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Design and synthesis of 4″,6″-unsaturated cyclic ADP-carbocyclic ribose as a Ca^{2+}-mobilizing agent

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Abstract

4″,6″-Didehydro-cADPcR (**3**), an unsaturated carbocyclic ribose analog of a Ca^{2+}-mobilizing second messenger cyclic ADP-ribose (cADPR), was designed and successfully synthesized using a key intramolecular condensation reaction forming the 18-membered pyrophosphate ring structure with a *S*-phenyl phosphorothioate-type substrate. Biological evaluation showed that 4″,6″-didehydro-cADPcR is a potent Ca^{2+}-mobilizing agent in T cells.

Cyclic ADP-ribose (cADPR, **1**, Fig. 1) is a general mediator involved in Ca^{2+} signaling,[1] and synthesis of its analogs has been extensively investigated, since these can be used in investigating the mechanism of cADPR-mediated Ca^{2+} signaling pathways and are also expected to be leads for the development of potential drug candidates.[2–5] In the synthesis of cADPR and its analogs, construction of the large 18-membered pyrophosphate ring structure is the key step, and we have developed an efficient method for forming the 18-membered ring by activating the *S*-phenyl phosphorothioate-type substrates with iodine or $AgNO_3$.[4]

cADPR is in a zwitterionic form with a positive charge around the N(1)–C(6)–N^6 moiety, making the molecule unstable, since the charged adenine moiety attached to the anomeric carbon of the N1-linked ribose can be an efficient leaving group to cleave the N1-ribosyl linkage. We previously designed and synthesized cyclic ADP-carbocyclic-ribose (cADPcR, **2**) as a stable mimic of cADPR, in which the oxygen atom in the N1-ribose ring of cADPR is replaced by a methylene group. cADPcR was shown to be actually resistant to both enzymatic and chemical hydrolysis, and it, like cADPR, effectively mobilizes intracellular Ca^{2+} in sea urchin eggs.[4c]

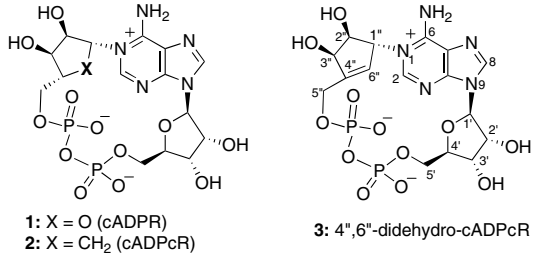

Fig. 1. cADPR (**1**), cADPcR (**2**), and 4″,6″-didehydro-cADPcR (**3**).

Our attention has been focused on developing cell-type selective cADPR analogs, which can be useful as biological tools and/or potential drug leads. Thus, we found that although cADPcR and its 3′-deoxy derivative are more

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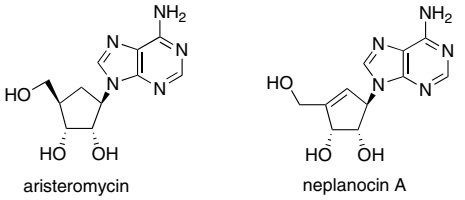


Fig. 2. Naturally occurring carbocyclic adenine nucleosides.

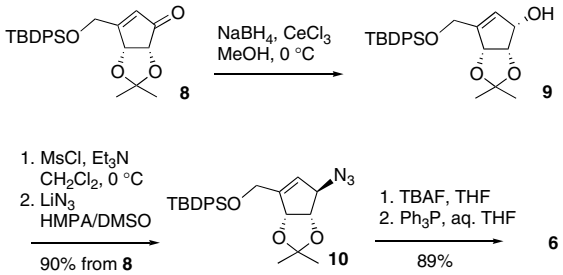
potent than cADPR in neuronal cells,[7] they are almost inactive in T cells, while cADPR works effectively as a Ca^{2+}-mobilizing second messenger both in T cell and in neuronal cell systems.[5a–c] These very important findings indicate that the target proteins and/or the mechanism of action of cADPR in T cells and neuronal cells are different.[5d]

Carbocyclic nucleosides are known as effective mimics of natural nucleosides.[6] An antibiotic aristeromycin (Fig. 2) is a naturally occurring mimic of adenosine,[7] in which the ribose of adenosine is replaced by a carbocyclic-ribose, and has antiviral and antitumor activities, due to its competitive inhibitory effect to adenosine against *S*-adenosylhomocysteine (AdoHcy) hydrolase.[8] Another nucleosidic antibiotic neplanocin A (Fig. 2), the structure of which corresponds to the 4′,6′-unsaturated derivative of aristeromycin, has significant antiviral and also antitumor effects, which clearly surpass those of aristeromycin.[8,9] Therefore, since an unsaturated carbocyclic-ribose may mimic the ribose more precisely than the corresponding saturated congener, we newly designed the unsaturated carbocyclic ribose analog of cADPR, that is, 4″,6″-didehydro-cADPcR (**3**). We describe here synthesis and biological effects of **3** to show that it is significantly active in T cells.

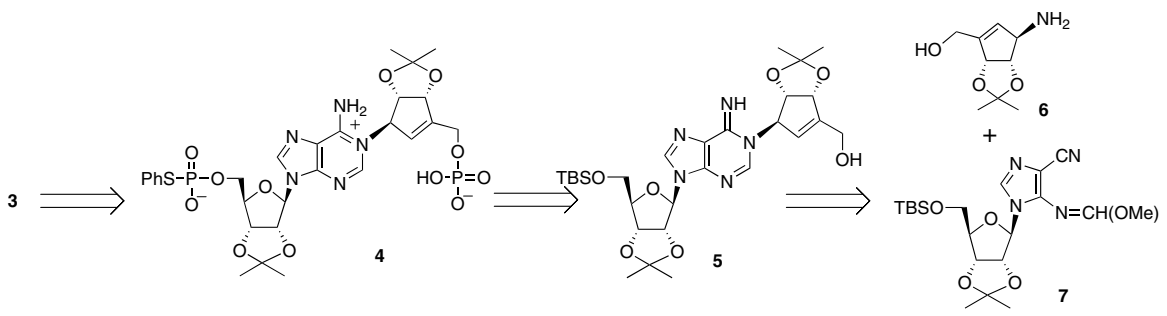
We planned to synthesize the target 4″,6″-didehydro-cADPcR (**3**) by a route, as summarized in Scheme 1, in which intramolecular condensation reaction with a *S*-phenyl phosphorothioate-type substrate **4** forming the 18-membered pyrophosphate structure was employed. It was key point whether the condensation could occur, since the 18-membered pyrophosphate structure containing an unsaturated carbocyclic ribose seemed to be strained compared with the corresponding saturated congener. Substrate **4** could be converted from the N1-unsaturated-carbocyclic-ribosyl adenosine derivative **5**, which would be constructed by condensation between the optically active cyclopentenyl amine **6** with a known imidazole nucleoside **7**.[4c]

The chiral cyclopentenyl amine **6** was synthesized from a cyclopentenone **8**, which was prepared by the method reported by Jeong and co-workers[10] (Scheme 2). 1,2-Reduction of the enone system of **8** with $NaBH_4/CeCl_3$ in MeOH stereoselectively gave the allylic α-alcohol **9**, of which mesylation and subsequent treatment with LiN_3 in HMPA/DMSO[11] afforded the β-azide **10**. Removal of the TBDPS group of **10** followed by reduction of the azido group with Ph_3P in aqueous THF gave the desired cyclopentenyl amine **6**.

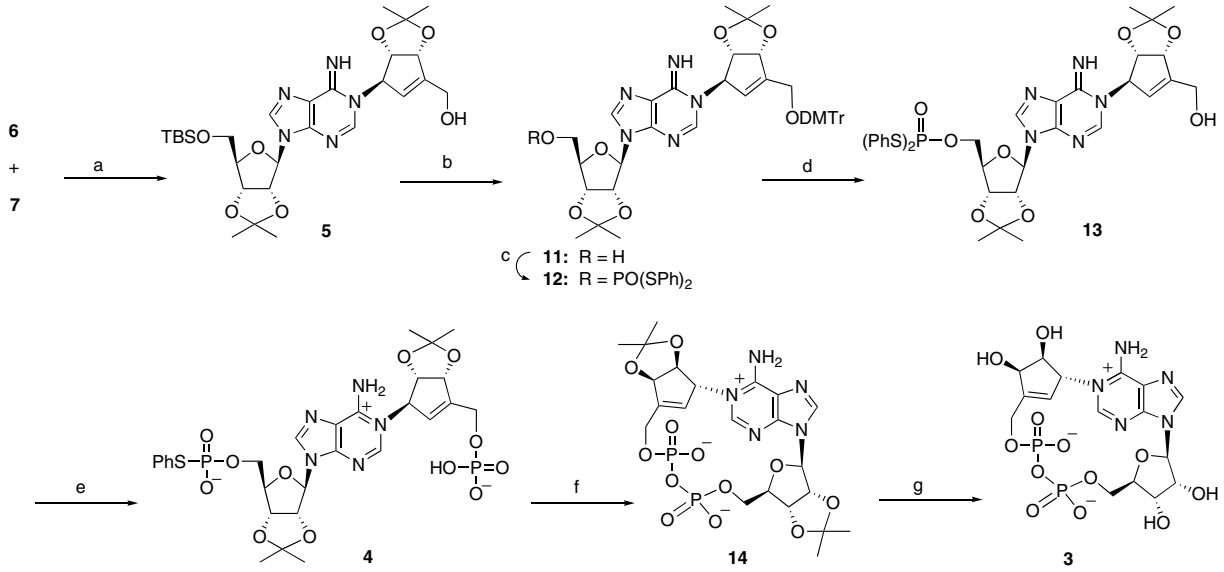
The target 4″,6″-didehydro-cADPcR (**3**) was successfully synthesized from the chiral cyclopentenyl amine **6** as shown in Scheme 3. The 4″,6″-didehydro-N1-carbocyclic-ribosyl adenosine derivative **5** was obtained in 58% yield by the treatment of a mixture of the amine **6** and the imidazole nucleoside **7**[4c] with K_2CO_3 in MeOH at room temperature. The 5″-hydroxy group of **5** was protected with a dimethoxytrityl (DMTr) group, and the 5′-*O*-TBS group of the product was removed with TBAF to give **11**. Treatment of **11** with an *S*,*S*′-diphenyl phosphorodithioate (PSS)/2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl)/pyridine system[12] gave the 5′-bis(phenylthio)phosphate **12**. After removal of 5′-*O*-DMTr group of **12** with aqueous AcOH, a phosphoryl group was introduced at the resulting 5″-primary hydroxyl by Yoshikawa's method with $POCl_3/(EtO)_3PO$,[13] followed by treatment of the product with H_3PO_2 and Et_3N[14] in the presence of *N*-methylmaleimide (NMM) in pyridine,[4c] to afford the 5″-*O*-phosphoryl *S*-phenyl phosphorothioate **4**, the substrate for the key intramolecular condensation.



Scheme 2.



Scheme 1.



Scheme 3. Reagents and conditions: (a) K_2CO_3, MeOH, rt, 58%; (b) (1) DMTrCl, pyridine, rt; (2) TBAF, THF, AcOH, rt, quant; (c) PSS, TPSCI, pyridine, rt, 74%; (d) aq 60% AcOH, rt, 80%; (e) (1) $POCl_3$, $(EtO)_3PO$, 0 °C; (2) H_3PO_2, Et_3N, NMM, pyridine, 0 °C to rt, 28%; (f) $AgNO_3$, MS 3 Å, Et_3N, pyridine, rt, 65%; (g) aq HCO_2H, rt; (2) aq NH_3, 86%.

When a solution of **4** in pyridine was added slowly to a mixture of a large excess of $AgNO_3$ and Et_3N in the presence of MS 3 Å in pyridine at room temperature,[4,15] the desired cyclization product **14** was successfully obtained in 65% yield, in spite of the strained 18-membered structure. Finally, removal of the isopropylidene groups of **14** with aqueous HCO_2H followed by treatment with aqueous ammonia furnished 4″,6″-didehydro-cADPcR (**3**).[17]

The Ca^{2+}-mobilizing effect of 4″,6″-didehydro-cADPcR (**3**) was evaluated in permeabilized Jurkat T cells. cADPR (**1**) released Ca^{2+} from intracellular stores as reported previously.[3d] While cADPcR (**2**) as compared to cADPR (**1**) is only a weak agonist for Ca^{2+} release in permeabilized Jurkat T cells, 4″,6″-didehydro-cADPcR (**3**) is much more potent than cADPcR (**2**) (Fig. 3). At 100 μM 4″,6″-didehydro-cADPcR induced a robust response while almost no effect of cADPcR was obtained. Interestingly, at 500 μM 4″,6″-didehydro-cADPcR a significantly higher amplitude was observed as compared to the natural second messenger cADPR. It is an important finding that, although the saturated carbocyclic analog cADPcR (**2**) is inactive, the corresponding 4″,6″-unsaturated analog **3** has a significant Ca^{2+}-mobilizing potency in T cells.

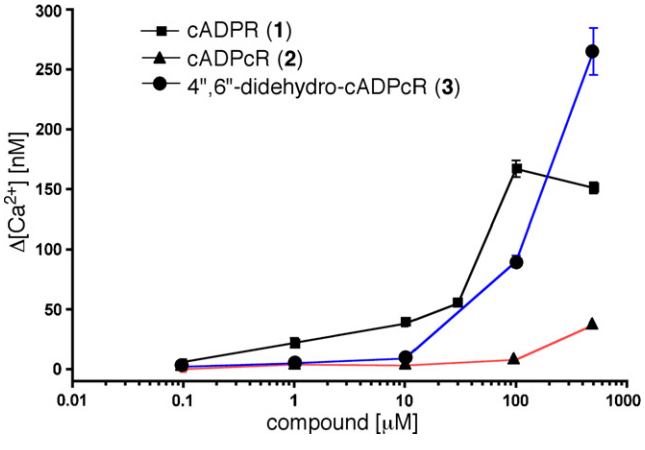


Fig. 3. Effects of cADPR (**1**), cADPcR (**2**), and 4″,6″-didehydro-cADPcR (**3**) in permeabilized Jurkat T cells. Jurkat T cells were permeabilized by saponin. Ca^{2+} stores were refilled by addition of ATP and ATP-regenerating system. [Ca^{2+}] was determined fluorimetrically using fura2/free acid. Compounds were added at the concentrations indicated. Data are differences between the Ca^{2+} concentration upon addition and before addition of compound. Mean ± SEM ($n = 3$–6).

In conclusion, 4″,6″-didehydro-cADPcR (**3**) designed as a novel stable mimic of cADPR was successfully synthesized using the key intramolecular condensation reaction with the *S*-phenyl phosphorothioate-type substrate. Evaluation of Ca^{2+}-mobilizing potency using permeabilized Jurkat T-lymphocytes showed that 4″,6″-didehydro-cADPcR is significantly active in T cells and therefore it may be useful as a biological tool and/or drug lead because of the biologically and chemically stable carbocyclic structure.[16]

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15. The intermolecular condensation between a *S*-phenyl phosphorothioate and a phosphomonoester is effectively promoted by I_2 or $AgNO_3$ to give the corresponding pyrophosphate compound: see Ref. 12.
16. In NG 108-15 neuronal cells,**3** did not show apparent Ca^{2+}-mobilizing activity, which suggests that **3** is selectively active in T cells.
17. Physical data of **3** (triethylammonium salt): ^{1}H NMR (D_2O, 500 MHz, K^+ salt) δ 8.63 (s, 1H), 8.49 (s, 1H), 6.31 (m, 1H), 6.07 (d, 1H, $J = 5.7$ Hz), 5.44 (m, 1H), 5.29 (m, 1H), 4.91 (m, 2H), 4.67 (m, 1H), 4.62 (m, 2H), 4.34 (m, 2H), 4.07 (m, 1H); ^{13}C NMR (D_2O, 125 MHz) δ 153.1, 151.6, 147.4, 146.0, 143.3, 124.4, 121.0, 91.3, 85.0, 74.1, 73.6, 73.2, 71.0, 69.0, 64.7, 61.7; ^{31}P NMR (D_2O, 202 MHz) δ −10.0 (d, $J = 15.3$ Hz), −10.6 (d, $J = 15.3$ Hz); HRMS (FAB, negative) calcd for $C_{16}H_{20}N_5O_{12}P_2$ 536.0589 [$(M-H)^-$], found 536.0577; UV (H_2O) λ_{max} 260 nm.