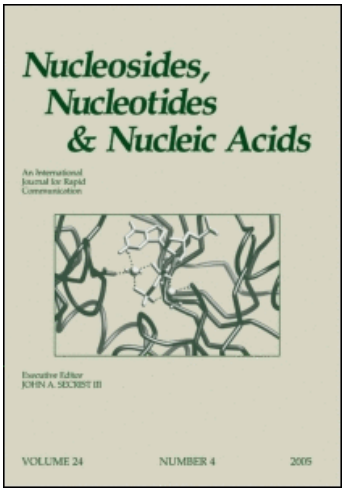

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Takashi Kudoh[a]; Masayoshi Fukuoka[a]; Satoshi Shuto[a]; Akira Matsuda[a]
[a] Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF CYCLIC ADP-CARBOCYCLIC-RIBOSE ANALOGS: STRUCTURE-ACTIVITY RELATIONSHIP AND CONFORMATIONAL ANALYSIS OF *N*-1-CARBOCYCLIC-RIBOSE MOIETY

Takashi Kudoh, Masayoshi Fukuoka, Satoshi Shuto, and Akira Matsuda
▫ *Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan*

▫ *Several cyclic ADP-carbocyclic-ribose analogs* ***3–10*** *modified in the N-1-carbocyclic-ribose moiety were synthesized. Their* Ca^{2+}*-releasing activity was estimated in sea urchin eggs to show that the 3″-deoxy analog* ***6*** *shows 5 times more potent activity than cADPcR, but the 2″,3″-didieoxy-2″,3″-unsunsaturated analog* ***3*** *has very weak activity. We also calculated their stable conformation and found that* ***3*** *and* ***6*** *were significantly different in their stable conformation.*

Keywords Cyclic ADP-Carbocyclic-Ribose, Ca^{2+}-Releasing Activity, Conformational Analysis

INTRODUCTION

Cyclic ADP-carbocyclic-ribose (cADPcR, **1**) was designed as a stable mimic of cyclic ADP-ribose (cADPR, **2**), an intracellular Ca^{2+}-mobilizing second messenger.[1] cADPcR has a carbocyclic-ribose bound to *N*-1 position of adenine moiety instead of D-ribose in cADPR, the synthesis of which had previously been completed using effective formation of the pyrophosphate linkage.[2] cADPcR is actually very stable under chemical and biological conditions and exhibits 3 times more potent Ca^{2+}-releasing activity than cADPR in sea urchin egg homogenates.

In this study, we focused on SAR of the *N*-1-carbocyclic-ribose moiety of cADPcR. Only a few analogs modified in this moiety have been synthesized, because of the difficulty in their synthesis by the classical enzymatic method.[3–5] So we designed and synthesized the eight cADPcR analogs **3–10** modified in the *N*-1-carbocyclic-ribose moiety (Figure 1). We estimated their Ca^{2+}-releasing activity and analyzed their stable conformation.

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Address correspondence to Satoshi Shuto, Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan.

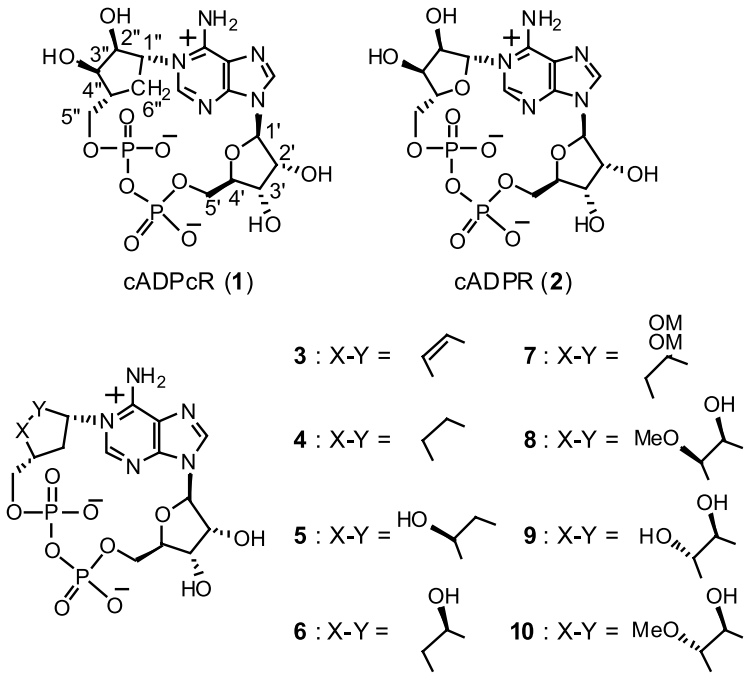


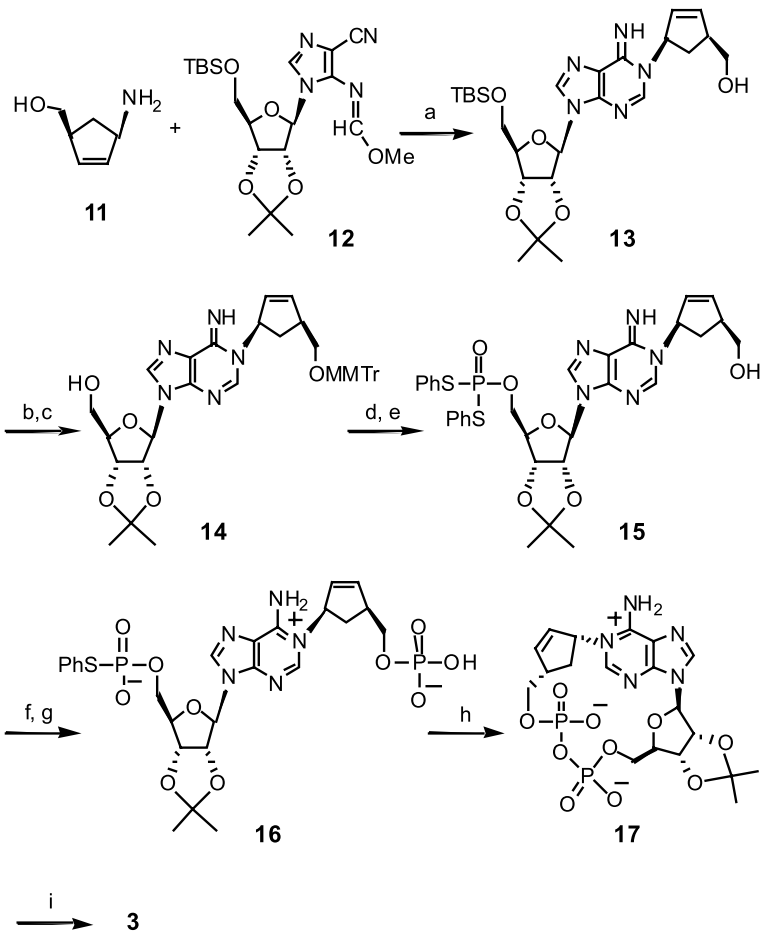
FIGURE 1

SYNTHESIS

Scheme 1 shows the synthetic route of the cADPcR analog **3**. The optically active cyclopentenylamine units **11**, corresponding to the carbocyclic-ribose moiety, were condensed with imidazole nucleoside **12** to construct the *N*-1-cyclopentenylenyladenosine derivative **13**. The 5″-hydroxy group of **13** was protected by MMTr group, and 5′-*O*-TBS protection was removed to give **14**. Bisphenylthiophosphate group was introduced to the 5′-position of **14** with *S*,*S*′-diphenylphosphorodithioate (PSS) and TPSCl, and then the 5″-*O*-MMTr group was removed to give **15**. The 5″-hydroxy group of **15** was phosphorylated by Yoshikawa's method, and one phenylthio group was removed by hypophosphorous acid to give the cyclization substrate **16**. The intramolecular condensation of two phosphates group was successfully proceeded by a silver nitrate/MS3A/pyridine systems to give the cyclic product **17**. Finally, remaining isopropylidene protection of hydroxy groups was removed by aqueous formic acid to complete the synthesis of **3**. Similarly, the other analogs **4–10** were synthesized using the corresponding optically active cyclopentylamine derivatives.

BIOLOGICAL ACTIVITY

We estimated Ca^{2+}-releasing activity of these analogs in sea urchin egg homogenates. The EC_{50} values of analogs were summarized in Figure 2. In this



SCHEME 1 (a) K_2CO_3, MeOH, 82%; (b) MMTrCl, pyridine, 84%; (c) TBAF, AcOH, THF, 96%; (d) PSS, TPSCl, pyridine, 54%; (e) 80% AcOH aq., 77%; (f) $POCl_3$, $(EtO)_3$ PO, 0°C; (g) H_3PO_2, Et_3N, *N*-methylmaleimide, pyridine, 0°C→r.t., 2 steps 64%; (h) $AgNO_3$, Et_3N, MS3A, pyridine, 84%; (i) 60% HCOOH aq., quant.

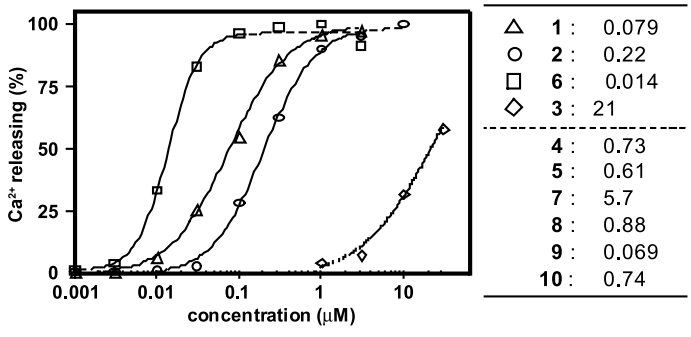


FIGURE 2 Ca^{2+} releasing activity in sea urchin egg homogenates. Maximum Ca^{2+} releasing by cADPR is 100%.

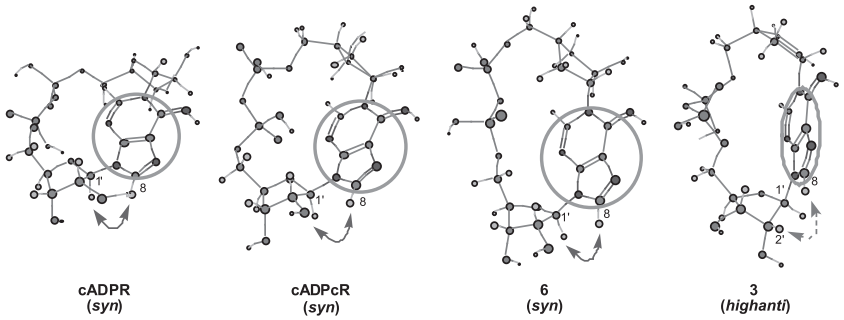


FIGURE 3 Conformational analysis by molecular dynamics: simulated annealing with NOESY constraints.

system, the EC_{50} value of cADPR was 0.22 μM, and cADPcR was 0.079 μM. As a result, 3″-deoxy-cADPcR (**6**) showed 5 times more potent activity than cADPcR. Xylose type analog **9** had potent activity equal as cADPcR, and 2″-deoxy (**5**), 2″,3″-dideoxy (**4**), 3″-*O*-methyl (**8**,**10**) analogs showed moderate activity. However, the unsaturated analog **3** showed very weak activity.

CONFORMATIONAL ANALYSIS

We calculated the stable conformation of cADPR, cADPcR and the cADPcR analogs **6** and **3** by molecular dynamics with a simulated annealing method, based on the NOE constraints of the intramolecular proton pairs measured in D_2O. Analogs with strong Ca^{2+}-mobilzing activity, such as cADPR, cADPcR, and **6** had similar *syn* conformations around *N*-9-glycosidic linkage, while the almost inactive analog **3** showed *highanti* conformation (Figure 3).

REFERENCES

1. Clapper, D.L.; Walseth, T.F.; Dargie, P.J.; Lee, H.C. Pyridine nucleotide metabolite stimulate calcium release from sea urchin egg microsomes desensitized to inositol triphosphate. J. Biol. Chem. **1987**, *262*, 9561–9568.
2. Shuto, S.; Fukuoka, M.; Manikowsky, A.; Ueno, Y.; Nakano, T.; Kuroda, R.; Kuroda, H.; Matsuda, A. Total synthesis of cyclic ADP-carbocyclic-ribose, a stable mimic of Ca^{2+}-mobilizing second messenger cyclic ADP-ribose. J. Am. Chem. Soc. **2001**, *123*, 8750–8759.
3. Zhang, F.-J.; Gu, Q.-M.; Sih, C.J. Bioorganic chemistry of cyclic ADP-ribose (cADPR). Bioorg. Med. Chem. **1999**, *7*, 653–664.
4. Shuto, S.; Fukuoka, M.; Kudoh, T.; Garnham, C.; Galione, A.; Potter, B.V.L.; Matsuda, A. Convergent synthesis and unexpected Ca^{2+}-mobilizing activity of 8-substituted analogues of cyclic ADP-carbocyclic-ribose, a stable mimic of the Ca^{2+}-mobilizing second messenger cyclic ADP-ribose. J. Med. Chem. **2003**, *46*, 4741–4749.
5. Shuto, S.; Matsuda, A. Chemistry of cyclic ADP-ribose and its analogs. Curr. Med. Chem. **2004**, *11*, 827–845.