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Ribosylation of Adenosine: An Orthogonally Protected Building Block for the Synthesis of ADP-Ribosyl Oligomers

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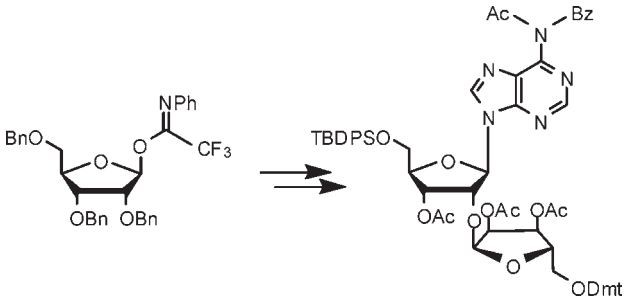
ABSTRACT



A method to ribosylate adenosine on the 2′ hydroxyl function in an α-selective fashion and in good yield is presented. Protective groups chosen for the acceptor and donor used in this glycosylation not only direct α-selectivity but also allow the construction of a fully orthogonally protected building block for the future assembly of oligo-ADP-ribosylated peptides and proteins.

Posttranslational modifications play crucial roles in numerous biological processes.[1,2] An important modification is ADP-ribosylation of proteins, which involves the transfer of ADP-ribose from β-NAD$^+$ to nucleophilic side chain residues in the target protein under the expulsion of nicotinamide. Both mono-ADP-ribosyl transferases (MARTs) and poly-ADP-ribosyl polymerases (PARPs) affect this reaction to form ADP-ribose monomers and polymers respectively (see Figure 1). Although ADP-ribosylation is involved in important cellular processes such as transcription regulation, cell proliferation, DNA repair, apoptosis, and immune response,[3−5] little is known of the molecular details that are behind these processes.

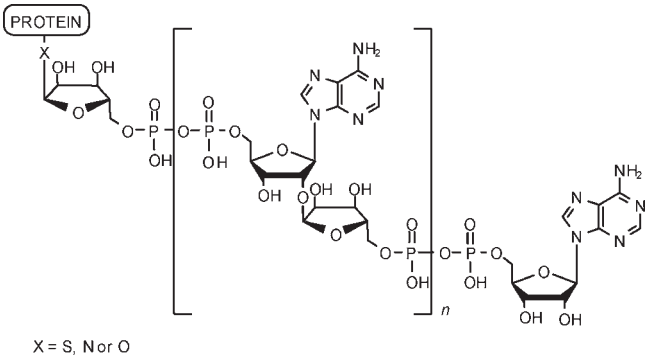


Figure 1. Poly-ADP-ribose polymer attached to target protein.

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It is recognized that well-defined ADP-ribosylated peptides and analogues thereof could help further the research on ADP-ribosylation.[6] Recently we have reported the synthesis of mono-ADP-ribosylated peptides, in which ADP-ribose is linked to a peptide via an asparagine or glutamine residue.[7] Others have prepared oxime linked analogues of mono-ADP-ribosyl peptides.[8] To enable the future synthesis of ADP-ribosyl derivatives containing more than one adenosine diphosphate ribose residue (see Figure 1, $n > 0$), the availability of a suitably protected ribosylated adenosine building block is crucial. Synthetic challenges in the construction of such a building block are the formation of the α-glycosidic bond between the anomeric center of ribofuranose and the 2′-hydroxyl of adenosine, and the design of a suitable, orthogonal protective group strategy.

O-Glycosylated nucleosides in general are difficult to synthesize.[9,18] The nucleobase is often more reactive as a nucleophile than the intended hydroxyl. As a result side reactions at the nucleobase may occur that can lead, in the case of purines, to depurination of the nucleoside. Consequently, yields are often low for such glycosylation reactions.[9] Shimofuridin,[10,11] bearing a 2′-*O*-β-fucopyranosyl moiety, and adenophostin,[12−14] bearing a 3′-*O*-α-glucopyranosyl moiety, are two examples of glycosylated nucleoside analogues, in which the nucleosides are equipped with pyranosyl moieties. Although several nucleosides decorated with *O*-β-linked furanosyl groups have been synthesized,[15−18] there is only one reported synthesis of 2′-*O*-α-ribosylated adenosine.[19] This reported procedure involves a 1,2-trans selective coupling of arabinofuranose to adenosine, assisted by the 2′-*O*-acyl group, followed by inversion of the stereochemistry at the 2-position of the arabinofuranose via an oxidation−reduction protocol.[19]

In order to synthesize 2′-*O*-α-ribosylated adenosine with orthogonal protective groups on the primary hydroxyl functions, we considered an approach involving α-glycosylation of adenosine with a suitable ribofuranosyl donor to be most convenient. We opted to use 2,3,5-tri-*O*-benzyl-D-ribofuranose since it is known from literature that nonparticipating benzyl protection on the ribofuranosyl donor in glycosylation reactions allows formation of mainly or exclusively the α-riboside.[20−22] In contrast, 2,3-isopropylidene[23] or 2,3-benzylidene[24] protection, which would also be convenient protection groups due to the ease of introduction and cleavage, leads to anomeric mixtures, while application of acyl based protective groups would result in exclusive formation of the unwanted β-riboside due to neighboring group participation. The high propensity for α-substitution of benzylated ribofuranosyl donors can be satisfactorily explained by the model advanced by Woerpel for nucleophilic attack on five-membered oxocarbenium ions.[22] It is accepted that such an oxocarbenium ion is a possible intermediate in Lewis acid promoted nucleophilic substitution on furanosyl donors.[25,26] Alternative explanations of the predisposition of ribofuranosyl donors, equipped with nonparticipating protections, to couple with high α-selectivity have also been offered.[21,27]

Having selected the donor, we chose 3,5-OTIPDS protected N^6-benzoyl adenosine **2** as the most suitable acceptor in this glycosylation (see Scheme 1).[28] Since adenosine may depurinate under strongly acidic conditions,[29] we considered that a mild glycosylation protocol would be required. Based on this reasoning we first attempted the procedure of Mukaiyama, who revealed that activation of **1** (see Scheme 1) with diphosphonium salts and ensuing base-assisted glycosylation resulted in high yielding and α-selective ribofuranosylation of unhindered alcohols.[21] However, when we used sterically demanding compound **2** as the acceptor under the reported conditions no product was formed.[30] When we performed the glycosylation with a larger excess of base a product different from the expected compound **4** was formed. The NMR data of the isolated product were consistent with **5**, in which 2,3,5-tri-*O*-Bn-ribofuranose was α-coupled to the N-1 of adenosine. Apparently, the excess of base increases the

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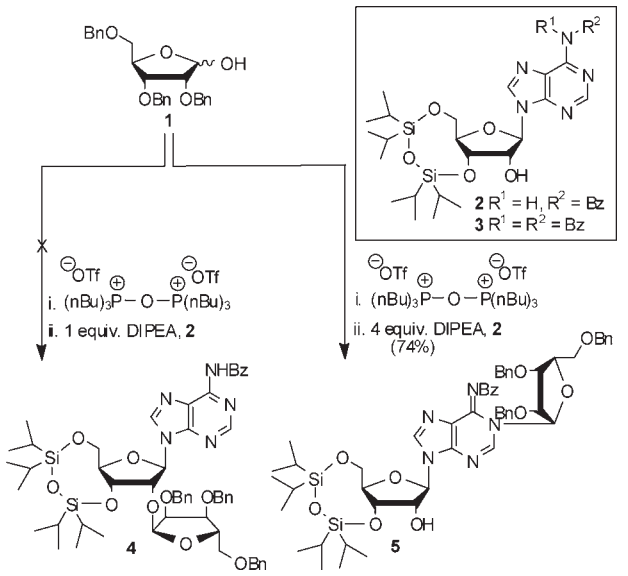
(30) When we adopted this procedure for the glycosylation of 3,5-OTIPDS protected uridine, we observed formation of 2′-*O*-(2,3,5-*O*-Bn-α-ribosyl)uridine, however in low yield (15%).

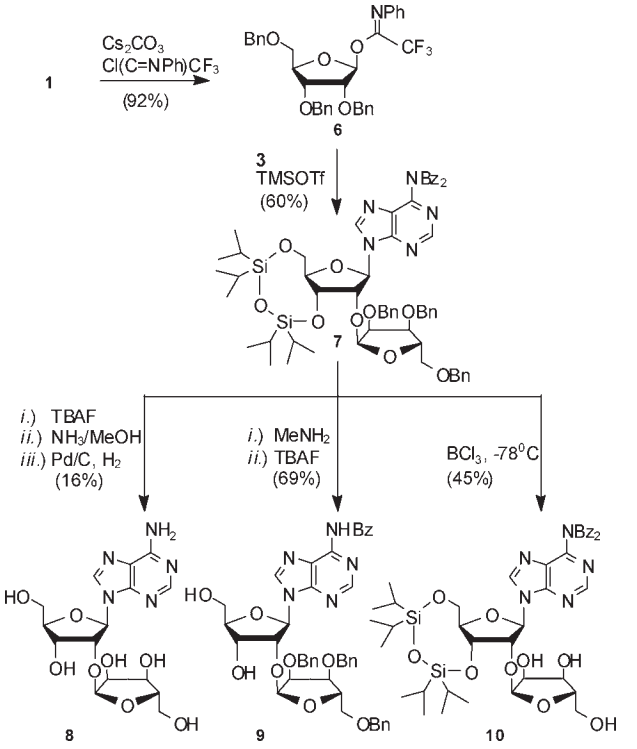
Scheme 1. Phosphonium Salt Mediated Glycosylations



nucleophilicity of the N-1 position of the adenine moiety by deprotonation of the exocyclic amide. This interesting side reaction resulting in compound **5** might prove to be valuable in the construction of cADPR analogues.[31] In an attempt to avoid *N*-glycosylation we applied N^6,N^6-dibenzoylated adenosine (**3**)[32] as an acceptor (see Scheme 1), but unfortunately, no glycosylation occurred.

We concluded that acceptor **3** was insufficiently reactive under these conditions. After screening a number of glycosylation methods[33] we found that imidate donor **6** in combination with TMSOTf as a promoter was the most efficient glycosylating reagent.[34] Imidate **6** was prepared in 92% yield by reacting 2,3,5-tri-*O*-Bn-ribofuranose with Cs_2CO_3 and Cl(C=NPh)CF_3 (see Scheme 2). Activation of imidate **6** with TMSOTf in the presence of acceptor **3** resulted in formation of **7**, containing the desired α-ribofuranosyl linkage in good yield.[35] Complete deprotection of **7** using TBAF and NH_3 and finally palladium catalyzed hydrogenation gave access to **8**, the spectroscopic and physical properties of which were in complete accordance with the published data.[19] The inherent orthogonality between silyl and benzyl ethers allows selective manipulation of the primary OH-groups in **7**. To exemplify this orthogonality we deprotected the adenosine hydroxyls by cleaving the silyl protective group using TBAF (1 M solution in THF). Due to the alkaline nature of TBAF one of the two benzoyl groups on the exocyclic amine of adenosine proved also susceptible to cleavage, resulting in the formation of the corresponding side product. To avoid this partial deprotection and the need for an extra purification step, we decided to first treat compound **7** with $MeNH_2$ to completely remove one benzoyl while leaving the other benzoyl in place and then perform the TBAF treatment to give **9**.[36] Similarly we deprotected the ribose moiety while leaving all the protective groups on adenosine in place. Palladium catalyzed hydrogenation with Pd/C in an alcoholic solvent proceeded sluggishly. Therefore we opted to treat compound **7** with BCl_3 at −78 °C to rapidly afford compound **10**.

Scheme 2. Key Glycosylation Using Imidate **6**



To generate the fully orthogonally protected building block **12** (see Scheme 3) we opted to use compound **10** as starting material. Straightforward conversion of **10** by protecting the primary hydroxyl of ribose with DMT and subsequent acetylation of the remaining secondary hydroxyls (see Scheme 3) gave **11**. Cleavage of one benzoyl group in **11**, removal of the TIPDS protection, introduction of a TBDPS on the primary hydroxyl of adenosine,

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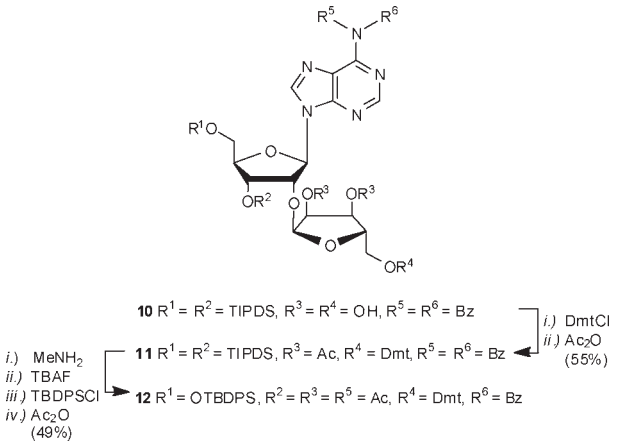
Scheme 3. Introduction of Orthogonal Protective Groups



and subsequent acetylation of the secondary hydroxyls and the exocyclic nitrogen of adenosine gave fully protected compound **12**. The thus formed compound **12** bears orthogonal protective groups on both primary hydroxyl functions, which allows the future synthesis of poly-ADP-ribosylated peptides using the methodology as reported before by us for *m*-ADP-ribosylated peptides.[7,37]

In conclusion, we have presented a method for α-selective ribosylation of adenosine at the 2′-OH. Protective groups chosen by us not only dictate α-selectivity but also allow site specific deprotection of primary hydroxyls and further functionalization toward a building block that can be used in the sequential synthesis of poly-ADP-ribosyl modified peptides.

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Supporting Information Available. Spectroscopic data and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.