Synthetic strategies for non-hydrolyzable pyrophosphate analogues

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Chapter 2

Design and Synthesis of Stable CDP-Glycerol and CDP-Ribitol Analogues as Potential Inhibitors of WTA Biosynthesis

Introduction

Teichoic acids (TAs) are major components of the cell wall in most Gram-positive bacteria, and they are involved in processes such as cell division, cell shape maintenance, peptidoglycan synthesis, bacteriophage interaction and virulence regulation.[1-2] They are anionic polymers composed of repeating alditol phosphate units linked through phosphodiester bonds. They can be covalently attached to the peptidoglycan layer (in wall teichoic acid, WTA), or embedded in the cell membrane through a glycolipid anchor (lipoteichoic acid, LTA).[3-4] WTA and LTA polymers exhibit remarkable structural diversity in their backbone composition, repeating unit length, and chemical modifications such as glycosylation and D-alanylation.[5-8] This variability gives rise to species-specific TA architectures that play distinct roles in bacterial physiology and host interactions.[5] Given their essential roles in bacterial pathogenesis, TAs have attracted considerable interest as potential targets for the development of novel therapeutics against antibiotic-resistant bacteria.[9-10]

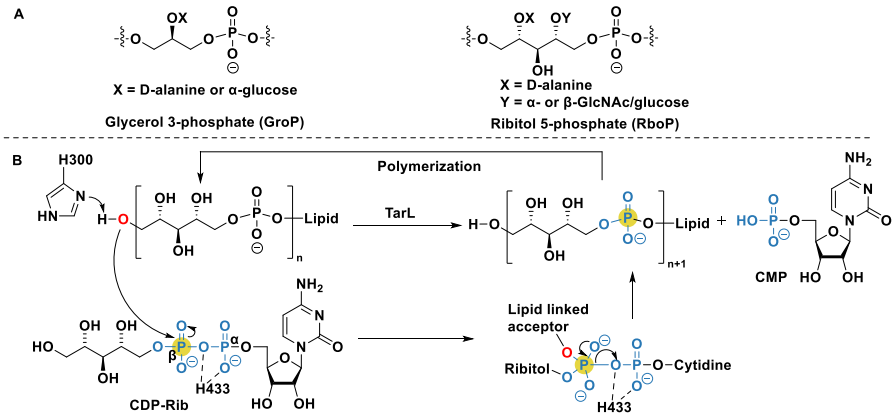


Figure 2.1. (A) WTA structures contain repeat units of glycerol 3-phosphate (GroP) or ribitol 5-phosphate (RboP). (B) Proposed mechanism for ribitol-phosphate polymerization catalyzed by TarL in *Staphylococcus aureus*.

The best-characterized WTA structures contain repeating units of ribitol 5-phosphate (RboP) or glycerol 3-phosphate (GroP) (Figure 2.1A).[11-12] The GroP-containing WTAs are synthesized by TagF-type polymerases, which catalyze the transfer of glycerol phosphate (GroP) units from CDP-glycerol to the growing WTA polymer chain.[13] The poly(GroP) WTA biosynthetic pathway has been primarily studied in *Bacillus subtilis* 168, while related enzymatic systems have been reported in other Gram-positive bacteria, including *Lactobacillus plantarum*, *Staphylococcus epidermidis*, and several *Streptomyces* species.[12]

The RboP-containing WTAs are assembled by TarL-type polymerases, which transfer ribitol phosphate (RboP) units from CDP-ribitol to extend the polymer chain.[14] The poly(RboP) WTA biosynthetic pathway has been elucidated in *B. subtilis* W23 and *Staphylococcus aureus*, both of which produce structurally similar poly(RboP) WTAs. Recent cryo-EM studies of *S. aureus* TarL

have revealed a catalytic mechanism for CDP-ribitol transfer (Figure 2.1B).[15] In this mechanism, the histidine residue H300, acting as an active-site base, deprotonates the terminal hydroxyl group of the growing RboP chain to generate the activated nucleophile. This enables the acceptor nucleophile to directly attack the *β*-phosphate of CDP-ribitol. Meanwhile, the imidazole side chain of H433 in contact with the α-phosphate group of CDP-ribitol to stabilize the resulting pentacoordinate transition state and facilitate CMP release by donating a proton thereby enabling chain extension. A similar catalytic strategy is proposed for TagF in CDP-glycerol transfer, leading to cleavage of the labile pyrophosphate (P–O–P) linkage and release of CMP.[13] Replacing the reactive P–O–P bond with a chemically stable mimic could block substitution and inhibit polymerization, providing a basis for the design of stable CDP-glycerol and CDP-ribitol analogues as potential inhibitors of wall teichoic acid biosynthesis. Considering the rise of antibiotic resistance, particularly in methicillin-resistant *Staphylococcus aureus* (MRSA),[16-17] such inhibitors can represent alternatives to conventional antibiotics. Moreover, as CDP-glycerol and CDP-ribitol are also key precursors in the biosynthesis of other bacterial polysaccharides, such as capsular polymers, the stable analogues can also be employed to investigate the catalytic mechanisms of biosynthetic enzymes involved in the assembly of these polysaccharides.[18-20]

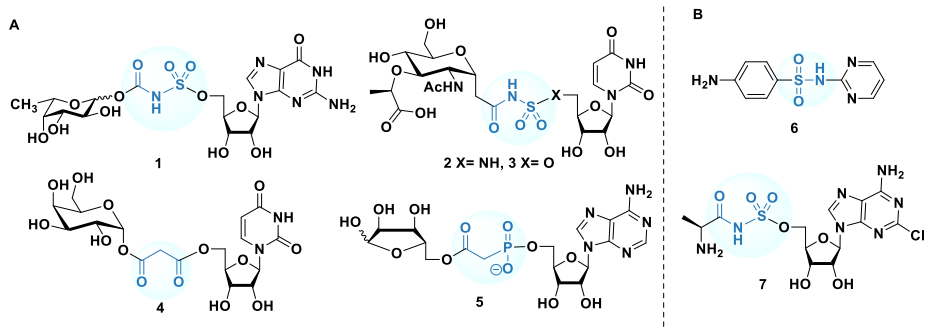


Figure 2.2. (A) Carbonyl and sulfamide functionalities as pyrophosphate analogues. (B) Antibacterial drugs containing sulfonamide functionalities.

In the design and synthesis of pyrophosphate analogues, the use of carbonyl and sulfamide functionalities as bioisosteric substitutes for the pyrophosphate group is a well-established strategy. Figure 2.2 shows a selection of examples, including sulfonylcarbamate **1**, designed to be an inhibitor for human fucosyltransferase (FucT-III),[21] sulfonylcarbamate inhibitors **2** and **3**, generated to inhibit MurC,[22] the malonate-based galactosyltransferase inhibitor **4**[23] and adenosine diphosphate ribose (ADPr) analogue **5**[24], among others.[25-28] Moreover, sulfamide-derived linkages are widely utilized in medicinal chemistry, particularly in the design of antibacterial agents, such as sulfadiazine **6**[29] and ascamycin **7**.[30]

Inspired by these precedents, this chapter describes the conception of CDP-glycerol and CDP-ribitol analogues, in which the chemically and enzymatically stable sulfonyl carbamate and phosphoryl carbamate linkers were introduced to replace the native pyrophosphate linkage, resulting in a series of CDP-Gro analogues **8-12** and CDP-Rbo analogues **13-17** (Figure 2.3). The

N-sulfonyl carbamate analogues were designed to broaden the set of target compound and because the O to NH replacement in the linker moieties imparts further stability. In addition, the NH-group enables different hydrogen-bonding interactions than the corresponding *O*-sulfonyl moieties.[31-32]

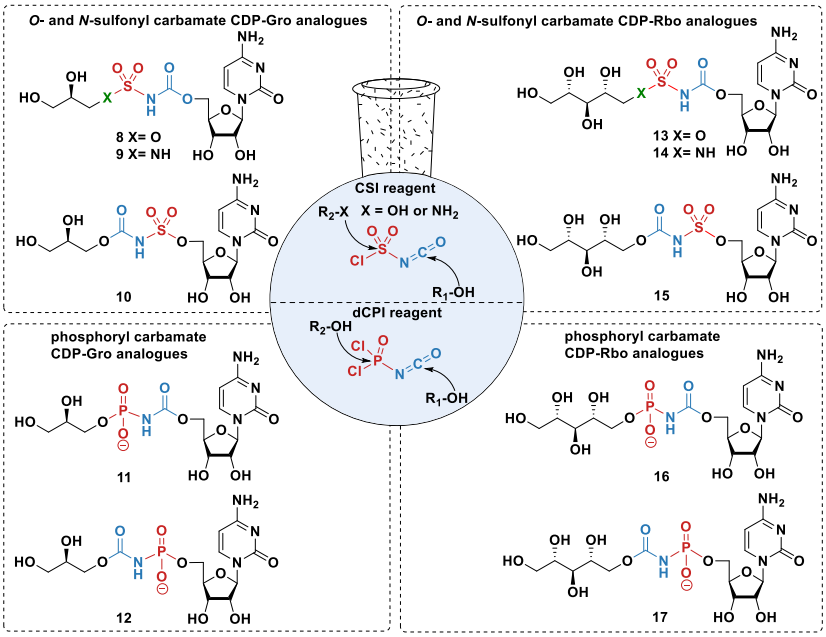


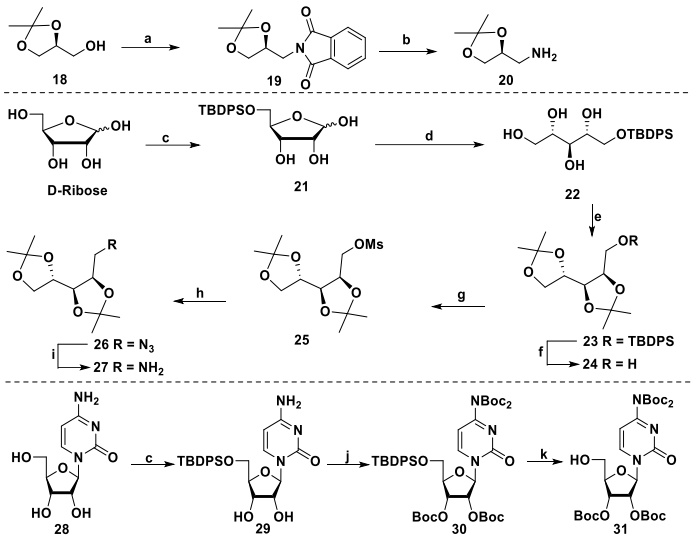
Figure 2.3. Designed various CDP-Gro and CDP-Rbo mimics and their synthetic strategies.

These CDP-Gro and CDP-Rbo analogues were generated using chlorosulfonyl isocyanate (CSI)[33] and dichlorophosphoryl isocyanate (dCPI)[34-35] reagents as versatile coupling agents (Figure 2.3). These reagents can be utilized in two synthetic directions—either by reacting first with the cytidine moiety and subsequently with the solketal or ribitol component, or *vice versa*—providing flexible routes to the desired CDP-Gro and CDP-Rib mimics. These analogues feature different pyrophosphate bioisosteres and may serve as WTA biosynthesis inhibitors.

Results and discussion

The syntheses of the CDP-Gro and CDP-Rib analogues were designed to employ building blocks that were protected with acid-labile groups, so that only a single acidic deprotection step would be required in the final stages. First, the required suitably protected 3-amino-1,2-propanediol, ribitol and 5-amino-1,2,3,4-pentaneteraol synthons were prepared (Scheme 2.1). Thus, following a reported procedure,[36] commercially available (*S*)-solketal **18** was treated with phthalimide under Mitsunobu condition to furnish the phthalimide derivative **19**. Subsequent hydrazinolysis of compound **19** gave the required primary amine **20** in 85% yield over two steps. Ribitol building blocks **24** and **27** were synthesized from D-ribose. Monosilylation of D-ribose afforded compound

21, which was reduced to give compound **22** in 60% yield over two steps. The four hydroxyls in **22** were protected with 2,2-dimethoxypropane to give compound **23** in 43% yield,[37] and desilylation using TBAF then afforded ribitol **24**. The alcohol was substituted for an azide, utilizing mesylate intermediate **25** to yield azide **26.** Hydrogenation of compound **26** with H_2, Pd/C in MeOH afforded the amine **27** in quantitative yield. With building block **18**, **20**, **24** and **27** in hand, attention then turned to the preparation of the cytidine derivative **31**, carrying *tert*-butylcarbonyl (Boc) protecting groups on the ribosyl hydroxyls as well as cytidine amine. Monosilylation of cytidine **28** was performed using a slight excess of TBDPSCl in the presence of 4-dimethylaminopyridine (DMAP) in pyridine to yield compound **29**. Subsequent treatment of **29** with di-*tert*-butyldicarbonate in the presence of DMAP and triethylamine gave the fully protected derivative **30**. Finally, removal of the TBDPS group afforded the desired building block **31** in 58% yield over three steps.

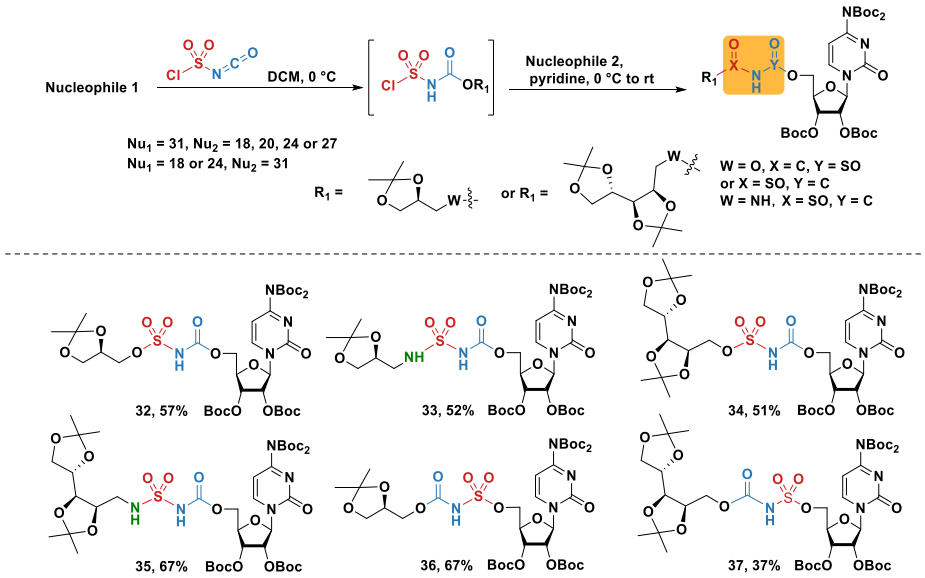


Scheme 2.1. Synthesis of key building blocks. Reagents and conditions. a) phthalimide, PPh_3, DIAD, THF, 88%. b) $NH_2NH_2{\cdot}H_2O$, MeOH, reflux, 85%. c) TBDPSCl, pyridine, DMAP, 0 °C to rt. d) $NaBH_4$, EtOH, 60% over 2 steps. e) 2,2-DMP, PTSA, acetone, 0 °C, 43%. f) TBAF, THF, 74%. g) MsCl, Et_3N, THF, 0 °C to rt, 2 h, 90%. h) NaN_3, DMF, 60 °C, 24 h, 90%. i) H_2, Pd/C, MeOH, rt, 48 h, quantitative. j) Et_3N, DMAP, Boc_2O, CH_2Cl_2. k) THF, TBAF, 58% over 3 steps.

With all building blocks available, the projected strategy for the construction of the *O*- and *N*-sulfonyl carbamate analogues had to be put into practice (Scheme 2.2). Following the reported procedure,[21, 38] the synthesis was performed in a two-step, one-pot sequence starting from commercially available chlorosulfonyl isocyanate (CSI). The condensation of Boc-protected cytidine **31** with CSI at 0 °C in dry dichloromethane afforded the corresponding sulfonyl chloride intermediate. Next, this intermediate was immediately reacted with compound **18**, **20**, **24** or **27** in the presence of pyridine to give protected CDP-Gro analogues **32** and **33** in 57% and 52%

yield, respectively, and protected CDP-Rbo analogues **34** and **35** in 51% and 67% yield, respectively.

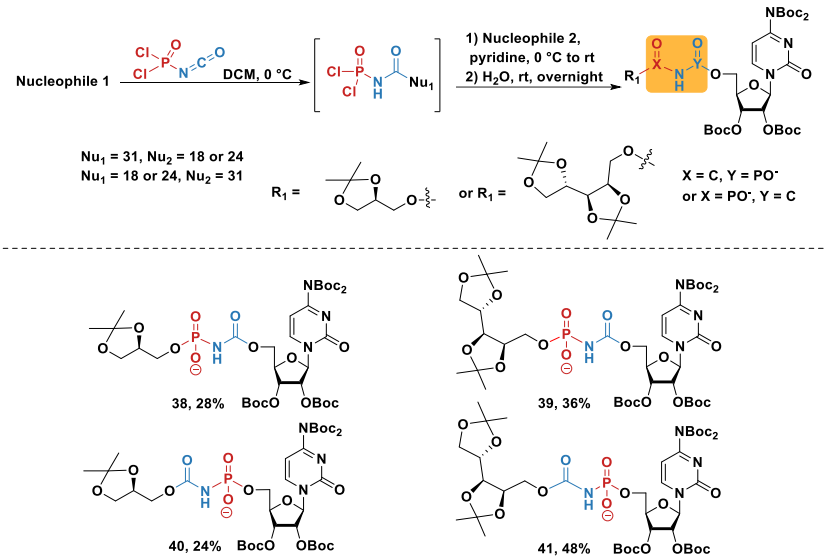
To access protected CDP-Gro and CDP-Rbo analogues **36** and **37**, the order of the addition of the nucleophiles was reversed and the CSI reagent was first treated with compound **18** or **24** to generate the corresponding sulfonyl chloride intermediates, which were then coupled with the cytidine derivative **31** to afford protected CDP-Gro analogue **36** and protected CDP-Rbo analogue **37** in 67% and 37% yields, respectively.



Scheme 2.2. Synthesis of *O*- and *N*-sulfonyl carbamate linked CDP-Gro and CDP-Rbo analogues.

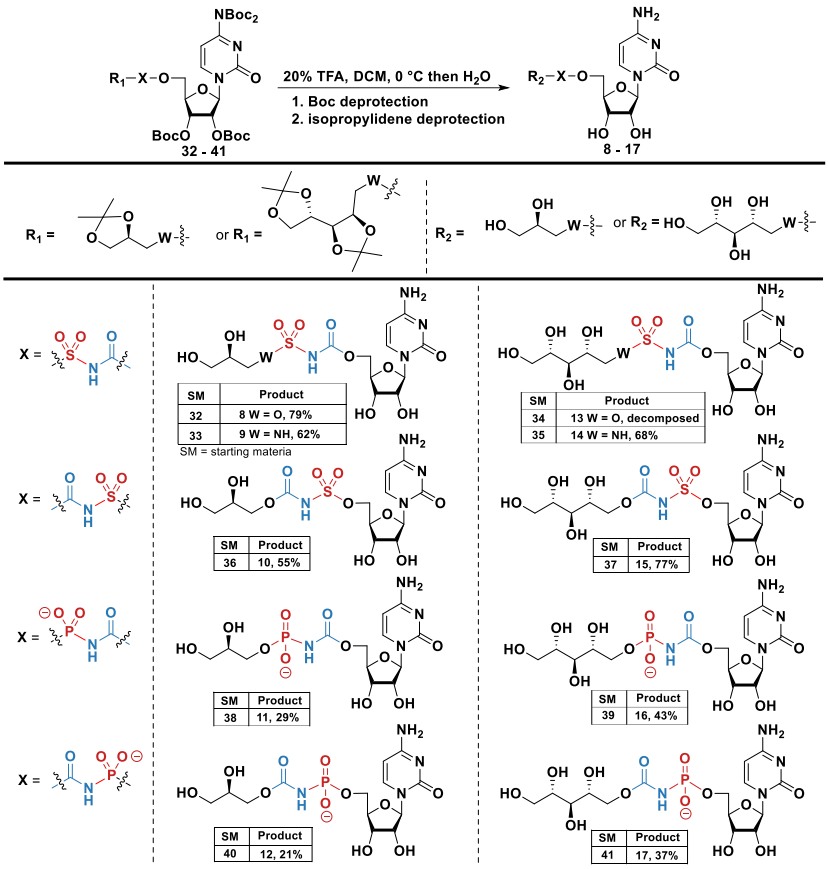
In a similar manner, the synthesis of the phosphoryl carbamate analogues was carried out using the dCPI reagent (Scheme 2.3), following the procedure reported by Steneker et. al.[35] Treatment of cytidine derivative **31** with dCPI afforded the corresponding cytidine phosphorodichloridate intermediate, which was subsequently reacted with solketal **18** or ribitol **24** in the presence of pyridine to give the coupled intermediates. After completion of the substitution reaction, the remaining P–Cl moeity was hydrolyzed to provide the crude CDP-Gro **38** and CDP-Rbo analogue **39**, respectively. Purification of these products proved challenging, because of charged nature of these compounds. This led to tailing during silica gel chromatography and broad elution profiles. In addition, the presence of partially coupled intermediates resulted in the co-elution with the desired products, further complicating the isolation of pure compounds. Despite repeated chromatographic and size-exclusion purification procedures, the protected CDP-Gro and CDP-Rbo analogues **38** and **39** were obtained only in moderate yield and with limited purity, as evidenced by ^{31}P NMR.

In a reversed sequence of reactions, solketal **18** or ribitol **24** were first treated with dCPI reagent to generate the corresponding phosphorodichloridate intermediate, which were then condensed with the cytidine derivative **31** to furnish, after hydrolysis of the intermediate phosphorchloridate, the corresponding oxyphosphonyl carbamates **40** and **41** in 24% and 48% yield, respectively, with similar difficulties encountered during purification.



Scheme 2.3. Synthesis of phosphoryl carbamate linked CDP-Gro and CDP-Rbo analogues.

With all the protected targets in hand, the synthesis of target compounds **8-17** was then undertaken (Scheme 2.4). The Boc and isopropylidene groups were all removed under acidic conditions using 20% trifluoroacetic acid (TFA) in dichloromethane at 0 °C. The reaction progress was monitored by HPLC, and upon completion, the solvent was removed under reduced pressure. For the sulfonyl carbamate analogues, the products could be purified by size-exclusion chromatography affording CDP-Gro analogues **8** (79%), **9** (62%) and **10** (55%) as well as CDP-Rbo analogues **14** (68%) and **15** (77%). Unfortunately, under these conditions, the ribitol analogue **13** was found to be unstable and underwent rapid cleavage as indicated by LC-MS analysis, even when milder acidic conditions were applied. The byproducts resulting from ribitol cleavage continued to undergo hydrolysis as the reaction progressed, and ultimately only fully deprotected cytidine could be isolated. In the case of the phosphoryl carbamate analogues, purification proved challenging, and pure products could not be obtained by size-exclusion chromatography, necessitating an additional HPLC purification step. Gratifyingly, this successfully afforded the desired pure CDP-Gro analogues **11** (29%) and **12** (21%) and CDP-Rbo analogues **16** (43%) and **17** (37%). The low yield can be attributed to the losses during size-exclusion and HPLC purification.



Scheme 2.4. Deprotection of CDP-Gro and CDP-Rbo analogues.

Conclusion

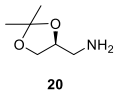
This chapter describes the straightforward synthesis of a series of CDP-Gro and CDP-Rbo mimics (**8**-**17**) as potential inhibitors of wall teichoic acid (WTA) polymerases. To streamline the synthetic route, acid-labile protecting groups (Boc and isopropylidene ketals), were employed, enabling a single-step deprotection. The sulfonyl carbamate and phosphoryl carbamate groups were introduced as pyrophosphate bioisosteres, providing enhanced chemical stability and resistance to enzymatic hydrolysis. The use of chlorosulfonyl isocyanate (CSI) and dichlorophosphoryl isocyanate (dCPI) reagents allowed efficient and versatile coupling reactions, generating a diverse set of analogues while preserving the structural features of the parent CDP-Gro and CDP-Rbo molecules. These analogues are attractive chemical tools for probing the catalytic mechanisms of WTA polymerases and can serve as lead compounds for the development of novel antibacterial compounds targeting cell wall biosynthesis.

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, and ^{31}P) were recorded with a Bruker AV-400, Bruker AV-400liq or Bruker AV-500. 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: 50 mM $HNEt_3OAc$ in Milli-Q® H_2O; solvent B: acetonitrile; linear gradient of B in A (0 → 20% A/B), 5 mL/min.

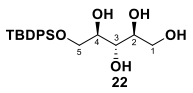
3-Amino-1,2-propanediol 20



20

(*S*)-Solketal (3.0 g, 22.8 mmol) was dissolved in anhydrous THF (230 mL), triphenylphosphine (7.2 g, 27.3 mmol) and phthalimide (3.4 g, 22.8 mmol) were added. The mixture was cooled to 0 °C and diisopropyl azodicarboxylate (5.4 mL, 27.3 mmol) was added dropwise. The resulting mixture was stirred for 20 h at room temperature under argon atmosphere. The reaction mixture was concentrated and the residue was purified by column chromatography on silica gel (R_f = 0.3, pentane/EtOAc 7:3, v/v) to give compound **19** as a white solid (5.2 g, 88%). To the solution of intermediate **19** (3.0 g, 11.5 mmol) in methanol (115 mL) was added hydrazine monohydrate (1.0 mL, 20.1 mmol). The reaction mixture was heated under reflux for 5 h. The resulting white precipitate was dissolved by adding a solution of KOH (0.9 g, 16.1 mmol) in 20 mL of methanol. After concentrating the solution, CH_2Cl_2 was added, and the mixture was filtered. The organic layer was washed with H_2O, dried over $MgSO_4$, and the solvent was evaporated to yield compound **20** (1.3 g, 9.8 mmol, 85%) as a pale-yellow liquid. Spectral data were in accordance with literature precedent.[36] ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 – 4.08 (m, 1H, H-2), 4.03 (dd, *J* = 8.1, 6.4 Hz, 1H, H-1), 3.66 (dd, *J* = 8.1, 6.4 Hz, 1H, H-1), 2.84 (dd, *J* = 13.2, 4.3 Hz, 1H, H-3), 2.77 (dd, *J* = 13.2, 6.3 Hz, 1H, H-3), 1.69 (bs, 2H, NH), 1.42 (d, *J* = 0.8 Hz, 3H, Me), 1.35 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 109.2 ($C(Me)_2$), 77.3 (C-2), 67.0 (C-1), 44.7 (C-3), 26.9 (Me), 25.4 (Me).

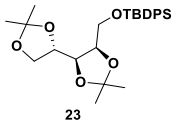
Ribitol 22



22

D-Ribose (3 g, 20 mmol) was dissolved in pyridine (60 mL), and DMAP (250 mg, 2 mmol) was added. The mixture was cooled to 0 °C. Tert-butyldiphenylsilyl chloride (TBDPSCl, 5.7 mL, 22 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was quenched with H_2O and then concentrated under reduced pressure. The resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give crude product as a yellow oil. Crude product was dissolved in anhydrous ethanol and cooled to 0 °C, $NaBH_4$ (1.1 g, 30 mmol) was added, and the reaction mixture was stirred at room temperature for 30 min. After the reaction was completed, the reaction mixture was quenched with acetic acid and concentrated *in vacuo*. The residue was diluted in EtOAc and washed with sat. aq $NaHCO_3$, H_2O, 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 **22** (4.7 g, 12 mmol, 60% over 2 steps) as a colorless oil. ^{1}H NMR (400 MHz, MeOD) δ 7.78 – 7.72 (m, 4H, CH_{arom}), 7.46 – 7.39 (m, 6H, CH_{arom}), 3.93 (dd, *J* = 9.9, 3.3 Hz, 1H, H-5), 3.90 – 3.85 (m, 1H, H-4), 3.83 – 3.76 (m, 3H, H-1, H-3, H-1), 3.71 (t, *J* = 6.1 Hz, 1H, H-2), 3.65 (dd, *J* = 12.2, 7.0 Hz, 1H, H-1), 1.08 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (101 MHz, MeOD) δ 135.4 (CH_{arom}), 133.3 ($C_{q\text{-}arom}$), 129.4, 127.4 (CH_{arom}), 73.2 (C-4), 72.9 (C-3), 72.5 (C-2), 65.6 (C-5), 63.0 (C-1), 26.0, 18.7 ($C(CH_3)_3$). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{21}H_{30}O_5SiNa$ 413.1758; Found 413.1755.

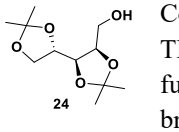
Ribitol 23



23

Compound **22** (6.7 g, 17 mmol) was co-evaporated thrice with dry toluene and dissolved in dry acetone (40 mL), 2,2-dimethoxypropane (40 mL) and *p*-TsOH (1.5 g, 8.5 mmol) were added at 0 °C. The reaction mixture was stirred for 3 h at 0 °C. After full conversion, the reaction was quenched with Et_3N and concentrated *in vacuo*. The residue was purified by column chromatography (R_f = 0.3, pentane/EtOAc 4:1, v/v) to give **23** (3.4 g, 7.3 mmol, 43%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.73 (m, 4H, CH_{arom}), 7.44 – 7.36 (m, 4H, CH_{arom}), 4.36 – 4.30 (m, 1H, H-4), 4.30 – 4.24 (m, 1H, H-3), 4.11 – 4.04 (m, 2H, H-2, H-5), 4.01 – 3.80 (m, 3H, H-1, H-1), 1.45 (s, 3H, Me), 1.38 (s, 6H, Me), 1.30 (s, 3H, Me), 1.09 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.8, 135.8 (CH_{arom}), 133.7, 133.6 ($C_{q\text{-}arom}$), 129.7, 127.7, 127.7 (CH_{arom}), 109.5, 108.7 ($C(Me)_2$), 78.3 (C-4), 78.1 (C-2), 73.6 (C-3), 67.9 (C-5), 62.9 (C-1), 27.7 (Me), 26.9 ($C(CH_3)_3$), 25.5, 25.4 (Me). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{27}H_{38}O_5SiNa$ 493.2381; Found 493.2386.

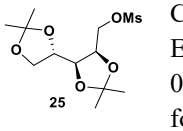
Ribitol 24



24

Compound **23** (1.5 g, 3.2 mmol) was dissolved in THF (10 ml), TBAF (1.0 M in THF; 4.8 ml, 4.8 mmol) was added and the mixture was stirred overnight. Upon full conversion was observed, the reaction was diluted with sat. aq. $NaHCO_3$ and brine, extracted with EtOAc, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (R_f = 0.3, pentane/EtOAc 4:1, v/v) to provide compound **24** (549 mg, 2.3 mmol, 74%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.39 – 4.32 (m, 1H, H-4), 4.20 – 4.12 (m, 2H, H-2, H-1), 4.05 (dd, *J* = 9.2, 5.9 Hz, 1H, H-3), 3.99 – 3.92 (m, 1H, H-1), 3.87 – 3.72 (m, 2H, H-5), 2.80 (dd, *J* = 8.8, 5.4 Hz, 1H, 5-OH), 1.41 (s, 3H, Me), 1.40 (s, 3H, Me), 1.36 (s, 3H, Me), 1.34 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 110.2, 108.9 ($C(Me)_2$), 78.2 (C-3), 77.4 (C-4), 73.4 (C-2), 68.2 (C-1), 60.7 (C-5), 27.9, 26.8, 25.5, 25.2 (Me). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{11}H_{20}O_5Na$ 255.1203; Found 255.1208.

Mesylate 25

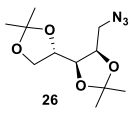


25

Compound **24** (94 mg, 0.40 mmol) was dissolved in THF (1 mL, 0.4 M), and Et_3N (64 µL, 0.48 mmol), Me-imidazole (38 µL, 0.48 mmol) and MsCl (38 µL, 0.48 mmol) were added to the solution at 0 °C. The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with EtOAc and H_2O. The aqueous layer was extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (R_f = 0.2, pentane/EtOAc 3:1, v/v) yielded compound **25** (112 mg, 0.36 mmol, 90%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.39 (dd, *J* = 11.4, 2.0 Hz, 1H, H-5), 4.28 (dd, *J* = 11.5, 5.2 Hz, 1H, H-5), 4.05 – 3.99 (m, 1H, H-4), 3.86 – 3.79 (m, 2H, H-2, H-1), 3.78 – 3.71 (m, 1H, H-1), 3.62 – 3.56 (m, 1H, H-3), 3.06 (s, 3H, Me Ms), 1.54 (s, 3H, Me), 1.50 (s, 3H, Me), 1.41 (s, 3H,

Me), 1.39 (s, 3H, Me). ^{13}C NMR (126 MHz, $CDCl_3$) δ 100.6, 100.4 ($C(Me)_2$), 70.6 (C-4), 68.6 (C-5), 67.5 (C-3), 66.8 (C-2), 62.9 (C-5), 37.7 (Me Ms), 29.2, 29.1, 19.8, 19.3 ($C(Me)_2$). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{12}H_{22}O_7SNa$ 333.3503; Found 333.3508.

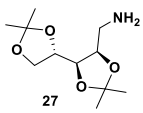
Azide 26



26

Mesylate **25** (112 mg, 0.36 mmol) was dissolved in anhydrous DMF (1 mL) and NaN_3 (117 mg, 1.8 mmol) was added, and the reaction was stirred for 24 h at 100 °C. The reaction mixture was cooled to rt and diluted with EtOAc and H_2O. The aqueous layer was extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (R_f = 0.2, Pentane/EtOAc 7:3, v/v) to obtain **26** (67 mg, 0.11 mmol, 71%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 – 3.94 (m, 1H, H-4), 3.88 – 3.73 (m, 3H, H-1, H-2), 3.66 – 3.58 (m, 1H, H-3), 3.43 (dd, *J* = 13.2, 2.5 Hz, 1H, H-5), 3.29 (dd, *J* = 13.2, 5.5 Hz, 1H, H-5), 1.56 (s, 3H, Me), 1.53 (s, 3H, Me), 1.44 (s, 3H, Me), 1.40 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 100.6, 100.3 ($C(Me)_2$), 72.0 (C-4), 68.9 (C-3), 66.9 (C-2), 63.0 (C-1), 51.0 (C-5), 29.3, 29.3, 20.0, 19.4 (Me). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{11}H_{19}N_3O_4Na$ 280.1268; Found 280.1269.

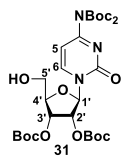
5-Amino-1,2,3,4-pentaneteraol 27



27

A catalytic amount of Pd/C (10 mol %) was added to a stirred solution of compound **26** (38 mg, 0.15 mmol) in MeOH (1 mL). The flask was purged with H_2 and the reaction mixture was stirred under H_2 atmosphere for 24 h at room temperature. The reaction mixture was filtered through a Celite bed. The filtrate was concentrated *in vacuo* to afford **27** (35 mg, 0.15 mmol, quantitative) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 – 3.67 (m, 4H, H-1, H-4, H-3), 3.57 – 3.41 (m, 1H, H-2), 2.93 (dd, *J* = 13.5, 3.2 Hz, 1H, H-5), 2.69 (dd, *J* = 13.4, 6.5 Hz, 1H, H-5), 1.53 (s, 3H, Me), 1.50 – 1.46 (m, 3H, Me), 1.39 (s, 3H, Me), 1.38 (s, 3H, Me). ^{13}C NMR (75 MHz, $CDCl_3$) δ 100.2, 100.1 ($C(Me)_2$), 73.9 (C-3), 69.9 (C-2), 66.9 (C-4), 63.2 (C-1), 43.3 (C-5), 29.5, 29.4, 20.1, 19.5 (Me). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{11}H_{21}NO_4SNa$ 232.1543; Found 232.1543.

Protected cytidine 31



31

Cytidine (4.8 g, 20 mmol) was dissolved in pyridine (25 mL), and DMAP (250 mg, 2.0 mmol) was added. The reaction mixture was cooled to 0 °C. TBDPSCl (6.3 mL, 24 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight under N_2 atmosphere. After full conversion, as indicated by TLC, the reaction was quenched with MeOH, diluted with sat. aq. $NaHCO_3$ and brine and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude 5'-*O*-TBDPS intermediate **29** was co-evaporated with toluene thrice and dissolved in DCM (200 mL). Et_3N (17 mL, 120 mmol) and DMAP (0.74 g, 6 mmol) were added, and the mixture was cooled on ice. Di-tert-butyl dicarbonate (26.5 g, 120 mmol) was added and the reaction mixture was stirred at room

temperature overnight. After full conversion, the reaction mixture was concentrated to afford crude product **30**. The crude **30** was dissolved in THF (80 ml), TBAF (1.0 M in THF; 75 ml, 75 mmol) was added and the mixture was stirred overnight. Upon full conversion, the reaction mixture was diluted with sat. aq. $NaHCO_3$ and brine, extracted with EtOAc, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (R_f= 0.3, pentane/EtOAc 2:1 v/v) to yield **31** (7.5 g, 11.6 mmol, 58% over 3 steps) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 7.6 Hz, 1H, H-6), 7.13 (d, *J* = 7.6 Hz, 1H, H-5), 5.72 – 5.66 (m, 2H, H-1', H-2'), 5.44 – 5.37 (m, 1H, H-3'), 4.35 – 4.29 (m, 1H, H-4'), 4.02 – 3.95 (m, 1H, H-5'), 3.84 – 3.76 (m, 1H, H-5'), 3.59 (dd, *J* = 8.3, 3.6 Hz, 1H, 5'-OH), 1.56 (s, 18H, $C(CH_3)_3$), 1.49 (s, 9H, $C(CH_3)_3$), 1.47 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.8 (C=O, cytosine), 154.0 ($C_{q\text{-}arom}$, cytosine), 152.3, 151.7 (C=O carbonate), 149.4 (C=O carbamate), 146.4 (C-6), 96.6 (C-5), 93.4 (C-1'), 85.2 ($C(CH_3)_3$), 83.6 (C-4'), 83.5, 83.3 ($C(CH_3)_3$), 74.5 (C-2'), 72.6 (C-3'), 61.6 (C-5'), 27.7, 27.7, 27.6 ($C(CH_3)_3$). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{29}H_{45}N_3O_{13}Na$ 666.2845; Found 666.2849.

General procedure A for the synthesis of sulfonyl carbamate linked analogues.

Nucleophile-**1** (1.0 eq) was dried by co-evaporation with anhydrous toluene three times (for low-boiling substrates, by treatment with fresh flame-dried 3 Å molecular sieves) prior to use. The dried nucleophile-**1** (1.0 eq) was then dissolved in dry DCM (0.1 M) under a nitrogen atmosphere. Fresh flame-dried molecular sieves (3 Å) were added, and the reaction mixture was cooled to 0 °C. Chlorosulfonyl isocyanate (1.0 eq) was added dropwise. The resulting mixture was stirred at 0 °C for 2-4 h until TLC analysis showed complete conversion of Nucleophile-**1**. Anhydrous pyridine (3 eq) was added, followed by the dropwise addition of Nucleophile-**2** (1.0 eq) after prior co-evaporation with toluene three times (for low-boiling substrates, by treatment with fresh flame-dried 3 Å molecular sieves). The reaction mixture was allowed to warm to room temperature and was stirred overnight. The mixture was concentrated and the residue was purified by flash column chromatography over silica gel and then by size-exclusion chromatography (LH-20) (DCM/MeOH 1:1, v/v) to obtain the product.

General procedure B global acidic deprotection of sulfonyl carbamate linked analogues.

The protected analogue was co-evaporated with dry toluene and dissolved in dry DCM. Trifluoroacetic acid (20%, v/v, final concentration 0.08 M) was added at 0 °C. The reaction mixture was stirred for 24 h, and 0.2 ml H_2O was added, and the reaction mixture was stirred for additional 5 h. The progress of the reaction was monitored by LC-MS until complete deprotection. The reaction mixture was then concentrated under reduced pressure. The residue was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in H_2O) to afford the deprotected analogue.

General procedure C: synthesis of protected phosphoryl carbamate linked analogues and global acidic deprotection of phosphoryl carbamate linked analogues.

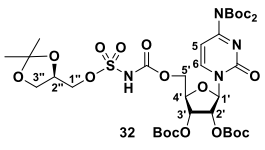
Step 1. Synthesis of protected phosphoryl carbamate linked analogues:

Nucleophile-**1** (1.0 eq) was dried by co-evaporation with anhydrous toluene three times (for low-boiling substrates, by treatment with activated 3 Å molecular sieves) prior to use. The dried nucleophile-**1** (1.0 eq) was then dissolved in dry DCM (0.1 M) under a nitrogen atmosphere. Fresh flame-dried molecular sieves (3 Å) were added, and the reaction mixture was cooled to 0 °C. Dichlorophosphoryl isocyanate (1.0 eq) was added dropwise. The resulting mixture was stirred at 0 °C for 2-4 h until TLC analysis showed complete conversion of the Nucleophile-**1**. Anhydrous pyridine (6.0 eq) was added, followed by the addition of the nucleophile-**2** (1.0 eq) after prior co-evaporation with toluene three times (for low-boiling substrates, by treatment with fresh flame-dried 3 Å molecular sieves). The reaction mixture was allowed to rise to room temperature and stirred overnight. After LC–MS analysis indicated formation of the desired chlorophosphoryl carbamate intermediate, H_2O (0.5 mL) was added, and the reaction mixture was stirred vigorously for 5 h to hydrolyze the P(O)–Cl bond. The mixture was filtered and concentrated under reduced pressure. The residue was purified by repeated flash column chromatography over silica gel and then by size-exclusion chromatography (LH-20) (DCM/MeOH 1:1, v/v) to obtain product. Due to the amphiphilic character of the phosphoryl carbamate intermediates, satisfactory NMR characterization was not feasible. Therefore, the phosphoryl carbamate intermediates were carried forward to the global deprotection without full characterization.

Step 2 – Global deprotection:

The protected analogue was co-evaporated with dry toluene and dissolved in dry DCM. Trifluoroacetic acid (20%, v/v, final concentration 0.05 M) was added at 0 °C. The reaction mixture was stirred for 24 h, H_2O was added and the stirring was continued for additional 5 h. The progress of the reaction was monitored by LC-MS until complete deprotection. The reaction mixture was then concentrated under reduced pressure. The residue was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in H_2O) and subsequent semiprep-HPLC purification afforded deprotected analogue.

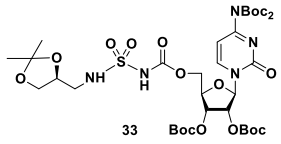
Protected *O*-sulfonyl carbamate linked CDP-Gro analogue 32



Following the general procedure A, the title compound was prepared from compound **31** (644 mg, 1.0 mmol) and chlorosulfonyl isocyanate (86 µL, 1.0 mmol, 1.0 eq) in dry DCM (5 mL). The reaction mixture was stirred at 0 °C for 3 h; and pyridine (242 µL, 3.0 mmol, 3.0 eq) and (*S*)-solketal (124 µL, 1.0 mmol, dried by molecular sieves (MS) 3 Å before using) were added at 0 °C. The resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo*, and the residue was purified by flash column chromatography over silica gel (R_f = 0.2, pentane/EtOAc 1:5, v/v) and then by size-

exclusion chromatography (LH-20) (DCM/MeOH 1:1, v/v) to afford **32** (502 mg, 0.57 mmol, 57%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 – 7.78 (m, 1H, H-6), 7.11 (d, *J* = 7.6 Hz, 1H, H-5), 5.83 (bs, 1H, NH), 5.47 – 5.32 (m, 2H, H-1', H-2'), 4.49 (d, *J* = 12.3 Hz, 1H, H-5'), 4.40 – 4.25 (m, 3H, H-3', H-4', H-5', H-2''), 4.23 – 4.09 (m, 2H, H-1''), 4.04 (dd, *J* = 8.8, 6.5 Hz, 1H, H-3''), 3.80 (dd, *J* = 8.8, 5.4 Hz, 1H, H-3''), 1.54 (s, 18H, $C(CH_3)_3$), 1.46 (s, 9H, $C(CH_3)_3$), 1.44 (s, 9H, $C(CH_3)_3$), 1.39 (s, 3H, Me), 1.31 (s, 3H, Me). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.0 (C=O, cytosine), 154.6 ($C_{q\text{-arom}}$, cytosine), 152.3, 152.2 (C=O carbonate), 149.5 (C=O carbamate), 145.5 (C-6), 110.1 ($C(Me)_2$), 97.5 (C-5), 85.6, 83.6, 83.5 ($C(CH_3)_3$), 79.7 (C-4'), 75.5 (C-1'), 73.4 (C-2''), 71.8 (C-2'), 70.4 (C-1'') 66.1 (C-3''), 64.0 (C-5'), 27.8, 27.8 ($C(CH_3)_3$), 26.8, 25.4 (Me). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{36}H_{56}N_4O_{19}S$ 881.3332; Found 881.3327.

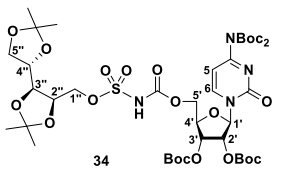
Protected *N*-sulfonyl carbamate linked CDP-Gro analogue 33



33

Following the general procedure A, the title compound was prepared from compound **31** (644 mg, 1 mmol) and chlorosulfonyl isocyanate (86 µL, 1 mmol, 1.0 eq) in dry DCM (5 mL). The reaction mixture was stirred at 0 °C for 3 h; and pyridine (242 µL, 6 mmol, 3.0 eq) and compound **20** (132 mg, 1 mmol, 1.0 eq) were added. The resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the residue was purified by flash column chromatography over silica gel and then by size exclusion (LH-20) (DCM/MeOH 1:1, v/v) to afford **33** (458 mg, 520 µmol, 52%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.6 Hz, 1H, H-6), 7.16 (d, *J* = 7.6 Hz, 1H, H-5), 6.29 (bs, 1H, NH), 5.69 (d, *J* = 3.5 Hz, 1H, H-1'), 5.60 – 5.48 (m, 2H, H-2', H-3'), 4.61 – 4.53 (m, 1H, H-5'), 4.39 – 4.31 (m, 2H, H-5', H-4'), 4.30 – 4.23 (m, 1H, H-2''), 4.04 (dd, *J* = 8.5, 6.3 Hz, 1H, H-3''), 3.70 (dd, *J* = 8.5, 5.9 Hz, 1H, H-3''), 3.32 – 3.23 (m, 1H, H-1''), 3.21 – 3.12 (m, 1H, H-1''), 1.55 (s, 18H, $C(CH_3)_3$), 1.47 (s, 18H, $C(CH_3)_3$), 1.39 (s, 3H, Me), 1.30 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.1, 154.0, 152.5, 152.1, 151.3, 149.3, 146.2 (C-6), 109.9 ($C(Me)_2$), 96.9 (C-5), 93.3 (C-1') , 85.5, 83.9, 83.6 ($C(CH_3)_3$), 79.0 (C-4'), 77.4 (C-2'), 77.1 (C-2''), 76.8, 75.4 (C-2'), 74.1 (C-2''), 71.2 (C-3'), 66.9 (C-3''), 63.9 (C-5'), 46.3 (C-1''), 27.7, 27.7 ($C(CH_3)_3$), 26.9 (Me), 25.4 (Me). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{36}H_{56}N_4O_{19}S$ 880.3492; Found 880.3486.

Protected *O*-sulfonyl carbamate linked CDP-Rbo analogue 34

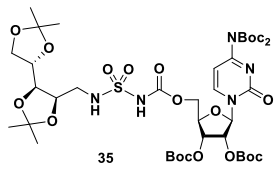


34

Following the general procedure A, the title compound was prepared from compound **31** (746 mg, 1.16 mmol) and chlorosulfonyl isocyanate (100 µL, 1.16 mmol, 1.0 eq) in dry DCM (5.8 mL). The reaction mixture was stirred at 0 °C for 3 h; and pyridine (281 µL, 3.48 mmol, 3.0 eq) and compound **24** (270 mg, 1 mmol, 1.0 eq) were added. The resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the residue was purified by flash column chromatography over silica gel and then by size-exclusion chromatography (LH-20) (DCM/MeOH 1:1, v/v) afforded **34** (580 mg, 592 µmol, 51%) as a white solid. ^{1}H NMR (400

MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.6 Hz, 1H, H-6), 7.16 (d, *J* = 7.6 Hz, 1H, H-5), 5.79 (dd, *J* = 13.8, 3.1 Hz, 1H, H-1'), 5.50 – 5.34 (m, 2H, H-2', H-3'), 4.76 – 4.41 (m, 4H, H-1'', H-5'), 4.40 – 4.34 (m, 1H, H-4'), 4.17 – 4.01 (m, 3H, H-2'', H-3'', H-5''), 3.98 – 3.76 (m, 2H, H-4'', H-5''), 1.57 (s, 18H, $C(CH_3)_3$), 1.51 – 1.46 (m, 18H, $C(CH_3)_3$), 1.42 (s, 6H, $C(Me)_2$), 1.35 (s, 6H, $C(Me)_2$). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{41}H_{64}N_4O_{21}S$ 981.3857; Found 981.3852.

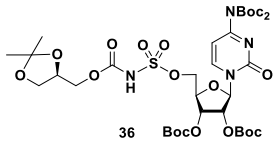
Protected *O*-sulfonyl carbamate linked CDP-Rbo analogue 35



35

Following the general procedure A, the title compound was prepared from **31** (96.5 mg, 0.15 mmol, 1.0 eq) and chlorosulfonyl isocyanate (13 µL, 0.15 mmol, 1.0 eq) in dry DCM (0.8 mL). The reaction mixture was stirred at 0 °C for 4 h; and pyridine (36 µL, 0.45 mmol, 3.0 eq) and compound **27** (35 mg, 0.15 mmol) were added. The resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the residue was purified by flash column chromatography over silica gel and then by size-exclusion chromatography (LH-20) (DCM/MeOH 1:1, v/v) afforded **35** (98.6 mg, 100 µmol, 67%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.6 Hz, 1H, H-6), 7.15 (d, *J* = 7.6 Hz, 1H, H-5), 5.78 (bs, 1H, NH), 5.64 (d, *J* = 3.0 Hz, 1H, H-1'), 5.54 (dd, *J* = 6.0, 3.1 Hz, 1H, H-2'), 5.51 – 5.44 (m, 1H, H-3'), 4.62 – 4.53 (m, 1H, H-5'), 4.42 – 4.29 (m, 2H, H-5', H-4'), 3.96 – 3.88 (m, 1H, H-2''), 3.85 – 3.68 (m, 3H, H-5'', H-4''), 3.58 – 3.50 (m, 1H, H-3''), 3.36 – 3.26 (m, 1H, H-1''), 3.24 – 3.13 (m, 1H, H-1''), 1.56 (s, 18H, $C(CH_3)_3$), 1.50 – 1.46 (m, 18H, $C(CH_3)_3$), 1.38 (s, 3H, Me), 1.37 (s, 3H, Me). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.1 (C=O, cytosine), 153.9 ($C_{q\text{-}arom}$, cytosine), 152.3, 152.0, 151.4 (C=O Boc), 149.4 (C=O carbamate), 146.0 (C-6), 125.4, 100.6, 100.4 ($C(Me)_2$), 96.8 (C-5), 93.9 (C-1'), 85.4, 83.7, 83.6 ($C(CH_3)_3$), 79.1 (C-4'), 75.4 (C-2'), 71.5 (C-3'), 70.4 (C-2''), 69.3 (C-3''), 66.6 (C-4''), 64.1 (C-5'), 63.0 (C-5''), 44.5 (C-1)'', 29.2 (Me), 29.2 (Me), 27.8, 27.7 ($C(CH_3)_3$), 20.0 (Me), 19.3 (Me). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{41}H_{65}N_5O_{20}S$ 980.4016; Found 980.4017.

Protected *O*-sulfonyl carbamate linked CDP-Gro analogue 36

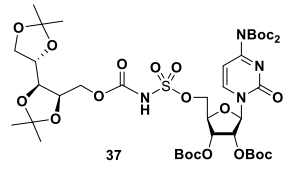


36

Following the general procedure A, the title compound was prepared from (*S*)-solketal (87 µL, 1 mmol, 1.0 eq, dried over activated 3 Å molecular sieves) and chlorosulfonyl isocyanate (124 µL, 1 mmol, 1.0 eq) in dry DCM (5 mL). The reaction mixture was stirred at 0 °C for 2 h; and pyridine (242 µL, 3 mmol, 3.0 eq) and compound **31** (644 mg, 1 mmol) were added The resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the residue was purified by flash column chromatography over silica gel (R_f = 0.2, pentane/EtOAc 1:5, v/v) and then by size exclusion (LH-20) (DCM/MeOH 1:1, v/v) afforded **36** (590 mg, 670 µmol, 67%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 7.6 Hz, 1H, H-6), 7.16 (d, *J* = 7.5 Hz, 1H, H-5), 5.89 (d, *J* = 3.7 Hz, 1H, H-1'), 5.48 (dd, *J* = 5.6, 3.8 Hz, 1H, H-2'), 5.36 (t, *J* = 5.9 Hz, 1H, H-3'), 4.60 (d, *J* = 11.3 Hz, 1H, H-5'), 4.51 (d, *J* = 11.7 Hz, 1H, H-5'), 4.45 (dt, *J* = 6.1, 3.0 Hz, 1H, H-4'), 4.33 (p, *J* = 5.9 Hz, 1H, H-2''), 4.20 (dd, *J* = 11.3, 4.8 Hz, 1H, H-1''), 4.11 – 4.04 (m, 2H, H-1'',

H-3''), 3.82 – 3.76 (m, 1H, H-3''), 1.57 (s, 18H, $C(CH_3)_3$), 1.51 – 1.46 (m, 18H, $C(CH_3)_3$), 1.43 (s, 3H, Me), 1.35 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.9 (C=O, cytosine), 154.3 ($C_{q\text{-}arom}$, cytosine), 152.1 (C=O Boc), 149.5 (C=O carbamate), 145.5 (C-6) 109.9 ($C(Me)_2$), 97.1 (C-5), 91.7 (C-1), 85.5, 83.8, 83.7 ($C(CH_3)_3$), 79.6 (C-4), 75.3 (C-2'), 73.6 (C-2''), 71.8 (C-3'), 66.9 (C-1''), 66.3 (C-3''), 27.8, 27.7 ($C(CH_3)_3$), 26.8, 25.4 (Me). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{36}H_{56}N_4O_{19}S$ 881.3332; Found 881.3330.

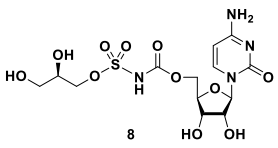
Protected *O*-sulfonyl carbamate linked CDP-Rbo analogue 37



37

Following the general procedure A, the title compound was prepared from **24** (270 mg, 1.16 mmol, 1.0 eq) and chlorosulfonyl isocyanate (100 µL, 1.16 mmol, 1.0 eq) in dry DCM (5.8 mL). The reaction mixture was stirred at 0 °C for 4 h; and pyridine (281 µL, 3.48 mmol, 3.0 eq) and compound **31** (746 mg, 1.16 mmol) were added. The resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the residue was purified by flash column chromatography over silica gel and then by size-exclusion chromatography (LH-20) (DCM/MeOH 1:1, v/v) afforded **37** (421 mg, 429 µmol, 37%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 7.7 Hz, 1H, H-6), 7.17 (d, *J* = 7.6 Hz, 1H, H-5), 5.96 (d, *J* = 3.4 Hz, 1H, H-1'), 5.39 (dd, *J* = 5.5, 3.3 Hz, 1H, H-2'), 5.27 (dd, *J* = 6.7, 5.5 Hz, 1H, H-3'), 4.74 (dd, *J* = 11.6, 2.6 Hz, 1H, H-1''), 4.68 – 4.58 (m, 2H, H-1'', H-5'), 4.52 – 4.39 (m, 2H, H-4', H-2''), 4.29 – 4.20 (m, 1H, H-5'), 4.15 – 4.09 (m, 1H, H-5''), 4.08 – 4.00 (m, 2H, H-3'', H-4''), 3.96 – 3.88 (m, 1H, H-5''), 1.56 (s, 18H, $C(CH_3)_3$), 1.48 (s, 18H, $C(CH_3)_3$), 1.43 (s, 3H, Me), 1.40 (s, 3H, Me), 1.33 (s, 6H, $C(Me)_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.9 (C=O, cytosine), 154.1 ($C_{q\text{-}arom}$, cytosine), 151.9, 151.9 (C=O Boc), 149.4 (C=O carbamate), 144.7 (C-6), 110.1, 109.7 ($C(Me)_2$), 96.9 (C-5), 91.1 (C-1), 85.2, 83.7, 83.5 ($C(CH_3)_3$), 79.2 (C-4'), 77.6 (C-4''), 75.5 (C-2'), 75.2 (C-2''), 73.2 (C-3'), 71.6 (C-3'), 70.8 (C-1''), 68.1 (C-5''), 65.8 (C-5'), 27.7, 27.7 ($C(CH_3)_3$), 27.7 (Me), 26.9 (Me), 25.4 (Me), 25.4 (Me). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{41}H_{64}N_4O_{21}S$ 981.3857; Found 981.3855.

***O*-sulfonyl carbamate linked CDP-Gro analogue 8**

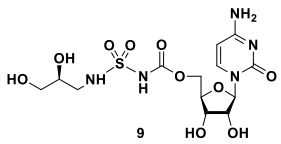


8

CDP-Gro analogue **8** was prepared according to general procedure B using compound **32** (33 mg, 37.5 µmol) and TFA (150 µL) in anhydrous CH_2Cl_2 (0.6 mL). After complete deprotection as monitored by LC-MS, the reaction mixture was directly concentrated *in vacuo*. The residue was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in H_2O) to afford compound **8** (9.1 mg, 29.8 µmol, 79%) as a white powder. ^1H NMR (400 MHz, D_2O) δ 7.79 (d, *J* = 7.6 Hz, 1H, H-6), 6.07 (d, *J* = 7.6 Hz, 1H, H-5), 5.91 (d, *J* = 4.0 Hz, 1H, H-1'), 4.45 – 4.37 (m, 1H, H-5'), 4.30 – 4.19 (m, 4H, H-2', H-3', H-4', H-5'), 4.11 (dd, *J* = 10.5, 4.1 Hz, 1H, H-1''), 4.04 (dd, *J* = 10.5, 5.9 Hz, 1H, H-1''), 3.97 – 3.87 (m, 1H, H-2''), 3.61 (dd, *J* = 11.8, 4.7 Hz, 1H, H-3''), 3.55 (dd, *J* = 11.8, 6.2 Hz, 1H, H-3''). ^{13}C NMR (101 MHz, D_2O) δ 165.0 (C=O, cytosine), 159.8 ($C_{q\text{-}arom}$, cytosine), 156.2 (C=O carbamate), 141.7 (C-6), 96.4 (C-5), 89.6 (C-1'), 81.8 (C-4'), 74.0 (C-2'),

70.2 (C-1''), 69.6 (C-2''), 69.4 (C-3'), 63.6 (C-5'), 61.9 (C-3''). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{13}H_{20}N_4O_{11}SNa$ 463.0741; Found 463.0744.

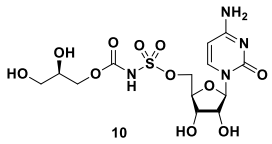
***N*-sulfonyl carbamate linked CDP-Gro analogue 9**



9

CDP-Gro analogue **9** was prepared according to general procedure B using compound **33** (85 mg, 97 µmol) and TFA (390 µL) in anhydrous CH_2Cl_2 (1.5 mL). After complete deprotection as monitored by LC-MS, the reaction mixture was directly concentrated *in vacuo*. The residue was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in H_2O) to afford compound **9** (26.3 mg, 60 µmol, 62%) as a white powder. ^{1}H NMR (400 MHz, D_2O) δ 7.75 (d, *J* = 7.6 Hz, 1H, H-6), 6.06 (d, *J* = 7.6 Hz, 1H, H-5), 5.89 (d, *J* = 4.0 Hz, 1H, H-1'), 4.46 – 4.39 (m, 1H, H-5'), 4.30 – 4.19 (m, 4H, H-2', H-3', H-4', H-5'), 3.81 – 3.74 (m, 1H, H-2''), 3.58 (dd, *J* = 11.8, 4.3 Hz, 1H, H-3''), 3.49 (dd, *J* = 11.8, 6.3 Hz, 1H, H-3''), 3.04 (dd, *J* = 13.2, 4.5 Hz, 1H, H-1''), 2.92 (dd, *J* = 13.2, 7.4 Hz, 1H, H-1''). ^{13}C NMR (101 MHz, D_2O) δ 165.4 (C=O, cytosine), 158.9 ($C_{q\text{-arom}}$, cytosine), 156.7 (C=O carbamate), 141.7 (C-6), 96.5 (C-5), 89.9 (C-1), 81.7 (C-4'), 73.9 (C-2'), 70.3 (C-2''), 69.4 (C-3'), 63.6 (C-5), 63.2 (C-3''), 45.4 (C-1''). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{13}H_{21}N_5O_{10}SNa$ 440.1082; Found 440.1083.

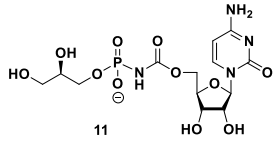
***O*-sulfonyl carbamate linked CDP-Gro analogue 10**



10

CDP-Gro analogue **10** was prepared according to general procedure B using compound **36** (60 mg, 68 µmol) and TFA (270 µL) in anhydrous CH_2Cl_2 (1.1 mL). After complete deprotection as monitored by LC-MS, the reaction mixture was directly concentrated *in vacuo*. The residue was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in H_2O) to afford compound **10** (16.5 mg, 37 µmol, 55%) as a white powder. ^{1}H NMR (400 MHz, D_2O) δ 7.79 (d, *J* = 7.7 Hz, 1H, H-6), 5.99 (d, *J* = 7.7 Hz, 1H, H-5), 5.80 (d, *J* = 2.9 Hz, 1H, H-1'), 4.35 – 4.27 (m, 1H, H-5'), 4.24 – 4.19 (m, 2H, H-5', H-4'), 4.19 – 4.14 (m, 2H, H-3', H-2'), 3.95 (dd, *J* = 11.4, 4.1 Hz, 1H, H-1''), 3.87 (dd, *J* = 11.4, 6.1 Hz, 1H, H-1''), 3.82 – 3.76 (m, 1H, H-2''), 3.52 (dd, *J* = 11.8, 4.5 Hz, 1H, H-3''), 3.45 (dd, *J* = 11.8, 6.2 Hz, 1H H-3''). ^{13}C NMR (101 MHz, D_2O) δ 164.16 (C=O, cytosine), 160.09 ($C_{q\text{-arom}}$, cytosine), 155.01 (C=O carbamate), 142.05 (C-6), 95.93 (C-5), 89.79 (C-1), 81.52 (C-4'), 74.07 (C-3'), 69.78 (C-2''), 69.14 (C-2'), 67.54 (C-5'), 66.16 (C-1''), 62.25 (C-3''). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{13}H_{20}N_4O_{11}SNa$ 463.0741; Found 463.0742.

Phosphoryl carbamate linked CDP-Gro analogue 11

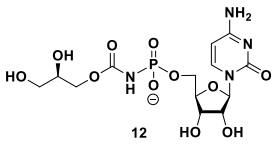


11

Following the general procedure C, the title compound was prepared from **31** (978 mg, 1.5 mmol, 1.0 eq) and dichlorophosphoryl isocyanate (150 µL, 1.5 mmol, 1.0 eq) in dry DCM (7.6 mL). The reaction mixture was stirred at 0 °C for 3 h; and pyridine (735 µL, 9.1 mmol, 6.0 eq) and (*S*)-solketal (200 mg, 1.5 mmol) were added. Flash

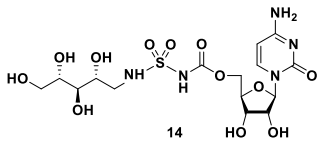
column chromatography over silica gel (R_f = 0.3, MeOH/CH_2Cl_2 5:95, v/v) and then by size exclusion (LH-20) (DCM/MeOH 1:1, v/v) afforded **38** (373 mg, 420 µmol, 28%) as a white solid. For the global deprotection step, CDP-Gro analogue **11** was prepared from compound **38** (29 mg, 33 µmol) and TFA (200 µL) in anhydrous CH_2Cl_2 (0.8 mL). Purification by size-exclusion chromatography (HW-40, NH_4OAc buffer) followed by semiprep-HPLC afforded **11** (4.2 mg, 9.5 µmol, 29%) as a white powder. ^{1}H NMR (400 MHz, D_2O) δ 7.73 (d, *J* = 7.6 Hz, 1H, H-6), 6.08 (d, *J* = 7.6 Hz, 1H, H-5), 5.88 (d, *J* = 4.0 Hz, 1H, H-1'), 4.45 (dd, *J* = 12.5, 2.5 Hz, 1H, H-5'), 4.37 – 4.28 (m, 2H, H-5', H-2'), 4.28 – 4.17 (m, 2H, H-3', H-4'), 3.92 – 3.77 (m, 3H, H-1'', H-2''), 3.59 (dd, *J* = 11.8, 4.1 Hz, 1H, H-3''), 3.52 (dd, *J* = 11.7, 5.7 Hz, 1H, H-3''). ^{13}C NMR (101 MHz, D_2O) δ 141.9 (C-6), 96.3 (C-5), 90.1 (C-1'), 81.3 (C-4'), 73.6 (C-2'), 70.7 (d, $J_{C\text{-}P}$ = 8.0 Hz, C-2''), 69.3 (C-3'), 66.4 (d, $J_{C\text{-}P}$ = 5.8 Hz, C-1''), 63.9 (C-5'), 62.1 (C-3''). ^{31}P NMR (162 MHz, D_2O) δ -4.52 (s). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{13}H_{22}N_4O_{11}PNa$ 441.1017; Found 441.1017.

Phosphoryl carbamate linked CDP-Gro analogue 12



Following the general procedure C, the title compound was prepared from (*S*)-solketal (200 mg, 1.5 mmol) and dichlorophosphoryl isocyanate (150 µL, 1.5 mmol, 1.0 eq) in dry DCM (7.6 mL). The reaction mixture was stirred at 0 °C for 2 h; and pyridine (735 µL, 9.1 mmol, 6.0 eq) and compound **31** (978 mg, 1.5 mmol, 1.0 eq) were added. Repeated flash column chromatography over silica gel (R_f = 0.3, MeOH/CH_2Cl_2 5:95, v/v) and then by size-exclusion chromatography (LH-20) (DCM/MeOH 1:1, v/v) afforded **40** (319 mg, 360 µmol, 24%) as a white solid. For the global deprotection step, CDP-Gro analogue **12** was prepared from compound **40** (42 mg, 48 µmol) and TFA (200 µL) in anhydrous CH_2Cl_2 (0.8 mL). Purification by size-exclusion chromatography (HW-40, NH_4OAc buffer) followed by semiprep-HPLC afforded **12** (4.5 mg, 10 µmol, 21%) as a white powder. ^{1}H NMR (400 MHz, D_2O) δ 7.73 (d, *J* = 7.6 Hz, 1H, H-6), 6.08 (d, *J* = 7.6 Hz, 1H, H-5), 5.88 (d, *J* = 4.0 Hz, 1H, H-1'), 4.45 (dd, *J* = 12.5, 2.5 Hz, 1H, H-5'), 4.37 – 4.28 (m, 2H, H-5', H-2'), 4.28 – 4.17 (m, 2H, H-3', H-4'), 3.92 – 3.77 (m, 3H, H-1'', H-2''), 3.59 (dd, *J* = 11.8, 4.1 Hz, 1H, H-3''), 3.52 (dd, *J* = 11.7, 5.7 Hz, 1H, H-3''). ^{13}C NMR (101 MHz, D_2O) δ 141.9 (C-6), 96.3 (C-5), 90.1 (C-1'), 81.3 (C-4'), 73.6 (C-2'), 70.7 (d, $J_{C\text{-}P}$ = 8.0 Hz, C-2''), 69.3 (C-3'), 66.4 (d, $J_{C\text{-}P}$ = 5.8 Hz, C-1''), 63.9 (C-5'), 62.1 (C-3''). ^{31}P NMR (162 MHz, D_2O) δ -4.53 (s). HRMS (ESI) M/Z: $[M+Na]^+$: Calcd for $C_{13}H_{22}N_4O_{11}PNa$ 441.1017; Found 441.1017.

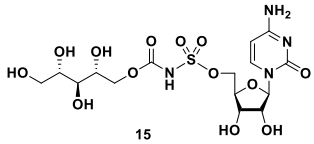
***N*-sulfonyl carbamate linked CDP-Rbo analogue 14**



CDP-Rbo analogue **14** was prepared according to general procedure B using compound **35** (60 mg, 61 µmol) and TFA (244 µL) in anhydrous CH_2Cl_2 (1.2 mL). After complete deprotection as monitored by LC-MS, the reaction mixture was directly concentrated *in vacuo*. The residue was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in H_2O) to afford **14**

(16.5 mg, 37 µmol, 68%) as a white powder. ^{1}H NMR (500 MHz, D_2O) δ 7.69 (d, *J* = 7.6 Hz, 1H, H-6), 5.97 (d, *J* = 7.6 Hz, 1H, H-5), 5.82 (d, *J* = 4.1 Hz, 1H, H-1'), 4.35 – 4.28 (m, 1H, H-5'), 4.20 – 4.09 (m, 4H, H-2', H-3', H-4', H-5'), 3.79 – 3.71 (m, 1H, H-2''), 3.69 – 3.60 (m, 2H, H-5'', H-4''), 3.59 – 3.46 (m, 2H, H-3'', H-5''), 3.10 (dd, *J* = 13.1, 3.2 Hz, 1H, H-1''), 2.89 (dd, *J* = 13.1, 8.3 Hz, 1H, H-1''). ^{13}C NMR (126 MHz, D_2O) δ 165.8 (C=O, cytosine), 159.8, 157.2, 141.4 (C-6), 96.5 (C-5), 89.6 (C-1'), 81.7 (C-4'), 73.9 (C-2'), 72.7 (C-3''), 71.9 (C-4''), 70.1 (C-2''), 69.4 (C-3'), 63.4 (C-5'), 62.4 (C-5''), 45.1 (C-1''). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{15}H_{26}N_5O_{12}S$ 500.1293; Found 500.1293.

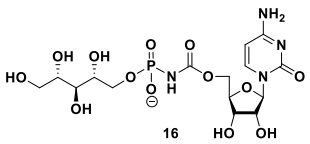
***O*-sulfonyl carbamate linked CDP-Rbo analogue 15**



15

CDP-Rbo analogue **15** was prepared according to general procedure B using compound **37** (59 mg, 59 µmol) and TFA (236 µL) in anhydrous CH_2Cl_2 (1.0 mL). After complete deprotection as monitored by LC-MS, the reaction mixture was directly concentrated *in vacuo*. The residue was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in H_2O) afforded compound **15** (22.7 mg, 45 µmol, 77%) as a white powder. ^{1}H NMR (400 MHz, D_2O) δ 7.82 (d, *J* = 7.6 Hz, 1H, H-6), 6.04 (d, *J* = 7.6 Hz, 1H, H-5), 5.90 (d, *J* = 3.3 Hz, 1H, H-1'), 4.43 – 4.37 (m, 1H, H-5'), 4.33 – 4.22 (m, 4H, H-2', H-3', H-4', H-5'), 4.19 (dd, *J* = 11.5, 2.9 Hz, 1H, H-1''), 4.05 (dd, *J* = 11.6, 6.7 Hz, 1H, H-1''), 3.93 (td, *J* = 6.5, 2.8 Hz, 1H, H-2''), 3.82 – 3.72 (m, 2H, H-4'', H-5''), 3.68 (t, *J* = 6.2 Hz, 1H, H-3''), 3.60 (dd, *J* = 11.7, 6.8 Hz, 1H, H-5''). ^{13}C NMR (101 MHz, D_2O) δ 165.7 (C=O, cytosine), 160.2 ($C_{q\text{-}arom}$, cytosine), 157.0 (C=O carbamate), 141.5 (C-6), 96.2 (C-5), 89.7 (C-1'), 81.4 (C-4'), 74.1 (C-2'), 72.0 (C-4''), 72.0 (C-3''), 70.0 (C-2''), 69.2 (C-3'), 67.6 (C-5'), 66.4 (C-1''), 62.4 (C-5''). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{15}H_{25}N_4O_{13}S$ 501.1133; Found 501.1135.

Phosphoryl carbamate linked CDP-Rbo analogue 16

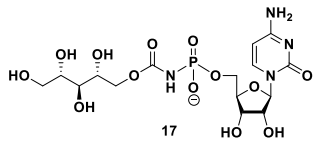


16

Following the general procedure C, the title compound was prepared from **31** (553 mg, 0.86 mmol, 1.0 eq) and dichlorophosphoryl isocyanate (85 µL, 0.86 mmol, 1.0 eq) in dry DCM (4.3 mL). The reaction mixture was stirred at 0 °C for 3 h, then pyridine (417 µL, 5.2 mmol, 6.0 eq) and compound **24** (200 mg, 0.86 mmol) were added. Purification of the crude product by repeated flash column chromatography over silica gel (R_f = 0.3, MeOH/CH_2Cl_2 5:95, v/v) followed by size-exclusion chromatography (LH-20) (DCM/MeOH 1:1, v/v) afforded **39** (306 mg, 312 µmol, 36%) as a white solid. For the global deprotection step, CDP-Rbo analogue **16** was prepared using compound **39** (103 mg, 105 µmol) and TFA (420 µL) in anhydrous CH_2Cl_2 (1.7 mL). Purification by size-exclusion chromatography (HW-40, NH_4OAc buffer) followed by semiprep-HPLC afforded **16** (22.5 mg, 45 µmol, 43%) as a white powder. ^{1}H NMR (500 MHz, D_2O) δ 7.76 (d, *J* = 7.6 Hz, 1H, H-6), 6.11 (d, *J* = 7.6 Hz, 1H, H-5), 5.89 (d, *J* = 4.1 Hz, 1H, H-1'), 4.48 – 4.42 (m, 1H, H-5'), 4.38 – 4.30 (m, 2H, H-5', H-2'), 4.29 – 4.20 (m, 2H, H-4', H-3'), 4.07 – 4.01 (m, 1H, H-1''), 3.94

(m, 1H, H-1''), 3.90 – 3.85 (m, 1H, H-2''), 3.82 – 3.77 (m, 1H, H-4''), 3.75 (dd, *J* = 11.9, 3.0 Hz, 1H, H-5''), 3.69 (t, *J* = 6.3 Hz, 1H, H-3''), 3.60 (dd, *J* = 11.8, 7.1 Hz, 1H, H-5''). ^{13}C NMR (126 MHz, D_2O) δ 164.8 (C=O, cytosine), 155.8 (C=O carbamate), 141.9 (C-6), 96.3 (C-5), 90.0 (C-1'), 81.3 (C-4'), 73.6 (C-2'), 72.0 (C-4''), 71.6 (C-3''), 70.8 (d, $J_{C\text{-}P}$ = 7.9 Hz, C-2''), 69.2 (C-3'), 66.6 (d, $J_{C\text{-}P}$ = 5.7 Hz, C-1''), 63.9 (C-5'), 62.3 (C-5''). ^{31}P NMR (202 MHz, D_2O) δ -4.65 (s). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{15}H_{26}N_4O_{13}P$ 501.1229; Found 501.1228.

Phosphoryl carbamate linked CDP-Rbo analogue 17



17

Following the general procedure C, the title compound was prepared from **24** (200 mg, 0.86 mmol, 1.0 eq) and dichlorophosphoryl isocyanate (85 µL, 0.86 mmol, 1.0 eq) in dry DCM (4.3 mL). The reaction mixture was stirred at 0 °C for 3 h; and pyridine (417 µL, 5.2 mmol, 6.0 eq) and compound **31** (553 mg, 0.86 mmol, 1.0 eq) were added. Purification of the crude product by repeated flash column chromatography over silica gel (R_f = 0.3, MeOH/CH_2Cl_2 5:95, v/v) afforded **41** (406 mg, 312 µmol, 48%) as a white solid. For the global deprotection step, CDP-Rbo analogue **17** was prepared using compound **41** (76 mg, 77 µmol) and TFA (310 µL) in anhydrous CH_2Cl_2 (1.2 mL). Purification by size-exclusion chromatography (HW-40, NH_4OAc buffer) followed by semiprep-HPLC afforded **17** (14.3 mg, 28 µmol, 37%) as a white powder. ^{1}H NMR (500 MHz, D_2O) δ 7.96 (d, *J* = 7.7 Hz, 1H, H-6), 6.08 (d, *J* = 7.6 Hz, 1H, H-5), 5.93 (d, *J* = 3.3 Hz, 1H, H-1'), 4.29 – 4.24 (m, 3H, H-1'', H-2', H-3'), 4.23 – 4.16 (m, 2H, H-4', H-5'), 4.15 – 4.06 (m, 2H, H-1'', H-5'), 3.97 – 3.93 (m, 1H, H-2''), 3.80 – 3.72 (m, 2H, H-5'', H-4''), 3.68 (t, *J* = 6.3 Hz, 1H, H-3''), 3.63 – 3.57 (m, 1H, H-5''). ^{13}C NMR (126 MHz, D_2O) δ 165.3 (C=O, cytosine), 156.6 ($C_{q\text{-}arom}$, cytosine), 156.1 (d, $J_{C\text{-}P}$ = 4.9 Hz, C=O carbamate), 141.7 (C-6), 96.2 (C-5), 89.4 (C-1'), 82.6 (d, $J_{C\text{-}P}$ = 9.4 Hz, C-4''), 74.2 (C-2'), 71.9 (C-4''), 71.8 (C-3''), 69.7 (C-2''), 69.3 (C-3'), 66.5 (C-1''), 64.3 (d, $J_{C\text{-}P}$ = 5.2 Hz, C-5'), 62.3 (C-5''). ^{31}P NMR (202 MHz, D_2O) δ -4.65 (s). HRMS (ESI) M/Z: $[M+H]^+$: Calcd for $C_{15}H_{26}N_4O_{13}P$ 501.1229; Found 501.1228.

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