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IP_3 Receptor-Ligand. 1 : Synthesis of Adenophostin A

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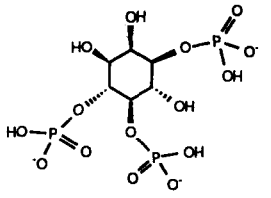
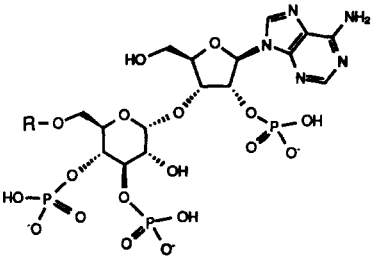
Abstract: *Adenophostin A, a potent IP_3 receptor-agonist, was synthesized. The basic skeleton with 3'-O-(α-D-glucosyl)adenosine was constructed by $AgClO_4$-γ-collidine-promoted glycosylation employing 2-O-benzyl-3,4,6-tri-O-acetyl-α-D-glucopyranosyl bromide as a glycosyl donor.*

In many cases, the activation of a variety of surface receptors leads to the mobilization of intracellular Ca^{2+} *via* the signal transduction mediated by D-*myo*-inositol 1,4,5-trisphosphate (**1**) (IP_3) as a second messenger.[1] Adenophostins A (**2**) and B (**3**), potent IP_3 receptor-agonists, were isolated from the cultured broth of *Penicillium brevicompactum* SANK 11991 and SANK 12177.[2] The IP_3 receptor-binding activity and Ca^{2+}-mobilizing activity of adenophostin A, as well as adenophostin B, were almost 100 times more potent than those of the natural ligand, IP_3. In this paper, the first synthesis of adenophostin A is presented.



1 (*IP_3*)

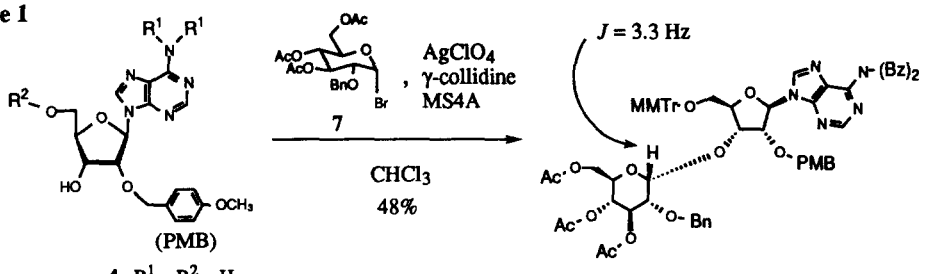


2 R = H (*adenophostin A*)
3 R = Ac (*adenophostin B*)

Adenophostin has a basic structure including 3'-*O*-(α-D-glucosyl)adenosine. In the synthesis of adenophostin A, the following two problems must be overcome: (i) construction of the basic skeleton with 3'-*O*-(α-D-glucosyl)adenosine; (ii) selective phosphorylation of three of four secondary hydroxyl groups. We chose 2'-*O*-(4-methoxybenzyl)adenosine[3] (**4**) as the starting material to glycosylate selectively the 3'-OH group of adenosine (Scheme 1). Dibenzoylation at the amino group of **4** followed by 4-methoxytritylation (MMTr) at the 5'-OH group in the conventional procedure[4] gave **6** in moderate yield.

Lichtenthaler et al.[5] synthesized 5'-*O*-(α-D-glucosyl)adenosine *via* the Ag_2CO_3-promoted glycosylation of 2',3'-*O*-isopropylidene-*N*,*N*-dibenzoyladenosine employing 2-*O*-benzyl-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide[6] (**7**) as a glycosyl donor. Glycosylation of **6** according to the Lichtenthaler's procedure proved to be ineffective because of steric hindrance around the 3'-OH group of **6**. The use of $AgClO_4$ and γ-collidine as promoters in place of Ag_2CO_3 gave 3'-*O*-(α-D-glucosyl)adenosine derivative **8** in 48% yield.[7] Under the conditions employed, no significant amounts of the corresponding β-glucosyl derivative were observed.

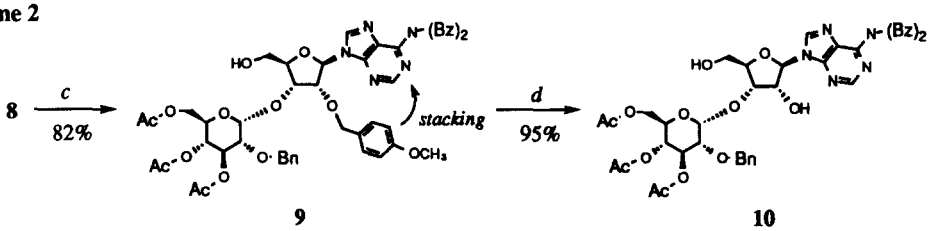
Scheme 1



Reagents :
(*a*) i) TMSCl, Pyridine; ii) BzCl; iii) H_2O; (*b*) MMTrCl, Pyridine.

The MMTr group of **8** was removed under acidic conditions to give **9** in 82% yield (Scheme 2). It was reported that the 4-methoxybenzyl (PMB) group bound to the 2'-OH group of adenosine could be removed by triphenylmethyl fluoroborate ($TrBF_4$).[3] In the case of compound **9**, the benzyl (Bn) group bound to the 2"-OH group was also thought to be susceptible to oxidation with $TrBF_4$. Consequently, 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)[8] was employed to remove the PMB group of **9** selectively to give **10** in 95% yield. In general, a PMB group can be removed upon oxidation with DDQ within 40 minutes.[9] The long reaction-time (*i. e.* 41 h) required for removal of the PMB group of **9** is attributable to the reduced electron density on the PMB group because of the stacking interaction between the PMB group and the adenine moiety.[10]

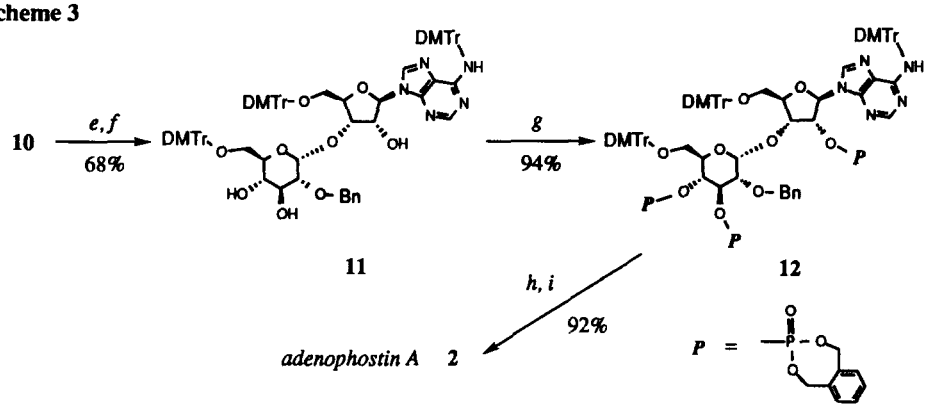
Scheme 2



Reagents and conditions : (*c*) 2% TFA-$CHCl_3$, 4 °C; (*d*) DDQ (1.7 eq.), CH_2Cl_2-H_2O (18 : 1), 41 h.

Deacylation of **10** followed by simultaneous 4,4'-dimethoxytritylation (DMTr) at the amino group of adenine and two primary hydroxyl groups gave triol **11** in 68% yield (Scheme 3). Triol **11** was then phosphitylated by Ozaki-Watanabe's procedure[11] using 4.5 eq. of *N,N*-diethyl-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amide and 7 eq. of 1*H*-tetrazole. Oxidation of the phosphite intermediate was tested under three independent conditions. Oxidation with I_2-H_2O led to partial cleavage of the benzylic esters of the phosphoryl groups.[12] Oxidation with *tert*-BuOOH was not successful as mentioned by Fraser-Reid.[13] Eventually, oxidation with mCPBA as described in the original report[11] gave fully protected **12**[14] in 94% yield. Deprotection of **12** was performed as follows: (i) detritylation by 80% AcOH; (ii) Hydrogenolysis under H_2 (1 atom) in the presence of Pd-black. Finally, adenophostin A was obtained without chromatographic purification in 92% yield. The use of Pd-C in place of Pd-black was not successful.

Scheme 3



Reagents and conditions : (*e*) 29% NH_4OH-Pyridine (4 : 1); (*f*) DMTrCl (3.5 eq.), Pyridine; (*g*) *N,N*-Diethyl-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amide (4.5 eq.), 1*H*-Tetrazole (7 eq.), CH_3CN; then mCPBA (9 eq.), -40 °C → rt; (*h*) 80% AcOH, 1h; (*i*) H_2 (1 atom), Pd-black, EtOH-H_2O (3 : 1), 24h.

Spectroscopic data on synthetic adenophostin A as well as the retention time on reversed-phase HPLC were identical with those of natural adenophostin A. The IP_3 receptor-binding activity of synthetic adenophostin A was the same as that of natural adenophostin A.

In conclusion, adenophostin A was successfully synthesized[15] *via* $AgClO_4$-γ-collidine-promoted glycosylation and selective phosphorylation.

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7. 347 mg (0.4 mmol) of compound **6** and 550.7 mg (1.2 mmol) of 2-*O*-benzyl-3,4,6-tri-*O*-acethyl-α-D-glucopyranosyl bromide **7** were dissolved in 4 mL of chloroform. To the mixture were added 2g of molecular sieves (MS4A), 160 μL (1.2 mmol) of 2,4,6-trimethylpyridine and 249 mg (1.2 mmol) of $AgClO_4$, and the total mixture was stirred at rt. After 24 h, the mixture was filtered through a celite and the filtrate was diluted with 100 mL of chloroform. The solution was washed with 100 mL each of 0.01N aq. HCl, 5% aq. $NaHCO_3$, then brine. The resulting organic layer was dried over anhydrous $MgSO_4$. The solvent was removed by evaporation and the residue was applied on a silica gel column (100 g, 70-230 mesh). Elution was performed by using n-hexane-EtOAc (3 : 2; v/v) to give 240.5 mg (48%) of the desired **8**.

   ^{1}H-NMR (270 MHz, $CDCl_3$, TMS as an internal standard) δ 8.37(s, 1H, H-8), 8.03(s, 1H, H-2), 7.87-6.67(m, 33H, Ar), 6.09(d, J = 6.6 Hz, 1H, H-1'), 5.45(t, J = 9.2 Hz, 1H, H-3"), 5.37(d, J = 3.3 Hz, 1H, H-1"), 5.04-4.98(m, 2H, H-2', H-4"), 4.66-4.32(m, 6H, H-3', H-4', $PhC\underline{H}_2$), 4.15-4.08(m, 2H, H-6"), 3.89(m, 1H, H-5"), 3.77(s, 3H, $C\underline{H}_3O$), 3.72(s, 3H, $C\underline{H}_3O$), 3.62-3.57(m, 1H, H-2"), 3.57-3.27(m, 2H, H-5'), 2.01(m, 9H, Ac).

   IR(KBr): 3062, 3032, 2937, 1752, 1706, 1600, 1576, 1512, 1450, 1368, 1240 cm^{-1}.

   FAB-MS: m/z 1246, $[M+H]^+$.
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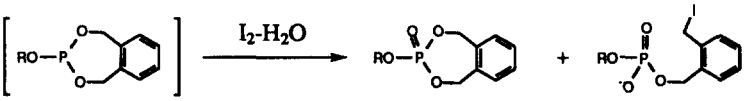
    We think that the phosphotriester may be partially cleaved as following scheme.



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14. ^{1}H-NMR (270 MHz, $CDCl_3$, TMS as an internal standard) δ 7.94(s, 1H, H-8), 7.88(s, 1H, H-2), 7.50-6.65(m, 56H, Ar), 6.42(d, J = 6.9 Hz, 1H, H-1'), 5.91(m, 1H, H-2'), 5.48-4.13(m, 21H, H-3', 4', 5', 1", 3", 4", $PhC\underline{H}_2$), 3.75-3.24(m, 22H, H-2", 5", 6", $C\underline{H}_3O$).

    IR(KBr): 3059, 3032, 2928, 2854, 2837, 1607, 1509, 1465, 1372, 1295, 1252 cm^{-1}.

    FAB-MS: m/z 1972, $[M+H]^+$.
15. The results were applied for Japanese Patent on September 7, 1994: Hotoda, H.; Kaneko, M.; Takahashi, M.; Tanzawa, K.; Takahashi, S.; application No. JP H6-213729.

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