Guanophostin A: Synthesis and evaluation of a high affinity agonist of the D-*myo*-inositol 1,4,5-trisphosphate receptor

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Guanophostin A, the guanosine counterpart of the inositol 1,4,5-trisphosphate receptor agonist adenophostin A, has been synthesized and is the first synthetic adenophostin A-like analogue to be equipotent to its parent in stimulating intracellular Ca^{2+} release; its nucleotide moiety is proposed to interact with the receptor binding core by guanine base cation-π stacking with Arg504 and hydrogen bonding with Glu505 and interaction of the ribosyl 2′-phosphate group with the helix-dipole of α_6.

Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] receptors [IP_3Rs] are Ca^{2+} channels located on the endoplasmic reticulum, the major intracellular Ca^{2+} store. Ins(1,4,5)P_3 (**1**, see Fig. 1) is a second messenger produced by the action of phospholipase C on phosphatidylinositol 4,5-bisphosphate in response to various extracellular signals. Ins(1,4,5)P_3 then opens the intrinsic calcium channel of the IP_3R and the resultant Ca^{2+} efflux causes an increase in cytosolic [Ca^{2+}], which regulates many cellular events.[1] To study the interaction of **1** with its receptor and to understand structure–activity relationships, many synthetic analogues of Ins(1,4,5)P_3 have been synthesized. None of these monomeric synthetic analogues has proven to be more potent than the natural ligand. In 1993, Takahashi *et al.* isolated two unusual agonists of IP_3Rs, adenophostin A (**2**) and adenophostin B (**3**) from culture broths of *Penicillium brevicompactum.*[2] Both were shown to bind to IP_3Rs with much greater affinity than Ins(1,4,5)P_3 and to be 10–100 times more potent than the natural ligand in evoking Ca^{2+} release *via* IP_3R.[3] This has stimulated many attempts to synthesize

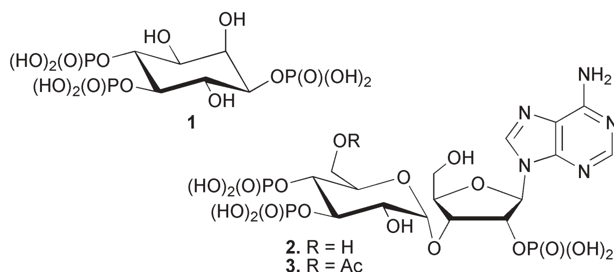

Fig. 1 Structure of Ins(1,4,5)P_3 (**1**), adenophostin A (**2**) and adenophostin B (**3**).

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various analogues of adenophostin and study their structure–activity relationship (SAR).[4] However, none of the synthetic analogs of adenophostins has achieved the potency of adenophostin itself. Herein, we report the first synthesis and biological characterisation of 'guanophostin A' in which the adenine of adenophostin A is replaced by guanine, the other naturally occurring ubiquitous nucleic acid and nucleotide purine base.

Although significant information has been gained from SAR studies on adenophostin analogs, some of the basic questions regarding the nature of the molecular interaction with the IP_3R remain unanswered. From SAR studies with synthetic analogues with and without a purine ring, it was evident that the presence of the adenine ring (or a surrogate) is crucial for enhanced affinity.[5] Two hypotheses have been proposed on the role of adenine to explain the enhanced potency of adenophostin over Ins(1,4,5)P_3. Either the adenosine moiety disposes the 2′-phosphate in a special spatial arrangement to strengthen the binding interactions with the receptor as an Ins(1,4,5)P_3 1-phosphate group surrogate (indirect role),[6] or the adenine moiety itself is involved in supplementary binding interactions with a nearby region of the binding site (direct role).[7] We have proposed a model for the interaction of **2** with the IP_3R binding core that involves a cation-π interaction of the adenine with an arginine residue of the IP_3R. To pursue these possibilities further, purine-modified adenophostin analogs are necessary. Although some purine-modified (predominantly at the C-6 position) adenophostin analogs have been synthesized, the important 2-position of this base is essentially unexplored. Our adenophostin-docked model of the IP_3R binding core also shows the carboxylate anion of Glu505 near the 2-position of the adenine base.[4a] Hence an H-bond donor or cationic residue at the 2-position may interact constructively with this carboxylate group. To explore this we chose to synthesise guanophostin A.

Ideally, Vorbrüggen condensation of a silylated purine with a properly protected disaccharide followed by deprotection of hydroxyl groups (to be later phosphorylated), phosphorylation and final deprotection are the important steps to be considered for the synthesis of adenophostin analogues. However, Vorbrüggen condensation of guanine or its derivatives with acylated sugars is known to be problematic[8] due to the formation of N-7 and N-9 regioisomers even under thermodynamic control and hence is not preferred synthetically. We therefore decided to use 2-amino-6-chloropurine (**7**) which is known[9] to undergo regiospecific coupling under Vorbrüggen conditions (Scheme 1). Moreover, a variety of substituted nucleosides could potentially be synthesized by manipulating the 6-chlorine motif after the glycosylation. Vorbrüggen condensation of 2-amino-6-chloropurine (**7**) and disaccharide **6**,[4a] synthesized from penta-*O*-acetyl-D-glucose (**4**)

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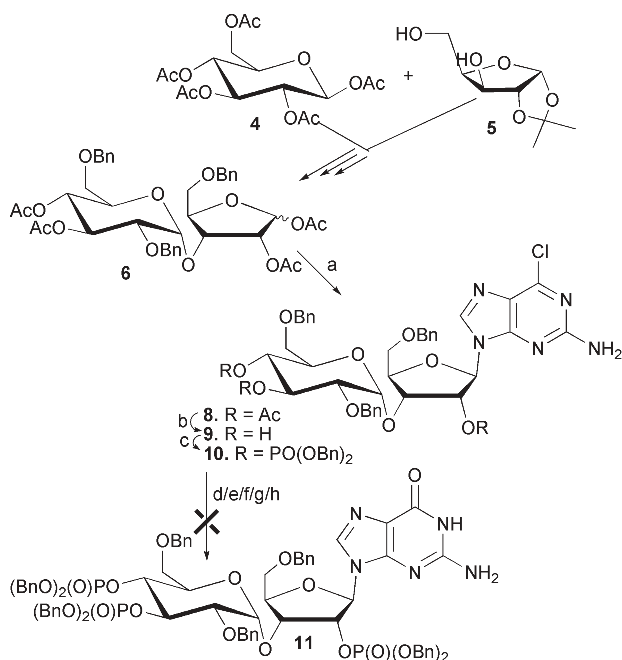


Scheme 1 *Reagents and conditions*: a. **7**, BSA, TMSOTf, MeCN, 70 °C; (b) NH_3, MeOH, rt, overnight (90%); (c) 1. $(BnO)_2PN(iPr)_2$, ImOTf, DCM, rt; 2. *m*-CPBA, −78 °C; (d) 4M NaOH, dioxane, 24 h, rt; (e) 3-hydroxypropiononitrile, DBU, Et_3N, DCM, 0 °C, 5d; (f) 3-hydroxypropiononitrile, NaH, THF, 40 °C, 4 h; (g) BnONa, DMF, rt, 30 min; (h) BnOH, K_2CO_3, 70 °C, 16 h.

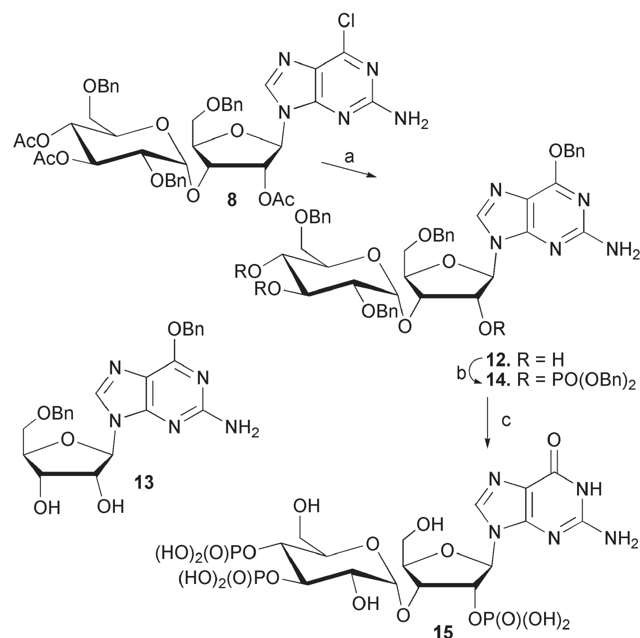
and 1,2-*O*-isopropylidene-D-xylose (**5**) in ten steps, using TMSOTf as the Lewis acid was initially investigated. Different silylating agents (for the persilylated purine) gave different yields for the subsequent condensation. For instance, the use of TMSOTf as both silylating agent and Lewis acid gave the expected nucleoside **8** in very low yield (27%) along with a dimeric compound (23%, possibly glycosylated at N-9 and C2-NH_2). The yield and product distribution could not be improved either by varying the solvent or the relative amounts of reactants. Similarly, the use of TMSCl–HMDS as silylating agent followed by the use of TMSOTf as catalyst also resulted in a low yield of the desired product. Use of *N*,*O*-bis-trimethylsilyl-acetamide (BSA) as silylating agent, however, followed by TMSOTf as catalyst for the condensation yielded **8** in quantitative yield. Although the exact reason for the dependence of the glycosylation reaction on the silylating agent is unclear, interference by the liberated acidic counter ions of the silylating agents (TfOH or HCl) cannot be ruled out. The high yield of condensation when BSA was used as the silylating agent, can be rationalized based on this argument, as neutral acetamide is the liberated by-product in this case.

Triacetate, **8** on ammonolysis yielded the expected triol **9** in 90% yield. Chemoselective phosphitylation[10] followed by *in situ* oxidation yielded the fully protected trisphosphate **10** in 94% yield. Various attempts (4M NaOH, dioxane; 3-hydroxypropiononitrile, DBU, DCM;[11] 3-hydroxypropiononitrile, NaH, THF;[9] BnOH, K_2CO_3[12]) to convert **10** to the guanosine derivative **11** failed. In some cases, **10** was inert and in others, dephosphorylation occurred. We therefore chose to generate the guanine moiety before phosphorylation. Since the O6 oxygen of guanine can compete with hydroxyl groups on the sugar for phosphorylation, generation of an *O*-protected guanine moiety is desirable and we chose an *O*-benzyl-guanine derivative to reduce the number of steps for deprotection. Thus, nucleoside **8** on treatment with *in situ*-generated sodium benzoxide [benzyl alcohol (6 eq.) and NaH (5 eq.)] in DMF at 80 °C for 3 h gave a mixture of **12** (43%) and de-glucosylated **13** (41%) (Scheme 2). Use of only two equivalents of sodium hydride and BnOH as solvent resulted in the exclusive formation of **12**. In both cases, the formation of 2-*N*-benzylated product was not observed.[13] Triol **12** on chemoselective phosphitylation followed by *in situ* oxidation gave the fully protected trisphosphate **14** in 82% yield. The benzyl protecting groups were removed by transfer hydrogenolysis and purification by ion exchange column chromatography provided guanophostin A **15**† quantitatively after assay using total phosphate analysis.

The ability of guanophostin A to stimulate the $InsP_3R$ was measured by using a low-affinity Ca^{2+} indicator trapped within the intracellular stores of chicken DT40 cells expressing only recombinant rat type I IP_3R as previously reported.[14] The results relative to adenophostin A and Ins(1,4,5)P_3 are shown in Table 1 and demonstrate that guanophostin A is *ca*. 18 fold more potent than Ins(1,4,5)P_3.

A molecular model of guanophostin A with the 2′-*endo* conformation of the ribose ring was built in Sybyl 7.1 (Tripos Associates) and docked into the mouse IP_3R1 binding core (PDB file No.1N4K) using GOLD[15] (Version 2.2). For a full account of the molecular modelling methods refer to Rosenberg *et al.*, 2003.[4a]

The highest scored binding mode of guanophostin closely resembled binding mode B of adenophostin A (described in ref. 4*a*). The interactions of the 4,5-bisphosphate motif of Ins(1,4,5)P_3 with the IP_3R binding core observed in the crystallographically determined structure were reproduced by the glucose 3″,4″-bisphosphate group of guanophostin A. Also, a cation-π interaction



Scheme 2 *Reagents and conditions*: (a) BnOH, NaH, rt; (b) 1. $(BnO)_2PN(iPr)_2$, ImOTf, DCM; (2) *m*-CPBA, −78 °C; (c) $Pd(OH)_2$, cyclohexene, MeOH, H_2O, 80 °C, overnight.

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Table 1 Ca^{2+} release *via* IP_3R evoked by guanophostin A[a]

	EC_{50}/nM	Hill coefficient	Ca^{2+} release (%)
$Ins(1,4,5)P_3$	23.7 ± 3.7	1.30 ± 0.14	81 ± 1
Adenophostin A	0.9 ± 0.1	1.20 ± 0.06	81 ± 2
Guanophostin A	1.3 ± 0.3	0.99 ± 0.08	83 ± 1

[a] Results (means ± SEM, n = 3–6) show the concentration of each ligand required to cause the half-maximal response (EC_{50}), the Hill coefficient, and the % of the intracellular stores released by a maximal concentration of each ligand.

between the guanidinium side chain of Arg504 and the guanine base of guanophostin was observed. An *ab initio* study of cation-π interactions in protein-DNA complexes revealed that in general those involving arginine and guanine are more stable than arginine–adenine pairs.[16] In addition to the base-protein interactions observed in binding mode B of adenophostin A, which include an N3–Arg269 hydrogen bond, additional potential hydrogen bonding interactions exist between the guanine 2-amino group and the binding core amino acid side chain of Glu505. The only 2-substituted adenophostin derivative so far synthesized on an adenine base is 2-methoxy-N^6-methyl-adenophostin.[4a] When evaluated for Ca^{2+} mobilization activity, this compound was *ca.* six-fold less potent than adenophostin A and only two-fold more potent than $Ins(1,4,5)P_3$.Thus, (with the caveat that the IP_3R binding core is not the whole, much larger, receptor) the higher potency of guanophostin A may illustrate the advantage of having a H-bond donor motif at the C-2 position of the purine.

In the X-ray structure[17] of the mouse IP_3R1 binding core, the 1-phosphate group of $Ins(1,4,5)P_3$ approaches the N-terminal of alpha helix 6 (α_6), comprising of residues 568–585 (α_9 in ref. 17). It is known that the dipole of an alpha helix can stabilise negatively charged groups such as phosphate at its N-terminal.[18] Thus, this is a likely interaction for the analogous 2′-phosphate of adenophostin and its analogues, which may even be better aligned with the axis of this alpha helix (Fig. 2). The fact that our model of guanophostin A binding places the 2′-phosphate closer to the

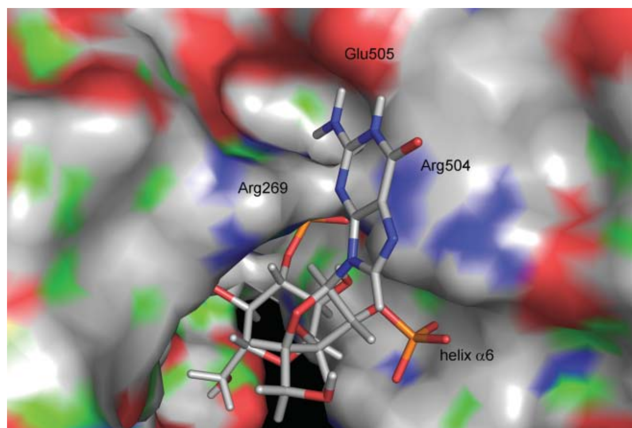

Fig. 2 The highest scored GOLD docked binding mode of guanophostin A with the 2′ *endo* conformation of the ribose ring shows the potential of this molecule to interact with Arg269 and Glu505 in addition to the previously proposed cation-π interaction between Arg504 and the purine ring. A new 2′-phosphate helix–dipole interaction is also illustrated.

N-terminus of helix α_6 is consistent with a stabilizing interaction with the helix dipole. This observation adds an extra element to our original model.[4a]

In conclusion, for the first time we have achieved the synthesis of a full agonist of the IP_3R that is equipotent to adenophostin A. This study reveals that the 2-position of a purine base is worthy of exploration for further design to the enhance affinity of $Ins(1,4,5)P_3R$ ligands. Further work in this area is underway.

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Notes and references

† Data for **15**; ^{1}H NMR (400 MHz, D_2O): δ 3.67–3.85 (m, 6H, H-5″, H-6_A″, H-6_B″, H-5_A′, H-5_B′, H-2″), 4.04 (dd, 1H, 16.0, 9.11 Hz, H-4″), 4.37 (m, 1H, H-4′), 4.40-4.45 (m, 1H, H-3″), 4.52 (m, 1H, H-3′), 5.16 (d, 1H, 3.73 Hz, H-1″), 5.20–5.26 (m, 1H, H-2′), 6.17 (d, 1H, 6.18 Hz, H-1′), 9.00 (s, 1H, H-8). ^{13}C NMR (100 MHz, D_2O): δ 60.03 (C-5′), 60.47 (C-6″), 70.21 (C-2″, ^{31}P coupled), 71.36 (C-5″, ^{31}P coupled), 72.95 (C-4″, ^{31}P coupled), 73.42 (C-3′, ^{31}P coupled), 75.39 (C-2′, ^{31}P coupled), 77.90 (C-3″, ^{31}P coupled), 84.46 (C-4′), 88.36 (C-1′, ^{31}P coupled), 98.04 (C-1″), 108.31 (C-5), 136.53 (C-8), 149.38 (C-4), 154.84 (C-6), 155.18 (C-2). ^{31}P NMR (161.94 MHz, D_2O with excess of TEA): δ 3.278, 3.54, 4.297. *m/z* (ES) = 684.1 [(M − H), 100%]; HRMS: Mass calcd for $C_{16}H_{25}N_5O_{19}P_3$ [M − H], 684.0362; Found, 684.0379.

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