Synthesis and biological activity of cyclic ADP-carbocyclic-ribose and its analogs as stable mimics of Ca\(^{2+}\)-mobilizing second messenger cyclic ADP-ribose

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ABSTRACT
Cyclic ADP-carbocyclic-ribose (cADPcR, 2) and its several analogs were designed and synthesized as stable mimics of Ca\(^{2+}\)-mobilizing second messenger cyclic ADP-ribose (cADPR, 1). cADPcR was stable and actually caused a significant release of Ca\(^{2+}\) stronger than that of cADPR.

INTRODUCTION
Cyclic ADP-ribose (cADPR, 1) is a general mediator involved in Ca\(^{2+}\) signaling. Due to their biological importance, the synthesis of cADPR analogs has been extensively studied by enzymatic and chemoenzymatic methods using ADP-ribosylcyclase. We have been working to develop flexible methods for chemically synthesizing cADPR analogs, since the analogs that can be obtained by existing methods are limited due to the substrate specificity of the enzyme.

We designed cyclic ADP-carbocyclic-ribose (cADPcR, 2) and its analogs 3a-d and 4a-d, as stable mimics of cADPR (Figure 1). cADPcR is readily hydrolyzed both enzymatically and non-enzymatically at the unstable N-1-glycosidic linkage of the adenine moiety. A stable analog of cADPR which exhibits Ca\(^{2+}\)-mobilizing activity in cells similar to that of cADPR is very useful as pharmacological tools and are urgently required.

We developed an efficient method to form an intramolecular pyrophosphate linkage by activating the phenylthiophosphate group with I\(_2\) or AgNO\(_3\) for the chemical synthesis of cADPR analogs. Using this method, we synthesized cADPcR (2) as well as its analogs 3a-d and 4a,b.

RESULT
Synthesis of cADPcR and its analogs. We synthesized cADPcR (2) as shown in Scheme 1. A sugar-protected imidazole nucleoside 5, prepared from AICAR, was converted into the methoxymethylene derivative 7. The pyrimidine ring-closure reaction of 7 with the chiral carbocyclic amine 9 by treating them with K\(_2\)CO\(_3\) in MeOH at room temperature gave the desired ring-closure product 10 in 83% yield. Compound 10 was further converted into the 5'-phenylthiophosphoryl-5'-phosphate derivative 12, the substrate for the intramolecular condensation reaction, in several reaction steps. When a solution of 12 in pyridine was added slowly to a mixture of a large excess of AgNO\(_3\) and Et\(_3\)N in the presence of MS 3A in pyridine at room temperature, the desired cyclization product 14 was obtained in 93% yield. Finally, the cyclic pyrophosphate 14 was treated with aqueous HCO\(_2\)H to give the target cADPcR (2) in 88% yield.

Figure 1

![Chemical structures of cADPR and cADPcR analogs.](https://academic.oup.com/nass/article-abstract/1/1/5/1069987)
Cyclic 8-chloro-ADPcR (3a) was similarly synthesized from the 2-chloroimidazole nucleoside 6, which was prepared by the chlorination at the 2-position of 5 (Scheme 1). The other 8-substituted cADPcR analogs 3b-d were synthesized from 3a via the nucleophilic replacement reaction at the 8-position. The analogs 4a-c modified at the N-1-carbocyclic-ribose moiety were also obtained by the synthetic route similar to that for cADPR, using the corresponding chiral carbocyclic amines, instead of 9.

Thus, this represents a general method for synthesizing cADPR related compounds.

Chemical and Biological Property of cADPcR. $^1$H NMR analysis of cADPcR suggested that its conformation in aqueous medium is similar to that of cADPR. cADPcR, unlike cADPR, was very stable under neutral and acidic conditions, where under basic conditions, it formed the Dimroth-rearranged $^N$-cyclized product. cADPcR was also stable in rat brain membrane homogenate which has cADPR degradation activity. Furthermore, cADPcR was resistant to the hydrolysis by CD38 cADPR hydrolase, while cADPR was rapidly hydrolyzed under the same conditions. When cADPcR was injected into sea urchin eggs, it caused a significant release of Ca$^{2+}$ in the cells, which was considerably stronger than that of cADPR. Thus, cADPcR was identified as a stable mimic of cADPR.$^6$

Biological evaluation of the other cADPcR analogs is now in progress.

REFERENCES