

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 278

www.rsc.org/obc

PAPER

Synthesis of cyclic adenosine 5'-diphosphate ribose analogues: a C2' *endo/syn* "southern" ribose conformation underlies activity at the sea urchin cADPR receptor†

Christelle Moreau,^a Gloria A. Ashamu,^a Victoria C. Bailey,^a Antony Galione,^b Andreas H. Guse^c and Barry V. L. Potter^{*a}

Received 7th July 2010, Accepted 14th September 2010

DOI: 10.1039/c0ob00396d

Novel 8-substituted base and sugar-modified analogues of the Ca²⁺ mobilizing second messenger cyclic adenosine 5'-diphosphate ribose (cADPR) were synthesized using a chemoenzymatic approach and evaluated for activity in sea urchin egg homogenate (SUH) and in Jurkat T-lymphocytes; conformational analysis investigated by ¹H NMR spectroscopy revealed that a C2' *endo/syn* conformation of the "southern" ribose is crucial for agonist or antagonist activity at the SUH-, but not at the T cell-cADPR receptor.

Introduction

Cyclic adenosine 5'-diphosphoribose (cADPR, **1**, Fig. 1), a metabolite of nicotinamide adenine dinucleotide (NAD⁺), was first discovered in 1987 by Lee and co-workers as a potent Ca²⁺ releasing second messenger.¹ Based on NMR and mass spectroscopy this dinucleotide was suggested to possess a cyclic structure with a glycosidic bond between N6 of the adenine ring and the anomeric carbon C1'' of the ribose linked to nicotinamide.² Later, the structure of cADPR was finally revealed by X-ray analysis to be a unique cyclic dinucleotide bearing two glycosidic

bonds between N9 and C1' of the ribose ring (or "southern" ribose) and N1 and C1'' of the second ribose moiety (or "northern" ribose). The structure also revealed both *N*-glycosidic bonds to be in the β -configuration and the "southern" ribose to be predominantly in the C2'-*endo* conformation.³ The cADPR/Ca²⁺ signalling system is active in diverse cellular systems such as animal cells *e.g.* smooth, skeletal and cardiac muscle, acinar cells, protozoa and plant cells.⁴ Pharmacological studies indicate that ryanodine receptors are the intracellular Ca²⁺ channels involved in cADPR-induced Ca²⁺ release.⁵⁻⁷

Many cADPR analogues have been synthesized since and their Ca²⁺ mobilising activities examined in various systems, but mainly in sea urchin egg homogenates (SUH) and Jurkat T cells (JTC).⁸⁻¹² Despite these efforts and although many useful synthetic tools have been developed, the structural features needed for both agonist/antagonist activities at the receptor still remain somewhat unclear. Early findings seemed to reveal that substitution at the 8-position of the adenine ring of cADPR (**2** and **3**, Fig. 1) converts a cADPR agonist into an antagonist in both SUH and JTC.^{13,14} However, it was later discovered that some 8-substituted cyclic

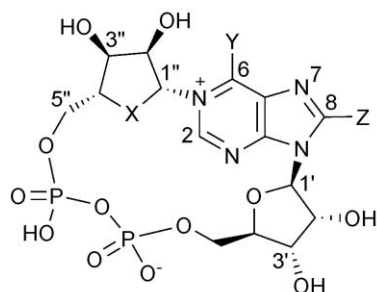
^aWolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Bath, UK, BA2 7AY. E-mail: b.v.l.potter@bath.ac.uk; Fax: +44 1225 386114; Tel: +44 1225 386639

^bDepartment of Pharmacology, University of Oxford, Mansfield Road, Oxford, UK, OX1 3QT

^cInstitute of Biochemistry and Molecular Biology I: Cellular Signal Transduction, University Medical Center Hamburg-Eppendorf, Germany

† This paper is part of an *Organic & Biomolecular Chemistry* web theme issue on chemical biology.

"Northern" ribose



"Southern" ribose

- X = O, Y = NH₂, Z = H, cADPR **1**
- X = O, Y = NH₂, Z = Br, 8-Bromo cADPR **2**
- X = O, Y = NH₂, Z = NH₂, 8-Amino cADPR **3**
- X = C, Y = NH₂, Z = H, cADPcR **4**
- X = C, Y = NH₂, Z = Br, 8-Bromo cADPcR **5**
- X = C, Y = NH₂, Z = N₃, 8-Azido cADPcR **6**
- X = C, Y = NH₂, Z = NH₂, 8-Amino cADPcR **7**
- X = O, Y = OH, Z = H, cIDPR **8**

Fig. 1 cADPR analogue structures and numbering system.

adenosine diphosphocarbocyclic ribose (cADPcR, 4–7, Fig. 1) analogues are agonists rather than antagonists in SUH, therefore suggesting that the oxygen atom of the “northern” ribose could be a crucial feature for antagonistic activity.¹⁵ A small number of 8-substituted cyclic inosine diphosphoribose (cIDPR, 8, Fig. 1) analogues have been synthesised in our laboratory. Some of these acted as agonists in T cells, suggesting the 6-amino group to be an important structural feature for antagonistic activity.^{16–18} Use of this template has led to structural biology insight on the cADPR hydrolase CD38.¹⁹ Modification of the base moiety of cADPR has produced an agonist analogue in 3-deaza cADPR, 70 times more potent than cADPR in SUH.²⁰ More radical structural modifications of the “northern” ribose led to agonist analogues.²¹ Agonistic activity was also observed when the pyrophosphate linkage was extended to a triphosphate.²² Further modifications of the “southern” ribose revealed that the 2'-OH has little effect on agonist activity in SUH, but that 3'-O-alkylation could generate an antagonist.²³ A recent NMR conformational study of this compound related the antagonism observed to an altered “backbone” conformation, but the evidence was not sufficient to establish this idea.²⁴

Recently, we successfully synthesized a series of 8-substituted 2'-deoxy analogues of cADPR.²⁵ Structure–activity relationship studies revealed that deletion of the 2'-OH group decreases antagonistic activity but, more importantly, some classical antagonist analogues unexpectedly showed agonistic activity at high concentrations in SUH. While some parallel trends were observed

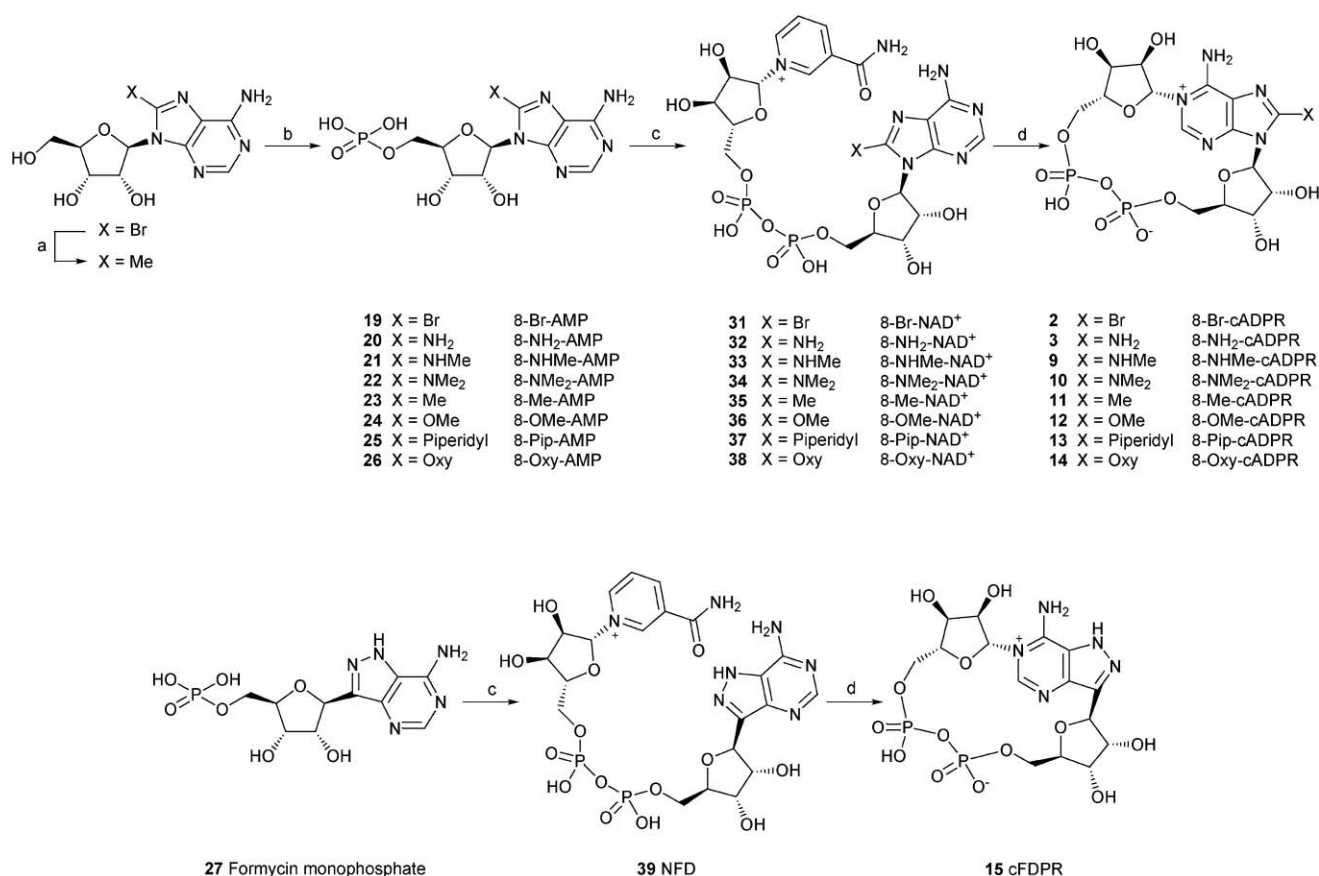
between analogues acting at both SUH and JTC cADPR receptors it is becoming clear, not surprisingly, that the receptors in these invertebrate and mammalian systems are different. In any case, these results illustrate that the global structural features required for cADPR agonism/antagonism are far from clear and are also different from system to system. There is clearly a need to rationalise the considerable range of activities now observed and deduce trends to underpin future design strategies in this area for chemical biological intervention.

Here, we report the synthesis of several new cADPR analogues (9–18) modified at the “southern” ribose and in the purine ring, as well as their biological evaluation in both SUH and JTC. Also, we report, for the first time, how agonism/antagonism in SUH may be linked to partial conformational preference in cADPR. A preliminary account of some of the synthetic work has appeared.¹⁴

Results and discussion

Chemistry

Synthesis of 8-modified cADPR analogues. All the cADPR analogues (2, 3, 9–15) described herein were synthesized chemoenzymatically; the last step being the enzymatic cyclisation reaction of the requisite NAD⁺ analogue catalyzed by ADP-ribosyl cyclase from *Aplysia californica*. Syntheses of the 8-modified cADPR analogues are summarised in Scheme 1.



Scheme 1 Synthesis of 8-modified cADPR analogues. *Reagents and conditions:* (a) PdCl₂, PPh₃, AlMe₃, THF, reflux, 2.5 h. (b) POCl₃, TEA, 0 °C. (c) β-NMN⁺, DCC, pyridine:water (4 : 1), 7 days, rt. (d) *Aplysia* cyclase, 25 mM HEPES (pH 6.8), 20 min, rt.

8-Bromo adenosine 5'-monophosphate (**19**) was prepared using established methodology developed by Yoshikawa *et al.*²⁶ Crude product was purified on an ion-exchange Q-Sepharose column eluted with a gradient of 1 M TEAB followed by a second column of activated charcoal to removed the inorganic phosphate contaminant. 8-Methyl adenosine 5'-monophosphate (8-Me-AMP, **23**) was prepared in 2 steps by a palladium-catalysed coupling reaction of 8-Br-adenosine with a methylating agent, followed by phosphorylation. Other AMP derivatives (**20–26**) were prepared by nucleophilic displacement on 8-bromo AMP by the appropriate agent. 8-modified NAD⁺ analogues (**31–39**) were synthesised by a method developed by Hughes *et al.* which consists of the coupling of β -nicotinamide mononucleotide (β -NMN⁺) with the corresponding monophosphate in the presence of dicyclohexylcarbodiimide (DCC) as a coupling reagent.²⁷ This method, however, was low yielding (7–61%) and lately we have used a better approach involving the coupling of a monophosphate with a nucleotide phosphoromorpholide in the presence of a Lewis acid.^{16,25}

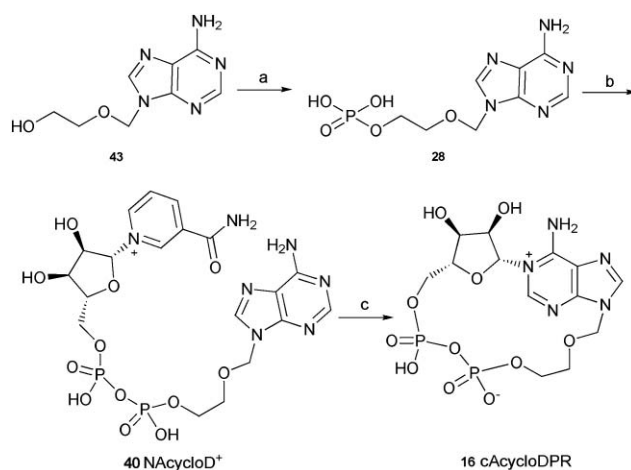
All of the final nucleotide pyrophosphates were carefully separated from any monophosphates and purified to homogeneity by ion-exchange chromatography. Subsequent incubation with the *Aplysia* enzyme, followed by purification by ion-exchange produced the desired 8-modified cADPR analogues. These were: 8-bromo cyclic adenosine diphosphoribose (8-Br-cADPR, **2**), 8-amino cyclic adenosine diphosphoribose (8-NH₂-cADPR, **3**), 8-methylamino cyclic adenosine diphosphoribose (8-NHMe-cADPR, **9**), 8-dimethylamino cyclic adenosine diphosphoribose (8-NMe₂-cADPR, **10**), 8-methyl cyclic adenosine diphosphoribose (8-Me-cADPR, **11**), 8-methoxy cyclic adenosine diphosphoribose (8-OMe-cADPR, **12**), 8-piperidyl cyclic adenosine diphosphoribose (8-Pip-cADPR, **13**), 8-oxy cyclic adenosine diphosphoribose (8-Oxy-cADPR, **14**) and 8-aza-9-deaza cyclic adenosine diphosphoribose (cFDPR, **15**).

Synthesis of ribose-modified cADPR. To further investigate the requirements of the adenosine ribose hydroxyls for Ca²⁺ mobilisation, three novel compounds were designed (i) cyclic acycloadenosine diphosphate ribose (cAcycloDPR, **16**) in which the ribose ring was replaced with a flexible ether chain, (ii) cyclic adenine 9- β -D-arabino ribofuranoside diphosphate ribose (cAraDPR, **17**) in which the stereochemistry of the 2'-OH was reversed and (iii) cyclic 2',3'-*O*-isopropylidene adenosine diphosphate ribose (cAcetDPR, **18**) in which the 2',3' diol was protected with an isopropylidene group.

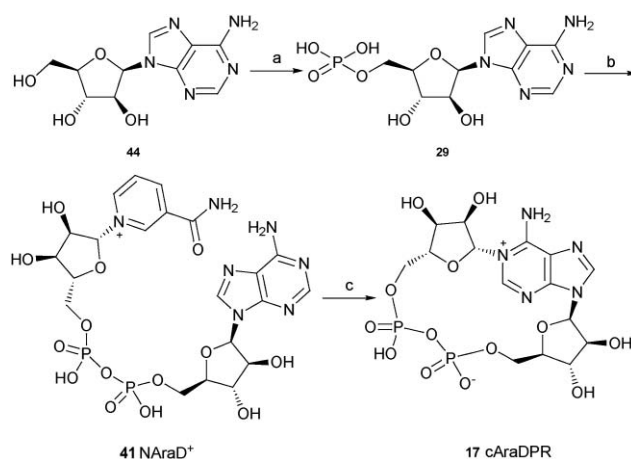
The synthesis of these three analogues **16**, **17** and **18** is summarised in Schemes 2, 3 and 4 respectively. Acyclic adenosine^{28,29} (**43**), arabinofuranoside (**44**) and isopropylidene protected adenosine (**45**) were selectively phosphorylated at the 5'-hydroxyl using a POCl₃, triethylphosphate and water mixture to give their respective monophosphates **28**, **29** and **30**. Subsequent pyrophosphate bond formation followed by cyclase incubation under the general conditions described for the 8-modified analogues, generated the cAcycloDPR (**16**), cAraDPR (**17**), cAcetDPR (**18**) analogues respectively.

Biology

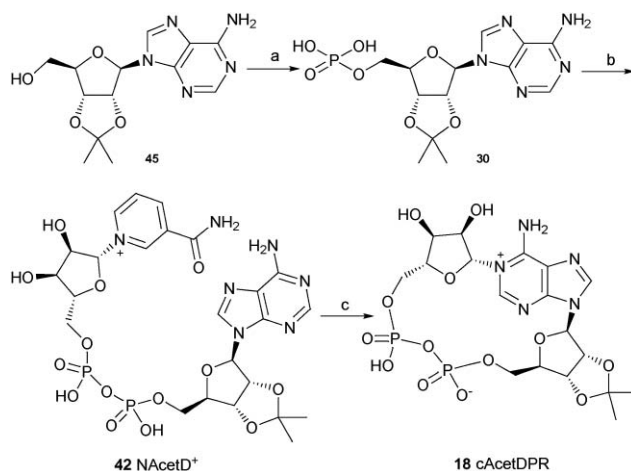
Biological evaluation of 8-modified analogues of cADPR on Ca²⁺-release in sea urchin egg homogenate. The first indication



Scheme 2 Synthesis of cAcycloDPR. Reagents and conditions: (a) POCl₃, TEPE, 0 °C, 1.5 h then ice/water. (b) β -NMN⁺, DCC, 7 days, rt. (c) *Aplysia* cyclase, 25 mM HEPES (pH 6.8), rt.



Scheme 3 Synthesis of cAraDPR. Reagents and conditions: (a) POCl₃, TEPE, 0 °C, 1.5 h then ice/water. (b) β -NMN⁺, DCC, 7 days, rt. (c) *Aplysia* cyclase, 25 mM HEPES (pH 6.8), rt.



Scheme 4 Synthesis of cAcetDPR. Reagents and conditions: (a) POCl₃, TEPE, 0 °C, 1.5 h then ice/water. (b) β -NMN⁺, DCC, 7 days, rt. (c) *Aplysia* cyclase, 25 mM HEPES (pH 6.8), rt.

that an exocyclic substitution in position 8 of the adenine ring of cADPR might be important in designing an antagonist of cADPR-induced Ca^{2+} release was demonstrated by Walseth & Lee.¹³ They found that substitution of H_A8 with an amino group converted the cADPR from an agonist into an antagonist.²⁵ Two other analogues, 8-bromo and 8-azido-cADPR were also found to be antagonists, but with lesser potency. The potency of 8-azido-cADPR was in between that of 8-amino-cADPR and 8-bromo-cADPR, although IC_{50} values were not reported. It was suggested that the size of the substituent (expressed in atomic units) may be responsible for the difference in potency of the three 8-substituent analogues synthesised as antagonists. The larger the size of the substituent at this position the lower the potency, as an increase in size from 16 ($-\text{NH}_2$) to 42 ($-\text{N}_3$) to 79 ($-\text{Br}$) resulted in a decrease in potency as an antagonist in this order. We have therefore synthesised various 8-substituted analogues of cADPR in order to further investigate this hypothesis.

8-Amino-cADPR (**3**) was also synthesised as a control to enable us to carry out comparative studies. 8-Amino-cADPR was confirmed as a potent antagonist in SUH with an IC_{50} value of 0.01 μM . Replacement of the amino group with a methyl group as in 8-methyl-cADPR (**11**) gave an antagonist with an IC_{50} value of 0.53 μM . The $-\text{CH}_3$ group is similar in atomic mass to $-\text{NH}_2$ and yet 8-Me-cADPR was 53 times less potent as an antagonist compared to 8- NH_2 -cADPR (**3**). Substitution with an oxy group (with an atomic mass of 17) was attempted. The analogue obtained (**14**) showed still weaker activity as an antagonist with an approximate IC_{50} of 2 μM . 8-“Hydroxy” AMP (**26a**) is known to exist predominantly in the keto form (**26b**) at physiological range $5 < \text{pH} < 9$,^{30,31} hence the nitrogen N7 is protonated (Fig. 2). It is reasonable to assume that 8-oxy cADPR is also predominantly in the keto form at the same pH range. The reduction in activity could be due to the protonation at N7 which may affect receptor binding. Substitution with groups of varying size such as $-\text{NHMe}$, $-\text{NMe}_2$ and $-\text{piperidyl}$ produced novel compounds (**9**, **10** and **13** respectively) with perturbations to the 8-amino motif, which were investigated for antagonist activity (Table 1). 8-NHMe-cADPR (**9**) was a much weaker antagonist compared to 8- NH_2 -cADPR (**3**) with an IC_{50} of ~ 40 μM , 8-NMe₂ cADPR (**10**) was weaker than 8-NHMe-cADPR (**9**) as an antagonist, but 8-piperidyl (**13**) was not active at all as an antagonist up to 50 μM . Other analogues such as 8-OCH₃-cADPR (**12**) and 8-Br-cADPR (**2**) were also synthesised for comparative study. These analogues were antagonists with IC_{50} values of 4.8 and 0.97 μM respectively. It appears, therefore, from this study, that the ability of the molecule to hydrogen bond at the 8-position with its target protein in SUH is not critical for activity as an antagonist as 8- CH_3 -cADPR (**11**) is a better antagonist compared to those that can form hydrogen bonds such as 8-OCH₃-cADPR (**12**) and 8-NHMe-cADPR (**9**).

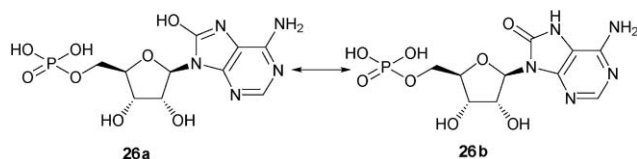


Fig. 2 Tautomeric form of 8-“hydroxyl” AMP (**26a**) vs. 8-oxy AMP (**26b**).

Table 1 Antagonistic activities^a of cADPR analogues **2**, **3**, **9–14**

8-X-cADPR	IC_{50} (μM) in SUH	IC_{50} (μM) in JTC
8- NH_2 -cADPR (3)	0.01	1
8-Me-cADPR (11)	0.53	n.d
8-Br-cADPR (2)	0.97	^b
8-Oxy-cADPR (14)	2	n.d
8-OMe-cADPR (12)	4.8	0.2
8-NHMe-cADPR (9)	<40	n.d
8-NMe ₂ -cADPR (10)	>40	n.d
8-Pip-cADPR (13)	No effect	n.d

^a Ca^{2+} mobilisation from sea urchin egg microsomes was evaluated fluorimetrically using 2.5% egg homogenates containing fluo-3 (3 μM) as previously described.^{25,33} Ca^{2+} release assay in Jurkat T cells was carried out as reported previously.^{34,35} ^b IC_{50} was not reached (partial antagonist). nd = active compound, IC_{50} was not determined.

Modification in the purine ring as in 8-aza-9-deaza cADPR (**15**), resulted in an analogue that is 10 times less potent as an agonist compared to cADPR ($\text{EC}_{50} = 0.31$ μM). The C8 carbon in the purine ring was replaced by a nitrogen atom and the N9 nitrogen was replaced by a carbon atom. Modification in the purine ring hence did not produce an antagonist compared to exocyclic modification as discussed above. The reduction in activity could be due to protonation of the N7 nitrogen which may affect receptor binding. A small modification at this position as in 7-deaza cADPR (**51**), has been reported to result in partial agonist activity.³²

Biological evaluation of sugar-modified analogues of cADPR on Ca^{2+} release in sea urchin egg homogenate. Preliminary work has already been published. The importance of the 2'-OH was investigated. It was found that 2'-deoxy-cADPR is a potent agonist in SUH but not in T cells.²³ Other groups reported that 2'-cADPRP is an agonist in T cells but is inactive in SUH.^{8,36} Moreover, 3'-deoxy cADPR was found to be a poor agonist in SUH,²³ therefore indicating the importance of the 3'-OH group for agonistic activity in SUH, whilst the 2'-hydroxyl group appeared to be less important.

In order to further study these interactions three compounds (**16**, **17** and **18**) were designed, synthesised and evaluated for their Ca^{2+} mobilising activities in SUH. Both cAcycloDPR (**16**) and cAraDPR (**17**) were inactive whilst cAcetDPR (**18**) was a poor agonist ($\text{EC}_{50} = 12$ μM). A careful study of the biological data obtained for these compounds suggested that instead of there being a relationship between the groups attached to the ribose ring and activity, the conformation of the ribose ring is of importance and will be discussed later.

Biological evaluation of 8-modified analogues of cADPR on Ca^{2+} -release in permeabilised Jurkat T-cells. In order to design antagonists for cADPR-induced mechanisms in Jurkat T-cells (JTC), we decided to investigate whether 8- NH_2 -cADPR (**3**) and 8-Br-cADPR (**2**) would have a similar effect to that seen in the SUH system. We found that these two analogues inhibited Ca^{2+} release mediated by cADPR. 8-Br-cADPR was less effective as an antagonist compared to 8- NH_2 -cADPR. We decided to investigate whether there is a link between the size of the 8-substituent and the potency of the antagonist as an aid to designing potent inhibitors of the cADPR-induced mechanism. The extension to mammalian cells is essential for the aim of defining a wider applicability for the

cADPR signalling system. Substitution of any group other than hydrogen into position 8 of the adenine ring resulted in analogues with antagonist activity in this system. Hence, all the 8-substituted analogues synthesized inhibited cADPR-induced Ca^{2+} -release. 8- NH_2 -, 8- CH_3 - and 8- NMe_2 -cADPR (**3**, **11** and **10**) are potent inhibitors. The activity of 8- NMe_2 -cADPR (**10**), although weaker than its parent (**3**), suggests that hydrogen bond donation *via* the 8 position to the receptor is not a requirement for antagonism. 8- CH_3 -cADPR (**11**) completely abolished cADPR induced release, suggesting further that hydrogen bond interaction between the substituent and the receptor site is unlikely to be responsible for antagonist activity, as the $-\text{CH}_3$ group in 8-Me-cADPR cannot form a hydrogen bond. There is, however, a possibility of a hydrophobic interaction taking place, supported by the fact that 8-NHMe-cADPR (**9**) antagonized at higher concentrations and shows less potent inhibition as compared to 8- NMe_2 -cADPR (**10**), the $-\text{NMe}_2$ substituent being more hydrophobic than the $-\text{NHMe}$ group. Moreover, and in strong contrast to the lack of effect in SUH, 8-piperidyl-cADPR antagonized at low concentrations, broadly comparable to 8- NH_2 -cADPR (**3**). The piperidyl group, though large, is also hydrophobic and flexible. Hence, it can change its conformation to fit a receptor site. 8-Br-cADPR (**2**) showed relatively poor inhibition, possibly due to the bulk of the bromo substituent and, unlike the piperidyl group, the bromo atom is a large rigid structure and hence is potentially hindered from fitting tightly into the receptor site. 8-Oxo-cADPR (**14**) showed poor inhibition even though the substituent is similar in size to a $-\text{NH}_2$ and $-\text{CH}_3$ substituent. There are potential multiple alterations in this analogue compared to cADPR as the 8-oxo form can tautomerise to the 8-hydroxy form. This change, though small, may be sufficient to affect the interaction of this analogue with the receptor. 8- OCH_3 -cADPR (**12**) showed good inhibition comparable to 8- CH_3 -cADPR (**11**). This compound has found application in a seminal study on the role of cADPR in T cells.³⁷ Overall, it appears that the presence of a hydrophobic group in position 8 enhances antagonist activity of the analogue in permeabilised JTC.

Modification in the purine ring in analogue (**15**) did not affect the Ca^{2+} release property of the analogue. This analogue showed a similar Ca^{2+} release profile compared to cADPR in JTC even though the 8-position is altered (although not outside the ring). These alterations appear to be unimportant for Ca^{2+} releasing activity in this system.

Conformational analysis

Despite significant past work by several groups, the structural features required for cADPR-mediated agonism/antagonism remain unclear. Recently, Shuto *et al.* reported that 2'',3''-dideoxydidehydro cADPcR (**62**), an inactive compound, adopted a major $\text{C3}'$ *endo* and a high *anti* conformation in aqueous solution,³⁸ therefore indicating that both the N9 -ribose moiety and the N9 -glycosidic bond conformations may be of crucial importance for the Ca^{2+} release activities of cADPR analogues.

In solution, nucleosides and nucleotides exist in conformational equilibrium between $\text{C2}'$ -*endo* and $\text{C3}'$ -*endo* forms (Fig. 3). In addition, the nucleobase can be oriented towards (*syn*) or away (*anti*) from the ribose ring. These local changes will affect the overall conformation of the cyclic dinucleotides. Extensive studies

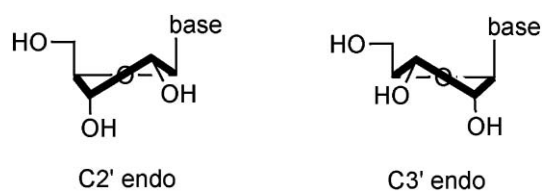


Fig. 3 Schematic representation of the ribofuranose ring in both $\text{C2}'$ *endo* and $\text{C3}'$ *endo* conformation.

developed by Altona and Sundaralingam have established that the $\text{C2}'$ -*endo*/ $\text{C3}'$ -*endo* ratio can be mathematically calculated from a ^1H NMR spectrum by adaptation of the equation $\text{C2}'$ -*endo* = $[J_{1,2'}/(J_{1,2'} + J_{3,4'})] \times 100$. Moreover, they have also shown that the sum $J_{1,2'} + J_{3,4'}$ is close to 10 and therefore the ratio $\text{C2}'$ -*endo*/ $\text{C3}'$ -*endo* can be estimated from the equation $10J_{1,2'}$.^{39,40}

In cADPR, the adenine base is oriented in the *syn* conformation about the glycosidic bond both in the crystal structure³ and in solution.^{24,38} Information on the conformation about the glycosidic bond has been well documented, and has revealed that the chemical shift of the H-2' proton could be used very effectively as a good indicator for the *syn/anti* equilibrium in nucleosides and nucleotides.^{41,42} Typically, purine nucleosides and nucleotides with a bulky substituent at C8 display a characteristic downfield shift of H-2' upon 8-substitution. The chemical shift values for the protons common to some AMP, NAD^+ and cADPR analogues prepared during the course of this study are listed in Table 2 and indeed, in agreement with early reports, we have found that the NMR resonance for H-2' can be used very effectively to assign the favoured glycosyl bond conformation. Therefore, AMP, 8-amino- and 8-aminomethyl AMP appear to be predominantly *anti* as previously reported.^{43,44} The H-2' resonance of 7-deaza AMP suggests that this nucleotide has significant *anti* glycosyl bond character, a result that is not surprising due to its resemblance to AMP. Our data show a similar trend for the H-2' resonance values in the NAD^+ series as in the AMP series. We had previously observed in the hypoxanthine series that the glycosyl bond conformation is unaffected by pyrophosphate bond formation.¹⁶ However, during the cyclization reaction, the NAD^+ analogues in the *anti* conformation *e.g.* NAD^+ , 8-amino-, 8-methylamino- and 7-deaza NAD^+ have their cyclic counterpart predominantly in the *syn* configuration.

We have therefore extracted information on the preferred conformation about the glycosidic bond based on NMR results, and have determined the conformation of the "southern" ribose using Altona's approach for all the cADPR analogues synthesized in our laboratory as well as those from other groups. The results of our analysis are summarized in Table 3. Structures of analogues **46–62** are shown in Fig. 4 and 5.

At first glance, it can be seen that most cADPR analogues adopt a *syn* conformation about the N9 -glycosyl linkage except cAcetDPR (**18**), cAraDPR (**17**) and 2'',3''-dideoxydidehydro cADPcR (**62**). These same compounds also adopt a $\text{C3}'$ *endo* form in the N9 -ribose moiety.

Additionally, 3'-deoxy cADPR, 2'-cADPRP and 3'-cADPRP also display a $\text{C3}'$ *endo* form, but with a predominantly *syn* glycosidic bond. It appears that these compounds are either inactive or are really poor agonists in SUH.²³ Conversely, all

Table 2 ¹H NMR chemical shifts (δ) and conformational analysis of 8-X-AMP, 8-X-NAD⁺ and 8-X-cADPR in D₂O

X	8-X-AMP				8-X-NAD ⁺				8-X-cADPR			
	H-1'	H-2'	Δ_{1-2} ^a	Conf ^b	H-1'	H-2'	Δ_{1-2}	Conf	H-1'	H-2'	Δ_{1-2}	Conf
H	6.1	4.8	1.3	<i>anti</i>	6.0	4.7	1.3	<i>anti</i>	5.8	5.2	0.6	<i>syn</i>
Br	5.8	5.1	0.7	<i>syn</i>	5.7	5.0	0.7	<i>syn</i>	6.3	5.6	0.7	<i>syn</i>
Me	5.8	4.8	1.0	<i>syn</i>	5.7	4.7	1.0	<i>syn</i>	5.8	5.3	0.5	<i>syn</i>
NH ₂	5.8	4.6	1.2	<i>anti</i>	5.9	4.6	1.2	<i>anti</i>	5.8	5.4	0.4	<i>syn</i>
NHMe	5.8	4.6	1.2	<i>anti</i>	5.7	4.5	1.2	<i>anti</i>	5.7	5.6	0.1	<i>syn</i>
NMe ₂	5.6	5.1	0.5	<i>syn</i>	5.9	5.4	0.5	<i>syn</i>	6.2	5.6	0.6	<i>syn</i>
Pip	5.6	5.1	0.5	<i>syn</i>	5.5	5.0	0.5	<i>syn</i>	5.9	5.6	0.3	<i>syn</i>
OMe	5.7	4.8	0.9	<i>syn</i>	5.7	4.8	0.9	<i>syn</i>	5.9	5.6	0.3	<i>syn</i>
Oxy	5.6	4.9	0.7	<i>syn</i>	5.5	4.9	0.6	<i>syn</i>	5.8	5.6	0.2	<i>syn</i>
7-Deaza	6.1	4.5	1.6	<i>anti</i>	6.1	4.4	1.7	<i>anti</i>	5.7	5.3	0.4	<i>syn</i>
7-Deaza-8-Br	6.0	5.1	0.9	<i>syn</i>	6.0	5.1	0.9	<i>syn</i>	6.0	5.4	0.6	<i>syn</i>

^a Difference in chemical shifts between H-1' and H-2'. ^b Favoured glycosidic bond conformation.

Table 3 N9 ribosyl moiety and N9 glycosidic bond conformation of cADPR analogues and their Ca²⁺ release activities in sea urchin egg homogenate

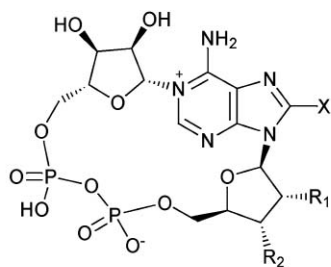
Compounds	$J_{1,2}$	$J_{3,4}$	C2' <i>endo</i>	H-1'	H-2'	Δ_{1-2}	Syn/Anti	Activity in SUH
Compounds synthesized by our group ^{14,23,25,32,45-50}								
cADPR (1)	5.6	3.2	64%	5.8	5.2	0.6	Syn	Agonist EC ₅₀ = 32 nM
8-Bromo-cADPR (2)	5.3	n.d ^a	53%	6.3	5.6	0.7	Syn	Antagonist IC ₅₀ = 0.97 μ M
8-NH ₂ cADPR (3)	5.6	n.d	56%	5.8	5.3	0.5	Syn	Antagonist IC ₅₀ = 0.01 μ M
8-NHMe cADPR (9)	5.5	n.d	55%	5.7	5.6	0.1	Syn	Weak antagonist IC ₅₀ = 40 μ M
8-NMe ₂ cADPR (10)	6.4	n.d	64%	6.2	5.6	0.6	Syn	Weak antagonist IC ₅₀ > 40 μ M
8-Me cADPR (11)	5.3	n.d	53%	5.8	5.3	0.5	Syn	Antagonist IC ₅₀ = 0.53 μ M
8-OMe cADPR (12)	5.9	n.d	59%	5.9	5.6	0.3	Syn	Antagonist IC ₅₀ = 4.8 μ M
8-Aza-9-deaza cADPR (15)	5.3	n.d	53%	5.9	5.0	0.9	Syn	Agonist EC ₅₀ = 0.3 μ M
<i>cAcylDPR</i> (16)	—	—	—	—	—	—	—	<i>Inactive</i>
<i>cAraDPR</i> (17)	7.3	8.1	47%	6.2	5.1	1.1	<i>Anti</i>	<i>Inactive</i>
<i>cAcetDPR</i> (18)	2.0	3.1	22%	6.2	5.1	1.1	<i>Anti</i>	<i>Poor agonist EC</i> ₅₀ = 12 μ M
2'-Deoxy cADPR (46)	7.0	n.d ^a	70%	—	—	—	Syn	Agonist EC ₅₀ = 58 nM
3'-Deoxy cADPR (47)	3.0	n.d	30%	5.9	5.2	0.7	<i>Syn</i>	<i>Poor agonist EC</i> ₅₀ = 5 μ M
2'-cADPRP (48)	4.0	n.d	40%	6.2	5.7	0.5	<i>Syn</i>	<i>Inactive</i>
3'-cADPRP (49)	3.7	n.d	37%	6.0	5.3	0.7	<i>Syn</i>	<i>Inactive</i>
3'-OMe cADPR (50)	5.6	2.7	67%	5.9	5.3	0.6	Syn	Antagonist IC ₅₀ = 4.8 μ M
7-Deaza cADPR (51)	6.4	n.d	64%	5.7	5.3	0.4	Syn	Agonist EC ₅₀ = 90 nM
7-Deaza-8-Br-cADPR (52)	5.9	3.5	63%	6.0	5.4	0.6	Syn	Antagonist IC ₅₀ = 0.73 μ M
8-Amino-2'-deoxy cADPR (53)	6.9-6.6	n.d	66-69%	6.06	—	—	Syn	Antagonist IC ₅₀ = 0.22 μ M
Compounds synthesized by Shuto's group ^{15,38,51,52}								
cADPcR (4)	6.1	2.6	70%	6.0	5.1	0.9	Syn	Agonist EC ₅₀ = 79 nM
8-Cl-cADPcR (5)	6.3	2.4	72%	6.1	5.2	0.9	Syn	Agonist EC ₅₀ = 19 μ M
8-NH ₂ -cADPcR (6)	6.3	n.d	63%	5.9	5.2	0.7	Syn	Agonist EC ₅₀ = 80 nM
8-N ₃ -cADPcR (7)	6.2	n.d	62%	5.9	5.2	0.7	Syn	Agonist EC ₅₀ = 3.9 μ M
3''-Deoxy cADPcR (54)	6.1	2.6	70%	6.1	5.2	0.9	Syn	Agonist EC ₅₀ = 14 nM
8-Cl-3''-deoxy cADPcR (55)	6.2	n.d	62%	6.1	5.2	0.9	Syn	Partial agonist EC ₅₀ = 0.19 μ M
8-NH ₂ -3''-deoxy cADPcR (56)	6.3	2.3	73%	5.9	5.3	0.6	Syn	Partial agonist EC ₅₀ = 17 nM
8-N ₃ -3''-deoxy cADPcR (57)	6.2	n.d	62%	5.9	5.2	0.7	Syn	Partial agonist EC ₅₀ = 0.49 μ M
2''-Deoxy cADPcR (58)	5.9	3.0	66%	6.1	5.2	0.9	<i>Syn</i>	Agonist EC ₅₀ = 0.61 μ M
2'',3''-Dideoxy cADPcR (59)	5.4	2.4	69%	6.1	5.2	0.9	Syn	Agonist EC ₅₀ = 0.73 μ M
2''-OMOM-3''-OMe cADPcR (60)	6.3	2.4	72%	6.0	5.1	0.9	Syn	Agonist EC ₅₀ = 0.88 μ M
2''-OMOM-3''-deoxy cADPcR (61)	5.3	2.2	70%	6.1	5.2	0.9	Syn	Agonist EC ₅₀ = 0.88 μ M
2'',3''-Dideoxy- didehydro cADPcR (62)	1.5	6.3	19%	6.1	5.0	1.1	<i>Anti</i>	<i>Poor agonist EC</i> ₅₀ > 20 μ M

^a Not determined. The compounds in italics do not display a C2' *endo/syn* conformation.

the other analogues in this table displaying a C2' *endo/syn* conformation are either agonists or antagonists in SUH.

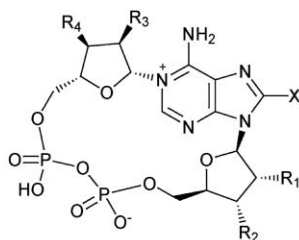
Whilst this analysis does not provide structural clues about relative agonistic/antagonistic activity or potency in either case, it does seem that the C2' *endo/syn* conformation may be a key requirement for activity in SUH only. Indeed, it has been demonstrated earlier that there are differences in ligand recognition by the protein between the sea urchin and T cell receptor. For example,

2'-deoxy cADPR (46) is inactive in T cells, but is a potent agonist in SUH, whereas 2'-cADPRP (48) is active in T cells but not in SUH (Table 4). 3'-OMe cADPR (50) was designed to investigate further the role of the hydroxyl group. 3'-Deoxy cADPR (47) can neither donate or accept a hydrogen bond at the 3' position, whilst 3'-OMe cADPR can only act as a hydrogen bond acceptor. This compound is an antagonist in SUH, thus showing that the oxygen atom must interact with the receptor in order to inhibit Ca²⁺ release. However,



X = H, Y = N, R ₁ = H, R ₂ = OH	2'-Deoxy cADPR 46
X = H, Y = N, R ₁ = OH, R ₂ = H	3'-Deoxy cADPR 47
X = H, Y = N, R ₁ = OPO ₃ ²⁻ , R ₂ = OH	2'-cADPR 48
X = H, Y = N, R ₁ = OH, R ₂ = OPO ₃ ²⁻	3'-cADPR 49
X = H, Y = N, R ₁ = OH, R ₂ = OMe	3'-OMe cADPR 50
X = H, Y = C, R ₁ = R ₂ = OH	7-Deaza cADPR 51
X = Br, Y = C, R ₁ = R ₂ = OH	7-Deaza 8-bromo cADPR 52
X = NH ₂ , Y = N, R ₁ = H, R ₂ = OH	8-NH ₂ -2'-deoxy cADPR 53

Fig. 4 Structures of the cADPR analogues mentioned herein – our group.



X = H, R ₁ = R ₂ = R ₃ = OH, R ₄ = H	3''-Deoxy cADPcR 54
X = Cl, R ₁ = R ₂ = R ₃ = OH, R ₄ = H	8-Cl-3''-deoxy cADPcR 55
X = NH ₂ , R ₁ = R ₂ = R ₃ = OH, R ₄ = H	8-NH ₂ -3''-deoxy cADPcR 56
X = N ₃ , R ₁ = R ₂ = R ₃ = OH, R ₄ = H	8-N ₃ -3''-deoxy cADPcR 57
X = H, R ₁ = R ₂ = R ₄ = OH, R ₃ = H	2''-Deoxy cADPcR 58
X = H, R ₁ = R ₂ = OH, R ₃ = R ₄ = H	2'',3''-Dideoxy cADPcR 59
X = H, R ₁ = R ₂ = OH, R ₃ = OMOM, R ₄ = OMe	2''-OMOM-3''-OMe cADPcR 60
X = H, R ₁ = R ₂ = OH, R ₃ = OMOM, R ₄ = H	2''-OMOM-3''-deoxy cADPcR 61

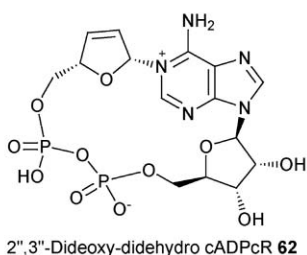


Fig. 5 Structures of the cADPR analogues mentioned herein – Shuto's group.

the same compound is an agonist in JTC, thus showing that the OMe group has little effect on the activity of cADPR. These results further highlight that there may be differences in the cADPR-Ca²⁺ release mechanism for the ryanodine receptor of SUH and JTC. This trend appears to be valid only with cADPR analogues and *not* with the cIDPR series. Indeed, some cIDPR derivatives (e.g. 8-Br cIDPR) show agonist activity in T cells but are apparently inactive in SUH.

We naturally need to invoke the caveat for all work of this nature that, while our study focuses upon cADPR analogue conformation in solution, we can draw no firm conclusions regarding actual ligand conformation as bound to the cADPR receptor. A recent trend in nucleoside/nucleotide work in general has been to employ the use of conformationally locked rigid ribose motifs using a

Table 4 Ca²⁺ release activities of cADPR analogues in SUH and JTC

Compounds	Activity in SUH	Activity in JTC	Ref.
cADPR (1)	Agonist	Agonist	1,34
8-Bromo cADPR (2)	Antagonist	Antagonist	14,25
8-NH ₂ cADPR (3)	Antagonist	Antagonist	13,34
8-NHMe cADPR (9)	Weak antagonist	Antagonist	50
8-NMe ₂ cADPR (10)	Weak antagonist	Antagonist	50
8-Me cADPR (11)	Antagonist	Antagonist	46
8-OMe cADPR (12)	Antagonist	Antagonist	37
8-Piperidyl cADPR (13)	Inactive	Antagonist	14
8-Aza-9-deaza cADPR (15)	Active	Active	50
cAcycloDPR (16)	Inactive	Not tested	49
cAraDPR (17)	Inactive	Not tested	49
cAcetDPR (18)	Poor agonist	Not tested	49
2'-Deoxy cADPR (46)	Agonist	Inactive	23
3'-Deoxy cADPR (47)	Poor agonist	Inactive	23
2'-cADPRP (48)	Inactive	Agonist	23,47
3'-cADPRP (49)	Inactive	Inactive	23,47
3'-OMe cADPR (50)	Antagonist	Not tested	23
7-Deaza-8-bromo cADPR (52)	Antagonist	Antagonist	37,48,49
8-Bromo cIDPR	Inactive	Agonist	17

variety of strategies.⁵³⁻⁵⁶ Taking our observations reported here into account it could be productive to apply such approaches also to the cADPR field and these could hopefully extend the work reported here to encompass receptor bound conformations.

Conclusion

A series of novel cADPR derivatives has been synthesized in order to investigate the determinants for both agonist and antagonist activity. These compounds were tested for their Ca²⁺ releasing activity in both SUH and JTC. A careful analysis of all the cADPR analogues synthesized over the past decade reveals that a C2' *endo/syn* conformation (Fig. 6) is crucial for activity (agonistic or antagonistic), whereas compounds in their C3' *endo* conformation

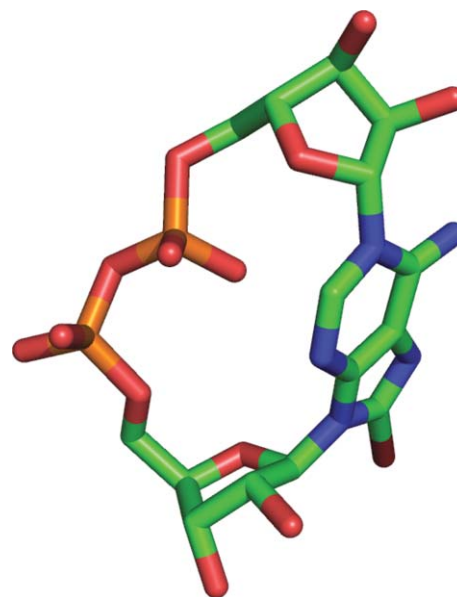


Fig. 6 Model of 8-Br cADPR (2) with its "southern" ribose in the C2' *endo/syn* conformation. Hydrogen atoms are not shown for clarity.

are either inactive or are poor agonists. This trend appears to be valid in sea urchin egg homogenate only (not in T cells) and with cADPR analogues only (not cIDPR).

Experimental

General Procedures

All reagents and solvents were of commercial quality and were used directly unless otherwise described. Pyridine was dried overnight with calcium hydride, distilled and stored over potassium hydroxide pellets. *Aplysia californica* ovotestis homogenate containing ADP-ribosyl cyclase was prepared as previously described.⁵⁷ The protein concentration of the *Aplysia* cyclase used in all cases was estimated as 10 mg ml⁻¹ using a Bio-rad protein estimation assay. Ion-exchange chromatography was performed on an LKB-Pharmacia medium pressure ion-exchange chromatograph using a Sepharose Q fast flow column with gradients of triethylammonium bicarbonate buffer (TEAB) pH 7.6 as eluent. 1 M TEAB was prepared by bubbling carbon dioxide gas into 1 M triethylamine solution. HPLC was performed on a Shimadzu LC-6A chromatograph with the UV detector operating at 259 nm using a combination of a Partisil 10 μ SAX guard column (10 \times 0.46 cm) and a Technicol (10 \times 0.46 cm) 10 μ SAX HPLC column, or using a Spherisorb 10 μ SAX (25 \times 0.46 cm) column with an isocratic elution using phosphate buffer (KH₂PO₄), pH 3.0 at a flow rate of 1 ml min⁻¹. ¹H NMR and ³¹P NMR spectra were recorded on either Jeol JNM GX-270 FT NMR or Jeol EX-400 FT NMR spectrometers. Chemical shifts were measured in ppm relative to deuterated water (D₂O) for ¹H NMR and to external 85% H₃PO₄ for ³¹P NMR. ³¹P NMR spectra were measured at 162 MHz or 109 MHz in D₂O. *J* values are given in Hertz (Hz). Mass spectra were recorded at the EPSRC Mass Spectrometry Service Center at the University of Swansea and at the University of Bath. Ultraviolet (UV) absorbance was measured with a Perkin-Elmer Lambda 3 UV/VIS spectrophotometer. Melting points were determined with a Reichert-Jung Thermo Galen Kofler block and are uncorrected.

Biology

Biological testing of cADPR analogues in SUH. Sea urchin egg homogenates were prepared as described.¹ Before use the homogenate was defrosted and diluted to give a 2.5% egg suspension using a buffer containing an ATP regenerating system, mitochondrial inhibitors and protease inhibitors. Finally, Fluo-3 fluorescent dye (3 μ M) was added and the homogenate was incubated at 17 °C. Extra-microsomal Ca²⁺ was measured by monitoring Fluo-3 fluorescence in a Perkin Elmer LS-50B fluorimeter.^{25,33}

Calcium release measurements in JTC. Permeabilized cells were prepared as follows: cells were transferred into an intracellular medium (nominal Ca²⁺ free, pH 7.2), permeabilized with 55 mg ml⁻¹ saponin for 20 min, and then washed 3 times to remove all saponin. Then the cells were left on ice for approximately 2 h to allow for re-sealing of intracellular Ca²⁺ stores. Finally, approximately 1.5 \times 10⁷ permeabilized cells were transferred into a quartz cuvette and placed into an F2000 fluorimeter (Hitachi Instruments). The cell suspension was warmed to 37 °C and stirred

slightly. Fura 2/free acid (1 μ M final concentration) was added and the free Ca²⁺ concentration was monitored at 340 and 380 nm as alternating excitation wavelengths and 495 nm as emission wavelength. Experiments were started by addition of creatine kinase (20 units/mL) and creatine phosphate (20 mM), followed by addition of 1 mM ATP to allow loading of the intracellular Ca²⁺ pools by sarcoplasmic/endoplasmic Ca²⁺ ATPases. Chelex resin was added generally to solutions of compounds to be tested for Ca²⁺ release to avoid any Ca²⁺ contamination. The quality of the permeabilized cell suspension was checked by its responsiveness to cADPR and D-*myo*-inositol 1,4,5-trisphosphate.

Chemistry

Synthesis of nucleoside analogues

Synthesis of 8-bromo-adenosine 5'-monophosphate (8-Br-AMP, 19). 8-Bromoadenosine (0.212 g, 0.61 mmol) was dissolved in triethylphosphate (4 mL) by heating with a heat gun. The flask was fitted with a CaCl₂ drying tube and the solution cooled to 0 °C in an ice bath. Phosphorus oxychloride (200 μ L, 2.16 mmol) was added dropwise and the flask was left stirring for 3 h at rt. HPLC analysis of the reaction mixture dissolved in water showed the presence of a phosphorylated material with the same retention time as an authentic sample of 8-Br-AMP. R, 8-Br-adenosine (1.2 min) 8-Br-AMP (1.8 min). The reaction mixture was quenched with 8 mL of pyridine:water (1 : 3, v/v) and left stirring for a further 30 min. The sample was dried *in vacuo* and ³¹P NMR spectroscopy showed the presence of inorganic phosphate and 8-Br-AMP at δ -0.1 and 0.4 ppm respectively. Inorganic phosphate was removed by passing the nucleotide mixture dissolved in 50 mL of water through a charcoal column. This column (25 \times 4 cm) was prepared by using *ca.* 1 cm of celite as a bed on top of which 7 cm of activated charcoal (Norit^B) were added. The nucleotide solution in water was poured unto the column and the eluent was collected. Water was used to flush inorganic phosphate off the column and a total of 75 mL of eluent was collected. The inorganic phosphate-free nucleotide was eluted off the column using ethanol:water:conc. ammonia (25 : 24 : 1, v/v, 2 \times 500 mL). δ_{H} (D₂O, 400 MHz) 3.8–3.9 (2H, m, H-5'a, H-5'b), 4.25 (1H, m, H-4'), 4.56 (1H, dd, *J*_{3,2'} 5.8, *J*_{3,4'} 4.2, H-3'), 5.19 (1H, dd, *J*_{2,3'} 5.8, *J*_{2,1'} 5.7, H-2'), 6.07 (1H, d, *J*_{1,2'} 5.8, H-1'), 8.14 (1H, s, H-2). δ_{C} (D₂O, 100 MHz) 65.3 (d, *J*_{CP} 3.7, C-5'), 70.5 (C-3'), 71.8 (C-2'), 84.4 (d, *J*_{CP} 9.2, C-4'), 89.2 (C-1'), 118.3 (C-5), 139.9 (C-8), 150.7 (C-4), 153.6 (C-2), 154.9 (C-6). δ_{P} (D₂O, 162 MHz, ¹H-decoupled) 3.4 (s, 1P) and δ_{P} (D₂O, 162 MHz, ¹H-coupled) 3.7 (s, *J* 5.7, 1P). λ_{max} 264 nm (ϵ 15100, pH 7.0).

8-Amino-adenosine 5'-monophosphate (8-NH₂-AMP, 20). To a solution of 8-azido AMP (25 mg, 59.2 μ mol) in 50 mM TEAB (7.5 mL, pH 8.0) was added dithiothreitol (14 mg, 90.6 μ mol) and the mixture was left stirring for 16 h at room temperature.⁵⁸ The reaction was judged to be complete by a change in UV absorption maximum from 282 to 274 nm. The solvent was removed *in vacuo* and the residue purified by ion-exchange chromatography using a gradient of 50–1000 mM TEAB pH 7.6. Pure 8-NH₂-AMP eluted as the triethylammonium salt between 310–390 mM TEAB (38.7 μ mol, 65%).

δ_{H} (D₂O, 270 MHz) 3.8–3.9 (2H, m, H-5'a, H-5'b), 4.2–4.1 (1H, m, H-4'), 4.3–4.4 (m, 1H, H-3'), 4.7 (1H, app.t, *J*_{2,1'} = *J*_{2,3'} 7.6,

H-2'), 5.9 (1H, d, $J_{1,2}$ 7.6, H-1'), 7.8 (1H, s, H-2). δ_{P} (D_2O , 162 MHz, ^1H -decoupled) 0.35 (s, 1P). λ_{max} 274 nm (ϵ 16000, pH 8.3).

8-Methylaminoadenosine 5'-monophosphate (8-NHMe-AMP, 21). A solution of 8-Br-AMP (270.8 μmol) in anhydrous 2 M methylamine in methanol (10 mL) was heated to 60–70 °C for 2 h. The crude sample was dried down *in vacuo*, the residue dissolved in milliQ water and the product purified by ion-exchange chromatography using a gradient of 0–350 mM TEAB. The product eluted between 200–300 mM TEAB to yield the title compound as a white solid (176 μmol , 65%). δ_{H} (D_2O , 400 MHz) 2.8 (3H, s, NMe), 3.8–3.9 (2H, m, H-5'a, H-5'b), 4.1 (1H, m, H-4'), 4.26 (1H, app.t, $J_{3,2} = J_{3,4}$ 5.5, H-3'), 4.6 (1H, app.t, $J_{2,1'} = J_{2,3'}$ 5.8, H-2'), 5.8 (1H, d, $J_{1,2}$ 8, H-1'), 7.8 (1H, s, H-2). δ_{P} (D_2O , 162 MHz, ^1H -decoupled) 3.2 (s, 1P). m/z (ES^-) 375.4 [100%, (M – H) $^-$]. λ_{max} 278 nm (ϵ 17700, pH 8.3). HPLC R_t = 2.3 mins.

8-Dimethylamino-adenosine 5'-monophosphate (8-NMe₂-AMP, 22). 8-Br-AMP (0.147 mmol) was added to an anhydrous solution of 2 M dimethylamine in methanol (2 M, 10 mL) and the solution was stirred under reflux overnight, after which the solvent was removed *in vacuo*. The crude sample was purified on an ion-exchange column using a TEAB gradient of 0–400 mM. Pure sample eluted off between 140–210 mM TEAB to afford the desired product (99.9 μmol , 68%). δ_{H} (D_2O , 400 MHz) 2.8 (6H, s, NMe₂), 3.8–3.9 (2H, m, H-5'), 4.1 (1H, m, H-4'), 4.3 (1H, app.t, $J_{3,2} = J_{3,4}$ 5.0, H-3'), 4.6 (1H, app.t, $J_{2,1'} = J_{2,3'}$ 6.4, H-2'), 5.6 (1H, d, $J_{1,2}$ 6.7, H-1'), 7.9 (1H, s, H-2). δ_{P} (D_2O , 162 MHz, ^1H -decoupled) 2.2 (s, 1P). m/z (FAB^-) 389 [90%, (M – H) $^-$]. λ_{max} 274 nm (ϵ 10900, pH 8.3). HPLC R_t = 4.9 mins.

8-Methyladenosine 5'-monophosphate (8-Me-AMP, 23). 8-Bromoadenosine (1 g, 2.89 mmol) was dissolved in hexamethyl-disilazane (20 mL) and dry dioxane (20 mL) in a three-necked flask. A catalytic amount of ammonium sulfate was added to the suspension and the mixture was left under reflux at 130 °C for 2–3 h. Triphenylphosphine (76 mg, 2.89 mmol), palladium dichloride (26 mg, 0.144 mmol) and trimethylaluminium (2.89 mL, 5.78 mmol) were added to the solution in dry THF under N₂. The reaction was left under reflux and a gentle stream of N₂ for 2.5 h (R_t 0.36, DCM–MeOH 9 : 1). The crude mixture was dried *in vacuo* to give a green residue which was dissolved in methanol (50 mL) and refluxed for 4 h with a small amount of ammonium chloride (R_t 0.05, DCM–MeOH 9 : 1). The crude sample was purified on a short silica gel column eluted with CHCl₃–EtOH (3 : 2) to give a white solid which was further recrystallised from water (0.39 g, 1.4 mmol, 48%). δ_{H} (D_2O , 270 MHz) 3.5–3.7 (2H, m, H-5'a, H-5'b), 4.0 (m, 1H, H-4'), 4.1 (m, 1H, H-3'), 4.8 (dd, $J_{2,1'} = 7.3$ and $J_{2,3'} = 5.1$ Hz, 1H, H-2'), 5.8 (1H, d, $J_{1,2}$ 7.3, H-1'), 8.0 (1H, s, H-2). m/z (FAB^+) 282 [100%, (M + H) $^+$]. HRMS (FAB^+) calcd for C₁₁H₁₆N₄O₄ 282.1207 (M + H) $^+$ found 282.1202. m.p. 208 °C.

Phosphorus oxychloride (100 μL , 1.08 mmol) was added dropwise at 0 °C to a solution of 8-methyl adenosine (170 mg, 0.6 mmol) in triethylphosphate (4 mL) under N₂. The reaction was left stirring for 3 h at rt after which HPLC analysis showed the presence of a new product at 2 min. The reaction mixture was quenched by stirring in 8 mL of pyridine:water (1 : 3) for 30 min and the solvents were removed *in vacuo*. The residue was dissolved in milliQ water (350 mL) and applied to an ion-exchange

Q-sepharose column eluted with 1 M TEAB. The product eluted off between 110–160 mM TEAB, and the inorganic phosphate impurity was removed by passing the solution through a charcoal column as previously described to afford the nucleotide as a white solid (0.38 mmol, 60.3%). δ_{H} (D_2O , 400 MHz) δ_{H} (D_2O , 400 MHz) 2.46 (3H, s, Me), 3.8–3.9 (2H, m, H-5'a, H-5'b), 4.1–4.21 (1H, m, H-4'), 4.3 (app.t, $J_{3,2} = J_{3,4} = 6.2$ Hz, 1H, H-3'), 4.8 (1H, app.t, $J_{2,1'} = J_{2,3'}$ 6.2, H-2'), 5.8 (1H, d, $J_{1,2}$ 6.8, H-1'), 7.9 (1H, s, H-2). δ_{P} (D_2O , 162 MHz, ^1H -decoupled) 4.0 (s, 1P) and δ_{P} (D_2O , 162 MHz, ^1H -coupled) 3.7 (s, J 5.6, 1P). λ_{max} 260 nm (ϵ 15300, pH 8.3).

8-Methoxy-adenosine 5'-monophosphate (8-OCH₃-AMP, 24). 8-Br-AMP (0.229 mmol) was treated with 0.5 M sodium methoxide in methanol (20 mL) under reflux overnight. The pH of the solution was adjusted to 7 with glacial acetic acid and the solvent was removed *in vacuo*. The residue was redissolved in milliQ water and purified by ion-exchange chromatography using a TEAB gradient from 50–350 mM. The pure sample eluted off between 210–250 mM TEAB and was obtained in 79% yield. δ_{H} (D_2O , 400 MHz) 3.7–3.0 (2H, m, H-5'), 4.0 (3H, s, OMe), 4.2 (1H, m, H-4'), 4.25 (1H, dd, $J_{3,2}$ 5.8, $J_{3,4}$ 4.2, H-3'), 4.8 (1H, dd, $J_{2,1'}$ 5.8, $J_{2,3'}$ 5.5, H-2'), 5.7 (1H, d, $J_{1,2}$ 5.5, H-1'), 7.8 (1H, s, H-2). δ_{P} (D_2O , 162 MHz, ^1H -decoupled) 3.7 (s, 1P). λ_{max} 260 nm (ϵ 13500, pH 8.3).

8-Piperidyl-adenosine 5'-monophosphate (8-pip-AMP, 25). A solution of 8-Br-AMP (111.3 μmol) in dry piperidine (0.4 mL) was stirred at 50 °C for 40 h after which HPLC analysis showed the presence of a new peak at 5.1 min. Piperidine was removed *in vacuo*, the residue dissolved in milliQ water and product purified by ion-exchange chromatography using a gradient of 0–300 mM TEAB. Pure 8-pip-AMP eluted between 30–65 mM TEAB (65% yield). δ_{H} (D_2O , 400 MHz) 1.6 (6H, m, 3 × CH₂), 3.2 (4H, m, 2 × CH₂), 3.8–3.9 (2H, m, H-5'), 4.0 (1H, m, H-4'), 4.34 (1H, app.t, $J_{3,2} = J_{3,4}$ 6.0, H-3'), 5.1 (1H, app.t, $J_{2,1'} = J_{2,3'}$ 6.0, H-2'), 5.6 (1H, d, $J_{1,2}$ 6.4, H-1'), 7.9 (1H, s, H-2). δ_{P} (D_2O , 162 MHz, ^1H -decoupled) 2.0 (s, 1P). m/z (FAB^-) 429 [85%, (M – H) $^-$]. λ_{max} 275 nm (ϵ 11660, pH 8.3). HPLC R_t = 5.1 mins.

8-Oxy-adenosine 5'-monophosphate (8-oxy-AMP, 26). 8-Br-AMP (128 mg, 301.9 μmol) was co-evaporated with pyridine (3 × 5 mL) and anhydrous sodium acetate (50 mg) and acetic anhydride (4.4 mL) were added. The solution was refluxed at 150–165 °C for 2 h and then stirred for 24 h at room temperature after which 2 mL of methanol were added. The solvents were removed, the residue was dissolved in 1 M NaOH (11 mL) and stirred for 24 h at rt after which it was neutralised with 1 M HCl. The sample was purified on an ion-exchange column using a gradient between 0–400 mM TEAB. Pure product eluted off between 300–360 mM TEAB in 51% yield (0.155 mmol). δ_{H} (D_2O , 400 MHz) 3.7–3.9 (2H, m, H-5'), 3.9 (1H, m, H-4'), 4.2 (1H, dd, $J_{3,2}$ 5.0, $J_{3,4}$ 4.6, H-3'), 4.9 (1H, dd, $J_{2,1'}$ 5.5, $J_{2,3'}$ 5.0, H-2'), 5.6 (1H, d, $J_{1,2}$ 5.5, H-1'), 8.0 (1H, s, H-2). δ_{P} (D_2O , 162 MHz, ^1H -decoupled) –0.4 (s, 1P). λ_{max} 270 nm (ϵ 10545, pH 8.3). HPLC R_t = 3.7 mins.

Acycloadenosine monophosphate (AcycloAMP, 28). 9-[(2-Hydroxyethoxy)methyl]adenine²⁹ 43 (50 mg, 0.24 mmol) was dissolved in triethylphosphate (2 mL), cooled to 0 °C and POCl₃ (75 μL , 0.53 mmol) was added slowly. The reaction was warmed to rt and stirred for 1.5 h after which it was quenched by the addition of iced water (5 g) and the product was purified by

ion-exchange chromatography eluting with a gradient of 1 M TEAB. The appropriate fractions were combined and the solvent removed *in vacuo* to give the title monophosphate in 55% yield. The inorganic phosphate was removed on a charcoal column as previously described with 95% recovery followed by precipitation using MeOH:acetone 1 : 10 (2 mL) to give the pure product 21. δ_{H} (400 MHz, D₂O) 3.57–3.60 (2H, m, POCH₂CH₂), 3.74–3.78 (2H, m, POCH₂), 5.66 (2H, s, OCH₂base), 8.16 (1H, s, H-2), 8.26 (1H, s, H-8), and. δ_{C} (D₂O, 100 MHz) 64.5 (d, J_{CP} 5.5, POCH₂), 69.8 (d, J_{CP} 9.2, POCH₂), 73.9 (OCH₂base), 119.2 (C-5), 143.4 (C-8), 149.7 (C-4), 153.6 (C-2), 156.3 (C-6). δ_{P} (D₂O, 161 MHz) 1.48. m/z (FAB⁺) 290.1 [100%, (M + H)⁺]. λ_{max} 259 nm (ϵ 15300). HPLC R_{t} = 2.5 mins.

Adenine 9- β -D-arabinofuranoside 5'-monophosphate (AraAMP, 29). Adenine 9- β -D-arabino-ribofuranoside 44 (177 mg, 0.66 mmol) was dissolved in triethylphosphate (2 mL), cooled to 0 °C and POCl₃ was added (140 μ L, 0.70 mmol). The reaction mixture was stirred overnight at 4 °C after which it was quenched with ice (10 g) and the material was purified by ion exchange chromatography eluting with a gradient of 1 M TEAB. The product obtained was passed through a charcoal column to remove the inorganic phosphate. The desired monophosphate was obtained in 58% yield. δ_{H} (270 MHz, D₂O) 4.3–4.0 (3H, m, H-4' and H-5'), 4.38 (1H, dd, $J_{3,2'}$ 6.6, $J_{3,4'}$ 6.4, H-3'), 4.59 (1H, dd, $J_{2,3'}$ 6.6, $J_{2,1'}$ 5.5, H-2'), 6.42 (1H, d, $J_{1,2'}$ 5.5, H-1'), 8.38 (1H, s, H-8), 8.53 (1H, s, H-2). δ_{C} (D₂O, 100 MHz) 63.1 (C-5'), 68.9 (C-3'), 80.8 (C-2'), 86.4 (d, J_{CP} 7.4, C-4'), 89.5 (C-1'), 123.3 (C-5), 149.0 (C-4 or C-8), 150.1 (C-4 or C-8), 153.5 (C-2), 155.1 (C-6). δ_{P} (D₂O, 161 MHz) 3.10. m/z (FAB⁺) 346 [100%, (M + H)⁺]. λ_{max} 259 nm (ϵ 15300). HPLC R_{t} = 2.21 mins.

2',3'-Isopropylidene adenosine monophosphate (AcetAMP, 30). To a cooled solution of dry 2',3'-*O*-isopropylidene adenosine 45 (200 mg, 0.65 mmol) suspended in triethylphosphate (2 mL) was added POCl₃ (140 μ L, 0.70 mmol) and the reaction stirred overnight at 4 °C. It was quenched by the addition of ice (10 g) and the mixture was purified by ion exchange chromatography eluted with a gradient of 1 M TEAB. The product was purified further on a charcoal column to remove the inorganic phosphate and the title nucleotide was obtained in 47% yield. δ_{H} (400 MHz, D₂O) 1.43 (3H, s, CH₃), 1.66 (3H, s, CH₃), 3.98 (2H, m, H-5'), 4.60 (1H, m, H-4'), 5.04 (1H, dd, $J_{3,2'}$ 5.9, $J_{3,4'}$ 2.0, H-3'), 5.35 (1H, dd, $J_{2,3'}$ 5.9, $J_{2,1'}$ 3.4, H-2'), 6.19 (1H, d, $J_{1,2'}$ 3.4, H-1'), 84.3 (C-3'), 8.12 (1H, s, H-8), 8.42 (1H, s, H-8). δ_{C} (D₂O, 100 MHz) 27.2, 29.0 (both CH₃), 67.3 (d, J_{CP} 3.7, C-5'), 86.7 (C-2'), 87.8 (d, J_{CP} 9.2, C-4'), 92.7 (C-1'), 117.6 (C), 121.1 (C-5), 143.0 (C-8), 151.4 (C-4), 158.5 (C-2), 158.1 (C-6). δ_{P} (D₂O, 161 MHz) 3.51. m/z (FAB⁺) 388.1 [100%, (M + H)⁺]. λ_{max} 259 nm (ϵ 15200). HPLC R_{t} = 2.98 mins.

Synthesis of NAD⁺ analogues

General method. All NAD⁺ analogues with the exception of 8-Br-NAD⁺ were prepared essentially by an adaptation of a literature method described by Hughes *et al.*²⁷ using dicyclohexylcarbodiimide as the coupling agent.

β -NMN⁺ and the appropriate AMP analogue were dissolved in water in a round bottom flask, dry pyridine was added to make a 4 : 1 pyridine:water mixture and excess DCC was subsequently added to the nucleotide mixture. The solution was left stirring at

room temperature for 7 days. The resulting mixture was poured into 100 mL of cold distilled water and left at 4 °C for 2 h to precipitate the DCU formed in the reaction. DCU was filtered off and the filtrate was extracted with 3 \times 50 mL aliquots of chloroform to remove other water insoluble organic impurities. The aqueous layer was collected and dried *in vacuo*. The residue was dissolved in milliQ water and purified by ion-exchange chromatography on Q-sepharose eluted with a gradient of 1 M TEAB.

Nicotinamide 8-bromoadenine dinucleotide (8-Br-NAD⁺, 31). β -NAD⁺ (200 mg, 0.3 mmol) free acid was dissolved in acetate buffer (5 mL, pH 3.9) and bromine (0.2 mL) was added dropwise. The reaction was left for 30 min after which a 1.25 M solution of NaHSO₃ was added dropwise to discharge the bromine colour and the unreacted bromine was extracted with CHCl₃. The sample was dried *in vacuo*, the residue dissolved in milliQ water and the product purified by ion-exchange chromatography using a gradient of 0–350 mM TEAB. Pure 8-Br-NAD⁺ eluted between 45–70 mM TEAB and was obtained in 61% yield (184.1 μ mol). δ_{H} (D₂O, 270 MHz) 4.0–4.4 (9H, m, ribose-H), 5.0 (1H, t, $J_{2,1'}$ = $J_{2,3'}$ 5.8, H-2'), 5.7 (1H, d, $J_{1,2'}$ 5.5, H-1'), 5.8 (1H, d, $J_{1',2''}$ 4.8, H-1''), 7.8 (1H, s, H_A2), 8.0 (1H, t, J 7, H_N5), 8.6 (1H, d, $J_{4,5}$ 8.2, H_N4), 8.9 (1H, d, $J_{6,5}$ 6.2, H_N6), 9.1 (1H, s, H_N2). δ_{P} (D₂O, 109 MHz, ¹H-decoupled) –11.54 and –11.92 (AB system, J_{AB} 21.2, 2P). m/z (ES[–]) 741.9 [50% (M)[–]]. λ_{max} 264 nm (ϵ 15500, pH 8.3). HPLC R_{t} = 2.4 mins.

Nicotinamide 8-aminoadenine dinucleotide (8-NH₂-NAD⁺, 32). 8-NH₂-AMP (14 mg, 38.7 μ mol) was coupled to β -NMN⁺ (8.08 mg, 24.2 μ mol) with 0.32 g of DCC in 6.5 mL of pyridine:water (4 : 1) as described. The pure dinucleotide was obtained in 16% yield. δ_{H} (D₂O, 400 MHz) 4.2–4.6 (9H, m, ribose-H), 4.7–4.8 (1H, m, H-2'), 5.86 (1H, d, $J_{1,2'}$ 7, H-1'), 6.1 (1H, d, $J_{1',2''}$ 4.7, H-1''), 8.0 (1H, s, H_A2), 8.2 (1H, t, $J_{5,6}$ = $J_{5,4}$ 6.6, H_N5), 8.7 (1H, d, $J_{4,5}$ 6.6, H_N4), 9.1 (1H, d, $J_{6,5}$ 6.6, H_N6), 9.3 (1H, s, H_N2). δ_{P} (D₂O, 162 MHz, ¹H-decoupled) –11.83 and –11.97 (AB system, J_{AB} 19.4, 2P). m/z (ES[–]) 677 [10% (M – H)[–]]. HPLC R_{t} = 4.1 mins.

Nicotinamide 8-methylaminoadenine dinucleotide (8-NHMe-NAD⁺, 33). 8-Methylamino-AMP (74.03 μ mol) was coupled to β -NMN⁺ as described above using 2 g of DCC. δ_{H} (D₂O, 400 MHz) 2.8 (3H, s, NHMe), 4.0–4.5 (10H, m, ribose-H), 5.7 (1H, d, $J_{1,2'}$ 7.0, H-1'), 5.9 (1H, d, $J_{1',2''}$ 4.6, H-1''), 7.9 (1H, s, H_A2), 8.0 (1H, t, J 7, H_N5), 8.6 (1H, d, J 7.9, H_N4), 9.0 (1H, d, J 5.8, H_N6), 9.1 (1H, s, H_N2). δ_{P} (D₂O, 109 MHz, ¹H-decoupled) –11.7 (s, 2P). m/z (FAB⁺) 693 [60%, [M + H]⁺]. λ_{max} 273 nm (ϵ 13000, pH 8.3). HPLC R_{t} = 4.7 mins.

Nicotinamide 8-dimethylaminoadenine dinucleotide (8-Me₂NH-NAD⁺, 34). 8-Dimethylamino-AMP (161 μ mol) was coupled to β -NMN⁺ (149.6 μ mol) with 2 g of DCC in 12.5 mL of pyridine:water (4 : 1) as described. The pure product was obtained in 11% (17.1 μ mol) yield. δ_{H} (D₂O, 400 MHz) 3.1 (3H, s, NMe₂), 4.4–5.0 (9H, m, ribose-H), 5.0 (1H, app.t, $J_{2,1'}$ = $J_{2,3'}$ 6.3, H-2'), 5.9 (1H, d, $J_{1,2'}$ 6.3, H-1'), 6.3 (1H, d, $J_{1',2''}$ 5.4, H-1''), 8.2 (1H, s, H_A2), 8.4 (1H, t, J 7, H_N5), 8.9 (1H, d, J 8.0, H_N4), 9.3 (1H, d, J 6.3, H_N6), 9.5 (1H, s, H_N2). δ_{P} (D₂O, 109 MHz, ¹H-decoupled) –11.42 and –11.82 (AB system, J_{AB} 21, 2P). m/z (FAB⁺) 707 [70% (M + H)⁺]. λ_{max} 273 nm (ϵ 16100, pH 8.3). HPLC R_{t} = 5.8 mins.

Nicotinamide 8-methyladenine dinucleotide (8-Me-NAD⁺, 35). 8-Me-AMP (139.2 μmol) was coupled to β-NMN⁺ (149.6 μmol) with 2 g of DCC in 12.5 mL of pyridine:water (4:1) as described. The pure dinucleotide was obtained in 12% yield after 2 purifications. δ_H (D₂O, 400 MHz) 2.5 (3H, s, Me), 4.2–4.6 (9H, m, ribose-H), 4.7 (1H, d, *J*_{2,3'} = *J*_{2,1'} 6.4, H-2'), 5.7 (1H, d, *J*_{1,2'} 6.4, H-1'), 5.8 (1H, d, *J*_{1,2''} 5.2, H-1''), 7.9 (1H, s, H_{A2}), 8.1 (1H, t, *J* 7.0, H_{N5}), 8.6 (1H, d, *J*_{4,5} 6.3, H_{N4}), 8.9 (1H, d, *J*_{6,5} 6.1, H_{N6}), 9.3 (1H, s, H_{N2}). δ_P (D₂O, 162 MHz, ¹H-decoupled) –10.96 and –11.34 (AB system, *J*_{AB} 20.5, 2P). *m/z* (ES[–]) 676 [100% (M – H)[–]]. λ_{max} 259 nm (ε 13755, pH 8.3). HPLC R_t = 2.46 mins.

Nicotinamide 8-methoxyadenine dinucleotide (8-OCH₃-NAD⁺, 36). 8-OMe-AMP (166.6 μmol) was coupled to β-NMN⁺ (50 mg, 149.6 μmol) with 2 g of DCC in 12.5 mL of pyridine:water (4:1) as described. The pure dinucleotide was obtained in 16% yield. δ_H (D₂O, 400 MHz) 4.0 (3H, s, OMe), 4.6–4.0 (9H, m, ribose-H), 4.8 (1H, dd, *J*_{2,3'} 5.8, *J*_{2,1'} 5.2, H-2'), 5.7 (1H, d, *J*_{1,2'} 5.2, H-1'), 5.8 (1H, d, *J*_{1,2''} 4.9, H-1''), 7.9 (1H, s, H_{A2}), 8.1 (1H, dd, *J*_{5,4} 7.9, *J*_{5,6} 6.1, H_{N5}), 8.6 (1H, d, *J*_{4,5} 7.9, H_{N4}), 9.0 (1H, d, *J*_{6,5} 6.1, H_{N6}), 9.1 (1H, s, H_{N2}). δ_P (D₂O, 162 MHz, ¹H-decoupled) –11.38 and –11.85 (AB system, *J*_{AB} = 20.9 Hz, 2P). *m/z* (ES[–]) 694 [100%, (M+H)⁺]. λ_{max} 259 nm (ε 13000, pH 8.3). HPLC R_t = 2.4 mins.

Nicotinamide 8-piperidyladenine dinucleotide (8-pip-NAD⁺, 37). 8-Pip-AMP (107.8 μmol) was coupled to β-NMN⁺ (45 mg, 134 μmol) with 1.8 g of DCC in 12.5 mL of pyridine:water (4:1) as described. The pure dinucleotide was obtained in 7% yield after 2 purifications. δ_H (D₂O, 400 MHz) 1.6 (6H, m, 3 × CH₂), 3.2 (4H, m, 2 × CH₂), 4.0–4.5 (9H, m, ribose-H), 5.0 (1H, d, *J*_{2,3'} 6.4, *J*_{2,1'} 6.1, 1H, H-2'), 5.5 (1H, d, *J*_{1,2'} 6.1, H-1'), 5.8 (1H, d, *J*_{1,2''} 5.5, H-1''), 7.9 (1H, s, H_{A2}), 8.1 (1H, dd, *J*_{5,4} 7.0, *J*_{5,4} 6.1, H_{N5}), 8.6 (1H, d, *J*_{4,5} 7.0, H_{N4}), 8.9 (1H, d, *J*_{6,5} 6.1, H_{N6}), 9.1 (1H, s, H_{N2}). δ_P (D₂O, 162 MHz, ¹H-decoupled) –11.44 and –11.84 (AB system, *J*_{AB} 20.8, 2P). *m/z* (ES[–]) 746 [50%, (M – H)[–]]. λ_{max} 274 nm (ε 12800, pH 8.3). HPLC R_t = 6.3 mins.

Nicotinamide 8-oxyadenine dinucleotide (8-oxy-NAD⁺, 38). 8-Oxy-AMP (155.3 μmol) was coupled to β-NMN⁺ (50 mg, 149.6 μmol) with 2 g of DCC in 12.5 mL of pyridine:water (4:1) as described. The pure dinucleotide was obtained in 9% yield after 2 purifications. δ_H (D₂O, 400 MHz) 4.4–4.0 (9H, m, ribose), 4.9 (1H, app.t, *J*_{2,3'} = *J*_{2,1'} 5.6, H-2'), 5.5 (1H, d, *J*_{1,2'} 5.6, H-1'), 5.85 (1H, d, *J*_{1,2''} 4.5, H-1''), 7.7 (1H, s, H_{A2}), 8.1 (1H, t, *J* 7.0, H_{N5}), 8.5 (1H, d, *J*_{4,5} 6.5, H_{N4}), 8.9 (1H, d, *J*_{6,5} 6.0, H_{N6}), 9.1 (1H, s, H_{N2}). δ_P (D₂O, 162 MHz, ¹H-decoupled) –11.44 and –11.92 (AB system, *J*_{AB} 21.0, 2P). *m/z* (ES[–]) 678 [100%, (M – H)[–]]. λ_{max} 266 nm (ε 13100, pH 8.3). HPLC R_t = 3.4 mins.

8-Aza-9-deaza nicotinamide adenine dinucleotide (NFD, 39). Formycin 5'-monophosphate monoammonium salt (205 μmol) was coupled to β-NMN⁺ (50 mg, 149.6 μmol) with 2 g of DCC in 12.5 mL of pyridine:water (4:1) as described. The pure dinucleotide was obtained in 9% yield. δ_H (D₂O, 400 MHz) 4.0–5.0 (10H, m, ribose-H), 5.2 (1H, d, *J*_{1,2'} 7.1, H-1'), 6.0 (1H, d, *J*_{1,2''} 5.3, H-1''), 8.0 (1H, s, H_{A2}), 8.1 (1H, m, H_{N5}), 8.6 (1H, d, *J*_{4,5} 8.0, H_{N4}), 9.05 (1H, d, *J*_{6,5} 5.3, H_{N6}), 9.2 (1H, s, H_{N2}). δ_P (D₂O, 162 MHz, ¹H-decoupled) –11.88 and –11.78 (AB system, *J*_{AB} = 21.6 Hz, 2P). *m/z* (ES[–]) 662 [30%, (M – H)[–]]. λ_{max} 275 nm (ε 4600, pH 8.3). HPLC R_t = 2.2 mins.

Nicotinamide 9-[(2-hydroxyethoxy)methyl] adenine dinucleotide (NAcycloD⁺, 40). AcycloAMP (14 μmol) was coupled to β-NMN⁺ (15 mg, 45 μmol) with 0.25 g of DCC in 1.5 mL of pyridine:water (4:1) as described. The pure dinucleotide was obtained in 12% yield. δ_H (D₂O, 400 MHz) 3.81 (2H, m, POCH₂CH₂), 4.07 (2H, m, POCH₂), 4.21–4.75 (5H, m, H-2'', H-3'', H-4'' and H-5''), 5.66 (2H, s, OCH₂base), 6.11 (1H, d, *J*_{1,2''} 5.5, H-1''), 8.18–8.26 (3H, m, H_{N5}, H_{A2} and H_{A8}), 8.89 (1H, d, *J*_{4,5} 6.9, H_{N4}), 9.20 (1H, d, *J*_{6,5} 6.2, H_{N6}), 9.36 (1H, s, H_{N2}). δ_P (D₂O, 162 MHz, ¹H-decoupled) –10.9 and –10.6 (AB system, *J*_{AB} 18.5, 2P). λ_{max} 259 nm (ε 17300, pH 8.3). HPLC R_t = 3.1 mins.

Nicotinamide adenine 9-β-D-arabinofuranoside dinucleotide (NAraD⁺, 41). AraAMP (240 μmol) was coupled to β-NMN⁺ (50 mg, 150 μmol) with 2 g of DCC in 12.5 mL of pyridine:water (4:1) as described. The pure dinucleotide was obtained in 10% yield. δ_H (D₂O, 400 MHz) 4.57–4.13 (10H, m, ribose-H), 6.05 (1H, d, *J*_{1,2'} 4.2, H-1'), 6.27 (1H, d, *J*_{1,2''} 5.7, H-1''), 8.13 (1H, s, H_{A2}), 8.19 (1H, m, H_{N5}), 8.34 (s1H, H_{A8}), 8.83 (1H, d, *J*_{4,5} 8.1, H_{N4}), 9.17 (1H, d, *J*_{6,5} 5.7, H_{N6}), 9.31 (1H, s, H_{N2}). δ_P (D₂O, 162 MHz, ¹H-decoupled) –11.44 and –11.82 (AB system, *J*_{AB} 20.8, 2P). *m/z* (FAB[–]) 663 [100%, (M – H)[–]]. λ_{max} 259 nm (ε 17300, pH 8.3). HPLC R_t = 2.32 mins.

Nicotinamide 2',3'-isopropylidene adenosine dinucleotide (NAcetD⁺, 42). AcetAMP (240 μmol) was coupled to β-NMN⁺ (50 mg, 150 μmol) with 2 g of DCC in 12.5 mL of pyridine:water (4:1) as described. The pure dinucleotide was obtained in 10% yield. δ_H (D₂O, 400 MHz) 1.31(3H, s, CH₃), 1.52 (3H, s, CH₃), 4.53–4.03 (8H, m, ribose-H), 5.04 (1H, dd, *J*_{3,2'} 5.8, *J*_{3,4'} 2.6, H-3'), 5.32 (1H, dd, *J*_{2,3'} 5.8, *J*_{2,1'} 2.8, H-2'), 6.04 (1H, d, *J*_{1,2''} 5.8, H-1''), 6.12 (1H, d, *J*_{1,2'} 2.8, H-1'), 8.07 (1H, s, H_{A2}), 8.15 (1H, dd, *J*_{5,4} 7.9, *J*_{5,6} 5.8, H_{N5}), 8.20 (1H, s, H_{A8}), 8.83 (1H, d, *J*_{4,5} 7.9, H_{N4}), 9.16 (1H, d, *J*_{6,5} 5.8, H_{N6}), 9.38 (1H, s, H_{N2}). δ_P (D₂O, 162 MHz, ¹H-decoupled) –11.8 (m, 2P). *m/z* (FAB[–]) 580 [M⁺ – nicotinamide]. λ_{max} 259 nm (ε 17300, pH 8.3). HPLC R_t = 4.18 mins.

Synthesis of analogues of cyclic-adenosine diphosphate ribose

General method. All cyclic dinucleotides were obtained by incubating the NAD⁺ analogues in HEPES buffer (25 mM, pH 6.8) with the crude *Aplysia* cyclase. The mixtures were left for 20 min after which they were purified on Q-sepharose eluted with a gradient of 1 M TEAB.

Cyclic adenosine 5'-diphosphate ribose (cADPR, 1). A solution of NAD⁺ (2.5 mg) in HEPES (2.5 mL) was incubated with the cyclase (10 μL) as described. cADPR was obtained in 60% yield. δ_H (D₂O, 400 MHz) 3.8 (1H, m, H-5'b), 4.0 (1H, m, H-5''b), 4.2 (1H, m, H-4'), 4.3 (1H, m, H-5''a), 4.4 (2H, m, H-3'' and H-5'a), 4.6 (2H, m, H-2'' and H-4''), 4.7 (1H, m, H-3'), 4.9 (1H, app.t, *J*_{2,3'} = *J*_{2,1'} 7.0, H-2'), 5.85 (1H, d, *J*_{1,2'} 7.0, H-1'), 6.0 (1H, d, *J*_{1,2''} 3.8, H-1''), 8.2 (1H, s, H_{A8}), 8.9 (1H, s, H_{A2}). δ_P (D₂O, 162 MHz, ¹H-decoupled) –11.36 and –11.84 (AB system, *J*_{AB} 14.9, 2P). *m/z* (FAB[–]) 540 [100%, (M – H)[–]]. λ_{max} 254 nm (ε 14300, pH 8.3).

8-Bromo cyclic adenosine 5'-diphosphate ribose (8-Br-cADPR, 2). A solution of 8-Br-NAD⁺ (2.25 mg) in HEPES (2.5 mL) was incubated with the cyclase (10 μL) as described. 8-Br-cADPR was obtained in 60% yield. δ_H (D₂O, 400 MHz) 4.2–5.0 (9H, m, ribose),

5.6 (1H, app.t, $J_{2,3'} = J_{2',1'}$ 5.0, H-2'), 6.0 (1H, d, $J_{1'',2''}$ 2.0, H-1''), 6.3 (1H, d, $J_{1',2'}$ 6.1, H-1'), 8.8 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -10.83 and -11.05 (AB system, J_{AB} 13.2, 2P). m/z (ES⁻) 619.9 [100%, (M - H)⁻]. λ_{max} 264 nm (ϵ 15730, pH 8.3). HPLC R_t = 3.9 mins.

8-Amino cyclic adenosine 5'-diphosphate ribose (8-NH₂-cADPR, 3). A solution of 8-NH₂-NAD⁺ (2.25 mg) in HEPES (1 mL) was incubated with the cyclase (10 μ L) as described. 8-NH₂-cADPR was obtained in 48% yield. δ_H (D₂O, 400 MHz) 4.2-5.0 (10H, m, ribose), 6.1 (1H, d, $J_{1',2'}$ 7.6, H-1'), 6.4 (1H, d, $J_{1'',2''}$ 5.0, H-1''), 9.0 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -13.35 and -14.05 (AB system, J_{AB} 17.5, 2P). m/z (ES⁻) 555.2 [100%, (M - H)⁻]. λ_{max} 274 nm (ϵ 15730, pH 8.3). HPLC R_t = 6.4 mins.

8-Methylamino cyclic adenosine 5'-diphosphate ribose (8-NHMe-cADPR, 9). A solution of 8-NHMe-NAD⁺ (2.5 mg) in HEPES (2.5 mL) was incubated with the cyclase (15 μ L) as described. 8-NHMe-cADPR was obtained in 42% yield. δ_H (D₂O, 400 MHz) 2.8 (3H, s, NHMe), 4.4-3.8 (9H, m, ribose), 5.6 (1H, app.t, $J_{2,3'} = J_{2',1'}$ 5.5, H-2'), 5.7 (1H, d, $J_{1',2'}$ 5.5, H-1'), 5.9 (1H, d, $J_{1'',2''}$ 4.0, H-1''), 8.6 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -10.87 and -11.58 (AB system, J_{AB} = 14.9 Hz, 2P). m/z (ES⁻) 569 [100%, (M - H)⁻]. λ_{max} 279 nm (ϵ 11050, pH 8.3). HPLC R_t = 7.8 mins.

8-Dimethylamino cyclic adenosine 5'-diphosphate ribose (8-NMe₂-cADPR, 10). A solution of 8-NMe₂-NAD⁺ (2.98 mg) in HEPES (2.5 mL) was incubated with the cyclase (15 μ L) as described. 8-NMe₂-cADPR was obtained in 62% yield. δ_H (D₂O, 400 MHz) 3.2 (6H, s, NMe₂), 4.2-5.0 (9H, m, ribose), 5.6 (1H, dd, $J_{2',1'}$ 6.4, $J_{2,3'}$ 5.0, H-2'), 6.2 (1H, d, $J_{1',2'}$ 6.4, H-1'), 6.3 (1H, d, $J_{1'',2''}$ 4.0, H-1''), 9.1 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -5.66 and -6.42 (AB system, J_{AB} 15.4, 2P). m/z (ES⁻) 583 [100%, (M - H)⁻]. λ_{max} 282 nm (ϵ 11800, pH 8.3). HPLC R_t = 9.7 mins.

8-Methyl cyclic adenosine 5'-diphosphate ribose (8-Me-cADPR, 11). A solution of 8-Me-NAD⁺ (3 μ mol) in HEPES (2 mL) was incubated with the cyclase (20 μ L) as described. 8-Me-cADPR was obtained in 84% yield. δ_H (D₂O, 400 MHz) 2.5 (3H, s, Me), 4.0-4.6 (9H, m, ribose), 5.3 (1H, app.t, $J_{2,3'} = J_{2',1'}$ 5.5, H-2'), 5.85 (1H, d, $J_{1',2'}$ 5.5, H-1'), 6.0 (1H, d, $J_{1'',2''}$ 4.0, H-1''), 8.8 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -8.3 and -9.1 (AB system, J_{AB} 16.1, 2P). m/z (ES⁻) 554 [100%, (M - H)⁻]. λ_{max} 260 nm (ϵ 10000, pH 8.3). HPLC R_t = 4.2 mins.

8-Methoxy cyclic adenosine 5'-diphosphate ribose (8-OCH₃-cADPR, 12). A solution of 8-OMe-NAD⁺ (4 μ mol) in HEPES (2 mL) was incubated with the cyclase (20 μ L) as described. 8-OMe-cADPR was obtained in 53% yield. δ_H (D₂O, 400 MHz) 4.1 (3H, s, OMe), 4.2-4.62 (9H, m, ribose), 5.6 (1H, app.t, $J_{2,3'} = J_{2',1'}$ 5.4, H-2'), 5.9 (1H, d, $J_{1',2'}$ 5.9, H-1'), 6.0 (1H, d, $J_{1'',2''}$ 4.0, H-1''), 8.8 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -10.9 (s, 2P). m/z (ES⁻) 570 [100%, (M - H)⁻]. λ_{max} 259 nm (ϵ 10300, pH 8.3). HPLC R_t = 4.0 mins.

8-Piperidyl cyclic adenosine 5'-diphosphate ribose (8-pip-cADPR, 13). A solution of 8-Pip-NAD⁺ (3.75 mg) in HEPES (3.3 mL) was incubated with the cyclase (10 μ L) as described. 8-Pip-cADPR was obtained in 49% yield. δ_H (D₂O, 400 MHz) 1.7 (6H, m, 3 \times CH₂), 3.2 (4H, m, 2 \times CH₂), 4.0-4.5 (9H, m, ribose), 5.6 (1H, app.t, $J_{2,3'} = J_{2',1'}$ 4.2, H-2'), 5.9 (1H, d, $J_{1',2'}$ 5.8, H-1'), 6.1 (1H, d, $J_{1'',2''}$ 4.0, H-1''), 8.9 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -10.92

and -11.76 (AB system, J_{AB} 15.9, 2P). m/z (ES⁻) 623 [100%, (M - H)⁻]. λ_{max} 282 nm (ϵ 12000, pH 8.3). HPLC R_t = 16.2 mins.

8-Oxy cyclic adenosine 5'-diphosphate ribose (8-oxy-cADPR, 14). A solution of 8-Oxy-NAD⁺ (3 μ mol) in HEPES (2.5 mL) was incubated with the cyclase (20 μ L) as described. 8-Oxy-cADPR was obtained in 49% yield. δ_H (D₂O, 400 MHz) 4.6-3.9 (9H, m, ribose), 5.6 (1H, app.t, $J_{2,3'} = J_{2',1'}$ 5.6, H-2'), 5.8 (1H, d, $J_{1',2'}$ 5.6, H-1'), 6.0 (1H, d, $J_{1'',2''}$ 4.0, H-1''), 8.8 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -10.85 and -11.3 (AB system, J_{AB} 16.1, 2P). m/z (ES⁻) 556 [20%, (M - H)⁻]. λ_{max} 279 nm (ϵ 9800, pH 8.3). HPLC R_t = 5.95 mins.

8-Aza-9-deaza cyclic adenosine 5'-diphosphate ribose (cFDPR, 15). A solution of NFD⁺ (6 μ mol) in HEPES (4 mL) was incubated with the cyclase (20 μ L) as described. cFDPR was obtained in 40% yield. δ_H (D₂O, 400 MHz) 4.0-5.0 (9H, m, ribose-H), 5.2 (1H, d, $J_{1',2'}$ 7.1, H-1'), 6.0 (1H, d, $J_{1'',2''}$ 5.3, H-1'') and 8.2 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -11.65 and -11.95 (AB system, J_{AB} 15.7, 2P). m/z (ES⁻) 540 [70%, (M - H)⁻]. λ_{max} 275 nm (ϵ 10300, pH 8.3). HPLC R_t = 5.9 mins.

Cyclic acycloadenosine 5'-diphosphate ribose (cAcycloDPR, 16). A solution of NAcycloD⁺ (1 μ mol) in HEPES (2.5 mL) was incubated with the cyclase (10 μ L) as described. cAcycloDPR was obtained in 21% yield. m/z (FAB⁺) 493 [100%, (M + H)⁺]. λ_{max} 257 nm (ϵ 14300, pH 8.3). HPLC R_t = 5.1 mins.

Cyclic adenine 9- β -D-arabino ribofuranoside 5'-diphosphate ribose (cAraDPR, 17). A solution of NAraD⁺ (7.5 μ mol) in HEPES (17.5 mL) was incubated with the cyclase (75 μ L) as described. cAcycloDPR was obtained in 54% yield. δ_H (D₂O, 400 MHz) 4.59-3.90 (8H, m, ribose), 4.65 (1H, dd, $J_{3',2'}$ 8.2, $J_{3',4'}$ 8.1, H-3'), 5.17 (1H, dd, $J_{2,3'}$ 8.2, $J_{2',1'}$ 7.3, H-2'), 6.03 (1H, d, $J_{1'',2''}$ 4.0, H-1''), 6.24 (1H, d, $J_{1',2'}$ 7.3, H-1'), 8.20 (1H, s, H_A8), 8.91 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -10.23 (br s, 1P) and -11.76 (br s, 1P). m/z (ES⁻) 540 [100%, (M - H)⁻]. λ_{max} 257 nm (ϵ 14300, pH 8.3). HPLC R_t = 4.34 mins.

Cyclic 2',3'-O-isopropylidene adenosine 5'-diphosphate ribose (cAcetDPR, 18). A solution of NAcetD⁺ (7.5 μ mol) in HEPES (17.5 mL) was incubated with the cyclase (75 μ L) as described. cAcetDPR was obtained in 46% yield. δ_H (D₂O, 400 MHz) 1.30 (3H, s, CH₃), 1.49 (3H, s, CH₃), 3.80-4.43 (8H, m, ribose), 4.65 (1H, dd, $J_{3',2'}$ 6.1, $J_{3',4'}$ 3.1, H-3'), 5.17 (1H, dd, $J_{2,3'}$ 6.1, $J_{2',1'}$ 2.0, H-2'), 6.03 (1H, d, $J_{1'',2''}$ 3.7, H-1''), 6.25 (1H, d, $J_{1',2'}$ 2.0, H-1'), 8.26 (1H, s, H_A8), 8.91 (1H, s, H_A2). δ_P (D₂O, 162 MHz, ¹H-decoupled) -10.14 and -11.52 (AB system, J_{AB} 13.9, 2P). m/z (ES⁻) 580 [100%, (M - H)⁻]. λ_{max} 257 nm (ϵ 14300, pH 8.3). HPLC R_t = 7.1 mins.

Acknowledgements

We thank the Wellcome Trust for Project Grant no. 084068 [to BVLP and AHG] and MRC for a studentship (VCB). Work in the Guse lab has been supported by the Deutsche Forschungsgemeinschaft, the Gemeinnützige Hertie-Stiftung and the Deutsche Akademische Austauschdienst. We thank Dr M Thomas for providing Fig. 6.

References

- 1 D. L. Clapper, T. F. Walseth, P. J. Dargie and H. C. Lee, *J. Biol. Chem.*, 1987, **262**, 9561–9568.
- 2 H. C. Lee, T. F. Walseth, G. T. Bratt, R. N. Hayes and D. L. Clapper, *J. Biol. Chem.*, 1989, **264**, 1608–1615.
- 3 H. C. Lee, R. Aarhus and D. Levitt, *Nat. Struct. Biol.*, 1994, **1**, 143–144.
- 4 A. H. Guse, *Curr. Mol. Med.*, 2004, **4**, 239–248.
- 5 A. Galione and G. C. Churchill, *Sci STKE*, 2000, **2000**, pe1.
- 6 A. H. Guse, *J. Mol. Med.*, 2000, **78**, 26–35.
- 7 H. C. Lee, *Physiol. Rev.*, 1997, **77**, 1133–1164.
- 8 F. J. Zhang, Q. M. Gu and C. J. Sih, *Bioorg. Med. Chem.*, 1999, **7**, 653–664.
- 9 B. V. L. Potter and T. F. Walseth, *Curr. Mol. Med.*, 2004, **4**, 303–311.
- 10 S. Shuto and A. Matsuda, *Curr. Med. Chem.*, 2004, **11**, 827–845.
- 11 A. H. Guse, *FEBS J.*, 2005, **272**, 4590–4597.
- 12 A. H. Guse, *Curr. Med. Chem.*, 2004, **11**, 847–855.
- 13 T. F. Walseth and H. C. Lee, *Biochim. Biophys. Acta*, 1993, **1178**, 235–242.
- 14 G. A. Ashamu, A. Galione and B. V. L. Potter, *Chem. Commun.*, 1995, 1359–1360.
- 15 S. Shuto, M. Fukuoka, T. Kudoh, C. Garnham, A. Galione, B. V. L. Potter and A. Matsuda, *J. Med. Chem.*, 2003, **46**, 4741–4749.
- 16 C. Moreau, G. K. Wagner, K. Weber, A. H. Guse and B. V. L. Potter, *J. Med. Chem.*, 2006, **49**, 5162–5176.
- 17 G. K. Wagner, S. Black, A. H. Guse and B. V. L. Potter, *Chem. Commun.*, 2003, 1944–1945.
- 18 G. K. Wagner, A. H. Guse and B. V. L. Potter, *J. Org. Chem.*, 2005, **70**, 4810–4819.
- 19 Q. Liu, I. A. Kriksunov, C. Moreau, R. Graeff, B. V. L. Potter, H. C. Lee and Q. Hao, *J. Biol. Chem.*, 2007, **282**, 24825–24832.
- 20 L. Wong, R. Aarhus, H. C. Lee and T. F. Walseth, *Biochim. Biophys. Acta.*, 1999, **1472**, 555–564.
- 21 L. J. Huang, Y. Y. Zhao, L. Yuan, J. M. Min and L. H. Zhang, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 887–889.
- 22 F. J. Zhang, S. Yamada, Q. M. Gu and C. J. Sih, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1203–1208.
- 23 G. A. Ashamu, J. K. Sethi, A. Galione and B. V. L. Potter, *Biochemistry*, 1997, **36**, 9509–9517.
- 24 S. M. Graham, D. J. Macaya, R. N. Sengupta and K. B. Turner, *Org. Lett.*, 2004, **6**, 233–236.
- 25 B. Zhang, G. K. Wagner, K. Weber, C. Garnham, A. J. Morgan, A. Galione, A. H. Guse and B. V. L. Potter, *J. Med. Chem.*, 2008, **51**, 1623–1636.
- 26 M. Yoshikawa, T. Kato and T. Takenishi, *Bull. Chem. Soc. Japan*, 1969, **42**, 3505–3508.
- 27 N. A. Hughes, G. W. Kenner and A. Todd, *J. Chem. Soc.*, 1957, 3733–3736.
- 28 M. Senkus, *J. Am. Chem. Soc.*, 1946, **68**, 734–736.
- 29 M. J. Robins and P. W. Hatfield, *Can. J. Chem.*, 1982, **60**, 547–553.
- 30 R. E. Holmes and R. K. Robins, *J. Am. Chem. Soc.*, 1965, **87**, 1772–1776.
- 31 J. O. Folayan and D. W. Hutchinson, *Biochim. Biophys. Acta, Nucleic Acids Protein Synth.*, 1977, **474**, 329–333.
- 32 V. C. Bailey, J. K. Sethi, S. M. Fortt, A. Galione and B. V. L. Potter, *Chem. Biol.*, 1997, **4**, 51–61.
- 33 P. J. Dargie, M. C. Agre and H. C. Lee, *Cell Regul.*, 1990, **1**, 279–290.
- 34 A. H. Guse, C. P. daSilva, F. Emmrich, G. A. Ashamu, B. V. L. Potter and G. W. Mayr, *J. Immunology*, 1995, **155**, 3353–3359.
- 35 A. H. Guse, E. Roth and F. Emmrich, *Biochem. J.*, 1993, **291**(Pt 2), 447–451.
- 36 R. Aarhus, R. M. Graeff, D. M. Dickey, T. F. Walseth and H. C. Lee, *J. Biol. Chem.*, 1995, **270**, 30327–30333.
- 37 A. H. Guse, C. P. da Silva, I. Berg, A. L. Skapenko, K. Weber, P. Heyer, M. Hohenegger, G. A. Ashamu, H. Schulze-Koops, B. V. L. Potter and G. W. Mayr, *Nature*, 1999, **398**, 70–73.
- 38 T. Kudoh, M. Fukuoka, S. Ichikawa, T. Murayama, Y. Ogawa, M. Hashii, H. Higashida, S. Kunerth, K. Weber, A. H. Guse, B. V. L. Potter, A. Matsuda and S. Shuto, *J. Am. Chem. Soc.*, 2005, **127**, 8846–8855.
- 39 C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, 1973, **95**, 2333–2344.
- 40 C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, 1972, **94**, 8205–8212.
- 41 R. Stolarski, L. Dudycz and D. Shugar, *Eur. J. Biochem.*, 1980, **108**, 111–121.
- 42 H. Rosemeyer, G. Toth, B. Golankiewicz, Z. Kazimierczuk, W. Bourgeois, U. Kretschmer, H. P. Muth and F. Seela, *J. Org. Chem.*, 1990, **55**, 5784–5790.
- 43 F. Jordan and H. Niv, *Biochim. Biophys. Acta*, 1977, **476**, 265–271.
- 44 F. E. Evans and N. O. Kaplan, *J. Biol. Chem.*, 1976, **251**, 6791–6797.
- 45 V. C. Bailey, J. K. Sethi, A. Galione and B. V. L. Potter, *Chem. Commun.*, 1997, 695–696.
- 46 B. Zhang, V. C. Bailey and B. V. L. Potter, *J. Org. Chem.*, 2007, **73**, 1693–1703.
- 47 A. H. Guse, C. P. daSilva, K. Weber, C. N. Armah, G. A. Ashamu, C. Schulze, B. V. L. Potter, G. W. Mayr and H. Hilz, *Eur. J. Biochem.*, 1997, **245**, 411–417.
- 48 J. K. Sethi, R. M. Empson, V. C. Bailey, B. V. L. Potter and A. Galione, *J. Biol. Chem.*, 1997, **272**, 16358–16363.
- 49 V. C. Bailey, *PhD Thesis*, University of Bath, 1997.
- 50 G. A. Ashamu, *PhD Thesis*, University of Bath, 1997.
- 51 S. Shuto, M. Fukuoka, A. Manikowsky, Y. Ueno, T. Nakano, R. Kuroda, H. Kuroda and A. Matsuda, *J. Am. Chem. Soc.*, 2001, **123**, 8750–8759.
- 52 T. Kudoh, T. Murayama, A. Matsuda and S. Shuto, *Bioorg. Med. Chem.*, 2007, **15**, 3032–3040.
- 53 E. Nandan, S. Y. Jang, S. Moro, H. O. Kim, M. A. Siddiqui, P. Russ, V. E. Marquez, R. Busson, P. Herdewijn, T. K. Harden, J. L. Boyer and K. A. Jacobson, *J. Med. Chem.*, 2000, **43**, 829–842.
- 54 P. A. Evans, K. W. Lai, H. R. Zhang and J. C. Huffman, *Chem. Commun.*, 2006, 844–846.
- 55 S. K. Singh, R. Kumar and J. Wengel, *J. Org. Chem.*, 1998, **63**, 10035–10039.
- 56 P. Nielsen and J. Wengel, *Chem. Commun.*, 1998, 2645–2646.
- 57 M. R. Hellmich and F. Strumwasser, *Cell Regul.*, 1991, **2**, 193–202.
- 58 I. L. Cartwright, D. W. Hutchinson and V. W. Armstrong, *Nucleic Acids Res.*, 1976, **3**, 2331–2339.