Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c5ob00427f

Aspartic acid based nucleoside phosphoramidate prodrugs as potent inhibitors of hepatitis C virus replication†

Munmun Maiti,[a] Mohitosh Maiti,[a] Jef Rozenski,[a] Steven De Jonghe[a,b] and Piet Herdewijn*[a,b]

Received 3rd March 2015,
Accepted 26th March 2015

DOI: 10.1039/c5ob00427f

www.rsc.org/obc

In view of a persistent threat to mankind, the development of nucleotide-based prodrugs against hepatitis C virus (HCV) is considered as a constant effort in many medicinal chemistry groups. In an attempt to identify novel nucleoside phosphoramidate analogues for improving the anti-HCV activity, we have explored, for the first time, aspartic acid (Asp) and iminodiacetic acid (IDA) esters as amidate counterparts by considering three 2′-*C*-methyl containing nucleosides, 2′-*C*-Me-cytidine, 2′-*C*-Me-uridine and 2′-*C*-Me-2′-fluoro-uridine. Synthesis of these analogues required protection for the vicinal diol functionality of the sugar moiety and the amino group of the cytidine nucleoside to regioselectively perform phosphorylation reaction at the 5′-hydroxyl group. Anti-HCV data demonstrate that the Asp-based phosphoramidates are ~550 fold more potent than the parent nucleosides. The inhibitory activity of the Asp-ProTides was higher than the Ala-ProTides, suggesting that Asp would be a potential amino acid candidate to be considered for developing novel antiviral prodrugs.

Introduction

Hepatitis C virus (HCV) infection represents a global health problem, affecting approximately 150 million people worldwide according to the World Health Organization (WHO).[1] HCV infection is the leading cause of life threatening liver diseases such as liver cirrhosis or hepatocellular carcinoma (HCC).[2] Previously as a standard of care (SOC), regular injection of pegylated interferon-α (Peg-INF) and oral administration of ribavirin (RBV) have been considered for the treatment, neither of which were specific inhibitors of HCV, and were associated with side effects and had limited efficacy in at least half of the patient population.[3,4] Consequently, the development of alternative treatment options was greatly necessary. Three newly approved protease inhibitors, telaprevir, boceprevir and simeprevir, have demonstrated improved efficacy in combination with Peg-INF and RBV, but their use is also associated with side effects.[5,6] Therefore, there has been an intense effort to develop alternative direct-acting antiviral agents (DAAs) that are more efficacious, have an improved safety profile and a high barrier to resistance, and are pan-genotypic.[7,8]

The RNA-dependent RNA polymerase (RdRp)[9] enzyme of HCV is essential for its replication, and thus represents a viable target for therapeutic intervention by designing specific inhibitors.[10] In this regard, nucleoside inhibitors (NIs) are found to be attractive due to their potency and high genetic barrier to resistance.[11] Several classes have been identified as potent anti-HCV agents,[12] in which *N*-nucleosides containing a 2′-*C*-Me branched sugar and their phosphate prodrugs were endowed with promising activity both *in vitro* and *in vivo*.[8,13,14] Among them, mericitabine, nucleotide prodrugs β-D-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyluridine (sofosbuvir/GS-7977), INX-08189 and IDX-184 have demonstrated high anti-HCV efficacy (Fig. 1).[15–17] In 2013, sofosbuvir (Sovaldi®) was approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with chronic HCV genotype 1–4 infection including those with hepatocellular carcinoma and those with HCV/HIV-1 co-infection. Mericitabine is currently being evaluated in phase II/III clinical trials. INX-08189 and IDX-184 were in clinical trials and are currently put on hold/discontinued due to toxicity issues.[17] These inhibitors are believed to act as non-obligate chain terminators where the 2′-*C*-Me group prevents an incoming nucleoside triphosphate from binding to the active site of NS5B polymerase.[8,18,19] One of the factors that

[a]*Rega Institute for Medical Research, Medicinal Chemistry, KU Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium.*
E-mail: Piet.Herdewijn@rega.kuleuven.be; Fax: +32 16 337340; Tel: +32 16 337387
[b]*Interface Valorisation Platform, KU Leuven, Kapucijnenvoer 33, 3000 Leuven, Belgium*

† Electronic supplementary information (ESI) available: Scheme S1, Fig. S1 and S2, NMR spectra and HPLC purity profile of the final compounds. See DOI: 10.1039/c5ob00427f

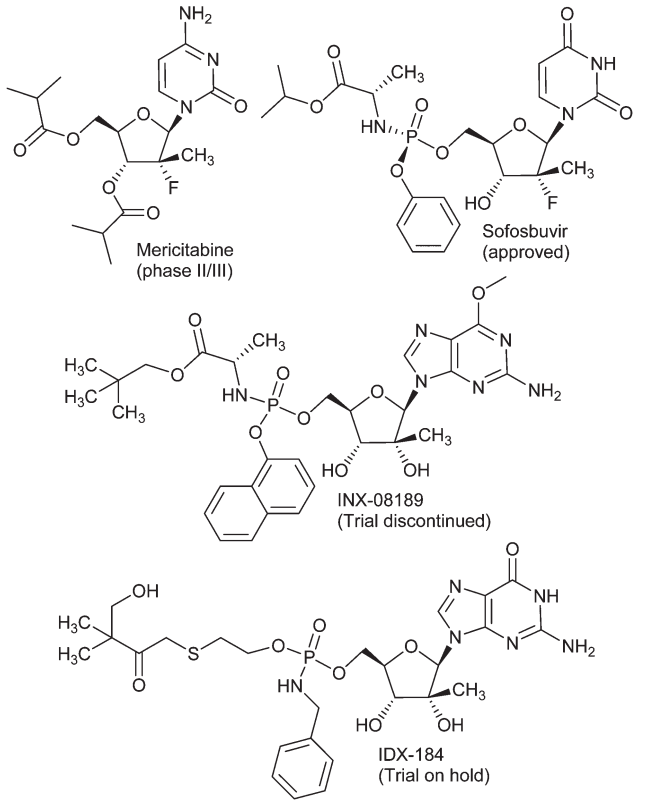


Fig. 1 Chemical structures of clinically advanced and approved anti-HCV drugs.

potentially limit the activity of such nucleoside inhibitors is their poor conversion into the pharmacologically active triphosphate derivative where the first phosphorylation step is rate-limiting. To circumvent this problem, application of the phosphoramidate ProTide approach, developed by McGuigan *et al.*,[20,21] has provided success to deliver the nucleoside monophosphate inside the cells.[22,23] The delivery of monophosphates inside the hepatic cells has an additional advantage in a sense that the first pass metabolism could be exploited where the liver enzymes would hydrolyze the carboxyl esters of the phosphoramidate moiety triggering a cascade of chemical and enzymatic events that would eventually produce the required monophosphate at the desired site of action, the liver.

As a part of our effort to identify novel nucleoside phosphoramidate analogues against HCV, we have explored aspartic acid (Asp) and iminodiacetic acid (IDA) esters as amidate counterparts. In known antiviral and antitumor prodrugs, L-alanine is the preferred amino acid motif. Other amino acids (such as L-aspartic acid) have not been explored in detail since in the original McGuigan report[20] L-alanine was found to be optimal for antiviral activity. Herein, three known 2′-methyl bearing nucleosides (Fig. 2), 2′-*C*-methyl-cytidine (**1**, NM107), 2′-*C*-methyl-uridine (**2**) and 2′-*C*-methyl-2′-fluoro-uridine (**3**, nucleoside of sofosbuvir), have been chosen as the nucleoside part to demonstrate the proof-of-concept, that is to identify the potency of Asp and IDA esters containing phosphoramidates when they are coupled to a moderately active (**1** or **2**) or an inactive (**3**) nucleoside. A comparative study is performed using L-alanine methyl ester of nucleosides **1** and **2**, because L-alanine is more commonly used for developing phosphoramidate analogues. In the present study, Asp and IDA have been chosen since it was previously shown by our group that their phosphoramidate analogues (that is phosphoramidic acid), for example L-Asp-dAMP,[24,25] L-Asp-d4TMP,[26] IDA-d4TMP,[26] can act as direct substrates for viral polymerase like HIV-1 RT in an *in vitro* enzymatic incorporation assay.[27] Apart from this, the advantage of Asp/IDA over alanine is that the side chain (β-COOH group) of aspartic acid could be functionalized, in other words two different ester functions can be introduced to design new bioconjugates to tune the bioavailability and targeted drug delivery simultaneously. Moreover, structurally altered new prodrug molecules with improved potency could be beneficial to lower the effective concentration required. In this context, we have synthesized a series of phenoxy phosphoramidate ProTide analogues, as shown in Fig. 2 and evaluated their inhibitory activity against hepatitis C virus. A brief structure–activity relationship (SAR) study has been performed by varying the ester functionality in the carboxyl acid groups of Asp and IDA. In addition, we have performed stability studies of two active congeners in human serum and investigated their metabolism in human liver S9 fractions. Antiviral activity, serum stability and liver metabolism studies together suggest that the aspartic acid ester is endowed with high potency to be considered for designing novel phosphoramidate-based antiviral prodrugs.

Results and discussion

Chemistry

The synthesis of various aspartic acid esters (**5–8**) required for the synthesis of phosphoramidate analogues was carried out using thionyl chloride and the respective alcohol starting from L-aspartic acid **4** (Scheme 1). Differentially protected ester **11** was prepared from commercially available protected aspartic acid **9** (Scheme 1) using a coupling reagent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), followed by the deprotection of the *tert*-butyloxycarbonyl (Boc) group at room temperature under acidic conditions.[28]

The synthesis of 2′-*C*-Me containing nucleosides **1** and **2** was accomplished *via* the Vorbrüggen glycosylation method starting from the commercially available benzoyl-protected 2′-*C*-Me ribose sugar **12**, followed by the deprotection of the benzoyl group in a methanolic ammonia solution at room temperature (Scheme 2).[29]

To assist in both the 5′-regioselectivity of phosphorylation and the general organic solubility of the nucleoside, isopropylidene protection was introduced for the 2′,3′-diol unit of the sugar moiety (Scheme 2) to obtain protected nucleosides **15** and **16**. They have been used for the synthesis of phosphoramidates **1b–d** and **2a–i** where the isopropylidene group was

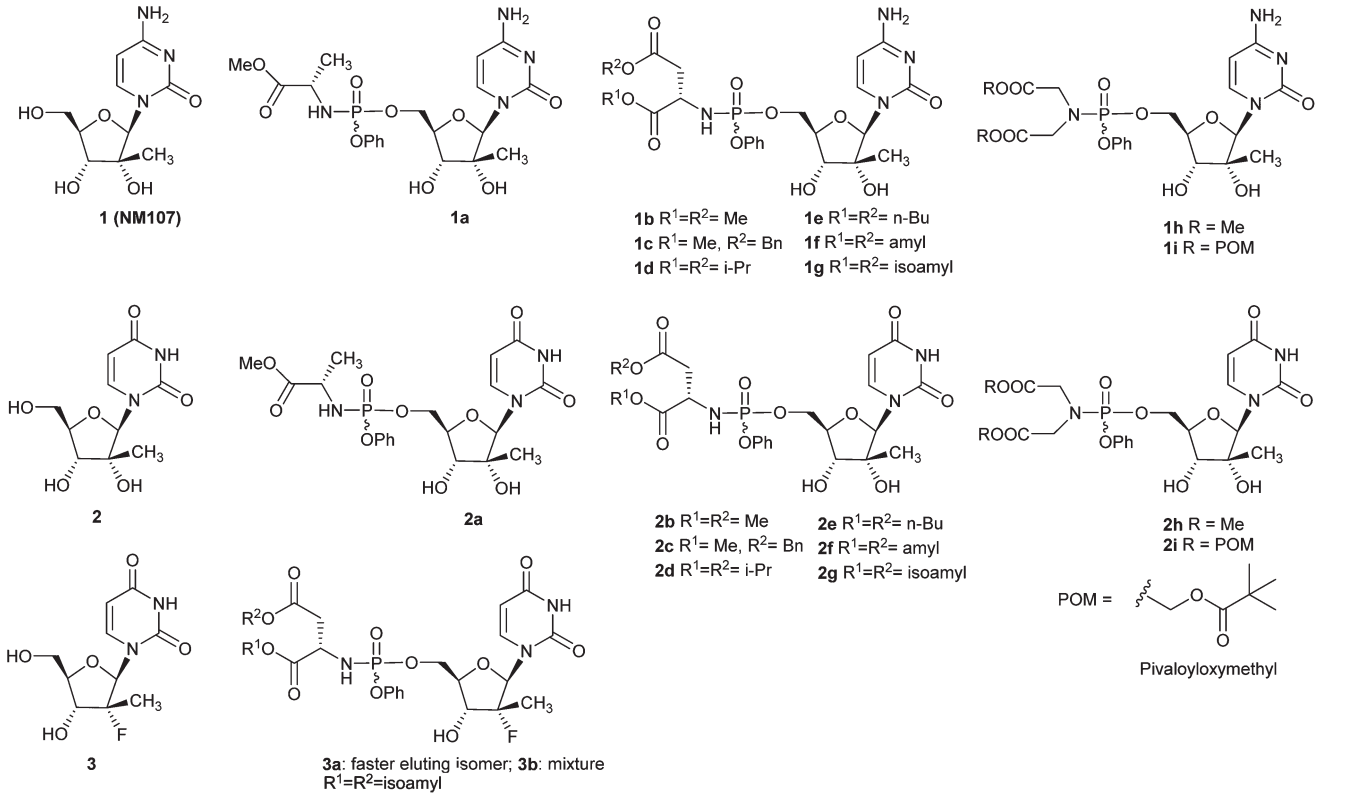
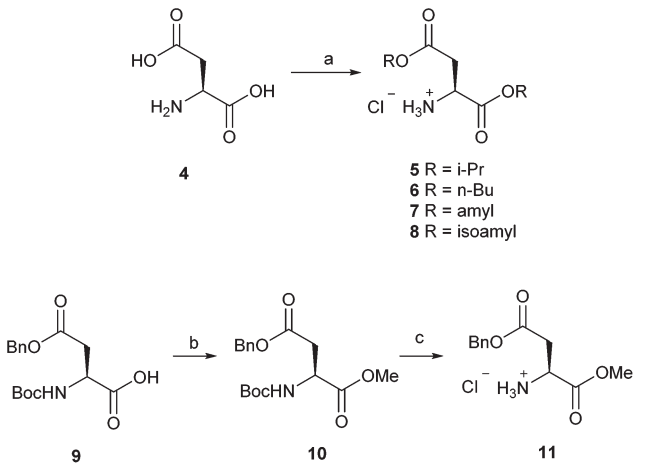
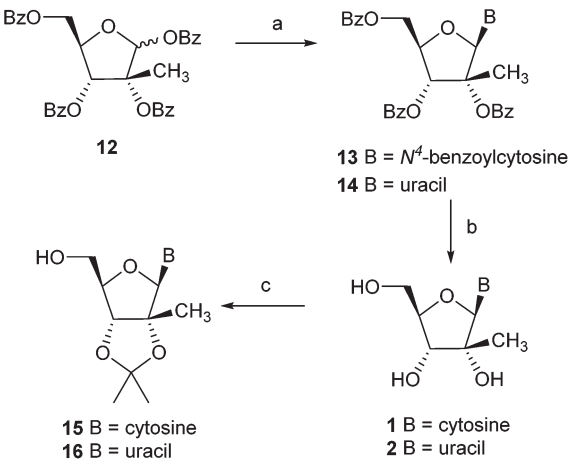


Fig. 2 Structures of nucleosides and their phosphoramidate analogues synthesized and evaluated in the study.



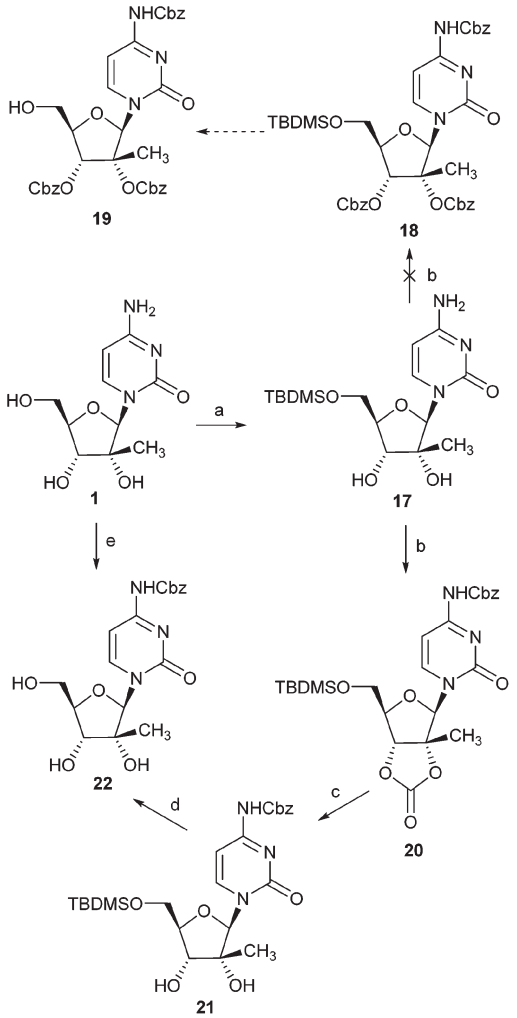
Scheme 1 Synthesis of aspartic acid esters used in the synthesis of phosphoramidate analogues. *Reagents and conditions*: (a) $SOCl_2$, i-PrOH/*n*-BuOH/amyl alcohol/isoamyl alcohol, 0 °C to rt, 12 h, then reflux, 3 h/heat to 50 °C for **8**, 40–94%; (b) EDC·HCl, Et_3N, MeOH, CH_2Cl_2, 0 °C to rt, 24 h, 72%; (c) 5–6 N HCl in i-PrOH, CH_2Cl_2, rt, 3–4 h, 75%.



Scheme 2 Synthesis of 2′-*C*-Me-cytidine (**1**) and 2′-*C*-Me-uridine (**2**) nucleosides and their corresponding 2′,3′-*O*-isopropylidene protected analogues **15** and **16**. *Reagents and conditions*: (a) N^4-benzoylcytosine/uracil, *N*,*O*-bis(trimethylsilyl)acetamide, MeCN, 80 °C, 1 h, then **12**, $SnCl_4$, reflux, 3 h, 80% for **13**, 90% for **14**; (b) sat. NH_3 in MeOH, rt, overnight, 80% for **1**, 91% for **2**; (c) *p*-TSA, acetone, 2,2-dimethoxypropane, rt, overnight, 90% for **15**, 80% for **16**.

deprotected using aqueous 80% TFA at room temperature without affecting the amino acid ester part (Scheme 6).[30]

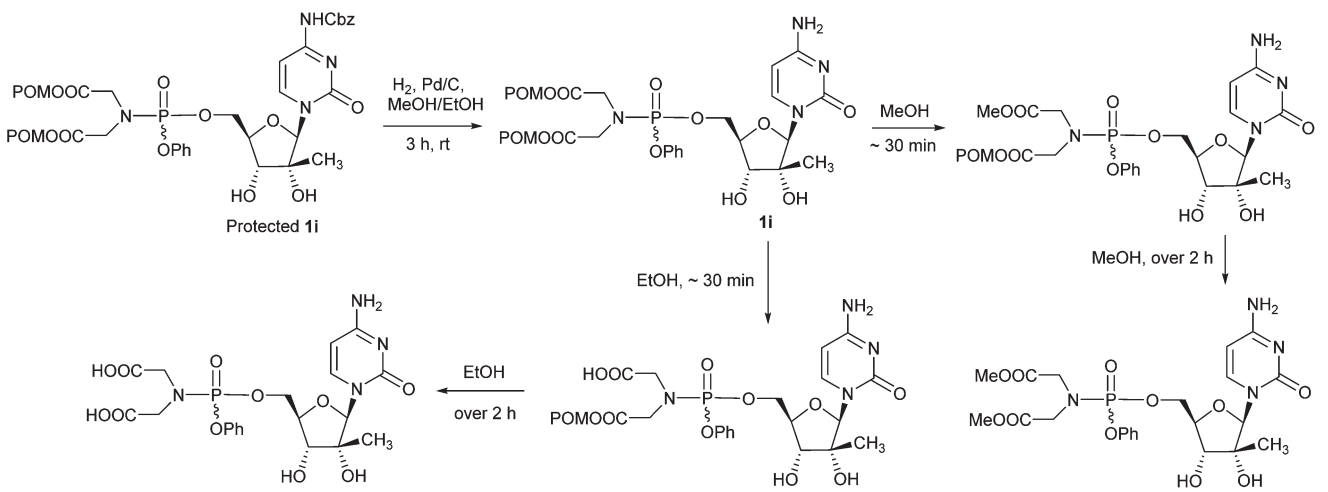
During the phosphoramidate synthesis of nucleoside **15**, the nucleophilicity of the amino group of the cytosine moiety was found to be a major issue to selectively perform reaction at the 5′-hydroxyl group. In most of the cases, an *N*-phosphorylated side product was formed in a large quantity. To overcome



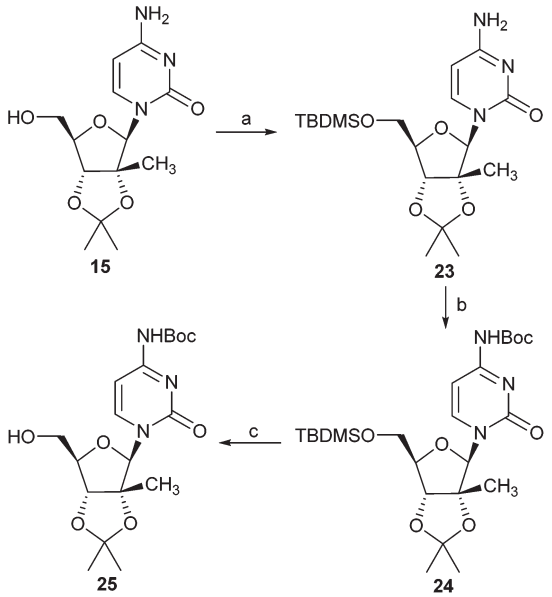
Scheme 3 (a) Imidazole, TBDMSCl, anh. pyridine, 0 °C to rt, overnight, 97%; (b) benzyl chloroformate, DMAP, dry CH_2Cl_2, 0 °C to rt, 24 h, 80%; (c) pyridine–H_2O (1 : 1), reflux, 2 h; (d) $Et_3N{\cdot}3HF$, dry THF, rt, overnight, 85%; (e) TMSCl, anh. pyridine, 0 °C to rt, 1.5 h, then benzyl chloroformate, 0 °C to rt, 2 h, 90%.

this difficulty, we first attempted to synthesize carboxybenzyl (Cbz)-protected nucleoside **19** (Scheme 3) to utilize the 5′-hydroxyl group selectively for the phosphoramidate synthesis (followed by the –Cbz deprotection under classical hydrogenation conditions) following a reported procedure which is described for the natural cytidine nucleoside.[31] However, cyclic carbonate **20** was the sole product formed under the reported reaction conditions for our substrate. This might be due to the presence of an additional methyl group at the 2′-position which is imposing a steric hindrance for a second carboxybenzyl protection, thus forming a cyclic carbonate under basic conditions, releasing benzyl alcohol as the leaving group. As a result, compound **22** was obtained by hydrolyzing the cyclic carbonate **20** followed by *tert*-butyldimethylsilyl group deprotection. On the other hand, compound **22** was also synthesized in one-pot reaction using trimethylsilyl (TMS) transient protection (Scheme 3)[32] and has been used to synthesize prodrugs **1a** and **1e–h** where in the final step the –Cbz group was removed by hydrogenation in the presence of Pd/C in ethanol (as shown in Scheme 6).

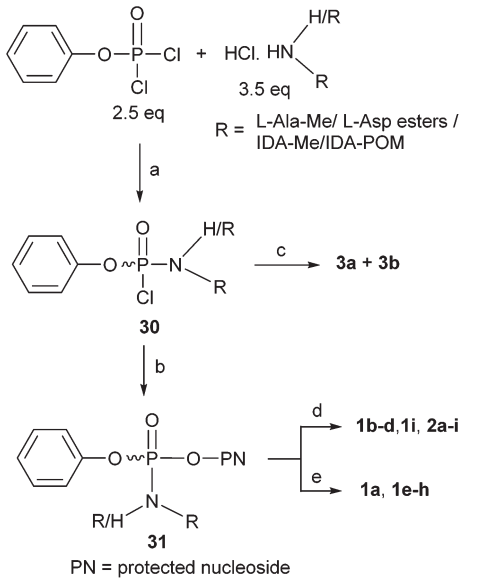
In the synthesis of POM-protected prodrug **1i**, rapid decomposition was observed during neutral deprotection of the –Cbz group under hydrogenation conditions in methanol or ethanol, as shown in Scheme 4. The progress of the reaction was monitored by TLC and the products were identified by mass spectroscopy (data not shown). To overcome the decomposition problem, acid labile protecting groups were considered (to make the amino group non-reactive by protonation) since the nucleophilicity of the amino group of the cytosine moiety was anticipated to be the reason for the lability of product **1i** under neutral deprotection conditions. Therefore, isopropylidene[30] and *tert*-butyloxycarbonyl (Boc)[33] groups were introduced to obtain the protected nucleoside **25** (Scheme 5) where an acidic deprotection strategy was followed to remove the protecting groups in the final step of phosphoramidate synthesis to obtain target compound **1i** (Scheme 6).



Scheme 4 Decomposition of the pivaloyloxymethyl (POM) group of compound **1i** under hydrogenation conditions.



Scheme 5 (a) Imidazole, TBDMSCl, anh. pyridine, 0 °C to rt, overnight, 95%, (b) di-*tert*-butyldicarbonate, THF–dioxane (1 : 1), reflux, 5 h, 77%; (c) $Et_3N{\cdot}3HF$, dry THF–pyridine (1 : 1), 0 °C to rt, 4 h, 92%.



Scheme 6 Synthesis of phenoxy phosphoramidate ProTide analogues. *Reagents and conditions*: (a) *N*-methylimidazole, dry CH_2Cl_2, –15 °C to rt, 10–12 h; (b) requisite protected nucleoside **15** (for phosphoramidates **1b–d**) or **16** (for phosphoramidates **2a–i**) or **22** (for phosphoramidates **1a**, **e–h**), or **25** (for phosphoramidate **1i**), dry CH_2Cl_2, –5 °C to rt, 4–6 h; (c) fluoro-nucleoside **3**, dry CH_2Cl_2, –5 °C to rt, 24 h; (d) TFA–H_2O (8 : 2, 0.1 M), 0 °C to rt, 3–6 h; (e) 10% Pd/C, H_2, EtOH, rt, 3 h.

The synthesis of fluoro-nucleoside **3** (PSI-6206, Fig. 2) was performed following the literature procedure[34,35] with modification in a few steps (Scheme S1 in the ESI†). Several trials for coupling the silylated uracil with protected sugar (**27**) to synthesize protected **3** (**29**) directly turned out to be unsuccessful under different glycosylation conditions. Therefore, nucleoside **3** was obtained *via* cytidine derivative (**28**). During the glycosylation reaction, silylation of N^4-benzoylcytosine was performed using *N*,*O*-bis(trimethylsilyl)acetamide (BSA) instead of previously reported hexamethyldisilazane (HMDS), thus avoiding the tedious removal of HMDS under inert conditions.

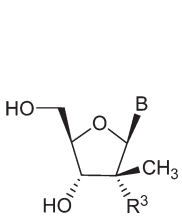
The synthesis of phenoxy phosphoramidate analogues **1a–i**, **2a–i**, **3a** and **3b** (Fig. 2) was achieved by using phosphorochloridate chemistry *via* a one-pot procedure,[36] as shown in Scheme 6. In the first step, a fresh chlorophosphoramidate reagent **30** was prepared by reacting phenyl dichlorophosphate [$PhOP(O)Cl_2$] with the requisite protected amino acids or protected iminodiacetic acids in the presence of *N*-methylimidazole (NMI) in anhydrous CH_2Cl_2. This freshly prepared chlorophosphoramidate reagent was then slowly added to a solution of appropriately protected nucleoside (**15**/**16**/**22**/**25**) to obtain protected nucleoside phosphoramidate analogue **31**. Subsequent removal of the protecting groups from the sugar and the nucleobase moiety provided the target prodrugs **1a–i** and **2a–i**, as shown in Scheme 6. The deprotection reactions were performed either under acidic conditions using trifluoroacetic acid (TFA) or by following hydrogenation using Pd/C 10% under neutral conditions depending on the protecting groups present in the molecule. Synthesis of the fluorine containing prodrugs **3a** and **3b** was performed analogously without using any protection for the fluoro-nucleoside **3**. The final prodrugs were purified by flash column chromatography and preparative HPLC, and were fully characterized by spectroscopy and analytical techniques. The final phosphoramidates were isolated as a diastereomeric mixture due to the stereochemistry arising at the phosphorus centre. The overall yield of the reaction was in the range of 42–86%.

Antiviral activity in the replicon assay

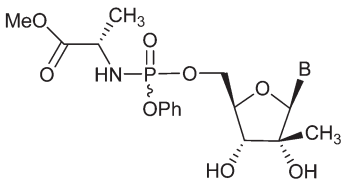
All phosphoramidates (**1a–i**, **2a–i**, **3a** and **3b**) and their parent nucleosides (**1**, **2** and **3**) have been assessed for antiviral activity against HCV 1b Con1 replicon in a 6 pts dose response assay. The anti-HCV drug 'interferon-α' was included in the assay as a positive control. For comparing the antiviral activity data of the L-aspartic acid di-ester prodrugs with the classical L-alanine methyl ester prodrugs, two phosphoramidates **1a** and **2a** were additionally synthesized and included in the study.

As indicated in Table 1, most of the L-aspartic acid containing analogues exhibited anti-HCV efficacy in the submicromolar range in a subgenomic replicon assay, and the EC_{50} values were found to be 25–1500 fold lower than those of the parent nucleosides. Interestingly, many of the Asp-ProTides, in particular those containing higher alkyl esters like *n*-butyl, amyl and isoamyl esters [**1f** (EC_{50}: 0.050 μM), **1g** (EC_{50}: 0.050 μM), **2f** (EC_{50}: 0.030 μM), **2g** (EC_{50}: 0.030 μM)] have demonstrated higher potency in comparison with the methyl alanine containing ProTides **1a** (EC_{50}: 0.45 μM) and **2a** (EC_{50}: 0.29 μM). These data indicate that the Asp-ProTides are also efficiently

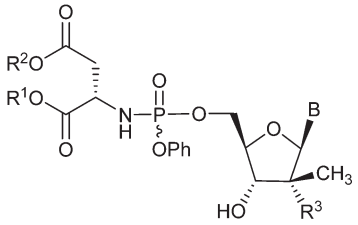
Table 1 Luciferase-based HCV **1b** replicon activity of phosphoramidate analogues



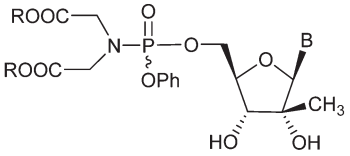
1, **2** R^3=OH
3 R^3=F



1a, **2a**



1b-g, **2b-g** R^3=OH
3a, **3b** R^3=F



1h, **1i**, **2h**, **2i**

ProTide	B	Amino acid/amine	Ester R, R^1 and R^2	EC_{90} (μM)	EC_{50} (μM)	CC_{50} (μM)	Selectivity index	*c* log *P*
1	Cytosine	—	—	6.89	1.34	>100	>74.6	−1.68
1a	Cytosine	Ala	Me	3.94	0.45	75.7	168	−0.75
1b	Cytosine	Asp	Me	29.2	3.71	>100	>27.0	−0.93
1c	Cytosine	Asp	Me (R^1) and Bn (R^2)	7.09	1.32	>100	>75.8	0.79
1d	Cytosine	Asp	i-Pr	6.42	0.96	>100	>104	0.74
1e	Cytosine	Asp	*n*-Bu	0.80	0.26	30.9	119	2.24
1f	Cytosine	Asp	Amyl	0.48	0.050	9.53	191	3.30
1g	Cytosine	Asp	Isoamyl	0.46	0.050	9.54	191	3.04
1h	Cytosine	IDA	Me	ND	>100	>100	>1.0	0.70
1i	Cytosine	IDA	POM	ND	44.197	>100	>2.26	2.95
2	Uracil	—	—	55.3	6.31	>100	>15.8	−1.69
2a	Uracil	Ala	Me	0.90	0.29	47.4	163	−0.77
2b	Uracil	Asp	Me	6.60	1.13	>100	>88.5	−0.95
2c	Uracil	Asp	Me (R^1) and Bn (R^2)	0.81	0.26	43.4	167	0.77
2d	Uracil	Asp	i-Pr	0.81	0.29	35.7	123	0.72
2e	Uracil	Asp	*n*-Bu	0.11	0.040	4.64	116	2.22
2f	Uracil	Asp	Amyl	0.12	0.030	7.11	237	3.28
2g	Uracil	Asp	Isoamyl	0.10	0.030	10.0	333	3.02
2h	Uracil	IDA	Me	ND	>10	ND	—	0.68
2i	Uracil	IDA	POM	ND	>10	ND	—	2.93
3	Uracil	—	—	>100	>100	>100	—	−0.92
3a (Faster eluting isomer)	Uracil	Asp	Isoamyl	0.55	0.06	27.7	462	3.79
3b (Mixture)	Uracil	Asp	Isoamyl	0.49	0.06	33.1	551	3.79
rINFα-2b				0.22	0.09	>2.0	>22.2	—

processed by the cellular enzymes to deliver the corresponding 5′-monophosphate that exhibits antiviral activity *via* formation of the corresponding triphosphate, thus able to bypass the first rate-limiting step in the kinase pathway.

In order to shed light on the Asp-ProTide strategy further, we have extended this approach on the nucleoside (**3**, PSI-6206) part of the recently approved prodrug sofosbuvir. It has been reported in the literature that the nucleoside **3** (PSI-6206) is completely devoid of activity (EC_{50}: >100 μM, data reproduced in the present study), whereas its phosphoramidate prodrug sofosbuvir is highly active and the activity was found to be entirely dependent on the intracellular phosphorylation by different cellular kinases.[15] Therefore, inclusion of this nucleoside and its Asp-phosphoramidate in the present study could clearly provide insight into the success of the Asp-ProTide approach. The anti-HCV data demonstrate that the Asp-ProTides **3a** and **3b** were highly potent (EC_{50}: 0.06 μM), while the corresponding nucleoside **3** is completely inactive. The favourable potency profile of the Asp-ProTides suggests that L-aspartic acid is a good alternative for developing potent antiviral phosphoramidate prodrugs.

An initial screening of the structure activity relationship (SAR) indicated a clear correlation between ProTide's lipophilicity and biological activity, that is bulkier and more lipophilic alkyl esters demonstrated higher potency. For example, while methyl esters of aspartic acid ProTides (**1b** and **2b**) showed activity in the micromolar range (EC_{50}: 3.71 μM and EC_{50}: 1.13 μM respectively), their amyl and isoamyl esters demonstrated activity in the submicromolar range. This is probably due to enhanced passive diffusion through the lipid-rich cell membrane.

The most interesting results were obtained in the case of Asp-ProTides of nucleoside **2** which was previously found to have lower activity compared to nucleoside **1**.[37] Therefore, not much attention has been paid so far to its development. Here, we have explored the apparently simple and easily synthesized nucleoside **2** for its further development as Asp-ProTide analogues in order to assess the efficacy of the aspartic acid esters as the amidate counterpart. We have observed that this Asp containing phosphoramidate prodrug approach is able to convert a moderately active nucleoside **2** or an inactive nucleoside **3** into a highly active antiviral compound. Antiviral data

demonstrate that while the parent nucleoside **2** displays an EC_{50} value of 6.31 μM, all its Asp-ProTides are highly active with an EC_{50} value of 1.13–0.03 μM. For the most potent analogues, the activity is higher than for the alanine containing ProTide **2a** (EC_{50}: 0.29 μM). A similar trend was observed for nucleoside **3** and its prodrug as mentioned earlier. These data support aspartic acid as a potential substitute for L-alanine in developing new phosphoramidate analogues.

In the case of higher alkyl esters bearing phosphoramidates (*n*-butyl, amyl and isoamyl), cytotoxicity was observed in the lower micromolar range, which was also reported recently by M. J. Sofia *et al.*[15] However, their higher potency still leads to favourable selectivity index values in the range of 100–550. One possible reason behind cytotoxicity could be the accumulation of fatty alcohols (long-chain alcohols) inside the cells that are generally released by the action of esterase-type enzymes during the activation of the phosphoramidate prodrugs.[38,39] Given the low concentration at which these ProTides are active (EC_{50}: 0.05–0.03 μM), this might not be the case here. On the other hand, it is also reported that fatty alcohols are effectively eliminated from the body when exposed.[40] Alternatively, cytotoxicity might arise due to the increased concentration and/or retention of the modified nucleotides inside the cells for a longer time, which can act as inhibitors for the cellular or mitochondrial polymerases due to their structural resemblance to the natural ones. Quite often, the mitochondrial toxicity becomes a major issue in pursuing an active nucleos(t)ide molecule for further development.[41]

Our attempt to explore IDA esters as the amidate part was not successful since none of these analogues (**1h**, **1i**, **2h** and **2i**) showed potency in the HCV replicon assay. Although previously the POM-IDA moiety provided indication of delivering monophosphates inside the cells in the case of herpesvirus infected embryonic lung cell lines,[42] no activity was observed here. It could be that these compounds have been poorly processed by hepatic enzymes to release monophosphates inside the cells. On the other hand, the further processing to the di- and triphosphate might be more efficient in the herpes infected lung cells than in the HCV infected hepatocytes, since it is generally known that the level of enzyme expression is highly dependent on the type of cell line.[43]

Among all 2′-*C*-Me-cytidine ProTides (**1a–i**), **1f** and **1g** (EC_{50}: 0.05 μM) are the most active analogues and compound **1g** has been chosen for further biochemical studies. In the 2′-*C*-Me-uridine series (**2a–i**), ProTides **2f** and **2g** are the most potent (EC_{50}: 0.03 μM) compounds. However, we have decided to select compound **2c** (EC_{50}: 0.26 μM) for further biochemical evaluation because the substitution profile (Me and benzyl ester) is different from that of compound **1g** (containing a di-isoamyl ester).

Stability study in human serum and a metabolism study in a human liver S9 fraction

The interesting antiviral profile of these analogues led us to consider a few preliminary biochemical studies for understanding their stability in the biological medium and in the hepatic system. The stabilities of two ProTides, **1g** and **2c**, were investigated at 37 °C in the presence of human serum using ^{31}P NMR spectroscopy. The ProTide was dissolved in a mixture of DMSO-d_6 (0.06 mL) and D_2O (0.18 mL), and then human serum (0.35 mL) was added. The reaction was monitored over a period of 16 h, as shown in Fig. 3.

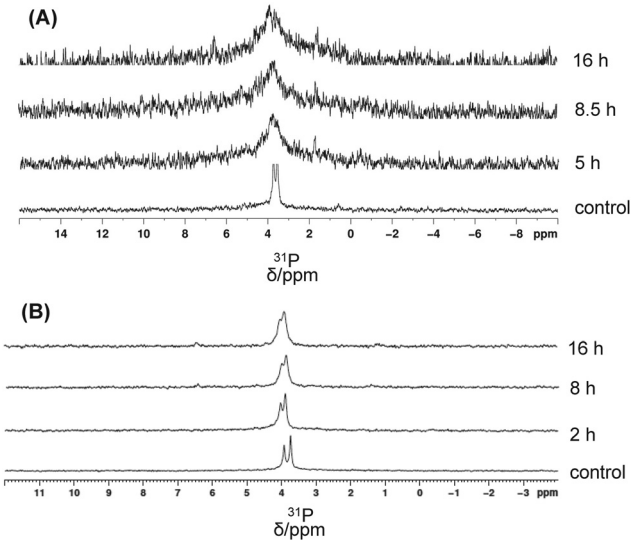


Fig. 3 Stack plot of ^{31}P NMR spectra depicting the stability of ProTides (A) **1g** and (B) **2c** in human serum at different time intervals. Control: no serum is added.

It was observed that both ProTides were reasonably stable under these conditions. In fact, nearly 80–90% of the parent compound was still present after 16 hours of incubation at 37 °C. In the case of compound **1g**, the quality of the spectra deteriorated due to the formation of a large amount of precipitate. However, the appearance of the parent phosphorus peaks reflects that not much decomposition has occurred in serum for this prodrug as well. Therefore, from the stability profile, it is clear that this type of phosphoramidate derivative is reasonably stable in human serum, which allows intact uptake by the target cells.

A metabolism study was performed in a human liver S9 fraction which was chosen as a surrogate *in vitro* model to test the release of monophosphates in hepatocytes. Studies showed that in the case of ProTide **2c**, first the β-carboxyl ester of the aspartate moiety was hydrolyzed (intermediate **32** identified by LC/MS, Fig. S1 and S2 in the ESI†) by the action of liver esterases, followed by the release of monophosphates slowly, probably through the α-carboxyl group deprotection, which is shown to be responsible for the nucleophilic attack at the phosphorus centre to displace the aryl group[44], subsequently releasing monophosphate **33** (Fig. S1 and S2†) by the action of phosphoramidase-type enzyme. Formation of **33** was additionally proved by co-injecting the chemically synthesized **33** (shown in Fig. 4) with the reaction aliquots during HPLC analysis. However, its quantification was not possible due to the difficulties in separation from the intermediate **32** under

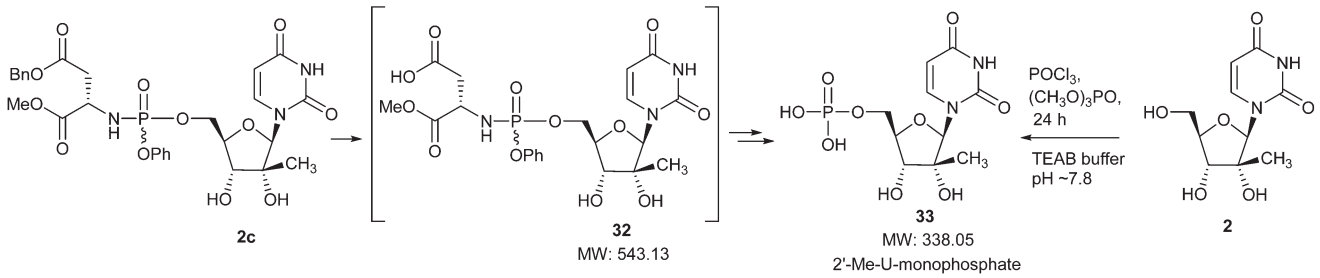


Fig. 4 Metabolism of ProTide **2c** in the human liver S9 fraction leading to the formation of 2′-Me-U monophosphate and its chemical synthesis from nucleoside **2**.

various RP-HPLC gradient conditions screened. Formation of the intermediate **32** and monophosphate **33** from ProTide **2c** in the liver S9 fraction is schematically represented in Fig. 4. These data indicate that the release of the nucleoside monophosphate is possible for aspartic acid based phosphoramidates where the liver enzymes are capable of processing these analogues.

Conclusions

We have demonstrated that the application of the phosphoramidate prodrug approach using aspartic acid was successful to deliver the nucleoside monophosphate inside the hepatic cells. For this purpose, a series of phosphoramidate ProTides of 2′-*C*-Me-cytidine, 2′-*C*-Me-uridine and 2′-*C*-Me-2′-F-uridine bearing alanine, aspartic acid and iminodiacetic acid esters have been prepared and evaluated for antiviral potency against hepatitis C virus (HCV) in a replicon assay. In particular, aspartic acid containing 2′-*C*-Me-uridine phosphoramidate analogues were found to be active in the replicon assay, often 5–200 fold more potent than the corresponding moderately active nucleoside 2′-*C*-Me-uridine. Interestingly, the Asp-ProTide strategy applied on the nucleoside of sofosbuvir was also successful in retaining the high antiviral potency. The present study thus indicates that it is also possible to bypass the rate limiting first phosphorylation step by using the Asp-ProTide strategy. In some cases, the activity is higher than that of the alanine containing ProTide, thus suggesting that aspartic acid is a potential amino acid alternative to be considered for designing antiviral phosphoramidate prodrugs. The advantage of using a bifunctional amino acid is that while the α-carboxyl group would play the role in aryl displacement (*via* neighbouring group assistance), the side chain functional group could be utilized to couple biomolecules for tuning the physicochemical properties of the ProTide. Other amino acids with a functional side chain could also be efficacious in this direction. Furthermore, it would also be beneficial to apply this prodrug approach for other modified nucleosides against other viruses. An effort for their synthesis and experimental evaluation is currently ongoing in our group to shed light on this rationale.

Experimental

General section

NMR spectra were recorded on a Bruker Avance II 300 MHz with a 5 mm broad band probe, a 500 MHz spectrometer equipped with a TXI-HCP Z gradient probe or on a Bruker Avance II 600 MHz spectrometer with a 5 mm TCI-HCN Z gradient cryo-probe. The spectra were processed with Bruker Topspin 2.1 software. Chemical shifts (δ) were expressed in parts per million (ppm). The ^{1}H and ^{13}C NMR chemical shifts were referenced relative to the TMS peak (δ = 0.00 ppm). ^{31}P NMR chemical shifts were referenced to an external 85% H_3PO_4 standard (δ = 0.00 ppm). All signals in proton and carbon 1D NMR spectra were assigned by 2D COSY, natural abundance ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC NMR spectra. Mass spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL min^{-1} and spectra were obtained in positive (or in negative) ionization mode with a resolution of 15 000 (FWHM) using leucine enkephalin as the lock mass. HPLC/MS was performed on a UPLC chromatograph (Acquity H Class, Waters, Milford, MA) coupled to a quadrupole time-of-flight mass spectrometer (Synapt G2, Waters, Milford, MA). Column: C18 Acquity, HSS T3 1.8 μM, 1.0 × 50 mm. Flow rate 150 μl min^{-1}. Mobile phase A: H_2O, mobile phase B: 50% acetonitrile. Gradient: 2% to 32% B in 5 minutes. Chemicals of analytical and synthetic grade were obtained from commercial sources and were used as such. Flash silica column chromatography was performed on silica gel 60 A, 0.035–0.070 mm (Acros Organics). The purity of the final compounds was determined by RP-HPLC analysis which is provided in the ESI.† All compounds were at least 95% pure. Human serum and human liver S9 fraction were purchased from Sigma Aldrich and Invitrogen respectively and stored at −80 °C until the time of use.

Chemistry

General procedure for synthesis of phosphoramidates (protected 1a–i and 2a–i). *Step*-1: A solution/suspension of the appropriate amine/amino acid ester hydrochloride salt (3.5 equiv.) in anhydrous CH_2Cl_2 was prepared and cooled to −15 °C. Phenyl dichlorophosphate (2.5 equiv.) was added

slowly. After 10 minutes, a solution of *N*-methylimidazole (10 equiv.) in dry CH_2Cl_2 was added dropwise. The mixture was allowed to reach room temperature slowly and left to stir for 10–12 h.

Step-2: In a separate flask, a solution/suspension of an appropriately protected nucleoside (1 equiv.) in anhydrous CH_2Cl_2 was cooled to −5 °C. With stirring, the solution prepared above was added slowly over a period of 1 h, keeping the temperature near −5 °C. The cooling bath was removed, and the reaction was left to stir at room temperature (for nearly 4–6 h) until TLC indicates a reasonable amount of product formation. The reaction mixture was then evaporated to dryness under reduced pressure, and the residue was purified by flash column chromatography eluting with CH_2Cl_2–MeOH or EtOAc–hexane in different proportions. The overall yield of the reaction is in the range of 30–90%.

General procedure for acetonide (and Boc) deprotection. The protected phosphoramidate was dissolved in a solution of TFA–H_2O (8 : 2, 0.1 M) and was stirred at room temperature until TLC shows no starting material (typically 3–6 h). The reaction mixture was evaporated to dryness and coevaporated with toluene thrice. The solid material was then dissolved in methanol and evaporated with silica gel and purified by flash column chromatography eluting with MeOH–CH_2Cl_2 in different proportions (generally 2–5% methanol in CH_2Cl_2) to obtain the required compound as a white solid. The overall yield of the reaction is in the range of 42–86%.

General procedure for *N*-Cbz deprotection. To a solution of Cbz-protected phosphoramidate in EtOH (5 mL $mmol^{-1}$) was added 10% Pd/C at room temperature. The mixture was stirred under a H_2 atmosphere for 3 h. The suspension was filtered and washed with methanol. The filtrate was evaporated to dryness and purified by flash column chromatography eluting with MeOH–CH_2Cl_2 in different proportions (generally 2–5% methanol in CH_2Cl_2) to furnish the required compound as a white solid. The overall yield of the reaction is in the range of 57–84%.

2′-*C*-Methyl-*N*^{4}-(benzyloxycarbonyl)cytidine-5′-[phenyl(methoxy-L-alaninyl)]phosphate (protected 1a). Yield: 50%; R_f = 0.4 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 2.91, 2.87 ppm; HRMS (ESI+) calcd for $C_{28}H_{34}N_4O_{11}P$ $[M + H]^+$ 633.1956, found 633.1963.

2′-*C*-Methyl-cytidine-5′-[phenyl(methoxy-L-alaninyl)]phosphate (1a). Yield: 77%; R_f = 0.2 (CH_2Cl_2–MeOH, 9.0 : 1.0); ^{1}H NMR (500 MHz, MeOD): δ = 7.72–7.69 (2 d, 1H, H-6), 7.39–7.18 (a series of multiplets, 5H, OPh), 6.06, 6.04 (2 s, 1H, H-1′), 5.85–5.82 (2 d, 1H, H-5), 4.58–4.34 (m, 2H, H-5′ & H-5″), 4.12–4.08 (m, 1H, H-4′), 4.01–3.93 (m, 1H, H-α-ala), 3.75 (d, 1H, H-3′), 3.69, 3.66 (2 s, 3H, OCH_3-ala), 1.36–1.32 (2 d, 3H, CH_3-ala), 1.10, 1.08 ppm (2 s, 3H, –CH_3-2′). ^{13}C NMR (125 MHz, MeOD): δ = 176.4 (d, $^3J_{CP}$ = 4.15 Hz, –CO), 176.1 (d, $^3J_{CP}$ = 5.34 Hz, –CO), 168.3 (C-4), 159.3 (C-2), 153.0 (phenyl C), 143.0, 142.9 (C-6), 131.7 (phenyl C), 127.1 (phenyl C), 122.3–122.1 (phenyl C), 97.1, 97.0 (C-5), 94.9, 94.7 (C-1′), 82.2 (C-4′), 80.6, 80.5 (C-2′), 74.9, 74.7 (C-3′), 67.1 (d, $^3J_{CP}$ = 5.32 Hz, C-5′), 66.7 (d, $^3J_{CP}$ = 4.74 Hz, C-5′), 53.6 (OCH_3-ala), 52.5, 52.3 (C-α-ala), 21.4 (CH_3-ala), 21.1 ppm (CH_3-2′); ^{31}P NMR (202 MHz, MeOD): δ = 3.83 and 3.70 ppm; HRMS (ESI+) calcd for $C_{20}H_{27}N_4O_9P$ $[M + H]^+$ 499.1588, found 499.1589.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-cytidine-5′-[phenylbis(methoxy-L-aspartyl)]phosphate (protected 1b). Yield: 30%; R_f = 0.25 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 2.98, 2.53 ppm; HRMS (ESI+) calcd for $C_{25}H_{34}N_4O_{11}P$ $[M + H]^+$ 597.1956, found 597.1965.

2′-*C*-Methyl-cytidine-5′-[phenylbis(methoxy-L-aspartyl)]phosphate (1b). Yield: 70%; R_f = 0.15 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (500 MHz, MeOD): δ = 7.72–7.69 (2 d, 1H, H-6), 7.39–7.18 (a series of multiplets, 5H, OPh), 6.06, 6.05 (2 s, 1H, H-1′), 5.88–5.84 (2 d, 1H, H-5), 4.61–4.36 (m, 2H, H-5′ & H-5″), 4.33–4.28 (m, 1H, H-α-asp), 4.12–4.08 (m, 1H, H-4′), 3.79–3.76 (2 d, 1H, H-3′), 3.70, 3.65, 3.63, 3.60 (4 s, 6H, OCH_3-asp), 2.86–2.72 (m, 2H, H-β-asp), 1.10, 1.08 ppm (2 s, 3H, –CH_3-2′); ^{13}C NMR (125 MHz, MeOD): δ = 174.4 (d, $^3J_{CP}$ = 4.44 Hz, –CO-α), 174.1 (d, $^3J_{CP}$ = 5.53 Hz, –CO-α), 173.2, 173.0 (–CO-β), 168.2 (C-4), 159.5 (C-2), 153.0, 152.9 (phenyl C), 143.1, 143.0 (C-6), 131.8 (phenyl C), 127.2 (phenyl C), 122.3–122.2 (phenyl C), 97.3 (C-5), 94.7 (C-1′), 82.2–82.1 (C-4′), 80.6, 80.5 (C-2′), 74.9, 74.7 (C-3′), 67.2 (d, $^3J_{CP}$ = 4.69 Hz, C-5′), 66.9 (d, $^3J_{CP}$ = 4.69 Hz, C-5′), 54.0, 53.9 (OCH_3-asp), 53.6, 53.5 (C-α-asp), 53.3 (OCH_3-asp), 40.1–40.0 (C-β-asp), 21.2 ppm (CH_3-2′); ^{31}P NMR (202 MHz, MeOD): δ = 3.65 and 3.52 ppm; HRMS (ESI+) calcd for $C_{22}H_{30}N_4O_{11}P$ $[M + H]^+$ 557.1643, found 557.1642.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-cytidine-5′-[phenyl-(α-methoxy-β-benzyloxy-L-aspartyl)]phosphate (protected 1c). Yield: 40%; R_f = 0.27 (CH_2Cl_2–MeOH, 9.5 : 0.5); HRMS (ESI+) calcd for $C_{31}H_{38}N_4O_{11}P$ $[M + H]^+$ 673.2269, found 673.2270.

2′-*C*-Methyl-cytidine-5′-[phenyl(α-methoxy-β-benzyloxy-L-aspartyl)]phosphate (1c). Yield: 71%; R_f = 0.22 (CH_2Cl_2–MeOH, 9.2 : 0.8); ^{1}H NMR (500 MHz, MeOD): δ = 7.84–7.81 (2 d, 1H, H-6), 7.38–7.16 (a series of multiplets, 10H, OPh & CH_2*Ph*), 6.03, 6.02 (2 s, 1H, H-1′), 5.98–5.92 (2 d, 1H, H-5), 5.08–5.05 (*CH*$_2$Ph), 4.60–4.31 (m, 3H, H-5′, H-5″ & H-α-asp), 4.15–4.08 (m, 1H, H-4′), 3.81–3.78 (2 d, 1H, H-3′), 3.65, 3.60 (2 s, 3H, OCH_3-asp), 2.91–2.76 (m, 2H, H-β-asp), 1.13, 1.12 ppm (2 s, 3H, –CH_3-2′); ^{13}C NMR (125 MHz, MeOD): δ = 174.3 (d, $^3J_{CP}$ = 4.86 Hz, –CO-α), 174.1 (d, $^3J_{CP}$ = 5.53 Hz, –CO-α), 172.5, 172.4 (–CO-β), 165.7 (C-4), 156.0 (C-2), 152.9, 152.8 (phenyl C), 144.3, 144.2 (C-6), 138.0 (phenyl C), 131.8, 130.4, 130.2, 130.1, 127.2, 122.3, 122.2 (phenyl C), 97.1 (C-5), 94.8 (C-1′), 82.5–82.3 (C-4′), 80.6 (C-2′), 74.7, 74.5 (C-3′), 68.6 (*CH*$_2$Ph), 67.1, 66.8 (C-5′), 54.0, 53.9 (OCH_3-asp), 53.6, 53.5 (C-α-asp), 53.3 (OCH_3-asp), 40.4–40.2 (C-β-asp), 21.1 ppm (CH_3-2′); ^{31}P NMR (202 MHz, MeOD): δ = 3.66 and 3.51 ppm; HRMS (ESI−) calcd for $C_{28}H_{34}N_4O_{11}P$ $[M - H]^-$ 631.1810, found 631.1801.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-cytidine-5′-[phenylbis(isopropyl-L-aspartyl)]phosphate (protected 1d). Yield: 44%; R_f = 0.42 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.13 and 2.67; HRMS (ESI+) calcd for $C_{29}H_{42}N_4O_{11}P$ $[M + H]^+$ 653.2582, found 653.2594.

2′-*C*-Methyl-cytidine-5′-[phenylbis(isopropyl-L-aspartyl)]phosphate (1d). Yield: 42%; R_f = 0.3 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (500 MHz, MeOD): δ = 7.84–7.81 (2 d, 1H, H-6), 7.39–7.19

(a series of multiplets, 5H, OPh), 6.03, 6.01 (2 s, 1H, H-1′), 5.97–5.93 (2 d, 1H, H-5), 4.99–4.90 (m, 2H, –*CH*$(CH_3)_2$), 4.63–4.37 (m, 2H, H-5′ & H-5″), 4.26–4.21 (m, 1H, H-α-Asp), 4.15–4.10 (m, 1H, H-4′), 3.80–3.78 (d, 1H, H-3′), 2.78–2.67 (m, 2H, H-β-Asp), 1.24–1.19 (m, 12H, –CH(*CH*$_3)_2$), 1.13 and 1.12 ppm (2 s, 3H, –CH_3-2′); ^{13}C NMR (125 MHz, MeOD): δ = 172.6, 172.3, 171.4, 171.3 (–CO-asp), 164.9 (C-4), 155.1 (C-2), 152.1, 152.0 (phenyl C), 143.5 (C-6), 130.9 (phenyl C), 126.3 (phenyl C), 121.4, 121.3 (phenyl C), 96.1 (C-5), 93.9 (C-1′), 81.6, 81.5 (C-4′), 79.7, 79.6 (C-2′), 73.9, 73.7 (C-3′), 70.8, 70.6, 69.8 (*CH*$(CH_3)_2$), 66.3, 66.1 (C-5′), 52.9, 52.8 (C-α-Asp), 39.9, 39.7 (C-β-Asp), 22.0–21.9 (–CH(*CH*$_3)_2$), 20.3 ppm (2′-CH_3); ^{31}P NMR (202 MHz, MeOD): δ = 3.8 and 3.5 ppm; HRMS (ESI–) calcd for $C_{26}H_{36}N_4O_{11}P$ $[M - H]^-$ 611.2123, found 611.2126.

2′-*C*-Methyl-N^4-(benzyloxycarbonyl)cytidine-5′-[phenylbis(*n*-butyl-L-aspartyl)]phosphate (protected 1e). Yield: 50%; R_f = 0.56 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.30 and 2.89 ppm; HRMS (ESI+) calcd for $C_{36}H_{48}N_4O_{13}P$ $[M + H]^+$ 775.2950, found 775.2947.

2′-*C*-Methyl-cytidine-5′-[phenylbis(*n*-butyl-L-aspartyl)]phosphate (1e). Yield: 57%; R_f = 0.22 (CH_2Cl_2–MeOH, 9.0 : 1.0); ^{1}H NMR (500 MHz, MeOD): δ = 7.69–7.67 (2 d, 1H, H-6), 7.39–7.18 (a series of multiplets, 5H, OPh), 6.05, 6.04 (2 s, 1H, H-1′), 5.84–5.82 (2 d, 1H, H-5), 4.62–4.36 (m, 2H, H-5′ & H-5″), 4.31–4.26 (m, 1H, H-α-Asp), 4.17–3.98 (m, 5H, H-4′ & –O*CH*$_2(CH_2)_2CH_3$), 3.76–3.74 (d, 1H, H-3′), 2.84–2.72 (m, 2H, H-β-Asp), 1.63–1.53 (m, 4H, –OCH_2*CH*$_2CH_2CH_3$), 1.41–1.29 (m, 4H, –$O(CH_2)_2$*CH*$_2CH_3$), 1.10 and 1.08 (2 s, 3H, –CH_3-2′), 0.93–0.89 ppm (m, 6H, –$O(CH_2)_3$*CH*$_3$); ^{13}C NMR (125 MHz, MeOD): δ = 174.0 (d, $^3J_{CP}$ = 5.13 Hz, –CO-α), 173.7 (d, $^3J_{CP}$ = 5.47 Hz, –CO-α), 172.8, 172.7 (–CO-β), 168.3 (C-4), 159.3 (C-2), 153.0 (phenyl C), 143.0 (C-6), 131.8 (phenyl C), 127.1 (phenyl C), 122.3, 122.2 (phenyl C), 97.1 (C-5), 94.8 (C-1′), 82.2 (C-4′), 80.5, 80.4 (C-2′), 75.0, 74.7 (C-3′), 67.5–66.8 (C-5′ & –O*CH*$_2(CH_2)_2CH_3$), 53.7, 53.6 (C-α-asp), 40.5–40.3 (C-β-asp), 32.6 (–OCH_2*CH*$_2CH_2CH_3$), 21.1–20.9 (CH_3-2′ & –$O(CH_2)_2$-*CH*$_2CH_3$), 14.9 ppm (–$O(CH_2)_3$*CH*$_3$); ^{31}P NMR (202 MHz, MeOD): δ = 3.71 and 3.48 ppm; HRMS (ESI–) calcd for $C_{28}H_{40}N_4O_{11}P$ $[M - H]^-$ 639.2436, found 639.2440.

2′-*C*-Methyl-N^4-(benzyloxycarbonyl)cytidine-5′-[phenylbis(amyl-L-aspartyl)]phosphate (protected 1f). Yield: 58%; R_f = 0.55 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.38 and 2.92 ppm; HRMS (ESI–) calcd for $C_{38}H_{50}N_4O_{13}P$ $[M - H]^-$ 801.3117, found 801.3133.

2′-*C*-Methyl-cytidine-5′-[phenylbis(amyl-L-aspartyl)]phosphate (1f). Yield: 70%; R_f = 0.57 (CH_2Cl_2–MeOH, 9.0 : 1.0); ^{1}H NMR (500 MHz, MeOD): δ = 7.69–7.66 (2 d, 1H, H-6), 7.39–7.18 (a series of multiplets, 5H, OPh), 6.06, 6.04 (2 s, 1H, H-1′), 5.87–5.82 (2 d, 1H, H-5), 4.61–4.37 (m, 2H, H-5′ & H-5″), 4.32–4.27 (m, 1H, H-α-Asp), 4.14–3.98 (m, 5H, H-4′ & –O*CH*$_2(CH_2)_3CH_3$), 3.77–3.74 (d, 1H, H-3′), 2.85–2.72 (m, 2H, H-β-Asp), 1.63–1.57 (m, 4H, –OCH_2*CH*$_2(CH_2)_2CH_3$), 1.34–1.29 (m, 8H, –$O(CH_2)_2$(*CH*$_2)_2CH_3$), 1.10 and 1.08 (2 s, 3H, –CH_3-2′), 0.91–0.88 ppm (m, 6H, –$O(CH_2)_4$*CH*$_3$); ^{13}C NMR (125 MHz, MeOD): δ = 173.9 (d, $^3J_{CP}$ = 5.21 Hz, –CO-α), 173.7 (d, $^3J_{CP}$ = 5.86 Hz, –CO-α), 172.8, 172.7 (–CO-β), 168.2 (C-4), 159.3 (C-2), 153.0, 152.9 (phenyl C), 143.0, 142.9 (C-6), 131.7 (phenyl C), 127.1 (phenyl C), 122.3, 122.2 (phenyl C), 97.1 (C-5), 94.9, 94.7 (C-1′), 82.2, 82.1 (C-4′), 80.5, 80.4 (C-2′), 74.9, 74.7 (C-3′), 67.8–66.9 (C-5′ & –O*CH*$_2(CH_2)_3CH_3$), 53.7, 53.6 (C-α-asp), 40.5–40.3 (C-β-asp), 30.2–29.9 (–OCH_2(*CH*$_2)_2CH_2CH_3$), 24.2 (–$O(CH_2)_3$*CH*$_2CH_3$), 21.2 (CH_3-2′), 15.2 ppm (–$O(CH_2)_4$*CH*$_3$); ^{31}P NMR (202 MHz, MeOD): δ = 3.72 and 3.48 ppm; HRMS (ESI+) calcd for $C_{30}H_{46}N_4O_{11}P$ $[M + H]^+$ 669.2895, found 669.2894.

2′-*C*-Methyl-N^4-(benzyloxycarbonyl)cytidine-5′-[phenylbis-(isoamyl-L-aspartyl)]phosphate (protected 1g). Yield: 58%; R_f = 0.59 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.32 and 2.93; HRMS (ESI+) calcd for $C_{38}H_{52}N_4O_{13}P$ $[M + H]^+$ 803.3263, found 803.3268.

2′-*C*-Methyl-cytidine-5′-[phenylbis(isoamyl-L-aspartyl)]phosphate (1g). Yield: 67%; R_f = 0.12 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (500 MHz, MeOD): δ = 7.69–7.67 (2 d, 1H, H-6), 7.39–7.18 (a series of multiplets, 5H, OPh), 6.05, 6.04 (2 s, 1H, H-1′), 5.85–5.82 (2 d, 1H, H-5), 4.62–4.36 (m, 2H, H-5′ & H-5″), 4.31–4.26 (m, 1H, H-α-Asp), 4.20–4.01 (m, 5H, H-4′ & –O*CH*$_2CH_2CH(CH_3)_2$), 3.77–3.73 (d, 1H, H-3′), 2.84–2.72 (m, 2H, H-β-Asp), 1.69–1.61 (m, 2H, –OCH_2CH_2*CH*$(CH_3)_2$), 1.53–1.45 (m, 4H, –OCH_2*CH*$_2CH(CH_3)_2$), 1.10, 1.08 (2 s, 3H, –CH_3-2′), 0.90–0.89 ppm (m, 12H, –OCH_2CH_2CH(*CH*$_3)_2$); ^{13}C NMR (125 MHz, MeOD): δ = 173.9 (d, $^3J_{CP}$ = 4.94 Hz, –CO-α), 173.7 (d, $^3J_{CP}$ = 5.88 Hz, –CO-α), 172.7, 172.6 (–CO-β), 168.2 (C-4), 159.3 (C-2), 153.0, 152.9 (phenyl C), 143.0 (C-6), 131.7 (phenyl C), 127.1 (phenyl C), 122.3, 122.2 (phenyl C), 97.1 (C-5), 94.8 (C-1′), 82.2 (C-4′), 80.5, 80.4 (C-2′), 74.9, 74.7 (C-3′), 67.3 (d, $^2J_{CP}$ = 4.97 Hz, C-5′), 66.9 (d, $^2J_{CP}$ = 4.81 Hz, C-5′), 66.2, 65.6, 65.5 (–O*CH*$_2CH_2CH(CH_3)_2$), 53.7, 53.5 (C-α-asp), 40.4–40.3 (C-β-asp), 32.2 (–OCH_2*CH*$_2CH(CH_3)_2$), 27.0, 26.9 (–OCH_2CH_2*CH*$(CH_3)_2$), 23.7, 23.6 (–OCH_2CH_2CH(*CH*$_3)_2$), 21.2 ppm (CH_3-2′); ^{31}P NMR (202 MHz, MeOD): δ = 3.71 and 3.47 ppm; HRMS (ESI–) calcd for $C_{30}H_{44}N_4O_{11}P$ $[M - H]^-$ 667.2750, found 667.2762.

2′-*C*-Methyl-N^4-(benzyloxycarbonyl)cytidine-5′-[phenylbis-(methoxyiminodiacetyl)]phosphate (protected 1h). Yield: 70%; R_f = 0.47 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 4.31 and 4.12; HRMS (ESI+) calcd for $C_{30}H_{36}N_4O_{13}P$ $[M + H]^+$ 691.2011, found 691.2014.

2′-*C*-Methyl-cytidine-5′-[phenylbis(methoxyiminodiacetyl)]-phosphate (1h). Yield: 84%; R_f = 0.26 (CH_2Cl_2–MeOH, 9.0 : 1.0); ^{1}H NMR (500 MHz, MeOD): δ = 7.69–7.60 (2 d, 1H, H-6), 7.40–7.20 (a series of multiplets, 5H, OPh), 6.07 (s, 1H, H-1′), 5.88–5.83 (2 d, 1H, H-5), 4.71–4.37 (m, 2H, H-5′ & H-5″), 4.13–3.96 (m, 5H, H-4′ & –CH_2-IDA), 3.76–3.67 (7H, H-3′ & OCH_3), 1.11, 1.06 ppm (2 s, 3H, –CH_3-2′); ^{13}C NMR (125 MHz, MeOD): δ = 171.8 (–CO-IDA), 167.4 (C-4), 158.4 (C-2), 152.1–151.9 (phenyl C), 142.3, 142.0 (C-6), 131.0, 130.9 (phenyl C), 126.4 (phenyl C), 121.3–121.1 (phenyl C), 96.3, 96.2 (C-5), 94.0, 93.7 (C-1′), 81.2–81.0 (C-4′), 79.6 (C-2′), 74.2, 73.7 (C-3′), 66.9 (d, $^2J_{CP}$ = 4.68 Hz, C-5′), 65.9 (d, $^2J_{CP}$ = 4.16 Hz, C-5′), 52.7 (–OCH_3), 49.1 (–CH_2-IDA), 20.3 ppm (CH_3-2′); ^{31}P NMR (202 MHz, MeOD): δ = 4.31 and 4.01 ppm; HRMS (ESI+) calcd for $C_{22}H_{30}N_4O_{11}P$ $[M + H]^+$ 557.1643, found 557.1642.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-N^4-(*t*-butyloxycarbonyl)cytidine-5′-[phenylbis(pivaloyloxymethyliminodiacetyl)]phosphate (protected 1i). Yield: 55%; R_f = 0.42 (EtOAc–hexane, 8.0 : 2.0); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.90 and 3.75 ppm; HRMS (ESI+) calcd for $C_{40}H_{58}N_4O_{17}P$ $[M + H]^+$ 897.3529, found 897.3510.

2′-*C*-Methyl-cytidine-5′-[phenyl-bis(pivaloyloxymethyliminodiacetyl)]phosphate (1i). Yield: 60%; R_f = 0.3 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (500 MHz, MeOD): δ = 7.64–7.57 (2 d, 1H, H-6), 7.40–7.20 (a series of multiplets, 5H, OPh), 6.05 (s, 1H, H-1′), 5.90–5.85 (2 d, 1H, H-5), 5.80–5.74 (m, –CH_2-POM), 4.69–4.39 (m, 2H, H-5′ & H-5″), 4.18–4.01 (m, 5H, H-4′ & –CH_2-IDA), 3.75–3.64 (2 d, 1H, H-3′), 1.17 (s, 18H, *t*-Bu), 1.09, 1.05 ppm (2 s, 3H, –CH_3-2′); ^{13}C NMR (125 MHz, MeOD): δ = 179.2 (–CO-POM), 171.1–170.9 (–CO-IDA), 168.1, 168.0 (C-4), 159.2 (C-2), 152.9–152.7 (phenyl C), 143.1, 142.8 (C-6), 131.9, 131.8 (phenyl C), 127.4 (phenyl C), 122.2–122.0 (phenyl C), 97.3 (C-5), 94.9, 94.6 (C-1′), 82.2–81.9 (C-4′ & –CH_2-POM), 80.5 (C-2′), 75.1, 74.6 (C-3′), 67.9 (d, $^2J_{CP}$ = 4.58 Hz, C-5′), 67.1 (d, $^2J_{CP}$ = 4.58 Hz, C-5′), 40.6 (quaternary C-*t*-Bu), 28.1 (*t*-Bu), 21.3 ppm (CH_3-2′); ^{31}P NMR (202 MHz, MeOD): δ = 3.97 and 3.70 ppm; HRMS (ESI+) calcd for $C_{32}H_{46}N_4O_{15}P$ $[M + H]^+$ 757.2692, found 757.2700.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-uridine-5′-[phenyl(methoxy-L-alaninyl)]phosphate (protected 2a). Yield: 90%; R_f = 0.67 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 2.70, 2.66 ppm; HRMS (ESI+) calcd for $C_{23}H_{31}N_3O_{10}P$ $[M + H]^+$ 540.1741, found 540.1747.

2′-*C*-Methyl-uridine-5′-[phenyl(methoxy-L-alaninyl)]phosphate (2a). Yield: 70%; R_f = 0.5 (CH_2Cl_2–MeOH, 9.0 : 1.0); ^{1}H NMR (500 MHz, MeOD): δ = 7.71–7.68 (2 d, 1H, H-6), 7.39–7.19 (a series of multiplets, 5H, OPh), 5.98, 5.97 (2 s, 1H, H-1′), 5.65–5.59 (2 d, 1H, H-5), 4.58–4.35 (m, 2H, H-5′ & H-5″), 4.12–4.09 (m, 1H, H-4′), 4.00–3.95 (m, 1H, H-α-ala), 3.81–3.79 (2 d, 1H, H-3′), 3.68, 3.66 (2 s, 3H, OCH_3-ala), 1.36–1.28 (2 d, 3H, CH_3-ala), 1.16, 1.14 ppm (2 s, 3H, –CH_3-2′); ^{13}C NMR (125 MHz, MeOD): δ = 176.3 (d, $^3J_{CP}$ = 4.40 Hz, –CO), 176.1 (d, $^3J_{CP}$ = 5.33 Hz, –CO), 166.7 (C-4), 153.1, 153.0, 152.9 (C-2 & phenyl C), 142.8, 142.6 (C-6), 131.7 (phenyl C), 127.1 (phenyl C), 122.2–122.1 (phenyl C), 103.7, 103.6 (C-5), 94.4, 94.2 (C-1′), 82.4 (C-4′), 80.5, 80.4 (C-2′), 74.8, 74.5 (C-3′), 67.1 (d, $^3J_{CP}$ = 4.97 Hz, C-5′), 66.6 (d, $^3J_{CP}$ = 4.84 Hz, C-5′), 53.7 (OCH_3-ala), 52.5, 52.3 (C-α-ala), 21.4–21.1 (CH_3-ala), 21.0 ppm (CH_3-2′); ^{31}P NMR (202 MHz, MeOD): δ = 3.82 and 3.69 ppm; HRMS (ESI–) calcd for $C_{20}H_{25}N_3O_{10}P$ $[M - H]^-$ 498.1283, found 498.1280.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-uridine-5′-[phenylbis(methoxy-L-aspartyl)]phosphate (protected 2b). Yield: 70%; R_f = 0.47 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.17, 2.70 ppm; HRMS (ESI–) calcd for $C_{25}H_{31}N_3O_{12}P$ $[M - H]^-$ 596.1651, found 596.1651.

2′-*C*-Methyl-uridine-5′-[phenylbis(methoxy-L-aspartyl)]phosphate (2b). Yield: 78%; R_f = 0.16 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (500 MHz, MeOD): δ = 7.69–7.67 (2 d, 1H, H-6), 7.37–7.20 (a series of multiplets, 5H, OPh), 5.98, 5.97 (2 s, 1H, H-1′), 5.65–5.59 (2 d, 1H, H-5), 4.62–4.36 (m, 2H, H-5′ & H-5″), 4.34–4.28 (m, 1H, H-α-asp), 4.12–4.09 (m, 1H, H-4′), 3.85–3.80 (2 d, 1H, H-3′), 3.70, 3.65, 3.63, 3.60 (4 s, 6H, OCH_3-asp), 2.85–2.72 (m, 2H, H-β-asp), 1.16, 1.14 ppm (2 s, 3H, –CH_3-2′); ^{13}C NMR (125 MHz, MeOD): δ = 173.5 (d, $^3J_{CP}$ = 4.77 Hz, –CO-α), 173.3 (d, $^3J_{CP}$ = 5.16 Hz, –CO-α), 172.3, 172.2 (–CO-β), 165.9 (C-4), 152.3 (C-2), 152.1 (phenyl C), 142.0, 141.9 (C-6), 130.9 (phenyl C), 126.3 (phenyl C), 121.4–121.3 (phenyl C), 102.8 (C-5), 93.5, 93.4 (C-1′), 81.6, 81.5 (C-4′), 79.6 (C-2′), 73.9, 73.7 (C-3′), 66.3 (d, $^2J_{CP}$ = 5.00 Hz, C-5′), 66.0 (d, $^2J_{CP}$ = 4.80 Hz, C-5′), 53.1 (OCH_3-asp), 52.7, 52.6 (C-α-asp), 52.5, 52.4 (OCH_3-asp), 39.3–39.1 (C-β-asp), 20.2 ppm (CH_3-2′); ^{31}P NMR (202 MHz, MeOD): δ = 3.68 and 3.60 ppm; HRMS (ESI+) calcd for $C_{22}H_{29}N_3O_{12}P$ $[M + H]^+$ 558.1483, found 558.1487.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-uridine-5′-[phenyl(α-methoxy-β-benzyloxy-L-aspartyl)]phosphate (protected 2c). Yield: 76%; R_f = 0.45 (CH_2Cl_2–MeOH, 9.5 : 0.5); HRMS (ESI–) calcd for $C_{31}H_{35}N_3O_{12}P$ $[M - H]^-$ 672.1964, found 672.1969.

2′-*C*-Methyl-uridine-5′-[phenyl-(α-methoxy-β-benzyloxy-L-aspartyl)]phosphate (2c). Yield: 63%; R_f = 0.33 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (500 MHz, MeOD): δ = 7.67–7.65 (2 d, 1H, H-6), 7.36–7.15 (a series of multiplets, 10H, OPh & CH_2*Ph*), 5.97, 5.96 (2 s, 1H, H-1′), 5.64–5.58 (2 d, 1H, H-5), 5.08–5.05 (*CH*$_2$Ph), 4.59–4.30 (m, 3H, H-5′, H-5″ & H-α-asp), 4.11–4.07 (m, 1H, H-4′), 3.83–3.78 (2 d, 1H, H-3′), 3.63, 3.59 (2 s, 3H, OCH_3-asp), 2.98–2.74 (m, 2H, H-β-asp), 1.15, 1.12 ppm (2 s, 3H, –CH_3-2′); ^{13}C NMR (125 MHz, MeOD): δ = 174.3 (d, $^3J_{CP}$ = 4.88 Hz, –CO-α), 174.1 (d, $^3J_{CP}$ = 5.65 Hz, –CO-α), 172.5, 172.4 (–CO-β), 166.7 (C-4), 153.1 (C-2), 152.9 (phenyl C), 142.8, 142.7 (C-6), 138.1, 138.0 (*CH*$_2$*Ph*), 131.8, 131.7, 130.4, 130.2, 127.2, 122.2, 122.1 (phenyl C), 103.7, 103.6 (C-5), 94.3, 94.2 (C-1′), 82.4, 82.3 (C-4′), 80.5, 80.4 (C-2′), 74.8, 74.6 (C-3′), 68.6 (*CH*$_2$Ph), 67.2 (d, $^2J_{CP}$ = 5.33 Hz, C-5′), 66.8 (d, $^2J_{CP}$ = 5.06 Hz, C-5′), 53.9 (OCH_3-asp), 53.6, 53.5 (C-α-asp), 40.4–40.2 (C-β-asp), 21.1 ppm (CH_3-2′); ^{31}P NMR (202 MHz, MeOD): δ = 3.66 and 3.53 ppm; HRMS (ESI–) calcd for $C_{28}H_{31}N_3O_{12}P$ $[M - H]^-$ 632.1651, found 632.1650.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-uridine-5′-[phenylbis(isopropyl-L-aspartyl)]phosphate (protected 2d). Yield: 83%; R_f = 0.75 (CH_2Cl_2–MeOH, 9.4 : 0.6); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.49 (s, 1H, –NH), 7.62–7.47 (2 d, 1H, H-6), 7.37–7.14 (a series of multiplets, 5H, OPh), 6.12, 6.08 (2 s, 1H, H-1′), 5.72–5.58 (2 d, 1H, H-5), 5.09–4.92 (m, 2H, CH-iPr), 4.53–4.19 (m, 5H, H-5′, H-5″, –CH-Asp, H-4′, H-3′), 2.94–2.50 (m, 2H, –CH_2-Asp), 1.59 (s, 3H, –CH_3), 1.39 (s, 3H, –CH_3), 1.25–1.19 ppm (m, 15H, –CH_3-iPr and –CH_3-2′); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.34 and 2.86 ppm; HRMS (ESI–) calcd for $C_{29}H_{39}N_3O_{12}P$ $[M - H]^-$ 652.2277, found 652.2269.

2′-*C*-Methyl-uridine-5′-[phenylbis(isopropyl-L-aspartyl)]phosphate (2d). Yield: 86%, R_f = 0.44 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (500 MHz, MeOD): δ = 7.68–7.65 (2 d, 1H, H-6), 7.38–7.18 (a series of multiplets, 5H, OPh), 5.98, 5.97 (2 s, 1H, H-1′), 5.65–5.60 (2 d, 1H, H-5), 5.03–4.83 (m, 2H, –*CH*(CH_3)$_2$), 4.64–4.40 (m, 2H, H-5′ & H-5″), 4.25–4.21 (m, 1H, -H-α-asp), 4.14–4.08 (m, 1H, H-4′), 3.84–3.82 (2 d, 1H, H-3′), 2.78–2.63 (m, 2H, -H-β-asp), 1.24–1.18 (m, 12H, –CH(*CH*$_3$)$_2$), 1.16, 1.14 ppm (2 s, 3H, –CH_3-2′); ^{13}C NMR (125 MHz, MeOD): δ = 172.5 (d, $^3J_{CP}$ = 5.07 Hz, –CO-α), 172.3 (d, $^3J_{CP}$ = 5.92 Hz, –CO-α), 171.3

(–CO-β), 165.7 (C-4), 152.2 (C-2), 152.1, 152.0 (phenyl C), 141.9, 141.8 (C-6), 130.9 (phenyl C), 126.3 (phenyl C), 121.3 (phenyl C), 102.9, 102.8 (C-5), 93.5, 93.3 (C-1′), 81.5, 81.4 (C-4′), 79.6, 79.5 (C-2′), 73.9, 73.7 (C-3′), 70.7, 69.8, 69.7 –*C*H(CH$_3$)$_2$), 66.4 (d, $^2J_{CP}$ = 4.52 Hz, C-5′), 65.9 (d, $^2J_{CP}$ = 4.73 Hz, C-5′), 52.8, 52.7 (–C-α-asp), 39.9–39.7 (–H-β-asp), 22.0–21.9 ppm (–CH(*C*H$_3$)$_2$), 20.2 (2′-CH$_3$); ^{31}P NMR (202 MHz, MeOD): δ = 3.82 and 3.59 ppm; HRMS (ESI–) calcd for $C_{26}H_{35}N_3O_{12}P$ [M – H]$^-$ 612.1964, found 612.1964.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-uridine-5′-[phenylbis(*n*-butyl-L-aspartyl)]phosphate (protected 2e). Yield: 86%; R_f = 0.45 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.24 and 2.77; HRMS (ESI–) calcd for $C_{31}H_{43}N_3O_{12}P$ [M – H]$^-$ 680.2590, found 680.2593.

2′-*C*-Methyl-uridine-5′-[phenylbis(*n*-butyl-L-aspartyl)]phosphate (2e). Yield: 80%; R_f = 0.2 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (500 MHz, MeOD): δ = 7.68–7.66 (2 d, 1H, H-6), 7.38–7.18 (a series of multiplets, 5H, OPh), 5.98, 5.97 (2 s, 1H, H-1′), 5.65–5.63 (2 d, 1H, H-5), 4.62–4.37 (m, 2H, H-5′ & H-5″), 4.32–4.26 (m, 1H, H-α-Asp), 4.16–3.98 (m, 5H, H-4′ & –O*C*H$_2$(CH$_2$)$_2$CH$_3$), 3.84–3.80 (2 d, 1H, H-3′), 2.84–2.69 (m, 2H, H-β-Asp), 1.62–1.53 (m, 4H, –OCH$_2$*C*H$_2$CH$_2$CH$_3$), 1.40–1.31 (m, 4H, –O(CH$_2$)$_2$*C*H$_2$CH$_3$), 1.16 and 1.13 (2 s, 3H, –CH$_3$-2′), 0.93–0.90 ppm (m, 6H, –O(CH$_2$)$_3$*C*H$_3$); ^{13}C NMR (125 MHz, MeOD): δ = 174.0 (d, $^3J_{CP}$ = 4.89 Hz, –CO-α), 173.7 (d, $^3J_{CP}$ = 5.91 Hz, –CO-α), 172.7 (–CO-β), 166.7 (C-4), 153.1 (C-2), 153.0, 152.9 (phenyl C), 142.8, 142.7 (C-6), 131.8 (phenyl C), 127.1 (phenyl C), 122.2, 122.1 (phenyl C), 103.7 (C-5), 94.3, 94.2 (C-1′), 82.4, 82.3 (C-4′), 80.5, 80.4 (C-2′), 74.8, 74.6 (C-3′), 67.5–66.7 (C-5′ & –O*C*H$_2$(CH$_2$)$_2$CH$_3$), 53.6, 53.5 (C-α-asp), 40.4–40.2 (C-β-asp), 32.5 (–OCH$_2$*C*H$_2$CH$_2$CH$_3$), 21.1–20.9 (CH$_3$-2′ & –O(CH$_2$)$_2$*C*H$_2$CH$_3$), 14.9 ppm (–O(CH$_2$)$_3$*C*H$_3$); ^{31}P NMR (202 MHz, MeOD): δ = 3.73 and 3.57 ppm; HRMS (ESI+) calcd for $C_{28}H_{39}N_3O_{12}P$ [M + H]$^+$ 642.2422, found 642.2429.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-uridine-5′-[phenylbis(amyl-L-aspartyl)]phosphate (protected 2f). Yield: 72%; R_f = 0.35 (EtOAc–hexane, 9.0 : 1.0); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.25 and 2.77 ppm; HRMS (ESI+) calcd for $C_{33}H_{47}N_3O_{12}P$ [M + H]$^+$ 710.3048, found 710.3059.

2′-*C*-Methyl-uridine-5′-[phenylbis(amyl-L-aspartyl)]phosphate (2f). Yield: 60%; R_f = 0.4 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (600 MHz, MeOD): δ = 7.68–7.66 (2 d, 1H, H-6), 7.38–7.18 (a series of multiplets, 5H, OPh), 5.98, 5.97 (2 s, 1H, H-1′), 5.65–5.61 (2 d, 1H, H-5), 4.62–4.38 (m, 2H, H-5′ & H-5″), 4.31–4.26 (m, 1H, H-α-Asp), 4.15–3.99 (m, 5H, H-4′ & –O*C*H$_2$(CH$_2$)$_3$CH$_3$), 3.83–3.80 (d, 1H, H-3′), 2.84–2.70 (m, 2H, H-β-Asp), 1.61–1.57 (m, 4H, –OCH$_2$*C*H$_2$(CH$_2$)$_2$CH$_3$), 1.35–1.27 (m, 8H, –O(CH$_2$)$_2$(*C*H$_2$)$_2$CH$_3$), 1.16 and 1.13 (2 s, 3H, –CH$_3$-2′), 0.91–0.88 ppm (m, 6H, –O(CH$_2$)$_4$*C*H$_3$); ^{13}C NMR (150 MHz, MeOD): δ = 173.9 (d, $^3J_{CP}$ = 4.70 Hz, –CO-α), 173.7 (d, $^3J_{CP}$ = 5.75 Hz, –CO-α), 172.7 (–CO-β), 166.6 (C-4), 153.1 (C-2), 153.0, 152.9 (phenyl C), 142.8, 142.7 (C-6), 131.8, 131.7 (phenyl C), 127.1 (phenyl C), 122.2 (phenyl C), 103.7 (C-5), 94.2 (C-1′), 82.4, 82.3 (C-4′), 80.5, 80.4 (C-2′), 74.8, 74.5 (C-3′), 67.7 (–O*C*H$_2$(CH$_2$)$_3$CH$_3$), 67.2 (d, $^2J_{CP}$ = 4.35 Hz, C-5′), 67.1, 67.0 (–O*C*H$_2$(CH$_2$)$_3$CH$_3$), 66.8 (d, $^2J_{CP}$ = 3.87 Hz, C-5′), 53.6, 53.5 (C-α-asp), 40.4–40.2 (C-β-asp), 30.2–29.9 (–OCH$_2$(*C*H$_2$)$_2$-CH$_2$CH$_3$), 24.2 (–O(CH$_2$)$_3$*C*H$_2$CH$_3$), 21.1, 21.0 (CH$_3$-2′), 15.2 ppm (–O(CH$_2$)$_4$*C*H$_3$); ^{31}P NMR (202 MHz, MeOD): δ = 3.74 and 3.57 ppm; HRMS (ESI+) calcd for $C_{30}H_{45}N_3O_{12}P$ [M + H]$^+$ 670.2735, found 670.2736.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-uridine-5′-[phenylbis-(isoamyl-L-aspartyl)]phosphate (protected 2g). Yield: 87%; R_f = 0.65 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.24 and 2.78 ppm; HRMS (ESI+) calcd for $C_{33}H_{49}N_3O_{12}P$ [M + H]$^+$ 710.3048, found 710.3050.

2′-*C*-Methyl-uridine-5′-[phenylbis(isoamyl-L-aspartyl)]phosphate (2g). Yield: 86%; R_f = 0.25 (EtOAc–hexane, 9.0 : 1.0); ^{1}H NMR (600 MHz, MeOD): δ = 7.68–7.66 (2 d, 1H, H-6), 7.38–7.18 (a series of multiplets, 5H, OPh), 5.98, 5.97 (2 s, 1H, H-1′), 5.65–5.61 (2 d, 1H, H-5), 4.62–4.38 (m, 2H, H-5′ & H-5″), 4.31–4.25 (m, 1H, H-α-Asp), 4.19–4.02 (m, 5H, H-4′ & –O*C*H$_2$CH$_2$CH(CH$_3$)$_2$), 3.84–3.80 (d, 1H, H-3′), 2.83–2.69 (m, 2H, H-β-Asp), 1.68–1.62 (m, 2H, –OCH$_2$CH$_2$*C*H(CH$_3$)$_2$), 1.52–1.46 (m, 4H, –OCH$_2$*C*H$_2$CH(CH$_3$)$_2$), 1.16, 1.13 (2 s, 3H, –CH$_3$-2′), 0.91–0.90 ppm (m, 12H, –OCH$_2$CH$_2$CH(*C*H$_3$)$_2$); ^{13}C NMR (150 MHz, MeOD): δ = 173.9 (d, $^3J_{CP}$ = 4.84 Hz, –CO-α), 173.7 (d, $^3J_{CP}$ = 5.68 Hz, –CO-α), 172.7, 172.6 (–CO-β), 166.8, 166.6 (C-4), 153.1 (C-2), 153.0, 152.9 (phenyl C), 142.8, 142.7 (C-6), 131.8, 131.6 (phenyl C), 127.1 (phenyl C), 122.2, 122.1 (phenyl C), 103.7 (C-5), 94.3, 94.2 (C-1′), 82.4, 82.3 (C-4′), 80.5, 80.4 (C-2′), 74.8, 74.5 (C-3′), 67.2 (d, $^2J_{CP}$ = 4.63 Hz, C-5′), 66.8 (d, $^2J_{CP}$ = 4.32 Hz, C-5′), 66.2, 65.6, 65.5 (–O*C*H$_2$CH$_2$CH(CH$_3$)$_2$), 53.6, 53.5 (C-α-asp), 40.4–40.2 (C-β-asp), 39.2, 39.1 (–OCH$_2$*C*H$_2$CH(CH$_3$)$_2$), 27.0, 26.9 (–OCH$_2$CH$_2$*C*H(CH$_3$)$_2$), 23.7, 23.6 (–OCH$_2$CH$_2$CH(*C*H$_3$)$_2$), 21.1 ppm (CH$_3$-2′); ^{31}P NMR (202 MHz, MeOD): δ = 3.72 and 3.56 ppm; HRMS (ESI+) calcd for $C_{30}H_{45}N_3O_{12}P$ [M – H]$^-$ 670.2735, found 670.2741.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-uridine-5′-[phenylbis(methoxyiminodiacetyl)]phosphate (protected 2h). Yield: 80%, R_f = 0.5 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.57–7.52 (2 d, 1H, H-6), 7.37–7.15 (a series of multiplets, 5H, OPh), 6.10, 6.09 (2 s, 1H, H-1′), 5.72–5.59 (2 d, 1H, H-5), 4.51–4.34 (m, 4H), 4.12–3.93 (m, 4H), 3.69 (m, 6H, –OCH$_3$), 1.60, 1.59 (s, 3H, –CH$_3$), 1.40, 1.39 (s, 3H, –CH$_3$), 1.21, 1.18 ppm (2 s, 3H, –CH$_3$-2′); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 4.28 and 4.05 ppm; HRMS (ESI+) calcd for $C_{25}H_{33}N_3O_{12}P$ [M + H]$^+$ 598.1796, found 598.1799.

2′-*C*-Methyl-uridine-5′-[phenylbis(methoxyiminodiacetyl)]phosphate (2h). Yield: 50%, R_f = 0.47 (CH_2Cl_2–MeOH, 9.3 : 0.7); ^{1}H NMR (500 MHz, MeOD): δ = 7.69–7.58 (2 d, 1H, H-6), 7.39–7.19 (a series of multiplets, 5H, OPh), 5.99, 5.98 (2 s, 1H, H-1′), 5.67–5.61 (2 d, 1H, H-5), 4.71–4.36 (m, 2H, H-5′ & H-5″), 4.13–3.95 (m, 5H, H-4′, –CH$_2$-IDA), 3.83–3.73 (2 d, 1H, H-3′), 3.68 (s, 6H, –OCH$_3$), 1.16 and 1.12 ppm (2 s, 3H, –CH$_3$-2′); ^{13}C NMR (125 MHz, MeOD): δ = 171.8 (–CO), 165.9, 165.8 (C-4), 152.3 (C-2), 152.0–151.9 (phenyl C), 142.1, 141.8 (C-6), 131.0, 130.9 (phenyl C), 126.4 (phenyl C), 121.2–121.1 (phenyl C), 103.0, 102.9 (C-5), 93.5, 93.2 (C-1′), 81.4–81.2 (C-4′), 79.6, 79.5 (C-2′), 74.1, 73.7 (C-3′), 66.8 (d, $^2J_{CP}$ = 4.90 Hz, C-5′), 65.9 (d, $^2J_{CP}$ = 4.35 Hz, C-5′), 52.6 (–OCH$_3$), 49.1 (–CH$_2$-IDA), 20.3, 20.2 ppm (2′-CH$_3$); ^{31}P NMR (202 MHz, MeOD): δ = 4.36 and

4.00 ppm; HRMS (ESI+) calcd for $C_{22}H_{29}N_3O_{12}P$ $[M + H]^+$ 558.1483, found 558.1494.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-uridine-5′-[phenylbis(pivaloyloxymethyliminodiacetyl)]phosphate (protected 2i). Yield: 45%; R_f = 0.66 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.39 (d, 1H, H-6), 7.53–7.48 (2 d, 1H, H-5), 7.35–7.16 (a series of multiplets, 5H, OPh), 6.09, 6.07 (2 s, 1H, H-1′), 5.74 (4H, CH_2-POM), 4.43–4.33 (m, 4H), 4.19–3.94 (m, 4H), 1.59 (s, 3H, –CH_3), 1.39 (s, 3H, –CH_3), 1.19 ppm (s, 21H, –CH_3 & *t*-Bu-POM); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 3.94 and 3.71 ppm; HRMS (ESI+) calcd for $C_{35}H_{48}N_3O_{16}P$ $[M + Na]^+$ 820.2664, found 820.2684.

2′-*C*-Methyl-uridine-5′-[phenylbis(pivaloyloxymethyliminodiacetyl)]phosphate (2i). Yield: 60%; R_f = 0.32 (CH_2Cl_2–MeOH, 9.5 : 0.5); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.60–7.43 (2 d, 1H, H-6), 7.34–7.17 (a series of multiplets, 5H, OPh), 6.03, 6.01 (2 s, 1H, H-1′), 5.75–5.71 (m, 4H, CH_2-POM), 5.66–5.64 (2 d, 1H, H-5), 4.57–4.49 (m, 2H, H-5′ & H-5″), 4.16–3.96 (m, 5H, CH_2-IDA & H-4′), 3.72–3.67 (m, 1H, H-3′), 1.18 ppm (s, 21H, 2′-CH_3 & *t*-Bu-POM); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.2, 177.1 (CO-POM), 168.7, 168.6 (CO-IDA), 163.6, 163.5 (C-4), 151.1, 151.0 (C-2), 150.5–150.4 (phenyl C), 140.2, 139.9 (C-6), 130.1, 129.9 (phenyl C), 125.6, 125.5 (phenyl C), 120.1–119.9 (phenyl C), 102.8 (C-5), 92.1, 91.9 (C-1′), 80.6, 80.2 (C-4′), 80.1 (CH_2-POM), 78.5 (C-2′), 73.2, 72.8 (C-3′), 65.1, 64.7 (C-5′), 48.0 (CH_2-IDA), 38.9 (*t*-Bu-quaternary C), 26.9 (*t*-Bu), 20.3 ppm (2′-CH_3); ^{31}P NMR (202 MHz, $CDCl_3$): δ = 4.20 and 4.16 ppm; HRMS (ESI+) calcd for $C_{32}H_{45}N_3O_{16}P$ $[M + H]^+$ 758.2532, found 758.2547.

2′-Deoxy-2′-fluoro-2′-*C*-methyl-uridine-5′-[phenyl-bis(isoamyl-aspartyl)]phosphate (3a, faster eluting diastereoisomer). Yield: 15%; R_f = 0.45 (MeOH–CH_2Cl_2, 9.5 : 0.5); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.56 (1H, NH), 7.36–7.33 (m, 3H, H-6 & OPh), 7.22–7.18 (m, 3H, OPh), 6.18 (d, J = 18.84 Hz, 1H, H-1′), 5.62 (d, J = 8.04 Hz, 1H, H-5), 4.59–4.54 (m, 2H, H-5′ & H-5″), 4.31–4.05 (m, 7H, H-α-Asp, NH-Asp, H-4′ & –O*CH*$_2$CH$_2$CH$(CH_3)_2$), 3.92–3.80 (m, 1H, H-3′), 3.64 (br s, 3′-OH), 2.96–2.55 (m, 2H, H-β-Asp), 1.65–1.59 (m, 2H, –OCH$_2$CH$_2$*CH*$(CH_3)_2$), 1.50–1.48 (m, 4H, –OCH$_2$*CH*$_2$CH$(CH_3)_2$), 1.36 (d, J = 22.4 Hz, 3H, –CH_3-2′), 0.91–0.89 (m, 12H, –OCH$_2$CH$_2$CH(*CH*$_3$)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.8 (d, $^3J_{CP}$ = 6.08 Hz, –CO-α), 171.1 (–CO-β), 162.5 (C-4), 150.5 (d, J_{CP} = 6.63 Hz, phenyl C), 150.2 (C-2), 139.1 (C-6), 130.1, 125.7, 120.2 (phenyl C), 103.1 (C-5), 100.5 (d, J = 182.12 Hz, C-2′), 89.3 (C-1′), 80.1 (C-4′), 71.7 (d, J = 17.8 Hz, C-3′), 65.1, 64.1 (–O*CH*$_2$CH$_2$CH$(CH_3)_2$), 63.9 (C-5′), 51.6 (C-α-Asp), 38.3 (d, J_{CP} = 4.12 Hz, C-β-Asp), 37.3, 37.2 (–OCH$_2$*CH*$_2$CH$(CH_3)_2$), 25.2, 25.1 (–OCH$_2$CH$_2$*CH*$(CH_3)_2$), 22.6, 22.5 (–OCH$_2$CH$_2$CH(*CH*$_3$)$_2$), 16.7 (d, J = 25.5 Hz, –CH_3-2′); ^{31}P NMR (202 MHz, $CDCl_3$): δ = 4.41; HRMS (ESI+) calcd for $C_{30}H_{42}F_1N_3O_{11}P$ $[M - H]^-$ 670.2746, found 670.2545.

2′-Deoxy-2′-fluoro-2′-*C*-methyl-uridine-5′-[phenyl-bis(isoamyl-aspartyl)]phosphate (NMR data of the later eluting diastereoisomer). R_f = 0.40 (MeOH–CH_2Cl_2, 9.5 : 0.5); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.61 (1H, NH), 7.46 (d, J = 8.23 Hz, 1H, H-6), 7.37–7.34 (m, 2H, OPh), 7.24–7.18 (m, 3H, OPh), 6.18 (d, J = 17.78 Hz, 1H, H-1′), 5.66 (d, J = 8.23 Hz, 1H, H-5), 4.57–4.46 (m, 2H, H-5′ & H-5″), 4.33–4.06 (m, 7H, H-α-Asp, NH-Asp, H-4′ & –O*CH*$_2$CH$_2$CH$(CH_3)_2$), 3.98–3.81 (m, 1H, H-3′), 3.68 (br s, 3′-OH), 2.92–2.67 (m, 2H, H-β-Asp), 1.66–1.59 (m, 2H, –OCH$_2$CH$_2$*CH*$(CH_3)_2$), 1.52–1.46 (m, 4H, –OCH$_2$*CH*$_2$CH$(CH_3)_2$), 1.42 (d, J = 22.4 Hz, 3H, –CH_3-2′), 0.91–0.89 (m, 12H, –OCH$_2$CH$_2$CH(*CH*$_3$)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 171.4 (d, $^3J_{CP}$ = 5.97 Hz, –CO-α), 170.7 (–CO-β), 162.6 (C-4), 150.6 (d, J_{CP} = 6.01 Hz, phenyl C), 150.2 (C-2), 139.4 (C-6), 130.1, 125.5, 120.2, 119.9 (phenyl C), 103.1 (C-5), 100.5 (d, J = 181.6 Hz, C-2′), 89.2 (C-1′), 80.0 (C-4′), 71.9 (d, J = 18.5 Hz, C-3′), 65.1, 64.2 (–O*CH*$_2$CH$_2$CH$(CH_3)_2$), 63.9 (C-5′), 51.4 (C-α-Asp), 38.6 (d, J_{CP} = 3.96 Hz, C-β-Asp), 37.3, 37.2 (–OCH$_2$*CH*$_2$CH$(CH_3)_2$), 25.1 (–OCH$_2$CH$_2$*CH*$(CH_3)_2$), 22.5, 22.4 (–OCH$_2$CH$_2$CH(*CH*$_3$)$_2$), 16.7 (d, J = 25.26 Hz, –CH_3-2′); ^{31}P NMR (202 MHz, $CDCl_3$): δ = 3.50; HRMS (ESI+) calcd for $C_{30}H_{42}F_1N_3O_{11}P$ $[M - H]^-$ 670.2746, found 670.2548.

Isopropyl ester of aspartic acid (5). To a suspension of aspartic acid (2.6 g, 20.0 mmol) in anhydrous isopropanol (100 mL) thionyl chloride (10 mL, 139 mmol) was added dropwise at 0 °C under an argon atmosphere. The mixture was allowed to come to RT and then refluxed for 8 h. After evaporation, the solid residue was triturated with diethyl ether. The white solid product was then filtered and washed with diethyl ether to obtain **5** as hydrochloride salt (94%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.67 (br s, 3H, –NH_3^+), 5.01–4.86 (m, 2H, –C*H*$(CH_3)_2$), 4.23 (t, 1H, H-α), 3.01–2.84 (dd, 2H, H-β′ & H-β″), 1.22–1.17 ppm (a series of singlet, 12H, –CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 168.7, 167.9, 70.1, 68.7, 48.6, 34.5, 21.6, 21.5, 21.4, 21.3 ppm; HRMS (ESI+) calcd for $C_{10}H_{20}NO_4$ $[M + H]^+$ 218.1387, found 218.1387.

***n*-Butyl ester of aspartic acid (6).** To a suspension of aspartic acid (1.6 g, 12.0 mmol) in anhydrous *n*-butanol (50 mL) thionyl chloride (6.2 mL, 85.2 mmol) was added dropwise at 0 °C under an argon atmosphere. The mixture was allowed to come to room temperature and stirred for 12 h. The clear solution was then refluxed for 4 h. After evaporation, the solid residue was triturated with diethyl ether. The off-white solid product was then filtered and washed several times with diethyl ether to obtain **6** as hydrochloride salt (94%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.77 (br s, 3H, –NH_3^+), 4.31 (t, 1H, H-α), 4.21–4.05 (m, 4H), 3.10–2.94 (2 dd, 2H, H-β′ & H-β″), 1.61–1.52 (m, 4H), 1.39–1.27 (m, 4H), 0.92–0.86 ppm (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.0, 169.1, 66.4, 65.4, 49.3, 35.0, 30.9, 30.8, 19.4, 19.3, 14.4, 14.3 ppm; HRMS (ESI+) calcd for $C_{12}H_{24}NO_4$ $[M + H]^+$ 246.1699, found 246.1697.

Amyl ester of aspartic acid (7). To a suspension of aspartic acid (1.0 g, 7.5 mmol) in anhydrous amyl alcohol (25 mL) thionyl chloride (4.0 mL, 53.3 mmol) was added dropwise at 0 °C under an argon atmosphere. The mixture was allowed to come to room temperature and stirred for 12 h. The suspension was then refluxed for 3 h. After evaporation, the solid residue was triturated with diethyl ether. The off-white solid product was then filtered and washed several times with diethyl ether to obtain **7** as hydrochloride salt (82%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.73 (br s, 3H, –NH_3^+), 4.31 (t, 1H, H-α), 4.20–4.03 (m, 4H), 3.09–2.93 (2 dd, 2H, H-β′ & H-β″),

1.61–1.54 (m, 4H), 1.31–1.26 (m, 8H), 0.90–0.85 ppm (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.1, 169.2, 66.6, 65.6, 49.3, 35.0, 28.5, 28.4, 28.3, 28.2, 22.6, 22.5, 14.7 ppm; HRMS (ESI+) calcd for $C_{14}H_{28}NO_4$ $[M + H]^+$ 274.2013, found 274.2007.

Isoamyl ester of aspartic acid (8). To a suspension of aspartic acid (1.0 g, 7.5 mmol) in anhydrous isoamyl alcohol (25 mL) thionyl chloride (4.0 mL, 53.3 mmol') was added dropwise at 0 °C under an argon atmosphere. The mixture was allowed to come to room temperature and stirred for 12 h. The suspension was then just heated at 50 °C until a clear solution was obtained. After evaporation, the crude yellow liquid was triturated with hexane and kept at −78 °C overnight. A jelly-type white precipitate was obtained and the hexane was immediately decanted carefully under cold conditions. Hexane was added again and kept at −78 °C until a jelly-type precipitate was formed and the above process was repeated several times to remove the impurities. The collective hexane was evaporated to one third and kept again at −78 °C and the aforementioned process is repeated to increase the final yield. Finally the white solid product was then washed several times with diethyl ether to obtain **8** as hydrochloride salt (40%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.60 (br s, 3H, –NH_3^+), 4.33 (t, 1H, H-α), 4.24–4.07 (m, 4H), 3.06–2.89 (2 dd, 2H, H-β′ & H-β″), 1.69–1.58 (m, 2H), 1.51–1.45 (m, 4H), 0.90–0.86 ppm (m, 12H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.1, 169.2, 65.2, 64.3, 49.3, 37.6, 37.4, 35.0, 25.3, 25.1, 23.2, 23.1, 23.0 ppm; HRMS (ESI+) calcd for $C_{14}H_{28}NO_4$ $[M + H]^+$ 274.2013, found 274.2018.

Boc-Asp-(OBzl)-OMe (10). Compound **9** (2 g, 6.2 mmol) was suspended in dry dichloromethane (50 mL) and allowed to cool to 0 °C in an ice bath. EDC·HCl (1.54 g, 8.0 mmol) was added and the reaction mixture was stirred for 30 min. Methanol (1 mL, 24.8 mmol) and Et_3N (2 mL) were then added to the mixture, and stirring was continued for 24 h at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed with water and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness to obtain the crude product which was then purified by silica gel column chromatography eluting with EtOAc–hexane (2 : 8) to obtain **10** (72%): R_f = 0.4 (EtOAc–hexane, 3 : 7). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38–7.26 (m, 5H, Phenyl ring), 5.62 (d, 1H, –NH), 5.15–5.06 (m, 2H, CH_2 of Bn), 4.65–4.58 (m, 1H, Hα), 3.66 (s, 3H, CH_3), 3.04–2.83 (m, 2H, Hβ), 1.43 ppm (s, 9H, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.5, 170.7, 155.4, 135.6, 128.6, 128.5, 128.4, 128.3, 80.0, 66.6, 52.6, 50.1, 36.9, 28.3 ppm; HRMS (ESI+) calcd for $C_{17}H_{23}NO_6Na$ $[M + Na]^+$ 360.1418, found 360.1418.

Asp-(OBzl)-OMe as hydrochloride salt (11). Compound **10** (1.5 g, 4.4 mmol) was dissolved in dichloromethane (15 mL). Approximately 5–6 N HCl in isopropanol (1.8 mL) was added and the mixture was stirred at room temperature for 3–4 h. Upon completion, the reaction mixture was evaporated to dryness and triturated with diethyl ether. The solid compound was then filtered and washed several times with diethyl ether to obtain compound **11** as a white solid (75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.82 (s, 3H, –NH_3^+), 7.31–7.27 (m, 5H, phenyl ring), 5.15 (s, 2H, CH_2), 4.66 (t, 1H, Hα), 3.65 (s, 3H, CH_3), 3.42–3.24 ppm (m, 2H, Hβ); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.2, 168.9, 135.6, 128.9, 128.7, 67.7, 53.8, 50.0, 34.5 ppm; HRMS (ESI+) calcd for $C_{12}H_{16}NO_4$ $[M + H]^+$ 238.1074, found 338.1072.

2′,3′,5′-Tri-*O*-benzoyl-2′-*C*-methyl-N^4-benzoyl-cytidine (13). The synthetic procedure is followed according to '*J. Org. Chem.* **1997**, 62, 1754–1759'. TLC (EtOAc–hexane, 1 : 1); R_f = 0.47. Yield: 80%; HRMS (ESI+) calcd for $C_{38}H_{31}N_3O_9Na$ $[M + Na]^+$ 696.1953, found 696.1946.

2′,3′,5′-Tri-*O*-benzoyl-2′-*C*-methyl-uridine (14). The synthetic procedure is followed according to '*J. Org. Chem.* **1997**, 62, 1754–1759' and the authenticity of the molecule was judged by comparing the NMR data with the literature values; TLC (EtOAc–hexane, 1 : 1): R_f = 0.55. Yield: 90%; HRMS (ESI+) calcd for $C_{31}H_{26}N_2O_9Na$ $[M + Na]^+$ 593.1531, found 593.1533.

2′-*C*-Methyl-cytidine (1). Saturated NH_3 in methanol (250 mL) was added to compound **13** (5.4 g, 8.0 mmol) and was stirred overnight at room temperature. The reaction mixture was evaporated with silica gel and chromatographed on a silica gel column eluting with CH_2Cl_2–MeOH–NH_3 (8.3 : 1.5 : 0.2) to obtain compound **1** as a white solid (80%). TLC (CH_2Cl_2–MeOH–NH_3, 8.3 : 1.5 : 0.2): R_f = 0.13. Yield: 80%. ^{1}H NMR (500 MHz, MeOD): δ = 8.13 (d, 1H, $J_{6,\ 5}$ = 7.5 Hz, H-6), 6.02 (s, 1H, H-1′), 5.89 (d, 1H, $J_{5,\ 6}$ = 7.5 Hz, H-5), 3.99–3.96 (dd, J = 1.9 Hz, 12.45 Hz, 1H, H-5′), 3.93–3.91 (m, 1H, H-4′), 3.82–3.77 (m, 2H, H-3′ & H-5″), 1.10 ppm (s, 3H, –CH_3). ^{13}C NMR (125 MHz, MeOD): δ = 167.5 (C-4), 158.5 (C-2), 143.1 (C-6), 95.9 (C-5), 93.9 (C-1′), 83.8 (C-4′), 80.2 (C-2′), 73.7 (C-3′), 60.8 (C-5′), 20.5 ppm (–CH_3); HRMS (ESI+) calcd for $C_{10}H_{15}N_3O_5Na$ $[M + Na]^+$ 280.0904, found 280.0901.

2′-*C*-Methyl-uridine (2). The synthetic procedure and purification are the same as described for **1**. TLC (CH_2Cl_2–MeOH–NH_3, 8.3 : 1.5 : 0.2); R_f = 0.39. Yield: 91%; ^{1}H NMR (600 MHz, MeOD): δ = 8.15 (d, 1H, $J_{6,\ 5}$ = 7.98 Hz, H-6), 5.95 (s, 1H, H-1′), 5.67 (d, 1H, $J_{5,\ 6}$ = 7.98 Hz, H-5), 3.99–3.96 (dd, J = 2.1 Hz, 12.5 Hz, 1H, H-5′), 3.93–3.91 (m, 1H, H-4′), 3.84 (d, J = 9.24 Hz, 1H, H-3′), 3.79–3.77 (dd, J = 2.1 Hz, 12.5 Hz, 1H, H-5″), 1.15 ppm (s, 3H, –CH_3); ^{13}C NMR (150 MHz, MeOD): δ = 166.1 (C-4), 152.4 (C-2), 142.5 (C-6), 102.3 (C-5), 93.1 (C-1′), 83.8 (C-4′), 80.0 (C-2′), 73.3 (C-3′), 60.4 (C-5′), 20.1 ppm (–CH_3); HRMS (ESI+) calcd for $C_{10}H_{15}N_2O_6$ $[M + H]^+$ 259.0925, found 259.0932.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-cytidine (15). The synthetic procedure is followed according to '*Bioorg. Med. Chem. Lett.* **2009**, 19, 1392–1395' and the authenticity of the molecule was judged by comparing the NMR data with the literature values. Yield: 90%; TLC (CH_2Cl_2–MeOH, 9.0 : 1.0): R_f = 0.48; ^{1}H NMR (300 MHz, MeOD): δ = 7.95 (d, 1H, $J_{6,\ 5}$ = 7.53 Hz, H-6), 6.16 (s, 1H, H-1′), 5.92 (d, 1H, $J_{5,\ 6}$ = 7.53 Hz, H-5), 4.49 (d, J = 2.97 Hz, 1H, H-3′), 4.26–4.23 (m, 1H, H-4′), 3.89–3.76 (ddd, 2H, H-5′ & H-5″), 1.57 (s, 3H, –CH_3), 1.40 (s, 3H, –CH_3), 1.24 ppm (s, 3H, –CH_3). ^{13}C NMR (75 MHz, MeOD): δ = 165.8, 156.4, 141.3, 113.2, 93.8, 93.6, 90.2, 85.9, 84.1, 61.1, 26.9, 25.9, 18.2 ppm; HRMS (ESI+) calcd for $C_{13}H_{20}N_3O_5$ $[M + H]^+$ 298.1397, found 298.1402.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-uridine (16). The synthetic procedure is followed according to '*Bioorg. Med. Chem. Lett.*

2009, 19, 1392–1395' except for quenching and purification methods. After completion of the reaction by TLC, the reaction mixture was quenched by the addition of Et_3N and evaporated to dryness with silica gel and chromatographed on a silica gel column eluting with EtOAc–hexane (50–90% EtOAc) to obtain compound **16** as a white solid (81%). TLC (CH_2Cl_2–MeOH, 9.0 : 1.0): R_f = 0.54; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.09 (br s, 1H, –NH), 7.86 (d, 1H, $J_{6,5}$ = 8.23 Hz, H-6), 6.12 (s, 1H, H-1′), 5.74 (d, 1H, $J_{5,6}$ = 8.23 Hz, H-5), 4.55 (d, J = 2.88 Hz, 1H, H-3′), 4.33–4.27 (m, 1H, H-4′), 3.99–3.85 (m, 3H, 5′-OH, H-5′ & H-5″), 1.59 (s, 3H, $-CH_3$), 1.42 (s, 3H, $-CH_3$), 1.32 ppm (s, 3H, $-CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.4, 150.7, 141.6, 114.4, 101.7, 93.7, 90.4, 86.0, 84.5, 62.3, 28.4, 27.4, 19.5 ppm; HRMS (ESI+) calcd for $C_{13}H_{19}N_2O_6$ $[M + H]^+$ 299.1238, found 299.1239.

2′-*C*-Methyl-N^4-benzyloxycarbonyl-cytidine (22). A suspension of compound **1** (60 mg, 0.23 mmol) in dry pyridine was prepared and cooled to 0 °C in an ice bath. Trimethylsilyl chloride (0.44 mL, 3.5 mmol) was added dropwise under an argon atmosphere. After 10 minutes the ice bath was removed and the solution was left to stir at room temperature for 1.5 h. The reaction mixture was then cooled to 0 °C and benzyl chloroformate (0.13 mL, 1.2 mmol) was added slowly. After 10 minutes the ice bath was removed and the solution was left to stir at room temperature for 2 h. Upon completion, the reaction was quenched by adding methanol (2 mL) at 0 °C and then left to stir at room temperature overnight. To the solution was added saturated sodium bicarbonate (0.5 mL) and evaporated to dryness with repeated coevaporation using toluene. The residue was dissolved in methanol and evaporated with silica gel. The crude product was purified by silica gel column chromatography eluting with 0–4.5% methanol in dichloromethane to yield compound **22** as a white solid (90%). TLC (CH_2Cl_2–MeOH, 9.0 : 1.0): R_f = 0.5; ^{1}H NMR (600 MHz, MeOD): δ = 8.59 (d, 1H, $J_{6,5}$ = 7.6 Hz, H-6), 7.42–7.29 (m, 6H, phenyl ring & H-5), 6.07 (s, 1H, H-1′), 5.22 (s, 2H, $-CH_2Ph$), 4.02–3.96 (m, 2H, H-5′ & H-4′), 3.86–3.80 (m, 2H, H-3′ & H-5″), 1.10 ppm (s, 3H, 2′-CH_3); ^{13}C NMR (150 MHz, MeOD): δ = 165.7 (C-4), 159.0 (C-2), 155.4 (*CO*-OCH_2Ph), 147.0 (C-6), 138.0 (phenyl C), 130.5, 130.3, 130.1 (phenyl C), 97.5 (C-5), 95.0 (C-1′), 84.8 (C-4′), 81.1 (C-2′), 74.0 (C-3′), 69.4 (–*C*H_2Ph), 61.2 (C-5′), 21.0 ppm (2′-CH_3); HRMS (ESI–) calcd for $C_{18}H_{22}N_3O_7$ $[M - H]^-$ 390.1307, found 390.1305.

5′-*O*-*t*-Butyldimethylsilyl-2′-*C*-methyl-2′,3′-*O*-isopropyliden-cytidine (23). To a solution of compound **15** (66 mg, 0.22 mmol) in anhydrous pyridine (3 mL) was added imidazole (22 mg, 0.33 mmol) and *tert*-butyldimethylsilyl chloride (100 mg, 0.67 mmol) at 0 °C under an argon atmosphere. The reaction mixture was stirred at room temperature overnight and then quenched with methanol. The solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography eluting with 0–5% methanol in dichloromethane to obtain compound **23** as a white solid (95%). TLC (CH_2Cl_2–MeOH, 9.0 : 1.0); R_f = 0.48; ^{1}H NMR (300 MHz, MeOD): δ = 7.93 (d, 1H, $J_{6,5}$ = 7.61 Hz, H-6), 6.15 (s, 1H, H-1′), 5.93 (d, 1H, $J_{5,6}$ = 7.61 Hz, H-5), 4.47 (d, J = 2.96 Hz, 1H, H-3′), 4.30 (q, 1H, H-4′), 4.0 (dd, 1H, J = 2.8, 11.6 Hz, H-5′), 3.89 (dd, 1H, J = 2.8, 11.6 Hz, H-5″), 1.56 (s, 3H, $-CH_3$), 1.41 (s, 3H, $-CH_3$), 1.24 (s, 3H, $-CH_3$), 0.97 (s, 9H, *t*-Bu-Si), 0.17, 0.16 ppm (2 s, 6H, $(CH_3)_2$-Si). ^{13}C NMR (75 MHz, MeOD): δ = 167.9, 158.2, 143.6, 115.8, 96.6, 96.5, 93.1, 88.6, 87.1, 65.5, 29.5, 28.5, 27.3, 21.3, 20.2, −4.4 ppm; HRMS (ESI+) calcd for $C_{19}H_{34}N_3O_5Si$ $[M + H]^+$ 412.2262, found 412.2260.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-5′-*O*-*t*-butyldimethylsilyl-N^4-*t*-butyloxycarbonyl-cytidine (24). Compound **23** (86 mg, 0.21 mmol) was dissolved in THF–dioxane (1 : 1, 4.0 mL) and di-*tert*-butyl dicarbonate (85 mg, 0.4 mmol) was added. The reaction mixture was then heated under reflux for 5 h. After evaporation, the residue was purified by silica gel column chromatography eluting with 0–1.5% methanol in CH_2Cl_2 to obtain compound **24** as a white solid (77%). TLC (CH_2Cl_2–MeOH, 9.5 : 0.5); R_f = 0.7; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.08 (d, 1H, $J_{6,5}$ = 7.5 Hz, H-6), 7.55 (br s, 1H, –NH), 7.10 (d, 1H, $J_{5,6}$ = 7.5 Hz, H-5), 6.24 (s, 1H, H-1′), 4.42 (d, J = 3.06 Hz, 1H, H-3′), 4.23 (q, 1H, H-4′), 3.96 (dd, 1H, J = 2.4, 11.6 Hz, H-5′), 3.81 (dd, 1H, J = 2.4, 11.6 Hz, H-5″), 1.58 (s, 3H, $-CH_3$), 1.48 (s, 9H), 1.40 (s, 3H, $-CH_3$), 1.20 (s, 3H, $-CH_3$), 0.91 (s, 9H, *t*-Bu-Si), 0.10, 0.09 ppm (2 s, 6H, $(CH_3)_2$-Si); HRMS (ESI+) calcd for $C_{24}H_{41}N_3O_7Si$ $[M + H]^+$ 512.2786, found 512.2790.

2′-*C*-Methyl-2′,3′-*O*-isopropyliden-N^4-*t*-butyloxycarbonyl-cytidine (25). To a solution of **24** in dry pyridine–THF (1 : 1, 2 mL) was added $Et_3N{\cdot}3HF$ (0.15 mL, 0.9 mmol) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 4 h. The solvent was evaporated under reduced pressure and the crude residue was purified by silica gel column chromatography eluting with 0–3% methanol in dichloromethane to yield compound **25** as a white solid (92%). TLC (CH_2Cl_2–MeOH, 9.5 : 0.5); R_f = 0.5; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.29 (d, 1H, $J_{6,5}$ = 7.6 Hz, H-6), 7.84 (br s, 1H, –NH), 7.24 (d, 1H, $J_{5,6}$ = 7.6 Hz, H-5), 6.23 (s, 1H, H-1′), 4.66 (d, J = 2.97 Hz, 1H, H-3′), 4.36 (q, 1H, H-4′), 4.00 (dd, 1H, J = 2.8, 12.1 Hz, H-5′), 3.92 (dd, 1H, J = 2.8, 12.1 Hz, H-5″), 1.61 (s, 3H, $-CH_3$), 1.50 (s, 9H, *t*-Bu), 1.43 (s, 3H, $-CH_3$), 1.26 ppm (s, 3H, $-CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.0, 155.9, 151.5, 145.5, 114.2, 95.4, 95.2, 91.3, 86.5, 85.5, 83.0, 62.2, 46.8, 28.6, 28.3, 27.6, 19.9, 9.1 ppm; HRMS (ESI+) calcd for $C_{18}H_{28}N_3O_7$ $[M + H]^+$ 398.1922, found 398.1919.

Synthetic procedures and characterization data for compounds **3** and **27–29** are provided in the ESI.†

HCV replicon assay

The test compounds were prepared as 10 mM dimethyl sulfoxide (DMSO) stock solutions and stored at −20 °C until being used in the assay. The antiviral activity and cytotoxicity of compounds were determined by using the HCV replicon cell line ET (luc-ubi-neo/ET), which is a Huh7 human hepatoma cell line that contains an HCV1b/Con1 replicon with a stable luciferase (Luc) reporter and three cell culture-adaptive mutations. Since the expression of the luciferase reporter gene is under the control of HCV RNA replication and the turnover of the luciferase protein is rapid, the luciferase activity is considered as a representative of the amount of HCV RNA present in the

cells. The HCV replicon antiviral evaluation assay examined the effects of compounds at six half-log concentrations each. Sub-confluent cultures of the ET line were plated in 96-well plates that were dedicated for the analysis of cell numbers (cytotoxicity) or antiviral activity and the next day compounds were added to the appropriate wells. Cells are processed 72 h later when the cells are still sub-confluent and the EC_{50} values (concentration inhibiting the HCV replicon by 50%) are determined from a graphical plot based on the luciferase activity. Each data point represents the average for four replicates in cell culture in a single experiment. The toxic concentration of the compound that reduces cell numbers is assessed by the CytoTox-1 cell proliferation assay (Promega) which is a colorimetric assay of cell numbers (and cytotoxicity).

Stability study in human serum

The experiment was carried out by dissolving ProTide **1g** and **2c** (5–6 mg) in DMSO-d_6 (0.06 mL) and D_2O (0.18 mL). After recording the ^{31}P NMR spectra at 37 °C as a control, human serum (0.35 mL) was added to the sample. Next, a series of ^{31}P NMR spectra were recorded at 37 °C over a period of 16 h. The ^{31}P NMR data were processed and analyzed with the Bruker Topspin 2.1 program.

Metabolism study in the human liver S9 fraction

A stock solution of ProTide (50 mM) was prepared in pure DMSO and stored at −20 °C until use. Reagents including NADPH and liver S9 fraction were thawed and immediately placed on ice. The reaction was performed in a total volume of 1 mL containing 5 mM of $MgCl_2$, 50 mM of K_2HPO_4 (pH 7.4), 5 mM NADPH, and 4 mg mL^{-1} human liver S9 fraction. The eppendorf was preincubated in a shaker at 37 °C for 10 min. The reaction was then initiated by addition of ProTide at a final concentration of 100 μM. The suspension was thoroughly mixed by pipetting. At the desired times (0, 0.5, 1, 2, 4, 6, 8, 24, and 51 h), 50 μL aliquots were taken and the reaction was stopped by mixing the reaction mixture sample with 150 μL of cold acetonitrile. The samples were centrifuged at 14 000 rpm for 30 min at 4 °C. Then 50 μL of the supernatant was taken from each sample and stored at −20 °C until LC/MS analysis.

Acknowledgements

This research was financed by the F.W.O. grant (G078014N). Mass spectrometry was made possible by the support of the Hercules Foundation of the Flemish Government (grant 20100225-7). Antiviral evaluation was performed by Southern Research Institute, USA. We thank Chantal Biernaux for her secretarial support.

Notes and references

1 http://www.who.int/en/.
2 I. M. Jacobson, G. L. Davis, H. El-Serag, F. Negro and C. Trepo, *Clin. Gastroenterol. Hepatol.*, 2010, **8**, 924–933.
3 J. H. Hoofnagle and L. B. Seeff, *N. Engl. J. Med.*, 2006, **355**, 2444–2451.
4 J.-M. Pawlotsky, *Curr. Top. Microbiol. Immunol.*, 2013, **369**, 321–342.
5 M. Capuozzo, A. Ottaiano, E. Nava, V. Scotti, S. Cascone, A. Vercellone, C. Cinque and R. V. Iaffaioli, *Front. Pharmacol.*, 2013, **4**, 114.
6 J. Kieran, S. Schmitz, A. O'Leary, C. Walsh, C. Bergin, S. Norris and M. Barry, *Clin. Infect. Dis.*, 2013, **56**, 228–235.
7 V. Soriano, E. Vispo, E. Poveda, P. Labarga, L. Martin-Carbonero, J. V. Fernandez-Montero and P. Barreiro, *J. Antimicrob. Chemother.*, 2011, **66**, 1673–1686.
8 L. Delang, J. Neyts, I. Vliegen, S. Abrignani, P. Neddermann and R. D. Francesco, *Curr. Top. Microbiol. Immunol.*, 2013, **369**, 289–320.
9 R. Bartenschlager, V. Lohmann and F. Penin, *Nat. Rev. Microbiol.*, 2013, **11**, 482–496.
10 S.-E. Behrens, L. Tomei and R. D. Francesco1, *EMBO J.*, 1996, **15**, 12–22.
11 S. S. Carroll and D. B. Olsen, *Infect. Disord.: Drug Targets*, 2006, **6**, 17–29.
12 M. J. Sofia, W. S. Chang, P. A. Furman, R. T. Mosley and B. S. Ross, *J. Med. Chem.*, 2012, **55**, 2481–2531.
13 C. Gardelli, B. Attenni, M. Donghi, M. Meppen, B. Pacini, S. Harper, A. Di Marco, F. Fiore, C. Giuliano and V. Pucci, *J. Med. Chem.*, 2009, **52**, 5394–5407.
14 C. McGuigan, A. Gilles, K. Madela, M. Aljarah, S. Holl, S. Jones, J. Vernachio, J. Hutchins, B. Ames, K. D. Bryant, E. Gorovits, B. Ganguly, D. Hunley, A. Hall, A. Kolykhalov, Y. Liu, J. Muhammad, N. Raja, R. Walters, J. Wang, S. Chamberlain and G. Henson, *J. Med. Chem.*, 2010, **53**, 4949–4957.
15 M. J. Sofia, D. Bao, W. Chang, J. Du, D. Nagarathnam, S. Rachakonda, P. G. Reddy, B. S. Ross, P. Wang, H.-R. Zhang, S. Bansal, C. Espiritu, M. Keilman, A. M. Lam, H. M. M. Steuer, C. Niu, M. J. Otto and P. A. Furman, *J. Med. Chem.*, 2010, **53**, 7202–7218.
16 M. Rodríguez-Torres, *Expert Rev. Anti-Infect. Ther.*, 2013, **11**, 1269–1279.
17 L. P. Jordheim, D. Durantel, F. Zoulim and C. Dumontet, *Nat. Rev. Drug Discovery*, 2013, **12**, 447–464.
18 G. Migliaccio, J. E. Tomassini, S. S. Carroll, L. Tomei, S. Altamura, B. Bhat, L. Bartholomew, M. R. Bosserman, A. Ceccacci, L. F. Colwell, R. Cortese, R. De Francesco, A. B. Eldrup, K. L. Getty, X. S. Hou, R. L. LaFemina, S. W. Ludmerer, M. MacCoss, D. R. McMasters, M. W. Stahlhut, D. B. Olsen, D. J. Hazuda and O. A. Flores, *J. Biol. Chem.*, 2003, **278**, 49164–49170.
19 E. Murakami, H. Bao, M. Ramesh, T. R. McBrayer, T. Whitaker, H. M. M. Steuer, R. F. Schinazi, L. J. Stuyver, A. Obikhod and M. J. Otto, *Antimicrob. Agents Chemother.*, 2007, **51**, 503–509.
20 K. G. Devine, C. McGuigan, T. J. O'Connor, S. R. Nicholls and D. Kinchington, *AIDS*, 1990, **4**, 371–373.

21 D. Cahard, C. McGuigan and J. Balzarini, *Mini-Rev. Med. Chem.*, 2004, **4**, 371–381.
22 E. Murakami, T. Tolstykh, H. Y. Bao, C. R. Niu, H. M. M. Steuer, D. H. Bao, W. Chang, C. Espiritu, S. Bansal, A. M. Lam, M. J. Otto, M. J. Sofia and P. A. Furman, *J. Biol. Chem.*, 2010, **285**, 34337–34347.
23 D. R. Bobeck, R. F. Schinazi and S. J. Coats, *Antiviral Ther.*, 2010, **15**, 935–950.
24 O. Adelfinskaya and P. Herdewijn, *Angew. Chem., Int. Ed.*, 2007, **46**, 4356–4358.
25 O. Adelfinskaya, M. Terrazas, M. Froeyen, P. Marliere, K. Nauwelaerts and P. Herdewijn, *Nucleic Acids Res.*, 2007, **35**, 5060–5072.
26 S. Q. Yang, C. Pannecouque, E. Lescrinier, A. Giraut and P. Herdewijn, *Org. Biomol. Chem.*, 2012, **10**, 146–153.
27 A. Giraut, X. P. Song, M. Froeyen, P. Marliere and P. Herdewijn, *Nucleic Acids Res.*, 2010, **38**, 2541–2550.
28 M. G. Nicolaou, C.-S. Yuan and R. T. Borchardt, *J. Org. Chem.*, 1996, **61**, 8636–8641.
29 X. Q. Tang, X. Liao and J. A. Piccirilli, *J. Org. Chem.*, 1999, **64**, 747–754.
30 M. Donghi, B. Attenni, C. Gardelli, A. D. Marco, F. Fiore, C. Giuliano, R. Laufer, J. F. Leone, V. Pucci and M. Rowley, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1392–1395.
31 J. H. Cho, F. Amblard, S. J. Coats and R. F. Schinazi, *Tetrahedron*, 2011, **67**, 5487–5493.
32 M. A. Gallop, *USA Pat*, US 8058260 B2, 2011.
33 S. Manfredini, N. Solaroli, A. Angusti, F. Nalin, E. Durini, S. Vertuani, S. Pricl, M. Ferrone, S. Spadari and F. Focher, *Antiviral Chem. Chemother.*, 2003, **14**, 183–194.
34 J. L. Clark, L. Hollecker, J. C. Mason, L. J. Stuyver, P. M. Tharnish, S. Lostia, T. R. McBrayer, R. F. Schinazi, K. A. Watanabe and M. J. Otto, *J. Med. Chem.*, 2005, **48**, 5504–5508.
35 P. Y. Wang, B. K. Chun, S. Rachakonda, J. F. Du, N. Khan, J. X. Shi, W. Stee, D. Cleary, B. S. Ross and M. J. Sofia, *J. Org. Chem.*, 2009, **74**, 6819–6824.
36 D. M. Lehsten, D. N. Baehr, T. J. Lobl and A. R. Vaino, *Org. Process Res. Dev.*, 2002, **6**, 819–822.
37 S. Benzaria, D. Bardiot, T. Bouisset, C. Counor, C. Rabeson, C. Pierra, R. Storer, A. G. Loi, A. Cadeddu, M. Mura, C. Musiu, M. Liuzzi, R. Loddo, S. Bergelson, V. Bichko, E. Bridges, E. Cretton-Scott, J. Mao, J. P. Sommadossi, M. Seifer, D. Standring, M. Tausek, G. Gosselin and P. La Colla, *Antiviral Chem. Chemother.*, 2007, **18**, 225–242.
38 G. Veenstra, C. Webb, H. Sanderson, S. E. Belanger, P. Fisk, A. Nielsen, Y. Kasai, A. Willing, S. Dyer and D. Penney, *Ecotoxicol. Environ. Saf.*, 2009, **72**, 1016–1030.
39 M. A. Keller, K. Watschinger, K. Lange, G. Golderer, G. Werner-Felmayer, A. Hermetter, R. J. A. Wanders and E. R. Werner, *J. Lipid Res.*, 2012, **53**, 1410–1416.
40 W. B. Rizzo, D. A. Craft, A. L. Dammann and M. W. Phillips, *J. Biol. Chem.*, 1987, **262**, 17412–17419.
41 A. J. White, *Sex. Transm. Infect.*, 2001, **77**, 158–173.
42 M. Maiti, L. Persoons, G. Andrei, R. Snoeck, J. Balzarini and P. Herdewijn, *ChemMedChem*, 2013, **8**, 985–993.
43 L. Guo, S. Dial, L. Shi, W. Branham, J. Liu, J.-L. Fang, B. Green, H. Deng, J. Kaput and B. Ning, *Drug Metab. Dispos.*, 2010, **39**, 528–538.
44 M. Ora, J. Ojanpera and H. Lohnberg, *Chem. – Eur. J.*, 2007, **13**, 8591–8599.