica-packed column for chromatographic purification. Option A (Step 45A) uses common laboratory protocols and equipment, is operationally simple and can be completed in several hours. However, we have found that on larger scales and for hydrophilic NAs such as **1**, this protocol leads to lower yields of isolated NA. Option B (Step 45B) requires neutralization of the reaction mixture with aqueous ammonium chloride followed by addition of silica gel. The reaction mixture is then frozen and lyophilized to give a powder, which may be dry-loaded directly onto a silica-packed column for chromatographic purification. Although this workup procedure requires prolonged wait times for solvent removal by lyophilization, it was associated with improved yields of isolated NA **1**, because no material was lost during aqueous workup, and therefore procedure B may be preferred for readers targeting hydrophilic NAs on gram scales.

In general, the research-scale protocol is fairly translatable to the process scale except for the requisite lyophilization steps. Although this can generally be done in a well-equipped pharmaceutical laboratory, readers wishing to perform these reactions on a process scale in less-well-equipped laboratories will have to follow the extraction and drying procedures described in the process-scale protocol herein. The process-scale extraction and drying procedures may lead to lower yields of **1** because of loss of material in the aqueous layers; however, the worked example **1** and intermediates **4a/b** and **5a/b** are particularly water soluble, and readers pursuing less polar NAs probably will not have such issues. In addition, the process-scale protocol described herein makes use of the combination of larger vessels with bottom drain valves and overhead stirrers to avoid the separatory funnels that worked well on the research scale but became cumbersome on the process scale.

Derivatization of C3′/C5′ protected uridine **1** may then be conducted by readers wishing to access C2′ functionalized NAs (Fig. 3a). For example, oxidation of the C2′ alcohol to a ketone followed by deprotection and alkyl Grignard addition provides access to C2′ 3° alcohols such as **6** (Fig. 3b)[9]. In addition, C2′ deoxy NAs (such as **7**) can be accessed through Barton-McCombie deoxygenation of the C2′ alcohol[9]. For further guidance and inspiration, readers may refer to ref. [9] for additional examples of C2′ derivatized NAs that were prepared from **1**. Imino nucleosides such as **8** (Fig. 3b) can be accessed by using a reductive amination instead of the *syn*-reduction step[9]. For readers wishing to access C4′ modified NAs (including LNAs), a Grignard addition to the fluorohydrin intermediate should be conducted instead of the *syn*-reduction using tetramethylammonium triacetoxyborohydride (Fig. 3a). To access LNAs, addition of alkynylmagnesium chloride to fluorohydrin intermediate affords diastereomeric products **9a** and **9b**, which can be separated by column chromatography. Cyclization of diastereomer **9a** by using 2 M aqueous sodium hydroxide then provides LNA **10**. Specifically, LNA **11** has been prepared in four steps by using this methodology, which compares favorably with the 23-step synthesis of the analogous uracil LNA, further highlighting the utility of this protocol[9,12]. To obtain C4′ modified D-configured NAs, the fluorohydrin intermediate can be treated with a desired Grignard reagent, and then the reaction can be quenched with methanol-ammonium chloride solution. Cyclization with Lewis acid then provides the desired C4′ modified D-configured NA **12** (specifically, NA **13** was prepared by using this methodology). Alternatively, if the Grignard reaction is gradually warmed to room temperature, in situ cyclization provides the C4′

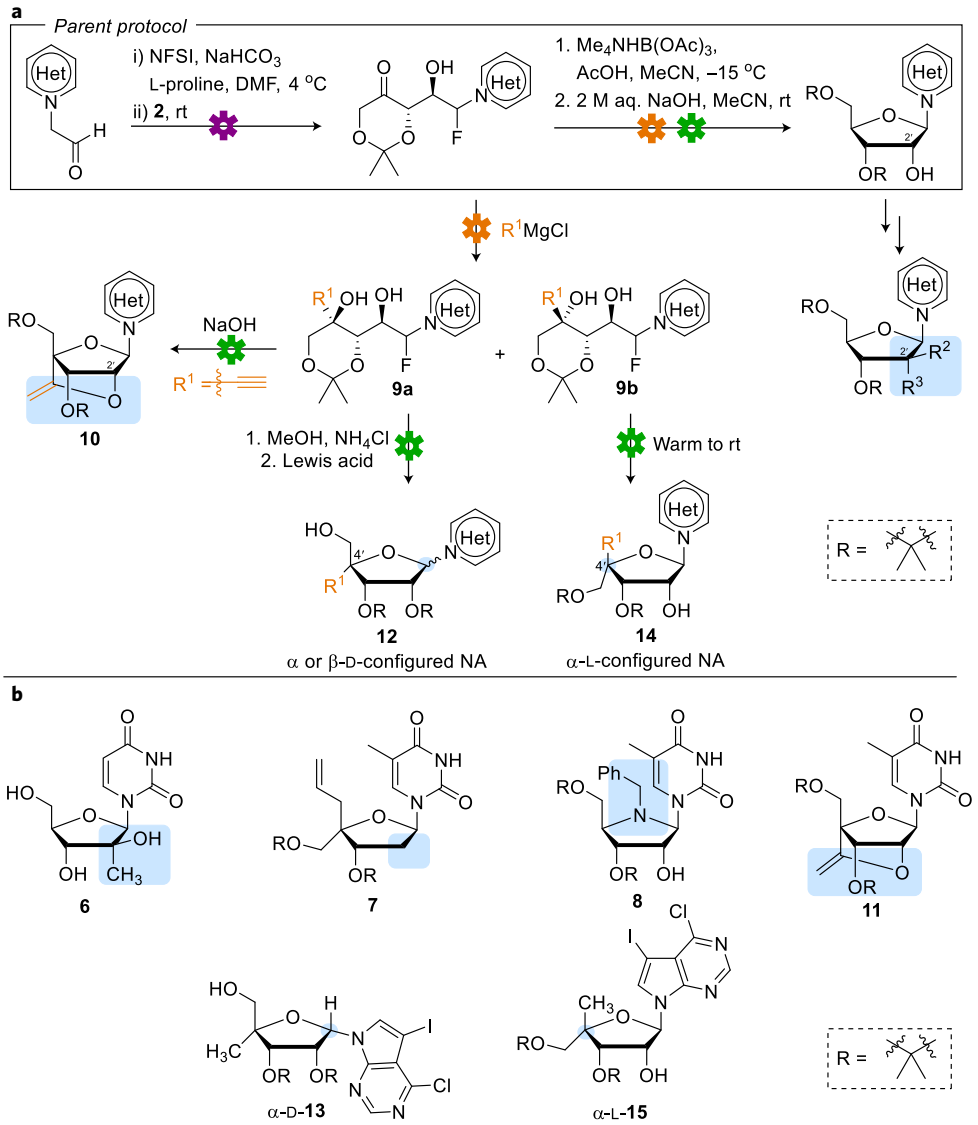

Fig. 3 | Possible variations on the parent protocol for the synthesis of NAs. a, Generic variations to the parent protocol. **b**, Specific examples of NAs prepared by using this protocol[6]. α-FAR (purple); reduction or organometallic addition (orange); AFD (green); blue boxes indicate positions of structural variations.

modified L-configured NA **14** (specifically, NA **15** was prepared by using this methodology). Although the protocols to access analogs **6**–**8** and **10**–**15** are not outlined as part of the parent protocol herein, procedures for these transformations may be accessed in ref. [9].

Limitations

The protocol has proven compatible with a wide range of nucleobases, including, but not limited to, uracil, thymine, trifluoromethyl uracil, adenosine, pyrazole, triazolyl, phthalimidyl and deazaadenine[9]. However, several heteroaryl-substituted acetaldehydes have been found unreactive in the α-FAR procedure; these include imidazole-, benzimidazole-, cytosine-, 6-chloropurine- and indazole-substituted acetaldehydes[6]. These unnatural nucleobase analogs afforded poor yields (<10%) of the corresponding fluorohydrins when using the optimized protocol described herein and as such, may require individual optimization efforts.

Generally speaking, the α-FAR exhibited good enantioselectivity for all aldehyde substrates (enantiomeric ratio (e.r.) of ≥95:5); however, in a few cases, particularly for the purine bases, the enantioselectivity of the α-FAR was lower[9]. The adenine aldehyde (en route to adenosine), for

example, exhibited an e.r. of 82:18 for the α-FAR reaction[9]. Although we did not investigate methods of improving the enantioselectivity of the α-FAR reaction in these cases, it was noticed that several of the aldol adducts were solids; thus, it is possible that the e.r. of these fluorohydrin's could be improved through crystallization[6].

Materials

Reagents

! CAUTION Many of the solvents and reagents are hazardous and/or flammable and should be handled with care in a ventilated fume hood by following good laboratory practice guidelines. A laboratory coat, gloves and safety glasses should be worn at all times when following this protocol.

- NFSI (98%; Matrix Scientific, cat. no. 003270) **! CAUTION** NFSI causes skin and eye irritation. Wear safety glasses, gloves and a laboratory coat when handling.
- L-Proline (99%; Oakwood Chemical, cat. no. M02947)
- Sodium bicarbonate ($NaHCO_3$, powder/certified American Chemical Society grade; Fisher Chemical, cat. no. S233-3)
- 2,2-Dimethyl-1,3-dioxan-5-one (**3**) (95%; Enamine, cat. no. EN300-136503) **! CAUTION** **3** can cause serious skin and eye irritation and may cause respiratory irritation.
- *N*,*N*-Dimethylformamide (DMF, ≥99.8%; ACP Chemicals, cat. no. D-5600) **! CAUTION** DMF is a flammable liquid and can cause acute oral, dermal, inhalation and reproductive toxicity.
- Ethyl acetate (99.5%; Fisher Chemical, cat. no. E145-4) **! CAUTION** Ethyl acetate is a highly flammable liquid and can cause serious eye irritation, and inhalation may cause drowsiness or dizziness.
- Brine solution (Milli-Q water, saturated with NaCl (Windsor coarse pickling salt))
- Sodium sulfate (Na_2SO_4, anhydrous, granular; Caledon Laboratory Chemicals, cat. no. 8220-1-80)
- Dichloromethane (99.5%; Fisher Chemical, cat. no. D37-4) **! CAUTION** Dichloromethane can cause serious skin and eye irritation and is a possible carcinogen.
- Methanol (≥99.8%; Sigma-Aldrich, cat. no. 179337) **! CAUTION** Methanol is a highly flammable liquid and can cause oral, dermal and inhalation toxicity.
- Silica gel 60 (40–63 μm; Merck-Millipore, cat. no. 1.09385)
- Sand (SiO_2, from Ottowa, beige granules; VWR Chemicals BDH, cat. no. BDH9274)
- Tetramethylammonium triacetoxyborohydride ($Me_4NHB(OAc)_3$, 95%; Sigma-Aldrich, cat. no. 317365) **! CAUTION** $Me_4NHB(OAc)_3$ causes skin and eye irritation. Wear safety glasses, gloves and a laboratory coat when handling.
- Acetic acid (glacial, 99.7%; Fisher Scientific Company, cat. no. 351270-212) **! CAUTION** Acetic acid causes severe skin burns and eye damage and may cause severe respiratory irritation if inhaled.
- Acetonitrile (HPLC grade, ≥99.9%; Sigma-Aldrich, cat. no. 34851) **! CAUTION** Acetonitrile is a highly flammable liquid and can cause acute oral, dermal and inhalation toxicity.
- Rochelle salt solution (Milli-Q water, saturated with potassium sodium L-tartrate tetrahydrate (98%, AK Scientific, cat. no. J92730))
- Sodium hydroxide (NaOH, ≥97%, beads; VWR Chemicals BDH, cat. no. BDH9292) **! CAUTION** Sodium hydroxide causes skin burns and eye damage; wear safety glasses, gloves and a laboratory coat when handling.
- Ammonium chloride solution (Milli-Q water, saturated with NH_4Cl (≥99.5%; Sigma-Aldrich, cat. no. 213330))
- *p*-Anisaldehyde (99%; Oakwood Chemical, cat. no. 098960)
- Sulfuric acid (H_2SO_4, 95–98%; Caledon Laboratory Chemicals, cat. no. 8825-1-29) **! CAUTION** Sulfuric acid causes severe skin burns and eye damage and may cause severe respiratory irritation if inhaled.
- Ethanol (anhydrous; Commercial Alcohols, Greenfield Global, cat. no. P016EAAN) **! CAUTION** Ethanol is a highly flammable liquid and can cause eye irritation and oral, dermal and inhalation toxicity.
- Acetone (≥99.5%; Sigma-Aldrich, cat. no. 179124) **! CAUTION** Acetone is a highly flammable liquid that can cause serious eye irritation, and inhalation may cause drowsiness or dizziness.
- Hexanes, mixure of isomers (>98.5% suitable for HPLC; Sigma-Aldrich, cat. no. 293253) **! CAUTION** Hexane is a highly flammable liquid and can cause skin and eye irritation, and chronic exposure can cause damage to the nervous system.
- Isopropanol (HPLC grade; Fisher, cat. no. A451-1) **! CAUTION** Isopropanol is a highly flammable liquid and can cause eye irritation and oral, dermal and inhalation toxicity.
- Methyl *tert*-butyl ether (>99.% ACS reagent; Sigma-Aldrich, cat. no. 443808) **! CAUTION** Methyl *tert*-butyl ether is a highly flammable liquid and can cause skin, eye and respiratory tract irritation.

- Dry ice (solid CO_2 pellets) **! CAUTION** CO_2 is an asphyxiant and may displace oxygen, causing rapid suffocation. Handle in a well-ventilated fume hood.
- $CDCl_3$ (deuterochloroform (D, 99.8%), Cambridge Isotope Laboratories, cat. no. DLM-7-100)
- Methanol-d_4 ((D, 99.8%), Cambridge Isotope Laboratories, cat. no. DLM-24-10)
- Acetone-d_6 ((D, 99.8%), Cambridge Isotope Laboratories, cat. no. DLM-9-10)

Equipment

- Aluminium-backed thin-layer chromatography (TLC) plates impregnated with silica gel 60 F_{254} (cut to size; Supelco, cat. no. 1.05554; or equivalent)
- Screw-capped TLC jar (any empty and clean glass, screw-cap jar with ~100–200-ml volume will be fit for purpose. Alternatively, a 100-ml glass beaker with a lid made from aluminum foil is also suitable.)
- Heat gun (ORS Nasco Milwaukee dual-temperature heat gun; Fisher Scientific, cat. no. 19-313-598; or equivalent)
- Graduated measuring cylinders: 10 ml (Thomas Scientific, cat. no. 1230F44; or equivalent) and 500 ml (Thomas Scientific, cat. no. 1230F49; or equivalent)
- pH paper (Fisher Scientific, cat. no. 13-640-508; or equivalent)
- Disposable borosilicate glass Pasteur pipettes (Fisher Scientific, cat. no. 13-678-20C; or equivalent)
- Pipette bulbs (1 ml; Fisher Scientific, cat. no. 03-448-21; or equivalent)
- Retort stand and clamps (Eisco four-piece metalware set containing one each of retort stand base, rod, boss head and clamp and retort ring; Fisher Scientific, cat. no. S02631; or equivalent)
- Disposable plastic syringes, various sizes (Henke-Ject two-part syringes: 1 ml, 2 ml Luer lock, 5 ml Luer lock and 50 ml Luer lock,; Henke Sass Wolf, cat. nos. 4010.200V0, 4020.X00V0, 4050.X00V0 and 4850003000, respectively; or equivalent)
- Disposable regular bevel needles, various sizes (BD precision glide hypodermic needle 22G × 1 1/2 inches, BD precision glide hypodermic needle 18G × 1 1/2 inches, and Air-Tite vet premium hypodermic needle 22G × 4 inches,; Air-Tite Products, cat. nos. BD305156, BD305196 and cat. no. N224, respectively; or equivalent)
- Teflon-coated magnetic egg-shaped stirrer bar (32 mm × 16 mm; Fisher Scientific, cat. no. 14-513-53; or equivalent)
- Magnetic stirrer plates (RCT basic stirrer; IKA, cat. no. 0009016401; or equivalent)
- Spatula (Eisco spoon and spatula tool; Fisher Scientific, cat. no. S50789A; or equivalent)
- Analytical balance (Mettler Toledo, Classic plus AB204-S/FACT analytical balance; or equivalent)
- Single-neck round-bottomed flasks, various sizes (100 ml 24/40, 250 ml 24/40, 500 ml 24/40 and 1 liter 24/40; Fisher Scientfic, cat. nos. 10-067C, 10-067E, 10-067G and 10-067H, respectively; or equivalent)
- Rubber septa (DWK Life Sciences Kimble Kontes hollow white rubber sleeve stoppers, 24/40; Fisher Scientific, cat. no. K774261-0024; or equivalent)
- Refrigerator possessing an internal power outlet and Dixell Prime temperature control panel (internal temperature set to −4 °C) (refer to Equipment setup for details and Supplementary Fig. 1, ESI).
- Freezer (internal temperature set to −15 °C) (Danby, model DUF419W; or equivalent)
- Round-bottomed flask support ring (Büchi evaporating flask support ring; Fisher Scientific, cat. no. 05403206; or equivalent)
- Disposable screw-cap 1-dram borosilicate glass vials (VWR, cat. no. 66011-041; or equivalent)
- Separatory funnels with plastic stoppers (Pyrex Squibb 250 and 500 ml; Thomas Scientific, cat. nos. 5246L25 and 5246L28, respectively; or equivalent)
- 600-ml glass beaker (Pyrex low-form Griffin; Sigma-Aldrich, cat. no. CLS1000600; or equivalent)
- 500-ml glass conical flask (Pyrex narrow-mouth graduated Erlenmeyer; Sigma-Aldrich, cat. no. CLS4980500; or equivalent)
- Solid addition funnel (powder funnel, 7.5-cm diameter; Fisher Scientific, cat. no. 10-500-2; or equivalent)
- Glass chromatography column with 5-cm i.d. (Synthware chromatography column with standard taper joint, reservoir and polytetrafluoroethylene stopcock; Fisher Scientific, cat. no. 31500895; or equivalent)
- Disposable borosilicate glass test tubes (28 ml) and rack (18 × 150 mm; Fisher Scientific, cat. no. 14-961-32; or equivalent)
- Rotary evaporator (Büchi Rotovapor R-100; or equivalent)
- Schlenk high-vacuum manifold
- Ultrapure water system (Milli-Q IQ 7000 ultra pure water system; MilliporeSigma, cat. no. ZIQ7000T0; or equivalent)

- Lyophilizer (Büchi Lyovapor L-200; or equivalent)
- NMR spectrometer (we recorded ^{1}H NMR spectra (500 MHz or 400 MHz, as specified) and ^{13}C NMR spectra (125.71 MHz) by using a Bruker AVANCE III 500 spectrometer or a Bruker AVANCE III 400 spectrometer with $CDCl_3$, methanol-d_4 or acetone-d_6 as solvent (as specified))
- NMR tubes (precision 5 mm; Deuterotubes, cat. no. Boro600-5-7; or as is required for your NMR spectrometer)
- High resolution mass spectrometer (we used an Agilent 6210 time-of-flight liquid chromatograph–mass spectrometer)
- Polarimeter (we used a PerkinElmer polarimeter 341 at 589 nm)

Additional equipment specific to the process-scale protocol

- 1,000-ml recovery flask (single-neck evaporating, 29/26 outer joint; Chemglass, cat. no. CG-1512-33; or equivalent)
- 3,000-ml round-bottomed flask (three-neck round-bottomed flask, half jacket, one center neck 29/42 outer, two side necks 24/40 outer, 6-mm drain valve; Chemglass, cat. no. CG-1539-07; or equivalent)
- 5,000-ml round-bottomed flask (three-neck, heavy wall, 45/50–24/40, with drain valve; Chemglass, cat. no. CG-911-A; or equivalent)
- Teflon-coated magnetic egg-shaped stirrer bar (76 × 19.1 mm and 12.7 × 25.4 mm; Fisher Scientific, cat. nos. 16800530 and 14-512-120, respectively; or equivalent)
- Overhead stirrer (J-KEM; cat. no. ohs-1-10mm; or equivalent)
- Glass stirrer shaft (polished glass stirrer shaft, 445 mm; SP Wilmad-LabGlass, cat. no. LG-9501-100; or equivalent)
- Stirring paddle (stirrer blades banana type, PTFE, 10-mm shaft; Ace Glass, cat. no. 8087-05; or equivalent)
- 2,000-ml conical flask (narrow-mouth glass Erlenmeyer; Borosil, cat. no. 4980030; or equivalent)
- Short-path distillation apparatus (distilling head, short path, 10/30 thermometer joint, 24/40 lower inner joint, ~195-mm width × 160-mm height; Chemglass, cat. no. CG-1239-03; or equivalent)
- RediSepRf filter column (125 g; Teledyne ISCO, cat. no. 69-2203-314)
- RediSepRf gold column (220 and 330 g; Teledyne ISCO, cat. nos. 69-2203-422 and 69-2203-330, respectively)
- CombiFlash automated chromatography system (CombiFlash Rf; Teledyne ISCO, cat. no. 69-5233-650)
- Chiller unit (Unistat 405 with Pilot ONE; Huber, cat. no. Unistat 405)
- Digital thermoprobe (Apollo; J-KEM, cat. no. APOLLO-J-S; or equivalent)
- 600-ml filter funnel (Buchner, fine frit; Chemglass, cat. no. CG-1402-28; or equivalent)

Reagent setup

2 M NaOH

Weigh out 8.0 g of NaOH into a 100-ml Pyrex glass bottle. Add 60 ml of ultrapure H_2O and mix gently until the NaOH is fully dissolved. Once the NaOH is completely dissolved, adjust the volume to 100 ml by using ultrapure H_2O. Store with a plastic cap at room temperature (20–23 °C) for ≤6 months.

p-Anisaldehyde TLC stain

Prepare by adding sulfuric acid (8 ml), acetic acid (2.4 ml) and *p*-anisaldehyde (6 ml) to ethanol (218 mL). The solution should be stirred vigorously and then stored in a wide-mouth, screw-capped jar wrapped in aluminum foil for ≤3 months.

Aldehyde starting material

The aldehyde starting material is aldehyde hydrate **2**. Research- and process-scale synthesis procedures for making this starting material are provided; Supplementary Procedures 1 and 2, respectively, and Supplementary Anticipated Results.

Equipment setup

Refrigeration for fluorination

To ensure a stable 4 °C environment for the duration of the fluorination step (overnight, typically 18 h), we set up a dedicated refrigerator containing several stirrer plates for the research-scale

procedure. A refrigerator possessing an internal power outlet and Dixell Prime temperature control panel was used for this purpose (Supplementary Fig. 1, ESI). However, if readers do not have a refrigerator with internal power outlets readily available, then we recommend feeding the power cable of a single stirrer plate through the door of a laboratory refrigerator; the rubber seal of the door should hold closed with the power cable protruding in most cases; however, duct tape may be used to hold the refrigerator door closed if necessary. For the process-scale procedure, a cold room was used to maintain a stable temperature of 4 °C for the duration of the α-FAR reaction.

Procedure: conducted on both research (2.8-g) and process (30-g) scales

Procedure 1: research-scale synthesis of C3′/C5′-acetonide protected uridine (1)

Synthesis of fluorohydrin 4a/b ● Timing Reaction ~2 d, purification ~3 h

1 Remove NFSI from the refrigerator and allow to warm to room temperature before opening (>15 min).

2 Weigh aldehyde hydrate **2** (2.80 g, 16.3 mmol) into a 100-ml round-bottomed flask, add DMF (22 ml) and a Teflon-coated magnetic stir bar and turn on the magnetic stirrer.
? TROUBLESHOOTING

3 Weigh L-proline (1.87 g, 16.3 mmol) and sodium hydrogen carbonate (1.37 g, 16.3 mmol) and add both solids sequentially to the suspension of **2** in single portions. Stopper the flask with a rubber septum.

4 Cool the mixture to 4 °C in the refrigerator with stirring. While the mixture is cooling, weigh NFSI (5.14 g, 16.3 mmol).
? TROUBLESHOOTING

5 Add NFSI portion-wise to the pre-cooled reaction mixture, then replace the rubber septum on the flask, return the reaction mixture to the refrigerator and stir overnight (~18 h).
! CAUTION Addition of NFSI is exothermic and may be accompanied by a temperature change. Ensure that the solution is cooled before adding NFSI. On scales larger than 3 g, we recommend actively cooling the reaction mixture in a 0 °C ice-water bath when adding NFSI.

6 Remove the flask from the refrigerator and add 2,2-dimethyl-1,3-dioxan-5-one (**3**) (1.30 ml, 10.8 mmol). Allow the mixture to come to room temperature and stir the mixture for 24 h.

7 To check completion of the reaction, carry out a 'mini workup' and check the 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 dioxanone **3** to determine completion of the reaction (eluting with 9:1 (vol/vol) dichloromethane/methanol; retention factor (R_f): fluorohydrin **4a/b** = 0.29, dioxanone **3** = 0.54, aldehyde hydrate **2** = 0.28), developing the TLC plate with *p*-anisaldehyde stain (fluorohydrin **4a/b** stains dark brown).
- If the reaction is incomplete, continue stirring until no starting material is observed by TLC.

8 Once complete, dilute the reaction mixture with ethyl acetate (300 ml) and transfer the mixture to a 500-ml separatory funnel, rinsing the round-bottomed flask with ethyl acetate (20 ml).

9 Add water (50 ml) and brine (50 ml), stopper the funnel and mix the layers by gentle shaking, pausing to vent the pressure every few seconds. Allow the layers to separate and drain out the bottom aqueous layer into a 600-ml beaker.

10 Repeat Step 9 three times (combining the aqueous layers).

11 Drain out the organic layer into a clean 500-ml conical flask, rinsing the separatory funnel with ethyl acetate (30 ml).

12 Add sufficient anhydrous sodium sulfate to the organic solution to absorb residual water, swirling the flask as you do so. This can be determined by visualizing clumps of sodium sulfate in the bottom of the conical flask. Continue to add sodium sulfate until clumps stop forming and the added sodium sulfate flows freely in the bottom of the flask.

13 Carefully decant the solution into a 1-liter round-bottomed flask, washing the conical flask and sodium sulfate with ethyl acetate (50 ml).

14 Remove the ethyl acetate on a rotary evaporator at 30 °C to provide a crude amber oil.
■ PAUSE POINT The crude material can be stored in the refrigerator overnight if desired.

15 Pack a chromatography column (5-cm i.d. × 16-cm silica length) by using 9:1 (vol/vol) dichloromethane/methanol.

16 Dissolve the crude material containing fluorohydrin **4a/b** in a minimum amount of 9:1 (vol/vol) dichloromethane/methanol and transfer the solution onto the top of the silica column by using a Pasteur pipette, being careful not to disturb the top of the silica. Allow the crude material to soak down onto the silica.

17 Cover the top of the silica with ~2 cm of sand and elute the column with 9:1 (vol/vol) dichloromethane/methanol by using gentle air pressure into 28-ml test tubes.

18 Identify fractions containing fluorohydrin **4a/b** by using TLC (see Step 7) and combine all pure fractions into a pre-weighed round-bottomed flask.

19 Concentrate the solvent on a rotary evaporator at 30 °C and then place the flask under high vacuum for 2–6 h to remove all residual solvent, providing fluorohydrin **4a/b** as a colorless foam.
? TROUBLESHOOTING

20 Weigh the flask and record the yield of fluorohydrin **4a/b**.

21 Confirm the identity of the product by using HRMS, ^{1}H NMR and ^{13}C NMR.

Synthesis of *syn*-diols 5a/5b ● Timing Reaction setup ~5 h, reaction duration ~17 h, purification ~3 h

22 Weigh fluorohydrin **4a/b** (1.99 g, 6.60 mmol) into a 250-ml round-bottomed flask and add acetonitrile (66 ml) and a Teflon-coated magnetic stir bar. Stopper the flask with a rubber septum and turn on the magnetic stirrer to effect dissolution.

23 Transfer the round-bottomed flask to the freezer and sit upright on a support ring until the solution has cooled to −15 °C (~30 min).

24 While the flask is cooling, weigh $Me_4NHB(OAc)_3$ (5.90 g, 22.4 mmol) into a screw-capped vial.
▲ CRITICAL STEP $Me_4NHB(OAc)_3$ is extremely hygroscopic; do not leave open to the air for more than ~5 s at a time. Weigh the empty capped screw-cap vial, then open the vial and quickly transfer a portion of $Me_4NHB(OAc)_3$ to the vial and recap both the vial and the reagent container. Check the mass of the vial and repeat until the desired mass of reagent has been transferred.

25 Once the solution containing **4a/b** has cooled to −15 °C, remove it from the freezer, reinitiate stirring on a stirrer plate and then add $Me_4NHB(OAc)_3$ in a single portion, followed by acetic acid (2.57 ml, 44.9 mmol) over ~1 min. Continue stirring for ~5 min until a homogeneous solution is obtained.
! CAUTION Addition of $Me_4NHB(OAc)_3$ is slightly exothermic and on large scales may be accompanied by a temperature change. Ensure that the solution is cooled before adding $Me_4NHB(OAc)_3$, and on large scales it may be necessary to actively cool the solution during the addition.
! CAUTION Acetic acid is viscous; use a Luer lock syringe to ensure safe addition.

26 Return the stoppered flask to the freezer and leave at −15 °C for 22 h without stirring.
▲ CRITICAL STEP While continuous magnetic stirring is not necessary, it is important to agitate the solution by vigorous manual swirling for ~30 s every hour for the first 5–6 h of the reaction to effect completion of the reaction.

27 To check completion of the reaction, run a TLC of the reaction mixture spotted next to fluorohydrin **4a/b** (eluting with 9:1 (vol/vol) dichloromethane/methanol; R_f: fluorohydrin **4a/b** = 0.29, *syn*-diols **5a/4b** = 0.20 and 0.15), developing the TLC plate with *p*-anisaldehyde stain (**5a/b** stain green-gray). If the reaction is incomplete, leave the reaction mixture at −15 °C and agitate every hour for ~30 s until TLC indicates complete consumption of the starting material.

28 Once complete, quench the reaction mixture with a saturated aqueous solution of Rochelle's salt (50 ml) and transfer the mixture to a 250-ml separatory funnel, rinsing the round-bottomed flask with dichloromethane (20 ml).
! CAUTION Aqueous quenching of $Me_4NHB(OAc)_3$ generates flammable hydrogen gas. Ensure that appropriate personal protective equipment (laboratory coat, gloves and safety glasses) is worn and that the reaction is quenched in a fume cupboard with adequate ventilation and away from any source of ignition.

29 Add dichloromethane to the separatory funnel (100 ml), stopper the funnel and mix the layers by gentle shaking, pausing to vent the pressure every few seconds. Allow the layers to separate and drain out the bottom organic layer into a 500-ml conical flask.
▲ CRITICAL STEP Ensure that the separatory funnel is vented frequently, because flammable hydrogen gas will be generated through quenching of $Me_4NHB(OAc)_3$, causing pressure to build during the extraction.

30 Repeat Step 29 three times (combining the organic layers).

31 Add sufficient anhydrous sodium sulfate to the organic solution in the conical flask to absorb residual water as described in Step 12.
32 Carefully decant the solution into a pre-weighed 1-liter round-bottomed flask, washing the conical flask and sodium sulfate with dichloromethane (50 ml).
33 Remove the solvent on a rotary evaporator at 30 °C to provide a crude yellow oil and weigh the round-bottomed flask to obtain a crude yield.
PAUSE POINT The crude material can be stored in the refrigerator overnight if desired.
? TROUBLESHOOTING
34 Pack a chromatography column (5-cm i.d. × 18-cm silica length) by using 9:1 (vol/vol) dichloromethane/methanol.
35 Dissolve the crude material containing diols **5a/b** in a minimum amount of 9:1 (vol/vol) dichloromethane/methanol and transfer the resulting solution onto the top of the silica column by using a Pasteur pipette, being careful not to disturb the top of the silica and allow the crude material to soak down onto the silica.
36 Cover the top of the silica with ~2 cm of sand and elute the column with 9:1 (vol/vol) dichloromethane/methanol by using gentle air pressure into 28-ml test tubes.
37 Identify fractions containing *syn*-diols **5a/b** by using TLC (see Step 27) and combine all fractions into a pre-weighed round-bottomed flask. S*yn*-diols **5a** and **5b** can be collected together and used as a mixture in the next step.
38 Concentrate the solvent on a rotary evaporator at 30 °C and then place the flask under high vacuum for 2–6 h to remove all residual solvent, providing *syn*-diols **5a/b** as an off-white foam.
39 Weigh the flask and record the yield of *syn*-diols **5a/b**.
40 Confirm the identity of the products by using HRMS, ^{1}H NMR and ^{13}C NMR.

Synthesis of acetonide protected uridine 1 ● **Timing Reaction duration ~1–5 h**
41 Weigh *syn*-diols **5a/b** (1.05 g, 3.45 mmol) into a 100-ml round-bottomed flask and add acetonitrile (35.5 ml) and a Teflon-coated magnetic stir bar and turn on the magnetic stirrer to effect dissolution.
42 Add 2 M aqueous sodium hydroxide solution (4.6 ml, 9.2 mmol) in a steady stream and stopper the flask with a rubber septum. Stir the solution for 2 h at room temperature (~23 °C).
43 Check completion of the reaction by running a TLC of the reaction mixture spotted next to *syn*-diols **5a/b** (eluting with 9:1 (vol/vol) dichloromethane/methanol; R_f: *syn*-diols **5a/b** = 0.20 and 0.15, uridine **1** = 0.22), developing the TLC plate with *p*-anisaldehyde stain (uridine **1** stains gray–brown). If the reaction is incomplete, leave the reaction mixture stirring for another hour at room temperature or until TLC indicates complete consumption of the starting material
? TROUBLESHOOTING
44 Once complete, add saturated aqueous ammonium chloride solution by using a Pasteur pipette until the pH of the solution reaches 7–8 (as indicated by pH paper) (~7 ml).
45 Work up the reaction and set up the chromatography column by using either method A or B below.
(A) **Aqueous workup: preferred method for small scales (<100 mg) and non-polar analogs** ● **Timing ~2 h**
(i) Transfer the mixture to a 250-ml separatory funnel, rinsing the round-bottomed flask with dichloromethane (20 ml).
(ii) Add dichloromethane to the separatory funnel (80 ml), stopper the funnel and mix the layers by gentle shaking, pausing to vent the pressure every few seconds. Allow the layers to separate and drain out the organic layer into a 500-ml conical flask.
(iii) Repeat Step 45A(ii) two times (combining the organic layers).
(iv) Add sufficient anhydrous sodium sulfate to the organic solution in the conical flask to absorb residual water as described in Step 12.
(v) Carefully decant the solution into a pre-weighed 500-ml round-bottomed flask, washing the conical flask and sodium sulfate with dichloromethane (30 ml).
(vi) Remove the solvent on a rotary evaporator at 30 °C to provide a crude yellow oil and weigh the round-bottomed flask to obtain a crude yield.
PAUSE POINT The crude material can be stored in the refrigerator overnight if desired.
(vii) Pack a chromatography column (5-cm i.d. × 12-cm silica length) by using 9:1 (vol/vol) dichloromethane/methanol.
(viii) Dissolve the crude material containing **1** in a minimum amount of 9:1 (vol/vol) dichloromethane/methanol and transfer the resulting solution onto the top of the silica

column by using a Pasteur pipette, being careful not to disturb the top of the silica and allow the crude material to soak down onto the silica.

(ix) Cover the top of the silica with ~2 cm of sand and elute the column with 9:1 (vol/vol) dichloromethane/methanol by using gentle air pressure into 28-ml test tubes.

(B) **Lyophilization and dry-load: improved yields for larger scales and polar analogs** ● Timing **~17 h**

(i) Depending on the volume of saturated aqueous ammonium chloride solution required for Step 44 above, add water or acetonitrile in the flask so that the approximate ratio of water/acetonitrile in the flask is 1:1 (vol/vol).

(ii) Add silica gel (~6 g) to the solution and stir vigorously for 1 min and then remove the stir bar from the round-bottomed flask.

(iii) Set up a −78 °C cooling bath in a cylindrical low-form vacuum Dewar (15–25-cm diameter) by using dry ice and acetone.

(iv) Freeze the reaction mixture by slowly rotating the round-bottomed flask at a 45° angle in the cold bath for ≥20 min to create a uniform shell coating the inside of the round-bottomed flask.

▲ CRITICAL STEP It is imperative that the reaction mixture be completely frozen before proceeding to the next step. If unsure, submerge the flask in a cylindrical low-form vacuum Dewar filled with liquid nitrogen for ~1 min.

(v) Lyophilize the reaction to obtain a fine off-white powder.

? TROUBLESHOOTING

(vi) Pack a chromatography column (5-cm i.d. × 12-cm silica length) by using 9:1 (vol/vol) dichloromethane/methanol.

(vii) Place a solid addition funnel onto the top of the column and pour in the crude solid powder containing uridine **1**, scraping the sides of the round-bottomed flask with a spatula if necessary to complete the transfer of the material to the top of the column. Gently tap the side of the column with your hand to settle the powder in an even layer.

(viii) Using a Pasteur pipette, gently transfer 9:1 (vol/vol) dichloromethane/methanol (~2–3 ml) dropwise onto the powder on the column until the top is evenly moistened.

(ix) Cover the top of the dry-load material with ~2 cm of sand and elute the column with 9:1 (vol/vol) dichloromethane/methanol by using gentle air pressure into 28-ml test tubes.

46 Identify fractions containing uridine **1** by using TLC (see Step 43) and combine all fractions into a pre-weighed round-bottomed flask.

47 Concentrate the solvent on a rotary evaporator at 30 °C, then place the flask under high vacuum for 2–6 h to remove all residual solvent, and provide uridine **1** as a colorless powder.

48 Weigh the flask and record the yield of uridine **1**.

49 Confirm the identity of uridine **1** by using HRMS, ^{1}H NMR, ^{13}C NMR and optical rotation.

Procedure 2: process-scale synthesis of 3′,5′-acetonide protected uridine NA 1

Synthesis of fluorohydrin 4a/b ● Timing **Reaction ~2 d, purification ~3 h**

1 Charge a 1-neck (29 joint) 1,000-ml round-bottomed flask containing a large Teflon-coated magnetic stir bar with aldehyde hydrate **2** (30.0 g, 174 mmol), $NaHCO_3$ (14.64 g, 174 mmol) and L-proline (20.1 g, 174 mmol).

2 Take the solids up in anhydrous DMF (243 ml), initiate stirring by using a magnetic stirrer and cool the resulting suspension to approximately −4 °C by using a saturated brine/ice bath.

3 Add NFSI (55.0 g, 174 mmol) portion-wise such that the internal temperature does not exceed −2 °C.

! CAUTION Addition of NFSI is exothermic and is accompanied by a temperature change.

4 Stir the resulting mixture in a 4 °C cold room for 21 h.

5 While still in the cold room, add 2,2-dimethyl-1,3-dioxan-5-one (**3**) (14.1 ml, 117 mmol).

6 After an additional 24 h, warm the reaction mixture to room temperature and transfer to a 5-liter extractor vessel containing a bottom drain valve, diluting the mixture with water (1,200 ml).

7 Fit the vessel with an overhead stirrer holding a glass stir rod terminated with a 10-cm stirring paddle.

8 Extract the aqueous DMF solution with ethyl acetate (500 ml).
▲ **CRITICAL STEP** Each extraction used stirring at 450 rpm for 5 min before the phases were allowed to separate.
9 Repeat Step 57 five times, combining the organic layers into two 2,000-ml conical flasks.
10 Transfer the aqueous layer to a conical flask, add 500 g of NaCl, mix vigorously with a metal stir rod and then decant the NaCl-saturated aqueous DMF solution back into the 5-liter extractor vessel.
11 Wash the residual solid NaCl with ethyl acetate (2 × 250 ml) and decant this solution into the extractor vessel.
12 After mixing the resulting biphasic mixture for 5 min at 450 rpm, separate the layers and combine the organic layer with the organic extracts from Step 28.
13 Repeat Step 57 once more.
14 Add sufficient anhydrous sodium sulfate to the combined organic extracts to absorb residual water as described in Step 12.
15 Filter the mixture through a fritted funnel and concentrate the filtrate on the rotary evaporator at 30 °C to afford a colored oil.
■ **PAUSE POINT** The crude material can be stored in the refrigerator overnight if desired.
16 Distill off residual DMF by using a short-path apparatus (pressure = 6–10 mbar; bath temperature = 55 °C).
▲ **CRITICAL STEP** Residual DMF will be present in the crude material, and as such, product of sufficient purity cannot be obtained by column chromatography on silica gel alone because of co-elution with DMF.
17 Take the remaining still pot residue up in 10–15 ml of $CHCl_3$ and load the resulting solution onto a dry 125-g ISCO rediSepRf filter column.
18 Using a Teledyne ISCO automated chromatography system, chromatograph through a 330-g ISCO RediSepRf gold column at 200 ml/min by using a gradient elution of 35:65 to 1:0 (vol/vol) ethyl acetate/hexanes over 10 min and hold at 1:0 (vol/vol) ethyl acetate/hexanes until product finishes eluting.
19 Identify fractions containing fluorohydrins **4a/b** by using TLC (refer to Step 7) and combine all relevant fractions into a pre-weighed round-bottomed flask.
20 Remove all volatiles on the rotary evaporator at 30 °C and dry the resulting off-white foam to a constant weight under high vacuum.
21 Weigh the flask and record the yield of fluorohydrins **4a/b**.
22 Confirm the identity of the product by using HRMS, ^{1}H NMR and ^{13}C NMR.

Synthesis of *syn*-diols 5a/5b ● Timing Reaction setup ~1 h, reaction duration ~48 h, purification ~3 h

23 Connect a 3-neck (24, 24 and 29 joint sizes) 3,000-ml jacketed round-bottom reaction vessel with a bottom drain valve to a Huber chiller unit.
! **CAUTION** In addition to using a standard clamp on the central neck, use a ring clamp to support the bottom of the vessel.
24 Fit the three necks with a septum housing a JKEM thermoprobe (left-most 24 joint), an overhead stirrer using a glass stir rod terminated with a large 10-cm stirring paddle (central 29 joint) and a N_2 inlet (right-most 24 joint).
25 Sweep the vessel with N_2 and turn the chiller on (terminal set to −25 °C).
26 Charge the vessel with acetonitrile (500 ml) and a solution of fluorohydrins **4a/b** (26.8 g, 89 mmol) in acetonitrile (390 ml).
27 Stir the solution (250 rpm) until an internal temperature of −20 °C is obtained.
28 Replace the N_2 inlet with a powder funnel to aid the portion-wise addition of $Me_4NHB(OAc)_3$ (117.0 g, 443 mmol, 4 × 25 g + 1 × 17 g batches). The solid borohydride should quickly dissolve, leaving a homogeneous yellow solution quickly after each addition.
! **CAUTION** Addition of $Me_4NHB(OAc)_3$ is slightly exothermic and will be accompanied by a temperature change. Ensure that the internal temperature of the reaction does not exceed −18 °C.
29 Replace the powder funnel with a 60-ml addition funnel charged with acetic acid (50.7 ml, 887 mmol).
30 Add the acetic acid over a period of 10 min.
31 Increase the internal temperature to −15 °C and stir the solution for 24 h. Check completion of the reaction by TLC as described for Step 27. If the reaction is complete, proceed to Step 83; if not, proceed to the next step.

32 Increase the internal temperature to −10 °C and stir the solution for 24 h. Check completion of the reaction by TLC as described for Step 27. If the reaction is complete, proceed to Step 83; if not, proceed to the next step.
33 Increase the internal temperature to 0 °C and stir the solution for 1 h.
34 Add saturated aqueous Rochelle's salt (600 ml) and dichloromethane (1,500 ml).
! CAUTION Aqueous quenching of $Me_4NHB(OAc)_3$ generates flammable hydrogen gas. Ensure that appropriate personal protective equipment (laboratory coat, gloves and safety glasses) is worn and that the reaction is quenched in a fume cupboard with adequate ventilation and away from any source of ignition.
35 Warm the biphasic mixture to room temperature by setting the chiller to 23 °C and stir rapidly (450 rpm) for 60 min.
36 Allow the phases to separate and collect the organic layer into two 2,000-ml conical flasks.
? TROUBLESHOOTING
37 Extract the aqueous layer with dichloromethane (1,000 ml) by stirring at 450 rpm for 5 min before the phases are allowed to separate. Separate the layers and collect the organic layer into the conical flask.
? TROUBLESHOOTING
38 Add 200 g of NaCl to the aqueous layer.
39 Repeat Step 86 two more times (with 750 ml of dichloromethane).
40 Add sufficient anhydrous sodium sulfate to the combined organic layers to absorb residual water as described in Step 12.
41 Pack a 600-ml glass filter funnel with 250 g of Celite and wet with dichloromethane. Filter the combined organic layers into a pre-weighed round-bottomed flask and concentrate the filtrate on the rotary evaporator at 30 °C to afford a colored solid.
42 Weigh the round-bottomed flask to obtain a crude yield.
■ PAUSE POINT The crude material can be stored in the refrigerator overnight if desired.
? TROUBLESHOOTING
43 Dissolve the residue in a minimal amount of 9:1 (vol/vol) dichloromethane/methanol and load the solution onto a dry 125-g ISCO RediSepRf filter cartridge.
44 Using a Teledyne ISCO automated chromatography system, chromatograph through a 330-g ISCO gold column at 200 ml/min by using a mobile phase gradient of 0:1 to 15:85 (vol/vol) methanol/dichloromethane over 10 min and hold at 15:85 (vol/vol) methanol/dichloromethane until product finishes eluting.
45 Identify fractions containing diols **5a** and **5b** by using TLC (refer to Step 27) and combine all relevant fractions into a pre-weighed round-bottomed flask.
46 Remove all volatiles on the rotary evaporator at 30 °C and then dry the residue to a constant weight under high vacuum to obtain *syn*-diols **5a** and **5b**.
47 Confirm the identity of the product by using HRMS, ^{1}H NMR and ^{13}C NMR.

Synthesis of acetonide protected uridine 1 ● Timing Reaction duration ~3 h, purification ~24 h
48 Charge a 1-neck (29 joint) 1,000-ml round-bottomed flask containing a large Teflon-coated magnetic stir bar with the mixture of **4a/4b** (13.9 g, 45.8 mmol).
49 Add acetonitrile (450 ml) and turn on the magnetic stirrer to effect dissolution.
50 Add a 2 M aqueous solution of sodium hydroxide (61.1 ml, 122 mmol) and stir the solution at room temperature (~23 °C).
51 After 2 h, check completion of the reaction by TLC as described for Step 43.
52 Once complete, quench the reaction by adding saturated aqueous ammonium chloride until a pH of 7–8 is reached (as indicated by pH paper) (~100 ml).
53 Pour the mixture into a 2-liter separatory funnel and collect the organic layer into a 2,000-ml conical flask.
54 With the aid of shaking, saturate the aqueous solution with NaCl (~150 g).
55 Extract the NaCl-saturated aqueous solution with 3:1 (vol/vol) dichloromethane/isopropanol (500 ml), combining all organic layers into a 2,000-ml conical flask.
56 Repeat Step 55 five more times, or until TLC of the aqueous layer indicates that no product remains.
57 Add sufficient anhydrous sodium sulfate to the combined organic layers to absorb residual water as described in Step 12.
58 Filter the solution through a 600-ml glass filter funnel and concentrate the solvent on a rotary evaporator at 30 °C to provide an off-white residue.
■ PAUSE POINT The crude material can be stored in the refrigerator overnight if desired.

59 Take the crude material up in 1:1 (vol/vol) isopropanol/methyl *tert*-butyl ether (160 ml) and stir the resulting slurry at 650 rpm for 30 min.

60 Collect the colorless precipitate in a 600-ml glass filter funnel by using vacuum, transfer the collected solids into a pre-weighed round-bottomed flask and dry to a constant weight under high vacuum (batch 1).

61 Concentrate the filtrate on a rotary evaporator at 30 °C, take the residue up in 1:19 (vol/vol) isopropanol/methyl *tert*-butyl ether (200 ml) and stir the resulting slurry at 650 rpm for 30 min.

62 Collect the colorless precipitate in a 600-ml glass filter funnel by using vacuum, transfer the collected solids into a pre-weighed round-bottomed flask and dry to a constant weight under high vacuum (batch 2).

63 Adsorb the filtrate with the aid of methanol (20 ml) onto silica (4 g of silica per gram of residue) and chromatograph through a 220-g ISCO gold column at 150 ml/min by using a mobile phase gradient of 0:1 to 15:85 (vol/vol) methanol/dichloromethane over 10 min and hold at 15:85 (vol/vol) methanol/dichloromethane until the product elutes.

64 Identify fractions containing acetonide **1** by TLC (refer to Step 43) and combine them in a round-bottomed flask.

65 Remove all volatiles on the rotary evaporator at 30 °C and combine with the solids from batches 1 and 2 in a pre-weighed round-bottomed flask.

66 Weigh the flask and record the yield for uridine **1**.

67 Confirm the identity of uridine **1** by HRMS, ^{1}H NMR, ^{13}C NMR and optical rotation.
? TROUBLESHOOTING

Troubleshooting

Troubleshooting advice can be found in Table 1.

Table 1 | Troubleshooting for the research- and process-scale protocols

Step	Problem	Possible reason	Solution
Procedure 1			
2	The starting material available is the aldehyde, not the hydrate	Reagent availability	The aldehyde can be used interchangeably with hydrate **1**, with no observable consequence to the procedural operation or outcome
4	The starting material does not dissolve in DMF	Hydrate **2** is only sparingly soluble in DMF	Proceed as described. In our experience the reaction will still proceed to completion even when the reaction is a heterogeneous mixture
19	After purification, an oil is obtained instead of a foam/solid	DMF not completely removed during workup/chromatography	Confirm the presence of DMF by ^{1}H NMR. If present, dissolve the oil in ethyl acetate and repeat the workup procedure (Steps 8–14) to remove DMF
33	Crude yield is substantially lower than expected	*Syn*-diol is water soluble, and some material was lost to the aqueous phase during workup	Perform TLC on the aqueous layer to determine whether it contains any *syn*-diol. If so, saturate the solution with NaCl and extract the mixture with dichloromethane (Step 29) until all *syn*-diol is removed from the aqueous phase as determined by TLC
43	After 5 h, the reaction is still incomplete	Uncertain/difficult substrate	Add an additional 2.5 equivalents of 2 M aqueous sodium hydroxide every 5 h until the reaction is determined to be complete by TLC
45B (v)	After lyophilization, the solid is clumpy and/or remains stuck to the walls of the flaks	Uncertain	Scrape the walls of the flask with a spatula, suspend the solid in dichloromethane/methanol (4:1, vol/vol), then remove the solvent on a rotary evaporator at 40 °C and dry the solid under high vacuum. Repeat if necessary until a powder is obtained
Procedure 2			
36	Despite using dichloromethane for the extraction, the top layer appears to be the organic layer	Uncertain	The top yellow layer is the organic phase, and the bottom heterogeneous and milky layer is the aqueous phase

Table continued

Table 1 (continued)

Step	Problem	Possible reason	Solution
37	A clean phase cut is not observed after stirring is ceased	Formation of emulsions	Filter the whole mixture through Celite to remove the milky particulate. Wash the cake with dichloromethane (500 ml) and return the mostly clear biphasic mixture back into the extraction vessel
42	Crude yield is significantly lower than expected	*Syn*-diol is water soluble, and some material was lost to the aqueous phase during workup	Perform TLC on the aqueous layer to determine whether it contains any *syn*-diol. If TLC indicates that product remains in the aqueous layer, extract with 3:1 (vol/vol) dichloromethane/isopropanol (4 × 500 ml). Combine the organic layers and return to Step 89
67	NMR of the final NA shows residual impurities after column chromatography	Coelution with byproducts	Suspend the product in hot isopropanol (80 °C) with stirring and then cool to room temperature and then to 4 °C. After 18 h, collect the colorless precipitate by vacuum filtration (batch A) Concentrate the filtrate and take the residue up in a minimal amount of 1:9 (vol/vol) methanol/dichloromethane and load the solution onto a dry 125-g ISCO RediSepRf filter cartridge and chromatograph through a 220-g ISCO gold column at 200 ml/min by using a mobile phase gradient of 0:1 to 15:85 (vol/vol) methanol/dichloromethane over 10 min and hold at 15:85 (vol/vol) methanol/dichloromethane until the product elutes. Identify fractions containing **1** by TLC (refer to Step 43), combine all relevant fractions into a round-bottomed flask and concentrate on a rotary evaporator at 30 °C (batch B) Combine batches A and B of **1** into a pre-weighed round-bottomed flask and repeat Steps 66 and 67

Timing

Procedure 1: research-scale synthesis of C3′/C5′-acetonide protected uridine (1)

Steps 1–21, synthesis of fluorohydrin **4a/b**: reaction ~2 d, purification ~3 h
Steps 22–40, synthesis of *syn*-diols **5a/5b**: reaction setup ~5 h, reaction duration ~17 h, purification ~3 h
Steps 41–44, synthesis of acetonide protected uridine **1**: reaction duration ~1–5 h
Step 45A, aqueous workup (preferred method for small scales (<100 mg) and non-polar analogs): ~2 h
Step 45B, lyophilization and dry-load (improved yields for larger scales and polar analogs): ~17 h

Procedure 2: process-scale synthesis of 3′,5′-acetonide protected uridine NA 1

Steps 1–22, synthesis of fluorohydrin **4a/b**: reaction ~2 d, purification ~3 h
Steps 23–47, synthesis of *syn*-diols **5a/5b**: reaction setup ~1 h, reaction duration ~48 h, purification ~3 h
Steps 48–67, synthesis of acetonide protected uridine **1**: reaction duration ~3 h, purification ~24 h

Anticipated results

Syn- and *anti*-fluorohydrins 4a* and 4b‡

This white foam is typically obtained as a 1.2:1 diasteromeric mixture (*syn*/*anti*, relative ratio determined by ^{1}H NMR spectroscopic analysis) at a 50–60% yield. Note: uracil *N*-*O* tautomers are also observed in ^{1}H NMR and ^{19}F NMR spectra.

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.33 (br s, 1.2H)*, 9.24 (br s, 1H)‡, 7.73 (d, J = 8.5 Hz, 1.2H)*, 7.65 (d, J = 8.1 Hz, 1H)‡, 6.71–6.62 (m, 2.3H), 5.80 (dd, J = 8.2, 1.7 Hz, 1.2H)*, 5.77 (d, J = 8.4 Hz, 1H)‡, 4.55–4.53 (m, 1H)‡, 4.40 (d, J = 9.2 Hz, 1.2H)*, 4.36–4.31 (m, 3H)‡, 4.15–4.03 (m, 3.6H)*, 3.81 (br s, 1.2H)*, 3.74 (br s, 1H)‡, 1.51 (s, 3H), 1.46 (s, 3H), 1.44 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 211.0 (C), 208.4 (C), 163.2 (C), 163.0 (C), 150.5 (C), 149.9 (C), 141.7 (d, J = 4.5 Hz, CH), 141.0 (CH), 103.3 (CH), 102.5 (CH), 102.1 (C), 101.8 (C), 90.7 (d, J = 207.5 Hz, CH), 90.3 (d, J = 210.0 Hz, CH), 73.8 (d, J = 4.5 Hz, CH), 71.6 (CH),

70.5 (d, J = 25.3 Hz, CH), 70.3 (d, J = 19.7 Hz, CH), 66.6 (CH_2), 66.5 (CH_2), 23.73 (CH_3), 23.67 (CH_3), 23.60 (CH_3), 23.4 (CH_3).

^{19}F NMR (470 MHz, $CDCl_3$): δ –162.0, –178.6.

IR (neat, cm^{-1}) 3223, 1679, 1457, 1379, 1222, 1083, 813, 728.

HRMS (EI+) calculated for $C_{12}H_{16}FN_2O_6$ $[M+H]^+$ 303.0987; found: 303.0963.

Syn-diols 5a/b

This white foam is typically obtained as a 1.2:1 diasteromeric mixture (*syn*/*anti*, relative ratio determined by ^{1}H NMR spectroscopic analysis) at a 50–60% yield.

NMR data for *syn*-diol, *syn*-fluorohydrin **5a**: ^{1}H NMR (500 MHz, methanol-d_4): δ 7.75 (d, J = 8.1 Hz, 1H), 6.46 (dd, J = 44.4, 4.9 Hz, 1H), 5.73 (d, J = 8.0 Hz, 1H), 4.03 (ddd, J = 18.3, 7.0, 5.0 Hz, 1H), 3.82 (dd, J = 11.4, 5.0 Hz, 1H), 3.71 (m, 2H), 3.60 (dd, J = 11.2, 8.9 Hz, 1H), 1.42 (s, 3H), 1.28 (s, 3H).

^{13}C NMR (125 MHz, methanol-d_4) δ 165.8 (C), 151.7 (C), 143.1 (d, J = 2.6 Hz, CH), 102.9 (CH), 100.1 (CH), 94.3 (d, J = 208.4 Hz, CH), 74.6 (d, J = 24.6 Hz, CH), 73.7 (d, J = 4.5 Hz, CH), 67.3 (CH), 65.3 (CH_2), 28.3 (CH_3), 19.7 (CH_3).

NMR data for *syn*-diol, *anti*-fluorohydrin **5b**: ^{1}H NMR (500 MHz, methanol-d_4) δ 7.90 (d, J = 8.2 Hz, 1H), 6.71 (dd, J = 44.0, 6.2 Hz, 1H), 5.74 (d, J = 8.1 Hz, 1H), 4.33–4.32 (m, 1H), 3.82–3.81 (m, 3H), 3.60–3.58 (m, 1H), 1.43 (s, 3H), 1.32 (s, 3H).

^{13}C NMR (125 MHz, MeOD) δ 165.8 (C), 152.2 (C), 143.0 (CH), 103.2 (CH), 100.2 (CH), 92.6 (d, J = 204.4 Hz, CH), 75.9 (d, J = 2.8 Hz, CH), 71.5 (d, J = 29.1 Hz, CH), 65.7 (CH), 64.5 (d, J = 2.2 Hz, CH_2), 28.5 (CH_3), 19.5 (CH_3).

IR (neat, cm^{-1}) 3384, 1674, 1458, 1380, 1268, 1199, 1069, 861, 814, 761.

HRMS (EI+) calculated for $C_{12}H_{18}FN_2O_6$ $[M+H]^+$ 305.1143; found: 305.1131.

Uridine 1

This white amorphous solid is typically obtained at a 60–80% yield (research scale) or 48% yield (process scale).

$[\alpha]_D$ = +10.2 (c 0.55 in methanol at 20 °C).

^{1}H NMR (400 MHz, acetone-d_6) δ 7.70 (d, J = 8.1 Hz, 1H), 5.81 (s, 1H), 5.61 (d, J = 8.3 Hz, 1H), 4.68 (d, J = 3.7 Hz, 1H) 4.46–4.44 (m, 1H), 4.20 (dd, J = 9.9 Hz, 4.8 Hz, 1H), 4.12 (dd, J = 9.9, 9.9 Hz, 1H), 3.90 (dd, J = 9.9, 4.5 Hz, 1H), 3.87 (ddd, J = 10.0, 9.7, 4.7 Hz, 1H), 1.57 (s, 3H), 1.43 (s, 3H).

^{13}C NMR (125 MHz, acetone-d_6) δ 164.1 (C), 151.8 (C), 142.4 (CH), 103.4 (CH), 102.3 (C), 94.5 (CH), 75.2 (CH), 74.6 (CH), 72.5 (CH), 66.0 (CH_2), 33.0 (CH_3), 22.8 (CH_3).

IR (neat, cm^{-1}) 3406, 2999, 1470, 1683, 1375, 1270, 1140, 1032, 842, 828.

HRMS (EI+) calculated for $C_{12}H_{17}N_2O_6$ $[M+H]^+$ 285.1081; found: 285.1079.

Data availability

The data described in Anticipated results were obtained from the materials prepared by using the protocol described herein and are concordant with the data that we have previously reported for compounds **4a/b**, **5a/b** and **1** (ref. [9]).

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Acknowledgements

The authors acknowledge Michael Smith Health Research BC for financial support in the form of a Research Trainee Award (E.K.D.). R.B. acknowledges support from the Canadian Glycomics Network (Strategic Initiatives Grant CD-81); the Consortium de Recherche Biopharmaceutique (CQDM Quantum Leap Grant); Merck & Co., Inc.; and the Natural Sciences and Engineering Research Council (NSERC) of Canada (Discovery Grant, RGPIN-2019-064680).

Author contributions

E.K.D. optimized the research-scale protocol and wrote the manuscript and supporting information. D.A.P. optimized the process-scale protocol and wrote the associated procedures. M.M. carried out initial discovery efforts for this protocol. M.B.N. optimized the synthesis of the aldehyde precursors. S.M.S., L.-C.C. and R.B. supervised the project.

Competing interests

Simon Fraser University and Merck & Co., Inc. have filed a patent application describing the synthesis of nucleoside analogs via the process presented in this manuscript—U.S. provisional patent application No. 62/994,349.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41596-022-00705-7.

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Peer review information *Nature Protocols* thanks Yongguirobin Chi and Henning Jacob Jessen for their contribution to the peer review of this work.

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Received: 1 November 2021; Accepted: 28 March 2022;
Published online: 4 July 2022

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