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An Approach to a Carbocyclic Analogue of Cyclic Adenosine 5'-Diphosphate Ribose. The Synthesis and Bisphosphorylation of N^1-[(1S, 3R)-3-(Hydroxymethyl)cyclopent-1-yl]inosine.

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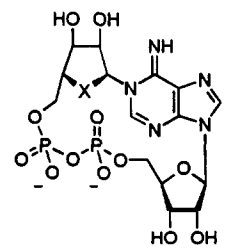
Abstract: The synthesis of N^1-[(1S, 3R)-3-(hydroxymethyl)cyclopent-1-yl]inosine (**11**) via a cyclocondensation route is reported. Regioselective bisphosphorylation of the primary 5'-hydroxyl groups leads to a carbocyclic, inosine analogue of *seco* adenosine 5'-diphosphate ribose (**3**). © 1997 Elsevier Science Ltd.

Cyclic adenosine 5'-diphosphate ribose (cADPR, **Fig.1**, **1**), a naturally occurring metabolite of nicotinamide adenine dinucleotide (NAD^+), is a candidate second messenger that mobilises Ca^{2+} from non-mitochondrial stores in a variety of mammalian and invertebrate tissues.[1] The compound is not only of pharmacological interest, being more effective than, and working independently of inositol trisphosphate in mobilising internal stores of Ca^{2+} in sea urchin eggs, but is also of chemical interest since it provides the first example of an unusual and new cyclic nucleotide.[2] A recent report has demonstrated that cADPR plays an important role in the Ca^{2+} entry process in T-lymphocytes.[3] The N6-ribosylated cyclic structure first proposed[4] has been corrected to N1 on the basis of UV spectroscopic[5] results and X-ray crystallography.[6]



X = O **1**
X = CH_2 **2**

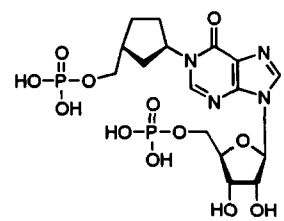


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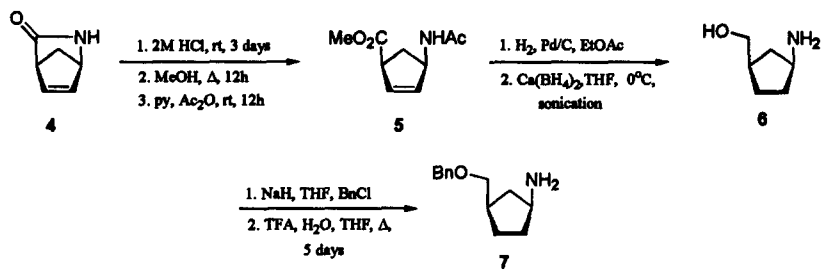
Fig. 1

The NAD^+ cyclising enzyme, cADPR cyclase, is isolable[7] and has fairly loose substrate specificity so that structurally modified analogues of cADPR have been obtained by chemo-enzymatic synthesis involving preparation of NAD^+ modified in the purine ring and enzyme mediated internal ribosylation at the N1-position.[8]

Such analogues have provided valuable pharmacological tools for probing the mechanism of cADPR modulated Ca^{2+} signalling pathways.[9] The chemical conversion of NAD^+ to cADPR by treatment with sodium bromide in DMSO has also been reported but offers no advantage over the enzyme mediated cyclisation and has not yet found application to the synthesis of cADPR analogues.[10] Other synthetic approaches to cADPR rely on the early, direct ribosylation of adenosine base moiety under either enzyme mediated[11] or phase transfer conditions[12] but are likely to be of limited value for the preparation of base modified cADPR analogues due to the need to individually develop and establish the correct regio- and stereoselectivity. This kind of problem is notably illustrated by the enzymatic or chemical conversion of NGD^+ to a product of ribosylation at the N7-position[13].

Since the nitrogen-carbon bond (at N1) in these types of compounds is also the site of a rapid, *in vivo*, enzymatic degradation that limits the pharmacological value of these analogues[14] we sought a synthesis of the carbocyclic analogue of cADPR (**Fig.1, 2**) as the first step in establishing a general, total, regio- and stereoselective chemical synthesis of compounds of this type. A different, carbocyclic analogue, in which the sugar in the lower part of the molecule is changed, has been derived from chemo-enzymatic manipulation of aristeromycin in this laboratory and has shown encouraging results as a long lived partial agonist in sea urchin eggs.[9b] Condensation type reactions used for the synthesis of a variety of nucleosides and in particular for the preparation of N1-alkylated adenosines and inosines[15] not only offer control over the site and stereochemistry of alkylation by incorporation of amine into purine ring synthesis but can also provide the multigram amounts of material needed in order to tackle the central synthetic challenge of phosphorylation and cyclisation. Herein we report our initial results concerning the preparation and phosphorylation of a N1-(carbocycle)alkylated inosine (**11**)[16] leading to an inosine analogue of *seco* ADPR (**Fig.1, 3**).

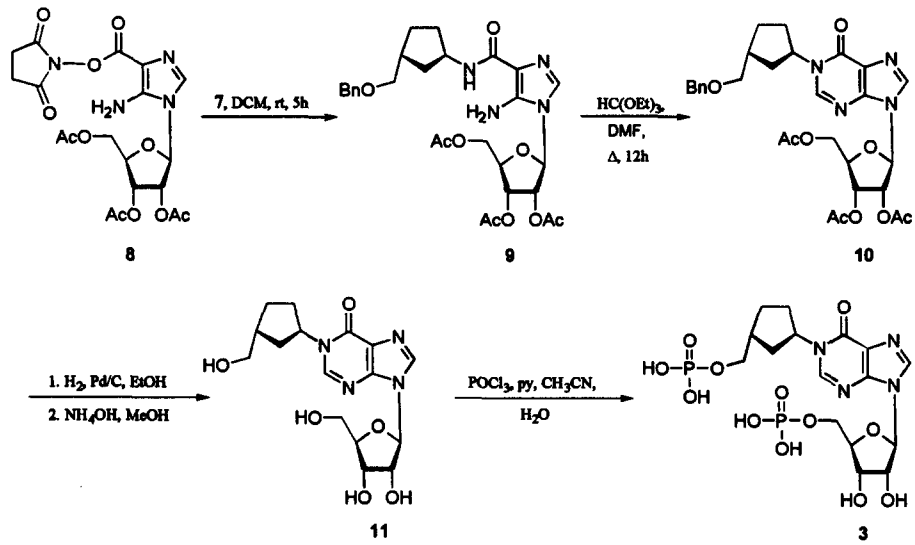
A carbocyclic amine of defined stereochemistry was derived by manipulation of (1R, 4S)-2-aza-bicyclo[2.2.1]hept-5-en-3-one (**4, Scheme 1**).[17]



Scheme 1

Optically pure methyl (1S, 4R)-4-acetamido-cyclopent-2-ene-1-carboxylate (**5**), a precursor to the allylic aminoalcohol used in the preparation of aristeromycin, was obtained by transformations first used by Vince et al.[18] and later by Roberts et al.[19] The selective reduction of the ester group to homoallylic alcohol is known but, after inserting an alcohol protection step (as benzyl ether), we, unlike these authors, found that the continued presence of the double bond led to complications in the hydrolysis of the amide group for the large scale isolation of allylic amine. Our best reaction conditions employed aqueous trifluoroacetic acid in THF at reflux and although this facilitated the isolation of the amine[20], epimerisation at the adjacent carbon centre also occured. Thus a modified reaction sequence (**5** through **7**) involving the early removal of the carbocyclic double bond by hydrogenation was employed.

The preparation of various N-[5-amino-1-(β-D-ribofuranosyl)imidazole-4-carbonyl]amino acids from 5(4)-amino-4(5)-imidazolecarboxamide (AICAR) reported by Srivastava et al.[21] provides the basis of a route to N1-alkylated inosines by substitution of amino acid for 1° amine and ring closure to purine by reaction with triethyl orthoformate (**Scheme 2**). Thus, manipulation of AICAR led to a key mixed hydroxamic acid anhydride (**8**) whose treatment with carbocyclic amine (**7**) in dichloromethane led rapidly to the 1° amide (**9**). Cyclisation to N^1-[(1S, 3R)-3-(benzyloxymethyl)cyclopentan-1-yl]inosine 2',3',5'-O-triacetate (**10**) required the forcing conditions of triethyl orthoformate in DMF at reflux but proceeded in good yield (71%). (The more usual conditions of triethyl orthoformate in acetic anhydride at reflux led only to the formimino ether). The stereochemical integrity of the cyclised material was established by ^{1}H NMR, IR and UV spectroscopy as well as by supercritical liquid chromatography in comparison with the stereoisomers derived from the cyclisation using epimerised allylic amine. Deprotection of the alcohol protecting groups was performed in a two step process involving first hydrogenolysis of the benzyl group on palladium-carbon (88%) and then hydrolysis of acetate by ammonia in methanol (90%) and gave the novel N^1-[(1S, 3R)-3-(hydroxymethyl)cyclopent-1-yl]inosine (**11**).



Scheme 2

The selective bisphosphorylation by phosphoryl chloride of 1° hydroxyl in the tetraol (**11**) was achieved by the method of Sowa and Ouchi[22] which was particularly successful for this purpose. The bisphosphate (**3**), which was isolated as the triethylammonium salt after ion-exchange chromatography (65%, Sepharose Q) and converted to the free acid by Dowex 50X (H^+ form), is characterised by two triplets in the ^{31}P-^{1}H coupled NMR spectrum. There was no evidence for *in situ* ring closure to pyrophosphate under these reaction conditions or those of Yoshikawa[23] as determined by high field ^{31}P NMR of the crude reaction mixture.

In conclusion, therefore, we have established a synthesis of gram quantities of a *seco* bisphosphate related to cADPR and can now address the difficult problem of ring closure by pyrophosphate bond formation.[24] Studies directed towards this end are in progress in this laboratory and will be reported in due course. The eventual modification of the carbocycle to include cis-hydroxyl functionality[25] and the substitution of carbocyclic amine by

the inherently more sensitive ribosylamine or other sugar amine is also envisaged. Such structurally more faithful analogues should serve to throw further light on the presently unknown nature of the receptor for cADPR and those that exhibit greater hydrolytic stability than cADPR are likely to be of significant pharmacological value.

Acknowledgements

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References and Notes

1 H. Koshiyama, H.C. Lee and A.H. Tashijian, *J. Biol. Chem.*, 1991, **266**, 16985; K. Currie, K. Swann, A. Galione and R.H. Scott, *Mol. Biol. Cell*, 1992, **3**, 1415; S. Takasawa, S. Nata, H. Yonekura and H. Okamoto, *Science*, 1993, **259**, 370.

2 A. Galione, *Trends Pharmacol. Sci.*, 1992, **13**, 304.

3 A.H. Guse, I. Berg, C.P. da Silva, B.V.L. Potter and G.W.Mayr, *J. Biol. Chem.*, 1997, **272**, 8546.

4 H.C. Lee, T.F. Walseth, G.T. Bratt, R.N. Hayes and D.L. Clapper, *J. Biol. Chem.*, 1989, **264**, 1608.

5 H. Kim, E.L. Jacobsen and M.K. Jacobsen, *Biochem. Biophys. Res. Comm.*, 1993, **194**, 1143.

6 H.C.Lee, R. Aarhus and D. Levitt, *Nature Struct. Biol.*, 1994, **1**, 143.

7 H.C. Lee and R. Aarhus, *Cell Reg., 1991*, **2**, 203.

8 G.A. Ashamu, A. Galione and B.V.L. Potter, *J. Chem. Soc., Chem. Commun.*, 1995, 1359.

9 a. T.F. Walseth and H.C. Lee, *Biochem. Biophys. Acta*, 1993, **1178**, 235; b. V.C. Bailey, S.M. Fortt, R.J. Summerhill, A.Galione and B.V.L. Potter, *FEBS Lett.*, 1996, **379**, 227; c. V.C. Bailey, J.K. Sethi, S.M. Fortt, A. Galione and B.V.L. Potter, *Chem. Biol.*, 1997, **4**, 51.

10 S. Yamada, Q-M. Gu and C.J. Sih, *J. Am. Chem. Soc.*, 1994, **116**, 10787.

11 C.J. Sih, and Q-M. Gu, *J.Am. Chem. Soc.*, 1994, **116**, 7481.

12 K. Aritomo, C. Urashima, T. Wada and M. Sekine, *Nucleosides Nucleotides*, 1996, **15**(1-3), 1.

13 Our unpublished observations and R.M. Graeff, T.F. Walseth, H.K. Hill and H.C. Lee, *Biochemistry*, 1996, **35**, 379.

14 H.C. Lee and R. Aarhus, *Biochem. Biophys. Acta*, 1993, **1164**, 68.

15 E.C. Taylor and P.K. Loeffler, J. Am. Chem., Soc., 1960, **82**, 3147.

16 Efforts to repeat the work of Imbach who reported N1-ribosylated adenosine from AICN by a condensation method failed (B. Rayner, C. Tapiero and J-L. Imbach, *Carbohyd. Res.*, 1977, **59**, 111). The use of ordinary 1° amines and triacetate protected AICN gives complex mixtures although other workers employing different protecting groups have been successful (E.J. Hutchinson, B.F. Taylor and G.M. Blackburn, unpublished results). The focus on a synthesis of an inosine analogue was also influenced by the known susceptibility of N1-alkylated adenosines to suffer Dimroth rearrangement (M.H. Wilson and J.A. McCloskey, *J. Org. Chem.*, 1973, **38**, 2247) as well as deamination by nucleophiles (T. Fuji, T. Saito and N.Terahara, *Chem. Pharm. Bull.*, 1986, **34**, 1094).

17 *Chiroscience Ltd*, Cambridge Science Park, Milton Road, Cambridge, CB4 4WE, UK

18 R. Vince and M. Hua, *J. Med. Chem.*, 1990, **33**, 17; S. Daluge and R. Vince, *J. Org. Chem.*, 1978, **43**, 2311

19 C.T. Evans, S.M. Roberts, K.A. Shoberu and A.G. Sutherland, *J. Chem. Soc., Perkin Trans.1*, 1992, 589.

20. The amine is readily derived from the trifluoroacetate salt by silica gel chromatography using NH_4OH-MeOH as eluant and ion exchange chromatography can be avoided.

21. P.C. Srivastava, R.W. Mancuso, R.J. Rousseau and R.K. Robins, *J. Med. Chem.*, 1974, **17**, 1207; J.A. Suggs and P.C. Srivastava, *J. Heterocyclic Chem.*, 1988, **25**, 1331.

22 T. Sowa and S. Ouchi, *Bull. Chem. Soc. Jpn.*, 1975, **48**, 2084.

23. M. Yoshikawa, T. Kato and T. Takenishi, *Bull Chem. Soc. Jpn.*, 1969, **42**, 3505.

24. Carbodiimide mediated intermolecular phosphate coupling reactions are of course well known but in our hands somewhat capricious. The mixed anhydride method using activation by diphenylphosphochloridate is in our experience superior (A.M. Michelson, *Biochim. Biophys. Acta*, 1964, **91**, 1). However, few examples of intramolecular couplings exist and these involve only proximal phosphates: Noble, N.J. and Potter, B.V.L., *J. Chem. Soc., Chem. Commun.*, 1989, 194; A. Berkessel, U. Geisel and D.A. Herault, *Tetrahedron Lett.*, 1996, **37**, 355.

25 R.C. Cermak and R.Vince, *Tetrahedron Lett.*, 1981, **22**, 2331.

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