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Novel P^1,P^2-Substituted Phosphonate Analogues of 2′-Deoxyadenosine and 2'-Deoxythymidine 5'-Triphosphates

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Abstract. P^1,P^2-Substituted methylene, fluoromethylene, and difluoromethylene analogues of the deoxynucleotides 2′-deoxyadenosine 5'-triphosphate and thymidine 5'-triphosphate have been prepared. by reaction between the bisphosphonic acid and the appropriate 5′-*O*-tosyl deoxynucleoside giving analogues of the 2'-deoxynucleoside 5′-diphosphate. The γ-phosphate is added subsequently either *via* activation of P-2 of the dNDP as its morpholidate followed by reaction with inorganic phosphate or using a novel, general reaction for the phosphorylation of nucleoside 5'-diphosphates employing *p*-nitrobenzyl phosphoromorpholidate.

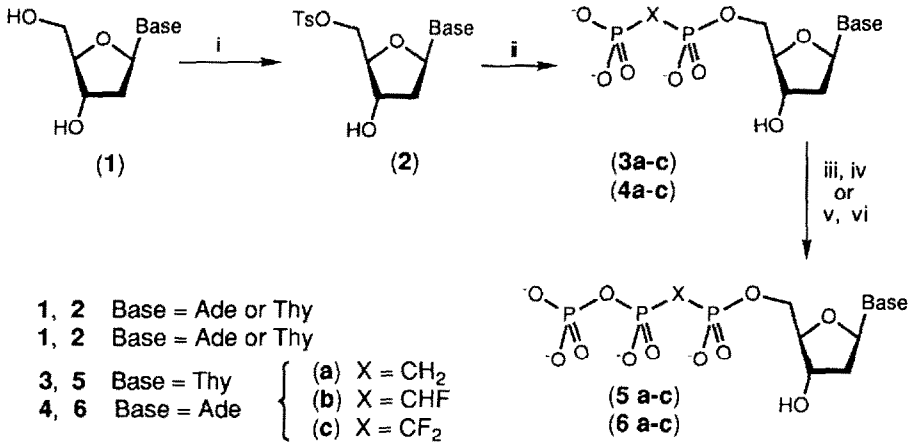
DNA replication is catalysed both by DNA polymerases and by reverse transcriptases and involves the cleavage of the P^1,P^2-bond of a deoxynucleotide triphosphate, dNTP, substrate with the release of pyrophosphate. The selection of the correct dNTP to be incorporated into the growing DNA strand is determined by complementation to the base in the template strand in accord with the rules of Watson Crick base-pairing. The net process is a powerful example of molecular recognition with an error rate estimated to be as low as 10^{-8} - 10^{-12} per nucleotide[1] during *in vivo* replication. This has been compared to a predicted error rate of 0.2 - 0.0006 per nucleotide based upon the differences in energy between correct and incorrect base pairs.[2] Considerable effort has been devoted to studies of this process, particularly using the Klenow fragment of DNA pol I from *E. coli*, for which an X-ray crystal structure is known[3] and the principal features of its kinetic mechanism have been described.[4] However, important non-covalent steps are yet to be resolved and call for the use of phosphonate deoxynucleotide analogues as polymerase inhibitors in order to arrest the covalent nucleotide addition step in the replication process.

Previously, we have used isosteric phosphonate analogues of nucleotides to good effect and we have established that isopolar α-halophosphonate analogues are capable of exhibiting improved biological action.[5-8] We here describe the synthesis of P^1,P^2-substituted methylene, fluoromethylene, and difluoromethylene analogues of the deoxynucleotides 2′-deoxyadenosine and thymidine 5'-triphosphates.

5′-*O*-p-Toluenesulphonyl-2'-deoxythymidine was prepared by the method of Goodman *et al.*[9] This procedure was effective for the direct tosylation of 2′-deoxyadenosine (65 % yield) after purification by flash chromatography (Figure 1). The method of Gibbs *et al.*[10] using 3 equivalents of tosyl chloride at room temperature was found to be unsatisfactory and gave large amounts of 3′,5′-di-*O*--tosyl-2'-deoxyadenosine. We found that the

2'-deoxynucleoside 5'-diphosphate analogues were best prepared from the 5'-*O*-tosylate and the appropriate bisphosphonic acid by essentially the procedure originally described by Stock [11] and by Poulter.[12] Yields were usually around 50-60% except for the fluoro- and difluoro-methylene analogues of 2'-deoxythymidine diphosphate compounds (**3b,c**) where the desired products were contaminated with by-products (*v.i.*). Product (**3b**) was purified by ion exchange chromatography (28% yield)[15] while purification of (**3c**) called for reverse phase h.p.l.c.

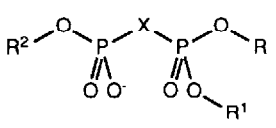
SCHEME



Reagents: (i) TosCl, 1.1 eq., -5°C, pyridine; (ii) $HO_3PXPO_3^{3-}$, $3Bu_4N^+$, CH_3CN; (iii) DCCD, morpholine, H_2O/Bu^tOH 1:1, reflux; (iv) $HOPO_3^{2-}$ $2Bu_3NH^+$, pyridine or DMSO; (v) *p*-nitrobenzyl phosphoromorpholidate, pyridine; (vi) H_2/5% $Pd/BaSO_4$, H_2O.

The by-products were purified, analysed by FAB MS, and found to contain either two or three equivalents of nucleoside per equivalent of bisphosphonate. The dimeric (**7**) and trimeric (**8**) 2'-deoxythymidine difluoromethylene species were isolated in 18% and 9% yields respectively while the dimeric 2'-deoxythymidine by-product for fluoromethylenebisphosphonate (**9**) was formed in 3% yield. In every case involving 2'-deoxythymidine, the two phosphorus atoms and the individual 2'-deoxynucleoside moieties were found to be non-equivalent (^1H, ^{31}P, and ^{19}F NMR), indicating that these molecules are not symmetrical. Structures (**7-9**) have been assigned consistent with all of the spectroscopic data (Figure). These by-products evidently arise from sequential displacement of the 5'-tosyloxy leaving groups by two oxygens on a single phosphonate: a somewhat unexpected result. By contrast, the reaction between difluoromethylenebisphosphonic acid and 5'-*O*-tosyl-2'-deoxyadenosine gave a by-product in 13% yield whose NMR spectra are consistent with the symmetrical dimeric species (**10**). None of these by-products, symmetrical or unsymmetrical, could be detected in reactions involving methylenebisphosphonate as nucleophile, which suggests that steric factors are less important than electronic ones in formation of these products

FIGURE



(**7**) R = R^1 = dThd, R^2 = H, X = CF_2
(**8**) R = R^1 = R^2 = dThd, X = CF_2
(**9**) R = R^1 = dThd, R^2 = H, X = CHF
(**10**) R = R^2 = dAdo, R^1 = H, X = CF_2

Finally, the dTDP analogues (**3a**) and (**3b**) were phosphorylated by an adaption of Moffatt 's morpholidate method for conversion of nucleoside 5'-monophosphates into their 5'-diphosphates.[13] With some difficulty, the dTDP analogues (**3a**) and (**3b**) were converted into their respective P^2-morpholidates which were then reacted without purification with inorganic phosphate to give (**5a**) and (**5b**) respectively in final yields of 32 and 28%. Attempts to apply this methodology to the more labile dADP analogues were unsuccessful.

We therefore devised a novel and convenient procedure for the phosphorylation of compounds (**3c**) and (**4a-c**) based upon *p*-nitrobenzyl phosphoromorpholidate. The dNDP analogues, as their tris-tributylammonium salts, were condensed with an excess of *p*-nitrobenzyl phosphoromorpholidate[14] in anhydrous pyridine. The P^1-deoxynucleotide-5'-yl P^3-*p*-nitrobenzyl (P^1,P^2-substituted)-triphosphates are produced in good yields (~70%), can readily be purified by ion exchange m.l.p.c., and are adequately stable. The *p*-nitrobenzyl group is best

Table Data for 2'-deoxyribonucleoside 5'-(P^1,P^2-substituted)-triphosphates

Compound	Yield %	% (M + Na^+)	% (M + H^+)[a]	δ_P (ppm)	δ_F (ppm)	$^2J_{PP}$	$^2J_{PF}$
dTPCH_2PP (**5a**)	32	90	55	P^1 19.1 P^2 7.1 P^3 - 5.2		P^1P^2 7.5 P^2P^3 24.2	
dTPCHFPP (**5b**)	28	90	100	P^1 11.24, P^2 11.20 P^3 0.1 - 5.0	- 219.1 - 219.4	P^1P^2 15.3 P^2P^3 25.4	60.4 60.2
dTPCF_2PP (**5c**)	56	95	100	P^1 4.5 P^2 - 6.6 P^3 - 4.8	- 118.4	P^1P^2 53.5 P^2P^3 28.0	83.0 81.0
dAPCH_2PP (**6a**)	58		100	P^1 19.2 P^2 6.4 P^3 - 5.7		P^1P^2 7.8 P^2P^3 24.5	
dAPCHFPP (**6b**)	53	*	*	P^1 11.3 P^2 - 0.6 P^3 - 5.1	- 219.2 - 219.4	P^1P^2 15.8 P^2P^3 25.4	60.9 60.1
dAPCF_2PP (**6c**)	67	50	100	P^1 4.6 P^2 - 5.9 P^3 - 4.4	- 119.4	P^1P^2 59.1 P^2P^3 27.3	82.1 80.6

[a] FAB MS, Kratos MS80

removed by catalytic hydrogenolysis (5% Pd on $BaSO_4$) in aqueous solution[15] to give the desired dNTP analogues in good yield (Table). This reaction appears to be general for the phosphorylation of most nucleoside phosphates and thus provides a convenient and high-yielding procedure for the preparation of nucleoside triphosphate analogues. For both of the monofluoromethylene analogues (**5b**) and (**6b**) the spectroscopic data show that these substances are mixtures of two diastereoisomers at the CHF centre in approximately equal abundance.

The six α,β-carbon-bridged dNTP analogues described here are under investigation as inhibitors of reverse transcriptase and further work is being directed to the production of (**5b**) and (**6b**) as discrete diastereoisomers.

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