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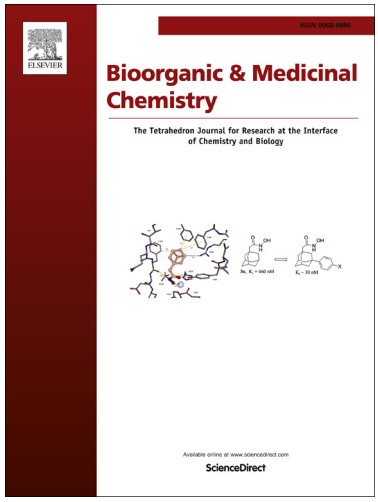

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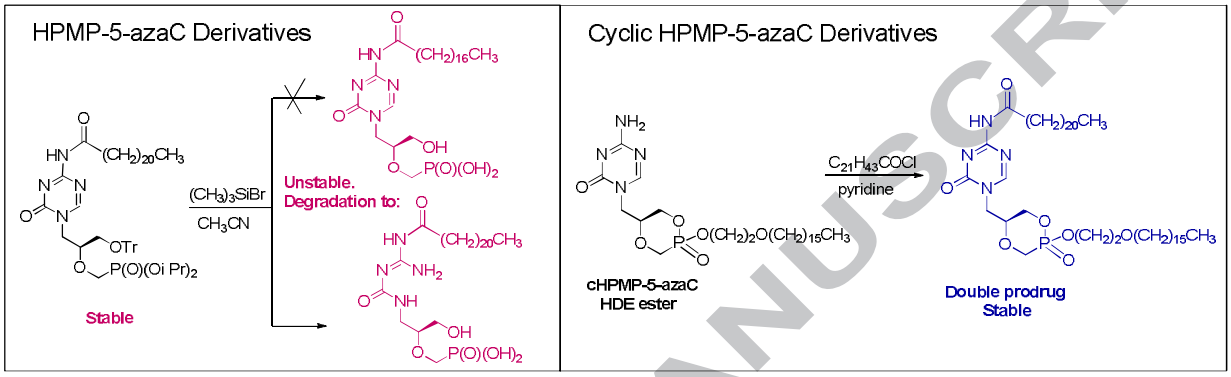

N^4-Acyl derivatives as lipophilic prodrugs of cidofovir and its 5-azacytosine analogue, (*S*)-HPMP-5-azaC: Chemistry and antiviral activity

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Abstract: Even number fatty acid residues - docosanoyl (behenoyl) and stearoyl were selected for introduction to the N^4-position of (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine) (HPMPC, cidofovir), and its 5-azacytosine counterpart, (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine) (HPMP-5-azaC) with the aim to prepare a new type of lipophilic prodrugs. The study on the influence of these modifications to the stability and biological activity of both antivirals was performed. Different reactivity of both systems towards acylation reactions was also found: the 4-NH_2 group of cidofovir was more reactive compared to that of HPMP-5-azaC. In 5-azacytosine derivatives, we found mostly a destabilizing effect of the N^4-acylation but this could be compensated by a positive influence of the esterification of the phosphonate group. Chemical stability of the 5-azacytosine moiety in the HPMP series is increasing in the following order: HPMP- 5-azaC < cyclic HPMP-5-azaC < HPMP-5-azaC esters. From the view of prodrug development, the best chemical stability was observed in case of the double prodrug **7:** the N^4-behenoyl derivative of the hexadecyloxyethyl ester of cyclic HPMP-5-azaC. The free phosphonic acid (N^4-behenoyl-HPMPC) appeared to be a more potent and selective inhibitor of herpesvirus replication than the parent HPMPC derivative.

Keywords: Acyclic nucleoside phosphonates; Cidofovir; 5-Azacytosine; HPMP-5-azaC; Prodrug; Phosphonate Ester ; Antivirals

1. Introduction

Cidofovir (HPMPC, Vistide™), (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine), a compound developed originally at the Institute of Organic Chemistry and Biochemistry in Prague, belongs to the most effective anti-DNA virus agents available (Fig. 1).[1] The compound was approved for the treatment of HCMV retinitis in AIDS patients in the form of intravenous injections.[2] “Off

label" cidofovir is also used as a topical gel in the treatment of acyclovir-resistant HSV and for the treatment of human papilloma virus (HPV) and poxvirus infections.[3] On the other hand, the low bioavailability of cidofovir and its side-effects, especially nephrotoxicity, remain the limiting factors in its widespread use. To improve its pharmacological properties, the main attention is currently paid to the development of its prodrugs, especially alkoxyalkyl esters. One of them, the HDP (hexadecyloxypropyl) ester, i.e. CMX001, was advanced to clinical trials by Chimerix Inc.[4] The randomized double-blind placebo controled study, CMX001-102 in healthy volunteers recently has concluded that CMX001 is orally bioavailable and can be well tolerated up to a dose of 140 mg in adults. This is the first demonstration of the use of phospholipid conjugation technology to achieve plasma drug concentrations that are expected to result in activity against multiple double-stranded DNA viruses.[5]

In our group, the search for new cidofovir derivatives was investigated in two directions: substitutions in the cytosine base (N^4-alkyl derivatives[6], C-5 alkyl/aryl derivatives[7]) and substitution of the cytosine moiety by a triazine conterpart.[8] Other data published on the modification of the cytosine moiety are rather scarce in literature.[9] While the first two substitutions lead to a complete loss of antiviral activity, preparation of a triazine (5-azacytosine) analogue of cidofovir, HPMP-5azaC, opened the way to a new class of acyclic phosphonate analogs that are extraordinary active against a variety of DNA viruses with decreased toxicity compared to cidofovir.[8]

Figure 1

In contrast to cidofovir, HPMP-5-azaC has a more complicated metabolic profile due to its chemical and enzymatic instability. In aqueous solutions ring opening between C-6 and N-1 of the triazine moiety occurs and HPMP-5-azaC is successively degraded to 2-{[(2*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]carbamoylguanidine. This final decomposition product has no cytotoxicity *in vitro* but is antivirally inactive. The hydrolytic degradation of the 5-azacytosine moiety is initiated by

the nucleophilic attack of a hydroxyl ion in position 6 whose electron density is much lower compared to cytosine. On the other hand, we found a large difference between hydrolytic instability of the 5-azacytosine free phosphonic acids and their corresponding esters. While HPMP-5-azaC is degraded rapidly under slightly basic (physiological) or neutral conditions, a targeted degradation of its esters (e.g. the diisopropyl ester) represents a several days lasting process requiring treatment with concentrated ammonia solution.[10] A stabilizing effect of the ester groups was observed also in a series of cyclic phosphonate esters of HPMP-5-azaC. Generally, in the HPMP series, the stability of the 5-azacytosine ring was found to decrease in the following order: esters of cyclic HPMP-5-azaC > cyclic HPMP-5-azaC > HPMP-5azaC. A detailed study of chemical degradation of diverse 5- acyclic azacytosine nucleoside phosphonates at different pH values was performed using NMR spectroscopy.[11] Besides chemical decomposition, HPMP-5-azaC undergoes also enzymatic deamination in cell culture, similarly like cytosine derivatives.[12]

To improve the stability towards the deamination process we tried to transform HPMP-5azaC in diverse N^4-acyl prodrugs on the level of free phosphonic acids as well as on the level of some ester prodrugs. As appropriate acyl groups we selected even number fatty acid residues (e.g. docosanoyl, stearoyl). Corresponding N^4-acyl derivatives were prepared also from HPMPC (cidofovir) and its esters. Thus far, the most important example of the use of N^4-acyl derivatives in cytosine-based drugs is N^4-behenoyl-1-(β-D-arabinofuranosyl)cytosine.[13] The compound was developed as a lipophilic prodrug of the commonly known cytostatic cytosine arabinoside (araC) drug.

Different reactivity of both systems towards the acylation process and the influence of N^4- acyl groups on the stability and biological activity of both types of ANP (the cidofovir and its 5-azacytosine analogue) will be also studied.

Acylation of 4-NH_2 group in cytosine derivatives is known as a simple process generally used in nucleic acid chemistry. In contrast, acylation of their 5-azacytosine counterparts is very difficult and it can be a real synthetic challenge. As witnessed by a literature search, from the outset of 5-azacytosine chemistry in 1960s (ref.[14]) there are only two references regarding this reaction. One of them concerns preparation

of N^4-acetyl-5-azacytidine by nucleosidation reaction from acetylated 5-azacytosine and tri-O-benzoyl-D-ribosyl chloride followed by deprotection of benzoyl groups. The mentioned compound was unstable and spontaneously hydrolyzed to *N*-acetyl-*N´*-formyl-amidourea.[15] The second hit is N^4-phenoxyacetyl-2´-deoxy-5-azacytidine described as a precursor in preparation of oligonucleotide analogues incorporating 5-azacytosine.[16] To understand the different reactivity of both systems, it is necessary to realize the fact that in aromatic amines, electrons are always withdrawn from the amino group into the aromatic ring with a strong decrease of basicity in the NH_2 group. In pyrimidine systems, e.g. in cytosine, it is primarily nitrogen atom N-3 that serves as the electron acceptor. As a consequence, this nitrogen becomes more basic than the $–NH_2$ group and is the major site of protonation. In 5-azacytosine, the presence of an additional nitrogen atom in the ring (N-5) thus causes further decrease of basicity of NH_2 group. Therefore, the reactivity of 5-azacytosine amino group towards acylation is much lower compared to 4-NH_2 group of cytosine. Besides this complication, we had to take in account also the fact that acylation of 5-azacytosine 4-NH_2 group decreases electron density on C-6 which can contribute to destabilization of the ring. From our previous studies, we know that the factor playing a key role in destabilization of the ring is a presence of free hydroxyl group(s) in a molecule. It concerns both nucleosides and acyclic derivatives including acyclic nucleoside phosphonates.[11] Moreover, also hydroxy groups of the phosphonate function were found to influence stability of the ring opening as witnessed by higher stability of cyclic phosphonates and esters compared to a free phosphonic acid.[10,11] Considering all these facts, we came to a conclusion that synthesis of some types of satisfactory stable N^4-acylated 5-azacytosine derivatives as prodrugs could be realizable, at least on a level of cyclic phosponates or phosphonate esters.

2. Chemistry

For the study of the N^4-acylation of HPMP-5-azaC derivatives we selected its trityl protected diisopropyl ester **1,** an intermediate easy to obtain in synthesis starting from (2*S*)-2-[(trityloxy]methyl]oxirane.[8] This compound is hydrolytically stable; moreover, as mentioned above,

targeted ring-opening in it is rather difficult, requiring harder conditions.[10] Introduction of fatty acid acyl groups was performed by reaction of **1** with appropriate even number acyl chlorides in pyridine: docosanoyl (behenoyl) chloride and stearoyl chloride. In both cases the desired N^4-acyl derivatives **2** and **3**, were formed exclusively in good preparative yield (Scheme 1). Preservation of the triazine ring is proven by the presence of typical H-6 signals in the ^{1}H NMR spectrum (singlet at 9.18 or 9.45 ppm, respectively) and C-6 in the ^{13}C NMR spectrum (163.30 ppm, 161.10 ppm). Interestingly, in contrast to 5-azacytosine compounds with a free amino group, the molecular peaks in the mass spectra of **2** and **3** are very small while the main peaks belong to their corresponding adduct with water (M+H_2O) whose supposed structure is shown in Fig. 2. This structure can be understood as the first step leading to decomposition of a triazine ring before its opening to a *N*-formyl derivative.[11] It witnesses a decreased stability of both derivatives, even during the ionization process in MS.

Scheme 1

Figure 2

Deprotection of the ester and trityl groups was performed with bromotrimethylsilane in acetonitrile under standard conditions used for acyclic nucleoside phosphonates.[6-8] In contrast to this procedure applied to **1** and leading successfully to HPMP-5-azaC, the reaction starting from N^4-behenoyl derivative **2** afforded a free phosphonic acid containing always a degraded triazine ring **4.** A similar result was found at the N^4-stearoyl derivative **3**. In this case we tried first to quickly remove a trityl group under mild conditions using trifluoroacetic acid, still before deprotection of the ester groups. Unfortunately, decrease of electron density at the C-6 by acyl groups was found so critical, that, instead of a detritylated 5-azacytosine phosphonate, we obtained only the fully protected open-ring derivative **5**, together with a small amount of its detritylated counterpart **6** (Scheme 1). The ring-opening reaction proved to be the fastest reaction step. Preparation of N^4-acyl derivatives of HPMP-5-azaC was thus found practically impossible without parallel ring-opening reactions.

The situation was slightly better when the free HPMP-5-azaC was substituted by its cyclic form (cHPMP-5-azaC) or substantially improved when some ester prodrugs of the cyclic form, e.g. the alkoxyalkyl ester of cHPMP-5-azaC, was used. These facts are in agreement with previously observed increased stability of cyclic forms[11]. Increased stability of the cyclic HPMP-5-azaC compared to HPMP-5-azaC is done by the absence of a free hydroxy group in the aliphatic chain which was found to participate in the ring-opening reaction. In our previous study of decomposition of acyclic 5-azacytosine nucleoside analogs we showed decomposition of HPMP-5-azaC as a process involving formation of the N-formiate intermediate which was not observed in the decomposition reaction of other derivatives. The explanation for this different reaction pathway is that the intermediary formylamido derivative can react in a transformylation reaction with a spatially close hydroxy group to form an O-formiate (Scheme 2). As a result, we can say that triazine can serve as a formylating agent when a free hydroxyl group is in a suitable close spatial arrangement.[11]

Scheme 2

Acylation of the alkoxyalkyl (hexadecyloxyethyl) ester of the cyclic HPMP-5-azaC with behenoyl chloride proceeded without any difficulties to give compound **7**, the derivative designed as a double prodrug of HPMP-5-azaC (Scheme 3). The aim of its synthesis was to prove an influence of the introduction of an additional lipophilic chain to the antiviral activity: the starting HDE cHPMP-5-azaC is known as a compound with extraordinary activity against a broad variety of of DNA viruses.[17] Acylation of free cHPMP-5-azaC using stearoyl chloride was more or less successful, affording the desired N^4-substituted derivative **8**. Unfortunately, this product is not sufficiently stable and, as witnessed by NMR spectra, it is accompanied also by two decomposition products. Both by-products are cHPMP-5-azaC bearing an opened 5-azacytosine moiety: one of them still containing stearamide moiety, one of them containing a free 4-NH_2 group. Open forms were deduced from missing C-6 in ^{13}C NMR of both by-products.

Scheme 3

Similarly, N^4-acyl compounds were prepared also from HPMPC (cidofovir) and some of its esters. Different reactivity of both systems (cytosine and 5-azacytosine) was observed: cidofovir was always much more reactive towards acylation reactions due to a higher basicity of its 4-NH_2 group. Despite this fact, direct acylation of a free cidofovir with behenoyl (or stearoyl) chloride was not successful. It is caused by its total insolubility in solvents usual for acylations (pyridine, acetonitrile, etc.) due to a highly polar character of a phosphonic acid residue. A more advantageous synthetic route was started from the fully protected phosphonate ester **9** using either carbonyldiimidazol mediated acylation with behenic acid, or classic acylation using acyl (behenoyl) chloride in pyridine (Scheme 4). Deprotection of the trityl group with trifluoroacetic acid, followed by the treatment with bromotrimethylsilane gave the free phosphonic acid (N^4-behenoyl-HPMPC) **12** or its monoisopropylester **13**, dependent on the reaction conditions: the reaction performed at 0°C afforded selectively monoester **13.** To preserve the N^4-acyl group during work-up and the purification process, utilization of Dowex 1 in CH_3COO^- form, an anion exchanger used commonly for purification of ANPs must be strictly avoided.

The different reactivity of cytosine *vs* 5-azacytosine base moiety was observed also in preparation of other types of prodrugs, e.g. pivaloyloxymethyl (POM) derivatives. Whereas cHPMP-5-azaC treated with chloromethyl pivalate afforded exclusively the POM ester of cHPMP-5-azaC[17], an analogous reaction performed with cyclic cidofovir afforded its POM ester bearing additionally the N^4-pivaloyl group **14** as a main reaction product (Scheme 5). This additional N^4 acylation is caused the strongly basic character of the cytosine N^4-amino group. Despite the fact that formation of the POM derivative of cHPMPC **15** is mentioned briefly in a patent, the course of reaction, yields or formation of other products are not described and full characterization of the product(s) is there also missing.[18] Anyway, promising antiviral activities of both POM derivatives, along with a low toxicity, moved us to include compounds **14** and **15** to this study and make comparison with their long-chain acylated counterparts.

3. Antiviral activity

The antiviral activity of the N^4-acyl derivatives was evaluated against various DNA viruses, including different herpesviruses [i.e. herpes simplex virus type 1 (HSV-1)] and type 2 (HSV-2), thymidine kinase-deficient HSV-1 (acyclovir-resistant, ACV^r), varicella-zoster virus (VZV), and human cytomegalovirus (HCMV) (Table 1). The compounds were also evaluated against several poxviruses, encompassing vaccinia virus (VACV), cowpox virus, camelpox virus and orf virus. Reference antiherpesvirus drugs (i.e. acyclovir, ganciclovir and brivudin) as well as cidofovir (HPMPC), HPMP-5-azaC and their cyclic forms were included in the antiviral and cytostatic assays. The N^4-acyl derivatives evaluated for their antiviral properties included compounds **7** [HDE ester (N^4-behenoyl-cHPMP-5-azaC)], **12** [free phosphonic acid (N^4-behenoyl-HPMPC), **13** [monoisopropylester (N^4-behenoyl-HPMPC)] and **14** [POM ester (N^4-pivaloyl-cHPMPC)] as well as the POM ester of cHPMPC (compound **15**). Both cyclic phosphonates **14** and **15** were tested as diastereoisomeric mixtures.

Compound **12** emerged as the most potent anti-HCMV compound evaluated in the present study with 20-30 fold lower EC_{50} values compared to HPMPC and an >11- to >17-fold gain in selectivity. In the case of HSV and VZV, the free phosphonic acid (N^4-behenoyl-HPMPC) was only 2- to 4.6-fold (HSV) and 4- to 6-fold (VZV) more active than the parent compound HPMPC. The monoisopropylester (N^4-behenoyl-HPMPC), i.e. compound **13**, proved to be 3- to 7-fold (HCMV) and 2- to 8-fold (VZV) less active than the free phosphonic acid (N^4-behenoyl-HPMPC). In the case of HSV, the difference in activity between these N^4-behenoyl-HPMPC derivatives was not higher than 2-fold. The activity of the HDE ester (N^4-behenoyl-cHPMP-5-azaC) did not significantly differ from the parent compound cyclicHPMP-5-azaC among the different herpesviruses.

When the N^4-acyl derivatives and the POM ester (cyclic-HPMPC) were evaluated against different poxviruses, their activities were not markedly different from those of the parent drugs, with a maximum of 4-fold decrease in EC_{50} value for compound **13** compared to HPMPC against the orf virus.

Table 1. Antiviral activity of some N^4-acyl derivatives against herpesviruses in HEL cells compared to cidofovir, HPMP-5-azaC and their cyclic forms as well as to reference antiherpesvirus drugs.

Compounds	Antiviral activity: EC_{50} (µM)[a] *(Selectivity index: ratio CC_{50} to EC_{50})*									Cytotoxicity (µM)	
	VZV				Herpes simplex virus			Human cytomegalovirus		Cell morphology	Cell growth
	TK^+ strains		TK^- strains		HSV-1	HSV-2	HSV-1 TK^-				
	YS	OKA	07/1	YS/R	KOS	G	KOS ACV^r	AD-169	Davis	MCC[b]	CC_{50}[c]
7	0.52 ± 0.09 ***>225***	1.21 ± 0.18 ***>97***	0.30 ± 0.13 ***>384***	0.36 ± 0.14 ***>327***	1.4 ± 0.2 ***>83***	1.4 ± 0.3 ***>83***	1.2 ± 0.2 ***>100***	0.21 ± 0.04 ***>570***	0.16 ± 0.10 ***>737***	20	>117
12	0.04 ± 0.01 ***>4162***	0.13 ± 0.27 ***>1241***	0.02 ± 0.02 ***>6961***	0.013 ± 0.003 ***>12973***	0.33 ± 0.71 ***>499***	0.33 ± 0.71 ***>499***	0.33 ± 0.71 ***>499***	0.027 ± 0.009 ***>6243***	0.046 ± 0.079 ***>3632***	20	>166
13	0.33 ***>475***	0.27 ± 0.31 ***>584***	0.05 ± 0 ***>3219***	0.08 ***>1996***	0.47 ± 0.22 ***>333***	0.93 ± 0.44 ***>166***	0.70 ± 0.11 ***>222***	0.20 ± 0.15 ***>768***	0.15 ± 0.05 ***>1050***	**≥31**	>155
14	0.04 ***>2500***	0.15 ± 0.07 ***>680***	0.11 ± 0.08 ***>885***	N.D.	1.4 ± 0.8 ***>71***	0.87 ± 0 ***>115***	0.98 ± 0.15 ***>102***	0.48 ± 0.05 ***>211***	0.24 ***>417***	>100	>100
15	0.2 ***230***	0.036 ± 0.030 ***1296***	0.023 ± 0.023 ***2044***	N.D.	1.6 ± 0.38 ***29***	0.93 ± 0.19 ***49***	2.1 ± 0 ***22***	0.42 ± 0 ***110***	0.4 ***115***	>100	46
HPMP-5-azaC	0.22 ± 0.18 ***1232***	0.35 ± 0.28 ***753***	0.42 ± 0.41 ***634***	0.09 ***2981***	0.80 ± 0.45 ***333***	0.94 ± 0.39 ***283***	0.88 ± 0.45 ***301***	0.42 ± 0.17 ***629***	0.65 ± 0.64 ***411***	>357	266 ± 251
cHPMP-5-azaC	0.35 ***732***	0.52 ± 0.37 ***485***	0.39 ± 0.30 ***656***	N.D.	1.3 ± 0.9 ***202***	1.7 ± 1.4 ***146***	1.4 ± 0.7 ***178***	0.20 ± 0.15 ***1241***	0.27 ± 0.13 ***954***	>381	254 ± 155
HPMPC	0.18 ± 0.19 ***1632***	0.30 ± 0.30 ***1002***	0.06 ± 0.08 ***4880***	0.06 ***4693***	1.5 ± 1.1 ***198***	2.1 ± 1.2 ***143***	1.4 ± 1.0 ***207***	0.81 ± 0.34 ***368***	0.92 ± 0.64 ***325***	≥1270	298 ± 207
cHPMPC	0.43 ± 0.21 ***580***	0.54 ± 0.37 ***458***	0.34 ± 0.37 ***731***	0.13 ***1835***	2.0 ± 1.2 ***124***	2.7 ± 1.6 ***92***	1.8 ± 1.3 ***139***	0.62 ± 0.39 ***397***	0.93 ± 0.36 ***267***	>336	247 ± 184
Acyclovir	*3.3 ± 1.7* ***≥338***	*2.4 ± 1.5* ***≥465***	*53 ± 31* ***≥21***	*62 ± 35.8* ***≥18***	*0.20 ± 0.12* ***≥5668***	*0.34 ± 0.09* ***≥3326***	*57 ± 35.6* ***≥20***	N.D.	N.D.	>1776	≥1123 ± 406
Brivudin	0.025 ± 0.029 ***≥33815***	0.019 ± 0.016 ***≥44362***	95.5 ± 60.0 ***≥9***	140 ± 17 ***≥6***	0.028 ± 0.004 ***≥30618***	121 ± 78 ***≥7***	35 ± 25 ***≥24***	N.D.	N.D.	≥1200	≥842 ± 377
Ganciclovir	N.D.	N.D.	N.D.	N.D.	0.070 ± 0.067 ***5071***	0.060 ± 0.058 ***5917***	4.2 ± 3.5 ***85***	8.3 ± 4.7 ***43***	7.1 ± 3.7 ***50***	≥1210	355 ± 392

[a] Effective concentration required to reduce virus-induced cytopathicity by 50%; [b] Minimum cytotoxic concentration required to cause a microscopically detectable alteration of cell morphology; [c] Cytotoxic concentration required to reduce cell growth by 50%; N.D. not determined.

Table 2. Antiviral activity of some N^4-acyl derivatives against herpesviruses in HEL cells compared to cidofovir and HPMP-5-azaC

Compounds	Antiviral activity: EC_{50} (µM)[a] *(Selectivity index: ratio CC_{50} to EC_{50})*						Cytotoxicity (µM)	
	Vaccinia virus		Cowpox virus	Camelpox virus		Orf virus	Cell morphology	Cell growth
	Lederle	WR	Brighton	CML-1	CML-14	NZ2	MCC[b]	CC_{50}[c]
7	8.2 ± 3.3 ***>14***	N.D.	N.D.	N.D.	N.D.	N.D.	≥23	>117
12	2.7 ± 1.8 ***>63***	10 ***>16***	7 ***>24***	5.5 ± 3.1 ***>30***	2.6 ± 1.5 ***>65***	0.53 ± 0.88 ***>312***	33	>166
13	5.2 ± 1.8 ***>30***	6.2 ± 0 ***>25***	5.2 ± 1.5 ***>30***	5.5 ± 2.0 ***>28***	2.3 ± 0.7 ***>67***	0.25 ***>624***	≥31	>155
14	16 ± 15 ***>6***	7.2 ± 5.3 ***>14***	8.1 ± 6.5 ***>12***	11 ± 7.1 ***>9***	5.7 ± 4.3 ***>18***	1.0 ***>98***	>100	>100
15	12 ***4***	10 ± 0.6 ***4***	13 ± 1.8 ***4***	13 ± 13.8 ***3***	7.9 ± 5.3 ***6***	0.83 ***57***	>100	46
HPMP-5-azaC	5.8 ± 1.2 ***46***	9.0 ± 0 ***29***	12 ± 3.7 ***23***	7.2 ± 4.1 ***37***	5.9 ± 2.5 ***45***	0.88 ± 0.32 ***302***	>357	266 ± 251
cHPMP-5-azaC	7.6 ± 5.4 ***33***	7.9 ± 0.6 ***32***	12 ± 4.0 ***20***	8.1 ± 4.2 ***32***	4.9 ± 2.4 ***52***	0.7 ± 0.5 ***360***	>381	254 ± 155
HPMPC	9.0 ± 6.1 ***33***	14 ± 5.2 ***21***	16 ± 3.7 ***18***	8.0 ± 3.8 ***37***	6.1 ± 4.1 ***49***	1.0 ± 0.6 ***290***	≥1270	298 ± 207
cHPMPC	12 ± 5 ***21***	16 ± 8 ***16***	15 ± 2.9 ***16***	8.9 ± 5.3 ***28***	4.8 ± 2.6 ***52***	1.1 ± 0.7 ***221***	>336	247 ± 184

[a] Effective concentration required to reduce virus-induced cytopathicity by 50%; [b] Minimum cytotoxic concentration required to cause a microscopically detectable alteration of cell morphology; [c] Cytotoxic concentration required to reduce cell growth by 50%; N.D. not determined.

4. Conclusions

N^4-Acyl derivatives as lipophilic prodrugs of cidofovir and its 5-azacytosine analogue (S)-HPMP-5-azaC have been prepared and their antiviral properties against herpes and poxviruses were evaluated *in vitro*. The free phosphonic acid (N^4-behenoyl-HPMPC) proved to be more potent and selective than its parent compound HPMPC against herpesviruses. Although the monoisopropylester (N^4-behenoyl-HPMPC) was less active than the free phosphonic acid (N^4-behenoyl-HPMPC), it inhibited herpesvirus replication with lower EC_{50} values than HPMPC. The potencies of the POM derivatives of cHPMPC and of N^4-pivaloyl-cHPMPC as well as that of the HDE ester with 5-azaC moiety (N^4-behenoyl-cHPMP-5-azaC ester, **7**) were not strikingly different than those of the parent compounds. Also the activities and selectivities of the N^4-acyl derivatives and the POM ester of cyclic-HPMPC against poxviruses were not markedly different compared to their respective parent drugs. In the 5-azacytosine series, N^4-acyl derivatives of free phosphonic acids (acyclic or cyclic) revealed as not appropriate prodrugs due to their low stability towards degradation of a triazine ring.

5. Experimental

5.1. General

Unless stated otherwise, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 13 Pa. Melting points were determined on a Kofler block and are uncorrected. Analytical TLC was performed on silica gel 60 F_{254} plates (Merck KGaA, Darmstadt, Germany); chromatographic systems are described in text. Column chromatography was performed on silica gel 60 μm (Fluka). Preparative reverse phase HPLC separations were performed on a Waters Delta 600 instrument with a Waters 2487 Dual λ Absorbance Detector using a column Luna Phenomenex ® C-18 (10 μm, 10x150 mm), flow 5 mL/min. Optical rotations were measured on Autopol IV polarimeter (Rudolph Research

Analytical, U.S.A.) at 20 °C, [▯]$_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H, ^{13}C and ^{31}P NMR spectra were measured on a Bruker Avance 500 spectrometer (^{1}H at 500.0 MHz, ^{13}C at 125.7 MHz and ^{31}P at 202.3 MHz) in CD_3OD, d_6-DMSO or $CDCl_3$. The spectra were referenced to TMS or residual solvent system (δ 3.31 and 49.0 for CD_3OD, δ 77.0 for $CDCl_3$, 2.5 and 39.7 for d_6-DMSO). ^{31}P NMR spectra were referenced to H_3PO_4 as an external standard. The assignment of chemical shifts was done with the help of 2D NMR experiments (H,H-COSY, H,C-HSQC and H,C-HMBC). Diastereoisomers of cyclic phosphonates were distinguished by their characteristic ^{31}P chemical shifts and by comparing H,H; H,P and C3´,P-coupling constants in chair conformation of the cyclic phosphonate esters that have characteristic values for each diastereoisomer. Determination of the relative configuration of cyclic phosphonate esters from NMR spectra (H,H-ROESY or comparison of ^{31}P (^{1}H dec.) NMR spectra) is explained in detail in ref.[17]. Mass spectra were measured on LCQ classic instrument using electrospray ionization (ESI) or ZAB-EQ (VG Analytical) spectrometer using FAB (ionization with xenon, accelerating voltage 8 kV, glycerol matrix). Most of chemicals and ion-exchange resins (Dowex 1X2-400) were purchased from Sigma-Aldrich.

5.2. 1-{(2*S*)-2-[(Diisopropoxyphosphoryl)methoxy-3-(triphenylmethoxy)]propyl}-*N*^{4}-docosa-noyl-5-azacytosine (2). Behenoyl chloride (1 g, 2.8 mmol) was added to a solution of **1** (900 mg, 1.5 mmol) in pyridine (5 mL), the mixture stirred for 2 days at room temperature and quenched by addition of methanol (1 mL). The solution was evaporated, the residue coevaporated with toluene and partitioned between water and ethyl acetate (150 mL each). The organic layer was dried over sodium sulfate, evaporated and chromatographed on a column of silica gel (200 mL) in system toluene – ethyl acetate (1:1). Yield: 750 mg (55 %) of **2** as a white foam. $[\alpha]_D$ −11.9 (*c* 0.298, $CHCl_3$). ^{1}H NMR ($CDCl_3$, ppm) δ: 0.89 (t, 3H, $J_{CH3,CH2}$ = 7.0, CH_3), 1.28 (m, 16H, CH_2), 1.69 (m, 2H, CH_2), 2.46 (t, 2H, $J_{CH2,CH2}$ = 7.6, CH_2), 1.33, 1.34, 1.37 and 1.38 (4xd, 12H, $J_{CH3,CH}$ = 6.1, CH_3), 4.68 (m, 1H, P-OCH), 4.78 (m, 1H, P-OCH), 3.30 (dd, 1H, $J_{P,CHa}$ = 11.1, J_{gem} = 13.2, PCH_a), 3.81 (dd, 1H, $J_{P,CHb}$= 9.7, J_{gem} = 13.2, PCH_b), 3.10 (dd, 1H, $J_{3´a,2´}$ = 2,5, J_{gem} = 13.1, H-3´a), 3.56 (dd, 1H, $J_{3´b,2´}$ =

4,0, J_{gem} = 13.1, H-3´b), 4.11 (dd, 1H, $J_{1´a,2´}$ = 7.2, J_{gem} = 14.8, H-1´a), 4.21 (dd, 1H, $J_{1´b,2´}$ = 3,3, J_{gem} = 13.1, H-1´b), 4.24 (m, 1H, H-2´), 7.21 (t, 3H, H-arom.), 7.27 (t, 6H, H-arom.), 7.41 (d, 6H, H-arom.), 9.18 (s, 1H, H-6), 10.90 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm) δ: 13.80 (CH_3), 22.37 (CH_2), 23.54 (d, $J_{P,C}$ = 4.4, CH_3), 23.72 (d, $J_{P,C}$ = 3.4, CH_3), 23.76 (d, $J_{P,C}$ = 4.4, CH_3), 23.92 (d, $J_{P,C}$ = 3.4, CH_3), 24.50, 28.92, 29.03, 29.04, 29.19, 29.30 (2H), 29.38 (10H), 31.60 and 36.80 (CH_2), 41.31 (C-1´), 65.60 ($J_{P,C}$ = 165.8, P-C), 66.14 (C-3´), 70.41 ($J_{P,C}$ = 6.4, P-O-C), 71.41 ($J_{P,C}$ = 6.4, P-O-C), 79.08 (d, $J_{P,C}$ = 9.8, C-2´), 86.97 (Tr), 126.68 (3C, Tr), 127.71 (6C, Tr), 128.37 (6C, Tr), 143.51 (3C, Tr), 159.14 (C-4), 162.76 (C-2), 163.30 (C-6), 176.87 (C=O). ^{31}P{^{1}H} NMR ($CDCl_3$, ppm) δ: 19.57. ESI MS, *m/z*: 969.6 $[M.H_2O+Na]^+$ (100), 951.7 $[M+Na]^+$ (16), 947.2 $[M.H_2O+H]^+$ (7), 929.3 $[M+H]^+$ (4). Anal. Calcd. for $C_{54}H_{81}N_4O_7P$. H_2O: C, 68.47; H, 8.83; N, 5.91; P, 3.27. Found: C, 68.51; H, 9.02; N, 5.81; P, 3.56.

5.3. 1-{(2*S*)-2-[(Diisopropoxyphosphoryl)methoxy-3-(triphenylmethoxy)]propyl}-N^4-octadecanoyl-5-azacytosine (3). The starting compound **1** (1.5 g, 2.5 mmol) was treated with stearoyl chloride (1.2 mL, 3.5 mmol) in pyridine (5 mL) and the mixture worked up analogously as described for **2**. Chromatography of the crude product in system toluene – ethyl acetate (2:1) gave 1.82 g (83 %) of **3** as a yellowish syrup. $[\alpha]_D$ −11.1 (*c* 0.225, $CHCl_3$). ^{1}H NMR (DMSO-d_6, ppm) δ: 084 (t, 3H, $J_{CH3,CH2}$ = 7.0, CH_3), 1.21 (overlay, 12H, CH_3), 1.21 (m, 12 H, CH_2), 1.54 (m, 2H, CH_2), 2.38 (t, 2H, $J_{CH2,CH2}$ = 7.6, CH_2), 2.99 (dd, 1H, $J_{3´a,2´}$ = 5.6, J_{gem} = 10.8, H-3´a), 3.05 (dd, 1H, $J_{3´b,2´}$ = 2.8, J_{gem} = 10.8, H-3´b), 3.57 (dd, 1H, $J_{1´a,2´}$ = 5.7, J_{gem} = 12.6, H-1´a), 3.78 (dd, 1H, $J_{1´b,2´}$ = 6.2, J_{gem} = 12.6, H-1´b), 3.80 (d, 2H, $J_{P,CH}$ = 9.0, PCH_2), 4.56 (m, 1H, H-2´), 4.60 (m, 2H, P-OCH), 7.24 (t, 3H, H-arom.), 7.29 (t, 6H, H-arom.), 7.39 (d, 6H, H-arom.), 9.45 (s, 1H, H-6), 10.71 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, ppm) δ: 14.07 (CH_3), 22.23 (CH_2), 23.78 (d, $J_{P,C}$ = 4.6, CH_3), 23.82 (d, $J_{P,C}$ = 4.6, CH_3), 23.93 (d, 2C, $J_{P,C}$ = 3.6, CH_3), 24.38, 28.79, 29.13 (2C), 29.15 (9C), 31.41 and 36.29 (CH_2), 46.20 (C-1´), 64.12 (d, $J_{P,C}$ = 164.6, P-C), 64.36 (C-3´), 70.33 (d, 2C, $J_{P,C}$ = 6.4, P-O-C), 78.56 (d, $J_{P,C}$ = 11.4, C-2´), 86.32 (Tr), 127.11 (3C, Tr), 127.93 (6C, Tr), 128.37 (6C, Tr), 143.87 (3C, Tr), 158.19

(C-4), 161.10 (C-2), 161.78 (C-6), 176.79 (C=O). $^{31}P\{^{1}H\}$ NMR ($CDCl_3$, ppm) δ: 19.62. ESI MS, *m/z*: 1802.9 $[2(M.H_2O)+Na]^+$ (56), 913.4 $[M.H_2O+Na]^+$ (100), 895.4 $[M+Na]^+$ (10), 890.9 $[M.H_2O+H]^+$ (3). HRMS (ESI): For $C_{50}H_{73}N_4O_7PNa$ $(MNa)^+$ calculated: 895.511460; found: 895.512663. Anal. Calcd. for $C_{50}H_{73}N_4O_7P$: C, 68.78; H, 8.43; N, 6.42; P, 3.55. Found: C, 68.71; H, 8.57; N, 6.20; P, 3.63.

(*S,E*)-(8-Amino-3-(hydroxymethyl)-6,10-dioxo-2-oxa-5,7,9-triazahentriacont-7-en-1-yl)phosphonic acid (4). A solution of **2** (204 mg, 0.22 mmol) in acetonitrile (10 mL) was kept in dark with bromotrimethylsilane (0.4 mL, 3 mmol) for 24 at room temperature and then evaporated. The residue was coevaporated with toluene (2x 20 mL), washed with diethylether (to remove TrOH) and crystallized from 96% ethanol. The crystalline product was filtered, washed with ethanol and ether and dried in vacuo. Yield: 90 mg (68%) of a mixture of **4**, octadecanoyl amide and final degradation product of HPMP-5-azaC (according ^{1}H and ^{13}C NMR) as a white solid. -ESI MS, *m/z*: 1183.3 $[2M-H]^-$ (100), 591.3 $[M-H]^-$ (56). HRMS (FAB): For $C_{28}H_{58}N_4O_7P$ $(MH)^+$ calculated: 593.404314; found: 593.406317.

(*S,E*)-Diisopropyl {8-amino-6,10-dioxo-3-[(trityloxy)methyl]-2-oxa-5,7,9-triazaheptacos-7-en-1-yl}phosphonate (5). 80% Trifluoroacetic acid (15 mL) was added to **3** (1.7 g, 2.0 mmol), the mixture shaken shortly (3-5 min) in stoppered flask and evaporated. The residue was coevaporated with toluene (50 mL) and then partitioned between ethyl acetate and saturated solution of sodium bicarbonate (150 mL each). An organic layer was dried over sodium sulfate and evaporated. The residue was chromatographed on a column of silica gel in ethyl acetate to afford **5** as a colorless oil (R_F 0.4). Yield: 590 mg (53%). $[\alpha]_D$ −10.6 (*c* 0.324, $CHCl_3$). ^{1}H NMR ($CDCl_3$, ppm) δ: 0.88 (t, 3H, $J_{CH3,CH2}$ = 7.0, CH_3), 1.26 (m, 12H, CH_2), 1.315 (d, 3H, $J_{CH3,CH}$ = 6.1, CH_3), 1.32 (d, 3H, $J_{CH3,CH}$ = 6.1, CH_3), 1.33 (d, 3H, $J_{CH3,CH}$ = 6.1, CH_3), 1.34 (d, 3H, $J_{CH3,CH}$ = 6.1, CH_3), 1.65 (m, 2H, CH_2), 2.33 (m, 2H, CH_2), 3.17 (dd, 1H, $J_{3'a,2'}$ = 5.4, J_{gem} = 10.2, H-3´a), 3.21 (dd, 1H, $J_{3'b,2'}$ = 4.3, J_{gem} = 10.2, H-3´b), 3.24 (ddd, 1H, $J_{1'a,2'}$ = 4.4, $J_{1'a,NH}$ = 7.9, J_{gem} = 14.0, H-1´a), 3.50 (ddd, 1H, $J_{1'b,2'}$ = 3.40,

$J_{1'b,NH}$ = 6.90, J_{gem} = 14.0, H-1´b), 3.67 (m, 1H, H-2´), 3.76 (dd, 1H, $J_{P,CHa}$ = 8.6, J_{gem} = 13.8, PCH_a), 3.96 (dd, 1H, $J_{P,CHb}$= 8.1, J_{gem} = 13.8, PCH_b), 4.75 (m, 2H, P-OCH), 6.10 (br, 1H, NH), 7.24 (t, 3H, H-arom.), 7.29 (t, 6H, H-arom.), 7.44 (d, 6H, H-arom.), 8.50 (br, 2H, NH_2), 13.00 (br, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm) δ: 14.11 (CH_3), 22.66 (CH_2), 23.95 (d, $J_{P,C}$ = 4.6, CH_3), 24.03 (d, $J_{P,C}$ = 4.9, CH_3), 24.035 (d, $J_{P,C}$ = 3.6, CH_3), 24.10 (d, $J_{P,C}$ = 3.9, CH_3), 24.83, 29.04, 29.24, 29.34, 29.41, 29.56, 29.61, 29.64 (2C), 29.67 (4C), 31.90 and 37.98 (CH_2), 41.45 (C-1´), 63.85 (C-3´), 65.37 (d, $J_{P,C}$ = 167.7, P-C), 71.02 (d, 2C, $J_{P,C}$ = 6.7, P-O-C), 71.33 (d, $J_{P,C}$ = 6.5, C-2´), 86.84 (Tr), 127.05 (3C, Tr), 127.83 (6C, Tr), 128.61 (6C, Tr), 143.64 (3C, Tr). FAB MS, *m/z*: 886 $[M+Na]^+$ (20), 864 $[M+H]^+$ (1), 243 $[(C_6H_5)_3C]^+$ (100). Anal. Calcd. for $C_{49}H_{75}N_4O_7P.1½\ H_2O$: C, 66.12; H, 8.83; N, 6.29; P, 3.48. Found: C, 66.09; H, 8.77; N, 6.43; P, 3.78.

(*S,E*)-Diisopropyl [8-amino-3-(hydroxymethyl)-6,10-dioxo-2-oxa-5,7,9-triazaheptacos-7-en-1-yl]phosphonate (6). The compound was eluted from the column as a more polar by-product (R_F 0.25) after isolation of **5**. Yield: 85 mg (7 %) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) δ: unsufficient quality of the spectrum for analysis. ^{13}C NMR ($CDCl_3$, ppm) δ: 14.10 (CH_3), 24.00 (d, 4C, $J_{P,C}$ = 4.0, 4xCH_3), 29.12, 29.27, 29.33, 29.42, 29.57, 29.67 (6C), 31.89 and 37.81 (CH_2), 39.41 (C-1´), 60.81 (C-3´), 64.43 (d, $J_{P,C}$ = 169.15, P-C), 71.46 (d, $J_{P,C}$) = 6.7, P-O-C), 71.48 (d, $J_{P,C}$) = 6.7, P-O-C), 81.85 (d, $J_{P,C}$ = 7.7, C-2´). FAB MS, *m/z*: 643 $[M+Na]^+$ (20), 621 $[M+H]^+$ (25).

***N*-(5-{[(5S)-2-(2-(Hexadecyloxy)ethoxy)-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl}-4-oxo-4,5-dihydro-1,3,5-triazin-2-yl)docosanamide (7).** A solution of HDE ester of cHPMP-5-azaC[17] (*trans*-isomer, 200 mg, 0.38 mmol) in pyridine (4 mL) was stirred with behenoyl chloride (500 mg, 1.39 mmol) at room temperature for 24 h, followed by 2 h at 40°C. The mixture was evaporated, the residue chromatographed on a column of silica gel (30 mL) in chloroform, followed by a gradient of MeOH in chloroform (1-4 %). Yield: 120 mg (37 %) as a white solid. $[\alpha]_D$ −5.9 (*c* 0.249, $CHCl_3$).

The isolated compound was a single pure diastereoisomer (Fig. 3). ^{1}H NMR ($CDCl_3$, ppm) δ: 0.88 (m, 6H, C*H*$_3$(CH$_2$)$_{14}$CH$_2$, C*H*$_3$(CH$_2$)$_{19}$CH$_2$), 1.21-1.40 (m, 62H, CH$_3$(C*H*$_2$)$_{13}$CH$_2$CH$_2$, CH$_3$(C*H*$_2$)$_{18}$CH$_2$CH$_2$), 1.59 (m, 2H, CH$_3$(CH$_2$)$_{13}$C*H*$_2$CH$_2$), 1.62 (m, 2H, CH$_3$(CH$_2$)$_{18}$C*H*$_2$CH$_2$), 2.42 (t, 2H, J_{vic} = 7.4, CH$_3$(CH$_2$)$_{18}$CH$_2$C*H*$_2$), 3.49 (m, 2H, CH$_3$(CH$_2$)$_{13}$CH$_2$C*H*$_2$), 3.64 (m, 1H, H-1´a), 3.68 (m, 2H, POCH$_2$C*H*$_2$), 3.87-3.95 (m, 3H, PCH$_a$, H-1´b,2´), 4.16-4.30 (m, 4H, PCH$_b$, H-3´a, POC*H*$_2$CH$_2$), 4.37 (ddd, 1H, J_{gem} = 11.7, $J_{P,3´b}$ = 10.0, $J_{3´b,2´}$ = 1.7, H-3´b), 9.13 (bs, 1H, NH), 9.59 (s, 1H, H-6). ^{13}C NMR ($CDCl_3$, ppm) δ: 14.11 (*C*H$_3$(CH$_2$)$_{14}$CH$_2$, *C*H$_3$(CH$_2$)$_{19}$CH$_2$), 22.68, 24.60, 26.06, 28.99, 29.23, 29.35, 29.40, 29.50, 29.57, 29.60, 29.65, 29.69, 31.92 (CH$_3$(*C*H$_2$)$_{14}$CH$_2$, CH$_3$(*C*H$_2$)$_{19}$CH$_2$), 37.72 (CH$_3$(CH$_2$)$_{19}$*C*H$_2$), 39.16 (C-1´), 63.75 (d, $J_{P,C}$ = 145.1, P-C), 64.76 (d, $J_{P,C}$ = 6.7, PO*C*H$_2$CH$_2$), 69.56 (d, $J_{P,C}$ = 5.4, POCH$_2$*C*H$_2$), 71.54 (CH$_3$(**C**H$_2$)$_{14}$CH$_2$), 73.04 (d, $J_{P,C}$ = 8.3, C-3´), 74.13 (d, $J_{P,C}$ = 5.2, C-2´), 158.13 (C-4), 162.31 (C-2), 164.42 (C-6), 175.53 (C=O). -ESI MS, *m/z*: 869.5 [M.H$_2$O-H]$^-$ (100). HRMS (FAB): For $C_{47}H_{90}N_4O_7P$ $(MH)^+$ calculated: 853.654716; found: 853.657447. HRMS (FAB): For $C_{47}H_{89}N_4O_7PNa$ $(M+Na)^+$ calculated: 875.636660; found: 875.635124. Anal. Calcd. for $C_{47}H_{89}N_4O_7P$: C, 66.16; H, 10.51; N, 6.57; P, 3.63. Found: C, 66.64; H, 10.23; N, 6.68; P, 3.68.

***N*-(5-{[(5S)-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl}-4-oxo-4,5-dihydro-1,3,5-triazin-2-yl)stearamide (8).** A mixture of cHPMP-5-azaC[17](100 mg, 0.38 mmol), 1M methanolic tetrabutylammonium hydroxide (0.38 mL) and methanol (50 mL) was evaporated to dryness and the residue coevaporated with pyridine. Pyridine (5 mL) was added, followed by stearoyl chloride (0.7 mL, 2 mmol) and the mixture stirred for 4 days at room temperature. Hexane (200 mL) was added, the mixture stirred for 10 min and let to stand. Hexane layer was removed after its complete separation. The operation with hexane was repeated still twice. The remaining product was purified by a reverse phase HPLC in gradient of water - acetonitrile. Approprite UV absorbing fractions were evaporated to give **8** as a white solid in yield 60 mg (30%). FAB MS, *m/z*: 551 $[M+Na]^+$ (10). HRMS (FAB): For $C_{25}H_{46}N_4O_6P$ $(MH)^+$ calculated: 529.315499; found: 529.316864. HRMS (FAB): For

$C_{25}H_{45}N_4O_6PNa$ $(M+Na)^+$ calculated: 551.297444; found: 551.295808. For records of ^1H, ^{13}C and ^{31}P NMR spectra see: Supporting information.

1-{(2*S*)-2-[(Diisopropoxyphosphoryl)methoxy-3-(triphenylmethoxy)]propyl}-*N*^{4}-docosanoylcytosine (10). Method A. Behenoyl chloride (1.13 g, 3.16 mmol) was added to a solution of **9** (ref. 19, 1.33 g, 2.2 mmol) in pyridine (25 mL) and the mixture was stirred at room temperature for 2 days. Methanol (2 mL) was added to quench the reaction. The solution was evaporated, the residue coevaporated with toluene and chromatographed on a column of silica gel (400 mL) in system ethyl acetate – acetone – ethanol – water (36:6:1:1). Yield: 1.64g (80%) of **10** as a white foam. $[\alpha]_D$ −18.0 (*c* 0.286, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm) δ: 0.88 (t, 3H, $J_{CH3,CH2}$ = 7.0, CH_3), 1.30 (m, 28 H, CH_2), 1.68 (m, 2H, CH_2), 2.49 (t, 2H, $J_{CH2,CH2}$ = 7.6, CH_2), 3.06 (dd, 1H, $J_{3´a,2´}$ = 3.4, J_{gem} = 10.6, H-3´a), 3.43 (dd, 1H, $J_{3´b,2´}$ = 3.2, J_{gem} = 10.6, H-3´b), 3.78 (dd, 1H, $J_{1´a,2´}$ = 8.6, J_{gem} = 13.6, H-1´a), 3.84 (d, 2H, $J_{P,CH}$ = 8.4, PCH_2), 3.98 (m, 1H, H-2´), 4.33 (dd, 1H, $J_{1´b,2´}$ = 3.2, J_{gem} = 13.6, H-1´b), 4.67 (m, 1H, P-OCH), 4.74 (m, 1H, P-OCH), 7.23 (t, 3H, Tr), 7.30 (t, 6H, Tr), 7.44 (d, 6H, Tr), 7.65 (d, 1H, $J_{5,6}$ = 7.4, H-5), 7.84 (d, 1H, $J_{6,5}$ = 7.4, H-6), 10.2 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm) δ: 14.03 (CH_3), 22.58 (CH_2), 23.92 (d, 2C, $J_{P,C}$ = 4.9, CH_3), 23.96 (d, 2C, $J_{P,C}$ = 3.9, CH_3), 24.91, 29.10, 29.26, 29.29, 29.43, 29.55 (2C), 29.60 (10C), 31.82 and 37.405 (CH_2), 51.76 (C-1´), 61.79 (C-3´), 64.39 (d, $J_{P,C}$ = 169.2, P-C), 70.77 (d, P-O-C), 71.06 (d, P-O-C), 78.48 (d, $J_{P,C}$ = 13.6, C-2´), 86.63 (Tr), 96.03 (C-5), 127.08 (3C, Tr), 127.84 (6C, Tr), 128.46 (6C, Tr), 143.38 (3C, Tr), 150.31 (C-6), 155.79 (C-2), 162.69 (C-4). ESI MS, *m/z*: 950.5 $[M+Na]^+$ (29). Anal. Calcd. for $C_{55}H_{82}N_3O_7P$: C, 71.17; H, 8.90; N, 4.53; P, 3.34. Found: C, 71.03; H, 9.13; N, 4.68; P, 3.52.

Method B. A suspension of behenic acid (425 mg, 1.25 mmol) and 1,1´-carbonyldiimidazole (162 mg, 1 mmol) in acetonitrile (5 mL) was stirred for 30 min at room temperature. Compound **9** (200 mg, 0.33 mmol) was added. The mixture was refluxed for 4 h and evaporated. The residue was chromatographed on a column of silica gel in system ethyl acetate – acetone – ethanol – water (36:6:1:1) to give **10** as a white foam. Yield: 190 mg (62%).

1-{(2*S*)-2-[(Diisopropoxyphosphoryl)methoxy]-3-hydroxypropyl}-N^4-docosanoylcytosine (11). A mixture of **10** (1.3 g, 1.4 mmol) and 80% trifluoroacetic acid (10 mL) was stirred vigorously in a stoppered flask for 5 min and evaporated. The residue was coevaporated with water (10 mL), dissolved in another portion of water (50 mL) and neutralized with Dowex 1 in HCO_3^- form to pH 7. Ethyl acetate was (150 mL) added, the mixture shaken well and Dowex 1 removed by suction. An organic layer was dried over magnesium sulfate and evaporated. The residue was chromatographed on a column of silica gel (100 mL) in system chloroform – methanol (9:1) to give **11** in yield 870 mg (91%) as a white solid. $[\alpha]_D$ −6.4 (*c* 0.234, $CHCl_3$). ^{1}H NMR ($CDCl_3$, ppm) δ: 0.88 (t, 3H, $J_{CH3,CH2}$ = 7.0, CH_3), 1.21-1.35 (m, 48H, CH_2, CH_3), 1.66 (m, 2H,CH_2), 2.46 (t, 2H, $J_{CH2,CH3}$ = 7.6, CH_2), 3.59 (dd, 1H, $J_{3´a,2´}$ = 3.4, J_{gem} = 12.6, H-3´a), 3.67 (dd, 1H, $J_{3´b,2´}$ = 5.5, J_{gem} = 12.6, H-3´b), 3.80 (dd, 1H, $J_{P,CHa}$ = 8.8, J_{gem} = 12.6, PCH_a), 3.85 (dd, 1H, $J_{P,CHb}$ = 8.8, J_{gem} = 12.6, PCH_b), 3.87 (m, 1H, H-2´), 4.05 (dd, 1H, $J_{1´a,2´}$ = 6.4, J_{gem} = 13.8, H-1´a), 4.17 (dd, 1H, $J_{1´b,2´}$ = 3.6, J_{gem} = 13.8, H-1´b), 4.70 (m, 1H, P-OCH), 4.75 (m, 1H, P-OCH), 7.43 (d, 1H, $J_{5,6}$ = 7.3, H-5), 7.77 (d, 1H, $J_{6,5}$ = 7.3, H-6), 9.34 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm) δ: 13.78 (CH_3), 22.35 (CH_2), 23.67 (d, 2C, $J_{P,C}$ = 4.9, CH_3), 23.71 (d, 2C, $J_{P,C}$ = 3.9, CH_3), 24.53, 29.02, 29.13, 29.20, 29.29, 29.32, 29.36 (2C), 30.15 (10C), 31.58 and 37.29 (CH_2), 50.56 (C-1´), 59.57 (C-3´), 64.44 (d, $J_{P,C}$ = 169.6, P-C), 71.19 (d, $J_{P,C}$ = 6.4, P-O-C), 71.29 (d, $J_{P,C}$ = 6.4, P-O-C), 79.88 (d, $J_{P,C}$ = 10.6, C-2´), 96.18 (C-5), 150.33 (C-6), 156.45 (C-2), 162.40 (C-4), 173.36 (C=O). ^{31}P{^{1}H} NMR ($CDCl_3$, ppm) δ: 20.17. ESI MS, *m/z*: 708.4 $[M+Na]^+$ (53), 686.3 $[MH]^+$ (100). Anal. Calcd. for $C_{36}H_{68}N_3O_7P$. H_2O : C, 61.43; H, 10.02; N, 5.97; P, 4.40. Found: C, 61.62; H, 10.09; N, 5.78; P, 4.70.

1-{(*S*)-N^4-Docosanoyl-[3-hydroxy-2-(phosphonomethoxy)propyl]}cytosine (12). Method A. Bromotrimethylsilane (1.3 mL, 10 mmol) was added at room temperature to a suspension of **11** (997 mg, 1.45 mmol) in acetonitrile (50 mL), the mixture stored in dark for three days and evaporated. The

residue was coevaporated with toluene (3x 30 mL). Methanol (50 mL) was added, the mixture neutralized with ammonia to pH 7 and evaporated. The solid residue was crystallized from water, washed with acetone, followed by diethyl ether and dried in vacuo. Yield: 380 mg (39 %) of **12** as a white solid.

Method B. A suspension of monoester **13** (120 mg, 0.2 mmol) in acetonitril (3 mL) was treated with bromotrimethylsilane (0.08 mL, 0.6 mmol) at room temperature for three days in dark. The further work up was the same as in Method A. Yield: 40 mg (35 %) of **12** as a white solid. Mp. 180-185 °C (methanol). ^{1}H NMR (CD_3OD, ppm) δ: 0.90 (t, 3H, $J_{CH3,CH2}$ = 7.0, CH_3), 1.29 (m, 36H, CH_2), 1.60 (m, 2H, CH_2), 2.25 (t, 2H, J_{CH2CH2} = 7.6, CH_2), 3.54 (dd, 1H, $J_{P,CHa}$ = 9.4, J_{gem} = 12.6, PCH_a), 3.66 (dd, 1H, $J_{P,CHb}$ = 10.6, J_{gem} = 12.6, PCH_b), 3.68 (dd, 1H, $J_{3´a,2´}$ = 3.8, J_{gem} = 12.4, H-3´a), 3.69 (m, 1H, H-2´), 3.77 (dd, 1H, $J_{3´b,2´}$ = 3.7, J_{gem} = 12.4, H-3´b), 3.92 (dd, 1H, $J_{1´a,2´}$ = 7.8, J_{gem} = 13.8, H-1´a), 4.23 (dd, 1H, $J_{1´b,2´}$ = 3.6, J_{gem} = 13.8, H-1´b), 7.41 (d, 1H, $J_{5,6}$ = 7.3, H-5), 8.10 (d, 1H, $J_{6,5}$ = 7.3, H-6). ^{13}C NMR (CD_3OD, ppm) δ: 12.67 (CH_3), 21.98, 24.33, 24.58, 28.44, 28.57, 28.68 (2C), 28.83, 29.08 (10C), 31.32 and 36.40 (CH_2), 50.56 (C-1´), 60.15 (C-3´), 66.68 (d, $J_{P,C}$ = 157.2, P-C), 79.79 (d, $J_{P,C}$ = 12.6, C-2´), 96.13 (C-5), 151.20 (C-6), 157.40 (C-2), 162.71 (C-4), 174.20 (C=O). ^{31}P{^{1}H} NMR (CD_3OD, t = 50°C, ppm) δ: 15.59. -ESI MS, *m/z*: 1201.4 $[2M-H]^-$ (16), 600.4 $[M-H]^-$ (100). HRMS (-ESI): For $C_{30}H_{55}N_3O_7P$ $(M-H)^-$ calculated: 600.3778; found: 600.3788. Anal. Calcd. for $C_{30}H_{56}N_3O_7P$: C, 59.88; H, 9.38; N, 6.98; P, 5.15. Found: C, 61.23; H, 9.59; N, 6.71; P, 4.91.

***N*^{4}-Docosanoyl-1-{(2*S*)-2-[(isopropoxyphosphoryl)methoxy]-3-hydroxypropyl}cytosine (13).** Bromotrimethylsilane (0.4 mL, 3 mmol) was added at 0 °C dropwise to a suspension of **11** (422 mg, 0.62 mmol) in acetonitrile (5 mL), the mixture kept in refrigerator overnight and evaporated. 0.5 % NH_4OH was added to a solid residue to neutralize the mixture to pH 7. The solid was filtered off, washed with water, followed by acetone and diethyl ether and dried in vacuo. Yield: 210 mg (53 %) of a white solid. Mp. 120 °C (CH_3OH). $[\alpha]_D$ −23.2 (*c* 0.241, CH_3OH). ^{1}H NMR (CD_3OD, t = 40°C, ppm) δ: 0.90 (m, 3H, $CH_3(CH_2)_{19}CH_2$), 1.18, 1.21 (2 × d, 2 × 3H, J_{vic} = 6.2, $(CH_3)_2CH$), 1.25-1.40 (m,

36H, $CH_3(CH_2)_{18}CH_2CH_2$), 1.66 (m, 2H, $CH_3(CH_2)_{18}CH_2CH_2$), 2.43 (t, 2H, $J_{CH2,CH2}$ = 7.4, $CH_3(CH_2)_{19}CH_2$), 3.53 (dd, 1H, $J_{3´a,2´}$ = 3.9, J_{gem} = 12.2, H-3´a), 3.54 (dd, 1H, $J_{P,CHa}$ = 9.2, J_{gem} = 12.8, PCH_a), 3.67 (dd, 1H, $J_{P,CHb}$ = 9.8, J_{gem} = 12.8, PCH_b), 3.73 (m, 1H, H-2´), 3.77 (dd, 1H, $J_{3´b,2´}$ = 4.1, J_{gem} = 12.2, H-3´b), 3.94 (dd, 1H, $J_{1´a,2´}$ = 7.6, J_{gem} = 13.7, H-1´a), 4.21 (dd, 1H, $J_{1´b,2´}$ = 3.4, J_{gem} = 13.7, H-1´b), 4.45 (dsep, 1H, J_{vic} = 6.2, $J_{H,P}$ = 8.1, P-OCH), 7.40 (d, 1H, $J_{5,6}$ = 7.3, H-5), 8.06 (d, 1H, $J_{6,5}$ = 7.3, H-6). ^{13}C NMR (CD_3OD, t = 40°C, ppm) δ: 14.40 ($CH_3(CH_2)_{19}CH_2$), 23.69 ($CH_3(CH_2)_{18}CH_2CH_2$), 24.81 (d, $J_{P,C}$ = 3.8, $(CH_3)_2CH$), 24.90 (d, $J_{P,C}$ = 4.1, $(CH_3)_2CH$), 26.08 ($CH_3(CH_2)_{18}CH_2CH_2$), 30.16, 30.40, 30.42, 30.56, 30.66, 30.71, 33.03 ($CH_3(CH_2)_{18}CH_2CH_2$), 38.18 ($CH_3(CH_2)_{18}CH_2CH_2$), 52.49 (C-1´), 61.62 (C-3´), 67.50 (d, $J_{P,C}$ = 160.4, P-C), 69.36 (d, $J_{P,C}$ = 6.1, $(CH_3)_2CH$), 81.15 (d, $J_{P,C}$ = 11.8, C-2´), 97.89 (C-5), 152.75 (C-6), 159.02 (C-2), 164.35 (C-4), 175.80 (C=O).

ESI MS, *m/z*: 1285.4 $[2M-H]^-$ (20), 642.4 $[M-H]^-$ (100). HRMS (-ESI): For $C_{33}H_{61}N_3O_7P$ $(M-H)^-$ calculated: 642.4247; found: 642.4256. Anal. Calcd. for $C_{33}H_{62}N_3O_7P$: C, 61.56; H, 9.71; N, 6.53; P, 4.81. Found: C, 61.67; H, 9.67; N, 6.68; P, 4.51.

Pivaloyloxymethylation of cyclic cidofovir (cHPMPC). A suspension of cHPMPC (400 mg, 1.53 mmol) in methanol (50 mL) was stirred with 1M methanolic tetrabutylammonium hydroxide (1.6 mL) till dissolution and evaporated. The residue was coevaporated with dioxane (2 x 20 mL), then dissolved in dioxane (15 mL) and stirred with chloromethyl pivalate (1.5 mL, 10.4 mmol) at 90°C for 1h. After cooling to room temperature, the reaction was quenched with methanol (5 mL) and the mixture evaporated. The residue was chromatographed on a column of silica gel (150 mL) in system chloroform – methanol (9:1) to give products **14** (R_F 0.65) and **15** (R_F 0.30).

({(5*S*)-2-Oxido-5-[(*N*^{4}-pivaloylcytosin-1-yl)methyl]-1,4,2-dioxaphosphinan-2-yl}oxy)methyl pivalate (14). Yield: 281 mg (40 %) as a white foam. According to NMR spectrum the compound is a mixture of 2 diastereoisomers (A:B ~ 5:3). ^{1}H NMR ($CDCl_3$, ppm) δ: 1.20 (s, 9H, $(CH_3)_3CCOO$-B), 1.23 (s, 9H, $(CH_3)_3CCOO$-A), 1.27 (s, 9H, $(CH_3)_3CCON$-A,B), 3.55 (dd, 1H, $J_{1´a,2´}$ = 7.7, J_{gem} = 14.0,

H-1´a-A), 3.64 (dd, 1H, $J_{1´a,2´}$ = 8.2, J_{gem} = 13.9, H-1´a-B), 3.83 (dd, 1H, $J_{P,CHa}$ = 1.8, J_{gem} = 14.2, PCH_a-B), 3.92 (d, 1H, J_{gem} = 14.7, PCH_a-A), 4.08-4.23 (m, 5H, H-2´-A,B, H-3´a-A, PCH_b-A,B), 4.38-4.46 (m, 3H, H-3´a-A, H-3´-B), 5.58 (dd, 1H, J_{gem} = 5.2, $J_{P,CHa}$ = 12.1, OCH_aH_bO-B), 5.69 (dd, 1H, J_{gem} = 5.2, $J_{P,CHa}$ = 11.9, OCH_aH_bO-B), 5.73 (dd, 1H, J_{gem} = 5.2, $J_{P,CHb}$ = 13.9, OCH_aH_bO-A), 5.75 (dd, 1H, J_{gem} = 5.2, $J_{P,CHb}$ = 13.9, OCH_aH_bO-B), 7.39 (d, 1H, $J_{5,6}$ = 7.3, H-5-A), 7.40 (d, 1H, $J_{5,6}$ = 7.3, H-5-B), 7.54 (d, 1H, $J_{6,5}$ = 7.3, H-6-A), 7.59 (d, 1H, $J_{6,5}$ = 7.3, H-6-B), 8.15 (bs, 2H, NH-A,B). ^{13}C NMR ($CDCl_3$, ppm) δ: 26.74 ($(CH_3)_3CCOO$-B), 26.80 ($(CH_3)_3CCOO$-A), 27.04 ($(CH_3)_3CCON$-A,B), 38.65 ($(CH_3)_3CCOO$-B), 38.72 ($(CH_3)_3CCOO$-A), 40.31 ($(CH_3)_3CCON$-A,B), 49.81 (C-1´-A), 49.91 (C-1´-B), 64.11 (d, $J_{P,C}$ = 144.3, P-C-A), 64.57 (d, $J_{P,C}$ = 147.2, P-C-B), 70.79 (d, $J_{P,C}$ = 6.9, C-3´-B), 72.23 (d, $J_{P,C}$ = 9.1, C-3´-A), 73.19 (d, $J_{P,C}$ = 4.8, C-2´-B), 73.54 (d, $J_{P,C}$ = 5.3, C-2´-A), 81.42 (d, $J_{P,C}$ = 6.2, OCH_2O-A), 81.77 (d, $J_{P,C}$ = 5.4, OCH_2O-B), 96.26 (C-5-A), 96.32 (C-5-B), 149.80 (C-6-A), 149.96 (C-6-B), 155.72 (C-2-A), 155.77 (C-2-B), 162.74 (C-4-A,B), 176.91 ($(CH_3)_3CCOO$-A), 176.93 ($(CH_3)_3CCOO$-B), 178.04 ($(CH_3)_3CCON$-B), 178.08 ($(CH_3)_3CCON$-A). $^{31}P\{^1H\}$ NMR ($CDCl_3$, ppm) δ: 10.71 (A), 12.89 (B). ESI MS, *m/z*: 940.7 $[2M+Na]^+$ (20), 482.1 $[M+Na]^+$ (82), 460.1 $[MH]^+$ (100). HRMS (-ESI): For $C_{19}H_{31}N_3O_8P$ $(MH)^+$ calculated: 460.1849; found: 460.1844. Anal. Calcd. for $C_{19}H_{30}N_3O_8P$: C, 49.67; H, 6.58; N, 9.15; P, 6.74. Found: C, 49.99; H, 6.72; N, 8.88; P, 6.61

({(5S)-5-[(Cytosin-1-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl}oxy)methyl pivalate (15). Yield: 109 mg (19 %) as a white foam. According to NMR the compound is a mixture of 2 diastereoisomers 3:1 (Fig.3). HPLC separation was performed with a linear gradient MeOH – water (0-100 %, flow rate 5 mL/min), diastereoisomer A was eluted at concentration 45 % MeOH, isomer B at 50 % MeOH. Major diastereoisomer A: ^{1}H NMR ($CDCl_3$, ppm) δ: 1.25 (s, 9H, $(CH_3)_3C$), 4.05 (dd, 1H, $J_{1´a,2´}$ = 7.7, J_{gem} = 14.4, H-1´a), 3.94 (d, 1H, J_{gem} = 14.7, PCH_a), 4.04-4.12 (m, 2H, H-1´b,2´), 4.15 (dd, 1H, $J_{P,CHb}$ = 11.2, J_{gem} = 14.7, PCH_b), 4.25 (t, 1H, $J_{3´a,2´}$ = J_{gem} = 11.5, H-3´a), 4.42 (ddd, 1H, $J_{P,3´b}$ = 17.0, J_{gem} = 11.5, $J_{3´b,2´}$ = 2.0, H-3´b), 5.71 (dd, 1H, J_{gem} = 5.2, $J_{P,CHa}$ = 11.8, OCH_aH_bO), 5.74

(dd, 1H, J_{gem} = 5.2, $J_{P,CHb}$ = 14.1, OCH_aH_bO), 5.75 (d, 1H, $J_{5,6}$ = 7.3, H-5), 7.22 (d, 1H, $J_{6,5}$ = 7.3, H-6). ^{13}C NMR ($CDCl_3$, ppm) δ: 26.81 ($(CH_3)_3C$), 38.71 ($(CH_3)_3C$), 49.21 (C-1´), 64.05 (d, $J_{P,C}$ = 144.9, P-C), 72.55 (d, $J_{P,C}$ = 9.1, C-3´), 74.30 (d, $J_{P,C}$ = 5.3, C-2´), 81.39 (d, $J_{P,C}$ = 6.2, OCH_2O), 94.31 (C-5), 146.45 (C-6), 156.42 (C-2), 166.19 (C-4), 176.98 (C=O). $^{31}P\{^1H\}$ NMR ($CDCl_3$, ppm) δ: 10.94.

Minor diastereoisomer B: ^{1}H NMR ($CDCl_3$, ppm) δ: 1.22 (s, 9H, $(CH_3)_3C$), 3.56 (dd, 1H, $J_{1´a,2´}$ = 8.3, J_{gem} = 14.6, H-1´a), 3.86 (dd, 1H, $J_{P,CHa}$ = 1.7, J_{gem} = 14.2, PCH_a), 4.09-4.12 (m, 2H, H-1´b,2´), 4.23 (dd, 1H, $J_{P,CHb}$ = 9.8, J_{gem} = 14.2, PCH_b), 4.40-4.49 (m, 2H, H-3´), 5.59 (dd, 1H, J_{gem} = 5.2, $J_{P,CHa}$ = 12.0, OCH_aH_bO), 5.68 (d, 1H, $J_{5,6}$ = 7.2, H-5), 5.77 (dd, 1H, J_{gem} = 5.2, $J_{P,CHb}$ = 14.0, OCH_aH_bO), 7.28 (d, 1H, $J_{6,5}$ = 7.2, H-6). ^{13}C NMR ($CDCl_3$, ppm) δ: 26.78 ($(CH_3)_3C$), 38.69 ($(CH_3)_3C$), 49.41 (C-1´), 64.60 (d, $J_{P,C}$ = 147.1, P-C), 71.03 (d, $J_{P,C}$ = 6.9, C-3´), 73.84 (d, $J_{P,C}$ = 4.9, C-2´), 81.78 (d, $J_{P,C}$ = 5.6, OCH_2O), 93.72 (C-5), 147.04 (C-6), 156.31 (C-2), 165.84 (C-4), 176.95 (C=O). $^{31}P\{^1H\}$ NMR ($CDCl_3$, ppm) δ: 12.86. ESI MS, *m/z*: 772.7 $[2M+Na]^+$ (15), 750.9 $[2M+H]^+$ (20), 398.0 $[M+Na]^+$ (22), 376.0 $[MH]^+$ (28). HRMS (ESI): For $C_{14}H_{22}N_3O_7PNa$ $(M+Na)^+$ calculated: 398.10876; found: 398.10888. Anal. Calcd. for $C_{14}H_{22}N_3O_7P$: C, 44.80; H, 5.91; N, 11.20; P, 8.25. Found: C, 44.99; H, 6.14; N, 10.96; P, 8.37.

Antiviral Activity Assays

The compounds were evaluated against different herpesviruses, including herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strain G, varicella-zoster virus (VZV) strains Oka and YS, TK- VZV strains 07-1 and YS-R, human cytomegalovirus (HCMV) strains AD-169 and Davis as well as poxviruses, including vaccinia virus Lederle and Western Reserve (WR) strains, cowpox virus strain Brighton, camelpox virus strains CML-1 and CML-14 and orf virus strain NZ2. The antiviral assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque

forming units (PFU). After a 1-2 h adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation (VZV) was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%.

Cytostatic and Cytotoxic Assays.

Cytotoxicity measurements were based on the inhibition of cell growth. HEL cells were seeded at a rate of 5 x 10^3 cells/well into 96-well microtiter plates and allowed to adhere and proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of further incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, or the compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Alternatively, cytotoxicity of the test compounds was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that caused a microscopically detectable alteration of cell morphology. Selectivity indexes were calculated as the ratio of CC_{50} to EC_{50}.

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Figure 1. Chemical structures of cidofovir and related 5-azacytosine derivatives

Figure 2. An example of the mass spectrum (electrospray ionization, ESI) of an N^4-acyl derivative of HPMP-5-azaC.

Figure 3. Structure of diastereoisomers of cyclic esters **15** and **7**.

Scheme 1. Synthesis and ring-opening reactions of N^4-acyl derivatives of HPMP-5-azaC esters.

Scheme 2. The course of hydrolytic decomposition of HPMP-5-azaC.

Scheme 3. Acylation of cyclic derivatives of HPMP-5-azaC.

Scheme 4. Preparation of N^4-docosanoyl (behenoyl) derivatives of cidofovir.

Scheme 5. Pivaloyloxymethylation of cyclic cidofovir

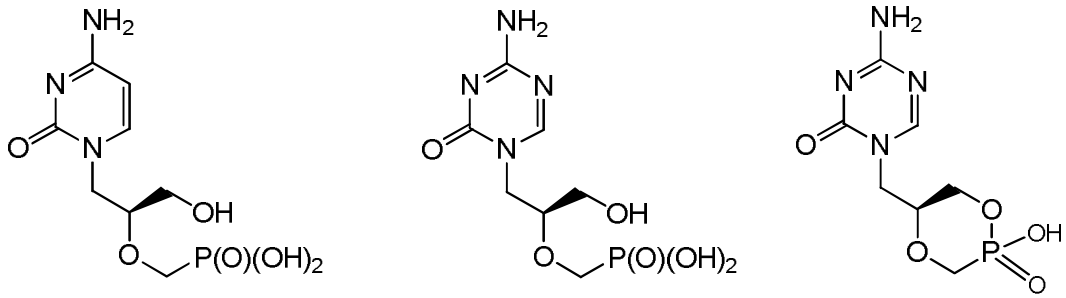


Figure 1.

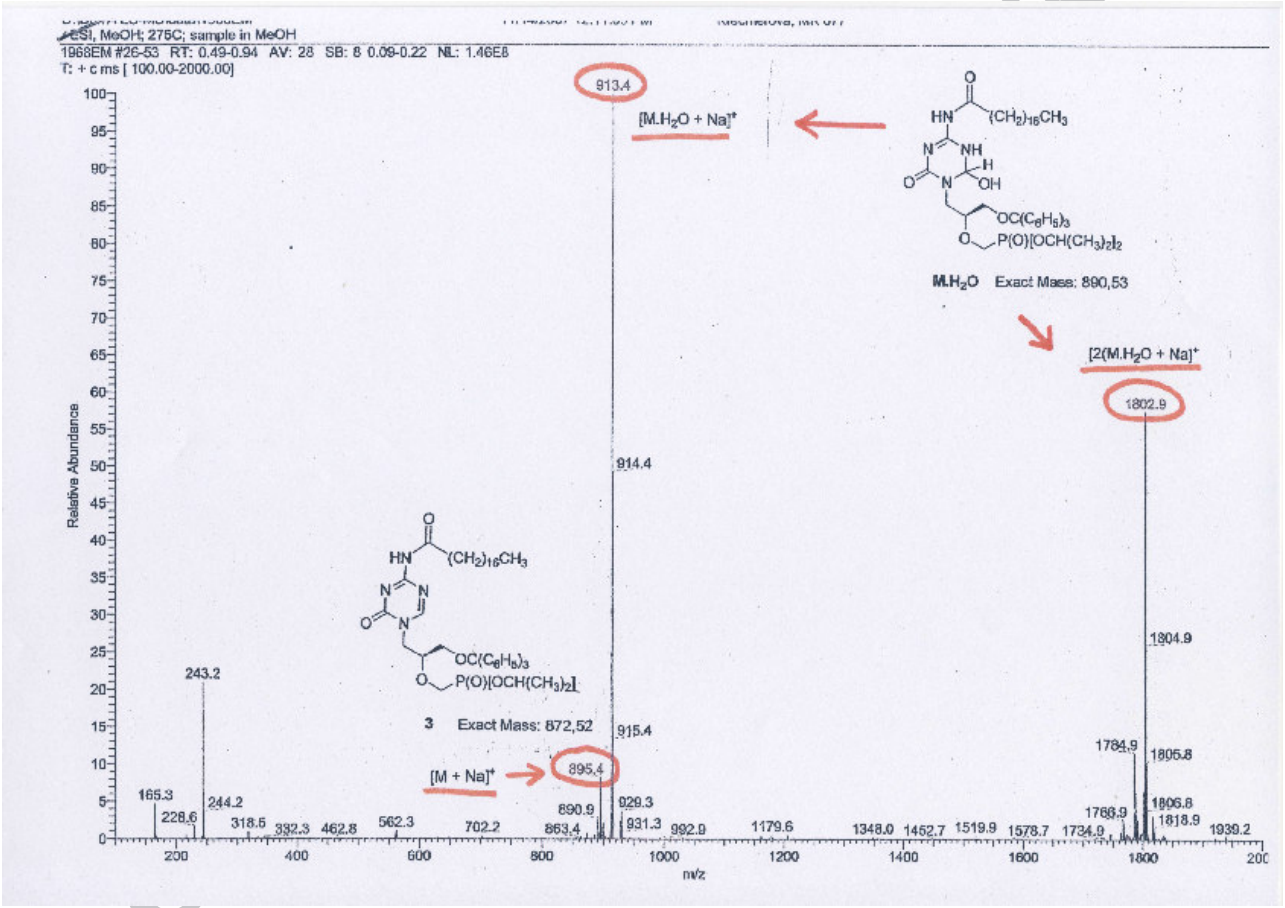
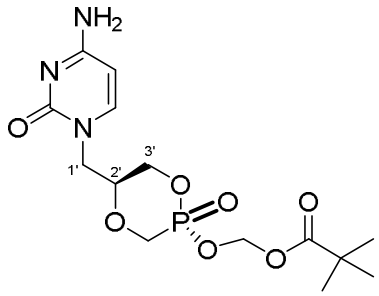
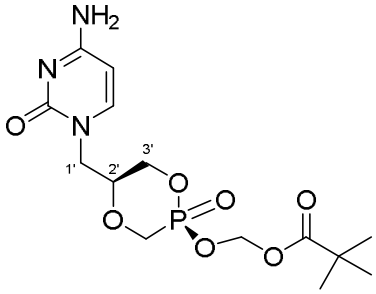


Figure 2.



15, major diastereoisomer A



15, minor diastereoisomer B

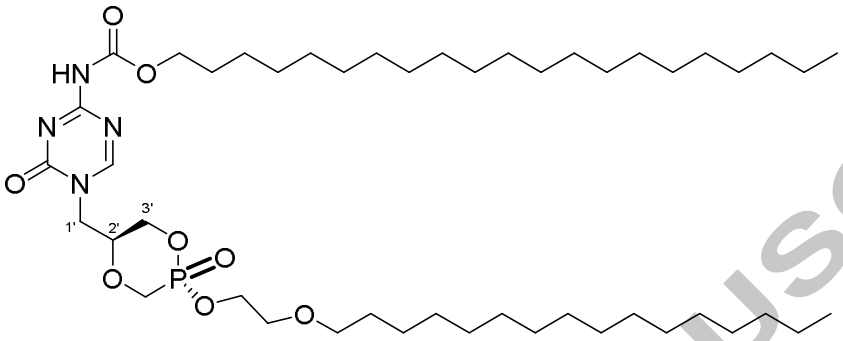


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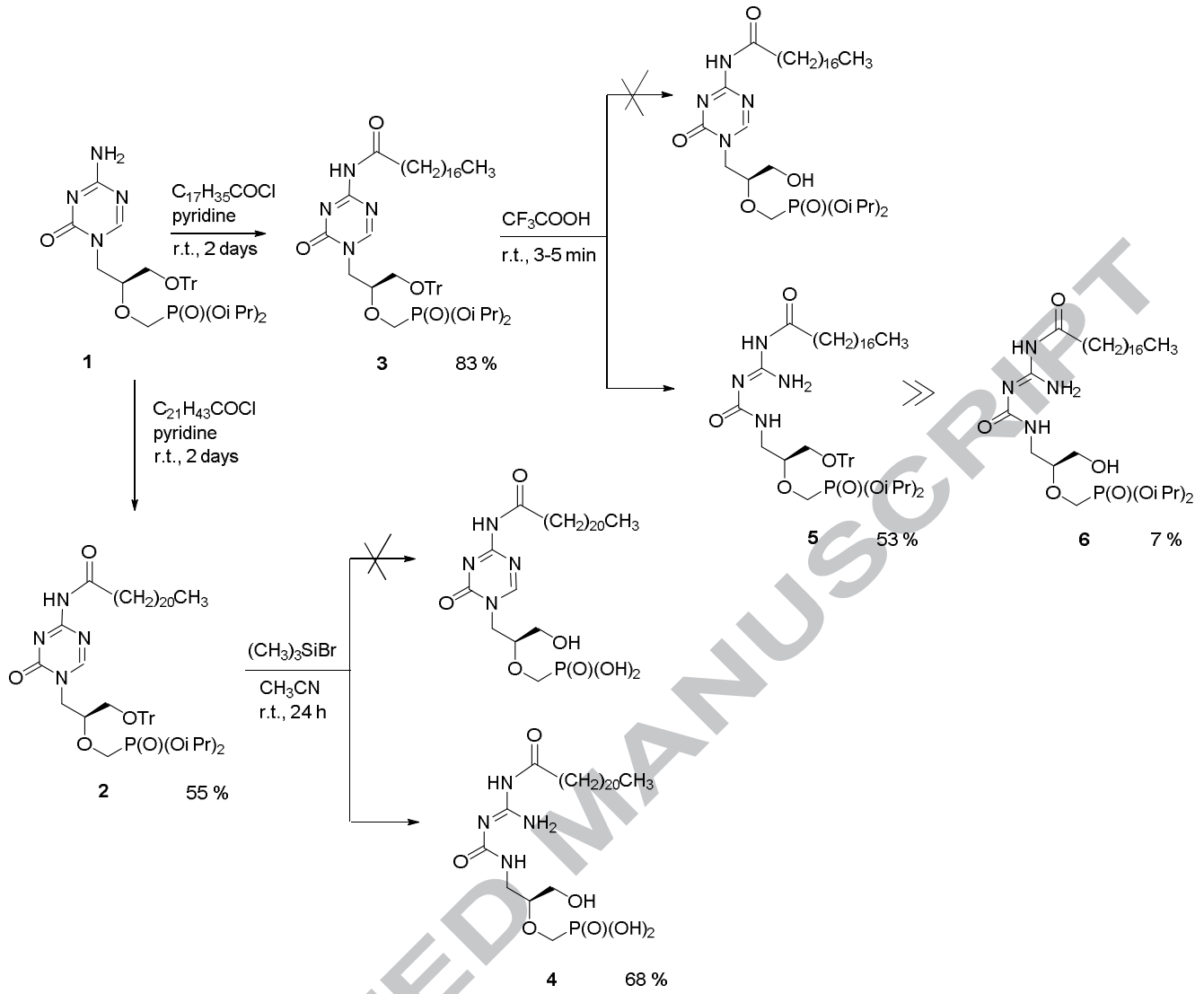
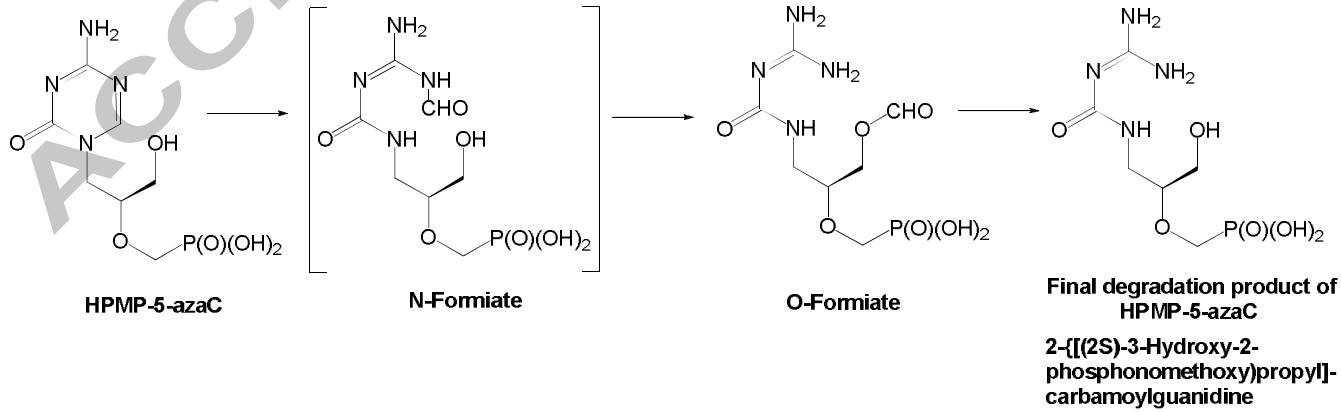
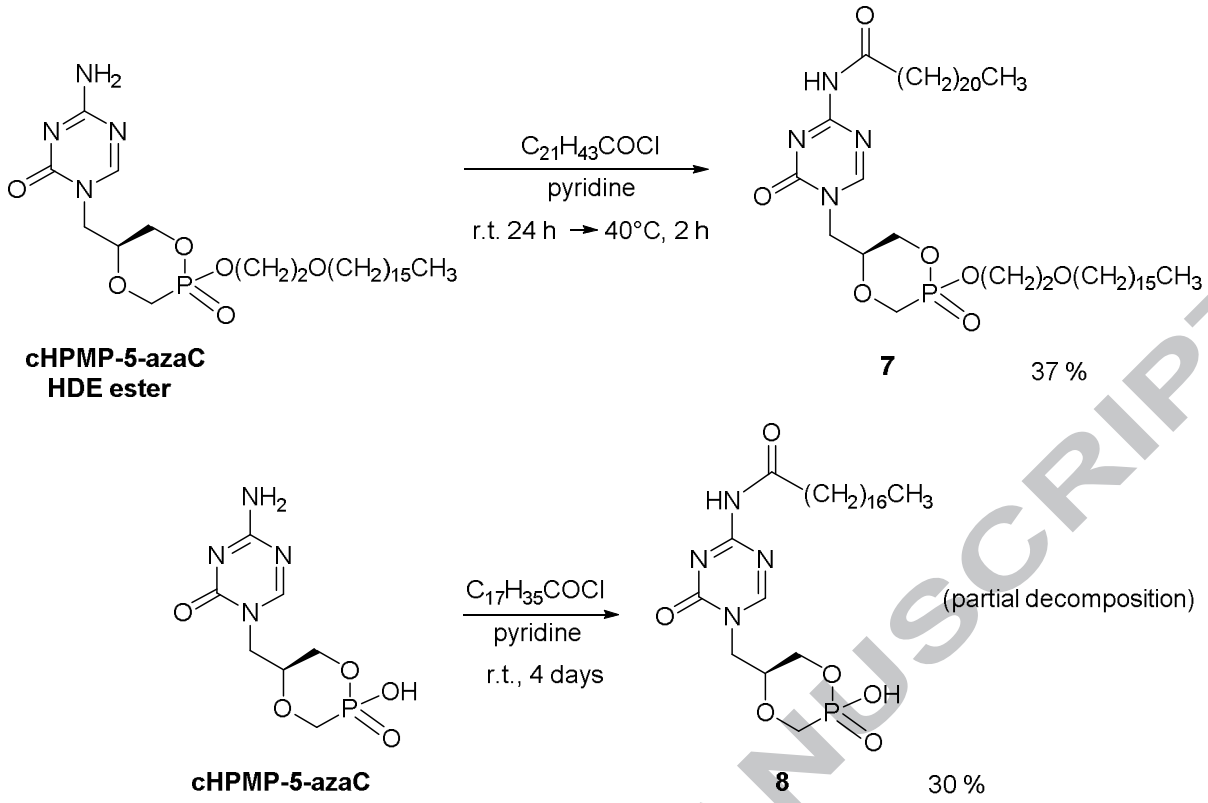
Figure 3.



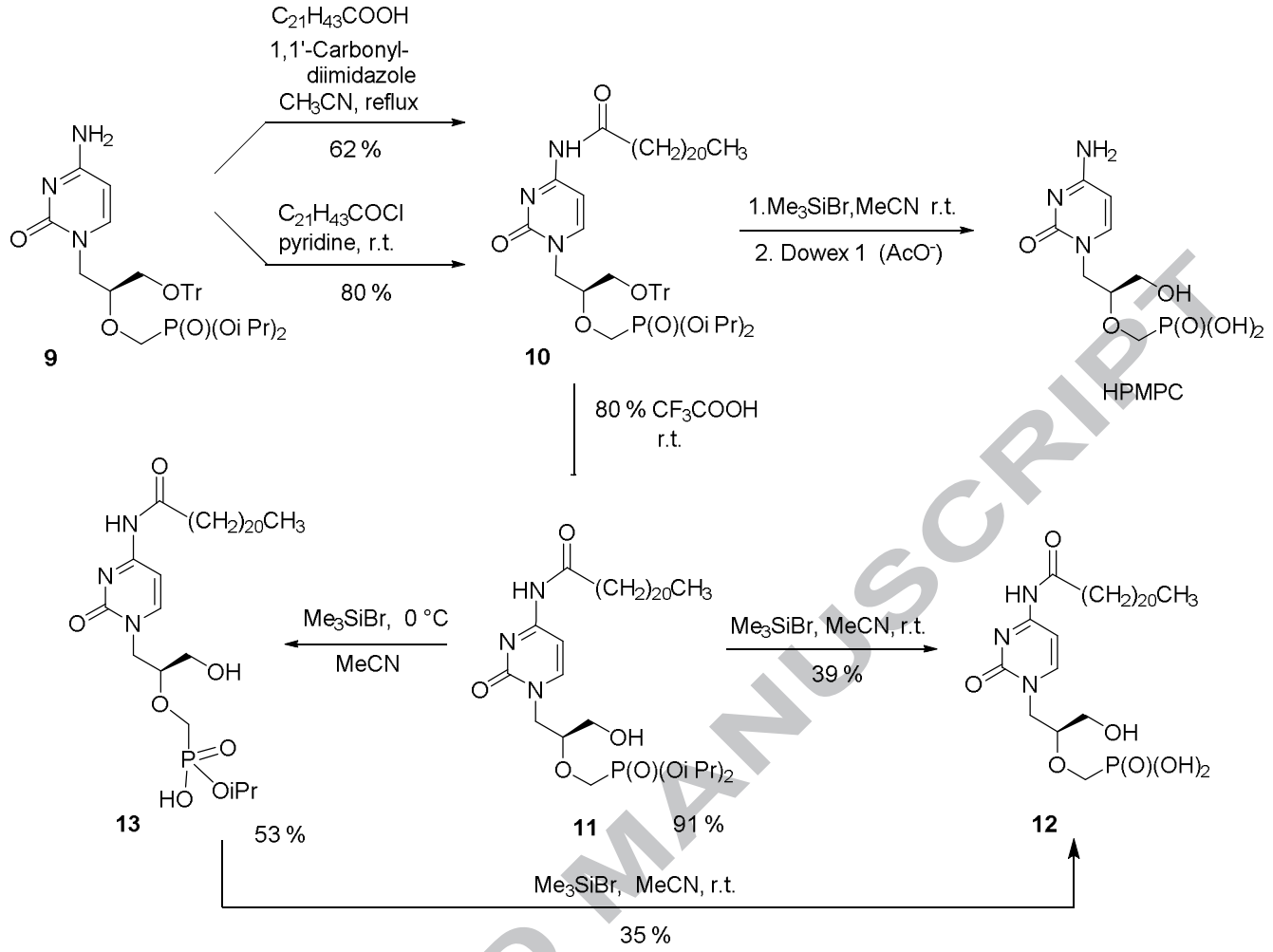
Scheme 1.



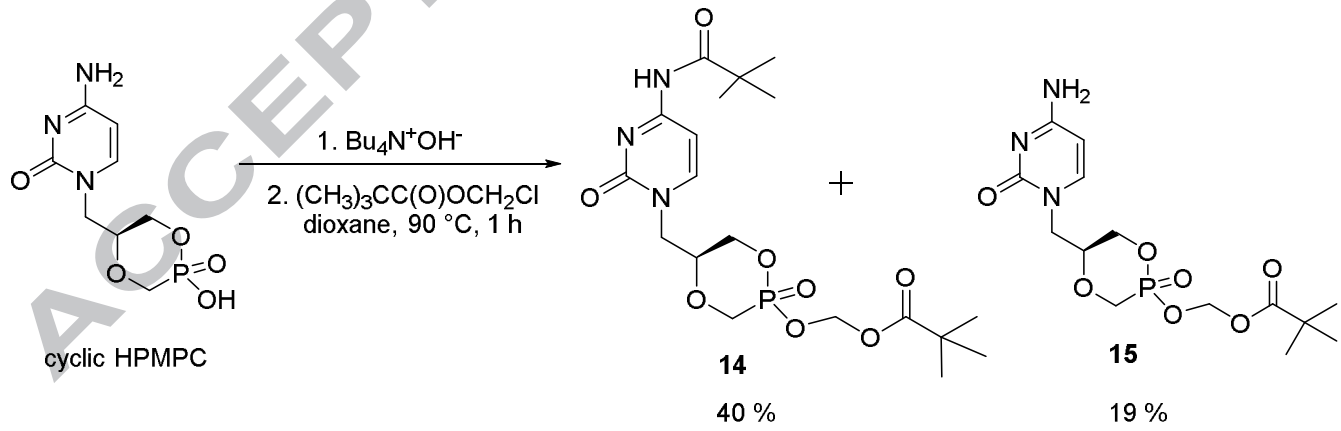
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.