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Total Synthesis of a New Stable Cyclic ADP-Ribose Mimic

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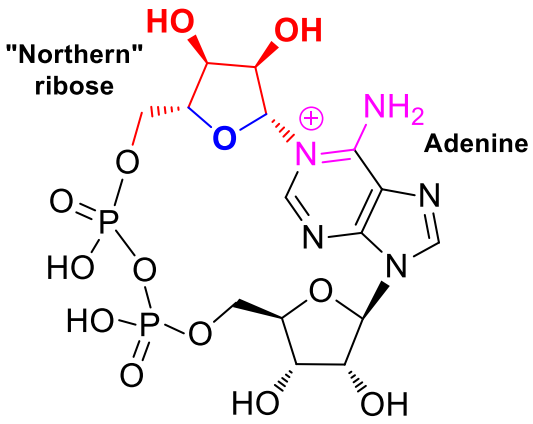
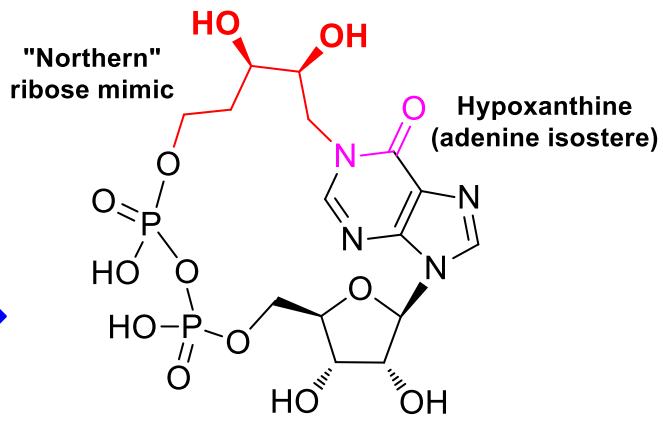
Total Synthesis of a New Stable Cyclic ADP-Ribose Mimic

Unstable Ca^{2+} second messenger



Active and stable analogue



This presentation

Abstract:

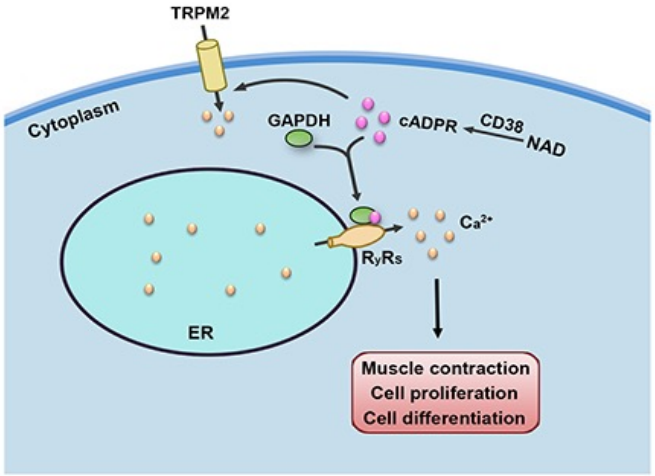
Cyclic ADP-ribose (cADPR) is a natural occurring metabolite of NAD^+, that mobilizes Ca^{2+} ions from intracellular stores. It was firstly isolated from sea urchin eggs extract, but it was later established that it is also produced in many other mammalian cells, such as pancreatic β-cells, T-lymphocytes, smooth and cerebellar neurons. cADPR is a dinucleotide in which a pyrophosphate bridge connects two ribose residues, bonded to adenine through N1 and N9 glycosidic bonds. As the N1 glycosidic bond is very labile, cADPR is rapidly hydrolyzed in neutral aqueous solution to ADP-ribose. In the light of the the poor knowledge of the cADPR receptor binding pocket, several stable and active derivatives have been synthesized. Among them, the cyclic inosine diphosphate ribose (cIDPR), in which the adenine is isosterically replaced by the hypoxanthine, was stable in physiological conditions and showed significant Ca^{2+} mobilizing activity. In our laboratories, we have synthesized several cIDPR analogues. In particular, the analogue with the “northern” ribose replaced by a pentyl chain (cpIDP) showed interesting Ca^{2+} mobilizing activity in the neuronal PC12 cell line. We report here on the total synthesis of a new stable cADPR analogue, in which the “northern” ribose is replaced by a 2”S,3”R dihydroxy pentyl chain. The new mimic elicites Ca^{2+} ions from intracellular stores in primary cortical neurons as effectively as cADPR.

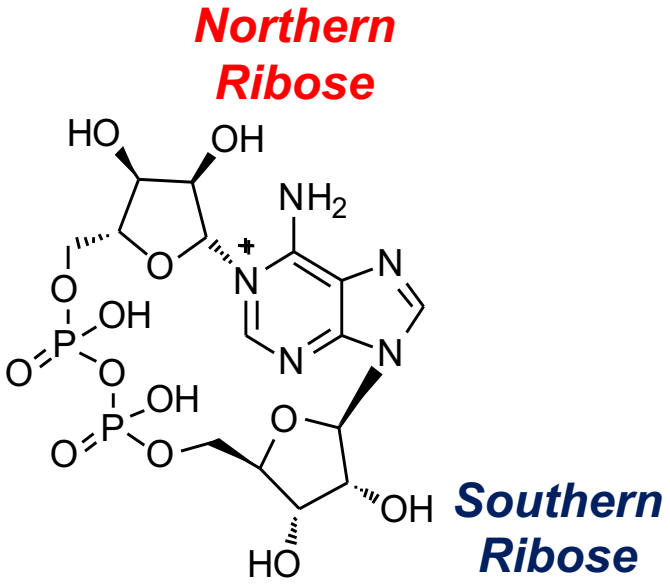
Keywords: cADPR; Ca^{2+} mobilization; primary cortical neurons; pyrophosphate bond formation

Introduction

Cyclic ADP-Ribose (cADRP)

Cyclic ADP-ribose (cADPR) was discovered in 1989 when Lee and co-workers examined various metabolites in sea urchin egg homogenates; it has been found also in various tissues of invertebrates and mammals: UNIVERSAL ENDOGENOUS METABOLITE



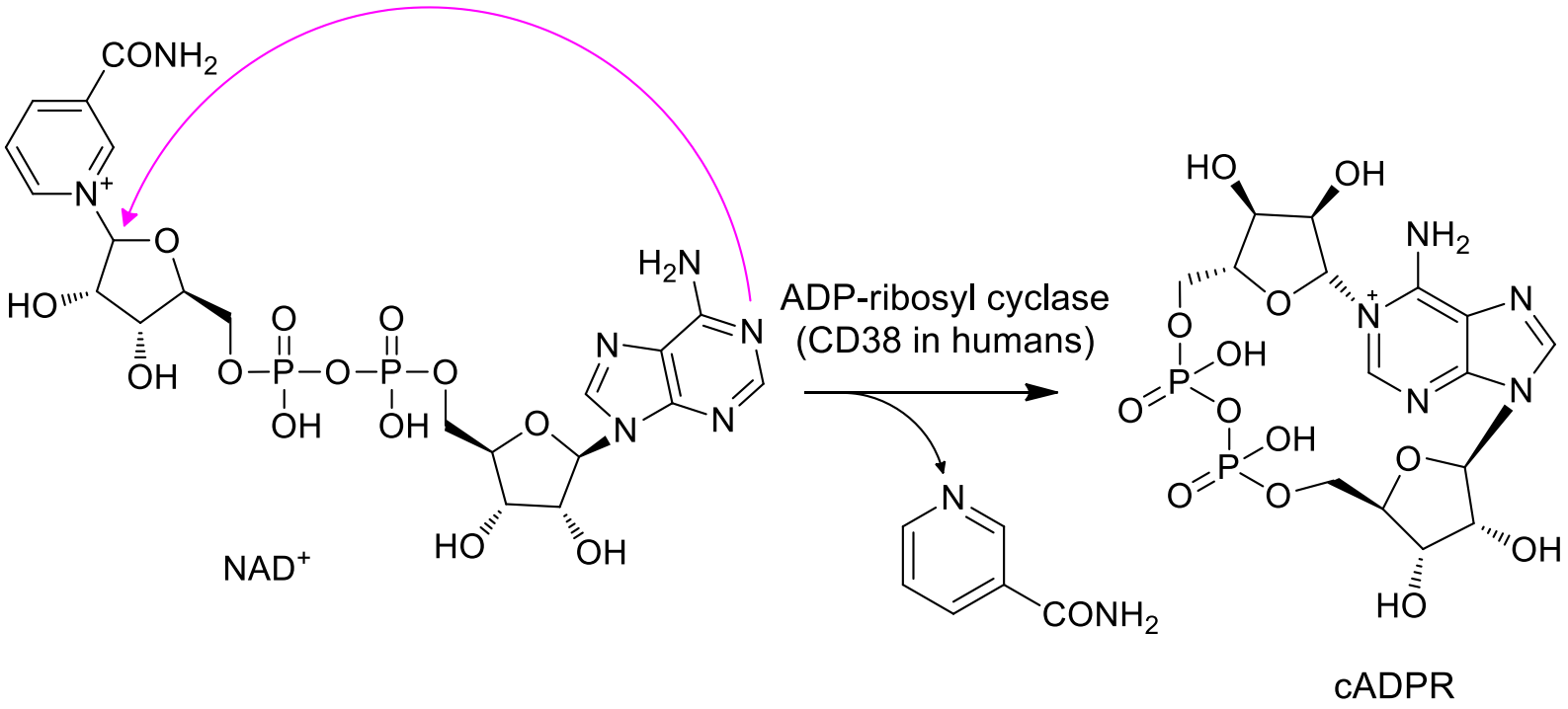


cyclic ADP-ribose (cADPR)

Lee, H.C.; Walseth, T.F.; Bratt, G.T.; Hayes, R.N.; Clapper, D.L. (1989). "Structural determination of a cyclic metabolite of NAD^+ with intracellular Ca^{2+}-mobilizing activity". *J. Biol. Chem*. **264** (3): 1608–15

Introduction

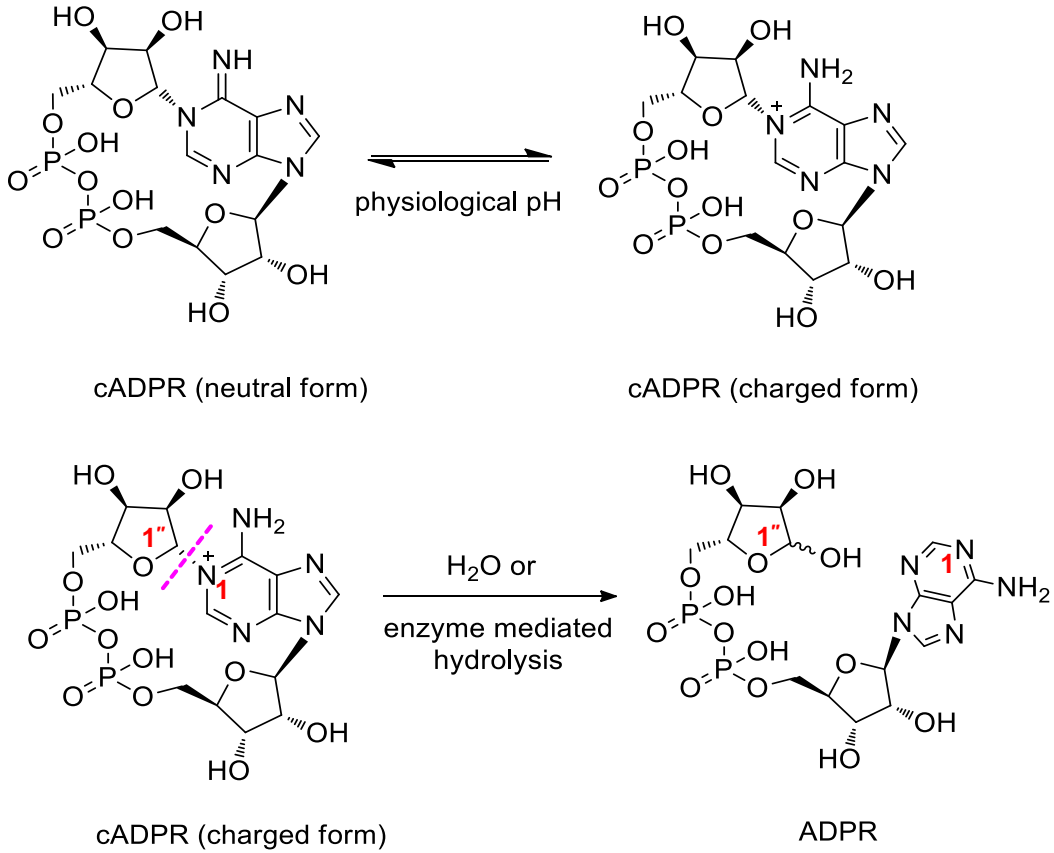
Biosynthesis of cADPR



Lee, H. C.; Aarhus, R.; Levitt, D. (1994). "The crystal structure of cyclic ADP-ribose". *Nat. Struct. Biol.* **1** (3): 143–4.

Introduction

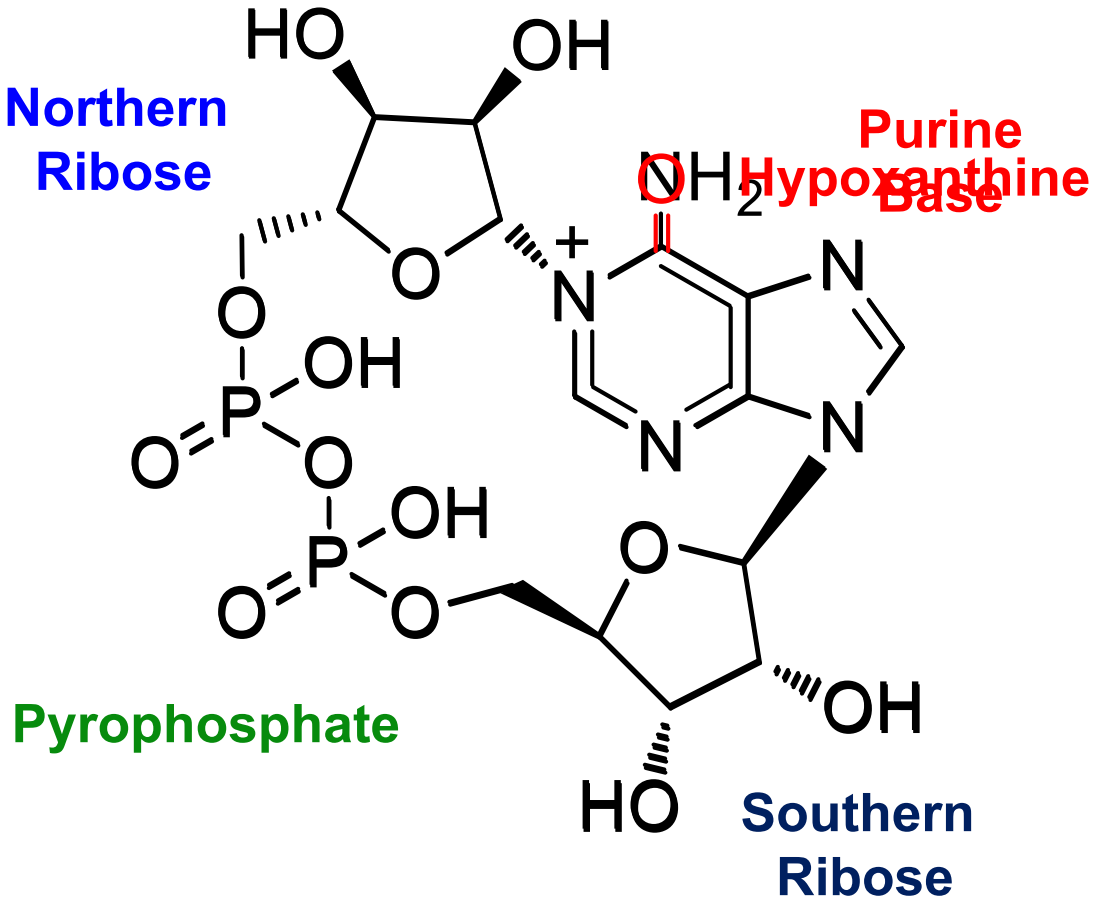
The Biological and Chemical Instability of cADPR



Potter, B. V.; Walseth, T. F. (2004) "Medicinal chemistry and pharmacology of cyclic ADP-ribose" *Curr. Mol. Med.* **4** (3): 303-311.

State of the Art

Generation of cADPR Analogues

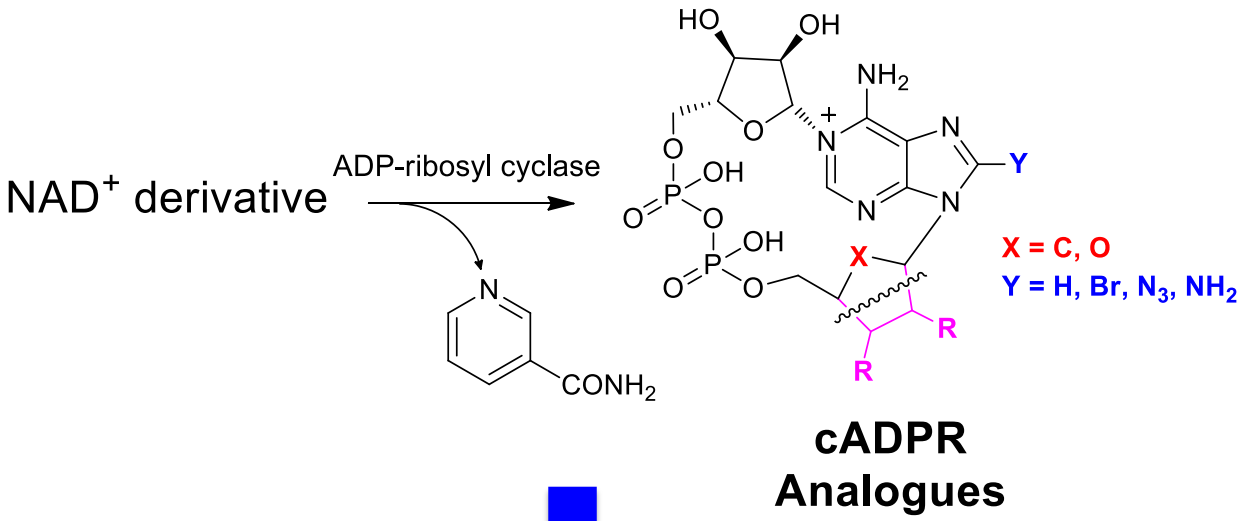


cyclic IDP Ribose (cIDPR)

- **Hydrolysis resistant thanks to the prevalent oxo form**
- **Intact Ca^{2+} releasing properties compared to cADPR**

State of the Art

Generation of cADPR Analogues: A Chemo-Enzymatic Approach



cADPR Analogues

Advantages

- **Broad substrate specificity**
- **Relative ease of the enzymatic steps**

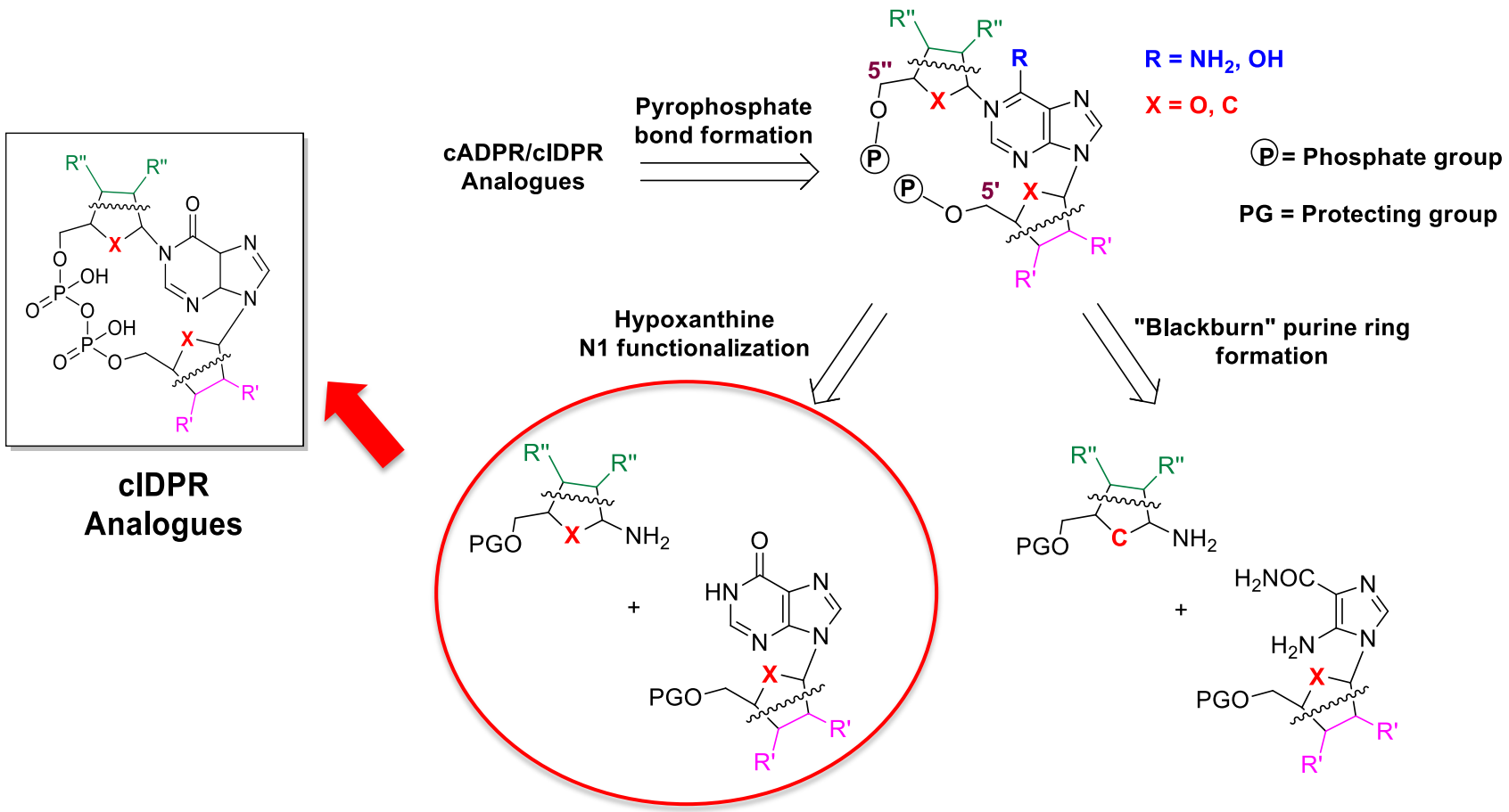
Disadvantages

- **Aberrant N7 cyclization for cIDPR congeners**
- **Analogues limited to intact "northern ribose"**

Guse A.H. (2004). "Regulation of calcium signaling by the second messenger cyclic adenosine diphosphoribose (cADPR)". *Curr. Mol. Med*. **4** (3): 239–248

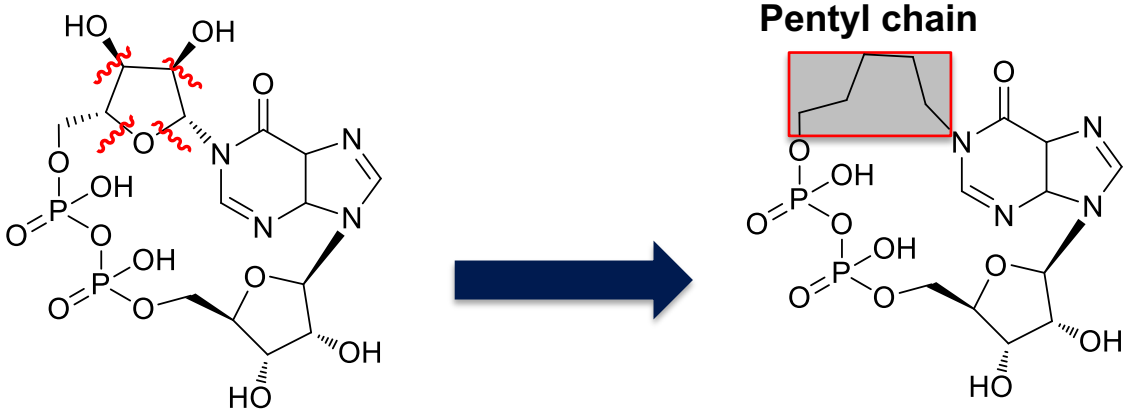
State of the Art

Generation of cADPR Analogues: The Total Synthesis

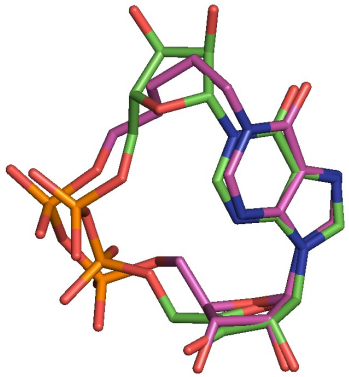


State of the Art

Generation of a Structurally Simplified cIDPR Analogue



cIDPR **cpIDP**



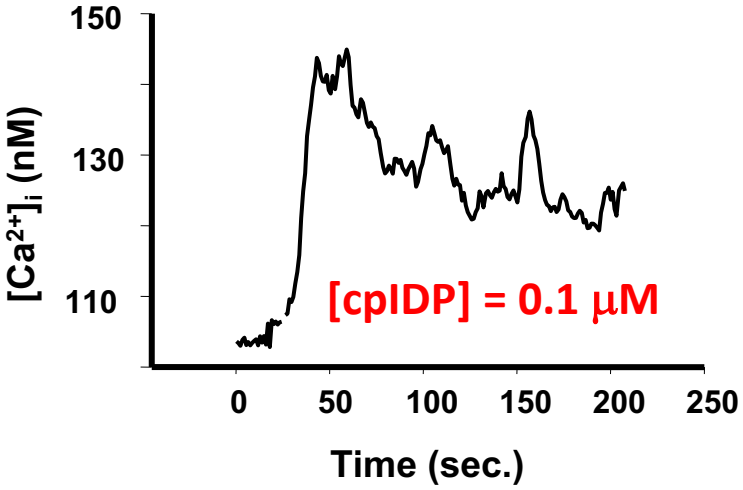
- **Green: cIDPR (extracted from PDB 2PGJ)**
- **Violet: low energy conformation of cpIDP**

(Macrocycle conformational sampling-Maestro 11.2)

D'Errico, S. et. al. (2015) "Synthesis of cyclic N1-pentylinosine phosphate, a new structurally reduced cADPR analogue with calcium-mobilizing activity on PC12 cells" *Beilstein J. Org. Chem.*, **11**: 2689–2695

State of the Art

Effect of cpIDP on Intracellular [Ca^{2+}] in NGF-Differentiated PC12 Cells



- ✓ ***Fast and transient increase in $[Ca^{2+}]_i$***
- ✓ ***The introduction of an alkyl chain in the N1 position of the purine renders the analogue membrane permeant***

BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

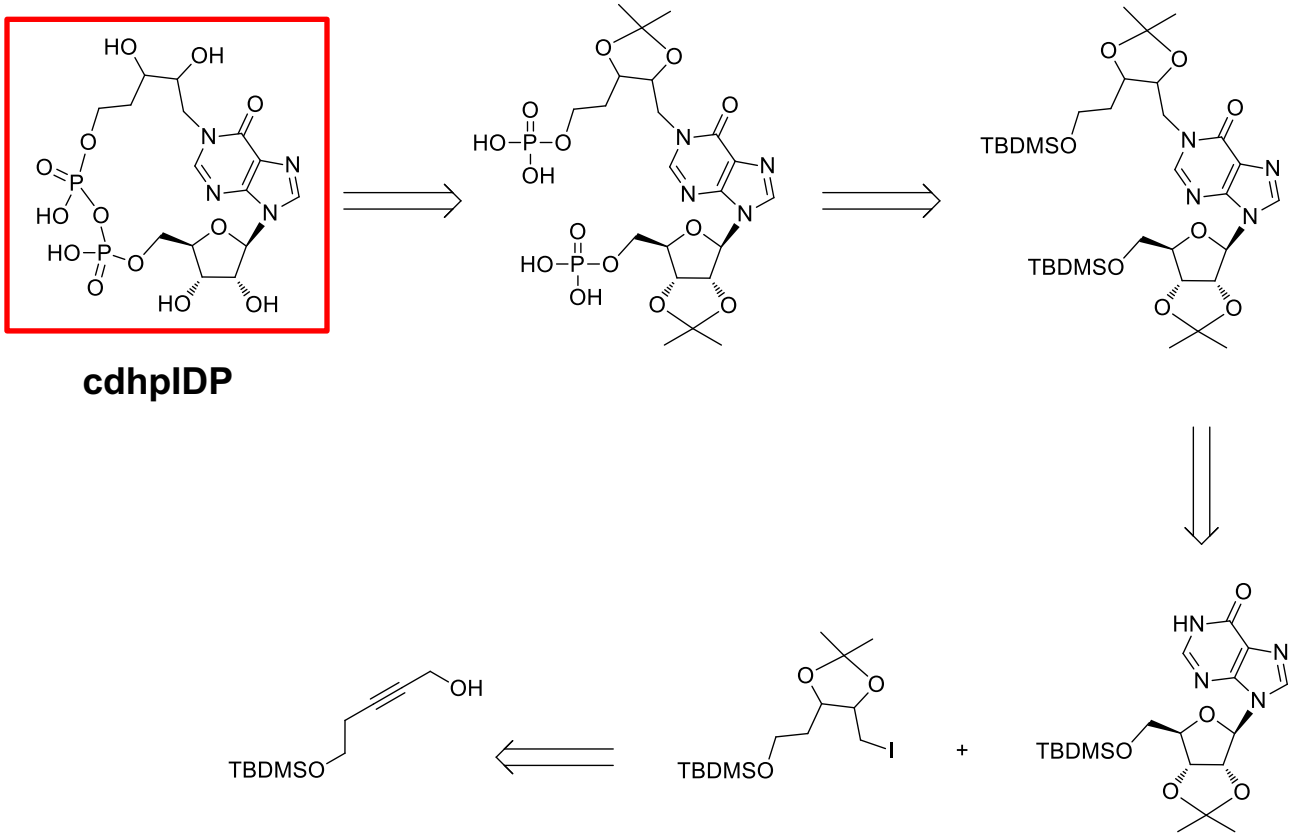
Synthesis of cyclic N^1-pentylinosine phosphate, a new structurally reduced cADPR analogue with calcium-mobilizing activity on PC12 cells

Ahmed Mahal[‡1], Stefano D'Errico[‡1], Nicola Borbone[1], Brunella Pinto[1], Agnese Secondo[2], Valeria Costantino[1], Valentina Tedeschi[2], Giorgia Oliviero[*1], Vincenzo Piccialli[3] and Gennaro Piccialli[1,4]

Beilstein J. Org. Chem. **2015,** *11,* 2689–2695.

Results and Discussion

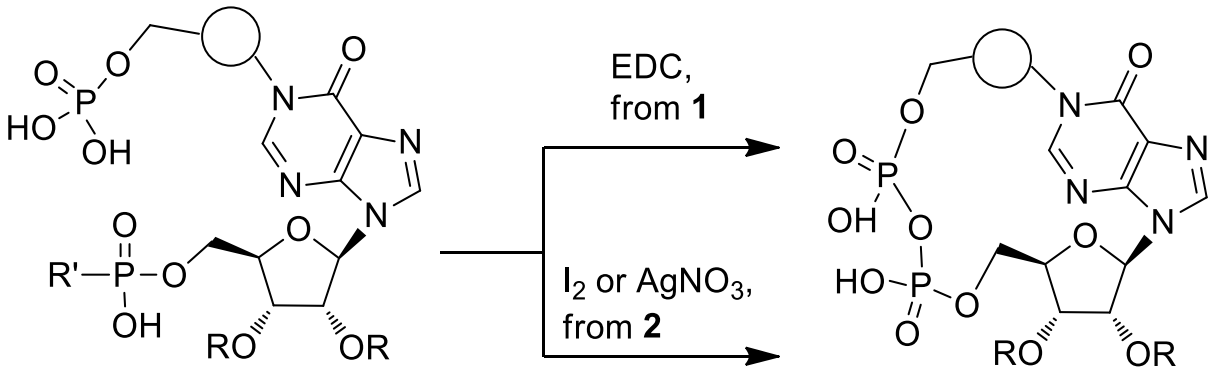
Synthesis of the Novel Stable cADPR Analogue – Retrosynthetic Analysis



D'Errico, S. et. al. "Probing the Ca^{2+} Mobilizing Properties on Primary Cortical Neurons of a New Stable cADPR Mimic" *Bioorg. Chem.*, accepted

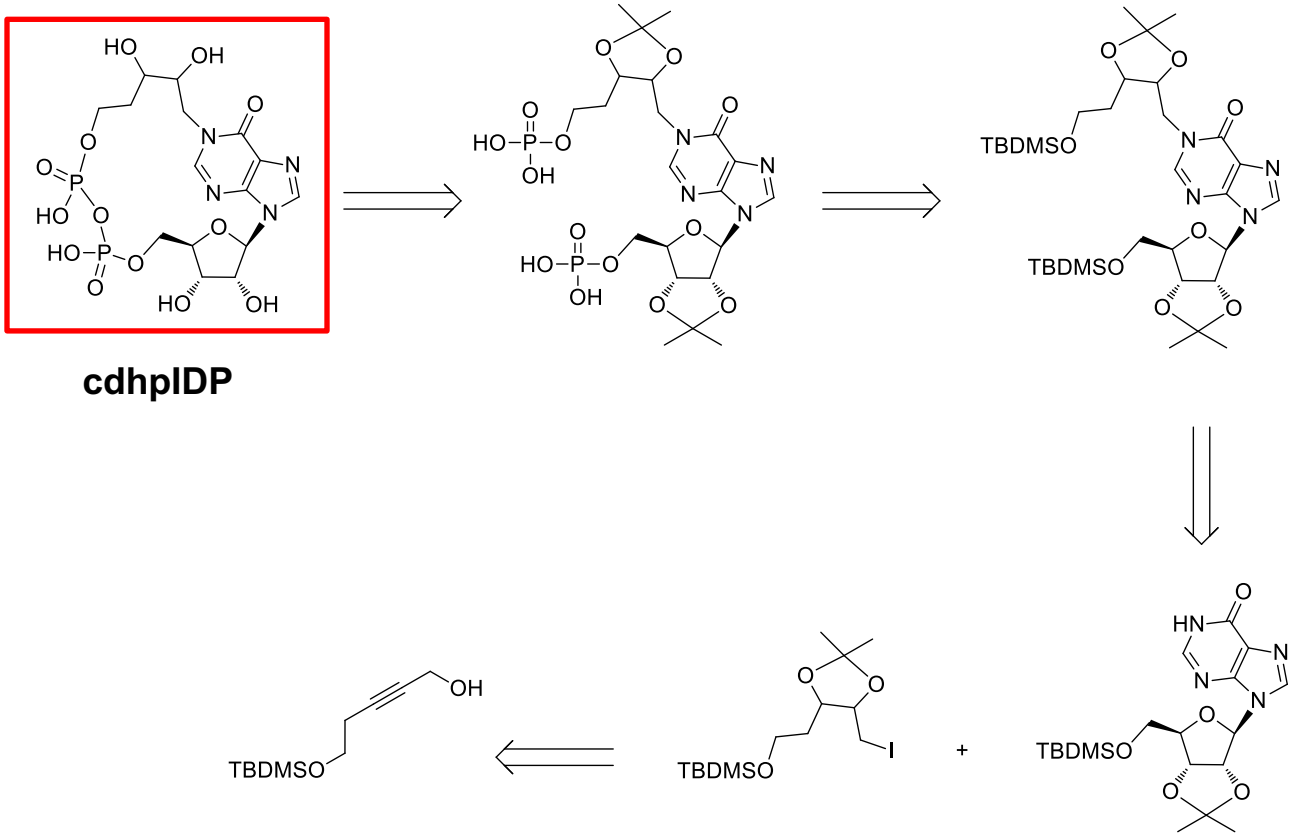
Results and Discussion

Strategies for the Pyrophosphate Bond Formation



Results and Discussion

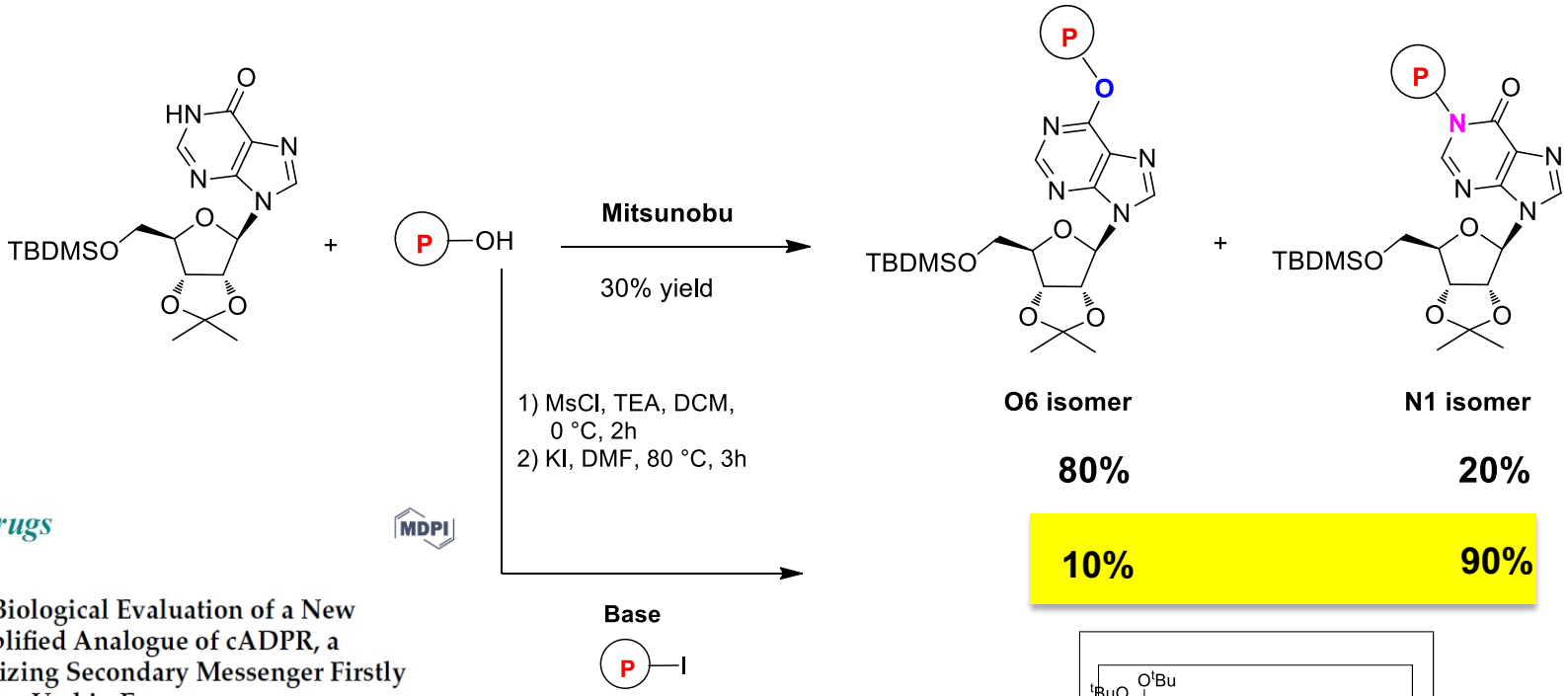
Synthesis of the Novel Stable cADPR Analogue – Retrosynthetic Analysis



D'Errico, S. et. al. "Probing the Ca^{2+} Mobilizing Properties on Primary Cortical Neurons of a New Stable cADPR Mimic" *Bioorg. Chem.*, accepted

Results and Discussion

Inosine N1 Alkylation – A Study of the Reaction Regioselectivity



marine drugs

MDPI

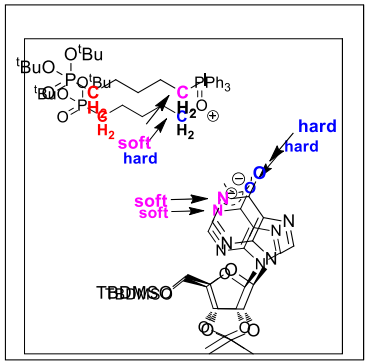
Article

Synthesis and Biological Evaluation of a New Structural Simplified Analogue of cADPR, a Calcium-Mobilizing Secondary Messenger Firstly Isolated from Sea Urchin Eggs

Stefano D'Errico [1,2], Nicola Borbone [1,2], Bruno Catalanotti [1], Agnese Secondo [3], Tiziana Petrozziello [3], Ilaria Piccialli [3], Anna Pannaccione [3], Valeria Costantino [1], Luciano Mayol [1], Gennaro Piccialli [1,3] and Giorgia Oliviero [2,4,*]

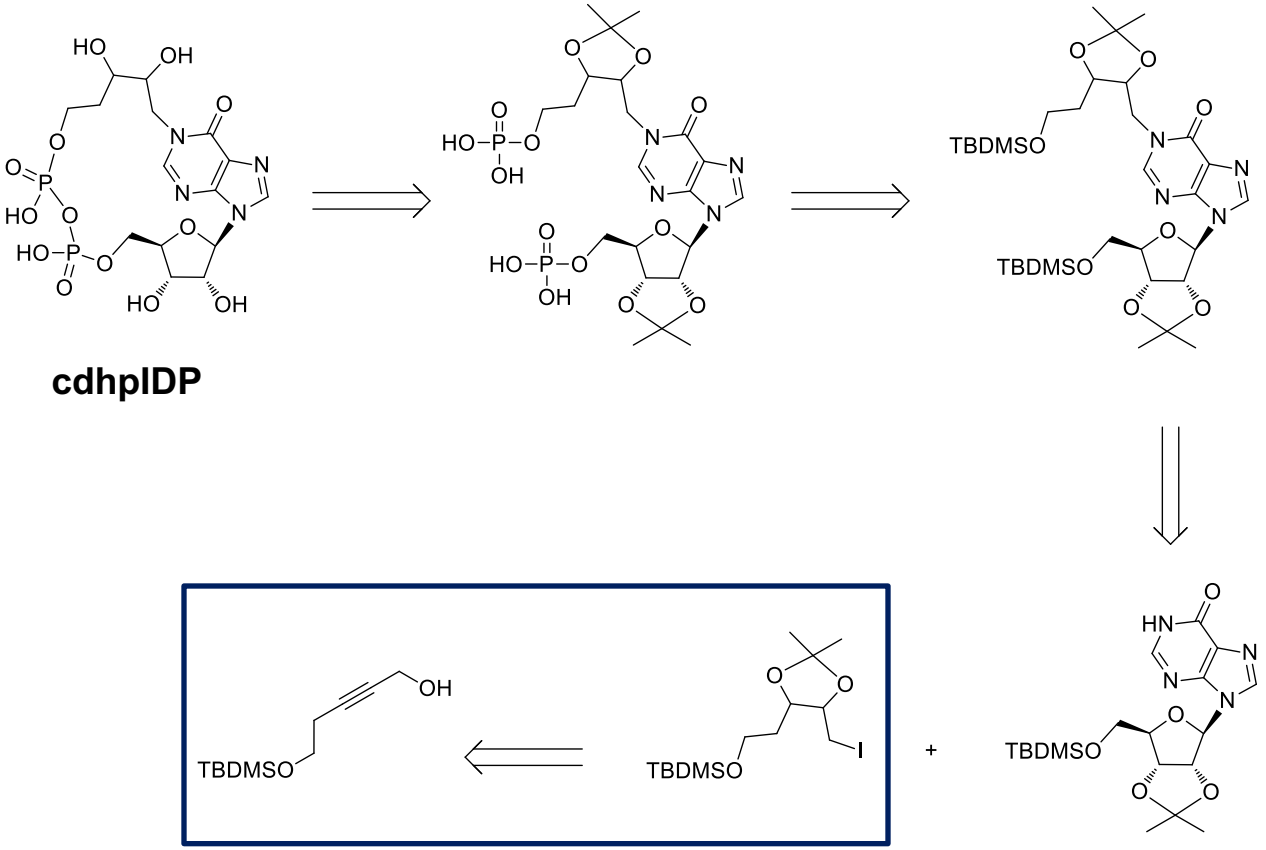
Mar. Drugs **2018**, *16*, 89; doi:10.3390/md16030089

Base	Yield (%)
DBU	14%
TEA	30%
K_2CO_3	90%



Results and Discussion

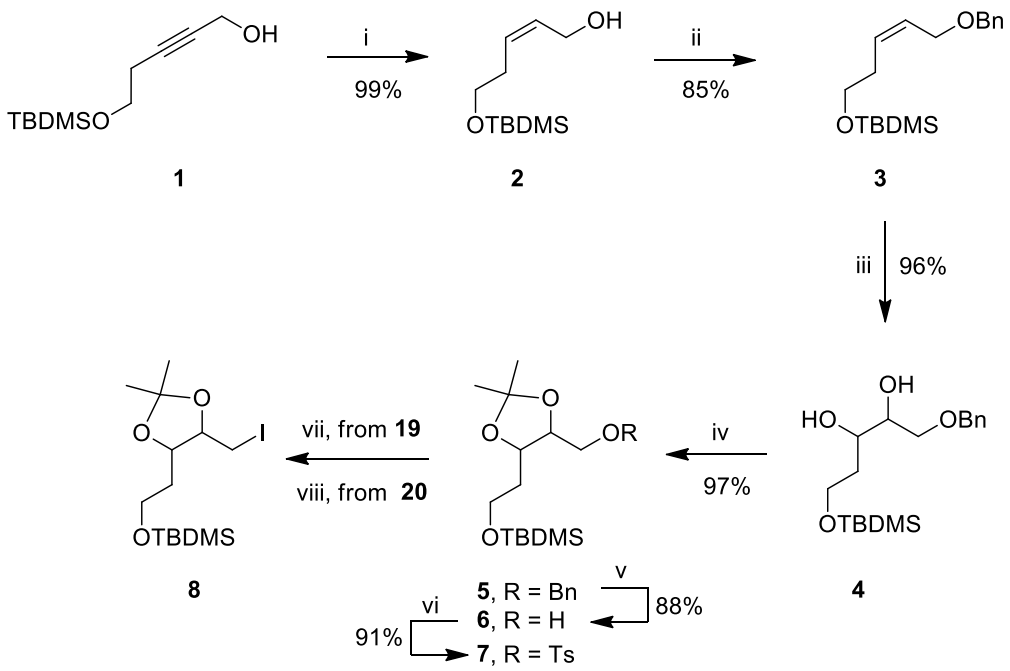
Synthesis of the Novel Stable cADPR Analogue – Retrosynthetic Analysis



D'Errico, S. et. al. "Probing the Ca^{2+} Mobilizing Properties on Primary Cortical Neurons of a New Stable cADPR Mimic" *Bioorg. Chem.*, accepted

Results and Discussion

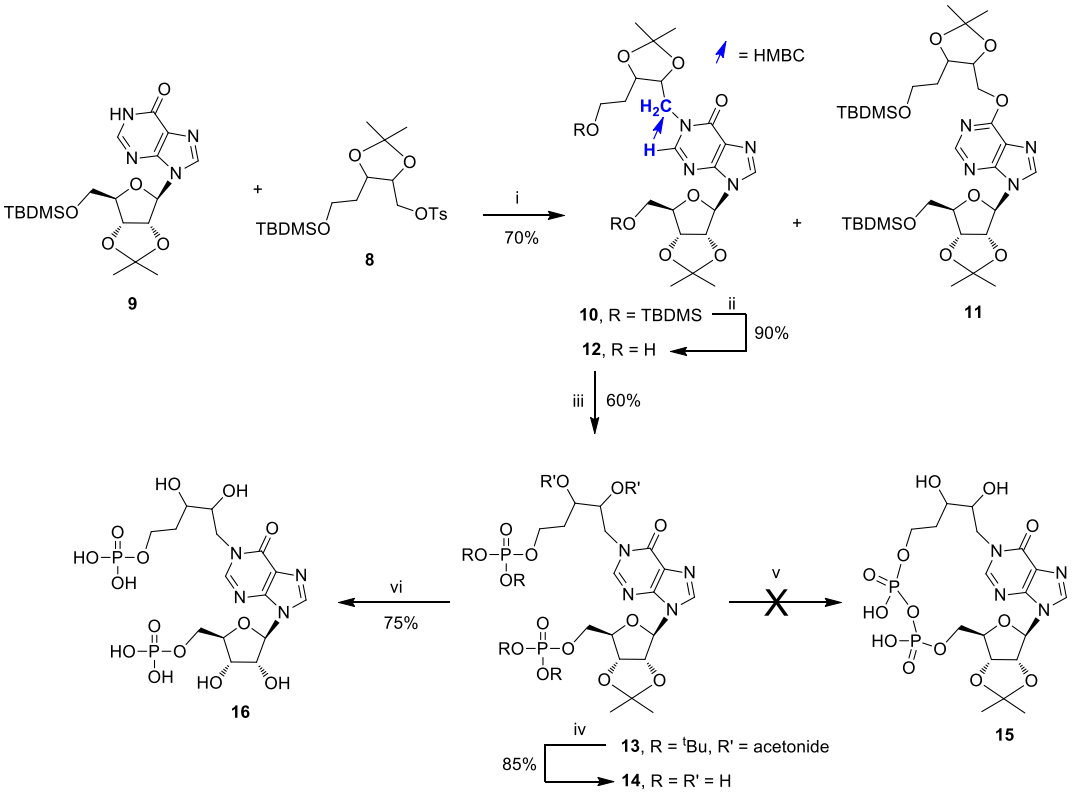
Synthesis of the Electrophile



Scheme. Reagents and Conditions: i) Lindlar's catalyst, Quinoline, r.t., 1 h 30 min.; ii) NaH, Benzyl bromide (BnBr), Tetrabutylammonium iodide (TBAI), THF, 0 °C→r.t., 16 h; iii) Osmium tetroxide (OsO_4), N-Methyl morpholine N-oxide (NMO), Acetone/H_2O, 9:1, 0 °C→r.t., 16 h; iv) PyOTs, 2,2-Dimethoxypropane (DMP), Acetone, 40 °C, 1 h; v) H_2, Pd/C, AcOEt, r.t., 2 h.; vi) *p*-Toluensulfonyl chloride (TsCl), Triethylamine (TEA), CH_2Cl_2, reflux, 16 h; vii) PPh_3, I_2, Imidazole, THF, reflux, 16 h or Methyltriphenoxyphosphonium iodide (MTPI), TEA, DMF, 60 °C, 16 h; viii) NaI, 2-Butanone, reflux, 16 h or KI, DMF, 80 °C, 5 h.

Results and Discussion

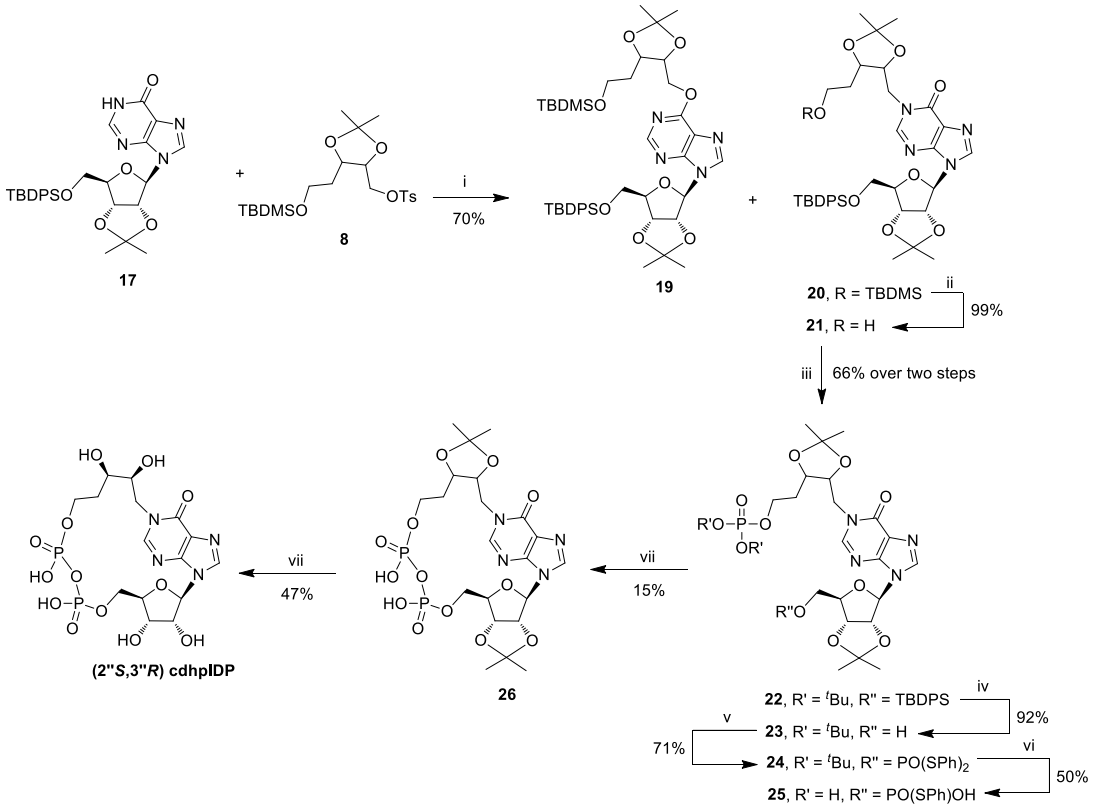
Synthesis of the Novel Stable cADPR Analogue – 1st Synthethic Route



Scheme. Reagents and Conditions: i) DBU, DMF, 80 °C, 12 h; ii) TBAF, THF, r.t., 2 h; iii) a) $((^{t}BuO)_2PN(^{i}Pr)_2)$, 1-*H*-tetrazole, THF, r.t., 6 h, b) $^{t}BuOOH$, THF, r.t., 1 h; iv) 50% TFA in CH_2Cl_2, 0 °C→r.t., 4 h; v) EDC, DMF, Pyridine, r.t., 72 h; vi) 50% TFA in H_2O, 0 °C→r.t., 4h.

Results and Discussion

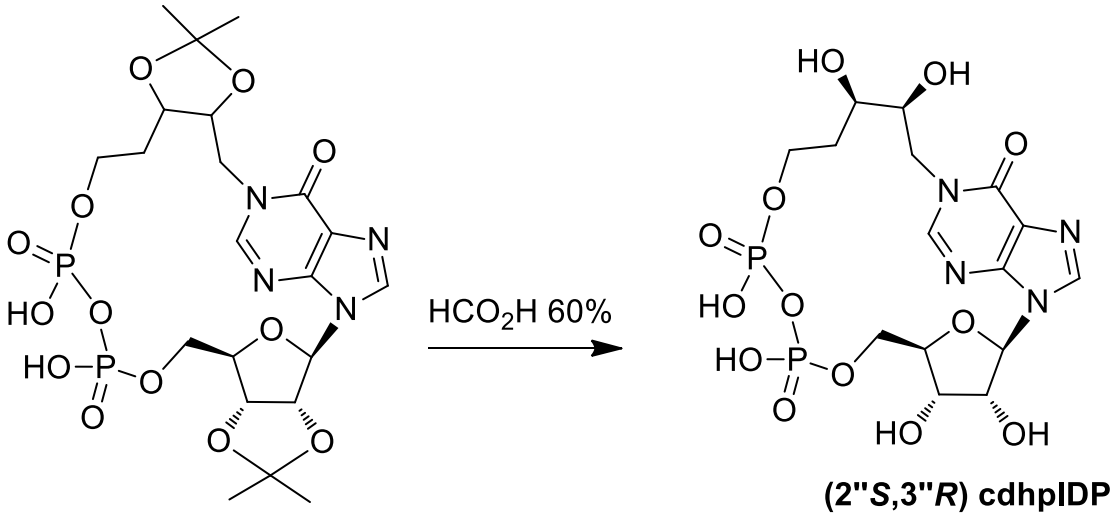
Synthesis of the Novel Stable cADPR Analogue – 2nd Synthethic Route



Scheme 5. Reagents and Conditions: i) DBU, DMF, 80 °C, 12 h; ii) PyOTs, EtOH, 40 °C, 1 h; iii) a) ((^{t}BuO)$_2$PN(^{i}Pr)$_2$), 1-*H*-tetrazole, THF, r.t., 6 h, b) ^{t}BuOOH, THF, r.t., 1 h; iv) TBAF, THF, r.t., 2 h; v) PSS, TPSCl, Pyridine, 40 °C, 16 h; vi) 50% TFA in CH_2Cl_2, 0 °C→r.t., 2 h; vii) I_2, Molecular sieves (MS) 3Å, Pyridine, r.t., 16 h; viii) 60% aqueous HCO_2H, r.t., 4 h.

Results and Discussion

Obtainment of the Target Compound

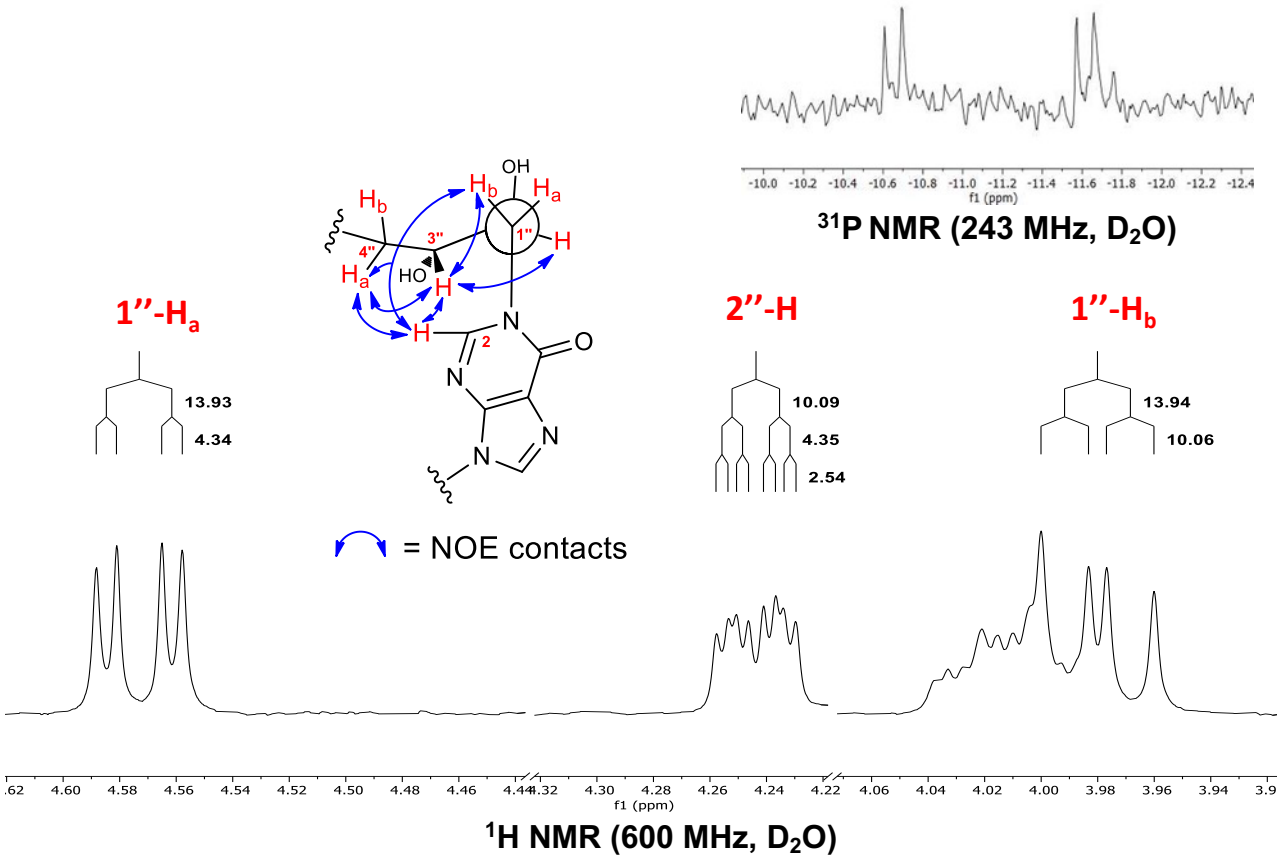


- ✓ ***The two main HPLC peaks eluted at t_R = 11.9 and 12.5 min. corresponded to the two cyclic fully deprotected diastereomers***

- ✓ ***The diastereomer eluted at lower t_R was recovered pure***

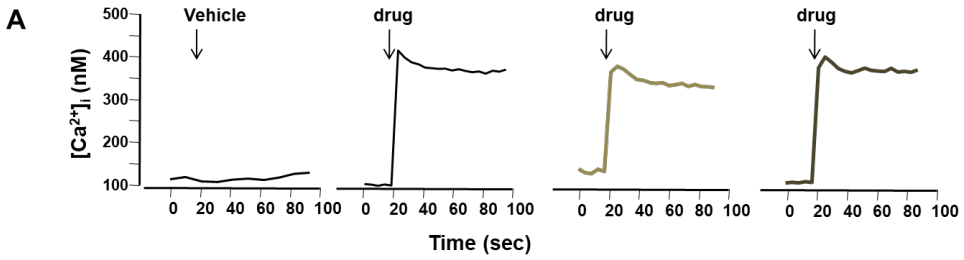
Results and Discussion

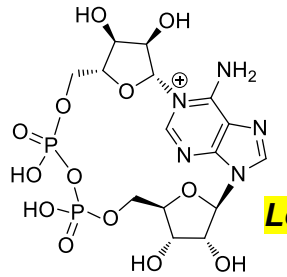
Determination of the Stereochemistry of 2'' and 3'' Carbon Atoms of the Pure Diastereomer



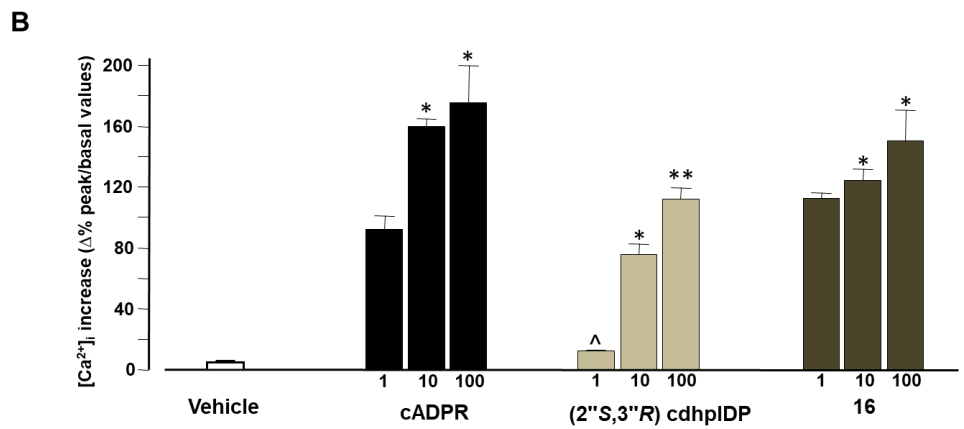
Results and Discussion

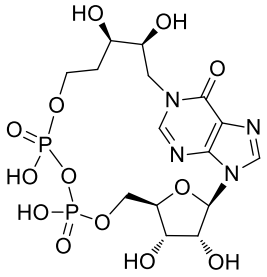
Effect of (2"S,3"R) cdhpIDP on Intracellular $[Ca^{2+}]_i$ in Primary Cortical Neurons



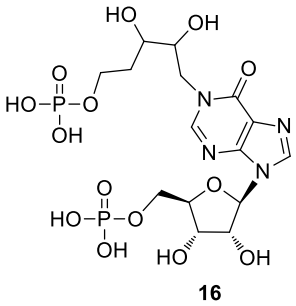


LogP = -3.95





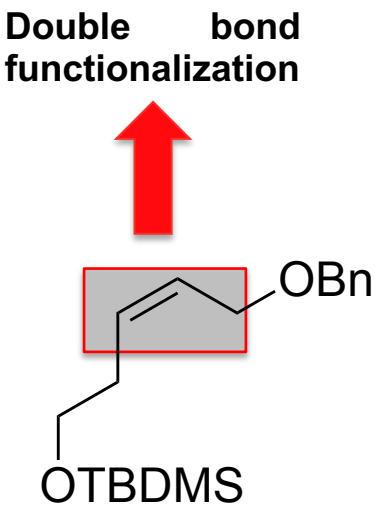
LogP = -3.64



LogP = -4.04

Figure. Effect of **cADPR**, **(2"*S*,3"*R*) cdhpIDP** and **16** on $[Ca^{2+}]_i$ in rat primary cortical neurons**.** Panel (A): representative single-cell trace of the effect of **cADPR** (100 nM), **(2"*S*,3"*R*) cdhpIDP** (100 nM) and **16** (100 nM) on $[Ca^{2+}]_i$. Panel (B): quantification of $[Ca^{2+}]_i$ increase calculated as the percentage change of plateau/basal value after the addition of each compound. Krebs-Ringer saline solution was used as vehicle. Calculated EC50 for **cADPR** was 0.9±0.005 nM; for **(2"*S*,3"*R*) cdhpIDP** 6.3±0.05 nM; for **16** 0.3±0.005 nM. *, $p < 0.05$ versus 1 nM; **, $p < 0.05$ versus previous concentration; ^, $p < 0.05$ versus 1 nM of **1**.

Future Perspective



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Expansion of the collection of new cADPR inspired modulators of intracellular Ca^{2+} concentration

Acknowledgements

Dipartimento di Farmacia - UNINA

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Dr. V. Tedeschi

SYSBIO
CENTRE OF SYSTEMS BIOLOGY

IBB-CNR, Napoli

Dr G. Roviello

IC-CNR, Bari

Dr M. Marzano

…and *YOU* for Your Kind Attention!