Synthetic peptides, nucleic acids and molecular probes to study ADP-Ribosylation

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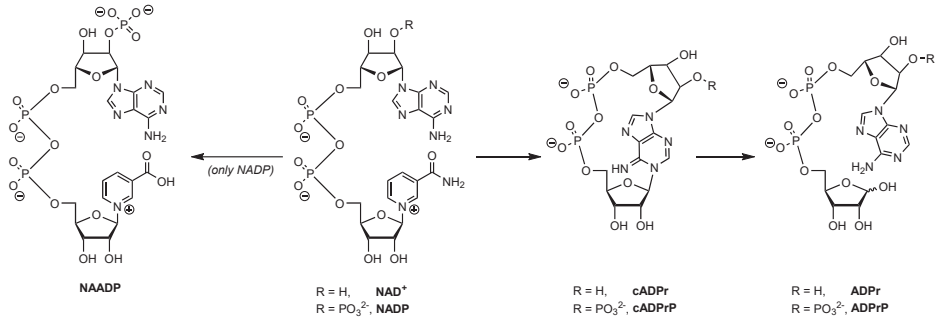
Chapter 7

Design and Synthesis of Potential Activity Based Probes for CD38 Based on Carba-Ribofuranose

Introduction

The cluster of differentiation 38 (CD38) proteins belong to the ADP-ribosyl cyclase family of which a type II, type III and a soluble type are known. While CD38 of type II is a glycoprotein with its catalytic site located outside the cell, type III is a non-glycosylated isoform with its catalytic site inside the cytosol. Although first discovered in thymocytes,[1] CD38 is ubiquitously expressed in most mammalian cells that have been examined.[2] CD38 can act as a membrane receptor[3,4] and binding with its ligand CD31 is, for example, involved in the cell adhesion of endothelial cells to lymphocytes and the modulation of cytoplasmic calcium fluxes.[5,6] CD38 is a multifunctional enzyme catalyzing not only the formation of cyclic ADPr (cADPr) out of nicotinamide adenine dinucleotide (NAD^+) but it can also cyclize NADP into cADPrP (Scheme 1). In addition, CD38 can hydrolyze cADPr and cADPrP into ADPr and ADPrP respectively.[7] Interestingly, it has also been established that CD38 can convert NAD phosphate (NADP) into nicotinic acid adenine dinucleotide (NAADP) in acidic environments.[8]



Scheme 1. Reactions catalyzed by CD38.

Both cADPr and NAADP are important signaling molecules that mobilize Ca^{2+},[9] a messenger that is involved in several pathways that regulate a variety of cellular processes.[10,11] In particular, Ca^{2+} homeostasis is of prime importance for immunity and the misregulation of Ca^{2+} signaling pathways in lymphocytes may lead to various autoimmune, inflammatory and immunodeficiency diseases. In this light, the importance of CD38 biology is underlined not only by its involvement in diseases such as diabetes,[12,13] AIDS[14,15] and chronic lymphocyte leukemia[16] but also in social behavior such as maternal care, which was severely reduced in CD38 knockout mice.[17,18] The importance of CD38 in health and disease and its role in multiple biological processes has resulted in a continued interest in elucidation of the corresponding biology that stands out for its complexity. In this framework, attention is directed

to the development of inhibitors of CD38. The catalysis by CD38 as an NAD^+ cyclase and hydrolase is thought to proceed *via* a non-covalent oxocarbenium intermediate. However, with substrates like 2-F-arabinose-nicotinamide mono nucleotide (2-ara-F-NMN), a covalent intermediate is formed whereby the glutamic acid (Glu) 226 residue in the CD38 active site performs a nucleophilic attack on 2-F-ara-NMN with concomitant expulsion of nicotinamide.[19–21] The latter proposed mechanism not only allows for the development of competitive[22,23] but also mechanism-based covalent inhibitors[24] modelled after its substrate NAD^+.[25] The availability of a covalent inhibitor allows for detection or even discovery of an active enzyme or enzyme family by activity-based protein profiling (ABPP) in the context of a biological system.[26–29] An activity-based probe (ABP) is a mechanism-based inhibitor provided with a tag to identify the corresponding enzyme. A fluorescent ABP has been reported by Hening Lin *et al.* that visualize CD38 on the cell membrane.[30] In addition, a cell-permeable ABP was developed that revealed the presence of CD38 in the cytoplasm albeit in significantly smaller quantities compared to its presence on the cell membrane.[31] These ABPs are based on the discovery of the group of Schramm that 2-F-ara-NMN can function as an electrophilic trap to form a covalent adduct with CD38. Likewise, the group of Withers has explored the use of 2'-deoxy-2'-fluoroglycosides as activity-based inhibitors and/or ABPs of glycosidases.[32–34] The group of Overkleeft discovered that differently configured cyclophellitol derivatives or similar configured cyclitols in which the epoxide is replaced by an aziridine moiety can function as efficient mechanism-based inhibitors and/or ABPs of several retaining glycosidases.[28,35–37]

Inspired by these achievements, candidate ABPs **1** and **2** derived from NAD^+ were designed (Figure 1A). The *carba*-ribosyl moiety in ABP **1** is functionalized with an aziridine electrophilic trap that is provided with an azide spacer as ligation handle. This enables the bio-orthogonal ligation with a reporter group such as a fluorophore or biotin tag via copper catalyzed, azide-alkyne cycloaddition (CuAAC). ABP **2** is functionalized with an epoxide as electrophilic trap and an alkyne spacer, positioned on the exocyclic amine of the adenine base, as ligation handle. The reporter groups ligated via CuAAC can provide a readout by in-gel fluorescence or mass spectrometry, applying either a pre-labeling protocol (Figure 1B) where the probe is already conjugated to the reporter group, or by a two-step labeling protocol in which the probe first reacts with the enzyme followed by an *in vitro* CuAAC with a suitable reporter tag.

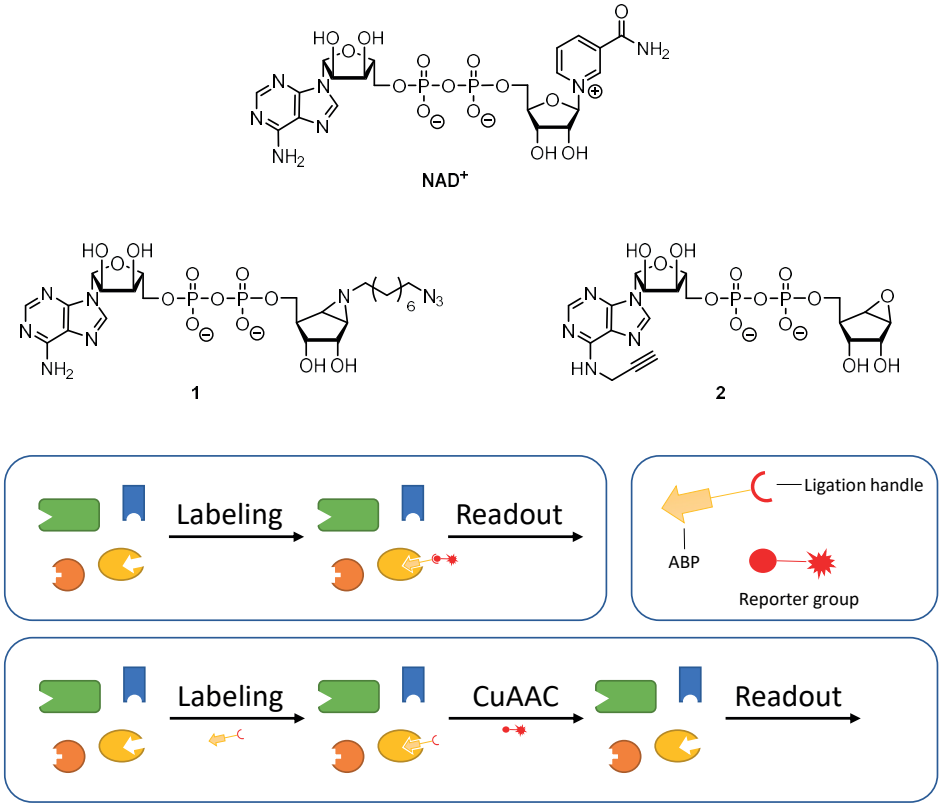
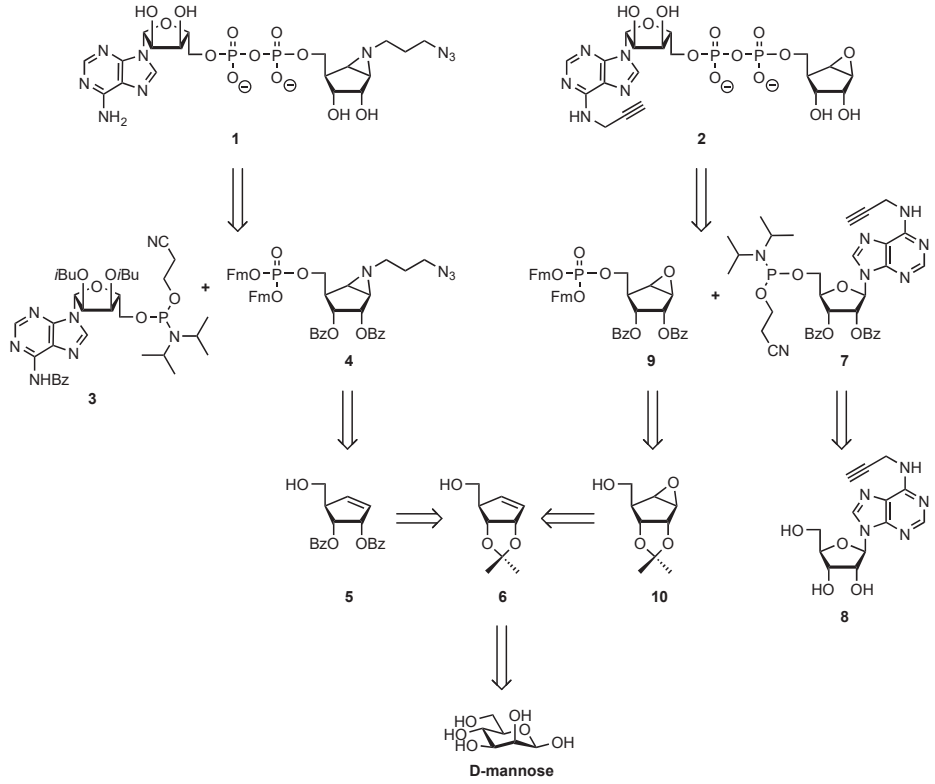


Figure 1. A) Design of ABPs **1** and **2** based on NAD^+. **B)** general workflow for one-step labeling (top) and two-step labeling (bottom). Readout is performed either by in-gel fluorescence or mass spectrometry analysis.

Results and discussion

The retrosynthetic analysis of ABPs **1** and **2** is depicted in Scheme 2. ABP **1** is provided with an azide as bio-orthogonal handle that is linked *via* a spacer at the aziridine moiety. ABP **2** bears a propargyl group placed at the exocyclic amine of adenosine. The pyrophosphate bridges in both **1** and **2** will be introduced using the established $P^{III} - P^{V}$ coupling method,[38] for which a suitably protected amidite and a phosphate monoester is needed. Thus, the four key building blocks required are: aziridine **4**, epoxide **9**, and adenosine phosphoramidites **3** and **7**. Known adenosine amidite **3**[39] is suitable to obtain aziridine-containing ABP **1**. Reagent **7**, decorated with the propargyl group, can be used to prepare ABP **2**. Phosphoramidite **7** is accessible *via* N^6-propargyladenosine **8**, which in turn can be obtained from adenosine by established procedures.[40,41] The phosphate monoesters needed for the pyrophosphate construction

are derived from warheads **4** and **9** after removal of the fluorenylmethyl (Fm) protecting groups. The phosphotriesters in **4** and **9** are accessible by phosphorylation with known bis-fluorenylmethyl phosphoramidite, followed by oxidation. Key to an efficient synthesis of aziridine **4** and epoxide **9** is the stereoselective introduction of the aziridine and the epoxide functional group. To achieve this, *carba*-ribose **6** is selected as suitable building block that can be efficiently obtained from D-mannose[42]. Compound **6** can be converted into epoxide **10** by $VO(acac)_2$ mediated epoxidation.[43,44] Upon standard protecting group manipulations on **6**, benzoylated cyclopentene **5** is conceivable. The homoallylic alcohol functionality in **5** can then be implemented in a four step reaction sequence, which has been successfully utilized before to synthesize several cyclophellitol-aziridines, [36,37,45] to give **4**.

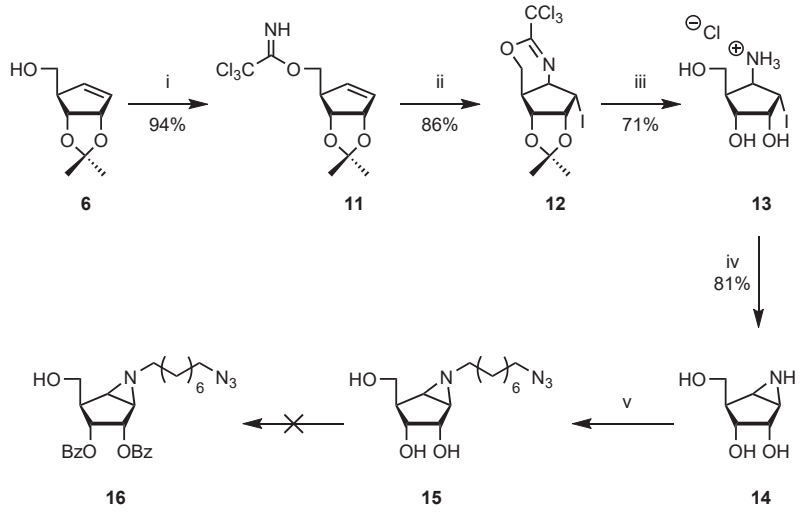


Scheme 2. Retrosynthetic analysis of ABPs **1** and **2** from D-mannose.

*Aziridine warhead (**4**)*

The first attempt to obtain *carba*-ribose **4**, containing an aziridine electrophilic trap is depicted in Scheme 3. The starting compound, isopropylidene protected **6**, was prepared from D-mannose according to a known 8-step procedure.[42] The installation of the aziridine

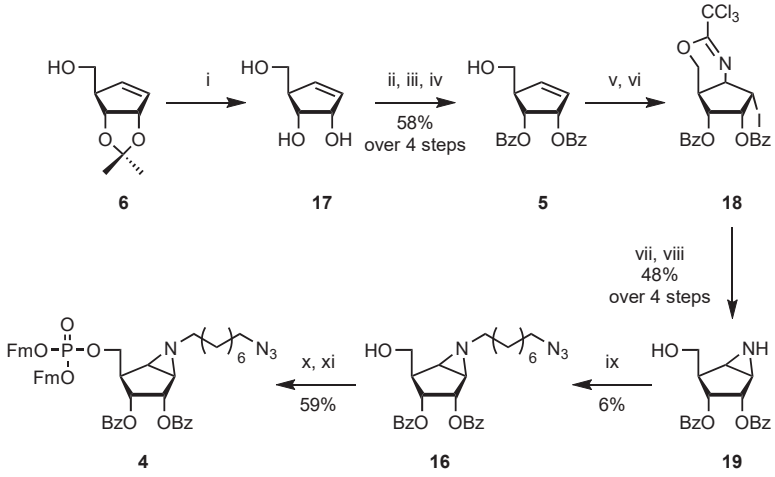
moiety was adopted from an established method to obtain aziridine cyclophellitols. [45] In the first step, the primary homo-allylic alcohol of **6** was transformed into trichloroacetimidate **11,** using DBU and trichloroacetonitrile. Subsequent iodocyclization by treatment of **11** with NIS gave imidate **12** in 86% yield. Simultaneous acid-mediated hydrolysis of both the imidate and the isopropylidene group resulted in HCl salt **13**. The crude product of this reaction was directly treated with basic Amberlite IRA67 resin to induce nucleophilic attack of the amine on the neighboring iodide, leading to the isolation of aziridine **14**. Subsequent chemoselective alkylation of the aziridine in **14** with 1-azido-8-iodooctane provided **15** with the prospected ligation handle. Unfortunately, all undertaken protecting group manipulations to make **15** suitable for the phosphorylation to give target **4** proofed to be cumbersome. Probably the aziridine group does not withstand the applied reaction conditions and thus it was decided to install the aziridine group later in the synthesis route.



Scheme 3. First attempt at the synthesis of the aziridine containing warhead. Reagents and conditions: i) Cl_3CCN, DBU, DCM, 0 °C. ii) NIS, MeCN, 0 °C. iii) HCl, MeOH, H_2O. iv) Amberlite IRA67 base resin, MeOH, 45 °C. v) 1-azido-8-iodooctane, K_2CO_3, DMF.

In order to facilitate the late-stage introduction of the aziridine moiety, the hydrolysis of isopropylidene group in **6** (Scheme 4) to afford triol **17** was followed by a three-step protecting group manipulation sequence, consisting of (i) regioselective introduction of a *tert*-butyl diphenyl silyl ether on the 5-OH, (ii) benzoylation of the 2- and 3-OH and (iii) F^- mediated removal of the silyl group to afford protected homoallylic alcohol **5**. Installation of the aziridine according to the procedure as described for the conversion of **11** into **14**,

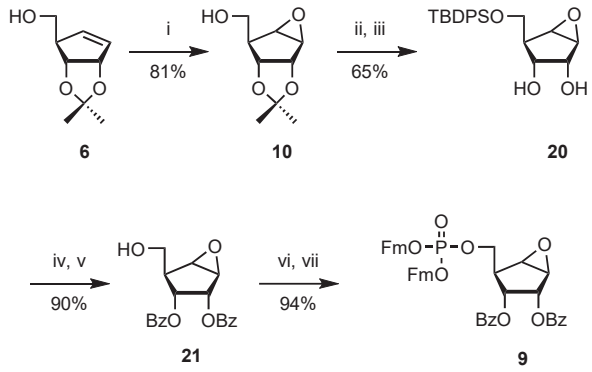
furnished the 2- and 3-*O*-Bz protected aziridine **19**. Subjection of **19** to the chemoselective alkylation as described in Scheme 3 provided **16** with the azide linker albeit in low yield. Finally, phosphitylation of the 5-OH with bis-fluorenylmethylphosphoramidite in the presence of activator ETT and oxidation of the phosphite intermediate with CSO yielded fully protected aziridine **4**, ready for pyrophosphate formation towards the target probe **1**.



Scheme 4. Synthesis of key aziridine containing warhead **4**. Reagents and conditions: i) AcOH, H_2O, 60 °C. ii) TBDPS-Cl, pyr. iii) BzCl, pyr. iv) TEA·3HF, THF. v) Cl_3CCN, DBU, DCM, 0 °C. vi) NIS, MeCN, 0 °C. vii) HCl, MeOH, H_2O. viii) TEA, MeOH, 45 °C. ix) 1-azido-8-iodooctane, K_2CO_3, DMF, 80 °C. x) $(FmO)_2PN(iPr)_2$, ETT, MeCN. xi) CSO, MeCN.

*Epoxide warhead (**9**)*

The route of synthesis towards epoxide building block **9** commenced with the $VO(acac)_2$ mediated, stereoselective epoxidation of homoallylic alcohol **6** to give **10** in 81% yield (Scheme 5). It is interesting to note that this epoxidation was unsuccessful with benzoylated alkene **5**. It can be postulated that the electron withdrawing character of the benzoyl protecting group inhibits the epoxidation. Acidolysis of the isopropylidene group in **10** after which the resulting triol was subjected to the same protecting group manipulations as described for the conversion of **17** into **5** (Scheme 4) furnished compound **21**. Phosphitylation and oxidation of **21** as described above, led to key epoxide **9** ready for use in the pyrophosphate formation towards target probe **2**.



Scheme 5. Synthesis of epoxide warhead **9**. Reagents and conditions: i) $VO(acac)_2$, *t*BuOOH, toluene, 60 °C. ii) AcOH, H_2O, 60 °C. iii) TBDPS-Cl, pyr, 65% over two steps. iv) BzCl, pyr. 90%. v) TEA·3HF, THF. vi) $FmO)_2PN(iPr)_2$, ETT, MeCN. vii) CSO, MeCN.

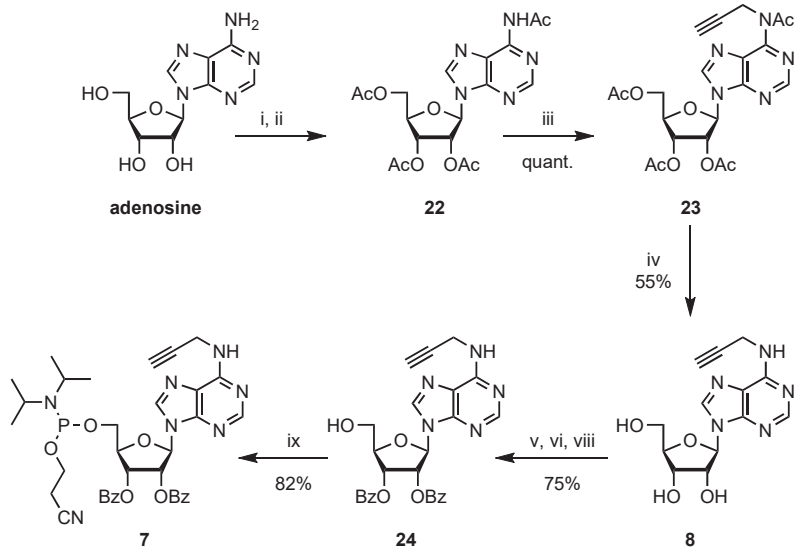
Adenosine building block

To execute the projected P^{III} – P^{V} coupling method for the pyrophosphate formation, the protected adenosine amidites **3** and **7** are needed for aziridine ABP **1** and epoxide APP **2**, respectively. Amidite **3** is a known compound[39] and the synthesis of amidite **7** is depicted in Scheme 6. The synthesis started with per-acetylation of commercially available adenosine with acetic anhydride in pyridine to give intermediate penta-acetate, bearing two acetyls on the exocyclic amine. One of the acetyl groups was removed by treatment with imidazole to give tetra-acetate **22**. After alkylation of **22** with propargyl bromide to afford **23** in quantitative yield, the remaining acetyl protecting groups were cleaved by sodium methoxide in methanol. The resulting triol **8** was subjected to the same three-step protecting group manipulation comprising of regioselective silylation of the 5-OH, benzoylation of the 2- and 3-OH and liberation of the 5-OH as described *en route* to aziridine **4** and epoxide **9** (Scheme 4 and 5 respectively), yielding primary alcohol **24** in 75% yield. Finally, reaction of the 5-OH in **24** with commercially available cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite in the presence of DIPEA furnished adenosine amidite **7**.

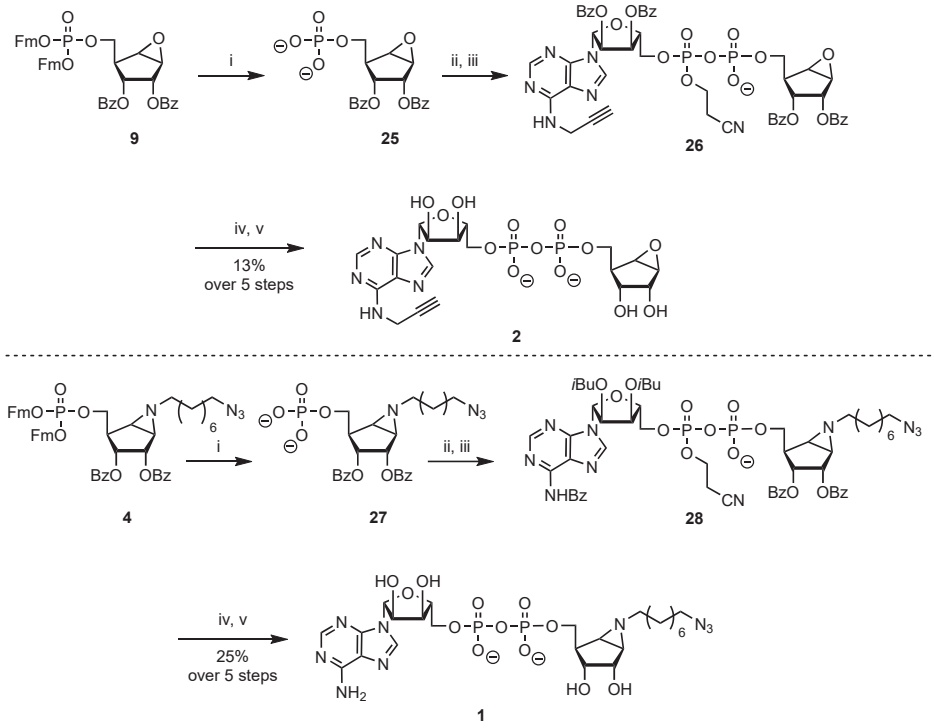
*Synthesis of ABPs **1** and **2***

With all building blocks in hand, the synthesis of ABPs **1** and **2** was undertaken by employing the P^{III} – P^{V} coupling strategy outlined in Scheme 7. Toward ABP **2**, the Fm protecting groups in phosphotriester **9** were removed by treatment with TEA to give the corresponding monoester **25**. The reaction was monitored by LC-MS analysis which indicated complete removal of the Fm groups after 24 hours. Co-evaporation of the reaction mixture with pyridine to convert the phosphomonoester into the more stable pyridinium salt. Next, **25** was treated with phosphoramidite **7** using ETT as an activator. The reaction was monitored

by ^{31}P-NMR, showing complete conversion into a phosphite-phosphate intermediate after 15 minutes. The intermediate was then oxidized by *t*BuOOH furnishing the partially protected pyrophosphate **26**. Finally, deprotection was performed by treating intermediate **26** with DBU to remove the cyanoethyl group and subsequently with ammonia to cleave all benzoyl groups. Purification with preparative HPLC led to the isolation of ABP **2** in 13% yield over 5 steps. For ABP **1** identical reaction conditions were applied with phosphotriester **4** and phosphoramidite **3**, furnishing ABP **1** in 25% yield after HPLC purification.



Scheme 6. Synthesis of adenosine amidite **7**. Reagents and conditions: i) Ac_2O, pyr. ii) Imidazole, MeOH. iii) propargyl bromide, DBU, MeCN. iv) NaOMe, MeOH. v) TBS-Cl, pyr. vi) BzCl, pyr. vii) PTSA, MeCN, H_2O. viii) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, DIPEA, DCM.



Scheme 7. Synthesis of ABPs **1** and **2**. Reagents and conditions: i) TEA, MeCN. ii) **3** or **7**, ETT, MeCN. iii) *t*BuOOH. iv) DBU, DMF. v) NH_4OH, H_2O.

Conclusion

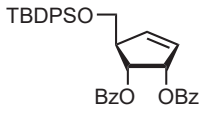
Two potential ABPs for the covalent inhibition of CD38 were designed and successfully synthesized. Both designs are based on NAD^+, the natural substrate of CD38. The probes bear electrophilic traps as a warhead to covalently bind the Glu residue in the active site of CD38. An aziridine was used as the warhead in case of ABP **1** and an epoxide-warhead was employed for ABP **2**. Both ABPs **1** and **2** are designed for one- or two-step labeling strategies (Figure 1B) and are equipped with bio-orthogonal ligation handles that would enable functionalization with a desired reporter group via CuAAC. Synthesis of both probes was executed *via* elaborate multistep procedures commencing with the deprotection of warheads **4** and **9**. Next, the 5-O-phosphates **25** and **27** were subject for the established P^{III} – P^{V} coupling procedure with appropriately protected adenosine phosphoramidites to furnish the prospected ABPs **1** and **2**. The activity of ABPs **1** and **2** towards CD38 will be evaluated *in vitro* in due course.

Experimental section

General synthetic procedures

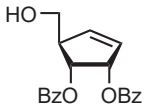
All reagents were of commercial grade and used as received unless stated otherwise. Solvents used in synthesis were dried and stored over 4Å molecular sieves, except for MeOH and MeCN which were stored over 3Å molecular sieves. Triethylamine (TEA) and diisopropylethylamine (DIPEA) were stored over KOH pellets. Column chromatography was performed on silica gel 60 Å (40-63 µm, Macherey-Nagel). TLC analysis was performed on Macherey-Nagel aluminium sheets (silica gel 60 F_{254}). TLC was used to visualize compounds by UV at wavelength 254 nm and by spraying with either cerium molybdate spray (25 g/L $(NH_4)_6Mo_7O_{24}$, 10 g/L $(NH_4)_4Ce(SO_4)_4{\cdot}H_2O$ in 10% H_2SO_4 water solution) or $KMnO_4$ spray (20 g/L $KMnO_4$ and 10 g/L K_2CO_3 in water) followed by charring at c.a. 250 °C. LC-MS analysis was performed on a Finnigan Surveyor HPLC system with a Nucleodur C18 Gravity 3 µm 50 x 4.60 mm column (detection at 200-600 nm) coupled to a Finnigan LCQ Advantage Max mass spectrometer with ESI or coupled to a Thermo LCQ Fleet Ion mass spectrometer with ESI. The method used was 10→90% 13.5 min (0→0.5 min: 10% MeCN; 0.5→8.5 min: 10% to 90% MeCN; 8.5→ 11 min: 90% MeCN; 11→13.5 min: 10% MeCN) or 0→50% 13.5 min. NMR spectra were recorded on a Bruker AV-400, AV-500 or AV-600 NMR. Chemical shifts (δ) are given in ppm relative to tetramethyl silane as internal standard. Coupling constants (*J*) are given in Hz. All given ^{13}C-APT spectra are proton decoupled. Proton and carbon numbering for NMR peak assignment, in case of the carbocyclic derivatives, was done similarly to their ribofuranosyl counterparts. Numbering starts at the 'anomeric' centre and progresses as for ribofuranose. 'H-6' or 'C-6' is used where the endocyclic oxygen is replaced for the substituted carbon.

(3*S*,4*R*,5*R*)-3,4,-*O*-dibenzoyl-5-(*O*-*tert*-butyldiphenylsilyl)-methylcyclopentene



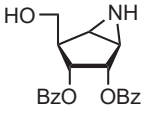
Compound **6**[42] (1.70 g, 10.0 mmol) was dissolved in a 80 v/v% solution of AcOH in H_2O (40 mL, 0.25 M). The reaction was stirred in an open flask at 60 °C for 2 hours. TLC indicated full conversion of the starting material to a lower running product (Rf = 0.3 in 10% EtOH in EtOAc). The reaction was concentrated *in vacuo* and thoroughly co-evaporated with a 1:1 mixture MeCN:H_2O followed by co-evaporation with 1,4-dioxane. The crude triol was than co-evaporated with pyridine thrice and dissolved in pyridine (100 mL, 0.1 M). TBDPS-Cl (2.86 mL, 11.0 mmol, 1.1 eq.) was added and the reaction was stirred overnight. Another portion of TBDPS-Cl (2.86 mL, 11.0 mmol, 1.1 eq.) was added and an additional 7 hours of stirring ensured full conversion of the triol into a higher running product. BzCl (4.64 mL, 40 mmol, 4.0 eq.) was added and the reaction was stirred overnight. The reaction was quenched with MeOH and concentrated *in vacuo*. The crude residue was taken up in EtOAc and the solution was washed with 1 M citric acid, sat. aq. $NaHCO_3$ and brine respectively. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (10 -> 15% Et_2O in pentane) yielded the title compound as a colorless oil (4.33 g, 7.51 mmol, 75%). **Rf:** 0.76 in 20% Et_2O in pentane. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.92 (ddd, J = 17.9, 8.1, 1.4 Hz, 4H, Bz arom.), 7.72 – 7.64 (m, 4H, TBDPS arom.), 7.54 – 7.44 (m, 2H, Bz arom.), 7.43 – 7.26 (m, 10H, Bz arom. + TBDPS arom.), 6.18 – 6.00 (m, 3H, H-1 + H-2 + H-6), 5.62 (dd, J = 6.6, 4.2 Hz, 1H, H-3), 3.87 (qd, J = 10.1, 5.0 Hz, 2H, H-5), 3.30 (q, J = 5.2 Hz, 1H, H-4), 1.05 (s, 9H, *t*Bu TBDPS). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.1, 166.0 (C=O Bz), 137.6 (C-6), 135.8, 135.7 (CH arom. TBDPS), 133.4, 133.4 (Cq arom. TBDPS), 133.0, 133.0 (CH arom. Bz), 130.2, 130.0 (Cq Bz), 129.9, 129.8, 129.7 (CH arom. Bz + CH arom. TBDPS), 129.4 (C-1), 128.3, 128.3, 127.9, 127.8 (CH arom. Bz + CH arom. TBDPS), 76.8 (C-2), 74.1 (C-3), 63.9 (C-5), 52.4 (C-4), 26.9 (CH_3 *t*Bu TBDPS), 19.4 (Cq *t*Bu TBDPS). **HRMS:** $[C_{36}H_{36}O_5Si + Na]^+$ found: 599.2221, calculated: 599.2224.

(3*S*,4*R*,5*R*)-3,4,-*O*-dibenzoyl-5-hydroxymethylcyclopentene (5)



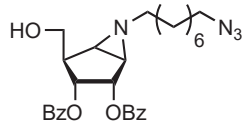
(3*S*,4*R*,5*R*)-3,4,-*O*-dibenzoyl-5-(*O*-*tert*-butyldiphenylsilyl)-methylcyclopentene (3.09 g, 5.36 mmol) was dissolved in THF (54 mL, 0.1 M) and the solution was cooled to 0 °C. TEA·3HF (17.5 mL, 107 mmol, 20.0 eq.) was added and the reaction was slowly allowed to warm to room temperature. After 4 days of stirring, the reaction was cooled to 0 °C and *carefully* quenched by the addition of sat. aq. $NaHCO_3$ until bubbling seized. The reaction was transferred into a separatory funnel and the water layer was extracted thrice with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (30 -> 40% EtOAc in pentane) yielded the title compound as a colorless oil (1.40 g, 4.12 mmol, 77%). **Rf:** 0.41 in 30% EtOAc in pentane. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.99 – 7.93 (m, 2H, Bz arom.), 7.93 – 7.87 (m, 2H, Bz arom.), 7.53 – 7.43 (m, 2H, Bz arom.), 7.33 (t, *J* = 7.8 Hz, 2H, Bz arom.), 7.30 – 7.23 (m, 2H, Bz arom.), 6.13 (dd, *J* = 5.4, 2.0 Hz, 1H, H-6), 6.11 – 6.04 (m, 2H, H-1 + H-2), 5.63 – 5.48 (m, 1H, H-3), 3.91 – 3.74 (m, 2H, H-5), 3.34 – 3.22 (m, 1H. H-4), 3.04 (bs, 1H, OH). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.5, 166.0 (C=O Bz), 137.2 (C-6), 133.2, 133.0 (CH arom. Bz), 129.9 (Cq Bz), 129.7, 129.7 (CH arom. Bz), 129.5 (Cq Bz), 129.4 (C-1), 128.3, 128.3 (CH arom. Bz), 76.7 (C-2), 74.1 (C-3), 62.8 (C-5), 52.9 (C-4). **HRMS:** $[C_{20}H_{18}O_5 + Na]^+$ found: 361.1047, calculated: 361.1046.

(1*R*,2*S*,3*R*,4*R*)-1,2-aziridine-3,4-*O*-dibenzoyl-5-hydroxymethylcyclopentane (19)



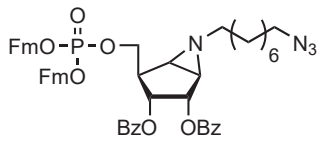
Compound **5** (1.25 g, 3.68 mmol) was dissolved in DCM (25 mL, 0.15 M) before DBU (0.22 mL, 1.47 mmol, 0.4 eq.) and trichloroacetonitrile (0.41 mL, 4.05 mmol, 1.1 eq.) were added. The reaction was stirred for 15 minutes before the reaction was diluted with DCM and the solution was washed with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude imidate was dissolved in MeCN (12.5 mL, 0.3 M) and NIS (4.14 g, 18.4 mmol, 5.0 eq.) was added to the solution. The flask was wrapped in aluminum foil to protect it from light and stirred overnight. The reaction was diluted with EtOAc and the solution was washed with sat. aq. $Na_2S_2O_3$. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting crude oxazoline was dissolved in 1,4-dioxane (37 mL, 0.1 M) and HCl (37% in H_2O, 11 mL) was added. The reaction was heated to 60 °C and stirred for 2 hours. The reaction was concentrated *in vacuo* and extensively co-evaporated with MeOH. The crude amine was dissolved in MeOH (37 mL, 0.1 M) and TEA (5.13 mL, 38.8 mmol, 10 eq.) was added. The reaction was stirred overnight at 55 °C. The reaction was concentrated *in vacuo* and re-dissolved in MeOH (37 mL, 0.1 M). Another portion of TEA (5.13 mL, 36.8 mmol, 10 eq.) was added and the reaction was refluxed for 14 hours to ensure full conversion of the amine to the aziridine. The reaction was concentrated *in vacuo* and flash column chromatography (5% MeOH in DCM) yielded the title compound as a 1:1.7 mixture product:TEA (calculated by ^{1}H-NMR) (938 mg, 1.78 mmol, 48%). **Rf**: 0.38 in 10% MeOH in DCM. **^{1}H NMR** (400 MHz, MeOD) δ 7.93 – 7.87 (m, 2H, Bz arom.), 7.87 – 7.79 (m, 2H, Bz arom.), 7.61 – 7.48 (m, 2H, Bz arom.), 7.40 (t, *J* = 7.8 Hz, 2H, Bz arom.), 7.31 (t, *J* = 7.8 Hz, 2H, Bz arom.), 5.68 (d, *J* = 5.1 Hz, 1H, H-2), 4.98 (dd, *J* = 8.6, 5.1 Hz, 1H, H-3), 3.92 – 3.78 (m, 2H, H-5), 3.07 (dd, *J* = 4.5, 2.6 Hz, 1H, H-6), 2.99 (d, *J* = 4.5 Hz, 1H, H-1), 2.76 (tdd, *J* = 8.3, 5.6, 2.6 Hz, 1H, H-4). **^{13}C NMR** (101 MHz, MeOD) δ 166.8, 166.7 (C=O Bz), 134.4, 134.3 (CH arom. Bz), 130.5 (Cq Bz), 130.3, 130.2, 129.5, 129.3 (CH arom. Bz), 74.8 (C-3), 73.1 (C-2), 62.0 (C-5), 46.4 (C-4), 36.3 (C-6), 35.7 (C-1).

(1*R*-2*S*-3*R*-4*R*)-1,2-(6-*N*-oct-(1-azide)-ane)aziridine-3,4-*O*-dibenzoyl-5-hydroxymethylcyclopentane (16)



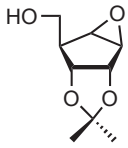
Compound **19** (930 mg, 1.78 mmol) was dissolved in DMF (6 mL, 0.3 M). 6-Iodo-1-azido-octane (1.00 g, 3.56 mmol, 2.0 eq.) and K_2CO_3 (296 mg, 2.14 mmol, 1.2 eq.) were added and the reaction was stirred overnight at 100 °C. The reaction was concentrated *in vacuo* and purification by flash column chromatography (3 -> 5% acetone in DCM) yielded the title compound (53 mg, 0.11 mmol, 6%). **Rf:** 0.48 in 5% acetone in DCM. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.02 – 7.93 (m, 2H, Bz arom.), 7.88 (dd, *J* = 8.3, 1.4 Hz, 2H, Bz arom.), 7.58 – 7.50 (m, 1H, Bz arom.), 7.50 – 7.44 (m, 1H, Bz arom.), 7.38 (t, *J* = 7.7 Hz, 2H, Bz arom.), 7.32 – 7.25 (m, 2H, Bz arom.), 5.69 (d, *J* = 5.2 Hz, 1H, H-2), 5.17 (dd, *J* = 8.3, 5.2 Hz, 1H, H-3), 4.03 (dd, *J* = 10.7, 4.5 Hz, 1H, H-5_a), 3.92 (dd, *J* = 10.7, 6.3 Hz, 1H, H-5_b), 3.27 (t, *J* = 6.9 Hz, 2H, RNCH$_2$CH$_2$(CH$_2$)$_4$CH$_2$C*H*$_2$N$_3$), 2.61 (dddd, *J* = 8.7, 6.7, 4.4, 2.6 Hz, 1H, H-4), 2.44 – 2.30 (m, 3H, H-1 + H-6 + RNC*H*$_{2a}$CH$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$), 2.21 – 2.02 (m, 1H, RNC*H*$_{2b}$CH$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$), 1.66 – 1.51 (m, 4H, RNC*H*$_2$CH$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$ + RNCH$_2$CH$_2$(CH$_2$)$_4$C*H*$_2$CH$_2$N$_3$), 1.38 – 1.29 (m, RNCH$_2$CH$_2$(C*H*$_2$)$_4$CH$_2$CH$_2$N$_3$ 8H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.0, 165.7 (C=O Bz), 133.2, 133.1 (CH arom. Bz), 129.9 (Cq Bz), 129.7, 129.7 (CH arom. Bz), 129.6 (Cq Bz), 128.4, 128.3 (CH arom. Bz), 74.1 (C-3), 72.3 (C-2), 62.2 (C-5), 58.4 (RNCH_2CH$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$), 51.5 (RNCH$_2$CH$_2$(CH$_2$)$_4$CH$_2$C*H*$_2$N$_3$), 45.5 (C-4), 44.1, 43.0 (C-1 + C-6), 29.7, 29.4, 29.1, 28.9 (RNCH$_2$CH$_2$(CH$_2$)$_4$C*H*$_2$CH$_2$N$_3$ + RNCH$_2$C*H*$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$ + RNCH$_2$CH$_2$(C*H*$_2$)$_4$CH$_2$CH$_2$N$_3$), 27.2, 26.7 (RNCH$_2$CH$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$). **HRMS:** $[C_{28}H_{34}N_4O_5 + H]^+$ found: 507.2607, calculated: 507.2602.

(1*R*-2*S*-3*R*-4*R*)-1,2- (6-*N*-oct-(1-azide)-ane)aziridine-3,4-*O*-dibenzoyl-5-(*O*-difluorenylmethyl phosphate)-methylcyclopentane (4)



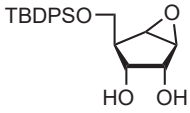
Compound **16** (53 mg, 0.11 mmol) was co-evaporated in toluene and dissolved in a solution of bis-(9H-fluoren-9-ylmethyl)-*N,N*-diisopropylamidophosphite (110 mg, 0.21 mmol, 2.0 eq.) and DCI (0.25 M, 0.42 mmol, 4.0 eq.) in MeCN (1.7 mL, 0.06 M). The reaction was stirred for 30 minutes. *t*BuOOH (5.5 M in nonane, 0.2 mL, 1.0 mmol, 10 eq.) was added and the reaction was stirred for an additional 45 minutes. The reaction was diluted with EtOAc and the solution was washed with sat. aq. $NaHCO_3$ and brine respectively. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (40% EtOAc in pentane) yielded the title compound (61 mg, 65 µmol, 59%). **Rf:** 0.64 in 40% EtOAc in pentane. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.98 – 7.90 (m, 2H, Bz arom.), 7.89 – 7.80 (m, 2H, Bz arom.), 7.74 – 7.63 (m, 4H, Fm arom.), 7.61 – 7.39 (m, 7H, Bz arom. + Fm arom.), 7.42 – 7.25 (m, 8H, Bz arom. + Fm arom.), 7.28 – 7.12 (m, 5H, Bz arom. + Fm arom.), 5.65 (d, *J* = 5.1 Hz, 1H, H-2), 4.90 (dd, *J* = 8.5, 5.1 Hz, 1H, H-3), 4.37 – 4.01 (m, 8H, H-5 + 2x CH Fm + 2x CH_2 Fm), 3.21 (t, *J* = 7.0 Hz, 2H, RNCH$_2$CH$_2$(CH$_2$)$_4$CH$_2$C*H*$_2$N$_3$), 2.69 (tdd, *J* = 8.7, 6.0, 2.6 Hz, 1H, H-4), 2.36 (d, *J* = 4.6 Hz, 1H, H-1), 2.31 – 2.15 (m, 2H, H-6 + RNC*H*$_{2a}$CH$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$), 2.09 – 1.93 (m, 1H, RNC*H*$_{2b}$CH$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$), 1.62 – 1.50 (m, 2H, RNCH$_2$CH$_2$(CH$_2$)$_4$C*H*$_2$CH$_2$N$_3$), 1.51 – 1.37 (m, 2H, RNCH$_2$C*H*$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$), 1.37 – 1.14 (m, 8H, RNCH$_2$CH$_2$(C*H*$_2$)$_4$CH$_2$CH$_2$N$_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.6, 165.5 (C=O Bz), 143.1, 143.1, 143.0, 143.0, 141.4, 141.4 (Cq Fm), 133.2, 133.1 (CH arom. Bz), 129.8 (Cq Bz), 129.7, 129.7 (CH arom. Bz), 129.3 (Cq Bz), 128.4, 128.3, 127.9, 127.2, 127.1 (CH arom. Bz + CH arom. Fm), 125.1, 125.1, 125.1, 120.0, 120.0 (CH arom. Fm), 74.1 (C-3), 72.1 (C-2), 69.4, 69.3, 69.3, 69.3 (2x CH_2 Fm), 67.3, 67.2 (C-5), 58.2 (RNCH_2CH$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$), 51.5 (RNCH$_2$CH$_2$(CH$_2$)$_4$CH$_2$C*H*$_2$N$_3$), 48.0, 48.0, 47.9, 47.9 (2x CH Fm), 44.2, 44.2 (C-4), 43.6 (C-1), 43.0 (C-6), 29.6, 29.4, 29.1, 28.8 (RNCH$_2$CH$_2$(CH$_2$)$_4$C*H*$_2$CH$_2$N$_3$ + RNCH$_2$C*H*$_2$(CH$_2$)$_4$CH$_2$CH$_2$N$_3$ + RNCH$_2$CH$_2$(C*H*$_2$)$_4$CH$_2$CH$_2$N$_3$), 27.1, 26.7 (RNCH$_2$CH$_2$(C*H*$_2$)$_4$CH$_2$CH$_2$N$_3$). **^{31}P NMR** (162 MHz, $CDCl_3$) δ -1.1. **HRMS:** $[C_{56}H_{55}N_4O_8P + H]^+$ found: 943.3833, calculated: 948.3830.

(1*R*,2*R*,3*R*,4*R*,5*S*)-1,2-epoxy-3,4-*O*-isopropylidene-5-hydroxymethylcyclopentane (10)



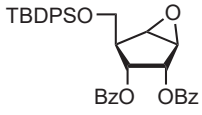
Compound **6**[42] (518 mg, 3.00 mmol) was co-evaporated with toluene and dissolved in toluene. To this solution, vanadyl acetylacetonate (80 mg, 0.3 mmol, 0.1 eq.) and *t*BuOOH (5.5 M in nonane, 2.2 mL, 12.0 mmol, 4.0 eq.) were added and the solution was stirred overnight at 60 °C. The reaction was concentrated *in vacuo*. Flash column chromatography (30 -> 50% EtOAc) furnished the title compound as a colorless oil (454 mg, 2.44 mmol, 81%). **Rf:** 0.21 in 30% EtOAc in pentane. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.65 (dd, *J* = 5.6, 0.8 Hz, 1H, H-2), 4.26 (dd, *J* = 5.7, 2.2 Hz, 1H, H-3), 3.91 – 3.76 (m, 2H, H-5), 3.70 (tt, *J* = 2.1, 1.0 Hz, 1H, H-6), 3.65 (d, *J* = 2.4 Hz, 1H, H-1), 2.41 (ddt, *J* = 7.3, 6.3, 2.3 Hz, 1H, H-4), 1.48 (s, 3H, CH_3 isopropylidene), 1.32 (s, 3H, CH_3 isopropylidene).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 112.3 (Cq isopropylidene), 83.0 (C-3), 79.9 (C-2), 61.9 (C-5), 60.2 (C-6), 58.2 (C-1), 49.8 (C-4), 27.1, 24.7 (CH_3 isopropylidine).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1,2-epoxy -5-(*O*-*tert*-butyldiphenylsilyl)-methylcyclopentane (20)



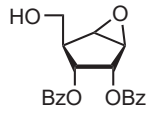
Compound **10** (393 mg, 2.11 mmol) was dissolved in an 80 v/v% solution of AcOH in H_2O (9 mL, 0.25 M). The reaction was stirred in an open flask at 60 °C for 2 hours. TLC indicated full conversion of the starting material to a lower running product (Rf = 0.3 in 10% EtOH in EtOAc). The reaction was concentrated *in vacuo* and thoroughly co-evaporated with a 1:1 mixture MeCN:H_2O followed by co-evaporation with 1,4-dioxane. The crude triol was than co-evaporated with pyridine thrice and dissolved in pyridine (21 mL, 0.1 M). TBDPS-Cl (1.08 mL, 4.22 mmol, 2.0 eq.) was added and the reaction was stirred overnight. The reaction was quenched by the addition of MeOH and the reaction was concentrated *in vacuo*. The resulting crude residue was taken up in EtOAc and the suspension was washed with 1 M HCl and brine respectively. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The title compound was obtained after flash column chromatography (30 -> 40% EtOAc in pentane) as a colorless oil (529 mg, 1.37 mmol, 65% over two steps). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.73 – 7.62 (m, 4H, TBDPS arom.), 7.46 – 7.29 (m, 6H, TBDPS arom.), 4.11 (d, J = 5.2 Hz, 1H, H-2), 3.98 – 3.81 (m, 2H, H-5), 3.59 – 3.50 (m, 2H, H-3 + H-6), 3.48 (dd, J = 2.8, 0.8 Hz, 1H, H-1), 2.92 (bs, 1H, OH), 2.22 (tdd, J = 8.0, 6.6, 1.2 Hz, 1H, H-4), 1.07 (s, 9H, *t*Bu TBDPS). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 135.6 (CH arom. TBDPS), 133.3, 133.2 (Cq arom. TBDPS), 129.9, 127.9 (CH arom. TBDPS), 72.6 (C-3), 69.9 (C-2), 63.4 (C-5), 57.2 (C-6), 56.2 (C-1), 47.8 (C-4), 26.9 (CH_3 *t*Bu TBDPS), 19.2 (Cq *t*Bu TBDPS).

(1*R*,2*R*,3*R*,4*R*,5*S*)-1,2-epoxy-3,4-*O*-di-benzoyl-5-(*O*-*tert*-butyldiphenylsilyl)-methylcyclopentane



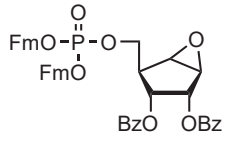
Compound **20** (442 mg, 1.15 mmol) was dissolved in pyridine (12 mL, 0.1 M). BzCl (0.53 mL, 4.6 mmol, 4.0 eq.) was added and the reaction was stirred 4 hours. The reaction was quenched by the addition of MeOH and concentrated *in vacuo*. The crude residue was taken up in EtOAc and the organic layer was washed with 1 M HCl and brine respectively, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (10 -> 20% Et_2O in pentane) yielded the title compound as a colorless oil (610 mg, 1.03 mmol, 90%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.95 – 7.89 (m, 2H, Bz arom.), 7.85 – 7.79 (m, 2H, Bz arom.), 7.72 – 7.62 (m, 4H, TBDPS arom.), 7.56 – 7.43 (m, 2H, Bz arom.), 7.43 – 7.31 (m, 8H, TBDPS arom. + Bz arom.), 7.31 – 7.24 (m, 2H, Bz arom.), 5.79 (d, J = 5.4, 0.8 Hz, 1H, H-2), 4.96 (dd, J = 8.2, 5.4 Hz, 1H, H-3), 4.04 – 3.93 (m, 2H, H-5), 3.87 (dd, J = 2.8, 1.2 Hz, 1H, H-6), 3.81 (dd, J = 2.7, 0.9 Hz, 1H, H-1), 2.84 – 2.73 (m, 1H, H-4), 1.06 (s, 9H, *t*Bu TBDPS). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.5, 165.5 (C=O Bz), 135.7, 135.6 (CH arom. TBDPS), 133.5 (Cq arom. TBDPS), 133.4 (CH arom. Bz), 133.3 (Cq arom. TBDPS), 133.2 (CH arom. Bz), 129.9, 129.8, 129.8, 129.7 (CH arom. Bz + CH arom. TBDPS), 129.5, 129.4 (Cq arom. Bz), 128.5, 128.3, 127.8, 127.8 (CH arom. Bz + CH arom. TBDPS), 72.6 (C-3), 70.9 (C-2), 62.4 (C-5), 57.0 (C-6), 54.8 (C-1), 46.2 (C-4), 26.9 (CH_3 *t*Bu TBDPS), 19.3 (Cq *t*Bu TBDPS). **HRMS:** $[C_{36}H_{36}O_6Si + H]^+$ found: 593.2350, calculated: 593.2354.

(1*R*,2*R*,3*R*,4*R*,5*S*)-1,2-epoxy-3,4-*O*-di-benzoyl-5-hydroxymethylcyclopentane (21)



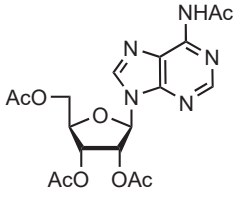
(3*S*,4*R*,5*R*)-1,2-epoxy-3,4-*O*-di-benzoyl-5-(*O*-tert-butyldiphenylsilyl)-methylcyclopentane (565 mg, 0.95 mmol) was dissolved in THF (9.5 mL, 0.1 M). TEA·3HF (1.56 mL, 9.53 mmol, 10 eq.) was carefully added and the reaction was stirred overnight. The reaction was cooled to 0 °C and carefully quenched with sat. aq. $NaHCO_3$. The reaction was transferred into a separatory funnel and the water layer was extracted thrice with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (20 -> 40% EtOAc) yielded the title compound as a colorless oil in quantitative yield. **Rf:** 0.22 in 30% EtOAc in pentane. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.02 – 7.95 (m, 2H, Bz arom.), 7.89 – 7.82 (m, 2H, Bz arom.), 7.60 – 7.54 (m, 1H, Bz arom.), 7.49 (ddt, *J* = 8.8, 7.4, 1.3 Hz, 1H, Bz arom.), 7.44 – 7.37 (m, 2H, Bz arom.), 7.33 – 7.27 (m, 2H Bz arom.), 5.83 (d, *J* = 5.2 Hz, 1H, H-2), 5.13 (dd, *J* = 8.1, 5.4 Hz, 1H, H-3), 4.12 – 3.89 (m, 2H, H-5), 3.87 – 3.77 (m, 2H, H-1 + H-6), 2.79 – 2.68 (m, 1H, H-4), 2.13 (bs, 1H, OH). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.0, 165.5 (C=O Bz), 133.6, 133.4, 129.9, 129.8 (CH arom. Bz), 129.5, 129.3 (Cq Bz), 128.6, 128.4 (CH arom. Bz), 72.8 (C-3), 71.0 (C-2), 61.3 (C-5), 57.2, 54.4 (C1 + C-6), 46.2 (C-4). **HRMS:** $[C_{20}H_{18}O_6 + H]^+$ found: 355.1174, calculated: 355.1176.

(1*R*,2*R*,3*R*,4*R*,5*S*)-1,2-epoxy-3,4-*O*-di-benzoyl-5-(*O*-difluorenylmethyl phosphate)methylcyclopentane (9)



Compound **20** (431 mg, 1.22 mmol) was co-evaporated with toluene. A solution of bis-(9H-fluoren-9-ylmethyl)-*N,N*-diisopropyl amido phosphite (698 mg, 1.34 mmol, 1.1 eq.) in MeCN (12 mL, 0.1 M) was added. To this solution, a 0.25 M DCI solution in MeCN (9.76 mL, 2.44 mmol, 2.0 eq.) was added and the reaction was stirred for 15 minutes. A 5.5 M *t*BuOOH in nonane (2.2 mL, 12.2 mmol, 10 eq.) was added and the reaction was stirred for an additional 1.5 hours. The reaction was taken up in EtOAc and the organic layer was washed with sat. aq. $NaHCO_3$ and brine respectively. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (30 -> 50% EtOAc in pentane) yielded the title compound as a white foam (905 mg, 1.14 mmol, 94%). **Rf:** 0.4 in 40% EtOAc in pentane. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.96 – 7.88 (m, 2H, Bz arom.), 7.87 – 7.80 (m, 2H, Bz arom.), 7.72 – 7.63 (m, 4H, Fm arom.), 7.57 – 7.50 (m, 3H, Bz arom + Fm arom.), 7.50 – 7.43 (m, 4H, Bz arom + Fm arom.), 7.40 – 7.18 (m, 13H, Bz arom + Fm arom.), 5.79 (d, *J* = 5.4 Hz, 1H, H-2), 4.95 (dd, *J* = 8.1, 5.4 Hz, 1H, H-3), 4.41 – 4.18 (m, 4H, 2x CH_2 Fm), 4.18 – 3.97 (m, 4H, H-5 + 2x CH Fm), 3.77 (dd, *J* = 2.7, 0.9 Hz, 1H, H-1), 3.62 (dd, *J* = 2.8, 1.2 Hz, 1H, H-6), 2.87 – 2.71 (m, 1H, H-4). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.4, 165.4 (C=O Bz), 143.1, 143.0, 143.0, 143.0, 141.4, 141.4, 141.4 (Cq Fm), 133.5, 133.4, 129.8, 129.7 (CH arom. Bz), 129.3, 129.0 (Cq Bz), 128.5, 128.4 (CH arom. Bz), 128.0, 128.0, 128.0, 127.9, 127.3, 127.2, 127.2, 127.2, 125.2, 125.1, 125.1, 125.1, 120.2, 120.1, 120.1, 120.1, 120.0 (CH arom. Bz + CH arom. Fm), 72.2 (C-3), 70.5 (C-2), 69.4, 69.4, 69.3 (2x CH_2 Fm), 65.7, 65.6 (C-5), 56.1 (C-6), 54.6 (C-1), 48.0, 48.0, 47.9, 47.9 (2x CH Fm), 44.4, 44.3 (C-4). **^{31}P NMR** (162 MHz, $CDCl_3$) δ -1.29. **HRMS:** $[C_{48}H_{39}O_9P + H]^+$ found: 791.2397, calculated: 791.2405.

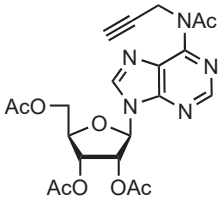
6-*N*-2',3',5'-tri-*O*-tetraacetyladenosine (22)



Adenosine (2.67 g, 10.0 mmol) was suspended in pyridine (50 mL, 0.2 M). Ac_2O (9.50 mL, 100 mmol. 10.0 eq.) was added and the reaction was stirred overnight. The now clear solution was quenched by the addition of EtOH and the reaction was concentrated *in vacuo* and co-evaporated with toluene. The resulting white solid was suspended in MeOH (25 mL, 0.4 M) and imidazole was added (408 mg, 6.0 mmol, 0.6 eq.) and the suspension was stirred overnight. The reaction was taken up in $CHCl_3$ and washed

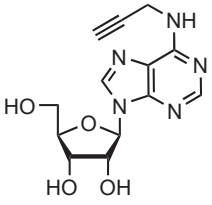
thrice with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* yielding the title compound in quantitative yield as a white foam which was used without further purification. Spectral data was in accordance with literary precedence.[40]

6-*N*-acetyl-6-*N*-propargyl-2',3',5'-tri-*O*-acetyladenosine (23)



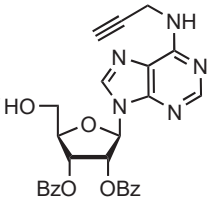
Compound **22** (1.31 g, 3.00 mmol) was dissolved in MeCN (10 mL, 0.3 M). DBU (0.95 mL, 6.34 mmol, 2.1 eq.) and propargyl bromide (0.49 mL, 4.52 mmol, 1.5 eq.) were added. The reaction was stirred for 1 hour after which the reaction was taken up in EtOAc. The organic layer was washed with 1 M HCl and brine respectively. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* yielding the title compound as a white foam in quantitative yield. **Rf:** 0.64 in EtOAc. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.84 (s, 1H, H-2), 8.28 (s, 1H, H-8), 6.28 (d, *J* = 5.0 Hz, 1H, H-1'), 5.99 (t, *J* = 5.2 Hz, 1H, H-2'), 5.71 (t, *J* = 5.3 Hz, 1H, H-3'), 5.09 (d, *J* = 2.5 Hz, 2H, CH_2 propargyl), 4.56 – 4.36 (m, 3H, H-4'+ H-5'), 2.39 (s, 3H, NAc), 2.19 – 2.15 (m, 4H, Ac + CH propargyl), 2.14 (s, 3H, Ac), 2.12 (s, 3H, Ac). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.9, 170.3, 169.6, 169.4 (C=O Ac), 152.6, 152.5 (C-4 + C-6), 152.2 (C-2), 142.4 (C-8), 126.9 (C-5), 86.8 (C-1'), 80.3 (C-4'), 79.2 (Cq propargyl), 73.1 (C-2'), 71.7 (CH propargyl), 70.4 (C-3'), 63.0 (C-5'), 36.4 (CH_2 propargyl), 24.4, 20.8, 20.5, 20.4 (CH_3 Ac). **HRMS:** $[C_{21}H_{23}N_5O_8 + H]^+$ found: 474.1618, calculated: 474.1619.

6-*N*-propargyladenosine (8)



Compound **23** (2.57 g, 5.45 mmol) was dissolved in MeOH (55 mL, 0.1 M) and a catalytic amount of sodium was added. The reaction was stirred 4 hours and white precipitate was filtered yielding the title compound (908 mg, 2.97 mmol, 55%). Spectral data was in accordance with literary precedence.[41] **^{1}H NMR** (400 MHz, DMSO) δ 8.41 (s, 1H), 8.28 (s, 1H), 5.90 (d, J = 6.1 Hz, 1H), 5.36 (s, 2H), 4.60 (dd, J = 6.1, 4.9 Hz, 1H), 4.25 (s, 2H), 4.15 (dd, J = 5.0, 3.1 Hz, 1H), 3.96 (q, J = 3.5 Hz, 1H), 3.68 (dd, J = 12.1, 3.7 Hz, 1H), 3.55 (dd, J = 12.1, 3.7 Hz, 1H), 3.11 – 2.99 (m, 1H). **HRMS:** $[C_{13}H_{15}N_5O_4 + H]^+$ found: 306.1195, calculated: 306.1197.

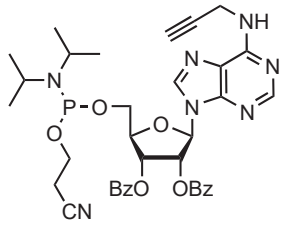
2',3'-*O*-dibenzoyl-6-*N*-propargyladenosine (24)



Compound **8** (445 mg, 1.46 mmol) was co-evaporated in pyridine and dissolved in pyridine (6 mL, 0.25 M). TBDMS-Cl (50 wt% in toluene, 0.76 mL, 2.19 mmol, 1.5 eq.) was added and the reaction was stirred for 2 hours after which TLC analysis showed full conversion of starting material into a higher running product (Rf = 0.35 in 5% MeOH in DCM). Bz-Cl (0.60 mL, 5.11 mmol, 3.5 eq.) was added to the reaction and after 1 hour of stirring TLC indicated full conversion of the intermediate. The reaction was taken up in EtOAc and the solution was washed with 1 M citric acid. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was dissolved in a 1:4 H_2O:MeCN mixture (6 mL total volume, 0.25 M) and PTSA monohydrate (417 mg, 2.19 mmol, 1.5 eq.) was added. The reaction was stirred for 3 hours. The reaction was taken up in EtOAc and the organic layer was washed with sat. aq. $NaHCO_3$ and brine respectively. The water layers were extracted with EtOAc and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (40% EtOAc in pentane) yielded the title compound as a white foam (581 mg, 1.09 mmol, 75%). **Rf:** 0.64 (40% EtOAc in pentane) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.46 (s, 1H, H-2), 8.11 – 8.01 (m, 2H, Bz arom.), 7.97 (s, 1H, H-8), 7.90 – 7.80 (m, 2H, Bz arom.), 7.59 (ddt, *J* = 8.7, 7.1, 1.3 Hz, 1H, Bz arom.), 7.54 – 7.39 (m, 3H, Bz arom.), 7.36 – 7.25 (m, 3H,

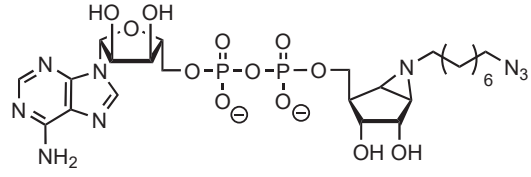
Bz arom.), 6.42 (dd, *J* = 7.5, 5.3 Hz, 1H, H-2'), 6.33 (d, *J* = 7.5 Hz, 1H, H-1'), 6.09 (dd, *J* = 5.3, 1.3 Hz, 1H, H-3'), 4.63 (q, *J* = 1.5 Hz, 1H, H-4'), 4.44 (s, 2H, CH_2 propargyl), 4.18 – 3.94 (m, 2H, H-5'), 2.25 (t, *J* = 2.5 Hz, 1H, CH propargyl). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.5, 164.8 (C=O Bz), 154.5 (C-4 + C-6), 152.9 (C-2), 139.9 (C-8), 133.7, 129.7 (CH arom. Bz), 129.2 (Cq Bz), 128.6, 128.5 (CH arom. Bz), 121.3 (C-5), 88.8 (C-1'), 86.5 (C-4'), 80.0 (Cq propargyl), 73.6 (C-3'), 73.5 (C-2'), 71.6 (CH propargyl), 62.7 (C-5'), 30.0 (CH_2 propargyl (signal taken from HSQC). **HRMS:** $[C_{27}H_{23}N_5O_6 + H]^+$ found: 514.1721, calculated: 514.1721.

5'-*O*-(*N*^{6}-propargyl-2',3'-di-*O*-benzoyladenosine)-2-cyanoethyl-*N*-*N*-diisopropylphosphoramidite (7)



Compound **24** (700 mg, 1.36 mmol) was co-evaporated with 1,4-dioxane and dissolved in DCM (4.5 mL, 0.3 M). DIPEA (0.59 mL, 3.40 mmol, 2.5 eq.) and 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphoramidite (0.33 mL, 1.50 mmol, 1.1 eq.) were added and the reaction was stirred for 1 hour. The reaction was diluted with DCM and the solution was washed with sat. aq. $NaHCO_3$ and brine respectively. The water layers were extracted with DCM and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (40% EtOAc in pentane, neutralized with 1% TEA) yielded the title compound as a white foam and a mixture of diastereomers (S_P/R_P) (800 mg, 1.12 mmol, 82%). **Rf:** 0.42 in 40% EtOAc in pentane. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.50 – 8.44 (m, 1H, CH adenine), 8.42 – 8.38 (m, 1H, CH adenine), 8.05 – 7.98 (m, 2H, Bz arom.), 7.95 – 7.86 (m, 2H, Bz arom.), 7.54 (dtd, J = 37.7, 7.4, 1.6 Hz, 2H, Bz arom.), 7.42 (td, J = 7.8, 2.4 Hz, 2H, Bz arom.), 7.32 (td, J = 7.9, 2.1 Hz, 2H, Bz arom.), 6.74 (bs, 1H, NH), 6.71 – 6.58 (m, 1H, H-1'), 6.25 – 6.14 (m, 1H, H-2'), 6.03 (ddd, J = 11.1, 5.5, 2.6 Hz, 1H, H-3'), 4.66 (q, J = 2.8 Hz, 1H, H-4'), 4.56 – 4.34 (m, 2H, CH_2 propargyl), 4.13 (ddq, J = 11.8, 6.9, 3.0 Hz, 1H, H-5'$_a$), 4.09 – 3.81 (m, 3H, H-5'$_b$ + O*C*H_2CH_2CN), 3.69 (dqq, J = 13.5, 6.8, 3.9, 2.7 Hz, 2H, CH N-*i*Pr), 2.73 (td, J = 6.4, 4.1 Hz, 2H, OCH_2CH_2CN), 2.28 (q, J = 2.6 Hz, 1H, CH propargyl), 1.29 – 1.17 (m, 12H, CH_3 N-*i*Pr). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 165.5, 165.4, 165.0, 165.0 (C=O Bz), 154.1 (Cq adenine), 153.3, 153.3, (CH adenine), 138.5 (CH adenine, signal taken from HSQC) 133.7, 133.6, 129.8, 129.8, 129.8 (CH arom. Bz), 129.0, 128.9 (Cq Bz/adenine), 128.5 (CH arom. Bz), 128.5, 128.5 (Cq Bz/adenine), 128.4 (CH arom. Bz), 120.1, 120.0, 117.7, 117.6 (Cq Bz/adenine), 85.4, 85.0 (C-1'), 83.6, 83.5, 83.3, 83.2 (C-4'), 80.1 (Cq propargyl), 74.6, 74.5 (C-2'), 72.7, 72.6 (C-3'), 71.6 (CH propargyl), 63.3, 63.2, 63.0 (C-5'), 58.9, 58.7, 58.6 (OCH_2CH_2CN), 43.3, 43.3, 43.2, 43.2 (CH N-*i*Pr), 24.8, 24.7, 24.7, 24.7, 24.7 (CH_3 N-*i*Pr), 20.4, 20.4, 20.3 (OCH_2CH_2CN). **HRMS:** *mass was detected as its corresponding H-phosphonate* $[C_{30}H_{27}N_6O_8P + H]^+$ found: 631.1698, calculated: 631.1701.

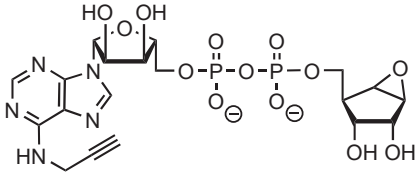
(1*R*,2*S*,3*R*,4*R*,5*R*)-(6-*N*-oct-(1-azide)-ane)azirdine-3,4-diol-5-*O*-(adenosine diphosphate) methylcyclopentane (1)



Aziridine **4** (61 mg, 65 µmol) was dissolved in MeCN (0.65 mL, 0.1 M) and TEA (127 µL, 0.91 mmol, 14 eq.) was added. The reaction was stirred overnight until LC-MS analysis showed full deprotection (gradient 10 -> 90% B in A, Rt = 6.09) from the Fm protecting groups. The reaction was co-evaporated with toluene, followed by co-evaporation with pyridine to convert the phosphate into the pyridinium salt followed by additional co-evaporation with toluene. Adenosine amidite **3** (56 mg, 78 µmol, 1.2 eq.) was co-evaporated separately with toluene and dissolved in 0.2 mL MeCN. This solution was added to the crude aziridine and the flask was rinsed with another 0.2 mL MeCN and added to the reaction. ETT (0.25 M in MeCN, 0.62 mL, 0.16 mmol, 2.4 eq.) was added

to the reaction (end volume 1.0 mL, 0.06 M). The reaction was stirred 15 minutes after which ^{31}P-NMR indicated full conversion of starting material (δ = 129.59, 127.36 P^{III} and -9.97 P^{V}). *t*BuOOH (5.5 M in nonane, 77 µL, 0.42 mmol, 6.5 eq.) was added and the reaction was stirred for 30 minutes after which ^{31}P-NMR (δ peaks shifted around -14) indicated full conversion of the phosphite species into the corresponding phosphate. DBU (0.5 M in DMF, 0.78 mL, 0.42 mmol, 6.0 eq.) was added and the reaction was stirred for 1 hour. Sat. aq. NH_4OH (1.6 mL) was added and the reaction was stirred overnight. The reaction was concentrated *in vacuo*. The residue was purified by gel filtration (HW40) followed by HPLC purification (buffered with NH_4OAc) where fractions containing product first were concentrated *in vacuo* and extensively co-evaporated with 1:1 MeCN:H_2O followed by lyophilization, yielding the title compound as a white solid (11.6 mg, 16.3 µmol, 25%). **^{1}H NMR** (850 MHz, D_2O) δ 8.50 (s, 1H, H-2), 8.20 (s, 1H, H-8), 6.08 (d, J = 5.6 Hz, 1H, H-1'), 4.68 (t, J = 5.3 Hz, 1H, H-2'), 4.47 (dd, J = 5.0, 3.8 Hz, 1H, H-3'), 4.35 (dt, J = 4.0, 2.4 Hz, 1H, H-4'), 4.29 – 4.23 (m, 2H, H-2 + H-5_a), 4.22 – 4.17 (m, 2H, H-5'), 4.15 (dt, J = 13.4, 5.0 Hz, 1H, H-5_b), 3.89 (s, 1H, H-3), 3.17 (t, J = 6.9 Hz, 2H, $RNCH_2CH_2(CH_2)_4CH_2CH_2N_3$), 2.95 (s, 1H, H-6), 2.59 (s, 1H, H-1), 2.46 (s, 1H, H-4), 1.54 – 1.44 (m, 2H, $RNCH_2CH_2(CH_2)_4CH_2CH_2N_3$), 1.39 (p, J = 7.0 Hz, 2H, $RNCH_2CH_2(CH_2)_4CH_2CH_2N_3$), 1.08 (p, J = 7.4 Hz, 2H, $RNCH_2CH_2(CH_2)_4CH_2CH_2N_3$), 1.03 – 0.88 (m, 8H, $RNCH_2CH_2(CH_2)_4CH_2CH_2N_3$). **^{13}C NMR** (214 MHz, D_2O) δ 155.5 (Cq adenine), 152.7 C-8 adenine), 148.9 (Cq adenine), 139.7 (C-2 adenine), 118.5 (Cq adenine), 87.0 (C-1'), 83.7, 83.7 (C-4'), 74.4 (C-2'), 70.2 (C-3'), 69.0 (C-2), 65.0, 65.0 (C-5'), 63.5 (C-5), 51.1 ($RNCH_2CH_2(CH_2)_4CH_2CH_2N_3$), 45.8, 44.4, 44.4 (C-1, C-4, C-6), 28.1, 28.0, 27.8, 26.7, 25.7, 25.7 ($RNCH_2CH_2(CH_2)_4CH_2CH_2N_3$). **^{31}P NMR** (202 MHz, D_2O) δ -10.2, -10.3, -10.6, -10.7. **LC-MS:** (0 -> 20% B in A) Rt = 8.49. **HRMS:** $[C_{24}H_{39}N_9O_{12}P_2 + H]^+$ found: 708.2263, calculated: 708.2266.

(1*R*,2*S*,3*R*,4*R*,5*R*)-1,2-epoxy-3,4-diol-5-*O*-((*N*^{6}-progargyladenosine)diphosphate)methylcyclopentane (2)



Epoxide **9** (158 mg, 0.2 mmol) was dissolved in MeCN (2.0 mL, 0.1 M) and TEA (0.4 mL, 2.8 mmol, 14 eq.) was added. The reaction was stirred overnight until LC-MS analysis showed full deprotection (gradient 10 -> 90% B in A, Rt = 5.13) from the Fm protecting groups. The reaction was co-evaporated with toluene, followed by co-evaporation with pyridine to convert the phosphate into the pyridinium salt followed by additional co-evaporation with toluene. Adenosine amidite **7** (171 mg, 0.24 mmol, 1.2 eq.) was co-evaporated separately with toluene and dissolved in 0.6 mL MeCN. This solution was added to the crude epoxide and the flask was rinsed with another 0.6 mL MeCN and added to the reaction. ETT (0.25 M in MeCN, 1.9 mL, 0.48 mmol, 2.4 eq.) was added to the reaction (end volume 3.1 mL, 0.06 M). The reaction was stirred 15 minutes after which ^{31}P-NMR indicated full conversion of starting material (δ = 127.49, 127.01 P^{III} and -11.30 P^{V}). *t*BuOOH (5.5 M in nonane, 0.24 mL, 1.3 mmol, 6.5 eq.) was added and the reaction was stirred for 30 minutes after which ^{31}P-NMR (δ = -12.95, -13.72, -13.86) and LC-MS analysis (gradient 10 -> 90% B in A, Rt = 6.50) indicated full conversion of the phosphite species into the corresponding phosphate. DBU (0.5 M in DMF, 2.4 mL, 1.2 mmol, 6.0 eq.) was added and the reaction was stirred 1 hour. Sat. aq. NH_4OH (5.0 mL) was added and the reaction was stirred overnight. The reaction was concentrated *in vacuo* and the residue was taken up in H_2O. The water layer was washed with EtOAc and the water layer was concentrated *in vacuo*. The residue was purified by gel filtration (HW40) followed by HPLC purification (buffered with NH_4OAc) where fractions containing product first were concentrated *in vacuo* and extensively co-evaporated with 1:1 MeCN:H_2O followed by lyophilization, yielding the title compound as a white solid (14.9 mg, 25.0 µmol, 13%). **^{1}H NMR** (850 MHz, D_2O) δ 8.37 (s, 1H, H-2 adenine), 8.18 (s, 1H, H-8 adenine), 6.00 (d, J = 5.7 Hz, 1H, H-1'), 4.64 (t, J = 5.4 Hz, 1H, H- 2'), 4.40 (dd, J = 5.1, 3.8 Hz, 1H, H-3'), 4.26 (dt, J = 3.8, 2.3 Hz, 1H, H-4'), 4.20 (d, J = 24.7 Hz, 2H, CH_2 propargyl),

4.13 – 4.06 (m, 2H, H-5'), 4.03 (dt, J = 10.7, 5.5 Hz, 1H, H-5_a), 3.96 (d, J = 5.2 Hz, 1H, H-2), 3.85 (ddd, J = 10.2, 8.4, 6.9 Hz, 1H, H-5_b), 3.53 (dd, J = 3.0, 1.2 Hz, 1H, H-6), 3.40 (dd, J = 2.9, 0.9 Hz, 1H, H-1), 3.30 (dd, J = 8.2, 5.2 Hz, 1H, H-3), 2.48 (t, J = 2.5 Hz, 1H, CH propargyl), 2.13 (tdd, J = 8.4, 5.3, 1.3 Hz, 1H, H-4). **^{13}C NMR** (214 MHz, D_2O) δ 153.7 (Cq adenine), 152.4 (C-8 adenine), 139.5 (C-2 adenine, signal taken from HSQC) 119.0 (Cq adenine), 86.8 (C-1'), 83.7, 83.6 (C-4'), 80.1 (Cq propargyl), 74.0 (C-2'), 71.6 (CH propargyl), 70.8 (C-3), 70.1, 70.1 (C-3'), 69.1 (C-2), 64.9, 64.9 (C-5'), 64.6, 64.6 (C-5), 57.4 (C-6), 55.9 (C-1), 44.8, 44.7 (C-4), 29.9 (CH_2 propargyl, signal taken from HSQC). **^{31}P NMR** (202 MHz, D_2O) δ -10.3, -10.4, -10.6, -10.7. **LC-MS:** (0 -> 20% B in A) Rt = 3.79. **HRMS:** $[C_{19}H_{25}N_5O_{13}P_2 + H]^+$ found: 594.0997, calculated: 594.0997.

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