Synthetic strategies for non-hydrolyzable pyrophosphate analogues

Guo, J.

Chapter 4

Synthesis of Unsymmetrical Difluoromethylene Bisphosphonates

Manuscript published as

Jianyun Guo, Pascal Balić, Vladimir S. Borodkin, Dmitri V. Filippov, and Jeroen D. C. Codée, *Org. Lett.* **2024**, *26*, 739–744.

Introduction

Naturally occurring pyrophosphates play important roles as building blocks in numerous vital biological processes.[1-3] For example, nicotinamide adenine dinucleotide (NAD^+, **1**) and flavin adenine dinucleotide (FAD, **2**) act as cofactors in a plethora of enzyme-catalyzed oxidations in living systems (Figure 4.1A).[4] NAD^+ is also consumed to install a post-translational modification (PTM), referred to as adenosine diphosphate ribosylation, in which protein nucleophilic side chains are decorated with an adenosine diphosphate ribose (ADPr) moiety (as in **3**). These PTMs play diverse roles in cellular metabolism, signal transduction, and DNA repair.[5-6] CDP-glycerol **4** and CDP-ribitol **5** are crucial building blocks for the biosynthesis of bacterial capsular polysaccharides and teichoic acids.[7] CDP-ribitol **5** also serves as the donor substrate to generate the linkage unit that connects the matriglycan to dystroglycan.[8] Nucleotide diphosphate (NDP) sugar donors, such as uridine diphosphate (UDP) galactose **6**, are Nature's building blocks to construct oligo- and polysaccharides and glycoconjugates.[9] The chemical modification of natural pyrophosphates is important to generate tools for structural studies,[10-11] and in drug design and development to generate inhibitors,[12-14] for example, through the generation of analogues that are (more) stable toward enzymatic and chemical hydrolysis. Various entities have been probed over the years to serve as close analogues of the pyrophosphate moiety,[15-16] including bisphosphonates (BPs),[17] imidodiphosphate (PNPs),[18-20] pyrophosphorothiolates,[21] selenophosphates,[22] boranophosphates,[23] and phosphonoacetates[24] (Figure 4.1B). Among these, the difluoromethylene bisphosphonate (P-CF_2-P) analogues stand out,[25-29] because they closely resemble the natural pyrophosphates, in terms of pK_a-value, as well as bond angles and lengths.[30-34] Furthermore, P-CF_2-P linked analogues have a very similar polarity and size as their natural congeners.[35] Several methods have been reported for synthesizing terminal difluoro phosphonates as pyrophosphate or triphosphate analogs, such as dicyclohexylcarbodiimide or other dehydrating agent-mediated condensations, or the electrophilic phosphorylation of nucleosides by cyclotriphosphate and nucleophilic substitution of leaving groups using difluoromethylenebisphosphonate tetrabutylammonium salts.[15, 28, 36-38] The latter approach has also been applied in a limited number of examples generating unsymmetrical difluoromethylene bisphosphonates.[13, 20, 39-40] These reactions proceed with relatively poor yield, require the installation of leaving groups on the substrates,which may lead to side-reactions, and generate charged intermediates which are challenging to purify.

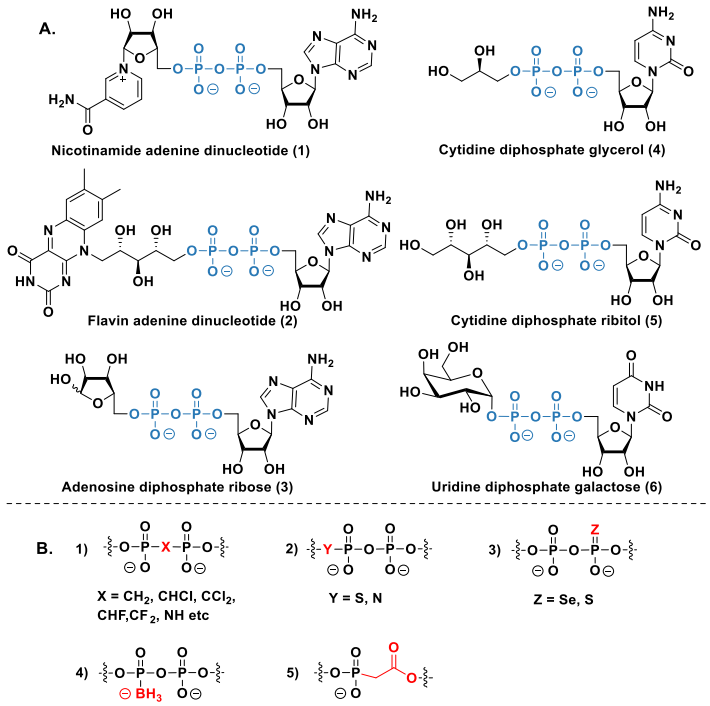
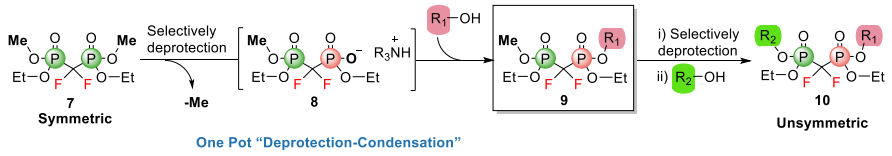


Figure 4.1. (A). Examples of natural unsymmetrical pyrophos-phate molecules. (B). Examples of previously reported stabilized pyrophosphate mimics.

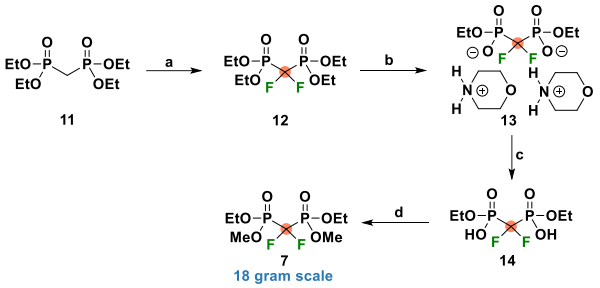
In this chapter, a synthetic strategy to generate difluoromethylene bisphosphonate analogues of various natural products using a desymmetrization strategy of diethyl(dimethyl) difluoromethylene bisphosphonate **7** was described. It was hypothesized that reagent **7** (Scheme 4.1) could be orthogonally deprotected, to set the stage for one or two consecutive condensation reactions through P^V condensation chemistry. Cleavage of a single methyl ester in **7** was expected to be selectively achieved because the bisfluoromethylene bisphosphonate monoanion is a better leaving group than the bisfluoromethylene bisphosphonate dianion, which would be generated after S_N2 displacement of the second methyl ester. After condensation with an alcohol of choice, intermediate **9** is obtained. Hereafter, the remaining methyl ester may be cleaved selectively, leaving the larger ethyl esters unscathed and setting the stage for the second condensation on the opposite side of the reagent, providing unsymmetric difluoromethylene bisphosphonates **10**.



Scheme 4.1. Strategy for the synthesis of unsymmetric difluoromethylene bisphosphonates.

Results and discussion

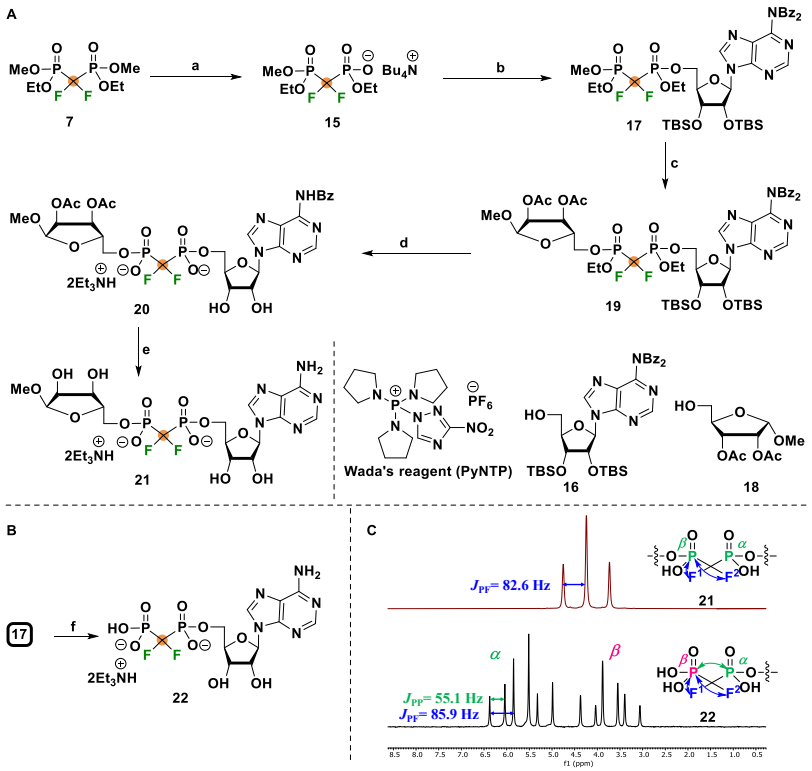
The required symmetrical diethyl(dimethyl) difluoromethylenebisphosphonate **7** was generated from commercially available tetraethyl methylenebisphosphonate **11**, which was fluorinated using *N*-fluorobenzenesulfonimide to obtain tetraethyl difluoromethylene bisphosphonate **12** in a 71% yield (Scheme 4.2).[41] Two ethyl groups of tetraester **12** were selectively removed using refluxing morpholine to furnish the bismorpholinium salt **13** in 84% yield,[42] which was converted to the corresponding acid **14** using Dowex ion exchange resin. Esterification of acid **14** using trimethyl orthoformate provided the desired symmetrical difluoromethylene bisphosphonate **7** in 87% yield.[43] The reagent could be readily generated on 18 gram scale.



Scheme 4.2. Synthesis of symmetrical diethyl(dimethyl) difluoromethylene bisphosphonate **7**. Reagent and conditions: a) NaHMDS, NFSI, 71%. b) morpholine, reflux, 84%. c) Dowex H^+ resin, MeOH, 90%. d) $CH(OCH_3)_3$, 95 °C, 87%.

With reagent **7** in hand, first the synthesis of the P-CF_2-P linked ADPr analogue **21** was undertaken (Scheme 4.3A). ADP ribosylation is a PTM that plays a vital role in regulating various biological processes,[44] and stable ADPr analogs are valuable tools to study the enzymes involved in ADP ribosylation as well as ADPr hydrolysis.[45] To investigate the strategy proposed in Scheme 4.1, optimization of the selective mono-*O*-dealkylation conditions was required. While treatment of **7** with thiophenol (1 equiv.) and triethylamine (1.5 equiv.), provided a mixture of the desired monomethyl product and fully demethylated diethyl ester, the use of tetrabutylammonium benzoate (1 equiv.) cleanly provided the desired mono tetrabutylammonium salt **15** (Scheme 4.3A). The use of MeCN as a solvent provided more selectivity than DMF (see experimental section, Figure 4.2). 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP, Wada's reagent)[46] has been reported as an effective reagent for the condensation of methylene bisphosphonates and alcohols,[17] and was therefore examined as a condensation agent for CF_2-substrates. Using this reagent in the condensation of adenosine **16** with the tetrabutylammonium salt **15** of the difluoromethylene bisphosphonate reagent provided adenosine bisphosphonate **17** in 65% yield over 2 steps. It is worth noting that the tetrabutylammonium salt can be used directly for the condensation reaction in a one-pot fashion without the need for conversion to the corresponding acid and/or isolation and purification. Next, the remaining methyl ester in compound **17** was removed to allow for coupling with protected ribose **18**,[47] resulting in the formation of protected ADPr analogue **19**. Purification by silica gel

column chromatography of this product proved difficult, as the compound is highly polar, with a polarity similar to the tetrabutylammonium hexafluorophosphate. Gratifyingly, size-exclusion chromatography (LH-20, MeOH/ CH_2Cl_2, 1:1 v/v) provided pure **19** in 77% yield. Deprotection of **19** was achieved by removal of the ethyl esters using thiophenol in MeCN/TEA,[17] desilylation with hydrogen fluoride in pyridine, and finally, cleavage of the acetyl esters and adenine benzoyl group using aqueous ammonia providing ADPr analogue **21**. However, partial cleavage of the ribose- and adenosine phosphonate linkages was observed, and the resulting side products could not be separated from the target compound **21**.[48] Therefore, the partially deprotected ADPr was purified after the desilylation step using preparative high-performance liquid chromatography (HPLC) to afford compound **20** in 48% yield. Subsequent deacylation then provided ADPr isostere **21** in 85% yield after isolation by size-exclusion chromatography.

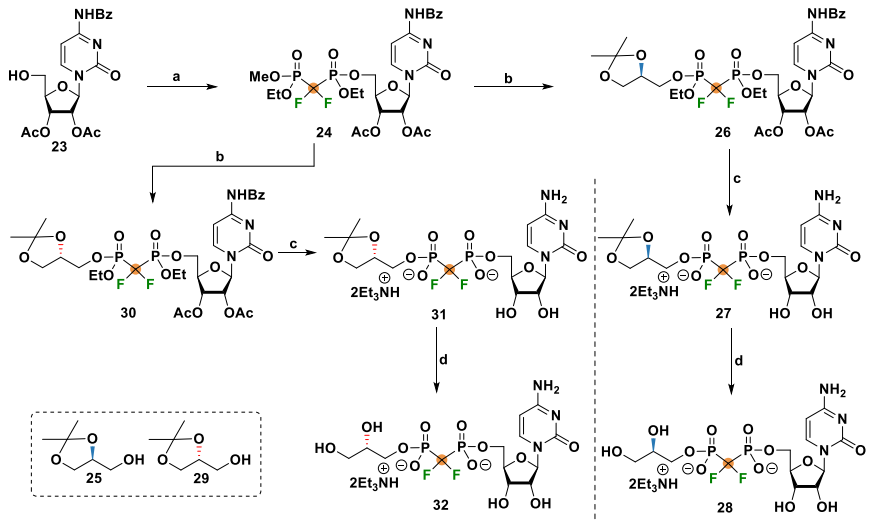


Scheme 4.3. (A) Synthesis of P-CF_2-P linked ADPr analogue. (B) Synthesis of P-CF_2-P linked ADP analogue. (C) Part of the ^{31}P NMR spectrum (in D_2O) of ADPr analogue **21** and ADP analogue **22**. Reagent and conditions: a) Bu_4NOBz, MeCN, rt. b) **16**, PyNTP, DIPEA, MeCN, rt, 65% over 2 steps. c) (i) Bu_4NOBz, MeCN, rt; (ii) **18**, PyNTP, DIPEA, MeCN, rt, 77% over 2 steps. d) (i) MeCN/TEA/PhSH (3:3:2), 35 °C; (ii) HF·pyridine, pyridine, 48% over 2 steps. e) 30% NH_4OH, 85%. f) (i) TMSBr, CH_2Cl_2, rt; (ii) HF·pyridine, pyridine, 0 °C- rt; (iii) 30% NH_4OH, 77% over 3 steps.

Compound **17** could also be used as a precursor to generate the P-CF_2-P analogue of adenosine diphosphate (ADP). To this end, the deprotection of the methyl and ethyl phosphonate esters was explored using bromotrimethylsilane (TMSBr) (Scheme 4.3B),[49-50] a commonly employed reagent for the deprotection of alkyl phosphate esters. Using 20 equivalents of TMSBr, incomplete conversion was observed, even after extended reaction time (7 days). However, by treating **17** with 20 equivalents of TMSBr, 30 equivalents of dry pyridine and prolonging the reaction time to two weeks, full conversion was achieved. Finally, desilylation and deacylation afforded ADP analogue **22** in 77% yield.

The ^{31}P NMR of CF_2-ADP **22** and CF_2-ADPr **21** exhibited notable differences (Scheme 4.3C). While the ^{31}P NMR spectrum of **22** reveals a coupling between the two phosphorus atoms ($J_{P\text{-}P}$ = 55.1 Hz) and a coupling of the two fluorine atoms with both the α-phosphorus (α-P) and β-phosphorus (β-P) atoms ($J_{P\text{-}F}$ = 85.9 Hz), leading to two triplet of doublets, the ^{31}P NMR of ADPr analogue **21** shows an apparent triplet. Apparently, the two phosphonates in **21** are magnetically very similar, because of their similar substituents, which leads to the disappearance of the P-P coupling, generating a relatively simple apparent triplet in the spectrum.

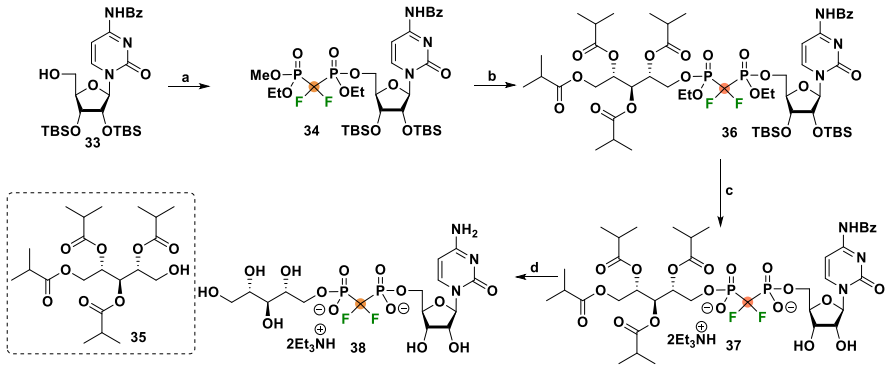
Next, the methodology was evaluated in the synthesis of CDP-glycerol (CDP-Gro) and CDP-ribitol (CDP-Rib) analogues (Scheme 4.4), which may be used to inhibit the enzymes that synthesize wall teichoic acids or aid in structural studies of these enzymes.[7] CDP-glycerol is also used for the assembly of poly(glycosylglycerol phosphate) capsule polymers in Gram-negative pathogens, which represent crucial virulence factors of various pathogenic Gram-negative



Scheme 4.4. Synthesis of P-CF_2-P linked CDP-glycerol analogues. Reagent and conditions: a) **15**, PyNTP, DIPEA, MeCN, rt, 55% over 2 steps. b) (i) Bu_4NOBz, MeCN, rt; (ii) PyNTP, DIPEA, MeCN, rt, **25**→**26,** 46% over 2 steps; **29**→**30**, 57% over 2 steps. c) (i) MeCN/Et_3N/PhSH (3:3:2), 35 °C; (ii) 30% NH_4OH, **27**, 58% over 2 steps; **31**, 65% over 2 steps. d) AcOH, H_2O, 35 °C, **28**, 68%; **32**, 48%.

bacteria.[51-52] Cytidine **23** was prepared via a three-step procedure (see experimental section), and devised such that only a single deprotection step is required to unmask the nucleoside. It was subjected to a condensation reaction with tetrabutyl ammonium phosponate **15** to give compound **24** in 55% yield over two steps. After demethylation of **24**, the resulting salt was condensed with commercially available (*S*)-solketal **25** under the agency of PyNTP to give the protected CDP-glycerol **26** in a 46% yield. The acyl and ethyl protecting groups in compound **26** were efficiently removed in a two-step process: first, removal of the ethyl groups was achieved with thiophenol in a mixture of MeCN/Et_3N, followed by deacylation using aqueous ammonia. Purification of the resulting crude product via preparative HPLC afforded intermediate **27** in 58% yield over 2 steps, of which the isopropylidene acetal was removed to provide the P-CF_2-P analogue of CDP-Gro **28**. In an analogous fashion the diastereomeric counterpart was generated starting from (*R*)-solketal **29**. It has been reported that some biosynthesis enzymes can actually accept both diasteroisomeric CDP-Gro substrates, incorporating either the *sn*-3 or *sn*-1 glycerolphosphates in poly(glycosylglycerol phosphate) polymers *in vitro*.[52] Both CF_2 CDP-Gro enantiomers may therefore be relevant as potential inhibitors of these enzymes. The assembly of the *sn*-1 CF_2 CDP-Gro **32** proceeded with similar yield as its *sn*-3 counterpart **28**.

Finally, assembly of CF_2 CDP-Rib **38** as was shown in Scheme 4.5. In line with the approach for the CDP-Gro analogue, cytidine building block **24** with ribitol **35** was combined to enable a two-step deprotection (*i.e.* removal of the ethyl esters followed by cleavage of all remaining acyl groups). However, this led to difficulties in purification, and the use of a cytidine building block carrying TBS-ethers was therefore adopted. As noted in the synthesis of ADPr analogue **21**, the lipophilicity of these groups can be beneficial during HPLC purification. Hence, to assemble CDP ribitol analogue **38**, building block **33** was combined with **15** under the action of PyNTP to give **34** in 63% yield. Demethylation and coupling with **35** then gave the fully protected CDP-ribitol. After removal of the ethyl groups, the TBS-groups were removed with HF·pyridine, followed by HPLC purification to deliver pure **37** in 47% yield. Ammonia treatment of **37** then delivered CDP ribitol analogue **38**.



Scheme 4.5. Synthesis of P-CF_2-P linked CDP-ribitol analogue. Reagents and conditions: a) **15**, PyNTP, DIPEA, MeCN, rt, 63% over 2 steps. b) (i) Bu_4NOBz, MeCN, rt; (ii) **35**, PyNTP, DIPEA, MeCN, rt, 40% over 2 steps. c) (i) MeCN/Et_3N/PhSH (3:3:2), 35 °C; (ii) HF·pyridine, pyridine, rt, 47% over 2 steps. d). 30% NH_4OH, 33%.

Conclusion

In conclusion, an efficient orthogonal deprotection-condensation strategy using diethyl(dimethyl) difluoromethylene bisphosphonate has been described for the synthesis of unsymmetric difluoromethylene bisphosphonates, which can serve as close analogues to naturally occurring pyrophosphate-containing molecules. Symmetrical difluoromethylene bisphosphonate **7** allows for a step-wise functionalization sequence in which one methyl can be selectively removed to attach the first alcohol coupling partner, after which the second methyl can be removed to connect a second alcohol. Wada's reagent was found to perform well in the construction of the difluorophosphonate ester linkages. The first synthesis of ADPr, CDP-glycerol and CDP-ribitol difluoromethylene bisphosphonate analogues demonstrates the applicability of the devised methodology, making these molecules available for structural and biochemical studies.

Experimental section

General information

All chemicals were commercial grade and used as received and all moisture sensitive reactions were performed under an argon or nitrogen atmosphere, at ambient temperature (21°C), unless stated otherwise. For TLC analysis were used aluminium sheets (Merck, TLC silica gel 60 F_{254}), sprayed with a solution of H_2SO_4 (20%) in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}$•$4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4$•$2H_2O$ (10g/L) in 10% aqueous H_2SO_4 or with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in H_2O and then heated at ~150°C. For column chromatography was used 60Å silica gel (40-63 μm, SD Screening Devices). NMR spectra (^1H, ^{13}C, ^{19}F and ^{31}P) were recorded with a Bruker AV-300, Bruker AV-400liq, Bruker AV-500 or Bruker AV-850. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard (^1H NMR in $CDCl_3$) or the residual signal of the deuterated solvent. Chemical shifts for ^{31}P spectra are indirectly referenced to H_3PO_4 (0.00 ppm) according to the IUPAC method. ^{31}P NMR spectra measured to monitor reactions were made by charging an NMR tube with an aliquot of the reaction mixture and fitting the tube with an acetone-d_6 capillary. Coupling constants (*J*) are given in Hz. All ^{13}C NMR and ^{31}P NMR spectra are proton decoupled. NMR peak assignments were made using COSY and HSQC experiments. LC/MS analysis was performed on a Surveyor HPLC system (Thermo Finnigan) equipped with a C18 column (Gemini, 4.6 mm x 50 mm, 5 μm particle size, Phenomenex), coupled to a LCQ Adventage Max (Thermo Finnigan) ion-trap spectrometer (ESI^+). Solvent system: A: Milli-Q H_2O, B: MeCN, C: 1% TFA (aq). High resolution mass spectra were recorded by direct injection on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive ion mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275°C) with resolution R = 60000 at m/z 400 (mass range m/z = 150 - 4000) and dioctyl phthalate (m/z = 391.28428) as a lock mass. Size-exclusion chromatography was performed on Sephadex™ LH-20 gel using CH_2Cl_2/MeOH (1:1, v/v) as the eluent, or on a TOYOPEARL HW-40-S resin column (16 × 600 mm) using an aqueous NH_4OAc buffer (0.15 M) containing 10% MeCN at a flow rate of 1 mL/min. Semi-preparative high-performance liquid chromatography (semiprep-HPLC) was performed on a Gilson system (322-H2 Pump, GX-281 Liquid Handler, UV/VIS-156 detector) equipped with a semi-preparative C18 column (NUCLEODUR C18 Gravity, 5 μm, 250 × 10 mm, Macherey Nagel) using Solvent A: 25 mM NH_4OAc in Milli-Q® H_2O; solvent B: acetonitrile; linear gradient of B in A (50 → 100% A/B), 5 mL/min.

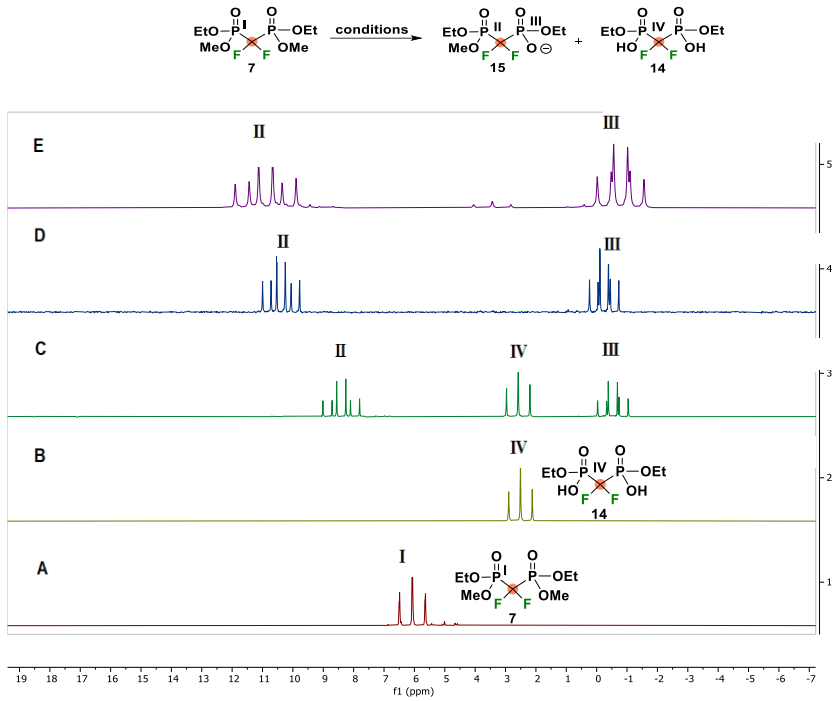
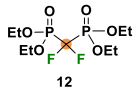


Figure 4.2. Optimization of the demethylation of compound **7**, monitored by ^{31}P NMR spectrometry. A: ^{31}P NMR spectrum (202 MHz, in $CDCl_3$) of compound **7**. B: ^{31}P NMR spectrum (202 MHz, in $CDCl_3$) of compound **14**. C: Overnight treatment of the reaction mixture with PhSH (1.0 eq) and TEA (1.5 eq) resulted in non-selective deprotection (monitored by ^{31}P NMR spectrometry (202 MHz, in $CDCl_3$)). D and E: Overnight treatment of the reaction mixture with Bu_4NOBz (1.0 eq) in either MeCN (D, monitored by ^{31}P NMR spectrometry (202 MHz, in $CDCl_3$)) or DMF (E, monitored by ^{31}P NMR spectrometry (122 MHz, in acetone-d_6 capillary)) at ambient temperature resulted in selective deprotection.

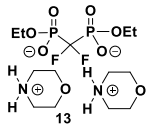
Tetraethyl difluoromethylenediphosphonate 12


12

Tetraethyl methylenediphosphonate (5 g, 17.3 mmol) was added in a flask under argon with a reflux condenser attached. NaHMDS (5.71 mL, 5.71 mmol of a 57.1 mL, 1.0 M solution (57.1 mmol) in THF) was added and the mixture was stirred for approximately 2 minutes. An anhydrous THF solution of *N*-fluorobenzenesulfonimide (NFSI) (5.19 mL, 5.19 mmol of a 51.9 mL, 1.0 M solution (51.9 mmol) in THF) was added and stirring was continued for approximately 2 minutes. Additional aliquots of NaHMDS (57.1 mL, 57.1 mmol) and NFSI (9.5 g in 51.9 mL THF, 51.9 mmol) were all added as described above. The reaction mixture was allowed to stir for 1h, then cooled to room temperature and the suspension was filtered. The residue was washed with hexane (20 mL) and the combined filtrate was concentrated *in vacuo* to give an oil. The oil was co-evaporated with hexane (30 mL) *in vacuo* to give an amber oil. The compound was purified with flash column chromatography, eluting with 10% acetone in dichloromethane (R*f* = 0.25), to give **12** as a colorless oil (4.0 g, 12.3 mmol,71%).

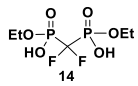
Spectroscopic data were in accord with those reported previously.[53] ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.40 – 4.22 (m, 8H, CH_2 Et, CH_2 Et, CH_2 Et, CH_2 Et), 1.37 (d, J = 8.0 Hz, 12H, CH_3 Et, CH_3 Et, CH_3 Et, CH_3 Et). ^{13}C NMR (101 MHz, D_2O): δ 120.7, 118.9, 118.0, 117.0, 116.1, 115.2, 114.2, 113.3 (m, P-CH_2-P), 65.5 (t, $J_{P\text{-}C}$ = 3.4 Hz, CH_2 Et), 16.4 (CH_3 Et). ^{31}P NMR (162 MHz, $CDCl_3$): δ 1.64 (t, $J_{P\text{-}F}$ = 85.9 Hz). ^{19}F NMR (471 MHz, $CDCl_3$): δ -121.85 (t, $J_{P\text{-}F}$ = 84.8 Hz). HRMS (ESI) M/Z: Calcd for $C_9H_{21}F_2O_6P_2$ 325.0775 $[M+Na]^+$; found 325.0778.

Morpholinium difluoromethylenebis(ethyl phosphonate) 13


13

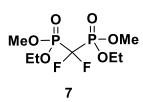
A solution of difluoromethylenebis(diethyl phosphonate) (35 g, 110 mmol) in morpholine (160 mL) was heated at reflux for 6 h. The reaction mixture was cooled to rt and concentrated in vacuo to remove unreacted morpholine. The residue was washed with anhydrous ether (200 mL), and the resulting solid was dried under vacuum to give morpholinium difluoromethylenebis (ethyl phosphonate) **13** (40.3g, 92 mmol, 84%) as a brown powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.11 – 4.06 (m, 4H, CH_2 Et, CH_2 Et), 3.94 – 3.87 (m, 8H, OCH_2CH_2), 3.17 – 3.09 (m, 8H, NCH_2CH_2), 1.28 (t, J = 7.1 Hz, 6H, CH_3 Et). ^{13}C NMR (101 MHz, $CDCl_3$): δ 65.0 (OCH_2CH_2), 62.9 (CH_2 Et), 40.6 (NCH_2CH_2), 16.4 (CH_3 Et). ^{31}P NMR (162 MHz, $CDCl_3$): δ 2.76 (t, $J_{P\text{-}F}$ = 76.1 Hz). ^{19}F NMR (376 MHz, $CDCl_3$): δ -121.71 (t, $J_{P\text{-}F}$ = 75.2 Hz). HRMS (ESI) M/Z: Calcd for $C_5H_{13}F_2O_6P_2$ 269.0149 $[M+H]^+$; found 269.0155.

P,P'-diethyl difluoromethylene bisphosphonic acid 14


14

Morpholinium difluoromethylenebis(ethyl phosphonate) **13** (35 g, 79 mmol) was dissolved in methanol (200 mL), Dowex 50W-X8 resin (30 g) was added and the resulting mixture stirred at rt for 4 h. The solution was filtered and the filtrate was concentrated under reduced pressure, giving the symmetrical P,P'-diethyl difluoromethylene bisphosphonic acid **14** (19.2 g, 71.1 mmol, 90%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.68 (bs, 2H, P(O)OH), 4.39 – 4.36 (m, 4H, CH_2 Et, CH_2 Et), 1.43 (t, J = 7.0 Hz, 6H, CH_3 Et, CH_3 Et). ^{13}C NMR (101 MHz, $CDCl_3$): δ 66.3 (CH_2 Et), 16.5 (CH_3 Et). ^{31}P NMR (162 MHz, $CDCl_3$): δ 1.90 (t, $J_{P\text{-}F}$ = 84.2 Hz). ^{19}F NMR (376 MHz, $CDCl_3$): δ -126.41 (t, $J_{P\text{-}F}$ = 86.4 Hz). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_5H_{13}F_2O_6P_2$ 269.0149; found 269.0157.

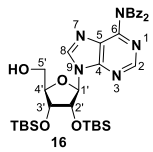
Diethyl(dimethyl) difluoromethylene bisphosphonate 7


7

A solution of P,P'-diethyl difluoromethylenebisphosphonic acid **14** (19 g, 71 mmol) in trimethyl orthoformate (200 mL) was heated to reflux in an oil-bath for 16 h. The reaction mixture was cooled to room temperature and concentrated under vacuum. Purification by silica gel column chromatography (R_f = 0.3, 100% EtOAc) yielded compound **7** as a colorless oil (18.2 g, 61.8 mmol, 87%). *A mixture of diastereoisomers:* ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.43 – 4.28 (m, 4H, CH_2 Et, CH_2 Et), 4.02 – 3.90 (m, 6H, P(O)OMe, P(O)OMe), 1.41 (t, J = 7.1 Hz, 6H, CH_3 Et, CH_3 Et). ^{13}C NMR (75 MHz, $CDCl_3$): δ 65.7 (d, J = 3.3 Hz, CH_2 Et), 55.3 (P(O)OMe), 16.4 (CH_3 Et). ^{31}P NMR (121 MHz, $CDCl_3$): δ 4.63, 4.62 (ABX, $J_{P\text{-}F}$ = 85.9 Hz). ^{19}F NMR (376 MHz, $CDCl_3$): δ -121.31, 121.33

(ABX, $J_{P\text{-}F}$ = 86.4 Hz). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_7H_{17}F_2O_6P_2$ 297.0462; found 297.0468.

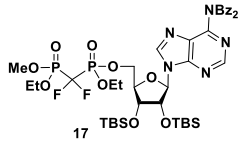
***N,N*-dibenzoyl-2,3-*bis*-*O*-*tert*-butyldimethylsilyladenosine 16**



16

Adenosine (15 g, 56 mmol) was co-evaporated with anhydrous pyridine three times, dissolved in anhydrous dimethylformamide (84 mL), and heated to 50 °C in an oil-bath. Imidazole (19 g, 280 mmol) and tert-butyldimethylsilyl chloride (42.2 g, 280 mmol) were added consecutively and the mixture was stirred overnight. The reaction mixture was quenched by the addition of H_2O (41 mL), diluted with Et_2O (200 mL), and washed with H_2O (100 mL). The aqueous layer was extracted twice with Et_2O (2 x 100 mL), and the resulting organic layers were combined, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was co-evaporated with toluene three times, placed under a nitrogen atmosphere and dissolved in pyridine (110 mL), whereafter benzoyl chloride (13 mL, 112 mmol) was added and the mixture stirred at room temperature for 5 hours. After analysis by TLC showed complete consumption of starting material, the reaction was quenched with sat. aq. $NaHCO_3$ (150 mL) at 0 °C, diluted with EtOAc (200 mL) and washed with water (150 mL) and brine (150 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was taken up in THF (600 mL), and aqueous TCA (318 g in 150 mL H_2O) was added at 0 °C. After stirring for 3 h at 0 °C, the reaction was quenched with sat. aq. $NaHCO_3$ and diluted with dichloromethane (500 mL). The water layer was back-extracted twice with dichloromethane (200 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (R_f = 0.3, pentane/EtOAc 3:1, v/v) to give **16** as a white solid (28.1 g, 43.1 mmol, 77%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.65 (s, 1H, H-2), 8.11 (s, 1H, H-8), 7.88 – 7.84 (m, 4H, CH_{arom}), 7.52 – 7.46 (m, 2H, CH_{arom}), 7.38 – 7.32 (m, 4H, CH_{arom}), 5.85 (d, *J* = 12.0 Hz, 1H, H-1'), 5.79 (d, *J* = 12.0 Hz, 1H, 5'-OH), 4.97 (dd, *J* = 6.0, 3.0 Hz, 1H, H-2'), 4.32 (d, *J* = 3.0 Hz, 1H, H-3'), 4.19 (t, *J* = 3.0 Hz, 1H, H-4'), 3.95 (dd, *J* = 9.0, 3.0 Hz, 1H, H-5'), 3.71 (t, *J* = 12.0 Hz, 1H, H-5'), 0.95 (s, 9H, $C(CH_3)_3$ TBS), 0.75 (s, 9H, $C(CH_3)_3$ TBS), 0.13 (s, 3H, CH_3 TBS), 0.11 (s, 3H, CH_3 TBS), -0.15 (s, 3H, CH_3 TBS), -0.71 (s, 3H, CH_3 TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.1 (C=O Bz), 153.0 (C-4), 152.0 (C-6), 151.6 (C-2), 145.3 (C-8), 134.0 ($C_{q\text{-}arom}$), 133.2, 129.6 (CH_{arom}), 129.5 (C-5), 128.9 (CH_{arom}), 91.3 (C-1'), 89.7 (C-4'), 74.1 (C-2'), 73.9 (C-3'), 63.0 (C-5'), 25.9, 25.8 ($C(CH_3)_3$ TBS), 18.2, 17.9 ($C(CH_3)_3$ TBS), -4.4, -4.4, -4.5, -5.7 (CH_3 TBS). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{36}H_{50}N_5O_6Si_2$ 704.3294; found 704.3291. $[\alpha]^{20}_D$ = +20 ° (c = 0.001, $CHCl_3$)

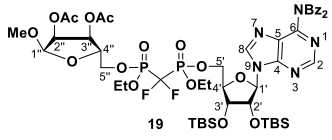
Protected CF_2 ADP analogue 17



17

Compound **7** (820 mg, 2.77 mmol) was co-evaporated with toluene and dissolved in dry MeCN (9.0 mL), whereupon tetrabutylammonium benzoate (1.0 g, 2.77 mmol) was added under nitrogen and the resulting mixture was cstirred overnight. Compound **16** (2.9 g, 4.16 mmol), dried prior to use by evaporation with toluene, DIPEA (4.7 mL, 27.7 mmol), and PyNTP (4.1 g, 8.31 mmol) were added consecutively. After 2 hours, the reaction was complete as indicated by TLC analysis. The reaction mixture was poured into DCM and washed with phosphate buffer (pH = 7). The water layer was extracted with DCM. The combined organics were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was purified by flash column chromatography over silica gel (R_f = 0.2, pentane/EtOAc 2:1, v/v) and then by size exclusion (LH-20) (DCM/MeOH 1:1) to obtain **17** as a yellow solid (178 mg, 1.8 mmol, 65%). *A mixture of diastereoisomers.* ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.65 (d, *J* = 1.4 Hz, 1H, H-2), 8.37 – 8.32 (m, 1H, H-8), 7.86 – 7.83 (m, 4H, CH_{arom}), 7.50 – 7.44 (m, 2H, CH_{arom}), 7.36 – 7.29 (m, 4H, CH_{arom}), 6.09 – 6.03 (m, 1H, H-1'), 4.82 – 4.72 (m, 1H, H-2'), 4.66 – 4.58 (m, 1H, 5'), 4.48 – 4.28 (m, 7H, H-3', H-4', H-5', CH_2 Et, CH_2 Et), 3.97 – 3.91 (m, 3H, P(O)OMe), 1.40 – 1.32 (m, 6H, CH_3 Et, CH_3 Et), 0.93 (s, 9H, $C(CH_3)_3$ TBS), 0.76 (s, 9H, $C(CH_3)_3$ TBS), 0.13 – 0.10 (m, 6H, CH_3 TBS, CH_3 TBS), -0.05 (s, 3H, CH_3 TBS), -0.36 (s, 3H, CH_3 TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.2 (C=O Bz), 153.0, 152.9 (C-4), 152.2, 152.1 (C-2,), 151.9 (C-6), 144.2, 144.0 (C-8), 134.1 ($C_{q\text{-}arom}$), 132.9, 129.5, 128.7 (CH_{arom}), 128.4, 128.2 (C-5), 88.8 (d, *J* = 2.8 Hz, C-1'), 88.5 (d, *J* = 2.8 Hz, C-1'), 83.5 (C-4'), 75.0 (d, *J* = 2.3 Hz, C-2'), 74.7 (d, *J* = 2.1 Hz, C-2'), 72.0, 71.9 (C-3'), 66.9, 66.7 (C-5'), 66.3, 66.1, 65.9 (CH_2 Et), 55.5, 55.4, 55.3 (P(O)OMe), 25.8, 25.6 ($C(CH_3)_3$ TBS), 18.1, 17.8 ($C(CH_3)_3$ TBS), 16.4 (CH_3 Et), -4.4, -4.4, -4.6, -4.8, -5.1, -5.2 (CH_3 TBS). ^{31}P NMR (202 MHz, $CDCl_3$): δ 5.35 - 4.36 (m). ^{19}F NMR (471 MHz, $CDCl_3$): δ -113.02 – -122.85 (m). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{42}H_{62}F_2N_5O_{11}P_2Si_2$ 968.3422; found 968.3430.

Protected CF_2 ADPr analogue 19

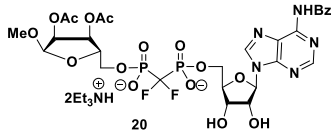


19

Compound **16** (200 mg, 0.21 mmol) was co-evaporated with toluene and dissolved in dry MeCN (0.7 mL), whereafter tetrabutylammonium benzoate (74 mg, 0.21 mmol) was added under nitrogen and stirred overnight. Compound **18** (77 mg, 0.31 mmol), dried by evaporation with toluene prior to use, DIPEA (0.35 mL, 2.06 mmol), and PyNTP (308 mg g, 0.62 mmol) were added successively. After 2 hours, the reaction was complete as indicated by TLC analysis. The reaction mixture was poured into DCM and washed with phosphate buffer (pH = 7). The water layer was extracted with DCM. The combined organics were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was purified by flash column chromatography over silica gel (R_f = 0.3, pentane/EtOAc 1:1, v/v) and then by size exclusion (LH-20) (DCM/MeOH 1:1) to obtain **19** (188 mg, 0.16 mmol, 77%) as a yellow solid. *A mixture of diastereoisomers.* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 – 8.60

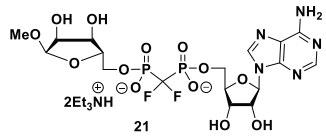
(m, 1H, H-2), 8.42 – 8.26 (m, 1H, H-8), 8.02 (dd, *J* = 7.2, 1.6 Hz, 1H, CH_{arom}), 7.87 – 7.78 (m, 3H, CH_{arom}), 7.33 (m, 5H, CH_{arom}), 6.12 – 6.03 (m, 1H, H-1'), 5.31 – 5.17 (m, 1H, H-3''), 5.05 – 4.89 (m, 1H, H-1''), 4.83 – 4.69 (m, 1H, H-2''), 4.67 – 4.56 (m, 1H, H-2'), 4.55 – 4.43 (m, 3H, H-4', H-5'), 4.43 – 4.18 (m, 7H, H-3', H-5'', CH_2 Et, CH_2 Et), 3.43 – 3.38 (m, 3H, OMe), 2.13 – 2.07 (m, 6H, CH_3 Et, CH_3 Et), 1.43 – 1.30 (m, 6H, OAc), 0.95 – 0.88 (m, 9H, $C(CH_3)_3$ TBS), 0.79 – 0.72 (m, 9H, $C(CH_3)_3$ TBS), 0.14 – 0.07 (m, 6H, CH_3 TBS, CH_3 TBS), -0.01 – -0.09 (m, 3H, CH_3 TBS), -0.23 – -0.42 (m, 3H, CH_3 TBS). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.2, 170.6, 170.5, 169.9, 169.8 (C=O Ac), 164.6 (C=O Bz), 153.0, 152.9 (C-4), 152.8 (C-2), 152.26, 152.2 (C-8), 151.9, 151.7 (C-6), 149.6 ($C_{q\text{-}arom}$), 144.3, 144.0, 142.3 (CH_{arom}), 134.1, 133.9 ($C_{q\text{-}arom}$), 132.9, 132.8, 129.5, 128.9, 128.9, 128.7 (CH_{arom}), 128.3 (C-5), 127.9, 127.9 (CH_{arom}), 101.8, 101.8, 101.7 (C-1''), 88.7, 88.7, 88.6, 88.5 (C-1'), 83.7, 83.6, 83.6, 83.3 (C-4'), 80.36 (C-4''), 75.2, 75.1, 75.0, 74.9 (C-2'), 72.0, 71.8 (C-3'), 71.0, 70.7, 70.9, 70.6 (C-2''), 69.9, 69.7, 69.6, 69.6 (C-3''), 67.8, 67.6 (C-5''), 67.1, 67.1, 67.0, 66.9 (C-5'), 66.5, 66.5, 66.3, 66.3, 66.2, 66.1, 66.1, 66.0 (CH_2 Et), 55.7, 55.7 (OMe), 25.8, 25.7, 25.7 ($C(CH_3)_3$ TBS), 20.9, 20.6 (OAc), 18.1, 17.9, 17.8 ($C(CH_3)_3$ TBS), 16.4 (CH_3 Et) , -4.4, -4.4, -4.6, -4.7, -4.7, -5.0, -5.1 (CH_3 TBS). ^{31}P NMR (162 MHz, $CDCl_3$): δ 5.24 – 2.44 (m). ^{19}F NMR (471 MHz, $CDCl_3$): δ -120.51 – -122.67 (m). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{51}H_{73}F_2N_5O_{17}P_2Si_2$ 1184.4056; found 1184.4019.

Protected CF_2 ADPr analogue 20



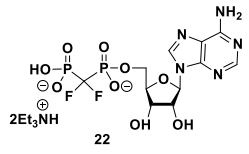
The protected ADPr analogue **19** (125 mg, 0.11 mmol) was dissolved in MeCN/TEA/PhSH (3:3:2, 1.0 mL) and stirred for 6 hours at 35 °C. Reaction progression was monitored by ^{31}P NMR and LC-MS, which indicated complete consumption of the starting material. The solution was concentrated *in vacuo*. The residue was dissolved in pyridine and cooled to 0 °C, after which HF·pyridine (70%, 86 µL 3.3 mmol) was added and stirred overnight at rt. The resulting solution was quenched with sat. aq. $NaHCO_3$ and concentrated. The resulting mixture was diluted with water then acidified to pH 6-7 using acetic acid. The mixture was washed with DCM (3x) to remove excess thiophenol and pyridine. The water layer was concentrated *in vacuo* then purified by semiprep-HPLC to give compound **20** as a white solid (40.5 mg, 40 µmol, 48% over 2 steps). ^{1}H NMR (400 MHz, D_2O): δ 8.79 (s, 1H, H-2), 8.72 (s, 1H, H-8), 7.98 – 7.96 (m, 2H, CH_{arom}), 7.69 – 7.64 (m, 1H, CH_{arom}), 7.57 – 7.53 (m, 2H, CH_{arom}), 6.25 (d, *J* = 5.5 Hz, 1H, H-1'), 5.24 (dd, *J* = 6.7, 2.4 Hz, 1H, C-3''), 5.14 (d, *J* = 4.6 Hz, 1H, H-1''), 5.02 (dd, *J* = 6.7, 4.6 Hz, 1H, H-2''), 4.77 – 4.75 (m, 1H, H-2'), 4.55 – 4.53 (m, 1H, H-3'), 4.38 – 4.36 (m, 1H, H-4'), 4.33 – 4.28 (m, 3H, H-4'', H-5'), 4.16 – 4.14 (m, 2H, H-5''), 3.34 (s, 3H, OMe), 2.02 (s, 3H, OAc), 1.99 (s, 3H, OAc). ^{13}C NMR (101 MHz, D_2O): δ 172.9 (C=O Ac), 172.1 (C=O Ac), 169.0 (C=O Bz), 152.0 (C-4), 151.9 (C-8), 148.9 (C-6), 133.4 (CH_{arom}), 132.7 ($C_{q\text{-}arom}$), 128.8, 128.1 (CH_{arom}), 123.6 (C-5), 101.4 (C-1''), 87.4 (C-1'), 84.1 (C-4'), 81.5 (C-4''), 74.4 (C-2'), 71.0 (C-2''), 70.5 (C-3''), 70.2 (C-3'), 65.8 (C-5''), 65.3 (C-5'), 55.3 (OMe), 20.0, 19.6 (OAc). ^{31}P NMR (162 MHz, D_2O): δ 3.52 (ABX, $J_{P\text{-}F}$ = 82.6 Hz). ^{19}F NMR (471 MHz, D_2O): δ -118.84 (t, $J_{P\text{-}F}$ = 80.1 Hz,). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{28}H_{34}F_2N_5O_{16}P_2$ 796.1438; found 796.1437.

CF_2 ADPr analogue 21



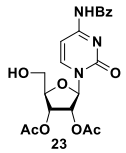
Compound **20** (40.5 mg, 40 µmol) was dissolved in aqueous ammonia (30 wt%, 5 mL) and stirred overnight. The excess ammonia was removed by stirring under vacuum. The crude product was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in water) followed by lyophilization to yield CF_2-ADPr analogue **21** (26.2 mg, 32 µmol, 85%) as a white powder. ^{1}H NMR (400 MHz, D_2O): δ 8.42 (s, 1H, H-2), 8.09 (s, 1H, H-8), 6.04 (d, J = 5.7 Hz, 1H, H-1'), 4.88 – 4.85 (m, 1H, H-1''), 4.69 (t, J = 5.5 Hz, 1H, H-2'), 4.48 (dd, J = 5.1, 3.6 Hz, 1H, H-3'), 4.33 – 4.31 (m, 1H, H-4'), 4.26 – 4.24 (m, 2H, H-5'), 4.12 – 4.09 (m, 3H, H-2'', H-3'', H-4''), 4.06 – 4.03 (m, 2H, H-5''), 3.34 (s, 3H, OMe). ^{13}C NMR (101 MHz, D_2O): δ 155.3 (C-4), 152.6 (C-8), 148.8 (C-6), 139.6 (C-2), 120.9, 119.2 (P-CH_2-P), 118.4 (C-5), 117.5, 116.5 (P-CH_2-P), 103.1 (C-1''), 86.7 (C-1'), 83.9 (C-4'), 83.4 (t, J = 2.7 Hz, C-4''), 74.1 (C-2'), 70.7 (C-2''), 70.2 (C-3'), 69.6 (C-3''), 65.9 (C-5''), 65.4 (C-5'), 55.3 (OMe). ^{31}P NMR (162 MHz, D_2O): δ 4.24 (t, $J_{P\text{-}F}$ = 82.6 Hz,). ^{19}F NMR (471 MHz, D_2O): δ -118.83 (t, $J_{P\text{-}F}$ = 84.8 Hz,). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{17}H_{26}F_2N_5O_{13}P_2$ 608.0964; found 608.0967.

CF_2 ADP analogue 22



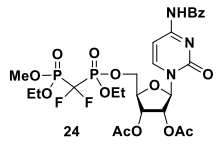
Compound **17** (200 mg, 0.21 mmol) was co-evaporated with toluene and dissolved in dry DCM (10 mL), whereupon pyridine (510 µL, 6.3 mmol) and trimethylsilyl bromide (0.45 mL, 4.2 mmol) were added under nitrogen and the reaction mixture was stirred for 2 weeks. After analysis by LC-MS showed complete consumption of starting material, pyridine (338 µL, 4.2 mmol) was added. The reaction was concentrated *in vacuo* to give a yellow-colored oil. The oil was dissolved in pyridine (10 mL), cooled to °C and HF·pyridine (70%, 109 µL, 4.2 mmol) was added. The reaction mixture was warmed to rt and stirred for 24 h until LC-MS showed complete consumption of starting material. The reaction was diluted with water (10 mL), quenched with sat. aq. $NaHCO_3$ (2 mL) and washed with DCM (3x 10 mL). Ammonia solution (30%) (5 mL) was added and the reaction was stirred overnight. The excess ammonia was removed by stirring under vacuum. The crude product was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in water) followed by lyophilization to yield ADP analogue **22** (77.0 mg, 0.16 mmol, 77%) as a white powder. Spectroscopic data were in accord with those reported previously.[37] ^{1}H NMR (400 MHz, D_2O): δ 8.48 (s, 1H, H-2), 8.16 (s, 1H, H-8), 6.07 (d, J = 4.0 Hz, 1H, H-1'), 4.71 – 4.68 (m, 1H, H-2'), 4.53 – 4.51 (m, 1H, H-3'), 4.36 – 4.33 (m, 1H, H-4'), 4.31 – 4.29 (m, 2H, H-5'). ^{13}C NMR (101 MHz, D_2O): δ 155.2 (C-4), 152.4 (C-8), 148.8 (C-6), 139.9 (C-2), 118.4 (C-5), 86.8 (C-1'), 83.9 (d, J = 7.1 Hz, C-4'), 83.8, 74.3 (C-2'), 70.1 (C-3'), 65.3 (d, J = 5.9 Hz, H-5'). ^{19}F NMR (471 MHz, D_2O): δ -118.88 (t, $J_{P\text{-}F}$ = 84.8 Hz,). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{11}H_{16}F_2N_5O_9P_2$ 462.0385; found 462.0396.

N^4-benzoyl-2', 3'-di-*O*-acetyl cytidine 23



Cytidine (5.0 g, 20.5 mmol) was co-evaporated with toluene three times and suspended in anhydrous pyridine (102 mL). Trimethylsilyl chloride (23.4 ml, 184 mmol) was slowly added and the mixture stirred for 2h, then BzCl (11.9 mL, 102.5 mmol) was added dropwise, and the mixture was stirred for an additional 2 h. The reaction mixture was cooled to 0 °C and quenched with water (20 mL) and warmed to room temperature. Aqueous NH_4OH (30 wt%,40 mL) was added, and the reaction mixture was concentrated *in vacuo* to give a white solid. The solid was partitioned between H_2O (280 mL) and EtOAc (80 mL), the organic layer was discarded, and the aqueous layer cooled to 0 °C. The formed white crystals were collected by filtration, rinsed with ice-cold Et_2O anddried under high vacuum at 60 °C to give 4-*N*-benzoyl-β-D-cytidine (6.2 g, 17.8 mmol, 87%). N^4-benzoyl cytidine (6.0 g, 17.3 mmol) was dissolved in dry pyridine (30 mL), after evaporation with pyridine, and *tert*-butyldimethylsilyl chloride (50 wt% in toluene, 12.0 mL, 34.4 mmol) was added and the reaction mixture was stirred overnight at room temperature. Then, acetic anhydride was added and the reaction mixture was stirred for 2 hours. After TLC analysis indicated complete acetylation, the reaction mixture was concentrated *in vacuo* and the residue was redissolved in EtOAc (170 mL) and washed with sat. aq. $NaHCO_3$ (50 mL), 5% Citric acid (50 mL), and H_2O (50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated *in vacuo* to give a yellow oil. The residue was dissolved in MeCN/H_2O (4:1, v/v, 170 mL) and excess *p*-TsOH (3.46 g, 28.4 mmol) was added after which the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated and taken up in EtOAc, washed with sat. aq. $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, filtered and evaporated *in vacuo*. The compound was purified with silica gel column chromatography DCM/MeOH (100/0 - 93/7) to yield **23** (5.9 g, 13.7 mmol, 80%). Spectroscopic data were in accord with those reported previously.[54] ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.77 (s, 1H, NH), 8.21 (d, J = 7.4 Hz, 1H, H-6), 7.89 (d, J = 7.6 Hz, 2H, CH_{arom}), 7.67 – 7.46 (m, 5H, H-5, CH_{arom}), 6.08 (d, J = 4.9 Hz, 1H , H-1'), 5.63 (t, J = 5.2 Hz, 1H, H-2'), 5.52 (t, J = 5.0 Hz, 1H, H-3'), 4.32 – 4.25 (m, 1H, H-4'), 4.04 (d, J = 12.2 Hz, 1H, H-5'), 3.87 (d, J = 12.3 Hz, 1H, H-5'), 3.09 (s, 1H, 5-OH), 2.11 (s, J = 5.6 Hz, 6H, OAc, OAc).

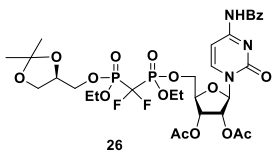
Cytidine 24



Compound **7** (200 mg, 0.68 mmol) was co-evaporated with toluene and dissolved in dry MeCN (3 mL). Tetrabutylammonium benzoate (247 mg, 0.68 mmol) was added under nitrogen, and the mixture was stirred overnight, whereafter the reaction was complete as indicated by ^{31}P NMR. N^4-benzoyl-2', 3'-di-*O*-acetyl cytidine **23** (440 mg, 1.02 mmol), dried prior to use by co-evaporation with toluene, DIPEA (0.79 mL, 10.0 eq) and PyNTP (1.02 g, 2.04 mmol) were added. After 2 h, the reaction was complete as indicated by TLC-analysis. The reaction mixture was poured into DCM and washed with phosphate buffer (pH = 7). The water layer was extracted with DCM. The combined organic was dried over Na_2SO_4 and evaporated *in vacuo*.

Purification by flash column chromatography (R_f = 0.2, 100% EtOAc) followed by size exclusion (LH-20) (DCM/MeOH 1:1) furnished **24** as a pale-yellow solid (258 mg, 0.37 mmol, 55%). *A mixture of diastereoisomers.* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (bs, 1H, NH), 8.27 – 8.11 (m, 1H, H-6), 7.95 – 7.86 (m, 2H, CH_{arom}), 7.66 – 7.47 (m, 4H, CH_{arom}, H-5), 6.36 – 6.31 (m, 1H, H-1'), 5.53 – 5.36 (m, 2H, H-2', H-3'), 4.67 – 4.53 (m, 2H, H-5'), 4.50 – 4.31 (m, 5H, H-4', CH_2 Et, CH_2 Et), 4.10 – 3.94 (m, 3H, P(O)OMe), 2.14 – 2.08 (m, 6H, OAc), 1.50 – 1.36 (m, 6H, CH_3 Et, CH_3 Et). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7, 169.6, 169.5, 169.5 (C=O Ac), 133.3, 129.1, 127.6 (CH_{arom}), 87.8, 87.4 (C-1'), 80.8 (C-4'), 73.7 (C-2'), 70.0, 69.7 (C-3'), 66.6, 66.5, 66.1, 66.0 (C-5', CH_2 Et), 55.7 (P(O)OMe), 20.6, 20.5 (OAc), 16.4 (CH_3 Et). ^{31}P NMR (162 MHz, $CDCl_3$): δ 6.25 – 2.07 (m). ^{19}F NMR (471 MHz, $CDCl_3$): δ -120.81 – -123.19 (m). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{26}H_{34}F_2N_3O_{13}P_2$ 696.1529; found 696.1549.

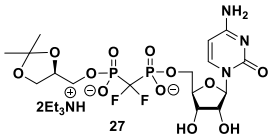
Protected *sn*-3 CF_2 CDP-Gro analogue 26



26

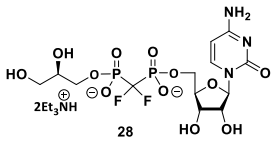
Compound **24** (430mg, 0.62 mmol) was co-evaporated with toluene, dissolved in dry MeCN and tetrabutylammonium benzoate (225 mg, 0.62 mmol) was added under nitrogen and the mixture stirred overnight. After reaction completion, as indicated by ^{31}P NMR, (*S*)-solketal **25** (245 mg, 1.86 mmol), dried prior to addition by co-evaporating with toluene, DIPEA (1.0 mL, 6.2 mmol), and PyNTP (1.02 g, 2.04 mmol) were added successively. After 2 h, the reaction was complete as indicated by TLC-analysis. The reaction mixture was poured into DCM and washed with phosphate buffer (pH = 7). The water layer was extracted with DCM, and the combined organics were dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by flash column chromatography over silica gel (R_f = 0.3, 100% EA) and then by size exclusion (LH-20) (DCM/MeOH 1:1) to obtain **26** (226 mg, 0.29 mmol, 46%) as a pale yellow solid. *A mixture of diastereoisomers.* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.83 (bs, 1H, NH), 8.26 – 8.06 (m, 1H, H-6), 7.95 – 7.84 (m, 2H, CH_{arom}), 7.68 – 7.45 (m, 4H, H-5, CH_{arom}), 6.37 – 6.27 (m, 1H, H-1'), 5.54 – 5.36 (m, 2H, H-3', H-2'), 4.70 – 4.51 (m, 2H, H-5'), 4.51 – 4.20 (m, 8H, H-4', H-1'', H-2'', CH_2 Et, CH_2 Et), 4.16 – 4.06 (m, 1H, H-3''), 3.94 – 3.81 (m, 1H, H-3''), 2.14 – 2.07 (m, 6H, OAc), 1.49 – 1.38 (m, 9H, CH_3 Et, CH_3 Et, $C(CH_3)_2$), 1.36 (s, 3H, $C(CH_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.6, 169.5, 169.5, 169.3, 169.3, 169.3 (C=O Ac), 162.5 (C=O Bz), 144.6 (C-6), 133.2 (C-5), 133.0 ($C_{q\text{-}arom}$), 129.0, 127.6 (CH_{arom}), 110.0, 109.9 ($C(CH_3)_2$), 87.8, 87.5 (C-1'), 80.7, 80.6 (C-4'), 74.1, 74.0, 74.0, 74.0 (C-2''), 73.6 (C-2'), 69.9, 69.8, 69.6 (C-3'), 69.0, 69.0, 68.8, 68.8, 68.5, 68.5, 68.4 (CH_2 Et), 66.6, 66.5, 66.5, 66.4 (C-5'), 66.2, 66.1 (C-1''), 65.9, 65.8, 65.8 (C-3''), 26.7, 26.6, 25.2 ($C(CH_3)_2$), 20.5, 20.5 (OAc), 16.4 (CH_3 Et). ^{31}P NMR (162 MHz, $CDCl_3$): δ 5.42 – 2.29 (m). ^{19}F NMR (471 MHz, $CDCl_3$): δ -120.56 – -123.13 (m). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{31}H_{42}F_2N_3O_{15}P_2$ 796.2087; found 796.2060.

Protected *sn*-3 CF_2 CDP-Gro analogue 27



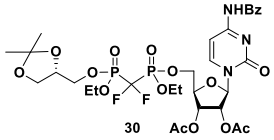
The protected *sn*-3 CF_2 CDP-Gro analogue **26** (30 mg, 0.11 mmol) was dissolved in MeCN/TEA/PhSH (3:3:2, 1 mL, v/v) and stirred for 6 hours at 35 °C. Reaction progression was monitored by ^{31}P NMR and LC-MS, which indicated complete consumption of the starting material. The solution was concentrated *in vacuo*. The residue was dissolved in aqueous ammonia (30 wt%, 1.5 mL) and stirred overnight. The reaction mixture was concentrated *in vacuo*. The resulting mixture was diluted with water then acidified to pH 6 -7 using acetic acid and poured into a separation funnel. The mixture was washed with DCM (3x) to remove excess thiophenol. The water layer was concentrated *in vacuo* and purified by semiprep-HPLC to give compound **27** as a white powder (12 mg, 21 µmol, 58% over 2 steps). ^1H NMR (400 MHz, D_2O): δ 7.97 (d, J = 8.0 Hz, 1H, H-6), 6.08 (d, J = 8.0 Hz, 1H, H-5), 5.94 (d, J = 4.0 Hz, 1H, H-1'), 4.40 – 4.34 (m, 1H, H-2''), 4.32 – 4.21 (m, 5H, H-2', H-3', H-4', H-5''), 4.13 – 4.09 (m, 1H, H-3''), 4.07 – 3.96 (m, 2H, H-1''), 3.84 (dd, J = 8.0, 4.0 Hz, 1H, H-3''), 1.41 (s, 3H, $C(CH_3)_2$), 1.34 (s, 3H, $C(CH_3)_2$). ^{13}C NMR (101 MHz, D_2O): δ 165.2 (C=O Ac), 156.5 ($C_{q\text{-}arom}$, cytosine), 141.8 (C-6), 110.1 ($C(CH_3)_2$), 96.3 (C-5), 89.1 (C-1'), 82.7 (C-4'), 74.8 (C-2''), 74.1 (C-2'), 69.1 (C-3'), 66.4 (C-1''), 65.2 (C-3''), 64.8 (C-5'), 25.4, 24.1 (OAc). ^{31}P NMR (162 MHz, D_2O): δ 3.47, 3.44 (ABX, $J_{P\text{-}F}$ = 82.6 Hz). ^{19}F NMR (376 MHz, D_2O): δ -118.88 (t, $J_{P\text{-}F}$ = 82.7 Hz). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{16}H_{26}F_2N_3O_{12}P_2$ 552.0954; found 552.0963.

***sn*-3 CF_2 CDP-Gro analogue 28**



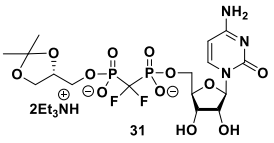
Compound **27** (12 mg, 21 µmol) was dissolved in water (0.5 mL) and acetic acid (0.2 mL) was added. The reaction mixture was stirred at 35 °C for 6 hours. After analysis by LC-MS showed complete consumption of the starting material, the mixture was concentrated *in vacuo*. The compound was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in water) followed by lyophilization to yield *sn*-3 CF_2-CDP-Gro **28** (8.8 mg, 0.29 mmol, 68%) as a white powder. ^1H NMR (400 MHz, D_2O): δ 8.09 (d, J = 8.0 Hz, 1H, H-6), 6.15 (d, J = 8.0 Hz, 1H, H-5), 5.93 (d, J = 4.0 Hz, 1H, H-1'), 4.35 – 4.22 (m, 5H, H-2', H-3', H-4', H-5'), 4.08 – 4.03 (m, 1H, H-1''), 4.01 – 3.95 (m, 1H, H-1''), 3.88 – 3.83 (m, 1H, H-2''), 3.64 (dd, J = 12.0, 4.0 Hz, 1H, H-3''), 3.56 (dd, J = 12.0, 8.0 Hz, 1H, H-3''). ^{13}C NMR (101 MHz, D_2O): δ 162.5 (C=O, cytosine), 152.9 ($C_{q\text{-}arom}$, cytosine), 142.9 (C-6), 121.8, 120.9, 119.1, 116.6 (m, P-CH_2-P), 95.8 (C-5), 89.3 (C-1'), 83.0 (t, J = 3.4 Hz, C-4'), 74.3 (C-2'), 70.8 (t, J = 3.1 Hz, C-2''), 69.1 (C-3'), 67.1 (t, J = 3.3 Hz, C-1''), 64.8 (t, J = 3.4 Hz, C-5'), 61.9 (C-3''). ^{31}P NMR (162 MHz, D_2O): δ 3.47, 3.44 (ABX, $J_{P\text{-}F}$ = 82.6 Hz). ^{19}F NMR (471 MHz, D_2O): δ -119.17 (t, $J_{P\text{-}F}$ = 84.8 Hz). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{13}H_{22}F_2N_3O_{12}P_2$ 512.0641; found 512.0654. $[\alpha]^{20}_D$ = +10 ° (c = 0.001, H_2O).

Protected *sn*-1 CF_2 CDP-Gro analogue 30



Compound **24** (150 mg, 0.22 mmol) was co-evaporated with toluene dissolved in dry MeCN, and tetrabutylammonium benzoate (78 mg, 0.22 mmol) was added under nitrogen and the mixture stirred overnight. The reaction was complete as indicated by ^{31}P NMR. (*R*)-solketal **29** (87 mg, 0.66 mmol) was added after being dried by evaporation with toluene together with DIPEA (0.37 mL, 2.2 mmol), and PyNTP (330 mg, 2.04 mmol). After 2 h, the reaction was complete as indicated by TLC-analysis. The reaction mixture was poured into DCM and washed with pH=7 phosphate buffer. The water layer was extracted with DCM. The combined organic was dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by flash column chromatography over silica gel (R_f = 0.3, 100% EA) and then by size exclusion (LH-20) (DCM/MeOH 1:1) to obtain **30** (279 mg, 0.35 mmol, 57%) as a yellow solid. *A mixture of diastereoisomers.* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.93 (s, 1H, NH), 8.26 – 8.06 (m, 1H, H-6), 7.96 – 7.83 (m, 2H, CH_{arom}), 7.67 – 7.43 (m, 4H, CH_{arom}), 6.33 – 6.27 (m, 1H, H-1'), 5.52 – 5.33 (m, 2H, H-3', H-2'), 4.67 – 4.49 (m, 2H, H-5'), 4.46 – 4.16 (m, 8H, H-4', H-1'', H-2'' , CH_2 Et, CH_2 Et), 4.13 – 3.96 (m, 1H, H-3''), 3.90 – 3.79 (m, 1H, H-3''), 2.12 – 2.03 (m, 6H, OAc, OAc), 1.47 – 1.36 (m, 9H, CH_3 Et, CH_3 Et, $C(CH_3)_2$), 1.34 (s, 3H, $C(CH_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.7, 169.6, 169.5, 169.4, 169.4 (C=O Ac), 162.6 (C=O Bz), 144.8 (C-6), 133.3 (C-5), 133.1 ($C_{q\text{-}arom}$), 129.1, 127.7 (CH_{arom}), 110.1, 110.09, 110.0 ($C(CH_3)_2$), 87.9, 87.6 (C-1'), 80.8, 80.7, 80.7 (C-4'), 74.2, 74.1, 74.1, 74.1, 74.0 (C-2''), 73.7, 73.7 (C-2'), 70.0, 69.9, 69.7 (C-3'), 69.0, 69.0, 68.9, 68.9, 68.6, 68.5, 68.5 (CH_2 Et), 66.7, 66.6, 66.6, 66.5, 66.5, 66.4 (C-5'), 66.3, 66.3, 66.2, 66.1 (C-1''), 65.9, 65.9, 65.9, 65.7 (C-3''), 26.8, 26.7, 26.7, 26.6, 25.3, 25.3 ($C(CH_3)_2$), 20.6, 20.5 (OAc), 16.6, 16.5, 16.5, 16.4 (CH_3 Et). ^{31}P NMR (162 MHz, $CDCl_3$): δ 5.41 – 2.29 (m). ^{19}F NMR (471 MHz, $CDCl_3$): δ -120.95 – -123.42 (m). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{31}H_{42}F_2N_3O_{15}P_2$ 796.2053; found 796.2057.

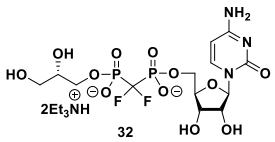
Protected *sn*-1 CF_2 CDP-Gro analogue 31



The protected *sn*-1 CF_2 CDP-Gro analogue **30** (88 mg, 0.11 mmol) was dissolved in MeCN/TEA/PhSH (3:3:2, 2 mL) and stirred for 6 hours at 35 °C. Reaction progression was monitored by ^{31}P NMR and LC-MS, which indicated complete consumption of the starting material. The solution was concentrated *in vacuo* redissolved in aqueous ammonia (30 wt%, 3 mL) and stirred overnight. The reaction mixture was concentrated *in vacuo*. The resulting mixture was diluted with water then acidified to pH 6 -7 using acetic acid and poured into a separation funnel. The mixture was washed with DCM (3x) to remove excess thiophenol. The water layer was concentrated *in vacuo*. The residue was purified by semiprep-HPLC to give compound **31** as a white powder (41 mg, 72 µmol, 65% over 2 steps). ^{1}H NMR (400 MHz, D_2O): δ 7.90 (d, *J* = 8.0 Hz, 1H, H-6), 6.03 (d, *J* = 8.0 Hz, 1H, H-5), 5.91 (d, *J* = 4.0 Hz, 1H, H-1'), 4.38 – 4.31 (m, 1H, H-2''), 4.31 – 4.15 (m, 5H, H-2', H-3', H-4', H-5'), 4.10 – 4.05 (m, 1H, H-3''), 4.05 – 3.99 (m, 1H, H-1''), 3.99 – 3.91 (m, 1H, H-1''), 3.81 (dd, *J* = 8.0, 4.0

Hz, 1H, H-3''), 1.37 (s, 3H, C(CH_3)$_2$), 1.31 (s, 3H, C(CH_3)$_2$). ^{13}C NMR (101 MHz, D_2O): δ 165.3 (C=O Ac), 156.6 ($C_{q\text{-arom}}$, cytosine), 141.8 (C-6), 121.2, 119.2, 117.2 (m, P-CH_2-P), 110.1 (*C*(CH_3)$_2$), 96.3 (C-5), 89.1 (C-1'), 82.7, 82.7, 82.7 (C-4'), 74.8, 74.8, 74.7 (C-2''), 74.2 (C-2'), 69.1 (C-3'), 66.3 (C-1''), 65.2 (C-3''), 64.8 (C-5'), 25.4, 24.1 (OAc). ^{31}P NMR (162 MHz, D_2O): δ 4.11, 4.08 (ABX, $J_{P\text{-}F}$ = 84.8 Hz). ^{19}F NMR (376 MHz, D_2O): δ -119.01 (t, $J_{P\text{-}F}$ = 82.7 Hz). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{16}H_{26}F_2N_3O_{12}P_2$ 552.0954; found 552.0961. $[\alpha]^{20}_D$ = +16 ^{o} (c = 0.001, H_2O).

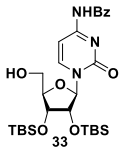
_sn_-1 CF_2 CDP-Gro analogue 32



32

Compound **31** (41mg, 72 µmol) was dissolved in water (0.5 mL) and acetic acid (0.2 mL) was added. The reaction mixture was stirred at 35 °C for 6 hours. After analysis by LC-MS showed complete consumption of the starting material, the mixture was concentrated *in vacuo*. The compound was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in water) followed by lyophilization to yield *sn*-1 CF_2 CDP-Gro analogue **32** (18 mg, 35 µmol, 48%) as a white powder. ^{1}H NMR (400 MHz, D_2O): δ 8.05 (d, *J* = 8.0 Hz, 1H, H-6), 6.13 (d, *J* = 8.0 Hz, 1H, H-5), 5.93 (d, *J* = 4.0 Hz, 1H, H-1'), 4.36 – 4.22 (m, 5H, H-2', H-3', H-4', H-5'), 4.10 – 4.03 (m, 1H, H-1''), 4.01 – 3.96 (m, 1H, H-1''), 3.89 – 3.83 (m, 1H, H-2''), 3.64 (dd, *J* = 12.0, 4.0 Hz, 1H, H-3''), 3.56 (dd, *J* = 12.0, 8.0 Hz, 1H, H-3''). ^{13}C NMR (101 MHz, D_2O): δ 163.1 (C=O, cytosine), 153.7 ($C_{q\text{-arom}}$, cytosine), 142.6 (C-6), 121.8, 120.8, 119.1, 117.4 (m, P-CH_2-P), 95.9 (C-5), 89.2 (C-1'), 82.9 (t, *J* = 3.3 Hz, C-4'), 74.2 (C-2'), 70.8 (t, *J* = 3.1 Hz, C-2''), 69.0 (C-3'), 67.1 (t, *J* = 3.3 Hz, C-1''), 64.79 (t, *J* = 3.5 Hz, C-5'), 61.9 (C-3''). ^{31}P NMR (162 MHz, D_2O): δ 3.66, 3.64 (ABX, $J_{P\text{-}F}$ = 82.6 Hz). ^{19}F NMR (471 MHz, D_2O): δ -119.13 (t, $J_{P\text{-}F}$ = 80.1 Hz). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{13}H_{22}F_2N_3O_{12}P_2$ 512.0641; found 512.0652.

N^6-benzoyl-2,3-*bis*-*O*-tert-butyldimethylsilylcytidine 33

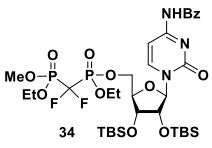


33

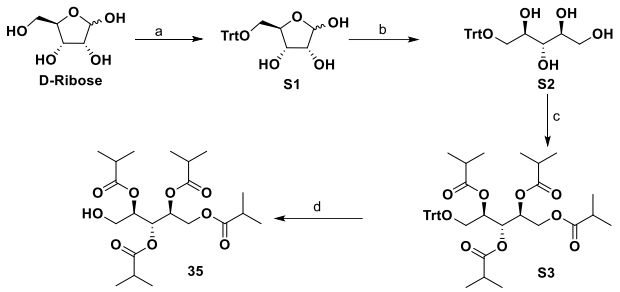
Commercially available cytidine (10 g, 41 mmol) was co-evaporated thrice with anhydrous pyridine, dissolved in anhydrous dimethylformamide (80 mL) and heated to 50 °C in an oil-bath, whereupon imidazole (14 g, 205 mmol) and *tert*-butyldimethylsilyl chloride (31 g, 205 mmol) were added consecutively and the resulting mixture stirred overnight. The reaction mixture was quenched by the addition of H_2O (30 mL), diluted with Et_2O (200 mL), and washed with H_2O (100 mL). The aqueous layer was back-extracted twice with Et_2O, and the resulting organic layers were combined, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was co-evaporated with toluene three times, placed under a nitrogen atmosphere and dissolved in pyridine (110 mL), whereupon benzoyl chloride (28.8 g, 205 mmol) was added and the mixture was stirred at room temperature for 5 hours. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with sat. aq. $NaHCO_3$ at 0 °C, diluted with EtOAc and washed with water and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was taken up in THF

(400 mL), and aqueous TCA (212 g in 100 mL H_2O) was added at 0 °C. After stirring for 3 h at 0 °C, the reaction was quenched with sat. aq. $NaHCO_3$ and diluted with dichloromethane (500 mL). The water layer was back-extracted twice with dichloromethane (200 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (R_f = 0.2, pentane/EtOAc 3:1, v/v) to give title nucleoside **33** as a white solid (18.7 g, 31.8 mmol, 77%). Spectral data was in accordance with literary precedence.[55] ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.84 (s, 1H, NH), 8.12 (d, *J* = 6.0 Hz, 1H, H-6), 7.96 – 7.86 (m, 2H, CH_{arom}), 7.66 – 7.44 (m, 4H, H-5, CH_{arom}), 5.48 (d, *J* = 6.0 Hz, 1H, H-1'), 4.73 (t, *J* = 6.0 Hz, 1H, H-2'), 4.23 – 4.16 (m, 2H, H-3', H-4'), 4.01 (dd, *J* = 12.0, 3.0 Hz, 1H, H-5'), 3.74 (d, *J* = 12.0 Hz, 1H, H-5'), 0.90 (s, 9H, $C(CH_3)_3$ TBS), 0.87 (s, 9H, $C(CH_3)_3$ TBS), 0.09 – 0.06 (m, 9H, CH_3 TBS, CH_3 TBS, CH_3 TBS), 0.04 (s, 3H, CH_3 TBS). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.5 (C=O, cytosine), 147.9 (C-6), 133.3, 129.1, 127.7 (CH_{arom}), 96.4 (C-5), 96.0 (C-1'), 86.2 (C-4'), 73.3 (C-2'), 71.3 (C-3'), 61.4 (C-5'), 25.9 ($C(CH_3)_3$ TBS), 18.1, 18.0 ($C(CH_3)_3$ TBS), -4.4, -4.7, -4.7, -4.8 (CH_3 TBS).

Difluoromethylenebisphosphonate cytidine 34

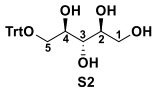


Compound **7** (100 mg, 0.34 mmol) was co-evaporated with toluene, dissolved in dry MeCN (11.5 mL) and treated with tetrabutylammonium benzoate (124 mg, 0.34 mmol) under a nitrogen atmosphere and stirred overnight. The reaction was complete as indicated by ^{31}P NMR. Compound **33** (358 mg, 0.51 mmol) was added after being dried by evaporation with toluene, DIPEA (0.58 mL, 3.4 mmol eq) and PyNTP (509 mg, 1.02 mmol) were added. After 2 h, the reaction was complete as indicated by TLC-analysis and was poured into DCM and washed with phosphate buffer (pH = 7). The water layer was extracted with DCM and the combined organics were dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by flash column chromatography over silica gel (R_f = 0.3, pentane/EtOAc 1:1, v/v) and then by size exclusion (LH-20) (DCM/MeOH 1:1, v/v) to obtain **34** (178 mg, 0.21 mmol, 63% over 2 steps) as a yellow solid. *A mixture of diastereoisomers.* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (s, 1H, NH), 8.39 – 8.18 (m, 1H, H-6), 7.95 – 7.81 (m, 2H, CH_{arom}), 7.65 – 7.46 (m, 4H, H-5, CH_{arom}), 5.85 – 5.77 (m, 1H, H-1'), 4.78 – 4.65 (m, 1H, H-5'), 4.51 – 4.27 (m, 6H, H-4', H-5', CH_2 Et, CH_2 Et), 4.27 – 4.21 (m, 1H, H-2'), 4.09 – 3.93 (m, 4H, H-3', P(O)OMe), 1.49 – 1.39 (m, 6H, CH_3 Et, CH_3 Et), 0.91 (s, 9H, $C(CH_3)_3$ TBS), 0.89 (s, 9H, $C(CH_3)_3$ TBS), 0.27 – 0.20 (m, 3H, CH_3 TBS), 0.17 – 0.11 (m, 3H, CH_3 TBS), 0.11 – 0.03 (m, 6H, CH_3 TBS, CH_3 TBS). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.5 (C=O, cytosine), 145.0 (C-6), 133.2, 129.1, 127.6 (CH_{arom}), 118.8, 117.8, 116.4, 116.1 (m, P-CH_2-P), 96.2 (C-5), 91.5 (C-1'), 81.2, 81.0 (C-4'), 75.7 (C-2'), 69.7, 69.6 (C-3'), 66.7, 66.6, 66.4, 66.1, 66.0, 66.0 (CH_2 Et), 65.8, 65.6 (C-5'), 55.6, 55.6 (OMe), 26.0, 25.9 ($C(CH_3)_3$ TBS), 18.1, 18.1 ($C(CH_3)_3$ TBS), 16.6, 16.5, 16.5 (CH_3 Et), -4.0, -4.1, -5.0, -5.0, -5.0 (CH_3 TBS). ^{31}P NMR (162 MHz, $CDCl_3$): δ 5.66 –2.45 (m). ^{19}F NMR (376 MHz, $CDCl_3$): δ -119.99 – -122.63. HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{34}H_{58}F_2N_3O_{11}P_2Si_2$ 840.3047; found 840.3061.



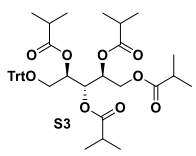
Scheme 4.6. Synthesis of 1,2,3,4-tetra-*O*-isobutyryl-D-ribitol **35**. Reagent and conditions: a) trityl chloride, pyridine, 50 °C, 6 h. b) $NaBH_4$, EtOH, 0 °C to rt, overnight, 89% over 2 steps. c) isobutyric anhydride, pyridine, DMAP, rt, overnight 88%. d) *p*-Toluenesulfonic acid, MeOH/DCM (1:1, v/v), rt, 3 h 62%.

5-*O*-trityl-D-ribitol S2



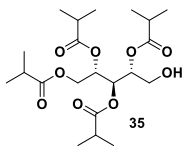
D-Ribose (5.1g, 34 mmol) was co-evaporated with toluene three times under nitrogen and dissolved in pyridine (110 mL), trityl chloride (9.4g, 34 mmol) was added and the reaction mixture was heated to 50 °C in an oil-bath for 6 h whereupon the solution became homogenous, the reaction mixture was cooled to room temperature and the pyridine was removed under reduced pressure. The resulting oil was subsequently redissolved in DCM, washed with water, The aqueous layer was extracted with DCM, and the combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was again taken up in DCM and added dropwise with stirring to a mixture of hexane (18 mL) and DCM (2 mL), the white crystals were collected by filtration and washed with hexane and water. Crystals were dried under a high vacuum at 50 °C to yield 5-*O*-triphenylchloromethyl-D-ribose (9.6g, 24.4 mmol, 73%). 5-*O*-triphenylchloromethyl-D-ribose (3g, 7.6 mmol) was dissolved in anhydrous ethanol and cooled to 0 °C, sodium tetrahydroborate (1.73g, 45.6 mmol) was slowly added. The reaction mixture was warmed to rt and stirred overnight. After analysis by TLC showed complete consumption of the starting material, the reaction mixture was quenched with acetic acid and concentrated *in vacuo*. The residue was diluted in EtOAc and washed with sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*, and the crude product was purified by flash column chromatography (R_f = 0.3, DCM/MeOH 10:1, v/v) to give **S2** (2.7 g, 6.8 mmol, 89%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 – 7.38 (m, 6H, CH_{arom}), 7.30 – 7.24 (m, 6H, CH_{arom}), 7.23 – 7.18 (m, 3H, CH_{arom}), 3.85 – 3.78 (m, 1H, H-2), 3.75 – 3.60 (m, 5H, H-3, H-4, H-5, 2-OH), 3.47 – 3.41 (m, 2H, H-1, 3-OH), 3.39 (d, *J* = 4.0 Hz, 1H, 4-OH), 3.37 – 3.33 (m, 1H, 1-OH), 3.30 (dd, *J* = 12.0, 8.0 Hz, 1H, H-1). ^{13}C NMR (101 MHz, $CDCl_3$): δ 143.5 ($C_{q\text{-}arom}$), 128.66, 128.1, 127.3 (CH_{arom}), 87.4 (CPh_3), 73.2 (C-4), 72.8 (C-3), 71.6 (C-2), 65.2 (C-5), 63.3 (C-1). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{24}H_{26}O_5Na$ 417.1672; found 417.1676.

1,2,3,4-tetra-*O*-isobutyryl-5-*O*-trityl-D-ribitol S3



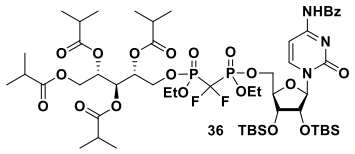
5-*O*-trityl-D-ribitol **S2** (3.5 g, 9.0 mmol) was co-evaporated with toluene three times, placed under a nitrogen atmosphere and dissolved in pyridine (36 mL). 4-Dimethylaminopyridine (4.95 g, 40.5 mmol) and isobutyric anhydride (9 mL, 54 mmol) were added and the mixture was stirred overnight at rt. The reaction mixture was concentrated *in vacuo* and taken up in DCM. After washing with 5% citric acid and water, the organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography (R_f = 0.2, pentane/EtOAc 20:1, v/v) to give **S3** as a colorless oil (4.8 g, 7.9 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 – 7.37 (m, 6H, CH_{arom}), 7.36 – 7.21 (m, 9H, CH_{arom}), 5.48 – 5.36 (m, 2H, H-2, H-3), 5.34 – 5.26 (m, 1H, H-4), 4.35 (dd, *J* = 12.0, 4.0 Hz, 1H, H-5), 4.15 (dd, *J* = 12.0, 8.0 Hz, 1H, H-5), 3.32 (dd, *J* = 8.0, 4.0 Hz, 1H, H-1), 3.23 (dd, *J* = 12.0, 8.0 Hz, 1H, H-1), 2.75 – 2.60 (m, 1H, $CH(CH_3)_2$), 2.59 – 2.47 (m, 1H, $CH(CH_3)_2$), 2.49 – 2.34 (m, 2H, $CH(CH_3)_2$, $CH(CH_3)_2$), 1.27 (d, *J* = 7.1 Hz, 3H, $CH(CH_3)_2$), 1.25 (d, *J* = 8.0 Hz, 3H, $CH(CH_3)_2$), 1.17 – 1.10 (m, 12H, $CH(CH_3)_2$), 1.07 (d, *J* = 8.0 Hz, 3H, $CH(CH_3)_2$), 1.04 (d, *J* = 8.0 Hz, 3H, $CH(CH_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.6, 176.0, 175.7, 175.1 (C=O $COCH(CH_3)_2$), 144.7, 144.0, 143.9, 143.7, 143.5 ($C_{q\text{-}arom}$), 128.7, 127.9, 127.1 (CH_{arom}), 86.7 (CPh_3), 70.7 (C-4), 69.6 (C-3), 69.4 (C-2), 61.9, 61.9 (C-5, C-1), 34.2, 33.9, 33.9, 33.9 ($CH(CH_3)_2$), 19.0, 19.0, 18.9, 18.9, 18.8, 18.7 ($CH(CH_3)_2$). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{40}H_{50}O_9Na$ 697.3347; found 697.3364.

1,2,3,4-tetra-*O*-isobutyryl-D-ribitol 35



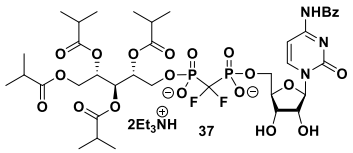
p-Toluenesulfonic acid (1.2 g, 6.4 mmol), dissolved in MeOH-DCM (1:1, 6 mL), was added dropwise over 1 h to a solution of **S3** (2.0 g, 3.2 mmol) in MeOH-DCM (1:1, 20 mL). The reaction mixture was stirred at rt for 3 h until complete conversion. Hereafter, solid $NaHCO_3$ was added, the turbid mixture filtered and the filtrate evaporated *in vacuo*. The residue was purified by flash column chromatography (R_f= 0.2, pentane /EtOAc, 3:1, v/v) to give compound **35** (867 mg, 1.98 mmol, 62 %) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.41 – 5.31 (m, 2H, H-2, H-3), 5.09 – 5.04 (m, 1H, H-4), 4.39 (dd, *J* = 12.0, 4.0 Hz, 1H, H-1), 4.20 – 4.11 (m, 1H, H-1), 3.83 (d, *J* = 16.0 Hz, 1H, H-5), 3.72 – 3.62 (m, 1H, H-5), 2.69 – 2.44 (m, 4H, $CH(CH_3)_2$), 1.70 (s, 1H, 5-OH), 1.22 – 1.11 (m, 24H, $CH(CH_3)_2$, $CH(CH_3)_2$, $CH(CH_3)_2$, $CH(CH_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.7, 176.5, 176.1, 175.9 (C=O $COCH(CH_3)_2$), 72.4 (C-4), 69.7 (C-2, C-3), 61.9 (C-1), 61.2 (C-5), 34.1 ($CH(CH_3)_2$), 19.0 ($CH(CH_3)_2$). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{21}H_{36}O_9Na$ 455.2251; found 455.2260.

Protected CF_2 CDP-Ribitol analogue 36



Compound **34** (400 mg, 0.48 mmol) was co-evaporated with toluene dissolved in dry MeCN (2.0 mL) and treated with tetrabutylammonium benzoate (175 mg, 0.48 mmol) under a nitrogen atmosphere and stirred overnight. Compound **35** (311 mg, 0.72 mmol), dried by evaporation with toluene, DIPEA (816 µl, 4.8 mmol) and PyNTP (719 mg, 1.44 mmol) were added successively. After 2 h, the reaction was complete as indicated by TLC-analysis. The reaction mixture was poured into DCM and washed with phosphate buffer (pH = 7). The water layer was extracted with DCM. The combined organics were dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by flash column chromatography over silica gel (R_f = 0.24, pentane/EtOAc 2:1, v/v) and then by size exclusion (LH-20) (DCM/MeOH 1:1, v/v) to obtain **36** (282 mg, 0.23 mmol, 48%) as a colorless oil. *A mixture of diastereoisomers.* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.72 (s, 1H), 8.34 – 8.17 (m, 1H, NH), 7.94 – 7.84 (m, 2H, CH_{arom}), 7.64 – 7.44 (m, 4H, H-5, CH_{arom}), 5.83 – 5.74 (m, 1H, H-1'), 5.42 – 5.25 (m, 3H, H-2'', 3'', 4''), 4.77 – 4.47 (m, 2H, H-5'), 4.47 – 4.25 (m, 8H, H-1'', H-3', H-4', CH_2 Et, CH_2 Et), 4.25 – 4.19 (m, 1H, C-2'), 4.17 – 4.09 (m, 1H, C-1''), 4.07 – 3.91 (m, 1H, C-3'), 2.68 – 2.45 (m, 4H, $CH(CH_3)_2$, $CH(CH_3)_2$, $CH(CH_3)_2$, $CH(CH_3)_2$), 1.48 – 1.34 (m, 6H, CH_3 Et), 1.22 – 1.07 (m, 24H, $CH(CH_3)_2$, $CH(CH_3)_2$, $CH(CH_3)_2$, $CH(CH_3)_2$), 0.93 – 0.88 (m, 9H, $C(CH_3)_3$ TBS), 0.90 – 0.85 (m, 9H, $C(CH_3)_3$ TBS), 0.26 – 0.18 (m, 3H, CH_3 TBS), 0.16 – 0.08 (m, 3H, CH_3 TBS), 0.09 – 0.02 (m, 6H, CH_3 TBS, CH_3 TBS). ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.5, 176.5, 175.9, 175.9, 175.8, 175.8, 175.2, 175.2, 175.1 (C=O $COCH(CH_3)_2$), 162.3 (C=O, cytosine), 144.9 (C-6), 133.2, 129.1, 127.6 (CH_{arom}), 119.4, 118.0, 116.1 (m, P-CH_2-P), 96.3 (C-5), 91.6, 91.4 (C-1'), 81.2, 80.9, 80.9 (C-4'), 75.7, 75.7 (C-2'), 69.8, 69.8, 69.7, 69.7, 69.6, 69.5, 69.5, 69.4, 69.4 (C-2', C-3', C-2'', C-4''), 68.7, 68.6 (C-3''), 66.8, 66.7, 66.7, 66.6, 66.5, 66.5, 66.4, 66.4, 66.3, 66.2, 66.2, 65.9, 65.7 (C-1'', C-5'), 61.7, 61.6 (C-5''), 34.0, 34.0, 34.0, 33.8 ($CH(CH_3)_2$), 25.9, 25.9 ($C(CH_3)_3$ TBS), 18.9, 18.9, 18.8, 18.8, 18.1 ($CH(CH_3)_2$), 18.0, 16.6 ($C(CH_3)_3$ TBS), 16.5, 16.5, 16.4, 16.4, 16.3 (CH_3 Et), -4.0, -4.1, -4.15, -4.1, -5.0, -5.0, -5.1 (CH_3 TBS). ^{31}P NMR (162 MHz, $CDCl_3$): δ 5.33 – 2.01 (m). ^{19}F NMR (376 MHz, $CDCl_3$): δ -120.30 – -123.12 (m). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{54}H_{90}F_2N_3O_{19}P_2Si_2$ 1240.5144; found 1240.5144.

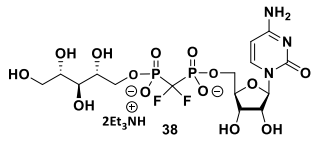
Protected CF_2 CDP-Ribitol analogue 37



The protected CDP-ribitol analogue **36** (55 mg, 0.11 mmol) was dissolved in MeCN/TEA/PhSH (3:3:2, 0.4 mL, v/v) and stirred for 6 hours at 35 °C. Reaction progression was monitored by ^{31}P NMR and LC-MS, which indicated complete consumption of the starting material. The solution was concentrated *in vacuo*. The residue was dissolved in pyridine and cooled to 0 °C, after which HF·pyridine (70 wt%, 31 µL, 1. 2 mmol) was added and stirred overnight at rt. The resulting solution was quenched with sat. aq. $NaHCO_3$ and concentrated. The resulting mixture was diluted

with water then acidified to pH 6-7 using acetic acid. The mixture was washed with DCM (3x) to remove excess thiophenol and pyridine. The water layer was concentrated and purified by semiprep-HPLC to give compound **37** (20 mg, 51 μmol, 47% over 2 steps) as a white powder. ^{1}H NMR (400 MHz, D_2O): δ 8.57 (d, J = 8.0 Hz, 1H), 7.92 – 7.85 (m, 2H, CH_{arom}), 7.70 – 7.61 (m, 1H, CH_{arom}), 7.58 – 7.48 (m, 3H, H-5, CH_{arom}), 5.95 (d, J = 4.0 Hz, 1H, H-1'), 5.31 – 5.19 (m, 3H, H-2'', H-3'', H-4''), 4.42 (d, J = 12.2 Hz, 1H, H-5'), 4.37 – 4.22 (m, 6H, H-1'', H-2', H-3', H-4', H-5', H-5''), 4.20 – 4.12 (m, 1H, H-1''), 4.08 (dd, J = 12.0, 4.0 Hz, 1H, H-5''), 2.66 – 2.38 (m, 4H, $CH(CH_3)_2$, $CH(CH_3)_2$, $CH(CH_3)_2$, $CH(CH_3)_2$), 1.11 – 0.97 (m, 24H, $CH(CH_3)_2$, $CH(CH_3)_2$, $CH(CH_3)_2$, $CH(CH_3)_2$). ^{13}C NMR (101 MHz, D_2O): δ 179.2, 178.8, 178.3, 177.7 (C=O $COCH(CH_3)_2$), 169.3 (C=O Bz), 163.2 (C=O, cytosine), 156.7 ($C_{q\text{-}arom}$, cytosine), 145.9 (C-6), 133.6 (CH_{arom}), 132.5 ($C_{q\text{-}arom}$), 128.9, 128.0 (CH_{arom}), 98.4 (C-5), 90.7 (C-1'), 82.5 (C-4'), 74.8 (C-2'), 71.2 (C-4''), 69.7 (C-2''), 68.9 (C-3''), 68.1 (C-3'), 64.0 (C-1''), 63.6 (C-5'), 61.5 (C-5''), 33.9, 33.8, 33.8, 33.7 ($CH(CH_3)_2$), 18.1, 17.9, 17.9 ($CH(CH_3)_2$). ^{31}P NMR (162 MHz, D_2O): δ 3.47 (dt, $J_{P\text{-}F}$ = 84.2 Hz, $J_{P\text{-}P}$ = 55.1 Hz, α -P), 2.77(dt, $J_{P\text{-}F}$ = 84.2 Hz, $J_{P\text{-}P}$ = 55.1 Hz, β-P). ^{19}F NMR (376 MHz, D_2O): δ -118.87 (td, $J_{P\text{-}F}$ = 82.7 Hz, $J_{F\text{-}H}$ = 7.5 Hz). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{38}H_{54}F_2N_3O_{19}P_2$ 956.27893; found 956.27971.

CF_2 CDP-Ribitol analogue 38



Compound **37** (18 mg, 0.019 mmol) was dissolved in aqueous ammonia (30 wt%, 2 mL) and stirred overnight. The excess ammonia was removed by stirring under vacuum. The crude product was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in water) followed by lyophilization to yield CF_2 CDP-ribitol analogue **38** (10.0 mg, 6.3 μmol, 33%) as a white powder. ^{1}H NMR (400 MHz, D_2O): δ 8.08 (d, J = 8.0 Hz, 1H, H-6), 6.15 (d, J = 8.0 Hz, 1H, H-5), 5.93 (d, J = 4.0 Hz, 1H, H-1'), 4.38 – 4.16 (m, 6H, H-1'', H-2', H-3', H-4', H-5'), 4.13 – 4.06 (m, 1H, H-1''), 3.91 – 3.80 (m, 2H, H-2'', H-4''), 3.76 (dd, J = 12.0, 4.0 Hz, 1H, H-5''), 3.74 – 3.69 (m, 1H, H-3''), 3.60 (dd, J = 12.0, 8.0 Hz, 1H, H-5''). ^{13}C NMR (101 MHz, D_2O): δ 162.7 (C=O, cytosine), 153.2 ($C_{q\text{-}arom}$, cytosine), 142.8 (C-6), 123.3, 121.8, 120.8, 119.1, 117.4 (m, P-CH_2-P), 95.9 (C-5), 89.2 (C-1'), 83.0 (d, J = 5.5 Hz, C-4'), 74.3 (C-2'), 72.0 (C-4''), 71.6 (C-3''), 70.9 (d, J = 4.9 Hz, C-2''), 69.1 (C-3'), 67.4 (d, J = 4.8 Hz, C-1''), 64.8 (d, J = 4.7 Hz, C-5'), 62.2 (C-5''). ^{31}P NMR (162 MHz, D_2O): δ 3.78，3.72 (ABX, $J_{P\text{-}F}$ = 82.6 Hz). ^{19}F NMR (471 MHz, D_2O): δ -119.21 (t, $J_{P\text{-}F}$ = 84.8 Hz,). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{15}H_{26}F_2N_3O_{14}P_2$ 572.0852; found 572.0867.

Appendix

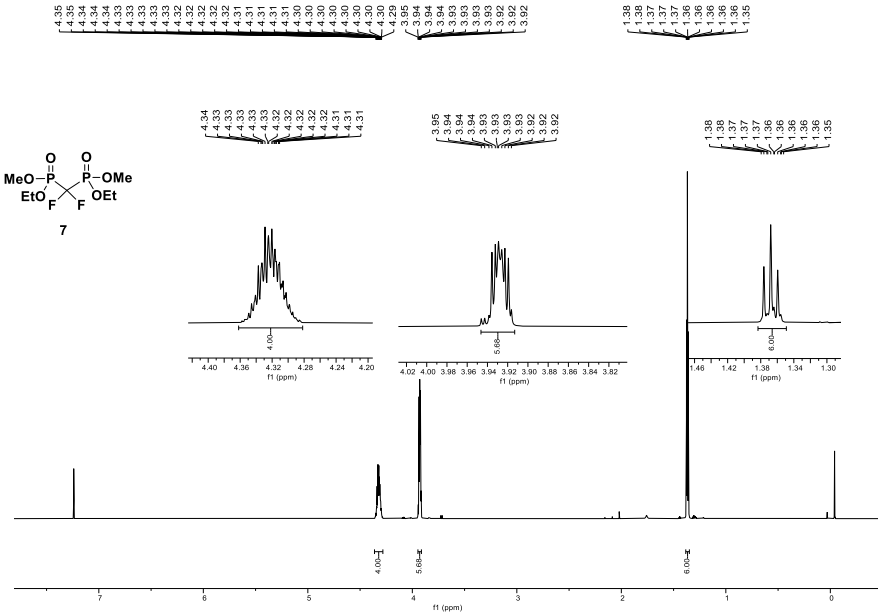


Figure 4.3. ^{1}H NMR (850 MHz, $CDCl_3$) spectra of compound **7**.

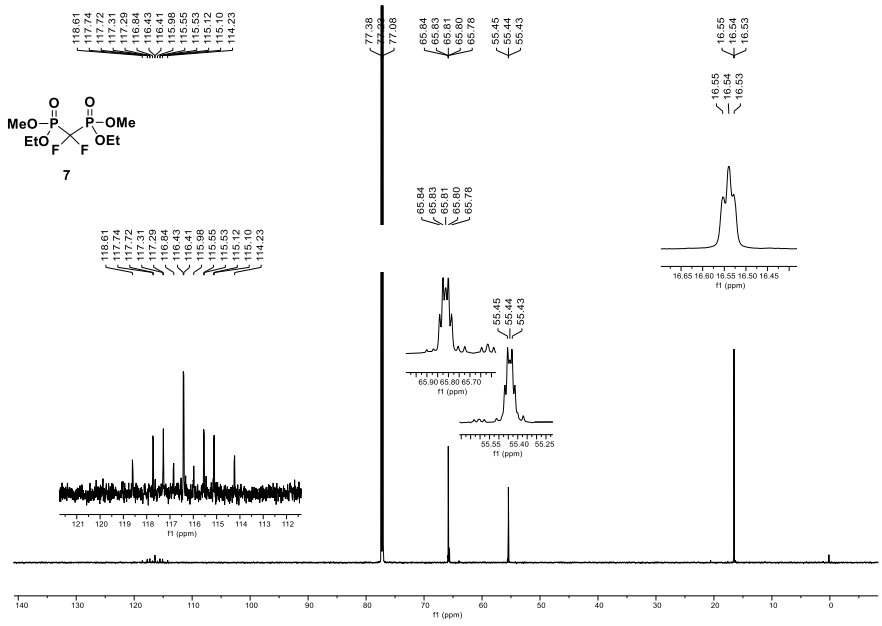


Figure 4.4. ^{13}C NMR (214 MHz, $CDCl_3$) spectra of compound **7** (broadband proton-decoupled).

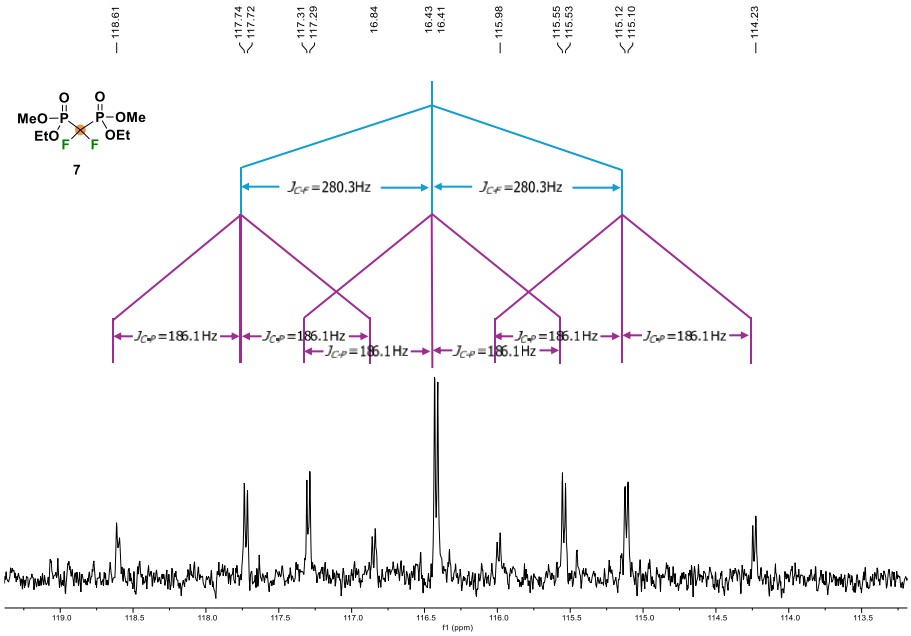


Figure 4.5. Part of ^{13}C NMR (214 MHz, $CDCl_3$) spectra of compound **7** (broadband proton-decoupled). The carbon signal of the P–CF_2–P unit is split by coupling with the two phosphorus atoms ($J_{C\text{-}P}$ = 186.1 Hz) and the two fluorine atoms ($J_{C\text{-}F}$ = 280.3 Hz), leading to a triplet of triplet. Signals corresponding to two diastereoisomers are visible in the spectrum.

References

(1) Kolodiazhnyi, O. I. Phosphorus compounds of natural origin: prebiotic, stereochemistry, application. *Symmetry* **2021**, *13*, 889.

(2) Frank, A.; Groll, M. The methylerythritol phosphate pathway to isoprenoids. *Chem. Rev.* **2017**, *117*, 5675-5703.

(3) Wilson, Miranda S. C.; Livermore, Thomas M.; Saiardi, A. Inositol pyrophosphates: between signalling and metabolism. *Biochem. J.* **2013**, *452*, 369-379.

(4) Covarrubias, A. J.; Perrone, R.; Grozio, A.; Verdin, E. NAD(+) metabolism and its roles in cellular processes during ageing. *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 119-141.

(5) Suskiewicz, M. J.; Prokhorova, E.; Rack, J. G. M.; Ahel, I. ADP-ribosylation from molecular mechanisms to therapeutic implications. *Cell* **2023**, *186*, 4475-4495.

(6) Lüscher, B.; Bütepage, M.; Eckei, L.; Krieg, S.; Verheugd, P.; Shilton, B. H. ADP-ribosylation, a multifaceted posttranslational modification involved in the control of cell physiology in health and disease. *Chem. Rev.* **2018**, *118*, 1092-1136.

(7) van der Es, D.; Hogendorf, W. F. J.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Teichoic acids: synthesis and applications. *Chem. Soc. Rev.* **2017**, *46*, 1464-1482.

(8) Kanagawa, M. Dystroglycanopathy: from elucidation of molecular and pathological mechanisms to development of treatment methods. *Int. J. Mol. Sci.* **2021**, *22*, 13162.

(9) Na, L.; Li, R.; Chen, X. Recent progress in synthesis of carbohydrates with sugar nucleotide-dependent glycosyltransferases. *Curr. Opin. Chem. Biol.* **2021**, *61*, 81-95.

(10) Lacabanne, D.; Wiegand, T.; Wili, N.; Kozlova, M. I.; Cadalbert, R.; Klose, D.; Mulkidjanian, A. Y.; Meier, B. H.; Böckmann, A. Atp analogues for structural investigations: case studies of a DnaB helicase and an ABC transporter. *Molecules* **2020**, *25*,

(11) Hager, A.; Wu, M.; Wang, H.; Brown Jr., N. W.; Shears, S. B.; Veiga, N.; Fiedler, D. Cellular cations control conformational switching of inositol pyrophosphate analogues. *Chem. Eur. J.* **2016**, *22*, 12406-12414.

(12) Park, J.; Pandya, V. R.; Ezekiel, S. J.; Berghuis, A. M. Phosphonate and bisphosphonate inhibitors of farnesyl pyrophosphate synthases: A structure-guided perspective. *Front. Chem.* **2021**, *8:612728*,

(13) Luo, X.; Li, M.; Zhan, K.; Yang, W.; Zhang, L.; Wang, K.; Yu, P.; Zhang, L. Selective inhibition of TRPM2 channel by two novel synthesized ADPR analogues. *Chem. Biol. Drug Des.* **2018**, *91*, 552-566.

(14) Elliott, T. S.; Slowey, A.; Ye, Y.; Conway, S. J. The use of phosphate bioisosteres in medicinal chemistry and chemical biology. *Med. Chem. Commun.* **2012**, *3*, 735-751.

(15) Dutta, A. K.; Captain, I.; Jessen, H. J. New synthetic methods for phosphate labeling. *Top. Curr. Chem.* **2017**, *375*, 51.

(16) Meltzer, D.; Nadel, Y.; Lecka, J.; Amir, A.; Sévigny, J.; Fischer, B. Nucleoside-(5′→P) methylenebisphosphonodithioate analogues: synthesis and chemical properties. *J. Org. Chem.* **2013**, *78*, 8320-8329.

(17) Engelsma, S. B.; Meeuwenoord, N. J.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V. Combined phosphoramidite-phosphodiester reagents for the synthesis of methylene bisphosphonates. *Angew. Chem. Int. Ed.* **2017**, *56*, 2955-2959.

(18) Kadina, A. P.; Kashemirov, B. A.; Oertell, K.; Batra, V. K.; Wilson, S. H.; Goodman, M. F.; McKenna, C. E. Two Scaffolds from Two Flips: (α,β)/(β,γ) CH2/NH "Met-Im" Analogues of dTTP. *Organic Letters* **2015**, *17*, 2586-2589.

(19) Singh, J.; Ripp, A.; Haas, T. M.; Qiu, D.; Keller, M.; Wender, P. A.; Siegel, J. S.; Baldridge, K. K.; Jessen, H. J. Synthesis of modified nucleoside oligophosphates simplified: fast, pure, and protecting group free. *J. Am. Chem. Soc.* **2019**, *141*, 15013-15017.

(20) Seebald, L. M.; Haratipour, P.; Jacobs, M. R.; Bernstein, H. M.; Kashemirov, B. A.; McKenna, C. E.; Imperiali, B. Uridine bisphosphonates differentiate phosphoglycosyl transferase superfamilies. *bioRxiv* **2023**, 09. 19. 558431.

(21) Eguaogie, O.; Cooke, L. A.; Martin, P. M. L.; Ravalico, F.; Conway, L. P.; Hodgson, D. R. W.; Law, C. J.; Vyle, J. S. Synthesis of novel pyrophosphorothiolate-linked dinucleoside cap analogues in a ball mill. *Org. Biomol. Chem.* **2016**, *14*, 1201-1205.

(22) Qi, N.; Jung, K.; Wang, M.; Na, L. X.; Yang, Z. J.; Zhang, L. R.; Guse, A. H.; Zhang, L. H. A novel membrane-permeant cADPR antagonist modified in the pyrophosphate bridge. *Chem. Commun.* **2011**, *47*, 9462-9464.

(23) Li, P.; Sergueeva, Z. A.; Dobrikov, M.; Shaw, B. R. Nucleoside and oligonucleoside boranophosphates: chemistry and properties. *Chem. Rev.* **2007**, *107*, 4746-4796.

(24) Baszczyński, O.; Watt, J. M.; Rozewitz, M. D.; Fliegert, R.; Guse, A. H.; Potter, B. V. L. Synthesis of phosphonoacetate analogues of the second messenger adenosine 5′-diphosphate ribose (ADPR). *RSC Adv.* **2020**, *10*, 1776-1785.

(25) Feldman, A. W.; Dien, V. T.; Romesberg, F. E. Chemical stabilization of unnatural nucleotide triphosphates for the *in vivo* expansion of the genetic alphabet. *J. Am. Chem. Soc.* **2017**, *139*, 2464-2467.

(26) Shevchuk, M.; Wang, Q.; Pajkert, R.; Xu, J.; Mei, H.; Röschenthaler, G.-V.; Han, J. Recent advances in synthesis of difluoromethylene phosphonates for biological applications. *Adv. Synth. Catal.* **2021**, *363*, 2912-2968.

(27) Shevchuk, M. V.; Röschenthaler, G.-V. Chemistry of difluoromethylene phosphonates: history, state of the art, and prospects. *Ukr. Bioorg. Acta* **2022**, *17*, 40-55.

(28) Surya Prakash, G. K.; Zibinsky, M.; Upton, T. G.; Kashemirov, B. A.; McKenna, C. E.; Oertell, K.; Goodman, M. F.; Batra, V. K.; Pedersen, L. C.; Beard, W. A.; Shock, D. D.; Wilson, S. H.; Olah, G. A. Synthesis and biological evaluation of fluorinated deoxynucleotide analogs based on bis-(difluoromethylene)triphosphoric acid. *Proc. Natl. Acad. Sci USA.* **2010**, *107*, 15693-15698.

(29) Hostachy, S.; Wang, H.; Zong, G.; Franke, K.; Riley, A. M.; Schmieder, P.; Potter, B. V. L.; Shears, S. B.; Fiedler, D. Fluorination influences the bioisostery of myo-inositol pyrophosphate analogues. *Chem. Eur. J.* **2023**, *29*, e202302426.

(30) Ivanova, M. V.; Bayle, A.; Besset, T.; Pannecoucke, X.; Poisson, T. New prospects toward the synthesis of difluoromethylated phosphate mimics. *Chem. Eur. J.* **2016**, *22*, 10284-10293.

(31) Blackburn, G. M.; England, D. A.; Kolkmann, F. Monofluoro- and difluoro-methylenebisphosphonic acids: isopolar analogues of pyrophosphoric acid. *J. Chem. Soc., Chem. Commun.* **1981**, 930-932.

(32) Crans, D. C.; Holder, A. A.; Saha, T. K.; Prakash, G. K. S.; Yousufuddin, M.; Kultyshev, R.; Ismail, R.; Goodman, M. F.; Borden, J.; Florián, J. Chelation of vanadium(V) by difluoromethylene bisphosphonate, a structural analogue of pyrophosphate. *Inorg. Chem.* **2007**, *46*, 6723-6732.

(33) Romanenko, V. D.; Kukhar, V. P. Fluorinated phosphonates: synthesis and biomedical application. *Chem. Rev.* **2006**, *106*, 3868-3935.

(34) O'Hagan, D. Understanding organofluorine chemistry. An introduction to the C–F bond. *Chem. Soc. Rev.* **2008**, *37*, 308-319.

(35) Saphier, S.; Katalan, S.; Yacov, G.; Berliner, A.; Redy-Keisar, O.; Fridkin, G.; Ghindes-Azaria, L.; Columbus, I.; Pevzner, A.; Drug, E.; Prihed, H.; Gershonov, E.; Eichen, Y.; Elias, S.; Parvari, G.; Zafrani, Y. Placing CF2 in the center: major physicochemical changes upon a minor structural alteration in gem-difunctional compounds. *Chem. Eur. J.* **2023**, *29*, e202202939.

(36) Romanenko, V. D.; Kukhar, V. P. Phosphonate analogues of nucleoside polyphosphates. *ARKIVOC* **2017**, *2018*, 1-49.

(37) Davisson, V. J.; Davis, D. R.; Dixit, V. M.; Poulter, C. D. Synthesis of nucleotide 5'-diphosphates from 5'-*O*-tosyl nucleosides. *J. Org. Chem.* **1987**, *52*, 1794-1801.

(38) Wang, G.; Boyle, N.; Chen, F.; Rajappan, V.; Fagan, P.; Brooks, J. L.; Hurd, T.; Leeds, J. M.; Rajwanshi, V. K.; Jin, Y.; Prhavc, M.; Bruice, T. W.; Cook, P. D. Synthesis of AZT 5‘-triphosphate mimics and their inhibitory effects on HIV-1 reverse transcriptase. *J. Med. Chem.* **2004**, *47*, 6902-6913.

(39) Lesiak, K.; Watanabe, K. A.; Majumdar, A.; Seidman, M.; Vanderveen, K.; Goldstein, B. M.; Pankiewicz, K. W. Synthesis of nonhydrolyzable analogues of thiazole-4-carboxamide and benzamide adenine dinucleotide containing fluorine atom at the C2' of adenine nucleoside: induction of K562 differentiation and inosine monophosphate dehydrogenase inhibitory activity. *J. Med. Chem.* **1997**, *40*, 2533-2538.

(40) Felczak, K.; Chen, L.; Wilson, D.; Williams, J.; Vince, R.; Petrelli, R.; Jayaram, H. N.; Kusumanchi, P.; Kumar, M.; Pankiewicz, K. W. Cofactor-type inhibitors of inosine monophosphate dehydrogenase via modular approach: Targeting the pyrophosphate binding sub-domain. *Bioorg. Med. Chem.* **2011**, *19*, 1594-1605.

(41) Boyle, N. A. Difluoromethylenediphosphonate: a convenient, scalable, and high-yielding synthesis. *Org. Lett.* **2006**, *8*, 187-189.

(42) Reddy, G. V.; Jacobs, H. K.; Gopalan, A. S.; Barrans, R. E.; Dietz, M. L.; Stepinski, D. C.; Herlinger, A. W. Synthesis of symmetrical methylenebis(alkyl hydrogen phosphonates) by selective cleavage of methylenebis(dialkyl phosphonates) with morpholine. *Synth. Commun.* **2004**, *34*, 331-344.

(43) Nicholson, D. A.; Cilley, W. A.; Quimby, O. T. Convenient method of esterification of polyphosphonic acids. *J. Org. Chem.* **1970**, *35*, 3149-3150.

(44) Gibson, B. A.; Kraus, W. L. New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 411-424.

(45) Madern, J. M.; Voorneveld, J.; Rack, J. G. M.; Kistemaker, H. A. V.; Ahel, I.; van der Marel, G. A.; Codée, J. D. C.; Filippov, D. V. 4-Thioribose analogues of adenosine diphosphate ribose (ADPr) peptides. *Org. Lett.* **2023**, *25*, 4980-4984.

(46) Oka, N.; Shimizu, M.; Saigo, K.; Wada, T. 1,3-Dimethyl-2-(3-nitro-1,2,4-triazol-1-yl)-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate (MNTP): a powerful condensing reagent for phosphate and phosphonate esters. *Tetrahedron* **2006**, *62*, 3667-3673.

(47) Kistemaker, H. A.; Lameijer, L. N.; Meeuwenoord, N. J.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V. Synthesis of well-defined adenosine diphosphate ribose oligomers. *Angew. Chem. Int. Ed.* **2015**, *54*, 4915-4918.

(48) Reese, C. B.; Titmas, R. C.; Valente, L. Action of toluene-*p*-thiol and triethylamine on fully protected thymidylyl-(3′→ 5′)-thymidine. Possible occurrence of thiolate ion-promoted internucleotide cleavage in the synthesis of oligonucleotides by the phosphotriester approach. *J. Chem. Soc., Perkin Trans. 1,* **1981**, 2451-2455.

(49) Campbell, D. A. The synthesis of phosphonate esters; an extension of the Mitsunobu reaction. *J. Org. Chem.* **1992**, *57*, 6331-6335.

(50) Harsági, N.; Keglevich, G. The hydrolysis of phosphinates and phosphonates: a review. *Molecules* **2021**, *26*, 2840.

(51) Litschko, C.; Oldrini, D.; Budde, I.; Berger, M.; Meens, J.; Gerardy-Schahn, R.; Berti, F.; Schubert, M.; Fiebig, T. A New Family of Capsule Polymerases Generates Teichoic Acid-Like Capsule Polymers in Gram-Negative Pathogens. *mBio* **2018**, *9*, 10.1128/mbio.00641-00618.

(52) Litschko, C.; Budde, I.; Berger, M.; Bethe, A.; Schulze, J.; Orozco, E. A. A.; Mahour, R.; Goettig, P.; Führing, J. I.; Rexer, T.; Gerardy-Schahn, R.; Schubert, M.; Fiebig, T. Mix-and-match system for the enzymatic synthesis of enantiopure glycerol-3-phosphate-containing capsule polymer backbones from *Actinobacillus pleuropneumoniae*, *Neisseria meningitidis*, and *Bibersteinia trehalosi*. *mBio* **2021**, *12*, e00897-00821.

(53) Blackburn, G. M.; Taylor, G. E. Syntheses of some fluorine-containing halomethanephosphonate and methylenebisphosphonate esters. *J. Organomet. Chem.* **1988**, *348*, 55-61.

(54) Beigelman, L. N.; Mikhailov, S. N. Transient protection in nucleoside synthesis using trityl groups: is it necessary to block hydroxyl groups? *Carbohydr. Res.* **1990**, *203*, 324-329.

(55) Huang, H.-S.; Kong, R.; Zheng, X.-A.; Chen, W.-J.; Han, S.-B.; Zeng, D.-Y.; Gong, S.-S.; Sun, Q. A practical method for regioselective 5′-*O*-tert-butyldimethylsilyl deprotection of persilylated nucleosides by methanolic phosphomolybdic acid. *Synlett* **2018**, *29*, 2437-2443.