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Lipophosphonoxins: New Modular Molecular Structures with Significant Antibacterial Properties

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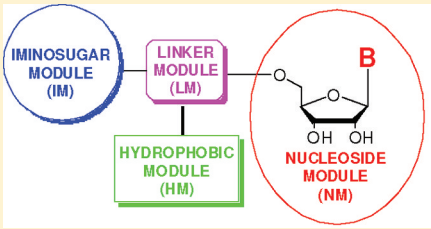
Supporting Information



	MIC μg/ml			
	E. faecalis CCM 4224	*S.aureus* CCM 4223	*B. subtilis*	*S.agalactiae*
12a	12.50	-	6.25	3.13
12b	6.25	25.00	3.13	3.13
12h	12.50	25.00-12.50	6.25	6.25
12i	6.25	12.50- 6.25	3.13	1.56

ABSTRACT: Novel compounds termed lipophosphonoxins were prepared using a simple and efficient synthetic approach. The general structure of lipophosphonoxins consists of four modules: (i) a nucleoside module, (ii) an iminosugar module, (iii) a hydrophobic module (lipophilic alkyl chain), and (iv) a phosphonate linker module that holds together modules i−iii. Lipophosphonoxins displayed significant antibacterial properties against a panel of Gram-positive species, including multiresistant strains. The minimum inhibitory concentration (MIC) values of the best inhibitors were in the 1−12 μg/mL range, while their cytotoxic concentrations against human cell lines were significantly above this range. The modular nature of this artificial scaffold offers a large number of possibilities for further modifications/exploitation of these compounds.

INTRODUCTION

The use of antibiotics has been generally beneficial for public health. However, very few new antibiotics have been marketed in the last 40 years.[1] Moreover, the advantages offered by antibiotics in the treatment of infectious diseases are endangered due to the increase in the number of antibiotic-resistant bacterial strains. This reduces the efficiency of antibiotic treatments and poses a serious health and economic problem. Currently, the need for novel antibiotics is becoming increasingly apparent.[2,3]

We previously synthesized an effective inhibitor of *Giardia* trophozoite growth termed phosphonoxin (**1**) (Figure 1) with an activity that rivaled existing therapeutics. Phosphonoxin was designed as a transition-state inhibitor of a glycosyl transferase, cyst wall synthase (CWS). CWS catalyzes synthesis of the chitin-like poly β-1−3-linked *N*-acetylgalactosamine [poly-(GalNAc)] that comprises about 63% of the giardia cyst wall utilizing UDP-GalNAc (**2**) as a substrate. Although phosphonoxin did not specifically inhibit cyst formation, it potently inhibited vegetative growth.[4]

Phosphonoxin bears structural similarities with several types of nucleoside antimicrobials: (i) polyoxins (**3**), (ii) muraymycins (**4**), and (iii) caprazamycins (**5**) (Figure 1).

The polyoxins of general formula 3 are a new group of antifungal antibiotics isolated from *Streptomyces cacaoi* that inhibit the growth of a number of mycelial fungi[5,6] by interfering with chitin synthesis.[7,8] Polyoxin D is a strong competitive inhibitor of chitin synthase in *Neurospora crassa*. Polyoxin D shares a gross structural similarity with uridine diphospho-*N*-acetyl-D-glucosamine (UDP-GlcNAc **2**), in agreement with its role as a competitive inhibitor.

The caprazamycins (CPZs) (**4**) were isolated from a culture broth of the actinomycete strain *Streptomyces* spp. MK730−

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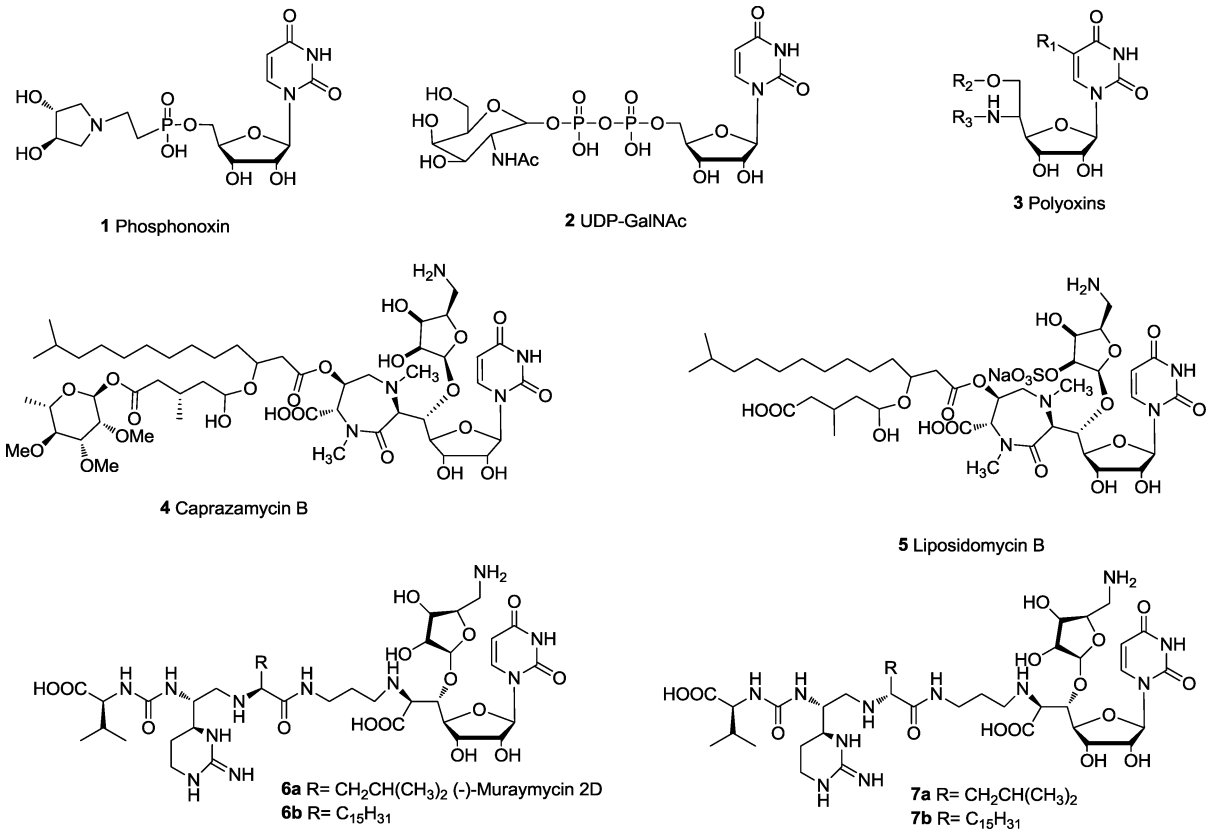

Figure 1. Structures of phosphonoxin, UDP-GlcNAc, and structurally related natural antimicrobials and their derivatives.

Table 1. Antibacterial Activity of Muraymycin Analogues

	MIC (μg/mL)					
compd	*S. aureus* ATCC 29213 (MSSA)	*S. aureus* SR3637 (MRSA)	*E. feacalis* ATCC 29212	*E. feacalis* SR7914 (VRE)	*E. faecium* ATCC 19434	*E. faecium* SR7917 (VRE)
6a	>64	>64	>64	>64	>64	>64
7a	>64	>64	>64	>64	>64	>64
6b	2	4	4	4	4	2
7b	2	4	2	4	0.5	0.25
vancomycin	1	1	1	>64	0.5	>64

62F2 in 2003[9,10] and represent the most recent members of the class of naturally occurring 6′-*N*-alkyl-5′-β-*O*-aminoribosyl-*C*-glycyluridine antibiotics that include the liposidomycins (**5**) (LPMs). The CPZs have shown excellent antimycobacterial activity *in vitro* not only against drug-susceptible (MIC = 3.13 μg/mL) but also multidrug-resistant *Mycobacterium tuberculosis* strains (MIC = 3.13 μg/mL) and exhibit no significant toxicity in mice. The biological target of the 6′-*N*-alkyl-5′-β-*O*-aminoribosyl-*C*-glycyluridine class of antibiotics is believed to be MraY translocase (IC_{50} = 0.05 μg/mL for LPMs).[11]

The muraymycins (**6a**), isolated from a culture broth of *Streptomyces* species,[12] are members of a class of naturally occurring 6′-*N*-alkyl-5′-β-*O*-aminorybosyl-*C*-glycyluridine antibiotics. Members of this family showed antibacterial activity against *Staphylococcus aureus* and *Enterococci* and were able to protect mice against *S. aureus* infection (ED_{50} = 1.1 mg/kg). The muraymycins inhibit the formation of lipid II and peptidoglycan and are believed to be inhibitors of phospho-MurNAc-pentapeptide translocase (MraY), which is responsible for the formation of lipid I in the peptidoglycan biosynthesis pathway.[13−17] Tanino recently described the synthesis of lipophilic muraymycin analogues **6b** and **7b** that differ in the stereochemistry at the carbon atom, where the lipophilic moiety is attached. These compounds exhibited significant activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) (Table 1).[18]

At the beginning of this study, we tested phosphonoxin for potential antibacterial properties. However, no significant activity was observed. We reasoned that the negative charge on the phosphonate moiety may hamper cellular uptake. Thus, to facilitate the cell entry, we introduced the lipophilic hexadecyloxypropyl ester group[19] to the structure and synthesized several variants based on this structure. The obtained structures were collectively termed lipophosphonoxins (LPPOs), and they showed promising activities against several Gram-positive bacterial species and low to moderate cytotoxicities. LPPOs represent an artificial modular structure that could be easily synthesized in few simple reaction steps.

Here we present synthesis, antibacterial activity, and cytotoxicity of several members of the lipophosphonoxin family.

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RESULTS AND DISCUSSION

General Structure of Lipophosphonoxins. LPPOs are modular molecules consisting of these parts: a nucleoside module (NM), a linker module (LM), an iminosugar module (IM), and a hydrophobic module (HM) (Figure 2).

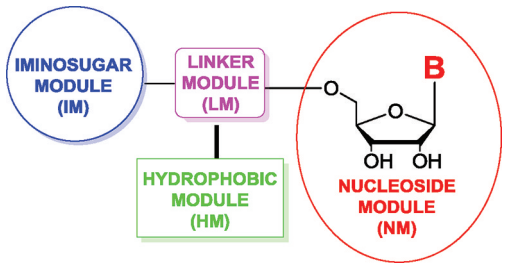
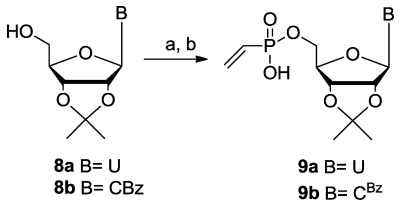


Figure 2. General structure of lipophosphonoxins.

The parent phosphonoxin (**1**) contained uridine as the NM. Cytidine was subsequently evaluated as an NM alternative. LPPOs containing hexadecyloxypropyl, ethyl, pivaloylthioethyl (SATE),[20] tetradecyl, hexadecyl, octadecyl, or eicosanyl ester groups as the HM were synthesized. Several hydroxylated pyrrolidines and piperidines were used as the IM. The LM was in all cases ethylphosphonic acid.

Chemistry. Vinylphosphonate synthons **9a–b** were prepared from nucleobase protected 2′,3′-isopropylidene nucleosides **8a–b** (Scheme 1). The phosphonate function was

Scheme 1[a]

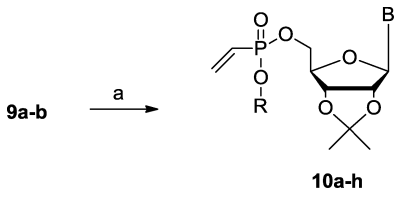


[a](a) CH_2=CHP(O)(OMe)OH, MeIm, TPSCl, DCM/MeCN; (b) 60% aq pyridine.

introduced by esterification of monomethyl vinylphosphonate[21,22] using triisopropylbenzensulfonylchloride (TPSCl) as a condensing agent (Scheme 1). Obtained methyl phosphonates were partially de-esterified by heating with 60% aqueous pyridine to remove the methyl ester group, affording nucleosidyl vinylphosphonates **9a–b**.

Next, esterification was performed with the appropriate alcohol using TPSCl as a condensing agent, yielding synthons **10a–h** (Scheme 2; Table 2).

Scheme 2[a]



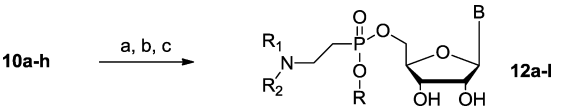
[a](a) ROH, MeIM, TPSCl, DCM/MeCN.

Table 2. Vinylphosphonates 10a–h

compd	B	R
10a	U	$PivSCH_2CH_2$
10b	U	CH_3CH_2
10c	U	$C_{14}H_{29}$
10d	U	$C_{16}H_{33}$
10e	U	$C_{18}H_{37}$
10f	U	$C_{20}H_{41}$
10g	U	$C_{16}H_{33}OCH_2CH_2CH_2$
10h	C^{Bz}	$C_{16}H_{33}OCH_2CH_2CH_2$

Finally, Michael addition of pyrrolidine derivatives **11a–d**[23,24] or piperidine derivatives **11e–f**[24,25] to vinylphosphonates **10a–d** was carried out, followed by deprotection, resulting in moderate to good yields of final lipophosphonoxins **12a–l** (Scheme 3, Tables 3 and 4). The deprotection consisted of the

Scheme 3[a]



[a](a) R_1R_2NH (**11a–f**), *n*BuOH, 90 °C; (b) $MeNH_2$/MeOH (B = C^{Bz}); (c) 0.2M HCl/MeOH.

removal of the nucleobase protecting group (in the case of cytosine derivative) with ethanolic methylamine and the subsequent cleavage of isopropylidene group with 0.2 M methanolic HCl. The final products were obtained by flash column chromatography on silica gel or using preparative reversed phase HPLC.

Antimicrobial Activity. The prepared compounds were tested against a panel of selected reference bacterial strains (Table 5). The minimal inhibitory concentration (MIC) values obtained by the standard microdilution method[26,27] were used to evaluate the antibacterial properties of the compounds.

The compound **12a**, consisting of the uridine NM, hexadecyloxypropyl HM, and (3*R*,4*R*)-3,4-dihydroxypyrrolidine IM, exhibited significant antibacterial properties against several Gram-positive bacteria, such as *Enterococcus faecalis* (12.50 μg/mL), *Bacillus subtilis* (6.25 μg/mL), and *Streptococcus agalactiae* (3.13 μg/mL), respectively (Table 5). The compound **12b**, wherein the uridine NM was replaced with cytidine, exhibited a slightly improved antibacterial activity: *E. faecalis* (6.25 μg/mL), *B. subtilis* (3.13 μg/mL), *S. agalactiae* (3.13 μg/mL), and *S. aureus* (25.00 μg/mL).

The replacement of the IM ((3*R*,4*R*)-3,4-dihydroxypyrrolidine) of **12a** with its enantiomeric (3*S*,4*S*)-3,4-dihydroxypyrrolidine (in the case of **12c**) did not change the activity. However, when (3*R*,4*S*,5*S*)-3,4-trihydroxypiperidine (in the case of **12d**) or (3*R*,5*R*)-3,4-dihydroxypiperidine (in the case of **12e**) were used as the IM, the antibacterial activity was almost abolished except for *S. agalactiae* (12.50 μg/mL).

The hexadecyloxypropyl HM of **12a** was replaced with either ethyl (in the case of **12g**), tetradecyl (in the case of **12h**), hexadecyl (in the case of **12i**), octadecyl (in the case of **12j**), or icosanyl (in the case of **12k**) ester group, respectively. The LPPO **12g** with ethyl ester HM did not exhibit any antibacterial activity. Compound **12k** exhibited the lowest activity; **12h** and **12j** exhibited activities comparable to their parent structure, **12a**. The LPPO **12i** with hexadecyl ester HM appeared to be

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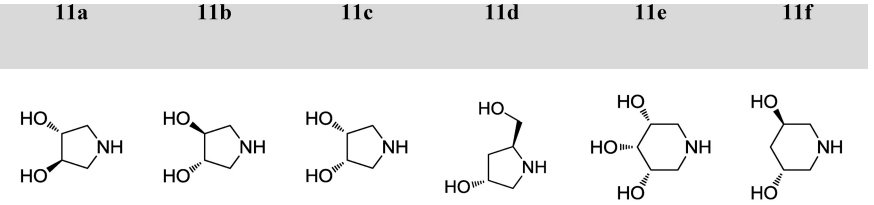
Table 3. Amines (Pyrrolidines and Piperidines) 11a−f



the most active compound of the tested series: *E. faecalis* (6.25 μg/mL), *B. subtilis* (3.13 μg/mL), *S. agalactiae* (1.56 μg/mL) and *S. aureus* (12.50−6.25 μg/mL). In the case of the LPPO **12f** with pivaloylthioethyl ester HM, no activity was observed.

None of the tested compounds exhibited MIC below 200 μg/mL against Gram-negative *Escherichia coli* or *Pseudomonas aeruginosa*. Possible explanations included (i) problems of these compounds with cell entry, (ii) their rapid degradation while in the cell, (iii) their rapid export from the cell, or (iv) the lack of molecular target(s) in Gram-negative bacteria. To better characterize these compounds, it would be useful to distinguish between (i) versus (ii−iv). To test the inhibitory potential of these compounds on a Gram-negative bacterium, we had to use permeabilized *E. coli* cells. Such cells have an enhanced ability to uptake molecules from their environment. Permeabilized *E. coli* cells were incubated with/without the tested compounds (see Experimental Section for details). If (i) were correct, at least some compound(s) would adversely affect the cell survival. If (ii), (iii), or (iv) were correct, no difference relative to the control would be observed.

Compounds **1**, **12a**, **12i**, and **12k** were tested on permeabilized *E. coli* cells. Compound **1** had no antibacterial effect on these cells. On the other hand, compounds **12a** and **12i** killed 90% of cells at 10 μM and **12k** at 25 μM (Table 6). These results suggest that the inefficiency of lipohposphonoxins against Gram-negative bacteria is likely due to their inability to cross the two plasmatic membranes of these species, and future modification may be attempted to allow their entry into the Gram-negative cell.

More importantly, these results imply that the HM may be required for the interaction of the compounds with their molecular target(s) because compound **1**, which lacks the HM, exhibited no activity. In other words, the HM may not be only an auxiliary module attached to the active molecule to create its prodrug form that is cleaved off upon the cell entry but an integral part of the active compound.

The most active compounds were further tested against clinically relevant multiresistant bacterial strains (Table 7). Compounds **12a**, **12b**, **12c**, **12i**, and **12l** showed significant antibacterial activities (6.25−3.13 μg/mL) against *Enterococcus feacium* VanA419/ana and *Staphylococcus epidermidis* 8700/B. Compound **12h** exhibited good activity against methicillin-resistant *S. aureus* (MRSA) 4591 (25.00 μg/mL), *Staphylococcus haemolyticus* 16568 (12.50 μg/mL), *E. faecium* VanA419/ana (6.25 μg/mL), and *S. epidermidis* 8700/B (6.25 μg/mL). Compound **12i** exhibited slightly stronger activity than **12h** against MRSA 4591 (12.50 μg/mL), *S. haemolyticus* 16568 (25.00−12.50 μg/mL), *E. faecium* VanA419/ana (3.13 μg/mL), and *S. epidermidis* 8700/B (3.13 μg/mL).

Viability, Cytotoxicity, and Apoptosis of Human Progenitor Cells. The effects of the newly synthesized lipophosphonoxins were tested on cell viability (metabolic activity), cytotoxicity (membrane integrity), and apoptosis (caspase 3/7 activation), using erythroid progenitor cells derived from human umbilical cord blood. Cell viability was measured 48 h after exposure, while cytotoxicity and apoptosis were measured 4 h after exposure to the compounds.

Compounds **12a**, **12b** and **12i**, shown to have the best antibacterial activity, had no detectable activity on normal primary cell viability and toxicity at the MIC concentrations (Figure 3). According to the cell viability results, these compounds showed to be toxic to these cells at concentrations that were well above their MIC values for most of the bacterial strains tested (Table 8). Finally, these compounds induced only a low level of apoptosis. Other tested compounds showed either higher toxicity to the primary cells or lower activity/selectivity against the selected bacterial strains (Supporting Information Figure 1), but this still does not exclude their potential antibacterial use, such as in topical applications.

Effect of the Diastereomeric Configuration at the Phosphorus Atom on Biological Activity. The compound **12i**, exhibiting the highest antibacterial activity, was separated into both diastereomers differing in the configuration at the phosphorus atom, yielding two pure diastereomers **12i-P1** and **12i-P2**. Figure 4 shows analytical HPLC records of the mixture and the two separated diastereomers. Diastereomer **12i-P1** with t_R = 8.20 min showed ^{31}P NMR shift at 31.27, while diastereomer **12i-P2** with t_R = 8.69 min showed ^{31}P NMR shift at 32.03.

Subsequently, the antibacterial activities of both diastereomers were evaluated and both diastereomers displayed almost the same MIC values, identical with MIC values of the original mixed compound **12i**. This suggests that the configuration at the phosphorus atom does not play an important role in the antibacterial activity. This is reminiscent of muraymycin analogues **6b** and **7b** (see Introduction) where the chirality at the atom of the lipophilic chain attachment did not significantly affect their antibacterial activity.[18] Nevertheless, in our case, the **12i-P1** diastereomer was slightly less toxic to erythroid progenitor cells (Figure 5).

Effect of LPPOs on Bacterial RNAP. An initial concern had been that LPPOs, due to their amphiphilic character, might function as nonspecific, detergent-like compounds. This concern was dispelled by the cytotoxicity tests: the used cell lines are highly sensitive to their environment and compounds acting in a detergent-like manner would severely compromise their viability, which was not the case. To further test whether LPPOs function in a nonspecific way, the effect of several LPPOs on the enzymatic activity of a key bacterial protein, RNA polymerase (RNAP), was evaluated (see Experimental Section for details). Multiple-round transcriptions with purified components were carried out in the absence or presence of high, 1 mM (~700 μg/mL) concentration of selected LPPOs (**12a**, **12c**, **12d**, and **12e**). None of these compounds significantly inhibited the enzymatic activity of RNAP, while two control detergents, cetyltrimethylammonium bromide and

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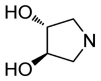
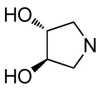
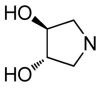
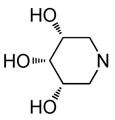
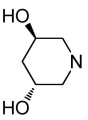
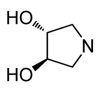
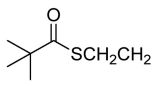
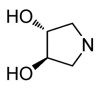
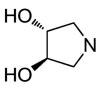
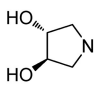
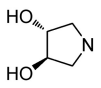
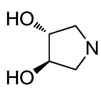
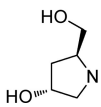
Table 4. Structures of Lipophosphonoxines 12a–l

Compound	R_1R_2N	B	R
12a		U	$C_{16}H_{33}OCH_2CH_2CH_2$
12b		C	$C_{16}H_{33}OCH_2CH_2CH_2$
12c		U	$C_{16}H_{33}OCH_2CH_2CH_2$
12d		U	$C_{16}H_{33}OCH_2CH_2CH_2$
12e		U	$C_{16}H_{33}OCH_2CH_2CH_2$
12f		U	
12g		U	CH_3CH_2
12h		U	$C_{14}H_{29}$
12i		U	$C_{16}H_{33}$
12j		U	$C_{18}H_{37}$
12k		U	$C_{20}H_{41}$
12l		U	$C_{16}H_{33}$

cetylpyridinium bromide, completely abolished transcription at 0.2 mM (~80 μg/mL) concentration.

CONCLUSIONS

We describe here a novel molecular scaffold leading to structures exhibiting significant antibacterial activities. The compounds based on this scaffold were collectively termed lipophosphonoxins (LPPOs). The most active LPPOs displayed good activities against a panel of Gram-positive bacteria strains at subcytotoxic concentrations. Importantly, the most active compounds also exhibited antibacterial activities against several multiresistant strains.

The molecular target(s) of LPPOs are unknown. On the basis of a gross structural resemblance (nucleobase, long alkyl chain), they may act in a similar way as, e.g., lipisidomycins or caprazamycins (Figure 1). In comparison with these com-

Table 5. Antibacterial Activity (against Reference Bacterial Strains)

	compd		MIC μg/mL	
	E. faecalis CCM 4224	*S.aureus* CCM 4223	*B. subtilis*	*S.agalactiae*
12a[a]	12.50		6.25	3.13
12b[a]	6.25	25.00	3.13	3.13
12c[a]	12.50		6.25	6.25
12d[a]				12.50
12e[a]	200		100	6.25
12f[a]				
12g[a]				
12h[a]	12.50	25.00−12.50	6.25	6.25
12i[a]	6.25	12.50−6.25	3.13	1.56
12j[a]	6.25	25.00−12.50	12.50−6.25	3.13
12k[a]	100			6.25
12l[a]	6.25	100	3.13	3.13
CMP[b]			16	2
TET[c]			2	1

[a]In all cases MIC = MBC. [b]CMP = chloramphenicol. [c]TET = tetracycline.

Table 6. Effect of Selected Compounds on Permeabilized *E. coli*

compd [10 μM]	killing power (log reduction[a])	
1	(4.4 μg/mL)	0[b]
12a	(7.2 μg/mL)	1
12i	(6.6 μg/mL)	1
12k	(7.2 μg/mL)	0.7

[a]One log is 90% reduction in colony forming units. [b]This compound had no effect also at 1 mM concentration.

Table 7. Antibacterial Activity (against Multiresistant Bacterial Strains)[a]

	MIC μg/mL			
compd	*S. aureus* MRSA 4591	*S. haemolyticus* 16568	*E. faecium* VanA419/ana	*S. epidermidis* 8700/B
12a			6.25	3.13
12b			6.25	6.25
12c			6.25	6.25
12h	25.00	12.5	12.50	12.50
12i	12.50	25−12.5	3.13	3.13
12j	200		6.25	6.25
12l			6.25	6.25

[a]In all cases MIC = MBC; multiresistant bacterial strains isolated from clinical isolates of patients from Teaching Hospital Olomouc: MRSA (methicilin-resistant *S. aureus* 4591); *S. haemolyticus* (fluoroquinolone-resistant strain 16568); *E. faecium* (vancomycin-resistant strain VanA419/ana); *S. epidermidis* (methicilin-resistant strain 8700/B).

pounds, however, LPPOs are significantly easier to synthesize. In summary, LPPOs appear to be specific antibacterial compounds and the molecular basis of their antibacterial activity has yet to be elucidated experimentally. Experiments to this effect are under way in our laboratory.

The modular composition of the scaffold and its simple synthesis will allow further optimization of its structure to enhance its antibacterial efficiency while decreasing its cytotoxicity. We will also focus on modifications of lipophosphonoxins to enhance their efficiency against Gram-negative bacteria. Results from these experiments as well as the research into the molecular mechanism of LPPOs will be reported in due course.

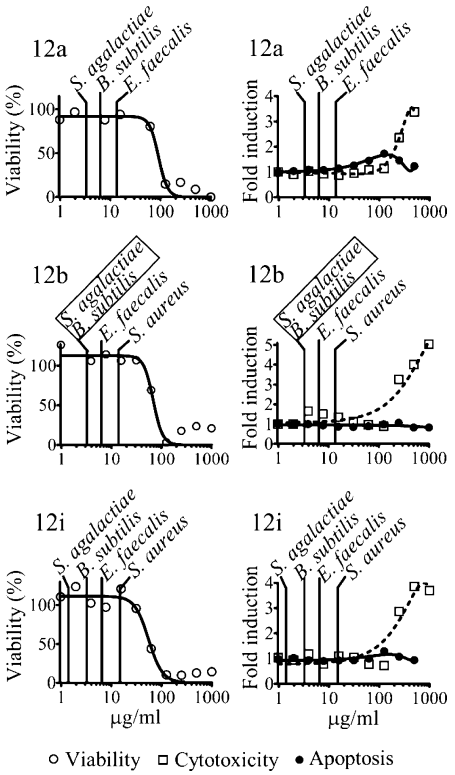


Figure 3. Cell viability, cytotoxicity, and apoptosis of erythroid progenitor cells after exposure to selected compounds (**12a**, **12b**, and **12i**). For comparison, the antibacterial activities (MIC values) of these compounds against selected bacterial species are indicated with vertical lines in the graphs (bacterial strains with the same MIC value are inside the boxes).

Table 8. Erythroid Progenitor Cells Viability

compd	IC_{50} (μg/mL)	maximum safe concentration
12a	91.00	62.50
12b	67.00	31.25
12c	47.00	31.25
12d	149.00	62.50
12e	53.00	31.25
12f	>1000	>1000
12g	nd	nd
12h	58.00	31.25
12i	56.00	31.25
12j	36.00	15.60
12k	31.00	15.60
12l	116.00	62.50

EXPERIMENTAL SECTION

Antibacterial Activity. Antimicrobial activity was assessed using the standard microdilution method determining the minimum inhibitory concentration (MIC) of tested samples leading to inhibition of bacterial growth.[26,27] Disposable microtitration plates were used for the tests. The samples were diluted in brain heart infusion broth (Himedia) to yield a concentration range between 200 and 1.56 μg/mL (in some cases, the lower concentration range was extended to 0.78 μg/mL). The plates were inoculated with a standard amount of the tested microbe; the inoculum density in each well was equal to 10^{5-6} CFU/ml. The MIC was read after 24/48 h of incubation at 37

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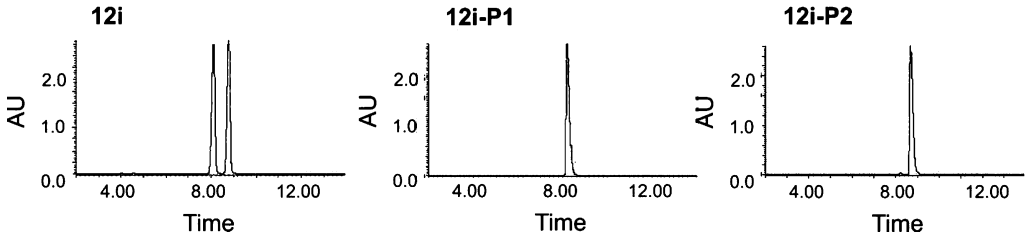

Figure 4. The analytical HPLC chromatograms of **12i**, **12i-P1**, and **12i-P2**. HPLC analysis was performed on Waters AutoPurification system with 2545 quarternary gradient module and 3100 single quadrupole mass detector using LUNA C18, column (Phenomenex, 100 mm × 4.6 mm, 3 μm) at flow rate 1 mL/min using gradient, A→B/15 min, (A, 50 mM NH_4HCO_3 in 50% aq CH_3CN; C, CH_3CN). AU, absorption units.

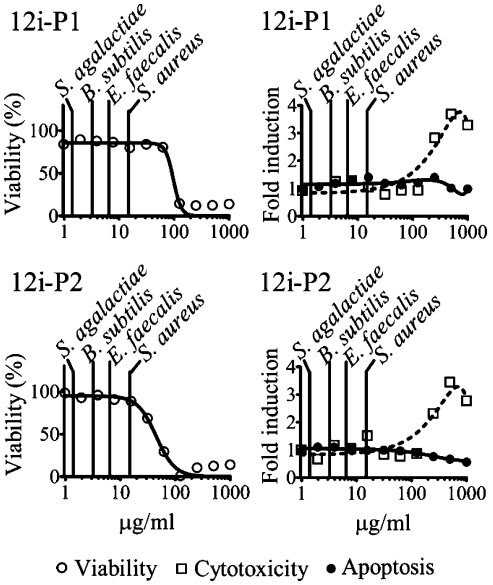

Figure 5. Cell viability, cytotoxicity, and apoptosis of erythroid progenitor cells after exposure to diastereomers **12i-P1** and **12i-P2**. Vertical lines in the graphs represent the MIC values for the respective bacterial strains.

°C as the minimum inhibitory concentration of the tested substance that inhibited the growth of the bacterial strains.

The minimum bactericidal concentration (MBC) is characterized as the minimum concentration of the sample required to achieve irreversible inhibition, i.e. killing the bacterium after a defined period of incubation. The MBC was examined by the inoculation method. With an applicator, 10 μL were transferred from microplate wells with defined concentrations of the tested sample and inoculated onto the surface of blood agar (Trios, Czech Republic). The MBC was determined as the lowest concentration that inhibited the visible growth of the used bacterium.

Standard reference bacterial strains (*E. faecalis* CCM 4224, *S. aureus* CCM 4223) from the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University Brno, and *S. agalactiae*, *B. subtilis*, methicilin-resistant *S. aureus* 4591, fluoroquinolone-resistant *S. haemolyticus* 16568, vancomycin-resistant *E. faecium* VanA419/ana, and methicilin-resistant *S. epidermidis* 8700/B strains obtained from the Teaching Hospital Olomouc were used. All tested microorganisms were stored in cryotubes (ITEST plus, Czech Republic) at −80 °C.

Antibacterial Activity against Competent *E. coli*. We mixed together 20 μL of *E. coli* (strain DH5α) competent cells (i.e., permeabilized, with an increased ability to uptake molecules from the outside),[28] pUC18 plasmid DNA (5 ng) (bearing amp^R as the selective marker after transformation) and a serially diluted tested compound/equal volume of "empty" buffer. The mixture was incubated for 30 min on ice followed by a heat shock performed at 42 °C for 90 s. The mixture was then allowed to sit on ice for 5 min. Subsequently, 1 mL of LB medium without antibiotics was added and the mixture was incubated for 1 h at 37 °C by vigorous shaking. Then the mixture was plated on LB agarose plates containing ampicillin at 100 μg/mL and incubated at 37 °C overnight. The number of colonies obtained from transformation with a tested compound was compared to the number of colonies obtained after transformation without inhibitor (positive control).

Cell Viability, Cytotoxicity, And Apoptosis. Culture and expansion of normal human erythroid progenitor cells was described earlier.[29] Cells were plated at 25000 cells/well/20 μL in 384-well plates immediately before compound addition. Cell viability and cytotoxicity have a fluorescent readout, and cells were plated into black 384-well plates (Corning, Cat. no. 3571), while apoptosis has a luminescent readout and cells were plated into white 384-well plates (Corning, Cat. no. 3570). Compounds were prediluted in the appropriate culture media and 5 μL of five times concentrated solution of compounds were added to the cell supernatant. Cytotoxicity and apoptosis were measured 4 h after compound addition using the CytoTox-ONE homogeneous membrane integrity assay (Promega, Cat. no. G7892) and the Caspase-Glo 3/7 assay (Promega, Cat. no. G8091). Cell viability was measured using the CellTiter-Blue cell viability assay (Promega, Cat. no. G8082) 48 h after compound addition. All assays were measured using the EnVision plate reader (PerkinElmer). Data were analyzed using GraphPad Prism 5.0 statistical software, and the data were normalized to the values of untreated control cells that were set as 100%.

Tests on Purified RNA Polymerase. *Bacillus subtilis* RNAP and σ^A were purified, and the holoenzyme was reconstituted as described.[30] As the template for transcriptions, we used supercoiled plasmid DNA bearing a fragment containing the *B. subtilis rrnB* P1 promoter (pLK7).[31] Multiple-round transcriptions were carried out in 10 μL reactions containing 6nM RNAP, 4 ng/μL supercoiled plasmid template, 40 mM Tris−HCl pH 8.0, 10 mM $MgCl_2$, 1 mM DTT, 0.1 mg/mL BSA, and 150 mM KCl. ATP, CTP, and GTP were 200 μM each. UTP was 10 μM plus 2 μM [α-^{32}P]-UTP purchased from Institute of Isotopes Co., Ltd. The transcriptions were carried out in the absence or presence (1 mM) of the tested compound. As a control, cetyltrimethylammonium bromide and cetylpyridinium bromide were used (0.2 mM).

Each sample was preincubated at 30 °C for 5 min followed by initiation with RNAP. The reaction was stopped after 15 min at 30 °C by 10 μL of formamide loading buffer (95% formamide, 20 mM EDTA pH 8.0) and briefly vortexed. Samples were loaded onto 7 M UREA 7% polyacrylamide gels and separated by electrophoresis. The dried gels were scanned with Molecular Imager_ FX (BIO-RAD). The amounts of the 145-nt-long transcript that originated from the *rrnB* P1 promoter were quantitated with Quantity One (BioRad).

Chemistry. Unless stated otherwise, all used solvents were anhydrous. Dimethyl vinylphosphonate, tetradecanol, hexadecanol, octadecanol, and eicosanol were purchased from Sigma Aldrich (Czech Republic). Protected nucleosides were prepared according to standard procedures. Hexadecyloxypropanol was prepared according to Hostetler et al.,[19] pivaloylthioethanol was prepared according to Lefebvre et al.,[23] dihydroxypyrrolidines **11a−c** were prepared according to Rejman et al.,[23] piperidine derivatives **11d** and **11e** were prepared according to Rejman et al.[25] and Kovačková et al.[24] respectively. All reactions were performed under an inert atmosphere

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of dry Ar or N_2. TLC was performed on silica gel precoated aluminum plates Silica gel/TLC-cards, UV 254 (Fluka), and compounds were detected by UV light (254 nm), by spraying with 1% ethanolic solution of ninhydrine to visualize amines, and by spraying with 1% solution of 4-(4-nitrobenzyl)pyridine in ethanol followed by heating and treating with gaseous ammonia (blue color, detection of alkylating agents, e.g. mesylderivatives, phosphonate esters). The purity of the final compounds was greater than 95%. Purity of prepared compounds was determined by LC-MS performed on Waters AutoPurification System with 2545 Quarternary Gradient Module and 3100 Single Quadrupole Mass Detector using LUNA C18, column (Phenomenex, 100 mm × 4.6 mm, 3 μm) at flow rate 1 mL/min. Typical conditions: mobile phase, A, 50 mM NH_4HCO_3; B, 50 mM NH_4HCO_3 in 50% aq CH_3CN; C, CH_3CN; A→B/10 min, B→C/10 min, C/5 min. Preparative RP HPLC was performed on LC5000 liquid chromatograph (INGOS-PIKRON, CR) using Luna C18 (2) column (250 mm × 21.2 mm, 5 μm) at flow rate of 10 mL/min by a gradient elution of methanol in 0.1 M TEAB pH 7.5 (A = 0.1 M TEAB; B = 0.1 M TEAB in 50% aq methanol; C = methanol) or without buffer. All final compounds were lyophilized from water. IR spectra were recorded on a FTIR spectrometer (Bruker Equinox 55, Germany). Mass spectra were recorded on LTQ Orbitrap XL (Thermo Fisher Scientific) using ESI ionization. NMR spectra were measured as DMSO-d_6 or D_2O solutions on Bruker AVANCE 400 (^{1}H at 400.0 MHz, ^{13}C at 100.6 MHz, ^{31}P at 162.0 MHz) and/or Bruker AVANCE 500 (^{1}H at 500.0 MHz, ^{13}C at 125.7 MHz, ^{31}P at 202.3 MHz) spectrometers. Chemical shifts (in ppm, δ scale) were referenced to the residual DMSO-d_6 signal (2.5 ppm for ^{1}H and 39.7 ppm for ^{13}C) or to 1,4-dioxane signal (3.75 ppm for ^{1}H and 69.3 ppm for ^{13}C) as internal standard in D_2O. ^{31}P NMR spectra were referenced to H_3PO_4 (0 ppm) as an external standard. Coupling constants (J) are given in Hz. Complete assignment of protons and carbons was done by analysis of correlated homonuclear H,H-COSY and heteronuclear H,C-HSQC and H,C-HMBC spectra. Relative configuration was checked using DPFGSE-NOE and 2D-ROESY techniques.

General Method A, Phosphonylation: Preparation of Compounds 9a–b. Dimethyl vinylphosphonate (0.53 mL, 1.5 mmol) was treated with 60% aqueous pyridine (10 mL/mmol) at 60 °C overnight to remove one methyl ester group. The reaction mixture was concentrated in vacuo and coevaporated with EtOH (2 × 50 mL/mmol). The residue was dissolved in the mixture of H_2O/EtOH 1:1 (10 mL/mmol) and passed through a column of Dowex 50 in H^+ form (10 mL/mmol). The resin was washed with additional mixture of H_2O/EtOH 1:1 (2 × 10 mL/mmol). The liquid was concentrated in vacuo. The obtained monomethyl vinylphosphonate was coevaporated with EtOH (2 × 10 mL/mmol) and toluene (2 × 10 mL/mmol). To the mixture of the monomethyl phosphonate, protected nucleoside (1 mmol) and methylimidazole (3 mmol) in DCM (10 mL/mmol), TPSCl (3 mmol) was added. The reaction mixture was stirred at rt overnight. The mixture was washed with a saturated aq $NaHCO_3$ (10–20 mL/mmol) followed by washing with 3% aq citric acid (10–20 mL/mmol) and dried over Na_2SO_4. Organic phase was concentrated in vacuo and monomethyl ester of nucleosidevinylphosphonic acid was obtained by flash chromatography on silica gel using linear gradient of ethanol in chloroform. The monomethyl ester intermediate in 60% aqueous pyridine (10 mL/mmol) was stirred at 60 °C overnight. The reaction mixture was concentrated in vacuo, coevaporated in EtOH (2 × 10 mL/mmol), and dissolved in the same solvent (10 mL/mmol). Dowex 50 in Et_3N form (10 mL/mmol) was added, and the suspension was stirred for 10 min. The resin was removed by filtration, and the filtrate was concentrated in vacuo.

General Method B, Esterification: Preparation of Compounds 10a–h. TPSCl (3 mmol) was added to the mixture of triethylamonium salt of nucleosidyl ester of vinylphosphonic acid (1 mmol), alcohol (2 mmol), and methylimidazole (3 mmol) in DCM (10 mL/mmol). The reaction mixture was stirred at rt overnight. The mixture was washed with saturated aq $NaHCO_3$ (10–20 mL/mmol), followed by washing with 3% aq citric acid (10–20 mL/mmol) and dried over Na_2SO_4. Organic phase was concentrated in vacuo and the product of esterification was obtained by flash chromatography on silica gel using linear gradient of ethanol in chloroform.

General Method C, Michael Addition and Deprotection: Preparation of Compounds 12a–l. The mixture of alkyl nucleosidyl mixed ester of vinylphosphonic acid (1 mmol) and amine (1.5 mmol) in *n*BuOH (10 mL/mmol) was stirred at 100 °C overnight. The mixture was concentrated in vacuo, and the protected intermediate was obtained by flash chromatography on silica gel using linear gradient of solvent system H1 (ethyl acetate:acetone:ethanol:-water 4:1:1:1) in ethyl acetate. In the case of *N*-4-Bz-Cyt, the intermediate was dissolved in 33% ethanolic methylamine, stirred at rt overnight, and concentrated in vacuo (this step is skipped in the case of uridine derivative). The intermediate was dissolved in 0.2 M methanolic HCl (10 mL/mmol) and stirred overnight. The reaction mixture was applied on a silica gel column. The final product was obtained by flash chromatography using linear gradient of solvent system H1 (ethyl acetate:acetone:ethanol:water 4:1:1:1) in ethyl acetate and lyophilized from water.

2, 3′-Isopropylideneuridin-5′-yl Vinylphosphonate (9a). The title compound was prepared according to general method A from 2′,3′-isopropylideneuridine (14 g, 50 mmol) in 48% yield (11.38 g, 23.93 mmol) as a yellowish thick oil. NMR of UrdPhN has shown free acid (not Et_3NH^+ salt). ^{1}H NMR (499.8 MHz, CD_3OD): 1.35, 1.54 (2 × q, 2 × 3H, ^4J = 0.5, $(CH_3)_2C$); 3.98 (dd, 2H, $J_{H,P}$ = 5.3, $J_{5',4'}$ = 3.4, H-5′); 4.35 (m, 1H, H-4′); 4.90 (m, 2H, H-2′,3′); 5.74 (d, 1H, $J_{5,6}$ = 8.1, H-5); 5.86 (ddd, 1H, $J_{H,P}$ = 46.0, J_{cis} = 12.3, J_{gem} = 3.1, $CH_{cis}\mathbf{H}_{trans}$=CHP); 5.95 (d, 1H, $J_{1',2'}$ = 2.5, H-1′); 6.00 (ddd, 1H, $J_{H,P}$ = 22.8, J_{trans} = 18.7, J_{gem} = 3.1, $C\mathbf{H}_{cis}H_{trans}$=CHP); 6.12 (ddd, 1H, $J_{H,P}$ = 19.7, J_{trans} = 18.7, J_{cis} = 12.3, =CHP); 7.86 (d, 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (125.7 MHz, CD_3OD): 25.53, 27.55 ($(\mathbf{C}H_3)_2C$); 65.31 (d, $J_{C,P}$ = 4.8, CH_2-5′); 82.49 (CH-3′); 85.67 (CH-2′); 86.56 (d, $J_{C,P}$ = 8.0, CH-4′); 93.40 (CH-1′); 102.98 (CH-5); 114.99 ($\mathbf{C}(CH_3)_2$); 130.74 ($\mathbf{C}H_2$=CHP); 132.70 (d, $J_{C,P}$ = 174.3, =CHP); 143.42 (CH-6); 152.16 (C-2); 166.15 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 13.16. HR-ESI: $C_{14}H_{18}O_8N_2P$ $(M - H)^-$ calcd 373.0806; found 373.0809

4 *N*-Benzoyl-2′,3′-isopropylidenecytidin-5′-yl Vinylphosphonate (9b). The title compound was prepared according to general method A from 4-*N*-benzoyl-2′,3′-isopropylidenecytidine (3.05 g, 7.87 mmol) in 52% yield (1.95 g, 4.1 mmol) as a yellowish thick oil. NMR **9b** has shown Et_3NH^+ salt (not free acid). ^{1}H NMR (600.1 MHz, CD_3OD): 1.31 (t, 9H, J_{vic} = 7.3, $C\mathbf{H}_3CH_2N$); 1.37, 1.57 (2 × q, 2 × 3H, ^4J = 0.7, $(CH_3)_2C$); 3.20 (q, 6H, J_{vic} = 7.3, $CH_3C\mathbf{H}_2N$); 4.02 (ddd, 1H, J_{gem} = 11.5, $J_{H,P}$ = 6.0, $J_{5'b,4'}$ = 3.4, H-5′b); 4.06 (ddd, 1H, J_{gem} = 11.5, $J_{H,P}$ = 4.5, $J_{5'a,4'}$ = 3.0, H-5′a); 4.50 (m, 1H, H-4′); 4.91 (dd, 1H, $J_{3',2'}$ = 6.0, $J_{3',4'}$ = 2.2, H-3′); 4.93 (dd, 1H, $J_{2',3'}$ = 6.0, $J_{2',1'}$ = 2.3, H-2′); 5.86 (ddd, 1H, $J_{H,P}$ = 46.0, J_{cis} = 12.4, J_{gem} = 3.1, $CH_{cis}\mathbf{H}_{trans}$=CHP); 6.00 (d, 1H, $J_{1',2'}$ = 2.3, H-1′); 6.01 (ddd, 1H, $J_{H,P}$ = 22.9, J_{trans} = 18.7, J_{gem} = 3.1, $C\mathbf{H}_{cis}H_{trans}$=CHP); 6.11 (ddd, 1H, $J_{H,P}$ = 19.6, J_{trans} = 18.7, J_{cis} = 12.4, =CHP); 7.55 (m, 2H, H-*m*-Ph); 7.62 (bd, 1H, $J_{5,6}$ = 7.5, H-5) 7.64 (m, 1H, H-*p*-Ph); 7.98 (m, 2H, H-*o*-Ph); 8.35 (d, 1H, $J_{6,5}$ = 7.5, H-6). ^{13}C NMR (150.9 MHz, CD_3OD): 9.18 ($\mathbf{C}H_3CH_2N$); 25.45, 27.47 ($(\mathbf{C}H_3)_2C$); 47.73 ($CH_3\mathbf{C}H_2N$); 65.09 (d, $J_{C,P}$ = 4.8, CH_2-5′); 82.47 (CH-3′); 87.16 (CH-2′); 87.91 (d, $J_{C,P}$ = 8.1, CH-4′); 95.59 (CH-1′); 98.54 (CH-5); 114.69 ($\mathbf{C}(CH_3)_2$); 129.16 (CH-*o*-Ph); 129.82 (CH-*m*-Ph); 130.80 ($\mathbf{C}H_2$=CHP); 132.71 (d, $J_{C,P}$ = 174.8, =CHP); 134.05 (CH-*p*-Ph); 134.78 (C-*i*-Ph); 147.24 (CH-6); 157.90 (C-2); 164.99 (C-4); 169.02 (CO). ^{31}P NMR (202.3 MHz, CD_3OD): 13.02. HR-ESI: $C_{21}H_{25}O_8N_3P$ $(M + H)^+$ calcd 478.13738; found 478.13719.

2 Pivaloylthioethyl 2′,3′-Isopropylideneuridin-5′-yl Vinylphosphonate (10a). The title compound was prepared according to general method B from **9a** (0.36 g, 1.2 mmol) and pivaloylthioethanol (0.39 g, 2.4 mmol) in 92% yield (0.57 g, 1.1 mmol) as a colorless oil. A mixture of diastereomers ~1:1. ^{1}H NMR (600.1 MHz, $CDCl_3$): 1.15 (m, 18H, $(CH_3)_3C$); 1.27, 1.28, 1.49 (3 × s, 12H, $(CH_3)_2C$); 3.05, 3.06 (2 × t, 2 × 2H, J_{vic} = 6.8, $SC\mathbf{H}_2CH_2O$); 3.97–4.09 (m, 4H, $SCH_2C\mathbf{H}_2O$); 4.17–4.25 (m, 4H, H-5′); 4.29 (m, 2H, H-4′); 4.80, 4.81 (2 × dd, 2 × 1H, $J_{3',2'}$ = 6.5, $J_{3',4'}$ = 3.7, H-3′); 4.88 (dd, 2H, $J_{2',3'}$ = 6.5, $J_{2',1'}$ = 2.3, H-2′); 5.655, 5.657 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.70, 5.73 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.3, H-1′); 5.99, 6.00 (2 × ddd, 2 ×

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1H, $J_{H,P}$ = 23.1, J_{trans} = 18.5, J_{cis} = 12.7, =CHP); 6.12, 6.14 (2 × ddd, 2 × 1H, $J_{H,P}$ = 52.4, J_{cis} = 12.7, J_{gem} = 1.8, $CH_{cis}\mathbf{H}_{trans}$=CHP); 6.28, 6.30 (2 × ddd, 2 × 1H, $J_{H,P}$ = 25.2, J_{trans} = 18.5, J_{gem} = 1.8, $C\mathbf{H}_{cis}H_{trans}$=CHP); 7.34, 7.38 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6); 10.34 (bs, 2H, NH). ^{13}C NMR (150.9 MHz, $CDCl_3$): 24.97, 24.99, 26.81, 26.82 ($(CH_3)_2C$); 27.00 ($(CH_3)_3C$); 28.43 (d, $J_{C,P}$ = 6.6, SCH_2CH_2O); 46.19 ($C(CH_3)_2$); 64.19, 64.21 (d, $J_{C,P}$ = 5.0, OCH_2CH_2S); 64.98, 65.06 (d, $J_{C,P}$ = 5.4, CH_2-5′); 80.39, 80.49 (CH-3′); 84.14, 84.22 (CH-2′); 85.16, 85.43 (d, $J_{C,P}$ = 6.9, CH-4′); 93.41, 93.82 (CH-1′); 102.25, 102.34 (CH-5); 114.16, 114.22 ($C(CH_3)_2$); 124.31, 124.36 (d, $J_{C,P}$ = 184.6, =CHP); 136.85 (CH_2=CHP); 141.69, 141.81 (CH-6); 150.03, 150.05 (C-2); 163.55, 163.57 (C-4); 205.41, 205.43 (CO). ^{31}P NMR (162.0 MHz, $CDCl_3$): 18.75, 18.88. HR-ESI: $C_{21}H_{32}O_9N_2PS$ $(M + H)^+$ calcd 519.1561; found 519.1562.

Ethyl 2′,3′-Isopropylideneuridin-5′-yl Vinylphosphonate (10b). The title compound was prepared according to general method B from **9a** (1.86 g, 6.2 mmol) and ethanol (1 g, 30 mmol) in 80% yield (2 g, 4.97 mmol) as a colorless oil. A mixture of diastereomers ~6:5. ^{1}H NMR (499.8 MHz, $CDCl_3$): 1.33, 1.34 (2 × td, 2 × 3H, J_{vic} = 7.1, $J_{H,P}$ = 0.3, $C\mathbf{H}_3CH_2O$); 1.35, 1.57 (2 × s, 2 × 6H, $(CH_3)_2C$); 4.09−4.17 (m, 4H, $CH_3C\mathbf{H}_2O$); 4.21−4.30 (m, 4H, H-5′); 4.34−4.39 (m, 2H, H-4′); 4.86, 4.87 (2 × dd, 2 × 1H, $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 3.7, H-3′); 4.92 (dd, 2H, $J_{2',3'}$ = 6.5, $J_{2',1'}$ = 2.4, H-2′); 5.71, 5.73 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.78, 5.805 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.4, H-1′); 6.05, 6.06 (2 × ddd, 2 × 1H, $J_{H,P}$ = 22.9, J_{trans} = 18.6, J_{cis} = 12.8, =CHP); 6.12, 6.14 (2 × ddd, 2 × 1H, $J_{H,P}$ = 51.6, J_{cis} = 12.8, J_{gem} = 2.0, $CH_{cis}\mathbf{H}_{trans}$=CHP); 6.34, 6.35 (2 × ddd, 2 × 1H, $J_{H,P}$ = 24.7, J_{trans} = 18.6, J_{gem} = 2.0, $C\mathbf{H}_{cis}H_{trans}$=CHP); 7.40, 7.44 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6); 10.15 (bs, 2H, NH). ^{13}C NMR (125.7 MHz, $CDCl_3$): 16.18 (d, $J_{C,P}$ = 6.2, $\mathbf{C}H_3CH_2O$); 25.10, 25.12, 26.95, 26.96 ($(CH_3)_2C$); 62.30 (d, $J_{C,P}$ = 5.5, CH_3CH_2O); 64.94, 64.99 (d, $J_{C,P}$ = 5.8, CH_2-5′); 80.54, 80.60 (CH-3′); 84.31, 84.39 (CH-2′); 85.32, 85.52 (d, $J_{C,P}$ = 7.1, CH-4′); 93.51, 93.78 (CH-1′); 102.36, 102.44 (CH-5); 114.34, 114.38 ($C(CH_3)_2$); 124.91, 124.95 (d, $J_{C,P}$ = 184.2, =CHP); 136.51, 136.53 (d, $J_{C,P}$ = 2.3, CH_2=CHP); 141.67, 141.73 (CH-6); 150.08, 150.11 (C-2); 163.58 (C-4). ^{31}P NMR (202.3 MHz, $CDCl_3$): 18.49, 18.66. HR-ESI: $C_{16}H_{24}O_8N_2P$ $(M + H)^+$ calcd 403.12648; found 403.12632.

Tetradecyl 2′,3′-Isopropylideneuridin-5′-yl Vinylphosphonate (10c). The title compound was prepared according to general method B from **9a** (2.45 g, 5.15 mmol) and tetradecanol (2.2 g, 10.31 mmol) in 40% yield (1.17 g, 2.05 mmol) as a colorless thick oil. A mixture of diastereomers ~6:5. ^{1}H NMR (499.8 MHz, $CDCl_3$): 0.88 (m, 6H, $C\mathbf{H}_3(CH_2)_{13}$); 1.20−1.35 (m, 44H, $CH_3(C\mathbf{H}_2)_{11}CH_2CH_2O$); 1.350, 1.354, 1.573, 1.576 (4 × q, 4 × 3H, ^4J = 0.7, $(CH_3)_2C$); 1.67 (m, 4H, $CH_3(CH_2)_{11}C\mathbf{H}_2CH_2O$); 4.03, 4.04 (2 × dt, 2 × 2H, $J_{H,P}$ = 7.3, J_{vic} = 6.7, $CH_3(CH_2)_{11}CH_2C\mathbf{H}_2O$); 4.19−4.29 (m, 4H, H-5′); 4.35−4.39 (m, 2H, H-4′); 4.85, 4.86 (2 × dd, 2 × 1H, $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 3.6, H-3′); 4.88, 4.89 (2 × dd, 2 × 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.3, H-2′); 5.71, 5.72 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.77, 5.81 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.3, H-1′); 6.03, 6.04 (2 × ddd, 2 × 1H, $J_{H,P}$ = 22.9, J_{trans} = 18.6, J_{cis} = 12.7, =CHP); 6.16, 6.19 (2 × ddd, 2 × 1H, $J_{H,P}$ = 51.6, J_{cis} = 12.7, J_{gem} = 2.0, $CH_{cis}\mathbf{H}_{trans}$=CHP); 6.33, 6.34 (2 × ddd, 2 × 1H, $J_{H,P}$ = 25.7, J_{trans} = 18.7, J_{gem} = 2.0, $C\mathbf{H}_{cis}H_{trans}$=CHP); 7.38, 7.43 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6); 9.34 (bs, 2H, NH). ^{13}C NMR (125.7 MHz, $CDCl_3$): 14.08 ($CH_3(CH_2)_{13}$); 22.64 ($CH_3(CH_2)_{11}CH_2CH_2O$); 25.23, 25.25 ($(CH_3)_2C$); 25.43 ($CH_3(CH_2)_{11}CH_2CH_2O$); 27.08, 27.09 ($(CH_3)_2C$); 29.10, 29.30, 29.46, 29.52, 29.59, 29.60, 29.62, 29.64 ($CH_3(CH_2)_{11}CH_2CH_2O$); 30.39 (d, $J_{C,P}$ = 6.2, $CH_3(CH_2)_{11}CH_2CH_2O$); 31.87 ($CH_3(CH_2)_{11}CH_2CH_2O$); 64.93, 65.01 (d, $J_{C,P}$ = 5.5, CH_2-5′); 66.47, 66.48 (d, $J_{C,P}$ = 5.7, $CH_3(CH_2)_{11}CH_2CH_2O$); 80.57, 80.66 (CH-3′); 84.45, 84.53 (CH-2′); 85.26, 85.55 (d, $J_{C,P}$ = 7.0, CH-4′); 93.43, 93.83 (CH-1′); 102.49, 102.58 (CH-5); 114.54, 114.59 ($C(CH_3)_2$); 124.97, 125.02 (d, $J_{C,P}$ = 184.3, =CHP); 136.60, 136.62 (CH_2=CHP); 141.47, 141.55 (CH-6); 149.96, 149.99 (C-2); 163.11 (C-4). ^{31}P NMR (202.3 MHz, $CDCl_3$): 18.59, 18.74. HR-ESI: $C_{28}H_{48}O_8N_2P$ $[M + H]^+$ calcd 571.31428, found 571.31445, $C_{28}H_{47}O_8N_2NaP$ $[M + Na]^+$ calcd 593.29622, found 593.29616.

Hexadecyl 2′,3′-Isopropylideneuridin-5′-yl Vinylphosphonate (10d). The title compound was prepared according to general method B from **9a** (1.7 g, 6 mmol) and hexadecanol (2.9 g, 12 mmol) in 38% yield (1.38 g, 2.3 mmol) as a colorless thick oil. A mixture of diastereomers ~6:5. ^{1}H NMR (499.8 MHz, $CDCl_3$): 0.88 (m, 6H, $C\mathbf{H}_3(CH_2)_{15}$); 1.22−1.35 (m, 52H, $CH_3(C\mathbf{H}_2)_{13}CH_2CH_2O$); 1.350, 1.354, 1.573, 1.577 (4 × q, 4 × 3H, ^4J = 0.7, $(CH_3)_2C$); 1.67 (m, 4H, $CH_3(CH_2)_{13}C\mathbf{H}_2CH_2O$); 4.03, 4.04 (2 × dt, 2 × 2H, $J_{H,P}$ = 7.3, J_{vic} = 6.7, $CH_3(CH_2)_{13}CH_2C\mathbf{H}_2O$); 4.19−4.30 (m, 4H, H-5′); 4.35−4.39 (m, 2H, H-4′); 4.85, 4.86 (2 × dd, 2 × 1H, $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 3.6, H-3′); 4.878, 4.8984 (2 × dd, 2 × 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.3, H-2′); 5.71, 5.72 (2 × dd, 2 × 1H, $J_{5,6}$ = 8.1, $J_{5,NH}$ = 2.2, H-5); 5.78, 5.81 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.3, H-1′); 6.03, 6.05 (2 × ddd, 2 × 1H, $J_{H,P}$ = 22.8, J_{trans} = 18.6, J_{cis} = 12.8, =CHP); 6.12, 6.13 (2 × ddd, 2 × 1H, $J_{H,P}$ = 51.7, J_{cis} = 12.8, J_{gem} = 2.0, $CH_{cis}\mathbf{H}_{trans}$=CHP); 6.33, 6.34 (2 × ddd, 2 × 1H, $J_{H,P}$ = 24.8, J_{trans} = 18.6, J_{gem} = 2.0, $C\mathbf{H}_{cis}H_{trans}$=CHP); 7.38, 7.43 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6); 9.15, 9.18 (2 × bs, 2 × 2H, NH). ^{13}C NMR (125.7 MHz, $CDCl_3$): 14.08 ($\mathbf{C}H_3(CH_2)_{15}$); 22.65 ($CH_3(\mathbf{C}H_2)_{13}CH_2CH_2O$); 25.23, 25.26 ($(\mathbf{C}H_3)_2C$); 25.43 ($CH_3(\mathbf{C}H_2)_{13}CH_2CH_2O$); 27.08, 27.10 ($(\mathbf{C}H_3)_2C$); 29.11, 29.32, 29.47, 29.53, 29.60, 29.61, 29.63, 29.65 ($CH_3(\mathbf{C}H_2)_{13}CH_2CH_2O$); 30.40 (d, $J_{C,P}$ = 6.3, $CH_3(CH_2)_{13}\mathbf{C}H_2CH_2O$); 31.88 ($CH_3(\mathbf{C}H_2)_{13}CH_2CH_2O$); 64.93, 65.00 (d, $J_{C,P}$ = 5.5, CH_2-5′); 66.47, 66.48 (d, $J_{C,P}$ = 5.7, $CH_3(CH_2)_{13}CH_2\mathbf{C}H_2O$); 80.57, 80.66 (CH-3′); 84.46, 84.54 (CH-2′); 85.25, 85.55 (d, $J_{C,P}$ = 7.1, CH-4′); 93.40, 93.80 (CH-1′); 102.49, 102.57 (CH-5); 114.55, 114.60 ($\mathbf{C}(CH_3)_2$); 124.99, 125.04 (d, $J_{C,P}$ = 184.2, =CHP); 136.62, 136.66 (d, $J_{C,P}$ = 2.0, $\mathbf{C}H_2$=CHP); 141.46, 141.55 (CH-6); 149.92, 149.95 (C-2); 163.05, 163.07 (C-4). ^{31}P NMR (202.3 MHz, $CDCl_3$): 18.59, 18.73. HR-ESI: $C_{30}H_{51}O_8N_2PNa$ $(M + Na)^+$ calcd 621.32752; found 621.32778.

Octadecyl 2′,3′-Isopropylideneuridin-5′-yl Vinylphosphonate (10e). The title compound was prepared according to general method B from **9a** (2.18 g, 4.58 mmol) and octadecanol (2.48 g, 9.17 mmol) in 43% yield (1.23 g, 1.96 mmol) as a colorless thick oil. A mixture of diastereomers ~6:5. ^{1}H NMR (499.8 MHz, $CDCl_3$): 0.88 (m, 6H, $C\mathbf{H}_3(CH_2)_{17}$); 1.20−1.40 (m, 60H, $CH_3(C\mathbf{H}_2)_{15}CH_2CH_2O$); 1.350, 1.354, 1.573, 1.576 (4 × q, 4 × 3H, ^4J = 0.7, $(CH_3)_2C$); 1.66 (m, 4H, $CH_3(CH_2)_{15}C\mathbf{H}_2CH_2O$); 4.03, 4.04 (2 × dt, 2 × 2H, $J_{H,P}$ = 7.3, J_{vic} = 6.7, $CH_3(CH_2)_{15}CH_2C\mathbf{H}_2O$); 4.19−4.29 (m, 4H, H-5′); 4.34−4.39 (m, 2H, H-4′); 4.85, 4.86 (2 × dd, 2 × 1H, $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 3.7, H-3′); 4.88, 4.89 (2 × dd, 2 × 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.3, H-2′); 5.71, 5.72 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.77, 5.81 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.3, H-1′); 6.03, 6.04 (2 × ddd, 2 × 1H, $J_{H,P}$ = 22.9, J_{trans} = 18.6, J_{cis} = 12.7, =CHP); 6.16, 6.19 (2 × ddd, 2 × 1H, $J_{H,P}$ = 51.7, J_{cis} = 12.7, J_{gem} = 2.0, $CH_{cis}\mathbf{H}_{trans}$=CHP); 6.33, 6.34 (2 × ddd, 2 × 1H, $J_{H,P}$ = 25.6, J_{trans} = 18.6, J_{gem} = 2.0, $C\mathbf{H}_{cis}H_{trans}$=CHP); 7.38, 7.43 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6); 9.37 (bs, 2H, NH). ^{13}C NMR (125.7 MHz, $CDCl_3$): 14.08 ($\mathbf{C}H_3(CH_2)_{17}$); 22.64 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 25.22, 25.25 ($(\mathbf{C}H_3)_2C$); 25.43 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 27.07, 27.09 ($(\mathbf{C}H_3)_2C$); 29.10, 29.31, 29.46, 29.53, 29.60, 29.61, 29.63, 29.65 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 30.39 (d, $J_{C,P}$ = 6.2, $CH_3(CH_2)_{15}\mathbf{C}H_2CH_2O$); 31.87 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 64.93, 65.01 (d, $J_{C,P}$ = 5.5, CH_2-5′); 66.46, 66.47 (d, $J_{C,P}$ = 5.7, $CH_3(CH_2)_{15}CH_2\mathbf{C}H_2O$); 80.57, 80.66 (CH-3′); 84.45, 84.53 (CH-2′); 85.27, 85.56 (d, $J_{C,P}$ = 7.0, CH-4′); 93.44, 93.83 (CH-1′); 102.49, 102.58 (CH-5); 114.53, 114.58 ($\mathbf{C}(CH_3)_2$); 124.97, 125.01 (d, $J_{C,P}$ = 184.2, =CHP); 136.62, 136.66 (d, $J_{C,P}$ = 2.0, $\mathbf{C}H_2$=CHP); 141.47, 141.56 (CH-6); 149.97, 150.00 (C-2); 163.14, 163.16 (C-4). ^{31}P NMR (202.3 MHz, $CDCl_3$): 18.59, 18.74. HR-ESI: $C_{32}H_{56}O_8N_2P$ $[M + H]^+$ calcd 627.37688, found 627.37738.

Icosanyl 2′,3′-Isopropylideneuridin-5′-yl Vinylphosphonate (10f). The title compound was prepared according to general method B from **9a** (2.35 g, 4.94 mmol) and icosanol (2.95 g, 9.9 mmol) in 34% yield (1.1 g, 1.68 mmol) as a colorless white wax. A mixture of diastereomers ~6:5. ^{1}H NMR (499.8 MHz, $CDCl_3$): 0.88 (m, 6H, $C\mathbf{H}_3(CH_2)_{19}$); 1.20−1.36 (m, 68H, $CH_3(C\mathbf{H}_2)_{17}CH_2CH_2O$); 1.351, 1.354, 1.574, 1.577 (4 × q, 4 × 3H, ^4J = 0.7, $(CH_3)_2C$); 1.66 (m, 4H, $CH_3(CH_2)_{17}C\mathbf{H}_2CH_2O$); 4.03, 4.04 (2 × dt, 2 × 2H, $J_{H,P}$ = 7.3, J_{vic} = 6.7, $CH_3(CH_2)_{17}CH_2C\mathbf{H}_2O$); 4.19−4.29 (m, 4H, H-5′); 4.35−4.39 (m, 2H, H-4′); 4.84, 4.86 (2 × dd, 2 × 1H, $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 3.4, H-3′); 4.87, 4.89 (2 × dd, 2 × 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.3, H-2′); 5.706, 5.714 (2

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× d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.77, 5.80 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.3, H-1′); 6.03, 6.06 (2 × ddd, 2 × 1H, $J_{H,P}$ = 22.8, J_{trans} = 18.6, J_{cis} = 12.7, =CHP); 6.17, 6.19 (2 × ddd, 2 × 1H, $J_{H,P}$ = 51.7, J_{cis} = 12.7, J_{gem} = 2.0, $CH_{cis}\mathbf{H}_{trans}$=CHP); 6.33, 6.34 (2 × ddd, 2 × 1H, $J_{H,P}$ = 25.6, J_{trans} = 18.6, J_{gem} = 2.0, $C\mathbf{H}_{cis}H_{trans}$=CHP); 7.37, 7.43 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6); 9.08 (bs, 2H, NH). ^{13}C NMR (125.7 MHz, $CDCl_3$): 14.10 ($\mathbf{C}H_3(CH_2)_{19}$); 22.66 ($CH_3(\mathbf{C}H_2)_{17}CH_2CH_2O$); 25.24, 25.26 ($(\mathbf{C}H_3)_2C$); 25.44 ($CH_3(\mathbf{C}H_2)_{17}CH_2CH_2O$); 27.09, 27.11 ($(\mathbf{C}H_3)_2C$); 29.12, 29.33, 29.48, 29.55, 29.62, 29.65, 29.67 ($CH_3(CH_2)_{17}CH_2CH_2O$); 30.41 (d, $J_{C,P}$ = 6.3, $CH_3(CH_2)_{17}\mathbf{C}H_2CH_2O$); 31.89 ($CH_3(\mathbf{C}H_2)_{17}CH_2CH_2O$); 64.92, 64.99 (d, $J_{C,P}$ = 5.5, CH_2-5′); 66.48, 66.50 (d, $J_{C,P}$ = 5.7, $CH_3(CH_2)_{17}CH_2\mathbf{C}H_2O$); 80.57, 80.66 (CH-3′); 84.47, 84.56 (CH-2′); 85.25, 85.54 (d, $J_{C,P}$ = 7.0, CH-4′); 93.44, 93.83 (CH-1′); 102.50, 102.59 (CH-5); 114.56, 114.62 ($\mathbf{C}(CH_3)_2$); 124.98, 125.02 (d, $J_{C,P}$ = 184.2, =CHP); 136.65, 136.70 (d, $J_{C,P}$ = 1.9, $\mathbf{C}H_2$=CHP); 141.41, 141.50 (CH-6); 149.90, 149.93 (C-2); 162.92, 162.95 (C-4). ^{31}P NMR (202.3 MHz, $CDCl_3$): 18.59, 18.74. HR-ESI: $C_{34}H_{60}O_8N_2P$ [M + H] calcd 655.40818, found 655.40857; $C_{34}H_{59}O_8N_2NaP$ [M + Na] calcd 677.39012, found 677.39032.

Hexadecyloxypropyl 2′,3′-Isopropylideneuridin-5′-yl Vinylphosphonate (10g). The title compound was prepared according to general method B from **9a** (1.8 g, 6.1 mmol) and hexadecyloxypropanol (1.45 g, 3.05 mmol) in 71% yield (1.42 g, 2.16 mmol) as a colorless white wax. A mixture of diastereomers ∼1:1. ^{1}H NMR (500.0 MHz, $CDCl_3$): 0.88 (m, 6H, $C\mathbf{H}_3(CH_2)_{15}$); 1.23−1.33 (m, 52H, $CH_3(\mathbf{CH}_2)_{13}CH_2CH_2O$); 1.348, 1.352 (2 × q, 2 × 3H, ^4J = 0.6, $(CH_3)_2C$); 1.54 (m, 4H, $CH_3(CH_2)_{13}C\mathbf{H}_2CH_2O$); 1.57 (s, 6H, $(CH_3)_2C$); 1.92, 1.94 (2 × p, 2 × 2H, J_{vic} = 6.1, $OCH_2C\mathbf{H}_2CH_2OC_{16}H_{33}$); 3.38, 3.39 (2 × t, 2 × 2H, J_{vic} = 6.7, $CH_3(CH_2)_{13}CH_2C\mathbf{H}_2O$); 3.48, 3.49 (2 × t, 2 × 2H, J_{vic} = 6.1, $OCH_2CH_2C\mathbf{H}_2OC_{16}H_{33}$); 4.13, 4.15 (2 × td, 2 × 2H, J_{vic} = 6.1, $J_{H,P}$ = 4.8, $OC\mathbf{H}_2CH_2CH_2OC_{16}H_{33}$); 4.20−4.30 (m, 4H, H-5′); 4.34−4.38 (m, 2H, H-4′); 4.85, 4.86 (2 × dd, 2 × 1H, $J_{3',2'}$ = 6.5, $J_{3',4'}$ = 3.6, H-3′); 4.92 (dd, 2H, $J_{2',3'}$ = 6.5, $J_{2',1'}$ = 2.3, H-2′); 5.700, 5.704 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1 H-5); 5.74, 5.76 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.3, H-1′); 6.03, 6.05 (2 × ddd, 2 × 1H, $J_{H,P}$ = 22.9, J_{trans} = 18.4, J_{cis} = 12.7, =CHP); 6.16, 6.18 (2 × ddd, 2 × 1H, $J_{H,P}$ = 51.8, J_{cis} = 12.7, J_{gem} = 1.9, $CH_{cis}\mathbf{H}_{trans}$=CHP); 6.33, 6.35 (2 × ddd, 2 × 1H, $J_{H,P}$ = 25.5, J_{trans} = 18.4, J_{gem} = 1.9, $C\mathbf{H}_{cis}H_{trans}$=CHP); 7.34, 7.39 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (125.7 MHz, $CDCl_3$): 14.07 ($\mathbf{C}H_3(CH_2)_{15}$); 22.62 ($CH_3(\mathbf{C}H_2)_{13}CH_2CH_2O$); 25.19, 25.21 ($(\mathbf{C}H_3)_2C$); 26.08 ($CH_3(\mathbf{C}H_2)_{14}CH_2O$); 27.04, 27.06 ($(\mathbf{C}H_3)_2C$); 29.29, 29.45, 29.55, 29.57, 29.58, 29.63 ($CH_3(\mathbf{C}H_2)_{14}CH_2O$); 30.66, 30.68 (d, $J_{C,P}$ = 6.4, $OCH_2\mathbf{C}H_2CH_2OC_{16}H_{33}$); 31.85 ($CH_3(\mathbf{C}H_2)_{13}CH_2CH_2O$); 63.56 63.57 (d, $J_{C,P}$ = 5.5, $O\mathbf{C}H_2CH_2CH_2OC_{16}H_{33}$); 64.92, 65.01 (d, $J_{C,P}$ = 5.5, CH_2-5′); 66.32, 66.36 ($OCH_2CH_2\mathbf{C}H_2OC_{16}H_{33}$); 71.15, 71.16 ($CH_3(CH_2)_{14}\mathbf{C}H_2O$); 80.60, 80.67 (CH-3′); 84.44, 84.53 (CH-2′); 85.38, 85.62 (d, $J_{C,P}$ = 7.1, CH-4′); 93.82, 94.12 (CH-1′); 102.57, 102.64 (CH-5); 114.42, 114.46 ($\mathbf{C}(CH_3)_2$); 124.75, 124.80 (d, $J_{C,P}$ = 184.0, =CHP); 136.75, 136.77 (d, $J_{C,P}$ = 1.9, $\mathbf{C}H_2$=CHP); 141.43, 141.48 (CH-6); 150.16 (C-2); 163.29, 163.32 (C-4). ^{31}P NMR (202.3 MHz, $CDCl_3$): 18.64, 18.80. HR-ESI: $C_{33}H_{58}O_9N_2P$ $(M + H)^+$ calcd 657.3874; found 657.3876.

Hexadecyloxypropyl 4-*N*-Benzoyl-2′,3′-isopropylidenecytidin-5′-yl Vinylphosphonate (10h). The title compound was prepared according to general method B from **9b** (0.65 g, 1.36 mmol) and hexadecyloxypropanol (0.81 g, 2.7 mmol) in 78% yield (0.81 g, 1.06 mmol) as a white foam. A mixture of diastereomers ∼1:1. ^{1}H NMR (600.1 MHz, CD_3OD): 0.895 (m, 6H, $C\mathbf{H}_3(CH_2)_{15}$); 1.24−1.35 (m, 52H, $CH_3(\mathbf{CH}_2)_{13}CH_2CH_2O$); 1.368, 1.371 (2 × q, 2 × 3H, ^4J = 0.6, $(CH_3)_2C$); 1.51 (m, 4H, $CH_3(CH_2)_{13}C\mathbf{H}_2CH_2O$); 1.57 (s, 6H, $(CH_3)_2C$); 1.89, 1.91 (2 × p, 2 × 2H, J_{vic} = 6.1, $OCH_2C\mathbf{H}_2CH_2OC_{16}H_{33}$); 3.38, 3.39 (2 × t, 2 × 2H, J_{vic} = 6.7, $CH_3(CH_2)_{13}CH_2C\mathbf{H}_2O$); 3.47 (td, 2H, J_{vic} = 6.1, $J_{H,P}$ = 1.2, $OCH_2CH_2C\mathbf{H}_2OC_{16}H_{33}$); 3.49 (t, 2H, J_{vic} = 6.1, $OCH_2CH_2C\mathbf{H}_2OC_{16}H_{33}$); 4.09−4.16 (m, 4H, $OC\mathbf{H}_2CH_2CH_2OC_{16}H_{33}$); 4.27−4.36 (m, 4H, H-5′); 4.48 (m, 2H, H-4′); 4.91 (dd, 2H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 3.6, H-3′); 5.06, 507 (2 × dd, 2 × 1H, $J_{2',3'}$ = 6.2, $J_{2',1'}$ = 1.8, H-2′); 5.87, 5.88 (2 × d, 2 × 1H, $J_{1',2'}$ = 1.8, H-1′); 6.145, 6.17 (2 × ddd, 2 × 1H, $J_{H,P}$ = 24.0, J_{trans} = 18.4, J_{cis} = 12.8, =CHP); 6.257, 6.261 (2 × ddd, 2 × 1H, $J_{H,P}$ = 52.0, J_{cis} = 12.8, J_{gem} = 2.1, $CH_{cis}\mathbf{H}_{trans}$=CHP); 6.30, 6.31 (2 × ddd, 2 × 1H, $J_{H,P}$ = 26.0, J_{trans} = 18.4, J_{gem} = 2.1, $C\mathbf{H}_{cis}H_{trans}$=CHP); 7.54 (m, 4H, H-*m*-Bz); 7.59 (d, 2H, $J_{5,6}$ = 7.7, H-5); 7.64 (m, 2H, H-*p*-Bz); 7.99 (m, 4H, H-*o*-Bz); 8.16, 8.17 (2 × d, 2 × 1H, $J_{6,5}$ = 7.7, H-5). ^{13}C NMR (150.9 MHz, CD_3OD): 14.48 ($CH_3(CH_2)_{15}$); 23.75 ($CH_3(\mathbf{C}H_2)_{13}CH_2CH_2O$); 25.46, 25.48 ($(\mathbf{C}H_3)_2C$); 27.27 ($CH_3(\mathbf{C}H_2)_{14}CH_2O$); 27.43 ($(\mathbf{C}H_3)_2C$); 30.49, 30.61, 30.78, 30.80, 30.81 ($CH_3(\mathbf{C}H_2)_{14}CH_2O$); 31.70 (d, $J_{C,P}$ = 6.5, $OCH_2\mathbf{C}H_2CH_2OC_{16}H_{33}$); 33.09 ($CH_3(\mathbf{C}H_2)_{13}CH_2CH_2O$); 64.96, 64.98 (d, $J_{C,P}$ = 5.7, $O\mathbf{C}H_2CH_2CH_2OC_{16}H_{33}$); 66.97, 67.04 (d, $J_{C,P}$ = 5.6, CH_2-5′); 67.42, 67.43 ($OCH_2CH_2\mathbf{C}H_2OC_{16}H_{33}$); 72.10 ($CH_3(CH_2)_{14}\mathbf{C}H_2O$); 82.64, 82.635, 82.644 (CH-3′); 86.57, 86.58 (CH-2′); 88.23, 88.30 (d, $J_{C,P}$ = 7.2, CH-4′); 97.60, 97.71 (CH-1′); 98.51, 98.54 (CH-5); 115.10, 115.11 ($\mathbf{C}(CH_3)_2$); 125.63, 125.67 (d, $J_{C,P}$ = 183.9, =CHP); 129.25 (CH-*o*-Bz); 129.83 (CH-*m*-Bz); 134.14 (CH-*p*-Bz); 134.70 (C-*i*-Bz); 138.01, 138.04 (d, $J_{C,P}$ = 1.9, $\mathbf{C}H_2$=CHP); 148.26, 148.34 (CH-6); 157.58, 157.60 (C-2); 165.58 (C-4); 169.19 (CO-Bz). ^{31}P NMR (202.3 MHz, CD_3OD): 19.62, 19.72. HR-EI: $C_{40}H_{62}N_3O_9P$ $(M + H)^+$ calcd 759.4224; found 759.4225.

Hexadecyloxypropyl Uridin-5′-yl 2-([3*R*,4*R*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12a). The mixture of dihydroxypyrrolidine **11a** (0.12 g, 1.18 mmol) and phosphonate **10g** (0.4 g, 0.61 mmol) in *n*BuOH (15 mL) was stirred at 105 °C overnight. The solvent was removed, and the isopropylidene intermediate was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate. This compound was without further characterization dissolved in 0.5 M methanolic HCl (30 mL), and the mixture was stirred at rt for 4 h. The desired product was obtained after evaporation of solvent in 34% overall yield (152 mg, 0.205 mmol) as a white amorphous solid. A mixture of diastereomers ∼6:5. ^{1}H NMR (499.8 MHz, CD_3OD): 0.90 (m, 6H, $C\mathbf{H}_3(CH_2)_{15}$); 1.25−1.38 (m, 52H, $CH_3(\mathbf{CH}_2)_{13}CH_2CH_2O$); 1.55 (m, 4H, $CH_3(CH_2)_{13}C\mathbf{H}_2CH_2O$); 1.93 (m, 4H, $OCH_2C\mathbf{H}_2CH_2OC_{16}H_{33}$); 2.12−2.25 (m, 4H, CH_2P); 2.71 (dd, 4H, J_{gem} = 10.6, J_{vic} = 3.0, H-2b,5b-pyrr); 2.86−3.00 (m, 4H, CH_2N); 3.116, 3.118 (2 × dd, 2 × 2H, J_{gem} = 10.6, J_{vic} = 5.0, H-2a,5a-pyrr); 3.42, 3.43 (2 × t, 2 × 2H, J_{vic} = 6.6, $CH_3(CH_2)_{13}CH_2C\mathbf{H}_2O$); 3.515, 3.522 (2 × t, 2 × 2H, J_{vic} = 6.1, $OCH_2CH_2C\mathbf{H}_2OC_{16}H_{33}$); 4.08 (m, 4H, $OC\mathbf{H}_2CH_2CH_2OC_{16}H_{33}$); 4.11−4.21 (m, 8H, H-3′,4′, H-3,4-pyrr); 4.216, 4.218 (2 × dd, 2 × 1H, $J_{2',3'}$ = 5.1, $J_{2',1'}$ = 4.1, H-2′); 4.26 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.8, $J_{5'b,4'}$ = 4.5, H-5′b); 4.30−4.34 (m, 2H, H-5′); 4.36 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.6, $J_{5'a,4'}$ = 2.9, H-5′a); 5.751, 5.753 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.845 (d, 2H, $J_{1',2'}$ = 4.1, H-1′); 7.70, 7.73 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (125.7 MHz, CD_3OD): 14.47 ($\mathbf{C}H_3(CH_2)_{15}$); 23.73 ($CH_3(\mathbf{C}H_2)_{14}CH_2O$); 24.75, 24.81 (d, $J_{C,P}$ = 139.6, CH_2P); 27.28, 30.47, 30.63, 30.76, 30.79 ($CH_3(\mathbf{C}H_2)_{14}CH_2O$); 31.75, 31.78 (d, $J_{C,P}$ = 6.2, $OCH_2\mathbf{C}H_2CH_2OC_{16}H_{33}$); 33.06 ($CH_3(\mathbf{C}H_2)_{14}CH_2O$); 50.81, 50.85 ($CH_2N$); 60.95, 60.98 ($CH_2$-2,5-pyrr); 64.86, 64.93 (d, $J_{C,P}$ = 6.7, $OCH_2\mathbf{C}H_2CH_2OC_{16}H_{33}$); 66.52, 66.54 (d, $J_{C,P}$ = 6.2, CH_2-5′); 67.49, 67.53 ($OCH_2CH_2\mathbf{C}H_2OC_{16}H_{33}$); 70.79, 70.86 (CH-3′); 72.14 ($CH_3(CH_2)_{14}\mathbf{C}H_2O$); 74.87, 74.93 (CH-2′); 78.12, 78.19 (CH-3,4-pyrr); 83.57, 83.58 (d, $J_{C,P}$ = 6.6, CH-4′); 91.75, 91.85 (CH-1′); 103.02 (CH-5); 142.58, 142.64 (CH-6); 152.18, 152.20 (C-2); 165.99 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 31.37, 31.64. HR-ESI: $C_{34}H_{63}O_{11}N_3P$ $(M + H)^+$ calcd 720.41947; found 720.41939.

Hexadecyloxypropyl Cytidin-5′-yl 2-([3*R*,4*R*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12b). The mixture of dihydroxypyrrolidine **11a** (0.05 g, 0.49 mmol) and benzoylcytosine vinylphosphonate **10h** (0.34 g, 0.45 mmol) in *n*BuOH (10 mL) was stirred at 100 °C for 12 h. The solvent was removed and the protected intermediate was purified by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate. White foam obtained after evaporation of solvents was without further characterization (only LC-MS confirmation) dissolved in 8 M ethanolic methylamine (10 mL) and left aside at rt overnight. The mixture was concentrated in vacuo, coevaporated with ethanol (2 × 20 mL), dissolved in 0.2 M methanolic HCl (10 mL), and the reaction mixture was left aside at rt overnight. After concentration in vacuo (note: temperature in the

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bath did not exceed 40 °C) and coevaporation with ethanol (3 × 20 mL), the final product was obtained by preparative HPLC in 27% overall yield (90.6 mg, 120 μM) as a white amorphous solid. A mixture of diastereomers ~8:7. ^{1}H NMR (600.1 MHz, CD_3OD): 0.90 (m, 6H, $CH_3(CH_2)_{15}$); 1.25−1.38 (m, 52H, $CH_3(CH_2)_{13}CH_2CH_2O$); 1.55 (m, 4H, $CH_3(CH_2)_{13}CH_2CH_2O$); 1.93 (m, 4H, $OCH_2CH_2CH_2OC_{16}H_{33}$); 2.10−2.18 (m, 4H, CH_2P); 2.56 (bm, 4H, H-2b,5b-pyrr); 2.75−2.87 (bm, 4H, CH_2N); 3.00 (bm, 4H, H-2a,5a-pyrr); 3.41, 3.43 (2 × t, 2 × 2H, J_{vic} = 6.7, $CH_3(CH_2)_{13}CH_2CH_2O$); 3.51, 3.53 (2 × t, 2 × 2H, J_{vic} = 6.0, $OCH_2CH_2CH_2OC_{16}H_{33}$); 4.04 (m, 4H, H-3,4-pyrr); 4.08−4.23 (m, 10H, H-2′,3′,4′-pyrr, $OCH_2CH_2CH_2OC_{16}H_{33}$); 4.25−4.42 (m, 4H, H-5′); 5.83, 5.84 (d, 2H, $J_{1',2'}$ = 3.0, H-1′); 5.93, 5.94 (2 × d, 2 × 1H, $J_{5,6}$ = 7.6, H-5); 7.77, 7.79 (2 × d, 2 × 1H, $J_{6,5}$ = 7.6, H-6). ^{13}C NMR (150.9 MHz, CD_3OD): 14.47 ($CH_3(CH_2)_{15}$); 23.75 ($CH_3(CH_2)_{14}CH_2O$); 25.09, 25.14 (d, $J_{C,P}$ = 139.1, CH_2P); 27.29, 27.30, 30.49, 30.64, 30.77, 30.80 ($CH_3(CH_2)_{14}CH_2O$); 31.76, 31.79 (d, $J_{C,P}$ = 6.3, $OCH_2CH_2CH_2OC_{16}H_{33}$); 33.08 ($CH_3(CH_2)_{14}CH_2O$); 50.55 ($CH_2N$); 60.99, 61.02 ($CH_2$-2,5-pyrr); 64.71, 64.79 (d, $J_{C,P}$ = 6.6, $OCH_2CH_2CH_2OC_{16}H_{33}$); 66.24, 66.31 (d, $J_{C,P}$ = 6.4, CH_2-5′); 67.51, 67.55 ($OCH_2CH_2CH_2OC_{16}H_{33}$); 70.52, 70.55 (CH-3′); 72.15 ($CH_3(CH_2)_{14}CH_2O$); 75.68, 75.70 (CH-2′); 78.61, 78.66 (CH-3,4-pyrr); 83.13, 83.16 (d, $J_{C,P}$ = 6.6, CH-4′); 92.89, 92.90 (CH-1′); 96.17, 96.18 (C-5); 142.56, 142.64 (CH-6); 158.26, 158.27 (C-2); 167.646, 167.653 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 32.67, 32.99. HR-ESI: $C_{34}H_{64}O_{10}N_4P$ $(M + H)^+$ calcd 719.4355; found 719.4357.

Hexadecyloxypropyl Uridin-5′-yl 2-([3*S*,4*S*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12c). The mixture of dihydroxypyrrolidine **11b** (0.26 g, 2.56 mmol) and phosphonate **10g** (0.84 g, 1.28 mmol) in *n*BuOH (30 mL) was stirred at 105 °C overnight. The solvent was removed, and the isopropylidene intermediate was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate. This compound was without further characterization dissolved in 0.5 M methanolic HCl (40 mL), and the mixture was stirred at rt for 4 h. After concentration in vacuo (note: temperature in the bath did not exceeded 40 °C) and coevaporation with ethanol (3 × 20 mL) the desired product was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate in 36% overall yield (344 mg, 0.46 mmol) as a white amorphous solid. A mixture of diastereomers ~6:5. ^{1}H NMR (499.8 MHz, CD_3OD): 0.90 (m, 6H, $CH_3(CH_2)_{15}$); 1.25−1.39 (m, 52H, $CH_3(CH_2)_{13}CH_2CH_2O$); 1.55 (m, 4H, $CH_3(CH_2)_{13}CH_2CH_2O$); 1.95 (m, 4H, $OCH_2CH_2CH_2OC_{16}H_{33}$); 2.37−2.52 (m, 4H, CH_2P); 3.16 (bm, 2H, H-2b,5b-pyrr); 3.42 (t, 2H, J_{vic} = 6.7, $CH_3(CH_2)_{13}CH_2CH_2O$); 3.425 (bm, 2H, H-2b,5b-pyrr); 3.43 (t, 2H, J_{vic} = 6.7, $CH_3(CH_2)_{13}CH_2CH_2O$); 3.515 (m, 4H, CH_2N); 3.52, 3.53 (2 × t, 2 × 2H, J_{vic} = 6.1, $OCH_2CH_2CH_2OC_{16}H_{33}$); 3.59, 3.92 (2 × bm, 2 × 2H, H-2a,5a-pyrr); 4.14 (m, 2H, H-4′); 4.16 (m, 2H, H-3′); 4.18−4.27 (m, 10H, H-2′, $OCH_2CH_2CH_2OC_{16}H_{33}$, H-3,4-pyrr); 4.31 (ddd, 1H, J_{gem} = 11.5, $J_{H,P}$ = 7.6, $J_{5'b,4'}$ = 5.4, H-5′b); 4.36 (m, 2H, H-5′); 4.40 (ddd, 1H, J_{gem} = 11.5, $J_{H,P}$ = 7.3, $J_{5'a,4'}$ = 2.9, H-5′a); 5.742, 5.746 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.78, 5.80 (2 × d, 2 × 1H, $J_{1',2'}$ = 3.9, H-1′); 7.68, 7.70 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (125.7 MHz, CD_3OD): 14.45 ($CH_3(CH_2)_{15}$); 23.32 (d, $J_{C,P}$ = 142.0, CH_2P); 23.74 ($CH_3(CH_2)_{14}CH_2O$); 27.29, 30.48, 30.64, 30.76, 30.79 ($CH_3(CH_2)_{14}CH_2O$); 31.70, 31.72 (d, $J_{C,P}$ = 6.1, $OCH_2CH_2CH_2OC_{16}H_{33}$); 33.07 ($CH_3(CH_2)_{14}CH_2O$); 52.18, 52.22 ($CH_2N$); 60.67, 60.75, 60.98, 61.01 (CH_2-2,5-pyrr); 65.33, 65.58 (d, $J_{C,P}$ = 6.7, $OCH_2CH_2CH_2OC_{16}H_{33}$); 67.16 (d, $J_{C,P}$ = 6.5, CH_2-5′); 67.44 ($OCH_2CH_2CH_2OC_{16}H_{33}$); 67.45 (d, $J_{C,P}$ = 4.3, CH_2-5′); 67.47 ($OCH_2CH_2CH_2OC_{16}H_{33}$); 70.77 (CH-3′); 72.16 ($CH_3(CH_2)_{14}CH_2O$); 74.55, 74.60 (CH-2′); 75.67, 75.68, 76.04 (CH-3,4-pyrr); 83.29 (d, $J_{C,P}$ = 6.2, CH-4′); 83.40 (d, $J_{C,P}$ = 5.8, CH-4′); 92.83, 92.85 (CH-1′); 103.03, 103.10 (CH-5); 143.12, 143.19 (CH-6); 152.13, 152.14 (C-2); 166.00 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 26.79, 27.26.

Hexadecyloxypropyl Uridin-5′-yl 2-([3*R*,4*S*,5*S*]-3,4-Trihydroxypiperidin-1-*N*-yl)ethylphosphonate (12d). The mixture of trihydroxypiperidine **11e** (0.04 g, 0.33 mmol) and phosphonate **10g** (0.1 g, 0.16 mmol) in *n*BuOH (5 mL) was stirred at 105 °C overnight. The solvent was removed and the isopropylidene intermediate was obtained by flash chromatography on silica gel using linear gradient of ethanol in chloroform. This compound was without characterization dissolved in 0.5 M methanolic HCl (20 mL), and the mixture was stirred at rt for 2 h. After concentration in vacuo (note: temperature in the bath did not exceeded 40 °C) and coevaporation with ethanol (3 × 20 mL), the desired product was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate in 68% overall yield (80 mg, 0.11 mmol) as a white amorphous solid. A mixture of diastereomers ~7:3. ^{1}H NMR (499.8 MHz, CD_3OD): 0.90 (m, 6H, $CH_3(CH_2)_{15}$); 1.25−1.38 (m, 52H, $CH_3(CH_2)_{13}CH_2CH_2O$); 1.55 (m, 4H, $CH_3(CH_2)_{13}CH_2CH_2O$); 1.93 (m, 4H, $OCH_2CH_2CH_2OC_{16}H_{33}$); 2.10−2.18 (m, 4H, CH_2P); 2.43, 2.54 (2 × bm, 2 × 4H, H-2,6-pip); 2.75 (m, 4H, CH_2N); 3.42, 3.43 (2 × t, 2 × 2H, J_{vic} = 6.6, $CH_3(CH_2)_{13}CH_2CH_2O$); 3.52, 3.53 (2 × t, 2 × 2H, J_{vic} = 6.1, $OCH_2CH_2CH_2OC_{16}H_{33}$); 3.68 (bm, 4H, H-3,5-pip); 3.81 (bm, 2H, H-4-pip); 4.11−4.22 (m, 10H, H-2′,3′,4′, $OCH_2CH_2CH_2OC_{16}H_{33}$); 4.23−4.38 (m, 4H, H-5′); 5.746, 5.749 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.84 (d, 2H, $J_{1',2'}$ = 4.1, H-1′); 7.71, 7.73 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (125.7 MHz, CD_3OD): 14.45 ($CH_3(CH_2)_{15}$); 23.55, 23.65 (d, $J_{C,P}$ = 138.5, CH_2P); 23.75 ($CH_3(CH_2)_{14}CH_2O$); 27.30, 30.49, 30.63, 30.77, 30.80 ($CH_3(CH_2)_{14}CH_2O$); 31.79, 31.82 (d, $J_{C,P}$ = 6.2, $OCH_2CH_2CH_2OC_{16}H_{33}$); 33.08 ($CH_3(CH_2)_{14}CH_2O$); 51.75 ($CH_2N$); 54.40 ($CH_2$-2,6-pip); 64.72, 64.78 (d, $J_{C,P}$ = 6.5, $OCH_2CH_2CH_2OC_{16}H_{33}$); 66.37 (d, $J_{C,P}$ = 6.3, CH_2-5′); 67.51, 67.55 ($OCH_2CH_2CH_2OC_{16}H_{33}$); 69.67 (CH-3,5-pip); 70.81, 70.83 (CH-3′); 71.83 (CH-4-pip); 72.17 ($CH_3(CH_2)_{14}CH_2O$); 74.97, 75.01 (CH-2′); 83.59, 83.63 (d, $J_{C,P}$ = 6.7, CH-4′); 91.84, 91.92 (CH-1′); 103.00 (CH-5); 142.56, 142.60 (CH-6); 152.18, 152.19 (C-2); 166.05 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 32.91, 33.23. HR-ESI: $C_{35}H_{65}O_{11}N_3P$ $(M + H)^+$ calcd 734.4347; found 734.4351.

Hexadecyloxypropyl Uridin-5′-yl 2-([3*R*,5*R*]-3,4-Dihydroxypiperidin-1-*N*-yl)ethylphosphonate (12e). The mixture of dihydroxypiperidine **11e** (0.05 g, 0.42 mmol) and phosphonate **10g** (0.25 g, 0.38 mmol) in *n*BuOH (5 mL) was stirred at 105 °C overnight. The solvent was removed, and the isopropylidene intermediate was obtained by flash chromatography on silica gel using linear gradient of ethanol in chloroform. This compound was without characterization dissolved in 0.5 M methanolic HCl (40 mL), and the mixture was stirred at rt for 3 h. After concentration in vacuo (note: temperature in the bath did not exceeded 40 °C) and coevaporation with ethanol (3 × 20 mL), the desired product was obtained by chromatography on silica gel using linear gradient of H1 in ethyl acetate in 76% overall yield (230 mg, 0.29 mmol) as a white amorphous solid. A mixture of diastereomers ~6:4. ^{1}H NMR (600.1 MHz, CD_3OD): 0.90 (m, 6H, $CH_3(CH_2)_{15}$); 1.25−1.38 (m, 52H, $CH_3(CH_2)_{13}CH_2CH_2O$); 1.55 (m, 4H, $CH_3(CH_2)_{13}CH_2CH_2O$); 1.70 (bm, 4H, H-4-pip); 1.93 (m, 4H, $OCH_2CH_2CH_2OC_{16}H_{33}$); 2.12−2.19 (m, 4H, CH_2P); 2.36, 2.57 (2 × m, 2 × 4H, H-2,6-pip); 2.66−2.76 (m, 4H, CH_2N); 3.42, 3.43 (2 × t, 2 × 2H, J_{vic} = 6.6, $CH_3(CH_2)_{13}CH_2CH_2O$); 3.51, 3.53 (2 × t, 2 × 2H, J_{vic} = 6.1, $OCH_2CH_2CH_2OC_{16}H_{33}$); 4.01 (m, 4H, H-3,5-pip); 4.12−4.24 (m, 10H, H-2′,3′,4′, $OCH_2CH_2CH_2OC_{16}H_{33}$); 4.24−4.40 (m, 4H, H-5′); 5.751, 5.754 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.854, 5.856 (2 × d, 2 × 1H, $J_{1',2'}$ = 4.1, H-1′); 7.71, 7.74 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (150.9 MHz, CD_3OD): 14.49 ($CH_3(CH_2)_{15}$); 23.49, 23.53 (d, $J_{C,P}$ = 138.4, CH_2P); 23.75 ($CH_3(CH_2)_{14}CH_2O$); 27.29, 27.30, 30.49, 30.65, 30.78, 30.81 ($CH_3(CH_2)_{14}CH_2O$); 31.76, 31.83 (d, $J_{C,P}$ = 6.3, $OCH_2CH_2CH_2OC_{16}H_{33}$); 33.08 ($CH_3(CH_2)_{14}CH_2O$); 40.63, 40.65 ($CH_2$-4-pip); 52.02, 52.04 (d, $J_{C,P}$ = 2.0, CH_2N); 60.07, 60.10 (CH_2-2,6-pip); 64.63, 64.75 (d, $J_{C,P}$ = 6.6, $OCH_2CH_2CH_2OC_{16}H_{33}$); 65.58 (CH-3,5-pip); 66.27, 66.52 (d, $J_{C,P}$ = 6.2, CH_2-5′); 67.51, 67.54 ($OCH_2CH_2CH_2OC_{16}H_{33}$); 70.77, 70.84 (CH-3′); 72.13, 72.15 ($CH_3(CH_2)_{14}CH_2O$); 74.99, 75.05 (CH-2′); 83.59, 83.60 (d, $J_{C,P}$ = 6.6, CH-4′); 91.65, 91.66 (CH-1′); 102.99 (CH-5); 142.45, 142.51 (CH-6); 152.16, 152.18 (C-2); 165.99 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 33.22, 33.53. HR-ESI: $C_{35}H_{65}O_{10}N_3P$ $(M + H)^+$ calcd 718.4402; found 718.4402.

2 Pivaloylthioethyl Uridin-5′-yl 2-([3*R*,4*R*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12f). The mixture of dihydrox-

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ypyrrolidine **11a** (0.25 g, 2.4 mmol) and phosphonate **10a** (0.57 g, 1.1 mmol) in *n*BuOH (12 mL) was stirred at 100 °C for 12 h. The solvent was removed, and the product was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate in 85% yield (0.58 g, 0.93 mmol) as white foam (HR-ESI: $C_{25}H_{39}O_{11}N_3PS$ $(M - H)^-$ calcd 620.2048; found 620.2049). The solution of this intermediate (0.58 g, mmol) in 0.2 M methanolic HCl (50 mL) was stirred at rt overnight. The mixture was concentrated in vacuo, and the product was obtained by preparative reverse phase HPLC in 26% overall yield (0.177 g, 0.29 mmol) as a white amorphous solid. A mixture of diastereomers ~5:3. ^{1}H NMR (499.8 MHz, CD_3OD): 1.23, 1.24 (2 × s, 2 × 9H, $(CH_3)_3C$); 2.46 (m, 4H, CH_2P); 3.12−3.24 (m, 4H, SCH_2CH_2O); 3.38 (bm, 4H, H-2b,5b-pyrr); 3.51 (m, 4H, CH_2N); 3.66 (bm, 4H, H-2a,5a-pyrr); 4.11−4.22 (m, 8H, H-3′,4′, OCH_2CH_2S); 4.26 (m, 6H, H-2′, H-3,4-pyrr); 4.34 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 8.0, $J_{5'b,4'}$ = 5.4, H-5′b); 4.36−4.40 (m, 2H, H-5′); 4.41 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 7.7, $J_{5'a,4'}$ = 2.8, H-5′a); 5.746, 5.750 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.797, 5.800 (2 × d, 2 × 1H, $J_{1',2'}$ = 4.0, H-1′); 7.68, 7.70 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (125.7 MHz, CD_3OD): 23.43, 23.49 (d, $J_{C,P}$ = 142.2, CH_2P); 27.66 ($(CH_3)_3C$); 29.52, 29.58 (d, $J_{C,P}$ = 6.6, SCH_2CH_2O); 47.56 ($C(CH_3)_3$); 52.13, 52.17 (CH_2N); 60.86, 60.89 (CH_2-2,5-pyrr); 66.20, 66.34 (d, $J_{C,P}$ = 6.6, OCH_2CH_2S); 67.40 (d, $J_{C,P}$ = 6.8, CH_2-5′); 67.50 (d, $J_{C,P}$ = 6.4, CH_2-5′); 70.77 (CH-3′); 74.54, 74.58 (CH-2′); 75.90 (CH-3,4-pyrr); 83.29, 83.34 (d, $J_{C,P}$ = 6.0, CH-4′); 92.65, 92.82 (CH-1′); 103.10, 103.12 (CH-5); 143.11, 143.14 (CH-6); 152.17 (C-2); 166.01 (C-4); 207.15 (COS). ^{31}P NMR (202.3 MHz, CD_3OD): 26.92, 27.39. HR-ESI: $C_{22}H_{37}O_{11}N_3PS$ $(M + H)^+$ calcd 582.18809; found 582.18811.

Ethyl Uridin-5′-yl 2-([3*R*,4*R*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12g). The mixture of dihydroxypyrrolidine **11a** (0.24 g, 2.3 mmol) and phosphonate **10b** (0.6 g, 1.5 mmol) in *n*BuOH (12 mL) was stirred at 100 °C for 12 h. The solvent was removed, and the intermediate was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate. The intermediate (0.76 g, 1.5 mmol) was dissolved in 0.5 M methanolic HCl (100 mL), and the mixture was stirred at rt overnight. The mixture was concentrated in vacuo, coevaporated with diethylether (3 × 50 mL) and ethylacetate (2 × 50 mL), and the product was obtained by crystallization from a mixture of ethanol and diethylether (1:1) in 77% yield (0.58 g, 1.16 mmol) as a white solid. Characterized by NMR as hydrochloride, a mixture of diastereomers ~1:1. ^{1}H NMR (600.1 MHz, DMSO-d_6): 1.235, 1.242 (2 × s, 2 × 3H, J_{vic} = 7.0, CH_3CH_2); 2.38 (m, 4H, CH_2P); 3.07 (bd, 2H, J_{gem} = 12.1, H-2b or 5b-pyrr); 3.30 (bm, 6H, CH_2N, H-2b or 5b-pyrr); 3.42, 3.67 (2 × bm, 2 × 2H, H-2a,5a-pyrr); 3.95, 3.97 (2 × t, 2 × 1H, $J_{3',2'}$ = $J_{3',4'}$ = 4.9, H-3′); 3.98 (m, 2H, H-4′); 4.05 (m, 4H, CH_3CH_2); 4.07 (m, 2H, H-2′); 4.09 (m, 4H, H-3,4-pyrr); 4.13, 4.20 (2 × m, 4H, H-5′); 5.38, 5.55 (2 × bs, 2 × 2H, OH-2′,3′); 5.67, 5.68 (2 × dd, 2 × 1H, $J_{5,6}$ = 8.1, $J_{5,NH}$ = 2.6, H-5); 5.76 (d, 2H, $J_{1',2'}$ = 5.2, H-1′); 5.83 (bs, 4H, OH-3,4-pyrr); 7.64, 7.66 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6); 10.61 (bs, 2H, NH-pyrr); 11.39 (s, 2H, NH-3). ^{13}C NMR (150.9 MHz, DMSO-d_6): 16.49 (d, $J_{C,P}$ = 5.1, CH_3CH_2); 22.05, 22.10 (d, $J_{C,P}$ = 139.2, CH_2P); 50.90 (CH_2N); 58.89, 58.95, 59.07 (CH_2-2,5-pyrr); 66.37, 66.44 (d, $J_{C,P}$ = 6.2, CH_2CH_3); 65.51, 65.56 (d, $J_{C,P}$ = 6.5, CH_2-5′); 69.67, 69.73 (CH-3′); 72.81, 72.85 (CH-2′); 74.41, 74.57 (CH-3,4-pyrr); 82.23 (d, $J_{C,P}$ = 5.2, CH-4′); 88.84, 88.94 (CH-1′); 102.36, 102.37 (CH-5); 141.21, 141.23 (CH-6); 150.95, 150.97 (C-2); 163.44 (C-4). ^{31}P NMR (202.3 MHz, DMSO-d_6): 27.31, 27.58. HR-ESI: $C_{17}H_{29}O_{10}N_3P$ $(M + H)^+$ calcd 466.1585; found 466.1585.

Tetradecyl Uridin-5′-yl 2-([3*R*,4*R*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12h). The mixture of dihydroxypyrrolidine **11a** (0.42 g, 4.1 mmol) and phosphonate **10c** (1.17 g, 2.05 mmol) in *n*BuOH (30 mL) was stirred at 100 °C overnight. The solvent was removed, and the isopropylidene intermediate was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate. This compound was without further characterization dissolved in 0.5 M methanolic HCl (50 mL), and the mixture was stirred at rt for 4 h. The reaction mixture was concentrated in vacuo and coevaporated with ethanol (3 × 40 mL). The desired product was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate in 66% overall yield (862 mg, 1.36 mmol) as a white amorphous solid. A mixture of two diastereomers ~6:5. ^{1}H NMR (600.1 MHz, CD_3OD): 0.90 (m, 6H, $CH_3(CH_2)_{13}$); 1.25−1.43 (m, 44H, $CH_3(CH_2)_{11}CH_2CH_2O$); 1.68 (m, 4H, $CH_3(CH_2)_{11}CH_2CH_2O$); 2.06−2.19 (m, 4H, CH_2P); 2.56 (m, 4H, H-2b,5b-pyrr); 2.74−2.88 (m, 4H, CH_2N); 3.00 (m, 4H, H-2a,5a-pyrr); 4.04 (m, 4H, H-3,4-pyrr); 4.06−4.13 (m, 4H, $CH_3(CH_2)_{11}CH_2CH_2O$); 4.13−4.16 (m, 4H, H-3′,4′); 4.200, 4.202 (2 × dd, 2 × 1H, $J_{2',3'}$ = 5.0, $J_{2',1'}$ = 4.0, H-2′); 4.24 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.7, $J_{5'b,4'}$ = 4.3, H-5′b); 4.29 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 7.2, $J_{5'b,4'}$ = 2.4, H-5′b); 4.31 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 5.0, $J_{5'a,4'}$ = 2.6, H-5′a); 4.35 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.6, $J_{5'a,4'}$ = 2.8, H-5′a); 5.736, 5.745 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.85, 5.86 (2 × d, 2 × 1H, $J_{1',2'}$ = 4.0, H-1′); 7.71, 7.74 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (150.9 MHz, CD_3OD): 14.46 ($CH_3(CH_2)_{13}$); 23.75 ($CH_3(CH_2)_{11}CH_2CH_2O$); 25.11 (d, $J_{C,P}$ = 139.5, CH_2P); 25.16 (d, $J_{C,P}$ = 139.0, CH_2P); 26.65, 30.27, 30.28, 30.50, 30.68, 30.71, 30.72, 30.78, 30.80, 30.82 ($CH_3(CH_2)_{11}CH_2CH_2O$); 31.55, 31.57 (d, $J_{C,P}$ = 6.0, $CH_3(CH_2)_{11}CH_2CH_2O$); 33.09 ($CH_3(CH_2)_{11}CH_2CH_2O$); 50.57, 50.60 (d, $J_{C,P}$ = 1.0, CH_2N); 61.01, 61.03 (CH_2-2,5-pyrr); 66.36, 66.42 (d, $J_{C,P}$ = 6.3, CH_2-5′); 66.60, 67.63 (d, $J_{C,P}$ = 6.8, $CH_3(CH_2)_{11}CH_2CH_2O$); 70.81, 70.88 (CH-3′); 74.95, 75.01 (CH-2′); 78.60, 78.65 (CH-3,4-pyrr); 83.63, 83.64 (d, $J_{C,P}$ = 6.6, CH-4′); 91.57, 91.75 (CH-1′); 102.95, 102.98 (CH-5); 142.56, 142.57 (CH-6); 152.18, 152.22 (C-2); 166.03 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 32.08, 32.38. HR-ESI: $C_{29}H_{53}O_{10}N_3P$ $(M + H)^+$ calcd 634.34631, found 634.34635.

Hexadecyl Uridin-5′-yl 2-([3*R*,4*R*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12i). The mixture of dihydroxypyrrolidine **11a** (0.36 g, 3.45 mmol) and phosphonate **10d** (1.38 g, 2.3 mmol) in *n*BuOH (20 mL) was stirred at 100 °C overnight. The solvent was removed and isopropylidene intermediate was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate. This compound was without further characterization dissolved in 0.5 M methanolic HCl (50 mL), and the mixture was stirred at rt for 4 h. The reaction mixture was concentrated in vacuo and coevaporated with ethanol (3 × 40 mL). The desired product was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate in 39% overall yield (590 mg, 0.89 mmol) as a white amorphous solid. A mixture of two diastereomers ~1:1. ^{1}H NMR (600.1 MHz, CD_3OD): 0.90 (m, 6H, $CH_3(CH_2)_{15}$); 1.24−1.43 (m, 52H, $CH_3(CH_2)_{13}CH_2CH_2O$); 1.68 (m, 4H, $CH_3(CH_2)_{13}CH_2CH_2O$); 2.06−2.18 (m, 4H, CH_2P); 2.55 (m, 4H, H-2b,5b-pyrr); 2.73−2.86 (m, 4H, CH_2N); 2.99 (m, 4H, H-2a,5a-pyrr); 4.04 (m, 4H, H-3,4-pyrr); 4.05−4.12 (m, 4H, $CH_3(CH_2)_{13}CH_2CH_2O$); 4.12−4.16 (m, 4H, H-3′,4′); 4.198, 4.200 (2 × dd, 2 × 1H, $J_{2',3'}$ = 5.1, $J_{2',1'}$ = 4.1, H-2′); 4.24 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.5, $J_{5'b,4'}$ = 4.2, H-5′b); 4.28 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 7.2, $J_{5'b,4'}$ = 2.4, H-5′b); 4.30 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 5.2, $J_{5'a,4'}$ = 2.7, H-5′a); 4.34 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.6, $J_{5'a,4'}$ = 2.8, H-5′a); 5.73, 5.74 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.85, 5.86 (2 × d, 2 × 1H, $J_{1',2'}$ = 4.1, H-1′); 7.71, 7.74 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (150.9 MHz, CD_3OD): 14.46 ($CH_3(CH_2)_{15}$); 23.75 ($CH_3(CH_2)_{13}CH_2CH_2O$); 25.14, 25.19 (d, $J_{C,P}$ = 139.3, CH_2P); 26.65, 30.27, 30.28, 30.50, 30.68, 30.69, 30.72, 30.73, 30.78, 30.81 ($CH_3(CH_2)_{13}CH_2CH_2O$); 31.55, 31.57 (d, $J_{C,P}$ = 6.0, $CH_3(CH_2)_{13}CH_2CH_2O$); 33.09 ($CH_3(CH_2)_{13}CH_2CH_2O$); 50.55, 50.59 (d, $J_{C,P}$ = 1.0, CH_2N); 61.02, 61.05 (CH_2-2,5-pyrr); 66.34, 66.42 (d, $J_{C,P}$ = 6.3, CH_2-5′); 66.60, 67.63 (d, $J_{C,P}$ = 6.8, $CH_3(CH_2)_{13}CH_2CH_2O$); 70.81, 70.89 (CH-3′); 74.96, 75.02 (CH-2′); 78.65, 78.69 (CH-3,4-pyrr); 83.64, 83.66 (d, $J_{C,P}$ = 6.6, CH-4′); 91.58, 91.76 (CH-1′); 102.94, 102.98 (CH-5); 142.57, 142.58 (CH-6); 152.18, 152.22 (C-2); 166.04 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 32.33, 32.63. HR-ESI: $C_{31}H_{57}O_{10}N_3P$ $(M + H)^+$ calcd 662.37761, found 662.37759.

Hexadecyl Uridin-5′-yl 2-([3*R*,4*R*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12i-P1). The title compound was obtained by preparative HPLC from **12i** using Waters AutoPurification system with 2545 quarternary gradient module and 3100 single quadrupole mass detector using LUNA C18, column (Phenomenex,

250 mm × 21.2 mm, 5 μm) at flow rate 10 mL/min using gradient: A, 50 mM NH_4HCO_3 in 50% aq CH_3CN; C, CH_3CN; A→A/10 min, A→B/50 min. ^{1}H NMR (600.1 MHz, CD_3OD): 0.90 (t, 3H, J_{vic} = 7.2, C**H**$_3$(CH$_2$)$_{15}$); 1.26−1.41 (m, 26H, CH$_3$(C**H**$_2$)$_{13}$CH$_2$CH$_2$O); 1.69 (m, 2H, CH$_3$(CH$_2$)$_{13}$C**H**$_2$CH$_2$O); 2.20 (m, 2H, CH$_2$P); 2.80 (bdd, 2H, J_{gem} = 10.5, J_{vic} = 1.9, H-2b,5b-pyrr); 3.00 (bm, 2H, CH$_2$N); 3.19 (bdd, 2H, J_{gem} = 10.9, J_{vic} = 4.5, H-2a,5a-pyrr); 4.05−4.16 (m, 6H, H-3′,4′, H-3,4-pyrr, CH$_3$(CH$_2$)$_{13}$CH$_2$C**H**$_2$O); 4.21 (dd, 1H, $J_{2',3'}$ = 5.1, $J_{2',1'}$ = 4.2, H-2′); 4.26 (ddd, 1H, J_{gem} = 11.3, $J_{H,P}$ = 7.0, $J_{5'b,4'}$ = 4.7, H-5′b); 4.36 (ddd, 1H, J_{gem} = 11.3, $J_{H,P}$ = 6.7, $J_{5'a,4'}$ = 2.6, H-5′a); 5.74 (d, 1H, $J_{5,6}$ = 8.1, H-5); 5.83 (d, 1H, $J_{1',2'}$ = 4.2, H-1′); 7.73 (d, 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (150.9 MHz, CD_3OD): 14.46 (**C**H$_3$(CH$_2$)$_{15}$); 23.76 (CH$_3$(**C**H$_2$)$_{13}$CH$_2$CH$_2$O); 24.66 (d, $J_{C,P}$ = 140.2, CH$_2$P); 26.64, 30.29, 30.50, 30.69, 30.74, 30.78, 30.79, 30.82 (CH$_3$(CH$_2$)$_{13}$CH$_2$CH$_2$O); 31.54 (d, $J_{C,P}$ = 6.0, CH$_3$(CH$_2$)$_{13}$CH$_2$CH$_2$O); 33.09 (CH$_3$(CH$_2$)$_{13}$CH$_2$CH$_2$O); 51.04 (CH$_2$N); 60.99 (CH$_2$-2,5-pyrr); 66.66 (d, $J_{C,P}$ = 6.4, CH$_2$-5′); 67.84 (d, $J_{C,P}$ = 6.8, CH$_3$(CH$_2$)$_{13}$CH$_2$**C**H$_2$O); 70.86 (CH-3′); 74.84 (CH-2′); 77.87 (CH-3,4-pyrr); 83.55 (d, $J_{C,P}$ = 6.4, CH-4′); 91.96 (CH-1′); 102.98 (CH-5); 142.74 (CH-6); 152.19 (C-2); 166.03 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 31.27.

Hexadecyl Uridin-5′-yl 2-([3*R*,4*R*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12i-P2). The title compound was obtained by preparative HPLC from **12i** using Waters AutoPurification system with 2545 quarternary gradient module and 3100 single quadrupole mass detector using LUNA C18, column (Phenomenex, 250 mm × 21.2 mm, 5 μm) at flow rate 10 mL/min using gradient: A, 50 mM NH_4HCO_3 in 50% aq CH_3CN; C, CH_3CN; A→A/10 min, A→B/50 min. ^{1}H NMR (600.1 MHz, CD_3OD): 0.90 (t, 3H, J_{vic} = 7.2, C**H**$_3$(CH$_2$)$_{15}$); 1.26−1.43 (m, 26H, CH$_3$(C**H**$_2$)$_{13}$CH$_2$CH$_2$O); 1.69 (m, 2H, CH$_3$(CH$_2$)$_{13}$C**H**$_2$CH$_2$O); 2.18 (m, 2H, CH$_2$P); 2.72 (bd, 2H, J_{gem} = 10.5, H-2b,5b-pyrr); 2.94 (bm, 2H, CH$_2$N); 3.13 (bdd, 2H, J_{gem} = 10.5, J_{vic} = 5.0, H-2a,5a-pyrr); 4.05−4.15 (m, 6H, H-3′,4′, H-3,4-pyrr, CH$_3$(CH$_2$)$_{13}$CH$_2$C**H**$_2$O);4.21 (dd, 1H, $J_{2',3'}$ = 5.0, $J_{2',1'}$ = 4.1, H-2′); 4.27−4.34 (m, 2H, H-5′); 5.73 (d, 1H, $J_{5,6}$ = 8.1, H-5); 5.84 (d, 1H, $J_{1',2'}$ = 4.1, H-1′); 7.70 (d, 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (150.9 MHz, CD_3OD): 14.46 (**C**H$_3$(CH$_2$)$_{15}$); 23.76 (CH$_3$(**C**H$_2$)$_{13}$CH$_2$CH$_2$O); 24.78 (d, $J_{C,P}$ = 139.8, CH$_2$P); 26.64, 30.28, 30.50, 30.68, 30.69, 30.73, 30.79, 30.81, 30.82 (CH$_3$(**C**H$_2$)$_{13}$CH$_2$CH$_2$O); 31.56 (d, $J_{C,P}$ = 6.0, CH$_3$(CH$_2$)$_{13}$**C**H$_2$CH$_2$O); 33.10 (CH$_3$(**C**H$_2$)$_{13}$CH$_2$CH$_2$O); 50.94 (CH$_2$N); 60.99 (CH$_2$-2,5-pyrr); 66.59 (d, $J_{C,P}$ = 6.1, CH$_2$-5′); 67.71 (d, $J_{C,P}$ = 6.8, CH$_3$(CH$_2$)$_{13}$CH$_2$**C**H$_2$O); 70.81 (CH-3′); 74.93 (CH-2′); 78.06 (CH-3,4-pyrr); 83.59 (d, $J_{C,P}$ = 6.6, CH-4′); 92.02 (CH-1′); 102.96 (CH-5); 142.69 (CH-6); 152.17 (C-2); 166.04 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 32.03.

Octadecyl Uridin-5′-yl 2-([3*R*,4*R*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12j). The mixture of dihydroxypyrrolidine **11a** (0.3 g, 2.94 mmol) and phosphonate **10e** (1.23 g, 1.96 mmol) in *n*BuOH (20 mL) was stirred at 100 °C overnight. The solvent was removed, and the isopropylidene intermediate was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate. This compound was without further characterization dissolved in 0.5 M methanolic HCl (50 mL), and the mixture was stirred at rt for 4 h. The reaction mixture was concentrated in vacuo and coevaporated with ethanol (3 × 40 mL). The desired product was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate in 68% overall yield (920 mg, 1.33 mmol) as a white amorphous solid. A mixture of two diastereomers ∼6:5. ^{1}H NMR (600.1 MHz, CD_3OD): 0.90 (m, 6H, C**H**$_3$(CH$_2$)$_{17}$); 1.25−1.43 (m, 60H, CH$_3$(C**H**$_2$)$_{15}$CH$_2$CH$_2$O); 1.68 (m, 4H, CH$_3$(CH$_2$)$_{15}$C**H**$_2$CH$_2$O); 2.06−2.19 (m, 4H, CH$_2$P); 2.55 (m, 4H, H-2b,5b-pyrr); 2.73−2.86 (m, 4H, CH$_2$N); 2.99 (m, 4H, H-2a,5a-pyrr); 4.04 (m, 4H, H-3,4-pyrr); 4.05−4.13 (m, 4H, CH$_3$(CH$_2$)$_{15}$CH$_2$C**H**$_2$O); 4.13−4.16 (m, 4H, H-3′,4′); 4.199, 4.201 (2 × dd, 2 × 1H, $J_{2',3'}$ = 4.8, $J_{2',1'}$ = 4.2, H-2′); 4.24 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.7, $J_{5'b,4'}$ = 4.2, H-5′b); 4.28 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 7.4, $J_{5'b,4'}$ = 2.5, H-5′b); 4.30 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 5.2, $J_{5'a,4'}$ = 2.7, H-5′a); 4.34 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.5, $J_{5'a,4'}$ = 2.7, H-5′a); 5.735, 5.745 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.85, 5.86 (2 × d, 2 × 1H, $J_{1',2'}$ = 4.2, H-1′); 7.71, 7.74 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (150.9 MHz, CD_3OD): 14.47 (**C**H$_3$(CH$_2$)$_{17}$); 23.75 (CH$_3$(**C**H$_2$)$_{15}$CH$_2$CH$_2$O); 25.13, 25.17 (d, $J_{C,P}$ = 139.3, CH$_2$P); 26.65, 30.28, 30.29, 30.49, 30.68, 30.69, 30.72, 30.73, 30.78, 30.79, 30.80 (CH$_3$(**C**H$_2$)$_{15}$CH$_2$CH$_2$O); 31.55, 31.57 (d, $J_{C,P}$ = 6.0, CH$_3$(CH$_2$)$_{15}$**C**H$_2$CH$_2$O); 33.09 (CH$_3$(**C**H$_2$)$_{15}$CH$_2$CH$_2$O); 50.55, 50.57 (d, $J_{C,P}$ = 1.1, CH$_2$N); 61.01, 61.04 (CH$_2$-2,5-pyrr); 66.34, 66.42 (d, $J_{C,P}$ = 6.3, CH$_2$-5′); 66.60, 67.62 (d, $J_{C,P}$ = 6.8, CH$_3$(CH$_2$)$_{15}$CH$_2$**C**H$_2$O); 70.80, 70.88 (CH-3′); 74.96, 75.02 (CH-2′); 78.63, 78.68 (CH-3,4-pyrr); 83.63, 83.65 (d, $J_{C,P}$ = 6.7, CH-4′); 91.56, 91.74 (CH-1′); 102.95, 102.98 (CH-5); 142.55, 142.57 (CH-6); 152.18, 152.22 (C-2); 166.03 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 32.12, 32.43. HR-ESI: $C_{33}H_{61}O_{10}N_3P$ $(M + H)^+$ calcd 690.40891, found 690.40891.

Icosanyl Uridin-5′-yl 2-([3*R*,4*R*]-3,4-Dihydroxypyrrolidin-1-*N*-yl)ethylphosphonate (12k). The mixture of dihydroxypyrrolidine **11a** (0.26 g, 2.52 mmol) and phosphonate **10f** (1.1 g, 1.68 mmol) in *n*BuOH (17 mL) was stirred at 100 °C overnight. The solvent was removed, and the isopropylidene intermediate was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate. This compound was without further characterization dissolved in 0.5 M methanolic HCl (50 mL), and the mixture was stirred at rt for 4 h. The reaction mixture was concentrated in vacuo and coevaporated with ethanol (3 × 40 mL). The desired product was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate in 70% overall yield (840 mg, 1.17 mmol) as a white amorphous solid. A mixture of two diastereomers ∼6:5. ^{1}H NMR (600.1 MHz, CD_3OD): 0.90 (m, 6H, C**H**$_3$(CH$_2$)$_{19}$); 1.25−1.43 (m, 68H, CH$_3$(C**H**$_2$)$_{17}$CH$_2$CH$_2$O); 1.69 (m, 4H, CH$_3$(CH$_2$)$_{17}$C**H**$_2$CH$_2$O); 2.06−2.23 (m, 4H, CH$_2$P); 2.68 (dd, 4H, J_{gem} = 10.6, J_{vic} = 2.9, H-2b,5b-pyrr); 2.84−2.97 (m, 4H, CH$_2$N); 3.10 (dd, 4H, J_{gem} = 10.6, J_{vic} = 5.0, H-2a,5a-pyrr); 4.07 (m, 4H, H-3,4-pyrr); 4.08−4.13 (m, 4H, CH$_3$(CH$_2$)$_{17}$CH$_2$C**H**$_2$O); 4.13−4.16 (m, 4H, H-3′,4′); 4.207, 4.210 (2 × dd, 2 × 1H, $J_{2',3'}$ = 5.0, $J_{2',1'}$ = 4.2, H-2′); 4.25 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.8, $J_{5'b,4'}$ = 4.4, H-5′b); 4.31 (m, 2H, H-5′); 4.35 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.6, $J_{5'a,4'}$ = 2.7, H-5′a); 5.735, 5.743 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.84, 5.85 (2 × d, 2 × 1H, $J_{1',2'}$ = 4.2, H-1′); 7.71, 7.74 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (150.9 MHz, CD_3OD): 14.46 (**C**H$_3$(CH$_2$)$_{19}$); 23.75 (CH$_3$(**C**H$_2$)$_{17}$CH$_2$CH$_2$O); 24.85, 24.91 (d, $J_{C,P}$ = 139.8, CH$_2$P); 26.64, 30.28, 30.29, 30.49, 30.69, 30.73, 30.74, 30.77, 30.79, 30.80 (CH$_3$(**C**H$_2$)$_{17}$CH$_2$CH$_2$O); 31.55, 31.57 (d, $J_{C,P}$ = 6.0, CH$_3$(CH$_2$)$_{17}$**C**H$_2$CH$_2$O); 33.09 (CH$_3$(**C**H$_2$)$_{17}$CH$_2$CH$_2$O); 50.81, 50.86 (CH$_2$N); 60.98, 61.02 (CH$_2$-2,5-pyrr); 66.52, 66.54 (d, $J_{C,P}$ = 6.1, CH$_2$-5′); 67.69, 67.73 (d, $J_{C,P}$ = 7.1, CH$_3$(CH$_2$)$_{17}$CH$_2$**C**H$_2$O); 70.80, 70.87 (CH-3′); 74.90, 74.95 (CH-2′); 78.19, 78.26 (CH-3,4-pyrr); 83.60 (d, $J_{C,P}$ = 6.5, CH-4′); 91.75, 91.93 (CH-1′); 102.97, 102.99 (CH-5); 142.65 (CH-6); 152.17, 152.20 (C-2); 166.03 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 31.29, 31.57. HR-ESI: $C_{35}H_{65}O_{10}N_3P$ $(M + H)^+$ calcd 718.44021, found 718.44030.

Hexadecyl Uridin-5′-yl 2-([3*R*,5*S*]-3-Hydroxy-5-(hydroxymethyl)pyrrolidin-1-*N*-yl)ethylphosphonate (12l). The mixture of dihydroxypyrrolidine **11d** (0.12 g, 1.0 mmol) and phosphonate **10d** (0.3 g, 0.5 mmol) in *n*BuOH (5 mL) was stirred at 95 °C overnight. The solvent was removed, and the isopropylidene intermediate was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate. This compound was without further characterization dissolved in 0.5 M methanolic HCl (50 mL) and the mixture was stirred at rt for 4 h. The reaction mixture was concentrated in vacuo and coevaporated with ethanol (3 × 40 mL). The desired product was obtained by flash chromatography on silica gel using linear gradient of H1 in ethyl acetate in 60% overall yield (200 mg, 0.3 mmol) as a white amorphous solid. A mixture of diastereomers ∼6:5. ^{1}H NMR (500.0 MHz, CD_3OD): 0.90 (m, 6H, C**H**$_3$(CH$_2$)$_{15}$); 1.24−1.43 (m, 52H, CH$_3$(C**H**$_2$)$_{13}$CH$_2$CH$_2$O); 1.70 (m, 4H, CH$_3$(CH$_2$)$_{13}$C**H**$_2$CH$_2$O); 2.03, 2.17 (2 × m, 2 × 1H, H-4-pyrr); 2.44−2.58 (m, 4H, CH$_2$P); 3.30 (m, 2H, H-2b-pyrr); 3.53 (m, 2H, CH$_a$**H**$_b$N); 3.70 (m, 2H, H-2a-pyrr); 3.73 (m, 2H, CH$_a$**H**$_b$OH); 3.78 (m, 2H, C**H**$_a$H$_b$N); 3.93 (m, 2H, C**H**$_a$H$_b$OH); 3.96 (m, 2H, H-5-pyrr); 4.07−4.18 (m, 8H, H-3′,4′, CH$_3$(CH$_2$)$_{13}$CH$_2$CH$_2$**H**$_2$O); 4.24 (m, 2H, H-2′); 4.28−4.42 (m, 4H, H-5′); 4.53 (m, 2H, H-3-pyrr); 5.75 (d,

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2H, $J_{5,6}$ = 8.1, H-5); 5.81, 5.83 (2 × d, 2 × 1H, $J_{1',2'}$ = 4.0, H-1′); 7.69, 7.71 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6). ^{13}C NMR (125.7 MHz, CD_3OD): 14.44 ($CH_3(CH_2)_{15}$); 23.17, 23.22 (d, $J_{C,P}$ = 140.5, CH_2P); 23.73 ($CH_3(CH_2)_{13}CH_2CH_2O$); 26.59, 30.28, 30.29, 30.47, 30.66, 30.73, 30.76, 30.79 ($CH_3(CH_2)_{13}CH_2CH_2O$); 31.51 (d, $J_{C,P}$ = 5.9, $CH_3(CH_2)_{13}CH_2CH_2O$); 33.07 ($CH_3(CH_2)_{13}CH_2CH_2O$); 37.35 (CH_2-3); 52.72, 52.76 (CH_2N); 60.82 (CH_2OH); 62.25, 62.28 (CH_2-2-pyrr); 67.18, 67.38 (d, $J_{C,P}$ = 6.3, CH_2-5′); 68.14, 68.37 (d, $J_{C,P}$ = 6.8, $CH_3(CH_2)_{13}CH_2CH_2O$); 70.14 (CH-3-pyrr); 70.40, 70.49 (CH-5-pyrr); 70.75, 70.79 (CH-3′); 74.66, 74.71 (CH-2′); 83.39, 83.45 (d, $J_{C,P}$ = 6.4, CH-4′); 92.40, 92.46 (CH-1′); 103.08, 103.12 (CH-5); 142.94, 142.97 (CH-6); 152.17, 152.19 (C-2); 166.00 (C-4). ^{31}P NMR (162.0 MHz, CD_3OD): 27.81, 28.26. HR-ESI: $C_{32}H_{59}O_{10}N_3P$ $(M + H)^+$ calcd 676.39326, found 676.39306.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Cell viability, cytotoxicity and apoptosis of erythroid progenitor cells after exposure to selected compounds and IR data of synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

C, cytosine; C^{Bz}, 4-*N*-benzoylcytosine; CPZ, caprazamycin; CWS, cyst wall synthase; ED_{50}, effective dose; HM, hydrophobic module; HPLC, high performance liquid chromatography; IM, an iminosugar module; LM, linker module; LPM, liposidomycin; LPPO, lipophosphonoxin; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; NM, nucleoside module; [poly(GalNAc)], poly β-1−3-linked *N*-acetylgalactosamine; SATE, pivaloylthioethyl; TPSCl, triisopropylbenzensulfonylchloride; U, uracil; UDP-GalNAc, uridinediphosphate-*N*-acetyl-alpha-D-galactosamine

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