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Design and Synthesis of 5′-Deoxy-5′-Phenyladenophostin A, a Highly Potent IP_3 Receptor Ligand[1]

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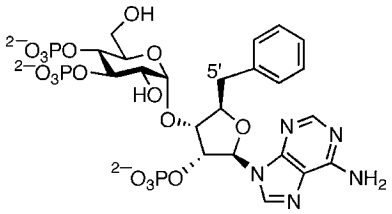
ABSTRACT



5′-Deoxy-5′-phenyladenophostin A (5), designed as a useful IP_3 receptor ligand based on the previous structure–activity relationship studies, was successfully synthesized via two key stereoselective glycosidation steps. This compound proved to be a highly potent IP_3 receptor agonist.

Adenophostin A (**2**),[2] isolated from *Penicillium brevicompactum*, is a very potent *myo*-inositol 1,4,5-trisphosphate (IP_3, **1**) receptor ligand, which is 10−100 times more potent than the endogenous ligand IP_3 in stimulating Ca^{2+} release and in binding to IP_3 receptors.[2−4] We previously synthesized an adenophostin A analogue **3** lacking the adenine moiety and its des-hydroxymethyl derivative **4**, and demonstrated that the compounds not only bind to IP_3 receptors but also stimulate Ca^{2+} mobilization with potencies comparable to IP_3 itself.[5] These results suggested that the pentofuranosyl structure of the D-ribose would not be essential for the activity but the tetrahydrofuran ring could effectively restrict the three-dimensional positioning of the D-glucose 3,4-bisphosphate, adenine, and the third phosphate moiety of the ring. Therefore, the 5′-hydroxymethyl moiety of adenophostin A seems to be unimportant for the binding and Ca^{2+} mobilization, suggesting that this moiety might be appropriate

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(1) This report constitutes Part 240 of Nucleosides and Nucleotides: for Part 239, see Kudoh, T.; Maruyama, T.; Ogawa, Y.; Matsuda, A.; Shuto, S. *Nucleosides Nucleotide Nucleic Acids* In press.

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(5) (a) Tatani, K.; Shuto, S.; Ueno, Y.; Matsuda, A. *Tetrahedron Lett.* **1998**, *39*, 5065−5068. (b) Shuto, S.; Tatani, K.; Ueno, Y.; Matsuda, A. *J. Org. Chem.* **1998**, *63*, 8815−8824. (c) Kashiwayanagi, M.; Tatani, K.; Shuto, S.; Matsuda, A. *Eur. J. Neurosci.* **2000**, *12*, 606−612.

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for further modification to develop novel adenophostin analogues of biological importance.

Bioactive ligands bearing an aromatic group, such as a fluorescent or photoreactive aromatic residue, are useful as biological tools in labeling target biomolecules and/or investigating the biological mechanisms of action.[6] Adenophostin A might be even more suitable than IP_3 itself as the lead for this kind of modification to develop such biological tools, because of its higher affinity for the receptor. Accordingly, identification of a site for the modification of adenophostin A without reducing the binding affinity is needed. Consequently, introduction of an aromatic group into an appropriate position of adenophostin A would be of biological interest.

On the basis of these findings and considerations, we designed a novel adenophostin A analogue **5** having a phenyl group at the 5′-position. Biological evaluation of this compound would clarify steric tolerability around the 5′-hydroxymethyl moiety for introducing a bulky aromatic group near the binding site of the IP_3 receptors.

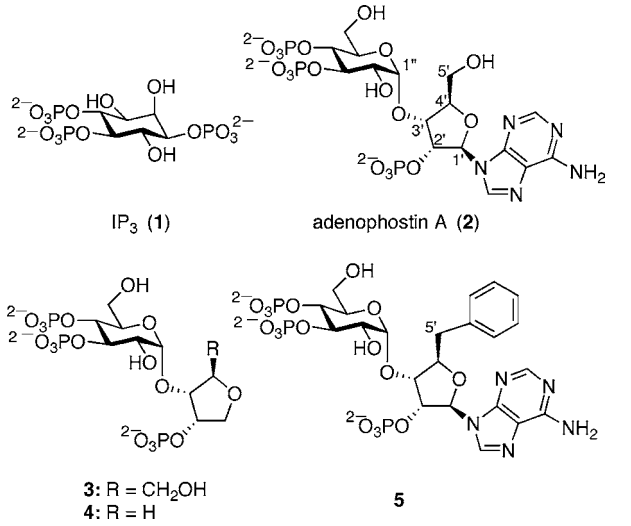
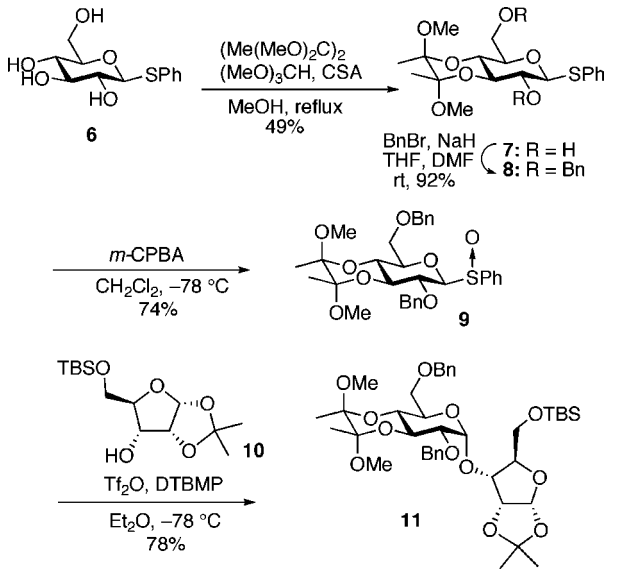


Figure 1. IP_3 receptor ligands.

The synthesis of the target compound **5**, shown in Schemes 1 and 2, includes the two key stereoselective glycosidation steps, constructing the α-disaccharide **11** with a sulfoxide donor and the *β*-nucleoside **16** by the Vorbrüggen glycosylation.[7]

The sulfoxide donor **9** and the acceptor **10** were used for the α-selective glycosidation preparing **11**. Sulfoxide glycosyl donors are known to be stable but can be activated under mild Lewis acidic conditions.[8,9] We recently developed highly α-selective *C*-glycosidation reactions based on the conformational restriction of the pyranosyl donor to the

Scheme 1



$^4C_1$ form by the 3,4-*O*-cyclic diketal protection.[10] In this restricted conformation, the kinetic anomeric effect is enhanced, resulting in the high α-selectivity in these *C*-glycosidation reactions.[10] On the basis of these results, we designed the sulfoxide donor **9** bearing a 3,4-*O*-cyclic diketal protecting group to realize the desired α-selective glycosidation due to the enhanced anomeric effect. The 3,4-*O*-cyclic diketal protecting group was thought to be also advantageous for selective phosphorylation of the hydroxyl groups at a later stage in the synthesis.[11]

The glycosyl donor **9** was prepared as shown in Scheme 1. Phenyl 1-thio-*β*-D-glucoside (**6**) was heated with 2,2,3,3-tetramethoxybutane, $(MeO)_3CH$, and (+)-camphorsulfonic acid (CSA) in MeOH under reflux[12] to give the 3,4-*O*-cyclic diketal derivative **7** in 49% yield, along with the corresponding 2,3-*O*-protected isomer. Benzylation of the 2- and 6-hydroxyls of **7** followed by *m*-chloroperbenzoic acid (*m*-CPBA) oxidation gave the sulfoxide donor **9**.

The glycosidation of the donor **9** and the acceptor **10** (1.0 equiv), prepared according to the previously reported method,[13] was investigated with Tf_2O and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as the promoter[9] under various conditions. The α/*β*-selectivity was significantly affected by the reaction conditions. Although the reaction with CH_2Cl_2 as solvent gave non-stereoselectively a mixture of α/*β*-glycosi-

(6) For an example, see: Nakanishi, W.; Kikuchi, K.; Inoue, T.; Hirose, K.; Iino, M.; Nagano, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 911−913.
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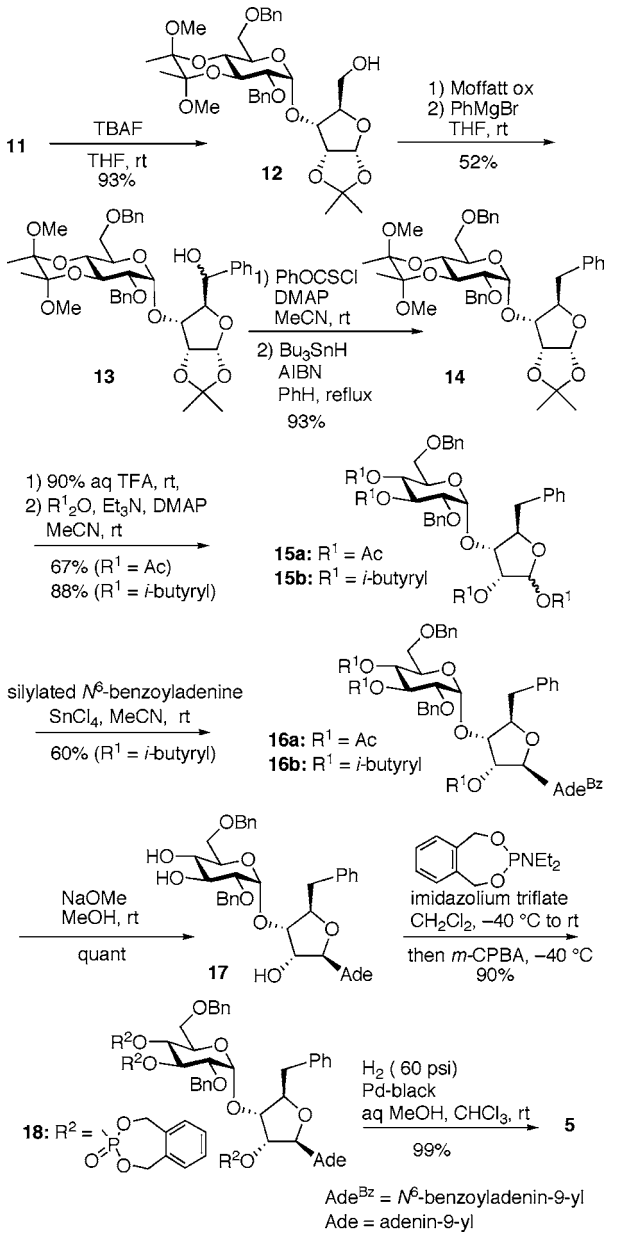
(10) Stereoselective synthesis of glycosides based on the conformational restriction strategy with the 3,4-*O*-cyclic diketal-protecting group (a) Abe, H.; Shuto, S.; Matsuda, A. *J. Am. Chem. Soc.* **2001**, *123*, 11870−11882. (b) Tamura, S.; Abe, H.; Matsuda, A.; Shuto, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 1021−1023. (c) Abe, H.; Terauchi, M.; Matsuda, A.; Shuto, S. *J. Org. Chem.* **2003**, *68*, 7439−7447. (d) Terauchi, M.; Abe, H.; Matsuda, A.; Shuto, S. *Org. Lett.* **2004**, *6*, 3751−3754.
(11) During our study, the synthesis of adenophostin analogues using a 3,4-*O*-cyclic diketal glycosyl donor has been reported: de Kort, M.; Regenbogen, A. D.; Overkleeft, H. S.; Challis, J.; Iwata, Y.; Miyamoto, S.; van der Marel, G. A.; van Boom, J. *Tetrahedron* **2000**, *56*, 5915−5928.
(12) Montchamp, J. L.; Tian, F.; Hart, M. E.; Frost, J. W. *J. Org. Chem.* **1996**, *61*, 3897−3899.
(13) Parr, I. B.; Horenstein, B. A. *J. Org. Chem.* **1997**, *62*, 7489−7494.

Scheme 2



dation products, we found that the stereoselectivity improved by using Et_2O as the solvent. Thus, the desired α-glucoside **11** was obtained as the sole glycosidation product in 78% yield, when the reaction was performed in Et_2O at −78 °C (Scheme 1).

Synthesis of the target compound **5** from the α-glucoside **11** was accomplished as summarized in Scheme 2. After removal of the 5-*O*-TBS group of **11**, the 5-hydroxymethyl moiety of the resulting **12** was oxidized by Moffatt oxidation conditions. The aldehyde obtained was immediately treated with PhMgBr in THF to give the 5-phenyl product **13**. Radical deoxygenation at the 5-position was performed by the treatment of **13** with PhOCSCl/4-(dimethyamino)pyridine (DMAP) in MeCN and then with Bu_3SnH/AIBN in hot benzene to give **14**. Acidic removal of the ketal protecting groups of **14** followed by acylation produced the 1,2,3′,4′-tetra-*O*-acetate **15a** or the 1,2,3′,4′-tetra-*O*-*i*-butyrylate **15b**.

The Vorbrüggen glycosylation of the acetate **15a** was examined (Table 1). The reaction was first carried out with

Table 1. Vorbrüggen Glycosylation with Donors **15a** and **15b**

entry	donor	Lewis acid	method[a]	solvent	yield (α/β)[b]
1	**15a**	TMSOTf	A	MeCN	18% (β)
2	**15a**	TMSOTf	A	$(ClH_2C)_2$	26% (β)
3	**15a**	$SnCl_4$	B	MeCN	66% (α/β)
4	**15b**	TMSOTf	A	MeCN	12% (β)
5	**15b**	$SnCl_4$	B	MeCN	60% (β)

[a] A: N^6-benzoyladenine (4 equiv), TMSOTf (10 equiv), and Et_3N (4 equiv) at room temperature. B: silylated N^6-Bz-adenine (5 equiv), prepared by heating *N*-benzoyladenine in hexamethyldisilazane/pyridine and $SnCl_4$ (6 equiv) at room temperature. [b] The α-anomer was not detected in the ^1H NMR spectrum in entries 1, 2, 4, and 5; and the α/β ratio was about 1:1 based on the ^1H NMR spectrum in entry 3.

N^6-benzoyladenine and TMSOTf/Et_3N in MeCN or dichloroethane (entries 1 and 2), where the silylated N^6-benzoyladenine was formed under the reaction conditions. Although these reactions selectively produced the expected β-nucleosidic product, the yield was low. The reaction using silylated N^6-benzoyladenine, prepared from N^6-benzoyladenine and hexamethyldisilazane/pyridine, as the acceptor and $SnCl_4$ as the promoter in MeCN improved the yield; however, the α/β-mixture was produced non-stereoselectively (entry 3).[14]

We recently reported that, in the Vorbrüggen glycosylation reaction with a sterically hindered ribosyl donor, high β-selectivity was realized when the hydroxyl groups of the donor were protected by *i*-butyryl groups.[15] Since glycosylation with the donor **15a** might not work well because of steric hindrance due to the 5-phenyl group, we therefore investigated reactions using the tetra-*O*-*i*-butyryl donor **15b**. Although the reaction using the donor **15b** under TMSOTf conditions was unsuccessful (Table 1, entry 4), the desired β-nucleoside **16b** was selectively obtained in 60% yield, when **15b** and silylated N^6-benzoyladenine were treated with $SnCl_4$ in MeCN at room temperature (entry 5).[16] Thus, like the previous case,[15] the *i*-butyryl protection of the sterically hindered ribosyl donor proved effective in this Vorbrüggen glycosylation.

The three *O*-*i*-butyryl and the *N*-benzoyl groups of **16b** were removed simultaneously with NaOMe/MeOH to give **17**. The phosphate units were selectively introduced into the three hydroxyls, using the phosphoramidite method with *o*-xylene *N*,*N*-diethylphosphoramidite (XEPA).[17] Thus, **17** was treated with XEPA in the presence of imidazolium triflate as a promoter[18] in CH_2Cl_2, followed by oxidation with

(14) For non-stereoselective examples of Vorbrüggen glycosylation, see: Azuma, T.; Isono, K. *Chem. Pharm. Bull.* **1977**, *25*, 3347−3353.

(15) Nomura, M.; Sato, T.; Washinosu, M.; Tanaka, M.; Asao, T.; Shuto, S.; Matsuda, A. *Tetrahedron* **2002**, *58*, 1279−1288.

(16) The regio- and stereochemistry of **16b** was confirmed by its NOE and HMBC spectra.

(17) Watanabe, Y.; Komoda, Y.; Ebisuya, K.; Ozaki, S. *Tetrahedron Lett.* **1990**, *31*, 255−256.

m-CPBA to give the desired 2′,3″,4″-trisphosphate derivative **18** in 90% yield. Finally, the benzyl protecting groups were all removed in one step by catalytic hydrogenation with Pd black in aqueous MeOH/$CHCl_3$ to furnish the target trisphosphate **5** as a sodium salt, after treatment with ion-exchange resin.

The Ca^{2+}-mobilizing activity of **5** was evaluated using recombinant rat type 1 IP_3 receptors expressed in DT40 cells lacking endogenous IP_3 receptors.[19] The results show that 5′-deoxy-5′-phenyladenophostin A (**5**) is a potent full agonist, mobilizing all of the IP_3-sensitive Ca^{2+} pool in a concentration-dependent manner. The half-maximally effective concentration (EC_{50}) for **5** was 2.1 ± 0.4 nM ($n = 5$; Hill slope = 1.62 ± 0.24), which is comparable to that for adenophostin A (2.1 ± 0.2 nM; $n = 12$; Hill slope = 1.54 ± 0.13), and is about 13-fold lower than the EC_{50} for the natural ligand IP_3 (24.8 ± 2.1 nM; $n = 11$; Hill slope = 1.21 ± 0.06) in parallel experiments.

In conclusion, the novel adenophostin A analogue **5** conjugated with a phenyl group at the 5′-position designed as a useful IP_3 receptor ligand was effectively synthesized via the two key stereoselective glycosidation steps. The analogue **5** proved to have significant Ca^{2+}-mobilizing potency. Thus, the 5′-position of adenophostin A is identified as a site suitable for the further modification to develop biological tools, which are useful for investigating biological mechanisms of action of Ca^{2+} mobilization.

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Supporting Information Available: Synthetic procedures and spectroscopic data of compounds, and NMR spectra (^1H, 2D and/or ^{13}C NMR) of the final compound **5** and the key intermediates **12** and **16b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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