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# Synthesis and antiviral properties of novel 7-heterocyclic substituted 7-deaza-adenine nucleoside inhibitors of Hepatitis C NS5B polymerase

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ABSTRACT

Previous investigations in our laboratories resulted in the discovery of a novel series of potent nucleoside inhibitors of Hepatitis C virus (HCV) NS5B polymerase bearing tetracyclic 7-substituted 7-deaza-adenine nucleobases. The planarity of such modified systems was suggested to play a role in the high inhibitory potency observed. This paper describes how we envisaged to maintain the desired planarity of the modified nucleobase by means of an intra-molecular H-bond, engaging a H-bond donor atom on an appropriately substituted 7-heterocyclic residue with the adjacent amino group of the nucleobase. The success of this strategy is reflected by the identification of several novel potent nucleoside inhibitors of HCV NS5B bearing a 7-heterocyclic substituted 7-deaza-adenine nucleobase. Amongst these, the 1,2,4-oxadiazole analog **11** showed high antiviral potency against HCV replication in replicon cells and efficient conversion to the corresponding NTP in vivo, with high and sustained levels of NTP measured in rat liver following intravenous and oral administration.

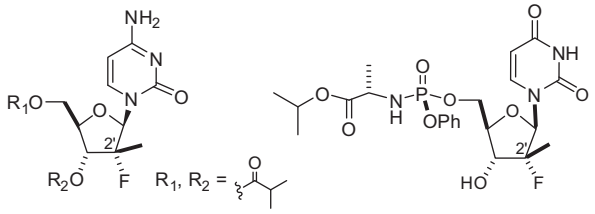


## 1. Introduction

Hepatitis C virus (HCV) infection is a serious epidemic which affects an estimated 170 million people.[1] In the majority of cases, the infection evolves in cirrhosis and ultimately in hepatocellular carcinoma.[2] The standard of care (SOC), a combination PEGylated interferon-α (PEG-IFNα) and ribavirin,[3] is poorly tolerated, requires long treatment periods and has suboptimal efficacy, with only 50% of genotype 1 infected patients achieving sustained virologic response (SVR).[4]

To overcome these issues significant efforts have been devoted to develop direct-acting antiviral (DAA) agents targeting essential virally-encoded non-structural enzymes (NS). The recent approval of the two HCV NS3 protease inhibitors telaprevir[6] and boceprevir[7] in combination with SOC holds great promise for improved efficacy and reduction of treatment time.[5]

A further area of intense research is the viral RNA-dependent RNA polymerase (RdRp) NS5B. HCV is a 9.6 kb positive single strand RNA virus that replicates mainly in the liver.[8] NS5B polymerase is essential to the replication process, producing first strand copy of the RNA genome and secondly catalyzing the synthesis of the positive strand RNA copies of the progeny virus.[9] Given its pivotal role in the viral life cycle, NS5B has been long recognized as a key target for antiviral intervention, and numerous inhibitors of NS5B have progressed to clinical studies, both in the nucleoside (NI) and non-nucleoside (NNI) classes.[10]




mericitabine, RG-7128 (**1**) PSI-7977 (**2**)

**Figure 1.** HCV nucleoside inhibitors in clinical trials.

NIs are at the heart of antiviral treatment for a variety of viral infections,[11] and the success of this class of compounds has provided a compelling rational for the discovery efforts towards nucleoside analogs to treat HCV infection. The significant challenges associated with the development of a NI for HCV are counterbalanced by the advantage offered by these agents.[12] In fact NIs target the highly conserved RdRp active site, being therefore pan-genotype inhibitors and offering a higher genetic barrier to resistance compared with NS3 inhibitors and NS5B non-nucleoside agents, a key feature for the success of any antiviral therapy.[12,13] Several nucleoside analogs have demonstrated significant efficacy in HCV infected patients and are currently in late stage clinical trials,

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including mericitabine (RG-7128, Fig. 1)[14] and the monophosphate prodrug PSI-7977[15] (**2**, Fig. 1).

To be efficacious as antiviral agents, NIs need to be converted into the corresponding nucleoside triphosphate (NTP) via a stepwise process catalyzed by cellular kinases.[12] Typically the NTP is then recognized by the RdRp and incorporated within the growing RNA chain where it acts as a chain terminator, preventing the addition of further nucleotides by means of a structural modification within the ribose unit.[16] Nucleoside analogs bearing a methyl group in the 2′-C-position of the ribose have long being recognized as potent and specific inhibitors of HCV NS5B.[17a,b] Amongst these, the 7-deaza-2′-C-methyladenosine analogue MK-0608 (**3**, Fig. 2)[18a] showed remarkably high antiviral potency in vitro (inhibition of RNA replication in a subgenomic replicon assay $EC_{50}$ = 0.3 μM) and achieved robust reduction of viral load in HCV infected chimpanzees ($\geqslant$3 log) with no emergency of resistant viral strain.[18b]

These findings prompted extensive investigations to identify novel analogs of MK-0608[19] and research in our laboratories resulted in the discovery of a novel series of potent 7-deaza-adenine-2′-C-methyladenosine analogues bearing a tetracyclic nucleobase (**4**, Fig. 2),[20] wherein the planarity of the modified nucleobase was suggested to play a key role in achieving high levels of intrinsic potency. This paper describes how we capitalized on these findings and envisaged an intra-molecular H-bond within a series of appropriately substituted 7-hetrocyclic-7-deaza-adenine analogs as an alternative way to achieve a similar planar arrangement (**5**, Fig. 2). Synthesis and in vitro antiviral properties of nucleosides belonging to this novel series will be described, as well as in vivo characterization of the most promising analogs. The high levels of selectivity and antiviral potency observed in vitro, combined with efficient and sustained conversion to NTP in rat liver, offer promise for future development of selected analogs within this series of novel NIs of HCV NS5B.

## 2. Synthesis

Investigation of 7-heterocyclic-7-deaza-adenine analogs started initially in the context of the 2′-C-methyl-2′-hydroxyribose series, and was further expanded to another widely represented class of 2′-C nucleoside inhibitors, the 2′-C-methyl-2′-deoxy-2′-fluoro ribose analogs.[14,15] Every novel nucleoside analog was also converted into the corresponding NTP, according to the efficient synthesis and purification platform we recently disclosed.[20] Selected compounds within the latter series were converted into a corresponding phosphoramidate monophosphate prodrug.[21]

### 2.1. Synthesis of 2′-C-methyl-ribose nucleoside analogs

Central to the synthesis of several 7-heterocyclic analogs was the versatile advanced iodinated intermediate **6** (Scheme 1), available in multi-gram amounts through a convergent synthetic route starting from 7-deaza-6-chloro-adenine and 3′,5′-bisbenzoyl-2′-C-methyl-ribose.[20] With practical amounts of **6** in hand, the pyrazole derivatives **7a** and **7b** were obtained in good yields (55–65%) via Suzuki coupling with the required boronic acids,[22a] while Stille cross coupling was employed to access pyrimidine **7c** and oxazole **7d**, albeit in more modest yields (11–19%).[22b] Cross-coupling of iodide **6** with trimethylsilyl acetylene in Sonogashira conditions followed by treatment with ammonium hydroxyde in methanol afforded alkyne **8** in 92%.[23] The latter was in turn reacted with trimethylsilylmethyl azide in the presence of in situ generated Cu(I) en route to the 1,2,3-triazole analogue **9**. Finally, intermediate **6** was reacted with zinc cyanide via a microwave promoted Pd(0) cross coupling to give the nitrile derivative **10** in 80%. The latter was converted to the 1,2,4-oxadiazole **11** in a 3-step sequence involving formation of the corresponding amideoxime, treatment of the latter with triethylorthoformate and deprotection of the resulting bis-ethoxy derivative with $BBr_3$. All the above described 7-heterocyclic analogs were synthesized in an efficient manner through convergent synthetic routes which completely avoided the use of protecting group manipulations, despite the highly functionalized nature of the nucleoside intermediates employed. [24]

Guided by the initial biological data on the analogs within this series, we proceeded to investigate further oxadiazole derivatives focusing on isomeric analogs of compound **11** (Scheme 2). Acid hydrolysis of the nitrile derivative **10** followed by acetylation of the ribose hydroxyl groups gave in 90% yields the carboxylic acid intermediate **12**. The latter was progressed to the 1,3,5-oxadiazole analog **13** by coupling with methyl amideoxime followed by cyclization and removal of the acetyl group. Notably, the same synthetic approach failed to produce the unsubstituted 1,3,5-oxadiazole analog. Finally, the 1,3,4-oxadiazole isomer **15** was prepared by coupling of the carboxylic acid intermediate **12** with Boc-hydrazide, followed by Boc-deprotection and cyclization with triethylorthoformate.

### 2.2. Synthesis of 2′-C-methyl-2′-deoxy-2′-F-ribose nucleoside analogs

Selected 7-heterocyclic substitutions were also investigated in the context of the 2′-C-methyl-2′-deoxy-2′-fluoro ribose series (Scheme 3). The required 2′-deoxy-2′-fluoro 7-iodinated intermediate **19** was prepared through a 3-step synthetic sequence starting from the fully protected ribose building block **16**.[25] Following selective 1-*O*-deacetylation with tributyltin methoxide,[26] nucleoside intermediate **18** was obtained in practical yields as a 1:1 mixture of α and β anomers following a glycosidic coupling in Mitsunobu conditions.[27] A slow gradient silica gel chromatographic separation of the two anomers and subsequent removal of the benzoate protecting groups afforded the required 7-iodo-2′-fluoro building block **19**. The latter was in turn converted to

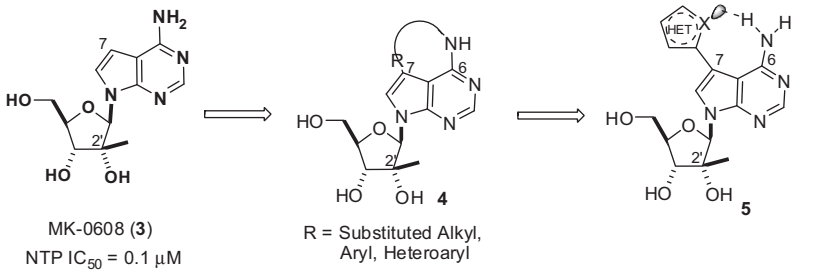
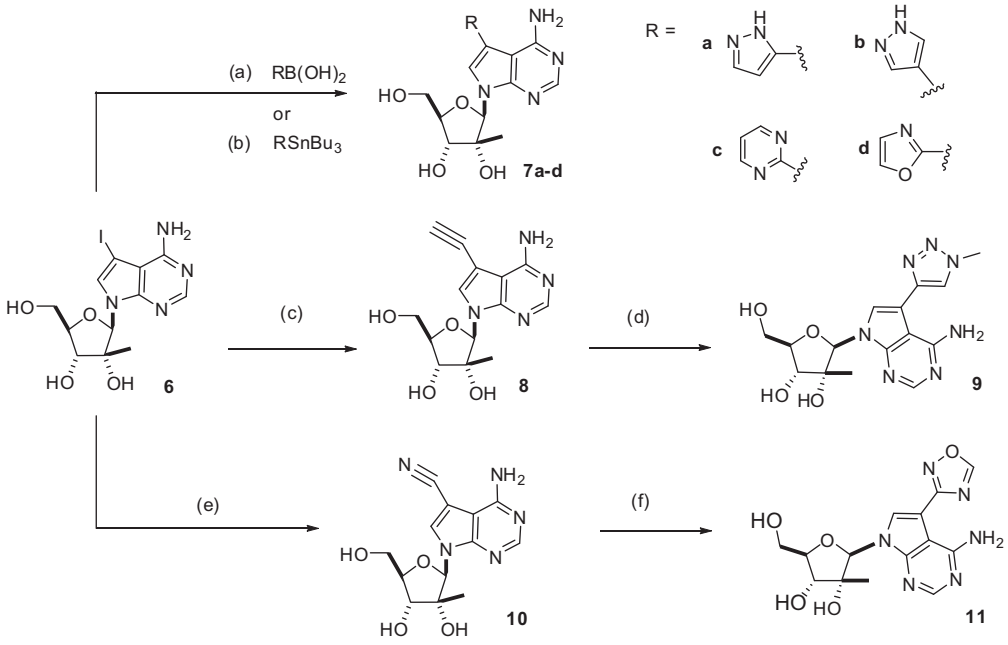
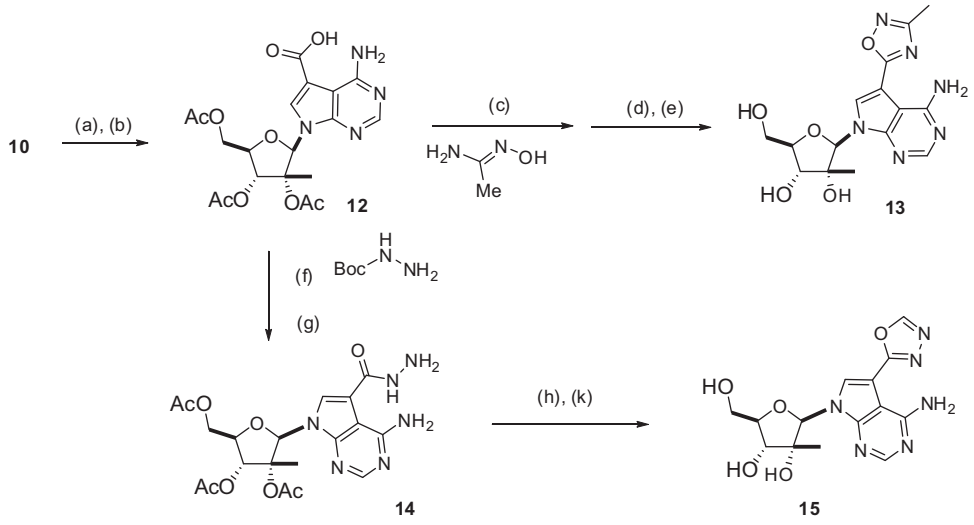



**Figure 2.** 7-Deaza-adenine analogs.




**Scheme 1.** Reaction conditions: (a) $Pd(PPh_3)_4$, $Na_2CO_3$, $H_2O$/dioxane, 100 °C, 55–65%; (b) $PdCl_2(PPh_3)_2$, 120 °C, DMF, microwave irradiation, 11–19%; (c) (i) TMS-acetylene, $Pd(PPh_3)_4$, $Et_3N$, CuI, DMF/THF; (ii) $NH_4OH$/MeOH; 92% over 2 steps; (d) (i)$TMSCH_2N_3$, $CuSO_4.5H_2O$, ascorbate, $^tBuOH$, $H_2O$, RT to 50 °C; (ii) 1 M aq NaOH/MeOH, 50 °C; (e) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 150 °C, microwave irradiation, 80%; (f) (i) $NH_2OH.HCl$, EtOH, 50 °C; (ii) $CH(OEt)_3$, $BF_3{\cdot}Et_2O$, 100 °C; (iii) $BBr_3$, DCM, 0 °C to RT; 25% over 3 steps.

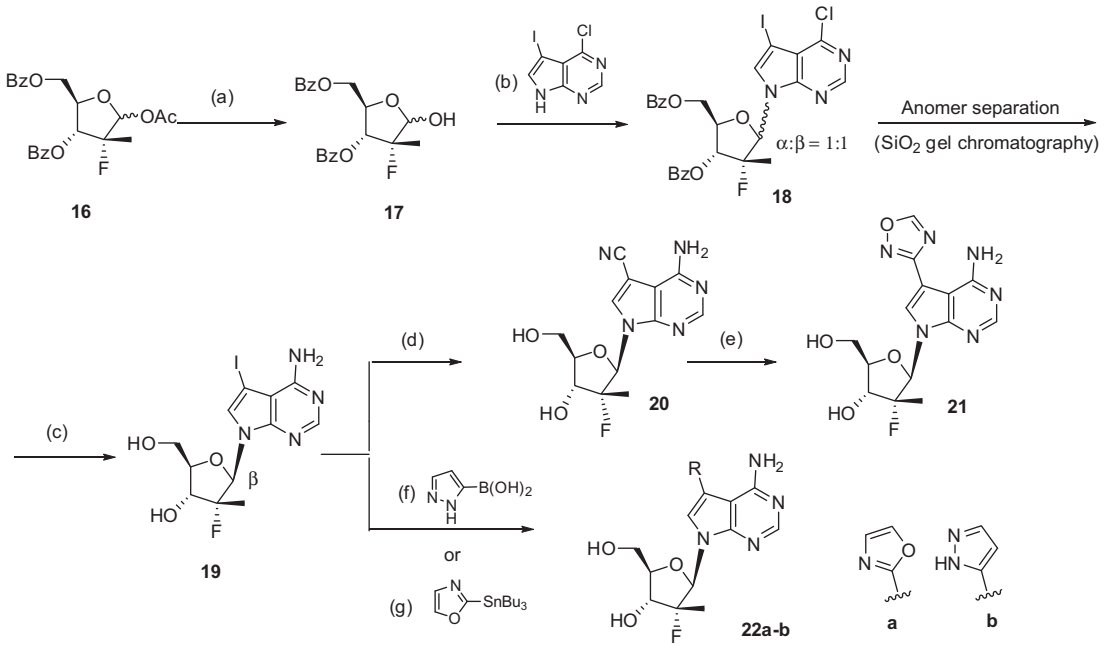



**Scheme 2.** Reagents and conditions: (a) 1.3 M aq HCl, 110 °C; (b) AcCl, AcOH; 96% over 2 steps; (c) TBTU, HOBt, $^iPr_2EtN$, DMF; (d) DMF, 140 °C; (e) 1 M NaOH, MeOH; 35% over 3 steps; (f) DCI, HOBt, NMM, THF, 0 °C to RT; (g) HCl/dioxane; (h) $HC(OEt)_3$, $BF_3{\cdot}Et_2O$, DMF, 70 °C; (k) 4 M HCl in dioxane, then 6 M aq $NH_4OH$; 13% over 4 steps.

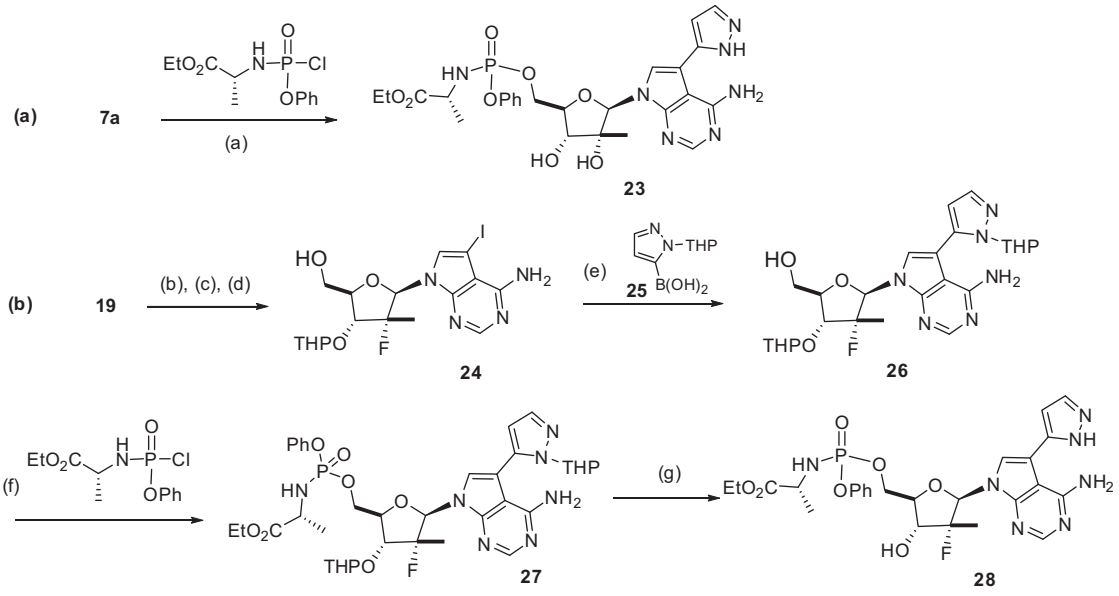
the 1,2,4-oxadiazole **21** and to the oxazole and pyrazole derivatives **22a** and **22b** with the similar synthetic protocols to those already described for the corresponding ribose analogs.

### 2.3. Synthesis of 2′-C-methyl-ribose and 2′-C-methyl-2′-deoxy-2′-F-ribose monophosphate prodrugs

Selected nucleoside analogs which combined very high levels of intrinsic potency against HCV NS5B with modest level of cellular antiviral potency, were converted into the corresponding monophosphate prodrugs.[21] The aim of this investigation was to assess if a by-pass of the often rate-determining first phosphorylation step could improve the efficiency of conversion to the corresponding NTP and hence the cellular antiviral potency. Synthesis of two representatives of monophosphate prodrugs belonging to the 2′-C-methyl-ribose and to the 2′-C-methyl-2′-deoxy-2′-fluorine structural classes are reported in Scheme 4a and b, respectively. Conversion of the pyrazole derivative **7a** into the phosphoramidate prodrug **23** was achieved in a straightforward manner by chemoselective *O*-phosphorylation of the unprotected nucleoside according to the Uchiyama protocol (Scheme 4a).[28] In contrast, synthesis of the 2′-deoxy-2′-fluoro analog 28 (Scheme 4b) proved more challenging. Following extensive optimization, it was found that protection of the secondary hydroxyl group of **19** was required and that the tetra-hydropyranyl group (THP) was the optimal choice. Suzuki coupling with the THP-protected pyrazole boronic acid **25** gave intermediate **26**, which was in turn *O*-phosphorylated with L-alanine-*N*-chlorophenoxyphosphinyl-ethyl ester and progressed




**Scheme 3.** Reagents and conditions: (a) $Sn(^nBu)_3OMe$, 70%; (b) DIAD, $Ph_3P$, 45%; (c) $NH_3$/MeOH, 40%; (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 150 °C, microwave irradiation, 30%; (e) (i) $NH_2OH.HCl$, EtOH, 50 °C; (ii) $CH(OEt)_3$, $BF_3{\cdot}Et_2O$, 100 °C; (iii) $BBr_3$, DCM, 0 °C to RT; 27% over 3 steps; (f) $Pd(PPh_3)_4$, $Na_2CO_3$, $H_2O$/dioxane, 100 °C, 68%; (g) $PdCl_2(PPh_3)_2$, 120 °C, DMF, microwave irradiation, 20%.




**Scheme 4.** Reaction conditions: Reagents and conditions: (a) $^t$BuMgCl, THF; (b) TBDMS-Cl, ImH; (c) 3,4-dihydro-2H-pyran, PTSA; (d) TBAF; 50% over 3 steps; (e) $Pd(PPh_3)_4$, $Na_2CO_3$, $H_2O$/dioxane, 100 °C, 70%; (f) $^t$BuMgCl, THF; (g) $HCO_2H$, 0 °C; 18% over 2 steps.

to prodrug **28** in practical yields after removal of the THP protecting groups.

## 3. Results and discussion

All the 7-heterocyclic nucleoside and nucleoside monophosphate prodrug analogs described above were evaluated for their antiviral potency against the replication of HCV RNA in HBI10A hepatoma cell lines containing a subgenomic HCV replicon.[29] The corresponding NTPs were tested for their inhibitory potency against NS5B polymerase.[30] The most promising candidates from the two inhibitor classes were profiled in rodent species to assess NTP formation in vivo.

### 3.1. Nucleoside analogs: in vitro and in vivo studies

Following our previous findings within the tetracyclic series (**4**, Fig. 2), which suggested that the overall planarity of the modified nucleobases could be instrumental to ensure high level of intrinsic potency,[20] we set out to investigate whether alternative modifications introduced in the 7-position of the 7-deaza-adenine base could maintain the desired planarity as well as the high intrinsic

potency, and potentially offer advantages in terms of improved antiviral cellular potency. Towards this end, we envisaged that with a series of appropriately substituted 7-hetrocyclic-7-deaza-adenine analogs we could engage the 6-amino group into a 7-membered ring intra-molecular H-bond with the 7-heterocyclic substituent, thus achieving the required system planarity (**5**, Fig. 2).

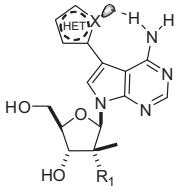
Indeed a head to head comparison between the phenyl analog **29**[20] and the pyrimidine **7c** (Table 1, entries 12 and 3, respectively) suggested that the introduction of a H-bond acceptor atom adjacent to the carbon linked to the 7-position to the 7-deaza-adenine base could prove beneficial to the inhibitory potency, with NTP **32** showing an $IC_{50}$ = 12 μM against NS5B while its phenyl analog **41** was devoid of activity (NTP $IC_{50}$ >100 μM).

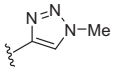
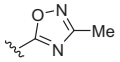
A much more significant effect was observed when we investigated the 7-substitution with 5-membered heterocycles bearing a H-bond acceptor adjacent to carbon linked to the deaza-adenine base. Indeed, both the oxazole analog **7d** and the 1,2,4-oxadiazole **11** showed significantly improved intrinsic potency, both inhibiting NS5B polymerase with NTP $IC_{50}$ = 0.5 μM (Table 1, entries 4 and 6). Both these analogs also displayed interesting levels of antiviral activity in the replicon assay, with $EC_{50}$ = 7.8 μM for the oxazole **7d** and a remarkable boost in cellular potency for oxadiazole **11**, with $EC_{50}$ = 1.9 μM. This value is comparable to that of past and current HCV NIs in the clinic,[14,15] and suggests an efficient conversion of **11** to the corresponding NTP species in the cellular media.

Encouraged by these results, we focused our efforts on further exploring 5-membered ring heterocyclic substituents, and expanded our investigations to the isomeric pyrazole analogs **7a** and **7b** (Table 1, entries 1 and 2). Analog **7a**, bearing a nitrogen atom adjacent to the carbon linked to the 7-position of the nucleobase, showed excellent levels of potency against HCV NS5B, with $IC_{50}$ = 0.1 μM for its corresponding NTP **30**. In contrast, the isomeric pyrazole **7b**, bearing the nitrogen atoms two positions away from the carbon linked to the 7-position and thus unable to engage the 6-amino group in the 7-membered ring intra-molecular H-bond, showed a dramatic loss in potency with $IC_{50}$ = 8.4 μM for NTP **31**.

These findings corroborated our initial hypothesis, and prompted us to find further evidence to support it. Towards this end, we turned to $^1$H NMR analysis to gain insight on the possible involvement of the protons of the 6-amino group in an

**Table 1**




| Entry | HET | $R_1$ | Compound nucleoside/NTP | HCV NSSB polymerase[b] $IC_{50}$ (μM) | HCV replicon[a] $EC_{50}$ (μM) |
|---|---|---|---|---|---|
| **1** |  | OH | **7a/30** | 0.1 | >100 |
| **2** |  | OH | **7b/31** | 8.4 | 30 |
| **3** |  | OH | **7c/32** | 12 | >100 |
| **4** |  | OH | **7d/33** | 0.5 | 7.8 |
| **5** |  | OH | **9/34** | 8.4 | 42 |
| **6** |  | OH | **11/35** | 0.5 | 1.9 |
| **7** |  | OH | **13/36** | 8.4 | 42 |
| **8** |  | OH | **15/37** | >95 | 46 |
| **9** |  | F | **21/38** | 0.4 | 41 |
| **10** |  | F | **22a/39** | 0.3 | 37 |
| **11** |  | F | **22b/40** | 0.07 | 35 |
| **12** |  | OH | **29/41** | >100 | 56 |

[a] Cellular activity determined in the HCV bicistronic replicon assay, using HBI10A cells stably transfected with genotype **1b** HCV replicon RNA, for details see Ref. 30; $EC_{50}$ are measured in the presence of 10% fetal bovine serum (FBS); $EC_{50}$ values are the mean of ⩾3 experiments, standard deviation was within ±20% of the reported value.

[b] Enzyme potency measured in a NS5B polymerization assay, using an heteromeric RNA template, for details see Ref. 31; $IC_{50}$ values are the mean of ⩾3 experiments, standard deviation was within ±20% of the reported value.

intra-molecular H-bond with an acceptor atom on the 7-substituent (Fig. 3). In fact, in solvents that do not form strong hydrogen bonds, significantly de-shielded chemical shift for NH-protons often indicate their participation in hydrogen bonding.[31a,b] We started our investigations from pyrazole **7b**, unable to engage in the intra-molecular H-bond, and set the chemical shift for its two isochronous $NH_2$ protons at 6.74 ppm in $CD_3CN$ as a reference value (Fig. 3a). When the isomeric pyrazole **7a**, bearing a hydrogen bond acceptor in the position adjacent to the carbon linked to the nucleobase, was analyzed in the same NMR conditions a dramatic downfield shift was observed for the chemical shift of the two $NH_2$-protons, found at 11.20 and 10.90 ppm, respectively (Fig. 3b). Similarly to **7a**, a significant downfield chemical shift was also observed for the 1,2,4-oxadiazole **11** (10.12 and 9.53 ppm, Fig. 3c), suggesting the involvement of 6-$NH_2$ protons in intra-molecular hydrogen-bond with acceptor atoms on the C-7 substituent.

The above findings, combined with the compelling in vitro potency observed for compounds **7d**, **11** and **7a**, prompted us to further investigate analogs within the series. Unexpectedly, both the 1,2,3-triazole derivative **9** and the isomeric oxadiazoles **13** and **15** showed a significant loss in inhibitory potency against NS5B (Table 1, entries 5, 7 and 8, NTP $IC_{50}$ = 8.4, 8.4 and >95 μM, respectively). Interestingly, similar NMR studies to those described above supported the presence of an intra-molecular hydrogen bond also for analogs **9**, **13** and **15** (data not shown), suggesting that the observed suboptimal potency is unlikely to be derived from a lack of planarity of the heterocycle-substituted nucleobase.

Our investigations within the series were next extended to structural variation within the ribose unit. The replacement of the 2′-hydroxyl group with a fluorine atom has been amongst the most successful ribose modifications reported in the nucleoside field, as exemplified by the two furthest advanced nucleoside clinical candidates in the HCV field (**1** and **2**, Fig. 1).[14,15] We were pleased to observe that by combining the 2′-C-methyl-2′-deoxy-2′-fluoro-ribose unit with the most promising 5-membered heterocyclic substituents identified from our previous efforts, we could maintain and even further improve the inhibitory potency against NS5B, as exemplified by oxadiazole **21**, oxazole **22a** and pyrazole **22b**, with NTP $IC_{50}$ = 0.4, 0.3 and, remarkably, 0.07 μM, respectively (Table 1, entries 9–11). Despite the excellent levels of enzymatic potency observed, all these 2′-deoxy-2′-fluorinated analogs showed only marginal levels of antiviral potency in the cellular assay ($EC_{50}$ = 35–41 μM). Noticeably, the 2′-fluoro oxadiazole analog **21** had a significantly reduced replicon potency compared to oxadiazole **11**, its direct analog in the 2′-C-methyl-ribose series, which showed high levels of antiviral potency with $EC_{50}$ = 1.9 μM.

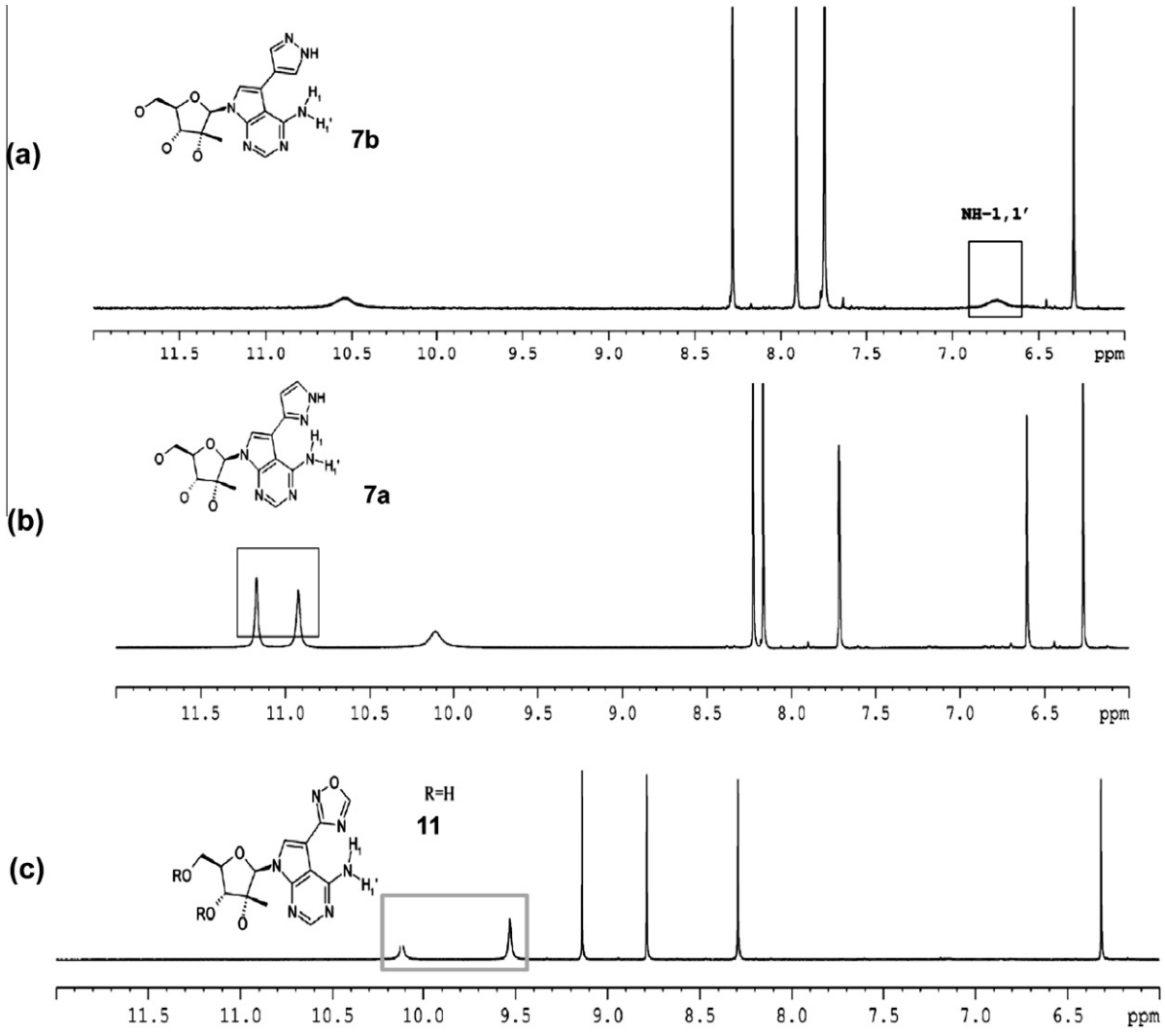



**Figure 3.** $^1H$ NMR experiments to investigate the presence of an intra-molecular H-bond with compounds **7a**, **7b** and **11**. For all the compounds: NMR experiments showed the NH protons chemical shift to be independent of concentration in the range 0.5–5 mM. The reported data were acquired at 1 mM concentration, $T$ = 283 K and acquired in $CD_3CN$ (chemical shift are reported in ppm using the residual proton signal of $CD_3CN$ at 1.94 ppm as internal reference).

Having identified novel and potent nucleoside inhibitors of NS5B, some of which showing also compelling antiviral cellular potency, we proceeded to further evaluate the most promising analogs, such as oxadiazole **11** and the two pyrazole analogs **7a** and **22b**, in the 2′-hydroxy and 2′-deoxy-2′-florine series, respectively. Their in vitro profile was further investigated by assessing the selectivity against the human DNA polymerases α, β, γ. Pleasingly, in all cases, the corresponding NTPs had $IC_{50}$ >10 μM.

We next pursued the initial evaluation of the pharmacokinetic properties of the above key compounds, with particular focus on the efficiency of their conversion to NTP in vivo, either as nucleoside analogs or following their derivatization into the corresponding monophosphate prodrugs as appropriate.

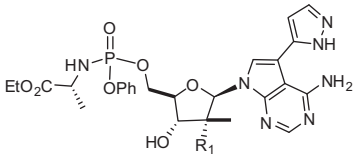
Within the selected nucleoside analogs, oxadiazole **11** appeared as the candidate of choice for further in vivo evaluation due to the high potency of the corresponding NTP combined with the remarkable antiviral activity observed in the cellular assay, suggestive of efficient intracellular conversion to the corresponding NTP ($IC_{50}$ = 0.5 μM, $EC_{50}$ = 1.9 μM, respectively). Initial evaluation of the pharmacokinetic profile of **11** was obtained following administration to Wistar Han (WH) Rats (1 mg/Kg intravenous and orally). Compound **11** showed high plasmatic clearance of the parent nucleoside (>hepatic flow), a volume of distribution of 7.0 L and a moderate bioavailability ($F$ = 14.7%). In a second experiment, **11** was dosed to WH rats intravenously at 20 mg/Kg, and levels of NTP formed in rat liver where measured up to 12 h from dosing.[32] Pleasingly, while the concentration of the parent nucleoside **11** in plasma was decreasing rapidly, in rat liver the corresponding NTP was formed efficiently, with NTP levels of 11.9, 3.9 and 0.5 nmol/g at 3, 6 and 12 h from dosing, respectively. The ratio between the concentration of the NTP in liver and of **11** in plasma was found in the range of 20–25 fold in favor of liver NTP at all timepoints, suggesting both a high hepatic extraction of the nucleoside **11** into the liver, target organ of HCV replication, and an efficient conversion to the NTP species, required for inhibition of NS5B polymerase. Following dosing of **11** at 20 mg/Kg orally, NTP liver levels at 12 h were found to be comparable to those of the intravenous administration (0.4 nmol/g) suggesting that significant levels of NTP in liver could be achieved also following oral administration of **11**. Further characterization of oxadiazole **11** in preclinical species is ongoing and results will be reported in due time.

### 3.2. Nucleoside monophosphate prodrug analogs: in vitro and in vivo studies

The other two selected analogs, pyrazole **22b** (2′-deoxy-2′-fluorine series) and oxazole **7a** (2′-C-methyl-ribose series) combining excellent potency against NS5B (NTP $IC_{50}$ = 0.07 and 0.1 μM, respectively) and modest level of cellular antiviral potency ($EC_{50}$ = 35 and >100 μM, respectively) were singled out to be evaluated as the corresponding monophosphate prodrugs (NMP). It is known that for many nucleosides the first phosphorylation is the rate limiting step, and, in such cases, a kinase bypass with a NMP prodrug might restore the efficient conversion to NTP and therefore the desired cellular antiviral potency.[21] In order to explore this approach, **22b** was converted into the corresponding phosphramidate prodrug **28**. No improvement in the antiviral potency was observed in the replicon assay (Table 2, entry 1, **28** $EC_{50}$ >50 μM), suggesting that the first intracellular phosphorylation might not be the only rate determining step towards the formation of the required NTP. Indeed, when prodrug **28** was dosed at 10 mg/Kg in hamster, no NTP formation was detected in liver, with the only species detected being the NMP.[†]

[†] Hamster was chosen instead of rat in view of the significantly higher plasma stability of prodrug **28** compared to rat.

**Table 2**



| Entry | $R_1$ | Compound | HCV replicon[a] $EC_{50}$ (μM) |
|---|---|---|---|
| 1 | F | **28** | >50 |
| 2 | OH | **23** | 1.8 |

[a] See note (a) in Table 1.

Remarkably, when the same approach was applied to the 2′-hydroxy analog **7a** (NTP $IC_{50}$ = 0.1 μM, replicon $EC_{50}$ = >100 μM) the corresponding NMP prodrug **23** offered a significant boost in cellular potency, with $EC_{50}$ = 1.8 μM (Table 2, entry 2). This finding is in line with the remarkable difference in replicon activity observed for nucleoside analogs belonging to the two series, as exemplified by the oxadiazole analogs **11** ($EC_{50}$ = 1.9 μM) and **21** ($EC_{50}$ = 41 μM) belonging to the 2′-C-methyl-ribose and to the 2′-deoxy-2′-fluorine series, respectively. These data suggest that further efforts to identify nucleoside or monophosphate prodrug with satisfactoty levels of antiviral potency within the 7-heterocyclic-substituted 7-deaza-adenine series should focus primarily on 2′-C-methyl-ribose analogs. Moreover, the high cellular potency observed for the NMP prodrug **23** warrants further investigations, including in vivo experiments to assess if efficient conversion to NTP is observed in hamster liver and the full evaluation of other NMP prodrugs of the most potent 2′-C-methyl-ribose analogs identified within this series.

## 4. Conclusions

On the basis of previous findings in a related series, we investigated novel 7-heteroaromatic substituted 7-deaza-nucloside analogs bearing a 2′-C-methyl substitution within the ribose ring. We envisaged that the presence of an appropriately situated H-bond acceptor within the heteroaromatic substituent could engage the adjacent amino group in an intra-molecular H-bond, thus imparting to the modified nucleobase a planar arrangement and maintaining high levels of intrinsic potency. The proposed hypothesis was supported by SAR studies and $^1$H NMR evidences. We identified several potent novel and potent nucleoside inhibitors of HCV NS5B polymerase, such as oxadiazole **11** and pyrazoles **7a** and **22b** (NTP $IC_{50}$ = 0.5, 0.1 and 0.07 μM, respectively). In particular oxadiazole **11** was found to be a potent inhibitor of HCV replication in vitro ($EC_{50}$ = 1.9 μM) and was efficiently phosphorylated in vivo, resulting in high and substained levels of NTP levels in rat liver following intravenous and oral administration. These properties, combined the high levels of antiviral potency of **11**, comparable or better to that of other HCV nucleoside inhibitors that have been or currently are in clinical studies, offer great promise for its further development. Moreover, successful application of the kinase by-pass approach resulted in restored cellular potency for analog **23**, the monophosphate prodrug of nucleoside **7a** ($EC_{50}$ = 1.8 μM). Future work will focus on further evaluation of the novel potent inhibitors identified, as well as on continued investigation of further 7-heterocyclic-7-deaza-adenine analogs within the 2′-C-methyl ribose series.

## 5. Experimental section

$^1$H NMR spectra were obtained on a Bruker 400 MHz spectrometer. The purity of the nucleoside analogs was determined by analytical RP-HPLC. Data were obtained by two methods: method (1) on an Acquity Waters UPLC system equipped with a ZQ micromass spectrometer, using a flow rate of 0.5 mL/min, a HSS T3, C18, 1.8 μm, 2.1 × 50 mm column as the stationary phase, and a mobile phase comprising MeCN +0.1% $HCO_2H$ (solvent A) and $H_2O$ +0.1% $HCO_2H$ (solvent B); method parameters were 0% solvent A (0.0 min) to 100% solvent A (2.7 min) and then 100% solvent A (0.2 min); method (2) on a Waters Alliance 2795 HPLC–MS system equipped with a diode array and a ZQ mass spectrometer, using a flow rate of 1.0 mL/min, an Atlantis T3, C18, 5 μm, 4.6 × 50 mm column as the stationary phase; method parameters were 0% solvent A (0.0 min) to 20% (5.2 min), then 95% (7.0 min) and 100% (8.5 min). All the nucleoside analogs showed purities higher than 95%. High resolving power accurate mass measurement electrospray (ES) mass spectral data were acquired by use of a Bruker Daltonics apex-Qe Fourier transform ion cyclotron resonance mass spetrometer (FT-ICR MS). External calibration was accomplished with oligomers of polypropylene.

### 5.1. General procedure for the Suzuki Coupling and selected examples

$Pd(PPh_3)_4$ (0.1 equiv) was added to a 0.1 M solution in dioxane of hetroaryl-boronic acid (1.5 equiv), $Na_2CO_3$ (2 M aq solution, 15 equiv) and iodide 6[20] or iodide 19 (vide infra). The reaction mixture was heated at 120 °C for 500 s under microwave irradiation and then filtered through a pad of celite. The filtrate was concentrated under reduced pressure and the residue was purified by preparative RP-HPLC eluting with MeCN/water containing 0.1% TFA to give the title compound as a solid.

#### 5.1.1. Pyrazole 7a

Solid (65%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 13.14 (s, 1H), 10.65 (br s, 1H), 8.60 (br s, 1H), 8.40 (s, 1H), 8.38 (s, 1H), 7.93 (br s, 1H), 6.63 (br s, 1H), 6.20 (s, 1H), 4.04 (d, $J$ = 9.1 Hz, 1H), 3.99–3.87 (m, 2H), 3.75 (m, 1H), 0.78 (s, 3H); MS (ES$^+$) $C_{15}H_{18}N_6O_4$ requires: 346. Found: 347 [M+H]$^+$. HRMS (ESI) $m/z$ Calcd for $C_{15}H_{19}N_6O_4$ 347.1462, measured 347.1457. RP-HPLC method 1, $t_R$ = 1.41 min; purity 99.3%; method 2, $t_R$ = 5.25 min; purity 98.2%.

#### 5.1.2. Pyrazole 7b

Solid, 55%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.44 (s, 1H), 7.91, (s, 1H), 7.73 (m, 1H), 7.80 (s, 1H), 6.22 (s, 1H), 4.02 (d, $J$ = 9.3 Hz, 1H), 3.96–3.81 (m, 2H), 3.69 (m, 1H), 0.78 (s, 3H); MS (ES$^+$) $C_{15}H_{18}N_6O_4$ requires: 346. Found: 347 [M+H]$^+$. HRMS (ESI) $m/z$ Calcd for $C_{15}H_{19}N_6O_4$ 347.1462, measured 347.1455. RP-HPLC method 1, $t_R$ = 1.26 min; purity 99.0%; method 2, $t_R$ = 4.27 min; purity 99.2%.

#### 5.1.3. Pyrazole 22b

(68%) $^1$H NMR (400 MHz, $CD_3CN/D_2O$) $\delta$ 8.19 (bd, $J$ = 17.1 Hz, 2H), 7.72 (br s, 1H), 6.63 (br s, 1H), 6.47 (d, $J$ = 17.1 Hz, 1H), 4.26 (bd, $J$ = 25.1 Hz, 1H), 4.05 (br s, 2H), 3.87 (m, 1H), 1.08 (d, $J$ = 22.4 Hz, 3H); $^{19}$F NMR (400 MHz, $CD_3CN/D_2O$) $\delta$ −162.64; MS (ES$^+$) $C_{15}H_{17}FN_6O_3$ requires: 348. Found: 349 (M+H$^+$). HRMS (ESI) $m/z$ Calcd for $C_{15}H_{18}FN_6O_3$ 349.1426, measured 349.1419. RP-HPLC method 1, $t_R$ = 1.52 min; purity 99.1%.

### 5.2. General procedure for the Stille coupling and selected examples

$PdCl_2(PPh_3)_2$ (0.1 equiv) was added to a 0.1 M solution of tri-*n*-butylstannyl)-heterocycle (3.0 equiv) and either iodide 6[20] or iodide 19 (vide infra) in DMF, and the resulting mixture was heated at 120 °C for 3 h under microwave irradiation. The reaction mixture was cooled to RT, partitioned between water/hexanes and the water phase was purified by preparative RP-HPLC eluting with MeCN/water containing 0.1% TFA to give the title compound as a solid.

#### 5.2.1. Oxazole 7d

Solid (11%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.88 (br s, 1H), 8.60 (s, 1H), 8.36 (s, 1H), 8.33 (br s, 1H), 8.23 (s, 1H), 7.45 (s, 1H), 6.21 (s, 1H), 4.06 (d, $J$ = 9.6 Hz, 1H), 3.99–3.87 (m, 2H), 3.73 (m, 1H), 0.77 (s, 3H); MS (ES$^+$) $C_{15}H_{17}N_5O_5$ requires: 347. Found: 348 [M+H]$^+$. RP-HPLC method 1, $t_R$ = 1.65 min; purity 99.6%; method 2, $t_R$ = 5.55 min; purity 98.13%.

#### 5.2.2. Pyrimidine 7c

Solid 19%; $^1$H NMR (300 MHz, DMSO-$d_6$, 300 K) $\delta$ 10.79 (br s, 1H), 8.89 (d, $J$ = 5.0 Hz, 2H), 8.82 (s, 1H), 8.48 (br s, 1H), 8.40 (s, 1H), 7.43 (t, $J$ = 5.0 Hz, 1H), 6.26 (s, 1H), 4.04 (d, $J$ = 9.1 Hz, 1H), 3.99–3.86 (m, 2H), 3.71 (m, 1H), 0.78 (s, 3H); MS (ES$^+$) $C_{16}H_{18}N_6O_4$ requires: 358. Found: 359 [M+H]$^+$.

#### 5.2.3. Oxazole 22a

20% isolated yield; $^1$H NMR (300 MHz, $CD_3CN/D_2O$) $\delta$ 8.43 (s, 1H), 8.26 (s, 1H), 7.78 (s, 1H), 7.27 (s, 1H), 6.45 (d, $J$ = 17.1 Hz, 1H), 4.23 (dd, $J$ = 24.0, 9.5 Hz, 1H), 4.06–3.99 (m, 2H), 3.83 (dd, $J$ = 12.0, 2.4 Hz, 1H), 1.06 (d, $J$ = 22.8 Hz, 3H); $^{19}$F NMR (300 MHz, $CD_3CN/D_2O$) $\delta$ −163.14; MS (ES$^+$) $C_{15}H_{16}FN_5O_4$ requires: 349. Found: 350 (M+H$^+$). HRMS (ESI) $m/z$ Calcd for $C_{15}H_{17}FN_5O_4$ 350.1266, measured 350.1261. RP-HPLC method 1, $t_R$ = 1.61 min; purity 95.3%.

### 5.3. Synthesis of triazole 9

Trimethylsilylmethyl azide (3.0 equiv) was added to a 0.25 M solution of 5-ethynyl-7-(2′-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 8[24] in a 1:1 v:v mixture of water and $^t$BuOH containing L-ascorbic acid sodium salt (0.5 equiv) and copper(II) sulfate pentahydrate (0.05 equiv). The heterogeneous mixture was stirred at 50 °C overnight, cooled to RT and concentrated under reduced pressure. The residue was treated with 1 M aq solution of NaOH (5.0 equiv) in a 1:1 v:v mixture of MeOH and $H_2O$. The resulting mixture was stirred at 50 °C for 2 h and then concentrated under reduced pressure. Purification by preparative RP-HPLC eluting with MeCN/water containing 0.1% TFA gave the title compound as a solid (21%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.48 (s, 1H), 8.40 (s, 1H), 8.20 (s, 1H), 6.23 (s, 1H), 4.17 (s, 3H), 3.98–3.85 (m, 3H), 3.75 (m, 1H), 0.79 (s, 3H); $^{19}$F NMR (300 MHz, DMSO-$d_6$) $\delta$ −73.90; MS (ES$^+$) $C_{15}H_{19}N_7O_4$ requires: 361. Found: 362 [M+H]$^+$. HRMS (ESI) $m/z$ Calcd for $C_{15}H_{20}N_7O_4$ 362.1571, measured 362.1563. RP-HPLC method 1, $t_R$ = 1.44 min; purity 97.6%; method 2, $t_R$ = 5.33 min; purity 96.3%.

### 5.4. Synthesis of carbonitrile intermediate 10

To a solution of iodide **6**[20](50 mg, 0.123 mmol) in DMF (0.8 mL) were added zinc cyanide (22 mg, 0.18 mmol) and $Pd(PPh_3)_4$ (14 mg, 0.012 mmol) and the resulting solution was heated for 15 min at 150 °C under microwave irradiation. The reaction mixture was then allowed to cool to RT, filtered through a pad of Celite and concentrated to dryness. The residue was purified by Silica gel chromatography eluting with 3–10% MeOH in DCM to afford the title compound (90%) as a solid; $^1$H NMR (400 MHz, $D_2O$) $\delta$ 8.45 (s, 1H), 8.43 (s, 1H), 6.40 (s, 1H), 4.22–4.16 (m, 1H), 4.15–4.08 (m, 2H), 3.99–3.92 (m, 1H), 0.94 (s, 3H); MS (ES$^+$) $C_{13}H_{15}N_5O_4$ requires 305.1. Found: 306 [M+H]$^+$.

### 5.5. Synthesis of oxadiazole 11

Hydroxylamine hydrochloride (1.5 equiv) and triethylamine (2.0 equiv) were added to a 0.2 M solution of carbonitrile **10** in ethanol. The resulting mixture was heated at 50 °C for 6 h, cooled to RT and concentrated under reduced pressure to yield 4-amino-*N*′-hydroxy-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine-5-carboximidamide as a solid [MS ($ES^+$) $C_{13}H_{18}N_6O_5$ requires: 338.1. Found: 339 $[M+H]^+$]. The solid residue was suspended in triethyl orthoformate (3.0 equiv), treated with $BF_3{\cdot}Et_2O$ (0.3 equiv) and heated at 100 °C for 15 min. After cooling to RT, the mixture was diluted with water and concentrated under reduced pressure. The residue was diluted with DCM (0.1 M), cooled to 0 °C and treated with a 1 M solution of $BBr_3$ in DCM (6.0 equiv). After stirring for 3 h at RT the reaction mixture was diluted at 0 °C with MeOH, treated with a 2 M solution of ammonia in MeOH and concentrated under reduced pressure. The residue was purified by preparative RP-HPLC eluting with MeCN/water containing 0.1% TFA to give the title compound as a solid (25%). $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.78 (s, 1H), 8.76 (s, 1H), 8.62–8.04 (m, 2H), 8.37 (s, 1H), 6.23 (s, 1H), 4.04 (d, $J$ = 9.2 Hz, 1H), 3.99–3.85 (m, 2H), 3.72 (m, 1H), 0.77 (s, 3H); MS ($ES^+$) $C_{14}H_{16}N_6O_5$ requires: 348. Found: 349 $[M+H]^+$. HRMS (ESI) $m/z$ Calcd for $C_{14}H_{17}N_6O_5$ 349.1255, measured 349.1259. RP-HPLC method 1, $t_R$ = 1.40 min; purity 96.6%; method 2, $t_R$ = 5.17 min; purity 95.8%.

### 5.6. Synthesis of carboxylic acid internediate 12

Carbonitrile **10** (140 mg, 0.46 mmol) was suspended in 3 M aq HCl (1.5 mL) and heated at reflux for a total of 18 h. The reaction mixture was concentrated under reduced pressure and the residue was co-evaporated several times with $Et_2O$ to yield the corresponding unprotected carboxylic acid as a yellow solid. The latter was then dissolved in acetic acid (925 μl) and treated with acetyl chloride (197 μl, 2.78 mmol), and the resulting reaction mixture was stirred at RT for 18 h. The mixture was concentrated under reduced pressure and the residue was partitioned between DCM and $H_2O$. The pH of the aqueous layer was adjusted to neutral by careful addition of s.s. $NaHCO_3$ and extracted with DCM. The organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated under reduced pressure to give the title compound (201 mg, 0.446 mmol, 96% over two steps) as a yellow solid; $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.19 (s, 1H), 8.00 (s, 1H), 6.61 (s, 1H), 5.53 (d, $J$ = 6.4 Hz, 1H), 4.48–4.32 (m, 3H), 2.16–2.06 (m, 9H), 1.33 (s, 3H); MS (ES+) $C_{19}H_{22}N_4O_9$ requires: 450. Found: 451 [M+H]+.

### 5.7. Synthesis of oxadiazole 13

To a mixture of carboxylic acid 12 (200 mg, 0.44 mmol), HOBT (1.36 mg, 8.88 μmol), DIPEA (388 μl, 2.220 mmol) and acetamide oxime (36.2 mg, 0.488 mmol) in DMF (4.5 mL) was added TBTU (150 mg, 0.466 mmol) and the resulting reaction mixture was stirred for 14 h at RT. The mixture was then quenched with water and extracted with DCM. The collected organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated under reduced pressure. The solid residue was dissolved in DMF (5.0 mL) and heated at 140 °C for 3 h. The reaction mixture was then cooled to RT, diluted with DCM and washed with brine. The organic layers were dried ($Na_2SO_4$) and concentrated under reduced pressure. The brown oil residue was dissolved in MeOH (10 mL) and treated with 1 M aq NaOH (1.33 mL, 1.33 mmol). After 2 h, the reaction mix was concentrated under reduced pressure, the pH was adjusted to neutrality by addition of 1 M aq HCl and purified by prep HPLC (Atlantis T3, 19 × 150 mm; eluent from 5% to 80% then to 95% $CH_3CN$ in water + 0.1% TFA) to afford the title compound as an off-white solid (74.7 mg, 35% over three steps); $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.00–10.63 (br s, 1H), 9.06–8.49 (br s, 1H), 8.38 (s, 1H), 8.22 (s, 1H), 6.24 (s, 1H), 4.01–3.81 (m, 3H), 3.74 (dd, $J$ = 5.1, 12.6 Hz, 1H), 0.79 (s, 3H); $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.02 (br s, 1H), 8.91 (s, 1H), 8.35 (s, 1H), 8.15 (br s, 1H), 6.23 (s, 1H), 4.12–3.85 (m, 3H), 3.70 (m, 1H), 2.45 (s, 3H), 0.78 (s, 3H); MS ($ES^+$) $C_{15}H_{18}N_6O_5$ requires 362. Found: 363 $[M+H]^+$. HRMS (ESI) $m/z$ Calcd for $C_{15}H_{19}N_6O_5$ 363.1411, measured 363.1405. RP-HPLC method 1, $t_R$ = 1.57 min; purity 98.2%.; method 2, $t_R$ = 6.15 min; purity 98.8%.

### 5.8. Synthesis of oxadiazole 15

Steps 1 and 2: 4-Methylmorpholine (1.0 equiv) was added to a cooled (0 °C) 0.2 M solution of carboxylic acid 12 in THF containing *tert*-butyl hydrazinecarboxylate (1.0 equiv) and 1*H*-benzotriazol-1-ol hydrate (2.0 equiv). After 5 min stirring at 0 °C, *N*,*N*′-dicyclohexylcarbodiimide (1.0 equiv) was added. The reaction mixture was stirred for 1 h at 0 °C and for further 12 h at RT. The mixture was then cooled to 0 °C and filtered through a short pad of Celite. The filtrate was diluted with DCM, washed with s.s. $NaHCO_3$, brine and dried over $Na_2SO_4$. The residue was evaporated to dryness under reduced pressure, dissolved in 4 M HCl in dioxane (25 equiv) and stirred at RT for 6 h. The reaction mixture was evaporated under reduced pressure and partitioned between water/$Et_2O$. The aqueous layer was brough to pH 7 by addition of s.s. $NaHCO_3$, extracted with $Et_2O$ and concentrated under reduced pressure. The residue was purified by RP-HPLC (Atlantis T3, 19 × 150 mm, 5 μm) eluting with MeCN/water containing 0.1% TFA to give carbohydrazide **14** as a white solid (67%); $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.00–10.63 (br s, 1H), 9.06–8.49 (br s, 1H), 8.38 (s, 1H), 8.22 (s, 1H), 6.24 (s, 1H), 4.01–3.81 (m, 3H), 3.74 (dd, $J$ = 5.1, 12.6 Hz, 1H), 0.79 (s, 3H); MS ($ES^+$) $C_{13}H_{18}N_6O_5$ requires 338.32. Found: 339 $[M+H]^+$.

Step 3: $BF_3{\cdot}Et_2O$ (0.2 equiv) was added dropwise to a 0.5 M solution of 4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbohydrazide (1.0 equiv) in DMF containing triethyl orthoformate (2.0 equiv). The reaction mixture was heated at 70 °C for 180 min, cooled to RT and diluted with water. The mixture was then treated with aqueous 1 M HCl stirring for 20 min at RT (pH ~2) and then treated with aq 6 M $NH_4OH$ and stirred for further 30 min at RT (pH ~8). The reaction mixture was evaporated under reduced pressure and purified by preparative RP-HPLC eluting with MeCN/water containing 0.1% TFA to give the title compound **15** (25%) as a white fluffy solid. $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.34 (s, 1H), 8.66 (s, 1H), 8.31 (s, 1H), 6.22 (s, 1H), 4.17–3.82 (m, 3H), 3.78–3.67 (m, 1H), 0.78 (s, 3H); (MS ($ES^+$) $C_{14}H_{16}N_6O_5$ requires 348. Found: 349 $[M+H]^+$.

### 5.9. Synthesis of 2′-deoxy-2′-fuoro-7-iodo intermediate 19

DIAD (2.8 equiv) was added to a 0.05 M solution of $Ph_3P$ (3.0 equiv) in acetonitrile and the resulting mixture was stirred for 30 min at 0 °C. The solution was then added via cannula into a 0.1 M solution of 2′-deoxy-2′-fluoro-2-methyl-D-ribofuranose intermediate **17**[25] (1.0 equiv) in acetonitrile at −40 °C. The resulting pale brown suspension was stirred overnight at RT. The reaction was quenched with AcOEt and the organic phase was washed with water, brine and dried over $Na_2SO_4$. The volatiles were removed under reduced pressure and the residue was purified by flash chromatography (gradient elution from 0% to 5% AcOEt/Hexane then isocratic elution 5% AcOEt/Hexane) to obtain 4-chloro-7-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-2-methyl-β-D-ribofuranosyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine 18-β as the first eluting anomer. The latter was dissolved in a 7 M solution of $NH_3$ in MeOH (0.01 M) and the resulting mixture was stirred overnight at 110 °C in a closed vessel. The volatiles were then

removed under reduced pressure and the residue was purified by flash chromatography eluting with MeOH:DCM 1:9 to give the title compound as white foam (24%). $^1$H NMR (400 MHz, $CD_3CN/D_2O$) $\delta$ 8.14 (s, 1H), 7.61 (s, 1H), 6.35 (d, $J$ = 18.5 Hz, 1H), 4.20 (dd, $J$ = 24.0, 9.4 Hz, 1H), 3.98–3.95 (m, 2H), 3.78 (dd, $J$ = 12.0, 2.8 Hz, 1H), 1.00 (d, $J$ = 22.6 Hz, 3H); $^{19}$F NMR (400 MHz, $CD_3CN/D_2O$) $\delta$ −160.89; MS ($ES^+$) $C_{12}H_{14}FIN_4O_3$ requires: 408. Found: 409 ($M+H^+$).

#### 5.9.1. Oxadiazole 21

The title compound was obtained in 27% isolated yield following the same procedure described for oxadiazole **11**, using 2′-deoxy-2′-fluoro-carbonitrile 19 instead of carbonitrile 10. $^1$H NMR (400 MHz, $CD_3CN/D_2O$) $\delta$ 9.07 (s, 1H), 8.53 (s, 1H), 8.18 (s, 1H), 6.38 (d, $J$ = 16.9 Hz, 1H), 4.13 (dd, $J$ = 24.0, 8.2 Hz, 1H), 3.94–3.88 (m, 2H), 3.71 (m, 1H), 0.96 (d, $J$ = 22.6 Hz, 1H); $^{19}$F NMR (400 MHz, $CD_3CN/D_2O$) $\delta$ −163.26; MS ($ES^+$) $C_{14}H_{15}FN_6O_4$ requires: 350. Found: 351 ($M+H^+$). HRMS (ESI) $m/z$ Calcd for $C_{14}H_{16}FN_6O_4$ 351.1218, measured 351.1213. RP-HPLC method 1, $t_R$ = 1.54 min; purity 96.5%.

### 5.10. Synthesis of NMP prodrug 23

To a 0.1 M solution of nucleoside **7a** in dry THF at −78 °C was added dropwise *t*-BuMgCl (1.0 M solution in THF, 2.2 equiv). The reaction mixture was stirred at −78 °C for 5 min and then at 0 °C for further 30 min. L-alanine-*N*-chlorophenoxyphosphinyl-ethyl ester was then added dropwise (1.0 M solution in dry THF, 1.5 equiv) at 0 °C, and the resulting mixture was stirred at RT for 60 min and quenched with 2 ml of s.s $NH_4Cl$. The volatiles were removed under reduced pressure and the residue was purified by RP-HPLC (Atlantis T3, 19 × 150 mm, 5 μm) eluting with $MeCN/H_2O$ containing 0.1% TFA to give the title compound. $^1$H NMR (600 MHz, $CD_3CN + D_2O$, 300 K) $\delta$ 11.86 (br s, 1H), 10.87 (br s, 1H), 8.96 (s, 1H), 8.18 (s, 1H), 7.86–7.82 (m, 1H), 7.58–7.54 (m, 1H), 7.36–7.31 (m, 2H), 7.25–7.15 (m, 3H), 6.81–6.76 (m, 1H), 6.32–6.30 (m, 1H), 4.55–4.49 (m, 1H), 4.47–4.40 (m, 1H), 4.18–4.13 (m, 1H), 4.18–3.97(m, 4H), 3.96–3.90 (m, 1H), 1.27–1.25 (m, 3H), 1.14–1.12 (m, 3H), 0.85 (s, 3H). $^{31}$P (243 MHz. $CD_3CN+D_2O$) $\delta$ 3.85, 3.49. MS ($ES^+$) $C_{26}H_{32}N_7O_8P$ requires 601.2. Found: 602 $[M+H]^+$.

### 5.11. Selected NTP examples (for a general procedure for the NTP synthesis see Ref. 31)

#### 5.11.1. Oxazole 32

$^1$H NMR (300 MHz, $D_2O$, 300 K) $\delta$ 8.37 (m, 1H), 8.03–7.91 (m, 2H), 7.18 (s, 1H), 6.16 (s, 1H), 4.67 (m, 1H), 4.44 (m, 1H), 4.32 (m, 1H), 4.19 (m, 1H), 3.26–3.10 (m, 6H), 3.02–2.81 (m, 18H), 1.86–1.66 (m, 6H), 1.50–1.26 (m, 18H), 1.00–0.86 (m, 9H), 0.81 (s, 3H); $^{31}$P NMR (121 MHz, $D_2O$, 300 K) $\delta$ −10.49 (d, $J$ = 19.4 Hz, 1P), −11.07 (d, $J$ = 19.4 Hz, 1P), −23.08 (t, $J$ = 19.4 Hz, 1P); MS ($ES^-$) $C_{15}H_{20}N_5O_{14}P_3$ requires: 587.0. Found: 586 $[M-H]^-$, 608 $[M+Na-H]^-$.

#### 5.11.2. Pyrazole 34

$^1$H NMR (300 MHz, $D_2O$, 300 K) $\delta$ 8.25 (br s, 1H), 7.79 (s, 1H), 7.71 (s, 1H), 7.00 (s, 1H), 6.11 (s, 1H), 4.71 (m, 1H), 4.43 (m, 1H), 4.25 (m, 2H), 3.16 (m, 6H), 2.91 (m, 18H), 1.83–1.66 (m, 6H), 1.47–1.27 (m, 18H), 0.97–0.85 (m, 9H), 0.77 (s, 3H); $^{31}$P NMR (121 MHz, $D_2O$, 300 K) $\delta$ −10.23 (d, $J$ = 19.5 Hz, 1P), −11.13 (d, $J$ = 19.5 Hz, 1P), −22.90 (t, $J$ = 19.5 Hz, 1P); MS ($ES^-$) $C_{15}H_{21}N_6O_{13}P_3$ requires: 586.0. Found: 585 $[M-H]^-$, 607 $[M+Na-H]^-$.

#### 5.11.3. Oxadiazole 33

$^1$H NMR (300 MHz, $D_2O$, 300 K) $\delta$ 9.33 (s, 1H), 8.41 (m, 1H), 8.09 (s, 1H), 6.23 (s, 1H), 4.61 (m, 1H), 4.55 (m, 1H), 4.33 (m, 1H), 4.16 (d, $J$ = 9.4 Hz, 1H), 3.17 (m, 6H), 2.92 (m, 18H), 1.82–1.68 (m, 6H), 1.48–1.29 (m, 18H), 0.98–0.87 (m, 9H), 0.84 (s, 3H); $^{31}$P NMR (121 MHz, $D_2O$, 300 K) $\delta$ −10.45, −11.15 (m, 2P), −22.92 (t, $J$ = 19.6 Hz, 1P); MS ($ES^-$) $C_{14}H_{19}N_6O_{14}P_3$ requires: 588.0. Found: 587 $[M-H]^-$.

#### 5.11.4. Oxadiazole 39

$^1$H NMR (300 MHz, $D_2O$, 300 K) $\delta$ 9.29 (s, 1H), 8.34 (br s, 1H), 8.03 (s, 1H), 6.42 (d, $J$ = 18.6 Hz, 1H), 4.61 (m, 1H), 4.44 (m, 1H), 4.31 (m, 1H), 3.14 (m, 6H), 2.89 (m, 18H), 1.78–1.66 (m, 6H), 1.43–1.27 (m, 18H), 1.04 (d, $J$ = 23.1 Hz, 3H), 0.95–0.84 (m, 9H); $^{31}$P NMR (121 MHz, $D_2O$, 300 K) $\delta$ −10.60 (d, $J$ = 19.4 Hz, 1P), −11.12 (d, $J$ = 19.4 Hz, 1P), −22.99 (t, $J$ = 19.4 Hz, 1H); $^{19}$F NMR (282 MHz, $D_2O$, 300 K) $\delta$ −161.35 (s, 1F); MS ($ES^-$) $C_{14}H_{18}FN_6O_{13}P_3$ requires: 590.0. Found: 589 $[M-H]^-$, 611 $[M+Na-H]^-$.

#### 5.11.5. Oxazole 40

$^1$H NMR (300 MHz, $D_2O$, 300 K) $\delta$ 8.28 (br s, 1H), 7.99 (s, 1H), 7.92 (s, 1H), 7.21 (s, 1H), 6.43 (d, $J$ = 17.8 Hz, 1H), 4.63 (m, 1H), 4.52–4.29 (m, 3H), 3.22–3.07 (m, 6H), 2.90 (m, 18H), 1.80–1.64 (m, 6H), 1.44–1.26 (m, 18H), 1.06 (d, $J$ = 22.8 Hz, 3H), 0.96–0.83 (m, 9H); $^{31}$P NMR (121 MHz, $D_2O$, 300 K) $\delta$ −10.36 (d, $J$ = 19.6 Hz, 1P), −11.23 (d, $J$ = 19.6 Hz, 1P), −23.07 (t, $J$ = 19.6 Hz, 1P); $^{19}$F NMR (282 MHz, $D_2O$, 300 K) $\delta$ −162.22 (s, 1F); MS ($ES^-$) $C_{15}H_{19}FN_5O_{13}P_3$ requires: 589.0. Found: 588 $[M-H]^-$.

#### 5.11.6. Pyrazole 41

$^1$H NMR (300 MHz, $D_2O$, 300 K) $\delta$ 8.23 (br s, 1H), 7.74 (s, 1H), 7.70 (s, 1H), 6.96 (s, 1H), 6.30 (d, $J$ = 17.2 Hz, 1H), 4.70 (m, 1H), 4.50–4.25 (m, 3H), 3.14 (m, 6H), 2.89 (m, 18H), 1.80–1.63 (m, 6H), 1.45–1.26 (m, 18H), 0.98 (d, $J$ = 22.6 Hz, 3H), 0.94–0.82 (m, 9H); $^{31}$P NMR (121 MHz, $D_2O$, 300 K) $\delta$ −9.88 (d, $J$ = 19.4 Hz, 1P), −11.24 (d, $J$ = 19.4 Hz, 1P), −22.82 (t, $J$ = 19.4 Hz, 1P); $^{19}$F NMR (282 MHz, $D_2O$, 300 K) $\delta$ −162.93 (s, 1F); MS ($ES^-$) $C_{15}H_{20}FN_6O_{12}P_3$ requires: 588.0. Found: 587 $[M-H]^-$.

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