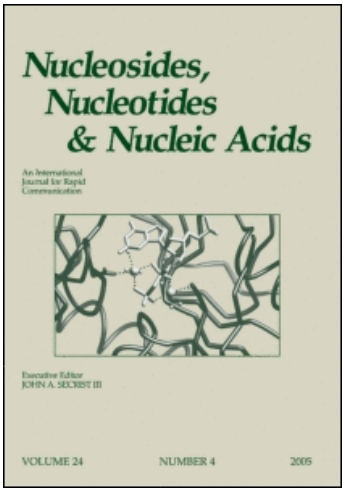

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INTRACELLULAR Ca^{2+}-MOBILIZING ADENINE NUCLEOTIDES. SYNTHESIS AND BIOLOGICAL ACTIVITY OF CYCLIC ADP-CARBOCYCLIC-RIBOSE AND *C*-GLYCOSIDIC ANALOG OF ADENOPHOSTIN A

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ABSTRACT

We designed novel Ca^{2+}-mobilizing purine nucleotides, cyclic ADP-carbocyclicribose **4**, and its inosine congener **5**, and *C*-glycosidic adenophostin A **6**. In the synthesis of cADPR analogs, the intramolecular condensation to form the pyrophosphate linkage should be the key step. We developed an efficient method for forming such an intramolecular pyrophosphate linkage by the activation of the phenylthiophosphate group with I_2 or $AgNO_3$. Using this method, we achieved to synthesize the target compounds **4** and **5**. The synthesis of *C*-glycosidic analog **6** of adenophostin A was achieved using a temporary silicon-tethered radical coupling reaction for constructing $(3'\alpha, 1''\alpha)$-*C*-glycosidic structure as the key step.

An elevation in intracellular levels of Ca^{2+} is known to be a key signaling event coupling cell activation by extracellular stimuli to a characteristic physiological response. These intracellular Ca^{2+} releases are mediated by two second messengers; one is *myo*-inositol trisphosphate (IP_3, **1**) (1) and the other is cyclic ADP-ribose (cADPR, **2**) (2). Adenophostin A (**3**), a 3′-*O*-glucosyladenosine

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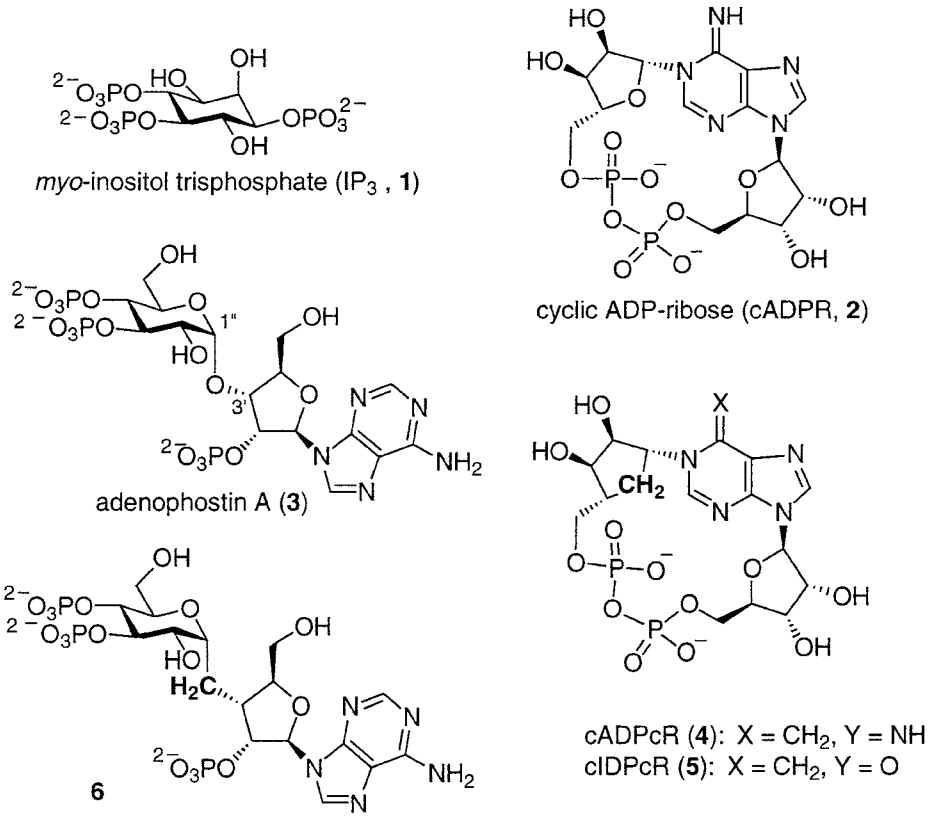

Figure 1.

trisphosphate, recently isolated from *Penicillium brevicompactum*, was identified as the strongest IP_3 receptor ligand yet known (3). We designed and synthesized novel Ca^{2+}-mobilizing purine nucleotides, cyclic ADP-carbocyclic-ribose **4**, and its inosine congener **5**, and *C*-glycosidic adenophostin A **6**, the structures of which are shown in Figure 1, based on the structural feature of cADPR and adenophostin A, since specific ligands for the receptors of the Ca^{2+}-mobilizing second messengers are very useful for proving the mechanism of Ca^{2+}-mobilizing signaling pathways.

THE STABLE MIMICS OF CADPR

Design of cADPcR and cIDPcR

cADPR (**2**) is a eighteen-membered cyclic pyrophosphate of *N*-1-ribosyladenosine (2b), and its Ca^{2+}-mobilizing activity was found by Lee in 1987 (2a). In cells, cADPR is synthesized from NAD^+ by ADP-ribosylcyclase and acts as a second messenger; it is hydrolyzed promptly by cADPR hydrolase to give ADP-ribose

and inactivated under physiological conditions (2c,d). cADPR is also known to be readily hydrolyzed non-enzymatically at the unstable *N*-1-glycosidic linkage of its adenine moiety to give ADP-ribose, even in neutral aqueous solution (2e). Although further intensive studies of cADPR are needed because of its biological importance, this biological as well as chemical instability of cADPR limits studies of its physiological role, at least to some extent. Therefore, stable analogs of cADPR that exhibit a Ca^{2+}-mobilizing activity in cells similar to that of cADPR are urgently required.

We designed cyclic ADP-carbocyclic-ribose (cADPcR, **4**) and the corresponding inosine congener, cIDP-carbocyclic-ribose (cIDPcR, **5**) as stable mimics of cADPR, in which an oxygen atom in the ribose ring of cADPR is replaced by a methylene group (4). The mimics **4** and **5** should be resistant to both enzymatic and chemical hydrolysis, since they lack the unstable *N*-1-glycosidic linkage of cADPR. These analogs preserve all of the functional groups of cADPR, except for this ring oxygen, and should have a conformation similar to that of cADPR. Therefore, we expected that these analogs would effectively mobilize intracellular Ca^{2+}, like cADPR, so that they could be used as pharmacological tools for studying the cADPR-modulated Ca^{2+} signaling pathways.

The First Synthesis of cIDPcR

We first tried to synthesize **5**, in which the intramolecular condensation of bisphosphate **7** to form the pyrophosphate linkage was a key step, but was difficult (Fig. 2) (4a,b,d). The difficulty of forming such an intramolecular pyrophosphate linkage has also been experienced by other groups (5). Sih reported that

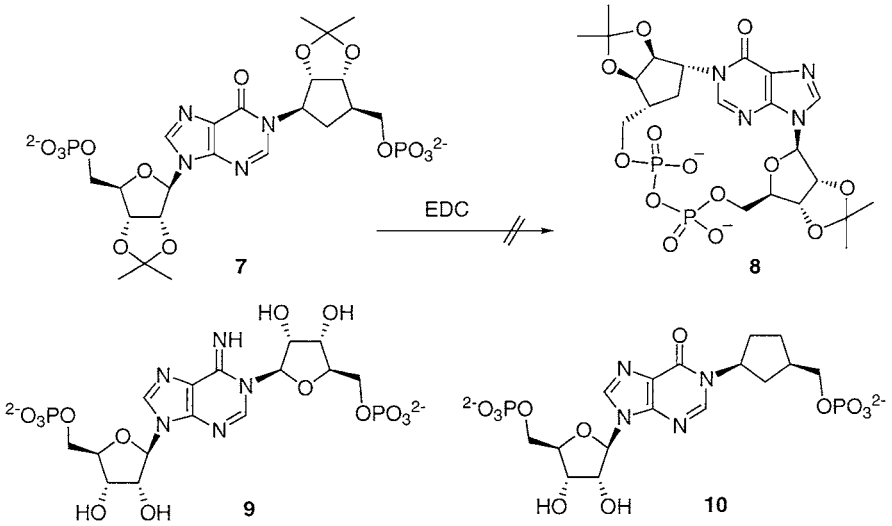

Figure 2.

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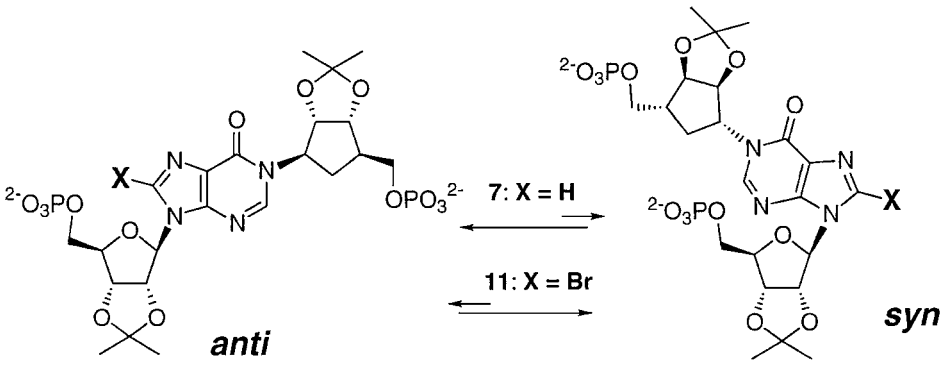

Figure 3.

condensation between the two phosphate groups of *N*-1-phosphoribosyl-AMP (**9**) with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was unsuccessful (5a). Later, ring-closure of bisphosphate **10** through the formation of a pyrophosphate linkage was examined by Fortt and Potter, but they also failed (5b).

We presumed that the conformation of the molecule determines whether or not intramolecular condensation occurs. The *syn-anti* conformation is an important determinant of the three-dimensional structure of nucleosides (6). As shown in Figure 3, in the *syn*-conformation, the two phosphate moieties are close to each other, however, the *anti*-conformer is predominant over the *syn*-conformers in usual nucleosides (6). This may be why these intramolecular condensations were unsuccessful.

It is well known that introducing a bulky substituent into the 8-position of purine nucleosides restricts the conformation in a *syn*-form through steric repulsion (6). We therefore designed the 8-bromo-substrate **11**. The 8-bromo group would restrict the conformation to the *syn*-form to facilitate the desired intramolecular condensation reaction.

An optically active carbocyclic trifrate **12** was prepared from cyclopentadiene by the previously reported method (4a). SN2 reaction between the carbocyclic trifrate **12** and this sugar-protected 8-bromoinosine **13** was performed with potassium bicarbonate in DME to give the desired *N*-1-substituted **14** in 44% yield, along with the corresponding O^6-substituted isomer in 9% yield. After removal of the silyl protecting groups, the desired bisphosphate **11** was successfully obtained by a phosphoramidite method with (2-cyanoethoxy)(*N*,*N*-diisopropylamino)chlorophosphine.

The intramolecular condensation reaction was investigated with EDC in *N*-methylpyrrolidone (NMP). When the bisphosphate was heated with EDC in NMP at 50°C, the desired cyclization product **15** was obtained in 23% yield. The 8-bromo group was removed reductively by catalytic hydrogenation. Finally, acidic treatment gave the target cIDPcR (**5**), as shown in Scheme 1 (4a).

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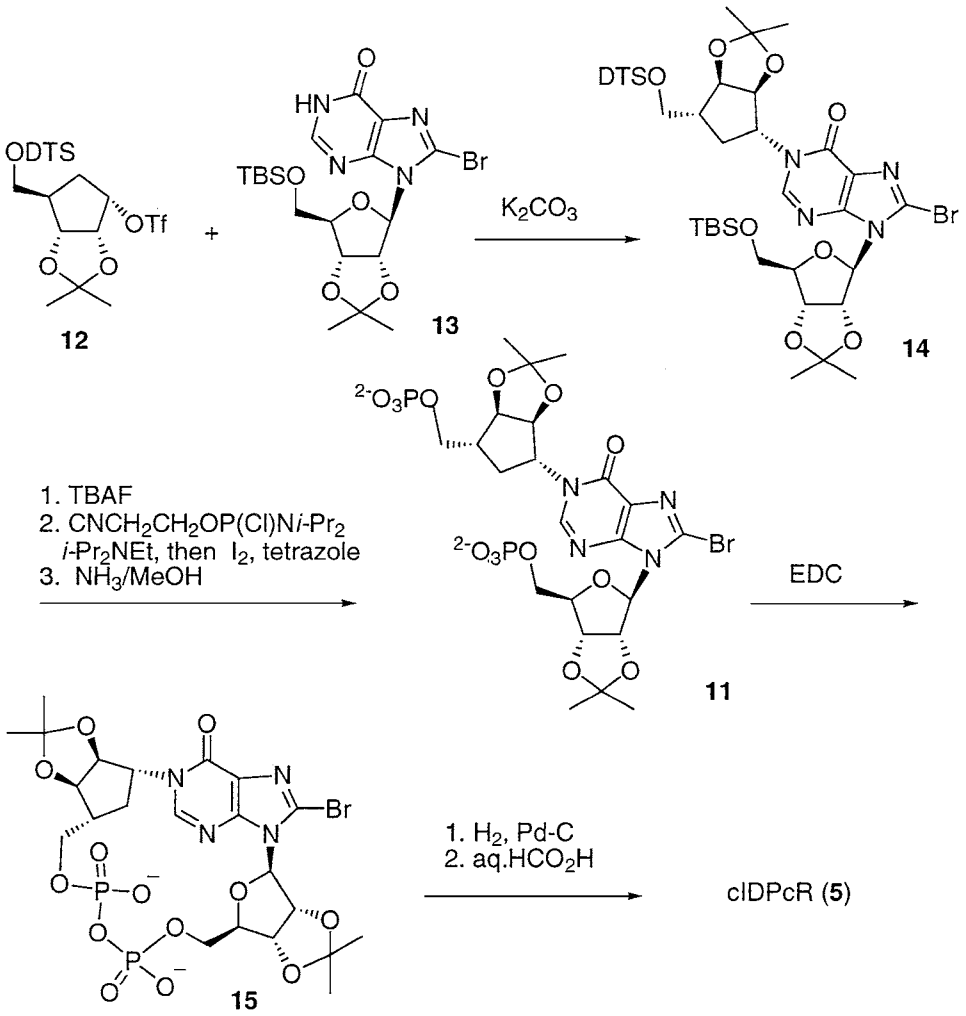
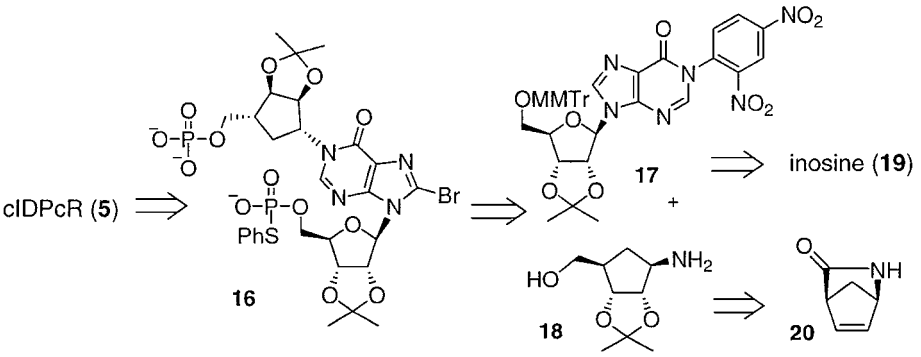


Scheme 1.

The Improved Synthesis of cIDPcR.

Although, as described above, we achieved synthesis of cIDPcR, which was the first total synthesis of a cADPR-related compound, the overall yield was very low. Therefore, we need to develop more efficient general methods for preparing various cADPR analogs. Especially, the development of both an efficient condensation to form the intramolecular pyrophosphate linkage and a straightforward construction of the *N*-1-carbocyclic structure were needed.

The improved synthetic plan is summarized in Scheme 2. Formation of the intramolecular pyrophosphate linkage is investigated by treating a 5′-phenylthiophosphate **16** as a substrate with silver nitrate or iodine (7). The *N*-1-carbocyclic-ribosyl-structure is constructed from *N*-1-(2,4-dinitrophenyl)inosine derivative **17**

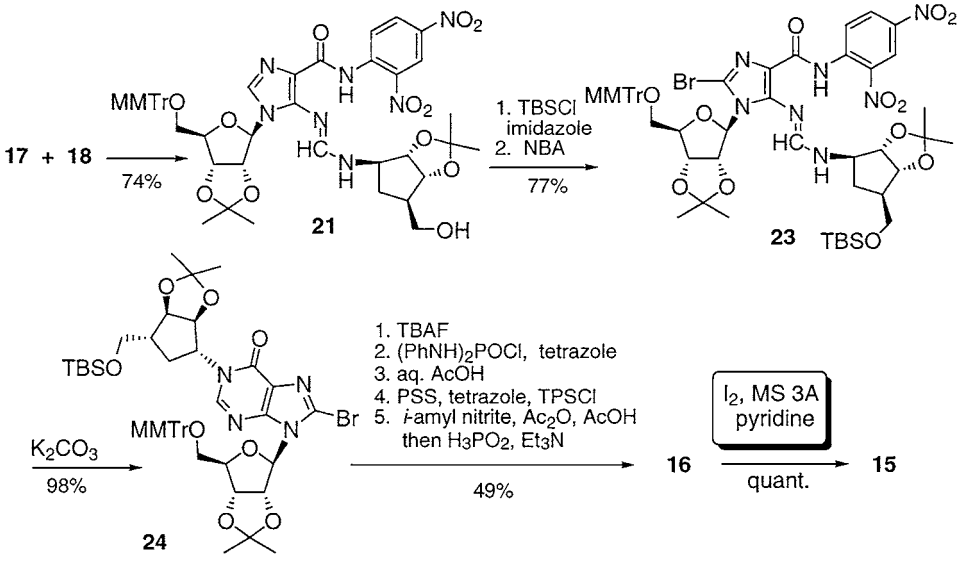


Scheme 2.

and optically active carbocyclic amine **18**, which is readily prepared from a commercially available bicyclic lactam **20** (8).

This *N*-1-dinitrophenylinosine derivative **17**, prepared according to Picciali's method (9), was heated with an excess of the carbocyclic amine **18** at 50°C in DMF to give the ring-cleaved product **21**. After the 5″-hydroxyl protection and bromination at the 2-position, the resulting **23** was heated with potassium carbonate at 50°C in DMF to give the desired ring-closed **24** in high yield. This method is clearly superior to our previous one using an SN2-type reaction. The compound **24** was converted into the thiophenylphosphate **16** in five steps.

The intramolecular condensation reaction of the substrate **16** was investigated under various conditions. We found that the desired cyclization product **15** was quantitatively obtained when **16** was treated with I_2 in the presence of molecular sieves 3A (MS 3A) in pyridine at room temperature (4c).



Scheme 3.

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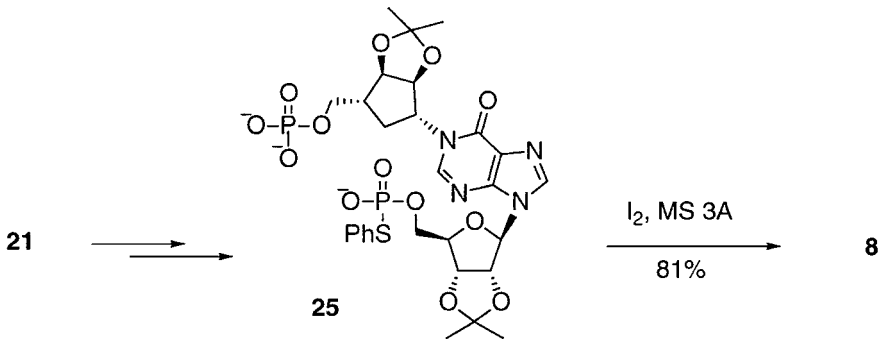
We next examined the use of the 8-unsubstituted substrate **25** in the intramolecular condensation reaction to investigate whether or not the 8-bromo substituent at the purine moiety actually facilitates intramolecular condensation. Thus, this 8-unsubstituted substrate **25**, prepared from **21** in several steps, was treated under the best condensation conditions described above to give the desired cyclization product **8** in 81%-isolated yield (4e).



Scheme 4.

These results suggest that the 8-bromo group facilitates the intramolecular condensation, at least to some extent, due to conformational restriction of the substrate in a *syn*-form.

In the similar way, the corresponding adenosine congener, cADPcR (**4**), was successfully synthesized (10).

C-GLUCOSIDE TRISPHOSPHATES AS IP_3 RECEPTOR LIGANDS

Design of *C*-Glycosidic Analog of Adenophostin A

Adenophostin A (**3**), the strongest IP_3 receptor agonist known so far, is a trisphosphate of 3′-*O*-α-glucosyladenosine. These interesting biological and structural features have prompted us (11) as well as other groups (12) to perform synthetic studies of novel IP_3 receptor ligands. We synthesized, for an example, the two compounds shown in Figure 4; one is an adenophostin analog **26** (11a-c) lacking the adenine moiety, and the other is a *C*-glucoside trisphosphate **27** (11g), a

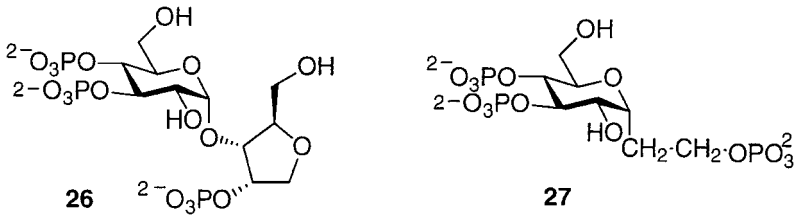


Figure 4.

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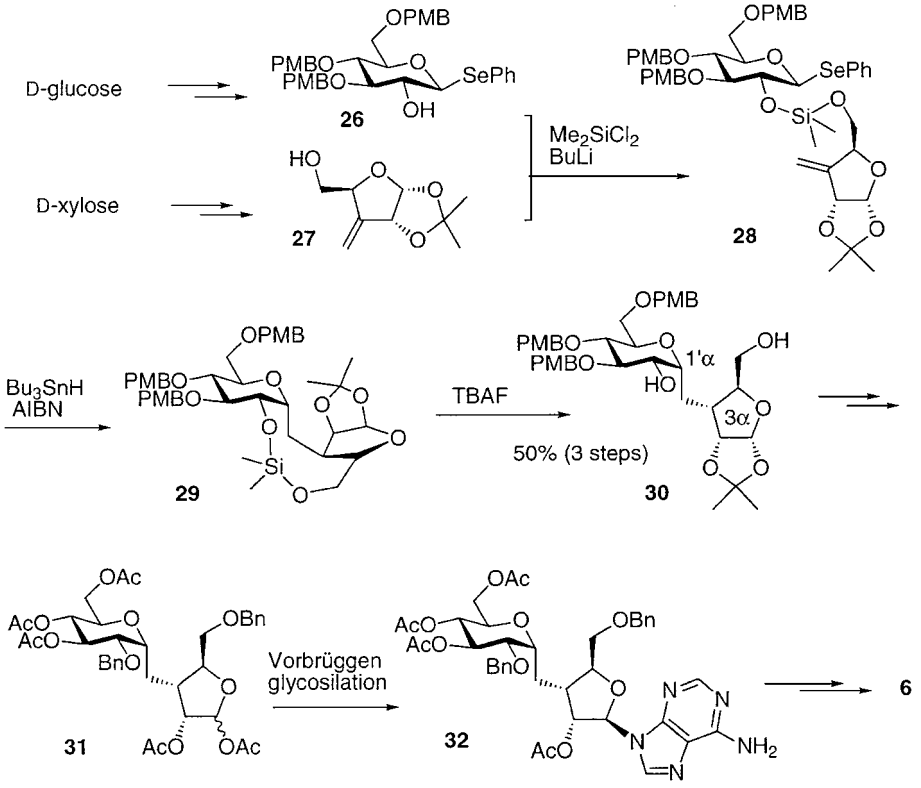
structurally simplified analog of adenophostin A. These analogs showed a potent binding affinity for the receptor with IC_{50} values of 25 and 36 nmol, respectively, which are comparable to IP_3 (11b, c). However, their activities are weaker than that of adenophostin A.

These results suggested that the α-D-glucopyranose structure is a good bioisostere of the *myo*-inositol backbone of IP_3; that the adenine moiety significantly enhances activity; and also that the *O*-glucosidic bond can be replaced with a *C*-glucosidic bond. Based on these results and considerations, we chose the *C*-glycosidic analog **6** of adenophostin A as a next target.

Synthesis of *C*-Glycosidic Analog of Adenophostin A

The synthesis is summarized in Scheme 5. The construction of the $(3'\alpha, 1''\alpha)$-*C*-glycosidic structure was considered to be the most important step. We planned to construct it by a temporary silicon-tethered radical coupling reaction.

Phenyl seleno-β-D-glucopyranoside **26** and 3-exomethylene-ribose derivative **27**, prepared from D-glucose and D-xylose, respectively, were connected with a dimethylsilyl tether to give the radical coupling reaction substrate **28**. Thus, the



Scheme 5.

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substrate **28** was successively treated with Bu_3SnH/AIBN in benzene and TBAF in THF to give the coupling product **30** with the desired (3α,$1'\alpha$)-configuration as the major product.

The target compounds **6** was synthesized from **30** via the introduction of adenine base by the Vorbrüggen method and phosphorylation of the hydroxyls by the phosphoramidite method (11d,f).

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