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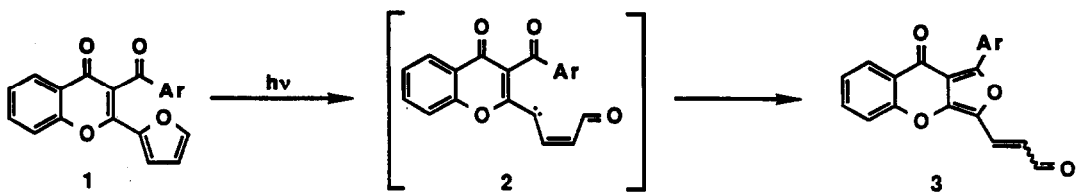
PHOTOACTIVABLE FLUOROPHORES. 2. SYNTHESIS AND PHOTOACTIVATION OF FUNCTIONALIZED 3-AROYL-2-(2-FURYL)-CHROMONES.[1]

Richard T. Cummings, James P. DiZio, and Grant A. Krafft*
Department of Chemistry, Syracuse University
Syracuse, New York 13244-1200

Summary: The synthesis and photochemistry of functionalized 3-aroyl-2-(2-furyl)-chromones **1** which are photoactivable fluorophores are described. Irradiation converts the non-fluorescent furyl chromones to highly fluorescent 1-arylfuro[3,4b]chromones **3**. Functionality has been incorporated to permit covalent attachment, impart aqueous solubility and to maximize the fluorescence of the ultimate 1-arylfuro[3,4b]chromone fluorophore. An efficient synthetic pathway to the furyl chromones via an intramolecular Dieckman cyclization also is described.

In the preceeding Letter[2], we described the synthesis and photoactivation of a new type of photoactivable fluorescent probe consisting of fluorophore-quencher conjugates which exhibit negligible fluorescence until photocleavage of a quenching chromophore. These photoactivable fluorphores (PAFs) serve as useful probes to study the transport and diffusion of macromolecules by the technique fluorescence photoactivation and dissipation developed by Ware and us.[3] In this Letter we describe a second type of PAF in which photoactivation occurs directly by a photochemically induced rearrangement that converts a cross-conjugated, non-fluorescent chromophore into a fully conjugated, highly fluorescent ultimate fluorophore.

This particular strategy for photochemical generation of a target fluorophore[4] is based on the interesting work of Huffman and co-workers[5] who initially described the photoisomerization of 3-aroyl- and 3-(2-furoyl)-2-(2-furyl)-chromones to the corresponding 1-aryl- or 1-(2-furyl)-furo[3,4b]chromones, as illustrated below. Although the quantum yields for this process were low (1-5%), the high fluorescence and absence of by-products prompted our studies to optimize this photoactive system to provide more efficient and usefully tailored PAFs.

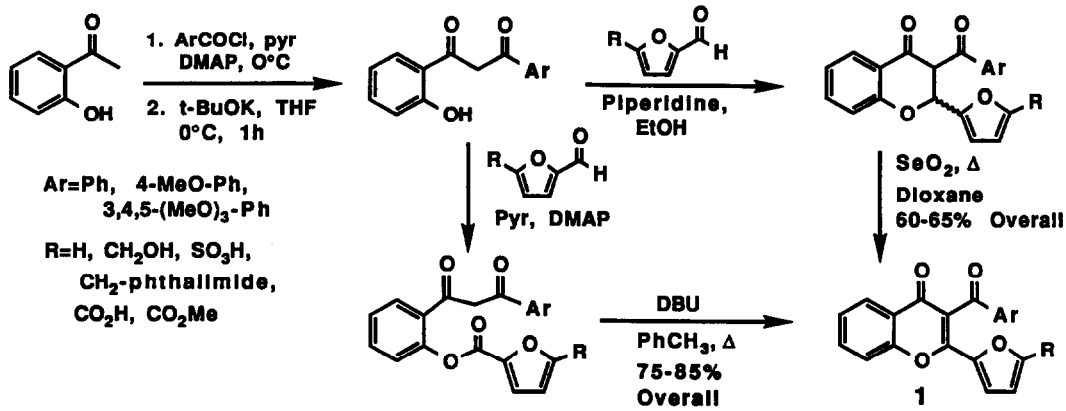


1 2 3

The postulated mechanism for this photoisomerization involves the carbene intermediate **2**, which cyclized to the furo[3,4b]chromone **3** by attack on the aroyl carbonyl oxygen.[5] The low quantum yields were attributed to competing attack of the carbene at the acrolein carbonyl to regenerate the original furyl chromone. Our attempts to optimize the photoisomerization centered on enhancing the nucleophilicity of the aroyl carbonyl and/or diminishing the nucleophilicity of the acrolein carbonyl, thereby promoting carbene cyclization to fluorescent product.

A series of 3-aroyl-2-(2-furyl)-chromones with substitution on the 3-aroyl and 2-furyl ring was prepared by the pathways outlined in Scheme I. The aldol condensation route is a modified and optimized pathway similar to the route reported by Huffman[5], while the DBU induced condensation is a simpler and more efficient route developed by us. This latter route provides the desired chromones in yields of 75-85% overall, starting with readily available 2'-hydroxyacetophenone.[6]

SCHEME I



Photoactivation of the parent compound (1a, Ar=Ph, R=H) at 350 nm for four minutes (3-5% reaction) provided a 60:40 ratio of the highly fluorescent E and Z olefin isomers 3a and 3b.[7] Extended irradiation (2.5 h) resulted in 50% conversion and a 97:3 ratio of 3a:3b.[8] The efficiency of fluorescence generation in the photoactivation of substituted furyl chromones was determined by measurement of the fluorescence intensity at 517 nm after irradiation of 5mM CH_2Cl_2 solutions using the 352-362 nm lines of a defocused argon ion laser for 1 minute.

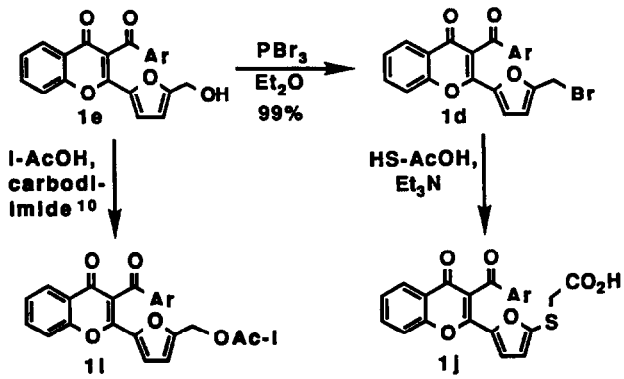
TABLE I

	Ar =	R =	Fluorescence Yield (517nm)[9]
1a	Phenyl	H	1.0
1b	4-(MeO)-Phenyl	H	2.9
1c	3,4,5-$(MeO)_3$-Phenyl	H	5.1
1d	3,4,5-$(MeO)_3$-Phenyl	CH_2Br	0.5
1e	3,4,5-$(MeO)_3$-Phenyl	CH_2OH	2.1
1f	3,4,5-$(MeO)_3$-Phenyl	CHO	0
1g	3,4,5-$(MeO)_3$-Phenyl	CO_2H (Me)	.02

Table I presents these data, expressed as fluorescence intensity relative to the fluorescence yield of **1a**, normalized to 1.0.[9] The greatest relative fluorescence intensity was generated when 3-(3,4,5-trimethoxybenzoyl)-2-(2-furyl)-chromone (**1c**) was photolyzed, while little or no fluorescence was observed upon irradiation of chromones with carbonyl substitution at the 5-position of the furan ring (**1f-1h**). The three electron donating methoxy groups did enhance the formation of the fluorescent furo[3,4b]chromone relative to the 4-methoxy or unsubstituted compound, but the furan ring carbonyl substituents, which were incorporated to reduce the nucleophilicity of the acrolein carbonyl towards the intermediate carbene, dramatically reduced the extent of photoisomerization. The 5-hydroxymethyl and 5-bromomethyl derivatives also photoactivated, but with a lower fluorescence yield relative to **1c**.

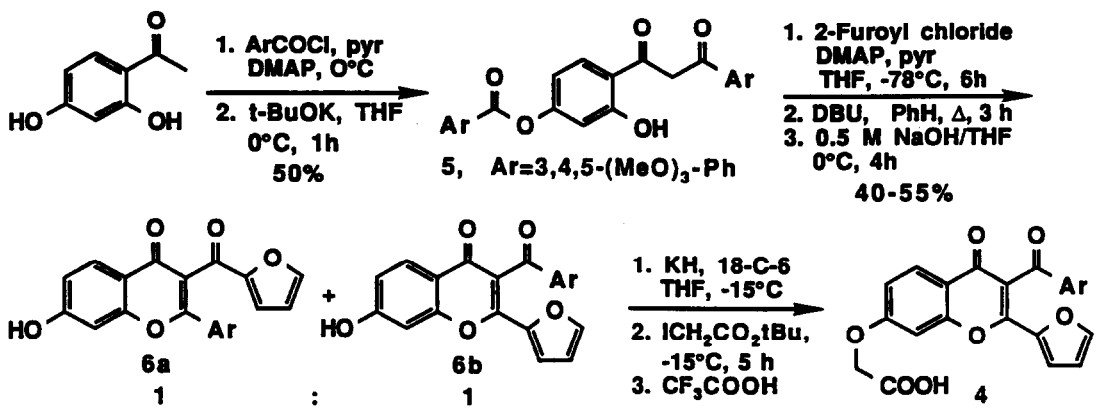
An alkyl group appended to the furan 5-position of the 3-(3,4,5-trimethoxyphenyl)-chromone was a suitable site for elaboration into water soluble target PAFs with a covalent linking group. **Scheme II** outlines the synthesis of functionalized chromones **1d**, **1i**, and **1j**. The hydroxymethyl derivative **1e** could be converted to iodoacetate ester **1i**, or to the bromide **1d**, providing access to the thioglycolic acid derivative **1j**. Compounds **1i** and **1j** were successfully attached to poly-L-lysine (24,000 MW) for diffusion studies.

SCHEME II



Scheme III outlines the synthesis of a furyl chromone (**4**) functionalized at the 7-position of the chromone ring with a glycolate ether group that provides an additional site for functionaliztion or covalent attachment. This synthesis capitalizes on the intramolecular acyl tranfer from the bis acylated 2,4-dihydroxyacetophenone to provide the monoprotected β-diketo resorcinol derivative **5**. Acylation of **5**, DBU-induced condensation, and saponification afforded a 1:1 isomer mixture, **6a** and **6b**, that were separable by HPLC. The 3-furoyl derivative **6a** had not been observed in any condensation reaction of the unsubstituted chromones (**Scheme I**) under similar conditions.

SCHEME III



Presence of the ester group at C-7 apparently facilitates trans-acylation prior to condensation/dehydration. Compound **6b** was converted to the glycolic acid ether **4** by O-alkylation with *tert*-butyliodoacetate, and subsequent removal of the tert-butyl group with CF_3COOH. Both compounds (**4** and **6b**) were water soluble at > 10 mM, and readily rearranged to generate fluorescent products. This series of C-7 functionalized molecules is another group of potentially useful chromone PAFs.

The 3-aroyl-2-(2-furyl)-chromones described in this paper represent a second class of PAF probe molecules that permits efficient photochemical generation of fluorescence, and incorporate specifically tailored functionality that facilitate covalent attachement to biological macromolecules. Successful studies of macromolecular transport and diffusion using these PAF molecules will be described in subsequent reports.

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REFERENCES AND NOTES

1. Preliminary aspects of this work were presented at the 192nd American Chemical Society Meeting, Anaheim, CA., September, 1986. Organic Abstract # 16.

2. Cummings, R.T. and Krafft, G. A. *Tetrahedron Lett.* **1987**, 28, preceding paper, this issue.

3. Krafft, G. A., Cummings, R., DiZio, J., Furukawa, R., Brvenik, L., Sutton, W. R., Ware, B. R. in Nucleo-cytoplasmic Transport, (Peters, R., Trendelenburg, M., eds.), pp. 35-52, Springer-Verlag, Berlin, 1986.

4. A number of photochemical reactions that generate fluorescent products have been reported. For an early review on the photochemical generation of fluorescence, see Zweig, A. *Pure and Applied Chem.* 1973, 33, 389-410.

5. Huffman, K. R., Kuhn, C. E., and Zweig, A. *J. Amer. Chem. Soc.* 1970, 599-605.

6. All new compounds exhibited satisfactory spectral data. Yields refer to isolated material purified to homogeneity by chromatography or recrystallization.

7. The ratios of olefin isomers were determined by HPLC and by ^{1}H-NMR.

8. Huffman *et. al.*[4] reported that they did not detect the presence of the *cis* isomer after short or extended intervals of irradiation. However, they did not fully characterize by NMR the product mixtures after photolysis, and obtained NMR spectra only on recrystallized *trans* material, from which small amounts of *cis* material likely would have been absent.

9. The quantity that was measured, "fluorescence yield", is a composite of the quantum yield for the photo-isomerization reaction and the fluorescence quantum yield for each particular furo[3,4b]chromone derivative generated. These quantities were not measured individually, since our goal involved optimization of the total fluorescent signal generated by irradiation of a given PAF for a fixed interval. Huffman *et. al.*[4] found little change in the absorption and emission characteristics of the furo[3,4b]chromones when the 3-phenyl group changed to a 3-(2-furyl) group, so that the variation in fluorescence yields obtained in our experiments can be attributed primarily to variation in the photoisomerization quantum yield for most of the derivatives. In the case of the bromomethyl derivative **1d** it also is possible (probable) that a reduction in the fluorescence quantum yield due to heavy atom vibronic quenching occurs.

10. The carbodiimide employed here was 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluene-sulfonate. The urea by-product of this reagent is water soluble and easily removed from the reaction mixture.

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