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ARTICLE

Phosphorothioate Anti-sense Oligonucleotides: The Kinetics and Mechanism of the Sulfurisation of Phosphites by Phenylacetyl Disulfide (PADS)

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In the pharmaceutical industry the sulfurisation of nucleotide-phosphites to produce more biologically stable thiophosphates is often achieved using 'aged' solutions of phenylacetyl disulfide (PADS) which consist of a mixture of polysulfides that are more efficient sulfur transfer reagents. However, both 'fresh' and 'aged' solutions of PADS are capable of the sulfurisation of phosphites. The rates of both processes in acetonitrile are first order in sulfurising agent, phosphite and a pyridine base, although with 'aged' PADS the rate becomes independent of base at high concentrations. The Brönsted β values for sulfurisation using 'fresh' and 'aged' PADS substituted pyridines are 0.43 and 0.26, respectively. With 'fresh' PADS the Brönsted β_{nuc} =0.51 for substituted trialkyl phosphites is consistent with a mechanism involving nucleophilic attack of the phosphite on the PADS disulfide bond to reversibly generate a phosphonium intermediate, the rate-limiting breakdown of which occurs by a base catalysed elimination process, confirmed by replacing the ionisable hydrogens in PADS with methyl groups. The comparable polysulfide phosphonium ion intermediate seen with 'aged' PADS presents a more facile pathway for product formation involving S-S bond fission as opposed to C-S bond fission.

Introduction

Anti-sense oligonucleotides (ASOs)[1] are usually single-stranded deoxy-ribonucleotides of 15-35 bases which bind to a specific section of mRNA to form a dimer that may then inhibit translation and therefore prevent synthesis of an unwanted target protein. ASOs are promising therapeutics[2] but the incorporation of a native phosphodiester link means they are rapidly hydrolysed by intracellular endonucleases and exonucleases[3]. To overcome this problem ASOs have been modified to decrease their susceptibility to nuclease catalysed hydrolysis and improve their pharmacokinetics[4]. One of the most common examples of these changes is the introduction of a phosphorothioate backbone (**1**)[5] to give derivatives which are poor substrates for enzyme catalysed hydrolysis and yet do not disrupt binding between the nucleobases[6]. The introduction of the relatively hydrophobic sulfur also increases cell uptake compared with the native P=O phosphodiester whilst still maintaining good water solubility as a polarisable anion. Although the introduction of one of the phosphate non-bridging oxygens by sulfur makes the phosphorus chiral, it is only the (*S*)-P phosphorothioate diastereomer that is nuclease resistant[7].

The successful large scale synthesis of oligonucleotides with phosphorothioate links requires the sulfurisation step to have a near quantitative yield, to be rapid and give a maximal P=S to P=O ratio. Sulfurisation of the protected nucleotide-phosphite (**2**), attached to a solid support, is often carried out with the sulfurising agent present in an organic solvent. Although there

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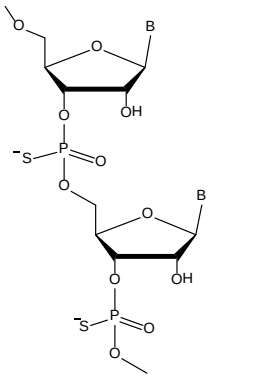
are a number of reagents available[8,9] phenylacetyl disulfide (PADS) (**3**) is commonly used in the pharmaceutical industry,[10,11,12]. This reagent is unusual in that it is best 'aged' in a basic acetonitrile solution to obtain optimal sulfurisation activity[13], often using 3-picoline as the base. We have recently shown that this is due to the formation of diacyl polysulfides (**4**) by an unusual elimination E1cB mechanism[14]. Although both 'fresh' and 'aged' PADS convert phosphite esters to the corresponding thiophosphate, 'ageing' improves the rate and efficiency of sulfurisation. Herein we address the reasons for this difference and report the kinetics and mechanism of both sulfurisation reactions.



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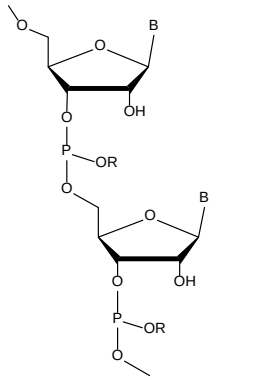

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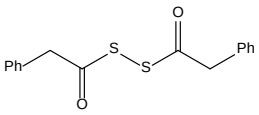

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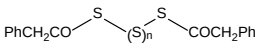

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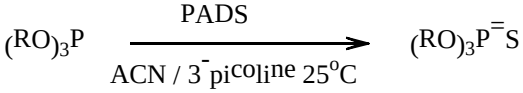
Results and Discussion

(i) Sulfurisation by 'fresh' PADS

Although a freshly made solution of PADS (**3**) is not an optimal sulfurising agent[13] it is still capable of converting phosphite triesters to the corresponding thiophosphate (**Scheme 1**).



Scheme 1

For example, a solution of 1.0 M PADS (**3**) and 2.0 M 3-picoline in acetonitrile sulfurises 0.1 M triphenyl phosphite to triphenyl thiophosphate at 25°C with a half-life of ~ 15 mins. The kinetics of this process were determined by following the reaction by ^{31}P NMR from which the rate law was found to be first order in each reagent (**eq.1**). With excess PADS and 3-picoline there is an exponential decrease in phosphite concentration from which the observed pseudo first-order rate constants were found to increase with increasing concentration of catalytic base, 3-picoline (**Fig. 1**) giving a second-order rate constant k_{cat} = 4.13 x 10^{-4} $M^{-1}s^{-1}$ at 25°C. Similarly the observed pseudo first-order rate constants increase with the concentration of PADS.

Rate = k [PADS].[picoline].[$(RO)_3P$] eq. 1

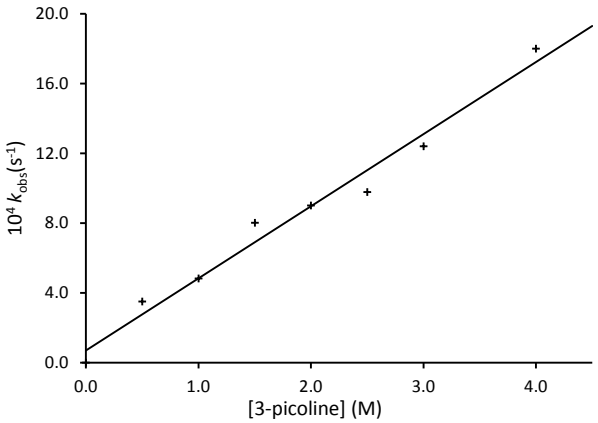


Figure 1 Pseudo first-order rate constants (k_{obs}) at 25°C as a function of the concentration of 3-picoline for the sulfurisation of 0.10 M triphenyl phosphite by 1.0 M PADS

Solutions of PADS in acetonitrile with 3-picoline slowly degrade to give polysulfides[14]. However, when using 'fresh' solutions of PADS for the sulfurisation of phosphites, the active sulfurising reagent must be the intact diacyl disulfide (**3**) as the rate of sulfurisation of phosphite esters by 'fresh' PADS are two orders of magnitude faster than the rate of degradation of PADS under similar conditions.

The sulfurisation process is likely to occur by an ionic mechanism as indicated by the dependency of the rate on a base, substituent

effects in all three reagents and on the polarity of the solvent. For example, the relative rates of sulfurisation of triphenyl phosphite in toluene, chloroform, acetonitrile and DMSO are 1: 4: 86: 230, respectively, and these ratios remain similar for the sulfurisation of substituted triaryl phosphites.

The mechanism of the reaction was investigated by determining the effect of substituents in the three reagents - the phosphite, the sulfurising agent and the catalytic base - on the rates of sulfurisation. Trialkyl phosphites undergo sulfurisation extremely rapidly in acetonitrile making kinetic investigations difficult, but the reactions are measurable in chloroform (**Table 1**). Electron-withdrawing substituents in trialkylphosphites $(RO)_3P$ decrease the rate of sulfurisation and there is a Brönsted-type linear dependence of the observed pseudo first-order rate constants (k_{obs}) on the pK_a of the corresponding alcohol in water generating an apparent β_{nuc} =0.51 (**Fig. 2**) indicative of positive charge development on phosphorus in the transition state and compatible with the phosphite acting as a nucleophile in the sulfurising reaction. Trialkyl phosphites have a high thiophilicity and their S-nucleophilicity is greater than that of sulfides.[15] There is little or no dependence of the rates of sulfurisation of triaryl phosphites on the aryl substituent (**Table 1**).

Table 1 The observed pseudo first-order rate constants (k_{obs}) for the sulfurisation of substituted trialkyl and triphenyl phosphites (0.10 M) by 'fresh' PADS (1.0 M) and 3-picoline (2.0 M), in $CDCl_3$ at 25°C.

$(RO)_3P$ R substituent	**pK_a ROH (H_2O)**	**k_{obs} (s^{-1})**
ethyl	16.0	1.43×10^{-2}
methyl	15.54	5.10×10^{-3}
2-chloroethyl	14.31	5.60×10^{-4}
2,2,2-trifluoroethyl	12.43	1.98×10^{-4}
4-methoxyphenyl	10.20	3.51×10^{-5}
4-methylphenyl	10.19	6.55×10^{-5}
phenyl	9.95	3.28×10^{-5}
4-fluorophenyl	9.95	2.66×10^{-5}
4-chlorophenyl	9.38	7.88×10^{-5}
3-chlorophenyl	9.02	4.62×10^{-5}

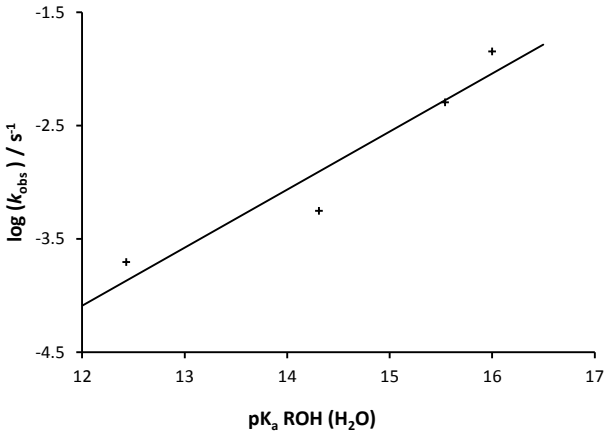


Figure 2 Dependence of the observed pseudo first-order rate constant (k_{obs}) for the sulfurisation of substituted trialkyl phosphites $(RO)_3P$ by PADS with 3-picoline in chloroform at 25°C on the pK_a of the corresponding alcohol (ROH) in water.

On the other hand, electron-withdrawing substituents in the aromatic rings of PADS (**3**) increase the rate of sulfurisation of triphenyl phosphite (0.10M) with 3-picoline (2.0M) in acetonitrile (**Table 2**). However, there is a non-linear dependence on the Hammett σ-values probably suggesting a change in rate-limiting step (**Figure 3**). For the 4-methoxy to the 4-chloro-substituted PADS the Hammett ρ-value is 2.34 indicative of significant generation of negative charge near the PADS residue in the transition state.

Table 2 The observed pseudo first-order rate constants (k_{obs}) for the sulfurisation of triphenyl phosphites (0.10 M) by 'fresh' aryl-substituted PADS (1.0 M) and 3-picoline (2.0 M), in acetonitrile at 25°C.

Ar substiuent in $(ArCH_2COS)_2$	**k_{obs} (s^{-1})**	**Hammett σ substituent**
4-MeO	2.56×10^{-4}	-0.27
H	7.70×10^{-4}	0
4-F	1.07×10^{-3}	0.06
4-Cl	4.01×10^{-3}	0.23
3-Cl	4.67×10^{-3}	0.37
4-CN	6.27×10^{-3}	0.66

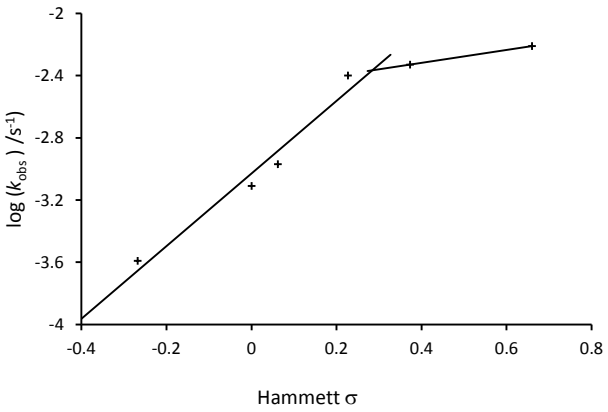


Figure 3 Dependence of the observed pseudo first-order rate constant (k_{obs}) for the sulfurisation of triphenyl phosphite (0.10 M) by aryl substituted PADS (1.0 M) with 3-picoline (2.0 M) in acetonitrile at 25°C on the Hammett σ-value of the aryl substituent.

The rates of sulfurisation of triphenyl phosphite by 'fresh' PADS (**3**) in acetonitrile also increase with the basicity of substituted pyridines. A Brönsted-type plot of the dependence of the observed pseudo first-order rate constants at 25°C on the pK_a of the conjugate acid of substituted pyridines in acetonitrile (**Table 3**) generates a slope β = 0.43 (**Figure 4**), compatible with positive charge development on the pyridine nitrogen in the transition state.

Table 3 The observed pseudo first-order rate constants (k_{obs}) for the sulfurisation of triphenyl phosphite (0.10 M) by 'fresh' PADS (1.0 M) and the pK_a of the catalysing base, substituted pyridines (2.0 M), in acetonitrile at 25°C

Pyridine substituent	**pK_a (ACN)**	**k_{obs} (s^{-1})**
4-CN	8.50	7.26×10^{-6}
3-CN	8.00	7.38×10^{-6}
3-Cl	9.77	2.27×10^{-5}
3-MeO	12.45	4.92×10^{-4}
H	12.60	2.96×10^{-4}
3-Me	13.70	8.81×10^{-4}
4-MeO	14.73	7.15×10^{-3}
2,6 diMe	14.70	8.75×10^{-4}

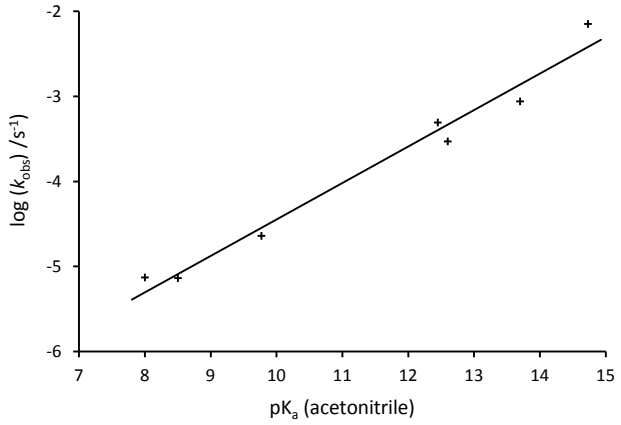
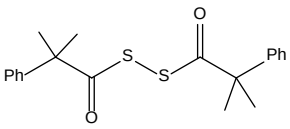


Figure 4 Dependence of the observed pseudo first-order rate constants (k_{obs}) for the sulfurisation of triphenyl phosphite by 'fresh' PADS on the pK_a of substituted pyridines in acetonitrile at 25°C

In order to distinguish between the role of the catalytic pyridines acting as general bases or as nucleophiles, sulfurisation was investigated with hindered bases and sulfurising agent. For example, the observed first-order rate constant for the sulfurisation of triphenyl phosphite in acetonitrile with PADS using sterically hindered 2,6-lutidine as the base is 3-fold greater than that for pyridine which is similar to the ratios seen for proton abstraction from carbon acids[16]. Whereas for pyridine acting as a nucleophilic catalyst such as in the hydrolysis of acetic anhydride[17] and acetyl fluoride[18], through the intermediate formation of the acetyl pyridinium ion, 2,6-lutidine is an ineffective catalyst despite its higher basicity[3, 4]. The sulfurising reaction with 'fresh' PADS therefore appears to involve the pyridine acting as a base abstracting a proton from the benzyl methylene in an elimination step. Such a role can be prevented by replacing these ionisable hydrogens and indeed the rate of sulfurisation of triphenyl phosphite with 2,2,2',2'- tetramethyl PADS (**5**) and 3-picoline in acetonitrile is an order of magnitude slower than with PADS (**3**) under the same conditions. With the sterically hindered 2,6-lutidine as the base and (**5**) there is no observable sulfurisation of triphenyl phosphite indicating that the mechanism of sulfurisation with tetramethyl PADS (**5**) and 3-picoline occurs by nucleophilic catalysis in contrast to the general base catalysed reaction of PADS (**3**) itself.

Finally, removing the methylenes of PADS to give dibenzoyl disulfide (DBDS) (**6**) generates a sulfurising agent which is 20-fold less effective than PADS under the same reaction conditions (k^{DBDS} = 3.82 x 10^{-5}, k^{PADS} = 7.70 x 10^{-4} s^{-1}). The dependence of the observed pseudo first-order rate constants (k_{obs}) for the sulfurisation of triphenyl phosphite by dibenzoyl disulfides (**6**) with substituted pyridines in acetonitrile at 25°C on on the pK_a of the conjugate acid of substituted pyridines in acetonitrile generates a $\beta = 0.16$ compared with β= 0.43 with 'fresh' PADS. The different β-values are consistent with different roles of the base in the sulfurisation reactions using dibenzoyl disulfides (**5**) and 'fresh' PADS (**3**).



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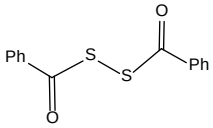

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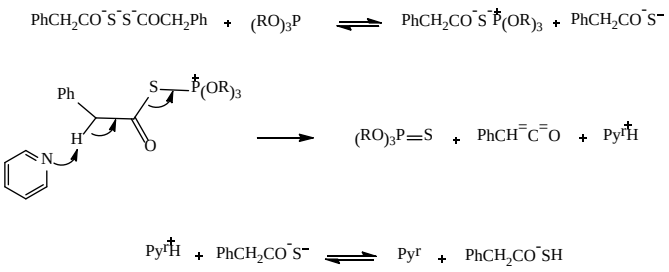
These observations are compatible with sulfurisation using 'fresh' PADS (**3**) occurring with the phosphite acting as a nucleophile and the pyridine acting as a general base suggesting the mechanism outlined in **Scheme 2**. Cleavage of the S-S bond in disulfides by nucleophiles is well established[19] and, in this case, initial nucleophilic attack of the phosphite on sulfur of the diacylsulfide generates an intermediate phosphonium ion and thioacetate anion in a reversible step; in acetonitrile these two species probably exist as an ion-pair. Rate-limiting breakdown of the phosphonium ion occurs by an elimination step involving proton abstraction by the pyridine base to give the product thiophosphate. This step is similar to that which we have suggested for the 'ageing' of PADS by trapping the ketene and D-exchange of the methylene hydrogens. In acetonitrile, the pK_a of the thioacid is ~ 20 and that for 3-picoline 13.7[20] so favouring formation of the neutral species (**Scheme 2**). Rate-limiting breakdown of the phosphonium ion is compatible with the LFER observations reported here which indicate that in the transition state the phosphorus and the catalytic pyridine base nitrogen are both relatively positively charged, whilst there is relative negative charge development transmitted to the aryl ring of PADS. There may be a change in rate-limiting step with electron-withdrawing substituents in the aryl ring of PADS due to the increased acidity of the methylene hydrogens facilitating proton abstraction causing the first step to become rate-limiting.



Scheme 2

(ii) Sulfurisation by 'aged' PADS

A freshly made solution of PADS (**3**) is not an optimal sulfurising agent[11] and a solution 'aged' for about 2 days in 50% v/v 3-picoline in acetonitrile improves their efficiency. For example, the pseudo first-order rate constants for the sulfurisation of 0.05 M triphenyl phosphite in acetonitrile by 0.5 M PADS with 1.0 M 3-picoline increase with the length of 'ageing' (**Fig. 5**), such that PADS 'aged' for 48 h. is more than an order of magnitude more active than a freshly prepared solution of PADS. We have shown that PADS degrades completely over this period of time due to its degradation to phenylacetyl polysulfides. Given that the molar concentration of the polysulfides is less than that derived from PADS they are thus much more efficient sulfurising agents.

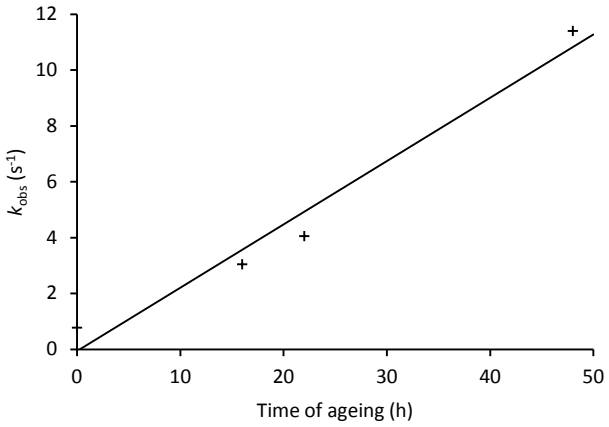


Figure 5 The pseudo first-order rate constants for the sulfurisation of triphenyl phosphite (0.05 M) to $(PhO)_3P=S$ by (0.5 M) PADS (**3**) as a function of the time that PADS has been 'aged' in acetonitrile with (1.0 M) 3-picoline at 25°C

The general procedure for preparing 'aged' PADS was to dissolve (**3**) (3M) in acetonitrile with 3-picoline (5M) maintained at 25°C for 48 h. This was then quenched with excess aqueous hydrochloric acid (2M) to remove excess 3-picoline and the mixture extracted using dichloromethane, which was then dried and removed under vacuum. The resultant yellow oil was then made up to the same volume as the original solution generating a solution with the same nominal concentration as the original PADS solution. A crude estimate of the totalconcentrations of the phenylacetyl polysulfides generated from the standard solution of PADS is 1.45 M based on HPLC data assuming similar extinction coefficients for all species.

The kinetics of the sulfurisation reactions of phosphites were determined by following the reaction by ^{31}P NMR usually with excess PADS and 3-picoline so there is an exponential decrease in phosphite concentration from which the observed first order constants could be obtained. The rate law using 'aged' PADS is first order in each reagent at low base concentrations, similar to that seen with 'fresh' PADS (**eq.1**). However, there is a non-linear dependence on base concentration (**Fig. 6**) indicative of a change in rate-limiting step with increasing concentration and therefore a stepwise reaction mechanism. In the region where there is a first-order dependence on 3-picoline concentration, the corresponding second-order rate constant k_{cat} = 6.72 x 10^{-3} $M^{-1}s^{-1}$ at 25°C which is 16 fold greater than that using 'fresh' PADS for which k_{cat} = 4.13 x 10^{-4} $M^{-1}s^{-1}$ However, the second-order rate constant for 'aged' PADS is based on the nominal concentration of PADS before it is degraded to a mixture of lower molar concentration of polysulfides and so the true constant is much higher than this.

The rates of sulfurisation of triphenyl phosphite by 'aged' PADS (**4**) in acetonitrile have a smaller dependency on the basicity of the catalytic base. A Brönsted-type plot of the dependence of the observed pseudo first-order rate constants at 25°C on the pK_a of the conjugate acid of substituted pyridines in acetonitrile (**Table 4**) generates a slope β = 0.26 compared with β = 0.43 using 'fresh' PADS. This indicates a transition state with less positive charge development on the pyridine nitrogen for 'aged'

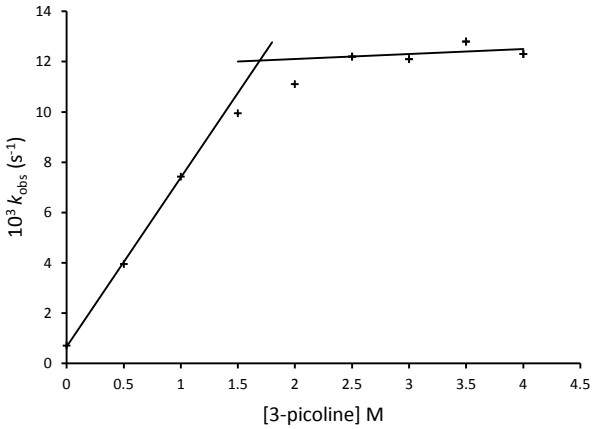


Figure 6 The dependence of the observed pseudo first-order rate constants for the sulfurisation of triphenyl phosphite (0.10 M) to $(PhO)_3P=S$ by (1.0 M) PADS (**3**) that has been 'aged' for 48 h in acetonitrile and 3-picoline (5.0 M) on the concentration of 3-picoline at 25°C.

PADS. One of the consequences of the smaller dependency of the rate of sulfurisation on the basicity of the pyridine catalyst using 'aged' PADS is that the rates of sulfurisation with 4-methoxypyridine using 'aged' and 'fresh' PADS are similar, based on the same starting concentration of PADS.

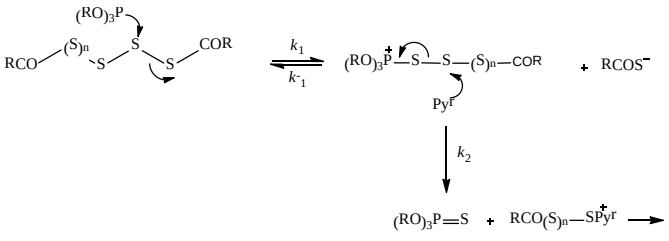
Table 4 The observed pseudo first-order rate constants (k_{obs}) for the sulfurisation of triphenyl phosphite (0.10 M) by 'aged' PADS (1.0 M) and the pK_a of the catalysing base, substituted pyridines (2.0 M), in acetonitrile at 25°C

Pyridine substituent	**pK_a (ACN)**	**k_{obs} (s^{-1})**
4-CN	8.50	3.92 x 10^{-4}
3-CN	8.00	4.09 x 10^{-4}
3-Cl	9.77	8.75 x 10^{-4}
3-MeO	12.45	4.78 x 10^{-3}
H	12.60	9.88 x 10^{-3}
3-Me	13.70	1.14 x 10^{-2}
4-MeO	14.73	1.28 x 10^{-2}

If the mechanism of sulfurisation using 'aged' PADS involved nucleophilic attack by trialkyl phosphite on the sulfur adjacent

to the acyl group of diacyl polysulfides (**4**), as occurs with 'fresh' PADS (**Scheme 2**), this would eliminate a sulfide anion compared with the better leaving group thiocarboxylate anion using 'fresh' PADS. Therefore this seems an unlikely process to explain the greater reactivity of 'aged' PADS as the pre-equilibrium constant K_1 would be reduced and the rate constant for the reverse step k_{-1} increased because of the greater nucleophilicity of sulfide anion compared with thioacetate ion.

It is proposed that the sulfurisation reaction with 'aged' PADS occurs with initial nucleophilic attack by trialkyl phosphite on a central sulfur of diacyl polysulfides (**4**) to liberate a thioacetate anion in a pre-equilibrium step similar to that suggested for 'fresh' PADS (**Scheme 2**). Nucleophilic attack by phosphite on the polysulfide (**4**) reversibly generates a phosphonium ion (**Scheme 3**) and both the magnitude of the rate (k_1 and k_{-1}) and equilibrium constants (K_1) for this process are similar to those for the comparable step using 'fresh' PADS (**3**). However, the pyridine base now acts as a nucleophile in the sulfurisation reaction with 'aged' PADS compared with its role as a base using 'fresh' PADS (**Scheme 2**).



Scheme 3

However, the polysulfide phosphonium ion intermediate presents a more facile pathway for product formation involving S-S bond fission as opposed to C-S bond fission. The bond dissociation energy is 226 kJ.mol^{-1} for the former compared with 272 kJ.mol^{-1} for the latter and the force constant for S-S stretch (440 Nm^{-1})[21] is significantly smaller leading to facile bond cleavage, especially in polysulfides[22]. There are many examples of S-S bond cleavage as a result of nucleophilic attack by nitrogen nucleophiles[19, 23]. The relatively small value for the Brönsted β_{nuc} = 0.26 for catalysis by substituted pyridines is also compatible with a facile process for the second step k_2 and the change in rate-limiting step[24] with increasing picoline concentration (**Fig. 6**) occurs when k_2[pic]>> k_{-1}.

Conclusion

Although an 'aged' solution of phenylacetyl disulfide (PADS), consisting of polysulfides, is a more efficient sulfur transfer reagent than 'fresh' PADS to convert trialkylphosphites to the corresponding thiophosphate 'fresh' PADS is still an effective sulfurising reagent. The differences in reactivity of the two solutions are reflected in their kinetic behaviour and the dependencies of their respective rates on variables such as substituent effects and concentration of the catalysing pyridine base. The rates of both processes in acetonitrile are first order in sulfurising agent, phosphite and a pyridine base. However, with an 'aged' solution of PADS the rate becomes independent of base at high concentrations. The Brönsted β values for sulfurisation using 'fresh' and 'aged' PADS substituted pyridines are different, 0.43 and 0.26, respectively. With 'fresh' PADS the Brönsted β_{nuc} =0.51 for substituted trialkyl phosphites is consistent with a mechanism involving nucleophilic attack of the phosphite on the PADS disulfide bond to reversibly generate a phosphonium intermediate. Replacing the ionisable hydrogens in PADS with methyl groups significantly reduces the rate of sulfurisation indicating that the rate-limiting breakdown of the phosphonium ion intermediate occurs by a base catalysed elimination process. The comparable polysulfide phosphonium ion intermediate seen with 'aged' PADS allows a more facile pathway for product formation involving S-S bond fission as opposed to C-S bond fission.

Experimental

General sulfurisation kinetics with 'aged' PADS A solution of PADS (1M) 50/50 v/v acetonitrile/3-picoline was left to 'age' for 48 hours. At T=*n* h a 1ml sample of this was removed and quenched with dilute hydrochloric acid (10ml, 2M) to remove the picoline. This was then washed with dichloromethane (DCM) (2 x 10ml), dried over magnesium sulfate and the solvent removed under vacuum. The resulting oil was then made up to 1ml using deuterated acetonitrile. To an NMR tube containing triphenyl

phosphite in deuterated acetonitrile (0.1M, 200 µL) and 3-picoline in deuterated acetonitrile (2M, 200 µL) was added 'aged' PADS solution (200 µL, 1M) and ^{31}P NMR spectrum recorded every 90 seconds.

Synthesis

The syntheses of the various arylacetyl disulfides have been previously reported[14].

Tris-(4-methoxyphenyl)phosphite: Into a 250 ml three-necked round-bottom flask a solution of 4-methoxyphenol (9.77 g, 78.7 mmol) and pyridine (8.10 ml, 7.92 g, 100 mmol) in diethyl ether (80 ml). To this, phosphorus trichloride (1.74 ml, 2.74 g, 20.0 mmol) was added dropwise at room temperature under nitrogen atmosphere and stirred for 1.5 h. When the reaction was complete, the reaction mixture was quenched with water (100 ml) and the organic phase then washed with water (50 ml) and brine (50 ml), dried over magnesium sulfate and the solvent removed under vacuum. The crude mixture was purified by silica gel column chromatography using chloroform as the eluent to give the product as a colourless oil (4.87 g, 4.03 ml, 12.2 mmol, 61 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.78 (s, 9H, CH_3), 6.87 (d, 6H, *J* 9.23 Hz, ArH), 7.11 (d, 6H, *J* 9.02 Hz, ArH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.6 (CH_3), 114.7 (CH), 121.7 (CH), 145.1 (Cq), 156.3 (Cq) ppm; ^{31}P NMR ($CDCl_3$, 400 MHz), δ 128.9 ppm, HRMS (m/z): $[M+H]^+$ for $C_{18}H_{12}O_3Cl_3P$, calculated 400.1076, measured 401.1150.

Tris-(3-chlorophenyl)phosphite: As above using 3-chlorophenol (14.04 g, 112.8 mmol) and pyridine (10.78 ml, 10.58 g, 141 mmol) in diethyl ether (120 ml), phosphorus trichloride (4.0 ml, 3.87 g, 28.2 mmol). The crude mixture was purified by silica gel column chromatography using chloroform as the eluent to give the product as a pale yellow oil (9.1 g, 6.8 ml, 22 mmol, 78 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.01 – 7.29 (m, 12H, ArH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 118.8 (d, $J_{C\text{-}P}$ 7.49 Hz, CH), 121.2 (d, $J_{C\text{-}P}$ 6.55 Hz, CH), 124.9 (CH), 130.6(CH), 135.1 (Cq), 151.8 (Cq) ppm; ^{31}P NMR ($CDCl_3$, 400 MHz), δ 126.5 ppm, HRMS (m/z): $[M+H]^+$ for $C_{18}H_{12}O_3Cl_3P$, calculated 411.9590, measured 411.9596.

Tris-(4-chlorophenyl)phosphite: As above using 4-chlorophenol (10.3 g, 80 mmol) and pyridine (8.10 ml, 7.92 g, 100 mmol) in diethyl ether (80 ml), phosphorus trichloride (1.74 ml, 2.74 g, 20.0 mmol). The crude mixture was purified by silica gel column chromatography using chloroform as the eluent to give the product as a white solid (5.06 g, 12.2 mmol, 61 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (d, *J* 8.93 Hz, 6H, ArH), 7.30 (d, *J* 8.96 Hz, 6H, ArH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 121.9 (d, $J_{C\text{-}P}$ 6.6 Hz, Cq), 129.9 (CH), 149.8 (d, $J_{C\text{-}P}$ 2.8 Hz, Cq) ppm; ^{31}P NMR ($CDCl_3$, 400 MHz), δ 126.8 ppm, HRMS (m/z): $[M+H]^+$ for $C_{18}H_{12}O_3Cl_3P$, calculated 411.9590, measured 411.9592.

Tris-(4-fluorophenyl)phosphite: To a solution of 4-fluorophenol (4.41 g, 39.3 mmol) and triethylamine (5.6 ml, 4.06 g, 40 mmol) in diethyl ether (40 ml) was added dropwise phosphorus trichloride (0.87 ml, 1.37 g, 10.0 mmol) then stirred at room temperature under nitrogen atmosphere for 24 h. The solid precipitate from the reaction was removed by vacuum filtration and washed with diethyl ether (2 x 10ml). The solvent from the filtrate was removed under vacuum and the crude product purified by silica gel column chromatography eluting with chloroform to give the product as a white solid (4.77 g, 13.1 mmol, 71% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.01 – 7.13 (m, 12H, ArH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) 116.4 (d, $J_{C\text{-}F}$ 23 Hz, CH), 122.0 (t, $J_{C\text{-}F}$ 7.4 Hz, $J_{C\text{-}P}$ 7.3 Hz, CH), 147.2 (d, $J_{C\text{-}F}$ 2.9 Hz, $J_{C\text{-}P}$ 3.0 Hz, Cq), 159.5 (d, $J_{C\text{-}F}$ 243 Hz, CF) ppm; ^{31}P NMR ($CDCl_3$, 400 MHz) δ 127.5 (127.8) ppm, HRMS (m/z): $[M+H]^+$ for $C_{18}H_{12}O_3F_3P$, calculated 364.0476, measured 364.0478.

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Notes and references

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