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Branching of poly(ADP-ribose): Synthesis of the Core Motif

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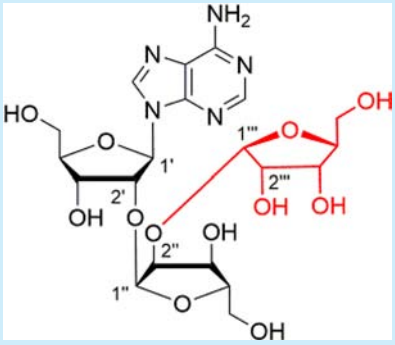
Supporting Information

ABSTRACT: The synthesis of the core motif of branched poly(adenosine diphosphate ribose) (poly(ADPr)) is described, and structural analysis reasserted the proposed stereochemistry for branching. For the synthesis, a ribose trisaccharide was first constructed with only α-*O*-glycosidic linkages. Finally, the adenine nucleobase was introduced via a Vorbrüggen-type glycosylation reaction. The orthogonality of the selected protecting groups was demonstrated, allowing for the construction of branched poly(ADPr) oligomers in the near future.



Poly(adenosine diphosphate ribose) (poly(ADPr)) is an intriguing biopolymer which is synthesized by poly(ADPr) polymerases (PARPs) for modifying various proteins and enzymes. This reversible post-translational modification (PTM) is associated with a wide variety of biological processes like DNA damage repair, mitosis, apoptosis, and transcription.[1] The first ADPr moiety was enzymatically transferred to an acceptor protein by a nucleophilic attack of the side chain of a specific amino acid (Glu, Asp, Asn, Arg) on nicotinamide adenine dinucleotide (NAD^+), thereby releasing nicotinamide. The resulting mono-ADPribosylated protein is the starting point for PARP enzymes to form *poly*-ADPr chains. PARP enzymes elongate the chains to over 200 units in size and also branch the polymer after every 20−50 elongation reactions (Figure 1).[2] In the linear part the characteristic pyrophosphates in the poly(ADPr) chain link the 5′-OH of adenosine with the 5′-OH of ribose, while the 2′-OH of adenosine is connected via an unique α *O*-glycosidic bond with ribose. At the branching point, the ribose moiety in the chain becomes trifunctionalized by linking its 2′-OH via an α-*O*-glycosidic linkage with another ribose.[2a,h]

Although the involvement of ADPribosylation in many cellular processes has become clear over the past decades, the mechanism of action and mode of interaction at a molecular level remains elusive. So far, the crystal structures of relevant enzymes have been studied with the use of enzymatically prepared linear ADPr fragments[3] and, recently, the first example of a synthetic ADPr dimer.[4] On the contrary, information on the manner of binding of branched ADPr is scarce. However, on the basis of the information obtained from crystal structure studies with poly(ADPribose) glycohydrolase (PARG) and fractionated poly(ADPr), it is suggested that branched ADPr cannot bind with the enzymes that recognize linear ADPr.[3b] Therefore, it is not excluded that there are unknown enzymes that specifically recognize these branched ADPr structures. Isolation of well-defined branched poly(ADPr) fragments has not been reported yet because of the limited number and the random positions of branching points in ADPr chains. In addition, enzymatic preparation followed by multiple chromatographic purifications produced nonhomogenous poly(ADPr) fragments.[2d,5] Recently, the first chemical syntheses of well-defined ADPr-related model compounds were reported.[4,6] Apart from the synthesis of mono-ADPribosylated oligopeptides,[7] we reported the synthesis of dimeric and trimeric ADPr-fragments in milligram amounts, suitable for detailed molecular studies.[6] As part of a program toward the synthesis and evaluation of structurally defined poly(ADPr) fragments, we here present the first synthesis of 2′-*O*-((2″-*O*-(α-D-ribofuranosyl)-α-D-ribofuranosyl)-β-D-ribofuranosyladenosine (**10**), the branching point in poly(ADPr) (Scheme 1).

Target **10** can be considered as a hyperglycosylated nucleoside, a structural element that is also found in a variety of biomolecules such as initiator t-RNA, complex nucleoside antibiotics, and neuroactive spider toxins.[8] The synthesis of this class of molecules presents a challenge as O-glycosylation of a protected nucleoside is accompanied by nearly unavoidable side reactions with nucleophilic positions in the nucleobase. In addition, en route to **10**, α-ribosyl linkages need to be installed. Two general routes for the synthesis of glycosylated nucleosides can be considered. In the first route, a suitably protected nucleoside is *O*-glycosylated, while in the second route introduction of the heterocyclic nucleobase is the final glycosylation event. Most of the reported syntheses to α-2′-*O*-ribosylated adenosine rely on the ribosylation of a suitably protected adenosine derivative.[4,8a,9] The approach to α-2′-*O*-ribosylated adenosine we reported proved to be inefficient during the scaling up procedure, in particular, for the O-glycosylation of adenosine. Therefore, we devised a new route

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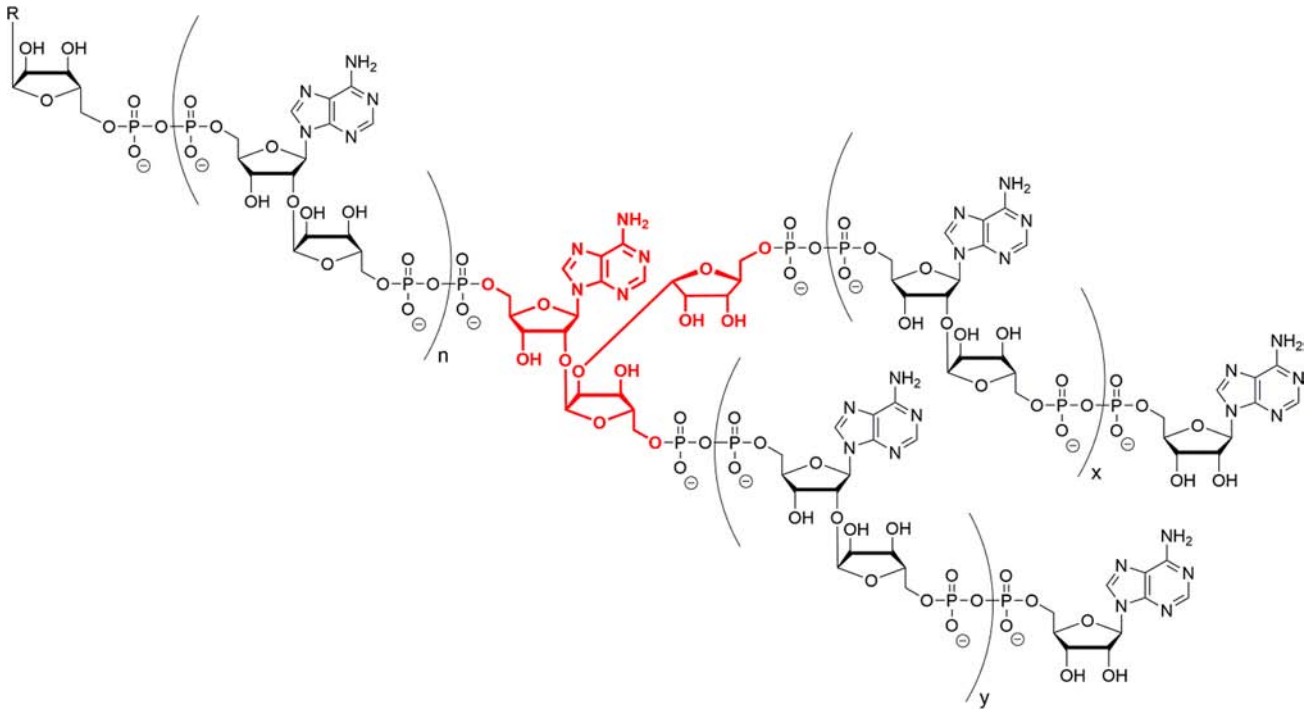
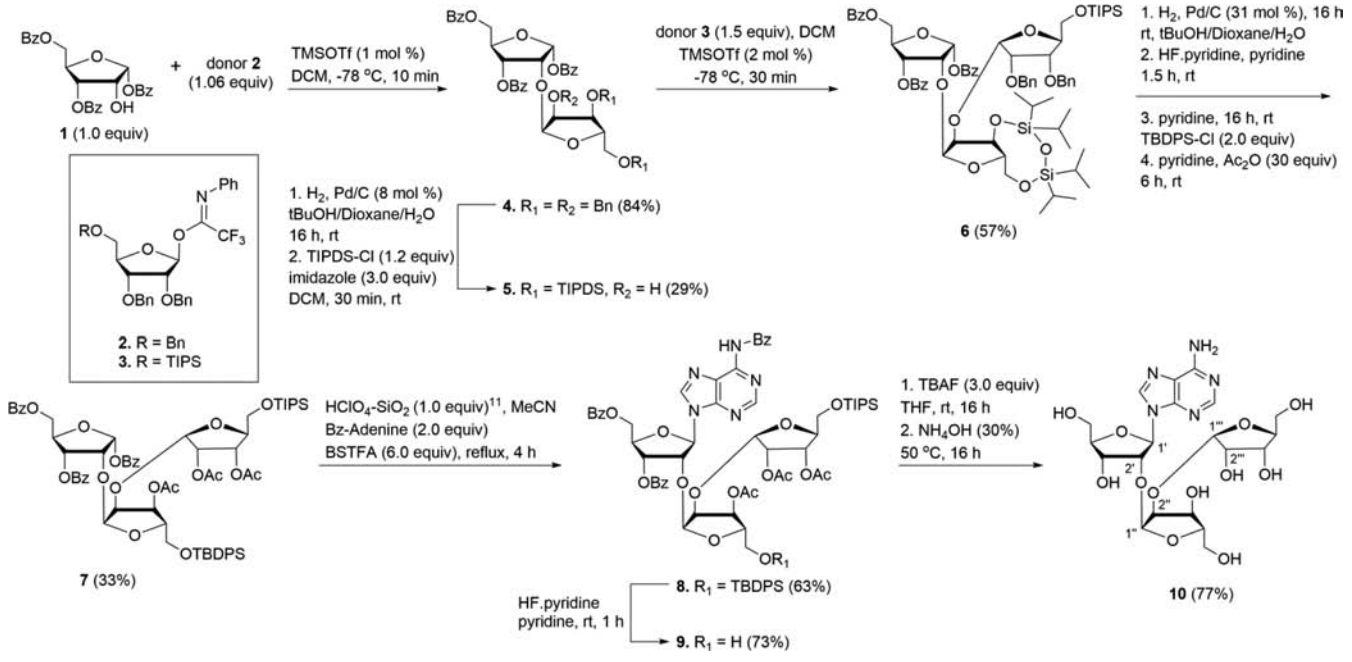


Figure 1. Schematic representation of branched poly(ADPr) with the core motif of branching depicted in red.

Scheme 1. Synthesis of 2′-*O*-((2″-*O*-(α-D-Ribofuranosyl)-α-D-ribofuranosyl)-β-D-ribofuranosyladenosine (10)



by initially forming a ribose disaccharide and introducing the adenine base at the anomeric center in a later stage of the synthesis.[6] This new route afforded α-2′-*O*-ribosylated adenosine in sufficient quantities and ultimately allowed the synthesis of linear ADPr-oligomers.

Guided by these results, we set out to assemble target compound **10** by postponing the crucial introduction of the adenine base at the trisaccharide stage of the synthesis (Scheme 1). The required ribose trisaccharide **7** is orthogonally protected and possesses only *cis*-glycosidic linkages. Although α-configured C-ribofuranoside linkages can be installed stereoselectively, introduction of similar O-glycosidic linkages usually require optimization experiments to attain acceptable stereoselectivity. First, α-configured ribose disaccharide **4** was obtained by TMSOTf-mediated condensation of commercially available tribenzoylribose **1** with known *N*-phenyltrifluoroacetimidate donor **2**.[9a] The glycosylation reaction was extremely fast (<1 min) with just a minimal amount of TMSOTf (1 mol %) and high yielding (84%), while the corresponding β-disaccharide could not be detected. In order to stereoselectively introduce the next ribose moiety at the 2″-OH in dimer **4**, the benzyl ethers had to be removed first. Unexpectedly,

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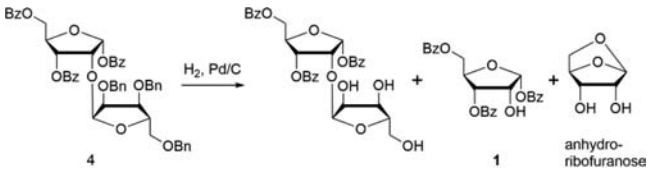
hydrogenolysis of the benzyl groups in **4** using catalytic amounts of Pd/C was accompanied by a significant degree of glycosidic bond cleavage. Apparently, besides the formation of the debenzylated dimer, an intramolecular side reaction occurred that resulted in a mixture of 1,5-anhydroribofuranose and starting compound **1** (Scheme 2). Treatment of the crude

Scheme 2. Products Formed during Hydrogenolysis of 4



mixture with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS) chloride yielded a sufficient amount of ribose disaccharide **5** (2 mmol). Alternatively, disaccharide **5** could uneventfully be obtained by our previously reported condensation of triisopropylsilyl (TIPS)-protected donor **3** with acceptor **1**.[6] Subsequent hydrogenation kept the TIPS group in place while glycosidic bond cleavage could not be detected. Potentially, removal of the TIPS group and installment of the TIPDS moiety should give **5** in higher yields.

The next glycosylation event, in which disaccharide acceptor **5** was reacted with TIPS donor **3**, proceeded in a complete α-selective manner to give trisaccharide **6** in 57% yield. At this stage, the removal of the benzyl-protecting groups was undertaken as we previously experienced that the presence of an adenine moiety complicates hydrogenolysis. Subjection of trisaccharide **6** to Pd/C-catalyzed hydrogenation not only removed the benzyl ethers but was also accompanied by partial opening of the TIPDS ring at the primary position. This drawback was negated by treatment of the crude mixture with HF·pyridine to completely remove the TIPDS while leaving the TIPS intact. Introduction of a TBDPS at the 5″-OH and finally acetylation of the secondary hydroxyl functionalities gave trisaccharide **7**. With building block **7** available, the crucial introduction of the adenine base by a Vorbrüggen glycosylation reaction was undertaken. Heating a mixture of trisaccharide **7** with N^6-benzoyladenine, $HClO_4$–SiO_2,[10,11] and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in acetonitrile gave fully protected2′-*O*-((2″-*O*-(α-D-ribofuranosyl)-α-D-ribofuranosyl)-β-D-ribofuranosyladenosine derivative (**8**) in a β-selective manner and in a yield of 63%. UV–vis spectroscopic analysis of the main product confirmed that adenine was correctly linked via its N9 position to the anomeric center, while the minor byproducts could be assigned to adenosine derivatives with incorrect N1, N3, and/or N7 linkages.[12] Compound **8** is orthogonally protected to allow complete control over the elongation reactions in future poly(ADPr) syntheses. To demonstrate the orthogonality, we selectively cleaved the TBDPS group without affecting the TIPS group to give compound **9** with a free hydroxyl group. Finally, all the protecting groups in **8** were removed by sequential treatment with TBAF and NH_4OH to afford target **10**, the core motif of branched ADPr.

Compound **10** was carefully analyzed with all relevant 1D and 2D NMR spectroscopic methods, and all the H and C atoms could be assigned (Figure 2, 6.20 (H1′), 5.25 (H1″), 4.96 (H1‴)). The anomeric configuration of the ribosyl residues was ascertained by measuring the value of J_{H1C2} using HSQC-HECADE experiments for compounds **6** and **8** (see the Supporting Information).[13] Having thus obtained the deprotected branched nucleoside of ADPr, we felt that a comparison of the spectroscopic data of our synthetic material with a compound isolated from natural sources was in order. The only available spectroscopic information on branched ADPr has been reported by Miwa and co-workers.[2a] They performed an enzymatic synthesis to generate poly(ADPr), purified the material, treated it with snake venom phosphodiesterase to hydrolyze all the pyrophosphate linkages, and finally fractionated the mixture using a weak anion-exchange column.[2h] By doing so, they isolated 2′-ribosyladenosine-5′,5″-bis-phosphate (Ado(P)-Rib-P) as the major product and a unknown minor byproduct which was later identified to be 2′-*O*-((2″-*O*-(α-D-ribofuranosyl)-α-D-ribofuranosyl)-β-D-ribofuranosyladenosine 5′,5″,5‴-*O*-tri-phosphate (Ado(P)-Rib-(P)-Rib-P). The observed ^{1}H NMR shifts for the anomeric protons (6.41 (H1′), 5.45 (H1″), and 5.21 (H1‴)) by Miwa and co-workers show the same relative shifts as we observed for compound **10**. The ^{1}H NMR shifts for compound **10** are, however, uniformly shifted approximately 0.2 ppm upfield because of the lack of phosphate monoesters at the primary hydroxyls.

In conclusion, we have successfully synthesized the core structure of branched poly(ADPr) with two ribose residues having α-glycosidic linkages via the preparation of an

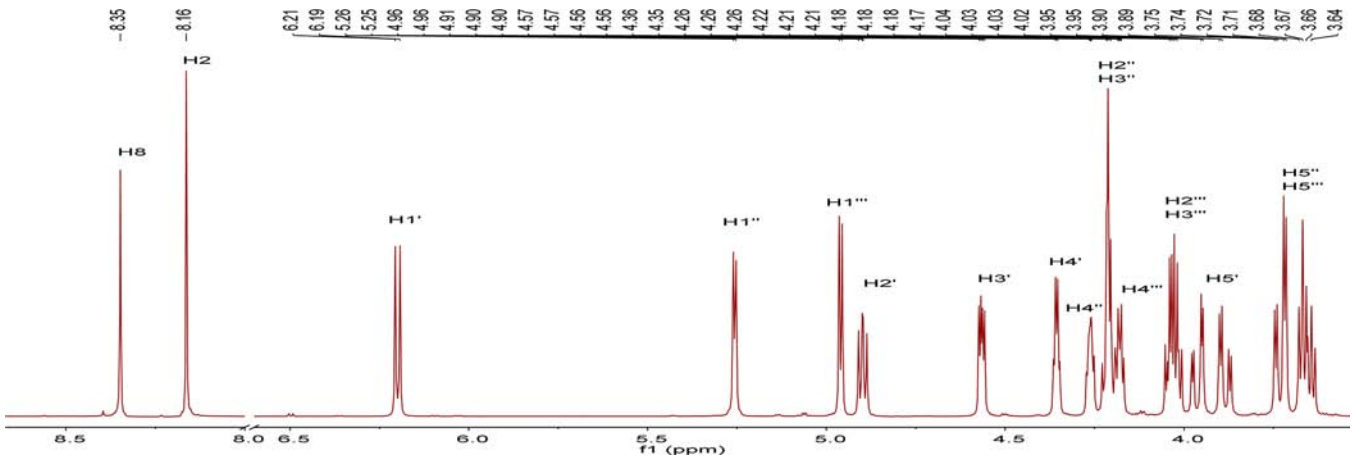


Figure 2. ^{1}H NMR spectrum compound **10** (500 MHz, D_2O).

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orthogonally protected trisaccharide and ensuing a Vorbrüggen glycosylation reaction for the regio- and stereoselective introduction of the adenine base. Spectroscopic analysis of core structure **10** supports the reported structure of the isolated naturally occurring poly(ADPr). The obtained result in combination with previously reported synthesis for linear ADPr oligomers is a basis to undertake the synthesis of branched poly(ADPr) oligomers. In order to obtain the required building block for the synthesis of branched poly-ADPr oligomers, the previously reported protecting group strategy will be applied to compound **8** as part of ongoing research.[6] The presented methodology is also a valuable asset for future syntheses of other glycosylated nucleosides, such as complex nucleoside antibiotics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02143.

Experimental procedures, ^{1}H, ^{13}C, and 2D NMR spectra and characterization of all compounds (PDF)

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Notes

The authors declare no competing financial interest.

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