Synthetic Study on ADP-ribosylation

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5 Total synthesis of branched ADP-ribose trimer

Introduction

ADP-ribosylation is an important post-translational modification, that plays a key role in many cellular events such as DNA repair, apoptosis, chromatin regulation.[1-3] This reversible modification arises through a covalent attachment, catalyzed by the enzymes of the PARP family, of mono-ADP ribose (MAR) and poly-ADP ribose (PAR) chains to the nucleophilic side chains of specific amino acids in target proteins. Hydrolases, such as PARG, catalyze the reverse reaction by removing MAR and PAR from these proteins. PAR, termed "the third nucleic acid", can exist as a linear or branched polymer.[4] Although it has been known for more than 40 years, the biological function and binding module of branched PAR remains elusive.[4-7] Few studies disclose some unique biological characteristics of the branched polymer such as a tighter binding with a histone[8, 9] and a higher resistance to PARG than its linear counterpart.[10] Recently, Chen *et al*[11] reported, that PBZ (PAR-binding zinc finger) domain of APLF (aprataxin polynucleotide-kinase-like factor), involved in DNA damage response, recognizes the branched PAR chain. However, the protein binding details at a molecular level are missing partly due to the lack of a structurally defined branched ADPr-chains that could serve as molecular tools. To date, almost all reported PAR-binding proteins/domains recognize only mono- or di-ADPr fragments,[12-14] suggesting that unknown proteins/domains which specifically recognize and bind branched tri-ADPr core might exist. The availability of well-defined branched PAR oligomers would facilitate such investigation and will contribute to a) the discovery of new branched PAR binding proteins;[11] b) the elucidation of the

binding mechanism via co-crystallization with proteins like APLF or PARG;[15] c) a better understanding of the bio-function of branched PAR. Branched core oligomer **1** (Figure 1), the smallest branched PAR, comprises three full ADPr units, making it a perfect tool for biological study.

Guided by the previous synthetic approaches towards linear oligo-ADPr (Chapter 4) and the core nucleotide of the branched ADPr[16, 17] (Chapter 2), the first total synthesis of minimal branched ADPr structure **1** is reported in this Chapter.

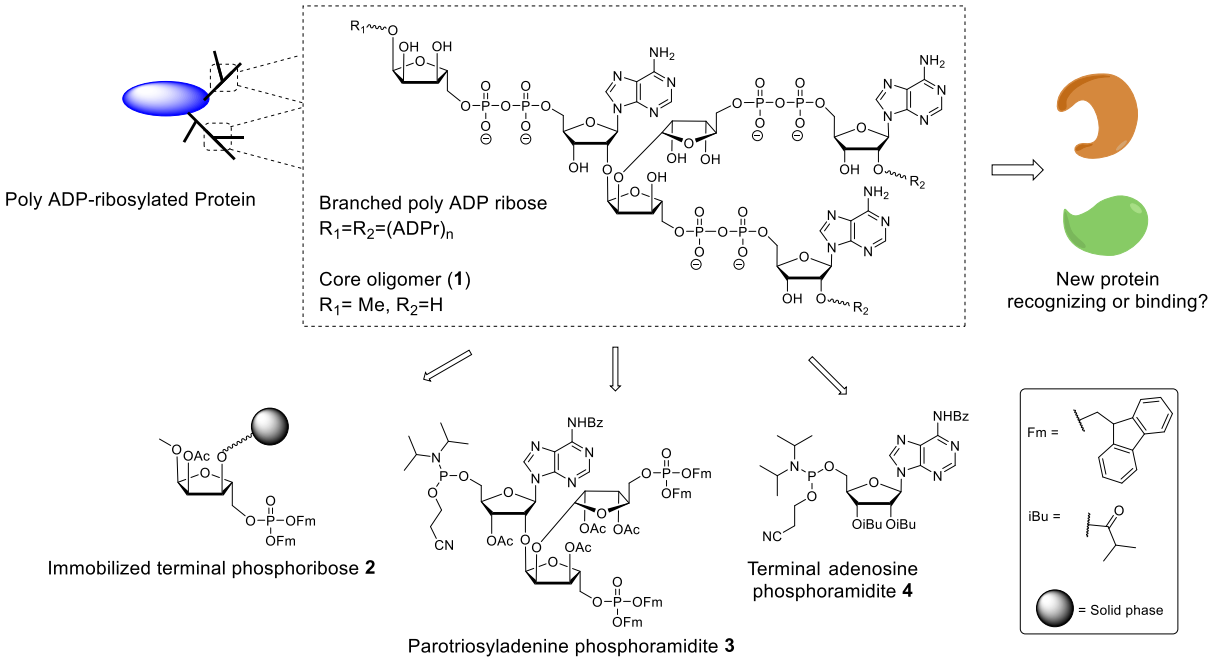
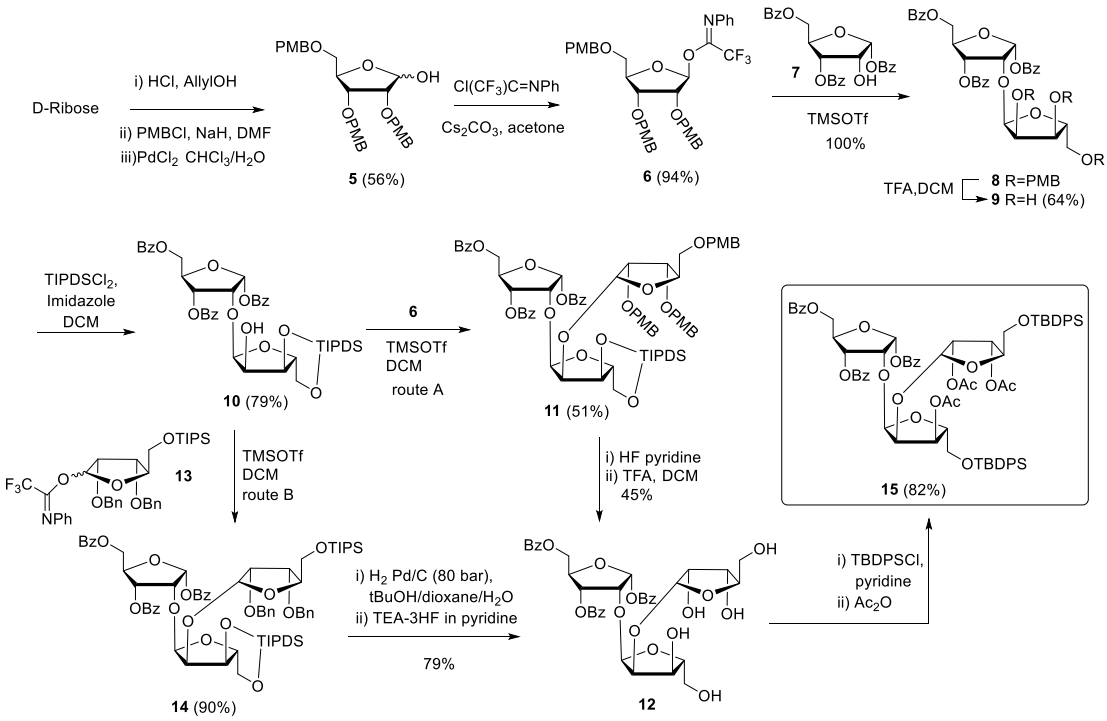


Figure 1. The branched ADP-ribose trimer and its retrosynthetic analysis

Results and discussion

The three anionic pyrophosphates functions in target ADPr-trimer **1** ensure that a solid phase approach is preferred over a solution phase approach. Retrosynthetically this means that oligomer **1** can be prepared with the aid of three components (Figure 1): (a) immobilized terminal phosphoribose **2**; (b) orthogonally protected phosphoramidite **3** of parotriosyl adenine and (c) known terminal adenosine phosphoramidite **4**.[18-21] The successful solid phase synthesis of linear ADPr oligomers, using a bis (9-fluorenyl)methyl (Fm) protected phosphoramidite (Chapter 4), was an incentive to use a similar strategy for the construction of branched oligomers using building blocks compounds **2**, **3** and **4** with solely base labile protecting groups. Notable in the synthetic scheme is the advanced key phosphoramidite **3** which should enable the first pyrophosphate construction. Not only the construction of phosphoramidite **3** is challenging but also its application in the P(III)-P(V) method for the first pyrophosphate introduction. An additional challenge represents the use of phosphoramidite

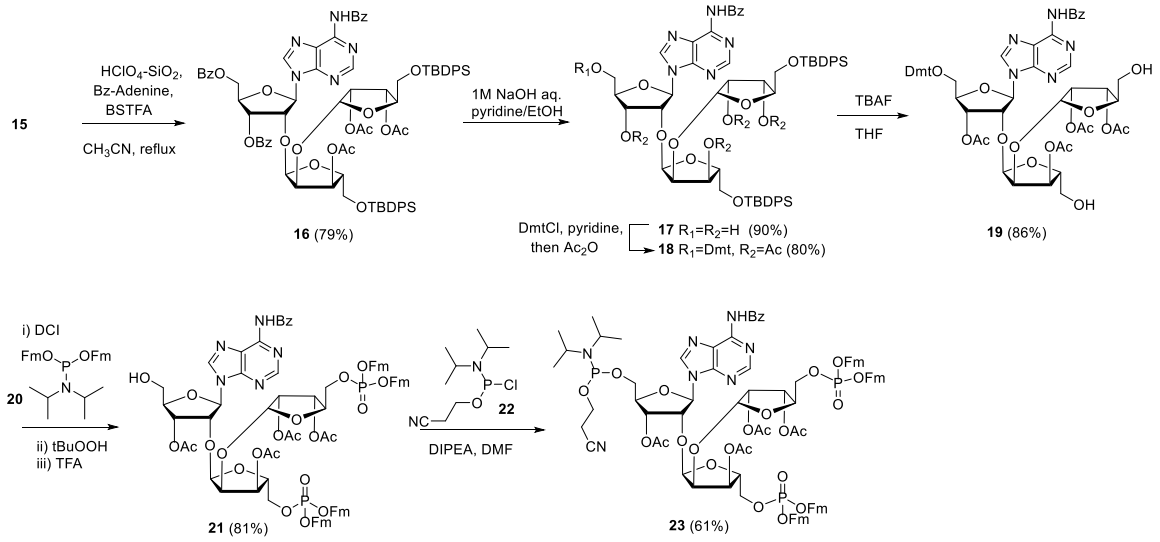
4 for the simultaneous introduction of two pyrophosphates in a molecule already provided with one pyrophosphate.



Scheme 1. Synthesis of protected parotriose **15**

The synthesis of immobilized terminal phosphoribose **2** and known terminal adenosine phosphoramidite **4** are described in Chapter 4. The synthesis of phosphoramidite **3** of a hyperglycosylated nucleoside with orthogonal protecting groups is depicted in Scheme 1 and 2. The key hurdle in the synthesis of **3** is the conversion of D-ribose into protected parotriose **15**, having two 1,2 cis-α-ribosidic bonds which are difficult to introduce (Scheme 1). Our previous method has enabled the selective construction of this core motif[16, 17] however the applied benzyl protective groups were difficult to remove. To circumvent this, PMB (p-methoxybenzyl) protected donor **6** was devised here in order to avoid hydrogenolysis and to preserve the α-selectivity. Firstly, D-ribose was allylated, per-methoxylbenzylated and finally de-allylated using $PdCl_2$ as a catalyst,[22, 23] to yield 2,3,5-tri-*O*-*p*-methoxylbenzyl-D-ribofuranose **5**. Conversion of **5** into the corresponding imidate donor **6** with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride and Cs_2CO_3 in acetone proceeded in good yield. Gratifyingly, the first TMSOTf mediated glycosylation using donor **6** and known acceptor **7**[19] (Chapter 4) furnished disaccharide **8** in high yield and excellent stereoselectivity (only α product). Subsequent rapid acidolysis using TFA removed all PMBs affording **9**. An attempted deprotection of the PMBs with DDQ resulted in a lower yield due to the formation of a 2,3-methoxybenzylidene side product. Replacement of the Bn groups by the PMB groups in the synthesis of key disaccharide **9** avoids hydrogenolysis while the overall

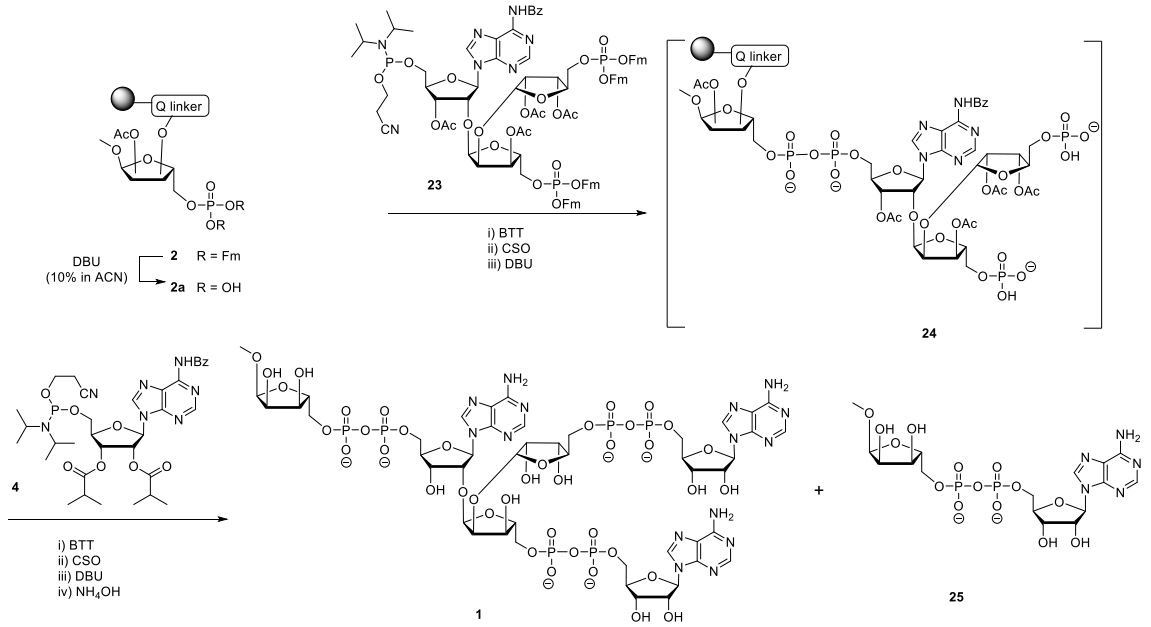
yield is slightly improved.[16] Next, the 3,5-OH was silylated to give TIPDS protected **10**, allowing a selective glycosylation on 2-OH. Condensation of donor **6** and acceptor **10** using the same TMSOTf activation conditions as applied for the synthesis of **8** gave trisaccharide **11** with excellent stereoselectivity (only α product) but in a moderate yield. Subsequently, all PMBs and silyl group need to be removed for upcoming protecting groups manipulation. Unfortunately, pentol **12** was obtained in only 45% yield after HF-pyridine mediated desilylation and TFA assisted removal of the PMBs. Switching the order of deprotection sequence does not give a better yield. The low yield could be attributed to acidic cleavage of one or both glycosidic bonds. To avoid the problematic acidolysis, known donor **13** (Chapter **2**)[16, 21] was used for the trisaccharide construction (route B). Condensation of donor **13** with acceptor **10** using the same TMSOTf/DCM condition gave trisaccharide **14** in a good yield and with excellent α stereoselectivity. Pd/C catalyzed high pressured (80 bar) hydrogenolysis followed by TEA/HF mediated desilylation produced **12** in 79% yield, compared with 45% from route A. Apparently, route B is more suitable for the large scale synthesis of **12**. Next, the protective group manipulation could be continued by selective silylation of both primary OHs in pentol **12** followed by acetylating of all secondary OHs to furnish protected parotriose **15** in 82% yield.



Scheme 2. Synthesis of phosphoramidite **23**

Now, the stage was set for the introduction of N^6-benzoylated adenine at the anomeric position of **15** via a Vorbrüggen type glycosylation catalyzed by $HClO_4$-SiO_2 to afford protected parotrosyl adenine **16** (Scheme 2).[16] The introduction of the two phosphotriesters and the phosphoramidite was attained by the following synthetic manipulations: 1M aq. NaOH was used for saponification of all acetyl and benzoyl esters to give **17** in a high yield. The released 5'-OH in **17** should be provided with cyanoethyl phosphoramidite whereas the Fm-protected phosphate triesters should be installed on the other two

primary positions, temporarily protected with TBDPS groups. Dimethoxytritylation of 5'-OH in **17**, followed by acetylation of the remaining secondary alcohols in the same reaction vessel gave **18**. Two silyl groups in **18** were carefully removed by TBAF to liberate terminal 5''-OH and 5'''-OH (**19**), allowing access to a high yielding single-operation cascade: a) DCI mediated phosphitylation of these OHs with Fm phosphoramidite **20**b) oxidation of the resulting phosphites to phosphate triesters by *t*BuOOH and c) treatment with stoichiometrical TFA to rapidly remove the DMT to obtain **21**. Finally, key phosphoramidite **23** was obtained by treatment of alcohol **21** with standard aminophosphorochloridite **22** and DIPEA in DMF. It is essential to use a neutralized silica gel column for the purification of **23** as it shares the base- and acid-sensitivity with its previously reported linear counterpart (Chapter 4). Eventually, starting from D-ribose, the advanced phosphoramidite **23** was prepared via the 14 step-sequence in sufficient amount (0.36 mmol) for the purposes of the solid phase synthesis of branched ADPr-oligomers.

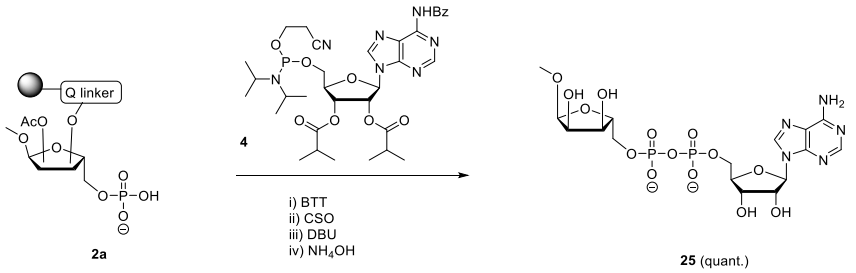


Scheme 3. Synthesis of branched core oligo-ADPr **1**

With sufficient amounts of branched amidite **23** at the disposal, the solid phase synthesis of target branched tri-ADPr **1**, could be undertaking on an automated oligonucleotide synthesizer (Scheme 3). The solid phase mediated introduction of pyrophosphate moieties using P(V)-P(III) chemistry requires, besides a suitable phosphoramidite, the availability of an immobilized phosphate monoester. Immobilized terminal ribose **2** was prepared by functionalization of Tentagel N, a solid support specifically intended for nucleotide synthesis, with a properly protected 5-O-phosphate of ribose equipped with Q linker, as described in Chapter 4. Removal of Fm groups in **2** by DBU treatment (10% in ACN) furnished immobilized phosphomonoester **2a**. The first pyrophosphate was introduced by the following three step procedure: a) BTT assisted P(V)-P(III) coupling of **2a** and phosphoramidite **23**, b)

CSO oxidation of resulting phosphite to phosphate c) DBU (10% in ACN) mediated cleavage of both cyanoethyl (CE) and terminal Fm groups. The produced immobilized intermediate **24** has two phosphate monoester function allowing the simultaneous introduction of the next two pyrophosphates by the same three step P(V)-P(III) coupling cycle using terminal phosphoramidite **4**. Final treatment of the obtained intermediate with aqueous NH_4OH removed all the protecting groups and cleaved the target molecule from solid support.

Analysis of the crude reaction mixture by anion exchange column chromatography and LC-MS showed target branched ADPr **1** together with mono ADPr **25** as a side product. Surprisingly, intermediate **24** (in which the protecting groups and Q-linker are lacking) could not be detected by LC-MS. This outcome suggests that the double pyrophosphate formation using amidite **4** was complete while the formation of monomer **25** probably results from the incomplete pyrophosphate coupling in the first step. Final anion exchange chromatography purification led to the isolation of 0.68 mg of target branched ADPr **1** and 2.83 mg of ADP-ribose (**25**). With a goal to suppress the formation of side product **25**, the use of CPG resin[21, 24] instead of Tentagel was attempted but did not show a better result, as evidenced by the analysis with anion exchange HPLC and ^{31}P-NMR. In addition, increasing the excess of amidite **23** or elongating coupling time of the reaction of **23** with **2a** also did not have a benefical effect. Finally, to estimate the reactivity of the immobilized phosphate monoester 2a, it was coupled with phosphoramidite **4** (Scheme 4), yielding only monomer **25** as gauged by LCMS and ^{31}P-NMR, while unreacted 1-methyl-5-phosphoribose side-product could not be detected. These data collectively show that the reactivity of phosphoramidite **23** for pyrophosphate formation is much lower than that of normal phosphoramidite such as **4**. The bulky phosphate triesters on **23,** containing four Fm groups might affect the reactivity and thereby the rate of the reaction of the phosphoramidite on immobilized phosphate monoester **2a**.



Scheme 4. Direct coupling between **2a** and **4**

Conclusion

In conclusion, this chapter describes the first total synthesis of the branched ADPr **1** that contains three ADPr units. The novel key phosphoramidite reagent **23**, functionalized with two phosphate

triesters was synthesized in 14 steps starting from D-ribose. The three pyrophoshates were introduced on solid support using P(V)-P(III) chemistry, a procedure which allowed the simultaneous introduction of two pyrophoshates. Although the yield of branched ADPr oligomer is low, sufficient amounts can be obtained for implementation in biochemical assays such as protein binding and co-crystallization with interesting target proteins such as PARG and APLF. The developed modular synthesis route also allows for the future modification of branched poly ADPr molecules, for example, clickable propargyl branched ADPr-oligomers.

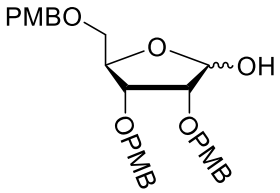
Acknowledgments

Nico Meeuwenoord is kindly acknowledged for his help in oligomer synthesis and final product purification.

Experimental section

General procedure

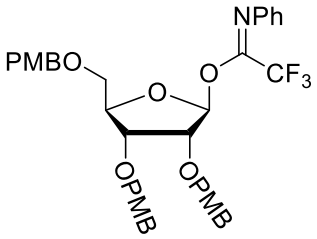
All chemicals were used as received unless stated otherwise. All solvents used in the reaction (including solid phase synthesis) were dried over 3Å molecular sieves. Solvents removal by rotary evaporation was under reduced pressure at 40°C. TLC, NMR, LCMS, anion exchange, HRMS, IR, optical rotation facilities were used as described in Chapter 2.



2,3,5-tri-*O*-*p*-methoxylbenzyl-D-ribofuranose (5)

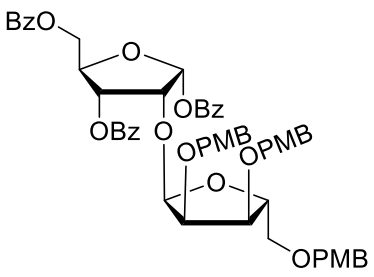
D-Ribose (6.0 g, 40 mmol), allyl alcohol (100 mL) and acetyl chloride (2.0mL, 28 mmol) were added into a flask and the mixture was stirred for 2.5 hours after which was quenched by $NaHCO_3$ solid and filtered over celite. The filtration was concentrated under reduced pressure and co-evaporated with toluene (2 x). To the residue, DMF (100 mL) was added and cooled down to 0 °C. NaH (60% in mineral oil, 6.4 g, 160 mmol) was added in 3 portions. After hydrogen generation ceased, 4-methoxybenzyl chloride (19 mL, 14 mmol) was added dropwise at 0°C under N_2. The mixture was stirred overnight after which was quenched by aqueous saturated NH_4Cl. EA and H_2O were added into the mixture and the organic layer was washed additionally by H_2O (2 x) and brine (1 x) and dried by $MgSO_4$. The mixture was concentrated under reduced pressure. To the residue, $CHCl_3$ (120 mL), H_2O (80 mL) and $PdCl_2$ (2.12 g, 12 mmol) were added and the mixture was vigorously stirred at 50 °C under O_2 atmosphere for 48 hours after which the mixture was concentrated under reduced pressure. EA (50 mL) and saturated aqueous $NaHCO_3$ (50 mL) were added into the residue and the organic layer was separated, dried by $MgSO_4$, filtered and concentrated under reduced pressure. Silica gel column chromatography (pentane/EA, 100/0 – 90/10 – 70/30) furnished **5** as light brown oil (11.44 g, 22.42 mmol, 56 %, α-product: 60%, β-product: 40%). ^{1}H-NMR and ^{13}C-NMR showed identical date with reported **5**.[22] ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.35 –

7.10 (m, 6H, arom.), 6.94 – 6.73 (m, 6H, arom.), 5.27 – 5.28 (m, 1H, H1), 4.67 – 4.37 (m, 5.6H, CH_2 PMB), 4.32 – 4.24 (m, 1.4H, H4, C*H*H PMB), 4.19 – 4.15 (m, 1.4H, OH, H3-β), 3.95 – 3.89 (m, 1.2H, H3-α, H2-β), 3.83 – 3.77 (m, 9.4H, OMe, H2-β), 3.61 (AB, *J* = 10.3, 2.8 Hz, 0.4H, H5-β), 3.47 – 3.37 (m, 1.6H, H5-αβ). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.55, 159.50, 159.49, 159.48, 159.37, 130.12, 130.02, 129.98 (cq. arom.), 129.82, 129.75 (arom.), 129.71 (Cq. arom.), 129.69, 129.68, 129.56 (arom.), 129.51(Cq. arom.), 129.34, 114.00, 113.97, 113.93, 113.91, 113.90 (arom.), 100.52 (C1-β), 96.38 (C1-α), 81.10 (C4-α), 81.08 (C4-β), 80.55 (C2-β), 77.48 (C2-α), 77.36 (C3-α), 76.89 (C3-β), 73.27, 72.48, 72.20, 72.15, 72.03 (CH2 PMB), 69.82 (C5-α), 69.16 (C5-β), 55.40 (OMe-α), 55.38 (OMe-β).



1-*O*-((*N*-Phenyl)-2,2,2-trifluoroacetimido)-2,3,4-tri-*O*-*p*-methoxylbenzyl-D-ribofuranose (6)

Compound **5** (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)



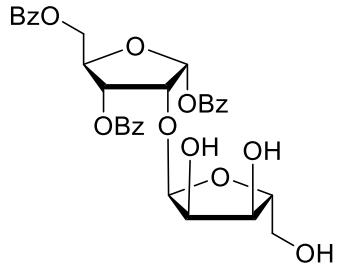
α-1,3,5-Tri-*O*-benzoyl-2',3',5'-tri-*O*-*p*-methoxylbenzyl-parobiose (8)

Compound **6** (5.9 g, 8.66 mmol) and α-D-ribofuranose 1,3,5-tribenzoate **7** (3.64 g, 7.87 mmol) were co-evaporated with toluene (1 x), 1,4-dioxane (2 x) and DCE (1 x), dissolved in dry DCM and stirred with freshly activated 3 Å molecular sieves at room temperature for 1 hour under N_2 to remove traces of water. The solution was then cooled to -78 ℃ and TMSOTf (28 µL, 0.16 mmol) was added to the reaction mixture. The reaction was being stirred at the same temperature for 10 minutes after which it was quenched by the addition of triethylamine. The reaction mixture was concentrated under reduced pressure and purified by silica gel chromatography (DCM/EtOAc, 97/3 – 95/5) to afford **8** as a white foam (7.51 g, 7.87 mmol, 100%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.17 – 8.14 (m, 4H, arom.), 8.10 – 8.02 (m, 2H, arom.), 7.58 – 7.46 (m, 3H, arom.), 7.44 – 7.40 (dd, *J* = 8.2, 7.0 Hz, 2H, arom.), 7.33 – 7.19 (m, 4H, arom.), 7.14 – 7.07 (m, 2H, arom.), 7.03 – 6.94 (m, 4H, arom.), 6.83 – 6.80 (m, 2H, arom., H1'), 6.69 – 6.58 (m, 4H, arom.), 5.68 (dd, *J* = 6.4, 1.9 Hz, 1H, H3'),

5.29 (d, *J* = 4.0 Hz, 1H, H1''), 4.77 – 4.70 (m, 2H, H4', H2'), 4.70 – 4.55 (m, 2H, H5'), 4.52 – 4.16 (m, 7H, CH_2 PMB, H4''), 3.89 – 3.79 (m, 2H, H2'', H3''), 3.79 (s, 3H, OMe), 3.73 (s, 3H, OMe), 3.72 (s, 3H, OMe), 3.42 (AB, *J* = 10.8, 3.3 Hz, 1H, H5''), 3.31 (AB, *J* = 10.8, 3.7 Hz, 1H, H5'').

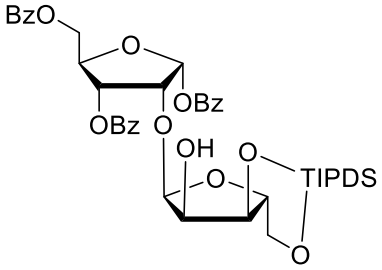
^{13}C NMR (101 MHz, $CDCl_3$) δ 166.32, 166.12, 165.76 (CO Bz), 159.19, 158.99, 158.96 (Cq. arom.), 133.24, 133.16 (Cq. arom.), 130.33 (arom.), 130.18 (Cq. arom.), 130.04, 130.00, 129.87 (arom.), 129.75 (Cq. arom.), 129.64 (arom.), 129.29, 129.22, 128.99, 128.53, 128.35, 128.31, 113.73, 113.53, 113.50 (arom.), 102.01 (C1''), 95.12 (C1'), 83.38 (C4'), 81.67 (C4''), 77.60 (C2''), 75.44 (C2'), 75.27 (C3''), 72.98 (CH_2 PMB), 72.24 (C3'), 72.07 (CH_2 PMB), 71.70 (CH_2 PMB), 69.05 (C5''), 64.35 (C5'), 55.25, 55.22, 55.21 (OMe). IR (film): 2934, 1721, 1612, 1513, 1451, 1266, 1248, 1175, 1111, 1068, 1026, 820, 710, 516 cm^{-1}. HRMS (ESI^+) calcd for $C_{55}H_{54}O_{15}Na$ (M+Na) 977.3355. Found 977.3357. $[\alpha]_D^{20}$ +84.0 (c = 1, in DCM)



α-1,3,5-tri-*O*-benzoylparobiose (9)

Compound **8** (7.0 g, 7.34 mmol), DCM (60 mL) and TFA (3.37 mL, 44 mmol) were added into a flask and the reaction was stirred for 90 minutes after which was quenched by saturated aqueous $NaHCO_3$. DCM extracted the mixture (4 x) and the organic layers were combined, dried ($MgSO_4$) and filtered. The filtration was concentrated under reduced pressure and purified by silica gel chromatography (DCM/acetone, 100/1 – 100/7 – 90/10 – 80/20) to furnish **9** as a white foam (3.22 g, 5.42 mmol, 74%).

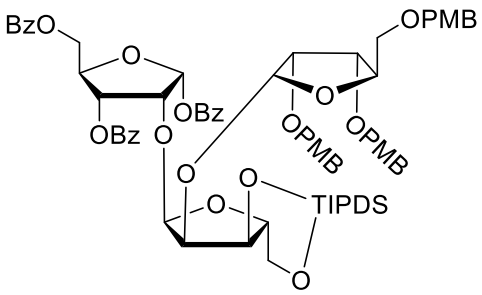
^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.13 – 8.03 (m, 6H, arom.), 7.63 – 7.55 (m, 3H, arom.), 7.47 (t, *J* = 7.8 Hz, 2H, arom.), 7.43 – 7.33 (m, 4H, arom.), 6.76 (d, *J* = 4.2 Hz, 1H, H1'), 5.74 (dd, *J* = 6.3, 2.0 Hz, 1H, H3'), 5.19 (d, *J* = 4.2 Hz, 1H, H1''), 4.87 (td, *J* = 3.8, 1.9 Hz, 1H, H4'), 4.75 (dd, *J* = 6.3, 4.2 Hz, 1H, H2'), 4.63 (AB, *J* = 12.1, 3.8 Hz, 2H, H5'), 4.00 – 3.97 (m, 2H, H2'', H4''), 3.86 (s, 1H, H3'), 3.64 (AB, *J* = 12.2, 3.1 Hz, 1H, H5''), 3.55 (AB, *J* = 12.1, 3.9 Hz, 1H, H5''), 2.73 (s, 1H, OH), 2.56 (s, 1H, OH), 2.04 (s, 1H, OH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.82, 166.14, 165.92 (CO Bz), 133.94, 133.66, 133.57, 130.04, 130.03, 129.79 (arom.), 129.66, 129.51, 129.05 (Cq. arom.), 128.71, 128.57 (arom.), 102.26 (C1''), 95.31 (C1'), 86.38 (C4''), 82.77 (C4'), 75.38 (C2'), 72.30 (C2''), 72.20 (C3'), 70.51 (C3''), 64.21 (C5'), 62.56 (C5'').



α-1,3,5-tri-*O*-benzoyl-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-parobiose (10)

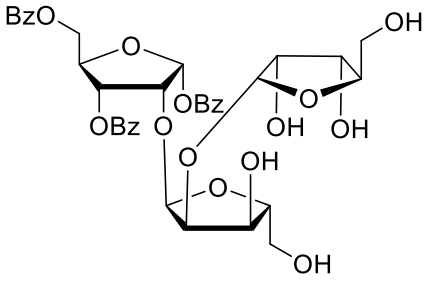
Compound **9** (2.48 g, 4.17 mmol) and imidazole (852 mg, 12.52 mmol) were co-evaporated with toluene (2 x), dissolved in DCM (41 mL) and then $TIPDSCl_2$ (1.6 mL, 5.01 mmol) was added. The reaction was stirred at room temperature for 16 hours and quenched upon the addition of H_2O (200 mL). The mixture was washed by DCM (3 x) and the organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/acetone, 100/0 – 97/3) to obtain **10**[16] as colorless foam (2.75 g, 3.29 mmol, 79%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.20 – 8.02 (m, 6H, arom.), 7.65 – 7.53 (m, 3H, arom.), 7.53 – 7.44 (m, 2H, arom.),

7.44 – 7.35 (m, 4H, arom.), 6.79 (d, *J* = 4.2 Hz, 1H, H1'), 5.67 (dd, *J* = 6.4, 2.1 Hz, 1H, H3'), 5.19 (d, *J* = 4.2 Hz, 1H, H1''), 4.81 – 4.73 (m, 2H, H2', H4'), 4.73 – 4.58 (m, 2H, H5'), 4.12 – 4.05 (ddd, *J* = 19.8, 7.7, 4.6 Hz, 2H, H2'', H3''), 3.93 (ddd, *J* = 8.4, 5.0, 3.5 Hz, 1H, H4''), 3.82 (dd, *J* = 11.7, 3.6 Hz, 1H, H5''), 3.66 (AB, *J* = 11.7, 8.3 Hz, 1H, H5''), 2.84 (d, *J* = 8.6 Hz, 1H, OH), 1.13 – 0.83 (m, 24H, CH_3 TBDPS), 0.80 (d, *J* = 7.3 Hz, 2H, CH TIPDS), 0.74 (d, *J* = 7.3 Hz, 2H, CH TIPDS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.15, 165.70 (CO Bz), 133.49, 133.46, 133.43, 130.16, 129.99, 129.82 (arom.), 129.71 (Cq. arom.), 128.67, 128.57, 128.49 (arom.), 101.96 (C1''), 95.14 (C1'), 83.87 (C4''), 83.32 (C4'), 75.67 (C2'), 71.95 (C3'), 71.08 (C2''), 70.84 (C3''), 64.31 (C5'), 63.50 (C5''), 17.55, 17.49, 17.46, 17.41, 17.07, 16.98, 16.83, 16.69, 13.46, 13.27, 13.04, 12.38 (CH, CH_3 TIPDS).



α-1,3,5-tri-*O*-benzoyl-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'',3'',5''-di-*O*-*p*-methoxylbenzyl-parobiose (11)

Compound **10** (1.43 g, 1.71 mmol) and **6** (1.40 g, 2.05 mmol) were co-evaporated with toluene (2 x), 1,4-dioxane (2 x) and DCE (1 x), dissolved in dry DCM and stirred with freshly activated 3 Å molecular sieves at room temperature for 1 hour under N_2 to remove traces of water. The solution was then cooled to -78 °C and TMSOTf (10 µL, 0.05 mmol) was added to the reaction mixture. The reaction was stirred at the same temperature for 30 minutes after which it was quenched by the addition of triethylamine. The reaction mixture was concentrated under reduced pressure and purified by silica gel chromatography (pentane/EtOAc, 100/0 – 90/10 – 80/20) to afford **11** as a white foam (1.16 g, 0.87 mmol, 51%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.18 – 8.11 (m, 2H, arom.), 8.07 (d, *J* = 6.9 Hz, 4H, arom.), 7.61 – 7.51 (m, 2H, arom.), 7.47 (td, *J* = 7.6, 3.9 Hz, 3H, arom.), 7.35 (t, *J* = 7.8 Hz, 2H, arom.), 7.27 – 7.16 (m, 4H, arom.), 7.12 – 7.02 (m, 4H, arom.), 6.90 – 6.67 (m, 7H, arom. H1'), 5.65 (dd, *J* = 6.2, 1.8 Hz, 1H, H3'), 5.38 (d, *J* = 3.5 Hz, 1H, H1''), 5.32 (d, *J* = 3.8 Hz, 1H, H1'''), 4.85 (dd, *J* = 6.2, 4.1 Hz, 1H, H2'), 4.75 – 4.60 (m, 3H, H4', H5', C*H*H PMB), 4.54 (AB, *J* = 12.0, 4.0 Hz, 1H, H5'), 4.48 – 4.45 (m, 2H, H2'', C*H*H PMB), 4.34 (dd, *J* = 32.6, 11.5 Hz, 2H, C*H*2, PMB), 4.26 – 4.00 (m, 4H, H4''', H3'', C*H*H PMB, H4''), 3.89 – 3.67 (m, 12H, H5'', C*H*H PMB, OMe PMB), 3.61 – 3.47 (m, 2H, H3''', H5'''), 3.47 – 3.33 (m, 2H, H2''', H5'''), 1.12 – 0.80 (m, 28H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.04, 165.87, 165.59 (CO Bz), 159.14, 159.05, 158.84 (Cq. arom.), 133.45, 133.39, 133.24 (arom.), 131.05, 130.43, 130.38 (Cq. arom.), 130.08 (arom.), 129.92 (Cq. arom.), 129.88, 129.81 (arom.), 129.65, 129.57, 129.43, 129.26, 129.21, 128.55, 128.52, 128.48, 113.73, 113.70, 113.66, 113.61, 113.49, 113.32 (arom.), 102.31 (C1''), 101.30 (C1'''), 94.98 (C1'), 83.35 (C4'), 80.89 (C4''), 79.10 (C4'''), 76.30 (C2'''), 75.51 (C3'''), 75.31 (C2'), 73.24 (C2''), 72.96 (CH2 PMB), 72.28 (C3'), 72.07, 71.10 (CH2 PMB), 68.92 (C3''), 68.73 (C5'''), 64.20 (C5'), 59.82 (C5''), 55.23, 55.21, 55.16 (OMe), 17.42, 17.37, 17.32, 17.17, 17.08, 17.05, 16.94 (CH_3 TIPDS), 13.48, 13.03, 12.67, 12.51 (CH TIPDS). IR (film): 2944, 2867, 1724, 1613, 1514, 1266, 1248, 1112, 1035, 711cm^{-1}. HRMS (ESI$^+$) calcd for $C_{72}H_{92}NO_{20}Si_2$ (M+NH_4) 1346.5746. Found 1346.5753. $[\alpha]_D^{20}$ +77.6 (c = 1, in DCM)

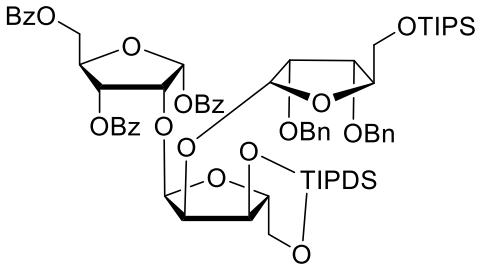


α-1,3,5-tri-*O*-benzoyl-2'',3''-di-*O*-benzylparotriose (12)

The procedure from **11**:

Compound **11** (430 mg, 0.32 mmol) and pyridine (3 mL) were added into a flask and the reaction was cooled to 0°C. Subsequently, HF-pyridine (0.25 mL, 9.62 mmol) was added at the same temperature under N_2. The reaction was stirred at room temperature for 3 hours and quenched by the addition of aqueous saturated $NaHCO_3$. The mixture was extracted by DCM (3 x) and the organic layers are combined and dried ($MgSO_4$). The mixture was filtered, concentrated under reduced pressure and co-evaporated with toluene (3 x). To the residue, DCM (3 mL) and TFA (0.25 mL, 3.27 mmol) were added and the reaction was stirred for 20 minutes after which was quenched by addition of aqueous saturated $NaHCO_3$. The mixture was extracted by DCM (3 x), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/Methanol, 100/0 – 99/1 – 97/3 – 95/5 – 96/4) to obtain **12** as a white foam (106 mg, 0.15 mmol, 47%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.17 – 7.97 (m, 6H, arom.), 7.63 – 7.50 (m, 3H. arom.), 7.50 – 7.32 (m, 6H, arom.), 6.76 (d, *J* = 4.1 Hz, 1H, H1'), 5.69 (dd, *J* = 6.3, 1.7 Hz, 1H, H3'), 5.27 (d, *J* = 4.0 Hz, 1H, H1''), 4.95 (d, *J* = 4.0 Hz, 1H, H1'''), 4.82 (td, *J* = 4.1, 1.6 Hz, 1H, H4'), 4.72 (dd, *J* = 6.3, 4.2 Hz, 1H, H2'), 4.61 (AB, *J* = 12.0, 4.1 Hz, 2H, H5'), 4.07 (dd, *J* = 5.8, 4.0 Hz, 1H, H2''), 3.98 – 3.93 (m, 3H, H3'', H4''', H4''), 3.72 – 3.35 (m, 7H, H3''', H2''', H5'', H5''', OH), 3.30 (s, 1H, OH), 3.22 – 3.08 (m, 1H, OH), 3.00 – 2.94 (m, 2H, OH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.67, 166.20, 165.96 (CO Bz), 133.85, 133.77, 133.52, 130.16, 129.79 (arom.), 129.56, 129.54, 129.14 (Cq. arom.), 128.67, 128.58, (arom.) 101.44 (C1''), 101.05 (C1'''), 95.37 (C1'), 86.33 (C4''), 85.75 (C4'''), 83.21 (C4'), 75.34 (C2'), 75.29 (C2''), 72.46 (C2'''), 72.16 (C3'), 70.91 (C3''), 70.85 (C3'''), 64.31 (C5'), 62.78 (C5'''), 62.19 (C5'').

Procedure from **14** to **12**



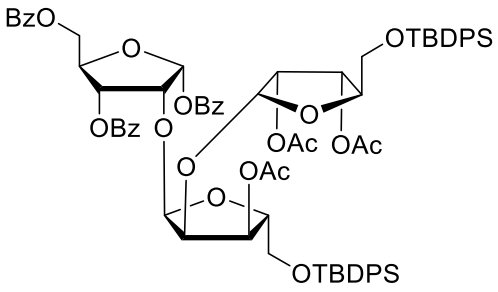
α-1,3,5-tri-*O*-benzoyl-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'',3''-di-*O*-benzyl-5''-*O*-triisopropylsilylparotriose (14)

Compound **14** was synthesized according to our previously reported procedure with minor modification.[16] Compound **10** (4.7 g, 5.61 mmol) and **13** (5.17 g, 7.86 mmol) were co-evaporated with toluene (1 x), 1,4-dioxane (2 x) and DCE (1 x). Dry DCM (94 mL) and freshly activated 3Å molecular sieves were added to the mixture. The reaction was stirred under N_2 at room temperature for 2 hours and then cooled to -78 °C. Next, TMSOTf (30 uL, 0.17 mmol) was added, the reaction mixture was stirred at the same temperature for 15 minutes and then was quenched by addition of triethylamine. The reaction mixture was concentrated under reduced pressure and purified by silica gel chromatography (pentane/EA, 100/0 – 100/3 – 90/10) to obtain **14** as a white foam (6.58 g, 5.04 mmol, 90%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.17 – 8.12 (m, 2H, arom.), 8.08 (ddd, *J* = 11.8, 8.4, 1.4 Hz, 4H, arom.), 7.59 – 7.53 (m, 2H, arom.), 7.52 – 7.43 (m, 3H, arom.), 7.36 (t, *J* = 7.8 Hz, 2H, arom.), 7.29 – 7.14 (m, 12H, arom.), 6.79 (d, *J* = 4.1 Hz, 1H, H1'), 5.60 (dd, *J* = 6.3, 1.9 Hz, 1H, H3'), 5.36 (d, *J* = 3.5 Hz, 1H, H1''), 5.27 (d, *J* = 3.8 Hz, 1H, H1'''), 4.84 (dd, *J* = 6.3, 4.1 Hz, 1H, H2'), 4.76 – 4.66 (m, 2H, CH_2 Bn, H4'), 4.62 (AB, *J* = 12.0,

3.4 Hz, 1H, H5'), 4.52 (AB, *J* = 12.0, 4.0 Hz, 1H, H5'), 4.46 – 4.43 (m, 1H, C*H*H Bn), 4.40 (dd, *J* = 5.3, 3.5 Hz, 1H, H2''), 4.32 (d, *J* = 11.8 Hz, 1H, CH_2, Bn), 4.22 – 4.14 (m, 2H, H3'', H4'''), 4.04 (dt, *J* = 8.8, 2.5 Hz, 1H, H4''), 3.97 (d, *J* = 11.4 Hz, 1H, C*H*H Bn), 3.82 (AB, *J* = 13.0, 2.4 Hz, 1H, H5''), 3.78 – 3.64 (m, 4H, H5'', H5''', H3'''), 3.44 (dd, *J* = 6.4, 3.8 Hz, 1H, C*H*H Bn), 1.13 – 0.85 (m, 49H, TIPS, TIPDS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.14, 165.99, 165.64 (CO Bz), 138.98, 138.71 (Cq. arom.), 133.48, 133.45, 133.28, 130.14 (arom.), 130.06 (Cq. arom.), 130.00, 129.92 (arom.), 129.80, 129.70 (Cq. arom.), 128.61, 128.55, 128.53, 128.12, 127.99, 127.84, 127.68, 127.34, 127.14 (arom.), 102.22 (C1''), 101.25 (C1'''), 95.08 (C1'), 83.33 (C4'), 81.18 (C4'''), 81.16 (C4''), 77.38 (C2'''), 75.61 (C3'''), 75.14 (C2'), 73.61 (C2''), 72.34 (C3'), 72.30 (CH_2, Bn), 71.78 (CH_2, Bn), 69.09 (C3'''), 64.25 (C5'), 62.64 (C5''), 60.02 (C5'''), 18.06, 17.51, 17.46, 17.41, 17.40, 17.21, 17.16, 17.08, 16.95, 13.60, 13.13, 12.78, 12.60, 12.03 (CH_3, CH, TIPDS, TIPS).

α-1,3,5-tri-*O*-benzoyl-2'',3''-di-*O*-benzylparotriose (12)

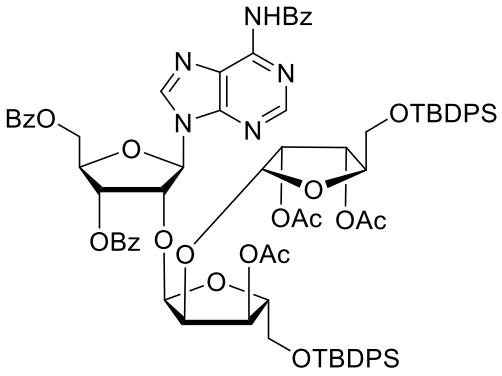
Compound **14** (3.21 g, 2.46 mmol) was dissolved in *t*BuOH/dioxane/H_2O (50 mL, 4/4/1; v/v/v), Pd/C (500 mg, 10% loading) and one drop of AcOH were added. The mixture was sonicated under N_2 for 10 minutes then was transferred into an autoclave. The reaction in the autoclave was stirred for 16 hours under 80 bar of H_2 after which was filtered and concentrated under reduced pressure. The residue was co-evaporated with toluene (3 x) and pyridine (3 x). To the intermediate, pyridine (12 mL), triethylamine (5.14 mL, 36.91 mmol) and $Et_3N{\cdot}3HF$ (6.02 mL, 36.91 mmol) were added successively under 0 °C. The reaction was allowed to warm up to room temperature and stirred for 16 hours after which was quenched carefully by addition of aqueous saturated $NaHCO_3$. DCM extracted (3x) the mixture and dried over $MgSO_4$. Purification by silica gel chromatography (DCM/Methanol, 100/0 – 95/5 – 90/10) to obtain **12**[16] as white foam (1.41 g, 1.94 mmol, 79%).



α-1,3,5-tri-*O*-benzoyl-3'-*O*-acetyl-5'-*O*-tertbutyldiphenylsilyl-2'',3''-di-*O*-acetyl-5''-*O*-tertbutyldiphenylsilylparotriose (15)

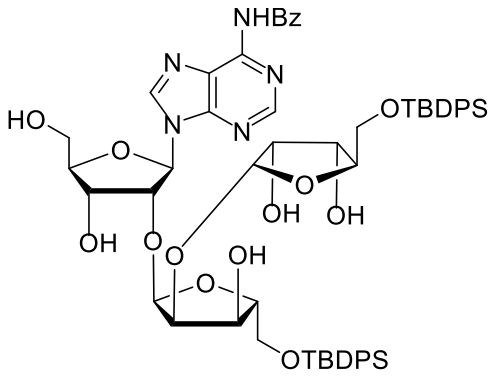
Compound **12** (2.36 g, 3.25 mmol) was co-evaporated with pyridine (1 x) and then N_2 was applied. Pyridine (32 mL) and TBDPSCl (2.54 mL, 9.75 mmol) were added and the mixture was stirred under N_2 at room temperature for 16 hours. Ac_2O (9.2 mL, 97.5 mmol) was added into the reaction and the mixture was stirred for 6 hours after which was quenched by addition of aqueous saturated $NaHCO_3$. The mixture was extracted by DCM (3 x), dried ($MgSO_4$) and concentrated under reduced pressure. Purification by silica gel chromatography (pentane/actone, 100/0 – 90/10 – 80/20) furnished **15**[16] as a white foam (3.54 g, 2.66 mmol, 82%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.23 – 8.17 (m, 2H, arom.), 8.17 – 8.13 (m, 2H, arom.), 8.11 – 8.04 (m, 2H, arom.), 7.65 (dq, *J* = 6.5, 1.5 Hz, 4H, arom.), 7.63 – 7.50 (m, 7H, 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'''), 5.31 (d, *J* = 4.2 Hz, 1H, H1''), 5.29 (d, *J* = 4.4 Hz, 1H, H1'''), 4.88 (dd, *J* = 7.0, 4.4 Hz, 1H, H2'''), 4.76 (td, *J* = 3.8, 1.8 Hz, 1H, H4'), 4.71 – 4.58 (m, 3H, H2', H5'), 4.35 (dd, *J* = 6.6, 4.2 Hz, 1H, H2'''), 4.09 – 4.06 (m, 2H, H4''', H4''), 3.81 (AB, *J* = 11.2, 2.8 Hz, 1H, H5''), 3.69 (AB, *J* = 11.2, 3.1 Hz, 1H, H5''), 3.67 – 3.58 (m, 2H, H5'''), 2.01 (s,

3H, Ac), 1.78 (s, 3H, Ac), 1.63 (s, 3H, Ac), 1.05 (s, 9H, CH_3 TBDPS), 0.97 (s, 10H, CH_3, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.70, 170.10, 169.70 (CO, Ac), 166.18, 166.15, 165.57 (CO, Bz), 135.76, 135.74, 135.72, 135.68, 133.51, 133.49 (arom.), 133.16, 133.11, 133.04, 132.98, 130.28 (Cq. arom.), 130.17, 130.13 (arom.), 129.99 (Cq. arom.), 129.96, 129.94, 129.90, 129.86, 129.81 (arom.), 129.75 (Cq. arom.), 128.70, 128.51, 127.93, 127.91, 127.90, 127.88 (arom.), 101.29 (C1''), 99.53 (C1'''), 95.17 (C1'), 83.74 (C4''), 83.67 (C4'), 83.14 (C4'''), 76.35 (C2'), 74.63 (C2''), 71.76 (C2'''), 71.67 (C3'), 71.26 (C3''), 69.84 (C3'''), 64.45 (C5'), 63.92 (C5''), 63.43 (C5'''), 26.91, 26.86 (CH_3 TBDPS), 20.70, 20.39, 20.17 (Ac), 19.38, 19.30 (Cq. TBDPS).



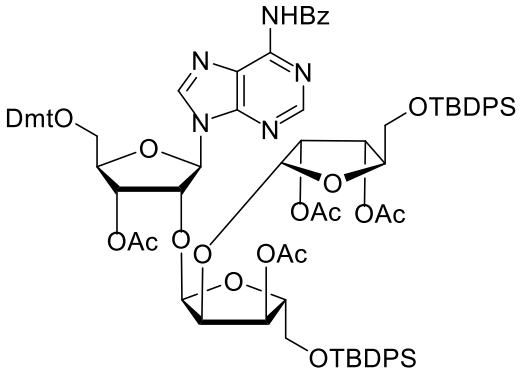
6-*N*-benzoyl-9-(3',5'-di-*O*-benzoyl-3''-*O*-acetyl-5''-*O*-tertbutyldiphenylsilyl-2''',3'''-di-*O*-acetyl-5'''-*O*-tertbutyldiphenylsilyl-β-parotriosyl)adenine (16)

Compound **15** (1.93 g, 1.48 mmol) and N^6-benzoyladenine (0.71 g, 2.97 mmol) were co-evaporated with 1,4-dioxane (2 x), ACN (1 x) and dissolved in dry ACN (24 mL) under N_2. N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (5.56 mL, 20.76 mmol) was added and the mixture was stirred at room temperature for 5 minutes. $HClO_4$-SiO_2 (7.4 g, 0.4 mmol/g, 2.96 mmol) was added and the mixture was refluxed for 16 hours. The reaction was quenched by aqueous saturated $NaHCO_3$ then filtered. The mixture was extracted with EtOAc (3 x), dried ($MgSO_4$) and concentrated under reduced pressure. Purification by silica gel chromatography (pentane/acetone, 100/0 – 85/15 – 80/20) gave **16**[16] as a white foam (1.82 g, 1.26 mmol, 85%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.99 (s, 1H, NH), 8.68 (d, *J* = 1.8 Hz, 1H, H2), 8.40 (d, *J* = 1.9 Hz, 1H, H8), 8.07 (tt, *J* = 6.6, 1.6 Hz, 4H, arom.), 8.01 – 7.93 (m, 2H, arom.), 7.65 – 7.47 (m, 13H, arom.), 7.46 – 7.25 (m, 16H, arom.), 6.32 (dd, *J* = 4.7, 1.8 Hz, 1H, H1'), 5.94 (td, *J* = 5.4, 1.8 Hz, 1H, H3'), 5.68 (t, *J* = 5.0 Hz, 1H, H2'), 5.43 (dt, *J* = 6.9, 2.2 Hz, 1H, H3''), 5.41 – 5.37 (m, 1H, H3'''), 5.24 (dd, *J* = 4.4, 1.8 Hz, 1H, H1''), 5.16 (dd, *J* = 4.5, 1.8 Hz, 1H, H1'''), 4.93 (ddd, *J* = 7.2, 4.3, 1.7 Hz, 1H, H2'''), 4.91 – 4.85 (m, 1H, H5'), 4.75 (q, *J* = 4.6 Hz, 1H, H4'), 4.70 (AB, *J* = 11.9, 5.0 Hz, 1H, H5'), 4.31 (ddd, *J* = 6.8, 4.3, 1.8 Hz, 1H, H2''), 4.09 (t, *J* = 2.9 Hz, 1H, H4'''), 4.00 (q, *J* = 2.7 Hz, 1H, H4'), 3.79 – 3.76 (m, 1H, H5'''), 3.71 – 3.68 (m, 1H, H5'''), 3.59 – 3.56 (m, 1H, H5''), 3.45 – 3.41 (m, 1H, H5''), 2.10 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.68 (s, 3H, Ac), 1.01 (s, 9H, CH_3 TBDPS), 0.95 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.57, 169.93, 169.83 (CO Ac), 166.30, 165.40, 164.49 (CO Bz), 152.94 (CH C2), 151.41 (Cq. arom.), 149.95 (CH C8), 149.80 (Cq. arom), 136.09, 135.67 (Cq. arom), 133.79 (Cq. arom), 133.63, 133.49 (arom.), 133.03, 132.98, 132.94 (Cq. arom.), 132.87 (arom.), 132.82 (Cq. arom.), 129.93, 129.90, 129.88 (aom.), 129.63, 129.60 (Cq. arom.), 128.97, 128.64, 128.59, 127.93, 127.91, 127.89 (arom.), 123.95 (Cq. arom.), 123.86 (arom.), 101.26 (C1''), 98.72 (C1'''), 89.14 (C1'), 83.11 (C4'''), 82.45 (C4''), 80.52 (C4'), 77.48 (C2'), 73.09 (C2''), 72.49 (C3'), 71.79 (C2'''), 71.02 (C3''), 69.86 (C3'''), 63.62 (C5'), 63.57 (C5''), 63.19 (C5'''), 26.87 (CH_3 TBDPS), 26.81 (CH_3 TBDPS), 20.77, 20.68, 20.43 (CH_3 Ac), 19.29, 19.26 (Cq. TBDPS).



6-*N*-benzoyl-9-(5'',5'''-di-*O*-tertbutyldiphenylsilyl-β-parotriosyl)adenine (17)

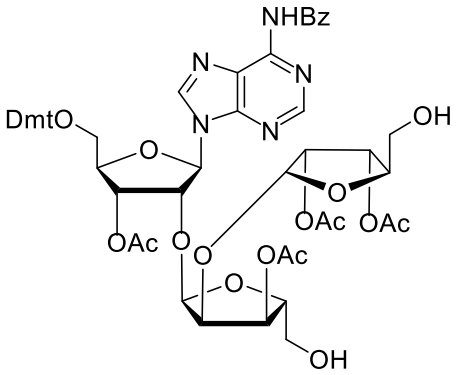
Compound **16** (1.82 g, 1.26 mmol) was dissolved in pyridine/EtOH (12.6 mL; 2/1 v/v), cooled to 0 °C after which aqueous NaOH (7.56 mL, 1 M solution) was slowly added. The reaction mixture was stirred for 2 hours at the same temperature after which Amberlite-H^+ was added until pH = 6. The mixture was filtered, concentrated under reduced pressure and purified by silica gel chromatography (DCM/methanol, 100/0 – 97/3 – 95/5) to obtain **17**[16] as a white foam (1.26 g, 1.13 mmol, 90%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.31 (s, 1H, NH), 8.82 (s, 1H, H2), 8.39 (s, 1H, H8), 8.02 – 7.95 (m, 2H, arom.), 7.67 – 7.54 (m, 8H, arom.), 7.48 (t, *J* = 7.8 Hz, 2H, arom.), 7.44 – 7.28 (m, 13H, arom.), 6.15 (d, *J* = 7.5 Hz, 1H, H1'), 6.07 (br, 1H, OH), 5.09 (d, *J* = 4.5 Hz, 1H, H1''), 5.00 (d, *J* = 4.0 Hz, 1H, H1'''), 4.94 (dd, *J* = 7.6, 4.6 Hz, 1H, H2'), 4.60 (d, *J* = 4.7 Hz, 1H, H3'), 4.41 (t, *J* = 4.8 Hz, 1H, H2''), 4.39 – 4.33 (m, 2H, H3''', H4'), 4.31 – 4.19 (m, 4H, H2''', H3''', H4'', H4'''), 4.00 (AB, *J* = 13.0, 1.8 Hz, 1H, H5'), 3.78 (d, *J* = 12.8 Hz, 1H, H5'), 3.75 – 3.64 (m, 4H, H5'', H5'''), 1.00 (s, 9H, TBDPS), 1.00 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.86 (CO Bz), 150.75, 150.45 (Cq. arom.), 135.69, 135.68, 135.66, 135.64 (arom.), 133.61, 133.09 (Cq. arom.), 133.05 (arom.), 132.89, 132.87, 132.71 (arom.), 130.09, 130.06, 130.04, 129.97, 129.00, 128.11, 127.99, 127.99, 127.97, 127.91 (arom.), 124.66 (Cq. arom.), 102.17 (C1'''), 101.26 (C1''), 89.74 (C1'), 88.39 (C4'), 86.56 (C4''), 86.18 (C4'''), 80.15 (C2'), 73.13 (C2'''), 73.01 (C3'), 72.10 (C3''), 71.34 (C3'''), 64.36 (C5'''), 64.19 (C5''), 63.47 (C5'), 26.95 (CH3 TBDPS), 26.93 (CH_3 TBDPS), 19.34 (Cq. TBDPS), 19.30 (Cq. TBDPS).



6-*N*-benzoyl-9-(3',3''2''',3'''-tetra-*O*-acetyl-5'-*O*-dimethoxyltrityl-5'',5'''-di-*O*-tertbutyldiphenylsilyl-β-parotriosyl)adenine (18)

Compound **17** (1.26 g, 1.13 mmol) was co-evaporated with pyridine (1 x), then N_2 was applied. Dry pyridine (5.6 mL) and 4,4'-dimethoxyltrityl chloride (DMTCl, 612 mg, 1.81 mmol) was added into the flask. 1 hour later, TLC showed incomplete conversion (DCM:methanol = 9.5:0.5 as eluent) and additional DMTCl (153 mg, 0.45 mmol) was added. The reaction was stirred for 1 hour after which it was cooled down to 0°C. Ac_2O (2.13 mL, 22.6 mmol) was added to the reaction flask. The mixture was stirred at 0°C for 5 h after which was quenched by aqueous saturated $NaHCO_3$. DCM extracted (3 x) the mixture and the organic layers were combined, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (pentane/acetone, 100/0 – 90/10 – 85/15 – 80/20 – 70/30) furnished **18** as a white foam (1.43 g, 0.90 mmol, 80%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.97 (s, 1H, NH), 8.67 (s, 1H, H2), 8.38 (s, 1H, H8), 8.03 – 7.94 (m, 2H, arom.), 7.68 – 7.56 (m, 9H, arom.), 7.56 – 7.48 (m, 2H, arom.), 7.45 – 7.17 (m, 21H, arom.), 6.82 – 6.75 (m, 4H, DMT), 6.27 (d, *J* = 4.3 Hz, 1H, H1'), 5.57 – 5.47 (m, 2H, H3', H3''), 5.42 (dd, *J* = 7.3, 3.3 Hz, 1H, H3'''), 5.35 (t, *J* = 4.9 Hz, 1H, H2'), 5.27 (d, *J* = 4.4 Hz, 1H, H1''),

5.25 (d, *J* = 4.4 Hz, 1H, H1’’’), 4.99 (dd, *J* = 7.3, 4.4 Hz, 1H, H2’’’), 4.41 (q, *J* = 4.3 Hz, 1H, H4’), 4.35 (dd, *J* = 6.8, 4.3 Hz, 1H, H2’’), 4.15 (q, *J* = 3.1 Hz, 1H, H4’’’), 4.10 (q, *J* = 2.9 Hz, 1H, H4’’), 3.85 – 3.65 (m, 10H, OMe DMT, H5’’’, H5’’), 3.64 – 3.53 (m, 2H, H5’), 3.46 (AB, *J* = 10.6, 4.5 Hz, 1H, H5’), 2.13 (s, 3H, CH_3 Ac), 2.08 (s, 6H, CH_3 Ac), 2.05 (s, 3H, CH_3 Ac), 1.03 (s, 10H, CH_3, TBDPS), 0.99 (s, 9H, CH_3 TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.59, 169.92, 169.72, 169.69 (CO Ac), 164.52 (CO Bz), 158.66, 158.64, 151.44, 149.62, 144.49 (Cq. arom.), 135.68, 135.65 (arom.), 135.58, 133.83, 133.03, 132.96, 132.85, 132.84 (Cq. arom.), 130.21, 130.14, 129.95, 129.92, 129.90, 128.96, 128.27, 127.99, 127.93, 127.90, 127.88, 127.85, 127.08 (arom.), 123.69 (Cq. arom.), 113.26 (arom.), 100.79 (C1’’), 99.15 (C1’’’), 88.41 (C1’), 86.75 (Cq. DMT), 83.19 (C4’’), 82.72 (C4’’’), 81.98 (C4’), 77.23 (C2’), 73.58 (C2’’), 71.92 (C3’), 71.67 (C2’’), 71.26 (C3’’), 69.91 (C3’’’), 63.79 (C5’’), 63.29 (C5’’’), 62.66 (C5’), 55.31 (OMe DMT), 26.86, 26.82 (CH_3 TBDPS), 21.08, 20.96, 20.82, 20.55 (CH_3 Ac), 19.29, 19.29 (Cq. TBDPS). IR (film): 2935, 1743, 1739, 1507, 1245, 1241, 1236, 1233, 1227, 1223, 1178, 1175, 1113, 1107, 1092, 1037, 1030, 703 cm^{-1}. HRMS (ESI^+) calcd for $C_{88}H_{96}N_5O_{19}Si_2$ (M+H) 1582.6311. Found 1582.6273. $[\alpha]_D^{20}$ +56.9 (c = 1, in $CHCl_3$)

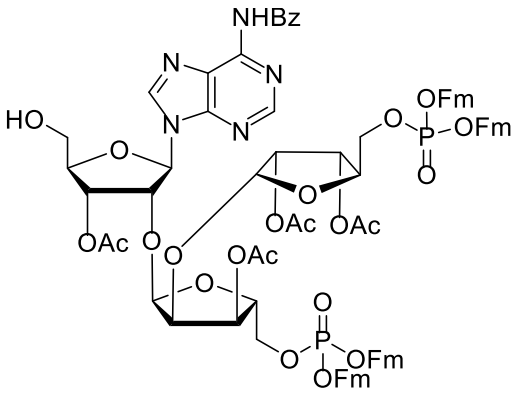


6-*N*-benzoyl-9-(3’,3’’2’’’,3’’’-tetra-*O*-acetyl-5’-*O*-dimethoxyltrityl-β-parotriosyl)adenine (19)

18 (1.43 g, 0.90 mmol), dry THF (9 mL) and TBAF (tetrabutylammonium fluoride solution 1.0 M in THF, 2.7 mL, 2.7 mmol) was added into a flask and the mixture was stirred for 16 hours at room temperature. Excess of EtOAc was added and the mixture was washed by H_2O (2 x) and brine (1 x). The organic layer was dried by $MgSO_4$. The mixture was filtered, concentrated under reduced pressure and purified by silica gel column chromatography (DCM/methanol, 100/0 – 100/1 – 100/2 – 100/3) to obtain **19** as a white foam (0.86 g, 0.78 mmol, 87%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.31 (s, 1H, NH), 8.61 (s, 1H, H2), 8.38 (s, 1H, H8), 8.08 – 7.99 (m, 2H, arom.), 7.63 – 7.55 (m, 1H, arom.), 7.55 – 7.47 (m, 2H, arom.), 7.45 – 7.40 (m, 2H, arom.), 7.36 – 7.28 (m, 4H, arom.), 7.28 – 7.18 (m, 3H, arom.), 6.79 (dd, *J* = 9.0, 3.0 Hz, 4H, DMT arom.), 6.24 (d, *J* = 5.6 Hz, 1H, H1’), 5.57 (dd, *J* = 5.4, 3.9 Hz, 1H, H3’), 5.45 (t, *J* = 5.5 Hz, 1H, H2’), 5.24 (dd, *J* = 7.3, 3.4 Hz, 1H, H3’’), 5.15 (dd, *J* = 7.3, 4.0 Hz, 1H, H3’’’), 5.11 (d, *J* = 4.3 Hz, 1H, H1’’), 5.03 (d, *J* = 4.4 Hz, 1H, H1’’’), 4.84 (dd, *J* = 7.3, 4.4 Hz, 1H, H2’’’), 4.37 (d, *J* = 4.0 Hz, 1H, H4’), 4.13 – 4.10 (m, 2H, H2’’, H4’’), 4.05 (q, *J* = 3.2 Hz, 1H, H4’’’), 3.81 – 3.61 (m, 10H, OMe DMT, H5’’, H5’’’), 3.56 (AB, *J* = 10.6, 3.9 Hz, 1H, H5’), 3.46 (AB, *J* = 10.6, 4.6 Hz, 1H, H5’), 3.07 (bs, 1H, OH), 2.53 (bs, 1H, OH), 2.14 (s, 3H, CH_3 Ac), 2.10 (s, 3H, CH_3 Ac), 2.08 (s, 3H, CH_3 Ac), 2.06 (s, 3H, CH_3 Ac). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.64, 170.16, 169.77, 169.73 (CO Ac), 165.07 (CO Bz), 158.64 (Cq. arom.), 152.75 (C2), 151.68, 149.74, 144.49, 135.65, 135.62, 133.55 (Cq. arom.), 132.95, 130.22, 130.19, 128.94, 128.28, 128.09, 127.97, 127.07 (arom.), 123.84 (Cq. arom), 113.24 (arom.), 101.14 (C1’’), 98.77 (C1’’’), 87.69 (C1’), 86.78 (Cq. arom.), 82.83 (C4’’), 82.56 (C4’’’), 82.39 (C4’), 76.93 (C2’), 73.04 (C2’’), 72.19 (C3’), 71.36 (C2’’’), 70.60 (C3’’), 69.74 (C3’’’), 62.97 (C5’), 62.24 (C5’’), 61.80 (C5’’’), 55.33 (OMe DMT), 21.03, 21.02, 20.77, 20.55 (CH_3 Ac).

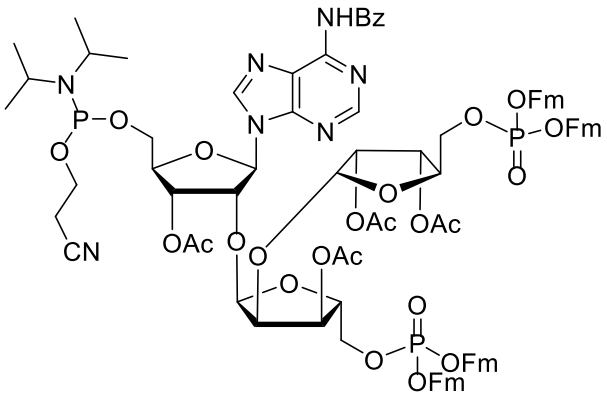
IR (film): 2935, 1739, 1734, 1730, 1609, 1607, 1583, 1507, 1456, 1448, 1369, 1238, 1227, 1224, 1176, 1090, 1030, 829, 734, 705 cm^{-1}. HRMS (ESI^+) calcd for $C_{56}H_{60}N_5O_{19}$ (M+H) 1106.3877. Found 1106.3896. $[\alpha]_D^{20}$ +68.0 (c = 1, in

$CHCl_3$)



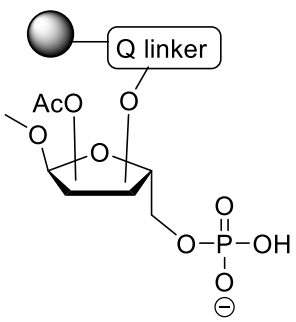
6-*N*-benzoyl-9-(3',3''2''',3'''-tetra-*O*-acetyl-5'',5'''-di-*O*-(di-fluorenyl)-β-parotriosyl)adenine (21)

Compound **19** (0.86 g, 0.78 mmol), DCI activator (4,5-dicyanoimidazole solution 0.25 M in ACN, 12.48 mL, 3.12 mmol) and freshly activated 3Å molecular sieves were added into flask. Compound **20** (0.2 M in ACN, 11.7 mL, 2.34 mmol) was added into the mixture and the reaction was stirred for 10 minutes at room temperature after which *tBu*OOH (5.5 M in decane, 1.42 mL, 7.80 mmol) was added at 0 ℃. The reaction was stirred at the same temperature for 45 minutes and quenched by aqueous saturated $NaHCO_3$. The mixture was filtered and excessive amount of EtOAc was added to the filtration. The organic layer was washed by H_2O (1 x) and brine (2 x) and was dried (Na_2SO_4). The mixture was filtered, concentrated under reduced pressure and co-evaporated with toluene (3 x). To the residue, DCM (10.4 mL) and TFA (0.15 mL, 1.95 mmol) were added and the reaction was stirred for 10 minutes at room temperature after which was quenched aqueous saturated $NaHCO_3$. DCM extracted (2 x) the mixture and the organic layers were combined and washed by H_2O (1 x) and brine (1 x). The organic layers were dried ($NaSO_4$), filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (DCM/methanol, 100/0 – 100/1 – 100/2 – 100/3) to obtain **21** as a white foam (1.01 g, 0.60 mmol, 77%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.07 (s, 1H, NH), 8.78 (s, 1H, H2), 8.55 (s, 1H, H8), 7.95 – 7.89 (m, 2H, arom.), 7.72 – 7.61 (m, 8H, arom.), 7.56 – 7.51 (m, 1H, arom.), 7.48 – 7.14 (m, 26H), 6.24 (dd, *J* = 11.9, 2.4 Hz, 1H, OH), 6.12 (d, *J* = 8.0 Hz, 1H, H1'), 5.66 (d, *J* = 5.4 Hz, 1H, H3'), 5.15 (dd, *J* = 8.0, 5.4 Hz, 1H, H2'), 5.10 – 5.06 (m, 2H, H3'', H3'''), 4.94 (d, *J* = 4.4 Hz, 1H, H1''), 4.84 (dd, *J* = 7.7, 4.4 Hz, 1H, H2''), 4.70 (d, *J* = 4.3 Hz, 1H, H1'''), 4.31 – 3.80 (m, 22H, CH/CH2 Fm, H4', H2''', H4'', H4''', H5', H5'', H5'''), 2.19 (s, 3H, CH_3 Ac), 2.14 (s, 3H, CH_3 Ac), 2.08 (s, 3H, CH_3 Ac), 2.05 (s, 3H, CH_3 Ac). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.21, 169.82, 169.55, 169.46 (CO Ac), 164.41 (CO Bz), 152.31 (C2), 150.59, 150.36 (Cq. arom.), 143.11, 143.07, 143.00, 142.91, 141.43, 141.39, 133.50 (Cq. arom.), 132.87, 128.89, 127.95, 127.20, 127.17, 127.15, 127.13, 125.16, 125.13, 125.10 (arom.), 124.61 (Cq. arom.), 120.11, 120.08, 120.05 (arom.), 101.26 (C1'''), 98.15 (C1''), 89.28 (C1'), 86.88 (C4'), 79.97, 79.90 (C4'''), 79.50, 79.44 (C4''), 77.69 (C2'), 74.23 (C3'), 71.45 (C2'''), 71.08 (C2'), 69.85 (C3'''), 69.48, 69.43, 69.37 (CH_2 Fm), 69.06 (C3''), 66.32, 66.29 (C5''), 66.09, 66.05 (C5'''), 62.95 (C5'), 47.95, 47.93, 47.89, 47.87, 47.83 (CH Fm), 21.15, 20.81, 20.69, 20.65 (CH_3 Ac). ^{31}P NMR (202 MHz, $CDCl_3$) δ -1.15, -1.20. IR (film): 2931, 1743, 1739, 1582, 1451, 1448, 1238, 1234, 1103, 1067, 1016, 991, 759, 740 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{91}H_{84}N_5O_{23}P_2$ (M+H) 1676.5027. Found 1676.5161. $[\alpha]_D^{20}$ +38.3 (c = 1, in $CHCl_3$)



6-*N*-benzoyl-9-(3',3''2''',3'''-tetra-*O*-acetyl-5'-*O*-(*N*,*N*-diisopropylamino-*O*-cyanoethyl)phosphoramidite)-5'',5'''-di-*O*-(di-flourenyl)-β-parotriosyl)adenine (23)

Compound **21** (1.01 g, 0.60 mmol), DMF (6 mL), DIPEA (0.21 mL, 1.2 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **22** (0.15 mL, 0.66 mmol) were added into the flask under N_2. The reaction was stirred at room temperature for 10 minutes after which was quenched by 0.3 mL methanol. Excessive amount of EtOAc was added and the mixture was washed with aqueous saturated $NaHCO_3$ (2 x), H_2O (1 x) and brine (1 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 (DCM/acetone, 100/0 – 90/10 – 80/20) to furnish **23** as a white foam (684 mg, 0.36 mmol, 60%). **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 9.39 – 9.15 (m, 1H, NH), 8.72 (d, *J* = 7.4 Hz, 1H, H2), 8.50 (d, *J* = 10.7 Hz, 1H, H8), 8.01 (d, *J* = 7.7 Hz, 2H, arom.), 7.68 – 7.13 (m, 35H), 6.24 (dd, *J* = 9.8, 4.8 Hz, 1H, H1'), 5.49 (dt, *J* = 15.0, 5.0 Hz, 1H, H3'), 5.20 (ddt, *J* = 13.5, 7.6, 3.9 Hz, 3H, H1'', H2', H3''), 5.14 – 5.05 (m, 2H, H1''', H3'''), 4.79 (td, *J* = 7.8, 4.4 Hz, 1H, H2'''), 4.40 (q, *J* = 4.1 Hz, 1H, H4'), 4.41 – 3.75 (m, 23H, CH_2/CH Fm, H4'', H4''', H2'', H5', H5'', H5''', O*C*H_2CH_2CN), 3.59 (dddd, *J* = 13.7, 9.5, 6.8, 2.5 Hz, 2H, $(CH_3)_2$*CH*), 2.72 (td, *J* = 6.2, 2.7 Hz, 1H, C*H*HCN), 2.66 (t, *J* = 6.3 Hz, 1H, C*H*HCN), 2.59 (dq, *J* = 6.2, 3.7, 3.0 Hz, 1H, C*H*HCN), 2.15 – 2.06 (m, 12H, CH_3 Ac), 1.22 – 1.10 (m, 12H, *(*CH_3*)*$_2$CHN). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.16, 170.15, 169.54, 169.52, 169.51, 169.47, 169.27, 169.23 (CO Ac), 164.62, 164.59 (CO Bz), 151.53, 151.43, 149.67, 142.99, 142.98, 142.95, 142.88, 142.87, 142.81, 141.27, 141.25, 141.24, 133.60, 132.58, 128.68, 128.66, 127.93, 127.84, 127.79, 127.04, 125.04, 125.02, 124.99, 124.94 (arom.), 123.82, 123.77 (Cq. arom.), 119.98, 119.97, 119.92, 119.88 (arom.), 117.86, 117.74 (CN), 100.70 (C1''), 98.80 (C1'''), 87.77, 87.48 (C1'), 82.25, 82.18, 82.07, 82.00 (C4'), 80.41, 80.35 (C4''), 80.30, 80.23 (C4'''), 77.64, 77.59 (C2'), 72.67 (C2''), 71.62, 71.52 (C3'), 70.81, 70.76 (C2'''), 69.84, 69.81 (C3''), 69.29, 69.24 (CH_2 Fm), 69.11 (C3'''), 66.41, 66.40, 66.37 (C5''), 66.19, 66.15 (C5'''), 62.13, 62.00, 61.87, 61.75 (C5'), 58.67, 58.62, 58.51, 58.45, 58.13, 58.08 (O*C*H_2CH_2CN), 47.81, 47.74 (CH Fm), 43.15, 43.12, 43.06, 43.02 ($(CH_3)_2$*CH*N), 24.65, 24.62, 24.59, 24.57, 24.54, 24.52 (*(*CH_3*)*$_2$CHN), 20.82, 20.76, 20.72, 20.49 (CH_3 Ac), 20.35, 20.30 (*C*H_2CN), 20.27 (CH_3 Ac). ^{31}P NMR (202 MHz, $CDCl_3$) δ 149.11, 148.96, 14.18 (H-phosphonate), -1.60, -1.62, -1.70, -1.73. IR (film): 2969, 1743, 1698, 1609, 1581, 1511, 1451, 1367, 1238, 1158, 1017, 984, 759, 742 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{94}H_{87}N_6O_{25}P_3$ ([H-phosphonate]+H) 1793.5007. Found 1793.5032. $[\alpha]_D^{20}$ +34.8 (c = 1, in DCM)



1-*O*-methyl-2-*O*-Q-Tentagel-3-*O*-acetyl-5-*O*-phosphate-α-D-ribofuranoside/1-*O*-methyl-2-*O*-acetyl-3-*O*-Q-Tentagel-5-*O*-phosphate-α-D-ribofuranoside (2a)

200 mg resin **2** 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 traces of water before use to furnish resin **2a**.

1-*O*-Methyl-α-branched core oligo-ADPr (1) and 1-*O*-methyl-α-mono ADPr (25)

50 mg (10 μmol) resin **2a** was transferred into a reaction column of a Mermade 6 oligonucleotide synthesizer and the complete synthesis was performed under an argon atmosphere.

Cycle A was performed 1 time and Cycle B was performed 1 time.

Cycle A:

The resin was rinsed with ACN (3 x) and drained. 5-(Benzylthio)-1*H*-tetrazole (BTT) (480 uL, 0.25 M in ACN) and **23** (400 uL, 0.1 M 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 with ACN (3 x). 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). 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) after which was drained and washed with ACN (3 x).

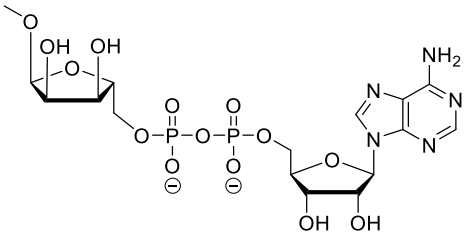
Cycle B:

The resin was rinsed with ACN (3 x) and drained. BTT (480 uL, 0.25 M in ACN) and **4** (400 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 another 3 times. The resin was rinsed by with ACN (3 x). 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). 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 after which was drained and washed with ACN (3 x).

After cycle A and B, the resin was transferred to a tube and treated with 10 mL NH_4OH (35%). The tube was sealed and stirred overnight, filtered and concentrated under reduced pressure. The crude was purified by anion exchange to obtain branched core oligomer **1** 0.68 mg (0.43 mmol, 4%) and mono-ADPr **25** 2.83 mg (4.94 mmol, 50%) as white solid.

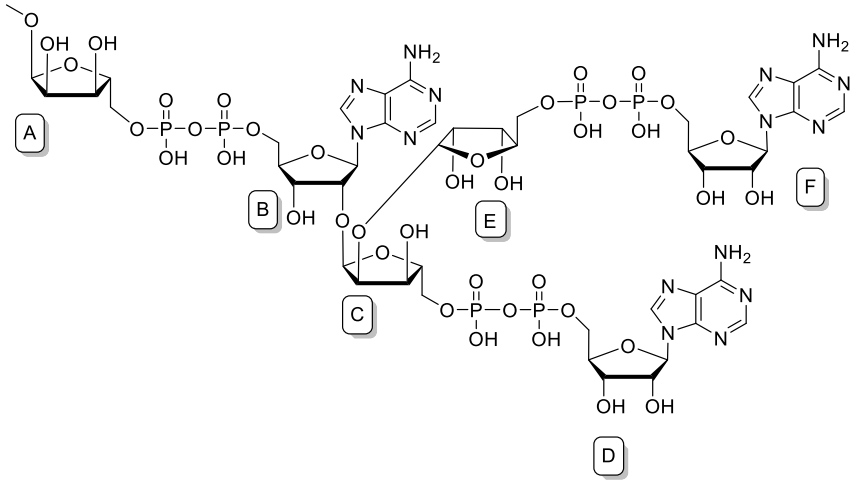
Column: Resource Q 6mL.

Gradient: 25% - 75%. (A: 10 mM NH_4OAc, B: 1 M NH_4OAc)



1-*O*-methyl-α-ADP-riboside (25)

^{1}H NMR (850 MHz, Deuterium Oxide) δ 8.53 (s, 1H, H2), 8.28 (s, 1H, H8), 6.15 (d, *J* = 5.9 Hz, 1H, H1'), 4.54 (d, *J* = 4.5 Hz, 2H, H3'), 4.40 (d, *J* = 2.9 Hz, 1H, H4'), 4.23 (t, *J* = 4.0 Hz, 2H, H5'), 4.18 (s, 1H, H4''), 4.13 – 4.12 (m, 2H, H2'', H3''), 4.01 (t, *J* = 4.7 Hz, 2H, H5''), 3.38 (s, 3H, OMe). ^{13}C NMR (214 MHz, D_2O) δ 155.72 (C4), 152.95 (C8), 149.21 (C6), 118.70 (C5), 103.26 (C1''), 86.83 (C1'), 83.99, 83.95 (C4'), 83.23, 83.18 (C4''), 74.27 (C2'), 70.83 (C2''), 70.43 (C3'), 69.68 (C3''), 65.62, 65.60 (C5''), 65.22, 65.20 (C5'), 55.46 (OMe). ^{31}P NMR (202 MHz, D_2O) δ -10.47, -10.57, -10.68, -10.78. LC-MS: Rt = 3.57 min. 0-50% NH_4OAc. ESI MS+ calc. 574.1 found 574.1 $[M+1]^+$. HRMS (ESI^+) calcd for $C_{16}H_{26}N_5O_{14}P_2$ (M+H) 574.0946. Found 574.0949.



1-*O*-Methyl-α-branched tri-ADP riboside (1)

^{1}H NMR (850 MHz, Deuterium Oxide) δ 8.35 (s, 2H, H2), 8.20 (s, 1H, H2), 8.06 – 8.05 (m, 2H, H8), 7.99 (s, 1H, H8), 6.01 (d, *J* = 4.6 Hz, 1H, H1-B), 5.92 (d, *J* = 11.4 Hz, 2H, H1-DF), 5.13 – 5.06 (m, 1H, H1-C), 4.94 (d, *J* = 4.3 Hz, 1H, H1-E), 4.83 (t, *J* = 2.3 Hz, 1H, H1-A), 4.60 (t, *J* = 5.3 Hz, 1H, H2-D), 4.58 – 4.56 (m, 2H, H2-BF), 4.46 (t, *J* = 5.0 Hz, 1H, H3-B), 4.42 (t, *J* = 4.5 Hz, 1H, H3-F), 4.39 (s, 1H, H3-D), 4.30 – 3.93 (m, 24H, the rest H2, H3, H5), 3.30 (s, 3H, OMe). ^{13}C NMR (214 MHz, D_2O) δ 119.06, 119.05 (C5), 104.15 (C1-A), 101.98 (C1-E), 101.08 (C1-C), 88.07 (C1-D), 87.91 (C1-F), 87.25 (C1-B), 85.04, 84.76, 84.69, 84.67, 84.09, 84.06 (C4-ABDCEF), 80.02, 80.00, 75.61, 75.44, 75.34, 72.44, 71.72, 71.30, 71.14, 70.56, 70.53, 70.48, 70.26 (C2, C3-ABCDEF), 66.52, 66.39, 66.33, 66.18, 66.09, 65.67 (C5-ABDCEF), 56.36 (OMe). ^{31}P NMR (202 MHz, D_2O) δ -11.04, -11.11, -11.14, -11.17, -11.19, -11.24, -11.33, -11.43. LC-MS: Rt = 3.54 min. 0-50% NH_4OAc. ESI MS+ calc. 1656.2 found 1656.3 $[M+1]^+$.
HRMS (ESI^+) calcd for $C_{46}H_{68}N_{15}O_{40}P_6$ (M+H) 1656.2168. Found 1656.2171.

Procedure for direct coupling between **2a** and **4 :**

50 mg (10 µmol) resin **2a** was transferred into a reaction column of a Mermade 6 oligonucleotide synthesizer

and the complete synthesis was performed under an argon atmosphere. Cycle B was performed 1 time. Then 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 under reduced pressure. The crude was analyzed by LC-MS and ^{31}P-NMR to confirm the complete conversion from **2a** to **25**.

Supporting Data:

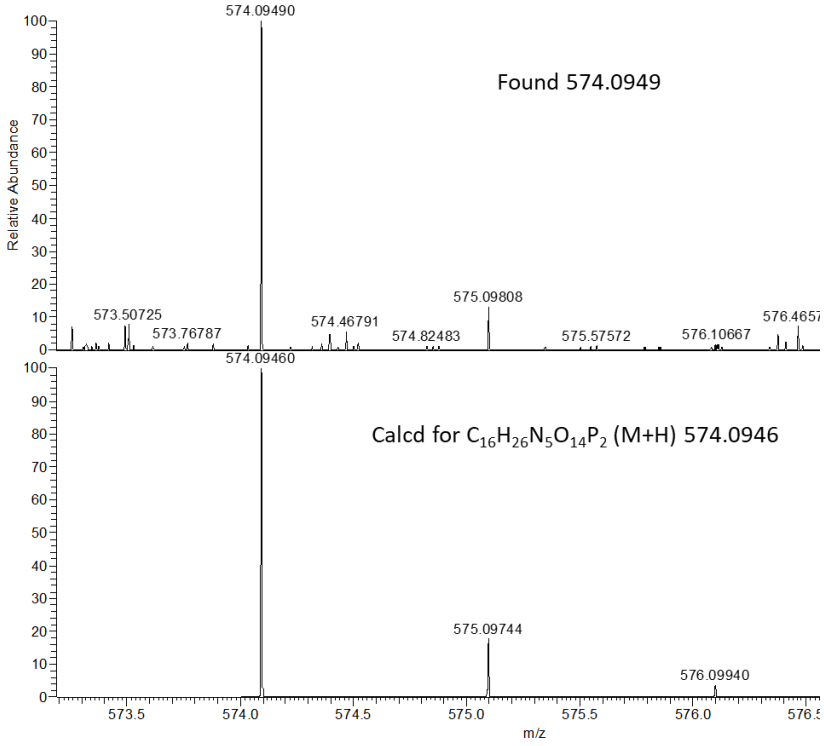


Figure 1S. HRMS data of mono ADPr **25**

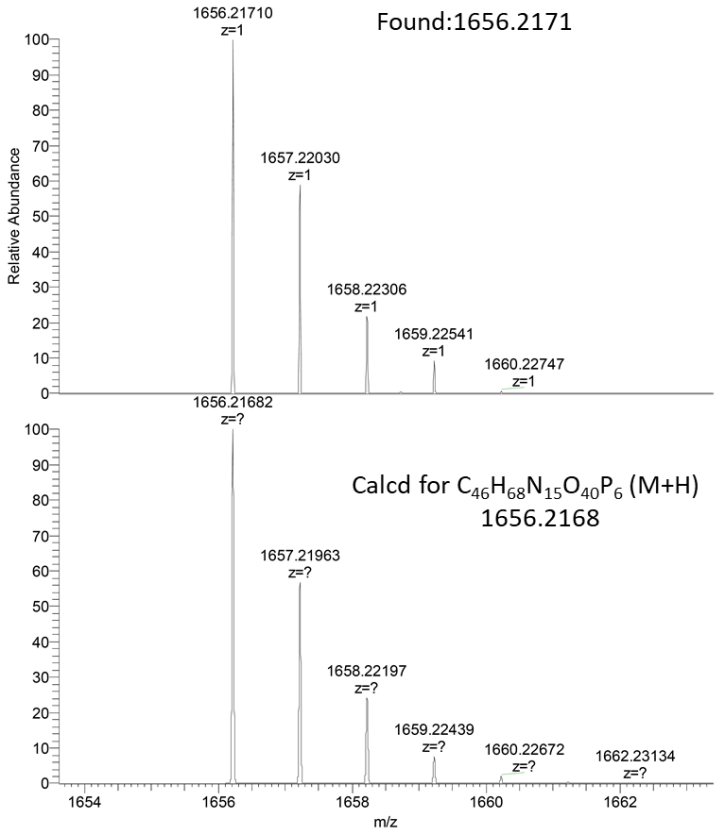


Figure 2S. HRMS data of branched tri-ADPr **1**

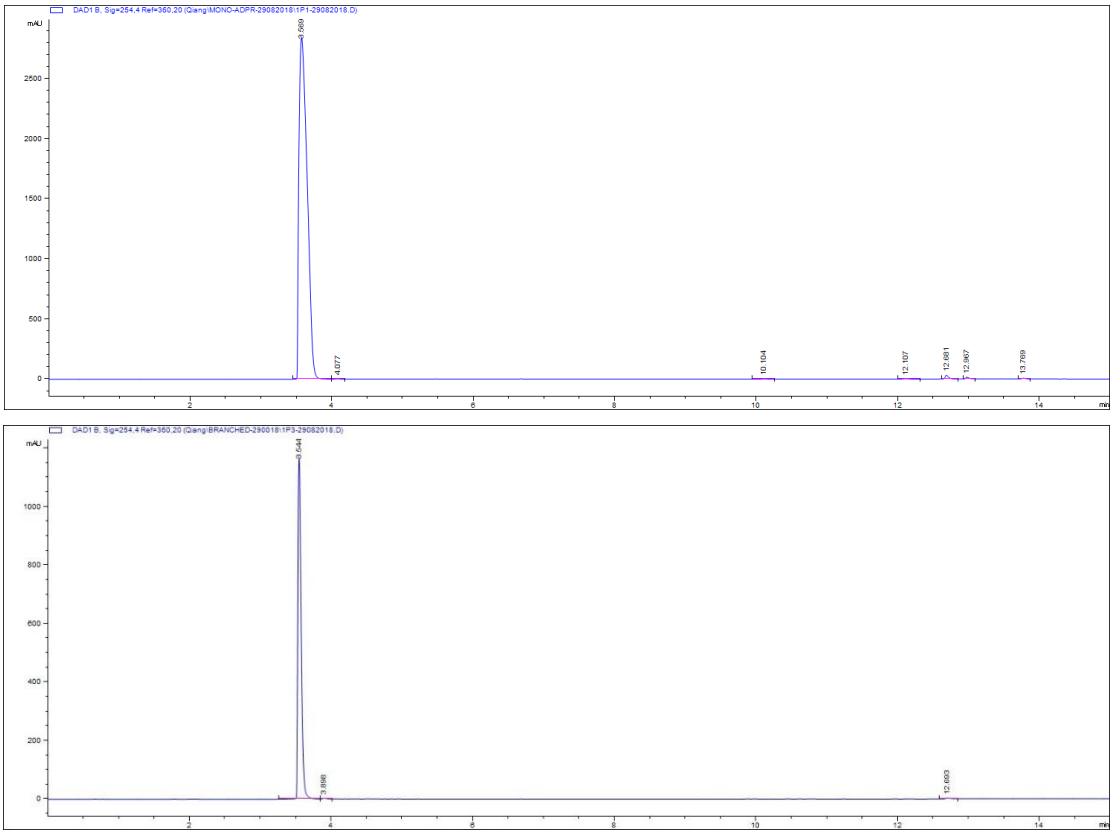


Figure 3S. UV spectra of LC-MS analysis of ADP-ribose **25** (upper) and branched ADPr **1** (lower)

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