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David Benito Alifonso
ISBN:978-84-691-9757-8/DL:T-1256-2008

Structural Determinants of Adenophostin A Activity. Proposal and Synthetic Approach to New Adenophostin A Analogues.

DAVID BENITO ALIFONSO

Dissertation presented to receive the degree of Doctor for the Universitat Rovira i Virgili, European Mention

UNIVERSITAT ROVIRA I VIRGILI

Departament de Química Analítica i Química Orgànica

Tarragona 2008

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Na. Maribel Matheu Malpartida, professora titular del Departament de Química Analítica i Química Orgànica de la Universitat Rovira i Virgili,

CERTIFICA:

Que el present treball titulat: “Structural determinants of Adenophostin A activity. Proposal and synthetic approach to new Adenophostin A analogues”, que presenta David Benito per a optar al grau de Doctor, ha estat realitzat sota la meva immediata direcció als laboratoris de Química Orgànica del departament de Química Analítica i Química Orgànica de la Universitat Rovira i Virgili.

Tarragona, Febrer de 2008

Maribel Matheu Malpartida

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En primer lloc, vull donar les gràcies a la meva directora de tesi, la Doctora Maria Isabel Matheu, pel seu suport i els seus consells durant tota la tesi, i molt especialment en l'última part d'aquest (llarg) procés, que és plasmar en paper tot el treball realitzat, gràcies. Voldria agraïr al Doctor Sergio Castillón per haver-me donat l'oportunitat de realitzar la tesi en el seu grup i per les experiències que se n'han derivat: sopars, dinars, barrancs….També voldria agraïr a la Doctora Yolanda Díaz pels consells i comentaris que m'han ajudat durant aquests anys.

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I, evidentment no em podia oblidar de les rates de laboratori que m'han acompanyat durant aquests anys, tant dels que van marxar quan nosaltres entràvem i ens van donar consells que no hem sabut aprofitar, com dels que han arribat i els vam donar consells que espero aprofitin (Pep i Isidre, que la força us acompanyi). I enmig de tots els passats i els futurs, voldria destacar els presents (tot i que uns quants ja són lluny): El nucli dur, que tants bons moments hem passat (Gerard l'eficient, Miguel el lògic i Omar l'Omar). Al final, bé o malament, tots hem acabat. A l'Antonio, que sempre ha tingut una resposta per qualsevol pregunta; Andrea, que sempre ha tingut una conversa per qualsevol pregunta; i a la Núria, amb qui he compartit tema. També voldria recordar als companys de polímers, al Dr. Dj. Andreu, el Lucas, la Lidia, la Marta, la Marisa, la cavereta i la resta de la colla. I a tu, Patri, gràcies per haver-me aguantat més que ningú al labo i al barco.

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I com no a la meva família, especialment als meus pares, que amb el seu esforç van començar el procés per a que jo ara pugui escriure aquestes línies, moltes gràcies.

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Abbreviations

AcOEt	Ethyl Acetate
AdA	Adenophostin A
AIBN	2,2'-Azobisisobutyronitrile
Arg	Arginine
bs	Broad singulet
BSA	Bovine Serum Albumin
CAN	Cerium ammonium nitrate
DAG	Diacylglycerol
DCM	Dichloromethane
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DMAP	*N,N*-dimethyl-4-aminopyridine
DMF	*N,N*-Dimethylformamide
d	Doublet
dd	Doublet of doublets
dt	Doublet of triplets
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmatic Reticulum
Gln	Glutamine
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single-Quantum Correlation
IP_3	1-D-*myo*-inositol-1,4,5-trisphosphate
IP_3R	Inositol Receptor

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m	Multiplet
M6P	Mannose-6-phosphate
MCPBA	*m*-Chloroperbenzoic Acid
MPLC	Medium-pressure Liquid Chromatography
MS	Molecular Sieves
n.d.	Not determined (yield)
NHS	Succinimide
NIS	*N*-Iodosuccinimide
NMO	*N*-methyl Morpholine N-oxide
NMR	Nuclear Magnetic Ressonance
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PEG	Polyetheleneglycol
PMB	*p*-Methoxybenzyl
ppm	Parts per Million
Pyr	Pyridine
R	Arginine
Q	Glutamine
SAR	Structure-Activity-Relationship
r.t.	Room Temperature
t	Triplet
TBAI	Tetrabutylammonium Iodide
TBAF	Tetrabutylammonium Fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDMSOTf	*tert*-butyldimethylsilyl Triflate

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TBDPS	*tert*-butyldiphenylsilyl
TEA	Triethylamine
TES	triethylsilyl
TEM	Tris/EDTA Medium
TfOH	Triflic acid
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin Layer Chromatography
TMS	Trimethyl Silyl
TMSOTf	Trimethyl Silyl Triflate
Tris	Tris-hydroxymethylaminomethane
UV	Ultraviolet Light

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6. Annex

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1. Introduction

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Ca^{2+} is the most common signal transduction element in cells[1] ranging from bacteria to specialized neurons.[2] Ca^{2+} is required for life, but prolonged high intracellular levels lead to cell death.[3] Why is Ca^{2+} used as a signalling element? There are several theories about the uniqueness of calcium. One theory is that Ca^{2+} must be maintained at low levels, because it precipitates phosphate, which is the energy currency of cells. So it is believed that cells evolved strategies for binding Ca^{2+}, firstly just to reduce its cytosolic levels, but at the end it turned into a valuable tool for signal transduction. Furthermore, Ca^{2+} cannot be metabolised like many other secondary-messenger molecules. Thus, cells need to regulate tightly intracellular levels using calcium stores like the endoplasmatic reticulum, numerous calcium-binding proteins like calmodulin, and also specialized proteins like calcium pumps.

1.1. Ca^{2+}: Function and Regulation.

Ca^{2+} is present in all organisms from life to death. During fertilization, mammalian eggs generate regular Ca^{2+} spikes that persist for about two hours and initiate development. During the next stage of development, the zygote proliferates rapidly to produce large groups of cells, and the Ca^{2+} signalling system controls specific processes responsible for pattern formation and cell differentiation, which culminates in the emergence of different types of specialized cells. A very important element determined during cell differentiation is the Ca^{2+} signalling toolkit, which each specialized cell requires in order to play its particular function.

Once cells have been assigned specific functions, they usually stop proliferating. In many cases, however, differentiated cells maintain the option of re-entering the cell cycle, and this usually occurs in response to growth factors. Ca^{2+} is one of the key regulators of cell proliferation, functioning in conjunction with other signalling pathways. For example, in the case of T-cells, the growth factor is an

[1] a) Berridge, M.J.; Bootman, M.D.; Lipp, P. *Nat. Rev. Mol. Cell. Biol.* **2000**, 1, 11; b) Clapham, D.E. *Cell.* **1995**, *80*, 259; c) Carafoli, E. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 1115; d) Brini, M.; Carafoli, E. *Cell. Mol. Life Sci.* **2000**, 354.
[2] Berridge, M.J. *Neuron* **1998**, *21*, 13.
[3] Berridge, M.J.; Bootman, M.D.; Lipp, P. *Nature*, **1998**, *395*, 645.

antigen that initiates a cascade of signals when bound to the T-cell receptor. One of the downstream effects is the activation of phospholipase Cγ1, which produces diacylglycerol and inositol trisphosphate. The latter subsequently activates the release of Ca^{2+} from cellular stores.

The main function of Ca^{2+} in controlling cell proliferation is to activate transcription factors either in the cytoplasm or within the nucleus. These Ca^{2+}-sensitive transcription factors activate numerous target genes. Some of these genes code for progression factors, while others produce components that trigger apoptosis. Thus, Ca^{2+} is central in establishing the signalling systems that enable cells to decide whether to grow or to die.

Regulatory mechanisms

Ca^{2+} regulation is critical for cell proper function. This is accomplished by the simultaneous interplay of several counteracting processes, which can be divided into Ca^{2+} 'on' and 'off' mechanisms depending on whether they serve to increase or decrease cytosolic Ca^{2+} (Figure 1).[4]

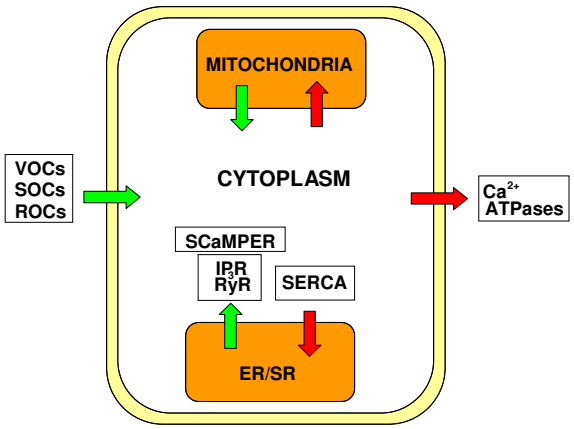

Figure 1. Schematic representation of Ca^{2+} 'on (green arrows)/off (red arrows)' mechanisms. The action of 'on mechanisms' implies an increase of cytoplasmatic Ca^{2+} levels, that of 'off mechanisms' implies a decrease.

Ca^{2+} 'on' mechanisms include channels located on the plasma membrane, which regulate the supply of Ca^{2+} from the extracellular space, and channels on the

[4] Berridge, M.J.; Bootman, M.D.; Roderick, H.L. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 517.

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endoplasmatic reticulum (ER) and sarcoplasmatic reticulum (SR), which release the Ca^{2+} from the intracellular stores. The 'off'-mechanism elements remove Ca^{2+} from the cytoplasm. These elements include Ca^{2+} ATPases on the plasma membrane, endoplasmatic reticulum, and sarcoplasmatic reticulum (SERCA), and exchangers that utilize the gradients of others ions to provide energy to transport Ca^{2+} out of the cell (i.e. Na^{+}/Ca^{2+} exchangers). Organelles other than the endoplasmatic reticulum and the sarcoplasmatic reticulum may also play a role in Ca^{2+} homeostasis by sequestering or releasing Ca^{2+}. For example, the mitochondria can limit the amplitude of cytosolic Ca^{2+} increase by rapid absorption and slow return of cytoplasmic Ca^{2+}. Using these 'on/off' mechanisms, cells are capable of changing their cytosolic Ca^{2+} concentrations from 100 nmol·L^{-1} at rest to greater than 1mol·L^{-1}.

Among the 'on' mechanisms, Ca^{2+} channels are of special interest. They can be divided into Ca^{2+} influx channels, which can be grouped on the basis on their activation into the following groups: voltage-operated Ca^{2+} channels (**VOCs**), receptor-operated Ca^{2+} channels (**ROCs**), mechanically-activated Ca^{2+} channels, and store-operated Ca^{2+} channels (**SOCs**); and Ca^{2+} release channels: Inositol 1,4,5-trisphosphate receptors **(IP_3Rs)**, ryanodine receptors **(RyRs)**, and sphingolipid Ca^{2+}-release mediating protein of endoplasmatic reticulum **(SCaMPER)**.

1.2. Inositol 1,4,5-trisphosphate (IP_3).

D-*Myo*-inositol 1,4,5-trisphosphate[5] (Figure 2) is a second messenger generated by the action of various extracellular stimuli (i.e. neurotransmitters, neuromodulators, hormones, etc.), that plays an important role in Ca^{2+} signalling.

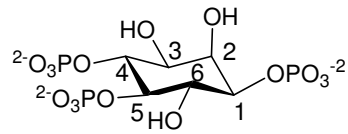

Figure 2. D-*Myo*-inositol 1,4,5-trisphosphate

[5] a) Berridge, M.J.; Irvine, R.F. *Nature* **1984**, *312*, 315; b) Berridge, M.J. *Nature* **1989**, *341*, 197; c) Berridge, M.J. *Nature* **1993**, *361*, 315; d) Marchant, J.S.; Taylor, C.W. *Biochemistry*, **1998**, *37*, 11524.

Inositol 1,4,5-trisphosphate release is activated by the following two energy-dependent (GTP or ATP) pathways: via G protein-linked receptors or via tyrosine kinase-linked receptors. When a first messenger signalling molecule interacts with a plasma membrane receptor (either G-protein-linked receptor or Tyrosine-kinase receptor) a cascade of processes is initiated (Figure 3).

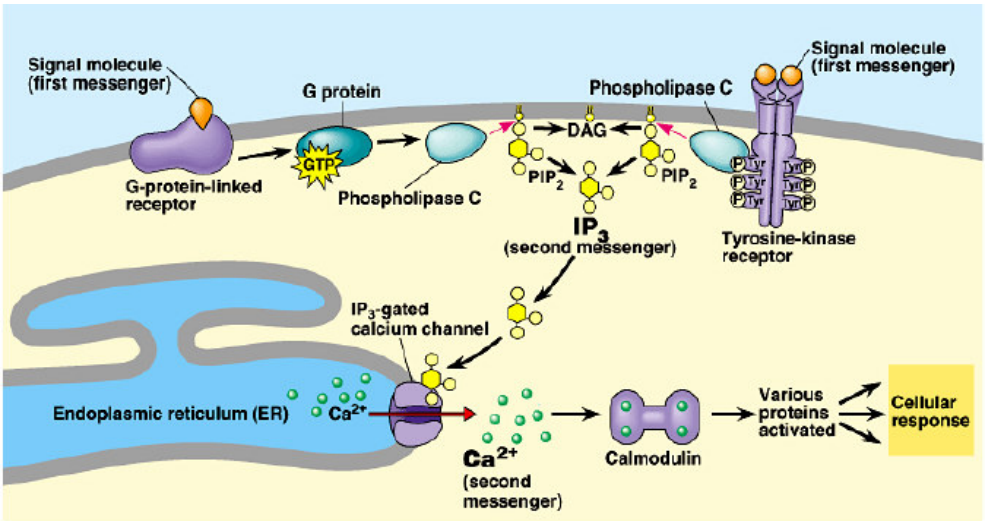

Figure 3. IP_3-calcium release pathway.

The final action of these two proteins results in the activation of phospholipase C, which catalyses the hydrolysis of phosphatidyl inositol 4,5 bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 diffuses into the cytoplasm and binds the inositol trisphoshate receptor (IP_3R). Binding induces a conformational change[7] that opens the channel and releases Ca^{2+} into the cytosol. The final consequence of this process is a cellular response to the original stimuli that initiated the cascade. This response is related not only to the increase in cytoplasmic Ca^{2+} but also to the frequency and intensity of the Ca^{2+} pulses produced.

[7] Hamada, K.; Miyata, T; Mayanagi, K; Hirota, J; Mikoshiba, K. *J. Biol. Chem.* **2002**, *277*, 21115.

Once generated, IP_3 is rapidly metabolised yielding inositol 1,4-bisphosphate, the product of 5-dephosphorylation, or inositol 1,3,4,5-tetrakisphosphate, the product of 3-phosphorylation, which can subsequently be 5-dephosphorylated to give inositol 1,3,4-trisphosphate.

Inositol 1,4,5-trisphosphate receptors (IP_3R)

The inositol 1,4,5-trisphosphate receptor[8] is a tetrameric ion channel that releases Ca^{2+} from intracellular stores in response to the binding of inositol 1,4,5-trisphosphate (IP_3). Each subunit has a single IP_3-binding site, lying towards the N-terminal domain (Figure 4, purple region). IP_3 binds independently to each subunit, and all four subunits must bind IP_3 before the channel can open.[9]

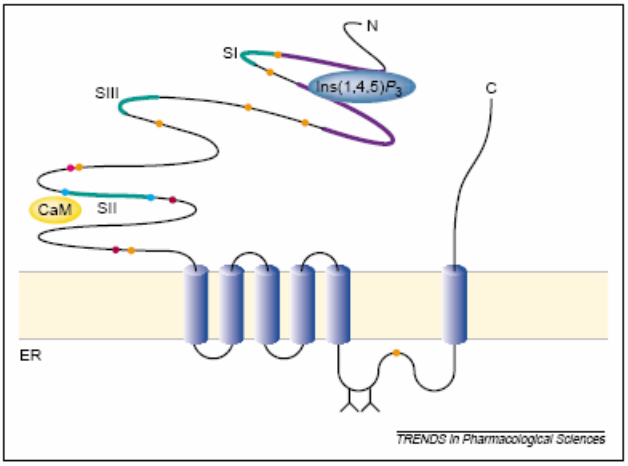

Figure 4. Structure of the inositol (1,4,5)-trisphosphate $IP_3$1 receptor.

Close to the C-terminal are located six transmembrane-spanning regions (Figure 4). These regions anchor the protein to the endoplasmatic reticulum membrane, mediate the assembly of the tetrameric receptor and play a major role in targeting IP_3 receptor to the endoplasmatic reticulum. In addition, the IP_3R pore resides within transmembrane regions 5 and 6. Residues located at C-terminal domain could occlude access to the IP_3 binding site, playing thus an inhibitory role in

[8] Mikoshiba, K. *Trends Pharmacol. Sci.* **1993**, *14*, 86.
[9] Taylor, C. W.; Laude, A. J. *Cell Calcium* **2002**, *32*, 321.

IP_3 binding.[10] Between the IP_3-binding site and the transmembrane region, there is a region formed by approximately 1600 residues where different elements bind modulating IP_3R activity. This region is known as the regulatory domain.[11]

The following are the principle elements that modulate the IP_3R activity:

- **cytoplasmatic nucleotides,** such as ATP, sensitise IP_3R at lower concentration by binding to an allosteric binding site, but inhibit IP_3 at higher concentration by binding to the IP_3 binding site (ATP-binding sites are shown as dark red circles in Figure 4);

- **calcium,** which at lower concentrations activates channel opening, has the opposite effect at higher concentrations and inhibits opening (calcium binding sites are despicted as orange circles in Figure 4);

- **phosphorylation** of different residues by a variety of different kinases allows for more efficient regulation (phosphorylation sites are shown as cyan circles in Figure 4);

- **proteins**, such as FK506 (whose binding site is depicted by a pink circle in Figure 4), are capable of interacting with and modulating IP_3Rs, although their action is not well known. In the case of calmodulin (CaM), however, binding of the protein close to one of the IP_3R phosphorylation sites is known to inhibit IP_3 from binding the receptor.

Three different IP_3R isoforms are expressed (Type I, II and III), and each is encoded by a distinct gene. The three isoforms are homologous and share 70% amino acid identity. They differ from one another in their affinity for IP_3[12] and in their tissue distribution.[13]

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[10] Morris, S. A.; Nerou, E. P.; Riley, A. M.; Potter, B. V. L.; Taylor, C. W. *Biochem. J.* **2002**, *367*, 113.
[11] Joseph, S.K. *Cell. Signal.* **1996**, *8*, 1.
[12] Nerou, E. P.; Riley, A. M.; Potter, B. V. L.; Taylor, C. W.; *Biochem J.* **2001**; *355*, 59.
[13] Taylor, C.W.; Genazzani, A. A.; Morris, S. A. *Cell Calcium,* **1999**, *26*, 237.

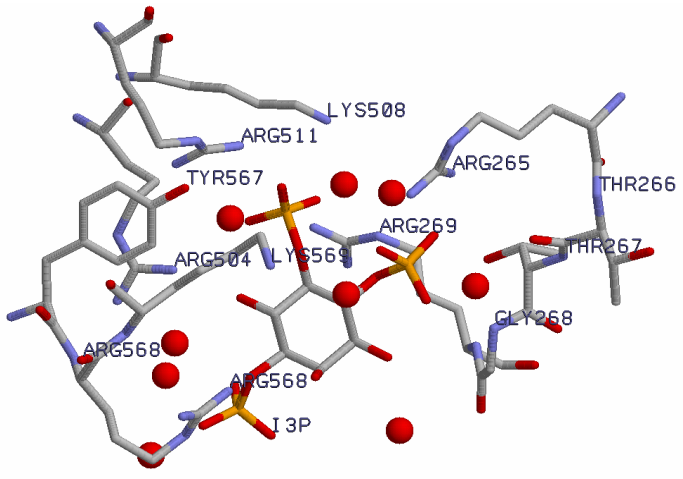

Figure 5. Binding site of the IP_3 receptor

The binding core of IP_3R is principally formed from basic residues, which coordinate the IP_3 phosphate groups either directly or indirectly via water molecules (Figure 5). Specific interactions include the following:

- 1-phosphate with Arg568 and Lys569.
- 4-phosphate with Arg265, Thr266, Thr267, Gly268, Arg269 and Lys569.
- 5-phosphate with Arg265, Arg269, Arg504, Lys508, Arg511 and Tyr567.

These are the main interactions, but the IP_3 phosphates engage in an extensive net of hydrogen bonds with both other residues and water molecules. Due to the important role of IP_3 in cell signalling, many IP_3 analogues have been synthesised.[14] Based on the different activities of the analogues tested, a

[14] a) Billington, D.C. *The Inositol Phosphates: Chemical Synthesis and Biological Significance*, VCH, Weinheim, **1993**, pp 1-153; b) Potter, B.V.L.; Nahorski, S.R. Synthetic inositol polyphosphates and analogues as molecular probes for neuronal second messengers receptors in *"Drug Design for Neuroscience",* Raven Press, New York, **1993**, vol. *14*, pp 383-416; c) Schmitt, L.; Spiess, B.; Schlewer, G. *Tetrahedron Lett.* **1992**, *33*,

pharmacophore[15] model has been proposed (Figure 6). In the context of this model, it has been demonstrated[16] that the vicinal bisphosphate moiety is crucial for IP_3 activity and that the 1-phosphate and 6-hydroxyl substituents enhance the molecule's affinity for the its receptor.

Among all the analogues tested, however, only IP_3 dimers[14x] have an affinity for IP_3R exceeded that of IP_3. These dimers consist of two IP_3 units linked with a polyethylene glycol spacer. It is believed that this structure allows the analogue to bind to two IP_3R subunits at same time, which results in increased activity.

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[16] a) Potter, B.V.L.; Lampe, D. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1933; b) Marchant, J.S.; Beecroft, M.D.; Riley, A.M.; Jenkins, D.J.; Marwood, R.D.; Taylor, C.W.; Potter, B.V.L. *Biochemistry* **1997**, *36*, 12780.

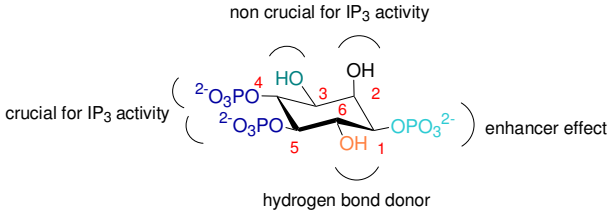

Figure 6. Pharmacophore model for IP_3

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1.3. Adenophostins.

In 1993, Takahashi *et al.*[17] isolated from a culture of *Penicillium brevicompactum* a class of glyconucleosides: Adenophostins A and B (Figure 7). These compounds have been shown to be the most potent agonists of IP_3R discovered to date with affinities between 10- and 100-fold higher than IP_3 itself.[18] Moreover, adenophostins are resistant to enzymes that metabolise IP_3, such as IP_3-5-phosphatase and IP_3-3-kinase. [21]

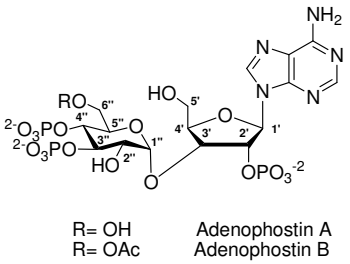
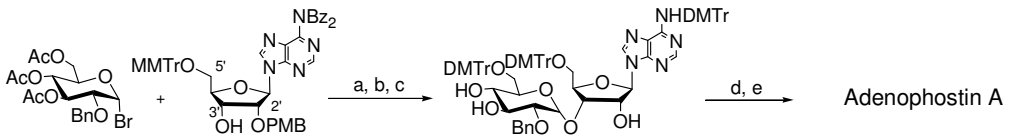


Figure 7. Adenophostins A and B.

From a chemical standpoint, adenophostins are similar to IP_3 in that they possess a *trans*-diequatorial configuration for the vicinal bisphosphate moiety flanked by a hydroxyl group at C-2''. Adenophostin structure was confirmed by total synthesis, which was conducted by Hotoda *et al* (Scheme 1).[19] Starting from

[17] Takahashi, M.; Kagasaki, T.; Hosoya, T.; Takahashi, S. *J. Antibiot.* **1993**, *46*, 1643.
[18] Takahashi, M.; Tanzawa, K.; Takahashi, S. *J. Biol. Chem.* **1994**, *269*, 369.
[19] Hotoda, H.; Takahashi, M.; Tanzawa, K.; Takahashi, S.; Kaneko, M. *Tetrahedron Lett.* **1995**, *36*, 5037.

adenosine and a conveniently protected glucosyl bromide, adenophostin A was obtained in 6 steps with 20% overall yield.



a) $AgClO_4$, γ -colidine, $CHCl_3$, 48%; b) i: 2% TFA, 82%, ii: DDQ, 95%; c) i: NH_4OH-Pyr, ii: DMTrCl, Pyr, 68%; d) i: N,N-diethyl-1,5-dihydro-2,4,3- benzodioxaphosphepin-3-amine, tetrazole, ii: MCPBA, 94%; e) i: 80% HOAc, ii: H_2, Pd-C, 92%

Scheme 1. First synthesis of adenophostin A.

Several other total syntheses have been developed in recent years,[20] mainly to avoid using an adenosine acceptor or to reduce unnecessary protection-deprotection steps.

Adenophostins analogues

In order to both uncover the structural features responsible for the adenophostins' activity and obtain more potent IP_3R agonists, many analogues have been synthesized. Figure 8 depicts the main structural modifications that have been made in an attempt to determine the underlying elements of adenophostin A activity.

As far as the riboside moiety is concerned, any modifications that increased conformational mobility and made optimal base positioning more difficult (**7,8**)[21] resulted in a dramatic decrease in activity. Moreover, whenever the base is absent, low or no affinity was observed (**3-6**,[22,23] **9-13**[24,14i]). Thus, it was not surprising that analogues of **1**[24] (with 0, 1, or 2 chain carbons) were the most active.

---

[20] a) Van Straten, N.C.R.; Van der Marel, G.A.; Van Boom. J.H. *Tetrahedron Lett*. **1996**, *37*, 3599; b) Marwood, R.D.; Correa, V.; Taylor, C.W.; Potter, B.V.L. *Tetrahedron Asymm*. **2000**, *11*, 397.

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[22] Hotoda, H.; Murayama, K.; Miyamoto, S.; Iwata, Y.; Takahashi, M.; Kawase, Y.; Tanzawa, K.; Kaneko, M. *Biochemistry*, **1999**, *38*, 9234.

[23] Shuto, S.; Tatani, K.; Ueno, Y.; Matsuda, A. *J. Org. Chem.* **1998**, *63*, 8815; b) Tatani, K.; Shuto, S.; Ueno, Y.; Matsuda, A. *Tetrahedron Lett.* **1998**, *39*, 5065.

[24] a) Mochizuki, T.; Kondo, Y.; Abe, H.; Taylor, C.W.; Potter, B.V.L.; Matsuda, A.; Shuto, S. *Org. Lett.* **2006**, *8*, 1455; b) Mochizuki, T.; Kondo, Y.; Abe, H.; Tovey, S.C.; Dedos, S.G.;

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Regarding to the adenine moiety it has been substituted with other heterocycles (**14**-**20**),[25,26,27,28,29] differently substituted naphthyl (**22**,**23**) or phenyl groups (**21**),[25,27] or linear alkyl ethers of different lengths (**24**-**26**).[30,3132] None of these substitutions afforded analogues with affinities superior to that of adenophostin A, and only guanophostin (**19**)[29] showed similar activity. It is worth noting that etheno-analogue (**17**)[28] also demonstrated somewhat high affinity, but it was only half as active as adenophostin A.

Concerning the glucose moiety, the only active analogue was **27**,[33] in which the pyranose ring was conserved and all substituents appeared in equatorial disposition. The only difference with adenophostin A was the absence of the 5'' hydroxymethylene group.

Not only structural changes in each subunit, but also regio and stereochemical considerations affect adenophostin activity. In order to determine the optimal glycosidic bond stereochemistry, four analogues were synthesized[34] (Figure 9). Evaluation of their activity showed that **31** was the most active, while **32**, **33** and **34** were almost completely inactive. This study concluded that, with respect to the glycosidic bond between the glucose and ribose moieties, the optimal stereochemistry and regiochemistry are α and 3', respectively.

Taylor, C.W.; Paul, M.; Potter, B.V.L.; Matsuda, A.; Shuto, S. *J. Med. Chem.* **2006**, *49*, 5750.

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[32] De Kort, M.; Valentijn, A.R.P.M.; Van der Marel, G.A.; van Boom, J.H. *Tetrahedron Lett.* **1997**, *38*, 7629.

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[34] Rosenberg, H.J.; Riley, A.M.; Marwood, R.; Correa, V.; Taylor, C.W.; Potter, B.V.L. *Carbohydr. Res.* **2001**, *332*, 53.

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Adenophostin analogues

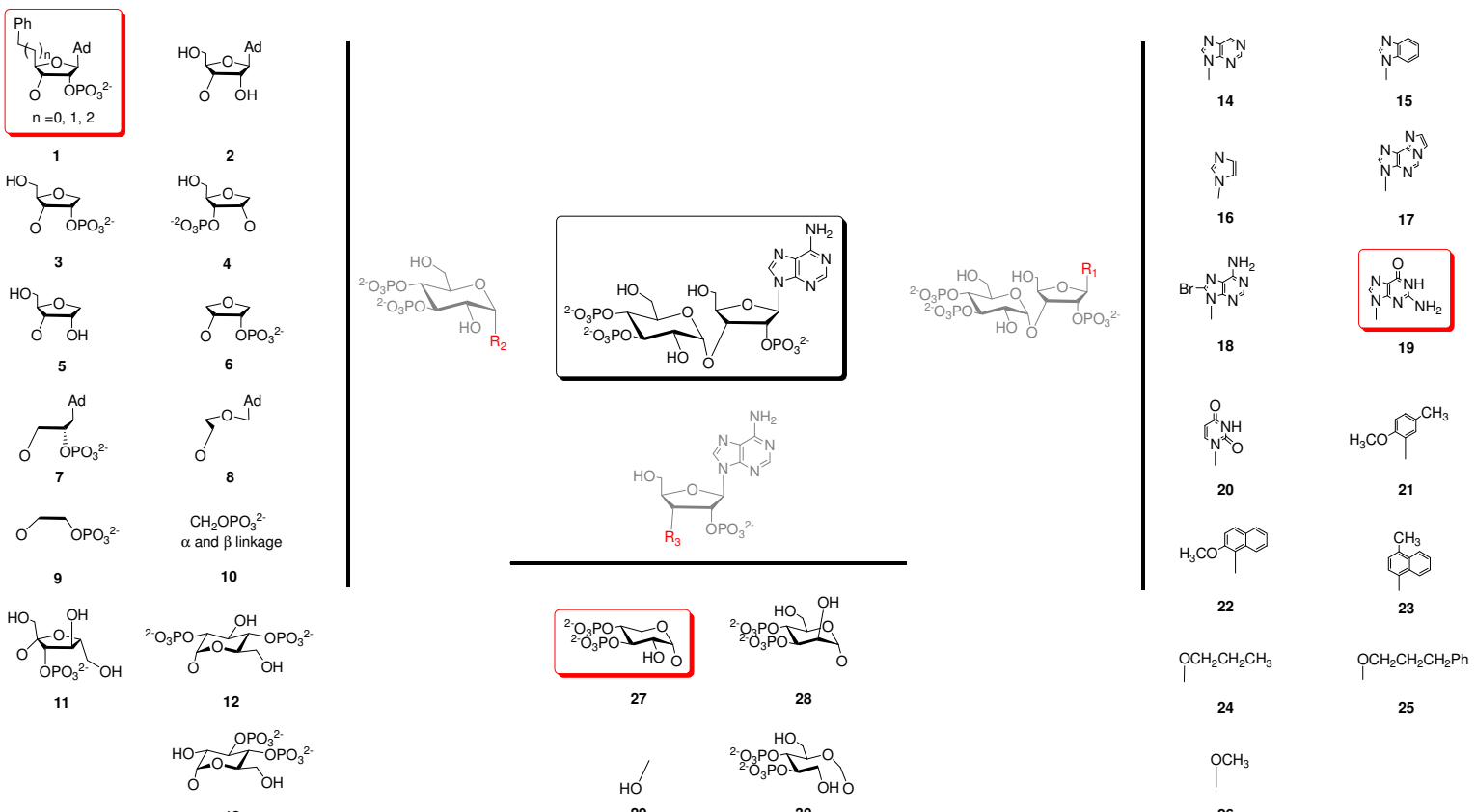

Figure 8. Structurally modified adenoophostin A analogues. The most effective modifications are boxed in red.

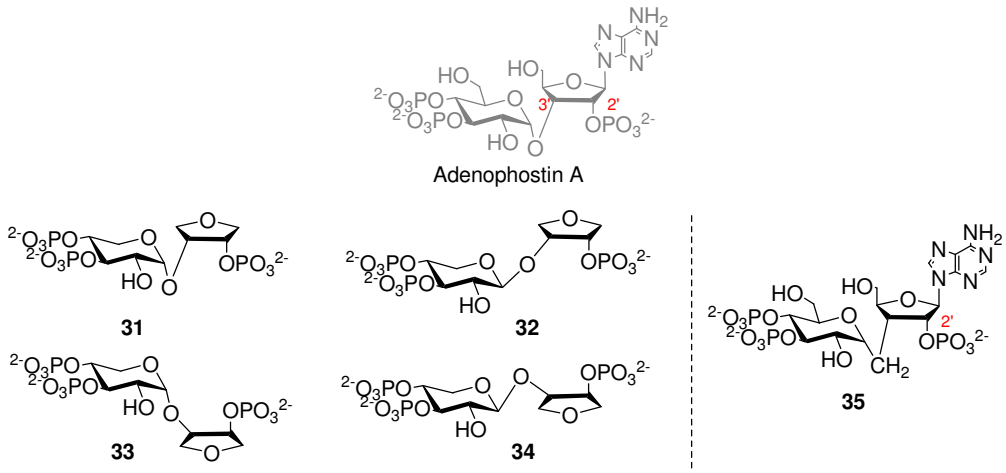

Figure 9. Glucose-ribose glycosidic linkage-modified analogues.

Adenophostin C-glycosidic analogue **35** was synthesized in order to clarify the role of the glycosidic oxygen.[35] This analogue was intended to be more stable to hydrolysis, but no prior accounts of its biological activity have been published. Many other analogues have been synthesized, some had different phosphorylation patterns,[14i,16b,36] and others had phosphotioate as a surrogate for phosphate,[37] conformationally restricted analogues,[38,39] multifunctional analogues.[31,32] None of these analogues, however, reached the same level of activity as adenophostin, and only cyclophostin **36** (Figure 10) showed slightly more affinity than IP_3. Cyclophostin is a 5',6"- tethered analogue which differs from Adenophostin only in that it possesses greater conformational restriction.

[35] Abe, H.; Shuto, S.; Matsuda, A. *J. Org. Chem.* **2000**, *65*, 4315; b) Abe, H.; Shuto, S.; Matsuda, A. *Tetrahedron Lett.* **2000**, *41*, 2391.
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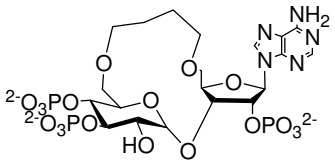

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Figure 10. Structure of cyclophostin

The synthesis of different adenophostin analogues and their biological evaluation elucidated the molecule's structure-activity relationship (SAR).[40] The main features responsible of adenophostin activity are as follows (Figure 11):

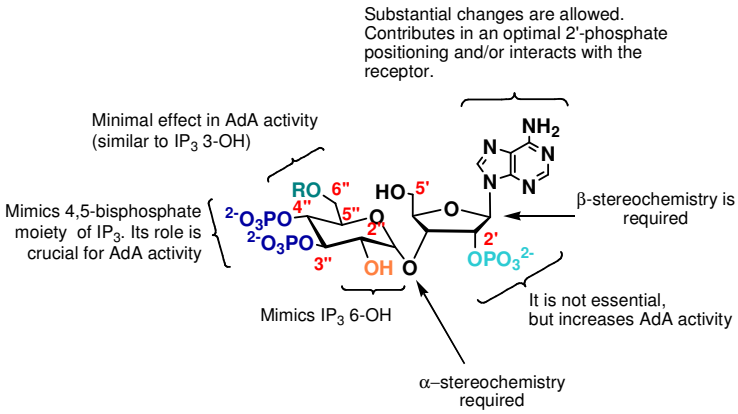

Figure 11. Pharmacophore model for adenophostins.

- A bisphosphate *trans*-diequatorial disposition flanked with 2''-OH, which mimics the 4,5-bisphosphate-6-OH moiety of IP_3, is a key feature for adenophostin activity.
- A phosphate group at position 2' increases but is not essential to adenophostin activity. Some studies[41] indicate that the optimal positioning of the adenophostin 2'-phospate is responsible for the molecule's high activity.

[40] a) Rosenberg, H.J.; Riley, A.M.; Laude, A.J.; Taylor, C.W.; Potter, B.V.L. *J. Med. Chem.* **2003**, *46*, 4860; b) Chretien, F.; Moitessier, N.; Roussel, F.; Mauger, J.-P.; Chapleur, Y. *Current Org. Chem.* **2000**, *4*, 513.

[41] Hotoda, H.; Takahashi, M.; Tanzawa, K.; Takahashi, S.; Miyamoto, S.; Kaneko, M. *Nucleic Acids Symp. Ser.* **1995**, *34*, 163.

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- Adenophostin analogues without adenine or other aromatic rings in their structure present very low activity. The exact role of the adenine moiety in adenophostin activity remains uncertain, but two effects have been suggested. The first theory is an indirect role, in which the adenine optimally positions the 2'-phosphate moiety.[22] The second theory involves more direct action, in which some receptor binding site residues engage in a cation-π interaction with the adenine. The latter effect is very common in protein-ligand interactions[42] and could occur between the positively-charged guanidinium side chain of Arg504 and the adenine of adenophostin.[40a]
- The hydroxyls at position 5' and 6'' are irrelevant to adenophostin activity.
- The glycosidic bond between glucopyranose and ribofuranose must be α.
- The glycosidic bond between ribofuranose and adenine must be β.

[42] Zacharias, N.; Dougherty, D. A. *Trends Pharmacol Sci*. **2002,** *23*, 281.

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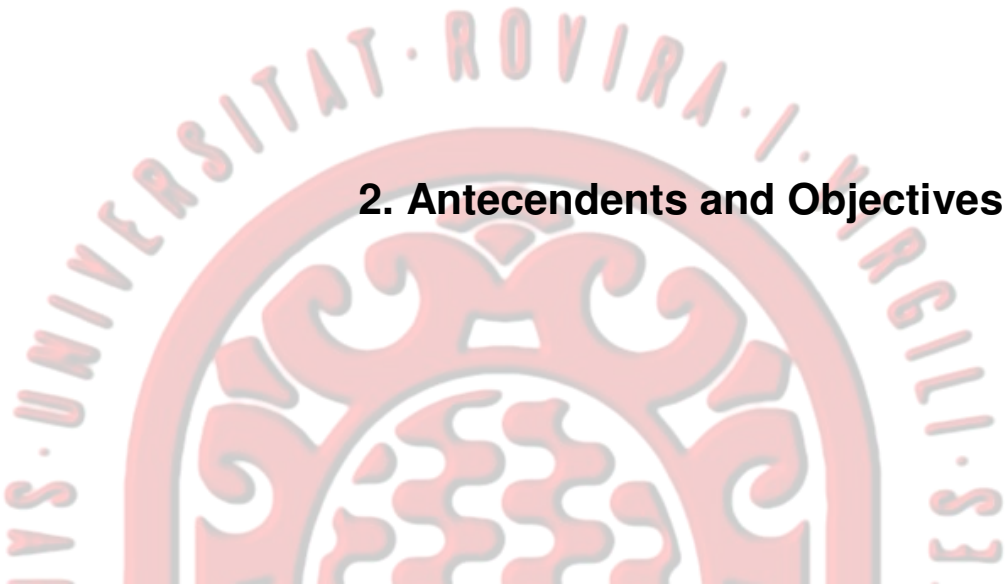

2. Antecendents and Objectives

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As previously described, the pharmacophore model for adenophostins leaves some unanswered questions. The real role of 2'-phosphate group remains to be seen, and the nature of the protein-ligand interactions with both the adenine moiety and the 2"-hydroxyl group is still indeterminate. The answer to these questions would aid in designing new, improved analogues.

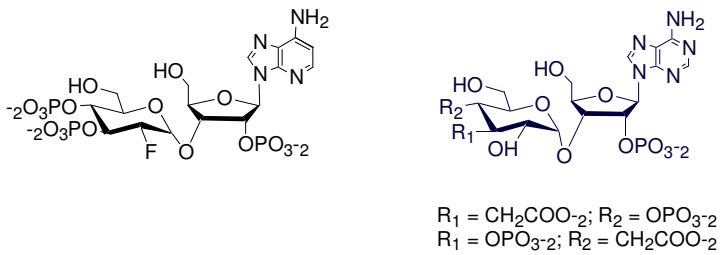
The first objective of this work is to perform biological studies with the following directives:

- **Study the role of the 2'-phosphate moiety of adenosphostine in relation to that of the 1-phosphate of IP_3.**
- **Determine whether the predominant role of the adenine moiety in adenophostin activity is to optimally position the 2'-phosphate or is to directly interact with the receptor.**

At the present, no previous analogue has ever surpassed adenophostin's activity. Thus, obtaining a more active compound is still an actively pursued topic in this field. From the structure-activity relationship studies, it is clear that any such analogue must at least bear the 3'',4''-bisphosphate moiety, the furanose ring, and an adenine group.

These findings, in combination with our group's experience in the field of fluorinated carbohydrates, led us to believe that incorporating a fluorine atom at the adenophostin 2''-position while maintaining all of the functionality essential for activity would create a good candidate analogue. It is well known that a fluorine at the 2-position of a glycoside increases glycosidic bond stability, especially against acidic hydrolysis. Thus, the resulting analogue would be more resistant to enzymes that metabolise IP_3. In addition, fluorine can act as an H-bond acceptor but not as an H-bond donor. Fluorination would therefore aid in elucidating the role of the natural product's 2''-OH.

The second objective of this work is to get inside into the synthesis of the to 2"-fluorinated adenophostin A analogue (Figure 12).



$R_1 = CH_2COO^{-2}$; $R_2 = OPO_3^{-2}$
$R_1 = OPO_3^{-2}$; $R_2 = CH_2COO^{-2}$

Figure 12

We also consider the possibility to access a new adenophostin analogues of adenophostin by substitution of 3",4"-bisphosphate moiety. In this sense, although a variety of phosphate surrogates have been introduced in biologically active compounds,[43] including vanadate-based phosphate analogues, a diversity of conjugated anions of acids such as carboxylic, tetronic, oxamic, difluoromethylenesulfonic and difluoromethylenephosphonic acids, and other acidic residues, the search for efficient phosphate analogues with desired binding and physicochemical properties is still very much ongoing.

In nature, different phosphate binding modes are present.[44] These include glycine-rich sequences, dinucleotide binding proteins, mononucleotide binding proteins, P-loops, novel P-loops, protein kinases, and the $C^{\alpha}NN$ structural motif. Knowledge of the binding site's structure and amino acid composition will aid in the design of a new ligand.

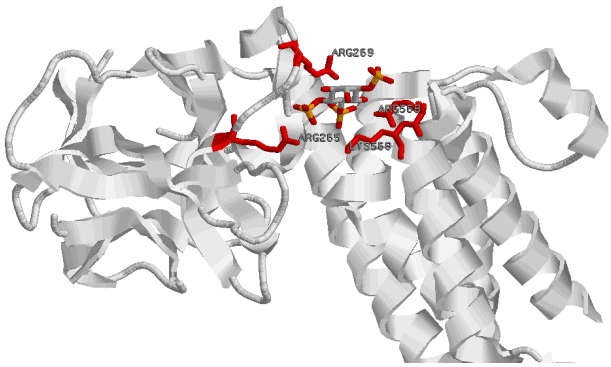
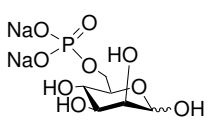


Figure 13. Tertiary structure of the IP_3R binding site with IP_3 bound. Basic residues are showed in red.

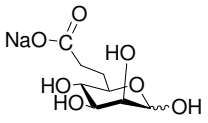
[43] a) L. Bialy, H. Waldmann, *Angew. Chem.* **2005**, *117*, 3880; b) L. Bialy, H. Waldmann, *Angew. Chem. Int. Ed.* **2005**, *44*, 3814.
[44] Hirsch, A. K. H.; Fischer, F. R.; Diederich, F. *Angew. Chem. Int.Ed.* **2007**, *46*, 338.

As previously described, the IP_3R binding core is principally composed of basic residues (Figure 13), which makes it a suitable environment for binding molecules with anionic moieties.

Morére *et al.*[45] described the use of carboxylate as an isostere of phosphate in the synthesis of mannose 6-phosphate analogues (Figure 14). Methylenecarboxylate analogues showed the same affinity for the mannose 6-phosphate receptor as the natural product. Moreover, these analogues were more stable to phosphatase action.



Mannose 6-phosphate



Mannose 6-methylenecarboxylate analogue

Figure 14

This precedent combined with the fact that the IP_3R binding site is suitable for anionic ligands, led us to believe that IP_3 receptors could recognize analogues bearing methylenecarboxylate moieties as phosphate isosters.

The third objective of this work is design an effective approach to synthesizing two adenophostin analogues incorporating 3" and 4"-methylenecarboxylate surrogate groups, respectively (Figure 12). These analogues would be more stable to the action of phosphatases. The proposed modifications would also lead to a differentiation between these two vicinal groups, which could contribute to some degree of understanding regarding the independent roles of 3"- and 4"-phosphates in mediating adenophostin activity.

[45] Vidal S; Garcia, M.; Montero, J.-L.; Morère, A. *Bioorganic Med. Chem.* **2002**, *10*, 4051.

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3. Results

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3.1. Biological assays.

Adenophostins have been found to be the most potent agonists of the IP_3 receptor.[18] As previously described, SAR studies have allowed for the development of a pharmacophore model, which has established the basic elements of adenophostin activity.

During initial investigations, adenophostin's 3'',4''-bisphosphate group was identified as being chemically equivalent to the 4,5-bisphosphate moiety of IP_3.[22] The equivalency of the adenophostin 2'-phosphate to the IP_3 1-phosphate was less evident (Figure 15).

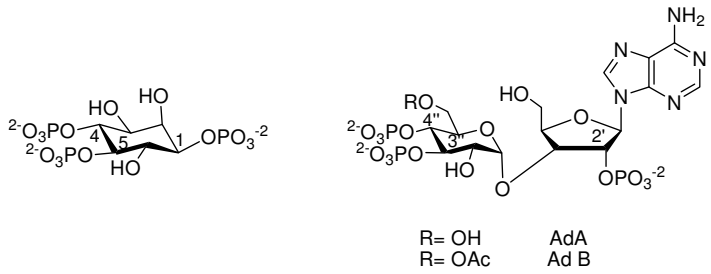

Figure 15. IP_3 and adenophostins A and B

In their structure elucidation work, Takahashi *et al.*[46] reported that adenophostin analogues lacking the 2'-phosphate group (2'-dephospho-adenophostin) showed decreased affinity that was three orders of magnitude lower than that of the natural product (15 nM vs. 0.18 nM). Nevertheless, this analogue still retained a weak Ca^{2+} mobilizing activity, indicating that the 2'-phosphate group might have an enhancing effect similar to that of the 1-phosphate group of IP_3. Later studies by Wilcox *et al.*[47] using computer-aided molecular modelling demonstrated that the adenophostin 2'-phosphate group might be relatively further from the 3'',4''-bisphosphate motif than the corresponding functional groups in 1,4,5-IP_3. This

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[47] Wilcox, R. A.; Erneux, C.; Primrose, W. U.; Gigg, R.; Nahorski, S.R. *Mol. Pharmacol.* **1995**, *47*, 1204.

suggested that adenophostin's high potency was probably due to optimal positioning of the 2'-phosphate group. Hotoda *et al.*[22] conducted NOESY experiments that confirmed that the relative position of the 2'-phosphoryl group was indeed optimal for IP_3R recognition.

When IP_3 binds to its receptor, the positively charge side chain of Arg568 interacts with the 1-phosphate (Figure 16). Changing this residue to an uncharged amino acid would remove this stabilizing interaction and thereby decreasing the ligands' affinities.

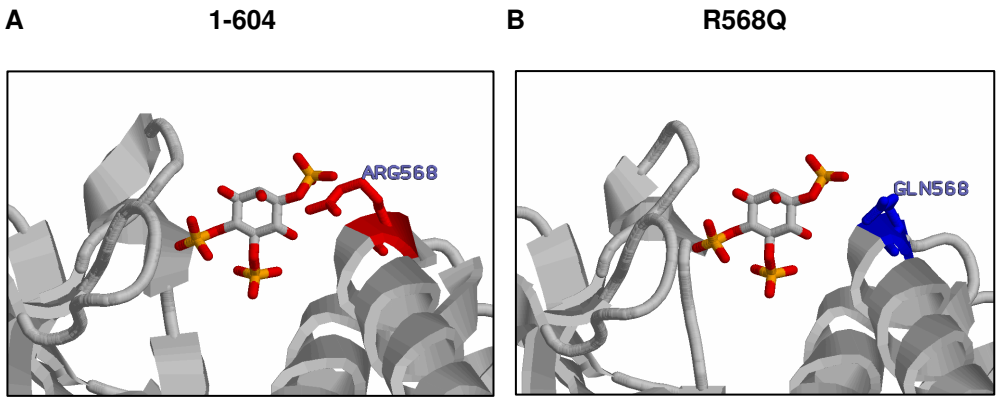
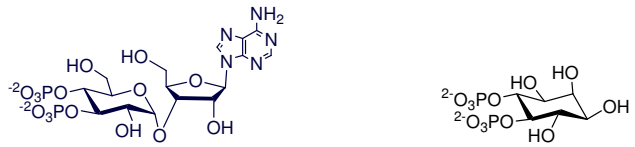


Figure 16. Cartoon representations of IP_3R binding core. A: Native receptor (named 1-604), Arg568 is shown in red (Structure taken from Protein Data Bank, 1N4K). B: R568Q construct, Gln568 is shown in blue (Fictitious representation of construct incorporating the mutation).

In order to study the role of the adenophostin 2'-phosphate relative to that of the 1-phosphate of IP_3, we evaluated the affinities of 2'-dephospho-adenophostin and 4,5-IP_2 (Figure 17) for the full length receptor, the N-terminal fragment (full receptor lacking C-terminal inhibitor domain, named 1-604), and the N-terminal fragment incorporating the mutation Arg568 to Gln (named R568Q).



2'-dephospho Adenophostin A

4,5-IP_2

Figure 17. Structures of analogues tested

The significance of this interaction would then be evaluated in terms of the ratio of the binding affinities before and after mutation.

Rosenberg *et al*[40a] noted that the adenophostin adenine moiety might directly interact with IP_3R, and this interaction might be crucial to the molecule's high activity. According to Rosenberg *et al.*, adenine would form a π-stacking interaction with the guanidinium moiety of Arg504. These types of interactions are involved in many molecular recognition processes.[48] For instance, similar π-stacking interactions have been implicated in the biological functions of the nicotinic acetylcoline receptor,[49] trimethylamine dehydrogenase,[50] human butyrylcholinesterase,[51] and voltage-gated Na^+ channels,[52] and they are also thought to stabilise membrane proteins.[53] In addition, recent studies have suggested that interactions between aromatic amino acids and various cationic centres are common motifs in protein structures.[54]

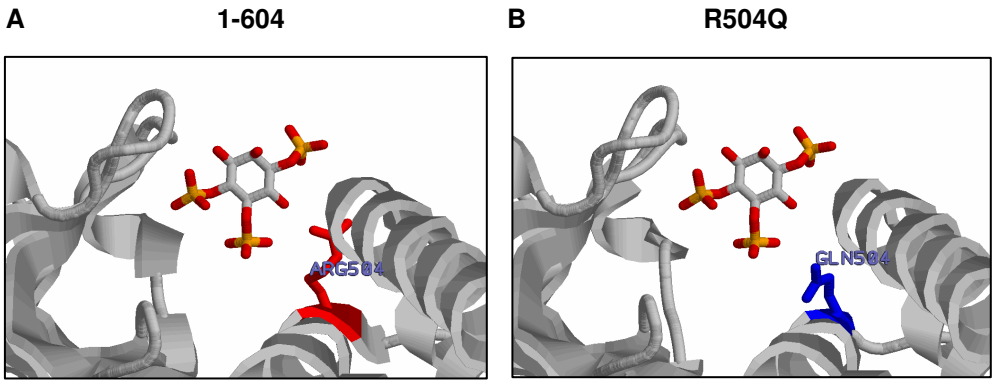


Figure 18. Cartoon representations of the IP_3R binding core with IP_3 bound. A: Native receptor, Arg504 is shown in red (Structure taken from Protein Data Bank, 1N4K). B: R504Q construct, Gln504 is shown in blue (Fictitious representation of construct incorporating the mutation).

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[48] Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303.
[49] Zhong, W.; Gallivan, J. P.; Zhang, Y.; Li, L.; Lester, H. A.; Dougherty, D. A. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 12088.
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[54] a) Minoux, H.; Chipot, C. *J. Am. Chem. Soc.* **1999**, *121*, 10366. b) Gallivan, J. P.; Dougherty, D. A. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 9459.

To better understand the adenine's role in adenophostin activity, and in order to evaluate the presence of these adenine-protein interaction and their contributions to adenophostin activity, the affinities of IP_3 and adenophostin for 1-604 and the N-terminal fragment incorporating the mutation Arg504 to Gln (named R504Q) were evaluated (Figure 18).

The aforementioned experiments would not only help clarify any adenophostin 2'-phosphate interactions and their respective contributions to adenophostin's affinity for IP_3R, but also would help identify the adenine's role in binding and where it interactions with the receptor. In order to develop the biological study, our work focused on two areas, obtaining the native and mutated receptors from bacterial expression or mouse cerebellar tissue, and measuring the ligands' affinities via radioligand binding assay.

All this work has been developed in the Calcium Signalling Laboratory of Pharmacology Department at University of Cambridge, under the supervision of Professor Colin W. Taylor.

Expression of fragments of type I IP_3 receptor in bacteria.

Proteins used during the biological assays were obtained from different sources. Full lenght receptors were obtained from mouse cerebellar homogenate. Whereas fragment corresponding to the binding domain (1-604) and mutated receptors were obtained from bacterial expression. Fragment 1-604 was obtained from a bacterial culture of *E.Coli* (strain BL21) transformed with a plasmid (pTrcHis) incorporating 1-604 cDNA fragment in its sequence. Proteins R568Q and R504Q were obtained from a bacterial culture of *E.Coli* (strain BL21) transformed with same plasmid (pTrcHis). This vector incorpored in its sequence the DNA obtained from site directed mutagenesis of the binding domain cDNA.

Equilibirum [^{3}H]-IP_3 binding.

Radioligand binding experiments were performed to measure both the rate and the extent that a radioligand (in this case the radioactively labelled drug, [^{3}H]-IP_3) bound to its receptor, transporter, enzyme, etc. This approach provides information as to both the number of binding sites and their affinities.

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When tritium decays, one of its neutrons is converted to a proton while concomitantly emitting an electron and a neutrino. The total energy released in this process is always the same, but it is randomly partitioned between the emitted electron and the emitted neutrino, which is not detected. If the electron has sufficient energy, it will travel far enough to encounter a molecule of the scintillation fluid. This fluid transduces and amplifies this signal by emitting photons, which the scintillation counter then detects. The intensity of the emission (i.e. the number of photons produced) is proportional to the energy of the electron.

Radioligand binding experiments are based on a model that is equivalent to the law of mass action for equilibrium reactions.

$$\mathbf{Ligand} + \mathbf{Receptor} \rightleftharpoons \mathbf{Ligand{\cdot}Receptor}$$

This model assumes the following:

- Binding occurs when ligand and receptor collide as a result of diffusion, provided that the collision takes place with the correct orientation and sufficient energy.

- Once binding has occurred, the ligand and receptor remain bound together for a random amount of time influenced by the binding affinity.

- After dissociation, the ligand and the receptor are the same as they were before binding.

- Equilibrium is reached when the rate at which new ligand·receptor complexes are formed equals the rate at which these complexes dissociate.

At equilibrium, ligand·receptor complexes are formed at the same rate that they dissociate, obeying the Equation 1:

$$\frac{\mathbf{[Ligand]{\cdot}[Receptor]}}{\mathbf{[Ligand{\cdot}Receptor]}} = \frac{\mathbf{k}_{off}}{\mathbf{k}_{on}} = \mathbf{K}_d$$

Equation 1

Where k_{on} and k_{off} are association and dissociation rate constants, respectively.

The K_d, expressed in units of concentration, is the equilibrium binding constant, and it represents the concentration of ligand at which half of the available receptors are occupied at equilibrium. A small K_d means that the receptor has a high affinity for the ligand, while a large K_d means that the receptor has a low affinity for the ligand.

Competitive binding experiments were performed measuring the binding of a small concentration of labelled ligand in the presence of various concentrations of unlabeled ligand.

Equilibrium competition binding curves were fitted to a four-parameter logistic equation using non-linear curve fitting software (Kaleidagraph, Synergy Software, Reading, PA, U.S.A.). The equilibrium dissociation constants (K_d) were determined (Equation 2):

$$B = N + \frac{T - N}{1 + \left(\frac{[L]}{IC_{50}}\right)^h}$$

Equation 2.

Where *T* is the total amount of radioligand bound in the absence of competing ligand, *N* is the amount of non-specific binding, *B* is the total amount of radioligand bound in the presence of a defined concentration of unlabeled ligand, [L], IC_{50} is the concentration of unlabeled ligand causing half-maximal displacement of the bound radioligand, and *h* is equivalent to the Hill coefficient.

The IC_{50} values derived from the curve fits were used to calculate the equilibrium dissociation constants (K_d) (Equation 3):

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$$K_d = \frac{IC_{50}}{1 + \frac{[radioligand]}{K_{d(radioligand)}}}$$

Equation 3

This equation is based on the following assumptions:

- Only a small fraction of both the labelled and unlabelled ligands has bound.
- The receptors are homogeneous and all have the same affinity for the ligands.
- There is no cooperativity, i.e. binding to one binding site does not alter the affinity of another site.
- The experiment has reached equilibrium.
- Binding is reversible and follows the law of mass action.
- K_d of the radioligand is known.

K_d of the radioligand was obtained from a homologous competitive binding assays (Equation 4):

$$K_d = IC_{50} - [L]$$

Equation 4.

Binding Assays Experiments.

Competitive binding curves were obtained when affinities of IP_3, Adenophostin A, 4,5-IP_2, and 2'-dephospho-Adenophostin A were evaluated for full length receptor. The logarithm of ligand concentration is plotted along the x-axes,

and the normalized specific binding (as a percentage) is plotted along the y-axes. Each curve represents the average for n independent experiments.

Evaluation of 2'-phosphate interactions.

The results shown in Figure 19 confirm that adenophostin A (K_d = 3.6 nM) is a full agonist of IP_3R with a 10-fold greater affinity for the receptor than IP_3 (K_d = 30.3 nM). A drastic decrease in binding affinity was also observed for dephosphorilated substrates IP_2 (K_d = 7490 nM) and 2'-dephospho-adenophostin A (K_d = 46.3 nM).

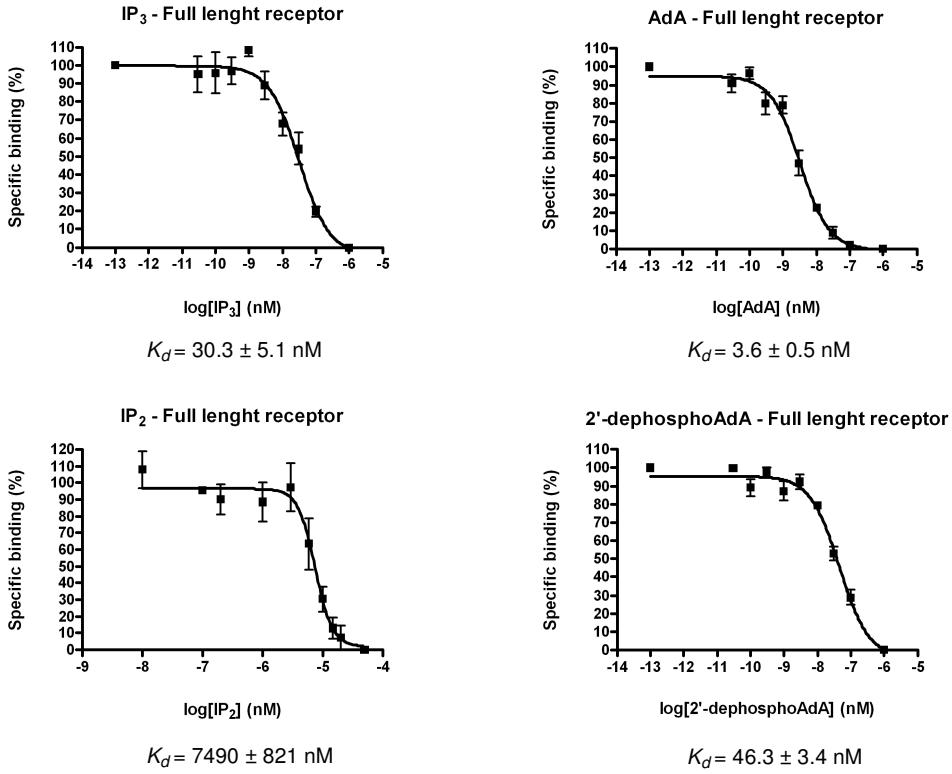


Figure 19. Binding curves for IP_3, adenophostin (AdA), IP_2, and 2'-dephospho-AdA using full-length receptor.

Plots corresponding to the binding assays performed with the same test ligands, but with the 1-604 protein in lieu of the full-length receptor are depicted in Figure 20. Again, dephosphorylated ligands show lower affinities than IP_3 and adenophostin A (K_d = 1090 nM for IP_2 and 12.2 nM for 2'-dephospho-Adenophostin).

Affinity of IP_3 for 1-604 (K_d = 30.3 nM) was increased 10-fold with respect to that for the full length receptor (K_d = 3.3 nM), but the affinity of adenophostin increased only slightly from 3.6 nM to 1.0 nM. Similar trends were observed for both IP_2 and 2'-dephospho-adenophostin A, and these molecules' K_d values went from 7500 to 1000 nM and from 46.3 to 12.2 nM, respectively.

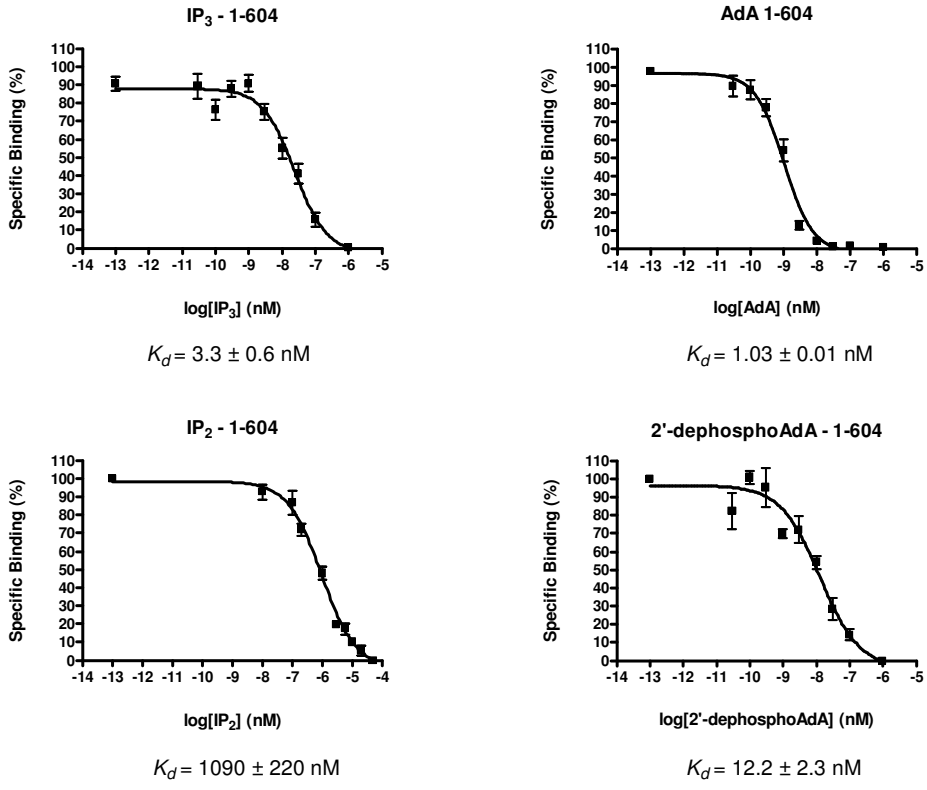


Figure 20. Binding curves for IP_3, AdA, IP_2, and 2'-dephosphoAdA using 1-604 (binding domain).

This expected increase in affinity was attributed to the absence of residues located at C-terminal domain. This residues, which are present in the full length receptor (Figure 19), but not in 1-604 fragment (Figure 20), are believed to occlude the binding core of IP_3R[10] and difficult IP_3 binding. However, in adenophostin case, affinity increase is less significative because adenophostin could partially avoid this inhibitory effect presumably by means of adenine interactions.[10,16b,56]

Binding curves corresponding to assays carried out with the R568Q fragment are represented in Figure 21. For these experiments, the differences in binding affinity between inositol derivatives and between adenophostin and its derivative were less marked.

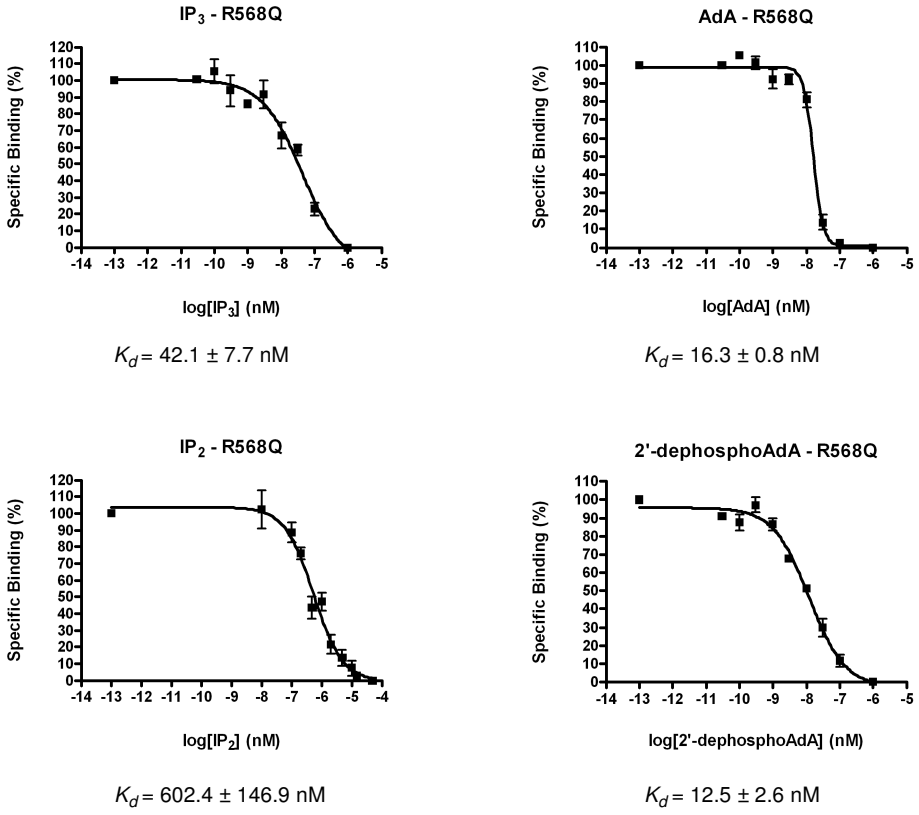


Figure 21. Binding curves for IP_3, AdA, IP_2, and 2'-dephospho-AdA using R568Q (binding domain modified at position 568).

[56] Hotoda, H.; Murayama, K.; Miyamoto, S.; Iwata, Y.; Takahashi, M.; Kawase, Y.; Tanzawa, K.; Kaneko, M. *Biochemistry*, **1999**, *38*, 9234.

Because a stabilising interaction with a positively charged amino acid was removed, the affinities of all ligands decreased (Table 1). The K_d values for IP_3 and adenophostin A were 42.1 nM and 16.3 nM, respectively.,and the K_d values for 4,5-IP_2 and 2'-dephospho-adenophostin were 602 nM and 12.5 nM, respectively. In addition, it seemed that mutation from Arg to Gln created a binding pocket that favoured the dephosphorylated compounds. This was probably because the dephospho-compounds were not capable of stabilizing the arginine side chain's positive charge, which was present in the native receptor.

Table 1. K_d (nM) values of tested ligands.

Protein	*IP_3*	*IP_2*	*AdA*	*2'-dephAdA*
Full length receptor	30.33	7490	3.26	46.30
1-604	3.27	1090	1.03	12.20
R568Q	42.10	602	16.29	12.49

The ratios comparing the various K_d values are listed in Table 2. For the wild-type receptors (full length and 1-604, entries 1 and 2), dephosphorylation of the inositol derivatives caused around 300-fold decrease in binding affinity. For adenophostin A and its 2'-dephosphoylated derivative, however, the decrease in affinity was only about 15-fold. In contrast, when the mutant receptor fragment, R568Q, was used (entry 3), the removal of phosphate interacting residue tends to equal ligands' affinities. The K_d value for IP_2 reflected a mere 14-fold decrease relative to their respective phosphorylated counterparts. Binding of the dephosphorylated adenophostin derivative was virtually identical to that of the native molecule. Comparing the ratios of both ligands for both 1-604 and R568Q (entry 4) gave an idea of the extent of the phosphates' contributions to binding. Thus, it appeared that the phosphate is slightly less significant for adenophostin.

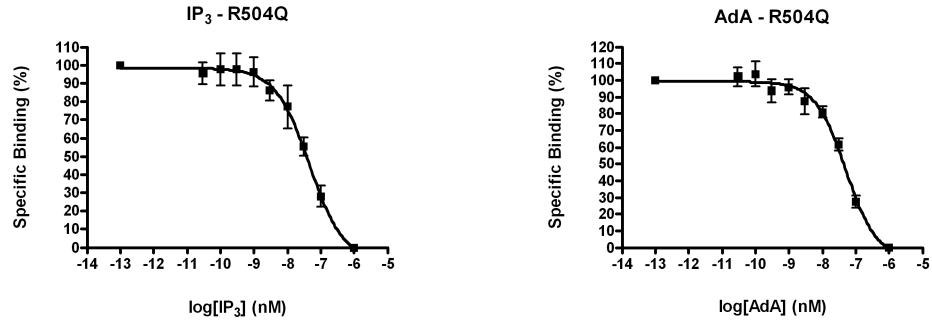
Table 2. K_d ratios of IP_3, IP_2, adenophostin A, and 2'-dephospho-adenophostin A against full length receptor, 1-604, and R568Q.

Entry	Protein	IP_2/IP_3	2'-dephAdA/AdA
1	***Full length***	247	14
2	***1-604***	333	12
3	***R568Q***	14	0.8
4	***1-604/R568Q***	**23**	**15**

In summary, the removal of a phosphate produces a smaller decrease in the adenophostin binding affinity than in the IP_3 binding affinity. Thus, identifying the 2'-phosphate of adenophostin with the 1-phosphate of IP_3 is not entirely accurate, because the significance of the phosphate with respect to binding affinity is slightly different between molecules.

Evaluation of adenine interactions.

For binding assays performed using full length receptor, the K_d of adenophostin (3.6 nM) was ten times lower than that of IP_3 (30.3 nM), In experiments using the R504Q mutant, however, no significant difference in binding affinity was detected between IP_3 and adenophostin A (K_{dIP3} =50.3, K_{dAdA} = 51.6).



K_d = 50.3 ±9.0 nM K_d = 51.6 ± 8.3 nM

Figure 22. Binding curves for IP_3, and AdA using R504Q. (binding domain modified at position 504).

Thus, when the residue capable of creating a cation-π interaction was removed, the affinity of adenophostin was decreased 15-fold.

Another effect associated with the R504Q mutation was a global decrease in affinity for both IP_3 and adenophostin (Figure 22) For both ligands, this loss of binding was most likely related to the role that Arg504 plays in the protein's tertiary structure. Arg504 is located at the base of an α-helix (α-6)[57], stabilizing its dipole moment. Replacing Arg with Gln probably causes a conformational change that disminishes the effectiveness of ligands interaction. If this were the only effect involved in the observed decrease in affinity, however, it would be the same for both ligands. Nevertheless, loss of adenophostin affinity is significantly greater than the loss of IP_3 affinity (). A direct interaction between the adenine ring and the Arg504 side chain, which would be completely absent from IP_3 binding, may explain this discrepancy.

Table 3. K_d values for IP_3 and adenophostin A with 1-604 and R504Q.

Protein	*IP_3*	*AdA*
1-604	3.27	1.08
R504Q	50.3	51.6

Ultimately, these results support the hypothesis for the existence of a significant cation-π binding interaction between the protein and the adenophostin adenine moiety. Thus, designing analogues to incorporate modifications both at the bisphosphate moiety and at the adenine ring to exploit this interaction may yield more active compounds in the future.

[57] Bosanac, I.; Alattia, J. R.; Mal, T. K.; Chan, J.; Talarico, S.; Tong, F.; Tong, K. I.; Yoshikawa, F.; Furuichi, T.; Iwai, M.; Michikawa, T.; Mikoshiba, K.; Ikura, M. *Nature*, **2002**, *420*, 696.

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3.2. Approach to the Synthesis of 2'-deoxy-2'-fluoro Adenophostin A .

The fluorination of biologically active organic compounds often leads to a dramatic changes in their biological activity, stability and bioavailability. Thus, many fluorine-containing pharmaceuticals have been developed over the past few decades.[58] Fluorinated carbohydrates derivatives, and particularly 2-deoxy-2-fluoro nucleosides, have received great attention because they exhibit fascinating biological activities: in antiviral therapies,[59,60c] cancer imaging diagnosis,[60a,c,61] and antitumor applications[60,62] (Figure 23). Fluorinated carbohydrates have been used as well to probe the mechanism and specificity of various enzymes[63] as they can modify the activity of substrates and stabilize the glycosidic linkage against hydrolysis.[64]

The fluorine van der Waals radius (1.47 Å) falls between that of oxygen (1.52 Å) and that of hydrogen (1.20 Å), making it a versatile element for bioisosteric replacements. A C–F moiety can mimic a C–OH moiety, because its polarity is similar, especially when fluorine and oxygen are involved in polar interactions with

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[64] Tsuchiya, T. Adv. Carbohydr. Chem. Biochem. **1990**, 48, 91.

strongly positively charged polarized centres, such as lateral chains of basic amino acids.[65]

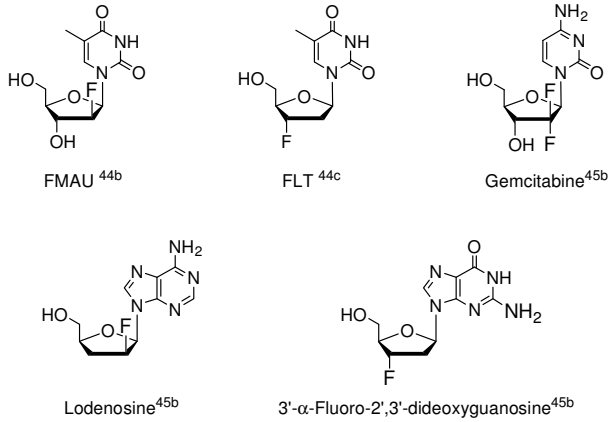

Figure 23. Fluorinated nucleosides with biological activity.

Although theoretical studies have indicated that C—H···F—C interactions are of no significant value[66] because bonding energy is low, with energies similar to those of van der Waals complexes, it has been suggested that O—H···F—C and N—H···F—C interactions of 2.5 Å or less may aid in stabilizing ligand-target interactions.[66] Moreover, F is the smallest substituent that can replace H, and, so a C—F bond can often substitute for a C—H bond with minimal structural consequences.[67]

The incorporation of fluorine into a drug allows for simultaneous modulation of electronic, lipophilic, and steric parameters, all of which can critically influence both the pharmacodynamic and the pharmacokinetic properties of a drug. The presence of fluorine in a nucleoside restricts its conformational freedom[68] and, when

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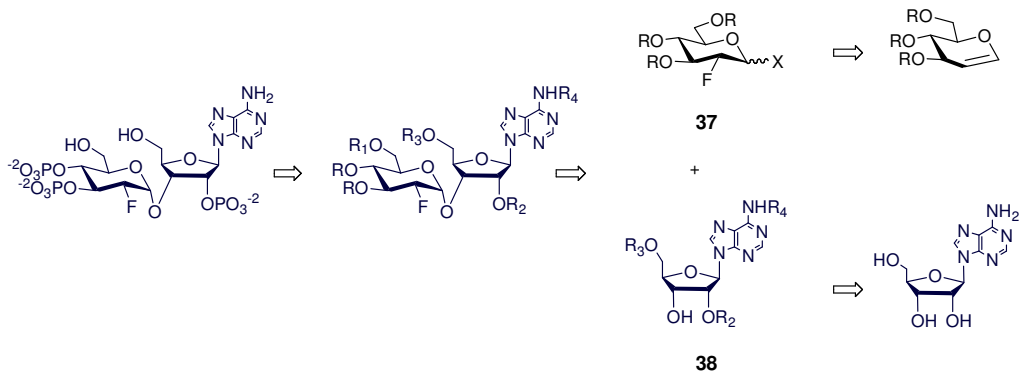
vicinal to a glycosidic bond, increases its stability towards the hydrolysis of the glycosidic linkage.[69]

As previously mentioned, one of the features that makes adenophostin so potent is its stability towards the enzymes that metabolise IP_3. The presence of fluorine at the adenophostin 2''- position would presumably increase the glycosidic bond's stability even further. Moreover, the adenophostin 2''-OH group is critical for binding, and so incorporating a fluorine at that position would provide information regarding the role of the 2''-OH (i.e. whether it is an H-bond acceptor or donor) in its interaction with the receptor.

Taking into account the possible benefits that the introduction of fluorine in Adenophostins structure could offer, we envisaged the synthesis of an adenophostin analogue incorporating fluorine at position 2''.

The proposed retrosynthetic analysis (Scheme 2) involves the stereoselective glycosylation of donor **37** with acceptor **38**. The donor could be obtained from an appropriately protected *D*-glucal via electrophilic fluorination. Product **38** can be obtained from the selective protection of adenosine.



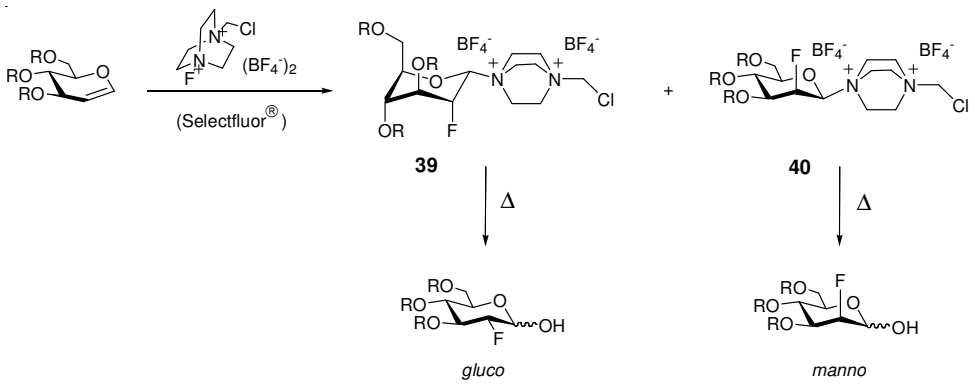
Scheme 2. Retrosynthetic scheme for the synthesis of 2'-deoxy-2'-fluoro-adenophostin A

[69] Namchuck, M. N.; McCarter, J. D.; Becalski, A.; Andrews, T.; Withers, S. G. *J. Am. Chem. Soc.* **2000**, *122*, 1270.

We envisaged that the 2-deoxy-2-fluoro derivative **37** could be obtained from the electrophilic addition of fluorine into the double bond of *D*-glucal using Selectfluor®[70] as a fluorine source. Application of this reagent has been shown to be a very mild, room-temperature method for the efficient fluorination of nucleophiles, such as glycals.[71]

3.2.1. Synthesis of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-glucosyl bromide.

Syn-addition of fluorine and its counter ion into the glucal double bound is the first step of the fluorination process (Scheme 3). The counter-ion is then displaced by a nucleophile present in the reaction medium. If water is used as a nucleophile, *manno* and *gluco-pyranoses* are obtained. The exact product distribution depends on a variety of factors, such as steric hindrance of the incoming nucleophile, carbohydrate ring substituents, and ring conformation.



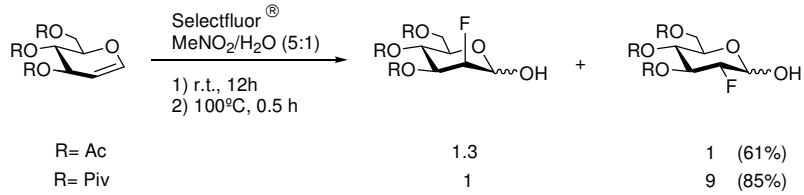
Scheme 3. Electrophilic fluorination of glucals with Selectfluor®

The relative rates of hydrolysis for the respective intermediate adducts controls the *gluco-manno* ratio. As the pre-*gluco* intermediate is less stable,

[70] (1-chloromethyl-4-fluoro-1,4-diazoniumbicycle[2.2.2]octane tetrafluoroborate)
[71] Burkart, M. D.; Zhang, Z.; Hung, S. Ch.; Wong, Ch. H. *J. Am. Chem. Soc.* **1997**, *119*, 11743.

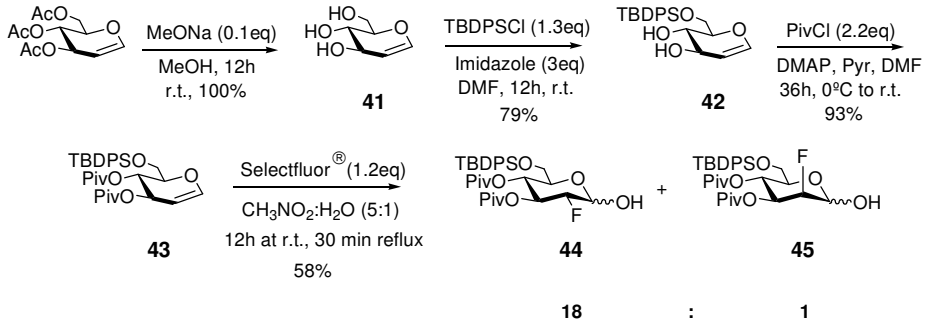
formation of this product is typically favored.[72] Thus, from a practical standpoint, control of heating time is crucial to obtaining reasonable diastereoselectivity.

Dax *et al.* reported that the stereoselectivity of this class of reactions mainly depended on the protecting groups present on the glycal.[72,73,] When acetyl ester protecting groups are employed, equimolar mixtures of diastereoisomers are obtained. When pivaloyl protecting groups are used, however, the formation of *gluco* derivatives is favoured (Scheme 4).



Scheme 4. Effect of protecting group substituents in *gluco*:*manno* fluorination ratio.

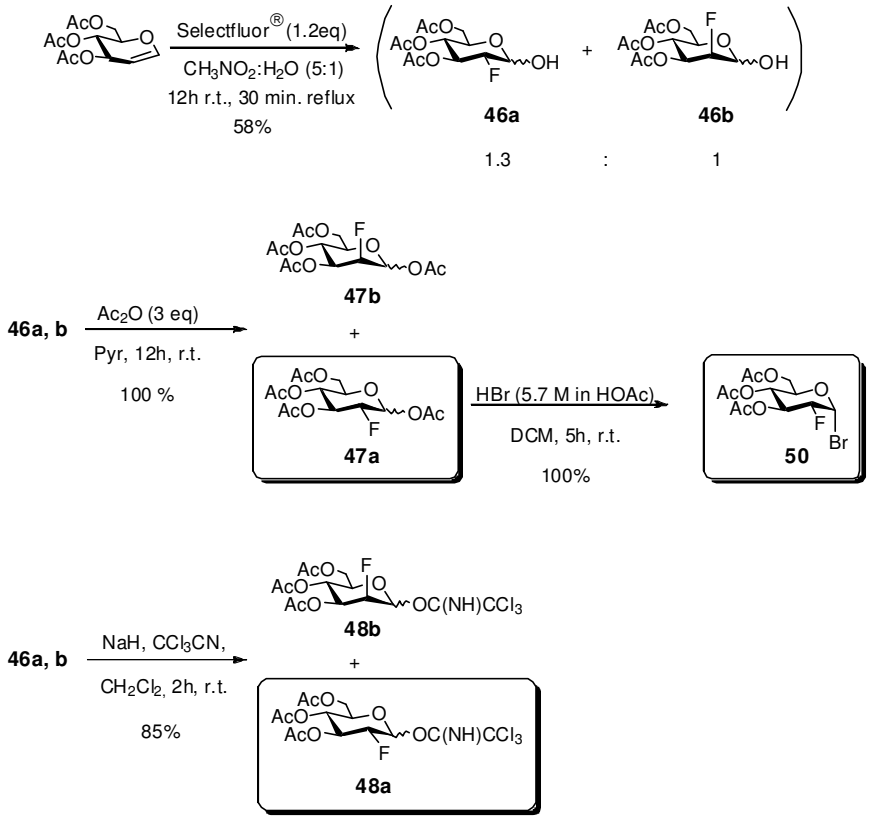
Our final goal was to obtain a glucoside with one type of protecting group at positions 3, 4 and an orthogonal to protecting group at position 6. This approach would greatly facilitate selective phosphorylation of these secondary hydoxyl groups during the final steps of the synthesis. In accordance with this strategy, we envisaged that fluorination of a glucal with bulky substituents would predominantly yield the desired *gluco* derivative.



Scheme 5. First approach to the synthesis of 2-deoxy-2-fluoro glycoside donor.

[72] Ortner, J.; Albert, M.; Weber, H.; Dax, K. *J. Carbohydr. Chem*. **1999**, *18*, 297
[73]. Dax, K.; Albert, M.; Ortner, J.; Paul, B. *J. Carbohydr. Res*. **2000**, *327*, 47.

The synthesis of 2-fluoro-2-deoxy glucoside **37** began with 3,4,6-tri-*O*-acetyl-*D*-glucal (Scheme 5). After quantitative deprotection, selective protection of the primary alcohol of **41** was afforded via reaction with *tert*-butyldiphenylsilyl chloride in DMF (79% yield). Reaction of the resulting product, **42,** with pivaloyl chloride produced the desired glucal **43** in **93** % yield. Subsequent electrophilic fluorination of **43** led to an anomeric mixture of diastereoisomers **44** and **45** in a *gluco*:*manno* ratio of 18:1. Although the final reaction's yield was moderate (58%), the diastereoisomeric ratio observed was slightly higher than that reported by Dax *et al.*[73] Unfortunately, the desired 2-fluoro-derivative could only be obtained following selective deprotection of the primary hydroxyl group, and this proved unfeasible.



Scheme 6

Our second approach attempted to avoid unnecessary protection-deprotection steps and to obtain the desired glucoside in a more straightforward manner.

The synthesis started with the same triacetylated D-glucal as before (Scheme 6), which after Electrophilic fluorination[71] gave a mixture of compounds **46a** and **46b** (*gluco*:*manno* ratio of 1.3:1), which was separable only after subsequent acetylation at the anomeric position.

The ^{19}F NMR spectrum of **47a** showed signals at –202.90 ppm (dd) and –201.65 ppm (ddd) with coupling constants $^{2}J_{F\text{-}H2}$, $^{3}J_{F\text{-}H3}$, and $^{3}J_{F\text{-}H1}$ of 49, 12, and 0 Hz, respectively, for the first signal and 50, 14, and 3 Hz for the second signal. These data confirmed the fluorine was in the equatorial position for both anomers.

With acetyl glycoside **47a** in hand, the next step was the introduction of bromine at the anomeric position. Treatment of a solution of **47a** in DCM with HBr (5.7 M in acetic acid)[74] for five hours afforded glycosyl bromide **50** quantitatively. The ^{1}H and ^{19}F NMR spectra confirmed that only the α isomer was formed (6.54 ppm, doublet, $^{3}J_{H1\text{-}H2}$ = 4.0 Hz).

In addition to glycosyl acetates **47a** and glycosyl bromide **50**, trichloroacetimidate[75] derivatives were also prepared in order to test the effect of different glycosyl donors.[76] Thus, a mixture of **46a** and **46b** was reacted with trichloroacetonitrile in the presence of NaH. As the result of the diastereomers' enhanced anomeric stability, which was imparted by the fluorine atom, the mixture of compounds could be purified, and an anomeric mixture of *gluco* derivatives (**48a)** was obtained with an 85 % yield.

[74] a) Shelling, J. G.; Dolphin, D.; Wirz, P.; Cobbledick, R. E.; Einstein, F. W. B. *Carohydr. Res.* **1984**, *132*, 241. b) McCarter, J. D.; Yeung, W.; Chow, J.; Dolphin, D.; Withers, S. G. *J. Am. Chem. Soc.* **1997**, *119*, 5792.
[75] Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212.
[76] Almacellas, N. Ph.D. Tesis, Universitat Rovira i Virgili **2007.**

3.2.2. Synthesis of N^6-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyladenosine.

The next step in the synthesis of 2'-deoxy-2'-fluoro adenophostin A required synthesizing building block **38**. Most of the syntheses of adenophostin or its analogues described in the literature use a suitable protected riboside as a glycosyl acceptor.[27,40a,77,78] The base moiety is usually incorporated later via Vorbrüggen glycosylation.[24a,26,79] We decided to perform the glycosylation with the corresponding protected adenosine directly. Using this methodology, the synthesis was simplified and was more convergent.

In all prior adenophostin syntheses, when adenosine was used as starting material, the 2'-position 2' was usually protected with a *p*-methoxybenzyl ether,[19,20a,35] and the primary alcohol was protected with either a monomethoxytrityl ether[19] or a benzyl ether.[20a,35] Finally, the base is typically protected with a benzoyl[19] or dimethoxytrityl group.[20b,35]

In this case, a *p*-methoxybenzyl group was chosen to protect the 2'-position, a benzoyl group was selected for protection of the N-6 amine, and a *tert*-butyldiphenylsilyl ether was employed for the primary alcohol. (Figure 24).

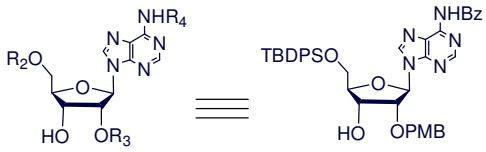


Figure 24. Protecting groups for glycoside acceptor.

Starting from commercially-available adenosine, selective protection of the 2'-hydroxyl was achieved using PMBCl and NaH,[80] which took advantage of the higher acidity of the 2'-hydroxyl.

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[77] Sureshan, K. M.; Trusselle, M.; Tovey, S. C.; Taylor. C.W.; Potter, B. V. L. *Chem. Commun.* **2006**, 2015.
[78] Marwood, R. D.; Jenkins, D. J.; Correa, V.; Taylor, C. W.; Potter, B. V. L. *J. Med. Chem.* **2000**, *43*, 4278.
[79] Shuto, S.; Mochizuki, T.; Abe, H.; Kondo, Y.; Matsuda, A. *Nucleic. Acids. Res. Supp.2* **2002**, 23.
[80] Takaku, H.; Kamaike, K. *Chem. Lett.* **1982**, 189.

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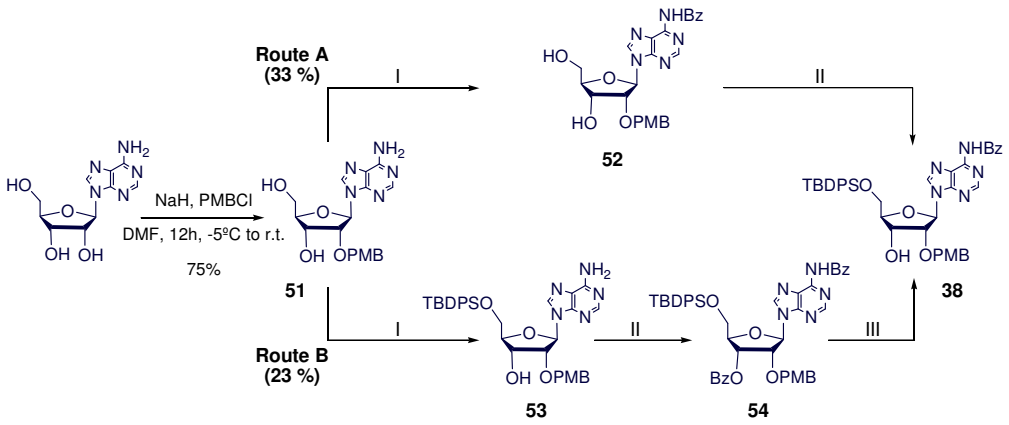
The synthesis of 2'-OPMB adenosine did not meet the expectations for efficiency set by the literature reports, and so some modifications were made to this methodology in order to increase the yield. The best yields were obtained when the reaction was held at room temperature for 12 h following the addition of the PMBCl (Scheme 7). Formation of **51** was confirmed by the ^{1}H NMR spectrum of the product, in which there appeared two doublets at 7.06 and 6.72 ppm that corresponded to the protecting group's aromatic protons coupled to the 3'-H (4.34 ppm) and the hydroxyl proton (5.32 ppm).

The formation of significant amounts of 3'-OPMB adenosine could not be avoided. Unfortunately, the product could not be crystallized as described in the literature, and purification via column chromatography was necessary.

Next the amine and the primary hydroxyl group were both selectively protected. Following the methodology of Takaku *et al.*,[80] *N*-6 amine benzoylation was achieved after reacting **51** with benzoyl chloride in pyridine and selectively hydrolyzing the benzoate ester, which was concomitantly formed from the exposed alcohol (Scheme 7, path A). Unfortunately, the selective alcohol deprotection led to a mixture of products and only modest yields (55 %). Finally, reaction of **52** with *tert*-butyldiphenylsilyl chloride gave the desired glycosyl acceptor **38** in 33 % overall yield from **51.**

An alternative route to obtain building block **38** was developed in an attempt to increase overall yields. Thus, after protection of the 2'-hydroxyl group, selective protection of primary alcohol was performed, obtaining silyl derivative **53** in 86 % yield. (Scheme 7, path B) Subsequent benzoylation and 3'-ester hydrolysis afforded the desired nucleoside. Unfortunately, selective hydrolysis again produced a mixture of compounds and low overall yield (23%).

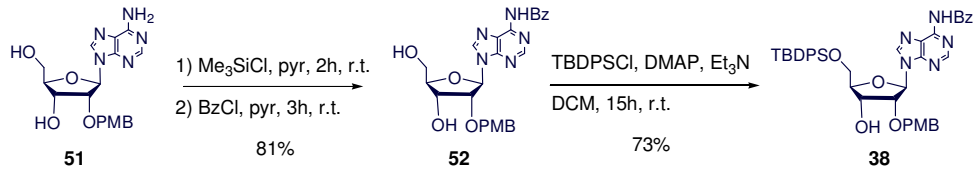


Route A I) a) BzCl, Pyr, 0 ºC to r.t., b) NaOH (1%), MeOH, 55 %; II) TBDPSCl, Imidazole, DMF, 0 ºC to r.t., 81 %

Route B I) TBDPSCl, Imidazole, DMF, 0 ºC to r.t., 86 %; II) BzCl, Pyr, 0 ºC to r.t., 81 %; III) NaOH (1%), MeOH, 44 %

Scheme 7

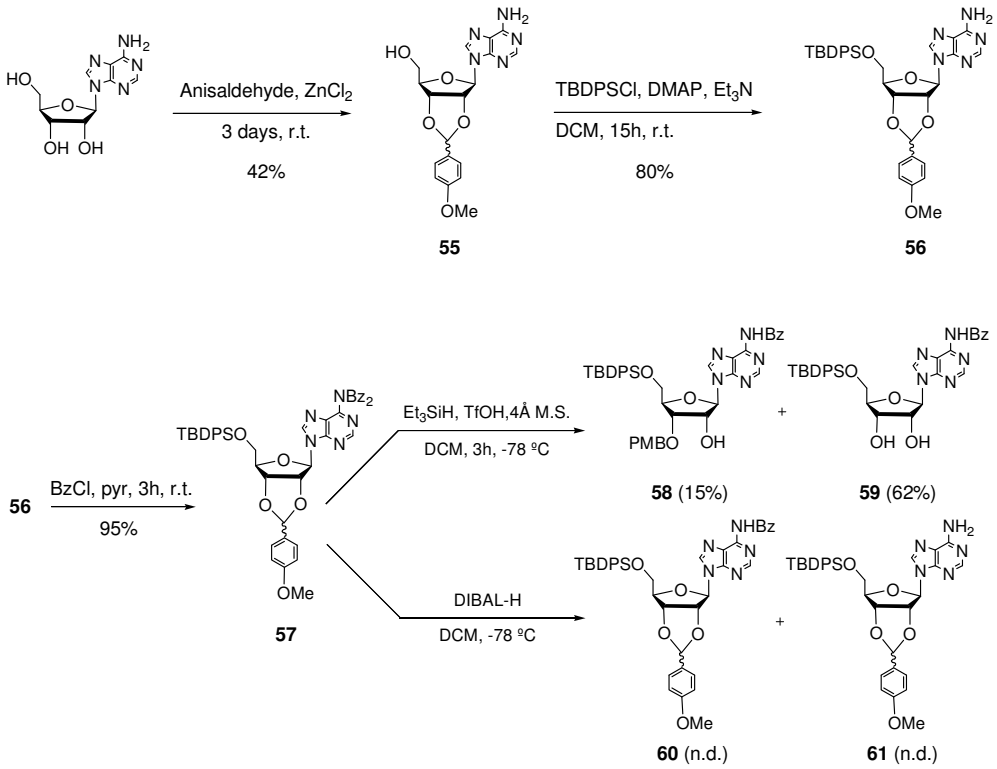
Finally, selective benzoylation of N-6 was accomplished using a temporary TMS-protecting group for hydroxyls 3' and 5'[81] (Scheme 8). After reaction of the alcohols with trimethylsilyl chloride in pyridine, benzoyl chloride was added. Treatment of the crude product mixture with DOWEX-H^+ afforded the N-6-protected adenosine derivative **52** in good yield (81%). Finally, selective protection of the 5'-OH was easily achieved using *tert*-butyldiphenylsilyl choride with a DMAP catalyst in DCM[82]. The desired glycosyl acceptor **38** was isolated in 73% yield (59% overall yield). The ^{1}H NMR spectrum possessed a signal at 1.08 ppm corresponding to *tert*-butyl moiety, and the integration of the aromatic region confirmed the presence of 19 aromatic protons. In addition, the signal corresponding to the 3'-OH at 2.87 ppm confirmed that the protection took place at the 5'-position.



Scheme 8

[81] Zhao, Y; Baranger; A. M. *J. Am. Chem. Soc.* **2003**, *125*, 2480.
[82] Prakash, T. P.; Kawasaki, A. M.; Fraser, A. S.; Vasquez, G.; Manoharan, M. *J. Org. Chem.* **2002**, *67*, 357.

Additional synthetic approaches were developed in order to increase overall yield even further and to avoid cumbersome purifications. We envisaged an alternative route to the desired glycosyl acceptor which involved the selective opening of 2',3'-*O*-*p*-methoxybenzylidene-protected adenosine. The direction of ring opening for five-membered benzylidene rings depends upon the stereochemistry at the acetal centre.[83] We hypothesized, however, that unlike typical five-membered benzylidene acetals, the presence of the adenine would dictate the selectivity of the ring opening rather than the *endo/exo* effect. To this end, commercial adenosine was treated with anisaldehyde and $ZnCl_2$ affording **55** as an inseparable mixture of *endo* and *exo* isomers (Scheme 9). The mixture of diastereoisomers was treated with *tert*-butyldiphenylsilyl chloride in DCM to yield compound **56**. Subsequent reaction with benzoyl chloride gave the desired N-6 protected nucleoside, **57**.



Scheme 9

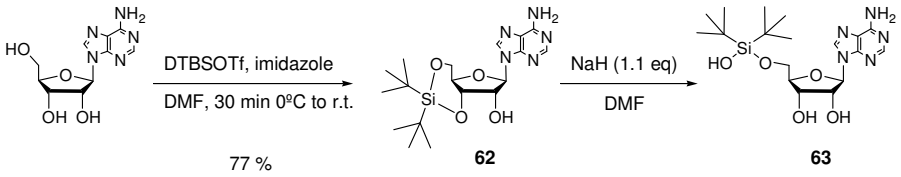
[83] Garegg, J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97.

The first attempt at *p*-methoxybenzylidene ring opening was made using triflic acid and triethylsilyl hydride. Normally, when this method is applied to a six-member benzylidene ring, the less hindered alcohol remains protected.[84]

Unfortunately, instead of the desired 2'-OPMB product, a mixture of the 3'-OPMB protected adenosine **58** and the diol **59** was obtained. When the amount of acid added was reduced to check if the 2'-OPMB product was hydrolysed after its formation, only 3'-OPMB and starting material were obtained.

Selective opening could also be achieved using DIBAL-H.[20b,33,85] Unfortunately, in this case, no 2'-OPMB product was detected, and N6-deprotected products **60** and **61** were observed. Thus, any time a selective ring opening occurred, only the undesired regioisomer was obtained.

An alternative approach was developed using a bifunctional protective group, such as di-*tert*-butylsilylene,[86] to protect the 5' and 3' hydroxyl groups simultaneously (Scheme 10). Reaction of commercially-available adenosine with di-*tert*-butylsilyl triflate and imidazole in DMF yielded silyl derivative **62**. Unfortunately, when compound **62** was treated with NaH to introduce a *p*-methoxybenzyl ether, deprotection at position 3' took place producing compound **63**. The opening of silanyl ring was confirmed by the presence of two doublets at 5.55 ppm (J = 6.0 Hz) and 5.19 ppm (J = 6.2 Hz) corresponding to hydroxylic protons. COSY experiment confirmed that this two protons were coupled with H-2' and H-3' respectively. Ultimately, we abandoned our attempts to find an alternative route to 2'-OPMB adenosine.



Scheme 10

[84] Sakagami, M.; Hamama, H. *Tetrahedron Lett.* **2000**, *41*, 5547.
[85] Riley, M. A.; Jenkins, D. J.; Marwood, R. D.; Potter, B. V. L. *Carbohydr. Res.* **2002**, *337*, 1067.
[86] a) Furusawa, K.; Ueno, K.; Katsura, T. *Chem. Lett.* **1990**, 97. b) Serebryany, V.; Beigelman, N. *Tetrahedron Lett.* **2002**, *43*, 1983.

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3.2.3. Glycosylation and further steps.

With glycoside acceptor **38** in hand, the next step in the synthesis of synthesis of 2'-deoxy-2'-fluoro adenophostin A was glycosylation. Experiments using acetate or trichloroacetimidate glycoside donors **47a** and **48a** failed, (Table 4, entries 1-4) so attention was focused on glycosyl bromide **50**.

The following activation reagents were tested: Ag_2CO_3,[87] Ag_2O,[87] silver salicilate,[87b] silver imidazolate, [88] $Hg(CN)_2$ and $HgBr_2$,[89] HgI_2,[90] $CdCO_3$,[91] $SnCl_4$,[92] $BF_3{\cdot}OEt_2$,[92] $ZnCl_2$,[93] $Sn(OTf)_2$,[94] $Cu(OTf)_2$,[95] and $InCl_3$.[96] Of all of the methods tested, $AgClO_4$[97] and AgOTf[98] were shown to be the most efficient. The best system was AgOTf with a Ag_2CO_3 base (Table 4, entries 5,7).

The effects of solvent on the stereochemical outcome of glycosylation reactions are well documented. The application of ethereal solvents such as dioxane or diethyl ether generally results in the formation of the thermodynamic product, the axial glycoside.[99] Thus, glycosylation reactions were performed in a 3:1 mixture of Et_2O and DCM,[100] which afforded compound **64** with exclusively α stereochemistry in 58 % yield (Table 4, entry 8). The H1"-H2" coupling constant (J =4.0 Hz)

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[87] a) Koenings, W.; Knorr, E. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 957. b) Wulff, G.; Röhle, G. *Angew. Chem. Int. Ed. Engl.* **1974**, *13*, 157. c) Igarashi, K. *Adv. Carbohydr. Chem. Biochem.* **1977**, *34*, 243.
[88] Garegg, P. J.; Johansson, R.; Samuelsson, B. *Acta Chem. Scand. B* **1982,** *36*, 249.
[89] a) Helferich, B.; Wedemeyer, K. F. *Liebigs Ann. Chem.* **1949**, *563*, 139. b) Helferich, B.; Jung, K. H.; *Liebigs Ann. Chem.* 1954, *589*, 77. c) Helferich, B.; Berger, A.; *Chem. Ber.* **1957**, *90*, 2492.
[90] Bock, K.; Meldal, M. *Acta Chem. Scand. B*, **1983**, *37*, 775.
[91] Conrow, R. B.; Bernstein, S. *J. Org. Chem.* **1971**, *36*, 863.
[92] Ogawa, T.; Matsui, M. *Carbohydr. Res.* **1976**, *51*, C13-C18.
[93] Higashi, K.; Nakayama, K.; Soga, T.; Shioya, E.; Uoto, K.; Kusama, T. *Chem. Pharm. Bull.* **1990**, *38*, 3280.
[94] a) Lubineau, A.; Malleron, A. *Tetrahedron Lett.* **1985**, *26*, 1713. b) Lubineau, A.; Le Gallic, J.; Malleron, A. *Tetrahedron Lett.* 1987, *28*, 5041.
[95] Yamada, H.; Hayashi, T. *Carbohydr. Res.* **2002**, *337*, 581.
[96] Mukherjee, D.; Ray, P. D.; Picione, J. *Synthesis*, **2001**, 323.
[97] a) Bredereck, H.; Wagner, A.; Faber, G.; Ott, H.; Rauther, J. *Chem. Ber.* **1959**, *92*, 1135. b) Bredereck, H.; Wagner, A.; Kuhn, H.; Ott, H. *Chem. Ber.* **1960**, *93*, 1201. c) Bredereck, H.; Wagner, A.; Geissel, D.; Gross, P.; Hutten, U.; Ott, H. *Chem. Ber.* **1962**, *95*, 3056. d) Bredereck, H.; Wagner, D.; Geissel, D.; Ott, H. *Chem. Ber.* **1962**, *95*, 3064.
[98] a) Lemieux, R. U.; Takeda, T.; Chung, B. Y.; *ACS Symp. Ser.* **1976**, *39*, 90. b) Hanessian, S.; Banoub, *J. Carbohydr. Res.* **1977**, *53*, C13.
[99] Wolff, G.; Röhle, G. *Angew. Chem. Int. Ed. Engl.* **1974**, *13*, 157.
[100] Demchenko, A.; Stauch, T.; Boons, G.J. *Synlett*, **1997**, 818.

confirmed the predicted stereochemistry. The ^{19}F NMR spectrum showed only a doublet of doublets at –201.25 ppm ($^2J_{F\text{-}H2''}$ = 48.8, $^3J_{F\text{-}H3''}$ = 12.0 Hz) with no indication of $F2''_{eq}$-$H1''_{eq}$ coupling, which confirmed a *trans*-diequatorial configuration. Amount of silver triflate was rised up to 1.1 eq in order to improve glycosylation yield, but no significant increase was observed (61%, Table 4, entry 9).

Table 4. Glycosylation assays

Entry	Glycosyl donor (X)	Activator	Base	Solvent	Yield (%)	α:β
1	OAc	$BF_3{\cdot}Et_2O$(3.5eq)		CH_2Cl_2	n.r.	
2[b]	OAc	$Sc(OTf)_2$[101]		Et_2O:CH_2Cl_2(3:1)	n.r.	
3	OAc	$BF_3{\cdot}Et_2O$(6eq)		Et_2O:CH_2Cl_2(3:1)	n.r.	
4[c]	$OC(NH)CCl_3$	TMSOTf		CH_2Cl_2	n.r.	
5[c]	Br	AgOTf(1.2eq)	NEt_3(1.2eq)	CH_2Cl_2	5	0:1
6[c]	Br	AgOTf(1.6eq)	DTBMP(3eq)	THF	n.r.	
7[c]	Br	AgOTf(1.5eq)	DTBMP(3eq)	Et_2O	30	1:0
8	Br	AgOTf(0.3eq)	Ag_2CO_3(1eq)	Et_2O:CH_2Cl_2(3:1)	58	1:0
9	Br	AgOTf(1.1eq)	Ag_2CO_3(1eq)	Et_2O:CH_2Cl_2(3:1)	61	1:0
10[b]	Br	AgOTf(1.1eq)	Ag_2CO_3(1eq)	Et_2O:CH_2Cl_2(3:1)	15	1:0

[a]All reactions were carried out with 4 M.S.

[b]3'-*O*-tributyltin derivative was used as glycosyl acceptor.

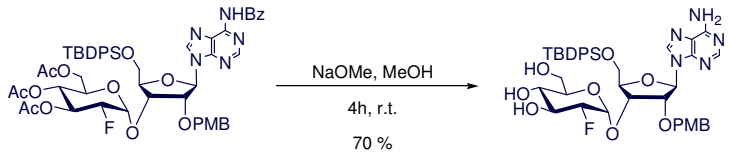
[c]Almacellas, N. Ph.D. Tesis, Universitat Rovira i Virgili **2007**

n.r.: No reaction

Reaction times were extended from 24 h to 5 days, but no increase in the yield was observed. Moreover, hydrolysed glycosyl donor was recovered after work-up. This indicated that activation of glycosyl bromide had taken place, but the glycosyl acceptor **38** was not sufficiently reactive to receive it. Since the major obstacle to glycosylation was the nucleophicity of the 3'-OH, the stannylether derivative was used as a glycosyl acceptor instead.[102] Formation of 3'-stannylether was carried out with either *bis*-tributyltin oxide in toluene in a Dean-Stark

[101] Yamanoi, T.; Yamazaki, I. *Tetrahedron Lett.* **2001**, *42*, 4009.
[102] Yamago, S.; Yamada, T.; Hara, O.; Ito, H.; Mino,Y.; Yoshida, J. I. *Org. Lett.* **2001**, *3*, 3867.

apparatus,[103] or TfOH-catalysed allyltriphenyltin in DCM.[104] Surprisingly, yields were low, and after 32 h, only 15 % of the glycosylated product was obtained (Table 4, entry 10). This result was attributed to the increased steric hindrance of stannylether formation.



64 **65**

Scheme 11

After treatment of fully-protected 2'-deoxy-2-fluoro adenophostin A with NaOMe/MeOH, compound **65** was obtained (Scheme 11). Selective protection of the 6''-OH was not similarly trivial. Initial efforts to introduce a *tert*-butyldiphenylsilyl protecting group at the desired position were unsuccessful (Table 5, entry 1). Thus, we looked for other reagents and conditions.

Table 5. 6''-OH silylation experiments with **65**.

Entry	Silylating agent	Base	Solvent	Temperature	Time	Yield
1	TBDPSCl	Et_3N, DMAP	CH_2Cl_2	r.t.	48 h	no react
2	TBDMSOTf	2,6-lutidine	CH_2Cl_2	r.t.	24 h	no react
3	TBDMSOTf	Pyridine	Pyridine	r.t.	24 h	no react
4	TBDMSCl	Et_3N, imidazole, DMAP	DMF	60 ºC	48 h	Traces
5	TESCl	Et_3N, imidazole, DMAP	DMF	60 ºC	48 h	6%

A first attempt was made using *tert*-butyldimethylsylyl triflate (TBDMSOTf) with 2,6-lutidine in DCM. Neither increasing reaction time nor increasing equivalents of TBDMSOTf (up to 3) generated any product, and starting material was recovered

[103] David, S.; Hanessian, S. *Tetrahedron*, **1985**, *41*, 643.
[104] Yamago, S.; Yamada, T.; Nishimura, R.; Ito, H.; Mino, Y.; Yoshida, J. *Chem. Lett.* **2002**, 152.

completely (Table 5, entry 2). Using a less hindered base, such as pyridine, in lieu of 2,6-lutidine (Table 5, entry 3), again resulted in no reaction.

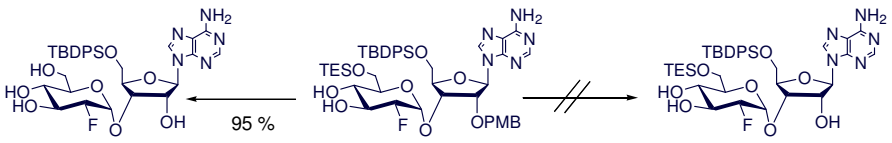
Next, we decided to increase reaction temperature (60ºC), so DMF and TBDMSCl were replaced with imidazole (Table 5, entry 4). Under these conditions, traces of a product with a higher R_f than that of the starting material were observed by TLC, but this approach was nonetheless abandoned.

Finally, experiments were performed to add a TES protecting group. The deacetylated 2''-deoxy-2''-fluoro-adenophostin derivative, **65**, was dissolved in DMF, TESCl and imidazole were added, and the reaction was left to run overnight at 60ºC (Table 5, entry 5). Again, the desired 6'-OTES product was obtained in very low 6% yield.

The instability of the TES group was particularly inconvenient for the next step of the synthesis, deprotection of 2'-OPMB. Christensen *et al.*[105] reported that the phenyl and adenine rings of 2'-*O*-*p*-methoxybenzyl adenosine derivatives engage in π-stacking interactions. Such an interaction would likely reduce the electron density present on the *p*-methoxybenzyl group. Unsuccessful deprotection attempts consistent with our aforementioned results have been described in the literature.[19]

In line with this hypothesis, the solvent was altered to disfavour putative π-stacking interactions and to favour oxidation. A 9:1 mixture of acetonitrile to water was tested first, which yielded excellent results when used with CAN. When this system was tried with our 6''-OTES protected substrate, however, complete hydrolysis of 6''-OTES was observed, presumably due to the acidity of CAN, affording compound **67** (Scheme 12). When deprotection was carried out with buffered CAN solution, no 2'-OH product was observed. Test reactions conducted with DDQ both with and without buffer did not afford the 2'-OH deprotected product. We therefore concluded that *p*-methoxybenzyl ether hydrolysis, which needed acidic media for deprotection, was inherently incompatible with TES stability.

[105] Christensen, L.F.; Broom, A.D. *J. Org. Chem.* **1972**, *37,* 3398.



67 **66** **66a**

Conditions: CAN, MeCN:H_2O (9:1), 5h, r.t.

Scheme 12. 2'-*O*-PMB deprotection with CAN.

Failure to achieve facile, selective protection of 6''-hydroxyl group prompted us to experiment with molecular mechanics (MM2) models to aid in understanding the observed reactivity. Calculation of the most stable conformation for **65** confirmed that the 6''-OH was pointing directly at the 5'-OTBDPS group (Figure 25), thereby making *tert*-butyldiphenylsilyl or TBDMS protection impossible.

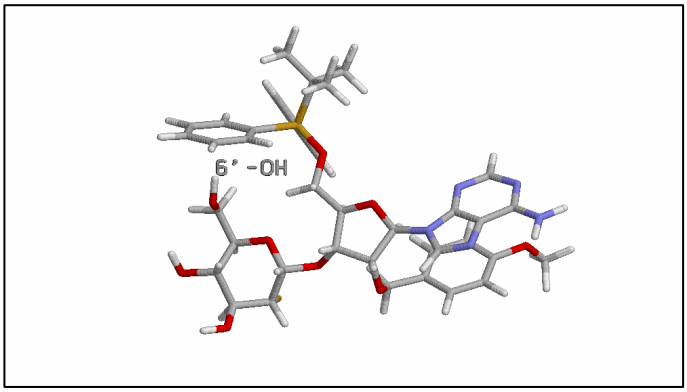


Figure 25. Geometry obtained from energy minimization studies using MM2.

Based on these findings, we decided to incorporate in the future phosphate groups directly without further protection steps. This way, we hoped to have access to a tetraphosphorylated derivative at the very least. It is worth noting that the 6''-OH group is not relevant to adenophostin's activity, and make it quite tolerant to modifications. Thus, a first attempt was made to phosphorylate all four positions (2', 3", 4", 6") non-selectively with the intention of removing the rest of the protecting groups at a later stage. This was cinfirmed by ^{31}P and ^{19}F NMR, appearing in ^{31}P spectrum 4 signals at phosphate region, and a double doublet in ^{19}F spectrum.

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However, the little amount used for this first attempt was insuficient to carry out any optimization of the reaction and also to provide optimal structural determination data.

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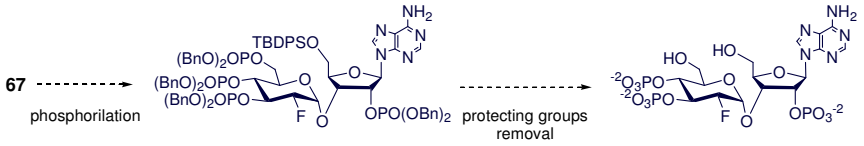

Scheme 13. Final steps of 2''-deoxy-2''-fluoro-adenophostin analogue synthesis.

Anyway, we have open an efficient synthetic approach to get a fluorinated analogue of Adenophostin A, resting only to be solved the removal of remaining protecting groups (Scheme 13).

ISBN:978-84-691-9757-8/DL:T-1256-2008

3.3. Approach to the Synthesis of 3'' and 4''-(2-methyl-2-ethanoate) analogues of Adenophostin A.

Bioisosteres are compounds or groups that possess nearly-equal molecular shapes and volumes, have approximately the same distribution of electrons, and exhibit similar physical properties. The use of bioisosteres has provided much useful information on the structure-activity-relationship of various pharmacologically active compounds.

The phosphate group is present in an enormous number of biological processes. Consequently, proteins that recognize the phosphate moiety have become attractive targets for therapeutic development. Nevertheless, the use of phosphate moieties in the synthesis of biologically active compounds is severely limited by the enzymatic lability and poor cellular bioavailability of this highly charged recognition element. The development of phosphate isosteres can help circumvent these difficulties. These isoteres can contain a non-scissile bond and/or less net charge while still being able to interact favourably with the target protein in much the same way a phosphate group does. Many phosphate mimics, including simple phosphonates analogues[106] and α-halogenated phosphonates (specifically α-fluorinated,[107] and α,α-difluorinated[108] phosphonates) retain the phosphorous atom. Sometimes one or more oxygen atoms within the phosphate groups are replaced

[106] a) Engel, R.; *Chem. Rev.* **1977**, *77*, 349. b) Wiemer, D. F. *Tetrahedron*, **1997**, *53*, 16609. c) Cipolla, L.; La Ferla, B.; Panza, L.; Nicotra, F. *J. Carbohydr. Chem.* **1998**, *17*, 1003. d) Page, P.; Blonski, C.; Périé, J. *Eur. J. Org. Chem.* **1999**, 2853. e) Vidal, S.; Vidil, C.; Morere, A.; Garcia, Montero, J.-L. *Eur.J. Org. Chem.* **2000**, 3433. f) Vidil, C.; Vidal, S.; Morere, A.; Montero, J.-L. *Phosphorus, Sulfur, and Silicon.* **2000**, *158*, 125. g) Borodkin, V. S.; Ferguson, A. J.; Nikolev, A. V. *Tetrahedron Lett.* **2001**, *42*, 5305; h) Khanjin, N. A.; Montero, J.-L. *Tetrahedron Lett.* **2002**, *43*, 4017. i) Meyer, O.; Grosdemange-Billiard, C.; Tritsch, D.; Rohmer, M. *Org. Biol. Chem.* **2003**, *1*, 4367. j) Vanek, V.; Budesinsky, M.; Kavenova, I.; Rinnova, M.; Rosenberg, I. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1065 k) Hirsch, G.; Grosdemange-Billiard, C.; Tritsch, D.; Rohmer,M. *Tetrahedron Lett.* **2004**, *45*, 519.

[107] a) McKenna, C. E.; Shen, P. *J. Org. Chem.* **1981**, *46*, 4573. b) Berkowitz, D. B.; Bose, M. *J. Fluor. Chem.* **2001**, *112*, 13.

[108] Gautier, A.; Garipova, G.; Salcedo, C.; Balieu, S.; Piettre, S. R. *Angew. Chem. Int. Ed.* **2004**, *43*, 5963.

with an isoelectronic sulfur atom[109] or with a borane moiety.[110] Others do not share the typical tetrahedral phosphate geometry and are based on the combination of one or more carboxylate groups, which generally reduces the overall charge of the molecule.[45,111] Yet another class of phosphate mimics completely replaces the phosphate moiety with a cyclic structure such as a sulfahydantoin.[112]

Until now, adenophostin modifications have primarily focused on structural modifications and, especially in recent years, on the adenine moiety. Little attention has been paid to phosphate bioisosterism, and only phosphorothioate surrogates have been used as phosphate mimics.[37] As previously stated, our main objective was the development of an effective approach for synthesizing two adenophostin analogues that incorporated methylenecarboxylate groups as phosphate isosters.[45,113] These would be the first reported adenophostin analogues with this particular functionality, and introduction of this group should afford more stable analogues with modified activities, which are resistant to the action of phosphatases.[113]

Given that the 2'-phosphate is not essential for adenophostin activity, our efforts focused on introducing this modification at the essential 3"- and 4"-positions (Scheme 14). In addition, these modifications would differentiate among the vicinal phosphates. Experiments exploring the differences between the two phosphates would impart greater understanding as to how they participate in adenophostin activity.

The 3"- and 4"-methylenecarboxylate adenophostin analogues would be synthesized from glycoside acceptor **38** and glycoside donors A and B (Scheme 14). The glycoside acceptor would be synthesized from adenosine in the same manner

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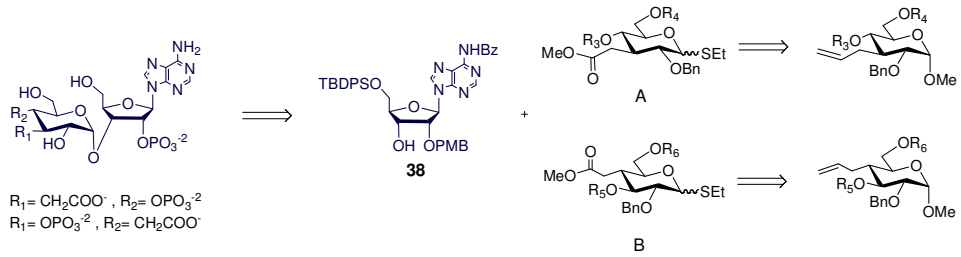
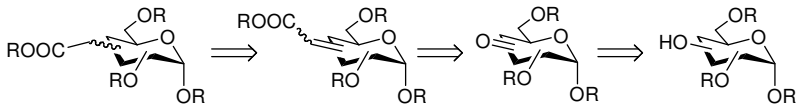
[109] Conway, S. J.; Miller, G. J. *Nat. Prod. Rep.* **2007**, *24*, 687.
[110] Nahum, V.; Fischer, B. *Eur. J. Org. Chem.* **2004**, 4124.
[111] a) Vyplel, H.; Scholz, D.; Macher, I.; Schindlmaier, K.; Schütze, E. *J. Med. Chem.* **1991**, *34*, 2759. b) Liu, C.; Thomas, N. F.; Potter, B. V. L. *J. Chem. Soc. Chem. Commun.* **1993**, 1687.
[112] Saunders, J. O.; Miknis, G. F.; Blake, J. F. PCT Int. Appl. **2004**.
[113] a) Widlanski, T. S.; Myers, J. K.; Stec, B.; Holtz, K. M.; Kantrowitz, E. R. *Chem. Biol.* **1997**, *4*, 489. b) Wipf, P.; Aslan, D. C; Luci, D. K.; Southwick, E. C.; Lazo, J. S. *Biotech. Bioeng.* **2000**, *71*, 58. c) Bergnes, G.; Gilliam, C. L.; Boisclair, M.D.; Blanchard, J. L.; Blake, K. V.; Epstein, D. M.; Pal, K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2849. d) Cao, X.; Moran, E. J.; Siev, D.; Lio, A.; Ohashi, C.; Mjalli, A. M. M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2953.

as described in the previous chapter. Based on previous results[114] showing that among the different adenophostin syntheses, the best glycosylation yields and α/β stereoselectivities were obtained with a thioglycoside donor and a NIS/TfOH activator, we chose thioglycosyl as a donor precursor. Furthermore, the carbohydrate unit should have a configuration of protecting groups that would allow for selective phosphorylation at 3- and 4-positions during the final stages of synthesis. Thus the 2- and 6-positions should bear the same protecting groups, which are themselves orthogonal to the protecting groups at positions 3 and 4. Ultimately, the approach outlined above was modified due to experimental obstacles.



Scheme 14

Different strategies and different precursors could be used in order to introduce a methylenecarboxylate group into a glucoside scaffold. For instance, the alkyl chain could be incorporated via Horner-Wadsworth-Emmons olefination of the corresponding 3- or 4-ulose, followed by double bond reduction (Scheme 15). Unfortunately, the glucose ring's stereochemistry makes it difficult to obtain the desired equatorial diastereoisomer. In both cases, the vicinal groups' configuration favours the axial stereochemistry for the reduced double bond.



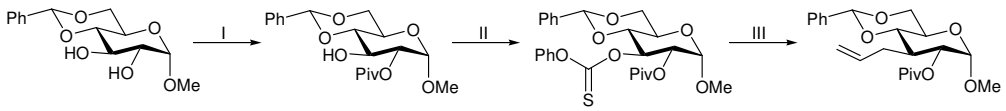
Scheme 15

[114] Van Straten, N. C. R.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron*, **1997**, *53*, 6509.

Either a nucleophilic or a radical addition at these positions, however, would result in the desired configuration. Thus, we thought that a radical allylation would provide a substrate, which, following oxidative cleavage, possessed an methylenecarboxylate group with the correct stereochemistry.

3.3.1. Synthesis of ethyl (4,6-di-*O*-acetyl-2-*O*-benzyl-3-deoxy-3-(2-methyl-2-ethanoate)-1-thio-α,β-glucoside.

Our initial attempt to synthesize the 3-methylenecarboxylate carbohydrate unit, employed the selective 2-*O*-pivaloylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside. This reaction proceeded with good yield (75%) and excellent regioselectivity, and, as a result of the steric hindrance around the 3-position, only the 2-*O*-pivaloyl isomer was obtained.



68 **69** **70**

I) PivCl, pyridine, 5h, 0 ºC, 75 %; II) PhOC(S)Cl, NHS, pyridine, toluene, 6h, r.t., 82 %; III) Allyl tri-*n*-butyltin, AIBN, toluene, 24h, reflux, 87 %

Scheme 16

Next, glucoside **68** was treated with phenyl chlorothionoformate, NHS, and pyridine in toluene. After 6 hours at room temperature, the radical precursor 3-tionocarbonate derivative **69**[115] was obtained in 82 % yield.

When a radical is formed at C-3, the stereochemistry of the carbons around the reaction centre controls from which face of the molecule the allyl group can approach (Figure 26). Introduction of the allyl group in an axial orientation would create an unfavourable 1,3-diaxial interaction with the methoxy group at C-1. Furthermore, the substituents at C-2 and C-4 are pointing towards the α face, which favours approximation of the incoming allyl at the β face.

[115] Postema, M. H. D.; Piper, J. L.; Liu, L.; Shen, J.; Faust, M.; Andreana, P. *J. Org. Chem.* **2003**, *68*, 4748.

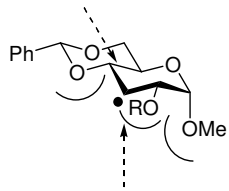
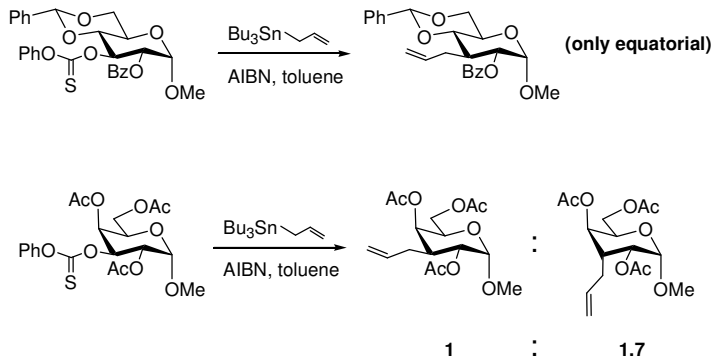


Figure 26. Stereoselectivity of radical allylation in *gluco* and *galacto* configurations. 1,2 -*cis*-interaction, more than 1,3 di-axial, are directing the stereochemistry of allyl group.

Postema *et al* (Scheme 17) [115,116] previously described the stereoselectivity of radical allylation at different positions of gluco- and galactopyranose rings in *C*-glycosides. When a radical was formed at the C-3 position of a α-glucoside, the configurations at C-4, C-2, and C-1 directed allyl addition and afforded only equatorial allylation product. When a radical was formed at the C-3 position of a α-galactoside, however, the axial configuration of the substituent at C-4 decreased the stereoselectivity of allylation, and an equatorial/axial ratio of 1:1.7 was obtained.



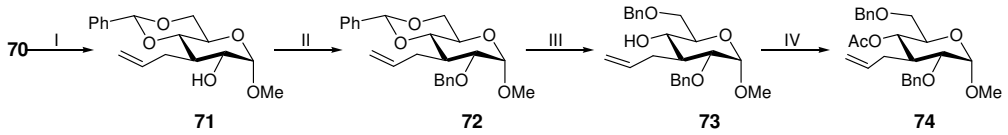
Scheme 17

Confident that radical allylation would occur with the same stereoselectivity, the 3-thionocarbonate **69** was converted into methyl-3-deoxy-3-allyl-2-*O*-pivaloyl-4,6-*O*-benzylidene-α-D-glucopyranoside **70** in 87 % yield after refluxing in toluene in the presence of allyl tri-*n*-butyltin and AIBN (Scheme 16). The ^{1}H NMR spectrum of **70** possessed a doublet of doublets at 4.68 ppm corresponding to H-2, and both the *cis* axial-equatorial coupling constant ($^{3}J_{H2\text{-}H1}$=3.2 Hz) and the *trans* diaxial coupling

[116] Liu, L.; Postema, M. H. D. *J. Am. Chem. Soc.* **2001**, *123*, 8602.

constant ($^3J_{H2-H3}$=10.4 Hz) confirmed that only the equatorial coupling product was obtained.

Following radical allylation, the pivaloyl ester needed to be replaced with a benzyl group so that the derivative would possess the same protecting groups at positions 2 and 6 after the benzylidene ring opening (Scheme 18). After removing the pivaloate ester with NaMeO (78 % yield), hydroxyl derivative **71** was benzylated, and compound **72** was afforded in excellent yield (88 %). Treatment of **72** with a Brønsted acid, such as TfOH, in presence of Et_3SiH at –78ºC generated the 6-*O*-protected product **73** in 70% yield.[84] Nearly quantitative acetylation of the free hydroxyl group yielded **74** and confirmed that the benzylidene ring opened in the desired fashion. The NMR spectrum of the acetylated product showed a doublet of doublets at 4.85 ppm, which corresponded to H-4 ($^3J_{H4-H3} = {}^3J_{H4-H5} = 12.0$ Hz).



I) MeONa/MeOH, 3h, r.t., 78 %; II) BnBr, TBAI, DMF, 12h, r.t., 88 %; III) Et_3SiH, TfOH, 4Å M.S., DCM, 1h, -78 ºC, 70 %; IV) Ac_2O, pyridine, 12h, r.t., 99 %.

Scheme 18

With allyl derivative **74** in hand, the next step was to oxidatively cleave the alkene to produce the key methylenecarboxylate unit. Initial experiments tested a combination of $RuCl_3$ and $NaIO_4$ oxidants in a CCl_4:H_2O:MeCN (1:1.5:1) solvent mixture.[117] During the course of this reaction, two spots appeared by TLC. One, with high R_f, corresponded to the aldehyde **75**, and the other, with low R_f, was initially believed to be the acid. Unfortunately, the ^{1}H NMR spectrum of the latter compound revealed a signal corresponding to 2 protons at 4.11 ppm, and the ^{13}C NMR spectrum contained two signals, at 208.3 and at 68 ppm. An HSQC experiment confirmed a correlation between these two protons and the carbon at 68 ppm, and an HMBC experiment revealed a correlation with the carbon at 208.3 ppm. Taken together, these results confirmed our suspicions, which were corroborated

[117] Shiozaki, M.; Deguchi, N.; Macindoe, W. M.; Arai, M.; Miyazaki, H.; Mochizuki, T.; Tatsuta, T.; Ogawa, J.; Maeda, H.; Kurukata, S. *Carbohydr. Res.* **1996**, *283*, 27.

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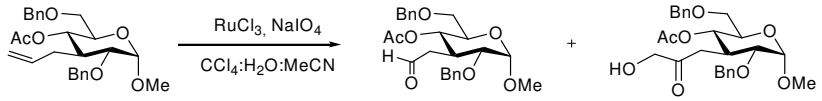
experimentally upon acetylation, that this by-product was the α-hydroxyketone **76** (Scheme 19). It was obtained in 40 % yield along with a 60 % yield of the aldehyde **75**. Substitution of OsO_4 for $RuCl_3$, produced similar results.

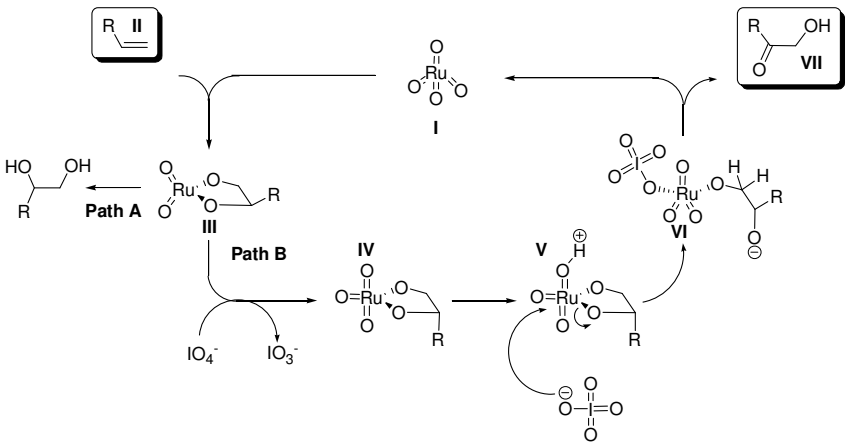


74 **75** **76**

60 % **40 %**

Scheme 19

Plietker[118] described the RuO_4–catalysed ketohydroxylation of olefins. Usually, nucleophilic attack of water at cyclic ruthenate esters' metal centre produced *syn*-diols (Scheme 20, path A). In the presence of "nucleophilic reoxidants," such as *tert*-butyl peroxide, hydrogen peroxide, sodium chlorite, oxone, or $NaIO_4$, however, the α-hydroxyketone (Scheme 20, path B) was formed.



Scheme 20. Proposed mechanism for Ru-catalyzed ketohydroxylation.[119]

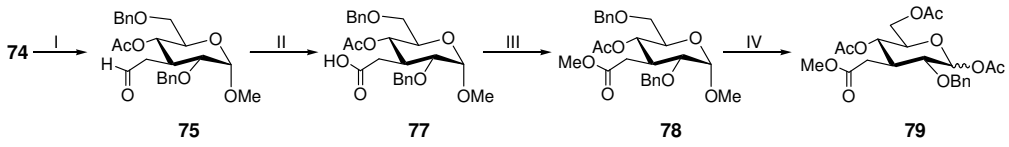
The mechanism of this oxidative fragmentation remains unclear. A simplified proposed mechanism is shown in Scheme 20. After a [3+2] cycloaddition of

[118] Plietker, B. *J. Org. Chem.* **2003**, *68*, 7123.

ruthenium tetraoxide (**I**) to the double bond, the resulting ruthenium(VI) compound **III** is oxidized to ruthenate **IV**. After proton activation, IO_4^- adds to the metal centre in **V** with concomitant cleavage of one of the two metal-oxygen bonds. The resulting mixed peroxoruthenate **VI** then rearranges to give acyloin **VII** and regenerate the active catalyst.

An alternative, two-step process of *syn*-dihydroxylation and concomitant mono-oxidation of the resulting diol could occur as opposed to the one-step mechanism involving the nucleophilic addition of IO_4^- at the metal centre. Previous studies[119] have revealed, however, that the process of ketohydroxylation is a direct transformation without the formation of intermediate diols or epoxides.

To avoid the formation of α-hydroxyketone, dihydroxylation and cleavage with $NaIO_4$ was separated into two chemical steps. Olefin **74** was dissolved in dioxane:water, and catalytic amounts of OsO_4 were added with stoichiomentric NMO acting as the reoxidizing agent.[120] The crude reaction product was treated with $NaIO_4$ to afford aldehyde **75** in 80% yield. The structure of the desired aldehyde was confirmed by the presence of a signal corresponding to the aldehydic proton at 9.58-9.56 ppm in the ^1H NMR spectrum. Subsequent oxidation with NaClO[120] afforded acid **77** in 70 % yield, which was confirmed by the presence of a signal corresponding to the acid carbonylic carbon at 178.3 ppm in the ^{13}C NMR spectrum. Product **77** was reacted with methyl iodide in the presence of Cs_2CO_3 to yield ester **78** quantitatively (Scheme 21).



I) 1) OsO_4, NMO, dioxane:H_2O, 3h, r.t. ; 2) $NaIO_4$, dioxane:H_2O, 24h, r.t., 80 %; II) NaClO, NaH_2PO_4, 2-methyl 2-butene, *t*-BuOH:H_2O, 5h,r. t., 70 %; III) MeI, Cs_2CO_3, DMF, 2h, r.t., 100 %; IV) Ac_2O:HOAc:H_2SO_4, 3h, 0 ºC, 86 %.

Scheme 21

[119] Plietker, B. *Eur. J. Org. Chem.* **2005**, 1919.
[120] Rozners, E.; Katkevica, D.; Bizdena, E.; Strömberg, R. *J. Am. Chem. Soc.* **2003**, *125*, 12125.

The final step prior to thioglycoside formation was the hydrolysis of the anomeric methoxy group. Initial hydrolysis experiments were conducted using a mixture of acetic anhydride, acetic acid, and sulphuric acid (50:25:1) at room temperature,[121] but the compound obtained was acetylated not only at the anomeric position but also at position 6 as well (Scheme 21, product **79**). A similar result was obtained when the temperature was decreased to 5ºC, and at lower temperatures the reaction mixture froze. Whenever anomeric hydrolysis occurred, deprotection of primary hydroxyl also occurred. Similar debenzylation-acetolysis of 1-methoxy and 6-benzyloxy groups of glucosides was reported by Sakai *et al*.[122] Later, Cao *et al.*[123] went on to describe the corrected reactivity ranking. According to the latter work, acetolysis takes places first at positions 1 and 6, followed by 3, 4, and finally 2. Thus, this reactivity suggested that the required transformation to generate the desired 2,6-*O*-dibenzyl glucoside was prohibited (Figure 27).

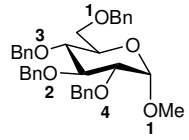


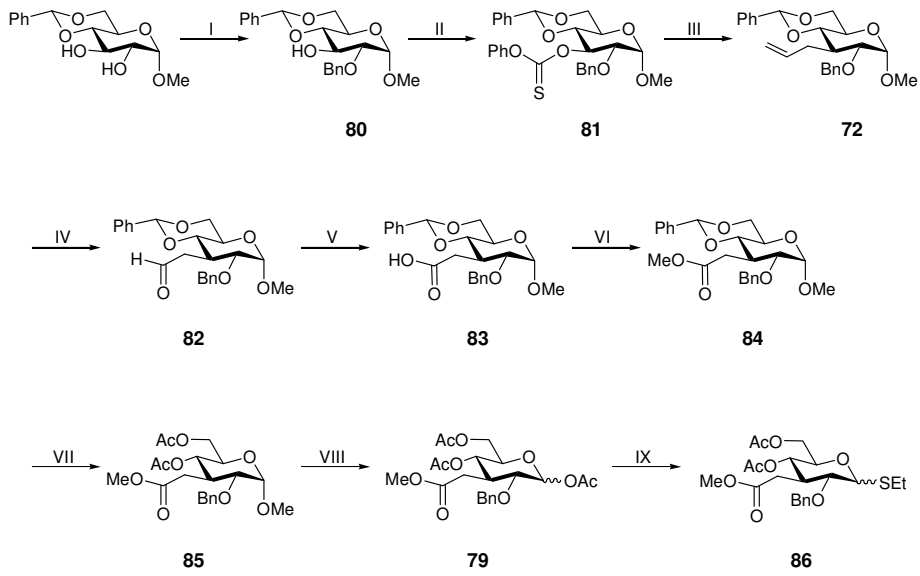
Figure 27. Reactivity ranking for debenzylation-acetolysis.

These results necessitated revision of our synthetic strategy. Selective opening of the dioxane ring of **72** was replaced with methanolysis of a benzylidene acetal in presence of catalytic amounts of iodine[124] and subsequent acetylation (Scheme 22). Following the methodology of Garegg for selective 2-*O*-benzylation of glucosides via stannylene acetal,[125] compound **80** was procured in good yields (80 %), and we were forced to reconsider the effectiveness of the previous pivaloylation-deprotection-benzylation strategy. Ultimately, these reactions were abandoned, and direct benzylation of (4,6-*O*-benzylidene)-methyl-α-D-glucopyranoside was chosen as an alternative.

[121] Fairweather, J. K.; Hrmova, M.; Rutten, S. J.; Fincher, G. B.; Driguez, H. *Chem. Eur. J.* **2003**, *9*, 2603.
[122] Sakai, J. I.; Takeda, T.; Ogihara, Y. *Carbohydr. Res.* **1981**, *95*, 125.
[123] Cao, Y.; Yamada, H. *Carbohydr. Res.* **2006**, *341*, 909.
[124] Sletten, E. M.; Liotta, L. J. *J. Org. Chem.* **2006**, *71*, 1335.
[125] Dasgupta, F.; Garegg, P. J. *Synthesis*, **1994**, 1121.

After direct benzylation, compound **80** was treated with phenyl chlorothionoformate, NHS, and pyridine to obtain **81** (Scheme 22) in near-quantitative yield (97 %). The subsequent allylation reaction proceeded with good yield (77%) and excellent stereoselectivity with only one diastereoisomer produced. The ^{1}H NMR spectrum of the allyl derivative, **72,** produced in this manner was identical to that of the product of benzylation of **71**. The oxidative cleavage of the olefin provided further confirmation of equatorial carbon-carbon bond formation, and aldehyde **82** was afforded in 78% yield (over two steps). Its ^{1}H NMR spectrum contained a signal at 3.56 ppm (dd, $^{3}J_{2,1}$= 3.2 Hz, $^{3}J_{2,3}$= 10.0 Hz) corresponding to H-2, and another at 3.32 ppm (dd, $^{3}J_{4,3}$= $^{3}J_{4,5}$= 10.0 Hz) corresponding to H-4.

Oxidation of aldehyde **82** with sodium chlorite in a buffered *t*-BuOH:H_2O mixture in the presence of a halogen scavenger, 2-methyl-2-butene, generated acid **83** in 78 % yield. This compound was then quantitatively protected forming methyl ester **84** via reaction with methyl iodide and caesium carbonate in DMF.



I) $(Bu_2Sn)_2O$, BnBr, toluene, 12h, reflux, 80 %; II) PhOC(S)Cl, NHS, pyridine, toluene, 5h, r.t., 97 %; III) Allyl tri-*n*-butyltin, AIBN, toluene, 24h, reflux, 77 %; IV) a) OsO_4, NMO, dioxane:H_2O, 3h, r.t. b) $NaIO_4$, dioxane:H_2O, 24h, r.t., 78 %; V) NaClO, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH:H_2O, 12h, r. t., 78 %; VI) MeI, Cs_2CO_3, DMF, 2h, r.t., 100 %; VII) a) I_2 cat., MeOH, 5h, reflux b) Ac_2O, Pyr, 12h, r.t., 95 %; VIII) Ac_2O:HOAc:H_2SO_4 (50:25:1), 3h, 0 ºC, 86 %; IX) EtSH, $BF_3{\cdot}Et_2O$, DCM, 15h, r.t.,80 %.

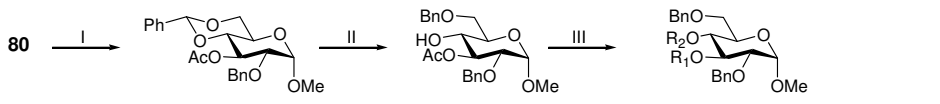
Scheme 22

Following methanolysis of **84** in catalytic amounts of iodine, compound **85** was generated from acetylation of the diol intermediate (not isolated) at 95 % yield over two steps.

Finally, treatment of acetyl glucoside **79** with ethanthiol and $BF_3{\cdot}Et_2O$[126] afforded the desired thioglycosyl derivative **86** (80%, α:β 7:1). ^1H NMR spectrum contained a doublet at 5.46 ppm with coupling constant of 5.2 Hz corresponding to the main α thioglycoside anomer.

3.3.2. Synthesis of ethyl (3,6-*O*-acetyl-2-*O*-benzyl-4''-deoxy-4''-(2-methyl-2-ethanoate)-1-thio-α,β-glucoside.

Like fragment A, our first approach to the synthesis of carbohydrate fragment B also aimed to incorporate the methylenecarboxylate fragment via radical allylation. Synthesis of 4-methylenecarboxylate building block B started from the same methyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-α-glucoside as before (Scheme 14). Compound **80** was quantitatively acetylated affording **91** (Scheme 23). Selective opening[84] of the benzylideneacetal generated **92** in 85 % yield, which was then reacted with phenylchlorothionocarbonate. Following the same procedure as was used to synthesize **81**, 4-thionocarbonate **93** was obtained in very low yields (Scheme 22), which was probably due to the electron withdrawing effect of vicinal acetyl group. Reaction conditions were modified, and the reaction was carried out in refluxing toluene in the presence of DMAP. Under these conditions, acetyl group migration was observed resulting in a 2:1 mixture of compounds **93** and **94**.



91 **92**

93 R_1= OAc R_2= OC(S)OPh (42 %)
94 R_1= OC(S)OPh R_2=OAc (21 %)

I) Ac_2O, pyridine, 12h, r.t. 100%; II) Et_3SiH, TfOH, 4Å M.S., DCM, 1h, -78 ºC, 85 %; III) PhOC(S)Cl, DMAP, pyridine, toluene, 24h, reflux, 63 %

Scheme 23

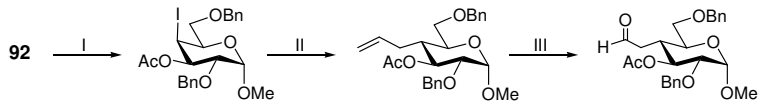
[126] Ferrier, R. J.; Furneaux, R. H. *Carbohydr. Res.* **1976**, *52*, 63.

Given these difficulties in efficiently obtaining the desired 4-tionocarbonate, synthesis of the corresponding 4-iodo derivative seemed as if it might be a good alternative. Treatment of glucoside **92** with triphenylphospine and iodine in refluxing toluene (Scheme 24) generated 4-iododerivative **95** in good yield (75 %). Iodation at position 4 was confirmed by ^{1}H NMR, which showed a signal at 4.79-4.78 ppm corresponding to H-4, and by ^{13}C NMR, which showed a peak at 39.0 ppm corresponding to C-4. Subsequent reaction of **95** with allyl tri-*n*-butyltin and AIBN result in a complex mixture of compounds, and after purification, compound **96** was obtained in low yield (35 %). Nevertheless, the desired stereoselectivity was confirmed by ^{1}H NMR experiments, in which a doublet of doublet corresponding to H-4 was observed at 5.36 ppm with *trans*-diaxial coupling constants $^{3}J_{H3-H2}=^{3}J_{H3-H4}$=10.0 Hz.

Wipf *et al.*[127] reported the use of allyltriphenyltin in order to avoid the formation complex mixtures commonly formed with allyl tri-*n*-butyltin. When applied to **95**, the reaction with allyltriphenyltin was more efficient, and the yield of **96** was nearly doubled (60 %). Postema *et al.*[115] found that radical allylation of a perbenzylated glucoside also led to a complex mixture of products, which was related to the cross-reactivity of the benzylic hydrogens near to the reaction centre. Postema *et al.* concluded that only the replacement of benzyl groups with acetates allowed one obtain the desired 4-allyl glucoside in reasonable yields. Nevertheless, in our case, the need to have the same protecting groups at positions 2 and 6, and an orthogonal protecting group at position 3 forced us to discard this option initially. (Later on, however, when it was realised that acetolysis of the 6-*O*-benyzl ether could not be avoided, the C-6 benzyl group was changed).

After olefin oxidation of **96** via the OsO_4/NMO system and subsequent diol cleavage with $NaIO_4$, aldehyde **97** was obtained in good yield (70 % over two steps).

[127] Wipf, P; Spencer, S. R. *J. Am. Chem. Soc.* **2005**, *127*, 225.

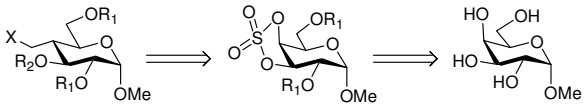


I) PhP_3, I_2, toluene, 12h, reflux, 75 %; II) Allyltriphenyltin, AIBN, 18h, 70 ºC, 60 %; III) 1) OsO_4, NMO, dioxane:H_2O, 3h, r.t.;
2) $NaIO_4$, dioxane:H_2O, 5h, r.t., 70 %.

Scheme 24

At this point, there was sufficient evidence to suggest that the radical reactions' modest yields and complex mixtures would make it difficult to obtain the final building block efficiently, the synthesis strategy for procuring this 4-methylenecarboxylate derivative fragment was modified.

Our second approach to the synthesis of 4-methylenecarboxylate building block B aimed to incorporate the alkylic chain via carbon-nucleophile opening of a cyclic sulphate (Scheme 25).



X = $CH{=}CH_2$, -COOMe, $-(COOEt)_2$

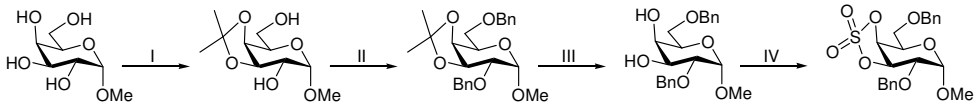
Scheme 25

Cyclic sulfates are important intermediates in organic synthesis.[128] They have high reactivity toward various nucleophiles and are more reactive than epoxides. They can also activate nucleophilic attack at one position while serving as a protecting group at a second position. Under more extreme conditions, cyclic sulfates can serve as activators in two sequential reactions. In addition, nucleophilic opening of five-membered cyclic sulfates with nucleophiles furnishes two contiguous stereocenters.

[128] a) Byun, H. S.; Bittman, R. *Tetrahedron*, **2000**, *56*, 7051. b) Lohray, B. *Synthesis*, **1992**, 1035.

Santoyo-González and Vargas-Berenguel[129] investigated thiolate ring-opening regioselectivity of methyl 4,6-*O*-sulfuryl galactosides. Nucleophilic attach at C-4 is favoured when the anomeric methoxy group is in an axial position. In contrast, when the methoxy group is equatorial, a C-3 attack is preferred. Thus, we experimented with nucleophiles for attacking cyclic sulfate **90** at position C-4.

But first, in order to obtain the desired sulfate, α-methyl galactopyranose (Scheme 26), was treated with 2-methoxypropene and catalytic toluenesulfonic acid in DMF to yield. **87.**[130] Benzylation of the 2- and 6-hydroxyl groups afforded **88** in good yield (85%). Hydrolysis of acetonide **88** with DOWEX-H^+ in MeOH generated diol **89** in 78% yield. Treatment of **89** with $SOCl_2$ in CCl_4 and subsequent oxidation with $NaIO_4$ led to the 3,4-cyclic sulphate, **90**.[131]



87 **88** **89** **90**

I) 2-methoxypropene, TsOH, DMF, 24h, r.t., 95 %; II) BnBr, NaH, TBAI, DMF, 12h, r.t., 85%; III) DOWEX-H^+, MeOH, 6h, r.t., 78 %;IV) a) $SOCl_2$, CCl_4, 1h, reflux; b) $NaIO_4$, $RuCl_3$, MeCN:H_2O, 2h, r.t., 91 %.

Scheme 26.

With cyclic sulfate **90** in hand, ring-opening experiments were conducted with different nucleophiles. The 3,4-sulfuryl derivative was treated with both the enolate of ethyl acetate and diethyl malonate (Table 6). The former treatment resulted in a complex mixture of products, while the latter did not react under the conditions tested. In order to find suitable reaction conditions, cyclic sulfate of L-diethyl tartrate **92** was used as a model substrate, and allyl cyano-organocuprate[132] was tested as the carboxylate precursor. Unfortunately, a complex mixture of products was obtained once again.

---

[129] Calvo-Asín, J. A.; Calvo-Flores, F. G.; Exposito-López, J. M.; Hernández-Mateo, F.; García-López, J. J.; Isac-García, J.; Santoyo-González, F.; Vargas-Berenguel, A. *J. Chem. Soc. Perkin Trans 1.* **1997**, 1079.

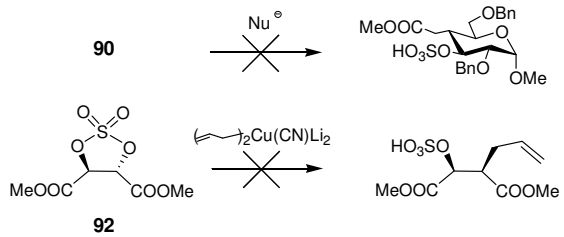
[130] Evans, M. E.; Parrish, F. W.; Long, L. Jr. *Carbohydr. Res.* **1967**, *3*, 453.

[131] Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 7538.

[132] Lipshutz, B. *Organocopper reagents, a pratical approach*. (ed. Richard J. K. Taylor), Oxford, **1994**, p.124.

ISBN:978-84-691-9757-8/DL:T-1256-2008

Table 6

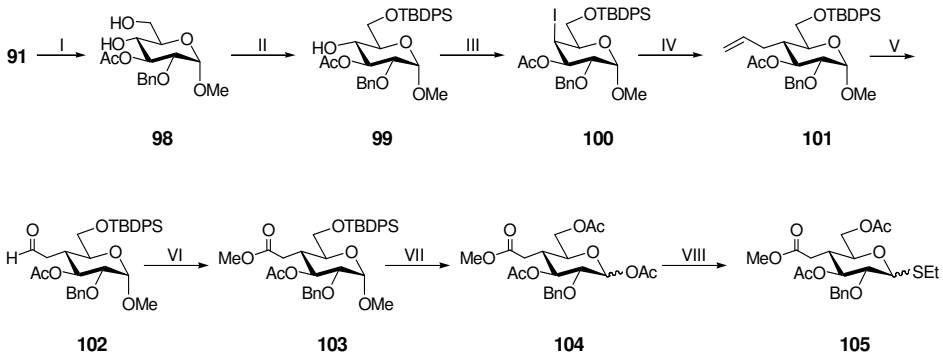


92

Nu	Substrate	Conditions	Result
CH_3COOMe	**90**	THF, LiHMDS, -78 ºC to r.t.	complex mixture
$EtOOCCH_2COOEt$	**90**	NaH, DME, r.t. to 70ºC	recovering of starting material
$(═)_2Cu(CN)Li_2$	**92**	THF, -78 ºC to r.t.	complex mixture

The combination of these initial failures, having obtained methyl [(4,6-*O*-benzylidene)-2-*O*-benzyl]-α-glucopyranoside in good yield, and the recollection that the 6-*O*-benzyloxy protecting group could not withstand anomeric hydrolysis conditions, prompted us to return to a modified radical allylation strategy.

In light of the 3-methylenecarboxylate fragment A synthesis, selective benzylidene acetal ring opening was abandoned. Instead, **91** was treated with catalytic amounts of iodine in refluxing methanol to afford diol **98** (Scheme 27).



I) I_2 cat., MeOH, 5h, reflux; II) TBDPSCl, DMAP, TEA, DCM, 5h, r.t., 73 % over two steps; III) PhP_3, I_2, imidazole,toluene, 12h, 87 %; IV) Allyl triphenyltin, AIBN, 18h, 70 ºC, 98 % (equatorial:axial 7.5:1); V) 1) OsO_4, NMO, dioxane:H_2O, 3h, r.t.; 2) $NaIO_4$, dioxane:H_2O, 5h, r.t., 74 %; VI) a) NaClO, NaH_2PO_4, 2-methyl 2-butene, *t*-BuOH:H_2O, 12h, r. t.; b) MeI, Cs_2CO_3, DMF, 2h, r.t., 99 % over two steps; VII) Ac_2O:HOAc:H_2SO_4, 3h, 0 ºC; VIII) EtSH, $BF_3{\cdot}Et_2O$, DCM, 15h, r.t., 72 % over two steps.

Scheme 27

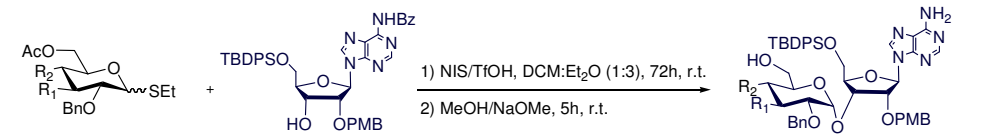
Selective protection of the 6-OH with *tert*-butyldiphenylsilyl chloride yielded **99**. Iododerivative **100** was synthesized following the same procedure that was used to generate **95**. When the radical reaction was performed on substrate **100**, yields rose to 98 %, which demonstrated the positive effect of changing from a benzyl to a *tert*-butyldiphenylsilyl protecting group. Allyl oxidative cleavage with OsO_4/NMO and $NaIO_4$ afforded aldehyde **102** in 74 % yield. This compound was converted to the corresponding acid using the same methodology as previously described. Finally, the acid was protected as a methyl ester via reaction with MeI and Cs_2CO_3 (99 % over two steps). Acetolysis of ester **103** and subsequent thioglycoside formation gave compound **105** in 72 % yield over two steps.

3.3.3. Synthesis of 3'-*O*-(3''-deoxy-3''-(2-methyl-2-ethanoate)-α-D-glucopiranosyl adenosine and 3'-*O*-(4''-deoxy-4''-(2-methyl-2-ethanoate)-α-D-glucopiranosyl adenosine derivatives.

Van Boom *et al.* reported the use of thioglycosides as glycosyl donors in the synthesis of adenophostin. In that case, however, the glycosyl acceptor used was 1,2-*O*-isopropilidene-5-*O*-*tert*-butyldiphenylsilyl ribose. It is worth noting that whenever protected adenosine was used as glycoside acceptor,[19] glycosylation never exceeded 50 % yield, presumably as a result of steric hindrance around 3'-OH. We believed that the combination of a thioglycoside glycoside donor and an adenosine acceptor would enhance the synthesis' overall yield.

Glycosylations using NIS/TfOH as an activator[133] (Scheme 28) were carried out in a 3:1 solvent mixture of Et_2O to DCM in order to favour α-stereoselectivity.[134] Glycosylations proceeded with excellent stereoselectivity, and only the α product was detected. After acetate protecting group removal, compounds **106a** and **106b** were obtained in 58% and 49% yield respectively. A portion of the unreacted donor and acceptor materials were also recovered.

[133] Demchenko, A.; Stauch, T.; Boons, G.-J. *Synlett* **1997**, 818.
[134] Wulff, G.; Röhle, G. *Angew. Chem. Int. Ed. Engl.* **1974**, *13*, 157.



86: R_1= CH_2COOMe
R_2= OAc

105: R_1= OAc
R_2=CH_2COOMe

38

106a: R_1 = CH_2COOMe
R_2 = OH 58 %

106b: R_1 = OH;
R_2 = CH_2COOMe 49 %

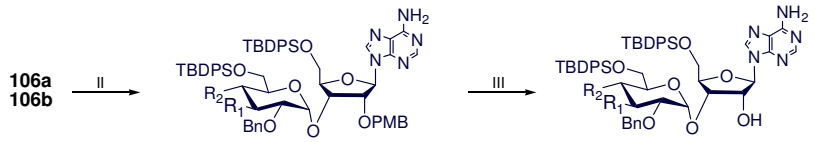
Scheme 28

Initially, a glycosyl donor-acceptor ratio of 1:1.2 was employed. We believed that increasing the proportion of the acceptor would increase yields, but the unreacted acceptor and glycosylation product had similar R_f values in a variety of different elution systems tested. Thus, purification of the glycosylation product was difficult, and obtaining pure fully-protected 3''-methylenecarboxylate and 4''-methylenecarboxylate adenophostin analogues became tiresome. The donor-acceptor ratio was therefore maintained at 1:1.2, which made it less difficult to purify away the acceptor in excess.

Treatment of the mixture of glycoside acceptor and adenophostin analogue with sodium methoxide allowed for greater purity glycosylation product to be isolated, and so these two steps were carried out in tandem. Together with acetate methanolysis, N6 amine deprotection was effected. This was not problematic, because amine phosphorylation was partially avoided using imidazolium triflate[135] instead of tetrazole as the promoter during the phosphorylation step.

The next synthetic step involved selective protection of the 6"-hydroxyl. Unlike 2-deoxy-2-fluoro adenophostin A, however, this was not particularly challenging, and even introduction of a *tert*-butyldiphenylsylil protecting group was possible. Thus, **106a** and **106b** were sylilated to obtain **107a** (67 %) and **107b** (78%).

[135] Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1998**, *120*, 12395.



107a R_1= CH_2COOMe R_2= OH 67%
107b R_1= OH R_2=CH_2COOMe 78%

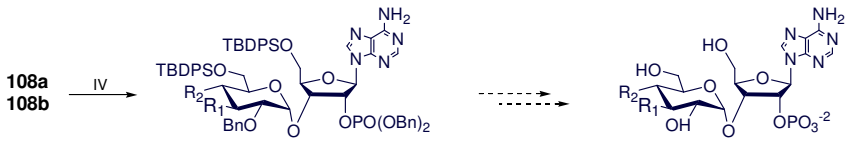
108a R_1= CH_2COOMe R_2= OH 79%
108b R_1= OH R_2=CH_2COOMe 84%

II) TBDPSCl, DMAP, Et_3N, DCM, 8h, 0 ºC to r.t.; III) CAN, MeCN:H_2O (9:1)

Scheme 29

This result supported our suspicions that the presence of the 2"-fluorine atom changes the conformation of the glucoside ring in such a way that makes 6''-OH inaccessible for sylilation reactions.

Subsequent deprotection of 2'-*O*-*p*-methoxybenzyl with CAN in acetonitrile afforded suitable substrates for phosphorylation, **108a** (79 %) and **108b** (84%). Initial phosphorylation attemps were made treating **108a** and **108b** with dibenzyl-N,N-diisopropyl phosphoramidite in the presence of imidazolium triflate thereby introducing phosphate precursors. Posterior oxidation of phosphite groups with MCPBA at -78ºC afforded the dibenzyloxy- protected phosphates **109a** and **109b** but in not enough amount to be properly caracterized..



109a R_1= CH_2COOMe R_2= $OPO(OBn)_2$
109b R_1= $OPO(OBn)_2$ R_1=CH_2COOMe

110a R_1= CH_2COO^{-2} R_2= OPO_3^{-2}
110b R_1= OPO_3^{-3} R_1=CH_2COO^{-2}

IV) a) $(BnO)_2PN^iPr$, imidazolium triflate, DCM, 12h, r.t.; b) MCPBA, 2h, -78 ºC to r.t

Further deprotection steps (silyl protecting group removal and debenzylation) of phosphorylated products will afford in the future the final products.

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4. Conclusions

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1. The absence of phosphate produces a minor effect in 2'-dephospho-Adenophostin than in IP_2. So, identificating 2'-phosphate of Adenophostin as an equivalent of 1-phosphate of IP_3 is not totally true. At least, its contribution to Adenophostin high affinity is not determining.

2. The results obtained from the biological study also show that adenine interacts directly into the binding core of IP_3R, and moreover, is very likely is through a cation-π interaction.

3. Regarding to the synthetic part, it has been shown that radical allylation/olefin oxidation is an appropriate methodology to incorporate a methylenecarboxylate moiety in good yield and stereoselectivites.

4. Glycosylation reactions made in order to get the framework of desired Adenophostin analogues were carried out either using thioglycosides activated with NIS/TfOH, or glycosyl bromides in the presence of AgOTf and Ag_2CO_3. In both cases good yields and excellent stereoselectivities were achieved. The obtained results are remarkable taking into account that glycosylations using nucleosides as glycosyl acceptors do not usually give excellent results. In that sense, an improvement of the glycosylation step with respect to the reported Adenophostin synthesis using adenosine derivatives as glycosyl donors have been made.

5. It has been showed as well that the presence of fluorine into Adenophostin backbone results in a conformational change in glucose ring, resulting in a geometry that makes almost impossible the introduction on a silyl protecting group at position 6''.

6. It has been stablished with success the synthetic route that will lead to three new analogues of Adenophostin A: 2'-deoxy-2'-fluoro Adenophostin A; 3''-deoxy-3''-(2-methyl-2-ethanoate) and (4''-deoxy-4''-(2-methyl-2-ethanoate) Adenophostin A, only resting the lasts deprotection steps that will be carried out in a near future.

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5. Experimental Section

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5.1. Experimental techniques and general methods.

^{1}H, ^{13}C, ^{31}P, and ^{19}F NMR spectra were recorded using VARIAN GEMINI 300 and VARIAN MERCURY 400 spectrometers. In all the ^{1}H NMR spectra, TMS was used as an internal reference. In the ^{13}C NMR spectra, the residual solvent signal was used as an internal reference ($CDCl_3$, triplet at 77.23 ppm) unless otherwise stated. All the ^{31}P and ^{19}F NMR spectra were referenced to 85% H_3PO_4 and $CFCl_3$, respectively, as external standards. Elemental analyses (C, H, N, and S) were performed with a Carlo Erba EA 1108 Analyser in the *Servei de Recursos Científics* (URV). Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter in a 1 dm cell at 20ºC. FT-IR was obtained with a FTIR 680PLUS spectrophotometer with a resolution of 4 cm^{-1}.

Flash column chromatography was performed with silica gel 60 (E. Merck, 40-63 μm). Medium-pressure liquid chromatography (MPLC) was carried out on silica gel 60 ACC (SDS, 6-35 μm). Radial chromatography was performed on 1, 2, or 4 mm plates of Kieselgel 60 PF_{254} silica gel (E. Merck), depending on the amount of product. Solvents were purified using standard procedures.136 For thin layer chromatography (TLC) aluminium sheets coated with silica gel 60 F_{254} (E. Merck) were used. Elution system A: hexane:ethyl acetate (7:3); elution system B DCM:MeOH (9:1). Compounds were visualized by UV (254 nm) and also by spraying the TLC plates with either 6% H_2SO_4 in ethanol, 1% anisaldehyde, 6% H_2SO_4 in ethanol, or 2% $PdCl_2$ and 15% H_2SO_4 in water, followed by charring at 150ºC for few minutes.

[^{3}H]Insositol-1,4,5-trisphosphate (58 Ci/mmol) was from Amersham (Little Chalfont, U. K.). EcoScint A scintillation cocktail was from National Diagnostics (Atlanta, GA), Inositol-1,4,5-trisphosphate was from American Radiolabeled Chemicals (St. Louis, MO). Adenophostin A, Inositol-2,4-bisphosphate, and 2'-dephospho-Adenophostin A were given by Prof. Barry V. L. Potter (School of Pharmacy and Pharmacology, University of Bath, U. K.).

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[136] D. D. Perrin, W. L. F. Armarego, *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, **1989**, 3rd Ed. (reimp.).

Preparation of rats cerebellar membranes.

The frozen cerebella from six rats (200-250g) were homogenized (twice for 10 sec at 4ºC) in homogenizing buffer (consisting of 20 mM Tris·HCl, 20 nM NaCl, 100 mM KCl, 1 mM EDTA, 1mg/ml BSA, pH 7.7) containing protease inhibitors (10 µl of leupeptin and 10 µl of pepstatin). After centrifugation (50.000 x g for 13 min at 4º), the pellet was resuspended in homogenizing buffer and homogenized as described above, and the protein content was adjusted to 5mg/ml.

Expression of fragments of type I IP_3 receptor in bacteria.

For 1-604 and R568Q fragments, constructs ligated in pTrcHis A vector at the *Xho*I/*Eco*RI sites were used, expressing N-terminally tagged hexa-His fusion proteins. Fragment corresponding to R504Q was amplified by PCR from the full-length receptor cDNA and then ligated into pTrcHis A vector at the *Xho*I/*Eco*RI. For expression of bacterial fusion proteins, the constructs were transformed into *E.Coli* strain Bl21(De3) and 1 ml of the culture was grown overnight in Luria-Bertani medium with 50 µg/ml ampicillin at 30ºC. This inoculum was then added to 100 ml of Luria-Bertani medium, cultured at 22ºC and when the D_{600} had reached 1.0-1.5, isopropyl β-*D*-thiogalactoside (0.5 mM) was added. After a further 20h at 15 ºC, the cells were harvested by centrifugation (5000 g, 15 min), and washed in PBS. The pellets were resuspended in 10 ml of Tris/EDTA medium (TEM; 50 mM Tris/1mM EDTA, pH 8.3) supplemented with 1mM β-mercaptoethanol and a protease inhibitor cocktail formulated for purification of poly-His-tagged proteins in bacteria. The suspension was incubated with lysozyme (100 µg/ml) for 4h at 4 ºC 30 min on ice. Protein concentrations were determined[137] using BSA as standard. Standard immunoblotting methods were used to identify the bacterial fusion proteins using a mouse anti-His_6 antibody, followed by a horseradish peroxidase-conjugate anti-mouse secondary antibody and Super Signal chemiluminescence reagent to visualize immunoreactive bands.

[137] Bradford, M.M. *Anal. Biochem.* **1976**, 72, 248.

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Equilibirum [^{3}H]IP_3 binding.

All equilibrium binding incubations were performed at 4ºC in TEM (final volume, 500 µl) containing [^{3}H]IP_3 (1 nM), receptor (full lenght, 1-604. R568Q or R504Q) and appropiate concentrations of competing ligands. After 5 min. reactions were terminated by addition of 200 µl of cold TEM containing 30% PEG 8000 and 200 µg of γ–globulin followed by centrifugation (20000 g, 5 min). Supernatants were removed by aspiration and the pellets were washed with 500 µl of cold TEM containing 15 % PEG and then resuspended in 1 ml of EcoScint A scintillation cocktail and their radioactivity was determined by liquid scintillation counting. Total [^{3}H]IP_3 binding was usually more than 2500 d.p.m. and non-specific binding was < 10 % of total binding.

General method for benzylation.

The corresponding glycoside (1 eq) is dissolved in DMF (7 ml/mmol). NaH (1.5 eq) is added and the solution is stirred for 0.5h at r.t. Then benzyl bromide (1.5 eq) and TBAI (0.02 eq) are added and the reaction mixture is stirred overnight. Then water is added to the solution and the product is extracted with DCM (x3). The combined organic phases are washed with water (x3), dried over Na_2SO_4 and concentrated.

General method for 4,6-*O*-benzylideneacetal selective opening.

A mixture of the corresponding glucoside (1 eq) and 4 Å M.S. (3.5 mg/mmol) in dry dichloromethane (12 ml/mmol) is stirred for 30 min at r. t. Then the reaccion flask is cooled to –78ºC. Triethylsilane hydride (3.2 eq) and triflic acid (3 eq) are added to the cooled mixture and the solution is stirred for further one hour at –78ºC. When TLC shows complete disappearance of the starting material, triethylamine (2 ml/mmol) and methanol (2 ml/mmol) are successively added in order to quench the reaction. Then the solution is diluted with DCM, filtered, and washed with a saturated aqueous solution of $NaHCO_3$. The organic layer is dried over Na_2SO_4 and evaporated to dryness.

General method for 4,6-O-benzylideneacetal hydrolysis.

Corresponding glucoside (1 eq) was dissolved in dry methanol (12ml/mmol), then I_2 (0.4 eq) is added. The mixture is refluxed for 4h. Then a solution of Na_2SO_3 (10%) is added to reaction. After 10 minutes stirring the solvents are evaporated under vacuo. The oil obtained is redissolved in water and extracted with DCM (x3), the organic layer is dried, the solvent is evaporated and the reaction crude obtained is used without further purification in the next step.

General procedure for *tert*-butyldiphenylsylil ether formation.

Corresponding substrate (1 eq) is dissolved in dry DCM (2ml/mmol). Then DMAP (0.1 eq) and dry triethylamine (6 ml/mmol) are added. The mixture is cooled to 0ºC and *tert*-Butyldiphenylsilyl chloride (1.5 eq) is added dropwise. The reaction is allowed to reach r.t. and stirred for 5h (or when TLC shows no further evolution). The mixture is diluted in DCM, washed with water (x3), and the organic layer is dried, filtered, and the solvent evaporated under vacuum.

General procedure for iodide formation.

A mixture of corresponding glucoside (1 eq), triphenyl phosphine (4 eq), I_2 (3 eq), and imidazole (4 eq) is refluxed in toluene (8 ml/mmol). Once TLC shows that the reaction is completed, the mixture is concentrated in vacuum, diluted with CH_2Cl_2 and washed with $NaHCO_3$ and Na_2SO_3 (sturated solutions). The organic phase is dried with $MgSO_4$, filtered and the solvents evaporated.

General procedure for thionocarbonate formation.

O-phenyl chlorothionoformate (2.1 eq) is added dropwise to a solution of corresponding glucoside (1 eq) and NHS (1 eq) in dry toluene (10ml/mmol). After the addition is completed, dry pyridine (2.80 ml, 34.70 mmol) is also added to the solution. The reaction is stirred for 5 h at r.t until completely disappearance of the starting material. Once the reaction is finished, the mixture is washed with HCl (1M)

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and a saturated solution of $NaHCO_3$. The organic layer is then dried with $MgSO_4$, filtered, and the solvent evaporated until dryness.

General methods for radical allylation.

Method A

AIBN (0.2 eq) and allyltributyltin (3.5 eq) are added to a solution of corresponding tionocarbonate or iodide (1 eq) in dry and deoxigenated toluene (1 ml/mmol). After 15h refluxing the solvent is evaporated under vacuum.

Method B

A mixture of corresponding iodide (1eq), allyltriphenyltin (10 eq) and AIBN (0.2 eq) is stirred at 70 ºC. After 18h the crude is applied to a silica gel column.

General procedure for oxidative allyl cleavage.

OsO_4 (0.02 eq) is added to a solution of the corresponding allyl derivative (1 eq) and NMO (1.1 eq) in dioxane/water (9/1) (3 ml/mmol). The solution protected from light is stirred for 3 h at r. t. When TLC shows complete consumption of the starting material, the reaction mixture is diluted in DCM and washed with a saturated aqueous solution of $NaHCO_3$. The organic phase is dried with $MgSO_4$ and concentrated in vacuo. The crude obtained is redissolved in dioxane/water (9/1) (3 ml/mmol) and $NaIO_4$ is added (1.1 eq). After 24 h stirring at r.t. the reaction mixture is diluted in DCM and washed with a saturated aqueous solution of $NaHCO_3$, dried with $MgSO_4$ and the solvent evaporated.

General procedure for aldehyde oxidation.

2-methyl 2-butene (5.4 eq) and $NaClO_2$ (9.5 eq) are added to a mixture of corresponding aldehyde (1 eq) and NaH_2PO_4 (9 eq) in *tert*-butanol/water (2/1) (35

ml/mmol). The reaction mixture is stirred for further 5h. Once TLC shows that the reaction is completed, the mixture is concentrated in vacuo, diluted with CH_2Cl_2 and washed with brine (x3). The organic layer obtained is dried with $MgSO_4$, filtered and the solvent evaporated.

General method for methyl ester formation.

$CsCO_3$ (2 eq) and methyl iodide (1.8 eq) is added to a solution of corresponding acid (1 eq) in dry DMF (1.5 ml/mmol). The reaction mixture is stirred 2h at r.t., diluted with DCM and washed with water (x3). The organic layer is dried with $MgSO_4$ and the solvents evaporated under vacuum.

General method for anomeric OMe hydrolysis.

Corresponding α-methyl glucoside (1 eq) is dissolved in a mixture of acetic anhydride/acetic acid/sulphuric acid (50/25/1) (20 ml/mmol) and stirred at 0ºC for 3 h. Then sodium acetate (16 eq) is added slowly and the solution is stirred for further 15 min. The reaction mixture is filtered and extracted with DCM. The organic layer is washed with a saturated solution of $NaHCO_3$ (x3), dried with $MgSO_4$ and the solvent evaporated under vacuum.

General method for thioglycoside formation.

$BF_3{\cdot}Et_2O$ (2.5 eq) is added to a solution of corresponding 1-*O*-acetate (1 eq) in dry DCM (50ml/mmol) and EtSH (1.1 eq) at 0 ºC. The reaction is allowed to reach r.t. and stirred for 15 h. Once TLC shows that the reaction is completed, the mixture is diluted with DCM and washed with a solution of $NaHCO_3$. Then the organic phase is dried with $MgSO_4$, filtered and the solvents are evaporated under vacuum.

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General method for acetylation.

A solution of corresponding glycoside (1 eq) and acetic anhydride (4 eq) in dry pyridine (8 eq) is stirred overnight at r.t. The reaction mixture is then poured into a water-ice mixture and extracted with CH_2Cl_2 (x3), dried with $MgSO_4$ and concentrated.

General method for deacetylation.

A solution of corresponding substrate (1 eq) is dissolved in dry methanol (1ml/mmol). Then sodium methoxide (1.1 eq/ester or amide group) is added to the solution. The evolution of the reaction is followed by TLC. When starting material is consumed, the solution is neutralized with acetic acid, and concentrated under vacuum. Then the crude is diluted and washed with an aqueous solution of $NaHCO_3$. The organic layer is dried, filtered and evaporated to dryness.

General method for glycosylation with thioglycosides.

Toluene (2 ml) is added into a flask with glycosyl donor (1 eq), glycosyl acceptor (2.2 eq), and evaporated until dryness. The process is repeated three times. Then 4 Å M.S (225 mg/mmol) is added and the flask is putted under vacuum overnight in a dryer equipped with P_2O_5. After 12h Et_2O (2.5 ml/mmol) and DCM (0.83 ml/mmol) are added and the mixture is stirred at r.t. for 30 min. Then the temperature is set to 0ºC and NIS (1.1 eq) and TfOH (0.1 eq) were introduced into the flask and the solution was stirred for 48h at r.t. When TLC (ethyl acetate/hexane, 6/4) showed no further evolution, Et_3N (1.6 ml/mmol) is added, and the mixture is filtered. The filtrate is diluted with DCM and washed with an aqueous solution of Na_2SO_3, then with $NaHCO_3$, and water. The organic layer is dried and evaporated under vacuum. The residue is applied to a silica gel column (elution system A).

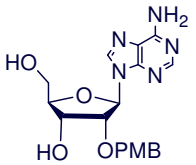
General procedure for PMB ether deprotection.

A solution of corresponding substrate and CAN (or DDQ) in acetonitrile:water (9:1) is stirred at r.t. Once TLC shows that the reaction is completed, the reaction mixture is diluted with DCM and washed with a saturated solution of $NaHCO_3$. The aqueous layer is extracted with DCM. The combined organic layers are dried, filtered, the solvents are evaporated under vacumm, and the reaction crude is purified by silica gel column chromatography.

5.2. Approach to the synthesis of 2''-deoxy-2''-fluoro Adenophostin A.

5.2.1. Synthesis of ^{6}*N*-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine.

Synthesis of 2'-*O*-*p*-methoxybenzyl adenosine (51)



A suspension of adenosine (2.00 g, 7.48 mmol) in DMF (67.5 ml) was cooled down to -5ºC. Then NaH (60% in mineral oil, 400 mg , 9.73 mmol) was added to the solution. The mixture was stirred for further 1h at –5ºC. Then 4-methoxybenzyl chloride (1.3 ml, 8.98 mmol) was added dropwise during 1h. After addition was complete, the reaction was let to reach r.t., and stirred for 12h. When TLC (DCM/MeOH, 9/1)showed no further evolution, DMF was evaporated under vacuum. The residual oil obtained was applied to a silica gel column, affording **51** (2.20 g, 75 % yield).

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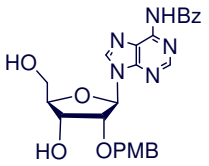
^{1}H NMR (400 MHz, DMSO-d_6, 25 ºC): δ = 8.31 (s, 1 H, H-8), 8.08 (s, 1 H, H-2), 7.38 (bs, 2 H, NH_2), 7.06 (d, 2 H, *J* = 8.8 Hz, Ar), 6.72 (d, 2 H, *J* = 8.8 Hz, Ar), 6.02 (d, 1 H, $J_{1',2'}$ = 6.4 Hz, H-1'), 5.51 (dd, 1 H, $J_{OH\text{-}5',5'a}$= 7.6 Hz, $J_{OH\text{-}5',5'b}$ = 4.4 Hz, OH-5'), 5.32 (d, 1 H, $J_{OH\text{-}3',3'}$= 5.2 Hz, OH-3'), 4.57 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.53 (dd, 1 H, $J_{2',1'}$= 6.4 Hz, $J_{2',3'}$= 4.8 Hz, H-2'), 4.36 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.34 (m, 1 H, H-3'), 4.02 (m, 1 H, H-4'), 3.68 (s, 3 H, OCH_3), 3.67 (m, 1 H, H-5'b), 3.56 (ddd, 1 H, $J_{5'a,5'b}$ = 12.0 Hz, $J_{5'a,4'}$ = 3.6 Hz, $J_{5'a,OH\text{-}5'}$ = 7.6 Hz, H-5'a) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 ºC): δ = 158.7 (C-5), 156.2 (C-6), 152.3 (C-2), 148.8 (C-4), 139.8 (C-8), 129.6, 129.1, 119.4, 113.4 (C Ar), 86.7 (C-1'), 86.3 (C-4'), 79.7 (C-2'), 70.7 (CH_2Ph), 69.0 (C-3'), 61.6 (C-5'), 55.0 (OCH_3) ppm. Anal. Calcd. for $C_{18}H_{21}N_5O_5$, 55.81 % C, 5.46 % H, 18.08 % N, found 55.82 % C, 5.44 % H, 18.12 % N.

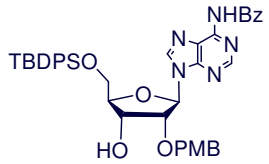
Synthesis of *N*^{6}-benzoyl-2'-*O*-*p*-methoxybenzyl adenosine (52).



Compound **51** (2.20 g, 5.68 mmol) was dissolved in dry pyridine. Then Me_3SiCl (4.7 ml, 37.49 mmol) was added. The reaction mixture was stirred for two hours. After that time, BzCl (1.98 ml, 7.04 mmol) was added, and the solution was stirred for further 3h. When TLC (system B) showed no further evolution the reaction was quenched with NH_4OH (30%, 15 ml) and the mixture was stirred for 15 min. After that time, the solution was poured into a water/ice bath, and then extracted with DCM. The organic phase was washed, dried with $MgSO_4$, and evaporated under vacuum. The reaction crude was redissolved in methanol and Dowex H^+ was added (4g). The suspension was stirred until complete disappearing of the starting material was observed. Then the solution was filtered, concentrated under vacuum, and the residue redissolved in DCM, washed with an aqueous solution of $NaHCO_3$ (x3), dried with $MgSO_4$, evaporated and purified by flash chromatography (system B) affording **52** (3.36 g, 81% yield).

^{1}H NMR (400 MHz, DMSO-d_6, 25 ºC): δ = 11.26 (s, 1 H, NH), 8.70 (s, 1 H, H-8), 8.67 (s, 1 H, H-2), 8.05-7.56 (m, 5H, Ar), 7.09 (d, 2 H, *J* = 8.7 Hz, Ar), 6.75 (d, 2 H, *J* = 8.7 Hz, Ar), 6.18 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, H-1'), 5.41 (d, 1 H, $J_{OH\text{-}3',3'}$= 5.0 Hz, OH-3'), 5.21 (t, 1 H, $J_{OH\text{-}5',5'a}$= $J_{OH\text{-}5',5'b}$ = 5.0 Hz, OH-5'), 4.63 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.59 (d, 1 H, $J_{2',1'}$= 6.0 Hz, H-2'), 4.44 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.38 (dd, 1 H, $J_{3',4'}$= 7.5 Hz, $J_{3',OH\text{-}3'}$= 5.0 Hz, H-3'), 4.04 (dd, 1 H, $J_{4',3'}$= 7.5 Hz, $J_{4',5'a}$= 3.8 Hz, H-4'), 3.72-3.59 (m, 2 H, H-5'a, H-5'b), 3.68 (s, 3 H, OCH_3) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 ºC): δ = 165,5 (C=O), 158.5 (C-5), 151.3 (C-6), 151.3 (C-2), 150.2 (C-4), 142.8 (C-8), 133.1,132.3, 128.3, 125.6, 129.2, 128.9, 116.0, 113.2 (C Ar), 86.2 (C-1'), 85.8 (C-4'), 79.6 (C-2'), 70.6 (CH_2Ph), 68.6 (C-3'), 60.9 (C-5'), 54.7 (OCH_3) ppm. Anal. Calcd. for $C_{25}H_{25}N_5O_6$, 61.09 % C, 5.13 % H, 14.25 % N, found 61.02 % C, 5.14 % H, 14.02 % N.

Synthesis of *N*^{6}-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine (38).



The title compound was prepared following the general procedure for silylation, starting from **52** (400 mg, 0.814 mmol) in dry DCM (1.6 ml), DMAP (10 mg, 0.08 mmol), dry triethylamine (670 µl), and *tert*-butyldiphenylsilyl chloride (310 µl, 1.22 mmol). After 15h stirring the reaction was treated as in the general procedure and purified by column chromatography (system B), affording **38** (4.89 g, 73 % yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 9.14 (bs, 1 H, NH), 8.77 (s, 1 H, H-8), 8.16 (s, 1 H, H-2), 8.04 (m, 2 H, Ar), 7.09 (d, 2 H, *J* = 8.4 Hz, Ar), 6.72 (d, 2 H, *J* = 8.4 Hz, Ar), 6.20 (d, 1 H, $J_{1',2'}$ = 4.8 Hz, H-1'), 4.64 (apparent singulet, 2 H, CH_2Ph), 4.56 (t, 1 H, $J_{2',1'}$= $J_{2',3'}$ = 4.8 Hz, H-2'), 4.46 (ddd, 1 H, $J_{3',2'}$= 4.8 Hz, $J_{3',4'}$= 9.6 Hz, $J_{3',OH\text{-}3'}$= 5.6 Hz, H-3'), 4.18 (m, 1 H, H-4'), 4.05 (dd, 1 H, $J_{5'a,5'b}$= 11.6 Hz, $J_{5'a,4'}$ = 3.2 Hz, H-5'a), 3.84 (dd, 1 H, $J_{5'b,5'a}$= 11.6 Hz, $J_{5'b,4'}$ = 3.2 Hz, H-5'b), 3.74 (s, 3 H, OCH_3), 2.87

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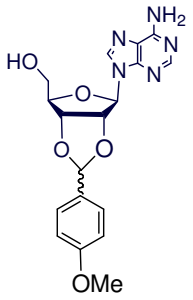
(d, 1 H, $J_{OH\text{-}3',3'}$= 5.6 Hz, OH-3'), 1.08 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 164.8 (C=O), 162.7 (Ar), 152.8 (C-2), 151.4 (C-6), 149.6 (C-4), 141.5 (C-8), 159.8, 135.8-128.0 (Ar), 123.4 (C5), 114.0 (Ar), 87.0 (C-1'), 85.5 (C-2'), 80.7 (C-4'), 72.9 (CH_2Ph), 69.5 (C-3'), 63.4 (C-5'), 55.4 (OCH_3), 27.1 (CH_3), 19.4 (C_{tBu}) ppm.

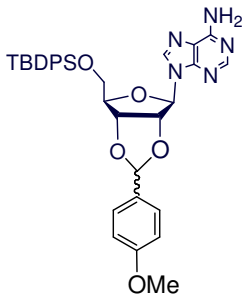
Synthesis of 2',3'-*O*-*p*-methoxybenzylydene adenosine (55).



A solution of adenosine (1g, 3.74 mmol) in benzaldehyde (3.5 ml) was stirred in the presence of $ZnCl_2$ at r.t. for 3 days. Then the solution was diluted in ethyl acetate and washed with water (x3). Aqueous layers were extracted with ehtyl acetate. The combined organic phases were dried and the solvent evaporated under vacuo. After purification on column silica gel (system B) product **55** was obtained as a *endo*/*exo* (2/3) mixture (600 mg, 42% yield).

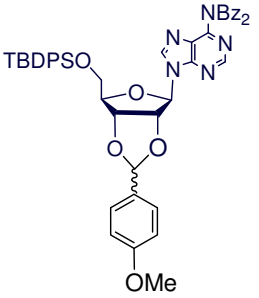
^1H NMR (400 MHz, d_6-DMSO, 25 ºC): δ = 8.39 (s, 2 H, H-8a, H-8b), 8.18 (s, 2 H, H-8a, H-8b), 7.52-7.39 (m, 4 H, NH_2, Ar), 7.02-6.95 (m, 2 H, Ar), 6.30 (d, 0.4 H, $J_{1',2'}$ = 2.9 Hz, H-1'b), 6.28 (d, 0.6 H, $J_{1',2'}$ = 2.9 Hz, H-1'a), 6.19 (s, 0.6 H, H_{ketal}a), 5.97 (s, 0.4 H, H_{ketal}b), 5.49-5.45 (m, 1 H, H-2'), 5.29 (t, 0.4 H, $J_{5',OH}$= 5.6 Hz, 5'-OHb), 5.16 (t, 0.6 H, $J_{5',OH}$= 5.6 Hz, 5'-OHa), 5.10-5.05 (m, 1 H, H-3'), 4.40-4.37 (m, 0.4 H, H-4'b), 4.31-4.26 (m, 0.6 H, H-4'a), 3.79 (s, 1.2 H, OCH_3b), 3.77 (s, 1.8 H, OCH_3a), 3.62-3.57 (m, 2 H, 2xH-5').

Synthesis of 5'-*O*-*tert*-butyldiphenylsilyl-2',3'-*O*-*p*-methoxybenzylydene adenosine (56).



The title compound was prepared following the general procedure for silylation, starting from **55** (600 mg, 1.55 mmol) in dry DCM (3.5 ml), DMAP (19 mg, 0.16 mmol), dry triethylamine (1.3 ml), and *tert*-butyldiphenylsilyl chloride (1.3 ml, 5.14 mmol). After 15h stirring the reaction was treated as in the general procedure and purified by column chromatography (system B), affording **56** (760 mg, 80 % yield).

Synthesis of N^6,N^6-dibenzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2',3'-*O*-*p*-methoxybenzylydene adenosine (57).



A solution of product **56** (760 mg, 1.22 mmol) and benzoyl chloride (425 ml, 3.66 mmol) in dry pyridine (9ml) was stirred for 3h at r.t. After that time, water was added to the rection mixture and then the solution was extracted with DCM (x3). Organic layer was dried and the solvent evaporated under vacuum. Upon silica gel column chromatography compound **57** was afforded (963 mg, 95% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 8.70 (s, 0.6 H, H-8a), 8.67 (s, 0.4 H, H-8b), 8.30 (s, 0.6 H, H-2a), 8.28 (s, 0.4 H, H-2b), 8.14-6.92 (m, 24 H, Ar), 6.35 (d, 0.6 H,

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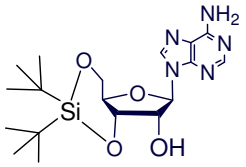
$J_{1'a,2'a}$ = 2.4 Hz, H-1'a), 6.29 (d, 0.4 H, $J_{1'b,2'b}$ = 3.8 Hz, H-1'b), 6.15 (s, 0.4 H, H_{ketal}b), 6.02 (s, 0.6 H, H_{ketal}a), 5.54 (dd, 0.6 H, $J_{2'a,1'a}$= 2.4, $J_{2',3'}$ = 6.4 Hz, H-2'a), 5.48 (dd, 0.4 H, $J_{2'b,1'b}$= 3.8, $J_{2'b,3'b}$ = 6.4 Hz, H-2'b), 5.28 (dd, 0.4 H, $J_{3'b,2'b}$= 6.4 Hz, $J_{3'b,4'b}$= 4.0 Hz, H-3'b), 5.12 (dd, 0.6 H, $J_{3'a,2'a}$= 6.4 Hz, $J_{3'a,4'a}$= 2.0 Hz, H-3'a), 4.66-4.63 (m, 0.6 H, H-4'a), 4.49-4.48 (m, 0.4 H, H-4'b), 3.98-3.80 (m, 5 H, OCH_3, 2xH-5'), 1.03 (s, 3.6 H, *t*Bu), 1.02 (s, 5.4 H, *t*Bu) ppm.

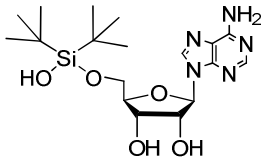
Synthesis of 3',5'-*O*-(di-*tert*butylsilandiyl) adenosine (62).



Di-*tert*-butylsilyl ditriflate (1.73 ml, 5.35 mmol) was added dropwise to a solution of adenosine (1.3 g, 4.87 mmol) in dry DMF (9.5 ml) at 0ºC for 15 min. The reaction mixture was allowed to reach r.t., and after 15 min NEt_3 was added to stop the reaction. Then, water was added and the solution was extracted with ethyl acetate (x2). The organic layer was washed with $NaCl_{(aq)}$, dried with $MgSO_4$ and the solvent evaporated to dryness. After purification of the reaction crude on silica gel column chromatography (elution system: ethyl acetate), compound **62** was afforded (1.52 g, 77% yield).

^{1}H RMN ($CDCl_3$, δ ppm, 400 MHz): 8.29 (s, 1 H, H-8), 7.86 (s, 1 H, H-2), 6.18 (bs, 2 H, NH_2), 5.98(s, 1 H, H-1'), 4.87 (dd, 1 H, $J_{3',4'}$ = 8.4 Hz, $J_{3',2'}$ = 5.2 Hz, H-3'), 4.70 (d, 1 H, $J_{2',3'}$ = 5.2 Hz, H-2'), 4,47 (dd, 1 H, $J_{5'a,5'b}$ = 8.2 Hz, $J_{5'a,4'}$ = 4.2 Hz, H-5'a), 4.11 (m, 2 H, H-4', H-5'b), 1.12 (s, 9 H, *t*-Bu), 1.06 (s, 9 H, *t*-Bu). ^{13}C RMN ($CDCl_3$, δ ppm, 100 MHz): 155.5 (C-6), 153.0 (C-2), 149.0 (C-4), 139.3 (C-8), 120.3 (C-5), 91.2 (C-1'), 75.8 (C-3'), 75.2 (C-4'), 74.0 (C-2'), 67.7 (C-5'), 27.6 (CH_3, *t*-Bu), 27.4 (CH_3, *t*-Bu), 23.0 (C, *t*-Bu), 20.6 (C, *t*-Bu).

Synthesis of 5'-*O*-(di-*tert*butylsilandiyl) adenosine (63).

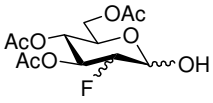


The title compound was prepared following the same methodology for 2'-*O*-*p*-methoxybenzyl protection used for compound **51**. Starting from a solution of compound **62** (493 mg, 1.21 mmol) in DMF (12 ml), NaH (60% in mineral oil, 63 mg, 1.57 mmol) was added. Inmediately, a product with low Rf appeared in the TLC (system B). At that point the reaction was quenched with water, diluted with DCM and the aqueous phase was washed with DCM (x3). The combined organic phases were dried and the solvents evaporated. Compund **63** was afforded in 90% yield (463 mg).

^{1}H RMN (d^6-DMSO, δ ppm, 400 MHz): 8.31 (s, 1 H, H-8), 8.13 (s, 1 H, H-2), 7.32 (bs, 2 H, NH_2), 5.90(d, 1 H, $J_{1',2'}$ = 5.6 Hz, H-1'), 5.55 (d, 1 H, $J_{2',OH'}$ = 5.6 Hz, OH-2'), 5.19 (d, 1 H, $J_{3',OH'}$ = 5.6 Hz, OH-3'), 4.60 (q, 1 H, $J_{2',1'} = J_{2',3'} = J_{2',OH'}$ = 5.6 Hz, H-2'), 4.22 (ddd, 1 H, $J_{3',2'} = J_{3',4'} = J_{3',OH'}$ = 5.6 Hz, H-3'), 3.40-3.95 (m, 2 H, H-4', H-5'a), 3.86 (t, 1 H, $J_{5'b,5'a} = J_{5'b,4'}$ = 6.0 Hz, H-5'b), 0.96 (s, 9 H, *t*-Bu), 1.06 (s, 18 H, 2 x *t*-Bu).

5.2.2.Synthesis of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-1-β-bromo glucoside.

Synthesis of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-*gluco* and *manno*pyranoses (46 and 47).



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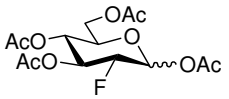
Selectfluor © (5g, 14.10 mmol) was added to a 10% solution of 3,4,6-tri-*O*-acetil-D-glucal (3.27g, 12.01 mmol) in CH_3NO_2:H_2O (5:1, 32 ml). The solution was stirred at r.t., and the reaction was followed by TLC (elution system A). After consumption of starting material (15h), the mixture was heated to reflux for 45 min. Then the solvents were evaporated under vacuum, the residue was redisolved in DCM. The resulting mixture was washed with an aqueous solution of $NaHCO_3$ (5%). The organic phase was dried with $MgSO_4$ and concentrated to dryness. The syrup was applied to a silica gel column. An inseparable mixture of anomers of *manno:gluco* (1:1.3) derivatives was obtained (2.08 g, 56% yield).

Data for α anomer extracted from the diastereoisomeric mixtue ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 5.60 (ddd, 1 H, $J_{3,F}$ = 12.4 Hz, $J_{3,2}$ = 9.4 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 5.48 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 5.04 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, H-4), 4.52 (ddd, 1 H, $J_{2,F}$ = 49.0 Hz, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 9.4 Hz, H-2), 4.27 (m, 2 H, H-6a, H-6b), 4.14 (m, 1 H, H-5), 2.10 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.05 (s, 3H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 171.2 (C=O), 170.5 (C=O), 170.0 (C=O), 90.2 (d, J_{C1-F} = 21.0 Hz, C-1), 87.9 (d, J_{C2-F} = 193.0 Hz, C-2), 70.6 (d, J_{C3-F} = 19.5 Hz, C-3), 68.1 (d, J_{C4-F} = 6.8 Hz, C-4), 67.2 (C-5), 62.0 (C-6), 21.2, 20.9, 20.8 (CH_3) ppm. ^{19}F NMR (376.4 MHz, $CDCl_3$, 25 ºC): δ = -200.42 (dd, $J_{F,H2}$ = 49.0 Hz, $J_{F,H3}$ = 12.4 Hz, F-2) ppm.

Data for β anomer extracted from the diastereoisomeric mixtue ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 5.33 (ddd, 1 H, $J_{3,F}$ = 14.0 Hz, $J_{3,2}$ = 9.2 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 5.05 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.0 Hz, H-4), 4.92 (dd, 1 H, $J_{1,2}$ = 7.6 Hz, $J_{1,F}$ = 3.0 Hz, H-1), 4.29 (ddd, 1 H, $J_{2,F}$ = 50.4 Hz, $J_{2,1}$ = 7.6 Hz, $J_{2,3}$ = 9.2 Hz, H-2), 4.27 (m, 1 H, H-6a), 4.12 (m, 1 H, H-6b), 3.78 (ddd, 1 H, $J_{5,4}$ = 10.0 Hz, $J_{5,6a}$ = 2.2 Hz, $J_{5,6b}$ = 4.4 Hz, H-5), 2.10 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.05 (s, 3H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 170.5 (C=O), 170.4 (C=O), 169.9 (C=O), 94.7 (d, J_{C1-F} = 23.3 Hz, C-1), 90.6 (d, J_{C2-F} = 190.6 Hz, C-2), 72.9 (d, J_{C3-F} = 19.8 Hz, C-3), 72.0 (C-5), 68.3 (d, J_{C4-F} = 6.8 Hz, C-4), 67.2 (C-6), 20.9, 20.8, 20.7 (CH_3) ppm. ^{19}F NMR (376.4 MHz, $CDCl_3$, 25 ºC): δ = -199.82 (dd, $J_{F,H1}$ = 3.0 Hz, $J_{F,H2}$ = 50.4 Hz, $J_{F,H3}$ = 14.0 Hz, F-2) ppm.

Synthesis of 1,3,4,6-*O*-tetra-*O*-acetyl-2-deoxy-2-fluoro-D-*gluco*pyranose (47).



The mixture of fluorinated compounds **46** were treated following the general procedure for acetylation, starting from 3.80 g (12.3 mmol) of the mixture dissolved in dry pyridine (40 ml) and acetic anhydride (5.8 ml, 61.6 mmol). The reaction was stirred overnight at r.t. and then treated as in the general procedure affording a diastereoisomeric mixtures of both *gluco* and *mano* derivatives quantitatively (1.33 g of *gluco*derivative, 31%).

Data for α anomer extracted from the anomeric mixture: ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 6.42 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 5.55(ddd, 1 H, $J_{3,F}$ = 12.4 Hz, $J_{3,2}$= 9.2 Hz, $J_{H3,H4}$ = 9.6 Hz, H-3), 5.10 (dd, 1 H, $J_{4,3}$ = 9.6 Hz, $J_{4,5}$ = 10.0 Hz, H-4), 4.67 (ddd, 1 H, $J_{2,F}$ = 48.9 Hz, $J_{2,1}$ = 4.0 Hz, $J_{2,3}$ = 9.2 Hz, H-2), 4.29 (dd, 1 H, $J_{6a,5}$= 4.0 Hz, $J_{6a,6b}$= 12.4 Hz, H-6a), 4.10 (ddd, 1 H, $J_{5,4}$ = 10.0 Hz, $J_{5,6a}$ = 4.0 Hz, $J_{5,6b}$ = 2.0 Hz, H-5), 4.06 (dd, 1 H, $J_{6b,5}$ = 2.0 Hz, $J_{6b,6a}$ = 12.4 Hz, H-6b), 2.21 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.05 (s, 3H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 170.6 (C=O), 170.0 (C=O), 169.6 (C=O), 169.0 (C=O), 88.3 (d, J_{C1-F} = 22.2 Hz, C-1), 86.2 (d, J_{C2-F} = 194.8 Hz, C-2), 70.6 (d, J_{C3-F} = 19.9 Hz, C-3), 69.5 (C-5), 67.4 (d, J_{C4-F} = 7.8 Hz, C-4), 61.4 (C-6), 21.0, 20.8, 20.7, 20.6 (CH_3) ppm. ^{19}F NMR (376.4 MHz, $CDCl_3$, 25 ºC): δ = -202.90 (dd, $J_{F,H2}$ = 48.9 Hz, $J_{F,H3}$ = 12.4 Hz, F-2) ppm.

Data for β anomer extracted from the anomeric mixture: ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 5.81 (dd, 1 H, $J_{1,2}$ = 8.2 Hz, $J_{1,F}$ = 3.0 Hz, H-1), 5.40(ddd, 1 H, $J_{3,F}$ = 14.0 Hz, $J_{3,2}$ = $J_{3,4}$ = 9.0 Hz, H-3), 5.08 (m, 1 H, H-4), 4.45 (ddd, 1 H, $J_{2,F}$ = 50.4 Hz, $J_{2,1}$ = 8.2 Hz, $J_{2,3}$ = 9.0 Hz, H-2), 4.30 (m, 2 H, H-6a, H-6b), 3.90 (ddd, 1 H, $J_{5,4}$ = 10.2 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 2.1 Hz, H-5), 2.18 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.04 (s, 3H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 171.2 (C=O), 170.0 (C=O), 169.6 (C=O), 169.0 (C=O), 91.2 (d, J_{C1-F} = 24.6 Hz, C-1), 88.3 (d, J_{C2-F} = 191.6 Hz, C-2), 72.7 (C-5), 72.7 (d, J_{C3-F} = 19.9 Hz, C-3), 67.6 (d, J_{C4-F} = 6.9 Hz,

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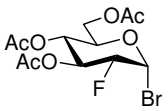
C-4), 60.5 (C-6), 21.2, 20.9, 20.8, 20.7 (CH_3) ppm. ^{19}F NMR (376.4 MHz, $CDCl_3$, 25 ºC): δ = -201.65 (ddd, $J_{F,H-1}$ = 3.0 Hz, $J_{F,H-2}$ = 50.4 Hz, $J_{F,H-3}$ = 14.0 Hz, F-2) ppm.

Synthesis of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-β-D-*gluco*pyranosyl bromide (50).

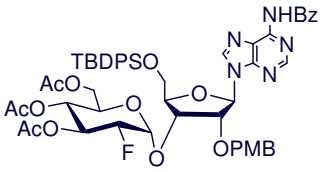


Compound **47a** (0.06 g, 0.18 ml) was dissolved in dry DCM (2ml). HBr (1ml, 5.7 M in acetic acid) was added, and the mixture was stirred at r.t. for further 5h. When TLC (Hexane/ Ethyl acetate, 1/1) showed total consumption of the starting material, the reaction mixture was transferred into an erlenmeyer flask and a saturated aqueous solution of $NaHCO_3$ (30 ml) was poured in. Product was extracted from the aqueous phase with DCM (x3), and the combined organic layers were dried with $MgSO_4$. The solvent was evaporated until dryness and the residue applied to a silica gel column chromatography affording **50** (64 mg, 100% yield).

^1H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 6.54 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 5.63(ddd, 1 H, $J_{3,F}$ = 10.8 Hz, $J_{3,2}$ = 9.2 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 5.12 (dd, 1 H, $J_{4,3}$ = 9.6 Hz, $J_{4,5}$ = 10.0 Hz, H-4), 4.55 (ddd, 1 H, $J_{2,F}$ = 49.0 Hz, $J_{2,1}$ = 4.0 Hz, $J_{2,3}$ = 9.2 Hz, H-2), 4.33 (m, 2 H, H-5, H-6a), 4.13 (dd, 1 H, $J_{6b,5}$= 2.0 Hz, $J_{6a,6b}$= 11.8 Hz, H-6b), 2.10 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.07 (s, 3H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 170.6 (C=O), 170.0 (C=O), 169.7 (C=O), 86.5 (d, J_{C2-F} = 198.3 Hz, C-2), 85.5 (d, J_{C1-F} = 25.2 Hz, C-1), 72.3 (C-5), 71.2 (d, J_{C3-F} = 19.0 Hz, C-3), 66.7 (d, J_{C4-F} = 7.6 Hz, C-4), 61.0 (C-6), 20.8, 20.7, 20.6 (CH_3) ppm. ^{19}F NMR (376.4 MHz, $CDCl_3$, 25 ºC): δ = -188.95 (dd, $J_{F,H-2}$ = 49.0 Hz, $J_{F,H-3}$ = 10.8 Hz, F-2) ppm.

5.2.3. Approach to the synthesis of 2''-deoxy-2''-fluoro-Adenophostin A.

Synthesis of 3'-*O*-(3'',4'',6''-tri-*O*-acetyl-2''-deoxy-2''-fluoro-α-D-glucopyranosyl)-N^6-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine (64).



Glycosyl acceptor (**38**) (280 mg, 0.38 mmol), Ag_2CO_3 (116mg, 0.42 mmol), and AgOTf (30 mg, 0.11 mmol) were placed in a flask, then 2 ml of dry toluene were added and evaporated to dryness (three times), finally 4 Å M.S. (200 mg) were put in and the flask was protected from light. The same procedure was followed for glycosyl donor (**50**, 200 mg). Then both acceptor and donor were placed in a dryer with P_2O_5 and left under vacuum for 24h. After that time dry Et_2O (1.2 ml) was added to acceptor flask. Then glycosyl bromide was dissolved in dry DCM (0.4 ml) and transferred to acceptor flask. The mixture was stirred for 48 h at r.t. After 2 days the solution was filtered through celite, and the solvents were evaporated to dryness. The crude reaction was applied to a silica gel column using a gradient of hexane/ethyl acetate (1/1, 1/2, 1/4) as elution system. After purification **64** was afforded (224 mg, 58 % yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 9.31 (s, 1 H, NH), 8.63 (s, 1 H, H2), 8.10 (s, 1 H, H8), 8.02-7.35 (m, 15 H, Ar), 7.06 (d, 2 H, *J* = 8.8 Hz, Ar), 6.66 (d, 2 H, *J* = 8.8 Hz, Ar), 6.15 (d, 1 H, $J_{H1'-H2'}$ =6.0 Hz, H-1'), 5.63 (dt, 1 H, $J_{H3"-F}$ = 12.0 Hz, $J_{H3"-H2"}$ = $J_{H3"-H4"}$ = 9.6 Hz, H-3"), 5.27 (d, 1 H, $J_{H1"-H2"}$ =4.0 Hz, H-1"), 5.06 (dd, 1 H, $J_{H4"-H5"}$ = 10.0 Hz, $J_{H4"-H3"}$ = 9.6 Hz, H-4"), 4.91 (dd, 1 H, $J_{H2'-H1'}$ = 6.0 Hz, $J_{H2'-H3'}$ = 5.2 Hz, H-2'), 4.62 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.58 (m, 1 H, H-3'), 4.54 (ddd, 1 H,$J_{H2"-F}$ = 48.8 Hz, $J_{H2"-H3"}$ = 9.6 Hz, $J_{H2"-H1"}$ = 4.0 Hz, H-2"), 4.42 (m, 1 H, H-4'), 4.40 (d, 1 H, J_{AB} =11.6 Hz, CH_2Ph), 4.17 (dd, 1 H, $J_{H6"a-H6"b}$ = 12.4 Hz, $J_{H6"a-H5"}$ = 4.4 Hz, H-6"a), 4.11 (m, 1 H, H-5'a),4.04 (ddd, 1 H, $J_{H5"-H4"}$ = 10.0 Hz, $J_{H5"-H6"a}$ = 4.4 Hz, $J_{H5"-H6"b}$ = 2.4 Hz, H-5"), 3.93 (dd, 1 H, $J_{H6"b-H6"a}$ =12.4 Hz, $J_{H6"b-H5"}$ = 2.4 Hz, H-6"b), 3.85 (dd, 1 H, $J_{H5'b-H5'a}$ = 11.6 Hz, $J_{H5'b-H4'}$ = 3.6 Hz, H-5'b), 3.72 (s,3 H, OCH_3), 2.11 (s, 3 H, CH_3),

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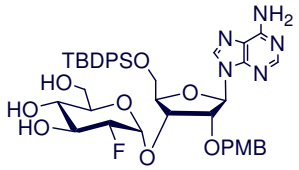
2.05 (s, 3 H, CH_3), 1.99 (s, 3 H, CH_3), 1.08 (s, 9 H, *t*-Bu). ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 170.6 (CO), 170.2 (CO), 169.8 (CO), 164.8 (CO), 159.5 (C-6),152.6 (C-2), 149.6 (C-4), 142.3 (C-8), 135.7-113.8 (Ar), 123.4 (C-5), 96.1 (d, $J_{C1''\text{-}F}$ = 20.5 Hz, C-1''),87.2 (d, $J_{C2''\text{-}F}$ = 196.8 Hz, C-2''), 87.5 (C-1'), 83.6 (C-4'), 78.0 (C-2'), 74.8 (C-3'), 72.4 (CH_2Ph), 70.6 (d, $J_{C3''\text{-}F}$ = 19.9 Hz, C-3''), 68.2 (C-5''), 67.9 (d, $J_{C4''\text{-}F}$ = 6.8 Hz, C-4''), 63.1 (C-5'), 61.6 (C-6''), 55.4 (OCH_3), 27.1 (CH_3, *t*-Bu), 20.9 (CH_3), 20.8 (CH_3), 20.8 (CH_3), 29.4 (C, *t*-Bu).

^{19}F RMN (376.4 MHz, $CDCl_3$, 25 ºC): δ = -201.25 (dd, $J_{F\text{-}H2''}$ = 48.8 Hz, $J_{F\text{-}H3''}$ = 12.0 Hz, F-2'').

Synthesis of 3'-*O*-(2''-deoxy-2''-fluoro-α-D-glucopiranosyl)-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine (65).



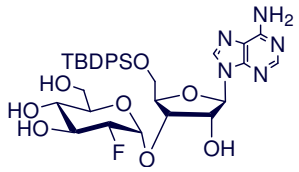
The title compound was prepared following the general procedure for ester/amide hydrolysis, starting from **64** (0.21 mmol) dissolved in dry methanol (0.2 ml), sodium methoxide (62 mg, 1.15 mmol) was added to the solution. The reaction mixture was stirred at r.t. for 4 h and then treated as in the general procedure and purified by column chromatography (elution system DCM/MeOH, 10/0, 9.5/0.5, 9/1) affording **65** (116 mg, 70% yield).

^{1}H NMR (300 MHz, CD_3OD, 25 ºC): δ = 8.25 (s, 1 H, H-2), 7.91 (s,1 H, H-8), 7.63-7.33 (m, 10 H, Ar), 7.06 (d, 1 H, *J* = 8.7 Hz, Ar), 6.68 (d, 1 H, *J* = 8.7 Hz, Ar), 6.19 (d, 1 H, $J_{H1'\text{-}H2'}$ = 6.3 Hz, H-1'), 5.85 (bs, 2 H, NH_2), 5.08 (d, 1 H, $J_{H1''\text{-}H2''}$ = 3.6 Hz, H-1''), 4.70 (m, 1 H, H-2'), 4.59 (d, 1 H, J_{AB} = 11.7 Hz, CH_2Ph), 4.51-4.16 (m, 4 H, CH_2Ph, H-3', H-4', H-2''), 4.01 (dd, 1 H, $J_{H5'a\text{-}H5'b}$ =11.4 Hz, $J_{H5'a\text{-}H4'}$ = 4.5 Hz, H-5'a), 3.80-3.65 (m, 6 H, H-5'b, H-6''a, H-6''b, OCH_3), 1.05 (s, 9 H, *t*-Bu). ^{13}C NMR (75.4 MHz, CD_3OD, 25 ºC): δ = 160.9 (C-6),151.8 (C-2), 149.6 (C-4), 143.2 (C-8), 136.9-128.9

(Ar), 123.4 (C-5), 114.6 (Ar), 98.1 (d, $J_{C1''\text{-}F}$ = 20.5 Hz, C-1''),91.6 (d, $J_{C2''\text{-}F}$ = 190.3 Hz, C-2''), 88.9 (C-1'), 86.4 (C-4'), 80.4 (C-2'), 75.2 (C-3'), 74.6 (CH_2Ph), 73.4 (C-5''), 73.0 (d, $J_{C3''\text{-}F}$ = 17.1 Hz, C-3''), 71.1 (d, $J_{C4''\text{-}F}$ = 7.3 Hz, C-4''), 65.3 (C-5'), 62.2 (C-6''), 55.9 (OCH_3), 27.6 (CH_3, *t*-Bu), 20.3 (C, *t*-Bu).

^{19}F RMN (376.4 MHz, CD_3OD, 25 ºC): δ = -202.77 (dd, $J_{F\text{-}H2''}$ = 50.4 Hz, $J_{F\text{-}H3''}$ = 13.9 Hz, F-2'').

Synthesis of 3'-*O*-(2''-deoxy-2''-fluoro-α-D-glucopiranosyl)-5'-*O*-*tert*-butyldiphenylsilyl adenosine (67).



The title compound was prepared following the general procedure for *p*-methoxybenzylether deprotection, starting from **65** (36 mg, 0.046 mmol) dissolved in 0.5 ml of acetonitrile:water (9:1) mixture, CAN (50 mg, 0.092 mmol) was added. The solution was stirred for 1 h and then treated as in the general procedure. Upon silica gel column purification (system B), **67** was afforded (29.2 mg) in 95 % yield.

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 8.19 (s, 1 H, H-2), 8.14 (s,1 H, H-8), 7.64 (m, 4 H, Ar), 7.37 (m, 6 H, Ar), 6.09 (d, 1 H, $J_{H1'\text{-}H2'}$ = 6.0 Hz, H-1'), 5.43 (d, 1 H, $J_{H1''\text{-}H2''}$ = 4.0 Hz, H-1''), 5.00 (m, 1 H, H-2''), 4.66 (t, 1 H, $J_{H3'\text{-}H4'}$ = $J_{H3'\text{-}H2'}$ = 4.4 Hz, H-3'), 4.31 (ddd, 1 H, $J_{H2''\text{-}F}$ = 49.6 Hz, $J_{H2''\text{-}H3''}$ = 9.6 Hz, $J_{H2''\text{-}H1''}$ = 4.0 Hz, H-2''), 4.36 (m, 1 H, H-4'), 4.02 (dd, 1 H, $J_{H5'a\text{-}H5'b}$ =12.0 Hz, $J_{H5'a\text{-}H4'}$ = 2.8 Hz, H-5'a), 4.00 (m, 1 H, H-3''), 3.92 (dd, 1 H, $J_{H5'b\text{-}H5'a}$ = 12.0 Hz, $J_{H5'b\text{-}H4'}$ =3.6 Hz, H-5'b), 3.73 (m, 2 H, H-6''a, H-6''b), 3.64 (ddd, 1 H, $J_{H5''\text{-}H4''}$ = 9.6 Hz, $J_{H5''\text{-}H6''a}$ = 3.6 Hz, $J_{H5''\text{-}H6''b}$ = 2.8 Hz, H-5''), 3.46 (t, 1 H, $J_{H4''\text{-}H3''}$ = $J_{H4''\text{-}H5''}$ = 9.6 Hz, H-4''), 1.03 (s, 9 H, *t*-Bu). ^{19}F RMN (376.4 MHz, $CDCl_3$, 25 ºC): δ = -202.68 (dd, $J_{F\text{-}H2''}$ = 49.6 Hz, $J_{F\text{-}H3''}$ = 13.0 Hz, F-2'').

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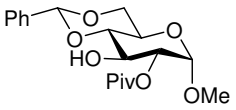
5.2. Approach to the synthesis of 3''-deoxy-3''-(2-methyl-ethanoate) and 4''-deoxy-4''-(2-methyl-ethanoate) Adenophostin A analogues.

5.2.1. Synthesis of 4,6-di-*O*-acetyl-2-*O*-benzyl-3-deoxy-3-(2-methyl-ethanoate) glucoside fragment.

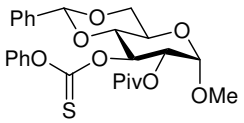
Synthesis of methyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-α-glucopyranoside (68).



Methyl 4,6-*O*-benzylidene α-glucopyranose (2 g, 7.08 mmol) was dissolved in dry pyridine (27 ml). Pivaloyl chloride (1.05 ml, 8.5 mmol) was added at –15 ºC. The reaction was stirred for 5h at 0ºC. Once TLC (elution system A) showed that the reaction was completed, the reaction was quenched with water and diluted with ethyl acetate. The organic phase was washed with HCl (10%), brine, water and dried with $MgSO_4$, filtered and the solvent evaporated affording **68** (1.95 g, 75% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.51-7.36 (m, 5 H, Ar), 5.56 (s, 1 H, H-7), 4.94 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.74 (dd, 1 H, $J_{2,1}$ = 4.0 Hz, $J_{2,3}$ = 9.4 Hz, H-2), 4.30 (dd, 1 H, $J_{6eq,5}$ = 4.8 Hz, $J_{6eq,6ax}$ = 9.6 Hz, H-6eq), 4.20 (dt, 1 H, $J_{3,2}$ = $J_{3,4}$ = 9.4 Hz, $J_{3,OH}$ = 2.0 Hz, H-3), 3.86 (dt, 1 H, $J_{5,6eq}$ = 4.8 Hz, $J_{5,6ax}$ = $J_{5,4}$ = 9.4 Hz, H-5), 3.77 (t, 1 H, $J_{6ax,5}$ = $J_{6eq,6ax}$ = 9.4 Hz, H-6ax), 3.57 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 9.4 Hz, H-4), 3.39 (s, 3 H, OCH_3), 1.25 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 178.4 (C=O), 137.2 (C Ar), 129.5-126.5 (CH Ar), 102.2 (C-7), 97.8 (C-1), 81.5 (C-4), 73.6 (C-2), 69.1 (C-6), 69.0 (C-3), 62.2 (C-5), 55.8 (OCH_3), 39.0 (C *t*Bu), 27.2 (3 CH_3) ppm.

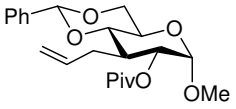
Synthesis of methyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(phenylthionocarbonate)-α-glucopyranoside (69).



The title compound was prepared following the general procedure for thionocarbonate formation, starting from **68** (900 mg, 2.46 mmol) in dry toluene (22 ml), NHS (243 mg, 2.46 mmol), and dry pyridine (990 ml, 12.30 mmol). After 6h stirring at r.t. the reaction mixture was treated as in the general procedure and purified by column chromatography (elution system A), affording **69** (1.02 g, 82% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.50-6.70 (m, 10 H, Ar), 6.24 (t, 1 H, $J_{3,2} = J_{3,4}$ = 9.6 Hz, H-3), 5.58 (s, 1 H, H-7), 5.05 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.98 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 4.34 (dd, 1 H, $J_{6eq,5}$ = 4.8 Hz, $J_{6eq,6ax}$ = 9.6 Hz, H-6eq), 4.01 (dt, 1 H, $J_{5,6eq}$ = 4.8 Hz, $J_{5,6ax} = J_{5,4}$ = 9.6 Hz, H-5), 3.87 (t, 1 H, $J_{6ax,5} = J_{6eq,6ax}$ = 9.6 Hz, H-6ax), 3.85 (t, 1 H, $J_{4,3} = J_{4,5}$ = 9.6 Hz, H-4), 3.43 (s, 3 H, OCH_3), 1.25 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 194.9 (C=S), 178.0 (C=O), 153.5, 137.2 (C Ar), 130.0-121.8 (CH Ar), 101.6 (C-7), 97.7 (C-1), 79.2 (C-4), 78.9 (C-3), 71.7 (C-2), 68.9 (C-6), 62.5 (C-5), 55.8 (OCH_3), 39.0 (C *t*Bu), 27.1 (3 CH_3) ppm.

Synthesis of methyl 3-allyl-4,6-*O*-benzylidene-3-deoxy-2-*O*-pivaloyl-α-glucopyranoside (70).

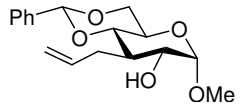


The title compound was prepared following the general procedure for radical allylation Method A, starting from **69** (1.02 g, 2.03 mmol) in dry and deoxygenated toluene (4 ml), allyl tri-*n*-butyltin (1.57 ml, 5.07 mmol), and AIBN (67 mg, 0.41 mmol).

After 24h refluxing the reaction was treated as in the general procedure and purified by column chromatography (system A), affording **70** (689 mg, 87% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.50-7.34 (m, 5 H, Ar), 5.85-5.75 (m, 1 H, H-9), 5.50 (s, 1 H, H-7), 5.11-5.01 (m, 2 H, H-10, H-11), 4.83 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.68 (dd, 1 H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 10.4 Hz, H-2), 4.26 (dd, 1 H, $J_{6eq,5}$ = 4.8 Hz, $J_{6eq,6ax}$ = 10.4 Hz, H-6eq), 3.83 (dt, 1 H, $J_{5,6eq}$ = 4.8 Hz, $J_{5,6ax}$ = $J_{5,4}$ = 10.4 Hz, H-5), 3.70 (t, 1 H, $J_{6ax,6eq}$ = $J_{6ax,5}$ = 10.4 Hz, H-6ax), 3.41 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.4 Hz, H-4), 3.38 (s, 3 H, OCH_3), 2.51-2.43 (m, 2 H, H-3, H-7), 2.29-2.23 (m, 1 H, H-7'), 1.24 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 178.0 (C=O), 137.6 (C Ar), 133.7 (C-9), 129.2-126.2 (CH Ar), 118.4 (C-10), 101.6 (C-7), 96.9 (C-1), 78.1 (C-4), 70.5 (C-2), 69.4 (C-6), 63.6 (C-5), 55.4 (OCH_3), 38.9 (C-*t*Bu), 38.4 (C-3), 29.6 (C-8), 27.2 (3 CH_3) ppm.

Synthesis of methyl 3-allyl-4,6-*O*-benzylidene-3-deoxy-α-glucopyranoside (71).

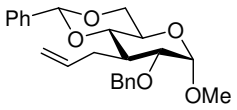


The title compound was prepared following the general procedure for ester/amide hydrolysis, starting from **70** (689 mg, 1.76 mmol) dissolved in dry methanol (2.0 ml), sodium methoxide (95 mg, 1.76 mmol) was added to the solution. The reaction mixture was stirred at r.t. for 4 h and then treated as in the general procedure and purified by column chromatography affording **71** (485 mg, 90 % yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.50-7.34 (m, 5 H, Ar), 5.99-5.88 (m, 1 H, H-9), 5.48 (s, 1 H, H-7), 5.18-5.10 (m, 2 H, H-10, H-10'), 4.68 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.27 (dd, 1 H, $J_{6eq,5}$ = 4.8 Hz, $J_{6eq,6ax}$ = 10.4 Hz, H-6eq), 3.77 (dt, 1 H, $J_{5,6eq}$ = 4.8 Hz, $J_{5,6ax}$ = $J_{5,4}$ = 10.4 Hz, H-5), 3.68 (t, 1 H, $J_{6ax,6eq}$ = $J_{6ax,5}$ = 10.4 Hz, H-6ax), 3.54 (ddd, 1 H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 10.4 Hz, $J_{2,OH}$ = 10.8 Hz, H-2), 3.45 (s, 3 H, OCH_3), 3.32 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.4 Hz, H-4), 2.51-2.46 (m, 2 H, H-8, H-8'), 2.13-2.05 (m, 1 H, H-3), 2.00 (d, 1 H, $J_{OH,2}$ = 10.8 Hz, 2-OH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 137.7 (C Ar), 134.8 (C-9), 129.1-126.2 (CH Ar), 118.0 (C-10), 101.5 (C-7),

99.5 (C-1), 78.2 (C-4), 69.9 (C-2), 69.4 (C-6), 63.8 (C-5), 55.5 (OCH_3), 42.2 (C-3), 30.0 (C-8) ppm.

Synthesis of methyl 3-allyl-2-*O*-benzyl-4,6-*O*-benzylidene-3-deoxy-α-glucopyranoside (72).



The title compound was prepared following the general procedure for benzylation, starting from **71** (198 mg, 0.65 mmol) in dry DMF (4.5 ml), NaH (40 mg, 0.97 mmol), benzyl bromide (115 μl, 0.97 mmol) and TBAI (5 mg, 0.01 mmol. The reaction was stirred overnight and then treated as in the general procedure and purified by column chromatography (9/1 hexane/ethyl acetate), affording **72** (224 mg, 88% yield).

The title compound was prepared following the general procedure for radical allylation, starting from **81** (208 mg, 0.41 mmol) in dry and deoxygenated toluene (0.8 ml), allyltributyltin (3.18 ml, 1.03 mmol), and AIBN (13 mg, 0.08 mmol). After 28h refluxing the reaction was treated as in the general procedure and purified by column chromatography (system A), affording **72** (142 mg, 88% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.50-7.35 (m, 10 H, Ar), 5.83-5.73 (m, 1 H, H-9), 5.47 (s, 1 H, H-7), 5.13-5.07 (m, 2 H, H-10, H-10'), 4.64 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.61 (apparent singulet, 2 H, CH_2Ph), 4.25 (dd, 1 H, $J_{6eq,5}$ = 4.8 Hz, $J_{6eq,6ax}$ = 10.4 Hz, H-6eq), 3.80 (dt, 1 H, $J_{5,6eq}$ = 4.8 Hz, $J_{5,6ax}$ = $J_{5,4}$ = 10.4 Hz, H-5), 3.65 (t, 1 H, $J_{6ax,6eq}$ = $J_{6ax,5}$ = 10.4 Hz, H-6ax), 3.41-3.31 (m, 5 H, H-2, H-4, OCH_3), 2.51-2.48 (m, 2 H, H-8, H-8'), 2.41-2.34 (m, 1 H, H-3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 138.1, 137.7 (C Ar), 134.5 (C-9), 130.1-126.2 (CH Ar), 118.0 (C-10), 101.5 (C-7), 97.6 (C-1), 78.3 (C-4), 76.5 (C-2), 72.5 (CH_2Ph), 69.5 (C-6), 63.5 (C-5), 55.4 (OCH_3), 39.8 (C-3), 29.5 (C-8) ppm.

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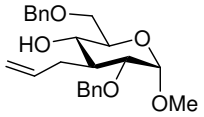
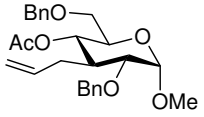
Synthesis of methyl 3-allyl-2,6-di-*O*-benzyl-3-deoxy-α-glucopyranoside (72).



The title compound was prepared following the general procedure for selective benzylidene acetal opening, starting from **72** (100 mg, 0.25 mmol) in dry DCM (3 ml), Et_3SiH (129 µl, 0.80 mmol), triflic acid (65 µl, 0.75 mmol) and 4Å M.S. (80 mg). The reaction was stirred at –78ºC for 1h and then treated as in the general procedure and the crude obtained was purified by column chromatography (system A), affording **73** (72 mg, 70% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.35-7.33 (m, 10 H, Ar), 5.90-5.79 (m, 1 H, H-8), 5.15-5.04 (m, 2 H, H-9, H-9'), 4.63 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.60 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.57 (apparent singulet, 2 H, CH_2Ph), 4.53 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 3.70-3.61 (m, 3 H, H-4, H-6, H-6'), 3.51-3.46 (m, 1 H, H-5), 3.37 (s, 3 H, OCH_3), 3.28 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 11.0 Hz, H-2), 2.59-2.36 (m, 2 H, H-7, H-7'), 2.16-2.09 (m, 1 H, H-3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 138.2, 137.9 (C Ar), 136.0 (C-8), 128.6-127.9 (CH Ar), 117.5 (C-9), 96.8 (C-1), 76.3 (C-2), 73.8, 72.4 (2 CH_2Ph), 70.7 (C-6), 70.1 (C-5), 69.9 (C-4), 55.3 (OCH_3), 42.0 (C-3), 31.3 (C-7) ppm. Anal. Calcd. for $C_{24}H_{30}O_5$, 72.34 % C, 7.59 % H, found 72.30 % C, 7.60 % H.

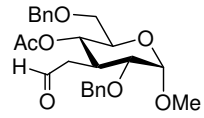
Synthesis of methyl 4-*O*-acetyl-3-allyl-2,6-di-*O*-benzyl-3-deoxy-α-glucopyranoside (74).



The title compound was prepared following the general procedure for acetylation, starting from **73** (72 mg, 0.18 mmol) in dry pyridine (0.5 ml) and acetic anhydride (66 µl, 0.70 mmol). The reaction was stirred overnight at r.t. and then treated as in the general procedure affording **74** (79 mg, 99% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.35-7.28 (m, 10 H, Ar), 5.84-5.74 (m, 1 H, H-8), 5.02-4.98 (m, 2 H, H-9, H-9'), 4.85 (t, 1 H, $J_{4,3} = J_{4,5}$ = 12.0 Hz, H-4), 4.64 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.60 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.56 (d, 1 H,J_{AB} = 12.0 Hz, CH_2Ph), 4.51 (apparent singulet, 2 H, CH_2Ph), 3.82-3.77 (m, 1 H, H-5), 3.46-3.39 (m, 5 H, H-6, H-6', OCH_3), 3.34 (dd, 1 H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 9.0 Hz, H-2), 2.33-2.23 (m, 3 H, H-3, H-7, H-7'), 1.92 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 170.2 (C=O), 138.2, 138.0 (C Ar), 135.3 (C-8), 128.6-127.8 (CH Ar), 117.1 (C-9), 96.7 (C-1), 76.5 (C-2), 73.8, 72.7 (2 CH_2Ph), 69.3 (C-6), 69.2 (C-5), 69.1 (C-4), 55.3 (OCH_3), 40.4 (C-3), 31.5 (C-7), 21.1 (CH_3) ppm. Anal. Calcd. for $C_{26}H_{32}O_6$, 70.89 % C, 7.32 % H, found 72.90 % C, 7.30 % H. $[\alpha]^{20}_D$ = +49.83.

Synthesis of methyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-deoxy-3-(2-ethanal)-α-glucopyranoside (75).



The title compound was prepared following the general procedure for allyl oxidative cleavage, starting from **74** (529 mg, 1.20 mmol), OsO_4 (6 mg, 0.02 mmol), and NMO (155 mg, 1.32 mmol) in dioxane/water (9/1) (3.6 ml). The reaction was stirred for 3h at r.t. and then treated as in the general procedure. Then the crude was redissolved in dioxane/water (9/1) (3.6 ml) and $NaIO_4$ (282 mg, 1.32 mmol) was added. The solution was stirred for 24 h and then treated as in the general procedure. The crude thus obtained was purified by column chromatography (system A) affording **75** (425 mg, 80% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 9.58-9.56 (m, 1 H, CHO), 7.33-7.26 (m, 10 H, Ar), 4.83 (t, 1 H, $J_{4,3} = J_{4,5}$ = 10.0 Hz, H-4), 4.61 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.53-4.43 (m, 4 H, 2 CH_2Ph), 3.83-3.80 (m, 1 H, H-5), 3.48-3.40 (m, 2 H, H-6, H-6'), 3.38 (s, 3 H, OCH_3), 3.32 (dd, 1 H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 2.75 (dt, 1 H, $J_{3,2} = J_{3,4}$ = 10.0 Hz, $J_{3,7}$ = 6.0 Hz, H-3), 2.44 (ddd, 1 H, $J_{7,3}$ = 6.0 Hz, $J_{7,7'}$ = 14.6 Hz, $J_{7,CHO}$ = 3.0 Hz, H-7), 2.79 (ddd, 1 H, $J_{7',3}$ = 6.0 Hz, $J_{7',7}$ = 14.6 Hz, $J_{7',CHO}$ = 2.2 Hz, H-7'), 1.87 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 200.6 (CHO),

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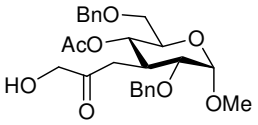
169.7 (C=O), 137.9, 137.6 (C Ar), 128.5-127.5 (CH Ar), 96.4 (C-1), 77.3 (C-2), 73.4, 72.4 (2 CH_2Ph), 69.6 (C-6), 68.9 (C-5), 68.7 (C-4), 55.0 (OCH_3), 43.6 (C-7), 37.0 (C-3), 20.5 (CH_3) ppm. Anal. Calcd. for $C_{25}H_{30}O_7$, 67.86 % C, 6.83 % H, found 67.86 % C, 6.86 % H.

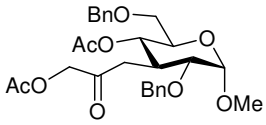
Synthesis of methyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-deoxy-3-(3-hydroxy-2-propanone)-α-glucopyranoside (76).



$RuCl_3$ (5 mg, 0.02 mmol) was added to a mixture of compound **74** (900 mg, 2.04 mmol) and $NaIO_4$ (918 mg, 4.28 mmol) in a CCl_4:H_2O:acetonitrile (1:1.5:1). The reaction mixture was stirred at r.t. for further 3h. After that time, an aqueous solution of $Na_2S_2O_3$ was added. The aqueous phase was extracted with ethyl acetate (x3). The combined organic phase was washed with brine, dried over $MgSO_4$ and the solvents evaporated. After purification on silica gel column, compound **76** was afforded (386 mg, 40%) together with aldehyde **75** (541 mg, 60%).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.35-7.26 (m, 10 H, Ar), 4.86 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.4 Hz, H-4), 4.67 (d, 1 H, $J_{1,2}$ = 2.8 Hz, H-1), 4.57-4.43 (m, 4 H, 2 CH_2Ph), 4.11 (apparent doublet, 2 H, H-9, H-9'), 3.83-3.79 (m, 1 H, H-5), 3.49-3.35 (m, 6 H, H-2, H-6, H-6', OCH_3), 3.01 (t, 1 H, $J_{OH,9}$ = $J_{OH,9}$ =4.8 Hz, OH), 2.75 (dddd, 1 H, $J_{3,2}$ = 10.4 Hz, $J_{3,4}$ = 10.5 Hz, $J_{3,7}$ = $J_{3,7'}$ = 6.0 Hz, H-3), 2.51 (dd, 1 H, $J_{7,3}$ = 6.0 Hz, $J_{7,7'}$ = 14.0 Hz, H-7), 2.35 (dd, 1 H, $J_{7',3}$ = 6.0 Hz, $J_{7',7}$ = 14.0 Hz, H-7'), 1.85 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 208.3, 170.2 (C=O), 137.8, 137.4 (C Ar), 128.7-127.8 (CH Ar), 96.3 (C-1), 77.4 (C-2), 73.7, 72.5 (2 CH_2Ph), 70.1 (C-4), 69.1 (C-5), 68.7 (C-6), 68.0 (C-9), 55.3 (OCH_3), 38.1 (C-7), 37.9 (C-3), 20.8 (CH_3) ppm. Anal. Calcd. for $C_{26}H_{32}O_8$, 66.09 % C, 6.83 % H, found 66.10 % C, 6.80 % H.

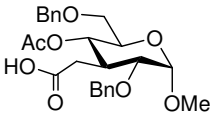
Synthesis of methyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-deoxy-3-(3-acetyl-2-propanone)-α-glucopyranoside.



The title compound was prepared following the general procedure for acetylation, starting from **76** (120 mg, 0.25 mmol) in dry pyridine (0.5 ml) and acetic anhydride (71 µl, 0.75 mmol). The reaction was stirred overnight at r.t. and then treated as in the general procedure(129 mg, 100% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.34-7.26 (m, 10 H, Ar), 4.85 (dd, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.4 Hz, H-4), 4.64 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.59-4.43 (m, 6 H, H-9, H-9', 2 CH_2Ph), 3.83-3.79 (m, 1 H, H-5), 3.46-3.37 (m, 6 H, H-2, H-6, H-6', OCH_3), 2.73 (dddd, 1 H, $J_{3,2}$ = 10.4 Hz, $J_{3,4}$ = 10.5 Hz, $J_{3,7}$ = $J_{3,7'}$ = 6.0 Hz, H-3), 2.52 (dd, 1 H, $J_{7,3}$ = 6.0 Hz, $J_{7,7'}$ = 14.0 Hz, H-7), 2.39 (dd, 1 H, $J_{7',3}$ = 6.0 Hz, $J_{7',7}$ = 14.0 Hz, H-7'), 2.13 (s, 3 H, CH_3) 1.85 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 208.3, 170.6, 170.4 (C=O), 137.9, 137.6 (C Ar), 129.0-127.9 (CH Ar), 96.5 (C-1), 77.4 (C-2), 73.7, 72.6 (2 CH_2Ph), 70.2 (C-4), 69.2 (C-5), 68.9 (C-6), 67.9 (C-9), 55.3 (OCH_3), 38.8 (C-7), 37.7 (C-3), 20.9, 20.7 (CH_3) ppm. Anal. Calcd. for $C_{28}H_{34}O_9$, 65.36 % C, 6.66 % H, found 65.35 % C, 6.70 % H.

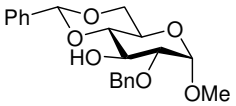
Synthesis of methyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-deoxy-3-(2-ethanoic acid)-α-glucopyranoside (77).



The title compound was prepared following the general procedure for aldehyde oxidation, starting from **75** (554 mg, 1.25 mmol), 2-methyl-2-butene (750 ml, 6.75 mmol), $NaClO_2$ (1.36 g, 12.00 mmol), and NaH_2PO_4 (1.37 g, 11.38 mmol) in *tert*-butanol/water (2/1) (45 ml). The reaction was stirred for further 5h at r.t., then treated as in the general procedure and purified by column chromatography (ethyl acetate/hexane, 1/1) affording **77** (400 mg, 70% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.33-7.30 (m, 10 H, Ar), 4.89 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.0 Hz, H-4), 4.64 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.57 (apparent singulet, 2 H, CH_2Ph), 4.52 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.46 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 3.84-3.79 (m, 1 H, H-5), 3.48-3.37 (m, 6 H, H-2, H-6, H-6', OCH_3), 2.65 (dddd, 1 H, $J_{3,2}$ = 10.8 Hz, $J_{3,4}$ = 10.0 Hz, $J_{3,7}$ = $J_{3,7'}$ = 5.6 Hz, H-3), 2.46 (dd, 1 H, $J_{7,3}$ = 5.6 Hz, $J_{7,7'}$ = 15.8 Hz, H-7), 2.36 (dd, 1 H, $J_{7',3}$ = 5.6 Hz, $J_{7',7}$ = 15.8 Hz, H-7'), 1.89 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 178.3, 170.3 (C=O), 137.8, 137.6 (C Ar), 128.6-127.8 (CH Ar), 96.4 (C-1), 77.1 (C-2), 73.6, 72.7 (CH_2Ph), 69.8 (C-4), 69.2 (C-5), 69.0 (C-6), 55.3 (OCH_3), 38.5 (C-3), 33.3 (C-7), 20.8 (CH_3) ppm. Anal. Calcd. for $C_{25}H_{30}O_8$, C 65.49 %; H 6.60 %; found 65.52 % C, 6.58 % H.

Synthesis of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-glucopyranoside (80).[125]

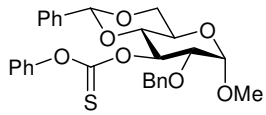


Methyl 4,6-*O*-benzylidene-α-glucopiranoside (450 mg, 1.59 mmol) was added to a flask with toluene (30 ml) equipped with a Dean-Stark apparatus. Then, 10 ml of toluene were removed by distillation and $(Bu_3Sn)_2O$ (1.78 ml, 0.99 mmol) was added. The reaction mixture was heated at reflux for 3 h. After that time 10 ml of toluene were removed, and Dean-Stark apparatus was replaced by a reflux system. The reaction solution was left at refluxing temperature for further 2 h. After that time, the solvent was evaporated until dryness and benzyl bromide (3.78 ml, 31.8 mmol) was added. The reaction mixture was stirred at 90 ºC for 2 days, then the benzyl bromide in excess was distilled under vacumm and the reaction crude was applied to a silica gel column. After purification using elution system A, compound **80** was afforded (473 mg, 80%).

^{1}H NMR ($CDCl_3$, 400 MHz, 25 ºC): δ = 7.48-7.11 (m, 10 H, Ar), 5.47 (s, 1 H, H-7), 4.75 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.65 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.57 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.22 (dd, 1 H, $J_{6eq,5}$ = 4.8 Hz, $J_{6eq,6ax}$ = 10.0 Hz, H-6eq), 4.11 (dt, 1 H, $J_{3,2}$ = $J_{3,4}$ = 10.0 Hz, $J_{3,OH}$ = 2.4 Hz, H-3), 3.78 (dt, 1 H, $J_{5,4}$ = $J_{5,6ax}$ =10.0 Hz,

$J_{5,6eq}$ = 4.8 Hz, H-5), 3.65 (t, 1 H, $J_{6ax,5}$ = $J_{6eq,6ax}$ = 10.0 Hz, H-6ax), 3.44 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.0 Hz, H-4), 3.43 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.33 (s, 3 H, OCH_3), 2.95 (d, 1 H, $J_{OH,3}$ = 2.4 Hz, 3-OH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 138.0, 137.1 (C Ar), 129.2-126.4 (CH Ar), 101.9 (C-7), 98.8 (C-1), 81.3 (C-4), 79.5 (C-2), 73.4 (CH_2Ph), 70.2 (C-3), 69.0 (C-6), 62.1 (C-5), 55.4 (OCH_3) ppm. Anal. Calcd. for $C_{21}H_{24}O_6$, 67.73 % C, 6.50 % H, found 67.70 % C, 6.55 % H.

Synthesis of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(phenylthionocarbonate)-α-glucopyranoside (81).



The title compound was prepared following the general procedure for thionocarbonate formation, starting from **80** (2.58 g, 6.94 mmol) in dry toluene (62 ml), NHS (690 mg, 6.94 mmol), and dry pyridine (2.80 ml, 34.70 mmol). After 5h stirring the reaction was treated as in the general procedure and the crude was purified by column chromatography (7/3 hexane/ethyl acetate, system A), affording **81** (3.42 g, 97% yield) as a solid.

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.48-6.99 (m, 15 H, Ar), 6.12 (t, 1 H, $J_{3,2}$ = $J_{3,4}$ = 9.6 Hz, H-3), 5.53 (s, 1 H, H-7), 4.79 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.73 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.71 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.30 (dd, 1 H, $J_{6eq,5}$ = 5.2 Hz, $J_{6eq,6ax}$ = 10.0 Hz, H-6eq), 3.95 (dt, 1 H, $J_{5,6eq}$ = 4.8 Hz, $J_{5,6ax}$ = $J_{5,4}$ = 10.0 Hz, H-5), 3.79-3.73 (m, 3 H, H-2, H-4, H-6ax), 3.42 (s, 3 H, OCH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 194.3 (C=S), 153.7, 137.6, 137.1 (C Ar), 129.6-122.0 (CH Ar), 101.6 (C-7), 98.9 (C-1), 80.7 (C-3), 79.6 (C-4), 77.7 (C-2), 73.4 (CH_2Ph), 69.1 (C-6), 62.4 (C-5), 55.7 (OCH_3) ppm. Anal. Calcd. for $C_{28}H_{28}O_7S$, 66.12 % C, 5.55 % H, 6.30 % S, found 66.13 % C, 5.56 % H, 6.28 % S. $[\alpha]^{20}_D$ = +8.75, m.p. =100.70 ºC.

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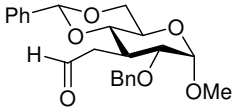
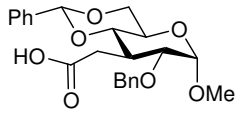
Synthesis of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-deoxy-3-(2-ethanal)-α-glucopyranoside (82).



The title compound was prepared following the general procedure for allyl oxidative cleavage, starting from **81** (1.19 g, 3.01 mmol), OsO_4 (15 mg, 0.06 mmol), and NMO (388 mg, 3.31 mmol) in dioxane/water (9/1) (16.5 ml). The reaction was stirred for 3h at r.t. and then treated as in the general procedure. Then the crude was redissolved in dioxane/water (9/1) (16.5 ml) and $NaIO_4$ (708 mg, 3.31 mmol) was added. The solution was stirred for 24 h and then treated as in the general procedure. The crude thus obtained was purified by column chromatography (system A) affording **82** (935 mg, 78% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 9.65 (m, 1 H, CHO), 7.42-7.31 (m, 10 H, Ar), 5.47 (s, 1 H, H-7), 4.60 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.56 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.52 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.26 (dd, 1 H, $J_{6eq,5}$ = 4.8 Hz, $J_{6eq,6ax}$ = 10.0 Hz, H-6eq), 3.82 (dt, 1 H, $J_{5,6eq}$ = 4.8 Hz, $J_{5,6ax}$ = $J_{5,4}$ = 10.0 Hz, H-5), 3.66 (t, 1 H, $J_{6ax,6eq}$ = $J_{6ax,5}$ = 10.0 Hz, H-6ax), 3.56 (dd, 1 H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.41 (s, 1 H, OCH_3), 3.32 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.0 Hz, H-4), 2.86-2.76 (m, 1 H, H-3), 2.65 (ddd, 1 H, $J_{8,CHO}$ = 2.0 Hz, $J_{8,3}$ = 5.8 Hz, $J_{8,8'}$ = 16.0 Hz, H-8), 2.46 (ddd, 1 H, $J_{8',CHO}$ = 3.6 Hz, $J_{8',3}$ = 7.4 Hz, $J_{8',8}$ = 16.0 Hz, H-8') ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 202.0 (CHO), 137.8, 137.4 (C Ar), 129.3-126.2 (CH Ar), 101.8 (C-7), 97.4 (C-1), 79.9 (C-2), 78.3 (C-4), 72.8 (CH_2Ph), 69.3 (C-6), 63.8 (C-5), 55.5 (OCH_3), 43.5 (C-8), 37.3 (C-3) ppm. Anal. Calcd. for $C_{23}H_{26}O_6$, 69.33 % C, 6.58 % H, found 69.30 % C, 6.57 % H.

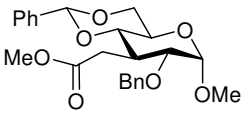
Synthesis of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-deoxy-3-(2-ethanoic acid)-α-glucopyranoside (83).



The title compound was prepared following the general procedure for aldehyde oxidation, starting from **82** (500 mg, 1.26 mmol), 2-methyl-2-butene (715 ml, 6.80 mmol), $NaClO_2$ (1.42 g, 12.10 mmol), and NaH_2PO_4 (1.38 g, 11.46 mmol) in *tert*-butanol/water (2/1) (45 ml). The reaction was stirred for 12h at r.t. and then treated as in the general procedure and purified by column chromatography (system A) affording **83** (406 mg, 78% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.45-7.25 (m, 10 H, Ar), 5.46 (s, 1 H, H-7), 4.63 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.58 (apparent singulet, 2 H, CH_2Ph), 4.23 (dd, 1 H, $J_{6eq,5}$ = 4.8 Hz, $J_{6eq,6ax}$ = 10.4 Hz, H-6eq), 3.79 (dt, 1 H, $J_{5,6eq}$ = 4.8 Hz, $J_{5,6ax}$ = $J_{5,4}$ = 10.4 Hz, H-5), 3.65 (t, 1 H, $J_{6ax,6eq}$ = $J_{6ax,5}$ = 10.4 Hz, H-6ax), 3.50 (dd, 1 H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 10.4 Hz, H-2), 3.43 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.4 Hz, H-4), 3.82 (s, 3 H, OCH_3), 2.43-2.66 (m, 1 H, H-3), 2.65-2.55 (m, 2 H, H-8, H-8') ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 178.7 (C=O), 137.7, 137.4 (C Ar), 129.0-126.2 (CH Ar), 101.6 (C-7), 97.4 (C-1), 79.1 (C-4), 77.6 (C-2), 72.6 (CH_2Ph), 69.4 (C-6), 63.8 (C-5), 55.3 (OCH_3), 38.1 (C-3), 32.0 (C-8) ppm.

Synthesis of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-deoxy-3-(2-methyl-ethanoate)-α-glucopyranoside (84).

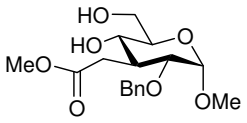


The title compound was prepared following the general procedure for esterification, starting from **83** (406 mg, 0.98 mmol), Cs_2CO_3 (638 mg, 1.96 mmol) and methyl iodide (110 µl, 1.76 mmol) in dry DMF (1.5 ml). The reaction was stirred for 2h at r.t. and then treated as in the general procedure affording **84** (420 mg, 100% yield).

ISBN:978-84-691-9757-8/DL:T-1256-2008

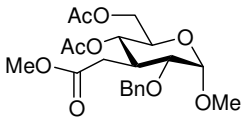
^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.46-7.31 (m, 10 H, Ar), 5.46 (s, 1 H, H-7), 4.63 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.59 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.55 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.23 (dd, 1 H, $J_{6eq,5}$ = 4.8 Hz, $J_{6eq,6ax}$ = 10.4 Hz, H-6eq), 3.79 (dt, 1 H, $J_{5,6eq}$ = 4.8 Hz, $J_{5,6ax}$ = $J_{5,4}$ = 10.4 Hz, H-5), 3.65 (t, 1 H, $J_{6ax,6eq}$ = $J_{6ax,5}$ = 10.4 Hz, H-6ax), 3.53-3.48 (m, 4 H, H-2, OCH_3), 3.42 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.4 Hz, H-4), 3.39 (s, 3 H, OCH_3), 2.74-2.66 (m, 1 H, H-3), 2.63-2.52 (m, 2 H, H-8, H-8') ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.9 (C=O), 137.8, 137.4 (C Ar), 129.0-126.2 (CH Ar), 101.6 (C-7), 97.4 (C-1), 79.5 (C-4), 77.7 (C-2), 72.6 (CH_2Ph), 69.3 (C-6), 63.7 (C-5), 55.3 (OCH_3), 51.5 (OCH_3), 38.3 (C-3), 32.3 (C-8) ppm. Anal. Calcd. for $C_{24}H_{28}O_7$, C 67.28 %; H 6.59 %; found 67.28 % C, 6.61 % H.

Synthesis of methyl 2-*O*-benzyl-3-deoxy-3-(2-methyl-ethanoate)-α-glucopyranoside.



The title compound was prepared following the general procedure for 4,6-*O*-benzylidene acetal hydrolysis, starting from **84** (555 mg, 1.30 mmol) and I_2 (132 mg, 0.518 mmol) in dry methanol (16ml). The reaction was refluxed for 4h and then treated as in the general procedure. The reaction crude was used without further purification in the next step.

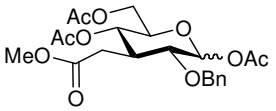
Synthesis of methyl 4,6-di-*O*-acetyl-2-*O*-benzyl-3-deoxy-3-(2-methyl-etanoate)-α-glucopyranoside (85).



The title compound was prepared following the general procedure for acetylation, starting from methyl 2-*O*-benzyl- 3-deoxy- 3-methyl propanoate-α-glucopyranoside (1.30 mmol) in dry pyridine (1.70 ml), and acetic anhydride (990 ml, 10.40 mmol). The reaction was stirred overnight at r.t. and then treated as in the general procedure and purified by column chromatography (system A), affording **85** (525 mg, 95% yield over two steps).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.35-7.32 (m, 5 H, Ar), 4.84 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.0 Hz, H-4), 4.64 (d, 1 H, $J_{1,2}$ = 3.3 Hz, H-1), 4.58 (apparent singulet, 2 H, CH_2Ph), 4.20 (dd, 1 H, $J_{6,5}$ = 5.4 Hz, $J_{6,6'}$ = 12.3 Hz, H-6), 3.99 (dd, 1 H, $J_{6',5}$ = 2.4 Hz, $J_{6',6}$ = 12.3 Hz, H-6'), 3.89-3.83 (m, 1 H, H-5), 3.58 (s, 3 H, OCH_3), 3.52 (dd, 1 H, $J_{2,1}$ = 3.3 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.38 (s, 3 H, OCH_3), 2.70-2.63 (m, 1 H, H-3), 2.50-2.34 (m, 2 H, H-7, H-7'), 2.06, 2.03 (s, 6 H, 2 CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.3, 170.6, 169.9 (C=O), 137.6 (C Ar), 128.4-128.0 (CH Ar), 96.5 (C-1), 76.9 (C-2), 72.6 (CH_2Ph), 68.9 (C-4), 68.1 (C-5), 62.5 (C-6), 55.1, 51.4 (OCH_3), 38.4 (C-3), 32.7 (C-7), 20.7, 20.6 (CH_3) ppm.

Synthesis of 1,4,6-tri-*O*-acetyl-2-*O*-benzyl-3-deoxy-3-(2-methyl-etanoate)-α,β-glucopyranose (79).



The title compound was prepared following the general procedure for anomeric-*O*-methyl hydrolysis and acetylation, starting from **85** (525 mg, 1.24 mmol), and a mixture of acetic anhydride/acetic acid/sulphuric acid (50/25/1) (24 ml). The reaction was stirred at 0ºC for 3 h and then treated as in the general procedure and purified by column chromatography (system A) affording **79** as a mixture of anomers (480 mg, 86% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.38-7.28 (m, 5 H, Ar), 6.39 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.92 (t, 1 H, $J_{4,3}$ = $J_{4,5}$ = 10.8 Hz, H-4), 4.66 (d, 1 H, J_{AB} = 11.2 Hz, CH_2Ph), 4.44 (d, 1 H, J_{AB} = 11.2 Hz, CH_2Ph), 4.22 (dd, 1 H, $J_{6,5}$ = 4.6 Hz, $J_{6,6'}$ = 16.6 Hz, H-

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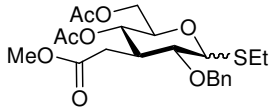
6), 4.01-3.96 (m, 2 H, H-5, H-6'), 3.62 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.8 Hz, H-2), 3.57 (s, 3 H, OCH_3), 2.64 (dddd, 1 H, $J_{3,2} = J_{3,4}$ = 10.8 Hz, $J_{3,7} = J_{3,7'}$ = 5.2 Hz, H-3), 2.48 (dd, 1 H, $J_{7,3}$ = 5.2 Hz, $J_{7,7'}$ = 15.6 Hz, H-7), 2.36 (dd, 1 H, $J_{7',3}$ = 5.2 Hz, $J_{7',7}$ = 15.6 Hz, H-7'), 2.18 (s, 3 H, CH_3), 2.07 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.4, 170.9, 170.0, 169.5 (C=O), 137.1 (C Ar), 128.7-128.0 (CH Ar), 88.3 (C-1), 75.7 (C-2), 72.8 (CH_2Ph), 70.7 (C-5), 68.5 (C-4), 62.3 (C-6), 51.8 (OCH_3), 38.6 (C-3), 32.7 (C-7), 21.3, 21.0, 20.8 (CH_3) ppm. Anal. Calcd. for $C_{22}H_{28}O_{10}$, C 58.40 %; H 6.24 %; found 58.38 % C, 6.21 % H.

Synthesis of ethyl 4,6-*O*-acetyl-2-*O*-benzyl-3-deoxy-3-(2-methyl-etanoate)-1-*thio*-α,β-glucopyranoside (86).

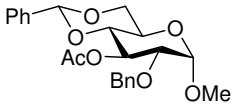


The title compound was prepared following the general procedure for thioglycoside formation, starting from **79** (80 mg, 0.18 mmol), $BF_3{\cdot}Et_2O$ (54µl, 0.45 mmol), and ethanethiol (15µl, 0.20 mmol) in dry DCM (9ml). The reaction was stirred at r.t. for 15 h and then treated as in the general procedure and purified by column chromatography (system A) affording **86** (65 mg, 80% yield, α:β 7:1).

Data for α: ^1H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.36-7.34 (m, 5 H, Ar), 5.45 (d, 1 H, $J_{1,2}$ = 5.4 Hz, H-1), 4.84 (t, 1 H, $J_{4,3} = J_{4,5}$ = 10.5 Hz, H-4), 4.67 (d, 1 H, J_{AB} = 11.4 Hz, CH_2Ph), 4.45 (d, 1 H, J_{AB} = 11.4 Hz, CH_2Ph), 4.35-4.30 (m, 2 H, H-5, H-6), 3.98 (dd, 1 H, $J_{6',5}$ = 1.8 Hz, $J_{6',6}$ = 11.7 Hz, H-6'), 3.73 (dd, 1 H, $J_{2,1}$ = 5.4 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 3.53 (s, 3 H, OCH_3), 2.64-2.27 (m, 5 H, H-3, H-7, H-7', SCH_2), 2.07 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 1.32-1.26 (m, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.0, 170.9, 170.5 (C=O), 137.5 (C Ar), 128.6-128.0 (CH Ar), 83.2 (C-1), 76.4 (C-2), 72.4 (CH_2Ph), 71.9 (C-4), 69.4 (C-5), 63.9 (C-6), 51.7 (OCH_3), 40.2 (C-3), 33.2 (C-7), 25.9 (SCH_2), 21.0, 20.8, 20.7 (CH_3), 15.1 (CH_3) ppm. Anal. Calcd. for $C_{22}H_{30}O_8S$, C 58.13 %, H 6.65 %, S 7.05; found 58.18 % C, 6.66 % H, 7.10 % S.

5.2.2. Synthesis of 3,6-di-*O*-acetyl-2-*O*-benzyl-4-deoxy-4-(2-methyl-etanoate) glucoside fragment.

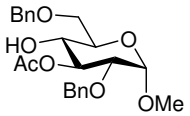
Synthesis of methyl 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-glucopyranoside (91).



The title compound was prepared following the general procedure for acetylation, starting from **80** (110 mg, 0.30 mmol) in dry pyridine (0.5 ml), and acetic anhydride (112 µl, 1.20 mmol). The solution was stirred overnight at r.t. and then treated as in the general procedure. No further purification was needed (123 mg, 100%).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.37-7.23 (m, 10 H, Ar), 5.56 (t, 1 H, $J_{3,2} = J_{3,4}$ = 10.0 Hz, H-3), 5.46 (s, 1 H, H-7), 4.69 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.66 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.63 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.27 (dd, 1 H, $J_{6eq,5}$ = 4.8 Hz, $J_{6eq,6ax}$ = 10.0 Hz, H-6eq), 3.89 (dt, 1 H, $J_{5,4} = J_{5,6ax}$ = 10.0 Hz, $J_{5,6eq}$ = 4.8 Hz, H-5), 3.70 (t, 1 H, $J_{6ax,5} = J_{6eq,6ax}$ = 10.0 Hz, H-6ax), 3.56 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.53 (t, 1 H, $J_{4,3} = J_{4,5}$ = 10.0 Hz, H-4), 3.40 (s, 3 H, OCH_3), 2.04 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 169.9 (C=O), 138.0, 137.2 (C Ar), 129.2-126.3 (CH Ar), 101.6 (C-7), 98.8 (C-1), 79.7 (C-4), 77.8 (C-2), 73.2 (CH_2Ph), 70.6 (C-3), 69.1 (C-6), 62.5 (C-5), 55.5 (OCH_3), 21.2 (CH_3) ppm. Anal. Calcd. for $C_{23}H_{26}O_7$, 66.65 % C, 6.32 % H, found 66.70 % C, 6.35 % H.

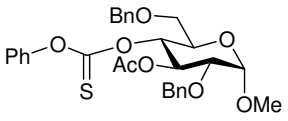
Synthesis of methyl 3-*O*-acetyl-2,6-di-*O*-benzyl-α-glucopyranoside (92).



The title compound was prepared following the general procedure for selective benzylidene acetal opening, starting from **91** (103 mg, 0.25 mmol) in dry DCM (3 ml), Et_3SiH (129 µl, 0.80 mmol), triflic acid (65 µl, 0.75 mmol) and 4Å M.S. (80 mg). The reaction was stirred at –78ºC for 1h and then treated as in the general procedure and purified by column chromatography (hexane/ethyl acetate, 2/8), affording **92** (88 mg, 85% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.34-7.31 (m, 10 H, Ar), 5.24 (t, 1 H, $J_{3,2} = J_{3,4}$ = 9.6 Hz, H-3), 4.68 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.66 (d, 1 H, J_{AB} = 12.8 Hz, CH_2Ph), 4.61 (d, 1 H, J_{AB} = 12.8 Hz, CH_2Ph), 4.60 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.56 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 3.75-3.62 (m, 4 H, H-4, H-5, H-6, H-6'), 3.53 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.38 (s, 3 H, OCH_3), 2.90 (d, 1 H, $J_{OH,4}$ = 4.8 Hz, OH), 2.09 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.3 (C=O), 138.0 (C Ar), 128.6-127.8 (CH Ar), 98.0 (C-1), 76.7 (C-2), 75.6 (C-3), 73.7, 73.2 (CH_2Ph), 70.4 (C-4, C-5), 69.3 (C-6), 55.4 (OCH_3), 21.2 (CH_3) ppm. Anal. Calcd. for $C_{23}H_{28}O_7$, 66.33 % C, 6.78 % H, found 66.30 % C, 6.80 % H.

Synthesis of methyl 3-*O*-acetyl-2,6-di-*O*-benzyl-4-*O*-(phenylthionocarbonate)-α-glucopyranoside (93).



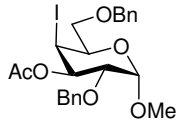
Method A: The title compound was prepared following the general procedure for tionocarbonate formation, starting from **92** (88 mg, 0.21 mmol) in dry toluene (3 ml), NHS (21 mg, 0.21 mmol) and dry pyridine (86 ml, 1.05 mmol). After 24h stirring the reaction mixture was treated as in the general procedure and purified by column chromatography (system A), affording **93** (48 mg, 42% yield), together with the acetate migration product **94** (24 mg, 21%).

Method B: A mixture of **92** (100 mg, 0.24 mmol) in dry toluene (5 ml), DMAP (30 mg, 0.21 mmol), and dry pyridine (98 ml, 1.20 mmol) were refluxed for 24h. After that time, the reaction mixture was treated as in the general procedure for

thionocarbonate formation, and purified by column chromatography (system A), affording **93** (84 mg, 63%).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.34-7.31 (m, 15 H, Ar), 5.24 (t, 1 H, $J_{3,2}$ = $J_{3,4}$ = 9.6 Hz, H-3), 4.68 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.66 (d, 1 H, J_{AB} = 12.8 Hz, CH_2Ph), 4.61 (d, 1 H, J_{AB} = 12.8 Hz, CH_2Ph), 4.60 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.56 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 3.75-3.62 (m, 4 H, H-4, H-5, H-6, H-6'), 3.53 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 3.38 (s, 3 H, OCH_3), 2.90 (d, 1 H, $J_{4,OH}$ = 4.8 Hz, OH), 2.09 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.3 (C=O), 138.0, 138.0 (C Ar), 128.6-127.8 (CH Ar), 98.0 (C-1), 76.7 (C-2), 75.6 (C-3), 73.7, 73.2 (CH_2Ph), 70.4 (C-4, C-5), 69.3 (C-6), 55.4 (OCH_3), 21.2 (CH_3) ppm. $[\alpha]^{20}_D$= +80.04. Anal. Calcd. for $C_{23}H_{28}O_7$, 66.33 % C, 6.78 % H, found 66.30 % C, 6.80 % H.

Synthesis of methyl 3-*O*-acetyl-2,6-di-*O*-benzyl-4-deoxy-4-iodo-α-glucopyranoside (95).



The title compound was prepared following the general procedure for iodide formation, starting from **92** (1.79 g, 4.31 mmol), triphenyl phosphine (4.52 g, 17.24 mmol), I_2 (3.28 g, 12.93 mmol), and imidazole (1.17 g, 17.24 mmol) in dry toluene (39 ml). The reaction mixture was stirred in refluxing toluene for 12h, and then treated as in the general procedure and purified by column chromatography (system A), affording **95** (1.67 g, 75% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.34-7.29 (m, 10 H, Ar), 4.79-4.78 (m, 1 H, H-4), 4.73 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.63 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.73 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.61 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.55 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.50 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.50 (dd, 1 H, $J_{3,2}$ = 10.0 Hz,

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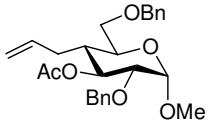
$J_{3,4}$ = 4.0 Hz, H-3), 3.90 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.62-3.57 (m, 1 H, H-6), 3.45-3.37 (m, 5 H, H-5, H-6', OCH_3), 2.09 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 170.0 (C=O), 138.1, 137.8 (C Ar), 128.5-127.7 (CH Ar), 98.7 (C-1), 76.2 (C-2), 74.1 (C-6), 73.7 (2 CH_2Ph), 70.5 (C-3), 66.8 (C-5), 55.4 (OCH_3), 39.0 (C-4), 21.2 (CH_3) ppm.

Synthesis of methyl 3-*O*-acetyl-4-deoxy-4-allyl-2,6-di-*O*-benzyl-α-glucopyranoside (96).



Method A

The title compound was prepared following the general procedure for radical allylation (Method A), starting from **95** (1.61 g, 3.05 mmol) in dry and deoxygenated toluene (6 ml), allyltributyltin (2.36 ml, 7.63 mmol), and AIBN (75 mg, 0.46 mmol). After 24h refluxing the reaction mixture was treated as in the general procedure and purified by column chromatography (system A), affording **96** (470 mg, 35% yield).

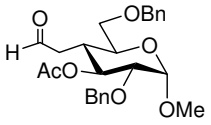
Method B

The title compound was prepared following the general procedure for radical allylation (Method B), starting from **95** (620 mg, 1.18 mmol), allyltriphenyltin (6.9 g, 17.55 mmol) and AIBN (37 mg, 0.36 mmol). After stirring at 70ºC for 18h the reaction mixture was treated as in the general procedure and purified by column chromatography (elution system hexane/ethyl acetate: 10/0, 9/1, 8/2, 7/3), affording **96** (313 mg, 60 %).

^1H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.35-7.28 (m, 10 H, Ar), 5.83-5.72 (m, 1 H, H-8), 5.36 (t, 1 H, $J_{3,2}$ = $J_{3,4}$ = 10.0 Hz, H-3), 4.95-4.84 (m, 2 H, H-9, H-9'), 4.75 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.65-4.45 (m, 10 H, 2 CH_2Ph), 3.71-3.67 (m, 1 H, H-5), 3.60-3.58 (m, 2 H, H-6, H-6'), 3.49 (dd, 1 H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.36 (s, 3 H, OCH_3), 2.01 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 170.0 (C=O), 138.0, 137.7 (C Ar), 134.2 (C-8), 128.4-127.4 (CH Ar), 116.7 (C-9), 98.0 (C-

1), 78.3 (C-2), 73.2, 72.3 (2 CH_2Ph), 70.9 (C-3), 69.4 (C-5), 68.7 (C-6), 55.0 (OCH_3), 40.1 (C-4), 31.1 (C-7), 20.9 (CH_3) ppm.

Synthesis of methyl 3-*O*-acetyl-2,6-di-*O*-benzyl-4-deoxy-4-(2-ethanal)-α-glucopyranoside (97).



The title compound was prepared following the general procedure for allyl oxidative cleavage, starting from **96** (620 mg, 1.41 mmol), OsO_4 (7 mg, 0.03 mmol), NMO (183 mg, 1.56 mmol) in dioxane/water (9/1) (8 ml) and treated as in the general procedure after stirring for 3h. Then the crude was redissolved in dioxane/water (9/1) (8 ml) and $NaIO_4$ (456 mg, 2.12 mmol) was added. The solution was stirred for 5 h at r.t. and then treated as in the general procedure. Purification on silica gel column (system A) afforded **97** (437 mg, 70% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 9.54 (t, 1 H, $J_{H,9}$ = 4.0 Hz, CHO), 7.34-7.26 (m, 10 H, Ar), 5.27 (t, 1 H, $J_{3,2} = J_{3,4}$ = 10.0 Hz, H-3), 4.74 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.59 (apparent singulet, 2 H, CH_2Ph), 4.52 (d, 2 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.39 (d, 2 H, J_{AB} = 12.0 Hz, CH_2Ph), 3.74 (dt, 1 H, $J_{5,4}$ = 10.0 Hz, $J_{5,6}$ = 3.2 Hz, H-5), 3.56-3.34 (m, H-2, 6 H, H-6, H-6', OCH_3), 2.54 (dt, 1 H, $J_{4,3} = J_{4,5}$ = 10.0 Hz, $J_{4,7}$ = 4.8 Hz, H-3), 2.34-2.22 (m, 2 H, H-7, H-7'), 1.98 (s, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 200.5 (CHO), 170.4 (C=O), 138.0, 137.6 (C Ar), 128.8-126.5 (CH Ar), 98.3 (C-1), 78.2 (C-2), 73.3, 72.7 (2 CH_2Ph), 72.2 (C-3), 69.8 (C-5), 69.6 (C-6), 55.4 (OCH_3), 42.1 (C-7), 37.2 (C-4), 21.0 (CH_3) ppm. Anal. Calcd. for $C_{25}H_{30}IO_7$, 67.86 % C, 6.83 % H, found 67.90 % C, 6.80 % H.

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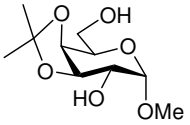
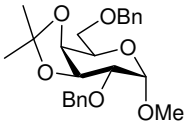
Synthesis of methyl 3,4-*O*-isopropilidene-α-galactopyranoside (87).



methyl-α-D-galactopyranose (2g, 9.42 mmol), was dissolved in dry DMF (28 ml). Methoxypropene (1.8 ml, 18.84 mmol) and *p*-toluenesulfonic acid (179 mg, 0.09 mmol) were added to the solution and stirred at r.t. After 24h stirring, TLC (CH_2Cl_2/MeOH, 9/1) showed that the starting material was consumed. The reaction was quenched with IRA402, and the mixture was filtered and extracted with H_2O/CH_2Cl_2. The organic layer was dried and the solvent evaporated. After purification on silica gel column, **87** was afforded (2.11 g, 95 %).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 4.79 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.26-4.23 (m, *2* H, H-3, H-4), 4.07-4.04 (m, 1 H, H-5), 3.96-3.78 (m, 3 H, H-2, H-6, H-6'), 3.46 (3 H, OCH_3), 2.74 (d, 1 H, *J* = 6.8 Hz, OH), 2.53 (d, 1 H, *J* = 6.0 Hz, OH), 1.51, 1.35 (s, 6 H, 2 CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 110.0 (C_{ketal}), 98.9 (C-1), 76.5 (C-3), 74.1 (C-4), 69.8 (C-2), 68.1 (C-6), 62.8 (C-5), 55.7 (OCH_3), 27.9, 26.1 (2 CH_3) ppm. Anal. Calcd. for $C_{10}H_{18}O_6$, 51.27 % C, 7.75 % H, found 51.30 % C, 7.80 % H

Synthesis of methyl 2,6-di-*O*-benzyl-3,4-*O*-isopropilidene-α-galactopyranoside (88).

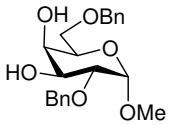


The title compound was prepared following the general procedure for benzylation, starting from **87** (2.11 g, 9.02 mmol) in dry DMF (63.4 ml), NaH (1.20 g, 29.77 mmol), benzyl bromide (3.97 ml, 33.37 mmol) and TBAI (43 mg, 0.09 mmol). The reaction was stirred overnight and then the reaction mixture was treated as in the

general procedure and purified by column chromatography (system A), affording **88** (3.18 g, 85% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.38-7.26 (m, 10 H, Ar), 4.82 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.71 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.67(d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.64 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.53 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.34 (dd, 1 H, $J_{3,2}$ = 7.8 Hz, $J_{3,4}$ = 5.2 Hz, H-3), 4.19-4.13 (m, 2 H, H-4, H-5), 3.76-3.68 (m, 2 H, H-6, H-6'), 3.51 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 7.8 Hz, H-2), 3.39 (s, 3 H, OCH_3), 1.37, 1.32 (s, 6 H, 2 CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 138.3, 138.2 (C Ar), 128.5-127.7 (CH Ar), 109.2 (C_{ketal}), 98.3 (C-1), 76.3 (C-3), 76.2 (C-4), 73.8, 72.4 (CH_2Ph), 73.5 (C-2), 69.6 (C-6), 66.5 (C-5), 55.6 (OCH_3), 28.2, 26.5 (2 CH_3) ppm. Anal. Calcd. for $C_{24}H_{30}O_6$, 69.54 % C, 7.30 % H, found 69.50 % C, 7.35 % H

Synthesis of methyl 2,6-di-*O*-benzyl-α-galactopyranoside (89).



Compound **88** (2.97 g, 7.16 mmol) was dissolved in MeOH (10 ml), and DOWEX-H$^{\oplus}$ (0.5 g) was added. After 6h stirring, the solution was filtered, concentrated and purified on a silica gel column (elution system: hexane/ethyl acetate (6/4)) **89** was afforded (2.08 g, 78 % yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.37-7.28 (m, 10 H, Ar), 4.70 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.69 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.65 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.59 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.56 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.05-4.03 (m, 1 H, H-3), 3.98-3.94 (m, 1 H, H-5), 3.92-3.89 (m, 1 H, H-4), 3.76-3.68 (m, 3 H, H-2, H-6, H-6'), 3.35 (s, 3 H, OCH_3), 2.95 (d, 1 H, J = 1.6 Hz, OH), 2.66 (d, 1 H, J = 4.0 Hz, OH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 138.2, 137.9 (C Ar), 128.7-127.9 (CH Ar), 98.1 (C-1), 76.8 (C-2), 73.8, 73.2 (CH_2Ph), 70.1 (C-3), 70.0 (C-6),

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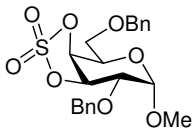
69.5 (C-5), 68.5 (C-4), 55.5 (OCH_3) ppm. Anal. Calcd. for $C_{21}H_{26}O_6$, 67.36 % C, 7.00 % H, found 67.40 % C, 7.05 % H

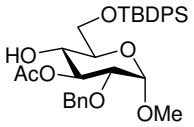
Synthesis of methyl 2,6-di-*O*-benzyl-3,4-*O*-sulphuryl-α-galactopyranoside (90).



Compound **89** (1.28 g, 3.42 mmol) was dissolved in dry CCl_4 (12 ml). Then $SOCl_2$ (297 µl, 4.10 mmol) was added. After 1h refluxing, the reaction flask was placed in a water/ice bath and acetonitrile (14 ml), $NaIO_4$ (1.09 g, 5.13 mmol), $RuCl_3$ (467 mg, 2.25 mmol) and finally water (20 ml) were added. The mixture was stirred for further 2h at r.t. Once the reaction was finished the mixture was diluted with Et_2O and washed with water, a saturated solution of $NaHCO_3$ (x2), and brine. The organic layer was filtered over a short silica path and concentrated. No further purification was needed (1.30 g, 91 % yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.37-7.30 (m, 10 H, Ar), 5.17 (dd, 1 H, $J_{4,3}$ = 5.0 Hz, $J_{4,5}$ = 2.0 Hz, H-4), 5.06 (dd, 1 H, $J_{3,4}$ = 5.0 Hz, $J_{3,2}$ = 8.6 Hz, H-3), 4.83 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.63 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.63(d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.58 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.53 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.14 (dt, 1 H, $J_{5,4}$ = 2.0 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 7.0 Hz, H-5), 4.03 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 8.6 Hz, H-2), 3.66-3.65 (m, 2 H, H-6, H-6'), 3.36 (s, 3 H, OCH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 137.4, 137.1 (C Ar), 128.8-128.0 (CH Ar), 98.2 (C-1), 84.1 (C-3), 80.5 (C-4), 74.3 (CH_2Ph), 74.0 (C-2, CH_2Ph), 67.8 (C-6), 65.3 (C-5), 56.2 (OCH_3) ppm. Anal. Calcd. for $C_{21}H_{24}O_8S$, 57.79 % C, 5.54 % H, 7.35 % S found 57.80 % C, 5.55 % H, 7.40 % S.

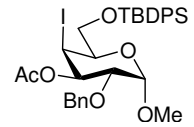
Synthesis of methyl 3-*O*-acetyl-2-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-glucopyranoside (99).



The title compound was prepared following the general procedure for silylation, starting from methyl 3-*O*-acetyl-2-*O*-benzyl-α-glucopyranoside **98** (4.00 g, 12.26 mmol) in dry DCM (8 ml), DMAP (150 mg, 1.23 mmol), dry triethylamine (10.25 ml, 73.56 mmol), and *tert*-butyldiphenylsilyl chloride (4.71 ml, 18.39 mmol). After 5h stirring the reaction was treated as in the general procedure and purified by column chromatography (1/1 hexane/ethyl acetate), affording **99** (4.89 g, 73 % yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.70-7.33 (m, 15 H, Ar), 5.28 (t, 1 H, $J_{3,2} = J_{3,4}$ = 9.6 Hz, H-3), 4.67 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.64 (apparent singulet, 2 H, CH_2Ph), 3.90-3.83 (m, 2 H, H-6, H-6'), 3.71-3.66 (m, 1 H, H-5), 3.59 (dt, 1 H, $J_{4,3} = J_{4,5}$ = 9.6 Hz, $J_{4,OH}$ = 4.4 Hz, H-4), 3.48 (dd, 1 H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 3.35 (s, 3 H, OCH_3), 2.81 (d, 1 H, $J_{OH,4}$ = 4.4 Hz, OH), 2.09 (s, 3 H, CH_3), 1.05 (s, 9H, *t*Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.1 (C=O), 138.2, 133.2 (C Ar), 135.8-127.9 (CH Ar), 97.8 (C-1), 77.1 (C-2), 75.4 (C-3), 73.2 (CH_2Ph), 71.4 (C-5), 71.2 (C-4), 64.4 (C-6), 55.2 (OCH_3), 27.0 (CH_3 *t*Bu), 21.3 (CH_3), 19.4 (C_{tBu}) ppm.

Synthesis of methyl 3-*O*-acetyl-2-*O*-benzyl-4-yodo-6-*O*-*tert*-butyldiphenylsilyl-4-deoxy-α-glucopyranoside (100).

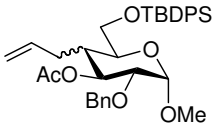


The title compound was prepared following the general procedure for iodide formation, starting from **99** (2.54 g, 4.50 mmol), triphenyl phosphine (4.75 g, 22.50 mmol), I_2 (3.45 g, 13.50 mmol), and imidazole (1.30 g, 22.50 mmol) in dry toluene

(40 ml). The reaction mixture was stirred in refluxing toluene for 12h, and then treated as in the general procedure and purified by column chromatography (system A), affording **100** (2.65 g, 87% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.66-7.34 (m, 15 H, Ar), 4.80-4.79 (m, 1 H, H-4), 4.73 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.61 (d, 1 H, J_{AB} = 12.0 Hz, CH_2Ph), 4.59 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.49 (dd, 1 H, $J_{3,2}$ = 10.0, $J_{3,4}$ = 4.0 Hz, H-3), 3.89 (dd, 1 H, $J_{2,1}$ = 3.6, $J_{2,3}$ = 10.0 Hz, H-2), 3.73 (dd, 1 H, $J_{6,5}$ = 6.4 Hz, $J_{6,6'}$ = 10.8 Hz, H-6), 3.57 (dd, 1 H, $J_{6',5}$ = 6.4, $J_{6',6}$ = 10.8 Hz, H-6'), 3.32 (s, 3 H, OCH_3), 3.25-3.22 (m, 1 H, H-5), 2.12 (s, 3 H, CH_3), 1.04 (s, 9H, *t*Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 170.2 (C=O), 138.2, 133.1 (C Ar), 135.8-128.0 (CH Ar), 98.9 (C-1), 76.4 (C-2), 73.8 (CH_2Ph), 70.8 (C-3), 68.4 (C-5), 67.9 (C-6), 55.4 (OCH_3), 38.8 (C-4), 27.0 (CH_3, *t*Bu), 21.4 (CH_3), 19.3 (C_{tBu}) ppm.

Synthesis of methyl 3-*O*-acetyl-4-deoxy-4-allyl-2-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-glucopyranoside (101).

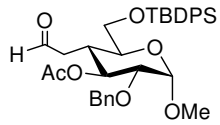


The title compound was prepared following the general procedure for radical allylation (Method B), starting from **100** (2.64 mg, 3.92 mmol), allyltriphenyltin (17.4 g, 58.80 mmol) and AIBN (130 mg, 0.78 mmol). After stirring at 70ºC for 18h the reaction mixture was treated as in the general procedure and purified by column chromatography (elution system hexane/ethyl acetate: 10/0, 9/1, 8/2, 7/3), affording **101** (2.26 mg equatorial:axial mixture 7.5:1, 98 %).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.70-7.33 (m, 15 H, Ar), 5.78-5.67 (m, 1 H, H-8), 5.37 (t, 1 H, $J_{3,2}$ = $J_{3,4}$ = 6.0 Hz, H-3), 4.90-4.81 (m, 2 H, H-9, H-9'), 4.76 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.64 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.60 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 3.83 (dd, 1 H, $J_{6,5}$ = 4.8 Hz, $J_{6,6'}$ = 10.8 Hz, H-6), 3.73 (dd, 1 H, $J_{6',5}$ = 6.4 Hz, $J_{6',6}$ = 10.8 Hz, H-6'), 3.63-3.59 (m, 1 H, 5H), 3.46 (dd, 1 H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 6.0 Hz, H-2), 3.35 (s, 3 H, OCH_3), 2.04-1.98 (m, 6 H, H-4, H-7, H-7', CH_3), 1.06 (s,

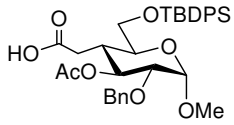
9H, *t*Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 170.4 (C=O), 138.5, 133.6, 133.5 (C Ar), 135.9-127.8 (CH Ar), 134.7 (C-8), 177.0 (C-9), 98.0 (C-1), 79.1 (C-2), 73.1 (CH_2Ph), 71.5 (C-3), 71.1 (C-5), 63.9 (C-6), 55.1 (OCH_3), 40.8 (C-4), 31.5 (C-7), 27.0 (CH_3 *t*Bu), 21.3 (CH_3), 19.5 (C_{tBu}) ppm.

Synthesis of methyl 3-*O*-acetyl-2-*O*-benzyl-4-deoxy-4-(2-ethanal)-6-*O*-*tert*-butyldiphenylsilyl-α-glucopyranoside (102).



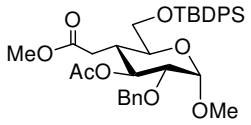
The title compound was prepared following the general procedure for allyl oxidative cleavage, starting from **101** (2.36 g, 4.01 mmol), OsO_4 (20 mg, 0.08 mmol), NMO (520 mg, 4.41 mmol) in dioxane/water (9/1) (22 ml) and stirred for 3h at r.t. The was treated as in the general procedure. The crude was redissolved in dioxane/water (9/1) (22 ml) and $NaIO_4$ (1.30 g, 6.02 mmol) was added. The reaction was stirred for 5h at r.t. and then treated as in the general procedure and purified by column chromatography (system A) affording **102** (1.75 g, 74% yield), and used directly in the next step.

Synthesis of methyl 3-*O*-acetyl-2-*O*-benzyl-4-deoxy-4-(2-methyl-etanoate)-6-*O*-*tert*-butyldiphenylsilyl-α-glucopyranoside.



The title compound was prepared following the general procedure for aldehyde oxidation, starting from **102** (1.56 g, 2.63 mmol), 2-methyl 2-butene (1.58 ml, 14.20 mmol), $NaClO_2$ (2.86 g, 25.25 mmol), and NaH_2PO_4 (2.87 g, 23.93 mmol) in *tert*-butanol/water (2/1) (90 ml). The reaction was stirred for 12h at r.t. and then treated as in the general procedure. The crude was used without further purification in the next step.

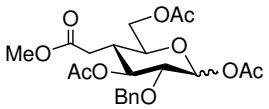
Synthesis of methyl 3-*O*-acetyl-2-*O*-benzyl-4-deoxy-4-(2-methyl-etanoate)-6-*O*-*tert*-butyldiphenylsilyl-α-glucopyranoside (103).



The title compound was prepared following the general procedure for methyl ester formation, starting from reaction crude of aldehyde **102** formation (2.63 mmol), Cs_2CO_3 (1.71 mg, 5.26 mmol) and methyl iodide (295 µl, 4.73 mmol) in dry DMF (3.8 ml). The reaction was stirred for 2h at r.t. and then treated as in the general procedure affording **103** (1.61 g, 99% yield over to steps).

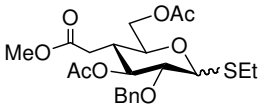
^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.68-7.29 (m, 15 H, Ar), 5.31 (t, 1 H, $J_{3,2} = J_{3,4}$ = 10.0 Hz, H-3), 4.72 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.60 (apparent singulet, 2 H, CH_2Ph), 3.75-3.68 (m, 3 H, H-6, H-6', H-5), 3.47 (dd, 1 H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.35 (s, 3 H, OCH_3), 3.33 (s, 3 H, OCH_3), 2.38-2.20 (m, 3 H, H-4, H-7, H-7'), 2.00 (s, 3 H, CH_3), 1.05 (s, 9H, *t*Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.3, 170.5 (C=O), 138.3, 133.4, 133.3 (C Ar), 135.8-127.8 (CH Ar), 98.0 (C-1), 78.7 (C-2), 72.7 (CH_2Ph), 72.4 (C-3), 71.4 (C-5), 64.7 (C-6), 55.1, 51.8 (OCH_3), 39.6 (C-4), 32.5 (C-7), 26.9 (CH_3 *t*Bu), 21.1 (CH_3), 19.3 ($C_{t\text{Bu}}$) ppm.

Synthesis of 1,3,6-tri-*O*-acetyl-2-*O*-benzyl-4-deoxy-3-(2-methyl-ethanoate)-1-α,β-*O*-glucopyranose (104).



The title compound was prepared following the general procedure for 1-O-methyl hydrolysis and acetylation, starting from **103** (1.72 g, 2.77 mmol), and a mixture of acetic anhydride/acetic acid/sulphuric acid (50/25/1, 55 ml). The reaction was stirred at 0ºC for 3 h and then treated as in the general procedure and used without further purification in the next step.

Synthesis of ethyl 3,6-di-*O*-acetyl-2-*O*-benzyl-4-deoxy-4-(2-methyl-ethanoate)-1-*thio*-α,β-glucopyranoside (105).



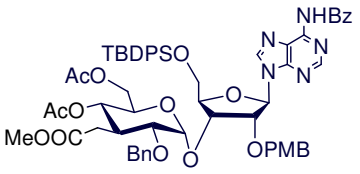
The title compound was prepared following the general procedure for thioglycoside formation, starting from **104** (2.77 mmol), $BF_3{\cdot}Et_2O$ (693 µl, 6.93 mmol), and ethanethiol (225µl, 3.05 mmol) in dry DCM (140 ml). The reaction was stirred at r.t. for 15 h and then treated as in the general procedure and purified by column chromatography (system A) affording **105** (907 mg, 72% yield over two steps, α:β 3.3:1).

Data for α anomer: ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 7.33-7.30 (m, 5 H, Ar), 5.46 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 5.20 (t, 1 H, $J_{3,2}$ = $J_{3,4}$ = 9.6 Hz, H-3), 4.67 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.52 (d, 1 H, J_{AB} = 12.4 Hz, CH_2Ph), 4.37-4.33 (m, 1 H, H-5), 4.29 (dd, 1 H, $J_{6',5}$ = 4.8 Hz, $J_{6,6'}$ = 12.0 Hz, H-6), 4.09 (dd, 1 H, $J_{6',5}$ = 2.4 Hz, $J_{6',6}$ = 12.0 Hz, H-6'), 3.74 (dd, 1 H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 3.66 (s, 3 H, OCH_3), 2.60-2.46 (m, 2 H, SCH_2), 2.34-2.26 (m, 3 H, H-4, H-7, H-7'), 2.08 (s, 3 H, CH_3), 2.02 (s, 3 H, CH_3), 1.32-1.26 (m, 3 H, CH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.1, 170.9, 170.4 (C=O), 137.8 (C Ar), 128.6-127.8 (CH Ar), 83.2 (C-1), 77.3 (C-2), 72.4 (C-3), 71.9 (CH_2Ph), 69.4 (C-5), 64.0 (C-6), 52.1 (OCH_3), 39.0 (C-4), 32.6 (C-7), 23.9 (SCH_2), 21.1, 21.0, 20.9 (CH_3), 14.8 (CH_3) ppm.

ISBN:978-84-691-9757-8/DL:T-1256-2008

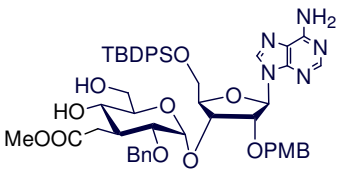
5.2.3. Synthesis of 3''-deoxy-3''-(2-methyl-ethanoate) and 4''-deoxy-4''(2-methyl-etanoate) Adenophostin A analogues.

Synthesis of 3'-*O*-(4'',6''-di-*O*-acetyl-2''-*O*-benzyl-3''-deoxy-3''-(2-methyl-ethanoate)-α-D-glucopiranosyl)-N^6-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine



The title compound was prepared following the general procedure for glycosylation with thioglycosides. Thus, starting from **38** (353 mg, 0.48 mmol), and **86** (100 mg, 0.22 mmol), 50 mg of 4 Å M.S., 0.55 ml of Et_2O, 0.55 ml of DCM, NIS (65 mg, 0.26 mmol) and TfOH (2.5 µl, 0.03 mmol) were introduced into the flask and the solution was stirred for 48h at r.t. Et_3N (35 µl) was added to stop the reaction, and then treated as in the general procedure an purified by column chromatography affording glycosylation product together with unreacted **38**. The mixture obtained was used in the next reaction without further purification.

Synthesis of 3'-*O*-(2''-*O*-benzyl-3''-deoxy-3''-(2-methyl-ethanoate)-α-D-glucopiranosyl)-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine (106a).

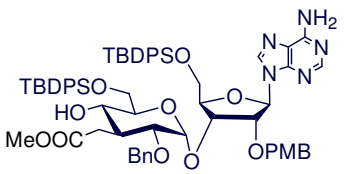


The title compound was prepared following the general procedure for ester/amide hydrolysis, starting from the crude obtained in the glycosylation reaction dissolved in

dry methanol (10 ml), sodium methoxide (20 mg) was added to the solution. The reaction mixture was stirred at r.t. for 4 h and then treated as in the general procedure and purified by column chromatography (elution system DCM/MeOH, 10/0, 9.5/0.5, 9/1) affording **106a** (132 mg, 58 % yield over two steps).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 8.20 (s, 1 H, H-2), 7.85 (s, 1 H, H-8), 7.68-7.23 (m, 15 H, Ar), 7.42 (d, 2 H, *J* = 8.8 Hz, Ar), 6.61 (d, 2 H, *J* = 8.8 Hz, Ar), 6.17 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, H-1'), 5.78 (s, 2 H, NH_2), 5.39 (d, 1 H, $J_{1'',2''}$ = 3.2 Hz, H-1''), 4.88 (dd, 1 H, $J_{2',1'}$ = 6.0, $J_{2',3'}$ = 4.8 Hz, H-2'), 4.67 (d, 1 H, J_{AB} = 11.2 Hz, CH_2Ph), 4.64-4.62 (m, 1 H, H-3'), 4.49 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.29-4.36 (m, 2 H, CH_2Ph, H-4'), 4.29 (d, 1 H, J_{AB} = 11.2 Hz, CH_2Ph), 4.06 (dd, 1 H, $J_{5'a,5'b}$ = 11.6 Hz, $J_{5'a,4'}$ = 4.4 Hz, H-5'a), 3.83-3.47 (m, 10 H, 2xOCH_3, H-5'b, H-4', H-6a'', H-6b'', H-4''), 3.28 (dd, 1 H, $J_{2'',1''}$ = 3.2 Hz, $J_{2'',3''}$ = 10.8 Hz, H-2''), 2.88-2.84 (m, 1 H, H-7a''), 2.52-2.43 (m, 2 H, H-3'', H-7b''), 1.08 (s, 9 H, *t*-Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.9 (C=O), 159.7 (C-5), 156.1 (C-6), 152.1 (C-2), 150.3 (C-4), 140.3 (C-8), 135.8-128.1 (Ar), 113.8 (Ar), 93.2 (C-1''), 87.1 (C-1'), 85.2 (C-4'), 80.0 (C-2'), 76.4 (C-2''), 75.1 (C-3'), 72.9 (CH_2Ph), 72.4 (CH_2Ph), 72.1 (C-4''), 70.2 (C-5''), 63.5 (C-5'), 62.6 (C-6''), 55.5 (OCH_3), 52.2 (OCH_3), 39.7 (C-3''), 33.9 (C-7''), 29.2 (CH_3), 21.4 (C_{tBu}) ppm.

Synthesis of 3'-*O*-(2''-*O*-benzyl-3''-deoxy-3''-(2-methyl-ethanoate)-6''-*O*-*tert*-butyldiphenylsilyl-α-D-glucopiranosyl)-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine (107a).



The title compound was prepared following the general procedure for silylation, starting from **106a** (98 mg, 0.05 mmol) in dry DCM (1ml), DMAP (25 mg, 0.01 mmol), dry triethylamine (100 µl, 0.05 mmol), and *tert*-butyldiphenylsilyl chloride (95 µl, 0.05 mmol). After 15h stirring the reaction was treated as in the general

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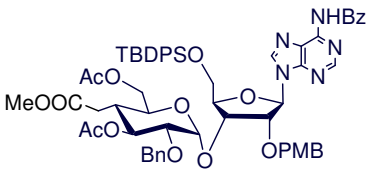
procedure and purified by column chromatography (system B), affording **107a** (75 mg, 67 % yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 8.18 (s, 1 H, H-2), 7.79 (s, 1 H, H-8), 7.68-7.14 (m, 25 H, Ar), 6.37 (d, 2 H, *J* = 8.4 Hz, Ar), 6.61 (d, 2 H, *J* = 8.4 Hz, Ar), 6.14 (d, 1 H, $J_{1',2'}$ = 6.4 Hz, H-1'), 5.72 (s, 2 H, NH_2), 5.32 (d, 1 H, $J_{1'',2''}$ = 3.2 Hz, H-1''), 4.81 (dd, 1 H, $J_{2',1'}$ = 6.4, $J_{2',3'}$ = 4.8 Hz, H-2'), 4.66 (d, 1 H, J_{AB} = 10.8 Hz, CH_2Ph), 4.54-4.49 (m, 2 H, CH_2Ph, H-3'), 4.35 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.34 (d, 1 H, J_{AB} = 10.8 Hz, CH_2Ph), 4.27-4.25 (m, 1 H, H-4'), 3.91 (dd, 1 H, $J_{5'a,5'b}$= 11.6 Hz, $J_{5'a,4}$ = 4.4 Hz, H-5'a), 3.85-3.53 (m, 10 H, 2xOCH_3, H-5'b, H-6''a, H-6''b, H-4''), 3.37 (dd, 1 H, $J_{2'',1''}$ = 3.2 Hz, $J_{2'',3''}$ = 11.2 Hz, H-2''), 2.73 (dd, 1 H, $J_{7''a,3''}$ = 4.8 Hz, $J_{7''a,7''b}$ = 15.6 Hz, H-7''a), 2.63-2.50 (m, 2 H, H-3'', H-7''b), 1.05 (s, 9 H, *t*-Bu), 1.04 (s, 9 H, *t*-Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.9 (C=O), 159.7 (C-5), 156.4 (C-6), 152.9 (C-2), 149.8 (C-4), 140.5 (C-8), 135.8-127.7 (Ar), 113.8 (Ar), 94.9 (C-1''), 86.7 (C-1'), 84.0 (C-4'), 80.0 (C-2'), 76.9 (C-2''), 73.1 (C-3'), 72.6 (CH_2Ph), 74.3 (C-4''), 71.6 (CH_2Ph), 70.5 (C-5''), 64.7 (C-6''), 63.6 (C-5'), 55.3 (OCH_3), 51.8 (OCH_3), 39.5 (C-3''), 33.4 (C-7''), 29.9 (CH_3), 27.1 (CH_3), 22.9 (C_{tBu}), 19.4 (C_{tBu}) ppm.

Synthesis of 3'-*O*-(3'',6''-di-*O*-acetyl-2-*O*-benzyl-4''-deoxy-4''-(2-methyl-ethanoate)-α-D-glucopiranosyl)-*N*^{6}-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine.

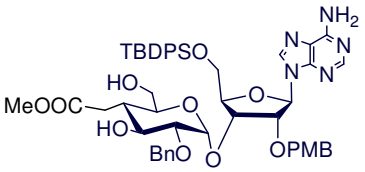


The title compound was prepared following the general procedure for glycosylation with thioglycosides. Thus, starting from **105** (315 mg, 0.69 mmol), and **38** (607 mg, 0.83 mmol), 150 mg of 4 Å M.S., 1.8 ml of Et_2O, 1.8 ml of DCM, NIS (203 mg, 0.83 mmol) and TfOH (7 µl, 0.08 mmol) were introduced into the flask and the solution was stirred for 48h at r.t. Et_3N (11 µl) was added to stop the reaction, and then the solution was treated as in the general procedure. The crude was purified by column

chromatography affording the desired glycoside together with unreacted **38**. The mixture obtained was used in the next reaction without further purification.

Synthesis of 3'-*O*-(2''-*O*-benzyl-4''-deoxy-4''-(2-methyl-ethanoate)-α-D-glucopiranosyl)-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine (106b).

The title compound was prepared following the general procedure for ester/amide hydrolysis, starting from the crude obtained in the glycosylation reaction dissolved in dry methanol (25 ml). Sodium methoxide (20 mg) was added to the solution. The reaction mixture was stirred at r.t. for 4 h and then treated as in the general procedure and purified by column chromatography (elution system DCM/MeOH, 10/0, 9.5/0.5, 9/1) affording **106b** (379 mg, 49 % yield over two steps).



^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 8.25 (s, 1 H, H-2), 7.82 (s, 1 H, H-8), 7.66-7.28 (m, 15 H, Ar), 6.94 (d, 2 H, *J* = 8.4 Hz, Ar), 6.61 (d, 2 H, *J* = 8.4 Hz, Ar), 6.19 (d, 1 H, $J_{1,2}$ = 6.4 Hz, H-1'), 5.72 (s, 2 H, NH_2), 5.52 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1''), 4.84 (dd, 1 H, $J_{2,1}$ = 6.0, $J_{2,3}$ = 4.8 Hz, H-2'), 4.73 (d, 1 H, J_{AB} = 10.8 Hz, CH_2Ph), 4.62-4.58 (m, 1 H, H-3'), 4.48 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.38-4.33 (m, 3 H, 2xCH_2Ph, H-4'), 4.02 (dd, 1 H, $J_{5'a,5'a}$ = 12.0 Hz, $J_{5',4'}$ = 4.8 Hz, H-5'), 3.83-3.54 (m, 10 H, 2xOCH_3, H-5'b, H-6''a,H-6''b, H-4'), 3.44 (dd, 1 H, $J_{2'',1''}$ = 3.2 Hz, $J_{2'',3''}$ = 9.6 Hz, H-2''), 2.97-2.91 (m, 1 H, H-7''a), 2.12-2.05 (m, 2 H, H-3'', H-7''b), 1.07 (s, 9 H, *t*-Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.6 (C=O), 159.8 (C-5), 156.4 (C-6), 152.7 (C-2), 150.1 (C-4), 141.0 (C-8), 135.7-128.0 (Ar), 114.1 (Ar), 92.8 (C-1''), 87.1 (C-1'), 85.3 (C-4'), 80.3 (C-2'), 76.8 (C-2''), 75.0 (C-3'), 72.9 (CH_2Ph), 72.5 (CH_2Ph), 71.9 (C-3''), 69.5 (C-5''), 63.4 (C-5'), 62.8 (C-6''), 55.4 (OCH_3), 52.2 (OCH_3), 38.4 (C-4''), 32.4 (C-7''), 27.0 (CH_3), 19.3 (C_{tBu}) ppm

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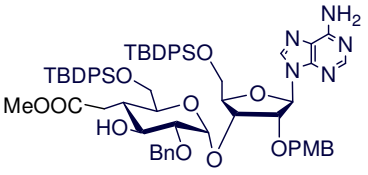
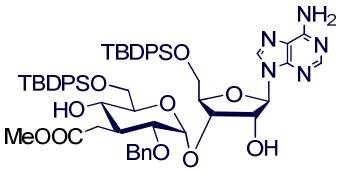
Synthesis of 3'-*O*-(2''-*O*-benzyl-4''-deoxy-4''-(2-methyl-ethanoate)-6''-*O*-*tert*-butyldiphenylsilyl-α-D-glucopiranosyl)-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine (107b).



The title compound was prepared following the general procedure for silylation, starting from **106b** (127 mg, 0.07 mmol) in dry DCM (1.3ml), DMAP (32 mg, 0.07 mmol), dry triethylamine (130 µl, 0.07 mmol), and *tert*-butyldiphenylsilyl chloride (123 µl, 0.07 mmol). After 13h stirring the reaction was treated as in the general procedure and purified by column chromatography (system B), affording **107b** (87 mg, 78 % yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 8.24 (s, 1 H, H-2), 7.82 (s, 1 H, H-8), 7.65-7.28 (m, 25 H, Ar), 6.93 (d, 2 H, *J* = 8.4 Hz, Ar), 6.61 (d, 2 H, *J* = 8.4 Hz, Ar), 6.19 (d, 1 H, $J_{1',2'}$ = 6.4 Hz, H-1'), 5.71 (s, 2 H, NH_2), 5.52 (d, 1 H, $J_{1'',2''}$ = 3.2 Hz, H-1''), 4.85 (dd, 1 H, $J_{2',1'}$ = 6.4 Hz, $J_{2',3'}$ = 5.2 Hz, H-2'), 4.73 (d, 1 H, J_{AB} = 10.8 Hz, CH_2Ph), 4.61-4.59 (m, 1 H, H-3'), 4.48 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.38-4.33 (m, 2 H, 2xCH_2Ph), 4.33-4.19 (m, 1 H, H-4'), 4.02 (dd, 1 H, $J_{5'a,5'b}$= 12.0 Hz, $J_{5'a,4}$ = 4.8 Hz, H-5'a), 3.82-3.57 (m, 12 H, 2xOCH_3, H-5'b, H-6''a, H-6''b, H-5'', H-3'', H-4''), 3.45 (dd, 1 H, $J_{2'',1''}$ = 3.2 Hz, $J_{2'',3''}$ = 9.6 Hz, H-2''), 2.75-2.68 (m, 1 H, H-7''a), 2.60-2.54 (m, 2 H, H-4'', H-7''b), 1.05 (s, 9 H, *t*-Bu), 1.04 (s, 9 H, *t*-Bu) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 ºC): δ = 172.9 (C=O), 159.6 (C-5), 156.5 (C-6), 153.2 (C-2), 149.7 (C-4), 139.6 (C-8), 135.7-128.1 (Ar), 113.8 (Ar), 96.1 (C-1''), 86.5 (C-1'), 84.1 (C-4'), 79.9 (C-2'), 79.9 (C-2''), 74.1 (C-3'), 72.7 (CH_2Ph), 71.5 (C-3''), 71.5 (CH_2Ph), 69.3 (C-5''), 64.7 (C-6''), 63.7 (C-5'), 55.3 (OCH_3), 51.8 (OCH_3), 39.4 (C-4''), 33.1 (C-7''), 29.9 (CH_3), 27.2 (CH_3), 22.7 (C_{tBu}), 19.4 (C_{tBu}) ppm.

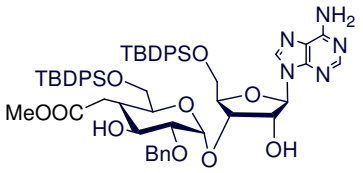
Synthesis of 3'-*O*-(2-*O*-benzyl-3''-deoxy-3''-(2-methyl-2-etanoate-6-*O*-*tert*-butyldiphenylsilyl -α-D-glucopiranosyl)-5'-*O*-*tert*-butyldiphenylsilyl adenosine (108a).



The title compound was prepared following the general procedure for *p*-methoxybenzylether deprotection, starting from **107a** (23 mg, 0.020 mmol) dissolved acetonitrile:water (9:1) 4 ml, CAN (24 mg, 0.044 mmol) was added. The solution was stirred for 2 h and then treated as in the general procedure. Upon silica gel column purification, **108a** was afforded (17 mg) in 79 % yield.

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 8.26 (s, 1 H, H-2), 7.99 (s, 1 H, H-8), 7.63-7.20 (m, 25 H, Ar), 6.07 (s, 2 H, NH_2), 5.97 (d, 1 H, $J_{1',2'}$ = 6.4 Hz, H-1'), 4.82 (d, 1 H, $J_{1'',2''}$ = 3.2 Hz, H-1''), 4.74 (t, 1 H, $J_{2',1'} = J_{2',3'}$ = 6.0 Hz, H-2'), 4.65 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.55 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.31-4.39 (m, 1 H, H-3'), 4.17-4.16 (m, 1 H, H-4'), 3.80 (dd, 1 H, $J_{5'a,5'b}$ = 12.0 Hz, $J_{5'a,4'}$ = 3.6 Hz, H-5'a), 3.75-3.57 (m, 8 H, OCH_3, H-5'b, H-6''a, H-6''b, H-5'', H-4''), 3.46 (dd, 1 H, $J_{2'',1''}$ = 3.2 Hz, $J_{2'',3''}$ = 11.2 Hz, H-2''), 2.71 (dd, 1 H, $J_{7''a,3''}$ = 4.8 Hz, $J_{7''a,7''b}$ = 15.2 Hz, H-7''a), 2.59 (dd, 1 H, $J_{7''b,3''}$ = 4.8 Hz, $J_{7''b,7''a}$ = 15.2 Hz, H-7''b), 2.52-2.44 (m, 1 H, H-3''), 1.01(s, 9 H, *t*-Bu), 0.98 (s, 9 H, *t*-Bu) ppm.

Synthesis of 3'-*O*-(2-*O*-benzyl-4''-deoxy-4''-(methyl)etanoate-6-*O*-*tert*-butyldiphenylsilyl -α-D-glucopiranosyl)-5'-*O*-*tert*-butyldiphenylsilyl adenosine (108b).



The title compound was prepared following the general procedure for *p*-methoxybenzylether deprotection, starting from **107b** (15 mg, 0.013 mmol) dissolved acetonitrile:water (9:1) 4 ml, CAN (15 mg, 0.029 mmol) was added. The solution

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was stirred for 2 h and then treated as in the general procedure. Upon silica gel column purification, **108b** was afforded in 84 % yield (11.5 mg).

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 8.24 (s, 1 H, H-2), 7.82 (s, 1 H, H-8), 7.72-7.29 (m, 25 H, Ar), 6.0 (d, 2 H, $J_{1',2'}$ = 5.6 Hz, H-1'), 5.92 (s, 2 H, NH_2), 4.80-4.77 (m, 2 H, CH_2Ph, H-1''), 4.67 (t, 1 H, $J_{2',1'} = J_{2',3'}$ = 5.6 Hz, H-2'), 4.65 (d, 1 H, J_{AB} = 11.6 Hz, CH_2Ph), 4.29 (dd, 1 H, $J_{3',2'}$ = 5.6, $J_{3',4'}$ = 9.2 Hz, H-3'), 4.22-4.19 (m, 1 H, H-4'), 3.77-3.44 (m, 10 H, OCH_3, H-5'a, H-5'b, H-6''a, H-6''b, H-5'', H-3'', H-2''), 2.59 (dd, 1 H, $J_{7''a,4''}$ = 4.8 Hz, $J_{7''a,7''b}$ = 16.0 Hz, H-7''a), 2.46 (dd, 1 H, $J_{7''b,4''}$ = 5.6 Hz, $J_{7''b,7''a}$ = 16.0 Hz, H-7''b), 2.25-2.19 (m, 1 H, H-4''), 1.05 (s, 9 H, *t*-Bu), 1.04 (s, 9 H, *t*-Bu) ppm.

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6. Annex

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6.1. Binding Data.

Full length-IP_3

	Data Set-A
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-4.025
TOP	99.67
LOGEC50	-7.501
HILLSLOPE	-0.9612
EC50	3.151e-008
Std. Error	
BOTTOM	8.003
TOP	3.320
LOGEC50	0.1158
HILLSLOPE	0.2125
95% Confidence Intervals	
BOTTOM	-20.19 to 12.14
TOP	92.97 to 106.4
LOGEC50	-7.735 to -7.268
HILLSLOPE	-1.391 to -0.5319
EC50	1.839e-008 to 5.401e-008
Goodness of Fit	
Degrees of Freedom	41
R^2	0.8888
Absolute Sum of Squares	7146
Sy.x	13.20
Data	
Number of X values	10
Number of Y replicates	5
Total number of values	45
Number of missing values	5

Full lenght-AdA

	Data Set-A
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.5338
TOP	94.85
LOGEC50	-8.489
HILLSLOPE	-1.002
EC50	3.241e-009
Std. Error	
BOTTOM	3.517
TOP	3.032
LOGEC50	0.07567
HILLSLOPE	0.1594
95% Confidence Intervals	
BOTTOM	-7.642 to 6.574
TOP	88.72 to 101.0
LOGEC50	-8.642 to -8.336
HILLSLOPE	-1.324 to -0.6796
EC50	2.279e-009 to 4.608e-009
Goodness of Fit	
Degrees of Freedom	40
R^2	0.9486
Absolute Sum of Squares	3505
Sy.x	9.361
Data	
Number of X values	10
Number of Y replicates	5
Total number of values	44
Number of missing values	6

Full lenght-IP_2

	Data Set-A
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	2.125
TOP	96.48
LOGEC50	-5.121
HILLSLOPE	-3.059
EC50	7.562e-006
Std. Error	
BOTTOM	9.948
TOP	4.688
LOGEC50	0.06011
HILLSLOPE	1.156
95% Confidence Intervals	
BOTTOM	-18.10 to 22.35
TOP	86.95 to 106.0
LOGEC50	-5.244 to -4.999
HILLSLOPE	-5.409 to -0.7088
EC50	5.706e-006 to 1.002e-005
Goodness of Fit	
Degrees of Freedom	34
R^2	0.8270
Absolute Sum of Squares	11836
Sy.x	18.66
Data	
Number of X values	10
Number of Y replicates	5
Total number of values	38
Number of missing values	12

Full lenght-2dephosphoAdA

	Data Set-A
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-4.036
TOP	95.38
LOGEC50	-7.339
HILLSLOPE	-0.9957
EC50	4.582e-008
Std. Error	
BOTTOM	5.320
TOP	1.881
LOGEC50	0.07626
HILLSLOPE	0.1487
95% Confidence Intervals	
BOTTOM	-14.77 to 6.699
TOP	91.59 to 99.18
LOGEC50	-7.493 to -7.185
HILLSLOPE	-1.296 to -0.6956
EC50	3.215e-008 to 6.530e-008
Goodness of Fit	
Degrees of Freedom	43
R^2	0.9464
Absolute Sum of Squares	2821
Sy.x	8.100
Data	
Number of X values	10
Number of Y replicates	5
Total number of values	47
Number of missing values	3

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1-604-AdA

	Data Set-A
Sigmoidal dose-response	
Best-fit values	
BOTTOM	-2.109
TOP	96.61
LOGEC50	-8.987
EC50	1.0294e-009
Std. Error	
BOTTOM	2.295
TOP	2.518
LOGEC50	0.06490
95% Confidence Intervals	
BOTTOM	-6.713 to 2.495
TOP	91.55 to 101.7
LOGEC50	-9.118 to -8.857
EC50	7.6275e-010 to 1.3893e-009
Goodness of Fit	
Degrees of Freedom	54
R^2	0.9475
Absolute Sum of Squares	5001
Sy.x	9.623
Data	
Number of X values	10
Number of Y replicates	6
Total number of values	57
Number of missing values	3

1-604 IP_2

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-4.328
TOP	98.51
LOGEC50	-6.007
HILLSLOPE	-0.7693
EC50	9.836e-007
Std. Error	
BOTTOM	4.961
TOP	3.043
LOGEC50	0.09244
HILLSLOPE	0.1046
95% Confidence Intervals	
BOTTOM	-14.47 to 5.818
TOP	92.29 to 104.7
LOGEC50	-6.196 to -5.818
HILLSLOPE	-0.9833 to -0.5554
EC50	6.365e-007 to 1.520e-006
Goodness of Fit	
Degrees of Freedom	29
R^2	0.9735
Absolute Sum of Squares	1246
Sy.x	6.555
Data	
Number of X values	10
Number of Y replicates	4
Total number of values	33
Number of missing values	7

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1-604 2 dephosphoAdA

	Data Set-A
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-4.176
TOP	96.34
LOGEC50	-7.891
HILLSLOPE	-0.7361
EC50	1.285e-008
Std. Error	
BOTTOM	8.551
TOP	4.205
LOGEC50	0.1592
HILLSLOPE	0.1746
95% Confidence Intervals	
BOTTOM	-21.62 to 13.27
TOP	87.77 to 104.9
LOGEC50	-8.216 to -7.566
HILLSLOPE	-1.092 to -0.3800
EC50	6.084e-009 to 2.715e-008
Goodness of Fit	
Degrees of Freedom	31
R^2	0.9057
Absolute Sum of Squares	4605
Sy.x	12.19
Data	
Number of X values	10
Number of Y replicates	4
Total number of values	35
Number of missing values	5

R568Q-IP_3

	Data Set-A
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-10.38
TOP	102.1
LOGEC50	-7.360
HILLSLOPE	-0.7642
EC50	4.369e-008
Std. Error	
BOTTOM	14.71
TOP	4.439
LOGEC50	0.2139
HILLSLOPE	0.2270
95% Confidence Intervals	
BOTTOM	-40.31 to 19.56
TOP	93.03 to 111.1
LOGEC50	-7.795 to -6.924
HILLSLOPE	-1.226 to -0.3020
EC50	1.603e-008 to 1.191e-007
Goodness of Fit	
Degrees of Freedom	33
R^2	0.8630
Absolute Sum of Squares	7151
Sy.x	14.72
Data	
Number of X values	10
Number of Y replicates	4
Total number of values	37
Number of missing values	3

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R568Q-AdA

	Data Set-A
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.6846
TOP	100.4
LOGEC50	-7.795
HILLSLOPE	-2.915
EC50	1.602e-008
Std. Error	
BOTTOM	2.815
TOP	1.589
LOGEC50	0.03457
HILLSLOPE	0.4098
95% Confidence Intervals	
BOTTOM	-5.028 to 6.397
TOP	97.15 to 103.6
LOGEC50	-7.865 to -7.725
HILLSLOPE	-3.747 to -2.084
EC50	1.363e-008 to 1.883e-008
Goodness of Fit	
Degrees of Freedom	36
R^2	0.9718
Absolute Sum of Squares	2120
Sy.x	7.673
Data	
Number of X values	10
Number of Y replicates	4
Total number of values	40
Number of missing values	0

R568Q-IP_2

	Data Set-A
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.7679
TOP	102.4
LOGEC50	-6.413
HILLSLOPE	-0.8897
EC50	3.862e-007
Std. Error	
BOTTOM	5.987
TOP	5.404
LOGEC50	0.1159
HILLSLOPE	0.2037
95% Confidence Intervals	
BOTTOM	-11.38 to 12.92
TOP	91.39 to 113.3
LOGEC50	-6.648 to -6.178
HILLSLOPE	-1.303 to -0.4764
EC50	2.247e-007 to 6.637e-007
Goodness of Fit	
Degrees of Freedom	36
R^2	0.8972
Absolute Sum of Squares	6296
Sy.x	13.22
Data	
Number of X values	11
Number of Y replicates	4
Total number of values	40
Number of missing values	4

R568Q-2dephosphoAdA

	Data Set-A
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-2.696
TOP	95.66
LOGEC50	-7.920
HILLSLOPE	-0.8110
EC50	1.203e-008
Std. Error	
BOTTOM	4.021
TOP	2.158
LOGEC50	0.07473
HILLSLOPE	0.09941
95% Confidence Intervals	
BOTTOM	-10.88 to 5.489
TOP	91.27 to 100.1
LOGEC50	-8.072 to -7.767
HILLSLOPE	-1.013 to -0.6086
EC50	8.478e-009 to 1.708e-008
Goodness of Fit	
Degrees of Freedom	33
R^2	0.9709
Absolute Sum of Squares	1359
Sy.x	6.418
Data	
Number of X values	10
Number of Y replicates	4
Total number of values	37
Number of missing values	3

R504-IP_3

	Data Set-A
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	181.6
TOP	1399
LOGEC50	-7.264
HILLSLOPE	-0.7921
EC50	5.447e-008
Std. Error	
BOTTOM	150.6
TOP	40.74
LOGEC50	0.1941
HILLSLOPE	0.2222
95% Confidence Intervals	
BOTTOM	-122.4 to 485.7
TOP	1317 to 1481
LOGEC50	-7.656 to -6.872
HILLSLOPE	-1.241 to -0.3434
EC50	2.210e-008 to 1.343e-007
Goodness of Fit	
Degrees of Freedom	42
R^2	0.8581
Absolute Sum of Squares	1.061e+ 006
Sy.x	158.9
Data	
Number of X values	10
Number of Y replicates	5
Total number of values	46
Number of missing values	4

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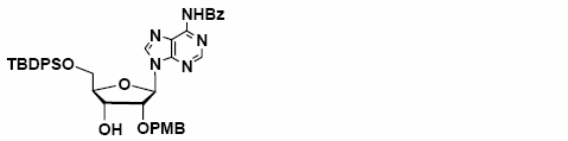
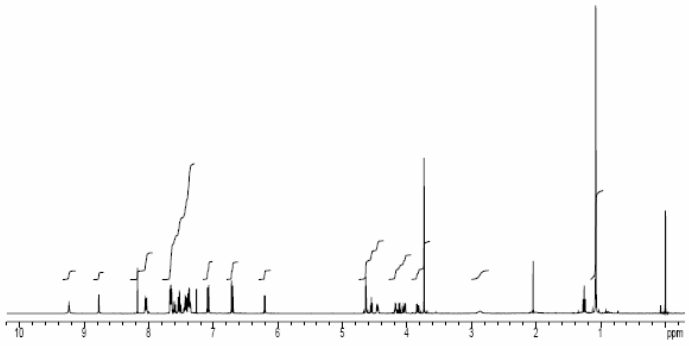
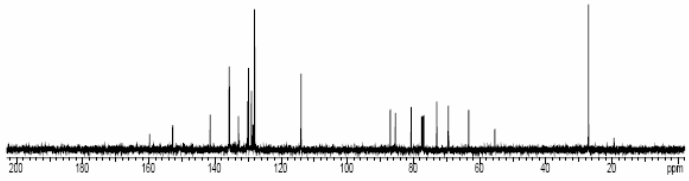
6.2. Selected NMR spectra

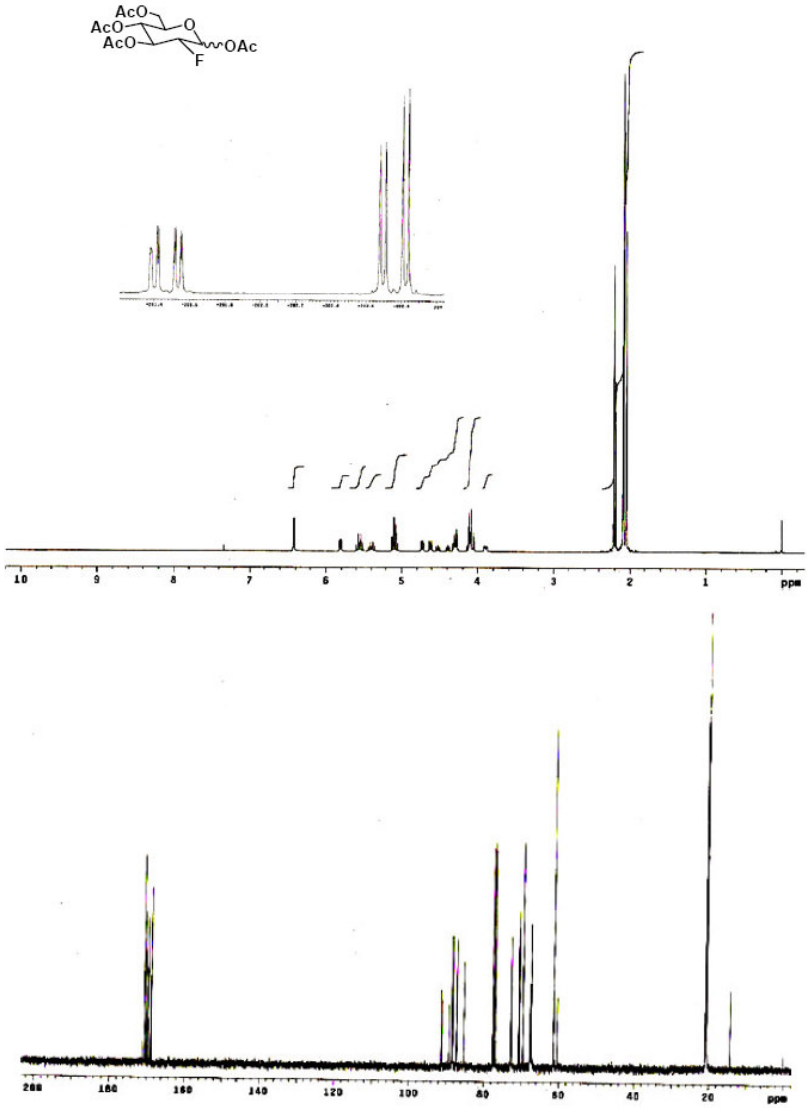
^{1}H, and ^{13}C NMR spectra of ^{6}*N*-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyladenosine.





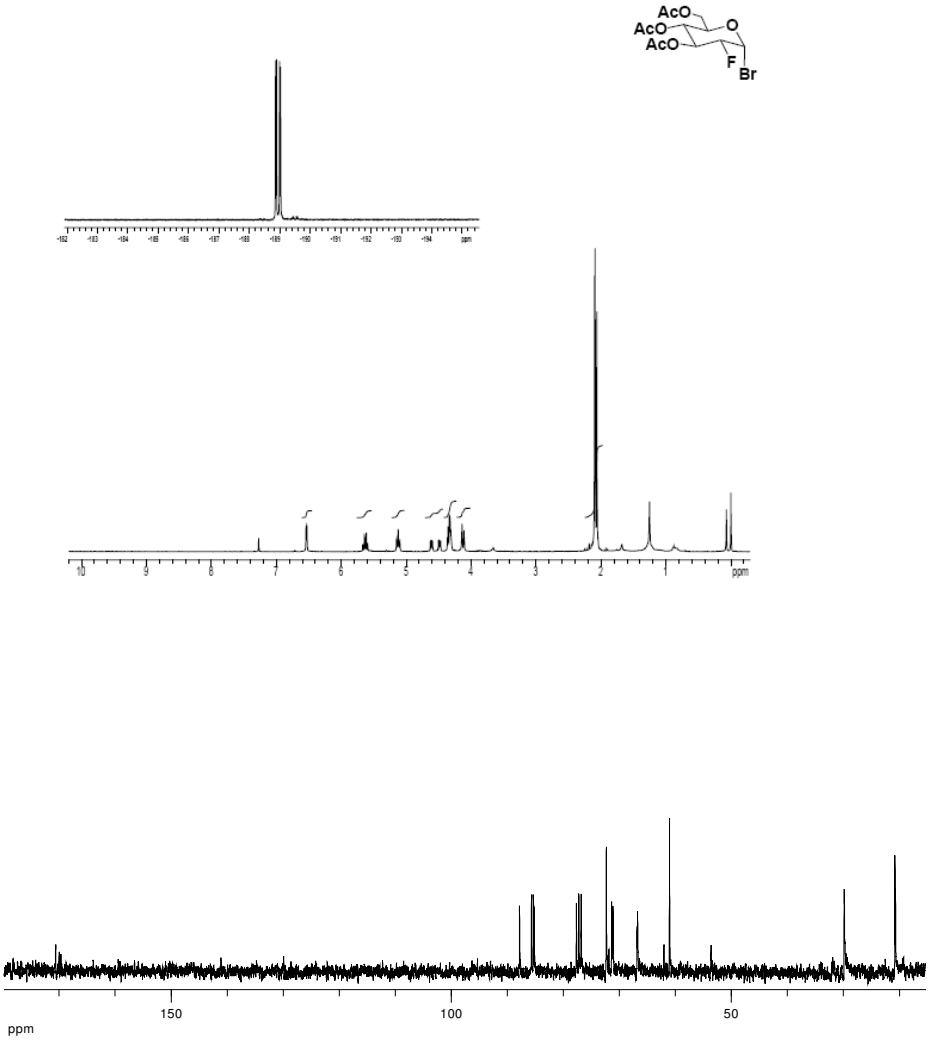


^{1}H, ^{19}F, ^{13}C and NMR spectra of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-fluoro-α,β-D-glucopyranose.



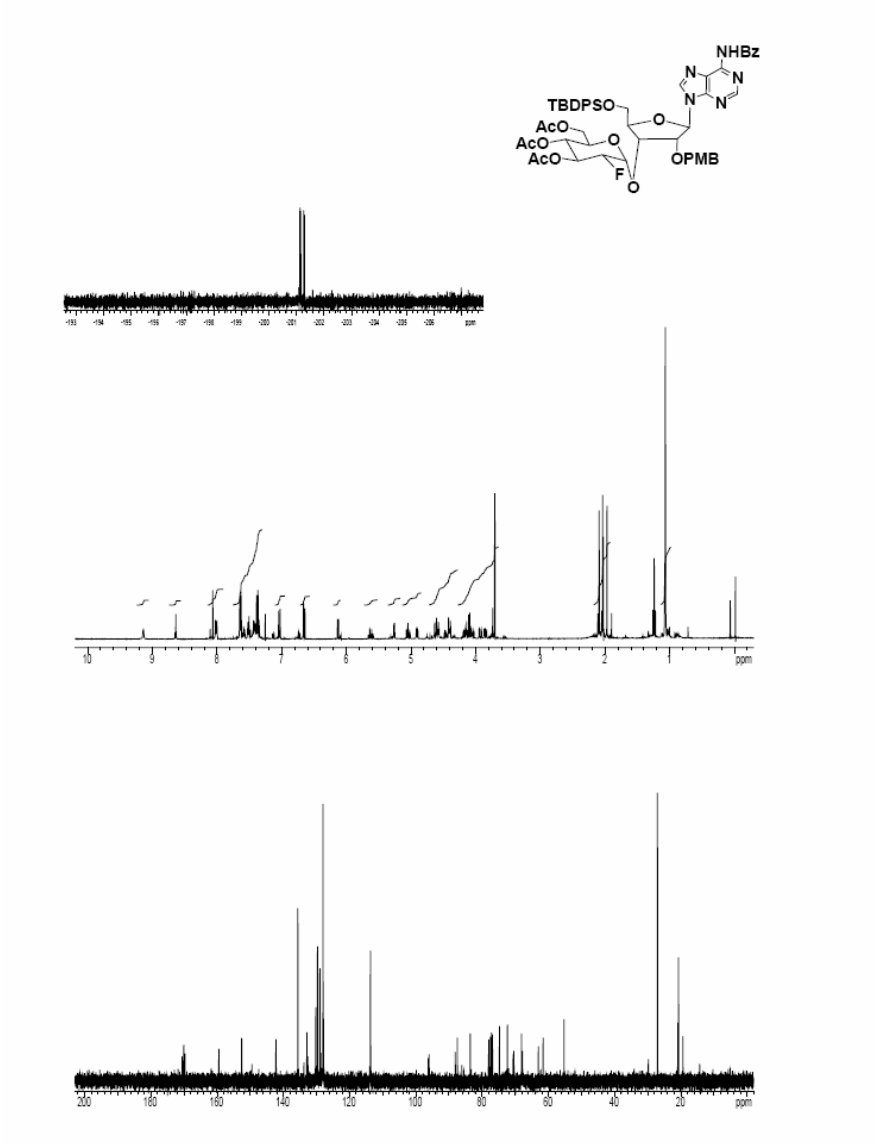
ISBN:978-84-691-9757-8/DL:T-1256-2008

^{1}H, ^{19}F and ^{13}C NMR spectra of 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-gluopyranosyl bromide.



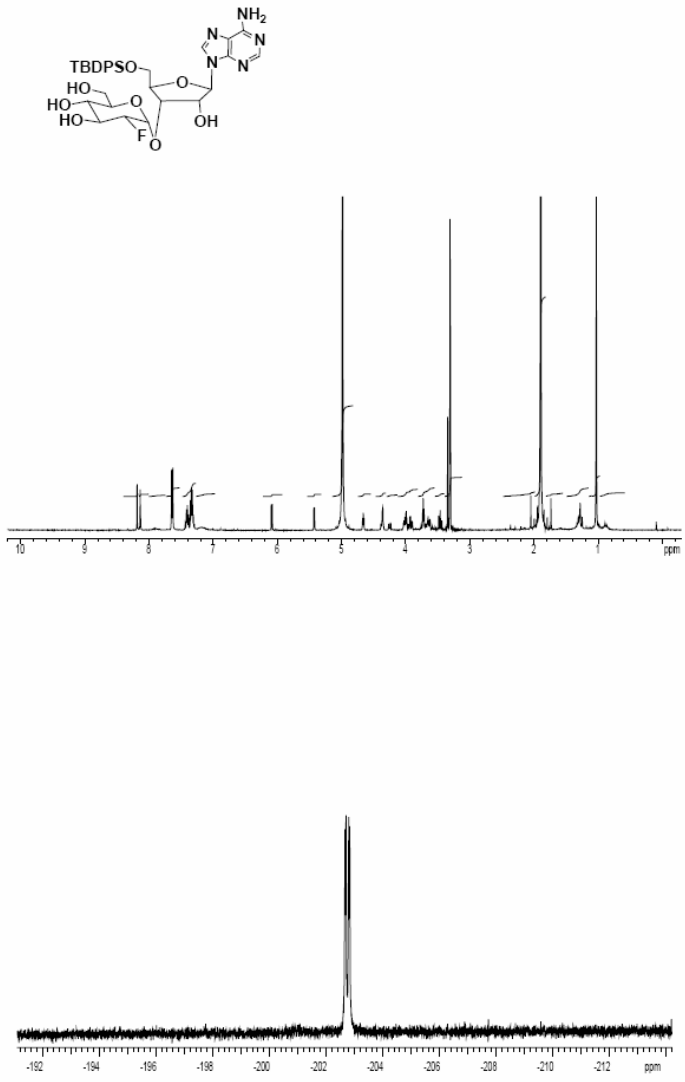
ISBN:978-84-691-9751-8/DL:T-1256-2008

^{1}H, ^{19}F and ^{13}C NMR spectra of 3'-*O*-(3'',4'',6''-tri-*O*-acetyl-2''-deoxy-2''-fluoro-α-D-glucopiranosyl)-*N*^{6}-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine.



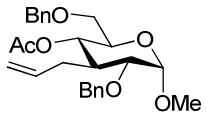
ISBN:978-84-691-9757-8/DL:T-1256-2008

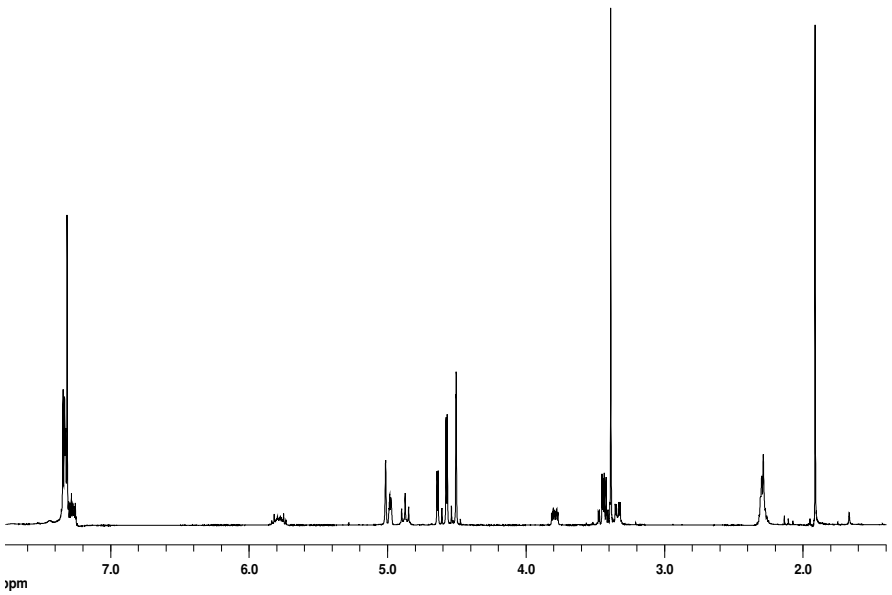
^{1}H and ^{19}F NMR spectra of 5'-*O*-*tert*-butyldiphenylsilyl-3'-*O*-2''-deoxy-2''-fluoro-α-D-glucopiranosyl) adenosine.

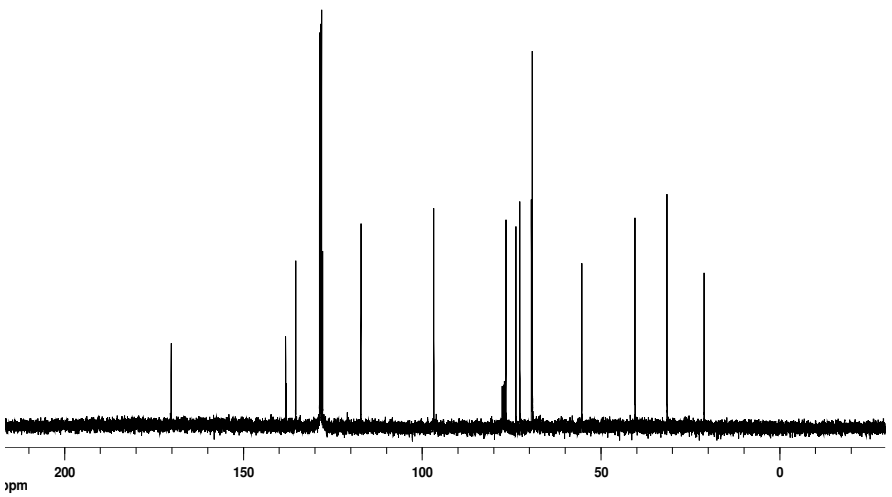


ISBN:978-84-691-9753-8/DL:T-1256-2008

^{1}H and ^{13}C NMR spectra of Methyl 4-*O*-acetyl-3-allyl-3-deoxy-2,6-di-*O*-benzyl-*α*-glucoside.

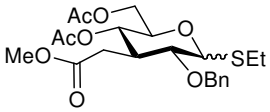


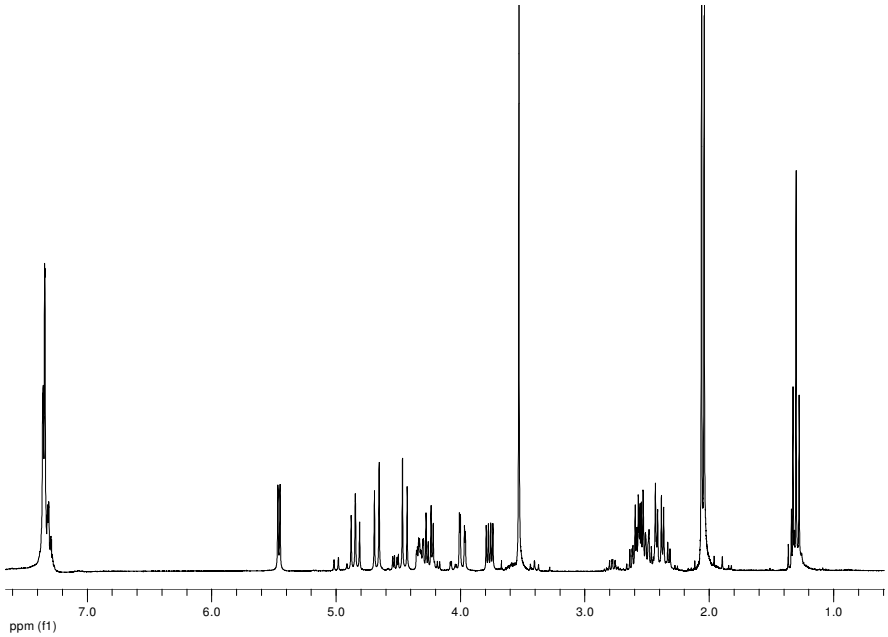




ISBN:978-84-691-9757-8/DL:T-1256-2008

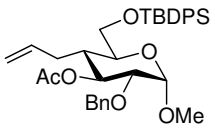
^{1}H NMR spectra of Ethyl 4,6-di-*O*-acetyl-2-*O*-benzyl-3-deoxy-3-(2-methyl-2-ethanoate)-1-*thio*-α,β-glucopyranoside.

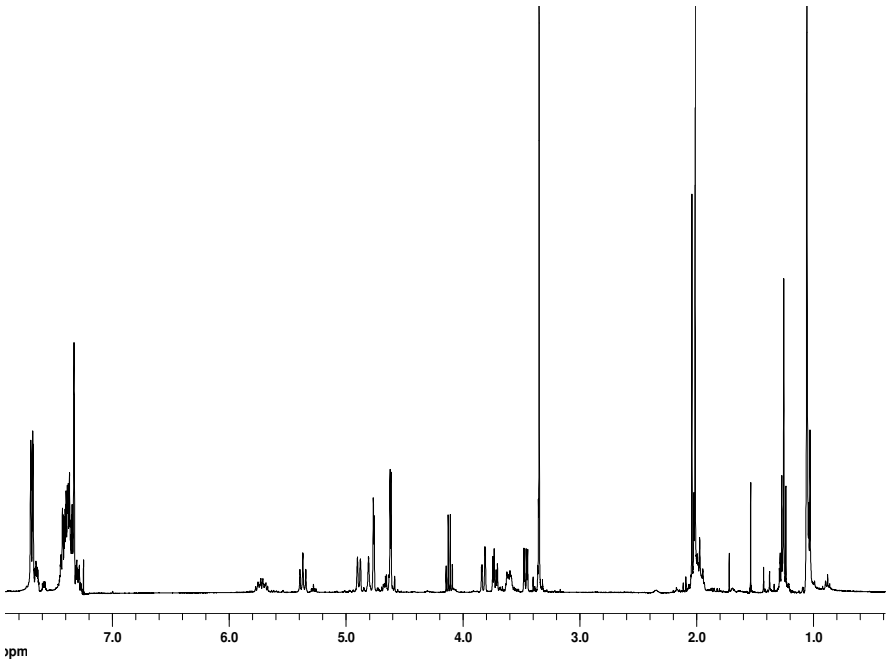


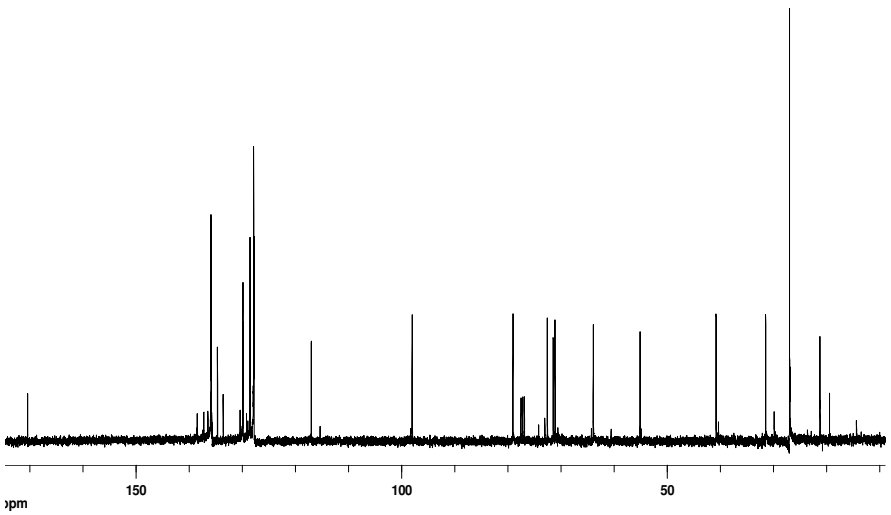


ISBN:978-84-691-9753-8/DL:T-1256-2008

^{1}H and ^{13}C NMR spectra of Methyl 3-*O*-acetyl-4-allyl-4-deoxy-2-*O*-benzyl-6-*O*-*tert*-butyldiphenilsilyl-α-glucoside.

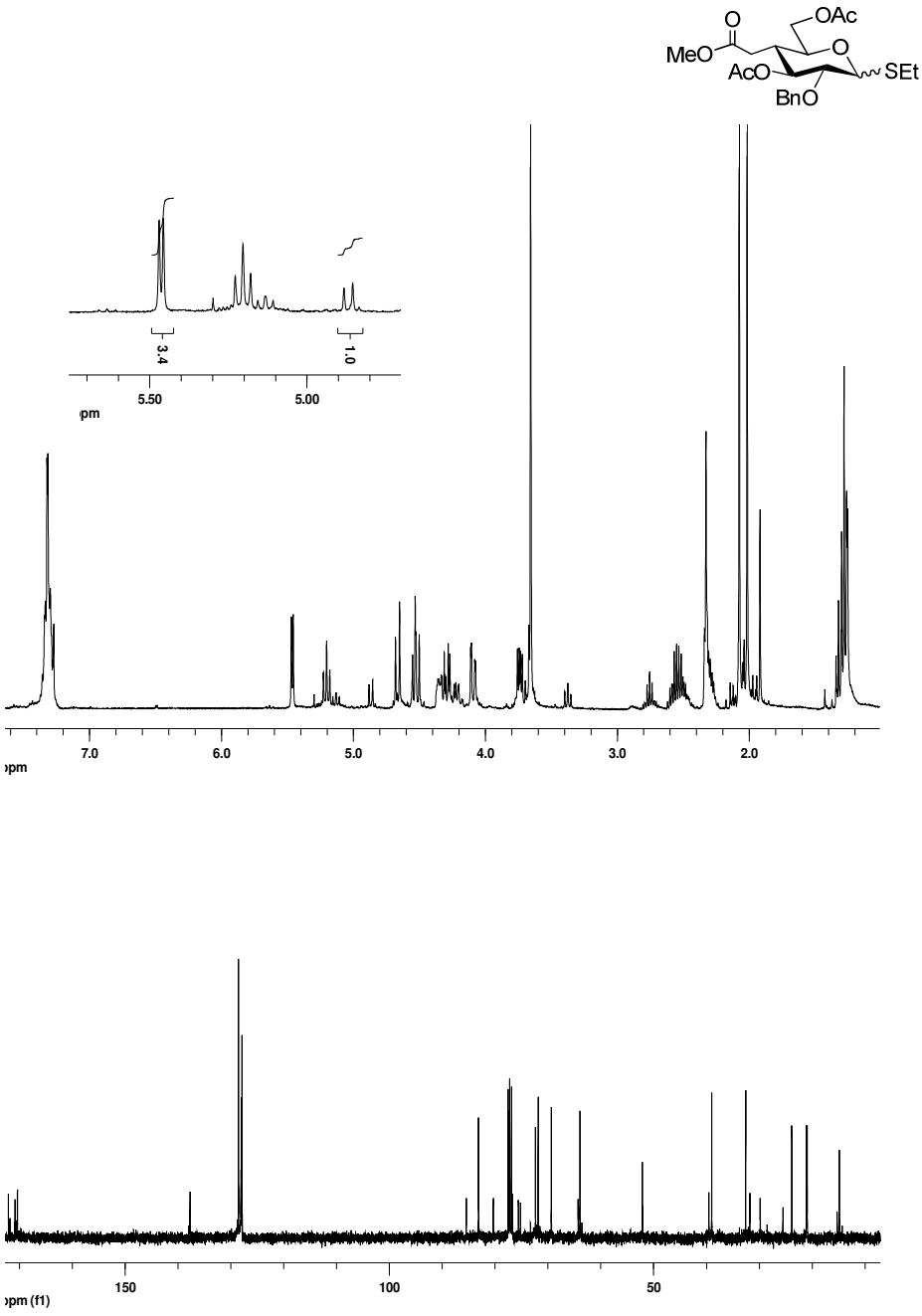






ISBN:978-84-691-9757-8/DL:T-1256-2008

^{1}H and ^{13}C NMR spectra of Ethyl 3,6-di-*O*-acetyl-2-*O*-benzyl-4-deoxy-4-(2-methyl-2-ethanoate)-1-*thio*-α,β-glucopyranoside.



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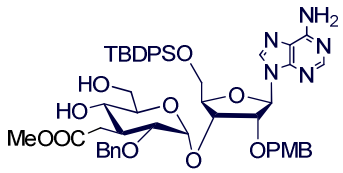
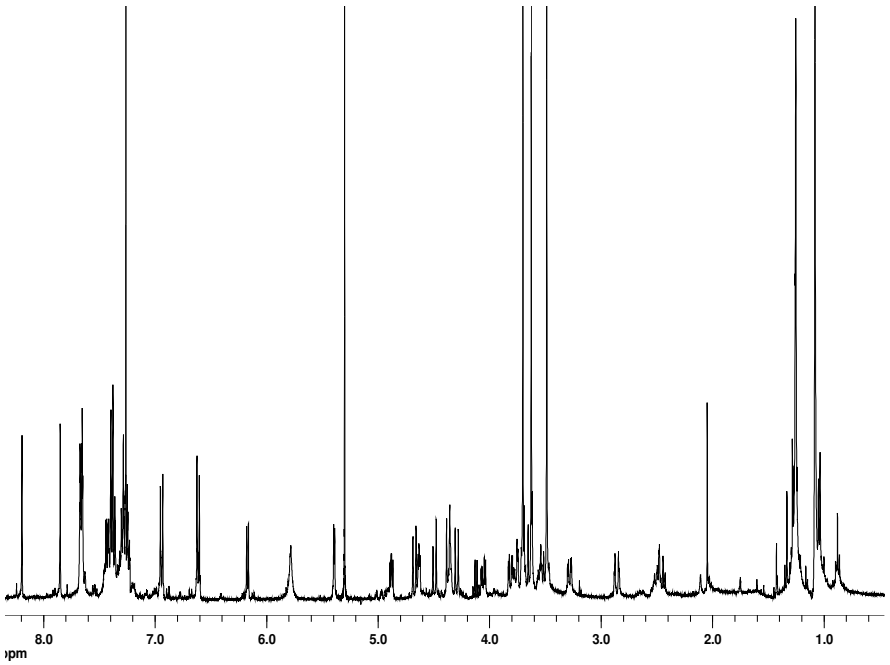
^{1}H NMR spectra of 3'-*O*-(2''-*O*-benzyl-3''-deoxy-3''-(2-methyl-2-ethanoate)-α-D-glucopyranosyl)-*N*^{6}-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine





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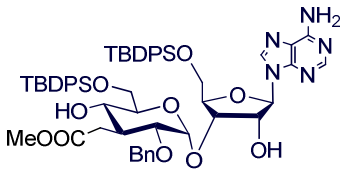
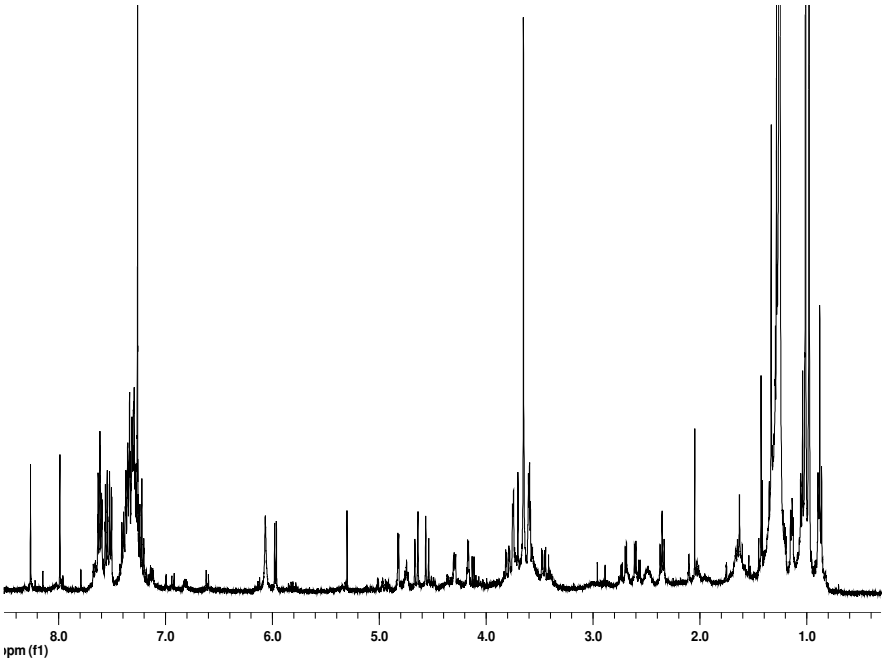
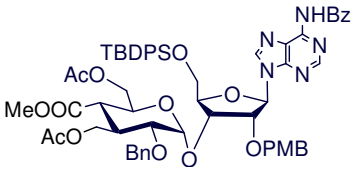
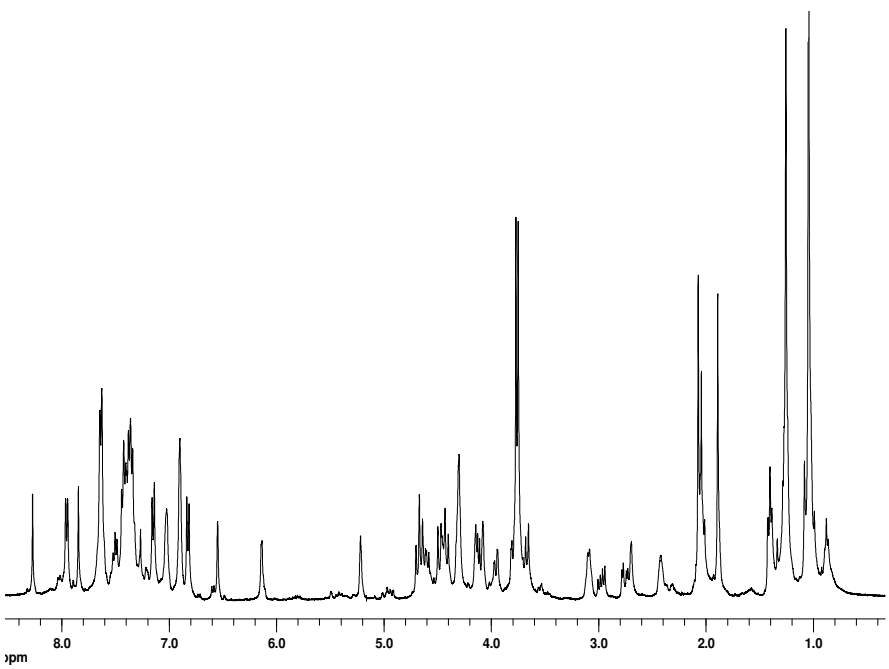
^{1}H NMR spectra of 3'-*O*-(2''-*O*-benzyl-3''-deoxy-3''-(2-methyl-2-ethanoate)-α-D-glucopyranosyl)-N^6-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl adenosine

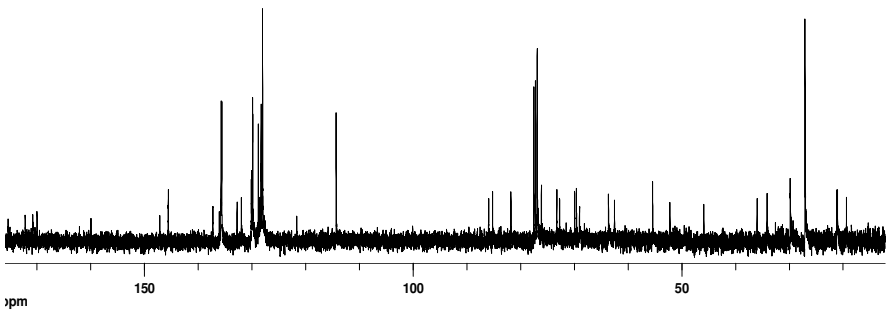




^{1}H, and ^{13}C NMR spectra of 3'-*O*-(3'',6''-di-*O*-acetyl-2''-*O*-benzyl-4''-deoxy-4''-(2methyl-2ethanoate)-α-D-glucopyranosyl)-N^{6}-benzoyl-5'-*O*-*tert*-butyldiphenylsilyl-2'-*O*-*p*-methoxybenzyl adenosine







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