Solid-Phase Synthesis

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Synthesis of Well-Defined Adenosine Diphosphate Ribose Oligomers**

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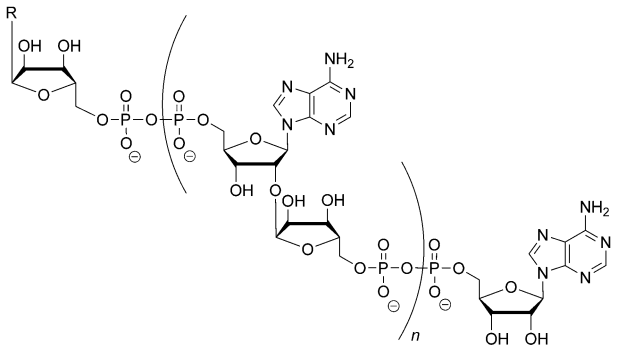
Abstract: *The post-translational modification of proteins that is known as adenosine diphosphate ribosylation (ADPr) regulates a wide variety of important biological processes, such as DNA-damage repair and cellular metabolism. This modification is also involved in carcinogenesis and the process of aging. Therefore, a better understanding of the function of ADP-ribosylation is crucial for the development of novel therapeutics. To facilitate the elucidation of the biology of ADPr, the availability of well-defined fragments of poly(ADP-ribose) is essential. Herein we report a solid-phase synthetic approach for the preparation of ADP-ribose oligomers of exactly defined length. The methodology is exemplified by the first reported synthesis of an ADP-ribose dimer and trimer.*

Poly-ADP-ribosylation (PARylation) is a post-translational modification in which numerous ADP-ribose molecules are transferred to an acceptor protein to form a poly(ADP-ribose) (PAR) polymer (Scheme 1). The size of the PAR chains can reach 400 units, and branching at the 2′-hydroxy group of the ribose moiety occurs once every 20 to 50 units.[1]



Scheme 1. Structure of linear PAR oligomers.

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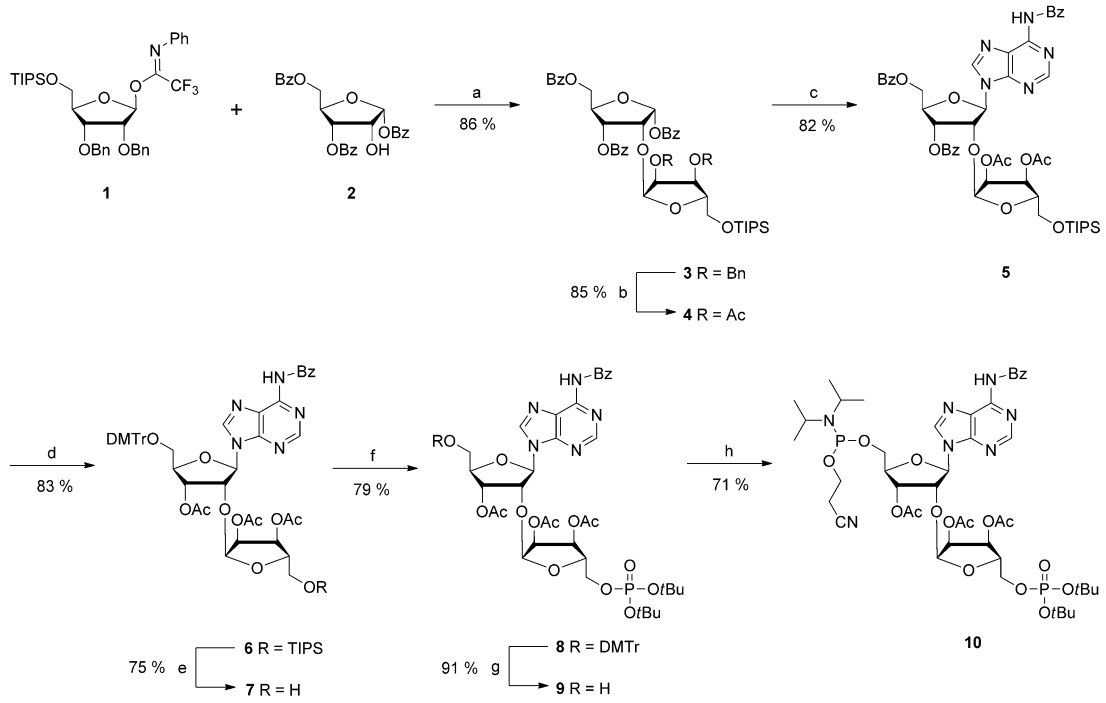
PAR polymers are associated with a wide variety of biological processes, including DNA-damage repair, mitosis, apoptosis, and transcription.[2] Elucidation of these processes at a molecular level requires the availability of sufficient amounts of structurally well defined PAR oligomers. PAR polymers and oligomers are currently prepared enzymatically, which leads to nonhomogenous products with a broad distribution in terms of their chain length and branching points. PAR fragments of some homogeneity can be obtained only by multiple chromatographic purification steps.[3] We thus considered the development of a completely chemical synthetic procedure as the most promising, and perhaps only, approach to the preparation of sufficient amounts of well-defined PAR oligomers for biological and structural studies.

For the development of an efficient synthetic route to PAR oligomers, several hurdles have to be overcome. The first requirement is a *cis*-selective (α-selective) ribosylation of adenosine in which the primary hydroxy functionalities of the reacting partners are orthogonally protected. Mikhailov et al. reported the first synthesis of α-linked 2-*O*-ribosyladenosine; however, orthogonality in the protecting-group pattern was lacking.[4a] Our research group devised a synthetic route to an α-linked 2-*O*-ribosyladenosine building block with the desired orthogonal protection.[4b] However, scaling up of this process proved to be unsatisfactory. A new route is presented herein. In this route, a Vorbrüggen-type reaction[5] is used to synthesize orthogonally protected 2-*O*-ribosylated adenosine on a relatively large scale (4 mmol).

The next challenge in synthesizing PAR oligomers is the introduction of pyrophosphate linkages in a molecule already containing one or more pyrophosphate bonds. The only reported method for such a transformation proved to be unsuccessful in our hands.[6] We therefore built upon our phosphoramidite procedure for the solution synthesis of sugar nucleotide pyrophosphates.[7] We anticipated that the synthesis of a pyrophosphate in the presence of preexisting pyrophosphate(s) would be difficult in solution. The adaptation of this method for pyrophosphate formation to a solid-phase procedure was plausible, as exemplified by the synthesis of nucleoside di- and triphosphates by Jessen and co-workers.[8] Herein we demonstrate the assembly of PAR oligomers with a well-defined length by using the phosphorylated 2-*O*-ribosyladenosine phosphoramidite **10** as a building block in an automated solid-phase process.

For the synthesis of 2-*O*-ribosyladenosine, we started out with the condensation of commercially available 1,3,5-tri-*O*-benzoylribose (**2**) and the *N*-phenyltrifluoroacetimidate donor **1** (Scheme 2).[9] The glycosylation reaction was fast, completely α-selective, and could be performed on an 8 mmol



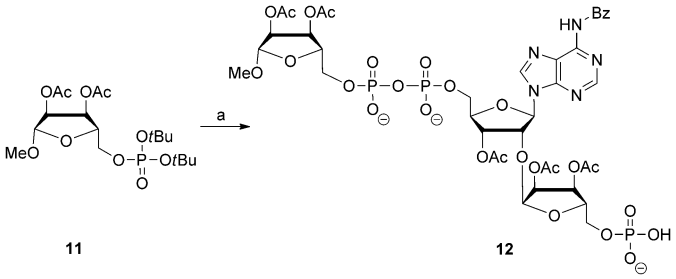
Scheme 2. Synthesis of the 2-*O*-ribosylated adenosine derivative **10**. a) TMSOTf (1 mol%), CH_2Cl_2, −78°C; b) i) H_2, Pd/C, *t*BuOH/dioxane/H_2O; ii) Ac_2O, pyridine; c) $HClO_4$–SiO_2 (0.5 equiv), MeCN, BSTFA, N^6-benzoyladenine, reflux; d) i) pyridine/EtOH/NaOH (1 M) (3:2:3, v/v/v); ii) pyridine, DMTrCl; iii) pyridine, Ac_2O; e) $Et_3N{\cdot}(HF)_3$, Et_3N, pyridine; f) i) $(tBuO)_2PN(iPr)_2$, 1-Me-Im·HCl (0.3 M), 1-Me-Im (0.2 M), DMF; ii) *t*BuOOH; g) TFA (1% v/v), CH_2Cl_2; h) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, CH_2Cl_2. Bn = benzyl, BSTFA = *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, DMTrCl = 4-4′-dimethoxytrityl chloride, Bz = benzoyl, DIPEA = *N*,*N*-diisopropyldiethylamine, DMF = *N*,*N*-dimethylformamide, TMSOTf = trimethylsilyl trifluoromethanesulfonate.

scale to give disaccharide **3** in good yield. At this stage, the benzyl groups were replaced with acetyl groups to circumvent the problematic removal of benzyl groups in the presence of adenine: The hydrogenolysis of **3**, followed by acetylation, gave disaccharide **4** in 85% overall yield.

For the introduction of the adenine moiety, the Vorbrüggen reaction was attempted under various conditions (with TMSOTf or $SnCl_4$ in MeCN or DCE), but all resulted in the formation of considerable amounts of N7- and N3-linked regioisomers and gave the target compound **5** in poor to moderate yield.[5,10] We also examined Brønsted acids as alternatives for Lewis acids.[11] The use of trifluoromethanesulfonic acid (TfOH) and pyridinium triflate resulted in slightly improved yields (40–60%), but the formation of N7- and N3-linked adenine regioisomers in the TfOH-mediated reaction could not be suppressed. The best results were obtained when compound **4** and persilylated N^6-benzoyladenine were heated at reflux in the presence of $HClO_4$ immobilized on silica gel[9,12] for 4 h. Under these conditions, N9-linked and β-configured **5** was obtained in 82% yield.[13] This procedure gave access to substantial amounts of 2-*O*-ribosylated adenosine, as the reaction was also successful on a larger scale (4 mmol). Subsequently, the benzoyl and acetyl esters in ribosylated adenosine **5** were hydrolyzed under mild conditions that left the benzoyl group at the exocyclic amine functionality of the adenine base intact. A DMTr group was introduced at the primary hydroxy functionality of the adenosine residue, and the secondary hydroxy groups were acetylated to give **6**. Finally, the removal of the triisopropylsilyl (TIPS) group in **6** afforded alcohol **7**.

The introduction of a di-*tert*-butylphosphate group did not proceed seamlessly, and the formation of the target di-*tert*-butyl phosphotriester was accompanied by the formation of various amounts of the H-phosphonate.[14] The combined use of 1-methylimidazole (1-Me-Im) and 1-methylimidazolium chloride (1-Me-Im·HCl) proved more successful than the use of other activators (tetrazole, dicyanoimidazole, 5-(ethylthio)-1*H*-tetrazole, benzimidazolium triflate) and reduced the formation of the H-phosphonate to a minimum (<5%). Oxidation with *t*BuOOH then generated phosphotriester **8**. Next, the DMTr group was selectively removed in the presence of the acid-labile *tert*-butylphosphate with a 1% (v/v) solution of trifluoroacetic acid (TFA) in CH_2Cl_2. Finally, a phosphoramidite group was installed on the 5′-hydroxy group of **9** to give the desired building block **10** in 19% overall yield (10 steps from **2**) and a practical quantity of 1 mmol.

To assess the feasibility of the intended solid-phase approach, phosphotriester **11** (for its synthesis, see the Supporting Information) and amidite **10** were used as starting compounds for a solution-phase study of pyrophosphate formation (Scheme 3). We adjusted our reported conditions in view of the projected synthesis on a solid support. The four-step procedure started with the TFA-mediated removal of the *tert*-butyl protecting groups in phosphotriester **11** and the conversion of the obtained phosphomonoester into its pyridinium salt. The crucial coupling of the obtained mono-

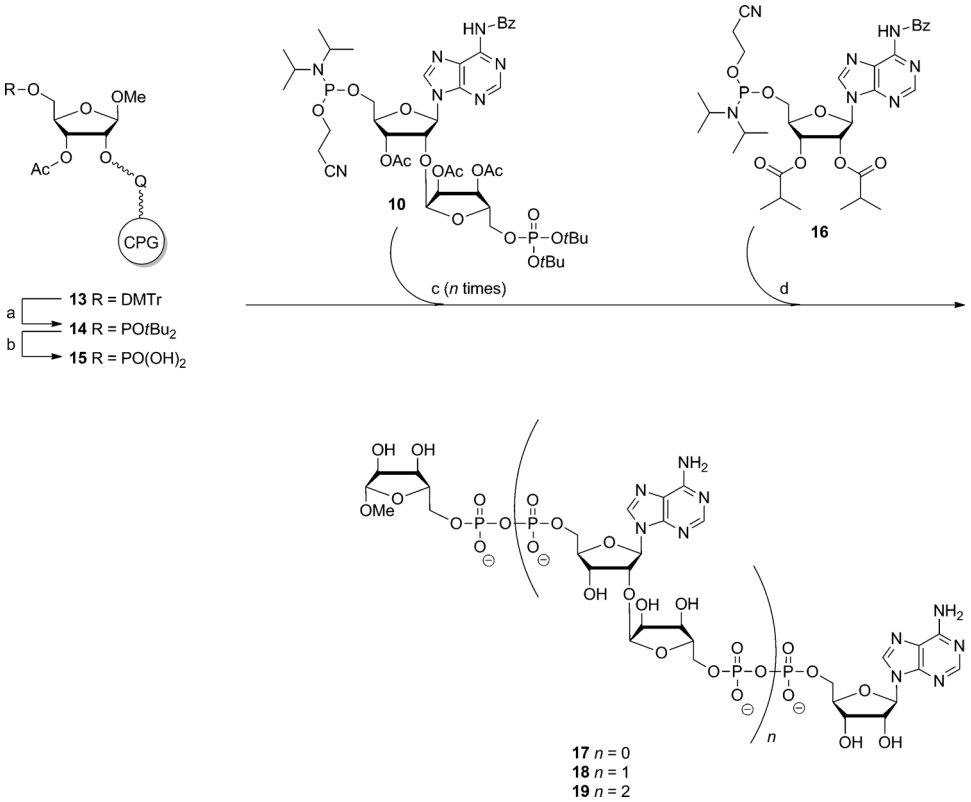


Scheme 3. Synthesis of pyrophosphate **12** in solution. a) i) TFA (10% v/v), CH_2Cl_2; ii) pyridine; iii) compound **10**, ETT, MeCN; iv) CSO, MeCN; v) DBU, DMF; vi) HCl, HFIP. DBU = 1,8-diazabicycloundec-7-ene, HFIP = hexafluoroisopropanol.

ester with the advanced amidite **10** was carried out with 5-ethylthiotetrazole (ETT) as an activator.[7] The formation of the labile phosphite–phosphate intermediate was fast (< 1 min) and quantitative, as monitored by ^{31}P NMR spectroscopy (see the Supporting Information). The ensuing oxidation of the phosphite–phosphate intermediate to the protected pyrophosphate intermediate was accelerated by exchanging *t*BuOOH for (1*S*)-(+)-(10-camphorsulfonyl)oxaziridine (CSO) as the oxidant (from 30 to 5 min). The intrinsic instability of the partially protected pyrophosphate intermediate was an incentive to remove the cyanoethyl group with a large excess of anhydrous DBU. Finally, the *t*Bu groups of the terminal phosphate were removed with HCl in HFIP to obtain **12** (Scheme 3).[9] The monitoring of all reactions en route to the desired pyrophosphate by ^{31}P NMR spectroscopy showed fast and quantitative conversion. Purification by reversed-phase (RP) silica-gel chromatography gave homogeneous **12.**

Having established this protocol for pyrophosphate formation, compound **10** was used as a building block in the solid-phase synthesis of PAR oligomers. Guided by the state of the art in automated oligonucleotide synthesis, we selected controlled pore glass (CPG) with long alkyl amine chains as the solid support. Hydroquinone-*O*,*O*′-diacetic acid (the "Q linker") was selected over the conventional succinyl linker for its improved resistance to cleavage by DBU.[15] The immobilized ribose **13** (see the Supporting Information) was treated with TFA to remove the DMTr group, and the di-*tert*-butyl phosphotriester functionality was introduced to furnish compound **14** (Scheme 4). The *t*Bu groups of phosphate **14** were removed with TFA, and the resin was neutralized with pyridine/H_2O to afford **15**. The resin (10 μmol) was loaded into an automated oligonucleotide synthesizer, and the above-described solution-phase protocol for pyrophosphate formation was applied to synthesize a monomer ($n = 0$), a dimer ($n = 1$), and a trimer ($n = 2$) (Scheme 4). One complete cycle (Scheme 4c or d) took 1 h, and after completion of the synthesis, the PAR oligomers were completely deprotected and released from the solid support by treatment with aqueous ammonia. The crude products were purified by gel filtration and anion-exchange chromatography to give compounds **17**, **18**, and **19**. The PAR dimer **18** and trimer **19** were obtained in good yields (35 and 29%, respectively).

In summary, we have described the first synthesis of a PAR dimer **18** and trimer **19**. For the synthesis of these compounds, a new scalable synthetic approach to the 2-*O*-ribosyladenosine **5** was developed with a Vorbrüggen coupling as the key step. The advanced building block **10**, with the desired phosphoramidite and protected-phosphate functionalities in place, was prepared in millimolar quantities, thus enabling the solution-phase development of an efficient



Scheme 4. Synthesis of the preloaded CPG resin and PAR oligomers ($n = 0$, 1, and 2). a) i) TFA (5% v/v), CH_2Cl_2; ii) $(tBuO)_2PNiPr_2$, 1-Me-Im·HCl (0.3 M), 1-Me-Im (0.2 M), DMF; iii) CSO, MeCN; b) i) TFA (10% v/v), CH_2Cl_2; ii) pyridine/H_2O (9:1, v/v); c) i) compound **10** (3 equiv), ETT (10 equiv), MeCN (2×); ii) CSO (100 equiv), MeCN (2×); iii) DBU (100 equiv), DMF, (2×); iv) HCl (5 equiv), HFIP (4×); v) pyridine (10% v/v), acetonitrile (2×); d) i) compound **16** (3 equiv),[16] ETT (10 equiv), MeCN (2×); ii) CSO (100 equiv), MeCN (2×); iii) DBU (100 equiv), DMF (2×); iv) NH_4OH (35%).

approach for pyrophosphate formation. The coupling and oxidation steps as well as the cleavage of the cyanoethyl group were fast and clean, as observed by ^{31}P NMR spectroscopy. A solid-support approach was essential for the introduction of multiple pyrophosphate entities, and milligram quantities of the PAR dimer and trimer were obtained. The PAR oligomers will be used for biological testing, such as binding studies or NMR spectroscopic experiments. We are currently testing our newly developed method in the construction of larger PAR oligomers as well as the synthesis of branched PAR derivatives. Furthermore, this completely chemical approach should also be suitable for the synthesis of PAR analogues with a chemical tag, such as a fluorophore or biotin.

Keywords: ADP-ribosylation · phosphorylation · poly(ADP-ribose) · pyrophosphates · solid-phase synthesis

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