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Synthesis of Clustered Disaccharide Polyphosphate Analogues of Adenophostin A

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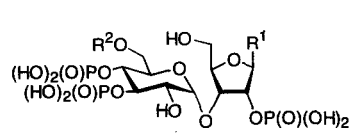
Abstract. Three new potential ligands for the IP_3 receptor (*i.e.* compounds **5-7**) were prepared by Sonogashira coupling of propargyl 2-*O*-acetyl-5-*O*-benzyl-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranoside (**15**) with iodobenzene, 1,2-diiodobenzene and 1,2,4,5-tetraiodobenzene, followed by deacetylation, phosphorylation and deprotection.

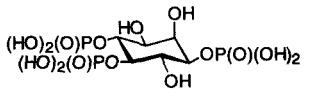
The adenophostins A and B (**1** and **2**, Fig. 1), isolated from the fermentation broth of *Penicillium brevicompactum*, are full agonists[1] of the mammalian D-*myo*-inositol 1,4,5-trisphosphate receptor (IP_3R). Interestingly, the binding affinity of both ligands for the IP_3R and the Ca^{2+}-mobilizing activity are 10-100 times higher in comparison with the natural ligand D-*myo*-inositol 1,4,5-trisphosphate (IP_3, **3**, Fig. 1).[2,3]

Earlier studies[4] indicated that the IP_3R, a glycoprotein spanning the membrane of the endoplasmic reticulum, harbors four independent ligand binding sites in a fourfold symmetrical spatial arrangement. It has been proposed[5] that the IP_3R forms a Ca^{2+}-channel upon the sequential binding of IP_3 to the four subunits (*i.e.* cooperative opening). On the other hand, the possibility that the binding of a *single* IP_3 molecule suffices to release Ca^{2+} into the cytosol (*i.e.* non-cooperative opening) is not excluded.[6]

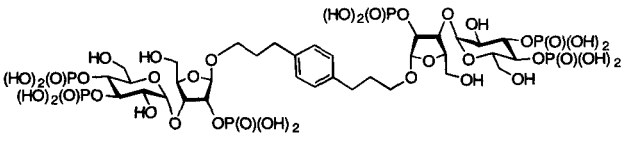
Figure 1



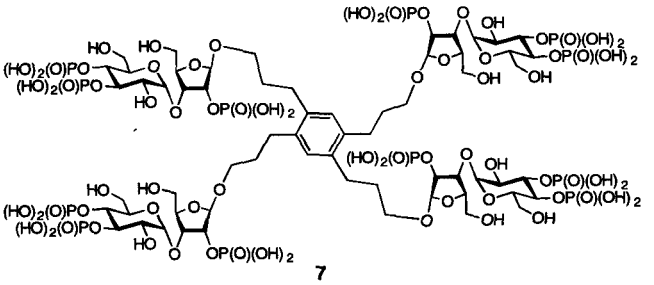
1 R^1= adenin-9-yl, R^2= H
2 R^1= adenin-9-yl, R^2= Ac
4 R^1= OCH_3, R^2= H
5 R^1= $OCH_2CH_2CH_2Ph$, R^2= H



3 IP_3



6



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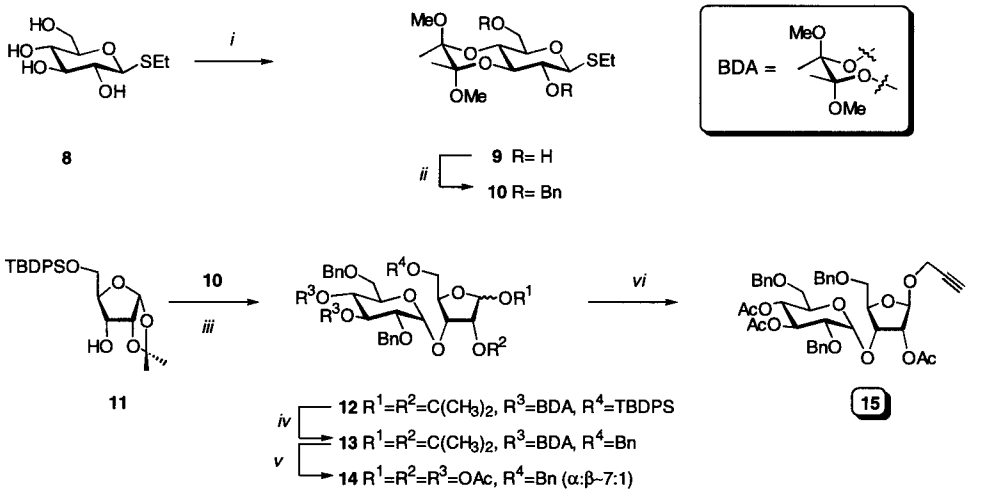
It occurred to us that a ligand, in which four IP_3 units are anchored *via* a spacer to the 1,2,4,5 positions of a central phenyl core moiety, would be a useful tool in solving the existing ambiguity concerning the precise mechanism of IP_3-mediated Ca^{2+}-channel opening. However, it may be expected[7] that the nature and orientation of the individual hydroxyl functions in *myo*-inositol will pose a formidable barrier in constructing a

D-*myo*-inositol derivative suitable for coupling to the core unit. Recently, Jenkins *et al.*[8] disclosed that the adenophostin A analogue **4** displayed Ca^{2+}-mobilizing potency similar to IP_3. The latter finding implies that a molecule in which the anomeric methyl group of compound **4** is replaced by an appropriate spacer would be an acceptable substitute for the corresponding IP_3-spacer containing derivative. On the basis of these considerations, we here present a route of synthesis to the mono-, di- and tetravalent adenophostin A analogues **5**, **6**, and **7**.

Target compounds **5-7** are composed of a central phenyl core, which is anchored *via* propyl spacers to one, two or four phosphorylated glucosyl α-1,3 ribose disaccharides. Retrosynthetic analysis reveals that the assembly of these mono-, di- and tetravalent molecules can be achieved by Sonogashira coupling[9] of iodobenzene, 1,4-diiodobenzene or 1,2,4,5-tetraiodobenzene with the common building block propargyl 2-*O*-acetyl-5-*O*-benzyl-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranoside (**15**). The α-glucosidic linkage in key disaccharide **15** can in principle be introduced by condensing, as reported[14] for the synthesis of adenophostin A, the ribose unit **11** (see Scheme 1) with ethyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-thio-β-D-glucopyranoside. In addition, it was established that the latter glucosyl donor could be replaced by the more easily accessible thioglucoside **10** (see Scheme 1).

Scheme 1

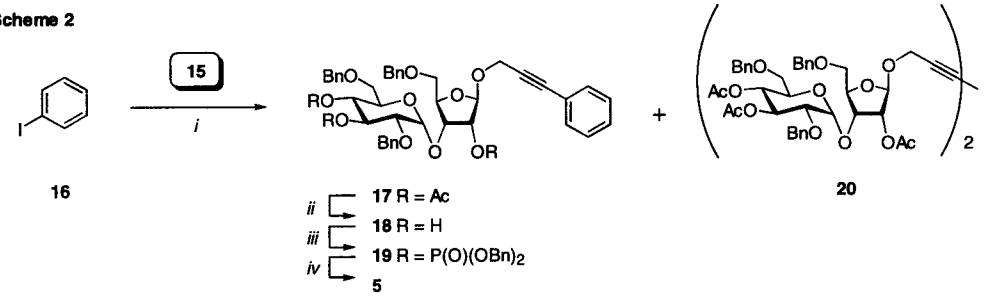


Reagents and conditions: (i) butane-2,3-dione (1.1 eq), $CH(OCH_3)_3$, cat. CSA, CH_3OH, reflux, 1h, 78% (2 regioisomers); (ii) BnBr, NaH, DMF, 98%; (iii) NIS/cat. TfOH, Et_2O, 30 min., 83% (α:β = 1:0); (iv) a. TBAF (1M in THF)/1,4-dioxane, 1/4, v/v, 50°C, 8h; b. BnBr, NaH, DMF, 92%; (v) a. $HOAc/H_2O/(HOCH_2)_2$, 14/6/3, v/v/v, reflux, 1h; b. Ac_2O, pyr, 16h, 81%. (vi) C_3H_3OH (2 eq), TMSOTf, $(CH_2Cl)_2$, 30 min., 81%.

The requisite ethyl thioglucoside **10** was easily available by the following two-step procedure (see Scheme 1). Protection of known[10] ethyl 1-thio-β-D-glucopyranoside (**8**) with 2,2,3,3-tetramethoxybutane,[11] prepared *in situ* by reaction of trimethyl orthoformate with butane-2,3-dione[12] in the presence of a catalytic amount of camphorsulfonic acid (CSA) gave, after purification[13] by silica gel column chromatography, 3,4-butane diacetal (BDA) **9**. Benzylation of **9** with benzyl bromide (BnBr) and sodium hydride (NaH) proceeded smoothly to give the fully protected glucosyl donor **10**. Glycosylation of known[14] ribose acceptor **11** with **10** in the presence of the promotor *N*-iodosuccinimide (NIS) and a catalytic amount of trifluoromethanesulfonic acid (TfOH) proceeded in a stereoselective fashion to give the α-linked disaccharide **12** in 83% yield. Removal of the 5'-*O*-*t*-butyldiphenylsilyl group in **12** with tetra-*n*-butylammonium fluoride (TBAF), followed by benzylation of the resulting primary hydroxyl function, yielded compound **13**. Removal of both the 3,4-

butane diacetal and isopropylidene groups in **13** under mild acid catalysed transacetalisation conditions[14] proceeded smoothly without any concomitant cleavage of the interglycosidic bond. Acetylation of the free hydroxyl functions afforded the fully protected dimer **14** as a mixture of anomers. Glycosidation of **14** with propargyl alcohol under the agency of a catalytic amount of trimethylsilyl triflate (TMSOTf) gave building block **15** in 50% yield based on **11**.

Scheme 2



Reagents and conditions: (i) 5 mol% $PdCl_2(PPh_3)_2$, 10 mol% CuI, Et_3N/DMF, 1/20, v/v, 16h, 80% (ii) $NaOCH_3$, CH_3OH, 1h, 100%; (iii) a. **21**, 1*H*-tetrazole, $(CH_2Cl)_2/CH_3CN$, 3/1, v/v, 30 min.; b. *t*-BuOOH, 0°C, 1h, 80%; (iv) Pd/C, H_2 (1 atm.), NaOAc, 1,4-dioxane/*iso*-propanol/H_2O, 4/2/1, v/v/v, 16h.

At this stage, attention was focused on the assembly of the target compounds **5-7**. Sonogashira coupling[9] (see Scheme 2) of terminal acetylene **15** (1.25 mmol) with iodobenzene **16** (1.00 mmol) in DMF (5 mL) under the influence of $PdCl_2(PPh_3)/CuI/Et_3N$ gave the phenyl acetylene derivative **17** and the cross coupling product **20** in a 2:1 ratio. Formation of the latter compound was prevented by adding a solution of **15** in DMF (2 mL) over a period of 1h to the iodobenzene/catalyst solution (3 mL). Zemplén deacetylation of **17** gave **18** which was phosphitylated with dibenzyl *N,N*-diisopropyl phosphoramidite[15] (**21**) followed by *in situ* oxidation of the resulting phosphite triesters with *tert*-butyl hydroperoxide to afford fully benzylated trisphosphate **19** (80% over the three steps). Debenzylation and contemporary reduction of the acetylene moiety was effected by hydrogenolysis (1 atm. H_2) over Pd-C in a buffered (NaOAc) solution to give, after purification by HW-40 gel filtration, the monovalent derivative **5** (Na^+-salt). In a similar way, di- and tetravalent derivatives **6** and **7** were readily available starting from 1,4-diiodobenzene and 1,2,4,5-tetraiodobenzene,[16] respectively. The identity and homogeneity of target compounds **5-7** were fully

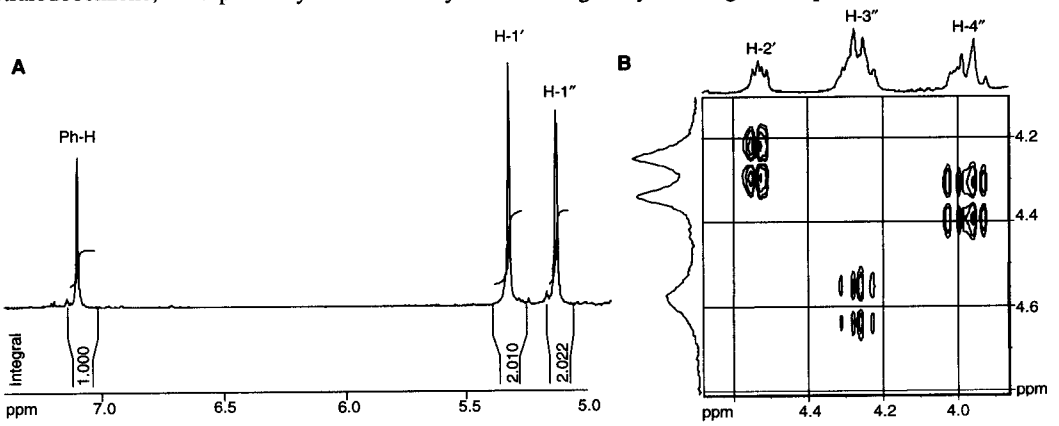


Figure 2. Part of the 600 MHz ^1H (A) and ^{31}P-^1H COSY (B) NMR spectra of the tetravalent adenophostin A analogue **7**.

ascertained by ^1H-, ^{13}C- and ^{31}P-NMR spectroscopy as well as ESI mass spectrometry. For example, the symmetrical substitution pattern of the central phenyl core with four disaccharide units in **7**, as well as the

position of the individual phosphate functions, was firmly established by ^{1}H- and ^{31}P-^{1}H COSY NMR spectroscopy (see Figure 2).

In conclusion, a straightforward and successful approach to clustered adenophostin A analogues has been presented. It is also of interest to note that coupling of the intermediate building block **14** with different terminal alkyn-1-ols allows adaptation of the spacer length. The latter possibility is in all likelyhood required for optimal binding of the clustered disaccharide to the IP_3R. The Ca^{2+}-releasing potential and mode of channel opening (*i.e.* cooperative or non-cooperative) by analogues **5-7** is currently under investigation.

Acknowledgement

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