N-Alkyl (Aryl) Sulfonylphosphoramidate Monoesters

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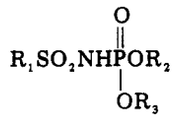
The syntheses and chemical and enzymatic properties of four N-substituted sulfonylphosphoramidate monoesters (I) are presented. These compounds, which were prepared from N-substituted sulfonylphosphoramidate diesters by dealkylation using sodium iodide, are electronic analogues of phosphomonoesters. Their first pKs lie in the range from 1 to 2 and the second in the range from 5.5 to 7. The compounds are not substrates for alkaline phosphatases from two different sources but are weak competitive inhibitors (K_is $\approx 10^{-3}$ M).

In the course of research on the synthesis and properties of analogues of phosphomonoesters, we recently investigated monoesters of N-substituted phosphoramidate (I).



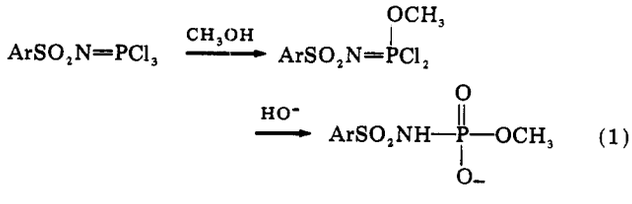
I, R_1 = alkyl or aryl; R_2 = alkyl; R_3 = H
II, R_1 = alkyl or aryl; R_2, R_3 = alkyl
III, R_1 = alkyl or aryl; R_2, R_3 = H

Gilyarov et al.[2] and Izako et al.[3] have shown that the corresponding diesters (II) have pK_a values ranging from 1.49 to 2.36; these pK_a values are in the range of the first pK_a for orthophosphoric acid, 2.12.[4] We expected, on the basis of electrostatic effects, that the removal of one ester group from the diesters would give monoesters with two ionization constants closely resembling those of monoesters of phosphoric acid; they should have pK_a values in the ranges 1–2 and 5.5–7.[4]

Although a variety of methods serve to synthesize N-substituted phosphoramidic acids (III) and their diesters (II),[5] no systematic approach to the series of monoesters (I) had previously been devised. The corresponding diesters are hydrolytically stable in neutral solution and decompose only very slowly in alkaline media.[6] Thus, although phosphoramidate monoesters can be prepared from the corresponding diesters by alkaline cleavage,[7] the N-substituted sulfonylphosphoramidate monoesters cannot be made in the same fashion. Undoubtedly the anionic character of the starting diesters severely inhibits nucleophilic attack by water or hydroxide ion on the phosphorus atom. The same inhibitory effect is observed in the alkaline hydrolysis of simple phosphodiesters.[8]

An alternative possibility for the preparation of N-substituted sulfonylphosphoramidate monoesters, namely, acid-catalyzed hydrolysis of diesters, yields mostly products resulting from P–N cleavage,[6] i.e., sulfonamide derivatives and diesters of phosphoric acid.

Kirsanov et al.[9] have published the only reported synthesis of a monoester of N-substituted sulfonylphosphoramidate. They obtained it from partial alcoholysis of a trichloro N-sulfonylphosphorimidate, followed by hydrolysis of the intermediate (eq 1). However, the difficulties inherent in partial



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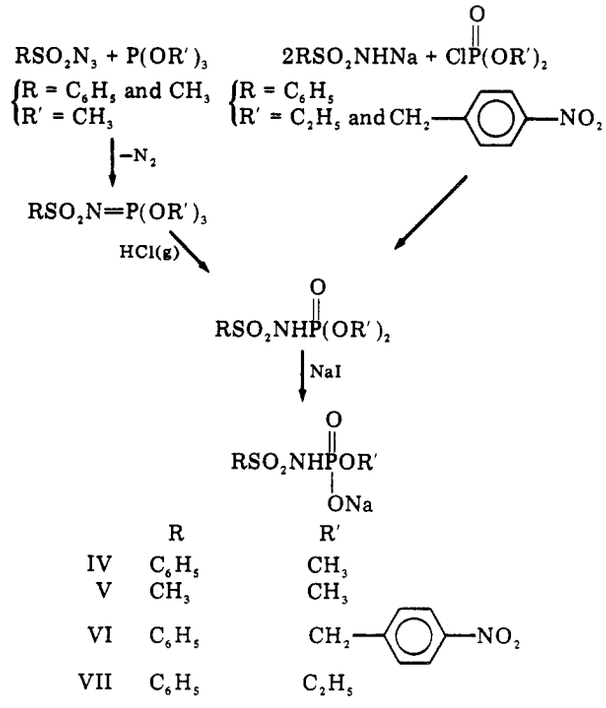
alcoholyses of trihalophosphoryl derivatives and the apparent limited applicability and low yields of Kirsanov's method prompted us to search for a more general and efficient synthetic method; ideally, such a method would use the readily accessible diesters II as intermediates.

This paper reports that dealkylation of four diesters of N-substituted sulfonylphosphoramidate by sodium iodide in acetone gives moderate to high yields of the easily purified monoesters and, in fact, constitutes an efficient method of entry into the monoester series. The new compounds were compared to simple phosphomonoesters with respect to their pK_a values and their effects as inhibitors for alkaline phosphatase were measured.

Results and Discussion

Scheme I presents the method of synthesis used for the preparation of the title compounds. The precursor diesters,

Scheme I



	R	R′
IV	C_6H_5	CH_3
V	CH_3	CH_3
VI	C_6H_5	CH_2–C_6H_4–NO_2
VII	C_6H_5	C_2H_5

two of which had previously been reported (see Experimental Section), were prepared by two different routes. The first route, used by Gilyarov et al.,[2] involved the reaction of trimethyl phosphite with the appropriate alkyl or arylsulfonyl azide to give the intermediate trimethyl N-substituted sulfonylphosphorimidates which were easily dealkylated by gaseous HCl to the diester; the second route involved the direct condensation of the appropriate diester chlorophosphate with the sodium salt of benzenesulfonamide and is based on the work of Rätz.[10]

The final dealkylation of the diester by sodium iodide was carried out following standard procedures for this reaction;[11]

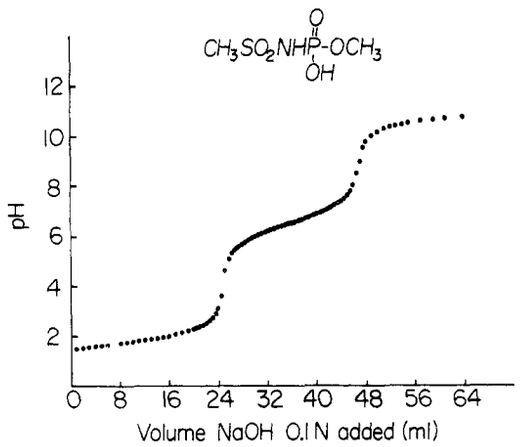


Figure 1. Titration curve for methyl *N*-methanesulfonylphosphoramidate (V). Conditions: 25 °C, aqueous solution.

Table I. Ionization Constants for N-Substituted Sulfonyl Phosphoramidate Monoesters and Selected Model Compounds

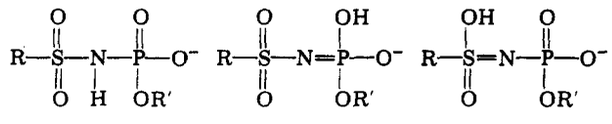
Compd	pK_a(I)	pK_a(II)
IV	1.05 ± 0.3	6.25 ± 0.2
V	1.16 ± 0.2	6.55 ± 0.2
VI	1.62 ± 0.1	5.90 ± 0.1
VII	1.80 ± 0.4	6.50 ± 0.4
Inorganic phosphate[4]	2.12	7.21
Methyl phosphate[4]	1.54	6.31
Ethyl phosphate[4]	1.60	6.62

the solvent of choice was acetone since both starting materials were soluble in it but the product monoester was not and conveniently precipitated in the course of the reaction. The success of this reaction depended critically on fully protonating the starting diester, since attempts to dealkylate the sodium salt of di-*p*-nitrobenzyl-*N*-benzenesulfonyl phosphoramidate met with failure. The inhibitory effect of a negative charge on the attack by iodide ion has been observed before[12] and can be explained on the basis of electrostatic repulsion.

Details of the synthetic procedures for new compounds are given in the Experimental Section.

The results in Table I and Figure 1 show that the newly prepared monoesters of *N*-alkyl (or aryl) sulfonylphosphoramidate behave as dibasic acids in aqueous solution. The pK_as for all four compounds are similar to the first two pK_as of inorganic phosphate and of phosphomonoesters. Thus, although a dialkyl phosphate group lowers the pK_a of methanesulfonamide (pK_a = 10.8[13]) by about 9 pK_a units,[2,3] a monoalkyl monoanion phosphate group lowers it only 4.3 units. This difference in acidity of about 5 powers of ten is consistent with the difference in acidity between the first ionization constant of phosphoric acid and the second one, or the first and second ionization constants of phosphate monoesters. It reflects the effect of a full negative charge on the loss of a second proton from the same molecule.

The microscopic site of protonation (or deprotonation) of the monoesters, however, is not defined by these data and will depend on the relative populations of three different tautomers:



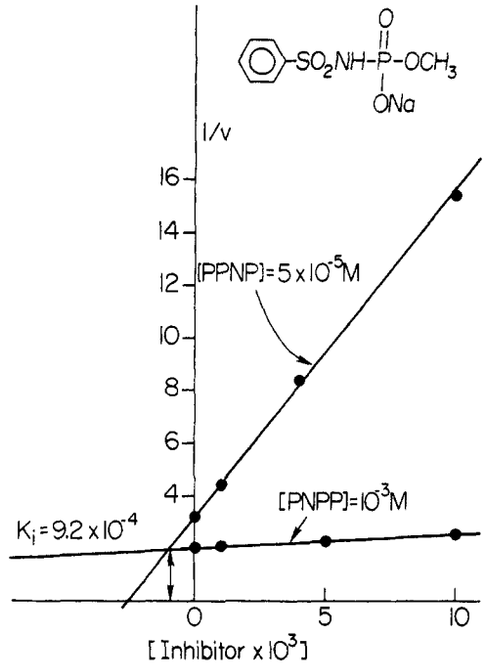


Figure 2. Dixon plot for the inhibition of alkaline phosphatase from *E. coli* by methyl *N*-benzenesulfonylphosphoramidate (IV). PNPP: *p*-nitrophenyl phosphate. Assays were run under standard conditions for the enzyme[21] in 1 M Tris HCl buffer, pH 8.0, at 25 °C.

Kabachnik et al.[14] and Matrosov et al.[15] have discussed the tautomeric distribution in N-substituted sulfonylphosphoramidate diesters and concluded, on the basis of infrared evidence, that the amide form [-NHP(=O)(OR)$_2$] predominates over the imidol form [-N=P(OH)(OR)$_2$] in these compounds. An analogous conclusion might be drawn for the monoester series, based on Kabachnik and Matrosov's assignment of infrared bands and our available spectroscopic data. However, since these authors failed to take into account possible effects on the frequencies of the sulfonyl group, and since their assignments depend on subtle differences in absorption in the region of 1200–1400 cm^{-1}, where the monoesters show two to three broad bands, we refrain here from reaching a definite conclusion on this subject.

We took advantage of the observation that the synthetic *N*-alkyl (or aryl) sulfonylphosphoramidate monoesters exist as dianions at pH 8 and tested their action as inhibitors for alkaline phosphatase from two different sources. These enzymes are phosphomonoesterases with a strict requirement that their substrates and competitive inhibitors[16] be dianions. Dixon plots[17] were used to discriminate between competitive and other forms of inhibition and to measure the inhibition constants. The results are presented in Table II and Figures 2 and 3.

The compounds are indeed recognized by the active sites as indicated by their behavior as competitive inhibitors. In addition, compounds IV, V, and VI were tested as pseudosubstrates for alkaline phosphatase from *E. coli*, by incubating them in the presence of enzyme and assaying for the release of alcohol in each case (VPC was used to detect the product from IV and V; UV was used for VI). The results were disappointingly negative since no alcohol could be detected in any of the experiments. Although the newly synthesized dianionic compounds bind at the active site, they do not fulfill the requirements for successful enzymatic P–O cleavage, and are thus not hydrolyzed.

Finally it should be pointed out that our method of preparation of N-substituted sulfonylphosphoramidate monoesters from the corresponding diesters will not be successful for ar-

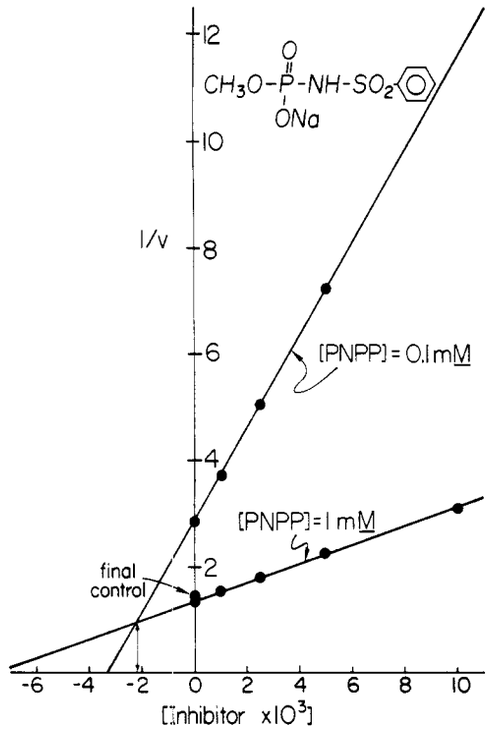


Figure 3. Dixon plot for the inhibition of alkaline phosphatase from chicken intestine by methyl *N*-benzenesulfonylphosphoramidate (IV). PNPP:*p*-nitrophenyl phosphate. Assays were run under analogous conditions as for the *E. coli* enzyme (see Figure 2).

Table II. Competitive Inhibition Constants for N-Substituted Sulfonylphosphoramidate Monoesters with Alkaline Phosphatases

Compd	K_i, M
IV[a]	9.2×10^{-4}
V[a]	2.4×10^{-2}
VI[a]	3.5×10^{-3}
VII[a]	4.8×10^{-3}
IV[b]	2.2×10^{-3}

[a] Enzyme from *E. coli*; $K_m = 2.65 \times 10^{-5}$ M. [b] Enzyme from chicken intestine; $K_m = 1.1 \times 10^{-4}$ M.

omatic residues, since the dealkylation reaction by iodide ion limits the method to aliphatic groups. This limitation, however, does not necessarily rule out the preparation of N-substituted sulfonylphosphoramidate aromatic monoesters. If the appropriate sulfonylphosphoramidate mixed aromatic–aliphatic diesters can be prepared, it should be possible, in principle, to apply our dealkylation reaction to them. The synthesis of asymmetric phosphate diesters by an improved method recently presented by Ramirez,[18] followed by standard chlorination procedures[19] to give the mixed chlorophosphate diesters, might yield the needed precursors.

Experimental Section

Melting points were taken on a Thomas-Hoover Uni-Melt apparatus and are uncorrected unless otherwise specified. pH was measured with a Radiometer pH meter Type TTT1C. Ultraviolet–visible spectra were taken on a Gilford 240 or a Cary 15 spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 137 sodium chloride spectrophotometer; spectra of aqueous samples were taken in Silanor (Merck). Vapor phase chromatography (VPC) was performed on an F & M Scientific, Hewlett-Packard 5750 research instrument. Elemental analyses were performed by Spang Microanalytical Laboratory and Dornis und Kolbe (West Germany).

Alkaline phosphatase from *E. coli* was obtained from Worthington and assayed by standard procedures.[20] Alkaline phosphatase from chicken intestine was Boehringer's. All commercial reagents and solvents were of the best available purity and were further purified in most cases by standard methods.[21]

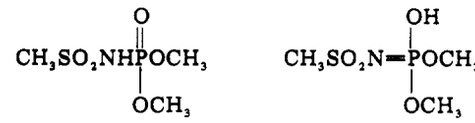
Trimethyl *N*-Benzenesulfonylphosphorimidate. Benzenesulfonyl azide in 50-g batches was prepared by the method of Boyer et al.,[22] using dry acetonitrile as solvent, instead of methanol. Freshly distilled trimethyl phosphite (1 equiv) was added drop by drop to a well-stirred solution of benzenesulfonyl azide in diethyl ether at room temperature. Gas evolution was strong during the first few minutes, but subsided upon addition of the last few drops of phosphite. The two phases which formed were separated and the lower one was crystallized by scratching it with a glass rod. The water-insoluble, wet-looking white solid was purified by pumping on it at 0.1 mmHg for 1 h: ^{1}H NMR (deuterioacetone) δ 3.83 (d, J = 12 Hz, 9 H), 7.95 ppm (m, 5 H); yield 60%.

Dimethyl *N*-Benzenesulfonylphosphoramidate. Dry HCl was bubbled into a solution of trimethyl *N*-benzenesulfonylphosphorimidate (30 g) in acetonitrile, in a three-necked round-bottom flask, equipped with a stirring bar, a gas bubbler, and a thermometer. The reaction, which was easily followed by noticing a rise and eventual fall in the temperature of the solution, was stopped after 45 min; the solvent was removed by rotoevaporation and the remaining oily residue was crystallized by scratching it for a few minutes with a glass rod. After pumping on it overnight at 0.1 mmHg, the resulting white solid was pure as judged by NMR spectroscopy: ^{1}H NMR (deuterioacetone) δ 3.70 (d, J = 12 Hz, 6 H), 7.71 and 8.13 ppm (m, 5 H); yield 90%.

Methyl *N*-Benzenesulfonylphosphoramidate Sodium Salt. Sodium iodide (11.32 g, Mallinckrodt), dissolved in a minimum amount of acetone, was added to a solution of 1 equiv of dimethyl *N*-benzenesulfonylphosphoramidate in acetone. The homogeneous solution was brought to reflux and after 10 min a white solid precipitated. Reflux was continued for another 30 min; the solution was then brought to room temperature and filtered. The white solid was thoroughly washed with fresh acetone and purified by dissolving it in hot water, decolorizing the aqueous solution with Norit, and precipitating the solid with 8 volumes of cold acetone. After filtration and drying, the solid melted at 220–223 °C: yield 76%; IR 1200 (P=O), 2700 (P–O–H or P–N–H), 3000 cm^{-1} (C–H); ^{1}H NMR (Silanor) δ 3.41 (d, J = 12 Hz, 3 H), 7.70 and 8.00 ppm (m, 5 H). Anal. Calcd for $C_7H_9NSPO_5Na$: C, 30.77; H, 3.32; N, 5.13; S, 11.74; P, 11.34; Na, 8.42. Found: C, 30.75; H, 3.70; N, 5.10; S, 11.71; P, 11.33; Na, 8.35.

Trimethyl *N*-Methanesulfonylphosphorimidate. Methanesulfonyl azide was prepared in 100-g batches by the method of Boyer et al.[22] using dry acetonitrile as solvent instead of methanol. The title compound was prepared following the method used for the *N*-benzenesulfonyl derivative (vide supra) and is essentially identical with that of Gilyarov et al.:[2] yield 84%; ^{1}H NMR (neat) δ 2.9 (d, J = 1.8 Hz, 3 H), 3.95 ppm (d, J = 12 Hz, 9 H).

Dimethyl *N*-Methanesulfonylphosphoramidate. The title compound was prepared following the method used for the *N*-benzenesulfonyl derivative (vide supra) and is essentially that of Gilyarov et al.[2] However, since the starting material is a liquid, gaseous hydrogen chloride was bubbled into it directly, without the need to carry out the reaction in solution. After 30 min of bubbling, the passage of gas was interrupted and the flask was put on ice. The wet, white solid which appeared was dissolved in a minimum of hot ethanol and crystallized by allowing the solution to cool slowly to room temperature. After filtration and drying under vacuum, the small needles melted at 111–112 °C (lit.[2] 111–112 °C): ^{1}H NMR (Silanor) δ 3.26 (s, 3 H), 3.86 (d, J = 12 Hz, 6 H), 4.8 ppm (s, HDO). The IR shows a characteristic doublet at 2710 and 2760 cm^{-1}. This band has been extensively discussed in the literature[14,15] and can be assigned either to the P–N–H stretch of the phosphoramidate or to the P–O–H stretch of the tautomeric phosphimidol:



Methyl *N*-Methanesulfonylphosphoramidate Sodium Salt. Sodium iodide (3.65 g) was dissolved in a minimum of acetone and added to a solution of dimethyl *N*-methanesulfonylphosphoramidate (5.2 g, 1 equiv) in 50 mL of dry acetone. The homogeneous solution was refluxed for 15 min; the white solid that precipitated at room temperature was filtered and washed thoroughly with fresh

acetone. The solid was then recrystallized from boiling methanol to yield 4 g of a dry, white, crystalline solid, which melted at 214–216 °C: yield 80%; ^{1}H NMR (Silanor) δ 3.20 (s, 3 H), 3.61 (d, J = 12 Hz, 3 H), 4.66 ppm (s, HDO). Anal. Calcd for $C_2H_7NPSO_5Na$: C, 11.38; H, 3.33; N, 6.64; S, 15.19; P, 14.67; Na, 10.89. Found: C, 11.50; H, 3.33; N, 6.60; S, 15.26; P, 14.83; Na, 10.84.

Di-*p*-nitrobenzyl *N*-Benzenesulfonylphosphoramidate. Di-*p*-nitrobenzyl chlorophosphate was prepared by the method of Zervas and Dilaris.[11] The sodium salt of benzenesulfonamide was prepared by titrating a suspension of benzenesulfonamide with 1 equiv of aqueous sodium hydroxide, until a homogeneous solution was obtained. This solution was then extracted several times with diethyl ether and lyophilized to yield sodium benzenesulfonamidate in 92% yield. The title compound was prepared by suspending sodium benzenesulfonamidate (1.85 g, 0.01 mol) in freshly distilled refluxing chloroform. To this suspension di-*p*-nitrobenzyl chlorophosphate (2.0 g, 0.005 mol[23]) was added. Reflux was continued for 28 h; the suspension was then allowed to come to room temperature and filtered. The solid obtained was washed thoroughly with fresh chloroform and dried under vacuum; its melting point was higher than 300 °C. The salt dissolved in 40 mL of hot water; on addition of an excess of 1 M HCl, the acid separated as an oil. After standing for 12 h at 4 °C it yielded a white solid, which was filtered, washed with water, and dried: yield 88%; mp 165–166 °C; IR 2700 cm^{-1} (P–N–H or P–O–H, vide supra for the IR spectrum of dimethyl *N*-methanesulfonylphosphoramidate); ^{1}H NMR (dimethyl sulfoxide-d_6) δ 4.86 (d, J = 9 Hz, 4 H), 7.20–8.06 ppm (m, 12 H).

***p*-Nitrobenzyl *N*-Benzenesulfonylphosphoramidate Disodium Salt.** Di-*p*-nitrobenzyl *N*-benzenesulfonylphosphoramidate (2.0 g, 0.004 mol) and sodium iodide (0.6 g, 0.004 mol) were dissolved in 50 mL of dry acetone. The homogeneous solution was refluxed for 45 min and then kept overnight at 4 °C. The resulting white precipitate was filtered, washed with acetone, and dried. The product was dissolved in 1 M NaOH; an insoluble impurity was removed by extraction with diethyl ether and methylene chloride. The clear, alkaline phase was cooled to 0–4 °C and titrated with concentrated HCl until no more white solid precipitated. The solid was collected by filtration and washed with water. It was then stirred overnight with 2 equiv of NaOH in water at 25 °C, after which the solution was extracted with diethyl ether and lyophilized to dryness. The resulting solid was twice crystallized from aqueous acetone, filtered, and dried under vacuum. Its melting point was higher than 300 °C: yield 52%; ^{1}H NMR (Silanor) δ 4.92 (d, J = 7–8 Hz, 2 H), 7.5–8.4 ppm (m, 9 H). Anal. Calcd for $C_{13}H_{11}N_2PSO_7Na_2$: C, 37.15; H, 3.60; N, 6.67; P, 7.37; S, 7.63; Na, 10.94. Found: C, 37.26; H, 3.60; N, 6.58; P, 7.37; S, 7.60; Na, 10.98.

Ethyl *N*-Benzenesulfonylphosphoramidate Sodium Salt. Diethyl *N*-benzenesulfonylphosphoramidate was prepared according to Rätz.[10] The title compound was prepared by dissolving diethyl *N*-benzenesulfonylphosphoramidate (0.6 g, 2.1 mmol) in 15 mL of dry acetone and adding solid sodium iodide (0.34 g, 2.1 mmol) to the solution. The homogeneous solution was refluxed for 13 h; the white solid that precipitated at room temperature was filtered and thoroughly washed with acetone. The solid was then dissolved in a minimum of water, precipitated with acetone, filtered through a Hirsch funnel, and dried under vacuum. This process yielded 0.41 g of a white, shiny, crystalline solid which melted at 235 °C: ^{1}H NMR (Silanor) δ 1.00 (t, J = 5 Hz, 3 H), 3.68 (q, J = 7 Hz, 2 H), 7.63 and 7.9 ppm (m, 5 H). Anal. Calcd for $C_8H_{11}NPO_5SNa$: C, 33.45; H, 3.86; N, 4.88; S, 11.16; P, 10.78; Found: C, 33.49; H, 3.96; N, 4.90; S, 11.29; P, 10.67.

Determination of pK_as. The compounds (mono- or disodium salts) were dissolved in deionized water to yield concentrations in the range 0.02–0.07 M and an equivalent amount of concentrated hydrochloric acid was added to prepare the diacid forms. These solutions were then titrated with 0.1 N sodium hydroxide at 25 °C. An example of a titration curve is shown in Figure 1; from such curves the two acid dissociation constants, pK_a(I) and pK_a(II), were calculated as follows.

pK_a(I). Since the values of K_a(I) are large, the ionization of the acid contributes importantly to the concentration of the monoanion, HA^-. For each acid, 5–12 values of K_a(I) were calculated, one for each increment of alkali, from the observed pH and the law of mass action. The concentration of the monoanion, HA^-, was set equal to the sum of that produced by neutralization of H_2A with sodium hydroxide and that produced by the self-ionization of the diacid; the latter, of course, is the same as the hydrogen ion concentration. The pK_a(I) values presented in Table I are calculated for each acid from the average of the K_as found as above. Although these low pK values necessarily are somewhat uncertain, the qualitative similarity of these pK_as to the first ionization constant of phosphates is unmistakable.

pK_a(II). This constant was set equal to the value of the pH at half-ionization for the second proton of the diacids. The pK_a values are not ionic strength corrected.

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Registry No.—IV, 62461-21-6; V, 62461-22-7; VI, 62461-23-8; VII, 62461-24-9; trimethyl *N*-benzenesulfonylphosphorimidate, 62461-25-0; benzenesulfonyl azide, 938-10-3; trimethyl phosphite, 121-45-9; dimethyl *N*-benzenesulfonylphosphoramidate, 4140-56-1; trimethyl *N*-methanesulfonylphosphorimidate, 7109-06-0; methanesulfonyl azide, 1516-70-7; dimethyl *N*-methanesulfonylphosphoramidate, 7109-15-1; di-*p*-nitrobenzyl *N*-benzenesulfonylphosphoramidate, 62461-26-1; sodium benzenesulfonamidate, 18522-93-5; di-*p*-nitrobenzyl chlorophosphate, 57188-46-2; *p*-nitrobenzyl *N*-benzenesulfonylphosphoramidate disodium salt, 62461-27-2; diethyl *N*-benzenesulfonylphosphoramidate, 1467-28-3.

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(23) Since the product has an acidic hydrogen (pK_a 1–2) it will readily protonate sulfonamidate anion. This exchange reaction causes 2 equiv of sulfonamidate to be consumed for each equivalent of product formed. Thus, the use of a twofold excess of sulfonamidate over chlorophosphate is critical in this reaction.