Synthesis of Thiophosphoramidates in Water: Click Chemistry for Phosphates

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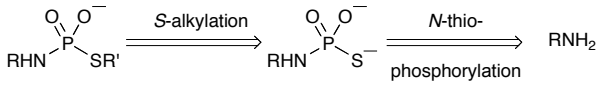
Abstract: **An aqueous method for the preparation of *N*,*S*-dialkyl thiophosphoramidates is reported. Thiophosphorylation of alkylamines was performed using $SPCl_3$ in aqueous reaction media, and the resulting thiophosphoramidate-*S*-anions were *S*-alkylated with soft electrophiles. Ranges of amines and electrophiles were explored.**

Phosphate esters and their structural analogues represent a major class of biomolecules that plays central roles in genetic transmission, membrane formation, signalling and metabolism. The synthesis of phosphate esters and their analogues has been revolutionized by the phosphoramidite method.[1] However, the preparation of phosphoramidites is often time-consuming, requiring global protection and anhydrous conditions. Thereafter, the formation of phosphate esters requires anhydrous conditions and a range of reagents that are highly effective in automated oligonucleotide synthesizers, but cumbersome in general laboratory usage.

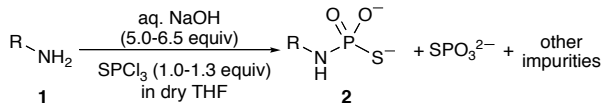
We sought to overcome these limitations by developing aqueous "click" methods that employ off-the-shelf reagents for the preparation of *N*,*S*-dialkyl thiophosphoramidates that represent simple mimics of phosphate diesters. Building on the use of phosphoryl chloride ($OPCl_3$) for the preparation of *N*-phosphorylated amines,[2-4] we began to investigate the use of thiophosphoryl chloride ($SPCl_3$) for the preparation of *N*-thiophosphorylated amines, which could then be elaborated, through *S*-alkylation, to give *N*,*S*-dialkyl thiophosphoramidates (Scheme 1). A key aim was to ensure clean conversions, where the requirement for time-consuming ion exchange or HPLC purifications that can often hamper the preparation of phosphate esters was significantly reduced or removed.



Scheme 1 Retrosynthetic strategy for the preparation of *N*,*S*-dialkyl thiophosphoramidates in aqueous solvent mixtures.

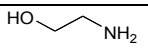
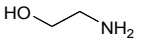
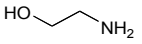
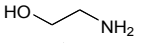
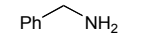
Our approach hinges on exploiting the greater intrinsic nucleophilicities of amines in comparison to water. Thus, through control of amine concentrations and other experimental parameters, we hoped to be able to induce selective *N*-thiophosphorylation of amines versus hydrolysis of the thiophosphorylating agent. Similar approaches have been used for the preparation of carboxylic amides and sulfonamides,[5] but, surprisingly, this approach has not been exploited significantly with (thio)phosphoric amide systems.

We first explored conditions for thiophosphorylation of the model amines ethanolamine and benzylamine with $SPCl_3$ (Scheme 2 and Table 1).



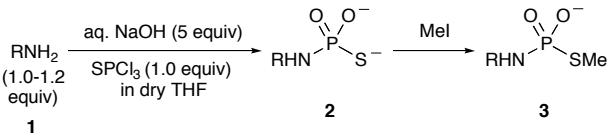
Scheme 2 Optimisation of aqueous *N*-thiophosphorylation.

Table 1 Screening thiophosphorylation conditions[a]

entry	substrate **1**	eq. of $SPCl_3$	**2**, %
1		1	98[b], 94[c]
2		1.1	96[b], 93[c]
3		1.2	92[b], 86[c]
4		1.3	91[b], 94[c]
5		1.0	97[b], >99[c]

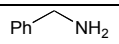
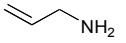
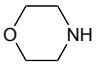
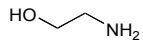
[a] See Supporting Information for details. [b] Determined by ^{31}P NMR. [c] Determined by ^{1}H NMR.

We used THF as a co-solvent given that $SPCl_3$ appears to display very limited solubility in water. In all cases, satisfactory conversions (mostly>90%) to thiophosphoramidate **2** were obtained. Varying the numbers of equivalents of $SPCl_3$, aiming to enhance conversions in the case of ethanolamine, afforded no improvements (entries 2-4). We also explored the number of equivalents of NaOH employed, but we found that 5 equiv represented the optimum number (data not shown). The only significant by-product from these *N*-thiophosphorylation procedures was inorganic thiophosphate, which likely arose because of the use of high concentrations of hydroxide ion causing competitive hydrolysis of the thiophosphorylating agent. When using non-polar amines, that could be readily extracted into organic solvents, the use of an excess of the amine substrate over $SPCl_3$ helped to mitigate against this problem (data not shown). Successful *N*-thiophosphorylation using 1 equiv of $SPCl_3$ with 1.0-1.2 equiv of amine substrate prompted us to explore *S*-alkylation of the *N*-thiophosphoramidate ions **2** with MeI in a one-pot procedure (Scheme 3). In all cases (Table 2, entries 1-4), conversions to *N*-alkyl *S*-methylthiophosphoramidate after simple extraction of excess MeI were high (>90%).



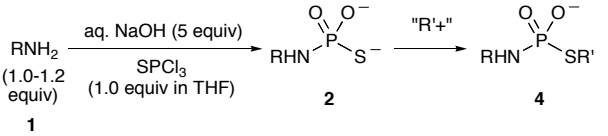
Scheme 3 Preliminary studies on *S*-alkylation of *N*-thiophosphoramidate ions using MeI.

Table 2 *N*-Thiophosphorylation of amines and *S*-methylation of *N*-thiophosphoramidates

entry	substrate **1**	conversion to *N*-alkyl *S*-methylthiophosphoramidate **3**, %
1		98[a], 94[b]
2		97[a], 96[b]
3		97[a], 91[b]
4		93[a], 80[b]

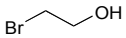
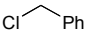
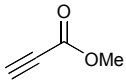
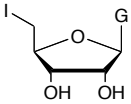
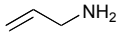
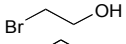
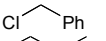
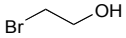
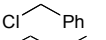
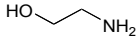
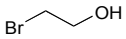
[a] Determined by ^{31}P NMR. [b] Determined by ^{1}H NMR.

Expanding the range of *S*-alkylating agents, we found that much less electrophilic alkylating agents were also effective (Scheme 4).



Scheme 4 Combined, one-pot *N*-thiophosphorylation/*S*-alkylation using ranges of amines and alkylating agents.

Table 3 *S*-alkylation of *N*-thiophosphoramidates

N-alkyl-thiophos-phoramidate	entry	alkylating agent	reaction time, h[a]	conversion to *N*,*S*-dialkyl thiophosphoramidate **4**
	1		17	>99[b], >99[c]
	2		22	>99[b], >99[c]
	3		25	>99[b], >99[c]
	4		96[d,e] (24)[e,f,g]	92[b], 92[c] (90[b], 89[c])
	5		5[h]	96[b], 97[c]
	6		19	90[b,i]
	7		5	93[b], 96[c]
	8		~80[d,f,j]	82[b]
	9		1.5	>99[b], 95[c]
	10		23	>99[b], >99[c]
	11		120	>99[b], 98[c]
	12		1	92[b], 87[c]
	13		4[g]	93[b], 85[c]
	14		7[g]	98[b], 99[c]
	15		17[e,f,g]	94[b], 89[c]
	16		20	91[b], 78[c]

[a] All alkylations were performed at room temperature except where stated. The amine was employed at 1.2 equiv except for entry 16, where 1.0 equiv was used. [b] Determined by ^{31}P NMR. [c] Determined by ^{1}H NMR. [d] Alkylation reaction heated at 50 °C. [e] Additional NaOH was added with alkylating agent. [f] Alkylating agent added over several portions. [g] Alkylation reaction heated at 80 °C. [h] Reaction mixture was neutralized with HCl prior to solvent removal. [i] A mixture of isomers (63:26) was obtained from opening of the epoxide ring, where attack at the less hindered end was favored. [j] Morpholine was used in 20% excess over $SPCl_3$ during the *N*-thiophosphorylation step. [j] Alkylating agent displays poor solubility in organic solvents, therefore, excess alkylating agent was removed by chromatography.

Pleasingly, across a range of amine substrates and alkylating agents, including halides, epoxides and conjugate acceptors, conversions were extremely good (mostly>90%). The only exception to this was the use of 5′-deoxy-5′-iodoguanosine, where a combination of steric, electronic and solubility factors make the 5′-iodide remarkably unreactive. Even in this unfavourable case, reasonable conversion (82%) was observed.

Our method allows for the facile, aqueous preparation of *N*,*S*-dialkyl thiophosphoramidates. Conversion levels are extremely high, and impurities are, in most cases, limited to small amounts of unconverted starting materials, and *S*-alkylated thiophosphate ions. Unlike many conventional organic-solvent-based methods, we have used a predominantly aqueous organic solvent mixture that supports the intrinsic solubility of the ionic phosphoramidate products. Furthermore, given this intrinsic solubility, there is no

requirement to remove the aqueous solvent after reactions, thus diminishing the environmental impact of the methodology. In addition, the reactions procedures are easy to perform using readily available materials, conversion levels are high, obviating the need for chromatography, and side-products (inorganic thiophosphate, NaCl and excess NaOH) are innocuous or readily neutralised. In summary, our method fulfils Sharpless's criteria for "Click" processes within the limits of the range of experiments that we have performed.[6] These factors should be taken in the context of phosphate ester mimics normally presenting significant synthetic challenges.[7-9]

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Notes and references

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† Electronic Supplementary Information (ESI) available: general experimental procedures and ^{1}H and ^{31}P NMR spectra of reaction products. See DOI: 10.1039/b000000x/

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