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Synthesis of 1-(8-Phosphonomethoxy-3,6-dioxaoctyl)pyrimidines and Purines, a Novel Series of Acyclonucleotide Analogues

Marie Charron, H. L. Allan Tse, and Tarek S. Mansour*

BioChem Therapeutic Inc., Laval, PQ, Canada H7V 4A7

David J. Knight, Corrine O'Sullivan, and Jonathan A. V. Coates

Department of Virology, Glaxo Research and Development Ltd., Greenford, Middlesex, United Kingdom, UB6 OHE

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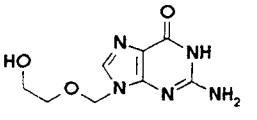
ABSTRACT

A convenient synthesis of 1-(8-phosphonomethoxy-3,6-dioxaoctyl) uracil, cytosine, adenine, and guanine nucleotide analogues **26**, **28**, **30**, *and* **32** *by Mitsunobu coupling of the nucleobases with suitably functionalized alcohol derivative* **24** *is described. The antiviral activity of this series of compounds against herpes simplex virus (HSV) types 1 and 2 and human cytomegalovirus is reported.*



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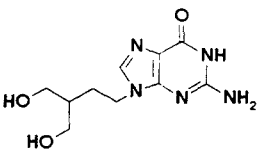

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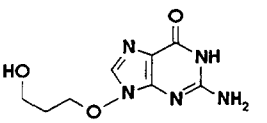

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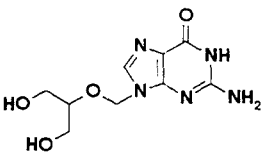

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INTRODUCTION

One of the most promising approaches in the design and synthesis of potential antiviral agents is to chemically modify compounds with demonstrable biological activity. Such modification of an active molecule has in many instances brought about a significant change in biological response. This is well illustrated by comparison of the guanine acyclic nucleosides **1**–**4**. Acyclovir (**1**) has potent activity against herpes simplex viruses (HSV) types 1 and 2, whereas **2** (BRL44385) is active against retroviruses and has antiherpetic activity [1,2]. Penciclovir (**3**) possesses potent activity against HSV 1 and 2 [3], whereas ganciclovir (**4**) is inhibitory to human cytomegalovirus [4].

The effectiveness of antiviral agents depends on selective conversion in infected cells by viral kinases to the corresponding monophosphate analogues and further phosphorylation by host cell enzymes to the triphosphates and incorporation into viral nucleic acid. To circumvent the dependence and selectivity of kinases toward nucleoside analogues, De Clercq et al. designed the phosphonomethoxy group as a stable phosphate mimic [5], which resulted in potent antiviral nucleotides having the 3-hydroxy-2-(phosphonylmethoxy)-propyl (HPMP) and 2-(phosphonylmethoxy)ethyl (PME) side chains [6]. Indeed, the cytosine and adenine nucleotides S-HPMPC (GS 504) and PMEA prodrug (GS 840) are currently under clinical evaluation against cytomegalovirus and human immunodeficiency viruses infections, respectively.

Acyclic analogues containing (1) flexible chains

*To whom correspondence should be addressed.

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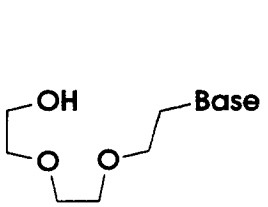
such as 2,3-dihydroxypropyl [7], 1,4-dihydroxybutyl [8], 3,5-dihydroxypentyl [9], and 1′,2′-seco[10,11],3′,4′-seco [12], (2) rigid unsaturated chains [13,14], and (3) a heteroatom (N,O) attached to the nitrogen atom of the base [1,15] have been described. It is likely that the ability of the acyclic chain, in mimicking the interaction of the carbohydrate portion of a nucleoside with the enzyme, plays a pivotal role in the phosphorylation or DNA polymerase inhibition mechanisms. With this in mind, we considered an approach to acyclonucleotides in which the PME moiety is separated from the nucleobase by a 3,6-dioxaoctanyl unit, resulting in crown-ether-like structures **5** and **6**. Synthetic routes of various analogues are presented.



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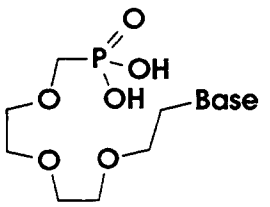

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RESULTS AND DISCUSSION

The syntheses of the uracil, 4-thiouracil, cytosine, and adenine acyclonucleosides are shown in Scheme 1.

The monosilyl derivative **8** was readily converted to the corresponding iodo intermediate **9**. Alkylation of uracil regiospecifically at N–1 with iodide **9** proceeds smoothly with DBU to afford the desired nucleoside **10**, which was readily desilylated with tetra-*n*-butylammonium fluoride (TBAF) to give **11** [16]. To overcome the low yield obtained in alkylating cytosine with iodide **9** under the previous conditions, the 4-thio derivative **13** (65% overall yield) was prepared by thionation of **10** with Lawesson's reagent, followed by desilylation with TBAF in THF. Aminolysis of nucleoside **13** in a sealed vessel furnished the cytosine containing nucleoside **14** in good yield. The alkylation of 6-chloropurine with **9** afforded a mixture of N–9 and N–7 regioisomers, **15** and **16**, in a ratio of 3:1, which were separated by chromatography on silica gel. Assignment of the structures was readily deduced from their UV and NMR spectra [17]. Desilylation of **15** followed by aminolysis produced the adenine derivative **17**.

With **11** in hand, we proceeded to prepare its PME derivative by alkylating the suitably N–3 protected uracil **20** with triflate **18** [18] (Scheme 2). In this case, alkylation proceeded in excellent yield to furnish the nucleotide **21**. However, attempted removal of the methoxybenzyl group oxidatively by ceric ammonium nitrate, hydrogenolysis with PdO-cyclohexene, NBS, or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) has failed. On the other hand, attempted deprotection by bromo- or iodotrimethylsilane [19] resulted in the formation of the phosphonic acid derivative **22**. Similar observations were obtained with the N–3 benzyl derivative. As no decomposition of the substrate was observed upon deprotection with different reagents, difficulty in deprotection of **21** is likely due to complexation of reagents with **19**.

As an alternative route, the PME moiety was introduced into the dioxaoctanyl chain prior to alkylation with the nucleobase [20]. Alkylation of alcohol **23** with triflate **18** followed by debenzylation afforded **24** in 73% overall yield. Under Mitsunobu conditions [21] [diethylazo dicarboxylate (DEAD)—triphenylphosphine (Ph_3P) mixtures], uracil, *N*-acetylcytosine, *N*-benzoyl adenine, and 2-amino-6-chloropurine were alkylated regiospecifically at N–1 or N–9 sites, respectively, albeit in low yields (Scheme 3).

Further progress to the ultimate compounds **26**, **28**, **30**, and **32** of the coupling products **25**, **27**, **29**, and **31** required dealkylation of ethyl groups of the phosphonates, which was achieved with bromotrimethylsilane in CH_3CN or CH_2Cl_2 (in the case of **29**) and purification of final products by HPLC techniques using YMC reverse phase C18 column (5 μ, 120 Å).

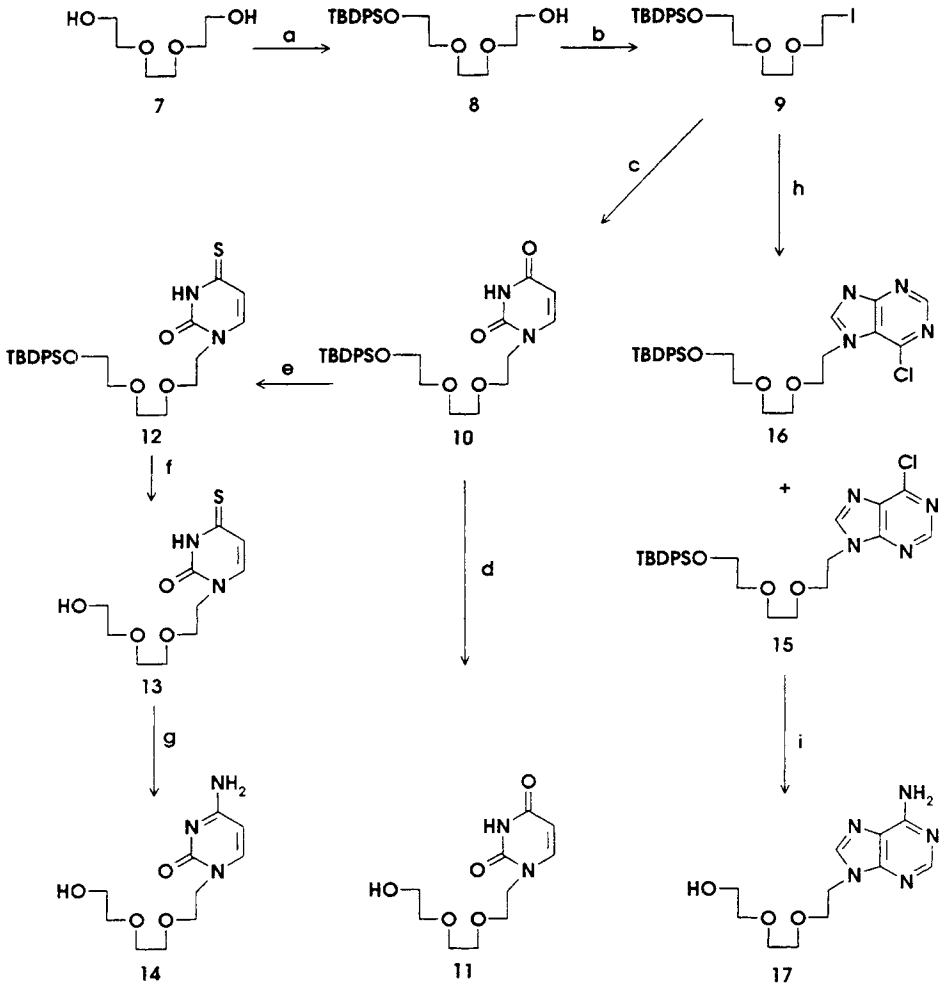
Compounds **11**, **13**, **14**, **26**, **28**, **30**, and **32** were assayed for activity against herpes simplex virus types 1 and 2 and human cytomegalovirus in Vero and flow 2002 cells, respectively, by plaque reduction assays [22]. None of the compounds exhibited appreciable activity up to 100 μg/ml, and none were found to be cytotoxic.

In conclusion, we have described an efficient route to the class of 8-phosphomethoxy-3,6-dioxaoctyl pyrimidines and purines based on Mitsunobu alkylation of the nucleobase with an acyclic chain already functionalized with a protected phosphonylmethyl ether group. The lack of antiherpetic activity is attributed to the inability of the triphosphates to inhibit the target enzymes or difficulty in forming the triphosphates.

EXPERIMENTAL

General Methods

Melting points were determined with a Mel-Temp II melting point apparatus and were uncorrected. TLC was performed on 0.20 mm thick Merck silica gel 60 F254. Components were located by spraying with 2% ceric sulfate in 2N sulfuric acid and heating with a hot gun until coloration took place. Flash column chromatography was performed on Merck silica gel particle size 0.04–0.063 mm (230–400 mesh) with the solvent systems specified. Evaporations were conducted in vacuo. The ^1H and ^{13}C



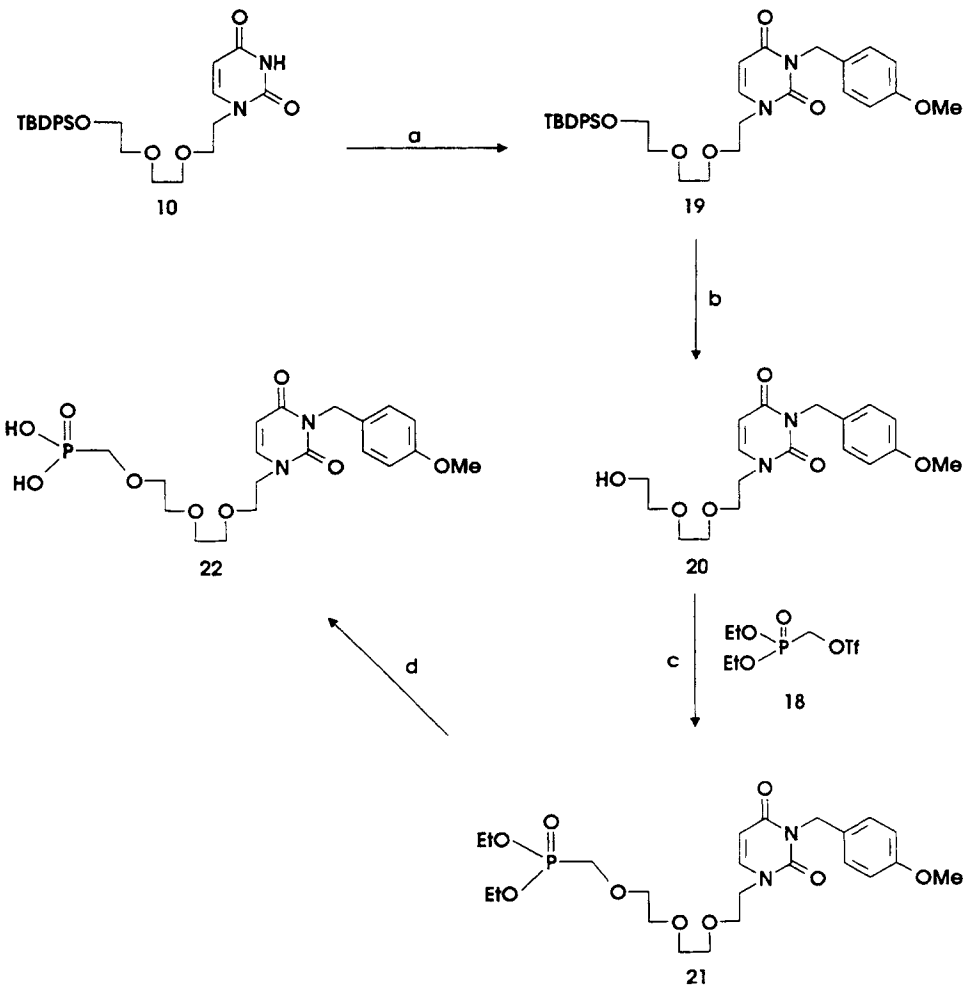
SCHEME 1 Synthesis of 8-hydroxy-3,6-dioxaoctyl uracil, 4'-thiouracil; cytosine, and adenine derivatives. (a) TBDPSCl, imidazole, DMF, RT, 16 hours, 55%; (b) Ph_3P, I_2, imidazole, CH_2Cl_2, 0°C, 25 minutes, 81%; (c) uracil, DBU, CH_3CN, 70°C, 16 hours 50%; (d) TBAF, THF, RT, 16 hours, 82%; (e) Lawesson's reagent, $(CH_2Cl)_2$, reflux, 20 hours, 72%; (f) TBAF, THF, RT, 1.5 hours, 93%; (g) NH_3 (g), CH_3OH, 4 hours, 83%; (h) 6-chloropurine, DBU, CH_3CN, reflux, 3 hours, 13% (N −7), 41% (N−9) after column chromatography; (i) 1. TBAF, THF, RT, 1.5 hours, 2. NH_3 (g), EtOH, 16 hours, 30%.

NMR spectra were recorded on a Varian Gemini 300 NMR spectrometer. Chemical shifts are reported in parts per million downfield from internal TMS. UV spectra were recorded on a Varian Cary 1 spectrometer.

8-O-Phosphonomethyl-3,6-dioxaoctyl-1-(N−1')-Uracil (**26**)

A solution of 8-oxybenzyldioxaoctanol (**23**) (702 mg, 2.92 mmol) in 2 mL of THF was added to NaH (128 mg of a 60% dispersion in oil, 3.21 mmol) in 1 mL THF at 0°C under argon, and the resulting solution was stirred for 15 minutes. A solution of diethyl phosphonomethyltriflate (**18**) (964 mg, 3.21 mmol) in 1 mL of THF was then added, and the reaction was allowed to warm up to room temperature while being stirred for 2 hours. The reaction mixture was then quenched with a few drops of MeOH, concentrated and purified by flash column chromatography (5% MeOH-EtOAc) to afford 912 mg (80%) of 8-*O*-diethylphosphonomethyl-1-oxybenzyl-3,6-dioxaoctane; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.32 (t, 6H, J = 6.2 Hz), 3.68 (br m, 10H), 3.78 (m, 2H), 3.88 (m, 2H), 4.17 (m, 4H), 4.66 (s, 2H), 7.34 (m, 5H).

Cyclohexene (1 mL) was added to a solution of8-*O*-diethylphosphonomethyl-1-oxybenzyl-3,6,dioxaoctane (912 mg, 2.34 mmol) and $PdO.H_2O$ (29 mg, 0.234 mmol) in 5 mL dry EtOH. The solution was refluxed for 1.5 hours, filtered through celite, concentrated and purified by flash column chromatography (15% MeOH-EtOAc) to afford 624 mg (89%) of 8-*O*-diethylphosphonomethyl-3,6-dioxaoctanol (**24**); ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.35 (t, 6H, J = 7.2 Hz), 2.88 (t, 1H, J = 6.0 Hz), 3.61 (m, 2H), 3.67 (m, 6H), 3.75 (m, 4H), 3.90 (d, 2H, J = 8.5 Hz), 4.18 (m, 4H).



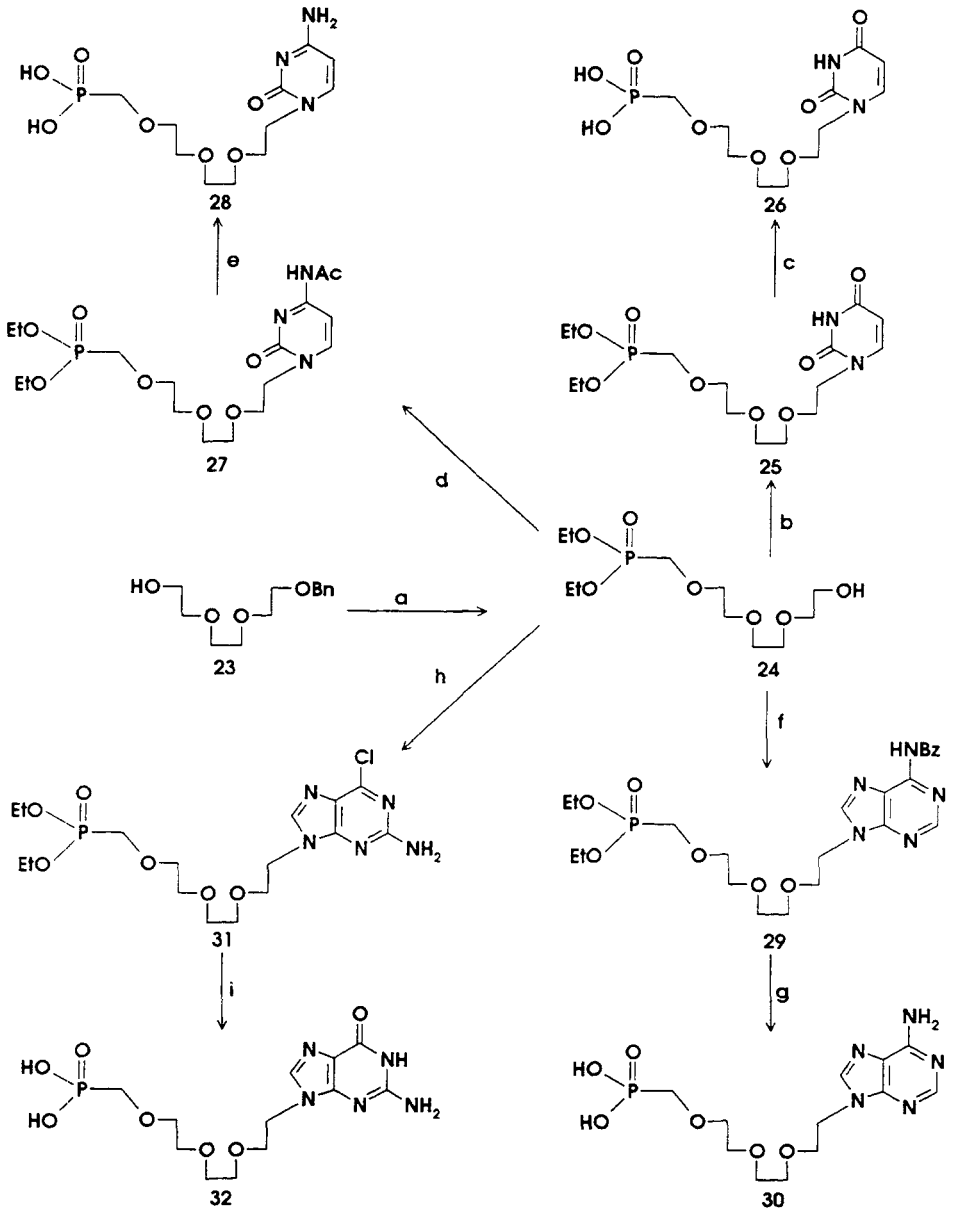
SCHEME 2 Synthesis of **22**. (a) 4-MeOPhCH$_2$Cl, DBU, CH$_3$CN, 70°C, 5 hours, 85%; (b) NH$_4$F, MeOH, 60°C, 1 hour, 86%; (c) 1. NaH, THF, 0°C, 2. **18**, 2 hours, 85%; (d) TMSBr, 2,6-lutidine, CH$_3$CN, RT, 16 hours, 80%.

Diethyl azodicarboxylate (315 μL, 2.0 mmol) was added to a suspension of **24** (501 mg, 1.67 mmol), uracil (224 mg, 2.0 mmol) and triphenylphosphine (525 mg, 2.0 mmol) in 4 mL of DMF. The resulting reaction mixture was stirred for 18 hours and then quenched with 2 drops of acetic acid, diluted with MeOH, filtered through a sand plug and concentrated. The residual DMF was then distilled under vacuum and the residue dissolved in MeOH, pre-adsorbed on silica gel and purified by flash column chromatography (10% MeOH-EtOAc) to afford 244 mg (37%) of 8-*O*-diethylphosphonomethyl-3,6-dioxaoctyl-1-(N-1′)-uracil (**25**); ^{1}H NMR (300 MHz) (DMSO-d$_6$): δ 1.22 (t, 6H, *J* = 7.2 Hz), 3.50 (m, 14H), 4.09 (m, 4H), 5.52 (d, 1H, *J* = 7.9 Hz), 7.55 (d, 1H, *J* = 7.9 Hz), 11.3 (br s, 1H).

Trimethylsilyl bromide (145 μL, 1.10 mmol) was added to a solution of **25** (62 mg, 0.157 mmol) and 2,6-lutidine (37 μL, 0.314 mmol) in 2 mL of CH$_3$CN. The reaction mixture was stirred under argon for 16 hours at room temperature. The resultant mixture was concentrated, redissolved in 1 mL of MeOH-H$_2$O, and stirred for 1 hour. Evaporation of the solvent gave 116 mg of a crude mixture, which afforded 18.3 mg (35%) of **26** after HPLC purification; mp: 169–170°C; UV (λ_{max}): 207.1, 264.4 nm (H$_2$O); ^{1}H NMR (300 MHz) (D$_2$O): δ 3.70 (m, 10H), 3.81 (t, 2H, *J* = 5.0 Hz), 4.01 (t, 2H, *J* = 5.1 Hz), 5.84 (d, 1H, *J* = 7.8 Hz), 7.67 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR (75.5 MHz) (D$_2$O): δ 55.2, 73.5, 75.0, 75.6, 76.8, 78.6, 78.8, 100.1, 108.2, 155.0.

8-O-Phosphonomethyl-3,6-dioxaoctyl-1-(N–1′)-cytosine (**28**)

Mp: 102.5–103.4°C; UV (λ_{max}): 200.9, 275.1 nm (H$_2$O); ^{1}H NMR (300 MHz) (D$_2$O): δ 3.68 (m, 10H), 3.80 (t, 2H, *J* = 4.7 Hz), 4.03 (t, 2H, *J* = 4.8 Hz), 6.07 (d, 1H, *J* = 7.4 Hz), 7.70 (d, 1H, *J* = 7.4 Hz); ^{13}C NMR (75.5 MHz) (D$_2$O): δ 56.4, 73.1, 74.8, 75.1, 76.6, 78.6, 78.7, 101.89, 155.7.



SCHEME 3 Synthesis of 3,6-dioxaoctyl PME nucleotides **26**, **28**, **30**, and **32**. (a) 1. NaH, THF, 0°C, 2. **18**, 2 hours, 82%, 3. PdO · H_2O, cyclohexene, EtOH, reflux, 1.5 hours, 89%; (b) uracil, DEAD, Ph_3P, DMF, RT, 18 hours, 34%; (c) 1. TMSBr, 2,6-lutidine, CH_3CN, RT, 16 hours, 2. HPLC purification, 35% overall; (d) *N*-acetylcytosine, DEAD, Ph_3P, DMF, RT, 16 hours, 20%; (e) 1. NH_3 (g), MeOH, 19 hours RT, 91%, 2. TMSBr, 2,6-lutidine, CH_3CN, RT, 16 hours, 3. HPLC purification, 26% overall; (f) 6-*N*-Benzoyladenine, DEAD, Ph_3P, THF, RT, 16 hours, 36%; (g) NH_3 (g), MeOH, 16 hours, RT, 2. TMSBr, 2,6-lutidine, CH_2Cl_2, RT, 16 hours, 3. HPLC purification, 27% overall; (h) 6-Chloro-2-aminopurine, DEAD, Ph_3P, THF, RT, 35%; (i) 1. TMSBr, 2,6-lutidine, CH_3CN, RT, 2. H_2O, reflux, 3. HPLC purification, 41% overall.

8-O-Phosphonomethyl-3,6-dioxaoctyl-1-(N–9′)-adenine (**30**)

Mp: 95.4–98.2°C; UV (λ_{max}): 214.9, 259.3 nm (MeOH); ^1H NMR (300 MHz) (D_2O): δ 3.30–3.80 (m, 10H), 3.94 (t, 2H, J = 4.7 Hz), 4.44 (t, 2H, J = 4.9 Hz), 8.20 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (75.5 MHz) (D_2O): δ 50.6, 73.2, 75.3, 76.5, 76.5, 76.6, 78.4, 78.6, 150.0, 159.3.

8-O-Phosphonomethyl-3,6-dioxaoctyl-1-(N–9′)-guanine (**32**)

Mp: 121.9–123.4°C; UV (λ_{max}): 208.0, 253.0 nm (MeOH); ^1H NMR (300 MHz) (D_2O): δ 3.51–3.58 (m, 2H), 3.58–3.71 (m, 8H), 3.90 (t, 2H, J = 5.1 Hz), 4.28 (t, 2H, J = 5.0 Hz), 7.89 (s, 1H); ^{13}C NMR (75.5 MHz) (D_2O): δ 50.3, 63.1, 73.0, 75.1, 75.5, 76.6, 76.7, 78.5, 78.7, 147.5.

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