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Synthetic Approaches for the Preparation of Phosphoramidate Prodrugs of 2'-Deoxypseudoisocytidine

Michaela Serpi,[a] Roberto De Biasi,[a, b] Fabrizio Pertusati,[a] Magdalena Slusarczyk,[a] and Christopher McGuigan[a]

In memory of Prof. Christopher McGuigan

A synthetic procedure for the preparation of phosphoramidate prodrugs of C-nucleosides is reported. Different phosphorochloridates were reacted with 3'-O-protected N-acetyl-2'-deoxypseudoisocytidine or 3'-O-protected 2'-deoxypseudoisocytidine, followed by acidic hydrolysis of the protecting group. In the presence of the N-acetyl moiety, the enolisable keto group of the nucleobase was able to react (like the 5'-OH) with the phosphorochloridates to give bisphosphorylated derivatives. Epimerisation [\(\beta\) to \(\alpha\)] occurred if the amino group of the nucleobase was unprotected. These side reactions demonstrate the peculiar behaviour of C-nucleosides compared to their nucleoside analogues. It was demonstrated that the first enzymatic activation step for this new class of prodrugs can be mediated by carboxypeptidase and that it follows the same pathway and rate reported for ProTides of more conventional nucleoside analogues. These new phosphoramidate derivatives deserve further investigation for their therapeutic potential as anti-cancer agents.

1. Introduction

The C-nucleosides represent a group of nucleoside analogues in which the sugar moiety is linked to the nucleobase by a carbon–carbon bond.[1] Several C-nucleosides are naturally occurring compounds. Among them, pseudouridine was the first to be isolated from yeast tRNA in 1957.[2] Subsequently, other C-nucleosides, including oxazinomycin,[3] pyrazomycin,[3] showdomycin,[4] and formycin A,[5] were isolated from culture filtrates of different bacterial strains. These compounds are antibiotics and exhibit anti-cancer and/or antiviral activity. Their advantageous properties arise from the presence of a C–C glycosidic bond, which gives a greater resistance than N-nucleosides towards chemical hydrolysis and enzymatic hydrolysis by phosphorylase and deaminase enzymes. On the basis of these interesting chemical and biological properties, a wide variety of synthetic analogues have been prepared thanks to the large array of novel synthetic methodologies developed in the last two decades. Several of these compounds have found numerous applications in medicinal chemistry and chemical biology.[11] Among them, pseudouridocytidine (PIC, 1), a nucleoside isostere of cytidine was developed as a candidate for anti-leukaemic therapy[6] (Figure 1). PIC was shown to be incorporated into both RNA and DNA and this incorporation was considered to be responsible for its therapeutic activity, which has been observed against several mouse leukaemias in vitro and in vivo.[7, 8] In addition, PIC was found to disrupt DNA methylation by inhibition of the enzyme DNA methyltransferase, most probably due to the presence of a nitrogen atom in the 5-position of the base.[9] However, the development of PIC was halted due to hepatotoxicity observed during phase I clinical evaluation.[10] The efficiency with which PIC is incorporated into RNA, and the rapid RNA turnover, associated with protein synthesis in the liver, were considered the main causes of its hepatotoxicity. This finding prompted the investigation of 2'-deoxypseudoisocytidine (2'-d-PIC, 2)[11] which, in preliminary tissue culture experiments, was found to exhibit inhibitory activity against P815 cell lines.[11a] PIC, 2'-d-PIC and their analogues were also used as novel base-pairing agents in oligonucleotides to investigate DNA and RNA structures and functions.[12] Although several C-nucleoside analogues have been described as anti-cancer and/or antiviral agents, none have ever been developed as anti-cancer or antiviral drugs. The recent advent of two novel C-nucleosides, BCX4430 (3)[13] and GS-6620 (4),[14] as potential therapeutic agents for the treatment of the Ebola virus and hepatitis C virus (HCV) infections, respectively, has stimulated renewed interest in this class of compounds (Figure 1).

As part of our current research we were interested to further investigate the potential utility of 2'-d-PIC (2) as an anti-leukaemic agent by preparing a series of phosphoramidate prodrugs for biological evaluation as anti-cancer agents. “ProTides” in
the clinic have consistently showed greater efficacy and more favourable safety profiles relative to the corresponding standard-of-care nucleoside analogues. Several pharmaceutical companies have already validated the phosphoramidate approach for antiviral applications. In 2014, Gilead launched on the market its anti-HCV ProTide, sofosbuvir (5), and in the following year tenofovir alafenamide (TAF, 6), NUC-1031 (7), and NUC-3373 (8).

2. Results and Discussion

2.1. Synthesis of 2′-Deoxypseudoisocytidine (2)

Several approaches have been developed for the preparation of C-glycosides and C-nucleosides. Among them, for the synthesis of 2′-d-PIC (2), we selected the methodology developed by Dave et al., which utilises a Pd-catalysed Heck-type coupling of aryl halides to cyclic enol ethers, either pyranoid or furanoid glycals. As outlined in Scheme 1, the protected furanoid glycal 12 and the halogenated N-acetyl pseudoisocytosine 11, served as starting materials for the Heck reaction. 2-N-Acetyl-5-iodoisocytosine (11) was synthesised in good yield in two steps from commercially available isocytosine (9), which was first iodinated with N-iodosuccinimide in acetic acid to afford the intermediate compound 10. Subsequent acetylation
of the exocyclic amino function of 10 using acetic anhydride yielded the desired nucleobase 11.\[22]\ Compound 12 was prepared from 3',5'-bis-O-(tert-butylimidemethylsilyl)thymidine\[23] by using typical silylation conditions first reported by Pedersen et al.\[24] and then applied by Hammer et al.\[25] for the preparation of furanose glycols with a wide range of O-silyl protections.

The coupling reaction of 5-ido base 11 with the protected ribofuranosyl glycal 12 using Pd(OAc)$_2$ as a catalyst, AsPh$_3$ as a soft ligand and N,N-disopropylethylamine as a base, formed selectively the β-C-nucleoside 13. After removal of the silyl groups with fluoride ions, the resulting 2'-deoxy-3'-keto C-nucleoside 14 was treated with sodium triacetoxyborohydride to reduce diastereoselectively the 3'-keto group from the β-face of the furanosyl ring, forming N-acetyl-2'-deoxypseudouridine 15.\[22] The cleavage of the acetyl group to afford nucleoside 2 was then accomplished by basic hydrolysis using NH$_3$ in MeOH. The assignment of the configuration at the 1'-position of 2 was based on the comparison of its $^1$H NMR spectrum with that reported in the literature.\[22]

### 2.2. Synthesis of N-Acetyl-2'-deoxypseudouridine Phosphoramidates

The two synthetic strategies commonly used for the preparation of phosphoramidate prodrugs (phosphorochloridate in the presence of either tert-butylimagemine chloride or N-methylimidazole as a base)\[26] failed when applied to 2, probably due to the low solubility of the starting material in the reaction medium, returning only unreacted starting materials. Attempts to improve the solubility of 2 using different solvents were unsuccessful. Application of the ProTide approach to precursors 14 and 15 also failed, indicating that development of a suitable synthetic strategy to afford phosphoramidates of 2 was more challenging than originally expected. These results prompted us to use a different synthetic methodology with compound 17 as the key intermediate (Scheme 2).

We envisaged that introduction of a tert-butylidemethylsilyl ether at the 3'-OH group in 15 would help to improve its solubility and to achieve exclusive phosphorylation at the 5'-position.

In order to prepare compound 17, the two hydroxy groups of deoxyribose present in N-acetyl-2'-deoxypseudouridine (15) were first protected with a tert-butylimidemethylsilyl group using tert-butylimidemethylsilyl chloride in DMF for 24 h at room temperature in the presence of 4-dimethylaminopyridine (DMAP) to provide, after flash chromatography, compound 16 in reasonable yield. Then, selective silyl group deprotection was achieved with aqueous trifluoroacetic acid to give, after isolation by silica gel chromatography, 17 with a free primary hydroxy group in moderate yield. Next, phosphorochloridates 18a-f, prepared as a mixture of $R$ and $S$ diastereoisomers according to a literature procedure,\[26] were reacted with 17 in the presence of tert-butylimagemine chloride (1.0 mol in THF), yielding 3'-O-tert-butylimidemethylsilyl phosphoramidates 20a-f (Scheme 2) as diastereoisomeric mixtures after column chromatography, except for 20d, which was isolated after purification as a single diastereoisomer. Despite the almost complete consumption of the starting material, the desired products 20a-f were recovered in low yields, which was ascribed in each case to the formation of a bisphosphorylated by-product, as exemplified in Figure 2. The bisphosphorylated compound 19f was isolated and its structure was characterised by mass spectrometry and $^1$H NMR analysis,\[31] which clearly suggested that the phosphorylation involved the oxygen atom of the pyrimidine ring rather than either one of the nitrogen atoms. N-Acetylisocytidine possesses an enolisable keto group which, like the 5'-OH group, is able to react with a phosphorochloridate to give an O-phosphorylated derivative. In support of this result, we found in the literature that the reaction of 2-acetylimidino-4-hydroxypyrimidines with phosphorochloridates gives O-phosphoryl rather than N-phosphoryl derivatives.\[27] The substantial steric requirement of the phosphoryl chloride and the steric hindrance exerted to some extent by the acetyl group

![Scheme 2. Synthesis of ProTides 21a-f. TBDMSCh, tert-butylimidemethylsilyl chloride; DMAP, 4-dimethylaminopyridine; TFA, trifluoroacetic acid.](image-url)
were considered to be the key features for preventing phosphorylation at either one of the ring nitrogen atoms.\textsuperscript{[27]}

Acidic deprotection of 20\textsubscript{a–f} afforded after preparative HPLC purification compounds 21\textsubscript{a–f} in moderate yields (Scheme 2 and Table 1). Attempts to remove the acetyl protection from 21\textsubscript{a–f} with Schwartz’s reagent as described by Ferrari et al.,\textsuperscript{[28]} failed due to the ring opening of the base. The difficulties encountered in removing the N-acetyl group from 21\textsubscript{a–f} using mild conditions, and the fact that the labile P–O bond of the ProTide would not tolerate other harsh de-acetylating agents such as methanolic ammonia, prompted us to abandon our attempts toward modification of 21\textsubscript{a–f}. We therefore continued our effort to conceive a more efficient route that would allow the preparation of the N-deacetylated analogues.

2.3. Synthesis of 2’-Deoxypseudoisocytidine Phosphoramidates

As shown in Scheme 3, compound 22, obtained by treatment of 16 with methanolic ammonia, underwent selective 5’-desilylation using aqueous trifluoroacetic acid in THF to afford the monosilyl compound 23 in excellent yield. Next, phosphoro-chloridates 18\textsubscript{a} and 18\textsubscript{g} were reacted with 23 in the presence of tert-butylmagnesium chloride (1.0 \text{M} in THF) to yield, after column chromatography, the 3’-O-tert-butyldimethylsilyl-protected phosphoramidates 24\textsubscript{a} and 24\textsubscript{g} in moderate yield as diastereomeric mixtures (Table 2). No traces of bisphosphorylated products either due to O- or N-phosphorylation were observed.

Acidic deprotection of the tert-butyldimethylsilyl moieties in 24\textsubscript{a} and 24\textsubscript{g} with trifluoroacetic acid in dichloromethane (1:2 v/v; room temperature, overnight), afforded the final compounds 25\textsubscript{a} and 25\textsubscript{g} as mixtures of \(\alpha\) and \(\beta\) isomers in a 3:1 ratio after column chromatography. The \(\beta\)-isomers of 25\textsubscript{a} and

Table 1. Reaction outcomes for the synthesis of precursors 20\textsubscript{a–f} and ProTides 21\textsubscript{a–f}.

| Cmpd | Ar R | Yield [\%] | d.r. | Cmpd | Yield [\%] | d.r. |
|------|------|------------|------|------|------------|------|
| 20\textsubscript{a} | Ph CH\textsubscript{2}Ph | 15:1 21 | 50 | 2.3:1 |
| 20\textsubscript{b} | Ph (CH\textsubscript{2})\textsubscript{5}CH\textsubscript{3} | 1.5:1 21 | 60 | 1:5:1 |
| 20\textsubscript{c} | Ph (CH\textsubscript{2})\textsubscript{4}CH\textsubscript{3} | 1:1 21 | 16 | 1:1 |
| 20\textsubscript{d} | Naph CH(CH\textsubscript{2})\textsubscript{3} | 12 | 1:0 21 | 37 | 1:0 |
| 20\textsubscript{e} | Naph CH\textsubscript{3}(CH\textsubscript{2})\textsubscript{3} | 1:1 21 | 46 | 2.3:1 |
| 20\textsubscript{f} | Naph CH\textsubscript{2}Ph | 1:5:1 21 | 25 | 4:1 |

Table 2. Reaction outcomes for the synthesis of 24\textsubscript{a} and 24\textsubscript{g} and ProTides 25\textsubscript{a} and 25\textsubscript{g} after HPLC purification.

| Cmpd | Ar R | d.r. | Yield [\%] | Cmpd | Yield [\%] |
|------|------|------|------------|------|------------|
| 24\textsubscript{a} | Ph CH\textsubscript{2}Ph | 2.3:1 21 | 41 | 25\textsubscript{a} | 62 |
| 24\textsubscript{g} | Ph CH(CH\textsubscript{2})\textsubscript{3} | 1:3 21 | 32 | 25\textsubscript{g} | 50 |

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25g were isolated in low yield after preparative HPLC purification (25a as a single diastereoisomer and 25g as a mixture; Scheme 3 and Table 2). Most probably, the presence of a dissociable proton on N-1 facilitates the α,β-epimerisation in acidic conditions through a ring opening–closure of the carbohydrate ring (Scheme 4) as previously reported for other C-nucleosides.\textsuperscript{[11b,c,29]}

If a mild procedure for the cleavage of tert-butyldimethylsilyl ethers to alcohols (based on an exchange reaction with trimethylsilyl triflate at –78 °C)\textsuperscript{[30]} was used, no epimerisation was observed.

2.4. Enzymatic Studies on the Activation of C-Nucleoside ProTides

To exert their biological activity, ProTides must be metabolised in vivo into the monophosphate form, which in turn generates the active triphosphate form by two consecutive phosphorylation reactions.\textsuperscript{[31]} In the process of intracellular activation of ProTides, the first step is catalysed by a carboxypeptidase-type enzyme, such as cathepsin A, which was shown to be responsible for the cleavage of the amino acid ester moiety.\textsuperscript{[32]} In order to demonstrate that the ProTides of C-nucleosides are activated in a similar manner, the interaction of compound 21e with a carboxypeptidase-type enzyme was investigated. Carboxypeptidase Y was used as a surrogate of cathepsin A because it behaves for its anti-leukaemic drug. Together with derivatives 25a and 25g, we plan to evaluate the N-acetylated derivatives 21a–f for their anti-tumour activity. We considered that the acetyl moiety would further enhance the lipophilicity of these compounds and remove the potential for their protonation in vitro, whereas in vivo the acetyl moiety would most probably be able to undergo cleavage (thus acting as a dual prodrug). The results of these investigations will be disclosed in due course.

3. Conclusions

An alternative route to C-nucleoside ProTides has been developed and used to prepare phosphoramidate derivatives of 2’d-PIC (2) and N-acetyl 2’d-PIC (15). Unexpected side reactions such as phosphorylation of the enolisable keto group of the nucleobase and epimerisation through ring opening highlighted the different reactivity of C-nucleosides compared to nucleoside analogues. The first carboxypeptidase-mediated bioactivation step for this new class of prodrugs followed the same pathway and rate as reported for ProTides of conventional nucleoside analogues. Biological evaluation of these novel nucleoside analogues should enhance our understanding of the potential of C-nucleosides as anti-tumour agents and in particular of 2’d-PIC as an anti-leukaemic drug. Together with derivatives 25a and 25g, we plan to evaluate the N-acetylated derivatives 21a–f for their anti-tumour activity. We considered that the acetyl moiety would further enhance the lipophilicity of these compounds and remove the potential for their protonation in vitro, whereas in vivo the acetyl moiety would most probably be able to undergo cleavage (thus acting as a dual prodrug). The results of these investigations will be disclosed in due course.

**Experimental Section**

**Chemistry**

All anhydrous solvents were purchased from Sigma-Aldrich and amino acid esters from Novabiochem. All commercially available reagents were used without further purification.
Precoated aluminium-backed plates (60F, 0.2 mm thickness, Merck) were used for thin-layer chromatography (TLC) and were visualised under both short- and long-wavelength UV light (254 and 366 nm, respectively). Flash column chromatography was performed using silica gel supplied by Fisher (60 A, 35–70 μm). Analytical HPLC analysis was performed using either a Thermo Scientific or a Varian Prostar system. \( ^{1}H \) NMR (500 MHz), \( ^{13}C \) (125 MHz), and \( ^{3}P \) NMR (202 MHz) spectra were recorded on a Bruker Avance 500 MHz spectrometer at 25 °C. Chemical shifts (δ) are quoted in parts per million (ppm) relative to internal references CD\(_{3}\)OD (δ = 3.34 ppm), \( ^{1}H \) NMR; δ = 49.86 ppm, \( ^{13}C \) NMR) and CDCl\(_{3}\) (δ = 7.26 ppm, \( ^{1}H \) NMR; δ = 77.4 ppm, \( ^{13}C \) NMR), or external 85% H\(_{2}\)PO\(_{4}\) (δ = 0.00 ppm, \( ^{3}P \) NMR). Coupling constants (J) are expressed in Hertz.

The following abbreviations are used in the assignment of NMR signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), brs (broad singlet), dd (doublet of doublet), dt (doublet of triplet).

Low-resolution mass spectrometry was performed on a Bruker Daltonics microOTOF–LC system.

For practical purposes, in some cases standard procedures are given. Procedures that differ from the standard are fully described.

N-(6-Oxo-1,6-dihydropyrimidin-2-yl)acetamide (10)

N-Iodosuccinimide (22.0 g, 98 mmol) was added to a solution of 2-aminopyrimidin-4(3H)-one (9, 10.0 g, 90 mmol) in acetic acid (170 mL) at 70 °C. The resulting suspension was heated at 100 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and H\(_{2}\)O (500 mL) was added. The solid was filtered, washed with \( 
\text{H}_2\text{O} \) and dried with a heat gun at 50 °C for 12 h to afford compound 10 as a light pink solid (12.8 g, 60 % yield).

The crude material was purified by column chromatography on silica gel (EtOAc/hexane 7:3) to give 13 as a light yellow solid (2.8 g, 52 % yield).

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N-[(2R,4S,5R)-4-[(tert-Butyldimethylsilyl)oxy]-5-[(tert-butyldimethylsilyl)oxy]methyl]tetrahydrofuran-2-yl]-6-oxo-1,6-dihydropyrimidin-2-yl]acetamide (16)

Imidazole (0.4 g, 5.8 mmol), tert-butyltrimethylsilyle chloride (0.44 g, 2.9 mmol) and DMAP (0.04 g, 0.33 mmol) were added to a solution (SiC(C(OH)Cl), 0.410 g, 65 % yield).

Phenyl-(benzoyl-L-alaninyl)-phosphorochloridate (18a)

Prepared according to standard procedure 1 in 92 % yield. 1H NMR (500 MHz, CDCl3): δ = 7.30–7.10 (m, 10 H; Ar), 5.20–5.16 (m, 2 H; OCH2Ph), 4.25–4.22 (m, 1 H; CH2), 3.51–3.48 (m, 1 H; NH), 1.54 (d, J = 7.3 Hz, 1.5 H; CH2), 3.12 ppm (d, J = 7.3 Hz, 1.5 H; CH2); 13C NMR (125 MHz, CDCl3): δ = 169.8 (d, J, ipso = 5.4 Hz; C=O), 135.0 (d, J, ipso = 6.6 Hz; ipso-C-Ph), 134.7 (ipso-OCH2Ph), 130.0, 129.8, 128.5, 128.4, 125.0, 124.5, 123.7, 151.0 (d, J, ipso = 6 Hz; CH2); 31P NMR (202 MHz, CDCl3): δ = 7.93 (0.5 P), 7.51 ppm (0.5 P).

Phenyl-(hexoy-L-alaninyl)-phosphorochloridate (18b)

Prepared according to standard procedure 1 in 87 % yield. 1H NMR (500 MHz, CDCl3): δ = 7.24–7.18 (m, 5 H; PH), 4.34–4.20 (m, 1 H; NH), 4.20–4.05 (m, 3 H; CH2, CH3), 4.03–3.94 (m, 2 H; OCH2Ph), 1.66–1.56 (m, 4 H; OCH2CH2CH2CH2), 1.59–1.53 (m, 3 H; CH2), 1.37–1.31 (m, 4 H; OCH2CH2), 0.94–0.87 ppm (CH2); 13C NMR (125 MHz, CDCl3): δ = 172.8 (d, J, ipso = 7.8 Hz; C=O), 172.7 (d, J, ipso = 7.8 Hz; Ar), 149.8 (d, J, ipso = 8.0 Hz; ipso-C-Ph), 149.8 (d, J, ipso = 8.0 Hz; ipso-C-Ph), 129.9, 129.8, 125.9, 125.9 (CH=Ph), 120.6 (d, J, ipso = 5.3 Hz; CH-Ph), 66.0 65.9 (OCH2), 50.8, 50.5 (C=CH2), 31.3 (OCH2CH2), 28.4 (OCH2CH2CH2), 22.5 (CH2), 22.4 (d, J, ipso = 5.6 Hz; CH2), 22.3 (d, J, ipso = 5.6 Hz; CH2); 13C NMR (CDCl3): δ = 7.96 (0.5 P), 7.64 ppm (0.5 P).

Phenyl-(pentoxy-L-alaninyl)-phosphorochloridate (18c)

Prepared according to standard procedure 1 in 96 % yield. 1H NMR (500 MHz, CDCl3): δ = 7.46–7.31 (m, 2 H; PH), 7.28–7.22 (m, 3 H; H-Ph), 4.68 (brs; NH), 4.18–4.09 (m, 3 H; OCH2, CH2), 1.73–1.71 (m, 2 H; OCH2CH2, CH2), 1.68–1.65 (m, 5 H; OCH2CH2CH2); 13C NMR (125 MHz, CDCl3): δ = 172.7 (d, J, ipso = 7.7 Hz; C=O), 172.6 (d, J, ipso = 7.3 Hz; C=O), 149.6 (d, J, ipso = 8.1 Hz; ipso-C-Ph), 149.4 (d, J, ipso = 8.0 Hz; ipso-C-Ph), 129.9, 129.8, 125.9, 125.8 (CH=Ph), 120.5 (d, J, ipso = 5.5 Hz; CH-Ph), 65.9 65.8 (OCH2), 50.5, 50.3 (C=CH2), 31.3 (OCH2CH2), 28.3 (OCH2CH2CH2), 22.4 (CH2), 22.4 (d, J = 5.8 Hz; CH2), 22.4 (d, J = 5.7 Hz; CH2); 31P NMR (202 MHz, CDCl3): δ = 7.92 (0.5 P), 7.61 ppm (0.5 P).
Prepared according to standard procedure 1 in 84% yield. H NMR (500 MHz, CDCl₃): δ = 8.20–8.30 (m, 1 H; CH-Naph), 7.78–7.82 (m, 1 H; CH-Naph), 7.62–7.76 (m, 1 H; H-Naph), 5.06–5.13 (m, 1 H; OCH(CH₃)₂), 4.54 (br s, 1 H; NH), 4.02–4.06 (m, 1 H; CH₂CH₂), 1.56 (d, J = 7.0 Hz, 1.5 H; CH₃CH₂), 1.54 (d, J = 7.0 Hz, 1.5 H; CH₃CH₂), 1.34–1.45 ppm (m, 6 H; OCH(CH₃)₂); ¹³C NMR (152 MHz, CDCl₃): δ = 171.8 (C=O), 149.9 (d, J₂₋₃ = 8.0 Hz; ipso-CH₆-C), 147.3 (ipso-CH₆-C), 134.9, 134.8 (C-Naph), 129.0, 127.6, 126.9, 126.7, 126.3 (CH-Naph), 126.1 (d, J₂₋₃ = 8.3 Hz; C-Naph), 125.5, 124.4, 122.0, 121.5, 121.4, 121.3, 116.2, 116.1, 115.2 (CH-Naph), 106.7, 70.9, 66.2 (OCH(CH₃)₂), 51.0, 50.7, 21.7, 21.5 (CH₃CH₂), 16.1 ppm (OCH(CH₃)₂); ³¹P NMR (202 MHz, CDCl₃): δ = 8.35 (0.5 P), 8.03 ppm (0.5 P).

Prepared according to standard procedure 2 from nucleoside 17 (0.048 g, 0.137 mmol), 18a (0.088 g, 0.27 mmol) in anhydrous THF (2.4 mL) and rBuMgCl in THF (1 mL, 0.16 mL). After workup, the crude product was purified by column chromatography on silica gel using CH₂Cl₂/CH₃OH (95:5) as eluent to provide 20a as a white solid (22 mg, 25% yield). H NMR (500 MHz, CDOD): δ = 7.89 (s, 1 H; H-6), 7.35–7.32 (m, 7 H; H-Ar), 7.22–7.14 (m, 3 H; H-Ar), 5.18–5.00 (m, 3 H; H-1′′, OCHOH), 4.40–4.39 (m, 1 H; H-3), 4.21–4.12 (m, 2 H; CH₂-C), 4.05–4.00 (m, 2 H; CH-4′′, C₂H₃), 2.33–2.23 (m, 1 H; H-C₂H₃), 2.21 (s, 1.2 H; COC₃H₇), 2.20 (s, 1.8 H; COC₃H₇), 1.81–1.71 (m, 1 H; H-C₂H₃), 1.37 (d, J = 7.1 Hz, 1.2 H; CH₂C₃H₇), 0.92 (s, 9 H; C(C₃H₇)₃), 0.11 (s, 1.5 H; Si(CH₃)₃), 0.10 (s, 1.5 H; Si(CH₃)₃), 0.09 ppm (3 H; Si(CH₃)₃); ³¹P NMR (202 MHz, CDOD): δ = 3.89 (0.6 P), 3.48 ppm (0.4 P); MS (ES⁺): m/z (%): 701 [M+H⁺]⁺ (30), 723.30 [M+Na⁺]⁺ (100).

Prepared according to standard procedure 2 from nucleoside 17 (0.090 g, 0.256 mmol), 18b (0.177 g, 0.512 mmol) in anhydrous THF (2.4 mL) and rBuMgCl in THF (1 mL, 0.30 mL). After workup, the crude product was purified by column chromatography on silica gel with CH₂Cl₂/CH₃OH (95:5) as eluent to provide 20b as a solid (34 mg, 21% yield). H NMR (500 MHz, CDOD): δ = 7.90 (s, 1 H; H-6), 7.36–7.31 (m, 2 H; H-Ph), 7.24–7.15 (m, 3 H; H-Ph), 5.11–5.04 (m, 1 H; H-1′), 4.44–4.42 (m, 1 H; H-3), 4.26–4.17 (m, 2 H; CH₂-C), 4.10–4.13 (m, 2 H; OCHOH), 4.04–4.03 (m, 1 H; H-4′′), 4.00–3.94 (m, 1 H; CH₂C₃H₇), 2.33–2.26 (m, 1 H; CH₂-C₂H₃), 2.23 (s, 1.5 H; COC₃H₇), 2.22 (s, 1.5 H; COC₃H₇), 1.86–1.78 (m, 1 H; CH₂-C₂H₃), 1.70–1.63 (m, 1 H; CH₂C₃H₇), 0.93 (s, 4.5 H; C(CH₃)₃), 0.92 (s, 4.5 H; C(CH₃)₃), 0.92–0.88 (m, 3 H; CH₂C₃H₇), 0.11 (s, 1.5 H; Si(CH₃)₃), 0.10 ppm (3 H; Si(CH₃)₃); ³¹P NMR (202 MHz, CDOD): δ = 3.80 (0.6 P), 3.48 ppm (0.4 P); MS (ES⁺): m/z (%): 695.85 [M+H⁺]⁺ (30), 717.50 [M+Na⁺]⁺ (100).

Prepared according to standard procedure 2 from nucleoside 17 (0.100 g, 0.284 mmol), 18c (0.189 g, 0.568 mmol) in anhydrous THF (2.6 mL) and rBuMgCl in THF (1 mL, 0.33 mL). After workup, the...
crude material was purified by column chromatography on silica gel with CH₂Cl₂/CH₃OH (95:5) as the eluent to provide 20c as a solid (50 mg, 28 % yield). ¹H NMR (500 MHz, CDCl₃; δ): δ = 7.88 (t, 1 H; H-6), 7.33–7.28 (m, 2 H; Ph-H), 7.21–7.12 (m, 3 H; H-Phe), 5.14–5.06 (m, 1 H; H-1), 4.40–4.38 (m, 1 H; H-3'), 4.25–4.16 (m, 2 H; CH₂­(S'), 4.13–4.10 (m, 2 H, OCH₃), 4.03–4.00 (m, 1 H; H-4'), 4.00–3.94 (m, 1 H; CH(OH)], 2.35–2.28 (m, 1 H, CH₂­(E')), 2.25 (s, 3 H, COOCH₃), 1.86–1.75 (m, 1 H; CH₂­(E), 1.73–1.70 (m, 2 H, OCH₂CH₃), 1.69–1.65 (m, 5 H; OCH₃CH₂CH₂CH₂CH₂), 1.36–1.32 (m, 2 H; CH₂CH₃), 0.94 (s, 4.5 H; C(CH₃)₃), 0.92 (s, 4.5 H; C(CH₃)₃), 0.91–0.89 (m, 3 H, CH₂(CH₃)₃), 0.11 (s, 1.5 H; Si(CH₃)₃), 0.11 (s, 1.5 H; Si(CH₃)₃), 0.10 ppm (s, 3 H; Si(CH₃)₃); ³¹P NMR (202 MHz, CDCl₃; δ): δ = 3.85 (0.5 P), 3.56 ppm (0.5 P); MS (ES⁻): m/z (%): 681.8 [M + H⁻] (30), 703.50 [M + Na⁻] (100).

(2S)-Isopropyl 2-(((2R,3'S,5'R)-5-(2-Acetamido-6-oxo-1,6-dihydropyrindin-5-yl)-3'-[(tert-butyldimethylsilyloxy)tetrahydrofuran-2'-yl)methoxy]napthalen-1-yl)oxy phosphoryl]amino)propanoate (20d)
Prepared according to standard procedure 2 from nucleoside 17 (0.095 g, 0.244 mmol). 18f (0.200 g, 0.495 mmol) in anhydrous THF (4 mL) and rBuMgCl in THF (1 mL, 0.37 mL). After workup, the crude material was purified by column chromatography on silica gel with CH₂Cl₂/CH₃OH (98:2) as the eluent to provide 19f as a solid (56 mg, 21 % yield). ¹H NMR (500 MHz, CDCl₃; δ): δ = 8.90 (brs, 1 H; NH), 8.88 (brs, 1 H; NH), 8.55 (s, 0.3 H; H-6), 8.49 (s, 0.3 H; H-6), 8.44 (s, 0.4 H; H-6), 7.81–7.17 (m, 22 H; H-Nap, H-Phe), 5.14–4.94 (m, 5 H; 2×OCH₂Ph, H-1'), 4.47–3.94 (m, 5 H; H₃=CH₂', 2×CH₂CH₂), 3.78–3.79 (m, 1 H; H-4'), 2.22 (s, 1.5 H; CO₂CH₃), 2.18 (s, 0.8 H; CO₂CH₃), 2.22 (s, 0.7 H; CO₂CH₃), 2.08–2.03 (m, 15 H; CH₂-2'), 1.88–1.86 (m, 0.5 H; CH₂-1'), 1.79–1.74 (m, 0.5 H; CH₂-2'), 1.65–1.59 (m, 0.5 H; CH₂-2'), 0.90 (s, 2.3 H; C(CH₃)₃), 0.88 (s, 2.3 H; C(CH₃)₃), 0.87 (s, 4.6 H; C(CH₃)₃), 0.07 (s, 1.1 H; Si(CH₃)₃), 0.03 (s, 1.1 H; Si(CH₃)₃), 0.15 (s, 1 H; Si(CH₃)₃), 0.00 (s, 2.2 H; 0.018 ppm (s, 1 H; Si(CH₃)₃); ³¹P NMR (202 MHz, CDCl₃; δ): δ = 3.96 (0.25 P), 3.25 (0.25 P), 3.19 (0.797), 3.06 ppm (0.3 P); MS (ES⁻): m/z (%): 1120.32 [M + H⁻] (60), 1142.30 [M + Na⁻] (100).

Further elution of the crude mixture with CH₂Cl₂/CH₃OH (95:5) yielded 20f as a solid (98 mg, 59 % yield). ¹H NMR (500 MHz, CDCl₃; δ): δ = 8.10 (d, J = 8.6 Hz, 1 H; H-Nap), 7.87 (d, J = 8.6 Hz, 16 H, H-Nap), 7.72–7.62 (m, 2 H, H-Nap), 7.53–7.39 (m, 4 H, H-Nap), 7.32–7.27 (m, 5 H, Ph-H), 7.52–7.05 (m, 2 H, OCH₂Ph), 4.98 (dd, J = 10.0, 5.5 Hz, 1 H, H₁'), 4.26–4.14 (m, 4 H, H₃', CH₂-S, CH₂-1', 3.97–3.92 (m, 2 H, H-4'), 2.22 (s, 3 H; CO₂CH₃), 2.12–2.06 (m, 1 H; CH₂-E'), 1.40–1.06 (m, 4 H; CH₂-2', CH₂-1'), 0.88 (s, 4.5 H; C(CH₃)₃), 0.87 (s, 4.5 H; C(CH₃)₃), 0.05 (s, 1.5 H; Si(CH₃)₃), 0.03 (s, 1.5 H; Si(CH₃)₃), 0.02 (s, 1.5 H; Si(CH₃)₃), –0.08 ppm (s, 1.5 H; Si(CH₃)₃); ³¹P NMR (202 MHz, CDCl₃; δ): δ = 4.09 (0.6 P), 3.97 ppm (0.4 P); MS (ES⁻): m/z (%): 751.85 [M + H⁻] (50), 773 [M + Na⁻] (100).

Standard Procedure 3: Synthesis of Phosphoramidates 21a-f, 25a and 25g
N-Acetyl-3'-O-silyl-pseudoisocytidine phosphoramidates 20a-f or 3'-O-silyl-pseudoisocytidine phosphoramidates 24a and 24g were treated with TFA/CH₂Cl₂ (1:1 v/v) at 0 °C. The resulting reaction mixture was stirred at 0°C for 6 h. After the reaction was completed the solvents were evaporated and the residue was purified by preparative HPLC to afford 21a-f and 25a and 25g, respectively.

(2S)-Benzyli(((2R,3’S,5'R)-5-(2-Acetamido-6-oxo-1,6-dihydropyrindin-5-yl)-3'-hydroxytetrahydrofuran-2'-yl)methoxy]-phenoxy)[phosphoryl]amino)propanoate (21a)
Prepared according to standard procedure 3 from compound 20a (0.022 g, 0.031 mmol) and TFA/CH₂Cl₂ (1:1 v/v, 0.3 mL). After workup, the crude material was purified by preparative HPLC (H₂O/CH₃CN from 90:10 to 0:100 in 30 min, flow = 20 mL/min⁻¹, λ = 280 nm) to yield 21a as a white solid (9.2 mg, 50 % yield). ¹H NMR (500 MHz, CDCl₃; δ): δ = 7.88 (s, 1 H; H-6), 7.35–7.29 (m, 7 H; H-Ar),...
(2S)-Hexyl 2-(((2R,3S,5R)-5-(2-Acetamido-oxo-1,6-dihydro-
pyrimidin-5-yl)-3-hydroxytetrahydrofuran-2-yl)methoxy)
(phenoxyl)phosphoryl)amino)propanoate (21 b)

Prepared according to standard procedure 3 from compound 20 b
(0.034 g, 0.049 mmol) and TFA/CHCl₃ (1:1 v/v, 0.5 mL). After
workup, the crude was purified by preparative HPLC (H₂O/CH₃CN
from 90:10 to 100:0 in 30 min, flow = 1 mL/min, λ = 280 nm, τᵣ = 12.97.

(2S)-Iso-pentyl 2-(((2R,3S,5R)-5-(2-Acetamido-oxo-1,6-dihydro-
pyrimidin-5-yl)-3-hydroxytetrahydrofuran-2-yl)methoxy)-
(naphthalen-1-yl)phosphoryl)amino)propanoate (21 d)

Prepared according to standard procedure 3 from compound 20 d
(40 mg, 0.057 mmol) and TFA/CH₂Cl₂ (1:1 v/v, 0.3 mL). After
workup, the crude was purified by preparative HPLC (H₂O/MeCN
from 90:10 to 100:0 in 30 min, flow = 20 mL/min, λ = 280 nm) to
yield 21 d as a white solid (12.6 mg, 37% yield). ¹H NMR (500 MHz,
CD₃OD): δ = 8.10–7.97 (m, 1H; H-Naph), 7.75–7.65 (2H, 2H; H-Naph,
H-6), 7.59–7.47 (m, 1H; H-Naph), 7.41–7.37 (m, 3H; H-Naph), 7.32
(d, J = 8.12 Hz, 0.57 Hz; H-Naph), 7.30 (d, J = 8.12 Hz, 0.25 Hz; H-Naph),
4.93–4.50 (m, 1H; H-7), 4.85–4.79 (m, 1H; O(CH₂)₃), 4.17–4.12
(2H, 2H; CH₂), 3.41–3.08 (m, 1H; H-3), 3.94–3.87 (2H, 2H;
CHO₃), 1.86–1.79 (m, 1H; CH₂-1), 2.12 (s, 3H; COCH₃), 1.68–1.99
(1H, 1H; CH₂), 1.24 (d, J = 7.5 Hz, 3H, CH₃), 1.09 (d, J = 6.4 Hz, 3H,
OCH(CH₃)₂), 1.08 ppm (d, J = 6.5 Hz, 3H, OCH(CH₃)₂); ¹C NMR
(125 MHz, CD₃OD): δ = 173.6 (COOCH₃), 173.4 (COOCH₃), 175.0
(CO₂-Phexyl), 165.5 (C-4), 151.2 (d, J₆₋₅ = 6.5 Hz; C-iso-
Phospho), 152.2 (d, J₆₋₅ = 7.5 Hz; C-iso-Phospho), 154.2 (C-6), 152.1 (C-2),
130.8, 126.2, 126.1 (CH-Ph), 124.2 (C-5), 124.2 (C-2), 121.5 (d, J₆₋₅
= 4.6 Hz; CH-Ph), 121.5 (d, J₆₋₅ = 4.7 Hz; CH-Ph), 86.3 (d, J₆₋₅ = 2.3 Hz;
C-4), 86.3 (d, J₆₋₅ = 2.3 Hz; C-4), 75.9 (C-1), 75.8 (C-1), 75.1 (C-4),
73.9 (C-3), 73.9 (C-3), 68.1 (d, J₆₋₅ = 5.9 Hz; C₅), 68.3 (d, J₆₋₅ = 5.2 Hz;
C-3), 66.5 (OCH₃), 66.5 (OCH₃), 51.7 (CH₃), 51.7 (CH₃), 41.4 (C-2),
41.7 (C-2), 32.6, 29.7, 26.6 (CH₂), 23.9 (COCH₃), 23.6 (CH₃), 20.6
(d, J₆₋₅ = 6.5 Hz; CH₃), 20.5 (d, J₆₋₅ = 7.3 Hz; CH₃), 14.3 ppm
(CH₃); ¹³P NMR (202 MHz, CD₃OD): δ = 3.38 (0.6P), 3.58 ppm
(0.4P); MS (ES⁺): m/z (%): 581.23 [M⁺H]⁺ (34), 603.57 [M⁺Na⁺]
(100); reversed-phase HPLC, eluting with H₂O/CH₃CN from 90:10
to 100:0 in 30 min, flow = 1 mL/min, λ = 254 nm, τᵣ = 18.72 min.

(2S)-Neopentyl 2-(((2R,3S,5R)-5-(2-Acetamido-oxo-1,6-dihydro-
pyrimidin-5-yl)-3-hydroxytetrahydrofuran-2-yl)methoxy)-
naphthalen-1-yl)phosphoryl)amino)propanoate (21 e)

Prepared according to standard procedure 3 from compound 20 e
(72 mg, 0.025 mmol) and TFA/CH₂Cl₂ (1:1 v/v, 0.5 mL). After
workup, the crude was purified by preparative HPLC (H₂O/CH₃CN
from 90:10 to 100:0 in 30 min, flow = 20 mL/min, λ = 280 nm) to
yield 21 e as a solid (16.0 mg, 46% yield). ¹H NMR (500 MHz,
CD₃OD): δ = 8.02–7.99 (m, 1H; H-Naph), 7.75–7.70 (m, 1H; H-Naph),
7.66 (s, 1H; H-6), 7.57 (d, J = 7.8 Hz, 0.7H; H-Naph), 7.52 (d, J = 7.8
Hz, 0.1H; H-Naph).
8.4 Hz, 0.3 H; H-Naph), 7.40–7.34 (m, 3 H; H-Naph), 7.32–7.28 (m, 0.7 H; H-Naph), 7.31–7.28 (m, 0.3 H; H-Naph), 4.89 (dd, J = 10.1, 6.2 Hz, 1 H; H-1), 4.20–4.12 (m, 2 H; CH2-S), 4.13–4.07 (m, 1 H; H-3), 4.00–3.96 (m, 1 H; CH2CH2), 3.92–3.90 (m, 1 H; H-4), 3.73, 3.66 (AB system, J = 10.5 Hz, 1 H; OCH2(CH2)3), 3.72, 3.62 (AB system, J = 10.5 Hz, 1 H; OCH2(CH2)3), 1.24–1.16 (m, 1 H; CH2-S), 1.20 (s, 2 H; COCH3), 2.09 (s, 1 H; COCH3), 2.16–2.10 (m, 1 H; CH2CH2), 1.26 (d, J = 7.18 Hz, 1.2 H; CH2CH2), 1.24 (d, J = 7.3 Hz, 1.8 H; CH2CH2), 0.79 (s, 3 H; OCH2(CH2)3), 0.81 ppm (s, 6 H; OCH2(CH2)3) 13C NMR (125 MHz, CD2OD): δ = 173.0 (COCH3), 171.2 (COCH2(CH2)3), 170.9 (COCH2(CH2)3), 159.2 (C-4), 153.5 (C-6), 152.1 (C-2), 146.7 (d, J, 3p, = 6.8 Hz; C-ipo-Naph), 134.9 (C-Naph), 127.4 (CH-Naph), 126.5 (d, J, 3p, = 5.2 Hz, C-Naph), 126.3, 126.1, 125.1, 124.6 (CH2-Naph), 123.1 (C-5), 115.0 (d, J, 3p, = 3.6 Hz; CH-Naph), 85.0 (C-4'), 74.4 (C-1), 74.4 (CCH3), 72.6 (C-3'), 66.9 (d, J, 3p, = 5.0 Hz; C-5), 66.9 (d, J, 3p, = 5.0 Hz; C-S), 50.5 (CHCH3), 50.3 (CHCH3), 40.8 (C-2), (C-OCH2(CH2)3), 22.5 (COCH3), 19.2 ppm (d, J, 3p, = 7.3 Hz; CH2CH2). MS (EI): m/z (%): 202 (M+H)−, 20.0 (M+Na)− (100); reversed-phase HPLC, eluting with H2O/CH3CN from 90:10 to 0:100 in 30 min, flow = 1 mL min−1, λ = 254 nm, tR = 17.45 min.

(25)-Benzy1 2-(((2R,3,S,5'R)-2-(32-3Acetamido-6-oxo-1,6-dihydropyr-1pyrimidin-5-yl)-3'-(tert-butyldimethylsilyloxy)loxymethyl) (naphthalen-1-yl)methyl)aminopropionate (21 f)

Prepared according to standard procedure 3 from the compound 20f (98 mg, 0.130 mmol) and to TFA/CH2Cl2 (1:1 v/v, 0.3 mL). After workup, the crude material was purified by preparative HPLC (H2O/CH3CN from 90:10 to 0:100 in 30 min, flow = 20 mL min−1, λ = 280 nm) to give 21 f as a white solid (20.1 mg, 25% yield). H NMR (500 MHz, CD2OD): δ = 8.13–8.11 (m, 1 H; H-11), 7.86–7.84 (m, 2H, H-Naph-6), 7.71–7.64 (m, 1 H; H-Naph), 7.51–7.46 (m, 3 H; H-Naph), 7.32 (d, J = 8.12 Hz, 0.65 Hz; H-Naph), 7.30 (d, J = 8.12 Hz, 0.35 Hz; H-Naph), 7.26–7.18 (m, 2H; H-Naph), 3.89–3.88 (m, 1H; OCH2CHO), 0.01 ppm (s, 6.3H; Si(C2 H3)3). 13C NMR (125 MHz, CD2OD): δ = 165.3 (C-4), 155.9 (C-2), 128.7 (C-6), 116.0 (C-6), 87.5 (C-4), 74.2 (C-1), 74.1 (C3), 63.4 (C-5), 140.0 (C-2), 25.1 (Si(CH3)3), 25.0 (Si(CH3)3), 17.8 (Si(CH3)3), 17.5 (Si(CH3)3), 5.7 (Si(CH3)3), 5.9 (Si(CH3)3), −6.6 (Si(CH3)3), −6.6 ppm (Si(CH3)3)). MS (ES): m/z (%): 456.3 (M + H)+ (100); reversed-phase HPLC, eluting with H2O/CH3CN from 90:10 to 0:100 in 30 min, flow = 1 mL min−1, λ = 254 nm, tR = 19.1 min.

2-Amino-5-(((2R,4'S,5'R)-4-(((tert-butyldimethylsilyloxy)loxymethyl)tetrahydrofuran-2-yl)pyrimidin-4(3H)-one (23)

A mixture of TFA and H2O (1:1 v/v, 2.0 mL) was added dropwise to a solution of 22 (0.244 g, 0.535 mmol) in THF (4 mL) at 0°C. The reaction was stirred at room temperature for 2 h under argon atmosphere. The reaction mixture was quenched with NaHCO3 and concentrated under reduced pressure to afford 23 as glassy solid, which was used in the next step without further purification (0.123 g, 67%). 1H NMR (500 MHz, CD2OD): δ = 7.61 (s, 1H; H-6), 4.92 (dd, J = 10.2, 6.0 Hz, 1H; H-1), 4.35–4.31 (m, 1H; H-3), 3.82–3.74 (m, 1H; H-4), 3.58 (dd, J = 11.5, 4.5 Hz, 1H; CH2S), 3.54 (dd, J = 11.5, 4.5 Hz, 1H; CH2S), 2.16–2.10 (m, 1H; CH2CH2), 1.88–1.82 (m, 1H; CH2CH2), 0.85 (s, 9H; Si(CH3)3), 0.06 (s, 3H; Si(CH3)3), 0.01 ppm (s, 3H; Si(CH3)3). 13C NMR (125 MHz, CD2OD): δ = 161.3 (C-4), 150.3 (C-6), 149.8 (C-2), 123.1 (C-5), 88.5 (C-4), 85.7 (C-1), 72.2 (C-3), 70.0 (C-5'), 37.4 (C-2), 24.0 (Si(CH3)3), 19.7 (Si(CH3)3), −6.1 (Si(CH3)3), −6.4 ppm (Si(CH3)3)). MS (ES): m/z (%): 559.2 (M + Na)+ (100); reversed-phase HPLC, eluting with H2O/CH3CN from 90:10 to 0:100 in 30 min, flow = 1 mL min−1, λ = 254 nm, tR = 10.8 min.
(25)-Isopropyl 2-(((2R,3'S,5'R)-5-(2-Amino-6-oxo-1,6-dihydropyrimidin-5-yl)-3-hydroxytetrahydrofuran-2'-yl)(methoxy)(phenoxy)phosphoryl)amino)propanoate (24g)

Prepared according to standard procedure 2 from nucleoside 23 (0.035 g, 0.1 mmol), 18g (0.067 g, 0.2 mmol) in anhydrous THF (1.5 mL) and bBuMgCl in THF (1 mL, 0.13 mL). After workup, the crude material was purified by column chromatography on silica gel using CHCl₃/CH₃OH (92:8) as an eluent to give 24g as a solid (0.020 g, 32%). ¹H NMR (500 MHz, CDCl₃): 8 = 7.56 (brs, 1H; H-6), 7.26–7.22 (2H, H-PH), 7.14–7.07 (3H, 3H-PH), 4.94 (dd, J = 10.0, 6.0 Hz, 1H; H-1'), 4.87–4.89 (1H, 1H; OCH(C₃H₇)), 4.38–4.31 (1H, H-3'), 4.12–4.03 (2H, 2H; CH₂-5'), 3.89–3.88 (1H, 1H; H-4'), 3.83–3.77 (1H, 1H; CH₂CH₃), 2.13–2.00 (1H, 1H; C₂H₂), 1.79–1.69 (1H, 1H; C₂H₂), 1.25 (d, 2J = 7.0 Hz, 0.7H; CH₂CH₃), 1.22 (d, 2J = 7.0 Hz, 2.3H; CH₂CH₃), 1.14 (d, 2J = 6.0 Hz, 3H; OCH(CH₃)), 1.12 (d, 2J = 6.0 Hz, 3H; OCH(CH₃)), 0.82 (t, 2J = 6.3H; SiC(CH₃)), 0.81 (t, 2J = 6.7H; SiC(CH₃)), 0.02 (s, 0.7H; Si(CH₃)), 0.05 (s, 0.7H; Si(CH₃)), −0.03 (s, 3.3H; Si(CH₃)), −0.07 ppm (2.3H; Si(CH₃)); ¹³C NMR (202 MHz, CDCl₃): 8 = 120.1 (d, 2C = 13.0, 5.5, 2.0 Hz, 1H; C₂H₂), 129.6, 125.6 (C₆H₅), 123.5 (C₂), 120.2 (d, 2J₃ = 5.7 Hz; CH-PH), 120.1 (d, 2J₃ = 4.5 Hz; CH-PH), 84.8 (d, 2J₃ = 8.4 Hz; C₂), 84.8 (d, 2J₃ = 8.4 Hz; C₂), 74.7 (C-1'), 72.8 (C-3'), 68.8 (OCH(CH₃)), 66.8 (d, 2J₃ = 5.7 Hz; C-5'), 50.5 (CH₂O), 40.5 (C-2'), 39.5 (C-2'), 20.6 (OCH(CH₃)), 19.5 (d, 2J₃ = 7.5 Hz; CH₂CH₃), 19.0 ppm (d, 2J₃ = 7.5 Hz; CH₂CH₃); ²⁵P NMR (202 MHz, CDCl₃): 8 = 3.91 (0.8P), 3.69 ppm (0.2P); MS (ES⁺): m/z (%) = 495.15 [M+H]+ (100); reversed-phase HPLC, eluting with H₂O/CH₃CN from 90:10 to 90:10 in 30 min, flow = 1 mL/min⁻¹, λ = 254 nm, tᵢ = 13.42 min.

Carboxypeptidase Y assay

Trizma buffer (300 µL, pH 7.6) was added to compound 21e (5 mg) dissolved in D₂O/acetone (150 µL). The ²⁵P NMR spectrum (202 MHz, 64–128 scans) was recorded at this stage as a reference (blank, tᵢ = 0). To this mixture, a stock solution of carboxypeptidase Y (Sigma–Aldrich, > 50 units mg⁻¹, dissolved in pH 7.6 Trizma buffer, to a concentration of 50 units mL⁻¹) was added.

²⁵P NMR spectra (128 scans) were recorded with a 3 min delay between experiments for 14 h at 25 ℃.

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Conflict of Interest

The authors declare no conflict of interest.

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Synthetic Approaches for the Preparation of Phosphoramidate Prodrugs of 2'-Deoxypseudoisocytidine

2'-Deoxypseudoisocytidine is a C-nucleoside with antileukemic activity. A series of phosphoramidate prodrugs of this C-nucleoside were synthesized. Their synthesis proved challenging due to unexpected side reactions (double phosphorylation and epimerization). Their enzymatic activation was found to be similar to that of N-linked nucleoside ProTides.