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p-Toluenesulfonyldiazoacetates: Reagents for Photoaffinity Labeling[1]

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p-Toluenesulfonyldiazoacetyl chloride and *p*-nitrophenyl *p*-toluenesulfonyldiazoacetate have been prepared and offer potential advantages as reagents for photoaffinity labeling. (i) The extinction coefficient for the sulfonyldiazo compounds at 370 nm is about 10 times that for the long wavelength absorption of other diazoesters; this absorption permits reasonably rapid photolysis in the presence of compounds that are destroyed by short wavelength uv radiation. (ii) The two derivatives named above are stable thermally; furthermore, since sulfonyldiazoesters are stable to acid and to weak base, photoaffinity labeling can be conducted over a wide range of pH. (iii) Photolysis of ordinary (i.e., oxygen) esters of sulfonyldiazo compounds in methanol or cyclohexane leads to insertion into the solvent to the exclusion of Wolff rearrangement; photolysis of thioesters at 350 nm in methanol gives about 25% insertion into solvent, accompanied by about 75% Wolff rearrangement; in contrast, photolysis of most thioesters of diazo derivatives leads exclusively to Wolff rearrangement [V. Chowdry and F. H. Westheimer, *J. Amer. Chem. Soc.* 100, 309 (1978)].

INTRODUCTION

Photoaffinity labeling was initiated over 15 years ago with the synthesis and photolysis of diazoacetyl chymotrypsin (*2*, *3*). Since then, many other reactions for photoaffinity labeling have been developed and applied to a variety of biochemical problems. In particular, the amino acid residues near the active site of enzymes have been located (*4–6*); the active regions of antibodies have been determined (*7–9*); and the binding sites for hormones (*10–12*), and for drugs on ribosomes (*13*, *14*) have been specified. The field has been extensively reviewed (*15–18*).

Diazo compounds have the advantage as photoaffinity labeling reagents that the carbenes to which they decompose are (presumably) short-lived and so react with whatever groups are close to the site that the reagents occupy at the moment when light is absorbed. But most diazoesters previously available suffer from disadvantages that diminish their utility; they are rather unstable thermally, decompose rapidly in acidic solution, and on photolysis undergo more or less Wolff rearrangement (*19*). This rearrangement produces a ketene that may be relatively longlived and so may fail to react at the site where it was produced; furthermore, it may (and in some instances does) react with water to yield a carboxymethyl derivative at the site of its original attachment; to the extent that this process occurs, no new and interesting products are

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obtained from photolysis. The Wolff rearrangement occurs quantitatively[3] for most diazoesters of thiols (*20*) and in such cases vitiates the utility of the reagents. The uv spectra of diazoesters are distinguished by a strong absorption band at 250 nm (log $\varepsilon \approx 4$) and (usually) a weak band around 360 nm (log $\varepsilon \approx 1$). When, for one reason or another, the photolysis must be conducted at a long wavelength (for example, to avoid destruction of a photosensitive protein or product), then the low extinction coefficient of this latter absorption is a decided disadvantage.

Recently, we found that 2-diazo-3,3,3-trifluoropropionates (*21*) are superior reagents for photoaffinity labeling (*22*) in that they obviate two of the principal disadvantages of other diazo esters: The trifluoro compounds are stable to heat and acid and undergo photolysis with much less Wolff rearrangement than do other diazoacetates. In particular, thioesters of 2-diazo-3,3,3-trifluoropropionic acid undergo photolysis with 40% insertion into solvent rather than with complete molecular rearrangement. However, the photolysis of thioesters is successful only at long wavelengths; at short wavelengths, the insertion product that is initially formed is photochemically destroyed. Unfortunately, however, the absorption coefficient for the trifluorodiazopropionates at long wavelengths is low.

Now we report the preparation and properties of *p*-toluenesulfonyldiazoacetates (*1*). These prove to be thermally and acid stable; simple *p*-toluenesulfonyldiazoesters undergo photolysis with high yields of insertion into methanol or cyclohexane and with no evidence of Wolff rearrangement. Although the corresponding thioesters unfortunately undergo this rearrangement, about 25% of the product is that of insertion into solvent, so that the reagents are useful, even if not ideal, for active thiols. The most interesting feature of the new compounds is their uv spectrum; the extinction coefficient at 360 nm is about 140; although this is not a large absorption, it is greater by about a factor of 10 than that reported for other diazoesters (*23*). Furthermore, the rates of photolysis are likewise greater, suggesting that the quantum yield for photolyses of the various diazoesters is similar, so that the larger extinction coefficient leads directly to more effective photolysis.

EXPERIMENTAL

Methods

Photolyses were usually carried out at room temperature with 100 to 200 μl of 0.1 to 0.3 *M* solutions in small water-jacketed Pyrex or quartz tubes in a Rayonet RPR 100 reactor. Sixteen RPR 2537-, RPR 3000-, or RPR 3500-Å lamps were used. The progress of photolysis was followed by recording the uv spectra of appropriately diluted aliquots of the photolysate. Gas chromatographic separation of products was conducted on a 6-ft × $\frac{1}{8}$-in. aluminum column packed with 3% Dexil 400 GC/Chromosorb WHP 80/100 with a Hewlett–Packard gas chromatograph, model 5750, equipped with thermal conductivity and flame ionization detectors; dry oxygen-free helium was used as the carrier gas. Small aliquots (3–8 μl) of photolyzed solutions were injected into the gas chromatographic system. A metallic stream-splitter (Hewlett–Packard, 10:1 ratio) was used to divert a small fraction of the product to the detector and the

[3] L. J. Crane, unpublished work in this laboratory.

major part to V-shaped collection bulbs for nmr or mass spectrographic identification. Detailed conditions are specified for individual products. Clean separation, often baseline separation, of the various products was achieved. ^1H Fourier–transform nmr spectra were obtained on a Varian XL-100-15 spectrometer. Mass spectra were obtained either with an AEI MS-9 high resolution spectrometer or from the NIH Mass Spectrometry Facility at MIT.

Analyses were carried out by Galbraith Laboratories (Knoxville, Tennessee).

Synthesis

p-Toluenesulfonyldiazomethane. *p*-Toluenesulfonyldiazomethane was synthesized from the ylid, $CH_3C_6H_4SO_2CH{=}P(C_6H_5)_3$, and *p*-carboxybenzene sulfonylazide (Eastman) by the method of VanLeusen (*24, 25*). Purification required repetition of the recommended rapid (15 min) chromatography over alumina; slow passage led to decomposition.

2-p-Toluenesulfonyl-2-diazoacetate, **A**. 1,8-Bis-(dimethylamino)-naphthalene (775 mg; "Proton Sponge"; Aldrich) was dissolved with stirring under nitrogen in 35 ml of dry methylene chloride in a flask equipped with a dry ice condenser. The solution was cooled in dry ice, and phosgene (0.9 ml) was transferred to it. The a solution of *p*-toluenesulfonyldiazomethane (710 mg) in cold dry methylene chloride (15 ml) was added, drop by drop, in the dark with concomitant stirring. The reaction mixture was stirred for 1 hr at dry ice temperature, by which time it had turned black. The mixture was allowed to warm to room temperature, stirred overnight, and then worked up in dim light as follows: Diethyl ether (20 ml) was added to the mixture, and the organic solution was washed successively with 10 ml each of 0.1 *M* hydrochloric acid, water, saturated bicarbonate, and water. The organic phase was dried over magnesium sulfate and evaporated to a brown solid that was purified by chromatography on a 15 × 2.5-cm column containing 35 g of Woelm grade II silica gel using toluene:hexane (3:1) as the eluant. The fractions were analyzed by tlc and the appropriate ones were pooled to obtain 475 mg of a yellow solid. Crystallization from methylene chloride–hexane yielded 370 mg of yellow crystals: mp 107–8°C; the yield (including a second crop of 60 mg) was 46%. The chromatography also yielded about 270 mg of a more slowly eluting pale yellow solid which was identified by ir, ^1H nmr, and mass spectrometry as *p*-toluenesulfonylmethyl chloride. The ir spectrum of the diazo acid chloride, **A**, has major bands at 2142 cm^{-1} (C=N_2), 1735 cm^{-1} (C=O), and 1347 and 1160 cm^{-1} (SO_2). The ^1H nmr spectrum ($CDCl_3$) has peaks at δ 7.90 (d, $J = 8$ Hz), 7.38 (d, $J = 8$ Hz), and 2.35 (s). The uv spectrum of **A** in cyclohexane is shown in Fig. 1a: log $\varepsilon_{240\,nm} = 4.25 \pm 0.02$; $\lambda_{max} = 366$ nm (log $\varepsilon = 2.22 \pm 0.02$). The mass spectrum showed the molecular ion for ^{35}Cl at *m/e* 258, accompanied by an ion at *m/e* 260, corresponding to the species with ^{37}Cl. Exact mass for the ^{35}Cl molecular ion for $C_9H_7ClN_2O_3S$. Calcd: 257.9866. Found: 257.9891. Elemental composition, Calcd: C, 41.78; H, 2.73; Cl, 13.70; N, 10.83; S, 12.39. Found: C, 41.86; H, 2.75; Cl, 13.67; N, 10.83; S, 12.31.

p-Nitrophenyl-2-p-toluenesulfonyl-2-diazoacetate, **B**. The diazoacid chloride, **A** (150 mg), was dissolved in freshly distilled dry methylene chloride (50 ml) under nitrogen. Dry sodium *p*-nitrophenoxide (112 mg, Aldrich, heated in a vacuum oven at 0.1 mm/140°C) was added with stirring at room temperature. The reaction mixture was allowed to stir overnight and then filtered; upon evaporation, the filtrate yielded a yellow

solid, which after recrystallization from methylene chloride–hexane gave 125 mg (first crop) and 50 mg (second crop) of the *p*-nitrophenyl ester, **B** Yield: 83%; mp 145.5–146.5°C. The ir spectrum shows bands at 2132 cm^{-1} ($C{=}N_2$) and 1726 cm^{-1} (C=O). The ^1H nmr spectrum (in CD_2Cl_2) has peaks at δ 8.3 (d, fine structure, J = 9.5 Hz), 7.96 (d, fine structure, J = 8.4 Hz), 7.46 (d, fine structure, J = 8.4 Hz), 7.3 (d, fine structure, J = 9.5 Hz), 2.5 (s). The mass spectrum shows a molecular ion at *m/e* 361. Calcd for $C_{15}H_{11}N_3SO_6$: C, 49.85; H, 3.07; N, 11.58; S, 8.87. Found: C, 49.99; H, 3.14; N, 11.58; S, 8.56.

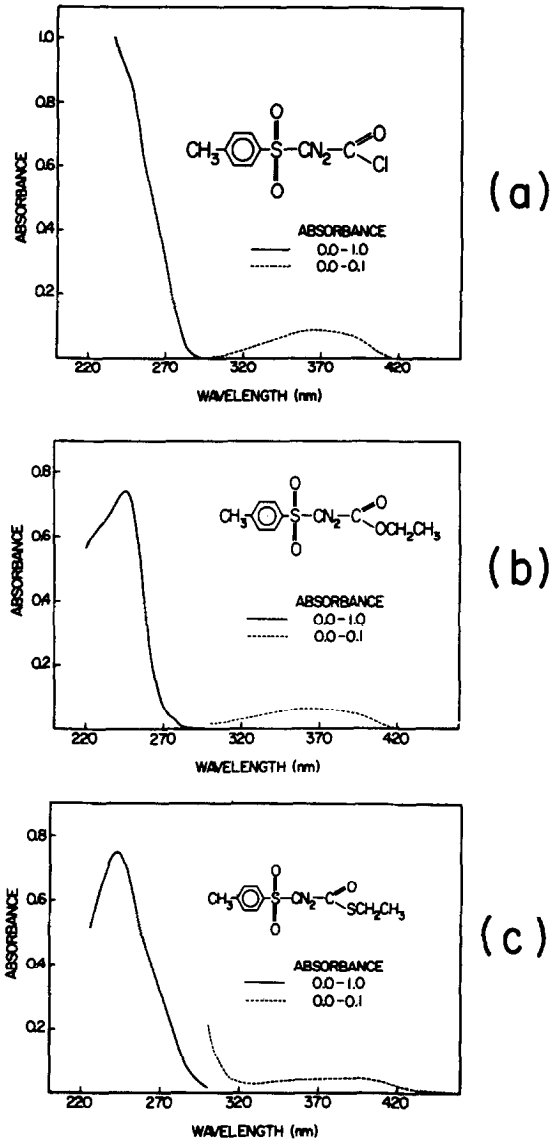


FIG. 1. Ultraviolet absorption spectra of approximately 5 × 10^{-5} *M* solutions of *p*-toluenesulfonyldiazoacetyl chloride, of ethyl *p*-toluenesulfonyldiazoacetate, and of thioethyl *p*-toluenesulfonyldiazoacetate. Note the change of scale for the long wavelength absorbance.

Ethyl 2-p-toluenesulfonyl-2-diazoacetate. Ethyl 2-p-toluenesulfonyl-2-diazoacetate, **C**, was synthesized according to Regitz and Bartz (*26*) from ethyl *p*-toluenesulfonylacetate (*27*) and *p*-toluenesulfonyl azide. The latter was prepared in our laboratories by Dr. J. A. Goldstein according to Regitz *et al.* (*28*). The ester melted at 65 to 65.5°C [lit. (*26*) mp 62–63°C]; its ir spectrum had peaks at 2116 cm^{-1} ($C{=}N_2$) and 1712 cm^{-1} (C=O) [lit. (*26*) for the diazo band: 2137 cm^{-1}). The ^1H nmr spectrum in $CDCl_3$ shows peaks at δ 7.88 (d, fine structure), 7.32 (d, fine structure), 4.20 (q, $J = 7.0$ Hz), 2.43 (s), and 1.23 (t, $J = 7.0$ Hz). The uv spectrum of C is shown in Fig. 1b.

Methyl 2-p-toluenesulfonyl-2-diazoacetate. Methyl 2-p-toluenesulfonyl-2-diazoacetate, **D**, was synthesized by procedures paralleling those for the corresponding ethyl ester. Methyl *p*-toluenesulfonylacetate was prepared from sodium *p*-toluenesulfinate and methyl chloroacetate following the procedure (*27*) published for the corresponding ethyl ester. The resulting crude methyl *p*-toluenesulfonylacetate was then allowed to react with *p*-toluenesulfonylazide under the same experimental conditions as those specified by Regitz and Bartz for the ethyl ester (*26*). Chromatography of the crude product (a viscous yellow oil, 1.6 g) on a 28 × 2.3-cm column of Woelm grade II silica gel yielded 720 mg of a yellow solid, which after crystallization from methylene chloride–hexane melted at 50 to 51°C. The ir spectrum shows peaks at 2138 cm^{-1} ($C{=}N_2$) and 1723 cm^{-1} (C=O). The ^1H nmr spectrum ($CDCl_3$) has peaks at δ 7.89 (d, $J = 8$ Hz), 7.34 (d, $J = 8$ Hz), 3.75 (s), and 2.43 (s). Exact mass for $C_{10}H_{10}N_2O_4S$, calcd: 254.0361 Found: 254.0396.

Thioethyl 2-p-toluenesulfonyl-2-diazoacetate, **E**. Ethane thiol (105 mg) freshly distilled under nitrogen from anhydrous calcium chloride was added under nitrogen with stirring to a solution of the acid chloride, **A** (220 mg), in dry ether (30 ml). Dry freshly distilled 2,6-lutidine (91 mg) was added, and the progress of the reaction at room temperature was monitored by tlc; the reaction was complete in 24 hr. The filtered yellow solution was washed with 10 ml of 0.01 *M* hydrochloric acid and then with water, dried, and evaporated to a yellow oil. This oil was chromatographed on an 18 × 2.5-cm column containing 35 g of Woelm grade II silica gel with toluene as the eluant. The yellow fractions were combined and evaporated to give 160 mg of a solid which in turn was recrystallized from methylene chloride–hexane to give crystals melting at 62.5 to 63.5°C in 65% yield. The ir spectrum showed bands at 2112 cm^{-1} ($C{=}N_2$) and 1651 cm^{-1} (C=O). The ^1H nmr spectrum in $CDCl_3$ shows peaks at δ 7.9 (d, fine structure $J = 8.5$ Hz), 7.35 (d, fine structure $J = 8.5$ Hz), 2.94 (q, $J = 7.4$ Hz), 2.43 (s), and 1.23 (t, $J = 7.4$ Hz). The uv spectrum (Fig. 1c) shows $\lambda_{max} = 243$ nm ($\log \varepsilon = 4.27 \pm 0.05$) and $\lambda_{max} = 390$ nm ($\log \varepsilon = 2.10 \pm 0.10$). *Anal.* calcd. for $C_{11}H_{12}N_2O_3S_2$: C, 46.45; H, 4.28; N, 9.85; S, 22.54. Found: C, 46.54; H, 4.30; N, 9.93; S, 22.57.

N-Acetyl-O-methylcysteinyl-2-p-toluenesulfonyl-2-diazoacetate, **F**. Di-*N*-acetylcystine dimethyl ester was prepared by acetylating cystine dimethyl ester dihydrochloride in anhydrous pyridine. The disulfide was reduced with zinc in methanol–acetic acid to yield *N*-acetylcysteine methyl ester, mp 79 to 80.5°C (*29*). This compound was acylated with the diazoacid chloride, **A**, by a procedure similar to that used for ethane thiol, see above. The product was purified by chromatography over Woelm grade II silica gel using acetonitrile as eluent. The yellow solid, obtained in 83% yield, was crystallized from methylene chloride–hexane (mp 123.5–124.5°C). The ir spectrum shows bands at 2094 cm^{-1} ($C{=}N_2$) and carbonyl bands at 1736, 1675, and 1642 cm^{-1}.

The ^1H nmr spectrum ($CDCl_3$) has peaks at δ 7.89 (d, fine structure, $J = 8$ Hz), 7.36 (d, fine structure, $J = 8$ Hz), 6.12 (broad doublet), 4.75 (multiplet), 3.68 (s), 3.43 (multiplet), 2.43 (s), and 1.89 (s). The uv spectrum shows λ_{max} at 241 nm.

Products

The products of the photolyses, separated by gas chromatography, were identified by their nmr and mass spectra. All the ^1H chemical shifts were calculated from the proton signal of the $CHCl_3$ in deuterochloroform at δ 7.25 as a reference. Small peaks at δ 1.50 and 1.22 in some spectra were caused by water and material bleeding from the column. A small peak sometimes seen between δ 2.05 and 2.15 arose from an impurity in the $CDCl_3$.

Ethyl p-toluenesulfonyl-2-methoxyacetate, **G**. This product was obtained by photolysis of ethyl 2-p-toluenesulfonyl-2-diazoacetate, **C**, in methanol; it shows a molecular ion, *m/e*, at 272.0742; calcd for $C_{12}H_{16}O_5S$: 272.0719. Its fragmentation pattern is shown in Scheme 1 and its nmr spectrum in Fig. 2a.

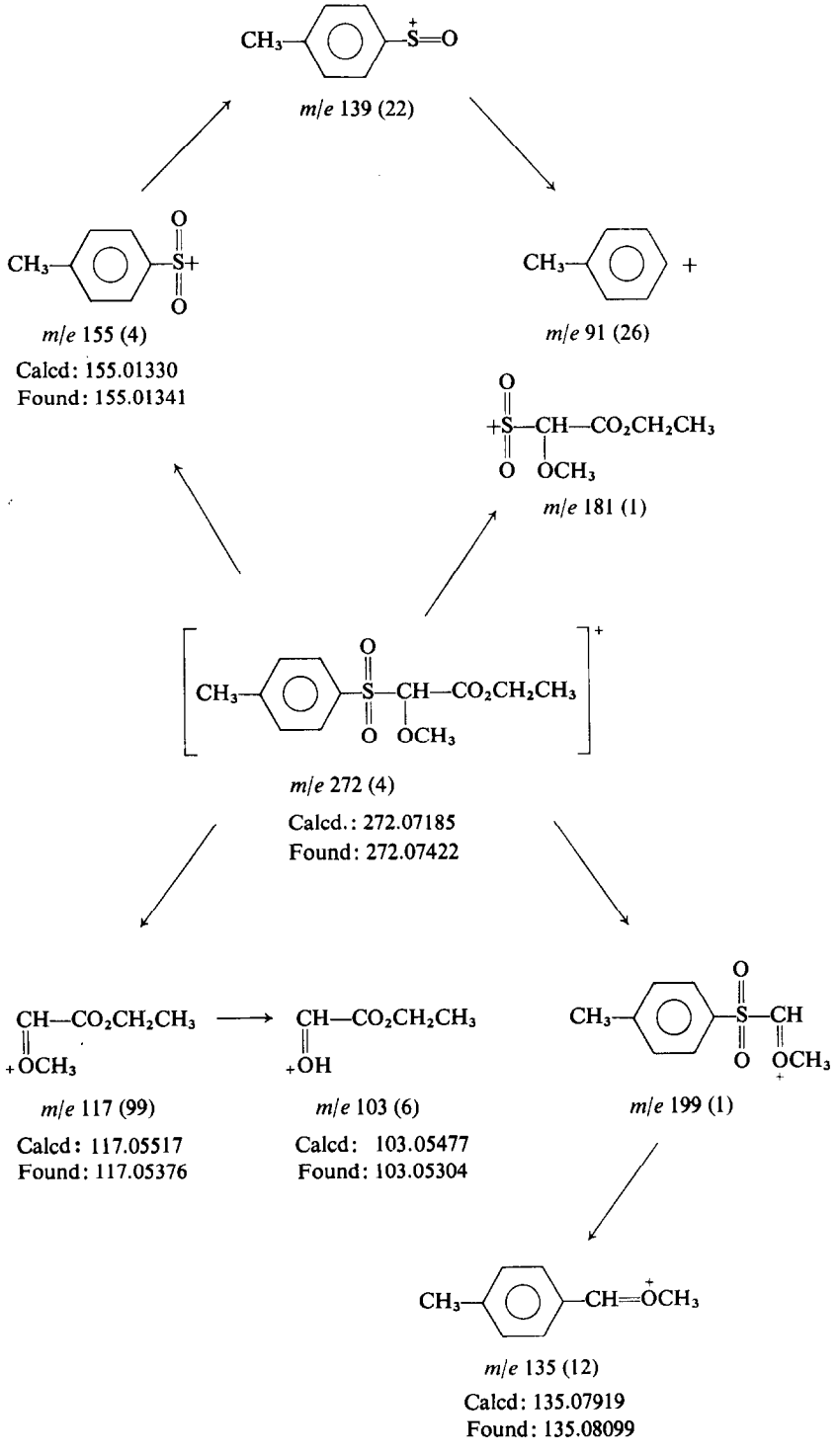
Methyl p-toluenesulfonyl-2-ethoxyacetate, **H**. This product was obtained by photolysis of methyl *p*-toluenesulfonyl-2-diazoactetate, **D**, in ethanol; it shows a molecular ion, *m/e*, at 272.0710 and major peaks at *m/e* 139, 117, and 89, corresponding to the assigned structure. Its nmr spectrum is shown along with that of its isomer, **G**, in Fig. 2b. In particular, the complex pattern from the diastereotopic protons of the methylene group of the 2-ethyl ether, **H**, contrasts with the clean quartet from the ethyl ester group of its isomer, **G**.

Ethyl-p-toluenesulfonyl-2-ethoxyacetate, **I**. This product, obtained in the photolysis of the ethyl diazo ester, **C**, in ethanol, gave a molecular ion at *m/e* 286.0904; calcd for $C_{13}H_{18}O_5S$: 286.0875. Its nmr spectrum is also shown in Fig. 2c; the signals from the diastereotopic methylene group and from the simple methylene group appear side by side.

Methyl p-toluenesulfonyl-2-methoxyacetate, **J**. This product, obtained in the photolysis of **D** in methanol, was identified by its nmr spectrum: δ 7.77 (d, $J = 8.0$ Hz), 7.36 (d, partially obscured by the signal from $CHCl_3$), 4.79 (s), 3.75 (s), 3.69 (s), and 2.43 (s). Its mass spectrum showed the molecular ion at *m/e* 258 and a fractionation pattern to yield the expected fragments at *m/e* 139, 135, 103, and 91.

Methyl p-toluenesulfonyl-2-thioethylacetate, **K**. This product was obtained by the photolysis of thioethyl *p*-toluenesulfonyl diazoacetate, **E**, in methanol. High resolution mass spectrometry gave *m/e* 288.0456; calcd for $C_{12}H_{16}S_2O_4$: 288.0490. The major fragmentation peaks are at *m/e* 91, 133, and 155. The ^1H nmr spectrum is shown in Fig. 3; note the pattern from the diastereotopic protons of the methylene group of the thio ether.

Thioethyl p-toluenesulfonyl-2-methoxyacetate, **L**. This product was also obtained by the photolysis of **E** in methanol. It was identified by its nmr spectrum: δ 7.77 (d, $J =$ 8.0 Hz), 7.39 (d, $J = 8.0$ Hz), 4.67 (s), 3.74 (s), 2.76 (q, $J = 7.2$ Hz), 2.41 (s), and 1.09 (t, $J = 7.2$ Hz). In sharp contrast to the spectrum of the thioethyl ether, **K**, that of the thioethyl ester, **L**, shows a simple quartet from the methylene group of the ethyl residue (no evidence for diastereotopic protons). The mass spectrum of **L** shows the molecular ion at *m/e* 288 together with prominent peaks at 139, 133, 105, and 91 that are consistent with the structure as assigned.



SCHEME I. Relative intensities of mass-spectral peaks are shown in parentheses.

Ethyl p-toluenesulfonyl-2-cyclohexylacetate, **M**. This product was obtained by the photolysis of ethyl *p*-toluenesulfonyl-2-diazoacetate, **C**, in cyclohexane. Its high resolution mass spectrum showed *m/e* of 324.1388; calcd for $C_{17}H_{24}O_4S$: 324.1395. The major peaks in the fragmentation pattern include those anticipated at *m/e* 242 and 169. The nmr spectrum shows peaks at δ 7.76 (d, fine structure), 7.30 (d, fine structure), 3.95 (q, J = 7.1 Hz), 3.79 (d, J = 8.2 Hz), 2.41 (s), 2.20 (broad d, J = 7.2 Hz), 1.64 (broad, s), 1.20 (broad, s), and 1.06 (t, J = 7.1 Hz).

Methyl p-toluenesulfinate, **N**. This arises as a by-product from the photolysis of either **C** or **D** in methanol. Its structure was established by its nmr and mass spectra. ^{1}H nmr:

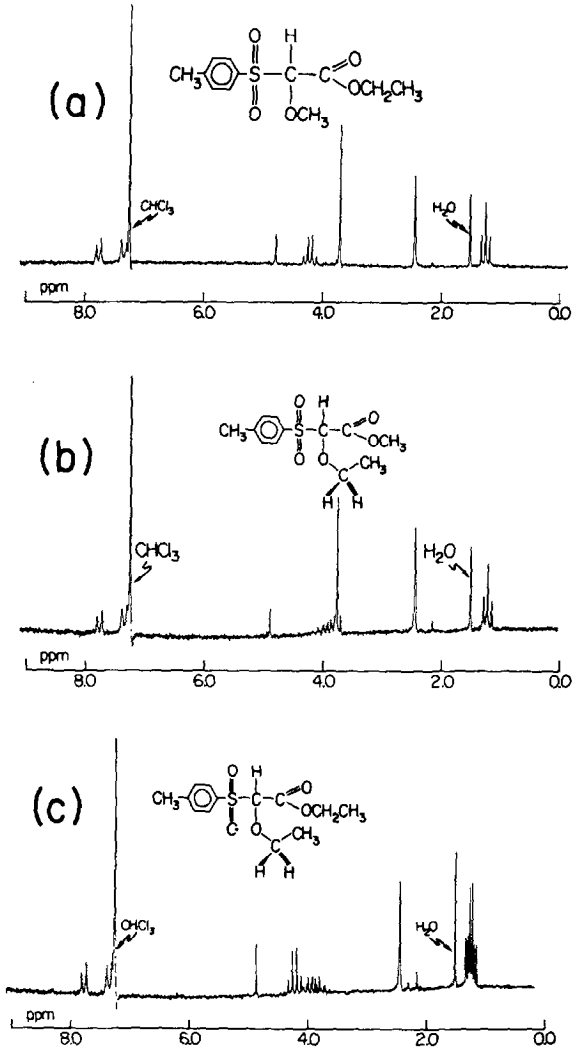


FIG. 2. One-hundred-megahertz Fourier-transform ^{1}H nmr spectra of ethyl *p*-toluenesulfonyl-2-methoxyacetate, **G**, of methyl *p*-toluenesulfonyl-2-ethoxyacetate, **H**, and of ethyl *p*-toluenesulfonyl-2-ethoxyacetate. The signals from the diastereotopic protons of the methylene groups of the latter two compounds appear at about δ 4.

FIG. 3. One-hundred-megahertz Fourier–transform ^1H nmr spectrum of methyl 2-thioethyl-*p*-toluenesulfonylacetate. The signals from the diastereotopic protons of the methylene group are centered at δ 2.86. The spectrum of the isomeric thioethyl ester is shown below for contrast.

δ 7.55 (d, J = 8.0 Hz), 7.28 (d, partially obscured by the signal from $CHCl_3$), 3.40 (s), and 2.36 (s). The mass spectrum showed the molecular ion at m/e 170.

Methyl p-toluenesulfonate, **O**. Methyl *p*-toluenesulfonate, another by-product of photolysis of **C** or **D** in methanol, was identified by a mass spectrum in agreement with that given in the literature (*30*) and by ^1H nmr spectrum: δ 7.78 (d, J = 8.0 Hz), 7.33 (d, J = 8.0 Hz), 3.71 (s), and 2.42 (s) (*31*).

Ethyl p-toluenesulfinate, **P**. This product, a by-product of the photolysis of **C** or **D** in ethanol, shows a parent ion in the mass spectrum at m/e 184 with major peaks at 139 and 91 as expected. Its nmr spectrum shows δ 7.59 (d, J = 8.0 Hz), 7.31 (d, J = 8.0 Hz), 3.89 to 4.27 and 3.47 to 3.75 (complex multiplets), 2.40 (s), and 1.25 (t, J = 7.0 Hz). The region from 3 to 5 ppm, shown in Fig. 4, highlights the signals from the diastereotopic protons of the methylene group.

Ethyl p-toluenesulfonate, **Q**. This product, also obtained from the photolysis of **C** or **D** in ethanol, shows a m/e of 200 and other major mass spectrographic peaks at 139 and 155. Its nmr spectrum, in agreement with that given in the literature (*31*), shows δ 7.77 (d, J = 8.6 Hz), 7.30 (d, partly obscured by $CHCl_3$ peak), 4.05 (q, J = 7.0 Hz), 2.41 (s), and 1.25 (t, J = 7.0 Hz). The region from 3 to 5 ppm is shown in Fig. 4.

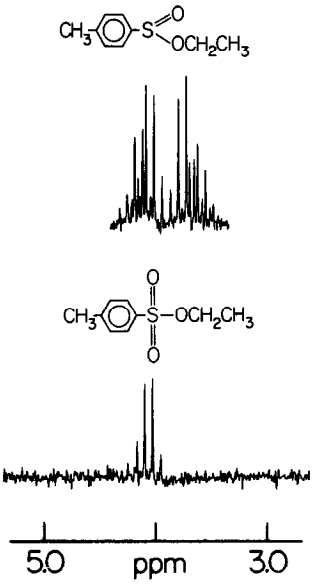


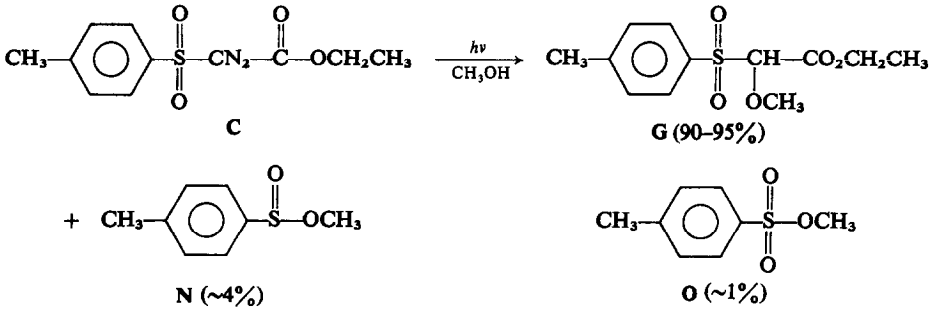
FIG. 4. One-hundred-megahertz Fourier–transform ^1H nmr of the δ 3.0 to 5.0 region of the spectra of ethyl *p*-toluenesulfinate, **P**, and ethyl *p*-toluenesulfonate, **Q**, contrasting the signals from the diastereotopic protons of the methylene group of the sulfinate with the simple quartet from that of the sulfonate.

Ethyl p-toluenesulfonylacetate. The nmr spectrum of synthetic material is superimposable upon that isolated by gas chromatography, although the signals from the aromatic region differ somewhat from those (*27*, *32*) in the literature (which, incidentally, are not in entire agreement with each other). ^1H nmr: δ 7.82 (m), 7.36 (m), 4.14 (q, $J = 7.1$ Hz), 4.08 (s), 2.44 (s), and 1.18 (t, $J = 7.1$ Hz).

RESULTS

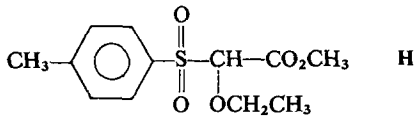
Photolyses in Alcohols

The photolysis of ethyl *p*-toluenesulfonyldiazoacetate, **C**, at 254 nm in methanol led to a rapid decrease in absorption, followed by a slower process. Gas chromatographic analysis at a column temperature of 235°C, injection port temperature of 295°C, and detector temperature of 250°C with helium flow of 25 ml/min showed **G** as the primary product; it was identified as ethyl *p*-toluenesulfonyl-2-methoxyacetate. At longer times,



G undergoes photolysis at 254 nm to **N** and **O**, which have been identified as methyl *p*-toluenesulfinate and methyl *p*-toluenesulfonate, respectively

Gas chromatography of an unphotolyzed solution in methanol of the ethyl diazoester, **C**, separated a mixture of two major products from some minor ones, presumably all formed by thermal reaction in the gas chromatographic system. The major products were the methoxyacetate, **G**, and the Wolff rearrangement product, methyl *p*-toluenesulfonyl-2-ethoxyacetate, **H**, identified by nmr spectroscopy of a 60 : 40 mixture.



H

The progress of the photolyses of **C** and of ethyldiazoacetate in 5×10^{-5} *M* solutions is shown in Fig. 5. The photolysis of the ethyl diazo ester, **C**, in ethanol led to products

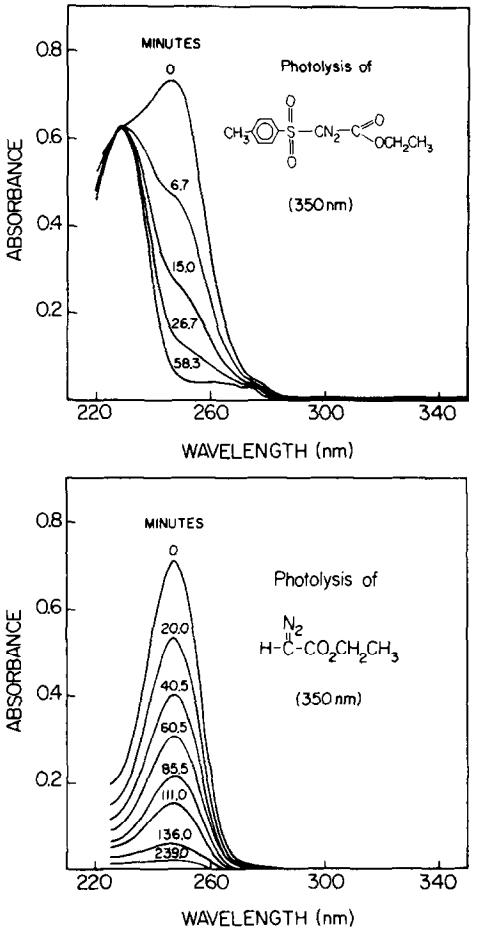


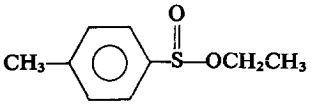
FIG. 5. Photolyses of approximately 2.5 ml of 5×10^{-5} *M* solutions of ethyl *p*-toluenesulfonyldiazoacetate and of ethyl diazoacetate in methanol. The photolyses were carried out at 350 nm, but the course of the photolyses was followed in the range of 220 to 280 nm where absorption is intense (and nearly equal for the two compounds). Note the difference in time scale for the two photolyses.

identified as **I** (insertion into the solvent), the ethyl sulfinate, **P**, and the ethyl sulfonate, **Q**, analogous to the corresponding methyl esters **N** and **O** produced upon photolysis of **C** in methanol. In addition, ethyl *p*-toluenesulfonylacetate, the reduction product of **C**, was isolated. The photoproducts are all stable to prolonged radiation at 350 nm, whereas further photolysis at 254 nm leads to a slow decrease in absorption, corresponding to some additional decomposition. The yields as a function of wave length are shown in Table 1.

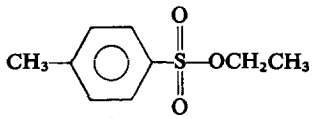
TABLE 1

PERCENTAGE COMPOSITION OF THE PRODUCTS FROM PHOTOLYSIS OF **C**, ETHYL-2-*p*-TOLUENESULFONYL-2-DIAZOACETATE IN ETHANOL USING RPR 2537, 3000, AND3500-Å LAMPS

	Wavelength		
	2537 Å	3000 Å	3500 Å
P (sulfinate)	23	7	14
Q (sulfonate)	4	2	3
R (reduction)	46	83	10
I (insertion)	26	10	73

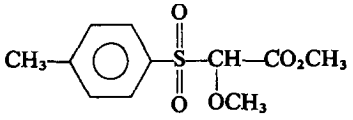


P



Q

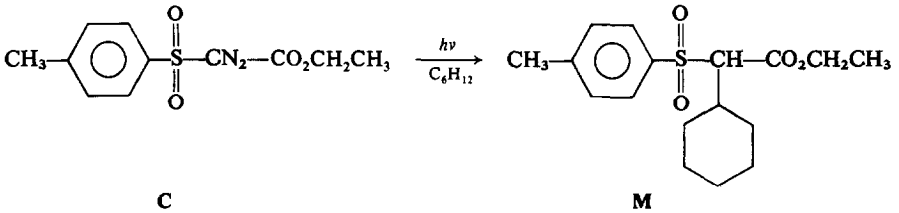
Photolysis of methyl *p*-toluenesulfonyldiazoacetate, **D**, in methanol and ethanol produced products paralleling those formed from ethyl *p*-toluenesulfonyldiazoacetate, **C**. In particular, the photolysis of **D** in methanol led to the formation of the insertion product, methyl *p*-toluenesulfonyl-2-methoxyacetate, **J**, and the photolysis in ethanol to the ethoxyacetate, **H**; in both cases, the yield of insertion product was high, and in the latter case (where the product of Wolff rearrangement could have been seen had it been present) no Wolff rearrangement was detected.



J

Photolysis in Cyclohexane

The photolysis of the ethyl diazo ester, **C**, in cyclohexane leads to the insertion

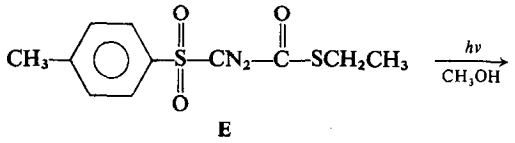


C **M**

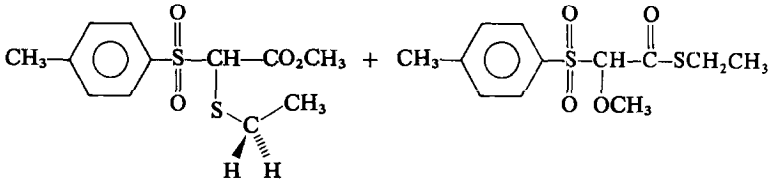
product, **M**, in yields of better than 90%. A small amount of material (by-product or by-products) has not been identified.

Photolysis of Thioesters

A 0.114 *M* solution of the thioethyl diazo ester, **E**, was photolyzed at 350 nm for 120 min, at which time the reaction (as monitored by tlc) was complete. Gas chromatographic analysis revealed four products, subsequently identified as thioethyl *p*-toluenesulfonyl-2-methoxyacetate, **L** (the product of insertion into the solvent), plus methyl *p*-toluenesulfonyl-2-thioethylacetate, **K** (the Wolff rearrangement product) and the methyl sulfinate, **N**, and the methyl sulfonate, **O**. They were present in 25, 68, 0.5 to 1, and 6 to 7% yields, respectively. The separation of the four products was carried out with an injector port temperature of 230°C, a flame ionization detector at 240°C, and a programmed increase of column temperature from 200 to 210°C at 1°C/min.



E



K (68%) + **N** (1%) + **O** (6–7%) **L** (25%)

The photolysis of methyl S-*p*-toluenesulfonyldiazoacetyl-*N*-acetylcysteine, **F**, in methanol at 350 nm was followed by changes in the ultraviolet absorption spectrum; the products in this case were not isolated. The disappearance of the absorption at 240 nm, ascribed to the thioester bond, occurred to the extent of about 75%, showing that the Wolff rearrangement here, as in the photolysis of the thioethyl ester **E**, is the major pathway but not the exclusive pathway for the photolysis, and implying that about 25% of the reaction led to insertion into the solvent.

DISCUSSION

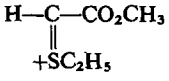
Nuclear Magnetic Resonance Identification of Products

^{1}H nmr spectroscopy was critical in the identification of the products from the photolyses. In every case, all of the protons in the molecules could be properly accounted for as to chemical shift, splitting pattern, and, at least approximately, as to the number of protons determined by integration. In particular, the signals from the methylene protons of the ethyl groups of ethyl esters and of thioethyl esters of the substituted toluenesulfonylacetates appear as clean quartets, while those of the methylene protons of the isomeric ethyl and thioethyl ether groups appear as complex multiplets. Both sets of methylene protons in **G**, **H**, **I**, **K**, **L**, and **M** are in principle diastereotopic, since the carbon atom at the 2-position of the acetate residue is

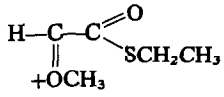
asymmetric. However, the asymmetric centers in the ester groups of **G, I, L,** and **M** are, apparently, too remote to produce an effectively different environment for the methylene protons, whereas in the 2-ether and 2-thioether groups of **H, I,** and **K** the asymmetric center is close by. The appearance of signals from diastereotopic protons constitutes a major support for the assignment of structures as given. In particular, the ethyl ester ethyl ether, **I,** shows signals from both types of methylene groups; the alternative structures for **G** and **H** are clearly apparent from the spectra shown in Fig. 2. Furthermore, the sulfur atom in ethyl *p*-toluenesulfinate, **P,** provides the asymmetric environment for the diastereotopic protons of the methylene group of the ester; the signals, showing all 16 expected peaks, are contrasted in Fig. 3 with the simple quartet from the methylene protons of ethyl *p*-toluenesulfonate, **Q.** Similar diastereotopic patterns arising from the asymmetry of trivalent sulfur have been reported for diethyl sulfite (*33*), for ethyl 3-(1-phenyl)-butynyl sulfide (*34*), and for ethyl benzenesulfinate (*35*).

Mass Spectroscopy

Strong additional support for the identification of the products of photolysis and especially of the isomeric structures **K** and **L** (methyl ester thioethyl ether and methyl ether thioethyl ester, respectively) arise from their mass spectra. Both compounds show molecular ions at *m/e* 288 and the expected fragmentation products at *m/e* 155, 139, and 91. The base peak, however, for the thioethyl ether has *m/e* 133, ascribed to the structure

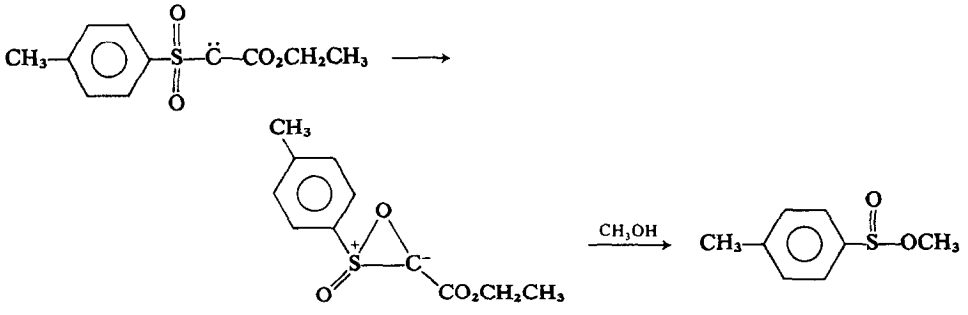


whereas the base peak from the thioethyl ester occurs at *m/e* 105 and presumably arises from a McLafferty rearrangement, leading to the loss of ethylene from the ion,



By-Products

The mechanisms of formation of the sulfinates **N** and **P** and sulfonates **O** and **Q** that accompany the formation of insertion products during photolysis have not yet been



determined. Possibilities include photolytic free radical cleavage of the diazosulfonates, followed by reaction with solvent, or formation of a carbene on photolysis followed by the formation of a three-membered (COS) ring intermediate, which in turn is cleaved by solvent. Whatever the mechanism for their formation, the structures of these by-products are secure. Photolysis of the diazoesters in ethanol leads, in addition to sulfonate and sulfinate, to a small quantity of reduction product, where the diazo group has been replaced by two hydrogen atoms. Photoreduction has been noted for benzophenone-sensitized photolysis of diazoesters and presumably occurs by way of triplet states (*20, 36*). Small quantities of products have been obtained that apparently result from photochemical ester exchange (*19, 37, 38*). A few other minor products, noted under Experimental, have not yet been identified. Despite the formation of these minor by-products, the photolyses in methanol and in cyclohexane are nearly clean and lead either to insertion into the solvent (for oxygen esters) or to insertion plus Wolff rearrangement for the thioesters.

Photochemistry

The primary objective of this research was to design and study reagents that might be useful for photoaffinity labeling. The *p*-toluenesulfonyl reagents here described are promising ones for that purpose.

First, the acid chloride and the *p*-nitrophenyl ester of *p*-toluenesulfonyldiazoacetic acid are stable solids that do not detonate on heating and are insensitive to shock. The thermal decomposition of the ethyl ester, **C**, is slow; Rigitz and Bartz (*26*) found a rate constant for decomposition in mesitylene of 2×10^{-5} sec^{-1} at 100°C. **C** is stable in base up to pH 10 and is stable to 1 *N* hydrochloric acid.

Second, the *p*-toluenesulfonyl derivatives such as **C** have a much stronger absorption at long wavelengths (350 nm) than do other diazo esters (Fig. 1). The rate of photochemical decomposition for the sulfonyldiazoacetate is correspondingly greater than that for ethyl diazoacetate (Fig. 5); this is what would be anticipated from the absorption spectra, provided that the quantum yields for decomposition at a long wavelength are similar for both esters. This property provides the most important advantage for the sulfonyldiazoacetates over other diazo reagents for photoaffinity labeling in cases involving biological materials that are damaged or destroyed by short wavelength ultraviolet radiation.

Finally, the *p*-toluenesulfonyldiazo esters and thioesters, offered here as models for their biochemical analogs, undergo photolysis with considerable or even predominant insertion into solvent. Ethyl *p*-toluenesulfonyl-2-diazoacetate undergoes photolysis in methanol with 90 to 95% insertion into the solvent regardless of the wavelength at which the photolysis is conducted. Similar photolysis in cyclohexane leads to insertion (here, of course, into a C—H bond) in greater than 90% yield, again regardless of the wavelength employed. Photolysis in ethanol gives smaller quantities of insertion and more by-products; the yields of insertion are however still good and are better at longer wavelengths of irradiation. Although some by-products are formed in all cases, none of the products of Wolff rearrangement were detected in these photolyses. The lack of Wolff rearrangement in the photolysis of these *p*-toluenesulfonyl diazoesters was foreshadowed by the relatively low yield of such products in the photolysis of α-acetyl-*p*-toluenesulfonyl diazomethane (*39*).

Photolysis of thioesters was also successful, although less so than that of the corresponding oxygen esters. The photolysis of thioethyl *p*-toluenesulfonyldiazoacetate in methanol led to 75% Wolff rearrangement and only 25% insertion into the solvent.

The photolyses of thioesters are prominent among those that must be conducted at long wavelengths, since the insertion products are themselves thioesters, absorb at 250 nm, and are destroyed by short wavelength uv radiation. It is then particularly with respect to examples where the affinity reagent is attached to sulfur that the *p*-toluenesulfonyl reagents are needed. Although a higher yield of insertion than 25% would be desirable, this yield is sufficient to permit the identification of photolysis products using radioactive tracer methods. Although the new reagents give somewhat less insertion into solvent than do the 2-diazo-3,3,3-trifluoropropionyl derivatives recently introduced (*21*), the higher extinction coefficient at long wavelengths is a compensatory advantage.

Testing of these reagents in enzymic systems is underway.

ACKNOWLEDGMENTS

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