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# A Preparative Synthesis of Lumiphore-Labeled Polymers

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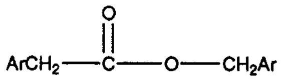
ABSTRACT: Polystyrene (PS), poly(methyl methacrylate), poly(vinyl acetate), and 1,2-poly(1,3-butadiene) were labeled with naphthyl groups using a focused UV lamp irradiation technique. Photolabelings of PS with anthracene, fluorene, and phenanthrene chromophores were also readily achieved. Fast labeling rates and high labeling efficiency (≥40 labels per PS chain) were achieved when PS was irradiated in the presence of 9-bromofluorene or 9-bromophenanthrene. No significant molecular-weight changes were observed in the PS photolabeling systems. The fluorescent labels attached to the PS chain were characterized by spectroscopic analysis.

## Introduction

In previous papers in this series[1] procedures have been described for phtolabeling macromolecules using photodissociation of compounds of the general structure



$$ArCH_2-\overset{\overset{O}{\|}}{C}-O-CH_2Ar$$

Photodissociation of these and similar molecules apparently leads to the formation of two $ArCH_2^\bullet$ radicals, one of which is capable of abstracting a proton from a polymer dissolved in an inert solvent such as benzene. Labeling occurs by the combination of the stable $ArCH_2^\bullet$ radical with a polymer radical $P^\bullet$.

$$ArCH_2^\bullet + P^\bullet \rightarrow P-CH_2Ar \qquad (1)$$

In principle, any sequence of reactions leading to both $P^\bullet$ and $ArCH_2^\bullet$ radicals should lead to the attachment of $ArCH_2$ groups to the polymer chain. For example, in early work, Holden and Guillet showed that anthryl groups could be attached to the end of polymer chains by thermal polymerization of methacrylate monomers in the presence of anthryl methyl bromide.[2] In this case the anthryl methyl radicals were produced by the attack of initiator radicals. These stable radicals then reacted with the growing polymer radicals by combination to terminate the chain.

This paper reports the use of a variety of aromatic reagents to attach fluorescent labels to polymer chains.

The use of luminescence techniques to study structural, conformational, and dynamic properties of polymers has become an increasingly important tool in polymer chemistry and physics. A number of books and review articles have been published within the last 10 years.[3] Labeled polymers have been used to determine micellization and micellar properties of water-soluble block copolymers,[4] local segmental dynamics of polymers in dilute solutions,[5] the effect of casting solvent on film homogeneity,[6] dopant site distributions in polymer films,[7] the diffusion of ionic reagents through networks,[8] cure processes in polymer systems,[9] the miscibility of polymer blends,[10] the end injection of triplet electronic excitation energy into polymer molecules,[11] the terminal segment mobility of polymers,[12] coalescence and film formation processes in nonaqueous dispersion particles,[13] polymer/surfactant interactions,[14] and the heat-induced phase separation of aqueous polymer solutions.[15] The formation of a triplet excimer of a labeled polymer in isopentane glass was also demonstrated.[16]

Realization of the full potential of luminescence techniques requires the availability of synthetic methods for incorporating fluorescent labels in preformed polymers. With this in mind, a laser photolabeling technique was developed.[1] However, this produces only a few milligrams of labeled polymer and requires expensive equipment.

A simple photolabeling system using only a 100-W UV lamp is reported here. It has been found useful in labeling gram quantities of typical vinyl polymers such as polystyrene (PS), poly(methyl methacrylate) (PMMA), poly(vinyl acetate) (PVAc), and 1,2-poly(1,3-butadiene) (PBD). It also seems likely that the procedure can be readily scaled up to prepare much larger quantities of labeled polymer.

## Experimental Section

**Materials.** PS ($\bar{M}_n$ 70 × $10^3$) and PMMA ($\bar{M}_n$ 130 × $10^3$) (both Aldrich), PVAc ($\bar{M}_n$ 70 × $10^3$) and PBD ($\bar{M}_n$ 95 × $10^3$) (both Polysciences), spectroscopic grades of benzene and tetrahydrofuran (THF) (Caledon), and high-purity naphthalene, anthracene, fluorene, and phenanthrene derivatives (Aldrich) were used as received. 1-Naphthylmethyl 1-naphthylacetate, $(1\text{-}NpCH_2)_2CO_2$, was synthesized using a modified literature method.[17]

Di-1-naphthylmethyl oxalate was synthesized by a reaction of 1-naphthalenemethanol and oxalyl bromide in the presence of triethylamine. To a solution of 1-naphthalenemethanol (1.266 g, 8 mmol) and triethylamine (1.1 mL) in benzene (25 mL) was carefully added 1.295 g of oxalyl bromide. After stirring at 0 °C for about 1 h, GC analysis showed that the 1-naphthalenemethanol was completely consumed. The product was isolated by recrystallization from THF/methanol. Yield: 1.1 g (74%). $^1$H NMR ($CDCl_3$): δ 5.68 (s, 4H, $CH_2$), 7.42–8.01 (m, 14H, $H_{ar}$). $^{13}$C NMR ($CDCl_3$): δ 67.1 ($CH_2$), 123.5–138.2 ($C_{ar}$), 157.8 (CO), MS: Calcd for $C_{24}H_{18}O_4$, *m/e* 370; found, *m/e* 370.

**Irradiation.** Polymer solutions for irradiation experiments were prepared by dissolving 1.0 g of polymer and 0.08 or 0.16 mmol of the chromophoric compound in 10 mL of benzene. Dissolved oxygen was removed and stirring achieved by bubbling nitrogen through the polymer solutions in a cylindrical quartz cell. With slow, continuous nitrogen bubbling, the full beam emitted from a 100-W Hg lamp (Photon Technology International A1010) was focused on an 8-mm-diameter spot in the center of the quartz reactor whose temperature was controlled at ca. 30 °C by a stream of air. The distance between the UV lamp and the quartz cell was 35 cm. The light intensity at the focus point was measured to be ~2.3 W/cm$^2$ by using a Molectron PR200 pyroelectric radiometer protected by a set of neutral-density filters.

**Characterization.** At various time intervals, a small amount of polymer solution was sampled from the quartz reactor and injected into a GPC analysis system (Waters 510) with a

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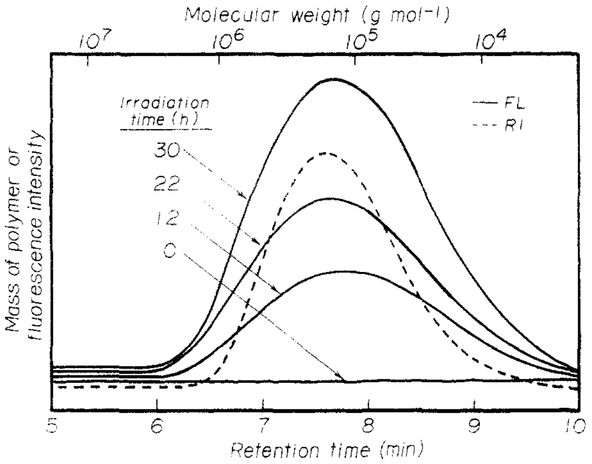

**Figure 1.** GPC curves of naphthyl-labeled PS ($\lambda_{ex}$ = 300 nm, $\lambda_{em} \geq$ 345 nm): (—) fluorescent response; (---) refractive index. A benzene solution (10 mL) containing 1 g of PS and 0.08 mmol of $(1\text{-NpCH}_2)_2CO_2$ was irradiated with a 100-W mercury discharge lamp under a nitrogen atmosphere.

differential refractometer (RI, Waters 410) and a fluorescence detector (FL, Applied Biosystem 980) to determine the photolabeling efficiency. The GPC system was operated at a flow rate of 1.0 mL min$^{-1}$, using THF as eluant. The excitation wavelength in the FL analysis was set at 300 nm for naphthalene, fluorene, and phenanthrene derivatives and a 345-nm cutoff filter was used to discriminate between scattered light and fluorescence emission. In the case of anthracene compounds, the excitation and emission wavelengths were set at 366 and 389 nm, respectively. All the GPC data were treated with a Commodore PC 20-III microcomputer.

Molecular-weight calibration curves were obtained from monodisperse samples of PS (MW 1250–900 000) and PMMA (MW 4100–1 300 000). The number-average molecular ($\bar{M}_n$) and polydispersity ratio ($\bar{M}_w/\bar{M}_n$) of PS and PBD were determined on the basis of the PS calibration, while those of PMMA and PVAc were on the basis of the PMMA calibration. Calibration curves for the calculation of polymer mass from the integrated area of the RI peak were drawn with a set of polymer solutions of known concentration. The numbers of naphthyl, anthryl, fluorenyl, and phenanthryl groups labeled on the polymer chains were calculated from the calibration curves obtained with standard solutions of methylnaphthalene, methylanthracene, fluorene, and phenanthrene, respectively.

The labeled polymers were purified by pouring the diluted polymer solutions into a large amount of methanol. The resulting precipitates were filtered on sintered glass disks, washed with methanol, and dried in vacuo for ca. 3 days. Ordinarily about 0.9 g of labeled polymer was isolated from each reaction.

THF solutions of the purified labeled PS were prepared, and their steady-state fluorescence spectra were recorded at room temperature on a SLM-4800S spectrofluorometer (SLM Instruments). The excitation wavelength for the fluorescence measurement of the napthyl-, fluorenyl-, or phenanthryl-labeled PS was set at 280 nm, and the anthryl-labeled PS was excited at 355 nm.

## Results and Discussion

**Photolabeling of Vinyl Polymers with Naphthylene Chromophores.** Figure 1 shows GPC fluorescence curves of PS before and after UV irradiation in the presence of $(1\text{-NpCH}_2)_2CO_2$. Before irradiation, no fluorescence emission was observed in the high-molecular-weight polymer region. After irradiation, fluorescence emission from the polymer was observed, indicating that the naphthyl labels were attached to the PS chain. As seen in Figure 1, irradiation for 30 h induced an intense fluorescence emission, corresponding to ca. 7 fluorophores per PS chain.

Photolabeling efficiencies for PS are plotted as a function of irradiation time in Figure 2a. A short time (ca. 5 h) of irradiation attached on average one naphthyl label per PS chain. The photolabeling efficiency increased linearly with irradiation time. Thus, polymers with a different number of labels could be prepared by simply changing the irradiation period.

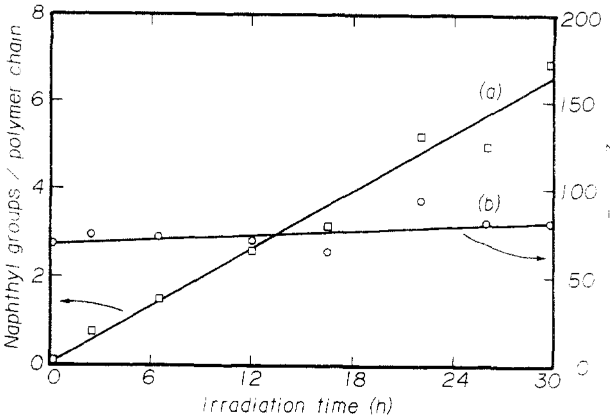

**Figure 2.** Photolabeling of PS. A 10% PS solution in benzene containing 8 mM $(1\text{-NpCH}_2)_2CO_2$ was irradiated under nitrogen for various times: (a) naphthyl labels per chain; (b) $\bar{M}_n/10^3$.

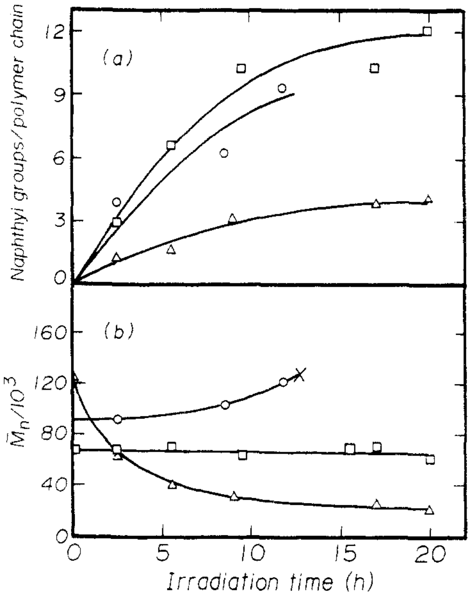

**Figure 3.** Photolabeling of (□) PVAc, (○) PBD, and (△) PMMA: (a) effect of irradiation time on the photolabeling efficiency; (b) molecular weight change during the photolabeling. Molecular weights of PVAc and PMMA were determined by GPC on the basis of a PMMA calibration, while a PS standard was used in the calculation of the molecular weight of PBD.

No significant molecular-weight change was observed, and the molecular-weight distribution remained unchanged ($\bar{M}_w/\bar{M}_n$ ca. 2) during the course of the photolabeling reaction (Figure 2b). Apparently, neither chain scission nor cross-linking took place to a significant extent, although later experiments with high-molecular-weight PS indicated that some chain scission does occur, presumably by $\beta$-scission of the polymer radical.

Photolabeling of other typical vinyl polymers was also attempted using the same system. In the case of PVAc, the photolabeling rate was quite high (Figure 3a). Irradiation for 3 h introduced more than 3 labels per PVAc chain. A PVAc having 12 naphthyl labels per polymer chain was readily prepared with a 20-h irradiation. Fast photolabeling was also observed in PBD, while the photolabeling reaction of PMMA was comparable to that in PS.

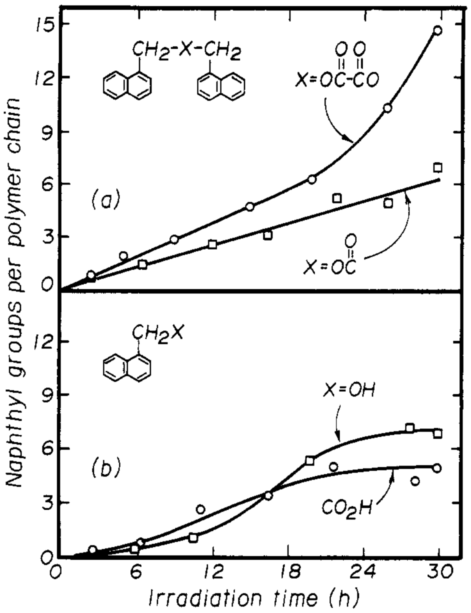

**Figure 4.** Effect of irradiation time on the photolabeling efficiency of PS. A 10% PS solution in benzene containing 8 mM $(1\text{-}N_pCH_2)_2X$ (X = $(CO_2)_2$ or $CO_2$) or 16 mM 1-$NpCH_2X$ (X = OH or $CO_2H$) was irradiated with a UV lamp under nitrogen.

The molecular weight of PVAc did not change during the photolabeling reaction (Figure 3b). However, in the PBD system cross-linking occurred after 12 h. In the photolabeling of PMMA, the molecular weight of the polymer gradually decreased, which we attribute to $\beta$-scission of the radical formed on the backbone carbons.

Additional studies of photolabeling were carried out on PS using a variety of other potential sources of radical generating compounds. Di-1-naphthylmethyl oxalate ($(1\text{-}NpCH_2CO)_2$) labeled the PS chain at a faster rate than $(1\text{-}NpCH_2)_2CO_2$ (Figure 4a). When PS was irradiated in the presence of 1-naphthalenemethanol (1-$NpCH_2OH$), which is commercially available, an induction period was observed, but prolonged irradiation steadily attached more than seven labels to a PS chain (Figure 5b). Similar results were obtained when PS was irradiated in the presence of 1-naphthylacetic acid (1-$NpCH_2CO_2H$).

**Photolabeling of PS with Other Chromophores.** Polymers labeled with different luminescent chromophores are required in many photochemical and photophysical studies. To expand the versatility of the present photolabeling technique, the possibility of labeling PS with chromophores other than naphthalene was examined. The results of a series of such experiments with PS are summarized in Table 1.

Irradiation in the presence of 9-anthracenemethanol failed to label PS efficiently. Even when the irradiation time was prolonged to 30 h, the photolabeling was as low as 0.1; that is, in ten PS molecules, only one molecule was labeled with an anthryl chromophore. On the other hand, when 9-(chloromethyl)anthracene was used, a 2.5-h irradiation time yielded a photolabeling higher than 0.8.

Photolabeling through the photolysis of 9-bromofluorene proceeded very fast (no. 3). Varying the irradiation time could change the photolabeling over a wide range, providing a very practical procedure for the synthesis of labeled polymer.

Photolabeling through photolysis of 2-acetylphenanthrene (2-AcPhe), however, was quite inefficient (no. 4). Irradiation of more than 20 h attached only about two phenanthryl labels to a PS chain. This might be due to the low efficiency of the generation of free radicals in the photolysis of 2-AcPhe. On the other hand, one might expect that 9-bromophenanthrene (9-PheBr) would photolyze easily, and indeed, the photolysis of 9-PheBr attached as many as 46 phenanthryl labels to a PS chain in a short period of irradiation (5 h).

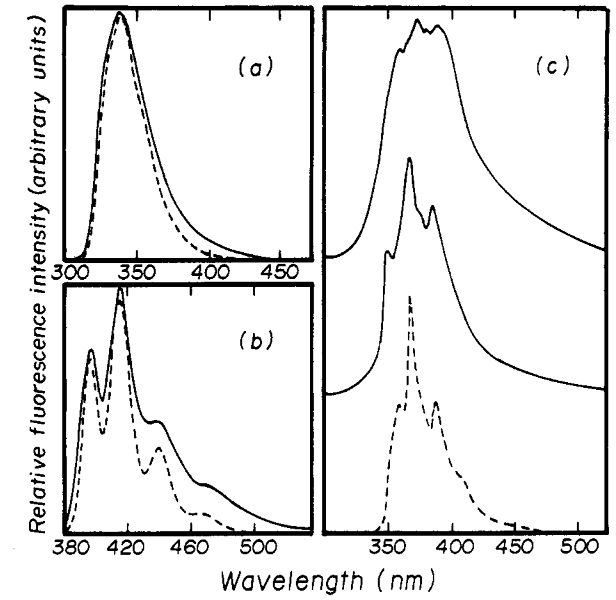

**Figure 5.** Steady-state fluorescence spectra (solid lines) of (a) naphthalene (7.0 naphthyls per chain), (b) anthracene (0.8 anthryl per chain), and (c) phenanthrene (2.3 and 46 phenanthryls per chain) labeled PS measured at room temperature. The naphthalene and phenanthrene labels were excited at 280 nm, and the anthracene label was excited at 355 nm. The dotted curves represent the normalized fluorescence emission spectra of the model compounds (a) 1-methylnaphthalene, (b) 9-methylanthracene, and (c) phenanthrene.

**Photolabeling Product Analysis.** Figure 5 shows the steady-state fluorescence spectra of THF solutions of naphthyl-, anthryl-, and phenanthryl-labeled PS. The fluorescence emission of the naphthyl-labeled PS (Figure 5a) was clearly naphthalene-like[18] though somewhat structureless because of the inhomogeneous broadening effect resulting from the variety of environmental conditions in which the chromophore might be found.[19] The anthryl- (Figure 5b) and phenanthryl-labeled PS's (Figure 5c) also showed emission profiles characteristic of the corresponding chromophores. The UV spectrum of the anthryl-labeled PS, exhibited signals at 392, 372, 354, and 336 nm due to absorption of the anthracene chromophore.

GPC fluorescence measurements have unambiguously demonstrated that the luminescent labels were attached to the polymer chains at all molecular weight levels (cf. Figure 1), and the spectroscopic analyses (Figure 5) further confirmed that the attached labels are the expected chromophores.

**Mechanism of the Photolabeling Reaction.** The photolabeling of vinyl polymers via laser photolysis of chromophoric compounds has been studied previously.[1] In the present procedure where compounds such as $ArCH_2X$ are used for photolabeling, it is not necessary to invoke a two-photon mechanism[1] for the reaction. When X is a halogen (Cl or Br), photolysis occurs readily to form $ArCH_2^{\bullet}$ and $X^{\bullet}$:

$$ArCH_2X \xrightarrow{h\nu} ArCH_2^{\bullet} + X^{\bullet} \qquad (2)$$

Both bromine and chlorine atoms readily abstract protons from the polymer chain via

**Table 1. Photolabeling of PS with Luminescent Chromophores**[a]

| | | luminescent labels per polymer chain[c] at irradiation time | | | | | |
|---|---|---|---|---|---|---|---|
| no. | chromophoric compd[b] | 0 | 2.5 h | 5.0 h | 9.0 h | 20.0 h | 30.0 h |
| 1 | 9-$AnCH_2OH$ | 0 | 0.01 | 0.02 | 0.04 | | 0.09 |
| 2 | 9-$AnCH_2Cl$ | 0 | 0.87 | 0.85 | 0.80 | | 0.84 |
| 3 | 9-FlBr | 0 | 24 | 29 | 30 | 40 | |
| 4 | 2-$PheCOCH_3$ | 0 | 0.21 | 0.51 | 0.98 | 2.3 | |
| 5 | 9-PheBr | 0 | 2.6 | 46 | 45 | | |

[a] A benzene solution of PS containing 16 mM of a chromophoric compound was irradiated with a 100-W UV lamp under a nitrogen atmosphere. [b] An, Fl, and Phe denote anthryl, fluorenyl, and phenanthryl groups, respectively. [c] Determined by GPC analysis.

$$P + X^{\bullet} \rightarrow HX + P^{\bullet} \qquad (3)$$

and labeling occurs by a combination of the stable $ArCH_2^{\bullet}$ radical with $P^{\bullet}$:

$$P^{\bullet} + ArCH_2^{\bullet} \rightarrow PCH_2Ar \qquad (4)$$

Because of the relative stability of $ArCH_2^{\bullet}$, its concentration will build up to a photostationary level many times greater than that of either $P^{\bullet}$ or $X^{\bullet}$, thus making reaction 4 very efficient. In our experiments, the bromide derivatives were much more efficient than the chlorides.

The slow rate of labeling of PMMA is presumably because there is no readily abstractable hydrogen in the PMMA chain, thus reducing the efficiency of reaction 3.

In earlier experiments, irradiation with unfocused UV lamps gave negligible amounts of fluorescent labeling. The much higher efficiencies observed in these experiments in highly focused beams is presumably because the much higher radical concentrations produced in the latter makes it more probable that an $ArCH_2^{\bullet}$ radical is available for reaction when a hydrogen is removed from a carbon on the polymer chain. These relatively reactive polymer radicals normally have a very short lifetime before they are terminated by chain transfer from the solvent or mutual recombination. $\beta$-Scission by the polymer radical leads to chain scission but does not preclude fluorescent labeling because the radical species produced may persist until terminated by $ArCH_2^{\bullet}$.

Benzene was chosen as the solvent for these studies because it is relatively inert to hydrogen abstraction by free radical species. Presumably, other inert solvents could also be used.

In conclusion, the present photolabeling technique has several noteworthy features.

(i) The experimental setup is very simple and does not need complicated or expensive equipment. The only requirement is a conventional focused UV irradiation system.

(ii) Almost all of the chromophoric compounds used in this study are commercially available. This obviates the difficult synthesis of complex compounds, making the present technique easily applicable.

(iii) Photolabelings of gram quantities of various typical vinyl polymers have been readily achieved, proving the photolabeling technique practical and versatile.

(iv) The method can be used with preformed polymers, thus avoiding extensive synthetic techniques using labeled monomers.

We expect that the facile preparative photolabeling technique developed in this work will find wide application.

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