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ARTICLE TYPE

Aqueous Synthesis of *N*,*S*-Dialkylthiophosphoramidates: Design, Optimisation and Application to Library Construction and Antileishmanial Testing

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We recently reported the use of $PSCl_3$ for the thiophosphorylation of alkylamines where the resulting *N*-thiophosphoramidate ions could be readily *S*-alkylated (*Chem. Commun.*, 2011, 47, 6156-6158.). Herein we report the development of this methodology using amino acid, amino sugar, aminonucleoside and aniline substrates. The hydrolysis properties of *N*-thiophosphoramidate ions and their reactivities towards alkylating agents are also explored. In addition, we demonstrate the application of our approach to the preparation of a small library of compounds, including quinoline-based *N*,*S*-dialkylthiophosphoramidates which were tested for antileishmanial activity.

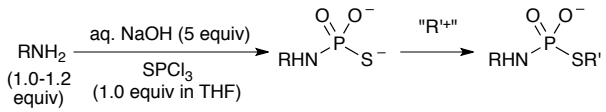
Introduction

Phosphate esters are key intermediates in the transmission of genetic material and many other critical cellular processes. Structural analogues of these systems have been used widely as tools for determining enzyme mechanisms and as inhibitors or activators of these enzymes. Sulfur-based analogues have been used in place of phosphodiesters to both increase and decrease the rate of ester bond cleavage. Accelerated cleavage rates are offered by *S*-bridging systems,[1] where the thiolate leaving group departs more readily than its alkoxide analogue, whereas reduced cleavage rates are seen for non-*S*-bridging systems.[2] Nitrogen-bridging phosphodiester mimics, where *N*-protonation becomes possible, have also been generated and studied.[3-10] A combination of *N*- and *S*-bridging systems have seen application in the form of phosphate triester mimics that show antiviral activity. These uncharged, nucleoside-based thiophosphoramidates serve as prodrogs which traverse cell membranes, however, within the cell, programmed hydrolysis occurs to reveal nucleoside monophosphates that go on to interefere with viral replication.

Recently, we reported a simple aqueous method for the preparation of *N*, *S*-thiophosphoramidates.[11] These phosphodiester mimics, with their *N*- and *S*-bridges, were assembled through the electrophilic action of the reactive phosphorylating agent $PSCl_3$ on nucleophilic primary alkylamines followed by *S*-alkylation of the resulting *N*-thiophosphoramidate ions—an approach that builds on our established use of reactive P species in aqueous systems.[12-14] Here we describe the development of our strategy for aminothiophosphorylation, including the use of alkyl, aryl, amino acid, amino-sugar and aminonucleoside substrates. We describe kinetic studies on the pH-dependent hydrolysis properties of *N*-thiophosphoramidate ions which were used to inform subsequent optimisation of *S*-alkylation steps. Studies on several *S*-reactive electrophile species and alkylation conditions are also reported, along with our efforts towards using bromoacetamides as generic amine-derived alkylating agents. Finally, our thiophosphorylation-alkylation conditions were applied to a library of lipophilic amines which were then alkylated with a quinoline derivative before being screened for activity against *Leishmania mexicana*, a causative agent of the Neglected Tropical Disease leishmaniasis.[15]

Combined thiophosphorylation-*S*-alkylation of alkyl-, aryl- and biomolecule-derived amines.

We demonstrated that simple alkylamines are effective substrates for $PSCl_3$ under aqueous conditions in the presence of NaOH. The resulting *N*-thiophosphoramidate ions can then be alkylated effectively using a range of soft alkylating agents (Scheme 1).



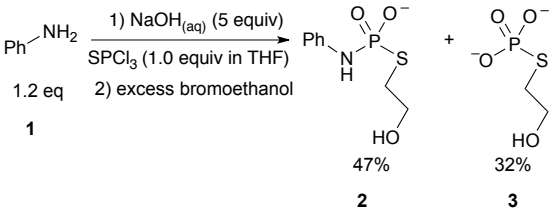
Scheme 1 Aqueous *N*-thiophosphorylation and *S*-alkylation.

With the aim of broadening the scope of amine substrate used in this method we explored the thiophosphorylation and *S*-alkylation of aniline, unprotected phenylalanine, glucosamine and two 5'-amino-5'-deoxynucleosides.

Aniline

We employed a 1.2:1 ratio of aniline **1** to $PSCl_3$ followed by alkylation of the expected *N*-thiophosphoramidate ion using bromoethanol (Scheme 2). Thiophosphorylation proceeded to a

reasonable extent of 62% as determined by ^{31}P NMR spectroscopy, with the key signal for thiophosphorylated amines usually appearing in the shift range 40-45 ppm. After addition of bromoethanol, a combination of ^{1}H and ^{31}P NMR methods revealed ~47% conversion to the *N*, *S*-thiophosphoramidate **2** (δ ~ 20-25 ppm), and 32% of *S*-alkylated thiophosphate ion **3** (δ ~ 15-20 ppm). The remainder of the product mixture was predominantly aniline **1** (19%). The use of higher concentrations of aniline may have improved conversion to the *N*-thiophosphoramidate, and our studies with morpholine have shown that despite the increased potential for *bis*- and *tris*-aminolysis of $PSCl_3$, this is likely possible. However, on the basis of this preliminary result, we did not pursue this optimisation.



Scheme 2 *N*-Thiophosphorylation and *S*-alkylation of aniline.

Phenylalanine

Post-translational phosphorylation of proteins represents a major signalling pathway, and access to phosphoproteins and their analogues supports the delineation of these key processes. In addition, phosphorylation of the carboxyl group of amino acids serves to activate the carbonyl group for substitution during coded protein biosynthesis. Furthermore, phosphonamide systems have been widely exploited as transition state mimics for the attack of water upon the cabonyl of amides (Figure 1). To date, a limited number of examples of aqueous amino acid phosphorylations have been reported. Metatriphosphate ion possesses an activated anhydride structure that has been shown to be an effective *N*-phosphorylating agent for amino acids. In addition to *N*-phosphorylation, in the context of an amino acid, the carboxylate group acts as an internal nucleophile, displacing pyrophosphate ion and forming a cyclic mixed anhydride-phosphoramidate species. Cyclic phosphate esters show enhanced electrophilicity over their acyclic counterparts, and in this guise, the cyclic mixed anhydride-phosphoramidates show electrophilicity towards water at the phosphoryl centre and amines at the carbonyl. Histidine side chains of proteins have also been successfully modified with thiophosphorylating agents ($PSCl_3$ and thiophosphoramidate ion) in order to prepare more hydrolytically stable analogues of phosphohistidyl proteins that are intermediates in a variety of signalling enzymes.[16-18]

With these ideas in mind, we hoped to apply $PSCl_3$ towards the primary amino function of phenylalanine and alkylate the resulting thiophosphoramidate ion to produce carboxamide hydrolysis transition state analogues (Figure 1).

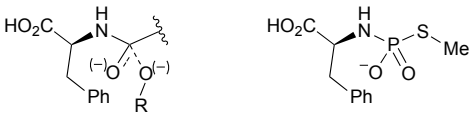
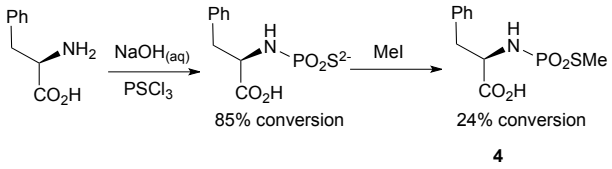


Figure 1 Structural resemblance of amide hydrolysis and amino acid-*N*-thiophosphoramidates.

Using 1.0 eq phenylalanine, 7.0 eq NaOH and 1.4 eq $PSCl_3$, 85% *N*-thiophosphoryaltion was observed by ^{31}P NMR spectroscopy (Scheme 3) after removal of inorganic thiophosphate through selective precipitation. The remainder of the P-containing impurites included *N*-phosphoramidate (~10%) and several unidentified species.

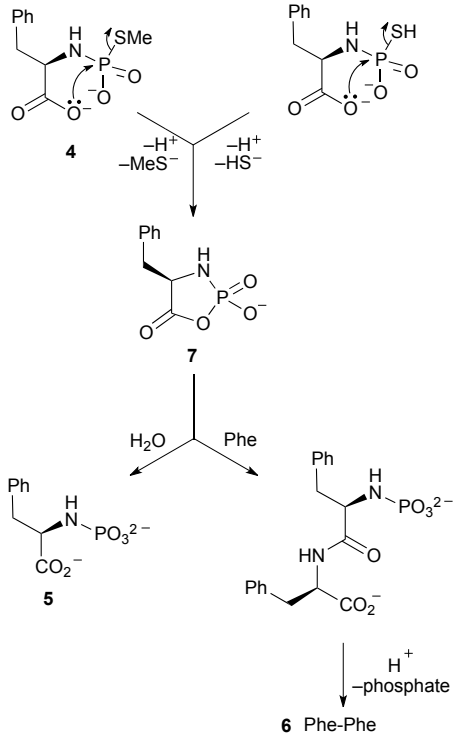
S-Alkylation was attempted in D_2O using methyl iodide, however, only 24% conversion to the *N,S*-dialkylthiophosphoramidate **4** was observed. The remainder of the P-containing materials included significant quantites of the *N*-phosphoramidate and phosphate ion.



Scheme 3 *N*-Thiophosphorylation–*S*-alkylation of phenylalanine.

Mass spectrometric analysis of the *N*-thiophosphorylation mixture also revealed significant quantities of *N*-phosphoramidate **5** plus Phe-Phe dimer **6**, however, the analysis was performed under acidic conditions, which were likely to encourage desulfurisation.

Taken together, these pieces of evidence strongly support the idea of intramolecular assistance of the carboxyl group in the decomposition of the *S*-alkylated thiophosphoramidate (or the protonated thiophosphoramidate ion), where the cyclic mixed anhydride intermediate **7** likely facilitates the formation of several of the decomposition products (Scheme 4).

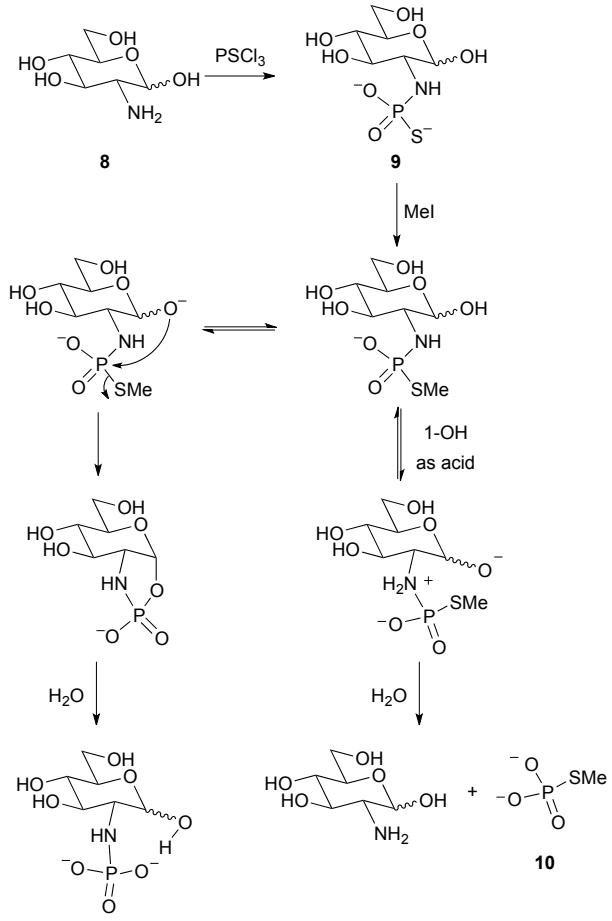


Scheme 4 Possible intramolecular reactions of phenylalanine-based thiophosphoramidate systems.

Glucosamine

Phospho-sugar systems play many biological roles and we hoped that we would be able to gain access to phospho-sugar mimics using our approach. Glucosamine was chosen as a readily avialable model substrate for our preliminary study. Others have investigated aqueous sugar phosphorylation procedures using metatriphosphate ion and its imino analogue, however, yields were low. The preparation of sugar phosphoramidates that go on to afford a phosphosugars has also been investigated, and good yields (79%) were reported.

A preliminary thiophosphorylation experiment using a 1:1 ratio of glucosamine **8** to $PSCl_3$ gave a rewarding ~90% conversion (estimated from a signal ~45.5 ppm in the ^{31}P NMR spectrum) to the *N*-thiophosphoramidate **9**. Over a time course of ~1 h, however, this signal dimished, with new signals appearing at similar chemical shifts. At present, we are not able to assign these, however, they are consistent with thiophosphoryl groups that have not been *S*-alkylated. Indeed, they may represent phosphorothioates that have arisen through intramolecular isomerisation. Despite this process, we proceeded with *S*-alkylation using MeI. This led to a majority of the P-containing product mixture being converted to *S*-methylated inorganic phosphate ion **10**. The large proportion of N-P bond scission suggests that once alkylated, intramolecular reaction facilitates this cleavage, unlike simple *N*,*S*-dialkyl thiophosphoramidates, which appear to be stable under the reaction conditions. A tentative decomposition mechanism for the glucosamine system is presented in Scheme 5. The key difference between this system and the Phe system is the potential for intramolecular acid catalysis via the 1-OH group, which, as an acetal, could act as an acid at the relatively high pHs used for thiophosphorylation (and alkylation). Smaller signals ~10-12 ppm are consistent with *N*-phosphoramidates that result from desulfurisation processes, where the two signals may signify the α- and β-anomers. On the basis of these preliminary results, we did not explore this system further, however, delivery of such a reactive phosphoryl donor to an enzyme active site may offer a useful tool for enzyme labelling or capture, and may also offer uses as a synthetic phosphorylation tool.



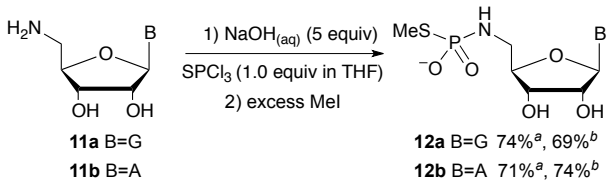
Scheme 5 *N*-Thiophosphorylation–alkylation of glucosamine and potential pathways for decomposition.

5'-Amino-5'-deoxyguanosine and 5'-Amino-5'-deoxyadenosine

Nucleoside phosphates are ubiquitous in biological systems, and a range of *N*-containing and *S*-containing phosphate mimics have been reported, with uses in mechanistic studies and antisense/siRNA applications. We have already reported the alkylation of *N*-thiophosphoramidate using a nucleoside-5'-iodide, and reasonable conversions were observed. Alkylation with the nucleoside-5'-iodide, however, proved to be very slow in comparison to other alkylating agents (see below). With this in mind, we sought to explore the *N*-thiophosphorylation of 5'-amino-5'-deoxynucleoside substrates and their subsequent alkylation.

We prepared adenosine and guanosine aminonucleosides **11a-b** using established procedures. The adenosine system **11b** was isolated as its hydrochloride salt, thus an additional equivalent of

NaOH was employed during thiophosphorylation. Alkylations were then performed using MeI, and, ion both cases, the *S*-alkylated aminonucleoside-*N*-thiophosphormaidates **12a-b** were formed at conversions levels ~70%. Given that unprotected nucleosides were employed, this level is impressive, however, chromatographic purification was necessary (see ESI) in order to confirm the identity of all the reaction products.



Scheme 6 *N*-Thiophosphorylation–alkylation on 5'-aminonucleosides.

The desired thiophosphoramidate products eluted ~35-40 min which correponded to ~100-140 mM TEAB. The areas under the absorbance–elution time profiles were also used to estimate conversion to the desired products, and the values were in agreement with the observations from ^{1}H and ^{31}P NMR spectroscopies.

S-Alkylation

In order to achieve effective alkylation, we explored the effects of pH, stoichiometry, reaction time and temperature on model substrates. In addition, we explored the kinetics of alkylation of a range of thiophosphoramidate ions and alkylating agents. The stability of the *S*-alkylated thiophosphoramidates was also explored. These results are summarised in the following sub-sections.

Stability of *N*-thiophosphoamidate ions

In line with *N*-phosphoramidates, we expected *N*-thiophosphoramidates to display greater stability at higher pHs. In order to explore this idea, we conducted ^{31}P NMR kinetic studies using ethanolamine-*N*-thiophosphoramidate **13** as substrate. The use of ^{31}P NMR spectroscopy allowed us to monitor the decomposition of the ethanolamine-*N*-thiophosphoramidate and to gain insight into the identities of the resulting hydrolysis products through the use of chemical shift and signal multiplicity data. The substrate was dissolved in 4 M or 0.5 M buffer, the water was removed by lyophilisation and the residue was redissolved in D_2O. This provided solutions where ~90% of the labile protons had been exchanged for deuterium to enable a deuterium lock signal to be used. The use of 4 M buffer solutions, ensured the pH changes observed during kinetic experiments were small, however, the presence of large concentrations of sodium ions caused problems with the measurement of pH. This manifested itself in the form of deviation from the expected gradient of –1 in the log k_{obs}-pH plot for pH>8. On this basis, some of the experminets at higher pHs were repeated using 0.5 M buffers. Under these conditions, greater changes in pH (0.2-0.5) were observed during the courses of the kinetic experiments, however, a gradient of –1 was observed for the log k_{obs}-pH data at higher pHs (Figure 2). Caution should also be taken in terms of the interpretation of measured pH values where the extent of deuteriation within the buffer is not clearly defined.

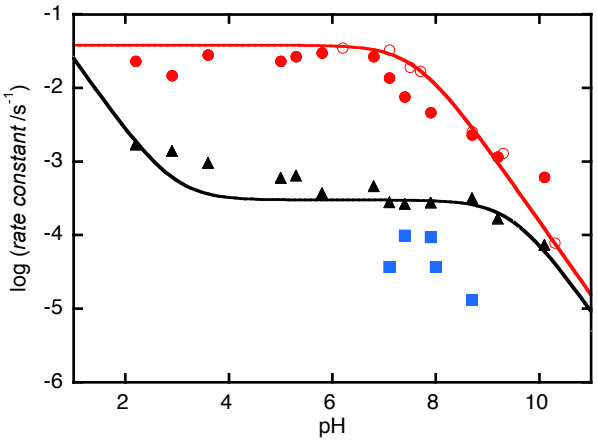
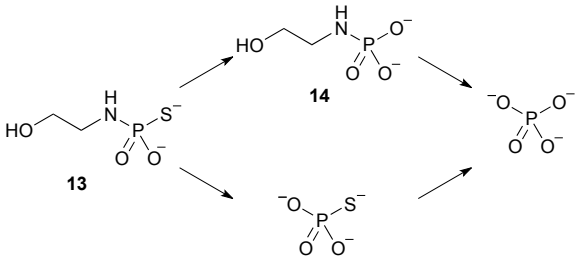


Figure 2 ^{31}P NMR spectroscopy study of the hydrolysis of ethanolamine-*N*-thiophosphoramidate ion as a function of pH. Red circles represent rate constants for the disappearance of ethanolamine-*N*-thiophosphoramidate ion (closed, stronger buffers; open, weaker buffers); black triangles represent the appearance of phosphate ion (a similar trace for the disappearance of thiophosphate ion was also observed; not shown); and blue squares represent the appearance of ethanolamine-*N*-phosphoramidate ion.

Closer analysis of the ^{31}P NMR spectra shows that in addition of P-N scission to give amine and inorganic thiophosphate ion, desulfurisation of both the *N*-thiophosphoramidate substrate **13** and thiophosphate ion occurs (Scheme 7) to give *N*-phosphoramidate **14** and phosphate ion, respectively.[9, 10, 19] Desulfurisation of the N-thiophosphoramidate ion, however, was only detectable for 7<pH<9, whereas desulfurisation of thiophosphate ion was seen across the profile. Rate constants for the processes discussed above have been estimated, however, the compromises made in terms of the use of buffers to facilitate the use of ^{31}P NMR spectroscopy, mean that these rate constants should only be considered on an order-of-magnitude basis. On the pH plateaux, disappearance of thiophosphoramidate shows a rate constant of ~ 4×10^{-2} s^{-1}; appearance of phosphate, 5×10^{-4} s^{-1}; and appearance of phosphoramidate, ~6×10^{-5} s^{-1}. Taken together, however, these data give clear evidence that *N*-thiophosphoramidate species display similar pH-reactivity properties to their oxy-analogues, and the use of higher pH would appear to be the most relaible pathway towards *S*-alkylation. In addition, these data align well with the findings of Ora *et al.* and their studies on closely related systems.



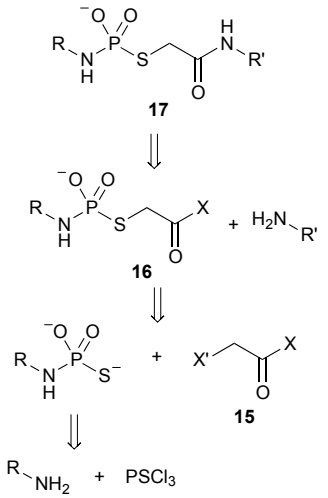
Scheme 7 Hydrolytic breakdown products of ethanolamine-*N*-thiophosphoramidate.

Stability of *N,S*-dialkylthiophosphoramidates

To gain an appreciation of the stability of *N*,*S*-dialkyl thiophosphoramidates, we performed ^{31}P NMR spectroscopy-based studies on *N*-benzylamino-*S*-*n*-propylthiophosphoramidate at pH ~7.5 and ~5.2. The pH of 7.5 was chosen to be close to physiological pH, whereas the pH 5.2 provides a situation where *N*-protonation is more likely, and reactivity is expected to be higher. In addition, the lower pH aligns with the conditions used for amastigote testing, which will be discussed below. The samples were incubated at 37 °C and ^{31}P NMR spectra were recorded periodically. No changes the forms of the spectra were observed over the course of 16 h. On the basis of these results, where we would expect to be able to detect 5% degradation reliably using the NMR method, we predict half-lives >200 h in both cases. Whilst this picture suffices for the development of our synthetic procedures, further detailed kinetic studies will be required.

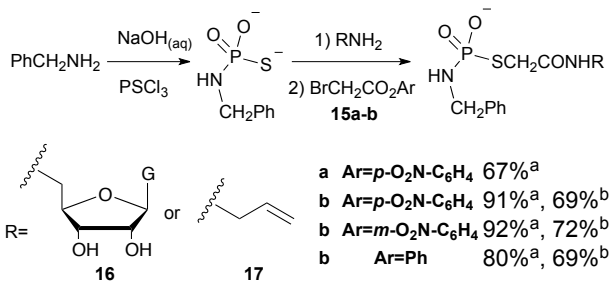
Bromoacetamides as alkylating agents

In order to expand the range of potential alkylating agents available for elaboration of *N*-thiophosphoramidate ions, we explored the use of a heterobifunctional cross-linking agents. We envisioned **15** being able to react selectively with thiophosphoramidate ions to produce activate acylating agents **16** that could be further reacted with readily accesible amines to produce mixed phosphoryl-acyl systems **17** that may serve as pyrophosphate mimics (Scheme 8). Our earlier experiences with a thiophosphate anion-based system (uridine-5'-monophosphorothioate, UMPS), suggested that this strategy could offer a convenient aqueous route to these species.[20]



Scheme 8 (A) Disconnection strategy for thiophosphoramidate-bromoacetate ester ligation of two amines. (B) Structural resemblance of thiophosphoryl-acetamide system to pyrophosphate.

Based on our earlier work with thiophosphates, we performed exploratory studies on the use of *p*-nitrophenyl-, *m*-nitrophenyl and phenyl-bromoacetate esters **15a-c** respectively. Our aim was to balance hydrolysis of the activated ester against the desired aminolysis process by tuning the reactivity of the phenolate leaving group. We used benzylamine as a model substrate for thiophosphorylation given that we had observed this process to proceed quantitatively. The second amine, RNH_2, was either the model system, allylamine, or the more challenging 5'-amino-5'-deoxyguanosine (Scheme 9).



Scheme 9 Amine-amine ligation via thiophosphorylation-bromoacetate ester cross-linking.

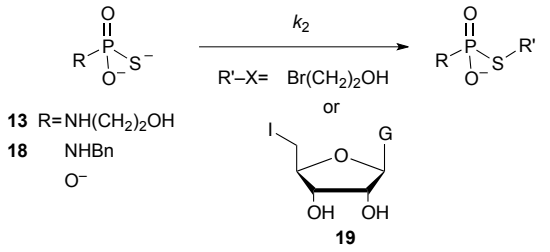
After each reaction, excess amine RNH_2 was removed by increasing pH followed by extraction with organic solvent. The pH was then reduced to facilitate protonation of the phenolate leaving groups by extraction into organic solvent. In all cases, the majority of material was converted to the desired thiophosphoramidate-acetamide products **16** and **17**.

In order to confirm the identity of the guanosine-derived product **16**, ion exchange chromatography was carried out. As seen for the *N*-thiophosphorylated aminonucleoside systems (see above), the desired thiophosphoramidate product **16** eluted ~35-40 min which corresponded ~90 mM TEAB (see ESI). The conversion level estimated by measuring the area under the absorbance curve in the elution profile correlated well with observations from ^{1}H and ^{31}P NMR spectroscopies.

These preliminary studies illustrate that the thiophosphorylation-bromoacetate route could offer a simple route towards nucleoside-based systems. Further optimisation of conditions, reaction times and the choice of phenolate leaving group should facilitate improvements.

Nucleophilicity of thiophosphoryl systems

During our alkylation studies, we observed that some alkylations appeared more sluggish than others, thus we sought to explore these observations through kinetic studies. In addition to being sensitive to the nature of the electrophile, we expected the kinetics of alkylation to vary as a function of the nature of the *N*-alkyl portion of the thiophosphramidate. We studied the progress of a series of alkylations reaction using ^{31}P NMR spectroscopy using ethanolamine-*N*-thiophosphoramidate **13**, benzylamine-*N*-thiophosphoramidate **18**, and, as a comparison, inorganic thiophosphate ion as nucleophiles. The added electrophiles were bromoethanol and 5'-deoxy-5'-iodoguanosine **1** (Scheme 10).



Scheme 10 Thiophosphoryl systems and alkylating agents used in *S*-alkylation kinetic study.

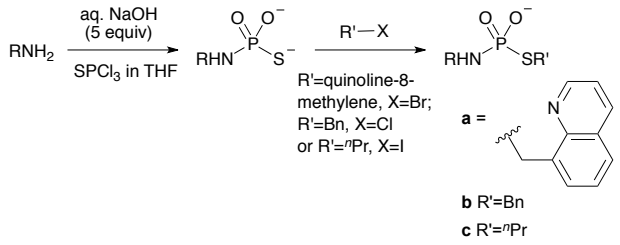
Alkylations were performed in the presence of a significant excess of alkylating agent to facilitate first order kinetic analyses. Using the reactive bromoethanol system, alkylations proceeded rapidly, thus we were unable to derive kinetic data. With the less reactive nucleoside system, however, bimolecular rate constants, k_2, of 4×10^{-5}, 2.5×10^{-4} and 3.3×10^{-4} $M^{-1}s^{-1}$ were obtained for ethanolamine-*N*-thiophosphoramidate **13**, benzylamine-*N*-thiophosphoramidate **18** and inorganic thiophosphate ion, respectively. These data confirm that the nature of the substituent on the thiophosphoryl group can have a significant effect on alkylation kinetics.

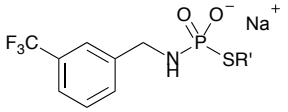
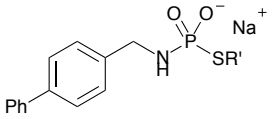
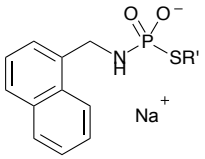
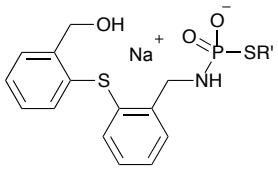
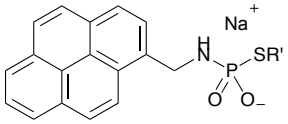
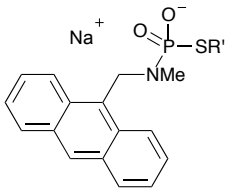
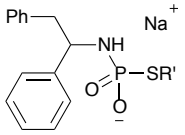
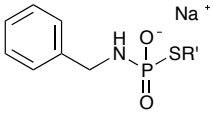
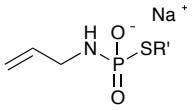
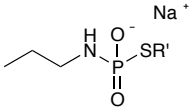
The nucleophilicity of thiolate ions can be measured quantitatively, and we would expect these values to be similar in nature to thiocarboxylate systems studies by Mayr and co-workers.[21] We are currently exploring these values.

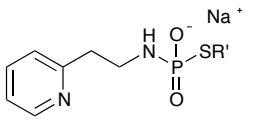
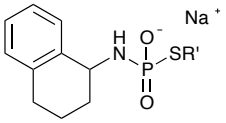
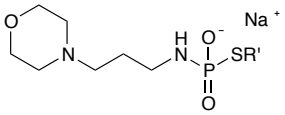
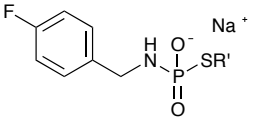
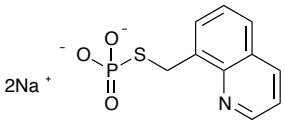
Preparation of *N,S*-dialkylthiophosphoramidate libraries using lipophilic alkylamines

To prove the general applicability of the method, we preparaed a small generic library of *N,S*-dialkyl thiophosphoramidates **20-33a-c** in a simple, rapid manner where the only form of purification was extraction of excess alkylating agent followed by removal of the aqueous solvent. All amines were hydrophobic in nature, and some of the reaction mixtures were heterogeneous.

Table 2. Preparation of a library of *N,S*-dialkyl thiophosphoramidates and control compound.



entry	product	conversion level, %
20		**a** 81[a], 60[b], 54[c] **b** 97[a], 97[b], 92[c] **c** 98[a], 100[b], 100[c]
21		**a** 79[a], 80[b] **b** 98[a], 96[b] **c** 98[a], 95[b]
22		**a** 75[a], 68[b] **b** 95[a], 97[b] **c** 98[a], 98[b]
23		**a** 90[a], 90[b] **b** 92[a], 94[b] **c** 93[a], 94[b]
24		**a** 93[a], 90[b] **b** 94[a], 97[b] **c** 97[a], 98[b]
25		**a** 61[a], 80[b] **b** 99[a], 100[b] **c** 97[a], 94[b]
26		**a** 65[a], 65[b] **b** 91[a], 90[b] **c** 67[a], 60[b]
27		**a** 91[a], 95[b]
28		**a** 93[a], 96[b]
29		**a** 94[a], 96[b] **b** 95[a], 92[b]

30		**a** 87[a], 75[b] **c** 95[a], 82[b]
31		**a** 75[a], 78[b] **c** 79[a], 87[b]
32		**a** 81[a], 67[b] **c** 91[a], 90[b]
33		**a** 90[a], 92[b],84[c] **c** 96[a], 96[b],100[c]
34		95[a], 93[b]

[a] Determined by ^{31}P NMR spectroscopy. [b] Determined by ^{1}H NMR spectroscopy. [c] Determined by ^{19}F NMR spectroscopy.

Alkylating agents were represented by benzyl chloride, *n*-propyl iodide and a quinoline system. The simple alkyl systems served to illustrate the usage of a reactive benzyl system and a simple alkyl system. The quinolines, on the other hand were designed by analogy with quinoline-based sulfamidates that have been successfully applied as anti-parasite agents. The syntheses of the sulfonamides were, however, by way of organic solvent-based procedures where laborious purification procedures were required. We hoped that the similar geometric properties of the thiophosphoramidate group may offer an alternative to the sulfonamide where product mixture could be used directly from aqueous synthetic procedures without isolation.

Testing Antileishmanial Activities

Quinoline-substituted sulfonamides have been reported as potential anti-leishmanial agents (Figure 4).[22, 23]

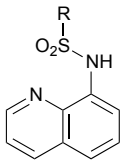
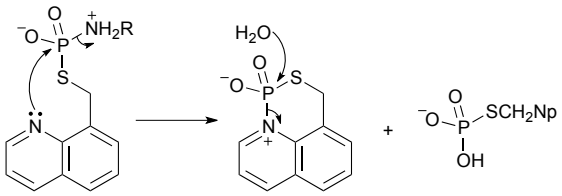


Figure 4 Quinoline-based sulfonamides used in antileishmanial testing studies.

Owing to the close structural homology of the thiophosphoramidate and sulfonamide groups, we prepared quinoline-based thiophosphoramidate derivatives **20-33a.** In addition, phosphorothiolate-quinoline system **34** was prepared as a control that represents the common hydrolysis product expected from P-N scission. We tested these systems for activity against both mammalian stage amastigote and insect stage promastigote forms of the Trypanosomatid *Leishmania mexicana* using established protocols.[24] Unfortunately, there were no clear signs of activity of these agents. In the case of amastigotes, we found that the quinoline systems were quite sensitive to the acidic nature of the specialist growth media, and showed significant decomposition over a timescale of hours. This contrasts with our findings for other systems, and we attribute this difference to the possibility of intramolecular general acid catalysis in the quinolines (Scheme 11).



Scheme 11 Potential intramolecular mechanism accounting for the instability of quinoline-based thiophosphoramidates.

Promastigote tesing also failed to demonstrate antileishmanial activity, thus we must conclude that *N*-alkyl-*S*-(methylene(8-quinolyl)) thiophosphoramidates are not effective against *Leishmania mexicana*, despite their structural resemblance to successful sulfonamide compounds.

Conclusions

Aqueous aminothiophosphorylation offers clean conversion to thiophosphoramidate anions when used in conjunction with nucleophilic, simple alkylamines, however, aryl systems have proven less successful. Unprotected amino acid, sugar and nucleoside systems showed varying degrees of effectiveness, with the aminonucleoside systems showing significant promise and scope for improved performance. In these cases, thiophosphorylations proceeded, in most cases, smoothly, however, on *S*-alkylation, decomposition was seen. In all cases, mechanisms involving intramolecular assistance can be postulated, and it is these that we believe lead to the decomposition in these systems.

The straightforward assembly of simple liphophilic systems using $PSCl_3$ allowed us to rapidly assemble a library of compunds, and, although the quinoline systems presented specific stability issues, the approach proved effective in facilitating swift access to aqueous solutions of library molecules that were amenable to biological testing without needing extensive purification.

Experimental

Attempted thiophosphorylation-alkylation of aniline (towards 2, 3)

Aniline (1.2 eq, 251 µL, 2.76 mmol) was mixed with aqueous sodium hydroxide (5 eq of a 1 M aqueous solution, 11.5 ml, 11.5 mmol) and water (1.48 ml) in a 50 mL round bottomed flask with indentations aimed towards inducing turbulent mixing. The mixture was cooled on an ice-water bath, thiophosphoryl chloride

(1.0 eq, 232 μL, 2.3 mmol) in THF (7 mL) was added dropwise over the course of 10 min. and the mixture was stirred for an additional 15 min. Bromoethanol (2 eq, 326 μL, 4.6 mmol) was added and the mixture was stirred for 22 h while maintaining pH~9 through periodic additions of 1 ml aliquots of 1 M NaOH solution. The mixture was then extracted with diethyl ether (3 × 10 mL) to remove excess aniline, $PSCl_3$, THF and bromoethanol, and the aqueous layer was concentrated by lyophilisation before being subjected to analysis. The conversion to *N*-thiophosphoramidate was estimated by ^{31}P NMR spectroscopy before addition of bromoethanol (see ESI). After addition of the alkylating agent, conversion was estimated using ^{31}P NMR spectroscopy (47%) and ^{1}H NMR spectroscopy (41%). The other impurities present were aniline 19% and alkylated inorganic thiophosphosphate 31% by both ^{31}P NMR and ^{1}H NMR spectroscopy. δ_H(400 MHz; D_2O) 7.26 (2 H, t, *J* 7.9, *m*-Ar-H), 7.09 (2 H, d, *J* 8.0, *o*-Ar-H), 6.99-6.90 (1 H, m, *p*-ArH), 3.56 (2 H, t, *J* 6.4, CH_2OH), 2.78-2.65 (2 H, m, SCH_2); δ_P(162 MHz; D_2O) 18.7 (t, $^3J_{H\text{-}P}$ 13.4, N*P*S); δ_C(101 MHz; D_2O) 141.7, 129.6, 121.3, 118.2 (d, $^3J_{C\text{-}P}$ 6.7, *C*HCNH), 61.6 (d, $^3J_{C\text{-}P}$ 4.7, *C*H_2OH), 32.5 (S*C*H_2); *m/z* (ES^-) 232.0203 (M–H. $C_8H_{11}NO_3PS$ requires 232.0203).

Attempted thiophosphorylation-alkylation of phenylalanine (towards 4)

D/L-phenylalanine (1 eq, 2.3 mmol, 380 mg) was dissolved in aqueous sodium hydroxide (7 eq of a 5 M aqueous solution, 3.22 ml, 16.1 mmol) in a 50 mL round bottomed flask with indents in an ice bath. Thiophosphoryl chloride (1.4 eq, 327 μL, 3.22 mmol) in THF (4 mL) was added dropwise to the mixture over the course of 10 min. After 1 h of stirring, inorganic thiophosphate ion arising from hydrolysis of the excess $PSCl_3$ was removed by applying methanol precipitation.[25] The residual supernatant solution was concentrated *in vacuo* before being freeze-dried to remove water and being subjected to NMR analysis. The crude phenylalanine thiophosphoramidate (0.5 mmol, 130.5 mg) was dissolved in D_2O (0.5 ml) and MeI (0.5 mmol, 31 μL) was added directly to the NMR. The sample was subjected to NMR analysis after 20 h. *Analysis after thiophosphorylation;* δ_H(400 MHz; D_2O) 7.34-7.02 (5 H, m, Ar-H), 3.76 (1 H, ddd, *J* 12.4, 7.9, 4.5, C*H*), 3.06 (1 H, dd, *J* 13.1 and 4.5, CH*H*), 2.79 (1 H, dd, *J* 13.1 and 7.9, C*H*H); δ_P(162 MHz; D_2O) 42.3 (d, $^3J_{H\text{-}P}$ 12.5, N*P*S); *m/z* (ES^+) 262.03 ($M + H^+$); *m/z* (ES^-) 244.0383 (phosphoramidate i.e. loss of S, (M–H). $C_9H_{11}NO_5P$ requires 244.0380). *Analysis after addition of MeI;* δ_H(400 MHz; D_2O) 7.53-7.05 (6 H, m, Ar-H), 3.90-3.68 (1 H, m, C*H*), 3.21-3.07 (2 H, m, CH_2), 1.67 (3 H, d, *J* 13.3, SCH_3); δ_P(162 MHz; D_2O) 25.1 (d, $^3J_{H\text{-}P}$ 12.8, N*P*S).

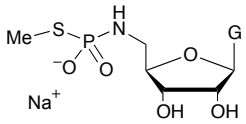
Attempted thiophosphorylation-alkylation of glucosamine (towards 9,10)

Glucosamine hydrochloride (1.0 eq, 496 mg, 2.3 mmol) was dissoved in aqueous sodium hydroxide (6 eq of a 1 M aqueous solution, 13.8 ml, 13.8 mmol) in a 50 mL round bottomed flask with indents in an ice bath. Thiophosphoryl chloride (1.0 eq, 232 μL, 2.3 mmol) in THF (7 mL) was added dropwise to the mixture over the course of 10 min. After 1 h of stirring, the conversion to thiophosphoramidate was estimated by ^{31}P NMR spectroscopy MeI (2.0 Eq, 4.6, 286 μL) was added and the mixture was stirred for a further 1 h and subjected to ^{31}P NMR spectroscopy analysis to assess *S-alkylation*. Analysis after thiophosphorylation; δ_P(162 MHz; D_2O) 45.6 (N*P*S). After addition of MeI, the majority of material appeared to be converted to *S*-methylthiophosphate **10**; δ_P(162 MHz; D_2O) 19.3 (d, $^3J_{H\text{-}P}$ 11.3, OPSMe).

Thiophosphorylation of 5'-amino-5'deoxyguanosine or 5'-amino-5'deoxyadenosine and alkylation with MeI or BnCl

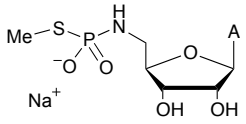
5'-Amino-5'-deoxyguanosine[25, 26] (1 Eq, 0.23 mmol) or 5'-Amino-5'-deoxyadenosine dihydrochloride[27] (1 Eq, 0.23 mmol) was dissolved in a mixture of aqueous sodium hydroxide (5 eq of a 1 M solution, 1.15 mmol for G; 7 eq, 1.61 mmol for A) and water (148 μL for G, 0 μL for A) in a 50 mL round bottomed flask with indents in an ice bath. Thiophosphoryl chloride (1 Eq, 23.2 μL, 0.23 mmol) in THF (0.7 mL) was added dropwise to the aqueous solution over the course of 10 min. and the mixture was then stirred for a further 1 h. Methyl iodide (2 Eq, 28.6 μL, 0.46 mmol) and additional aqueous sodium hydroxide solution (1 Eq) were added to the flask and stirring was continued for 1 h. The excess of alkylating agent was removed by ether extraction (3 × 10 mL). The residual aqueous solution was then lyophilised and the residues were analysed (see crude ^{1}H and ^{31}P NMR spectra in ESI). The crude samples were dissolved in a 50 mM TEAB buffer, pH 7.5 (5 mL) and purified on DEAE Sepharose® FF column (50 mL, 10 × 3 mm, 3 mL/min), running TEAB buffer gradient 50-200 mM. Fractions were pooled and lyophilised before confirmation of their identities by ^{1}H and ^{31}P NMR spectroscopies. The triethylammonium salts of the compounds were dissolved in water (5 mL) and passed through a Na-Dowex® 50W×2, 200-400 (50 mL, 30 × 2 mm, 3 mL/min) column, with water as the mobile phase. The fractions containing products, detected *via* UV trace (254 nm), were collected, lyophilised and spectroscopic analyses were performed on the residues.

11a



δ_H(700 MHz; D_2O) 7.71 (1 H, s, 8-*H*), 5.66 (1 H, d, *J* 7.8, 1'-C*H*), 4.98-4.94 (1 H, m, 2'-C*H*OH), 4.31-4.28 (1 H, m, 3'-C*H*OH), 4.22-4.19 (1 H, m, 4'-C*H*), 3.06-2.99 (2 H, m, 5'-CH_2), 1.97 (3 H, d, *J* 13.0, CH_3S); $\delta_P[^1H]$(283 MHz; D_2O) 26.4-26.1 (m, NH*P*S); δ_C(176 MHz; D_2O) not assigned owing to low spectrum intensity; *m/z* (ES^-) 391.0594 (M–H. $C_{11}H_{16}N_6O_6PS$ requires 391.0595).

11b



δ_H(700 MHz; D_2O) 8.20 (1 H, s, 2-*H*), 8.08 (1 H, s, 8-*H*), 5.88 (1 H, d, *J* 6.6, 1'-C*H*), 4.76 (1 H, t, *J* 5.4, 2'-C*H*OH), 4.33-4.29 (1 H, m, 3'-C*H*OH), 4.17-4.13 (1 H, m, 4'-C*H*), 3.13-3.00 (2 H, m, 5'- NH_2CH_2), 1.95 (3 H, d, *J* 13.1, CH_3S); $\delta_P[^1H]$(283 MHz; D_2O) 26.1-25.8 (m, NH*P*S); δ_C(176 MHz; D_2O) 155.6, 152.8,

148.9, 140.8, 140.6, 119.0, 87.7 (1'-*C*H), 85.1 (d, $^{3}J_{C\text{-}P}$ 8.7, 4'-*C*H), 73.1 (2'-*C*HOH), 71.1 (3'-*C*HOH), 43.3 (5'-NH_2CH_2), 11.6 (d, $^{2}J_{C\text{-}P}$ 3.4, CH_3S); *m/z* (ES^-) 375.0643 (M–H. $C_{11}H_{16}N_6O_5PS$ requires 375.0646).

Kinetic studies on the decomposition of ethanolamine-*N*-thiophosphoroamidate 13

Buffers were prepared using CAPS (pH 10.5 and 10.17), CHES (pH 9.81, 9.44 and 9.06), EPPS (pH 8.44 and 8.00), HEPES (pH 7.50 and 7.10), MES (pH 6.60, 6.00 and 5.88) and acetate (pH 4.80 and 4.66) systems where the pHs were adjusted by the addition of hydrochloric acid or hydroxide solutions (see ESI). Crude, lyophilised ethanolamine thiophosphoroamidate **13** (30 mg) was dissolved in a buffer solution (0.5 M, 4 mL or 0.5 mL, see ESI) and the mixture was lyophilised. The lyophilised solid was then dissolved in D_2O (0.5 mL), a pH meter reading was taken and the mixture was transferred to a NMR tube. Owing to the fact that a rigorous deuterium exchange was not performed, the measured pD value could not be converted directly to a pD value, however, for the purposes of this preliminary study, the uncertainty in these values (~0.1 pD units) was deemed acceptable. The NMR tube containing the buffered substrate was then heated to 50 °C in the NMR machine magnet, and spectra were acquired every 30 (CAPS, CHES, EPPS, HEPES), 15 (MES), 10 (acetate buffer) or 8 (citric buffer) minutes.

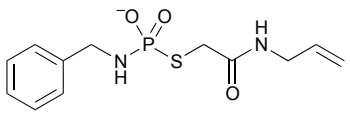
The intensities of the peaks corresponding to the thiophosphoroamidate, normalised with the highest intensity peak in the spectra, set to have the value 1, were plotted as a pseudo first order function of time and least squares fittings were performed against an exponential decay curve $I_t = I_0 e^{-kt}$.

Bromoacetamide cross-linker

Use of benzylamine-*N*-thiophosphoramidate 18 with phenylbromoacetates 15a-b and allyl amine or 5'-amino-5'-deoxyguanosine

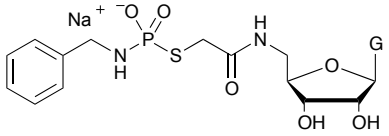
Benzylamine (1 Eq, 25 μL, 0.23 mmol) was thiophosphorylated using our established procedure. Allylamine (2 Eq, 32 μL, 0.46 mmol) or 5'-amino-5'deoxyguanosine (1 Eq, 65 mg, 0.23 mmol) was added to aqueous/THF solution of the thiophosphorylated benzylamine and mixed for several minutes, before the phenylbromoacetate ester (1 Eq, 0.23 mmol) was added. After 15 minutes of vigorous stirring, the pH of the mixture was adjusted using 50 mM hydrochloric acid to the approximately the pK_a of the phenol leaving group. The solution was extracted with ethyl acetate (3 × 10 mL), the pH was adjusted to pH 9 and the extraction was performed using chloroform (3 × 10 mL) in an atempt to remove excess amine. The aqueous sample was lyophilized and the dry solid was analysed and purified (nucleoside).

17



δ_H(400 MHz; D_2O) 7.45-7.22 (5 H, m, C_6H_5), 5.85-5.74 (1 H, m, CH_2=C*H*), 5.20-5.10 (2 H, m, CH_2=CH), 4.00 (2 H, d, *J* 10.9, CH_2NH), 3.72 (2 H, dt, *J* 5.1 and 1.6, NHCH_2), 3.33 (2 H, d, *J* 12.9, SCH_2); δ_P[^{1}H](162 MHz; D_2O) 22.0-21.6 (m, NH*P*S); δ_C(101 MHz; D_2O) 172.4 (d, $^{3}J_{C\text{-}P}$ 3.6, *C*=O), 140.6 (d, $^{3}J_{C\text{-}P}$ 7.6, *C*CH_2NH), 133.6 (*C*H=CH_2), 128.9, 128.8, 127.4, 116.4 (CH_2=CH), 45.6 (PhCH_2NH), 42.2 (CH_2CH=CH_2), 33.7 (SCH_2); *m/z* (ES^-) 299.0627 (M–H. $C_{12}H_{16}N_2O_3PS$ requires 299.0624).

16



The crude sample (50 mg) was dissolved in a 50 mM TEAB buffer, pH 7.5 (5 mL) and purified on DEAE Sepharose® FF column (50 mL, 10 × 3 mm, 3 mL/min), running TEAB buffer gradient 50-200 mM. Fractions were pooled and lyophilised, and the main peak in the UV trace was found to contain the desired product (87% by ^{31}P NMR spectroscopy, 78% by ^{1}H NMR spectroscopy). The triethylammonium salt of the compound was dissolved in water (5 mL) and passed through a Na-Dowex® 50W×2, 200-400 (50 mL, 30 × 2 mm, 3 mL/min) column, with water as the mobile phase. The fractions containing product, detected *via* UV trace (254 nm), were collected and lyophilised. The purity after cation exchange chromatography was estimated to be 80 % by ^{31}P NMR spectroscopy and 68% by ^{1}H NMR spectroscopy). δ_H(700 MHz; D_2O) 7.74 (1 H, s, 8-*H*), 7.10-6.99 (5 H, m, Ar-H), 5.58 (1 H, d, *J* 4.5, 1'-C*H*), 4.47 (1 H, app t, *J* 5.0, 2'-C*H*OH), 4.17 (1 H, app t, *J* 5.3, 3'-C*H*OH), 4.11-4.07 (1 H, m, 4'-C*H*), 3.71-3.60 (2 H, m, CH_2NH), 3.49 (1 H, dd, *J* 14.3 and 7.4, 5'-CH_2), 3.43 (1 H, dd, *J* 14.6 and 3.4, 5'-CH_2), 3.29-3.17 (2 H, m, SCH_2); δ_P[^{1}H](162 MHz; D_2O) 22.7-22.5 (m, NH*P*S); δ_C(101 MHz; D_2O) 172.9 (d, $^{3}J_{C\text{-}P}$ 2.6, *C*=O), 158.7, 153.7, 151.1, 140.2, 137.4, 128.8, 128.5, 128.2, 127.5, 127.3, 126.9, 126.7, 116.5, 87.7 (1'-*C*H), 82.0 (4'-*C*H), 73.4 (2'-*C*HOH), 70.9 (3'-*C*HOH), 45.0 (*C*H_2NH), 41.4 (5'-*C*H_2), 33.4 (d, $^{2}J_{C\text{-}P}$ 12.4, S*C*H_2); *m/z* (ES^-) 524.1127 (M–H. $C_{19}H_{23}N_7O_7PS$ requires 524.1123).

Kinetic study of the alkylation of thiophosphate ion using 5'-deoxy-5'-iodoguanosine 19.

A stock solution of 100 mM NaOH with 10 % D_2O was made with NaOH (0.5 ml, 1 M), H_2O (4 ml) and D_2O (0.5 ml). 5'-iodo 5'-deoxyguanosine (19 mg, 0.05 mmol) and tribasic sodium thiophosphate (0.09 g, 0.5 mmol) were dissolved in the stock NaOH solution (0.5 ml). The solution was transferred into a NMR tube and reaction progress at 50 °C was monitored in the NMR spectrometer by ^{31}P NMR spectroscopy (202 MHz, 128 repetitions). Two runs were performed with time points being taken either every 1 h or every 10 min.

Kinetic studies of the alkylations of benzylamine-*N*-thiophosphoroamidate 18 and ethanolamine-N-thiophosphoroamidate 13 ion with bromoethanol and 5'-deoxy-5'-iodoguanosine 19.

5'-iodo-5'-deoxyguanosine **19** (19 mg, 0.05 mmol) or 2-bromoethanol (3.5 μL, 0.05 mmol) was measured directly into an NMR tube. Crude benzylamine-*N*-thiophosphoroamidate (101.5 mg) or ethanolamine-*N*-thiophosphoroamidate (78.5 mg) was dissolved in D_2O (0.5 mL) and added to the alkylating agent. The mixture was then subjected to ^{31}P NMR spectroscopic analyses at

50 °C over a period of 12 h, with spectra being collected every 30 minutes. All the signals appearing in the spectra were integrated. The normalised peak area for the signal at 25 ppm (quintet in the coupled spectra, J=10.7 Hz), corresponding to the alkylated product, was then plotted against time and these data were used for kinetic fittings.

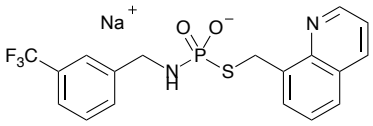
_N_-thiophosphorylation of simple hydrophobic amine library and _S_-alkylation of the resulting _N_-thiophosphoramidate anions 20-33a-c.

Details of quantities are summarized in tabular format in the Electronic Supplementary Information.

An amine (ESI table 3, RNH_2) was mixed with sodium hydroxide solution (5 Eq of a 1 M aqueous solution: **20-33a**: 0.9 mL, 0.9 mmol; **20-33b,c**: 2.425 mL, 2.425 mmol) and water (**20-33a**: 0.116 mL; **20-33b,c**: 0.312 mL) in a round-bottomed flask with indentations that aim to ensure turbulent mixing. Thiophosphoryl chloride (1 Eq, **20-33a**: 0.18 mmol, 0.018 mL; **20-33b,c**: 0.049 mL, 0.485 mmol) dissolved in THF (**20-33a**: 0.548 mL; **20-33b,c**: 1.476 mL) was added dropwise to the aqueous mixture over the course of 10 min. After 1 h of vigorous mixing to allow _N_-thiophosphorylation to take place, an alkylating agent was added (ESI table 3) along with additional sodium hydroxide solution (ESI table 3) and vigorous mixing was continued fo either 1h (**20-33a**) or overnight (**20-33b,c**). Then, ether extraction was performed (**20-33a**: 3 × 5 mL; **20-33b,c**: 3 × 20 mL) and the aqueous layer was lyophilized. In the examples where a white precipitate appeared during the extraction, the sample was centrifuged and the precipitate was dried overnight in a vacuum desiccator before being analysed. The crude material was then subjected to ^{1}H and ^{31}P NMR analyses to assess conversion levels, and ^{13}C NMR analyses were used to confirm the identity of the major product.

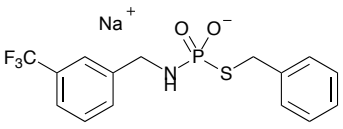
Summary of Spectroscopic Data.

20a



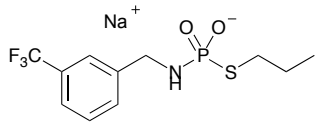
δ_H(700 MHz; CD_3OD) 8.78-8.75 (1 H, m, Ar-H), 8.20-8.17 (1 H, m, Ar-H), 7.82 (1 H, d, J 7.0, Ar-H), 7.73-7.67 (2 H, m, Ar-H), 7.50 (1 H, br s, F_3CCCHC), 7.48-7.32 (4 H, m, Ar-H) 4.56 (2 H, d, J 11.2, CH_2NH), 3.80 (2 H, d, J 8.6, SCH_2); $\delta_P[^1H]$(283 MHz; CD_3OD) 23.7-23.4 (m, NH_P_S); δ_F(376 MHz; CD_3OD) –63.8 (s, CF_3); δ_C(176 MHz; CD_3OD) 149.2, 145.7, 142.2 (d, $^{3}J_{C\text{-}P}$ 10.8, CCH_2NH), 138.0 (m, $^{3}J_{C\text{-}P}$ not resolved, SCH_2C), 136.5, 130.9, 129.7, 128.7, 128.4, 126.9, 126.2-126.0 (m, $^{2}J_{C\text{-}F}$ not resolved, Ar), 124.9 ($^{1}J_{C\text{-}F}$ 272, CF_3), 123.9-123.7 (m, $^{3}J_{C\text{-}F}$ not resolved), 122.8 (q, $^{3}J_{C\text{-}F}$ 3.0, Ar), 121.0, 45.6 (CH_2NH), 30.0 (SCH_2), the other peaks have not been resolved; m/z (ES^-) 411.0547 (M–H. $C_{18}H_{15}N_2O_2F_3PS$ requires 411.0549).

20b



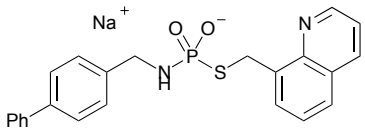
δ_H(700 MHz; CD_3OD) 7.61 (1 H, s, $Ar(CF_3)$), 7.52 (1 H, d, J 7.6, $Ar(CF_3)$), 7.46 (1 H, d, J 7.6, $Ar(CF_3)$), 7.43 (1 H, t, J 7.6, $Ar(CF_3)$), 7.32 (2 H, d, J 7.6, Ar-H), 7.23 (2 H, app t, J 7.5, Ar-H), 7.15 (1 H, t, J 7.3, Ar-H), 3.91 (2 H, d, J 9.3, SCH_2), 3.86 (2 H, d, J 10.1, CH_2NH); $\delta_P[^1H]$(283 MHz; CD_3OD) 22.3 (app qn, J 9.3, NH_P_S); δ_F(376 MHz; CD_3OD) –63.9 (s, CF_3); δ_C(176 MHz; CD_3OD) 142.8-142.7 (m, Ar), 140.0-139.9 (m, Ar), 131.0, 130.0 (q, $^{2}J_{C\text{-}F}$ 31), 128.45, 128.40, 127.9, 126.3, 124.4 (q, $^{1}J_{C\text{-}F}$ 272, CF_3), 123.8-123.7 (m, $^{3}J_{C\text{-}F}$, Ar), 122.9 (q, $^{3}J_{C\text{-}F}$ 3.8, Ar), 45.4 (CH_2NH), 34.6 (SCH_2); m/z (ES^-) 360.04420 (M–H. $C_{15}H_{14}NO_2F_3PS$ requires 360.04405).

20c



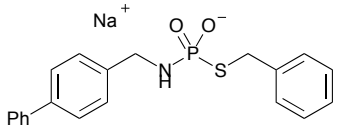
δ_H(700 MHz; CD_3OD) 7.83 (1 H, br s, Ar-H), 7.65 (1 H, d, J 6.8, Ar-H), 7.51-7.46 (2 H, m, Ar-H), 4.12 (2 H, d, J 10.1, SCH_2), 2.61 (2 H, dt, J 10.2 and 7.4, SCH_2), 1.60 (2 H, app sx, J 7.4, CH_2CH_3), 0.99 (3 H, t, J 7.4, CH_2CH_3); $\delta_P[^1H]$(283 MHz; CD_3OD) 23.7 (app qn, J 10.2, NH_P_S); δ_F(376 MHz; CD_3OD) –63.9 (s, CF_3); δ_C(176 MHz; CD_3OD) 142.9 (d, $^{3}J_{C\text{-}P}$ 7.4, CCH_2NH), 131.0 (4-_C_H), 130.0 (q, $^{2}J_{C\text{-}F}$ 31.5, CCF_3), 128.4 (5-_C_H), 124.3 (q, $J_{C\text{-}F}$ 271, CF_3), 123.9 (q, $^{3}J_{C\text{-}F}$ 3.2, 2-_C_H), 122.8(q, $^{3}J_{C\text{-}F}$ 3.6, 6-_C_H), 45.4 (CH_2NH), 32.3 (SCH_2), 24.2 (d, $^{3}J_{C\text{-}P}$ 6.8, CH_2CH_3), 12.7 (CH_2CH_3); m/z (ES^-) 312.04392 (M–H. $C_{11}H_{14}NO_2F_3PS$ requires 312.04405).

21a



δ_H(500 MHz; CD_3OD) 8.80 (1 H, dd, J 4.2 and 1.8, Ar-H), 8.21 (1 H, dd, J 8.2 and 1.8, Ar-H), 7.81 (1 H, d, J 7.0, Ar-H), 7.74 (1 H, dd, J 8.2 and 1.3, Ar-H), 7.59-7.24 (8 H, m, Ar-H), 7.22-7.20 (2 H, m, Ar-H), 4.56 (2 H, d, J 11.2, CH_2NH), 3.80 (2 H, d, J 8.2, SCH_2); $\delta_P[^1H]$(283 MHz; CD_3OD) 23.8-23.6 (m, NH_P_S); δ_C(125 MHz; CD_3OD) 149.2, 145.8, 140.8, 140.1 (d, $^{3}J_{C\text{-}p}$ 10.5, CCH_2NH), 139.3, 138.0 (d, $^{3}J_{C\text{-}P}$ 4.4, SCH_2C), 136.6, 129.7, 128.6, 128., 127.8, 126.8, 126.4, 126.3, 126.2, 126.0, 120.8, 45.5 (CH_2NH), 30.1 (SCH_2); m/z (ES^-) 419.0993 (M–H. $C_{23}H_{20}N_2O_2PS$ requires 419.0988).

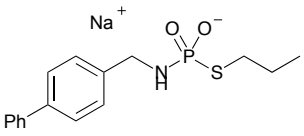
21b



δ_H(700 MHz; CD_3OD) 7.57 (2 H, d, J 7.8, Ar-H), 7.50 (2 H, d, J 7.8, Ar-H), 7.40 (2 H, t, J 7.6, Ar-H), 7.35 (2 H, d, J 7.8, Ar-H), 7.32-7.27 (3 H, m, Ar-H), 7.21 (2 H, d, J 7.5, Ar-H), 7.14 (1 H, t, J 7.5, Ar-H), 3.91 (2 H, d, J 9.0, NCH_2), 3.85 (2 H, d, J 9.6, CH_2S); $\delta_P[^1H]$(283 MHz; CD_3OD) 22.5 (app qn, J 9.3, NH_P_S); δ_C(176 MHz; CD_3OD) 140.9, 140.5 (d, $^{3}J_{C\text{-}p}$ 8.5, CCH_2NH), 139.8 (d, $^{3}J_{C\text{-}P}$ 5.1, CCH_2S), 139.5, 128.5, 128.4, 127.9, 127.8, 126.7, 126.4, 126.4, 126.2, 45.6 (CH_2NH), 34.6 (SCH_2); m/z (ES^-

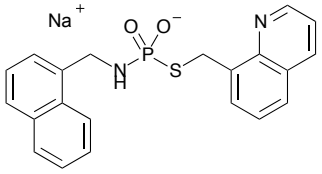
) 368.08796 (M – H. $C_{20}H_{19}NO_2PS$ requires 368.08796).

21c



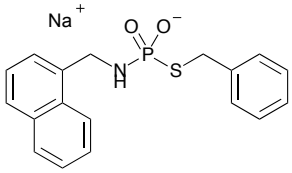
δ_H(700 MHz; CD_3OD) 7.57-7.56 (2 H, m, Ar-H), 7.53-7.51 (2 H, m Ar-H), 7.47-7.45 (2 H, m, Ar-H), 7.39 (1 H, t, *J* 7.7, Ar-H), 7.30-7.27 (1 H, m, Ar-H), 4.08 (2 H, d, *J* 9.8, C*H*$_2$NH), 2.63 (2 H, dt, *J* 10.5 and 7.4, SC*H*$_2$), 1.61 (2 H, app sx, *J* 7.4, C*H*$_2$CH$_3$), 0.95 (3 H, t, *J* 7.4, CH$_2$C*H*$_3$); δ_P[^{1}H](283 MHz; CD_3OD) 23.8 (app qn, J 9.7, NH*P*S); δ_C(176 MHz; CD_3OD) 140.9, 140.5 (d, $^3J_{C\text{-}p}$ 8.5, *C*CH$_2$NH), 139.5, 128.4, 127.8, 126.7, 126.4, 126.4, 45.6 (*C*H$_2$NH), 32.4 (SCH$_2$), 24.2 (d, $^3J_{C\text{-}P}$ 6.7, *C*H$_2$CH$_3$), 12.8 (CH$_2$*C*H$_3$); *m/z* (ES$^-$) 320.08799 (M–H. $C_{16}H_{19}NO_2PS$ requires 320.08796).

22a



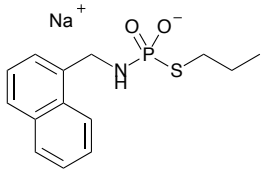
δ_H(700 MHz; CD_3OD) 8.72 (1 H, dd, *J* 4.2 and 1.7, Ar-H), 8.12 (1 H, dd, *J* 8.3 and 1.7, Ar-H), 8.02 (1 H, d, *J* 8.1, Ar-H), 7.79 (1 H, d, *J* 7.0, Ar-H), 7.78 (1 H, d, *J* 7.9, Ar-H), 7.68-7.60 (2 H, m, Ar-H), 7.37-7.31 (4 H, m, Ar-H), 7.27-7.24 (1 H, m, Ar-H), 7.18 (1 H, d, *J* 6.8, Ar-H), 4.57 (2 H, d, *J* 11.1, C*H*$_2$NH), 4.18 (2 H, d, *J* 7.0, SC*H*$_2$); δ_P[^{1}H](283 MHz; CD_3OD) 23.6-23.5 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 149.4, 146.0, 138.3 (d, $^3J_{C\text{-}P}$ 3.3, SCH$_2$*C*), 136.9), 136.1 (d, $^3J_{C\text{-}P}$ 11, *C*CH$_2$NH), 133.7, 131.4, 129.6, 128.5, 127.9, 127.1, 126.9, 126.0, 125.4, 125.1, 125.0, 124.9, 123.5, 43.6 (*C*H$_2$NH), 32.2 (SCH$_2$); *m/z* (ES$^-$) 393.0834 (M–H. $C_{21}H_{18}N_2O_2PS$ requires 393.0832).

22b



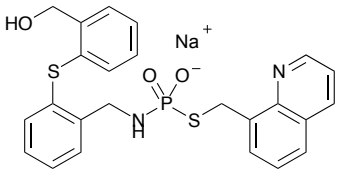
δ_H(700 MHz; CD_3OD) 8.15 (1 H, d, *J* 8.5, Ar-H), 7.82 (1 H, d, *J* 8.0, Ar-H), 7.72 (1 H, d, *J* 8.1, Ar-H), 7.48-7.42 (2 H, m, Ar-H), 7.40-7.38 (1 H, m, 2-C*H*), 7.37-7.33 (3 H, m, Ar-H), 7.23 (2 H, app t, *J* 7.6, Ar-H), 7.15 (1 H, t, *J* 7.4, Ar-H), 4.30 (2 H, d, *J* 7.4, C*H*$_2$NH), 4.30 (2 H, d, *J* 10.2, SC*H*$_2$); δ_P[^{1}H](283 MHz; CD_3OD) 22.4-22.2 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 140.2 (d, $^3J_{C\text{-}p}$ 4.0, CH$_2$S), 136.3 (d, $^3J_{C\text{-}P}$ 9.0, CH$_2$NH), 133.9, 131.5, 128., 128.0, 127.2, 127.1, 126.3, 125.5, 125.23, 125.15, 125.0, 123.5, 43.6 (*C*H$_2$NH), 34.6 (SCH$_2$); *m/z* (ES$^-$) 342.07236 (M–H. $C_{18}H_{17}NO_2PS$ requires 342.07231).

22c



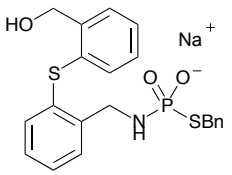
δ_H(700 MHz; CD_3OD) 8.24 (1 H, d, *J* 8.4, Ar-H), 7.84 (1 H, d, *J* 8.1, Ar-H), 7.75 (1 H, d, *J* 8.3, Ar-H), 7.57 (1 H, d, *J* 7.1, Ar-H), 7.50 (1 H, ddd, *J* 8.3, 6.8 and 1.3, 7-C*H*), 7.43 (1 H, ddd, *J* 8.4, 6.8 and 1.3, Ar-H), 7.40 (1 H, dd, *J* 8.1, 7.1, Ar-H), 4.51 (2 H, d, *J* 7.7, C*H*$_2$NH), 2.64 (2 H, dt, *J* 10.5 and 7.4, SC*H*$_2$), 1.63 (2 H, app sx, *J* 7.4, C*H*$_2$CH$_3$), 0.95 (3 H, t, *J* 7.4, CH$_2$C*H*$_3$); δ_P[^{1}H](283 MHz; CD_3OD) 23.8-23.6 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 136.3 (d, $^3J_{C\text{-}P}$ 9.0, *C*CH$_2$NH), 133.9, 131.5, 128.1, 127.2, 125.5, 125.2, 125.1, 125.0, 123.5, 43.6 (*C*H$_2$NH), 32.2 (SCH$_2$), 24.0 (d, $^3J_{C\text{-}P}$ 6.5, *C*H$_2$CH$_3$), 12.5 (CH$_2$*C*H$_3$); *m/z* (ES$^-$) 294.07232 (M–H. $C_{14}H_{17}NO_2PS$ requires 294.07231).

23a



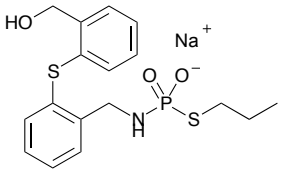
δ_H(700 MHz; CD_3OD) 8.77-8.75 (1 H, m, Ar-H), 8.18-8.16 (1 H, m, Ar-H), 7.76 (1 H, d, *J* 7.1, Ar-H), 7.68 (1 H, d, *J* 8.1, Ar-H), 7.52-7.48 (2 H, m, Ar-H), 7.41-7.37 (2 H, m, Ar-H), 7.24 (1 H, t, *J* 7.5, Ar-H), 7.14 (1 H, t, *J* 7.5, Ar-H), 7.10 (1 H, t, *J* 7.5, Ar-H), 7.06 (1 H, t, *J* 7.6, Ar-H), 6.96-6.92 (2 H, m, Ar-H), 4.62 (2 H, s, C*H*$_2$OH), 4.52 (2 H, d, *J* 11.0, C*H*$_2$NH), 3.92 (2 H, d, *J* 8.9, SC*H*$_2$); δ_P[^{1}H](283 MHz; CD_3OD) 23.9-23.8 (m, NH*P*S); δ_C(125 MHz; CD_3OD) 149.2, 145.7, 141.9, 136.6, 133.0, 132.8, 141.3 (d, $^3J_{C\text{-}P}$ 11.2, *C*CH$_2$NH), 129.7, 129.1, 128.4, 127.9, 127.5, 127.5, 127.4), 127.3, 127.1, 120.9, 61.7 (*C*H$_2$OH), 44.0 (*C*H$_2$NH), 30.1 (SCH$_2$); *m/z* (ES$^-$) 481.0822 (M–H. $C_{24}H_{22}N_2O_2PS$ requires 481.0815).

23b



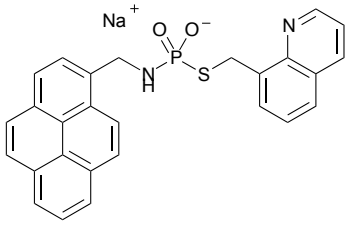
δ_H(700 MHz; CD_3OD) 7.56 (1 H, d, *J* 7.7, Ar-H), 7.54 (1 H, d, *J* 7.7, Ar-H), 7.28-7.10 (9 H, m, Ar-H), 7.06-7.02 (2 H, m, Ar-H), 4.71 (2 H, s, C*H*$_2$OH), 4.06 (2 H, d, *J* 9.4, C*H*$_2$NH), 3.81 (2 H, d, *J* 9.4, SC*H*$_2$); δ_P[^{1}H](283 MHz; CD_3OD) 22.6 (app qn, *J* 9.4, NH*P*S); δ_C(176 MHz; CD_3OD) 141.9 (*C*CH$_2$OH), 139.9 (*i*-*C*$_6$H$_5$CH$_2$S), 133.0, 132.8, 131.3 (d, $^3J_{C\text{-}P}$ 8.1, *C*CH$_2$NH), 128.8–126.4 (12 × s), 126.5, 61.3 (*C*H$_2$OH), 46.1 (*C*H$_2$NH), 35.4 (SCH$_2$); *m/z* (ES$^-$) 430.07103 (M–H. $C_{21}H_{21}NO_3PS_2$ requires 430.07059).

23c



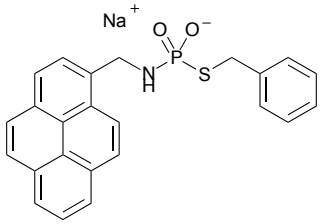
δ_H(700 MHz; CD_3OD) 7.64 (1 H, d, *J* 7.7, Ar-H), 7.54 (1 H, d, *J* 7.7, Ar-H), 7.28 (1 H, t, *J* 7.5, Ar-H), 7.25 (1 H, t, *J* 7.5, Ar-H), 7.17 (1 H, t, *J* 7.5, Ar-H), 7.14 (1 H, t, *J* 7.5, Ar-H), 7.06 (1 H, d, *J* 7.7, Ar-H), 7.05 (1 H, d, *J* 7.7, Ar-H), 4.72 (2 H, s, CH_2OH), 4.17 (2 H, d, *J* 9.4, CH_2NH), 2.56 (2 H, dt, *J* 10.5 and 7.4, SCH_2), 1.60 (2 H, app sx, *J* 7.4, CH_2CH$_3$), 0.90 (3 H, t, *J* 7.4, CH$_2$CH_3); δ_P[^{1}H](283 MHz; CD_3OD) 23.9 (app qn, *J* 9.8, NH*P*S); δ_C(176 MHz; CD_3OD) 141.9 (*C*CH$_2$OH), 133.0, 132.8, 131.3 (d, $^3J_{C\text{-}P}$ 11.2, *C*CH$_2$NH), 129.1, 127.9, 127.5, 127.4, 127.3, 62.3 (*C*H$_2$OH), 46.1 (*C*H$_2$NH), 35.4 (S*C*H$_2$), 27.2 (d, $^3J_{C\text{-}P}$ 6.2, *C*H$_2$CH$_3$), 16.8 (CH$_2$*C*H$_3$); *m/z* (ES$^-$) 382.07098 (M–H. $C_{17}H_{21}NO_3PS_2$ requires 382.07060).

24a



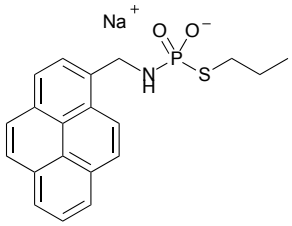
δ_H(700 MHz; CD_3OD) 8.66 (1 H, dd, *J* 4.1 and 1.7, Ar-H), 8.24 (1 H, d, *J* 9.2, Ar-H), 8.11 (1 H, d, *J* 7.5, Ar-H), 8.10 (1 H, d, *J* 7.6, Ar-H), 7.99-7.89 (6 H, m, Ar-H), 7.76 (1 H, d, *J* 7.0, Ar-H), 7.73 (1 H, d, *J* 7.7, Ar-H), 7.53 (1 H, d, *J* 8.2, Ar-H), 7.32-7.29 (1 H, m, Ar-H), 7.21 (1 H, dd, *J* 8.2 and 4.1, Ar-H), 4.58 (2 H, d, *J* 11.7, CH_2NH), 4.44 (2 H, d, *J* 7.4, SCH_2); δ_P[^{1}H](283 MHz; CD_3OD) 23.4-23.2 (m, NH*P*S); δ_C(125 MHz; CD_3OD) 149.3, 145.9, 137.8 (d, $^3J_{C\text{-}P}$ not resolved, SCH$_2$*C*), 134.0 (d, $^3J_{C\text{-}P}$ 11.2, *C*CH$_2$NH), 136.6, 131.3, 130.8, 130.5, 128.5, 129.5, 128.4, 127.0, 126.7, 126.3, 126.1, 125.4, 125.8, 124.4, 124.4, 124.2, 123.2, 120.7, 44.0 (*C*H$_2$NH), 30.1 (S*C*H$_2$); *m/z* (ES$^-$) 467.0996 (M–H. $C_{27}H_{20}N_2O_2PS$ requires 467.0988).

24b



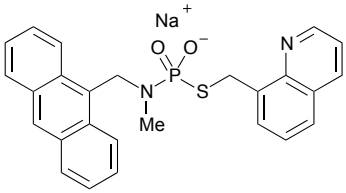
δ_H(700 MHz; CD_3OD) 8.39 (1 H, d, *J* 9.2, Ar-H), 8.14 (1 H, t, *J* 7.5, Ar-H), 8.09-8.05 (1 H, m, Ar-H), 8.06 (1 H, d, *J* 7.7, Ar-H), 8.00 (1 H, s, Ar-H), 7.97-7.93 (1 H, m, Ar-H), 7.30 (1 H, d, *J* 7.3, Ar-H), 7.18 (1 H, t, *J* 7.6, Ar-H), 7.12 (1 H, t, *J* 7.4, Ar-H), 4.56 (2 H, d, *J* 7.7, CH_2NH), 3.89 (2 H, d, *J* 10.2, SCH_2); δ_P[^{1}H](283 MHz; CD_3OD) 22.6-22.3 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 140.1 (*C*CH$_2$NH), 134.2 (CH$_2$S), 131.3, 130.8, 130.5, 128.5, 128.4, 127.9, 127.0, 126.9, 126.5, 126.4, 126.2, 125.5, 124.5, 124.4, 123.1, 43.7 (*C*H$_2$NH), 34.5 (S*C*H$_2$); *m/z* (ES$^-$) 416.08815 (M–H. $C_{24}H_{19}NO_2PS$ requires 416.08796).

24c



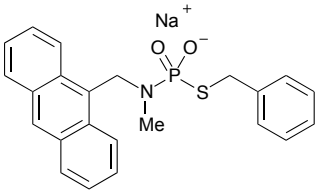
δ_H(700 MHz; CD_3OD) 8.39 (1 H, d, *J* 9.2, ArH), 8.16-8.13 (2 H, m, Ar-H), 8.12-8.11 (2 H, m, Ar-H), 8.09 (1 H, d, *J* 9.2, Ar-H), 8.01-7.99 (2 H, m, Ar-H), 7.96 (1 H, t, *J* 7.6, Ar-H), 4.46 (2 H, d, *J* 7.7, CH_2NH), 2.63 (2 H, dt, *J* 9.8 and 7.4, SCH_2), 1.60 (2 H, app sx, *J* 7.4, CH_2CH$_3$), 0.94 (3 H, t, *J* 7.4, CH$_2$CH_3); δ_P[^{1}H](283 MHz; CD_3OD) 23.7-23.5 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 134.4 (d, $^3J_{C\text{-}P}$ 11.7, *C*CH$_2$NH), 131.3, 130.8, 130.5, 128.5, 127.0, 126.9, 126.5, 126.4, 125.5, 124.5, 124.4, 124.3, 44.1 (*C*H$_2$NH), 32.5 (S*C*H$_2$), 24.2 (d, $^3J_{C\text{-}P}$ 6.7, *C*H$_2$CH$_3$), 12.7 (CH$_2$*C*H$_3$); *m/z* (ES$^-$) 368.08837 (M–H. $C_{20}H_{19}NO_2PS$ requires 368.08796).

25a



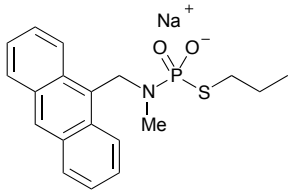
δ_H(700 MHz; CD_3OD) 8.67 (1 H, dd, *J* 4.1 and 1.7, Ar-H), 8.23 (1 H, d, *J* 9.1, Ar-H), 8.08 (2 H, t, *J* 7.7, Ar-H), 7.99-7.89 (6 H, m, Ar-H), 7.76 (1 H, d, *J* 7.0, Ar-H), 7.74 (1 H, d, *J* 7.7, Ar-H), 7.53 (1 H, d, *J* 8.1, Ar-H), 7.32-7.29 (1 H, m Ar-H), 7.20 (1 H, dd, *J* 8.2 and 4.1, Ar-H), 4.59 (2 H, d, *J* 11.5, CH_2N), 4.44 (2 H, d, *J* 7.4, SCH_2), 3.30-3.28 (3 H, m, NCH_3); δ_P[^{1}H](283 MHz; CD_3OD) 23.3-23.1 (m, N*P*S); δ_C(176 MHz; CD_3OD) aromatic signals could not be assigned owing to the level of heterogeneity of this particular sample, 43.8 (*C*H$_2$N), 38.6-37.8 (m, N*C*H$_3$), 29.9 (S*C*H$_2$); *m/z* (ES$^-$) 457.1153 (M–H. $C_{26}H_{22}N_2O_2PS$ requires 457.1145).

25b



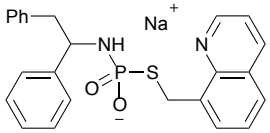
δ_H(700 MHz; CD_3OD) 8.60 (2 H, d, *J* 8.8, Ar-H), 8.40 (1 H, s, Ar-H), 7.97 (2 H, d, *J* 8.5, Ar-H), 7.52 (2 H, d, *J* 7.5, Ar-H), 7.49-7.45 (2 H, m, Ar-H*J*, 7.43-7.40 (2 H, m, Ar-H), 7.33 (2 H, t, *J* 7.6,Ar-H), 7.26 (1 H, t, *J* 7.4, Ar-H), 5.01 (2 H, d, *J* 3.8, CH_2NH), 4.15 (2 H, d, *J* 10.1, SCH_2), 2.21 (3 H, d, *J* 12.2, NCH_3); δ_P[^{1}H](283 MHz; CD_3OD) 23.7-23.4 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 140.9 (d, $^3J_{C\text{-}p}$ 5.4), 131.7, 131.6, 129.7 (d, $^3J_{C\text{-}P}$ 10.7), 128.7, 128.5, 127.6, 127.3, 126.3, 125.5, 124., 124.7, 44.5 (*C*H$_2$NH), 34.3 (SCH$_2$), 32.4 (NCH$_3$); *m/z* (ES$^-$) 406.10419 (M–H. $C_{23}H_{21}NO_2PS$ requires 406.10361).

25c



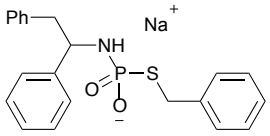
δ_H(700 MHz; CD_3OD) 8.74 (2 H, d, *J* 8.9, Ar-H), 8.42 (1 H, s, Ar-H), 7.99 (2 H, d, *J* 8.4, Ar-H), 7.50 (2 H, 2 × t, *J* 7.4, 2- and 7-C*H*), 7.51-7.48 (2 H, m, Ar-H), 7.44-7.41 (2 H, m, Ar-H) 5.17 (2 H, d, *J* 3.7, CH_2NH), 2.85 (2 H, dt, *J* 9.8 and 7.4, SCH_2), 2.30 (3 H, d, *J* 12.1, NCH_3), 1.75 (2 H, app sx, *J* 7.4, CH_2CH_3), 1.04 (3 H, t, *J* 7.4, CH_2CH_3); δ_P[^{1}H](283 MHz; CD_3OD) 24.9-24.6 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 131.5, 131.4, 129.4 (d, $^3J_{C\text{-}P}$ 9.1), 128.5, 127.1, 125.3, 124.8, 124.4, 44.2 (CH_2NH), 32.4 (SCH_2), 31.8 (NCH_3), 24.7 (d, $^3J_{C\text{-}P}$ 5.5, CH_2CH_3), 12.7 (CH_2CH_3); *m/z* (ES^-) 358.10394 (M–H. $C_{19}H_{21}NO_2PS$ requires 358.10361).

26a



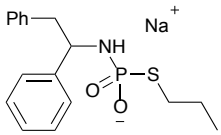
δ_H(700 MHz; CD_3OD) 8.79 (1 H, dd, *J* 4.2 and 1.8, Ar-H), 8.21 (1 H, dd, *J* 8.2 and 1.8, Ar-H), 7.71 (1 H, dd, *J* 8.2 and 1.3, Ar-H), 7.62-7.60 (1 H, m, Ar-H), 7.43 (1 H, dd, *J* 8.2 and 4.2, Ar-H), 7.39 (1 H, dd, *J* 8.1 and 7.2, Ar-H), 7.10-6.96 (8 H, m, Ar-H), 6.81-6.79 (2 H, m, Ar-H), 4.51 (1 H, *J* 12.7 and 9.6, SCH_2), 4.40-4.35 (1 H, m, CH_2CH), 4.31 (1 H, *J* 12.7 and 10.3, SCH_2), 3.19 (1 H, *J* 13.1 and 4.6, CH_2CH), 2.74 (1 H, *J* 13.1 and 9.5, CH_2CH); δ_P[^{1}H](283 MHz; CD_3OD) 21.7-21.5 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 149.2, 149.0, 145.9, 144.1-144.0 (unresolved, *C*CHNH), 138.5, 137.8-137.7 (unresolved, SCH_2C), 136.6, 136.4, 129.7-125.8 (11 × s), 120.9, 120.7, 57.9-57.6 (unresolved, CH_2CH), 45.1-44.9 (unresolved, CH_2CH), 30.1-29.8 (unresolved, SCH_2); *m/z* (ES^-) 433.1143 (M–H. $C_{24}H_{22}N_2O_2PS$ requires 433.1145).

26b



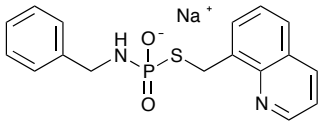
δ_H(700 MHz; CD_3OD) 7.19-7.03 (13 H, m, Ar-H), 6.95-6.92 (2 H, m, Ar-H), 4.46 (1 H, ddd, *J* 11.2, 9.2 and 5.2, CH_2CH), 3.60 (1 H, *J* 12 and 6.9, SCH_2), 3.47 (1 H, *J* 12 and 7.7, SCH_2), 3.22 (1 H, *J* 13.1 and 5.2, CH_2CH), 2.88 (1 H, *J* 13.1 and 9.0, CH_2CH); δ_P[^{1}H](283 MHz; CD_3OD) 20.9-20.8 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 144.4 (d, $^3J_{C\text{-}P}$ 3.7, *C*CHNH), 139.2 (d, $^3J_{C\text{-}P}$ 7.8, SCH_2C), 138.5, 129.5-125.5 (6 × s), 57.7 (CH_2CH), 45.7-45.4 (unresolved, CH_2CH), 34.5-34.3 (unresolved, SCH_2); *m/z* (ES^-) 382.10338 (M – H. $C_{21}H_{21}NO_2PS$ requires 382.10361).

26c



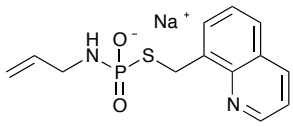
δ_H(700 MHz; CD_3OD) 7.18-7.03 (8 H, m, Ar-H), 6.97-6.94 (2H, m, Ar-H), 4.45 (1 H, ddd, *J* 14.0, 11.2 and 5.6, CH_2CH), 3.22 (1 H, *J* 13.2 and 5.3, CH_2CH), 2.93 (1 H, CH_2CH), 2.37-2.22 (m, SCH_2), 1.42-1.32 (2 H, m, CH_2CH_3), 0.79 (3 H, t, *J* 7.4, CH_3); δ_P[^{1}H](283 MHz; CD_3OD) 22.4-22.3 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 144.4 (d, $^3J_{C\text{-}P}$ not resolved, *C*CHNH), 138.5, 129.4, 127.4 (2 × s), 126.8, 126.0, 125.5, 57.6 (CH_2CH), 45.5 (d, $^3J_{C\text{-}P}$ 5.6, CH_2CH), 32.0 (SCH_2), 23.6 (d, $^3J_{C\text{-}P}$ 7.4, CH_2CH_3), 12.5 (CH_3); *m/z* (ES^-) 334.10399 (M–H. $C_{17}H_{21}NO_2PS$ requires 335.10361).

27a



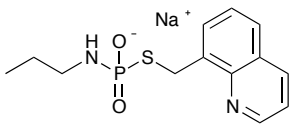
δ_H(700 MHz; CD_3OD) 8.80 (1 H, dd, *J* 4.2 and 1.7, Ar-H), 8.25 (1 H, dd, *J* 8.2 and 1.7, Ar-H), 7.82 (1 H, d, *J* 7.1, Ar-H), 7.76 (1 H, d, *J* 8.1, Ar-H), 7.49-7.42 (2 H, m, Ar-H), 7.20-7.11 (5 H, m, Ar-H), 4.55 (2 H, d, *J* 11.2, SCH_2), 3.74 (2 H, d, *J* 7.7, CH_2NH); δ_P[^{1}H](283 MHz; CD_3OD) 23.7-23.5 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 149.2, 145.8, 141.0-140.8 (m, $^3J_{C\text{-}P}$ not resolved, CCH_2NH), 138.2-138.0 (m, $^3J_{C\text{-}P}$ not resolved, SCH_2C), 136.6, 129.7, 128.6, 127.7, 127.2, 126.9, 126.1 (2 × s), 120.9, 45.8 (CH_2NH), 29.8 (SCH_2); *m/z* (ES^-) 343.0678 (M–H. $C_{17}H_{16}N_2O_2PS$ requires 343.0678).

28a



δ_H(500 MHz; CD_3OD) 8.89 (1 H, dd, *J* 4.2 and 1.8, Ar-H), 8.30 (1 H, dd, *J* 8.3 and 1.7, Ar-H), 7.90-7.86 (1 H, m, Ar-H), 7.82 (1 H, dd, *J* 8.2 and 1.3, Ar-H), 7.56-7.49 (2 H, m, Ar-H), 5.83-5.72 (1 H, m, CH_2=C*H*), 4.99 (1 H, dq, *J* 17.1 and 1.7, C*H*H=CH), 4.91-4.87 (1H, m, CH*H*=CH), 4.57 (2 H, d, *J* 11.1, SCH_2), 3.23 (2 H, ddt, *J* 8.8, 5.7 and 1.5, CH_2NH); δ_P[^{1}H](283 MHz; CD_3OD) 23.8-23.6 (m, NH*P*S); δ_C(125 MHz; CD_3OD) 149.5, 146.0, 138.3 (d, $^3J_{C\text{-}P}$ 4.6, SCH_2C), 137.5 (d, $^3J_{C\text{-}P}$ 9.9, *C*HCH$_2$), 136.9, 130.0, 128.9, 127.2, 126.4, 121.2, 113.7 (CH_2=CH), 44.8 (CH_2NH), 30.0 (d, $^2J_{C\text{-}P}$ 2.7, SCH_2); *m/z* (ES^-) 293.0522 (M–H. $C_{13}H_{14}N_2O_2PS$ requires 293.0519).

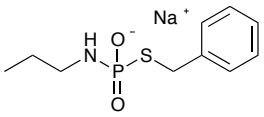
29a



δ_H(500 MHz; CD_3OD) 8.89 (1 H, dd, *J* 4.2 and 1.8, Ar-H), 8.30 (1 H, dd, *J* 8.3 and 1.7, Ar-H), 7.89 (1 H, dd, *J* 7.1 and 1.0, Ar-

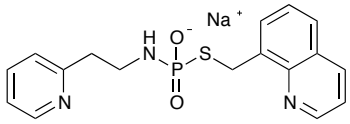
H), 7.82 (1 H, dd, *J* 8.2 and 1.2, Ar-H), 7.56-7.49 (2H, m, Ar-H), 4.56 (2 H, d, *J* 11.1, SCH_2), 2.57 (2 H, dt, *J* 8.7 and 7.4, CH_2NH), 1.30 (2 H, app sx, *J* 7.4, CH_3CH_2), 0.77 (3 H, t, *J* 7.4, CH_3CH_2); δ_P[^{1}H](283 MHz; CD_3OD) 24.2-24.0 (m, NH*P*S); δ_C(125 MHz; CD_3OD) 149.5, 146.1, 138.3 (d, $^3J_{C\text{-}P}$ 4.6, SCH_2C), 136.9, 129.9, 128.8, 127.2, 126.4, 121.2, 43.8 (*C*H_2NH), 30.1 (d, $^2J_{C\text{-}P}$ 2.7, S*C*H_2), 24.4 (d, $^2J_{C\text{-}P}$ 9.1, CH_3CH_2), 10.7 (CH_3CH_2); *m/z* (ES^-) 295.0677 (M–H. $C_{13}H_{16}N_2O_2PS$ requires 295.0675)

29b



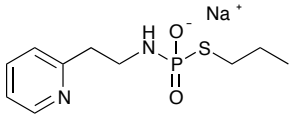
δ_H(700 MHz; CD_3OD) 7.38 (2 H, d, *J* 7.3, Ar-H), 7.28 (2 H, t, *J* 7.6, Ar-H), 7.21 (1 H, t, *J* 7.3, Ar-H), 3.89 (2 H, d, *J* 9.9, SCH_2), 2.68 (2 H, dt, *J* 8.7 and 7.4, CH_2NH), 1.39 (2 H, app sx, *J* 7.4, CH_3CH_2), 0.86 (3 H, t, *J* 7.4, CH_3CH_2); δ_P[^{1}H](283 MHz; CD_3OD) 23.1 (app qn, *J* 9.5, NH*P*S); δ_C(176 MHz; CD_3OD) 140.2 (d, $^3J_{C\text{-}P}$ 6.1, SCH_2C), 128.7, 128.2, 126.5, 43.8 (*C*H_2NH), 34.6 (d, $^2J_{C\text{-}P}$ 2.8, S*C*H_2), 24.5 (d, $^3J_{C\text{-}P}$ 8.8, CH_3CH_2), 10.7 (CH_3CH_2); *m/z* (ES^-) 244.05622 (M–H. $C_{10}H_{15}NO_2PS$ requires 244.05666).

30a



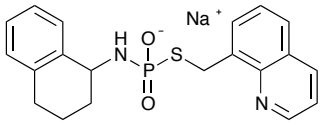
δ_H(700 MHz; CD_3OD) 8.81 (1 H, dd, *J* 4.2 and 1.6, Ar-H), 8.37 (1 H, d, *J* 4.3, Ar-H), 8.23 (1 H, dd, *J* 8.2 and 1.4, Ar-H), 7.82 (1 H, d, *J* 7.0, Ar-H), 7.74 (1 H, d, *J* 8.0, Ar-H), 7.65 (1 H, td, *J* 7.7 and 1.7, Ar-H), 7.47-7.42 (2 H, m, Ar-H), 7.20 (1 H, d, *J* 7.8, Ar-H), 7.17 (1 H, dd, *J* 6.9 and 5.5, Ar-H), 4.50 (2 H, d, *J* 10.4, SCH_2), 3.05-2.99 (2 H, m, CH_2CH_2NH), 2.81 (2 H, t, *J* 7.2, CH_2CH_2NH); δ_P[^{1}H](283 MHz; CD_3OD) 23.6 (app qn, *J* 10.1, NH*P*S); δ_C(176 MHz; CD_3OD) 160.0, 149.2, 148.2, 145.8, 137.9-137.8 (m, $^3J_{C\text{-}P}$ not resolved, SCH_2C), 137.0, 136.6, 129.7, 128.5, 126.9, 126.1, 123.6, 121.5, 120.9, 41.6 (*C*H_2NH), 39.1 (d, $^3J_{C\text{-}P}$ 7.6, *C*H_2CH_2NH), 30.2 (S*C*H_2); *m/z* (ES^-) 358.0786 (M–H. $C_{17}H_{17}N_3O_2PS$ requires 358.0784).

30c



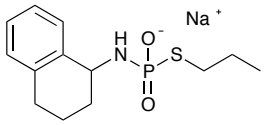
δ_H(700 MHz; CD_3OD) 8.42 (1 H, ddd, *J* 5.0, 1.7 and 0.9, Ar-H), 7.74 (1 H, td, *J* 7.7 and 1.8, Ar-H), 7.37 (1 H, d, *J* 7.8, Ar-H), 7.24 (1 H, ddd, *J* 7.5, 5.0 and 1.0, Ar-H), 3.22 (2 H, dt, *J* 9.8 and 7.1, CH_2CH_2NH), 2.98 (2 H, t, *J* 7.1, CH_2CH_2NH), 2.55 (2 H, dt, *J* 10.3 and 7.3, SCH_2), 1.58 (2 H, app sx, *J* 7.4, CH_2CH_3), 0.93 (3 H, t, *J* 7.4, CH_2CH_3); δ_P[^{1}H](283 MHz; CD_3OD) 24.0 (app qn, *J* 10.1, NH*P*S); δ_C(176 MHz; CD_3OD) 159.9, 148.2, 137.1, 123.8, 121.5, 41.6 (CH_2CH_2NH), 39.2 (d, $^3J_{C\text{-}P}$ 8.0, *C*H_2CH_2NH), 32.1 (S*C*H_2), 23.9 (d, $^3J_{C\text{-}P}$ 6.4, *C*H_2CH_3), 12.5 (CH_2CH_3); *m/z* (ES^-) 259.06778 (M–H. $C_{10}H_{16}N_2O_2PS$ requires 259.06756).

31a



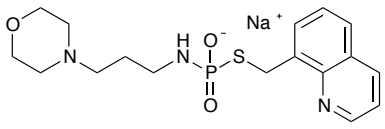
δ_H(700 MHz; CD_3OD) 8.83 (1 H, dd, *J* 4.0 and 1.3, Ar-H), 8.27-8.24 (1 H, m, Ar-H), 7.88 (1 H, d, *J* 7.0, Ar-H), 7.76 (1 H, d, *J* 8.0, Ar-H), 7.57 (1 H, d, *J* 7.6, Ar-H), 7.51-7.43 (2 H, m, Ar-H), 7.01-6.98 (1 H, m, Ar-H), 6.95-6.91 (2 H, m, Ar-H), 4.64-4.61 (2 H, m, SCH_2), 4.30-4.25 (1 H, m, C*H*NH), 2.70-2.55 (2 H, m, 3-CH_2), 1.95–1.87 (1 H, m, CH*H*), 1.79-1.71 (1 H, m, CH*H*), 1.68–1.55 (2 H, m, CH_2); δ_P[^{1}H](283 MHz; CD_3OD) 21.3 (app q, *J* 10.3, NH*P*S); δ_C(176 MHz; CD_3OD) 149.2, 146.0, 140.0-139.8 (m, $^3J_{C\text{-}P}$ not resolved, *C*CHNH), 138.0-137.9 (m, $^3J_{C\text{-}P}$ not resolved, SCH_2C), 136.8, 136.5, 129.7, 128.9, 128.6, 128.0, 126.9, 126.2, 125.9, 125.2, 120.9, 49.7 (*C*HNH), 31.9, 30.3 (d, $^3J_{C\text{-}P}$ 2.6), 29.0 (S*C*H_2), 19.7; *m/z* (ES^-) 383.0987 (M–H. $C_{20}H_{20}N_2O_2PS$ requires 383.0988).

31c



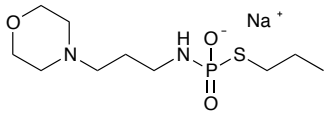
δ_H(700 MHz; CD_3OD) 7.63 (1 H, d, *J* 7.2, Ar-H), 7.09-7.03 (2 H, m, Ar-H), 6.99 (1 H, d, *J* 7.3, Ar-H), 4.34-4.29 (1 H, m, C*H*NH), 2.80-2.64 (4 H, m, 3-CH_2 and SCH_2), 2.11-2.04 (1 H, m, 4-CH_2), 1.96-1.89 (1 H, m, 4-CH_2), 1.89-1.82 (1 H, m, 2-CH_2), 1.78-1.62 (4 H, m, 2-CH_2 and CH_2CH$_3$), 0.99 (3 H, t, *J* 7.4, CH_2CH_3); δ_P[^{1}H](283 MHz; CD_3OD) 22.2-22.0 (m, NH*P*S); δ_C(176 MHz; CD_3OD) 140.1-140.0 (m, *C*CHNH), 136.7, 128.9, 128.1, 126.0, 125.2, 49.7 (*C*HNH), 32.5 (2-*C*H), 32.3 (S*C*H_2), 29.0 (4-*C*H), 23.9 (d, $^3J_{C\text{-}P}$ 7.0, *C*H_2CH_3), 19.8 (3-*C*H), 12.6 (CH_2CH_3); *m/z* (ES^-) 284.08826 (M–H. $C_{13}H_{19}NO_2PS$ requires).

32a



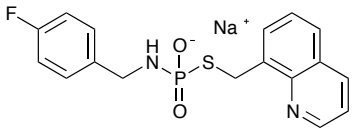
δ_H(700 MHz; CD_3OD) 8.86 (1 H, dd, *J* 4.2 and 1.7, Ar-H), 8.26 (1 H, dd, *J* 8.2 and 1.7, Ar-H), 7.87 (1 H, d, *J* 7.0, Ar-H), 7.78 (1 H, d, *J* 8.2, Ar-H), 7.52-7.46 (2 H, m, Ar-H), 4.54 (2 H, d, *J* 10.8, SCH_2), 3.60 (4 H, t, *J* 4.6, O(CH_2)$_2$), 2.67 (2 H, dt, *J* 9.3 and 7.0, PNHCH_2), 2.30 (4 H, br s, (CH_2)$_2$N), 2.24-2.20 (2 H, m, NCH_2), 1.50-1.44 (2 H, m, $CH_2CH_2CH_2$); δ_P[^{1}H](283 MHz; CD_3OD) 23.8 (app qn, *J* 10.1, NH*P*S); δ_C(176 MHz; CD_3OD) 149.2, 145.9, 138.2-138.0 (m, SCH_2C), 136.6, 129.6, 128.5, 126.9, 126.1, 121.0, 66.2 (O(*C*H_2)$_2$), 56.6 (N*C*H_2), 53.3 ((*C*H_2)$_2$N), 40.2 (*C*H_2NH), 29.9 (S*C*H_2), 27.3 (d, $^3J_{C\text{-}P}$ 7.5, *C*H_2CH_2NHP); *m/z* (ES^-) 380.1202 (M–H. $C_{17}H_{23}N_3O_3PS$ requires 380.1203).

32c



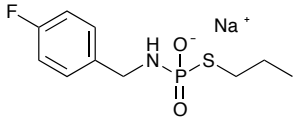
δ_H(700 MHz; CD_3OD) 3.70-3.66 (4 H, m, O(CH_2)$_2$), 2.91 (2 H, dt, *J* 9.6 and 6.8, SCH_2), 2.63 (2 H, dt, *J* 10.3 and 7.3, CH_2NH), 2.46 (4 H, br s, (CH_2)$_2$N), 2.44-2.40 (2 H, m, NCH_2), 1.75-1.65 (2 H, m, $CH_2CH_2CH_2$), 1.65-1.60 (2 H, m, CH_2CH$_3$), 0.97 (3 H, t, *J* 7.4, CH_2CH_3); δ_P[^{1}H](283 MHz; CD_3OD) 24.2 (app qn, *J* 9.9, NH*P*S); δ_C(176 MHz; CD_3OD) details for major conformer 66.3 (O(*C*H$_2$)$_2$), 56.8 (N*C*H$_2$), 53.4 ((*C*H$_2$)$_2$N), 40.2 (*C*H$_2$NH), 32.1 (S*C*H$_2$), 27.8-27.4 (m, $CH_2CH_2CH_2$), 24.2-23.8 (m, *C*H$_2$CH$_3$), 12.7-12.5 (m, CH_2CH_3); *m/z* (ES$^-$) 281.10967 (M–H. $C_{10}H_{22}N_2O_3PS$ requires 281.10943).

33a



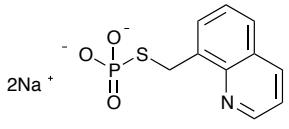
δ_H(700 MHz; CD_3OD) 8.79 (1 H, dd, *J* 4.2 and 1.7, Ar-H), 8.23 (1 H, dd, *J* 8.2 and 1.7, Ar-H), 7.81 (1 H, d, *J* 7.0, Ar-H), 7.74 (1 H, d, *J* 8.2, Ar-H), 7.47-7.44 (1 H, m, Ar-H), 7.42 (1 H, dd, *J* 8.2 and 4.2, Ar-H), 7.16-7.12 (2 H, m, Ar-H), 6.98 (2 H, t, *J* 8.8, Ar-H), 4.54 (2 H, d, *J* 11.1, CH_2NH), 3.73 (2 H, d, *J* 8.3, SCH_2); δ_P[^{1}H](283 MHz; CD_3OD) 23.6-23.4 (m, NH*P*S); δ_F(376 MHz; CD_3OD) –(118.8-119.0) (m, Ar-*F*); δ_C(176 MHz; CD_3OD) 162.4, 149.2, 145.8, 138.1-137.9 (m, PSCH$_2$*C*), 137.0-136.8 (m, *C*CH$_2$NHP), 136.6, 129.7, 129.0 (d, $^3J_{C\text{-}F}$, 8.0), 128.6, 126.9, 126.1, 121.0, 114.2 (d, $^2J_{C\text{-}F}$, 21.4), 45.0 (*C*H$_2$NH), 29.8 (S*C*H$_2$); *m/z* (ES$^-$) 361.0583 (M–H. $C_{17}H_{15}N_2O_2FPS$ requires 361.0581).

33c



δ_H(700 MHz; CD_3OD) 7.41-4.37 (2 H, m, Ar-H), 7.00-6.95 (2 H, m, Ar-H), 4.02 (2 H, d, *J* 9.4, CH_2NH), 2.61 (2 H, dt, *J* 10.3 and 7.3, SCH_2), 1.61 (2 H, app sx, *J* 7.4, CH_2CH$_3$), 0.95 (3 H, t, *J* 7.4, CH$_2$CH_3); δ_P[^{1}H](283 MHz; CD_3OD) 23.8 (app qn, *J* 9.8 NH*P*S); δ_F(376 MHz; CD_3OD) –(118.9-119.1) (m, Ar-*F*); δ_C(176 MHz; CD_3OD) 161.8 (d, $J_{C\text{-}F}$ 242.5, F*C*), 137.4 (d, $^3J_{C\text{-}P}$ 8.0, *C*CH$_2$NH), 129.0 (d, $^3J_{C\text{-}F}$, 8.0, 3-*C*H and 5-*C*H), 114.2 (d, $^2J_{C\text{-}F}$, 21.5, 2-*C*H and 6-*C*H), 45.0 (*C*H$_2$NH), 32.1 (S*C*H$_2$), 24.0 (d, $^3J_{C\text{-}P}$ 6.4, *C*H$_2$CH$_3$), 12.5 (CH$_2$*C*H$_3$); *m/z* (ES$^-$) 262.04744 (M – H. $C_{10}H_{14}NO_2FPS$ requires 262.04724)

34



δ_H(500 MHz; CD_3OD) 8.87-8.84 (1 H, m, Ar-H), 8.29-8.25 (1 H, m, Ar-H), 8.00-7.96 (1 H, m, Ar-H), 7.77-7.74 (1 H, m, Ar-H), 7.53-7.44 (2 H, m, Ar-H), 4.66-4.62 (2 H, m, SCH_2); δ_P[^{1}H](283 MHz; CD_3OD) 18.1 (t, $^3J_{H\text{-}P}$ 6.9, NH*P*S); δ_C(125 MHz; CD_3OD) 149.1, 146.1, 138.8 (d, $^3J_{C\text{-}P}$ 7.0, SCH$_2$*C*), 136.7, 130.1, 128.5, 126.4, 126.3, 120.7, 43.4 (*C*H$_2$NH); *m/z* (ES$^-$) 254.0047 (M–H. $C_{10}H_9NO_2PS$ requires 254.0046).

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Notes and references

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† Electronic Supplementary Information (ESI) available: [details of kinetic studies; ^{31}P NMR, ^{1}H, ^{31}P and ^{13}C NMR spectra of reported compounds; summary of biological testing data]. See DOI: 10.1039/b000000x/

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