Universiteit
Leiden
The Netherlands

Synthetic Study on ADP-ribosylation

Liu, Q.

Citation
Liu, Q. (2019, November 27). *Synthetic Study on ADP-ribosylation*. Retrieved from https://hdl.handle.net/1887/80840

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/80840

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

The following handle holds various files of this Leiden University dissertation:
http://hdl.handle.net/1887/80840

Author: Liu, Q.
Title: Synthetic Study on ADP-ribosylation
Issue Date: 2019-11-27

4 Synthesis of well-defined linear ADPr oligomers and biotinylated derivatives thereof

Introduction

ADP-ribosylation is a post-translational modification that plays a crucial role in various important cellular events.[1] ADP-ribosylation is catalyzed by a family of PARP enzymes, mediating the transfer of a single ADPr moiety from NAD^+ to the nucleophilic side chains of specific amino acids in target proteins.[2] Some of these enzymes can also add additional ADPr units, generating poly ADP ribose (PAR) chains up to a length of hundreds of ADP ribose units.[3] Poly ADP-ribosylation is involved in several processes including, regulation of chromatin structure, apoptosis-inducing factor (AIF)-dependent cell death and DNA repair. Several types of proteins participate in PARP dependent pathways, such as "readers" (e.g. macrodomain proteins, WWE domain and PBZ domains), that recognize and bind with PAR and "erasers" (e.g. PARG and ARH1/3), that hydrolyze PAR.[2-4] It can be assumed that most of these readers and erasers only interact with relatively small parts of the PAR chain. Since the interaction at a molecular level between PAR and the associated proteins or enzymes is still elusive, the availability of ADPr oligomers is of prime importance. Although enzymatically prepared PAR fragments have been widely applied for biological experiments, the enzymatic synthesis suffers from low yields, inhomogeneous samples and insufficient quantities, in particular concerning short oligomers (2-10 units).[5-7]

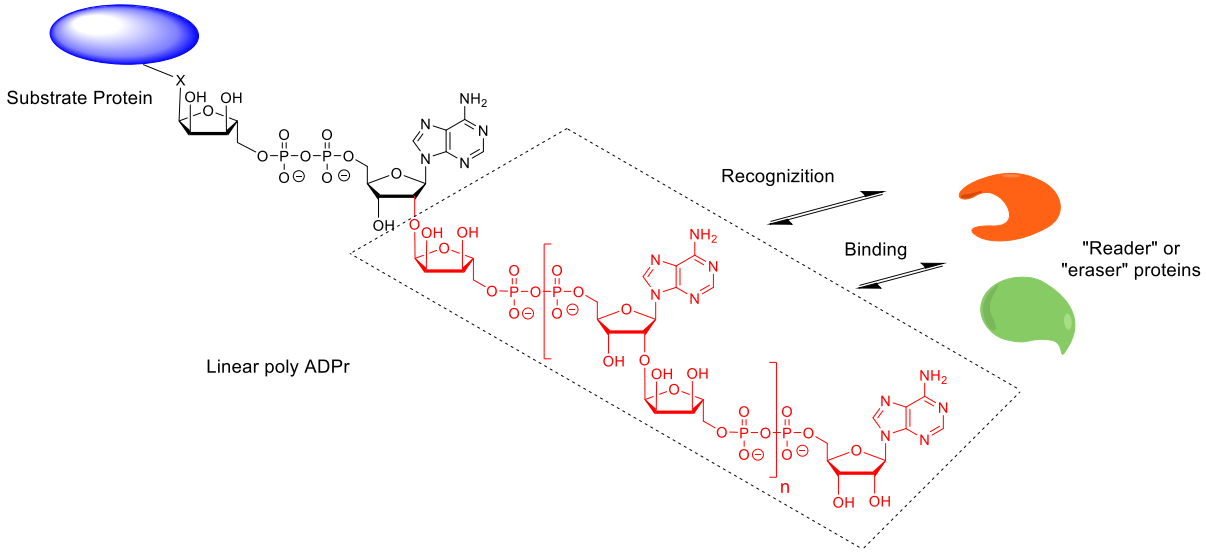


Figure 1. The structure poly-ADPr and “reader”/"eraser” proteins.

Chemical synthesis of well-defined ADPr oligomers is, therefore, a relevant alternative to tackle the problems of insufficient purity and limited supply, mentioned above.[8-10] The group of Hergenrother presented a solution phase synthesis of ADPr dimers by which a PAR–protein binding assay was performed and the first human PARG substrate-enzyme co-crystal structure was elucidated. Biotinylated or fluorescently labeled derivatives of di-ADPr have been prepared as well.[10] In Leiden, Kistemaker *et al.*[8] developed a solid phase synthesis approach, culminating in the isolation of an ADPr trimer. Ensuing experiments showed that the ADPr trimer, in contrast to the dimer and monomer, binds and activates a chromatin remodeler protein ALC1 which is involved in oncogenesis.[9] These encouraging breakthroughs demonstrate the potential of synthetic oligo-ADPr for the elucidation of the interaction of relevant proteins with PAR in a length-dependent manner.[11] It is to be expected that the availability of such well-defined ADPr fragments will contribute to future insights in oncology,[9, 12] neurodegeneration[13] and inflammation.[14] The development of methods for the synthesis of ADP-ribose oligomers and derivatives thereof (such as biotinylated and fluorescently labelled) is a valuable approach to obtain molecular tools for both protein binding assays[10, 15] and proteomics studies.[16-19]

This Chapter describes a solid phase synthesis of a series of linear ADPr oligomers (varying in length from dimer to pentamer), using a new protecting group strategy. This procedure builds upon the method described in Chapter 3 and involves the application of base-labile fluorenylmethyl (Fm) group as the temporary protection to avoid repetitive exposure of the growing ADPr-chain to acidic conditions that were used in an original method for the solid phase synthesis of ADPr-chains developed by Kistemaker *et al.*[8] In addition, ADPr mono-, di-, and trimer, provided with a propargyl handle, were prepared via the Fm-based chemistry and subsequently functionalized via a click reaction to furnish the corresponding biotinylated ADPr oligomers.

Results and discussion

The synthesis of ADPr oligomers is highly demanding due to the presence of both a 1,2-cis-α-ribosylated adenosine and an anionic pyrophosphate in the repeating unit. Both moieties are not only difficult to construct but also possess a certain degree of acid lability (Figure 2). Kistemaker *et al.* has reported a solid phase synthesis of an ADPr dimer and trimer by a combination of P(III) and P(V) chemistry, where in the key step a phosphomonoester at the ribose reacted with a phosphoramidite at the adenosine residue.[8, 20] Prior to the formation of this phosphite-phosphate intermediate the *tBu* protecting groups of the ribose phosphotriester should be released to give the phosphomonoester. We reasoned that the repeated acidic deprotection of the *tBu* groups exerts an adverse effect on the

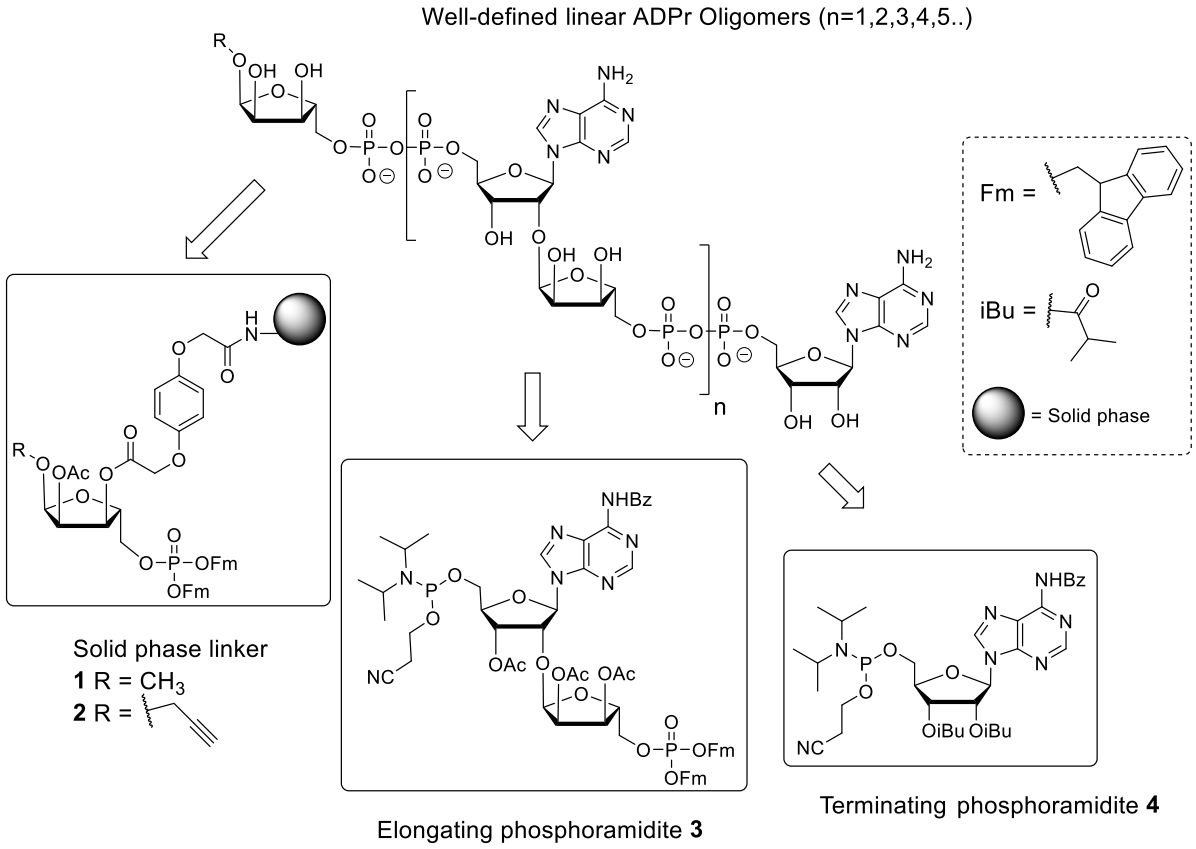
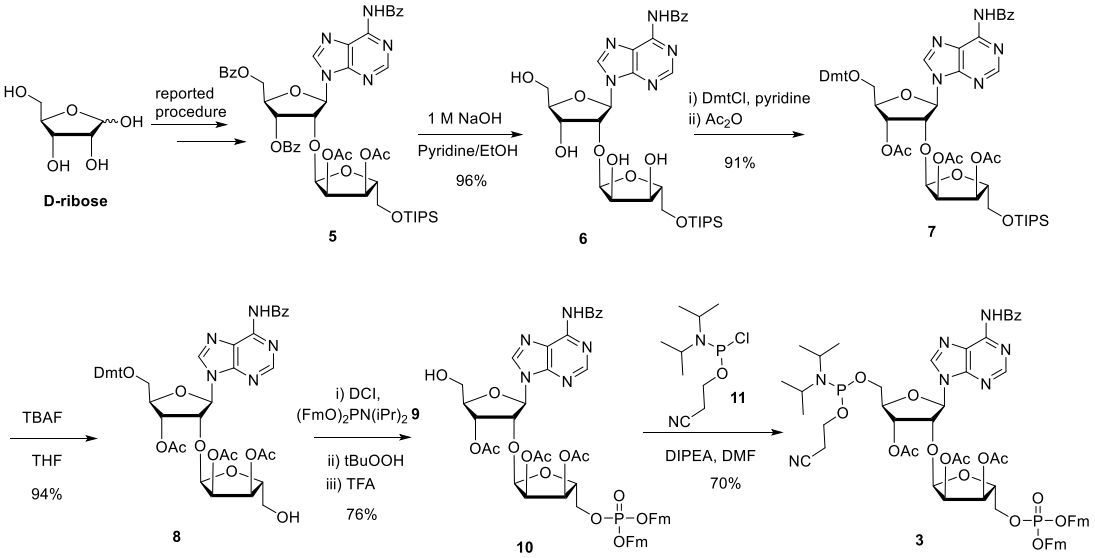


Figure 2. Retrosynthetic analysis of linear ADPr oligomers.

maximal length of oligomer, that can be reached. On the basis of these considerations a synthetic route with only base labile protecting groups was designed. The *tBu* groups in the phosphotriester precursor were replaced by the base labile fluorenylmethyl (Fm) protecting groups, leading to key building block **3** for repeated introduction of the pyrophosphate function.[21, 22] Furthermore, the first α-configured 1-*O*-methyl or 1-*O*-propargyl phosphorylated ribose residue was immobilized via the alkali labile Q linker on either LCAA-CPG or on Tenta Gel N (TG) resin (**1** or **2**).[8, 10, 23, 24] The hydroxyl groups of **1** and **2** and

both the hydroxyl groups and exocyclic amine in terminal phosphoramidite **4** are all protected with alkali sensitive protective groups.

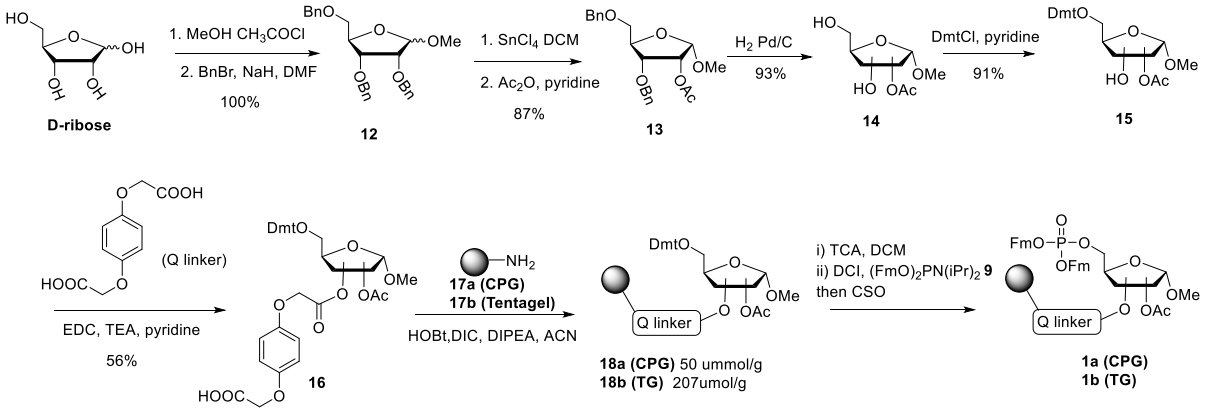
The route of synthesis to key building block **3** is outlined in Scheme 1 and commences with the construction of 1,2-cis-α-ribosylated nucleoside **5** by adoption of the method reported by Kistemaker *et al.*[8] Of note, this method is characterized by the post-glycosylation introduction of the adenine base via a Vorbrüggen coupling and can be performed on a scale that gives access to substantial amount of **5**.



Scheme 1. Synthesis of key repeating phosphoramidite **3**.

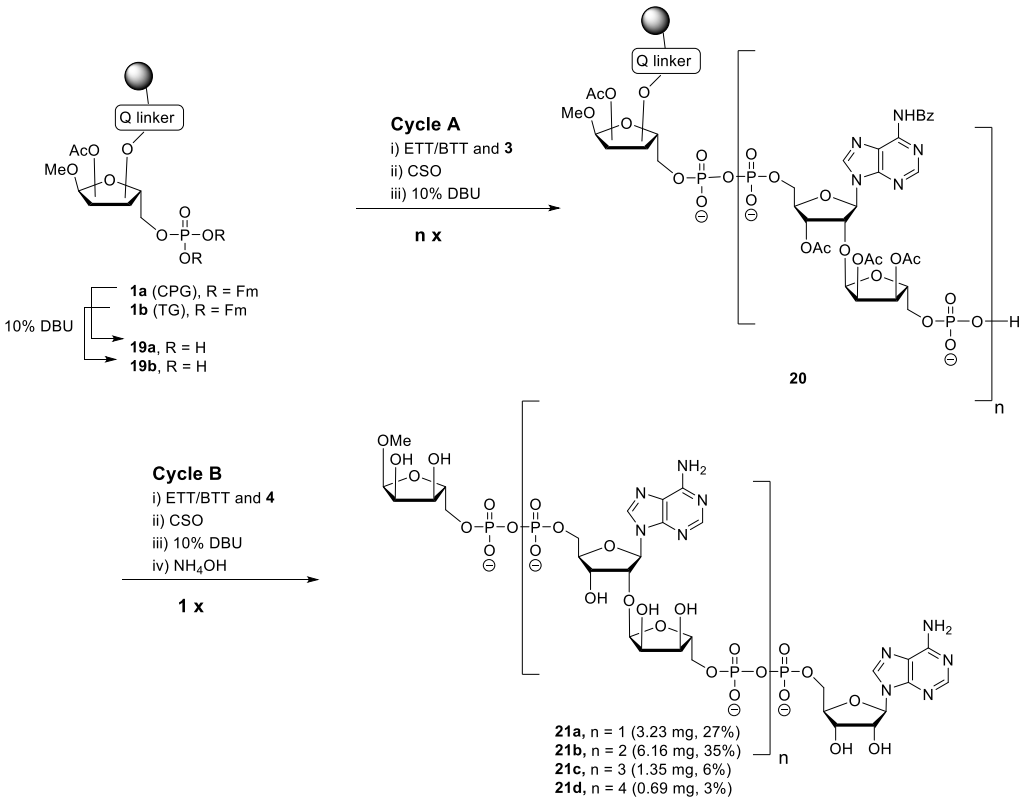
The protecting group manipulations *en route* to orthogonally protected intermediate **7** entail a saponification of the benzoyl and acetyl esters in **5** with aqueous NaOH (1 M) to give after column chromatography tetraol **6** in an excellent yield (96%). Subsequent selective dimethoxytritylation of the primary hydroxyl followed by acetylation of the remaining secondary hydroxyls gave **7** in high yield and in sufficient amount (2.3 mmol). This adaptation of the earlier described one pot 3-step procedure (saponification, DMT introduction and acetylation) turned out to be more favorable.[8] The bis-Fm triester is introduced by the removal of 5''-TIPS group of **7** with TBAF in THF, followed by the treatment of the liberated primary OH in **8** with bis-(*9H*-fluoren-9-ylmethyl)-diisopropylamidophosphite **9**[25, 26] in the presence of 4,5-dicyanoimidazole (DCI), *in situ* oxidation using *t*BuOOH and, finally, careful work-up to remove the excessive DCI. The obtained crude phosphotriester was treated with TFA in DCM to selectively remove DMT, furnishing **10** in 76% yield (3 steps). The phosphoramidite functionality was introduced using 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite **11** in the presence of DIPEA in DMF to provide key phosphoramidite **3** in a good yield. This reaction also requires a careful work-up procedure for the removal of DIPEA, which is capable to cleave one of the base labile Fm groups. In

addition, the simultaneous occurrence of an acid labile (phosphoramidite) and a base labile (Fm) group in compound **3** requires column chromatography with high-quality IRR silica gel (see experimental section) instead of the traditional TEA-neutralized one.



Scheme 2. Synthesis of the solid supports **1a** and **1b** with a ribose phosphotriester immobilized via Q linker.

The projected solid phase synthesis of ADPr oligomers requires a solid support to which the first protected ribose phosphotriester is connected via the base labile Q-linker (Scheme 2). To this end, D-ribose was converted into 1-*O*-methyl-2,3,5-tris-O-benzyl-ribofuranoside (**12**) in high yield, which was isolated as a mixture of anomers. The 2-*O*-benzyl in **12** was selectively removed with $SnCl_4$/DCM[27] and the released hydroxyl was acetylated, after which the pure α-anomer **13** could be isolated. The isomerization of β- to the desired α-anomer can be explained by the opening and closing of the furanose ring under Lewis acidic ($SnCl_4$) conditions. Pd/C catalyzed hydrogenolysis of the benzyl groups in **13** was accompanied by acetyl migration to give **14** as a mixture of regio-isomers. After protection of the primary OH with the DMT group to give **15**, the Q-linker was introduced[8] with EDC/TEA in pyridine. The obtained acid **16** was used to functionalize both LCAA-CPG **17a** and TentaGel N (TG) resin **17b** to yield **18a** and **18b** respectively with desired loadings (50 µmol/g for **18a** and 207 µmol/g for **18b**). Apart from the usual CPG support, the TG resin was selected on the basis of its polystyrene matrix grafted with a polyethylene glycol (PEG) linker. Finally, the Fm-protected phosphotriester was installed by the following reaction sequence. At first, the DMT group was removed with TCA in DCM, then phosphitylation of the primary OH with phosphoramidite **9** and oxidation by CSO were performed to give either CPG **1a** or TG **1b**. The successful introduction of phosphate function was confirmed by ^{31}P-NMR analysis of the mixture obtained by NH_4OH treatment of the supports **1a** and **1b**.



Scheme 3. Synthesis of linear ADPr oligomers

At this stage, the functionalized solid supports (**1a**, **1b**) and elongating phosphoramidite (**3**) were available, and the terminating phosphoramidite (**4**) was prepared as well according to a known procedure.[8]

To test the feasibility and efficiency of the pyrophosphate formation via the proposed method, the synthesis of an ADPr dimer was undertaken in a fritted syringe via manual couplings (Scheme 3). The influence of the solid support was determined by the application of both CPG **1a** and TG **1b** supports. Removal of the Fm groups of **1a**/**b** by DBU treatment (10% in ACN, 15 min x 2) gave phosphomonoester (**19a**/**b**) suitable for pyrophosphate coupling. Guided by the protocol of Kistemaker *et al*,[8] the following revised 3-step elongation cycle A was executed: a) treatment of immobilized phosphate monoester **19a**/**b** with phosphoramidite **3** using 5-(ethylthio)-1*H*-tetrazole (ETT) as activator (10 min x 2); b) oxidation of the phosphate-phosphite (P^V-P^{III}) to a phosphate-phosphate (P^V-P^V) bridge with CSO (5 min x 2); c) treatment the immobilized and partly protected pyrophosphate with DBU (10% in ACN, 15 min x 2) to simultaneously remove both the cyanoethyl (CE) group on the pyrophosphate and the Fm groups on the terminal phosphate. Completion of the first elongation cycle gave the immobilized phosphomonoester **20** (n = 1), ready for the introduction of the second pyrophosphate. To obtain the ADPr dimer **21a** the synthesis was continued with similar cycle B in which phosphoramidite **4** was used in "step a" to form the phosphate-phosphite (P^V-P^{III}) intermediate. After completion of cycle B, the

resin was treated with NH_4OH to remove all the protecting groups and to release the target ADPr dimer from the resin. Subsequent anion exchange chromatography purification yielded 0.32 mg (3%) and 3.23 mg (27%) of dimer **21a** from CPG and TG resin respectively. This result suggests that for this manual oligo-ADPr synthesis TG resin is more preferable than traditional CPG resin.[8, 28] In both syntheses, we found 1-*O*-methyl mono-ADPr side product which may be attributed to an inefficient pyrophosphate coupling of the cycle A.

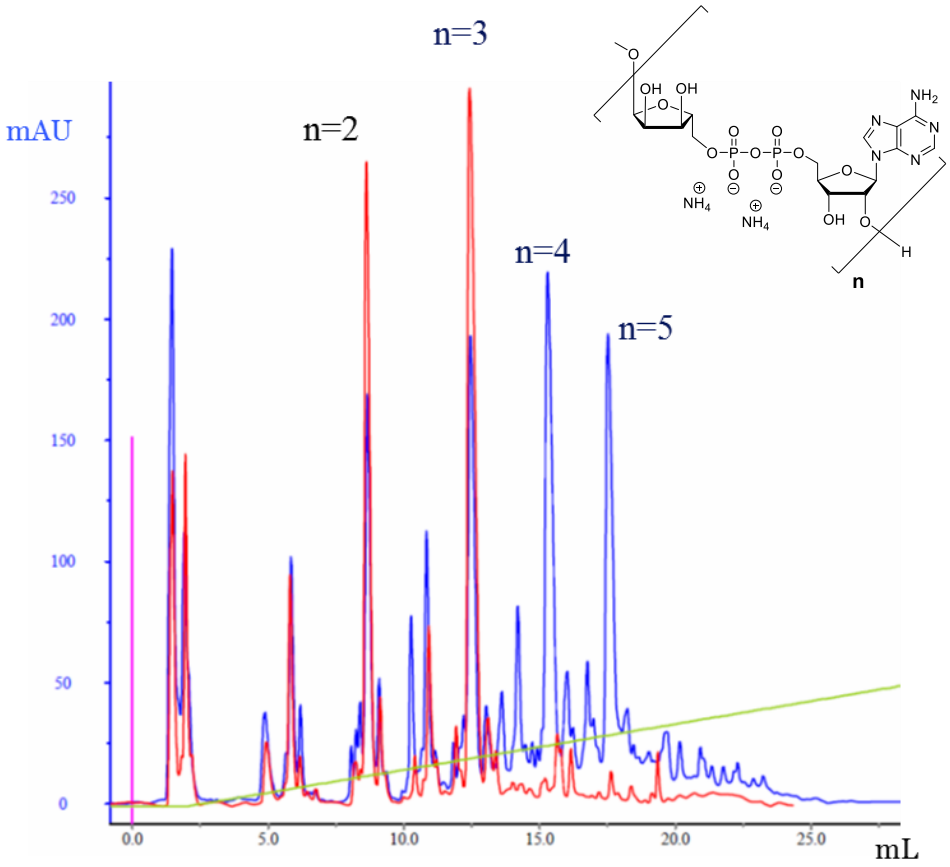
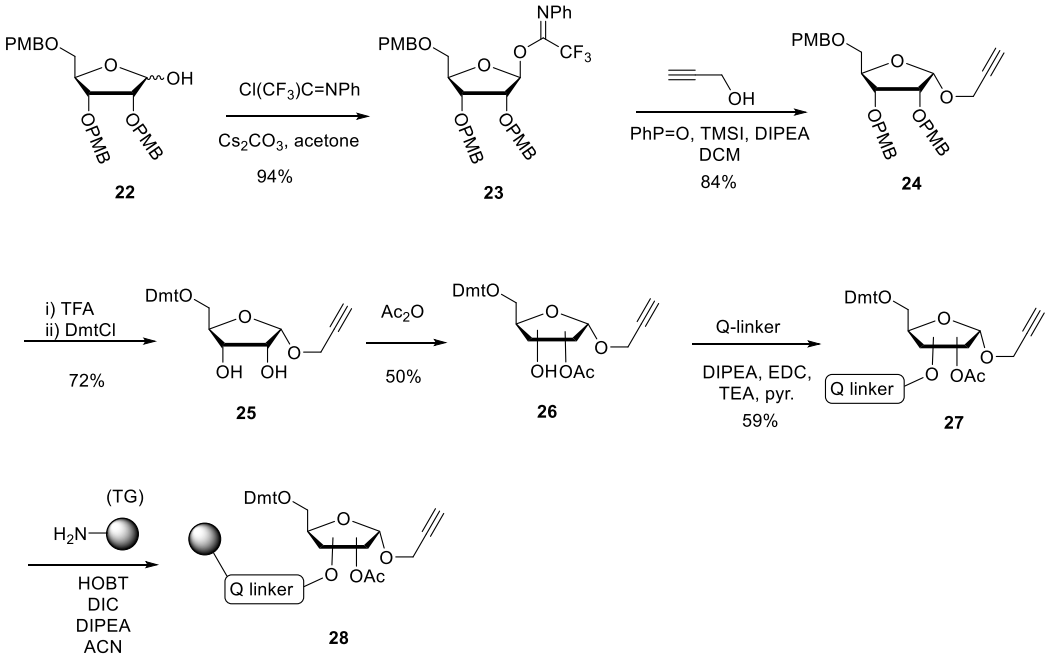


Figure 3. Analytical anion exchange chromatography[a] UV spectra for the crude sample of the synthesis of ADPr trimer (red) and pentamer (blue)

a Column: DNAPac PA-100, 4*250 mm. Gradient: NH_4OAc, 0%-20%. In 10 column volume.

The successful manual synthesis of dimer **21a** was an incentive to explore the synthesis of ADPr oligomers using an automated DNA synthesizer. On the basis of the above experiment, TG resin was chosen for synthesis while other pilot studies showed that 5-(benzylthio)-1*H*-tetrazole (BTT)[29, 30] is a better activator than ETT and an elongated DBU treatment time (10 min x 4) is necessary to completely cleave the Fm groups (Scheme 3). With these adaptations, an ADPr trimer was successfully synthesized on a DNA synthesizer (Figure 3, red line) with 35% yield, which is higher than the method in which *tBu*

groups were used for phosphate monoester protection.[8] Next, a more ambitious pentamer synthesis was performed using same method. After cleavage from resin, crude sample was analyzed by anion exchange chromatography showing the target pentamer peak together with tetramer and trimer peaks (Figure 3, blue line). Purification by anion exchange chromatography and gel filtration furnished the individual ADPr trimer **21b** (1.18 mg), tetramer **21c** (1.35 mg) and pentamer **21d** (0.69 mg), respectively. The unequalled synthesis of the tetra- and pentamer was followed by an attempt to assemble a decamer. However, the crude reaction mixture showed substantial side-products which made isolation of pure oligomers impossible. The formation of side-products and the shorter oligomers could be attributed to the incomplete coupling of cycle A and possibly the absence of a capping procedure.



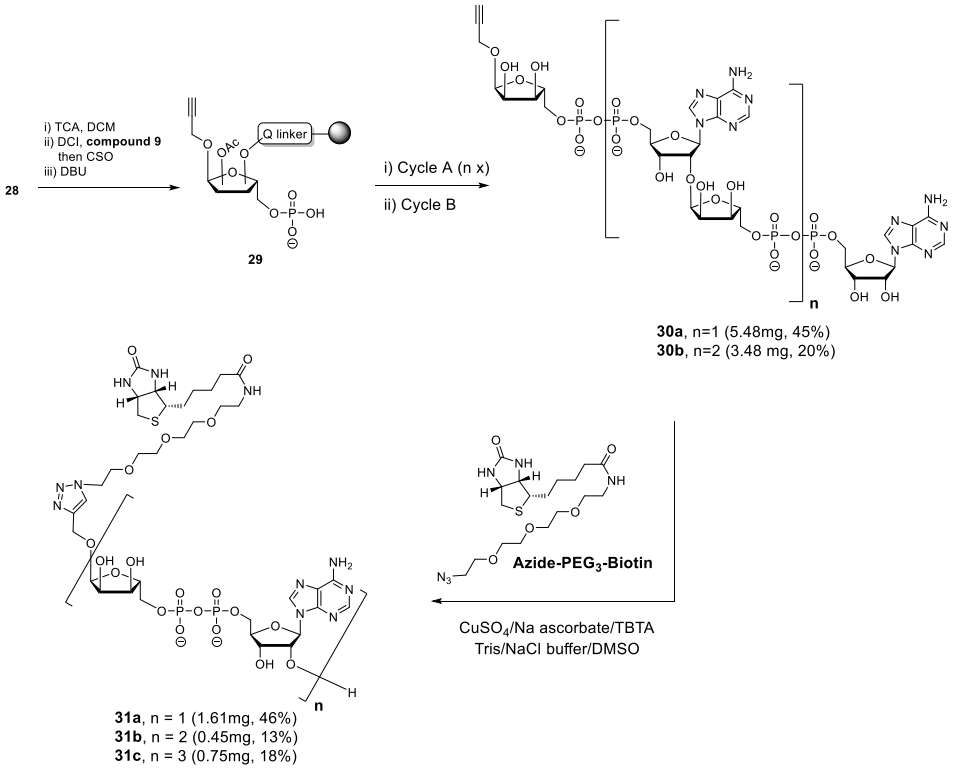
Scheme 4. Synthesis of resin **28** with an immobilized propargyl functionalized ribose triester

Next, the attention was directed to the synthesis of biotinylated ADPr oligomers by the synthesis of TG resin **28** on which a ribose with a propargyl group at the anomeric position is immobilized (Scheme 4). In this way, ADPr oligomers with a terminal alkyne can be prepared, which after solid phase synthesis can be functionalized with labels and handles (such as biotin) via copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. The synthesis of resin **28** started with the conversion of known 2,3,5-tri-*O*-*p*-methoxylbenzyl-ribofuranose **22**[31] into imidate donor **23**, using $Cl(CF_3)C=NPh$, Cs_2CO_3 in acetone (Scheme 4). Coupling of donor **23** with propargyl alcohol under the influence of TMSOTf/DCM gave **24** as an anomeric mixture ($\alpha : \beta = 1.35 : 1$). Fortunately, activation of **23** with a combination of TMSI and $Ph_3P=O$ (6 equivalents), as reported for the introduction of α-glucosidic

linkages, yielded solely α product **24** in high yield.[32, 33] In this ribosylation reaction DIPEA was added to prevent PMB cleavage.[31,34, 35] Subsequent protective group manipulation comprised the removal of the PMB groups in **24** by TFA assisted acidolysis, the regioselective tritylation of the primary hydroxyl function and, finally, acetylation of either the 2- or 3-OH in **25** with Ac_2O (0.9 equivalent) to give ribofuranoside **26**. Resin **28** was prepared by the installation of the Q-linker on either the 2- or 3-OH of **26** to give **27** that was subsequently coupled to TG resin (loading: 165 µmol/g) using the same procedures as described for the conversion of **15** into supports **1a**/**1b** (Scheme 2).

The assembly of biotinylated ADPr oligomers is depicted in scheme 5 and starts with the solid phase synthesis of propargylated ADPr dimer **30a** and trimer **30b**, using the same procedures and elongation cycles as used for the above described ADPr oligomers **21a**-**d** (Scheme 3). Fully protected resin **28** was detritylated, phosphitylated with reagent **9**, oxidized to the corresponding phosphotriester and finally treated with DBU to yield 1-*O*-propargyl-α-phosphoriboside resin **29**, which is suitable for oligomer synthesis. The solid phase procedures toward propargylated ADPr dimer **30a** and trimer **30b** used cycle A, one and two times respectively (n = 1 or 2) and ended each synthesis with cycle B. After purification by anion exchange chromatography, ADPr dimer **30a** and trimer **30b** were isolated in reasonable yield and quantity.

The thus prepared propargylated mono ADPr,[24] dimer **30a** and trimer **30b** were conjugated to commercially available azide-PEG_3-biotin, using a modified copper(I)-catalyzed alkyne-azide



Scheme 5. Assembly of biotinylated ADPr oligomers via copper(I)-catalyzed cycloaddition. The cycles A and B are the same as in scheme 4.

cycloaddition method (catalytic cocktail: $CuSO_4$/Na ascorbate/TBTA). The cycloaddition was monitored by LC-MS analysis, which showed of complete consumption of propargyl oligomers after 1 hour. HPLC purification yielded biotinylated mono-, di- and tri-ADPr (**31a-c**), structures and purity of which were confirmed by ^{1}H-NMR, ^{31}P-NMR, LC-MS and HRMS.

Conclusion

A new solid phase procedure towards well-defined linear ADPr oligomers is discussed. The novelty of the procedure entails the application of base sensitive temporary and permanent protective groups. In particular, protection of the intermediate phosphate monoester, that has to be liberated repeatedly, with fluorenylmethyl (Fm) groups facilitated the construction of multiple pyrophosphates linkages. To put the solid phase procedure into practice, a highly advanced Fm protected elongating phosphoramidite (**3)** was synthesized in good yield and the resin was provided with the alkali labile Q-linker. ADPr oligomers up to a pentamer were assembled in sufficient quantities. A similar procedure resulted in a propargylated ADPr dimer and trimer allowing the formation of the corresponding biotinylated-ADPr oligomers. The latter will be used as valuable tools for future protein binding and proteomics studies.

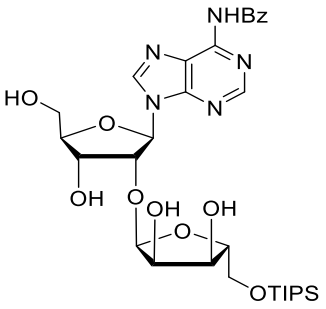
Acknowledgments

Nico Meeuwenoord is kindly acknowledged for his help in oligomer synthesis and final product purification. Stanley Tsui is kindly acknowledged for part of building block synthesis.

Experimental section

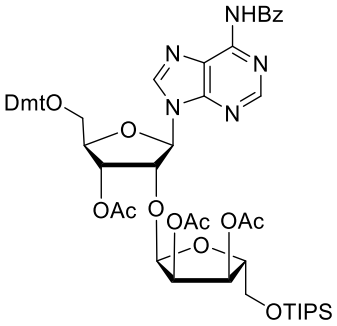
General procedure

All chemicals were used as received unless stated otherwise. All solvents used in reaction (including solid phase synthesis) were dried over 3Å molecular sieves. Solvents removal by rotary evaporation was under reduced pressure at 40 ℃. TLC, NMR, LCMS, anion exchange, HRMS, IR, optical rotation facilities were used as described in Chapter 2. Azide-PEG_3-biotin and LCAA-CPG resin were purchased from Sigma-Aldrich. Tenta Gel N resin was purchased from Rapp Polymere.



N^6-benzoyl-9-(5-O-triisopropylsilyl-β-parobiosyl) adenine (6)

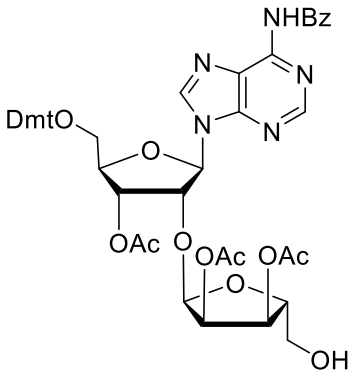
To a round-bottom flask, compound **5** (2.48 g, 2.63 mmol), pyridine (18 mL) and EtOH (9 mL) were added in sequence. The mixture was cooled to 0 °C after which aqueous NaOH (15.78 mL, 1 M solution) was slowly added. The reaction was stirred for 1 h at 0 °C after which Amberlite-H^+ was added until pH = 6. The mixture was filtered, concentrated and purified by silica gel column chromatography (DCM/MeOH, 100/0 – 100/3 – 100/5) to obtain **6** as a white foam (1.66 g, 2.52 mmol, 96%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.69 (s, 1H, NH), 8.69 (s, 1H, H2), 8.32 (s, 1H, H8), 8.00 (d, *J* = 7.1 Hz, 2H, arom), 7.57 (t, *J* = 7.4 Hz, 1H, arom.), 7.47 (t, *J* = 7.7 Hz, 2H, arom.), 6.11 (d, *J* = 7.0 Hz, 1H, H1'), 5.78 (brs, 1H, OH), 5.01 (d, *J* = 4.1 Hz, 1H, H1''), 4.95 (dd, *J* = 7.1, 4.6 Hz, 1H, H2'), 4.76-4.41 (m, 4H, H3', OH x 3), 4.29 (s, 1H, H4'), 4.23-4.19 (m, 3H, H2'', H3'', H4''), 3.93-3.67 (m, 4H, H5', H5''), 0.98-0.97 (m, 21H, TIPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.29 (CO, Bz), 150.84, 150.20, 133.44 (Cq. arom.), 133.04, 128.88, 128.23 (arom.), 124.07 (cq, arom.), 101.73 (C1''), 89.09 (C1'), 87.81 (C4'), 86.67 (C4''), 79.39 (C2'), 73.35 (C2''), 72.69 (C3'), 71.72 (C3''), 63.95 (C5''), 63.06 (C5'), 18.03, 18.01, 11.92 (TIPS). IR (film): 3337, 2942, 2866, 1704, 1614, 1584, 1459, 1252, 1093, 1042, 883, 709, 686 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{31}H_{46}N_5O_9Si$ (M+H) 660.3059. Found 660.3061. $[\alpha]_D^{20}$ +5.2 (c = 1, in DCM)



N^6-benzoyl-9-(3',2'',3''-tris-*O*-acetyl-5'-*O*-dimethoxyltrityl-5''-*O*-triisopropylsilyl-β-parobiosyl) adenine (7)

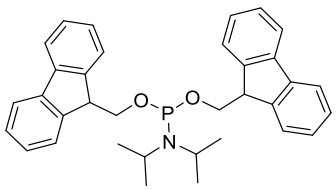
Compound **6** (1.66 g, 2.52 mmol) was co-evaporated with pyridine (1 x), then N_2 was applied. Dry pyridine (12 mL) and 4,4'-dimethoxyltrityl chloride (DMTCl, 1.36 g, 4.01 mmol) was added into the flask. 40 minutes later, TLC showed complete conversion and the mixture was cool to 0°C after which Ac_2O (1.42 mL, 15 mmol) was added. The mixture was stir at 0°C for 5 h and was quenched by aq. $NaHCO_3$ (sat.). DCM extracted (3 x) the mixture and the organic layers were combined and dried ($MgSO_4$). The mixture was filtered, concentrated and purified by silica gel column chromatography (pentane/acetone, 100/0 – 90/10 – 85/15 – 80/20 – 70/30) to obtain **7** as a white foam (2.50 g, 2.30 mmol, 91%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.37 (s, 1H, NH), 8.70 (s, 1H, H2), 8.17 (s, 1H, H8), 8.01(d, *J* = 7.3 Hz, 2H, arom.), 7.58 – 7.51 (m, 1H, arom. Bz), 7.47 – 7.43 (m, 4H, arom. Bz, DMT), 7.33 – 7.19 (m, 7H, DMT), 6.81 (d, *J* = 9 Hz, 4H, arom. DMT), 6.26 (d, *J* = 6.0 Hz, 1H, H1'), 5.62 (dd, *J* = 5.2, 3.6 Hz, 1H, H3'), 5.38 (dd, *J* = 7.0, 2.7 Hz, 1H, H3''), 5.35 (d, *J* = 4.7 Hz, 1H, H1''), 5.26 (t, *J* = 5.6 Hz, 1H, H2'), 4.86 (dd, *J* = 7.0, 4.6 Hz, 1H, H2''), 4.35 (q, *J* = 3.5 Hz, 1H, H4'), 4.15 (q, *J* = 2.8 Hz, 1H, H4''), 3.86 (AB, ddd, *J* = 11.0, 2.9 Hz, 2H, H5''), 3.76 – 3.75 (m, 6H, CH3, DMT), 3.52 (AB, *J* = 10.6, 3.5 Hz, 2H, H5'), 2.16 (s, 3H, Ac), 2.11 (s, 3H, Ac), 1.84 (s, 3H, Ac), 1.07 – 1.03 (m, 21H, TIPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.13, 169.49, 169.40 (CO, Ac), 164.65 (CO, Bz), 158.57 (Cq. arom.), 152.68 (CH, C2), 151.84 (C4), 149.63 (C6), 144.26 (Cq. arom.), 141.38 (CH, C8), 135.26 (Cq. arom.), 133.59, 132.61, 130.04, 128.68, 128.10, 127.84, 127.82, 126.98 (arom.), 123.41 (C5), 113.15 (Cq. arom.),

101.23 (C1''), 86.83 (Cq, DMT), 86.34 (C1'), 83.24 (C4''), 82.49 (C4'), 77.90 (C2'), 72.20 (C3'), 71.45 (C2''), 70.06 (C3''), 63.10 (C5''), 62.86 (C5'), 55.09 (CH_3, DMT), 20.77, 20.73, 20.10 (CH3, Ac), 17.79, 17.76, 11.76 (TIPS).



N^6-benzoyl-9-(3',2'',3''-tris-*O*-acetyl-5'-*O*-dimethoxyltrityl-β-parobiosyl) adenine (8)

Compound **7** (2.50 g, 2.30 mmol), dry THF (23 mL) and TBAF (tetrabutylammonium fluoride solution 1.0 M in THF, 4.60 mL, 4.60 mmol) was added into a flask and the mixture was stirred for 24 h. Excessive amount of EtOAc was added and the mixture was washed by H_2O (2 x) and brine (2 x). The organic layer was dried ($MgSO_4$), filtered, concentrated and purified by silica gel column chromatography (DCM/methanol, 100/0 – 99/1 –99/2) to obtain **8** as a white foam (2.01g, 2.16 mmol, 94%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.40 (s, 1H, NH), 8.72 (s, 1H, H2), 8.20 (s, 1H, H8), 8.04 (d, *J* = 7.1 Hz, 2H, arom. Bz), 7.63 – 7.54 (m, 1H, arom. Bz), 7.54 – 7.46 (m, 2H, arom. Bz), 7.45 – 7.36 (m, 2H, arom. DMT), 7.36 – 7.15 (m, 7H, arom. DMT), 6.81 (d, *J* = 9.0 Hz, 4H, arom. DMT), 6.24 (d, *J* = 5.9 Hz, 1H, H1'), 5.55 (dd, *J* = 5.2, 3.7 Hz, 1H, H3'), 5.33 (d, *J* = 4.6 Hz, 1H, H1''), 5.25 – 5.16 (m, 2H, H2'), 4.78 (dd, *J* = 7.3, 4.5 Hz, 1H, H3''), 4.33 (q, *J* = 3.6 Hz, 1H, H4'), 4.12 (q, *J* = 3.4 Hz, 1H, H4''), 3.82 – 3.66 (m, 8H, CH_3 DMT, H5''), 3.50 (AB, *J* = 10.7, 3.7 Hz, 2H, H5'), 2.86 (s, 1H, OH), 2.13 (s, 3H, CH_3 Ac), 2.10 (s, 3H, CH_3 Ac), 1.83 (s, 3H, CH_3 Ac). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.43, 169.86, 169.70(CO, Ac), 158.67 (Cq. arom.), 151.82 (C4), 149.78 (C6), 144.34 (Cq. arom.), 135.43, 133.68 (Cq. arom), 132.85, 130.17, 128.87, 128.21, 128.03, 127.15 (arom.), 123.24 (C5), 113.30 (arom.), 101.32 (C1''), 86.96 (Cq. DMT), 86.42 (C1'), 82.68 (C4''), 82.50 (C4'), 78.23 (C2'), 72.12 (C3'), 71.24 (C2''), 69.72 (C3''), 62.95 (C5'), 61.93 (C5''), 55.31 (CH_3, DMT), 20.95, 20.82, 20.23 (CH_3 Ac). IR (film): 2931, 1743, 1734, 1609, 1583, 1508, 1448, 1247, 1227, 1178, 1091, 1030 cm^{-1}. HRMS (ESI^+) calcd for $C_{49}H_{50}N_5O_{14}$ (M+H) 932.3349. Found 932.3369. $[\alpha]_D^{20}$ +28.6 (c = 1, in $CHCl_3$)

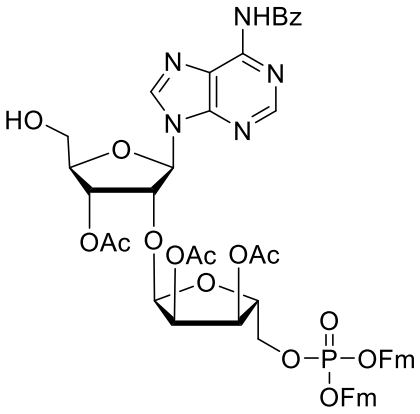


Bis(*9H*-fluoren-9-ylmethyl)-diisopropylamidophosphite (9)

Note: The title compound was made according to reported procedure with some modifications.[25] TLC used for this reaction was pre-run in 5% Et_3N in pentane. $CDCl_3$ used for NMR analysis was filtered through a layer of basic aluminum oxide before use.

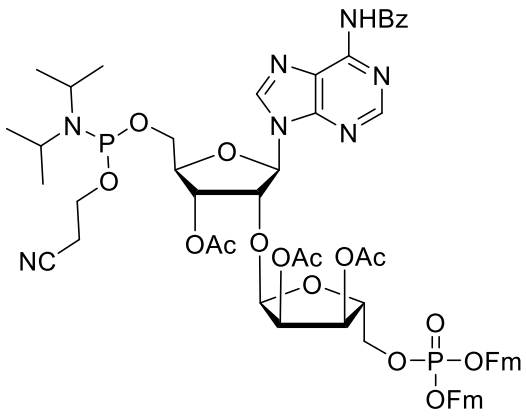
Diisopropylaminophosphodichloridite (2.28 g, 11.3 mmol) was added to a 0 °C solution of 9-fluorenylmethanol (4.44 g, 22.6 mmol) and EtN*iPr*$_2$ (5.88 mL, 41.6 mmol) in THF (25 mL). The mixture was stirred for 1 h at room temperature, then quenched by 1M pH 7 phosphate buffer (200 mL). The mixture was extracted with EtOAc (200 mL x 2), dried (Na_2SO_4), filtered and concentrated. The residue was further purified by flash silica gel column chromatography (prepared with 1% Et_3N in pentane and eluting with pentane/ethyl acetate/Et_3N 100:5:1) to give the **9** as a light yellow oil (4.10 g, 7.9 mmol, 70%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.77 (ddt, *J* = 8.3, 7.5, 1.0 Hz, 4H, arom.), 7.67 (ddq, *J* = 11.9, 7.5, 0.9 Hz, 4H, arom.), 7.47 – 7.36 (m, 4H, arom.), 7.36 – 7.24 (m, 4H, arom.), 4.21 (t, *J* = 7.1 Hz, 2H, CH Fm), 4.03 (dt, *J* = 9.9, 6.8 Hz, 2H, CH_2 Fm), 3.84 (dt, *J* = 9.9, 7.3 Hz, 2H, CH_2 Fm), 3.68 (dp, *J* = 10.1, 6.8 Hz, 2H, NC*H*(CH_3)$_2$), 1.19 (d, *J* = 6.8 Hz, 12H, NCH*(CH$_3$)$_2$*). ^{13}C

NMR (126 MHz, $CDCl_3$) δ 145.07, 144.79, 141.50, 141.39 (Cq. arom.), 127.53, 127.49, 126.96, 126.94, 125.58, 125.34, 119.96, 119.89 (arom.), 66.11, 65.97 (CH_2 Fm), 49.35, 49.29 (CH Fm), 43.22, 43.12 (N*CH*(CH_3)$_2$), 24.78, 24.73 (NCH*(CH$_3$)$_2$*). ^{31}P NMR (202 MHz, $CDCl_3$) δ 146.72.



N^6-benzoyl-9-(3',2'',3'''-tris-*O*-acetyl-5''-*O*-(di-flourenylphosphoryl)-β-parobiosyl)adenine (10)

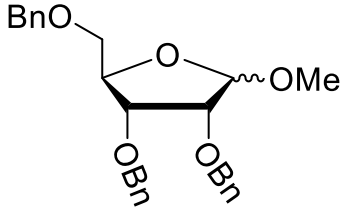
Compound **8** (1.99 g, 2.14 mmol), DCI activator (4,5-dicyanoimidazole solution 0.25 M in ACN, 17 mL, 4.28 mmol) and freshly activated 3Å molecular sieves were added in to flask. **9** (0.2 M in ACN, 16 mL, 3.21 mmol) were added into the mixture and the reaction was stirred for 10 minutes at room temperature after which *tBu*OOH (5.5 M in decane, 3.89 mL, 21.40 mmol) was added at 0°C. The reaction was stirred at same temperature for 30 minutes and quenched by aq. $NaHCO_3$ (sat.). The mixture was filtered and EtOAc was added to the filtration. The mixture was washed by H_2O (1 x) and brine (2 x) and the organic layer was dried (Na_2SO_4), filtered, concentrated and co-evaporated with toluene (3 x). To the residue, DCM (28 mL) and TFA (0.41 mL, 5.35 mmol) were added and the reaction was stirred for 10 minutes at room temperature after which was quenched by aq. $NaHCO_3$ (sat.). DCM extracted (2 x) the mixture and the organic layers are combined and washed by H_2O (1 x) and brine (1 x). The organic layers were dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel column chromatography (DCM/methanol, 100/0 – 100/1 – 100/2 – 100/3) to obtain **10** as a white foam (1.72 g, 1.62 mmol, 76%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.08 (s, 1H, NH), 8.78 (s, 1H, H2), 8.05 (s, 1H, H8), 8.03 – 7.99 (m, 2H, arom. Bz), 7.68 (ddd, *J* = 15.0, 7.6, 3.5 Hz, 4H, arom.), 7.64 – 7.59 (m, 1H, arom.), 7.56 – 7.41 (m, 6H), 7.39 – 7.19 (m, 8H), 6.00 (d, *J* = 11.5 Hz, 1H, 5'-OH), 5.96 (d, *J* = 7.8 Hz, 1H, H1'), 5.64 (d, *J* = 5.2 Hz, 1H, H3'), 5.13 (dd, *J* = 7.8, 5.2 Hz, 1H, H2'), 5.05 (dd, *J* = 7.3, 4.1 Hz, 1H, H3''), 4.95 (d, *J* = 4.6 Hz, 1H, H1''), 4.62 (dd, *J* = 7.3, 4.6 Hz, 1H, H2''), 4.31 (d, *J* = 1.8 Hz, 1H, H4'), 4.28 – 4.14 (m, 4H, CH_2 Fm), 4.12 – 4.03 (m, 3H, H4'', CH Fm), 4.00 – 3.80 (m, 4H, H5', H5''), 2.15 (s, 3H, CH_3 Ac), 2.09 (s, 3H, CH_3 Ac), 1.96 (s, 3H, CH_3 Ac). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.02, 169.62, 169.35(CO, Ac), 164.48(CO, Bz), 152.47(CH, C2), 150.66, 150.59, 143.16, 143.12, 143.09, 143.00, 141.50, 141.46, 133.54(Cq. arom.), 133.11, 129.07, 128.05, 128.03, 127.99, 127.26, 125.25, 125.24, 125.21(arom.), 124.73 (Cq, arom.), 120.13, 120.12, 120.10, 120.09 (arom.), 101.12 (C1''), 89.59 (C1'), 86.74 (C4'), 80.40 (C4''), 77.77 (C2'), 73.75 (C3'), 70.99 (C2''), 69.51, 69.46 (CH_2 Fm), 69.30 (C3''), 66.26 (C5'), 62.90 (C5'), 47.99, 47.93 (CH Fm), 21.04, 20.78, 20.40 (CH_3 Ac). ^{31}P NMR (202 MHz, $CDCl_3$) δ -1.21. IR (film): 2924, 1743, 1609, 1507, 1452, 1448, 1229, 1219, 1078, 1030, 830, 740 cm^{-1}. HRMS (ESI^+) calcd for $C_{56}H_{53}N_5O_{15}P$ (M+H) 1066.3270. Found 1066.3298. $[\alpha]_D^{20}$ +5.8 (c = 1, in $CHCl_3$)



N^6-benzoyl-9-(3',2''3'''-tris-*O*-acetyl-5'-*O*-(*N*,*N*-diisopropylamino-*O*-cyanoethyl)phosphoramidite)-5''-*O*-(di-flourenylphosphoryl)-β-parobiosyl)adenine (3)

Compound **10** (1.38 g, 1.30 mmol), DMF (13 mL), DIPEA (0.56 mL, 3.24 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **11** (0.32 mL, 1.43 mmol) were added into the flask and stirred at room temperature for 15 minutes. Methanol (0.2 mL) was added to quench the excessive phosphorimidite after which excessive amount EtOAc was added and the mixture was washed by aq. $NaHCO_3$ (sat. 1 x), H_2O (1 x) and brine (2 x). The organic layer was dried (Na_2SO_4) and filtered. The filtration was co-evaporated with toluene (1 x) then purified by automatic column (pentane/EtOAc, 20/80 – 0/100) to furnish **3** as a white foam (1.15 g, 0.91 mmol, 70%). **Note**: Careful wash was needed for the work-up because the DIPEA in the reaction could cleave the Fm group. Automatic column was performed on Biotage Isolera Specktra Four machine using High-quality IRR silica gel column (40-63 µm). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.16 (s, 1H, NH), 8.79 (d, *J* = 7.6 Hz, 1H, H2), 8.45 (d, *J* = 8.5 Hz, 1H, H8), 8.10 – 7.93 (m, 2H, arom.), 7.76 – 7.18 (m, 19H, arom.), 6.37 – 6.21 (m, 1H, H1'), 5.48 (ddd, *J* = 20.8, 5.1, 3.2 Hz, 1H, H3'), 5.24 (d, *J* = 4.5 Hz, 1H, H1''), 5.11 (ddd, *J* = 7.5, 6.0, 3.7 Hz, 1H, H3''), 4.94 (dt, *J* = 6.3, 4.6 Hz, 1H, H2'), 4.65 (ddd, *J* = 10.9, 7.3, 4.5 Hz, 1H, H2''), 4.35 (dd, *J* = 3.1, 1.7 Hz, 1H, H4'), 4.29 – 3.72 (m, 12H, Fm, H5', H5'', O*CH*$_2$CH$_2$CN), 3.67 – 3.44 (m, 2H, *CH*(CH$_3$)$_2$), 2.71 – 2.59 (m, 2H, *CH*$_2$CN), 2.15 (d, *J* = 8.8 Hz, 3H, CH$_3$ Ac), 2.09 (s, 3H, CH$_3$ Ac), 1.83 (d, *J* = 9.3 Hz, 2H, CH$_3$ Ac), 1.20-1.14 (m, 12H, CH*(CH$_3$)$_2$*). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.09, 170.08, 169.77, 169.73, 169.41, 169.38 (CO Ac), 164.67, 164.64 (CO Bz), 151.99, 151.89 (C4), 149.69 (C6), 143.05, 143.03, 142.96, 142.94, 141.42, 141.40, 133.79 (Cq. arom.), 132.84, 132.82, 128.94, 128.91, 127.94, 127.19, 125.13, 125.10 (arom.), 123.19, 123.07 (C5), 120.06, 120.03 (arom.), 117.98, 117.76 (CN), 101.59 (C1''), 86.19, 85.84 (C1'), 83.13, 83.06, 82.82, 82.75 (C4'), 80.50, 80.43 (C4''), 79.46, 79.43 (C2'), 72.39, 72.17(C3'), 70.80, 70.77 (C2''), 69.41, 69.37 (CH_2 Fm), 69.29, 69.26 (C3''), 66.41, 66.37, 66.32 (C5''), 62.76, 62.66, 62.62, 62.54 (C5'), 58.80, 58.66, 58.63, 58.50 (O*CH2*CH$_2$CN), 47.92, 47.90, 47.86, 47.83 (CH Fm), 43.29, 43.24, 43.19, 43.15 ((CH$_3$)$_2$*CH*N), 24.78, 24.72(*(CH$_3$)$_2$*CHN), 20.95, 20.88, 20.74 (CH$_3$ Ac), 20.47, 20.41 (OCH$_2$*CH$_2$*CN), 20.19, 20.16(CH$_3$ Ac). ^{31}P NMR (202 MHz, $CDCl_3$) δ 150.02, 149.62, 14.79 (H-phosphate), -1.05, -1.07. IR (film): 2968, 1744, 1609, 1451, 1236, 1074, 1025, 981, 741 cm^{-1}.

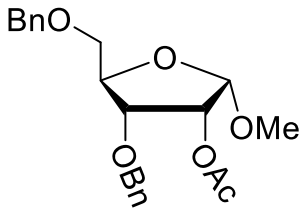
HRMS (ESI$^+$) calcd for $C_{59}H_{57}N_6O_{17}P_2$ ([H-phosphonate]+H) 1183.3250. Found 1183.3246. $[\alpha]_D^{20}$ +13.9 (c = 1, in DCM)



1-*O*-Methyl-2,3,5-tris-*O*-benzyl-αβ-D-ribofuranoside (12)

D-Ribose (5 g, 33.30 mmol), methanol (120 mL) and acyl chloride (0.62 mL, 10.99 mmol) were added into a flask and the reaction was stirred at room temperature for 5 hours after which was quenched by $NaHCO_3$ (6 g). The mixture was filtered and concentrated. The residue was then co-evaporated with toluene (3 x) and DMF (160 mL) was added into the flask. The mixture

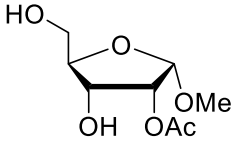
was cooled down to 0 °C then NaH (5.4 g, 166.5 mmol, 60% in mineral oil) was added. After gas generation was ceased, BnBr (15 mL, 166.5 mmol) was added in 3 portions over 10 min. The mixture was allowed to warm up to room temperature carefully and stirred for 16 h. MeOH (15 mL) was added to quench the reaction and H_2O and EtOAc were added. The water layer was washed with EtOAc then all the organic layers were combined and dried ($MgSO_4$). The mixture was filtered, concentrated and purified by silica gel column chromatography (pentane/EtOAc, 19/1 – 2/1) to obtain **12** as a colorless oil (14.5 g, 33.30 mmol, 100%). Spectroscopic data was identical with the reported same compound.[36] ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.44 – 7.12 (m, 20H, arom. αβ), 4.91 (d, *J* = 1.2 Hz, 1H, H1-β), 4.87 (d, *J* = 4.3 Hz, 0.3H, H1-α), 4.69 – 4.39 (m, 8H, CH_2-Bn-αβ), 4.35 (ddd, *J* = 7.1, 5.8, 3.7 Hz, 1H, H4-β), 4.27 – 4.21 (m, 0.3H, H4-α), 4.02 (dd, *J* = 7.1, 4.7 Hz, 1H, H3-β), 3.86 – 3.80 (m, 1.3H, H2-β, H3-α), 3.77 (dd, *J* = 6.8, 4.3 Hz, 0.3H, H2-α), 3.60 (AB, *J* = 10.6, 3.8 Hz, 1H, H5-β), 3.51 (AB, *J* = 10.6, 5.8 Hz, 1H, H5-β), 3.46 (s, 1H, OMe-α), 3.40 (AB, *J* = 10.4, 4.1 Hz, 0.3H, H5-α), 3.34 (AB, *J* = 10.4, 4.2 Hz, 0.3H, H5-α), 3.30 (s, 3H, OMe-β). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.37, 138.31, 137.98, 137.89, 137.87 (Cq. arom. αβ), 128.44, 128.41, 128.39, 128.35, 128.34, 128.31, 128.05, 127.99, 127.95, 127.91, 127.84, 127.80, 127.71, 127.69, 127.67, 127.63, 127.61, 127.54 (arom. αβ), 106.40 (C1-β), 102.53 (C1-α), 82.15(C4-α), 80.51 (C4-β), 79.75 (C2-β), 78.44 (C3-β), 77.86 (C2-α), 75.03 (C3-α), 73.48, 73.19, 72.47, 72.45, 72.35, 72.34 (CH_2 Bn-αβ), 71.37 (C5-β), 70.19 (C5-α), 55.57 (OMe-α), 55.09 (OMe-β).



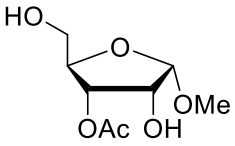
1-*O*-Methyl-2-*O*-acetyl-3,5-di-*O*-benzyl-α-D-ribofuranoside (13)

Compound **12** (8.3 g, 19.10 mmol) and DCM (95 mL) were added into a flask after which the solution was cooled down to 0 °C. $SnCl_4$ (19.1 mL, 19.1 mmol, 1M solution in DCM) was added to the reaction and the mixture was stirred at 4 °C for 16 hours. The reaction was quenched by aq. $NaHCO_3$ (sat.) and filtered. The organic filtration was washed by H_2O (1 x), brine (1 x) and dried ($MgSO_4$). The mixture was filtered, concentrated and co-evaporated with toluene (3 x). The residue was re-dissolved in pyridine (95 mL), added DMAP (117 mg, 0.96 mmol) and acetic anhydride (18.0 mL, 191.0 mmol). The reaction was stirred at room temperature for 3 hours after which was quenched by aq. $NaHCO_3$ (sat.). EtOAc was added to extract the mixture and the organic layer was further washed by H_2O (1 x) and brine (1 x). The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel column chromatography (pentane/EtOAc, 95/5 – 80/20 – 70/30) to obtain **13** as a colorless oil (6.4 g, 16.57 mmol, 87 %). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.40 – 7.22 (m, 10H), 5.12 (d, *J* = 4.5 Hz, 1H, H1), 4.91 (dd, *J* = 7.1, 4.5 Hz, 1H, H2), 4.68 (d, *J* = 12.4 Hz, 1H, C*H*H Bn), 4.53 (d, *J* = 12.1 Hz, 1H, C*H*H Bn), 4.47 (dd, *J* = 12.3, 10.4 Hz, 2H, 2xC*H*H Bn), 4.21 (d, J = 4.0 Hz, 1H, H4), 4.04 (dd, *J* = 7.1, 4.2 Hz, 1H, H3), 3.51 – 3.43 (m, 4H, OMe, H5), 3.33 (AB, *J* = 10.5, 4.2 Hz, 1H, H5), 2.20 (s, 3H, Ac). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.22 (CO Ac), 137.75, 137.74 (Cq. arom.), 128.21, 128.17, 127.97, 127.67, 127.50 (arom.), 101.64 (C1), 81.36 (C4), 75.01 (C3), 73.23, 72.90 (CH_2 Bn), 71.99 (C2), 69.25 (C5), 55.40 (OMe), 20.61 (Me Ac). IR (film): 2928, 1740, 1453, 1372, 1238, 1124, 1096, 1065, 1027, 739, 698 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{22}H_{26}O_6Na$ (M+Na) 409.1622. Found 409.1622. $[\alpha]_D^{20}$ +95.0 (c = 1, in DCM)

1-*O*-Methyl-2-*O*-acetyl-α-D-ribofuranoside (14A)/



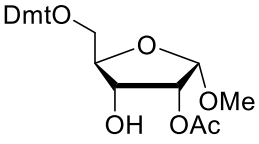
14A



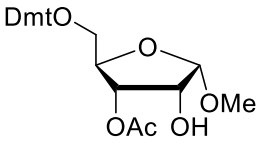
14B

1-*O*-Methyl-3-*O*-acetyl-α-D-ribofuranoside (14B)

Compound **13** (1.24 g, 3.21 mmol) was dissolved in tBuOH/Dioxane/H_2O (12 mL, 4/4/1; v/v/v). Pd/C (124 mg, 10 wt % Pd) were added and H_2 bubbled through the mixture for 24 hours at room temperature after which the reaction mixture was filtered over celite. The filtration was concentrated under reduced pressure and co-evaporated with toluene (1 x). The residue was purified by silica gel column chromatography (DCM/methanol, 100/2 – 100/5) to obtain **14** as a colorless oil (615 mg, 2.98 mmol, 93 %). (80% 2-OAC product and 20% 3-OAc product) ^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.10 (d, *J* = 4.1 Hz, 1H, H1-A), 4.96 (dd, *J* = 7.2, 3.3 Hz, 0.25H, H3-B), 4.92 (d, *J* = 4.6 Hz, 0.25H, H1-B), 4.75 (dd, *J* = 6.3, 4.1 Hz, 1H, H2-A), 4.19 (td, *J* = 7.2, 6.8, 3.5 Hz, 1H, H3-AB), 4.13 (td, *J* = 3.6, 2.4 Hz, 1H, H4-A), 4.05 (q, *J* = 3.4 Hz, 0.25H, H4-B), 3.81 – 3.73 (m, 1.5H, H5-A, H5-B), 3.69 (AB, *J* = 11.5, 3.4 Hz, 1H, H5-A), 3.45 (s, 0.75H, OMe-B), 3.41 (s, 3H, OMe-A), 2.84 (d, *J* = 9.6 Hz, 1H, 3-OH-A), 2.74 (d, *J* = 10.8 Hz, 0.25H, 3-OH-B), 2.40 (bs, 1.25H, 5-OH-AB), 2.15 (s, 3H, Ac-A), 2.11 (s, 0.75H, Ac-B). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.92, 170.47 (CO, Ac), 102.57 (C1-B), 101.96 (C1-A), 86.03 (C4-A), 83.07 (C4-B), 73.24 (C2-A), 71.49 (C3-B), 71.35 (C2-B), 70.04 (C3-A), 62.65 (C5-A), 62.51 (C5-B), 55.57 (OMe-B), 55.37 (OMe-A), 21.00 (CH_3 Ac-B), 20.77 (CH_3 Ac-A). IR (film): 3444, 2932, 1735, 1374, 1234, 1081, 1028, 964, 899, 5002, 607, 479 cm^{-1}. HRMS (ESI$^+$) calcd for $C_8H_{14}O_6Na$ (M+Na) 229.0683. Found 229.0685. $[\alpha]_D^{20}$ +112.3 (c = 1, in DCM)



15A



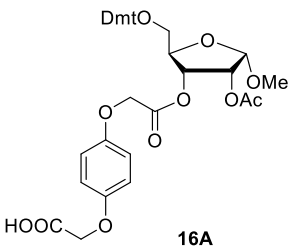
15B

1-*O*-Methyl-2-*O*-acetyl-5-O-(4,4'-di-methoxyltrityl)-α-D-ribofuranoside (15A) /

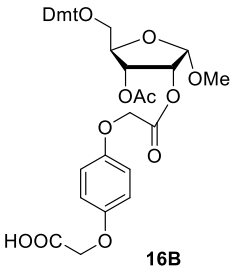
1-*O*-Methyl-3-*O*-acetyl-5-O-(4,4'-di-methoxyltrityl)-α-D-ribofuranoside (15B)

Compound **14** (584 mg, 2.83 mmol), 4,4'-dimethoxyltrityl chloride (DMTCl, 1.01 g, 2.98 mmol), and pyridine were added into a flask and the solution was stirred for 16 hours at room temperature after which was concentrated. The residue was dissolved in EtOAc and washed by aq. $NaHCO_3$ (sat.). The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel column chromatography (pentane/EtOAc, 100/0 – 90/10 – 80/20 – 60/40) to obtain **15** as a light yellow foam (1.31 g, 2.58 mmol, 91 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.46 – 7.42 (m, 2H, DMT arom.), 7.38 – 7.16 (m, 7H, DMT arom.), 6.88 – 6.80 (m, 4H, DMT arom.), 5.22 (d, *J* = 4.1 Hz, 0.25H, H1-B), 5.16 (dd, *J* = 7.0, 2.8 Hz, 0.75H, H3-A), 5.06 – 5.04 (m, 1H, H1-A, H2-B), 4.44 (dd, *J* = 7.0, 4.7 Hz, 0.75H, H2-A), 4.27 – 4.24 (m, 0.5H, H3-B, H4-B), 4.16 (q, *J* = 3.3 Hz, 0.75H, H4-A), 3.79 (s, 6H, OMe DMT), 3.51 (s, 2.25H, OMe-A), 3.47 (s, 0.75H, OMe-B), 3.39 – 3.33 (m, 1H, H5), 3.25 – 3.16 (m, 1H, H5), 2.72 (s, 1H, OH), 2.20 (s, 0.75H, Ac-B), 2.10 (s, 2.25H, Ac-A). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.44(CO Ac-A), 170.30 (CO Ac-B), 158.55, 144.83, 144.69, 136.03, 135.96, 135.87, 135.76 (Cq. arom.), 130.40, 130.13, 128.22, 127.91, 126.87, 113.21 (arom.), 102.59 (C1-A), 101.94(C1-B), 86.27, 86.26 (Cq. DMT), 85.50 (C4-B), 81.97 (C4-A), 73.34 (C2-B), 72.08 (C3-A), 71.42 (C2-A), 70.73 (C3-B), 63.68 (C5-A), 63.67 (C5-B), 55.70 (OMe), 55.34 (OMe DMT-B), 55.25 (OMe DMT-A), 21.01 (CH_3 Ac-A), 20.85 (CH_3 Ac-B).

IR (film): 3507, 2933, 2837, 1741, 1608, 1509, 1446, 1300, 1248, 1177, 1077, 1035, 830, 596 cm^{-1}. HRMS (ESI^+) calcd for $C_{29}H_{32}O_8Na$ (M+Na) 531.1989. Found 531.1992. $[\alpha]_D^{20}$ +53.1 (c = 1, in DCM)



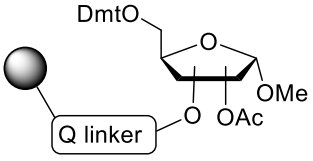
16A



16B

1-*O*-Methyl-2-*O*-acetyl-3-*O*-hydroquinone-*O,O'*-diacetylhemiester-5-O-(4,4'-di-methoxyltrityl)-α-D-ribofuranoside (16A) / 1-*O*-Methyl-2-*O*-hydroquinone-*O,O'*-diacetylhemiester-5-*O*-(4,4'-di-methoxyltrityl)-3-*O*-acetyl-α-D-ribofuranoside (16B)

Compound **15** (1.22 g, 2.40 mmol) was dissolved in pyridine (12 mL). DMAP (29 mg, 0.24 mmol), EDC (446 mg, 2.88 mmol), Et_3N (0.24 mL, 1.73 mmol) and hydroquinone-O,O'-diacetic acid (Q-linker) (650 mg, 2.88 mmol) were added and the reaction was stirred at room temperature for 16 hours. The reaction mixture was concentrated, diluted with $CHCl_3$ and washed with H_2O. The water layer was extracted with $CHCl_3$ and the combined organic layers were dried ($MgSO_4$), concentrated and purified by silica gel chromatography neutralized with 1% Et_3N (DCM/methanol, 100/0 – 99/1 – 95/5 – 90/10) to obtain **16** as a white foam (962 mg, 1.34 mmol, 56%). ^1H NMR (500 MHz, Chloroform-*d*) δ 7.38 – 7.32 (m, 2H, arom.), 7.27 – 7.18 (m, 6H, arom.), 7.17 – 7.10 (m, 1H, arom.), 6.85 – 6.73 (m, 8H, arom.), 5.35 (dq, *J* = 7.5, 2.6 Hz, 1/3H, H3-B), 5.28 – 5.22 (m, 2/3H, H3-A), 5.19 (dd, *J* = 5.4, 3.7 Hz, 4/3H, H1-A, H2-A), 5.17 – 5.12 (m, 2/3H, H1-B, H2-B), 4.63 – 4.48 (m, 2H, CO*CH*$_2$O), 4.39 – 4.37 (m, 2H, *CH*$_2$COOH), 4.15 (q, *J* = 3.4 Hz, 1H, H4), 3.72 – 3.71 (m, 6H, OMe DMT), 3.38 (s, 3H, OMe), 3.33 – 3.29 (m, 1H, H5), 3.20 – 3.13 (m, 1H, H5), 2.01 (s, 1H, CH_3 Ac-B), 1.97 (s, 2H, CH_3 Ac-A). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.30(COOH-A), 174.28 (COOH-B), 170.49 (CO Ac-A), 170.03(CO Ac-B), 168.82(CH_2*CO*O-B), 168.45(CH_2*CO*O-A), 158.66, 158.65, 153.83, 152.15, 152.13, 144.75, 136.00, 135.96, 135.85, 135.78 (Cq. arom.), 130.21, 130.19, 128.29, 128.01, 126.98, 126.97, 116.00, 115.91, 115.71, 113.31 (arom.), 101.66 (C1-B), 101.64 (C1-A), 86.47 (Cq. DMT-B), 86.43(Cq. DMT-A), 81.21 (C4-B), 81.03 (C4-A), 71.78 (C2-A), 71.64 (C3-B), 71.42 (C2-B), 70.60 (C3-A), 67.62 (*CH*$_2$COOH), 66.25 (*CH*$_2$COO-B), 66.05 (*CH*$_2$COO-A), 63.45 (C5-B), 63.41 (C5-A), 55.88 (OMe-A), 55.76 (OMe-B), 55.36 (OMe DMT), 20.93 (CH_3 Ac-A), 20.75 (CH_3 Ac-B). IR (film): 2934, 1738, 1607, 1507, 1445, 1246, 1178, 1073, 1032, 828cm^{-1}. HRMS (ESI^+) calcd for $C_{39}H_{40}O_{13}Na$ (M+Na) 739.2361. Found 739.2364. $[\alpha]_D^{20}$ +33.0 (c = 1, in DCM)



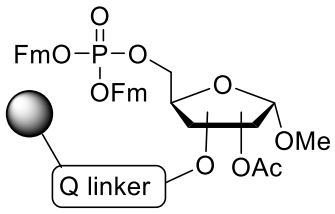
18a (CPG)
18b (TG)

1-*O*-Methyl-2-*O*-acetyl-3-*O*-Q-CPG-5-*O*-(4,4'-di-methoxyltrityl)-α-ribofuranoside/ 1-*O*-Methyl-2-*O*-Q-CPG-3-*O*-acetyl-5-*O*-(4,4'-di-methoxyltrityl)-α-D-ribofuranoside (18a, CPG)

1-*O*-Methyl-2-*O*-acetyl-3-*O*-Q-TG-5-*O*-(4,4'-di-methoxyltrityl)-α-ribofuranoside/ 1-*O*-Methyl-2-*O*-Q-TG-3-*O*-acetyl-5-*O*-(4,4'-di-methoxyltrityl)-α-D-ribofuranoside (18b, TG)

To a 20 mL reaction syringe with filter frit was added LCAA-CPG (3 g, 0.20 mmol) or Tentagel N NH2 (800 mg, 0.20 mmol), MeCN (12 mL), compound **16** (430 mg, 0.6 mmol), HOBT (12 mg, 0.08 mmol), DIC (0.28 mL, 1.8 mmol) and DIPEA (0.52 mL, 3 mmol). The mixture was shaken at room temperature for 16 hours. The reaction mixture was drained and the CPG/Tentagel was washed with ACN (2 x), DMF (2 x) and DCM (3 x) under N_2. The remaining

unmodified amine groups were capped by adding a mixture of CAP 1 (6 mL) and CAP 2 (6 mL). The mixture was shaken for 2 hours, drained and washed with DMF (3 x) and DCM (3 x) under N_2. The CPG/Tentagel was dried under reduced pressure and the loading was determined by trityl analysis at 503 nm. The loadings for **18a** (CPG) and **18b** (TG) are 50 µmol/g and 207 µmmol/g respectively.



1a (CPG)
1b (TG)

1-*O*-Methyl-2-*O*-acetyl-3-*O*-Q-CPG-5-*O*-(di-*O*-fluorenylmethylphosphoryl)-α-D-ribofuranoside/ 1-*O*-Methyl-2-*O*-Q-CPG-3-*O*-acetyl-5-*O*-(di-*O*-fluorenylmethylphosphoryl)-α-D-ribofuranoside (1a, CPG)

1-*O*-Methyl-2-*O*-acetyl-3-*O*-Q-TG-5-*O*-(di-*O*-fluorenylmethylphosphoryl)-α-D-ribofuranoside/ 1-*O*-Methyl-2-*O*-Q-TG-3-*O*-acetyl-5-*O*-(di-*O*-fluorenylmethylphosphoryl)-α-D-ribofuranoside (1b, TG)

To a 20 mL reaction syringe with filter frit was added **18a** (3 g) or **18b** (900 mg). Dichloroacetic acid (5 %, v/v, in DCM) was added repeatedly until no yellow color was observed. The resin was extensively washed with DCM (3 x), ACN (5 x) under N_2. ETT (0.25M in ACN, 12 eq) and **9** (0.2 M in ACN, 4 eq) were added into the resin and the mixture was shaken under N_2 for 10 minutes and drained. Repeat this coupling 1 more time and the resin was drained and washed with ACN (5 x). 9 mL (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) (0.5 M in ACN) was added and the mixture was shaken for 30 minutes under N_2. The resin was drained, washed with ACN (5 x) under N_2 and dried under reduced pressure to obtain 1a or 1b which was stored at 4 °C before use. A test sample of **1a** (60 mg) or **1b** (20 mg) was added into a 2 mL reaction syringe. To this syringe was added 1 mL DBU solution (10%, v/v, in ACN) and was shaken for 10 min to remove Fm groups on 5-phosphate after which was drained and washed with ACN (3 x). Treatment with 1mL NH_4OH (35%) for 1 hour to cleave the product from resin and the filtration was concentrated. ^{31}P NMR (162 MHz, D_2O): **1a** δ 5.28 (s); **1b** δ 5.30 (s)

1-*O*-methyl-α-ADPr dimer (21a, manual synthesis)

To a 5 mL reaction syringe with filter frit, **1a** (200 mg, 10 µmol) or **1b** (50 mg, 10 µmol) was added, washed with ACN (3 x) under N_2. 2 mL DBU solution (10%, v/v, in ACN) and was shaken for 15 min (two times) to remove Fm groups on 5-phosphate after which was drained and washed with ACN (5 x) to give **19a/b**. Then:

Cycle A: ETT (0.48 mL, 0.25 M in ACN) and **3** (0.4 mL, 0.2M in ACN) were added into the resin and the mixture was shaken for 10 minutes, drained and followed by a second addition of ETT and **3**. The mixture was drained and the resin was washed with ACN (5 x) under N_2. The intermediate phosphate-phosphite was oxidized with (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) solution (2 mL, 0.5 M in ACN) for 5 minutes (2 x) and washed with ACN (5 x). DBU solution (2 mL, 10%, v/v, in ACN) was added into the syringe and was shaken for 15 min (2 x) to remove Fm groups on 5'-phosphate and CE group after which was drained and washed with ACN (5 x) under N_2.

Cycle B: ETT (0.48 mL, 0.25 M in ACN) and **4** (0.4 mL, 0.2M in ACN) were added to the resin and the mixture was shaken for 10 minutes, drained and followed by a second addition of ETT and **4**. The mixture was drained and the resin was washed with ACN (5 x) under N_2. The intermediate phosphate-phosphite was oxidized with (1S)-

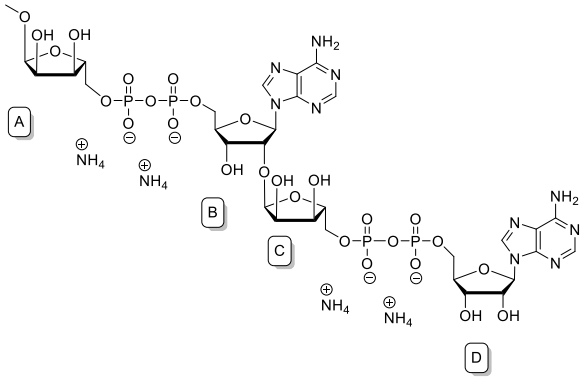
(+)-(10-camphorsulfonyl)-oxaziridine (CSO) solution (2 mL, 0.5 M in ACN) for 5 minutes (2 x) and washed with ACN (5 x). DBU solution (2 mL, 10%, v/v, in ACN) was added into the syringe and was shaken for 10 min to remove CE group after which the syringe was drained and the resin was washed with ACN (5 x) under N_2. The resin was treated with NH_4OH (35%) overnight to cleave the product from the resin and remove all the protecting groups. The mixture was filtered and the filtrate was concentrated.

The crude material was purified by anion exchange to obtain ADPr dimer **21a** (0.32 mg, 0.27 µmol, 3%, from CPG; 3.23 mg, 2.73 µmol, 27%, from TG) as a white solid.

Column: Resource Q 6mL.

Gradient: 30% - 70%. (A: 10 mM NH_4OAc, B: 1 M NH_4OAc)

Dimer **21a**:



^{1}H NMR (500 MHz, Deuterium Oxide) δ 8.47 (s, 2H, H2), 8.27 (d, *J* = 2.7 Hz, 2H, H8), 6.19 (d, *J* = 3.1 Hz, 1H, H1-B), 5.98 (d, *J* = 5.9 Hz, 1H, H1-D), 5.30 (d, *J* = 4.3 Hz, 1H, H1-C), 4.93 – 4.90 (m, 1H, H1-A), 4.69 – 4.63 (m, 1H, H2-D), 4.55 (dd, *J* = 5.3, 3.1 Hz, 1H, H2-B), 4.53 – 4.43 (m, 2H, H3-B, H3-D), 4.39 – 4.25 (m, 4H, H4-BCD, H5-B), 4.25 – 4.15 (m, 6H, H2-A, H3-A, H4-A, H5-B, H5-D), 4.15 – 4.10 (m, 2H, H2-C, H3-C), 4.03 – 4.00 (m, 4H, H5-A, H5-C), 3.36 (s, 3H, OMe). ^{13}C NMR (126 MHz, D_2O) δ 152.43, 152.30 (C4), 148.37, 147.92 (C6), 118.40, 118.19 (C5), 103.20 (C1-A), 101.24 (C1-C), 87.27 (C1-D), 87.09 (C1-B), 84.19, 84.15, 84.13 (C4-C, C4-D), 83.17, 83.10, 83.02 (C4-B, C4-A), 78.87 (C2-B), 74.57 (C2-D), 71.33 (C2-A), 70.78 (C2-C), 70.49 (C3-D), 69.78 (C3-A), 69.61 (C3-C), 68.72 (C3-B), 65.59, 65.55 (C5-A, C5-C), 65.23, 65.21 (C5-D), 64.29, 64.25 (C5-B), 55.40 (OMe). ^{31}P NMR (202 MHz, D_2O) δ -11.08, -11.18, -11.26, -11.32, -11.37, -11.44, -11.54. LC-MS: Rt = 2.98 min. 0-50% NH_4OAc. ESI MS+ calc. 1115.2 found 1115.2 $[M+1]^+$. HRMS (ESI^+) calcd for $C_{31}H_{47}N_{10}O_{27}P_4$ (M+H) 1115.1557. Found 1115.1558.

1-*O*-methyl-α-ADPr trimer 21b (DNA synthesizer)

200 mg **1b** was added into a 5 mL reaction syringe with filter frit and the resin was washed with ACN (5 x) under N_2. 3 mL DBU solution (10%, v/v, in ACN) was added into the syringe and was shaken for 20 minutes to remove Fm groups on 5-phosphate after which was drained. The DBU treatment was repeated for another 20 minutes. The resin was washed with ACN (5 x) and dried under reduced pressure to remove traceless water before use. 50 mg resin from above was transferred into a reaction column of a Mermade 6 oligonucleotide synthesizer and the complete synthesis was performed under an argon atmosphere. For trimer synthesis, Cycle A was performed 2 times and Cycle B was performed 1 time.

Cycle A: The resin was rinsed with ACN (3 x) and drained. BTT (600 uL, 0.25 M in ACN) and LIU-78 (300 uL, 0.1M in ACN) were added into the resin and the mixture was left to stand for 10 minutes, drained. Repeat this coupling for two more times. The resin was rinsed by with ACN (3 x). The intermediate phosphate-phosphite was oxidized with (1*S*)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) solution (2 mL, 0.5 M in MeCN) for 5 minutes (2 x). The resin was drained and washed with ACN (3x). DBU solution (2 mL, 10%, v/v, in ACN) was added into the resin and was left to stand for 10 minutes (4 x) to remove Fm groups on 5'-phosphate and CE group after which was drained and washed with ACN (3 x).

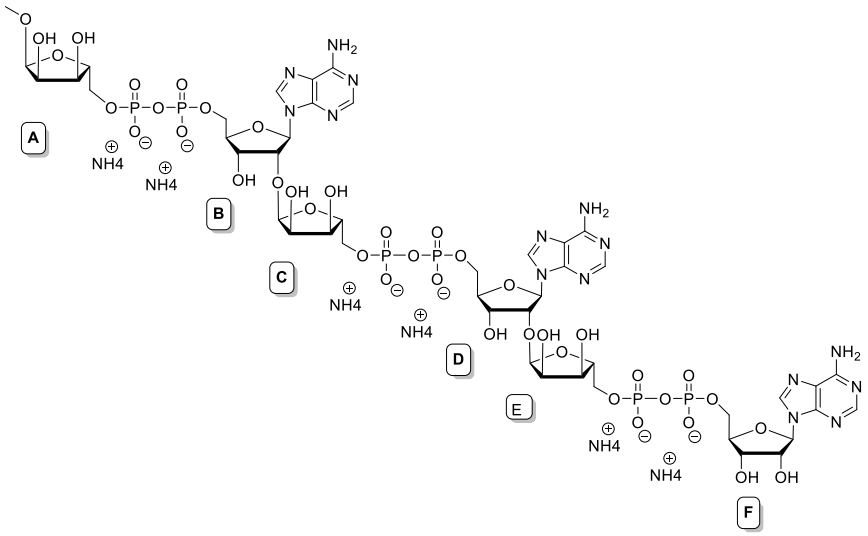
Cycle B: The resin was rinsed with ACN (3x) and drained. BTT (600 uL, 0.25 M in ACN) and **4** (300 uL, 0.1M in ACN) were added into the resin and the mixture was left to stand for 10 minutes, drained. Repeat this coupling for two more times. The resin was rinsed by with ACN (3x). The intermediate phosphate-phosphite was oxidized with CSO solution (2 mL, 0.5 M in MeCN) for 5 minutes (2 x). The resin was drained and washed with ACN (3 x). DBU solution (2 mL, 10%, v/v, in ACN) was added into the resin and was left to stand for 10 minutes to remove CE group after which was drained and washed with ACN (3 x).

The resin was transferred to a tube and treated with 10 mL NH_4OH (35%). The mixture was stirred overnight in a sealed condition, filtered and concentrated. The crude was purified by anion exchange to obtain ADPr trimer **21b** (6.16 mg, 3.51 µmol, 35%) and ADPr dimer **21a** (4.5 mg, 3.81 µmol, 38%) as white solid.

Column: Resource Q 6mL.

Gradient: 30% - 70%. (A: 10 mM NH_4OAc, B: 1 M NH_4OAc)

Trimer **21b**:



^{1}H NMR (500 MHz, Deuterium Oxide) δ 8.36 – 8.24 (m, 3H, H2), 8.09 – 7.97 (m, 3H, H8), 6.13 (d, *J* = 3.2 Hz, 1H, H1-B), 5.97 (d, *J* = 3.1 Hz, 1H, H1-D), 5.94 (d, *J* = 5.9 Hz, 1H, H1-F), 5.30 (d, *J* = 4.2 Hz, 1H, H1-C), 5.19 (d, *J* = 4.1 Hz, 1H, H1-E), 4.85 – 4.82 (m, 1H, H1-A), 4.63 (t, *J* = 5.5 Hz, 1H, H2-F), 4.57 (dd, *J* = 5.3, 3.3 Hz, 1H, H2-B), 4.54 (t, *J* = 5.7 Hz, 1H, H3-B), 4.48 – 4.40 (m, 3H, H2-D, H3-F, H3-D), 4.34 – 3.94 (m, 24H), 3.33 (s, 3H, OMe). ^{13}C NMR (214 MHz, D_2O) δ 155.66, 155.55, 155.45 (C4), 149.44, 149.14, 148.84 (C6), 119.28, 119.11, 119.00 (C5), 104.13 (C1-A), 102.35 (C1-E), 102.24 (C1-C), 87.87 (C1-F), 87.71 (C1-D), 87.40 (C1-B), 85.18, 85.14, 85.10, 84.82, 84.78, 84.09, 84.05, 84.01, 83.96, 83.81, 83.78 (C4-ABCDEF), 80.26 (C2-D), 79.69 (C2-B), 75.38 (C2-F), 72.33, 72.30, 71.70, 71.41,

70.82, 70.81, 70.80, 70.55, 70.05, 69.87 (the rest C2, C3), 66.50, 66.19, 65.51 (C5-ABCDEF), 56.33, 56.32 (OMe). ^{31}P NMR (202 MHz, D_2O) δ -10.45, -10.51, -10.55, -10.61, -10.67, -10.69, -10.71, -10.78, -10.80, -10.82. LC-MS: Rt = 2.96 min. 0-50% NH_4OAc. ESI MS+ calc. 1656.2 found 1656.2 $[M+1]^+$. HRMS (ESI^+) calcd for $C_{46}H_{68}N_{15}O_{40}P_6$ (M+H) 1656.2168. Found 1656.2196.

1-*O*-methyl-α-ADPr pentamer/tetramer/trimer (21b-d, from pentamer synthesis)

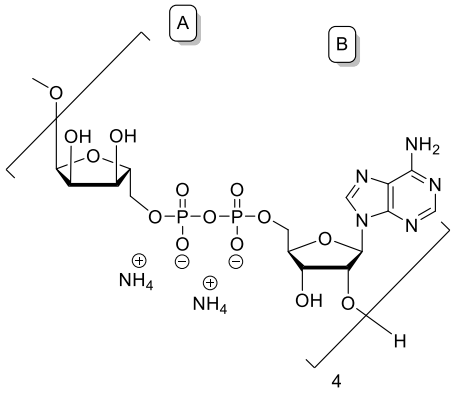
21b-d was synthesized according to the similar procedure as that of **21a**. Cycle A was performed 4 times and cycle B was performed 1 time after which the resin was transferred into a tube and treated with NH_4OH (35%) overnight in a sealed condition. The mixture was filtered, concentrated under reduced pressure and purified by anion exchange and gel filtration to obtain ADPr trimer **21b** (1.18 mg, 0.71 μmol, 7%), tetramer **21c** (1.35 mg, 0.61 μmol, 6%) and pentamer **21d** (0.69 mg, 0.25 μmol, 3%) as white solid.

Anion exchange column: Resource Q 6mL. Gradient: 50% - 80%. (A: 10 mM NH_4OAc, B: 1 M NH_4OAc)

Gel filtration gradient: 20% ACN in 0.15 M aq. NH_4HCO_3.

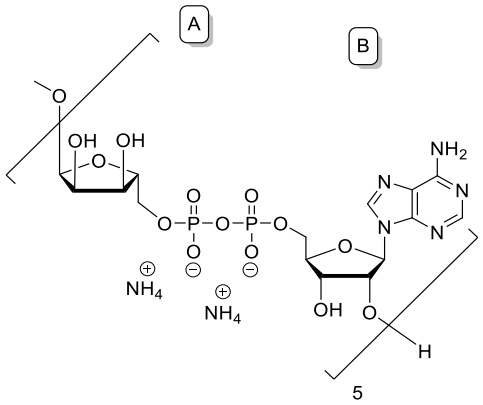
Trimer **21b**: Identical data with manual synthesis.

Tetramer **21c**:

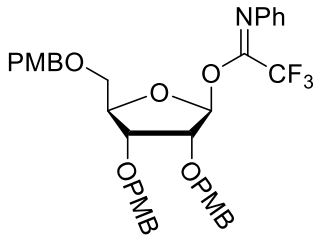


^{1}H NMR (850 MHz, Deuterium Oxide) δ 8.39 (s, 1H), 8.38 (s, 1H), 8.32 (s, 1H), 8.31 (s, 1H, H2-ade), 8.10 (d, *J* = 1.2 Hz, 1H), 8.08 (d, *J* = 1.2 Hz, 1H), 8.06 (d, *J* = 1.1 Hz, 1H), 8.02 (d, *J* = 1.1 Hz, 1H, H8-ade), 6.17 (d, *J* = 3.5 Hz, 1H, H1-B), 6.03 (d, *J* = 3.0 Hz, 2H, H1-B), 5.99 (d, *J* = 5.8 Hz, 1H, H1-B), 5.34 (d, *J* = 4.3 Hz, 1H, H1-A), 5.27 (t, *J* = 4.5 Hz, 2H, H1-A), 4.92 – 4.89 (m, 1H, H1-A), 4.66 – 4.65 (m, 2H), 4.58 (t, *J* = 5.7 Hz, 1H), 4.55 (dd, *J* = 5.3, 3.8 Hz, 1H), 4.52 (t, *J* = 5.5 Hz, 1H), 4.50 – 4.45 (m, 3H), 4.39 – 4.01 (m, 32H), 3.38 (d, *J* = 1.0 Hz, 3H, OMe). ^{31}P NMR (202 MHz, D_2O) δ -11.10, -11.21, -11.24, -11.31, -11.34, -11.43, -11.47. LC-MS: Rt = 2.83 min. 0-50% NH_4OAc. ESI MS+ calc. 1099.1 found 1099.8 $[M+2]^+$. HRMS (ESI^+) calcd for $C_{61}H_{90}N_{20}O_{53}P_8$ (M+2H)/2 1099.1426. Found 1099.1446.

Pentamer **21d**:



^{1}H NMR (850 MHz, Deuterium Oxide) δ 8.38 (s, 1H), 8.33 (s, 1H), 8.31 – 8.26 (m, 3H, H2-ade), 8.12 (s, 1H), 8.02 (d, *J* = 3.7 Hz, 2H), 7.98 (d, *J* = 5.0 Hz, 2H, H8-ade), 6.19 (d, *J* = 3.8 Hz, 1H, H1-B), 6.08 – 6.05 (m, 2H, H1-B), 6.04 (t, *J* = 1.8 Hz, 1H, H1-B), 5.98 (d, *J* = 5.9 Hz, 1H, H1-B), 5.31 (d, *J* = 4.2 Hz, 1H, H1-A), 5.26 (d, *J* = 3.6 Hz, 1H, H1-A), 5.24 (s, 1H, H1-A), 5.22 (d, *J* = 4.2 Hz, 1H, H1-A), 4.87 (d, *J* = 2.5 Hz, 1H, H1-A), 4.65 (dt, *J* = 10.4, 4.9 Hz, 2H), 4.59 (t, *J* = 5.5 Hz, 1H), 4.52 (d, *J* = 3.3 Hz, 5H), 4.49 – 4.46 (m, 1H), 4.39 – 3.98 (m, 41H), 3.37 (s, 3H, OMe). ^{31}P NMR (202 MHz, D_2O) δ -11.10, -11.10, -11.12, -11.21, -11.25, -11.31, -11.36, -11.45. LC-MS: Rt = 2.84 min. 0-50% NH_4OAc. ESI MS+ calc. 913.4 found 913.1 $[M+3]^+$. HRMS (ESI^+) calcd for $C_{61}H_{90}N_{20}O_{53}P_8$ (M+2H)/2 1369.6732. Found 1369.6742.



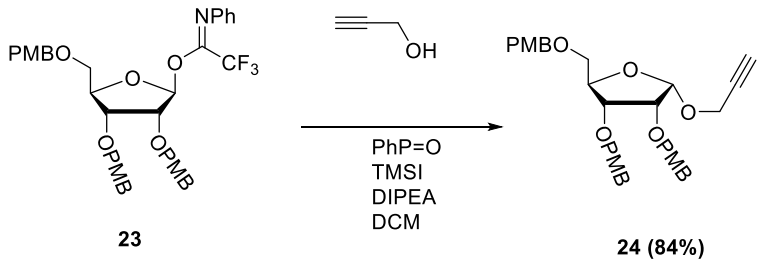
1-*O*-((*N*-Phenyl)-2,2,2-trifluoroacetimido)-2,3,5-tri-*O*-*p*-methoxylbenzyl-β-D-ribofuranose (23)

Compund **22**[31] (9.3 g, 18.23 mmol) was dissolved in acetone (93 mL). Cs_2CO_3 (8.89 g, 27.34 mmol,) and 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (3.23 mL, 20.05 mmol) were added and the reaction mixture was stirred at room temperature for 3 hours. After filtered over celite, the solvent was removed and the residue was purified using silica gel column chromatography neutralized with 1% Et_3N (Pentane/EA, 100/0 – 90/10 – 80/20) to afford the title compound as a light yellow oil (11.67 g, 17.13 mmol, 94 %).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.35 – 7.04 (m, 9H, arom.), 6.89 – 6.75 (m, 8H, arom), 6.27 (s, 1H, H1), 4.77 – 4.31 (m, 7H, CH_2 PMB, H4), 4.08 (t, *J* = 6.1 Hz, 1H, H3), 4.01 (d, *J* = 4.6 Hz, 1H, H2), 3.82 – 3.76 (m, 9H, OMe), 3.68 – 3.65 (m, 1H, H5), 3.54 (AB, *J* = 11.2, 5.2 Hz, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.60, 159.54, 159.28, 143.97, 130.42 (Cq. arom.), 129.96 (arom.), 129.76 (Cq. arom.),, 129.66 (arom.), 129.58 (arom.), 129.53 (Cq. arom.), 129.34, 129.32, 128.85, 113.96, 113.92, 113.84, 113.80 (arom.), 102.65 (C1), 82.34 (C4), 78.17 (C2), 77.05 (C3), 73.27, 73.04, 72.42, 72.22, 72.01 (CH_2 PMB-αβ), 69.89, 69.54 (C5-αβ), 55.40, 55.39 (OMe, αβ). IR (film): 2935, 2837, 1709, 1612, 1512, 1302, 1246, 1205, 1156, 1110, 1033, 819, 755, 695, 515 cm^{-1}. HRMS (ESI^+) calcd for $C_{29}H_{34}O_8Na$ (M+Na) 533.2146. Found 533.2147. $[\alpha]_D^{20}$ +41.6 (c = 1, in DCM)

1-*O*-Propargyl-2,3,5-tri-*O*-*p*-methoxylbenzyl-α-D-ribofuranose (24)

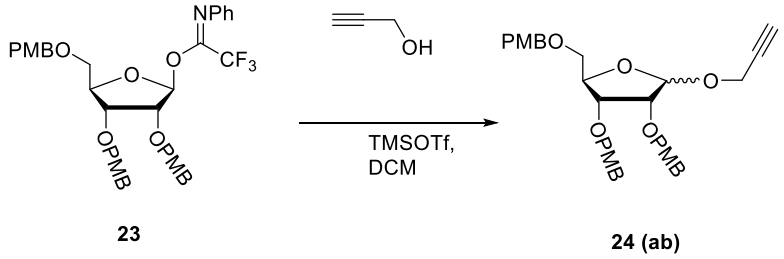
Method 1:



23 **24 (84%)**

Compound **23** (3.07 g, 4.50 mmol) were added into a flask and co-evaporated with toluene (3 x). $Ph_3P=O$ (7.51 g, 27 mmol), DCM (45 mL), freshly flame-dried 3Å molecular sieves, DIPEA (0.78 mL, 4.50 mmol) and propargyl alcohol (0.52 mL, 9.00 mmol) were added into the flask contain **23** after which it was cooled down to 0 °C. TMSI (0.64 mL, 4.5 mmol) was then added into the reaction under N_2 and the mixture was stirred for 1 hour before it was quenched by aq. $Na_2S_2O_3$ (sat.). The reaction was diluted with DCM and the organic layer was washed with H_2O (1 x) and brine (1 x), dried ($MgSO_4$), filtered and concentrated. Silica gel chromatography purification (pentane/EtOAc, 100/0 – 80/20) afforded **24** as a light yellow oil (2.07 g, 3.78 mmol, 84%).

Method 2:



23 **24 (ab)**

Compound **23** (1.64 g, 2.41 mmol), propargyl alcohol (0.14 mL, 2.41 mmol, **23** and propargyl alcohol were co-evaporated with dioxane for 2 times), DCM (24 mL) and freshly flame-dried 3Å molecular sieves were added into a flask and stirred under N_2 for 1 hour. The mixture was cooled down to -78 °C and TMSOTf (8.7 µL, 48 µmol) was added. The reaction was stirred at same temperature for 20 minutes after which it was quenched by addition of excessive amount of TEA and concentrated. Silica gel chromatography purification (pentane/EtOAc, 95/5 – 75/25) afforded **24** (α and β anomer) as a light yellow oil. (α-anomer: 272 mg, 0.50 mmol, 21%; β-anomer: 205 mg, 0.37 mmol, 15%).

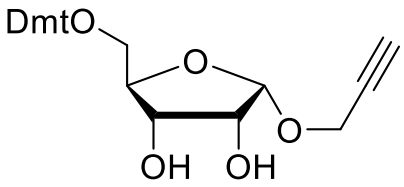
α anomer:

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.31 – 7.24 (m, 2H), 7.24 – 7.18 (m, 2H), 7.17 – 7.09 (m, 2H), 6.91 – 6.78 (m, 6H, arom. PMB), 5.31 (dd, *J* = 2.9, 1.4 Hz, 1H, H1), 4.66 – 4.31 (m, 8H, CH_2 PMB, O*CH*$_2$CCH), 4.20 (td, *J* = 4.1, 2.2 Hz, 1H, H4), 3.85 – 3.74 (m, 11H, H2, H3, OMe), 3.37 (AB, *J* = 10.5, 4.0 Hz, 1H, H5), 3.31 (AB, *J* = 10.5, 4.2 Hz, 1H, H5), 2.39 (t, *J* = 2.4 Hz, 1H, OCH_2C*CH*). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.37, 159.28, 159.24, 130.40, 130.05, 129.93 (Cq. arom.), 129.86, 129.74, 129.33, 113.80, 113.70 (arom.), 98.89 (C1), 82.56 (C4), 79.67 (Cq. CH_2*C*CH), 77.22 (C2), 74.77 (C3), 74.32 (CH_2C*CH*), 73.15, 71.99, 71.87 (CH2 PMB), 69.75 (C5), 55.33 (CH3 PMB), 54.24 (O*CH*$_2$CCH). IR (film): 3281, 2910, 1611, 1585, 1512, 1247, 1032, 819, 750 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{32}H_{40}O_8N$ (M+NH_4) 566.2748. Found 566.2745. $[\alpha]_D^{20}$ +68.6 (c = 1, in DCM)

β anomer:

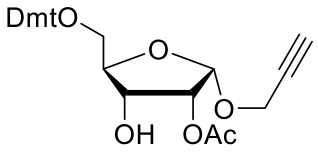
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.35 – 7.13 (m, 6H), 6.92 – 6.77 (m, 6H, arom. PMB), 5.16 (s, 1H, H1), 4.71 – 4.26 (m, 7H, CH_2 PMB, H4), 4.16 (dd, *J* = 3.4, 2.4 Hz, 1H, O*CH*$_2$CCH), 4.01 (dd, *J* = 7.5, 4.7 Hz, 1H, H3), 3.86 (d, *J* =

4.7 Hz, 1H, H2), 3.82 – 3.71 (m, 9H, OMe PMB), 3.57 (AB, *J* = 10.7, 3.4 Hz, 1H, H5), 3.44 (AB, *J* = 10.7, 5.7 Hz, 1H, H5), 2.39 (t, *J* = 2.4 Hz, 1H, $OCH_2C\mathit{CH}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.43, 159.36, 159.18, 130.43, 129.96, 129.90 (Cq. arom.), 129.76, 129.56, 129.25, 113.89, 113.80, 113.77 (arom.), 103.34 (C1), 80.83 (C4), 79.28 (Cq. $CH_2\mathit{C}CH$), 79.21 (C2), 77.82 (C3), 74.55 ($CH_2C\mathit{CH}$), 72.81, 72.11, 72.05 (CH_2 PMB), 70.70 (C5), 55.34 (CH_3 PMB), 55.32 (CH_3 PMB), 54.07 ($O\mathit{CH_2}CCH$). IR (film): 3281, 2931, 2836, 1611, 1585, 1512, 1301, 1244, 1173, 1094, 1030, 818, 516cm^{-1}. HRMS (ESI$^+$) calcd for $C_{32}H_{40}O_8N$ (M+NH_4) 566.2748. Found 566.2747. $[\alpha]_D^{20}$ +17.4 (c = 1, in DCM)

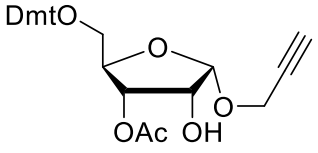


1-*O*-Propargyl-*O*-(4,4'-di-methoxyltrityl)-α-D-ribofuranose (25)

Compound **24** (2.07 g, 3.78 mmol), DCM (37 mL) and TFA (1.74 mL, 22.68 mmol) were added into the flask and the mixture was stirred for 1 hour after which it was diluted with toluene and concentrated. The residue was co-evaporated with toluene (2 x), pyridine (2 x) before pyridine (25 mL) and DMTCl (2.56 g, 7.56 mml) was added. The reaction was stirred for 1 hour and quenched by addition of aq. $NaHCO_3$ (sat.). DCM extracted the mixture (3 x) and the organic layers were combined, dried ($MgSO_4$), filtered and concentrated. Silica gel chromatography purification (silica gel was neutralized with 2% TEA in pentane before use, pentane/EtOAc, 100/0 – 70/30 – 50/50) afforded **25** as a colorless oil. (1.34 g, 2.73 mmol, 72%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 – 7.37 (m, 2H), 7.36 – 7.15 (m, 9H, arom. DMT), 6.87 – 6.78 (m, 4H, DMT), 5.32 (d, *J* = 4.5 Hz, 1H, H1), 4.44 – 4.35 (m, 2H, $O\mathit{CH_2}CCH$), 4.35 – 4.28 (m, 1H, H2), 4.17 (td, *J* = 3.6, 2.3 Hz, 1H, H4), 4.02 (dd, *J* = 4.8, 2.9 Hz, 1H, H3), 3.78 (s, 6H, OMe DMT), 3.34 (AB, *J* = 10.2, 3.6 Hz, 1H, H5), 3.14 (AB, *J* = 10.2, 3.7 Hz, 1H, H5), 2.95 (d, *J* = 9.5 Hz, 1H, 2-OH), 2.55 (d, *J* = 8.0 Hz, 1H, 3-OH), 2.46 (t, *J* = 2.4 Hz, 1H, $CH_2C\mathit{CH}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.58, 144.81, 136.03, 135.84 (Cq. arom.), 130.16, 130.14, 128.21, 127.97, 126.92, 113.26, 113.25 (arom.), 100.42 (C1), 86.27 (Cq. DMT), 85.05 (C4), 79.00 (Cq. $OCH_2\mathit{C}CH$), 75.10 ($CH_2C\mathit{CH}$), 72.27 (C2), 71.54 (C3), 63.74 (C5), 55.31 (OMe DMT), 54.89 ($O\mathit{CH_2}CCH$). IR (film): 3467, 3284, 2931, 2835, 1608, 1445, 1248, 1176, 1088, 1032, 828, 754 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{29}H_{30}O_7Na$ (M+Na) 513.1884. Found 513.1882. $[\alpha]_D^{20}$ +65.9 (c = 1, in DCM)



26A

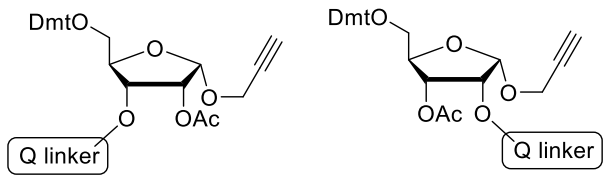


26B

1-*O*-Propargyl-2-*O*-acetyl-5-O-(4,4'-di-methoxyltrityl)-α-D-ribofuranoside (26A) / 1-*O*-Propargyl-3-*O*-acetyl-5-O-(4,4'-di-methoxyltrityl)-α-D-ribofuranoside (26B)

Compound **25** (1.04 g, 2.12 mmol) and pyridine (10 mL) were added into a flask after which it was cooled down to 0 °C. Ac_2O (0.18 mL, 1.91 mmol) was added to the flask slowly and the mixture was stirred overnight at room temperature. The mixture was then concentrated and diluted with EtOAc and H_2O. The aqueous layer was separated washed with EtOAc (1 x). All organic layers are combined, dried ($MgSO_4$), filtered and concentrated. Silica gel chromatography purification (silica gel was neutralized with 2% TEA in pentane before use, pentane/EtOAc, 90/10 – 80/20 – 50/50) afforded **26** (A+B) as a colorless oil. (568 mg, 1.07 mmol, 50%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.46 – 7.37 (m, 2H), 7.36 – 7.24 (m, 6H), 7.24 – 7.17 (m, 1H), 6.86 – 6.79 (m, 4H, arom. DMT), 5.52 (d, *J* = 4.1 Hz, 0.25H, H1-B), 5.35 (d, *J* = 4.7 Hz, 0.75H, H1-A), 5.15 (dd, *J* = 7.0, 2.6 Hz, 0.75H, H3-A), 5.08 (dd, *J* = 5.9, 4.2 Hz, 0.25H, H3-B), 4.48 (ddd, *J* = 11.4, 7.0, 4.6 Hz, 0.75H, H2-A), 4.43 – 4.31 (m, 2H, O*CH*$_2$CCH), 4.29 – 4.21 (m, 0.5H, H3-B, H4-B), 4.17 – 4.11 (m, 0.75H, H4-A), 3.79 (s, 6H, OMe DMT), 3.38 – 3.32 (m, 1H, H5-AB), 3.23 – 3.14 (m, 1H, H5-AB), 2.69 (d, *J* = 10.4 Hz, 0.25H, OH-B), 2.63 (d, *J* = 11.2 Hz, 0.75H, OH-A), 2.46 (q, *J* = 2.5 Hz, 1H, CH$_2$C*CH*), 2.20 (s, 0.75H, OMe-B), 2.09 (s, 2.25H, OMe-A). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.48 (CO Ac), 158.63, 144.84, 144.72, 136.06, 135.99, 135.90, 135.79 (Cq. arom.), 130.24, 130.20, 128.31, 128.29, 128.01, 127.99, 126.97, 113.29 (arom.), 99.78 (C1-A), 99.43 (C1-B), 86.38 (OCH$_2$*C*CH), 86.01 (C4-B), 82.72 (C4-A), 79.21 (Cq. arom.), 74.99 (OCH$_2$C*CH*-B), 74.84 (OCH$_2$C*CH*-A), 73.24 (C2-B), 72.04 (C3-A), 71.67 (C2-A), 70.72 (C3-B), 63.65 (C5), 55.36 (OMe DMT), 54.78 (O*CH*$_2$CCH-A), 54.67 (O*CH*$_2$CCH-B), 21.09 (Me Ac-A), 20.92 (Me Ac-B). IR (film): 3283, 2932, 1741, 1608, 1509, 1301, 1249, 1177, 1084, 830, 751 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{31}H_{32}O_8Na$ (M+Na) 555.1989. Found 555.1991. $[\alpha]_D^{20}$ +65.0 (c = 1, in DCM)

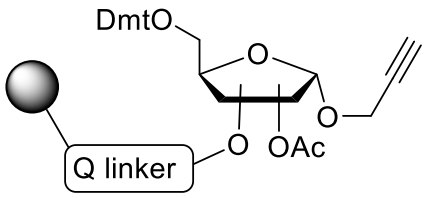


27A **27B**

1-*O*-Propargyl-2-*O*-acetyl-3-*O*-hydroquinone-*O,O*'-diacetylhemiester-5-O-(4,4'-di-methoxyltrityl)-α-D-ribofuranoside (27A) /

1-*O*-Propargyl-2-*O*-hydroquinone-*O,O*'-diacetylhemiester-5-O-(4,4'-di-methoxyltrityl)-3-*O*-acetyl-α-D-ribofuranoside (27B)

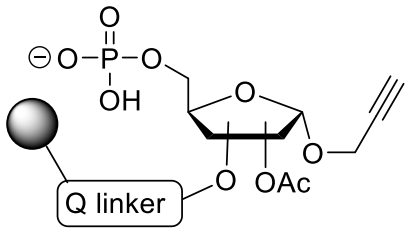
Compound **26** (544 mg, 1.02 mmol) was dissolved in pyridine (5.5 mL). DMAP (12 mg, 0.10 mmol), EDC (235 mg, 1.23 mmol), Et_3N (103 uL, 0.74 mmol) and hydroquinone-O,O'-diacetic acid (Q-linker) (277 mg, 1.23 mmol) were added and the reaction was stirred at room temperature for 16 hours. The reaction mixture was concentrated, diluted with $CHCl_3$ and washed with H_2O. The water layer was extracted with $CHCl_3$ and the combined organic layers were dried ($MgSO_4$), concentrated under reduced pressure and purified by silica gel chromatography (silica gel was neutralized with 2% TEA in pentane before use, then DCM/methanol, 100/0 – 99/1 – 95/5 – 90/10) to obtain **27** as a white foam (445 mg, 0.60 mmol, 59%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 – 7.36 (m, 2H), 7.36 – 7.24 (m, 6H), 7.24 – 7.17 (m, 1H), 6.94 – 6.78 (m, 8H, arom.), 5.62 – 5.53 (m, 1H, H1), 5.44 (dd, *J* = 6.8, 2.6 Hz, 0.25H, H2-B), 5.36 – 5.25 (m, 1.75H, H2-A, H3), 4.71 – 4.57 (m, 2H, CH2 Q linker), 4.48 (d, *J* = 7.0 Hz, 2H, CH_2 Q linker), 4.33 (t, *J* = 2.2 Hz, 2H, O*CH*$_2$CCH), 4.25 (dd, *J* = 4.5, 2.7 Hz, 1H, H4), 3.78 (s, 6H, DMT), 3.38 (AB, *J* = 10.2, 3.2 Hz, 1H, H5), 3.28 – 3.18 (m, 1H, H5), 2.46 (dt, *J* = 13.0, 2.4 Hz, 1H, OCH$_2$C*CH*), 2.07 – 2.02 (m, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.87 (CO-Q), 170.49 (CO Ac-A), 169.91 (CO Ac-B), 168.80 (CO-Q-B), 168.39 (CO-Q-A), 158.64, 153.60, 152.28, 152.23, 144.68, 135.93, 135.76 (Cq. arom.), 130.20, 130.17, 128.26, 128.02, 126.98, 116.01, 115.97, 115.72, 113.30 (arom.), 98.74 (H1-B), 98.65 (H1-A), 86.45 (Cq. DMT), 81.74 (C4-B), 81.56 (C4-A), 79.03 (OCH$_2$*C*CH), 75.01 (OCH$_2$C*CH*-A), 74.80 (OCH$_2$C*CH*-B), 71.63 (C2-A), 71.29 (C2-B), 70.50 (C3), 67.27 (CH2-Q), 66.19 (CH2-Q-B), 66.04 (CH2-Q), 63.32 (C5), 55.35 (OMe DMT), 54.86 (O*CH*$_2$CCH-A), 54.78 (O*CH*$_2$CCH-B), 20.87 (Ac-A), 20.71 (Ac-B). IR (film): 2933, 1742, 1608, 1508, 1249, 1180, 1033, 829, 597 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{41}H_{40}O_{11}Na$ (M+Na) 931.2463. Found 731.2444. $[\alpha]_D^{20}$ +53.2 (c = 1, in DCM)



1-*O*-Propargyl-2-*O*-acetyl-3-*O*-Q-TG-5-*O*-(4,4'-di-methoxyltrityl)-α-D-ribofuranoside /

1-*O*-Propargyl-2-*O*-Q-TG-3-*O*-acetyl-5-*O*-(4,4'-di-methoxyltrityl)-α-D-ribofuranoside (28)

To a 20 mL reaction syringe with filter frit was added Tenta Gel N resin (700 mg, 0.17 mmol), ACN (5 mL), compound **27** (392 mg, 0.53 mmol), HOBT (11 mg, 0.08 mmol), DIC (0.25 mL, 1.59 mmol) and DIPEA (0.46 mL, 2.65 mmol). The mixture was shaken at room temperature for 16 hours. The reaction mixture was drained and the Tentagel was washed with ACN (2 x), DMF (2 x) and DCM (3 x) under N_2. The remaining unmodified amine groups were capped by adding a mixture of CAP 1 (6 mL) and CAP 2 (6 mL). The mixture was shaken for 2 hours, drained and washed with DMF (3 x) and DCM (3 x) under N_2. The Tentagel was dried under reduced pressure and the loading was determined by trityl analysis at 503 nm. The loading for **28** is 165 µmol/g.



1-*O*-Propargyl-2-*O*-acetyl-3-*O*-Q-TG-5-*O*-phosphoryl-α-D-ribofuranoside/

1-*O*-Propargyl-2-*O*-Q-TG-3-*O*-acetyl-5-*O*-phosphoryl-α-D-ribofuranoside (29)

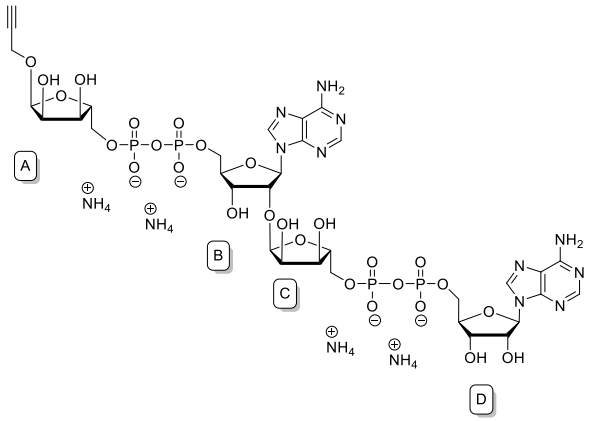
To a 20 mL reaction syringe with filter frit was added **28** (700 mg). Dichloroacetic acid (5 %, v/v, in DCM) was added repeatedly until no yellow color was observed. The resin was extensively washed with DCM (3 x), ACN (5 x) under N_2. ETT (5.54 mL, 0.25M in ACN, 12 eq) and **9** (2.31 mL, 0.2 M in ACN, 4 eq) were added into the resin and the mixture was shaken under N_2 for 10 minutes and drained. Repeat this coupling 1 more time and the resin was drained and washed with ACN (5 x). 9 mL (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) (0.5 M in ACN) was added and the mixture was shaken for 30 minutes under N_2. The resin was drained, washed with ACN (5 x) under N_2 and dried under reduced pressure to obtain Fm protected initiator. A test sample of it (30 mg) was added into a 2 mL reaction syringe. To this syringe was added 1 mL DBU solution (10%, v/v, in ACN) and was shaken for 10 minutes to remove Fm groups on 5-phosphate after which was drained and washed with ACN (3 x). Treatment with 1mL NH_4OH (35%) for 1 hour to cleave the product from resin and the filtration was concentrated. ^{31}P NMR (162 MHz, D_2O) showed single peak at 4.58 ppm which suggest the Fm protected phosphate was successfully introduced. 450 mg Fm protected resin was added into a 20 mL reaction syringe with filter frit and the resin was washed with ACN (5 x) under N_2. DBU solution (5 mL, 10%, v/v, in ACN) was added into the syringe and was shaken for 25 minutes to remove Fm groups after which it was drained. The DBU treatment was repeated for another 25 minutes. The resin was washed with ACN (5 x) and dried under reduced pressure furnished initiator **29**.

1-*O*-Propargyl-α-ADPr dimer (30a) and 1-*O*-Propargyl-α-ADPr trimer (30b)

61 mg (10 µmol) initiator resin **29** was transferred into a reaction column of a Mermade 6 oligonucleotide synthesizer and the complete synthesis was performed under an argon atmosphere.

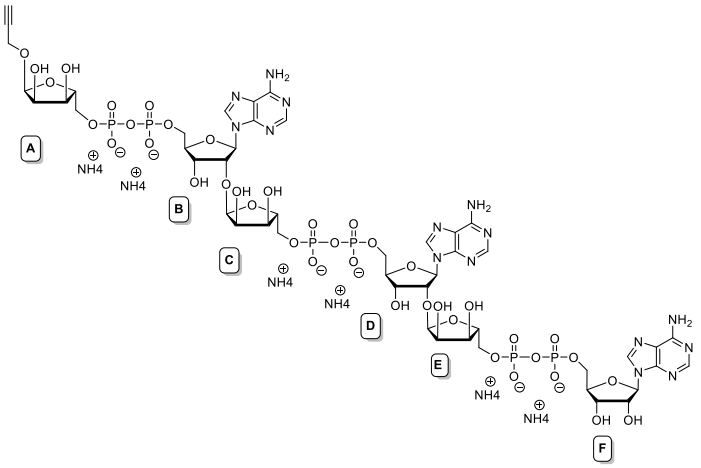
For **30a** synthesis, Cycle A was performed once and Cycle B was performed once, yielding **30a** (5.48 mg, 4.54 µmol, 45%) after anion exchange chromatography purification (20% - 70%; A: 10 mM NH_4OAc, B: 1 M NH_4OAc). For **30b** synthesis, Cycle A was performed twice and Cycle B was performed once, yielding **30b** (3.48 mg, 1.95 µmol, 20%) and **30a** (2.44 mg, 2.02 µmol, 20%) after anion exchange chromatography purification (25% - 75%, A: 10 mM NH_4OAc, B: 1 M NH_4OAc).

30a:



^{1}H NMR (850 MHz, Deuterium Oxide) δ 8.41 (s, 1H, H2), 8.38 (s, 1H, H2), 8.17 (s, 1H, H8), 8.15 (s, 1H, H8), 6.19 (d, *J* = 3.6 Hz, 1H, H1-B), 6.01 (d, *J* = 5.8 Hz, 1H, H1-D), 5.29 (d, *J* = 4.3 Hz, 1H, H1-C), 5.14 (d, *J* = 4.2 Hz, 1H, H1-A), 4.67 (t, *J* = 5.5 Hz, 1H, H2-D), 4.64 (dd, *J* = 5.3, 3.7 Hz, 1H, H2-B), 4.59 (t, *J* = 5.6 Hz, 1H, H3-B), 4.48 (dd, *J* = 5.2, 3.5 Hz, 1H, H3-D), 4.40 – 4.35 (m, 3H, H4-BCD), 4.33 – 3.99 (m, 15H, H4-A, H5-ABCD, H2-BC, H3-BC, CH_2-prop.), 2.82 (t, *J* = 2.4 Hz, 1H, CH-prop.). ^{13}C NMR (214 MHz, D_2O) δ 155.91, 155.87 (C4), 153.23, 153.12 (C8), 149.60, 149.26 (C6), 119.36, 119.23 (C5), 102.37, 102.34 (C1-A), 101.58, 101.55 (C1-C), 87.96, 87.95 (C1-B), 87.46, 87.44 (C1-D), 85.14, 85.12, 85.10, 85.08, 84.84, 84.81, 84.59, 84.56, 84.55, 84.52, 84.01, 83.97 (C4-ABCD), 80.03 (Cq. Prop.), 79.90 (H2-B), 76.61, 76.57 (CH-prop.), 75.41 (C2-D), 72.32, 71.84, 71.39, 70.78, 70.42, 70.12 (C2-AC, C3-ABCD), 66.59, 66.57, 66.48, 66.45, 66.20, 66.18, 65.55, 65.51 (C5-ABCD), 55.79, 55.77 (CH_2-prop.). ^{31}P NMR (202 MHz, D_2O) δ -10.35, -10.45, -10.52, -10.60, -10.62, -10.68, -10.78. LC-MS: Rt = 3.02 min. 0-50% NH_4OAc. ESI MS+ calc. 1139.2 found 1139.2 $[M+1]^+$. HRMS (ESI^+) calcd for $C_{33}H_{47}N_{10}O_{27}P_4$ (M+H) 1139.1557. Found 1139.1566.

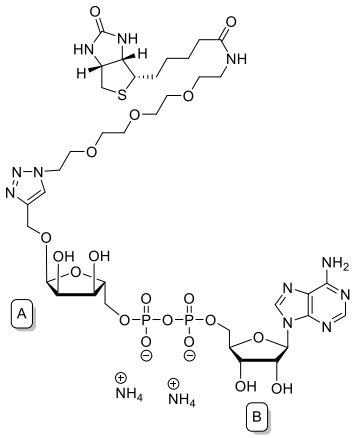
30b:



^{1}H NMR (850 MHz, Deuterium Oxide) δ 8.43 (s, 1H, H2), 8.42 (s, 1H, H2), 8.36 (s, 1H, H2), 8.18 (s, 1H, H8), 8.16 (s, 1H, H8), 8.13 (s, 1H, H8), 6.18 (d, *J* = 3.2 Hz, 1H, H1-B), 6.04 (d, *J* = 3.4 Hz, 1H, H1-D), 6.00 (d, *J* = 5.9 Hz, 1H, H1-F), 5.34 (d, *J* = 4.3 Hz, 1H, H1-C), 5.26 (d, *J* = 4.3 Hz, 1H, H1-E), 5.17 (d, *J* = 4.3 Hz, 1H, H1-A), 4.68 (t, *J* = 5.5 Hz, 1H, H2-F), 4.60 (dt, *J* = 4.4, 2.2 Hz, 1H, H2-B), 4.56 (t, *J* = 5.8 Hz, 1H, H3-B), 4.52 (dd, *J* = 5.2, 3.4 Hz, 1H, H2-D), 4.51 – 4.46 (m, 2H, H3-DF), 4.41 – 3.99 (m, 24H, H2-ACE, H3-ACE, H4-ABCDEF, H5-ABCDEF, CH_2-prop.), 2.83 (t, *J* = 2.4 Hz, 1H, CH-prop.). ^{13}C NMR (214 MHz, D_2O) δ 154.45, 154.35, 154.28 (C4), 149.36, 148.95, 148.76 (C6), 119.29, 119.14, 119.03 (C5), 102.30 (C1-C), 102.18 (C1-E), 101.58 (C1-A), 88.08 (C1-F), 87.87 (C1-D), 87.75 (C1-B), 85.14, 85.12, 85.10, 85.09, 84.92, 84.88, 84.59, 84.55, 84.11, 84.07, 83.88, 83.84 (C4-ABCDEF), 80.19 (C2-D), 80.04 (Cq. prop.), 79.75 (C2-B), 76.59 (CH, prop.), 75.42 (C2-F), 72.31, 72.30, 71.86, 71.38, 70.80, 70.77, 70.41, 69.96, 69.72 (C2-ACE, C3-ABCDEF), 66.55, 66.52, 66.49, 66.47, 66.19, 66.17, 65.46, 65.44, 65.36, 65.34 (C5-ABCDEF), 55.78 (CH_2-prop.). ^{31}P NMR (202 MHz, D_2O) δ -10.44, -10.47, -10.50, -10.54, -10.56, -10.63, -10.72. LC-MS: Rt = 2.96 min. 0-50% NH_4OAc. ESI MS+ calc. 1680.2 found 1680.1 $[M+1]^+$. HRMS (ESI^+) calcd for $C_{48}H_{69}N_{15}O_{40}P_6$ (M+2H)/2 840.6120. Found 840.6115.

Biotin-PEG$_3$-mono-ADPr (**31a)**

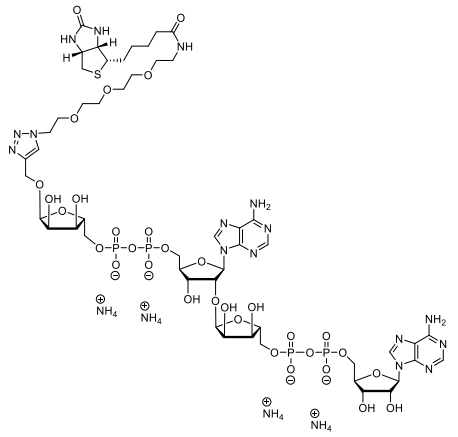
α-1-O-propargyl-mono ADPr[24](200 uL, 1 eq, 10 mg/mL in H_2O) was added to azide-PEG_3-biotin (125 uL, 2 eq, 25 mg/mL in ACN). Subsequently, 500 uL buffer (20 mM TRIS/150 mM NaCl, pH 7.6) was added to the mixture. Next, 120 uL click cocktail was added (1:1:1 v/v/v, CuSO4 (26 mg/mL in water): Sodium Ascorbate (120 mg/mL in water): TBTA ligand (52 mg/mL in DMSO)) after which 100 uL DMSO was added to increase solubility. The mixture was shaken for 30 minutes at room temperature followed using LC-MS analysis. The reaction should be finished in 1 hour (determined by total conversion of propargy-ADPr) and was quenched using 30 uL EDTA (0.5 M). The mixture was purified by HPLC (0% - 30%, A:25 mM NH_4OAc in H_2O, B: ACN) to furnish **31a** (1.61 mg, 1.55 µnol, 46%) as white solid after repeated lyophilization.



^{1}H NMR (850 MHz, Deuterium Oxide) δ 8.55 (s, 1H, H2), 8.27 (s, 1H, H8), 8.02 (s, 1H, triazole), 6.15 (d, *J* = 6.0 Hz, 1H, H1-B), 5.13 – 5.06 (m, 1H, H1-A), 4.65 – 4.60 (m, 3H, CH2-PEG, C*H*H-triazole), 4.58 (ddd, *J* = 8.0, 5.1, 0.8 Hz, 1H, CH-biotin), 4.54 (dd, *J* = 5.1, 3.5 Hz, 1H, H3-B), 4.40 (ddt, *J* = 12.4, 8.0, 3.8 Hz, 2H, H4-B, CH-biotin), 4.26 – 4.21 (m, 2H, H5-B), 4.20 (dq, *J* = 3.8, 2.1, 1.5 Hz, 1H, H4-A), 4.17 – 4.12 (m, 2H, H2-A, H3-A), 4.07 – 4.01 (m, 2H, H5-A), 3.97 (dd, *J* = 5.6, 4.5 Hz, 2H, CH2-PEG), 3.67 – 3.57 (m, 10H, CH2-PEG), 3.37 (dd, *J* = 6.0, 4.8 Hz, 2H, CH2-PEG), 3.28 (ddd, *J* = 9.6, 5.7, 4.5 Hz, 1H, CH-S), 2.96 (AB, *J* = 13.1, 5.0 Hz, 1H, CH2-S), 2.79 – 2.71 (m, 1H, CH2-S), 2.25 (t, *J* = 7.3 Hz, 2H, CH2-biotin), 1.74 – 1.51 (m, 4H, CH2-biotin), 1.41 – 1.33 (m, 2H, CH2-biotin). ^{13}C NMR (214 MHz, D_2O) δ 178.13 (CO, biotin), 166.55 (CONH), 156.27 (C4), 150.29 (Cq. triazole), 145.16 (C6), 126.47 (CH, triazole), 119.81 (C5), 102.69 (C1-A), 88.12 (C1-B), 85.26, 85.22 (C4-B), 84.59, 84.55 (C4-A), 75.59 (C2-B), 72.22 (C2-A), 71.66 (C3-B), 70.89 (PEG), 70.83 (PEG), 70.75 (C3-A), 70.71, 70.64, 70.07, 69.99, 69.97, 69.95 (PEG), 66.84, 66.82 (C5-A), 66.43, 66.41 (C5-B), 63.28 (CH-biotin), 61.68 (PEG), 61.46 (CH-biotin), 56.54 (CH-S), 51.23 (CH2-trizole), 40.90 (CH2-S), 40.14 (PEG), 36.66, 29.07, 28.88, 26.34 (CH2-biotin). ^{31}P NMR (202 MHz, D_2O) δ -11.14, -11.24, -11.34, -11.44. LC-MS: Rt = 4.81 min. 0-50% NH_4OAc. ESI MS+ calc. 1042.3 found 1042.3 $[M+1]^+$. HRMS (ESI^+) calcd for $C_{36}H_{58}N_{11}O_{19}P_2S$ (M+H) 1042.3101. Found 1042.3111.

Biotin-PEG$_3$-di-ADPr (31b)

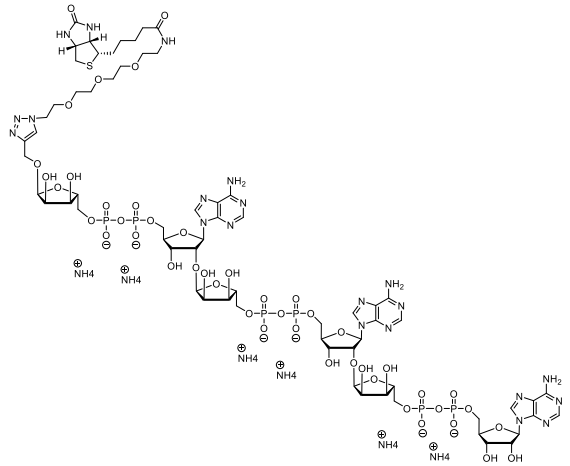
31b was synthesized using same procedure as **31a** but using 244 uL propargy di-ADPr **30a**. Purification by HPLC (0% - 30%, A:25 mM NH_4OAc in H_2O, B: ACN) to furnish **31b** (0.45 mg, 0.27 µmol, 13%) as white solid after repeated lyophilization.



^{1}H NMR (500 MHz, Deuterium Oxide) δ 8.41 (s, 1H, H2), 8.38 (s, 1H, H2), 8.15 (s, 1H, H8), 8.14 (s, 1H, H8), 8.01 (s, 1H, CH-triazole), 6.18 (d, *J* = 3.6 Hz, 1H, H1-B), 6.01 (d, *J* = 5.9 Hz, 1H, H1-D), 5.27 (d, *J* = 4.2 Hz, 1H, H1-C), 5.13 – 5.07 (m, 1H, H1-A), 4.70 – 4.56 (m, 6H), 4.51 – 4.47 (m, 1H), 4.41 – 3.93 (m, 18H), 3.62 (ddd, *J* = 15.5, 8.3, 4.6 Hz, 10H, PEG), 3.37 (t, *J* = 5.3 Hz, 2H, PEG), 3.27 (dt, *J* = 9.8, 5.2 Hz, 1H, CH-S), 2.96 (AB, *J* = 13.0, 5.0 Hz, 1H, CH_2-S), 2.79 – 2.72 (m, 1H, CH_2-S), 2.24 (t, *J* = 7.3 Hz, 2H), 1.83 – 1.31 (m, 6H, CH_2-biotin). ^{31}P NMR (202 MHz, D_2O) δ -11.11, -11.21, -11.29, -11.34, -11.37, -11.45, -11.48. LC-MS: Rt = 4.53 min. 0-50% NH_4OAc. ESI MS+ calc. 1583.4 found 1583.3 $[M+1]^+$. HRMS (ESI^+) calcd for $C_{51}H_{80}N_{16}O_{32}P_4S$ (M+2H)/2 792.1892. Found 792.1889.

Biotin-PEG$_3$-tri-ADPr (31c)

31c was synthesized using same procedure as **31a** but using 300 uL propargyl tri-ADPr **30b**. Purification by HPLC (0% - 30%, A:25 mM NH_4OAc in H_2O, B: ACN) to furnish **31c** (0.69 mg, 0.31 µmol, 18%) as white solid after repeated lyophilization.



^{1}H NMR (850 MHz, Deuterium Oxide) δ 8.38 (s, 1H, H2), 8.35 (s, 1H, H2), 8.29 (s, 1H, H2), 8.09 (s, 1H, H8), 8.05 (s, 1H, H8), 8.04 (s, 1H, H8), 8.00 (s, 1H, CH-triazole), 6.18 (d, *J* = 3.7 Hz, 1H, H1-B), 6.03 (d, *J* = 3.6 Hz, 1H, H1-D), 5.99 (d, *J* = 5.9 Hz, 1H, H1-F), 5.31 (d, *J* = 4.3 Hz, 1H, H1-C), 5.21 (d, *J* = 4.2 Hz, 1H, H1-E), 5.11 – 5.07 (m, 1H, H1-A), 4.67 (t, *J* = 5.5 Hz, 2H), 4.64 – 4.54 (m, 8H), 4.53 – 4.46 (m, 4H), 4.40 – 3.92 (m, 36H), 3.68 – 3.55 (m, 10H, PEG), 3.37 (t, *J* = 5.3 Hz, 2H, PEG), 3.26 (dt, *J* = 9.7, 5.1 Hz, 1H, CH-S), 2.95 (dd, *J* = 13.1, 5.0 Hz, 1H, CH_2-S), 2.78 – 2.71 (m, 1H, CH_2-S), 2.24 (t, *J* = 7.3 Hz, 2H), 1.72 – 1.30 (m, 6H, CH_2-biotin). ^{31}P NMR (202 MHz, D_2O) δ -11.12, -11.14, -11.22, -11.24, -11.29, -11.34, -11.38, -11.41, -11.51. LC-MS: Rt = 5.74 min. 0-20% NH_4OAc. ESI MS+ calc. 1062.7 found 1063.4 $[M+2]^+$. HRMS (ESI^+) calcd for $C_{66}H_{101}N_{21}O_{45}P_6S$ (M+2H)/2 1062.7198. Found 1062.7216.

References

1. B. Luscher, M. Butepage, L. Eckei, S. Krieg, P. Verheugd and B. H. Shilton, *Chem. Rev.*, 2018, **118**, 1092-1136.
2. B. A. Gibson and W. L. Kraus, *Nat. Rev. Mol. Cell Biol.*, 2012, **13**, 411-424.
3. J. O'Sullivan, M. Tedim Ferreira, J. P. Gagne, A. K. Sharma, M. J. Hendzel, J. Y. Masson and G. G. Poirier, *Nat. Commun.*, 2019, **10**, 1182.
4. R. Gupte, Z. Liu and W. L. Kraus, *Genes Dev.*, 2017, **31**, 101-126.
5. K. Y. Lin, D. Huang and W. Lee Kraus, *Methods Mol. Biol.*, 2018, **1813**, 91-108.
6. E. Barkauskaite, A. Brassington, E. S. Tan, J. Warwicker, M. S. Dunstan, B. Banos, P. Lafite, M. Ahel, T. J. Mitchison, I. Ahel and D. Leys, *Nat. Commun.*, 2013, **4**, 2164.

7. E. S. Tan, K. A. Krukenberg and T. J. Mitchison, *Anal. Biochem.*, 2012, **428**, 126-136.
8. H. A. Kistemaker, L. N. Lameijer, N. J. Meeuwenoord, H. S. Overkleeft, G. A. van der Marel and D. V. Filippov, *Angew. Chem. Int. Ed. Engl.*, 2015, **54**, 4915-4918.
9. H. R. Singh, A. P. Nardozza, I. R. Moller, G. Knobloch, H. A. V. Kistemaker, M. Hassler, N. Harrer, C. Blessing, S. Eustermann, C. Kotthoff, S. Huet, F. Mueller-Planitz, D. V. Filippov, G. Timinszky, K. D. Rand and A. G. Ladurner, *Mol. Cell*, 2017, **68**, 860-871 e867.
10. M. J. Lambrecht, M. Brichacek, E. Barkauskaite, A. Ariza, I. Ahel and P. J. Hergenrother, *J. Am. Chem. Soc.*, 2015, **137**, 3558-3564.
11. J. Fahrer, R. Kranaster, M. Altmeyer, A. Marx and A. Burkle, *Nucleic Acids Res.*, 2007, **35**, e143.
12. D. Ahel, Z. Hořejší, N. Wiechens, S. E. Polo, E. Garcia-Wilson, I. Ahel, H. Flynn, M. Skehel, S. C. West, S. P. Jackson, T. Owen-Hughes and S. J. Boulton, *Science*, 2009, **325**, 1240.
13. T. I. Kam, X. Mao, H. Park, S. C. Chou, S. S. Karuppagounder, G. E. Umanah, S. P. Yun, S. Brahmachari, N. Panicker, R. Chen, S. A. Andrabi, C. Qi, G. G. Poirier, O. Pletnikova, J. C. Troncoso, L. M. Bekris, J. B. Leverenz, A. Pantelyat, H. S. Ko, L. S. Rosenthal, T. M. Dawson and V. L. Dawson, *Science*, 2018, **362**.
14. K. A. Krukenberg, S. Kim, E. S. Tan, Z. Maliga and T. J. Mitchison, *Chem. Biol.*, 2015, **22**, 446-452.
15. D. Slade, M. S. Dunstan, E. Barkauskaite, R. Weston, P. Lafite, N. Dixon, M. Ahel, D. Leys and I. Ahel, *Nature*, 2011, **477**, 616-620.
16. F. M. Narendja and G. Sauermann, *Anal. Biochem.*, 1994, **220**, 415-419.
17. H. Jiang, J. H. Kim, K. M. Frizzell, W. L. Kraus and H. Lin, *J. Am. Chem. Soc.*, 2010, **132**, 9363-9372.
18. K. Kalesh, S. Lukauskas, A. J. Borg, A. P. Snijders, V. Ayyappan, A. K. L. Leung, D. O. Haskard and P. A. DiMaggio, *Sci. Rep.*, 2019, **9**, 6655.
19. C. M. Daniels, S. E. Ong and A. K. Leung, *Mol. Cell*, 2015, **58**, 911-924.
20. H. A. Kistemaker, A. P. Nardozza, H. S. Overkleeft, G. A. van der Marel, A. G. Ladurner and D. V. Filippov, *Angew. Chem. Int. Ed. Engl.*, 2016, **55**, 10634-10638.
21. G. S. Cremosnik, A. Hofer and H. J. Jessen, *Angew. Chem. Int. Ed. Engl.*, 2014, **53**, 286-289.
22. I. Pavlovic, D. T. Thakor, J. R. Vargas, C. J. McKinlay, S. Hauke, P. Anstaett, R. C. Camuna, L. Bigler, G. Gasser, C. Schultz, P. A. Wender and H. J. Jessen, *Nat. Commun.*, 2016, **7**, 10622.
23. L. Li, Q. Li, S. Ding, P. Xin, Y. Zhang, S. Huang and G. Zhang, *Molecules*, 2017, **22**.
24. Q. Liu, H. A. V. Kistemaker, S. Bhogaraju, I. Dikic, H. S. Overkleeft, G. A. van der Marel, H. Ovaa, G. J. van der Heden van Noort and D. V. Filippov, *Angew. Chem. Int. Ed. Engl.*, 2018, **57**, 1659-1662.
25. L. Bialy and H. Waldmann, *Chemistry*, 2004, **10**, 2759-2780.
26. Y. Watanabe, T. Nakamura and H. Mitsumoto, *Tetrahedron Lett.*, 1997, **38**, 7407-7410.
27. N.-S. Li, J. Lu and J. A. Piccirilli, *Org. Lett.*, 2007, **9**, 3009-3012.
28. H. A. V. Kistemaker, N. J. Meeuwenoord, H. S. Overkleeft, G. A. van der Marel and D. V. Filippov, *Eur. J. Org. Chem.*, 2015, **2015**, 6084-6091.
29. X. Wu, *Nucleic Acids Res.*, 1998, **26**, 4315-4323.
30. S. Pitsch, P. A. Weiss, L. Jenny, A. Stutz and X. Wu, 2001, **84**, 3773-3795.
31. N. Minakawa, Y. Kato, K. Uetake, D. Kaga and A. Matsuda, *Tetrahedron*, 2003, **59**, 1699-1702.
32. L. Wang, H. S. Overkleeft, G. A. van der Marel and J. D. C. Codee, *J. Am. Chem. Soc.*, 2018, **140**, 4632-4638.
33. L. Wang, H. S. Overkleeft, G. A. van der Marel and J. D. C. Codee, *Eur. J. Org. Chem.*, 2019, **2019**, 1994-2003.
34. N. Oka, A. Mori and K. Ando, *Eur. J. Org. Chem.*, 2018, **2018**, 6355-6362.
35. N. Oka, R. Kajino, K. Takeuchi, H. Nagakawa and K. Ando, *J. Org. Chem.*, 2014, **79**, 7656-7664.
36. P. Finch, G. M. Iskander and A. H. Siriwardena, *Carbohydr. Res.*, 1991, **210**, 319-325.