

Isopolar Phosphonate Analogue of Adenosine Diphosphate Ribose

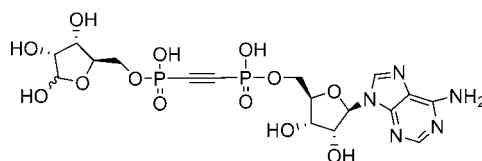
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ABSTRACT



from D-ribose

The synthesis of the bisphosphonate ADP-ribose, in which acetylene has replaced the oxygen of the pyrophosphate linkage, is reported.

Adenosine diphosphate (ADP) ribose (1, Figure 1) is a ubiquitous cellular metabolite. It is the turnover product in metabolic pathways of key cellular components such as protein-bound poly-(ADP-ribose), nicotinamide adenine dinucleotide (NAD⁺) and cyclic ADP-ribose. Through its reducing ribosyl moiety, ADP-ribose can damage cells by acting as a nonspecific protein-glycating agent with affinity for the numerous cellular enzymes possessing nucleotide-binding sites.¹ Recently, ADP-ribose was shown to exhibit intracellular signaling regulatory functions by mediating cellular Ca²⁺-gating.² This latest discovery has renewed interest in the biology of ADP-ribose.³ Intracellular levels of ADP-ribose are thought to be regulated by divalent cation-dependent pyrophosphatases that cleave ADP-ribose to yield adenosine monophosphate and 5-ribosyl phosphate.⁴ Pyrophosphatases that selectively hydrolyze ADP-ribose belong to a vast and widely studied family of enzymes, called NUDIX enzymes, capable of hydrolyzing numerous nucleoside pyrophosphate derivatives.⁴ Recent investigations of the

biological importance of ADP-ribose pyrophosphatases and that of their mechanisms, based on crystallographic data obtained from *Escherichia coli*,⁵ *Mycobacterium tuberculosis*,⁶ and human⁷ enzymes, have provided a new momentum in the study of this subfamily of NUDIX enzymes.

Our current research efforts are directed toward the synthesis of novel NAD⁺ analogues to probe the binding site of NAD(P)⁺-dependent enzymes⁸ that do not possess the commonly encountered Rossmann fold.⁹ During the

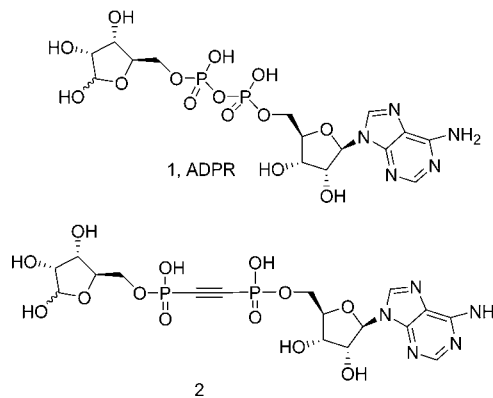


Figure 1. Adenosine diphosphate ribose **1** and its pyrophosphonate mimic **2**.

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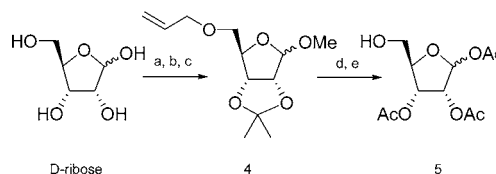
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development of divergent syntheses of NAD(P) analogues, it came to our attention that very few ADP-ribose derivatives possessing modified pyrophosphate linkages had been reported in the literature.¹⁰ In addition, Blackburn reported the only pyrophosphonate derivatives of ADP and ATP, in which the oxygen of a P—O—P bond had been replaced by an alkynyl moiety.^{11–13} In the ATP analogue, the substitution took place between the β - and the γ -phosphorus atoms and the synthesis involved the condensation of AMP-morpholide with acetylene-1,2-bisphosphonic acid obtained from the Michaelis–Arbusov reaction between dichloroethyne and triethyl phosphite. Such alkynyl-containing bisphosphonates were shown to have lower pK_a s than the corresponding pyrophosphates due to the lack of hydrogen bond between the P_β —O—H and the P_γ —O[−] caused by the linear acetylenic linkage.¹¹ It can be anticipated that at physiological pH, an acetylenic-containing pyrophosphonate ADP-ribose analogue could have equal capabilities for ionic interactions with enzyme binding residues as its parent pyrophosphate. However, weaker complexes are to be expected between this type of pyrophosphonate and Mg^{2+} present in the pyrophosphatase binding pocket. This assumption is based on Blackburn's work with alkynyl-containing ATP analogues and Ca^{2+} .¹¹ Yet, long-range interactions between acetylene bisphosphonate-containing nucleotides and binding residues and metals through enzyme bond–water molecules can also be anticipated.^{14,15}

As a consequence, we decided to synthesize the ADP-ribose analogue (**2**, Figure 1) in which the bridging oxygen has been exchanged for an alkynyl moiety. This analogue can be viewed as a mimic of the transition state occurring during the enzymatic cleavage of the P—O—P bond as the distance between the two phosphorus atoms has been calculated to differ by 2 Å (calculated P—P distance; 2.8 Å in P—O—P and 4.8 Å in P—CC—P).¹⁶ In addition to the binding interactions anticipated to occur between enzyme and analogue, it is hoped that the acetylenic pyrophosphonate will participate in some new enzymatic chemistry. Indeed, in the presence of a strong nucleophile, tetraethyl 1,2-ethynyl bisphosphonate was shown to decompose via the rupture of one of the P—C bonds.¹⁷ Such chemical behavior could lead

Scheme 1. Synthesis of α/β 1,2,3-Tri-*O*,*O*,*O*-acetyl D-Ribose **5**^a

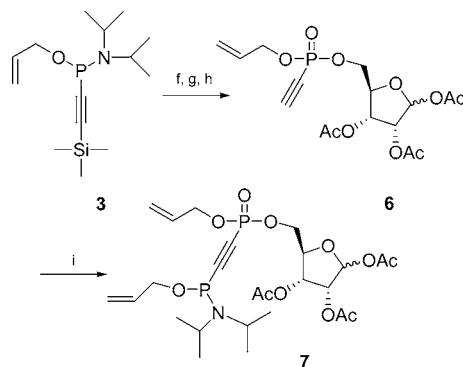


^a Conditions: (a) CH_3OH , H-Dowex resin, rt, 48 h. (b) $(CH_3O)_2C(CH_3)_2$, H-Dowex resin, rt, 12 h. (c) $CH_3CH_2CH_2Br$, BaO, wet DMF, rt, 12 h, 92%. (d) CH_3COOH glacial, $(CH_3CO)_2O$, H_2SO_4 cat., 0 °C, 12 h. (e) $PdCl_2$, DCM/ CH_3OH (1/1), 0 °C to room temperature, 4 h, 75%.

to an unprecedented mode of irreversible inhibition of pyrophosphatases. Similarly, in the presence of an acidic residue, addition of an enzyme nucleophilic residue across the triple bond can be anticipated,¹⁷ once again, leading to covalent bonding and irreversible inhibition. To the authors' knowledge, no analogue for which an alkynyl moiety has been introduced between the α - and β -phosphorus atoms of a sugar nucleoside diphosphate has been synthesized. Here, we report a versatile synthesis of the bisphosphonate acetylene analogue of ADP-ribose (**2**, Figure 1).

The synthesis of the ADP-ribose analogue **2** was achieved via the early introduction of an alkynyl-phosphonate moiety (Scheme 1). Allyloxy diisopropylaminotrimethylsilylethynylphosphine (**3**, Scheme 2) was prepared from trichlorophosphine and allyl alcohol in the presence of pyridine to yield allyloxy dichlorophosphine (³¹P NMR 178.3 ppm), which was then converted to the allyloxy chlorodiisopropylaminophosphine (³¹P NMR 182.0 ppm).¹⁸ Subsequent treatment with trimethylsilyl acetylene in the presence of *n*-BuLi in THF at −78 °C gave **3**. The use of an allyl-protecting group was essential for completing the synthesis of **2**, as other alkoxy groups were initially employed unsuccessfully. While the methyl-protected diisopropylaminotrimethylsilylethynylphosphine was a suitable reagent for the synthesis of the

Scheme 2. Synthesis of Intermediate **7**^a



^a Conditions: (f) **5**, 2,4-DNP, CH_3CN , rt, 2 h. (g) H_2O_2 , DCM, 0 °C, 7 min. (h) 50% CsF, CH_3CH_2OH , rt, 1 h. (i) Allyloxychlorodiisopropylaminophosphine, *n*-BuLi, THF, −78 °C, 1 h, 53%.

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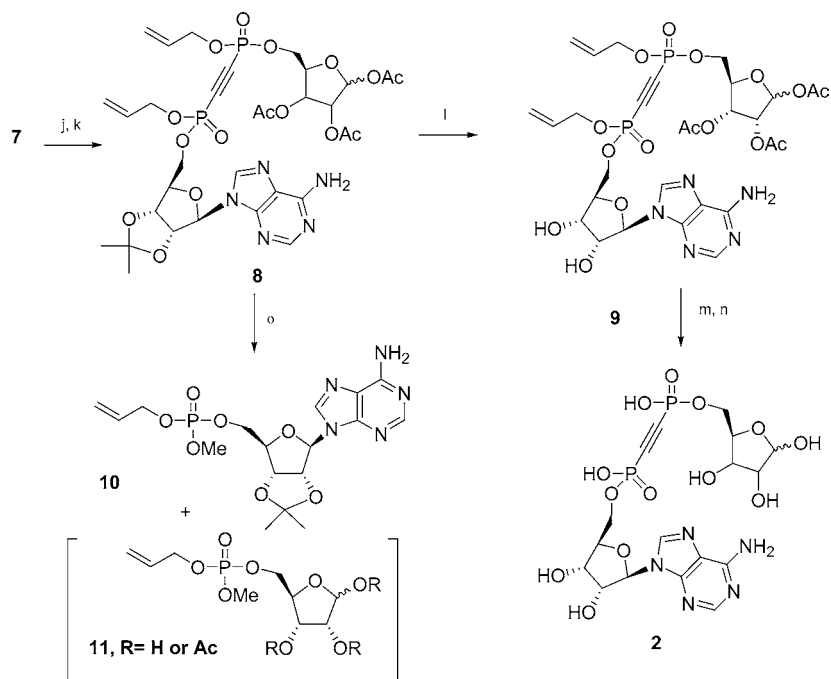
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Scheme 3. Synthesis of **2**^a

^a Conditions: (j) 2',3'-*O*,*O*-isopropylidene adenosine, 2,4-DNP, CH₃CN, rt, 12 h. (k) *t*-BuOOH, -40 °C, 7 min, 31%. (l) H₂O/TFA, rt, 1 h. (m) Pd[PPh₃]₄, PPh₃, TEA, HCOOH, THF, rt, 8 h, 85%. (n) NaOMe, methanol, rt, 30 min. (o) NH₃, CH₃OH, rt, 12 h, 68%.

fully protected ADP-ribose acetylene analogue, only partial deprotection of the methyl groups could be achieved in the final step of the synthesis. Upon more forceful conditions, only decomposition products could be detected. Cyanoethyl was not a suitable protecting group due to its lability under basic conditions, while *p*-methoxybenzyloxy chlorodiisopropylaminophosphine was easily prepared but was inappropriate to complete the synthesis, as the PMB group was readily oxidized in a subsequent step. Finally, *p*-chlorophenylxy diisopropylaminotrimethylsilylethynylphosphine proved to be a synthetic precursor difficult to obtain in reasonable purity and yields. Consequently, our endeavor focused on synthesizing **2** starting from ribose and allyloxy diisopropylaminotrimethylsilylethynylphosphine **3**.

The alkynylphosphonate **6** was obtained in 71% yield over two steps by coupling 1,2,3-*O*,*O*-triacetyl-D-ribose **5**, prepared in five steps from D-ribose (Scheme 1), with **3** in the presence of 2,4-dinitrophenol (2,4-DNP),¹⁹ followed by careful phase transfer oxidation of the resulting phosphonite and desilylation with CsF. Initial attempts to catalyze the coupling of **5** with **3** with either tetrazole²⁰ or imidazolium triflate offered poor to moderate yields over the two steps (<30%). The sugar-containing alkynylphosphonate **6** was obtained as a mixture of diastereoisomers (³¹P NMR -6.47,

-6.69 ppm). The fully protected ADP-ribose acetylene-containing analogue **8** (Scheme 3) was obtained in 31% isolated yield over two steps from the 2,4-DNP-catalyzed coupling of 2',3'-*O*,*O*-isopropylidene adenosine with P¹-[allyloxy diisopropylamino]-P²-[(1'',2'',3''-*O*,*O*,*O*-acetyl)-D-ribofuranose-5-yl, allyl]-acetylene-(1-phosphine, 2-phosphonate) **7** (Scheme 2), followed by oxidation of the resulting phosphinate to the phosphonate with *t*-BuOOH. The intermediate **7** had been obtained by adding *n*-BuLi to a THF solution of allyloxy chlorodiisopropylaminophosphine and **6**, cooled to -78 °C. One should note that initial preparation of the alkynyl anion and subsequent addition of the chlorophosphine resulted in complete decomposition of **6**.

The acetonide protecting group of the fully protected ADP-ribose analogue **8** was hydrolyzed by a mixture of H₂O and TFA (v/v; 1/1.9) to give **9** in 65% yield after preparative thin-layer chromatography. Unreacted starting material was recovered in 23% yield. The synthetic intermediate **9** was then treated with a mixture of Pd[PPh₃]₄, PPh₃, Et₃N, and formic acid²¹ to remove the allyl group. An alternative sequence, which offered similar overall yields, started with the deallylation reaction immediately followed by the acetonide removal. Finally, the acetate esters were hydrolyzed by a solution of sodium methoxide in methanol to obtain the ADP-ribose analogue **2** in 5% overall yield. It should be

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mentioned that **8** rapidly degraded to **10** and another derivative thought to be **11**, if first treated with a saturated methanolic ammonia solution. This observation came as no surprise, as Maier reported that the P–C bond of tetraethyl 1,2-ethynyl bisphosphonate was cleaved upon basic treatment.¹⁷ In addition, when **9** was first deallylated then treated with a saturated methanolic ammonia solution, a mixture of three partially deacetylated products were obtained. They were the 1''-*O*-, 2''-*O*-, or 3''-*O*- monoacetylated derivatives of compound **2** (detected by LCIMS, ¹H NMR, and RP-HPLC). Attempts to remove the remaining acetate with a KCN solution or an NH₄OH/methanol solution were unsuccessful, even after heating at 50 °C overnight. While unsatisfactory from a synthetic perspective, these experiments confirmed that hydrolysis of the pyrophosphonate diester P–CC–P bond did not occur under these conditions. Consequently, the stability to nucleophilic attack of the phosphonate monoester P–CC–P bond is greater than that of the P–CC–P bond of the phosphonate triester.

Due to our interest in probing enzymes that bind NAD-(P)⁺, we wanted this synthetic route to be as flexible as possible with regard to the introduction of other residues than the terminal ribose of ADP-ribose. For this reason, we investigated the feasibility of carrying out a similar sequence starting instead from 2',3'-*O*,*O*-isopropylidene adenosine to the triacetyl ribose **5**. While the synthetic route remains broadly identical, some slight modifications had to be introduced. For instance, neither tetrazole nor imidazolium triflate was suitable to promote any reaction between allyloxy diisopropylaminotrimethylsilyl-ethynylphosphine **3** and 2',3'-*O*,*O*-isopropylidene adenosine. For this synthetic sequence, only 2,4-DNP was an appropriate activator for the phosphoramidite coupling reaction. Furthermore, protection of the N⁶-adenosine was required in order to form the P–CC–P linkage in reasonable yields via the *n*-BuLi-catalyzed nucleophilic displacement by the acetylene anion of the chlorine

on allyloxy-chlorodiisopropylaminophosphine. While this route is somewhat lengthier than the one described above, it still provides facile access to ADP and ATP analogues that incorporate an acetylenic group between the α- and the β-phosphorus atoms. Further work on the deprotection sequence to yield ADP analogues is currently in progress, as well as work on the stereoselective reduction of the alkyne pyrophosphonate derivative to their (*E*)- and (*Z*)-alkene pyrophosphonate equivalents. The ADP-ribose analogues reported herein are being evaluated for binding affinity to a variety of ADPR-binding enzymes that include pyrophosphatases, dehydrogenases, and glycohydrolases, and their biological activity will be reported in due course.

To summarize, we have developed a novel and potentially versatile synthetic route for the preparation of a new type of modified ADP-ribose in which the oxygen of the pyrophosphate linkage has been replaced by an acetylene moiety. This bisphosphonate is stable under a broad range of pH conditions and is expected to be relatively resistant to nonenzymatic cleavage under aqueous conditions. We expect that this analogue will offer further information on the catalytic abilities of ADP-ribose-pyrophosphatases, behave as a potential inhibitor of these enzymes, and finally provide new tools to investigate the biological importance of the enzyme-catalyzed hydrolysis of ADP-ribose. In time, this route developed for the synthesis of ADP-ribose analogues will be applied to the synthesis of other sugar and nucleoside derivatives to probe pyrophosphatase specificity.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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