# "RS$^+$" as a Coupling Reagent for Phosphorylation and Carboxylic Acid Activation

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*Dedicated to Prof. Sho Ito on the occasion of his 77th birthday*

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Phenylsulfenyl chloride, a sulfenium ion transfer reagent, promotes the formation of pyrophosphate from tetrabutylammonium phosphate, under controlled "pH" conditions, in yields of up to 85%. Dichloroacetic and succinic acids, in the presence of phenylsulfenyl chloride and a tertiary amine, form the corresponding anhydrides in very good yields. The treatment of dichloroacetic acid or *p*-nitrobenzoic acid with phenylsulfenyl chloride in the presence of a tertiary amine, followed by the addition of aniline, affords the corresponding anilides in yields of up to 42%.

## Introduction

The activation of acids (carboxylic acids or phosphoric acids) towards the formation of ester or amide bonds is of great interest both for chemists and for biologists. Although the non-enzymatic formation of the amide bond has been studied intensely, research in this field is still very active even now.[1] Our ongoing studies on the chemistry of electrophilic sulfenylating species prompted us to investigate the activation of acids using sulfenium ion transfer reagents.[2] In the case of carboxylic acids, the general reaction is the transfer of a sulfenium ion to the acid anion, resulting in the formation of a reactive sulfenic-carboxylic anhydride[3] (or sulfenyl carboxylate), which in turn reacts with the desired nucleophile to give the final product (Scheme 1).

$$\text{R-COO}^- + [\text{R'S}^+] \longrightarrow \text{R-COO-SR'} \xrightarrow{\text{HNu}} \text{R-CO-Nu} + \text{R'SOH}$$

Scheme 1

The first synthesis of a sulfenyl acetate was reported by Kharasch in 1956.[4] The product was obtained by the treatment of 2,4-dinitrobenzenesulfenyl chloride with sodium acetate, and this synthesis was then extended to other sulfenyl carboxylates.[5] Later, Barton showed that the sulfenyl group of sulfenyl acetate derivatives may be considered as a protecting group for carboxylic acids, since it can be removed by photolysis under neutral and mild conditions.[6] Since then the reaction of sulfenyl halides with carboxylates has been neglected. We argued that if more reactive sulfenium ions were used, the obtained sulfenyl carboxylates might be so reactive that they could be considered as intermediates. Sulfenyl halides are a very common and easily accessible source of sulfenium ions and we decided to explore the potential of sulfenic-carboxylic anhydrides as intermediates by using phenylsulfenyl chloride.

Non-enzymatic phosphorylation, the general formation of P−O−P bonds, has been much less widely investigated than the activation of carboxylic acids. For the transformation of AMP into ADP, or of ADP into ATP, an activated form of phosphate, such as the acetyl phosphate, in the presence of a catalyst is generally used. Hosseini and Lehn, for example, used a protonated macrocyclic polyamine as a catalyst.[7] In addition, $Fe^{III}$ ions have been shown to catalyze the formation of ATP from ADP in the presence of acetyl phosphate.[8]

Here we report our preliminary results on the treatment of phenylsulfenyl chloride: (i) with orthophosphoric acid to form pyrophosphoric acid, and (ii) with carboxylic acids to give either the corresponding anhydrides or, by subsequent addition of aniline, the corresponding amides.

## Results and Discussion

### Formation of the PO-P bond

To avoid water, the reactions were carried out using tetrabutylammonium phosphate, which is soluble in organic solvents, and phenylsulfenyl chloride in a dry acetonitrile/tetrahydrofuran mixture.[9] The hypothetical reaction sequence is reported in Scheme 2.

1) $H_2PO_4NBu_4 + PhSCl \longrightarrow Ph{-}S{-}O{-}\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}{-}O^- \; Bu_4N^+ + HCl$

2) $Ph{-}S{-}O{-}\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}{-}O^- \; Bu_4N^+ + H_2PO_4NBu_4 \longrightarrow$

$$^-O{-}\overset{\overset{O}{\|}}{\underset{\underset{O}{|}}{P}}{-}O{-}\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}{-}O^- \; 2\,Bu_4N^+ + PhSOH$$

3) $PhSOH + PhSCl \longrightarrow Ph{-}S{-}\overset{\overset{O}{|}}{S}{-}Ph + HCl$

Scheme 2

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Assuming that two equivalents of hydrochloric acid are formed and that pyrophosphoric acid ($H_4P_2O_7$) is a stronger acid than orthophosphoric acid ($H_3PO_4$),[10] we decided to control the "pH" by adding a base to ensure the presence of the orthophosphate anion. All the reactions were monitored by $^{31}P$ NMR spectroscopy with selective proton decoupling. In a typical experiment, phenylsulfenyl chloride (1.0 equiv. or more, as reported in Table 1) dissolved in 1.0 mL of acetonitrile was added dropwise to a solution of tetrabutylammonium phosphate (1.0 mmol) in 10 mL of dry 3:1 acetonitrile/tetrahydrofuran. At the same time a base was added in order to maintain a formal "pH" above 5.[11] The amounts of the various compounds present in the sample at different times were determined by integration of the $^{31}P$ NMR signals. In some experiments the yield was confirmed by preparative TLC on SIL-HR.

Table 1. Treatment of tetrabutylammonium dihydrogenphosphate (0.1 M) with phenylsulfenyl chloride in the presence of trioctylamine in acetonitrile/tetrahydrofuran (3:1) at room temperature

| # | PhSCl, equiv. | "pH" | Pyrophosphate yield %[a] |
|---|---|---|---|
| 1 | 1.0 | 6.0 | 57.0[b] |
| 2 | 0.5 | 6.5 | 31.0 |
| | 1.0 | 6.5 | 56.0 |
| | 1.5 | 6.5 | 65.6[c] |
| 3 | 0.5 | 7.0 | 26.0 |
| | 1.0 | 7.0 | 48.0 |
| | 1.5 | 7.0 | 60.0 |
| | 2.0 | 7.0 | 85.0[c] |

[a] The yield was determined by $^{31}P$ NMR spectroscopy. – [b] The yield was determined by preparative TLC on glass plates of silica gel SIL-HF. – [c] Traces of polyphosphates were observed in the $^{31}P$ NMR spectrum.

We tested a number of different bases: ammonium hydroxide, tetrabutylammonium hydroxide, tetramethylammonium hydroxide, pyridine, 2,6-di-*tert*-butylpyridine, trioctylamine, 1,8-bis(dimethylamino)naphthalene, sodium hydrogencarbonate and potassium carbonate. Of these, trioctylamine proved to be the best, and the data obtained with this base are reported in Table 1. The other bases were rejected due to various problems: ammonium hydroxide, for example, is too hygroscopic, 2,6-di-*tert*-butylpyridine is unable — even in large excess — to control the "pH", and in the presence of carbonate anions the phosphates were adsorbed onto the solid salt.

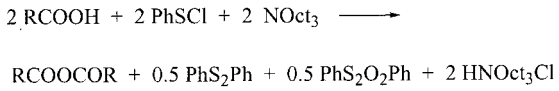
The data reported in Table 1 show a very modest effect of the pH on the product yield in the "pH" range 6–7. However, it is of fundamental importance to buffer the reaction mixture with an appropriate base, because in its absence the HCl produced protonates the orthophosphate, thus sequestering the nucleophile. Indeed, experiments carried out without the addition of trioctylamine produced very low yields of pyrophosphate (<20%).

Very good yields of pyrophosphate (up to 85%) were obtained by using an excess of phenylsulfenyl chloride. This clearly indicates that a proportion of this reagent is consumed in side reactions, such as reaction with any water present. Indeed, reactions carried out in the presence of added water showed no pyrophosphate formation.

It is worth pointing out that, at room temperature, phenylsulfenyl chloride reacts instantaneously with the reagent (disappearance of the orange color in the time taken for mixing) and that, on using two equiv. of reactant, traces of polyphosphates are observed in the $^{31}P$ NMR spectrum as the pyrophosphate competes with the orthophosphate as a nucleophile at high degrees of conversion of orthophosphate.

### Formation of C(O)–OR and C(O)–NHR bonds

The activation of carboxylic acid towards the formation of the symmetric anhydride was tested with dichloroacetic acid (p$K_a$ 1.48) and succinic acid (p$K_{a1}$ 4.16, p$K_{a2}$ 5.61; Scheme 3).




Scheme 3

The reactions were carried out as described for the phosphorylation, using trioctylamine or diisopropylethylamine for "pH" control. Under these conditions, the dichloroacetic acid was present in large part as the anion, which may act as a nucleophile. The results are reported in Table 2. For the succinic acid, proximity effects favored the intramolecular formation of the succinic anhydride, which, when an excess of phenylsulfenyl chloride was used, was obtained in very good yields of up to 88%. Attempts to obtain acetic anhydride failed, probably because the concentration of acetate was too low at a formal pH of 5–6.

Table 2. Treatment of carboxylic acids (0.1 M) with phenylsulfenyl chloride in the presence of trioctylamine in dichloromethane, at room temperature

| # | Carboxylic Acid | PhSCl, equiv. | Anhydride Yield %[a] |
|---|---|---|---|
| 1 | Dichloroacetic acid | 1.0 | 20.1 |
| 2 | Succinic acid | 1.0 | 41.2 |
| 3 | Succinic acid | 2.0 | 88.7 |

[a] The yield was determined from the isolated product.

If the activation of the carboxylic acid is followed by the addition of a nucleophilic amine, the corresponding amide is obtained (Scheme 4). Table 3 reports the data relating to reactions carried out using aniline as nucleophile. In this process, a key factor is the order of addition of the reagents. The nucleophilic amine has to be added after the formation of the reactive anhydride, otherwise only the phenylsulfenamide is formed.

$$2\ RCOOH + 2\ PhSCl + 3\ NOct_3 + PhNH_2 \longrightarrow$$

$$RCONHPh + RCOONOct_3H + 0.5\ PhS_2Ph + 0.5\ PhS_2O_2Ph + 2\ NOct_3HCl$$

Scheme 4

Table 3. Treatment of carboxylic acids (0.1 M, 1 equiv.) with phenylsulfenyl chloride in the presence of trioctylamine or diisopropylethylamine and subsequent addition of aniline (1 equiv.) in dichloromethane, at room temperature

| # | Carboxylic Acid | PhSCl equiv. | Amide Yield %[a] |
|---|---|---|---|
| 1 | Dichloroacetic acid | 1.0 | 18.0 |
| 2 | Dichloroacetic acid | 2.0 | 42.0 |
| 3 | 4-Nitrobenzoic acid | 1.0 | 19.0 |
| 4 | 4-Nitrobenzoic acid | 2.0 | 38.0[b] |

[a] The yield was determined from the isolated product. – [b] The reaction was carried out in the presence of diisopropylethylamine.

## Conclusion

The data reported show that "PhS$^{+}$" is a very good coupling agent for forming the P–O–P bond probably via the intermediate sulfenic-phosphoric anhydride. Analogously, the sulfenium ion is able to promote the formation of anhydrides from carboxylic acids and, in the presence of amines, the corresponding amides.

An alternative source of "RS$^{+}$" also suitable for this type of reaction is represented by the disulfide radical cations readily prepared by one-electron oxidation of the parent disulfides.[12] It has been reported, for example, that disulfide radical cations may be used in electrophilic aromatic substitutions.[13] These reagents are probably preferable to sulfenyl halides for carboxylate and phosphate activation in laboratory preparations, and studies in this direction are in progress.

If our results confirm this hypothesis, then this finding may also be relevant for the biological activation of phosphates. Indeed, in biological systems there are a large number of disulfides present in hydrophilic or hydrophobic pockets, and these compounds may easily be oxidized to disulfide radical cations by several oxidizing systems. We may therefore speculate that the activation of phosphates by a disulfide radical cation might also be operative in natural processes.

## Experimental Section

**General:** $^{1}$H NMR spectra were recorded on Bruker AC 200 or Bruker AC 250 spectrometers operating at 200.13 and 250.18 MHz, respectively; the signal of the residual protons in the deuterated solvent was used as reference. $^{31}$P NMR spectra were measured with a Bruker AC 200 operating at 81.0 MHz and the signal of 85% $H_3PO_4$ was used as external reference ($\delta = 0$); $^{13}$C NMR spectra were measured with a Bruker AC 250 operating at 62.9 MHz and the signals of the solvent were used as reference. The pH was measured with a Metrohom pH meter. Chromatographic separation of monophosphates and diphosphates for quantitative determination was carried out on 20 × 20 SIL G 25 HR Macherey–Nagel glass plates. Commercial reagents and known compounds were purchased from standard chemical suppliers or prepared according to literature procedures and purified to match the reported physical and spectroscopic data. Solvents were purified according to standard procedures.

**General Procedure for the Treatment of Tetrabutylammonium Dihydrogenphosphate with Phenylsulfenyl Chloride:** The reactions were carried out under nitrogen in a reactor equipped with a magnetic bar, a dropping funnel, and a glass electrode, using dry solvents at room temperature. Benzenesulfenyl chloride dissolved in acetonitrile (1.0 mmol in 1 mL) was added dropwise to a solution of tetrabutylammonium dihydrogenphosphate (0.34 g, 1.0 mmol) in acetonitrile/tetrahydrofuran (3:1). The formal pH was kept constant at about 6 by dropwise addition of trioctylamine. The reaction was analyzed by $^{31}$P NMR spectroscopy, sampling the reaction mixture without further dilution. The yields were determined taking account of the fact that the sum of all the integrated phosphorus signals had to be constant. For determination of the yield from isolated products the reaction mixture was washed with water (2 × 20 mL). The organic phase was then washed to neutrality with a solution of 5% $KHSO_4$ (3 × 20 mL), brine (20 mL), and deionized water. The product was then isolated by preparative TLC on SIL-HR silica, following the procedure reported in the literature,[14] and was obtained in 57% yield (0.19 g).

**$Bu_4NH_2PO_4$:** $^{1}$H NMR (200 MHz, $D_2O$): $\delta = 0.90$ (t, $J = 7.2$ Hz, 3 H), 1.31 (m, 2 H), 1.60 (m, 2 H), 3.15 (m, 2 H). – $^{31}$P NMR (81 MHz, $D_2O$): $\delta = 3.17$ (s).

**$(Bu_4N)_2HPO_4$:** $^{1}$H NMR (200 MHz, $D_2O$): $\delta = 0.90$ (t, $J = 7.2$ Hz, 3 H), 1.31 (m, 2 H), 1.60 (m, 2 H), 3.15 (m, 2 H). – $^{31}$P NMR (81 MHz, $D_2O$): $\delta = 3.39$ (s).

**General Procedure for the Treatment of Carboxylic Acids with Phenylsulfenyl Chloride:** The reactions were carried out in dry dichloromethane, using the same apparatus as described above. Benzenesulfenyl chloride was added dropwise to a solution of acid (1.0 mmol) and trioctylamine (1.0 mmol; or diisopropylethylamine, DIEA) in 10 mL of dry dichloromethane. Concomitant addition of trioctylamine (or DIEA) allowed the formal pH to be controlled at a value of about 6.5–7.0. The reactions were monitored by $^{1}$H NMR spectroscopy, suppressing the solvent peak, and by TLC. After workup (as described above) the products were purified by radial chromatography on silica gel (Merck 60 TLC PF 254). Dichloroacetic anhydride was isolated in 20.1% yield (0.022 g), using one equivalent of PhSCl. Succinic anhydride was obtained in 41.2% yield (0.045 g) and 88.7% yield (0.097 g), respectively, when using 1 equivalent and 2 equivalents of PhSCl.

When aniline (1.0 equivalent with respect to PhSCl) was added to the reaction mixture five minutes after the addition of phenylsulfenyl chloride, anilides were obtained. Dichloroacetic anilide was obtained in 18% yield (0.037 g) and in 42% yield (0.085 g) , respectively, when using 1 and 2 equivalents of PhSCl. The anilide of 4-nitrobenzoic acid was isolated in 19% yield (0.046 g) and in 38% yield (0.092 g), respectively, using 1 and 2 equivalents of PhSCl.

**2,2,-Dichloro-*N*-phenylacetamide:** $^{1}$H NMR (200 MHz, $CD_3CN$): $\delta = 6.25$ (s, 1 H, $CHCl_2$), 7.18 (m, 1 H), 7.37 (m, 2 H), 7.56 (m, 2

H), 8.80 (broad s, 1 H, NH). – $^{13}$C NMR (62.9 MHz, $CD_3CN$): δ = 67.6, 120.7, 125.5, 129.4, 137.6, 162.4.

***N*-Phenyl-4-nitrobenzamide:** $^1$H NMR (250 MHz, DMSO): δ = 7.35 (t, *J* = 7.13 Hz, 1 H), 7.37 (dd, *J* = 7.35 Hz, *J* = 8.05 Hz, 2 H), 7.76 (d, *J* = 8.05 Hz, 2 H), 8.17 (d, *J* = 8.70 Hz, 2 H), 8.35 (d, *J* = 8.70 Hz, 2 H), 10.55 (s, 1 H, NH). – $^{13}$C NMR (62.9 MHz, $CD_3CN$): δ = 120.6, 123.7, 124.4, 128.9, 129.4, 138.9, 140.8, 149.3, 164.0.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