Desulphurisation of Vicinal Bisphosphorothioates: a Novel Synthetic Route to Substituted Cyclic Pyrophosphates

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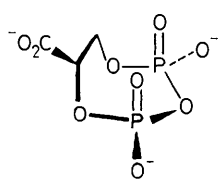
N-Bromosuccinimide-mediated desulphurisation of vicinal bisphosphorothioates offers a novel route for the synthesis of P^1, P^2-disubstituted 7-membered cyclic pyrophosphates.

Considerable work has been carried out on the formation of pyrophosphate linkages during the development of nucleotide synthesis,[1] and much of the classical chemistry has involved the use of activating agents such as phosphorochloridates[1] and carbodiimides.[2] Recently , the novel metabolite, D-2,3-bisphosphoglycerate cyclic pyrophosphate (**1**), a seven-membered cyclic pyrophosphate, has been found to be the major phosphate component in *Methanobacterium thermoautotrophicum* and certain *methanobrevibacteria,*[3,4] and is the first biological example of this system.[3] Its function is, however, still uncertain. It may act as a phosphate reserve or, more likely, be a chelator of potassium ions. We report here a novel route directed towards the synthesis of such 7-membered cyclic pyrophosphates.

Phosphorothioate analogues are of considerable use in nucleotide chemistry and enzymology.[5] Recent work in this laboratory has addressed the synthesis and exploitation of novel molecules of biological interest possessing vicinal bisphosphorothioates.[6,7] *N*-Bromosuccinimide (NBS) *inter alia* has been used to desulphurise phosphorothioate esters and anhydrides to form oxygen isotope-labelled phosphates.[5,8] We reasoned that if a phosphorothioate moiety in a vicinal bisphosphorothioate were to be activated, *e.g.* by NBS, then a neighbouring phosphorothioate might effectively compete with the solvent and couple with the activated system to form a cyclic P^1,P^2-disubstituted pyrophosphate.

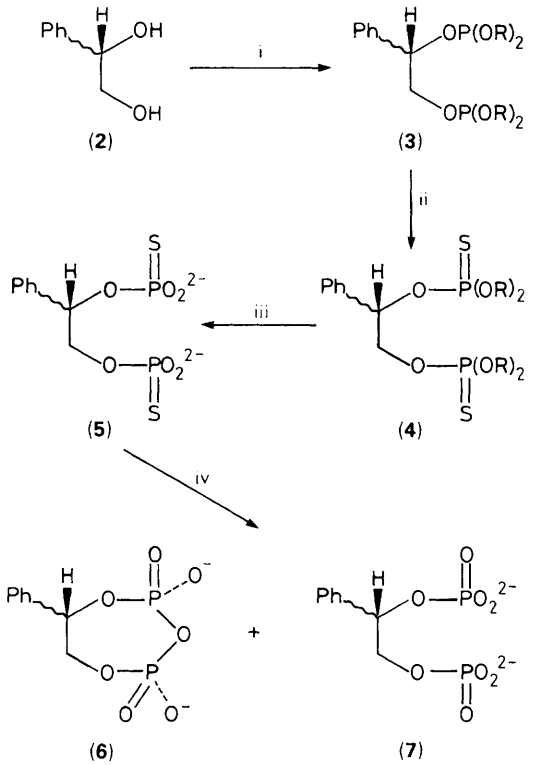
An NBS-mediated desulphurisation was carried out on two model systems, 1-phenylethane-1,2-bisphosphorothioate (**5**) and *trans*-cyclohexane-1,2-bisphosphorothioate (**8**), which were synthesised according to Scheme 1 from their respective diols using a P^{III} approach.[6,9] 1-Phenylethane-1,2-diol (**2**) was phosphitylated with *N*,*N*-di-isopropylamino(2-cyanoethoxy)chlorophosphine to the bisphosphoramidite (^{31}P n.m.r., δ_P 147.4 p.p.m.) and reaction with tetrazole and 3-hydroxypropionitrile produced 1-phenylethane-1,2-bis(di-2-cyanoethoxy)phosphite (**3**) (δ_P 138.5 p.p.m.). Addition of sulphur gave 1-phenylethane-1,2-bis(di-2-cyanoethoxy)-phosphorothioate (**4**) in 65% yield after purification by flash chromatography (δ_P 66.5 p.p.m.). Treatment with aqueous ammonia removed the cyanoethyl groups to give the ammonium salt of 1-phenylethane-1,2-bisphosphorothioate (**5**) (δ_P 43.4 p.p.m.). Subsequent treatment of the free acid with cyclohexylamine generated the triscyclohexylammonium salt of (**5**). *trans*-Cyclohexane-1,2-bisphosphorothioate (**8**) was prepared in a similar fashion.



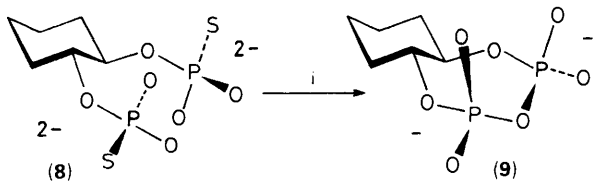
(**1**)

Figure 1

The desulphurisation reaction was rapid and simple to execute. The 1,2-bisphosphorothioate (**5**) was dissolved in water and dioxane (1 : 4) and an eight-fold excess of NBS was added (other desulphurising agents were tried and were found to be less efficient). The mixture was shaken and after 1 min excess of NBS was reduced by 2-mercaptoethanol. After a further minute the reaction mixture was diluted with triethylammonium hydrogen carbonate (TEAB) buffer and solvent was removed *in vacuo* to yield a crude mixture of pyrophos-



Scheme 1. Synthesis and desulphurisation of 1-phenylethane-1,2-bisphosphorothioate. R = $-CH_2CH_2CN$.
Reagents: i, $ClP(NPr^i_2)OR$, $HNPr^i_2$ (2 equiv. of each in dry MeCN, 1 h at 0 °C) then ROH–tetrazole (2.4 equiv.); ii, S_8–dry pyridine, 2 h at room temp.; iii, NH_4OH_{aq}, 3 h at 65 °C; iv, *N*-bromosuccinimide (8 equiv.)–dioxane–water, 1 min at room temp., then $HSCH_2CH_2OH$, 10 mM TEAB, pH 7.1.



Scheme 2. Synthesis of cyclohexane-1,2-cyclic-pyrophosphate.
Reagents: i, *N*-bromosuccinimide (8 equiv.)–dioxane–water, 1 min at room temp., then $HSCH_2CH_2OH$, 10 mM TEAB, pH 7.1.

phate (**6**) (δ_P −10.7, −11.5 p.p.m., $^2J_{PP}$ 18 Hz), [f.a.b., 279 (M − H)$^-$] and the bisphosphate (**7**) (δ_P 2.8, 3.0 p.p.m.) as a glass. The products were purified by anion exchange chromatography using DEAE-Sephadex (DEAE = diethylaminoethyl) eluted with TEAB buffer.

The relative yield of pyrophosphate depended on which bisphosphorothioate was used. When (**5**) was desulphurised the pyrophosphate (**6**) was formed in *ca.* 40% yield, the remaining 60% being the bisphosphate (**7**) resulting from double desulphurisation. Yields were estimated by ^{31}P n.m.r. spectroscopy and quantitative phosphate analysis. Use of cyclohexane-1,2-bisphosphorothioate (**8**), however, increased the yield of the cyclic pyrophosphate (**9**) (δ_P, −11.4 p.p.m.), [f.a.b., 257(M−H)$^-$] to 85—90% (Scheme 2). The ratio of pyrophosphate to phosphate formed is presumably a function of the relative conformation of the two phosphorothioate groups. Owing to stereochemical constraints imposed by the cyclohexane ring, the juxtaposition of the two phosphorothioate groups is presumably more favourable for pyrophosphate formation.

Using isotopic labelling, a preliminary investigation of the mechanism of this novel coupling reaction was undertaken. When desulphurisation was carried out in $H_2{}^{18}O$ and the ^{18}O-labelled pyrophosphate was examined by ^{31}P n.m.r. spectroscopy, a significant proportion (45%) of the ^{18}O was found in the bridging P–O–P linkage, as determined by $^{31}P^{18}O$ isotope shift analysis (data not shown). This indicates that although the situation is undoubtedly complex and several mechanisms are possible, significant desulphurisation at one centre must have occurred, followed by torsional rotation of the resulting ^{18}O-labelled phosphate, before its capture by the other activated phosphorothioate.

Seven-membered cyclic pyrophosphates are stable to both acid and alkaline conditions in the cold. Treatment of (**9**) with 1 M HCl at room temperature for three days yielded little hydrolysis, but boiling at 100 °C hydrolysed the pyrophosphate, within 15 min. Similarly, with 1 M NaOH at room temperature no hydrolysis was detected. Boiling at 100 °C for 5 h resulted in total hydrolysis to the bisphosphate. It is clear, however, that intracellular enzymes are capable of efficiently hydrolysing such pyrophosphates, as (**1**) is rapidly hydrolysed in neutral cell extracts to 2,3-bisphosphoglycerate.[10] These systems could thus be interesting 'pro drug' precursors of biologically active molecules possessing vicinal bisphosphates and exploitation of this reaction could lead to the synthesis of less hydrophilic biologically interesting cyclic pyrophosphates such as those which might be derived from second messenger polyphosphates of *myo*-inositol.[11]

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