Pergamon

Tetrahedron Letters 39 (1998) 7341–7344

TETRAHEDRON LETTERS

Synthetic Studies of Carbocyclic Analogs of Cyclic ADP-Ribose. Formation of a Cyclic Dimer, a 36-Membered-ring Product, in the Condensation Reaction of an 8-Bromo-N^1-[5-(phenylthiophosphoryl)carbocyclic-ribosyl]inosine 5'-Phosphate Derivative Mediated by $AgNO_3$

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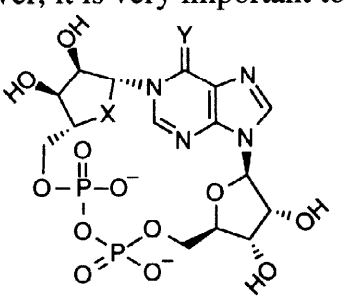
Received 17 June 1998; revised 21 July 1998; accepted 23 July 1998

Abstract: Formation of symmetric 36-membered-ring product **14** was observed in the synthetic study of a stable mimic of cyclic ADP-ribose (cADPR, **1**). When 8-bromo-N^1-[5-(phenylthiophosphoryl)carbocyclic-ribosyl]inosine 5'-phosphate derivative **5** was treated with $AgNO_3$ and Et_3N in *N*-methyl-2-pyrrolidinone-HMPA (3:1), the substrate was dimerized and cyclized to give **14** in 39% yield.

Keywords: Nucleic acids analogues; Cyclopentanes; Phosphorylation; Dimerisation

Cyclic ADP-ribose (cADPR, **1**), a newly discovered cyclic nucleotide [1], mobilizes intracellular Ca^{2+} in various cells more actively than inositol 1,4,5-trisphosphate (IP_3) although a mechanism is completely independent of that of IP_3 [2,3], indicating that it 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 chemo-enzymatic methods [4-15]. ADP-ribosyl cyclase from *Aplysia california* catalyzes the internal ribosylation at the N^1-position of a purine moiety of modified NAD^+ that has been chemically or enzymatically prepared to give the corresponding cADPR analogs [4-15]. However, it is very important to develop more flexible synthetic methods for cADPR and its analogs, since the analogs that can be obtained by the above method are limited due to the substrate specificity of the enzyme.

We designed carbocyclic analogs **2** and **3** as stable mimics of cADPR, since cADPR is readily hydrolyzed both enzymatically [2,3] and non-enzymatically at the unstable N^1-glycosidic linkage of the adenine moiety.[1] Stable analogs of cADPR which have Ca^{2+}-mobilizing activity in cells similar to that of cADPR are very useful as pharmacological tools and are urgently required. Recently,



1: X = O, Y = NH
2: X = CH, Y = NH
3: X = CH, Y = O

This paper constitutes part 178 of Nucleosides and Nucleotides. Part 177: Matsuda A, Kosaki H, Saitoh Y, Yoshimura Y, Minakawa N, Nakata H. J. Med. Chem., in press.

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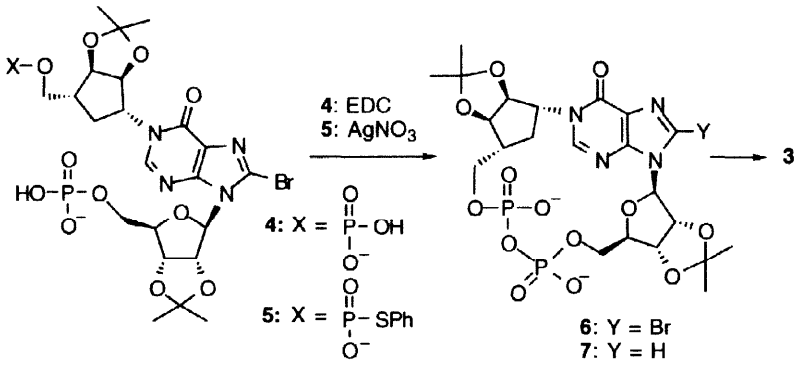
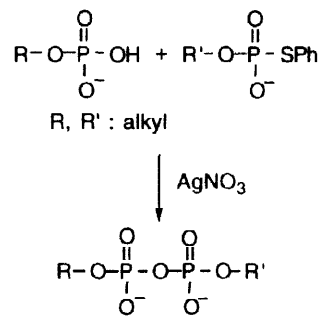
0040-4039/98/$ - see front matter
PII: S0040-4039(98)01575-5

we achieved the total synthesis of the inosine congener **3** [16] which is the first chemical synthesis of a cADPR analog and may lead to the development of general methods for synthesizing cyclic nucleosides of this type. During that study, we also found that the key intramolecular condensation reaction between the two phosphate groups of **4** (Scheme 1) could be significantly facilitated by introducing a bromo substituent at the 8-position of the hypoxanthine ring, probably because the molecule is conformationally restricted in a *syn*-form around its glycosyl linkage.[2] However, the yield of the key step, namely, the intramolecular condensation reaction between the two phosphate groups of **4**, was not sufficient (yield 23%).

Scheme 1



Scheme 2

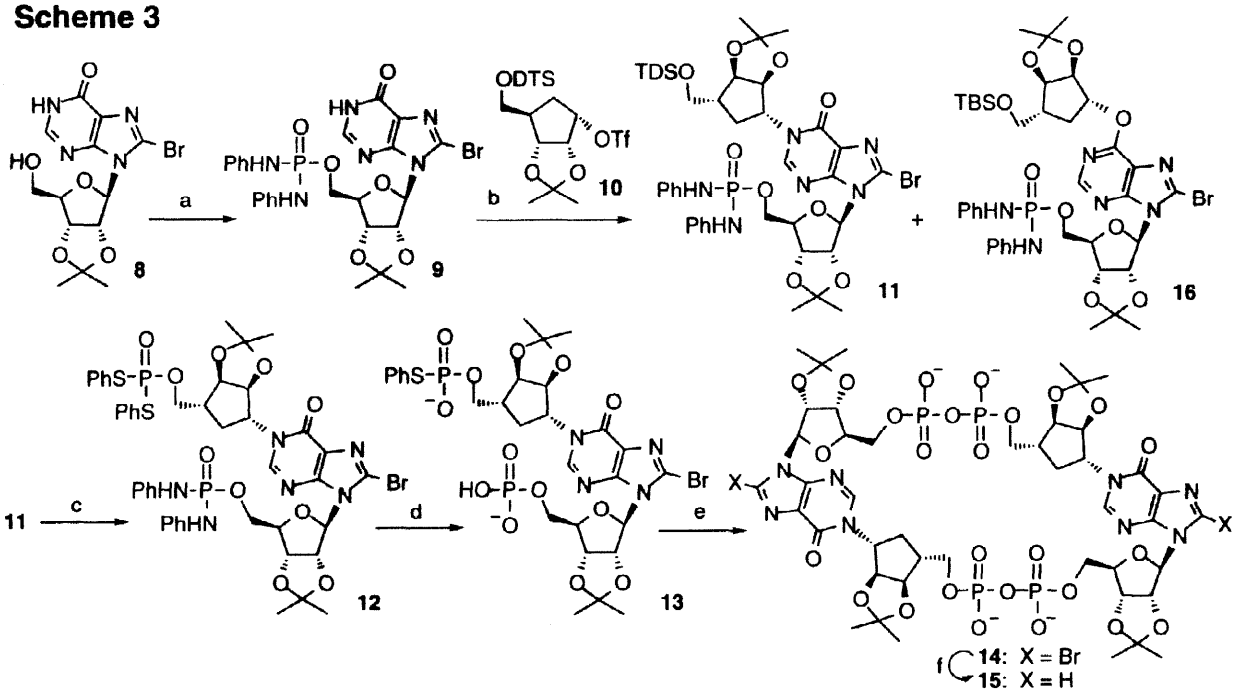


Hata and co-workers have found that $AgNO_3$ effectively mediates the condensation between an *S*-phenyl phosphorothioate and a phosphomonoester to give the corresponding pyrophosphate compounds, as shown in Scheme 2, and successfully synthesized the 5'-cap structure of mRNA using this method [17,18]. We hoped to develop an alternative method for obtaining sufficient cADPR analogs for biological evaluations by using Hata's method. We introduced a dianilinophosphoryl group [19] to the nucleoside unit as a phosphate group equivalent. 8-Bromo-2',3'-*O*-isopropylideneinosine (**8**) was treated with $(PhNH)_2POCl$ and tetrazole in pyridine [19] to give 5'-*O*-phosphorodianilidate **9** in 75% yield. The S_N2 substitution reaction between carbocyclic unit **10** [16] and **9** in the presence of K_2CO_3 and 18-crown-6 in DME gave the desired N^1-substituted product **11** in 31% yield along with the corresponding O^6-regioisomer **16** in 10% yield. The regio- and stereochemistries were confirmed by their ^{1}H-NMR spectra and NOE experiments.[3] The DTS group of **11** was removed with TBAF, and a bis(phenylthio)phosphoryl group was then introduced at the resulting 5"-primary hydroxyl of the carbocyclic-ribose moiety by treating it with cyclohexylammonium *S,S'*-diphenyl phosphorodithioate (PSS), triisopropylbenzenesulfonyl chloride (TPSCl), and tetrazole in pyridine [20] to give **12** in 60% yield. Successive treatment of **12** with isoamyl nitrite in a mixed solvent of pyridine-AcOH-Ac_2O, and H_3PO_2 in pyridine [21] gave **13**, the substrate for the intramolecular condensation reaction, in 49% yield as a triethylammonium salt.

When a solution of **13** in *N*-methyl-2-pyrrolidinone (MPD)-HMPA (3:1) was added slowly over 15 h to an excess of $AgNO_3$ and Et_3N in the same solvent, a cyclized product was isolated in 39% yield as a sodium salt, after purification by ion-exchange column chromatography and C18-HPLC. However, the product **14**[4] was different from the previously

1. cADPR is unstable even in neutral aqueous solution ($t_{1/2}$ = 40 h): Lee HC, Aarhus R. Biochim. Biophys. Acta. 1993;1164:68-74.
2. When the reaction was performed with a substrate which lacked the 8-bromo substituent, none of the desired intramolecular cyclization product was obtained: ref. 7.
3. **11**; irradiated at H-2, obserbed at H-1" (12%). **16**; irradiated at H-2, obserbed at H-1" (0.6%).

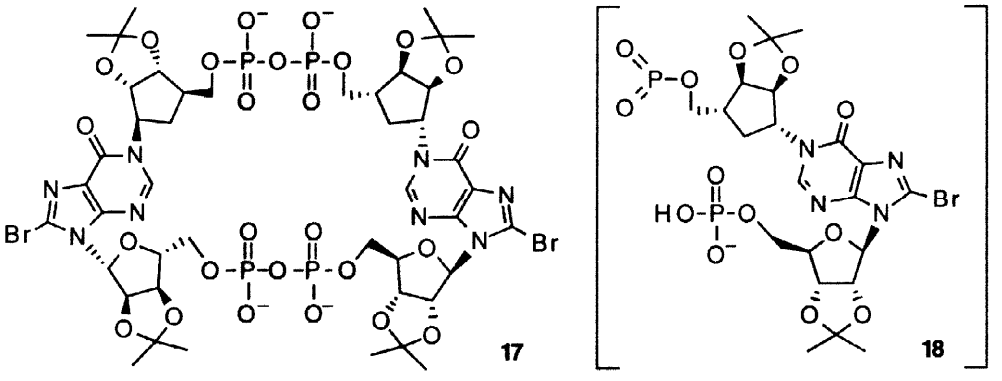
Scheme 3



Reagents: a) $(PhNH)_2POCl$, tetrazole, pyridine, rt, 75%; b) K_2CO_3, 18-crown-6, DME, 50 °C, 31% (**11**), 10% (**16**); c) 1) TBAF, THF, rt, 73%; 2) PSS, TPSCl, tetrazole, pyridine, rt, 60%; d) 1) isoamy nitrite, Ac_2O, AcOH, pyridine, rt, 2) H_3PO_2, Et_3N, pyridine, rt, 49%; e) $AgNO_3$, Et_3N, MPD, HMPA, rt, 39%; f) H_2, Pd-C, aq. EtOH, 48%.

identified compound **6** that was obtained by treating **4** with EDC [16]. The presence of a pyrophosphate linkage in the molecule was suggested by the following two points: 1) in its ^{31}P-NMR spectrum, two signals (-10.5 ppm, J = 22.0 Hz and -10.9 ppm, J = 22.0 Hz) were observed in the area typical for pyrophosphate groups, and 2) the product was completely resistant to hydrolysis by alkaline phosphatase, which generally hydrolyzes phosphomonoester linkages.[5] Although a molecular-ion peak of **14** was not obtained in its MS analysis, after reductive removal of the bromo group, a molecular-ion peak corresponding to the structure of the symmetric cyclic dimer **15** was observed at *m/z* 1329.1976 (calcd for $C_{44}H_{57}N_8O_{26}P_4Na_4$, 1329.1925). The MS data also suggested the formation of another cyclic-dimer **17**. However, it would be improbable because 1) the two phosphorus signals were coupled mutually in the ^{31}P-NMR spectrum (J = 22.0 Hz), indicating nonequivalence of the two phosphorus atoms of the pyrophosphate moiety, and 2) formation of a pyrophosphate linkage between the phosphates groups of the nucleoside 5'- and carbocyclic moiety 5"-positions was suggested by a reaction mechanism via intermediate **18**. In the ^{31}P-NMR spectra, while the signals of the phosphorus atoms of dimers **14** and **15** were observed around -10 ppm (area typical for pyrophosphate groups), similar to those of **6** and **7**, the J values of dimers **14** and **15** (ca. 22 Hz) were different from those of monomers **6** and **7** as well as **1** (ca. 15 Hz) [16], probably due to the difference in ring size. It was difficult to distinguish between the cyclic monomer **6** (or **7**) and the dimer **14** (or **15**) by their ^{1}H- and ^{13}C-NMR spectra because of the symmetric structure of **14** (or **15**).

4. ^{1}H-NMR data of **14** (500 MHz, D_2O) δ 8.45 (s, 1 H), 6.30 (d, 1 H, J = 2.7 Hz), 5.47 (dd, 1 H, J = 2.7, 6.6 Hz), 5.20 (dd, 1 H, J = 6.6, 4.8 Hz), 5.16-5.10 (m, 1 H), 4.85-4.80 (m, 1 H), 4.70 (dd, 1 H, J = 6.3, 5.9 Hz), 4.51-4.47 (m, 1 H), 4.31-4.25 (m,1 H), 4.22-4.17 (m, 1 H), 4.09 (t, 2 H, J = 5.1 Hz), 2.57-2.52 (m, 1 H), 2.45-2.40 (m, 1 H), 2.24-2.17 (m, 1 H), 1.63, 1.55, 1.27, 1.21 (each s, each 3 H).
5. Compound **14** (0.1 OD_{256} units) was incubated with calf intestine alkaline phosphatase (6 units) in Tris-HCl buffer (500 mM, pH 9.0) containing 10 mM of $MgCl_2$ at 37 °C for 12 h, and the reaction mixture was analyzed by HPLC.



17 18

Therefore, the condensation reaction product was determined to be the symmetric cyclic dimer **14** based on the above data.

Synthetic studies of cADPR analogs have been extensively studied because of their biological importance. This study suggests a possibility of producing cyclic dimers in the synthesis of compounds of this type. Therefore, attention should be paid to the structural determination of cyclized products. The careful analysis of compounds by ^{31}P-NMR, especially of *J* values, as well as mass spectrometry would be needed.

Most recently, De Flora and Lee found that CD38 and ADP-ribosyl cyclase catalyze the synthesis of a linear dimer of ADP-ribose that potentiates the Ca^{2+}-mobilizing activity of cADPR [22]. Accordingly, the biological effects of these cyclic dimers may also be interesting.

Acknowledgment: This investigation was supported in part by a Grant from the Ministry of Education, Science, Sports, and Culture of Japan. We are grateful to Prof. K. Ikeda (Center for Instrumental Analysis, Hokkaido University) and Dr. T. Yamamoto (Asahi Chemical Industry) for their help with the mass spectra measurements.

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