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 biphosphorylated derivatives. Epimerisation (β to α) 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,[4] showdomycin,[4] and formycin A,[5] were isolated from culture filtrates of different bacterial strains. These compounds are antibacterials 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.[6] Among them, pseudoisocytidine (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.[11] 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

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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 Daves 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. Compound 12 was pre-
pared from 3',5'-bis-O-(tert-butyldimethylsilyl)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 glycalcs with a wide range of O-silyl protections.

The coupling reaction of 5-iodo base 11 with the protected ribofuranosyl glycal 12 using Pd(OAc)₂ as a catalyst, AsPh₃ 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 triacetoxysilane to reduce diastereoselectively the 3'-keto group from the β-face of the furanosyl ring, forming N-acetyl-2'-deoxypseudoisocytidine 15.[22] The cleavage of the acetyl group to afford nucleoside 2 was then accomplished by basic hydrolysis using NH₃ in MeOH. The assignment of the configuration at the 1'-position of 2 was based on the comparison of its ¹H NMR spectrum with that reported in the literature.[22]

2.2. Synthesis of N-Acetyl-2'-deoxypseudoisocytidine Phosphoramidates

The two synthetic strategies commonly used for the preparation of phosphoramidate prodrugs (phosphorochloridate in the presence of either tert-butylmagnesium 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-butyldimethylsilyl 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'-deoxypseudoisocytidine (15) were first protected with a tert-butyldimethylsilyl group using tert-butyldimethylsilyl 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 R₅ and S₅ diastereoisomers according to a literature procedure,[26] were reacted with 17 in the presence of tert-butylmagnesium chloride (1.0 m in THF), yielding 3'-O-tert-butyldimethylsilyl phosphoramidates 20a-f (Scheme 2) as diastereomeric 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 ³¹P and ¹H 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-acetylimino-4-hydroxy pyrimidines 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. TBDMSCI, tert-butyldimethylsilyl 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.\(^{[27]}\)

Acidic deprotection of 20 a–f afforded after preparative HPLC purification compounds 21 a–f in moderate yields (Scheme 2 and Table 1). Attempts to remove the acetyl protection from 21 a with Schwartz’s reagent as described by Ferrari et al.,\(^{[28]}\) failed due to the ring opening of the base. The difficulties encountered in removing the N-acetyl group from 21 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 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, phosphorochloridates 18 a and 18 g were reacted with 23 in the presence of tert-butyllmagnesium chloride (1.0 M in THF) to yield, after column chromatography, the 3’-O-tert-butyldimethylsilyl-protected phosphoramidates 24 a and 24 g in moderate yield as diastereoisomeric 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 a and 24 g with trifluoroacetic acid in dichloromethane (1:2 v/v; room temperature, overnight) afforded the final compounds 25 a and 25 g as mixtures of α and β isomers in a 3:1 ratio after column chromatography. The β-isomers of 25 a and

<table>
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<th>Cmpd</th>
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<th>Yield [%]</th>
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<td>Ph</td>
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<td>Naph</td>
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<td>CH₂Ph</td>
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<td>62</td>
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<tr>
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<td>CH(CH₂)₂</td>
<td>1:3</td>
<td>32</td>
<td>25 g</td>
<td>50</td>
<td>4:1</td>
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### Table 2. Reaction outcomes for the synthesis of 24 a and 24 g and ProTides 25 a and 25 g after HPLC purification.

1) 1M (BuMgCl), THF

2) 18 a–g, rt, 24 h

32-41%

16

NH₂MeOH

0°C to rt, 12 h

90%

22

TFA/H₂O/THF (1:1:4)

0°C to rt, 2 h

67%

23

TFA, CH₂Cl₂

0°C to rt, 6 h

or

CH₂SIOTI, CH₂Cl₂

-78°C to rt, 2 h

50-62%

24 a, g

25 a, g

Figure 2. Proposed rationale for the O-phosphorylation side reaction.

Figure 3. Synthesis of ProTides 25 a and 25 g.
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]}

![Scheme 4. Proposed mechanism for the epimerisation of C-nucleoside phosphoramidates under acidic conditions.](image)

If a mild procedure for the cleavage of tert-butyldimethylsilyl ethers to alcohols (based on an exchange reaction with trimethylsilyl triflate at $-78 \, ^\circ\text{C}$)\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 belongs to the same family of C-type carboxypeptidases and it was reported to share similarities in the active site.\textsuperscript{[33]}

Compound 21e in [D$_6$]acetone was therefore incubated in an NMR tube with carboxypeptidase Y in Trizma buffer (pH 7.6), and the progress of the reaction was monitored by $^{31}$P NMR analysis over 14 h. The stacked spectra (Figure 3) show the formation of a new peak after 10 min of incubation, which corresponds to intermediate I ($\delta_p = 5.06$ ppm, $t = 10$ min). Complete conversion of the ProTide 21e (which in [D$_6$]acetone appears as a single peak at $\delta_p = 4.26$ ppm) into the corresponding aminoacyl phosphoramidate ester (II: $\delta_p = 7.19$ ppm) was observed in 40 min. In vivo, the aminoacyl phosphoramidate ester metabolite is then believed to undergo P–N bond cleavage, mediated by a phosphoramidase-type enzyme to eventually release the parent drug in its monophosphate form.

![Figure 3. Deconvoluted $^{31}$P NMR spectra (202 MHz, [D$_6$]acetone/pH 7.6 Trizma buffer) to show the carboxypeptidase-mediated activation of compound 21e.](image)

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 visualized 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 ThermoScientific or a Varian Prostar system. 1H NMR (500 MHz), 13C (125 MHz), and 31P 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 CD3OD (δ = 3.34 ppm), 1H NMR; δ = 49.86 ppm, 13C NMR) and CDC13 (δ = 7.26 ppm, 1H NMR; δ = 77.4 ppm, 13C NMR) or external 85% H3PO4 (δ = 0.00 ppm, 31P NMR). Coupling constants (J) are expressed in Hz. 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).

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

N-lodosuccinimide (22.0 g, 98 mmol) was added to a solution of 2-(6-Oxo-1,6-dihydropyrimidin-2-yl)acetamide (10) (11.8 g, 49.8 mmol) and acetic anhydride (200 mL). The organic layer was dried over MgSO4, filtered and concentrated under reduced pressure. The residue was dissolved in MeOH (0.2 mL) at 0 °C and the mixture was stirred for 2 h. The mixture was evaporated to dryness under reduced pressure and the residue was purified by flash column chromatography (EtOAc/hexane 7:3) to give 13 as a light yellow solid (2.8 g, 52% yield). 1H NMR (500 MHz, CDCl3): δ = 12.30 (brs, 1 H; NH), 9.86 (brs, 1 H; NH), 8.04 (s, 1 H; H-6), 5.75 (s, 1 H; H-1), 4.92 (s, 1 H; H-2), 4.53–4.51 (m, 1 H; H-4), 3.84 (dd, δ = 11.5, 25.5 Hz, 1 H; H-5’a), 3.69 (dd, δ = 11.5, 4.0 Hz, 1 H; H-5’b), 0.92 (s, 9 H, C(CH3)3), 0.90 (s, 9 H, C(CH3)j), 0.99 (s, 6 H, Si(CH3)2), 0.02 ppm (2 H, SiH2CH3); 13C NMR (125 MHz, CDCl3): δ = 174.2 (C=O), 161.3 (C=O), 151.4 (C=O), 148.1 (C-2), 123.0 (C-5), 101.3 (C-2’), 85.4 (C-4’), 77.9 (C-1’), 64.7 (C-5’), 26.5 (Si(CH3)2), 26.1 (Si(CH3)2), 22.5 (COCH3), 19.4 (Si(CH3)2), 18.8 (Si(CH3)2), -6.1 (Si(CH3)2), -63 ppm (Si(CH3)2); MS (ES+): m/z (%): 496 [M+H]+ (40), 518.26 [M+Na]+ (100).

2-Amino-5-((2R,4’S,5’S)-4’-hydroxy-5’-(hydroxymethyl)tetrahydrofur-2’-yl)-6-oxo-1,6-dihydropyrimidin-2-yl)acetamide (15)

The reaction was stirred at room temperature for 12 h under an argon atmosphere. The suspension was diluted with acetic acid (30 mL) and the volatiles removed under reduced pressure to obtain crude compound 14, which was used in the next step without further purification. 1H NMR (500 MHz, CD3OD): δ = 8.1 (s, 1 H; H-6), 5.17 (dd δ = 10.2, 6.8 Hz, 1 H; H-1), 4.04 (t, δ = 3.5 Hz, 1 H; H-4’), 3.65 (dd, δ = 12.2, 2.6 Hz, 1 H; H-5’a), 3.61 (dd, δ = 12.2, 3.5 Hz, 1 H; H-5’b), 2.89–2.86 (m, 1 H; H-2’a), 2.53–2.50 (m, 1 H; H-2’b), 2.23 ppm (3 H; CH3); MS (ES−, negative mode): m/z: found 266.20 [M−H]− 100%; reversed-phase HPLC, eluting with H2O/CH2CN from 98/2 to 0/100 in 45 min, flow = 1.0 min−1, λ = 254 nm, tR = 7.75 min.

The residue was dissolved in a mixture of acetic acid/CH3CN (1:1 v/v, 200 mL) and the mixture was cooled to −15 °C, followed by the portionwise addition of NaBH4(OAc)2 (3.0 g, 14.1 mmol). After 2 h, the mixture was evaporated to dryness under reduced pressure and the residue was purified by flash column chromatography (CH3Cl2/CH3OH/8.2) to give 15 as a white solid (1.3 g, 80% yield). 1H NMR (500 MHz, CD3ODMSO): δ = 12.09 (brs, 1 H; NH), 11.73 (brs, 1 H; NH), 8.1 (s, 1 H; H-6), 5.14 (brs, 1 H; S-OH), 4.95–4.90 (m, 1 H; H-1), 4.09 (t, δ = 3.3 Hz, 1 H; H-4’), 3.65 (dd, δ = 12.0, 4.2, 2.5 Hz, 1 H; H-5’a), 3.61 (dd, δ = 12.5, 7.3, 2.5 Hz, 1 H; H-5’b), 3.17 (d, δ = 5.4 Hz, 1 H; 3’-OH), 2.76 (dd, δ = 17.6, 6.6 Hz, 1 H; H-2’a), 2.42 (dd, δ = 17.5, 10.0 Hz, 1 H; H-2’b), 2.16 ppm (3 H; CH3); 13C NMR (125 MHz, CDCl3): δ = 175.1 (C=O), 159.6 (C-4), 153.6 (C-6), 152.7 (C-2), 123.4 (C-5), 88.9 (C-4’), 75.7 (C-1’), 73.4 (C-3’), 64.1 (C-4’), 41.7 (C-2’), 23.9 ppm (CH3); MS (ES−): m/z (%): calcd for C18H18N4O2: 269 [M]; found: 291.09 [M+Na]+ (100); reversed-phase HPLC, eluting with H2O/CH3OH from 90:10 to 0:100 in 30 min, flow = 1.0 min−1, λ = 254 nm, tR = 4.87 min.

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3.94–3.92 (m, 1 H; H-4'), 3.83 (dd, J = 12.2, 3.6 Hz, 1 H; H-5'a), 3.63 (dd, J = 12.7, 4.0 Hz, 1 H; H-5'b), 2.23–2.17 (m, 1 H; H-2'a), 2.10 ppm (dd, J = 13.0, 6.0 Hz, 1 H; H-2'b); 13C NMR (125 MHz, CDCl3): δ = 166.1 (C-4), 156.3 (C-1), 148.6 (C-6), 114.6 (C-5), 87.5 (C-4'), 76.0 (C-1'), 73.4 (C-3'), 62.6 (C-5'), 40.3 ppm (C-2'); MS (ES+): m/z (%): 228.04 [M+H+] (50), 200.01 [M+Na+] (100); reversed-phase HPLC, eluting with H2O/CH3OH from 90:10 to 0:100 in 30 min, flow = 1 mL/min−1, λ = 254 nm, tR = 45.7 min.

**Standard Procedure 1: Synthesis of Phosphorochloridates 18a–g**

Anhydrous triethylamine (2.0 mol equiv.) was added dropwise at −78 °C to a stirred solution of the appropriate amino ester hydrochloride/tosylate salt (1.0 mol equiv.) and the appropriate dichlorophosphate (1.0 mol equiv.) in anhydrous dichloromethane (61.6 mol) under an argon atmosphere. After 1 h the reaction mixture was warmed to room temperature and was stirred for an additional 1–2 h. Formation of the desired phosphorochloridate was monitored by 31P NMR spectroscopy. After the reaction was completed, the solvent was removed under reduced pressure and the resulting residue was re-dissolved in anhydrous diethyl ether and the triethylammonium salt was removed by filtration. The filtrate was evaporated to dryness and the crude material was purified by flash column chromatography with ethyl acetate/hexane (1:1 v/v) as the eluent to give the desired phosphorochloridate as an oil.

**Phenyl-(benzoxyl-α-l-alaninyl)-phosphorochloridate (18a)**

Prepared according to standard procedure 1 in 92% yield. 1H NMR (500 MHz, CDC13); δ = 7.30–7.10 (m, 10 H; H-Ar), 5.20–5.16 (m, 2 H; OCH2Ph), 4.25–4.22 (m, 1 H; CH(O)); 3.51–3.48 (m, 1 H; NH); 1.54 (d, J = 7.3 Hz, 1.5 H; CH2Ph); 1.52 ppm (d, J = 7.3 Hz, 1.5 H; CH2Ph); 13C NMR (125 MHz, CDCl3); δ = 169.8 (d, Jc–p = 5.4 Hz; C-O); 135.0 (d, Jc–p = 6.8 Hz; ipso-C); 134.7 (ipso-OCH2Ph); 130.0, 129.8, 128.7, 128.7, 128.4, 128.3, 126.0 (CH-Ph), 120.6 (d, Jc–p = 2.5 Hz; Ch-Ph); 120.5 (d, Jc–p = 2.5 Hz; Ch-Ph), 68.1, 67.7 (OCH2Ph), 50.8, 50.5 (CH2CH2); 20.5 ppm (d, J = 5.6 Hz; CH3); 31P NMR (202 MHz, CDCl3); δ = 7.93 (0.5 P), 7.51 ppm (0.5 P).

**Phenyl-(hexoxyl-α-l-alaninyl)-phosphorochloridate (18b)**

Prepared according to standard procedure 1 in 87% yield. 1H NMR (500 MHz, CDC13); δ = 7.24–7.18 (m, 5 H; H-Ph), 4.34–4.20 (m, 1 H; NH), 4.20–4.05 (m, 3 H; CH2CH2 and OCH2); 4.03–3.94 (m, 2 H; OCH2CH2); 1.66–1.56 (m, 4 H; OCH2CH2CH2CH2); 1.59–1.53 (m, 3 H; CH2); 1.37–1.31 (m, 4 H; CH2CH2CH2); 0.94–0.87 ppm (CH3); 13C NMR (125 MHz, CDCl3); δ = 172.8 (d, Jc–p = 7.8 Hz; C-O); 172.7 (d, Jc–p = 7.8 Hz; C-O), 149.8 (d, Jc–p = 8.0 Hz; ipso-C-Ph); 149.8 (d, Jc–p = 8.0 Hz; ipso-C-Ph); 129.9, 129.8, 125.9, 125.9 (CH-Ph); 120.6 (d, Jc–p = 5.3 Hz; CH-Ph), 66.0 65.9 (OCH2); 50.8, 50.5 (CH2CH2); 31.3 (OCH2CH2), 28.4 (OCH2CH2CH2); 22.5 (CH2); 22.4 (d, Jc–p = 5.6 Hz; CH2); 22.3 (d, Jc–p = 5.6 Hz; CH2); 13C (ipso CH3); 13P NMR (202 MHz, CDCl3); δ = 7.96 (0.5 P), 7.64 ppm (0.5 P).

**Phenyl-(pentoxyl-α-l-alaninyl)-phosphorochloridate (18c)**

Prepared according to standard procedure 1 in 96% yield. 1H NMR (500 MHz, CDC13); δ = 7.46–7.31 (2 H; Ph-H), 7.28–7.22 (2 H; Ph-H), 4.68 (brs; NH), 4.18–4.09 (m, 3 H; CH2CH2O); 1.73–1.71 (m, 2 H; OCH2CH2); 1.68–1.65 (m, 5 H; CH2CH2OCH2CH2); 1.36–1.32 (m, 2 H; CH2CH2); 0.92–0.89 ppm (m, 3 H; CH2); 13C NMR (125 MHz, CDC13); δ = 172.7 (d, Jc–p = 7.7 Hz; C-O); 172.6 (d, Jc–p = 7.3 Hz; C-O), 149.6 (d, Jc–p = 8.1 Hz; ipso-C-Ph); 149.4 (d, Jc–p = 8.0 Hz; ipso-C-Ph); 129.9, 129.8, 125.9, 125.8 (CH-Ph); 120.5 (d, Jc–p = 5.5 Hz; CH-Ph), 65.9 65.8 (OCH2); 50.5, 50.3 (CH2CH2); 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); 13C (ipso CH3); 13P NMR (202 MHz, CDCl3); δ = 7.92 (0.5 P), 7.61 ppm (0.5 P).
1-Naphthyl-(isopropoxy-\(-\)alaninyl)-phosphorochloridate (18 d)

Prepared according to standard procedure 1 in 84% yield. 1H NMR (500 MHz, CDCl₃): δ = 8.10–8.07 (m, 1 H; CH-Ph), 7.88–7.82 (m, 1 H; CH-Ph), 7.76–7.76 (m, 1 H; H-Nap), 7.64–7.63 (m, 1 H; H-Nap), 5.13–5.09 (m, 1 H; OCH₂(CH₃)₂), 4.54 (brs, 1 H; NH), 4.26–4.22 (m, 1 H; CH₂CH₂), 1.56 (d, J = 7.0 Hz, 1.5H; CH₃CH₂), 1.54 (d, J = 7.0 Hz, 1.5H; CH₃CH₂), 1.34–1.25 ppm (m, 6 H; OCH(CH₃)₂); 13C NMR (125 MHz, CDCl₃): δ = 171.3 (C-β), 149.8 (d, J₂₋₃ crawler = 8.0 Hz; ipso-C-Ph), 147.3 (ipso-C-Ph), 134.9, 134.8 (C-Naph), 129.0, 127.6, 126.9, 126.7, 126.3 (CH-Naph), 126.1 (d, J₂₋₃ crawler = 8.3 Hz; C-Naph), 125.5, 124.4, 122.0, 121.5, 121.4, 121.3, 116.2, 115.2 (CH₂-Naph), 70.6, 69.2 (OCH(CH₃)₂), 51.0, 50.7 (CH(.CH₃)₂), 21.7, 21.5 (CH(CHOH)₂), 16.1 ppm (OCH(CH₃)₂); 31P NMR (202 MHz, CDCl₃): δ = –8.35 (0.5 P), 8.03 ppm (0.5 P).

Standard Procedure 2: Synthesis of 20a–f, 24a and 24g

A solution of 8BuMgCl in THF (1.0, 1.2 mol equiv.) was added at 0°C to a stirred solution of 17 or 23 (1 mol equiv) in THF. The reaction mixture was allowed to warm to room temperature and the appropriate phosphorochloridate (2.0 mol equiv) dissolved in anhydrous THF was added. The reaction mixture was stirred for 16–18 h and then evaporated under vacuum to give a crude residue that was purified by column chromatography on silica gel, eluting with a gradient of CH₂OH (0–5%) in CH₂Cl₂ to afford products 20a–f, 24a and 24g as white solids.

2-(Benzyloxy)-1H-benzo[\(d\)]imidazole (18 e)

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 8BuMgCl in THF (1.0, 0.16 mL). After workup, the crude product was purified by column chromatography on silica gel using CH₂Cl₂/CH₂OH (95:5) as eluent to provide 20a as a white solid (22 mg, 25% yield). 1H NMR (500 MHz, CDOD): δ = 7.89 ppm (1 H, 2H), 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-1H; OCH₂CH₂), 4.40–4.39 (m, 1 H; H-3), 4.21–4.12 (m, 2 H; H-C₅), 4.05–4.00 (m, 2 H; H-4', CH₂CH₂), 2.33–2.23 (m, 1 H; CH₂CH₂), 2.21 (s, 1.2H; COCH₂), 2.20 (s, 1.8H; COCH₂), 1.85–1.71 (m, 1 H; CH₂CH₂), 1.37 (d, J = 7.1 Hz, 1.2H; CH₂CH₂), 0.92 (s, 9 H; C(CH₃)₃), 0.11 (s, 1.5H; Si(CH₃)₂), 0.10, (s, 1.5H; Si(CH₃)₂), 0.09 ppm (3 H; Si(CH₃)₂); 31P NMR (202 MHz, CDCl₃): δ = 3.89 (0.6 P), 3.48 ppm (0.4P); MS (ES⁺): m/z (%): 701 [M + H]⁺ (100), 702 [M + Na]⁺ (100).

PhenyI-(isopropoxy-\(-\)alaninyl)-phosphorochloridate (18 g)

Prepared according to standard procedure 1 in 93% yield. 1H NMR (500 MHz, CDCl₃): δ = 7.41–7.36 (m, 2 H; Ph-Ph), 7.31–7.27 (m, 3 H; H-Ph), 5.21–5.01 (m, 1 H; OCH₂CH₂), 4.65 (brs, 1 H; NH), 4.21–4.06 (m, 1 H; CH₂CH₂), 1.51 (d, J = 7.0 Hz, 3 H; CH₃CH₂), 1.26–1.19 ppm (m, 6 H; OCH₂CH₂); 13C NMR (125 MHz, CDCl₃): δ = 172.2 (d, J₂₋₃ crawler = 8.2 Hz; C-O), 172.1 (d, J₂₋₃ crawler = 9.1 Hz; C-C = C > O), 149.8 (d, J₂₋₃ crawler = 8.4 Hz; ipso-C-Ph), 149.8 (d, J₂₋₃ crawler = 8.3 Hz; ipso-C-Ph), 129.9 (CH-Ph), 126.0 (CH-Ph), 120.5 (d, J₂₋₃ crawler = 5.4 Hz; CH₃CH₂), 69.8, 69.8 (OCH₂CH₂), 50.9, 50.6 (CH₂CH₂), 21.6, 21.5 (OCH₂CH₂), 20.4 (d, J₂₋₃ crawler = 4.3 Hz; CH₂CH₂), 20.6 ppm (d, J₂₋₃ crawler = 4.3 Hz; CH₂CH₂); 31P NMR (202 MHz, CDCl₃): δ = 8.08 (0.5 P), 7.71 ppm (0.5 P).

PhenyI-(isopropoxy-\(-\)alaninyl)-phosphorochloridate (18 g)

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 8BuMgCl in THF (1.0, 0.33 mL). After workup, the

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crude material was purified by column chromatography on silica gel with CH$_2$Cl$_2$/CH$_3$OH (95:5) as the eluent to provide 20c as a solid (50 mg, 28% yield).$^1$ H NMR (500 MHz, CD$_2$OD): $\delta$ = 7.88 (s, 1 H; H-6), 7.35–7.28 (m, 2 H; H-Ph), 7.21–7.12 (m, 3 H; H-Ph), 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; CH$_2$-5), 4.13–4.10 (m, 2 H; OCH$_3$), 4.03–4.00 (m, 1 H; H-4), 4.00–3.94 (m, 1 H; CH$_2$H), 2.35–2.28 (m, 1 H; CH$_2$-2), 2.25 (s, 3 H; COCH$_3$), 1.86–1.75 (m, 1 H; CH$_2$-2), 1.73–1.70 (m, 2 H; OCH$_2$CH$_3$), 1.69–1.65 (m, 5 H; OCH$_2$CH$_2$CH$_2$CH$_3$), 1.36–1.32 (m, 2 H; CH$_2$CH$_3$), 0.94 (s, 4.5 H; C(CH$_3$_3)$_2$), 0.92 (s, 4.5 H; C(CH$_3$_3)$_2$), 0.91–0.89 (m, 3 H; C(CH$_3$_3)$_2$), 0.11 (s, 1.5 H; Si(CH$_3$_3)$_2$), 0.11 (s, 1.5 H; Si(CH$_3$_3)$_2$), 0.10 ppm (s, 3 H; Si(CH$_3$_3)$_2$); $^{13}$P NMR (202 MHz, CD$_2$OD): $\delta$ = 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).

(25)-Isopropyl 2-(((2R,3'S,5'R)-5-(2-Acetamido-6-oxo-1,6-dihydropyrimidin-5-yl)-3'-(tert-butyl dimethylsilyloxy)tetrahydrofuran-2'-ylmethoxy)(naphthalen-1-yloxy)phosphoryl)(amino)propanoate (20d)

Prepared according to standard procedure 2 from nucleoside 17 (0.095 g, 0.248 mmol), 18f (0.200 g, 0.495 mmol) in anhydrous THF (4 mL) and tertBuMgCl in THF (1 mL, 0.37 mL). After workup, the crude material was purified by column chromatography on silica gel with CH$_2$Cl$_2$/CH$_3$OH (98:2) as the eluent to provide 19f as a solid (56 mg, 21% yield).$^1$ H NMR (500 MHz, CD$_2$Cl$_2$): $\delta$ = 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$_2$-H$_2$Ph), 5.14–4.94 (m, 5 H; 2xOCH$_2$Ph, H-1'), 4.47–3.94 (m, 5 H; H$^3$, CH$_3$-5', 2xCH$_2$CH$_3$), 3.78–3.79 (m, 1 H; H-4'), 2.22 (s, 1.5 H; COCH$_3$), 2.18 (s, 0.8 H; COCH$_3$), 2.22 (s, 0.7 H; COCH$_3$), 2.08–2.03 (m, 0.5 H; CH$_2$-2'), 1.88–1.86 (m, 0.5 H; CH$_2$-2'), 1.79–1.74 (m, 0.5 H; CH$_2$-2'), 1.65–1.59 (m, 0.5 H; CH$_2$-2'), 0.90 (s, 2.3 H; C(CH$_3$_3)$_2$), 0.88 (s, 2.3 H; C(CH$_3$_3)$_2$), 0.87 (s, 4.6 H; C(CH$_3$_3)$_2$), 0.70 (s, 1 H; Si(CH$_3$_3)$_2$), 0.05 (s, 1 H; Si(CH$_3$_3)$_2$), 0.15 (s, 1 H; Si(CH$_3$_3)$_2$), 0.00 ppm (s, 2 H; Si(CH$_3$_3)$_2$); $^{13}$P NMR (202 MHz, CD$_2$OD): $\delta$ = 3.96 (0.25 P), 3.25 (0.25 P), 3.19 (0.7 F), 3.06 ppm (0.3 F); MS (ES$^-$): $m/z$: 1120.32 [M+H]$^-$ (60), 1142.30 [M+Na$^+$] (100).

Further elution of the crude mixture with CH$_2$Cl$_2$/CH$_3$OH (95:5) yielded 20f as a solid (98 mg, 59% yield).$^1$ H NMR (500 MHz, CD$_2$Cl$_2$): $\delta$ = 8.10 (d, J = 8.6 Hz, 1 H; H-Naph), 7.87 (d, J = 8.6 Hz, 1 H; H-Naph), 7.72–7.62 (m, 2 H, H-Naph-H$_6$), 7.33–7.39 (m, 4 H; H-Naph), 7.32–7.27 (m, 5 H; H$_2$Ph), 5.12–5.05 (m, 2 H; OCH$_2$Phi), 4.98 (dd, J = 10.0, 5.5 Hz, 1 H; H-1'), 4.26–4.14 (m, 4 H; H$_3'$, CH$_3$-5', CH$_3$-5', 2xCH$_2$CH$_3$), 3.97–3.92 (m, 1 H; H-4'), 2.22 (s, 3 H; COCH$_3$), 2.12–2.06 (m, 1 H; CH$_2$-2'), 1.40–1.06 (m, 4 H; CH$_2$-2', CH$_2$-2', C(CH$_3$_3)$_2$), 0.98 (s, 4.5 H; C(CH$_3$_3)$_2$), 0.87 (s, 4.5 H; C(CH$_3$_3)$_2$), 0.05 (s, 1.5 H; Si(CH$_3$_3)$_2$), 0.03 (s, 1.5 H; Si(CH$_3$_3)$_2$), 0.02 (s, 1.5 H; Si(CH$_3$_3)$_2$); $^{13}$P NMR (202 MHz, CD$_2$OD): $\delta$ = 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-pseudoisocytidyl phosphoramidates 20a-f or 3'-O-silyl-pseudoisocytidyl phosphoramidates 24a and 24g were treated with TFA/CH$_2$Cl$_2$ (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.

(25)-Benzy1 2-(((2R,3'S,5'R)-5-(2-Acetamido-6-oxo-1,6-dihydropyrimidin-5-yl)-3'-hydroxytetrathydrofuran-2'-yilmethoxy)(naphthalen-1-yloxy)phosphoryl)(amino)propanoate (21a)

Prepared according to standard procedure 3 from compound 20a (0.022 g, 0.031 mmol) and TFA/CH$_2$Cl$_2$ (1:1 v/v, 0.3 mL). After workup, the crude material was purified by preparative HPLC (H$_2$O/CH$_3$CN from 90:10 to 0:100 in 30 min, flow = 20 mL/min$^{-1}$), $\lambda$ = 280 nm) to yield 21a as a white solid (92 mg, 50% yield).$^1$ H NMR (500 MHz, CD$_2$OD): $\delta$ = 7.88 (s, 1 H; H-6), 7.35–7.29 (m, 7 H; H-Ar),

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(2S)-Hexyl 2-(((2R,3’R,5’R)-5-(2-Acetamido-6-oxo-1,6-dihydropyrimidin-5-yl)-3’-hydroxytetrahydrofuran-2’-yl)methoxy)(phenoxypophosphoryl)amino)propanoate (2b)

Prepared according to standard procedure 3 from compound 20b (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, t₁₅ = 11.79.

(dd, J = 9.9, 5.9 Hz, 0.5H; H-1’), 4.35–4.32 (m, 1H; H-3’), 4.30–4.21 (m, 2H; CH₂-5’), 4.15–4.05 (m, 3H; H-4’, OCH₃), 4.00–3.94 (m, 1H; H-CH₃), 3.23–3.21 (m, 1H; CH₂-CH₂), 2.23 (s, 1.5H; COCH₃), 2.22 (s, 1.5H; COCH₃), 1.84–1.75 (m, 1H; CH₂-2’), 1.37 (d, J = 7.1 Hz, 1.2H; CHCH₂), 1.33 ppm (d, J = 7.2 Hz, 1.2H; CHCH₃). ¹¹C NMR (125 MHz, CDCl₃): δ = 175.0 (COCH₃), 175.0 (COCH₃), 175.0 (CO₂H-exyl), 175.0 (CO₂H-exyl), 165.5 (C-4’), 152.3 (d, J = 6.5 Hz; C- ipso-Pho), 152.2 (d, J = 7.5 Hz; C-ipso-Pho), 154.2 (C-6’), 152.1 (C-2’), 130.8, 126.2, 126.1 (CH-Ph), 124.2 (C-5’), 124.2 (C-5’), 121.5 (d, J = 6.4 Hz; CH-Ph), 121.5 (d, J = 4.7 Hz; CH-Ph), 86.3 (d, J = 2.3 Hz; C-4’), 86.3 (d, J = 2.3 Hz; C-4’), 75.9 (C-1’), 75.9 (C-1’), 74.5 (C-3’), 74.5 (C-3’), 67.9 ppm (d, J = 5.6 Hz, C-6’), 67.8 (d, J = 5.6 Hz, C-6’), 67.9 (OCH₃), 67.7 (OCH₃), 51.7 (CHCH₃), 51.1 (CHCH₃), 42.8 (C-2’), 42.0 (C-2’), 23.9 (CH₃), 20.4 (d, J = 7.1 Hz; CH₃), 20.4 ppm (d, J = 7.4 Hz; CH₃); ¹³P NMR (202 MHz, CDCl₃): δ = 3.94 (0.7P), 3.52 ppm (0.3P); MS (ES’): m/z (%): 587.18 [M]+ [H]+ (30), 609.53 [M+N]+ (100); reversed-phase HPLC, eluting with H₂O/CH₃CN from 90:10 to 100:0 in 30 min, flow = 1 mL/min, λ = 254 nm, t₁₅ = 17.44.

(2S)-Isopropyl 2-(((2R,3’S,5’R)-5-(2-Acetamido-6-oxo-1,6-dihydropyrimidin-5-yl)-3’-hydroxytetrahydrofuran-2’-yl)methoxy)(naphthalen-1-yl)phosphoryl)amino)propanoate (21 d)

Prepared according to standard procedure 3 from compound 20d (40 mg, 0.057 mmol) and TFA/CHCl₃ (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). ¹¹H NMR (500 MHz, CDCl₃): δ = 8.10–7.97 (m, 1H; H-Naph), 7.75–7.65 (m, 2H; H-Naph, H-6), 7.59–7.47 (m, 1H; H-Naph), 7.41–7.37 (3H, 1H; H-Naph), 7.32 (d, J = 8.12 Hz, 0.75 Hz; H-Naph), 7.30 (d, J = 8.12 Hz, 0.25 Hz; H-Naph), 4.93–4.90 (m, 1H; H-1’), 4.85–4.79 (m, 1H; OCH₃(CH₂)), 4.17–4.12 (m, 2H; CH₂(H₂)), 3.94–3.87 (m, 2H; CH₂(CH₃)), 2.97–2.31 (1H, CH₂-C₆), 2.21 (s, 3.0H; COCH₃), 1.89–1.78 (m, 3H; CH₂-2’), 1.20 (s, 3H; COCH₃), 1.20–1.24 (m, 1H; H-CH₂-C₆), 1.15 (d, J = 7.5 Hz, 3H, CH₃), 1.09 (d, J = 6.4 Hz, 3H, OCH(CH₃)), 1.08 ppm (d, J = 6.5 Hz, 3H, OCH(CH₃)); ¹¹C NMR (125 MHz, CDCl₃): δ = 173.6 (COCH₃), 173.4 (CO₂H), 173.1 (C(O)), 173.1 (CO), 166.2 (C-4’), 154.2 (C-6’), 152.2 (C-2’), 146.7 (d, J = 8.3 Hz; C- ipso-Pho), 134.9 (C-Naph), 127.5 (CH-Naph), 126.5 (d, J = 5.2 Hz; C-Naph), 126.0, 126.1, 125.1, 124.6 (CH-Naph), 123.5 (C-Naph), 114.9 (d, J = 3.6 Hz; CH₂-CH₂), 85.0 (C-4’), 74.4 (C-1’), 72.6 (C-3’), 68.8 (OCO₂H), 66.9 (d, J = 5.02 Hz; C-5’), 50.6 (CH₂(CH₂)), 40.2 (C-2’), 22.5 (COCH₃), 20.5 (OCH₂CH₃), 20.4 (OCH₂CH₃); ¹³P NMR (202 MHz, CDCl₃): δ = 3.43 ppm (MS (ES’): m/z (%): 611.20 [M+Na]+ (100); reversed-phase HPLC, eluting with H₂O/CH₃CN from 90:10 to 100:0 in 30 min, flow = 1 mL/min, λ = 280 nm, t₁₅ = 18.72 min.

(2S)-Nepentyl 2-(((2R,3’S,5’R)-5-(2-Acetamido-6-oxo-1,6-dihydropyrimidin-5-yl)-3’-hydroxytetrahydrofuran-2’-yl)methoxy)(naphthalen-1-yl)phosphoryl)amino)propanoate (21 e)

Prepared according to standard procedure 3 from compound 20e (42 mg, 0.056 mmol) and TFA/CHCl₃ (1:1 v/v, 0.3 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). ¹¹H NMR (500 MHz, CDCl₃): 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 =...
(25)-Benzyli 2-(((R)3,5,5′R)-5′-2-Amino-6-oxo-1,6-dihydro-
pyrimidin-5-yl)-3′-hydroxytetrahydrofuran-2′-ylmethoxy-
(naphthalen-1-yl)oxy)phosphorylaminopropanoate (21 f)

Prepared according to standard procedure 3 from the compound 20 f (98 mg, 0.130 mmol) and to TFA/CHCl₃ (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, CDCl₃): δ = 8.13–8.11 (m, 1 H; H-1), 7.86–7.84 (m, 2 H; H-Naph); 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 H; H-Naph), 7.30 (d, J = 8.12 Hz, 0.35 H; H-Naph), 7.33–7.28 (m, 5 H; H-Ph), 5.14–5.07 (m, 2 H; OCH₂Ph), 5.03 (dd, J = 8.6, 5.5 Hz, 1 H; H-1), 4.25–4.20 (m, 3 H; CH₂-5′, H-3′), 4.15–4.09 (m, 1 H; CH₂OCH₃), 4.03–4.00 (m, 1 H; H-2′), 2.21 (3 H; COCH₃), 2.19–2.15 (m, 1 H; CH₂Ph), 1.49–1.36 (m, 1 H; CH₂Ph), 1.37 (d, J = 7.5 Hz, 3 H; CH₃Naph); 13C NMR (125 MHz, CDCl₃): δ = 173.6 (COCH₃), 173.6 (C-O), 173.5 (C-O), 166.2 (C-4), 154.2 (C-6), 150.6 (C-2), 146.7 (δ, Jᵣ₋₅′ = 8.3 Hz; Cis–Naph), 146.6 (δ, Jᵣ₋₅′ = 8.3 Hz; Cis–Naph), 135.8, 134.9, 134.8 (C-12Naph), 128.2 128.1, 127.9, 127.9, 127.8, 127.4 (CH-Naph), 126.4, 126.4, 126.1, 125.1 (CH-Naph), 124.6 (C-5), 124.5 (C-5), 121.3, 121.3 (CH-Naph), 115.1 (δ, Jᵣ₋₅′ = 3.6 Hz; CH₃Naph), 115.0 (δ, Jᵣ₋₅′ = 3.6 Hz; CH₃Naph), 85.1 (C-4′), 85.0 (C-4′), 74.3 (C-1′), 74.2 (C-1′), 72.7 (C-3′), 72.6 (C-3′), 69.0 (δ, Jᵣ₋₅′ = 5.0 Hz; C-5′), 66.9 (δ, Jᵣ₋₅′ = 5.0 Hz, C-5′), 66.6 (OCH₂Ph), 66.6 (OCH₂Ph), 50.5 (CH₂Nap), 50.4 (CH₂Nap), 40.7 (C-4′, 40.5 (C-2′), 22.5 (COCH₃), 19.0 (δ, Jᵣ₋₅′ = 7.3 Hz; CH₃C₆H₄), 18.9 ppm (Jᵣ₋₅′ = 7.3 Hz; CH₃C₆H₄); 19P NMR (202 MHz, CDCl₃): δ = –4.33 (8.8 P), 3.98 (28.2 P); MS (ES⁺): m/z (%): 659.20 (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.

2-Amino-5-(((R)2,4,4,5,5′R)-5′-2-Amino-6-oxo-1,6-dihydro-
pyrimidin-5-yl)-3′-(tetrahydrofuran-2′-yl)methoxy-
(phenoxyl)phosphorylaminopropanoate (24 a)

Prepared according to standard procedure 2 from nucloside 23 (0.035 g, 0.11 mmol), 18a (0.071 g, 0.2 mmol) in anhydrous THF (2 mL) and rBuMgCl in THF (1 mL, 0.13 mL). After workup, the crude was purified by column chromatography on silica gel using CH₂Cl₂/CH₃OH (92:8) as the eluent to give 24a as a solid (0.0223 g, 41%).

H NMR (500 MHz, CDCl₃): δ = 7.56 (s, 1 H; H-6), 7.26–7.19 (m, 7 H; H-Ph), 7.12–7.06 (m, 3 H; H-Ph), 5.07–5.01 (m, 2 H; OCH₂Ph), 4.94 (dd, J = 10.0, 6.0 Hz, 1 H; H-1), 4.29–4.21 (m, 1 H; H-3), 4.05–4.01 (m, 2 H; CH₂-5′), 3.96–3.88 (m, 1 H; CH₂-5′), 3.86–3.83 (m, 1 H; H-4′), 2.10–2.06 (m, 0.3 H; H-2′a), 2.04–2.01 (m, 0.7 H; H-2′a), 1.74–1.79 (m, 1 H; H-2′b), 1.29–1.25 (m, 3 H; CH₂OH), 0.84 (s, 2.7 H; Si(C₆H₅)₂), 0.82 (s, 6.3 H; Si(CH₃)₂), 0.01 (s, 1.8 H; Si(CH₃)₂), 0.00 (p, 4.2 H; Si(CH₂-5′); 19P NMR (202 MHz, CDCl₃): δ = 3.79 (0.7 P), 3.49 ppm (0.3 P).
(25)-Isopropyl 2-(((2R,3’S,5’S)-5-((2-Amino-6-oxo-1,6-dihydro-
pyrimidin-5-yl)-3’-(tet-butylmethylsilyl)oxy)tetracydrofur-
an-2’-yl)methoxy)(phenoxy)phosphoryl)aminopropanoate (24g)

Prepared according to standard procedure 3 from compound 24a (10 mg, 0.016 mmol) and TFA/CH₂Cl₂ (1:1 v/v, 0.6 mL). After workup, the crude material was purified by preparative HPLC to yield 24g as a white solid (4.0 mg, 50%). ¹H NMR (500 MHz, CDCl₃): δ = 7.68 (s, 0.8 H; H-6), 7.67 (s, 0.2 H; H-6), 7.20–7.20 (m, 2 H; H-Ph), 7.15–7.02 (m, 3 H; H-Ph), 5.07 (dd, J = 10.0, 5.5 Hz, 1 H; H-1), 4.98–

4.96 (m, 1 H; OCH(CH₂)), 4.33–4.28 (m, 1 H; H-3), 4.23–4.19 (m, 2 H; CH₂-S), 4.05–4.01 (m, 1 H; H-4), 3.92–3.87 (m, 1 H; CH₃CH₂), 2.32–

2.20 (m, 2 H; CH₂-S), 1.90–1.80 (m, 2 H; CH₃), 1.37 (d, J = 7.0 Hz, 0.6 H; CH₂), 1.34 (d, J = 7.0 Hz, 2.4 H; CH(CH₃)), 1.25 (d, J = 6.5 Hz, 3 H; OCH(CH₂)), 1.23 ppm (d, J = 6.5 Hz, 3 H; OCH(CH₂)); ³¹C NMR (125 MHz, CDCl₃): δ = 170.2 (COCH₂CH₂), 159.2 (C-4), 153.5 (C-6), 152.0 (C-1), 141.1 (δ, J = 4.5 Hz; C ipsoPh), 129.4, 126.8 (CH-Ph), 123.5 (C-5), 120.2 (δ, J = 5.7 Hz; CH-Ph), 120.1 (δ, J = 4.5 Hz; CH-Ph), 84.8 (δ, J = 8.4 Hz; C-4), 8.48 (δ, J = 8.4 Hz; C-4), 74.7 (C-1), 72.8 (C-3), 68.8 (OCH(CH₂)), 66.8 (δ, J = 5.7 Hz; C-5), 50.5 (CH₃CH₂), 40.5 (C-2), 39.5 (C-2), 20.6 (OCH(CH₂)), 19.5 (δ, J = 7.5 Hz; CH₂CH₃), 19.0 ppm (δ, J = 7.5 Hz; CH₂CH₃); ³¹P NMR (202 MHz, CDCl₃): δ = 3.91 (0.8P) ppm, MS (ES⁻): m/z (%) = 495.15 (M⁻H⁻) (100); reversed-phase HPLC, eluting with H₂O/H₃OCHCN from 90:10 to 0:100 in 30 min, flow = 1 mL/min⁻¹, λ = 254 nm, tᵣ = 13.42 min.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: 2’-deoxypseudosioctydine · anticancer · C-
nucleosides · phosphoramidates · prodrugs


These are not the final page numbers!
Synthetic Approaches for the Preparation of Phosphoramidate Prodrugs of 2'-Deoxypseudoisocytidine

C the difference: 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.