(Dansyldiazomethyl)phosphinates: Fluorescent Reagents for Photoaffinity Labeling

Joseph Stackhouse and F. H. Westheimer*

James Bryant Conant Laboratory of Chemistry, Harvard University, Cambridge, Massachusetts 02138

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Esters and thioesters of (dansyldiazomethyl)methylphosphinic acid (1) have been prepared and photolyzed; photolysis leads to high yields of the product of insertion into solvent. The reagents have excellent potential for photoaffinity labeling since they are highly fluorescent, can easily be prepared with high specific radioactivity (36 Ci/mmol), and have large (4000 M^{-1} cm^{-1}) extinction coefficients at long wavelengths (350 nm).

Photoaffinity labeling[1] has become one of the more important tools in the investigation of biological systems.[2,3] It has been used to mark specific amino acids near the active sites of enzymes,[4] to mark the binding sites in antibodies,[5] to identify specific receptors in membranes[6,7] and ribosomes,[8,9] and for numerous other investigations.[2,10,11] Diazo compounds have been among the more serviceable of the reagents for photoaffinity labeling, but simple irradiation at long wavelengths is restricted by their low extinction coefficients, whereas irradiation at short wavelengths can destroy protein; in addition, the photolysis of esters of diazoacetates and malonates is accompanied by extensive Wolff rearrangement;[12] diazothioacetates undergo this wasteful reaction exclusively.[13] More recently, two new reagents were developed in thse laboratories where the esters undergo photolysis with minimal Wolff rearrangement and where the thioesters undergo photolysis with only partial rearrangement.[14] One class of these reagents—*p*-tosyldiazoacetates—has a moderate extinction coefficient for photolysis at 350 nm.

In the present paper, the synthesis and photolysis of esters and thioesters of (dansyldiazomethyl)phosphinic acid are introduced. (Dansyldiazomethyl)phosphinates (more precisely named as [[[5-(dimethylamino)-naphth-1-yl]sulfonyl]diazomethyl]methylphosphinates) have several advantages over prior diazo reagents for photoaffinity labeling. The extinction coefficient at 350 nm is around 4000 M^{-1} cm^{-1}, some 20 times that of the best previous reagent. The products of the photolysis of these reagents are highly fluorescent and can therefore be identified, as can other dansyl [i.e., 5-(dimethylamino)-1-naphthylsulfonyl] derivatives,[15] at very low concentrations. Dansyl chloride, the starting material for the synthesis, is commerically available (from New England Nuclear or from Amersham) at up to 40 Ci/mmol specific activity (tritium label in the dimethylamino group or in the aromatic ring). Photolysis of the ordinary esters of (dansyldiazomethyl)-phosphinic acid in 2-propanol leads to insertion into the solvent in high yield, with little Wolff rearrangement. Photolysis of the corresponding thioesters yields products both of insertion into solvent and of Wolff rearrangement in the ratio of about 1:3; although this not ideal, it is adequate and as good as that observed with any other reagent. In addition, the Wolff rearrangement product itself may be a highly reactive intermediate, which could form stable ester or amide linkages upon further reaction.

The synthesis of the reagents is outlined in Scheme I.

Experimental Section

Methods. ^1H Fourier transform NMR spectra were obtained either with a Varian FT-80 or a Varian XL-100-15 spectrometer equipped for proton and phosphorus decoupling; Me_4Si dissolved in $CDCl_3$ or the proton signal at δ 7.25 of the $CHCl_3$ in $CDCl_3$ was used as a reference peak. Mass spectra were determined with an AEI MS-9 high-resolution spectrometer at 150–180 °C and 70 eV, and precise mass measurements were determined with a Kratos-MS-50 high-resolution spectrometer. UV spectra were taken on a Cary 15 UV–visible spectrometer, infrared spectra on

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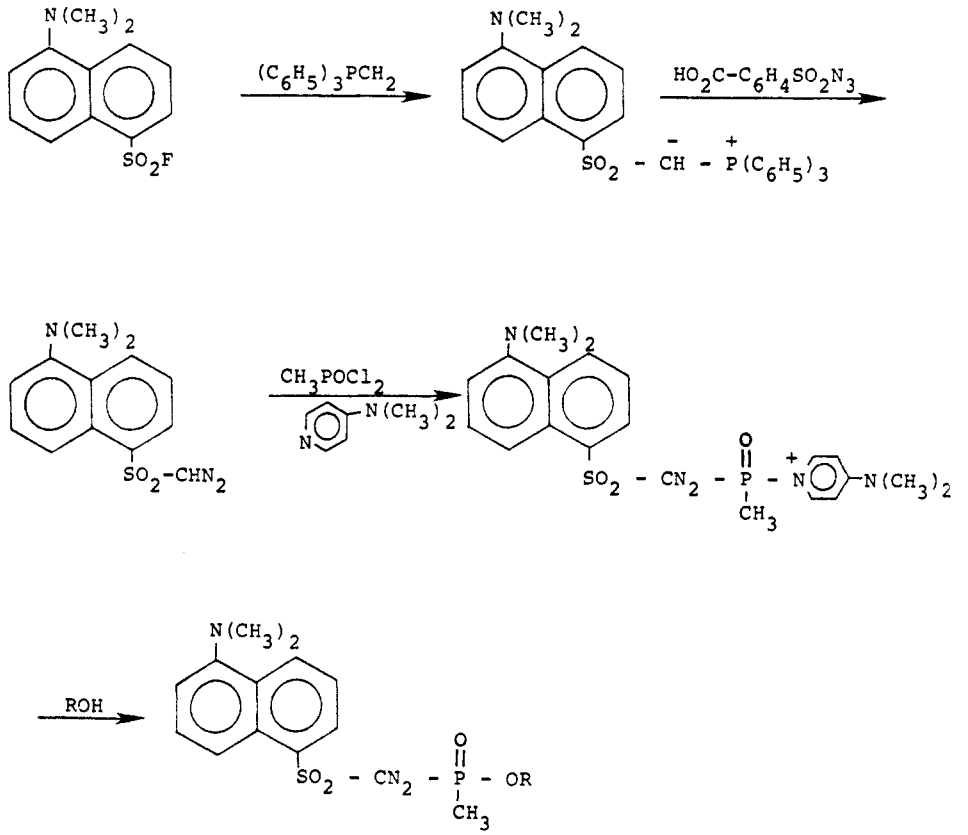
Scheme I



a Perkin-Elmer 137 infrared spectrometer, and fluorescence spectra on a Farrand MK-1 fluorimeter with a corrected exitation module. High-pressure liquid chromatography (LC) was conducted with a Waters Model ALC 202/401 chromatograph, using two 2 mm × 60 cm columns packed with Corasil. Combustion analyses were performed by Galbraith Laboratories.

Photolyses were executed in a Rayonet RPR-100 reactor equipped with 16 RPR 2537-Å or 16 RPR 3500-Å lamps. Preparative photolyses were carried out in a 25-mm-i.d. quartz tubes, with magnetic stirring. Products were separated by thin-layer chromatography (TLC) on E. Merck 60-F 254 silica gel plates with methylene chloride or ether–tetrahydrofuran (THF) (75:25) for phosphinate esters or toluene–ether (9:1) for acetate esters as eluent; the spots were visualized by the fluorescence of the TLC plates or of the products. Each TLC plate was eluted three or four times, so as to achieve effective separation of the spots despite the low R_f's that were obtained with relatively nonpolar solvents. This procedure gave better separations than single elutions with more polar solvents. Product yields were determined for photolyses carried out in quartz cuvettes of 1-mm path length. UV spectra were obtained at various stages of the photolyses by measurements on the solutions in these cuvettes, and finally, the yields of products were confirmed for these solutions by high-pressure LC and NMR spectroscopy and by radiochemical counting.

Preparative photolyses were generally conducted with 20–25 mg of material dissolved in 150 mL of solvent, with irradiation for 5–10 min at 254 nm or 20–40 min at 350 nm. 2-Propanol was distilled before use from calcium hydride and sodium borohydride to avoid the possibility of contamination by acetone, which, if present, might have functioned as a photosensitizer.

Photolyses with 2-propanol-2-^{13}C (Merck Sharp and Dohme) were done in a 6-mm-i.d. quartz tube with 5-mm o.d. Pyrex rod insert. This tube was attached by a ground-glass joint and three-way stopcock to a vacuum manifold. A sample of several milligrams of the compound to be photolyzed was loaded into the quartz tube, the Pyrex rod was inserted, and the tube was attached to the manifold, cooled in liquid nitrogen, and evacuated on the manifold. The 2-propanol-2-^{13}C was distilled from a side arm into the photolysis tube. The tube was then warmed to room temperature and shaken to dissolve solids, and the contents were photolyzed. After the photolysis, the tube was chilled in liquid nitrogen and evacuated to remove the nitrogen formed by photolysis, and then the residual 2-propanol-2-^{13}C was distilled from the sample and recovered. The products of several such photolyses were combined prior to thin-layer chromatography. The recovered 2-propanol-2-^{13}C was reused.

Scintillation counting was performed on a Packard Tri-carb Model 3320 scintillation counter using Fisher Scintiverse or New England Nuclear Aquasol scintillation fluid and counting at a 40% efficiency. Radiochromatograms were run on E. Merck 60 F-254 silica gel, Quantum Industries alumina, or Eastman Chromagram 13254 cellulose TLC plates and scanned on a Packard 385 recording ratemeter.

Materials. Dansyltriphenylphosphonium methylide was synthesized by a modification of the general method of Van Leusen et al.[16] Dansyl fluoride[17] (9.9 g) in 40 mL of dry THF was added over a 0.5-h period with rapid stirring at −78 °C to a solution of methylenetriphenylphosphorane.[18] The reaction mixture was stirred for an additional 1 h at −78 °C and then for 0.5 h at room temperature. It was then centrifuged, the solid washed with THF, and the solution centrifuged again. Solvent was removed from the combined THF solutions by rotoevaporation, and the residue was dissolved in about 200 mL of chlorobenzene and washed three times with 100-mL portions of water. The chlorobenzene solution was dried over magnesium sulfate, and the solvent was removed by rotoevaporation to yield an oil that crystallized on being allowed to stand. This material was recrystallized from methylene chloride–hexanes to give 15.6 g (78% yield) of crude product. An analytical sample was obtained by chromatography over a short silica gel column (Woelm, activity grade III) with acetone–ether (1:1) as eluent and/or by crystallization from methylene chloride–ether: mp 195.5–196 °C (cor); ^1H NMR ($CDCl_3$) δ 2.85 (s, 6 H), 3.17 (d, J = 12 Hz, 1 H), 7.25–7.62 (m, 18 H), 8.10–8.50 (m, 3 H). Anal. Calcd for $C_{31}H_{28}NO_2PS$:

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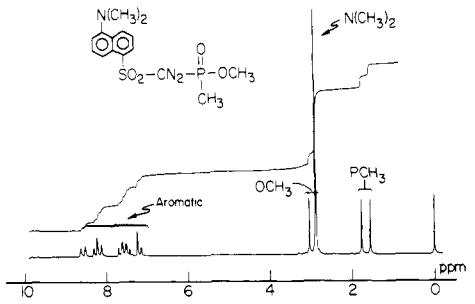

Figure 1. 80-MHz ^{1}H NMR spectrum of methyl (dansyldiazomethyl)methylphosphinate in $CDCl_3$ with tetramethylsilane as the reference.

C, 73.06; H, 5.54. Found: C, 73.20, 73.29; H, 5.73, 5.76.

Dansyldiazomethane was prepared by the general method of Van Leusen et al.[19] Dansyltriphenylphosphonium methylide (11.77 g) in 20 mL of dry methylene chloride was dripped into a stirred slurry of *p*-carboxylbenzenesulfonyl azide (5.37 g; prepared by the method of Hendrickson and Wolf[20] or commercially available from Eastman Kodak Co.) in 80 mL of methylene chloride over 2 h at 0 °C. The reaction mixture turned bright yellow immediately. (New Teflon-coated stirring bars should be used, since particles of iron, occasionally embedded in old magnetic stirring bars, catalyze the slow decomposition of the product.) The reaction mixture was stirred for 1 h at 0 °C and then washed three times with 100-mL portions of water. The solution was dried over sodium sulfate and filtered, and the solvent was removed by rotoevaporation. The residue was chromatographed on a 4 × 30 cm column of silica gel (Woelm, activity grade III) with toluene–ether (9:1) as eluent. The yellow oil, after it had been dried under vacuum, slowly crystallized: mp 72–75 °C (cor); yield 4.33 g (68% of theory). No special precautions were taken (and none were needed) to protect the compound or any of its derivatives from decomposition from exposure to ordinary room lighting: ^{1}H NMR ($CDCl_3$) δ 2.89 (s, 6 H), 5.40 (s, 1 H), 7.25–8.51 (m, 6 H); UV (4×10^{-5} M in ether) λ_{max} 345 nm (ϵ 4.7×10^3), 255 (1.5×10^4); IR 2100 cm^{-1} (s); mass spectrum, *m/e* 275 (P). Anal. Calcd for $C_{13}H_{13}N_3O_2S$: C, 56.71; H, 4.76; N, 15.26. Found: C, 56.41; H, 4.76; N, 15.02. Dr. Norman Kudo of the Harvard School of Public Health subjected dansyldiazomethane to the Ames test; it was not significantly mutagenic.

Methyl (Dansyldiazomethyl)methylphosphinate (1). 4-(Dimethylamino)pyridine (144 mg, Schering) in 0.4 mL of dry methylene chloride was added under nitrogen at dry ice temperature to methylphosphonic dichloride (0.052 mL, Alpha) dissolved in 0.4 mL of dry methylene chloride. The mixture was stirred, and dansyldiazomethane (102.5 mg) in 0.4 mL of dry methylene chloride was added. The material in the flask solidified; it melted at room temperature and was then stirred for 10 min. The reaction mixture was again cooled in dry ice, and methanol (0.30 mL) was added. The reaction mixture was allowed to warm to room temperature and was stirred for 10 min, and most of the solvent was evaporated under vacuum. The residue was chromatographed on 20 g of silica gel (Woelm, activity grade III) with ether as eluent. The product was a yellow oil which crystallized readily under vacuum: yield 88 mg (64% of theory); mp 102–104 °C (cor); ^{1}H NMR ($CDCl_3$) Figure 1; IR 2120 cm^{-1} (s); UV (4.65×10^{-5} M in ether) λ_{max} 255 nm (ϵ 1.29×10^4), 355 (4.1×10^3); mass spectrum, *m/e* 367 (P). Anal. Calcd for $C_{15}H_{18}N_3O_4PS$: C, 49.04; H, 4.94; N, 11.44. Found: C, 49.40; H, 5.25; N, 11.35.

Isopropyl (Dansyldiazomethyl)phosphinate. This compound was prepared as a yellow oil in 53% yield (31 mg) by a procedure that paralleled that for the corresponding methyl ester: ^{1}H NMR ($CDCl_3$) δ 0.87 (d, J = 6 Hz, 3 H), 1.21 (d, J = 6 Hz, 3 H), 1.55 (d, J = 16 Hz, 3 H), 2.89 (s, 6 H), 4.10–4.63 (m, 1 H), 7.17–8.66 (m, 6 H); IR 2120 cm^{-1} (s); UV (4.55×10^{-5} M in ether) λ_{max} 255 nm (ϵ 1.37×10^4), 350 (4.3×10^3); mass spectrum, *m/e* 395 (P). Anal. Calcd for $C_{17}H_{22}N_3O_4PS$: C, 51.64; H, 5.35. Found: C, 51.39; H, 5.68.

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***p*-Nitrophenyl (Dansyldiazomethyl)phosphinate.** The compound was prepared by the method used for the methyl ester with solid *p*-nitrophenol in place of methanol. The yield of oil was 162 mg, from which yellow crystals were obtained on addition of ether: 123 mg (46% of theory); mp 129–132 °C (cor); ^{1}H NMR ($CDCl_3$) δ 2.04 (d, J = 17 Hz, 3 H), 2.83 (s, 6 H), 6.68–8.51 (m, 10 H); IR (thin film) 2120 cm^{-1} (s); mass spectrum, *m/e* 474 (P). Anal. Calcd for $C_{20}H_{19}N_4O_6PS$: C, 50.63; H, 4.04. Found: C, 50.59; H, 4.12.

[^{3}H]Dansyl Derivatives. The preparation of tritiated compounds paralleled that already described for the corresponding nonradioactive materials. Since the preparations, however, were on a micro scale, special precautions were usually necessary to keep flasks closed (with rubber stopples or glass seals), and to dry all solvents scrupulously.

Methyl ([^{3}H]Dansyldiazomethyl)phosphinate. [^{3}H]Dansyl chloride was obtained from Amersham (toluene solution, 13.7 Ci/mmol) or New England Nuclear (pentane solution, 36.7 Ci/mmol). The reaction to prepare dansyl fluoride[17] was carried out with 63 μCi of [^{3}H]dansyl chloride from which solvent had been evaporated with a stream of dry nitrogen and 30 mg of potassium fluoride dihydrate in 50–100 μL of deionized water and 100–150 μL of acetone. The mixture was stirred for 4 h in a sealed polyethylene centrifuge tube and evaporated with a stream of dry nitrogen, and the product was extracted with methylene chloride. The product (dansyl fluoride) was analyzed by TLC on silica gel.[17] For chromatography, a spot of cold dansyl fluoride was applied to the base of a silica gel TLC plate. The radioactive material was spotted over this, and the plate was promptly dipped in dry pyridine to put the solvent front just ahead of the spot and then eluted immediately with toluene. The tritiated and nontritiated materials cochromatographed; residual dansyl chloride reacted with the pyridine and was held at the origin on the silica gel. Almost all the radioactivity was in the dansyl fluoride; the yield, determined radiochemically, varied from 40% to 90%. Methylenetriphenylphosphorane was prepared in 1.5 mL of dry THF from 280 mg of triphenylmethylphosphonium bromide and 0.95 equiv of fresh butyllithium.

This solution (100 μL) was injected onto 48 μCi of dry dansyl fluoride in a capped flask at dry ice temperatures. The mixture was allowed to warm, maintained at room temperature for 5 min, and twice partitioned between ethyl ether (5 mL) and water (3.5 mL). After evaporation of the ether, the product in methylene chloride was chromatographed over Woelm silica gel (activity grade III) in a Pasteur pipet (7 cm × 6 mm column) eluted first with 4.5 mL of THF/ether (20/80) and then with 5 mL of THF/ether (75/25); yield 55%. The elution volumes for the chromatography had to be calibrated for each batch of silica gel prepared; this was done on similar columns with nonradioactive material. On elution with THF/ether (20/80), the product cochromatographed on a silica gel TLC plate with nonradioactive dansyltriphenylphosphonium methylide.

p-Carboxylbenzenesulfonyl azide (5 mg) was dissolved in 200 μL of dry dimethyl sulfoxide, and 1–2 μL of this solution was added to [^{3}H]dansyltriphenylphosphonium methylide in 100 μL of methylene chloride. The methylene chloride was removed, and the film was allowed to stand for 20 min at room temperature. The product was loaded with methylene chloride onto a 7 cm × 6 mm column of Woelm silica gel (activity grade III, packed in a Pasteur pipet) and eluted with the same solvent. The yield was 55%, and since no carrier was added, the specific activity of the product can be assumed to be that of the starting dansyl chloride, 13.7 or 36.7 Ci/mmol. The product, dissolved in dry toluene, was stored at −20 °C in the dark; thin-layer chromatography on silica gel showed it to be unchanged after 2 months.

The material cochromatographed with nonradioactive dansyldiazomethane on silica gel (5/95 ether/methylene chloride eluent) and alumina (50/50 toluene/methylene chloride eluent), TLC plates showing a single radioactive band in both systems. The material (at a specific activity of 13.7 Ci/mmol) was partially eluted on silica gel or cellulose TLC plates with THF, photolyzed while wet at 254 nm for 30 s, and then immediately reeluted with THF. This led to about 50% binding of the radioactivity to the plate in either case. Nonradioactive dansyldiazomethane was run in parallel to the tritiated material on the TLC plates. No radioactivity was bound in a control (nonphotolysis) experiment.

4-(Dimethylamino)pyridine (58 mg, Schering) in dry methylene chloride (300 μL) was added under nitorgen at room temperature to methylphosphonic dichloride (20 μL, Alfa), and the mixture was stirred for 5 min. A toluene solution (10 μL) of [^{3}H]-dansyldiazomethane was evaporated to dryness, and the methylphosphonic dichloride–4-(dimethylamino)pyridine solution (20 μL) was added. After 10 min, methanol (20 μL) was added, and after 5 min more, the solvent was evaporated and the residue chromatographed on a 5 cm × 6 mm column of E. Merck silica gel 60 (230–400 mesh). The column was flushed with ether/THF/glacial acetic acid (49.5/49.5/1), loaded with the reaction product in methylene chloride, and eluted with the ether/THF/acetic acid mixture and then with THF/acetic acid (99/1). The solvent was evaporated and the product dissolved in methylene chloride (100 μL). Alliquots were taken immediately for scintillation counting and TLC. Yields of methyl ([^{3}H]dansyldiazomethyl)phosphinate varied from 30 to 50%. The material cochromatographed with nonradioactive methyl (dansyldiazomethyl)phosphinate on silica gel with ether/THF/acetic acid (78/19/3) as eluent and on aluminia with ether/acetic acid (97/3) as eluent and showed only a single radioactive spot in both systems.

[^{14}C]Methyl (dansyldiazomethyl)phosphinate was prepared by the procedure used for the nonradioactive product, but with [^{14}C]methanol (3.0 μL, 3.4 mCi/mmol, New England Nuclear) plus nonradioactive methanol (12 μL). The yield was 57% (specific activity 0.74 mCi/mmol).

S-Methyl (dansyldiazomethyl)methylphosphinothioate (6) was prepared by a procedure parallel to that used for the corresponding *O*-methyl ester (1). Methanethiol (Matheson) was condensed in dry ice/acetone and transferred with a syringe that had been cooled in dry ice. The crude reaction product was chromatographed on unhydrated Woelm silica gel with ether–acetic acid (19:1) as eluent. The product, after being dried under vacuum, was a yellow oil: yield 58 mg (25% of theory); ^{1}H NMR ($CDCl_3$) δ 1.51 (d, J = 14 Hz, 3 H), 1.98 (d, J = 15 Hz, 3 H), 2.89 (s, 6 H), 7.15–8.65 (m, 6 H); IR (thin film) 2100 cm^{-1} (s); UV (3.9 × 10^{-5} M in ether) λ_{max} 255 nm (ε 1.47 × 10^4), 350 (4.2 × 10^3); mass spectrum, m/e 383 (P). Anal. Calcd for $C_{15}H_{18}N_3O_3PS_2$: C, 46.99; H, 4.73. Found: C, 46.60; H, 4.82.

***N*-Methyl (dansyldiazomethyl)phosphinamide** was prepared by the same method as that used for the *S*-methyl ester above, except that dry methylamine was substituted for methanthiol. The crude product was chromatographed on silica gel (Woelm, activity grade III) with ether and then acetone as eluents. The yellow crystalline product (24 mg, 55% of theory) melted at 147–148 °C dec: ^{1}H NMR ($CDCl_3$) δ 1.65 (d, J = 16 Hz, 3 H), 1.97 (dd, J = 14 Hz, 5 Hz, 3 H), 2.89 (s, 6 H), 2.35–2.89 (m, 1 H), 7.26–8.53 (m, 6 H); IR (thin film) 2100 cm^{-1} (s); UV (4.10 × 10^{-5} M in ether) λ_{max} 255 nm (ε 1.32 × 10^4), 350 (4.2 × 10^3); mass spectrum, m/e 366 (P). Anal. Calcd for $C_{15}H_{19}N_4O_3PS$: C, 49.17; H, 5.23. Found: C, 49.00; H, 5.44.

Dansyldiazoacetyl Chloride. Dry triethylamine (1.43 mL) in 25 mL of dry methylene chloride was added to phosgene (15 mL) at dry ice temperature. Dansyldiazomethane (1.42 g) was added, and the reaction mixture was stirred for 1 h and then held at room temperature for 20 h. (Thin-layer chromatography showed that most of the diazo compound had reacted in 2 h.) Methylene chloride and excess phosgene were removed under vacuum at room temperature, and residue was dissolved in several millititers of methylene chloride and rapidly forced at 5 psi of nitrogen through a column packed with 15 g of silica gel (Woelm, activity grade III). The solvent was rotoevaporated to a small volume and the product crystallized by the addition of several millititers of dry ether. The crystals were again dissolved in methylene chloride, most of the solvent was removed by rotoevaporation, and then ether was slowly added to yield crystalline product. This crystallization was repeated several times: yield 1.15 g (66% of theory); mp 134 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.90 (s, 6 H), 7.16–8.70 (m, 6 H); IR 2150 cm^{-1} (s); mass spectrum, m/e 337 (P), 335 (P); exact mass m/e 337.02908 (theory, 337.02879). Anal. Calcd for $C_{14}H_{12}N_3O_3ClS$: C, 49.78; H, 3.58; N, 12.44. Found: C, 49.77; H, 3.64; N, 12.31.

Ethyl Dansyldiazoacetate (11). First dry triethylamine (0.270 mL) and then absolute ethanol (0.220 mL) were added to dansyldiazoacetyl chloride (0.320 g) dissolved in 5 mL of dry methylene chloride. The solution was mixed, sealed, and allowed to stand for 1 h at room temperature. Methylene chloride (15 mL) was added, and the solution was washed three times with 15-mL portions of water. The solvent was removed by rotary evaporation and the residue chromatographed on silica gel (Woelm, activity grade III) with toluene–ether (9:1) as eluent. After the oily yellow product had been dried under vacuum (0.1 mm, 25 °C), it slowly crystallized: yield 0.296 g (90% of theory); mp 80–82.5 °C (cor); ^{1}H NMR ($CDCl_3$) δ 1.11 (t, J = 7 Hz, 3 H), 2.88 (s, 6 H), 4.08 (q, J = 7 Hz, 2 H), 7.14–8.66 (m, 6 H); IR 2100 cm^{-1} (s); UV (3.15 × 10^{-5} M in ether) λ_{max} 250 nm (ε 1.53 × 10^4), 355 (3.8 × 10^3); mass spectrum, m/e 347 (P). Anal. Calcd for $C_{16}H_{17}N_3O_4S$: C, 55.32; H, 4.93. Found: C, 55.70; H, 4.94.

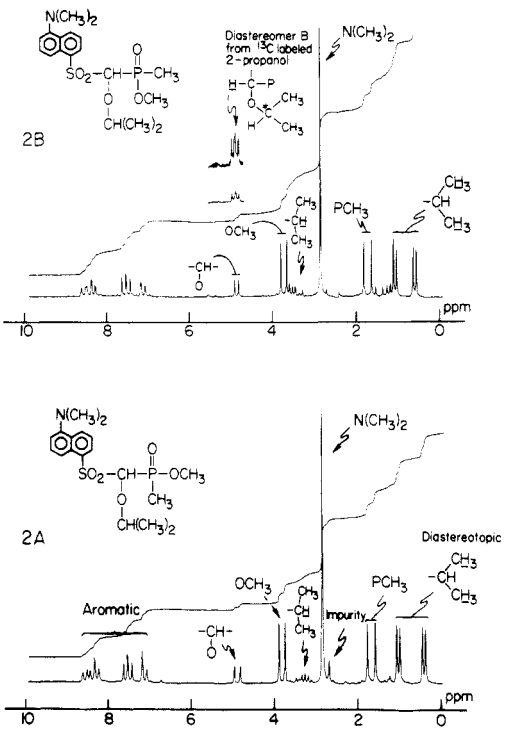


Figure 2. 80-MHz ^{1}H NMR spectra in $CDCl_3$ of the diastereomeric insertion product, methyl (dansylisopropoxymethyl)-methylphosphinate, formed by photolysis of methyl (dansyldiazomethyl)methylphosphinate in 2-propanol. The insert in Figure 2B shows the signal from the methine proton in an experiment where the solvent was 2-propanol-2-^{13}*C*; the upper half of the insert shows this signal on an expanded scale. The additional splitting caused by the ^{13}C is apparent. The two methyl groups of the isopropyl residue give rise to separate signals, since they are diastereotopic; each signal is, of course, split into a doublet by the adjacent single proton.

Identification of Products. The products were separated by TLC. The spots were scraped from the thin-layer plates and the products extracted with THF. After the solvent had been evaporated, the products were identified by their NMR and mass spectra.

Products of Photolysis of 1. Methyl (dansylisopropoxymethyl)methylphosphinate (2) is comprised of two diastereomers that arise because of the asymmetric centers at carbon and phosphorus. These diastereomers, designated **2A** and **2B**, separated on TLC with R_f's of 0.19 and 0.14; they are formed in a ratio of 1:2 by photolysis either at 254 or 350 nm. The first, **2A**, gave a precise mass of m/e 399.12665 (theory m/e 399.12692) and major peaks at m/e 235, 218, 171, 170, 168, 154, 123, and 93. The second, **2B**, gave a precise mass of m/e 399.126 48 (theory m/e 399.126 92) and the same major peaks. The NMR spectra of **2A** and **2B** are shown in Figure 2. Although these spectra are quite similar, as would be expected, the chemical shifts for one of the two diastereotopic methyl groups of the isopropoxy group differ by 0.16 ppm, and the positions of other signals are slightly but clearly different. The assignment of peaks was partially confirmed by ^{31}P decoupling, where the signals for the *P*-methyl group, for the *O*-methyl group, and for the methylene hydrogen atom of the methylene group collapsed, but no change was seen in that from

the tertiary hydrogen atom of the isopropyl group.

The most significant NMR spectra were those of the insertion product obtained when the photolyses were carried out in 2-propanol-2-^{13}C. Here the signal from the methine hydrogen atom, $ArSO_2CH[OC^*H(CH_3)_2]P(O)(CH_3)OCH_3$, of isomer **2B** appears as a clear pesudotriplet, with obvious coupling of the proton spin both to that of the phosphorus atom (J = 7.5 Hz) and to that of the ^{13}C (J = 6.1 Hz). The signal from the methylene hydrogen atom of isomer **2A** appears as a multiplet, a rather indistinct quartet, again providing evidence for the coupling of the methylene hydrogen atom with both ^{31}P and ^{13}C.[21] This provides proof that the products are those of insertion and not of Wolff rearrangement. The rearrangement product, $ArSO_2CH(OCH_3)P(O)(CH_3)OC^*H(CH_3)_2$, presumably would not show coupling between the methylene hydrogen atom and the ^{13}C atom, since they would be separated by four bonds.

Both diastereomers **2A** and **2B** show similar UV spectra in $CHCl_3$, with λ_{max} of 255 nm (ϵ 1.1 × 10⁴) and 345 (3 × 10³). Both are intensely fluorescent, with an emission band centered at 500 nm. The quantum yield for fluorescence, determined by the method of Himel and Mayer,[22] is the same for both isomers; in dioxane with excitation at 255 nm, the yield is 0.30, whereas with excitation at 350 nm, it is 0.23.

In addition to the two diastereomeric insertion products, a number of minor reaction products were separated by TLC; tentative structures have been assigned on the basis of their ^1H NMR and mass spectra.

Compound **5**, tentatively identified as methyl [[5-(dimethylamino)-1-naphthyl]isopropoxymethyl]methylphosphinate, could not be completely separated from diastereomer **2B**. In high-resolution mass spectroscopy, it showed an exact mass of m/e 335.165 06 (theory m/e 335.165 02) and major peaks at m/e 242, 201, 200 and 122. Its ^1H NMR spectrum exhibited peaks at δ 1.21 (d, J = 7 Hz, 6 H), 1.45 (d, J = 14 Hz, 3 H), 2.88 (s, 6 H), 3.34–3.65 (m, 1 H), 3.56 (d, J = 11 Hz, 6 H), 5.45 (d, J = 14 Hz, 1 H), and 7.14–8.58 (m, 6 H).

Methyl (dansylmethyl)methylphosphinate (**3**), a minor product with R_f of 0.06, presumably arises by reduction of the carbene produced on irradiation of the diazo compound **1**: molecular ion at m/e 341, major peaks at m/e 184, 169, 168, and 154; ^1H NMR ($CDCl_3$) δ 1.86 (d, J = 16 Hz, 3 H), 2.90 (s, 6 H), 3.62 (d, J = 12 Hz, 3 H), 3.89 (d, J = 12 Hz, 1 H), 3.91 (d, J = 15 Hz, 1 H), 7.17–8.58 (m, 6 H), in addition to two extraneous peaks (water plus an impurity) in the region of δ 1.5.

A minor product, R_f 0.10, has tentatively been identified as an internal insertion product, with the structure **4**. The unseparated diastereomers of this minor product have a mass spectrum with a molecular ion at m/e 339 and major peaks at m/e 275, 260, 246, 245, 230, 215, 202, 200, 198, 197, 182, and 168: ^1H NMR ($CDCl_3$; tentative assignment of the spectrum to the two diastereomers in the ratio of 3:2) δ 1.51 (d, J = 15 Hz, 3 H), 2.97 (s, 6 H), 3.55–4.10 (m, 1 H), 4.02 (d, J = 11 Hz, 3 H), 5.04 (d, J = 18 Hz, 1 H), and 7.10–8.43 (m, 6 H), and δ 1.85 (d, J = 15 Hz, 3 H), 2.97 (s, 6 H), 3.55–4.10 (m, 1 H), 3.42 (d, J = 11 Hz, 3 H), 4.98 (d, J = 12 Hz, 1 H), and 7.10–8.43 (m, 6 H).

Dansylisopropoxymethane and dansyldiazomethane, minor products with $R_f \approx 0.9$, were identified by comparision of their mass and NMR spectra with those of authentic samples. The sample of dansylisopropoxymethane (unpurified) was obtained by photolysis of dansyldiazomethane in 2-propanol at 254 nm. In one experiment, 3 mg of **1** was dissolved in 11 mL of 2-propanol and photolyzed, in a 1-mm cuvette, in 0.4-mL portions. The combined product from many photolyses was evaporated to dryness, and the product was taken up in $CDCl_3$ and examined by ^1H NMR spectroscopy. Only minor amounts of material could not be accounted for by the products here identified.

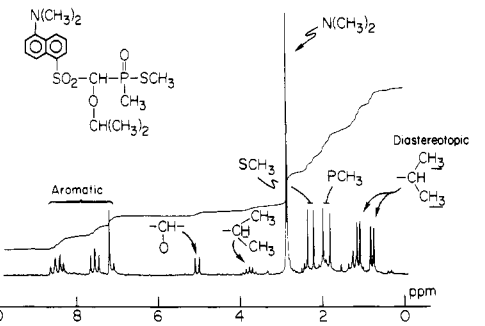


Figure 3. 80-MHz ^1H NMR spectrum of the mixture of the diastereomeric insertion products obtained on photolysis of *S*-methyl (dansyldiazomethyl)phosphinothioate. The spectrum is consistent with that expected for the diastereomers of *S*-methyl (dansylisopropoxymethyl)methylphosphinothioate, where one diastereomer is formed in a much greater amount than the other. The distinction between this compound and the Wolff rearrangement product (see Figure 4) was made by carrying out the photolysis in 2-propanol-2-^{13}C. Here (as in the spectrum shown in the insert of Figure 2) the signal from the methine proton was split by the ^{13}C, which shows that the isopropyl group is attached at the methine carbon atom.

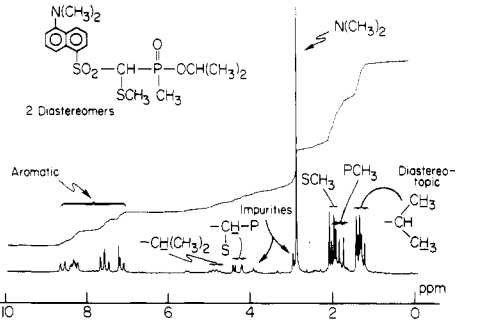


Figure 4. 80-MHz ^1H NMR spectrum of the Wolff rearrangement product obtained by photolyzing *S*-methyl (dansyldiazomethyl)methylphosphinothioate in 2-propanol. The distinction between this compound and the insertion product (see Figure 3) was made by carrying out the photolysis in 2-propanol-2-^{13}C. The signal from the methine proton was not subject to additional splitting by the ^{13}C; the isopropyl group, then, was not attached at the methine carbon atom.

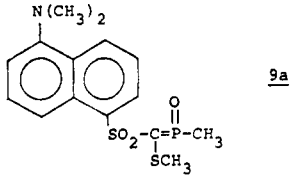
Photolysis of 6. A mixture of the two diastereomers, **8A** and **8B**, of *S*-methyl (dansylisopropoxymethyl)methylphosphinothioate was formed in 10:1 ratio (estimated by NMR spectroscopy) by the photolysis of *S*-methyl (dansyldiazomethyl)phosphinothioate (**6**) at 254 nm in 2-propanol; the mixture had an R_f of 0.24. The compounds gave an exact mass of m/e 415.103 99 (theory m/e 415.104 09), with major peaks at m/e 246, 235, 219, 218, 202, 174, 171, 170, 168, 139, and 93. The ^1H NMR spectrum ($CDCl_3$), shown in Figure 3, clearly identifies the structure as that of the insertion product into solvent.

The diastereomeric Wolff rearrangement products **10A** and **10B**, with R_f values of 0.19, were formed in about 2.5 times the yield of the insertion products **8A** and **8B**. The mixture of diastereomers showed an exact mass of m/e 415.104 16 (theory m/e 415.104 09) with major peaks at m/e 230, 181, 171, 170, 168, 141, 139, 123, and 120. The appropriate ^1H NMR spectrum is shown in Figure 4. The peaks from the two diastereomers are generally quite distinct, although those from the dimethylamino group and from the *S*-methyl group are coincident for the two isomers. The diastereotopic methyl groups of the isopropyl group would be expected to give rise to eight signals (two diastereomers, each with

(21) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; p 348.

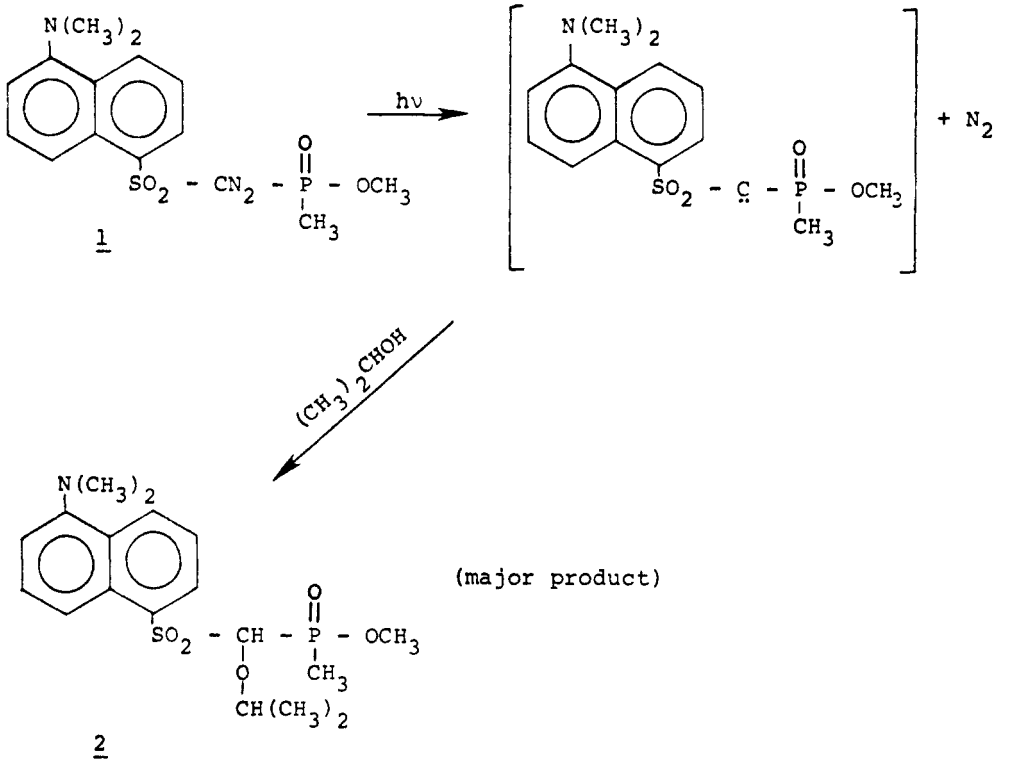
(22) Himel, C. M.; Mayer, R. T. *Anal. Chem.* **1970**, *42*, 130. Fletcher, A. N. *J. Mol. Spectrosc.* **1967**, *23*, 221. Turner, G. K. *Science* **1964**, 146, 183.

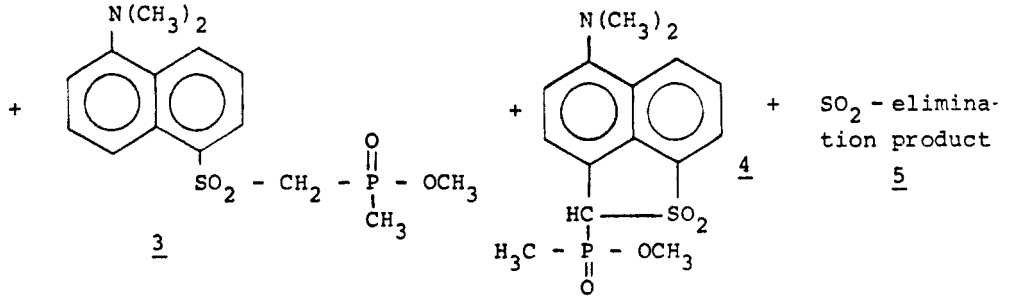
(23) See Scheme III. This formula represents a possible intermediate in the Wolff rearrangement. Alternatively, the rearrangement may be concerted, with no true intermediate, or may proceed by way of the metaphosphate analgoue **9a**.



9a

Scheme II





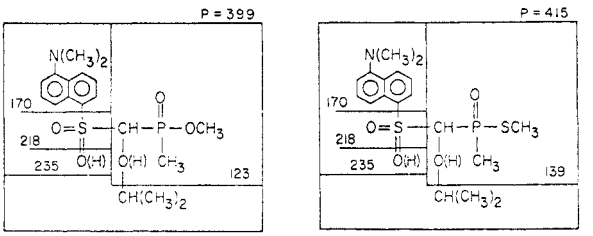
two diastereotopic methyl groups, each split by the methine hydrogen atom); apparently two of the signals are coincident. The assignments shown in Figure 4 were confirmed by both proton and ^{31}P decoupling; in particular, the two doublets for the methine hydrogen atom of the isopropyl group collapse to a single doublet under ^{31}P decoupling. This places the isopropyl group adjacent to phosphorus, as appropriate to the Wolff rearrangement product.

Additional confirmation of the structures of the products of insertion and of Wolff rearrangement was obtained by conducting the photolyses in 2-propanol-2-^{13}C. The ^1H NMR spectrum of the insertion product showed clear evidence of coupling between the ^{13}C atom and the methylene hydrogen atom, whereas the spectrum for the Wolff rearrangement product showed no such coupling. Both the isertion product and the Wolff rearrangement product are intensely fluorescent.

Other minor products were not investigated.

Ethyl dansylmethoxyacetate (12) was obtained by the photolysis of ethyl dansyldiazoacetate (**11**, 15–29 mg) for 5–10 min at 254 nm in 100 mL of methanol. The compound showed an R_f of 0.13 and a mass spectrum with parent ion at m/e 351: ^1H NMR ($CHCl_3$) δ 1.10 (t, J = 7 Hz, 3 H), 2.85 (s, 6 H), 3.57 (s, 3 H), 4.05 (q, J = 7 Hz, 2 H), 5.00 (s, 2 H), 7.05–8.65 (m, 6 H). The compound is only weakly fluorescent and exhibits pronounced photolability on irradiation at 254 nm in methanol. The maximum yield obtained was about 30%. The corresponding Wolff rearrangement product was separated from the insertion product chromatographically and showed an R_f of 0.33. Its parent peak in the mass spectrum also comes at m/e 351: ^1H NMR ($CDCl_3$) δ 1.10 (t, J = 7 Hz, 3 H), 2.84 (s, 6 H), 3.35 (s, 6 H), 3.35 (s, 3 H), 4.07 (q, J = 7 Hz, 2 H), 4.10 (q, J = 7 Hz, 2 H), 5.27 (s, 1 H), 6.95–8.65 (m, 6 H).

Chart I

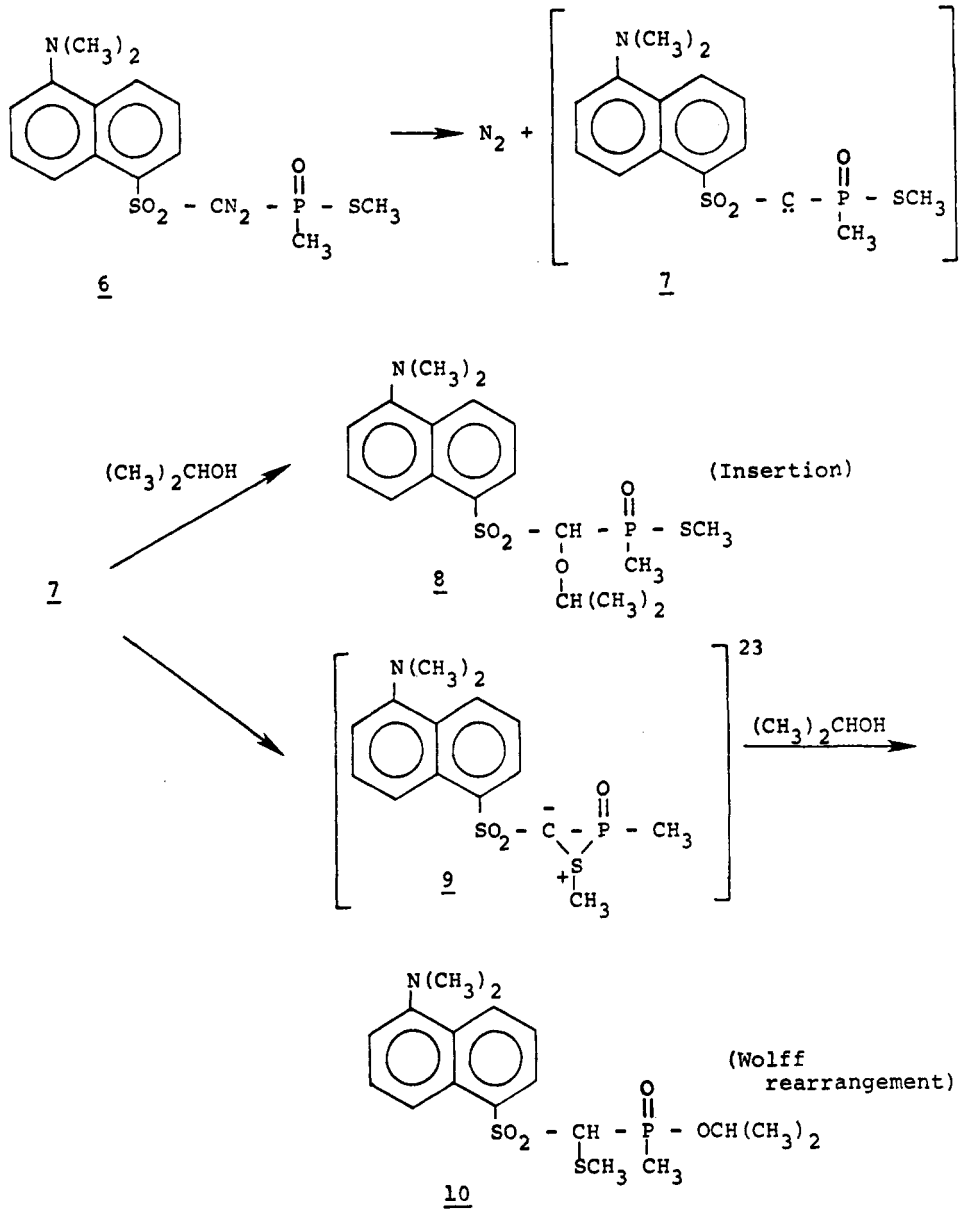


Results

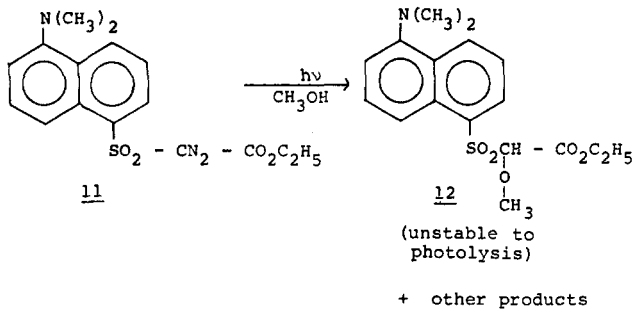
The chemistry of the photolyses is shown in Schemes II–IV.

The identifications of the starting materials and of the products are based on the methods of synthesis, analyses or precise mass spectra, fragmentation patterns in the mass spectra, and IR and ^1H NMR spectra; the details are given in the Experimental Section. In particular, each of the diazo compounds showed the intense, sharp band in its IR

Scheme III



Scheme IV



spectrum around 2100 cm^{-1} that is characteristic of aliphatic diazo groups. The analyses and exact masses agree with theoretical values within experimental error, and the fragmentation patterns are reasonable for the assigned structures of the products. The rationale of the fragmentation patterns for products **2** and **8** is illustrated in Chart I; the others can be similarly analyzed.

The ^1H NMR spectra of the starting materials are in all cases clean; Figure 1 (the spectrum of **1**) is illustrative. The ^1H NMR spectra of the products clearly establish their structure, although in every case evidence for small amounts of impurities appears in the spectra of major products, and evidence for somewhat more impurities, as might be expected, appears in the spectra of the minor products. Spectra of the major products (**2**, **7**, and **8**) are shown in Figures 2–4.

The yields of the insertion products **2A** and **2B**, obtained on photolysis of methyl (dansyldiazomethyl)phosphinate, are time dependent, since the photoproducts themselves are somewhat photosensitve. Similarly, the yields of the insertion products and Wolff rearrangement products, **8** and **10**, obtained on photolysis of *S*-methyl (diazomethyl)phosphinothioate, are time dependent. Since the rate of photochemical decomposition at 254 nm of the products **2A** and **2B** is only one-tenth that of the diazo starting material, **1**, the loss through decomposition is not severe. The rate of decomposition for photolysis at 350 nm, however, although slower, is half as great as that of **1** at the same wavelength.

The yields can be estimated by separating the products by high-pressure liquid chromatography. The results are not precise but suggest that at 254 nm the combined yield of the two diastereomeric insertion products, **2A** and **2B**,

is about 60 ± 5% and that for the thiomethyl products, **8** and **10**, is about 45 ± 5%. The yield of **2A** and **2B** estimated from radiochromatograms of the photolysate of the methyl-^{14}C ester agrees with the high-pressure LC value. On the other hand, yields estimated from NMR spectra are substantially higher. The ^1H NMR spectra of the reaction mixture show only the compounds here identified, with **2A** and **2B** as the dominant products from the photolysis of **1**, and the insertion and Wolff rearrangement products **8** and **10** as the dominant products from the photolysis of **6**; although the precise yields have not been determined, the yields of useful products are ample.

Discussion

The major advantages of the dansyldiazo reagents for photoaffinity labeling include the following. (A) Intense fluorescence of the photoproducts is observed, allowing easy identification of chromatographic fractions that contain the dansyl group. The level at which fluorescent photoproducts can be detected depends upon the intensity of the excitation, and upon the solvent. Since, however, the fluorescence yield is high, the products can presumably be seen, as with other intensely fluorescent materials, at the subnanogram level. (B) Since dansyl chloride is available at a specific activity of up to 40 Ci/mmol, intensely radioactive reagent can be prepared, which could provide an accurate measure of minute amounts of insertion. (C) The reagents have an intense absorption (ϵ 4000 M^{-1} cm^{-1}) at 350 nm and so allow photolysis at wavelengths that are not absorbed by protein as well as a convenient assay for bound reagent. (D) Finally, the synthesis is quite straightforward, and the reagents are stable to room lighting. The acid chloride of the (dansyldiazomethyl)phosphinate is best used for labeling in situ, with 4-(dimethylamino)pyridine as the catalyst for condensation, but the *p*-nitrophenyl ester is a stable crystalline solid that can probably be used in many syntheses. Esters and thioesters have been prepared here for illustrative purposes. Dansyldiazomethane with tritium incorporated in its dimethylamino group has also been prepared at a specific activity of 36.7 Ci/mmol and has been converted to methyl (dansyldiazomethyl)phosphinate. One further possible advantage of the (dansyldiazomethyl)phosphinates has not yet been investigated. The ^{31}P NMR signal from the reagents in $CDCl_3$ (43 ppm downfield from 85% H_3PO_4, external reference) will be well separated from those of phosphate esters and might provide an additional tool for analyzing and following the compounds in biological systems.

Initially we had thought that dansyldiazoacetates such as **11** would prove excellent reagents for photoaffinity labeling. Unfortunately, such is not the case. The reagent has several defects that make it unsuitable for the purpose. First, and most important, the product of insertion into solvent, **12**, is photolabile. Only a relatively low yield can be obtained of product. Second, the product of insertion of the diazo compound into 2-propanol is not highly fluorescent; although it could be made highly radioactive, at least one of the virtues anticipated for dansyl reagents is absent. Fortunately, these defects do not apply to the corresponding methylphosphinates, which are no more difficult to prepare than the acetates and which embody all the desirable properties anticipated for dansyl derivatives.

The photolysis of a methyl ester in 2-propanol proceeds to give a good yield of the product of insertion into the solvent; **2A** and **2B** dominate in the product mixture formed. The compounds are reasonably stable to further photolysis, even at 254 nm, and the reaction shown in Scheme II presumably serves as a model of photolytic insertion by various esters into neighboring nucleophiles. Some minor products were also picked up; these would represent loss of reagent, since they did not incorporate 2-propanol (the solvent).

Similarly, the photolysis of the *S*-methyl ester in 2-propanol gave a reasonable yield of insertion, accompanied by about twice as much of the product of Wolff rearrangement. Although Wolff rearrangement is usually undesirable, it may, for phosphinate esters, serve as an additional route to trapping. Rearrangement has so far always proved the major pathway in the photolysis of diazo thioesters; the yield of insertion product obtained in this work is about as good as any so far achieved.

Incidentally, the use of 2-propanol-2-^{13}C has proved to be an excellent method of determining the details of the structure of photoproducts and in particular of distinguishing between insertion and Wolff rearrangement products.

Acknowledgment. The authors thank Mr. Leo Letendre for assistance with the high-resolution mass spectrometry and Mr. Richard Beatty and Mr. Stephen Buchwald for help with the ^{31}P NMR spectroscopy. This research was supported by a grant from the Institute of General Medical Sciences (GM 04712-24).

Registry No. 1, 76631-12-4; **2A**, 76631-13-5; **2B**, 76631-14-6; **3**, 76631-15-7; **4** (isomer 1), 76631-16-8; **4** (isomer 2), 76631-17-9; **5**, 76631-18-0; **6**, 76631-19-1; **8A**, 76631-20-4; **8B**, 76631-21-5; **10A**, 76631-22-6; **10B**, 76631-23-7; **11**, 76631-24-8; **12**, 76631-25-9; dansyltriphenylphosphonium methylide, 76631-26-0; (dansylmethylene)triphenylphosphorane, 76631-27-1; dansyl fluoride, 34523-28-9; methylenetriphenylphosphorane, 3487-44-3; dansyldiazomethane, 76631-28-2; isopropyl (dansyldiazomethyl)methylphosphinate, 76631-29-3; *p*-nitrophenyl (dansyldiazomethyl)-methylphosphinate, 76631-30-6; methyl ([^3H]dansyldiazomethyl)-methylphosphinate, 76631-35-1; [^{14}C]methyl (dansyldiazomethyl)-methylphosphinate, 76631-31-7; *N*-methyl (dansyldiazomethyl)-methylphosphinamide, 76631-32-8; dansyldiazoacetyl chloride, 76631-33-9; methyl dansylethoxyacetate, 76631-34-0.