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FULL PAPER

A Click-Chemistry Linked 2'3'-cGAMP Analog

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Abstract: 2'3'-cGAMP is an uncanonical cyclic dinucleotide where one A and one G base are connected *via* a 3'-5' and a unique 2'-5' linkage. The molecule is produced by the cyclase cGAS in response to cytosolic DNA binding. cGAMP activates STING and hence one of the most powerful pathways of innate immunity. cGAMP analogs with uncharged linkages that feature better cellular penetrability are currently highly desired. Here, we report the synthesis of a cGAMP analog with one amide and one triazole linkage. The molecule is best prepared *via* a first Cu(I) catalysed click reaction which establishes the triazole, while the cyclization is achieved by macrolactamization.

Introduction

Cyclic dinucleotides (CDNs) are important cellular messenger molecules in a variety of organisms.[1] The compounds play a crucial role in a wide range of biological processes, such as signal transduction, control of biofilm formation or quorum sensing.[2] Bacteria produce molecules in which two purine bases are linked *via* two 3'-5' phosphate linkages to give symmetrical cyclophane structures.[3] One main example for such a molecule is the c-di-GMP compound **1** shown in Fig. 1.[4,5] Biochemically the compound is generated from the corresponding nucleotide-5'-triphosphates. Recently, an unsymmetrical cyclic dipurine molecule (cGAMP, **2**) was discovered in mammalian cells.[6,7] In this molecule, the two purines are connected *via* one 3'-5' and another 2'-5' linkage.[8] The dinucleotide **2** is produced by the cyclase cGAS (cyclic GMP-AMP synthase). cGAS is a cytosolic DNA sensor and part of the innate immune system.[9,10] 2'3'-cGAMP (**2**) binds to the transmembrane receptor STING (stimulator of interferon genes) with nanomolar affinity (k_d = 4.59 nM),[11] which activates the type 1 interferon (IFN) pathway.[12-14] Subsequent degradation of cGAMP **2** occurs by the specific cleavage of the 2'-5' phosphodiester bond by ENPP1 highlighting the importance of this unusual connection.[15,16]
There is currently tremendous interest to develop synthetic routes towards analogs of cGAMP **2** as potential agonists or antagonists for cGAS and STING.[17-19] The bisphosphorothioate cGAMP derivative **3**,[20,21] for example, is already in clinical trials.[22,23] Alternative targeting of STING with small molecules is also known.[24-26] Particularly, compounds which lack the negatively charged phosphodiester linkages are discussed as new immune-regulatory pharmaceuticals.[27] While such derivatives are known for symmetric 3'-5' dinucleotides[28-32], to the best of our knowledge, uncharged cGAMP **2** analogs do not exist.
In this article, we describe the modular synthesis of a neutral cGAMP analog **4** that features one triazole and one amide linkage. The triazole was generated by a Cu(I) catalysed alkyne-azide click reaction (CuAAC) that was found to be particularly efficient on nucleotides and oligonucleotides.[33-35]

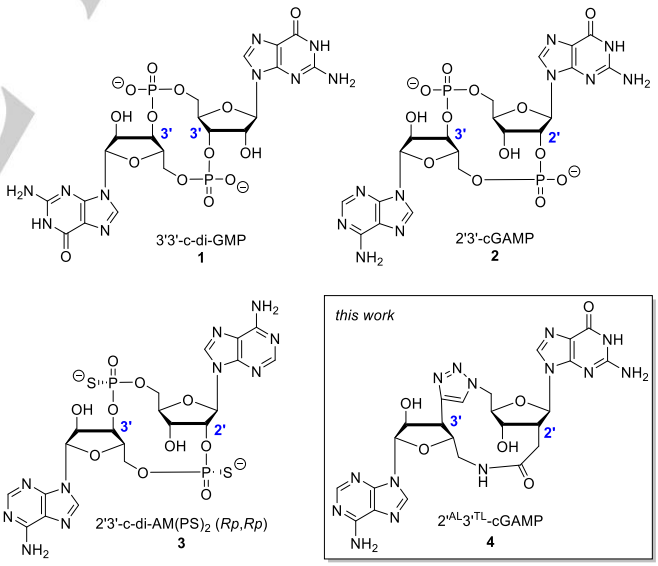

Figure 1: Depiction of the symmetrical microbial c-di-GMP **1**, the unsymmetrical STING activator cGAMP **2**, as well as the bisphosphorothioate analog **3**, together with the molecule **4** targeted here.

Results and Discussion

We decided to start our synthetic study by synthesizing the cGAMP analog **4**, in which the 5'-G-3'-A linkage is replaced by a triazole unit and the 2'-G-5'-A linkage is substituted by an amide bond.

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Molecular modeling (Fig. 2) showed that the analog **4** is able to adopt a conformation that is similar to the natural ligand bound to STING.[11,36]

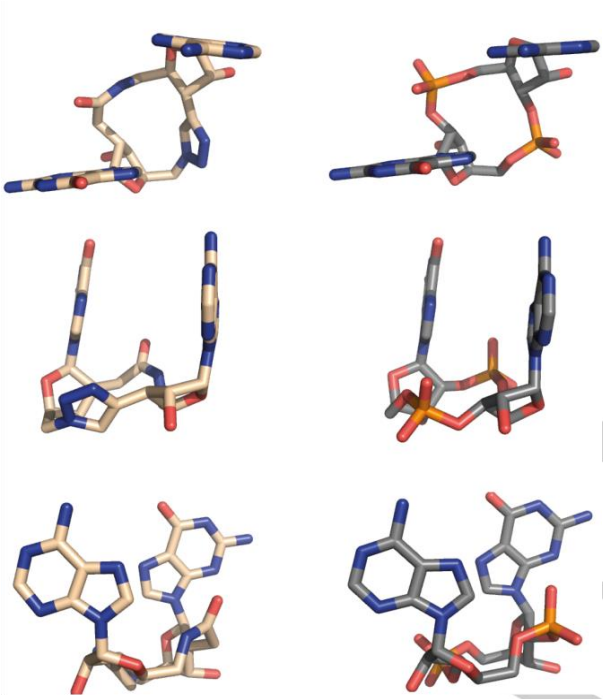


Figure 2: 3D representation showing the potential conformational similarity between compound **4** (left) and natural 2'3'-cGAMP (right, conformation of **2** bound to STING, PDB: 4LOH).

In both cases, the macrocycle is thought to force the bases into a shifted parallel orientation with the imidazole part of the nucleobases pointing towards each other. This requires *anti*-conformations of both glycosidic bonds. The preferred conformation of compound **4** will be governed by aromatic the triazole unit. For the conformation of the amide we assume a *syn*-conformation due to the small ring size.

Analysis of potential synthetic accesses of **4** shows that it can be generated by Cu(I) catalyzed azide alkyne reaction plus a preceding or following lactamization. We developed the synthesis based on the A-half **5** and the corresponding G-half **6** as depicted in Fig. 3. For the synthesis of the A-half **5**, we started with the commercially available 1,2-acetonide protected xylofuranoside **7** (two steps from D-xylose), which we converted in three steps into the 5-TBS-1,2-acetonide protected 3-methylene xylofuranoside **8**.

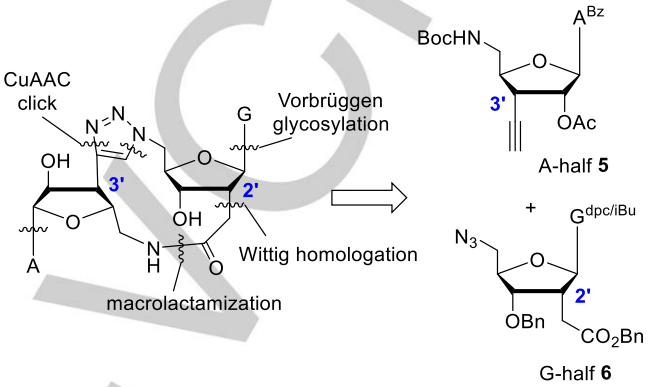
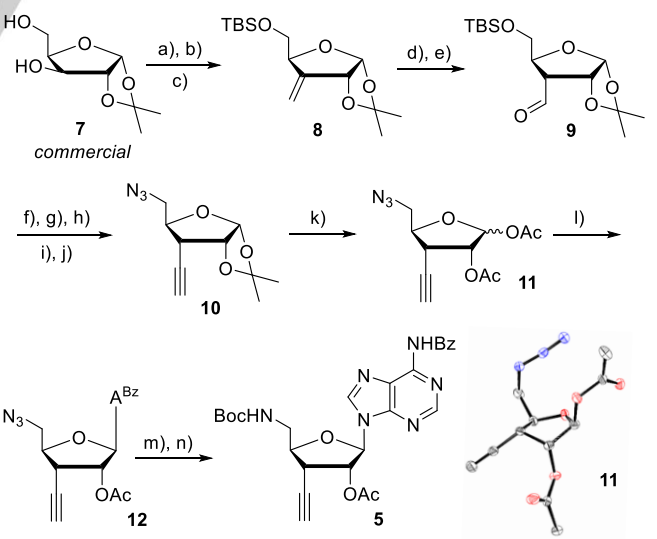


Figure 3: Synthetic strategy towards compound **4**. dpc = diphenylcarbamoyl, iBu = isobutyryl.

After stereoselective hydroboration (BH_3·DMS, dr: 9:1) of **8** and Swern oxidation, we obtained the carbonyl compound **9**, which we subjected to a Corey-Fuchs alkinylation (CBr_4, BuLi). TBS deprotection and conversion of the primary hydroxyl group into the azide gave the key intermediate **10**. X-ray analysis of the structure of **10** proved the right configuration of the compound (recrystallisation from isohexanes/ethyl acetate).

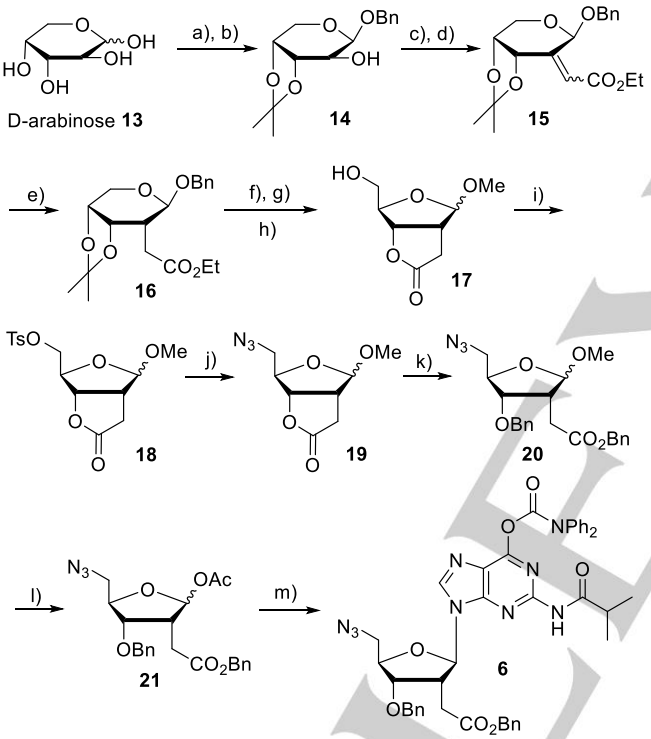


Scheme 1: Synthesis of the A-half **5** in 14 steps. a) TBSCl, Py, RT, 2h, 97%; b) $(COCl)_2$, DMSO, NEt_3, DCM, -60°C, 3h; c) CH_3PPh_3Br, BuLi, THF, RT, 6h, 81% (over two steps); d) BH_3*DMS, THF, RT, 12h then 30% H_2O_2, 2N NaOH, RT, 2h, 76%; e) $(COCl)_2$, DMSO, NEt_3, DCM, -60°C, 3h, 93%; f) CBr_4, PPh_3, DCM, 0°C, 1h then RT, 12h, 85%; g) BuLi, THF, -78°C, 1.5h, 83%; h) TBAF, THF, RT, 4h, 95%; i) TsCl, Py, RT, 18h, 87%; j) NaN_3, DMF, 80°C, 3h, 94%; k) HOAc/Ac_2O, H_2SO_4 (cat.), RT, 5h, 78%; l) 6-*N*-Benzoyladenine, BSA, TMSOTf, DCE, 80°C, 4h, 61%; m) PMe_3, H_2O, THF, 40 °C, then RT, 12h, 66%; n) Boc_2O, NEt_3, DCM, RT, 16h, 64%. Overall yield starting from **7**: 6%

Subsequent cleavage of the isopropylidene group and acetyl protection of the hydroxyl groups provided compound **11**, which

10.1002/chem.201805409

was the sugar building block for the following glycosylation step. The Vorbrüggen reaction to **12** was found to be most efficient under BSA/TMSOTf conditions with a benzoyl protected A-heterocycle (α/β: 1:12). Finally, we converted the azide *via* a Staudinger reduction (PMe_3 worked better than PPh_3) into the corresponding amine, which was Boc-protected afterwards to give the A-half **5**.



Scheme 2. Synthesis of the G-half **6** in 13 steps. a) AcCl, BnOH, 60°C, 5h, 80%; b) $Me_2C(OMe)_2$, Me_2CO, *p*-TsOH (cat.), 60°C, 2h, 84%; c) $(COCl)_2$, DMSO, NEt_3, DCM, -60°C, 3h; d) Ph_3PCHCO_2Et, DCM, RT, 12h, 86% (over two steps); e) H_2, Raney-Ni, EtOH, RT, 20h, 90%; f) H_2, Pd/C, EtOH/THF, 36h, 88%; g) 80% HOAc, RT, 24h; h) H_2SO_4 (cat.), MeOH, 4°C, 3d, 72% (over two steps); i) TsCl, Py, RT, 18h, 76%; i) NaN_3, DMF, 80°C, 3h, 75%; k) BnBr, KOH, THF, reflux, 5h, 91%; l) HOAc/Ac_2O, H_2SO4 (cat.), RT, 3h, 85%; m) 6-*O*-(Diphenylcarbamoyl)-2-*N*-isobutyrylguanine ($G^{dpc/iBu}$), BSA, TMSOTf, DCE, 80°C, 2h, 72%. Overall yield starting from **13**: 10%.

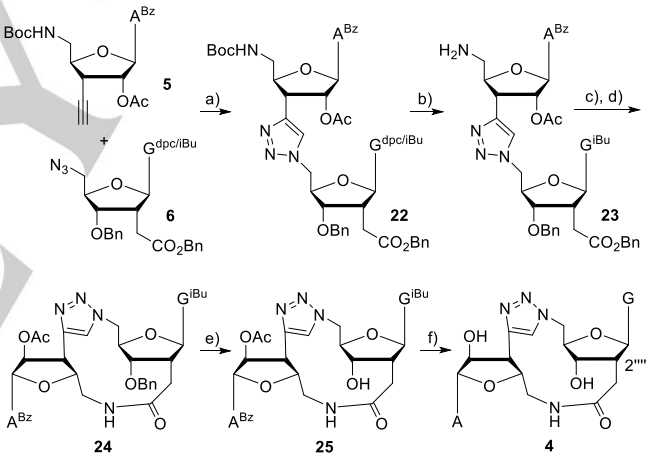
The desired G-half (Scheme 2) was synthesized starting from D-arabinose (**13**). 1-*O*-Benzyl and 3,4-acetonide protection yielded alcohol **14**.

Subsequent Swern oxidation and Wittig homologation provided the intermediate **15** (E/Z: 4:1). Employing the acetonide protective group as a stereoselective directing group, compound **16** was almost exclusively obtained in *R*-configuration *via* a Raney-Ni-assisted hydrogenation (dr: 20:1).

Under these reduction conditions the 1-*O*-benzyl group remained unaffected – keeping the sugar in its pyranoside configuration. Removal of the protective groups and treatment with catalytic amounts of acid furnished at 4 °C selectively the ribofuranoside **17**. This was followed by an *in situ* lactonization. The resulting alcohol **17** was tosylated and reacted with NaN_3 to give azide **19**. The absolute configuration of the compounds was again proven with a crystal structure of **18** (SI).

We subsequently opened the lactone ring to compound **20** *via* hydroxide-mediated benzyl protection and converted it into its 1-*O*-acetyl derivative **21**. The glycosylation reaction to the G-half **6** was performed by a so far unreported Vorbrüggen pattern in high β-selectivity (α/β: 1:14) and good yields (79%).

The assembly of nucleoside building blocks A (**5**) and G (**6**) was initiated by a CuAAC reaction. This reaction went smoothly and provided the dinucleotide **22** in fair yield of 80% (Scheme 3). We noticed that click-approaches with the Boc-deprotected amine compound A gave rise of several side products as monitored by thin-layer chromatography (TLC).



Scheme 3. The assembly towards cyclic dinucleotide **4** in 6 steps. a) $CuSO_4$, Na-Ascorbate, THF/tBuOH/H_2O, RT, 24h, 80%; b) TFA/DCM (1:1), 0°C, 1h, 81%; c) H_2, Pd/C, EtOH, 36h; d) HATU, DIPEA, DMF (1mM), RT, 24h, 52% (over two steps); e) BCl_3, DCM, -40°C, 3d; f) NH_3, H_2O/MeOH, 50°C, 20h, 48% (over two steps). Overall yield starting from **5** and **6**: 16%.

TFA treatment of dinucleotide **22** resulted in the cleavage of both the Boc and the diphenylcarbamoyl (dpc) group. Besides, this was the last step of the consecutive synthesis where purification could be easily conducted by flash column chromatography (DCM/MeOH, 10:1) due to the increasing polarity of the following compounds. A palladium catalyzed hydrogenation reaction deprotected the benzyl ester by leaving the secondary 3''''-*O*-benzyl ether intact. Final macrolactamization with HATU furnished the cyclized dinucleotide **24**.

Deprotection of the 3''''-*O*-benzyl ether under BCl_3/DCM conditions (-40°C) proved to be the best option even though solubility in organic solvents decreased with ongoing removal of protective groups. Final ammonolysis revealed our target molecule **4** in 2% overall yield starting from the G-pathway (19 steps) and 1% starting from the A-pathway (20 steps), respectively. Compounds **24**, **25** and **4** were purified by RP-HPLC and subjected to further NMR-studies.

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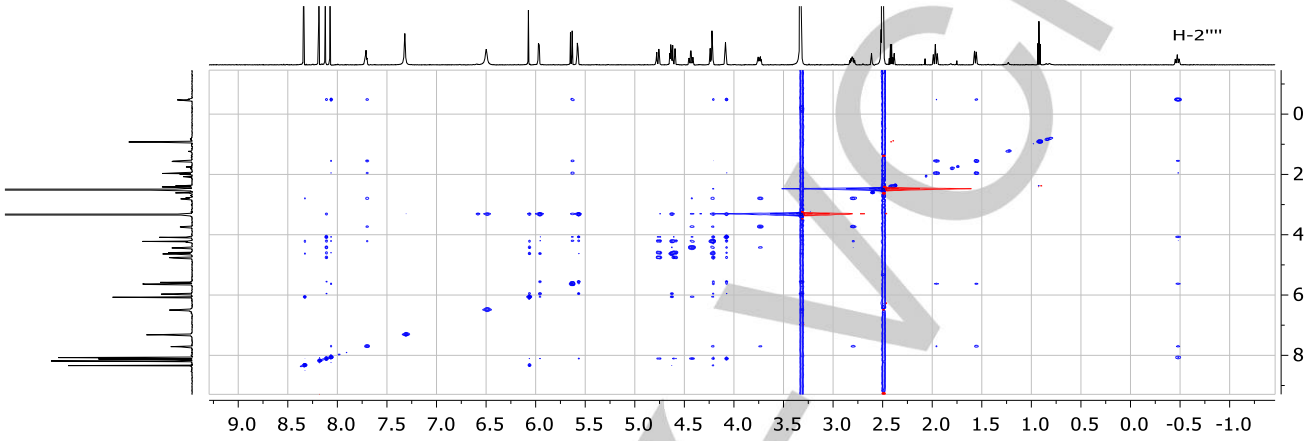

Figure 4: NOESY spectrum of the final compound **4** in DMSO-d_6

Conformational Analysis and Conclusion

We performed detailed NOESY experiments in order to determine the conformation preferences of target compound **4** in respect to potential STING binding. The spectrum is shown in Fig. 4. The most informative NOE contacts together with a depiction of the modelling results of **4** in solution is shown in Fig. 5. The NMR data confirm the overall structure with two β-configured glycosidic bonds both in anti-conformation. Most interesting, however, is the large shielding of proton H-2'''', which shifts from δ(compound **23**) = 4.12 ppm to δ(compound **4**) = -0.47 ppm. This dramatic shift indicates that the proton is positioned just on top of the aromatic triazole ring.

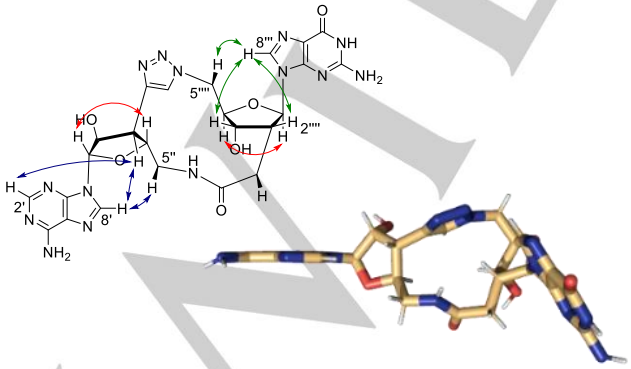

Figure 5: Selected NOE contacts of compound **4** (in DMSO-d_6) and modelling of the preferred conformation based on the NOE data.

According to this low chemical shift, it is assumed that H-2'''' points directly to the triazole ring within the cyclized structures of compounds **24**, **25** and **4**. Unraveling of the conformation just based on the NOE data shows that compound **4** likely adopts a more open conformation in solution (DMSO-d_6) compared to cGAMP **2**, with the two heterocycles being not parallel to each other.

Potential binding of compound **4** to STING was tested *in vitro* by nanoDSF assays and analysis of thermal unfolding of the STING constructs hSTING_L139 (human STING AA139-379) and mSTING_L138 (mouse STING AA138-378). We used the physiological ligand 2′3′-cGAMP and a ligand with lower affinity, 3′3′-cGAMP, as positive controls. As expected after the conformational analysis of compound **4**, binding to hSTING or mSTING could not be detected. This result was confirmed with ITC experiments (see supporting information). Based on the more open structure of the here prepared compound **4**, we believe that interaction studies with cGAS or ENPP1 may be more promising. Investigations in this direction are on the way.

In summary, we report the first synthesis of a 2'3'-cGAMP analog which features uncharged bridges that should provide membrane crossing properties. The synthetic strategy involved first linking of the two nucleotides by a Cu(I)-catalyzed click reaction followed by a macrolactamization to close the cycle. The synthesis of medium size ring structures is always difficult. We believe that the here described strategy will open the access to a variety of derivatives of **4**. This allows systematic scanning of the conformational space of the two nucleobases relative to each other regarding the binding to the involved proteins STING, cGAS and ENPP1.

Experimental Section

Unless otherwise specified, all reactions were magnetically stirred under an N_2 atmosphere. Reaction vessels were dried under high vacuum at 550 °C prior to use. Dry solvents and reagents were purchased from commercial suppliers, such as Sigma-Aldrich, Acros Organics, Carbosynth, TCI Europe, ABCR, VWR, stored under septum over molecular sieves and used as received. The reaction progress and fractions during column chromatography were monitored by TLC on silica gel 60-F_{254} plates purchased from Merck and visualized by irradiation with UV-light (254 nm or 366 nm) and *p*-anisaldehyde staining solution (*p*-anisaldehyde (3.7 mL), EtOH (135 mL), conc. H_2SO_4 (5 mL), conc. AcOH (1.5 mL)). Purification was performed using flash column chromatography with silica gel (Merck, particle size 0.063 – 0.200 mm). The eluents used were determined by TLC. Purification of the crude dinucleotides **24**, **25** and **4** was operated by

10.1002/chem.201805409

Waters 2695 reversed phase high performance liquid chromatography (RP-HPLC) using Nucleosil columns (250/4 mm, C18ec, particle size 3 µm for analysis or 250/10 mm, C18ec, 5 µm for purification) from Machery-Nagel with a bufferfree H_2O/MeCN eluent system. Water was purified by a Milli-Q Plus system from Merck Millipore. NMR-spectra were measured on a *Bruker Ascend 400* or *Bruker ARX 600* at room temperature operating at 400 MHz or 600 MHz for ^{1}H-nuclei and at 101 MHz or 151 MHz for ^{13}C-nuclei. The chemical shift (δ) in the NMR-spectra is reported in parts per million (ppm) and referenced by the residual solvent signal. Measurements were performed in $CDCl_3$ and DMSO-d_6. The spectra were referenced to the residual protons and carbons of the solvent ($CHCl_3$: δ(^{1}H) =7.26 ppm, δ(^{13}C) = 77.16 ppm; DMSO-d_6: δ(^{1}H) =2.50 ppm, δ(^{13}C) = 39.52 ppm). Proton-spectra also show the integral intensity, the multiplicity, abbre*via*ted with s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and the coupling constant (*J* in Hz). Assignments of the signals were performed using 2D-NMR techniques such as homonuclear correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond coherence (HMBC). All spectra were analysed with the software MestReNOVA 10.0 from Mestrelab Research S. L. Atom labelling and nomenclature are not in correspondence with IUPAC. High resolution mass spectra (HRMS) were measured on a *Thermo Finnigan MAT 95* (EI) and a *Thermo Finnigan LTQ FTICR* (ESI). IR-measurements were performed on a Perkin Elmer Spectrum BX FT-IR spectrometer with a diamond-ATR (Attenuated Total Reflection) setup. Uncorrected melting points were determined with an automated Stanford Research Systems *EZ-Melt* apparatus (digital image processing technology). Samples were loaded in open capillary tubes. X-ray crystallography of single crystals was performed on an *Oxford XCalibur diffractometer* and further analysis by the software Ortep-3.[37] The structure of the synthesized analog **4** in Fig. 2 was obtained using the geometry optimization tool of the open source software Avogadro and visualized by PyMol.

Nano differential scanning fluorimetry (nanoDSF): Thermal melting experiments of STING constructs were performed using a Tycho NT.6 instrument (NanoTemper Technologies). In brief, the samples were heated up in a glass capillary and while heating, the internal fluorescence at 330 nm and 350 nm was recorded. Data analysis, data smoothing and calculation of derivatives was done using the internal evaluation features of the NT.6 instrument. All measurements were repeated to confirm robustness of the assay. **Isothermal titration calorimetry:** ITC experiments were performed using a Malvern PEAQ-ITC system with 20 µM protein in ITC-buffer (20 mM HEPES pH = 7.5, 150 mM NaCl) in the cell. The positive controls of cGAMP ligands (Biolog) were titrated in a concentration of 200 µM into the cell by 19 injections of 2 µL, spaced 150 s apart, at 25°C. Compound **4** was used in a concentration of 291 µM for titration. The results were analyzed using the MicroCal PEAQ-ITC analysis software provided with the instrument. All titrations were repeated to confirm robustness of the assay. **Cloning, Expression and Purification:** Human STING AA139-379 and mouse STING AA138-378 constructs were cloned according to previous studies.[38] The plasmids were used to transform *E. coli* Rosetta (DE3) protein expression strain cells (Novagen). The cells were grown in 1 L of Turbo Broth™ media (Molecular Dimensions) supplemented with Kanamycin (50 mg/L) and Chloramphenicol (34 mg/L) at 37°C to an OD_{600}=1.3 and expression was induced by adding IPTG to a final concentration of 0.2 mM. Purification of the STING constructs has been performed as described previously.[38]

5-*O*-(*tert*-Butyldimethylsilyl)-3,3-deoxymethylene-1,2-*O*-isopropylidene-α-D-xylofuranose (8): The title compound was prepared according to a modified procedure of Betkekar et al.[39] To a solution of oxalyl chloride (12.3 mL, 17.9 g, 141 mmol, 1.10 eq.) in dry DCM (450 mL) was slowly added DMSO (20.0 mL, 22.0 g, 282 mmol, 2.20 eq.) under N_2 at -78 °C. The temperature was maintained below -60 °C and evolving gas was purged. After the mixture was stirred for 1 h at -60 °C, a solution of 5-*O*-(*tert*-butyldimethylsilyl)-1,2-*O*-isopropylidene-α-D-xylofuranose[40] (39.0 g, 128 mmol, 1.00 eq.) in dry DCM (125 mL) was added to the reaction mixture over 5 min and stirred for 2 h. Triethylamine (53.6 mL, 38.9 g, 384 mmol, 3.00 eq.) was added and the suspension was stirred for a further hour at -60 °C. The reaction mixture was warmed to RT, quenched with saturated aqueous $NaHCO_3$ (200 mL) and extracted with DCM (3x200 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to obtain 5-*O*-(*tert*-butyldimethylsilyl)-3-oxo-1,2-*O*-isopropylidene-α-D-xylofuranose[41] as a waxy yellow solid. The compound was used in the next step without further purification.

Methyl triphenyl phosphonium bromide (86.2 g, 241 mmol, 2.00 eq.) was suspended in THF (360 mL) and cooled to -78 °C under N_2. *n*-Butyl lithium (96.5 mL, 241 mmol, 2.5M in hexanes, 2.00 eq.) was carefully added dropwise and the resulting red suspension (LiBr precipitates) was stirred for 1 h at 0°C. Subsequent addition of a solution of the crude ketone (36.5 g, 121 mmol, 1.00 eq.) in THF (60 mL) over 10 min gave a slurry which was stirred at RT for 6h. The reaction mixture was quenched with saturated aqueous NH_4Cl (50 mL) and then extracted with ethyl acetate (3 x 200 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 9:1→4:1) to yield compound **8** as a colorless syrup (31.1 g, 104 mmol, 81% over 2 steps). R_f = 0.68 (silica, isohexanes/EtOAc = 4:1). IR (ATR): $\tilde{\nu}$ = 2930, 1463, 1372, 1252, 1071, 1018, 775 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 5.85 (d, ^3J = 4.1 Hz, 1H, H-1), 5.42 (dd, ^4J = 2.2 Hz, ^4J = 1.1 Hz, 1H, C=CH^a), 5.26 (m, 1H, C=CH^b), 4.88 (dd, ^3J = 4.1 Hz, ^4J = 1.4 Hz, 1H, H-2), 4.75 (ddd, ^3J = 4.2 Hz, ^3J = 3.8 Hz, ^4J = 2.2 Hz, 1H, H-4), 3.75 (dd, ^2J = 10.6 Hz, ^3J = 4.2 Hz, 1H, H$_a$-5), 3.67 (dd, ^2J = 10.6 Hz, ^3J = 3.8 Hz, 1H, H$_b$-5), 1.49 (s, 3H, C(CH_3)$_2$), 1.38 (s, 3H, C(CH_3)$_2$), 0.87 (s, 9H, SiC(CH_3)$_3$), 0.043 (s, 3H, Si(CH_3)$_2$), 0.040 (s, 3H, Si(CH_3)$_2$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 147.7 (C-3),112.6 (*C*(CH$_3$)$_2$), 111.7 (C=*C*H$_2$), 105.1 (C-1), 82.1 (C-2), 81.0 (C-4), 65.9 (C-5), 27.7 (C(*C*H$_3$)$_2$), 27.5 (C(*C*H$_3$)$_2$), 26.0 (SiC(*C*H$_3$)$_3$), 18.4 (Si*C*(CH$_3$)$_3$), -5.24 (Si(*C*H$_3$)$_2$), -5.31 (Si(*C*H$_3$)$_2$) ppm. ESI-HRMS calcd. for [$C_{15}H_{28}O_4Si$ + NH_4]$^+$ 318.2095, found: 318.2098.

5-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-(hydroxymethyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (9a): The title compound was prepared according to a modified procedure of Betkekar et al.[39] To a solution of vinyl compound **8** (29.5 g, 98.2 mmol, 1.00 eq.) in dry THF (300 mL) was added borane dimethyl sulfide complex (73.6 mL, 147 mmol, 2M in THF, 1.50 eq.) at 0 °C. After the solution was stirred for 12 h at RT, aqueous 2 N NaOH (225 mL) was carefully added under strong gas evolution at 0 °C to give a turbid suspension. The reaction mixture was treated slowly with 30% aqueous hydrogen peroxide (98.0 mL) at the same temperature to avoid heat development. The suspension was stirred for further 2 h at RT, quenched with saturated aqueous $Na_2S_2O_3$ solution (200 mL) at 0 °C and finally extracted with EtOAc (3 x 300 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 4:1→2:1→1:1) to afford alcohol **9a** (23.8 g, 74.7 mmol, 76%) as a colorless oil. R_f = 0.76 (isohexanes/EtOAc = 1:1). IR (ATR): $\tilde{\nu}$ = 3456, 2931, 1463, 1381, 1253, 1105, 1019, 778 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 5.79 (d, ^3J = 3.7 Hz, 1H, H-1), 4.75 (dd, ^3J = 4.9 Hz, ^3J = 3.7 Hz, 1H, H-2), 4.08 (ddd, ^3J = 9.9 Hz, ^3J = 6.6 Hz, ^3J = 3.5 Hz, 1H, H-4), 3.88 (dd, ^2J = 10.6 Hz, ^3J = 3.5 Hz, 1H, H$_a$-5), 3.86 (d, ^3J = 6.2 Hz, 2H, CH_2OH), 3.66 (dd, ^2J = 10.6 Hz, ^3J = 6.6 Hz, 1H, H$_b$-5), 3.06 (s, 1H, O*H*), 2.19-2.09 (m, 1H, H-3), 1.52 (s, 3H, C(CH_3)$_2$), 1.32 (s, 3H, C(CH_3)$_2$), 0.90 (s, 9H, SiC(CH_3)$_3$), 0.091 (s, 3H, Si(CH_3)$_2$), 0.087 (s, 3H, Si(CH_3)$_2$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 112.2 (*C*(CH$_3$)$_2$), 105.0 (C-1), 82.6 (C-2), 80.7 (C-4), 64.0 (C-5), 59.6 (*C*H$_2$OH), 50.0 (C-3), 26.9 (C(*C*H$_3$)$_2$), 26.4 (C(*C*H$_3$)$_2$), 26.0 (SiC(*C*H$_3$)$_3$), 18.4 (Si*C*(CH$_3$)$_3$), -5.32 (Si(*C*H$_3$)$_2$) ppm. ESI-HRMS calcd. for [$C_{15}H_{30}O_5Si$ + NH_4]$^+$: 336.2201, found: 336.2204.

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5-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-formyl-1,2-*O*-isopropylidene-α-D-ribofuranose (9): The title compound was prepared according to a modified procedure of Parr et al.[41] To a solution of oxalyl chloride (6.91 mL, 10.1 g, 79.6 mmol, 1.10 eq.) in dry DCM (380 mL) was slowly added DMSO (11.3 mL, 12.4 g, 159 mmol, 2.20 eq.) under N_2 at -78 °C. The temperature was maintained below -60 °C and evolving gas was purged. After the mixture was stirred for 1 h at -60 °C, a solution of alcohol **9a** (23.0 g, 72.2 mmol, 1.00 eq.) in dry DCM (70 mL) was added to the reaction mixture over 5 min and stirred for 2 h. Triethylamine (30.2 mL, 21.9 g, 217 mmol, 3.00 eq.) was added and the suspension was stirred for a further hour at -60 °C. The reaction mixture was warmed to RT, quenched with saturated aqueous $NaHCO_3$ (150 mL) and extracted with DCM (3x150 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and volatile components removed under reduced pressure. Purification of the crude product was performed by flash-column chromatography (silica gel, isohexanes/EtOAc, 9:1→4:1) to give aldehyde **9** (21.2 g 67.0 mmol, 93%) as a colorless oil. It was also possible to use the crude product in the next step without further purification. R_f = 0.61 (isohexanes/EtOAc = 4:1). IR (ATR): ṽ = 2931, 1726, 1472, 1382, 1253, 1100, 1019, 778 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 9.78 (d, ^4J = 1.2 Hz, 1H, C*H*O), 5.87 (d, ^3J = 3.7 Hz, 1H, H-1), 5.03 (dd, ^3J = 5.2 Hz, ^3J = 3.7 Hz, 1H, H-2), 4.55 (ddd, ^3J = 9.5 Hz, ^3J = 3.6 Hz, ^3J = 3.2 Hz, 1H, H-4), 3.86 (dd, ^2J = 11.3 Hz, ^3J = 3.6 Hz, 1H, H_a-5), 3.78 (dd, ^2J = 11.3 Hz, ^3J = 3.2 Hz, 1H, H_b-5), 3.02 (dd, ^3J = 9.5 Hz, ^3J = 5.2 Hz, ^4J = 1.2 Hz, 1H, H-3), 1.48 (s, 3H, C(C*H*$_3$)$_2$), 1.33 (s, 3H, C(C*H*$_3$)$_2$), 0.86 (s, 9H, SiC(C*H*$_3$)$_3$), 0.04 (s, 3H, Si(C*H*$_3$)$_2$), 0.03 (s, 3H, Si(C*H*$_3$)$_2$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 198.0 (*C*HO), 113.1 (*C*(CH$_3$)$_2$), 105.7 (C-1), 80.8 (C-2), 78.2 (C-4), 62.8 (C-5), 56.6 (C-3), 26.8 (C(*C*H$_3$)$_2$), 26.6 (C(*C*H$_3$)$_2$), 26.0 (SiC(*C*H$_3$)$_3$), 18.5 (Si*C*(CH$_3$)$_3$), 5.31 (Si(CH$_3$)$_2$), -5.31 (Si(*C*H$_3$)$_2$) ppm. EI-HRMS calcd. for $[C_{15}H_{28}O_5Si - CH_3]^+$: 301.1466, found: 301.1475.

5-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-(2,2-dibromovinyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (10a): The title compound was prepared according to a modified procedure of Betkekar et al.[39] A solution of tetrabromomethane (43.0 g, 130 mmol, 2.00 eq.) in DCM (350 mL) was mixed with triphenylphosphine (68.0, 259 mmol, 4.00 eq.) under N_2 at 0 °C and stirred for 1 h at this temperature. The resulting orange solution was treated with a solution of aldehyde **9** (20.5 g, 64.8 mmol, 1.00 eq.) in DCM (120 mL). The dark suspension was stirred at RT for 12 h. After removal of volatile materials, the crude product was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 9:1→4:1) to provide dibromo compound **10a** (26.1 g, 55.3 mmol, 85%) as a slightly yellow oil. R_f = 0.83 (isohexanes/EtOAc = 4:1). IR (ATR): ṽ = 2929, 1471, 1382, 1252, 1097, 1020, 777 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 6.49 (d, ^3J = 9.4 Hz, 1H, Br$_2$CC*H*), 5.82 (d, ^3J = 3.5 Hz, 1H, H-1), 4.68 (dd, ^3J = 4.5 Hz, ^3J = 3.5 Hz, 1H, H-2), 4.03 (ddd, ^3J = 9.9 Hz, ^3J = 3.6 Hz, ^3J = 3.4 Hz, 1H, H-4), 3.81 (dd, ^2J = 11.5 Hz, ^3J = 3.4 Hz, 1H, H_a-5), 3.66 (dd, ^2J = 11.5 Hz, ^3J = 3.6 Hz, 1H, H_b-5), 3.00 (td, ^3J = 9.9 Hz, ^3J = 4.5 Hz, 1H, H-3), 1.53 (s, 3H, C(C*H*$_3$)$_2$), 1.32 (s, 3H, C(C*H*$_3$)$_2$), 0.89 (s, 9H, SiC(C*H*$_3$)$_3$), 0.06 (s, 6H, Si(C*H*$_3$)$_2$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 133.2 (Br$_2$*C*CH), 112.2 (*C*(CH$_3$)$_2$), 105.2 (C-1), 91.8 (Br$_2$*C*CH), 81.7 (C-2), 80.9 (C-4), 62.3 (C-5), 49.2 (C-3), 26.9 (C(*C*H$_3$)$_2$), 26.4 (C(*C*H$_3$)$_2$), 26.1 (SiC(*C*H$_3$)$_3$), 18.5 (Si*C*(CH$_3$)$_3$), 5.16 (Si(*C*H$_3$)$_2$), -5.22 (Si(*C*H$_3$)$_2$) ppm. EI-HRMS calcd. for $[C_{16}H_{28}Br_2O_4Si - CH_3]^+$: 454.9884, found: 454.9882

5-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-ethynyl-1,2-*O*-isopropylidene-α-D-ribofuranose (10b): The title compound was prepared according to a modified procedure of Betkekar et al.[39] Dibromo compound **10a** (25.0 g, 52.9 mmol, 1.00 eq.) was dissolved in dry THF (270 mL) and cooled to -78 °C under N_2. *n*-Butyl lithium (48.7 mL, 122 mmol, 2.5M in hexanes, 2.30 eq.) was added dropwise over a period of 10 min until a red solution was formed. After stirring for 1.5 h at this temperature, the reaction mixture was quenched with saturated aqueous NH_4Cl (100 mL) and extracted with EtOAc (3 x 200 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue was conducted by flash-column chromatography (silica gel, isohexane/EtOAc, 9:1→4:1) to afford ethynyl compound **10b** (13.7 g 43.8 mmol, 83%) as a yellowish oil. R_f = 0.60 (isohexanes/EtOAc, 4:1). IR (ATR): ṽ = 3279, 2930, 1472, 1373, 1252, 1215, 1110, 1017, 815, 777 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 5.81 (d, ^3J = 3.6 Hz, 1H, H-1), 4.72 (dd, ^3J = 4.1 Hz, ^3J = 3.6 Hz, 1H, H-2), 4.11 (ddd, ^3J = 10.1 Hz, ^3J = 2.9 Hz, ^3J = 2.0 Hz, 1H, H-4), 3.97 (dd, ^2J = 12.0, ^3J = 2.0 Hz, 1H, H_a-5), 3.79 (dd, ^2J = 12.0, ^3J = 2.9 Hz, 1H, H_b-5), 2.97 (ddd, ^3J = 10.1 Hz, ^3J = 4.1 Hz, ^4J = 2.6 Hz, 1H, H-3), 2.21 (d, ^4J = 2.6 Hz, 1H, CC*H*), 1.57 (s, 3H, C(C*H*$_3$)$_2$), 1.36 (s, 3H, C(C*H*$_3$)$_2$), 0.89 (s, 9H, SiC(C*H*$_3$)$_3$), 0.08 (s, 3H, Si(C*H*$_3$)$_2$), 0.07 (s, 3H, Si(C*H*$_3$)$_2$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 112.3 (*C*(CH$_3$)$_2$), 105.1 (C-1), 81.9 (C-4), 81.2 (C-2), 78.2 (*C*CH), 72.3 (C*C*H) , 61.1 (C-5), 36.3 (C-3), 26.8 (*C*(CH$_3$)$_2$), 26.5 (C(*C*H$_3$)$_2$), 26.1 (SiC(*C*H$_3$)$_3$), 18.6 (Si*C*(CH$_3$)$_3$), -5.1 (Si(CH$_3$)$_2$), -5.2 (Si(CH$_3$)$_2$) ppm. ESI-HRMS calcd. for $[C_{16}H_{28}O_4Si + NH_4]^+$: 330.2095, found: 330.2098.

3-Deoxy-3-ethynyl-1,2-*O*-isopropylidene-α-D-ribofuranose (10c): The title compound was prepared according to a modified procedure of Betkekar et al.[39] To a yellow solution of ethynyl compound **10b** (13.3 g, 42.6 mmol, 1.00 eq.) in THF (200 mL) was added TBAF (55.3 mL, 55.3 mmol, 1M in THF, 1.30 eq.) at RT. The resulting dark solution was stirred for 4 h at this temperature. The reaction mixture was quenched with silica and the solvent was concentrated under reduced pressure. The crude product was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 2:1→1:1→2:3) to yield alcohol **10c** (8.03 g, 40.5 mmol, 95%) as colorless crystals. M.p. = 43 – 45 °C. R_f = 0.38 (isohexanes/EtOAc = 1:1). IR (ATR): ṽ = 3456, 3279, 2936, 1375, 1249, 1215, 1105, 1007, 871 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 5.83 (d, ^3J = 3.5 Hz, 1H, H-1), 4.75 (dd, ^3J = 4.1 Hz, ^3J = 3.5 Hz, 1H, H-2), 4.17 (ddd, ^3J = 10.3 Hz, ^3J = 3.2 Hz, ^3J = 3.0 Hz, 1H, H-4), (4.00 (dd, ^2J = 12.2 Hz, ^3J = 3.2 Hz, 1H, H_a-5), 3.71 (ddd, ^2J = 12.2 Hz, ^3J = 8.8 Hz, ^3J = 3.0 Hz, 1H, H_b-5), 2.96 (ddd, ^3J = 10.3 Hz, ^3J = 4.1 Hz, ^4J = 2.5 Hz, 1H, H-3), 2.23 (d, ^4J = 2.5 Hz, 1H, CC*H*), 1.84 (dd, ^3J = 8.8 Hz, 4.2 Hz, 1H, O*H*), 1.58 (s, 3H, C(C*H*$_3$)$_2$), 1.37 (s, 3H, C(C*H*$_3$)$_2$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 112.7 (*C*(CH$_3$)$_2$), 105.1 (C-1), 81.4 (C-4), 81.3 (C-2), 77.6 (*C*CH), 72.6 (C*C*H), 60.6 (C-5), 36.3 (C-3), 26.7 (C(*C*H$_3$)$_2$), 26.5 (C(CH$_3$)$_2$) ppm. EI-HRMS calcd. for $[C_{10}H_{14}O_4 - CH_3]^+$: 183.0652, found: 183.0652.

3-Deoxy-3-ethynyl-1,2-*O*-isopropylidene-5-*O*-tosyl-α-D-ribofuranose (10d): *p*-Toluenesulfonyl chloride (15.5 g, 56.8 mmol, 1.50 eq.) was dissolved in dry pyridine (50.0 mL) and added to a solution of alcohol **10c** (7.5 g 37.8 mmol, 1.00 eq.) in pyridine (150 mL) at 0 °C. The reaction mixture was stirred for 18 h at RT and finally quenched with MeOH (10 mL). After removal of volatile components, purification of the residue by flash-column chromatography (silica gel isohexanes/EtOAc, 2:1→1:1) gave tosyl compound **10d** (11.7 g, 33.2 mmol, 87%) as colorless crystals. Crystallization from isohexanes/EtOAc (vapor diffusion) provided suitable single crystals for X-ray characterization. M.p. = 113 – 114 °C. R_f = 0.73 (isohexanes/EtOAc = 1:1). IR (ATR): ṽ = 3296, 2990, 1598, 1450, 1360, 1176, 1097, 958, 813 665 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 7.80 (d, ^3J = 8.2 Hz, 2H, aryl-C*H*-CSO$_3$), 7.34 (d, ^3J = 8.2 Hz, 2H, aryl-C*H*-CCH$_3$), 5.72 (d, ^3J = 3.5 Hz, 1H, H-1), 4.68 (dd, ^3J = 4.0 Hz, ^3J = 3.5 Hz, 1H, H-2),, 4.40 – 4.31 (m, 1H, H_a-5), 4.22 – 4.14 (m, 2H, H-4, H_b-5), 2.84 (ddd, ^3J = 9.7 Hz, ^3J = 4.0 Hz, ^4J = 2.4 Hz, 1H, H-3), 2.44 (s, 3H, aryl-C*H*$_3$), 2.21 (d, ^4J = 2.4 Hz, 1H, CC*H*), 1.52 (s, 3H, C(C*H*$_3$)$_2$), 1.34 (s, 3H, C(C*H*$_3$)$_2$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 145.1 (aryl-*C*-SO$_3$), 132.7 (aryl-*C*-CH$_3$), 130.0 (aryl-CH-CCH$_3$), 128.2 (aryl-CH-CSO$_3$), 112.8 (*C*(CH$_3$)$_2$), 105.0 (C-1), 80.7 (C-2), 78.5 (C-4), 76.5 (*C*CH), 73.3 (C*C*H), 67.4 (C-5), 37.0 (C-3), 26.7 (C(CH$_3$)$_2$), 26.4 (C(CH$_3$)$_2$), 21.8 (aryl-*C*H$_3$) ppm. ESI-HRMS calcd. for $[C_{17}H_{20}O_6S + H]^-$: 353.1054, found: 353.1057. ESI-HRMS calcd. for $[C_{17}H_{20}O_6S + NH_4]^+$: 370.1319, found: 370.1317.

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5-Azido-3,5-dideoxy-3-ethynyl-1,2-*O*-isopropylidene-α-D-ribofuranose (10): A mixture of tosyl compound **10d** (10.5 g, 29.8 mmol, 1.00 eq.) and sodium azide (8.86 g, 95.3 mmol, 3.20 eq.) was suspended in DMF (300 mL) and stirred under N_2 at 80 °C for 3 h. The yellow suspension was diluted with brine (200 mL) and extracted with EtOAc (4 x 300 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* Purification of the crude product by flash-column chromatography (silica gel, isohexanes/EtOAc, 9:1→4:1) yielded azide compound **10** (6.25 g, 28.0 mmol, 94%) as a colorless oil. R_f = 0.67 (isohexanes/EtOAc = 2:1). IR (ATR): ṽ = 3280, 2989, 2100, 1375, 1216, 1166, 1105, 1012, 871 cm^{-1}. ^1H NMR, COSY (400 MHz, $CDCl_3$): δ = 5.86 (d, ^3J = 3.6 Hz, 1H, H-1), 4.75 (dd, ^3J = 4.1 Hz, ^3J = 3.6 Hz, 1H, H-2), 4.24 (ddd, ^3J = 10.2 Hz, ^3J = 3.9 Hz, ^3J = 2.7 Hz, 1H, H-4), 3.76 (dd, ^2J = 13.6 Hz, ^3J = 2.7 Hz, 1H, H_a-5), 3.37 (dd, ^2J = 13.6 Hz, ^3J = 3.9 Hz, 1H, H_b-5), 2.88 (ddd, ^3J = 10.2 Hz, ^3J = 4.1 Hz, ^4J = 2.5 Hz, 1H, H-3), 2.25 (d, ^4J = 2.5 Hz, 1H, CC*H*), 1.57 (s, 3H, C(C*H*$_3$)$_2$), 1.37 (s, 3H, C(C*H*$_3$)$_2$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 112.8 (*C*(CH$_3$)$_2$), 105.1 (C-1), 81.0 (C-2), 79.7 (C-4), 76.9 (*C*CH), 73.2 (C*C*H), 50.7 (C-5), 37.9 (C-3), 26.7 (C(*C*H$_3$)$_2$), 26.4 (C(*C*H$_3$)$_2$) ppm. EI-HRMS calcd. for $[C_{10}H_{13}N_3O_3 - CH_3]^+$: 208.0717, found: 208.0717.

1,2-di-*O*-Acetyl-5-azido-3,5-dideoxy-3-ethynyl-D-ribofuranose (11): A stirred solution of azide compound **10** (6.00 g, 26.9 mmol, 1.00 eq.) in acetic acid (100 mL) and acetic anhydride (50 mL) was treated with concentrated sulfuric acid (96%, 1.30 mL) at 0 °C. The reaction mixture turned dark and was stirred for 5 h at RT. After careful quenching with saturated aqueous $NaHCO_3$ solution (200 mL) and solid $NaHCO_3$ until CO_2 evolution stopped, the reaction was extracted with DCM (4 x 200 mL). The organic phase was washed with brine (200 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The crude product was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 9:1) to afford diacetate compound **11** (5.61 g, 21.0 mmol, 78%) as colorless crystals. α/β = 1:6. β anomer could be isolated for analysis. Crystallization from isohexanes/EtOAc (vapor diffusion) provided suitable single crystals of the β anomer for X-ray characterization. M.p. = 81 – 82 °C. R_f (α anomer) = 0.55 (isohexanes/EtOAc = 4:1). R_f (β anomer) = 0.46 (isohexanes/EtOAc, 4:1). IR (ATR, β anomer): ṽ = 3281, 2934, 2099, 1743, 1438, 1371, 1205, 1097, 1024, 959, cm^{-1}. Major β anomer: ^1H NMR, COSY (400 MHz, $CDCl_3$): δ = 6.12 (s, 1H, H-1), 5.37 (d, ^3J = 4.5 Hz, 1H, H-2), 4.38 (ddd, ^3J = 10.0 Hz, ^3J = 3.2 Hz, ^3J = 3.0 Hz, 1H, H-4), 3.75 (dd, ^2J = 13.7 Hz, ^3J = 3.0 Hz, 1H, H_a-5), 3.39 (ddd, ^3J = 10.0 Hz, ^3J = 4.5 Hz, ^4J = 2.5 Hz, 1H, H-3), 3.24 (dd, ^2J = 13.7 Hz, ^3J = 3.2 Hz, 1H, H_b-5), 2.16 (d, ^4J = 2.5 Hz, 1H, CC*H*), 2.14 (s, 3H, C-2-OCOC*H*$_3$), 2.08 (s, 3H, C-1-OCOC*H*$_3$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 169.5 (C-2-O*C*OCH$_3$), 169.2 (C-1-O*C*OCH$_3$), 98.7 (C-1), 83.7 (C-4), 76.8 (C-2), 76.0 (*C*CH), 73.6 (C*C*H), 51.0 (C-5), 34.5 (C-3), 21.12 (C-1-OCO*C*H$_3$), 20.73 (C-2-OCO*C*H$_3$) ppm. ESI-HRMS calcd. for $[C_{11}H_{13}N_3O_5 + H]^+$: 268.0928, found: 268.0930. ESI-HRMS calcd. for $[C_{11}H_{13}N_3O_5 + NH_4]^+$: 285.1193, found: 285.1196.

6-Benzoylamino-9-(2-*O*-acetyl-5-azido-3,5-dideoxy-3-ethynyl-β-D-ribofuranosyl)-9*H*-purine (12): *N*,*O*-Bis(trimethylsilyl)acetamid (BSA) (3.66 mL, 3.05 g, 15.0 mmol, 4.00 eq.) was added under N_2 to a stirred suspension of diacetate compound **11** (1.00 g, 3.74 mmol, 1.00 eq.) and 6-*N*-benzoyladenine (1.79 g, 7.48 mmol, 2.00 eq.) in dichloroethane (40 mL) and heated to 80 °C for 1 h until a clear solution was obtained. The reaction mixture was brought to RT and treated with trimethylsilyl triflate (TMSOTf) (1.36 mL, 1.66 g, 7.48 mmol, 2.00 eq.). The dark red solution was stirred at 80 °C for 4 h and additional 8 h at RT. The reaction was quenched with saturated aqueous $NaHCO_3$ (30 mL) and extracted with DCM (4 x 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 2:1→1:1→1:2→EtOAc) to provide nucleoside **12** (1.02 g, 2.29 mmol, 61%) as a colorless foam. The reaction could also be performed on a 4 g scale of the diacetate starting material **11** (yield: 50%). M.p. = 110 °C (decomp.). R_f = 0.20 (isohexanes/EtOAc = 1:2). R_f = 0.24 (DCM/MeOH, 100:5). IR (ATR): ṽ = 3296, 3060, 2932, 2103, 1747, 1698, 1581, 1218, 1072, 797 cm^{-1}. ^1H NMR, COSY (400 MHz, $CDCl_3$): δ = 9.27 (s, 1H, N*H*), 8.74 (s, 1H, H-2), 8.16 (s, 1H, H-8), 7.98 (d, ^3J = 7.7 Hz, 2H, aryl-o-C*H*), 7.58 – 7.53 (m, 1H, aryl-p-C*H*), 7.46 (t, ^3J = 7.7 Hz, 2H, aryl-m-C*H*), 6.09 (s, 1H, H-1'), 5.87 (d, ^3J = 5.7 Hz, 1H, H-2'), 4.38 (ddd, ^3J = 10.0 Hz, ^3J = 4.8 Hz, ^4J = 2.7 Hz, 1H, H-4'), 4.07 (ddd, ^3J = 10.0 Hz, ^3J = 5.7 Hz, ^4J = 2.4 Hz, 1H, H-3'), 3.79 (dd, ^2J = 13.6 Hz, ^3J = 2.7 Hz, 1H, H_a-5'), 3.57 (dd, ^2J = 13.6 Hz, ^3J = 4.8 Hz, 1H, H_b-5'), 2.26 (d, ^4J = 2.4 Hz, 1H, CC*H*), 2.18 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 169.6 (OCOCH$_3$), 164.8 (N-*C*O-aryl), 152.8 (C-2), 151.2 (C-4), 149.9 (C-6), 142.0 (C-8), 133.6 (aryl-*C*-CO-N), 132.9 (aryl-p-*C*H), 128.9 (aryl-m-*C*H), 128.0 (aryl-o-*C*H), 123.7 (C-5), 89.7 (C-1'), 83.0 (C-4'), 76.9 (C-2'), 75.7 (*C*CH), 74.4 (C*C*H), 51.2 (C-5'), 36.2 (C-3'), 20.7 (OCO*C*H$_3$) ppm. ESI-HRMS calcd. for $[C_{21}H_{18}N_8O_4 + H]^+$: 447.1524, found: 447.1529.

6-Benzoylamino-9-(2-*O*-acetyl-5-amino-3,5-dideoxy-3-ethynyl-β-D-ribofuranosyl)-9*H*-purine (5a): Trimethylphosphine (4.48 mL, 4.48 mmol, 1.0M in THF, 2.00 eq.) was added to a stirred solution of nucleoside **12** (1.00 g, 2.24 mmol, 1.00 eq.) in THF (25 mL). After 5 min the reaction mixture turned turbid under N_2 evolution and was heated to 40 °C for 1.5 h. The reaction mixture was treated with water (0.44 mL, 24.6 mmol, 11.0 eq.) and stirred for 10 h at RT. Volatile materials were removed under reduced pressure and the residue was purified by flash-column chromatography (silica gel, DCM/MeOH, 100:2→100:5) to give amino compound **5a** (0.62 g, 1.48 mmol, 66%) as a colorless foam. The reaction could also be performed on a 4 g scale of the azide starting material **12** (yield: 56%). R_f = 0.34 (DCM/MeOH = 5:1). IR (ATR): ṽ = 3366, 3275, 2918, 1747, 1640, 1422, 1296, 1138, 943, 860 cm^{-1}. ^1H NMR, COSY (400 MHz, $CDCl_3$): δ = 8.75 (s, 1H, H-2), 8.27 (s, 1H, H-8), 8.00 (d, ^3J = 7.7 Hz, 2H, aryl-o-C*H*), 7.61 – 7.55 (m, 1H, aryl-p-C*H*), 7.49 (t, ^3J = 7.7 Hz, 2H, aryl-m-C*H*), 6.08 (s, 1H, H-1'), 5.81 (d, ^3J = 5.9 Hz, 1H, H-2'), 4.27 (ddd, ^3J = 9.9 Hz, ^3J = 4.8 Hz, ^4J = 3.0 Hz, 1H, H-4'), 4.02 (ddd, ^3J = 9.9 Hz, ^3J = 5.9 Hz, ^4J = 2.4 Hz, 1H, H-3'), 3.24 (dd, ^2J = 14.0 Hz, ^3J = 3.0 Hz, 1H, H_a-5'), 3.01 (dd, ^2J = 14.0 Hz, ^3J = 4.8 Hz, 1H, H_b-5'), 2.23 (d, ^4J = 2.4 Hz, 1H, CC*H*), 2.19 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 169.8 (O*C*OCH$_3$), 164.9 (N-*C*O-aryl), 152.8 (C-2), 151.3 (C-4), 149.9 (C-6), 142.2 (C-8), 133.6 (aryl-*C*-CO-N), 132.9 (aryl-p-*C*H), 128.9 (aryl-m-*C*H), 128.0 (aryl-o-*C*H), 123.7 (C-5), 89.5 (C-1'), 85.4 (C-4'), 77.4 (C-2'), 76.7 (*C*CH), 73.9 (C*C*H), 42.6 (C-5'), 35.8 (C-3'), 20.9 (OCO*C*H$_3$) ppm. ESI-HRMS calcd. for $[C_{21}H_{20}N_6O_4 + H]^+$: 421.1619, found: 421.1623.

6-Benzoylamino-9-(2-*O*-acetyl-5-(*tert*-butoxycarbonyl)amino-3,5-dideoxy-3-ethynyl-β-D-ribofuranosyl)-9*H*-purine (5): A mixture of amine compound **5a** (2.00 g, 4.76 mmol, 1.00 eq.), triethylamine (1.99 mL, 1.44 g, 14.3 mmol, 3.00 eq.) and di-*tert*-butyldicarbonate (1.53 mL, 1.56 g, 7.14 mmol, 1.50 eq.) in dry DCM (40 mL) was stirred at RT for 16 h. MeOH (3 mL) was added and volatile materials were removed *in vacuo*. The residue was purified by flash-column chromatography (silica gel, DCM/MeOH, 100:2→100:3) to give the title compound **5** as a colorless foam (1.59 g, 3.05 mmol, 64%). M.p. = 143 °C (decomp.). R_f = 0.20 (DCM/MeOH = 100:5). IR (ATR): ṽ = 3265, 2977, 1749, 1699, 1610, 1516, 1455, 1248, 1227, 1086 cm^{-1}. ^1H NMR, COSY (400 MHz, $CDCl_3$): δ = 9.15 (s, 1H, N*H*Bz), 8.79 (s, 1H, H-2), 8.03 (s, 1H, H-8), 8.01 (d, ^3J = 7.7 Hz, 2H, aryl-o-C*H*), 7.63 – 7.56 (m, 1H, aryl-p-C*H*), 7.49 (t, ^3J = 7.7 Hz, 2H, aryl-m-C*H*), 6.26 (dd, ^3J = 7.0 Hz, ^4J = 3.7 Hz, 1H, N*H*Boc), 5.99 (d, ^3J = 2.4 Hz, 1H, H-1'), 5.66 (dd, ^3J = 6.6 Hz, ^3J = 2.4 Hz, 1H, H-2'), 4.43 (ddd, ^3J = 9.4 Hz, ^3J = 3.7 Hz, ^3J = 3.2 Hz, 1H, H-4'), 4.03 (ddd, ^3J = 9.4 Hz, ^3J = 6.6 Hz, ^4J = 2.4 Hz, 1H, H-3'), 3.67 (ddd, ^2J = 14.5 Hz, ^3J = 7.0 Hz, ^3J = 3.7 Hz, 1H, H_a-5'), 3.57 (ddd, ^2J = 14.5 Hz, ^3J = 7.0 Hz, ^3J = 3.2 Hz, 1H, H_b-5'), 2.28 (d, ^4J = 2.4 Hz, 1H, CC*H*), 2.18 (s, 3H, OCOC*H*$_3$), 1.46 (s, 9H, C(C*H*$_3$)$_3$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 170.0 (O*C*OCH$_3$), 164.7 (N-*C*O-aryl), 156.5 (N-*C*O-O), 152.9 (C-2), 151.1 (C-4), 150.2 (C-6), 142.4 (C-8), 133.5 (aryl-*C*-CO-N), 133.0 (aryl-p-*C*H), 129.0

(aryl-m-*C*H), 128.1 (aryl-o-*C*H), 124.2 (C-5), 90.5 (C-1'), 83.5 (C-4'), 79.6 (*C*(CH_3)$_3$), 77.4 (C-2'), 76.4 (*C*CH), 74.4 (C*C*H), 41.6 (C-5'), 35.8 (C-3'), 28.6 (C(CH_3)$_3$), 20.8 (OCO*C*H$_3$) ppm. ESI-HRMS calcd. for [$C_{26}H_{28}N_6O_6$ + H]$^+$: 521.2143, found: 521.2150.

Benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside (14): The title compound was prepared according to a modified procedure of Shing et al.[42] Benzyl β-D-arabinopyranoside (48.0 g, 200 mmol, 1.00 eq.) was suspended in acetone (500 mL) and 2,2-dimethoxypropane (49.0 mL, 41.6 g; 400 mmol, 2.00 eq.). After addition of *p*-toluenesulfonic acid monohydrate (1.14 g, 5.99 mmol, 0.03 eq.), the reaction mixture was stirred at 60 °C for 2 h to obtain a clear solution. The reaction was neutralized by treatment with triethylamine (0.84 mL, 0,61 g, 5.99 mmol, 1.00 eq.). Volatile components were removed *in vacuo* and the residue was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 2:1→3:2→1:1, gradient elution) to furnish the title compound **14** (46.8 g, 167 mmol, 84%) as a colorless oil. R_f = 0.54 (isohexanes/EtOAc = 1:1). IR (ATR): ṽ = 3221, 2938, 1499, 1453, 1315, 1252, 1048, 1001, 848, 783, 701 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 7.42 – 7.28 (m, 5H, aryl-H), 4.94 (d, ^3J = 3.6 Hz, 1H, H-1), 4.79 (d, ^2J = 11.7 Hz, 1H, PhCH_2O), 4.55 (d, ^2J = 11.7 Hz, 1H, PhCH_2O), 4.24 (ddd, ^3J = 6.2 Hz, ^3J = 2.6 Hz, ^3J = 1.2 Hz, 1H, H-4), 4.21 (q, ^3J = 6.2 Hz, 1H, H-3), 4.01 (dd, ^2J = 13.2 Hz, ^3J = 2.6 Hz, 1H, H_a-5), 3.93 (dd, ^2J = 13.2 Hz, ^3J = 1.2 Hz, 1H, H_b-5), 3.80 (dd, ^3J = 6.2 Hz, 3.6 Hz, 1H, H-2), 2.22 (br s, 1H, O*H*), 1.53 (s, 3H, CH_3), 1.36 (s, 3H, CH_3) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 137.1 (aryl-*C*-CH$_2$), 128.7 (aryl-m-*C*H), 128.22 (aryl-p-*C*H), 128.15 (aryl-o-*C*H), 109.4 (*C*(CH_3)$_2$), 97.0 (C-1), 76.1 (C-4), 73.1 (C-3), 70.1 (C-2), 69.9 (Ph*C*H$_2$O), 59.9 (C-5), 28.0 (*C*H$_3$), 26.1 (CH$_3$) ppm. ESI-HRMS calcd. for [$C_{15}H_{20}O_5$ + NH_4]$^+$: 298.1649, found: 298.1651. EI-HRMS calcd. for [$C_{15}H_{20}O_5$ - CH_3]$^+$: 265.1071, found: 265.1084.

Benzyl 2-deoxy-2-*C*-[(ethoxycarbonyl)methylene]-3,4-*O*-isopropylidene-β-D-arabinofuranoside (15): The title compound was prepared according to a modified procedure of Kaiya et al.[43] Oxalyl chloride (15.3 mL, 22.4 g, 176 mmol, 1.15 eq.) was dissolved in dry DCM (600 mL). After cooling to −78 °C, dry DMSO (25.1 mL, 27.6 g, 352 mmol, 2.30 eq.) was added dropwise and the mixture was stirred at −60 °C for 1 h until no further gas development was observed. Subsequently, a solution of acetonide compound **14** (43.0 g, 153 mmol, 1.00 eq.) in dry DCM (150 mL) was added slowly over 10 min and the mixture was stirred at -60 °C for 2 h. The reaction mixture was treated with triethylamine (64.1 mL, 46.6 g, 460 mmol, 3.00 eq.), stirred at -60 °C for 1 h, quenched upon addition of saturated aqueous $NaHCO_3$ (300 mL) and extracted with DCM (3 x 300 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Ketone **15a** was obtained as a waxy syrup which was used in the next step without further purification. EI-HRMS calcd. for [$C_{15}H_{18}O_5$ - CH_3]$^+$: 263.0914, found: 263.0914.

A mixture of crude ketone **15a** (42.0 g, 151 mmol, 1.00 eq.) and (carbethoxymethylene)triphenylphosphorane[44] (68.3 g, 196 mmol, 1.30 eq.) in DCM (400 mL) was stirred at RT for 12 h. Volatile components were evaporated and the residue was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 4:1) to afford the title compound **15** as a colorless oil (46.0 g, 132 mmol, 86% over 2 steps). E/Z = 4:1 (inseparable mixture by fcc). R_f = 0.84 (isohexanes/EtOAc = 2:1). IR (ATR, E/Z-mixture): ṽ = 2983, 1718, 1372, 1214, 1150, 1020, 853, 736, 699 cm^{-1}. Major E-Isomer: ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 7.41 – 7.32 (m, 5H, aryl-H), 6.41 (d, ^4J = 1.8 Hz, 1H, C=C*H*), 6.06 (d, ^3J = 7.5 Hz, 1H, H-3), 5.44 (d, ^4J = 1.8 Hz, 1H, H-1), 4.86 (d, ^2J = 11.9 Hz, 1H, OCH_2Ph), 4.62 (d, ^2J = 11.9 Hz, 1H, OCH_2Ph), 4.34 (dd, ^3J = 7.5 Hz, ^3J = 1.7 Hz, 1H, H-4), 4.20 (q, ^3J = 7.1 Hz, 2H, OCH_2CH$_3$), 3.70 (d, ^3J = 1.7 Hz, 2H, H-5), 1.53 (s, 3H, C(CH_3)$_2$), 1.40 (s, 3H, C(CH_3)$_2$), 1.30 (t, ^3J = 7.1 Hz, 3H, OCH$_2$CH_3) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 165.5 (*C*=O), 147.9 (C-2), 137.6 (aryl-*C*-CH$_2$), 128.6 (aryl-m-*C*H), 128.1 (aryl-p-*C*H), 128.0 (aryl-o-*C*H), 124.4 (C=*C*H), 110.6 (*C*(CH_3)$_2$), 95.8 (C-1), 75.2 (C-4), 69.5 (Ph*C*H$_2$O), 68. 6 (C-3), 63.2 (C-5), 60.8 (O*C*H$_2$CH$_3$), 26.4 (C(*C*H$_3$)$_2$), 25.3 (C(*C*H$_3$)$_2$), 14.3 (OCH$_2$*C*H$_3$) ppm. ESI-HRMS calcd. for [$C_{19}H_{24}O_6$ + NH_4]$^+$: 366.1911, found: 366.1911.

Benzyl 2-deoxy-2-*C*-[(ethoxycarbonyl)methyl]-3,4-*O*-isopropylidene-β-D-ribopyranoside (16): The title compound was prepared according to a modified procedure of Kaiya et al.[43] Vinyl compound **15** (45.0 g, 129 mmol, 1.00 eq.) was dissolved in EtOH (300 mL) and Raney-Ni (ca. 15 mL) was added to the solution at RT. The reaction vessel was evacuated and flushed with hydrogen three times. Subsequently, the mixture was stirred under hydrogen atmosphere for 20 h. Upon completion of the reaction as monitored by TLC, the reaction mixture was filtered through celite. Volatile materials were removed *in vacuo* and the residue was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 4:1) to yield reduced compound **16** (40.8 g, 116 mmol, 90%) as a colorless oil. dr = 13:1. R_f = 0.33 (isohexanes/EtOAc = 4:1). IR (ATR): ṽ = 2983, 1732, 1455, 1370, 1212, 1071, 1021, 870, 738, 699 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 7.38 – 7.25 (m, 5H, aryl-H), 4.82 (d, ^3J = 11.7 Hz, 1H, OCH_2Ph), 4.64 (d, ^3J = 8.0 Hz, 1H, H-1), 4.49 (dd, ^3J = 7.4 Hz, ^3J = 2.8 Hz, 1H, H-3), 4.48 (d, ^3J = 11.7 Hz, 1H, OCH_2Ph), 4.24 (ddd, ^3J = 7.4 Hz, ^3J = 2.6 Hz, ^3J = 2.3 Hz, 1H, H-4), 4.10 (q, ^3J = 7.2 Hz, 2H, OCH_2CH$_3$), 3.84 (dd, ^2J = 12.8 Hz, ^3J = 2.6 Hz, 1H, H_a-5), 3.63 (dd, ^2J = 12.8 Hz, ^3J = 2.3 Hz, 1H, H_b-5), 2.60 – 2.55 (m, 2H, CH_2COO), 2.28 (ddd, ^3J = 8.0 Hz, ^3J = 6.5 Hz, ^3J = 2.8 Hz, 1H, H-2), 1.47 (s, 3H, C(CH_3)$_2$), 1.31 (s, 3H, C(CH_3)$_2$), 1.22 (t, ^3J = 7.2 Hz, 3H, OCH$_2$CH_3) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 172.2 (*C*=O), 138.1 (aryl-*C*-CH$_2$), 128.5 (aryl-m-*C*H), 128.1 (aryl-p-*C*H), 127.8 (aryl-o-*C*H), 109.1 (*C*(CH_3)$_2$), 99.0 (C-1), 73.2 (C-4), 72.6 (C-3), 69.7 (Ph*C*H$_2$O), 62.6 (C-5), 60.7 (O*C*H$_2$CH$_3$), 37.2 (C-2), 33.6 (*C*H$_2$COO), 26.7 (C(*C*H$_3$)$_2$), 25.0 (C(*C*H$_3$)$_2$), 14.3 (OCH$_2$*C*H$_3$) ppm. EI-HRMS calcd. for [$C_{19}H_{26}O_6$ - CH_3]$^+$:335.1489, found: 335.1481.

2-Deoxy-3,4-*O*-isopropylidene-2-*C*-[(ethoxycarbonyl)methyl]-D-ribopyranose (17a): To a stirred solution of ester compound **16** (38.0 g, 108 mmol, 1.00 eq.) in EtOH (250 mL) and THF (100 mL) was added Pd/C (10 wt.%, 1.70 g) under N_2 at RT. The reaction vessel was evacuated and flushed with hydrogen three times. The mixture was stirred under hydrogen atmosphere for 24 h and then filtered through celite. The solution was concentrated to dryness under reduced pressure and the residue was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 4:1→1:1) to furnish anomeric alcohol **17a** as a colorless oil (24.8 g, 95.3 mmol, 88%). α/β = 1:9 (inseparable mixture by fcc). R_f = 0.36 (isohexanes/EtOAc = 1:1). IR (ATR, α/β-mixture): ṽ = 2984, 1731, 1458, 1371, 1213, 1109, 1056, 1020, 868 cm^{-1}. Major β anomer: ^{1}H NMR, COSY (400 MHz, CDCl3): δ = 4.88 (dd, ^3J = 8.3 Hz, ^3J = 4.8 Hz, 1H, H-1), 4.44 (dd, ^3J = 7.1 Hz, ^3J = 2.9 Hz, 1H, H-3), 4.23 (ddd, ^3J = 7.1 Hz, ^3J = 3.7 Hz, ^3J = 3.4 Hz, 1H, H-4), 4.16 (q, ^3J = 7.1 Hz, 2H, OCH_2CH$_3$), 3.88 (dd, ^2J = 12.6 Hz, ^3J = 3.4 Hz, 1H, H_a-5), 3.60 (dd, ^2J = 12.6, ^3J = 3.7 Hz, 1H, H_b-5), 3.26 (d, ^3J = 4.8 Hz, 1H, O*H*), 2.66 (dd, ^2J = 16.9 Hz, ^3J = 5.9 Hz, 1H, CH_2COO), 2.59 (dd, ^3J = 16.9 Hz, ^2J = 8.3 Hz, 1H, CH_2COO), 2.20 (ddd, ^3J = 8.3 Hz, ^3J = 5.9 Hz, ^3J = 2.9 Hz, 1H, H-2), 1.45 (s, 3H, (CH_3)$_2$), 1.30 (s, 3H, C(CH_3)$_2$), 1.26 (t, J = 7.1 Hz, 3H, OCH$_2$CH_3) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 172.8 (*C*=O), 109.2 (*C*(CH_3)$_2$), 94.4 (C-1), 73.4 (C-3), 72.7 (C-4), 63.3 (C-5), 61.0 (O*C*H$_2$CH$_3$), 38.5 (C-2), 33.5 (*C*H$_2$COO), 27.0 (C(*C*H$_3$)$_2$), 25.27 (C(*C*H$_3$)$_2$), 14.3 (OCH$_2$*C*H$_3$) ppm. EI-HRMS calcd. for [$C_{12}H_{20}O_6$ - CH_3]$^+$: 245.1020, found: 245.1023.

Methyl 2-*C*-carboxymethyl-2-deoxy-2,3-lactone-D-ribofuranoside (17): The title compound was prepared according to a modified procedure of Li et al.[45] Anomeric alcohol **17a** (20.7 g, 79.5 mmol, 1.00 eq.) was dissolved in AcOH/H_2O (v/v 4:1, 400 mL) and stirred at RT for 24 h. Tthe mixture was heated to 40 °C for additional 2 h. Volatile materials were evaporated and the crude product **17b** was co-evaporated with toluene (3 x 300 mL) and used in the next step without further purification.

R_f = 0.05 (isohexanes/EtOAc = 1:1). EI-HRMS calcd. for $[C_{12}H_{20}O_6 - 2 \times H_2O]^+$: 184.0730, found: 184.0726.
Concentrated sulfuric acid (0.56 mL) was added to a stirred solution of triol compound **17b** in dry methanol (450 mL) at 0 °C. The reaction mixture was stirred at 4 °C for 72 h and then neutralized by addition of solid sodium bicarbonate. The resulting suspension was filtered through celite and the filtrate was concentrated to dryness under reduced pressure. Purification by flash-column chromatography (silica gel, isohexanes/EtOAc, 2:1→1:1→1:3) yielded lactone **17** (10.8 g, 57.4 mmol, 72% over 2 steps) as colorless crystals. α/β = 2:3. β anomer could be isolated for analysis. M.p. = 45 – 47 °C. R_f (α anomer) = 0.44 (DCM/MeOH = 100:5). R_f (β anomer) = 0.30 (DCM/MeOH = 100:5). IR (ATR, β anomer): ṽ = 3442, 2940, 1775, 1172, 1102, 1031, 1003, 932 cm^{-1}. Major β anomer: ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 5.16 (dd, ^3J = 7.0 Hz, ^3J = 0.9 Hz, 1H, H-3), 4.90 (d, ^3J = 1.4 Hz, 1H, H-1), 4.49 – 4.44 (m, 1H, H-4), 3.73 (dd, ^2J = 12.8 Hz, ^3J = 2.9 Hz, 1H, H_a-5), 3.68 (dd, ^2J = 12.8, ^3J = 3.6 Hz, 1H, H_b-5), 3.41 (s, 3H, OCH_3), 3.13 – 3.05 (m, 1H, H-2), 2.87 (dd, ^2J = 18.6 Hz, ^3J = 11.0 Hz,1H, CH_2COO), 2.55 (dd, ^2J = 18.6 Hz, ^3J = 3.7 Hz, 1H, CH_2COO) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 175.6 (C=O), 111.8 (C-1), 86.9 (C-4), 84.8 (C-3), 63.7 (C-5), 55.8 (OCH_3), 46.6 (C-2), 32.4 (CH_2COO) ppm. EI-HRMS calcd. for $[C_8H_{12}O_5 - CH_2OH]^+$: 157.0495, found: 157.0494.

Methyl 2-*C*-carboxymethyl-2,5-dideoxy-2,3-lactone-5-tosyl-D-ribofuranoside (18): To a stirred solution of lactone compound **17** (10.0 g, 53.1 mmol, 1.00 eq.) in dry pyridine (300 mL) was added a solution of *p*-toluenesulfonyl chloride (13.2 g, 69.1 mmol, 1.30 eq.) in pyridine (40 mL) at 0 °C. After stirring at RT for 18 h, the reaction was quenched by treatment with MeOH (20 mL). Solvents were removed *in vacuo* and the residue was purified by flash-column chromatography (silica gel, hexane/EtOAc, 3:2→1:1→1:3) to give tosyl compound **18** as colorless crystals (13.9 g, 40.6 mmol, 76%). α/β = 2:3 (inseparable mixture by fcc). Crystallization from isohexanes/EtOAc (vapor diffusion) provided suitable β single crystals for X-ray characterization. M.p. = 78 – 80 °C. R_f = 0.36 (isohexanes/EtOAc = 1:1). IR (ATR, α/β-mixture): ṽ = 2938, 1781, 1598, 1358, 1173, 1111, 979, 815, 665 cm^{-1}. β anomer: ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 7.82 – 7.75 (m, 2H, aryl H-2-2'), 7.40 – 7.32 (m, 2H, aryl H-3,-3'), 4.98 (dd, ^3J = 7.0 Hz, ^3J = 0.9 Hz, 1H, H-3), 4.86 (d, ^3J = 1.1 Hz, 1H, H-1), 4.41 – 4.34 (m, 1H, H-4), 4.10 (dd, ^2J = 10.3 Hz, ^3J = 7.6 Hz, 1H, H_a-5), 4.06 (dd, ^2J = 10.3 Hz, ^3J = 6.4 Hz, 1H, H_b-5), 3.24 (s, 3H, OCH_3), 3.13 – 3.05 (m, 1H, H-2), 2.82 (dd, ^3J = 18.6 Hz, ^3J = 11.0 Hz, 1H, CH_2COO), 2.56 – 2.47 (m, 1H, CH_2COO), 2.45 (s, 3H, aryl CH_3) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 175.3 (C=O), 145.5 (aryl C-4), 132.5 (aryl C-1), 130.13 (aryl C-3,-3'), 128.02 (aryl C-2,-2'), 111.6 (C-1), 83.9 (C-3), 82.2 (C-4), 68.3 (C-5), 55.5 (OCH_3), 45.5 (C-2), 31.6 (CH_2COO), 21.8 (aryl CH_3) ppm. α anomer: ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 7.82 – 7.75 (m, 2H, aryl H-2-2'), 7.40 – 7.32 (m, 2H, aryl H-3-3'), 4.99 (d, ^3J = 3.8 Hz, 1H, H-1), 4.82 (dd, ^3J = 7.6 Hz, ^3J = 2.7 Hz, 1H, H-3), 4.32 – 4.28 (m, 1H, H-4), 4.23 (dd, ^2J = 11.0 Hz, ^3J = 3.1 Hz, 1H, H_a-5), 4.20 (dd, ^2J = 11.0 Hz, ^3J = 3.4 Hz, 1H, H_b-5), 3.31 (s, 3H, OCH_3), 3.05 – 2.98 (m, 1H, H-2), 2.67 (dd, ^3J = 17.7 Hz, ^3J = 1.5 Hz, 1H, CH_2COO), 2.56 – 2.47 (m, 1H, CH_2COO), 2.45 (s, 3H, aryl CH_3) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 176.2 (C=O), 145.4 (aryl C-4), 132.4 (aryl C-1), 130.11 (aryl C-3,-3'), 128.03 (aryl C-2,-2'), 104.5 (C-1), 83.1 (C-3), 80.2 (C-4), 69.0 (C-5), 55.4 (OCH_3), 44.1 (C-2), 29.0 (CH_2COO), 21.8 (aryl CH_3) ppm. ESI-HRMS calcd. for $[C_{12}H_{20}O_6 + NH_4]^+$: 360.1111, found: 360.1109.

Methyl 5-azido-2-*C*-carboxymethyl-2,5-dideoxy-2,3-lactone-D-ribofuranoside (19): A mixture of tosyl compound **18** (13.0 g, 38.0 mmol, 1.00 eq.) and sodium azide (14.1 g, 152 mmol, 4.00 eq.) was suspended in DMF (300 mL) and stirred under N_2 at 80 °C for 3 h. The yellow suspension was diluted with brine (200 mL) and extracted with EtOAc (4x300 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 4:1→2:1→1:1) to afford azide compound **19** (6.08 g, 28.5 mmol, 75%) as a colorless oil. α/β = 2:3 (inseparable mixture by fcc). R_f = 0.46 (isohexanes/EtOAc = 1:1). IR (ATR, α/β-mixture): ṽ = 2936, 2100, 1776, 1444, 1282, 1160, 1110, 1031, 920 cm^{-1}. β anomer: ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 4.95 (dd, ^3J = 7.3 Hz, ^3J = 1.3 Hz, 1H, H-3), 4.92 (d, ^3J = 1.3 Hz, 1H, H-1), 4.41 – 4.36 (m, 1H, H-4), 3.53 (dd, ^2J = 12.7 Hz, ^3J = 7.2 Hz, 1H, H_a-5), 3.39 (s, 3H, OCH_3), 3.38 (m, 1H, H_b-5), 3.18 – 3.11 (m, 1H, H-2), 2.86 (dd, ^2J = 18.6 Hz, ^3J = 10.9 Hz, 1H, CH_2COO), 2.56 (dd, ^2J = 18.6 Hz, ^3J = 4.7 Hz, 1H, CH_2COO) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 175.3 (C=O), 111.9 (C-1), 84.7 (C-3), 84.0 (C-4), 55.7 (OCH_3), 53.1 (C-5), 45.8 (C-2), 31.8 (CH_2COO) ppm. α anomer: ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 5.11 (d, ^3J = 5.2 Hz, 1H, H-1), 4.81 (dd, ^3J = 7.8 Hz, ^3J = 2.9 Hz, 1H, H-3), 4.37 – 4.33 (m, 1H, H-4), 3.68 (dd, ^2J = 13.2 Hz, ^3J = 3.4 Hz, 1H, H_a-5), 3.43 (dd, ^2J = 13.2 Hz, ^3J = 3.8 Hz, 1H, H_b-5), 3.38 (s, 3H, OCH_3), 3.12 – 3.05 (m, 1H, H-2), 2.73 (dd, ^2J = 17.7 Hz, ^3J = 1.6 Hz, 1H, CH_2COO), 2.54 (dd, ^2J = 17.7 Hz, ^3J = 9.1 Hz, 1H, CH_2COO) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 176.3 (C=O), 104.4 (C-1), 83.8 (C-3), 81.6 (C-4), 55.3 (OCH_3), 52.1 (C-5), 44.4 (C-2), 29.0 (CH_2COO) ppm. ESI-HRMS calcd. for $[C_8H_{11}N_3O_4 + NH_4]^+$: 231.1088, found: 231.1088.

Methyl 5-azido-3-*O*-benzyl-2,5-dideoxy-2-*C*-[(benzyloxycarbonyl)methylene]-D-ribofuranoside (20): The title compound was prepared according to a modified procedure of Webber et al.[46] Azide compound **19** (5.05 g, 23.7 mmol, 1.00 eq.) was mixed with KOH (10.6g, 190 mmol, 8.00 eq.) in THF (250 mL). The stirred suspension was treated with benzyl bromide (28.1 mL, 40.5 g, 237 mmol, 10.0 eq.) and refluxed for 5 h. After cooling to 0 °C, the reaction was diluted with water (250 mL) and extracted with EtOAc (3 x 300 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 9:1→4:1→2:1) to furnish benzylated compound **20** as a colorless oil (8.89 g, 21.6 mmol, 91%). α/β = 2:3. β anomer could be isolated for analysis. R_f (α anomer) = 0.57 (isohexanes/EtOAc = 4:1). R_f (β anomer) = 0.48 (isohexanes/EtOAc = 4:1). IR (ATR, β anomer): ṽ = 2931, 2100, 1733, 1455, 1282, 1168, 1057, 910, 738, 698 cm^{-1}. β anomer: 1H NMR, COSY (400 MHz, $CDCl_3$): δ = 7.40 – 7.26 (m, 10H, aryl H), 5.11 (d, ^2J = 12.3 Hz, 1H, COOCH_2Ph), 5.06 (d, ^2J = 12.3 Hz, 1H, COOCH_2Ph), 4.82 (d, ^3J = 2.3 Hz, 1H, H-1), 4.43 (s, 2H, OCH_2Ph), 4.16 – 4.11 (m, 1H, H-3), 4.14 – 4.08 (m, 1H, H-4), 3.37 (s, 3H, OCH_3), 3.32 (dd, ^2J = 12.7 Hz, ^3J = 6.2 Hz, 1H, H_a-5), 3.25 (dd, ^2J = 12.7 Hz, ^3J = 4.3 Hz, 1H, H_b-5), 2.86 – 2.79 (m, 1H, H-2), 2.74 (dd, ^2J = 16.5 Hz, ^3J = 7.6 Hz, 1H, CH_2COO), 2.43 (dd, ^2J = 16.5 Hz, ^3J = 7.4 Hz, 1H, CH_2COO) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 172.2 (C=O), 137.5 (OCH_2*Ph*-C-1), 135.9 (COOCH_2*Ph*-C-1), 128.7, 128.6, 128.41, 128.38, 128.08, 127.8 (aryl 10C), 109.4 (C-1), 81.4 (C-4), 80.3 (C-3), 72.6 (OCH_2Ph), 66.6 (COOCH_2Ph), 55.8 (OCH_3), 54.3 (C-5), 44.2 (C-2), 30.5 (CH_2COO) ppm. ESI-HRMS calcd. for $[C_{22}H_{25}N_3O_5 + NH_4]^+$: 429.2132, found: 429.2138.

Acetyl 5-azido-3-*O*-benzyl-2,5-dideoxy-2-*C*-[(benzyloxycarbonyl)methylene]-D-ribofuranoside (21): To a solution of benzylated compound **20** (8.02 g, 19.5 mmol, 1.00 eq.) in AcOH (70 mL) and Ac_2O (70 mL), was added concentrated H_2SO_4 (0.20 mL) at 0 °C. The solution was warmed to RT and stirred for 3 h. After careful quenching with saturated $NaHCO_3$ solution (150 mL) and solid $NaHCO_3$ until CO_2 evolution stopped, the reaction was extracted with DCM (4 x 200 mL), washed with brine (400 mL), dried over anhydrous $MgSO_4$ and filtered. Volatile materials were removed *in vacuo* and the residue was co-evaporated with toluene (2 x 100 mL). The crude product was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 9:1→4:1→2:1) to obtain acetylated compound **21** as a colorless oil (7.27 g, 16.5 mmol, 85%). α/β = 3:2 (inseparable mixture by fcc). R_f = 0.25 (isohexanes/EtOAc = 4:1). IR (ATR): ṽ = 2101, 1733, 1455, 1366, 1230, 1170, 1007, 899, 738, 698 cm^{-1}. β anomer: ^{1}H NMR, COSY (400 MHz,

$CDCl_3$): δ = 7.26 (s, 10H, aryl H), 6.09 (d, *J* = 2.4 Hz, 1H, H-1), 5.09 (d, ^2J = 12.2 Hz, 1H, COOCH_2Ph), 5.04 (d, ^2J = 12.2 Hz, 1H, COOCH_2Ph), 4.45 (s, 2H, OCH_2Ph), 4.28 – 4.24 (m, 1H, H-3), 4.16 (ddd, ^3J = 9.6 Hz, ^3J = 5.0 Hz, ^3J = 4.6 Hz, 1H, H-4), 3.43 (dd, ^2J = 13.2 Hz, ^3J = 4.6 Hz, 1H, H_a-5), 3.23 (dd, ^2J = 13.2 Hz, ^3J = 5.0 Hz, 1H, H_b-5), 3.00 – 2.92 (m, 1H, H-2), 2.78 (dd, ^2J = 16.9 Hz, ^3J = 8.4 Hz, 1H, CH_2COO), 2.61 (dd, ^2J = 16.9 Hz, ^3J = 7.1 Hz, 1H, CH_2COO), 2.07 (s, 3H, CH_3COO) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 171.8 (*C*OOBn), 170.1 (*C*OOCH$_3$), 137.3 (OCH$_2$*Ph*-C-1), 135.7 (COOCH$_2$*Ph*-C-1), 128.71, 128.64, 128.51, 128.47, 128.20, 127.84 (aryl 10C), 101.3 (C-1), 82.2 (C-4), 78.9 (C-3), 72.8 (O*C*H$_2$Ph), 66.8 (COO*C*H$_2$Ph), 52.5 (C-5), 43.7 (C-2), 30.3 (*C*H$_2$COO), 21.3 (*C*H$_3$COO) ppm. α anomer: ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 7.41 – 7.22 (m, 10H, aryl H), 6.36 (d, ^3J = 4.8 Hz, 1H, H-1), 5.10 (s, 2H, COOCH_2Ph), 4.46 (d, ^2J = 12.0 Hz, 1H, OCH_2Ph), 4.42 (d, ^2J = 12.0 Hz, 1H, OCH_2Ph), 4.32 – 4.27 (m, 1H, H-4), 4.01 (dd, ^3J = 7.0, ^3J = 2.3 Hz, 1H, H-3), 3.35 (dd, ^2J = 12.9 Hz, ^3J = 5.1 Hz, 1H, H_a-5), 3.15 (dd, ^2J = 12.9 Hz, ^3J = 4.2 Hz, 1H, H_b-5), 2.89 – 2.82 (m, 1H, H-2), 2.77 (dd, ^2J = 16.7 Hz, ^3J = 6.6 Hz, 1H, CH_2COO), 2.61 (dd, ^2J = 16.7 Hz, ^3J = 5.8 Hz, 1H, CH_2COO), 2.05 (s, 3H, CH_3COO) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 172.0 (*C*OOBn), 170.5 (CH$_3$*C*OO), 137.8 (OCH$_2$*Ph*-C-1), 135.8 (COOCH$_2$*Ph*-C-1), 128.74, 128.59, 128.52, 128.51, 128.05, 127.80 (aryl 10C), 98.2 (C-1), 84.4 (C-4), 79.2 (C-3), 72.6 (O*C*H$_2$Ph), 66.7 (COO*C*H$_2$Ph), 52.7 (C-5), 43.1 (C-2), 28.5 (*C*H$_2$COO) 21.3 (*C*H$_3$COO) ppm. ESI-HRMS calcd. for $[C_{23}H_{25}N_3O_6 + NH_4]^+$: 457.2081, found: 457.2082.

9-{5-Azido-3-*O*-benzyl-2,5-dideoxy-2-*C*-[(benzyloxycarbonyl)methylene]-β-D-ribofuranosyl}-6-*O*-(diphenylcarbamoyl)-2-*N*-isobutyrylguanine (6): *N*,*O*-Bis(trimethylsilyl)acetamide (BSA) (2.23 mL, 1.85 g, 9.10 mmol, 4.00 eq.) was added under N_2 to a stirred suspension of compound **21** (1.00 g, 2.28 mmol, 1.00 eq.) and 6-*O*-(diphenylcarbamoyl)-2-*N*-isobutyrylguanine[47,48] (1.90 g, 4.55 mmol, 2.00 eq.) in dichloroethane (30 mL) and heated to 80 °C for 30 min until a clear solution was obtained. The reaction mixture was brought to RT and treated with trimethylsilyl triflate (TMSOTf) (1.07 mL, 1.32 g, 5.92 mmol, 2.60 eq.). The dark red solution was stirred at 80 °C for 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (30 mL) at RT and extracted with DCM (4 x 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash-column chromatography (silica gel, isohexanes/EtOAc, 4:1→2:1→1:1) to give nucleoside **6** (1.31 g, 1.65 mmol, 72%) as a colorless foam. The reaction could also be performed on a 5 g scale of the starting material **21** (yield: 59%). R_f = 0.68 (isohexanes/EtOAc = 1:1). IR (ATR): ṽ = 3321, 2933, 2102, 1731, 1584, 1492, 1268, 1166, 1047, 694 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 7.99 (s, 1H, H-8), 7.92 (s, 1H, N*H*), 7.47 – 7.18 (m, 20H, aryl-H), 6.00 (d, ^3J = 8.2 Hz, 1H, H-1'), 4.94 (d, ^2J = 12.3 Hz, 1H, COOCH_2Ph), 4.88 (d, ^2J = 12.3 Hz, 1H, COOCH_2Ph), 4.58 (d, ^2J = 11.6 Hz, 1H, OCH_2Ph), 4.443 (d, ^2J = 11.6 Hz, 1H, OCH_2Ph), 4.441 (d, ^3J = 5.8 Hz, ^3J = 2.1 Hz, 1H, H-3'), 4.27 – 4.21 (m, 1H, H-4'), 3.80 (dd, ^2J = 13.0 Hz, ^3J = 6.3 Hz, 1H, H_a-5), 3.74 – 3.63 (m, 1H, H-2'), 3.70 (dd, ^2J = 13.0 Hz, ^3J = 5.3 Hz, 1H, H_b-5), 2.85 (dd, ^2J = 16.7 Hz, ^3J = 8.3 Hz, 1H, CH_2COO), 2.80 – 2.70 (m, 1H, C*H*(CH$_3$)$_2$), 2.48 (dd, ^2J = 16.7 Hz, ^3J = 6.5 Hz, 1H, CH_2COO), 1.26 (s, 3H, CH(CH_3)$_2$), 1.24 (s, 3H, CH(CH_3)$_2$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 174.7 (*C*ONH), 171.3 (*C*OOBn), 156.3 (C-2), 154.6 (C-4), 151.9 (O*C*ONPh$_2$), 150.4 (C-6), 143.4 (C-8), 141.8 (OCON-*Ph*-C1), 137.4 (OCH$_2$*Ph*-C-1), 135.5 (COOCH$_2$*Ph*-C-1), 129.3, 128.70, 128.67, 128.46, 128.41, 128.28, 128.17 (aryl 20C), 122.0 (C-5), 89.5 (C-1'), 82.8 (C-4'), 80.1 (C-3'), 72.4 (OCH$_2$Ph), 66.8 (COO*C*H$_2$Ph), 52.6 (C-5'), 42.7 (C-2'), 36.5 (CH(CH$_3$)$_2$), 30.2 (*C*H$_2$COO), 19.5 (CH(*C*H$_3$)$_2$), 19.4 (CH(*C*H$_3$)$_2$) ppm. ESI-HRMS calcd. for $[C_{43}H_{41}N_9O_7 + H]^+$: 796.3202, found: 796.3214.

4-{6'-Benzoylamino-9'-[2''-*O*-acetyl-5''-(*tert*-butoxycarbonyl)amino-3'',5''-dideoxy-β-D-ribofuranosyl]-9'*H*-purin-3''-yl}-1-{9'''-{3''''-*O*-benzyl-2'''',5''''-dideoxy-2''''-*C*-[(benzyloxycarbonyl)methylene]-β-D-ribofuranosyl}-6'''-*O*-(diphenylcarbamoyl)-2'''-*N*-isobutyrylguanin-5''''-yl}-1,2,3-triazole (22): The title compound was prepared according to a modified procedure of Singh et al.[49] A-half **5** (1.30 g, 2.50 mmol, 1.00 eq.) and G-half **6** (2.39 g, 3.00 mmol, 1.20 eq.) were dissolved in THF/*tert*-BuOH/H_2O (2:2:1, 80 mL) under N_2 at RT. Subsequently, a solution of sodium ascorbate (0.41 g, 2.00 mmol, 0.80 eq.) in water (3 mL) and a solution of copper(II) sulfate (0.16 g, 1.00 mmol, 0.40 eq.) in water (2 mL) was added. The mixture was stirred at RT for 12 h. Volatile components were evaporated and the residue was purified by flash-column chromatography (silica gel, DCM/MeOH, 100:2→100:5→10:1) to provide dinucleotide **22** (2.62 g, 2.00 mmol, 80%) as a colorless foam. M.p. = 183 °C (decomp.). R_f = 0.46 (DCM/MeOH = 10:1). IR (ATR): ṽ = 3268, 2976, 2106, 1738, 1707, 1584, 1452, 1216 1167, 732 cm^{-1}. ^{1}H NMR, COSY (400 MHz, $CDCl_3$): δ = 9.14 (s, 1H, N*H*Bz), 8.86 (s, 1H, H-2'), 8.25 (s, 1H, N*H*^{i}Bu), 8.09 (s, 1H, H-8'), 8.02 (d, ^3J = 7.6 Hz, 1H, Bz-o-C*H*), 7.85 (s, 1H, H-8'''), 7.82 (s, 1H, H-5), 7.62 – 7.56 (m, 1H, Bz-p-C*H*), 7.50 (t, ^3J = 7.6 Hz, 2H, Bz-m-C*H*), 7.48 – 7.12 (m, 20H, aryl-H), 6.81 (dd, ^3J = 7.3 Hz, ^4J = 3.2 Hz, 1H, N*H*Boc), 6.19 (d, ^3J = 3.9 Hz, 1H, H-1''), 5.95 (d, ^3J = 8.2 Hz, 1H, H-1''''), 5.71 (dd, ^3J = 7.3 Hz, ^3J = 3.9 Hz, 1H, H-2''), 5.15 (dd, ^2J = 14.0 Hz, ^3J = 6.5 Hz, 1H, H_a-5''''), 4.93 (dd, ^2J = 14.0 Hz, ^3J = 8.0 Hz, 1H, H_b-5''''), 4.88 (d, ^2J = 12.3 Hz, 1H, COOCH_2Ph), 4.87 – 4.83 (m, 1H, H-4''''), 4.83 – 4.78 (m, 1H, H-4''), 4.79 (d, ^2J = 12.3 Hz, 1H, COOCH_2Ph), 4.61 (d, ^2J = 11.6 Hz, 1H, OCH_2Ph), 4.40 (d, ^3J = 5.3 Hz, 1H, H-3''''), 4.37 – 4.32 (m, 1H, H-3''), 4.32 (d, ^2J = 11.6 Hz, 1H, OCH_2Ph), 4.15 – 4.04 (m, 1H, H-2''''), 3.61 – 3.51 (m, 1H, H_a-5''), 3.53 – 3.44 (m, 1H, H_b-5''), 2.80 (dd, ^2J = 16.6 Hz, ^3J = 8.3 Hz, 1H, CH_2COO), 2.59 (hept, ^3J = 6.9 Hz, 1H, C*H*(CH$_3$)$_2$), 2.45 (dd, ^2J = 16.7 Hz, ^3J = 7.1 Hz, 1H, CH_2COO), 1.70 (s, 3H, OCOCH_3), 1.45 (s, 9H, C(CH_3)$_3$), 1.25, (d, ^3J = 6.9 Hz, 3H, CH(CH_3)$_2$), 1.21, (d, ^3J = 6.9 Hz, 3H, CH(CH_3)$_2$) ppm. ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$): δ = 174.3 (iBu-*C*ONH), 171.0 (*C*OOBn), 169.8 (O*C*OCH$_3$), 164.7 (N-*C*O-Ph), 156.7 (N-*C*O-OC(CH$_3$)$_3$), 156.4 (C-2'''), 154.1 (C-4'''), 152.7 (C-2'), 151.6 (O*C*ONPh$_2$), 151.1 (C-4'), 150.4 (C-6'), 150.1 (C-6'''), 144.4 (C-8'''), 142.7 (C-8'), 141.7 (OCON-*Ph*$_2$-C1), 140.7 (C-4), 137.1 (OCH$_2$*Ph*-C-1), 135.4 (COOCH$_2$*Ph*-C-1), 133.5 (Bz-*C*-CO-N), 133.0 (Bz-p-*C*H), 129.3, 129.0, 128.61, 128.55, 128.42, 128.25, 128.17, 128.03, 127.98 (aryl 25C), 125.2 (C-5), 124.2 (C-5'), 122.8 (C-5'''), 90.9 (C-1''''), 90.2 (C-1''), 83.3 (C-4''), 82.1 (C-4''''), 79.6 (C-3'''), 79.4 (*C*(CH$_3$)$_3$), 77.1 (C-2''), 71.8 (OCH$_2$Ph), 66.7 (COO*C*H$_2$Ph), 51.2 (C-5''''), 42.4 (C-5''), 40.5 (C-2''''), 39.9 (C-3''), 36.9 (*C*H(CH$_3$)$_2$), 30.3 (CH$_2$COO), 28.6 (C(*C*H$_3$)$_3$), 20.3 (OCO*C*H$_3$), 19.5 (CH(*C*H$_3$)$_2$), 19.40 (CH(*C*H$_3$)$_2$) ppm. ESI-HRMS calcd. for $[C_{69}H_{69}N_{15}O_{13} + H]^+$: 1316.5272, found: 1316.5330. ESI-HRMS calcd. for $[C_{69}H_{69}N_{15}O_{13} + Na]^+$: 1338.5091, found: 1338.5151.

4-[6'-Benzoylamino-9'-(2''-*O*-acetyl-5''-amino-3'',5''-dideoxy-β-D-ribofuranosyl)-9'*H*-purin-3''-yl]-1-{9'''-[3''''-*O*-benzyl-2'''',5''''-dideoxy-2''''-*C*-carboxymethyl-β-D-ribofuranosyl]-2'''-*N*-isobutyrylguanin-5''''-yl}-2'''',5''-lactame-1,2,3-triazole (24): To a stirred solution of dinucleotide **22** (2.12 g, 1.61 mmol, 1.00 eq.) in dry DCM (40 mL) was added TFA (20 mL) at 0 °C under N_2. The mixture was stirred for 1 h at this temperature and then concentrated *in vacuo*. The brown residue was purified by flash-column chromatography (silica gel, DCM/MeOH, 100:2→100:5 →5:1) to give amino compound **23** as a colorless solid (1.33 g, 1.30 mmol, 81%). M.p. = 128 °C (decomp.). R_f = 0.39 (DCM/MeOH = 5:1). ESI-HRMS calcd. for $[C_{51}H_{52}N_{14}O_{10} + H]^+$: 1021.4064, found: 1021.4038. ESI-HRMS calcd. for $[C_{51}H_{52}N_{14}O_{10} - H]^-$: 1019.3918, found: 1019.3918.

To a solution of amino compound **23** (1.08 g, 1.06 mmol, 1.00 eq.) in EtOH (50 mL) was added Pd/C (10 wt.%, 0.30 g) under nitrogen stream at RT. The reaction vessel was evacuated and flushed with hydrogen three times. The mixture was stirred under hydrogen atmosphere for 36 h and then filtered through celite. The solution was concentrated to dryness under reduced pressure. The residue was used in the next step without further purification. ESI-HRMS calcd. for $[C_{44}H_{46}N_{14}O_{10} + H]^+$: 931.3594, found: 931.3594. ESI-HRMS calcd. for $[C_{44}H_{46}N_{14}O_{10} - H]^-$: 929.3448, found: 929.3450.

Finally, the title compound was prepared according to a modified procedure of Horne et al.[50] and Kinzie et al.[51] To a yellow solution of the hydrogenated compound **23** and HATU (0.60 g, 1.58mmol, 1.50 eq.) in dry DMF (1000 mL) was added DIPEA (0.72 mL, 0.54 g, 4.21 mmol, 4.00 eq.) at RT. The solution turned orange and was stirred at RT for 24 h. After addition of MeOH (5 mL), volatile materials were removed under reduced pressure and the crude product was purified by flash-column chromatography (silica gel, DCM/MeOH, 100:2→100:5→5:1) to yield cyclized compound **24** as a colorless solid (506 mg, 0.55 mmol, 52% over 2 steps). An analytical sample was provided by RP-HPLC. M.p. = 185 °C (decomp.). R_f = 0.57 (DCM/MeOH = 5:1). R_t = 16.1 min (RP-HPLC, 15% to 80% MeCN gradient elution). IR (ATR): ṽ = 3220, 1682, 1608, 1454, 1403, 1222, 1049, 797, 708 cm^{-1}. ^1H NMR, COSY, NOESY (600 MHz, DMSO-d_6): δ = 12.06 (s, 1H, N*H*), 11.59 (s, 1H, N*H*), 11.27 (s, 1H, N*H*), 8.83 (s, 1H, H-2‘), 8.68 (s, 1H, H-8‘), 8.54 (s, 1H, H-5), 8.35 (s, 1H, H-8‘‘‘), 8.08 – 8.03 (m, 2H, Bz-o-C*H*), 8.06 – 8.03 (s, 1H, $CH_2CONHCH_2$), 7.68 – 7.63 (m, 1H, Bz-p-C*H*), 7.59 – 7.53 (m, 2H, Bz-m-C*H*), 7.52 – 7.49 (m, 2H, Bn-o-C*H*), 7.44 – 7.40 (m, 2H, Bn-m-C*H*), 7.38 – 7.34 (m, 1H, Bn-p-C*H*), 6.46 (d, ^3J = 1.1 Hz, 1H, H-1‘‘), 5.92 (dd, ^3J = 5.9 Hz, ^3J = 1.1 Hz, 1H, H-2‘‘), 5.66 (d, ^3J = 10.2 Hz, 1H, H-1‘‘‘), 4.85 (dd, ^2J = 15.0 Hz, ^3J = 3.3 Hz, 1H, H_a-5’‘‘), 4.80 – 4.77 (m, 1H, H_b-5’‘‘), 4.80 (d, ^2J = 10.9 Hz, 1H, OCH_2Ph), 4.76 (dd, ^3J = 10.6 Hz, ^3J = 6.0 Hz, 1H, H-3’‘), 4.68 (d, ^2J = 10.9 Hz, 1H, OCH_2Ph), 4.68 – 4.66 (m, 1H, H-4’‘‘), 4.57 (td, ^3J = 10.5 Hz, ^3J = 4.1 Hz, 1H, H-4‘‘), 4.15 (d, ^3J = 3.4 Hz, 1H, H3‘‘‘), 3.87 – 3.81 (m, 1H, H_a-5’‘), 2.91 – 2.83 (m, 1H, H_b-5’‘), 2.67 (hept, ^3J = 6.9 Hz, 1H, C*H*$(CH_3)_2$), 2.11 (s, 3H, $OCOCH_3$), 2.04 (t, ^3J = 12.0 Hz, 1H, CH_2CONH), 1.76 – 1.70 (m, 1H, CH_2CONH), 1.07, (d, ^3J = 6.9 Hz, 3H, $CH(CH_3)_2$), 1.06, (d, ^3J = 6.9 Hz, 3H, $CH(CH_3)_2$), -0.19 – -0.26 (m, 1H, H-2‘‘‘‘) ppm. ^{13}C NMR, HSQC, HMBC (151 MHz, DMSO-d_6): δ = 180.1 (iBu-*C*ONH), 170.8 ($CH_2CONHCH_2$), 169.1 ($OCOCH_3$), 165.7 (N-*C*O-Ph), 154.8 (C-2‘‘‘), 151.8 (C-2‘), 151.5 (C-4‘), 150.7 (C-6‘), 149.1 (C-6‘‘‘), 148.30 (C-4‘‘‘), 144.0 (C-8‘), 142.8 (C-4), 138.2 (OCH_2Ph-C-1), 137.9 (C-8‘‘‘), 133.3 (Bz-*C*-CO-N), 132.5 (Bz-p-*C*H), 128.53 (Bz-o-*C*H), 128.49 (Bz-m-*C*H), 128.47 (Bn-m-*C*H), 128.3 (Bn-o-*C*H), 127.9 (Bn-p-*C*H), 126.8 (C-5), 126.2 (C-5‘), 119.7 (C-5‘‘‘), 89.3 (C-1‘‘), 83.3 (C-1‘‘‘‘), 81.2 (H-4‘‘), 79.9 (H-3‘‘‘‘), 79.8 (H-4‘‘‘‘), 77.5 (C-2‘‘), 72.0 (OCH_2Ph), 53.0 (C-5‘‘‘‘), 46.9 (C-2‘‘‘‘), 44.5 (C-3‘‘), 42.4 (C-5‘‘), 34.7 (C*H*$(CH_3)_2$), 29.7 (CH_2CONH), 20.7 ($OCOCH_3$), 18.81 ($CH(CH_3)_2$), 18.79 ($CH(CH_3)_2$) ppm. ESI-HRMS calcd. for $[C_{44}H_{44}N_{14}O_9 + H]^+$: 913.3489, found: 913.3495. ESI-HRMS calcd. for $[C_{44}H_{44}N_{14}O_9 - H]^-$: 911.3343, found: 911.3348.

4-[6’-Benzoylamino-9’-(2’’-*O*-acetyl-5’’-amino-3’’,5’’-dideoxy-β-D-ribofuranosyl)-9’*H*-purin-3’’-yl]-1-{9’’’-[2’’’’,5’’’’-dideoxy-2’’’’-*C*-carboxymethyl-β-D-ribofuranosyl]-2’’’-*N*-isobutyrylguanin-5’’’’-yl}-2’’’’,5’’-lactame-1,2,3-triazole (25): To a solution of dinucleotide **24** (340 mg, 0.37 mmol, 1.00 eq.) in dry DCM (300 mL) was added BCl_3 (5.96 mL, 5.96 mmol, 1M in DCM, 16.0 eq.) at -40°C. The mixture was stirred for 3 days at this temperature, quenched by addition of MeOH (5 mL) and extracted with saturated sodium bicarbonate (20 mL) and DCM (4 x 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The compound was used in the next step without further purification. An analytical sample was prepared by RP-HPLC to yield a colorless solid. M.p. = 258 °C (decomp.). R_t = 12.5 min (RP-HPLC, 15% to 80% MeCN gradient elution). IR (ATR): ṽ = 3234, 1756, 1677, 1613, 1460, 1403, 1220, 1047, 796, 707 cm^{-1}. ^1H NMR, COSY, NOESY (600 MHz, DMSO-d_6): δ = 12.07 (s, 1H, N*H*), 11.69 (s, 1H, N*H*), 11.26 (s, 1H, N*H*), 8.82 (s, 1H, H-2‘), 8.66 (s, 1H, H-8‘), 8.43 (s, 1H, H-5), 8.33 (s, 1H, H-8‘‘‘), 8.08 – 8.03 (m, 1H, Bz-o-C*H*), 7.93 – 7.87 (m, 1H, $CH_2CONHCH_2$), 7.68 – 7.63 (m, 1H, Bz-p-C*H*), 7.59 – 7.53 (m, 2H, Bz-m-C*H*), 6.42 (d, ^3J = 1.1 Hz, 1H, H-1‘‘), 5.88 (dd, ^3J = 5.9 Hz, ^3J = 1.1 Hz, 1H, H-2‘‘), 5.76 (d, J = 10.2 Hz, 1H, H-1‘‘‘‘), 5.68 (d, J = 3.4 Hz, 1H, O*H*-3‘‘‘‘), 4.78 (dd, ^2J = 15.0 Hz, ^3J = 3.3 Hz, 1H, H_a-5’‘‘), 4.71 (dd, ^3J = 10.6 Hz, ^3J = 6.0 Hz, 1H, H-3’‘), 4.68 (dd, ^2J = 15.0 Hz, ^3J = 1.8 Hz, 1H, H_b-5’‘‘), 4.50 (td, ^3J = 10.4 Hz, ^3J = 3.9 Hz, 1H, H-4‘‘), 4.32 – 4.29 (m, 1H, H-4’‘‘), 4.17 – 4.13 (m, 1H, H3‘‘‘‘), 3.82 – 3.74 (m, 1H, H_a-5’‘), 2.89 – 2.79 (m, 1H, H_b-5’‘), 2.73 (hept, ^3J = 6.8 Hz, 1H, C*H*$(CH_3)_2$), 2.09 (s, 3H, $OCOCH_3$), 2.02 (t, ^3J = 12.0 Hz, 1H, CH_2CONH), 1.67 – 1.60 (m, 1H, CH_2CONH), 1.11, (d, ^3J = 6.8 Hz, 3H, $CH(CH_3)_2$), 1.10, (d, ^3J = 6.8 Hz, 3H, $CH(CH_3)_2$), -0.39 – -0.49 (m, 1H, H-2‘‘‘‘) ppm. ^{13}C NMR, HSQC, HMBC (151 MHz, DMSO-d_6): δ = 180.1 (iBu-*C*ONH), 171.0 ($CH_2CONHCH_2$), 169.2 ($OCOCH_3$), 165.7 (N-*C*O-Ph), 154.8 (C-2‘‘‘), 151.8 (C-2‘), 151.5 (C-4‘), 150.7 (C-6‘), 149.1 (C-6‘‘‘), 148.30 (C-4‘‘‘), 144.0 (C-8‘), 142.6 (C-4), 138.2 (C-8‘‘‘), 133.3 (Bz-*C*-CO-N), 132.5 (Bz-p-*C*H), 128.52 (Bz-o-*C*H), 128.49 (Bz-m-*C*H), 126.6 (C-5), 126.2 (C-5‘), 119.7 (C-5‘‘‘), 89.3 (C-1‘‘), 83.2 (C-1‘‘‘‘),83.0 (C-4‘‘‘‘), 81.0 (C-4‘‘), 77.4 (C-2‘‘), 70.6 (C-3‘‘‘‘), 52.5 (C-5‘‘‘‘), 47.1 (C-2‘‘‘‘), 44.5 (C-3‘‘), 42.5 (C-5‘‘), 34.8 (C*H*$(CH_3)_2$), 29.2 (CH_2CONH), 20.6 ($OCOCH_3$), 18.85 ($CH(CH_3)_2$), 18.83 ($CH(CH_3)_2$) ppm. ESI-HRMS calcd. for $[C_{37}H_{38}N_{14}O_9 + H]^+$: 823.3019, found: 823.3015. ESI-HRMS calcd. for $[C_{37}H_{38}N_{14}O_9 - H]^-$: 821.2873, found: 821.2873.

4-[6’-Amino-9’-(5’’-amino-3’’,5’’-dideoxy-β-D-ribofuranosyl)-9’*H*-purin-3’’-yl]-1-{9’’’-[2’’’’,5’’’’-dideoxy-2’’’’-*C*-carboxymethyl-β-D-ribofuranosyl]-guanin-5’’’’-yl}-2’’’’,5’’-lactame-1,2,3-triazole (4): The crude compound **25** was dissolved in MeOH (15 mL) and aqueous ammonia (25%, 15 mL) in a sealed vessel at RT. The mixture was stirred at 50 °C for 20 h. Volatile components were removed under reduced pressure. The residue was purified by preparative RP-HPLC to provide the final compound **4** as a colorless solid (109 mg, 0.18 mmol, 48% over 2 steps). M.p. = 270 °C (decomp.). R_t = 7.8 min (RP-HPLC, 15% to 80% MeCN gradient elution). IR (ATR): ṽ = 3338, 1639, 1599, 1477, 1419, 1209, 1089, 1047, 1005, 730 cm^{-1}. ^1H NMR, COSY, NOESY (600 MHz, DMSO-d_6): δ = 10.60 (s, 1H, Guanine-N*H*), 8.34 (s, 1H, H-8‘), 8.19 (s, 1H, H-2‘), 8.12 (s, 1H, H-5), 8.07 (s, 1H, H-8‘‘‘), 7.73 – 7.69 (m, 1H, $CH_2CONHCH_2$), 7.32 (s, 2H, A-NH_2), 6.50 (s, 2H, G-NH_2), 6.07 (d, ^3J = 1.1 Hz, 1H, H-1‘‘), 5.97 (d, ^3J = 5.1 Hz, 1H, H-2‘‘), 5.64 (d, J = 10.3 Hz, 1H, H-1‘‘‘‘), 5.58 (d, J = 3.6 Hz, 1H, O*H*-3‘‘‘‘), 4.77 (dd, ^2J = 14.9 Hz, ^3J = 3.1 Hz, 1H, H_a-5’‘‘), 4.65 – 4.62 (m, 1H, O*H*-2‘‘), 4.61 (dd, ^2J = 14.9 Hz, ^3J = 1.5 Hz, 1H, H_b-5’‘‘), 4.43 (td, ^3J = 10.5 Hz, ^3J = 4.1 Hz, 1H, H-4‘‘), 4.23 – 4.21 (m, 1H, H-4’‘‘), 4.23 (dd, ^3J = 10.6 Hz, ^3J = 5.6 Hz, 1H, H-3’‘), 4.10 – 4.07 (m, 1H, H-3‘‘‘‘), 3.77 – 3.71 (m, 1H, H_a-5’‘), 2.84 – 2.77 (m, 1H, H_b-5’‘), 1.97 (m, 1H, CH_2CONH), 1.56 (dd, ^2J = 12.0 Hz, ^3J = 2.2 Hz,1H, CH_2CONH), -0.44 – -0.41 (m, 1H, H-2‘‘‘‘) ppm. ^{13}C NMR, HSQC, HMBC (151 MHz, DMSO-d_6): δ = 170.9 ($CH_2CONHCH_2$), 156.8 (C-2‘‘‘), 156.2 (C-6‘), 153.8 (C-6‘‘‘), 152.6 (C-2‘), 151.7 (C-4‘‘‘), 148.7 (C-4‘), 143.7 (C-4), 139.81 (C-8‘), 136.0 (C-8‘‘‘), 126.8 (C-5), 119.5 (C-5‘), 116.1 (C-5‘‘‘), 91.9 (C-1‘‘), 83.7 (C-4‘‘‘‘), 82.7 (C-1‘‘‘‘), 80.8 (C-4‘‘), 75.9 (C-2‘‘), 70.6 (C-3‘‘‘‘), 52.2 (C-5‘‘‘‘), 46.7 (C-2‘‘‘‘), 45.9 (C-3‘‘), 42.7 (C-5‘‘), 29.3 (CH_2CONH) ppm. ESI-HRMS calcd. for $[C_{24}H_{26}N_{14}O_6 + H]^+$: 607.2233, found: 607.2231. ESI-HRMS calcd. for $[C_{24}H_{26}N_{14}O_6 - H]^-$: 605.2087, found: 605.2090.

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Conflict of interest

The authors declare no conflict of interest.

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Keywords: cGAMP analog, cGAS, STING, ENPP1, cyclophane, CuAAC, macrolactamization

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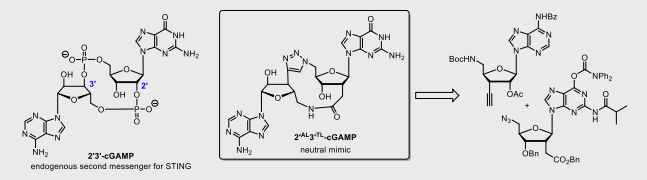

2’3’-cGAMP is an uncanonical cyclic dinucleotide which consists of one A and one G nucleobase connected *via* a 3’-5’ and a unique 2’-5’ phosphodiester linkage. Here, we report a convergent synthesis of a cGAMP analog with one amide and one triazole replacing the natural phosphodiester backbone. The molecule is best prepared by a first CuAAC reaction followed by macrolactamization.

A Click-Chemistry Linked 2’3’-cGAMP Analog

*Clemens R. Dialer, Samuele Stazzoni, David J. Drexler, Felix M. Müller, Simon Veth, Alexander Pichler, Hidenori Okamura, Gregor Witte, Karl-Peter Hopfner and Thomas Carell**