Synthetic Study on ADP-ribosylation

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2 Synthesis of a native branched ADPr fragment and its biotinylated derivatives

Part of this chapter has been published:

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Introduction

Poly ADP-ribosylation (PARylation) is an important post-translational modification in which negatively charged ADP-ribose chains are transferred to an acceptor protein using NAD^+ (nicotinamide adenine dinucleotide) as a donor and PARPs (poly ADP ribose polymerases) as the involved enzymes[1] (Figure 1.). PARylation and the resulted polymers (PARs) are involved in many biological events such as DNA repair, transcriptional regulation, cell death and apoptosis.[2] PAR chains can be either linear or branched[3]. Linear PAR can grow to over 200 units in size, with a branching site occurring on average once every 20 to 50 elongation reactions.[1] While the knowledge on linear PAR is steadily growing, less progress is made with the role of branched PAR and its function is still unclear. There are a few reports

on branched PAR after its discovery by Miwa *et al*[3] at the end of the 1970s. For example, the branched and not the linear PAR chains bind most preferably to histones[4] and other nuclear proteins[5]. The branching point is reported not to be the endoglycosidic cleavage site of poly-ADP-ribose glycohydrolase (PARG)[6] which indicates that there might be undiscovered enzymes that specifically recognize the branched PAR structure.[7, 8] In 1981, the chemical structure of the branching point of PAR was established as *O*-α-D-ribofuranosyl-(1'''→2'')-*O*-α-D-ribofuranosyl-(1''→2')-adenosine-5',5'',5'''-tris(phosphate) (Figure 1**, 1**) by Miwa *et al*.[9] They performed an enzymatic synthesis using NAD^+ and calf thymus nuclei, to get PAR *in vitro*. Subsequent hydrolysis of all the pyrophosphate linkages in PAR by treatment with snake venom phosphodiesterase led to the isolation of branched PAR fragment **1**. The configuration of **1** was determined by derivatization and with the aid of physicochemical techniques including gas chromatography, mass spectrometry, and ^{1}H-NMR spectroscopy[9]. Shortly after the structure elucidation, two different groups[10, 11] reported the existence of branched PAR in *vivo,* indicating that the branched PAR fragment made from enzymatic synthesis is indeed the naturally occurring product. Furthermore, enzymatic synthesis is widely applied to simulate in *vivo* conditions and to produce PAR[12-14]. In this respect, the organic synthesis of branched ADPr fragment **1** is a challenging and valuable goal that can confirm this structure elucidation and will support future biological studies.

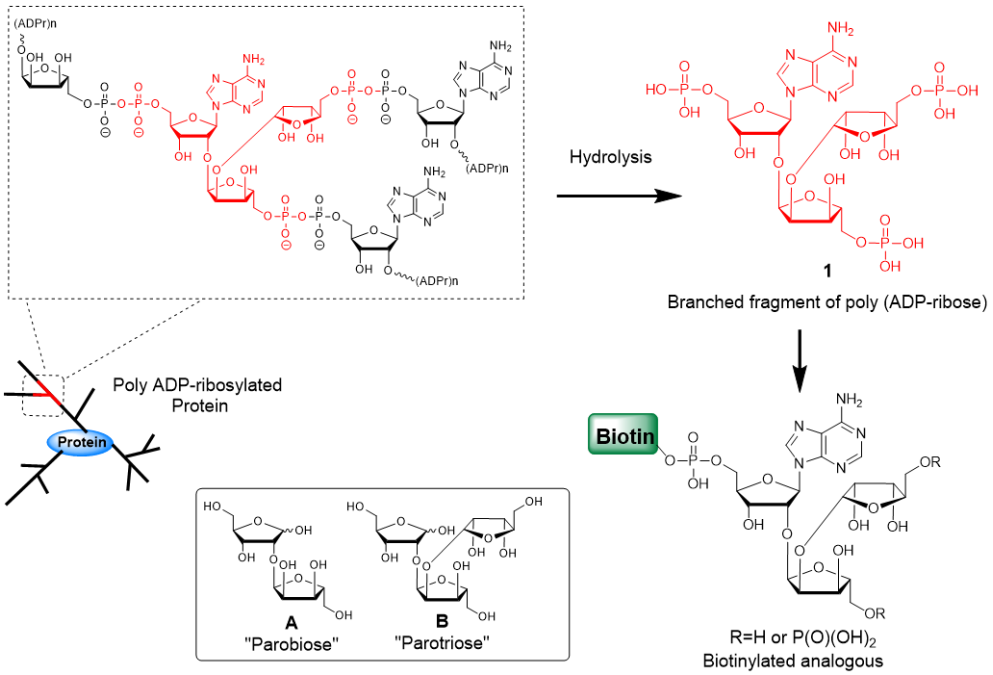
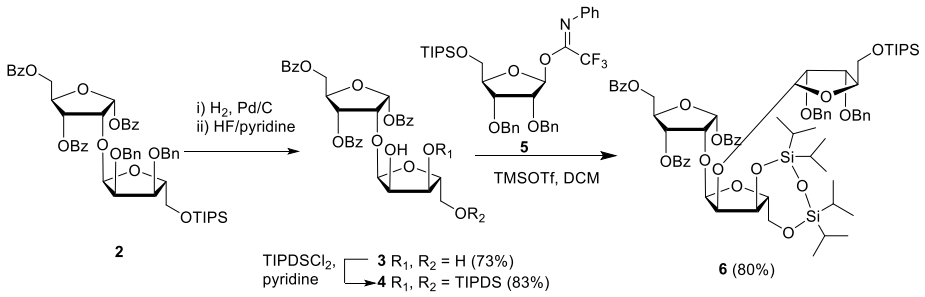


Figure 1. Structure of branched PAR fragment **1** and branched biotinylated analogous. Disaccharide **A** is "parobiose" and trisaccharide **B** is called "parotriose".

Up to now, synthetic methodologies have been developed towards PAR related molecules such as ribosylated amino acids[15], mono-ADP ribosylated peptides[16, 17] and a linear ADP ribose dimer and

trimer.[18] This chapter describes the synthesis of branched ADPr fragment **1** and also its structural analysis in comparison with the enzymatically prepared compound. The synthetic route toward **1** is guided by the earlier reported synthesis of the core motif of branched PAR[19] by adaptation of the protective group strategy and simultaneous introduction of three phosphotriester functions on the 5',5'', 5'''-primary hydroxyls of a suitably protected branched trisaccharide with phosphoramidite chemistry. Furthermore, this chapter describes the use of similar approach to the synthesis of biotinylated derivatives of branched and linear ADPr fragments (Figure 1), which could be valuable tools for searching for new proteins capable to bind branched PAR in proteomic studies.[20]

Results and discussion



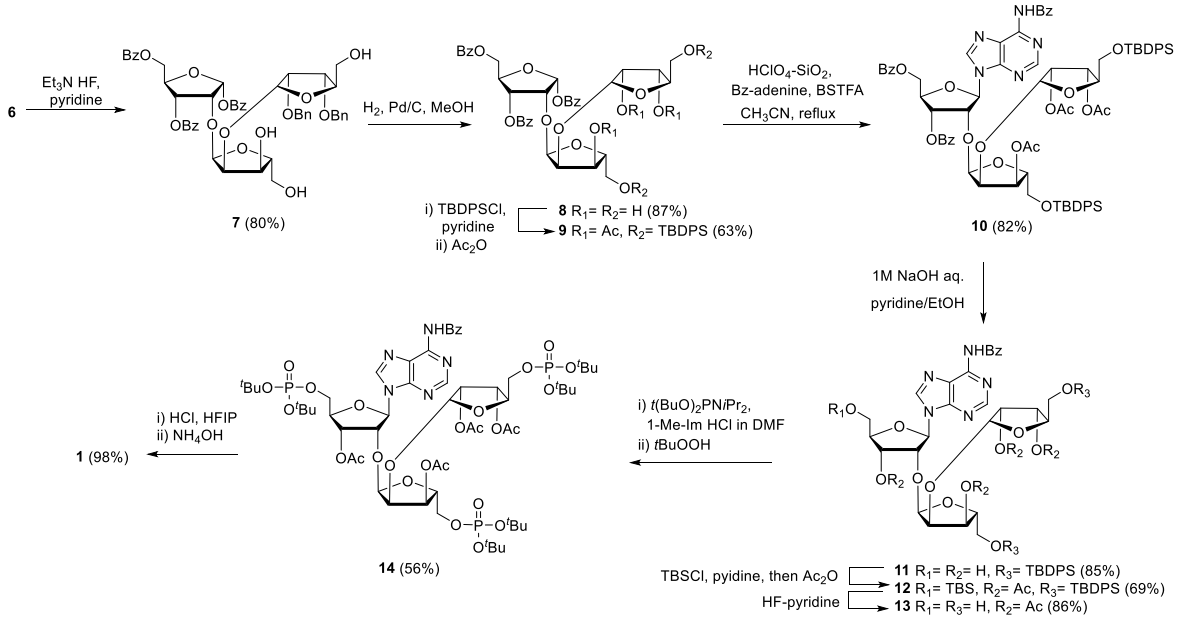
Scheme 1. Synthesis of protected parotriose **6** from parobiose **2**

The first stage of the route to target **1** comprises the preparation of protected parotriose **6**, provided with two challenging 1,2 cis-α-glycosidic linkages[21] (Scheme 1). Hydrogenolysis of the benzyl groups in α-configured and protected parobiose **2**, obtained according to an earlier reported method,[18] was followed by the removal of TIPS (triisopropylsilyl) group with HF·Pyridine to give 2'',3'',5''-OH parobiose **3** in good yield. It is interesting that the presence of the TIPS instead of the benzyl group at the 5'-OH of compound **2,** avoided glycosidic bond cleavage during hydrogenolysis as reported in previous study[19], resulting in a significantly improved yield and making large scale synthesis possible.

The 3'',5''-OH functions in triol **3** were selectively masked with the diol protecting TIPDS group by treatment with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) in pyridine to get alcohol **4**. Coupling of partially protected parobiose **4** with *N*-phenyl trifluoroacetimidate donor **5** afforded the fully protected parotriose **6** with complete α-selectivity and improved yield.[19]

The subsequent introduction of adenine base required a number of protective group manipulations (Scheme 2). The benzyl-protecting groups in **6** had to be replaced because the adenine moiety would complicate hydrogenolysis.[21] However, the removal of benzyl ethers in **6** by Pd/C-catalyzed hydrogenation was tedious (took more than 4 days) and was accompanied by ring opening of the TIPDS group. This side reaction could not be prevented by the use of other catalysts such as $Pd(OH)_2$. Although

the lability of the TIPDS group on the trisaccharides presumably made the hydrogenolysis problematic, selective removal of the TIPDS in **6** could not be attained**.** Therefore, **6** was treated with an excess of $Et_3N{\cdot}HF$ for 24 h to remove all silyl groups. Hydrogenolysis of the thus obtained compound **7** using Pd/C in methanol for 24 h afforded compound **8**, provided with five hydroxyl functions, in high yield. Readjusting the protection by the installation of TBDPS groups on the primary hydroxyls of compound **8** and acetylation of the remaining secondary hydroxyls set the stage for the introduction of N^6-benzolyadenine on the reducing end of parotriose **9**. Vorbrüggen type glycosylation using $HClO_4$-SiO_2 as catalyst and persilylated N^6-benzolyadenine proceeded completely β selective and furnished **10** in high yield.[19] The selective glycosylation on the *N*-9 position and not *N*-3 or *N*-7 was ascertained by UV-spectroscopy. Before three identical phosphate triesters could be installed on the 5',5'', 5'''-primary hydroxyls, protective group manipulation was required to ensure regioselective phosphorylation. Thus, saponification of the acetyl and benzoyl esters with aqueous NaOH in pyridine/ethanol gave intermediate **11,** allowing protection of the remaining free 5'-OH groups with TBDPS groups.



Scheme 2. Synthesis of the branched portion of poly-ADPr **1**

Surprisingly, the reaction of the 5'-OH in **11** with TBDPSCl failed, but fortunately the equally suitable TBS group was introduced successfully using the more reactive TBSCl in pyridine. Subsequent acetylation of this intermediate gave fully protected **12**. After removal of the silyl ethers by HF·pyridine, all primary hydroxyl functions were released to give triol **13**, amenable to the simultaneous introduction of three di-*tert*-butylphosphotriesters. Treatment of **13** with 10 equivalents di-*tert*-butyl-*N*,*N*–diisopropylphosphoramidite using 1-methylimidazole and 1-methylimidazolium chloride as activators under strictly anhydrous conditions[22] and subsequent oxidation of the intermediate

phosphite triesters gave **14** in moderate yield. The low reactivity of 5'-OH of the adenosine moiety as noticed in the silylation of **11** also decreases the yield of the phosphitylation reaction as the formation of target **14** was accompanied by bis-phosphitylated product. In the final stage, the *tert*-butyl groups of the phosphotriester in fully protected **14** were removed by HCl/HFIP in 1 h, followed by ammonolysis of the acyl groups to furnish tris-phosphorylated parotriosyladenine **1** in excellent yield.

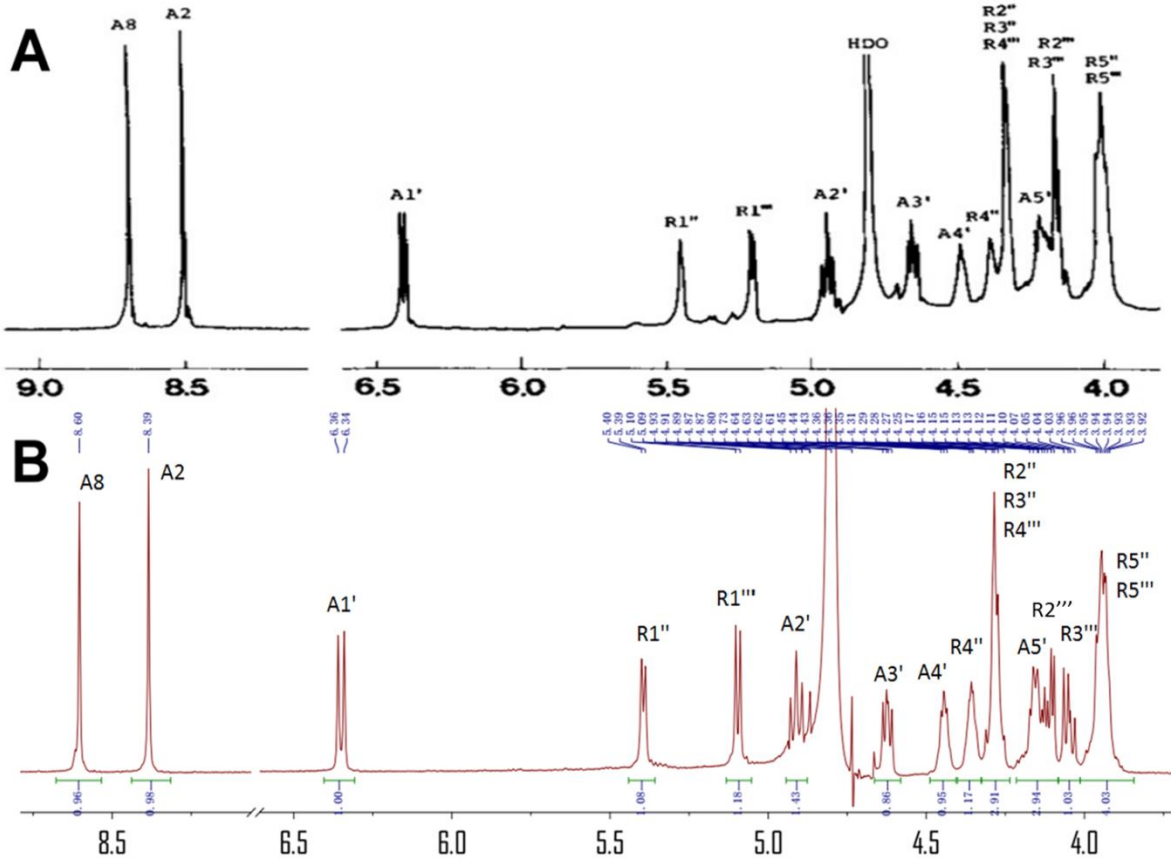
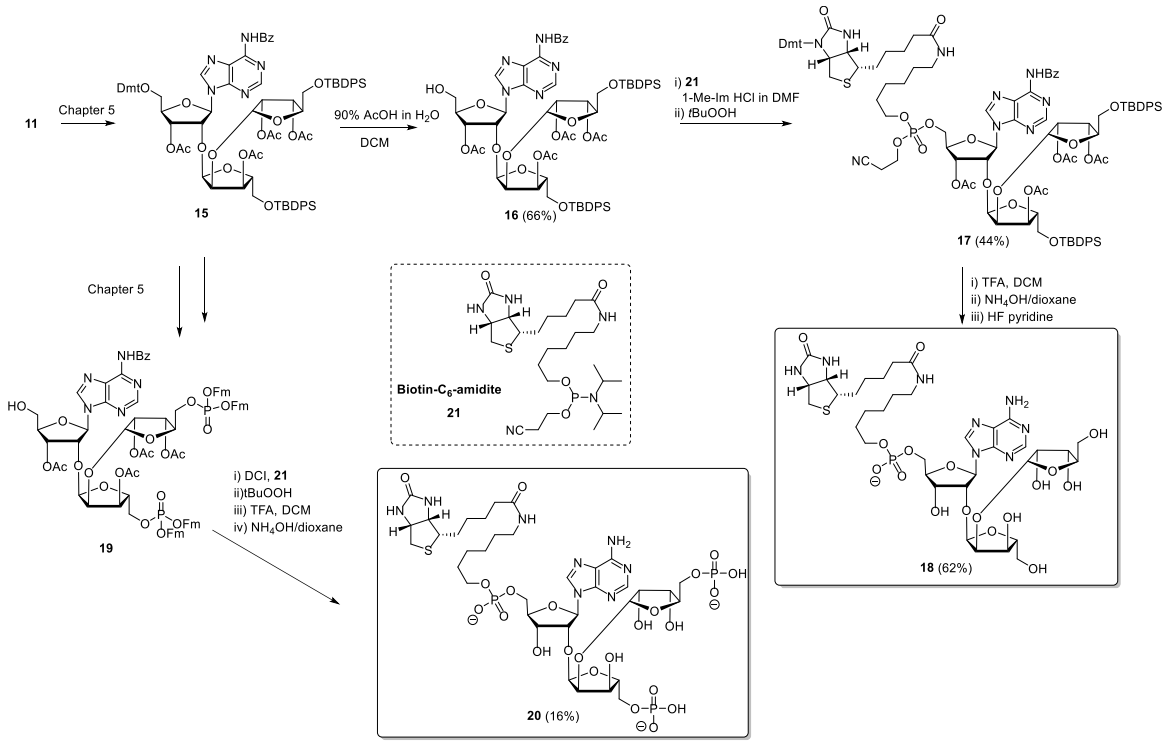


Figure 2. Comparison of ^{1}H-NMR-spectra of the branching portion of PAR. (**A**) Isolated compound **1**, 270 MHz, in D_2O (pH=3) as reported by Miwa et al. (**B**) Synthetic compound **1** from this work, 300 MHz, in CD_3COOD and D_2O (pH=3).

Next, the spectroscopic data of the just obtained target compound **1** were compared with those reported by Miwa[9] for the enzymatically prepared product (Figure 2A). In the first instance, significant differences between the ^{1}H-NMR spectra were observed, that may be attributed to a pH difference of the NMR samples, which in turn may be due to different isolation procedures. The isolation of the synthetic branched ADPr fragment **1** involved global deprotection by ammonia treatment, followed by purification by HW-40 gel filtration using 0.15 M NH_4OAc in H_2O as eluent under essentially neutral conditions. Contrary, Miwa firstly desalted the isolated product by DEAE-cellulose column chromatography, followed by column chromatography on phosphocellulose. By doing this, Miwa

obtained branched ADPr fragment as an acidified sample (pH=3) while the synthetic product occurs in the neutralized form (pH=7) as an ammonium salt. To get a more accurate comparison, the pH of the sample with synthetic fragment **1** in D_2O was reduced from 7 to 3 by adding CD_3COOD. Under these conditions, the NMR spectrum of the synthetic branched ADPr fragment **1** and the enzymatically prepared one of Miwa proved to be virtually identical (Figure 2A vs Figure 2B). A small difference in the multiplicity at R2''' and R3''' could be attributed to the slight difference in the applied field. Overall the chemical shifts of all protons in the synthetic compound **1** are approximately 0.1 ppm upfield from those of enzymatically prepared compound mainly because Miwa used DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) as a reference. Taken together, it was concluded that the synthetic branched ADPr fragment and the enzymatically prepared one have the same chemical structure.

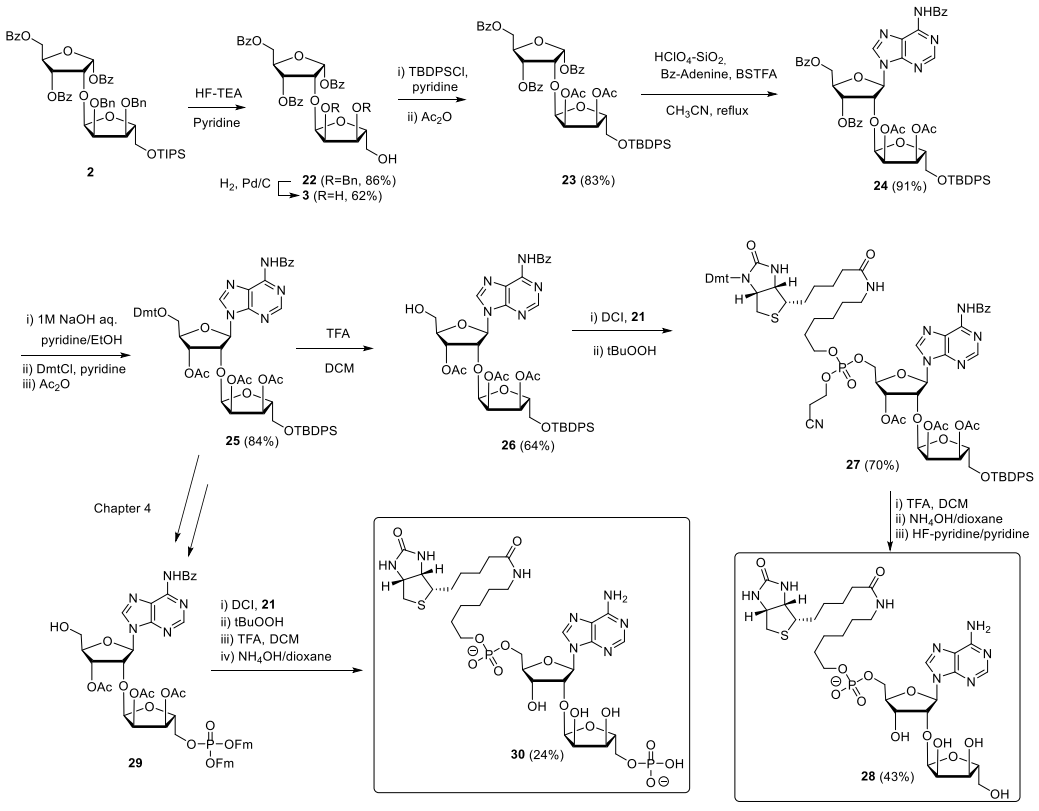


Scheme 3. Synthesis of biotinylated branched ADPr fragment

Biotinylated ADPr derivatives can act as valuable tools in biochemical studies on PAR. Although the synthesis of biotinylated ADPr dimers[8] has been reported, the synthesis of a similar biotinylated branched ADPr fragment is unknown. Recently Chen *et al*[23] reported that the PBZ domain of APLF protein binds to branched PAR by recognizing fragment **1** in the PAR chain, thereby regulating chromatin remodeling in DNA repair. Based on this result, it was reasoned that the search for the yet undiscovered proteins capable of binding to branched PAR would be facilitated by the availability of biotinylated branched ADPr fragments. The successful synthesis approach to compound **1** can be adapted for both the synthesis of biotinylated branched ADPr fragments (**18** and **20**, Scheme 3) and

the biotinylated linear counterparts, as negative controls (**28** and **30**, Scheme 4).

The synthesis of both the phosphorylated (**20**) and unphosphorylated (**18**) biotinylated branch points of PAR started with bis-ribosylated adenosine building block **11** (Scheme 3). Dimethoxytritylation of the 5'-OH in **11** and acetylation of the remaining secondary hydroxyls yielded **15** (details discussion in Chapter 5). Selective removal of the DMT group with 90% AcOH/H_2O in DCM furnished **16,** the liberated hydroxyl of which was amenable for the installation of the biotin moiety by phosphorylation with biotin-C_6-amidite (**21**)[24] under the activation of DCI and subsequent oxidation by *t*-BuOOH to give fully protected **17**. The DMT group in **17** was removed using TFA in DCM, all acetyl esters and the benzamide were cleaved with aqueous NH_4OH and finally HF·pyridine treatment removed the silyl groups to furnish target biotinylated branched ADPr fragment **18** in 62% yield after HW-40 purification. En route to 5'',5'''-phosphorylated biotinylated branched ADPr fragment **20**, compound **15** was desilylated, phosphorylated by coupling with bis (*9H*-fluoren-9-ylmethyl)-diisopropylamidophosphite and subsequently oxidized. Finally, detritylation gave **19** in good yield (see details discussion in Chapter 5). Fluorenylmethyl (Fm) groups[25] were selected to protect both terminal phosphates to achieve the simultaneous removal of the Fm, acetyls and benzoyl groups using NH_4OH treatment. The free hydroxyl in **19** was coupled with Biotin-C_6-amidite **21** under the activation of DCI, followed by oxidation by *t*-BuOOH, as described above for the formation of **17**. The protecting groups were removed by sequential treatment with TFA and aqueous ammonia to yield **20** after RP-HPLC purification.



Scheme 4. Linear biotinylated ADPr fragment.

The synthesis of biotinylated portions of linear PAR (**28** and **30**, Scheme 4) commenced with known protected parobiose **2**[18] and its conversion into **3**. In contrast to the same transformation, described in scheme 1 of this chapter, the TIPS protection in **2** was removed first by the treatment with HF·TEA in pyridine followed by removal of the benzyl groups in the thus obtained **22** by hydrogenolysis with Pd/C and H_2 under high pressure to yield triol **3** after overnight treatment. This reversal of reactions gave a lower yield but the reaction time is markedly reduced from several days to 16 hours. The more acid-resistant TBDPS, instead of the TIPS, was introduced at 5''-OH of **3** and the other secondary alcohols were acetylated to furnish suitably protected parobiose **23**. N^6-benzoyl adenine was installed using the Vorbrüggen glycosylation method,[18] furnishing **24** in excellent yield. The synthetic route was continued by the following one-pot sequence of protecting group manipulations: aqueous NaOH mediated saponification, DMT introduction at 5'-OH and acetylation of the remaining secondary hydroxyls to yield orthogonally protected **25**. To obtain biotinylated linear fragment **28**, building block **25** was successively subjected to TFA mediated detritylation and reaction of the resulting **26** with phosphoramidite **21**, followed by oxidation, as described above, to furnish fully protected **27**. With the aid of the same three-step deprotection protocol as described for the formation of **18**, biotinylated linear fragment **28** was isolated. The final biotinylated iso-ADPr **30** was obtained by subjecting **25** to protective group manipulation and phosphorylation to give **29** (see Chapter 4). Conversion of intermediate **29** into biotinylated target **30** was accomplished using the same method as described for the isolation of **20.**

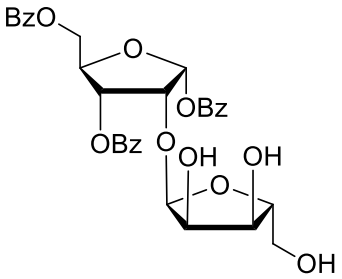
Conclusion

For the first time, O-α-D-ribofuranosyl-(1'''→2'')-O-α-D-ribofuranosyl-(1''→2')-adenosine-5',5'',5'''-tris(phosphate) (**1**), a tris-phosphorylated branched PAR fragment was obtained by organic synthesis. Comparison of the ^{1}H-NMR spectra of this fragment with the naturally occurring product showed the same chemical shifts which means that the structure of **1** was identical to the naturally occurring compound[9] and that the regio- and stereochemistry of branching point of PAR was correctly elucidated by Miwa et al. In addition, key elements of the synthetic methodology presented in this Chapter also give access to biotinylated branched/linear PAR fragments synthesis. These analogous will be a valuable asset for future biological studies toward the discovery of BBPs (branched PAR binding protein) and elucidating biological function of branched ADPr.

Experimental section

General procedure

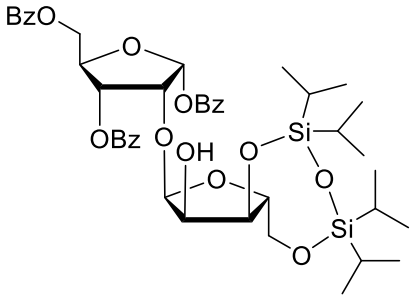
All solvents used were stored over molecular sieves and all reactions were carried out in oven or flame-dried glassware. Unless stated otherwise, all solvents were removed by rotary evaporation under reduced pressure at 40 °C. Reactions were monitored by TLC-analysis using Merk 25 DC plastikfolien 60 F254 with detection by spraying with 20% H_2SO_4 in MeOH or $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ in 10% sulfuric acid, followed by charring at approx. 150°C. LC-MS analysis was performed on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer with an electrospray ion source coupled to Surveyor HPLC system (Thermo Finnegan) using an analytical Gemini C18 column (Phenomex, 50 x 4.60 mm, 3 micron) in combination with eluents A: H_2O; B: MeCN and C: 1% aq. TFA as the solvent system. High resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylpthalate (m/z = 391.2842) as a "lock mass". The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were measured on Brüker DPX-300, Brüker AV-400/500/600/850 and all individual signal was assigned using 2D-NMR spectroscopy. Chemical shifts were given in ppm (δ) relative to TMS (0 ppm) or indirectly referenced to H_3PO_4 (0.00 ppm) in D_2O via the solvent residual signal and coupling constants were given in Hz. Infrared (IR) spectra were record on a Shimadzu FT-IR 8300. Optical rotation was measured by MCP 100 Modular Circular Polarimeter using methanol as solvent.



α-1,3,5-Tri-*O*-benzoylparobiose (3)

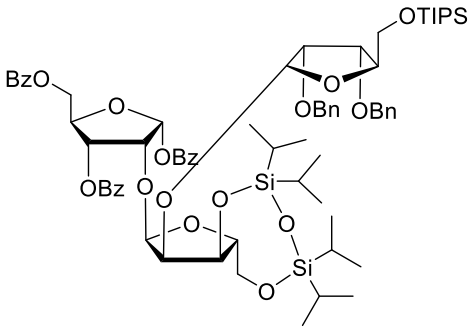
Compound **2** (6.90 g, 7.41 mmol) was dissolved in *t*BuOH/Dioxane/H_2O (120 ml, 4/4/1; v/v/v) and Pd/C (370 mg, 10% loading) was added. H_2 was bubbled through the solution for 72 h. TLC analysis showed an incomplete conversion, therefore, 300 mg Pd/C was added and the reaction was stirred under H_2 for 4 days after which the reaction mixture was filtered over celite, concentrated under reduced pressure and co-evaporated with pyridine (1 x) and toluene (1 x). 60 mL pyridine was added and the mixture was cooled to 0 °C. Et_3N (15.5 mL, 111.0 mmol) and $Et_3N\cdot 3HF$ (18 mL, 111.0 mmol) were added. The mixture was stirred for 18 h at room temperature and quenched by aq. $NaHCO_3$ (sat.). The mixture was extracted with EtOAc (3 x 240 mL) and the combined organic layers were dried over $MgSO_4$. After concentration under reduced pressure, the crude was purified by silica gel chromatography (pentane/EtOAc, 25/75 – 20/80) to obtain **3** as a white foam (3.20 g, 5.38 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 – 8.05 (m, 6H, arom.), 7.62 – 7.57 (m, 3H, arom), 7.49 – 7.46 (m, 2H, arom), 7.42 – 7.36 (m, 4H, arom), 6.76 (d, *J* = 4.2 Hz, 1H, H1'), 5.74 (dd, *J* = 6.3, 2.0 Hz, 1H, H3'), 5.20 (d, *J* = 4.2 Hz, 1H, H1''), 4.88 (td, *J* = 3.7, 1.9 Hz, 1H, H4'), 4.75 (dd, *J* = 6.3, 4.2 Hz, 1H, H2'), 4.63 (AB, *J* = 12.1, 3.8 Hz, 2H, H5'), 4.04 – 3.94 (m, 2H, H2'', H4''), 3.89 – 3.84 (m, 1H, H3'), 3.66 (AB, *J* = 12.1, 3.8 Hz, 1H, H5''), 3.56 (AB, *J* = 12.1, 3.8 Hz, 1H, H5''), 2.70 (d, *J* = 9.6 Hz, 1H, OH), 2.52 (d, *J* = 9.1 Hz, 1H, OH), 1.85 (s, 1H, OH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.84 (CO Bz), 166.14 (CO Bz), 165.93(CO Bz), 133.98, 133.69, 133.60, 130.06, 130.04, 129.80 (arom.), 129.66, 129.51, 129.04 (cq. arom.), 128.74, 128.73, 128.59 (arom.), 102.27 (C1''), 95.31 (C1'), 86.39 (C4''), 82.77 (C4'), 77.48, 77.16 76.84, 75.40(C2'), 72.33(C2''), 72.21 (C3'), 70.53 (C3''), 64.22 (C5'), 62.61 (C5''). IR (film): 3482 (br), 2930, 1717, 1267, 1117, 1093, 1068, 1022, 710 cm^{-1}. HRMS (ESI$^+$) calcd for

$C_{31}H_{30}O_{12}Na$ (M+Na) 617.1629. Found 617.1627. $[\alpha]_D^{20}$ +102.8 (c = 1, in MeOH)



α-1,3,5-Tri-*O*-benzoyl-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-parobiose (4)

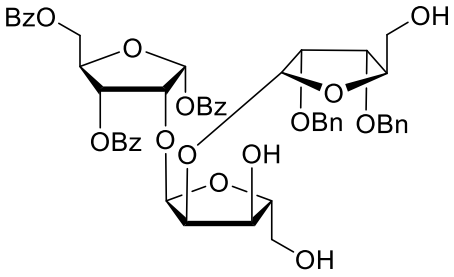
Compound **3** (6.66 g, 11.2 mmol) and imidazole (2.29 g, 33.6 mmol) were co-evaporated with toluene (2 x), dissolved in DCM (66 mL) and then TIPDCl (4.3 mL, 13.4 mmol) was added. The reaction was stirred at room temperature for 15 h and quenched upon the addition of H_2O (200 mL). The mixture was washed by DCM (3 x 100 mL) and the organic layer was dried by $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/acetone, 100/0 – 97/3) to obtain **4** as colorless foam (7.83 g, 9.35 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 – 8.03 (m, 6H, arom.), 7.63 – 7.53 (m, 3H, arom.), 7.48 (t, *J* = 7.6 Hz, 2H, arom), 7.44 – 7.34 (m, 4H, arom.), 6.79 (d, *J* = 4.2 Hz, 1H, H1'), 5.67 (dd, *J* = 6.3, 2.0 Hz, 1H, H3'), 5.19 (d, *J* = 4.0 Hz, 1H, H1''), 4.77 (m, 2H, H2', H4'), 4.65 (AB, *J* = 12.1, 3.5 Hz, 2H, H5'), 4.14 – 4.01 (m, 2H, H2'' H3''), 3.95 – 3.90 (m, 1H, H4''), 3.81 (AB, *J* = 11.8, 3.5 Hz, 1H, H5''), 3.66 (AB, *J* = 11.6, 8.3 Hz, 1H, H5''), 2.85 (d, *J* = 8.2 Hz, 1H, OH), 1.03 (m, 6H, CH_3, TIPDS), 1.00 – 0.83 (m, 18H, CH_3, TIPDS), 0.80 (d, *J* = 7.3 Hz, 2H, CH, TIPDS), 0.73 (d, *J* = 7.2 Hz, 2H, CH, TIPDS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.15, 165.70 (CO Bz), 133.50, 133.48, 133.45, 130.14, 129.97 (arom.), 129.92 (cq. arom.), 129.80 (arom.), 129.63 (cq. arom.), 128.67, 128.57, 128.49 (arom.), 101.93 (C1''), 95.13(C1'), 83.81(C4''), 83.32(C4'), 75.67 (C2'), 71.93 (C3'), 71.03 (C2''), 70.77 (C3''), 64.30 (C5'), 63.39 (C5''), 17.55, 17.49, 17.45, 17.41, 17.06, 16.98, 16.82, 16.68, 13.44, 13.24, 13.01, 12.33 (CH, CH_3, TIPDS).



α-1,3,5-Tri-*O*-benzoyl-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'',3''-di-*O*-benzyl-5''-*O*-triisopropylsilylparotriose (6)

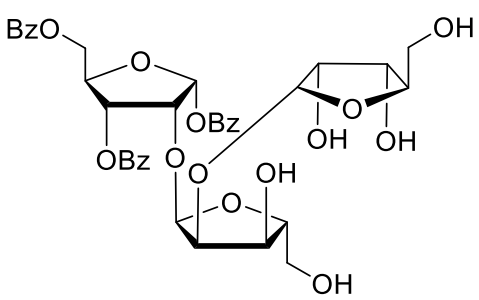
Compounds **4** (7.8 g, 9.32 mmol) and **5** (7.36 g, 11.18 mmol) were co-evaporated with toluene (2 x), 1,4-dioxane (2 x) and DCE (1 x). Dry DCM (150 mL) and freshly activated 4Å molecular sieves were added to the mixture. The mixture was stirred under argon at room temperature for 2 h and then cooled to -78°C. Next, TMSOTf (50 μL, 0.28 mmol) was added, the reaction mixture was stirred at the same temperature for 30 minutes and then was quenched by addition of triethylamine. The reaction mixture was concentrated under reduced pressure and purified by silica gel chromatography (pentane/EtOAc, 70/30 – 50/50) to obtain **6** as a white foam (9.7 g, 7.43 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 7.6 Hz, 2H, arom.), 8.08 (t, *J* = 7.6 Hz, 4H, arom.), 7.59 – 7.54 (m, 2H, arom), 7.48 (q, *J* = 7.4 Hz, 3H), 7.37 (t, *J* = 7.7 Hz, 2H, arom.), 7.29 – 7.11 (m, 12H, arom.), 6.79 (d, *J* = 4.0 Hz, 1H, H1'), 5.59 (dd, *J* = 6.3, 1.7 Hz, 1H, H3'), 5.36 (d, *J* = 3.4 Hz, 1H, H1''), 5.28 (d, *J* = 3.9 Hz, 1H, H1'''), 4.85 (dd, *J* = 6.2, 4.1 Hz, 1H, H2'), 4.76 – 4.67 (m, 2H, CH_2 Bn, H4'), 4.62 (AB, *J* = 12.0, 3.5 Hz, 1H, H5'), 4.52 (AB, *J* = 12.0, 4.0 Hz, 1H, H5'), 4.49 – 4.36 (m, 2H, CH_2 Bn, H2''), 4.32 (d, *J* = 11.8 Hz, 1H, CH_2 Bn), 4.25 – 4.01 (m, 4H, H3'', H4''', H4'', CH_2 Bn), 3.95 (d, *J* = 11.4 Hz, 1H, CH_2 Bn), 3.83 (AB, *J* = 13.1, 2.3 Hz, 1H, H5''), 3.78 – 3.63 (m, 4H, H5'',H5''', H3'''), 3.43 (dd, *J* = 6.4, 3.9 Hz, 1H, H2'''), 1.11 – 0.94 (m, 42H,

CH_3, TIPS, TIPDS,), 0.93–0.88 (m, 7H, CH, TIPS, TIPDS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.15, 165.99, 165.66 (CO Bz), 138.94, 138.68 (cq. arom.), 133.50, 133.47, 133.30, 130.14 (arom.), 130.04 (cq. arom.), 129.99, 129.93 (arom.), 129.77, 129.68 (cq. arom.), 128.62, 128.55, 128.13, 128.00, 127.85, 127.69, 127.35, 127.16 (arom.), 102.21 (C1''), 101.23 (C1'''), 95.07 (C1'), 83.34 (C4'), 81.18 (C4'''), 81.12 (C4''), 77.36 (C2'''), 75.56 (C3'''), 75.12 (C2'), 73.58 (C2''), 72.35 (C3'), 72.30 (CH_2 Bn), 71.78 (CH_2 Bn), 69.04 (C3''), 64.25 (C5'), 62.61 (C5''), 59.95 (C5'''), 18.07, 17.52, 17.47, 17.42, 17.22, 17.18, 17.09, 16.96, 13.61, 13.13, 12.77, 12.59, 12.03 (CH_3, CH_2, TIPDS, TIPS).



α-1,3,5-Tri-*O*-benzoyl-2'',3''-di-*O*-benzylparotriose (7)

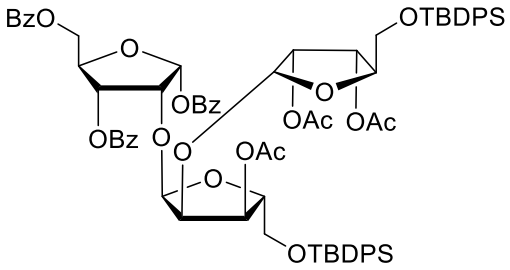
Compound **6** (7.7 g, 5.90 mmol) and 60 mL pyridine were added into a flask and cooled to 0℃. Subsequently, $Et_3N{\cdot}3HF$ (14.5 mL, 88.53 mmol) was added under argon. The reaction was stirred at room temperature for 24 h and then additional 1.5 ml $Et_3N \cdot 3HF$ was added at 0℃. The mixture was stirred for 5 h at room temperature and quenched by addition of aq. $NaHCO_3$ (sat.). 50 mL H_2O was added and the mixture was extracted by EtOAc (3 x 80 mL). The combined organic layers were dried over $MgSO_4$. The mixture was filtered and then concentrated under reduced pressure. Purification by silica gel chromatography (pentane/actone, 70/30 – 50/50) furnished **7** as a white foam (4.3 g, 4.74 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 – 8.04 (m, 6H, arom.), 7.60 – 7.52 (m, 3H,), 7.47 – 7.43 (m, 2H), 7.42 – 7.29 (m, 4H), 7.29 – 7.19 (m, 8H), 7.19 – 7.10 (m, 2H), 6.80 (d, *J* = 4.1 Hz, 1H, H1'), 5.64 (dd, *J* = 6.3, 2.4 Hz, 1H, H3'), 5.37 (d, *J* = 3.6 Hz, 1H, H1''), 5.31 (d, *J* = 3.7 Hz, 1H, H1'''), 4.79 (dd, *J* = 6.3, 4.1 Hz, 1H, H2'), 4.75 (td, *J* = 3.9, 2.4 Hz, 1H, H4'), 4.62 (AB, *J* = 12.0, 3.6 Hz, 1H, H5'), 4.57 (d, *J* = 11.9 Hz, 1H, CH_2, Bn), 4.52 (AB, *J* = 12.0, 4.3 Hz, 1H, H5'), 4.43 – 4.36 (m, 2H, CH_2, Bn), 4.30 (dd, *J* = 5.4, 3.6 Hz, 1H, H2'), 4.10 (q, *J* = 3.3 Hz, 1H, H4''), 4.04 (d, *J* = 11.4 Hz, 1H, CH_2, Bn), 4.01 – 3.90 (m, 2H, H4'', H3''), 3.70 (dd, *J* = 5.9, 3.1 Hz, 1H, H3'''), 3.65 – 3.43 (m, 5H, OH, H2''', H5'', H5'''), 3.36 – 3.30 (m, 1H, H5'''), 1.76 (d, *J* = 6.1 Hz, 1H, OH), 1.53 (dd, *J* = 8.4, 4.8 Hz, 1H, OH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.14 (CO, Bz), 165.70 (CO, Bz), 137.85, 137.73 (cq. arom.), 133.64, 133.61, 133.50, 130.27, 130.10 (arom.), 129.94 (cq. arom), 129.89 (arom.), 129.70, 129.66 (cq. arom.), 128.65, 128.61, 128.57, 128.51, 128.48, 128.46, 128.41, 128.30, 127.98, 127.80, 127.77 (arom.), 102.66 (C1''), 99.56 (C1'''), 95.65 (C1'), 84.40 (C3''), 83.72 (C4''), 82.94 (C4'), 79.44 (C2'''), 76.07 (C3'''), 75.63 (C2'), 72.99 (cq. CH2, Bn), 72.86 (C2''), 72.32 (CH2, Bn), 71.97 (C3'), 70.30 (C4''), 64.19 (C5'), 62.60 (C5'''), 62.03 (C5''). IR (film): 3456 (bs), 2920, 1722, 1267, 1096, 1069, 1024, 712 cm^{-1}. HRMS (ESI^+) calcd for $C_{50}H_{50}O_{16}Na$ (M+Na) 929.2991. Found 929.2999. $[\alpha]_D^{20}$ +98.3 (c = 1, in MeOH)



α-1,3,5-Tri-*O*-benzoylparobiose (8)

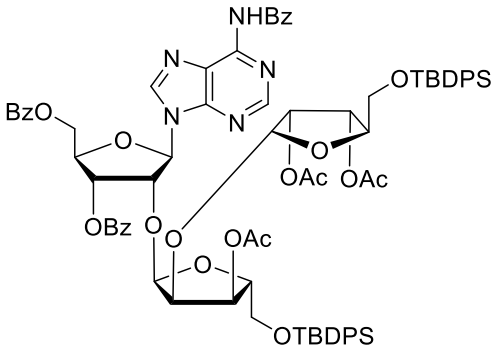
Compound **7** (420 mg, 0.46 mmol) was dissolved in MeOH (10 mL), Pd/C (100mg, 10% loading) and few drops of AcOH were added. The mixture was sonicated under argon for 5 minutes then H_2 was bubbled for 24 h. The reaction was filtered over celite and the residue was concentrated under reduced pressure and purified by silica gel chromatography (DCM/MeOH, 95/5 – 92/8)

to obtain **8** as a white foam (292 mg, 0.40 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 – 8.00 (m, 6H), 7.62 – 7.52 (m, 3H, arom), 7.49 – 7.33 (m, 6H, arom.), 6.76 (d, *J* = 4.2 Hz, 1H, H1'), 5.69 (dd, *J* = 6.3, 1.7 Hz, 1H, H3'), 5.27 (d, *J* = 3.9 Hz, 1H, H1''), 4.95 (d, *J* = 3.9 Hz, 1H, H1'''), 4.87 – 4.79 (m, 1H, H4'), 4.72 (dd, *J* = 6.3, 4.2 Hz, 1H, H2'), 4.67 – 4.53 (m, 2H, H5'), 4.10 – 4.02 (m, 1H, H2''), 3.96 – 3.93 (m, 3H, H3'', H4''', H4''), 3.73 – 3.35 (m, 7H, H3''', H2''', H5'', H5''', OH), 3.29 (d, *J* = 7.6 Hz, 1H, OH), 3.14 (d, *J* = 9.6 Hz, 1H, OH), 3.04 – 2.98 (m, 2H, OH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.65 (CO, Bz), 166.17 (CO, Bz), 165.92 (CO, Bz), 133.85, 133.76, 133.52, 130.13, 129.76 (arom.), 129.50, 129.47, 129.07 (cq. arom.), 128.65, 128.56 (arom.), 101.41 (C1''), 100.98 (C1'''), 95.33 (C1'), 86.31 (C4''), 85.71 (C4'''), 83.18 (C4'), 75.30 (C2'), 75.21 (C2''), 72.45 (C2'''), 72.14 (C3'), 70.91 (C3''), 70.84 (C3'''), 64.29 (C5'), 62.76 (C5'''), 62.16 (C5''). IR (film): 3466 (bs), 2934, 1717, 1269, 1119, 1094, 1069, 1024, 710 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{36}H_{38}O_{16}Na$ (M+Na) 749.2052. Found 749.2051. $[\alpha]_D^{20}$ +115.8 (c = 1, in MeOH)



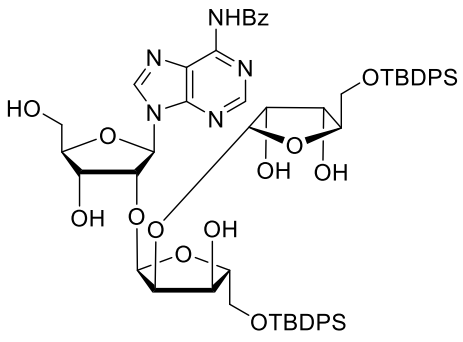
α-1,3,5-Tri-*O*-benzoyl-3'-*O*-acetyl-5'-*O*-tertbutyldiphenylsilyl-2'',3''-di-*O*-acetyl-5''-*O*-tertbutyldiphenylsilylparotriose (9)

Compound **8** (2.7 g, 3.72 mmol) was co-evaporated with pyridine (2 x) and then applied argon. Pyridine (38 mL) and TBDPSCl (4 mL, 15.15 mmol) were added and the mixture was stirred under argon at room temperature for 6 h. Ac_2O (11 mL, 113.4 mmol) was added into the reaction and the mixture was stirred for 16 h after which the reaction was quenched by addition of aq. $NaHCO_3$ (sat.). The mixture was extracted by DCM (3 x 50 mL) and dried by $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel chromatography (pentane/actone, 100/0 – 80/20) obtained **9** as a white foam (3.1 g, 2.33 mmol, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 – 8.18 (m, 2H, arom.), 8.18 – 8.12 (m, 2H, arom.), 8.10 – 8.05 (m, 2H, arom.), 7.69 – 7.49 (m, 11H, arom.), 7.45 – 7.30 (m, 18H, arom.), 6.80 (d, *J* = 4.3 Hz, 1H, H1'), 5.72 (dd, *J* = 6.3, 1.8 Hz, 1H, H3'), 5.46 (dd, *J* = 6.6, 1.9 Hz, 1H, H3''), 5.39 (dd, *J* = 7.0, 3.2 Hz, 1H, H3'''), 5.32 (d, *J* = 4.2 Hz, 1H, H1''), 5.29 (d, *J* = 4.5 Hz, 1H, H1'''), 4.89 (dd, *J* = 7.0, 4.4 Hz, 1H, H2'''), 4.77 (td, *J* = 3.7, 1.7 Hz, 1H, H4'), 4.72 – 4.56 (m, 3H, H2', H5'), 4.36 (dd, *J* = 6.6, 4.2 Hz, 1H, H2''), 4.09 – 4.06 (m, 2H, H4''', H4''), 3.81 (AB, *J* = 11.2, 2.7 Hz, 1H, H5''), 3.74 – 3.57 (m, 3H, H5'', H5'''), 2.01 (s, 3H, Ac), 1.79 (s, 3H, Ac), 1.64 (s, 3H, Ac), 1.05 (s, 9H, CH_3, TBDPS), 0.97 (s, 9H, CH_3, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.70, 170.09, 169.70 (CO, Ac), 166.17, 166.14, 165.56 (CO, Bz), 135.75, 135.72, 135.70, 135.67, 133.51, 133.49 (arom.), 133.13, 133.09, 133.02, 132.95, 130.25 (cq. arom.), 130.15, 130.12, 129.95, 129.93, 129.89, 129.85, 129.79 (arom.), 129.73 (cq. arom), 128.69, 128.50, 127.92, 127.90, 127.87 (arom.), 101.29 (C1''), 99.52 (C1'''), 95.16 (C1'), 83.73 (C4''), 83.66 (C4'), 83.14 (C4'''), 76.35 (C2'), 74.62 (C2''), 71.75 (C2'''), 71.66 (C3'), 71.26 (C3''), 69.82 (C3'''), 64.44 (C5'), 63.90 (C5''), 63.42 (C5'''), 26.90 (CH_3, TBDPS), 26.85 (CH_3, TBDPS), 20.70 (Ac), 20.39 (Ac), 20.17 (Ac), 19.38 (cq. TBDPS), 19.29 (cq. TBDPS). IR (film): 1728, 1265, 1252, 1112, 1067, 1042, 1026, 709 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{74}H_{80}O_{19}Si_2Na$ (M+Na) 1351.4725. Found 1351.4734. $[\alpha]_D^{20}$ +72.8 (c = 1, in MeOH)



6-*N*-benzoyl-9-(3',5'-di-*O*-benzoyl-3''-*O*-acetyl-5''-*O*-tertbutyldiphenylsilyl-2''',3'''-di-*O*-acetyl-5'''-*O*-tertbutyldiphenylsilyl-β-parotriosyl)adenine (10)

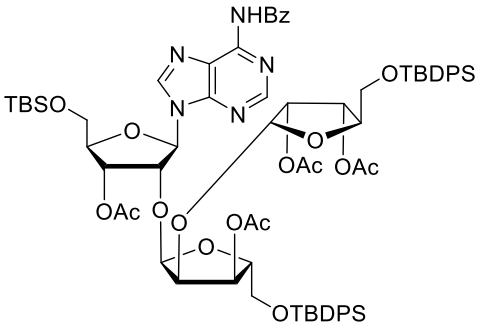
Compound **9** (1.1 g, 0.83 mmol) and N^6-benzoyladenine (0.41 g, 1.71 mmol) were co-evaporated with toluene (2 x), 1,4-dioxane (2 x), MeCN (1 x) and dissolved in dry MeCN (14 mL) under argon. *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (3.2 mL, 12 mmol) was added and the mixture was stirred at room temperature until everything was dissolved. $HClO_4$-SiO_2 (4.3 g, 0.4 mmol/g, 1.71 mmol) was added and the mixture was refluxed for 48 h. Then the reaction was quenched by aq. $NaHCO_3$ (sat.) then filtered. The mixture was extracted with EtOAc (3 x 100 mL), dried by $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel chromatography (pentane/acetone, 100/0 – 85/15 – 75/25 – 70/30) obtained **10** as a white foam (0.99 g, 0.68 mmol, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.04 (s, 1H, NH), 8.68 (s, 1H, H2), 8.41 (s, 1H, H8), 8.08 (tt, *J* = 6.6, 1.4 Hz, 4H, arom.), 8.00 – 7.93 (m, 2H, arom.), 7.65 – 7.45 (m, 13H, arom.), 7.45 – 7.27 (m, 16H, arom.), 6.32 (d, *J* = 4.6 Hz, 1H, H1'), 5.95 (t, *J* = 5.3 Hz, 1H, H3'), 5.70 (t, *J* = 5.0 Hz, 1H, H2'), 5.44 (dd, *J* = 6.9, 2.4 Hz, 1H, H3''), 5.40 (dd, *J* = 7.4, 3.5 Hz, 1H, H3'''), 5.25 (d, *J* = 4.4 Hz, 1H, H1''), 5.17 (d, *J* = 4.3 Hz, 1H, H1'''), 4.94 (dd, *J* = 7.3, 4.4 Hz, 1H, H2'''), 4.89 (AB, *J* = 12.0, 3.8 Hz, 1H, H5'), 4.79 – 4.74 (m, 1H, H4'), 4.70 (AB, *J* = 12.0, 4.9 Hz, 1H, H5'), 4.32 (dd, *J* = 6.9, 4.3 Hz, 1H, H2''), 4.10 (q, *J* = 3.1 Hz, 1H, H4'''), 4.01 (q, *J* = 2.8 Hz, 1H, H4'), 3.78 (AB, *J* = 11.4, 2.7 Hz, 1H, H5'''), 3.70 (AB, *J* = 11.3, 3.2 Hz, 1H, H5'''), 3.58 (AB, *J* = 11.2, 2.8 Hz, 1H, H5''), 3.44 (AB, *J* = 11.2, 3.3 Hz, 1H, H5''), 2.11 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.68 (s, 3H, Ac), 1.01 (s, 9H, CH_3, TBDPS), 0.96 (s, 9H, CH_3, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.53, 169.88, 169.79 (CO, Ac), 166.25, 165.35, 164.45 (CO, Bz), 152.87 (CH, C2), 151.35, 149.74 (cq. arom.), 135.61, 135.59 (arom.), 133.71 (aq. arom.), 133.59, 133.44 (arom.), 132.96, 132.91, 132.87 (cq. arom,), 132.82 (arom.), 132.75 (aq. arom.), 129.90, 129.88, 129.85, 129.83 (arom.), 129.57, 129.53 (cq. arom.), 128.92, 128.59, 128.54, 127.88, 127.86, 127.82, 127.81, 127.79 (arom.), 123.92 (cq. arom.), 101.19 (C1''), 98.61 (C1'''), 89.09 (C1'), 83.02 (C4'''), 82.38 (C4''), 80.46 (C4'), 72.98 (C2''), 72.44 (C3'), 71.74 (C2'''), 70.96 (C3''), 69.79 (C3'''), 63.58 (C5'), 63.52 (C5''), 63.13 (C5'''), 26.83, 26.76 (CH_3, Ac), 20.75, 20.66, 20.40 (CH_3, TBDPS), 19.25 (cq. TBDPS). IR (film): 2930, 1728, 1238, 1111, 1069, 1038, 1028, 702 cm^{-1}. HRMS (ESI^+) calcd for $C_{79}H_{84}N_5O_{18}Si_2$ (M+H) 1446.5344. Found 1446.5344. $[\alpha]_D^{20}$ +31.4 (c = 1, in MeOH)



6-*N*-benzoyl-9-(5'',5'''-di-*O*-tertbutyldiphenylsilyl-β-parotriosyl)adenine (11)

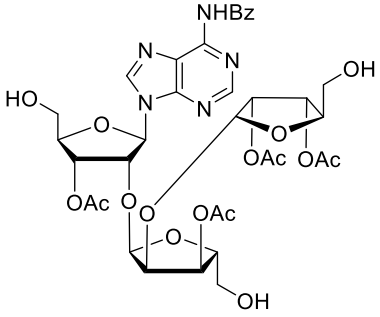
Compound **10** (984 mg, 0.68 mmol) was dissolved in pyridine/EtOH (7 mL; 2/1 v/v), cooled to 0°C after which aqueous NaOH (4.1 mL, 1 M) was slowly added. The reaction mixture was stirred for 2 h at the same temperature after which Amberlite-H^+ was added until pH = 6. The mixture was filtered, concentrated under reduced pressure and purified by silica gel chromatography (DCM/methanol, 100/0 – 97/3 – 95/5) to obtain **11** as a white foam (641 mg, 0.58 mmol, 85%). ^{1}H NMR (400

MHz, $CDCl_3$) δ 9.52 (s, 1H, NH), 8.80 (s, 1H, H2), 8.53 (s, 1H, H8), 8.03 – 7.92 (m, 2H, arom.), 7.62 – 7.59 (m, 8H), 7.56 – 7.49 (m, 1H, arom.), 7.46 – 7.23 (m, 14H, arom.), 6.23 (d, *J* = 7.3 Hz, 1H, H1'), 5.12 (d, *J* = 4.4 Hz, 1H, H1''), 4.99 (d, *J* = 4.0 Hz, 1H, H1'''), 4.94 (dd, *J* = 7.4, 4.7 Hz, 1H, H2'), 4.60 (d, *J* = 4.7 Hz, 1H, H3'), 4.44 (t, *J* = 4.8 Hz, 2H, H2''), 4.39 – 4.22 (m, 5H, H3''', H4', H2''', H3''', H4''), 4.20 (q, *J* = 2.8 Hz, 1H, H4'''), 3.97 (AB, *J* = 13.0, 1.8 Hz, 1H, H5'), 3.77 – 3.65 (m, 5H, H5', H5'', H5'''), 3.73 – 3.63 (m, 4H), 0.99 (s, 9H, TBDPS), 0.98 (s, 9H, TBDPS) ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.04 (CO, Bz), 152.21 (C2), 150.66, 150.30 (cq. arom.), 144.26 (C8), 135.61, 135.59, 135.56 (arom.), 133.58, 133.06 (cq. arom.), 132.91 (arom.), 132.83, 132.65 (cq. arom), 130.00, 129.97, 129.93, 129.86, 128.85, 128.09, 127.91, 127.87, 127.82 (arom.), 124.36 (cq. arom.), 101.94 (C1'''), 101.08 (C1''), 89.48 (C1'), 88.17 (C4'), 86.40 (C4''), 86.19 (C4'''), 80.01 (C2'), 76.99 (C2''), 73.22 (C2'''), 72.92 (C3'), 72.15 (C3''), 71.16 (C3'''), 64.32 (C5'''), 64.13 (C5''), 63.27 (C5'), 26.88 (CH_3, TBDPS), 26.86 (CH_3, TBDPS), 19.26 (cq. TBDPS), 19.23 (cq. TBDPS). IR (film): 3329 (bs), 2930, 2857, 1701, 1612, 1458, 1105, 1072, 1037, 702 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{59}H_{70}N_5O_{13}Si_2$ (M+H) 1112.4503. Found 1112.4511. $[\alpha]_D^{20}$ +48.7 (c = 1, in MeOH)



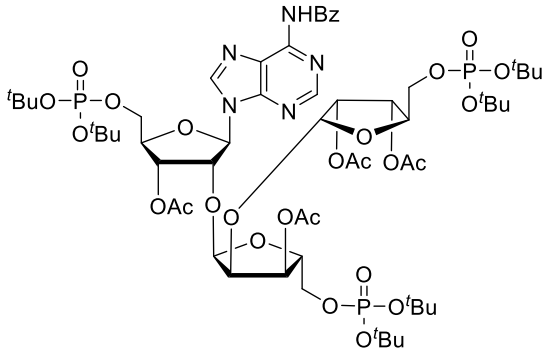
6-*N*-benzoyl-9-(3',3''2''',3'''-tetra-*O*-acetyl-5'-*O*-tertbutyldimethylsilyl-5'',5'''-di-*O*-tertbutyldiphenylsilyl-β-parotriosyl)adenine (12)

Compound **11** (146 mg, 0.13 mmol) was dissolved in dry pyridine (1.3 mL), TBSCl (50 mg, 0.32 mmol) was added and the reaction was stirred for 6 hours at room temperature. TLC showed an incomplete conversion and additional TBSCl (100 mg, 0.66 mmol) was added. The mixture was stirred at room temperature for 5 h after which Ac_2O (0.37 ml, 3.9 mmol) was added. The mixture was stirred at 0°C for 10 h then quenched by aq. $NaHCO_3$ (sat.). 20 mL H_2O was added and the mixture was extracted with DCM (3 x 15 mL), dried over $MgSO_4$, concentrated under reduced pressure and purified by silica gel chromatography (pentane/actone, 100/0 – 90/10 – 85/15 – 80/20) to obtain **12** as a white foam (133 mg, 0.09 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H, NH), 8.80 (s, 1H, H2), 8.51 (s, 1H, H8), 8.05 – 7.95 (m, 2H, arom.), 7.69 – 7.54 (m, 9H, arom.), 7.50 (t, *J* = 7.6 Hz, 2H, arom.), 7.45 – 7.29 (m, 12H, arom.), 6.33 (d, *J* = 3.8 Hz, 1H, H1'), 5.53 (dd, *J* = 6.8, 2.1 Hz, 1H, H3''), 5.45 (dd, *J* = 7.3, 3.0 Hz, 1H, H3'''), 5.41 (t, *J* = 5.5 Hz, 1H, H3'), 5.34 – 5.32 (m, 2H, H1'', H1'''), 5.10 (t, *J* = 4.6 Hz, 1H, H2'), 5.05 (dd, *J* = 7.1, 4.5 Hz, 1H, H2'''), 4.39 – 4.34 (m, 2H, H2'', H4'), 4.18 (t, *J* = 2.9 Hz, 1H, H4'''), 4.10 – 4.07 (m, 2H, H4'', H5'), 3.93 – 3.68 (m, 5H, H5', H5'', H5'''), 2.14 (s, 3H, Ac), 2.11 (s, 6H, 2Ac), 2.06 (s, 3H, Ac), 1.04 (s, 9H, TBDPS), 1.01 (s, 9H, TBDPS), 0.93 (s, 9H, CH_3, TBS), 0.12 (s, 6H, CH_3, TBS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.53, 169.94, 169.71, 169.62 (CO, Ac), 164.61 (CO, Bz), 152.86 (C2), 151.42, 149.58 (cq. arom.), 141.76 (C8), 135.65, 135.62, 135.59 (arom.), 133.83, 133.00, 132.96, 132.83 (cq. arom.), 132.74, 129.89, 129.85, 128.87, 127.93, 127.85, 127.82 (arom), 123.51 (cq. arom.), 100.71 (C1''), 99.37 (C1'''), 88.06 (C1'), 83.30 (C4''), 82.85 (C4', C4'''), 77.99 (C2'), 73.86 (C2''), 71.60 (C2'''), 71.30 (C3''), 71.04 (C3'), 69.97 (C3'''), 63.77 (C5''), 63.34 (C5'''), 62.11 (C5'), 26.82, 26.80 (CH_3, TBDPS), 26.02 (CH_3, TBS), 21.04, 20.86, 20.78, 20.46 (CH_3, Ac), 19.27 (cq. TBDPS), 18.53 (cq. TBS), -5.31, -5.40 ($SiCH_3$, TBS). IR (film): 2951, 2930, 2859, 1746, 1236, 1113, 1043, 702 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{73}H_{92}N_5O_{17}Si_3$ (M+H) 1394.5790. Found 1394.5789. $[\alpha]_D^{20}$ +70.0 (c = 1, in MeOH).



[6-*N*-benzoyl-9-(3',3''2''',3'''-tetra-*O*-acetyl-β-parotriosyl)adenine] (13)

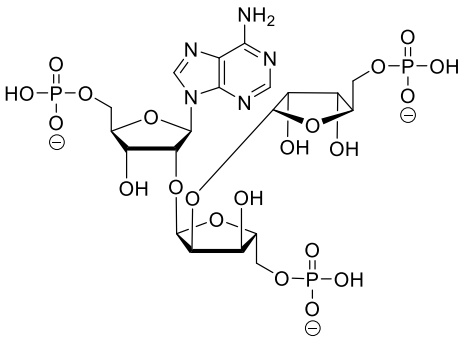
Compound **12** (133 mg, 0.09 mmol) was dissolved in pyridine (1 mL), cooled to 0°C after which HF·pyridine (0.12 mL, 4.3 mmol) was added. The reaction was stirred for 1.5 hours at 0°C after which was quenched by aq. $NaHCO_3$ (sat.) then extracted with EtOAc (4 x 10 mL), dried over $MgSO_4$, concentrated under reduced pressure and purified by silica gel chromatography (DCM/methanol, 100/0 – 100/1 – 96/4) to obtain **13** as a white foam (62 mg, 77 µmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H, NH), 8.64 (s, 1H, H2), 8.59 (s, 1H, H8), 8.02 (d, *J* = 7.4 Hz, 2H, arom.), 7.62 – 7.53 (m, 1H, arom.), 7.49 (t, *J* = 7.6 Hz, 2H, arom.), 6.24 (d, *J* = 10.9 Hz, 1H, OH), 6.10 (d, *J* = 7.8 Hz, 1H, H1'), 5.61 (d, *J* = 5.4 Hz, 1H, H3'), 5.17 (dd, *J* = 7.3, 4.2 Hz, 1H, H3''), 5.12 – 5.08 (m, 2H, H2', H3'''), 4.96 (d, *J* = 4.4 Hz, 1H, H1''), 4.91 (dd, *J* = 7.3, 4.5 Hz, 1H, H2''), 4.68 (d, *J* = 4.2 Hz, 1H, H1'''), 4.24 (s, 1H, H4'), 4.03 – 3.98 (m, 3H, H4'', H2''', H4'''), 3.91 (AB, *J* = 12.6 Hz, 1H, H5'), 3.82 – 3.48 (m, 5H, H5', H5'', H5'''), 3.41 (bs, 1H, OH), 2.99 (bs, 1H, OH), 2.14 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.06 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.56, 170.03, 169.71 (CO, Ac), 165.25 (CO, Bz), 152.04 (C2), 150.72, 150.52 (cq. arom.), 144.26 (C8), 133.28 (cq, arom), 133.06, 128.90, 128.21 (arom.), 124.72 (cq. arom.), 101.58 (C1'''), 98.42 (C1''), 89.08 (C1'), 86.54 (C4'), 82.24 (C4''), 82.09 (C4'''), 77.68 (C2'), 73.89 (C3'), 72.12 (C2''), 71.44 (C2'''), 70.41 (C3''), 69.68 (C3'''), 62.79 (C5'), 61.79 (C5''), 61.54 (C5'''), 21.15, 20.97, 20.76, 20.72 (CH_3, Ac). IR (film): 3352 (bs), 2932, 1738, 1612, 1584, 1456, 1369, 1238, 1092, 1043 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{35}H_{42}N_5O_{17}$ (M+H) 804.2570. Found 804.2573. $[\alpha]_D^{20}$ +78.3 (c = 1, in MeOH)



6-*N*-benzoyl-9-(3',3''2''',3'''-tetra-*O*-acetyl-5',5'',5'''-tri-*O*-(ditertbutylphosphoryl)-β-parotriosyl)adenine (14)

1-Methyl-imidazole·HCl (200 mg, 1.68 mmol) and 1-methyl-imidazole (88 µL, 1.1 mmol) were co-evaporated with dry CH_3CN (3 x), then N_2 was applied. To this mixture, freshly activated molecular sieves and dry DMF (0.9 mL) were added and the activator solution was stirred at room temperature for 2 hours under N_2. Next, compound **13** (73 mg, 91 µmol) was co-evaporated with dry 1,4-dioxane (3 x) and mixed with the activator solution, after which di-tert-butyl-*N*,*N*-diisopropylphosphoramidite (0.28 mL, 0.9 mmol) was added and the reaction was stirred at room temperature for 1 hour. Then *t*BuOOH in decane (0.56 mL, 5.5 M, 3.08 mmol) was added at 0°C and the reaction mixture was stirred for 1 hour at room temperature. The reaction was quenched by aq. $NaHCO_3$ (sat.) and extracted with EtOAc (3 x 10 mL), dried over $MgSO_4$, concentrated under reduced pressure. Purification by silica gel chromatography (DCM/MeOH, 100/0 – 95/5) then LH-20 gel filtration (DCM/methanol, 50/50) obtained **14** as a white foam (70 mg, 51 µmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 1H, NH), 8.81 (s, 1H, H2), 8.45 (s, 1H, H8), 8.10 – 7.99 (m, 2H, arom.), 7.67 – 7.58 (m, 1H, arom.), 7.53 (dd, *J* = 8.3, 6.7 Hz, 2H, arom.), 6.26 (d, *J* = 4.7 Hz, 1H, H1'), 5.50 (t, *J* = 5.1 Hz, 1H, H3'), 5.37 (dd, *J* = 7.1, 2.8 Hz, 1H, H3''), 5.33 – 5.26 (m, 1H, H2'), 5.23 (dd, *J* =

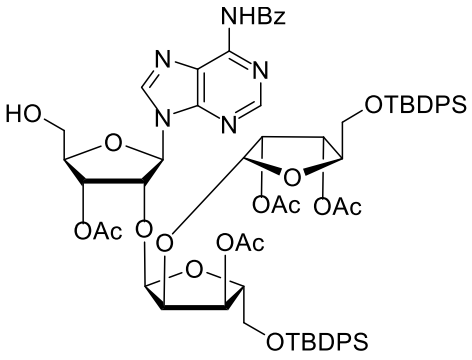
7.4, 3.6 Hz, 1H, H3'''), 5.19 (d, *J* = 4.3 Hz, 1H, H1''), 5.17 (d, *J* = 4.4 Hz, 1H, H1'''), 4.86 (dd, *J* = 7.4, 4.4 Hz, 1H, H2'''), 4.44 (tt, *J* = 4.2, 2.1 Hz, 1H, H4'), 4.38 – 4.33 (m, 1H, H5'), 4.26 – 4.18 (m, 4H, H5', H4'', H4''', H2''), 4.15 – 3.97 (m, 4H, H5'', H5'''), 2.17 (s, 3H, Ac), 2.14 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.56 – 1.39 (m, 54H, *t*Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.46, 169.70, 169.56, 169.49 (CO, Ac), 164.61 (CO, Bz), 152.88 (C2), 151.45, 149.79 (cq. arom.), 142.46 (C8), 133.82 (cq. arom.), 132.85, 128.96, 127.99 (arom.), 123.80 (cq. arom.), 100.58 (C1''), 98.85 (C1'''), 88.10 (C1'), 83.22, 83.17, 83.15, 83.10, 82.96, 82.92, 82.90, 82.85, 82.82 (cq. *t*Bu), 81.20, 81.12, 81.04, 80.86, 80.77 (C4', C4'', C4'''), 77.02, 72.94 (C2''), 71.51 (C3'), 71.23 (C2'''), 70.48 (C3''), 69.56 (C3'''), 65.85, 65.79 (C5'''), 65.55, 65.50 (C5''), 65.03, 64.97 (C5'), 29.97, 29.93, 29.88 (CH_3, *t*Bu), 21.00, 20.97, 20.76, 20.52 (CH_3, Ac). ^{31}P NMR (162 MHz, $CDCl_3$) δ -9.85, -9.99, -10.04. IR (film): 2980, 1746, 1371, 1244, 1040, 997.2 cm^{-1}. HRMS (ESI^+) calcd for $C_{59}H_{92}N_5O_{26}P_3$ (M+H) 1379.5243. Found 1380.5339. $[\alpha]_D^{20}$ +41.1 (c = 1, in MeOH)



_O_-α-D-ribofuranosyl-(1'''→2'')-_O_-α-D-ribofuranosyl-(1''→2')-adenosine-5',5'',5'''-tris(phosphate)
[Parotriosyladenine-5',5'',5'''-tri-_O_-phosphate] (1)

Compound **14** (20 mg, 14.5 µmol) was dissolved in HFIP (0.6 mL), concentrated HCl was added (7.2 µL, 87 µmol) and the reation mixture was stirred at room temperature for 1 h and ^{31}P-NMR spectroscopy showed complete cleavage of the tert-butyl groups. 80 µL NH_4OH (35%) was added to quench the reaction and concentrated under reduced pressure. Co-evaporating the residue with 1,4-dioxane (3 x) then 2 mL NH_4OH (35%) was added and the mixture was stirred at room temperature for 3 days. LCMS showed complete reaction and then concentrated under reduced pressure. The residue was purified by HW-40 gel filtration (0.15 M, NH_4OAc in Miliq H_2O). Repeated lyophilization obtained **1** as a white solid (11.0 mg, 14.2 µmol, 98%). ^{1}H NMR (400 MHz, D_2O) δ 8.60 (s, 1H, H8), 8.26 (s, 1H, H2), 6.27 (d, *J* = 6.3 Hz, 1H, H1'), 5.36 (d, *J* = 3.8 Hz, 1H, H1''), 4.98 (d, *J* = 4.4 Hz, 1H, H1'''), 4.93 (dd, *J* = 6.3, 5.1 Hz, 1H, H2'), 4.60 (dd, *J* = 5.1, 3.0 Hz, 1H, H3'), 4.39 – 4.38 (m, 1H, H4'), 4.35 – 4.30 (m, 1H, H4''), 4.29 – 4.19 (m, 3H, H2'', H3'', H4'''), 4.05 (dd, *J* = 6.2, 3.0 Hz, 1H, H3'''), 4.02 – 4.00 (m, 2H, H5'), 3.95 (dd, *J* = 6.3, 4.3 Hz, 1H, H2'''), 3.86 – 3.74 (m, 4H, H5'', H5'''). ^{13}C NMR (101 MHz, D_2O) δ 155.66 (cq. arom. C6), 153.00 (cq. arom. C2), 149.11 (cq. arom. C4), 140.29 (cq. arom. C8), 118.61 (cq. arom. C5), 101.46 (C1'''), 101.12 (C1''), 85.25 (C1'), 85.17 (C4'), 84.46 (C4''), 84.22 (C4'''), 80.25 (C2'), 75.55 (C2''), 71.29 (C2'''), 70.71 (C3'), 69.95 (C3''), 69.78 (C3'''), 63.90 (C5'', C5'''), 63.76 (C5'). ^{31}P NMR (162 MHz, D_2O) δ 3.53, 3.48, 3.46. IR (film): 3180 (bs), 1686, 1647, 1420, 1034, 930, 795, 783, 719 cm^{-1}. HRMS (ESI^+) calcd for $C_{20}H_{33}N_5O_{21}P_3$ (M+H) 772.0875. Found 772.0874. $[\alpha]_D^{20}$ +29.6 (c = 1, in MeOH)

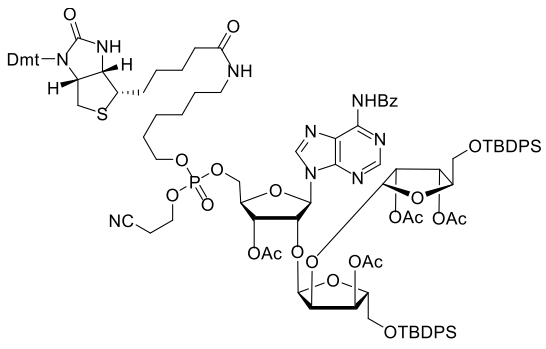
Synthesis of compound **15** will be described in Chapter 5



6-*N*-benzoyl-9-(3',3''2''',3'''-tetra-*O*-acetyl-5'',5'''-di-*O*-tertbutyldiphenylsilyl-β-parotriosyl)adenine (16)

Compound **15** (65mg, 41 µmol), DCM (0.4 mL) and AcOH (0.8 mL, 90% in H_2O) were added into a flask. The reaction was stirred for 3 hours after which it was quenched with aq. $NaHCO_3$ (sat.), extracted with DCM (3 x), dried (Na_2SO_4) concentrated and purified by silica gel column chromatography (DCM/acetone, 100/0 – 75/15) to furnish **16** as a white foam (34 mg, 27 µmol, 66 %).

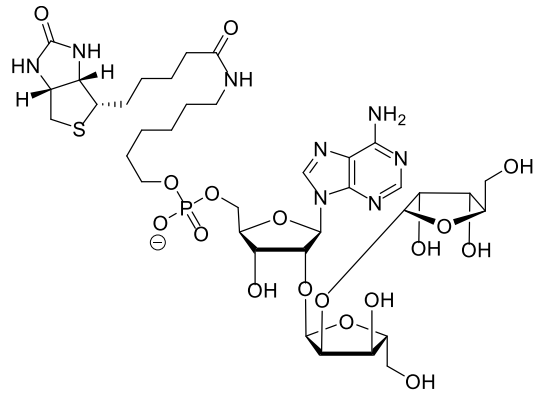
^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.07 (s, 1H, NH), 8.86 (s, 1H, H2), 8.59 (s, 1H, H8), 7.99 – 7.91 (m, 2H, Ar), 7.67 – 7.54 (m, 9H, Ar), 7.54 – 7.47 (m, 2H, Ar), 7.44 – 7.23 (m, 12H, Ar), 6.38 – 6.26 (m, 1H, OH), 6.14 (d, *J* = 7.9 Hz, 1H, H1'), 5.66 (d, *J* = 5.5 Hz, 1H, H3'), 5.42 (ddd, *J* = 14.8, 7.3, 3.5 Hz, 2H, H3'', H3'''), 5.17 (dd, *J* = 7.9, 5.5 Hz, 1H, H2'), 5.06 (d, *J* = 4.3 Hz, 1H, H1'''), 5.01 (dd, *J* = 7.4, 4.3 Hz, 1H, H2'''), 4.82 (d, *J* = 4.5 Hz, 1H, H1''), 4.34 – 4.25 (m, 2H, H4', H2''), 4.10 (q, *J* = 3.2 Hz, 1H, H4'''), 4.05 – 3.98 (m, 2H, H4'', H5'), 3.88 (t, *J* = 12.3 Hz, 1H, H5'), 3.81 – 3.66 (m, 4H, H5''', H5''), 2.22 (s, 3H, Ac), 2.15 – 2.14 (m, 6H, Ac), 2.10 (s, 3H, Ac), 1.03 (s, 9H, TBDPS), 0.96 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.54, 170.08, 169.71, 169.65 (CO Ac), 164.28 (CO Bz), 150.54, 150.37 (Cq. Ar), 135.71, 135.69, 135.68, 135.66 (Ar), 133.68, 133.06 (Cq. Ar), 133.02 (Ar), 132.95, 132.91, 132.86 (Cq. Ar), 129.99, 129.95, 129.06, 127.94, 127.92, 127.89, 127.84 (Ar), 124.54 (Cq. Ar), 101.20 (C1''), 98.35 (C1'''), 89.84 (C1'), 87.20 (C4'), 82.51 (C4''), 82.24 (C4'''), 77.55 (C2'), 74.46 (C3'), 72.33 (C2''), 71.80 (C2'''), 70.97 (C3''), 69.74 (C3'''), 63.63 (C5''), 63.12 (C5'''), 63.01 (C5'), 26.94, 26.87 (CH_3 TBDPS), 21.19, 21.07, 20.86, 20.83 (CH_3 Ac), 19.33, 19.29 (Cq. TBDPS). IR (film): 2931, 1743, 1739, 1609, 1447, 1427, 1235, 1227, 1112, 1039, 701 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{67}H_{77}N_5O_{17}Si_2$ (M+H) 1280.4926. Found 1280.4965. $[\alpha]_D^{20}$ +56.9 (c = 1, in Methanol)



6-*N*-benzoyl-9-(3',3''2''',3'''-tetra-*O*-acetyl-5'-O-{6-[(2-Cyanoethoxy)phosphoryl]-[1-N-(4,4'-dimethoxytrityl)biotinyl]aminohexane}-5'',5'''-di-*O*-tertbutyldiphenylsilyl-β-parotriosyl)adenine (17)

1-Methyl-imidazole·HCl (19 mg, 0.16 mmol) and 1-methyl-imidazole (9 µL, 0.11 mmol) were co-evaporated with dry CH_3CN (3 x), then N_2 was applied. To this mixture, freshly activated molecular sieves and dry DMF (0.5 mL) were added and the activator solution was stirred at room temperature for 2 hours under N_2. Next, compound **16** (34 mg, 27 µmol) was co-evaporated with dry 1,4-dioxane (3 x) after which the activator solution above was added. Subsequently, biotin-C_6-phosphoramidite **21** (46 mg, in 0.5 mL ACN, 54 µmol) was added and the reaction was stirred at room temperature for 20 minutes. *t*BuOOH in decane (29 µL, 5.5 M, 0.16 mmol) was added at 0 °C and the reaction mixture was stirred for 1 hour at room temperature. The reaction was quenched by aq. $NaHCO_3$ (sat.), extracted with DCM (3 x), dried ($MgSO_4$), concentrated, purified by LH-20 gel filtration (DCM/methanol, 50/50) to obtain **17** as a white foam (25 mg, 12 µmol, 44 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.37 (d, *J* = 17.4

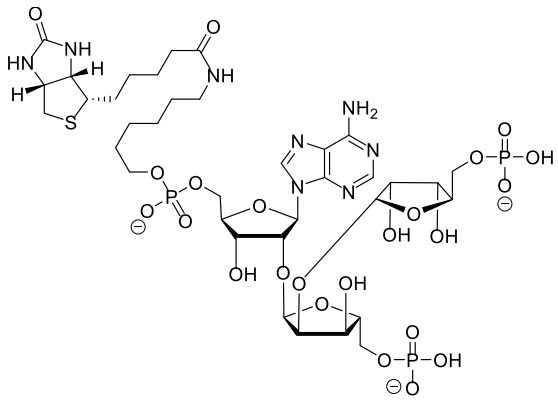
Hz, 1H, NH Ade), 8.79 (s, 1H, H2), 8.44 (d, *J* = 3.2 Hz, 1H, H8), 7.99 (d, *J* = 17.4 Hz, 2H, Ar), 7.72 – 7.08 (m, 27H, Ar), 6.79 (dd, *J* = 8.9, 2.0 Hz, 4H, Ar DMT), 6.27 (d, *J* = 4.1 Hz, 1H, H1'), 6.05 (d, *J* = 6.3 Hz, 1H, NH Biotin), 5.60 – 5.38 (m, 4H, H3', H3'', H3'''), 5.30 – 5.23 (m, 3H, H2', H1'', H1'''), 5.01 (dt, *J* = 7.4, 2.4 Hz, 1H, H2''), 4.56 – 4.29 (m, 6H, H4', H5', H2''', CH Biotin), 4.27 – 3.98 (m, 6H, H4'', H4''', $CH_2OP=O$), 3.90 – 3.62 (m, 10H, CH_3 DMT, H5'', H5'''), 3.29 – 3.00 (m, 3H, CHS, CH_2S), 2.68 (dt, *J* = 9.0, 6.1 Hz, 2H, *CH*$_2$NH), 2.42 (AB, *J* = 13.0 Hz, 1H, *CH*$_2$CONH), 2.26 (AB, *J* = 13.0, 5.5 Hz, 1H, *CH*$_2$CONH), 2.22 – 1.96 (m, 12H, CH_3 Ac), 1.74 – 1.17 (m, 14H, CH_2), 1.03 – 0.99 (m, 18H, CH_3 TBDPS). ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.11, -1.15. IR (film): 3250, 2931, 1743, 1695, 1447, 1428, 1363, 1237, 1113, 1035, 1006, 737, 703 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{86}H_{110}N_9O_{22}PSSi_2$ (M+2H)/2 869.8376 (-DMT). Found 869.8372. $[\alpha]_D^{20}$ +30.7 (c = 1, in $CHCl_3$)



9-(5'-O-(6-O-biotinylaminohexany)-phosphoryl)-β-parotriosyladenine (18)

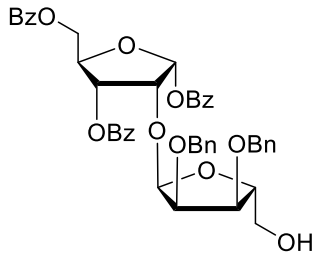
Compound **17** (25 mg, 12 µmol), DCM (0.5 mL) and TFA (2 µL, 24 µmol) were added into the flask and the reaction was stirred at room temperature for 30 minutes. TLC showed no complete reaction and more TFA (3 µL, 36 µmol) was added. 1 hour later the reaction was quenched by NH_4OH (160 µL) and concentrated. To the residue, NH_4OH (0.4 mL) and 1,4-dioxane (0.4 mL) were added and stirred for 24 hours at room temperature. The reaction was concentrated, co-evaporated with 1,4-dioxane (2 x), toluene (3 x) and pyridine (2 x). The residue was dissolved in pyridine (0.2 mL) and HF·pyridine (56 µL, 70% HF pyridine solution, 2.16 mmol base on HF) was added at 0 ℃. The mixture was stirred for 16 hours after which it was quenched by NH_4OH, concentrated, purified by HW-40 gel filtration [25% ACN in aqueous NH_4OAc (0.15 M)]. Repeated lyophilization obtained **18** as a white solid (6.96 mg, 7.43 µmol, 62 %). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.54 (s, 1H, H2), 8.29 (s, 1H, H8), 6.28 (d, *J* = 6.2 Hz, 1H, H1'), 5.38 (d, *J* = 3.5 Hz, 1H, H1''), 5.02 – 4.93 (m, 2H, H2', H1'''), 4.62 (dd, *J* = 5.1, 3.0 Hz, 1H, H3'), 4.54 (dd, *J* = 8.0, 4.8 Hz, 1H, CH Biotin), 4.39 (t, *J* = 2.8 Hz, 1H, H4'), 4.34 (dd, *J* = 8.0, 4.5 Hz, 1H, CH Biotin), 4.20 (dt, *J* = 17.2, 2.6 Hz, 3H, H2'', H3'', H4''), 4.14 – 4.02 (m, 3H, H4''', H5'), 3.94 (dd, *J* = 6.3, 3.2 Hz, 1H, H3'''), 3.86 (dd, *J* = 6.3, 4.3 Hz, 1H, H2'''), 3.75 – 3.54 (m, 6H, H5'', H5''', *CH*$_2$OP=O), 3.21 (dt, *J* = 9.8, 5.3 Hz, 1H, CHS), 3.11 – 2.87 (m, 3H, $CONHCH_2$, CH_2S), 2.71 (d, *J* = 13.0 Hz, 1H, CH_2S), 2.17 (t, *J* = 7.1 Hz, 2H, CH_2CONH), 1.75 – 1.00 (m, 14H, CH_2). ^{13}C NMR (101 MHz, D_2O) δ 176.77 (CO Biotin), 154.88, 149.43 (Cq. Ar), 101.63 (C1'''), 101.33 (C1''), 85.76 (C1'), 85.72 (C4''), 85.43 (C4'''), 85.02, 84.93 (C4'), 79.88 (C2'), 75.86 (C2''), 71.92 (C2'''), 70.99 (C3'), 70.23 (C3''), 69.93 (C3'''), 66.57 (C5''), 65.17 (C5'), 62.39 (CH Biotin), 61.74 ($CH_2OP=O$), 61.71 (C5'''), 60.55 (CH Biotin), 55.73 (CHS), 40.02 (CONH*CH*$_2$), 39.46 (CH_2S), 35.78 (*CH*$_2$CONH), 29.99, 29.91, 28.49, 28.13, 27.98, 26.04, 25.53, 24.88 (CH_2). ^{31}P NMR (162 MHz, D_2O) δ 1.04. HRMS (ESI$^+$) calcd for $C_{36}H_{58}N_8O_{17}PS$ (M+H) 937.3373. Found 937.3372.

Synthesis of compound **19** is described in Chapter 5



9-(5'-O-(6-O-biotinylaminohexany)-phosphoryl-5'',5'''-di-*O*-phosphoryl)-β-parotriosyladenine (20)

Compound **19** (20 mg, 12 µmol), DCI (96 µL, 0.25 M in ACN, 24 µmol) and freshly flame-dried 3Å molecular sieves were added into a flask and the mixture was stirred under N_2 at room temperature. **21** (20 mg, 24 µmol) was added and it was stirred for 1 hour. *t*BuOOH in decane (11 µL, 5.5 M, 60 µmol) was added at 0 °C and the reaction mixture was stirred for 1 hour at room temperature. The reaction was quenched by aq. $NaHCO_3$ (sat.), extracted with DCM (3 x), dried (Na_2SO_4) concentrated and purified by LH-20 gel filtration (DCM/methanol, 50/50). The fractions containing product were collected and concentrated. To the residue, DCM (0.2 mL) and TFA (1.8 µL, 24 µmol) were added and the mixture was stirred for 1 hour after which it was quenched by NH_4OH. The mixture was concentrated, added 1,4-dioxane (0.4 mL) and NH_4OH (0.4 mL). The reaction was stirred for 24 hours and concentrated. HPLC purification (0 - 30%, A: 25mM NH_4OAc/H_2O B: ACN) and repeated lyophilization yielded **20** (2.10 mg, 1.92 µmol, 16 %) as a white solid. ^{1}H NMR (600 MHz, Deuterium Oxide) δ 8.54 (s, 1H, H2), 8.32 (s, 1H, H8), 6.29 (d, *J* = 5.7 Hz, 1H, H1'), 5.38 (d, *J* = 3.9 Hz, 1H, H1''), 5.05 (d, *J* = 4.4 Hz, 1H, H1'''), 4.96 (t, *J* = 5.5 Hz, 1H, H2'), 4.61 (dd, *J* = 5.2, 3.6 Hz, 1H, H3'), 4.53 (dd, *J* = 8.0, 4.9 Hz, 1H, CH Biotin), 4.40 – 4.36 (m, 1H, H4'), 4.36 – 4.29 (m, 2H, H4''', CH Biotin), 4.28 – 4.20 (m, 3H, H2'', H3'', H4''), 4.10 – 4.02 (m, 3H, H5', H3'''), 3.98 (dd, *J* = 6.3, 4.4 Hz, 1H, H2'''), 3.95 – 3.84 (m, 4H, H5'', H5'''), 3.73 – 3.60 (m, 2H, CH_2OP=O), 3.21 (ddd, *J* = 9.8, 5.7, 4.5 Hz, 1H, CHS), 3.09 – 2.96 (m, 2H, CONH*CH*$_2$), 2.91 (dd, *J* = 13.1, 5.0 Hz, 1H, CH_2S), 2.70 (d, *J* = 13.0 Hz, 1H, CH_2S), 2.17 (t, *J* = 7.1 Hz, 2H, *CH*$_2$CONH), 1.69 – 1.38 (m, 6H, CH_2), 1.29 (dtd, *J* = 14.5, 7.5, 3.4 Hz, 4H, CH_2), 1.15 – 1.02 (m, 4H, CH_2). ^{13}C NMR (151 MHz, D_2O) δ 177.44 (CO), 166.24 (CO), 154.37 (C4), 150.82 (C8), 149.83 (C6), 141.92 (C2), 119.62 (C5), 102.43 (C1'''), 102.02 (C1''), 86.87 (C1'), 85.44, 85.38 (C4'), 85.06, 85.00 (C4'''), 84.98, 84.92 (C4''), 80.52 (C2'), 76.48 (C2''), 72.41 (C2'''), 71.26 (C3'), 70.80 (C3''), 70.63 (C3'''), 67.21, 67.18 (*CH*$_2$OP=O), 65.67, 65.63, 65.62, 65.59 (C5', C5'', C5'''), 63.00 (CH Biotin), 61.15 (CH Biotin), 56.33 (CHS), 40.62 (CONH*CH*$_2$), 40.08 (CONH*CH*$_2$), 36.38 (CH_2S), 30.58, 30.54, 29.09, 28.73, 28.58, 26.65, 26.14, 25.50 (CH_2). ^{31}P NMR (202 MHz, D_2O) δ 1.08, 1.00. HRMS (ESI$^+$) calcd for $C_{36}H_{60}N_8O_{23}P_3S$ (M+H) 1097.2699. Found 1097.2716.



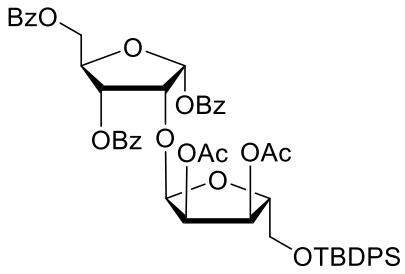
α-1,3,5-Tri-*O*-benzoyl-2',3'-di-*O*-benzyl-parobiose (22)

Compound **2** (3 g, 3.22 mmol), pyridine (15 mL) and TEA (6.7 mL) were added into a flask and cooled to 0°C. TEA·3HF was added and the reaction was stirred at room temperature for 24 h. The reaction was quenched by additioin of aq. $NaHCO_3$ (sat.). 50 mL H_2O was added and the mixture was extracted by EtOAc (3 x). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification by silica gel chromatography (pentane/EtOAc, 80/20 – 60/40) furnished **22** as a white foam (2.14 g, mmol, 86 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.20 – 7.95 (m, 6H, arom.), 7.61 – 7.38 (m, 5H, arom.), 7.32 – 6.99 (m, 14H, arom.), 6.81 (t, *J* = 3.4 Hz, 1H, H1'), 5.69 (dd, *J* = 6.4, 2.0 Hz, 1H, H4'), 5.34 – 5.22 (m,

1H, H1''), 4.82 – 4.53 (m, 5H, H3', H2', H5', CH_2 Bn), 4.46 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.34 (dd, *J* = 15.8, 12.0 Hz, 2H, CH_2 Bn), 4.18 – 4.08 (m, 1H, H4''), 3.89 – 3.78 (m, 2H, H3'', H2''), 3.64 (AB, *J* = 12.0, 3.0 Hz, 1H, H5''), 3.43 (AB, *J* = 12.0 Hz, 1H, H5''), 1.96 (s, 1H, OH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.37, 166.11, 165.77 (CO Bz), 138.17, 138.05 (Cq. arom.), 133.36, 133.24, 130.15, 130.12 (arom.), 129.90 (Cq. arom.), 129.76(arom.), 129.64 (Cq. arom.), 128.57, 128.42, 128.33, 128.25, 128.16, 127.69, 127.54, 127.39, 127.30 (arom.), 102.27 (C1''), 95.13 (C1'), 83.36 (C4''), 82.51 (C3'), 78.07 (C2''), 75.60, 75.51 (C3'', C2'), 72.64, 72.28 (CH_2 Bn), 72.07 (C4'), 64.33 (C5'), 62.06 (C5''). IR (film): 1733, 1715, 1268, 1091, 1069, 1018, 1011, 710, 697 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{45}H_{42}O_{12}Na$ (M+Na) 797.2568. Found 797.2569. $[\alpha]_D^{20}$ +82.3 (c = 1, in Methanol)

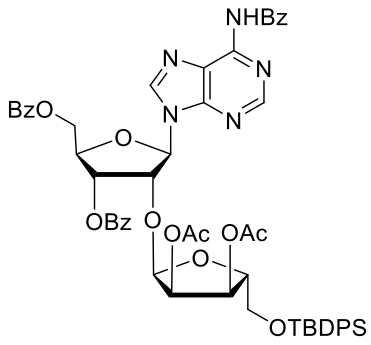
Compound 3 (from compound **22**)

Compound **22** (2.14 g, mmol), MeOH (13 mL), Pd/C (107 mg, 10% loading), and few drops of AcOH were added into reactor, then H_2 replaced air residue 3 times. The mixture was stirred under 88 mbar H_2 for 16 h and filtered over celite. The residue was concentrated, purified by silica gel column chromatography (pentane/EtOAc, 70/30 - 30/70) to obtain **3** as a white foam (1.02 g, mmol, 62%).



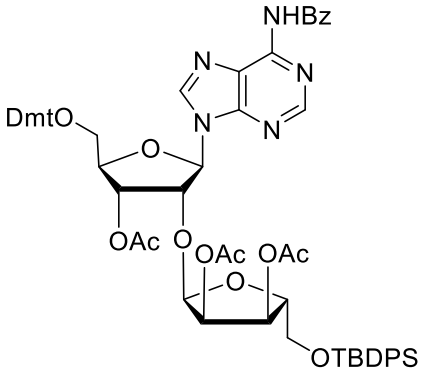
α-1,3,5-Tri-*O*-benzoyl-2',3'-di-*O*-acetyl-5'-*O*-tertbutyldiphenylsilyl-parobiose (23)

Compound **3** (0.95 g, 1.59 mmol), pyridine (16 mL) and TBDPSCl (0.5 mL, 1.92 mmol) were added into a flask and the mixture was stirred at room temperature for 16 h and TLC showed incompletely conversion. Another portion of TBDPSCl was added. 5 h later, Ac_2O was added and the reaction was stirred at room temperature for 3 h after which the reaction was quenched by aq. $NaHCO_3$ (sat.). The mixture was extracted by DCM (3 x) and dried ($MgSO_4$). The mixture was filtered, concentrated, purified by silica gel chromatography (pentane/acetone, 95/5 – 90/10 – 80/20) to furnish **23** as a white foam (1.22 g, mmol, 83 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.16 (ddt, *J* = 10.8, 7.2, 1.4 Hz, 4H, arom.), 8.10 – 8.02 (m, 2H, arom.), 7.66 – 7.52 (m, 7H, arom.), 7.46 – 7.30 (m, 12H, arom.), 6.82 (d, *J* = 4.3 Hz, 1H, H1'), 5.75 (dd, *J* = 6.4, 1.7 Hz, 1H, H3'), 5.46 (d, *J* = 4.5 Hz, 1H, H1'), 5.38 (dd, *J* = 7.0, 2.5 Hz, 1H, H3''), 4.99 (dd, *J* = 7.0, 4.6 Hz, 1H, H2''), 4.82 (td, *J* = 3.7, 1.7 Hz, 1H, H4'), 4.72 – 4.57 (m, 3H, H2', H5'), 4.10 (q, *J* = 2.8 Hz, 1H, H4''), 3.75 – 3.60 (m, 2H, H5''), 1.62 (s, 3H, CH_3 Ac), 1.44 (s, 3H, CH_3 Ac), 1.01 (s, 9H, CH_3 TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.36, 169.75 (CO Ac), 166.18, 165.91, 165.14 (CO Bz), 135.72, 135.66, 133.52, 133.48 (arom.), 132.94, 132.88 (Cq. arom.), 130.19, 130.15 (arom.), 130.00 (Cq. arom.), 129.93, 129.90, 129.77 (arom.), 129.60 (Cq. arom.), 128.71, 128.47, 128.44, 127.89, 127.88 (arom.), 101.10 (C1''), 95.17 (C1'), 83.47 (C4'), 83.32 (C4''), 75.94 (C2'), 71.74 (C3'), 71.47 (C2''), 70.03 (C3''), 64.38 (C5'), 63.54 (C5''), 26.83 (CH_3 TBDPS), 19.84, 19.83 (CH_3 Ac), 19.27 (Cq. TBDPS). IR (film): 1721, 1715, 1451, 1265, 1249, 1222, 1111, 1104, 1067, 1024, 956, 701 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{51}H_{52}O_{14}SiNa$ (M+Na) 939.3019. Found 939.3020. $[\alpha]_D^{20}$ +82.9 (c = 1, in $CHCl_3$)



6-*N*-benzoyl-9-(3',5'-Tri-*O*-benzoyl-2'',3''-di-*O*-acetyl-5''-*O*-tertbutyldiphenylsilyl-β-parobiosyl) adenine (24)

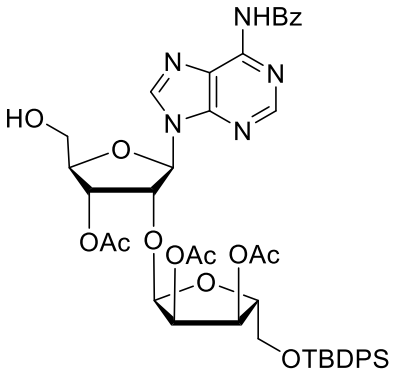
Compound **23** (1.16 g, 1.26 mmol) and N^6-benzoyladenine (452 mg, 1.89 mmol) were co-evaporated with dry 1,4-dioxane and dissolved in dry ACN. *N*,*O*-bis(trimethylsilyl)trifluorioacetamide (BSTFA) (1.69 mL, 6.30 mmol) was added and the mixture was stirred at room temperature until everything was dissolved. $HClO_4$-SiO_2 was added and the mixture was refluxed for 16 h after which was quenched by aq. $NaHCO_3$ (sat.). The filtrate was extracted by DCM and dried by $MgSO_4$. The mixture was filtered and concentrated and purified by silica gel chromatography (pentane/acetone, 100/0 – 80/20 – 70/30) furnished **24** as a white foam (1.19 g, 1.15 mmol, 91%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.07 (s, 1H, NH), 8.68 (s, 1H, H2), 8.17 (s, 1H, H8), 8.10 (ddd, *J* = 11.4, 8.4, 1.4 Hz, 4H, arom.), 8.05 – 7.97 (m, 2H, arom.), 7.64 – 7.48 (m, 9H, arom.), 7.48 – 7.29 (m, 10H, arom.), 6.27 (d, *J* = 5.5 Hz, 1H, H1'), 5.93 (dd, *J* = 5.4, 4.0 Hz, 1H, H3'), 5.53 (t, *J* = 5.5 Hz, 1H, H2'), 5.44 – 5.29 (m, 2H, H1'', H3''), 4.96 – 4.78 (m, 2H, H2''', H5'), 4.78 – 4.59 (m, 2H, H4', H5'), 4.00 (q, *J* = 2.8 Hz, 1H, H4''), 3.61 (AB, *J* = 11.4, 2.6 Hz, 1H, H5''), 3.50 (AB, *J* = 11.3, 3.1 Hz, 1H, H5''), 1.87 (s, 3H, CH_3 Ac), 1.77 (s, 3H, CH_3 Ac), 0.98 (s, 9H, CH_3 TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.29, 169.67 (CO Ac), 166.21, 165.36, 164.60 (CO Bz), 152.99 (arom.), 151.73, 149.80 (Cq. arom.), 142.03, 135.64, 135.60, 133.71 (arom.), 133.64 (Cq. arom.), 133.56, 132.92 (arom.), 132.89, 132.76 (Cq. arom.), 129.94, 129.90, 129.87, 129.79 (arom.), 129.48, 129.37 (Cq. arom.), 128.97, 128.69, 128.62, 127.94, 127.85, 127.83 (arom.), 123.82 (Cq. arom.), 101.60 (C1''), 87.80 (C1'), 83.13 (C4''), 80.93 (C4'), 77.77 (C2'), 72.45 (C3'), 71.62 (C2''), 70.02 (C3''), 63.67 (C5'), 63.25 (C5''), 26.78 (CH_3 TBDPS), 20.39, 20.35 (CH_3 Ac), 19.22 (Cq TBDPS). IR (film): 2938, 1722, 1609, 1583, 1451, 1263, 1244, 1111, 1069, 1026, 700 cm^{-1}. HRMS (ESI^+) calcd for $C_{56}H_{56}N_5O_{13}Si$ (M+H) 1034.3638. Found 1034.3646. $[\alpha]_D^{20}$ +8.1 (c = 1, in Methanol)



6-*N*-benzoyl-9-(3',2'',3''-tris-*O*-acetyl-5'-*O*-dimethoxyltrityl-5''-*O*-tertbutyldiphenylsilyl-β-parobiosyl) adenine (25)

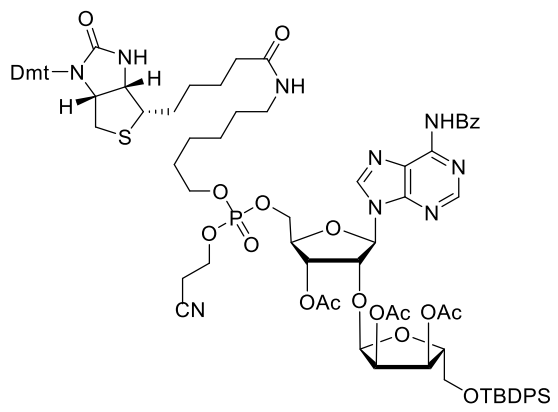
Compound **24** (196 mg, 0.19 mmol) was dissolved in pyridine/EtOH (2.1 mL; 2/1, v/v), cooled to 0°C after which aq. NaOH (1.1 mL, 1 M) was slowly added. The reaction was stirred for 1.5 hours at same temperature after which Amblite-H^+ was added in portions until pH=6. The mixture was filtered, concentrated under reduced pressure and co-evaporated with toluene (2 x), pyridine (2 x) and dissolved in pyridine (1.9 mL). 4,4'-dimethoxyltrityl chloride (DmtCl, 77 mg, 0.23 mmol) was added. The mixture was stirred for 16 h and TLC showed incomplete conversion after which the reaction was concentrated under reduced pressure. Pyridine and DmtCl (192 mg, 0.57 mmol) were added into the residue and the mixture was stirred for 1 h. The reaction was cooled to 0°C and Ac_2O (0.36 mL, 3.79 mmol) was added. The mixture was stirred at 0 °C for 2.5 h and quenched by aq. $NaHCO_3$ (sat.). DCM extracted (3 x) the mixture and the organic layers were combined and dried ($MgSO_4$), filtered, concentrated and purified by silica gel column chromatography (pentane/EtOAc/acetone, 90/10/0 – 70/30/0 – 80/0/20 – 70/0/30) to obtain **25** as a colorless oil (182 mg, 0.16 mmol, 84%). ^{1}H NMR (400

MHz, Chloroform-*d*) δ 9.05 (s, 1H, NH), 8.75 (s, 1H, H2), 8.16 (s, 1H, H8), 8.06 – 7.94 (m, 2H, arom.), 7.71 – 7.15 (m, 22H, arom.), 6.88 – 6.74 (m, 4H, DMT), 6.27 (d, *J* = 5.9 Hz, 1H, H1'), 5.58 (dd, *J* = 5.2, 3.6 Hz, 1H, H3'), 5.45 (dd, *J* = 7.1, 2.8 Hz, 1H, H3''), 5.37 (d, *J* = 4.6 Hz, 1H, H1''), 5.23 (t, *J* = 5.5 Hz, 1H, H2'), 4.94 (dd, *J* = 7.0, 4.6 Hz, 1H, H2''), 4.34 (d, *J* = 3.5 Hz, 1H, H4'), 4.14 (d, *J* = 2.8 Hz, 1H, H4''), 3.83 – 3.68 (m, 8H, OMe DMT, H5''), 3.51 (AB, *J* = 10.7, 3.5 Hz, 2H, H5'), 2.10 (d, *J* = 1.0 Hz, 6H, CH_3 Ac), 1.85 (s, 3H, CH_3 Ac), 1.02 (s, 9H, CH_3 TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.33, 169.74, 169.65 (CO Ac), 164.59 (CO Bz), 158.76, 151.96, 149.69, 144.42 (Cq. arom.), 135.68, 135.65 (arom.), 135.42, 133.79, 132.93 (Cq. arom.), 132.91 (arom.), 132.73 (Cq. arom.), 130.24, 130.22, 129.97, 129.92, 129.00, 128.29, 128.09, 127.93, 127.91, 127.23 (arom.), 123.35 (Cq. arom.), 113.36 (arom.), 101.61 (C1''), 87.06 (Cq. DMT), 86.49 (C1'), 83.14 (C4''), 82.66 (C4'), 78.49 (C2'), 72.36 (C3'), 71.62 (C2''), 70.25 (C3''), 63.50 (C5''), 63.03 (C5'), 55.32 (OMe DMT), 26.82 (CH_3 TBDPS), 20.96, 20.95, 20.34 (CH_3 Ac), 19.28 (Cq. TBDPS). IR (film): 2935, 1743, 1739, 1506, 1244, 1219, 1092, 1030, 703 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{65}H_{68}N_5O_{14}Si$ (M+H) 1170.4527. Found 1170.4518. $[\alpha]_D^{20}$ -135.0 (c = 1, in Methanol)



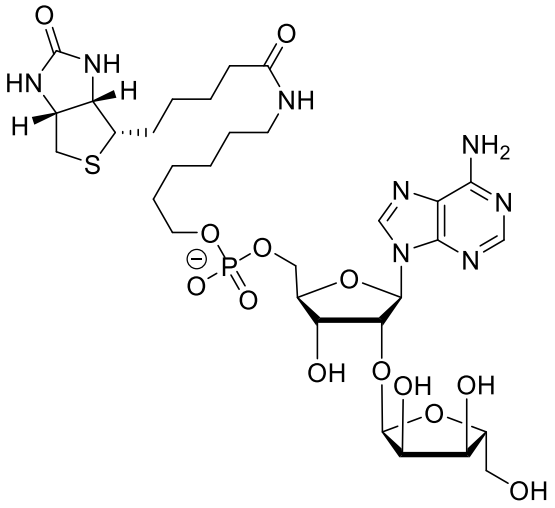
6-*N*-benzoyl-9-(3',2'',3''-tris-*O*-acetyl-5''-*O*-tertbutyldiphenylsilyl-β-parobiosyl) adenine (26)

Compound **25** (71 mg, 61 µmol), DCM (0.61 mL) and TFA (9.3 µL, 122 µmol) were added into a flask and the mixture was stirred at room temperature for 1 hour. TLC showed complete conversion and the reaction was quenched by aq. $NaHCO_3$ (sat.). DCM extracted (3 x) the mixture and the organic layers were combined, dried ($MgSO_4$), filtered, concentrated and purified by silica gel column chromatography (DCM/acetone, 95/5 – 90/10 – 85/15) to furnish **26** as a white foam (34 mg, 39 µmol, 64 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.08 (s, 1H, NH), 8.82 (s, 1H, H2), 8.11 (s, 1H, H8), 8.05 – 7.94 (m, 2H, arom.), 7.66 – 7.48 (m, 7H, arom.), 7.46 – 7.30 (m, 6H, arom.), 6.11 (d, *J* = 11.3 Hz, 1H, OH), 6.02 (d, *J* = 7.8 Hz, 1H, H1'), 5.68 (d, *J* = 5.3 Hz, 1H, H, H3'), 5.37 (dd, *J* = 7.0, 2.9 Hz, 1H, H3''), 5.14 (dd, *J* = 7.8, 5.3 Hz, 1H, H2'), 5.09 (d, *J* = 4.7 Hz, 1H, H1''), 4.92 (dd, *J* = 7.0, 4.7 Hz, 1H, H2''), 4.32 (q, *J* = 1.4 Hz, 1H, H4'), 4.06 – 3.94 (m, 2H, H4'', H5'), 3.87 (t, *J* = 11.8 Hz, 1H, H5'), 3.76 – 3.62 (m, 2H, H5''), 2.15 (s, 3H, CH_3 Ac), 2.13 (s, 3H, CH_3 Ac), 1.97 (s, 3H, CH_3 Ac), 0.99 (s, 9H, CH_3 TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.24, 169.65, 169.53 (CO Ac), 164.46 (CO Bz), 150.58, 150.51 (Cq. arom.), 135.66 (arom.), 133.50 (Cq. arom.), 133.12 (arom.), 132.85, 132.69 (Cq. arom.), 129.98, 129.95, 129.07, 127.98, 127.92, 127.89 (arom.), 124.64 (Cq. arom.), 101.23 (C1''), 89.80 (C1'), 86.89 (C4'), 83.09 (C4''), 77.61 (C2'), 73.78 (C3'), 71.58 (C2''), 70.24 (C3''), 63.43 (C5''), 62.89 (C5'), 26.83 (CH_3 TBDPS), 21.03, 20.94, 20.44 (CH_3 Ac), 19.24 (Cq. TBDPS). IR (film): 2931, 2857, 1743, 1609, 1584, 1456, 1360, 1235, 1113, 1048, 703 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{44}H_{50}N_5O_{12}Si$ (M+H) 868.3220. Found 868.3230. $[\alpha]_D^{20}$ +5.6 (c = 1, in $CHCl_3$)



6-*N*-benzoyl-9-(3',2'',3''-tri-*O*-acetyl-5'-*O*-{6-[(2-cyanoethoxy)phosphoryl]-[1-*N*-(4,4'-dimethoxytrityl)biotinyl]aminohexanyl}-5''-*O*-tertbutyldiphenylsilyl-β-parotriosyl)adenine (27)

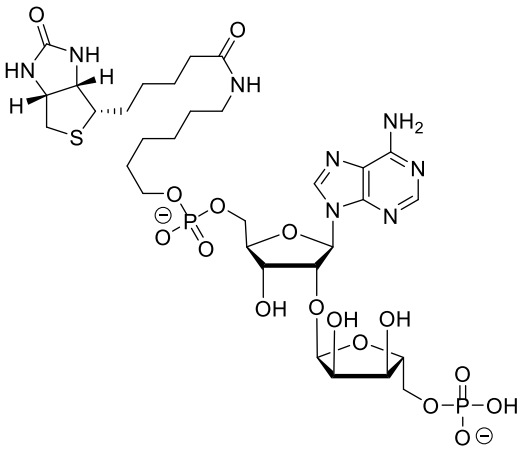
1-Methyl-imidazole·HCl (36 mg, 0.30 mmol) and 1-methyl-imidazole (16 µL, 0.20 mmol) were co-evaporated with dry CH_3CN (3 x), then N_2 was applied. To this mixture, freshly activated molecular sieves and dry DMF (0.5 mL) were added and the activator solution was stirred at room temperature for 2 hours under N_2. Next, compound **26** (29 mg, 33 µmol) was co-evaporated with dry 1,4-dioxane (3 x) after which the activator solution above was added. Subsequently, **21** (113 mg, in 1 mL ACN, 0.9 mmol) was added and the reaction was stirred at room temperature for 20 minutes. *t*BuOOH in decane (60 µL, 5.5 M, 0.33 mmol) was added at 0 ℃ and the reaction mixture was stirred for 1 hour at room temperature. The reaction was quenched by aq. $NaHCO_3$ (sat.), extracted with EtOAc (3 x), dried ($MgSO_4$), concentrated, purified by LH-20 gel filtration (DCM/methanol, 50/50) and silica gel chromatography (DCM/MeOH, 100/0 – 97/3) to obtain **27** as a white foam (38 mg, 23 µmol, 70%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.47 (d, *J* = 14.8 Hz, 1H, NH), 8.80 (d, *J* = 1.7 Hz, 1H, H2), 8.31 (d, *J* = 1.2 Hz, 1H, H8), 8.06 – 7.92 (m, 2H, arom.), 7.68 – 7.54 (m, 5H, arom.), 7.52 – 7.33 (m, 8H, arom.), 7.33 – 7.21 (m, 5H, arom.), 7.21 – 7.07 (m, 4H, arom.), 6.86 – 6.71 (m, 4H, aom.), 6.21 (dd, *J* = 5.1, 3.9 Hz, 1H, H1'), 6.02 (d, *J* = 18.0 Hz, 1H, NH Biotin), 5.59 (d, *J* = 9.7 Hz, 1H, NH Biotin), 5.54 – 5.41 (m, 2H, H3', H3''), 5.36 (dd, *J* = 4.6, 1.9 Hz, 1H, H1''), 5.18 – 5.06 (m, 1H, H2'), 4.95 (dd, *J* = 7.1, 4.6 Hz, 1H, H2''), 4.51 – 4.27 (m, 5H, CH Biotin, H4', H5'), 4.25 – 4.00 (m, 5H, CH_2OP=O, H4''), 3.85 – 3.66 (m, 9H, OMe DMT, H5''), 3.25 – 3.03 (m, 3H, CONH*CH₂*, CH Biotin), 2.68 (dt, *J* = 12.3, 5.9 Hz, 2H, CH_2OCN), 2.46 – 2.37 (m, 1H, CH_2S), 2.25 (AB, *J* = 13.1, 5.7 Hz, 1H, CH_2S), 2.18 – 2.02 (m, 8H, CH_3 Ac, *CH₂*CONH), 1.91 (d, *J* = 3.1 Hz, 3H, CH_3 Ac), 1.73 – 1.19 (m, 14H, CH_2), 1.02 (s, 9H, CH_3 TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.99, 172.96, 170.29, 169.80, 169.79, 169.74, 169.72 (CO), 165.06, 165.02, 161.60, 158.45, 151.93, 150.09, 143.86, 135.92, 135.84 (Cq. arom.), 135.69, 135.65 (Cq. arom.), 133.39, 133.37 (Cq. arom.), 132.92 (arom.), 132.85, 132.74 (Cq. arom.), 131.39, 130.00, 129.97, 129.81, 128.90, 128.19, 128.17, 127.92, 127.90, 127.64, 127.00 (arom.), 124.07, 124.02, 116.81, 116.74 (Cq. arom.), 112.90 (arom.), 101.52 (C1''), 87.41, 87.28 (C1'), 83.13 (C4''), 81.17, 81.09 (C4'), 78.00 (C2'), 72.79 (CN), 71.55 (C2''), 71.05 (C3'), 70.21 (C3''), 68.71, 68.64 (CH_2OP=O), 66.45, 66.39, 66.33, 66.28 (C5'), 65.53, 65.52 (CH Biotin), 63.49 (C5''), 62.32, 62.27, 62.23 (CH_2OP=O), 59.77, 59.75 (CH Biotin), 55.33 (OMe DMT), 54.33, 54.32 (*CH*CH_2 Biotin), 39.28, 39.26 (CONH*CH₂*), 39.09, 39.05 (CH_2S), 36.05, 36.03, 35.30, 34.50, 33.82, 30.98, 29.92, 29.86, 29.80, 29.28, 29.22, 28.70, 28.30, 28.28 (CH_2), 26.83 (CH_3 TBDPS), 26.06, 26.00, 25.54, 25.49, 24.85, 24.81 (CH_2), 20.95, 20.87, 20.40 (CH_3 Ac), 19.80, 19.73 (CH_2CN), 19.27 (Cq. arom.). ^{31}P NMR (122 MHz, $CDCl_3$) δ -1.56, -1.67. IR (film): 2930, 1743, 1700, 1695, 1653, 1616, 1507, 1456, 1290, 1238, 1181, 1034, 1030, 703 cm^{-1}. HRMS (ESI^+) calcd for $C_{84}H_{99}N_9O_{19}PSSi$ (M+H) 1628.6279. Found 1628.6376. $[\alpha]_D^{20}$ +32.5 (c = 1, in $CHCl_3$)



9-[5'-*O*-(6-*O*-biotinylaminohexany)-phosphoryl-β-parobiosyl]adenine (28)

Compound **27** (14 mg, 8.60 µmol), DCM (0.4 mL) and TFA (4 µL, 51.60 µmol) were added into the flask and the reaction was stirred at room temperature for 1 hour. TLC showed no complete reaction and more TFA (2 µL, 25.80 µmol) was added. 1 hour later the reaction was quenched by NH_4OH (55 µL) and concentrated. To the residue, NH_4OH (0.4 mL) and 1,4-dioxane (0.4 mL) were added and stirred for 24 hours at room temperature. The reaction was concentrated, co-evaporated with 1,4-dioxane (2 x), toluene (3 x) and pyridine (2 x). The residue was dissolved in pyridine (0.4 mL) and HF·pyridine (60 µL, 70% HF pyridine solution, 2.31 mmol base on HF) was added at 0 ℃. The mixture was stirred for 16 hours after which it was quenched by NH_4OH, concentrated, purified by HW-40 gel filtration [25% ACN in aqueous NH_4OAc (0.15 M)]. Repeated lyophilization obtained **28** as a white solid (3.01 mg, 3.74 µmol, 43%). ^{1}H NMR (850 MHz, Deuterium Oxide) δ 8.62 (d, *J* = 2.7 Hz, 1H, H2), 8.41 (d, *J* = 3.3 Hz, 1H, H8), 6.34 (d, *J* = 5.2 Hz, 1H, H1'), 5.28 (d, *J* = 4.3 Hz, 1H, H1''), 4.96 (t, *J* = 5.2 Hz, 1H, H2'), 4.67 (t, *J* = 4.5 Hz, 1H, H3'), 4.60 (dd, *J* = 8.0, 4.9 Hz, 1H, CH Biotin), 4.45 (q, *J* = 3.0 Hz, 1H, H4'), 4.41 (dd, *J* = 8.0, 4.4 Hz, 1H, CH Biotin), 4.25 (q, *J* = 3.5 Hz, 1H, H4''), 4.19 – 4.06 (m, 4H, H5', H2'', H3''), 3.83 – 3.71 (m, 3H, H5'', CH_2OP=O), 3.67 (AB, *J* = 12.4, 4.6 Hz, 1H, H5''), 3.29 (dt, *J* = 9.7, 5.1 Hz, 1H, CHS), 3.16 – 3.06 (m, 2H, CONH*CH_2*), 2.98 (AB, *J* = 13.0, 4.9 Hz, 1H, CH_2S), 2.77 (d, *J* = 13.1 Hz, 1H, CH_2S), 2.24 (t, *J* = 7.7 Hz, 2H, *CH_2*CONH), 1.76 – 1.48 (m, 6H, CH_2), 1.45 – 1.32 (m, 4H, CH_2), 1.25 – 1.14 (m, 4H, CH_2). ^{13}C NMR (214 MHz, D_2O) δ 177.39 (CO), 166.21 (CO), 153.32 (C4), 149.60 (C6), 119.61 (C5), 102.68 (C1''), 87.34 (C1'), 86.41 (C4''), 85.35, 85.31 (C4'), 80.26 (C2'), 72.45 (C2''), 71.34 (C3'), 70.69 (C3''), 67.20, 67.17 (CH_2OP=O), 65.51, 65.49 (C5'), 62.98 (CH Biotin), 62.38 (C5''), 61.13 (CH Biotin), 56.30 (CHS), 40.58 (CH_2S), 40.05 (CONH*CH_2*), 36.37 (*CH_2*CONH), 30.58, 30.54, 29.08, 28.71, 28.56, 26.62, 26.10, 25.51 (CH_2). ^{31}P NMR (122 MHz, D_2O) δ -0.33. HRMS (ESI^+) calcd for $C_{31}H_{50}N_8O_{13}PS$ (M+H) 805.2950. Found 805.2972.

Synthesis of compound **29** will be described in Chapter 4



9-[5'-O-(6-O-biotinylaminohexanyl)-phosphoryl-5''-*O*-phosphoryl-β-parotriosyl]adenine (30)

Compound **29** (11 mg, 10 µmol), DCI (0.12 mL, 0.25 M in ACN, 30 µmol) and freshly flame-dried 3Å molecular sieves were added into a flask and the mixture was stirred under N_2 at room temperature. **21** (34 mg, 40 µmol) was added and it was stirred for 1 hour. *t*BuOOH in decane (9 µL, 5.5 M, 50 µmol) was added at 0 ℃ and the reaction mixture was stirred for 1 hour at room temperature. The reaction was quenched by aq. $NaHCO_3$ (sat.), extracted with DCM (3 x), dried (Na_2SO_4) concentrated and purified by LH-20 gel filtration (DCM/methanol, 50/50). The fractions containing product were collected and concentrated. To the residue, DCM (0.4 mL) and TFA (1.6 µL, 20 µmol) were added and the mixture was stirred for 1 hour after which it was quenched by NH_4OH. The mixture was concentrated, added 1,4-dioxane (0.4 mL) and NH_4OH (0.4 mL). The reaction was stirred for 24 hours and concentrated. HPLC purification (0 - 30%, A: 25mM NH_4OAc/H_2O B: ACN) and repeated lyophilization yielded **30** (2.1 mg, 2.37 µmol, 24%) as a white solid. ^{1}H NMR (850 MHz, Deuterium Oxide) δ 8.51 (s, 1H, H2), 8.30 (s, 1H, H8), 6.27 (d, *J* = 5.1 Hz, 1H, H1'), 5.25 (d, *J* = 4.3 Hz, 1H, H1''), 4.91 (t, *J* = 5.2 Hz, 1H, H2'), 4.62 (dd, *J* = 5.2, 4.1 Hz, 1H, H3'), 4.54 (ddd, *J* = 8.0, 5.0, 0.8 Hz, 1H, CH Biotin), 4.38 (t, *J* = 3.5 Hz, 1H, H4'), 4.34 (dd, *J* = 8.0, 4.5 Hz, 1H, CH Biotin), 4.32 (tt, *J* = 3.8, 1.9 Hz, 1H, H4''), 4.18 – 4.13 (m, 2H, H2'', H5'), 4.11 – 4.04 (m, 2H, H5', H3''), 3.93 (AB, *J* = 11.5, 6.0, 3.8 Hz, 1H, H5''), 3.89 (AB, *J* = 11.4, 5.0, 3.5 Hz, 1H, H5''), 3.71 – 3.63 (m, 2H, CH_2OP=O), 3.22 (ddd, *J* = 9.7, 5.7, 4.5 Hz, 1H, CHS), 3.03 (ddt, *J* = 40.2, 13.4, 6.7 Hz, 2H, CONH*CH*$_2$), 2.91 (dd, *J* = 13.1, 5.0 Hz, 1H, CH_2S), 2.71 (d, *J* = 13.0 Hz, 1H, CH_2S), 2.21 – 2.14 (m, 2H, *CH*$_2$CONH), 1.69 – 1.45 (m, 4H, CH_2), 1.42 (p, *J* = 6.8 Hz, 2H, CH_2), 1.33 – 1.27 (m, 4H, CH_2), 1.17 – 1.02 (m, 4H, CH_2). ^{13}C NMR (214 MHz, D_2O) δ 177.41 (CO), 166.23 (CO), 154.93 (C4), 149.79 (C6), 119.63 (C5), 102.74 (C1''), 87.15 (C1'), 85.29, 85.25 (C4'), 85.14, 85.10 (C4''), 80.12 (C2'), 72.31 (C2''), 71.19 (C3'), 70.78 (C3''), 67.19, 67.16 (CH_2OP=O), 65.69, 65.67 (C5''), 65.50, 65.48 (C5'), 62.99, 61.14 (CH Biotin), 56.31 (CHS), 40.60 (CH_2S), 40.07 (CONH*CH*$_2$), 36.37 (*CH*$_2$CONH), 30.56, 30.52, 29.08, 28.71, 28.56, 26.64, 26.12, 25.49 (CH_2). ^{31}P NMR (162 MHz, D_2O) δ 1.10, 0.97. HRMS (ESI$^+$) calcd for $C_{31}H_{51}N_8O_{16}P_2S$ (M+H) 885.2613. Found 885.2628.

References

1. B. A. Gibson and W. L. Kraus, *Nat. Rev. Mol. Cell Biol.*, 2012, **13**, 411-424.
2. M. S. Cohen and P. Chang, *Nat. Chem. Biol.*, 2018, **14**, 236-243.
3. M. Miwa, N. Saikawa, Z. Yamaizumi, S. Nishimura and T. Sugimura, *Proc. Natl. Acad. Sci. U. S. A*, 1979, **76**, 595-599.
4. P. L. Panzeter, C. A. Realini and F. R. Althaus, *Biochemistry*, 1992, **31**, 1379-1385.
5. T. Nozaki, M. Masutani, T. Akagawa, T. Sugimura and H. Esumi, *Biochem. Biophys. Res. Commun.*, 1994, **198**, 45-51.
6. S. A. Braun, P. L. Panzeter, M. A. Collinge and F. R. Althaus, *Eur. J. Biochem.*, 1994, **220**, 369-375.
7. S. Ramsinghani, D. W. Koh, J. C. Ame, M. Strohm, M. K. Jacobson and J. T. Slama, *Biochemistry*, 1998, **37**, 7801-7812.
8. M. J. Lambrecht, M. Brichacek, E. Barkauskaite, A. Ariza, I. Ahel and P. J. Hergenrother, *J. Am. Chem. Soc.*, 2015, **137**, 3558-3564.

9. M. Miwa, M. Ishihara, S. Takishima, N. Takasuka, M. Maeda, Z. Yamaizumi, T. Sugimura, S. Yokoyama and T. Miyazawa, *J. Biol. Chem.*, 1981, **256**, 2916-2921.
10. H. Juarezsalinas, V. Levi, E. L. Jacobson and M. K. Jacobson, *J. Biol. Chem.*, 1982, **257**, 607-609.
11. M. Kanai, M. Miwa, Y. Kuchino and T. Sugimura, *J. Biol. Chem.*, 1982, **257**, 6217-6223.
12. R. Alvarez-Gonzalez and M. K. Jacobson, *Biochemistry*, 1987, **26**, 3218-3224.
13. C. M. Daniels, S. E. Ong and A. K. Leung, *J. Proteome Res.*, 2014, **13**, 3510-3522.
14. C. C. Kiehlbauch, N. Aboul-Ela, E. L. Jacobson, D. P. Ringer and M. K. Jacobson, *Anal. Biochem.*, 1993, **208**, 26-34.
15. H. A. Kistemaker, G. J. van Noort, H. S. Overkleeft, G. A. van der Marel and D. V. Filippov, *Org. Lett.*, 2013, **15**, 2306-2309.
16. 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.
17. G. J. van der Heden van Noort, M. G. van der Horst, H. S. Overkleeft, G. A. van der Marel and D. V. Filippov, *J. Am. Chem. Soc.*, 2010, **132**, 5236-5240.
18. 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.
19. H. A. Kistemaker, H. S. Overkleeft, G. A. van der Marel and D. V. Filippov, *Org. Lett.*, 2015, **17**, 4328-4331.
20. C. M. Daniels, S. E. Ong and A. K. Leung, *Mol. Cell*, 2015, **58**, 911-924.
21. G. J. van der Heden van Noort, H. S. Overkleeft, G. A. van der Marel and D. V. Filippov, *Org. Lett.*, 2011, **13**, 2920-2923.
22. H. Tsukamoto and D. Kahne, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 5050-5053.
23. Q. Chen, M. A. Kassab, F. Dantzer and X. Yu, *Nat. Commun.*, 2018, **9**, 3233.
24. R. T. Pon, *Tetrahedron Lett.*, 1991, **32**, 1715-1718.
25. Y. Watanabe, T. Nakamura and H. Mitsumoto, *Tetrahedron Lett.*, 1997, **38**, 7407-7410.