Synthetic affinity-based probes for ADP-Ribose interactome studies

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Phosphoadenosyl-ADP-ribose as a key component in the synthesis of oligo-ADP-ribosylated peptides

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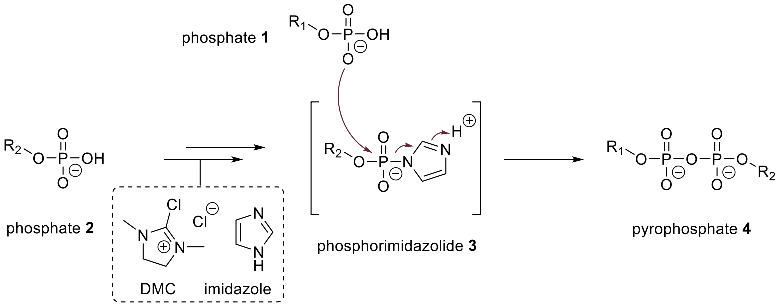
Introduction

As detailed in earlier parts of this work, adenosine diphosphate ribosylation (ADP-ribosylation) is an essential post-translational modification that is involved in various cellular processes and occurs in three distinct forms: mono-ADP-ribosylation, linear poly-ADP-ribosylation, and branched poly-ADP-ribosylation.[1–8] The complexity of the ADP-ribosylome is further increased by the variety of amino acid residues to which ADP-ribose can be conjugated: arginine[9,10], aspartate[11], cysteine[12], glutamate[11], histidine[12,13], lysine[14–16], serine[17], threonine[12] and tyrosine[9,18]. To fully understand the signaling pathways regulated by (poly-)ADP-ribosylation, it is essential to identify the readers of this modification in the specific context of the proteins they modify.[19]

One approach for identifying ADP-ribose readers is through the use of chemical tools. Recent studies have shown progress by using biotinylated ADP-ribose oligomers as affinity probes in proteomic analyses to identify readers of mono-ADP-ribose[20], linear poly-ADP-ribose[20,21], and, most recently, branched poly-ADP-ribose (Chapter 3). However, a significant limitation of these probes is their lack of a peptide backbone. For example, the study by Kistemaker *et al.*[22] demonstrated that the binding of a macrodomain to mono-ADP-ribose depends on the peptide sequence to which it is conjugated. This raises the question of whether the readers of oligo-ADP-ribose can distinguish between the amino acid residues to which ADP-ribose is conjugated, such as a serine versus a glutamate, or whether there are readers that recognize ADP-ribose fragments only when they are conjugated to a peptide backbone. To gain a more clear understanding of these nuances in ADP-ribose signaling, there is thus a need for oligo-ADP-ribosylated peptides that more closely resemble the natural post-translational modification.

Mono-ADP-ribosylated peptides can be obtained using chemoenzymatic and fully synthetic approaches.[23] However, only chemoenzymatic synthetic procedures are available for oligo- or poly-ADP-ribosylated peptides.[24] This hampers understanding the nuances in ADP-ribosylation signaling, as chemoenzymatic methods do not yield well-defined ADP-ribose structures, which are crucial for this purpose. Moreover, fully synthetic methods offer other significant advantages compared to chemoenzymatic approaches, such as the possibility to add bio-orthogonal handles or stabilize the specific positions in oligo-ADP-ribosylated peptides. Therefore, a fully synthetic method to obtain oligo-ADP-ribosylated peptides is highly desired, as this would enable the creation of customized, well-defined structures that can serve as valuable molecular probes.

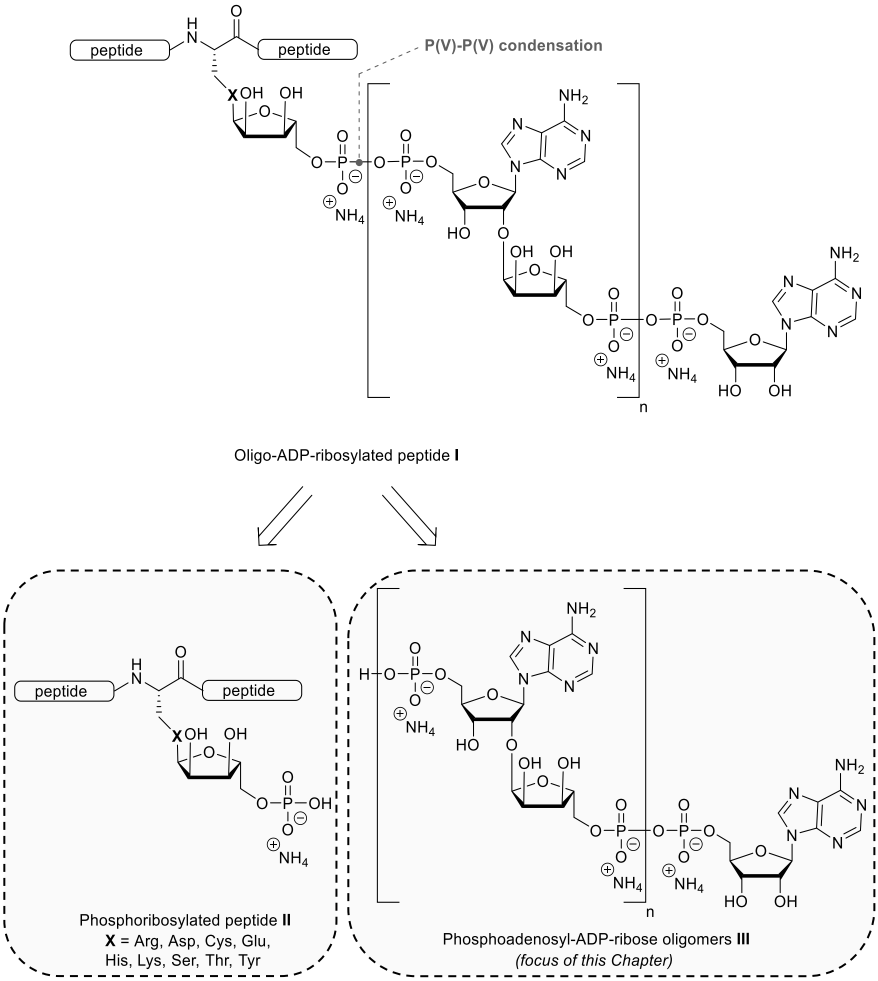
The main challenge in developing a fully synthetic method for oligo-ADP-ribosylated peptides is combining an α-glycosylated peptide backbone with the multiple pyrophosphates found in oligo-ADP-ribose. As described in the preceding Chapters, the currently used method for synthesizing pyrophosphates in oligo-ADP-ribose is based on P(V)-P(III) couplings, where a phosphate acts as the nucleophile, and a phosphoramidite, after activation by an azole activator, serves as the electrophile.[25,26] However, since the P(V)-P(III) coupling strategy requires protecting all amino- and hydroxyl-functionalities, problems may occur when combining it with peptide chemistry due to incompatible protecting group strategies. Therefore, a new approach was sought for the preparation of oligo-ADP-ribosylated peptides. A possible solution can be found by applying a phosphate condensation method described by Tanaka and coworkers for the construction of pyrophosphates.[27] In this reaction, two phosphates are used; wherein one serves as the nucleophile, and the other phosphate, after being activated as a phosphorimidazolide, acts as the electrophile (Scheme 1). Such a P(V)-P(V) condensation reaction can be carried out on the unprotected substrates in aqueous media and has successfully produced pyrophosphates in various contexts, including nucleotides[28], sugar nucleotides[27], peptides[29] and more recently, mono-ADP-ribosylated peptides[30].



Scheme 1. Abbreviated mechanism of the P(V)-P(V) condensation for pyrophosphate formation, highlighting the most important transformations. The reaction requires two phosphates: one of which functions as the nucleophile (1), whereas the other one (2), after activation by 2-chloro-1,3-dimethylimidazolinium chloride (DMC) and imidazole, is transformed into a reactive phosphorimidazolide (3), which functions as the electrophile to effectively form a pyrophosphate bridge (4) between the two components.

To apply this P(V)-P(V) condensation reaction for the generation of oligo-ADP-ribosylated peptides, two key components are required: a phosphoribosylated peptide and a phosphoadenosyl-ADP-ribose fragment (Scheme 2). Established methods can be used to obtain peptides phosphoribosylated at specific amino acid residues. For example, Kistemaker *et al.* described the phosphoribosylation at glutamine[22] and asparagine[22] residues, while Voorneveld *et al.* reported that of arginine[31], serine[32], threonine[32], tyrosine[33] and cysteine[32]. Additionally, Wijngaarden *et al.* investigated aspartate[34] and glutamate[34], and Minnee *et al.* specifically studied the phosphoribosylation of histidine.[35]

While the phosphoribosylated peptides can be obtained using these procedures, generating the phosphoadenosyl-ADP-ribose fragments requires a novel synthetic route. This Chapter provides such a route by introducing a methodology for producing phosphoadenosyl-ADP-ribose oligomers of different lengths, thereby enabling the synthesis of well-defined oligo-ADP-ribosylated peptides through the aforementioned P(V)-P(V) condensation reaction. The method presented employs a 'traditional' solid-phase approach that utilizes P(V)-P(III) couplings while introducing modified building blocks, facilitating the straightforward preparation of ADP-ribose fragments phosphoadenosylated at the anomeric center of the distal ribose. Using this approach, both phosphoadenosyl-ADP-ribose and phosphoadenosyl-di-ADP-ribose fragments were synthesized. Furthermore, this Chapter demonstrates the condensation of the phosphoadenosyl-ADP-ribose fragment with an H2B1C-derived phosphoribosylated peptide to generate the first synthetic well-defined di-ADP-ribosylated peptide using the P(V)-P(V) strategy.



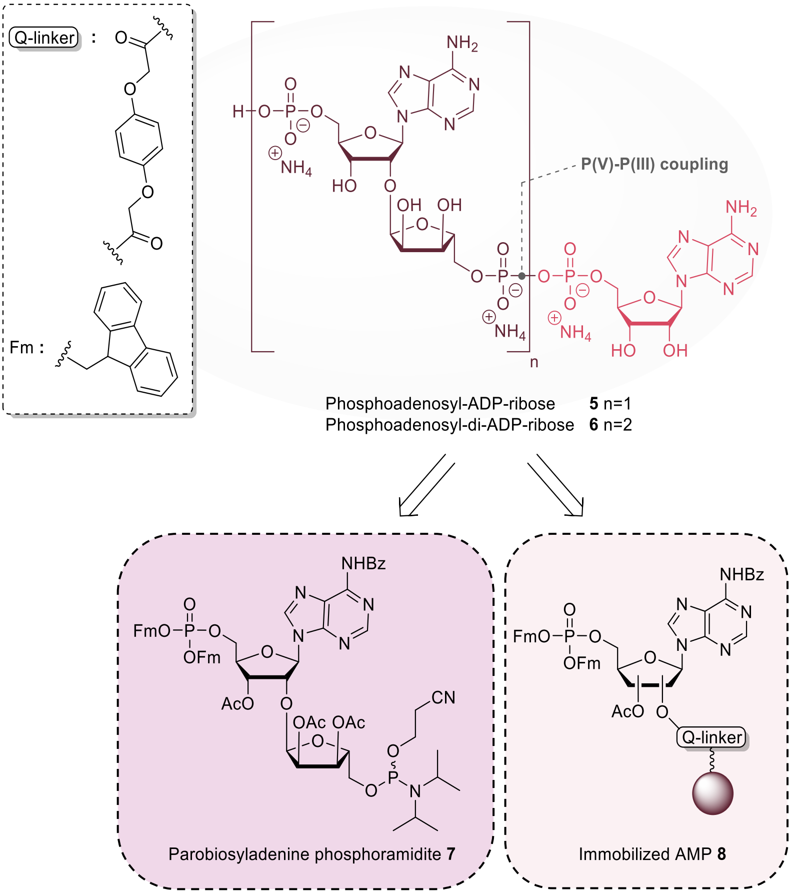
Scheme 2. Retrosynthetic strategy for obtaining oligo-ADP-ribosylated peptides (I) via the P(V)-P(V) condensation reaction using phosphoribosylated peptides (II) and phosphoadenosyl-ADP-ribose oligomers (III) as key components.

Results and Discussion

Synthesis of phosphoadenosyl-ADP-ribose oligomers

To produce oligo-ADP-ribosylated peptides through a P(V)-P(V) condensation reaction, a new synthetic method for generating phosphoadenosyl-ADP-ribose oligomers was developed (Scheme 3). This method employs a solid-phase approach using P(V) -P(III) couplings to obtain the pyrophosphate linkages within the phosphoadenosyl-ADP-ribose oligomers. Although this strategy shares similarities with previously described methodologies by Kistemaker *et al.*[25] and Liu *et al.*[26] (further detailed in Chapter 1), the key distinction lies in the direction of the chain assembly. Instead of initiating the synthesis from the ribose end of the ADP-ribose oligomer, this approach begins at the adenosine end, ensuring that the required phosphoadenosyl moiety can be installed at the anomeric center of the distal ribose in the ADP-ribose oligomer. While a fairly standard controlled-pore glass (CPG) solid support with immobilized adenosine monophosphate (AMP) **8** served as the starting point for the solid-phase synthesis, a novel key building block named ''parobiosyladenine phosphoramidite **7**'' was designed to implement this strategy (the term ''parobiosyl'' refers to the characteristic disaccharide core found in poly-ADP-ribose chains).[36] Building block **7** was employed to extend the oligomer chain to the desired length while, at the same time, facilitating the effective installation of the phosphoadenosyl-end.

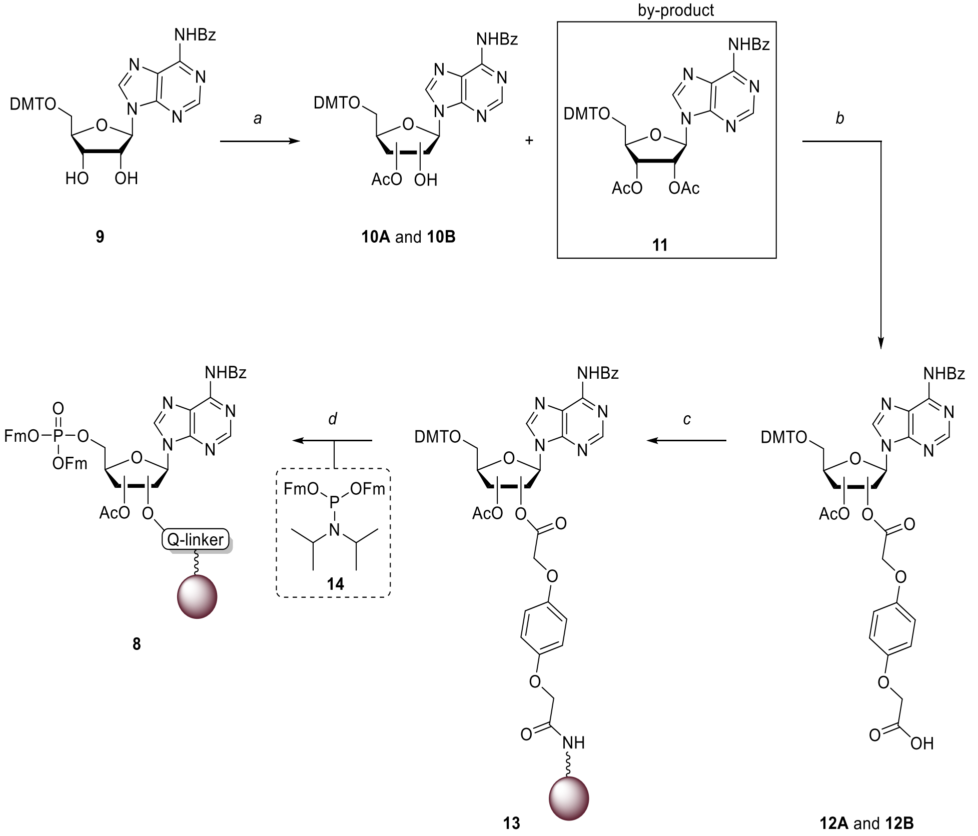
Notably, compound **7** features a phosphoramidite at the 5'' position of the 2'-*O*-ribosyl moiety alongside a 9-fluorenylmethyl (Fm)-protected phosphate at the 5' position of the adenosine, in contrast to the conventional approach, in which the phosphoramidite moiety is located at the 5' position of the adenosine and the protected phosphate at the 5'' position of the 2'-*O*-ribosyl moiety.[25,26] In other words, the phosphoramidite and phosphate moieties are interchanged in building block **7** to enable a direct solid-phase synthesis of phosphoadenosyl-ADP-ribose oligomers. To demonstrate the feasibility of this method in generating phosphoadenosyl-ADP-ribose oligomers, phosphoadenosyl-ADP-ribose **5** and phosphoadenosyl-di-ADP-ribose **6** were synthesized as precursors to generate di-ADP-ribosylated and tri-ADP-ribosylated peptides, respectively.



Scheme 3. Retrosynthesis of phosphoadenosyl-ADP-ribose oligomers, phosphoadenosyl-ADP-ribose 5 *and phosphoadenosyl-di-ADP-ribose* 6*, via P(V)-P(III) couplings on solid-phase using parobiosyladenine phosphoramidite* 7 *and immobilized AMP* 8 *as key building blocks.*

Synthesis of immobilized AMP 8

First, immobilized AMP **8** was synthesized starting from commercially available *N*^{6}-benzoyl adenosine **9**, which was protected at the 5'-hydroxyl with a dimethoxytrityl (DMT) group (Scheme 4). The first step involved monoacetylation of the secondary hydroxyls, resulting in a regioisomeric mixture of **10a** and **10b** with a total yield of 42%, where either the 2'-hydroxyl (**10b**) or 3'-hydroxyl (**10a**) was acetylated. By-product **11**, with both 2'- and 3'-hydroxyls acetylated, was also obtained in 22% yield due to the lack of regioselectivity in this reaction. After obtaining **10a** and **10b**, the hydroquinone-*O*,*O*'-diacetic acid (Q-linker) was installed on the remaining free hydroxyls via esterification using EDC·HCl and DMAP, resulting in a regioisomeric mixture of **12a** and **12b** in 46% yield. Subsequently, the Q-linker was conjugated to the aminopropyl-CPG solid support through a (7-azabenzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (PyAOP)-mediated coupling to acquire immobilized adenosine **13** with a loading of 36.7 µmol/g (54%). Finally, the 5'-hydroxyl position was phosphorylated in a three-step procedure involving removal of the DMT group using TCA, phosphitylation using bis-Fm-*N*,*N*-diisopropylphosphoramidite **14**[37] and 5-(ethylthio)-1H-tetrazole (ETT) as activator and oxidation using camphorsulfonyl-oxaziridine (CSO) to yield immobilized AMP **8**.



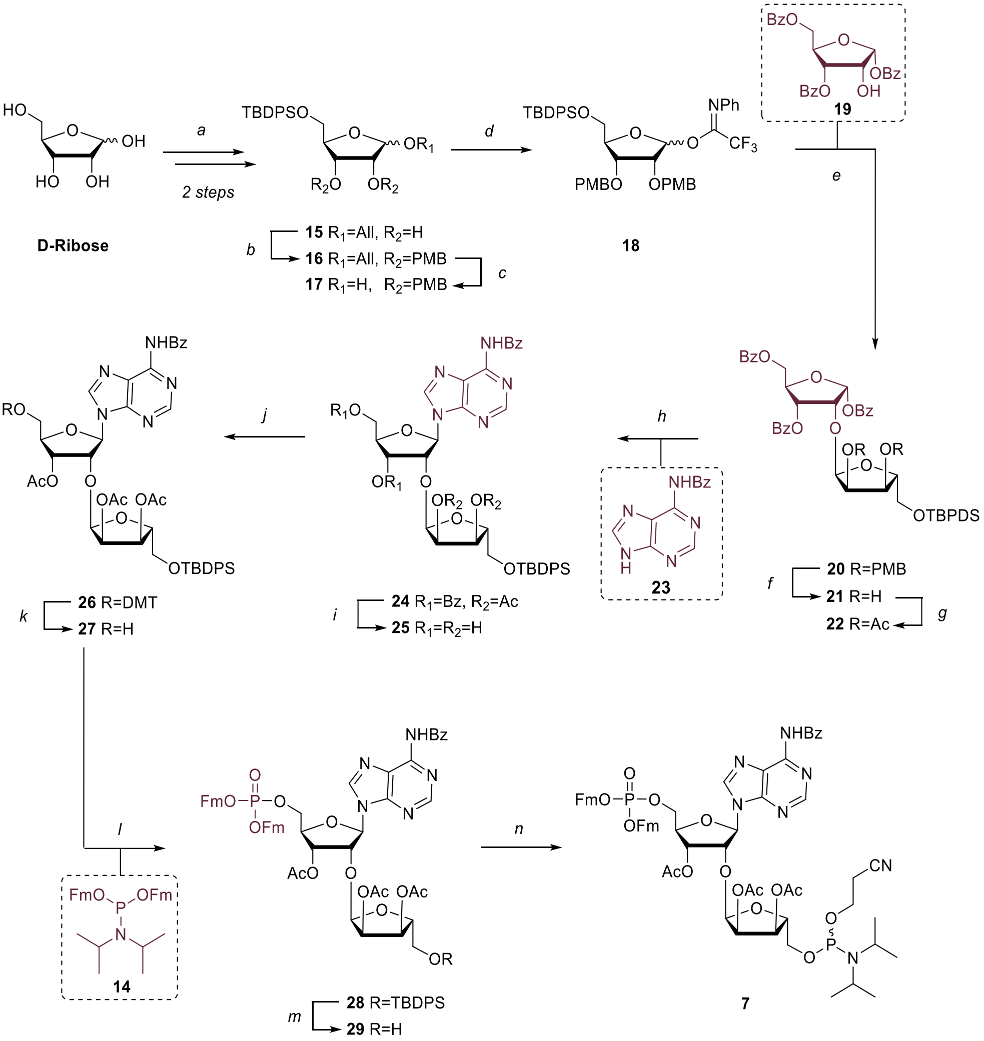
Scheme 4. Synthesis of immobilized AMP **8** *for the synthesis of phosphoadenosyl-ADP-ribose oligomers. Reagents and conditions: a) Ac$_2$O, pyridine, 0 °C → rt, overnight, 42% for* **10a** *and* **10b***, 22% for* **11***. B) DMAP, EDC·HCl, Et$_3$N, Q-linker, pyridine, rt, overnight, 46%. C) PyAOP, DiPEA, DMF, rt, 10 min; then aminopropyl-CPG, DMF, rt, overnight, loading of 36.7 µmol/g (54%). D) i. TCA, DCM, rt. ii. ETT, (FmO)$_2$PN(iPr)$_2$* **14**[37]*, ACN, rt, 10 min (3x). iii. CSO, ACN, rt, 5 min (2x).*

Synthesis of parobiosyladenine phosphoramidite 7

Next, key parobiosyladenine phosphoramidite 7 was synthesized through a 15-step sequence starting from commercially available D-ribose (Scheme 5). First, partially protected ribofuranose 15 was synthesized, featuring an allyl group on the C1-hydroxyl and an acid-stable TBDPS group on the C5-hydroxyl via the two-step procedure of Kistemaker *et al.*[22] In the following step, non-participating *para*-methoxbenzyl (PMB) protecting groups were installed on the C2- and C3-hydroxyl groups to enable effective acidic deprotection later in the route. This resulted in fully protected ribofuranose 16 in 89% yield using PMB-Br and sodium hydride. Next, the allyl group on the C1-hydroxyl of 16 was removed through an isomerization of the allyl into the vinyl ether using an iridium catalyst, followed by oxidative cleavage with iodine and aqueous $NaHCO_3$, yielding hemiacetal 17 in 86% yield. The hemiacetal was then converted into an imidate using phenyltrifluoroacetimidoyl chloride (PTFAI-Cl) and Cs_2CO_3, resulting in glycosyl donor 18 with a yield of 92%.

With glycosyl donor **18** prepared, a TMSOTf-mediated glycosylation was performed using commercially available 1,3,5-tri-***O***-benzoyl-α-D-ribofuranose **19** as the glycosyl acceptor, successfully generating disaccharide **20** in 77% yield and with excellent α-stereoselectivity. The PMB groups were then removed via acidic deprotection using TFA, leading to **21** in 79% and replaced with acetyl groups to quantitatively afford **22**. The adenine moiety was introduced through a Vörbruggen glycosylation using ***N***^{6}-benzoyl adenine 23, BSTFA as the silylating agent and $HClO_4$-SiO_2 as the acid catalyst. After overnight reflux, nucleoside **24** was obtained with a yield of 66%.

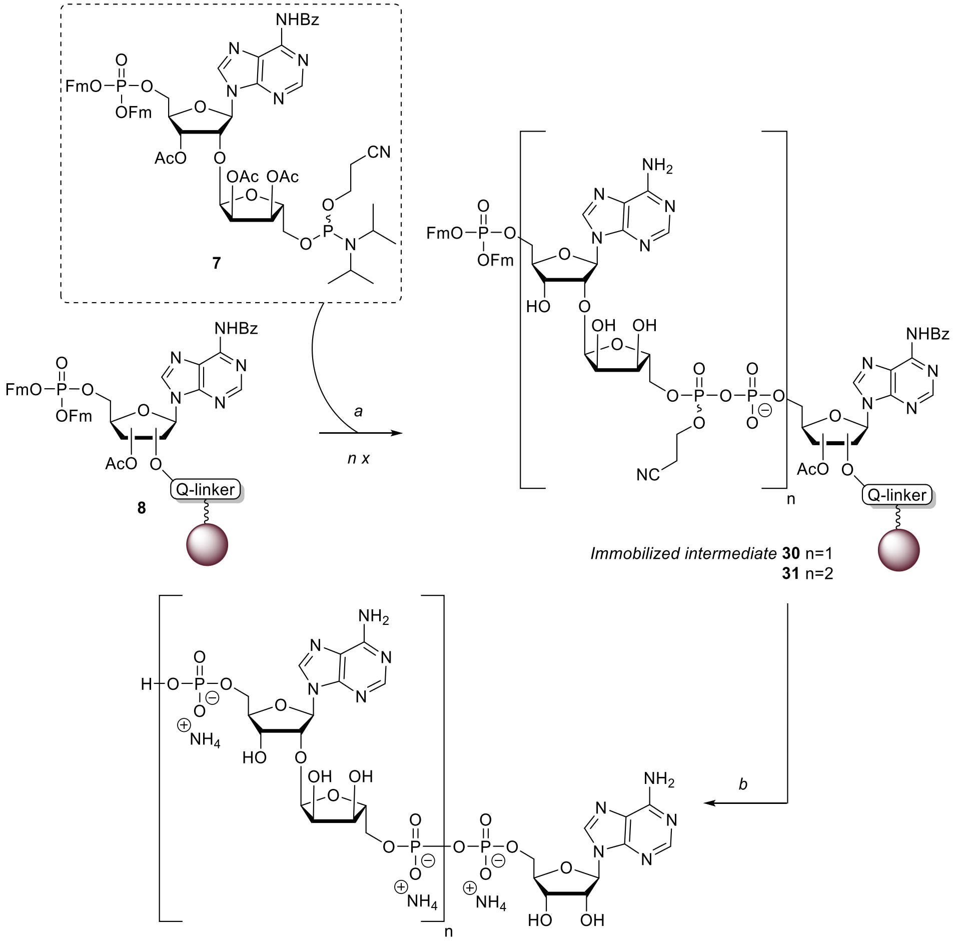
To selectively introduce the desired Fm-protected phosphotriester and phosphoramidite at the 5'- and 5''-hydroxyls, respectively, of the parobiosyladenine building block, the benzoyl and acetyl esters in 24 were removed in the next step by saponification, generating 25 in 92% yield. Subsequently, the DMT group was selectively installed on the 5'-OH and the remaining hydroxyls were protected with acetyl esters in a one-pot reaction, yielding compound 26, with a DMT group on the 5'-hydroxyl and a TBDPS group on the 5''-hydroxyl, in 90% yield over two steps. This allowed for the orderly installation of the desired P(V) and P(III) functionalities. First, the DMT group was removed using TFA, resulting in 27 in 63% yield, after which the liberated 5'-hydroxyl group was phosphitylated using bis-Fm-*N*,*N*-diisopropylphosphoramidite 14[37] and dicyanoimidazole (DCI) as the activator. The resulting phosphite triester was oxidized in one pot with *t*BuOOH, yielding the Fm-protected phosphotriester 28 in excellent yield over two steps. Subsequently, the TBDPS group was removed using HF·pyridine, resulting in alcohol 29 in 88% yield. Finally, the liberated 5''-hydroxyl group was phosphitylated using 2-cyanoethyl (CNE) *N*,*N*-diisopropylchlorophosphoramidite, leading to the formation of key parobiosyladenine phosphoramidite 7 in 69% yield.



Scheme 5. Synthesis of key parobiosyladenine phosphoramidite 7 *for the synthesis of phosphoadenosyl-ADP-ribose oligomers. Reagents and conditions: a)* 15 *was synthesized in 2 steps according to the procedures of Kistemaker et al.*[22] *b) PMB-Br, NaH, DMF/THF, 0 °C → rt, overnight, 89%. C) [Ir(COD)(PmePh$_2$)$_2$]PF$_6$, H$_2$, THF, rt; then* 16*, THF, rt, 2 days; then sat. aq. NaHCO$_3$, I$_2$, rt, 30 min, 86%. D) PTFAI-Cl, Cs$_2$CO$_3$, acetone, rt, 3 h, 92%. E) 1,3,5-tri-O-benzoyl-α-D-ribofuranose* 19*, MS 3Å, DCM, rt, 1 h; then TMSOTf, -78 °C, 10 min, 77%. F) TFA, DCM, rt, 1 h, 79%. G) DMAP, Ac$_2$O, pyridine, rt, 3.5 h, quant. H) N^6-benzoyladenine* 23*, BSTFA, ACN, rt, 30 min; then HClO$_4$-SiO$_2$, reflux, overnight, 66%. I) NaOH, pyridine/EtOH/H$_2$O, 0 °C, 1 h, 92%. J) DMTCl, pyridine, rt, 2 h; then Ac$_2$O, 0 °C, 5 h, 90% over 2 steps. K) TFA, DCM, rt, 10 min, 63%. L) MS 3Å, DCI, (FmO)$_2$PN(iPr)$_2$* 14[37]*, ACN, rt, 10 min; then tBuOOH, decane, 0 °C, 30 min, 92% over 2 steps. M) HF·pyridine, THF, 0 °C → rt, 2 h, 88%. N) DiPEA, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, DMF, rt, 10 min, 69%.*

Construction of phosphoadenosyl-ADP-ribose 5 and phosphoadenosyl-di-ADP-ribose 6

With both immobilized and protected AMP 8 and key parobiosyladenine phosphoramidite 7 in hand, attention was turned to the synthesis of phosphoadenosyl-ADP-ribose derivatives 5 and 6 (Scheme 6). The synthesis involved a solid-phase strategy with a repeated three-step cycle to achieve the desired oligomer length.



Phosphoadenosyl-ADP-ribose **5** n=1
Phosphoadenosyl-di-ADP-ribose **6** n=2

Scheme 6. Synthesis of phosphoadenosyl-ADP-ribose 5 and phosphoadenosyl-di-ADP-ribose 6 on solid phase. Reagents and conditions: a) i. DBU, ACN, rt, 5 min (4x). ii. 7, ETT, ACN, rt, 10 min (3x). iii. CSO, ACN, rt, 5 min (2x). b) i. DBU, ACN, rt, 5 min (4x). ii. NH_4OH, rt, overnight, 14% for 5 (n=1), 12% for 6 (n=2).

The cycle began with the deprotection of the base-labile Fm groups of immobilized AMP **8** using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to liberate the phosphomonoester for the subsequent P(III)-P(V) coupling. In the second step, phosphoramidite **7** and ETT were added to form the P(III)-P(V) intermediate. Finally, CSO was used to oxidize the P(III)-P(V) intermediate into the desired pyrophosphate moiety. The desired oligomer length was achieved by applying this three-step procedure once for phosphoadenosyl-ADP-ribose **5** or twice for phosphoadenosyl-di-ADP-ribose **6**, yielding immobilized intermediate **30** or **31**, respectively. The immobilized oligomers were then treated with DBU to remove the base-labile CNE- and Fm-groups from the pyrophosphate and the phosphate, respectively. After the final treatment with NH_4OH to cleave the oligomers from the solid support and remove the benzoyl- and acetyl-protective groups, **5** (14% yield) and phosphoadenosyl-di-ADP-ribose **6** (12% yield) were successfully obtained.

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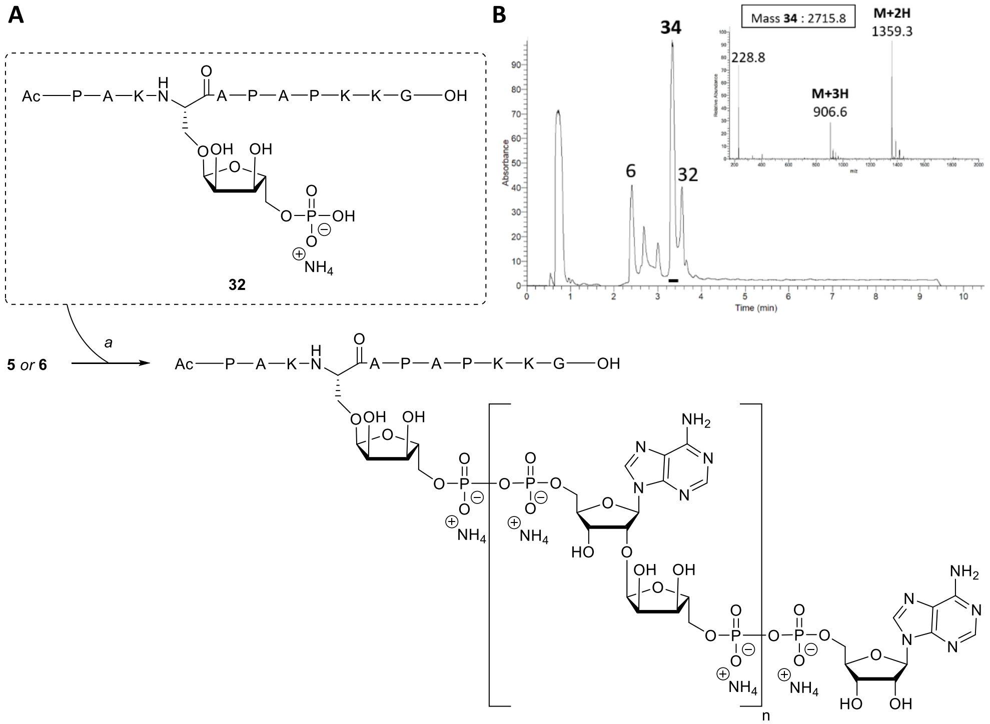
P(V)-P(V) condensation to produce oligo-ADP-ribosylated peptides

To explore the applicability of the phosphorimidazolide-driven P(V)-P(V) condensation reaction in the generation of oligo-ADP-ribosylated peptides, phosphoadenosyl-ADP-ribose **5** was conjugated to H2B1C-based peptide **32**[38] which was phosphoribosylated on the serine residue, to form di-ADP-ribosylated peptide **33** (Scheme 7A). To accomplish this, phosphoadenosyl-ADP-ribose **5** was first subjected to a mixture of DMC-HCl and imidazole in D_2O to form the activated phosphorimidazolide species. Then, phosphoribosylated peptide **32** was reacted with the electrophilic ADP-ribose phosphorimidazolide and an excess of $ZnCl_2$[39] to effectuate the P(V)-P(V) condensation. After purification by size exclusion chromatography, followed by strong anion exchange chromatography, di-ADP-ribosylated peptide **33** was obtained with a yield of 12%.

Building upon this first successful synthesis of di-ADP-ribosylated peptide **33**, an attempt was made to condense phosphoribosylated peptide **32** and phosphoadenosyl-di-ADP-ribose **6** to form trimer **34**. After stirring overnight under the abovementioned reaction conditions used for the preparation of **33**, LC-MS analysis confirmed the formation of tri-ADP-ribosylated peptide **34** (Scheme 7B). This product, however, could not be isolated due to unforeseen degradation of the pyrophosphate groups during strong anion exchange chromatography. Suitable purification conditions thus remain to be found for trimer **34**. Once appropriate purification conditions are found, peptide **34** may be synthesized, along with other potentially longer oligo-ADP-ribosylated peptides by varying the phosphoribosylated peptide or the phosphoadenosyl-ADP-ribose fragment.



H2B1C Ser6(ADPr)$_2$: Ac-Pro-Ala-Lys-Ser(O$_\gamma$-[ADPr$_2$])-Ala-Pro-Ala-Pro-Lys-Lys-Gly-OH **33** n=1
H2B1C Ser6(ADPr)$_3$: Ac-Pro-Ala-Lys-Ser(O$_\gamma$-[ADPr$_3$])-Ala-Pro-Ala-Pro-Lys-Lys-Gly-OH **34** n=2

Scheme 7. A) Synthesis of di-ADP-ribosylated peptide 33 and tri-ADP-ribosylated peptide 34 via the P(V)-P(V) condensation reaction. Reagents and conditions: a) DMC-HCl, imidazole, D$_2$O, rt, 5 min; then 5 or 6, rt, 45 min; then 32, ZnCl$_2$, D$_2$O, 40 °C, overnight, 12% for 33 (n=1), n/a for 34 (n=2). B) LC-MS analysis of the crude reaction mixture of tri-ADP-ribosylated peptide 34 after overnight stirring. The LC-MS gradient is 0 → 50% ACN [0.1% TFA]. Total wavelength scan (200–680 nm) LC chromatogram, showing peaks for starting materials 6 and 32, as well as product 34. Extracted ESI-MS spectra of the LC peak for 34 (retention time: 3.3 – 3.4 min, MS range: 160-2000 m/z).

Conclusion

The development of synthetic methodology for the generation of fully synthetic oligo-ADP-ribosylated peptides has important implications for advancing the ADP-ribose research field. For instance, the effect of the peptide backbone on binding of ADP-ribose-recognizing proteins remains understudied because of the lack of well-defined oligo-ADP-ribosylated peptides. To this end, a new strategy was developed here to obtain synthetic oligo-ADP-ribosylated peptides through a P(V)-P(V) condensation reaction between a phosphoribosylated peptide and a phosphoadenosyl-ADP-ribose fragment. To synthesize phosphoadenosyl-ADP-ribose oligomers of desired lengths, this Chapter introduced a novel solid-phase procedure based on P(V)-P(III) couplings. By incorporating new solid-phase building blocks 7 and 8, this method allowed for the effective generation of both phosphoadenosyl-ADP-ribose 5 and phosphoadenosyl-di-ADP-ribose 6, which can be directly applied to generate oligo-ADP-ribosylated peptides. To explore this approach, this Chapter presented the P(V)-P(V) coupling of phosphoadenosyl-ADP-ribose oligomer 5 to H2B1C-derived phosphoribosylated peptide 32, which resulted in the first fully synthetic di-ADP-ribosylated peptide 33. Overall, this work outlined a practical strategy for generating phosphoadenosyl-ADP-ribose oligomers of desired lengths and showed their application in synthesizing well-defined oligo-ADP-ribosylated peptides, which in turn can act as powerful chemical tools to enhance the understanding of the ADP-ribose signaling pathways.

Acknowledgments

Ioanna Tsoumani is acknowledged for her work in setting up the synthetic route to parobiosyladenine phosphoramidite 7 and for her assistance in the solid phase synthesis, Daisy Robinson for her help in building block synthesis, Nico Meeuwenoord for his assistance in solid-phase synthesis and purification of the final compounds. Sven Wijngaarden is acknowledged for setting up the methodology of the P(V)-P(V) condensation and for the condensation of the phosphoadenosyl-ADP-ribose oligomers to phosphoribosylated peptides.

Experimental

General

All chemicals were of reagent grade and were used without further purification unless stated otherwise. Dry solvents were stored over flame dried 4Å molecular sieves before use, except for ACN which was stored over 3Å molecular sieves. All reactions were performed under an N_2 atmosphere unless stated otherwise. Reactions were monitored by analytical thin layer chromatography (TLC) using Merck aluminum sheets pre-coated with silica gel 60Å with detection by UV-absorption (254 nm) and by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot2H_2O$ (10 g/L) in 10% sulfuric acid followed by charring. Additional analysis with TLC-MS was used when needed. Column chromatography was performed manually using Macherey-Nagel silica gel 60Å (40-63 µm) or a Biotage IsoleraTM flash purification system using silica gel cartridges (Screening Device SilicaSep HP, particle size 15-40 µm, 60Å) in the indicated solvents. For HW-40 gel filtration purifications an ÄKTA explorer system equipped with a Superdex-30-HR column (16 mm x 100 cm, flow 1 mL/min) was used. For strong anion exchange chromatography purifications an ÄKTA pure system equipped with a Source-15Q column (16x100 mm, flow 4 mL/min) was used.

NMR spectra were recorded on a Bruker AV-300, AV-400, AV-500 or AV-850 spectrometer. Chemical shifts are given in ppm (∂) relative to the chloroform or deuterium oxide residual solvent peak or tetramethylsilane (TMS) as an internal standard. Coupling constants (*J*) are given in Hz. All given ^{13}C-NMR spectra are proton decoupled. 2D NMR experiments (HSQC, COSY) were carried out to assign protons and carbons of the synthesized structures and numbering and assignation follows the general numbering shown in Figure S1.

High resolution mass spectra (HRMS) of the new compounds were recorded with a Q-Exactive HF Orbitrap (Thermo Scientific) equipped with an electrospray ion source (ESI) and injection of 2 µl of a 1 µM solution via an Ultimate 3000 nano UPLC (Dionex) system with an external calibration (Thermo Scientific), source voltage of 3,5 kV, capillary temperature of 275 °C, no sheath gas, resolution R = 240.000 at m/z=400 (mass range m/z=160-2000 or until a maximum of 6000) and ACN:H_2O (1:1 v/v) supplemented with 0.1% formic acid as eluent. LC-MS analysis was performed on an LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a C18 column (Gemini, 4.6 mm x 50 mm, 3 µM particle size, Phenomenex) equipped with buffers A: H_2O, B: ACN, C: 1% TFA or on an Agilent technologies 1260 infinity LC-MS with a 6120 Quadrupole MS system equipped with buffers A: H_2O, B: ACN and C: 100 mM NH_4OAc.

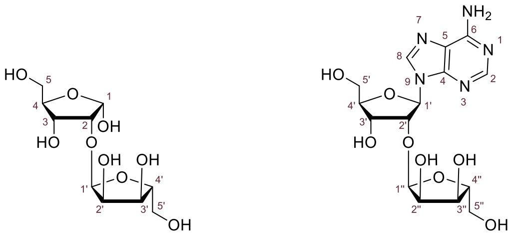
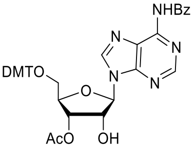
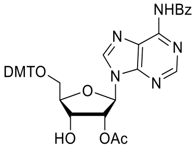


Figure S1. Numbering system for parobiose (left) and parobiosyladenine (right).

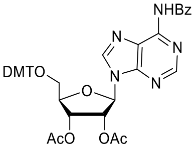
N^6-Benzoyl-3’-*O*-acetyl-5’-*O*-dimethoxytrityl-β-D-adenosine (10a) / *N*^{6}-Benzoyl-2’-*O*-acetyl-5’-*O*-dimethoxytrityl-β-D-adenosine (10b) / *N*^{6}-Benzoyl-2’, 3’-di-*O*-acetyl-5’-*O*-dimethoxytrityl-β-D-adenosine (11)



10a



10b

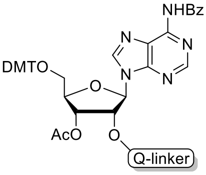


11

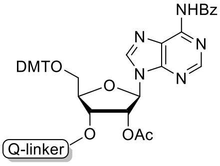
Diol **9** (1.38 g, 2.05 mmol) was dissolved in pyridine (10 mL). Ac$_2$O (174 µL, 1.84 mmol, 0.9 eq) was added dropwise at 0 °C and the reaction mixture was stirred at room temperature overnight. Then, the reaction mixture was concentrated under reduced pressure, diluted with EtOAc and the organic phase was washed with H$_2$O (1x). The aqueous phase was extracted with EtOAc (3x) and the combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (0% → 5% MeOH in DCM) afforded esters **10a** and **10b** as a white foam and as a regioisomeric mixture (0.62 g, 0.86 mmol, **10a**:**10b** ratio; 0.59:0.41, with an overall yield of 42%) and ester **11** as a white foam (0.35 g, 0.46 mmol, 22%). Data for regioisomeric mixture of **10a** and **10b**: **^{1}H NMR (400 MHz, CDCl$_3$)** δ 9.25 – 9.11 (m, 1H, NH-AB), 8.79 – 8.65 (m, 1H, H2-AB), 8.25 (s, 0.59H, H8-A), 8.20 (s, 0.41H, H8-B), 8.02 (d, *J* = 7.8 Hz, 2H, arom.-AB), 7.63 – 7.58 (m, 1H, arom.-AB), 7.54 – 7.49 (m, 2H, arom.-AB), 7.43 – 7.38 (m, 1H, arom.-AB), 7.33 – 7.17 (m, 8H, arom.-AB), 6.81 – 6.75 (m, 4H, DMT arom.-AB), 6.28 (d, *J* = 4.7 Hz, 0.41H, H1’-B), 6.09 (d, *J* = 6.7 Hz, 0.59H, H1’-A), 5.88 (t, *J* = 5.1 Hz, 0.41H, H2’-B), 5.47 (dd, *J* = 5.5, 2.1 Hz, 0.59H, H3’-A), 5.13 (t, *J* = 5.9 Hz, 0.59H, H2’-A), 4.86 (t, *J* = 5.1 Hz, 0.41H, H3’-B), 4.40 (q, *J* = 3.2 Hz, 0.59H, H4’-A), 4.27 (q, *J* = 3.8 Hz, 0.41H, H4’-B), 3.78 – 3.75 (m, 6H, CH$_3$ DMT-AB), 3.55 – 3.35 (m, 2H, H5’-AB), 2.18 (s, 1.77H, CH$_3$ Ac-A), 2.13 (s, 1.23H, CH$_3$ Ac-B), 1.95 (bs, 1H, OH-AB). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 170.5 (CO Ac-A), 170.2 (CO Ac-B), 164.7 (CO Bz-AB), 158.7 (Cq. Arom.-AB), 153.0, 152.6 (C2-AB), 151.7 (C4-B), 151.5 (C4-A), 149.8, 149.7 (C6-AB), 144.4, 144.3 (Cq. Arom.-AB), 141.7 (C8-B), 141.5 (C8-A), 135.6, 135.6, 135.5, 135.4, 133.6 (Cq. Arom.-AB), 133.0, 132.9, 130.2, 130.1, 130.1, 129.0, 129.0, 128.3, 128.1, 128.0, 127.1 (arom.-AB), 123.4 (C5-B), 123.2 (C5-A), 113.3 (arom.-AB), 89.6 (C1’-A), 87.0, 86.9 (Cq. DMT-AB), 86.4 (C1’-B), 84.0 (C4’-B), 83.9 (C4’-A), 76.0 (C2’-B), 74.5 (C2’-A), 74.1 (C3’-A), 70.5 (C3’-B), 63.4, 63.1 (C5’-AB), 55.4 (CH$_3$ DMT-AB), 21.0 (CH$_3$ Ac-A), 20.8 (CH$_3$ Ac-B). **HRMS** (ESI) [M+H]$^+$ calculated for C$_{40}$H$_{38}$N$_5$O$_8$ 716.27149; found 716.27105.

Data for **11**: **^{1}H NMR (400 MHz, CDCl$_3$)** δ 9.21 (s, 1H, NH), 8.75 (s, 1H, H2), 8.19 (s, 1H, H8), 8.02 (d, *J* = 7.2 Hz, 2H, arom.), 7.61 – 7.57 (m, 1H, arom.), 7.53 – 7.48 (m, 2H, arom.), 7.45 – 7.42 (m, 2H, arom.), 7.34 – 7.20 (m, 7H, arom.), 6.83 – 6.80 (m, 4H, DMT arom.), 6.39 (d, *J* = 6.8 Hz, 1H, H1’), 6.16 (dd, *J* = 6.9, 5.3 Hz, 1H, H2’), 5.72 (dd, *J* = 5.3, 2.8 Hz, 1H, H3’), 4.38 (q, *J* = 3.0 Hz, 1H, H4’), 3.78 – 3.77 (m, 6H, CH$_3$ DMT), 3.53 (AB, *J* = 10.7, 3.1 Hz, 1H, H5’), 3.47 (AB, *J* = 10.7, 3.4 Hz, 1H, H5’), 2.15 (s, 3H, CH$_3$ Ac), 2.06 (s, 3H, CH$_3$ Ac). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 169.8, 169.5 (CO Ac), 164.8 (CO Bz), 158.7 (Cq. Arom.), 153.0 (C2), 152.2 (C4), 149.8 (C6), 144.1 (Cq. Arom.), 141.2 (C8), 135.3, 135.3, 133.6 (Cq. Arom.), 132.9, 130.2, 128.9, 128.3, 128.1, 128.0, 127.2 (arom.), 123.3 (C5), 113.4 (arom.), 87.2 (Cq. DMT), 84.9 (C1’), 82.8 (C4’), 73.3 (C2’), 71.9 (C3’), 63.1 (C5’), 55.3 (CH$_3$ DMT), 20.8, 20.5 (CH$_3$ Ac). **HRMS** (ESI) [M+H]$^+$ calculated for C$_{42}$H$_{40}$N$_5$O$_9$ 758.28205; found 758.28200.

N^6-Benzoyl-2’-*O*-hydroquinone-*O*,*O*’-diacetylhemiester-3’-*O*-acetyl-5’-*O*-dimethoxytrityl-β-D-adenosine (12a) / *N*^{6}-Benzoyl-2’-*O*-acetyl-3’-*O*-hydroquinone-*O*,*O*’-diacetylhemiester-5’-*O*-dimethoxytrityl-β-D-adenosine (12b)



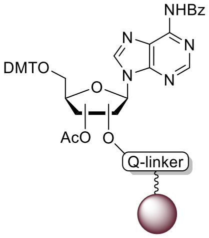
12a



12b

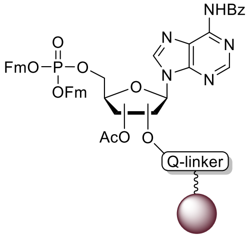
A regiosiomeric mixture of **10a** and **10b** (2.85 g, 3.98 mmol, 10a:10b ratio; 0.5:0.5) was dissolved in pyridine (21 mL). DMAP (49 mg, 0.40 mmol, 0.1 eq), EDC·HCl (0.92 g, 4.78 mmol, 1.2 eq), Et_3N (0.4 mL, 2.91 mmol, 0.73 eq) and Q-linker (1.08 g, 4.78 mmol, 1.2 eq) were added and the reaction mixture was stirred at room temperature overnight. Then, the reaction mixture was concentrated under reduced pressure, diluted with $CHCl_3$ and the organic phase was washed with H_2O (1x). The aqueous phase was extracted with $CHCl_3$ (1x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by automated silica gel column chromatography using high-quality IRR silica gel (40-63 μm) (0% → 10% MeOH in DCM) afforded compounds **12a** and **12b** as a white foam and as a regioisomeric mixture (1.68 g, 1.82 mmol, **12a**:**12b** ratio; 0.5:0.5, with an overall yield of 46%). Data for regioisomeric mixture: **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.68 (s, 0.5H, H2-B), 8.67 – 8.58 (m, 1.5H, NH-AB, H2-A), 8.23 (s, 0.5H, H8-B), 8.18 (s, 0.5H, H8-A), 8.06 – 8.02 (m, 2H, arom.-AB), 7.83 (tt, *J* = 7.8, 1.7 Hz, 0.5H, arom.-A/B), 7.58 – 7.53 (m, 1H, arom.-AB), 7.48 – 7.40 (m, 5H, arom.-AB), 7.32 – 7.20 (m, 5.5H, arom.-AB), 6.87 – 6.79 (m, 6H, arom.-AB), 6.74 – 6.67 (m, 2H, arom.-AB), 6.33 (d, *J* = 7.0 Hz, 0.5H, H1'-A), 6.27 (d, *J* = 6.9 Hz, 0.5H, H1'-B), 6.26 – 6.19 (m, 1H, H2'-AB), 5.82 (dd, *J* = 5.0, 2.6 Hz, 0.5H, H3'-B), 5.71 (dd, *J* = 5.2, 2.5 Hz, 0.5H, H3'-A), 4.66 (d, *J* = 1.5 Hz, 1H, CH_2 Q-AB), 4.59 – 4.52 (m, 3H, CH_2 Q-AB), 4.39 – 4.36 (m, 1H, H4'-AB), 3.77 – 3.74 (m, 6H, CH_3 DMT-AB), 3.54 (AB, *J* = 10.8, 2.6 Hz, 1H, H5'-AB), 3.47 (AB, *J* = 10.8, 2.6 Hz, 1H, H5'-AB), 2.11 (s, 1.5H, CH_3 Ac-A), 1.96 (s, 1.5H, CH_3 Ac-B). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 172.5, 172.4 (CO Q carboxylic acid-AB), 169.9 (CO Ac-A), 169.5 (CO Ac-B), 168.3 (CO Q ester-B), 168.1 (CO Q ester-A), 165.5, 165.3 (CO Bz-AB), 158.7 (Cq. Arom.-AB), 153.1 (C2-B), 152.9 (C2-A), 152.5 (Cq. Arom.-AB), 152.3 (C4-B), 152.1 (C4-A), 149.9 (C6-AB), 147.3 (arom.-AB), 144.1, 144.0 (Cq. Arom-AB), 141.7 (C8-B), 141.5 (C8-A), 138.5 (arom.-AB), 135.3, 135.2, 135.2, 135.2, 133.2 (Cq. Arom.-AB), 132.9, 130.2, 130.0, 129.2, 128.8, 128.8, 128.3, 128.3, 128.2, 128.1, 127.9, 127.9, 127.2, 124.7 (arom.-AB), 123.5 (C5-B), 123.4 (C5-A), 116.0, 115.9, 115.7, 115.6, 113.4, 113.2, 113.0 (arom.-AB), 87.3, 87.2 (Cq. DMT-AB), 85.1 (C1'-B), 84.9 (C1'-A), 83.0, 82.6 (C4'-AB), 73.8, 72.9 (C2'-AB), 72.7 (C3'-B), 71.8 (C3'-A), 66.0, 65.9, 65.8 (CH_2 Q-AB), 62.9 (C5'-AB), 55.3 (CH_3 DMT-AB), 20.7 (CH_3 Ac-A), 20.4 (CH_3 Ac-B). **HRMS** (ESI) $[M+H]^+$ calculated for $C_{50}H_{46}N_5O_{13}$ 924.30866; found 924.30792.

N^6-Benzoyl-2'-*O*-acetyl-3'-Q-CPG-5'-*O*-dimethoxytrityl-**β**-D-adenosine / *N^6*-Benzoyl-2'-Q-CPG-3'-*O*-acetyl-5'-*O*-dimethoxytrityl-**β**-D-adenosine (13)



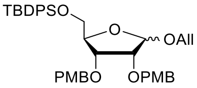
A regiosiomeric mixture of adenosine derivative **12a** and **12b** (0.38 g, 0.41 mmol, 2.0 eq) was dissolved in DMF (2 mL). PyAOP (0.21 g, 0.41 mmol, 2.0 eq) and DiPEA (142 μL, 0.82 mmol, 4.0 eq) were added and the reaction mixture was stirred at room temperature for 10 minutes. Then, the reaction mixture was added to a 20 mL reaction syringe with filter frit containing aminopropyl-CPG solid support (loading 68 μmol/g, 3.00 g, 0.20 mmol, 1.0 eq) in DMF (5.5 mL) and the resulting mixture was shaken at room temperature overnight. The solid support was drained and rinsed with DMF (5x) followed by ACN (5x). Then, capping mix (1:2:7 v/v/v, Ac_2O:pyridine:ACN, 10 mL) was added and the mixture was shaken for 1 hour to cap the remaining unmodified amine groups. The solid support was drained, rinsed with ACN (1x) and the cap treatment was repeated (1x). Then, the solid support was rinsed with ACN (2x) followed by DMF (2x) and DCM (5x). The CPG solid support was dried under N_2 pressure and the loading was determined by spectrophotometric trityl analysis at 503 nm. The loading for immobilized adenosine **13** was 36.7 μmol/g (54%).

N^6-Benzoyl-2'-*O*-acetyl-3'-Q-CPG-5'-*O*-(di-fluorenyl)-phosphoryl-β-D-adenosine / N^6-Benzoyl-2'-Q-CPG-3'-*O*-acetyl-5'-*O*-(di-fluorenyl)-phosphoryl-β-D-adenosine (8)



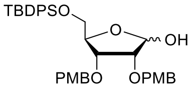
Functionalized CPG solid support **13** (loading 36.7 μmol/g, 1.09 g, ~40 μmol, 1.0 eq) was added to a 20 mL reaction syringe with filter frit. TCA (3% m/v in DCM) was added repeatedly until no yellow color was observed. Then, the solid support was rinsed with DCM (3x) followed by ACN (5x) and dried under N_2 pressure. ETT (0.25 M in ACN, 2.4 mL, 0.60 mmol, 15.0 eq) and bis(9H-fluoren-9-methyl)-diisopropylaminophosphite **14**[37](0.1 M in ACN, 1.2 mL, 0.12 mmol, 3.0 eq) were added and the resulting mixture was shaken at room temperature for 10 minutes. Then, the solid support was drained and the coupling was repeated (2x). Next, the solid support was rinsed with ACN (3x). CSO (0.5 M in ACN, 4.0 mL, 2.00 mmol, 50.0 eq.) was added and the mixture was shaken for 5 minutes. The solid support was drained and the oxidation was repeated (1x). Then, the solid support was rinsed with ACN (5x) and dried under N_2 pressure to afford immobilized phosphotriester **8**. For analytical purposes, a small portion of immobilized phosphotriester **8** (100 mg, ~3.7 μmol) was added to a 5 mL reaction syringe with filter frit. DBU (10% v/v in ACN, 1 mL, 0.67 mmol, 181 eq.) was added and the mixture was left to stand for 5 minutes. The solid support was drained and the DBU treatment was repeated (3x). Then, the solid support was rinsed with ACN (5x). NH_4OH (35%, 3 mL) was added and the reaction mixture was shaken at room temperature overnight to cleave the product from the solid support and remove all other protecting groups. Then, the filtrate was collected, concentrated under reduced pressure and used for ^{31}P-NMR spectroscopy. **^{31}P NMR (162 MHz, D_2O)** δ 4.53 (phosphate).

1-*O*-Allyl-2,3-di-*O*-*p*-methoxybenzyl-5-*O*-tert-butyldiphenylsilyl-α, β-D-ribofuranose (16)



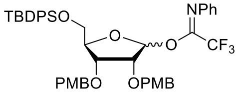
Diol **15**[22] (2.14 g, 5.00 mmol) was co-evaporated with toluene (1x) and dissolved in a mixture of dry DMF/THF (7/1, 50 mL). PMB-Br (2.2 mL, 15.00 mmol, 3.0 eq) and sodium hydride (60% dispersion in mineral oil, 0.60 g, 15.00 mmol, 3.0 eq.) were slowly added at 0 °C and the reaction mixture was stirred at room temperature overnight. Then, the reaction mixture was quenched with H_2O, diluted with Et_2O and the organic phase was washed with H_2O (2x) and brine (1x). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (10% → 30% Et_2O in pentane) afforded ether **16** as a yellow oil and as an anomeric mixture (2.96 g, 4.42 mmol, α : β ratio; 0.17:0.83, with an overall yield of 89%). *<u>Note</u>: The α and β anomers were partially separated by silica gel column chromatography purification for clean NMR assignment.* Data for α anomer: **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.62 – 7.53 (m, 4H, arom.), 7.43 – 7.32 (m, 6H, arom.), 7.30 – 7.28 (m, 2H, arom.), 7.25 – 7.21 (m, 2H, arom.), 6.90 – 6.86 (m, 2H, arom.), 6.83 – 6.79 (m, 2H, arom.), 5.98 (ddt, *J* = 17.1, 11.1, 6.5, 4.8 Hz, 1H, $CH_2\mathit{CH}CH_2$), 5.37 – 5.17 (m, 2H, $CH_2CH\mathit{CH_2}$), 5.03 (d, *J* = 4.3 Hz, 1H, H1), 4.61 – 4.58 (m, 3H, CH_2 PMB), 4.49 (d, *J* = 12.3 Hz, 1H, CH_2 PMB), 4.33 – 4.25 (m, 1H, $O\mathit{CH_2}CH$), 4.17 – 4.10 (m, 2H, H4, $O\mathit{CH_2}CH$), 3.95 (dd, *J* = 6.5, 3.0 Hz, 1H, H3), 3.80 – 3.78 (m, 4H, H2, CH_3 PMB), 3.78 (s, 3H, CH_3 PMB), 3.61 (AB, *J* = 11.1, 3.6 Hz, 1H, H5), 3.51 (AB, *J* = 11.1, 3.3 Hz, 1H, H5), 0.95 (s, 9H, CH_3 TBDPS). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 159.4, 159.2 (Cq. Arom.), 135.8 (arom.), 135.0 (CH_2CHCH_2), 133.3, 130.6, 130.2 (Cq. Arom.), 129.9, 129.7, 127.8 (arom.), 117.3 (CH_2CHCH_2), 113.9, 113.8 (arom.), 100.3 (C1), 83.5 (C4), 77.8 (C2), 74.9 (C3), 72.2, 72.0 (CH_2 PMB), 68.7 (OCH_2CH), 64.3 (C5), 55.4, 55.4 (CH_3 PMB), 26.9 (CH_3 TBDPS), 19.3 (Cq. TBDPS). Data for β anomer: **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.70 – 7.64 (m, 4H, arom.), 7.44 – 7.32 (m, 6H, arom.), 7.30 – 7.25 (m, 2H, arom.), 7.19 (d, *J* = 8.6 Hz, 2H, arom.), 6.86 (d, *J* = 8.6 Hz, 2H, arom.), 6.81 (d, *J* = 8.6 Hz, 2H, arom.), 5.81 (ddt, *J* = 16.7, 10.9, 5.7 Hz, 1H, $CH_2\mathit{CH}CH_2$), 5.23 – 5.10 (m, 2H, $CH_2CH\mathit{CH_2}$), 5.05 (s, 1H, H1), 4.60 (d, *J* = 11.7 Hz, 1H, CH_2 PMB), 4.54 (d, *J* = 11.7 Hz, 1H, CH_2 PMB), 4.46 (d, *J* = 11.4 Hz, 1H, CH_2 PMB), 4.38 (d, *J* = 11.4 Hz, 1H, CH_2 PMB), 4.27 – 4.21 (m, 1H, H4), 4.19 – 4.10 (m, 2H, H3, $O\mathit{CH_2}CH$), 3.93 (dd, *J* = 12.9, 6.2 Hz, 1H, $O\mathit{CH_2}CH$), 3.87 (d, *J* = 4.8 Hz, 1H, H2), 3.82 (d, *J* = 3.9 Hz, 1H, H5), 3.80 (s, 3H, CH_3 PMB), 3.78 (s, 3H, CH_3 PMB), 3.69 (AB, *J* = 11.1, 4.6 Hz, 1H, H5), 1.03 (s, 9H, CH_3 TBDPS). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 159.5, 159.4 (Cq. Arom.), 135.8 (arom.), 134.3 (CH_2CHCH_2), 133.6, 130.2, 130.2 (Cq. Arom.), 129.8, 129.5, 127.8 (arom.), 117.3 (CH_2CHCH_2), 113.9, 113.9 (arom.), 104.6 (C1), 82.2 (C4), 79.8 (C2), 77.6 (C3), 72.1, 72.1 (CH_2 PMB), 68.6 (OCH_2CH), 64.6 (C5), 55.4, 55.4 (CH_3 PMB), 27.0 (CH_3 TBDPS), 19.4 (Cq. TBDPS). Spectral data were in accordance with those reported in the literature. [22]

2,3-Di-*O*-*p*-methoxybenzyl-5-*O*-tert-butyldiphenylsilyl-**α , β**-D-ribofuranose (17)



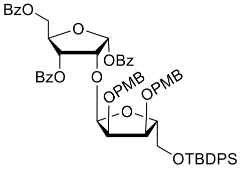
$[Ir(COD)(PmePh_2)_2]PF_6$ (93 mg, 0.11 mmol, 0.01 eq.) was added to a flame-dried flask and dissolved in dry THF (11 mL). The resulting solution was purged with Ar, followed with H_2 until the solution became clear and then again with Ar. Allyl riboside **16** (7.37 g, 11.0 mmol, 1.0 eq) was co-evaporated with toluene (3x), dissolved in dry THF (55 mL) and slowly added to the flask containing the iridium catalyst. The resulting mixture was stirred at room temperature for 2 days. After complete conversion into the vinyl ether, sat. aq. $NaHCO_3$ (66 mL) and I_2 (3.35 g, 13.2 mmol, 1.2 eq) were added and the reaction mixture was stirred for 30 minutes. Then, the reaction was quenched with sat. aq. $Na_2S_2O_3$ and the aqueous phase was extracted with EtOAc (1x). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (20% → 50% Et_2O in pentane) afforded hemiacetal **17** as a yellow oil and as an anomeric mixture (5.95 g, 9.46 mmol, α **:** β ratio; 0.74:0.26, with an overall yield of 86%). Data for anomeric mixture: **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.64 – 7.58 (m, 4H, arom.), 7.44 – 7.35 (m, 6H, arom.), 7.32 – 7.17 (m, 4H, arom.), 6.89 – 6.82 (m, 4H, arom.), 5.30 – 5.25 (m, 1H, H1-α, H1-β), 4.66 – 4.45 (m, 4H, CH_2 PMB), 4.29 – 4.25 (m, 1.26H, H4-α, H3-β, H4-β), 4.17 (d, *J* = 11.3 Hz, 0.74H, OH-α), 4.05 (dd, *J* = 4.9, 1.7 Hz, 0.74H, H3-α), 3.98 – 3.95 (m, 0.74H, H2-α), 3.86 – 3.83 (m, 0.52H, H2-β, H5-β), 3.80 – 3.78 (m, 6.26H, CH_3 PMB, H5-β), 3.62 – 3.58 (m, 1.48H, H5-α), 3.11 (d, *J* = 6.5 Hz, 0.26H, OH-β), 1.01 (s, 2.3H, CH_3 TBDPS-β), 0.99 (s, 6.7H, CH_3 TBDPS-α). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 159.5, 159.5 (Cq. Arom.), 135.8, 135.7, 135.7, 135.6 (arom.), 133.2, 133.0, 132.7 (Cq. Arom.), 130.1, 130.0, 130.0, 129.9, 129.9 (arom.), 129.8, 129.7 (Cq. Arom.), 129.7, 129.6, 129.6, 128.0, 127.9, 127.9, 114.0, 114.0, 113.9 (arom.), 100.2 (C1-β), 96.4 (C1-α), 82.6 (C4-α), 82.3 (C4-β), 80.2 (C2-β), 77.8 (C3-β), 77.5 (C2-α), 76.1 (C3-α), 72.6, 72.1, 72.1, 72.0 (CH_2 PMB), 64.1 (C5-β), 63.3 (C5-α), 55.4 (CH_3 PMB), 26.9 (CH_3 TBDPS), 19.3 (Cq. TBDPS). Spectral data were in accordance with those reported in the literature.[22]

1-*O*-((*N*-Phenyl)-2,2,2-trifluoroacetimido)-2,3-di-*O*-*p*-methoxybenzyl-5-*O*-tert-butyldiphenylsilyl-**α , β**-D-ribofuranose (18)



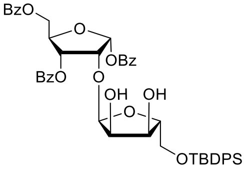
Hemiacetal **17** (5.47 g, 8.71 mmol) was dissolved in acetone (44 mL). Cs_2CO_3 (4.26 g, 13.07 mmol, 1.5 eq) and PTFAI-Cl (1.55 mL, 9.58 mmol, 1.1 eq) were added and the reaction mixture was stirred at room temperature for 3 hours. Then, the reaction mixture was filtered over Celite and concentrated under reduced pressure. Purification by silica gel column chromatography neutralized with 1% Et_3N (5% → 20% Et_2O in pentane) afforded imidate **18** as a yellow oil and as an anomeric mixture (6.43 g, 8.00 mmol, α **:** β ratio; 0.45:0.55, with an overall yield of 92%). Data for anomeric mixture: **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.71 – 7.64 (m, 2H, arom.), 7.59 – 7.55 (m, 2H, arom.), 7.42 – 7.34 (m, 7H, arom.), 7.28 – 7.22 (m, 5H, arom.), 7.11 – 7.06 (m, 1H, arom.), 6.86 – 6.80 (m, 6H, arom.), 6.43 (bs, 0.45H, H1-α), 6.27 (bs, 0.55H, H1-β), 4.63 (d, *J* = 13.2 Hz, 2H, CH_2 PMB), 4.56 – 4.38 (m, 2H, CH_2 PMB), 4.37 – 4.29 (m, 1H, H4-α, H4-β), 4.26 – 4.16 (m, 0.55H, H3-β), 4.13 – 4.04 (m, 1.45H, H2-α, H2-β, H3-α), 3.89 (d, *J* = 9.2 Hz, 0.55H, H5-β), 3.79 – 3.77 (m, 6.55H, CH_3 PMB, H5-β), 3.67 (d, *J* = 10.6 Hz, 0.45H, H5-α), 3.56 (d, *J* = 10.3 Hz, 0.45H, H5-α), 1.04 (s, 4.95H, CH_3 TBDPS-β), 0.96 (s, 4.05H, CH_3 TBDPS-α). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 159.6, 159.5, 159.3, 144.0 (Cq. Arom.), 135.8, 135.7, 135.7, 135.7 (arom.), 133.5, 133.2, 133.1, 132.8, 130.5 (Cq. Arom.), 130.0, 130.0, 130.0, 129.8, 129.8 (arom.), 129.8 (Cq. Arom.), 129.6, 129.5, 129.4, 128.8, 128.8, 127.9, 127.9, 127.8, 127.8, 126.5, 124.3, 120.6, 119.7, 114.0, 114.0, 113.9, 113.8 (arom.), 102.9 (C1-β), 85.8 (C4-α), 83.5 (C4-β), 78.8 (C2-α), 78.5 (C2-β), 76.1 (C3-β), 75.4 (C3-α), 73.0, 72.4, 72.3, 72.0 (CH_2 PMB), 63.8 (C5-α), 63.4 (C5-β), 55.4, 55.3 (CH_3 PMB), 26.9, 26.8 (CH_3 TBDPS), 19.3, 19.3 (Cq. TBDPS). Spectral data were in accordance with those reported in the literature.[22]

α-1,3,5-Tri-*O*-benzoyl-2',3'-di-*O*-*p*-methoxybenzyl-5'-*O*-tert-butyldiphenylsilyl-parobiose (20)



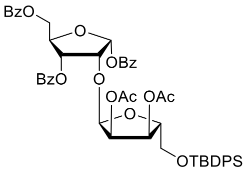
1,3,5-Tri-*O*-benzoyl-α-D-ribofuranose **19** (0.97 g, 2.10 mmol) and imidate **18** (1.84 g, 2.30 mmol, 1.1 eq) were co-evaporated with toluene (1x), 1,4-dioxane (2x) and DCE (1x) and dissolved in dry DCM (42 mL). Freshly activated 3Å molecular sieves were added and the resulting mixture was stirred at room temperature for 1 hour. Next, the mixture was cooled to -78 °C, TMSOTf (8.3 µL, 46 µmol, 0.02 eq) was added and the reaction mixture was stirred at the same temperature for 10 minutes. The reaction was carefully quenched with Et$_3$N (3.2 mL) and concentrated under reduced pressure. Purification by silica gel column chromatography (20% → 50% Et$_2$O in pentane) afforded disaccharide **20** as a white foam (1.89 g, 1.76 mmol, 77%). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 8.15 (ddt, *J* = 7.8, 2.6, 1.2 Hz, 4H, arom.), 8.06 – 8.03 (m, 2H, arom.), 7.58 – 7.48 (m, 7H, arom.), 7.40 – 7.28 (m, 10H, arom.), 7.24 – 7.20 (m, 2H, arom.), 7.04 – 6.99 (m, 4H, arom.), 6.80 (d, *J* = 4.2 Hz, 1H, H1), 6.68 – 6.62 (m, 4H, arom.), 5.64 (dd, *J* = 6.3, 1.9 Hz, 1H, H3), 5.26 (d, *J* = 4.2 Hz, 1H, H1'), 4.76 – 4.73 (m, 1H, H4), 4.71 (dd, *J* = 6.4, 4.3 Hz, 1H, H2), 4.65 (AB, *J* = 12.0, 3.4 Hz, 1H, H5), 4.58 (AB, *J* = 12.0, 4.3 Hz, 1H, H5), 4.50 (d, *J* = 11.7 Hz, 1H, CH$_2$ PMB), 4.36 (d, *J* = 11.7 Hz, 2H, CH$_2$ PMB), 4.27 (d, *J* = 11.7 Hz, 1H, CH$_2$ PMB), 4.12 (q, *J* = 3.2 Hz, 1H, H4'), 3.97 (dd, *J* = 6.2, 3.8 Hz, 1H, H3'), 3.90 (dd, *J* = 6.2, 4.2 Hz, 1H, H2'), 3.74 (s, 3H, CH$_3$ PMB), 3.72 (s, 3H, CH$_3$ PMB), 3.60 (AB, *J* = 11.3, 3.4 Hz, 1H, H5'), 3.50 (AB, *J* = 11.4, 2.8 Hz, 1H, H5'), 0.93 (s, 9H, CH$_3$ TBDPS). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 166.5, 166.2, 165.9 (CO Bz), 159.1, 159.0 (Cq. Arom.), 135.7, 135.6, 133.3, 133.3, 133.2 (arom.), 133.1, 130.6 (Cq. Arom.), 130.3 (arom.), 130.3, 130.2, 130.0 (Cq. Arom.), 129.9, 129.9, 129.8 (arom.), 129.8 (Cq. Arom.), 129.3, 129.1, 128.6, 128.6, 128.4, 128.4, 127.8, 127.8, 113.7 (arom.), 102.0 (C1'), 95.3 (C1), 83.6 (C4'), 83.4 (C4), 78.4 (C2'), 75.5 (C3'), 75.2 (C2), 72.4 (C3), 72.1, 72.0 (CH$_2$ PMB), 64.5 (C5), 63.7 (C5'), 55.3, 55.3 (CH$_3$ PMB), 26.9 (CH$_3$ TBDPS), 19.3 (Cq. TBDPS).

α -1,3,5-Tri-*O*-benzoyl-5'-*O*-tert-butyldiphenylsilyl-parobiose (21)



Compound **20** (2.17 g, 2.33 mmol) was dissolved in dry DCM (23 mL). TFA (1.2 mL, 15.13 mmol, 6.5 eq) was added and the reaction mixture was stirred at room temperature for 1 hour. Then, the reaction was carefully quenched with sat. aq. NaHCO$_3$ and the aqueous phase was extracted with DCM (3x). The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (40% Et$_2$O in pentane) afforded diol **21** as a white foam (1.53 g, 1.83 mmol, 79%). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 8.15 – 8.01 (m, 6H, arom.), 7.66 – 7.52 (m, 7H, arom.), 7.48 – 7.29 (m, 12H, arom.), 6.78 (d, *J* = 4.2 Hz, 1H, H1), 5.73 (dd, *J* = 6.3, 1.9 Hz, 1H, H3), 5.20 (d, *J* = 4.2 Hz, 1H, H1'), 4.89 – 4.85 (m, 1H, H4), 4.77 (dd, *J* = 6.3, 4.3 Hz, 1H, H2), 4.67 (AB, J = 12.1, 3.5 Hz, 1H, H5), 4.60 (AB, J = 12.2, 3.9 Hz, 1H, H5), 4.23 – 4.10 (m, 1H, H2'), 4.10 – 4.00 (m, 2H, H3', H4'), 3.70 – 3.61 (m, 2H, H5'), 2.66 (d, *J* = 10.3 Hz, 1H, 2'-OH), 2.53 (d, *J* = 9.6 Hz, 1H, 3'-OH), 1.00 (s, 9H, CH$_3$ TBDPS). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 166.9, 166.1, 165.9 (CO Bz), 135.7, 135.6, 133.9, 133.6, 133.5 (arom.), 133.0, 132.9 (Cq. Arom.), 130.1, 130.0, 129.9, 129.8 (arom.), 129.5, 129.0 (Cq. Arom.), 128.7, 128.7, 128.6, 127.9, 127.9 (arom.), 102.5 (C1'), 95.2 (C1), 87.0 (C4'), 82.8 (C4), 75.2 (C2), 72.7 (C2'), 72.5 (C3), 71.1 (C3'), 64.3 (C5), 63.9 (C5'), 26.9 (CH$_3$ TBDPS), 19.3 (Cq. TBDPS).

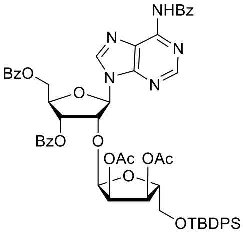
α -1,3,5-Tri-*O*-benzoyl-2',3'-di-*O*-acetyl-5'-*O*-tert-butyldiphenylsilyl-parobiose (22)



Diol **21** (2.39 g, 1.99 mmol) was co-evaporated with toluene (2x) and dissolved in dry pyridine (13 mL). DMAP (24 mg, 0.20 mmol, 0.1 eq) and Ac$_2$O (1.9 mL, 19.91 mmol, 10.0 eq) were added and the reaction mixture was stirred at room temperature for 3.5 hours. Then, the reaction mixture was concentrated under reduced pressure and co-evaporated with toluene (3x). The resulting residue was diluted with EtOAc and the organic phase was washed with sat. aq. NaHCO$_3$ (2x) and brine (1x). The organic layer was dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (40% Et$_2$O in pentane) afforded title compound **22** as a white foam (1.82 g, 1.99 mmol, quantitative). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 8.16 (ddt, *J* = 9.4, 8.1, 1.1 Hz, 4H, arom.), 8.09 – 8.05 (m, 2H, arom.), 7.67 – 7.50 (m, 7H, arom.), 7.48

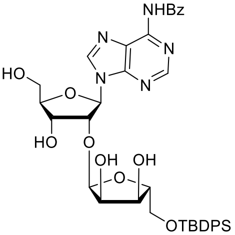
– 7.29 (m, 12H, arom.), 6.82 (d, *J* = 4.2 Hz, 1H, H1), 5.75 (dd, *J* = 6.4, 1.7 Hz, 1H, H3), 5.46 (d, *J* = 4.6 Hz, 1H, H1'), 5.38 (dd, *J* = 7.0, 2.5 Hz, 1H, H3'), 4.99 (dd, *J* = 7.0, 4.5 Hz, 1H, H2'), 4.82 (td, *J* = 3.7, 1.6 Hz, 1H, H4), 4.73 – 4.57 (m, 3H, H2, H5), 4.10 (q, *J* = 2.8 Hz, 1H, H4'), 3.74 – 3.62 (m, 2H, H5'), 1.62 (s, 3H, CH_3 Ac), 1.45 (s, 3H, CH_3 Ac), 1.01 (s, 9H, CH_3 TBDPS). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.3, 169.7 (CO Ac), 166.2, 165.9, 165.1 (CO Bz), 135.7, 135.7, 133.5, 133.5 (arom.), 133.0, 132.9 (Cq. Arom), 130.2, 130.1 (arom.), 130.0 (Cq. Arom.), 129.9, 129.9, 129.8 (arom.), 129.6 (Cq. Arom.), 128.7, 128.5, 128.4, 127.9, 127.9 (arom.), 101.1 (C1'), 95.2 (C1), 83.5 (C4), 83.3 (C4'), 75.9 (C2), 71.7 (C3), 71.5 (C2'), 70.0 (C3'), 64.4 (C5), 63.5 (C5'), 26.8 (CH_3 TBDPS), 19.8 (CH_3 Ac), 19.3 (Cq. TBDPS).

N^6-Benzoyl-9-(3',5'-di-*O*-benzoyl-2'',3''-*O*-di-acetyl-5''-*O*-tert-butyldiphenylsilyl-β-parobiosyl)adenine (24)



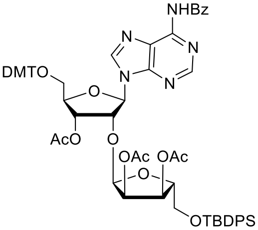
Protected disaccharide **22** (3.79 g, 4.13 mmol) and *N*^{6}-benzoyladenine **23** (1.98 g, 8.26 mmol, 2.0 eq) were co-evaporated with 1,4-dioxane (2x) and ACN (1x) and dissolved in dry ACN (69 mL). BSTFA (15.4 mL, 57.82 mmol, 14.0 eq) was added and the resulting mixture was stirred at room temperature for 30 minutes. Next, $HClO_4$-SiO_2 (20.65 g, 8.26 mmol, 2.0 eq) was added and the reaction mixture was refluxed overnight. Then, the reaction was carefully quenched with sat. aq. $NaHCO_3$ and filtered over Celite. The aqueous phase was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (20% → 25% acetone in pentane) afforded nucleoside **24** as a white foam (2.80 g, 2.71 mmol, 66%). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 9.00 (s, 1H, NH), 8.69 (s, 1H, H2), 8.17 (s, 1H, H8), 8.10 (dd, *J* = 10.6, 7.9 Hz, 4H, arom.), 8.02 (d, *J* = 7.4 Hz, 2H, arom.), 7.61 – 7.51 (m, 9H, arom.), 7.46 – 7.33 (m, 10H, arom.), 6.27 (d, *J* = 5.5 Hz, 1H, H1'), 5.92 (t, *J* = 4.5 Hz, 1H, H3'), 5.51 (t, *J* = 5.5 Hz, 1H, H2'), 5.39 – 5.32 (m, 2H, H1'', H3''), 4.91 – 4.82 (m, 2H, H2'', H5'), 4.73 – 4.65 (m, 2H, H4', H5'), 3.99 (q, *J* = 2.6 Hz, 1H, H4''), 3.60 (AB, *J* = 11.3, 2.4 Hz, 1H, H5''), 3.50 (AB, *J* = 11.3, 3.0 Hz, 1H, H5''), 1.87 (s, 3H, CH_3 Ac), 1.77 (s, 3H, CH_3 Ac), 0.98 (s, 9H, CH_3 TBDPS). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.3, 169.7 (CO Ac), 166.3, 165.4, 164.6 (CO Bz), 153.1 (arom.), 151.8, 149.8 (Cq. Arom.), 135.7, 135.7, 133.8 (arom.), 133.7 (Cq. Arom.), 133.6, 133.0 (arom.), 133.0, 132.8 (Cq. Arom.), 130.0, 130.0, 129.9, 129.9 (arom.), 129.5, 129.4 (Cq. Arom.), 129.1, 128.8, 128.7, 128.0, 127.9, 127.9 (arom.), 123.8 (Cq. Arom.), 101.7 (C1''), 87.8 (C1'), 83.2 (C4''), 81.0 (C4'), 77.9 (C2'), 72.5 (C3'), 71.7 (C2''), 70.1 (C3''), 63.7 (C5'), 63.3 (C5''), 26.8 (CH_3 TBDPS), 20.4, 20.4 (CH_3 Ac), 19.3 (Cq. TBDPS).

N^6-Benzoyl-9-(5''-*O*-tert-butyldiphenylsilyl-β-parobiosyl)adenine (25)



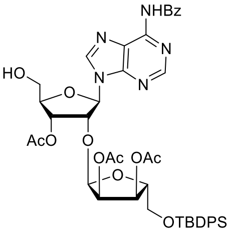
Nucleoside **24** (4.12 g, 3.99 mmol) was dissolved in a mixture of pyridine/EtOH (2/1, 40 mL). Aq. NaOH (1 M, 24 mL) was slowly added at 0 °C and the reaction mixture was stirred at the same temperature for 1 hour. Then, the reaction mixture was quenched with Amberlite-H^+ until pH 6, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (0% → 7.5% MeOH in DCM) afforded tetraol **25** as a white foam (2.73 g, 3.67 mmol, 92%). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 9.53 (s, 1H, NH), 8.73 (s, 1H, H2), 8.27 (s, 1H, H8), 7.98 (d, *J* = 7.4 Hz, 2H, arom.), 7.59 – 7.51 (m, 5H, arom.), 7.43 – 7.33 (m, 8H, arom.), 6.10 (d, *J* = 7.0 Hz, 1H, H1'), 5.06 (d, *J* = 3.2 Hz, 1H, H1''), 4.98 (dd, *J* = 6.8, 4.8 Hz, 1H, H2'), 4.58 (d, *J* = 4.6 Hz, 1H, H3'), 4.31 – 4.23 (m, 4H, H4', H2'', H3'', H4''), 3.93 (d, *J* = 12.1 Hz, 1H, H5'), 3.75 (d, *J* = 12.1 Hz, 1H, H5'), 3.64 (d, *J* = 2.4 Hz, 2H, H5''), 0.97 (s, 9H, CH_3 TBDPS). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.2 (CO Bz), 152.3 (C2), 150.8, 150.2 (Cq. Arom.), 143.6 (C8), 135.7, 135.6 (arom.), 133.4 (Cq. Arom.), 133.1 (arom.), 132.9, 132.7 (Cq. Arom.), 130.0, 128.9, 128.2, 128.0 (arom.), 124.2 (Cq. Arom.), 101.8 (C1''), 89.2 (C1'), 87.9 (C4'), 86.4 (C4''), 79.5 (C2'), 73.4 (C2''/C3''), 72.8 (C3'), 71.9 (C2''/C3''), 64.2 (C5''), 63.1 (C5'), 26.9 (CH_3 TBDPS), 19.3 (Cq. TBDPS).

N^6-Benzoyl-9-(3',2'',3''-tri-*O*-acetyl-5'-*O*-dimethoxytrityl-5''-*O*-tert-butyldiphenylsilyl-β-parobiosyl)adenine (26)



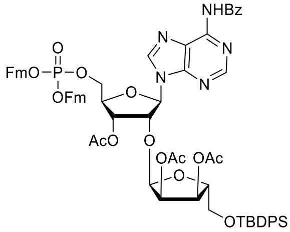
Tetraol **25** (1.77 g, 2.38 mmol) was co-evaporated with pyridine (1x) and dissolved in dry pyridine (12 mL). DMTCl (0.97 g, 2.86 mmol, 1.2 eq) was added and the reaction mixture was stirred at room temperature for 2 hours. After complete conversion into the dimethoxytrityl ether, Ac$_2$O (1.4 mL, 14.28 mmol, 6.0 eq) was added at 0 °C and the reaction mixture was stirred at the same temperature for 5 hours. Then, the reaction was carefully quenched with sat. aq. NaHCO$_3$ and the aqueous phase was extracted with DCM (3x). The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (0% → 30% acetone in pentane) afforded title compound **26** as a white foam (2.52 g, 2.15 mmol, 90% over 2 steps). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 9.14 (s, 1H, NH), 8.76 (s, 1H, H2), 8.16 (s, 1H, H8), 8.04 – 8.02 (m, 2H, arom.), 7.65 – 7.61 (m, 5H, arom.), 7.55 – 7.52 (m, 2H, arom.), 7.42 – 7.30 (m, 14H, arom.), 7.23 – 7.21 (m, 1H, arom.), 6.82 – 6.79 (m, 4H, DMT), 6.27 (d, *J* = 5.9 Hz, 1H, H1'), 5.58 (dd, *J* = 5.0, 3.8 Hz, 1H, H3'), 5.45 (dd, *J* = 7.0, 2.8 Hz, 1H, H3''), 5.37 (d, *J* = 4.6 Hz, 1H, H1''), 5.21 (t, *J* = 5.5 Hz, 1H, H2'), 4.95 (dd, *J* = 7.0, 4.6 Hz, 1H, H2''), 4.34 (q, *J* = 3.4 Hz, 1H, H4'), 4.14 (q, *J* = 2.7 Hz, 1H, H4''), 3.78 – 3.75 (m, 8H, CH$_3$ DMT, H5''), 3.55 (AB, *J* = 10.6, 3.1 Hz, 1H, H5'), 3.47 (AB, *J* = 10.7, 3.6 Hz, 1H, H5'), 2.11 (s, 3H, CH$_3$ Ac), 2.10 (s, 3H, CH$_3$ Ac), 1.85 (s, 3H, CH$_3$ Ac), 1.02 (s, 9H, CH$_3$ TBDPS). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 170.4, 169.8, 169.7 (CO Ac), 164.7 (CO Bz), 158.8 (Cq. Arom.), 153.1 (C2), 151.9, 149.7, 144.4 (Cq. Arom.), 141.4 (C8), 135.7, 135.7 (arom.), 135.4, 133.8 (Cq. Arom.), 132.9 (arom.), 132.7 (Cq. Arom.), 130.2, 130.0, 129.9, 129.0, 128.3, 128.1, 128.0, 127.9, 127.9, 127.3 (arom.), 123.3 (Cq. Arom.), 113.4 (arom.), 101.6 (C1''), 87.1 (Cq. DMT), 86.5 (C1'), 83.2 (C4''), 82.7 (C4'), 78.6 (C2'), 72.4 (C3'), 71.6 (C2''), 70.3 (C3''), 63.5 (C5''), 63.0 (C5'), 55.3 (CH$_3$ DMT), 26.8 (CH$_3$ TBDPS), 21.0, 20.4 (CH$_3$ Ac), 19.3 (Cq. TBDPS).

N^6-Benzoyl-9-(3',2'',3''-tri-*O*-acetyl-5''-*O*-tert-butyldiphenylsilyl-β-parobiosyl)adenine (27)



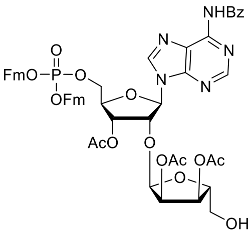
Compound **26** (2.03 g, 1.74 mmol) was dissolved in DCM (17 mL). TFA (270 µL, 3.48 mmol, 2.0 eq) was added and the reaction mixture was stirred at room temperature for 10 minutes. Then, the reaction was carefully quenched with sat. aq. NaHCO$_3$ and the aqueous phase was extracted with DCM (3x). The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (0% → 30% acetone in DCM) afforded alcohol **27** as a white foam (0.94 g, 1.09 mmol, 63%). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 9.10 (s, 1H, NH), 8.82 (s, 1H, H2), 8.11 (s, 1H, H8), 8.06 – 7.98 (m, 2H, arom.), 7.65 – 7.49 (m, 7H, arom.), 7.46 – 7.30 (m, 6H, arom.), 6.11 (dd, *J* = 11.6, 2.5 Hz, 1H, OH), 6.02 (d, *J* = 7.9 Hz, 1H, H1'), 5.68 (d, *J* = 5.4 Hz, 1H, H3'), 5.37 (dd, *J* = 7.0, 2.9 Hz, 1H, H3''), 5.14 (dd, *J* = 7.8, 5.3 Hz, 1H, H2'), 5.09 (d, *J* = 4.8 Hz, 1H, H1''), 4.92 (dd, *J* = 7.0, 4.7 Hz, 1H, H2''), 4.32 (q, *J* = 1.5 Hz, 1H, H4'), 4.04 – 3.97 (m, 2H, H4'', H5'), 3.87 (t, *J* = 11.5 Hz, 1H, H5'), 3.70 (AB, J = 2.6 Hz, 1H, H5''), 3.68 (AB, J = 3.0 Hz, 1H, H5''), 2.15 (s, 3H, CH$_3$ Ac), 2.13 (s, 3H, CH$_3$ Ac), 1.97 (s, 3H, CH$_3$ Ac), 0.99 (s, 9H, CH$_3$ TBDPS). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 170.2, 169.6, 169.5 (CO Ac), 164.5 (CO Bz), 152.4 (C2), 150.6, 150.5 (Cq. Arom.), 143.1 (C8), 135.7 (arom.), 133.5 (Cq. Arom.), 133.1 (arom.), 132.9, 132.7 (Cq. Arom.), 130.0, 129.9, 129.1, 128.0, 127.9, 127.9 (arom.), 124.6 (Cq. Arom.), 101.2 (C1''), 89.8 (C1'), 86.9 (C4'), 83.1 (C4''), 77.6 (C2'), 73.8 (C3'), 71.6 (C2''), 70.2 (C3''), 63.4 (C5''), 62.9 (C5'), 26.8 (CH$_3$ TBDPS), 21.0, 20.9, 20.4 (CH$_3$ Ac), 19.2 (Cq. TBDPS).

N^6-Benzoyl-9-(3',2",3"-tri-*O*-acetyl-5'-*O*-(di-fluorenylphosphoryl)-5"-*O*-tert-butyldiphenylsilyl-**β**-parobiosyl)-adenine (28)



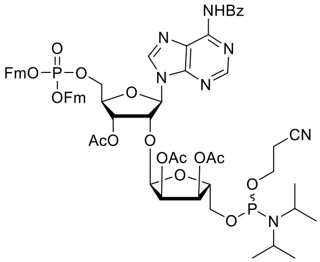
Alcohol **27** (1.07 g, 1.23 mmol) was co-evaporated with ACN (3x). Freshly activated 3Å molecular sieves, DCI (0.25 M in dry ACN, 9.8 mL, 2.46 mmol, 2.0 eq) and difluorenylmethyl *N*,*N*-diisopropylphosphoramidite **14**[37] (0.2 M in dry ACN, 9.2 mL, 1.85 mmol, 1.5 eq) were added and the reaction mixture was stirred at room temperature for 10 minutes. After complete conversion into the phosphite triester indicated by ^{31}P-NMR, *t*BuOOH (5.5 M in decane, 2.2 mL, 12.30 mmol, 10.0 eq) was added at 0 °C and the reaction mixture was stirred at the same temperature for 30 minutes. After complete conversion into the phosphotriester, the reaction was carefully quenched with sat. aq. NaHCO$_3$ and filtered. Then, the resulting mixture was diluted with EtOAc and the organic phase was washed with H$_2$O (1x) and brine (2x). The organic layer was dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (0% → 25% acetone in DCM) afforded phosphotriester **28** as a white foam (1.48 g, 1.13 mmol, 92% over 2 steps). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 8.91 (s, 1H, NH), 8.78 (s, 1H, H2), 8.23 (s, 1H, H8), 8.01 – 7.95 (m, 2H, arom.), 7.71 – 7.15 (m, 29H, arom.), 6.18 (d, *J* = 5.4 Hz, 1H, H1'), 5.44 – 5.38 (m, 2H, H3', H3"), 5.28 (d, *J* = 4.6 Hz, 1H, H1"), 4.98 (t, *J* = 5.4 Hz, 1H, H2'), 4.92 (dd, *J* = 7.0, 4.6 Hz, 1H, H2"), 4.33 – 4.23 (m, 5H, H4', CH$_2$ Fm), 4.15 – 4.05 (m, 5H, H5', H4", CH Fm), 3.73 (AB, *J* = 11.3, 2.7 Hz, 1H, H5"), 3.66 (AB, *J* = 11.3, 3.3 Hz, 1H, H5"), 2.09 (s, 6H, CH$_3$ Ac), 1.86 (s, 3H, CH$_3$ Ac), 1.01 (s, 9H, CH$_3$ TBDPS). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 170.3, 169.7 (CO Ac), 164.4 (CO Bz), 153.1 (C2), 151.6, 149.7, 143.0, 143.0, 142.9, 142.9, 141.5, 141.4 (Cq. Arom.), 141.4 (C8), 135.7, 135.7 (arom.), 133.7 (Cq. Arom.), 133.0 (arom.), 132.9, 132.7 (Cq. Arom.), 130.0, 130.0, 129.1, 128.1, 128.0, 128.0, 127.9, 127.3, 127.2, 125.1, 125.1, 125.1 (arom.), 123.3 (Cq. Arom.), 120.2, 120.1, 120.1 (arom.), 101.5 (C1"), 87.0 (C1'), 83.2 (C4"), 81.2 (d, $^3J_{cp}$ = 8.1 Hz, C4'), 78.2 (C2'), 71.6 (C2"), 71.3 (C3'), 70.3 (C3"), 69.7, 69.6, 69.6 (CH$_2$ Fm), 66.2 (d, $^2J_{cp}$ = 2.0 Hz, C5'), 63.5 (C5"), 48.0, 48.0, 47.9, 47.9 (CH Fm), 26.9 (CH$_3$ TBDPS), 20.9, 20.9, 20.4 (CH$_3$ Ac), 19.3 (Cq. TBDPS). **^{31}P NMR (162 MHz, CDCl$_3$)** δ -1.06 (phosphate). **HRMS** (ESI) [M+H]$^+$ calculated for $C_{72}H_{71}N_5O_{15}PSi$ 1304.44481; found 1304.44407.

N^6-Benzoyl-9-(3',2",3"-tri-*O*-acetyl-5'-*O*-(di-fluorenylphosphoryl)-**β**-parobiosyl)adenine (29)



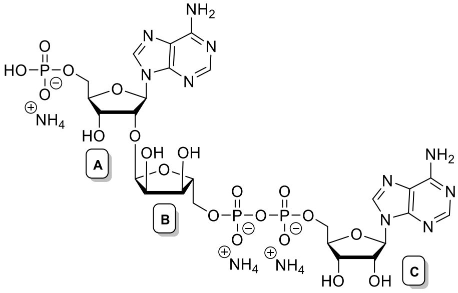
Phosphotriester **28** (0.33 g, 0.25 mmol) was co-evaporated with 1,4-dioxane (6x) and dissolved in dry THF (2.5 mL). HF·pyridine (~70% HF, 2.25 mL, 25.00 mmol, 100.0 eq) was added at 0 °C and the reaction mixture was stirred at room temperature for 2 hours. Then, the reaction was diluted with DCM and carefully quenched with sat. aq. NaHCO$_3$. Next, the aqueous phase was extracted with DCM (1x) and the combined organic layers were washed with H$_2$O (1x) and brine (1x), dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (0% → 2% MeOH in DCM) afforded compound **29** as a clear oil (0.23 g, 0.22 mmol, 88%). **^{1}H NMR (300 MHz, CDCl$_3$)** δ 9.46 (s, 1H, NH), 8.81 (s, 1H, H2), 8.27 (s, 1H, H8), 8.03 – 7.98 (m, 2H, arom.), 7.73 – 7.08 (m, 19H, arom.), 6.24 (d, *J* = 6.8 Hz, 1H, H1'), 5.47 (dd, *J* = 5.2, 2.9 Hz, 1H, H3'), 5.28 (d, *J* = 4.6 Hz, 1H, H1"), 5.14 (dd, *J* = 7.3, 3.6 Hz, 1H, H3"), 5.02 (t, *J* = 6.4 Hz, 1H, H2'), 4.68 (dd, *J* = 7.3, 4.6 Hz, 1H, H2"), 4.35 – 4.23 (m, 5H, H4', CH$_2$ Fm), 4.13 – 3.99 (m, 5H, H5', H4", CH Fm), 3.58 – 3.48 (m, 2H, H5"), 3.41 – 3.27 (m, 1H, 5"-OH), 2.16 (s, 3H, CH$_3$ Ac), 2.09 (s, 3H, CH$_3$ Ac), 1.68 (s, 3H, CH$_3$ Ac). **^{13}C NMR (75 MHz, CDCl$_3$)** δ 170.4, 169.7, 169.6 (CO Ac), 164.8 (CO Bz), 153.3 (C2), 151.9, 149.9, 143.0, 142.9, 141.4, 141.4 (Cq. Arom.), 141.0 (C8), 133.9 (Cq. Arom.), 132.9, 128.9, 128.1, 128.0, 127.3, 127.3, 127.2, 125.1, 125.1, 125.0 (arom.), 122.8 (Cq. Arom.), 120.2, 120.1, 120.1 (arom.), 102.1 (C1"), 85.5 (C1'), 83.0 (C4"), 81.6 (d, $^3J_{cp}$ = 7.5 Hz, C4'), 78.3 (C2'), 71.8 (C3'), 71.3 (C2"), 69.9 (C3"), 69.8, 69.7 (CH$_2$ Fm), 66.5 (d, $^2J_{cp}$ = 6.0 Hz, C5'), 61.8 (C5"), 48.0, 48.0, 47.9, 47.9 (CH Fm), 20.9, 20.9, 20.1 (CH$_3$ Ac). **^{31}P NMR (121 MHz, CDCl$_3$)** δ -1.23 (phosphate). **HRMS** (ESI) [M+H]$^+$ calculated for $C_{56}H_{53}N_5O_{15}P$ 1066.32703; found 1066.32694.

N^6-Benzoyl-9-(3’,2”,3”-tri-*O*-acetyl-5’-*O*-(di-fluorenylphosphoryl)-5”-*O*-(*N*,*N*-diisopropylamino-*O*-cyano-ethyl)phosphoramidite)-**β**-parobiosyl)adenine (7)



Compound **29** (0.74 g, 0.69 mmol) was co-evaporated with 1,4-dioxane (6x) and dissolved in dry DMF (6.9 mL). DiPEA (0.3 mL, 1.73 mmol, 2.5 eq) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (170 µL, 0.76 mmol, 1.1 eq) were added and the reaction mixture was stirred at room temperature for 10 minutes. Then, the reaction mixture was quenched with MeOH (100 µL), diluted with EtOAc and the organic phase was washed with sat. aq. $NaHCO_3$ (1x), H_2O (1x) and brine (2x). The organic layer was dried over Na_2SO_4, filtered, diluted with toluene and concentrated under reduced pressure. Purification by automated silica gel column chromatography using high-quality IRR silica gel (40-63 µm) (0% → 40% acetone in DCM) afforded phosphoramidite **7** as a white foam and as a mixture of two P(III) diastereomers (0.60 g, 0.47 mmol, 69%). Data for diastereomeric mixture: **^{1}H NMR (400 MHz, $CDCl_3$)** δ 9.28 (s, 1H, NH), 8.73 (d, *J* = 3.8 Hz, 1H, H2), 8.25 (d, *J* = 5.1 Hz, 1H, H8), 7.97 (d, *J* = 7.9 Hz, 2H, arom.), 7.71 – 7.63 (m, 4H, arom.), 7.55 (t, *J* = 7.4 Hz, 1H, arom.), 7.50 – 7.41 (m, 6H, arom.), 7.37 – 7.15 (m, 8H, arom.), 6.18 (d, *J* = 5.7 Hz, 1H, H1’), 5.50 – 5.43 (m, 1H, H3’), 5.33 – 5.25 (m, 2H, H1”, H3”), 5.04 (t, *J* = 5.3 Hz, 1H, H2’), 4.83 (dd, *J* = 7.0, 4.7 Hz, 1H, H2”), 4.33 – 4.22 (m, 5H, CH_2 Fm, H4’), 4.18 – 4.15 (m, 1H, H4”), 4.13 – 4.05 (m, 4H, CH Fm, H5’), 3.82 – 3.65 (m, 4H, O$CH_2$$CH_2$CN, H5”), 3.57 – 3.49 (m, 2H, $(CH_3)_2$*CH*N), 2.57 (dt, *J* = 16.8, 6.2 Hz, 2H, CH_2CN), 2.16 (s, 3H, CH_3 Ac), 2.10 (s, 3H, CH_3 Ac), 1.84 (d, *J* = 8.0 Hz, 3H, CH_3 Ac), 1.16 – 1.09 (m, 12H, $(CH_3)_2$CHN). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.0, 170.0, 169.4, 169.3 (CO Ac), 164.5 (CO Bz), 152.6 (C2), 151.5, 151.4, 149.6, 142.7, 142.6, 142.6 (Cq. Arom.), 141.4, 141.3 (C8), 141.2, 141.1, 133.4 (Cq. Arom.), 132.6, 128.6, 127.7, 127.7, 127.0, 124.8, 124.8 (arom.), 123.3 (Cq. Arom.), 119.9, 119.8 (arom.), 117.7, 117.6 (CN), 101.1, 101.1 (C1”), 86.6, 86.4 (C1’), 82.1, 82.1, 82.0, 81.9 (C4”), 81.1, 81.0, 81.0 (C4’), 77.7, 77.6 (C2’), 71.2, 71.1 (C3’), 71.0 (C2”), 70.0, 69.9 (C3”), 69.3, 69.3, 69.3, 69.3 (CH_2 Fm), 66.1, 66.0, 65.9 (C5’), 63.0, 62.9, 62.8 (C5”), 58.3, 58.2, 58.1, 58.0 (O$CH_2$$CH_2$CN), 47.6, 47.6 (CH Fm), 43.0, 42.9, 42.9, 42.8 ($(CH_3)_2$*CH*N), 24.5, 24.4, 24.4, 24.3 ($(CH_3)_2$CHN), 20.6 (CH_3 Ac), 20.2, 20.1, 20.1, 20.1 (CH_2CN), 20.0 (CH_3 Ac). **^{31}P NMR (162 MHz, $CDCl_3$)** δ 149.96, 149.67 (phosphoramidite), -1.12, -1.13 (phosphate). **HRMS** (ESI) [(H-phosphonate)+H]$^+$ calculated for $C_{59}H_{57}N_6O_{17}P_2$ 1183.32499; found 1183.32488.

Phosphoadenosyl-ADP-ribose (5)



Pre-loaded CPG solid support **8** (loading 35.4 µmol/g, 282 mg, ~10 µmol, 1.0 eq) was placed in a Mermade 6 oligonucleotide synthesizer and the complete synthesis was performed under an argon atmosphere. Cycle A was performed (1x).

Cycle A

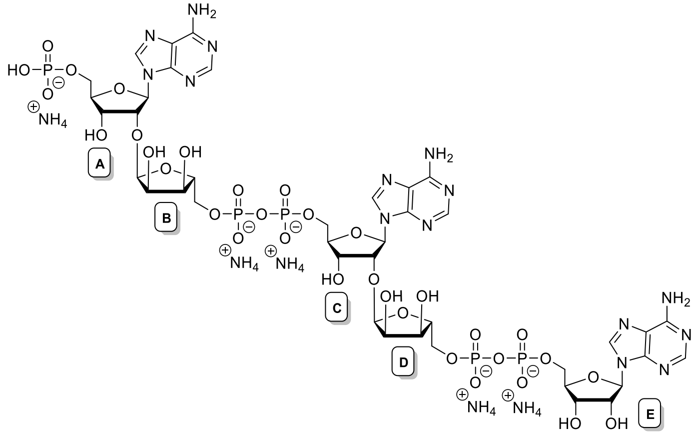
The solid support was rinsed with ACN (5x). DBU (10% v/v in ACN, 2 mL, 1.34 mmol, 133.7 eq.) was added and the mixture was left to stand for 5 minutes. The solid support was drained and the DBU treatment was repeated (3x). Then, the solid support was rinsed with ACN (3x) and phosphoramidite **7** (0.1 M in ACN, 300 µL, 30 µmol, 3.0 eq) and ETT (0.25 M in ACN, 600 µL, 150 µmol, 15.0 eq) were added. The mixture was left to stand for 10 minutes, drained and the coupling was repeated (2x). Next, the solid support was rinsed with ACN (3x).

CSO (0.5 M in ACN, 1 mL, 500 µmol, 50.0 eq.) was added and the mixture was left to stand for 5 minutes. The solid support was drained and the oxidation was repeated (1x).

After Cycle A, the solid support was rinsed with ACN (3x). DBU (10% v/v in ACN, 2 mL, 1.34 mmol, 133.7 eq.) was added and the mixture was left to stand for 5 minutes. The solid support was drained and the DBU treatment was repeated (3x). Then, the solid support was rinsed with ACN (3x). NH_4OH (35%, 10 mL) was added and the reaction mixture was shaken at room temperature overnight to cleave the product from the solid support and remove all the protecting groups. Then, the filtrate was collected, concentrated under reduced pressure and purification by HW-40 gel filtration (0.15 M NH_4OAc in 10% ACN/MilliQ) afforded phosphoadenosyl-ADP-ribose **5** as a white solid (1.30 mg, 1.36 µmol, 13.6%) after repeated lyophilization.

^{1}H NMR (500 MHz, D_2O) δ 8.42 (s, 1H, H2), 8.41 (s, 1H, H2), 8.19 (s, 1H, H8), 8.18 (s, 1H, H8), 6.19 (d, *J* = 4.0 Hz, 1H, H1'-A), 6.01 (d, *J* = 6.0 Hz, 1H, H1'-C), 5.25 (d, *J* = 4.1 Hz, 1H, H1-B), 4.68 (dd, *J* = 6.0, 5.1 Hz, 1H, H2'-C), 4.65 (dd, *J* = 5.3, 4.0 Hz, 1H, H2'-A), 4.55 (t, *J* = 5.3 Hz, 1H, H3'-A), 4.48 (dd, *J* = 5.1, 3.3 Hz, 1H, H3'-C), 4.39 – 4.36 (m, 1H, H4'-A), 4.35 – 4.33 (m, 2H, H4-B, H4'-C), 4.24 – 4.18 (m, 5H, H5'-A, H2-B, H3-B, H5-B), 4.13 – 4.09 (m, 1H, H5'-A), 4.07 – 4.03 (m, 2H, H5'-C). **^{13}C NMR (126 MHz, D_2O)** δ 154.2, 154.1 (C4-A, C4-C), 151.2, 151.0 (C8-A, C8-C), 148.6, 148.3 (C6-A, C6-C), 140.2, 140.1 (C2-A, C2-C), 118.4, 118.3 (C5-A, C5-C), 101.5 (C1-B), 87.1 (C1'-C), 86.6 (C1'-A), 84.2, 84.2, 84.0, 84.0 (C4-B, C4'-C), 83.5 (d, $^3J_{cp}$ = 8.8 Hz, C4'-A), 79.1 (C2'-A), 74.5 (C2'-C), 71.3 (C2-B), 70.5 (C3'-C), 69.8 (C3-B), 69.5 (C3'-A), 65.6 (d, $^2J_{cp}$ = 5.0 Hz, C5'-C), 65.2 (d, $^2J_{cp}$ = 2.5 Hz, C5-B), 63.8 (d, $^2J_{cp}$ = 3.8 Hz, C5'-A). **^{31}P NMR (202 MHz, D_2O)** δ 0.33 (phosphate), -11.20, -11.31, -11.37, -11.47 (pyrophosphate). **LC-MS** (0→50% ACN [0.1% TFA]): Rt = 2.5 min, m/z: 889.2. **HRMS** (ESI) $[M+H]^+$ calculated for $C_{25}H_{36}N_{10}O_{20}P_3$ 889.13147; found 889.13112.

Phosphoadenosyl-di-ADP-ribose (6)



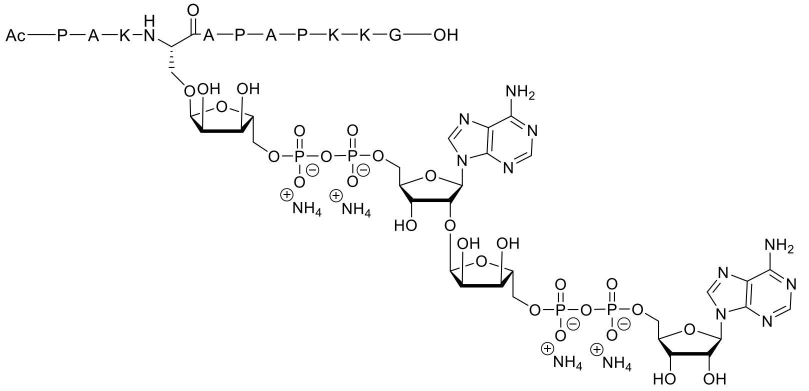
Pre-loaded CPG solid support **8** (loading 36.7 µmol/g, 272 mg, ~10 µmol, 1.0 eq) was placed in a Mermade 6 oligonucleotide synthesizer and the complete synthesis was performed under an argon atmosphere. Cycle A was performed (2x).

After Cycle A, the solid support was rinsed with ACN (3x). DBU (10% v/v in ACN, 2 mL, 1.34 mmol, 133.7 eq.) was added and the mixture was left to stand for 5 minutes. The solid support was drained and the DBU treatment was repeated (3x). Then, the solid support was rinsed with ACN (3x). NH_4OH (35%, 10 mL) was added and the reaction mixture was shaken at room temperature overnight to cleave the product from the solid support and remove all the protecting groups. Then, the filtrate was collected, concentrated under reduced pressure and purification by strong anion exchange chromatography (A: 10 mM NH_4OAc in MilliQ, B: 1 M NH_4OAc in MilliQ, gradient: 25-40% B) followed by HW-40 gel filtration (0.15 M NH_4HCO_3 in 10%

ACN/MilliQ) afforded phosphoadenosyl-di-ADP-ribose **6** as a white solid (1.88 mg, 1.23 µmol, 12.3%) after repeated lyophilization.

^{1}H NMR (850 MHz, D_2O) δ 8.45 (s, 1H, H2), 8.44 (s, 1H, H2), 8.39 (s, 1H, H2), 8.22 (s, 1H, H8), 8.18 (s, 1H, H8), 8.15 (s, 1H, H8), 6.20 (d, *J* = 3.7 Hz, 1H, H1'-A), 6.07 (d, *J* = 3.4 Hz, 1H, H1'-C), 6.00 (d, *J* = 5.7 Hz, 1H, H1'-E), 5.32 (d, *J* = 4.3 Hz, 1H, H1-B), 5.29 (d, *J* = 4.0 Hz, 1H, H1-D), 4.69 (t, *J* = 5.4 Hz, 1H, H2'-E), 4.67 – 4.65 (m, 1H, H2'-A), 4.55 (t, *J* = 5.4 Hz, 1H, H3'-A), 4.54 – 4.51 (m, 1H, H2'-C), 4.50 – 4.48 (m, 2H, H3'-CE), 4.40 – 4.38 (m, 1H, H4'-A), 4.37 – 4.33 (m, 4H, H4-BD, H4'-CE), 4.32 – 4.29 (m, 1H, H5/H5'), 4.27 – 4.20 (m, 8H, H2-BD, H3-BD, H5'-A, H5/H5'), 4.14 – 4.12 (m, 1H, H5'-A), 4.09 – 4.03 (m, 4H, H5/H5'). **^{31}P NMR (202 MHz, D_2O)** δ 1.06 (phosphate), -10.60 (pyrophosphate). **LC-MS** (0→50% ACN [0.1% TFA]): Rt = 2.5 min, m/z: 1430.6. **HRMS** (ESI) $[M+2H]^{2+}$ calculated for $C_{40}H_{58}N_{15}O_{33}P_5$ 715.59993; found 715.60076.

H2B1C Ser6(ADPr)$_2$: Ac-Pro-Ala-Lys-Ser(O$^{\gamma}$-[ADPr$_2$])-Ala-Pro-Ala-Pro-Lys-Lys-Gly-OH (33)



DMC-HCl (169 mg, 1.0 mmol) and imidazole (136 mg, 2.0 mmol) were dissolved in D_2O (500 µl), and the resulting mixture was shaken at room temperature for 5 minutes. Then, the pre-mixed activation cocktail (7.55 µL) was added to an Eppendorf tube containing phosphoadenosyl-ADP-ribose **5** (1.3 mg, 1.36 µmol, 1.2 eq.), and the reaction mixture was shaken at room temperature for 45 minutes. After conversion into the activated phosphorimidazolide, the resulting solution was added to an Eppendorf tube containing phosphoribosyl-peptide **32**[38] (1.5 mg, 1.13 µmol, 1 eq.) and $ZnCl_2$ (3.1 mg, 23 µmol, 20 eq.) in D_2O (38 µl), and the reaction mixture was shaken at 40 °C overnight. Next, the reaction mixture was diluted with MilliQ (230 µL) and purification by HW-40 gel filtration (0.15 M NH_4OAc in 10% ACN/MilliQ) followed by strong anion exchange chromatography (A: 20 mM NH_4OAc in MilliQ, B: 1.0 M NH_4OAc in MilliQ, gradient: 0-100% B) afforded di-ADP-ribosylated peptide **33** as a white solid (0.28 mg, 0.13 µmol, 12%) after repeated lyophilization.

^{1}H-NMR (500 MHz, D_2O) δ 8.36 (s, 1H, H2), 8.34 (s, 1H, H2), 8.12 (s, 1H, H8), 8.10 (s, 1H, H8), 6.14 (d, *J* = 3.6 Hz, 1H, H1'), 5.96 (d, *J* = 6.0 Hz, 1H, H1'), 5.24 (d, *J* = 4.3 Hz, 1H, H1''), 4.95 (bs, 1H, H1''). **^{31}P-NMR (162 MHz, D_2O)** δ -11.14 – -11.61 (pyrophosphate). **LC-MS** (0→20% ACN [0.1% TFA]): Rt = 5.4 min, m/z: 1088.8. **HRMS** (ESI) $[M+2H]^{2+}$ calculated for $C_{79}H_{126}N_{24}O_{40}P_4$ 1088.3830; found 1088.3856.

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