Stereospecific synthesis of 1,9-bis(β-D-glycosyl)adenines: a chemical route to stable analogues of cyclic-ADP ribose (cADPR)

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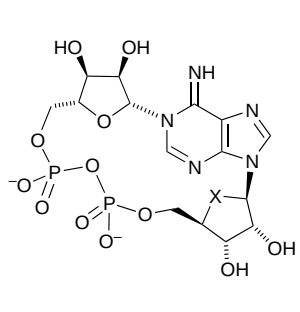
Stereospecific syntheses of two hydrolysis-resistant analogues of the 1,9-bis(β-D-ribosyl)adenine moiety of cyclic-ADP ribose are described.

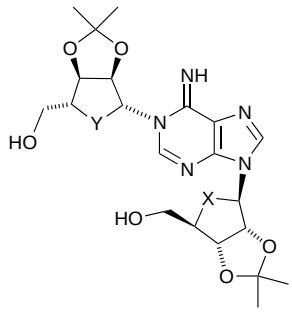
Cyclic-ADP Ribose, cADPR **1**, is a naturally occurring metabolite of NAD^+ and is deemed to be a secondary messenger involved in intracellular calcium mobilisation.[1] The precise role of this compound and the mechanism by which it acts at receptor sites other than those activated by inositol-1,4,5-triphosphate[2] remain unclear. cADPR is formed through the action of ADP-ribosyl cyclase, an enzyme found in many mammalian and invertebrate tissues, which brings about displacement of the nicotinamide group of NAD^+ by N^1 of its own adenine residue with overall retention of configuration.[3] The labile $C^{1''}$–N^1 glycosylic bond formed in this process is easily hydrolysed to give adenosine diphosphate ribose, ADPR **2**, by either enzymatic or spontaneous hydrolysis, thus accounting for the extremely low concentrations (sub-micromolar) of cADPR observed in cells.

As part of an ongoing investigation of the detailed function of cADPR, we are seeking to make novel analogues of cADPR for use in competitive enzyme inhibition studies as well as in investigations of agonist/antagonist action at the ryanodine receptor.[4] In a recent report,[5] we described the synthesis of a carbocyclic NAD^+ analogue containing a stable methylenebisphosphonate linkage which is designed to inhibit the formation of cADPR and thus can be employed in structural studies in a binary complex with the enzyme.

Hitherto, analogues of cADPR have generally been formed by enzymatic cyclisation[6] of an analogue of NAD^+, though Sekine has described a chemical procedure which gave the α-configuration at N^1 for a cADPR derivative.[7] Both Lee[8] and Potter[9] have described antagonist activity from 8-substituted cADPR derivatives whilst Sih[10] has used a part enzyme, part chemical synthesis to generate cADPR species with changes in the pyrophosphate linkage. Potter[11] has employed aristeromycin to prepare a longer-acting cADPR analogue **3** with a carbaribose moiety at N^9 though necessarily retaining a ribofuranose moiety at N^1.



1 X = O, cADPR
3 X = CH_2

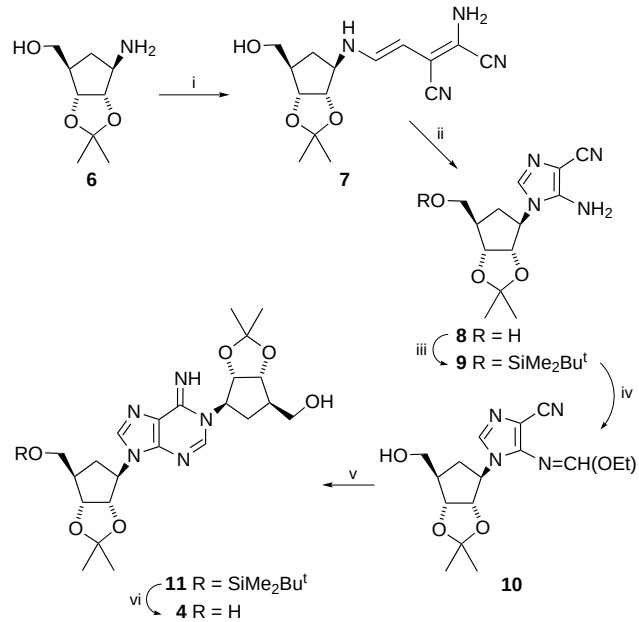


4 X = CH_2, Y = CH_2
5 X = O, Y = CH_2

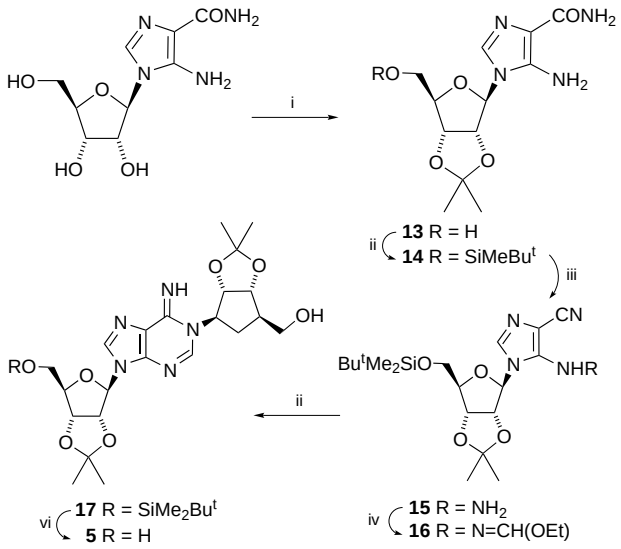
No group has as yet sought directly to address the inherent instability of the labile (O)$C^{1''}$–N^1 glycosylic bond. Carbocyclic sugars have been employed in many nucleoside analogues to achieve stability of the glycosylic linkage,[12] and the incorporation of a carbaribose at N^1 in cADPR seemed a logical target for this application. Because we predicted† that a nicotinamide carbocyclic riboside NAD^+ derivative would have insufficient reactivity at the glycosylic position to be a substrate for enzymatic cyclisation, the choice of a chemical route to carbaribose analogues of cADPR became unavoidable.

We here report the stereospecific synthesis of the nucleoside framework of two analogues of cADPR, both having a carbocyclic sugar moiety at the $N^{1'}$ position, namely the derivative **4** and the 1-β-D-carbaribosyl-9-β-D-ribofuranosyladenine derivative **5**. This route can also be employed to prepare the parent 1,9-bis(β-D-ribofuranosyl)adenine. We are currently developing both of these carbocyclic species *via* 5′,5″-bisphosphorylation and 5′,5″-methylenebisphosphorylation to generate novel, hydrolytically-stable analogues of cADPR.

The synthetic scheme is built around the repeated use of the cyclopentylamine **6** of defined β-configuration at C^1 (Scheme 1). Amine **6** is obtained in five high-yielding steps from commercially available (−)-2-azabicyclo[2.2.1]hept-5-en-3-one as outlined in a previous report.[5] Imidazole **9** was prepared *via* amidine **7** using diaminomaleonitrile as described by Booth,[13] which proved much superior to alternative reagents explored. Intermediate **7** was purified by silica gel chromatography, although prolonged exposure to air and light resulted in extensive darkening and decomposition. The required amino



Scheme 1 *Reagents and conditions*: i, diaminomaleonitrile, $PhNH_3^+Cl^-$ (cat), EtOAc, room temp., dark, 18 h, 65%; ii, DBU (2 equiv.), H_2O, room temp., 2 h, 100%; iii, Bu^tMe_2SiCl, imidazole, DMF, room temp., 18 h, 92%; iv, $HC(OEt)_3$, heat, 4 h, 94%; v, **6**, EtOH, EtO^- (cat), 50 °C, 40%; vi, Bu_4NF, THF, room temp., 18 h, 96%



Scheme 2 *Reagents and conditions*: i, acetone, HC(OEt)$_3$, TsOH, room temp., 2 h, 78%; ii, Bu^{t}Me$_2$SiCl, imidazole, DMF, room temp., 18 h, 84%; iii, TsCl, pyridine, room temp., 3 h, 83%; iv, HC(OEt)$_3$, heat, 4 h, 100%; v, **6**, EtOH, EtO$^-$ (cat), 50 °C, 40%; vi, Bu$_4$NF, THF, room temp., 18 h, 94%

imidazole **8** was duly formed by base-induced cyclisation with excess DBU and followed by silyl protection with Bu^{t}Me$_2$SiCl to give imidazole **9**. Reaction of **9** with neat triethyl orthoformate resulted in near quantitative conversion into imidate **10**, the key precursor for cyclisation. Addition of amine **6** to an ethanolic solution of imidate **10** in the presence of catalytic sodium ethoxide for 48 h at 50 °C provided 1,9-bis(carbaribosyl)adenine **11** in 40% yield. Deprotection of the 5′-hydroxy group with Bu$_4$NF gave the required diol **4** in an overall yield of 14% after twelve synthetic steps.

Attempts to prepare the corresponding imidazole **15** using this procedure with commercially available 2,3-*O*-isopropylidene-1-β-D-ribofuranosylamine were unsuccessful. It appeared that the intermediate amidine is considerably more labile than its carbocyclic counterpart **7** and only degradation fragments were obtained. In an alternative synthesis (Scheme 2), commercially available AICAR **12** was protected as its 2′,3′-*O*-isopropylidene derivative using acetone–triethyl orthoformate–TsOH to give **13** in 78% yield. Following silylation of the 5′-hydroxy group, dehydration of the amide to nitrile **15** was achieved using TsCl in pyridine in 84% yield. Refluxing **15** in triethyl orthoformate produced imidate **16** which was condensed with carbocyclic amine **6** and cyclised as before. Removal of the 5′-*O*-SiMe$_2$Bu^t protecting group with Bu$_4$NF gave the required 1-β-D-carbaribosyl-9-β-D-ribofuranosyladenine derivative **5** in 21% overall yield.

The 1,9-dialkyladenine structure for the analogues rests not only on synthesis but is confirmed by their pK_a and spectroscopic characteristic. The pK_a values of 8.36 for the 1,9-bis(β-D-carbaribosyl)adenine derivative **4** and 7.93 for the 1-β-D-carbaribosyl-9-β-D-ribosyladenine derivative **5** are close to pK_a 8.30 for cADPR[3] and differ greatly from the pK_as for 6,9-dialkyladenines,[3,16] which lie in the range 3.5–4. The UV absorption spectra for analogues **4** and **5** exhibit the strong shoulder at 300 nm characteristic of both cADPR and of 1,9-dialkyl adenines and absent in 6,9-dialkyl adenines.[15] Compounds **4** and **5** and the related intermediates described have been fully characterised by UV,‡ NMR and mass spectral analysis.§ In both analogues, the N^1–C$^{1''}$ bond appears to have the desired level of stability to hydrolysis, with no cleavage of the N^1–C$^{1'}$ bond over a week in aqueous solution under ambient conditions. In fact, the slow transformations of **4** and **5** observed at higher pH appear to result from isomerisation by a Dimroth rearrangement to give *N*^{6},*N*^{9}-disubstituted adenines, as characterised by markedly changed UV spectral properties.[15] Such rearrangements have been shown[16] to be accelerated by a β-D-ribofuranosyl group at the 9-position. This process appears to be very slow in the case of analogues **4** and **5** (half life ⩾1 day at pH 9) and is the subject of further investigations.

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Footnotes and References

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† Recent results using NAD$^+$ cyclase and a synthetic methylenebisphosphonate carbaNAD$^+$ analogue (ref. 5) have confirmed this prediction.

‡ pK_a Determinations: For both analogues **4** and **5**, optical density at 300 nm was recorded at 25.0 °C at various pH values in the range 5.5–11.5 using a range of buffers (0.05 M buffer + 0.1 M NaCl where buffer = MES, EPPS, CHES, of CAPS). Absorbance values were plotted against the corresponding pH values and the pK_a for each analogue was determined using a Kaleidagraph™ curve-fit analysis.

§ *Selected data* for **4**: mp 110–112 °C; λ_{max}/nm (3t) 260 (11 230), 300 (sh) (2458) (H_2O); FAB$^+$ [M + H]$^+$, 476.2506. $C_{23}H_{33}N_5O_6$ requires [M + H], 476.2509; δ_H($CDCl_3$) 7.85 (1 H, br s), 7.72 (1 H, s), 5.31 (1 H, dd, *J* 5.5 and 5.5), 4.85 (1 H, dd, *J* 6.5 and 6), 4.76 (1 H, dd, *J* 6 and 2), 4.75–4.69 (1 H, m), 4.67 (1 H, dd, *J* 6.5 and 4 Hz), 4.65–4.55 (1 H, m), 3.84 (1 H, dd, *J* 10.8 and 6), 3.82 (1 H, dd, *J* 10.6 and 7.5), 3.79 (1 H, dd, *J* 10.6 and 5), 3.72 (1 H, dd, *J* 10.8 and 2.5), 2.62–2.42 (5 H, m), 2.32 (1 H, m), 1.60 (6 H, s), 1.31 (3 H, s), 1.30 (3 H, s). For **5**: mp 98–100 °C. For **17**: λ_{max}/nm (ε) 260 (11 570), 300 (sh) (3475) (MeOH); FAB$^+$ [M + H]$^+$, 478.2259. $C_{22}H_{31}N_5O_7$ requires [M + H], 478.2301; δ_H($CDCl_3$) 7.78 (1 H, br s), 7.76 (1 H, s), 5.81 (1 H, d, *J* 4.5), 5.27 (1 H, dd, *J* 5.7), 5.03 (1 H, dd, *J* 6 and 1.5), 4.99 (1 H, dd, *J* 6 and 4.5), 4.73 (1 H, dd, *J* 6 and 2.4), 4.67–4.58 (1 H, br m), 4.48 (1 H, ddd, *J* 1.6, 1.6 and 1.6), 3.92 (1 H, dd, *J* 12.5 and 1.6), 3.81 (1 H, dd, *J* 11 and 3) 3.75 (1 H, dd, *J* 12.5 and 1.6), 3.72 (1 H, dd, *J* 11 and 3), 2.61–2.53 (2 H, br m), 2.52–2.47 (1 H, br m), 1.62 (3 H, s,), 1.55 (3 H, s), 1.36 (3 H, s), 1.30 (3 H, s).

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