Operationally Simple Regioselective 5'-Phosphorylation of Unprotected 5-Ethynyl-2'-deoxyuridine Analogues

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Here, we present the development of a straightforward methodology to regioselectively phosphorylate the 5'-OH group of unprotected nucleosides. We employ cyclosaligenyl phosphoramidite reagents together with pyridinium trifluoroacetate as activator, followed by in situ oxidation to prepare a panel of novel nucleoside-based chemical probes, ProLabel compounds **4–15**. Alternative procedures for this transformation are available, but are limited in number and scope. Furthermore, the benefits of the new methodology include milder reaction conditions, wider solvent applicability, and, by avoiding sensitive reagents, a more straightforward handling of reagents, reactions, and workup processes. The panel of novel cyclosaligenyl phosphotriester uridine ProLabels have variable 2' substituents (H, F, Cl, Br, I) as well as four different cyclosaligenyl groups that would span a range of half-lives for in vitro applications.

Modified nucleoside monophosphates are indispensable for drug discovery research, yet are challenging to synthesise. We have developed a straightforward method to regioselectively phosphorylate the 5'-OH group of unprotected nucleosides, overcoming some of the limitations of existing procedures for this transformation. These 5'-cyclosaligenyl phosphotriesters also have a variable 2' substituent.

Introduction

Nucleotide phosphotriester prodrugs, or pronucleotides (ProTides), are used for the treatment of viral infections and cancer.[1,2] The influence of the ProTide structure on drug efficacy is 2-fold. First, ProTides are designed to allow cell entry by passive membrane diffusion, whereas the corresponding free nucleoside monophosphate would typically experience poor passive membrane diffusion and thus poor cellular uptake. Second, the ProTide structure bypasses the need for phosphorylation by the often rate-limiting and substrate-specific nucleoside kinase that is necessary for most nucleoside drugs. ProTides can thus progress along the endogenous metabolic pathway for incorporation into newly synthesised DNA more efficiently than their underlying nucleoside. In a different application, modified nucleosides are employed as chemical probes to visualise and track DNA synthesis in proliferating cells.[3,4] Similarly to ProTides, the mechanism of action is to utilise endogenous metabolic pathways; however, instead of killing the cell or microbe, the purpose of a nucleoside-based chemical probe is to covalently position an unobtrusive and latent reporter group into DNA of living cells.[3] 5-Ethynyl-2'-deoxyuridine (EdU, **1**) is a widely used chemical probe for the metabolic labelling of DNA. Once incorporated into DNA, the alkyne reporter group of **1** is reacted with a small-molecule fluorescent azide using copper-catalysed azide–alkyne cycloaddition (CuAAC); this enables detection of newly synthesised DNA using fluorescent imaging techniques.[3] These

two distinct concepts: ProTides and nucleoside-based chemical probes, have recently been integrated by us, and then others, to afford ProLabel compounds.[5-8] Our rationale for this deliberate merging of strategies was to transfer the benefits of the ProTide structure on optimal in vitro activity to nucleoside-based chemical probes (Fig. 1).[6]

There are numerous published methods to phosphorylate the 5'-OH group of protected nucleosides, whereas the regioselective 5'-OH phosphorylation of unprotected nucleosides is less common.[9] Even so, three distinct classes of phosphorylation reagents have been used: P^V phosphorochloridates;[5,7,10] P^{III} chlorophosphites,[6,10-12] and P^{III} phosphoramidites[5,13-15] (Fig. 2a). Phosphorochloridate reagents are straightforward to prepare and are stable on storage, however exhibit substrate-dependent reactivity and fail to react with some nucleoside substrates.[9] In contrast, chlorophosphite reagents react rapidly; however, they lack chemoselectivity and are incompatible with *N*-unprotected nucleosides; their high reactivity may also be counterproductive with respect to ease of preparation and manipulation.[16] Lastly, phosphoramidite reagents are straightforward to synthesise, exhibit excellent long-term stability in cold storage (−20°C),[17] and have a reliable reactivity profile such that they are routinely used for automated oligonucleotide synthesis.[13-15] Our group first reported the synthesis and successful application of the ProLabel strategy using 5'-cyclosaligenyl EdU (**2**) as a ProLabel to study DNA synthesis in proliferating cells (Fig. 2b).[6] Compound **2** is neutral, membrane-permeable, and once inside the cell is hydrolysed by pH-driven mechanisms to release the negatively charged monophosphate of **1** (Fig. 1).[6] Ito and coworkers then showed that 5'-bis-pivaloyloxymethyl EdU (**3**, Fig. 2b) can also be used to label proliferating cells by generating the monophosphate of **1**.^[7] The ProLabels **2** and **3** were synthesised from **1** using a chlorophosphite reagent and a phosphorochloridate reagent respectively.

Our goal was to further develop the ProLabel strategy to provide alternative 5'-cyclosaligenyl nucleoside chemical probes for the study of DNA synthesis and cell proliferation. An imperative when designing the ProLabels is the requirement for a neutral and sufficiently lipophilic phosphate protecting group to allow the ProLabel to readily diffuse through cell membranes. The phosphate protecting group must be hydrolysable under physiological conditions while balanced with a half-life appropriate for the biological application. The underlying nucleoside must have minimal toxicity. Our experience with the synthesis of **2** indicated that 5'-cyclosaligenyl groups were appropriate for use as ProLabels but that wider application of chlorophosphite reagents to regioselectively synthesise other 5'-*O*-phosphoesters of **1** would be challenging. Purification of the chlorophosphite requires a cumbersome short-path vacuum distillation, while use of the crude chlorophosphite lacked synthetic reproducibility. Herein, we describe the development of a convenient and reliable synthetic route that enables the regioselective synthesis of **2** in addition to a panel of 12 novel 5'-cyclosaligenyl phosphotriester uridine analogues **4–15** (Fig. 3).

Results and Discussion

Kato and coworkers^[13] demonstrated excellent regioselectivity for phosphitylation of the 5'-OH group of thymidine **16** at room temperature (rt) when using the sterically hindered phosphoramidite di-*tert*-butyl *N,N*-diethylphosphoramidite **17** in conjunction with pyridinium hydrochloride (Py·HCl) as activator and pyridine as solvent at rt (Scheme 1a). Although product **18** is likely not viable as a chemical probe (the *tert*-butyl phosphoester group is not labile under physiological conditions), we were encouraged to explore the capacity of cyclosaligenyl phosphoramidites as a phosphitylating reagents with **1** as substrate. Indeed treatment of 2',3'-dideoxythymidine (**19**) with cyclosaligenyl phosphoramidite **20** (Scheme 1b) gave the 5'-cyclosaligenyl derivative **21** in good yield, albeit that reaction with 2',3'-dideoxyribose as substrate does not present a concern in relation to regioselectivity.^[16] Although the cyclosaligenyl moiety in **20** offers a less hindered phosphoramidite than **17**, we anticipated regioselectivity might be achieved by employing a lower reaction temperature to compensate for any reduction in kinetic control.

The reported synthesis for the cyclosaligenyl phosphoramidite reagent **20** proceeds in two steps (Scheme 2). First, treatment of saligenyl alcohol **22** with PCl₃ and Et₃N in Et₂O generates cyclosaligenyl chlorophosphite **23**.^[18] After removal of the Et₃N hydrochloride salt by-product by vacuum filtration, **23** is treated with *i*Pr₂NH in Et₂O to generate the target cyclosaligenyl phosphoramidite **20** as a yellow oil; no yield was provided.

With no reagent compatibility concerns evident, we proposed performing the synthesis of the target cyclosaligenyl phosphoramidite reagents of the present study, including **20** and **24–26**, in a one-pot procedure, without workup of the cyclosaligenyl chlorophosphite intermediate. To test the approach, saligenyl alcohol **22** was treated with PCl₃ and Et₃N in Et₂O, then reacted with *i*Pr₂NH (Scheme 3). The desired product **20** was obtained in 65 % yield following purification by basic alumina chromatography. The developed one-pot methodology was then extended to the synthesis of the remaining target substituted cyclosaligenyl phosphoramidites **24–26**. Compounds were obtained from the corresponding saligenyl alcohols **27–29** in moderate isolated yields following purification (Scheme 3). The panel of four phosphoramidite reagents **20**, **24–26** were selected to provide a coverage of in vitro half-life values suited to chemical probe use, with the reported hydrolysis in pH 7.29 buffer ranging from 0.9 to 7 h.^[12] Phosphoramidites **20**,^[18] **24**,^[16] **26**^[19] are known whereas phosphoramidite **25** is novel. To the best of our knowledge, only the purification of the chlorinated cyclosaligenyl phosphoramidite (**26**) is reported (silica gel filtration with *i*Pr₂NH and hexane as eluant) before use as a phosphorylating reagent.

Phosphorylation of **1** with the panel of cyclosaligenyl phosphoramidites **20** and **24–26** was then assessed using Py·HCl as activator (Scheme 4). When using the reported reaction conditions (Scheme 1b),^[16] we observed 3',5'-bis-phosphorylation of **1** as the major product. We

attributed the lack of regioselectivity to the poor solubility of **1** in CH₃CN. We next trialled pyridine in place of CH₃CN as solvent; although **1** was soluble in pyridine, we found that Py·HCl was not soluble in pyridine and that residual water in the Py·HCl interfered with the reaction and no phosphorylation was observed. Finally, we solubilised the Py·HCl in CH₃CN and dried the solution over 3-Å molecular sieves and added this to a solution of **1** in pyridine, followed by the cyclosaligenyl phosphoramidite in CH₃CN. These mixed-solvent conditions at –20°C gave acceptable 5' regioselectivity. We sought to further optimise reaction conditions, however, as the hygroscopic nature of the Py·HCl salt and the antisolvent behaviour of CH₃CN for solubilising **1** was a dual concern in relation to synthetic reproducibility when extending the synthetic method to phosphitylation of other 2'-modified nucleosides. Specifically, we sought an alternative activator with improved solubility. For this purpose, we evaluated three commonly employed activators for phosphoramidites: dicyanoimidazole (DCI), imidazolium triflate (Imi·Tf), and 1*H*-tetrazole; however, no reaction was observed with these activators. We then tried pyridinium trifluoroacetate (Py·TFA) in pyridine and found that reactivity was similar to Py·HCl, while Py·TFA had excellent solubility in pyridine (>1.5 M) whereas Py·HCl had very low solubility. Moreover, Py·TFA was readily formed in situ by the addition of TFA to a solution of the nucleoside in pyridine, giving the added benefit of reducing operational complexity associated with the need to manipulate several anhydrous solutions, and lowering the overall solvent volume required. Furthermore, we found that the 2' derivatives of **1** (**30–35**), which are acid-sensitive,^[20] could be stored in the Py·TFA solution over molecular sieves for extended periods of time (>24 h) without degradation. ³¹P NMR analysis of the phosphitylation of **1** in [D5]pyridine confirmed complete conversion of the phosphoramidite reagent **20** to the corresponding phosphite intermediate following 20 min reaction at –20°C, with a chemical shift change from 135.3 to ~119.8 and 118.8 ppm in the ³¹P NMR spectrum (1 : 1 mixture of diastereomers). After in situ oxidation with *t*-BuOOH, the relative intensity of ³¹P species in the NMR spectrum indicated regioselectivity preference for the 5'-*O*-cyclosaligenyl phosphoester diastereomers over the 3' and 3',5' *O*-cyclosaligenyl phosphoester diastereomers (Supplementary Material). Additionally, we acquired a ³¹P{H} NMR spectrum of pure compound **2** in [D5] pyridine. The intensity and chemical shifts of the ³¹P signals in these spectra confirm that the 5'-*O*-cyclosaligenyl phosphoester diastereomers are the major reaction products over the 3'- and 3',5'-*O*-cyclosaligenyl phosphoester diastereomers. Characterisation of pure compound **2** by 2D ¹H NMR established the 5' regioselectivity. For all reactions, TLC analysis was consistent with formation of a preferred product; the product was subsequently characterised by NMR analysis as the 5' regioisomer. Characterisation by 2D ¹H NMR established the regioselectivity. This degree of regioselectivity is comparable with that obtained using the chlorophosphite methodology.^[10,12] Finally, to remove residual Py·TFA, we employed ion exchange resins as **2** was susceptible to hydrolysis in common aqueous workup solvents. A formulation of a mixed-bed resin consisting of a 2 : 1 ratio of Amberlyst® A21 and Amberlite®

IR 120 resins effectively removed both pyridine and TFA while maintaining a neutral pH (as determined by universal pH paper). The Py·TFA-free product was purified by silica gel flash chromatography to obtain **2** in 40 % yield.

The optimised synthesis and purification methodology was then applied to 5-ethynyl precursors **1** and **30–35** with the target compounds **2** and **4–15** prepared in yields between 20 and 46 % (Scheme 5). For all reactions, and consistent with the synthesis of **2**, TLC analysis indicated the preferential formation of the 5' regioisomer over other possible regioisomers. The 5' regioisomers were subsequently isolated from the crude reaction mixture and characterised by 1D and 2D NMR analysis. Unfortunately, as for compound **2**, substantial compound loss occurred during purification of cyclosaligenyl phosphotriesters. This is responsible for the variable recovered yields of product. Nevertheless, all target compounds were able to be prepared in useful quantities and demonstrate the wide scope of this synthetic methodology.

Conclusion

In summary, an efficient regioselective 5'-OH phosphorylation of unprotected uridine nucleoside ProLabel compounds has been developed. We employ cyclosaligenyl phosphoramidite reagents together with Py·TFA as activator for 5'-OH phosphitylation, followed by in situ oxidation. In comparison with the few alternative reported regioselective phosphitylation or phosphorylation reactions, we identify several benefits of this new methodology including straightforward handling of reagents, reactions and workup complete within 2 h, and wider solvent compatibility. Furthermore, as reaction conditions are mild, including a nearly neutral pH, this methodology can be extended to nucleoside substrates that are acid or base labile. We propose that the simplicity of the reaction conditions combined with the inexpensive, stable, and readily available activator will facilitate wider accessibility to this important class of compounds for future chemical probe or drug discovery applications.

Experimental

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 % sulfuric acid). Silica gel flash chromatography was performed using silica gel (60 Å, 230–400 mesh). NMR (^1H , ^{13}C , ^{19}F , COSY (correlation spectroscopy), NOESY, HSQC (heteronuclear single-quantum correlation) and HMBC (Heteronuclear Multiple Bond Correlation) spectra were recorded on a Bruker Avance III HD 500 MHz NMR spectrometer equipped with a broadband observe (BBO) probe at 25°C. Chemical shifts for ^1H and ^{13}C NMR obtained in [D₆]DMSO are reported in parts per million relative to residual solvent proton (δ 2.50 ppm) and carbon (δ 39.5 ppm) signals respectively.

Chemical shifts for ^1H and ^{13}C NMR obtained in CDCl_3 are reported in parts per million relative to residual solvent proton (δ 7.26 ppm) and carbon (δ 77.16 ppm) signals respectively. The ^{31}P NMR and ^{19}F NMR chemical shifts are reported in parts per million using neat $(\text{EtO})_3\text{P}$ (–1 ppm) and $\text{F}_3\text{CCO}_2\text{H}$ (–78.5 ppm) as the external references respectively, employing coaxial insert tubes. Signal splitting multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), br (broad signal). Assignments of ^1H and ^{13}C chemical shifts were established by COSY, HSQC, HMBC, and NOESY experiments. In the case of cyclosaligenyl phosphoester derivatives **2** and **4–15**, not all ^{13}C chemical shifts could be assigned owing to a combination of these samples being a mixture of diastereomers and the signal splitting caused by heteroatom coupling (^{31}P and/or ^{19}F). Two-dimensional experiments displayed the expected correlations between signals observed in ^1H and ^{13}C spectra. Coupling constants are reported in hertz (Hz). Low-resolution mass spectrometry (LRMS) electrospray ionisation (ESI) data were acquired on a Thermo Fisher MSQ Plus single quadrupole ESI mass spectrometer using electrospray as the ionisation technique in positive or negative mode as stated. High-resolution (HR) MS (ESI) data were acquired on a Bruker MaXis II Quadrupole Time-of-Flight mass spectrometer using electrospray as the ionisation technique in positive-ion or negative mode as stated. All MS analysis samples were prepared as solutions in either methanol or acetonitrile. Purity of the compounds was >95 % as determined by Thermo Fisher Dionex Ultimate 3000 series HPLC with UV detection at 254 nm. The melting points are uncorrected. Proton and carbon atom numbering for NMR chemical shifts are designated as shown below. 2'-Halogenated nucleoside starting materials **31–33** were synthesised as reported previously whereas **30** is commercially available. Compound **30** was, however, synthesised using adapted literature procedures, with compound characterisation consistent with reported values.^[21] Fluorinated nucleosides **34** and **35** are novel and their synthesis is reported in the Supplementary Material. Saligenyl alcohols **20** and **25** were purchased from commercial suppliers whereas **24** and **26** were synthesised according to literature procedures.^[22]

General Procedure 1: Phosphitylation of Free Nucleoside Derivatives with Cyclosaligenyl Phosphoramidites

A solution of the nucleoside derivative in pyridine was kept on 3-Å molecular sieves for 16 h. TFA (4 equiv.) was added and the mixture left to stand for at least 1 h. The mixture was then removed from the molecular sieves by syringe, added to a round-bottom flask, and cooled to –20°C. The cyclosaligenyl phosphoramidite (1.1 equiv.) was dissolved in a minimum amount of CH_2Cl_2 (4 equiv.) and added to the nucleoside substrate slowly with vigorous stirring. The mixture was allowed to warm to rt over 30 min, at which point the mixture was cooled to –5°C, and *t*-BuOOH (5–6 M in decane, 2.4 equiv.) was added and the mixture allowed to again warm to rt over 30 min. The reaction mixture was concentrated under vacuum and the residue dissolved in MeOH (10 mL). Amberlite® IR-120 H^+ and Amberlite® IRA-21 resins (both

prewashed MeOH and dried) were combined in a 1 : 2 ratio, then mixed by vortexing. The mixed resin was then added to the MeOH solution of crude product and stirred until no pyridine was detectable by TLC (UV) and universal pH indicator paper indicated neutral pH. The mixture was then filtered and the resin washed with MeOH (10 mL). The filtrate was concentrated under vacuum and the crude product purified by silica gel flash chromatography as described for each compound.

General Procedure 2: Synthesis of Cyclosaligenyl Phosphoramidite Reagents

The substrate saligenyl alcohol was dissolved in Et₂O, then cooled to –78°C. PCl₃ (1 equiv.) and Et₃N (2 equiv.) were dissolved in Et₂O separately, then added to the reaction mixture slowly. After stirring for 2 h and warming to 0°C, the reaction mixture was again cooled to –78°C and *i*Pr₂NH (2 equiv.) added. The reaction mixture was allowed to warm to 0°C in a dry ice/acetone bath. The precipitated amine salts were removed by filtration under an N₂ atmosphere and with the receiver flask cooled to 0°C. The resulting cloudy white solution was then concentrated under vacuum without heating. A short alumina chromatography column (plug) was prepared from basic alumina that had been stored in a 150°C oven overnight and conditioned with CH₂Cl₂ (stabilised with 0.1 % EtOH, acid-free). The purified compound was concentrated under vacuum without heating and stored at –20°C.

5'-O-Cyclosaligenyl-5-ethynyl-2'-deoxyuridine monophosphate (2)

Compound **2** was synthesised from **1** (0.0500 g, 0.1982 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.0355 g, 40 %). ³¹P NMR and liquid chromatography (LC)-MS analysis indicate an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. The spectroscopic data were consistent with literature values. *R*_f 0.43 (10 % MeOH in CH₂Cl₂). δ_H (500 MHz, [D₆]DMSO) 11.66 (br s, 2H, 2 × NH), 7.94 (s, 1H, H-6), 7.92 (s, 1H, H-6), 7.41–7.33 (m, 2H, Ar), 7.30–7.24 (m, 2H, Ar), 7.23–7.17 (m, 2H, Ar), 7.17–7.09 (m, 2H, Ar), 6.09 (dd, *J* 6.4, 2H, 2 × H-1'), 5.58–5.40 (m, 6H, 2 × ArCH₂– and 2 × OH-3'), 4.42–4.25 (m, 4H, 2 × H-5'(_α and _β)), 4.22–4.15 (m, 2H, 2 × H-3'), 4.12 (s, 1H, ≡CH), 4.10 (s, 1H, ≡CH), 3.96–3.89 (m, 2H, 2 × H-4'), 2.24–2.12 (m, 1H, 2 × H-2'(_α or _β)), 2.10 (ddd, *J* 13.5, 6.4, 3.6, 2H, 2 × H-2'(_α or _β)). δ_C (126 MHz, [D₆]DMSO) 161.50 (C-4), 161.49 (C-4), 149.4 (d, *J* 7.1, 2 × C-2_{Ar}), 149.31 (C-4), 149.32 (C-4), 144.31 (C-6), 144.29 (C-6), 129.82–129.71 (m, 2 × C-4_{Ar}), 126.1 (2 × C-6_{Ar}), 124.4 (2 × C-5_{Ar}), 121.0 (apparent (a) t, *J* 10.1, 2 × C-1_{Ar}), 118.22 (d, *J* 8.9, C-3_{Ar}), 118.19 (d, *J* 8.7, C-3_{Ar}), 98.1 (C-5), 98.0 (C-5), 85.0 (C-1'), 84.9 (C-1'), 84.50 (a t, *J* 7.7, 2 × C-4'), 83.93 (≡C–H), 83.87 (≡C–H), 76.10 (C≡CH), 76.07 (C≡CH), 69.8 (C-3'), 69.7 (C-3'), 68.6 (d, *J* 7.1, ArCH₂), 68.5 (d, *J* 7.3, ArCH₂) 67.6 (d, ²*J*_{CP} 5.5), 67.4 (d, ²*J*_{CP} 5.7, C-5'), 38.8 (C-2'), 38.7 (C-2'). ³¹P{¹H} NMR (202 MHz, [D₆]DMSO) –9.5, –9.6. *m/z* (LRMS ESI) 443 [M + Na]⁺, 419 [M – H][–]. *m/z* (HRMS ESI⁺) [M + H + H₂O]⁺ 439.0879; calcd for C₁₈H₂₀N₂O₉P 439.0901.

5'-O-[3-Methylcyclosaligenyl]-5-ethynyl-2'-deoxyuridine monophosphate (4)

Compound **4** was synthesised from **1** (0.100 g, 0.3965 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.0530 g, 31 %). ³¹P NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. *R_f* 0.53 (10 % MeOH in CH₂Cl₂). δ_{H} (500 MHz, [D₆]DMSO) 11.67 (br s, 2H, 2 $\times$ NH), 7.93 (s, 1H, H-6), 7.92 (s, 1H, H-6), 7.29–7.21 (m, 2H, Ar), 7.13–7.02 (m, 4H, Ar), 6.10 (a td, *J* 6.8, 3.6 Hz, 2H, 2 $\times$ H-1'), 5.53–5.36 (m, 6H, 2 $\times$ ArCH₂– and 2 $\times$ OH-3'), 4.45–4.24 (m, 4H, 2 $\times$ H-5'(α and β)), 4.24–4.15 (m, 2H, 2 $\times$ H-3'), 4.11 (s, 1H, $\equiv$ CH), 4.09 (s, 1H, $\equiv$ CH), 3.97–3.88 (m, 2H, 2 $\times$ H-4'), 2.27–2.06 (m, 10H, 2 $\times$ H-2'(α and β) and 2 $\times$ CH₃). δ_{C} (126 MHz, [D₆]DMSO) 161.5 (2 $\times$ C-4), 149.35 (C-2), 149.34 (C-2), 147.88 (d, *J* 7.2, 2 $\times$ C-2_{Ar}), 144.34 (C-6), 144.26 (C-6), 130.9 (2 $\times$ C-6_{Ar}), 126.88 (d, *J* 7.8, 2 $\times$ C-3_{Ar}), 123.9 (2 $\times$ C-4_{Ar}), 123.6 (2 $\times$ C-5_{Ar}), 120.91 (a t, *J* 9.7, 2 $\times$ C-1_{Ar}), 98.07 (C-5), 98.05 (C-5), 85.0 (C-1'), 84.9 (C-1'), 84.51 (a t, *J* 6.7, 2 $\times$ C-4'), 83.9 ($\equiv$ C–H), 83.8 ($\equiv$ C–H), 76.09 (C $\equiv$ CH), 76.05 (C $\equiv$ CH), 69.77 (C-3'), 69.73 (C-3'), 68.45 (a t, *J* 6.2, 2 $\times$ ArCH₂), 67.7 (d, *J* 5.6, C-5'), 67.4 (d, *J* 5.7, C-5'), 38.8 (C-2'), 38.7 (C-2'), 14.94 (CH₃), 14.90 (CH₃). ³¹P{H} NMR (202 MHz, [D₆]DMSO) –8.9, –9.0. *m/z* (LRMS ESI) 457 [M + Na]⁺, 435 [M + H]⁺, 433 [M – H][–]. *m/z* (HRMS ESI⁺) 457.0765 [M + Na]⁺; calcd for C₁₉H₁₉N₂NaO₈P 457.0771.

5'-O-[3-Methoxycyclosaligenyl]-5-ethynyl-2'-deoxyuridine monophosphate (5)

Compound **5** was synthesised from **1** (0.0500 g, 0.1982 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5–10 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.0300 g, 33 %). ³¹P NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. *R_f* 0.46 (10 % MeOH in CH₂Cl₂). δ_{H} (500 MHz, [D₆]DMSO) 11.67 (br s, 2H, 2 $\times$ NH), 7.97 (s, 1H, H-6), 7.91 (s, 1H, H-6), 7.19–7.06 (m, 4H, Ar), 6.81 (a ddd, *J* 7.5, 2.9, 1.6, 2H, Ar), 6.13–6.06 (m, 2H, Ar), 5.54–5.38 (m, 6H, 2 $\times$ ArCH₂– and 2 $\times$ OH-3'), 4.43–4.24 (m, 4H, 2 $\times$ H-5'(α and β)), 4.24–4.16 (m, 2H, 2 $\times$ H-3'), 4.10 (s, 1H, $\equiv$ CH), 4.09 (s, 1H, $\equiv$ CH), 3.97–3.89 (m, 2H, 2 $\times$ H-4'), 3.83 (d, *J* 1.0, 6H, 2 $\times$ MeO), 2.27–2.02 (m, 4H, 2 $\times$ H-2'(α and β)). δ_{C} (126 MHz, [D₆]DMSO) 161.51 (C-4), 161.48 (C-4), 149.33 (C-2), 149.30 (C-2), 148.27 (d, *J* 7.2, C-3_{Ar}), 148.25 (d, *J* 7.0, C-3_{Ar}), 144.46 (C-6), 144.22 (C-6), 138.52 (d, *J* 6.8, C-2_{Ar}), 138.49 (d, *J* 6.8, C-2_{Ar}), 124.57 (C-5_{Ar}), 124.53 (C-5_{Ar}), 122.00 (d, *J* 8.6, C-1_{Ar}), 121.92 (d, *J* 8.6, C-1_{Ar}), 116.98 (C-6_{Ar}), 116.95 (C-6_{Ar}), 112.63 (C-4_{Ar}), 112.58 (C-4_{Ar}), 98.06 (C-5), 98.03 (C-5), 85.00 (C-1'), 84.92 (C-1'), 84.55 (d, *J* 6.8, C-4'), 84.46 (d, *J* 6.8, C-4'), 83.86 ($\equiv$ C–H), 83.83 ($\equiv$ C–H), 76.07 (C $\equiv$ CH), 76.04 (C $\equiv$ CH), 69.81 (C-3'), 69.72 (C-3'), 68.56–68.41 (m, 2 $\times$ ArCH₂), 67.67 (d, *J* 5.7, C-5'), 67.51 (d, *J* 5.3, C-5'), 55.92 (OCH₃), 55.91 (OCH₃), 38.85 (C-2'), 38.62 (C-2'). ³¹P{H} NMR (202 MHz, [D₆]DMSO) –9.0, –9.1. *m/z* (LRMS ESI) 473 [M +

Na]⁺, 451 [M + H]⁺, 449 [M – H][–]. *m/z* (HRMS ESI⁺) 473.0698 [M + Na]⁺; calcd for C₁₉H₁₉N₂NaO₉P 473.0720.

5'-O-[5-Chlorocyclosaligenyl]-5-ethynyl-2'-deoxyuridine monophosphate (6)

Compound **6** was synthesised from **1** (0.100 g, 0.3965 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.0380 g, 22 %). 2D NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. *R_f* 0.44 (10 % MeOH in CH₂Cl₂). δ_{H} (500 MHz, [D₆]DMSO) 11.66 (s, 2H, 2 × NH), 7.94 (s, 1H, H-6), 7.92 (s, 1H, H-6), 7.48–7.38 (m, 4H, Ar), 7.22–7.15 (m, 2H, Ar), 6.10 (dd, *J* 6.9, 2H, 2 × H-1'), 5.55–5.37 (m, 6H, 2 × ArCH₂– and 2 × OH-3'), 4.41–4.26 (m, 4H, 2 × H-5'(α and β)), 4.24–4.15 (m, 2H, 2 × H-3'), 4.13 (s, 1H, $\equiv$ CH), 4.11 (s, 1H, $\equiv$ CH), 3.97–3.89 (m, 2H, 2 × H-4'), 2.26–2.16 (m, 2H, 2 × H-2'(α or β)), 2.16–2.06 (m, 2H, 2 × H-2'(α or β)). δ_{C} (126 MHz, [D₆]DMSO) 161.49 (2 × C-4), 149.32 (C-2), 149.31 (C-2), 148.23 (d, *J* 6.9, C-2_{Ar}), 148.21 (d, *J* 7.0, C-2_{Ar}), 144.34 (2 × C-6), 129.57 (C-4_{Ar}), 129.52 (C-4_{Ar}), 128.24 (C-5_{Ar}), 128.22 (C-5_{Ar}), 126.02 (C-6_{Ar}), 126.00 (C-6_{Ar}), 122.93 (d, *J* 10.0, C-1_{Ar}), 122.89 (d, *J* 9.9, C-1_{Ar}), 120.14 (d, *J* 8.9, C-3_{Ar}), 120.10 (d, *J* 8.8, C-3_{Ar}), 98.06 (C-5), 98.03 (C-5), 85.00 (C-1'), 84.97 (C-1'), 84.4 (a t, *J* 7.3, 2 × C-4'), 83.91 ($\equiv$ C–H), 83.86 ($\equiv$ C–H), 76.12 (C $\equiv$ CH), 76.07 (C $\equiv$ CH), 69.7 (2 × C-3'), 68.0 (d, *J* 7.2, ArCH₂), 67.90 (d, *J* 7.2, ArCH₂), 67.8 (d, *J* 5.2, C-5'), 67.6 (d, *J* 5.4, C-5'), 38.8 (C-2'), 38.7 (C-2'). ³¹P{H} NMR (202 MHz, [D₆]DMSO) –10.0. *m/z* (LRMS ESI) 477, 479 [M + Na, ³⁵Cl, ³⁷Cl]⁺, 453, 455 [M – H, ³⁵Cl, ³⁷Cl][–]. *m/z* (HRMS ESI⁺) 495.0314 [M + Na + H₂O, ³⁵Cl]⁺; calcd for C₁₈H₁₈ClN₂NaO₉P 495.0331.

5'-O-Cyclosaligenyl-5-ethynyl-2'-deoxy-2'-difluorouridine monophosphate (7)

Compound **7** was synthesised from compound **35** (0.050 g, 0.1735 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.0160 g, 20 %). ³¹P NMR and LC-MS analysis indicates an approximate equimolar mixture of diastereomers around the phosphorus stereocentre. *R_f* 0.30 (5 % MeOH in CH₂Cl₂). δ_{H} (500 MHz, [D₆]DMSO) 11.95 (br s, 2H, 2 × NH), 7.95 (s, 1H, H-6), 7.93 (s, 1H, H-6), 7.43–7.33 (m, 2H, Ar), 7.33–7.25 (m, 2H, Ar), 7.24–7.18 (m, 2H, Ar), 2H), 7.18–7.09 (m, 2H, Ar), 6.50 (a t, *J* 6.2, 2H, 2 × OH-3'), 6.09 (a td, *J* 8.6, 3.9, 2H, H-1'), 5.61–5.39 (m, 4H, 2 × ArCH₂–), 4.60–4.35 (m, 4H, 2 × H-5'(α and β)), 4.34–4.21 (m, 2H, 2 × H-3'), 4.20 (s, 1H, $\equiv$ CH), 4.19 (s, 1H, $\equiv$ CH), 4.14–4.03 (m, 2H, 2 × H-4'). δ_{C} (126 MHz, [D₆]DMSO) 161.1 (2 × C-4), 149.39 (d, *J* 7.1, 2 × C-2_{Ar}), 149.0 (2 × C-2), 144.23–143.97 (m, 2 × C-6), 129.83–129.73 (m, 2 × C-4_{Ar}), 126.13 (2 × C-6_{Ar}), 124.5 (2 × C-5_{Ar}), 121.02 (d, *J* 9.9, C-1_{Ar}), 120.92 (d, *J* 9.9, C-1_{Ar}), 118.21 (d, *J* 8.8, C-3_{Ar}), 118.18 (d, *J* 8.8, C-3_{Ar}), 98.76 (C-5), 98.74 (C-5), 84.52 ($\equiv$ C–H), 84.46 ($\equiv$ C–H), 84.28–83.52 (br m, 2 × C-1') 78.55–78.05 (m, 2 × C-4'), 75.52 (C $\equiv$ CH), 75.51 (C $\equiv$ CH), 69.74–69.18 (m, 2 × C-3'), 68.67 (d,

J 7.1, ArCH₂), 68.57 (d, *J* 7.1, ArCH₂), 66.48 (d, *J* 5.4, C-5'), 66.41 (d, *J* 5.3, C-5') (C-2' not observed). ³¹P{H} NMR (202 MHz, [D₆]DMSO) –9.5, –9.6. ¹⁹F{H} NMR (202 MHz, [D₆]DMSO) –114.9, –115.1, –115.4, –115.6, –116.2. *m/z* (LRMS ESI) 479 [M + Na]⁺, 457 [M + H]⁺, 455 [M – H][–]. *m/z* (HRMS ESI⁺) 479.0412 [M + Na]⁺; calcd for C₁₈H₁₅F₂N₂ NaO₈P 479.0426.

5'-O-[3-Methylcyclosaligenyl]-5-ethynyl-2'-deoxy-2'-difluorouridine monophosphate (8)

Compound **8** was synthesised from compound **35** (0.1000 g, 0.3474 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.0450 g, 26 %). ³¹P NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. *R_f* 0.31 (5 % MeOH in CH₂Cl₂). δ_{H} (500 MHz, [D₆]DMSO) 11.95 (br s, 2H, 2 $\times$ NH), 7.92 (s, 1H, H-6), 7.91 (s, 1H, H-6), 7.29–7.20 (m, 2H, Ar), 7.13–7.07 (m, 4H, Ar), 6.50 (s, 2H, OH-3'), 6.08 (t, *J* 8.0, 2H, 2 $\times$ H-1'), 5.55–5.34 (m, 4H, 2 $\times$ ArCH₂–), 4.55–4.35 (m, 4H, 2 $\times$ H-5'(α and β)), 4.33–4.23 (m, 2H, 2 $\times$ H-3'), 4.17 (s, 1H, $\equiv$ CH), 4.16 (s, 1H, $\equiv$ CH), 4.09–4.02 (m, 2H, 2 $\times$ H-4'), 2.23 (s, 3H, Me), 2.21 (s, 3H, Me). δ_{C} (126 MHz, [D₆]DMSO) 161.06 (C-4), 161.04 (C-4), 149.08 (2 $\times$ C-2), 147.87 (d, *J* 7.2, 2 $\times$ C-2_{Ar}), 144.34–143.88 (m, 2 $\times$ C-6), 130.89 (d, *J* 4.1, 2 $\times$ C-4_{Ar}), 126.89 (d, *J* 7.8, C-3_{Ar}), 126.88 (d, *J* 7.8, C-3_{Ar}), 124.01–123.95 (m, 2 $\times$ C-5_{Ar}), 123.59 (d, *J* 5.2, 2 $\times$ C-6_{Ar}), 120.97 (d, *J* 9.5, C-1_{Ar}), 120.87 (d, *J* 9.5, C-1_{Ar}), 98.76 (2 $\times$ C-5), 84.45 ($\equiv$ C–H), 84.43 ($\equiv$ C–H), 84.22–83.57 (br m, 2 $\times$ C-1'), 78.52–78.19 (m, 2 $\times$ C-4'), 75.52 (C $\equiv$ CH), 75.50 (C $\equiv$ CH), 69.76–69.19 (m, 2 $\times$ C-3'), 68.61 (d, *J* 7.1, ArCH₂), 68.53 (d, *J* 7.0, ArCH₂), 66.65–66.38 (m, 2 $\times$ C-5'), 14.92 (CH₃), 14.89 (CH₃) (C-2' not observed). ³¹P{H} NMR (202 MHz, [D₆]DMSO) –8.9, –9.1. ¹⁹F{H} NMR (202 MHz, [D₆]DMSO) –114.6, –114.8, –115.1, –115.3, –115.8. *m/z* (LRMS ESI) 493 [M + Na]⁺, 471 [M + H]⁺, 469 [M – H][–]. *m/z* (HRMS ESI⁺) 493.0572 [M + Na]⁺; calcd for C₁₉H₁₇F₂N₂NaO₈P 493.0583.

5'-O-[3-Methoxycyclosaligenyl]-5-ethynyl-2'-deoxy-2'-difluorouridine monophosphate (9)

Compound **9** was synthesised from compound **35** (0.1000 g, 0.3474 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.0430 g, 26 %). ³¹P NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. *R_f* 0.30 (5 % MeOH in CH₂Cl₂). δ_{H} (500 MHz, [D₆]DMSO) 11.95 (br s, 2H, 2 $\times$ NH), 7.97 (s, 1H, H-6), 7.94 (s, 1H, H-6), 7.18–7.02 (m, 4H, Ar), 6.85–6.78 (m, 2H, Ar), 6.51 (s, 2H, 2 $\times$ OH-3'), 6.09 (dd, *J* 8.7, 2H, 2 $\times$ H-1'), 5.56–5.39 (m, 4H, 2 $\times$ ArCH₂–), 4.57–4.34 (m, 4H, 2 $\times$ H-5'(α and β)), 4.34–4.22 (m, 2H, 2 $\times$ H-3'), 4.17 (s, 1H, $\equiv$ CH), 4.16 (s, 1H, $\equiv$ CH), 4.10–4.02 (m, 2H, 2 $\times$ H-4'), 3.82 (s, 3H, MeO), 3.82 (s, 3H, MeO). δ_{C} (126 MHz, [D₆]DMSO) 161.1 (2 $\times$ C-4), 149.1 (2 $\times$ C-2), 148.27 (d, *J* 7.1, C-2_{Ar}), 148.24 (d, *J* 7.1, C-2_{Ar}),

144.37–143.92 (m, $2 \times \text{C-6}$), 138.61–138.32 (m, $2 \times \text{C-1}_{\text{Ar}}$), 125.59 (C-4_{Ar}), 125.58 (C-4_{Ar}), 122.00 (d, J 10.0, C-1_{Ar}) 121.99 (d, J 10.3, C-1_{Ar}), 116.99 (C-5_{Ar}), 116.96 (C-5_{Ar}), 112.66 (C-6_{Ar}), 112.58 (C-6_{Ar}), 99.75 (C-5), 99.72 (C-5), 84.3 ($2 \times \equiv\text{CH}$), 84.28–83.54 (m, $2 \times \text{C-1}'$), 78.41–78.11 (m, $2 \times \text{C-4}'$), 75.5 (br s, $2 \times \text{C}\equiv\text{CH}$), 69.76–69.23 (m, $2 \times \text{C-3}'$), 68.63 (ArCH₂), 68.58 (ArCH₂), 66.57 (d, J 4.7, $2 \times \text{C-5}'$), 56.93 (OMe), 56.90 (OMe) (C-2' not observed). ³¹P{H} NMR (202 MHz, [D₆]DMSO) –9.2, –9.3. ¹⁹F{H} NMR (202 MHz, [D₆]DMSO) –114.7, –115.2, –115.8, –116.3. m/z (LRMS ESI) 487 [M + H]⁺, 485 [M – H][–]. m/z (HRMS ESI⁺) 509.0559 [M + Na]⁺; calcd for C₁₉H₁₇F₂N₂NaO₉P 509.0532.

5'-O-[5-Chlorocyclosaligenyl]-5-ethynyl-2'-deoxy-2'-difluorouridine monophosphate (10)

Compound **10** was synthesised from compound **35** (0.1000 g, 0.3474 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.0410 g, 24 %). ³¹P NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. R_f 0.31 (5 % MeOH in CH₂Cl₂). δ_{H} (500 MHz, [D₆]DMSO) 11.96 (br s, 2H, $2 \times \text{NH}$), 7.95 (s, 1H, H-6), 7.93 (s, 1H, H-6), 7.47–7.39 (m, 4H, Ar), 7.21–7.16 (m, 2H, Ar), 6.50 (t, J 5.8, 2H, $2 \times \text{OH-3}'$), 6.13–6.05 (m, 2H, $2 \times \text{H-1}'$), 5.56–5.42 (m, 4H, $2 \times \text{ArCH}_2$ –), 4.61–4.37 (m, 4H, $2 \times \text{H-5}'_{(\alpha \text{ and } \beta)}$), 4.33–4.22 (m, 2H, $2 \times \text{H-3}'$), 4.20 (s, 1H, $\equiv\text{CH}$), 4.20 (s, 1H, $\equiv\text{CH}$), 4.13–4.03 (m, 2H, H-4'). δ_{C} (126 MHz, [D₆]DMSO) 161.1 ($2 \times \text{C-4}$), 149.1 ($2 \times \text{C-2}$), 148.25 (d, J 6.9, C-2_{Ar}), 148.23 (d, J 6.9, C-2_{Ar}), 144.42–143.95 (m, $2 \times \text{C-6}$), 129.64 (C-4_{Ar}), 129.58 (C-4_{Ar}), 128.35 (C-5_{Ar}), 128.34 (C-5_{Ar}), 126.0 ($2 \times \text{C-6}_{\text{Ar}}$), 122.97 (a t, J 9.5, $2 \times \text{C-1}_{\text{Ar}}$), 120.19 (d, J 9.1, C-3_{Ar}), 120.16 (d, J 8.8 Hz C-3_{Ar}), 98.81 (C-5), 98.90 (C-5), 84.57 ($\equiv\text{CH}$), 84.51 ($\equiv\text{CH}$), 84.34–83.59 (m, $2 \times \text{C-1}'$), 78.49–78.08 (m, $2 \times \text{C-4}'$), 75.57 (C $\equiv\text{CH}$), 75.54 (C $\equiv\text{CH}$), 69.73–69.21 (m, $2 \times \text{C-3}'$), 68.22 (d, J 7.2, ArCH₂), 68.08 (d, J 7.3, ArCH₂), 66.70 (d, J 4.7, C-5'), 66.61 (d, J 4.9, C-5') (C-2' not observed). ³¹P{H} NMR (202 MHz, [D₆]DMSO) –10.16, –10.17. ¹⁹F{H} NMR (202 MHz, [D₆]DMSO) –114.6, –114.7, –115.1, –115.3, –115.8, –117.3. m/z (LRMS ESI) 491, 493 [M + H, ³⁵Cl, ³⁷Cl]⁺, 489, 491 [M – H, ³⁵Cl, ³⁷Cl][–]. m/z (HRMS ESI⁺) 513.0054 [M + Na, ³⁵Cl]⁺; calcd for C₁₈H₁₄ClF₂N₂NaO₈P 513.0037.

(2'S)-5'-O-Cyclosaligenyl-5-ethynyl-2'-deoxy-2'-fluorouridine monophosphate (11)

Compound **11** was synthesised from compound **30** (0.100 g, 0.370 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.0749 g, 46 %). ³¹P NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. R_f 0.21 (5 % MeOH in CH₂Cl₂). δ_{H} (500 MHz, [D₆]DMSO) 11.87 (s, 2H, $2 \times \text{NH}$), 7.88 (d, J 1.8, 1H, H-6), 7.87 (d, J 1.8, 1H, H-6), 7.42–7.36 (m, 2H, $2 \times \text{Ar}$), 7.32–7.25 (m, 2H, $2 \times \text{Ar}$), 7.24–7.17 (m, 2H, $2 \times \text{Ar}$), 7.18–7.13 (m, 2H, $2 \times \text{Ar}$), 6.18–6.10 (m, 2H, $2 \times \text{H-2}$), 6.10–6.06 (m, 2H, $2 \times \text{OH-3}'$), 5.57–5.41 (m, 4H, $2 \times \text{ArCH}_2$ –), 5.11–4.95 (m, 2H, $2 \times$

H-2') 4.50–4.35 (m, 4H, $2 \times \text{H-5}'_{(\alpha \text{ and } \beta)}$), 4.28–4.17 (m, 2H, $2 \times \text{H-3}'$), 4.14 (s, 1H, $\equiv\text{CH}$), 4.13 (s, 1H, $\equiv\text{CH}$), 4.07–4.00 (m, 2H, $2 \times \text{H-4}'$). δ_{C} (126 MHz, [D6]DMSO) 161.31 ($2 \times \text{C-4}$), 149.44 (d, J 6.9, $2 \times \text{C-2}_{\text{Ar}}$), 149.01 ($2 \times \text{C-2}$), 144.58 (a dd, J 7.3, 2.8, $2 \times \text{C-6}$), 129.81 ($2 \times \text{C-4}_{\text{Ar}}$), 126.13 ($2 \times \text{C-6}_{\text{Ar}}$), 124.48 ($2 \times \text{C-5}_{\text{Ar}}$), 120.96 (a t, J 9.6, $2 \times \text{C-1}_{\text{Ar}}$), 118.20 (d, J 8.8, $2 \times \text{C-3}_{\text{Ar}}$), 97.72 (C-5), 97.70 (C-5), 94.83 (a dd, J 191.3, 16.8, $2 \times \text{C-2}'$), 84.08 ($\equiv\text{CH}$), 84.03 ($\equiv\text{CH}$), 83.29 (a t, J 15.7, $2 \times \text{C-1}'$), 81.65–81.04 (m, $2 \times \text{C-4}'$), 75.77 (C $\equiv\text{CH}$), 75.75 (C $\equiv\text{CH}$), 73.22 (a dd, J 25.1, 7.5, $2 \times \text{C-3}'$), 68.50 (a t, J 7.2, $2 \times \text{ArCH}_2$), 66.93 (d, J 4.8, $2 \times \text{C-5}'$). $^{31}\text{P}\{\text{H}\}$ NMR (202 MHz, [D6]DMSO) –9.52, –9.55. $^{19}\text{F}\{\text{H}\}$ NMR (202 MHz, [D6]DMSO) –198.4, –198.6. m/z (LRMS ESI) 471 $[\text{M} + \text{H}^+ \text{MeOH}]^+$, 470 $[\text{M} - \text{H} + \text{MeOH}]^-$. m/z (HRMS ESI $^+$) 461.0518 $[\text{M} + \text{Na}]^+$; calcd for $\text{C}_{18}\text{H}_{16}\text{FN}_2\text{NaO}_8\text{P}$ 461.0521.

(2'S)-5'-O-Cyclosaligenyl-5-ethynyl-2'-deoxy-2'-chlorouridine monophosphate (12)

Compound **12** was synthesised from compound **31** (0.0716 g, 0.250 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH_2Cl_2) to afford the *title compound* as a colourless glass (0.0362 g, 32 %). ^{31}P NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. R_f 0.38 (5 % MeOH in CH_2Cl_2). δ_{H} (500 MHz, [D6]DMSO) 11.86 (br s, 2H, $2 \times \text{NH}$), 7.91 (s, 1H, H-6), 7.91 (s, 1H, H-6), 7.42–7.33 (m, 2H, Ar), 7.32–7.27 (m, 2H, Ar), 7.25–7.18 (m, 2H, Ar), 7.18–7.13 (m, 2H, Ar), 6.29 (br s, 2H, $2 \times \text{OH-3}'$), 6.20 (s, 1H, H-1'), 6.19 (s, 1H, H-1'), 5.57–5.41 (m, 4H, $2 \times \text{ArCH}_2$), 4.61–4.55 (m, 2H, $2 \times \text{H-2}'$), 4.53–4.39 (m, 4H, $2 \times \text{H-5}'_{(\alpha \text{ and } \beta)}$), 4.21 (dd, J 4.9, 2H, $2 \times \text{H-3}'$), 4.14 (s, 1H, $\equiv\text{CH}$), 4.13 (s, 1H, $\equiv\text{CH}$), 4.05–3.98 (m, 2H, $2 \times \text{H-4}'$). δ_{C} (126 MHz, [D6]DMSO) 161.36 ($2 \times \text{C-4}$), 149.44 (d, J 7.1, $2 \times \text{C-2}_{\text{Ar}}$), 148.96 ($2 \times \text{C-2}$), 144.23 (C-6), 144.18 (C-6), 129.80 (C-4 $_{\text{Ar}}$), 129.80 (C-4 $_{\text{Ar}}$), 126.12 ($2 \times \text{C-6}_{\text{Ar}}$), 124.48 ($2 \times \text{C-5}_{\text{Ar}}$), 121.05 (d, J 9.9, C-1 $_{\text{Ar}}$), 120.93 (d, J 9.9, C-1 $_{\text{Ar}}$), 118.27–118.13 (m, $2 \times \text{C-3}_{\text{Ar}}$), 97.49 ($2 \times \text{C-5}$), 84.33 (C-1'), 84.24 (C-1'), 84.12 ($\equiv\text{CH}$), 84.08 ($\equiv\text{CH}$), 81.66 (a d, J 6.4, C-4'), 81.51 (a d, J 6.4, C-4'), 76.04–75.59 (m, $2 \times \text{C}\equiv\text{CH}$ and $2 \times \text{C-3}'$), 68.59 (d, J 7.1, ArCH_2), 68.49 (d, J 7.2, ArCH_2), 63.46 (d, J 4.9, $2 \times \text{C-5}'$), 63.48 (C-2'), 63.44 (C-2'). $^{31}\text{P}\{\text{H}\}$ NMR (202 MHz, [D6]DMSO) –9.3, –9.4. m/z (LRMS ESI) 455, 457 $[\text{M} + \text{H}, ^{35}\text{Cl}, ^{37}\text{Cl}]^+$, 453, 455 $[\text{M} - \text{H}, ^{35}\text{Cl}, ^{37}\text{Cl}]^-$. m/z (HRMS ESI $^+$) 477.0210 $[\text{M} + \text{Na}, ^{35}\text{Cl}, ^{37}\text{Cl}]$; calcd for $\text{C}_{18}\text{H}_{16}\text{ClN}_2\text{NaO}_8\text{P}$ 477.0225.

(2'S)-5'-O-Cyclosaligenyl-5-ethynyl-2'-deoxy-2'-bromouridine monophosphate (13)

Compound **13** was synthesised from compound **32** (0.0600 g, 0.1812 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH_2Cl_2) to afford the *title compound* as a colourless glass (0.0362 g, 32 %). ^{31}P NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. R_f 0.59 (10 % MeOH in CH_2Cl_2). δ_{H} (500 MHz, [D6]DMSO) 11.86 (br s, 2H, $2 \times \text{NH}$), 7.88 (s, 1H, H-6), 7.88 (s, 1H, H-6), 7.44–7.33 (m, 2H, Ar), 7.33–7.26 (m, 2H,

Ar), 7.24–7.18 (m, 2H, Ar), 7.18–7.12 (m, 2H, Ar), 6.30 (br s, 2H, 2 × OH-3'), 6.11 (s, 1H, H-1'), 6.09 (s, 1H, H-1'), 5.60–5.39 (m, 4H, 2 × ArCH₂-), 4.65–4.58 (m, 2H, 2 × H-2'), 4.55–4.39 (m, 4H, 2 × H-5'(_α and _β)), 4.31 (dd, *J* 5.4, 2H, 2 × H-2'), 4.14 (s, 1H, ≡CH), 4.13 (s, 1H, ≡CH), 4.02–3.93 (m, 2H, 2 × H-4'). δ_C (126 MHz, [D₆]DMSO) 161.3 (2 × C-2), 149.44 (d, *J* 7.1, 2 × C-2_{Ar}), 148.91 (2 × C-2), 144.33–143.88 (m, 2 × C-6), 129.8 (2 × C-4_{Ar}), 126.1 (2 × C-6_{Ar}), 124.5 (2 × C-5_{Ar}), 121.06 (d, *J* 9.8, C-1_{Ar}), 120.95 (d, *J* 9.9, C-1_{Ar}), 118.25 (d, *J* 8.7, C-3_{Ar}), 118.22 (d, *J* 8.7, C-3_{Ar}), 97.5 (2 × C-5), 84.59–83.47 (m, 2 × ≡CH and 2 × C1'), 81.96 (d, *J* 6.5, C-4'), 81.84 (d, *J* 6.5, C-4'), 75.99–75.58 (m, 2 × C≡CH and 2 × C-3'), 68.61 (d, *J* 7.0, ArCH₃), 68.50 (d, *J* 7.0, ArCH₃), 67.01 (d, *J* 5.0, 2 × C-5'), 55.07 (2 × C-2'). ³¹P{H} NMR (202 MHz, [D₆]DMSO) –9.4, –9.5. *m/z* (LRMS ESI) 499, 501 [M + H]⁺ ⁷⁹Br, ⁸¹Br]⁺, 497, 499 [M – H, ⁷⁹Br, ⁸¹Br][–]. *m/z* (HRMS ESI⁺) 520.9702 [M + Na, ⁷⁹Br, ⁸¹Br]⁺; calcd for C₁₈H₁₆BrN₂NaO₈P 520.9720.

(2'S)-5'-O-Cyclosaligenyl-5-ethynyl-2'-deoxy-2'-iodouridine monophosphate (14)

Compound **14** was synthesised from compound **33** (0.0350 g, 0.0926 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.0125 g, 25 %). ³¹P NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. *R_f* 0.28 (5 % MeOH in CH₂Cl₂). δ_H (500 MHz, [D₆]DMSO) 11.84 (s, 2H, 2 × NH), 7.85–7.81 (m, 2H, 2 × H-6), 7.40–7.34 (m, 2H, Ar), 7.31–7.26 (m, 2H, Ar), 7.24–7.18 (m, 2H, Ar), 7.18–7.12 (m, 2H, Ar), 6.24–6.14 (m, 2H, 2 × OH-3'), 5.88–5.85 (m, 2H, 2 × H-1'), 5.59–5.40 (m, 4H, 2 × ArCH₂-), 4.55 (td, *J* 6.7, 3.2, 2H, 2 × H-1'), 4.52–4.38 (m, 4H, 2 × H-5'(_α and _β)), 4.37–4.29 (m, 2H, 2 × H-3'), 4.15 (s, 1H, ≡CH), 4.13 (s, 1H, ≡CH), 3.95–3.87 (m, 2H, 2 × H-4'). δ_C (126 MHz, [D₆]DMSO) 161.3 (2 × C-4), 149.44 (d, *J* 7.2, 2 × C-2_{Ar}), 148.94 (2 × C-2), 144.19–143.51 (m, 2 × C-6) 129.81 (2 × C-4_{Ar}), 126.14 (2 × C-6_{Ar}), 124.48 (2 × C-5_{Ar}), 121.07 (d, *J* 9.9, C-1_{Ar}), 120.97 (d, *J* 9.9, C-1_{Ar}), 118.26 (a dd, *J* 8.5, 7.3, 2 × C-3_{Ar}) 97.80 (2 × C-5), 85.00–84.52 (br m, 2 × C-1'), 84.22 (≡CH), 84.17 (≡CH), 82.60–82.10 (m, 2 × C-4'), 76.76 (C-3'), 76.74 (C-3'), 75.89 (2 × C≡CH), 68.62 (d, *J* 7.0, ArCH₃), 68.51 (d, *J* 7.0, ArCH₃), 67.10–66.86 (m, 2 × C-5'), 32.01–31.77 (m, 2 × C-2'). ³¹P{H} NMR (202 MHz, [D₆]DMSO) –9.46, –9.48. *m/z* (LRMS ESI) 547 [M + H]⁺, 545 [M – H][–]. *m/z* (HRMS ESI⁺) 568.9574 [M + Na]; calcd for C₁₈H₁₆FN₂NaO₈P 568.9581.

(2'R)-5'-O-Cyclosaligenyl-5-ethynyl-2'-deoxy-2'-fluorouridine monophosphate (15)

Compound **15** was synthesised from compound **34** (0.100 g, 0.370 mmol) according to General Procedure 1. The crude product was purified by silica gel flash chromatography (5 % MeOH in CH₂Cl₂) to afford the *title compound* as a colourless glass (0.040 g, 25 %). ³¹P NMR and LC-MS analysis indicates an approximately equimolar mixture of diastereomers around the phosphorus stereocentre. *R_f* 0.28 (5 % MeOH in CH₂Cl₂). δ_H (500 MHz, [D₆]DMSO) 11.74 (br s, 2H, 2 × NH), 7.93 (s, 1H, H-6), 7.92 (s, 1H, H-6), 7.40–7.33 (m, 2H, Ar), 7.31–7.23 (m, 2H,

Ar), 7.23–7.11 (m, 4H, Ar), 5.88–5.73 (m, 4H, 2 × H-1', 2 × OH-3'), 5.56–5.41 (m, 4H, 2 × ArCH₂-), 5.24–5.04 (m, 2H, 2 × H-2'), 4.53–4.39 (m, 2H, 2 × H-5'(_α or _β)), 4.38–4.28 (m, 2H, 2 × H-5'(_α or _β)), 4.26–4.13 (m, 1H, 2 × H-3'), 4.11 (s, 1H, ≡CH), 4.09 (s, 1H, ≡CH), 4.01 (dd, *J* 6.1, 2.9, 2H, 2 × H-4'). δ_{C} (126 MHz, [D6]DMSO) 161.7 (2 × C-4), 149.43 (d, *J* 7.0, C-2_{Ar}), 149.39 (d, *J* 7.1, C-2_{Ar}), 149.15 (C-2), 149.11 (C-2), 145.05 (C-6), 145.01 (C-6), 129.84–129.63 (m, 2 × C-4_{Ar}), 126.1 (C-6_{Ar}), 126.09 (C-6_{Ar}), 124.43 (C-5_{Ar}), 124.37 (C-5_{Ar}), 121.06 (d, *J* 9.9, C-1_{Ar}), 120.88 (d, *J* 9.8, C-1_{Ar}), 118.21 (a t, *J* 8.4, 2 × C-3_{Ar}), 97.99 (C-5), 97.98 (C-5), 92.64 (a dd, *J* 184.9, 3.5, 2 × C-2'), 89.55 (a dd, *J* 36.5, 13.2, 2 × C-1'), 84.0 (≡CH), 83.9 (≡CH), 80.27 (a dd, *J* 12.0, 7.1, 2 × C-4'), 75.89 (C≡CH), 75.88 (C≡CH), 68.57 (d, *J* 7.2, 2 × ArCH₂), 67.55 (a t, *J* 17.1, 2 × C-3'), 66.69 (d, *J* 5.4, C-5'), 66.30 (d, *J* 5.4, C-5'). ³¹P{H} NMR (202 MHz, [D6]DMSO) –9.5, –9.6. ¹⁹F{H} NMR (202 MHz, [D6]DMSO) –199.7, –199.8. *m/z* (LRMS ESI) 439 [M + H]⁺, 437 [M – H][–]. *m/z* (HRMS ESI⁺) 461.0503 [M + Na]⁺; calcd for C₁₈H₁₆FN₂O₈P 461.0521.

Cyclosaligenyl-N,N-diisopropylaminophosphoramidite (20)

Compound **20** was prepared from saligenyl alcohol **22** (0.500 g, 4.027 mmol) according to General Procedure 2. The *title compound* was afforded as white solid (0.663 g, 65 %). The NMR spectroscopic data were consistent with literature values.^[18] δ_{H} (500 MHz, CDCl₃) 7.21–7.15 (m, 1H), 7.01–6.86 (m, 3H), 5.14 (dd, ²*J*_{HH} 14.2, ³*J*_{PH} 5.0, 1H), 4.87 (dd, ³*J*_{PH} 19.6, ²*J*_{HH} 14.2, 1H), 3.63 (dp, *J* 10.6, 6.8, 2H), 1.24 (dd, *J* 10.6, 6.8, 12H). δ_{P} (202 MHz, CDCl₃) 135.3.

3-Methylcyclosaligenyl-N,N-diisopropylaminophosphoramidite (24)

Compound **24** was prepared from 3-methylsaligenyl alcohol **27** (1.000 g, 7.237 mmol) according to General Procedure 2. The *title compound* was afforded as white solid (0.600 g, 31 %). δ_{H} (500 MHz, CDCl₃) 7.08–7.04 (m, 1H, Ar), 6.85–6.79 (m, 2H, Ar), 5.12 (dd, ²*J*_{HH} 14.0, ³*J*_{PH} 5.0, 1H, H-7), 4.84 (dd, ³*J*_{PH} 19.9, ²*J*_{HH} 14.0, 1H, H-7), 3.63 (dp, *J* 10.5, 6.8, 2H, 2 × ((H₃C)₂CHN)), 2.22 (d, *J* 0.7, 3H, Me), 1.24 (dd, *J* 12.4, 6.8, 12H, 2 × ((H₃C)₂CHN)). δ_{C} (126 MHz, CDCl₃) 151.6 (d, *J* 4.7, C-2), 129.9, 128.0, 124.0 (d, *J* 13.0, C-1), 122.9, 120.6, 64.4 (d, *J* 4.4, C-7), 44.4 ((H₃C)₂C), 44.3 ((H₃C)₂C), 24.83 ((H₃C)₂C), 24.81 ((H₃C)₂C), 24.8 ((H₃C)₂C), 24.7 ((H₃C)₂C), 15.8 (Me). δ_{P} (202 MHz, CDCl₃) 135.2.

3-Methoxycyclosaligenyl-N,N-diisopropylaminophosphoramidite (25)

Compound **25** was prepared from 3-methoxysaligenyl alcohol **28** (0.500 g, 3.2432 mmol) according to General Procedure 2. The *title compound* was afforded as white solid (0.496 g, 54 %). δ_{H} (500 MHz, CDCl₃) 6.89–6.79 (m, 2H, Ar), 6.63–6.58 (m, 1H, Ar), 5.09 (dd, ²*J*_{HH} 14.0, ³*J*_{PH} 5.0, 1H, H-7), 4.82 (dd, ³*J*_{PH} 19.2, ²*J*_{HH} 14.0, 1H, H-7), 3.85 (s, 3H, MeO), 3.61 (dhept, *J* 10.4, 6.7, 2H, 2 × ((H₃C)₂CHN)), 1.24 (dd, *J* 20.7, 6.8, 12H, 2 × ((H₃C)₂CHN)). δ_{C} (126 MHz, CDCl₃) 149.9 (d, *J* 0.9, C-3), 142.75 (d, *J* 5.0, C-2), 125.88 (d, *J* 12.6, C-1), 120.9 (Ar), 117.6

(Ar), 111.9 (Ar), 64.0 (d, J 3.7, C-7), 56.4 (MeO), 44.6 ((H₃C)₂C), 44.5 ((H₃C)₂C), 24.9 ((H₃C)₂C), 24.8 ((H₃C)₂C), 24.7 ((H₃C)₂C), 24.6 ((H₃C)₂C). δ_P (202 MHz, CDCl₃) 135.5.

5-Chlorocyclosaligenyl-N,N-diisopropylaminophosphoramidite (26)

Compound **26** was prepared from 5-chlorosaligenyl alcohol **29** (0.500 g, 3.153 mmol) according to General Procedure 2. The *title compound* was afforded as white solid (0.590 g, 65 %). The NMR spectroscopic data were consistent with literature values.^[19] δ_H (500 MHz, CDCl₃) 7.13 (dd, J 8.6, 2.6, 1H, Ar), 6.99–6.92 (m, 1H, Ar), 6.83 (d, J 8.6, 1H, Ar), 5.09 (ddt, $^2J_{HH}$ 14.4, $^3J_{PH}$ 4.8, $^4J_{HH}$ 0.9, 1H, H-7), 4.81 (dd, $^3J_{PH}$ 20.1, $^2J_{HH}$ 14.5, 1H, H-7), 3.61 (dp, J 10.7, 6.8, 2H, 2 × ((H₃C)₂CHN)), 1.24 (dd, J 9.7, 6.8, 12H, 2 × ((H₃C)₂CHN)). δ_C (126 MHz, CDCl₃) 152.1 (d, J 4.2, C-2), 128.6 (Ar), 126.0 (C-5), 125.7 (d, J 13.6, C-1), 125.3 (Ar), 120.5 (Ar), 64.0 (d, J 4.8, C-7), 44.5 ((H₃C)₂C), 44.4 ((H₃C)₂C), 24.8 ((H₃C)₂C), 24.7 ((H₃C)₂C), 24.6 ((H₃C)₂C). δ_P (202 MHz, CDCl₃) 136.5.

Supplementary Material

¹H, ¹³C, ¹⁹F, and ³¹P NMR data and the syntheses of compounds **34** and **35** are available on the Journal's website.

Conflicts of Interest

The authors declare no conflicts of interest.

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Fig. 1. (a) Ethynyl-2'-deoxyuridine (EdU, **1**) is a widely used chemical probe for the metabolic labelling of DNA. (b) EdU ProLabels avoid two drawbacks of **1**, the requirement for nucleoside transporters for cell entry and the requirement for processing by thymidine kinase.

Fig. 2. (a) Generic structures of P^V chlorophosphite, P^{III} phosphorochloridate, and P^{III} phosphoramidite reagents. (b) 5-Ethynyl-2'-deoxyuridine (EdU, **1**) and ProLabels of EdU monophosphate: cyclosaligenyl EdU **2** and 5'-bis-pivaloyloxymethyl EdU **3**.^[6,7]

Fig. 3. Target cyclosaligenyl phosphotriester uridine analogues **2** and **4–15**.

Fig. 4. Numbering system for pyrimidine nucleosides using uridine as an example.

Scheme 1. (a) Regioselective phosphitylation of thymidine **16** with di-*tert*-butyl *N,N*-diethylphosphoramidite **17** to yield thymidine 5'-*O*-di-*tert*-butyl phosphite **18**.^[13] (b) Phosphitylation of 2',3'-dideoxythymidine **19** with cyclosaligenyl phosphoramidite **20** followed by oxidation with *tert*-butyl hydrogen peroxide to yield cyclosaligenyl phosphotriester **21**.^[16] Py·HCl is used as activator for the phosphoramidite in both (a) and (b).

Scheme 2. The reported synthesis of cyclosaligenyl phosphoramidite **20** proceeds in two steps from saligenyl alcohol **22**, via the intermediate cyclosaligenyl chlorophosphite **23**.^[18]

Scheme 3. Development of a one-pot synthesis for cyclosaligenyl phosphoramidites **20** and **24–26** from the corresponding saligenyl alcohol **22** and **27–29**. The reaction proceeds through in situ formation of a cyclosaligenyl chlorophosphite intermediate.

Scheme 4. Synthesis of cyclosaligenyl phosphotriester **2** from treatment of EdU (**1**) with cyclosaligenyl phosphoramidite **20** (1.5 equiv.), employing either pyridinium chloride (Py·HCl); pyridinium trifluoroacetate (Py·TFA); tetrazole; 4,5-dicyanoimidazole (DCI); or imidazolium trifluoromethanesulfonate (ImH⁺TfO⁻) as activator and CH₃CN or pyridine as solvent.

Scheme 5. Synthesis of cycloSal ProLabels of EdU monophosphate (**2**, **4–15**) from the corresponding unprotected EdU derivative (**1**, **30–35**) as substrate in reaction with cyclosaligenyl phosphoramidites (**20**, **24–26**) (1.5 equiv., dissolved in CH₂Cl₂), employing pyridinium trifluoroacetate (Py·TFA) as activator and pyridine as solvent.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

A

B

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Scheme 5.

Supporting information

Operationally simple regioselective 5'-phosphorylation of unprotected 5-ethynyl-2'-deoxyuridine analogues

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Compound S6 ^1H and $^{19}\text{F}\{\text{H}\}$ NMR (CDCl_3)	S54
Compound S7 ^1H and ^{13}C NMR (CDCl_3)	S55
Compound S7 $^{19}\text{F}\{\text{H}\}$ NMR (CDCl_3)	S56
Compound S8 ^1H and ^{13}C NMR (CDCl_3)	S57
Compound S8 $^{19}\text{F}\{\text{H}\}$ NMR (CDCl_3)	S58
Compound 35 ^1H and ^{13}C NMR (DMSO- d_6)	S59
Compound 35 $^{19}\text{F}\{\text{H}\}$ NMR (DMSO- d_6)	S60

Stacked spectra of Compound 2 $^{31}\text{P}\{\text{H}\}$ NMR and $^{31}\text{P}\{\text{H}\}$ NMR Crude reaction mixture for synthesis of compound 2 (pyridine- d_5)	S61
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Fluorinated nucleosides **34** and **35** are novel and their synthesis is reported below. To the best of our knowledge, intermediate compounds **S3**, **S4** and **S8** are novel while spectroscopic data for **24** has not been reported previously.

We found that deamination of commercially available gemcitabine employing diazotisation conditions proceeded efficiently at 0 °C to afford 2'-dideoxy-2'-difluoro-uridine (157) in 98% yield (**Scheme S1**). Compound **S1** was subsequently acetylated with Ac₂O, employing pyridine as base and solvent, to give the 3',5'-protected intermediate **S2** in quantitative yield. The synthesis then follows the synthetic strategies previously developed for the synthesis of EdU. This involves iodination of the 5 position of **S2** to give **S3**, followed by Sonogashira coupling with ethynyl-2-trimethylsilane to give **S4**, and lastly deprotection to afford dF-EdU (**35**) in 67% yield.

Scheme S1. Synthesis of dF-EdU (**35**) from gemcitabine.

2'-Dideoxy-2'-difluoro-uridine (**S1**)

Gemcitabine (1.00 g, 3.093 mmol) was dissolved in H₂O (1.8 mL) and AcOH (3.5 mL) and cooled to 0 °C. NaNO₂ dissolved in a minimum amount of H₂O was slowly added to the reaction mixture with stirring. The mixture was stirred for 3 h at 0 °C at which point TLC analysis indicated complete conversion of starting material to a single product. The mixture was allowed to warm to rt and was then poured into acetone (100 mL). The precipitated salts were filtered off and the solvent removed *in vacuo*. The residue was co-evaporated with EtOH (2×) then the residue dissolved in MeOH and adsorbed onto silica gel. The crude product was purified by silica gel flash chromatography to afford the title compound as a clear oil (quantitative). Spectroscopic data was consistent with literature values.¹ *R*_f = 0.23 (10% MeOH in CH₂Cl₂). ¹H NMR (500 MHz, MeOD-*d*₄) δ_H = 7.87 (d,

$J = 8.2$ Hz, 1H), 6.17 – 6.12 (m, 1H), 5.73 (d, $J = 8.2$ Hz, 1H), 4.28 (td, $J = 12.1$, 8.1 Hz, 1H), 3.96 – 3.87 (m, 2H), 3.78 (dd, $J = 12.5$, 3.1 Hz, 1H). ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 11.57$ (br s, 1H, NH), 7.79 (d, $J = 8.2$ Hz, 1H, H-6'), 6.06 (t, $J = 7.9$ Hz, 1H, H-1'), 5.72 (d, $J = 8.1$ Hz, 1H, H-5), 4.18 (td, $J = 12.8$, 8.4 Hz, 1H, H-3'), 3.83 (ddd, $J = 8.4$, 3.6, 2.5 Hz, 1H, H-4'), 3.76 (dd, $J = 12.7$, 2.4 Hz, 1H, H-5'(α or β)), 3.62 (dd, $J = 12.7$, 3.6 Hz, 1H, H-5'(α or β)). ^{19}F NMR (470 MHz, MeOD- d_4) $\delta = -118.6$ (dd, $J = 13.2$, 4.8 Hz), -119.2 (dd, $J = 13.3$, 5.0 Hz), -120.0, -120.5. LRMS (ESI): $m/z = 265$ [$\text{M} + \text{H}$] $^+$, 263 [$\text{M} - \text{H}$] $^-$, 309 [$\text{M} + \text{HCO}_2$] $^-$.

3',5'-*O*-Bis-acetyl-2'-dideoxy-2'-difluoro-uridine (**S2**)

Compound **S1** was dissolved in pyridine (3.3 mL, 30.93 mmol, 10 equiv) and Ac₂O (0.73 mL, 7.73 mmol, 2.5 equiv) and the mixture stirred at rt for 8 h. EtOAc (50 mL) was added and the organic material washed with 5% HCl_(aq) (20 mL), NaHCO_{3(satd. aq)} (20 mL), brine (20 mL) and dried over MgSO₄. The solvent was removed to give the title compound as a yellow gum (quantitative). $R_f = 0.16$ (30% EtOAc in CH₂Cl₂). ^1H NMR (500 MHz, CDCl₃) $\delta_{\text{H}} = 9.40$ (br s, 1H, NH), 7.36 (dd, $J = 8.2$, 2.4 Hz, 1H, H-6), 6.26 (dd, $J = 11.9$, 6.3 Hz, 1H, H-1'), 5.81 (d, $J = 8.2$ Hz, 1H, H-5), 5.25 (ddd, $J = 13.8$, 5.5, 3.0 Hz, 1H, H-3'), 4.39 (d, $J = 4.2$ Hz, 2H, H-5'(α and β)), 4.29 (q, $J = 4.4$ Hz, 1H, H-4'), 2.20 (s, 3H, Ac), 2.12 (s, 3H, Ac). ^{13}C NMR (126 MHz, CDCl₃) $\delta_{\text{C}} = 170.4$ (H₃CCO₂), 169.1 (H₃CCO₂), 162.6 (C-4), 150.2 (C-2), 139.8 (d, $J = 3.7$ Hz, C-6), 120.5 (dd, $J = 266.4$, 259.1 Hz, C-2'), 103.4 (C-5), 83.1 (dd, $J = 38.0$, 20.8 Hz, C-1'), 78.0 (dd, $J = 4.6$, 2.5 Hz, C-4'), 70.7 (dd, $J = 34.2$, 17.0 Hz, C-3'), 62.1 (C-5'), 20.8 (H₃CCO₂), 20.5 (H₃CCO₂). ^{19}F NMR (470 MHz, CDCl₃) $\delta_{\text{F}} = -116.8$ (dt, $J = 247.4$, 12.3 Hz), -121.4 (d, $J = 250.4$ Hz). LRMS (ESI): $m/z = 349$ [$\text{M} + \text{H}$] $^+$, 347 [$\text{M} - \text{H}$] $^-$. HRMS (ESI $^+$) m/z [$\text{M} + \text{Na}$] $^+$ calcd. for C₁₃H₁₄F₂N₂NaO₇ 371.0661, found 371.0634.

3',5'-*O*-Bis-acetyl-2'-dideoxy-2'-difluoro-5-iodo-uridine (**S3**)

Compound **S3** was synthesised from compound **S2** (0.980 g, 2.815 mmol) according to adapted literature procedures.² The crude product was purified by silica gel flash chromatography (5 – 20% EtOAc in CH₂Cl₂) to afford the title compound as a pale yellow foam/gum (1.20 g, 90%). $R_f = 0.52$ (30% EtOAc in CH₂Cl₂). ^1H NMR (500 MHz, CDCl₃) $\delta_{\text{H}} = 8.90$ (br s, 1H, NH), 7.80 (d, $J = 2.2$ Hz, 1H, H-6), 6.24 (dd, $J = 10.5$, 6.9 Hz, 1H, H-1'), 5.29 (ddd, $J = 13.3$, 5.7, 3.9 Hz, 1H, H-3'), 4.42 (d, $J = 3.5$ Hz, 2H, H-5'(α and β)), 4.35 – 4.29 (m, 1H, H-4'), 2.21 (2× s, 6H, Ac). ^{13}C NMR (126 MHz, CDCl₃) $\delta_{\text{C}} = 170.4$ (H₃CCO₂), 169.1 (H₃CCO₂), 159.3 (C-4), 149.5 (C-2), 144.1 (H-6), 120.6 (dd, $J = 265.7$,

260.0 Hz, C-2'), 83.4 (dd, $J = 38.2, 20.7$ Hz, C-1'), 78.3 (d, $J = 2.1$ Hz, H-4'), 70.5 (dd, $J = 33.8, 17.0$ Hz, H-3'), 69.4 (C-5), 61.9 (C-5'), 21.0 (H₃CCO₂), 20.5 (H₃CCO₂). ¹⁹F NMR (470 MHz, CDCl₃) $\delta_F = -116.3$ (dt, $J = 247.9, 11.8$ Hz), -120.6 (d, $J = 247.7$ Hz). LRMS (ESI): $m/z = 475$ [M + H]⁺, 473 [M - H]⁻. HRMS (ESI⁺) m/z [M + Na]⁺ calcd. for C₁₃H₁₃F₂IN₂NaO₇ 496.9628, found 496.9590.

3',5'-O-Bis-acetyl-2'-dideoxy-2'-difluoro-5-(ethynyl(2-trimethylsilyl))-uridine (**S4**)

Compound **S4** was synthesised from compound **S3** (1.400 g, 2.953 mmol) using Sonagashira conditions. **S3** was combined with CuI (0.056 g, 0.295 mmol, 0.1 equiv), Pd(PPh₃)₄ (0.154 g, 0.148 mmol, 0.05 equiv) under an atmosphere of argon. The mixture was suspended in anhydrous CH₂Cl₂ (10 mL) and ethynyl-2-trimethylsilane (2 mL, 14.7 mmol, 5 equiv) added immediately, followed by Et₃N (0.8 mL, 5.9 mmol, 2 equiv). Upon addition of Et₃N the reaction mixture clarified and developed a yellow-orange colour. The mixture was stirred at rt until complete consumption of **S3** was evident by TLC (2 – 3 h). The solvent was removed *in vacuo* and the residue dissolved in EtOAc. The EtOAc fraction was washed with 5% HCl_(aq) (20 mL), NaHCO_{3(satd. aq)} (20 mL), 1.0 M EDTA-Na (3 × 20 mL) and brine (20 mL) then dried over MgSO₄ and concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography (10 – 20% EtOAc in CH₂Cl₂) to afford the title compound as a colourless foam (1.246 g, 95 %). $R_f = 0.66$ (30% EtOAc in CH₂Cl₂). ¹H NMR (500 MHz, CDCl₃) $\delta_H = 8.98$ (br s, 1H, NH), 7.66 (d, $J = 2.0$ Hz, 1H, H-6), 6.26 (t, $J = 8.4$ Hz, 1H, H-1'), 5.31 (dt, $J = 12.9, 5.8$ Hz, 1H, H-3'), 4.44 (dd, $J = 12.7, 3.1$ Hz, 1H, H-5'(α or β)), 4.38 (dd, $J = 12.7, 3.5$ Hz, 1H, H-5'(α or β)), 4.31 (dt, $J = 6.4, 3.3$ Hz, 1H, H-4'), 2.20 (s, 3H, Ac), 2.18 (s, 3H, Ac), 0.22 (s, 9H, Si(CH₃)₃). ¹³C NMR (126 MHz, CDCl₃) $\delta_C = 170.2$ (H₃CCO₂), 169.1 (H₃CCO₂), 160.3, 148.9 (C-2), 142.4 (d, $J = 2.7$ Hz, C-6), 120.6 (dd, $J = 264.4, 261.3$ Hz, C-2'), 101.5 (C-5), 100.6 (C≡C), 94.6 (C≡C), 83.7 (dd, $J = 39.5, 21.6$ Hz, C-1'), 78.0 (d, $J = 5.6$ Hz, C-4'), 70.3 (dd, $J = 32.9, 17.0$ Hz, C-3') 61.8 (C-5'), 20.9 (H₃CCO₂), 20.5 (H₃CCO₂), -0.1 (Si(CH₃)₃). ¹⁹F NMR (470 MHz, CDCl₃) $\delta_F = -115.9$ (dt, $J = 247.6, 10.8$ Hz), -119.6 (d, $J = 249.6$ Hz). LRMS (ESI): $m/z = 445$ [M + H]⁺, 443 [M - H]⁻. HRMS (ESI⁺) m/z [M + Na]⁺ calcd. for C₁₈H₂₂F₂N₂NaO₇Si 467.1057, found 467.1018.

2'-dideoxy-2'-difluoro-5-ethynyl-uridine (**35**)

Compound **S4** (0.466 g, 1.049 mmol) was dissolved in MeOH (15 mL) and sodium methoxide (4.6 M) (0.187 mL, 0.0404 mmol) added. The mixture was stirred at rt for 3 hours after which TLC analysis indicated complete consumption of the starting material

and formation of a single product. The mixture was neutralised with Amberlite IRA-120 H⁺ resin and filtered. The crude compound was adsorbed onto silica and purified by solid addition silica gel flash chromatography (10 % MeOH in CH₂Cl₂) to afford the title compound as a pale yellow solid (0.257 g, 85%). Spectroscopic data was consistent with literature.¹ *R*_f = 0.11 (5% MeOH in CH₂Cl₂). Darkens at 190-205 °C, mp = 210-215 °C. ¹H NMR (500 MHz, DMSO) δ_H = 11.91 (br s, 1H, NH), 8.26 (s, 1H, H-6), 6.32 (d, *J* = 6.6 Hz, 1H, OH-3'), 6.04 (t, *J* = 7.3 Hz, 1H, H-1'), 5.42 (t, *J* = 5.2 Hz, 1H, OH-5'), 4.22 (tdd, *J* = 12.4, 10.6, 8.3, 5.6 Hz, 1H, H-3'), 4.17 (s, 1H), 3.86 (dt, *J* = 8.6, 2.7 Hz, 1H, H-4'), 3.79 (dt, *J* = 12.8, 3.0 Hz, 1H, H-5'(_α or _β)), 3.64 (ddd, *J* = 12.7, 5.4, 3.1 Hz, 1H, H-5'(_α or _β)). ¹⁹F NMR (470 MHz, DMSO) δ_F = -116.3, -116.5 (d, *J* = 12.3 Hz), -116.83 (d, *J* = 13.5 Hz), -117.00 (d, *J* = 13.1 Hz), -117.18, -117.70. LRMS (ESI): *m/z* = 289 [M + H]⁺, 287 [M - H]⁻.

Scheme S2. Synthesis of 2'-F-ribo-EdU (**34**) from commercially available 2'-deoxy-2'-fluorodeoxyuridine (**S5**) employing an adapted procedure for the synthesis of EdU.

(2'*R*)-3',5'-*O*-Bis-acetyl-2'-deoxy-2'-fluoro-uridine (**S6**)

Compound **S6** was synthesised from commercially available (2'*R*)-2'-deoxy-2'-fluorouridine (**S5**) (2.000 g, 8.127 mmol). Compound **S5** was dissolved in pyridine (8.8 mL, 81.0 mmol, 10 equiv) then Ac₂O (1.92 mL, 20.3 mmol, 2.5 mmol) was added and mixture stirred for at rt 16 h. The reaction mixture was dissolved in EtOAc (50 mL) and washed with 5% HCl_(aq) (20 mL), NaHCO_{3(satd. aq)} (20 mL), brine (20 mL) and dried over MgSO₄ to give the title compound as a yellow gum (2.623 g, 98%). The spectroscopic data was consistent with literature values.²⁻³ *R*_f = 0.21 (30% EtOAc in CH₂Cl₂). ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 11.47 (s, 1H, NH), 7.72 (d, *J* = 8.1 Hz, 1H, H-6), 5.87 (dd, *J* = 22.4, 2.1 Hz, 1H, H-1'), 5.68 (d, *J* = 8.0 Hz, 1H, H-5), 5.53 (ddd, *J* = 52.6, 5.3, 2.1 Hz,

1H, H-2'), 5.26 (ddd, $J = 17.2, 7.9, 5.3$ Hz, 1H, H-3'), 4.34 (dd, $J = 12.1, 2.9$ Hz, 1H, H-5'(α or β)), 4.27 (ddd, $J = 8.2, 5.7, 2.9$ Hz, 1H, H-4'), 4.16 (dd, $J = 12.1, 5.7$ Hz, 1H, H-5'(α or β)), 2.11 (s, 3H, Ac), 2.04 (s, 3H, Ac). ^1H NMR (500 MHz, CDCl_3) $\delta_{\text{H}} = 9.30$ (br s, 1H), 7.39 (d, $J = 8.1$ Hz, 1H), 5.83 – 5.74 (m, 2H), 5.37 (ddd, $J = 52.2, 4.9, 1.7$ Hz, 1H), 5.15 (ddd, $J = 17.8, 8.3, 5.0$ Hz, 1H), 4.44 (dd, $J = 12.3, 2.6$ Hz, 1H), 4.40 (ddd, $J = 7.6, 4.5, 2.6$ Hz, 1H), 4.30 (dd, $J = 12.3, 4.6$ Hz, 1H), 2.15 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (126 MHz, DMSO) $\delta = 170.1, 169.5, 150.6, 142.5, 102.1, 90.8$ (d, $J = 187.0$ Hz), 90.5 (d, $J = 36.4$ Hz), 77.9, 69.8 (d, $J = 14.8$ Hz), 62.67, 20.5, 20.3. ^{19}F NMR (470 MHz, CDCl_3) $\delta = 198.76 - -199.06$ (m). LRMS (ESI): $m/z = 353$ $[\text{M} + \text{Na}]^+$, 331 $[\text{M} + \text{H}]^+$, 329 $[\text{M} - \text{H}]^-$.

(2'*R*)-3',5'-*O*-Bis-acetyl-2'-deoxy-2'-fluoro-5-iodo-uridine (**S7**)

Compound **S7** was synthesised from compound **S6** (2.68 g, 8.11 mmol) according to literature procedures.² Briefly, compound **S6**, ceric ammonium nitrate (4.45 g, 8.11 mmol, 1 equiv), and I_2 (1.64 g, 6.49 mmol 0.8 equiv) were combined and dissolved in CH_3CN (40 mL) and heated to 50 °C for 16 h. The solvent was then removed *in vacuo* and the residue dissolved in EtOAc (20 mL) and washed with NaHCO_3 (satd. aq.) (20 mL) and brine (20 mL) then dried over MgSO_4 . The crude product was purified by silica gel flash chromatography (10 – 30% EtOAc in CH_2Cl_2) to afford the title compound as a pale red solid (3.330 g, 90%). $R_f = 0.52$ (30% EtOAc in CH_2Cl_2). mp 135-140 °C. The spectroscopic data was consistent with literature values.³ ^1H NMR (500 MHz, CDCl_3) $\delta = 8.97$ (s, 1H, NH), 7.89 (s, 1H, H-6), 5.89 (dd, $J = 18.2, 1.7$ Hz, 1H, H-1'), 5.30 (ddd, $J = 51.7, 4.7, 1.7$ Hz, 1H, H-2'), 5.10 (ddd, $J = 18.1, 8.0, 4.8$ Hz, 1H, H-3'), 4.49 – 4.41 (m, 2H, H-5'(α or β) and H-4'), 4.36 (dd, $J = 13.2, 4.0$ Hz, 1H, H-5'(α or β)), 2.22 (s, 3H, Ac), 2.16 (s, 3H, Ac). ^{13}C NMR (126 MHz, CDCl_3) $\delta_{\text{C}} = 170.5$ (H_3CCO_2), 170.0 (H_3CCO_2), 160.0 (C-4), 149.6 (C-2), 144.5 (C-6), 91.1 (d, $J = 193.0$ Hz, C-2'), 90.3 (d, $J = 35.6$ Hz, C-1'), 78.9 (C-4'), 69.3 (d, $J = 15.6$ Hz, C-3'), 61.9 (C-5'), 21.3 (H_3CCO_2), 20.5 (H_3CCO_2). ^{19}F NMR (470 MHz, CDCl_3) $\delta = -200.2$ (dt, $J = 51.9, 18.2$ Hz). LRMS (ESI): $m/z = 457$ $[\text{M} + \text{H}]^+$, 455 $[\text{M} - \text{H}]^-$.

(2'*R*)-3',5'-*O*-Bis-acetyl-2'-deoxy-5-(ethynyl(2-trimethylsilyl))-2'-fluoro-uridine (**S8**)

Compound **S8** was synthesised from compound **S7** (1.700 g, 3.728 mmol) under Sonagashira conditions, similarly to **S4** described above. The crude product was purified by silica gel flash chromatography (10 – 20% EtOAc in CH_2Cl_2) to afford the title compound as an off-white solid (0.921 g, 58%). mp 195-198 °C. $R_f = 0.69$ (30% EtOAc in CH_2Cl_2). ^1H NMR (500 MHz, CDCl_3) $\delta_{\text{H}} = 8.74$ (s, 1H, NH), 7.79 (s, 1H, H-6), 5.95

(dd, $J = 17.6, 1.5$ Hz, 1H, H-1'), 5.26 (ddd, $J = 51.6, 4.7, 1.5$ Hz, 1H, H-2'), 5.07 (ddd, $J = 18.9, 8.4, 4.7$ Hz, 1H, H-3'), 4.49 – 4.43 (m, 2H, H-5'(α or β) and H-4'), 4.38 – 4.31 (m, 1H, H-5'(α or β)), 2.19 (s, 3H, Ac), 2.16 (s, 3H, Ac), 0.21 (s, 9H, Si(CH₃)₃). ¹³C NMR (126 MHz, CDCl₃) $\delta_C = 170.2$ (H₃CCO₂), 170.0 (H₃CCO₂), 160.6 (C-4), 148.7 (C-2), 142.5 (C-6), 101.1 (C $\equiv$ C-Si), 100.6 (C-5), 94.8 (C $\equiv$ C-Si), 91.2 (d, $J = 193.4$ Hz, C-2'), 89.9 (d, $J = 35.3$ Hz, C-1'), 78.8, 69.1 (d, $J = 15.7$ Hz, C-3'), 61.6 (C-5'), 21.1 (H₃CCO₂), 20.5 (H₃CCO₂), -0.1 (Si(CH₃)₃). ¹⁹F NMR (470 MHz, CDCl₃) $\delta_F = -201.4$ (dt, $J = 51.8, 18.2$ Hz). LRMS (ESI): $m/z = 427$ [M + H]⁺, 425 [M - H]⁻. HRMS (ESI⁺) m/z [M + Na]⁺ calcd. for C₁₈H₂₃FN₂NaO₇Si 449.1151, found 449.1122.

(2'*R*)-2'-Deoxy-5-ethynyl-2'-fluoro-uridine (**34**)

Compound **S8** (0.880 g, 1.049 mmol) was dissolved in MeOH (30 mL) and sodium methoxide (4.6 M) (0.368 mL, 1.70 mmol) added. The mixture was stirred at rt for 2 h after which TLC analysis indicated complete consumption of the starting material and formation of a single product. The mixture was neutralised with Amberlite IRA-120 H⁺ resin and filtered. Silica gel was added to the filtrate and the solvent removed *in vacuo*. The compound was purified by solid addition silica gel flash chromatography (10% MeOH in CH₂Cl₂) to afford the title compound as an off-white foam (0.280 g, 50%). $R_f = 0.27$ (10% MeOH in CH₂Cl₂). ¹H NMR (500 MHz, DMSO-*d*₆) $\delta_H = 11.69$ (br s, 1H, NH), 8.41 (s, 1H, H-6), 5.86 (dd, $J = 16.9, 1.3$ Hz, 1H, H-1'), 5.60 (d, $J = 6.6$ Hz, 1H, OH-3'), 5.36 (t, $J = 4.7$ Hz, 1H, OH-5'), 5.03 (ddd, $J = 53.1, 4.3, 1.4$ Hz, 1H, H-1'), 4.24 – 4.11 (m, 1H, H-3'), 4.08 (s, 1H, $\equiv$ C-H), 3.89 (dt, $J = 8.3, 2.5$ Hz, 1H, H-4'), 3.81 (ddd, $J = 12.4, 4.4, 2.3$ Hz, 1H, H-5'(α or β)), 3.60 (ddd, $J = 12.3, 4.4, 2.5$ Hz, 1H, H-5'(α or β)). ¹³C NMR (126 MHz, DMSO-*d*₆) $\delta_C = 162.0$ (C-4), 149.5 (C-2), 144.5 (C-6), 97.6 (C-5), 93.9 (d, $J = 185.3$ Hz, C-2'), 87.7 (d, $J = 34.2$ Hz, C-1'), 83.7 (C $\equiv$ C-H), 83.2 (C-4'), 76.5 (C $\equiv$ C-H), 66.9 (d, $J = 16.3$ Hz, C-3'), 58.7 (C-5'). ¹⁹F NMR (470 MHz, DMSO-*d*₆) $\delta_F = -202.5$ (ddd, $J = 53.0, 23.5, 16.8$ Hz). LRMS (ESI): $m/z = 293$ [M + Na]⁺, 271 [M + H]⁺, 269 [M - H]⁻. HRMS (ESI⁺) m/z [M + Na]⁺ calcd. for C₁₁H₁₁FN₂NaO₅ 293.0544, found 293.0542.

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Compound **2** ^1H and ^{13}C NMR (DMSO- d_6)

Compound **2** $^{31}\text{P}\{\text{H}\}$ NMR (DMSO- d_6)

Compound **4** ^1H and ^{13}C NMR ($\text{DMSO-}d_6$)

Compound **4** $^{31}\text{P}\{\text{H}\}$ NMR ($\text{DMSO}-d_6$)

Compound **5** ^1H and ^{13}C NMR (DMSO- d_6)

Compound **5** $^{31}\text{P}\{\text{H}\}$ NMR ($\text{DMSO}-d_6$)

Compound **6** ^1H and ^{13}C NMR ($\text{DMSO-}d_6$)

Compound **6** $^{31}\text{P}\{\text{H}\}$ NMR ($\text{DMSO-}d_6$)

Compound **7** ^1H and ^{13}C NMR (DMSO- d_6)

Compound **7** $^{31}\text{P}\{\text{H}\}$ NMR ($\text{DMSO-}d_6$)

Compound **7** $^{19}\text{F}\{\text{H}\}$ NMR ($\text{DMSO-}d_6$)

Compound **8** ^1H and ^{13}C NMR ($\text{DMSO-}d_6$)

$\text{C}_{19}\text{H}_{17}\text{F}_2\text{N}_2\text{O}_8\text{P}$

Compound **8** $^{31}\text{P}\{\text{H}\}$ NMR ($\text{DMSO-}d_6$)

Compound **8** $^{19}\text{F}\{\text{H}\}$ NMR ($\text{DMSO-}d_6$)

Compound **9** ^1H and ^{13}C NMR (DMSO- d_6)

$\text{C}_{19}\text{H}_{17}\text{F}_2\text{N}_2\text{O}_9\text{P}$

Compound **9** $^{31}\text{P}\{\text{H}\}$ NMR (DMSO- d_6)

Compound **9** $^{19}\text{F}\{\text{H}\}$ NMR (DMSO- d_6)

Compound **10** ^1H and ^{13}C NMR ($\text{DMSO}-d_6$)

Compound **10** $^{31}\text{P}\{\text{H}\}$ NMR (DMSO- d_6)

Compound **10** $^{19}\text{F}\{\text{H}\}$ NMR (DMSO- d_6)

Compound **11** ^1H and ^{13}C NMR ($\text{DMSO-}d_6$)

Compound **11** $^{31}\text{P}\{\text{H}\}$ NMR (DMSO- d_6)

Compound **11** $^{19}\text{F}\{\text{H}\}$ NMR (DMSO- d_6)

Compound **12** ^1H and ^{13}C NMR ($\text{DMSO-}d_6$)

Compound **12** $^{31}\text{P}\{\text{H}\}$ NMR ($\text{DMSO-}d_6$)

Compound **13** ¹H and ¹³C NMR (DMSO-*d*₆)

Compound **13** ^3P (h) NMR (MMSO- d_6)

Compound **14** $^{31}\text{P}\{\text{H}\}$ NMR (DMSO- d_6)

Compound **15** ^1H and ^{13}C NMR (DMSO- d_6)

Compound **15** $^{31}\text{P}\{\text{H}\}$ NMR ($\text{DMSO-}d_6$)

Compound **115** $^{19}\text{F}\{\text{H}\}$ NMR ($\text{DMSO-}d_6$)

Compound **20** ^1H and ^{31}P NMR (CDCl_3)

Compound **24** ^1H and ^{13}C NMR (CDCl_3)

Compound **24** ^{31}P NMR (CDCl_3)

Compound **25** ^1H and ^{13}C NMR (CDCl_3)

Compound **25** ^{31}P NMR (CDCl_3)

Compound **26** ^1H and ^{13}C NMR (CDCl_3)

Compound **26** ^{31}P NMR (CDCl_3)

Compound **S1** ^1H (MeOD- d_4) and ^{13}C NMR (DMSO- d_6)

Compound **S1** $^{19}\text{F}\{\text{H}\}$ NMR (MeOD- d_4)

Compound **S2** ^1H and ^{13}C NMR (CDCl_3)

Compound **S2** ^{19}F NMR (CDCl_3)

Compound **S3** ^1H and ^{13}C NMR (CDCl_3)

Compound **S3** ^{19}F NMR (CDCl_3)

Compound **S4** ^1H and ^{13}C NMR (CDCl_3)

Compound **S4** ^{19}F NMR (CDCl_3)

Compound **34** ^1H and ^{19}F NMR (DMSO- d_6)

Compound **S6** ^1H and ^{19}F NMR (CDCl_3)

Compound **S7** ^1H and ^{13}C NMR (CDCl_3)

Compound **S8** ^1H NMR (CDCl_3)

Compound **S8** ^{19}F NMR (CDCl_3)

Compound **35** ^1H and ^{13}C NMR (DMSO- d_6)

Compound **35** ^{19}F NMR ($\text{DMSO-}d_6$)

Compound **2** $^{31}\text{P}\{\text{H}\}$ NMR ($\text{pyridine-}d_5$)