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Stereoselective synthesis of highly functionalised arabinosyl nucleosides through application of a *N*-nitro protecting group

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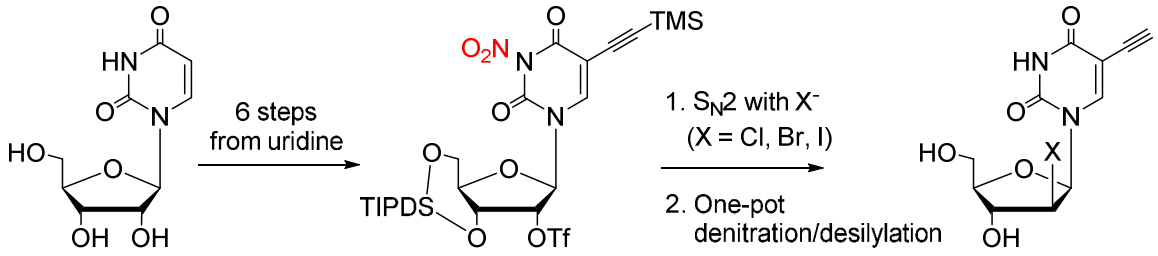


Abstract

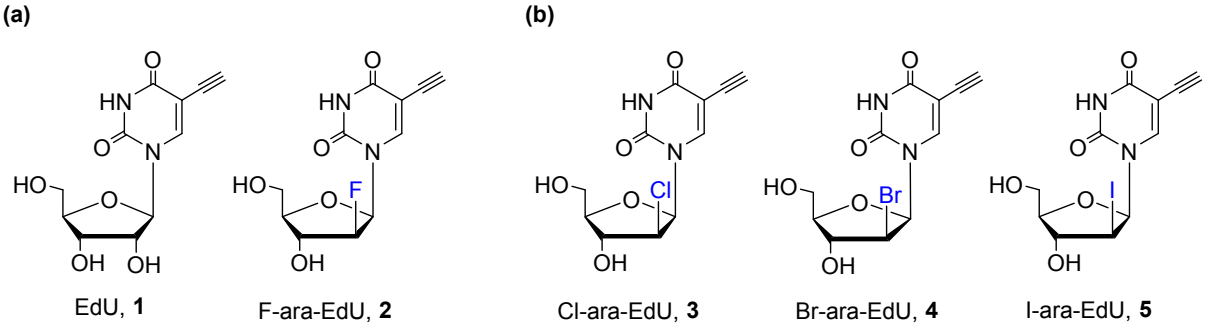
2′-Deoxy-2′,5-disubstituted arabinosyl uridine derivatives bearing a halogen (Cl, Br or I) at C2′ and an ethynyl group at C5 have been synthesized in 6 steps from 2′,3′,5′-tri-*O*-acetyl-5-iodo-uridine in overall yields 61% (compound **3**, Cl), 47% (compound **4**, Br), 19% (compound **5**, I). Stabilisation of a 2′-*O*-triflyl leaving group intermediate to overcome spontaneous intramolecular 2,2′-anhydro uridine formation was pivotal to the synthesis. Specifically, to favor S_N2 reaction with a halogen nucleophile over intramolecular cyclisation, the nucleophilicity of O-2 oxygen was reduced by incorporation of an adjacent electron withdrawing nitro substituent at N-3. The introduction of the 3-*N*-Nitro group proceeded rapidly (nitronium trifluoroacetate, 1 min) and in quantitative yield. A one-pot method to remove the 3-*N*-nitro group by reductive nitration (zinc metal in acetic acid, 5 min) and the silyl protecting groups of the alkyne and 3′,5′ hydroxyls (fluoride reagent, 16 h) was established as the final synthetic step. This application of the 3-*N*-nitro protecting group addresses the significant shortfalls of the conventional approach to synthesis of 2′ modified nucleosides, wherein condensation of a 2′ modified sugar fragment with a pyrimidine base provides poor stereocontrol of *N*-glycosylation, low yields and incompatibility with 2′ iodo sugars.

Introduction

Nucleoside derived chemical probes are small molecule reagents that have had a profound impact on our ability to answer fundamental questions about DNA synthesis, a process central to all proliferating cells. An exemplar nucleoside chemical probe is 5-ethynyl-2′-deoxyuridine (EdU, **1**), Figure 1(a), wherein the 5-ethynyl group of **1** replaces the 5-methyl group of thymidine.[1] When proliferating mammalian cells are treated with **1** this probe molecule is incorporated into DNA. Subsequent conjugation of the alkyne moiety in the modified DNA to a fluorescent azide molecule enables detection of cellular DNA using fluorescence microscopy.[1] The cytotoxicity of **1** does however restrict its applications to short exposure and low dose experiments[2-6] such that next generation chemical probes with improved properties are desirable to extend our current capabilities for studying DNA synthesis. Luedtke and colleagues reported that the mammalian cell toxicity of **1** is mitigated (i.e. little/no cellular arrest for up to 60 days treatment) by modification of the ribose of **1** to the fluorinated arabinosyl derivative, (2′*S*)-2′-deoxy-2′-fluoro-5-ethynyluridine (F-ara-EdU, **2**) Figure 1(a).[7] Probe compound **2** retains DNA labelling capability in proliferating cells similarly to **1**. The 2′-halo-ara-EdU analogues Cl-ara-EdU (**3**), Br-ara-EdU (**4**) and I-ara-EdU (**5**) are not known, but may also have potential as next generation chemical probes or as therapeutics, Figure 1(b). Herein we describe the stepwise development of a stereoselective synthesis of 2′-halo-ara-EdU analogues **3-5**.

2′-Deoxy-2′-substituted arabinosyl nucleosides are not found in nature. The conventional synthesis of 2′ modified nucleosides proceeds by condensation of the sugar fragment with a purine or pyrimidine base. Poor stereocontrol of this *N*-glycosylation reaction, irrespective of the anomeric configuration of the sugar precursor, is a well-known flaw of this synthetic

method. For example, the reported synthesis of 2′-deoxy-2′-fluoro-arabinosyl pyrimidine nucleosides from 2′-deoxy-2′-fluoro-3′,5′-bis-acyl-α-D-arabinofuranosyl bromide proceeds to form the nucleoside product as a mixture of α and β anomers.[8,9] Moreover, reaction yields with 2′-chloro-2′-deoxy-arabinosyl or 2′-bromo-2′-deoxy-arabinosyl sugars are considerably reduced compared to the 2′-deoxy-2′-fluoro-arabinosyl sugar, while there are no reports of this reaction with a 2′-deoxy-2′-iodo-arabinosyl sugar.[10,11] Reported syntheses of 5-substituted-2′-deoxy-2′-halo-arabinosyl pyrimidine nucleosides are even fewer than that for 2′-deoxy-2′-halo-arabinosyl pyrimidine nucleosides but are approached similarly. Stereocontrol of the *N*-glycosylation reaction of the 2′ sugar fragment with the 5-substituted pyrimidine remains a problem, while the reactions are slower than with the corresponding unsubstituted pyrimidines and typically require many days to reach completion.[7,11,12] Thus it was anticipated that employing this sugar-base condensation approach to the synthesis of the target 2′-halo-ara-EdU compounds **3-5**, even if successful, would suffer from poor stereocontrol, extended reaction times and low yields. The motivation for the current study was to overcome the existing obstacles inherent in approaches to 2′ modified nucleosides to provide a reliable synthetic methodology to the synthesis of 2′,5-bisfunctionalised nucleosides, target compounds **3-5**.

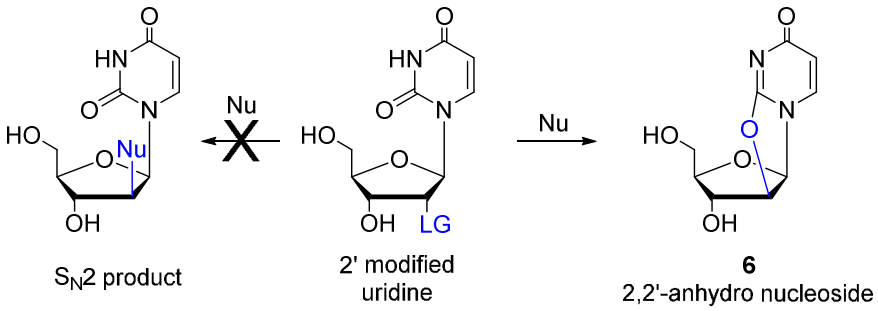


EdU, **1** F-ara-EdU, **2** Cl-ara-EdU, **3** Br-ara-EdU, **4** I-ara-EdU, **5**

Figure 1. (a) Nucleoside chemical probes in current use: 5-ethynyl-2′-deoxyuridine (EdU, **1**) and (2′*S*)-2′-deoxy-2′-fluoro-5-ethynyluridine (F-ara-EdU, **2**). (b) Target compounds: 2′-halo modified EdU nucleosides: Cl-ara-EdU, **3**; Br-ara-EdU, **4**; and I-ara-EdU, **5**.

Results and Discussion

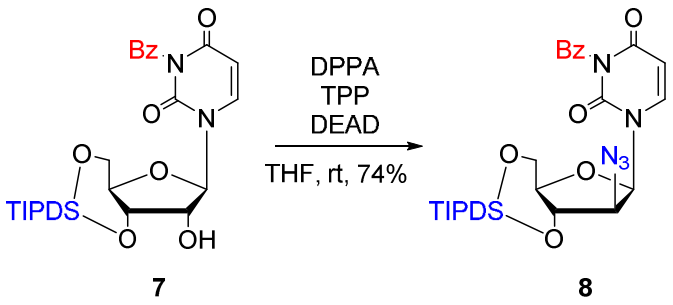
In principle, an alternative synthesis of 2′-deoxy-2′-halo-arabinosyl pyrimidines is S_N2 displacement of an activated 2′-hydroxyl group on the native pyrimidine scaffold with a halide ion nucleophile. Here the *N*-glycosidic bond configuration is both predefined and maintained, while an inversion of configuration at 2′ is expected. In practice however this S_N2 product does not form and instead an intramolecular cyclisation proceeds to the 2,2′-anhydro nucleoside **6**, Scheme 1.



Scheme 1. Formation of 2,2′-anhydrouridine **6** by intramolecular cyclisation of a 2′ leaving group (LG) modified uridine is favoured over S_N2 displacement. Nu = nucleophile (e.g. halide anion).

An approach to favour S_N2 over intramolecular cyclisation is to decrease the nucleophilicity of the precursor pyrimidine nucleoside O-2 oxygen by incorporating an electron withdrawing substituent on N-3. The first successful application of this approach was accomplished by utilising a benzoyl group at N-3 with azide anion as the nucleophile. Displacement of the 2′ hydroxyl of 3-*N*-benzyol-3′,5′-*O*-tetraisopropyldisiloxane-1,3-diyl)-uridine **7** with the azide ion and inversion of configuration was achieved under Mitsunobu conditions, Scheme 2. [13]

The desired S_N2 product, arabinosyl derivative **8** was formed as product.[13] To the best of our knowledge no attempt to perform a S_N2 displacement on **7** with other nucleophiles under these conditions has since been reported. Furthermore, attempts to isolate the 2′-triflate derivative of **7** were also unsuccessful, this derivative is not stable and converts to the 2,2′-intramolecular cyclisation product.[14]

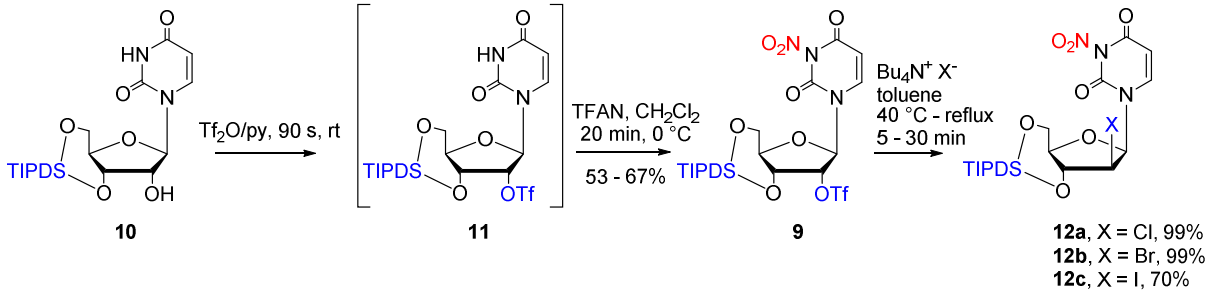


7 **8**

Scheme 2. Synthesis of 2′-deoxy-2′-azido-arabinosyl uridine derivative **8** by displacement of the 2′ hydroxyl of 3-*N*-benzoyl protected uridine precursor **7** with azide ion under Mitsunobu conditions. TPP: triphenylphosphine, TIPDS: tetraisopropyldisilyloxane, DPPA: diphenyl phosphoryl azide.

A more detailed exploration of this concept found that a nitro group at N-3 in combination with a triflate moiety as the 2′ leaving group overcame competing intramolecular cyclisation. The introduction of the 3-*N*-nitro protecting group on to the native pyrimidine bases, uracil and thymine uses nitronium trifluoroacetate (TFAN)[14,15] as the nitrating reagent. TFAN is generated from TFAA and a nitrate salt such as NH_4NO_3. There is little known about the mechanism of nitration in pyrimidine systems, while the mechanism of nitration of purines using TFAN is disputed; with both ionic[16] and radical[17,18] processes proposed. The 3-*N*-nitro, 2′-triflate nucleoside **9** is a stable compound and is reported to undergo reaction with

chloride, bromide or iodide ions via a S_N2 displacement mechanism to furnish the corresponding 3-*N*-nitro-2′-deoxy-2′-halo-arabinosyl uridines **12a-12c** in high yield, Scheme 3.[14] However subsequent denitration of **12a-12c** is not reported. To synthesise **9**, the precursor nucleoside **10** is first treated with pyridine and Tf_2O to briefly form the 2′ triflate intermediate **11**, which is then trapped *in situ* by 3-N-nitration with added TFAN. In our hands attempts to reproduce the synthesis of **9** gave inconsistent results and were not successful. We note that the literature specified significant care with addition of TFAN (time, rate of addition and temperature) to successfully trap intermediate **11** without degradation first occuring.[14]

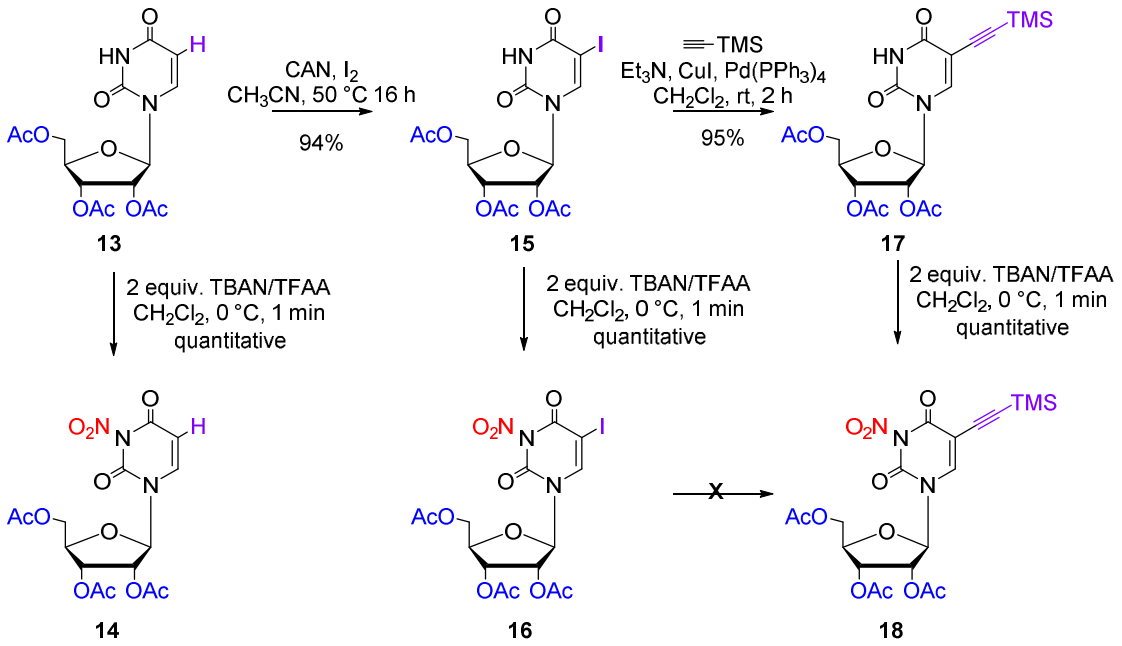


Scheme 3. Synthesis of 3-*N*-nitro-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-triflyl-uridine **9** from 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine **10** and subsequent S_N2 displacement of the 2′-triflate with halide ions to yield 2′-deoxy-2′-halo-3-*N*-nitro-3′,5′-*O*-tetraisopropyldisiloxane-1,3-diyl)-arabinofuranosyl uridines **12a-12c**.[14] Reproduced with permission from Tetrahedron Lett. Vol. 39. Copyright 1998 Elsevier.

The application of a 3-*N*-nitro group to our target nucleoside derivatives **3-5** necessitates that conditions for the introduction and removal of this group are compatible with either a 5-iodo

or 5-ethynyl group in the nucleoside precursor. To evaluate if introduction of a 3-*N*-nitro group is feasible with these groups in place 2′,3′,5′-tri-*O*-acetyl-5-iodo-uridine **15** and 2′,3′,5′-tri-*O*-acetyl-5-(ethynyl(2-trimethylsilyl))-uridine **17** were treated with the TFAN reagent. As a positive control for the reaction we employed nitration conditions known for 2′,3′,5′-tri-*O*-acetyl-uridine **13** (8 equiv. KNO_3/TFAA, 20 min, 0 ^oC, CH_2Cl_2).[15] The reaction with **13** gave the expected 3-*N*-nitro compound **14** in quantitative yield, consistent with the literature.[15] The desired 3-*N*-nitro product **16** was obtained from 5-iodo derivative **15**, however deiodination of **15** was also observed, leading to **14** as a significant side product. Reaction of 5-ethynyl derivative **17** produced a complex mixture of products with no analytical evidence to indicate formation of the desired product **18**. As the TFAN reagent is both an oxidant and is acidic, we speculate that these properties and/or the presence of a radical species[17] may have attributed to the observed deiodination of **15** and the competing side reactions with alkyne **17**.[16] TFAN prepared from TFAA and inorganic nitrate salts requires a 4-8 fold excess of reagent for nitration of pyrimidines to reach completion (~20 min).[19] In contrast, TFAN prepared from TFAA and tetrabutylammonium nitrate (TBAN) is reported as an efficient *C*-nitrating system in purine nucleosides, requiring less than two-fold TFAN excess for rapid and complete reaction.[17,18] When we applied 2 equivalents of TBAN/TFAA to the 5-iodo derivative **15** nitration proceeded to completion within 1 min as evidenced by TLC. This is a remarkable 20-fold faster than with either KNO_3 or NH_3NO_3 as the nitrate source. Furthermore no deiodination product **14** was observed. Similarly, the reaction of **17** with TBAN/TFAA (2 equiv. TBAN/TFAA, 1 min, 0 ^oC, CH_2Cl_2) also proceeded rapidly, with full conversion to **18**, as evidenced by TLC. The reactions were quenched with MeOH and purified directly by silica gel flash chromatography without further workup. The products **16** and **18** were obtained in quantitative yield, Scheme 4. Next, to establish if conversion of 5-iodo derivative **16** to the 5-ethynyl derivative **18** could be achieved, compound **16** was

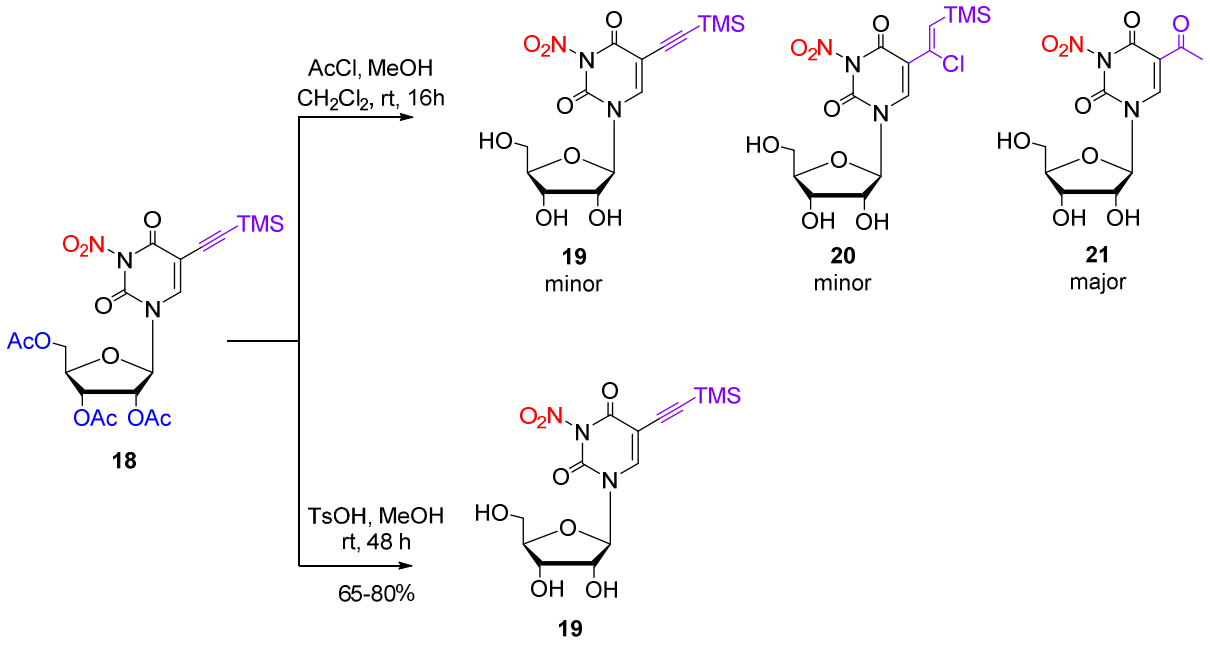
subjected to the Sonagashira reaction, however all attempted conditions failed to give **18** and instead resulted in formation of a complex mixture, Scheme 4. These findings indicated that the 5-ethynyl group of the target compounds **3-5** would need to be in place prior to installation of the 3-*N*-nitro group. Compound **18** was thus the focus for further synthetic development, specifically to ready the ribose moiety for installation of a 2′ leaving group.



Scheme 4. 3-N-Nitration of 2′,3′,5′-tri-*O*-acetyl-uridine **13** and 5-subsituted-2′,3′,5′-tri-*O*-acetyl-uridines **15** and **17**.

The *N*-nitro group is known to be base labile and is incompatible with the more usual basic deacetylation conditions of NaOMe in MeOH or ammonolysis.[15,20] The reported deacetylation conditions for **14** are acidic (HCl catalysed methanolysis[19,21]), however acid catalysed hydration and electrophilic addition of HCl to the 5-ethynyl group of 5-ethynyl uridine derivatives is known[22-25] and this presents a potential hurdle to deacetylation of the TMS protected 5-ethynyl derivative **18**. When compound **18** was subjected to HCl in

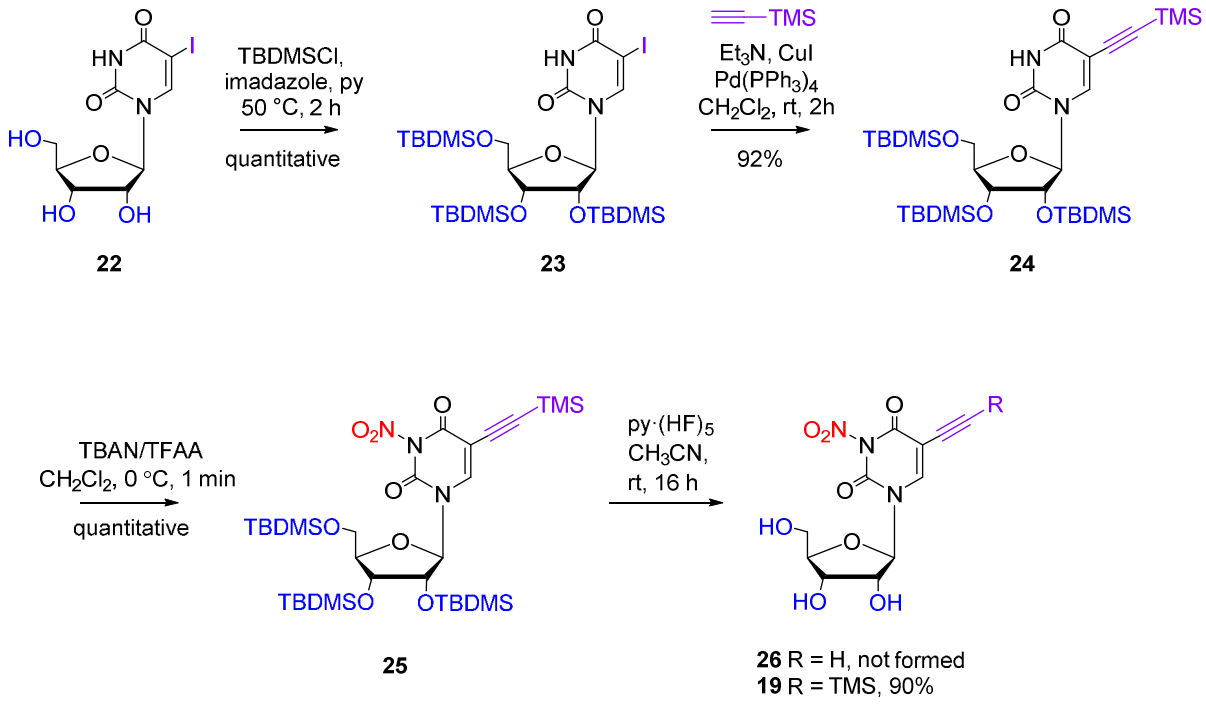
methanol the desired free sugar product **19** was observed by LC-MS only as a minor product. The HCl electrophilic addition product, vinyl chloride **20**, was also detected as a minor product, while the major product is compound **21**, consistent with loss of the TMS group and hydration of the alkyne, Scheme 5. In an endeavour to limit electrophilic addition and/or hydration we investigated deacetylation of **18** using a panel of five sulfonic acids: trifluoromethanesulfonic acid (TfOH); H_2SO_4; methanesulfonic acid (MsOH); *para*-toluenesulfonic acid (TsOH); and polymeric vinylbenzenesulfonic acid (amberlite IRA-120 H^+). The reactions using homogeneous acids produced as the major product, the desired product **19** as evidenced by TLC and LC-MS. The relative reaction rate order as estimated by TLC was H_2SO_4 > TfOH > MsOH > TsOH >> IRA-120 H^+. Side reactions, including loss of the 5-ethynyl TMS group, were comparatively slower than removal of the acetate groups, with all except IRA-120 H^+ providing kinetic control. Reaction workup proved problematic however as neutralisation using either $CaCO_3$ or weakly basic resins (Amberlyst A-21 and Amberlite IRA-67) led to substantial decomposition of **19**. Direct adsorption of the crude reaction mixture onto silica gel also caused substantial product degradation (recovered yield of **19** <20% yield) with the exception of the TsOH reaction, which provided **19** in 65 – 80% yield after purification. We speculate that removal of the solvent under reduced pressure at room temperature during adsorption onto silica concentrated the sulfonic acids and initiated decomposition of **19**, including loss of the ethynyl TMS group. This effect may have been less pronounced with TsOH, which in combination with being the weakest of the acids, gave a solid when concentrated (mp ~105.3 ˚C). The latter heterogeneous solution may have contributed to less degradation of **19** during adsorption onto silica in the presence of TsOH, Scheme 5.



Scheme 5. Deacetylation of nucleoside **18** under acidic conditions to give the target product **19**. Hydration, loss of the TMS group and electrophilic addition of HCl to the 5-ethynyl group gives by-products **20** and **21** using AcCl.

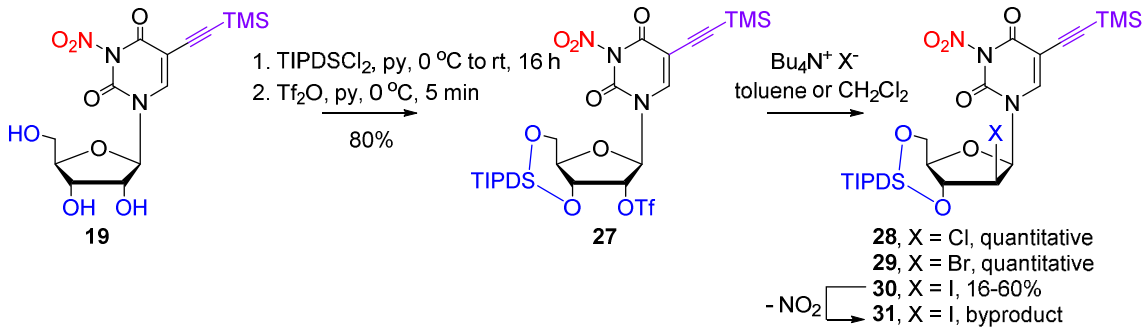
In addition to the acetate group, the TBDMS group has successfully been utilised as a hydroxyl protecting group for 3-N-nitration of uridines.[14,19] The TBDMS protected derivative **23** was prepared from 5-iodo uridine **22** in quantitative yield,[26] Scheme 6. The Sonogashira reaction of **23** with ethynyl-2-trimethylsilane to give **24** followed by 3-N-nitration (2 equiv TBAN/TFAA, 1 min, 0 ^{o}C, CH_2Cl_2) to give **25** both proceeded in excellent yield, Scheme 6. Triethylamine trihydrofluoride ($Et_3N{\cdot}(HF)_3$) was initially chosen for desilylation of **25** as it is considered a mild acidic source of fluoride.[27,28] Indeed, the reaction of **25** with $Et_3N{\cdot}(HF)_3$ yielded the fully desilylated product **26**, although upon purification by solid addition silica gel chromatography complete degradation of **26** was observed. Olah's reagent (~70% HF,

~30% pyridine w/w or py·$(HF)_5$) was employed as an alternative mild fluoride source for desilylation of **25**. The HF in Olah's reagent forms immobile fluorosilicates on addition of the reaction residue to silica gel, enabling the HF to be quenched on workup with silica gel chromatography. This avoids the product degradation found with $Et_3N·(HF)_3$. Unexpectedly the 5-ethynyl TMS group of **25** proved stable towards Olah's reagent and only the TBDMS groups were removed to give **19** as the product, Scheme 6. ^{19}F NMR confirmed no fluorine contamination in **19**. The deprotection of the acetyl groups of **18** and the silyl groups of **25** each gave the free ribose **19** as product. The acetylation pathway was selected in the synthetic route toward the target final compounds **3-5** as the reagents required are less hazardous than handling and disposal of HF reagents.



Scheme 6. Application of the TBDMS group as a 2′, 3′,5′ hydroxyl protecting group for 3-N-nitration of uridines.

With the deprotected ribose nucleoside **19** in hand, the 3′ and 5′ hydroxyl groups of **19** were selectively protected with the tetraisopropyldisiloxane (TIPDS) protecting group and the 2′-hydroxyl converted to the 2′-triflate with trifluoromethanesulfonic anhydride (triflylic anhydride, Tf_2O). This hydroxyl group protection was performed as a one pot procedure and gave **27** in 80% yield, Scheme 7. Similarly to 3-*N*-nitro-2′-triflate **9**,[14] purified **27** was stable in solution at room temperature for >12 h and stable as a solid at 4 ˚C for several weeks. It was expected that **27** would undergo efficient nucleophilic substitution with Cl^-, Br^-, and I^- similarly to triflate **9** (Scheme 3) as they differ only by a TMS protected ethynyl group in position 5 of the pyrimidine ring. Nucleophilic displacement of the 2′-triflate of **27** with tetrabutylammonium chloride (TBAC) or bromide (TBAB) proceeded readily at room temperature in both toluene and CH_2Cl_2 as solvent to give **28** and **29**, respectively, in quantitative yield, Scheme 7. Displacement of the 2′-triflate of **27** with tetrabutylammonium iodide (TBAI) to form the 2′-iodo species **30** gave variable yields. The maximum yield of **30** was 60%, with yields as low as 16% obtained. Decomposition to multiple products (including the denitrated compound **31**) occurred with longer reaction times, higher temperatures or increased equivalents of TBAI, Scheme 7. In an attempt to eliminate the variability in iodination of **27** we assessed alternative iodide sources (Bu_4NI, LiI, NaI, and KI) and solvents (toluene, CH_3CN, acetone, CH_2Cl_2, EtOAc, and THF). Only one combination, TBAI with acetone, provided any rate enhancement. No combinations prevented formation of I_2 and denitration to give **31** as evident by TLC. Consequently, we continued with TBAI in toluene and kept reaction times to a minimum to favour the production of **30** over **31**.

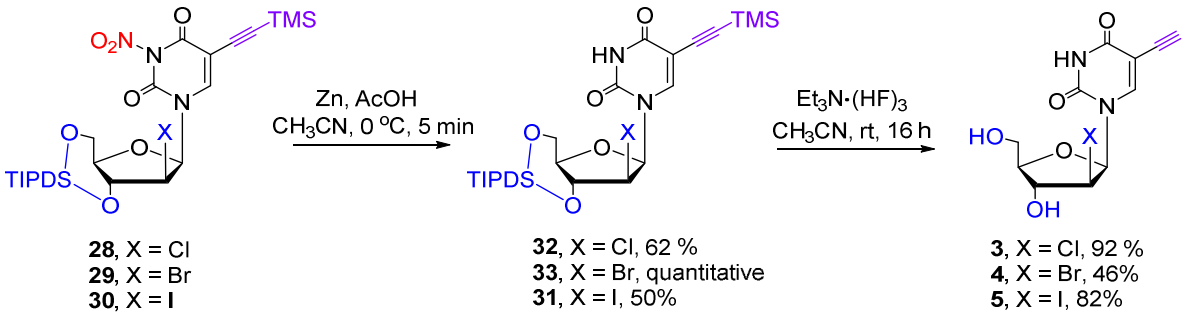


Scheme 7. One-pot conversion of deprotected ribose nucleoside **19** to 2′-triflate precursor **27** followed by nucleophilic substitution and inversion of configuration of the 2′-triflate with tetrabutylammonium halide salts to give target 2′-halo compounds **28-30**.

There are three reported methods for denitration of 3-*N*-nitro-uridines: catalytic hydrogenation over palladium,[14] NaI in acetone,[20] and Bu_3SnH with AIBN.[29] Each method has drawbacks for application to our compounds including (i) potential for reduction of the alkyne and halides of our compounds using hydrogenation, (ii) NaI in acetone is a slow reaction (c.a. 24 hours) where side reactions are prevalent with time causing a reported lack of reproducibility, and (iii) use of Bu_3SnH and AIBN is described for radical reductive elimination of 2′-deoxy-2′-halo-uridines, this would be an unwanted competing reaction.[30] We thus sought to develop alternative denitration conditions that would be compatible with both 5-ethynyl and 2′-halo nucleosides.

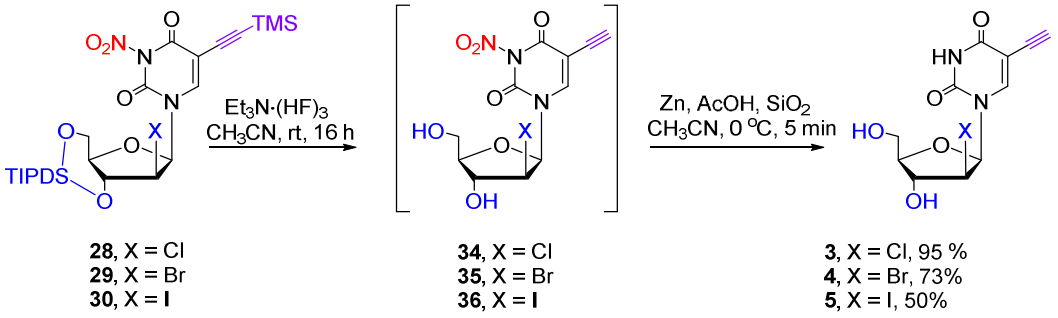
The only reported reduction of the *N*-nitro functionality other than in pyrimidines is the reduction of *N*-nitrourethane and *N*-nitroguanidine using Zn metal in acid.[31-33] Although the reported conditions are compatible with alkyne functionality[34] we proceeded cautiously with

reduction of **28-30** as alkyne **18** was sensitive to HCl. Additionally, reductive elimination at higher temperatures is known to occur with these reagents, posing a potential competing reaction to nitro group reduction.[35,36] We first assessed Zn and Fe with acetic acid in CH_3CN to reduce the nitro group of **14**. Both reactions were successful and gave **13** as product, however with Zn the reaction was rapid (completion within 5 min at 0 ˚C) while with Fe the reaction was incomplete after more than 2 h at room temperature. The conditions of Zn and acetic acid in CH_3CN were next explored for reductive denitration of per-*O*-acetylated TMS-alkyne **18**. This gave the expected denitrated product **17** in quantitative yield. The now optimal reductive denitration conditions were applied to 2′-halo derivatives **28-30** and afforded the expected 2′-halo denitrated compounds **31-33** in good to excellent yields, Scheme 8. The discrepancy between the yield of **32** and **33** was attributed to product loss during the physical manipulation of **32** on workup as both reactions were complete and clean as evidenced by TLC. Some degradation occurred with the iodo compound **30**, which worsened if reaction times were extended or the reaction temperature raised to room temperature. The final step in the synthesis of the target 2′-halo-ara-EdU compounds was removal of the silyl protecting groups of **31-33**. To accomplish this, compounds were treated with $Et_3N{\cdot}(HF)_3$ in CH_3CN followed by aqueous workup and silica gel chromatography to give the corresponding target fully deprotected 2′-halo-ara-EdU derivatives **3**, **4** and **5** in 92%, 46%, and 82% yields respectively, Scheme 8. $Et_3N{\cdot}(HF)_3$ is a more basic fluoride source than Olah's reagent and is able to remove the TMS group. NOESY confirmed correct stereochemistry at the 2′ position, with H-2′/H-4′ and H-3′/H-6 correlations observed, consistent with the desired 2′*S* configuration. Furthermore, absence of correlation between H-2′/H-6 confirms arabinosyl configuration.



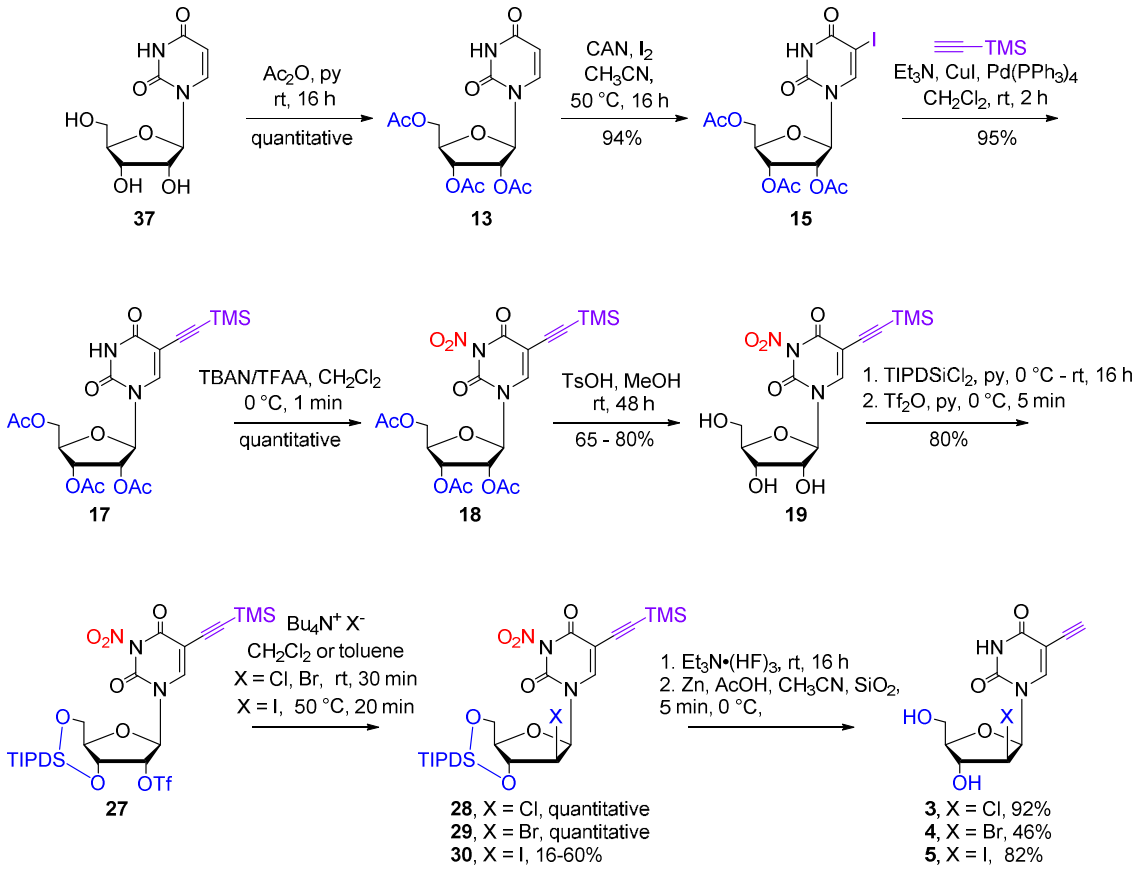
Scheme 8. Reductive denitration of **28-30** followed by desilylation of **31-32** gave the final target compounds **3-5**.

To further improve the overall yield of **3-5** we investigated combining the proceeding two steps of desilylation and reductive denitration in a one-pot reaction, Scheme 9. Desilylation was carried out first using $Et_3N{\cdot}(HF)_3$ in CH_3CN to give the desilylated *N*-nitro intermediates **34-36**. The subsequent addition of excess Zn reduced the nitro group and quenched any remaining fluoride by formation of insoluble ZnF_2. The final products **3**, **4** and **5** were purified by solid addition silica gel flash chromatography without further workup and obtained in 50-95% yield in one-pot, Scheme 9.



Scheme 9. One-pot desilyation and reductive denitration of compounds **28-30** to give the final target compounds **3-5**.

The overall 8-step synthetic approach developed to access the target 2′-halo-ara-EdU analogues, compounds **3**, **4** and **5** from uridine **37**, is shown in Scheme 10. This synthesis required the development of a 3-*N*-nitro protecting group strategy to overcome 2,2′-anhydro nucleoside formation and that was compatible with a C5-alkyne and late-stage 2′ nucleophilic displacement. The 3-*N*-nitro protecting group in combination with the 2′-triflate leaving group are required to install a halogen at 2′ with inversion of configuration. This has not been established previously with a substituent at the 5-position of uridine or with a 2′ iodo group. To successfully utilise the 3-*N*-nitro protecting group strategy we developed new synthetic methodology for its installation and removal that was both rapid (minutes) and compatible with the multi-functionalised uridine scaffold. The final synthesis proceeds in 6 steps from the commercially available **15** to give arabinosyl EdU analogues in overall yields 61%, 47%, 19% for **3**, **4** and **5**, respectively. Yields for the 8 step synthesis from the natural product uridine **37** are similar as acetylation and iodination proceed with high efficiency to give **13** and **15**, respectively. In summary, in the course of this synthesis we have developed several synthetic methodologies and protecting group strategies that we anticipate will be a welcome addition to a synthetic chemist's repertoire[37] when tackling functionalised nucleosides or other important nitrogen containing compounds.



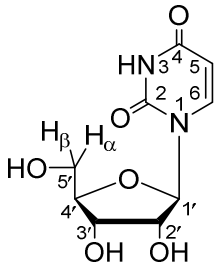
Scheme 10. Overall synthesis developed for target 2′-halo-ara-EdU **3**, **4** and **5** compounds commencing from uridine **37**.

Experimental Section

General Chemistry

All reactions were carried out in dry solvents under anhydrous conditions unless otherwise stated. All chemicals were purchased from commercial suppliers and used without further purification. All reactions were monitored by TLC using silica plates with visualisation of eluted bands by UV fluorescence (λ = 254 nm) and charring with vanillin stain (6 g vanillin in 100 mL of EtOH containing 1% v/v 98% H_2SO_4). Silica gel flash chromatography was performed using silica gel 60 Å

(230-400 mesh). NMR (^{1}H, ^{13}C, ^{19}F, COSY, NOESY, HSQC and HMBC) spectra were recorded on a Bruker AVANCE III HD 500 MHz NMR spectrometer equipped with a BBO probe at 25 ˚C. Chemical Shifts for ^{1}H and ^{13}C NMR obtained in DMSO-d_6 are reported in ppm relative to residual solvent proton (δ = 2.50 ppm) and carbon (δ = 39.5 ppm) signals, respectively. Chemical Shifts for ^{1}H and ^{13}C NMR obtained in $CDCl_3$ are reported in ppm relative to residual solvent proton (δ = 7.26 ppm) and carbon (δ = 77.2 ppm) signals, respectively. Signal splitting multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), br (broad signal). Coupling constants are reported in hertz (Hz). LRMS (ESI) data were acquired on a waters ZQ or Thermo Fisher MSQ Plus single quadruple ESI mass spectrometers using electrospray as the ionisation technique in positive and/or negative mode as stated. HRMS data were acquired on a 12 T SolariX XR FT-ICR-MS using electrospray as the ionisation technique in positive-ion and/or negative mode as stated. All MS analysis samples were prepared as solutions in either methanol or acetonitrile. Purity of the compounds were >95% as determined by Thermo Fisher Dionex Ulitmate 3000 series HPLC via UV detection at 254 nm. The melting points are uncorrected. Proton and carbon atom numbering for NMR chemical shifts are designated as shown below for uridine (**37**).



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General procedure 1. *N*-nitration of uridine nucleosides with TBAN/TFAA

Uridine derivative and TBAN (2 equiv) were combined dry and placed under an atm of Ar. The mixture was dissolved in anhydrous CH_2Cl_2 (approx. 3.4 mL/mmol) with vigorous stirring. The resulting mixture was cooled in an ice bath to 0 ˚C then TFAA (2 equiv) added. After approximately 1 min a few drops of MeOH was added to quench the fuming mixture. The resulting pale yellow

mixture was allowed to warm to rt then applied directly to a preconditioned silica gel flash column chromatography column for purification as described for each compound.

General procedure 2. *N*-Nitration of uridine nucleosides with KNO_3/TFAA

To prepare the nitrating mixture TFAA (8 equiv) was added to powdered KNO_3 (8 equiv) previously cooled to 0 ˚C under an atm of argon and the mixture stirred until a white paste formed (~10 min). Next, the uridine derivative, dissolved in a minimum amount of CH_2Cl_2, was added to the nitrating mixture. The reaction was stirred for 20 min at 0 ˚C or until completion by as evidenced TLC. The reaction mixture was poured into 1.0 M phosphate buffer solution (20 mL, pH ≈7) then CH_2Cl_2 (20 mL) added. The organic and aqueous phases were separated and the aqueous phase extracted with CH_2Cl_2 (3 × 20 mL). The organic extracts were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography as described for each compound.

General procedure 3. Sonogashira coupling of 5-iodo-uridine derivatives with ethynyl-2-trimethysilane

5-Iodo-uridine derivative, CuI (0.1 equiv) and $Pd(PPh_3)_4$ (0.05 equiv) were combined and placed under an atm of argon. The mixture was dissolved/suspended in anhydrous CH_2Cl_2 followed by immediate addition of ethynyl-2-trimethysilane followed by Et_3N. Upon addition of Et_3N the reaction mixture clarifies and develops a yellow-orange in colour. The mixture was stirred at rt until complete consumption of the 5-iodo-uridine derivative, as evidenced by TLC (2 – 3 h). The solvent was removed *in vacuo* and the residue dissolved in EtOAc. The organic phase was washed with 5% $HCl_{(aq)}$ (20 mL), $NaHCO_{3(sat.\ aq)}$ (20 mL), 1.0 M EDTA-Na (3 × 20 mL) and brine, then dried over $MgSO_4$ and concentrated *in vacuo.* The crude product was purified as described for each compound.

General procedure 4. *N*-Denitration of uridine derivatives

The *N*-nitro uridine analogue was dissolved in CH_3CN and cooled to 0 ˚C. Acetic acid (10 equiv), silica gel (~0.8 g/mol) and Zn (5 equiv) were added sequentially. The mixture was stirred vigorously at 0 ˚C until complete conversion of the starting material as evidenced by TLC. The mixture was filtered through cotton wool and a silica plug. The eluant was dissolved in EtOAc (20 mL) and washed with $NaHCO_{3(aq.\ sat.)}$(20 mL) and brine (20 mL) then dried over $MgSO_4$. The crude product was purified by silica gel flash chromatography as described for each compound.

General procedure 5. Desilylation with $Et_3N{\cdot}(HF)_3$

The silylated substrate was dissolved in CH_3CN and $Et_3N{\cdot}(HF)_3$ (10 equiv) added. The reaction was stirred at rt until desilylation was complete as evidenced by TLC. The crude product was purified by silica gel flash chromatography as described for each compound.

General procedure 6. One-pot desilylation and *N*-denitration

The silyl protected *N*-nitro-uridine analogue was dissolved in CH_3CN then $Et_3N{\cdot}(HF)_3$ (10 equiv) added and the mixture stirred at rt. After complete desilylation, as evidenced by TLC, the mixture was cooled to 0 ˚C then acetic acid (20 equiv), silica gel (~0.8 g/mol), and Zn (5 equiv) was added sequentially. After approximately 5 min the mixture was filtered through a plug of cotton wool and silica gel. The crude product was purified by solid addition silica gel flash chromatography as described for each compound.

2′,3′,5′-Tri-*O*-acetyl-3-*N*-nitro-uridine (14)

Compound **14** was synthesised from **13** employing both general procedure 1 and general procedure 2 to give spectroscopically identical compounds (quantitative yields from both methods). The NMR spectra for compounds obtained by both methods were consistent with literature values.[15] ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.44 (d, J = 8.4 Hz, 1H), 5.97 (d, J = 5.0 Hz, 1H), 5.92 (d, J = 8.4 Hz, 1H), 5.39 – 5.28 (m, 2H), 4.42 – 4.30 (m, 3H), 2.14 (s, 4H), 2.14 (s, 3H), 2.12 (s, 3H). LRMS (ESI): *m/z* = 460 $[M - H + HCOOH]^-$.

2′,3′,5′-Tri-*O*-acetyl-5-iodo-3-*N*-nitrouridine (16)

Compound **16** was synthesised from **15** (0.200 g, 0.403 mmol) by two methods: Method A: according to general procedure 1. Method B: according general procedure 2. The crude product was purified by silica gel flash chromatography (Method A: 20% - 60 EtOAc in n-hexane, Method B: 0-20% EtOAc in CH_2Cl_2) to afford the title compound as a colourless solid (general procedure 1: quantitative, general procedure 2: 50 %). Mp = 145-150 ˚C R_f = 0.64 (5% MeOH in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.92 (s, 1H, H-6), 6.05 – 6.01 (m, 1H, H-1′), 5.36 – 5.28 (m, 2H, H-2′ and H-3′), 4.45 – 4.39 (m, 2H, H-4′ and H-5′$_{(\alpha \text{ or } \beta)}$), 4.35 (dd, J = 13.4, 3.3 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 2.24 (s, 3H, AcO), 2.14 (s, 3H, AcO), 2.12 (s, 3H, AcO). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 170.1 (H_3CCOO), 169.8 (H_3CCOO), 169.7 (H_3CCOO), 152.5 (C-4), 145.3 (C-2), 143.2 (C-6), 88.4 (C-1′), 81.1 (C-4′), 73.3 (C-2), 70.2 (C-3′), 66.8 (C-5), 62.9 (C-5′), 21.2 (H_3CCOO), 20.6 (H_3CCOO), 20.5 (H_3CCOO). LRMS (ESI): *m/z* = 586 $[M - H + HCOOH]^-$. HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{15}H_{16}IN_3O_{11}Na$ 563.9721, found 563.9719.

2′,3′,5′-Tri-*O*-acetyl-5-(ethynyl(2-trimethylsilyl))-uridine (17)

Compound **17** was synthesised from **15** (1.700 g, 3.426 mmol) according to general procedure 3. The crude product was purified by silica gel flash chromatography to give the title compound as an off-white foam (1.520 g, 95%). R_f = 0.37 (20% EtOAc in CH_2Cl_2). The NMR spectroscopic data was consistent with literature values.[39, 41] ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 8.93 (br s, 1H, NH), 7.76 (s, 1H, H-6), 6.10 (d, J = 4.9 Hz, 1H, H-1′), 5.36 – 5.28 (m, 2H, H-2′ and H-3′), 4.42 – 4.32 (m, 3H, H-4′ and H-5′$_{(\alpha \text{ and } \beta)}$), 2.21 (s, 3H, AcO), 2.12 (s, 3H, AcO), 2.10 (s, 3H, AcO), 0.21 (s, 9H, $(Si(CH_3)_3)$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 170.2 (H_3CCOO), 169.9 (H_3CCOO), 169.7 (H_3CCOO), 160.8 (C-4), 149.3 (C-2), 142.2 (C-6), 101.6 (C-5), 100.5 (C≡*C*–Si), 95.0 (*C*≡C–Si), 87.3 (C-1′), 80.4 (C-4′), 73.4 (C-2′), 70.3 (C-3′), 63.2 (C-5′), 21.2 (H_3CCOO), 20.7 (H_3CCOO), 20.6 (H_3CCOO), 0.0 ($Si(CH_3)_3$). LRMS (ESI): *m/z* = 465 $[M - H]^-$, 467 $[M + H]^+$, 489 $[M + Na]^+$.

2′,3′,5′-Tri-*O*-acetyl-5-(ethynyl(2-trimethylsilyl))-3-*N*-nitro-uridine (18)

Compound **18** was synthesised from **17** (1.5200 g, 3.2582 mmol) according to general procedure 1. The crude product was purified by silica gel flash chromatography (5% EtOAc in CH_2Cl_2) to afford title compound as a white solid (quantitative). Mp = 135–137 ˚C. R_f = 0.36 (100% CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.78 (s, 1H, H-6), 6.08 – 6.05 (m, 1H, H-1′), 5.36 – 5.32 (m, 2H, H-2′ and H-3′), 4.42 (ddd, J = 2.6, 0.8 Hz, 1H, H-4′), 4.38 (d, J = 2.6 Hz, 2H, H-5′$_{(\alpha \text{ and } \beta)}$), 2.21 (s, 3H, AcO), 2.13 (s, 3H, AcO), 2.12 (s, 3H, AcO), 0.22 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 169.9 (H_3CCOO), 169.7 (H_3CCOO), 169.7 (H_3CCOO), 153.8 (C-4), 144.7 (C-2), 140.9 (C-6), 102.4 (C≡*C*–Si), 101.3 (C-5), 93.2 (*C*≡C–Si), 88.3 (C-1′), 80.9 (C-4′), 73.4 (C-2′), 70.1 (C-3′), 62.9 (C-5′), 21.0 (H_3CCOO), 20.6 (H_3CCOO), 20.5 (H_3CCOO), -0.2($Si(CH_3)_3$). LRMS (ESI): *m/z* = 556 [M – H + HCOOH]$^-$, 534 [M + Na]$^+$. HRMS (ESI+) *m/z* [M + Na]$^+$ calcd for $C_{20}H_{25}N_3O_{11}SiNa$ 534.1150, found 534.1133.

2′,3′,5′-Tri-*O*-(*tert*-butyldimethylsilyl)-5-iodo-uridine (23)

5-Iodo-uridine (**22**) (1.10 g, 2.97 mmol), *tert*-butyl-dimethylsilyl chloride (TBDMSCl) (1.79 g, 11.88 mmol, 4 equiv), and imidazole were combined and placed under an atm of argon. The mixture was treated with a minimum amount of pyridine (~1 mL) to solubilise the starting materials. The resulting mixture was heated to 50 ˚C with stirring for 2 h at which point a single product was evident by TLC. To quench the excess TBDMSCl the mixture was allowed to cool to rt then MeOH (0.5 mL) added with stirring. After 5 min the mixture was dissolved in CH_2Cl_2 and poured into 5% $HCl_{(aq)}$ (20 mL) then extracted with CH_2Cl_2 (3 × 20 mL). The CH_2Cl_2 extracts were combined and washed with 5% $HCl_{(aq)}$ (20 mL). The 5% $HCl_{(aq)}$ (20 mL) phase was extracted with CH_2Cl_2 (20 mL). The combined CH_2Cl_2 extracts were washed with sat. $NaHCO_{3(aq)}$ and brine, then dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography (1-5 % MeOH in CH_2Cl_2) to give the title compound as a colourless foam (quantitative). R_f = 0.17 (100% CH_2Cl_2), 0.42 (5% EtOAc in CH_2Cl_2), 0.81 (40% EtOAc in *n*-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 8.17 (s, 1H, NH), 8.05 (s, 1H, H-6), 6.03 (d, J = 6.7 Hz, 1H, H-1′), 4.11 (dd, J = 6.7, 4.4

Hz, 1H, H-2′), 4.07 – 4.03 (m, 2H, H-3′ and H-4′), 3.90 (dd, J = 11.6, 2.0 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.74 (dd, J = 11.7, 1.9 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 0.99 (s, 9H, $(CH_3)_2SiC(CH_3)_3$), 0.91 (S, 9H, $(CH_3)_2SiC(CH_3)_3$), 0.86 (s, 9H, $(CH_3)_2SiC(CH_3)_3$), 0.20 (s, 6H, $(CH_3)_2SiC(CH_3)_3$), 0.10 (s, 3H, $(CH_3)_2SiC(CH_3)_3$), 0.07 (s, 3H, $(CH_3)_2SiC(CH_3)_3$), 0.03 (s, 3H, $(CH_3)_2SiC(CH_3)_3$), -0.05 (s, 3H, $(CH_3)_2SiC(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 159.7 (C-4), 150.0 (C-2), 144.7 (C-6), 87.6 (C-1′), 86.7 (C-4′), 76.0 (C-2′), 72.8 (C-3′), 68.7 (C-5), 63.3 (C-5′), 26.5 ($(CH_3)_2SiC(CH_3)_3$), 25.9 ($(CH_3)_2SiC(CH_3)_3$), 25.8 ($(CH_3)_2SiC(CH_3)_3$), 18.8 ($(CH_3)_2SiC(CH_3)_3$), 18.2 ($(CH_3)_2SiC(CH_3)_3$), 18.0 ($(CH_3)_2SiC(CH_3)_3$), -4.31 ($(CH_3)_2SiC(CH_3)_3$)), -4.33 (($CH_3)_2SiC(CH_3)_3$)), -4.5 (($CH_3)_2SiC(CH_3)_3$), -4.7 (($CH_3)_2SiC(CH_3)_3$), -4.8 (($CH_3)_2SiC(CH_3)_3$), -4.9 (($CH_3)_2SiC(CH_3)_3$). LRMS (ESI): m/z = 712 $[M + H]^+$, 710 $[M - H]^-$. HRMS (ESI+) m/z $[M + Na]^+$ calcd for $C_{27}H_{53}IN_2O_6Si_3Na$ 735.2148, found 735.2178.

2′,3′,5′-Tri-*O*-(*tert*-butyldimethylsilyl)-5-(ethynyl(2-trimethylsilyl))-uridine (24)

Compound **24** was synthesised from **23** (1.90 g, 2.66 mmol) according to general procedure 3. The crude product was purified by silica gel flash chromatography (Gradient: 10-30% EtOAc in *n*-hexane to afford the title compound as faint yellow foam (1.675 g, 92%). R_f = 0.09 (100% CH_2Cl_2), 0.42 (5% EtOAc in CH_2Cl_2), 0.82 (40% EtOAc in *n*-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 8.06 (s, 1H, NH), 7.95 (s, 1H, H-6), 6.02 (d, J = 6.3 Hz, 1H, H-1′), 4.13 (dd, J = 6.3, 4.4 Hz, 1H, H-2′), 4.06 (dt, J = 7.1, 2.2 Hz, 2H, H-3′ and H-4′), 3.91 (dd, J = 11.6, 2.0 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.74 (dd, J = 11.6, 1.8 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 0.98 (s, 9H, $(CH_3)_2SiC(CH_3)_3$), 0.91 (s, 9H, $(CH_3)_2SiC(CH_3)_3$), 0.86 (s, 9H, $(CH_3)_2SiC(CH_3)_3$), 0.22 (s, 9H, $Si(CH_3)_3$), 0.19 (s, 3H, $(CH_3)_2SiC(CH_3)_3$), 0.18 (s, 3H, $(CH_3)_2SiC(CH_3)_3$), 0.09 (s, 3H, $(CH_3)_2SiC(CH_3)_3$), 0.07 (s, 3H, $(CH_3)_2SiC(CH_3)_3$), 0.03 (s, 3H, $(CH_3)_2SiC(CH_3)_3$), -0.04 (s, 3H, $(CH_3)_2SiC(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 161.0 (C-4), 149.3 (C-2), 142.8 (C-6), 100.7 (C-5), 100.0 (C≡*C*–Si), 95.3 (*C*≡C–Si), 88.0 (C-1′), 86.4 (C-4′), 76.2 (C-2′), 72.6 (C-3′), 63.1 (C-5′), 26.4 ($(CH_3)_2SiC(CH_3)_3$), 25.9 ($(CH_3)_2SiC(CH_3)_3$), 25.8 ($(CH_3)_2SiC(CH_3)_3$), 18.7 ($(CH_3)_2SiC(CH_3)_3$), 18.2 ($(CH_3)_2SiC(CH_3)_3$), 18.0 ($(CH_3)_2SiC(CH_3)_3$), 0.0 ($Si(CH_3)_3$), -4.3 (($CH_3)_2SiC(CH_3)_3$), -4.4 (($CH_3)_2SiC(CH_3)_3$), -4.6 (($CH_3)_2SiC(CH_3)_3$), -4.6

((CH_3)$_2$SiC(CH_3)$_3$), -4.9 ((CH_3)$_2$SiC(CH_3)$_3$), -5.2 ((CH_3)$_2$SiC(CH_3)$_3$). LRMS (ESI): *m/z* = 682 [M - H]$^-$, 684 [M + H]$^+$. HRMS (ESI+) *m/z* [M + Na]$^+$ calcd for $C_{32}H_{62}N_2O_6Si_4Na$ 705.3577, found 705.3553.

2′,3′,5′-Tri-*O*-(*tert*-butyldimethylsilyl)-5-(ethynyl(2-trimethylsilyl)-3-*N*-nitro-uridine (25)

Compound **25** was synthesised from **24** (0.3200 g, 0.4684 mmol) according to general procedure 1. The crude product was purified by silica gel flash chromatography (100% CH_2Cl_2) to afford the title compound as a colourless foam (quantitative). R_f = 0.91 (40% EtOAc in *n*-hexane), 0.88 (100% CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.99 (s, 1H, H-6), 6.02 (d, *J* = 6.3 Hz, 1H, H-1′), 4.14 (dd, *J* = 6.4, 4.4 Hz, 1H, H-1′), 4.09 (q, *J* = 1.9 Hz, 1H, H-4′), 4.06 (dd, *J* = 4.5, 2.0 Hz, 1H, H-3′), 3.93 (dd, *J* = 11.6, 2.1 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.75 (dd, *J* = 11.7, 1.8 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 0.98 (s, 9H, ($CH_3$)$_2$SiC(C*H*$_3$)$_3$), 0.91 (s, 9H, ($CH_3$)$_2$SiC(C*H*$_3$)$_3$), 0.87 (s, 9H, ($CH_3$)$_2$SiC(C*H*$_3$)$_3$), 0.23 (s, 9H, Si($CH_3$)$_3$), 0.19 (d, 6H, (C*H*$_3$)$_2$SiC($CH_3$)$_3$), 0.10 (s, 3H, (C*H*$_3$)$_2$SiC($CH_3$)$_3$), 0.08 (s, 3H, (C*H*$_3$)$_2$SiC($CH_3$)$_3$), 0.03 (s, 3H, (C*H*$_3$)$_2$SiC($CH_3$)$_3$), -0.05 (s, 3H, (C*H*$_3$)$_2$SiC($CH_3$)$_3$)). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 154.4 (C-4), 144.8 (C-2), 141.5 (C-6), 102.1 (C≡*C*–Si), 100.5 (C-5) , 93.6 (*C*≡C–Si), 89.1 (C-1′), 87.2 (C-4′), 76.5 (C-2′), 72.7 (C-3′), 63.1 (C-5′), 26.4 ((CH_3)$_2$SiC(*C*H$_3$)$_3$), 25.9 ((CH_3)$_2$SiC(*C*H$_3$)$_3$), 25.8 ((CH_3)$_2$SiC(*C*H$_3$)$_3$), 18.7 ((CH_3)$_2$Si*C*(CH_3)$_3$), 18.2 ((CH_3)$_2$Si*C*(CH_3)$_3$), 18.0 ((CH_3)$_2$Si*C*(CH_3)$_3$), -0.2 (Si(CH_3)$_3$), -4.3 ((CH_3)$_2$SiC(CH_3)$_3$), -4.4 ((CH_3)$_2$SiC(CH_3)$_3$), -4.6 ((CH_3)$_2$SiC(CH_3)$_3$), -4.7 ((CH_3)$_2$SiC(CH_3)$_3$), -5.0, -5.2 ((CH_3)$_2$SiC(CH_3)$_3$). LRMS (ESI): *m/z* = 746 [M + H_3O]$^+$, HRMS (ESI+) *m/z* [M + Na]$^+$ calcd for $C_{32}H_{61}N_3O_8Si_4Na$ 750.3427, found 750.3433.

5-(Ethynyl(2-trimethylsilyl))-3-*N*-nitro-uridine (19)

Compound **19** was synthesised by two methods**. Method A**: desilylation of **25**. Method B: deacetylation of **18**. Method A: Within a polypropylene tube **25** (0.3000 g, 0.412 mmol) was dissolved in CH_3CN (1.5 mL) and cooled to -40 ˚C in a dry ice/CH_3CN bath. Then py·$(HF)_5$ (0.1606 mL, 6.179 mmol, 15 equiv) previously cooled to -20 ˚C was added to the mixture and allowed to warm to rt. After 18 h at rt the mixture was cooled to -40 ˚C then added directly to a silica gel flash chromatography column preconditioned with 5% MeOH in CH_2Cl_2. The pcolumn was eluted using a

gradient of 5-10% MeOH in CH_2Cl_2 to afford the title compound as an orange/yellow foam (0.1420 g, 90%). Method B: **18** (0.6000 g, 1.173 mmol) and *p*-toluenesulfonic acid (0.127 g, 1.1730 mmol, 1 equiv) were placed under an atm of argon and dissolved in MeOH (3 mL) and a minimum amount of CH_2Cl_2. The mixture was stirred at rt for 48 h then purified by solid addition silica gel flash chromatography (Gradient: 5-8% MeOH in CH_2Cl_2) to give the title compound as a colourless foam/gum (80 %, 0.3602 mg). R_f = 0.59 (10% MeOH in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 8.66 (s, 1H, H-6), 5.66 (d, *J* = 2.9 Hz, 1H, H-1′), 5.60 (d, *J* = 5.0 Hz, 1H, OH-2′), 5.37 (t, *J* = 4.4 Hz, 1H, OH-5′), 5.11 (d, *J* = 6.3 Hz, 1H, OH-3′), 4.14 (td, *J* = 4.8, 2.7 Hz, 1H, H-2′), 4.01 (q, *J* = 6.2 Hz, 1H, H-3′), 3.92 (dt, *J* = 6.6, 2.4 Hz, 1H, H-4′), 3.78 (ddd, *J* = 12.1, 4.5, 2.6 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.61 (ddd, *J* = 12.2, 2.2 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 0.20 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 154.8 (C-4), 145.0 (C-6), 144.5 (C-2), 98.9 (C≡*C*–Si), 97.3 (C-5), 95.5 (*C*≡C–Si), 90.9 (C-1′), 84.6 (C-4′), 74.0 (C-2′), 68.1 (C-3′), 59.1 (C-5′), -0.2 ($Si(CH_3)_3$). LRMS (ESI): *m/z* = 384 $[M - H]^-$, 430 $[M - H + HCOOH]^-$. HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{14}H_{19}N_3O_8SiNa$ 408.0834, found 408.0818.

5-(Ethynyl(2-trimethylsilyl))-3-*N*-nitro-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-triflyl-uridine (27)

Compound **19** (0.300 g, 0.778 mmol) was dissolved in pyridine (2 mL) and cooled to 0 ˚C then 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDSiCl_2) (0.28 mL, 1.1 equiv) added slowly. The reaction was allowed to warm to rt spontaneously overnight, then cooled again to 0 ˚C and Tf_2O (1.0 M CH_2Cl_2 solution) (1.2 mL, 1.5 equiv) added dropwise and stirred for 5 min at 0 ˚C. The mixture was dissolved in EtOAc (20 mL) and washed with 5% $HCl_{(aq)}$ solution (20 mL) and phosphate buffer (20 mL, pH ≈ 7, 1.0 M), with the aqueous phases back extracted with EtOAc (2 × 20 mL). The organic extracts were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography (100% CH_2Cl_2) to afford the title compound as a waxy solid (0.4726 g, 80%). Mp = 98 – 100 ˚C (decomp, darkens at 70 ˚C). R_f = 0.88 (20% EtOAc in *n*-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.92 (s, 1H, H-6), 5.89 (s, 1H, H-1′), 5.23 (d, *J* = 4.2 Hz, 1H, H-2′), 4.45 (dd, *J* = 9.5, 4.3 Hz, 1H, H-3′), 4.33 (d, *J* = 13.9 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 4.17 (dd, *J* = 9.5,

2.5 Hz, 1H, H-4′), 4.03 (dd, J = 14.0, 2.7 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 1.18 – 0.97 (m, 28H, (Si(*i*-Pr)$_2$)$_2$), 0.22 (s, 9H, Si(CH$_3$)$_3$). ^{13}C NMR (125 MHz, CDCl$_3$) δ_C = 153.9 (C-4), 144.2 (C-2), 140.1 (C-6), 102.8 (C≡*C*–Si), 100.9 (C-5), 92.9 (*C*≡C–Si), 88.8 (C-1′), 87.3 (C-2′), 82.7 (C-4′), 66.9 (C-3′), 58.6 (C-5′), 17.6 SiCH(*C*H$_3$)$_2$, 17.6 SiCH(*C*H$_3$)$_2$, 17.4 SiCH(*C*H$_3$)$_2$, 17.3 SiCH(*C*H$_3$)$_2$, 17.0 SiCH(*C*H$_3$)$_2$, 16.9 SiCH(*C*H$_3$)$_2$, 16.9 SiCH(*C*H$_3$)$_2$, 16.8 SiCH(*C*H$_3$)$_2$, 13.7 Si*C*H(CH$_3$)$_2$, 13.0 Si*C*H(CH$_3$)$_2$, 12.9 Si*C*H(CH$_3$)$_2$, 12.7 Si*C*H(CH$_3$)$_2$, -0.2 (Si(CH$_3$)$_3$). LRMS (ESI): *m/z* = 688 [M-TMS+H]$^+$, 564 [M-TfO+H]$^+$. HRMS (ESI+) *m/z* [M + Na]$^+$ calcd for $C_{27}H_{44}F_3N_3O_{11}SSi_3Na$ 782.1848, found 782.1829.

(2′S)-2′-Chloro-2′-deoxy-5-(ethynyl(2-trimethylsilyl))-3-*N*-nitro-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine (28)

Compound **27** (0.3000 mg, 0.3950 mmol) and TBAC (0.1317 g, 0.474 mmol, 1.2 equiv) were combined and placed under an atm of argon. The mixture was dissolved in CH_2Cl_2 (2 mL) and stirred at rt for 30 min. The crude product was purified by silica gel flash chromatography (100% CH_2Cl_2) to afford the title compound as a white solid (quantitative). Mp = 145 – 150 ˚C. R_f = 0.68 (20% EtOAc in *n*-hexane). ^{1}H NMR (500 MHz, CDCl$_3$) δ_H = 7.77 (s, 1H, H-6), 6.27 (d, J = 6.4 Hz, 1H, H-1′), 4.57 (dd, J = 8.1, 6.4 Hz, 1H, H-2′), 4.39 (t, J = 8.2 Hz, 1H, H-3′), 4.15 (dd, J = 13.2, 2.5 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 4.06 (dd, J = 13.3, 3.1 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.84 (dt, J = 8.2, 2.7 Hz, 1H, H-4′), 1.17 – 0.95 (m, 28H, (Si(*i*-Pr)$_2$)$_2$), 0.23 (s, 9H, Si(CH$_3$)$_3$). ^{13}C NMR (125 MHz, CDCl$_3$) δ_C = 154.3 (C-4), 144.8 (C-2), 141.3 (C-6), 102.4 (C≡*C*–Si), 100.5 (C-5), 93.5 (*C*≡C–Si), 84.6 (C-1′), 83.0 (C-4′), 75.4 (C-3′), 62.7 (C-2′), 60.2 (C-5′), 17.84 (SiCH(*C*H$_3$)$_2$), 17.75 (SiCH(*C*H$_3$)$_2$), 17.6 (SiCH(*C*H$_3$)$_2$), 17.5 (SiCH(*C*H$_3$)$_2$), 17.3 (SiCH(*C*H$_3$)$_2$), 17.24 (SiCH(*C*H$_3$)$_2$), 17.22 (SiCH(*C*H$_3$)$_2$), 14.1 (Si*C*H(CH$_3$)$_2$), 13.3 (Si*C*H(CH$_3$)$_2$), 12.75 (Si*C*H(CH$_3$)$_2$), 12.67 (Si*C*H(CH$_3$)$_2$), 0.0 (Si(CH$_3$)$_3$). LRMS (ESI): *m/z* = 690, 692 [M – H + HCOOH, ^{35}Cl, ^{37}Cl]$^-$, 468 [M + H]$^+$, 668, 670 [M + Na, ^{35}Cl, ^{37}Cl]. HRMS (ESI+) *m/z* [(M + Na)$^+$, ^{35}Cl] calcd for $C_{26}H_{44}ClN_3O_8Si_3Na$ 668.2016, found 668.2009.

(2′S)-2′-Bromo-2′-deoxy-5-(ethynyl(2-trimethylsilyl))-3-*N*-nitro-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine (29)

Compound **27** (0.3340g, 0.439 mmol) and TBAB (0.2126 g, 0.6596 mmol, 1.5 equiv) were combined and placed under an atm of argon. The mixture was dissolved in toluene (2.5 mL) and stirred at rt for 40 min. The solvent was removed *in vacuo* and the residue dissolved in CH_2Cl_2. The crude product was purified by silica gel flash chromatography (100 % CH_2Cl_2) to afford the title compound as a white solid (0.296 mg, 98%). Mp = 142 – 145 ˚C. R_f = 0.35 (10% EtOAc in *n*-hexane), 0.67 (20% EtOAc in *n*-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.77 (s, 1H, H-6), 6.24 (d, *J* = 6.4 Hz, 1H, H-1′), 4.58 (dd, *J* = 8.4, 6.5 Hz, 1H, H-2′), 4.50 (t, *J* = 8.3 Hz, 1H, H-3′), 4.16 (dd, *J* = 13.3, 2.4 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 4.06 (dd, *J* = 13.3, 3.0 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.82 (dt, *J* = 8.1, 2.7 Hz, 1H, H-4′), 1.17 – 0.99 (m, 28H, (Si(*i*-Pr)$_2$)$_2$), 0.23 (s, 9H, Si(CH$_3$)$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 154.3 (C-4), 144.8 (C-2), 141.2 (C-6), 102.4 (C≡*C*–Si), 100.5 (C-5), 93.5 (*C*≡C–Si), 84.5 (C-1′), 83.7(C-4′), 75.5(C-3′), 60.2 (C-5′), 53.1 (C-2′), 17.9 (SiCH(*C*H$_3$)$_2$), 17.7 (SiCH(*C*H$_3$)$_2$), 17.6 (SiCH(*C*H$_3$)$_2$), 17.5 (SiCH(*C*H$_3$)$_2$), 17.4 (SiCH(*C*H$_3$)$_2$), 17.32 (SiCH(*C*H$_3$)$_2$), 17.30 (SiCH(*C*H$_3$)$_2$), 17.28 (SiCH(*C*H$_3$)$_2$), 14.3 (Si*C*H(CH$_3$)$_2$), 13.3 (Si*C*H(CH$_3$)$_2$), 12.70 (Si*C*H(CH$_3$)$_2$), 12.67 (Si*C*H(CH$_3$)$_2$), 0.0 (Si(CH$_3$)$_3$). LRMS (ESI): *m/z* = 688, 690 [M - H, ^{79}Br, ^{81}Br]$^-$, 712, 714 [M + Na, ^{79}Br, ^{81}Br]$^+$. HRMS (ESI+) *m/z* [M + Na, ^{79}Br]$^+$ calcd for $C_{26}H_{44}BrN_3O_8Si_3Na$ 712.1511, found 712.1514.

(2′S)-2′-Deoxy-5-(ethynyl(2-trimethylsilyl))-2′-iodo-3-*N*-nitro-3′,5′-*O*-tetraisopropyldisiloxane-1,3-diyl)-uridine (30)

Compound **27** (0.1500 g, 0.1975 mmol) and TBAI (0.0875 g, 0.237 mmol, 1.2 equiv) were combined and placed under an atm of argon. Next the mixture was dissolved in toluene (1 mL) and heated to 50 ˚C for 20 min. The mixture was rapidly cooled in a water bath to rt. The solvent was removed *in vacuo* without heating and the residue dissolved CH_2Cl_2 and the crude product purified by silica gel flash chromatography (100% CH_2Cl_2) to afford the title compound as a colourless solid (0.0871 g, 60%). Mp = 149 – 152 ˚C (darkens at 70 ˚C). R_f = (0.71 20% EtOAc in *n*-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.75 (s, 1H, H-6), 6.11 (d, *J* = 6.6 Hz, 1H, H-1′), 4.63 – 4.52 (m, 2H, H-2′ and H-3′), 4.17 (dd, *J* = 13.4, 2.0 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 4.06 (dd, *J* = 13.4, 3.0 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.77 (ddd, *J* = 8.0, 2.9, 1.9 Hz, 1H, H-4′), 1.18 – 0.99 (m, 28H, (Si(*i*-Pr)$_2$)$_2$), 0.24 (s, 9H, Si(CH$_3$)$_3$). ^{13}C NMR (125

MHz, $CDCl_3$) δ_C = 154.2 (C-4), 144.6 (C-2), 140.6 (C-6), 102.3 (C≡*C*–Si), 100.6 (C-5), 93.4 (*C*≡C–Si), 85.1 (C-1′), 84.6 (C-4′), 76.2 (C-3′), 59.8 (C-5′), 29.4 (C-2′), 17.7 (SiCH(*C*H_3)$_2$), 17.5 (SiCH(*C*H_3)$_2$), 17.4 (SiCH(*C*H_3)$_2$), 17.4 (SiCH(*C*H_3)$_2$), 17.3 (SiCH(*C*H_3)$_2$), 17.3 (SiCH(*C*H_3)$_2$), 17.2 (SiCH(*C*H_3)$_2$), 17.2 (SiCH(*C*H_3)$_2$), 14.4 (Si*C*H(CH$_3$)$_2$), 13.1(Si*C*H(CH$_3$)$_2$), 12.5 (Si*C*H(CH$_3$)$_2$), 12.4 (Si*C*H(CH$_3$)$_2$), -0.2 (Si(CH$_3$)$_3$). LRMS (ESI): *m/z* = 735 [M - H]$^-$, 781 [M - H + HCOOH]$^-$, 759 [M + Na]$^+$. HRMS (ESI+) *m/z* [M + Na]$^+$ calcd for $C_{26}H_{44}IN_3O_8Si_3Na$ 760.1373, found 760.1351.

(2′S)-2′-Chloro-2′-deoxy-5-(ethynyl(2-trimethylsilyl))-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine (32)

Compound **32** was synthesised from **28** (0.2000 g, 0.263 mmol) according to general procedure 4. The crude product was purified by silica gel flash chromatography (1% MeOH in CH_2Cl_2) to afford the title compound as a white foam (0.098 g, 62%). R_f = 0.23 (20% EtOAc in *n*-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 8.57 (s, 1H, NH), 7.75 (s, 1H, H-6), 6.27 (d, *J* = 6.4 Hz, 1H, H-1′), 4.54 (dd, *J* = 7.5, 6.4 Hz, 1H, H-2′), 4.41 (t, *J* = 7.7 Hz, 1H, H-3′), 4.13 (dd, *J* = 13.1, 3.0 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 4.06 (dd, *J* = 13.1, 3.1 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.80 (dt, *J* = 7.9, 3.0 Hz, 1H, H-4′), 1.21 – 0.94 (m, 28H, (Si(*i*-Pr)$_2$)$_2$), 0.23 (s, 9H, Si(CH$_3$)$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 160.9 (C-4), 149.0 (C-2), 142.6 (C-6), 100.5 (C-5), 100.1 (C≡*C*–Si), 95.1 (*C*≡C–Si), 83.5 (C-1′), 82.5 (C-4′), 76.0 (C-3′), 62.9 (C-2′), 60.4 (C-5′), 17.64 (SiCH(*C*H_3)$_2$), 17.57 (SiCH(*C*H_3)$_2$), 17.5 (SiCH(*C*H_3)$_2$), 17.4 (SiCH(*C*H_3)$_2$), 17.2 (SiCH(*C*H_3)$_2$), 17.1(SiCH(*C*H_3)$_2$), 13.9 (Si*C*H(CH$_3$)$_2$), 13.2 (Si*C*H(CH$_3$)$_2$), 12.6 (Si*C*H(CH$_3$)$_2$), 12.5 (Si*C*H(CH$_3$)$_2$), 0.0 (Si(CH$_3$)$_3$). LRMS (ESI): *m/z* = 599, 601 [M - H, ^{35}Cl, ^{37}Cl]$^-$, 601, 603 [M + H, ^{35}Cl, ^{37}Cl]$^+$. HRMS (ESI+) *m/z* [M + Na, ^{35}Cl]$^+$ calcd for $C_{26}H_{45}ClN_2O_6Si_3Na$ 623.2166, found 623.2149.

(2′S)-2′-Bromo-2′-deoxy-5-(ethynyl(2-trimethylsilyl))-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine (33)

Compound **33** was prepared from **29** (0.300 g, 0.905 mmol) according general procedure 4. The crude product was purified by silica gel flash chromatography (10% EtOAc in CH_2Cl_2) to afford the

title compound as a colourless foam (260 mg, quantitative). R_f = 0.16 (20% EtOAc in *n*-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = δ 8.54 (s, 1H, NH), 7.75 (s, 1H, H-6), 6.22 (d, *J* = 6.0 Hz, 1H, H-1′), 4.61 – 4.48 (m, 2H, H-2′ and H-3′), 4.14 (dd, *J* = 13.1, 3.0 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 4.07 (dd, *J* = 13.1, 3.1 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.78 (dt, *J* = 7.5, 3.0 Hz, 1H, H-4′), 1.18 – 0.99 (m, 28H, (Si(*i*-Pr)$_2$)$_2$), 0.23 (s, 9H, Si(CH_3)$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 160.9 (C-4), 149.0 (C-2), 142.4 (C-6), 100.5 (C-5), 100.1 (C≡*C*–Si), 95.1 (*C*≡C–Si), 83.3 (C-4′), 83.2 (C-1′), 76.1 (C-3′), 60.4 (C-5′), 53.7 (C-2′), 17.7 (SiCH(*C*H$_3$)$_2$), 17.6 (SiCH(*C*H$_3$)$_2$), 17.5 (SiCH(*C*H$_3$)$_2$), 17.4 (SiCH(*C*H$_3$)$_2$), 17.19 (SiCH(*C*H$_3$)$_2$), 17.17 (SiCH(*C*H$_3$)$_2$), 17.15 (SiCH(*C*H$_3$)$_2$), 17.11 (SiCH(*C*H$_3$)$_2$), 14.0, (Si*C*H(CH$_3$)$_2$) 13.2, (Si*C*H(CH$_3$)$_2$) 12.6 (Si*C*H(CH$_3$)$_2$), 12.5 (Si*C*H(CH$_3$)$_2$), 0.0 (Si(CH$_3$)$_3$). LRMS (ESI): *m/z* = 643, 645 [M - H, ^{79}Br, ^{81}Br]$^-$, 667, 669 [M + Na, ^{79}Br, ^{81}Br]$^+$. HRMS (ESI+) *m/z* [M + Na, ^{79}Br]$^+$ calcd for $C_{26}H_{45}BrN_2O_6Si_3Na$ 667.1661, found 667.1643.

(2′S)-2′-Deoxy-5-(ethynyl(2-trimethylsilyl))-2′-iodo-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine (31)

Compound **31** was synthesised by two methods. Method A: reductive denitration of **30** (0.0820 mg, 0.1084 mmol) according to general procedure 4. Method B: as a by-product of iodination of **27**. Method A: (0.0385 g, 50%). Method B: Compound **27** (0.7350 g, 0.967 mmol) and tetrabutylammonium iodide (1.786 g, 4.835 mmol, 5 equiv) were combined and placed under an atm of argon. Next the mixture was dissolved in acetone (5.5 mL) and heated to 50 ˚C for 5 min. The solvent was removed *in vacuo* and crude product was purified by silica gel flash chromatography (20% EtOAc in *n*-hexane) to give the title compound as a yellow oil (0.125 g, 17 %). R_f = (0.26 20% EtOAc in *n*-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 8.18 (s, 1H, NH), 7.73 (s, 1H, H-6), 6.05 (dd, *J* = 4.5, 1.7 Hz, 1H, H-1′), 4.62 – 4.56 (m, 2H, H-2′ and H-3′), 4.15 (dd, *J* = 13.2, 2.5 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 4.07 (dd, *J* = 13.1, 3.0 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.74 (dq, *J* = 5.4, 2.7 Hz, 1H, H-4′), 1.17 – 0.96 (m, 28H, Si(*i*-Pr)$_2$)$_2$), 0.23 (s, 9H, Si(CH$_3$)$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 160.7 (C-4), 149.0 (C-2), 142.0 (C-6), 100.8 (C-5), 100.2 (C≡*C*–Si), 95.1 (*C*≡C–Si), 84.3 (C-4′), 84.0 (C-1′), 76.9 (C-3′ coincident with solvent residual) 60.2 (C-5′), 30.5 (C-2′), 17.7 (SiCH(*C*H$_3$)$_2$), 17.6 (SiCH(*C*H$_3$)$_2$), 17.4

(SiCH(CH$_3$)$_2$), 17.4 (SiCH(CH$_3$)$_2$), 17.3 (SiCH(CH$_3$)$_2$), 17.3 (SiCH(CH$_3$)$_2$), 17.2 (SiCH(CH$_3$)$_2$), 14.3 (SiCH(CH$_3$)$_2$), 13.2 (SiCH(CH$_3$)$_2$), 12.5 (SiCH(CH$_3$)$_2$), 12.5 (SiCH(CH$_3$)$_2$), 0.0 (Si(CH$_3$)$_3$). (ESI): *m/z* = 693 [M + H]$^+$. HRMS (ESI+) *m/z* [M + Na]$^+$ calcd for $C_{26}H_{45}IN_2O_6Si_3Na$ 715.1522, found 715.1506.

(2′S)-Chloro-2′-deoxy-5-ethynyl-uridine (Cl-ara-EdU) (3)

Compound **3** was synthesised by two methods. Method A: By a one-pot desilylation and *N*-denitration of compound **28** (0.080 g, 0.124 mmol) according to general procedure 6. Method B: by desilylation of compound **32** (0.3720 mg, 0.6186 mmol) according to general procedure 5. The crude product produced by both methods was purified by solid addition silica gel flash chromatography (10% MeOH in CH_2Cl_2) to afford the title compound as a white solid (method A: 0.0345 g, 95%, Method B: 0.1630 g, 92%). Mp = 227-232 ˚C (decomp, darkens at 210 ˚C). R_f = 0.32 (10% in CH_2Cl_2). NMR (500 MHz, DMSO-d_6) δ_H = 11.80 (s, 1H, NH), 8.31 (s, 1H, H-6), 6.19 (d, J = 5.9 Hz, 1H, H-1′), 6.11 (br s, 1H, OH-3′), 5.37 (br t, J = 5.1 Hz, 1H, OH-5′), 4.62 (t, J = 6.1 Hz, 1H, H-2′), 4.19 (t, J = 6.5 Hz, H-3′), 4.13 (s, 1H, ≡CH), 3.82 – 3.70 (m, 2H, H-4′ and H-5′$_{(\alpha \text{ or } \beta)}$), 3.63 (dt, J = 12.3, 4.1 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C = 161.5 (C-4), 149.2 (C-2), 144.3 (C-6), 97.3 (C-5), 83.8 (C≡CH), 83.7 (C-4′), 83.4 (C-1′), 76.1 (C≡CH), 74.3 (C-3′), 64.0 (C-2′), 59.0 (C-5′). LRMS (ESI): *m/z* = 285, 287 [M - H, ^{35}Cl, ^{37}Cl]$^-$, 287, 289 [M + H, ^{35}Cl, ^{37}Cl]$^+$. HRMS (ESI+) *m/z* [M + Na, ^{35}Cl]$^+$ calcd for $C_{11}H_{11}ClN_2O_5Na$ 309.0248, found 309.0236.

(2′S)-2′-Bromo-2′-deoxy-5-ethynyl-uridine (Br-ara-EdU) (4)

Compound **4** was synthesised by two methods. Method A: By a one-pot desilylation and *N*-denitration of compound **29** (0.100 g, 0.145 mmol) according to general procedure 6. Method B: by desilylation of compound **33** (0.259 g, 0.375 mmol) according to general procedure 5. The crude product produced by both methods was purified by solid addition silica gel flash chromatography (10% MeOH in CH_2Cl_2) to afford the title compound as an off-white solid (method A: 0.0350 g, 73%, Method B: 0.065 g, 46%). Mp = 225-235 ˚C (decomp, darkens at 185 ˚C). R_f = 0.33 (10% in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 11.80 (s, 1H NH), 8.30 (s, 1H, H-6), 6.13 (d, J = 6.0 Hz, 1H, H-1′),

6.11 – 6.08 (br S, 1H, OH-3′), 5.39 – 5.35 (br m, 1H, OH-5′), 4.65 (dd, J = 6.8, 6.0 Hz, 1H, H-2′), 4.30 (t, J = 6.7 Hz, 1H, H-3′), 4.13 (s, 1H, ≡CH), 3.79 – 3.71 (m, 2H, H-4′ and H-5′$_{(\alpha \text{ or } \beta)}$), 3.64 (dt, J = 13.3, 4.2 Hz, 1H, H-5′$_{(\alpha \text{ or } \beta)}$). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C = 161.4 (C-4), 149.2 (C-2), 144.2 (C-6), 97.4 (C-5), 84.1 (C-4′), 83.8 (C≡*C*H), 83.2 (C-1′), 76.1 (*C*≡CH), 74.4 (C-3′), 59.1 (C-5′), 55.8 (C-2′). LRMS (ESI): *m/z* = 328, 330 [M - H, ^{79}Br, ^{81}Br]$^-$, 330, 332 [M + H] ^{79}Br, ^{81}Br]$^+$. HRMS (ESI+) [M + Na$^+$, ^{79}Br]$^+$ *m/z* calcd for $C_{11}H_{11}BrN_2O_5Na$ 352.9743, found 352.9726.

(2′S)-2′-Deoxy-5-ethynyl-2′-iodo-uridine (l-ara-EdU) (5)

Compound **5** was synthesised by two methods. Method A: By a one-pot desilylation and *N*-denitration of compound **30** (0.0870 g, 0.118 mmol) according to general procedure 6. Method B: by desilylation of compound **31** (0.259 mg, 0.375) according to general procedure 5. The crude product produced by both methods was purified by solid addition silica gel flash chromatography (10% MeOH in CH_2Cl_2) to afford the title compound as a tan solid (method A: 0.0221 g, 50%, method B: 0.0352 g, 82%). Mp = 205-210 ˚C (decomp, darkens at 195 ˚C). R_f = 0.37 (10% in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 11.80 (br s, 1H, NH), 8.29 (s, 1H, H-6), 6.02 (br s, 1H, OH-3′), 5.96 (d, J = 6.5 Hz, 1H, H-1′), 5.38 (br s, 1H,OH-5′), 4.58 (dd, J = 8.0, 6.5 Hz, 1H, H-2′), 4.35 (t, J = 7.6 Hz, 1H, H-3′), 4.14 (s, 1H, ≡CH) 3.78 – 3.72 (m, 1H, H-5′$_{(\alpha \text{ or } \beta)}$), 3.70 – 3.62 (m, 2H, H-4′ and H-5′$_{(\alpha \text{ or } \beta)}$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 161.4 (C-4), 149.2 (C-2), 144.0 (C-6), 97.6 (C-5), 84.8 (C-4′), 83.8 (C-1′ and C≡*C*H), 76.2 (*C*≡CH), 75.3 (C-3′), 58.9 (C-5′), 33.0 (C-2′). LRMS (ESI): *m/z* = 376 [M - H]$^-$, 422 [M + HCOO]$^-$, 378 [M + H]$^+$. HRMS (ESI+) *m/z* [M + Na]$^+$ Calcd for $C_{11}H_{11}IN_2O_5Na$ 400.9604, found 400.9591.

Supporting Information. Synthetic procedures for compounds 10, 13, and 15. ^{1}H and ^{13}C NMR spectra for compounds **3-5**, **10**, **13-19**, **23-25**, **27-33**. NOESY NMR spectra for compounds **3-5**.

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