Bioorganic & Medicinal Chemistry Letters 20 (2010) 4850–4854

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and evaluation of a novel double pro-drug: INX-08189. A new clinical candidate for hepatitis C virus

Christopher McGuigan [a,*], Karolina Madela [a], Mohamed Aljarah [a], Arnaud Gilles [a], Andrea Brancale [a], Nicola Zonta [a], Stanley Chamberlain [b], John Vernachio [b], Jeff Hutchins [b], Andrea Hall [b], Brenda Ames [b], Elena Gorovits [b], Babita Ganguly [b], Alexander Kolykhalov [b], Jin Wang [b], Jerry Muhammad [b], Joseph M. Patti [b], Geoffrey Henson [b]

[a] Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3NB, UK
[b] Inhibitex Inc, 9005 Westside Parkway, Alpharetta, GA 30009, USA

ARTICLE INFO

Article history:
Received 13 May 2010
Revised 16 June 2010
Accepted 17 June 2010
Available online 20 June 2010

Keywords:
HCV
ProTide
Phosphoramidate
Nucleoside
Nucleotide
Antiviral pro-drug
Polymerase
NS5B

ABSTRACT

We herein report a novel double pro-drug approach applied to the anti-HCV agent 2′-β-*C*-methyl guanosine. A phosphoramidate ProTide motif and a 6-*O*-methoxy base pro-drug moiety are combined to generate lipophilic prodrugs of the monophosphate of the guanine nucleoside. Modification of the ester and amino acid moieties lead to a compound INX-08189 that exhibits 10 nM potency in the HCV genotype 1b subgenomic replicon, thus being 500 times more potent than the parent nucleoside. The potency of the lead compound INX-08189 was shown to be consistent with intracellular 2′-*C*-methyl guanosine triphosphate levels in primary human hepatocytes. The separated diastereomers of INX-08189 were shown to have similar activity in the replicon assay and were also shown to be similar substrates for enzyme processing. INX-08189 has completed investigational new drug enabling studies and has been progressed into human clinical trials for the treatment of chronic HCV infection.

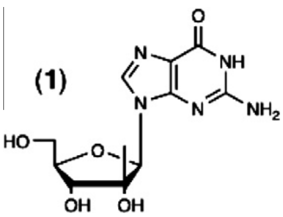
Over 180 million people are chronically infected with hepatitis C virus (HCV) and at risk of developing life threatening liver disease.[1] The current therapy consists of pegylated interferon and ribavirin.[2] Neither agent is specific for HCV, side effects are common, and efficacy is limited in certain genotypes.[2] As with antivirals in general, nucleoside analogues are amongst the leading classes of compounds being developed as new agents for the treatment of HCV infection. Several 2′-*C*-ribonucleoside analogues, including 2′-*C*-methyladenosine and 2′-*C*-methyl guanosine, (**1**) have been shown to possess activity against HCV in the replicon assay as well as antiviral activity against several members of the *Flavivirus* family[3,4] 2′-*C*-methyl guanosine was evaluated further in a series of nonclinical studies, which indicated the absence of detectable cytotoxicity, potent inhibition of the HCV RNA-dependent RNA polymerase as its triphosphate, and oral bioavailability in rats of 82%.[5,10] Unfortunately, its potential as a therapeutically useful nucleoside was limited due to low oral bioavailability in non-rodent species, inefficient cellular uptake and poor intracellular metabolism of 2′-*C*-methyl guanosine to its active triphosphate form.[5] We have previously reported the application of our ProTide, phosphoramidate pro-drug approach[6] to 2′-*C*-methyl guanosine (**1**) to overcome these limitations.[7,8] In this publication we describe a series of novel double pro-drugs of 2′-*C*-methyl guanosine for HCV therapy.

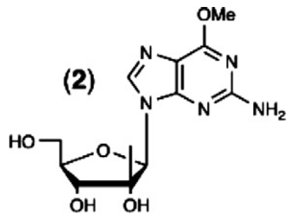
The HCV antiviral activities of our phosphoramidates were evaluated against HCV genotype 1b, in a Huh7 cell line expressing a stable, bicistronic subgenomic replicon encoding the *Renilla* luciferase reporter gene.[9] HCV replication in this cell line was monitored by measuring the luminescence produced by luciferase activity. From the initial series of compounds, the naphthyl benzylalanine phosphoramidate of (**1**), in the assay described above, is active at 0.062 μM, being ca. 16 times more active than (**1**) which has an EC_{50} of 1.0 μM. However, subsequent work to address the rodent plasma instability of these compounds lead to L-valine phosphoramidate derivatives of (**1**) such as the naphthyl benzyl L-valine phosphoramidate, which demonstrated much improved rodent plasma stability.[8] Unfortunately, with the improved rodent plasma stability of the branched amino acids, came a significant decrease in HCV replicon activity. Extensive modification of the ester functionality did not improve HCV potency significantly.[8] We then turned to modifications of the purine base as a means of

* Corresponding author. Tel./fax: +44 2920874537.
E-mail addresses: mcguigan@cf.ac.uk, mcguigan@cardiff.ac.uk (C. McGuigan).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.06.094

potentially affecting potency without changing the inherent plasma stability of the L-valine phosphoramidates. Modifications were made at the C-6 and C-2 positions using an HCV polymerase model as a guide.[10] SAR development is underway at the C-2 position and will be discussed elsewhere. We considered whether simple C-6 modifications could maintain binding of the corresponding triphosphate to HCV RNA polymerase, and in particular, whether a 6-*O*-methoxy substituent as in **2** could be tolerated in the model. It was considered that the likely increase in lipophilicity of (**2**) could enhance the poor cell uptake of (**1**).

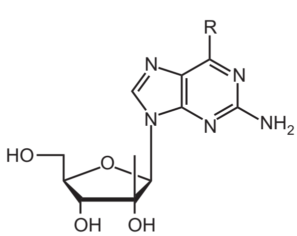




To test this, an HCV polymerase model was built according to the literature[10] and docking studies with the phosphorylated forms of various C-6 substituted derivatives were performed. These studies showed that the triphosphate of nucleoside **2**, docks only poorly into the NS5b active site, suggesting that it would be a poor inhibitor of the NS5B polymerase.[11] The replicon activity of 6-*O*-methyl-2′-*C*-methyl guanosine (**2**) was determined and is reported, for the first time, in Table 1 along with the modelling results and replicon activity of a number of other C-6 substituted analogues. The modest replicon activity observed for these derivatives may be ascribed to their slow intracellular conversion to 2′-*C*-methyl guanosine by deaminase activity, which might be at the nucleoside level (e.g., ADA, EC 3.5.4.4),[12] or at the nucleotide level.

Table 1
Comparison of modelling predictions of the nucleoside triphosphate and HCV replicon activity of C-6 substituted derivatives of 2′-*C*-methyl guanosine

R	Modelling prediction	Replicon activity (μM)
OH	Good binding	1
OCH3	Poor binding	5
OEt	Poor binding	9
SMe	Poor binding	11
NHMe	Poor binding	13
NHBn	Poor binding	27
Cl	Poor binding	8



Compounds in Table 1 were synthesized from the C-6 chloro 2-amino 2′-*C*-methyl purine riboside, and phosphoramidate derivatives were made of each. Full details of this work will be reported elsewhere.

In spite of the reduced replicon activity of 6-*O*-methyl-2′-*C*-methyl guanosine, and the prediction from the modelling that the corresponding triphosphate would be inactive, we sought to prepare a series of ProTides of (**2**) to investigate the effect of the phosphorylation by-pass strategy on the replicon activity of this modified purine. The hope was that phosphoramidates of **2** would show improved cellular uptake, and would be metabolically converted to the active 2′-*C*-methyl guanosine triphosphate.[13]

Target compound (**2**) was prepared in an overall yield of 60% via the 6-chloro nucleoside generated by the TMS triflate mediated condensation of the tetrabenzoyl 2-*C*-methyl sugar and chloro base (Fig. 1).

Compound (**2**) was converted to 5′-ProTides following our established methods.[14] In brief, 1-naphthol and $POCl_3$ were reacted to generate the naphthyloxy phosphorodichloridate and this was allowed to react with various amino acid ester salts to generate the phosphorochloridates (**3**) as key synthons. As shown in Figure 2, reaction of (**3**) with nucleoside (**2**) in THF in the presence of *N*-methyl imidazole gave the target compounds (**4a**–**m**) in moderate yield. Notably, use of the 6-O-methylated nucleoside as opposed to the guanine nucleoside, allows coupling with the chlorophosphoramidate to proceed without prior protection of the nucleoside sugar hydroxyl groups, saving a deprotection step in the linear synthetic sequence and saving two steps in the overall synthesis.

Compounds (**4**) were purified by flash column chromatography and HPLC as necessary. They were routinely isolated as roughly 1:1 mixtures of phosphate diastereoisomers as evidenced by splitting of HPLC peaks and ^{31}P NMR signals. Compounds were tested as mixtures of diastereomers in the first instance. ^{13}C NMR and mass spectrometry data also confirmed the structure and purity of (**4a**–**m**).[15] Compounds (**4a**–**h**), being the alanine series, were evaluated versus HCV in replicon assay, with data shown in Table 2.

Thus, in general the data in Table 2 show a significant increase in the cell based potency from this family of ProTides, in comparison to the parent nucleoside (**2**). The most active ester is the neopentyl (**4g**) with EC_{50} of 0.01 μM and EC_{90} of 0.04 μM. This is ca. 500–550-fold more active than the parent nucleoside (**2**). Notably, comparing the Ala benzyl ester ProTide of (**2**), compound **4a**, with its equivalent ProTide of the guanine parent (**1**)[7] shows a ca. fourfold potency boost for the 6-methoxy analogue. In part, this may be due to the enhanced lipophilicity and consequent cellular uptake for **4a**; calculated *C* log *P* values are 3.1 and 1.9, respectively. The much reduced activity of the isopropyl ester derivative (**4c**) highlights the importance of synthesizing multiple phosphoramidate derivatives.

Given the high potency of the neopentyl ester **4g** of the 6-methoxy nucleoside **2**, we also prepared the equivalent ProTide of **1**. The data on this compound, **5** are presented alongside **4g** in Table 3.

Thus, the 6-methoxy analogue shows a calculated lipophilicity some 100 times that of the guanine parent. This translates into a ⩾5-fold enhancement in membrane transport as measured by Caco-2 permeation and a >5-fold boost in HCV potency. This supports the idea that phosphoramidates of **2** have improved cellular uptake over phosphoramidates of **1**.

To pursue this family of phosphoramidates further, we embarked on selective amino acid variation, while retaining the neopentyl ester of the lead (**4g**). Data are shown in Table 4.

From the data in Table 4, it is clear that L-Ala (**4g**) is strongly (24×) preferred over D-Ala (**4i**). This highlights the importance of intracellular metabolism in the activity of these phosphoramidates because both have very similar *C* log *P* values. Increasing the overall size of the amino acid side chain as for the L-Met (**4j**) and L-Leu (**4k**) derivatives decreases HCV activity somewhat, but the most dramatic decrease in activity comes with branching at the amino acid beta carbon as in L-Ile (**4l**) and L-Val (**4m**). Overall, the 6-*O*-methyl modification consistently improves the HCV replicon activity relative to the guanine derivatives for the different amino acid derivatives in Table 4. For example, the corresponding guanine version of the L-Val derivative, **4m**, is 10-fold less active (EC_{50} = 1.5 μM) in the replicon assay than is the 6-*O*-methyl L-Val derivative.[8]

From this survey of amino acid and ester variations, the neopentyl alanine ProTide (**4g**) emerged as one of the more interesting compounds. To further characterize **4g** as a lead compound and to more fully define its potency, it was repeated multiple times in the HCV replicon assay. As shown in Figure 3, replicate assays revealed a

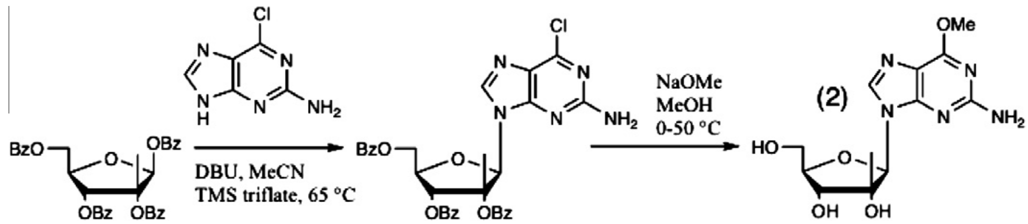


Figure 1. Synthesis of 6-OMe nucleoside (**2**)

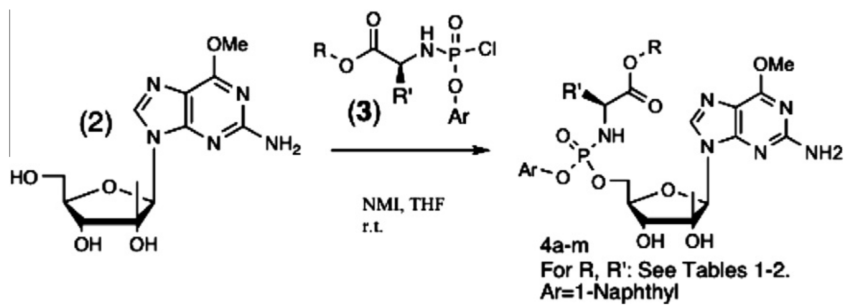


Figure 2. Synthesis of ProTides of (**2**).

Table 2
Anti-HCV activity (EC_{50}, EC_{90}) and cell cytotoxicity (CC_{50}) of alanine ProTides of (**2**)

Compd	R	Genotype 1b replicon		Huh7 CC_{50} (μM)
		EC_{50} (μM)	EC_{90} (μM)	
4a	Bn	0.03	0.10	12
4b	*n*-Pr	0.03	0.10	13
4c	*i*-Pr	0.54	1.70	>100
4d	cHex	0.03	0.09	6
4e	cPnt	0.03	0.08	9
4f	S-PhEt	0.04	0.13	14
4g	*t*-$BuCH_2$	0.01	0.04	7
4h	*t*-$BuCH_2CH_2$	0.02	0.08	14
2	—	5.2	21	>100

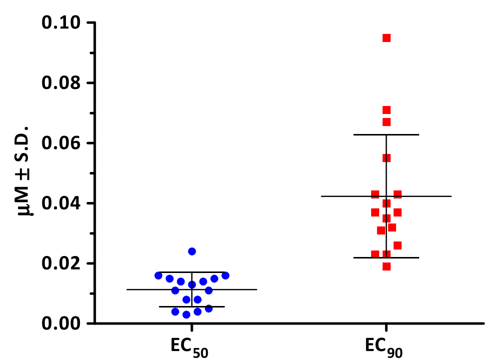


Figure 3. Potency of **4g** in the HCV genotype 1b replicon assay. Data represent 16 replicates.

Table 3
Anti-HCV activity, cytotoxicity, calculated lipophilicity and observed Caco-2 permeation for **4a** and **5**

Compd	Replicon		CC_{50} (μM)	*C* log *P*[a]	Caco-2%[b]
	EC_{50} (μM)	EC_{90} (μM)			
4g	0.010	0.038	7	3.3	2.7
5	0.072	0.27	>100	1.3	<0.5

[a] log *P* from Chemdraw Ultra 11.0.1.
[b] % Transport in 80 min across Caco-2 cells grown for 27 days (resistance 600 ohms/cm^2), measured apical to basal. Permeability measured for **4a** = 4.02 ± 0.1 × 10^{-6} cm/s.

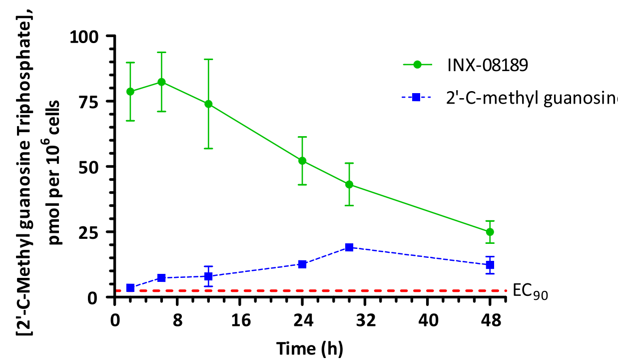


Figure 4. Conversion of 2 μM Phosphoramidate **4g** and **1** into 2′-*C*-methyl guanosine triphosphate in primary human hepatocytes. EC_{90} line is defined as the amount of intra cellular triphosphate necessary to achieve 90% inhibition in the HCV replicon assay.

Table 4
Anti HCV activity (EC_{50}, EC_{90}) and cell cytotoxicity (CC_{50}) of ProTides of (**2**) with amino acid variations

Compd	AA	Genotype 1b replicon		Huh7 CC_{50} (μM)
		EC_{50} (μM)	EC_{90} (μM)	
4g	L-Ala	0.01	0.04	7
4i	D-Ala	0.24	0.58	51
4j	L-Met	0.06	0.19	28
4k	L-Leu	0.07	0.23	14
4l	L-Ile	0.86	2.53	18
4m	Val	0.19	0.64	33
2	—	5.71	24.57	>100

highly potent inhibitor of HCV replication with EC_{50} and EC_{90} values ranging from 0.003–0.024 μM (mean = 0.01 μM) and 0.019–0.095 μM (mean = 0.04 μM), respectively. These data confirm **4g** as

Table 5
Data on separated diasteromers of **4g**

Inx	Chirality	Peak	Replicon EC_{50} (μM)	δ_P	$T_{1/2}$[a] (min)
4g–1	S_P	1st	0.044	4.28 (D)	20
4g–2	R_P	2nd	0.019	4.23 (E)	17

[a] Half-life in carboxypeptidase assay—see Figure 5.

the most potent nucleoside analogue based inhibitor of the HCV NS5b RNA dependent RNA polymerase reported to date. In addition, **4g** has also been tested against HCV genotypes 1a and 2 and shown to be very active, 12 and 1 nM, respectively.

To confirm that the phosphoramidate, **4g**, was being converted to the known HCV NS5b enzyme inhibitor 2′-*C*-methyl guanosine triphosphate, **4g** was incubated in primary human hepatocytes at an arbitrary concentration of 2 μM, and triphosphate levels were measured over a period of 48 h. In addition, the triphosphate levels resulting from incubation with 2 μM 2′-*C*-methyl guanosine were also measured (Fig. 4).

The data indicates that **4g** produces a substantial C_{max} of 2′-C-methyl guanosine triphosphate of 80 pmol/10^6 cells within the first 8 h, then decays with a half-life estimated to be over 24 h. The triphosphate of 6-*O*-methyl-2′-*C*-methyl guanosine (**2**) was synthesized (CarboSynth, Inc.), and used as an analytical standard, but none of this triphosphate was detected in multiple experiments. The nucleoside (**1**), on the other hand, slowly builds to a much delayed and lower C_{max} at 32 h compared to **4g**, consistent with its modest activity. Further work to understand the metabolism of the 6-*O*-methyl-2′-C-methyl guanosine phosphoramidates is underway and will be discussed in later publications. Similar to for the anti-leukaemic agent nelarabine,[13] it appears that a 6-methoxy base group is a good pro-drug for a guanine nucleoside analogue, but here at the monophosphate metabolic level, possibly utilizing adenylate deaminase (EC 3.5.4.6) or some other cellular

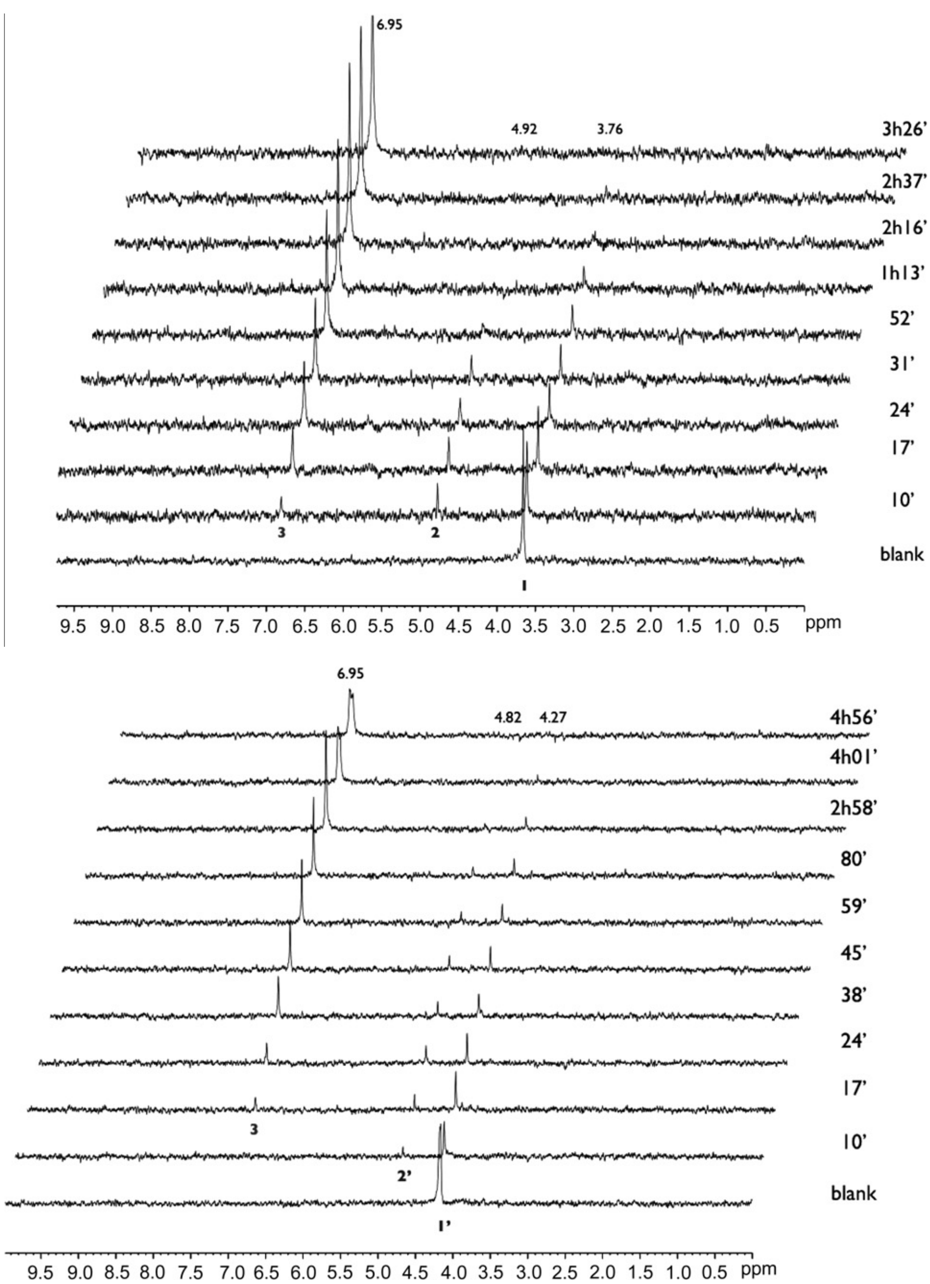


Figure 5. CPY assay on separated isomers of **4g**. Upper isomer **4g–1**, lower isomer **4g–2**. Conditions: 5 mg of compound in 200 μl of acetone + 400 μl of Trizma buffer 0.3–0.5 mg of carboxypeptidase A (purchased from SIGMA) in 200 μl of Trizma buffer.

deaminase[16] These data, together with the potent HCV replicon inhibition data, demonstrate that **4g** can be metabolised rapidly in vitro to the active 2′-*C*-methyl guanosine triphosphate; a direct inhibitor of the HCV RNA dependent RNA polymerase NS5b.

Given the considerable interest in **4g**, the two diastereoisomers were separated on a preparative scale using a Chiral Pak AD chiral chromatography column with 1:1 ethanol/hexane as eluant (Chiral Technologies, West Chester, PA). The absolute configuration of each of the two diastereomers was determined by Vibrational Circular Dichroism (VCD) (BioTools, Inc., Jupiter, FL). Replicon data on the separate isomers are given in Table 5.

Interestingly the HCV replicon activities of the two separated diastereomers are within twofold, suggesting that the initial cleavage of the amino acid ester and subsequent elimination of the naphthol, to give the common amino acid monophosphate metabolite, happens at similar rates. A Carboxypeptidase A assay has proved a useful in vitro model of the initial metabolism of such ProTides.[7] Thus, we carried out such an assay on the separated isomers of **4g**. The data shown in Figure 5 is a series of ^{31}P NMRs taken over a 3.5 h incubation of each diastereomer (10 mM) with Carboxypeptidase A in Trizma buffer.

The ^{31}P NMR traces show the parent phosphoramidate near 4.2 ppm at time zero, is rapidly cleaved by Carboxypeptidase A to the free amino acid carboxylate (peak 2/2′) with a ^{31}P NMR shift near 5 ppm, and then to the aminoacyl phosphate intermediate (peak 3/3′) with a ^{31}P NMR shift near 7.0 ppm. The formation of this intermediate is considered essential for the activity of ProTides.[6,7] Consistent with the replicon assay data, the estimated $T_{1/2}$ values of **4g**–**1** and **4g**–**2** are similar, 20 and 17 min, respectively. Further work is underway on **4g**–**1** and **4g**–**2** that may distinguish the two diastereomers, and will be reported elsewhere.

Based on the exceptional HCV antiviral activity of **4g** (INX-08189), along with other advantageous properties, such as cell permeability, ease of synthesis, and bioavailability, it was advanced into in vivo studies supporting its selection as a clinical candidate for HCV. Full details on the DMPK studies used to support the selection of **4g** will be reported elsewhere. The agent has now progressed into human clinical trials for HCV.

Acknowledgements

This study was supported by a grant from Inhibitex, Inc. to C.M. C.M. is a board member and shareholder of Inhibitex, Inc. All authors from Inhibitex, Inc. own options and are shareholders. We would like to thank CiVentiChem, RTP, NC for scaling up INX-08189 for separation of the diastereomers and Dr. Mark Gumbleton and Mr. Mathew Smith, Cardiff University for initial Caco-2 studies. We would like to thank Helen Murphy for secretarial assistance.

References and notes

1. Bostan, N.; Mahmood, T. *Crit. Rev. Microbiol.* **2010**, *36*, 91.
2. Hoofnagle, J. H.; Seef, L. B. *N. Engl. J. Med.* **2006**, *355*, 2444.
3. Soriano, V.; Madejon, A.; Vispo, E.; Labarga, P.; Garcia-Samaniego, J.; Martin-Carbonero, L.; Sheldon, J.; Bottecchia, M.; Tuma, P.; Barreiro, P. *Expert Opin. Emerg. Drugs* **2008**, *13*, 1.
4. Eldrup, A. B.; Prhavc, Ml; Brooks, J.; Bhat, B.; Prakash, T. P.; Song, Q.; Bera, S.; Bhat, N.; Dande, P.; Cook, P. D.; Bennett, C. F.; Carroll, S. S.; Ball, R. G.; Bosserman, M.; Burlein, C.; Colwell, L. F.; Fay, J. F.; Flores, O. A.; Getty, K.; LaFemina, R. L.; Leone, J.; MacCoss, M.; McMasters, D. R.; Tomassini, J. E.; Langen, D. V.; Wolanski, B.; Olsen, D. B. *J. Med. Chem.* **2004**, *47*, 5284.
5. Eldrup, A. B.; Allerson, C. R.; Bennett, C. F.; Bhat, S. B. B.; Bhat, N.; Bosserman, M. R.; Brooks, J.; Burlein, C.; Carroll, S. S.; Cook, P. D.; Getty, K. L.; MacCoss, M.; McMasters, D. R.; Olsen, D. B.; Prakash, T. P.; Prhavc, M.; Song, Q.; Tomassini, J. E.; Xia, J. *J. Med. Chem.* **2004**, *47*, 2283.
6. Cahard, D.; McGuigan, C.; Balzarini, J. *Mini-Rev. Med. Chem.* **2004**, *4*, 371.
7. McGuigan, C.; Perrone, P.; Madela, K.; Neyts, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 431.
8. McGuigan, C.; Gilles, A.; Madela, K.; Aljarah, M.; Holl, S.; Jones, S.; Vernachio, J.; Hutchins, J.; Bryant, D.; Gorovits, E.; Ganguly, B.; Hall, A.; Kolykhalov, A.; Muhammad, J.; Chamberlain, S.; Henson, G. *J. Med. Chem.* **2010**, *53*. doi:10.1021/jm1003792.
9. Blight, K.; Kolykhalov, A. A.; Rice, C. M. *Science* **2000**, *290*, 1972.
10. Migliaccio, G.; Tomassini, J. E.; Carroll, S. S.; Tomei, L.; Altamura, S.; Bhat, B.; Bartholomew, L.; Bosserman, M. R.; Ceccacci, A.; Colwell, L. F.; Cortese, R.; ele De Francesco, R.; Eldrup, A. B.; Getty, K. L.; Hou, D. S.; LaFemina, R. L.; Ludmerer, S. W.; MacCoss, M.; McMasters, D. R.; Stahlhut, M. W.; Olsen, D. B.; Hazuda, D. J.; Flores, O. A. *J. Biol. Chem.* **2003**, *278*, 49164.
11. Docking results were scored based on the RMSD value calculated between the GTP base present in the original model and the base of the docked compounds.
12. Gupta, M.; Nair, V. *Collect. Czech. Chem. Commun.* **2006**, *71*, 769.
13. Lambe, C. U.; Averett, D. R.; Paff, M. T.; Reardon, J. E.; Wilson, J. G.; Krenitsky, T. A. *Cancer Res.* **1995**, *55*, 3352.
14. Derudas, M.; Carta, D.; Brancale, A.; Vanpouille, C.; Lisco, A.; Margolis, L.; Balzarini, J.; McGuigan, C. *J. Med. Chem.* **2009**, *52*, 5520.
15. Brief synthetic and analytical details for the preparation of **2** and **4g.**
To a pre-cooled (0 °C) solution of 2,3,4,5-tetra-*O*-benzoyl-2-*C*-methyl-*beta*-D-ribofuranose (10.0 g, 17.22 mmol), 2-amino-6-chloropurine (3.2 g, 18.87 mmol), and 1,8-diazabicycl[5.4.0]undec-7-ene (DBU) (7.7 ml, 51 mmol) in anhydrous acetonitrile (200 ml) trimethysilyl triflate (12.5 ml, 68.8 mmol) was added dropwise. The reaction mixture was heated at 65 °C for 4–6 h, allowed to cool down to room temperature, poured into saturated aqueous sodium bicarbonate (300 ml), and extracted with dichloromethane (3 × 150 ml). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The residue was precipitated from dichloromethane and methanol. Precipitate was filtered and washed with methanol, to give the desired compound (8.5 g, 79%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (dd, *J* = 1.2, 8.3, 2H), 8.02–7.94 (m, 5H), 7.65–7.60 (m, 1H), 7.58–7.45 (m, 4H), 7.35 (q, *J* = 7.7, 4H), 6.65 (s, 1H), 6.40 (d, *J* = 6.7, 1H), 5.31 (s, 2H), 5.08 (dd, *J* = 4.2, 11.6, 1H), 4.79 (dd, *J* = 6.4, 11.6, 1H), 4.74 (td, *J* = 4.2, 6.5, 1H), 1.60 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.31 (C=O), 165.38 (C=O), 165.32 (C=O), 159.13 (C2), 152.87 (C6), 152.06 (C4), 141.42 (C8), 133.77 (C–H Bn), 133.69 (C–H Bn), 133.28 (C–H Bn), 129.90 (C–H Bn), 129.82 (C–H Bn), 129.78 (C Bn), 129.70 (C–H Bn), 129.41 (C Bn), 128.78 (C Bn), 128.61 (C–H Bn), 128.50 (C–H Bn), 128.41 (C–H Bn), 126.00 (C5), 88.84 (C1′), 85.68 (C2′), 79.43 (C4′), 76.07 (C3′), 63.57 (C5′), 17.77 (2′-*Me*).
Synthesis of 2-amino-6-methoxy-9-(2-C-methyl-β-D-ribofuranosyl) purine
To a suspension of 2-amino-6-chloro-9-(2-*C*-methyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)purine (3.0 g, 4.78 mmol) in anhydrous methanol (36 ml) at 0 °C NaOMe in methanol (5.4 ml, 25% w/w) was added. The mixture was stirred at room temperature for 24 h then quenched by addition of amberlite (H^+). The mixture was then filtrated and solvent was removed under reduced pressure. The resultant residue was dissolved in water (50 ml) and extracted with hexane (50 ml). The organic layer was then extracted with water (50 ml), and the combined water fractions were concentrated under reduced pressure. The residue was purified by silica gel chromatography ($CHCl_3$/MeOH 85:15) to give the pure compound (1.125 g, 76%) as a white solid. ^{1}H NMR (500 MHz, MeOD) δ 8.26 (s, 1H, H_8), 5.99 (s, 1H), 4.24 (d, *J* = 9.1, 1H), 4.08 (s, 3H), 4.04 (ddd, *J* = 2.3, 5.7, 8.6, 2H), 3.87 (dd, *J* = 3.0, 12.4, 1H), 0.96 (s, 3H). ^{13}C NMR (126 MHz, MeOD) δ 162.75 (C6), 161.86 (C2), 154.50 (C4), 139.35 (C8), 115.36 (C5), 93.00 (C1′), 84.15 (C4′), 80.34 (C2′), 73.57 (C3′), 61.17 (C5′), 54.25 (6-O*Me*), 20.35 (2′-*Me*). HPLC: t_R = 9.00 min. (solvent gradient needed O→100% ACN in H_2O 30 mins). HRMS (ESI): calcd for $C_{12}H_{17}N_5O_5$+Na^+ 334.1127, found 334.1125. Calcd for $C_{12}H_{17}N_5O_5$·0.75 H_2O: C, 44.37; H, 5.74; N, 21.56. Found: C, 44.24; H, 5.49; N, 20.83.
Synthesis of 2-amino-6-methoxy-9-(2-C-methyl-β-D-ribofuranosyl) purine 5′-O-[α-naphthyl-(2,2-dimethylpropoxy-L-alaninyl)] phosphate **4g**
To 2-amino-6-methoxy-9-(2-*C*-methyl-β-D-ribofuranosyl) purine (1.35 g, 4.34 mmol) in THF (35 ml), phosphorochloridate (10.83 mmol, 1 M solution in THF)) in THF was added, followed by addition of *N*-methyl-imidazole (1.71 ml, 21.7 mmol). The mixture was stirred overnight and the solvent was removed under reduced pressure. To remove the *N*-methyl-imidazole, the phosphoramidate was dissolved in chloroform and washed 3 times with hydrochloric acid (HCl 0.5 N). The organic layer was then dried over sodium sulfate and evaporated under reduce pressure. The residue was then purified on silica gel using $CHCl_3$ to $CHCl_3$/MeOH 95:5 as an eluent, to give the pure phosphoramidate as a white solid (510 mg, 18%). ^{31}P NMR (202 MHz, $CDCl_3$) δ 4.33, 4.29. ^{1}H NMR (500 MHz, MeOD) δ 8.19–8.15 (m, 1H, H_8-naph), 7.98, 7.96 (2 × s, 1H, H_8), 7.86–7.82 (m, 1H, H_5-napht), 7.68, 7.65 (2 × d, *J*= 7.0 Hz, 1H, H_4-napht), 7.53–7.44 (m, 3H, H_2, H_7, H_6-napht), 7.39, 7.37 (2 × t, *J*= 8.0 Hz, 1H, H_3-napht), 6.01, 6.00 (2 × s, 1H, $H_{1'}$), 4.67–4.64 (m, 1H, $H_{5'}$), 4.63- 4.59 (m, 1H, $H_{3'}$, $H_{4'}$), 4.09–4.05 (m, 1H, Ha), 4.04 (s, 3H, 6OCH_3), 3.75, 3.72, 3.64, 3.58 (2 × AB, J_{AB} = 10.5 Hz, 2H, CH_2 ester), 1.33 (d, *J* = 7.5 Hz, 3H, CH_3 Ala), 0.98, 0.96 (2 × s, 3H, 2′CCH_3), 0.85, 0.84 (2 × s, 9H, 3 × CH_3 ester). ^{13}C NMR (126 MHz, MeOD) δ 175.06, 174.80 (2 × d, $^3J_{C–C–N–P}$ = 5.0 Hz, C=O ester), 162.73 (C6), 161.86 (C2), 154.55, 154.51 (C4), 148.00, 147.95 (d, $^2J_{C–O–P}$ = 3.8 Hz, ipso naph), 139.36, 139.08 (CH8), 136.27, 136.25 (C10-naph), 128.86, 128.80 (CH-naph), 127.88, 127.73 (2 × d, $^3J_{C–C–O–P}$ = 6.3 Hz, C9-naph), 127.48, 126.53, 126.49, 125.97, 122.81, 122.76 (CH-naph), 116.24, 116.22 (C2-naph), 116.19, 115.63 (C5), 93.34, 93.18 (C1′), 82.32, 82.16 (2 × d, $^3J_{C–C–O–P}$ = 8.8 Hz, C4′), 79.99, 79.95 (C2′), 75.52, 75.37 (CH_2 ester), 74.95, 74.70 (C3′), 68.11, 67.62 (2 × d, $^2J_{C–O–P}$ = 5.0 Hz, C5′), 54.28, 54.07 (6OCH_3)51.79, 51.71 (Ca Ala), 32.26, 32.22 (C ester), 26.74, 26.71 (3 × CH_3 ester), 20.89, 20.69 (2 × d, $^3J_{C–C–N–P}$ = 6.3 Hz, CH_3 Ala), 20.39, 20.35 (2′CCH_3). HPLC R_t = 20.95, 21.48 min.
16. Faletto, M. B.; Miller, W. H.; Garvey, E. P.; St. Clair, M. H.; Daluge, S. M.; Good, S. S. *Antimicrobial Agents Chemother.* **1997**, *41*, 1099.