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Design, synthesis and evaluation of carbamate-linked uridyl-based inhibitors of human ST6Gal I

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Abstract

© 2020 Elsevier Ltd Sialic acid at the terminus of cell surface glycoconjugates is a critical element in cell-cell recognition, receptor binding and immune responses. Sialyltransferases (ST), the enzymes responsible for the biosynthesis of sialylated glycans are highly upregulated in cancer and the resulting hypersialylation of the tumour cell surface correlates strongly with tumour growth, metastasis and drug resistance. Inhibitors of human STs, in particular human ST6Gal I, are thus expected to be valuable chemical tools for the discovery of novel anticancer drugs. Herein, we report on the computationally-guided design and development of uridine-based inhibitors that replace the charged phosphodiester linker of known ST inhibitors with a neutral carbamate to improve pharmacokinetic properties and synthetic accessibility. A series of 24 carbamate-linked uridyl-based compounds were synthesised by coupling aryl and hetaryl α-hydroxyphosphonates with a 5'-amino-5'-deoxyuridine fragment. The inhibitory activities of the newly synthesised compounds against recombinant human ST6Gal I were determined using a luminescent microplate assay, and five promising inhibitors with Ki's ranging from 1 to 20 μM were identified. These results show that carbamate-linked uridyl-based compounds are a potential new class of readily accessible, non-cytotoxic ST inhibitors to be further explored.

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Design, Synthesis and Evaluation of Carbamate-linked Uridyl-based Inhibitors of Human ST6Gal I

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ABSTRACT

Sialic acid at the terminus of cell surface glycoconjugates is a critical element in cell-cell recognition, receptor binding and immune responses. Sialyltransferases (ST), the enzymes responsible for the biosynthesis of sialylated glycans are highly upregulated in cancer and the resulting hypersialylation of the tumour cell surface correlates strongly with tumour growth, metastasis and drug resistance. Inhibitors of human STs, in particular human ST6Gal I, are thus expected to be valuable chemical tools for the discovery of novel anticancer drugs. Herein, we report on the computationally-guided design and development of uridine-based inhibitors that replace the charged phosphodiester linker of known ST inhibitors with a neutral carbamate to improve pharmacokinetic properties and synthetic accessibility. A series of 24 carbamate-linked uridyl-based compounds were synthesised by coupling aryl and hetaryl α-hydroxyphosphonates with a 5′-amino-5′-deoxyuridine fragment. The inhibitory activities of the newly synthesised compounds against recombinant human ST6Gal I were determined using a luminescent microplate assay, and five promising inhibitors with K_i's ranging from 1-20 μM were identified. These results show that carbamate-linked uridyl-based compounds are a potential new class of readily accessible, non-cytotoxic ST inhibitors to be further explored.

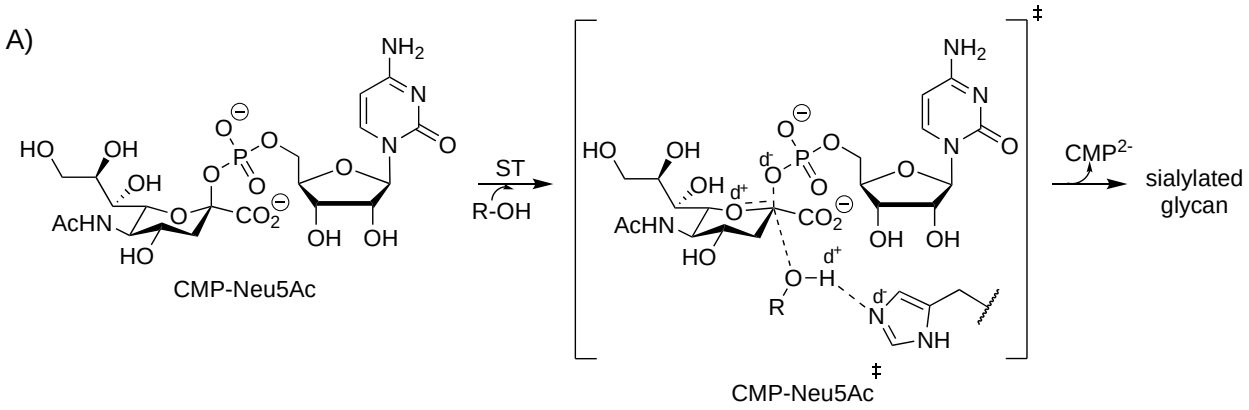
INTRODUCTION

Sialic acid (*N*-acetylneuraminic acid, Neu5Ac) is one of the human body's most important sugars next to glucose.[1] These negatively charged nine-carbon α-keto aldonic acids are located at the terminal end of glycan chains on cell surface and secreted molecules, where they play essential roles in cellular biology.[2, 3] These functions are mainly related to cellular and molecular recognition events, such as activation or inhibition of intracellular and intramolecular interactions, cell-cell recognition, receptor binding, protein-lectin interactions, protein targeting, cell adhesion, and immune responses.[4-7] The biosynthesis of sialic acid containing glycoconjugates in humans is mediated by 20 sialyltransferases (STs) anchored within the Golgi apparatus' membrane with the catalytic domain present within the lumen.[4-5] Sialylation catalysed by STs uses the sugar nucleotide donor CMP-Neu5Ac and an oligosaccharide or glycoconjugate terminated by a galactose (Gal), *N*-acetylgalactosamine (GalNAc), or another sialic acid residue as the acceptor.[4, 8]

Upregulation of ST activity and the resulting modified cell surface sialylation is strongly associated with cancer, with hypersialylation of 30–50% observed in several cancers.[9] This has been directly correlated with an increased metastatic potential of tumours, facilitation of apoptotic avoidance mechanisms and poor patient prognosis.[10, 11] Hypersialylation as a result of ST upregulation is also linked to chemo-resistance and thus reduced treatment efficacy in ovarian,[12-14] colorectal,[15] and cervical cancer,[16] hepatocellular carcinoma,[17] pancreatic ductal adenocarcinoma[18] and myeloid leukaemia.[19] Exposure to radiation induces an increased expression of human ST6Gal I (hST6Gal I) and reduces radiation-induced cell death in colon cancer.[20] Thus, the critical role of STs in tumour growth, progression and resistance to both radio- and

chemotherapy demonstrates the importance of developing small molecule modulators of this family of enzymes as tools to help understand a potential new anti-cancer drug target.

A number of ST inhibitors have been reported ranging from those isolated from natural sources (e.g. lithocholic acid and soyasaponin) and high throughput screening to those specifically designed to mimic ST substrates as reviewed recently.[9] Of these, analogues mimicking the proposed oxocarbenium ion-like transition state of the donor (Figure 1A) pioneered by RR Schmidt, exhibit the highest affinity to STs.[21, 22] The most potent are those incorporating a 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (Neu5Ac2en) moiety, where the C2–C3 double bond mimics the planar anomeric carbon; and an extra carbon between the anomeric carbon and CMP leaving group mimics the elongated distance in the transition state.[21, 23] Of the various modifications, substituting the glycerol side chain of the Neu5Ac2en moiety with a phenoxy group gave derivative **1** with a K_i value of 29 nM against rat liver ST6Gal in a HPLC-based assay (Figure 1B).[24] More synthetically accessible derivatives that replace the Neu5Ac2en with an aryl moiety have been developed.[21, 22, 25] For example, the (*R*)-isomer of the 3-phenoxy derivative **2** (Figure 1B) is a potent inhibitor of both rat liver ST6Gals (K_i = 70 nM)[21] and recombinant human ST6Gal I (K_i = 19 nM),[26] along with cyclopentyl[26] (**3**) and amide[27] (**4**) derivatives with K_i values of 28 nM and 16 nM respectively against hST6Gal I, also in a HPLC-based assay. It has also been demonstrated that the size of the aryl substituent does not impact binding to human ST6Gal I, with fluorescein-labelled derivatives such as *(R)*-**5** and *(S)*-**5**, exhibiting K_d values of 22 nM and 9 nM, respectively, against human ST6Gal I.[28] These fluorescein-labelled derivatives have since been reported as important high-throughput in vivo screening tools to assist in the development of inhibitors of human oligo- and polysialyltransferases.[29]



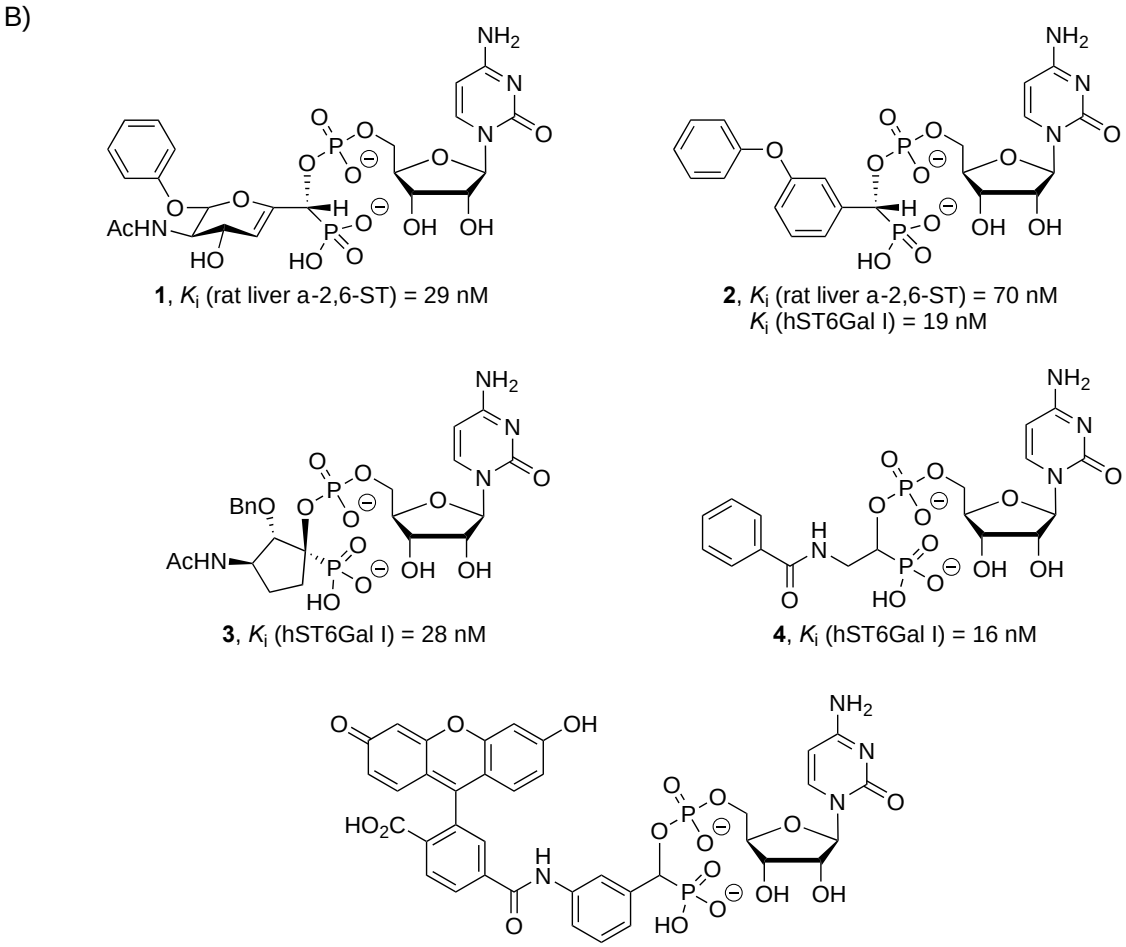


Figure 1. (A) General mechanism of sialylation catalysed by sialyltransferases. (B) Reported transition-state analogue inhibitors **1**–**4** active against rat and human ST6Gal enzymes as determined using a HPLC-based assay[21, 24, 26, 27] and reported fluorescent cell-permeable chemical probe **5**.[28]

The charged phosphodiester linkage present in the majority of known ST inhibitors (e.g. Figure 1B) is considered essential for activity.[21, 22, 25, 30] Yet it may also lead to low bioavailability in vivo and loss of activity due to cleavage by phosphatases or instability in the ST active site.[31, 32] The charged nature of the linker may also lead to poor cellular permeability, although recent reports of phosphodiester derivatives bearing a fluorescent probe (such as **5**, Figure 1B) have suggested these charged compounds may be able to cross cellular membrane via a vesicular uptake mechanism.[28] In addition to the potential pharmacokinetic issues, the synthesis of the phosphodiester-linked inhibitors utilises a capricious condensation of an α-hydroxyphosphonate and an highly air-sensitive cytidine phosphitamide.[21, 26, 27]

To improve synthetic accessibility to ST inhibitors, we have replaced the phosphodiester linker with an uncharged carbamate, which can be synthesised using a wide range of alkoxy carbonylating agents.[33] Structurally, the carbamate functionality is related to an amide-ester hybrid and in general displays very good chemical and proteolytic stability. Their capability to permeate cell membranes has resulted in carbamates being widely utilised as peptide bond isosteres,[33] which would also provide advantages in this case. More specifically a carbamate moiety would have similar hydrogen bonding capabilities to a phosphodiester group and maintain the three-atom distance between the nucleoside (Figure 2) and the Neu5Ac mimic observed in reported phosphodiester-linked ST inhibitors such as **1**–**4** (Figure 1B).

We have demonstrated previously using docking and molecular dynamics (MD) simulations that carbamate- and 1,2,3-triazole-linked derivatives have comparable interactions to their phosphodiester-linked counterparts with the hST6Gal I active site (PDB ID: 4JS2).[34, 35] Using free energy perturbation (FEP) calculations we have shown that these compounds can successfully mimic the charged phosphodiester linkage with a comparable binding affinity through an

enthalpy-entropy compensation.[36] We have also explored replacing the cytidine moiety with uridine, which has the advantage of requiring less protecting groups enabling inhibitors to be produced more readily and in fewer steps. In addition, as CMP-Neu5Ac is the common natural donor for all ST subtypes, the replacement of the cytidine moiety with uridine could be a hitherto unexplored route to selectivity.

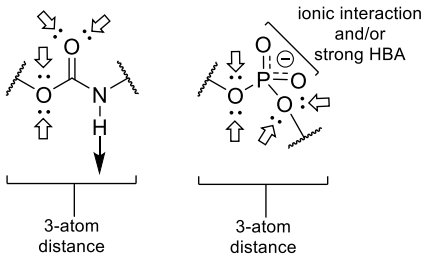


Figure 2. The possible binding interactions of a carbamate and phosphodiester linker. White arrows represent hydrogen bond acceptors and black arrows represent hydrogen bond donors.

Herein, the phosphodiester linker of classical ST inhibitors has been replaced with a carbamate, along with uridine in place of cytidine and the effects investigated both computationally and experimentally. Additionally, the difference of diastereomers and various substituents of the aryl sialic acid mimic, particularly at the 3-position, were also explored. The target compounds were prepared in 7 steps from two protected building blocks: the 5′-amino-5′-deoxynucleoside and an α-hydroxyphosphonate. The inhibitory activity of the newly synthesised compounds was then evaluated against recombinant hST6Gal I in a luminescence microplate assay, along with cellular toxicity assessment in a pancreatic cancer cell line.

RESULTS AND DISCUSSION

1. Free Energy Calculations. Building on previous computational studies demonstrating the feasibility of using carbamates and 1,2,3-triazoles as phosphodiester isosteres,[34-37] we have

performed FEP calculations, which are one of the most rigorous calculation methods available to compare the relative binding affinity of cytidine- and uridine-based carbamate inhibitors with hST6Gal I (PDB ID: 4JS2).[38, 39] Alchemical transformation of the cytidine-based inhibitor ***(R)*-6** to the uridine-based inhibitor ***(R)*-7** in complex with hST6Gal I and in solution was performed (Figure 3).[40, 41] Combining the data from all simulations, the $\Delta\Delta G_b$ value observed for this transformation was −1.2 ± 0.3 kcal/mol (see Supporting Information, Table S1 and S2). As state-of-the-art computational studies report a root-mean-square-deviation of ~1.0 kcal/mol for the relative binding free energies when comparing to experimental data,[39] our results suggests that replacing the cytidine in carbamate-linked inhibitors with a uridine would not have a significant impact on binding to hST6Gal I, with uridine potentially being slightly preferential.

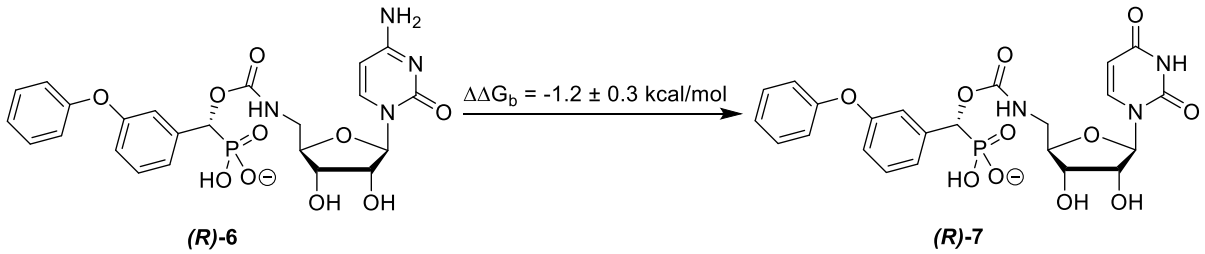


Figure 3. The perturbation of cytidine-based ***(R)*-6** to uridine-based ***(R)*-7** performed during FEP calculation of $\Delta\Delta G_b$ to hST6Gal I (PDB ID: 4JS2).

To rationalise the result observed during the FEP calculation an additional 15 ns of MD simulation was performed for uridine-based inhibitor ***(R)*-7**. From these trajectories the hydrogen bonds and hydrophobic contacts of the uridine component and the binding pocket were analysed and compared to those previously reported[36] for the cytidine component of ***(R)*-6** (Figure 4). This comparison showed that a majority of interactions were maintained between the two nucleoside components in support of the FEP calculations and suggesting that hST6Gal I can accommodate uridine-based derivatives. Based on the outcomes of these calculations we prepared a series of uridine-based compounds (see Table 1).

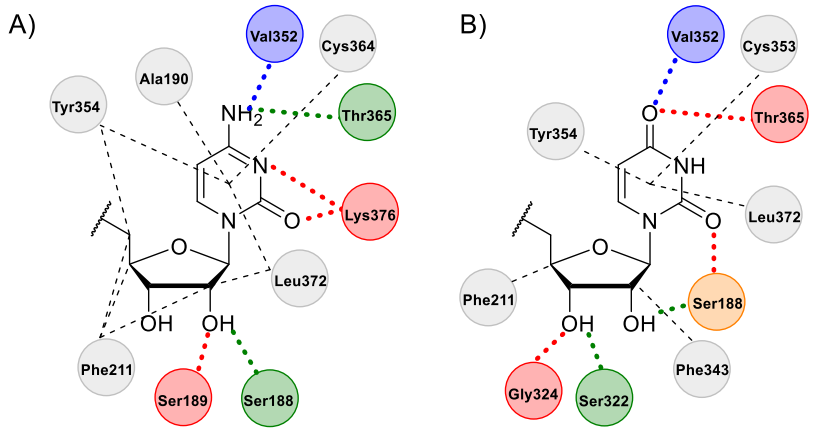
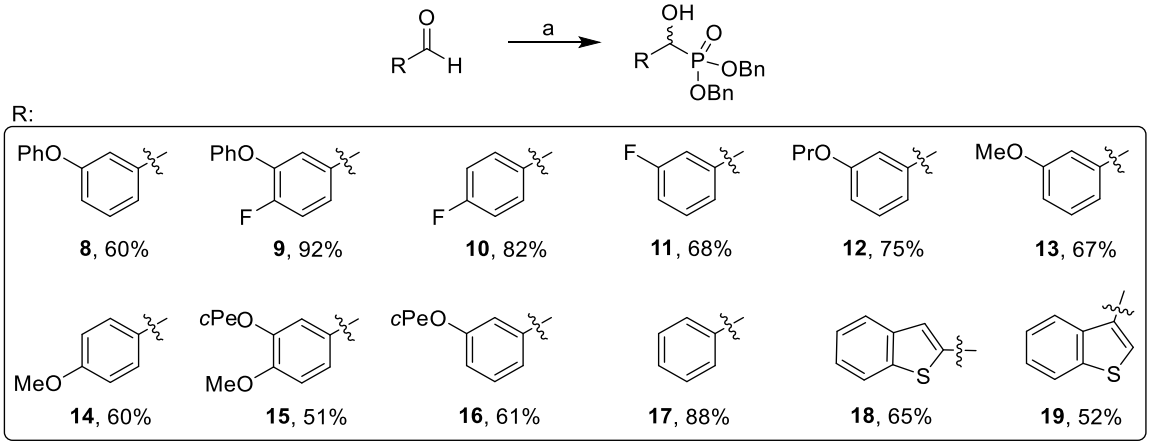


Figure 4. Comparison of interactions with hST6Gal I between the (A) cytidine component of ***(R)*-6**[36] and the (B) uridine component of ***(R)*-7**. Consistent interactions were observed between the remaining components of ***(R)*-6** and ***(R)*-7**. Interacting hST6Gal I residues (PDB ID: 4JS2) are represented as circles which are colour coded based on interaction type as follows: Red = hydrogen bond donor; Green = hydrogen bond acceptor; Blue = water bridged hydrogen bond; Orange = hydrogen bond donor and acceptor; Grey = hydrophobic contact. Dashed lines indicate hydrophobic contacts and dotted lines represent the hydrogen bonds shown in Supplementary Table 3 and 4.

2. Synthesis of Sialic Acid Mimic. The sialic acid mimics for the target compounds synthesised in this work are α-hydroxyphosphonates that are derived from commercially available aldehydes. The benzyl protecting group was selected for the phosphonate as it can be easily removed under catalytic hydrogenation conditions. Based on reported methods,[23, 42] the aldehydes were reacted with dibenzyl phosphite and triethylamine in dichloromethane (CH_2Cl_2) to give the requisite α-hydroxyphosphonates (**8–19**) in good to excellent yields of 51–92% as racemic mixtures (Scheme 1). As the diastereoisomeric final products were readily separable by RP-HPLC, racemic mixtures of the α-hydroxyphosphonates were used in the following synthetic steps.[21, 26, 27]

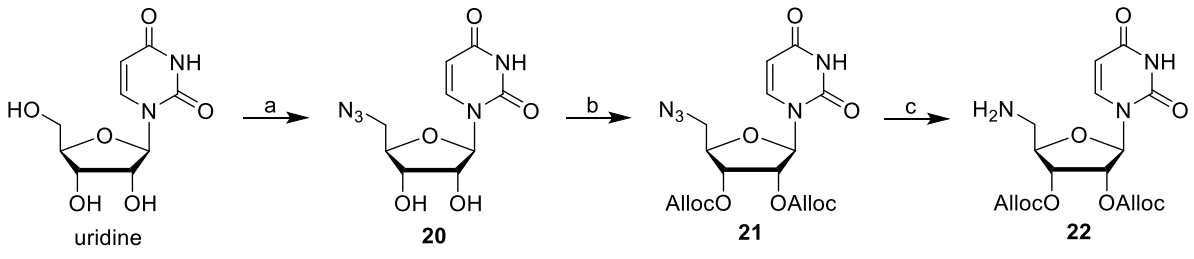
Scheme 1. Synthesis of the α-hydroxyphosphonates **8**–**19**.[a]



[a]**Reagents and conditions:** (a) Dibenzyl phosphite, triethylamine, CH_2Cl_2, rt, overnight.

3. Synthesis of the Nucleoside Fragment. The nucleoside fragments were prepared from cytidine and uridine. Several protecting groups were trialled with the allyloxycarbonyl (Alloc) protecting group favourable for uridine, and benzyloxycarbonyl (Cbz) preferred for cytidine to facilitate global deprotection. Thus, Alloc protected uridine derivatives were prepared by the initial 5′-selective installation of an azide via a Mitsunobu reaction to give **20**.[43] This was followed by the reaction with allyl chloroformate to give the Alloc protected 5′-azidouridine **21** that was then reduced to give the protected 5′-amino-5′-deoxyuridine **22** in 61% overall yield (Scheme 2).

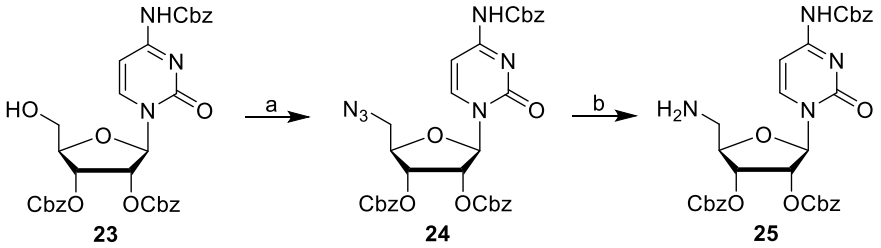
Scheme 2. Synthesis of the Alloc protected 5′-amino-5′-deoxyuridine **22**.[a]



[a]**Reagents and conditions:** (a) Triphenylphosphine, DIAD, HN_3, THF, 0ºC–rt, 17 h, 97%; (b) allyl chloroformate, pyridine, 20ºC–rt, 30 min, 90%; (c) (i) triphenylphosphine, THF, rt, 2 h; (ii) H_2O, reflux, 6 h, 70% over two steps.

The Cbz protected 5′-hydroxycytidine derivative **23** was synthesised from cytidine as reported.[44] The *N/O*-protected compound **23** was subjected to azidonation under Mitsunobu conditions to yield **24**, then reduced to produce the protected 5′-amino-5′-deoxycytidine fragment **25** in 33% overall yield in five steps from cytidine (Scheme 3).

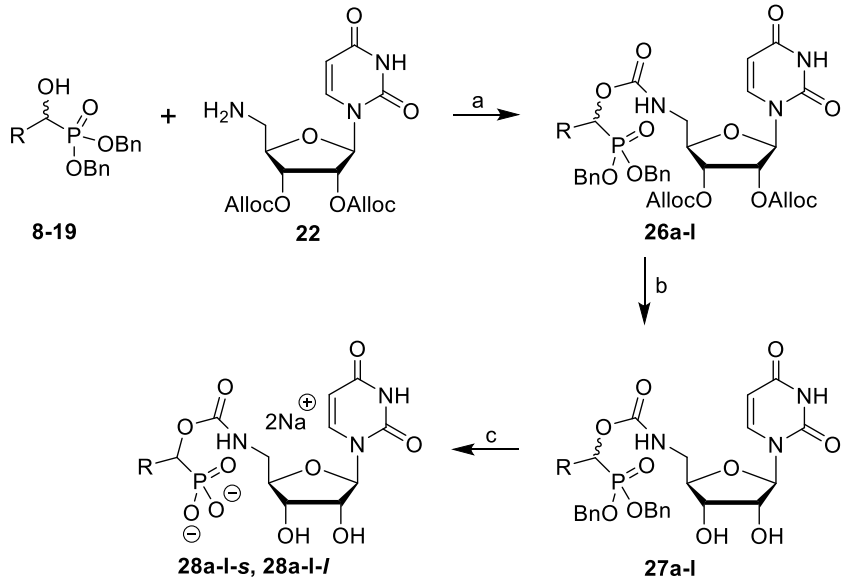
Scheme 3. Synthesis of Cbz protected 5′-amino-5′-deoxycytidine **25**.[a]



[a]**Reagents and conditions:** (a) Triphenylphosphine, DIAD, HN_3, THF, 0°C–rt, 18 h, 97%; (b) (i) triphenylphosphine, THF, rt, 2 h; (ii) H_2O, reflux, 5 h, 50% over two steps.

4. Synthesis of Target Molecules. Coupling of the α-hydroxyphosphonate building blocks **8–19** and the 5′-amino-derivatives **22** or **25** was performed using 4-nitrophenyl chloroformate (4-NPC) and catalytic DMAP (Schemes 4 and 5). The protected carbamate coupled derivatives were obtained as a 1:1 mixture of diastereoisomers in 22–73% yield (Table 1). The fully protected uridine-based derivatives **26a–l** required two deprotection steps (Scheme 4), both performed in excellent yields (Table 1). The first deprotection step involved removal of the Alloc protecting groups using $Pd(PPh_3)_4$, with dimedone as an allyl scavenger. The partially deprotected derivatives **27a–l** were subjected to hydrogenation (10% Pd/C and one atmosphere of H_2) to remove the benzyl protecting groups to produce the crude mixture of target uridine-based compounds **28a–l**.

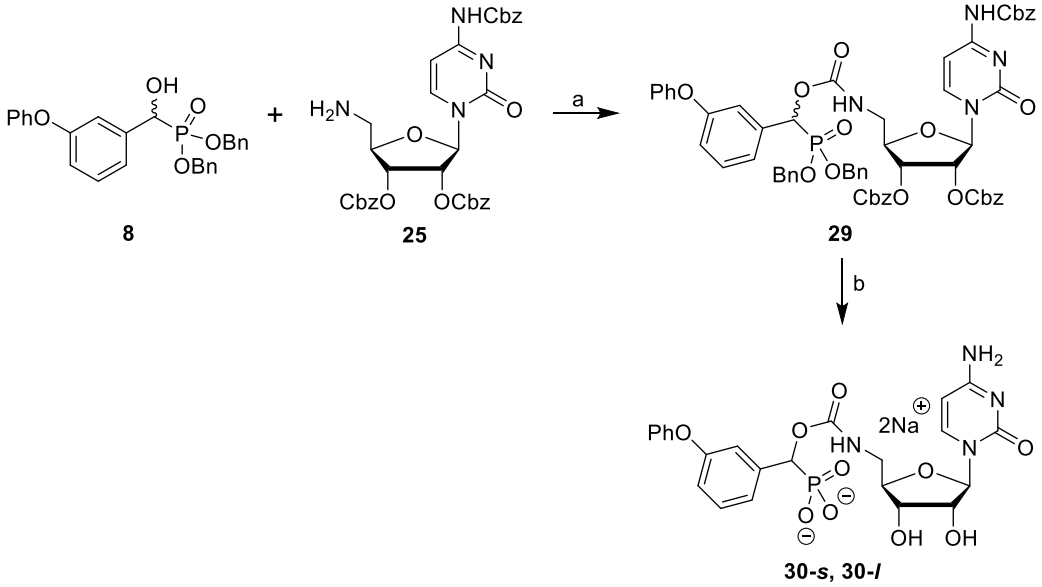
Scheme 4. Synthesis of uridine-based target compounds **28a–l**.[a, b]



[a]**Reagents and conditions:** (a) (i) Compounds **8–19**, 4-NPC, triethylamine, DMAP, CH_2Cl_2, 0ºC–rt, 3 h; (ii) Compound **22**, rt, 24 h; (b) $Pd(PPh_3)_4$, dimedone, THF, rt, 30 min; (c) (i) 10% Pd/C, 1 atm. H_2, rt, 1 h; (ii) RP-HPLC; (iii) IR 120 Na^+. [b]Refer to Table 1 for R group identity.

The Cbz and benzyl protected cytidine-based derivative **29** underwent a global deprotection (10% Pd/C/H_2) to produce a crude mixture of the target cytidine-based compound **30** (Scheme 5). The 1:1 diastereomeric mixture of compounds **28a–l** and **30** were separated by preparative RP-HPLC, converted to their corresponding sodium salt via ion exchange, and lyophilised to produce the target compounds in excellent yields (Table 1). The separated diastereomers are designated as (***s***) and (***l***), representing the shorter and longer HPLC retention times respectively, as the chemical shift differences between the diastereomers were too small to use predictive computational methods to assign the stereoisomers.[45, 46] All final compounds were stable in their salt form at room temperature in a sealed vial for over a year with negligible loss of activity, and stable for up to 6 months in solution at -18°C.

Scheme 5. Synthesis of cytidine-based target compounds **30-(*s*)** and **30-(*l*)**.[a]

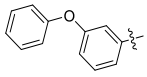
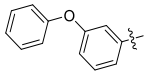
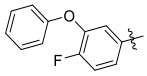
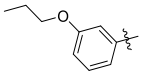
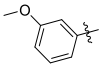
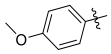
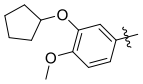
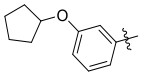
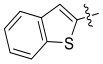


[a]**Reagents and conditions:** (a) (i) Compound **8**, 4-NPC, triethylamine, DMAP, CH_2Cl_2, 0ºC–rt, 3 h; (ii) Compound **25**, rt, 24 h; (b) (i) 10% Pd/C, 1 atm. H_2, rt, 1 h; (ii) RP-HPLC; (iii) IR 120 Na^+.

5. Enzymatic Assay. The majority of ST inhibition data reported to-date has been obtained using a HPLC-based assay that relies on quantification of the transferred sialic acid onto a UV-labelled acceptor and reported in terms of their K_i inhibitory constant values.[25] In recent years, more rapid and sensitive microplate assays have also been developed.[47] Herein, the ST inhibitory activity was determined using the CMP-Glo™ luminescence microplate assay from Promega as detailed by Das et al.[48] for the uridine-based compounds **28a–l** and cytidine-based compound **30** against recombinant hST6Gal I (aa 44–406) from R&D Systems. CMP-Neu5Ac was used as the sialic acid donor and *N*-acetyllactosamine (LacNAc) used as the acceptor. CMP produced as a by-product of the ST reaction was detected using a luciferase-based reaction with a linear response to the concentration of CMP. The luminescence produced was detected using a POLARstar Omega plate reader. With acceptor concentration set at 1 mM, the K_m for CMP-Neu5Ac was calculated to be 37.2 ± 5.4 μM (see Supporting Information, Figure S15), which was comparable to reported

data.[26, 49, 50] The inhibition rates of all compounds at 10 μM (with 1 mM of acceptor and 100 μM CMP-Neu5Ac) was initially obtained (Table 1), and for those compounds with over 30% inhibition, K_i values were also determined (Table 2). As shown in Table 1 all compounds tested were active at 10 μM, with the Lineweaver–Burk double reciprocal plots suggesting a mixed mode of inhibition as reported previously for ST inhibitors (see Supporting Information, Figure S16–S22).[9, 49] As the vast majority of ST inhibitors are reported in terms of their K_i inhibition constants, the same measure has been used herein for comparison purposes. Future studies of the most promising compounds using labelled substrates would also allow determination of the K_d dissociation constants.

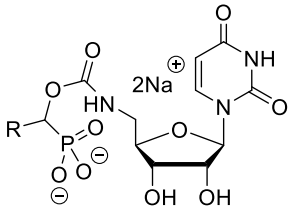
Table 1. Yields for the preparation of the carbamate-linked uridine-based compounds **28a–l** and the cytidine-based compound **30** and inhibition data against recombinant hST6Gal I. [a]

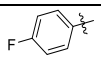
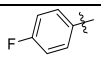
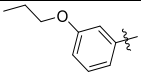
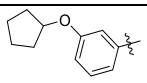
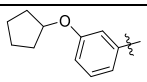
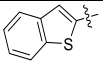
R	Coupling Product	Alloc Deprotection	Cbz/Bn Deprotection[b]	Inhibition at 10 µM
	**29**: 23%	-	**30-(*s*)**: 89%	2.2%
			30-(*l*): 92%	6.3%
	**26a**: 49%	**27a**: 81%	**28a-(*s*)**: 90%	19.9%
			28a-(*l*): 92%	28.2%
	**26b**: 38%	**27b**: 84%	**28b-(*s*)**: 96%	1.1%
			28b-(*l*): 97%	1.8%
	**26c**: 40%	**27c**: 76%	**28c-(*s*)**: 80%	34.6%
			28c-(*l*): 83%	58.9%
	**26d**: 22%	**27d**: 68%	**28d-(*s*)**: 72%	36.6%
			28d-(*l*): 70%	18.0%
	**26e**: 36%	**27e**: 91%	**28e-(*s*)**: 83%	7.2%
			28e-(*l*): 93%	30.9%
	**26f**: 38%	**27f**: 87%	**28f-(*s*)**: 94%	29.8%
			28f-(*l*): 82%	23.6%
	**26g**: 62%	**27g**: 85%	**28g-(*s*)**: 85%	18.1%
			28g-(*l*): 88%	7.1%
	**26h**: 51%	**27h**: 71%	**28h-(*s*)**: 93%	12.7%
			28h-(*l*): 81%	7.9%
	**26i**: 38%	**27i**: 92%	**28i-(*s*)**: 87%	19.0%
			28i-(*l*): 84%	78.0%
	**26j**: 44%	**27j**: 80%	**28j-(*s*)**: 90%	7.1%
			28j-(*l*): 81%	15.2%
	**26k**: 36%	**27k**: 97%	**28k-(*s*)**: 86%	66.4%
			28k-(*l*): 88%	13.2%
	**26l**: 73%	**27l**: 70%	**28l-(*s*)**: 68%	5.9%
			28l-(*l*): 70%	6.9%

[a]For details of the procedures, see Supporting Information. [b]Yield of sodium salts displayed.

Comparing the activity of the cytidine-based (**30**) and uridine-based (**28a**) inhibitors demonstrates that substituting the nucleoside is tolerated and even beneficial. This validates the FEP calculations discussed above (Figure 3) and provides a rationale for exploring other alternatives to cytidine.[21, 22, 25, 26] The most active inhibitors in this series have K_i values that range from 1–20 μM, comparable to many of the reported phosphodiester-based ST inhibitors.[26, 27]

Table 2. Affinity of CMP Neu5Ac (K_m) to recombinant hST6Gal I and inhibition data[a]



Compound	R	Inhibition at 10 μM	K_m or K_i (μM)	K_m/K_i
CMP-Neu5Ac	-	-	37.2 ± 5.4	-
28c-(*s*)		34.6%	306 ± 132	0.1
28c-(*l*)		58.9%	1.1± 0.1	32.5
28d-(*s*)		36.6%	19.2 ± 2.1	1.9
28e-(*l*)		30.9%	20.3 ± 2.0	1.8
28i-(*s*)		19.0%	55.5 ± 26.9	0.7
28i-(*l*)		78.0%	11.5 ± 5.6	3.2
28k-(*s*)		66.4%	8.5 ± 1.0	4.4

[a]For details of the procedures, see Supporting Information.

The importance of the nature and position of the substituent on the α-hydroxyphosphonate fragment is seen by comparing the activity of the phenyl parent compound **28j** (***s***: 7.1%; ***l***: 15.2%) to the various substituted derivatives from which several key trends emerged. For 3-substituted derivatives the alkyl ethers were more active, with the 3-cyclopentoxy derivative **28i-*l*** being one

of the most active inhibitors overall (K_i = 11.5 ± 5.6 μM). The 3-phenoxy derivatives **28a**, along with the 3-propoxy (**28e**) and 3-methoxy (**28f**) derivatives showed moderate activity of up to 31% inhibition. When 3-substitution was coupled with a substituent in the 4-position a decrease in activity resulted as seen by comparing the 3-phenoxy derivative **28a** (***s***: 19.9%; ***l***: 28.2%) to the 4-fluoro-3-phenoxy derivative **28b** (***s***: 1.1%; ***l***: 1.8%) or the 3-cyclopentoxy derivative **28i** (***s***: 19.0%; ***l***: 78.0%) to the 3-cyclopentoxy-4-methoxy derivative **28h** (***s***: 12.7%; ***l***: 7.9%). However, when there was only the single substituent at the 4-position activity was maintained as seen by comparing the 4-methoxy derivative **28g** (***s***: 18.1%; ***l***: 7.7%) to the 3-cyclopentoxy-4-methoxy derivative **28h** (***s***: 12.7%; ***l***: 7.9%) or improved in the case of the 4-fluoro derivative **28c** (***s***: 34.6%; ***l***: 58.9%) compared to the 4-fluoro-3-phenoxy derivative **28b** (***s***: 1.1%; ***l***: 1.8%). Thus, substituents at either the 3- or 4-position are beneficial for ST inhibition in this series of compounds, but not when substituted at both positions presumably for steric reasons. Overall, the 4-fluoro derivative **28c-*l*** was the most active compound with a K_i value of 1.1 ± 0.1 μM.

The second most promising compound was the 2-benzothiophene derivative **28k-*s*** (K_i = 8.5 ± 1.0 μM). To our knowledge heterocycles such as **28k** have not been explored in the context of ST inhibition and therefore warrant further investigation. The difference in activity between the 2- and 3-benzothiophene derivatives **28k** and **28l** highlights the importance of regiochemistry for these compounds. Regarding stereochemistry, for the five most active compounds there were significant differences in activity between stereoisomers. For the benzothiophenyl derivative **28k**, the *s*-isomer was more active with 66.4% inhibition compared to 13.2% for the *l*-isomer. For the two most active aryl compounds, the *l*-isomer of the cyclopentyl and *p*-fluoro-derivative **28i** and **28c** exhibited 78.0 and 58.9% inhibition respectively, compared to 19.0 and 34.6% for their diastereomers. The same trend was observed in their K_i values with a 5-fold difference in activity

for **28i** *s*- and *l*-isomers and up to 300-fold difference for the **28c** *s*- and *l*-isomers. The large difference activity is observed for the most active compounds (**28c-*l*, 28d-*s*, 28e-*l*, 28i-*l* and 28k-*s***), whereas all other compounds showed comparable activity between their diastereomers. It has previously been found that stereochemistry does not play a major role for cytidine-based ST inhibitors,[9] therefore this is a particularly interesting and warrants further in-depth calculations or structural studies such as crystallisation of the inhibitors with hST6Gal 1 to rationalise the findings.

All final compounds were also evaluated in the MTS cell viability assay on a MiaPaCa-2 cancer cell line at 200 μM and observed to be non-toxic. This is expected as inhibiting sialylation affects cell adhesion and migration rather than cell viability. All compounds were assessed *in silico* as being free from any pan-assay interference compounds (PAINS) as described by Baell et al., using a publicly available filter (http://zinc15.docking.org/patterns/home).[51, 52] The ADME parameters and PK properties were computed for the uridyl final compounds using SwissADME (www.swissadme.ch) and compared to their phosphodiester/cytidine-containing counterparts, indicating improved properties including reduced size, H-bond acceptors, lipophilicity, molar refractivity and polarity, along with greater synthetic accessibility. Along with screening against other ST enzyme subtypes (ST3Gal I and ST8Sia II) for selectivity, efforts are currently underway to further improve PK properties and cell permeability of our most promising compounds using phosphonate ester prodrugs. Phosphonate dibenzyl esters (as present in the partially protected compounds **27a-l)**, have been explored by others as prodrugs but drug release is less efficient than from other prodrugs such as pivaloyloxymethyl (POM) and isopropyloxycarbonlyoxymethyl (POC) derivatives, to be investigated in future work.[53, 54]

CONCLUSIONS

Herein, we examined the replacement of the phosphodiester linker and cytidine of classical ST inhibitors[26, 27] with a carbamate linker and uridine respectively. Additionally, the difference of diastereomers and various substituents of the aryl sialic acid mimic were also explored. FEP calculations demonstrated that for binding to hST6Gal I, uridine was a beneficial alternative to cytidine. Thus, 26 carbamate-linked compounds were synthesised by coupling various α-hydroxyphosphonates with 5′-aminonucleosides and evaluated against recombinant hST6Gal I in a luminescence-based microplate assay, giving five compounds (**28c-*l***, **28d-*s***, **28e-*l***, **28i-*l*** and **28k-*s***) with *K*i values in the 1–20 μM range. The identification of the benzothiophene inhibitor **28k-*s*** as one of the best compounds of this series suggests that various heterocycles should be further explored in the future, including against a wider panel of ST enzymes as recently reported by Moremen and Jarvis, as well for cross-reactivity towards UDP-sugar glycosyltransferases due to the introduction of uridine.[55, 56]

Overall, these results show that both the carbamate and uridine modifications are viable changes to the classical model of ST inhibitors. The promising activity of this first generation of carbamate-linked, uridyl-based inhibitors provide a strong starting point for the development of more drug-like and selective ST inhibitors as chemical tools to fully uncover role of STs in cancer progression, metastasis and drug resistance. The facile 7-step synthesis developed is highly amenable to scale-up to provide larger quantities of pure material for both crystallisation studies and cell-based and animal studies, which is important for the field. Further studies are underway to generate an asymmetric route to the most active fluorinated and heterocyclic diastereomers to be evaluated in cell-based models of ST upregulation in pancreatic, ovarian and hepatic cancer.

EXPERIMENTAL SECTION

Free Energy Calculation. The FEP calculation describing the alchemical transformation[40, 41] of the cytidine-based inhibitor ***(R)*-6** to the uridine based inhibitor ***(R)*-7** was prepared using VMD version 1.9.2.[57] and carried out using the NAMD 2.10 package.[58] The change in the binding free energy with the perturbation from cytidine to uridine (Figure 5) can be expressed as:

$$\begin{aligned}\Delta\Delta G_b &= \Delta G_b^{Uri} - \Delta G_b^{Cyt} \\ &= \Delta G_2 - \Delta G_1 \\ &= \Delta\Delta G_{ex} \\ &= \sum_i \Delta\Delta G_{ex,i}\end{aligned} \qquad \textbf{Eq. 1}$$

In the FEP method, one introduces a hybrid Hamiltonian, $\mathcal{H}(\lambda)=(1-\lambda)\,\mathcal{H}_0 + \lambda\mathcal{H}_1$, where H_0 represents the Hamiltonian for the initial state and H_1 for the final state. The interval between 0 and 1 is divided into n subintervals (λ_i, where i = 1,…, n – 1), and for each subinterval the free energy difference is calculated from the ensemble average, $\Delta\Delta G_{ex,i} = -k_B T \ln\langle\exp[-(\mathcal{H}(\lambda_{i+1}) - \mathcal{H}(\lambda_i))/k_B T]\rangle_{\lambda i}$. The free energy difference between initial and final states is obtained from the sum, $\Delta\Delta G = \Sigma_i\, \Delta\Delta G_{ex,i}$. Alchemical transformations were carried out with 20 uniformly distributed λ values between 0 and 1 (0.0, 0.05, 0.1, … , 0.9, 0.95, 1.0), with the transformation simulated in both the forward (0 ⟶ 1) and backward (1 ⟶ 0) directions. To prevent the occurrence of singularities at values of λ where an atom disappears/appears, a soft-core potential with α = 4.0 Å^2 was used to reshape the Lennard-Jones potential into a form devoid of singularity when λ approaches either 0 or 1.[59, 60]

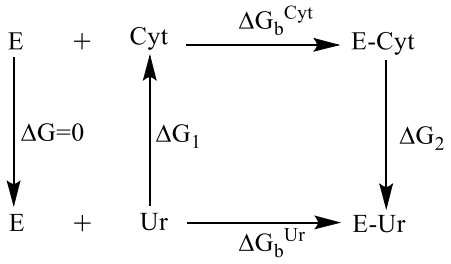


Figure 5. The thermodynamic cycle used in the cytidine to uridine FEP simulations to calculate $\Delta\Delta G_b$ (see Eq. 1). Cyt represents the *(R)*-diastereoisomer of the cytidine-based inhibitor ***(R)*-5**, Uri represents the *(R)*-diastereoisomer of the uridine-based inhibitor ***(R)*-6** and E represents hST6Gal I.

The starting structure for each λ window was generated from the hST6Gal I-inhibitor MD simulations of the cytidine-based inhibitor ***(R)*-6**, described in our previous work (PDB ID: 4JS2).[36] Each λ window simulation for both the complex and ligand only systems were solvated in an 82 Å × 82 Å × 82 Å cubic TIP3P[61] water box. Each box was neutralised with counter ions of Na^+ and Cl^-, with the salt (NaCl) concentration set to 0.15 mol/L. The protein was represented with the non-polarisable CHARMM PARAM36 force field.[62] The force field used for the ligands were those optimised in our previous work using the GAAMP method.[36, 63] All systems were simulated in periodic boundary conditions using the Langevin algorithm for maintaining the temperature at 298.15 K, and the Langevin Piston Nose–Hoover method[64, 65] for maintaining a constant pressure of 1.0 bar. The electrostatic interactions were calculated using the Particle Mesh Ewald method.[66] The van der Waals forces were treated with a cut-off of 12 Å and a smoothening function between 10 and 12 Å. All of the covalent bonds involving hydrogen were kept rigid with the RATTLE algorithm.[67] The integration time step was set to 1.0 fs, 2.0 fs and 4.0 fs for bonded, non-bonded and long-range electrostatics, respectively.

Each λ window underwent an initial 5000 energy minimisation steps followed by 1 ns equilibration simulation to provide starting coordinates and then simulated for 2 ns with a harmonic restraint placed on the centre of mass of hST6Gal I for the complex simulations. A harmonic restraint was also applied to one atom from the ligand in bulk simulations. Both restraints used a force constant of 32 kcal/mol/$Å^2$.

$\Delta\Delta G_b$ and the associated errors were calculated using the ParseFEP plugin[68] in VMD, which was used to combine the results of the forward and backward simulations with the Bennett acceptance-ratio.[69]

To examine the H-bond interactions between the uridine-based inhibitors and the hST6Gal I active site the λ window 1.0 from the backwards simulation was extended to 15 ns. H-bond interactions were analysed according to a geometric criterion. A H-bond was defined by a minimum donor–hydrogen–acceptor angle of 120° and a maximum heavy atom distance of 3 Å. Hydrophobic contacts were also analysed according to a geometric criterion of a maximum distance of 4 Å between hydrophobic atom pairs. To identify these interactions VMD version 1.9.2.[57] was utilised.

General Chemistry. All solvents and reagents were purchased from Sigma Aldrich (USA), Carbosynth (UK) or ChemSupply (SA) and used as supplied or purified by standard procedures. Unless otherwise noted all reactions were performed under anhydrous conditions. Reactions were monitored by TLC using Merck Silica Gel 60 F_{254} aluminium backed plates and components visualised by UV light or staining. Solvents were removed under reduced pressure at a max. of 40ºC (water bath). Column chromatography was performed under 'flash' conditions on silica gel 60 (40–63 μm mesh). ^{1}H, ^{13}C, ^{31}P, ^{19}F and 2D NMR spectra were acquired on a Varian VNMRS PS54 500 MHz and Inova 300 MHz, or Bruker Advance Neo 500 MHz and 400 MHz NMR instruments. ^{1}H and ^{13}C NMR chemical shifts (in ppm) were calibrated against residual solvent peaks or TMS; ^{31}P NMR shifts against an external reference of 85% H_3PO_4 in D_2O; and ^{19}F shifts against an external reference of 0.05% α,α,α-trifluorotoluene in $CDCl_3$. High resolution electrospray ionisation mass spectra (HRMS) were performed on a Waters XEVO G2 Q-TOF spectrometer with leucine enkephalin (LeuEnk) as an internal standard. Infrared spectra were obtained on either a Shimadzu FTIR Affinity or Bruker Vortex 70 FTIR spectrophotometer. Optical rotation was determined using a Jasco P-2000 polarimeter. Melting points were obtained using a Büchi Melting Point M-560 apparatus and are uncorrected. Analytical RP-HPLC was performed with a Shimadzu Prominence-I LC-2030C system with a PDA detector (190–800 nm), using a Luna C18 (2) 100Å (Phenomenex, 3 μm, 4.6 x 150 mm) or Shim-Pack GIS (Shimadzu, 5 μm, 4.6 x 150 mm) column.

Preparative RP-HPLC was performed on a Shimadzu Prominence LC-20AP system with a PDA detector (190–800 nm) using a Prep C18 (Shimadzu, 5 μm, 20 x 150 mm) column. The purity of all final compounds was determined to be ≥ 95% based on ^{1}H NMR data and analytical HPLC (see Supporting Information, Figures S1–S13).

Synthesis of α-Hydroxyphosphonates. The synthesis of all α-hydroxyphosphonates was based on the method of Wong et al.[42] To a mixture of dibenzyl phosphite (1.1 equiv.) and the desired benzaldehyde (1 equiv.) in CH_2Cl_2, was added triethylamine (2 equiv.). The reaction mixture was stirred at rt for 14 h and monitored by TLC. Upon completion, the reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution and brine. The organic phase was separated, dried with anhydrous $MgSO_4$, filtered and the filtrate evaporated, with the resultant product purified by column chromatography.

*Dibenzyl α-hydroxy(3-phenoxy)benzylphosphonate (**8**)*. From 3-phenoxybenzaldehyde (300 mg, 1.51 mmol): purification by column chromatography (EtOAc : hexane, 1:1, R_f = 0.43) to give **8** (417 mg, 60%) as a white solid. mp 65.0–68.0ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24–7.31 (m, 12H), 7.19–7.22 (m, 3H), 7.13 (br s, 1H), 7.08 (t, J = 7.5 Hz, 1H), 6.95 (d, J = 8.5 Hz, 2H), 5.02 (d, $J_{H,P}$ = 10.0 Hz, 1H), 4.89–5.00 (m, 4H). The spectral data match those reported.[26]

*Dibenzyl α-hydroxy(4-fluoro-3-phenoxy)benzylphosphonate (**9**)*. From 4-fluoro-3-phenoxyben-zaldeh yde (500 mg, 2.31 mmol): purification by column chromatography (EtOAc : pet. spirit, 2:3, R_f = 0.14) to give **9** (1.11 g, 92%) as a white solid. mp 71.0–73.0ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04–7.30 (m, 16H), 6.90 (d, J = 8.0 Hz, 2H), 4.90–5.00 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.3, 154.2 (dd, $J_{C,F}$ = 247.9 Hz, $J_{C,P}$ = 3.8 Hz), 143.7 (d, $J_{C,F}$ = 12.4 Hz), 136.0 (d, $J_{C,P}$ = 6.3 Hz), 133.05–133.16 (m), 129.8, 128.8, 128.72, 128.69, 128.12, 128.06, 123.5–123.6 (m), 123.4, 120.7–120.8 (m), 117.5, 117.1, 116.9, 70.5 (d, $J_{C,P}$ = 158.4 Hz), 68.8–68.9 (m), 68.7 (d, $J_{C,P}$ = 6.6 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.54 (d, $J_{P,F}$ = 6.5 Hz).^{19}F NMR (235.3 MHz, $CDCl_3$) δ −68.81 (d, $J_{F,P}$ = 2.4 Hz). HRMS (ESI) calcd for $C_{27}H_{25}FO_5P$ $[M + H]^+$, 479.1424; Found 479.1425. IR (FTIR) ν_{max} 3307, 1505, 1489, 1208, 991, 740, 724, 698.

*Dibenzyl α-hydroxy(4-fluoro)benzylphosphonate (**10**)*. From 4-fluorobenzaldehyde (1.00 g, 8.06 mmol): purification by column chromatography (EtOAc : pet. spirit, 7:3, R_f = 0.55) to give **10** (2.54 g, 82%) as a white solid. mp 102.0–104.0ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.40 (m, 2H), 7.18–7.28 (m, 10H), 6.94–6.97 (m, 2H), 5.04 (d, $J_{H,P}$ = 10.0 Hz, 1H), 4.88–5.00 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.7 (dd, $J_{C,F}$ = 245.3 Hz, $J_{C,P}$ = 3.8 Hz), 136.12 (d, $J_{C,P}$ = 3.4 Hz), 136.07 (d, $J_{C,P}$ = 3.4 Hz), 132.29–132.33 (m), 129.1 (dd, $J_{C,P}$ = 8.4 Hz, $J_{C,F}$ = 5.5 Hz), 128.7, 128.63, 128.58, 128.5, 128.04, 127.99, 115.3 (dd, $J_{C,F}$ = 21.3 Hz, $J_{C,P}$ = 2.8 Hz), 70.5 (d, $J_{C,P}$ = 159.1 Hz), 68.8 (d, $J_{C,P}$ = 7.1 Hz), 68.5 (d, $J_{C,P}$ = 7.4 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.74 (d, $J_{P,F}$ = 4.9 Hz). ^{19}F NMR (235.3 MHz, $CDCl_3$) δ −50.94 (d, $J_{F,P}$ = 2.4 Hz). HRMS (ESI) calcd for $C_{21}H_{21}O_4PF$ $[M + H]^+$, 387.1161; Found 387.1160. IR (FTIR) ν_{max} 3230, 1601, 1506, 1220, 101, 732, 695.

*Dibenzyl α-hydroxy(3-fluoro)benzylphosphonate (**11**)*. From 3-fluorobenzaldehyde (500 mg, 4.03 mmol): purification by column chromatography (EtOAc : pet. spirit, 1:1 R_f = 0.44) to give **11** (1.06 g, 68%) as a white solid. mp 78.0–79.0ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16–7.33 (m, 13H), 6.96–7.01 (m, 1H), 5.05 (d, $J_{H,P}$ = 12.0 Hz, 1H), 4.91–5.02 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3 (dd, $J_{C,F}$ = 244.0 Hz, $J_{C,P}$ = 3.0 Hz), 139.1 (dd, $J_{C,F}$ = 8.0 Hz, $J_{C,P}$ = 3.0 Hz), 136.03 (d, $J_{C,P}$ = 3.0 Hz), 135.97 (d, $J_{C,P}$ = 3.0 Hz), 129.9 (dd, $J_{C,F}$ = 8.0 Hz, $J_{C,P}$ = 3.0 Hz), 128.73, 128.71, 128.69, 128.67, 128.2, 128.1, 122.9 (dd, $J_{C,P}$ = 6.0 Hz, $J_{C,F}$ = 3.0 Hz), 115.2 (dd, $J_{C,F}$ = 21.0 Hz, $J_{C,P}$ = 3.0 Hz), 114.3 (dd, $J_{C,F}$ = 23.0 Hz, $J_{C,P}$ = 6.0 Hz), 70.7 (dd, $J_{C,P}$ = 158.0 Hz, $J_{C,F}$ = 2.0 Hz), 68.9 (d, $J_{C,P}$ = 7.3 Hz), 68.7 (d, $J_{C,P}$ = 7.3 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.43 (d, $J_{P,F}$ = 8.1 Hz). ^{19}F NMR (235.3 MHz, $CDCl_3$) δ −49.90 (d, $J_{F,P}$ = 23.5 Hz). HRMS (ESI) calcd for $C_{21}H_{21}O_4FP$ $[M + H]^+$, 387.1161; Found 387.1161. IR (FTIR) ν_{max} 3231, 1243, 1224, 1202, 734, 690.

*Dibenzyl α-hydroxy(3-propoxy)benzylphosphonate (**12**)*. From 3-propoxybenzaldehyde (300 mg, 1.82 mmol): purification by column chromatography (EtOAc : hexane, 1:1, R_f = 0.21) to give **12** (587 mg, 75%) as a white solid. mp 56.0–57.5ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21–7.32 (m, 11H), 7.01 (br s, 2H), 6.85 (d, J = 8.5 Hz, 1H), 5.03 (d, $J_{H,P}$ = 10.0 Hz, 1H), 4.87–4.98 (m, 4H), 3.81 (td, J =

6.5 Hz, J = 1.5 Hz, 2H), 3.17 (br s, 1H), 1.76 (sxt, J = 7.1 Hz, 2H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.1 (d, $J_{C,P}$ = 3.8 Hz), 137.6 (d, $J_{C,P}$ = 2.3 Hz), 136.0–136.1 (m), 129.2 (d, $J_{C,P}$ = 2.3 Hz), 128.5, 128.4, 128.33, 128.30, 127.90, 127.87, 119.3 (d, $J_{C,P}$ = 6.0 Hz), 114.9 (d, $J_{C,P}$ = 2.3 Hz), 112.7 (d, $J_{C,P}$ = 5.3 Hz), 69.3, 71.1 (d, $J_{C,P}$ = 158.3 Hz), 68.6 (d, $J_{C,P}$ = 8.3 Hz), 68.3 (d, $J_{C,P}$ = 8.3 Hz), 22.5, 10.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.00. HRMS (ESI) calcd for $C_{24}H_{27}O_5PNa$ $[M + Na]^+$, 449.1494; Found 449.1515. IR (FTIR) ν_{max} 3430, 2978, 1232, 999, 729, 692.

*Dibenzyl α-hydroxy(3-methoxy)benzylphosphonate (**13**)*. From 3-methoxybenzaldehyde (300 mg, 2.22 mmol): purification by column chromatography (EtOAc : hexane, 2:3, R_f = 0.45) to give **13** (584 mg, 67%) as a white solid. mp 77.5–80.0ºC (lit. mp 76–78ºC).[70] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21–7.31 (m, 11H), 7.01–7.03 (m, 2H), 6.81 (d, J = 8.0 Hz, 1H), 5.04 (d, $J_{H,P}$ = 11.0 Hz, 1H), 4.97–4.84 (m, 4H), 3.64 (s, 3H). The spectral data match those reported.[70]

*Dibenzyl α-hydroxy(4-methoxy)benzylphosphonate (**14**)*. From 4-methoxybenzaldehyde (500 mg, 3.67 mmol): purification by column chromatography (EtOAc : hexane, 1:1, R_f = 0.34) to give **14** (884 mg, 60%) as a white solid. mp 103.0–105.0ºC (lit. mp 104–105ºC).[70] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, J = 7.3 Hz, 2H), 7.31 (br s, 10H), 6.86 (d, J = 8.5 Hz, 2H), 5.87 (br s, 1H), 5.16 (d, $J_{H,P}$ = 10.5 Hz, 1H), 4.88–5.03 (m, 4H), 3.73 (s, 3H). The spectral data match those reported.[70]

*Dibenzyl α-hydroxy(3-cyclopentoxy-4-methoxy)benzylphosphonate (**15**)*. From 3-cyclopentoxy-4-met hoxybenzaldehyde (700 mg, 3.18 mmol): purification by column chromatography (EtOAc : pet. spirit, 1:1, R_f = 0.23) to give **15** (789 mg, 51%) as a white solid. mp 116.5–119.2ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.32 (m, 8H), 7.20–7.22 (m, 2H), 7.01 (d, J = 8.0 Hz, 1H), 6.95 (d, J = 8.0 Hz, 1H), 6.80 (d, $J_{H,P}$ = 8.0 Hz, 1H), 4.85–4.99 (m, 5H), 4.61–4.62 (m, 1H), 3.83 (s, 3H), 1.75–1.81 (m, 6H), 1.54 (br s, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 150.2 (d, $J_{C,P}$ = 3.0 Hz), 147.8 (d, $J_{C,P}$ = 2.0 Hz), 136.2–136.3 (m), 128.68, 128.63, 128.52, 128.55, 128.11, 128.06, 119.8 (d, $J_{C,P}$ = 8.0 Hz), 113.8–113.9 (m), 111.8 (d, $J_{C,P}$ = 2.0 Hz), 80.4, 71.1 (d, $J_{C,P}$ = 159.0 Hz), 68.5–68.6 (m), 56.2, 32.92, 32.87, 24.2. ^{31}P NMR (162

MHz, $CDCl_3$) δ 22.20. HRMS (ESI) calcd for $C_{27}H_{31}O_6PNa$ $[M + Na]^+$, 505.1756; Found 505.1769. IR (FTIR) ν_{max} 3309, 2962, 1515, 1233, 989, 738, 698.

*Dibenzyl α-hydroxy(3-cyclopentoxy)benzylphosphonate (**16**)*. From 3-cyclopentoxybenzaldeh-yde (973 mg, 5.11 mmol): purification by column chromatography (EtOAc : pet. spirit, 3:2, R_f = 0.30) to give **16** (1.42 g, 61%) as a white solid. mp 55.7–59.3ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15–7.27 (m, 11H), 6.98–7.01 (m, 2H), 6.78 (d, J = 8.2 Hz, 1H), 4.85–5.07 (m, 5H), 4.59–4.62 (m, 1H), 1.68–1.81 (m, 6H), 1.49–1.56 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.1 (d, $J_{C,P}$ = 2.4 Hz), 137.9, 136.22 (d, $J_{C,P}$ = 1.5 Hz), 136.18 (d, $J_{C,P}$ = 1.5 Hz), 129.3 (d, $J_{C,P}$ = 2.0 Hz), 128.51, 128.47, 128.4, 128.3, 128.0, 127.9, 119.2 (d, $J_{C,P}$ = 5.9 Hz), 115.8 (d, $J_{C,P}$ = 2.8 Hz), 114.1 (d, $J_{C,P}$ = 5.7 Hz), 79.1, 71.1 (d, $J_{C,P}$ = 159.0 Hz), 68.7 (d, $J_{C,P}$ = 6.8 Hz), 68.4 (d, $J_{C,P}$ = 7.3 Hz), 32.83, 32.78, 24.1. ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.31. HRMS (ESI) calcd for $C_{26}H_{29}O_5PNa$ $[M + Na]^+$, 475.1650; Found 475.1629. IR (FTIR) ν_{max} 3290, 2965, 1599, 1255, 989, 736, 694.

*Dibenzyl α-hydroxybenzylphosphonate (**17**)*. From benzaldehyde (500 mg, 4.72 mmol): purification by column chromatography (EtOAc : hexane, 1:1, R_f = 0.24) to give **17** (1.53 g, 88%) as a white solid. mp 115.0–116.0ºC (lit. mp 117–118ºC).[70] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20–7.46 (m, 15H), 5.04 (d, $J_{H,P}$ = 10.5 Hz, 1H), 4.84–4.96 (m, 4H). The spectral data match those reported.[70]

*Dibenzyl α-hydroxy(benzo[b]thiophen-2-yl)methylphosphonate (**18**)*. From benzo[*b*]thiophene-2-carb oxaldehyde (1.00 g, 6.16 mmol): recrystallisation from EtOAc gave **18** (1.67 g, 65%) as a white crystalline solid. mp 155.3–156.6ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–7.80 (m, 1H), 7.66–7.68 (m, 1H), 7.22–7.36 (m, 13H), 5.33 (d, $J_{H,P}$ = 12.0 Hz, 1H), 4.99–5.10 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.0 (2 x s), 139.8 (2 x s), 139.5 (2 x s), 135.95–136.06 (m), 128.72, 128.70, 128.67, 128.3, 128.2, 124.59–124.60 (2 x s), 124.5, 123.89–123.91 (2 x s), 123.1–123.2 (2 x s), 122.5, 69.0–69.1 (m), 68.1 (d, $J_{C,P}$ = 164.0 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.02. HRMS (ESI) calcd for $C_{23}H_{21}O_4PSNa$ $[M + Na]^+$, 447.0796; Found 447.0804. IR (FTIR) ν_{max} 3189, 1457, 1200, 1006, 727, 695.

Dibenzyl α-hydroxy(benzo[b]thiophen-3-yl)methylphosphonate (***19***). From benzo[*b*]thiophene-3-carb oxaldehyde (250 mg, 1.54 mmol); recrystallisation from EtOAc gave **19** (339 mg, 52%), as a white crystalline solid. mp 117.7–110.3ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82–7.85 (m, 2H), 7.67 (d, J = 3.4 Hz, 1H), 7.22–7.36 (m, 10H), 7.11–7.13 (m, 2H) 5.44 (d, $J_{H,P}$ = 10.6 Hz, 1H), 4.80–5.07 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.5, 137.4 (d, $J_{C,P}$ = 6.1 Hz), 136.1 (d, $J_{C,P}$ = 5.8 Hz), 135.9 (d, $J_{C,P}$ = 5.8 Hz), 131.4, 128.71, 128.65, 128.63, 128.59, 128.2, 128.1, 125.9 (d, $J_{C,P}$ = 7.5 Hz), 124.8, 124.4, 122.8, 122.7, 68.82 (d, $J_{C,P}$ = 3.3 Hz), 68.75 (d, $J_{C,P}$ = 3.3 Hz), 66.6 (d, $J_{C,P}$ = 164.1 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.66. HRMS (ESI) calcd for $C_{23}H_{21}O_4PSNa$ $[M + Na]^+$, 447.0796; Found 447.0808. IR (FTIR) ν_{max} 3274, 1497, 1254, 989, 764, 696.

Synthesis of Nucleoside Fragments.

*5′-Azido-5′-deoxyuridine (**20**).* A solution of hydrazoic acid (HN_3, ~2.5 M) was prepared by the slow addition of sulfuric acid (4.50 mL) to a suspension of NaN_3 (10.6 g, 164 mmol) in H_2O (5.30 mL) and toluene (64.8 mL) at 0ºC. The mixture was stirred for 30 min, at which time Na_2SO_4 was observed as a precipitate. The toluene layer was decanted, and used immediately in the following reaction. Uridine (2.00 g, 8.19 mmol) was suspended in dry THF (80 mL) under an atmosphere of N_2. Triphenylphosphine (6.44 g, 24.6 mmol) and the freshly prepared HN_3 (49 mL, 123 mmol) were added to the suspension, which had been cooled to 0ºC, followed by the dropwise addition of diisopropyl azodicarboxylate (DIAD, 5.20 mL, 24.6 mmol). Subsequently the reaction mixture was allowed to warm to rt and stir for 17 h. After this time the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in minimal amount of CH_2Cl_2 and extracted into H_2O. After washing with CH_2Cl_2 the crude product was obtained by concentrating the aqueous phase, with the aid of a toluene azeotrope. The crude product was purified via column chromatography (CH_2Cl_2:methanol (MeOH), 9:1, R_f = 0.44) to afford **20** (2.11 g, 97%) as a white foam. mp 151.2–152.8ºC, (lit. mp 151.5–152.5ºC).[71] ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.71 (d, J = 8.0 Hz, 1H), 5.84 (d, J = 4.0 Hz, 1H), 5.74 (d, J = 8.0 Hz, 1H), 4.22 (t, J

= 5.6 Hz, 1H), 4.10 (t, *J* = 5.6 Hz, 1H), 4.05–4.08 (m, 1H), 3.69 (dd, *J* = 13.5 Hz, *J* = 3.0 Hz, 1H), 3.60 (dd, *J* = 13.5 Hz, *J* = 4.5 Hz, 1H). The spectral data match those reported.[43]

*2′,3′-O-Bis(allyloxycarbonyl)-5′-azido-5′-deoxyuridine (**21**).* Allyl chloroformate (9.81 g, 82.0 mmol) was added dropwise over a 10 min interval to a solution of **20** (2.20 g, 8.20 mmol) in pyridine (13.1 mL, 164 mmol), which had been cooled to −20ºC. The reaction mixture was allowed to warm to rt and was stirred for 30 min. Volatiles were removed under reduced pressure and the residue was dissolved in CH_2Cl_2. The product was partitioned between EtOAc and H_2O. The organic layer was washed with brine and was then dried with $MgSO_4$ and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (CH_2Cl_2:EtOAc, 3:2, R_f = 0.75) to give **21** (3.23 g, 90%) as an off-white gum. mp 43.2–46.8ºC. $[\alpha]_D^{25}$ +30.6 (*c* = 1.5, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (br s, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 5.97 (d, *J* = 5.0 Hz, 1H), 5.86–5.96 (m, 2H), 5.81 (d, *J* = 8.0 Hz, 1H), 5.26–5.40 (m, 6H), 4.59–4.68 (m, 4H), 4.30 (d, *J* = 2.5 Hz, 1H), 3.78 (dd, *J* = 13.5 Hz, *J* = 2.5 Hz, 1H), 3.67 (dd, *J* = 13.5 Hz, *J* = 4.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.9, 153.9, 153.8, 150.2, 140.4, 131.01, 130.96, 119.70, 119.68, 103.7, 88.6, 80.2, 75.6, 73.6, 69.6, 69.5, 51.7. HRMS (ESI) calcd for $C_{17}H_{19}N_5O_9Na$ $[M + Na]^+$, 460.1075; Found 460.1078. IR (FTIR) ν_{max} 2107, 1750, 1688, 1232, 1080, 908.

*2′,3′-O-Bis(allyloxycarbonyl)-5′-amino-5′-deoxyuridine (**22**).* To a solution of **21** (3.23 g, 7.39 mmol) in dry THF, triphenylphosphine (3.88 g, 14.8 mmol) was added with the resulting solution allowed to stir at rt. After 2 h a few drops H_2O was added and the solution was heated at reflux for 6 h. Upon completion volatiles were removed under reduced pressure and the subsequent crude purified by column chromatography (CH_2Cl_2 : MeOH, 9:1, R_f = 0.33) to yield **22** (2.13 g, 70%) as a white solid. mp 48.2–49.5ºC. $[\alpha]_D^{25}$ −4.0 (*c* = 1.0, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.93 (d, *J* = 8.0 Hz, 1H), 5.86–5.98 (m, 3H), 5.69 (d, *J* = 8.0 Hz, 1H), 5.49 (t, *J* = 5.0 Hz, 1H), 5.24–5.37 (m, 5H), 4.56–4.69 (m, 4H), 4.11–4.14 (m, 1H), 2.98 (dd, *J* = 15.0 Hz, *J* = 3.5 Hz, 1H), 2.92 (dd, *J* = 15.0 Hz, *J* = 3.5 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.9, 153.3, 153.1, 150.5, 142.1, 131.75, 131.63, 118.7, 102.4, 87.4,

81.3, 75.1, 73.9, 68.7, 68.5, 41.8. HRMS (ESI) calcd for $C_{17}H_{21}N_3O_9$ $[M + H]^+$, 412.1356; Found 412.1351. IR (FTIR) ν_{max} 2107, 1750, 1688, 1232, 1080, 902.

*2′,3′-O-N⁴-Tris(benzyloxycarbonyl)-5′-deoxycytidine (**23**).* Cytidine (1.00 g, 4.12 mmol) and imidazole (700 mg, 10.3 mmol) were dissolved in a minimum of DMF. Once dissolved *tert*-butyldimethylsilyl (TBDMS) chloride (682 mg, 4.52 mmol) was slowly added. The resulting mixture was stirred at rt under an atmosphere of N_2 and judged to be complete by TLC after 16 h. Upon completion the reaction solution was poured onto crushed ice with NaCl added, with the subsequent precipitate collected via vacuum filtration. The crude 5′-*O*-TBDMS-protected cytidine and DMAP (1.38 g, 12.3 mmol) were dissolve in CH_2Cl_2 and the resulting suspension was cooled to 0ºC. Benzyl chloroformate (1.8 mL, 12.3 mmol) was added dropwise and the suspension stirred at rt. After 16 h the solution was diluted with CH_2Cl_2 and washed with H_2O, followed by $NaHCO_3$ and brine. The organic phase was collected, dried with $MgSO_4$ and concentrated under reduced pressure. The resulting crude product was purified by column chromatography ($CHCl_3$:EtOAc, 4:1, R_f = 0.58) to produce the completely protected cytidine derivative (2.18 g, 70%) as a clear solid. The clear solid (1.96 g, 2.58 mmol) was dissolved in THF (15 mL) and glacial acetic acid (0.2 mL, 3.10 mmol) was added. The reaction mixture was cooled to 0ºC and 1.0 M TBAF in THF (2.8 mL, 2.84 mmol) was added in a dropwise manner. The resulting reaction mixture was allowed to gradually warm to rt over 2 h and left to stir at rt for an additional 16 h. After such time, the volatile reactants were removed under reduced pressure with the resulting crude product was purified by column chromatography ($CHCl_3$: EtOAc, 4:1, R_f = 0.26) to produce **23** (1.60 g, 96%) as a white solid. mp 96.0–97.1ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (br s, 1H), 8.07 (d, *J* = 7.0 Hz, 1H), 7.26–7.37 (m, 16H), 5.94 (d, *J* = 4.6 Hz, 1H), 5.73 (t, *J* = 4.6 Hz, 1H), 5.52 (t, *J* = 5.0 Hz, 1H), 5.13–5.18 (m, 2H), 5.04–5.11 (m, 4H), 4.28 (br s, 1H), 4.20 (br s, 1H), 3.97 (d, *J* = 12.5 Hz, 1H), 3.81 (d, *J* = 12.5 Hz, 1H). Spectral data match those reported.[44]

*5′-Azido-2′,3′-O-N⁴-tris(benzyloxycarbonyl)-5′-deoxycytidine (**24**).* **23** (1.60 g, 2.48 mmol) was suspended in dry THF (50 mL), with triphenylphosphine (1.95 g, 7.43 mmol) and the freshly prepared

HN_3 (14.9 mL, 37.1 mmol; see **20** for details) were added to the suspension, which had been cooled to 0ºC. To the cooled suspension, DIAD (1.57 mL, 7.43 mmol) was added slowly in a dropwise manner. Subsequently the reaction mixture was allowed to warm to rt and stirred for 18 h. After this time, the reaction mixture was concentrated under reduced pressure and the crude product was purified via column chromatography (CH_2Cl_2 : EtOAc, 4:1, R_f = 0.68) to afford **24** (1.62 g, 97%) as a white foam. mp 56.5–59.2ºC. $[\alpha]_D^{25}$ +13.2 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (br s, 1H), 7.84 (d, *J* = 7.5 Hz, 1H), 7.28–7.37 (m, 16H), 5.93 (d, *J* = 2.5 Hz, 1H), 5.51–5.52 (m, 1H), 5.34 (t, *J* = 5.5 Hz, 1H), 5.20 (s, 2H), 5.05–5.12 (m, 4H), 4.32 (br s, 1H), 3.77 (dd, *J* = 13.0 Hz, 2.5 Hz, 1H), 3.67 (dd, *J* = 13.0 Hz, 4.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.0, 154.6, 153.93, 153.91, 152.3, 145.1, 135.0, 134.66, 134.63, 128.9, 128.8, 128.74, 128.69, 128.54, 128.47, 128.48, 128.46, 95.7, 91.2, 80.1, 76.6, 73.4, 70.6, 68.2, 51.3. HRMS (ESI) calcd for $C_{33}H_{30}N_6O_{10}$ $[M + Na]^+$, 693.1921; Found 693.1931. IR (FTIR) ν_{max} 3263, 1739, 1669, 1262, 1194.

*5′-Amino-2′,3′-O-N^4-tris(benzyloxycarbonyl)-5′-deoxycytidine (**25**)*. To a solution of **24** (1.62 g, 2.48 mmol) in dry THF, triphenylphosphine (1.30 g, 4.96 mmol) was added with the resulting solution allowed to stir at rt. After 2 h a few drops H_2O was added and the solution was heated at 60ºC for 5 h. Upon completion volatiles were removed under reduced pressure and the subsequent crude purified by column chromatography (CH_2Cl_2 : MeOH, 95:5, R_f = 0.28) to yield **25** as a white solid (800 mg, 50%). mp 68.0–71.3ºC. $[\alpha]_D^{25}$ −18.4 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, *J* = 7.4 Hz, 1H), 7.28–7.38 (m, 15H), 7.20 (d, *J* = 7.4 Hz, 1H), 6.29 (d, *J* = 4.0 Hz, 1H), 5.52–5.54 (m, 1H), 5.32–5.35 (m, 1H), 5.20 (s, 2H), 5.04–5.12 (m, 4H), 4.48–4.51 (m, 1H), 3.58 (d, *J* = 16.1 Hz, 1H), 3.38 (d, *J* = 16.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 155.0, 154.0, 153.9, 152.4, 145.6, 136.0, 134.71, 134.69, 128.7, 128.61, 128.56, 128.4, 128.34, 128.28, 95.0, 88.1, 81.4, 77.6, 74.2, 70.3–70.4 (m), 67.9, 50.7. HRMS (ESI) calcd for $C_{33}H_{32}N_4O_{10}Na$ $[M + Na]^+$, 667.2016; Found 667.2050. IR (FTIR) ν_{max} 3288, 2924, 1749, 1649, 1555, 1495, 1271, 1197, 1060, 784, 736, 696.

Carbamate Coupling. 4-NPC (1.1 equiv.) was added dropwise to a solution of dibenzyl α-hydroxyphosphonate (1 equiv.), an excess of triethylamine (2 equiv.) and a catalytic amount of DMAP (0.1 equiv.) in dry CH_2Cl_2 at 0°C. The ice bath was removed and the solution stirred at rt for 3 h. Upon completion, 5′-amino-5′-deoxynucleoside (1.2 equiv.; **22** or **25**) was added and the reaction was stirred at rt for 24 h. Upon completion the reaction mixture was washed with water and brine. The organic phase was separated and then dried with anhydrous $MgSO_4$, filtered and evaporated, with the resultant crude product purified by column chromatography.

*(Bis(benzyloxy)phosphoryl)(3-phenoxyphenyl)methyl(2', 3'-O-bis(allyloxycarbonyl)uridin-5'-y-l)carb amate (**26a**).* From **8** (234 mg, 638 μmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.35) to afford **26a** (281 mg, 49%) as a white solid. mp 52.5–55.0ºC. $[\alpha]_D^{25}$ −8.1 (*c* = 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06–7.32 (m, 18H), 6.91–6.96 (m, 2H), 6.06 (d, $J_{H,P}$ = 14.0 Hz, 1H), 5.83–5.96 (m, 2H), 5.59–5.74 (m, 2H), 5.47–5.53 (m, 2H), 5.25–5.37 (m, 5H), 4.85–5.05 (m, 4H), 4.56–4.64 (m, 4H), 4.14–4.20 (m, 1H), 3.39–3.60 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.8, 157.4 (2 x d, $J_{C,P}$ = 2.0 Hz), 156.96–157.03 (2 x s), 155.0–155.3 (m), 153.85–153.90 (m), 149.95–149.99 (2 x s), 142.0–142.1 (2 x s), 136.0–136.2 (m), 135.7–135.8 (2 x s), 131.11–131.14 (2 x s), 131.0 (m), 129.9, 128.67, 128.65, 128.62, 128.56, 128.5, 128.07, 128.05, 128.01, 123.5–123.6, 122.6–122.7, 119.7, 119.46–119.50 (2 x s), 119.1–119.2, 118.2–118.3 (m), 103.2, 92.4–92.5 (2 x s), 80.3, 75.93–75.97 (2 x s), 73.1–73.2 (2 x s), 71.4–71.5 (2 x d, $J_{C,P}$ = 170.0 Hz), 69.5, 69.4, 68.6–68.9 (m), 41.85–42.01 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.51–19.56 (2 x s). HRMS (ESI) calcd for $C_{45}H_{44}N_3O_{15}PNa$ $[M + Na]^+$, 920.2402; Found 920.2404. IR (FTIR) ν_{max} 1689, 1231, 1007, 739.

*(Bis(benzyloxy)phosphoryl)(4-fluoro-3-phenoxyphenyl)methyl(2', 3'-O-bis(allyloxycarbony-l) uridin-5'-yl)carbamate (**26b**).* From **9** (281 mg, 588 μmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.50) to afford **26b** (206 mg, 38%) as an off-white solid. mp 70.0–73.0ºC. $[\alpha]_D^{25}$ −9.8 (*c* = 1.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (br s, 1H), 7.03–7.30 (m, 17H), 6.90 (d, *J* = 8.3 Hz, 2H), 5.98 (d, $J_{H,P}$ = 12.4 Hz, 1H), 5.83–5.94 (m, 2H), 5.65–5.74 (m, 2H), 5.43–5.54 (m, 2H),

5.25–5.38 (m, 5H), 4.86–5.05 (m, 4H), 4.55–4.66 (m, 4H), 4.14–4.19 (m, 1H), 3.40–3.58 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.86–162.91 (2 x s), 157.17–157.21 (2 x s), 154.9–155.1 (m), 154.5 (d, $J_{C,F}$ = 249.0 Hz), 153.8–153.9 (m), 149.96–150.02 (2 x s), 143.6–143.8 (m), 142.2–142.4 (m), 135.9–136.1 (m), 131.08–131.15 (2 x s), 130.96–131.02 (2 x s), 130.7–130.8 (2 x s), 129.8–129.9 (2 x s), 128.6–128.8 (m), 128.0–128.1 (m), 124.3, 123.3–123.4 (2 x s), 121.5, 119.73, 119.48–119.54 (2 x s), 117.35–117.40 (2 x s), 117.2 (d, $J_{C,F}$ = 18.0 Hz), 103.2, 92.7–92.8 (2 x s), 80.2–80.3 (2 x s), 75.8–75.9 (2 x s), 73.1–73.4 (2 x s), 70.8–70.9 (2 x d, $J_{C,P}$ = 171. 0 Hz), 69.5, 69.4, 68.6–69.0 (m), 42.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.38–19.46 (2 x d, $J_{P,F}$ = 4.9 Hz). ^{19}F NMR (235.3 MHz, $CDCl_3$) δ −67.57 – −67.79 (2 x d, $J_{F,P}$ = 1.9 Hz). HRMS (ESI) calcd for $C_{45}H_{43}N_3O_{15}PFNa$ $[M + Na]^+$, 938.2308; Found 938.2311. IR (FTIR) ν_{max} 1685, 1231, 986, 734.

*(Bis(benzyloxy)phosphoryl)(4-fluorophenyl)methyl(2', 3'-O-bis(allyloxycarbonyl)uridin-5'-yl) carbamate (**26c**)* From **10** (196 mg, 506 μmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 8:2, R_f = 0.48) to afford **26c** (165 mg, 40%) as a white solid. mp 80.9–81.6ºC. $[\alpha]_D^{25}$ −5.4 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (br s, 1H), 7.38–7.41 (m, 2H), 7.23–7.31 (m, 8H), 7.12–7.18 (m, 3H), 6.94–6.99 (m, 2H), 6.00–6.07 (m, 2H), 5.83–5.93 (m, 2H), 5.63–5.68 (2 x d, *J* = 8.0 Hz, 1H), 5.56–5.57 (m, 1H), 5.49–5.54 (m, 1H), 5.24–5.36 (m, 5H), 4.80–5.09 (m, 4H), 4.54–4.65 (m, 4H), 4.17–4.19 (m, 1H), 3.40–3.56 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 162.96–163.00 (2 x d, $J_{C,F}$ = 246.0 Hz), 155.1–155.3 (m), 153.79–153.84 (m), 150.2, 142.0–142.2 (2 x s), 135.8–136.1 (m), 131.0–131.1 (2 x s), 130.9, 129.7–130.0 (m), 129.45–129.54 (m), 128.61, 128.58, 128.55, 128.51, 128.04, 128.00, 127.97, 127.95, 119.61–119.63 (2 x s), 119.38–119.43 (2 x s), 115.6 (d, $J_{C,F}$ = 21.0 Hz), 103.2, 92.0–92.1 (2 x s), 80.2, 75.7–75.9 (2 x s), 73.2–73.3 (2 x s), 70.9–71.0 (2 x d, $J_{C,P}$ = 173.0 Hz), 69.5, 69.3, 68.6–68.9 (m), 41.9–42.1 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.84–19.91 (m). ^{19}F NMR (235.3 MHz, $CDCl_3$) δ −49.98 – −50.22 (2 x d, $J_{F,P}$ = 2.4 Hz). HRMS (ESI) calcd for $C_{39}H_{39}N_3O_{14}FPNa$ $[M + Na]^+$, 846.2051; Found 846.2077. IR (FTIR) ν_{max} 1751, 1222, 994, 871.

*(Bis(benzyloxy)phosphoryl)(3-fluorophenyl)methyl(2', 3'-O-bis(allyloxycarbonyl)uridin-5'-yl) carbamate (**26d**)*. From **11** (299 mg, 755 μmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.52) to afford **26d** (136 mg, 22%) as a white solid. mp 76.0–77.5ºC. $[\alpha]_D^{25}$ −3.1 (*c* = 6.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (br s, 1H), 7.10–7.30 (m, 14H), 6.97–7.01 (m, 1H), 6.06 (d, $J_{H,P}$ = 16.0 Hz, 1H), 5.83–5.94 (m, 3H), 5.63–5.67 (2 x d, *J* = 8.0 Hz, 1H), 5.49–5.54 (m, 2H), 5.25–5.38 (m, 5H), 4.84–5.07 (m, 4H), 4.55–4.66 (m, 4H), 4.16–4.19 (m, 1H), 3.42–3.58 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 162.75–162.78 (2 x d, $J_{C,F}$ = 245.0 Hz), 155.0–155.2 (m), 153.8–153.9 (m), 150.1, 142.1–142.2 (2 x s), 135.8–136.3 (m), 131.08–131.12 (2 x s), 131.0, 130.1 (d, $J_{C,F}$ = 8.0 Hz), 128.67, 128.65, 128.62, 128.58, 128.11, 128.09, 128.05, 123.4–123.7 (m), 119.7, 119.42–119.47 (2 x s), 115.6–115.9 (m), 114.8–115.0 (2 x d, $J_{C,F}$ = 22.0 Hz), 103.2, 92.4, 80.2, 75.8–75.9 (2 x s), 73.2, 71.05–71.14 (2 x d, $J_{C,P}$ = 170.0 Hz), 69.5, 69.3, 68.7–69.1 (m), 41.9–42.1 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.26–19.29 (2 x s). ^{19}F NMR (235.3 MHz, $CDCl_3$) δ −49.92 – −50.03 (2 x s). HRMS (ESI) calcd for $C_{39}H_{40}N_3O_{14}PNa$ $[M + Na]^+$, 828.2140; Found 828.2144. IR (FTIR) ν_{max} 1677, 1207, 947, 781, 734, 688.

*(Bis(benzyloxy)phosphoryl)(3-propoxyphenyl)methyl(2', 3'-O-bis(allyloxycarbonyl)uridin-5'-y-l)carb amate (**26e**)*. From **12** (372 mg, 617 μmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.26) to afford **26e** (268 mg, 36%) as a white solid. mp 60.1–61.1ºC. $[\alpha]_D^{25}$ −2.2 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (br s, 1H), 7.15–7.31 (m, 11H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.96–7.01 (m, 1H), 6.95–6.97 (m, 1H), 6.82–6.88 (m, 1H), 6.06–6.07 (2 x d, $J_{H,P}$ = 16.0 Hz, 1H), 5.84–5.94 (m, 2H), 5.78–5.81 (m, 1H), 5.67 (d, *J* = 8.0 Hz, 0.5H), 5.59 (d, *J* = 8.0 Hz, 0.5H), 5.51–5.54 (m, 1H), 5.46–5.50 (m, 1H), 5.26–5.36 (m, 5H), 4.79–5.07 (m, 4H), 4.55–4.66 (m, 4H), 4.17 (br s, 1H), 3.75–3.87 (s, 2H), 3.39–3.60 (m, 2H), 1.75 (sxt, *J* = 7.4 Hz, 2H), 0.990–0.994 (2 x t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.89–162.93, 159.2, 155.1–155.4 (m), 153.86–153.9 (m), 150.0, 142.0–142.1 (2 x s), 136.0–136.2 (m), 134.9–135.0 (2 x s), 131.07–131.10 (2 x s), 130.9, 129.6, 128.63, 128.59, 128.56, 128.50, 128.47, 128.1, 128.04, 127.99, 120.0–120.1 (2 x d, $J_{C,P}$ = 5.5 Hz), 119.7, 119.46–119.49 (2 x s), 115.2–115.3 (2 x s), 113.7–114.0 (2 x d, $J_{C,P}$ = 5.6 Hz), 103.2, 92.2–92.3 (2 x s), 80.2, 75.95–

76.03 (2 x s), 73.0–73.2 (2 x s), 71.6–71.7 (2 x d, $J_{C,P}$ = 170.0 Hz), 69.53, 69.40, 69.3, 68.6–68.9 (m), 41.7–42.0 (2 x s), 22.6, 10.6. ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.94. HRMS (ESI) calcd for $C_{42}H_{46}N_3O_{15}PNa$ $[M + Na]^+$, 886.2564. Found 886.2590. IR (FTIR) ν_{max} 2963, 1693, 1224, 1094, 808, 732.

*(Bis(benzyloxy)phosphoryl)(3-methoxyphenyl)methyl(2', 3'-O-bis(allyloxycarbonyl)uridin-5'-y-l)carb amate (**26f**)*. From **13** (400 mg, 1.06 mmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.26) to afford **26f** (317 mg, 38%) as a white solid. mp 64.7–65.5ºC. $[\alpha]_D^{25}$ −9.7 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (br s, 1H), 7.17–7.31 (m, 11H), 7.10 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.98 (s, 1H), 6.83–6.86 (m, 1H), 6.07 (d, $J_{H,P}$ = 14.0 Hz, 1H), 5.85–5.93 (m, 2H), 5.79 (bs, 1H), 5.67 (d, *J* = 8.0 Hz, 0.5H), 5.60 (d, *J* = 8.0 Hz, 0.5H), 5.47–5.50 (m, 2H), 5.26–5.37 (m, 5H), 4.80–5.07 (m, 4H), 4.58–4.64 (m, 4H), 4.16–4.20 (m, 1H), 3.72 (s, 3H), 3.42–3.61 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.8–162.9, 159.6–159.7, 155.2–155.3 (2 x d, $J_{C,P}$ = 11.4 Hz), 153.8–153.9 (m), 150.0, 142.1–142.2 (2 x s), 135.9–136.2 (m), 135.0–135.1 (2 x s), 131.09–131.07 (2 x s), 130.9, 129.6–129.7 (2 x d, $J_{C,P}$ = 1.5 Hz), 128.7, 128.62, 128.59, 128.55, 128.54, 128.51, 128.11, 128.06, 128.03, 128.00, 120.2–120.3 (2 x d, $J_{C,P}$ = 5.5 Hz), 119.7, 119.5, 114.5–114.6 (2 x s), 113.2–113.5 (2 x d, $J_{C,P}$ = 5.5 Hz), 103.18–103.21 (2 x s), 92.6–92.7 (2 x s), 80.2, 76.0–76.1 (2 x s), 72.9–73.0 (2 x s), 71.5–71.6 (2 x d, $J_{C,P}$ = 170.0 Hz), 69.5, 69.4, 68.7–69.0 (m), 55.29–55.33 (2 x s), 41.6–41.8 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.89. HRMS (ESI) calcd for $C_{40}H_{42}N_3O_{15}PNa$ $[M + Na]^+$, 858.2251; Found 858.2214. IR (FTIR): ν_{max} 2930, 1688, 1236, 1028, 808, 733.

*(Bis(benzyloxy)phosphoryl)(4-methoxyphenyl)methyl(2', 3'-O-bis(allyloxycarbonyl)uridin-5'-y-l)carb amate (**26g**)*. From **14** (454 mg, 1.14 mmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.50) to afford **26g** (592 mg, 62%) as a white solid. mp 48.3–51.7ºC. $[\alpha]_D^{25}$ −1.5 (*c* = 1.6, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.41 (br s, 1H), 7.36–7.37 (m, 2H), 7.26–7.32 (m, 8H), 7.15–7.19 (m, 2H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 2H), 6.00–6.04 (m, 1H), 5.84–5.92 (m, 3H), 5.59–5.67 (m, 1H), 5.47–5.51 (m, 2H), 5.33–5.37 (m, 4H), 5.26–5.30 (m, 1H), 4.74–5.10 (m, 4H), 4.56–4.66

(m, 4H), 4.18 (br s, 1H), 3.78–3.79 (2 x s, 3H), 3.39–3.58 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.9, 160.1–160.2 (2 x s), 155.3–155.4 (m), 153.85–153.92 (m), 150.1, 142.1–142.2 (2 x s), 135.9–136.3 (m), 131.06–131.09 (2 x s), 130.9, 129.5–129.7 (2 x d, $J_{C,P}$ = 6.3 Hz), 128.5–128.7 (m), 128.1, 128.0, 127.9, 125.4–125.5 (2 x s), 119.7, 119.50–119.54 (2 x s), 114.1, 103.21–103.24 (2 x s), 92.6, 80.2, 75.9–76.1 (2 x s), 72.9–73.1 (2 x s), 71.27–71.30 (2 x d, $J_{C,P}$ = 172. 5 Hz), 69.0, 68.9, 68.6–68.8 (m), 55.38–55.40 (2 x s), 41.6–41.9 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) 20.43. HRMS (ESI) calcd for $C_{40}H_{42}N_3O_{15}PNa$ $[M + Na]^+$, 858.2246; Found 858.2241. IR (FTIR) ν_{max} 2917, 2848, 1701, 1236, 1081, 808.

*(Bis(benzyloxy)phosphoryl)(3-cyclopentoxy-4-methoxyphenyl)methyl(2', 3'-O-bis(allyloxy-car-bonyl) uridin-5'-yl)carbamate (**26h**)*. From **15** (226 mg, 469 μmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.38) to afford **26h** (220 mg, 51%) as an off-white solid. mp 74.7–75.3ºC. $[\alpha]_D^{25}$ −1.0 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.10 (br s, 1H), 7.27–7.31 (m, 8H), 7.09–7.20 (m, 3H), 6.96–7.00 (m, 2H), 6.79 (d, *J* = 8.0 Hz, 1H), 6.00–6.02 (d, $J_{H,P}$ = 15.0 Hz, 1H), 5.85–5.94 (m, 2H), 5.62–5.69 (m, 2H), 5.48–5.51 (m, 2H), 5.27–5.38 (m, 5H), 4.76–5.09 (m, 4H), 4.60–4.65 (m, 5H), 4.18 (br s, 1H), 3.82–3.83 (2 x s, 3H), 3.37–3.64 (m, 2H), 1.77–1.85 (m, 6H), 1.54 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.69–162.74 (2 x s), 155.2–155.4 (m), 153.85–153.93 (m), 150.5–150.6 (2 x d, $J_{C,P}$ = 2.5 Hz), 149.97–150.01 (2 x s), 147.7, 142.1–142.2 (2 x s), 136.0–136.3 (m), 131.06–131.10 (2 x s), 130.9, 128.7, 128.60, 128.56, 128.51, 128.46, 128.11, 128.05, 128.03, 127.97, 125.7 (d, $J_{C,P}$ = 6.3 Hz), 120.7–120.9 (2 x d, $J_{C,P}$ = 7.5 Hz), 119.8, 119.5–119.6 (2 x s), 114.5–114.7 (2 x d, $J_{C,P}$ = 5.0 Hz), 111.8, 103.3, 92.6–92.8 (2 x s), 80.39–80.44 (2 x s), 80.21–80.24 (2 x s), 76.0–76.1 (2 x s), 72.99–73.03 (2 x s), 71.5–71.6 (2 x d, $J_{C,P}$ = 172.5 Hz), 69.5, 69.4, 68.5–68.7 (m), 56.1–56.2 (2 x s), 41.7–41.9 (2 x s), 32.8, 24.2. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.38. HRMS (ESI) calcd for $C_{45}H_{51}N_3O_{16}P$ $[M + H]^+$, 920.3007; Found 920.3010. IR (FTIR) ν_{max} 2928, 1692, 1235, 1028, 808.

*(Bis(benzyloxy)phosphoryl)(3-cyclopentoxyphenyl)methyl(2', 3'-O-bis(allyloxycarbonyl)uridin-5'-yl)carbamate (**26i**)*. From **16** (221 mg, 487 μmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.67) to afford **26i** (165 mg, 38%) as a white solid. mp 71.2–73.6ºC. $[\alpha]_D^{25}$ −1.5 (*c* =

1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 9.55 (br s, 1H), 7.12–7.33 (m, 12H), 6.94–6.99 (m, 2H), 6.79–6.83 (m, 1H), 6.06–6.07 (2 x d, $J_{H,P}$ = 13.9 Hz, 1H), 5.85–5.93 (m, 3H), 5.59–5.70 (m, 1H), 5.53–5.55 (m, 1H), 5.48 (br s, 1H), 5.25–5.36 (m, 5H), 4.78–5.10 (m, 4H), 4.56–4.66 (m, 5H), 4.16–4.20 (m, 1H), 3.40–3.58 (m, 2H), 1.72–1.87 (m, 6H), 1.56 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.1, 158.2, 155.2–155.4 (m), 153.8–153.9 (m), 150.1, 141.9–142.0 (2 x s), 135.9–136.2 (m), 134.8–134.9, 131.06–131.08 (2 x s), 130.9, 129.6, 128.60, 128.56, 128.53, 128.46, 128.4, 128.1, 128.01, 127.99, 127.96, 119.74–119.78 (m), 119.66, 119.45–119.4 (2 x s), 116.1–116.2 (2 x s), 114.8–115.0 (2 x d, $J_{C,P}$ = 5.7 Hz), 103.2, 92.0–92.2 (m), 80.1, 79.25–79.28, 75.9–76.0 (2 x s), 73.0–73.2 (2 x s), 71.56–71.63 (2 x d, $J_{C,P}$ = 170.9 Hz), 69.47–69.50 (2 x s), 69.3, 68.6–68.9 (m), 41.7–42.0 (2 x s), 32.8, 32.9, 24.1. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.05. HRMS (ESI) calcd for $C_{44}H_{48}N_3O_{15}PNa$ $[M + Na]^+$, 912.2721; Found 912.2706. IR (FTIR) ν_{max} 3299, 2960, 1689, 1233, 991, 782, 734, 696.

(Bis(benzyloxy)phosphoryl)(phenyl)methyl(2′, 3′-O-bis(allyloxycarbonyl)uridin-5′-yl)carbama-te ***(26j)***. From **17** (310 mg, 843 μmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.40) to afford **26j** (301 mg, 44%) as a white solid. mp 70.0–72.0ºC. $[\alpha]_D^{25}$ −2.5 (*c* = 2.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 8.78 (br s, 1H), 7.43 (br s, 2H), 7.24–7.33 (m, 11H), 7.20 (br s, 1H), 7.16 (br s, 1H), 7.08–7.10 (m, 1H), 6.075–6.080 (2 x d, $J_{H,P}$ = 14.0 Hz, 1H), 5.87–5.93 (m, 2H), 5.58–5.68 (m, 2H), 5.47–5.50 (m, 2H), 5.27–5.37 (m, 5H), 4.78–5.06 (m, 4H), 4.60–4.67 (m, 4H), 4.18 (br s, 1H), 3.40–3.61 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.20–163.23 (2 x s), 155.2–155.4 (2 x d, $J_{C,P}$ = 11.3 Hz), 153.79–153.82 (m), 150.1, 141.8–142.0 (2 x s), 135.9–136.2 (m), 133.5–133.6 (2 x s), 131.0, 130.9, 128.9, 128.8, 128.6, 128.51, 128.45, 128.01, 127.95, 127.9, 119.6, 119.39–119.43 (2 x s), 103.1, 91.6–91.8 (2 x s), 80.13–80.09 (2 x s), 75.8–75.9 (2 x s), 73.1–73.2 (2 x s), 71.5–71.6 (2 x d, $J_{C,P}$ = 170.0 Hz), 69.4, 69.3, 68.5–69.0 (m), 41.8–42.1 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.97. HRMS (ESI) calcd for $C_{39}H_{40}N_3O_{14}PNa$ $[M + Na]^+$, 828.2140; Found 828.2144. IR (FTIR) ν_{max} 1681, 1208, 980, 734, 693.

(Bis(benzyloxy)phosphoryl)(benzo[b]thiophen-2-yl)methyl(2′, 3′-O-bis(allyloxycarbonyl)uridin-5′-yl) carbamate ***(26k)***. From **18** (229 mg, 540 μmol): purification by column chromatography (CH_2Cl_2 :

CH_3CN, 7:3, R_f = 0.40) to afford **26k** (257 mg, 36%) as a white solid. mp 96.6–99.0ºC. $[\alpha]_D^{25}$ −2.6 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 9.23 (br s, 1H), 7.76–7.78 (m, 1H), 7.65–7.70 (m, 1.5H), 7.53–7.56 (m, 0.5H), 7.44–7.48 (m, 1H), 7.35–7.38 (m, 1H), 7.17–7.33 (m, 11H), 7.10–7.11 (2 x d, *J* = 10.0 Hz, 1H), 6.41 (d, $J_{H,P}$ = 15.0 Hz, 1H), 5.83–5.94 (m, 2H), 5.66 (d, *J* = 8.0 Hz, 0.5H), 5.58–5.60 (m, 1.5H), 5.47–5.52 (m, 2H), 5.24–5.38 (m, 4H), 4.92–5.14 (m, 4H), 4.54–4.65 (m, 4H), 4.16–4.21 (m, 1H), 3.42–3.66 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.8, 155.0–155.2 (m), 153.8–153.9 (m), 150.0–150.1 (2 x s), 142.0–142.1 (2 x s), 140.3, 139.23 (d, $J_{C,P}$ = 5.0 Hz), 139.16, 135.8–136.2 (m), 133.0, 132.2–132.3 (2 x s), 132.09–132.07 (2 x s), 131.05–131.10 (2 x s), 130.9, 128.7, 128.61, 128.58, 128.57, 128.20, 128.17, 128.14, 128.12, 125.1–125.3 (m), 124.5–124.9 (m), 124.2, 122.4, 119.7, 119.47–119.52 (2 x s), 103.21–103.24 (2 x s), 92.2–92.5 (2 x s), 80.15–80.21 (2 x s), 75.9–76.0 (2 x s), 73.1–73.2 (2 x s), 69.5, 69.3–69.4 (2 x s), 69.0–69.1 (m), 68.5 (d, $J_{C,P}$ = 177.5 Hz), 41.8–42.1 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 18.26. HRMS (ESI) calcd for $C_{41}H_{40}N_3O_{14}PSNa$ $[M + Na]^+$, 884.1866; Found 884.1908. IR (FTIR) ν_{max} 2929, 1693, 1200, 1007, 733, 661.

*(Bis(benzyloxy)phosphoryl)(benzo[b]thiophen-3-yl)methyl(2', 3'-O-bis(allyloxycarbonyl)uridin-5'-yl) carbamate (**26l**)*. From **19** (258 mg, 608 μmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.49) to afford **26l** (384 mg, 73%) as a white solid. mp 80.0–83.0ºC. $[\alpha]_D^{25}$ −20.0 (*c* = 0.1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) 7.93–7.98 (m, 1H), 7.78–7.83 (m, 1H), 7.72–7.73 (m, 1H), 7.00–7.33 (m, 13H), 6.54 (d, $J_{H,P}$ = 14.2 Hz, 1H), 5.81–5.99 (m, 3H), 5.43–5.56 (m, 3H), 5.52–5.36 (m, 5H), 4.96–5.12 (m, 4H), 4.51–4.64 (m, 4H), 4.14–4.19 (m, 1H), 3.39–3.57 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 155.3–155.5 (m), 153.9, 153.8, 150.1, 141.8–141.9 (2 x s), 140.1–140.2 (2 x s), 137.4–137.5 (2 x s), 135.6–136.2 (m), 131.07–131.10 (2 x s), 130.9, 128.6, 128.51, 128.47, 128.4, 128.13, 128.05, 128.0, 127.9, 127.7–127.8 (2 x s), 124.76–124.81 (2 x s), 124.5–124.6 (2 x s), 122.7–122.8 (m), 119.6, 119.4, 103.1, 91.8–91.9 (2 x s), 80.1–80.2 (2 x s), 75.8–75.9 (2 x s), 73.16–73.19 (2 x s), 69.5, 69.3, 68.7–69.0 (m), 66.4 (d, $J_{C,P}$ = 176.6 Hz), 41.9–42.0 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.57–

19.61 (2 x s). HRMS (ESI) calcd for $C_{41}H_{40}N_3O_{14}PSNa$ $[M + Na]^+$, 884.1866; Found 884.1888. IR (FTIR) ν_{max} 3064, 1753, 1688, 1232, 991.

*(Bis(benzyloxy)phosphoryl)(3-phenoxyphenyl)methyl(N^4, 2', 3'-O-bis(benzyloxycarbonyl)cytidin-5'-yl)carbamate (**29**)*. From **8** (423 mg, 918 μmol): purification by column chromatography (CH_2Cl_2 : CH_3CN, 7:3, R_f = 0.91) to afford **29** (242 mg, 23%) as a white solid. mp 57.7–60.0ºC. $[\alpha]_D^{25}$ −10.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (br s, 1H), 7.50–7.54 (m, 1H), 7.13–7.32 (m, 31H), 7.02–7.05 (m, 1H), 6.87–6.92 (m, 3H), 6.48–6.52 (m, 1H), 6.08–6.10 (2 x d, $J_{H,P}$ = 14.0 Hz, 1H), 5.68 (br s, 1H), 5.52–5.55 (2 x d, *J* = 2.5 Hz, 1H), 5.35–5.42 (m, 1H,), 5.15 (s, 2H), 4.79–5.08 (m, 8H), 4.25 (br s, 1H), 3.46–3.61 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.4, 157.3, 156.88–156.90 (2 x s), 155.1–155.3 (m), 154.78–154.80 (2 x s), 153.92–153.95 (m), 152.4–152.5 (2 x s), 146.5–146.6 (2 x s), 135.9–136.2 (m), 135.8, 135.1–135.2, 134.8, 134.6, 129.9, 129.8, 128.3–128.7 (m), 127.8–128.0 (m), 123.4, 122.4–122.6 (2 x d, $J_{C,P}$ = 5.1 Hz), 119.0–119.1 (2 x s), 118.75–118.80 (2 x s), 118.1–118.2 (m), 95.8, 94.2–94.3 (2 x s), 80.3–80.5 (2 x s), 76.3–76.4 (2 x s), 73.9–74.1 (2 x s), 71.16–71.19 (2 x d, $J_{C,P}$ = 170.0 Hz), 70.4, 70.27–70.31 (2 x s), 68.8–69.0 (2 x d, $J_{C,P}$ = 6.6 Hz), 68.5–68.7 (2 x d, $J_{C,P}$ = 6.5 Hz), 67.86–67.90 (2 x s), 42.2. ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.74–19.77 (2 x s). HRMS (ESI) calcd for $C_{61}H_{55}N_4O_{16}PNa$ $[M + Na]^+$, 1153.3248; Found 1153.3279. IR (FTIR) ν_{max} 2919, 1754, 1666, 1566, 1510, 1254, 1034.

Alloc Deprotection: A suspension of Alloc protected carbamate (**26a–l**), $Pd(PPh_3)_4$ (15mol%) and dimedone (8 equiv.) in dry THF was stirred at rt. Upon consumption of starting material (generally after 30 min) volatiles were removed under reduced pressure and the subsequent crude product purified by column chromatography.

*(Bis(benzyloxy)phosphoryl)(3-phenoxyphenyl)methyl(uridin-5'-yl)carbamate (**27a**)*. From **26a** (174 mg, 194 μmol): purification by column chromatography (CH_2Cl_2 : MeOH, 95:5; R_f = 0.05) to afford **27a** (138 mg, 81%) as a white solid. mp 66.0–67.0ºC. $[\alpha]_D^{25}$ +4.8 (*c* = 4.4, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.20 (br s, 1H), 7.01–7.26 (m, 18H), 6.84–6.92 (m, 2H), 6.25–6.29 (m, 1H), 6.04–6.10 (m, 1H), 5.54–5.62 (m, 2H), 4.78–5.00 (m, 4H), 4.19–4.22 (m, 1H), 4.04–4.10 (m, 1H), 3.99–4.00 (m, 1H),

3.29–3.49 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8, 157.4–157.5 (2 x s), 156.88–156.93 (2 x s), 155.5 (d, $J_{C,P}$ = 12.1 Hz), 151.0, 141.5–141.8 (2 x s), 135.6–135.9 (m), 130.0, 128.74, 128.69, 128.0, 127.9, 123.7, 122.5–122.6 (2 x s), 119.0–119.2 (2 x s), 118.2–118.3 (m), 102.8, 92.6–93.1 (2 x s), 82.65–82.69 (2 x s), 73.9–74.0 (2 x s), 71.2–71.3 (2 x d, $J_{C,P}$ = 173.0 Hz), 70.8–71.0 (2 x s), 68.9–69.1 (m), 42.7–43.0 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.74–19.77 (2 x s). HRMS (ESI) calcd for $C_{37}H_{36}N_3O_{11}PNa$ $[M + Na]^+$, 752.1985; Found 752.1980. IR (FTIR) ν_{max} 3313, 2924, 1665, 1245, 1053, 734, 689.

*(Bis(benzyloxy)phosphoryl)(4-fluoro-3-phenoxyphenyl)methyl(uridin-5′-yl)carbamate (**27b**)*. From **26b** (152 mg, 162 μmol): purification by column chromatography (CH_2Cl_2 : MeOH, 95:5; R_f = 0.27) to afford **27b** (105 mg, 84%) as a white solid. mp 70.0–71.5 C. $[\alpha]_D^{25}$ −1.4 (*c* = 3.6, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.19 (br s, 1H), 7.02–7.26 (m, 17H), 6.82–6.85 (m, 2H), 6.18 (br s, 1H), 5.96–6.02 (m, 1H), 5.52–5.63 (m, 2H), 4.79–5.01 (m, 4H), 4.22 (br s, 1H), 4.08 (bs, 1H), 3.99 (bs, 1H), 3.32–3.55 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.9, 157.1, 155.3 (d, $J_{C,P}$ = 10.9 Hz), 154.4 (d, $J_{C,F}$ = 250.8 Hz), 151.0–151.1 (2 x s), 143.5–143.7 (m), 141.5–141.7 (2 x s), 135.5–135.7 (m), 130.5, 129.9, 128.74, 128.69, 128.02, 127.95, 124.3, 123.39–123.42 (2 x s), 121.4–121.5 (2 x s), 117.3–117.4 (m), 102.7, 92.5–92.8 (2 x s), 82.5–82.6 (2 x s), 73.9, 70.88–70.92 (2 x s), 70.57–70.61 (2 x d, $J_{C,P}$ = 172.1 Hz), 68.9–69.1 (m), 42.9–43.0 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.29–19.47 (2 x d, $J_{P,F}$ = 4.0 Hz). ^{19}F NMR (235.3 MHz, $CDCl_3$) δ −67.14 – −67.18 (2 x s). HRMS (ESI) calcd for $C_{37}H_{35}N_3O_{11}PFNa$ $[M + Na]^+$, 770.1891; Found 770.1873. IR (FTIR) ν_{max} 3313, 2924, 1665, 1245, 1053, 986, 734.

*(Bis(benzyloxy)phosphoryl)(4-fluorophenyl)methyl(uridin-5′-yl)carbamate (**27c**)*. From **26c** (165 mg, 200 μmol): purification by column chromatography (EtOAc : MeOH, 95:5; R_f = 0.24) to afford **27c** (100 mg, 76%) as a white solid. mp 91.6–92.7ºC. $[\alpha]_D^{25}$ +3.9 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.32–10.37 (2 x br s, 1H), 7.16–7.34 (m, 11H), 7.06–7.07 (m, 2H), 6.85–6.92 (m, 2H), 6.41 (br s, 1H), 6.03–6.09 (m, 1H), 5.55–5.64 (m, 2H), 4.60–5.03 (m, 4H), 4.20 (br s, 1H), 4.01–4.05 (m, 2H), 3.33–3.49 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.2, 162.9–163.0 (2 x d, $J_{C,F}$ = 245.0 Hz), 155.5

(d, $J_{C,P}$ = 11.3 Hz), 151.08–151.13, 141.5, 135.8 (d, $J_{C,P}$ = 5.0 Hz), 135.6 (d, $J_{C,P}$ = 6.3 Hz), 129.8–130.0 (m), 129.4, 128.7, 128.6, 128.02, 127.99, 127.95, 115.6–115.7 (2 x d, $J_{C,F}$ = 21.3 Hz), 102.6, 91.6–91.8 (2 x s), 82.51–82.55 (2 x s), 74.0, 70.98–71.02 (2 x s), 70.79–70.84 (2 x d, $J_{C,P}$ = 172.5 Hz), 68.9–69.0 (m), 42.9–43.1 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.95–20.01 (2 x s). ^{19}F NMR (235.3 MHz, $CDCl_3$) δ −49.57 – −49.68 (2 x d, $J_{F,P}$ = 2.4 Hz). HRMS (ESI) calcd for $C_{31}H_{31}N_3O_{10}PFNa$ $[M + Na]^+$, 678.1629; Found 678.1628. IR (FTIR) ν_{max} 3312, 1702, 1245, 1094, 1030, 808, 732.

*(Bis(benzyloxy)phosphoryl)(3-fluorophenyl)methyl(uridin-5'-yl)carbamate (**27d**)*. From **26d** (193 mg, 234 μmol): purification by column chromatography (CH_2Cl_2 : MeOH, 95:5; R_f = 0.17) to afford **27d** (101 mg, 68%) as a white solid. mp 88.8–91.6ºC. $[\alpha]_D^{25}$ +3.6 (*c* = 1.0, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.28–10.34 (br s, 1H), 7.05–7.30 (m, 14H), 6.86–6.94 (m, 1H), 6.42–6.46 (m, 1H), 6.03–6.11 (m, 1H), 5.54–5.66 (m, 2H), 4.76–5.07 (m, 4H), 4.20–4.22 (m, 1H), 3.95–4.07 (m, 2H), 3.30–3.50 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.16–164.19 (2 x s), 162.7 (d, $J_{C,F}$ = 246.8 Hz), 155.5 (d, $J_{C,P}$ = 10.8 Hz), 150.1–151.2 (2 x s), 142.3–142.5 (2 x s), 136.09–136.13 (2 x s), 135.7–135.8 (m), 135.6 (d, $J_{C,P}$ = 5.9 Hz), 130.2–130.3 (m), 128.72, 128.67, 128.1, 128.0, 123.5, 115.8–115.9 (m), 114.7–115.0 (m), 102.6, 91.7–92.0 (2 x s), 82.5, 74.0, 70.9–71.0 (2 x s), 70.9 (d, $J_{C,P}$ = 165.0 Hz), 69.0–69.1 (m), 42.8–43.1 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.50–19.56 (2 x s). ^{19}F NMR (235.3 MHz, $CDCl_3$) δ −49.27 – −49.38 (2 x s). HRMS (ESI) calcd for $C_{31}H_{31}N_3O_{10}PFNa$ $[M + Na]^+$, 678.1629; Found 678.1626. IR (FTIR) ν_{max} 3312, 1702, 1245, 1015, 734.

*(Bis(benzyloxy)phosphoryl)(3-propoxyphenyl)methyl(uridin-5'-yl)carbamate (**27e**)*. From **26e** (205 mg, 237 μmol): purification by column chromatography (CH_2Cl_2 : MeOH, 95:5; R_f = 0.15) to afford **27e** (150 mg, 91%) as a white solid. mp 56.3–57.7ºC. $[\alpha]_D^{25}$ +6.8 (*c* = 1.0, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.27–10.31 (2 x br s, 1H), 7.08–7.29 (m, 12H), 6.95–6.99 (m, 1H), 6.89–6.90 (m, 1H), 6.76–6.81 (m, 1H), 6.35 (br s, 1H), 6.05–6.11 (m, 1H), 5.33–5.67 (m, 2H), 4.73–4.98 (m, 4H), 4.19 (br s, 1H), 4.02 (br s, 2H), 3.66–3.78 (m, 2H), 3.32–3.48 (m, 2H), 1.65–1.73 (m, 2H), 0.91–0.96 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1, 159.2, 155.6 (d, $J_{C,P}$ = 10.5 Hz), 151.0, 141.2–141.3 (2 x s), 135.7–135.9

(m), 134.9, 129.7, 128.64, 128.56, 128.5, 128.00, 127.96, 127.9, 119.8–119.9 (m), 115.2–115.3 (2 x s), 113.9–114.0 (m), 102.7, 91.6–91.7 (2 x s), 82.5–82.6 (2 x s), 74.0, 71.4–71.5 (2 x d, $J_{C,P}$ = 169.0 Hz), 70.9–71.0 (2 x s), 69.5, 68.8–69.0 (m), 42.8–43.0 (2 x s), 22.6, 10.6. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.05–20.07 (2 x s). HRMS (ESI) calcd for $C_{34}H_{38}N_3O_{11}PNa$ $[M + Na]^+$, 718.2142; Found 718.2175. IR (FTIR) ν_{max} 3299, 2934, 1679, 1239, 992, 735, 693.

(Bis(benzyloxy)phosphoryl)(3-methoxyphenyl)methyl(uridin-5'-yl)carbamate ***(27f)***. From **26f** (267 mg, 319 μmol): purification by column chromatography (CH_2Cl_2 : MeOH, 9:1; R_f = 0.42) to afford **27f** as a white solid (184 mg, 87%). mp 79.8–81.4ºC. $[\alpha]_D^{25}$ −0.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.34 (br s, 1H), 7.08–7.28 (m, 12H), 6.91–6.99 (m, 2H), 6.74–6.80 (m, 1H), 6.38–6.41 (m, 1H), 6.05–6.12 (m, 1H), 5.62–5.66 (m, 1H), 5.58 (d, *J* = 8.0 Hz, 0.5H), 5.51 (d, *J* = 8.0 Hz, 0.5H), 4.73–4.97 (m, 4H), 4.18 (br s, 1H), 4.01–4.04 (m, 2H), 3.59–3.61 (2 x s, 3H), 3.35–3.45 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.21–164.24 (2 x s), 159.6–159.7 (m), 155.6 (d, $J_{C,P}$ = 11.0 Hz), 151.0–151.1 (2 x s), 141.3, 135.8 (d, $J_{C,P}$ = 6.0 Hz), 135.7 (d, $J_{C,P}$ = 6.0 Hz), 134.9, 129.67–129.72, 128.64, 128.58, 128.0, 127.9, 120.0–120.2 (m), 114.4–114.6 (2 x s), 113.4–113.5 (m), 102.5–102.6 (2 x s), 91.5–91.7 (2 x s), 82.5–82.6 (2 x s), 74.0, 71.3–71.4 (2 x d, $J_{C,P}$ = 170.0 Hz), 70.9–71.0 (2 x s), 68.9–69.0 (m), 55.21–55.24 (2 x s), 42.8–43.0 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.03–20.07 (2 x s). HRMS (ESI) calcd for $C_{32}H_{34}N_3O_{11}PNa$ $[M + Na]^+$, 690.1829; Found 690.1827. IR (FTIR) ν_{max} 3312, 1702, 1245, 1094, 808, 732.

(Bis(benzyloxy)phosphoryl)(4-methoxyphenyl)methyl(uridin-5'-yl)carbamate ***(27g)***. From **26g** (147 mg, 176 μmol): purification by column chromatography (EtOAc : MeOH, 9:1, R_f = 0.62) to afford **27g** (99.5 mg, 85%) as an off-white solid. mp 72.0–75.0ºC. $[\alpha]_D^{25}$ +5.4 (*c* = 0.8, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.12–10.21 (2 x br s, 1H), 7.30–7.33 (m, 2H), 7.20–7.25 (m, 8H), 7.09–7.10 (m, 3H), 6.76–6.80 (2 x d, *J* = 8.0 Hz, 2H), 6.25–6.30 (m, 1H), 6.02–6.06 (2 x d, $J_{H,P}$ = 13.5 Hz, 1H), 5.51–5.60 (m, 2H), 4.68–5.02 (m, 4H), 4.20 (br s, 1H), 4.04–4.08 (m, 1H), 3.98–4.00 (m, 1H), 3.70–3.73 (2 x s, 3H), 3.31–3.41 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.05–164.10 (2 x s), 160.1–160.2 (2 x s),

155.7 (d, $J_{C,P}$ = 11.0 Hz), 151.05–151.11 (2 x s), 141.4, 136.0 (d, $J_{C,P}$ = 6.3 Hz), 135.8 (d, $J_{C,P}$ = 6.3 Hz), 129.5–129.7 (2 x d, $J_{C,P}$ = 5.0 Hz), 128.7, 128.6, 128.5, 127.98, 127.95, 127.9, 125.4, 114.1–114.2 (m), 102.6–102.7 (2 x s), 91.8–91.9 (2 x s), 82.6, 73.96–74.02 (2 x s), 71.1–71.2 (2 x d, $J_{C,P}$ = 175.0 Hz), 70.9–71.0 (2 x s), 68.7–68.9 (m), 55.3–55.4 (2 x s), 42.7–43.0 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.32–20.46 (2 x s). HRMS (ESI) calcd for $C_{32}H_{34}N_3O_{11}PNa$ $[M + Na]^+$, 690.1846; Found 690.1829. IR (FTIR) ν_{max} 3312, 1702, 1245, 1081, 812.

(Bis(benzyloxy)phosphoryl)(3-cyclopentoxy-4-methoxyphenyl)methyl(uridin-5'-yl)carbamate ***(27h)***. From **26h** (189 mg, 205 μmol): purification by column chromatography (CH_2Cl_2 : MeOH, 95:5; R_f = 0.18) to afford **27h** (109 mg, 71%) as a white solid. mp 95.1–96.8ºC. $[\alpha]_D^{25}$ +18.7 (*c* = 1.0, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.37 (br s, 1H), 7.19–7.33 (m, 9H), 7.08–7.10 (m, 2H), 6.90–6.96 (m, 2H), 6.72–6.75 (m, 1H), 6.37 (br s, 1H), 6.01–6.07 (m, 1H), 5.62–5.66 (m, 1.5H), 5.57 (d, *J* = 8.0 Hz, 0.5H), 4.74–5.02 (m, 4H), 4.53 (br s, 1H), 4.21 (br s, 1H), 4.00–4.06 (m, 2H), 3.74–3.75 (2 x s, 3H), 3.30–3.54 (m, 2H), 1.72 (br s, 6H), 1.48 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.2, 155.67–155.71 (2 x d, $J_{C,P}$ = 10.5 Hz), 151.08–151.11 (2 x s), 150.47–150.54 (2 x d, $J_{C,P}$ = 2.5 Hz), 147.5–147.6 (2 x s), 141.2–141.4 (2 x s), 135.9 (d, $J_{C,P}$ = 6.3 Hz), 135.7 (d, $J_{C,P}$ = 6.3 Hz), 128.64, 128.56, 128.53, 128.00, 128.95, 128.91, 128.90, 125.5, 120.6–120.7 (m), 114.65–114.69 (m), 111.8, 102.6–102.7 (2 x s), 91.5–91.8 (2 x s), 82.5, 80.4, 74.0, 71.29–71.34 (2 x d, $J_{C,P}$ = 172.5 Hz), 70.8–71.0 (2 x s), 68.7–68.9 (m), 56.0–56.1 (2 x s), 42.8–43.0 (2 x s), 32.7, 24.1. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.49–20.52 (2 x s). LRMS (ESI) calcd for $C_{37}H_{42}N_3O_{12}PNa$ $[M + Na]^+$, 774.2404; Found 774.2398. IR (FTIR) ν_{max} 3329, 2935, 1685, 1237, 1034, 736, 695.

(Bis(benzyloxy)phosphoryl)(3-cyclopentoxyphenyl)methyl(uridin-5'-yl)carbamate ***(27i)***. From **26i** (145 mg, 163 μmol): purification by column chromatography (CH_2Cl_2 : MeOH, 95:5; R_f = 0.15) to afford **27i** (107 mg, 92%) as a white solid. mp 63.6–67.0ºC. $[\alpha]_D^{25}$ −27.1 (*c* = 1.0, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.26 (br s, 1H), 7.10–7.29 (m, 12H), 6.94–6.97 (m, 1H), 6.89–6.90 (m, 1H), 6.75–6.79 (m, 1H), 6.29 (br s, 1H), 6.05–6.11 (m, 1H), 5.55–5.65 (m, 2H), 4.74–4.98 (m, 4H), 4.58 (br s, 1H), 4.21 (br

s, 1H), 4.01–4.08 (m, 2H), 3.31–3.50 (m, 2H), 1.70–1.77 (m, 6H), 1.52 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.0, 158.2, 155.5–155.6 (2 x d, $J_{C,P}$ = 10.0 Hz), 151.1, 141.3–141.5 (2 x s), 135.7–135.9 (m), 134.8, 129.66–129.69 (2 x s), 128.7, 128.6, 128.5, 128.01, 127.98, 127.9, 119.7, 116.1–116.2 (m), 115.0–115.1 (m), 102.65–102.72 (2 x s), 91.8–92.1 (2 x s), 82.5–82.6 (2 x s), 79.3, 74.0, 71.37–71.45 (2 x d, $J_{C,P}$ = 161.2 Hz), 70.8, 68.8–69.0 (m), 42.8–43.0 (2 x s), 32.8–32.9 (2 x s), 24.1. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.08. HRMS (ESI) calcd for $C_{36}H_{40}N_3O_{11}PNa$ $[M + Na]^+$, 744.2298; Found 744.22920. IR (FTIR) ν_{max} 3304, 1681, 1240, 991, 733, 694.

(Bis(benzyloxy)phosphoryl)(phenyl)methyl(uridin-5'-yl)carbamate ***(27j)***. From **26j** (214 mg, 266 μmol): purification by column chromatography (CH_2Cl_2 : MeOH, 95:5; R_f = 0.10) to afford **27j** (137 mg, 80%) as a white solid. mp 112.2–114.1ºC. $[\alpha]_D^{25}$ −7.4 (*c* = 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.25–10.31 (2 x s, 1H), 7.36–7.38 (m, 2H), 7.18–7.26 (br s, 12H), 7.05–7.06 (br s, 2H), 6.39–6.41 (m, 1H), 6.06–6.14 (m, 1H), 5.49–5.64 (m, 2H), 4.68–4.96 (m, 4H), 4.17 (br s, 1H), 4.00–4.04 (m, 2H), 3.33–3.46 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1–164.2 (2 x s), 155.7 (d, $J_{C,P}$ = 11.3 Hz), 151.09–151.14 (2 x s), 141.35–141.40 (2 x s), 135.8 (d, $J_{C,P}$ = 5.7 Hz), 135.7 (d, $J_{C,P}$ = 6.1 Hz), 133.5, 128.7, 128.6, 128.02, 127.97, 127.9, 102.6, 91.6–91.9 (2 x s), 82.5, 74.0, 70.8–71.0 (2 x s), 71.5–71.6 (2 x d, $J_{C,P}$ = 170.0 Hz), 68.8–69.0 (m), 42.7–43.0 (2 x s).^{31}P NMR (162 MHz, $CDCl_3$) δ 20.19–20.25 (2 x s). HRMS (ESI) calcd for $C_{31}H_{32}N_3O_{10}PNa$ $[M + Na]^+$, 660.1718; Found 660.1719. IR (FTIR) ν_{max} 3288, 1678, 1239, 1012, 810, 735, 694.

(Bis(benzyloxy)phosphoryl)(benzo[b]thiophen-2-yl)methyl(uridin-5'-yl)carbamate ***(27k)***. From **26k** (54.2 mg, 62.9 μmol): purification by column chromatography (CH_2Cl_2 : MeOH, 95:5; R_f = 0.10) to afford **27k** (42.3 mg, 97%) as a white solid. mp 104.0–105.0ºC. $[\alpha]_D^{25}$ +1.4 (*c* = 1.0, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.04 (br s, 1H), 7.66–7.72 (m, 1H), 7.57–7.63 (m, 1H), 7.10–7.30 (m, 14H), 6.40–6.47 (m, 1H), 6.33 (br s, 1H), 5.51–5.59 (m, 2H), 4.85–5.06 (m, 4H), 4.22–4.24 (m, 1H), 4.10–4.16 (m, 1H), 4.00–4.03 (m, 1H), 3.37–3.50 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.8–163.9 (2 x s), 155.4 (d, $J_{C,P}$ = 10.3 Hz), 151.1, 141.8, 141.5, 140.17–140.21 (m), 139.0–139.1 (m), 135.5–136.0 (m), 128.7,

128.6, 128.2, 128.12, 128.09, 128.05, 125.0–125.3 (m), 124.6–124.7 (2 x s), 124.1–124.2 (2 x s), 122.4–122.5 (2 x s), 102.7, 92.4–93.0 (2 x s), 82.5–82.6 (2 x s), 73.9, 70.7–70.9 (2 x s), 69.2–69.4 (m), 67.8–67.9 (2 x d, $J_{C,P}$ = 176.3 Hz), 42.7–43.0 (2 x s). ^{31}P NMR (162 MHz, $CDCl_3$) δ 18.36–18.45 (2 x s). HRMS (ESI) calcd for $C_{33}H_{32}N_3O_{10}PSNa$ $[M + Na]^+$, 716.1444; Found 716.1439. IR (FTIR) ν_{max} 3280, 2929, 1687, 1220, 994, 733, 696.

*(Bis(benzyloxy)phosphoryl)(benzo[b]thiophen-3-yl)methyl(uridin-5′-yl)carbamate (**27l**).* From **26l** (300 mg, 348 μmol): purification by column chromatography (CH_2Cl_2 : MeOH, 98:2; R_f = 0.05) to afford **27l** (170 mg, 70%) as a white solid. mp 88.0–92.0ºC. $[\alpha]_D^{25}$ +1.2 (*c* = 2.0, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.17 (br s, 1H), 7.68–7.88 (m, 3H), 7.18–7.27 (m, 11H), 6.96 (br s, 2H), 6.51–6.58 (m, 1H), 6.24–6.31 (m, 1H), 5.44–5.57 (m, 2H), 4.67–5.00 (m, 4H), 4.07–4.19 (m, 2H), 3.98 (br s, 1H), 3.31–3.48 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.8, 155.7, 151.1, 141.7, 140.1, 137.3, 135.8, 135.5, 128.7, 128.6, 128.1, 128.0, 127.7, 125.0, 124.7, 122.8, 122.6, 102.7, 82.6, 73.9, 70.7, 69.0, 66.1–66.3 (2 x d, $J_{C,P}$ = 175.0 Hz), 42.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.74. IR (FTIR) 3301, 2923, 1678, 1242, 993. HRMS (ESI) calcd for $C_{33}H_{32}N_3O_{10}PSNa$ $[M + Na]^+$, 716.1444; Found 716.1468.

Preparation of Sodium Salts. To remove both Cbz and benzyl protecting groups the following procedure was utilised. To a solution of protected compound (**27a–l** or **29**) in MeOH, 10% Pd/C by mass was added. The mixture was stirred at rt, with H_2 gas bubbled through. Upon consumption of starting material (~1 h) the reaction mixture was filtered through a bed of Celite® and the filtrate concentrated under reduced pressure to yield a crude mix of diastereoisomers that were separated by preparative RP-HPLC (buffer system: CH_3CN/0.05 M triethylammonium bicarbonate (TEAB) buffer, pH 7.2−7.5 unless otherwise stated) and converted to their sodium salt form by ion exchange resin (IR 120 Na^+) and lyophilised from water to give compound ***s*** and ***l*** as amorphous solids. Where ***s*** and ***l*** stands for shorter and longer RP-HPLC retention time, respectively.

*Disodium [(3-phenoxy)phenylphosphonatomethyl]-uridin-5′-yl-carbamate (**28a**).* From **27a** (250 mg, 343 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and

lyophilised from water to afford diastereomers **28a-*s*** (91.8 mg, 90%) and **28a-*l*** (93.8 mg, 92%) as white powders. **28a-*s***: RP-HPLC, 0−40 min linear gradient 13−18% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 15.60 min; mp decomp. > 400.0ºC. $[\alpha]_D^{25}$ +39.3 (*c* = 0.1, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.58 (d, *J* = 7.7 Hz, 1H), 7.45−7.50 (m, 3H), 7.37 (d, *J* = 7.6 Hz, 1H), 7.23–7.28 (m, 2H), 6.99−7.05 (m, 3H), 5.95 (d, *J* = 3.8 Hz, 1H), 5.89 (d, *J* = 7.7 Hz, 1H), 5.62 (d, $J_{H,P}$ = 13.8 Hz, 1H), 4.16−4.19 (m, 1H), 3.96−3.99 (m, 1H), 3.88–3.91 (m, 1H), 3.71 (dd, *J* = 15.0 Hz, *J* = 5.2 Hz, 1H), 3.55 (dd, *J* = 15.0 Hz, *J* = 3.1 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 174.7, 159.5 (d, $J_{C,P}$ = 12.8 Hz), 157.9, 156.5, 142.5, 140.5, 130.8, 130.2, 123.9, 123.7 (d, $J_{C,P}$ = 3.4 Hz), 118.8, 118.5, 117.7, 103.6, 89.9, 82.5, 76.5 (d, $J_{C,P}$ = 146.1 Hz), 74.5, 70.4, 41.9. ^{31}P NMR (162 MHz, $CDCl_3$) δ 12.46. HRMS (ESI) calcd for $C_{23}H_{24}N_3O_{11}PNa$ $[M + Na]^+$, 572.1046; Found 572.1063. IR (FTIR) ν_{max} 3270, 1664, 1484, 1251, 1071, 968, 749, 690. **28a-*l***: RP-HPLC, 0−40 min linear gradient 13−18% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 16.90 min; mp decomp. > 400.0ºC. $[\alpha]_D^{25}$ +5.6 (*c* = 0.2, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.47−7.56 (m, 4H), 7.31−7.39 (m, 2H), 7.26 (s, 1H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.03 (d, *J* = 8.0 Hz, 1H), 5.93 (d, *J* = 4.2 Hz, 1H), 5.65 (d, $J_{H,P}$ = 13.7 Hz, 1H), 5.57 (d, *J* = 8.0 Hz, 1H), 4.21−4.28 (m, 3H), 3.80 (dd, *J* = 15.6 Hz, *J* = 2.8 Hz, 1H), 3.48 (dd, *J* = 15.6 Hz, *J* = 4.8 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 168.2, 159.6 (d, $J_{C,P}$ = 12.0 Hz), 157.6, 157.5, 153.5, 142.6, 141.7, 131.0, 130.5, 124.8, 123.4 (d, $J_{C,P}$ = 3.0 Hz), 119.8, 118.1, 117.7 (d, $J_{C,P}$ = 3.0 Hz), 103.3, 89.9, 83.4, 76.7 (d, $J_{C,P}$ = 145.0 Hz), 74.5, 70.8, 42.4. ^{31}P NMR (162 MHz, $CDCl_3$) δ 12.51. HRMS (ESI) calcd for $C_{23}H_{24}N_3O_{11}PNa$ $[M + Na]^+$, 572.1046; Found 572.1071. IR (FTIR) ν_{max} 3286, 1690, 1486, 1252, 1071, 968, 692.

*Disodium [(4-fluoro-3-phenoxy)phenylphosphonatomethyl]-uridin-5'-yl-carbamate (**28b**)*. From **27b** (371 mg, 496 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28b-(*s*)** (146 mg, 96%) and **28b-(*l*)** (148 mg, 97%) as white powders. **28b-(*s*)**: RP-HPLC, 0−40 min linear gradient 13−18% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 17.40 min; mp decomp. > 240.0ºC. $[\alpha]_D^{25}$ −10.3 (*c* = 0.4, H_2O). ^{1}H NMR

(400 MHz, D_2O) δ 7.65 (d, *J* = 8.0 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 2H), 7.26−7.39 (m, 4H), 7.03 (d, *J* = 8.0 Hz, 2H), 5.92–5.94 (m, 2H), 5.57 (d, $J_{H,P}$ = 13.6 Hz, 1H), 4.17−4.21 (m, 1H), 3.95−3.97 (m, 1H), 3.87–3.90 (m, 1H), 3.75 (dd, *J* = 15.1 Hz, *J* = 5.5 Hz, 1H), 3.55 (dd, *J* = 15.1 Hz, *J* = 3.1 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 169.8, 159.7 (d, $J_{C,P}$ = 12.6 Hz), 158.1, 154.3, 154.0 (d, $J_{C,F}$ = 244.0 Hz), 142.5 (d, $J_{C,F}$ = 13.0 Hz), 141.1, 137.9, 131.0, 125.6, 124.1, 120.8, 117.4 (d, $J_{C,F}$ = 18.7 Hz), 116.9, 103.3, 89.8, 82.9, 76.2 (d, $J_{C,P}$ = 146.4 Hz), 74.7, 70.5, 42.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 12.34 (d, $J_{P,F}$ = 3.5 Hz). ^{19}F NMR (235.3 MHz, $CDCl_3$) δ −70.70. HRMS (ESI) calcd for $C_{23}H_{23}N_3O_{11}PFNa$ $[M + Na]^+$, 590.0952; Found 590.0977. IR (FTIR) ν_{max} 3244, 1689, 1633, 1590, 1460, 1270, 1207, 1072, 970. **28b-*l***: RP-HPLC, 0−40 min linear gradient 13−18% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 25.10 min; mp decomp. > 240.0ºC. $[\alpha]_D^{25}$ +23.7 (*c* = 0.3, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.59 (d, *J* = 7.9 Hz, 1H), 7.43–7.54 (m, 5H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 8.0 Hz, 2H), 5.99 (d, *J* = 4.1 Hz, 1H), 5.73–5.76 (m, 2H), 4.35 (br s, 1H), 4.29 (br s, 2H), 3.84 (d, *J* = 12.0 Hz, 1H), 3.56 (d, *J* = 14.3 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 169.1, 158.9 (d, $J_{C,P}$ = 11.4 Hz), 157.4, 154.0, 153.5 (d, $J_{C,F}$ = 245.0 Hz), 142.9 (d, $J_{C,F}$ = 11.0 Hz), 141.3, 137.1, 130.6, 124.7, 124.2, 120.7, 117.5, 117.2 (d, $J_{C,F}$ = 18.2 Hz), 103.0, 89.9, 82.6, 75.6 (d, $J_{C,P}$ = 146.7 Hz), 74.1, 70.4, 42.0. ^{31}P NMR (162 MHz, $CDCl_3$): δ 12.68 (d, $J_{P,F}$ = 2.1 Hz). ^{19}F NMR (235.3 MHz, $CDCl_3$) δ −70.96. HRMS (ESI) calcd for $C_{23}H_{23}N_3O_{11}PFNa$ $[M + Na]^+$, 590.0952; Found 590.0959. IR (FTIR) ν_{max} 3205, 1680, 1634, 1508, 1490, 1270, 1208, 1070, 967.

*Disodium [(4-fluoro)phenylphosphonatomethyl]-uridin-5'-yl-carbamate (**28c**).* From **27c** (100 mg, 152 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28c-*s*** (31.4 mg, 80%) and **28c-*l*** (33.0 mg, 83%) as white powders. **28c-*s***: RP-HPLC, 0−50 min linear gradient 5−10% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 19.60 min; mp decomp. > 280.0ºC. $[\alpha]_D^{25}$ +2.2 (*c* = 0.1, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 7.50 (d, *J* = 7.8 Hz, 1H), 7.42–7.44 (m, 2H), 7.06–7.09 (m, 2H), 5.97 (d, *J* = 5.7 Hz, 1H), 5.74 (d, *J* = 7.8 Hz, 1H), 5.47 (d, $J_{H,P}$ = 13.2 Hz, 1H), 4.24–4.26 (m, 1H), 4.13–4.16 (m, 1H), 4.03–4.05 (m, 1H),

3.57 (dd, J = 14.7 Hz, J = 7.4 Hz, 1H), 3.45 (dd, J = 14.7 Hz, J = 3.7 Hz, 1H). ^{13}C NMR (125 MHz, D_2O/MeOH-d_4) δ 174.4, 162.4 (d, $J_{C,F}$ = 243.2 Hz), 159.7 (d, $J_{C,P}$ = 12.5 Hz), 158.1, 141.2, 136.0, 129.2–129.3 (m), 115.4 (d, $J_{C,F}$ = 21.0 Hz), 103.7, 89.4, 83.3, 76.7 (d, $J_{C,P}$ = 166.3 Hz), 74.1, 71.3, 43.0. ^{31}P NMR (162 MHz, D_2O) δ 12.56. ^{19}F NMR (235.3 MHz, D_2O) δ −53.7 (d, $J_{F,P}$ = 3.4 Hz). HRMS (ESI) calcd for $C_{17}H_{18}N_3O_{10}PF$ $[M - H]^-$, 474.0714; Found 474.0720. IR (FTIR) ν_{max} 3268, 1690, 1510, 1269, 1207, 1075, 970. **28c-*l***: RP-HPLC, 0−50 min linear gradient 5−10% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 24.80 min; mp decomp. > 280.0ºC. $[\alpha]_D^{25}$ −1.9 (c = 0.2, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.53–7.56 (m, 2H), 7.47 (d, J = 7.8 Hz, 1H), 7.21–7.25 (m, 2H), 5.98 (d, J = 4.8 Hz, 1H), 5.65 (d, J = 7.8 Hz, 1H), 5.60 (d, $J_{H,P}$ = 13.3 Hz, 1H), 4.27–4.32 (m, 2H), 4.20 (br s, 1H), 3.57 (dd, J = 14.8 Hz, J = 2.6 Hz, 1H), 3.50 (dd, J = 14.8 Hz, J = 5.1 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 172.5, 162.4 (d, $J_{C,F}$ = 241.0 Hz), 159.4 (d, $J_{C,P}$ = 14.9 Hz), 156.7, 141.3, 135.9, 129.2 (d, $J_{C,F}$ = 6.5 Hz), 115.6 (d, $J_{C,F}$ = 21.0 Hz), 103.5, 89.6, 83.3, 76.3 (d, $J_{C,P}$ = 148.0 Hz), 74.1, 71.0, 42.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ 12.77 (d, $J_{P,F}$ = 1.6 Hz). ^{19}F NMR (235.3 MHz, D_2O) δ −53.55 (d, $J_{F,P}$ = 2.8 Hz). HRMS (ESI) calcd for $C_{17}H_{19}N_3O_{10}PFNa$ $[M + Na]^+$, 498.3119; Found 498.3125. IR (FTIR) ν_{max} 3382, 1687, 1264, 1079, 963.

*Disodium [(3-fluoro)phenylphosphonatomethyl]-uridin-5'-yl-carbamate (**28d**)*. From **27d** (102 mg, 154 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28d-*s*** (28.8 mg, 72%) and **28d-*l*** (28.0 mg, 70%) as white powders. **28d-*s***: RP-HPLC, 0−50 min linear gradient 5−10% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 18.10 min; mp decomp. > 260.0ºC. $[\alpha]_D^{25}$ +1.5 (c = 0.1, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 7.48–7.52 (m, 1H), 7.35–7.42 (m, 1H), 7.27 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 12.3 Hz, 1H), 7.00–7.07 (m, 1H), 6.03 (d, J = 5.9 Hz, 1H), 5.71 (d, J = 8.1 Hz, 1H), 5.60 (d, $J_{H,P}$ = 13.7 Hz, 1H), 4.28–4.31 (m, 1H), 4.17–4.21 (m, 1H), 4.10–4.12 (m, 1H), 3.47–3.64 (m, 2H). ^{13}C NMR (125 MHz, D_2O/MeOH-d_4) δ 172.0, 163.1 (d, $J_{C,F}$ = 243.8 Hz), 159.5–159.7 (m), 156.4, 142.6–142.8 (m), 130.3–130.4 (m), 123.3, 113.9–114.7 (m), 103.5, 92.8, 81.0, 76.6 (d, $J_{C,P}$ = 140.0 Hz), 74.1, 71.3, 43.4. ^{31}P NMR (162 MHz, D_2O) δ

12.30. ^{19}F NMR (235.3 MHz, D_2O): δ −51.79. HRMS (ESI) calcd for $C_{17}H_{19}N_3O_{10}PF$ $[M + Na]^+$, 498.3119; Found 498.3115. IR (FTIR) ν_{max} 3346, 1679, 1631, 1265, 1080, 971. **28d-*l***: RP-HPLC, 0−50 min linear gradient 5−10% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 25.80 min; mp decomp. > 280.0ºC. $[\alpha]_D^{25}$ −1.6 (*c* = 0.2, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.42–7.47 (m, 2H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.24 (d, *J* = 10.4 Hz, 1H), 7.09–7.13 (m, 1H), 5.94 (d, *J* = 5.1 Hz, 1H), 5.62 (d, *J* = 7.9 Hz, 1H), 5.55 (d, $J_{H,P}$ = 13.6 Hz, 1H), 4.23–4.29 (m, 2H), 4.15–4.18 (m, 1H), 3.66 (dd, *J* = 15.6 Hz, *J* = 4.0 Hz, 1H), 3.47 (dd, *J* = 14.8 Hz, *J* = 5.4 Hz, 1H). ^{13}C NMR (125 MHz, D_2O/MeOH-d_4) δ 172.0, 163.3 (d, $J_{C,F}$ = 237.5 Hz), 159.3 (d, $J_{C,P}$ = 11.3 Hz), 156.2, 142.7, 141.3, 130.5–130.6 (m), 123.3, 114.5 (d, $J_{C,F}$ = 29.0 Hz), 114.0 (d, $J_{C,F}$ = 13.0 Hz), 103.4, 89.4, 83.3, 76.3 (d, $J_{C,P}$ = 148.8 Hz), 74.2, 71.0, 42.5. ^{31}P NMR (162 MHz, D_2O) δ 12.53 (d, $J_{P,F}$ = 13.9 Hz). ^{19}F NMR (235.3 MHz, D_2O) δ −51.43 (d, $J_{F,P}$ = 9.1 Hz). HRMS (ESI) calcd for $C_{17}H_{18}N_3O_{10}PF$ $[M - H]^-$, 474.0714; Found 474.0710. IR (FTIR) ν_{max} 3359, 1635, 1262, 1077, 970.

*Disodium [(3-propoxy)phenylphosphonatomethyl]-uridin-5'-yl-carbamate (**28e**)*. From **27e** (150 mg, 216 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28e-*s*** (48.2 mg, 83%) and **28e-*l*** (54.0 mg, 93%) as white powders. **28e-*s***: RP-HPLC, 0−50 min linear gradient 9−14% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 20.20 min; mp decomp. > 230.0ºC. $[\alpha]_D^{25}$ +4.8 (*c* = 0.2, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.59 (d, *J* = 7.8 Hz, 1H), 7.42 (t, *J* = 7.9 Hz, 1H), 7.18 (d, *J* = 7.1 Hz, 1H), 7.11 (s, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 6.10 (d, *J* = 5.7 Hz, 1H), 5.73 (d, *J* = 7.8 Hz, 1H), 5.59 (d, $J_{H,P}$ = 13.8 Hz, 1H), 4.30–4.31 (m, 2H), 4.10–4.18 (m, 3H), 3.72 (dd, *J* = 13.8 Hz, *J* = 6.9 Hz, 1H), 3.56 (d, *J* = 14.4 Hz, 1H), 1.89 (sxt, *J* = 6.8 Hz, 2H), 1.13 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 171.4, 159.4 (d, $J_{C,P}$ = 12.7 Hz), 158.6, 155.8, 141.8, 141.1, 129.7, 120.6 (d, $J_{C,P}$ = 3.8 Hz), 113.6, 113.2, 103.3, 89.0, 83.2, 76.7 (d, $J_{C,P}$ = 146.3 Hz), 74.3, 71.1, 70.8, 42.9, 22.6, 10.5. ^{31}P NMR (162 MHz, D_2O) δ 12.88. HRMS (ESI) calcd for $C_{20}H_{25}N_3O_{11}P$ $[M - H]^-$, 514.1227; Found 514.1225. IR (FTIR) ν_{max} 3365, 1611, 1264, 1019, 690. **28e-*l***: RP-HPLC, 0−50 min linear gradient 9−14% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow;

t_R = 25.90 min; mp decomp. > 230.0ºC. $[\alpha]_D^{25}$ −2.8 (*c* = 0.3, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.59 (t, *J* = 7.9 Hz, 1H), 7.48 (d, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.24 (s, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 6.09 (d, *J* = 5.4 Hz, 1H), 5.76 (d, $J_{H,P}$ = 13.8 Hz, 1H), 5.49 (d, *J* = 7.9 Hz, 1H), 4.41–4.44 (m, 1H), 4.34–4.37 (m, 2H), 4.24 (t, *J* = 6.6 Hz, 1H), 3.91 (dd, *J* = 15.2 Hz, *J* = 2.5 Hz, 1H), 3.62 (dd, *J* = 15.2 Hz, *J* = 4.4 Hz, 1H), 1.97 (sxt, *J* = 7.0 Hz, 2H), 1.19 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 169.0, 159.2 (d, $J_{C,P}$ = 11.2 Hz), 158.5, 154.1, 141.6, 140.9, 130.0, 120.5 (d, $J_{C,P}$ = 3.6 Hz), 113.8, 113.6, 103.0, 88.8, 83.5, 76.3 (d, $J_{C,P}$ = 146.8 Hz), 74.2, 70.9, 70.6, 42.2, 22.4, 10.5. ^{31}P NMR (162 MHz, D_2O) δ 13.05. HRMS (ESI) calcd for $C_{20}H_{25}N_3O_{11}P$ $[M-H]^-$, 514.1227; Found 514.1241. IR (FTIR) ν_{max} 3373, 1663, 1264, 1066, 696.

*Disodium [(3-methoxy)phenylphosphonatomethyl]-uridin-5'-yl-carbamate (**28f**).* From **27f** (107 mg, 160 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28f-*s*** (40 mg, 94%) and **28f-*l*** (35 mg, 82%) as white powders. **28f-*s***: RP-HPLC, 0−50 min linear gradient 4−9% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 24.90 min; mp decomp. > 260.0ºC. $[\alpha]_D^{25}$ +3.9 (*c* = 0.1, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.53 (d, *J* = 7.7 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.14 (d, *J* = 7.6 Hz, 1H), 7.08 (s, 1H), 6.94 (d, *J* = 7.6 Hz, 1H), 6.07 (d, *J* = 5.9 Hz, 1H), 5.71 (d, *J* = 7.7 Hz, 1H), 5.55 (d, $J_{H,P}$ = 13.6 Hz, 1H), 4.25–4.31 (m, 2H), 4.12–4.15 (m, 1H), 3.92 (s, 3H), 3.68 (dd, *J* = 14.7 Hz, *J* = 8.1 Hz, 1H), 3.53 (dd, *J* = 14.3 Hz, *J* = 3.1 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 174.1, 159.5 (d, $J_{C,P}$ = 12.1 Hz), 159.1, 157.8, 141.8, 140.9, 129.7, 120.5, 112.8–113.1 (m), 103.5, 89.1, 83.1, 76.9 (d, $J_{C,P}$ = 146.1 Hz), 74.2, 71.2, 56.0, 43.0. ^{31}P NMR (162 MHz, D_2O) δ 12.76. HRMS (ESI) calcd for $C_{18}H_{21}N_3O_{11}P$ $[M-H]^-$, 486.0919; Found 486.0921. IR (FTIR) ν_{max} 3299, 2928, 1687, 1246, 1010, 739, 695. **28f-*l***: RP-HPLC, 0−50 min linear gradient 4−9% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 31.90 min; mp decomp. > 260.0ºC. $[\alpha]_D^{25}$ −0.8 (*c* = 0.2, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.46 (t, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 7.7 Hz, 1H), 7.19 (d, *J* = 7.6 Hz, 1H), 7.15 (s, 1H), 7.03 (d, *J* = 7.7 Hz, 1H), 6.00 (d, *J* = 5.2 Hz, 1H), 5.61 (d, $J_{H,P}$ = 13.7 Hz, 1H), 5.54 (d, *J* = 7.7 Hz, 1H), 4.21–4.31 (m, 3H), 3.95 (s, 3H), 3.75 (dd, *J* = 15.2 Hz, *J* = 2.8 Hz,

1H), 3.51 (dd, J = 15.0 Hz, J = 4.8 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) 175.4, 159.3–159.5 (m), 158.8, 141.7, 140.7, 130.1, 120.5, 113.2, 103.5, 89.2, 83.4, 76.7 (d, $J_{C,P}$ = 145.0 Hz), 74.2, 71.0, 56.2, 42.6. ^{31}P NMR (162 MHz, D_2O) δ 12.73. HRMS (ESI) calcd for $C_{18}H_{21}N_3O_{11}P$ $[M - H]^-$, 486.0919; Found 486.0922. IR (FTIR) ν_{max} 3342, 2929, 1687, 1260, 1010, 738, 696.

*Disodium [(4-methoxy)phenylphosphonatomethyl]-uridin-5′-yl-carbamate (**28g**)*. From **27g** (298 mg, 446 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28g-*s*** (100 mg, 85%) and **28g-*l*** (104 mg, 88%) as white powders. **28g-*s***: RP-HPLC, 0−40 min linear gradient 3−8% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 20.10 min; mp decomp. > 215.0ºC. $[\alpha]_D^{25}$ −63.6 (c = 0.1, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.66 (d, J = 7.8 Hz, 1H), 7.57 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 6.12 (d, J = 5.2 Hz, 1H), 5.90 (d, J = 7.8 Hz, 1H), 5.66 (d, $J_{H,P}$ = 13.1 Hz, 1H), 4.35–4.44 (m, 2H), 4.16–4.18 (m, 1H), 4.02 (s, 3H), 3.76 (dd, J = 15.0 Hz, J = 7.5 Hz, 1H), 3.63 (d, J = 15.0 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 172.1, 159.3 (d, $J_{C,P}$ = 12.6 Hz), 158.3, 156.4, 141.0, 132.2, 128.8 (d, $J_{C,P}$ = 3.2 Hz), 113.9, 103.3, 89.1, 83.0, 76.3 (d, $J_{C,P}$ = 159.9 Hz), 74.0, 70.9, 56.1, 42.6. ^{31}P NMR (162 MHz, D_2O) δ 13.57. HRMS (ESI) calcd for $C_{18}H_{22}N_3O_{11}PNa$ $[M + Na]^+$, 510.0890; Found 510.0900. IR (FTIR) ν_{max} 3278, 1682, 1635, 1511, 1458, 1247, 1071, 972. **28g-*l***: RP-HPLC, 0−40 min linear gradient 3−8% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 24.60 min. mp decomp. > 215.0ºC. $[\alpha]_D^{25}$ −73.4 (c 0.1, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.64 (d, J = 7.9 Hz, 2H), 7.49 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 8.6 Hz, 2H), 6.06 (d, J = 4.4 Hz, 1H), 5.74 (d, $J_{H,P}$ = 13.2 Hz, 1H), 5.57 (d, J = 8.0 Hz, 1H), 4.35 (br s, 3H), 4.05 (s, 3H), 3.89 (d, J = 14.8 Hz, 1H), 3.61 (dd, J = 14.8 Hz, J = 3.2 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 166.9, 159.2 (d, $J_{C,P}$ = 11.7 Hz), 158.5, 152.5, 141.3, 132.3, 128.9 (d, $J_{C,P}$ = 2.6 Hz), 114.3, 103.0, 88.9, 83.5, 76.0 (d, $J_{C,P}$ = 149.1 Hz), 74.1, 70.5, 56.3, 42.1. ^{31}P NMR (162 MHz, D_2O) δ 13.58. HRMS (ESI) calcd for $C_{18}H_{21}N_3O_{11}P$ $[M - H]^-$, 486.0919; Found 486.0930. IR (FTIR) ν_{max} 3225, 1690, 1512, 1461, 1273, 1247, 1073, 974.

Disodium [(3-cyclopentoxy-4-methoxy)phenylphosphonatomethyl]-uridin-5'-yl-carbamate ***(28h)***. From **27h** (109 mg, 145 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28h-*s*** (41.6 mg, 93%) and **28h-*l*** (36.2 mg, 81%) as white powders. **28h-*s***: RP-HPLC, 0−50 min linear gradient 10−16% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 20.00 min; mp decomp. > 230.0ºC. $[\alpha]_D^{25}$ +2.2 (*c* = 0.1, H_2O). ^1H NMR (400 MHz, D_2O) δ 7.58 (d, *J* = 7.8 Hz, 1H), 7.18 (s, 1H), 7.05–7.12 (m, 2H), 6.01 (d, *J* = 4.9 Hz, 1H), 5.71 (d, *J* = 7.8 Hz, 1H), 5.51 (d, $J_{H,P}$ = 13.2 Hz, 1H), 4.98 (br s, 1H), 4.22–4.28 (m, 2H), 4.10–4.12 (m, 1H), 3.93 (s, 3H), 3.67 (dd, *J* = 14.8 Hz, *J* = 8.4 Hz, 1H), 3.52 (dd, *J* = 14.8 Hz, *J* = 3.2 Hz, 1H), 1.70–1.93 (m, 8H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 171.7, 159.5, 156.0–156.1 (m), 148.5, 146.6, 141.2, 133.0, 120.9 (d, $J_{C,P}$ = 3.7 Hz), 114.3, 112.2, 103.2, 89.3, 83.1, 81.9, 76.7 (d, $J_{C,P}$ = 149.7 Hz), 74.6, 71.3, 56.5, 43.0, 33.0, 32.9, 24.32, 24.27. ^{31}P NMR (162 MHz, D_2O) δ 13.13. HRMS (ESI) calcd for $C_{23}H_{29}N_3O_{12}P$ $[M - H]^-$, 570.1489; Found 570.1500. IR (FTIR) ν_{max} 3279, 2928, 1690, 1631, 1512, 1261, 1075, 973. **28h-*l***: RP-HPLC, 0−50 min linear gradient 10−16% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 24.00 min; mp decomp. > 240.0ºC. $[\alpha]_D^{25}$ +0.5 (*c* = 0.2, H_2O). ^1H NMR (400 MHz, D_2O) δ 7.41 (d, *J* = 8.0 Hz, 1H), 7.22 (s, 1H), 7.15 (s, 2H), 5.93 (d, *J* = 4.1 Hz, 1H), 5.59 (d, $J_{H,P}$ = 13.1 Hz, 1H), 5.34 (d, *J* = 7.7 Hz, 1H), 5.01 (br s, 1H), 4.21–4.28 (m, 3H), 3.96 (s, 3H), 3.80 (dd, *J* = 15.0 Hz, *J* = 2.4 Hz, 1H), 3.48 (dd, *J* = 15.0 Hz, *J* = 4.4 Hz, 1H), 1.70–2.11 (m, 8H). ^{13}C NMR (125 MHz, D_2O/MeOH-d_4) δ 166.8, 158.9–159.1 (m), 149.1, 146.8, 141.3, 131.6, 120.8, 114.4, 112.6, 102.9, 89.5, 83.6, 81.8, 75.3 (d, $J_{C,P}$ = 153.8 Hz), 74.5, 70.6, 56.6, 42.1, 32.9, 32.8, 24.2. ^{31}P NMR (162 MHz, D_2O) δ 13.02. HRMS (ESI) calcd for $C_{23}H_{29}N_3O_{12}P$ $[M - H]^-$, 570.1489; Found 570.1465. IR (FTIR) ν_{max} 3365, 2929, 1690, 1512, 1261, 1075.

Disodium [(3-cyclopentoxy)phenylphosphonatomethyl]-uridin-5'-yl-carbamate ***(28i)***. From **27i** (118 mg, 164 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28i-*s*** (41.8 mg, 87%) and **28i-*l*** (40.3 mg, 84%) as white powders. **28i-*s***: RP-HPLC, 0−50 min linear gradient 13−18% CH_3CN, 0.05 M TEAB buffer, 15

mL/min flow; t_R = 18.30 min; mp decomp. > 250.0ºC. $[\alpha]_D^{25}$ +2.7 (*c* = 0.2, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.64 (d, *J* = 8.0 Hz, 1H), 7.41–7.44 (m, 1H), 7.18 (d, *J* = 7.2 Hz, 1H), 7.09 (s, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 6.09 (br s, 1H), 5.75 (d, *J* = 6.7 Hz, 1H), 5.59 (d, $J_{H,P}$ = 13.8 Hz, 1H), 5.01 (br s, 1H), 4.34 (br s, 2H), 4.19 (br s, 1H), 3.72–3.77 (m, 1H), 3.57–3.60 (m, 1H), 2.12 (br s, 2H), 1.80–1.87 (m, 6H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 171.0, 161.9 (d, $J_{C,P}$ = 10.7 Hz), 157.5, 141.9, 141.4, 129.7, 120.7, 114.8, 114.3, 103.3, 89.0, 83.3, 81.2, 76.8 (d, $J_{C,P}$ = 141.0 Hz), 74.3, 71.1, 42.9, 33.0, 32.9, 24.24, 24.21. ^{31}P NMR (162 MHz, D_2O) δ 12.80. HRMS (ESI) calcd for $C_{22}H_{27}N_3O_{11}P$ $[M - H]^-$, 540.1383; Found 540.1407. IR (FTIR) ν_{max} 3137, 2964, 1604, 1357, 1262, 1080, 832, 795. **28i-*l***: RP-HPLC, 0−50 min linear gradient 13−18% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 21.60 min; mp decomp. > 250.0ºC. $[\alpha]_D^{25}$ −3.4 (*c* = 0.1, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 7.45 (t, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 7.09 (s, 1H), 7.00 (d, *J* = 8.0 Hz, 1H), 5.95–5.96 (m, 1H), 5.63 (d, $J_{H,P}$ = 13.3 Hz, 1H), 5.32 (d, *J* = 7.7 Hz, 1H), 5.00 (br s, 1H), 4.25–4.31 (m, 3H), 3.81 (d, *J* = 15.0 Hz, 1H), 3.50 (d, *J* = 15.0 Hz, 1H), 2.06 (br s, 2H), 1.76–1.83 (m, 6H). ^{13}C NMR (125 MHz, D_2O/MeOH-d_4) δ 174.6, 159.5 (d, $J_{C,P}$ = 12.5 Hz), 157.7, 141.9, 141.2, 130.1, 120.7, 115.0, 114.5, 103.0, 89.1, 83.7, 81.3, 76.5 (d, $J_{C,P}$ = 152.5 Hz), 74.6, 70.8, 42.3, 33.0, 32.9, 24.2. ^{31}P NMR (162 MHz, D_2O) δ 12.68. HRMS (ESI) calcd for $C_{22}H_{27}N_3O_{11}P$ $[M - H]^-$, 540.1383; Found 540.1389. IR (FTIR) ν_{max} 3237, 2960, 1687, 1260, 1010, 967.

*Disodium [phenylphosphonatomethyl]-uridin-5'-yl-carbamate (**28j**)*. From **27j** (147 mg, 231 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28j-*s*** (52 mg, 90%) and **28j-*l*** (47 mg, 81%) as white powders. **28j-*s*:** RP-HPLC, 0−50 min linear gradient 3−8% CH_3CN, 0.05 M TEAB buffer, 19 mL/min flow; t_R = 22.20 min; mp decomp. > 320.0ºC. $[\alpha]_D^{25}$ +20.1 (*c* = 0.2, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 7.71 (d, *J* = 7.5 Hz, 1H), 7.46−7.57 (m, 5H), 6.10 (d, *J* = 3.9 Hz, 1H), 5.89 (d, *J* = 7.7 Hz, 1H), 5.66 (d, $J_{H,P}$ = 13.4 Hz, 1H), 4.45 (br s, 1H), 4.33 (br s, 1H), 4.23 (br s, 1H), 3.70−3.74 (m, 1H), 3.53 (d, *J* = 13.8 Hz, 1H). ^{13}C NMR (125 MHz, D_2O/MeOH-d_4) δ 166.6, 159.3 (d, $J_{C,P}$ = 13.1 Hz), 152.3, 142.0, 139.3, 128.6,

127.9, 127.2, 103.2, 88.8, 83.5, 76.6 (d, $J_{C,P}$ = 148.8 Hz), 73.7, 71.0, 42.8. ^{31}P NMR (162 MHz, D_2O) δ 13.34. HRMS (ESI) calcd for $C_{17}H_{20}N_3O_{10}PNa$ $[M + Na]^+$, 480.0784; Found 480.0800. IR (FTIR) ν_{max} 3373, 1636, 1264, 1078, 970. **28j-*l*:** RP-HPLC, 0−50 min linear gradient 3−8% CH_3CN, 0.05 M TEAB buffer, 19 mL/min flow; t_R = 26.40 min; mp decomp. > 310.0ºC. $[\alpha]_D^{25}$ +1.5 (*c* 0.2, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 7.52−7.59 (m, 5H), 7.47 (d, *J* = 7.0 Hz, 1H), 5.98 (d, *J* = 5.0 Hz, 1H), 5.64–5.66 (m, 2H), 4.32−4.37 (m, 2H), 4.24−4.26 (m, 1H), 3.76 (dd, *J* = 15.2 Hz, *J* = 3.1 Hz, 1H), 3.53 (dd, *J* = 15.2 Hz, *J* = 5.3 Hz, 1H). ^{13}C NMR (125 MHz, D_2O/MeOH-d_4) δ 167.0, 159.5 (d, $J_{C,P}$ = 11.3 Hz), 152.6, 141.9, 139.9, 129.0, 128.0, 127.5, 103.3, 89.4, 83.6, 76.8 (d, $J_{C,P}$ = 147.5 Hz), 74.0, 70.9, 42.4. ^{31}P NMR (162 MHz, D_2O) δ 13.03. HRMS (ESI) calcd for $C_{17}H_{19}N_3O_{10}P$ $[M - H]^-$, 456.0808; Found 456.0809. IR (FTIR) ν_{max} 3397, 1681, 1266, 1077, 962.

*Disodium [(benzo[b]thiophen-2-yl)phosphonatomethyl]-uridin-5'-yl-carbamate (**28k**).* From **27k** (342 mg, 493 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28k-*s*** (118 mg, 86%) and **28k-*l*** (121 mg, 88%) as white powders. **28k-*s***: RP-HPLC, 0−50 min linear gradient 8−13% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 19.20 min; mp decomp. > 200ºC. $[\alpha]_D^{25}$ −10.0 (*c* = 0.1, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 7.84 (d, *J* = 7.5 Hz, 1H), 7.74 (d, *J* = 7.5 Hz, 1H), 7.34–7.41 (m, 3H), 7.30 (d, *J* = 2.8 Hz, 1H), 5.88 (d, *J* = 5.8 Hz, 1H), 5.79 (d, $J_{H,P}$ = 14.0 Hz, 1H), 5.48 (d, *J* = 7.9 Hz, 1H), 4.17–4.21 (m, 1H), 4.09–4.11 (m, 1H), 4.00–4.02 (m, 1H), 3.65 (dd, *J* = 14.6 Hz, *J* = 9.1 Hz, 1H), 3.43 (dd, *J* = 14.6 Hz, *J* = 3.7 Hz, 1H). ^{13}C NMR (125 MHz, D_2O/MeOH-d_4) δ 167.6, 159.7 (d, $J_{C,P}$ = 12.0 Hz), 153.2, 143.9, 141.3, 140.1, 139.9, 125.2, 124.9, 123.7, 122.9, 122.2 (d, $J_{C,P}$ = 6.0 Hz), 102.7, 88.7, 83.6, 74.6, 73.6 (d, $J_{C,P}$ = 147.3 Hz), 71.3, 43.2. ^{31}P NMR (162 MHz, D_2O) δ 12.25. HRMS (ESI) calcd for $C_{19}H_{19}N_3O_{10}PS$ $[M - H]^-$, 512.0529; Found 512.0537. IR (FTIR) ν_{max} 3380, 2978, 2602, 2496, 1686, 1172, 1036, 907. **28k-*l***: RP-HPLC, 0−50 min linear gradient 8−13% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 26.90 min; mp decomp. > 200ºC. $[\alpha]_D^{25}$ +46.6 (*c* = 0.1, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 7.99 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.45–7.53 (m, 3H), 7.35 (d, *J* = 2.8 Hz, 1H), 5.99 (d, $J_{H,P}$ = 13.6 Hz,

1H), 5.90 (d, *J* = 4.6 Hz, 1H), 5.29 (d, *J* = 7.7 Hz, 1H), 4.33 (br s, 1H), 4.26–4.27 (m, 2H), 3.83 (d, *J* = 15.2 Hz, 1H), 3.52 (d, *J* = 15.2 Hz, 1H). ^{13}C NMR (125 MHz, D_2O/MeOH-d_4) δ 167.5, 159.3 (d, $J_{C,P}$ = 11.1 Hz), 153.1, 143.9, 140.9, 140.2, 139.7, 125.3, 124.9, 124.1, 123.1, 122.4, 102.6, 88.9, 83.8, 74.5, 73.2 (d, $J_{C,P}$ = 148.2 Hz), 70.7, 42.2. ^{31}P NMR (162 MHz, D_2O) δ 11.47. HRMS (ESI) calcd for $C_{19}H_{19}N_3O_{10}PS$ $[M - H]^-$, 512.0529; Found 512.0532. IR (FTIR) ν_{max} 3379, 2978, 2602, 2496, 1475, 1172, 1036, 907.

*Disodium [(benzo[b]thiophen-3-yl)phosphonatomethyl]-uridin-5'-yl-carbamate (**28l**)*. From **27l** (89.0 mg, 128 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **28l-*s*** (24.3mg, 68%) and **28l-*l*** (25.0 mg, 70%) as white powders. **28l-*s***: RP-HPLC, 0−60 min linear gradient 8−13% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 22.20 min; mp decomp. > 205ºC. $[\alpha]_D^{25}$ −3.6 (*c* = 0.1, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 8.10 (d, *J* = 8.1 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 7.63 (s, 1H), 7.46 (t, *J* = 7.4 Hz, 1H), 7.40 (t, *J* = 7.3 Hz, 1H), 7.31 (d, *J* = 7.9 Hz, 1H), 5.95 (d, $J_{H,P}$ = 13.5 Hz, 1H), 5.81 (d, *J* = 5.1 Hz, 1H), 5.22 (d, *J* = 8.0 Hz, 1H), 4.09–4.14 (m, 2H), 3.96–3.98 (m, 1H), 3.56–3.62 (m, 1H), 3.41 (d, *J* = 15.0 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 167.8, 159.9 (d, $J_{C,P}$ = 12.5 Hz), 153.2, 141.4, 140.6, 138.9, 135.0, 125.1, 124.7, 123.9, 123.5, 102.7, 89.1, 83.5, 74.5, 72.3 (d, $J_{C,P}$ = 152.0 Hz), 71.1, 42.8. ^{31}P NMR (162 MHz, D_2O) δ 12.56. HRMS (ESI) calcd for $C_{19}H_{19}N_3O_{10}PS$ $[M - H]^-$, 512.0529; Found 512.0547. IR (FTIR) ν_{max} 3362, 1634, 1073. **28l-*l***: RP-HPLC, 0−60 min linear gradient 8−13% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 26.50 min; mp decomp. > 205ºC. $[\alpha]_D^{25}$ +3.2 (*c* = 0.05, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 8.17 (d, *J* = 8.3 Hz, 1H), 7.97 (d, *J* = 7.9 Hz, 1H), 7.65 (s, 1H), 7.43–7.54 (m, 2H), 7.09 (d, *J* = 7.8 Hz, 1H), 6.01 (d, $J_{H,P}$ = 13.5 Hz, 1H), 5.82 (d, *J* = 5.4 Hz, 1H), 4.98 (d, *J* = 7.9 Hz, 1H), 4.17–4.19 (m, 1H), 4.10–4.12 (m, 1H), 4.02–4.05 (m, 1H), 3.64–3.71 (m, 1H), 3.37–3.42 (m, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 174.1, 159.7 (d, $J_{C,P}$ = 9.5 Hz), 154.8, 141.0, 139.0, 136.7, 135.1, 125.3, 124.9, 124.1, 123.8, 103.0, 89.2, 83.8, 74.4, 72.4 (d, $J_{C,P}$ = 153.1 Hz), 70.9, 42.3. ^{31}P NMR (162

MHz, D_2O) δ 12.54. HRMS (ESI) calcd for $C_{19}H_{19}N_3O_{10}PS$ $[M - H]^-$, 512.0529; Found 512.0534. IR (FTIR) ν_{max} 3363, 1660, 1078.

*Disodium cytidin-5'-yl-[(3-phenoxy)phenylphosphonatomethyl]-carbamate (**30**)*. From **29** (324 mg, 287 μmol): purification by RP-HPLC, converted to its sodium salt by ion exchange (IR 120 Na^+) and lyophilised from water to afford diastereomers **30-*s*** (70.0 mg, 89%) and **30-*l*** (72.4 mg, 92%) as white powders. **30-*s***: RP-HPLC, 0−60 min linear gradient 12−17% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 15.20 min; mp decomp. > 230ºC. $[\alpha]_D^{25}$ −10.3 (*c* = 0.1, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.63 (d, *J* = 7.5 Hz, 1H), 7.37−7.42 (m, 3H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.13–7.19 (m, 2H), 6.92–6.94 (m, 3H), 6.05 (d, *J* = 7.5 Hz, 1H), 5.85 (d, *J* = 3.1 Hz, 1H), 5.53 (d, $J_{H,P}$ = 13.9 Hz, 1H), 4.07−4.13 (m, 1H), 3.83−3.85 (m, 1H), 3.67–3.76 (m, 2H), 3.50 (dd, *J* = 15.2 Hz, *J* = 3.0 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 166.7, 159.8 (d, $J_{C,P}$ = 12.0 Hz), 158.3, 158.0, 156.5, 142.8, 141.0, 130.8, 130.3, 124.1 (d, $J_{C,P}$ = 4.0 Hz), 123.8, 119.0, 118.1, 117.9 (d, $J_{C,P}$ = 3.5 Hz), 97.2, 90.4, 82.5, 76.8 (d, $J_{C,P}$ = 145.8 Hz), 75.1, 70.2, 41.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ 12.18. HRMS (ESI) calcd for $C_{23}H_{24}N_4O_{10}P$ $[M - H]^-$, 547.1225; Found 547.1241. IR (FTIR) ν_{max} 3292, 1647, 1487, 1250, 1069, 968, 783, 692. **30-*l***: RP-HPLC, 0−50 min linear gradient 12−17% CH_3CN, 0.05 M TEAB buffer, 15 mL/min flow; t_R = 20.80 min; mp decomp. > 220ºC. $[\alpha]_D^{25}$ +6.8 (*c* = 0.2, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.46–7.53 (m, 4H), 7.37 (d, *J* = 7.5 Hz, 1H), 7.31 (t, *J* = 8.1 Hz, 1H), 7.24 (s, 1H), 7.11 (d, *J* = 8.1 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 1H), 5.91 (d, *J* = 4.0 Hz, 1H), 5.70 (d, *J* = 7.5 Hz, 1H), 5.64 (d, $J_{H,P}$ = 13.9 Hz, 1H), 4.19 (br s, 3H), 3.82 (d, *J* = 14.0 Hz, 1H), 3.49 (dd, *J* = 15.8 Hz, *J* = 4.0 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/MeOH-d_4) δ 166.6, 159.5 (d, $J_{C,P}$ = 12.0 Hz), 158.2, 157.43, 157.36, 142.5, 141.5, 130.8, 130.3, 124.6, 123.4 (d, $J_{C,P}$ = 3.0 Hz), 119.6, 117.9, 117.4, 96.9, 90.4, 82.9, 76.5 (d, $J_{C,P}$ = 144.0 Hz), 74.9, 70.6, 42.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 12.45. HRMS (ESI) calcd for $C_{23}H_{24}N_4O_{10}P$ $[M - H]^-$, 547.1225; Found 547.1240. IR (FTIR) ν_{max} 3332, 1647, 1487, 1249, 1067, 967, 783, 692.

Enzyme Inhibition Assay. The assay was performed based on the CMP-Glo™ assay developed by Promega and as detailed by Das et al.[48] For details of the procedures, see Supporting Information.

Cell Viability Assay. MIA PaCa-2 human pancreatic cancer cells (from American Type Culture Collection, ATCC, USA) were grown in DMEM cell culture medium with L-glutamine, supplemented with 10% FBS. Cell culture medium and supplements were obtained from Invitrogen (Australia). The cells were incubated at 37°C under a humidified atmosphere containing 5% CO_2 in a Heracell incubator (Kendro Laboratory Products Germany). Cell viability was determined using the CellTiter 96 Aqueous One Solution (MTS) Cell Proliferation colorimetric assay, as described previously.[72] Cells were plated in 96-well plates at a density of 60,000 cells/mL and allowed to attach overnight (24 h). Samples were added at a final concentration of 200 μM in media and the plates incubated at 37°C for 24–48 hrs. The MTS reagent [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] (50 μg) was added and the cells were incubated for another 3 h, and absorbance determined on a plate reader at 490 nm. IC_{50} values were calculated from logarithmic sigmoidal dose response curves generated using GraphPad Prism v6 software (GraphPad Inc.). The data were obtained from ≥2 independent experiments performed in triplicate as the mean ± the standard deviation (SD).

ASSOCIATED CONTENT

Supporting Information.
The following files are available free of charge.
HPLC purity of the target compounds, details of biological assays, and NMR spectral data (PDF). Molecular formula strings (SMILES).

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest

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ABBREVIATIONS

ST, sialyltransferase; Neu5Ac, *N*-acetylneuraminic acid; Gal, galactose; GalNAc, *N*-acetylgalactosamine; CMP, cytidine 5′-monophosphate; CMP-Neu5Ac, cytidine 5′-monophosphate *N*-acetylneuraminic acid; hST6Gal I, human β-galactoside α-2,6-sialyltransferase I; MD, molecular dynamics; FEP, free energy perturbation; RP-HPLC, reverse phase HPLC; Alloc, allyloxycarbonyl; Cbz, benzyloxycarbonyl; DIAD, diisopropyl azodicarboxylate; LacNAc, *N*-acetyllactosamine; PAINS, pan-assay

interference assay; MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt; MiaPaCa-2, human pancreatic cancer cell line; VMD, Visual Molecular Dynamics; NAMD, Nanoscale Molecular Dynamics; CHARMM, Chemistry at Harvard Macromolecular Mechanics; GAAMP, General Automated Atomic Model Parameterisation; Equiv., equivalents; 4-NPC, 4-nitrophenylchloroformate; Neu5Ac2en, 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid; TBDMS, *tert*-butyldimethylsilyl; TEAB, triethylammonium bicarbonate.

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