Potential mechanism-based tyrosine kinase inhibitors. Part 1. Phosphorylation chemistry of pyridine *N*-oxides

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Phosphorylated derivatives of 4-picoline *N*-oxide have been observed on treatment with both phosphorylating and phosphitylating agents. These intermediates were trapped by external nucleophiles. Propane-1-thiol reacted preferentially at carbon to yield a propylsulfanylpyridine whereas propylamine reacted preferentially at phosphorus. This chemistry carries implications for the design of mechanism-based tyrosine kinase inhibitors.

Phosphorylation of tyrosine residues of specific proteins is a key step in cell proliferation.[1] Inhibitors of tyrosine kinases are of interest both for the study of these enzymes and for therapeutic purposes. A range of reversible inhibitors of these enzymes have been described[2] as well as reactive active-site labels[3] but, to our knowledge, no mechanism-based irreversible inhibitors[4] have been reported. Such compounds might provide therapeutic advantages over those already described. Appropriately substituted pyridine *N*-oxides are analogues of tyrosine and their phosphorylation chemistry might provide the basis of mechanism-based tyrosine kinase inhibitors (see Fig. 1). Were such compounds phosphorylated by tyrosine kinases the resulting phosphorylated intermediate **1** would be electrophilic and may react with nucleophilic active-site residues of the enzyme leading to inhibition. We elected to study the phosphorylation chemistry of a model *N*-oxide, 4-picoline *N*-oxide **4**, and to evaluate the possibility of interception of *O*-phosphoryl *N*-oxides with external nucleophiles.

It is well documented that phosphorylating agents bearing nucleophilic leaving groups can be used to generate 2-substituted pyridines.[5] For example 2-chloropyridines can be formed by treatment of the parent pyridine *N*-oxide with $POCl_3$; 2-cyanopyridines can likewise be prepared from phosphoryl cyanide derivatives.[6] These reactions are believed to proceed *via* *O*-phosphorylated intermediates[7] by analogy with other Reissert–Henze type reactions.[8] However, in contrast with related reactions,[9] there appear to be no reports of the detection of such intermediates and only limited reports of reactions of this type utilising added nucleophiles.[10] We here report the detection of such *O*-phosphoryl intermediates and their reaction with biologically relevant nucleophiles: amines and thiols.[11]

Results and discussion

As a prelude to studies on intermediates in the phosphorylation chemistry of *N*-oxides we carried out phosphorylation reactions on *p*-cresol to provide comparative spectroscopic data. We employed both phosphorylating agents (reaction with diphenyl chlorophosphate to form **3c**) and phosphitylating agents {tetrazole-mediated reaction with bis(benzyloxy)(diisopropylamino)phosphane $(PhCH_2O)_2P[N(CHMe_2)_2]$ and reaction with chloro(diisopropylamino)methoxyphosphane, $MeOP[N(CHMe_2)_2]Cl$ were both evaluated}. In the case of phosphitylation reactions we determined the spectroscopic properties of the intermediate phosphorus(III) species **2a**, **b** (δ_P 161 and 148, respectively; phosphorus NMR shift values are quoted relative to 85% phosphoric acid) *in situ* prior to oxidation of the crude phosphite products to the corresponding phosphates **3a**, **b** (δ_P −5.1 and +5.7, respectively). The NMR spectra produced in

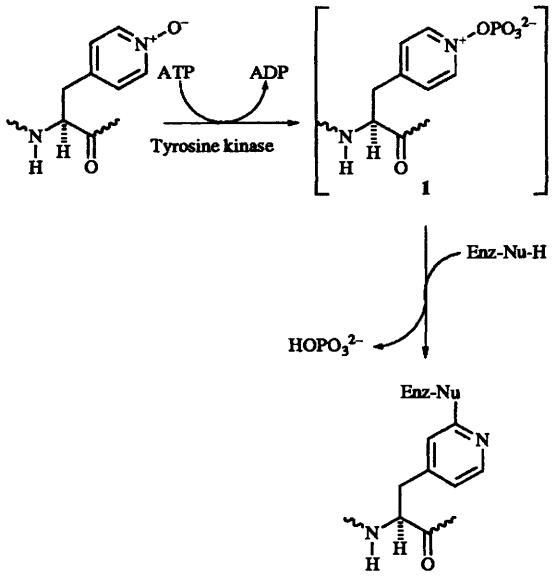
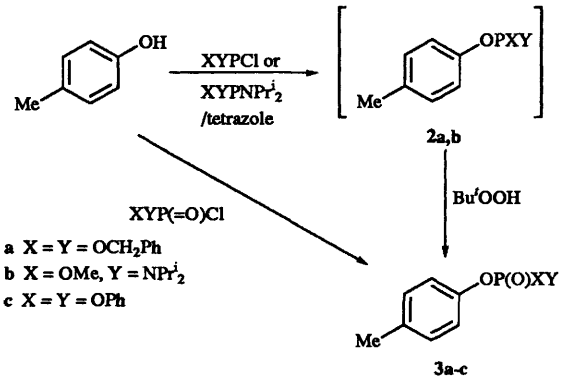


Fig. 1



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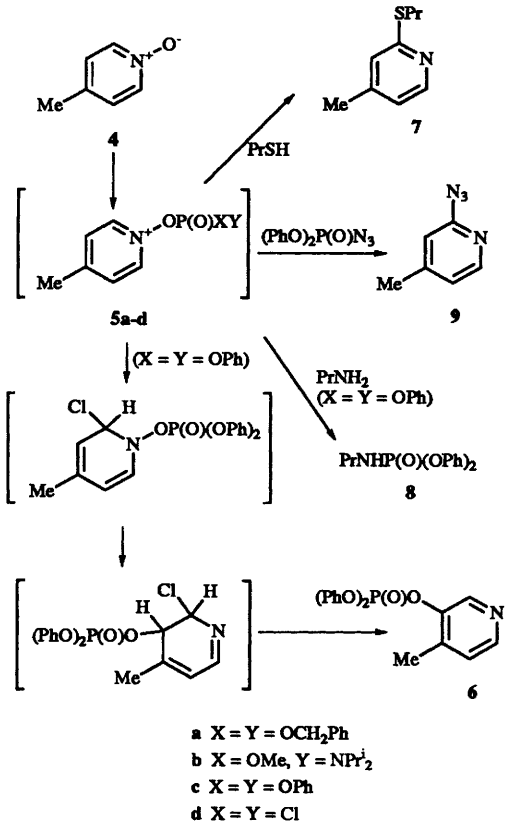

these studies provided the basis for the study of the transient intermediates formed in the equivalent reactions of 4-picoline *N*-oxide.

The reaction of 4-picoline *N*-oxide **4** with diphenyl chlorophosphate in dry $CDCl_3$ was followed by ^1H and ^{31}P NMR spectroscopy. Over the course of 12 h at room temperature the characteristic resonances of the starting materials were replaced by new resonances consistent with the accumulation of **5c**; in particular a new phosphorus(v) species (δ_P −11.1) accumulated as judged by ^{31}P NMR spectroscopy. That this species retained the symmetry properties of the starting 4-picoline *N*-oxide was shown by ^1H NMR (two 2 H doublets due to the pyridine ring protons). Diphenyl 4-methyl-3-pyridyl phosphate **6** accumulated in the reaction mixture at a slower rate. On standing **5c** isomerised to **6** which was isolated. The assignment of regiochemistry of this derivative rests on NOE experiments in particular irradiation of the resonance due to the methyl group gives rise to an enhancement of one aromatic resonance; no enhancement of the resonance due to the proton adjacent to the phosphoryl group was observed. The formation of a 3-*O*-phosphoryl pyridine parallels the formation of analogous 3-substituted products in reactions of pyridine *N*-oxides with arylsulfonyl chlorides.[12] The latter may arise *via* rearrangement of an intermediate formed by chloride ion attack on the *O*-phosphoryl *N*-oxide as illustrated. Reaction of **4** with $POCl_3$ at low temperature led to a similar *O*-phosphoryl *N*-oxide intermediate, **5d** (δ_P −3.9), although it did not accumulate to the same extent. With diphenylphosphoryl azide as the phosphorylating agent the phosphorylated *N*-oxide was not detected, instead only accumulation of 2-azido-4-methylpyridine **9**, was observed.

We investigated the possibility that phosphitylating agents might provide a route to *O*-phosphoryl *N*-oxides. When MeOP[$N(CHMe_2)_2$]Cl was mixed with **4** there was an immediate reaction to generate, in a 1:1 ratio, 4-picoline and a phosphorus(v) species (δ_P 13.1) assigned as *O*-phosphoryl *N*-oxide derivative **5b**. In this process **4** is acting as both nucleophile and oxidant. A similar process, resulting in **5a** (δ_P −12.2), was observed upon tetrazole-mediated reaction of **4** with bis(benzyloxy)(diisopropylamino)phosphane.

We next turned to an examination of the reactions of *O*-phosphoryl *N*-oxides with added nucleophiles. Phosphorylated intermediates **5b** and **5c**, generated *in situ*, were trapped at room temperature by one equivalent of propane-1-thiol to generate 4-methyl-2-propylsulfanylpyridine **7** which accumulated *in situ* (as judged by ^1H NMR spectroscopy) and was isolated in up to 22% overall yield. Since both 2- and 3-substituted pyridines can result from Reissert–Henze type reactions[13] we took the precaution of confirming the regiochemistry of this product by an NOE experiment. When **5c** was the reacting *O*-phosphoryl species the product of thiol trapping, **7**, was accompanied by a similar quantity of the rearranged 3-*O*-phosphorylpyridine **6**. The latter compound was not an intermediate in the formation of **7** since it did not react with propane-1-thiol under the reaction conditions.

In contrast, the reaction of **5c** with one equivalent of propylamine under similar conditions, led to $PrNHP(O)(OPh)_2$, the product of nucleophilic substitution at phosphorus rather than attack at the pyridine ring. As with thiol trapping experiments, the rearranged phosphorylated intermediate **6** was also isolated.

These results indicate that *O*-phosphoryl derivatives of pyridine *N*-oxides do accumulate and can be trapped by external nucleophiles in competition with rearrangement chemistry. The exact nature of the trapping process is dependent upon the nucleophile. In a succeeding paper[14] we report the application of these results to the design and testing of potential inhibitors of epidermal growth factor receptor tyrosine kinase.

Experimental

Melting points were determined on a Gallenkamp hot stage apparatus, and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1750 Fourier transform instrument. ^1H NMR spectra were recorded on a Gemini 200 (at 200 MHz) or on a Brüker AM500 (at 500 MHz). ^{13}C NMR spectra were recorded on a Gemini 200 (at 50 MHz) or on a Brüker AM500 (at 125 MHz). ^{31}P NMR spectra were recorded on a Brüker AM250 at 101 MHz and referenced to 85% phosphoric acid. *J* values are given in Hz. Mass spectra were recorded on a VG Micromass ZAB IF (FAB^+, DCI^+) and VG Trio 1 (GCMS-CI^+, EI^+) by Drs R. Aplin and R. Procter. High resolution mass spectra were recorded on a VG Autospec. All solvents were purified by conventional methods and moisture sensitive reactions were carried out under an anhydrous inert atmosphere.

Reaction of *p*-cresol with bis(benzyloxy)(diisopropylamino)-phosphane and subsequent oxidation

Bis(benzyloxy)(diisopropylamino)phosphane (0.11 cm^3, 0.31 mmol) was added to a solution of dry *p*-cresol (33 mg, 0.31 mmol) in dry deuteriated chloroform (2 cm^3) containing tetrazole (64 mg, 0.92 mmol) and 3 drops of dry dimethylformamide (DMF) at 0 °C. The resulting solution showed spectroscopic features consistent with the presence of *dibenzyl p-tolyl phosphite* **2a**: $\delta_H(CDCl_3$; 200 MHz) 2.29 (3 H, s, CH_3), 4.99 (4 H, d, *J* 8, CH_2), 6.93 (2 H, d, *J* 8, ArH), 7.07 (2 H, d, *J* 8, ArH) and 7.30–7.40 (10 H, m, ArH); $\delta_P(CDCl_3)$ 161.0; *m/z* [CI^+ (NH_3)] 353 (MH^+, 20%).

After stirring for 3 h at room temperature, anhydrous *tert*-butyl hydroperoxide (305 mm^3 of a 3 mol dm^{-3} solution in hexanes, 0.92 mmol) was added to the above solution and stirring continued for 14 h. Purification by flash column chromatography (dichloromethane, R_f product 0.4) afforded *dibenzyl p-tolyl phosphate* **3a** as a colourless oil (28 mg, 38%); $\delta_H(CDCl_3$; 500 MHz) 2.32 (3 H, s, CH_3), 5.12 (4 H, d, *J* 8, CH_2),

7.05 (2 H, dd, *J* 2 and 8, ArH), 7.09 (2 H, dd, *J* 2 and 8, ArH) and 7.30–7.40 (10 H, m, ArH); $\delta_C(CDCl_3$; 125 MHz) 20.7, 69.8 (d, J_P 5), 119.8 (d, J_P 4), 128.0, 128.6 (2 C), 130.1, 134.7, 135.6 (d, J_P 7) and 148.4; $\delta_P(CDCl_3$; 110 MHz) −5.10; $\nu_{max}(CHCl_3)$/cm^{-1} 1278; *m*/*z* [CI$^+$(NH$_3$)] 369 (MH$^+$, 100%) (Found: M, 369.1258. Calc. for $C_{21}H_{22}O_4P$: *M*, 369.1256).

Reaction of *p*-cresol with chloro(diisopropylamino)methoxyphosphane and subsequent oxidation

Diisopropylethylamine (49 mm^3, 0.28 mmol) and then chloro(diisopropylamino)methoxyphosphane (55 mm^3, 0.28 mmol) were added to a solution of dry *p*-cresol (31 mg, 0.28 mmol) in dry dichloromethane (1.5 cm^3) or deuteriated chloroform at 0 °C. The resulting solution exhibited spectroscopic features consistent with the presence of (diisopropylamino)methoxy(*p*-tolyloxy)phosphane **2b**: $\delta_H(CDCl_3$; 500 MHz) 1.12 (6 H, d, *J* 7, $NCCH_3$), 1.16 (6 H, d, *J* 7, $NCCH_3$), 2.23 (3 H, s, $ArCH_3$), 3.43 (3 H, d, *J* 13, OCH_3), 3.65 (2 H, m, NCH), 6.88 (2 H, dd, *J* 2 and 8, ArH) and 6.99 (2 H, dd, *J* 2 and 8, ArH); $\delta_P(CDCl_3)$ 148.0; *m*/*z* [CI$^+$(NH$_3$)] 270 (MH$^+$, 40%).

The above solution was stirred at room temperature for 45 min and then the mixture was cooled to 0 °C and a solution of anhydrous *tert*-butyl hydroperoxide (93 mm^3 of a 3 mol dm^{-3} solution in hexanes, 0.28 mmol) was added at 0 °C and stirring continued for 1 h. Purification by flash column chromatography (dichloromethane–ethyl acetate, 4:1, R_f product 0.5) afforded *methyl* p-*tolyl* N,N-*diisopropylamidophosphate* **3b** (38 mg, 47%) as an oil; $\delta_H(CDCl_3$; 500 MHz) 1.18 (6 H, d, *J* 7, $NCCH_3$), 1.26 (6 H, d, *J* 7, $NCCH_3$), 2.30 (3 H, s, CH_3), 3.50 (2 H, dq, *J* 7 and 14, NCH), 3.73 (3 H, d, *J* 11, OCH_3), 7.09 (2 H, d, *J* 8, ArH) and 7.14 (2 H, d, *J* 8, ArH); $\delta_C(CDCl_3$; 125 MHz) 20.7, 22.3, 22.6, 46.2, 52.8 (d, J_P 6), 119.8 129.8, 133.6 and 149.2; $\delta_P(CDCl_3)$ 5.7; $\nu_{max}(CHCl_3)$/cm^{-1} 1260; *m*/*z* [CI$^+$(NH$_3$)] 286 (MH$^+$, 100%) (Found: M, 286.1572. Calc. for $C_{14}H_{25}NO_3P$: *M*, 286.1572).

Diphenyl *p*-tolyl phosphate 3c

Diphenyl chlorophosphate (2.40 cm^3, 11.6 mmol) was added to a solution of dry *p*-cresol (1.250 g, 11.6 mmol) in dry dichloromethane (14 cm^3) containing triethylamine (1.61 cm^3, 11.6 mmol) at 0 °C. After stirring at room temperature for 3 h, the white precipitate was filtered off and the solution left at 5 °C overnight. The crystals that precipitated were filtered and the liquors reduced in volume on a rotary evaporator. Purification by flash column chromatography (dichloromethane, R_f product 0.3) afforded a colourless oil (1.200 g, 31%) which solidified on cooling to give the *title compound* mp 18 °C (lit.,[15] 18–20 °C); $\delta_H(CDCl_3$; 200 MHz) 2.33 (3 H, s, CH_3), 7.12 (2 H, d, *J* 9, ArH), 7.14 (2 H, d, *J* 9, ArH) and 7.20–7.37 (10 H, m, ArH); $\delta_C(CDCl_3$; 125 MHz) 20.7, 119.8 (d, J_P 5), 120.1 (d, J_P 4), 125.5, 129.8, 130.2, 135.2, 148.3 (d, J_P 8) and 150.5 (d, J_P 7); $\delta_P(CDCl_3)$ −16.7; $\nu_{max}(CHCl_3)$/cm^{-1} 1295; *m*/*z*[CI$^+$(NH$_3$)] 341 (MH$^+$, 100%) (Found: M, 341.0942. Calc. for $C_{19}H_{18}O_4P$: *M*, 341.0942).

Reaction of 4-picoline *N*-oxide (4-methylpyridine *N*-oxide) with diphenyl chlorophosphate

Diphenyl chlorophosphate (488 mm^3, 2.35 mmol) was added dropwise to a stirred solution of dry 4-picoline *N*-oxide (257 mg, 2.35 mmol) in dry deuteriated chloroform (1.5 cm^3) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirring continued for 20 h. The tan solution showed spectroscopic properties consistent with the accumulation of diphenyl 4-methylpyridinium phosphate **5c**: $\delta_H(CDCl_3$; 200 MHz) 2.58 (3 H, s, CH_3), 7.14–7.42 (10 H, m, 2 × C_6H_5), 7.63 (2 H, d, *J* 7, PyH) and 8.76 (2 H, d, *J* 7, PyH); $\delta_P(CDCl_3)$ −11.1.

Reaction of 4-picoline *N*-oxide with phosphorus oxychloride

Phosphorus oxychloride (17 mm^3, 0.18 mmol) was added dropwise to a solution of dry 4-picoline *N*-oxide (20 mg, 0.18 mmol) in dry deuteriated chloroform (0.5 cm^3) at −78 °C. The solution showed spectroscopic properties consistent with the accumulation of 4-methylpyridinium dichlorophosphate **5d**: $\delta_H(CDCl_3$; 200 MHz) 2.67 (3 H, s, CH_3), 7.93 (2 H, d, *J* 7, PyH) and 9.81 (2 H, d, *J* 7, PyH); $\delta_P(CDCl_3)$ −3.9.

Tetrazole-mediated reaction of bis(benzyloxy)(diisopropylamino)phosphane with 4-picoline *N*-oxide

A solution containing bis(benzyloxy)(diisopropylamino)phosphane (128 mm^3, 0.38 mmol), tetrazole (79 mg, 1.14 mmol) and 2 drops of dry DMF in dry deuteriated chloroform (2 cm^3) was added to a solution of dry 4-picoline *N*-oxide (41 mg, 0.38 mmol) in dry deuteriated chloroform (1 cm^3). The resulting solution showed spectroscopic properties consistent with the presence of a 1:1 mixture of 4-picoline (confirmed by doping) and dibenzyl 4-methylpyridinium phosphate **5a**: $\delta_H(CDCl_3$; 500 MHz) 2.52 (3 H, s, CH_3), 4.96 (4 H, d, *J* 9, CH_2), 7.20–7.40 (10 H, m, 2 × C_6H_5), 7.68 (2 H, d, *J* 6, PyH) and 8.87 (2 H, d, *J* 6, PyH); $\delta_P(CDCl_3)$ −12.2.

Reaction of 4-picoline *N*-oxide with chloro(diisopropylamino)-methoxyphosphane

Chloro(diisopropylamino)methoxyphosphane (54 mm^3, 0.278 mmol) was added to a solution of dry 4-picoline *N*-oxide (30 mg, 0.278 mmol) in dry deuteriated chloroform (1 cm^3). The spectroscopic properties of the solution were consistent with the presence of a 1:1 mixture of 4-picoline (confirmed by doping) and methyl 4-methylpyridinium *N*,*N*-diisopropylamidophosphate **5b**: $\delta_H(CDCl_3$; 200 MHz, partial) 2.74 (3 H, s, $ArCH_3$), 8.28 (2 H, d, *J* 7, ArH) and 9.30 (2 H, d, *J* 7, PyH); $\delta_P(CDCl_3)$ 13.1.

4-Methyl-2-propylsulfanylpyridine 7

Method 1. Diphenyl chlorophosphate (506 mm^3, 2.44 mmol) was added dropwise to a stirred solution of dry 4-picoline *N*-oxide (266 mg, 2.44 mmol) in dry deuteriated chloroform (1.5 cm^3) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirring continued for 20 h. Propane-1-thiol (221 mm^3, 2.44 mmol) was added to the tan coloured solution and stirring continued at room temperature. After 10 days of stirring, the solution was taken up in dichloromethane (10 cm^3) and washed with saturated aqueous sodium carbonate (10 cm^3). The organic extract was reduced in volume and the *title compound* was isolated after flash column chromatography (dichloromethane–ethyl acetate, 3:2, R_f product 0.7) as an oil (20 mg, 5%); $\delta_H(CDCl_3$; 500 MHz) 1.05 (3 H, t, *J* 7, CH_3), 1.74 (2 H, m, CH_2Me), 2.28 [3 H, s, $ArCH_3$; irradiation gave NOE enhancements of the resonances at δ 6.78 (*ca.* 4%) and 7.01 (*ca.* 6%)], 3.14 (2 H, t, *J* 7, SCH_2), 6.78 (1 H, d, *J* 5, ArH), 7.01 [1 H, s, Py-3-H; irradiation gave NOE enhancements of the resonances at δ 1.05 (*ca.* 5%), 1.74 (*ca.* 3.5%), 2.28 (*ca.* 5.5%) and 3.14 (*ca.* 5%)] and 8.28 (1 H, d, *J* 5, ArH); *m*/*z* [CI$^+$(NH$_3$)] 168 (MH$^+$, 100%) (Found: M, 168.0847. Calc. for $C_9H_{14}NS$: *M*, 168.0847).

Diphenyl (4-*methyl*-3-*pyridyl*) *phosphate* **6** was also isolated by chromatography (R_f 0.4) as a colourless oil (80 mg, 10%); $\delta_H(CDCl_3$; 500 MHz) 2.24 [3 H, s, $ArCH_3$; irradiation gave an NOE enhancement of the resonance at δ 7.16 (*ca.* 7%)], 7.16 [1 H, d, *J* 5, PyH, irradiation gave an NOE enhancement of the resonances δ 2.24 (*ca.* 5%) and 8.34 (*ca.* 3.5%)], 7.24–7.43 (10 H, m, ArH), 8.34 (1 H, d, *J* 5, PyH) and 8.57 (1 H, s, PyH); δ_C 15.8, 120.1, 125.8, 126.2, 129.9 (2 C), 138.8, 141.7, 146.7 and 150.3; $\delta_P(CDCl_3)$ −16.37; $\nu_{max}(CHCl_3)$/cm^{-1} 1297; *m*/*z* [CI$^+$(NH$_3$)] 342 (MH$^+$, 100%) (Found: M, 342,0892. Calc. for $C_{18}H_{17}NO_4P$: *M*, 342.0895).

Method 2. Chloro(diisopropylamino)methoxyphosphane (221 mm^3, 1.14 mmol) was added to a solution of dry 4-picoline

N-oxide (124 mg, 1.14 mmol) in dry dichloromethane (4 cm^3) at 0 °C. After stirring for 5 h at room temperature, *tert*-butyl hydroperoxide was added (378 mm^3 of a 3 mol dm^{-3} solution in hexanes, 1.14 mmol) at 0 °C and stirring continued at room temperature for 30 min. Propane-1-thiol (103 mm^3, 1.14 mmol) was added to the tan coloured solution and stirring continued at room temperature. After 10 days of stirring, the solution was taken up in dichloromethane (10 cm^3) and washed with saturated aqueous sodium carbonate (10 cm^3). The organic extract was reduced in volume and purified by flash column chromatography (dichloromethane–ethyl acetate, 3:2, R_f product 0.7) to give 4-methyl-2-propylsulfanylpyridine as an oil (20 mg, 22%) having identical ^1H NMR and mass spectra to that obtained from method 1.

Diphenyl propylamidophosphate 8

Diphenyl chlorophosphate (846 mm^3, 408 mmol) was added dropwise to a stirred solution of dry 4-picoline *N*-oxide (445 mg, 4.08 mmol) in dry deuteriated chloroform (2.0 cm^3) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirring continued for 20 h. Propylamine (336 mm^3, 4.08 mmol) was added to the tan coloured solution and stirring continued at room temperature. After 10 days of stirring, the solution was taken up in dichloromethane (10 cm^3) and washed with saturated aqueous sodium carbonate (10 cm^3). The organic extract was reduced in volume and purified by flash column chromatography (dichloromethane–ethyl acetate, 1:1, R_f product 0.5). The title compound was crystallised from dichloromethane (232 mg, 28%), mp 52–53 °C (lit.,[16] 56–57 °C); $\delta_H(CDCl_3; 200\ MHz)$ 0.85 (3 H, t, *J* 7, CH_3), 1.42 (2 H, m, CH_2) 2.97–3.06 (2 H, m, NCH_2) and 7.10–7.38 (10 H, m, ArH); *m*/*z* [$CI^+(NH_3)$] 242 (MH^+, 100%).

Also isolated from the column was a compound identified as diphenyl 4-methyl-3-pyridyl phosphate **6**, a colourless oil (R_f 0.3, 77 mg, 6%), identical by ^1H, ^{31}P NMR and mass spectra to the previous sample.

2-Azido-4-methylpyridine 9

To a stirred solution of dry 4-picoline *N*-oxide (127 mg, 1.17 mmol) in dry deuteriated chloroform (1 cm^3) at 0 °C was added diphenylphosphoryl azide (251 mm^3, 1.17 mmol). The reaction mixture was allowed to warm to room temperature and then heated at reflux for 3 days.

The tan coloured solution was taken up in dichloromethane (10 cm^3) and washed with saturated aqueous sodium carbonate (10 cm^3) The organic extract was concentrated under reduced pressure. Flash column chromatography (dichloromethane–ethyl acetate, 1:1, R_f product 0.4) afforded the *title compound* (35 mg, 22%); $\delta_H(CDCl_3; 200\ MHz)$ 2.53 [3 H, s, CH_3; irradiation gave NOE enhancement of the resonances at δ 7.04 (*ca.* 7.5%) and 7.73 (*ca.* 8.5%)], 7.04 (1 H, dd *J* 1 and 7, PyH), 7.73 [1 H, s, PyH; irradiation gave NOE enhancement of the resonance at δ 2.53 (*ca.* 5%)] and 8.68 (1 H, d, *J* 7, PyH); $\delta_C(CDCl_3; 125\ MHz)$ 21.6, 113.7, 119.3, 124.2, 143.8 and 148.8; *m*/*z* [$CI^+(NH_3)$] 135 (MH^+, 40%) (Found: M, 135.0671. Calc. for $C_6H_7N_4$: *M*, 135.0671).

Acknowledgements

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