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Synthesis of 1-*O*-[(3*S*,4*R*)-3-Hydroxytetrahydrofuran-4-yl]-α-D-glucopyranoside 3,4,3'-Trisphosphate as a Novel Potent IP_3 Receptor Ligand

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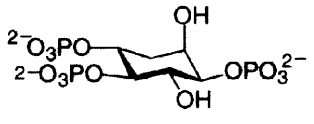
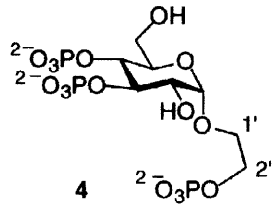
Abstract: 1-*O*-[(3*S*,4*R*)-3-Hydroxytetrahydrofuran-4-yl]-α-D-glucopyranoside 3,4,3'-trisphosphate (**5**) was designed and synthesized as a novel IP_3 receptor ligand. This compound bound strongly to IP_3 receptor from porcine cerebella with an affinity comparable to that of IP_3.

Keyword : Glycosidation; Inositols; Receptors; Structure-activity

Considerable attention has been focused on D-*myo*-inositol 1,4,5-trisphosphate (IP_3), an intracellular Ca^{2+}-mobilizing second messenger, because of its significant biological importance [1,2]. Therefore, analogs of IP_3 have been synthesized extensively to develop specific ligands for the IP_3 receptors, which are very useful for proving the mechanism of IP_3-mediated Ca^{2+} signaling pathways. However, none of these analogs has surpassed IP_3 itself either in binding affinity for IP_3 receptor or Ca^{2+}-mobilizing activity [3]. Recently, Takahashi and co-workers isolated adenophostin A and B from *Penicillium brevicompactum* and found that these are very strong IP_3 receptor ligands; **2** and **3** are 10 ~100 times more potent than IP_3 with regard to both the affinity for IP_3 receptor and Ca^{2+}-mobilizing ability in cells [4-6]. These findings suggested that the α-D-glucopyranose structure may be a bioisostere of the D-*myo*-inositol moiety in IP_3, and the three phosphate groups of adenophostins, which are essential for their biological activities [3], may be superimposed in the same positions as those of IP_3 in their three-dimensional location.



IP_3 (**1**)



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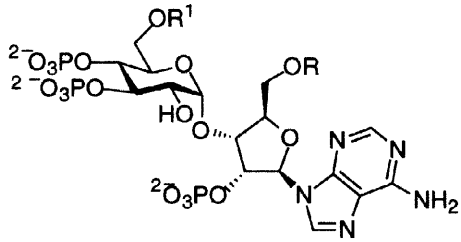
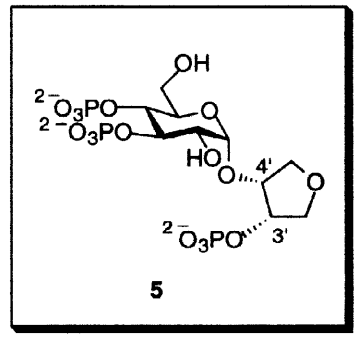


adenophostin A (**2**): R = H

adenophostin B (**3**): R = Ac



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Recently, two groups [7,8] designed and synthesized 2-hydroxyethyl-α-D-glucopyranoside 3,4,2'-trisphosphate (**4**) as a simplified analog of adenophostins, and showed that it was an agonist at IP_3 receptors with ~10-fold lower potency than IP_3. It has been suggested that the lower affinity of **4** for the receptor compared to those of adenophostins may be due to the conformational flexibility of the side-chain moiety of **4**. Based on this consideration, we designed 1-*O*-[(3*S*,4*R*)-3-hydroxytetrahydrofuran-4-yl]-α-D-glucopyranoside 3,4,3'-trisphosphate (**5**) as a novel IP_3 receptor ligand in which the location of the 3'-phosphate group in space is restricted by a tetrahydrofuran ring as in adenophostins. In this communication, we describe the synthesis and preliminary biological effects of **5**.

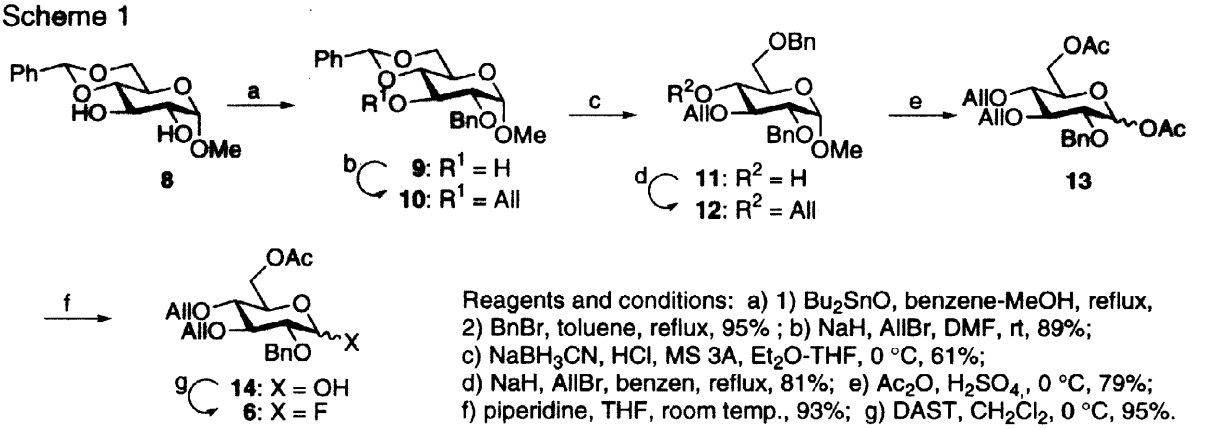
We planned to synthesize the target compound **5** via glycosidation reaction with fluoro-glycosyl donor **6** and tetrahydrofurandiol derivative **7**. The preparation of the glycosyl donor is shown in Scheme 1. We found that the 2-hydroxyl group of 4,6-*O*-benzylidene-glucose derivative **8** was very selectively benzylated by heating its 2,3-*O*-stannylidene derivative with BnBr to give **9** in 95% yield when toluene was used as a solvent [9]. After the 3-hydroxyl of **9** was protected with an allyl group, the benzylidene moiety of **10** was reductively cleaved with $NaBH_3CN$/HCl in THF [10] to give 6-*O*-benzyl derivative **11** in 61% yield along with the corresponding 4-*O*-benzyl isomer (5%). The 4-hydroxyl of **11** was allylated, and then acetolysis of the resulting fully protected sugar **12** with Ac_2O and H_2SO_4 gave **13** in 79% yield. In this reaction, both the 6-*O*-Bn and 1-*O*-Me groups were replaced by an acetoxy group. After the 1-*O*-acetyl group of **13** was selectively removed with piperidine in THF, the product **14** was treated with DAST in CH_2Cl_2 to give fluoro-glycoside **6**.

The glycosyl acceptor **7** was synthesized from known enol ether **15**, which was readily prepared from D-isoascorbic acid by a previously reported method [11], as shown in Scheme 2. Successive treatment of **15** with $LiAlH_4$ in Et_2O and TrCl in pyridine gave tetraol derivative **16**. After the free secondary hydroxyl of **16** was protected with an allyl group, the isopropylidene and trityl groups were simultaneously removed with TsOH in MeOH to give **18**. Intramolecular condensation of **18** was investigated under various conditions. When **18** was treated with Tf_2O in MeCN, the result was the most desirable to give tetrahydrofuran derivative **7** in 41% yield.

The glycosidation reaction with fluoro-glycosyl donor **6** and acceptor **7** was performed next. When **6** and **7** were treated with TMSOTf in the presence of Et_3N at room temperature in Et_2O, the desired α-glycoside was mainly produced and was obtained as an inseparable mixture with the corresponding β-anomer (**19**, yield 78%, α:β = 94:6 from its ^1H NMR spectrum). The 6-*O*-acetyl group of **19** was replaced with a benzyl group by a usual method to give the α-glycoside **20** in a pure form, after silica gel column chromatography. The three allyl groups of **20** were simultaneously deprotected by heating it with Pd-C and TsOH under reflux in aqueous EtOH [12], and the product was isolated as the corresponding tri-*O*-acetate **21**. Phosphate units were introduced using the phosphoramidite method. Thus, after the three acetyl groups of **21** were removed, the resulting trihydroxy product was treated with dibenzyldiisopropylphosphoramidite and tetrazole in CH_2Cl_2 followed by oxidation with *m*-CPBA [13] to give the desired triphosphate derivative **22** in 46% yield. Finally, all of the benzyl groups of **22** were simultaneously removed by catalytic hydrogenation with Pd-C in EtOH to give the target compound **5** in 89% yield as a sodium salt after treatment with ion-exchange resin.

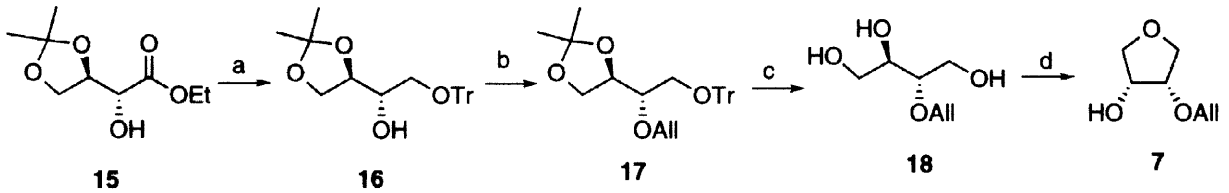
The binding affinity of the resulting compound for the IP_3 receptor of porcine cerebella was evaluated *in vitro* with [^3H] IP_3 as a radioligand [14]. As a result, **5** significantly

Scheme 1



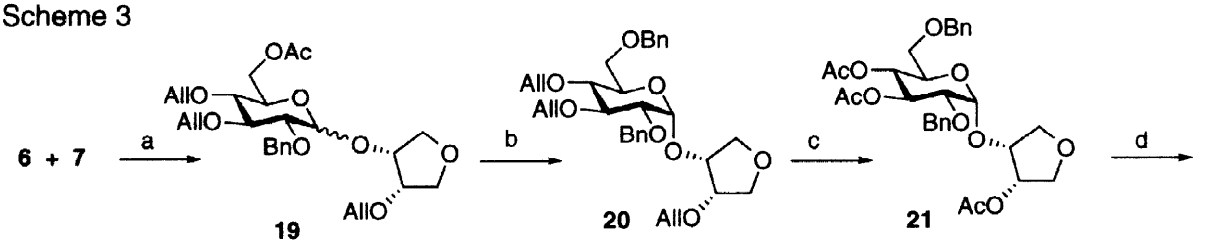
Reagents and conditions: a) 1) Bu_2SnO, benzene-MeOH, reflux, 2) BnBr, toluene, reflux, 95% ; b) NaH, AllBr, DMF, rt, 89%; c) $NaBH_3CN$, HCl, MS 3A, Et_2O-THF, 0 °C, 61%; d) NaH, AllBr, benzen, reflux, 81%; e) Ac_2O, H_2SO_4, 0 °C, 79%; f) piperidine, THF, room temp., 93%; g) DAST, CH_2Cl_2, 0 °C, 95%.

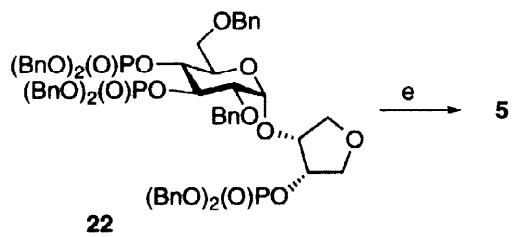
Scheme 2



Reagents and conditions: a) 1) $LiAlH_4$, Et_2O, reflux, 2) TrCl, py., room temp., 65%; b) NaH, AllBr, DMF, room temp., 57%; c) TsOH, MeOH, room temp, 50%; d) Tf_2O, Et_3N, DMAP, MeCN, room temp., 41%.

Scheme 3





Reagents and conditions: a) TMSOTf, Et_3N, Et_2O, room temp., 78% (α:β = 94:6); b) 1) NaOMe, MeOH, room temp., 2) NaH, BnBr, DMF, room temp., 94%; c) 1) Pd-C, TsOH, aq.EtOH, reflux, 2) Ac_2O, py., room temp., 77%; d) 1) NaOMe/ MeOH, room temp., 2) $(BnO)_2PN^iPr_2$, tetrazole, CH_2Cl_2, room temp., then *m*-CPBA, -40 °C to room temp., 46%; e) 1) H_2, Pd-C, EtOH, room temp., 2) Diaion WK-30 (Na^+), 89% (sodium salt).

inhibited the binding of [^{3}H] IP_3 with an IC_{50} value of 27 nM which is comparable to the affinity of IP_3 itself (IC_{50} = 19 nM). This result is notable since all of the IP_3 analogs synthesized so far showed only weaker affinity for the receptor than IP_3 itself [3].

Thus, this study indicates that the α-D-glucopyranose structure is a good bioisostere of the *myo*-inositol backbone of IP_3, and also adequate conformational restriction of the phosphate group of the side-chain moiety attached at the 1α-position [15] improves the binding affinity for IP_3 receptor.

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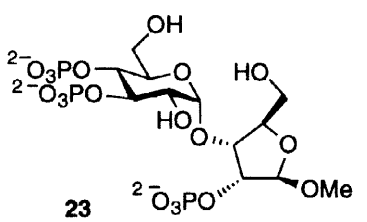
[15] The synthesis of 3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranoside 2,3'4'-trisphosphate **23**, which was also designed to restrict the conformation of the side-chain moiety of **4**, has also been reported recently: Jenkins DJ, Potter BVL. Chem. Commun. 1997:449-450.



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