

# Alternative Synthesis of Cyclic IDP-Carbocyclic Ribose. Efficient Cyclization of an 8-Bromo-*N*<sup>1</sup>-[5-(phosphoryl)carbocyclic- ribosyl]inosine 5'-Phenylthiophosphate Derivative Mediated by Iodine<sup>1</sup>

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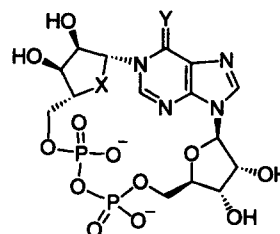
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**Abstract:** An efficient synthesis of cyclic IDP-carbocyclic-ribose, as a stable mimic for cyclic ADP-ribose, was achieved. *N*<sup>1</sup>-Carbocyclic-ribosylinosine derivative **15**, prepared from *N*<sup>1</sup>-(2,4-dinitrophenyl)inosine derivative **10** and an optically active carbocyclic amine **11**, was converted to 8-bromo-*N*<sup>1</sup>-carbocyclic-ribosylinosine bis-phosphate derivative **20**. Treatment of **20** with I<sub>2</sub> in the presence of molecular sieves in pyridine gave the desired cyclic product **8** quantitatively, which was deprotected and reductively debrominated to give the target cyclic IDP-carbocyclic ribose (**3**). © 1999 Elsevier Science Ltd. All rights reserved.

**Keyword:** Nucleotides; Nucleic acid analogs; Phosphorylation; Thiophosphoryl compounds

Cyclic ADP-ribose (cADPR, **1**)<sup>2</sup> is a newly discovered general mediator involved in Ca<sup>2+</sup> signaling.<sup>3</sup> Due to their biological importance, the synthesis of cADPR analogs has been extensively studied by enzymatic and chemo-enzymatic methods using ADP-ribosylcyclase.<sup>4</sup> It is very important to develop flexible methods for synthesizing cADPR and its analogs, since the analogs that can be obtained by existing methods are limited due to the substrate specificity of the enzyme.

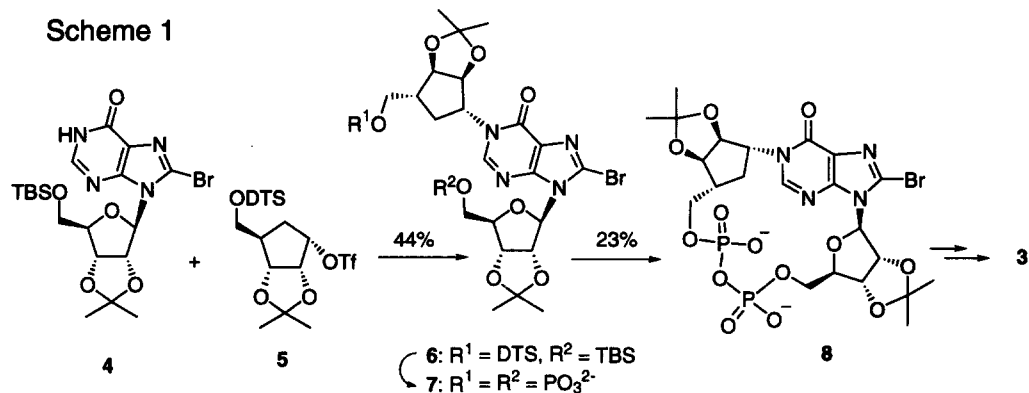
We designed carbocyclic analogs **2** and **3** as stable mimics of cADPR,<sup>5</sup> since cADPR is readily hydrolyzed both enzymatically<sup>3</sup> and non-enzymatically<sup>6</sup> at the unstable *N*<sup>1</sup>-glycosidic linkage of the adenine moiety. Stable analogs of cADPR which exhibit Ca<sup>2+</sup>-mobilizing activity in cells similar to that of cADPR are very useful as pharmacological tools and are urgently required. We previously achieved the total synthesis of the inosine congener **3**<sup>5</sup> which is the first chemical synthesis of a cADPR analog<sup>7</sup> and may lead to the development of general methods for synthesizing cyclic nucleotides of this type. During that study, we also found that the key intramolecular condensation reaction between the two phosphate groups of **7** (Scheme 1) proceeded only when a bromo substituent was introduced at the 8-position of the hypoxanthine ring of the substrate, probably because the molecule is conformationally restricted in a *syn*-form around its glycosidic linkage. However, the overall yield was very low, and its biological evaluation has not been



**1** (cADPR): X = O, Y = NH  
**2**: X = CH<sub>2</sub>, Y = NH  
**3**: X = CH<sub>2</sub>, Y = O

done. In this communication, we describe an efficient alternative method for preparing **3**.

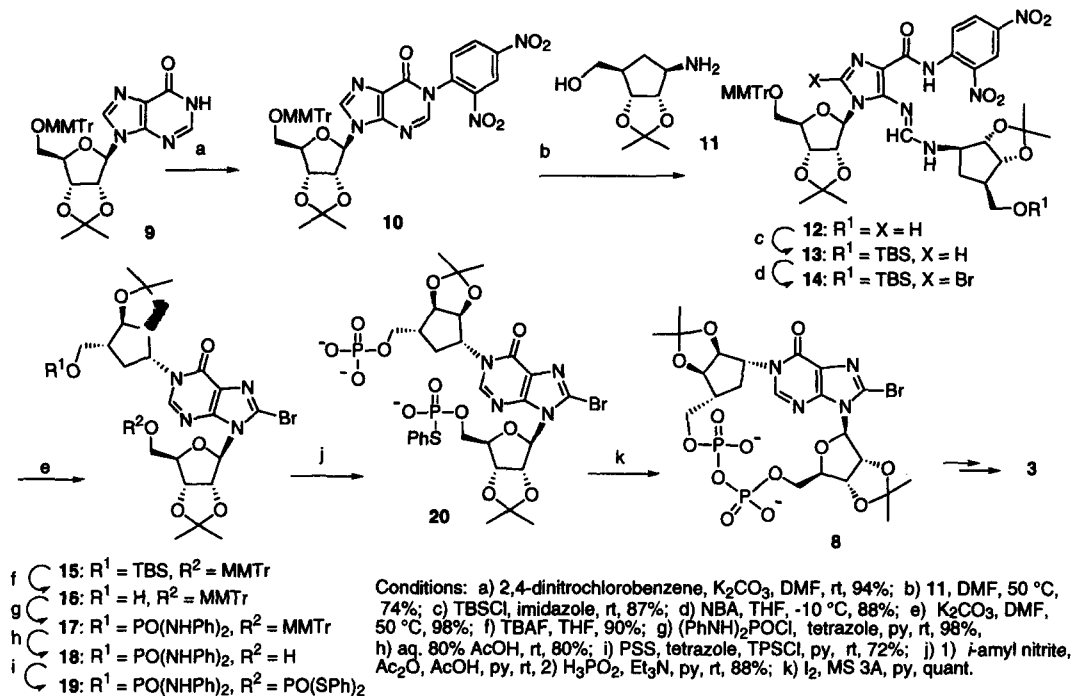
Our previous synthetic route (Scheme 1) had two main problems: 1) rather long reaction steps including an enzymatic optical resolution to construct the optically active carbocyclic unit **5** from cyclopentadiene, and 2) the yields of the two key steps, namely the coupling between inosine unit **4** and carbocyclic unit **5** and the intramolecular condensation reaction between the two phosphate groups of **7**, were insufficient (44% and 23%, respectively). Accordingly, the development of both a more straightforward method to construct the *N*<sup>1</sup>-carbocyclic-ribosyl-inosine structure and an efficient condensation method for forming the intramolecular pyrophosphate linkage is needed.



The improved synthesis of **3** is shown in Scheme 2. We planned to construct the *N*<sup>1</sup>-carbocyclic-ribosyl-structure by Piccialli's procedure<sup>8</sup> for preparing *N*<sup>1</sup>-alkylinosines from *N*<sup>1</sup>-(2,4-dinitrophenyl)inosine and alkylamines. *N*<sup>1</sup>-(2,4-Dinitrophenyl)inosine derivative **10** was prepared by treating 2',3'-*O*-isopropylidene-5'-*O*-MMTr-inosine (**9**) with 2,4-dinitrochlorobenzene and  $\text{K}_2\text{CO}_3$  in DMF.<sup>8</sup> The optically active carbocyclic amine **11** was readily prepared from commercially available (1*R*)-(-)-azabicyclo[2.2.1]hept-5-en-3-one by Blackburn's method.<sup>7b</sup> Heating **10** with **11** (10 equiv) at 50 °C in DMF gave the ring-cleaved product **12** in 74% yield.<sup>9</sup> After the 5"-hydroxyl of **12** was protected with a TBS group, it was treated with *N*-bromoacetamide (NBA) in THF to give 2-bromo derivative **14**. When **14** was heated in the presence of  $\text{K}_2\text{CO}_3$  at 50 °C in DMF, the desired ring-closure product **15** was obtained in 98% yield. The TBS group of **15** was removed with TBAF, and then a di(anilino)phosphoryl group was introduced at the resulting 5"-primary hydroxyl of **16** by treating it with  $(\text{PhNH})_2\text{POCl}$  and tetrazole in pyridine<sup>10</sup> to give **17** in high yield. After the 5'-*O*-MMTr group was removed with aqueous AcOH, a bis(phenylthio)phosphoryl group was introduced at the primary hydroxyl of the ribose moiety<sup>11</sup> with a cyclohexylammonium *S,S*-diphenylphosphorodithioate (PSS)/tetrazole/pyridine system to give **19**. Successive treatment of **19** with isoamyl nitrite in a mixed solvent of pyridine-AcOH-Ac<sub>2</sub>O, and  $\text{H}_3\text{PO}_2$  in pyridine<sup>12</sup> gave **20** in 88% yield as a triethylammonium salt. The intramolecular condensation reaction of **20** was investigated under various conditions. When a solution of **20** in pyridine was added slowly over 15 h, using a syringe-pump, to a mixture of  $\text{I}_2$  (20 equiv) and molecular sieves 3 A in pyridine at room temperature,<sup>13</sup> the most desirable result was achieved. The HPLC chart of the reaction mixture at 20 h is shown in Figure 1.<sup>14</sup> After purification

by C18-column chromatography, the desired cyclic product **8** was obtained quantitatively<sup>15</sup> as a triethylammonium salt, which was readily converted to target compound **3** by a previously described method.<sup>5</sup>

## Scheme 2



In conclusion, we have developed a very efficient method for synthesizing cyclic IDP-carbocyclic-ribose. The key intramolecular cyclization reaction occurred in high yield by an  $I_2$ -mediated method, in which conformational restriction of the substrate in a *syn*-form around its glycosyl linkage due to the substituent at the 8-position was very important. This may be a general method for synthesizing cyclic nucleotides of this type.

## Acknowledgment.

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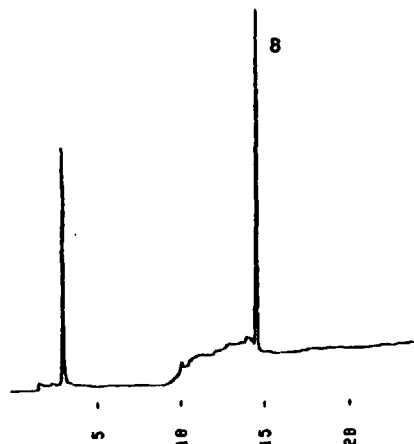


Figure 1. HPLC analysis of the reaction of **20** and  $I_2$  in the presence of molecular sieves in pyridine at 20 h.

## References and Notes

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14. After the addition of TEAA buffer (2 N, pH 7.0, 10  $\mu$ L), the reaction mixture (30  $\mu$ L) was evaporated, and then co-evaporated with water. The residue was partitioned between water and CHCl<sub>3</sub>. The aqueous layer was filtered with a syringe filter (cellulose acetate), and then analyzed by HPLC [column, YMS-ODS-M-80, 4.6 x 150 mm; 5 – 80% MeCN/0.1 M TEAA buffer (pH 7.0), 30 min; 254 nm]. Compound **8** was eluted at 14.7 min which was identified with the authentic sample synthesized previously, and a peak observed at 3.1 min was unknown non-nucleosidic compound due to its UV spectrum.
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