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Short communication

Cyclic phosphoramidates as prodrugs of 2′-*C*-methylcytidine

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ABSTRACT

The currently approved treatment for hepatitis C virus infections is a combination of Ribavirin and pegylated Interferon. It leads to a sustained virologic response in approximately only half of the patients treated. For this reason there is an urgent need of new therapeutic agents. 2′-*C*-Methylcytidine is the first nucleoside inhibitor of the HCV NS5B polymerase that was efficacious in reducing the viral load in patients infected with HCV. The application of a monophosphate prodrug approach based on unprecedented cyclic phosphoramidates is reported. Our SAR studies led to compounds that are efficiently converted to the active triphosphate in human hepatocytes.

1. Introduction

Hepatitis C virus is a 9.5 kb positive-strand RNA virus that undergoes rapid mutation, making treatment difficult. Chronic hepatitis C viral infection can lead to cirrhosis and hepatocellular carcinoma. Estimates of the total number of infected individuals are currently 170–200 million worldwide [1]. While the incidence of new infections is declining, mortality is expected to increase in the middle of the next decade [2a]. The currently approved treatment available for patients with chronic hepatitis C is a combination of Ribavirin and pegylated Interferon, leading to a sustained virologic response (SVR) in approximately half of the patients treated. Side effects such as fatigue, flu-like symptoms, depression, and hemolytic anemia can not only be dose limiting but also lead to a premature discontinuation of therapy prior to achievement of sustained viral response [2a,b].

HCV inhibition can be achieved by blocking essential virally-encoded non-structural enzymes (NS2-3 and NS3-4A proteases, the NS3 helicase as well as the NS5B RNA-dependent RNA polymerase) [2c,d]. The NS3-4A protease and NS5B RNA enzymes have been the focus of intense drug discovery efforts over the years [2a]. These have culminated in the identification of antiviral compounds with which significant reductions in HCV viral load have been demonstrated in the clinic [2a,b].

The RNA-dependent RNA polymerase (RdRp) is at the heart of the viral replication complex. Nucleoside as well as non-nucleoside inhibitors of this enzyme have been described. The former offer the advantage of pan-genotype inhibition due to the high conservation of the RdRp active site across all HCV genotypes. A nucleoside needs to be converted efficiently into the corresponding nucleotide triphosphate anabolite via cellular kinases. This newly synthesized nucleotide must be a substrate for the HCV RdRp, and needs to be incorporated into the nascent nucleic acid chain causing chain termination.

The efficiency of NTP formation of a given nucleoside often rests on the rate-limiting first phosphorylation which converts the nucleoside into its corresponding monophosphate anabolite by cellular kinases (Fig. 1; from **4** to **5**). Several approaches have been

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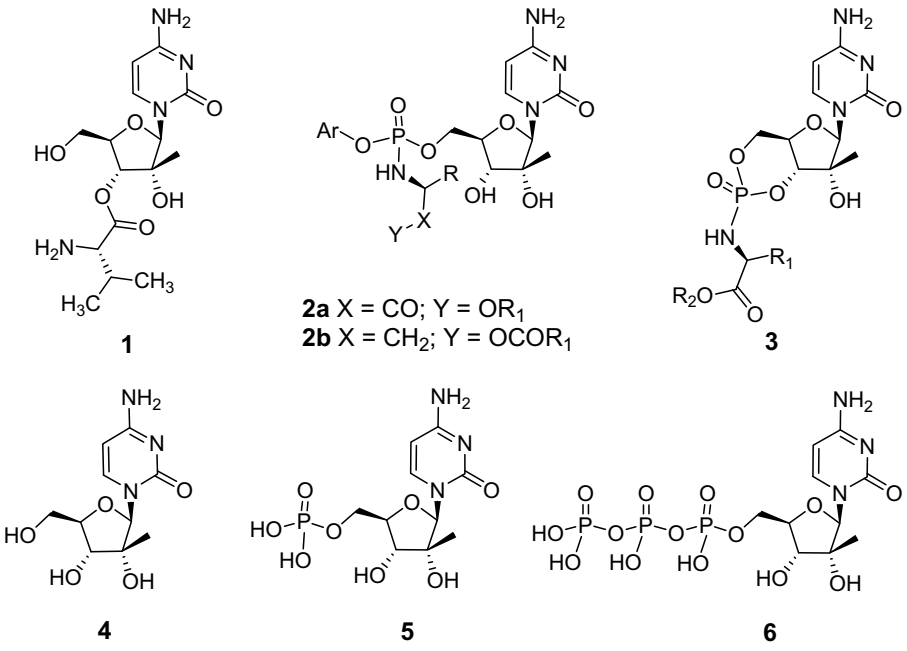


Fig. 1. 2′-*C*-Methylcytidine (**4**), its prodrugs (**1**, **2a**, **2b**, **3**) and anabolites (**5** and **6**).

described in the literature which address this problem by delivering a masked nucleoside monophosphate (SATE approach [3a], cycloSal approach [3b], HepDirect approach [3c] as well as the use of phosphoramidates [3d]).

We have recently been involved in a prodrug based nucleoside inhibitor program focusing on 2′-*C*-methylcytidine **4**, a potent and selective inhibitor of HCV replication in cell culture. We used Valopicitabine **1** [4], the 3′-*O*-L-valine ester derivative of 2′-*C*-methylcytidine, as our reference compound. Although **1** has been evaluated in Phase IIb clinical studies, further development has been put on hold due to an unfavourable risk/benefit profile [2c]. Initially we based our approach on Protide type structures **2a** [5a,5b], and on acyloxy ethylamino phosphoramidates **2b** [5c], as we were aware that initial, first phosphorylation to **5** was the rate-limiting step in cellular processing to form active triphosphate **6** [6a,b]. This approach in literature has been addressed as 'kinase-bypass-effect' [3c,7a–c].

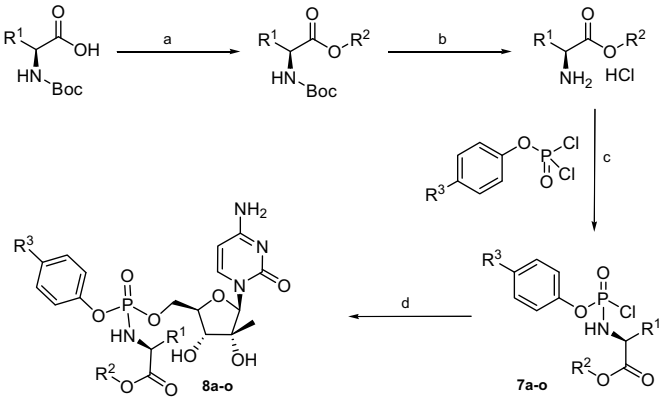
Over the course of our program, we could eliminate the need for a phenolic leaving group from our prodrugs, designing the cyclic phosphoramidates of type **3** (Fig. 1) [8]. This generated an additional level of diversity in our SAR studies and this approach might have two additional advantages: the cyclisation reduces the degree of rotational freedom as compared to generally described Protide prodrugs **2a**, possibly allowing for improved entry into the cell, and less phenol related cytotoxic side effects [9a,b] might occur.

In this communication we describe the synthesis and structure–activity relationship studies around cyclic phosphoramidates **3** and show that they efficiently generate high levels of 2′-methylcytidineTP **6** in hepatocytes.

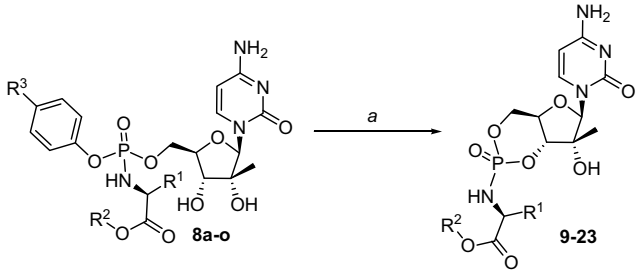
2. Results and discussion

2.1. Chemistry

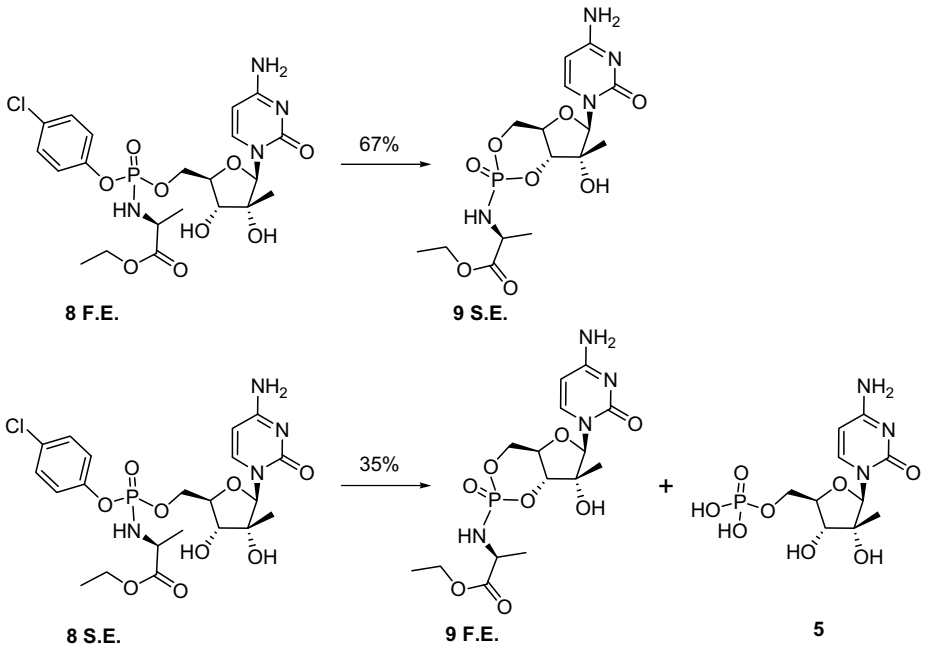
The synthesis of the precursors **8a**–**o** is described in Scheme 1 [5b]. The ester coupling between amino acids and alcohols as well as derivatisation to form phosphoramidate chlorides **7a**–**o** were modified literature procedures giving generally good yields [10]. Selective *t*BuMgCl-mediated reaction between phosphoramidate chlorides **7a**–**o** and 2′-*C*-methylcytidine [11] to form intermediate nucleoside derived phosphoramidates **8a**–**o** proceeded in discrete to moderate yields as a mixture of diastereomers that were separated or taken on as a mixture to the cyclisation step.



Scheme 1. Reagents and conditions: (a) R_2OH, TEA, DMAP, DCM 80–97%; (b) 4 N HCl dioxane, 95–100%; (c) Et_3N, DCM, −78 °C to rt, 72–91%; (d) *t*BuMgCl, **4**, THF, −78 °C to rt, 5–36%.



Scheme 2. Reagents and conditions: (a) KO*t*Bu, DMSO, 41–78%.



Scheme 3. **F.E.** (fast eluting) and **S.E.** (slow eluting) allude to relative retention time on an UPLC system.

The low yield of the Grignard mediated addition was probably due to the low solubility of the starting nucleoside under the reaction conditions.

The cyclisation proceeded smoothly by treatment of the diastereomeric mixtures of **8a–o** with KO*t*Bu in DMSO, to give a diastereomeric mixture of cyclisation products **9–23**, which were separated by RP-HPLC, whenever possible, Scheme 2. Yields depended largely on the nucleofugality of the aromatic group. An electron withdrawing group on the aromatic moiety (e.g. $R^3 = Cl$) gave cleaner and higher yielding cyclisations than an unsubstituted phenol ($R^3 = H$). Another observation we made was the formation of varying amounts of a side product upon cyclisation, which was identified as monophosphate **5** by HPLC and MS spectroscopy in all cases.

To elucidate the cyclisation step further, we synthesized and separated the diastereomers **8 F.E.** (fast eluting) and **8 S.E.** (slow eluting) according to Scheme 1 and exposed them to the cyclisation conditions as described in Scheme 2. We noted a pronounced difference in the behaviour of the two open phosphoramidates, Scheme 3: whereas phosphoramidate **8 F.E.** cyclised cleanly to yield single diastereomer **9 S.E.** in 67% isolated yield, the corresponding diastereomer **8 S.E.** formed not only selectively the other diastereomer **9 F.E.** (35% isolated yield), but also monophosphate **5**. Depending on the substituents R^1 and R^2 of open phosphoramidates **8a–o** (Scheme 2), the corresponding cyclised, fast eluting diastereomer could not always be isolated, as the major pathway was formation of undesired monophosphate **5**.

For both **9 F.E.** as well as **9 S.E.** the absolute stereochemistry on phosphorous could be assigned by measuring the internuclear distances of the NH to the protons indicated by NOESY experiments (Fig. 2). Molecular modeling was used to show that the measured distances for **9 F.E.** are only compatible with the *R*-configuration on phosphorous, placing the amino acid residue in a pseudo-axial conformation. These interactions are absent for **9 S.E.** where the only weak crosspeak was observed between the NH and the hydrogen at C-4, suggesting an *S*-configuration on phosphorous, positioning the amino acid residue in an equatorial position. It is of interest to note that the difference in phosphorous shifts in the ^{31}P NMR is always the same (i.e. the fast eluting diastereomer appears more upfield than the slow eluting diastereomer) as exemplified for **9 F.E.** and **9 S.E.** (Fig. 2) [12,13]. An analogous behaviour in terms of ^{31}P shift (i.e. the more polar fast eluting diastereomer is *R* with

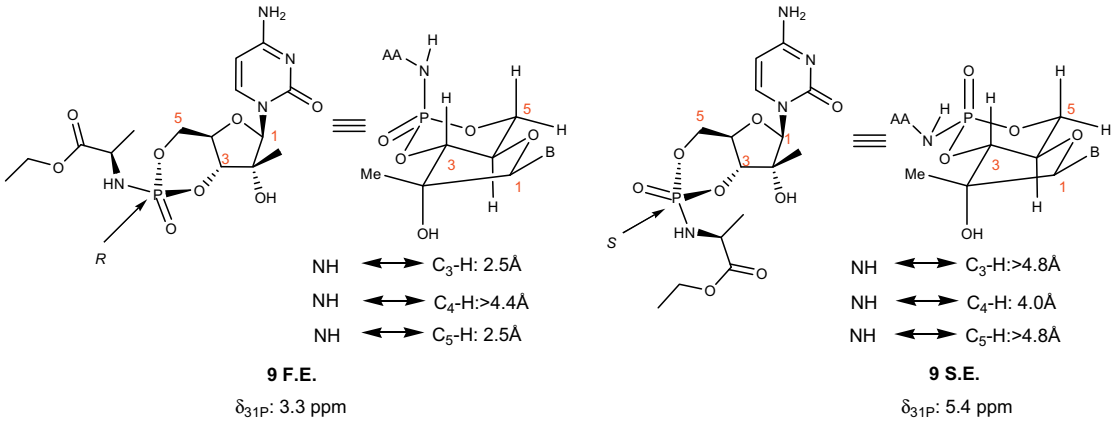


Fig. 2. Assignment of absolute stereochemistry of **8 F.E.** and **8 S.E.** on phosphorous; AA: amino acid; B: base.

Table 1
Antiviral activity, cytotoxicity and conversion to NTP **6** in human hepatocytes of compounds **1**, **9 F.E.** and **9 S.E.**.

Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)	[NTP], AUC_{0-2h}[c] (μM × h)
1	7.6	>100	16 ± 8
9 F.E.	>20	>100	23 ± 3
9 S.E.	4	>100	6 ± 1

[a] In 10% fetal cow serum (FCS).
[b] Concentration of prodrug reducing the cell viability by 50%.
[c] Intracellular 2′-*C*-methylcytidine triphosphate (NTP) after incubation at 10 μM with cryopreserved human hepatocytes; all experiments were conducted in duplicate; LLOQ = 2 μM.

a more upfield ^{31}P shift, whereas the more lipophilic slow eluting diastereomer was assigned the *S*-configuration with a more downfield ^{31}P shift) can be observed for open chain naphthyl phosphoramidate derivatives of BVdU (brivudin) [14].

2.2. Pharmacology

Compounds **9** were tested on the cell-based subgenomic replicon assay [15] against compound **1** as the benchmark. Compound **9 F.E.** was essentially inactive, whereas **9 S.E.** showed a 2-fold improvement with respect to compound **1**. No cytotoxicity was observed up to 100 μM concentration (Table 1). To evaluate if cyclic phosphoramidates can be converted to the active 2′-*C*-methylcytidineTP **6**, we incubated them with cryopreserved human hepatocytes for 2 h. Interestingly, the inactive diastereomer (as judged by replicon activity) **9 F.E.** was shown to produce an equal if not higher amount of NTP with respect to **1**, while the more active **9 S.E.** produced less nucleoside triphosphate in 2 h time frame of this assay. These results show that cyclic phosphoramidates can be converted to NTP **6**, and thus are valid structures for a kinase-bypass approach. We speculate that the difference between the low replicon activity and the reasonable levels of NTP observed in human hepatocytes might be due to the presence of enzymes in human hepatocytes, which are not expressed in the HUH-7 replicon [15] cells.

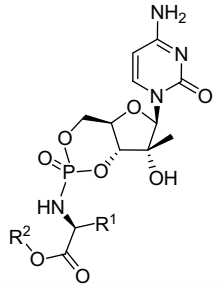
We explored the SAR of cyclic phosphoramidates, scanning a variety of structural motifs on the amino acid portion by changing ester as well as amino acid functionality according to Schemes 1 and 2. When it was not possible to isolate the fast eluting cyclised diastereomer due to the reactivity issue as described above, only the data for the slow eluting diastereomer has been reported. We routinely measured replicon activity, but were more interested in the NTP levels in hepatocytes, which were determined at 2 h in screening mode [16].

From the SAR studies several observations can be made. First, none of the cyclic compounds gave good activity in the replicon assay, indicating that the structural changes do not lead to efficient turnover. There is also no correlation between the activity in the replicon assay and levels of NTP **6** in human hepatocytes. The levels of NTP in the hepatocytes depend on the structure of the amino ester. The extension of the ester group to more lipophilic groups yielded compounds that gave higher levels of the corresponding triphosphate (**9 F.E.** versus **12 S.E.**). A *tert*-butyl ester is also processed (**11 S.E.**).

Cyclic esters are tolerated and seem to follow the trend as described above: more lipophilic esters yield higher levels of **6** (**13**–**15 S.E.**). The introduction of an oxygen into the ester side chain has a positive effect in terms of NTP measured (compound **17 S.E.**) whereas the substitution with branched esters did not have any large consequence on NTP formation (**18 F.E.**–**20 S.E.**).

Having established general trends in terms of ester properties we decided to alter the amino acid functionality, leaving the ethyl-ester intact, again taking **9 F.E.** as our reference compound.

Table 2
Antiviral activity and conversion to NTP **6** in human hepatocytes of compounds **9**–**23**.



Compound[a]	R^1/R^2	EC_{50}[b] (μM)	[NTP], AUC_{0-2h}[c] (μM × h)
9 F.E.	Me/Et	>20	23 ± 3
9 S.E.	Me/Et	4	6 ± 1
10 F.E.	Me/*n*-Bu	>20	5 ± 0
10 S.E.	Me/*n*-Bu	15	23 ± 1
11 S.E.	Me/*t*-Bu	>20	38 ± 3
12 F.E.	Me/*n*-heptyl	15	21 ± 5
12 S.E.	Me/*n*-heptyl	10.5	113 ± 53
13 (**1**:**3**)[d]	Me/*c*-pentyl	>20	2 ± 1
14 F.E.	Me/*c*-hexyl	>20	25 ± 2
14 S.E.	Me/*c*-hexyl	>20	3 ± 1
15 S.E.	Me/*c*-heptyl	>10	94 ± 27
16 F.E.	Me/3-methoxypropyl	>20	12 ± 7
16 S.E.	Me/3-methoxypropyl	28	1 ± 1
17 S.E.	Me/2-(hexyloxy)-ethyl	14	94 ± 14
18 F.E.	Me/3-methyl-butyl	>20	18 ± 2
18 S.E.	Me/3-methyl-butyl	>20	4 ± 1
19 S.E.	Me/2-ethyl-butyl	>20	3 ± 0
20 F.E.	Me/2-propyl-pentyl	>20	20 ± 7
20 S.E.	Me/2-propyl-pentyl	14	34 ± 13
21 S.E.	Et/Et	5.6	15 ± 1
22 S.E.	2-Methyl-propyl/Et	>50	BLQ
23 (**1**:**1.4**)[d]	*n*-Propyl/Et	>20	3 ± 0

[a] **F.E.**: fast eluting diastereomer; **S.E.**: slow eluting diastereomer.
[b] Concentration of prodrug reducing the cell viability by 50%.
[c] Intracellular 2′-*C*-methylcytidine triphosphate (NTP) after incubation at 10 μM with cryopreserved human hepatocytes; all experiments were conducted in duplicate; LLOQ = 2 μM.
[d] **F.E.**:**S.E.**.

Interestingly the ABU-amino acid derivative **21 S.E.** showed comparable efficiency in terms of triphosphate formation as compared to parent **9 F.E.** with better cell-based activity in the replicon assay. Further increase of steric incumbrance, however, had a detrimental effect on in vitro triphosphate formation (**22 S.E.** and **23**).

The interpretation of the data is complicated by the different behaviours of the pairs of diastereomers. There does not seem to be a preference in terms of diastereomer (fast or slow eluting) with respect to triphosphate formation. The observed SAR could reflect two trends: ease of cell penetration of the cyclic phosphoramidates and/or their subsequent processing to monophosphate **5**. Generally, more lipophilic esters should give compounds with better cell permeability and hence a higher concentration in cells that is

Table 3
Plasma stability of compounds **21 S.E.** and **19 S.E.** in different species with corresponding in vivo measurement of NTP **6**.

Compound	Human	Dog	Rat	Hamster	[NTP][a] (nmol/g)
21 S.E.	++	++	−−	++	<0.2
19 S.E.	−	++	−−	+−	0.32

++: stable; +−: 80% of the dose after 2 h; −: 30% of the dose after 1 h; −−: 0% of the dose after 18 min.
[a] NTP level after P.O. dose (30.3 μmol of compound in PEG; LLOQ = 0.2 nmol/g) in hamster liver.

Table 4
In vivo formation of NTP **6** in hamster liver.

Compound	[NTP][a] (nmol/g)
1	<0.2
12 S.E.	5.9
15 S.E.	10.3
17 S.E.	2.6
19 S.E.	8.9
20 S.E.	6.8

[a] NTP level after subcutaneous dose (1.5 μmol of compound in PEG; LLOQ = 0.2 nmol/g) in hamster live at 6 h.

processed to **5**. Inside the cells it is not known how these cyclic structures are converted to monophosphate **5**, and assumptions about the SAR versus hydrolytic enzyme(s) are purely speculative.

To see if these compounds could also deliver NTP in vivo, we needed to select a small animal model. Unfortunately, these compounds are not stable in the rat plasma, as the examples in Table 3 show. Much better stability was observed in hamster, which was used for the in vivo experiments. Good stability was also observed in human and dog plasma.

From the experimentally established species-dependent stability of the cyclic prodrugs in plasma, we decided to perform in vivo p.o. experiments in hamster. Neither **21 S.E.** nor **19 S.E.**, however, yielded satisfactory exposure levels (Table 3), as measured by the presence of **6** in hamster livers after 6 h [17]. The reason could be poor absorption and/or instability of the prodrugs in the gastrointestinal tract. To circumvent these problems we dosed a range of different prodrugs subcutaneously, to measure triphosphate formation of injected compounds (Table 4).

All cyclic phosphoramidate prodrugs do show better triphosphate formation in vivo as compared to benchmark compound **1**. It is interesting to note that the in vitro results measured by using human hepatocyte (Table 2) do not track with the good conversion to active species **6** in hamster (**15 S.E.**: $NTP_{Hum.Hep.}$ AUCO-2h 94 μM × h; $NTP_{Hamster}$: 10.3 nmol/g and **17 S.E.**: $NTP_{Hum.Hep.}$ AUCO-2h 94 μM × h; $NTP_{Hamster}$: 2.6 nmol/g).

From Table 4 we can deduce that all administered compounds show much higher NTP formation in vivo as compared to benchmark compound **1**, indeed, both **15 S.E.** and **19 S.E.** display exceptional efficiency to form **6** after subcutaneous administration in a preclinical species.

3. Conclusions

We have reported a new class of cyclic phosphoramidate prodrugs, that is only weakly active in the cell-based replicon assay but is converted efficiently to active species **6** in situ in cryopreserved human hepatocytes. A brief assessment of in vivo behaviour of our prodrugs, choosing hamster as experimental species for p.o. administration, based on plasma-stability studies, indicates the need for further structural modifications in order to obtain a more favourable PK profile upon oral dosing. We could, however, show that subcutaneous administration of this novel class of prodrugs can form appreciable levels of **6** in vivo, depending on the type of ester employed.

4. Experimental section

4.1. Chemistry

4.1.1. General

^{1}H NMR spectra were obtained on a Bruker 300 MHz spectrometer at 300 K. MS analyses were performed on a Waters micromass ZQ that was equipped with a Waters Acquity UPLC system. The solvent system used was MeCN/H_2O with 0.1% formic acid. All reagents were obtained from commercial suppliers. All animal procedures were done in accordance with guidelines from the Institutional Animal Care and Use Committee at Merck.

4.1.2. *Ethyl* N-*[chloro(4-chlorophenoxy)phosphoryl]-L-alaninate* ***7a*** *($R^1 = Me$, $R^2 = Et$, $R^3 = Cl$)*

A solution of L-alanine ethyl ester (5 g, 32.6 mmol) in DCM (180 ml) was treated at room temperature with 4-chlorophenyl dichlorophosphate (5.30 ml, 32.6 mmol). The resulting mixture was cooled down to −78 °C and treated dropwise with triethylamine (9.07 ml, 65.2 mmol). The reaction was allowed to reach room temperature overnight. Solvent was evaporated in vacuo under a constant stream of dry nitrogen and the resulting yellow solid residue was redissolved in anhydrous diethyl ether (30 ml). The supernatant was carefully removed with a syringe, and filtered under nitrogen into a preweighed flask fitted with a rubber seal. All volatiles of this colorless solution were removed in vacuo, making sure that no moisture could enter into the system, to furnish the title compound **7a** as a colorless oil (10.5 g, 99%) as a 1:1 mixture of diastereomers and was used without further purification. ^{31}P NMR ($CDCl_3$): 9.43 and 9.11 ppm.

4.1.3. *Ethyl (2S)-2-{[{[(2R,3R,4R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl]methoxy}(4-chlorophenoxy) phosphoryl]amino}propanoate: (**8 F.E.** and **8 S.E.**)*

A solution of 2′-methylcytidine (4.0 g, 15.55 mmol; previously triturated and then concentrated in vacuo with toluene) in THF (160 ml) was treated at −78 °C with *tert*-butyl magnesium chloride (34.2 ml, 34.2 mmol, as 1 M solution in THF). The mixture was stirred at −78 °C for 10 min and then at 0 °C for 1 h. The reaction was again cooled down to −78 °C and treated dropwise with freshly prepared ethyl *N*-[chloro(4-chlorophenoxy)phosphoryl]-L-alaninate solution (31.1 ml, 1 M in THF). The mixture was allowed to go to rt and stirred at that temperature overnight. The resulting suspension was quenched by the addition of water at 0 °C, and extracted with EtOAc. The combined organic phases were washed with brine. All volatiles were removed in vacuo and the crude product was purified by column chromatography (eluent DCM:MeOH from 98:2 to 90:10) to yield 659 mg of compound. This compound was redissolved in a minimum amount of DMSO and purified by RP-HPLC using a Thermoquest Preparative HPLC, Phenomenex Luna 5 μm $C_{18}(2)$ 250 × 21.10 mm reverse phase column, eluent: H_2O (containing 5 mM ammonium bicarbonate)/MeCN. The fractions containing the pure compounds were combined and freeze dried to afford the title compounds as a white powder: 1.27 g of **8 F.E.** (15% yield) and 2.46 g of **8 S.E.** (19% yield). **8 F.E.**: ^{1}H NMR (CD_3OD) δ 8.03 (d, $J = 7.74$ Hz, 1H), 7.47–7.36 (m, 2H), 7.35–7.20 (m, 2H), 6.12–6.04 (m, 1H), 6.02 (s, 1H), 4.67–4.53 (m, 1H), 4.53–4.37 (m, 1H), 4.25–4.11 (m, 3H), 4.06–3.93 (m, 1H), 3.80 (d, $J = 4.22$ Hz, 1H), 1.44–1.35 (m, 3H), 1.32–1.24 (m, 3H), 1.21 (m, 3H); ^{31}P NMR: (DMSO-d_6) δ 3.77; MS (ES^+) m/z 547 $(M + H)^+$. **8 S.E.**: ^{1}H NMR (CD_3OD) δ 8.01 (d, $J = 7.74$ Hz, 1H), 7.43 (d, $J = 8.85$ Hz, 2H), 7.31 (d, $J = 8.85$ Hz, 2H), 6.06 (d, $J = 7.74$ Hz, 1H), 6.02 (s, 1H), 4.58 (ddd, $J = 11.89$ Hz, $J = 6.25$ Hz, $J = 1.82$ Hz, 1H), 4.47–4.38 (m, 1H), 4.24–4.12 (m, 3H), 4.06–3.92 (m, 1H), 3.81 (d, $J = 9.28$ Hz, 1H), 1.41 (d, $J = 6.85$ Hz, 3H), 1.27 (t, $J = 7.18$ Hz, 3H), 1.21 (s, 3H); ^{31}P NMR (DMSO-d_6) δ 3.92; MS (ES^+) m/z 547 $(M + H)^+$.

4.1.4. *Ethyl* N-*[(2R,4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl]-L-alaninate (**9 F.E.**)*

A solution of **8 S.E.** (27.9 mg, 0.051 mmol) in DMSO (2 ml) was treated at room temperature with potassium *tert*-butoxide (5.72 mg, 0.051 mmol) and the resulting mixture was stirred for

30 min, when the mixture was cooled to 0 °C and treated dropwise with 1 N HCl to bring the pH to 6. Purification by Thermoquest Preparative HPLC, using a Symmetry C_{18}, 19 × 300 mm reverse phase column, eluent: H_2O + 0.1%TFA/MeCN + 0.1%TFA. Fractions containing the pure compound were combined and freeze dried to afford the title compound **9 F.E.** in 35% yield. ^{1}H NMR (CD_3OD) δ 7.90 (d, J = 7.74 Hz, 1H), 6.19–6.08 (m, 2H), 4.70–4.51 (m, 2H), 4.40–4.29 (m, 1H), 4.23 (q, J = 7.16 Hz, 2H), 4.19–4.09 (m, 1H), 4.03–3.89 (m, 1H), 1.44 (d, J = 7.07 Hz, 3H), 1.32 (t, J = 6.97 Hz, 3H), 1.30 (s, 3H); ^{31}P NMR: (DMSO-d_6) δ 3.33 ppm; MS (ES^+) m/z 419 $(M + H)^+$.

4.1.5. Ethyl N-*[(2S,4a*R,6R,7R,*7a*R)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl]-L-alaninate (**9 S.E.**)*

A solution of **8 F.E.** (44.6 mg, 0.082 mmol) in DMSO (2 ml) was treated at room temperature with potassium *tert*-butoxide (9.15 mg, 0.082 mmol) and the resulting mixture stirred for 30 min, then the mixture was cooled to 0 °C and treated dropwise with 1 N HCl to bring the pH to 6. Purification by Thermoquest Preparative HPLC, using a Symmetry C_{18}, 19 × 300 mm reverse phase column, eluent: H_2O + 0.1%TFA/MeCN + 0.1%TFA. Fractions containing the pure compound were combined and freeze dried to afford the title compound **9 S.E.** (23 mg, 67%). ^{1}H NMR (CD_3OD) δ 7.89 (d, J = 7.83 Hz, 1H), 6.19–6.09 (m, 2H), 4.72–4.56 (m, 2H), 4.40–4.29 (m, 1H), 4.23 (q, J = 7.15 Hz, 2H), 4.19–4.12 (m, 1H), 4.03–3.92 (m, 1H), 1.44 (d, J = 7.07 Hz, 3H), 1.32 (t, J = 7.07 Hz, 3H), 1.30 (s, 3H); ^{31}P NMR: (DMSO-d_6) δ 5.39 ppm; MS (ES^+) m/z 419 $(M + H)^+$.

References

[1] M.J. Alter, D. Kruszon-Moran, O.V. Nainan, G.M. McQuillan, F. Gao, L.A. Moyer, R.A. Kaslow, H.S. Margolis, N. Engl. J. Med. 341 (1999) 556–562.

[2] (a) U. Koch, F. Narjes, Curr. Top. Med. Chem. 7 (2007) 1302–1329;
(b) T. Wong, S.S. Lee, Can. Med. Assoc. J. 174 (2006) 649–659;
(c) R. De Francesco, A. Carfi, Adv. Drug Deliv. Rev. 59 (2007) 1242–1262;
(d) N. Yao, T. Hesson, M. Cable, Z. Hong, A.D. Kwong, H.V. Le, P.C. Weber, Nat. Struct. Biol. 4 (6) (1997) 463–467.

[3] (a) I. Lefebvre, C. Périgaud, A. Pompon, A.-M. Aubertin, J.-L. Girardet, A. Kirn, G. Gosselin, J.L. Imbach, J. Med. Chem. 38 (1995) 3941–3950;
(b) C. Meier, Angew. Chem. Int. Ed. Engl. 35 (1996) 70–72;
(c) M.D. Erion, D.A. Bullough, C.-C. Lin, Z. Hong, Curr. Opin. Investig. Drugs 7 (2) (2006) 109–117;
(d) C. McGuigan, D. Cahard, H.M. Sheeka, E. De Clercq, J. Balzarini, J. Med. Chem. 39 (1996) 1748–1753.

[4] C. Pierra, A. Amador, S. Benzaria, E. Cretton-Scott, M. D'Amours, J. Mao, S. Mathieu, A. Moussa, E.G. Bridges, D.N. Standring, J.-P. Sommadossi, R. Storer, G. Gosselin, J. Med. Chem. 49 (2006) 6614–6620.

[5] (a) C. McGuigan, S.A. Harris, S.M. Daluge, K.S. Gudmundsson, E.W. McLean, T.C. Burnette, H. Marr, R. Hazen, L.D. Condreay, L. Johnson, E. De Clercq, J. Balzarini, J. Med. Chem. 48 (2005) 3504–3515;
(b) C. Gardelli, B. Attenni, M. Donghi, M. Meppen, B. Pacini, S. Harper, A. Di Marco, F. Fiore, C. Giuliano, V. Pucci, R. Laufer, N. Gennari, I. Marcucci, J.F. Leone, D.B. Olsen, M. MacCoss, M. Rowley, F. Narjes, J. Med. Chem., in press. (c) M. Donghi, B. Attenni, C. Gardelli, A. Di Marco, F. Fiore, C. Giuliano, R. Laufer, J.F. Leone, V. Pucci, M. Rowley, F. Narjes, Bioorg. Med. Chem. Lett. 19 (2009) 1392–1395.

[6] (a) S.S. Carroll, D.B. Olsen, Infect. Disord. Drug Targets 6 (2006) 17–29;
(b) E. Murakami, H. Bao, M. Ramesh, T.R. McBrayer, T. Whitaker, H.M. Micolochik Steyer, R.F. Schinazi, L.J. Stuyver, A. Obikhod, M.J. Otto, P.A. Furman, Antimicrob. Agents Chemother. 51 (2) (2007) 503–509.

[7] (a) W.Y. Sun, A. Zhou, L.I. Wiebe, E.E. Knaus, Nucleosides Nucleotides Nucleic Acids 22 (12) (2003) 2121–2132;
(b) J.P. Krise, V.J. Stella, Adv. Drug Deliv. Rev. 19 (1996) 287–310;
(c) C. Schultz, Bioorg. Med. Chem. 11 (2003) 885–898.

[8] E. Gunic, J.-L. Girardet, K. Ramasamy, V. Stoisavljevic-Petkov, S. Chow, L.-T. Yeh, R.K. Hamatake, A. Raney, Z. Hong, Bioorg. Med. Chem. Lett. 17 (9) (2007) 2452–2455.

[9] (a) G. Schüürmann, A.O. Aptula, R. Kühne, R.-U. Ebert, Chem. Res. Toxicol. 16 (2003) 974–987;
(b) T. Sawahata, R.A. Neal, Mol. Pharmacol. 23 (1983) 453–460.

[10] C. McGuigan, J.-C. Thiery, F. Daverio, W.G. Jiang, D. Davies, M. Mason, Bioorg. Med. Chem. Lett. 13 (2005) 3219–3227.

[11] M. Uchiyama, Y. Aso, R. Noyori, Y. Hayakawa, J. Org. Chem. 58 (1993) 373–379.

[12] The internuclear distances were measured using a Bruker 600 MHz NMR spectrometer by NOESY experiments. A conformational analysis was performed using the Maestro suite and the MMFF potential. This analysis revealed that in the *R*-configuration at the phosphorus the nitrogen assumes an axial orientation whereas in the *S*-configuration it assumes an equatorial orientation. Comparison with NOESY data showed **9 F.E.** to have the *R*-configuration and **9 S.E.** the *S*-configuration.

[13] For all available pairs of cyclic phosphoramidates, the fast eluting diastereomer appears more upfield than the slow, more lipophilic diastereomer in the ^{31}P NMR.

[14] C. Congiatu, A. Brancale, M.D. Mason, W.G. Jiang, C. McGuigan, J. Med. Chem. 49 (2006) 452–455.

[15] V. Lohmann, F. Korner, J.O. Koch, U. Herian, L. Theilmann, R. Bartenschlager, Science 285 (1999) 110–113.

[16] (a) Meppen Malte, Narjes Frank, Pacini Barbara, Gardelli Cristina, Durette Philippe L, WO 2008079206.
(b) The data obtained from the use of cryopreserved human hepatocytes shows variability with batches.

[17] V. Pucci, C. Giuliano, R. Zhang, K.A. Koeplinger, J.F. Leone, E. Monteagudo, F. Bonelli, J. Sep. Sci. 32 (2009) 1275–1283.