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RESEARCH ARTICLE

Synthesis of novel 3',3'-cyclic dinucleotide analogues targeting STING protein

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Abstract: The Stimulator of Interferon Genes (STING) plays an important role in innate immunity by inducing type I interferons in response to sensing viral or bacterial cytosolic DNA. Cyclic dinucleotides (CDNs) are known to activate STING. Here, we describe the synthesis and biological evaluation of two new 3',3'-CDN, in which the internucleotide linkages were replaced, respectively, by a 1,2,3-triazole moiety (as more stable isosteres of phosphate linkers) and an unsaturated carbon chain (as flexibility can led to crucial binding affinity and specificity). Thus, CuAAC and macrocyclization by RCM are the two key steps.

Introduction

STING (STimulator of INterferon Genes) protein[1,2] is a unique and pivotal protein of the cGAS-STING signaling pathway[3,4] that has attracted increased interest as target for antiviral therapy.[5] It is localized on the endoplasmic reticulum[6] in quiescent cells. When cytosolic DNA is detected,[7,8] the cGAS (cyclic guanosine monophosphate-adenosine monophosphate *synthase*) protein is activated to produce 2',3' cyclic GMP-AMP (2',3'-cGAMP), the natural agonist of STING in human cells. Activated STING then induces type I interferons (IFNs) and pro-inflammatory cytokines secretion, innate immune responses that are the first defense mechanims to fight infectious diseases. However, several viruses evolved to inhibit the cGAS-STING pathway at different stages in order to circumvent the host innate defense and allow their replication. Thus, boosting the host innate immunity by small analogs of 2',3'-cGAMP is recognized to be a very promising approach to treat multiple viral diseases including COVID-19.[9-12]

2',3'-cGAMP is a cyclic dinucleotide (CDN) formed by GMP and AMP linked through phosphate diesters from 3'-5' and 2'-5' positions, respectively. However, the negative charged phosphodiester linkages lead to a poor cellular penetration and low enzymatic stability, such as with plasma phosphatases and hydrolases (ENPP1)[13,14] that cleave the phosphate backbone preferentially at the 2'-5' position. To overcome these limitations, several synthetic CDN analogues modified either at the heterocyclic moiety, at the sugar and at the internucleotide linker, were reported,[15-22] some of them being evaluated in clinical trials.[23] Among them, ADU-S100 (dithio-[Rp,Rp]-cyclic-[A(2'-,5'-)pA(3'-,5'-)p), developed by Aduro Biotech Inc., was the first CDN STING activator evaluated in clinical trial in patients with advanced/metastatic solid tumors,[24] (Figure 1).

The 3',3'-dithio analog of ADU-S100 **3** was also developed;[25] it strongly induces the production of type I IFN and is stable to "e.g. phosphodiesterases" which cleave the phosphorodiester moiety. Other modifications were brought either at the nucleobase (purines and pyrimidines) such as analog **4** developed by Sperovie Biosciences,[26] or at the sugar in which the oxygen was replaced by a methylene such as analog **5** bearing a non-canonical nucleobase.[27] Finally, various neutral non-phosphate backbone based CDNs were reported[28-32] such as the derivative **6**, developed by IFM Therapeutics, a 3',3'-CDN in which the internucleotide linkage was replaced by the neutral and biological stable urea moiety while maintaining its hydrogen *bonding* ability. Also, it has been demonstrated by Ablasser group and others,[33,34] that the natural 2',3'-cGAMP induces stronger binding affinity to STING and IFN responses compared to c-di-GMP, 3',2'-cGAMP and 3',3'-cGAMP. The interaction between STING and a CDN is probably due to multiple factors such as specific interactions of the nucleobase with the STING protein (π-stacking, charge-charge interactions (the guanidinium group of arginine residue, especially R232) and H-bonds),[35,36] which suggest that (1) the sugar moiety and especially the oxygen of, have few specific interactions with STING, (b) the imidazole moiety of adenine ring has low impact on the binding, contrary to the pyrimidine moiety which has strong π−π interaction with Tyr167 meanwhile the *N7* of the guanine moiety is important for the recognition by H-bonds. The phosphate moiety is use only to stabilize the active conformation. Altogether, these findings suggested that more modifications can be done of the CDN moiety either at the sugar, nucleobase or internucleotide moiety, keeping above mentioned important features.

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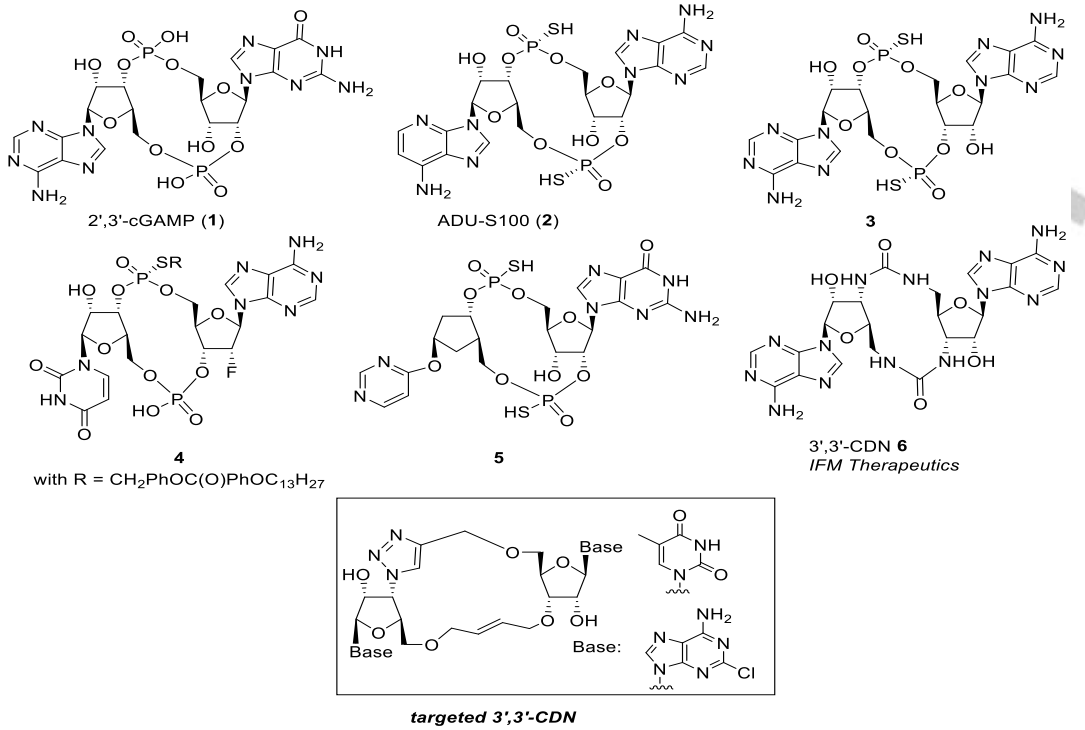


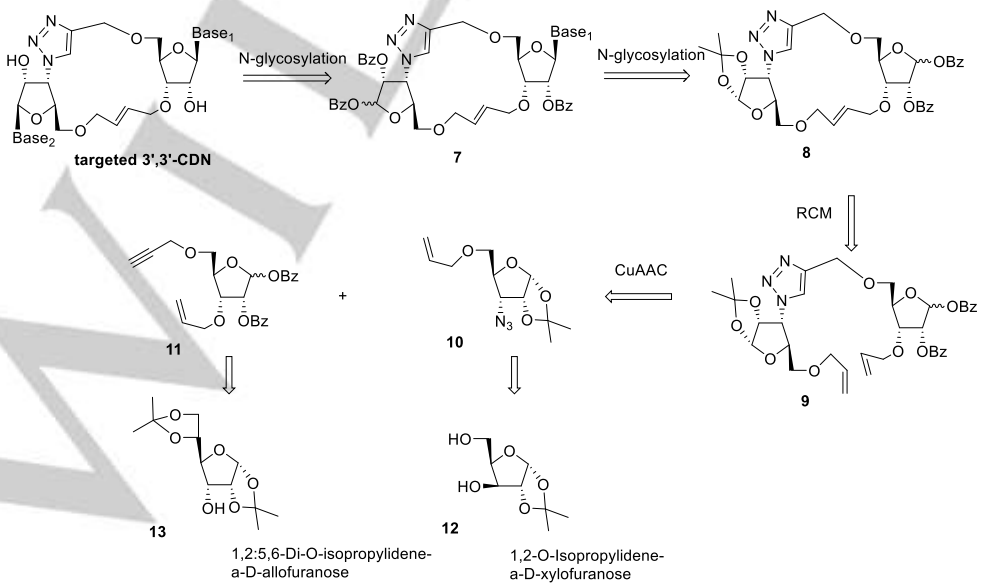
Figure 1. Structures of representative CDN agonists of STING and target compounds

Thus, herein, we present the synthesis of two novel 3',3'-cGAMP analogues bearing as internucleotides linkage a 1,2,3-triazole moiety as more stable isosteres of phosphate linkers[37] and an unsaturated carbon chain since flexibility can led to crucial binding affinity and specificity[38] Nucleobases were chosen between thymine (pyrimidine), as a model and the 2-amino-6-chloropurine, a guanine analog.

Results and Discussion

2.1 Chemistry

*2.1.1 Retrosynthesis of targeted 3',3'-CDN***.** The pathway proposed aimed to reach either homodimeric (similar nucleobases) or heterodimeric (different nucleobases) CDN. Thus, our retrosynthetic analysis identified the intermediate **7** bearing a nucleobase ($Base_1$) as the last step in the synthesis of desired CDN, (Scheme 1). This compound **7** was expected to be accessed by a *N*-glycosylation under Vorbrüggen conditions[39] from the macrocycle **8** with a nucleobase ($Base_1$). The derivative **8** containing an alkene would be generated *via* a ring-closing metathesis reaction, a tool to generate products containing large rings,[40,41] of the substrate **9**. Finally, the 1,2,3-triazolo derivative **9** can arise from a *CuAAC* reaction[42-47] between the azido analog **10** (obtained from commercially available 1,2-*O*-*iso*propylidene-α-D-ribufuranose (**12**)) and the ribosyl derivative **11** (obtained from commercially available 1,2:5,6-di-*O*-*iso*propylidene-α-D-allofuranose (**13**)), respectively.



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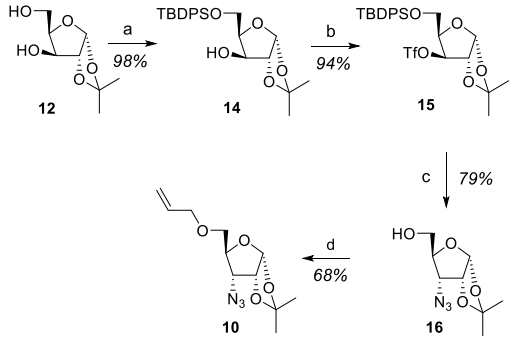
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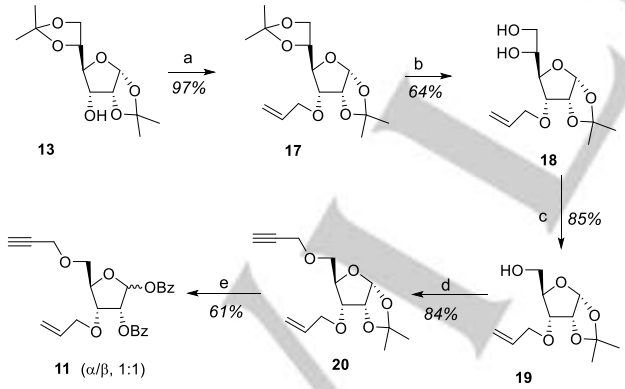
Scheme 1. Retrosynthesis of desired 3',3'-CDN

*2.1.2. Synthesis of the key precursor **10**.* The synthesis (Scheme 2) began with the selective silylation of the 5'-OH of α-D-xylofuranose derivative **12** (98% yield) followed by the triflatation of the 3'-OH to generate the activated **15** in 94% yield.[48-50] The subsequent azidation was realized in presence of nBu$_4$NBr in DMF, which favoured the nucleophilic substitution at lower temperatures, thus decreasing the elimination product ratio. The following deprotection of the silyl ether with TBAF afforded the desired azidosugar **16** in 79% yield (two steps). Finally, **16** was reacted with allyl bromide in NaH to afford the desired compound **10** (68% yield).



Scheme 2. Reagents and conditions: (a) TBDPSCl, imidazole, DMAP, DCM, r.t., 2 h; (b) Tf_2O, pyr., DMAP, DCM, r.t., 2 h; (c)-i) NaN_3, nBu_4NBr, DMF, M.W. 80 °C (25 W), 10 min; -ii) TBAF, THF, r.t., 30 min; (d) NaH, allyl bromide, THF, r.t., 2 h.

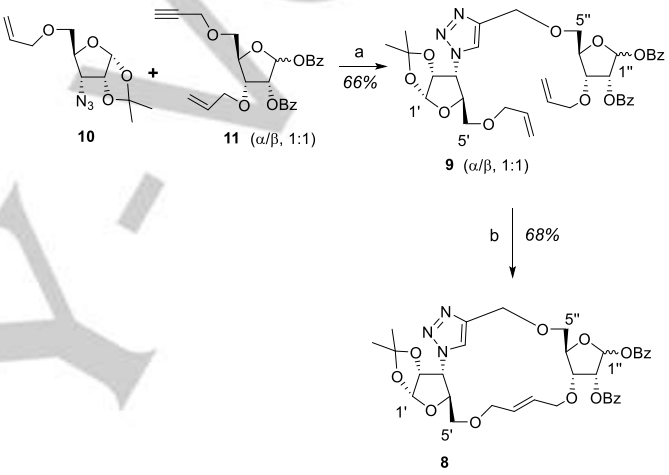
*2.1.3. Synthesis of the key precursor **11**.* The synthesis of desired **11** (Scheme 3) began with the *O*-allylation of the 3'-OH of α-D-allofuranose derivative **13** (97% yield). The regioselective removal of 5,6-*iso*propylidene of obtained **17**was realized in a mixture of acetic acid and formic acid in water to **18** (64% yield). The cleavage of the diol was mediated by $NaIO_4$ and the subsequent reduction with $NaBH_4$ afforded the **19** in 85% yield (two steps). By reacting with propargyl bromide in NaH, **19** afforded the desired sugar **20** (84% yield).



Scheme 3. Reagents and conditions: (a) NaH, allyl bromide, THF, r.t., 3 h; (b) Acetic ac./formic ac./H_2O, r.t.; (c)-i) $NaIO_4$, EtOH/H_2O, r.t., 1 h; -ii) $NaBH_4$, EtOH, r.t., 30 min; (d) NaH, propargyl bromide, THF, r.t., 3 h; (e)-i) Formic ac./H_2O, 60 °C, 1 h; -ii) BzCl, Pyr., DMAP, DCM, r.t., 2 h.

Finally, in order to prepare the sugar for stereoselective *N*-glycosylation under Vorbrüggen conditions, the acetal group of **20** was removed under mild acidic hydrolysis; after coevaporations with pyridine/toluene to eliminate acidic traces, the crude intermediate was engaged with benzoyl chloride to yield to di-benzoate protected compound **11** as a mixture of anomers (α/β: 1:1) -determined by NMR (SI) in 61% yield. The benzoyl groups make easier the monitoring reactions on TLC and LC-MS analyses. Also, since the anomeric configuration has no impact on the stereoselectivity of the *N*-glycosylation under Vorbrüggen conditions, the anomeric α/β mixture at **11** does not need to be separated. Thus, till the introduction of the nucleobase, all next compounds are obtained as an equimolar mixtures of α and β diastereisomers.

*2.1.4. Synthesis of the macrocycle **8**.* The two building blocks **10** and **11** were then reacted through a copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) to provide the desired 1,2,3-triazolo analogue **9** in *66%* yield, (Scheme 4). The macrocyclization ended *via* a Ru-catalyzed ring-closing metathesis with 5 mol % of second-generation Grubbs catalyst under high dilution (7 µmol/mL) to provide the macrocyclic compound **8** in *68%* yield (α/β: 1:1).



Scheme 4. Reagents and conditions: (a) $CuSO_4$, Na-Ascorbate, *t*BuOH/H_2O (2:1), 40 °C, 3 h; (b) Grubbs II (5mol%), DCM, argon, 35 °C, 2.5 h.

It should be emphasized that the 1,2,3-triazole does not preclude the RCM cyclization in reasonable chemical yields despite the fact that the amino group has deleterious effect on Grubb's catalyst.[51] We could hypothesize that the 1,2,3-triazolo ring acts as a shackle which forces the two ribose units to get closer together and favours the meeting of the two allylic chains during this reaction step. A noteworthy aspect is that the RCM seems to have a complete control of the stereoselectivity (>99% *E*) which we attribute to the strong preference of the unsaturated 17-membered ring to exist in only one stereoisomeric form (thermodynamic control). The ^{1}H NMR (700 MHz ($CDCl_3$)) clearly shows the multiplet disappearance at 5.83 ppm of both allyl moieties of (**9**) and confirms the *E* stereochemistry of the alkene formed by a coupling constant of 15.5 Hz between the two alkenyl hydrogens, (see SI).

*2.1.5. Synthesis of the final CDN **7**.* With the carbon framework complete, the only task left in hand was the introduction by *N*-glycosylation of nucleobases. Firstly, we envisioned to obtain heterodimeric form of CDN, (Scheme 5). Thus, the compound **8** was reacted with the N^6-benzoylated adenine under the conditions of *N*-glycosylation of Vorbrüggen using *N,O*-bis(trimethylsilyl)acetamide (BSA) as silylating agent and TMSOTf as Lewis acid, leading to the desired CDN **21** as a single isomer in low 34% yield. In order to introduce a second different nucleobase under similar Vorbrüggen conditions, the deprotection of the acetonide protecting group and successive benzoylation of released hydroxyl groups led to **22**. After a tedious and rough purification, **22** was submitted to a Vorbrüggen condensation with silylated 6-chloropurine and TMSOTf.

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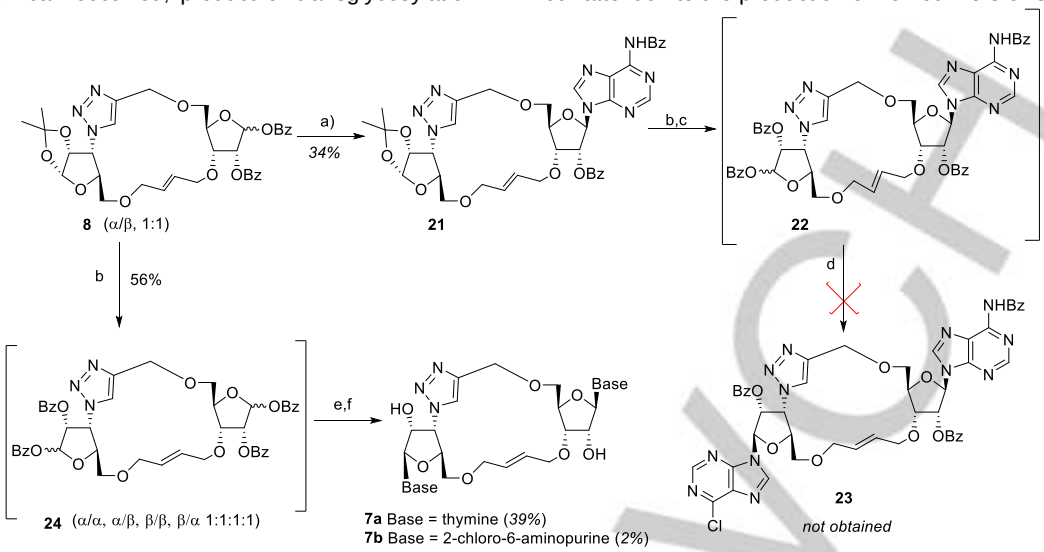
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Unfortunately, we were unable to observe any traces of the products corresponding to the targeted CDN; among side-reactions which can occurred, product of transglycosylation[52] was observed by LC-MS. Altogether, this approach is not suitable to produce heterodimer forms of CDNs. Thus, we turned our attention to the production of homodimers of CDNs.



Scheme 5. Reagents and conditions: (a) N^6-Bz-adenine, BSA, TMSOTf, MeCN, 80 °C; (b) i) Formic ac./H_2O, 60 °C, 1 h; -ii) BzCl, Pyr., DMAP, DCM, r.t., 2 h; (d) 6-Chloropurine, BSA, TMSOTf, MeCN, 80 °C, 4h; (e) thymine (for 7a) and 2,6-dichloropurine (for 7b), BSA, TMSOTf, MeCN, 80 °C; (f) NH_3 in MeOH (7N), r.t., 48 h.

Thus, the 1,2-*iso*propylidene group of **8** was hydrolyzed under mild acidic conditions and the obtained hydroxyls were protected by benzoylation to provide **24** as a complex anomeric mixture of α/α, α/β, β/α and β/β isomers. The thymine was first introduced on the macrocycle **24** under Vorbrüggen conditions to afford, after 2'-*O*-benzoate deprotection by a treatment of a 7 *N* ammonia solution in MeOH, the 3',3'-cyclic di-thymine analogue **7a** in *39%* yield (two steps). Similarly, the 2,6-dichloropurine was introduced under Vorbrüggen conditions and the obtained residue was directly submitted to an aminolysis (for Bz removal and SNAr of 6-chloro group at purine) to afford the desired 3',3'-cyclic di-2-chloro-6-aminopurine derivative **7b** in *2%* yield (two steps). The final compounds were fully characterized by various ^1H and ^{13}C NMR techniques (COSY, TOCSY, HSQC, HMBC, and NOESY, (see SI) and spectra suggest that these molecules existed in a single conformation on the NMR timescale, and that, as expected, only the β/β anomers were obtained.

2.2 Biological evaluation

Both final synthesized compounds **7a** and **7b** were evaluated to determine their activity as STING agonist or antagonist, using as reference compounds commercial STING agonist cGAMP and STING antagonist H151. Murine bone marrow-derived macrophages (BMDM) were stimulated either directly by the compounds, or first with cGAMP in the absence or in presence of compounds **7a** and **7b**. The secretion of type I interferon-induced CXCL10/IP-10 by macrophages was measured. The potential cytotoxic effect was assessed by MTT assays. While cGAMP induced CXCL10 secretion at concentrations 3 to 14 uM, compounds **7a** and **7b** did not induce CXCL10 response at concentrations up to 14uM. Conversely, while the STING antagonist H151 at 3-10 uM inhibited by ca 80% the induction of CXCL10 in response to cGAMP, compounds **7a** and **7b** did not reduce cGAMP-induced CXCL10 response at concentrations up to 10 uM. Thus, **7a** and **7b** are not cytotoxic, but they displayed no activity as STING agonist or antagonist. Differential Scanning Fluorimetry (DSF) assay was done for **7a** on STING protein. Using this method, STING protein is observed to have a T_m of 51.1 °C (with 1% DMSO) taken as a control, T_m of 57.6 °C with the natural ligand 3',3'-cGAMP and only T_m 51.3 °C in presence of **7a**, respectively, prooving that this compound has no measurable effect on STING protein.

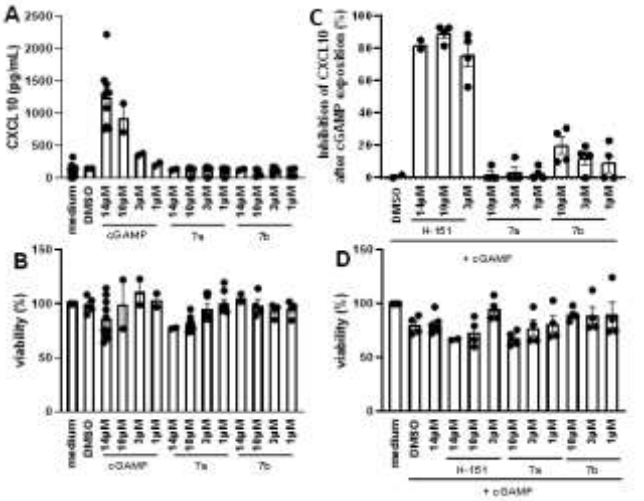


Figure 2. Biological evaluation of compound 7a and 7b tested as STING agonist (A, B) or antagonist (C, D) on bone marrow derived macrophages. Reference agonist cGAMP and antagonist H151 are shown. In C, D the compounds are added 3hr prior to macrophage stimulation with cGAMP (14uM). Compounds cytotoxicity is evaluated by MTT assay. Data are from 4 (A, B) or 3 (C, D) independent experiments, each point representing an independent bone marrow culture from an individual mouse.

Conclusion

In conclusion, we have developed for the first time the synthesis of two new cyclic dinucleotide analogues. The developed methodology is restricted to homodimeric CDNs and is based on a CuAAC and RCM reactions to obtain the macrocycle. Nucleobases were introduced stereoselectively under Vorbrüggen *N*-glycosylation conditions. The synthesis of di-purine analogues or heterodimers closer to the natural c-GAMP, remains challenging *via* the explored *N*-glycosylation conditions. Further studies to overcome these current limitations are underway. Although non cytotoxic, none of the two compounds displayed activity as STING agonist or antagonist.

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Experimental Section

Chemistry

Commercially available chemicals were provided as reagent grade and used as received. Some reactions requiring anhydrous conditions were carried out using oven-dried glassware and under an atmosphere of dry Argon. All anhydrous solvents were provided from commercially sources as very dry reagents. The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel precoated plates (Kieselgel 60F254, E. Merck). Compounds were visualized by UV irradiation and/or spraying with sulfuric acid (H_2SO_4 5% in ethanol) stain followed by charring at average 150 °C. Flash column chromatography was performed on Silica Gel 60 M (0.040-0.063 mm, E. Merck). The infrared spectra were measured with Perkin–Elmer Spectrometer. The ^1H and ^{13}C NMR spectra were recorded on BrukerAvance DPX 250 or BrukerAvance 400 Spectrometers. Chemical shifts are given in ppm and are referenced to the deuterated solvent signal or to TMS as internal standard and multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Carbon multiplicities were assigned by distortion less enhancement by polarization transfer (DEPT) experiments. ^1H and ^{13}C signals were attributed on the basis of H–H and H–C correlations. High Resolution Mass spectra were performed on a Bruker Q-TOF MaXis spectrometer by the "Fédération de Recherche" ICOA/CBM (FR2708) platform. LC-MS data was acquired on a Thermo-Fisher UHPLC-MSQ system equipped with an electron spray ionization source (ESI). The temperature of the source was maintained at 350 °C. Initially, the cone voltage was set at 35 V and after 5 min was increased to 75V. In full scan mode, data was acquired between 100 and 1000 m/z in the positive mode with a 1.00 S scan time. In addition, a UV detection was performed with a Diode array detector at three wavelengths 273, 254 and 290 nm, respectively. A water/methanol (70%/30%) solution mixture with 0.1% formic acid was used as mobile phase. The composition of the mobile phase was increased to 100% methanol with 0.1% formic acid with a 7% ramp. The flow rate was set at 0.300 mL.min-1. Samples diluted in the mobile phase were injected (3 µL) on a C18 column (X-terra, Waters), 2.1 mm internal diameter, and 100 mm length placed into an oven at 40 °C. Electronic extraction of ions was performed and the subsequent areas under the corresponding chromatographic peaks determined.

General procedure for acetonide deprotection and subsequent benzoylation. The acetonide intermediate (c = 0.18 mol/L, 1.0 eq.) was dissolved in formic acid/H_2O (85:15 v/v) and the resulting mixture was heated for 1 hour at 60 °C. Toluene was added and all volatiles were evaporated under reduced pressure. After an aqueous workup with sat. aq. $NaHCO_3$ and extractions with EtOAc, the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting yellowish residue was co-evaporated with dry pyridine/toluene (1:1) and then dissolved in DCM under a dried and inert atmosphere (argon). Dry pyridine (4.0 eq.) and DMAP (5 mol%) were added followed by benzoyl chloride (2.5 eq.) at 0 °C. The reaction mixture was allowed to warm at room temperature for 3 hours. The reaction was quenched by addition of H_2O (10 mL), and the resulting mixture was extracted by DCM (2 x 30 mL). The organic layer was subsequently washed with 1 *N* HCl solution and sat. aq. $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure.

General procedure for N-glycosylation under Vorbrüggen conditions. *N,O-Bis*(trimethylsilyl)acetamide (10.0 eq.) was added under argon to a stirred suspension of the nucleobase (c = 0.18 mol/L, 6.0 eq.) in dry MeCN (8 mL) and heated to 80 °C for 30 minutes until a clear solution was obtained. A solution of activated sugar (200 mg, 0.24 mmol, 1.0 eq.) in dry MeCN (2 mL) was added and the resulted mixture was stirred at 80 °C for additional 30 minutes. The reaction mixture was brought to room temperature and treated with trimethylsilyl triflate (8.0 eq.). The dark red solution was stirred at 80 °C for 4 hours. The reaction was quenched with sat. aq. $NaHCO_3$ and extracted with EtOAc. The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was filtered through silica (silica gel, DCM 100% -> DCM/MeOH, 98:2). After concentration under reduced pressure, the obtained material was diluted with a solution of NH_3 (7*N*) in MeOH (15 mL) and the resulted mixture was let stirred at room temperature for 48 hours until complete conversion of the intermediate material. The volatiles were evaporated under reduced pressure and the residue was purified by flash-column chromatography (silica gel, DCM 100% -> DCM/MeOH, 98:2 -> DCM/MeOH, 95:5) to provide the final compound**.**

1) Synthesis of precursor 10

5-*O*-(*tert*-Butyldiphenylsilyl)-1,2-*O*-*iso*propylidene-*α*-*D*-xylofuranose (14). Under a dried and inert atmosphere (nitrogen), was added imidazole (1.15 g, 16.83 mmol, 1.6 eq.), a catalytic amount of DMAP (5 mol%) and dropwise TBDPSCl (3.3 mL, 12.62 mmol, 1.2 eq) to a stirred solution of 1,2-*O*-isopropylidene-α-D-xylofuranose **(12)** (2.00 g, 10.52 mmol, 1.0 eq.) in dry DCM (40 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature for 2 h. The reaction was quenched by addition of sat. aq. $NaHCO_3$ and then the mixture was extracted with DCM. The combined organic layers were washed with water and brine, then dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash-column chromatography (silica gel, PE/EtOAc, 4:1) to afford compound **(14)** as a white solid (4.42 g, 10.31 mmol) in 98% yield. Rf= 0.26 (silica, PE/EtOAc, 4:1). ^1H NMR (400 MHz, DMSO-d6): δ 7.65 (m, 4H, *o*-Ph), 7.45 (m, 6H, *m*-, *p*-Ph), 5.82 (d, ^3J = 3.6 Hz, 1H, H_1), 5.22 (d, ^3J = 5.0 Hz, 1H, -OH), 4.37 (d, ^3J = 3.6 Hz, 1H, H_2), 4.12 (m, 1H, H_4), 3.98 (dd, ^3J = 4.4 Hz, ^3J = 5.0 Hz, 1H, H_3), 3.89 (dd, ^3J = 5.0 Hz, ^3J = 10.6 Hz, 1H, H_{5b}), 3.73 (dd, ^3J = 6.6 Hz, ^3J = 10.6 Hz, 1H, H_{5a}), 1.37 (s, 3H, C(C*H*$_3$)2), 1.23 (s, 3H, C(C*H*$_3$)$_2$), 1.00 (s, 9H, SiC(C*H*$_3$)$_3$). ^{13}C NMR (100 MHz, DMSO-d6): δ□135.6 (2 x CH, -Ph), 135.5 (2x CH, -Ph), 133.5 (2x quat C, -Ph), 130.3 (2x CH, -Ph), 128.3 (4x CH, -Ph), 110.9 (quat C, *C*(CH$_3$)$_2$), 104.9 (CH, C_1), 85.4 (CH, C_2), 81.5 (CH, C_4), 73.9 (CH, C_3), 62.5 (CH_2, C_5), 27.2 (3 x CH_3, SiC(*C*H$_3$)$_2$), 27.1 (CH_3, C(*C*H$_3$)$_2$), 26.6 (CH_3, C(*C*H$_3$)$_2$), 19.3 (quat C, Si*C*(CH$_3$)$_2$). HRMS (ESI$^+$) calcd for $C_{24}H_{32}NaO_5Si$, 451.1911 [M +Na]$^+$; found, 451.1929. The spectroscopic data are in accordance with those reported in literature (**CAS #** 114861-14-2).

5-O-(tert-Butyldiphenylsilyl)-1,2-O-isopropylidene-3-O-trifluoromethane sulfonyl-α-D-xylofuranose (15). To an ice cooled and stirred solution of alcohol **(14)** (1.50 g, 3.50 mmol, 1.0 eq.) in dry DCM (40 mL) was added anhydrous pyridine (0.5 mL, 6.18 mmol, 1.8 eq.) and dropwise triflic anhydride (0.7 mL, 4.15 mmol, 1.2 eq) under inert atmosphere (nitrogen). The reaction mixture was allowed to warm up to room temperature and stirred for 2 h. The reaction was quenched by addition of sat. aq. $NaHCO_3$ and then the mixture was extracted with DCM. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude material was purified by flash-column chromatography (silica gel, DCM 100%) to afford compound **(15)** as a colourless oil (1.84 g, 3.29 mmol) in 94% yield. Rf= 0.78 (silica, DCM 100%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.68 (m, 4H, *o*-Ph), 7.42 (m, 6H, *m*-, *p*-Ph), 5.96 (d, ^3J = 3.8 Hz, 1H, H_1), 5.35 (d, ^3J = 2.8 Hz, 1H, H_3), 4.75 (d, ^3J = 3.8 Hz, 1H, H_2), 4.33 (td, ^3J = 2.8 Hz, ^3J = 6.8 Hz, 1H, H_4), 3.89 (dd, ^3J = 6.8 Hz, 2H, $H_{5a\text{-}b}$), 1.48 (s, 3H, C(C*H*$_3$)2), 1.34 (s, 3H, C(C*H*$_3$)$_2$), 1.08 (s, 9H, SiC(C*H*$_3$)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ□135.6 (4x CH, -Ph), 132.9-132.6 (2x quat C, -Ph), 130.0 (2x CH, -Ph), 127.8 (4x CH, -Ph), 113.0 (quat C, *C*(CH$_3$)$_2$), 104.6 (CH, C_1), 87.9 (CH, C_3), 82.8 (CH, C_2), 78.8 (CH, C_4), 60.0 (CH_2, C_5), 26.7 (3x CH_3, SiC(*C*H$_3$)$_2$), 26.6 (CH_3, C(*C*H$_3$)$_2$), 26.3 (CH_3, C(*C*H$_3$)$_2$), 19.1 (quat C, Si*C*(CH$_3$)$_2$). HRMS (ESI$^+$) calcd. for $C_{25}H_{31}F_3NaO_7SSi$, 583.1404 [M +Na]$^+$; found, 583.1400. The spectroscopic data are in accordance with those reported in literature (**CAS #** 114861-16-4).

3-Azido-3-deoxy-1,2-O-isopropylidene-α-D-ribofuranose (16). A mixture of triflate compound **(15)** (1.10 g, 1.96 mmol, 1.0 eq.), *n*Bu$_4$NBr (7 mg, 1 mol%) and sodium azide (255 mg, 3.92 mmol, 2.0 eq.) was suspended in dry DMF (20 mL) and stirred under inert atmosphere (nitrogen) at 80 °C for 10 min under microwave irradiation (25 W). The yellow suspension was diluted with brine and extracted with EtOAc. The resulting organic layer was dried over Na_2SO_4 and concentrated *under reduced pressure*. The brownish residue was diluted in dry THF (8 mL) and treated with 1 M tetrabutylammonium fluoride (2.4 mL, 2.35 mmol, 1.2 eq.) for 30 min under inert atmosphere at room temperature. The volatiles were evaporated under reduced pressure and the residue was purified by flash-column chromatography (silica gel, DCM 100% -> DCM/MeOH, 98:2), to give **(16)** as a colourless oil (330 mg, 1.55 mmol) in 79% yield (over the two steps). Rf= 0.4 (silica, DCM/MeOH, 98:2). ^1H NMR (400 MHz, $CDCl_3$): δ 5.82 (d, ^3J = 4.1 Hz, 1H, H_1), 4.76 (t, ^3J = 4.1 Hz, 1H, H_2), 4.13 (dt, ^3J = 2.7 Hz, ^3J = 9.6 Hz, 1H, H_4), 4.02 (ddd, ^3J = 2.7 Hz, ^3J = 4.0 Hz, ^3J = 12.6 Hz, 1H, H_{5b}), 3.71 (ddd, ^3J = 3.0 Hz, ^3J = 8.8 Hz, ^3J = 12.6 Hz, 1H, H_{5a}), 3.61 (dd, ^3J = 4.1 Hz, ^3J = 9.6 Hz, 1H, H_3), 2.20 (dd, ^3J = 4.4 Hz, ^3J = 8.8 Hz, 1H, -O*H*), 1.58 (s, 3H, C(C*H*$_3$)$_2$), 1.38 (s, 3H, C(C*H*$_3$)$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 113.3 (quat C, *C*(CH$_3$)$_2$), 104.2 (CH, C_1), 80.2 (CH, C_2), 78.2 (CH, C_4), 60.0 (CH_2, C_5), 59.4 (CH, C_3), 26.4 (2x CH_3, C(*C*H$_3$)$_2$). HRMS (ESI$^+$) calcd. for $C_8H_{13}N_3NaO_4$, 238.0798 [M +Na]$^+$; found, 238.0792. The spectroscopic data are in accordance with those reported in literature (CAS # 23345-80-4).

5-O-Allyl-3-azido-3-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (10). Under a dried and inert atmosphere (argon), a 60% emulsion of sodium hydride (70 mg, 1.74 mmol, 1.5 eq.) in mineral oil was added in three portions to a mixture of alcohol **(16)** (250 mg, 1.16 mmol, 1.0 eq.) in anhydrous THF (12 mL) stirred at 0 °C. After 15 min at this temperature, allyl bromide (0.17 mL, 1.97 mmol, 1.7 eq.) was added dropwise, and the

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resulting mixture was then stirred at room temperature for 2 hours. The reaction was quenched by adding EtOH (5 mL) and solvents were evaporated to give a grey viscous oil which was then diluted in EtOAc. After an aqueous workup with sat. aqueous $NaHCO_3$, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, a yellow sirup was obtained and purified by flash-column chromatography (silica gel, PE/EtOAc, 8:2) to afford **(10)** as a colourless oil (202 mg, 0.79 mmol) in 68% yield. Rf= 0.26 (silica, EP/EtOAc, 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.91 (ddt, ^3J = 5.9 Hz, $^3J_{cis}$= 10.3 Hz, $^3J_{trans}$= 17.2 Hz, 1H, CH_2=C*H*-CH_2-O), 5.83 (d, ^3J = 4.0 Hz, 1H, H_1), 5.29 (ddd, ^4J = 1.6 Hz, J_{gem}= 3.0 Hz, $^3J_{trans}$= 17.2 Hz, 1H, C*H*$_2$=CH-CH_2-O), 5.21 (ddd, ^4J = 1.6 Hz, J_{gem}= 3.0 Hz, $^3J_{trans}$= 10.3 Hz, 1H, C*H*$_2$=CH-CH_2-O), 4.72 (t, ^3J = 4.0 Hz, 1H, H_2), 4.19 (ddd, ^3J = 2.6 Hz, ^3J = 3.7 Hz, ^3J = 9.7 Hz, 1H, H_4), 4.10 (ddd, ^4J = 1.5 Hz, ^3J = 5.7 Hz, $^2J_{gem}$= 12.8 Hz, 1H, CH_2=CH-C*H*$_2$-O), 4.03 (ddd, ^4J = 1.5 Hz, ^3J = 5.7 Hz, $^2J_{gem}$= 12.8 Hz, 1H, CH_2=CH-C*H*$_2$-O), 3.78 (dd, ^3J = 2.6 Hz, $^2J_{gem}$= 11.2 Hz, 1H, H_{5a}), 3.62 (dd, ^3J = 3.7 Hz, $^2J_{gem}$= 11.2 Hz, 1H, H_{5b}), 3.60 (dd, ^3J = 4.0 Hz, ^3J = 9.7 Hz, 1H, H_3), 1.58 (s, 3H, C(C*H*$_3$)$_2$), 1.37 (s, 3H, C(C*H*$_3$)$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 134.2 (CH, CH_2=*C*H-CH_2-O), 117.7 (CH_2, *C*H_2=CH-CH_2-O), 113.0 (*quat* C, *C*$(CH_3)_2$), 104.2 (CH, C_1), 79.9 (CH, C_2), 77.2 (CH, C_4), 72.7 (CH_2, CH_2=CH-*C*H_2-O), 67.6 (CH_2, C_5), 60.4 (CH, C_3), 26.4 (2x CH_3, C(*C*H_3)$_2$). HRMS (ESI$^+$) calcd for $C_{11}H_{17}N_3NaO_4$, 278.1111 [M +Na]$^+$; found, 278.1112.

2) Synthesis of precursor 11

3-O-Allyl-1,2:5,6-bis-O-isopropylidene-α-D-allofuranose (17). Under a dried and inert atmosphere (argon), a 60% emulsion of sodium hydride (430 mg, 10.75 mmol, 1.4 eq.) in mineral oil was added in three portions to a mixture of 1,2:5,6-Di-*O*-isopropylidene-*α-D*-allofuranose (**13**) (2.00 g, 7.68 mmol, 1.0 eq.) in anhydrous THF (40 mL) stirred at 0 °C. After 15 min at this temperature, allyl bromide (1.0 mL, 11.52 mmol, 1.5 eq.) was added dropwise, and the resulting mixture was then stirred at room temperature for 2 hours. The reaction was quenched by adding EtOH (20 mL) and solvents were evaporated to give a grey viscous oil which was diluted in EtOAc. After an aqueous workup with sat. aq. $NaHCO_3$, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. A viscous oil was obtained and purified by flash-column chromatography (silica gel, PE/EtOAc, 4:1), to afford **(17)** as a colourless oil (2.24 g, 7.45 mmol) in 97% yield. Rf= 0.22 (silica, PE/EtOAc, 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.96 (ddt, ^3J = 5.9 Hz, $^3J_{cis}$= 10.3 Hz, $^3J_{trans}$= 17.2 Hz, 1H, CH_2=C*H*-CH_2-O), 5.78 (d, ^3J = 4.0 Hz, 1H, H_1), 5.34 (ddd, ^4J = 1.6 Hz, J_{gem}= 3.0 Hz, $^3J_{trans}$= 17.2 Hz, 1H, C*H*$_2$=CH-CH_2-O), 5.26 (ddd, ^4J = 1.6 Hz, J_{gem}= 3.0 Hz, $^3J_{cis}$= 10.3 Hz, 1H, C*H*$_2$=CH-CH_2-O), 4.62 (t, ^3J = 4.1 Hz, 1H, H_2), 4.39 (td, ^3J = 3.1 Hz, ^3J = 7.0 Hz, 1H, H_5), 4.23 (ddd, ^4J = 1.4 Hz, ^3J= 5.9 Hz, $^2J_{gem}$= 12.3 Hz, 1H, CH_2=CH-C*H*$_2$-O), 4.09 (ddd, ^4J = 1.4 Hz, ^3J = 5.9 Hz, $^2J_{gem}$= 12.3 Hz, 1H, CH_2=CH-C*H*$_2$-O; dd, ^3J = 3.1 Hz, ^3J = 8.7 Hz, 1H, H_4), 4.01 (d, ^3J = 7.0 Hz, 2H, $H_{6\text{-}6'}$), 3.88 (dd, ^3J = 4.5 Hz, ^3J = 8.7 Hz, 1H, H_3), 1.58, 1.45, 1.38, 1.35 (4x s, 12H, 2x C(C*H*$_3$)$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 134.6 (CH, CH_2=*C*H-CH_2-O), 118.0 (CH_2, *C*H_2=CH-CH_2-O), 112.9 (*quat* C, *C*$(CH_3)_2$), 109.7 (*quat* C, *C*$(CH_3)_2$), 103.8 (CH, C_1), 78.0 (CH, C_2), 77.8 (CH, C_4), 77.6 (CH, C_3), 74.8 (CH, C_5), 71.6 (CH_2, CH_2=CH-*C*H_2-O), 65.0 (CH_2, C_6), 26.8, 26.6, 26.2, 25.1 (4x CH_3, C(*C*H_3)$_2$). HRMS (ESI$^+$) calcd for $C_{15}H_{28}NO_6$, 318.1911 [M +NH_4]$^+$; found, 318.1912. The spectroscopic data are in accordance with those reported in literature (CAS # 33746-39-3).

3-O-Allyl-1,2-O-isopropylidene-α-D-allofuranose (18). Diacetonide **(17)** (6.79 g, 22.6 mmol, 1.0 eq.) was diluted in a mixture of acetic acid/formic acid/water (24:10:12 v/v/v) and the resulted mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then concentrated and the colourless oil was diluted in EtOAc. After an aqueous workup with sat. aq. $NaHCO_3$, the resulting organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. A colourless oil was obtained and purified by flash-column chromatography (silica gel, PE/EtOAc, 1:4) to afford the diol **(18)** as a colourless oil (5.47 g, 21.02 mmol) in 93% yield. Rf= 0.20 (silica, PE/EtOAc, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.96 (ddt, ^3J= 5.9 Hz, $^3J_{cis}$= 10.3 Hz, $^3J_{trans}$= 17.2 Hz, 1H, CH_2=C*H*-CH_2-O), 5.78 (d, ^3J = 4.0 Hz, 1H, H_1), 5.34 (ddd, ^4J = 1.6 Hz, J_{gem}= 3.0 Hz, $^3J_{trans}$= 17.2 Hz, 1H, C*H*$_2$=CH-CH_2-O), 5.26 (ddd, ^4J = 1.6 Hz, J_{gem}= 3.0 Hz, $^3J_{cis}$= 10.3 Hz, 1H, C*H*$_2$=CH-CH_2-O), 4.65 (t, ^3J = 4.0 Hz, 1H, H_2), 4.26 (ddd, ^4J = 1.4 Hz, ^3J = 5.6 Hz, $^2J_{gem}$= 12.3 Hz, 1H, CH_2=CH-C*H*$_2$-O), 4.07 (m, 3H, H_4, H_5 CH_2=CH-C*H*$_2$-O), 3.92 (dd, ^3J = 4.0 Hz, ^3J = 8.6 Hz 1H, H_3), 3.73 (m, 2H, $H_{6\text{-}6'}$), 2.48 (d, ^3J = 3.7 Hz, 1H, C_5-OH), 2.43 (dd, ^3J = 5.8 Hz, ^3J = 7.8 Hz, 1H, C_6-OH), 1.59 (s, 3H, C(C*H*$_3$)$_2$), 1.36 (s, 3H, C(C*H*$_3$)$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 133.6 (CH, CH_2=*C*H-CH_2-O), 118.9 (CH_2, *C*H_2=CH-CH_2-O), 113.3 (*quat* C, *C*$(CH_3)_2$), 104.1 (CH, C_1), 79.0 (CH, C_4), 77.3 (CH, C_2), 76.8 (CH, C_3), 71.3 (CH, CH_2=CH-*C*H_2-O), 70.8 (CH, C_5), 63.1 (CH_2, C_6), 26.8, 26.5 (2x CH_3, C(*C*H_3)$_2$). HRMS (ESI$^+$) calcd for $C_{12}H_{21}NO_6$, 261.1333 [M +H]$^+$; found, 261.1334. The spectroscopic data are in accordance with those reported in literature (CAS # 156742-37-9).

3-O-Allyl-1,2-O-isopropylidene-α-D-ribofuranose (19). NaIO4 (495 mg, 2.30 mmol, 1.2 eq.) in water (3.2 mL) was added to a solution of the diol compound **(18)** (500 mg, 1.92 mmol, 1.0 eq.) in ethanol (8 mL) cooled at 0 °C. The reaction was let to stir at room temperature for 1 hour; a white solid precipitated. The mixture was filtered on Celite® and the white solid rinsed with ethanol. The resulting organic layer was concentrated under reduced pressure and the residue (yellowish oil) was then diluted in ethanol (12 mL). $NaBH_4$ (81 mg, 2.11 mmol, 1.1 eq.) was added portionwise to the mixture cooled at 0 °C and the reaction was stirred at room temperature for 30 minutes. The solvents were evaporated under reduced pressure and the residue was diluted in EtOAc. After an aqueous workup with sat. aq. $NaHCO_3$, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude oil was purified by flash-column chromatography (silica gel, DCM 100% -> DCM/MeOH, 98:2) to afford **(19)** (375 mg, 1.63 mmol) as a colourless oil in 85% yield. Rf= 0.32 (silica, DCM/MeOH, 98:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.95 (ddt, ^3J= 5.9 Hz, $^3J_{cis}$= 10.3 Hz, $^3J_{trans}$= 17.2 Hz, 1H, CH_2=C*H*-CH_2-O), 5.75 (d, ^3J= 4.0 Hz, 1H, H_1), 5.32 (ddd, ^4J= 1.6 Hz, $^2J_{gem}$= 3.2 Hz, $^3J_{trans}$= 17.2 Hz, 1H, C*H*$_2$=CH-CH_2-O), 5.23 (ddd, ^4J=1.6 Hz, $^2J_{gem}$= 3.2 Hz, $^3J_{cis}$= 10.3 Hz, 1H, C*H*$_2$=CH-CH_2-O), 4.62 (d, ^3J= 4.0 Hz, 1H, H_2), 4.20 (ddd, ^4J= 1.6 Hz, ^3J= 5.8 Hz, $^2J_{gem}$= 12.7 Hz, 1H, CH_2=CH-C*H*$_2$-O), 4.09 (ddd, ^3J= 2.3 Hz ^3J= 3.8 Hz, ^3J= 9.0 Hz, 1H, H_4), 4.09 (ddd, ^4J= 1.6 Hz, ^3J= 5.8 Hz, $^2J_{gem}$= 12.7 Hz, 1H, CH_2=CH-C*H*$_2$-O), 3.95 (dd, ^3J= 2.5 Hz, ^3J= 4.0 Hz, $^2J_{gem}$= 12.7 Hz, 1H, H_{5a}), 3.84 (dd, ^3J= 4.0 Hz, ^3J= 9.1 Hz, 1H, H_3), 3.68 (dd, ^3J= 3.1 Hz, ^3J= 9.0 Hz, $^2J_{gem}$= 12.7 Hz, 1H, H_{5b}), 1.81 (dd, ^3J= 4.0 Hz, ^3J= 9.0 Hz, 1H, -OH), 1.57 (s, 3H, -CH_3), 1.35 (s, 3H, -CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ□134.4 (CH, CH_2=*C*H-CH_2-O), 118.1 (CH_2, *C*H_2=CH-CH_2-O), 113.1 (*quat* C, *C*$(CH_3)_2$), 104.0 (CH, C_1), 78.7 (CH, C_4), 77.8 (CH, C_2), 76.8 (CH, C_3), 71.7 (CH_2, CH_2=CH-*C*H_2-O), 60.6 (CH_2, C_5), 26.8, 26.5 (2x CH_3, C(*C*H_3)$_2$). HRMS (ESI$^+$) calcd for $C_{11}H_{22}NO_5$, 248.1492 [M +NH_4]$^+$; found, 248.1495. The spectroscopic data are in accordance with those reported in literature (CAS # 158895-35-3).

1,2-O-isopropylidene-5-O-2-propynyl-3-O-2-propen-1-yl-α-D-ribofuran ose (20). Under a dried and inert atmosphere (argon), a 60% emulsion of sodium hydride (275 mg, 6.88 mmol, 1.4 eq.) in mineral oil was added in three portions to a mixture of **(19)** (1.13 g, 4.90 mmol, 1.0 eq.) in anhydrous THF (25 mL) stirred at 0 °C. After 15 min at this temperature, propargyl bromide (0.8 mL, 7.4 mmol, 1.5 eq.) was added dropwise, and the resulting mixture was then stirred at room temperature for 3 hours. The reaction was quenched by adding EtOH (20 mL) and solvents were evaporated to give a grey viscous oil which was diluted in DCM. After an aqueous workup with sat. aqueous $NaHCO_3$, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. A viscous oil was obtained and purified by flash-column chromatography (silica gel, DCM 100% -> DCM/MeOH 98:2 -> DCM/MeOH 95:5), to afford **(20)** as a yellowish oil (1.10 g, 4.12 mmol) in 84% yield. Rf= 0.78 (silica, DCM/MeOH, 95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.94 (ddt, ^3J= 5.9 Hz, $^3J_{cis}$= 10.3 Hz, $^3J_{trans}$= 17.2 Hz, 1H, CH_2=C*H*-CH_2-O), 5.76 (d, ^3J= 4.0 Hz, 1H, H_1), 5.32 (ddd, ^4J= 1.6 Hz, $^2J_{gem}$= 3.2 Hz, $^3J_{trans}$= 17.2 Hz, 1H, C*H*$_2$=CH-CH_2-O), 5.22 (ddd, ^4J=1.6 Hz, $^2J_{gem}$= 3.2 Hz, $^3J_{cis}$= 10.3 Hz, 1H, C*H*$_2$=CH-CH_2-O), 4.59 (d, ^3J= 4.0 Hz, 1H, H_2), 4.26 (dd, ^4J= 2.4 Hz, $^2J_{gem}$= 15.9 Hz, 1H, HCC-C*H*$_2$-O), 4.19 (ddd, ^4J= 1.6 Hz, ^3J= 5.9 Hz, $^2J_{gem}$= 12.8 Hz, 1H, CH_2=CH-C*H*$_2$-O), 4.17 (dd, ^4J= 2.4 Hz, $^2J_{gem}$= 15.9 Hz, 1H, HCC-C*H*$_2$-O), 4.13 (ddd, ^3J= 2.3 Hz ^3J= 3.8 Hz, ^3J= 9.0 Hz, 1H, H_4), 4.10 (ddd, ^4J= 1.6 Hz, ^3J= 5.9 Hz, $^2J_{gem}$= 12.8 Hz, 1H, CH_2=CH-C*H*$_2$-O), 3.85 (dd, ^3J= 4.0 Hz, ^3J= 9.0 Hz, 1H, H_3), 3.83 (dd, ^3J= 2.3 Hz, $^2J_{gem}$= 11.2 Hz, 1H, H_{5a}), 3.71 (dd, ^3J= 3.8 Hz, $^2J_{gem}$= 11.2 Hz, 1H, H_{5b}), 2.44 (t, ^4J= 2.4 Hz, 1H, *H*CC-CH_2-O), 1.57 (s, 3H, C(C*H*$_3$)$_2$), 1.35 (s, 3H, C(C*H*$_3$)$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ□134.1 (CH, CH_2=*C*H-CH_2-O), 118.1 (CH_2, *C*H_2=CH-CH_2-O), 112.9 (*quat* C, *C*$(CH_3)_2$), 104.4 (CH, C_1), 79.4 (*quat* C, HC*C*-CH_2-O), 77.6 (CH, C_4), 77.5 (CH, C_2), 77.2 (CH, C_3), 74.7 (CH, HC*C*-CH_2-O), 71.7 (CH_2, CH_2=CH-*C*H_2-O), 67.6 (CH_2, C_5), 58.7 (CH_2, HCC-*C*H_2-O), 26.8, 26.5 (2x CH_3, C(*C*H_3)$_2$). HRMS (ESI$^+$) calcd for $C_{14}H_{24}NO_5$, 286.1649 [M +NH_4]$^+$; found, 286.1649.

5-O-2-propynyl-3-O-2-propen-1-yl-1,2-dibenzoate-α/β-D-ribofuranose (11). According to procedure 1, the derivative **(20)** (2.0 g, 7.45 mmol, 1.0 eq.) was deprotected under acidic conditions (formic ac., 34 mL + H_2O, 6 mL) and subsequently protected into benzoates under basic conditions: DCM (50 mL), pyridine (2.4 mL, 30 mmol, 4.0 eq.), DMAP (5 mol%) and benzoyl chloride (2.2 mL, 18.63 mmol, 2.5 eq.). The title compound **11** was obtained after purification by flash-column chromatography (silica gel, PE/EtOAc, 4:1) as a yellowish oil (1.98 g, 4.54 mmol) in 61% yield (over the two steps). α/β = 1:1. β anomer could be isolated for analysis. Rf = 0.3 (silica, PE/EtOAc, 4:1). Major β anomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (m, 4H, *o*-Ph), 7.60 (m, 2H, *p*-Ph), 7.46 (m, 4H, *m*-Ph), 6.54 (s, 1H, H_1), 5.83 (ddt, ^3J= 5.7 Hz, $^3J_{cis}$= 10.3 Hz, $^3J_{trans}$= 17.2 Hz, 1H, CH_2=C*H*-CH_2-O), 5.68 (d, ^3J= 4.3 Hz, 1H, H_2), 5.26 (ddd, ^4J= 1.6 Hz, $^2J_{gem}$= 3.2 Hz, $^3J_{trans}$=

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17.2 Hz, 1H, CH_2=CH-CH$_2$-O), 5.15 (ddd, ^4J=1.6 Hz, $^2J_{gem}$= 3.2 Hz, $^3J_{cis}$= 10.3 Hz, 1H, CH_2=CH-CH$_2$-O), 4.46 (dd, $^3J_{cis}$= 4.3 Hz, ^3J= 7.7 Hz, 1H, H_3), 4.38 (ddd, ^3J= 3.2 Hz ^3J= 4.2 Hz, ^3J= 7.7 Hz, 1H, H_4), 4.18 (dd, ^4J= 2.4 Hz, $^2J_{gem}$= 15.9 Hz, 1H, HCC-CH_2-O), 4.16 (ddd, ^4J= 1.6 Hz, ^3J= 5.7 Hz, $^2J_{gem}$= 12.5 Hz, 1H, CH$_2$=CH-CH_2-O), 4.13 (dd, ^4J= 2.4 Hz, $^2J_{gem}$= 15.9 Hz, 1H, HCC-CH_2-O), 4.07 (ddd, ^4J= 1.6 Hz, ^3J= 5.7 Hz, $^2J_{gem}$= 12.5 Hz, 1H, CH$_2$=CH-CH_2-O), 3.82 (dd, ^3J= 3.2 Hz, $^2J_{gem}$= 11.0 Hz, 1H, H_{5a}), 3.77 (dd, ^3J= 4.2 Hz, $^2J_{gem}$= 11.0 Hz, 1H, H_{5b}), 2.35 (t, ^4J= 2.4 Hz, 1H, *H*CC-CH$_2$-O). ^{13}C NMR (100 MHz, CDCl$_3$): δ 165.4 (*quat* C, Ph-*C*O), 164.7 (*quat* C, Ph-*C*O), 133.9 (CH, CH$_2$=*C*H-CH$_2$-O), 133.5 (CH, Ph-CO), 133.4 (CH, Ph-CO), 130.0 (2x CH, Ph-CO), 129.9 (2x CH, Ph-CO), 129.6 (*quat* C, Ph-CO), 129.4 (*quat* C, Ph-CO), 128.5 (2x CH, Ph-CO), 128.4 (2x CH, Ph-CO), 117.9 (CH$_2$, *C*H$_2$=CH-CH$_2$-O), 99.2 (CH, C$_1$), 81.6 (CH, C$_4$), 79.4 (*quat* C, HC*C*-CH$_2$-O), 76.7 (CH, C$_3$), 74.6 (CH, HC*C*-CH$_2$-O), 74.1 (CH, C$_2$), 72.2 (CH$_2$, CH$_2$=CH-*C*H$_2$-O), 68.9 (CH$_2$, C$_5$), 58.7 (CH$_2$, HCC-*C*H$_2$-O). HRMS (ESI$^+$) calcd for $C_{25}H_{28}NO_7$, 454.1860 [M +NH$_4$]$^+$; found, 454.1862.

3) Synthesis of macrocycle 8.

[4α-allyloxy-5β-[[1-[(3aα,5β,6α,6aα)-5-(allyloxymethyl)-2,2-dimethyl-3a,5,6,6a-tetrahydrofuro[2,3-d][1,3]dioxol-6-yl]triazol-4-yl]methoxymethyl]-2α/β-benzoyloxy-tetrahydrofuran-3α-yl]benzoate (9). The azido derivative **(10)** (500 mg, 1.96 mmol, 1.0 eq.) and the α/β mixture of analogue **11** (1.03 g, 2.35 mmol, 1.2 eq.) were dissolved in *t*BuOH/H$_2$O (2:1, 20 mL) under nitrogen at room temperature. Subsequently, a solution of sodium ascorbate (154 mg, 0.78 mmol, 0.40 eq.) in water (2 mL) and a solution of copper(II) sulfate pentahydrate (50 mg, 0.20 mmol, 0.10 eq.) in water (2 mL) was added. The reaction mixture was stirred at 40 °C for 3 hours. Volatiles were evaporated and the residue was purified by flash-column chromatography (silica gel, PE/EtOAc, 4:1 -> PE/EtOAc, 1:1) to provide **(9)** as a colourless oil (892 mg, 1.29 mmol) in 66% yield. α/β = 1:1. Rf (α anomer) = 0.44 (DCM/MeOH = 100:5). Rf (β anomer) = 0.30. Rf= 0.4 (silica, PE/EtOAc, 1:1). β anomer could be partially isolated for analysis. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.08 (d, ^3J= 7.6 Hz, 2H, *o*-Ph), 8.01 (d, ^3J= 7.6 Hz, 2H, *o*-Ph), 7.72 (s, 1H, =C*H*-N), 7.59 (m, 2H, *p*-Ph), 7.46 (m, 4H, *m*-Ph), 6.53 (s, 1H, $H_{1'}$), 5.96 (d, ^3J= 3.8 Hz, 1H, $H_{1'}$), 5.83 (m, 2H, CH$_2$=C*H*-CH$_2$-O), 5.27 (d, ^3J= 3.0 Hz, 1H, $H_{2'}$), 5.22-5.10 (m, 5H, C*H*$_2$=CH-CH$_2$-O, $H_{3'}$), 4.72 (t, ^3J= 3.0 Hz, 1H, $H_{2'}$), 4.70 (s, 2H, O-C*H*$_2$-triazolyl), 4.51 (dt, ^3J= 3.0 Hz, ^3J= 10.0 Hz, 1H, $H_{4'}$), 4.40 (m, 2H, $H_{3''}$, $H_{4''}$), 4.14 (dd, ^3J= 5.5 Hz, $^2J_{gem}$= 12.5 Hz, 1H, CH$_2$=CH-C*H*$_2$-O), 4.04 (dd, ^3J= 5.5 Hz, $^2J_{gem}$= 12.5 Hz, 1H, CH$_2$=CH-C*H*$_2$-O), 3.99 (d, ^3J= 5.5 Hz, 1H, CH$_2$=CH-C*H*$_2$-O), 3.86 (dd, ^3J= 1.7 Hz, $^2J_{gem}$= 11.2 Hz, 1H, $H_{5''a}$), 3.75 (dd, ^3J= 3.9 Hz, $^2J_{gem}$= 11.2 Hz, 1H, $H_{5''b}$), 3.70 (dd, ^3J= 2.4 Hz, $^2J_{gem}$= 11.5 Hz, 1H, $H_{5'a}$), 3.46 (dd, ^3J= 3.8 Hz, $^2J_{gem}$= 11.5 Hz, 1H, $H_{5'b}$), 1.58 (s, 3H, -CH$_3$), 1.32 (s, 3H, -CH$_3$). ^{13}C NMR (175 MHz, CDCl$_3$): δ165.4 (*quat* C, Ph-*C*O), 164.7 (*quat* C, Ph-*C*O), 144.8 (*quat* C, N-*C*=CH-N), 134.1 (CH, CH$_2$=*C*H-CH$_2$-O), 133.8 (CH, CH$_2$=*C*H-CH$_2$-O), 133.5 (CH, Ph-CO), 133.4 (CH, Ph-CO), 130.0 (2x CH, Ph-CO), 129.9 (2x CH, Ph-CO), 129.6 (*quat* C, Ph-CO), 129.4 (*quat* C, Ph-CO), 128.5 (2x CH, Ph-CO), 128.4 (2x CH, Ph-CO), 123.0 (CH, N-C=*C*H-N), 117.9 (CH$_2$, *C*H$_2$=CH-CH$_2$-O), 117.6 (CH$_2$, *C*H$_2$=CH-CH$_2$-O), 113.6 (*quat* C, *C*(CH$_3$)$_2$), 104.4 (CH, C$_{1'}$), 99.3 (CH, C$_{1''}$), 81.7 (CH, C$_{4''}$), 78.7 (CH, C$_{2'}$), 77.9 (CH, C$_{4'}$), 76.9 (CH, C$_{3''}$), 74.1 (CH, C$_{2''}$), 72.7 (CH$_2$, CH$_2$=CH-*C*H$_2$-O), 72.1 (CH$_2$, CH$_2$=CH-*C*H$_2$-O), 70.3 (CH$_2$, C$_{5''}$), 67.0 (CH$_2$, C$_{5'}$), 64.9 (CH$_2$, O-*C*H$_2$-triazolyl), 61.1 (CH, C$_{3'}$), 26.6, 26.3 (2x CH$_3$, C(*C*H$_3$)$_2$). HRMS (ESI$^+$) calcd for $C_{36}H_{42}N_3O_{11}$, 692.2814 [M +H]$^+$; found, 692.2815.

(2α,3α,7α,9β,17α,18α,19α/β,21β)-[(13E)-19-benzoyloxy-5,5-dimethyl-4,6,8,11,16,20,23-heptaoxa-1,26,27-triazapentacyclo[23.2.1.02,9.03,7.017,21] octacosa-13,25(28),26-trien-18-yl] benzoate (8). To a stirred solution of derivative **(9)** (500 mg, 0.72 mmol,1.00 equiv.) in dry DCM (120 mL) was added dropwise a solution of Grubbs II catalyst (68 mg, 0.08 mmol, 5 mol%) in dry DCM (2 mL) at 35 °C under argon over 2 hours. The mixture was then stirred for additional 30 minutes at this temperature and then concentrated under reduced pressure. The dark residue was washed through a pad of silica with DCM and then filtered by PE/EtOAc, 1:1. Volatiles were evaporated and the greenish residue was filtered through a plug of activated charcoal with EtOAc to give crude macrocycle compound **(8)** as a white foam (325 mg) which was used in the next step without further purification. α/β = 1:1. β anomer could be partially isolated for analysis. Rf= 0.2 (silica, PE/EtOAc, 1:1). ^{1}H NMR (700 MHz, CDCl$_3$): δ 8.12 (dd, ^4J= 1.1 Hz, ^3J= 8.1 Hz, 2H, *o*-Ph),8.06 (dd, ^4J= 1.1 Hz, ^3J= 8.1 Hz, 2H, *o*-Ph), 7.73 (s, 1H, =C*H*-N), 7.60 (m, 2H, *p*-Ph), 7.50 (t, ^3J= 7.8 Hz, 2H, *m*-Ph), 7.45 (t, ^3J= 7.8 Hz, 2H, *m*-Ph), 6.54 (s, 1H, $H_{1'}$), 5.98 (d, ^3J= 3.9 Hz, 1H, $H_{1'}$), 5.60 (d, ^3J= 4.4 Hz, 1H, $H_{2'}$), 5.47 (ddd, ^3J= 4.3 Hz, ^3J= 6.8 Hz, $^3J_{trans}$= 15.5 Hz, 1H, CH'$_2$-CH'=C*H*''-CH''$_2$), 5.37 (ddd, ^3J= 4.3 Hz, ^3J= 7.1 Hz, $^3J_{trans}$= 15.5 Hz, 1H, -CH'$_2$-C*H*'=CH''-CH''$_2$), 5.23 (dd, ^3J= 3.9 Hz, ^3J= 10.2 Hz, 1H, $H_{3'}$), 4.84 (t, ^3J= 3.9 Hz, 1H, $H_{2'}$), 4.75 (d, $^2J_{gem}$= 12.8 Hz, 1H, O-C*H*$_2$-triazolyl), 4.52 (d, $^2J_{gem}$= 12.8 Hz, 1H, O-C*H*$_2$-triazolyl), 4.47 (ddd, ^3J= 3.4 Hz, ^3J= 4.4 Hz, ^3J= 10.2 Hz, 1H, $H_{4'}$), 4.40 (dd, ^3J= 4.4 Hz, ^3J= 8.0 Hz, 1H, $H_{3''}$), 4.22 (ddd, ^3J= 3.6 Hz, ^3J= 4.2 Hz, ^3J= 8.0 Hz, 1H, $H_{4''}$), 4.18 (dd, ^3J= 4.3 Hz, $^3J_{gem}$= 12.0 Hz, 1H, -C*H*'$_2$-CH'=CH''-CH''$_2$), 4.04 (dd, ^3J= 4.3 Hz, $^3J_{gem}$= 13.1 Hz, 1H, -CH'$_2$-CH'=CH''-C*H*''$_2$), 3.77 (dd, ^3J= 7.1 Hz, $^3J_{gem}$= 12.0 Hz, 1H, -C*H*'$_2$-CH'=CH''-CH''$_2$), 3.72 (dd, ^3J= 6.8 Hz, $^3J_{gem}$= 13.1 Hz, 1H, -CH'$_2$-CH'=CH''-C*H*''$_2$), 3.71 (dd, ^3J= 4.2 Hz, $^2J_{gem}$= 10.0 Hz, 1H, $H_{5''}$), 3.57 (dd, ^3J= 4.4 Hz, $^2J_{gem}$= 10.8 Hz, 1H, $H_{5'}$), 3.57 (dd, ^3J= 3.6 Hz, $^2J_{gem}$= 10.0 Hz, 1H, $H_{5''}$), 3.50 (dd, ^3J= 3.4 Hz, $^2J_{gem}$= 10.8 Hz, 1H, $H_{5'}$), 1.65 (s, 3H, -CH$_3$), 1.35 (s, 3H, -CH$_3$). ^{13}C NMR (175 MHz, CDCl$_3$): δ 165.3 (*quat* C, Ph-*C*O), 164.9 (*quat* C, Ph-*C*O), 144.0 (*quat* C, N-*C*=CH-N), 133.5 (CH, Ph-CO), 133.4 (CH, Ph-CO), 130.3 (CH, CH'$_2$-CH'=C*H*''-CH''$_2$), 130.1 (2x CH, Ph-CO), 129.9 (2x CH, Ph-CO), 129.5 (*quat* C, Ph-CO), 129.4 (*quat* C, Ph-CO), 128.6 (CH, CH'$_2$-CH'=CH''-CH''$_2$), 128.5 (2x CH, Ph-CO), 128.5 (2x CH, Ph-CO), 123.4 (CH, N-C=*C*H-N), 113.6 (*quat* C, *C*(CH$_3$)$_2$), 104.3 (CH, C$_{1'}$), 99.2 (CH, C$_{1''}$), 80.9 (CH, C$_{4''}$), 78.6 (2x CH, C$_{2'}$, C$_{3''}$), 76.7 (CH, C$_{4'}$), 74.2 (CH, C$_{2''}$), 72.0 (CH$_2$, -C*H*'$_2$-CH'=CH''-CH''$_2$), 70.3 (CH$_2$, -CH'$_2$-CH'=CH''-C*H*''$_2$), 67.7 (CH$_2$, C$_{5''}$), 65.6 (CH$_2$, C$_{5'}$), 63.3 (CH$_2$, O-*C*H$_2$-triazolyl), 62.5 (CH, C$_{3'}$), 26.5, 26.2 (2x CH$_3$, C(*C*H$_3$)$_2$). HRMS (ESI$^+$) calcd for $C_{34}H_{38}N_3O_{11}$, 664.2501 [M +H]$^+$; found, 664.2505.

4) Synthesis of 3',3'-CDN.

(2α,3α,7α,9β,17α,18α,19β,21β)-[(13E)-19-((N6-benzoyl)adenin-9-yl)-5,5-dimethyl-4,6,8,11,16,20,23-heptaoxa-1,26,27-triazapentacyclo [23.21.02,9.03,7.017,21]octacosa-13,25(28),26-trien-18-yl] benzoate (21). A solution of *N,O*-Bis(trimethylsilyl)acetamide (1M) in dry MeCN (0.36 mL, 0.36 mmol, 4.0 eq.) was added under argon to a stirred suspension of N^6-benzoyladenine (43 mg, 0.18 mmol, 2.0 eq.) in dry MeCN (2 mL) and heated to 80 °C for 30 minutes until a clear solution was obtained. A solution of intermediates mixture **(14)** (60 mg, 0.09 mmol, 1.0 eq.) in MeCN (2 mL) was added and the resulted mixture was let stirred at 80 °C for additional 30 minutes. The reaction mixture was brought to room temperature and treated with a solution of trimethylsilyl triflate (1M) in dry ACN (0.22 mL, 0.22 mmol, 2.5 eq.). The dark red solution was stirred at 80 °C for 2 hours. The reaction was quenched with sat. aq. NaHCO$_3$ and extracted with EtOAc. The organic layers were dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography (DCM 100% -> DCM/MeOH, 97:3) to provide the nucleoside (**21**) as a white solid (24 mg, 0.031 mmol) in *34%* yield, in 90% purity. Rf= 0.7 (DCM/MeOH, 9:1). ^{1}H NMR (700 MHz, DMSO-d_6): δ 11.26 (br s, 1H, -N*H*Bz), 8.75 (s, 1H, H_2), 8.73 (s, 1H, H_8), 8.28 (s, 1H, =C*H*-N), 8.06 (d, ^3J= 7.3 Hz, 2H, *o*-Ph), 8.01 (d, ^3J= 7.3 Hz, 2H, *o*-Ph), 7.70 (t, ^3J= 7.3 Hz, 1H, *p*-Ph), 7.65 (t, ^3J= 7.3 Hz, 1H, *p*-Ph), 7.56 (m, 4H, *m*-Ph), 6.47 (d, ^3J= 3.0 Hz, 1H, $H_{1''}$), 5.99 (dd, ^3J= 3.0 Hz, ^3J= 5.3 Hz, 1H, $H_{2''}$), 5.93 (d, ^3J= 3.6 Hz, 1H, $H_{1'}$), 5.39 (dt, ^3J= 5.0 Hz, $^3J_{trans}$= 15.5 Hz, 1H, CH'$_2$-CH'=C*H*''-CH''$_2$), 5.33 (dt, ^3J= 5.0 Hz, $^3J_{trans}$= 15.5 Hz, 1H, CH'$_2$-C*H*'=CH''-CH''$_2$), 5.15 (dd, ^3J= 4.7 Hz, ^3J= 10.2 Hz, 1H, $H_{3'}$), 4.86 (t, ^3J= 4.2 Hz, 1H, $H_{2'}$), 4.75 (d, $^2J_{gem}$= 13.0 Hz, 1H, O-C*H*$_2$-triazolyl), 4.69 (ddd, ^3J= 4.2 Hz, ^3J= 5.0 Hz, ^3J= 10.2 Hz, 1H, $H_{4'}$), 4.53 (d, $^2J_{gem}$= 13.0 Hz, 1H, O-C*H*$_2$-triazolyl), 4.38 (dd, ^3J= 5.3 Hz, ^3J= 7.0 Hz, 1H, $H_{3''}$), 4.22 (ddd, ^3J= 3.3 Hz, ^3J= 3.8 Hz, ^3J= 7.0 Hz, 1H, $H_{4''}$), 4.04 (dd, ^3J= 5.0 Hz, $^2J_{gem}$= 13.3 Hz, 1H, CH'$_2$-CH'=CH''-C*H*''$_2$), 3.96 (dd, ^3J= 5.0 Hz, $^2J_{gem}$= 13.2 Hz, 1H, C*H*'$_2$-CH'=CH''-CH''$_2$), 3.77 (m, 3H, C*H*'$_2$-CH'=CH''-CH''$_2$, CH'$_2$-CH'=CH''-C*H*''$_2$, $H_{5''}$), 3.54 (dd, ^3J= 3.3 Hz, $^2J_{gem}$= 11.0 Hz, 1H, $H_{5''}$), 3.51 (dd, ^3J= 4.6 Hz, $^2J_{gem}$= 10.8 Hz, 1H, $H_{5'}$), 3.47 (dd, ^3J= 3.7 Hz, $^2J_{gem}$= 10.8 Hz, 1H, $H_{5'}$), 1.54 (s, 3H, -CH$_3$), 1.25 (s, 3H, -CH$_3$). ^{13}C NMR (175 MHz, DMSO-d_6): δ 165.1 (2x *quat* C, Ph-*C*O), 152.3 (*quat* C, C$_6$), 152.2 (CH, C$_2$), 150.9 (*quat* C, C$_4$), 143.1 (*quat* C, N-C=CH-N, CH, C$_8$), 134.3 (CH, Ph-CO), 133.0 (CH, Ph-CO), 129.9 (2x CH, Ph-CO), 129.6 (CH, CH'$_2$-CH'=CH''-CH''$_2$), 129.5 (CH, CH'$_2$-CH'=CH''-CH''$_2$), 129.4 (2x CH, Ph-CO), 129.0 (*quat* C, 2x CH, Ph-CO), 129.0 (*quat* C, 2x CH, Ph-CO), 126.2 (*quat* C, C$_5$), 125.5 (CH, N-C=*C*H-N), 112.9 (*quat* C, *C*(CH$_3$)$_2$), 104.6 (CH, C$_{1'}$), 87.0 (CH, C$_{1''}$), 81.3 (CH, C$_{4''}$), 78.6 (CH, C$_{2'}$), 76.5 (CH, C$_{3''}$), 75.8 (CH, C$_{4'}$), 74.9 (CH, C$_{2''}$), 71.1 (CH$_2$, -C*H*'$_2$-CH'=CH''-CH''$_2$), 69.7 (CH$_2$, -CH'$_2$-CH'=CH''-CH''$_2$), 67.0 (CH$_2$, C$_{5'}$), 66.8 (CH$_2$, C$_{5''}$), 62.9 (CH$_2$, O-CH$_2$-triazolyl), 62.5 (CH, C$_{3'}$), 26.7, 26.7 (2x CH$_3$, C(*C*H$_3$)$_2$). HRMS (ESI$^+$) calcd for $C_{39}H_{41}N_8O_{10}$, 781.2940 [M +H]$^+$; found, 781.2942.

(*2α,3α,4α,6β,14α,15α,16α/β,18β*)-[(10Z)-4,15,16-tribenzoyloxy-5,8,13,17,20-pentaoxa-1,23,24-triazatetracyclo[20.2.1.02,6.014,18]pentacosa-10,22(25),23-trien-3-yl]benzoate (24). According to procedure 1, the derivative **(8)** (600 mg, 0.90 mmol, 1.0 eq.) was deprotected under acidic conditions (formic ac., 5.5 mL + H$_2$O, 1 mL) and subsequently protected into benzoates under basic conditions: DCM (10 mL), pyridine (0.3 mL, 3.60 mmol, 4.0 eq.), DMAP (5 mol%) and benzoyl chloride (0.26 mL, 2.25 mmol, 2.5 eq.). After the successive aqueous work-ups, volatile materials were removed under reduced pressure, the obtained residue was filtered through silica (silica gel, PE/EtOAc, 1:1 -> PE/EtOAc, 1:4) and isolated as a white foam (416

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mg) used in the next step without further purification. α-α/α-β/β-α/β-β = 0.25:0.25:0.25:0.25 (inseparable mixture by flash column chromatography). Rf= 0.5 (silica, PE/EtOAc, 1:4). HRMS (ESI$^+$) calcd for $C_{38}H_{36}N_3O_{11}$, 710.2344 [M - OBz]$^+$; found, 710.2343.

3',3'-Thymine-CDN or (2α,3α,4β,6β,14α,15α,16β,18β)–(10*E*)-5,8,13,17,20-pentaoxa-4,16-di-thymin-1-yl-1,23,24-triazatetracyclo[20.2.1.02,6.014,18] pentacosa-10,22(25),23-triene-3,15-diol (7a). The title compound **7a** was prepared according to procedure 2, starting from thymine (180 mg, 1.43 mmol, 6.0 eq.) as nucleobase. After aqueous treatments and filtration over silica, the crude dibenzoate intermediate CDN was isolated as a whitish foam (143 mg) - Rf= 0.5 (silica, DCM/MeOH, 95:5) - and was directly engaged under the basic deprotection conditions. After the completion of the reaction, all volatiles were evaporated under reduced pressure and the residue was purified by flash-column chromatography (silica gel, DCM 100% -> DCM/MeOH, 98:2 -> DCM/MeOH, 95:5) to provide the final 3',3'-thymine-CDN compound **7a** as a white foam (24 mg, 0.039 mmol) in 39% yield. Rf= 0.39 (silica, DCM/MeOH, 9:1). ^{1}H NMR (700 MHz, DMSO-d_6): δ 11.40 (s, 1H, -NH), 11.32 (s, 1H, -NH), 8.26 (s, 1H, =C*H*-N), 6.18 (d, ^3J= 5.4 Hz, 1H, -OH'), 5.83 (d, ^3J= 2.0 Hz, 1H, H$_{1'}$), 5.73 (d, ^3J= 5.0 Hz, 1H, H$_{1''}$), 5.48 (ddd, ^3J= 4.8 Hz, ^3J= 5.9 Hz, $^3J_{trans}$= 15.4 Hz, 1H, CH'$_2$-CH'=C*H''*-CH''$_2$), 5.42 (m, 3H, -CH'$_2$-C*H*'=CH''$_2$-, H$_{3'}$, -OH''), 4.81 (d, $^2J_{gem}$= 13.0 Hz, 1H, O-C*H*$_2$-triazolyl), 4.72 (dt, ^3J= 4.3 Hz, ^3J= 9.7 Hz, 1H, H$_{4'}$), 4.45 (d, $^2J_{gem}$= 13.0 Hz, 1H, O-C*H*$_2$-triazolyl), 4.44 (td, ^3J= 2.0 Hz, ^3J= 5.7 Hz, 1H, H$_{2'}$), 4.11 (dd, ^3J= 5.0 Hz, ^3J= 10.7 Hz, 1H, H$_{2''}$), 4.08 (dd, ^3J= 4.8 Hz, $^2J_{gem}$= 13.7 Hz, 1H, C*H*'$_2$-CH'=CH''-CH''$_2$), 4.00 (dd, ^3J= 5.3 Hz, $^2J_{gem}$= 12.9 Hz, 1H, CH'$_2$-CH'=CH''-C*H*''$_2$), 3.94 (dt, ^3J= 2.5 Hz, ^3J= 4.8 Hz, 1H, H$_{4''}$), 3.86 (dd, ^3J= 6.0 Hz, $^2J_{gem}$= 12.9 Hz, 1H, CH'$_2$-CH'=CH''-C*H*''$_2$), 3.78 (dd, ^3J= 5.9 Hz, $^2J_{gem}$= 13.7 Hz, 1H, C*H*'$_2$-CH'=CH''-CH''$_2$), 3.65 (dd, ^3J= 2.8 Hz, $^2J_{gem}$= 11.0 Hz, 1H, H$_{5''}$), 3.63 (dd, ^3J= 4.7 Hz, $^2J_{gem}$= 10.8 Hz, 1H, H$_{5'}$), 3.54 (dd, ^3J= 3.8 Hz, $^2J_{gem}$= 10.8 Hz, 1H, H$_{5'}$), 3.49 (dd, ^3J= 5.0 Hz, ^3J= 9.7 Hz, 1H, H$_{3''}$), 3.33 (dd, ^3J= 2.2 Hz, $^2J_{gem}$= 11.0 Hz, 1H, H$_{5''}$), 1.83 (d, ^4J= 1.0 Hz, 3H, -CH$_{3b}$), 1.81 (d, ^4J= 1.0 Hz, 3H, -CH$_{3a}$). ^{13}C NMR (175 MHz, CDCl$_3$): δ□164.3-164.2 (2x *quat* C, C$_{4a-b}$), 151.1 (*quat* C, C$_{2b}$), 150.8 (*quat* C, C$_{2a}$), 142.7 (*quat* C, N-*C*=CH-N), 137.2 (CH, C$_{6a}$), 136.3 (CH, C$_{6b}$), 130.8 (CH, CH'$_2$-CH'=C*H*''-CH''$_2$), 128.7 (CH, CH'$_2$-CH'=CH''-CH''$_2$), 126.1 (CH, N-C=C*H*-N), 109.9 (*quat* C, C$_{5a}$), 109.6 (*quat* C, C$_{5b}$), 91.9 (CH, C$_{1'}$), 88.7 (CH, C$_{1''}$), 81.3 (CH, C$_{4''}$), 78.5 (CH, C$_{4'}$), 76.7 (CH, C$_{3''}$), 73.4 (CH, C$_{2'}$), 72.9 (CH, C$_{2''}$), 70.2 (CH$_2$, *C*H'$_2$-CH'=CH''-CH''$_2$), 69.5 (CH$_2$, CH'$_2$-CH'=CH''-C*H*''$_2$), 67.3 (CH$_2$, C$_{5'}$), 66.6 (CH$_2$, C$_{5''}$), 62.6 (CH$_2$, O-*C*H$_2$-triazolyl), 61.3 (CH, C$_{3'}$), 13.0 (CH$_3$, CH$_{3b}$), 12.7 (CH$_3$, *C*H$_{3a}$). HRMS (ESI$^+$) calcd for $C_{27}H_{34}N_7O_{11}$, 632.2311 [M +H]$^+$; found, 632.2308. HPLC (isocratic, 80% water-20% MeCN, 20 min): t_R=4.006 min (97% purity).

3',3'-(2-Chloro-6-aminopurine)-CDN or (2α,3α,4β,6β,14α,15α,16β,18β)-(10*E*)-5,8,13,17,20-pentaoxa-4,16-(2-chloro-6-aminopurin-9-yl)-1,23,24-triazatetracyclo[20.2.1.02,6.014,18] pentacosa-10,22(25),23-triene-3,15-diol (7b). The title compound **7b** was prepared according to procedure 2 starting from 2,6-dichloropurine (270 mg, 1.43 mmol, 6.0 eq.) as nucleobase. After aqueous treatments and filtration over silica, the crude dibenzoate intermediate CDN was isolated as a whitish foam (181 mg) - Rf= 0.52 (silica, DCM/MeOH, 95:5) - and was directly engaged under the basic deprotection conditions. After the completion of the reaction, all volatiles were evaporated under reduced pressure and the residue was purified by flash-column chromatography (silica gel, DCM 100% -> DCM/MeOH, 98:2 -> DCM/MeOH, 95:5) to provide the final 3',3'-(2-amino-6-chloropurine) CDN **7b** as a white foam (3.4 mg, 4.8 µmol) in *2*% yield. Rf= 0.28 (silica, DCM/MeOH, 9:1). ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.46 (s, 1H, H$_{8a}$), 8.36 (s, 1H, H$_{8b}$), 8.33 (s, 1H, =C*H*-N), 7.89 (br s, 2H, -NH$_{2a}$), 7.84 (br s, 2H, -NH$_{2b}$), 6.37 (br s, 1H, -OH'), 6.09 (d, ^3J= 2.0 Hz, 1H, H$_{1'}$), 5.81 (d, ^3J= 5.0 Hz, 1H, H$_{1''}$), 5.73 (dd, ^3J= 6.0 Hz, ^3J= 9.5 Hz, 1H, H$_{3'}$), 5.58 (d, ^3J= 5.9 Hz, 1H, -OH''), 5.52 (dt, ^3J= 5.0 Hz, $^3J_{trans}$= 15.4 Hz, 1H, CH'$_2$-CH'=C*H''*-CH''$_2$), 5.48 (dt, ^3J= 5.0 Hz, $^3J_{trans}$= 15.4 Hz, 1H, CH'$_2$-C*H*'=CH''-CH''$_2$), 4.86 (dt, ^3J= 5.0 Hz, ^3J= 9.7 Hz, 1H, H$_{4'}$), 4.77 (m, $^2J_{gem}$= 13.0 Hz, 2H, H$_{2'}$, O-C*H*$_2$-triazolyl), 4.53 (dd, ^3J= 5.0 Hz, ^3J= 10.4 Hz, 1H, H$_{2''}$), 4.49 (d, $^2J_{gem}$= 13.0 Hz, 1H, O-C*H*$_2$-triazolyl), 4.12 (dd, ^3J= 5.0 Hz, $^2J_{gem}$= 14.3 Hz, 1H, CH'$_2$-CH'=CH''-C*H*''$_2$), 4.05 (dt, ^3J= 3.2 Hz, ^3J= 4.8 Hz, 1H, H$_{4''}$), 3.96 (dd, ^3J= 5.0 Hz, $^2J_{gem}$= 13.0 Hz, 1H, C*H*'$_2$-CH'=CH''-CH''$_2$), 3.89 (dd, ^3J= 5.0 Hz, $^2J_{gem}$= 13.0 Hz, 1H, C*H*'$_2$-CH'=CH''-CH''$_2$), 3.86 (dd, ^3J= 5.0 Hz, $^2J_{gem}$= 14.3 Hz, 1H, CH'$_2$-CH'=CH''-C*H*''$_2$), 3.49 (dd, ^3J= 4.8 Hz, 1H, H$_{3''}$), 3.66 (m, 3H, H$_{5'}$, H$_{5''-5''}$), 3.43 (dd, ^3J= 3.2 Hz, $^2J_{gem}$= 10.8 Hz, 1H, H$_{5'}$). ^{13}C NMR (175 MHz, CDCl$_3$): δ□157.4-157.3 (2x *quat* C, C$_{6a-b}$), 153.7 (2x *quat* C, C$_{2a-b}$), 150.9 (*quat* C, C$_{4b}$), 150.4 (*quat* C, C$_{4a}$), 143.0 (*quat* C, N-*C*=CH-N), 140.4 (CH, C$_{8a}$), 139.6 (CH, C$_{8b}$), 130.8 (CH, CH'$_2$-CH'=C*H*''-CH''$_2$), 128.7 (CH, CH'$_2$-C*H*'=CH''-CH''$_2$), 126.0 (CH, N-C=C*H*-N), 118.7 (*quat* C, C$_{5a}$), 118.4 (*quat* C, C$_{5b}$), 90.3 (CH, C$_{1'}$), 88.0 (CH, C$_{1''}$), 81.7 (CH, C$_{4''}$), 78.8 (CH, C$_{4'}$), 77.2 (CH, C$_{3''}$), 74.0 (CH, C$_{2'}$), 73.5 (CH, C$_{2''}$), 70.3 (CH$_2$, CH'$_2$-CH'=CH''-C*H*''$_2$), 69.6 (CH$_2$, *C*H'$_2$-CH'=CH''-CH''$_2$), 68.4 (CH$_2$, C$_{5'}$), 67.3 (CH$_2$, C$_{5''}$), 62.9 (CH$_2$, O-*C*H$_2$-triazolyl), 62.3 (CH, C$_{3'}$). HRMS (ESI$^+$) calcd for $C_{27}H_{30}Cl_2N_{13}O_7$, 718.1763 [M +H]$^+$; found, 718.1765. HPLC (isocratic, 75% water-25% MeCN, 20 min): t_R=7.224 min (95% purity).

Biological activity

1) Primary macrophage cultures. Murine bone marrow cells were isolated from femurs and cultivated (10^6/mL) for 7 days in Dulbecco's minimal essential medium (DMEM) supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco, Australia), 2 mM L-glutamine, 25mM Hepes (Gibco, Australia), 20% horse serum and 30% L929 cell-conditioned medium as source of M-CSF. After further three days in fresh medium, the bone marrow-derived macrophages (BMDM; 10^5 cells/well) in DMEM supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin, 2 mM L-glutamine, 25mM Hepes and 0.1% FCS were incubated with the compounds. The lyophilised compounds were solubilised in DMSO and used at a non-cytotoxic, ~~1%~~0.3% DMSO final concentration in DMEM supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin, 2 mM L-glutamine, 25mM Hepes. Absence of cytotoxicity was controlled using MTT incorporation. The compounds were tested as agonists at concentrations of 1, 3, 10 or 14µM, as compared with reference STING agonist cGAMP (1-14 µM, InvivoGen, San Diego, CA, USA) or DMSO vehicle controls. For testing their potential antagonist activity, the compounds were tested at 1, 3, or 10uM, and compared with reference STING antagonist H151 (3 - 14µM, InvivoGen, San Diego, CA, USA) or DMSO vehicle controls , added 3 hr prior to the STING stimuli cGAMP (at 14uM). Cell culture supernatants were harvested after 18h and the concentration of CXCL10 (IP-10) in the supernatant measured by ELISA (R&D System, Minneapolis, USA).

2) Differential Scanning Fluorimetry (DSF) assay *:* Measurements were performed with wt hSTING as described earlier.[53] Briefly, the reactions were performed in 384-well q-PCR white plates (Roche) in reaction buffer (50 mM TrisHCl [pH 7.4], 150 mM NaCl). The reactions contained 5 µM wt hSTING, 100 µM compound (**7a**) and 1:5000 (v/v) SYPRO Orange (Thermo Fisher Scientific, USA) in final volume of 10 µl. The negative control contained only the solvent (1% DMSO), the positive control contained 100 µM 3'3'cGAMP. Samples were analyzed in a LightCycler 480 II (Roche) system. The first derivative of the fluorescence intensity associated with thermal denaturation of the protein was evaluated and then calculated as the melting temperature (Tm).

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Conflict of Interest

The authors declare no conflict of interest

Keywords: CuAAC • 3',3'-cyclic dinucleotides • macrocyclization • ring-closing metathesis • STING protein • 1,2,3-triazole •

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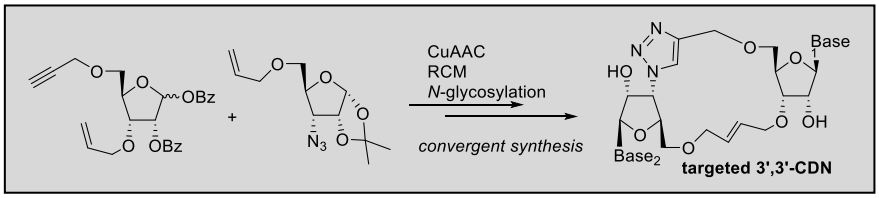
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Starting from chiral sugars, new cyclic dinucleotides are described; the synthesis involves (1) the coupling of both sugar moieties through CuAAC reaction, (2) the macrocyclization of resulting intermediate under ring-closing metathesis and (3) the introduction of nucleobases by N-glycosylation under Vorbrüggen conditions. These compounds are targeting the STING protein.

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