Scrambling of the label in a fluorenylphosphonamidic [^{18}O]-sulfonate during dissociative nucleophilic substitution (elimination–addition): a measure of the importance of preassociation

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When $R_2CHP(O)(NEt_2)OS^{18}O_2Ar$ (R_2CH = 9-fluorenyl, Ar = *p*-tolyl) undergoes nucleophilic substitution (elimination–addition) with Et_2NH (0.4 mol dm^{-3} in $CHCl_3$) the phosphene intermediate $R_2C{=}P(O)NEt_2$ recombines with the sulfonate leaving group (internal return), causing scrambling of the ^{18}O label, more quickly than it diffuses away; efficient conversion into $R_2CHP(O)(NEt_2)_2$ therefore depends on preassociation between the substrate and the nucleophile.

Phosphoryl transfer is an essential part of many biological processes[1] and phosphate mimics, notably phosphonates,[2] are important for probing the mechanisms of biological phosphorylation reactions and also for influencing metabolic processes. The mechanism is generally associative [$S_N2(P)$], with a five-coordinate intermediate or transition state,[3] although for substrates with an acidic ligand (usually HO, HS, HNR) a dissociative pathway may be competive, elimination of HX (X = leaving group) generating a reactive (metaphosphate-like) three-coordinate P^v intermediate.[3,4] Alkyl groups in general are not acidic but fluorenyl is unusual and evidence suggests that 9-fluorenylphosphonamidic chlorides such as **1** (X = Cl) undergo substitution with amines by an elimination–addition (EA) mechanism and a reactive phosphene intermediate **2**.[5] However, the stereochemistry (incomplete and concentration-dependent non-stereospecificity) cannot be reconciled with a planar phosphene as the sole product-forming species unless it is supposed that preassociation[6] is important,[7] and that will only be the case if the phosphene recombines very rapidly with the chloride leaving group; then some of the phosphene may still diffuse away from the leaving group and become free (symmetrically solvated) but the rest will just recombine with chloride ion and return to substrate unless the nucleophile is already in place (preassociation) to trap it as it is formed. Such internal return is unlikely to be properly revealed by configurational change (racemisation) of the substrate – the phosphene intermediate would have to survive long enough to allow tumbling – and little if any is seen in the reactions of the fluorenylphosphonamidic chlorides. To establish the reality of return, and so to justify invoking preassociation, it is necessary to examine a process faster than tumbling, in particular the equilibration of potentially equivalent sites in the leaving group. Chloride lacks such sites and carboxylate and phosphate are relatively poor leaving groups so we had to make use of sulfonate.

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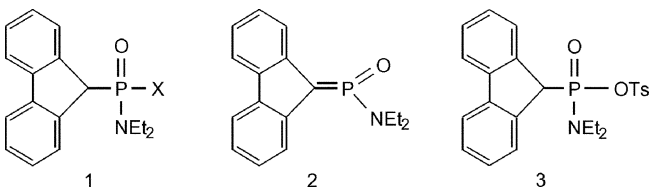
Treatment of the phosphonamidic acid **1** (X = OH) [obtained by hydrolysis of **1** (X = Cl)] with a slight excess of Et_3N and TsCl in $CDCl_3$ (δ_P 18 → 26) gave the mixed anhydride without appreciable pyrophosphonate formation.† Aqueous work up (mildly acidic) and crystallisation afforded the pure anhydride **3** [mp 102–104 °C, *m*/*z* (FAB) 456 (M + H)$^+$; δ_P 26.1; δ_H 8.1–7.25 (12 H), 4.76 (1 H, d, J_{PH} 32 Hz), 2.57 (4 H, m), 2.48 (3 H, s) and 0.46 (6 H, t, J_{HH} 7 Hz)]. Use of ^{18}O-labelled TsCl afforded **3** having an ^{18}O content of 106 atom% (ES MS: 15.5% no ^{18}O, 63% one ^{18}O, 21.5% two ^{18}O).‡ Provided only a very slight excess of Et_3N was used and the reaction was quenched immediately on completion the ^{18}O was almost entirely confined to the SO_2 group (^{31}P NMR: only *ca.* 2% P–^{18}O).



The anhydride **3** is more reactive than the corresponding chloride **1** (X = Cl) and it also contains an alternative site – the S atom – at which the nucleophile can attack.[8] With Et_2NH, however, the product was overwhelmingly the phosphonic amide **1** (X = NEt_2) (δ_P 34.1), the alternative sulfonic amide (Et_2NTs) amounting only to 1–2% (^{1}H NMR; GC-MS).§

The reaction of the [^{18}O]-labelled anhydride **3** with Et_2NH (13 equiv.; 0.4 mol dm^{-3}) in $CHCl_3$ (containing 6% C_6D_6 as NMR lock) was monitored by ^{31}P NMR spectroscopy at 27 °C. Fig. 1 shows the signal due to the substrate in selected spectra. At first (t = 0) the high field ^{18}O-shifted peak is barely visible (*ca.* 2% of the substrate signal) but at 5% completion of the substitution reaction

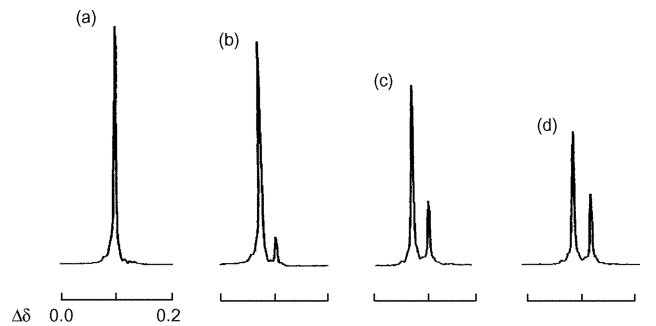
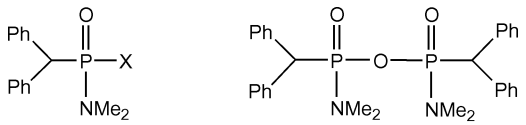


Fig. 1 ^{31}P NMR spectrum (162 MHz) of ^{18}O-labelled substrate **3** in reaction with Et_2NH: (a) initially (t = 0), (b) at 5% completion of substitution (t = 20 min), (c) at 17% completion (t = 50 min), (d) at 43% completion (t = 100 min).

($t = 20$ min) it is conspicuous (11%) and at 17% completion ($t =$ 50 min) it is important (25%). The substrate contains 106 atom% ^{18}O so scrambling of the label between the SO_2 and the bridging O atom can only give 35–36% of the substrate molecules with a P–^{18}O bond and that situation was attained by 43% completion ($t = 100$ min) (Fig. 1). Scrambling is therefore not only important but some five times faster than substitution. By implication the phosphene intermediate recombines with the leaving group and returns to the substrate much more often than it adds Et_2NH to form the substitution product.

For our present purposes it is important to distinguish between internal return, where the phosphene recombines specifically with the sulfonate to which it was bonded in the substrate, and external return, where it combines with any sulfonate ion present in the reaction medium. We therefore examined the nosylate analogue of the tosylate **3**. As expected it proved to be much more reactive even though the phosphene intermediate it forms is of course the same. In $CHCl_3$ containing Et_2NH (15 equiv.) and $Et_2NH_2^{+-}OTs$ (1 equiv.) it rapidly formed the amide **1** (X = NEt_2) and the tosylate **3** (which reacts much less quickly) in a 9 : 1 ratio, implying only a small preference (1.6-fold) for reaction with TsO^- rather than Et_2NH. In the experiment with the labelled tosylate the isotope scrambling was well advanced by the time substitution was 10% complete. At that stage the concentration of $Et_2NH_2^{+-}OTs$ (0.1 equiv.) will still have been less than one-hundredth that of the amine (13 equiv.), so any external return will have been negligible. It must therefore be internal return that is responsible for the scrambling and internal return that occurs five times faster than reaction of the phosphene with the amine. This is important. At very low concentrations of amine the amide product might be derived largely from liberated phosphene, but at all other concentrations it will be derived almost entirely from phosphene that is generated within a preassociation complex where the nucleophile is already in place to trap it.

There is evidence that the diphenylmethylphosphonamidic chloride **4** (X = Cl) also reacts with amines by elimination–addition[9] so our study was extended to include the phosphonamidic-sulfonic anhydride **4** (X = OTs). Both the unlabelled compound [mp 160–



4 **5**

162 °C; δ_P 28.3; *m/z* (FAB) 430 $(M + H)^+$] and its ^{18}O-labelled counterpart (ES MS: 106 atom% ^{18}O; 15.5% no ^{18}O, 63% one ^{18}O, 21.5% two ^{18}O) were prepared in the same way as the fluorenyl compounds but with longer reaction times. The reaction of labelled **4** (X = OTs) with 0.4 mol dm^{-3} Et_2NH in $CHCl_3$ (containing 6% C_6D_6) was very slow ($t_{1/2} \approx 80$ h at 27 °C) and was not accompanied by extensive scrambling of the label although some P–^{18}O substrate ($\Delta\delta_P$ 0.03 ppm) could just be detected in the later stages (Fig. 2). This is not a very sensitive test for return, however, because most of the substrate is consumed in ways that do not involve the phosphene. Thus the phosphonic diamide **4** (X = NEt_2) (δ_P 31.1) accounts for only 10% of the product, the dominant products being the phosphonamidic acid **4** (X = OH) (salt with Et_2NH) (δ_P 18.2), formed alongside Et_2NTs as a result of nucleophilic attack at sulfur, and the pyrophosphonate **5** (δ_P 22.7 and 21.8,

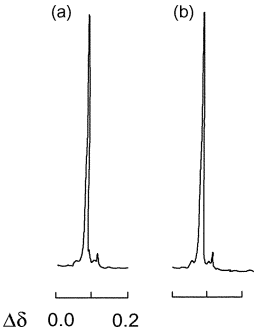


Fig. 2 ^{31}P NMR spectrum (162 MHz) of ^{18}O-labelled substrate **4** (X = OTs): (a) with Et_2NH at 90% completion ($t = 16$ days), (b) with Et_2NH + DBU at 75% completion ($t \approx 18$ h).

diastereoisomers), resulting from attack [most likely $S_N2(P)$] of the acid (salt) at the P atom of the substrate.

To encourage elimination–addition some of the relatively strong base DBU was included in the reaction mixture. The amount of DBU was only one-fifteenth the amount of Et_2NH but its effect was quite dramatic: the rate increased 10-fold ($t_{1/2} \approx 8$ h) and the yield of the phosphonic diamide **4** (X = NEt_2) was increased to 80%.¶ Now with phosphene formation undoubtedly the dominant process, scrambling could be detected at an earlier stage of reaction but was still not ever extensive (Fig. 2).

The contrasting behaviour of the fluorenyl and diphenylmethyl systems as regards ^{18}O scrambling and internal return is not, we think, due to differences in the stabilities of the phosphene intermediates or the rates at which they diffuse away from the sulfonate leaving group. Rather is it due to the differing stabilities of the carbanions (conjugate bases of the substrates) formed when the phosphene intermediates recombine with the leaving group in the first stage of return. In both cases the charge will be delocalised but in the fluorenyl case the carbanion also benefits from aromaticity. It is therefore reasonable that internal return should be important (faster than diffusion) in the fluorenyl case but unimportant (slower than diffusion) in the diphenylmethyl case. Preassociation between the substrate (or its conjugate base) and the nucleophile removes the need for diffusion and allows product formation to compete directly with internal return. It will therefore be important where internal return is important (fluorenyl substrates) but unimportant where it is not (diphenylmethyl substrates).

Most substitution reactions that proceed *via* metaphosphate-like three-coordinate P^v intermediates involve substrates with OH, SH or NH ligands on the P atom. These will usually be at least as acidic as the α-CH of a fluorenyl ligand, and the conjugate base at least as stable. It is therefore reasonable to expect preassociation to be generally important in these reactions.

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Notes and references

† It is not generally possible to prepare a phosphonic-sulfonic mixed anhydride by sulfonylation of the phosphonate anion because the anion immediately attacks the product, displacing sulfonate and forming the pyrophosphonate. In the case of the anhydride **3**, however, such attack will be retarded by the bulky ligands on the P atom.

‡ Labelled TsCl was prepared by hydrolysis of the unlabelled compound using [^{18}O]water (1.1 equiv.) in pyridine (2.5 equiv.) (15 min at 60 °C) followed by conversion of the resulting pyridinium salt into the more tractable *tert*-butylammonium salt and treatment of this with oxalyl chloride (DMF catalyst). One third of the ^{18}O is lost (as $C^{18}O_2$) in the reaction with oxalyl chloride so the product was taken through the labelling sequence again to increase the ^{18}O content.

§ The yield of the acid **1** (X = OH) was somewhat greater but it is not only the by-product of Et_2NTs formation but also the product of reaction of the substrate with traces of moisture.

¶ The remaining 20% was made up equally of the phosphonamidic acid (salt) and two DBU-derived products, δ_P 34.5 and 28.4, the latter seemingly being formed from the former.

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