Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Bioorganic & Medicinal Chemistry 12 (2004) 475–487
Bioorganic & Medicinal Chemistry

Aplysia californica mediated cyclisation of novel 3′-modified NAD^+ analogues: a role for hydrogen bonding in the recognition of cyclic adenosine 5′-diphosphate ribose

Christopher J. W. Mort,[a] Marie E. Migaud,[a] Antony Galione[b] and Barry V. L. Potter[a,*]

[a]*Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK*
[b]*University Department of Pharmacology, Oxford University, Mansfield Road, Oxford OX1 3QT, UK*

Received 21 August 2003; accepted 10 October 2003

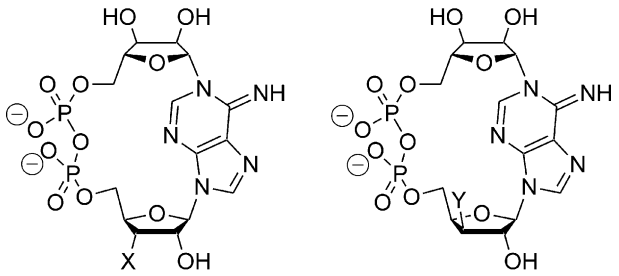
Abstract—Cyclic ADP-ribose mobilizes intracellular Ca^{2+} in a variety of cells. To elucidate the nature of the interaction between the C3′ substituent of cADP-ribose and the cADPR receptor, three analogues of NAD^+ modified in the adenosine ribase (xyloNAD$^+$ 3′F-xyloNAD$^+$ and 3′F-NAD$^+$ were chemically synthesised from D-xylose and adenine starting materials. 3′F-NAD$^+$ was readily converted to cyclic 3′F-ADP ribose by the action of the cyclase enzyme derived from the mollusc *Aplysia californica*. XyloNAD$^+$ and 3′F-xyloNAD$^+$ were cyclised only reluctantly and in poor yield to afford unstable cyclic products. Biological evaluation of cyclic 3′F-ADP ribose for calcium release in sea urchin egg homogenate gave an EC_{50} of 1.5 ± 0.5 μM. This high value suggests that the ability of the C3′ substituent to donate a hydrogen bond is crucial for agonism.

1. Introduction

Recently, the calcium-releasing nucleotide cyclic adenosine diphosphate ribose (cADPR **1**, Fig. 1)[1] has been identified as a second messenger[2] in a wide variety of cell types including mammalian smooth muscle[3] cells and neurons.[4] Initiated by activation of the cADPR receptor, cyclic ADP ribose mediated Ca^{2+} release occurs independently of both the inositol 1,4,5-trisphosphate (IP_3) and the nicotinic acid adenosine diphosphate (NAADP) Ca^{2+} release pathways.[5–7] In mammalian cells, the biosynthesis of cADPR from NAD^+ is catalysed by the ubiquitous membrane-bound glycoprotein CD38,[8,9] implicated in the endogenous production of cADPR[10,11] and responsible also for catalysing the hydrolytic degradation of NAD^+ and cADPR to ADPR.[12–15] In contrast, it has been known since 1991 that the ADP ribosyl cyclase enzyme derived from the sea mollusc *Aplysia californica* possesses very little hydrolase activity.[16] Together with the loose substrate specificity inherent in the *A. californica* cyclase, this has permitted the synthesis of numerous purine- and sugar-modified cADPR analogues.[17] ADP-ribosyl cyclase catalyses the synthesis of cADPR from NAD^+ by removing the nicotinamide moiety and forming a β-glycosidic link between the N1 of adenine and the C1″ of the nicotinamide ribose.[18] The mechanism has been investigated by co-crystallisation of the enzyme with nicotinamide,[12,19,20] mutagenesis of the enzyme,[21] mass-spectroscopy of an enzyme-inhibitor intermediate[22] and by kinetic and inhibition studies.[23–25] Through their altered biological properties, synthetic analogues of cADPR have provided extensive information on the structure–activity relationship (SAR) of the cADPR receptor. Modification of the purine moiety has produced cADPR receptor antagonists,[26] membrane-permeant antagonists,[27,28] partial agonists,[29] and agonists.[17,30] The *Aplysia* cyclase has also been found to tolerate modifications to the adenine ribose component of the NAD^+ substrate.[17,31–33] Among the cADPR analogues chemo-enzymatically synthesised were 3′-deoxy-cADPR **2**, 3′-OMe-cADPR **3** and 3′-OPO_3^{2-}-cADPR **4** (Fig. 1).[34] Sea-urchin egg homogenate (SUH) calcium-release assays performed on these three analogues defined a crucial role for the 3′-hydroxyl group in biological activity.[33] The introduction of the 3′-phosphate group (**4**) resulted in a complete loss of binding affinity for the cADPR receptor, while complete

* Corresponding author. Tel.: +44-1225-386639; fax: +44-1225-386114; e-mail: b.v.l.potter@bath.ac.uk

0968-0896/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2003.10.012



1 X=OH (cADPR)
2 X=H
3 X=OMe
4 X=OPO_3^{2-}
5 X=F

6 X=OH
7 X=F

Figure 1. Previously synthesised (**2–4**) and proposed (**5–7**) 3′-modified analogues of cyclic ADP ribose **1**.

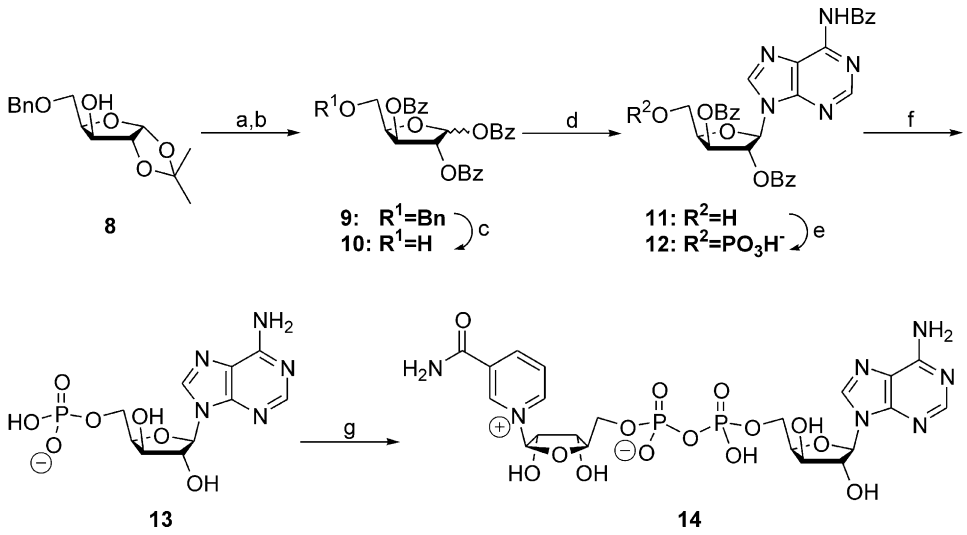
removal of the 3′-hydroxyl (**2**) caused a 100-fold decrease in agonism. Methylation of the C3′ (**3**) yielded an antagonist, thus far the only carbohydrate-modified cADPR antagonist. These observations underline the importance of the C3′-hydroxyl group of cADPR for binding to the receptor and initiating Ca^{2+} release. To further elucidate the nature of the interaction between the C3′ substituent and the receptor, the synthesis of three novel cADPR analogues (**5–7**) was undertaken. Biological evaluation of these analogues would reveal whether the stereochemistry of the C3′-hydroxyl is important for Ca^{2+} release, and clarify the hydrogen-bonding requirements at the C3′ for agonism and antagonism. We present here the synthesis of adenosine ribose-modified 3′-modified analogues of NAD^+ and examine their behaviour to incubation with *A. californica* cyclase. In particular, we have in **5** replaced the 3′-hydroxyl group of the adenosine motif of cADPR by the widely recognised surrogate fluorine to explore H-bond donor/acceptor status with respect to the cADPR receptor. The Ca^{2+} release profile of **5** is presented in SUH.

2. Chemistry

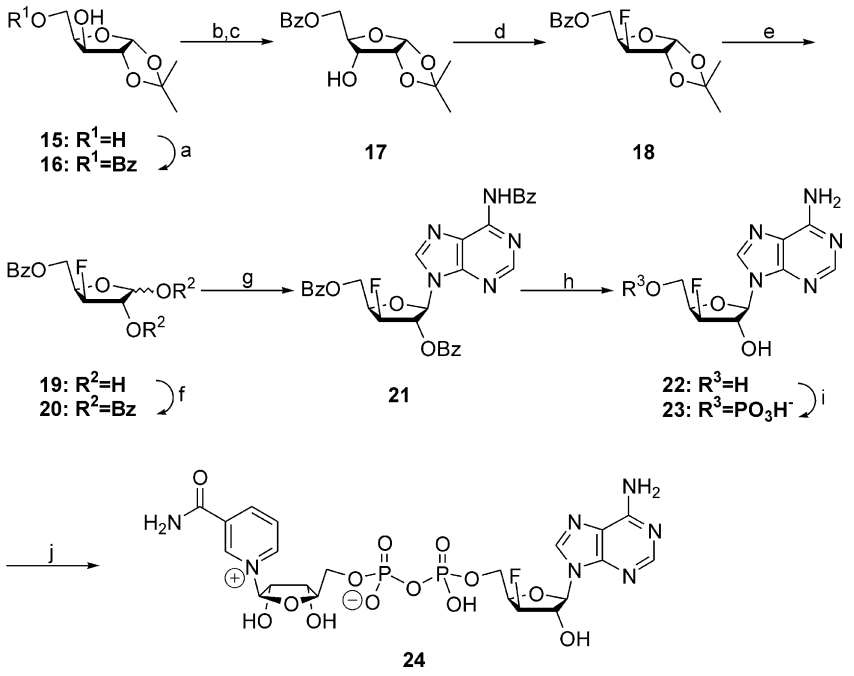
In order to explore the potential effect of replacing the adenine ribose by a xylose sugar xyloNAD$^+$ **14** was synthesised as shown in Scheme 1. 5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose **8** was synthesised from commercially available 1,2-*O*-isopropylidene-α-D-xylofuranose **15** via the dialkylstannylene intermediate.[35] Hydrolysis of the unusually stable 1,2-*O*-acetonide group was accomplished in good yield using aqueous TFA at 0 °C to avoid concomitant deprotection of the benzyl ether. The resultant diol was benzoylated to yield **9**. Catalytic transfer hydrogenation using Pearlman's reagent[36] rapidly and quantitatively deprotected the primary benzyl ether affording an inseparable anomeric mixture of **10**. The reaction of 1,2,3-tri-*O*-benzoyl-D-xylofuranose **10** with persilylated N6-benzoyladenine proceeded smoothly to yield one main product,[37] the required N6-benzoyl-9-(2′,3′-di-*O*-benzoyl-β-D-xylofuranosyl)-adenine **11**. The relative hydrophobicity of the partially protected nucleoside permitted facile purification via conventional silica gel chromatography. Application of Yoshikawa's methodology[38] cleanly converted the partially protected nucleoside into the 5′-phosphate ester, as monitored by anion-exchange HPLC. ^{31}P and ^{1}H NMR spectroscopy identified this material as the phosphorylated nucleoside **12** contaminated only with triethylphosphate. Removal of the benzoyl protecting groups was accomplished with methanolic ammonia. Chromatographic purification of the residue on AG-MP1 anion-exchange resin yielded the requisite 9-(β-D-xylofuranosyl)-adenine-5′-monophosphate **13**. Conventional DCC-mediated phosphate condensation[39,40] of the modified adenine nucleotide with nicotinamide mononucleotide to yield **14** was expected to be low yielding since DCC might also facilitate intramolecular condensation of **13** to yield cyclic xylo-3′,5′-AMP.[41] Hence, the alternative DPPC-activation methodology[42] was employed to promote nucleophilic attack of the adenine nucleoside phosphate ester at the activated NMN pyrophosphate centre. Saponification of the 2″,3″-*O*-Ac groups of the nicotinamide moiety with methanolic ammonia followed by anion-exchange purification yielded the free acid of xyloNAD$^+$ **14** in modest yield. No cyclic xyloAMP or symmetrical dinucleotide by-products were detected.

Scheme 2 illustrates the synthesis of the fluorinated analogue 3′F-xyloNAD$^+$. Careful kinetic control permitted the regioselective benzoylation of **15**,[43] to proceed in high yield. Inversion of the 3′-OH was performed by a two-step oxidation and reduction sequence to yield ribofuranose **17**. The use of aqueous THF in the reduction step obviated the transesterification as witnessed by Imbach et al.[43] Fluorine was introduced with expected inversion of C3′ stereochemistry by DAST and confirmed by the large geminal splitting ($J_{3\text{-}F}$ 50.4 Hz) of the ^{1}H NMR H-3 doublet and by the presence of a 'ddd' signal at around −200 ppm in the ^{19}F NMR spectrum of the product. The 1,2-*O*-isopropylidene group of **18** was hydrolysed using aqueous acetic acid and the resultant diol **19** benzoylated and chromatographed to yield an inseparable mixture (1:1 α/β) of 1,2,5-tri-*O*-benzoyl-3-deoxy-3-fluoro-D-xylofuranose **20**. Glycosidation and phosphorylation were performed in a manner analogous to the synthesis of xyloAMP to yield the fluorinated nucleotide **23**. DPPC activation of acetylated NMN followed by condensation with **23** yielded the required dinucleotide **24** after deprotection of the acetate protecting groups.

The synthesis of the prime fluorinated target 3′F-NAD$^+$ **29** is outlined in Scheme 3. 1-*O*-Acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-fluoro-D-ribofuranose **25** was prepared from D-xylose by the reliable 12-step procedure of Mikhailopulo et al.[44] Reaction of **25** under Vorbrüggen conditions[37] afforded the C1′-N7 β-nucleoside which was then deprotected using methanolic ammonia



Scheme 1. Synthesis of xyloNAD$^+$ **14**: (a) TFA/H_2O (9:1 v/v), 0 °C; (b) BzCl, py, 0 °C→rt; (c) $Pd(OH)_2$/C, EtOH/cyclohexene (2:1 v/v), reflux; (d) N6-Bz-adenine-$(TMS)_2$, TMS-OTf, DCE, reflux; (e) $POCl_3$, $PO(OEt)_3$, 0 °C→rt; (f) NH_3, MeOH, 50 °C; (g) activated NMN, py/DMF (2:1 v/v).

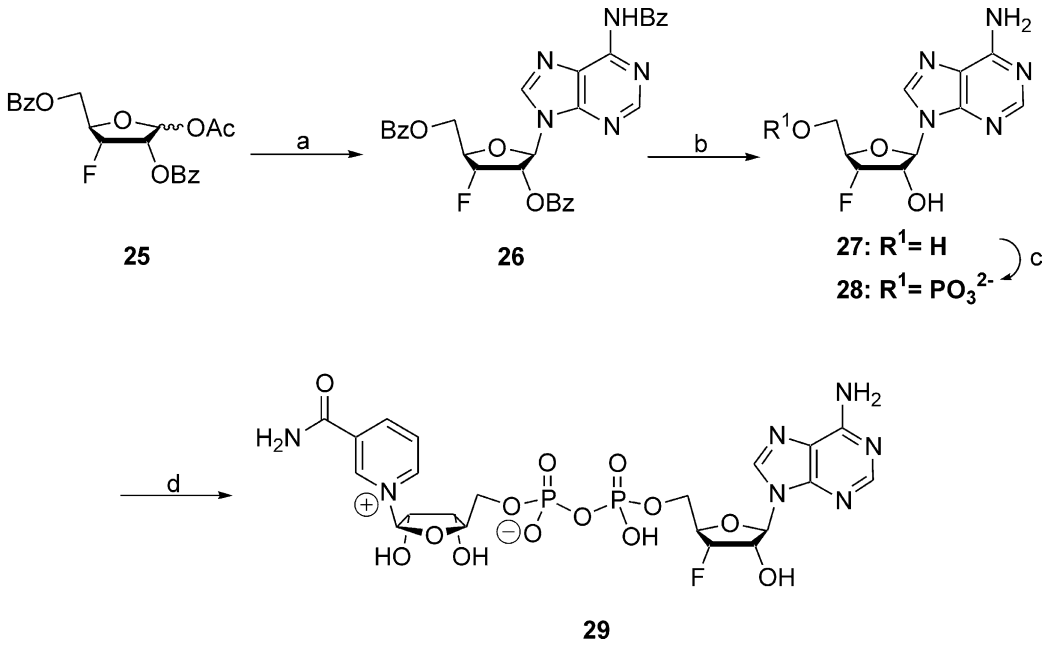


Scheme 2. Synthesis of 3′F-xyloNAD$^+$ **24**: (a) BzCl, py, −20 °C; (b) CrO_3, py, Ac_2O, 0 °C; (c) $NaBH_4$, THF/H_2O (5:4 v/v), 0 °C; (d) DAST, py, DCM, −10 °C→rt; (e) AcOH/H_2O (4:1 v/v), 80 °C; (f) BzCl, py, DMAP; (g) N6-Bz-adenine-$(TMS)_2$, TMS-OTf, DCE, reflux; (h) NH_3, MeOH, 50 °C; (i) $POCl_3$, $PO(OEt)_3$, 0 °C→rt; (j) activated NMN, py/DMF (2:1 v/v).

to give the fluorinated nucleoside **27**. Yoshikawa phosphorylation[38] followed by ion-exchange purification yielded 3′-deoxy-3′-fluoroadenosine 5′-monophosphate **28** in good yield. The coupling of NMN to the adenine nucleotide **28** was again performed using the DPPC coupling to afford 3′F-NAD$^+$ **29**. All three NAD$^+$ analogues were then treated with ADP-ribosyl cyclase and their substrate properties examined.

3. Results and discussion

ADP-Ribosyl cyclase isolated from the mollusc *A. californica* is known to have high cyclase activity and minimal hydrolase activity.[16] The commercial availability and loose substrate specificity of the enzyme has enabled the synthesis of a large number of cADPR analogues. When modifications were made to the purine ring of the substrate, the main kinetic effect observed was a slower turnover.[45] Exchange of the 2′- or 3′-hydroxyl groups of the adenosine ribose moiety for charged groups such as phosphate[31] or more hydrophobic moieties such as methoxy was also of little consequence to the cyclase.[33] Upon incubation of xyloNAD$^+$ with the *Aplysia* cyclase, three new entities could be detected by HPLC. As expected, the first peak corresponded to nicotinamide, the second possessed a retention time very similar to that of cADPR. It was



Scheme 3. Synthesis of cyclic-3′F-ADP ribose **29**: (a) N6-Bz-adenine-$(TMS)_2$, TMS-OTf, DCE, reflux; (b) NH_3, MeOH, 50 °C; (c) $POCl_3$, $PO(OEt)_3$, 0 °C→rt; (d) activated NMN, py/DMF (2:1 v/v).

therefore inferred that this latter product was the desired cyclic nucleotide. However, after 60 min a large proportion of the xyloNAD$^+$ **14** starting material remained unconverted. In an attempt to drive the reaction to completion, a further quantity of cyclase was added. No increase in the quantity of cyclised product was detected, but an increase in the amount of a third product was observed. This third product displayed a retention time analogous to that of ADPR, the linear product of NAD$^+$ or cADPR hydrolysis. These observations seemed to indicate that although cyclisation was occurring, the cyclic product was rapidly hydrolysed. Indeed, purification of the reaction mixture by anion-exchange chromatography failed to isolate the cyclic product, which readily decomposed to xyloADPR over the course of the purification. The attempted cyclisation of 3′F-xyloNAD$^+$ **24** followed a similar pattern and no stable cyclic product was isolated. The reluctance of xyloNAD$^+$ to cyclise may be rationalised by the following arguments. In purine nucleosides, a correlation exists between the *syn* and *anti* equilibrium around the glycosidic bond and the C3′-endo-C2′-*endo* conformations of the carbohydrate fragment.[46] Analysis of the solution conformation of several purine nucleosides by ^{13}C NMR spectroscopy has demonstrated that population of the C3′-endo conformation of the furanose ring of xylo-adenosine should result in repulsion between the C3′ substituent on the upper face of the sugar and the base moiety. This repulsion is maximal when the base lies in the *syn* conformation. Thus, it can be envisaged that the C3′-*endo* conformation changes the *syn*/*anti* equilibrium toward the *anti* form to a greater degree in xylo-adenosine than in the parent adenosine. Indeed, this has been demonstrated by ^1H-coupled ^{13}C NMR experiments.[47] Using X-ray crystallographic data to refine previous observations,[48] Birnbaum et al. confirmed that for xylo-adenosine, a strong preference (population ~80–87%) exists for an N-type (C3′-*endo*) sugar ring conformation with an *anti* conformation around the glycosidic C1′–N7 bond. In most natural ribonucleosides, the C2′-endo *syn* base conformation is more energetically favourable than the C3′-*endo syn* base conformation (Fig. 2).[49] However, in contrast to xylo-adenosine, the barrier to pseudorotation in adenosine between C3′-*endo* and C2′-*endo* is low (~3 kcal/mol) (i.e., fairly equal population of both N and S states) permitting fairly rapid interconversion. A low energy pathway for *anti*/*syn* interconversion is important in the cyclisation of NAD$^+$ to yield cADPR since the purine must be *syn* before ribosylation can occur at the N1. X-ray crystallography[18] and NMR spectroscopy of cADPR[50] have established the conformation of the adenosine furanose ring as C2′-*endo*, also observed in the adenosine furanose ring of NAD$^+$.[51] Enzymatic cyclisation of xyloNAD$^+$ **14** may therefore be impeded by the strong tendency of the xylofuranose ring to adopt a C3′-endo conformation with the concomitant *anti* orientation of the purine moiety. The instability of the proposed cyclised products observed by HPLC might also be explained by the tendency of the adenine nucleoside moiety to adopt an *anti* conformation, thus weakening the newly-formed N1–C1″ bond to yield the linear unstrained ADPR analogue.

Enzyme kinetic[23–25] and mass spectroscopy experiments[22] have shown that *Aplysia* cyclase initially binds the nicotinamide ribose moiety of the NAD$^+$ substrate. The enzyme is then believed to bind the second aromatic purine terminus. It therefore seems plausible that the cyclase first binds the nicotinamide riboside terminus of xyloNAD$^+$ and cleaves the C1′–N1 glycosidic link to form a covalent bond[22] between the enzyme and the C1′ of the nicotinamide ribose. The inflexible C3′-*endo anti* conformation of the xylo nucleoside unit may prevent the enzyme from binding the purine terminus of the dinucleotide. Alternatively, successful recognition may

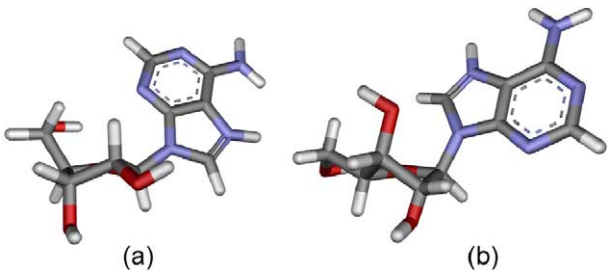


Figure 2. (a) C2′-endo conformation of adenosine — *syn* and *anti* rotomers are readily interconverted; (b) C3′-*endo* conformation of xylo adenosine — *anti* rotomer favoured due to repulsion between purine and C3′-OH.

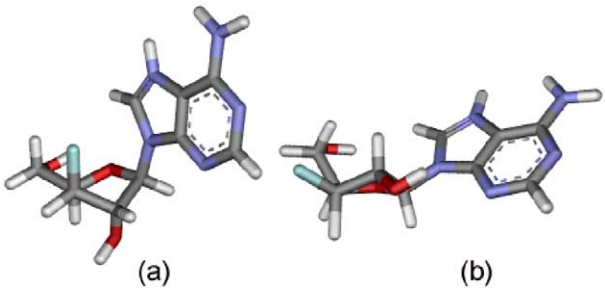


Figure 3. C3′-*endo* (a) and C2′-*endo* conformations (b) of 3′F-xylo-adenosine sighting along the C3′–C4′ bond. The favourable gauche interaction between the C3′–F and C4′–O4′ bonds (a) forces the nucleoside into the C3′-*endo* conformation.

occur at both aromatic termini but rotation of the purine into the required *syn* conformation cannot take place. The presence of water in the enzyme active site would then permit the hydrolysis of the proposed covalent xyloADPR–enzyme complex resulting in the release of xyloADPR from the enzyme.

In nucleosides that incorporate one fluorine substituent on the sugar moiety, the *gauche* effect arising from the interaction between the various electronegative substituents is the predominant effect that drives the N/S equilibrium strongly towards one of the puckered forms.[52] In 3′F-xylo-adenosine, the strong electronegativity of the C3′ fluorine atom located above the plane of the sugar ring pushes the equilibrium towards a conformation in which the C–F bond adopts a preferential gauche orientation with the C4′-ring oxygen bond. This gauche orientation only occurs in the C3′-*endo* (N-type) conformation and this form is therefore preferred. The conformational analogy with the xylo-adenosine nucleoside unit allows similar arguments to be applied in rationalising the difficulty in cyclising 3′F-xyloNAD$^+$ **24**. In 3′F-adenosine, however, the fluorine atom is located below the plane of the sugar ring. The ring must adopt a C2′-endo conformation in order to attain a favourable gauche orientation between the C–F bond and the C4′-ring oxygen bond, thus driving the overall equilibrium towards the S-type conformation[52] (Fig. 3).

It was therefore anticipated that *anti*/*syn* interconversion in the prime target analogue 3′F-NAD$^+$ **29** would occur more readily than in either of the two xylo analogues **14** and **24** permitting enzymatic cyclisation to occur. Indeed, upon incubation of 3′F-NAD$^+$ **29** with the *Aplysia* enzyme, cyclic 3′F-ADP ribose **5** was successfully formed in yields comparable with the cyclisation of the natural substrate NAD$^+$ and the product was stable.

Cyclic 3′F-ADP ribose **5** was evaluated for Ca^{2+} release activity in SUH in accordance with a previously published procedure.[33] Upon incubation of **5** and SUH in the presence of Fluo-3, a dye that exhibits fluorescence upon sequestering calcium, the release of Ca^{2+} was observed in the SUH. Cyclic 3′F-ADP ribose was therefore shown to be an agonist of the cADPR receptor.

The concentration of cyclic 3′F-ADP ribose required to elicit half-maximal Ca^{2+} release (EC_{50}) was calculated as 1.5 ± 0.5 μM (Fig. 4). By comparison, the EC_{50} values for cyclic ADP ribose and cyclic 2′-deoxy-ADP ribose have previously been reported as 32 and 58 nM, respectively.[33] The high value obtained for the EC_{50} of **5** compares with that determined for the agonist cyclic 3′-deoxy-ADP ribose **2** ($EC_{50} = 5$ μM) and with that reported for the antagonist cyclic 3′-OMe-ADP ribose **3** ($IC_{50} = 5$ μM).[33] Both **3** and **5** possess a substituent at the 3′-position that functions only as a hydrogen bond acceptor. While the importance of the 3′-OH group in the binding of cADPR to the receptor has been previously established by the biological evaluation of **2** and **3**, the low binding affinity exhibited by the 3′-modified cADPR congeners **2**, **3**, and **5** can now be ascribed, at least in part, to their inability to act as hydrogen bond donors. Furthermore, the antagonist activity observed for **3** can be tentatively attributed to the larger steric volume of the methyl group. Unfortunately, for the other analogues **6** and **7** the influence upon receptor recognition and Ca^{2+} release by a reversal in the stereochemistry of the 3′-group (with any potential change in nucleoside/macrocycle conformation) could not be assessed since cyclic xyloADP ribose and cyclic 3′F-xyloADP ribose were considered too unstable for biological assay.

4. Conclusion

Starting from simple sugar precursors and adenine, we have prepared analogues of adenosine monophosphate that were coupled to diphenyl phosphate-activated nicotinamide mononucleotide. XyloNAD$^+$ **14**, 3′F-xyloNAD$^+$ **24** failed to cyclise in a productive fashion when incubated with *A. californica* ADP-ribosyl cyclase while 3′F-NAD$^+$ **29** yielded cyclic 3′F-ADP ribose **5**. The inability of **14** and **24** to cyclise was attributed to restricted purine rotation around the glycosidic bond by steric repulsion between the 3′-substituent on the β-face and the purine moiety. This repulsion was exacerbated by the C3′-*endo* conformation of the xylo-nucleosides bringing the C3′-group into closer proximity with the purine. Calcium-release assay revealed **5** to be a relatively weak agonist. In conjunction with biological data previously obtained for cyclic 3′-deoxy-ADP ribose **2** and cyclic 3′-OMe-ADP ribose **3**, this result suggests

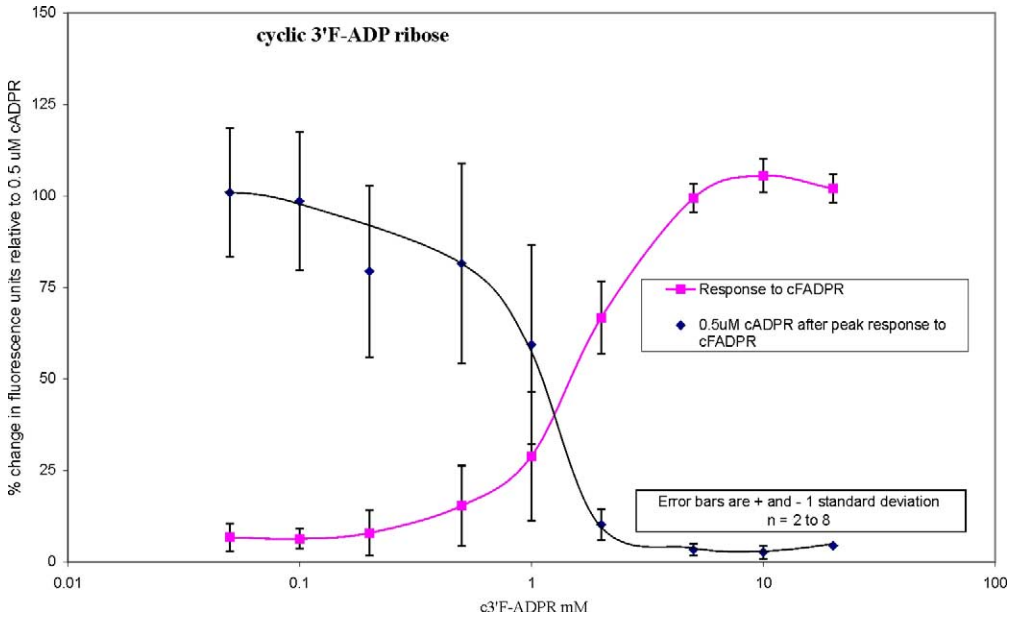


Figure 4. Concentration–response curves for cyclic 3′F-ADP ribose for calcium release in sea urchin egg homogenate.

that the ability of a 3′-substituent of cADPR or an analogue to donate a hydrogen bond is crucial for good cADPR receptor recognition.

5. Experimental

5.1. General

Thin layer chromatography (TLC) was carried out using percolated plates (Merck TLC aluminium sheet silica 60 F_{254}Art no. 5554). TLC plates were visualised by ultraviolet light (254 nm) and immersion in anisaldehyde stain (by volume: 93% ethanol, 3.5% sulfuric acid, 1% acetic acid and 2.5% anisaldehyde) followed by heating. Flash chromatography (SGC) utilised Merck Sorbsil C60 silica gel and Merck GPR solvents used without distillation. Ion exchange chromatography was performed using an unjacketed Glass Econo-column FPLC column (20×1.0 cm internal diameter) and BioRad AG-MP1 strong anion-exhange resin (functional group $RCH_2Me_3N^+$; matrix styrene divinylbenzene) with a concave gradient (0 min: 100% MQ→20 min: 100% MQ→40 min: 96% MQ→60 min: 84% MQ→75 min: 68% MQ→105 min: 0% MQ→120 min: 0% MQ) of 150 mM TFA. HPLC was performed on a Shimadzu LC-6A chromatograph with UV detector operating at 254 nm or on a Varian ProStar system with UV detector operating at 254 or 314 nm. HPLC chromatography used a combination of a Partisil 10 μm SAX guard column (10×0.46 cm) and either a Supelco (20×0.46 cm) 10 μm SAX HPLC column with an isocratic elution using phosphate buffer (50 mM), pH 3 containing 5% MeOH at a flow rate of 2 mL/min or a Technicol (10×0.46 cm) 10 μm SAX HPLC column with an isocratic elution using at 1 mL/min of the same buffer.

All chemicals including solvents, reagents, buffers and enzymes were purchased from Aldrich/Sigma. Reactions that required anhydrous conditions were conducted under dry nitrogen. Dichloromethane and pyridine were distilled over CaH_2 and stored over molecular sieves and KOH, respectively. Dioxane was distilled over BaO and stored over activated molecular sieves, as was DMF. THF was freshly distilled over Na and benzophenone. NMR analyses were carried out on a 400 MHz Varian spectrometer. The δ values are stated in ppm and J values are given in Hertz (Hz). For ^{1}H NMR, the values are referenced on the TMS signal in $CDCl_3$ (0 ppm), on the HOD signal in D_2O (4.65 ppm), on the CHD_2COCD_3 in acetone-d_6 (2.09 ppm) and on CHD_2SOCD_3 in DMSO-d_6 (2.40 ppm). For ^{13}C NMR, the values are referenced on the carbon signal in $CDCl_3$ (77.00 ppm) and on added CH_3CN in D_2O (1.30 ppm). The signal of $CFCl_3$ is used to reference ^{19}F NMR (0.00 ppm). For ^{31}P NMR, the values are referenced on the H_3PO_4 signal in D_2O (0.00 ppm). Mass spectra were recorded at the University of Bath. Ultraviolet (UV) absorbance was measured with a Perkin-Elmer Lambda 3 UV/VIS spectrophotometer. Melting points were determined using a Reichert-Jung Thermo Galen Kofler Block and are uncorrected.

5.2. Chemistry

5.2.1. 5-*O*-Benzyl-1,2,3-tri-*O*-benzoyl-D-xylofuranose (9). 5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose[35] (1.10 g, 3.92 mmol) was added to a pre-cooled (0 °C) mixture of trifluoroacetic acid (36 mL) and water (4 mL). The solution was stirred at 0 °C for 45 min after which TLC (4:1 $CHCl_3$/Me_2CO) indicated the formation of two major products (R_f = baseline, R_f = 0.09), a trace of SM (R_f = 0.57) and a second minor product (R_f = 0.38). The reaction mixture was poured into toluene (100 mL) and concentrated. The residue was azeotroped with toluene a further five times to yield a colourless viscous oil (1.15 g).

The oil was dissolved in dry pyridine (25 mL), cooled to 0 °C under an atmosphere of dry N_2 and benzoyl chloride (3.1 equiv, 12.20 mmol, 1.41 mL) added dropwise. After 18 h at rt, TLC (3:1 hexane/EtOAc) indicated the formation of several products. Benzoyl chloride (2.0 equiv, 7.84 mmol, 0.90 mL) was added and the solution stirred for a further 11 h. TLC indicated mainly one product (R_f = 0.38) but a trace of SM was still present. The addition of benzoyl chloride (0.5 equiv, 1.96 mmol, 0.23 mL) with stirring for a further 13 h resulted in the complete conversion to one spot (R_f = 0.38). The reaction mixture was concentrated and co-evaporated from toluene (3×). The residue was dissolved in DCM and washed with saturated aqueous $CuSO_4$ and water. The organic phase was dried ($MgSO_4$) and concentrated to yield an orange oil (4.31 g). Purification by SGC [linear gradient; 15:1→4:1 (v/v) hexane/EtOAc + 0.5% (v/v) MeOH] yielded the title compound as a 2:1 mixture of α- and β-anomers (1.65 g, 76%). α: ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 3.69, 3.78 (2H, ABX, $^2J_{AB}$ = 9.8, $^3J_{AX}$ = 4.9, $^3J_{BX}$ = 4.4, H-5, H-5′), 4.51 (2H, s, CH_2–Ar), 4.90–4.94 (1H, m, H-4), 5.97 (1H, dd, J_{2-1} = 4.9, J_{2-3} = 5.4, H-2), 6.13 (1H, dd, J_{3-4} = 6.3, J_{3-2} = 5.9, H-3), 6.95 (1H, d, J_{1-2} 4.9, H-1), 7.10–7.60 (14H, m, Ar), 7.95–8.10 (6H, m, Ar). ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 68.57 (C-5), 74.42 (CH_2–Ar), 75.72 (C-3), 77.00 (C-2), 78.06 (C4), 94.54 (C-1). β: ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 3.85–3.92 (2H, m, H-5, H-5′), 4.44, 4.52 (2H, AB, J_{AB} = 12.2, CH_2–Ar), 4.94–4.98 (1H, m, H-4), 5.79 (1H, s, H-2), 5.91 (1H, d, J_{3-4} = 4.9, H-3), 6.67 (1H, s, H-1), 7.10–7.60 (14H, m, Ar), 7.95–8.10 (6H, m, Ar). ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 68.83 (C-5), 74.26 (CH_2–Ar), 75.03 (C-3), 80.69 (C-2), 82.54 (C-4), 100.06 (C-1). α + β 128.96, 129.03, 129.07, 129.12, 129.27, 129.38, 129.52, 129.69, 129.87, 130.04, 130.09, 130.46, 130.51, 130.57, 130.64, 134.11, 134.19, 134.32, 134.48, 138.23, 138.28 (24xAr), 165.42, 165.61, 165.73, 165.99, 166.08, 166.45 (6×C=O).

5.2.2. 1,2,3-tri-*O*-Benzoyl-D-xylofuranose (10). 5-*O*-Benzyl-1,2,3-tri-*O*-benzoyl-D-xylofuranose **9** (1.652 g, 2.99 mmol) was dissolved in a mixture of EtOH (30 mL) and cyclohexene (15 mL). Palladium hydroxide on carbon [0.4 g, ~1/4 (w/w)] was added and the suspension refluxed for 1 h. TLC (3:1 hexane/EtOAc) indicated the formation of one product (R_f = 0.12) and the complete consumption of starting material. The $Pd(OH)_2$/C was filtered off through Celite, washed thoroughly with $CHCl_3$, and the combined organic fraction concentrated. Purification by SGC [linear gradient; 9:1→3:1 (v/v) hexane/EtOAc + 0.5% (v/v) MeOH] yielded the title compound as an inseparable anomeric mixture (β/α) (1:2 ratio) (1.271 g, 92%). β: ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 2.57 (1H, br s, OH), 3.80–4.03 (2H, m, H-5, H-5′), 4.84–4.88 (1H, m, H-4), 5.81 (2H, br s, H-2, H-3), 6.63 (1H, s, H-1), 7.30–7.65 (9H, m, Ar), 7.90–8.15 (6H, m, Ar); ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 61.48 (C-5), 75.05 (C-3), 80.60 (C-2), 83.98 (C-4), 99.61 (C-1), 164.99, 165.09, 165.79 (3×C=O). α: ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 2.57 (1H, br s, OH), 3.80–3.88 (2H, m, H-5, H-5′), 4.80 (1H, 'dt' (ddd), $J_{4-5} \sim J_{4-5'}$ = 4.4, J_{4-3} = 6.7, H-4), 5.91 (1H, 't' (dd), J_{2-1} = 4.7, J_{2-3} = 5.6, H-2), 6.07 (1H, 't' (dd), J_{3-2} = 5.6, J_{3-4} = 6.4, H-3), 6.91 (1H, d, J_{1-2} = 4.7, H-1), 7.30–7.65 (9H, m, Ar), 7.90–8.15 (6H, m, Ar); ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 60.86 (C-5), 75.46 (C-3), 76.72 (C-2), 79.06 (C-4), 93.94 (C-1), 128.62, 128.68, 128.82, 128.86, 128.92, 129.02, 129.43, 129.48, 130.01, 130.07, 130.17, 130.19, 130.22, 133.73, 133.78, 133.85, 134.03, 134.07 (18×Ar), 165.24, 165.53, 166.37 (3×C=O); MS m/z (relative intensity) FAB^+ 341 (100, $M+H^+$–C_6H_5COOH), 462 (1, $M+H^+$); HRMS (FAB^+) calcd $C_{26}H_{22}O_8$ ($M+H^+$) 462.1315, found 462.1285.

5.2.3. N6-Benzoyl-9-(2′,3′-di-*O*-benzoyl-β-D-xylofuranosyl)-adenine (11). A suspension of N6-benzoyladenine (1.15 equiv, 2.49 mmol, 597 mg) in HMDS (15 mL) and pyridine (0.4 mL) was refluxed under an atmosphere of dry N_2. After 68 h, the homogeneous yellow solution was concentrated and azeotroped with dry toluene (2×) to yield a gummy residue. A solution of dry 1,2,3-tri-*O*-benzoyl-D-xylofuranose **10** (1.005 g, 2.17 mmol) in DCE (20 mL) was added to the bis-silylated benzoyladenine with the careful exclusion of moisture. TMS-OTf (2.2 equiv, 4.67 mmol, 0.93 mL) was added under dry N_2 and the solution heated under reflux. After 1 h, TLC (1:1 $CHCl_3$/Me_2CO) indicated the formation of a major product (R_f = 0.55) and a minor product (R_f = 0.13). 2-D TLC was helpful due to streaking. The solution was heated under reflux for a further 3 h but no change in composition occurred. The solution was allowed to cool and diluted with DCM. Washing with water (2×), drying ($MgSO_4$) and concentration yielded an orange foam (1.72 g). Purification by SGC [$CHCl_3$ followed by a linear gradient of 7:1→1:1 (v/v) $CHCl_3$/Me_2CO] yielded the protected nucleoside as a yellow foam (820 mg, 65%). ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 3.90, 4.05 (2H, ABX, $^2J_{AB}$ = 12.9, $^3J_{AX}$ = 3.5, $^3J_{BX}$ = 2.9, H-5′$_A$, H-5′$_B$), 4.79 (1H, 'dt' (ddd), $J_{4-5} \sim J_{4-5'}$ = 3.2, J_{4-3} = 6.4, H-4′), 6.00 (1H, 't' (dd), J_{3-4}–J_{3-2} = 6.0, H-3′), 6.27 (1H, d, J_{1-2} = 5.3, H-1′), 6.56 (1H, 't' (dd), J_{2-1}–J_{2-3} = 5.6, H-2′), 7.35–7.55 (9H, m, Ar), 7.83 (1H, br d, NH), 7.98 (2H, d, J 7.4, CHCC=O), 8.02 (2H, d, J = 9.4, CHCC=O), 8.12 (2H, d, J = 8.8, CHCC=O), 8.26 (1H, d, J = 2.3, H-2), 8.68 (1H, s, H-8); ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 60.91 (C-5′), 75.07 (C-3′), 77.52 (C-2′), 81.04 (C-4′), 87.69 (C-1′), 127.59 (C-2), 127.86, 128.48, 128.58, 128.73, 128.87, 130.14, 130.17, 130.69, 131.86, 132.28, 133.21, 133.98, 135.76, 136.07, 141.33 (Ade), 150.47 (Ade), 151.38 (Ade, C-8), 160.08 (Ade), 163.17, 165.08, 166.19, 170.37; MS m/z (relative intensity) FAB^+ 341(100, $M+H^+$–C_6H_5COOH), 580(59, $M+H^+$); HRMS (FAB^+) calcd $C_{26}H_{22}O_8$ ($M+H^+$) 580.1832, found 580.1851. UV λ_{MAX} = 279 nm (EtOH).

5.2.4. xylo-Adenosine-5′-phosphate (13). N6-Benzoyl-9-(2′,3′-di-*O*-benzoyl-β-D-xylofuranosyl) adenine **11** (446 mg, 0.77 mmol) was dissolved in triethylphosphate (3 mL) and the yellow solution heated gently under N_2 until some vapour was seen to condense on the neck of the flask. The solution was cooled to 0 °C and phosphorous oxychloride (1.1 equiv, 0.85 mmol, 0.079 mL) added dropwise with stirring. After 1 h, TLC (1:1 $CHCl_3$/Me_2CO) indicated the complete consumption of starting material and the formation of a very polar UV active spot on the TLC baseline. MQ water (90 mL) was

added to the reaction mixture followed by ether (90 mL). The resultant white precipitate, identified by TLC as the polar product was filtered off, washed with water and dried under vacuum. MS for **12** *m*/*z* (relative intensity) FAB$^+$ 240(100), 556(61, M$^+$–C_6H_5CO–), 659 (62, M$^+$); HRMS (FAB$^+$) calcd $C_{31}H_{26}N_5O_{10}P_1$ (M$^+$) 659.1417, found 659.1424.

The crude protected nucleotide was dissolved in a solution of methanol (50 mL) through which ammonia gas had been bubbled for 10 min. The yellow solution was cautiously heated at ~50 °C in a sealed tube for 36 h. Monitoring by HPLC (10 μm Partisil SAX anion-exchange column (15 cm), isocratic elution 0.05 M KH_2PO_4/5% MeOH/pH 3) revealed the relatively rapid formation of a peak with $R_T = 3.3$ min (N6-benzoyl-xyloAMP) and the gradual formation of a peak with $R_T = 2.6$ min. The reaction mixture was concentrated, leaving a yellow solid. The solid was redissolved in MQ water (80 mL) and brought to pH 8 with 1 M NaOH. Purification was effected by ion-exchange chromatography on BioRad AG-MP1 resin using a concave gradient of 150 mM TFA (0 min: 100% MQ→20 min: 100% MQ→40 min: 96% MQ→60 min: 84% MQ→75 min: 68% MQ→105 min: 0% MQ→120 min: 0% MQ). The product eluted at 28–45% TFA. Fractions containing >95% of the product (as determined by HPLC) were pooled. Concentration and co-evaporation (3×) from MQ water yielded the product as a colourless, vitreous solid. $\lambda_{MAX} = 258$ nm. ^{1}H NMR (399.8 MHz; D_2O) δ_H 3.95–4.04 (2H, m, H-5′$_A$, H-5′$_B$), 4.30 (1H, s, H-3′), 4.42 (1H, d, $J_{4'-5'} = 4.7$, H-4′), 4.52 (1H, s, H-2′), 5.90 (1H, s, H-1′), 8.00 (1H, s, H-8), 8.30 (1H, s, H-2); ^{31}P NMR (161.9 MHz; D_2O) δ_P 5.31 (s, decoupled); 5.24 (t, $J_{P-C-H5'} = 6.3$, coupled). MS *m*/*z* (relative intensity) FAB$^+$ 348 (40); HRMS (FAB$^+$) calcd $C_{10}H_{15}N_5O_7P$ (M$^+$) 348.0709, found 348.0680.

5.2.5. xylo-Adenosine 5′-diphospho-5″-ribofuranoside-1′-nicotinamide (xyloNAD$^+$) (14). Activation of NMN. A solution of NMN (50 mg, 0.15 mmol, free acid) in MQ water (0.3 mL) was added dropwise to a pre-cooled (0 °C), rapidly stirred, mixture of acetic anhydride (6 mL) in pyridine (10 mL). After 48 h at 0 °C, the reaction was concentrated ($T < 20$ °C) and the residue stirred with a mixture of MQ water and pyridine [20:1 (v/v), 1 mL total volume] for 20 min at rt. The solvent was removed in vacuo, and the residue evaporated from dry DMF (3×). Diphenyl phosphorochloridate (2.0 equiv, 0.30 mmol, 0.062 mL) and tri-*n*-butylamine (2.0 equiv, 0.30 mmol, 0.072 mL) were added to a solution of the freshly prepared 2,3-di-*O*-acetyl-NMN in a mixture of DMF (1.0 mL) and dioxane (2.0 mL) under an atmosphere of dry N_2. The solution was stirred at rt for 1 h after which the solvent was removed in vacuo. Excess diphenyl phosphorochloridate was removed by repeated trituration (3×) with cold, dry ether before finally co-evaporating with dry DMF (3×).

Coupling. Tri-*n*-octylamine (2.0 equiv, 0.334 mmol, 0.118 g, 0.146 mL) was added dropwise to a suspension of xylo-adenosine-5′-phosphate (58 mg, 0.167 mmol, free acid) in dry MeOH (5 mL). The resulting solution was stirred at rt for 30 min, concentrated and then co-evaporated from dry DMF (3×). The off-white solid was added to a solution of the activated 2′,3′-di-*O*-acetyl-NMN in a mixture of dry DMF (1.0 mL) and pyridine (2.0 mL). After 36 h at rt, the reaction mixture was concentrated and dissolved in a pre-cooled (0 °C) mixture of MQ water and concentrated ammonia [20:1 (v/v), 2 mL total volume]. The flask was maintained at 0 °C for 6 h and concentrated. The residue was dissolved in MQ water, washed with $CHCl_3$ (2×) and concentrated ($T < 20$ °C). The solid was re-dissolved in MQ water and the pH of the solution brought carefully to pH8 with 1 M NaOH. Purification was achieved by ion-exchange chromatography on BioRad AG-MP1 resin using a concave gradient of 150 mM TFA (0 min: 100% MQ→20 min: 100% MQ→40 min: 96% MQ→60 min: 84% MQ→75 min: 68% MQ→105 min: 0% MQ→120 min: 0% MQ). The title compound eluted at 17–25% TFA. Fractions containing >95% of the product (as determined by HPLC) were pooled. Concentration and co-evaporation (3×) from MQ water, followed by lyophilisation yielded the product (free acid) as a white, fluffy solid in 12% yield. ^{1}H NMR (399.8 MHz; D_2O) δ_H 4.20–4.75 (10H, m, xylo- and ribofuranoside protons), 6.13 (1H, s, H-1′), 6.16 (1H, d, $J = 5.0$, H-1″), 8.28 (1H, 't' (dd), $J = 6.4$, H_{NIC}-5), 8.41 (1H, s, H2), 8.57 (1H, br s, H8), 8.92 (1H, d, $J = 7.9$, H_{NIC}-4), 9.28 (1H, d, $J = 5.3$, H_{NIC}-6), 9.41 (1H, s, H_{NIC}-2); ^{31}P NMR (161.9 MHz; D_2O) δ_P −10.57→−10.02 (m, P-*O*-P). MS *m*/*z* (relative intensity) FAB$^+$ 665(30).

5.2.6. 5-*O*-Benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose (16).[43] A solution of 1,2-*O*-isopropylidene-α-D-xylofuranose **15** (25.0 g, 0.131 mol) in dry pyridine (125 mL) was cooled to −20 °C under dry N_2. A solution of benzoyl chloride (1.0 equiv, 0.131 mol, 18.48 g, 15.3 mL) in dry pyridine (70 mL) was added dropwise with rapid stirring over 2 h at −20 °C. TLC (9:1 $CHCl_3$/Me_2CO) indicated a very small quantity of starting material ($R_f = 0.07$), one main product ($R_f = 0.52$) and a small side-product ($R_f = 0.89$). The orange suspension was diluted with DCM and washed with aqueous ammonium chloride and water. The organic phase was separated, dried ($MgSO_4$) and concentrated. The residue was co-evaporated from toluene (3×) to remove traces of pyridine. The clear orange oil (50.1 g > 100%) was purified by SGC ($CHCl_3$ followed by a linear gradient of 20:1→15:1 (v/v) $CHCl_3$/Me_2CO) to yield an off-white solid (27.1 g, 70.2%). Mp [pentane/ether, 1:1 (v/v)] 83–85 °C. ^{1}H NMR data consistent with literature data.[43] MS *m*/*z* (relative intensity) FAB$^+$ 237 (92, M + H$^+$–Me_2CO), 295 (100, M + H$^+$); HRMS (FAB$^+$) calcd $C_{15}H_{19}O_6$ (M + H$^+$) 295.1182, found 295.1186.

5.2.7. 5-*O*-Benzoyl-1,2-*O*-isopropylidene-α-D-ribofuranose (17). Chromium trioxide (4.0 equiv, 0.190 mol, 19.03 g) was added to a stirred solution of pyridine (8.0 equiv, 0.386 mol, 30.8 mL) in DCM (400 mL) at rt. The red solution was stirred rapidly at rt for 15 min and then cooled to 0 °C. 5-*O*-Benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose **16** (14.0 g, 0.048 mol, 1.0 equiv) was added, followed immediately by acetic anhydride (4.0

equiv, 0.190 mol, 18.0 mL). The suspension was stirred at 0 °C for 10 min. A small aliquot of the reaction mixture was removed, co-evaporated from toluene (3×) and re-dissolved in DCM. TLC (9:1 $CHCl_3/Me_2CO$) indicated that all the starting material ($R_f = 0.52$) had been consumed and that only a less polar streaking spot was present. The reaction mixture was poured into EtOAc (1 L) and the precipitate filtered off through a Büchner funnel containing a layer of silica (2 cm) over Celite (1 cm). The precipitate was washed thoroughly with EtOAc and the combined organic fraction concentrated ($T < 25$ °C) to yield a brown mobile oil (20.5 g > 100%).

The crude 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-3-ulose was dissolved in THF (120 mL), cooled to 0 °C and water (96 mL) added. Sodium borohydride (1.1 equiv, 0.052 mol, 1.98 g) was added in five small portions over 15 min. TLC (9:1 $CHCl_3/Me_2CO$) indicated the formation of a new material ($R_f = 0.61$) and the complete consumption of the 3-ulose intermediate. The reaction was diluted with $CHCl_3$ and washed with water. The aqueous phase was extracted repeatedly (3×) with $CHCl_3$ and the combined organic fractions dried ($MgSO_4$) and concentrated. Purification by SGC [$CHCl_3$ followed by 9:1 (v/v) $CHCl_3/Me_2CO$] yielded the product as a white solid (11.37 g, 81%). Mp (ether/pentane 1:1 v/v) = 81–82 °C. ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 1.37 (3H, s, isopropylidene CH_3), 1.59 (3H, s, isopropylidene CH_3), 2.84 (1H, d, $J_{OH\text{-}3} = 9.1$, OH), 3.90–3.96 (1H, m, H-3), 4.11 (1H, ddd, $J_{4-5} = 2.8$, $J_{4-5'} = 5.6$, $J_{4-3} = 11.7$, H-4), 4.45, 4.70 (2H, ABX, $^2J_{AB} = 12.3$, $^3J_{AX} = 4.1$, $^3J_{BX}$ 2.3, H-5, H-5′) 4.61, (1H, 't' (dd), $J_{2-1} = 4.1$, $J_{2-3} = 4.7$, H-2), 5.84 (1H, d, $J_{1-2} = 3.9$, H-1), 7.42 (2H, 't' (dd), $J_{Ar2-Ar1} = 7.6$, $J_{Ar2-Ar3} = 7.6$, Ar-2), 7.55 (1H, 't' (dd), $J_{Ar3-Ar2} = 7.6$, Ar-3), 8.05 (2H, d, $J_{Ar1-Ar2} = 7.6$, Ar-1); ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 26.40 (2× isopropylidene CH_3), 63.22 (C-5), 71.87 (C-3), 78.12 (C-2 + C-4), 103.74 (C-1), 112.49 (isopropylidene $C(CH_3)_2$), 128.04 (Ar), 129.38 (Ar), 129.43 (Ar), 132.84 (Ar quat.), 166.07 (C=O); MS *m*/*z* (relative intensity) FAB^+ 237 (100, $M+H^+$-CH_3COCH_3), 295(40, $M+H^+$); HRMS (FAB^+) calcd $C_{15}H_{18}O_6$ ($M+H^+$) 294.1104, found 294.1097.

5.2.8. 5-*O*-Benzoyl-3-deoxy-3-fluoro-1,2-*O*-isopropylidene-D-xylofuranose (18). Diethylaminosulfur trifluoride (1.1 equiv, 0.262 mol, 4.22 g, 3.46 mL) was added slowly to a mixture of 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-ribofuranose (7.00 g, 23.8 mmol) and pyridine (3.6 equiv, 85.8 mmol, 6.79 g, 6.94 mL) in dry DCM (120 mL) at −10 °C under an atmosphere of dry N_2. After 4 days at rt, TLC (9:1 $CHCl_3/Me_2CO$) indicated only a small quantity of SM and formation of one main product ($R_f = 0.86$). The orange solution was very cautiously washed with water, dried ($MgSO_4$) and concentrated to yield an orange oil (8.1 g, >100%). Purification by SGC [linear gradient; 15:1→11:1 (v/v) hexane/EtOAc + 0.5% (v/v) MeOH] yielded the title compound as a pale yellow oil (5.71 g, 81%).^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 1.33 (3H, isopropylidene CH_3), 1.50 (3H, isopropylidene CH_3), 4.50–4.63 (3H, m, H-5, H-5′, H-4), 4.73 (1H, dd, $J_{2-F} = 11.1$, $J_{2-1} = 3.8$, H-2), 5.05 (1H, dd, $J_{3-F} = 50.4$, J_{3-4} 1.8, H-3), 6.03 (1H, d, $J_{2-1} = 3.8$, H-1), 7.43 (2H, 't' (dd), $J_{Ar2-Ar1} = 7.9$, $J_{Ar2-Ar3} = 7.2$, Ar-2), 7.56 (1H, 't' (dd), $J_{Ar3-Ar2}$ 7.2, Ar-3), 8.05 (2H, d, $J_{Ar1-Ar2}$ 7.6, Ar-1); ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 26.07 (isopropylidene CH_3), 26.56 (isopropylidene CH_3), 61.20 (d, $J_{C-5-F} = 10.7$, C-5), 77.53 (d, $J_{C-4-F} = 18.3$, C-4), 82.28 (d, $J_{C-2-F} = 32.0$, C-2), 93.81 (d, $J_{C-3-F} = 183.9$, C-3), 104.82 (C-1), 112.08 (isopropylidene $C(CH_3)_2$), 128.07 (Ar), 129.30 (Ar), 129.45 (Ar), 132.89 (Ar quat.), 165.67 (C=O); ^{19}F NMR (376.2 MHz; $CDCl_3$) δ_F −208.89 (ddd, $J_{F-3} = 50.1$, $J_{F-4} = 30.8$, $J_{F-2} = 11.0$); MS *m*/*z* (relative intensity) FAB^+ 239 (77, $M+H^+$ –CH_3COCH_3), 297 (100, $M+H^+$); HRMS (FAB^+) calcd $C_{15}H_{17}O_5F_1$ ($M+H^+$) 296.1060, found 296.1051.

5.2.9. 5-*O*-Benzoyl-3-deoxy-3-fluoro-D-xylofuranose (19). 5-*O*-Benzoyl-3-deoxy-3-fluoro-1,2-*O*-isopropylidene-D-xylofuranose **18** (3.24 g, 19.0 mmol) was dissolved in a mixture of acetic acid (80 mL) and water (20 mL) and heated to 80 °C. After 72 h, TLC (9:1 $CHCl_3/Me_2CO$) indicated only a small quantity of SM ($R_f = 0.84$) and one main product ($R_f = 0.16$). The reaction was concentrated and co-evaporated from toluene (3×). Purification by SGC [linear gradient; 15:1→7:1 (v/v) $CHCl_3/Me_2CO$] yielded the product as a colourless oil (2.10 g, 75%). α: ^{1}H NMR (399.8 MHz; CD_3COCD_3) δ_H 2.97–2.03 (1H, br s, OH), 4.18–4.30 (1H, m, H-2), 4.41–4.60 (3H, m, H-5, H-5′, H-4), 5.02 (1H, dm, $J_{3-F} = 52.6$, H-3), 5.70 (1H, d, $J_{1-2} = 6.4$, H-1), 7.47 (2H, 't' (dd), $J_{Ar2-Ar1} = 7.3$, $J_{Ar2-Ar3} = 7.6$, Ar-2), 7.59 (1H, 't' (dd), $J_{Ar3-Ar2} = 7.2$, Ar-3), 7.99 (2H, d, $J_{Ar1-Ar2} = 7.4$, Ar-1); α ^{19}F NMR (376.2 MHz; $CDCl_3$) δ_F −201.95 (ddd, $J_{F-2} = 12.4$, $J_{F-4} = 28.9$, $J_{F-3} = 51.0$); α ^{13}C NMR (100.5 MHz; CD_3COCD_3) δ_C 64.60 (d, $J_{C-5-F} = 13.7$, C-5), 76.37 (d, $J_{C-2-F} = 25.9$, C-2), 79.5 (d, $J_{C-4-F} = 19.1$, C-4), 97.52 (d, $J_{C-3-F} = 185.4$, C-3), 97.61 (d, $J_{C-1-F} = 8.4$, C-1); β: ^{1}H NMR (399.8 MHz; CD_3COCD_3) δ_H 2.97–2.03 (1H, br s, OH), 4.18–4.30 (1H, m, H-4), 4.37–4.62 (3H, m, H-2, H-5, H-5′), 5.10 (1H, d, $J_{3-F} = 53.0$, H-3), 5.46 (1H, s, H-1), 7.47 (2H, 't' (dd), $J_{Ar2-Ar1} = 7.3$, $J_{Ar2-Ar3} = 7.6$, Ar-2), 7.59 (1H, 't' (dd), $J_{Ar3-Ar2} = 7.2$, Ar-3), 7.99 (2H, d, $J_{Ar1-Ar2} = 7.4$, Ar-1); β ^{19}F NMR (376.2 MHz; $CDCl_3$) δ_F −204.22 (ddd, $J_{F-2} = 15.2$, $J_{F-4} = 26.2$, $J_{F-3} = 51.0$); β ^{13}C NMR (100.5 MHz; CD_3COCD_3) δ_C 63.50 (d, $J_{C-5-F} = 12.2$, C-5), 76.50 (d, $J_{C-2-F} = 19.0$, C-2), 80.82 (d, $J_{C-4-F} = 26.2$, C-4), 98.53 (d, $J_{C-3-F} = 182.3$, C-3), 104.77 (C-1); α + β 129.62, 129.64, 130.48, 131.17, 131.25, 134.20, 134.24, 166.64, 166.7.; MS *m*/*z* (relative intensity) FAB^+ 239 (100, $M+H^+$ –H_2O), 257 (100, $M+H^+$); HRMS (FAB^+) calcd $C_{12}H_{14}O_5F_1$ ($M+H^+$) 257.0825, found 257.0804.

5.2.10. 1,2,5-Tri-*O*-benzoyl-3-deoxy-3-fluoro-D-xylofuranose (20). Benzoyl chloride (2.0 equiv, 0.164 mol, 2.30 g, 1.90 mL) was added to a pre-cooled (0 °C) solution of 5-*O*-benzoyl-3-deoxy-3-fluoro-D-xylofuranose **19** (2.10 g, 8.20 mmol) and DMAP (75 mg, cat.) in dry pyridine (100 mL) in four portions over a period of 8 h under an atmosphere of dry N_2. After a further 16 h at rt, TLC (3:1 hexane/EtOAc) indicated the formation of several products. DMAP (100 mg) was added to the reaction mixture followed by benzoyl chloride (1.0 equiv, 8.20 mmol, 0.85 mL) dropwise and the reaction stirred for a

further 24 h at rt. TLC indicated mainly two products (R_f = 0.38 and 0.43). The reaction mixture was concentrated and co-evaporated from toluene (2×). The resultant orange solid was re-dissolved in $CHCl_3$ and washed with saturated aqueous $CuSO_4$ and water. Drying ($MgSO_4$) and concentration yielded a dark orange oil (6.81 g, >100%). Purification by SGC [linear gradient; 15:1→3:1 (v/v) hexane/EtOAc + 0.5% (v/v) MeOH] yielded a 1:1 mixture of α- and β-anomers as a colourless oil (1.61 g, 42%).

α: ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 4.57–4.96 (3H, m, H-5, H-5′, H-4), 5.60 (1H, ddd, $J_{3\text{-F}}$ = 53.0, J_{3-4} = 4.7, J_{3-2} = 3.2, H-3), 5.82 (1H, ddd, $J_{2\text{-F}}$ = 22.2, J_{2-3} = 5.0, J_{2-1} = 3.2, H-2), 6.91 (1H, d, J_{1-2} = 5.0, H-1), 7.30–8.13 (15H, m, Ar); α ^{19}F NMR (376.2 MHz; $CDCl_3$) δ_F −202.92 ('dt' (ddd), $J_{F\text{-}4}$ = 21.4, $J_{F\text{-}2}$ = 22.9, $J_{F\text{-}3}$ = 53.4); α: ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 61.5 (d, $J_{C\text{-}5\text{-}F}$ = 11.6, C-5), 77.30 (2C, 2d, $J_{C\text{-}2\text{-}F}$ = $J_{C\text{-}4\text{-}F}$ 28.0, C-2, C-4), 93.95 (d, $J_{C\text{-}3\text{-}F}$ = 189.4, C-3), 94.26 (C-1); β ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 4.57–4.96 (3H, m, H-5, H-5′, H-4), 5.38 (1H, dd, $J_{3\text{-F}}$ 50.4, J_{3-4} 4.1, H-3), 5.80 (1H, d, $J_{2\text{-F}}$ 11.0, H-2), 6.67 (1H, s, H-1), 7.30–8.13 (15H, m, Ar); β: ^{19}F NMR (376.2 MHz; $CDCl_3$) δ_F −203.58 (ddd, $J_{F\text{-}2}$ = 10.7, $J_{F\text{-}4}$ = 25.9, $J_{F\text{-}3}$ = 48.8); β: ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 62.37 (d, $J_{C\text{-}5\text{-}F}$ = 12.9, C-5), 79.26 (d, $J_{C\text{-}2\text{-}F}$ = 30.3, C-2), 81.08 (d, $J_{C\text{-}4\text{-}F}$ = 20.0, C-4), 92.37 (d, $J_{C\text{-}3\text{-}F}$ = 191.3, C-3), 99.08 (C-1); α + β: 128.21, 128.23, 128.29, 128.36, 128.38, 128.50, 129.37, 129.60, 129.67, 129.79, 129.87, 130.00, 133.09, 133.12, 133.34, 133.48, 133.54, 133.83, 164.42, 164.50, 164.59, 164.81, 165.95, 167.27. MS *m*/*z* (relative intensity) Electrospray 343 (70, M–PhCOO), 487 (100, M + Na^+), 951 (90, 2 M + Na+).

5.2.11. N6-Benzoyl-9-(2′-*O*-benzoyl-3′-deoxy-3′-fluoro-β-D-xylofuranosyl)-adenine (21). Compound **21** was obtained as described above for compound **11** starting from 452 mg (0.973 mmol) of **20**, yielding a pale yellow oil (125 mg, 22%). R_f = 0.44 (95:5 $CHCl_3$/MeOH). λ_{MAX} = 280 nm (EtOH). ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 4.73–4.86 (3H, m, H-4′, H-5′$_A$, H-5′$_B$), 5.42 (1H, dd, $J_{3'\text{-F}}$ = 50.1, $J_{3'-4'}$ = 2.0, H-3′), 5.92 (1H, d, $J_{2'\text{-F}}$ = 13.3, H-2′), 6.52 (1H, d, $J_{1'-2'}$ = 1.5, H-1′), 7.25–7.50 (7H, m, Ar), 7.54–7.65 (2H, m, Ar), 8.03–8.06 (6H, m, Ar), 8.30 (1H, s, H-8), 8.76 (1H, s, H-2), 9.31 (1H, br s, NH); ^{19}F NMR (376.2 MHz; $CDCl_3$) δ_F −201.80 (ddd, $J_{F\text{-}2'}$ = 12.4, $J_{F\text{-}4'}$ = 30.3, $J_{F\text{-}3'}$ = 49.6); ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 61.43 (d, $J_{C\text{-}5'\text{-}F}$ = 10.3, C-5′), 80.21 (d, $J_{C\text{-}4'\text{-}F}$ = 31.1, C-4′), 80.39 (d, $J_{C\text{-}2'\text{-}F}$ = 19.3, C-2′), 87.39 (C-1′), 93.44 (d, $J_{C\text{-}3'\text{-}F}$ = 197.5, C-3′), 128.83, 128.23, 128.66, 128.69, 128.87, 129.46, 129.96, 130.20, 131.64, 133.63, 134.34, 136.09, 139.80 (Ade, C-8), 139.88 (Ade), 151.20 (Ade), 151.64 (Ade, C-2), 160.04 (Ade), 163.06, 164.78, 166.26 (3×C=O); MS *m*/*z* (relative intensity) FAB$^+$ 581 (100, M$^+$);

5.2.12. 3′-Deoxy-3′-fluoro-xylo-adenosine (22). N6-Benzoyl-9-(2′-*O*-benzoyl-3′-deoxy-3′-fluoro-β-D-xylofuranosyl)adenine (**21**) (582 mg, 1.00 mmol) was dissolved in a solution of methanol (50 mL) through which ammonia gas had been bubbled for 10 min. The yellow solution was cautiously heated at ~50 °C in a sealed tube for 36 h after which TLC (4:1 $CHCl_3$/Me_2CO) indicated the formation of one main product (R_f = 0.30). The reaction mixture was concentrated and the residue dissolved in MQ water. The solution was washed with ether (1×) and the nucleoside-containing aqueous phase concentrated to yield the title compound as a yellow powder (160 mg, 59%). λ_{MAX} = 259 nm (EtOH). ^{1}H NMR (399.8 MHz; $CD_3SO_2CD_3$) δ_H 3.20–3.50 (1H, br s, OH), 3.71, 3.77 (2H, ABX, $^2J_{AB}$ = 11.4, $^3J_{AX}$ = 6.1, $^3J_{BX}$ = 5.5, H-5′$_A$, H-5′$_B$), 4.32 (1H, dsex, $J_{4'\text{-F}}$ = 28.4, $J_{4'-5A'}$ = 6.1, $J_{4'-5B'}$ = 6.0, $J_{4'-3'}$ 3.5, H-4′), 4.76 (1H, 'dt' (ddd), $J_{2'\text{-F}}$ = 15.8, $J_{2'-3'}$ = 4.4, $J_{2'-1'}$ = 2.1, H-2′), 5.11 (1H, ddd, $J_{3'\text{-F}}$ = 51.8, $J_{3'-4'}$ = 3.2, $J_{3'-2'}$ = 1.8, H-3′), 5.95 (1H, d, $J_{1'-2'}$ = 2.3, H-1′), 7.36 (1H, br s, NH), 8.10 (1H, s, H-8), 8.17 (1H, s, H-2); ^{19}F NMR (376.2 MHz; $CD_3SO_2CD_3$) δ_F −201.26 (ddd, $J_{F\text{-}3'}$ = 51.0, $J_{F\text{-}4'}$ = 27.5, $J_{F\text{-}2'}$ = 15.1); ^{13}C NMR (100.5 MHz; $CD_3SO_2CD_3$) δ_C 58.17 (d, $J_{C\text{-}5'\text{-}F}$10.3, C-5′), 77.47 (d, $J_{C\text{-}2'\text{-}F}$ = 25.7, C-2′), 81.80 (d, $J_{C\text{-}4'\text{-}F}$ = 19.3, C-4′), 88.57 (d, $J_{C\text{-}1'\text{-}F}$ = 1.9, C-1′), 95.50 (d, $J_{C\text{-}3'\text{-}F}$ = 183.0, C-3′), 118.63 (C-5), 138.26 (C-8), 149.02 (C-4), 152.62 (C-2), 155.88 (C-6); MS *m*/*z* (relative intensity) FAB$^+$ 270 (100, M + H$^+$); HRMS (FAB$^+$) calcd $C_{10}H_{12}N_5O_3F_1$ (M$^+$) 269.0924, found 269.0930.

5.2.13. 3′-Deoxy-3′-fluoro-xylo-adenosine monophosphate (23). 3′-Deoxy-3′-fluoro-xylo-adenosine **22** (120 mg, 0.446 mmol) was dissolved in triethylphosphate (4 mL) by gentle warming with a heat-gun until some vapour was seen to condense on the neck of the round-bottom flask. The stirred solution was cooled to 0 °C and phosphorous oxychloride (1.2 equiv, 0.535 mmol, 82 mg, 0.050 mL) added dropwise under dry N_2. The solution was allowed to warm slowly to rt and stirred overnight. HPLC (SAX) indicated the formation of a new peak (R_T = 3.1 min) and approximately 50% starting material (R_T = 2.8 min). The yellow solution was cooled to 0 °C and phosphorous oxychloride (0.6 equiv, 0.268 mmol, 41 mg, 0.025 mL) added dropwise. After 18 h at rt, HPLC revealed the composition of the reaction mixture to still consist of ~20% of the nucleoside SM. Further addition of phosphorous oxychloride (0.2 equiv, 0.089 mmol, 14 mg, 8.3 μL) and stirring for 12 h at rt failed to drive the reaction to completion. MQ water (150 mL) was cooled to 0 °C with rapid stirring and the reaction mixture added dropwise. After 30 min, the aqueous phase was washed with EtOAc (6×) to remove triethylphosphate. The solution was brought to pH = 11 with 1.0 M NaOH and purified by chromatography using AG-MP1 strong ion-exchange resin. The title compound eluted at 52–70% of 150 mM TFA and was isolated as a vitreous, colourless solid (104mg, 67%) λ_{MAX} = 259 nm (water, pH = 7). ^{1}H NMR (399.8 MHz; D_2O) δ_H 4.18–4.31 (2H, m, H-5′$_A$, H-5′$_B$), 4.68 (1H, 'dtt' (dddd), $J_{4'\text{-F}}$ = 29.0, $J_{4'\text{-C5'-}O\text{-P}}$ = 6.7, $J_{4'\text{-5A'}}$ ~ $J_{4'-5B''}$ = 4.1, H-4′), 4.79 (1H, d, $J_{2'\text{-F}}$ = 13.1, H-2′), 5.22 (1H, d, $J_{3'\text{-F}}$ 50.7, H-3′), 6.14 (1H, s, H-1′), 8.36 (1H, s, H-8), 8.37 (1H, s, H-2); ^{19}F NMR (376.2 MHz; D_2O) δ_F −202.36 (ddd, $J_{F\text{-}3'}$ = 51.0, $J_{F\text{-}4'}$ = 28.9, $J_{F\text{-}2'}$ = 13.8); ^{31}P NMR (161.9 MHz; D_2O) δ_P 1.55 (s, decoupled); MS *m*/*z* (relative intensity) FAB$^+$ 329(100, M−2H$^+$–H_2O), 350 (13, M + H$^+$); HRMS (FAB$^+$) calcd $C_{10}H_{13}N_5O_6F_1P_1$ (M$^+$) 349.0587, found 349.0586.

5.2.14. 3′F-xylo-Adenosine 5′-diphospho-5″-ribofuranoside-1″-nicotinamide (3′F-xyloNAD$^+$) (24). Compound **24** was obtained as described above for compound **14** starting from 50.0 mg (0.143 mmol, free acid) of **23** to give a white, fluffy solid (22.8 μmol, 16%, quantified by UV); λ_{MAX} = 258 nm. ^{1}H NMR (399.8 MHz; D_2O) δ_H 4.18–4.50 (8H, m, F-xylo- and ribofuranoside protons), 4.63–4.74 (1H, m), 5.26 (1H, ddd, $J_{3'\text{-}F}$ = 51.0, $J_{3'-4'}$ = 3.2, $J_{3'-2'}$ = 1.8, H-3′), 6.00 (1H, d, $J_{1'-2'}$ = 2.3, H-1′), 6.04 (1H, d, J = 5.5, H-1″), 8.13 (1H, s, H-8), 8.18 (1H, dd, J = 6.1, J = 7.9, H_{NIC}-5), 8.20 (1H, s, H-2), 8.80 (1H, d, J = 8.2, H_{NIC}-6), 9.15 (1H, d, J = 6.1, H_{NIC}-4), 9.29 (1H, d, J = 5.3, H_{NIC}-2), ^{19}F NMR (376.2 MHz; D_2O) δ_F −202.03 (ddd, $J_{F\text{-}3'}$ = 51.0, $J_{F\text{-}4'}$ = 26.6, $J_{F\text{-}2'}$ = 13.2); ^{31}P NMR (161.9 MHz; D_2O) δ_P −10.3 (d, $J_{P\text{-}O\text{-}P}$ = 20.6), −10.6 (d, $J_{P\text{-}O\text{-}P}$ = 21.3); MS m/z (relative intensity) FAB$^+$ 666 (35, M + H$^+$).

5.2.15. 1-*O*-Acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-fluoro-D-ribofuranose (25). Compound **25** was synthesised in 12 steps according to the procedure of Mikhailopulo et al.[44] β: TLC (3:1 hexane/EtOAc) R_f = 0.35, colourless oil (210 mg, quant); ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 1.97 (3H, s, $COCH_3$), 4.45–4.71 (2H, m, H-5), 4.72 (1H, 'dq' (dddd), $J_{4\text{-}F}$ = 19.0, J_{4-5} = 4.4, $J_{4-5'}$ = 4.4, J_{4-3} = 4.1, H-4), 5.38 (1H, ddd, $J_{3\text{-}F}$ = 55.0, J_{3-4} = 5.5, J_{3-2} = 1.5, H-3), 5.60 (1H, ddd, $J_{2\text{-}F}$ = 8.2, J_{2-3} = 5.0, J_{2-1} = 2.3, H-2), 6.41 (1H, 't' (dd), $J_{1\text{-}F}$ = 2.1, J_{1-2} = 1.8, H-1), 7.35–7.62 (6H, m, Ar), 8.04–8.11 (4H, m, Ar); ^{19}F NMR (376.2 MHz; $CDCl_3$) δ_F −209.25 (ddd, $J_{F\text{-}3}$ = 51.0, $J_{F\text{-}4}$ = 17.9, $J_{F\text{-}2}$ = 8.3); ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 21.1 ($COCH_3$), 63.17 (d, $J_{C\text{-}5\text{-}F}$ = 5.1, C-5), 75.00 (d, $J_{C\text{-}2\text{-}F}$ = 14.2, C-2), 82.31 (d, $J_{C\text{-}4\text{-}F}$ = 25.1, C-4), 89.24 (d, $J_{C\text{-}3\text{-}F}$ = 194.6, C-3), 98.13 (C-1), 128.36 (Ar), 128.41 (Ar), 128.50 (Ar), 129.49 (Ar), 129.57 (Ar), 129.75 (Ar), 129.82 (Ar), 133.27 (Ar), 133.34 (Ar), 133.57 (Ar), 133.61 (Ar), 164.97 (C=O), 165.08 (C=O), 165.67 (C=O), 165.71 (C=O), 168.91 (C=O), 169.66 (C=O); MS m/z (relative intensity) FAB$^+$ 343 (100, M + H$^+$-AcOH), 402 (1, M$^+$); HRMS (FAB$^+$) calcd $C_{21}H_{19}F_1O_7$ (M$^+$) 402.1115, found 402.1131.

α: TLC (3:1 hexane/EtOAc) R_f = 0.39, white foam (715 mg, 91%); ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 1.97 (3H, s, $COCH_3$), 4.45–4.70 (2H, m, H-5), 4.85 (1H, 'ddt' (dddd), $J_{4\text{-}F}$ = 26.1, J_{4-5} = 3.8, $J_{4-5'}$ = 3.8, J_{4-3} = 1.5, H-4), 5.36 (1H, 'dt' (ddd), $J_{2\text{-}F}$ = 21.7, J_{2-3} = 4.7, J_{2-1} = 5.0, H-2), 5.47 (1H, 'dt' (ddd), $J_{3\text{-}F}$ = 57.0, J_{3-2} = 4.7, J_{3-4} = 1.5, H-3), 6.65 (1H, d, J_{1-2} = 4.4, H-1), 7.35–7.62 (6H, m, Ar), 8.04–8.11 (4H, m, Ar); ^{19}F NMR (376.2 MHz; $CDCl_3$) δ_F −195.64 (ddd, $J_{F\text{-}3}$ = 56.5, $J_{F\text{-}4}$ = 26.0, $J_{F\text{-}2}$ = 22.0); ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 20.79 ($COCH_3$), 61.30 (d, $J_{C\text{-}5\text{-}F}$ = 9.7, C-5), 71.61 (d, $J_{C\text{-}2\text{-}F}$ = 15.5, C-2), 80.72 (d, $J_{C\text{-}4\text{-}F}$ = 25.1, C-4), 88.30 (d, $J_{C\text{-}3\text{-}F}$ = 191.3, C-3), 93.89 (C-1), 128.36 (Ar), 128.41 (Ar), 128.50 (Ar), 129.49 (Ar), 129.57 (Ar), 129.75 (Ar), 129.82 (Ar), 133.27 (Ar), 133.34 (Ar), 133.57 (Ar), 133.61 (Ar), 164.97 (C=O), 165.08 (C=O), 165.67 (C=O), 165.71 (C=O), 168.91 (C=O), 169.66 (C=O); MS m/z (relative intensity) FAB$^+$ 343 (100, M + H$^+$–AcOH), 402 (1, M$^+$); HRMS (FAB$^+$) calcd $C_{21}H_{19}F_1O_7$ (M$^+$) 402.1115, found 402.1131.

5.2.16. N6-Benzoyl-9-(2′-*O*-benzoyl-3′-deoxy-3′-fluoro-β-D-ribofuranosyl) adenine (26). Compound **26** was obtained as described above for compound **11** starting from 265 mg (6.59 mmol) of **25**, yielding a yellow foam (194 mg, 51%). R_f = 0.74 (9:1 $CHCl_3$/MeOH); λ_{MAX} = 280 nm (EtOH); ^{1}H NMR (399.8 MHz; $CDCl_3$) δ_H 4.55–4.88 (3H, m, H-4, H-5), 6.00 (1H, ddd, $J_{3\text{-}F}$ = 53.6, J_{3-4} = 4.7, J_{3-2} = 2.3, H-3), 6.37 (1H, ddd, $J_{2\text{-}F}$ = 17.6, J_{2-1} = 5.7, J_{2-3} = 5.0, H-2), 6.46 (1H, d, J_{1-2} = 6.4, H-1), 7.35–7.62 (6H, m, Ar), 8.04–8.11 (4H, m, Ar); ^{19}F NMR (376.2 MHz; $CDCl_3$) δ_F −199.91 (ddd, $J_{F\text{-}3}$ = 53.7, $J_{F\text{-}4}$ = 23.4, $J_{F\text{-}2}$ = 17.9); ^{13}C NMR (100.5 MHz; $CDCl_3$) δ_C 63.33 (d, $J_{C\text{-}5\text{-}F}$ = 8.4, C-5), 73.64 (d, $J_{C\text{-}2\text{-}F}$ = 14.8, C-2), 81.49 (d, $J_{C\text{-}4\text{-}F}$ = 24.5, C-4), 86.23 (C-1), 89.55 (d, $J_{C\text{-}3\text{-}F}$ = 190.7, C-3), 123.89 (Ar), 128.03 (Ar), 128.41 (Ar), 128.65 (Ar), 128.78 (Ar), 128.87 (Ar), 129.06 (Ar), 129.32 (Ar), 129.83 (Ar), 129.93 (Ar), 130.20 (Ar), 133.03 (Ar), 133.62 (Ar), 133.79 (Ar), 134.15 (Ar), 142.01 (Ade), 149.89 (Ade), 151.94 (Ade), 153.07 (Ade), 164.58 (C=O), 165.40 (C=O), 166.14 (C=O); MS m/z (relative intensity) FAB$^+$ 343 (53, M + H$^+$–(Bz-N^6-Ade)), 582 (100, M + H$^+$), 583 (33, M + 2H$^+$);

5.2.17. 3′-Deoxy-3′-fluoro-adenosine (27). Compound **27** was obtained as described above for compound **22** starting from 220 mg (0.378 mmol) of **26**, to yield an orange powder (132 mg, >100%). R_f = 0.37 (4:1 $CHCl_3$/MeOH); λ_{MAX} = 259nm (EtOH); ^{1}H NMR (399.8 MHz; CD_3COCD_3) δ_H 3.61–3.68 (2H, m, H-5′), 4.29 (1H, 'dt' (ddd), $J_{4'\text{-}F}$ = 27.5, $J_{4'-3'}$ = 3.5, $J_{4'-5'}$ = 3.5, H-4′), 4.95 (1H, ddd, $J_{2'\text{-}F}$ = 36.4, $J_{2'-1'}$ = 7.8, $J_{2'-3'}$ = 4.1, H-2′), 5.10 (1H, dd, $J_{3'\text{-}F}$ = 54.8, $J_{3'-4'}$ = 4.4, H-3′), 5.95 (1H, d, $J_{1'-2'}$ = 8.2, H-1′), 7.44 (1H, br s, NH), 8.15 (1H, s, H-8), 8.39 (1H, s, H-2); ^{19}F NMR (376.2 MHz; CD_3COCD_3) δ_F −197.54 (ddd, $J_{F\text{-}3}$ = 53.7, $J_{F\text{-}4}$ = 26.2, $J_{F\text{-}2}$ = 26.2); ^{13}C NMR (100.5 MHz; CD_3COCD_3) δ_C 61.02 (d, $J_{C\text{-}5\text{-}F}$ = 10.9, C-5), 71.87 (d, $J_{C\text{-}2\text{-}F}$ = 16.1, C-2), 83.98 (d, $J_{C\text{-}4\text{-}F}$ = 21.2, C-4), 86.79 (C-1), 93.15 (d, $J_{C\text{-}3\text{-}F}$ = 181.7, C-3), 119.28 (Ade), 140.00 (Ade), 149.01 (Ade), 152.32 (Ade), 156.11 (Ade); MS m/z (relative intensity) Electrospray 270 (100, M + H$^+$).

5.2.18. 3′-Deoxy-3′-fluoroadenosine 5′-monophosphate (28). Compound **28** was obtained as described above for compound **23** starting from 90 mg (0.334 mmol) of **27**, to yield an vitreous, colourless solid (97mg, 83%). λ_{MAX} = 258nm (H_2O, pH = 6). ^{1}H NMR (399.8MHz; D_2O) δ_H 4.07–4.21 (2H, m, H-5′), 4.62–4.72 (1H, m, $J_{4'\text{-}F}$ = 26.9, H-4′), 4.93 (1H, ddd, $J_{2'\text{-}F}$ = 24.6, $J_{2'-1'}$ = 4.7, $J_{2'-3'}$ = 4.1, H-2′), 5.32 (1H, dd, $J_{3'\text{-}F}$ = 53.3, $J_{3'-4'}$ = 4.1, H-3′), 6.21 (1H, d, $J_{1'-2'}$ = 7.9, H-1′), 8.39 (1H, s, H-8), 8.59 (1H, s, H-2); ^{19}F NMR (376.2 MHz; D_2O) δ_F −198.97 (ddd, $J_{F\text{-}3}$ = 50.9, $J_{F\text{-}4}$ = 24.8, $J_{F\text{-}2}$ = 24.8); ^{31}P NMR (161.9 MHz; D_2O) δ_P 1.34 (s, decoupled); MS m/z (relative intensity) FAB$^+$ 349 (0.5,M$^+$), 369 (5, M−3H$^+$ + Na$^+$); HRMS (FAB$^+$) calcd $C_{10}H_{13}N_5O_6F_1P_1$ (M$^+$) 349.0588, found 349.0582.

5.2.19. 3′F-Adenosine 5′-diphospho-5″-ribofuranoside-1″-nicotinamide (3′F-NAD$^+$) (29). Compound **29** was obtained as described above for compound **14** starting from 61.0 mg (0.175 mmol, free acid) of **23** to give a white, fluffy solid (0.055mmol, 31%, quantified by UV);

λ_{MAX} = 259 nm (H_2O, pH = 7); ^1H NMR (399.8MHz; D_2O) δ_H 4.20–4.75 (9H, m, ribofuranoside protons), 5.05–5.20 (1H, m, H-3′), 5.88 (1H, m, H-1′), 5.90 (1H, m, H-1″), 7.55 (1H, s, H8), 8.00 (1H, 't' (dd), J = 6.4, H_{NIC}-5), 8.27 (1H, br s, H2), 8.63 (1H, d, J = 7.9, H_{NIC}-4), 8.91 (1H, d, J = 5.3, H_{NIC}-6), 9.12 (1H, s, H_{NIC}-2); ^{19}F NMR (376.2 MHz; D_2O) δ_F −198.97 (ddd, $J_{F\text{-}3}$ = 50.9, $J_{F\text{-}4}$ = 24.8, $J_{F\text{-}2}$ = 24.8); ^{31}P NMR (161.9 MHz; D_2O) δ_P −10.57→ −10.02 (m, P-*O*-P).

5.2.20. Cyclic 3′F-adenosine 5′-diphosphate ribose (30). The conditions developed for the preparation of the cADPR analogues were also employed for the synthesis of cyclic 3′-deoxy-3′-fluoro-ADP ribose. Incubation of the NAD^+ analogue **29** (5 μmol, quantified by UV) in 25 mM HEPES buffer (10 mL) at pH 6.8 in the presence of *Aplysia* cyclase (20 μL of a 25 mM HEPES solution of enzyme, ~2 units) was carried out at rt and the formation of product was followed by analytical anion exchange HPLC. HPLC revealed the formation of a new peak (R_T = 4.4 min) along with the nicotinamide peak (R_T = 2.2 min). Purification was achieved by ion-exchange chromatography on BioRad AG-MP1 resin using a concave gradient of 150 mM TFA (0 min: 100% MQ→20 min: 100% MQ→40 min: 96% MQ→60 min: 84% MQ→75 min: 68% MQ→105 min: 0% MQ→120 min: 0% MQ). The title compound eluted at 15–17% TFA. Fractions containing >95% of the product (as determined by HPLC) were pooled. Concentration and co-evaporation (3×) from MQ water, followed by lyophilisation yielded the product (free acid) as a white, fluffy solid (2.3μmol, 46%, quantified by UV); ^1H NMR (399.8 MHz; D_2O) δ_H 3.30–4.75 (9H, m, fluororibo- and ribofuranoside protons), 5.36 (1H, m, H-3′), 5.74 (1H, m, H-2′ or H-4′), 6.13 (1H, d, J 7.9, H-1′), 6.16 (1H, d, J 3.5, H-1″), 8.41 (1H, s, H8), 9.02 (1H, s, H2); ^{19}F NMR (376.2 MHz; D_2O) δ_F −200.99 (ddd, $J_{F\text{-}3}$ = 53.7, $J_{F\text{-}4}$ = 26.1, $J_{F\text{-}2}$ = 27.6); ^{31}P NMR (161.9 MHz; D_2O) δ_P −9.00→ −8.50 (m, P-*O*-P).

Acknowledgements

We acknowledge Project Grant support from the Wellcome Trust (No 055709)

References and notes

1. Guse, A. H. *J. Mol. Med.* **2000**, *78*, 26.
2. Guse, A. H. *Cell. Signal.* **1999**, *11*, 309.
3. Prakash, Y. S.; Kannan, M. S.; Walseth, T. F.; Sieck, G. C. *Am. J. Physiol.* **1998**, *274*, C1653.
4. Higashida, H.; Hashii, M.; Yokoyama, S.; Hoshi, N.; Asai, K.; Kato, T. *J. Neurochem.* **2001**, *76*, 321.
5. Cancela, J. M.; Gerasimenko, O. V.; Gerasimenko, J. V.; Tepikin, A. V.; Petersen, O. H. *EMBO J.* **2000**, *19*, 2549.
6. da Silva, C. P.; Guse, A. H. *Biochim. Biophys. Acta* **2000**, *1498*, 122.
7. Bootman, M. D.; Collins, T. J.; Peppiatt, C. M.; Prothero, L. S.; MacKenzie, L.; De Smet, P.; Travers, M.; Tovey, S. C.; Seo, J. T.; Berridge, M. J.; Ciccolini, F.; Lipp, P. *Semin. Cell Devel. Biol.* **2001**, *12*, 3.
8. Franco, L.; Guida, L.; Bruzzone, S.; Zocchi, E.; Usai, C.; De Flora, A. *FASEB J.* **1998**, *14*, 1507.
9. Okamoto, H. *Mol. Cell. Biochem.* **1999**, *193*, 115.
10. Lee, H. C.; Munshi, C.; Graeff, R. *Mol. Cell. Biochem.* **1999**, *193*, 89.
11. De Flora, A.; Guida, L.; Franco, L.; Zocchi, E. *Inter. J. Biochem. Cell Biol.* **1997**, *29*, 1149.
12. Graeff, R.; Munshi, C.; Aarhus, R.; Johns, M.; Lee, H. C. *J. Biol. Chem.* **2001**, *276*, 12169.
13. Matsumura, N.; Tanuma, S. *Biochem. Biophys. Res. Commun.* **1998**, *253*, 246.
14. Cakir-Kiefer, C.; Muller-Steffner, H.; Schuber, F. *Biochem. J.* **2000**, *349*, 203.
15. Tohgo, A.; Munakata, H.; Takasawa, S.; Nata, K.; Akiyama, T.; Hayashi, N.; Okamoto, H. *J. Biol. Chem.* **1997**, *272*, 3879.
16. Lee, H. C.; Aarhus, R. *Cell. Regul.* **1991**, *2*, 203.
17. Zhang, F. J.; Gu, Q. M.; Sih, C. J. *Bioorg. Med. Chem.* **1999**, *7*, 653.
18. Lee, H. C.; Aahrus, R.; Levitt, D. *Nat. Struct. Biol.* **1994**, *1*, 143.
19. Prasad, G. S.; Levitt, D. G.; Lee, H. C.; Stout, C. D. *Proteins* **1996**, *24*, 138.
20. Prasad, G. S.; McRee, R. E.; Stura, E. A.; Levitt, D. G.; Lee, H. C.; Stout, C. D. *Nat. Struct. Biol.* **1996**, *3*, 957.
21. Munshi, C.; Aarhus, R.; Graeff, R.; Lee, H. C. *J. Biol. Chem.* **2000**, *275*, 21566.
22. Sauve, A. A.; Deng, H. T.; Angeletti, R. H.; Schramm, V. L. *J. Am. Chem. Soc.* **2000**, *122*, 7855.
23. Sauve, A. A.; Munshi, C.; Aarhus, R.; Lee, H. C.; Schramm, V. L. *Biochemistry* **1998**, *37*, 13239.
24. Migaud, M. E.; Pederick, R. L.; Bailey, V. C.; Potter, B. V. L. *Biochemistry* **1999**, *38*, 9105.
25. Wall, K. A.; Klis, M.; Kormet, J.; Coyle, D.; Ame, J. C.; Jacobson, M. K.; Slama, J. T. *Biochem. J.* **1998**, *335*, 631.
26. Ashamu, G. A.; Galione, A.; Potter, B. V. L. *J. Chem. Soc. Chem. Commun.* **1995**, *18*, 1929.
27. Sethi, J. K.; Empson, R. M.; Bailey, V. C.; Potter, B. V. L.; Galione, A. *J. Biol. Chem.* **1997**, *272*, 16358.
28. Bailey, V. C.; Sauve, A. A.; Galione, A.; Potter, B. V. L. *J. Chem. Soc. Chem. Commun.* **1997**, *7*, 695.
29. Bailey, V. C.; Sethi, J. K.; Fortt, S. M.; Potter, B. V. L.; Galione, A. *Chem. Biol.* **1997**, *4*, 51.
30. Wong, L.; Aarhus, R.; Lee, H. C. *Biochim. Biophys. Acta* **1999**, *1472*, 555.
31. Vu, C. Q.; Lu, P. J.; Chen, C. S.; Sih, C. *J. Biol. Chem.* **1996**, *271*, 4747.
32. Bailey, V. C.; Fortt, S. M.; Summerhill, R. J.; Potter, B. V. L.; Galione, A. *FEBS Lett.* **1996**, *379*, 227.
33. Ashamu, G. A.; Sethi, J. K.; Galione, A.; Potter, B. V. L. *Biochemistry* **1997**, *36*, 9505.
34. Ashamu, G. A.; Galione, A.; Potter, B. V. L. *J. Chem. Soc. Chem. Commun.* **1995**, *13*, 1359.
35. Marwood, R. D.; Shuto, S.; Jenkins, D. J.; Potter, B. V. L. *J. Chem. Soc. Chem. Commun.* **2000**, 219.
36. Hanessian, S.; Liak, T. *J. Synthesis* **1981**, 396.
37. Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.
38. Yoshikawa, M.; Kato, T.; Takenishi, T. *Tetrahedron Lett.* **1967**, *50*, 5068.
39. Hughes, N. A.; Kenner, G. W.; Todd, A. R. *J. Chem. Soc.* **1957**, *735*, 3733.
40. Fawcett, C. P.; Agre, N. O. *J. Biol. Chem.* **1961**, *2237*, 1709.
41. Lee, W. J. *J. Heterocycl. Chem.* **1971**, *8*, 179.
42. Colman, R. F. *Methods Enzymol.* **1997**, *280*, 186.
43. Gosselin, G.; Puech, F.; Genudellac, C.; Imbach, J. L. *Carbohydr. Res.* **1993**, *249*, 1.
44. Mikhailopulo, I. A.; Poopeiko, N. E.; Pricota, T. I.; Sivets, G. G.; Kvasyuk, E. I.; Balzarini, J.; DeClercq, E. *J. Med. Chem.* **1991**, *34*, 2195.

45. Ashamu, G. A. PhD Thesis, University of Bath, 1997.
46. Ludemann, H. D. *Biophys. Struct. Mech.* **1975**, *1*, 121.
47. Akhrem, A. A. *Org. Mag. Res.* **1979**, *12*, 247.
48. Ekiel, I. *Acta Biochim. Pol.* **1979**, *26*, 435.
49. Pearlman, D. A.; Kim, S. H. *J. Biomol. Struct. Dyn.* **1985**, *3*, 99.
50. Wada, T.; Inageda, K.; Aritomo, K.; Nishina, H.; Takahashi, K.; Katada, T.; Sekine, M. *Nucleosides Nucleotides* **1995**, *14*, 1301.
51. Smith, P. E.; Tanner, J. J. *J. Mol. Recog.* **2000**, *13*, 27.
52. Thibaudeau, C.; Plavec, J.; Chattopadhyaya, J. *J. Org. Chem.* **1998**, *63*, 4967.