Design and Synthesis of 5'-Deoxy-5'-Phenyladenophostin A, a Highly Potent IP₃ Receptor Ligand¹

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ABSTRACT



5'-Deoxy-5'-phenyladenophostin A (5), designed as a useful IP₃ 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 IP₃ receptor agonist.

Adenophostin A (2),² isolated from *Penicillium brevicompactum*, is a very potent *myo*-inositol 1,4,5-trisphosphate (IP₃, **1**) receptor ligand, which is 10–100 times more potent than the endogenous ligand IP₃ in stimulating Ca²⁺ release and in binding to IP₃ 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 IP₃ receptors but also stimulate Ca^{2+} mobilization with potencies comparable to IP₃ 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 3,4bisphosphate, 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 Ca²⁺ mobilization, suggesting that this moiety might be appropriate

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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 IP₃ 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 IP₃ receptors.



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



 ${}^{4}C_{1}$ 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 reflux¹² 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 Tf₂O and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as the promoter⁹ under various conditions. The α/β -selectivity was significantly affected by the reaction conditions. Although the reaction with CH₂Cl₂ as solvent gave non-stereoselectively a mixture of α/β -glycosi-

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dation products, we found that the stereoselectivity improved by using Et₂O 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 Et₂O 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 PhOCSCI/4-(dimethyamino)pyridine (DMAP) in MeCN and then with Bu₃SnH/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 $(\alpha/\beta)^b$
1	15a	TMSOTf	A	MeCN	18% (β)
2	15a	TMSOTf	Α	$(ClH_2C)_2$	26%~(eta)
3	15a	$SnCl_4$	В	MeCN	$66\% (\alpha/\beta)$
4	15b	TMSOTf	А	MeCN	12%~(eta)
5	15b	${ m SnCl}_4$	В	MeCN	60%~(eta)

^{*a*} A: N^6 -benzoyladenine (4 equiv), TMSOTf (10 equiv), and Et₃N (4 equiv) at room temperature. B: silylated N^6 -Bz-adenine (5 equiv), prepared by heating *N*-benzoyladenine in hexamethyldisilazane/pyridine and SnCl₄ (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/Et₃N 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 SnCl₄ 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 SnCl₄ 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 promoter¹⁸ in CH₂Cl₂, 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/CHCl₃ to furnish the target trisphosphate **5** as a sodium salt, after treatment with ion-exchange resin.

The Ca²⁺-mobilizing activity of **5** was evaluated using recombinant rat type 1 IP₃ receptors expressed in DT40 cells lacking endogenous IP₃ receptors.¹⁹ The results show that 5'-deoxy-5'-phenyladenophostin A (**5**) is a potent full agonist, mobilizing all of the IP₃-sensitive Ca²⁺ pool in a concentration-dependent manner. The half-maximally effective concentration (EC₅₀) 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 EC₅₀ for the natural ligand IP₃ (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 IP₃ 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 ¹³C 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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