Syntheses of Reactive Fluorescent Stains Derived from 5(2)-Aryl-2(5)-(4-pyridyl)oxazoles and Bifunctionally Reactive Linkers

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Several bifunctionally reactive linkers containing halide or sulfonate ester groups were prepared. The linkers were used to quaternize 5-(4-methoxyphenyl)-2-(4-pyridyl)oxazole and 2-(6-chromanyl)-5-(4-pyridyl)oxazole to produce fluorescent stains that contained a reactive group such as an isothiocyanate, an *N*-hydroxysuccinimidyl ester, a maleimide, or an oxirane. The stains were derivatized with either 1-propylamine, 1-propanethiol, or piperidine, as appropriate, to help in characterization. The stains may serve as more photostable alternatives to fluoresceins or coumarins.

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Introduction.

Fluorescence microscopy is of value in both research and clinical assays. The method employs a microscope with an ultraviolet light source which excites the fluorophore in the sample and allows visualization and/or quantification of the fluorescent emission. The fluorophore is usually covalently attached to a biological molecule, such as an antibody, to impart specificity to the fluorophore. Attachment of the fluorophore to the antibody can be done through several types of reactive groups including isothiocyanate, *N*-hydroxysuccinimide ester, or maleimide. The labeled antibody can then be used for the detection and localization of the antigen-antibody reaction. This technique has been used to identify numerous disease causing microorganisms, such as toxoplasmosis, malaria, anthrax, bubonic plague, typhus, polio, and rabies. In clinical assays, the technique is used to screen for diseases such as syphilis, neurosyphilis, lupus, rheumatoid arthritis, myasthenia gravis, rickettsia, typhus, chlamydia, psittacosis, Epstein-Barr virus, Herpes Simplex viruses, and streptococcal infections [1]. Other uses of reactive stains include enzyme assays [2], staining proteins separated by gel electrophoresis, fluorescence activated cell sorting [3], and DNA sequence analysis [4].

The most common fluorophores used to label antibodies are fluorescein and rhodamines, which suffer from several shortcomings. Both have a small Stokes' shift between absorption and emission causing them to suffer from self absorption [5], limiting their linearity of response and light output, and preventing effective filtering of excitation light to only the wavelengths matching their absorption maxima. The fluorescence quantum yield (Φ) of both is *p*H-sensitive. In the case of fluorescein, the Φ is highest only above *p*H 8 [6]. Under physiological conditions, the Φ of fluorescein isothiocyanate is reduced to ~0.1 upon conjugation [7]. Additionally, the Φ can fall below 1% in some regions of biological cells [6]. The Φ of rhodamines is also *p*H-dependent: Φ is lowered by alkaline *p*H. The greatest disadvantage of fluorescein is its rapid fading [8]. Fluorescein is photochemically reactive, decomposes readily, and its fluorescence changes from green to blue. This often forces rapid error-prone judgements based on the appearance of slides. It also makes storage of slides for re-examination difficult. Rhodamines also suffer from the same problem to a lesser extent. Despite these shortcomings, stains based on these two fluorophores continue to be used primarily because of their large extinction coefficients and the lack of a suitable alternative.

Discoveries in the field of laser dye research have led to the synthesis of compounds with unprecedented service lifetimes as laser dyes, orders of magnitude greater than those of fluorescein, coumarins, and rhodamines (Table 1). Laser dyes are compounds which, when dissolved in a suitable solvent, and excited with a suitable light source in an appropriate optical setting, emit laser light. Fluorescence of a dye is a necessary but not sufficient condition for lasing to occur. One of the most powerful excitation sources is the xenon flashlamp, but degradation or 'fading' of the dye is always a serious concern with this source. The most generally accepted method for expressing resistance to fading, called 'dye lifetime', a measure of

Table 1

Photochemical Stability of Selected Fluorescent Dyes

Dye Name(s)	Photochemical Stability [a]
Fluorescein, Disodium Salt [b]	900 [f]
Rhodamine 6G; Rhodamine 590 [c]	50,000 [f]
Coumarin C6H [d]	2,400,000 [g]
4PyMPO-MePTs [e]	>10,000,000,000 [h]

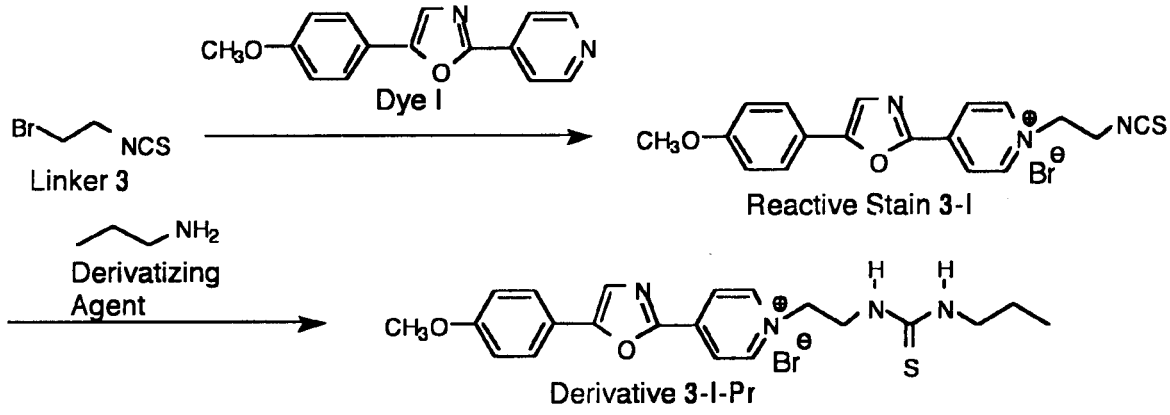
[a] Determined on a comparative basis as service lifetime when dye is used as a flashlamp pumped laser dye in either a Phase-R DL-10Y or DL-1200 laser. [b] Dye contains same fluorophore as fluorescein isothiocyanate. [c] Dye contains a fluorophore closely related to that of rhodamine B isothiocyanate. [d] This is an unusually stable 'butterfly-amino' coumarin [9]. [e] The quaternary salt of Dye I with methyl p-toluenesulfonate. [f] See [10]. [g] See [9]. [h] See [11].

photochemical stability, is to measure the total energy in joules delivered to the flashlamp, usually in pulses, until the circulating solution of laser dye has decomposed to the point where the energy output is half the initial value. The total energy in joules divided by the volume of the dye solution in liters is the 'lifetime' or photochemical stability of the dye [12].

Dye I (Table 2), also exhibits an unusually large Stokes' shift (>150 nm), is not sensitive to *p*H values normally found in physiological conditions, has a quantum yield of 0.77 in an aqueous environment [13], and has an excitation maximum that is a good match for the common excitation lamp of a fluorescence microscope, which has peak output near 365 nm [15]. The combination of photostabil-

Table 2

General Scheme for the Synthesis of Reactive Stains and Their Derivatives



Linker	Dye	Reactive Stain	Derivatizing Agent	Derivative(s)
1 (Figure 1)	**I** (See above)	**1-I**	n-$C_3H_7NH_2$	**1-I-Pr**
2 (Figure 1)	**I**	**2-I**	"	**2-I-Pr**
3 (Figure 2)	**I**	**3-I** (See above)	"	**3-I-Pr** (See Above)
5 (Figure 4)	**I**	**5-I** (Figure 12)	"	**5-I-Pr** (Figure 12)
6 (Figure 5)	**I**	**6-I**	"	**6-I-Pr**
7 (Figure 6)	**I**	**7-I**	"	**7-I-Pr**
8 (Figure 7)	**I**	**8-I** (Figure 14)	n-C_3H_7SH	**8-I-Pr** (Figure 14)
9 (Figure 7)	**I**	**9-I**	"	**9-I-Pr**
10 (Figure 8)	**I**	**10-I** (Figure 15)	"	**10-I-Pr** (Figure 15)
			$(CH_2)_5NH$	**10-I-Pip**
1	**II** (Figure 9)	**1-II**	n-$C_3H_7NH_2$	**1-II-Pr**
3	**II**	**3-II**	"	**3-II-Pr**
7	**II**	**7-II** (Figure 13)	"	**7-II-Pr** (Figure 13)
9	**II**	**9-II**	n-C_3H_7SH	**9-II-Pr**
10	**II**	**10-II** (Figure 15)	"	**10-II-Pr** (Figure 15)
			$(CH_2)_5NH$	**10-II-Pip** (Figure 17)

Fletcher *et al.* reported on a group of water-soluble cationic pyridinium salts, some of which have the greatest lifetimes as laser dyes ever observed [11]. Their 4PyMPO-MePTs (**2A** in [13]), for example, has >10,000,000 times the lifetime of fluorescein, and its emission peak is in the desirable 570 nm range. A good fluorescent stain should have an emission maximum above 480 nm to avoid the autofluorescence present in many biological samples which usually appears 'blue' (430-470 nm) [14]. This dye, the basis for our choice of ity, water solubility, large Stokes' shift, and *p*H tolerance solve most of the shortcomings associated with fluorescein. In addition, this class of compounds can be converted easily into reactive stains by taking advantage of the quaternizable pyridine group.

A major shortcoming of these pyridinium salts is their small extinction coefficients (ε), slightly >20,000 in ethanol. Hall *et al.*, however, have described the synthesis of a related compound, 2-(4-methoxyphenyl)-5-(4-pyridyl)oxazole (**14a** in [13]), which differs only in the

position of the nitrogen in the oxazole ring, but has ε >30,000. The emission wavelength of **14a**, 495 nm, was judged too short for our purposes, so we prepared a new dye (**Dye II**, Figure 9) in which the methoxy group, an auxofluor, was replaced by a cyclic ether group, bathochromically shifting the emission wavelength to 520 nm. Kauffman *et al.* reported a bathochromic shift of fluorescence emission, accompanied by a decrease in ε, by a similar change of auxofluor with terphenyls [16], in which no electron-attracting auxofluor was present. However, we had observed a 20 nm bathochromic shift of fluorescence of the methanesulfonate salts of related 5-aryl-2-(4-pyridyl)oxazoles (**12a** *vs.* **2a** in [13]), with little change in ε, when a cyclic ether replaced the methoxy group as the electron-releasing auxofluor and an electron-attracting auxofluor, the pyridinium moiety, was present. As we anticipated, the high ε and bathochromic shift of emission both appeared in the quaternary salts of **Dye II**.

Various bifunctionally reactive linkers were synthesized to quaternize **Dyes I** and **II** to obtain the reactive stains. All the linkers contain a halide or sulfonate ester for reaction with the pyridine moiety of the dyes and either a *N*-hydroxysuccinimide ester, isothiocyanate, maleimide, or oxirane group to react with biologically significant groups such as amines and thiols. To the best of our knowledge, an oxirane has never been used as a reactive group for the covalent labeling of proteins. A variety of linkers was synthesized to determine what affect the structure of the linkers had on the solubility, reactivity, and photophysical properties of the stains.

The details of the syntheses of the various bifunctional linkers, and of the reactive stains based on quaternization of 5-(4-methoxyphenyl)-2-(4-pyridyl)oxazole (**Dye I**) and of the new 2-(6-chromanyl)-5-(4-pyridyl)oxazole (**Dye II**) are now presented, along with their 1-propylamine, 1-propanethiol, and piperidine derivatives, which were prepared to aid in the characterization of the stains. We prepared the 1-propylamine derivative of each isothiocyanate and *N*-hydroxysuccinimide ester, the 1-propanethiol derivative of each maleimide, and the 1-propanethiol and piperidine derivative of each oxirane. The photophysical properties of the derivatives and the utility of these fluorescent stains will be reported separately. The reactive stains should have broad biochemical application, and their large Stokes' shift should make them suitable for fluorescence polarization immunoassays.

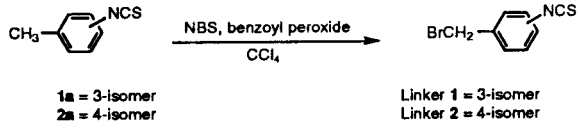
Discussion and Results.

Syntheses of Linkers.

The syntheses of the isothiocyanate linkers were accomplished either by free radical bromination of the methyl group of a commercially available phenyl isothiocyanate (Linkers **1** and **2**) or preparation of the amine for conversion to the corresponding isothiocyanate (Linkers **3-6**).

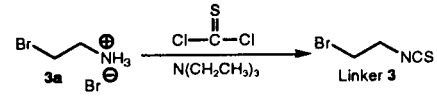
Both 3- and 4-(bromomethyl)phenyl isothiocyanates (Linkers **1** and **2**, Figure 1) were prepared by the procedure described by Gonda *et al.* for the free radical bromination of the 2-isomer by *N*-bromosuccinimide [17]. However, we found the reaction time of 1.5 hours described by Gonda *et al.* insufficient to complete the reaction with our isomers, which needed approximately 5 hours of reflux in carbon tetrachloride (when all solid floated [18]) to obtain yields similar to those cited by Gonda *et al.* (48-56%).

Figure 1. Synthesis of 3 and 4-Bromomethylphenyl Isothiocyanates (Linkers 1 and 2)



The other isothiocyanate linkers were prepared by conversion of the amine to the isothiocyanate as described by Garmaise *et al.* [19] for the conversion of aliphatic amines (or their hydrohalides) to isothiocyanates using thiophosgene and triethylamine in chloroform. A change we made to the procedure was to increase the molar equivalents of triethylamine used from slightly greater than two to three, since three moles of mineral acid are liberated during the conversion of 1 mole of hydrohalide. The 2-bromoethyl isothiocyanate (Linker **3**, Figure 2) was synthesized in one step from commercially available 2-bromoethylamine hydrobromide **3a** in 64% yield. One other isothiocyanate

Figure 2. Synthesis of 2-Bromoethyl Isothiocyanate (Linker 3)



linker, 4-(2-chloroethoxy)phenyl isothiocyanate (Linker **4**, Figure 3) and an intermediate isothiocyanate **5e** (Figure 4) were also synthesized using this procedure. The yields

Figure 3. Synthesis of 4-(2-Chloroethoxy)phenyl Isothiocyanate (Linker 4)

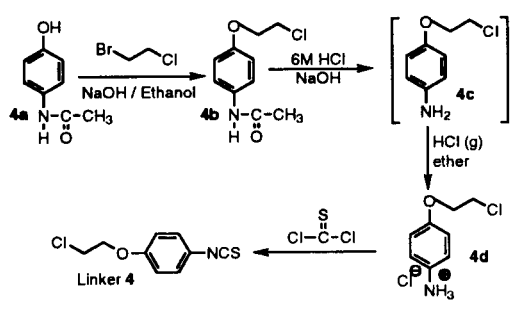
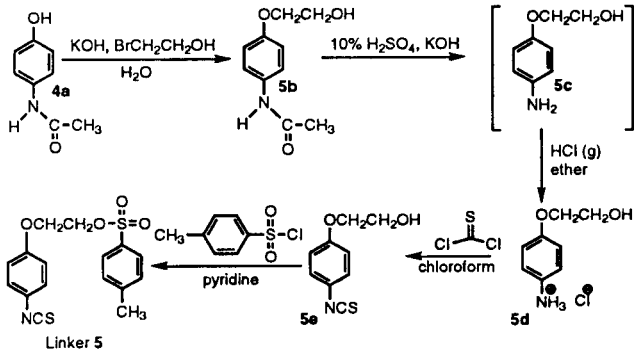


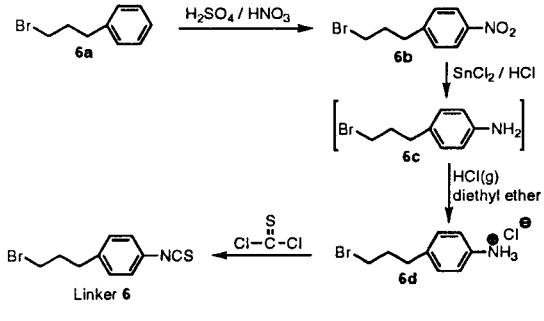
Figure 4. Synthesis of 4-(2-Hydroxyethoxy)phenyl Isothiocyanate 4-Toluenesulfonate Ester (Linker 5)



were similar to that of 2-bromoethyl isothiocyanate (Linker **3**) and the aliphatic isothiocyanates described by Garmaise *et al.*, showing the utility of this method for preparing both aliphatic and aromatic isothiocyanates.

The amines, or their hydrochlorides, were synthesized from either of two starting materials. The 4-(3-bromopropyl)benzeneamine hydrochloride **6d** (Figure 5) was synthesized from 1-bromo-3-phenylpropane **6a** by nitration followed by reduction. The nitration was carried out using concentrated sulfuric and nitric acids [20]. The purification of the nitro compound **6b** took advantage of the high boiling and melting point of the 4-isomer compared with those of the other isomers formed. The mixture of isomers was separated by vacuum distillation and pure compound was obtained by recrystallization at low temperature from methanol. The nitro compound **6b** was reduced to the amine **6c** with tin(II) chloride [21]. Other methods, such as catalytic hydrogenation, were not tried to avoid dehalogenating the compound. Attempts at isolating the amine **6c** as the free base resulted in polymerization, therefore, the hydrochloride **6d** was isolated.

Figure 5. Synthesis of 4-(3-Bromopropyl)phenyl Isothiocyanate (Linker 6)



The 4-(3-bromopropyl)phenyl isothiocyanate (Linker **6**, Figure 5) was prepared from the corresponding amine hydrochloride **6d** using a modification of an older procedure, described by Dyer *et al.* [22], which converted the amine to the isothiocyanate with thiophosgene in refluxing cyclohexane using 3 equivalents of the starting amine to form one equivalent of the isothiocyanate. The other two equivalents served as the proton acceptor for the liberated mineral acid. This seemed a waste of valuable starting material, so triethylamine was instead used as the proton acceptor. The yield, 60%, was similar to those attained with the Garmaise *et al.* procedure. The carmaise procedure was milder and easier, since it did not require reflux, and thus it was used in subsequent isothiocyanate preparations.

The 4-(2-chloroethoxy)- **4d** (Figure 3) and the 4-(2-hydroxyethoxy)benzeneamine **5d** (Figure 4) were both synthesized from *N*-(4-hydroxyphenyl)acetamide **4a** using Williamson's ether synthesis with either 1-bromo-2-chloroethane or 2-bromoethanol, respectively. The yields of the reactions were not high, but purification was not difficult since any unreacted starting materials were either volatile or could be removed with a basic wash. The melting points of the two ethers **4b** and **5b** agreed with reported values [23a,b]. The amides **4b, 5b** were hydrolyzed either with 6*M* hydrochloric acid or with 10% sulfuric acid to give the corresponding amines **4c** and **5c**. In both cases isolation of the amines as the hydrochlorides **4d** and **5d** proved easier than isolation of the free amine. The chloro isomer **4c** polymerized when concentrated, although Ashford *et al.* [24] was able to isolate it. The melting point of the hydrochloride **4d** agreed with the literature value [24]. A portion of the hydroxy isomer was isolated as the free amine **5c**. However, recrystallization on a larger scale usually resulted in oils and required a large amount of solvent. Isolation as the hydrochloride **5d** proved easier, and melting points of both the amine **5c** and its hydrochloride **5d** agreed with reported values [25]. The last step in the synthesis of Linker **5** was conversion of the hydroxy group of 4-(2-hydroxyethoxy)phenyl isothiocyanate **5e** to the tosylate ester, which was carried out in high yield, 93%, using 4-toluenesulfonyl chloride in pyridine [26].

The *N*-hydroxysuccinimide ester linker **7** (Figure 6) was synthesized from 3-methylbenzoic acid **7a** by means of a free radical bromination similar to the one described for the previous isothiocyanates [17], followed by a dicyclohexylcarbodiimide catalyzed reaction with *N*-hydroxysuccinimide [27]. The melting point of the 3-(bromomethyl)benzoic acid **7b** agreed with the literature value of 150° [28]. Glover *et al.* synthesized **7b** by hydrolysis of the corresponding nitrile but gave no details [27]. Our attempt at acid hydrolysis of the nitrile using a general procedure [29], however, resulted only in the isolation of tar.

The maleimide linkers **8** and **9** (Figure 7) were synthesized from *N*-(2-hydroxyethyl)maleimide **8a** by conver-

Figure 6. Synthesis of 3-Bromomethylbenzoic Acid N-Hydroxysuccinimide Ester (Linker 7)

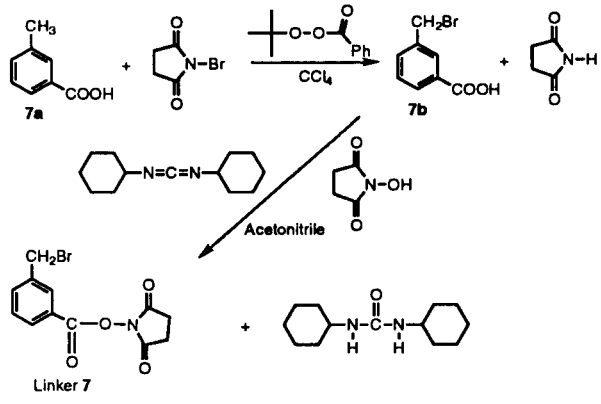
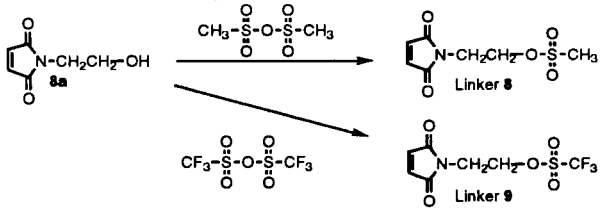


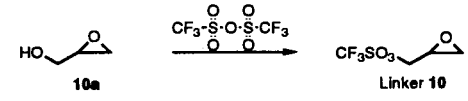
Figure 7. Synthesis of N-(2-Hydroxyethyl)maleimide Methyl and Trifluoromethylsulfonates (Linkers 8 and 9)



sion of the hydroxy group to either a methyl- or trifluoromethylsulfonate. The usual method of sulfonate ester formation, from a sulfonyl chloride and pyridine, resulted in the formation of tars, presumably caused by the base-catalyzed polymerization of the maleimide. The conversion was done by heating neat methanesulfonic anhydride with the maleimide. The methanesulfonic acid liberated during the reaction did not cause any decomposition. The trifluoromethane analog (Linker **9**) was also synthesized from the corresponding anhydride. This conversion did not require any heat and was performed at room temperature with ether as the solvent. The starting material, *N*-(2-hydroxyethyl)maleimide **8a** was synthesized in two steps using the procedures described by Yamada *et al.* and Michadera *et al.* [30]. In neither step was the reported yield obtained and work-up in both proved difficult. Some alternate methods such as those described by Trammer *et al.*, Weber *et al.*, and Keller *et al.* may be good alternatives [31].

The oxirane linker **10** (Figure 8) was synthesized in one step from glycidol **10a** and trifluoromethanesulfonic anhydride using the method described by Vedejs *et al.* for the synthesis of prop-2-ynyl trifluoromethanesulfonate [32]. The liquid product was purified by vacuum distillation, and its boiling point agreed with the reported value [33].

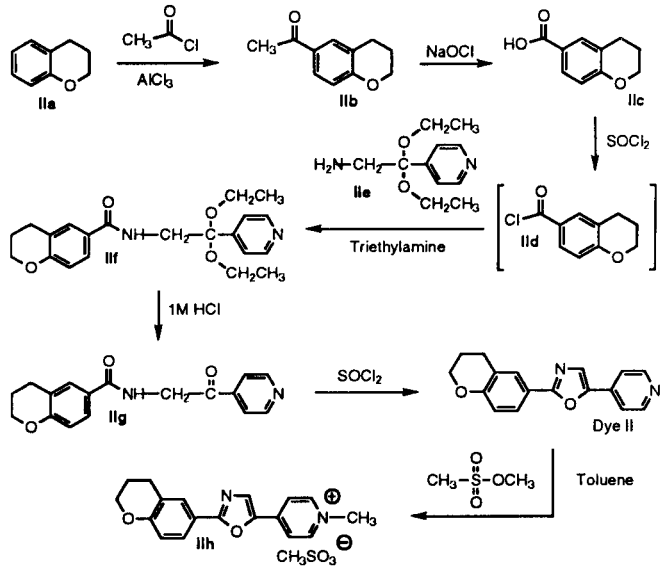
Figure 8. Synthesis of Glycidol Trifluoromethylsulfonate (Linker 10)



Syntheses of 5(2)-Aryl-2(5)-(4-pyridyl)oxazoles, The Dyes.

The simplest of the three dyes, 5-(4-methoxyphenyl)-2-(4-pyridyl)oxazole (**Dye I**, Table 2), was synthesized using the methods described by Hall *et al.* [13] as was 2-(6-chromanyl)-5-(4-pyridyl)oxazole (**Dye II**, Figure 9). Chroman **IIa** was made from 3,4-dihydrocoumarin by reduction followed by acid catalyzed cyclization as described by Hall *et al.* The yield of chroman **IIa**, 17%, was much lower than that described by Hall *et al.*, 82-84%, due to the formation of an unexpected by-product. Vacuum distillation of the crude chroman resulted in two major fractions. The lower boiling fraction was the desired chroman **IIa** while the higher boiling fraction was identified as 6-*t*-butylchroman by its boiling point [34] and pmr spectrum. The *t*-butyl group must have been introduced during one of the two acid quenches in the presence of *t*-butylmethyl ether by an electrophilic substitution mechanism. Borowitz *et al.* reported an acid catalyzed intramolecular rearrangement involving chroman and a *t*-butyl group [34]. Any syntheses of chroman using this procedure should avoid using *t*-butylmethyl ether during work-up.

Figure 9. Synthesis of 2-(6-Chromanyl)-5-(4-pyridyl)oxazole (Dye II)

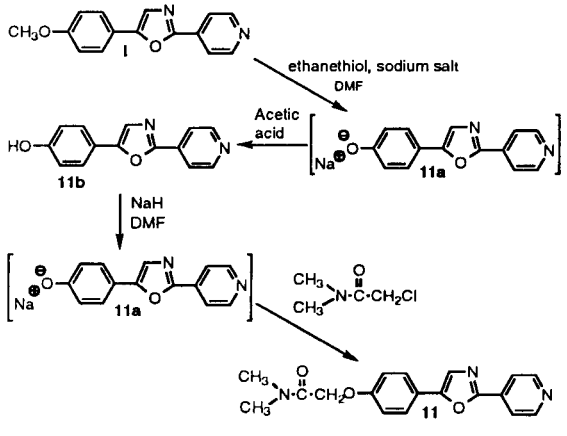


The remaining steps in the synthesis of **Dye II** all gave high yields. The chroman **IIa** was acetylated using a pro-

cedure described by Hall *et al.* for the synthesis of 6-(2-bromoacetyl)chroman. A haloform reaction [35] was used to convert the acetyl **IIb** to the corresponding acid **IIc**. The melting points of both compounds **IIb** and **IIc** agreed with reported values [36a,b]. The acid **IIc** was converted to the acid chloride **IId** using thionyl chloride in high yield with vacuum distillation providing a convenient purification method. The acid chloride **IId** was quickly reacted with 2,2-diethoxy-2-(4-pyridyl)ethylamine **IIe** [37] to form the ketal amide **IIf** in toluene with triethylamine as the proton acceptor. The ketal amide **IIf** was surprisingly soluble in toluene, making it possible to remove the residual ammonium salts by filtration. The ketal was hydrolyzed in boiling 1*M* hydrochloric acid, and the ketoamide **IIg** was then cyclized with refluxing thionyl chloride. Purification of the oxazole free base **II** by chromatography through an Ace-Kauffman column was an effective purification procedure.

A potentially more water soluble version of **Dye I** was also synthesized (**11** in Figure 10). The synthesis took advantage of the method of Feutrill *et al.* by cleaving the aromatic methoxy group [38] of **Dye I** using the sodium salt of ethanethiol in refluxing dimethylformamide. The progress of the cleavage can be monitored by the disappearance of the blue fluorescence of **Dye I** and the appearance of the red fluorescence of the sodium salt of the phenolate **11a**. Isolation of the phenol **11b** proved difficult so the crude pruduct was used in the subsequent Williamson reaction with *N,N*-dimethyl-2-chloroacetamide. The progress of this reaction can also be monitored by the color of fluorescence. This time the disappearance of the red fluorescence of the sodium salt and a return of the blue fluorecence of the ether indicates completion of reaction. Although not tried in this sequence, the *N,N*-dimethylacetamido ether **11** probably can be made in one pot from **Dye I** in dimethylformamide. We have not quaternized this base as yet.

Figure 10. Synthesis of 2-(4-Pyridyl)-5-[4-(N,N-dimethylacetamidooxyphenyl)] oxazole **11**

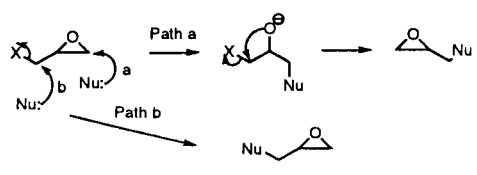


Synthesis of Reactive Stains (Table 2).

The nature of the leaving group and of the amine and/or thiol reactive group of the linker determined the conditions under which it was used to quaternize the free base. The reactions took advantage of the solubility of the starting materials in fairly nonpolar solvents and the insolubility of the polar ionic products in the same solvent to simplify the work-up. In general, the quaternizing reaction consisted of mixing the linker with the free base in a fairly nonpolar solvent followed by filtration of the pyridinium salt product. In some cases this resulted in products which did not require further purification. In those cases where purification was required, non-nucleophilic solvents such as acetonitrile, dimethylformamide, or ethyl acetate were used to avoid reaction with the biologically reactive group.

The isothiocyanate group was found be stable under elevated temperatures and all stains containing an isothiocyanate as the reactive group were prepared in refluxing toluene (**1-**, **2-**, **3-**, **5-**, and **6-I** as well as **1-** and **3-II**). The maleimides also were stable under these conditions. Quaternizations of the free bases (**Dyes I** and **II**) with the methanesulfonate ester (Linker **8**) were carried out in refluxing toluene. The trifluoromethanesulfonate ester (Linker **9**) was so reactive that diethyl ether at room temperature sufficed. The oxirane stains **10-I** and **10-II** were also synthesized at room temperature in diethyl ether. It was particularly important to us that the oxirane linker **10** was capable of quaternizing the free base under mild conditons. There are two possible sites for nucleophilic attack on the oxiranyl linker, the C1 and C3 carbons (Figure 11) [39]. It was felt that without a good leaving group more stringent conditions would be necessary to complete the reactions. Under these conditions attack at the C1 carbon may effectively compete with attack at the desired C3 position. Attack at the C1 carbon can still lead to the desired product by subsequent intramolecular attack of the leaving group by the anion intermediate [33], but this second mechanism may also lead to the formation of byproducts. The *N*-hydroxysuccinimde ester stains **7-I** and **7-II** were both synthesized at room temperature in ethyl acetate [27]. The reaction required several days to be complete but it was feared that the ester would decompose under more stringent conditions.

Figure 11. Possible Nucleophilic Substitution Mechanisms for Substituted Oxiranes



The nature of the free base and the leaving group were the major factors influencing the yields of the reactions. The yields of reactions in which benzyl bromides or trifluoromethylsulfonates, both good leaving groups, were used were generally high. The benzyl bromides generally gave yields near 90% when using equimolar amounts of starting materials in toluene, while triflates gave nearly quantitative yields. In the case of the *N*-hydroxysuccinimide ester Linker **7**, also a benzyl bromide, the yields were slightly lower due to the mild conditions. But we found that the yields could be increased to acceptable levels, >60%, by longer reaction times and using a 2-fold excess of the linker. Linkers containing less reactive leaving groups such as bromide (not benzyl bromide), chloride, tosylate, and methanesulfonate, often resulted in a poor yield of the pyridinium salt. The chloride (Linker **4**) did not yield any product even when such high boiling solvents as benzonitrile or dimethyl sulfoxide were used as solvents for the reaction, and thus was not pursued further. The tosylate analog (Linker **5**) was synthesized to overcome this problem but it too gave modest yields. Similar to the *N*-hydroxysuccinimide ester Linker **7**, the yields of these reactions could be increased by longer reaction times and using a 2-fold excess of the linker.

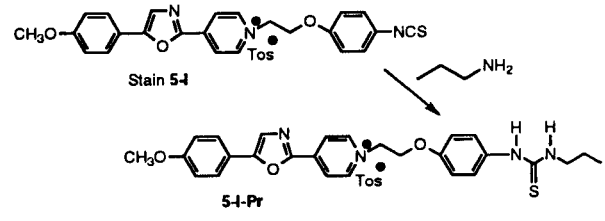
The other factor influencing the yields of the quaternizing reaction was the nucleophilicity of the free base. It has become apparent that the two free bases vary considerably in reactivity. The yields of reactions with **Dye I** were lower than their **Dye II** counterparts; 60% versus 75%, respectively, under nearly identical conditions using the *N*-hydroxysuccinimide ester Linker **7** and 78% for both using 2-bromoethyl isothiocyanate (Linker **3**), but the reaction time and molar excess of linker were greater in the reaction with **Dye I**. The linkers containing triflate as the leaving group were sufficiently reactive to overwhelm any differences in reactivity between the free bases. The reason for the difference in reactivity is believed to be the result of the position of the oxazole nitrogen with respect to the pyridine ring. The nitrogen side of the ring acts as a strong electron withdrawing group capable of decreasing the electron density of the pyridine ring, making the pyridine nitrogen less nucleophilic. Therefore, the pyridine nitrogen of **Dye I** is less nucleophilic than that of **Dye II**. The pmr spectra of the compounds corroborate this hypothesis. The 3,5 hydrogens of the pyridine ring in **Dye I** and its salts are always shifted down field from their **Dye II** counterparts. For example, the 3,5-pyridyl hydrogens of **Dye I** have a chemical shift of 8.76 ppm [13] compared with 8.36 for those in the **Dye II**. Generally, the more electron withdrawing the substituents on the aromatic ring, the further downfield the chemical shift [40]. Hall *et al.* also observed a large difference between the melting points of **Dye I** and 2-(4-methoxyphenyl)-5-(4-pyridyl)oxazole (**14a** in [13]), the latter melting some 60° higher. This difference was attributed to the weight of a resonance form which made the methoxyphenyl and oxazole rings coplanar in the 2-(4-methoxyphenyl)-5-(4-pyridyl)oxazole [13]. This seems likely only if the nitrogen side of the oxazole ring acts as a strong electron withdrawing group.

Synthesis of 1-Propylamine, 1-Propanethiol, and Piperidine Derivatives of the Reactive Stains (Table 2).

Initially, attempts were made at synthesizing either the γ-lysine or the cysteine derivatives of the stains, but these derivatives had solubility similar to that of the amino acids, *i.e.* good aqueous solubility and poor solubility in most organic solvents, making it difficult to remove unreacted starting material. They also formed gums making purification even more difficult. Additionally, there have been reports that the cysteine derivatives of maleimides hydrolyze to the succinamic acid under mildly basic conditions [41], which can further complicate purification. To avoid some of the problems associated with the amino acid derivatives, the stains were reacted with 1-propylamine, 1-propanethiol, or piperidine. These reagents had the advantage of being volatile and soluble in relatively nonpolar solvents, which facilitated their removal and simplified purification of the derivatives.

The 1-propylamine derivatives of the isothiocyanate stains (thioureas), (**1-**, **2-**, **3-**, **5-**, and **6-I-Pr** as well as **1-**, **3-II-Pr**, Figure 12) were generally synthesized in crude yields >91% using a large excess of 1-propylamine in either acetone or acetonitrile at room temperature. Neither the stains nor the derivatives were very soluble in the reaction solvents chosen. Nevertheless, these solvents proved very useful since the purity of the crude products was very high. Usually the stain would go into solution slowly after the addition of the 1-propylamine to the mixture. Shortly after complete solution of the stain the thiourea would precipitate, which could be collected by filtration.

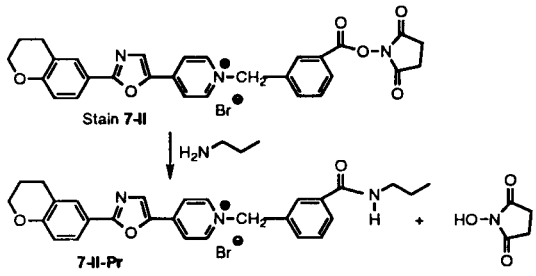
Figure 12. General Scheme for the Synthesis of 1-Propylamine Derivatives of Isothiocyanate Stains (Thioureas)



The derivatives of the *N*-hydroxysuccinimide ester stains **7-I-Pr** and **7-II-Pr** (Figure 13) were synthesized in a manner analogous to the thioureas. The yields were

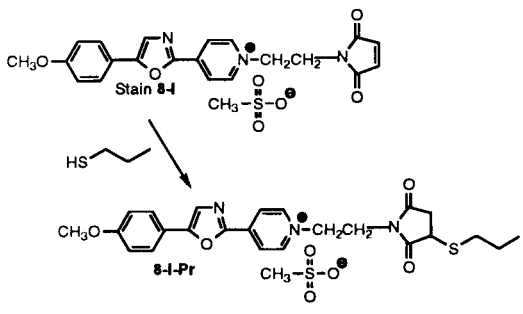
generally lower, 96 and 73% for Stains **7-I** and **7-II**, respectively, and the crude derivatives were not as pure, the major impurity being *N*-hydroxysuccinimide, a by-product of the reaction.

Figure 13. General Scheme for the Synthesis of 1-Propylamine Derivatives of N-Hydroxysuccinimide Ester Stains



The thioether derivatives of the maleimides **8-**, **9-I-Pr**, and **9-II-Pr** (Figure 14) were synthesized in a mixture of acetonitrile:water::1:1 using an excess of 1-propanethiol. Attempts at synthesizing the derivatives in acetonitrile alone failed to give the desired product. The nucleophilic substitution of the maleimide required a basic catalyst. The water in the reaction mixture, p*K*a = 15.74 [42], is considerably less acidic than 1-propanethiol, p*K*a = 10.65 [43], and served as a good catalyst. The yields of the reaction ranged from 68-96%, with the lower yields representing a purified yield or yields resulting after reprocessing of starting material due to incomplete reaction. Recrystallization from 2-propanol was an effective means of purification, despite the fact that the derivatives often formed gums and resulted in products with relatively broad melting ranges, probably due to formation of *R* and *S* isomers.

Figure 14. General Scheme for the Synthesis of 1-Propanethiol Derivatives of Maleimide Stains

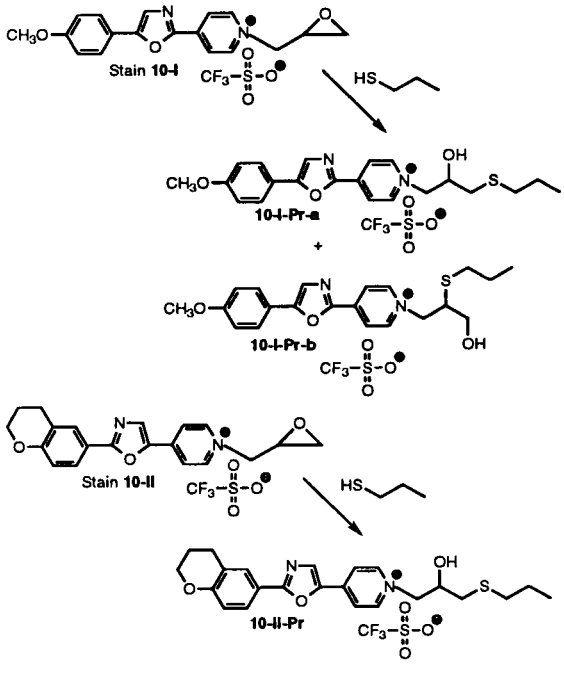


An hplc method using a reverse phase column was developed for separating the derivatives, but it proved useful only as an analytical technique. The derivatives bound the C18 column (Econosphere, Alltech) very tightly and required a mixture of methanol, dimethylformamide, and trifluoroacetic acid (97.5:2.5:0.1) to elute the compound. Omission of either dimethylformamide or trifluoroacetic acid resulted in a solvent system that would not elute the compounds. Stronger solvents such as tetrahydrofuran were also ineffective. It is believed the compounds interacted very strongly with the silanol matrix of the C18 column. Silanols can interact strongly with some compounds by hydrogen bonding and ion exchange [44]. Chromatographic separation of similar pyridinium salts on a C8 reverse phase column (Econosphere, Alltech) (silanols more accessible) resulted in longer retention times, while separation with a more completely capped C8 column (Alltima, Alltech) resulted in shorter retention times [45] corroborating this hypothesis. The addition of the trifluoroacetic acid disrupted ion exchange, while dimethylformamide disrupted hydrogen bonding between the derivatives and the silanol matrix. The method was not used preparatively because small amounts of compound overwhelmed the semi-preparative column and because of the possibility of anion scrambling due to the presence of trifluoroacetic acid in the solvent system.

The 1-propanethiol derivatives of the oxirane stains (**10-I-Pr-a, -b** and **10-II-Pr**, Figure 15) were synthesized in fair yield in acetonitrile with triethylamine as a basic catalyst. Stain **10-II** reacted in the expected least hindered position of the oxirane to give a single isomer (*R* and *S* isomers notwithstanding), while Stain **10-I** gave a mixture of isomers, the predominant isomer resulting from nucleophilic attack at the more hindered position of the oxirane. The ratio of isomers (determined from pmr spectrum) was found to be ~4:1::**10-I-Pr-b**:-**a** after purification (Figure 16). This result was unexpected since the stains are so similar in structure and the reaction was done under identical conditions. It is suspected that the orientation of the oxazole nitrogen with respect to the pyridine ring is again responsible for the difference in reactivity between the two closely related stains. As stated before, the pyridine nitrogen of **Dye I** has a lower electron density than **Dye II**. The difference in electron density between the two compounds must be sufficient to alter the reactivity of the oxirane, even though the inductive effect has a transmission factor of only 0.41 through a methylene group [46].

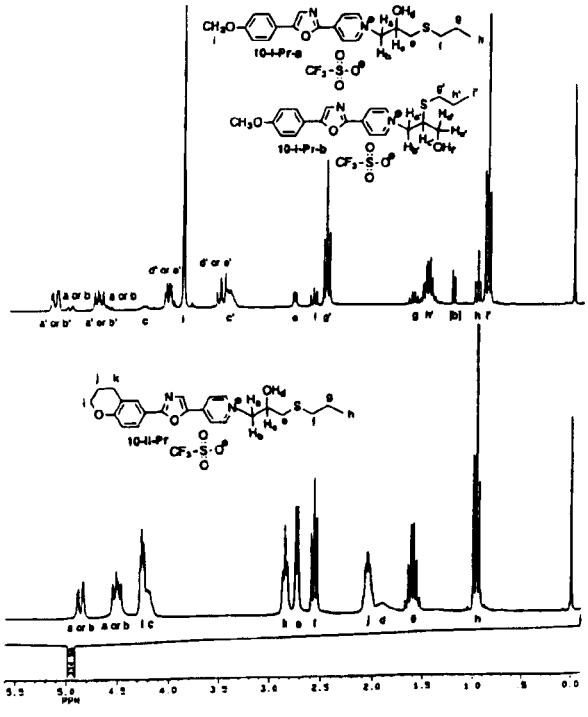
Nucleophilic substution of an oxirane under basic conditions nearly always results in products formed by attack at the least hindered position [47]. No other examples of reaction at the more hindered position were found unless conjugative effects were involved. Even trifluoromethyl substituted oxiranes react in the least hindered position [48]. Nucleophilic attack at the more substituted carbon can occur through an SN1 or 'borderline' SN2 mechanism (bond breaking is more nearly complete in the transition state than bond formation) under acidic conditions [47]. However, oxiranes with strong electron withdrawing sub-

Figure 15. Synthesis of 1-Propanethiol Derivatives of Oxirane Stains (10-I-Pr-a, 10-I-Pr-b, and 10-II-Pr)



stituents such as a trifluoromethyl group still react in the least hindered position under acidic conditions. The already electron-poor, most-substituted carbon cannot support the formation of a carbocation at its position [48].

Figure 16. Pmr spectrum of aliphatic region of 10-I-Pr-a, 10-I-Pr-b, and 10-II-Pr [a]

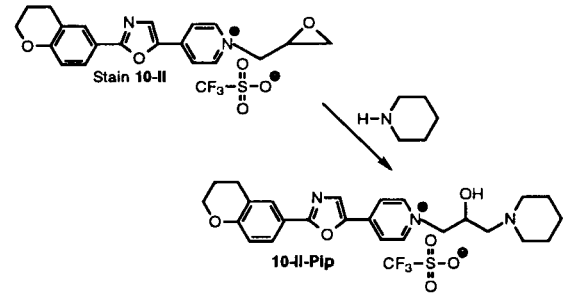


[a] For complete interpretation see experimental. [b] Trace of recrystallization solvent 2-propanol.

This makes the reaction of Stain **10-I** truly unusual.

Attempts at synthesizing the 1-propylamine derivatives of the oxirane stains resulted in the isolation of gums which were difficult to purify, probably due to the formation of a mixture of isomers and possibly dimerization. Dimers can potentially form when the secondary amine derivative reacts with another mole of stain to form the tertiary amine derivative. Instead, piperidine derivatives were synthesized, which had the advantage of forming more crystalline and higher melting derivatives, (**10-I-Pip** and **10-II-Pip**, Figure 17) as well as eliminating the possiblity of dimerization. Piperidine is also more sterically hindered than 1-propylamine and reduced the possibility of reaction at the more hindered position of the oxirane; this was especially a concern with the stain **10-I**. The piperidine derivative of Stain **10-II** was made in high yield, 83%, in acetonitrile with excess piperidine. Recrystallization from 2-propanol was an effective means of purification. The piperidine of derivative of Stain **10-I** proved more difficult to synthesize. An equimolar amount of piperidine was

Figure 17. General Scheme for the Synthesis of Piperidine Derivatives of Oxirane Stains



used in methylene chloride, since the use of excess piperidine caused much decomposition. Recrystallization of the solid was ineffective and usually resulted in the formation of a gum. A blue fluorescent impurity was noticed in the sample, most likely **Dye I**, possibly indicating that this compound is somewhat unstable. The presence of **Dye I** indicates that it may serve as a leaving group in an elimination reaction or in a substitution reaction in which the anion intermediate attacks the electropositive methylene group of the oxiranyl linker, similar to path a in Figure 11. The result of this mechanism would be the liberation of trifluoromethanesulfuric acid, which can protonate the piperidine nitrogen in **10-I-Pip**. Such an impurity would account for the elemental assays found. A pmr spectrum showed that only the expected isomer was isolated.

Experimental

General.

All melting points were determined in unsealed capillary tubes with 76 mm immersion thermometers and needed no cor-

rection. Those below 260° were determined with a heated oil bath (Thomas-Hoover Unimelt, Arthur H. Thomas Co.). Those above 260° were determined in a heated aluminum block (Mel-Temp, Laboratory Devices Co.). Infrared spectra were determined with a Perkin Elmer 1600 series FTIR in potassium bromide using a diffuse reflectance cell (DRC) with frequencies given in inverse centimeters. Proton magnetic resonance (pmr) spectra were determined with either a Varian EM-360L 60 MHz instrument, a Bruker NR-80 80 MHz instrument, or a Bruker 250 MHz instrument using various deuterated solvents with tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (ppm) from TMS. Most stirring in round bottomed flasks was performed with teflon coated prolate spheroid shaped magnets driven by a Corning PC-133 unit. Rotary evaporation and drying of compounds was usually performed with water aspirator vacuum, approximately 30 torr, unless otherwise noted, in which case a mechanical pump was used. Chromatographic purification by means of an Ace-Kauffman column means that a solid sample was extracted with hot solvent, passing through adsorbent into refluxing solvent in a special apparatus [49] (Ace Glass Co.). Thin-layer chromatography (tlc) was performed with Whatman MK6F silica plates visualized with short- and long-wave ultraviolet light. High pressure liquid chromatography (hplc) was performed with a Milton Roy Constametric 3000 pump, Econosphere C18 column, and monitored with a Milton Roy spectroMonitor 3100 uv detector. Elemental analysis was performed by either Desert Analytics, Tucson, AZ, or by Oneida Research Services, Whitesboro, NY. The decolorizing carbon used was Norit, neutral (Fisher C-170) and indicates a hot filtration was performed. All solvents and reagents used were obtained from one of the large chemical distributors (Sigma, Aldrich, Fisher, etc.) and used without further purification unless otherwise noted. Freon TF is 1,1,2-trichlorotrifluoroethane.

1-Bromomethyl-3-[(thiocarbonyl)amino]benzene (Linker **1**, Figure 1).

A mixture of 20.0 g (0.134 mole) of m-tolyl isothiocyanate **1a**, 23.9 g (0.134 mole) of *N*-bromosuccinimide, and 3.24 g (0.0134 mole) of benzoyl peroxide in 30 ml of carbon tetrachloride was boiled under reflux until all *N*-bromosuccinimide was converted to succinimide (all precipitate floated, approximately 5 hours). The mixture was vacuum filtered while still warm, and the precipitate washed with three 10 ml portions of carbon tetrachloride. The filtrate was concentrated and dissolved in 300 ml of hexane and passed through a chromatographic column (5.2 cm diameter packed to a height of 10 cm with Davisil 60-200 mesh Silica Gel) followed by 900 ml of hexane. The eluate was concentrated to yield a yellow oil, which was distilled under vacuum through a 15 cm Vigreaux fractionating column wrapped with heating tape. The column was heated to approximately 70° prior to distillation. Starting material was collected between 46-49° @ 0.10 torr. The column was then heated to approximately 100° and a second fraction, a yellow oil, was collected between 89-114° @ 0.07 torr. The second fraction was dissolved in a minimum of methanol at room temperature and the mixture was placed in an acetone/ice bath to crystallize. After two recrystallizations 12.54 g (41%) of colorless crystals, mp 35.8-36.8° were obtained. An additional 2.49 g (8.1%) of slightly less pure compound, mp 34.7-36.0°, was obtained by concentrating the filtrates of the first recrystallizations and recrystallizing them in the same manner; tlc: R_f (hexane) = 0.23; ir (carbon tetrachloride): ν 2050 (NCS); pmr, (80 MHz, deuteriochloroform): δ 4.44 (2H, s, CH_2Br), 7.25 ppm (4H, m, ArH).

Anal. Calcd. for C_8H_6BrNS: C, 42.12; H, 2.65; N, 6.14. Found: C, 42.25; H, 2.63; N, 5.97.

1-Bromomethyl-4-[(thiocarbonyl)amino]benzene (Linker **2**, Figure 1).

The procedure was the same as that used for Linker **1**, using *p*-tolyl isothiocyanate **2a**, which was treated like compound **1a** above. The filtrate was cooled to room temperature and enough carbon tetrachloride was added to dissolve any precipitate (approximately 200 ml). The mixture was purified by means of a chromatographic column (5.2 cm diameter packed to a 10 cm height with Davisil 60-200 mesh Silica Gel) with 1000 ml of carbon tetrachloride as eluent. The eluate was concentrated and the resulting orange solid was recrystallized twice from hexane. After vacuum drying (mechanical pump), 13.90 g (46%) of yellow crystals was recovered, mp 98.8-99.8°; tlc: Rf = 0.48 (carbon tetrachloride); ir (carbon tetrachloride): ν 2046 (NCS); pmr, (80 MHz, 6%, deuteriochloroform): δ 4.47 (2H, s, CH_2Br), 7.18 (2H, d, J = 8.47 Hz, ArH *ortho* to NCS), 7.39 ppm (2H, d, J = 8.47 Hz, ArH *meta* to NCS).

Anal. Calcd. for C_8H_6BrNS: C, 42.12; H, 2.65; N, 6.14. Found: C, 42.25; H, 2.36; N, 5.89.

1-Bromo-2-[(thiocarbonyl)amino]ethane (Linker **3**, Figure 2).

Chloroform (170 ml) was cooled to 0° at which time 20.0 g (0.098 mole) of 2-bromoethylamine hydrobromide **3a**, and 11.27 g (0.098 mole) of thiophosgene were added. Triethylamine, 29.7 g (0.294 mole), was then added to the reaction mixture over a 2 hour period, then the reaction mixture was stirred at room temperature for an additional 2 hours. The mixture was washed twice with each of the following, 150 ml of 5% sodium hydroxide, 150 ml of 5% hydrochloric acid, and 150 ml of water, respectively. The organic layer was dried over magnesium sulfate, and concentrated to yield a brown oil. The oil was vacuum distilled, bp 43-46° @ 0.15 torr to yield 8.65 g (64%) of a light yellow liquid . A second vacuum distillation (liquid turned brown upon standing) yielded 7.16 g (53%) of a colorless liquid bp 48-44° @ 0.35-0.20 torr, respectively (lit [50] 102-108° @ 15 torr); tlc: Rf = 0.25 (cyclohexane); ir (neat): ν 2087 (NCS); pmr (80 MHz, deuteriochloroform): δ 3.54 (2H, t, J = 5.42 Hz, CH_2Br), 3.92 ppm (2H, t, J = 5.30 Hz, CH_2NCS).

N-[4-(2-Chloroethoxy)phenyl]ethanamide (**4b** in Figure 3).

A solution containing 5.0 g (0.089 mole) of potassium hydroxide and 10.0 g (0.066 mole) of 4-acetamidophenol **4a** in 50 ml of absolute ethanol was added over a 40 minute period to a solution of 15 g (0.105 mole) of 1-bromo-2-chloroethane in 100 ml of refluxing absolute ethanol. A white precipitate formed almost immediately. The mixture was refluxed for an additional 1.25 hours after addition was complete. The mixture was cooled, vacuum filtered, and the filtrate concentrated; a tan solid resulted. The solid was dissolved in diethyl ether and washed with 1.5 *M* sodium hydroxide. The ether layer was dried over magnesium sulfate and concentrated to yield a colorless solid. The solid was recrystallized from toluene (Norit). After vacuum drying (80°/1.25 hours) 4.17 g (30%, 34% in a later run) of fluffy, colorless crystals were obtained, mp 126-127° (lit [23a] 126-127°); ir: ν 3312 (NH), 1663 (C=O), 1255 (C-O-C); pmr (80 MHz,

deuteriochloroform): δ 2.14 (3H, s, CH_3), 3.79 (2H, t, J = 5.34 Hz, CH_2Cl), 4.21 (2H, t, J = 5.88 Hz, OCH_2), 6.87 (2H, d, J = 8.87 Hz, ArH *ortho* to OCH_2), 7.26 (1H, s, NH), 7.40 ppm (2H, d, J = 8.59 Hz, ArH *ortho* to NH).

4-(2-Chloroethoxy)benzenaminium Chloride (**4d** in Figure 3).

A solution of 6*M* hydrochloric acid (150 ml) containing 15.0 g (70 mmoles) of *N*-[4-(2-chloroethoxy)phenyl]ethanamide **4b**, was boiled under reflux for 3.5 hours. A colorless precipitate developed when the mixture was cooled to room temperature. Diethyl ether (150 ml) was added to the mixture, which was then made basic with 6 *M* sodium hydroxide. The layers were separated and the aqueous layer washed with two additional 300 ml portions of diethyl ether. The combined ether layers were dried over sodium sulfate. Hydrogen chloride was bubbled through the ether solution resulting in the precipitation of the ammonium salt **4d** as a pink solid. The solid was vacuum filtered, and washed with 100 ml of diethyl ether. After vacuum drying (80°/4 hours), 13.87 g (95%) of light pink solid was obtained, mp 207-209° (lit [24] 208-210°); ir: ν 2867, 2610 (NH_3), 1264 (C-O-C); pmr (60 MHz, dimethyl sulfoxide-d_6, 10%): δ 3.94 (2H, m, CH_2Cl), 4.24 (2H, m, OCH_2), 7.05 (2H, d, J = 9 Hz, ArH *ortho* to OCH_2), 7.42 (2H, d, J = 8Hz, ArH *meta* to OCH_2), 10.10 ppm (3H, s, broad, NH_3).

1-(2-Chloroethoxy)-4-[(thiocarbonyl)amino]benzene (Linker **4**, Figure 3).

A mixture of 11.0 g (53.1 mmoles) of 4-(2-chloroethoxy)benzenaminium chloride **4d**, 6.11 g (53.1 mmoles) of thiophosgene, and 16.1 g (159 mmoles) of triethylamine in 65 ml of chloroform were reacted using the procedure described for 2-bromoethyl isothiocyanate (Linker **3**). However, the purification of product was considerably different. The reddish-brown colored reaction mixture was washed twice with each of the following; 75 ml of 5% sodium hydroxide, 75 ml of 5% hydrochloric acid, and 75 ml of water, respectively. The organic layer was dried over magnesium sulfate, and passed through a chromatographic column (9 cm high by 5 cm diameter packed with Davisil 62, 60-200 mesh Silica Gel) using chloroform as the eluent. Removal of solvent gave a brown solid, which was dissolved in chloroform and passed through another Silica Gel column. After concentration, an orange solid resulted. Recrystallization did not remove the remaining contaminants, so the solid was passed through a medium Ace-Kauffman column (packed with 7 cm of Davisil 62, 60-200 mesh Silica Gel) using Freon TF as the eluent. After concentration, approximately 6.5 g (crude) of a yellow solid was obtained. This was recrystallized from 50 ml of hexane (seeding was necessary to prevent the isothiocyanate (Linker **4**) from oiling) to give 6.45 g (57%) of yellow solid, mp 54-56.5°; tlc: R_f (chloroform) = 0.73; ir: ν 2127 (NCS), 1248 (C–O–C); pmr (60 MHz, deuteriochloroform, 8%): δ 3.94 (2H, t, J = 5 Hz, CH_2Cl), 4.20 (2H, t, J = 5 Hz, OCH_2), 6.84 (2H, d, J = 10 Hz, ArH *meta* to NCS), 7.20 ppm (2H, d, J = 9 Hz, ArH *ortho* to NCS).

Anal. Calcd. for C_9H_8ClNOS: C, 50.59; H, 3.77; N, 6.56. Found: C, 50.62; H, 3.74; N, 6.45.

N-[4-(2-Hydroxyethoxy)phenyl]ethanamide (**5b** in Figure 4).

A solution of 12.2 g (0.20 mole) of potassium hydroxide and 30.24 g (0.20 mole) of 4-acetamidophenol **4a** in 200 ml of water was placed on a steam bath and heated for 5 minutes at which time 28.6 g of 2-bromoethanol was added with the aid of 100 ml of water. The solution turned from purple to brown shortly after the addition. The mixture was heated for three hours and then allowed to cool overnight. The solution was saturated with solid sodium chloride resulting in the separation of a brown oil. The mixture was extracted twice with chloroform resulting in three layers. The chloroform layer was separated and dried over magnesium sulfate and concentrated, resulting in a brown oil. The oil was dissolved in 60 ml of water and potassium hydroxide was added, causing the formation of a colorless precipitate. The mixture was cooled to 0°, filtered, and washed with 50 ml of a saturated sodium chloride solution; 16.4 g (42%) of a nearly colorless solid **5b** resulted after drying, mp 119-122°; tlc: R_f (ethyl acetate) = 0.29.

An analytical sample was prepared by recrystallization from chloroform (~1g/70 ml), mp 121-122° (lit [23b] 119-120°); ir: ν 3230 (NH, OH), 1670 (C=O), 1245 (C-O-C); pmr (60 MHz, Unisol™, 10%): δ 2.07 (3H, s, CH_3), 3.90 (4H, m, CH_2CH_2), 4.45 (1H, s, OH), 6.80 (2H, d, J = 9 Hz, ArH *ortho* to OCH_2), 7.45 (2H, d, J = 9 Hz, ArH *meta* to OCH_2), 9.40 ppm (1H, s, broad, NH).

4-(2-Hydroxyethoxy)benzenaminium Chloride (**5d** in Figure 4).

A 10% (v/v) solution of concentrated sulfuric acid (150 ml) containing 15.0 g (76.8 mmoles) of 4-(2-hydroxyethoxy)-acetanilide **5b** was boiled under reflux for two hours, resulting in a clear yellow solution. After cooling the mixture was made basic (*p*H 10 -11) with a potassium hydroxide solution, saturated with solid sodium chloride, and extracted 3 times with 100 ml portions of chloroform. The combined extracts were dried over magnesium sulfate and concentrated at 30°, resulting in 8.0 g (68%) of a buff colored solid **5c**, mp 71-75°; R_f (ethyl acetate) = 0.39. Recrystallization of the free amine **5c** proved difficult, so the hydrochloride **5d** was isolated. The amine **5c** was dissolved in 200 ml of 2:1::diethyl ether:tetrahydrofuran, dried over magnesium sulfate, and dry hydrogen chloride gas was bubbled through it. After filtration and a wash with diethyl ether, 8.8 g (60%) of a buff precipitate resulted, mp 210-213° dec (lit [25] 200-205° dec); ir: ν 3404 (OH), 2594 (NH_3), 1261(C-O-C); pmr (60 MHz, dimethyl sulfoxide-d_6, 10%): δ 3.75 (2H, m, CH_2OH), 3.98 (2H, m, OCH_2), 5.0 (1H, s, broad, OH), 7.01(2H, d, J = 9 Hz, ArH *ortho* to OCH_2), 7.36 (2H, d, J = 8 Hz, ArH *meta* to OCH_2), 10.28 ppm (3H, s, broad, NH_3).

A sample of the free amine was prepared by passing a portion of the product through a chromatographic column (Davisil 62) using ethyl acetate as the eluent, mp 73.5-75.5° (lit [25] 71-72°).

2-{4-[(Thiocarbonyl)amino]phenoxy}ethanol (**5e** in Figure 4).

Using the procedure described for Linker **2**, reacted 8.0 g (42.2 mmoles) of 4-(2-hydroxyethoxy)benzenaminium chloride **5d** and 4.85 g (42.2 mmoles) of thiophosgene in 50 ml of chloroform. The mixture was cooled to 0° and 12.8 g (127 mmoles) of triethylamine was added over approximately 2 hours, during which time the reaction mixture turned from tan to brown. After addition was complete, the mixture was stirred an additional 2.5 hours at room temperature, then diluted with 50 ml of chloroform and washed twice with each of the following, respectively; 75 ml of 5% sodium hydroxide, 75 ml of 5% hydrochloric acid, and 75 ml of a saturated salt solution. The organic layer was dried over magnesium sulfate, filtered through a funnel containing 1 cm of Silica Gel (Davisil 62) and washed with 300 ml of

ethyl acetate. The filtrate was concentrated to yield 5.8 g (70%) of a buff solid, which was recrystallized from 250 ml of cyclohexane (Norit) to yield 5.41 g (67%) of yellow plates after drying, mp 99.3-102°; R_f (ethyl acetate) = 0.62.

An analytical sample was prepared by passing a small portion of the product through a chromatographic column (Davisil 62) using ethyl acetate as the eluent. Concentration yielded a light yellow solid which was recrystallized from ethanol:water::1:4 to yield colorless needles, mp 100-102°; ir: ν 3403 (OH), 2135 (NCS), 1252 (C-O-C); pmr (80 MHz, deuteriochloroform): δ 2.10 (1H, m, OH), 4.03 (4H, m, CH_2CH_2), 6.86 (2H, d, J = 8.86 Hz, ArH *ortho* to OCH_2), 7.17 ppm (2H, d, J = 8.94 Hz, ArH *ortho* to NCS).

Anal. Calcd. for $C_9H_9NO_2S$: C, 55.39; H, 4.65; N, 7.18. Found: C, 55.31; H, 4.64; N, 7.12.

2-{4-[(Thiocarbonyl)amino]phenyl}ethyl 4-Toluenesulfonate (Linker **5**, Figure 4).

A mixture of 5.00 g (25.5 mmoles) of 4-(2-hydroxyethoxy)-phenyl isothiocyanate **5e** and 8.0 g of dry pyridine was cooled to 5-10° and 5.34 g (28.0 mmoles) of *p*-toluenesulfonyl chloride was added in portions over a 20 minute period below 20°. An additional 11 ml of pyridine was added to aid stirring. The mixture was stirred an additional 2.5 hours at 25°, then poured into 100 ml of cold (0°) 12 *M* hydrochloric acid:water::3:7, filtered, and washed with a copious amount of water, to yield 8.30 g (93%) of an off-white solid after drying, mp 107.5-109.5°, R_f (ethyl acetate) = 0.81. The solid was recrystallized from ~ 500 ml of cyclohexane (Norit) to yield nacreous needles, after drying, 6.86 g (77%), mp 110-112°; tlc: R_f (chloroform) = 0.50; ir: ν 2118 (NCS), 1253 (C-O-C), 1183 (sulfonate); pmr (80 MHz, deuteriochloroform): δ 2.46 (3H, s, $ArCH_3$), 4.15 (2H, m, CH_2OSO_2), 4.35 (2H, m, $ArOCH_2$), 6.74 (2H d, J = 8.90 Hz, ArH *ortho* to OCH_2), 7.13 (2H, d, J = 8.95, ArH *ortho* to NCS), 7.34 (2H, d, J = 8.14 Hz, ArH *ortho* to CH_3), 7.80 ppm (2H, d, J = 8.26 Hz, ArH *ortho* to SO_3).

Anal. Calcd. for $C_{16}H_{15}NO_4S_2$: C, 55.00; H, 4.33; N, 4.01. Found: C, 55.50; H, 4.41; N, 3.96.

1-(3-Bromopropyl)-4-nitrobenzene **6b** (Figure 5).

A mixture of 39.25 g (0.436 mole) of concentrated nitric acid and 51.95 g of concentrated sulfuric acid was added over 6 hours, with brisk stirring, to 101 g (0.508 mole) of cold (0°) 1-bromo-3-phenylpropane **6a**. The mixture was warmed to 100° over the next 4 hours and kept at that temperature for an additional two hours. It was then cooled to room temperature and extracted with methylene chloride. The methylene chloride was washed with 10% w/w potassium hydroxide and dried over anhydrous magnesium sulfate, and concentrated. The resulting orange oil was distilled under vacuum through a 25 cm helical Vigreaux fractionating column heated with heating tape and fractions collected in a Noonan receiver. Starting material was collected between 59-100°, the 2-isomer between 101-113°, and the 4-isomer between 114-142°, all at 0.13 torr. The highest boiling liquid was dissolved in a minimum amount of methanol at room temperature. The mixture was then cooled to -65° to crystallize. The liquid was decanted and the colorless crystals were washed several times with additional cold methanol. When warmed to room temperature, the crystals melted into a pale yellow oil which was placed under vacuum (mechanical pump) to remove any excess methanol, yielding 26.58 g (25%); ir (neat): ν 1518 and 1344 (NO_2); pmr (80 MHz, 10%, deuteriochloroform): δ 2.18 (2H, m, CH_2CH_2Br), 2.91 (2H, t, J = 7.78 Hz, $ArCH_2$), 3.40 (2H, t, J = 6.33 Hz, CH_2Br), 7.36 (2H, d, J = 8.58 Hz, ArH *meta* to NO_2), 8.13 ppm (2H, d, J = 8.61 Hz, ArH *ortho* to NO_2).

Anal. Calcd. for $C_9H_{10}BrNO_2$: C, 44.29; H, 4.13; N, 5.74. Found: C, 44.37; H, 4.16; N, 6.07.

4-(3-Bromopropyl)benzenaminium Chloride **6d** (Figure 5).

To a 1000 ml beaker, equipped with magnetic stirring, were added 72.2 ml of concentrated hydrochloric acid and 54.2 g (0.286 mole) of tin(II) chloride dihydrate. Upon solution of the tin(II) chloride, 19.5 g (0.0799 mole) of 1-bromo-3-(4-nitrophenyl)propane **6b** was added and the mixture stirred rapidly. A slow temperature rise was observed followed by a large exotherm. Once the temperature began to fall the mixture was placed in an ice bath and 100 ml of cold water was added followed by 250 ml of diethyl ether. While being stirred, the reaction mixture was made basic with 6 *M* sodium hydroxide. A heavy pink precipitate formed, but it redissolved when the *p*H reached 10-11. The layers were separated and the aqueous layer washed with an additional 150 ml of diethyl ether. The combined ether layers were dried over magnesium sulfate. Hydrogen chloride gas was bubbled through the ether solution. A white precipitate quickly formed, which was vacuum filtered, and placed under house vacuum over sodium hydroxide pellets overnight to yield 16.06 g (75%) of **6d**, mp 194-196°; ir (potassium bromide): ν 2850 and 2596 (NH_3); pmr (250 MHz, dimethyl sulfoxide-d_6): δ 2.10 (2H, m, CH_2CH_2Br), 2.75 (2H, t, J = 6.25 Hz, $ArCH_2$), 3.53 (2H, t, J = 3.75 Hz, CH_2Br), 7.32 (2H, d, J = 6.25 Hz, ArH *ortho* to CH_2), 7.39 ppm (2H, d, J = 6.25 Hz, ArH *meta* to CH_2), (NH_3, not observed).

1-(3-Bromopropyl)-4-[(thiocarbonyl)amino]benzene (Linker **6**, Figure 5).

A mixture of 14.0 g (52.6 mmoles) of 4-(3-bromopropyl)aniline hydrochloride **6d** and 6.10 g (53.0 mmoles) of thiophosgene in 250 ml of cyclohexane was heated to reflux, at which time 14.94 g of dry triethylamine in 40 ml of cyclohexane were added over a 40 minute period. A brown precipitate began to develop immediately. The mixture was boiled under reflux for an additional 30 minutes, and allowed to cool slightly before vacuum filtration. The precipitate was washed with 100 ml of cyclohexane, and the filtrate was purified by passage through a chromatographic column (5.2 cm diameter column packed with 2 cm of Davisil 60-200 mesh Silica Gel) using cyclohexane as the eluent. The eluate was concentrated, resulting in an orange oil, which was crystallized from methanol (Norit) at 0°. After vacuum drying (mechanical pump), 8.14 g (60%) of pale tan crystals resulted, mp 38.5-39.7°; tlc: R_f = 0.32 (cyclohexane); ir (neat): ν 2108 (NCS); pmr (80 MHz, deuteriochloroform): δ 2.15 (2H, m, CH_2CH_2Br), 2.81 (2H, t, J = 6.62 Hz, $ArCH_2$), 3.39 (2H, t, J = 6.37 Hz, CH_2Br), 7.19 ppm (4H, s, br, ArH).

Anal. Calcd. for $C_{10}H_{10}BrNS$: C, 46.88; H, 3.94; N, 5.47. Found: C, 47.16; H, 3.72; N, 5.29.

3-(Bromomethyl)benzoic Acid (**7b** in Figure 6).

A mixture of 30.0 g (0.220 mole) of 3-toluic acid **7a**, 39.22 g (0.220 mole) of *N*-bromosuccinimide, and 4.27 g (0.022 mole) of *t*-butyl benzoyl peroxide in 100 ml carbon tetrachloride was boiled under reflux for 4 hours. The mixture was cooled, and concentrated by rotary evaporation. The solid was resuspended

with 400 ml of water, stirred, filtered, washed with a copious amount of water, and vacuum dried overnight, resulting in 46.4 g of off-white solid, mp 127-144°, softens 115°. The solid was recrystallized from dibutyl ether (Norit), filtered, washed with dibutyl ether, resulting in 25.9 g of buff solid, mp 150-154°, after drying (75°/overnight). Some decomposition did occur at the boiling point of dibutyl ether. The solid was recrystallized from 500 ml of carbon tetrachloride (Norit), concentrated by boiling to 250 ml, filtered, washed with cyclohexane, and dried overnight, resulting in 21.0 g (44%) of colorless solid, mp 154-155°, (lit [28] 150°); tlc: R_f (ethyl acetate) = 0.37; ir: ν 2800 (OH), 1694 (C=O); pmr (60 MHz, deuteriochloroform): δ 4.57 (2H, s, CH_2Br), 7.55 (2H, m, ArH *meta* and *para* to COOH), 8.10 (2H, m, ArH *ortho* to COOH), 11.75 ppm (1H, s, COOH).

1-[(3-Bromomethylbenzoyl)oxy]-2,5-pyrrolidinedione (Linker 7, Figure 6).

A mixture of 10.80 g (50.2 mmoles) of 3-(bromomethyl)benzoic acid **7b**, 6.94 g (60.4 mmoles) of *N*-hydroxysuccinimide, 10.60 g (51.4 mmoles) of dicyclohexylcarbodiimide in 200 ml of dry acetonitrile was stirred at room temperature overnight and filtered to remove most of the dicyclohexylurea, which was washed with 100 ml of acetonitrile. The filtrate was concentrated resulting in 17.2 g of nearly colorless solid, which was recrystallized from 300 ml of absolute ethanol, yielding 11.29 g (72%) of small colorless needles, mp 158.5-161.5°; tlc: R_f (ethyl acetate) = 0.69.

An analytical sample was prepared by another recrystallization from absolute ethanol. A different crystalline form may have been isolated, mp 154-155.5°; ir: ν 1769 and 1735 (C=O); pmr (60 MHz, deuteriochloroform): δ 2.90 (4H, s, succinimidyl Hs), 4.53 (2H, s, CH_2Br), 7.57 (2H, m, ArH *meta* and *para* to C=O), 8.05 ppm (2H, m, ArH *ortho* to C=O).

Anal. Calcd. for $C_{12}H_{10}BrNO_4$: C, 46.18; H, 3.23; N, 4.49. Found: C, 46.18; H, 3.17; N, 4.47.

2-(2,5-Dioxo-1*H*-pyrrol-1-yl)ethyl Methanesulfonate (Linker **8**, Figure 7).

A mixture of 2.00 g (14.0 mmoles) of *N*-(2-hydroxyethyl)-maleimide **8a** [30] and 2.68 g (15.4 mmoles) of methanesulfonic anhydride was heated to 100-110° in an ethylene glycol bath for 3.5 hours. The mixture was cooled and dissolved in 50 ml of ethyl acetate and washed 4 times with a saturated sodium bicarbonate/salt solution. The combined aqueous layers were washed with 50 ml of ethyl acetate. The combined organic layers were dried over magnesium sulfate and concentrated with rotary evaporation, resulting in 3.08 g (100%) of pale yellow oil. The oil solidified when placed at -20°, mp 63-65.5°. The solid was recrystallized from 100 ml of *t*-butyl methyl ether (Norit) resulting in a colorless solid, mp 66-68.5°, softens 64°; tlc: R_f (ethyl acetate) = 0.59.

A second run on the same scale resulted in 2.89 g (94%) of oil which contained a significant amount of polar impurity, tlc: R_f (ethyl acetate) = 0.25. The impurity was removed by passing the sample through a 2.5 cm diameter chromatographic column packed with Silica Gel (Aldrich 24,219-7) using 200-250 ml of methylene chloride/ethyl acetate (9:1) as the eluent. After concentration, 1.85 g (60%) of colorless solid was recovered, mp 67-72°, softens 63°. The solid was recrystallized from *t*-butyl methyl ether at -20°, resulting in 1.63 g (53%) of colorless solid, mp 67.5-70.5°; ir: ν 1702 (C=O), 1167 (sulfonate); pmr (60 MHz, deuteriochloroform): δ 3.04 (3H, s, CH_3), 3.88 (2H, t, J = 6 Hz, CH_2N), 4.39 (2H, t, J = 6 Hz, OCH_2), 6.75 ppm (2H, s, CH=CH).

Anal. Calcd. for $C_7H_9NO_5S$: C, 38.35; H, 4.14; N, 6.39. Found: C, 38.44; H, 4.08; N, 6.38.

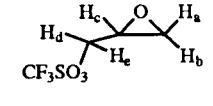
2-(2,5-Dioxo-1*H*-pyrrol-1-yl)ethyl Trifluoromethanesulfonate (Linker **9**, Figure 7).

To a suspension of 3.00 g (21.3 mmoles) of *N*-(2-hydroxyethyl)maleimide **8a** [30] in 50 ml of dry diethyl ether under nitrogen, was added 2.20 g (22.0 mmoles) of trifluoromethanesulfonic anhydride in portions with the aid of 10 ml of diethyl ether. An exotherm to 35° was observed and all reactants went into solution. The solution was stirred for an additional hour at room temperature at which time 50 ml of diethyl ether was added to the mixture. The mixture was washed once with 100 ml of ice cold water, followed by 100 ml of a saturated salt solution. The ether layer was dried over magnesium sulfate and concentrated resulting in 5.27 g (91%) of a colorless oil. The oil was stored at -20° under argon, during which time it solidified. It remained solid at room temperature but melted when held in the hand. The product was used without further purification in subsequent reactions.

Vacuum distillation was used to prepare a pure sample, bp 84-87° @ 0.1 torr. The compound discolored readily in the presence of air. Recrystallization from Freon TF at -20° was tried to remove the impurity with little success; more decomposition occured. The identity of the compound was proven with a pmr spectrum and conversion to **9-I** and **9-II**; tlc: R_f (ethyl acetate) = 0.59; pmr (60 MHz, deuteriochloroform): δ 3.95 (2H, t, J = 5 Hz, CH_2N), 4.65 (2H, t, J = 5 Hz, OCH_2), 6.84 ppm (2H, s, CH=CH).

Oxiranylmethyl Trifluoromethanesulfonate (Linker **10**, Figure 8).

A solution of 4.57 g (57.8 mmoles) of dry pyridine in 100 ml of methylene chloride was cooled to -25° (under nitrogen with mechanical stirring) with a dry ice/methylene chloride/acetone bath, and 16.30 g (54.2 mmoles) of trifluoromethanesulfonic anhydride was added over a 30 minute period, causing a slight exotherm and turning the solution orange. A heavy precipitate formed toward the end of the addition. To this suspension 6.64 g (49.1 mmoles) of glycidol was added over a 30 minute period with the aid of 15 ml of methylene chloride, causing the dissolution of some of the precipitate. The suspension was allowed to warm to 10° over the next two hours; it was filtered and washed with a few ml of methylene chloride. The filtrate was concentrated with rotary evaporation, resulting in 13.7 g of brown oil. The oil was dissolved in 100 ml of diethyl ether resulting in the precipitation of a solid. The solid was removed by filtration, and washed with diethyl ether; the filtrate was concentrated at 20° by rotary evaporation, resulting in 10.2 g of brown oil, which was distilled, bp 33° @ 0.2 torr to give 5.34 g (53%) of colorless liquid. The identity of the compound was ultimately proven by pmr spectra and conversion to **10-I** and **10-II**; pmr (60 MHz, deuteriochloroform): δ 2.72 (1H, m, H_a or H_b), 2.96 (1H, m, H_a or H_b), 3.35 (1H, m, H_c), 4.32 (1H, dd, H_d or H_e), 4.79 ppm (1H, dd, H_d or H_e).



1-[6-(3,4-Dihydro-2*H*-1-benzopyranyl)]ethanone (**IIb** in Figure 9).

A solution of 25.3 g (0.192 mole) of chroman **IIa** [13] in 200 ml of dichloroethane (under nitrogen) was cooled to 0° in a methanol/ice bath. In another flask, 28.13 g (0.211 mole) of aluminum chloride, 30.14 g (0.384 mole) of acetyl chloride were dissolved in 300 ml of dichloroethane, capped with a drying tube, and cooled to -20°. The acetyl chloride solution was added to the chroman solution over approximately an hour period at ~0°, resulting in a burgundy solution, which was stirred for an additional hour at 0°. The solution was quenched with a mixture of 200 ml of water, 200 ml of ice, and 100 ml of concentrated hydrochloric acid, stirred for one half hour, and the layers separated. The organic layer was washed with 500 ml of water followed by 500 ml of a saturated sodium bicarbonate solution, and then dried over magnesium sulfate. Concentration of the solution resulted in 35.5 g of oil. The oil solidified in an ice bath. Further drying (mechanical pump) resulted in 32.2 g (96%) of solid, mp 38-42.5°. The solid was recrystallized from 50 ml of Freon TF at -20°, filtered, washed twice with 10 ml portions of cold Freon TF, resulting in 29.5 g (88%) of colorless solid after drying, mp 42-44° (lit [36a] 44.5-45.5°); ir: ν 1664 (C=O); pmr (60 MHz, deuteriochloroform, 10%): δ 2.03 (2H, m, $ArCH_2CH_2$), 2.54 (3H, s, CH_3), 2.83 (2H, t, J = 6 Hz, $ArCH_2$), 4.22 (2H, t, J = 5 Hz, OCH_2), 6.83 (1H, d, J = 9 Hz, ArH *ortho* to OCH_2), 7.70 ppm (2H, m, ArH *meta* to OCH_2).

6-(3,4-Dihydro-2*H*-1-benzopyranyl)carboxylic Acid (**IIc** in Figure 9).

A 5.25% sodium hypochlorite solution was warmed to 55°, at which time 29.50 g (0.170 mole) of 6-acetylchroman **IIb** was added and the solution vigorously mechanically stirred. An exotherm was observed, which was kept below 70° with an ice bath. After the exotherm had subsided, the mixture was stirred for an additional half hour at room temperature. The solution was quenched with a solution of 20 g of sodium metabisulfite in 60 ml of water. An acidified potassium iodide test was used to ensure that all sodium hypochlorite was destroyed. The mixture was acidified with concentrated hydrochloric acid, during which time a colorless precipitate formed. The solid was filtered and washed with a copious amount of water, resulting in 28.45 g (95%) of oily solid after drying. The solid was dissolved in 400 ml of 10% potassium hydroxide (w/v) and washed twice with 100 ml portions of carbon tetrachloride. The aqueous layer was acidified with concentrated hydrochloric acid, resulting in 21.22 g (71%) of colorless solid after filtration and drying, mp 149-151° (lit [36b] 144-146°); pmr (60 MHz, deuteriochloroform): δ 2.01 (2H, m, $ArCH_2CH_2$), 2.83 (2H, t, J = 7 Hz, $ArCH_2$), 4.23 (2H, t, J = 5 Hz, OCH_2), 6.80 (1H, d, J = 10 Hz, ArH *ortho* to OCH_2), 7.79 (2H, m, ArH *ortho* to COOH), 12.20 ppm (1H, s, COOH).

Concentration of the carbon tetrachloride solution resulted in the recovery of 7.59 g (26%) of starting material **IIb**.

N-[2,2-Diethoxy-2-(4-pyridyl)ethyl]-6-(3,4-dihydro-2*H*-1-benzopyranyl)carboxamide (**IIf** in Figure 9).

A mixture of 20.24 g (0.115 mole) of 6-carboxychroman **IIc** and 23.27 g (0.196 mole) of thionyl chloride was gently boiled under reflux for 1.5 hours; after the first half hour all solid had dissolved and the solution turned green. The solution was allowed to cool and the air condenser was changed to a distillation head with air condenser. Vacuum was applied initially without heating at which time the mixture turned solid. The solid was then vacuum distilled resulting in 21.10 g (94%) of colorless acid chloride **IId**, which quickly solidified; pmr (60 MHz, deuteriochloroform): δ 2.03 (2H, m, $ArCH_2CH_2$), 2.86 (2H, t, J = 6 Hz, $ArCH_2$), 4.27 (2H, t, J = 5 Hz, OCH_2), 6.87 (1H, d, J = 10 Hz, ArH *ortho* to OCH_2), 7.87 ppm (2H, m, ArH *meta* to OCH_2).

The acid chloride **IId**, 21.1 g (0.108 mole), was added to a solution of 22.8 g (0.108 mole) of 2,2-diethoxy-2-(4-pyridyl)ethylamine **IIe** [37], and 21.82 g (0.216 mole) of dry triethylamine in 250 ml of toluene. A precipitate formed immediately and an exotherm to 63° was observed. The mixture was stirred overnight at room temperature. The suspension was filtered and the precipitate washed with 50 ml of toluene. The filtrate was washed with two 150 ml portions of water, followed by 150 ml of a saturated sodium bicarbonate solution, dried over magnesium sulfate, and concentrated with rotary evaporation, resulting in 37.58 g (95%) of yellow oil after drying. The oil solidified upon standing, mp 101-105°, softens 98°. The solid was recrystallized from 500 ml of cyclohexane (Norit), resulting in 37.4 g (94%) of nearly colorless solid, mp 97-102°, softens 93°, after thorough drying.

An analytical sample was prepared by passing ~1 g of the compound through a chromatographic column (11 cm high x 2 cm diameter) packed with Silica Gel (Aldrich 24,217-9) using 300 ml of ethyl acetate as the eluent. Removal of the solvent gave 0.9 g of colorless oil, which solidified with scratching. The solid was recrystallized from 30 ml of cyclohexane, resulting in colorless needles, mp 103-105°, after thorough drying; tlc: R_f (ethyl acetate) = 0.27; ir: ν 3254 (NH), 1648 (C=O), 1605 (C=N); pmr (60 MHz, deuteriochloroform): δ 1.23 (6H, t, J = 8 Hz, OCH_2CH_3), 2.03 (2H, m, $ArCH_2CH_2$), 2.76 (2H, t, J = 7 Hz, $ArCH_2$), 3.49 (4H, m, OCH_2CH_3), 3.86 (2H, d, J = 6 Hz, $NHCH_2$), 4.20 (2H, t, J = 6 Hz, $ArOCH_2$), 5.80 (1H, s, br, NH), 6.78 (1H, d, J = 8 Hz, ArH *ortho* to OCH_2), 7.30 (2H, d, J = 9 Hz, ArH *meta* to OCH_2), 7.50 (2H, d, J = 5 Hz, ArH *meta* to PyrN), 8.70 ppm (2H, d, J = 5 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{21}H_{26}N_2O_4$: C, 69.09; H, 7.07; N, 7.56. Found: C, 68.24; H, 7.04; N, 7.51.

N-[2-Oxo-2-(4-pyridyl)ethyl]-6-(3,4-dihydro-2*H*-1-benzopyranyl)carboxamide (**IIg** in Figure 9).

To a 1000 ml erlenmyer flask, equipped with magnetic stirring, were added 35.7 g (97 mmoles) of the ketal **IIf** and 360 ml of 1*M* hydrochloric acid. The mixture was heated to boiling, during which time the solution turned from clear to yellow and a yellow precipitate began to develop. The mixture was boiled for a half hour, cooled to room temperature and made basic with concentrated ammonia. The mixture was filtered, washed with a copious amount of water, and dried, resulting in 25.66 g (90%) of buff solid, mp 179-183.5°. The solid was recrystallized from 350 ml of 2-propanol, filtered, washed with 75 ml of 2-propanol, and dried, resulting in 24.40 g (86%) of colorless needles, mp 188 -191°, yellow melt; tlc: R_f (ethyl acetate) = 0.13. An analytical sample was prepared by a second recrystallization from 2-propanol (Norit), mp 188-190.5°, yellow melt; ir: ν 3361 (NH), 1702, 1630 (C=O), 1263 (C-O-C); pmr (60 MHz, deuteriochloroform): δ 1.89 (2H, m, $ArCH_2CH_2$), 2.67 (2H, t, J = 7 Hz, $ArCH_2$), 4.06 (2H, t, J = 5 Hz, OCH_2), 4.79 (2H, d, J = 4 Hz, $NHCH_2$), 6.67 (1H, d, J = 9 Hz, ArH *ortho* to OCH_2), 6.94 (1H, s, br, NH), 7.45 (2H, d, J = 7 Hz, ArH *meta* to OCH_2), 7.63 (2H,

d, J = 5 Hz, ArH *meta* to PyrN), 8.72 ppm (2H, d, J = 5 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 68.86; H, 5.36; N, 9.32.

2-[6-(3,4-Dihydro-2*H*-1-benzopyranyl)]-5-(4-pyridyl)oxazole (**Dye II**, Figure 9).

A suspension of 23.37 g (79.5 mmoles) of the amide **IIg** in 470 ml of thionyl chloride was gently boiled under reflux for 45 minutes, during which time all the solid dissolved, resulting in a clear orange solution. The mixture was vacuum concentrated to 150-200 ml using an aspirator and gentle heating, and then quenched with 1000 ml of ice. Once the quench solution had cooled, it was made basic with concentrated ammonia, cooled, filtered, and washed with a copious amount of water. After drying, 20.87 g (95%) of yellow solid was recovered, mp 157-159.5°. The solid was passed through a medium Ace-Kauffman column packed with 3 cm of Silica Gel (Aldrich 24,217-9) over 4 cm of Alumina (Aldrich 19,997-5) using cyclohexane as the eluent. Once all fluorescent material had eluted, the solution was cooled to room temperature and the product filtered, resulting in 17.97 g (82%) of colorless solid, mp 158-160.5°; tlc: R_f (ethyl acetate) = 0.39.

An analytical sample was prepared by recrystallization from cyclohexane, resulting in colorless plates, mp 157.5-160°; ir: ν 1613 (C=N), 1262 (C-O-C); pmr (60 MHz, deuteriochloroform): δ 1.90 (2H, m, $ArCH_2CH_2$), 2.70 (2H, t, J = 6 Hz, $ArCH_2$), 4.08 (2H, t, J = 6 Hz, OCH_2), 6.67 (1H, d, J = 10 Hz, ArH *ortho* to OCH_2), 7.36 (3H, m, ArH *meta* to PyrN and oxazole H), 7.67 (2H, m, ArH *meta* to OCH_2), 8.51 ppm (2H, d, J = 6 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.58; H, 5.07; N, 10.04.

1-Methyl-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Methanesulfonate (**IIh** in Figure 9).

A solution of 1.50 g (5.43 mmoles) of Dye **II** and 0.60 g (5.43 mmoles) of methyl methanesulfonate in 50 ml of dry toluene was boiled under reflux overnight, during which time a yellow precipitate developed. The suspension was filtered and washed with 100 ml of ethyl acetate, resulting in 1.91 g (91%) of solid after drying, mp >260°. The solid was dissolved in 30 ml of hot 2-propanol, filtered hot, concentrated to 20 ml and allowed to crystallize. After drying, 1.80 g (86%) of yellow needles were recovered, mp 265-269° dec. An analytical sample was prepared by another recrystallization from 50 ml of 2-propanol to give 1.54 g (73%), mp 267-270°; ir: ν 1635 (C=N), 1272 (C-O-C), 1196 (SO_3); pmr (60 MHz, dimethyl sulfoxide-d_6): δ 1.95 (2H, m, $ArCH_2CH_2$), 2.30 (3H, s, CH_3SO_3), 2.83 (2H, t, J = 6 Hz, $ArCH_2$), 4.25 (5H, m, NCH_3 and OCH_2), 6.88 (1H, d, J = 10 Hz, ArH *ortho* to OCH_2), 7.86 (2H, m, ArH *meta* to OCH_2), 8.40 (2H, d, J = 6 Hz, ArH *meta* to PyrN), 8.50 (1H, s, oxazole H), 8.96 ppm (2H, d, J = 5 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{19}H_{20}N_2O_5S$: C, 58.75; H, 5.19; N, 7.21. Found: C, 58.69; H, 5.21; N, 7.17.

5-(4-Hydroxyphenyl)-2-(4-pyridyl)oxazole (**11b** in Figure 10).

A solution of 5.0 g (19.8 mmoles) of Dye **I** in 50 ml of dry dimethylformamide was added to a solution of 4.48 g (53.3 mmoles) of ethanethiol sodium salt in 60 ml of dry dimethylformamide under nitrogen. The mixture was boiled under reflux for 3 hours during which time the fluorescence of the solution turned from violet to red. The mixture was cooled to room temperature overnight and acidified with a few ml of acetic acid, resulting in a bright blue fluorescence. The mixture was concentrated and vacuum dried to 4.65 g (99%) of a yellow solid, mp ~ 300-305° with some decomposition. Several recrystalization solvents were tried but the compound was insoluble in most. The higher boiling solvents would dissolve the compound but only impure orange solid was obtained after the recrystallization. Since purification was difficult the crude solid was used in the reaction below. An attempt was made to purify the solid as its hydrochloride salt. The salt was recrystallized from ethanol to give two distinct crystalline forms, large red needles and fine yellow needles.

N,N-Dimethyl-2-{4-[2-(4-pyridyl)-5-oxazolyl]phenyloxy}ethanamide (**11**, Figure 10).

The crude phenol **11b**, 3.0 g (12.6 mmoles), was dissolved in 50 ml of dry dimethylformamide under nitrogen. Next, 0.52 g (13.0 mmoles, 60% oil dispersion) of sodium hydride was added resulting in a small exotherm (1-2°), formation of hydrogen gas, and changing the fluorescence from blue to red. To this mixture, 1.57 g (12.9 mmoles) of 2-chloro-*N,N*-dimethylacetamide (Pfaltz and Bauer) were added over a half hour period with the aid of 25 ml of dry dimethylformamide. The mixture was stirred at room temperature for an additional half hour and then boiled under reflux for three hours, during which time the fluorescence became violet. The mixture was concentrated by rotary evaporation, washed with water, filtered, and dried to yield 2.85 g (70% crude) of pink solid. Recrystallization from toluene (Norit) resulted in a buff solid, mp ~ 146-156°. The solid was passed through a medium Ace-Kauffman column with 5 cm of neutral Alumina (Aldrich 19,997-5) and dry toluene as the solvent. Concentration of the eluate resulted in ~ 0.8 g (20%) of pale yellow solid, mp 174-176°, cloudy melt. An analytical sample was prepared by means of another recrystallization from toluene; ir: ν 1659 (C=O), 1605 (C=N), 1262 (C-O-C); pmr (60 MHz, deuteriochloroform): δ 2.95 (3H, s, NCH_3), 3.08 (3H, s, NCH_3), 4.70 (2H, s, CH_2), 6.98 (2H, d, J = 9 Hz, ArH *ortho* to OCH_2), 7.34 (1H, s, oxazole H), 7.62 (2H, d J = 9 Hz, ArH *meta* to OCH_2), 7.86 (2H, d, J = 6 Hz, ArH *meta* to PyrN), 8.70 ppm (2H, d , J = 5 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.87; H, 5.47; N, 12.87.

1-{3-[(Thiocarbonyl)amino]phenylmethyl}-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Bromide (Stain **1-I**).

A solution of 2.0 g (7.94 mmoles) of Dye **I** and 1.81 g (7.94 mmoles) of 3-(bromomethyl)phenyl isothiocyanate (Linker 1) in 35 ml of dry toluene was boiled under reflux during which time a yellow precipitate developed. After two hours of reflux the mixture was cooled, vacuum filtered and the precipitate washed with 15 ml of toluene. After vacuum drying (80°/2 hours), 2.10 g (55%) of stain **1-I** was recovered as a bright yellow solid, mp 225-226.5° dec. Overnight reflux of the filtrate, yielded an additional 1.29 g (34%), mp 224.5-226.5° dec. Total yield 3.39 g (89%). An analytical sample was prepared from the product of a later run by washing the solid with hot ethyl acetate, mp 231-233°; ir: ν 2112 (NCS), 1637 (C=N), 1264 (C-O-C); pmr (80 MHz, deuteriochloroform): δ 3.90 (3H, s, OCH_3), 6.41 (2H, s, CH_2), 7.05 (2H, d, J = 8.38 Hz, ArH *ortho* to OCH_3), 7.26 (2H,

m, ArH *para* to CH_2 and *meta* to NCS), 7.45 (1H, s, ArH *ortho* to CH_2 and NCS), 7.58 (1H, s, oxazole H), 7.71 (3H, m, ArH *meta* to OCH_3 and *para* to NCS), 8.45 (2H, d, J = 5.35 Hz, ArH *meta* to PyrN), 9.59 ppm (2H, d, J = 5.70 Hz, ArH *ortho* to PyrN).

Desert Analytics dried the sample at 100° before obtaining a satisfactory analysis.

Anal. Calcd. for $C_{23}H_{18}BrN_3O_2S$: C, 57.51; H, 3.78; N, 8.75. Found: C, 57.68; H, 3.64; N, 8.63.

1-{4-[(Thiocarbonyl)amino]phenylmethyl}-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Bromide (Stain **2-I**).

Dye **I**, 2.0 g (7.94 mmoles), and 1.81 g (7.94 mmoles) of 4-(bromomethyl)phenyl isothiocyanate (Linker **2**) in 40 ml of toluene were reacted using the procedure for Stain **1-I**. After 2 hours of reflux 2.23 g (59%) of Stain **2-I** was obtained as an amorphous orange solid, mp 248 -251° dec. The filtrate was placed back into the reaction vessel and boiled under reflux overnight. An additional 1.11 g (29%) of solid was obtained, mp 256-259° dec, total yield, 3.34 g (88%). An analytical sample was prepared by washing the solid in hot chloroform. The solid was filtered, washed with hot chloroform, and dried overnight under high vacuum, mp 258-260°. The sample changed color from yellow to reddish orange upon exposure to air but an increase in mass was not noticed; ir: ν 2095 (NCS), 1636 (C=N), 1268 (C-O-C); pmr (80 MHz, dimethyl sulfoxide-d_6): δ 3.84 (3H, s, OCH_3), 5.90 (2H, s, CH_2), 7.11 (2H, d, J = 8.82 Hz, ArH *ortho* to OCH_3), 7.58 (4H, m, ArH *ortho* and *meta* to NCS), 7.93 (2H, d, J = 8.86 Hz, ArH *meta* to OCH_3), 8.07 (1H, s, oxazole H), 8.86 (2H, d, J = 4.90 Hz, ArH *meta* to PyrN), 9.24 ppm (2H, d, J = 6.18 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{23}H_{18}BrN_3O_2S$: C, 57.51; H, 3.78; N, 8.75. Found: C, 57.25; H, 3.58; N, 8.76.

1-{2-[(Thiocarbonyl)amino]ethyl}-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Bromide (Stain **3-I**, Table 2).

A solution of 3.0 g (12.0 mmoles) of Dye **I**, and 3.30 g (20.0 mmoles) of 2-bromoethyl isothiocyanate (Linker **3**) in 50 ml of dry toluene was boiled under reflux for two days during which time a yellow precipitate developed. The reaction mixture was cooled to room temperature and vacuum filtered and the filtrate was placed back into the reaction vessel along with an additional 1.65 g (10.0 mmoles) of Linker **3** and boiled under reflux for an additional 2 days. The precipitate was washed with 80 ml of ethyl acetate followed by 40 ml of *t*-butyl methyl ether, and dried, resulting in 1.82 g of solid. After an additional two days, the mixture was filtered, and the filtrate and precipitate were treated as before. After vacuum drying 1.13 g of solid were recovered. An additional 3 days of reflux of the filtrate yielded 0.99 g of product. All three fractions melted at 236-239°. The fractions were combined, washed in 150 ml of boiling ethyl acetate, filtered while still hot, and washed with an additional 200 ml of hot ethyl acetate. After drying (70°/overnight), 3.91 g (78%) of solid was recovered, mp 238-240°. An analytical sample was prepared from the product of a previous run by washing the solid with hot ethyl acetate, mp 229-231° dec; ir: ν 2108 (NCS), 1643 (C=N), 1264 (C-O-C); pmr (250 MHz, dimethyl sulfoxide-d_6): δ 3.86 (3H, s, OCH_3), 4.44 (2H, t, J = 5.23 Hz, CH_2NCS), 4.98 (2H, t, J = 5.35 Hz, CH_2CH_2NCS), 7.14 (2H, d, J = 8.75 Hz, ArH *ortho* to OCH_3), 7.98 (2H, d, J = 8.70 Hz, ArH *meta* to OCH_3), 8.14 (1H, s, oxazole H), 8.76 (2H, d, J = 6.53 Hz, ArH *meta* to PyrN), 9.25 ppm (2H, d, J = 6.48 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{18}H_{16}BrN_3O_2S$: C, 51.66; H, 3.85; N, 10.08. Found: C, 51.85; H, 3.84; N, 10.00.

1-[2-{4-[(Thiocarbonyl)amino]phenoxy}ethyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium 4-Toluenesulfonate (Stain **5-I**, Figure 12).

A solution of 0.72 g (2.85 mmoles) of Dye **I** and 1.00 g (2.85 mmoles) of 2-(4-isothiocyanophenoxy)ethyl tosylate Linker **5** in 20 ml of dry toluene was boiled under reflux overnight during which time a yellow precipitate developed. It was cooled to room temperature, filtered, washed with toluene to yield 0.55 g (32%) of a yellow solid, mp 228-230° dec. An analytical sample was prepared by first washing the solid in hot *t*-butyl methyl ether, followed by recrystallization from acetonitrile (filtered hot), mp 230-232.5° dec; ir: ν 2126 (NCS), 1645 (C=N), 1266 (C-O-C), 1206 (sulfonate); pmr (250 MHz, deuteriochloroform/dimethyl sulfoxide-d_6): δ 2.33 (3H, s, $ArCH_3$), 3.89 (3H, s, OCH_3), 4.55 (2H, m, OCH_2), 5.29 (2H, m, $PyrNCH_2$), 6.82 (2H, d, J = 8.95 Hz, ArH *ortho* to OCH_2), 7.03 (2H, d, J = 9.00 Hz, ArH *ortho* to OCH_3), 7.12 (4H, m, ArH *ortho* to CH_3 and NCS), 7.64 (1H, s, oxazole H), 7.72 (2H, d, J = 8.80 Hz, ArH *meta* to OCH_3), 7.78 (2H, d, J = 8.10 Hz, ArH *ortho* to SO_3), 8.46 (2H, d, J = 4.90 Hz, ArH *meta* to PyrN), 9.42 ppm (2H, d, J = 3.90 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{31}H_{27}N_3O_6S_2$: C, 61.88; H, 4.52; N, 6.98. Found: C, 61.83; H, 4.50; N, 6.95.

1-[3-{4-[(Thiocarbonyl)amino]phenyl}propyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Bromide (Stain **6-I**).

Dye **I**, 2.0 g (7.94 mmoles) and 2.03 g (7.94 mmoles) of 4-(3-bromopropyl)phenyl isothiocyanate (Linker **6**) in 35 ml of dry toluene were reacted using the procedure described for Stain **1-I**, except with different reaction times. After boiling under reflux overnight, 1.59 g (39%) of bright yellow solid was recovered, mp 234-236° dec. An additional 0.36 g (9%) of solid was recovered after another overnight reflux, mp 241.5-243.5° dec, bringing the total yield to 1.95 g (48%). An analytical sample was prepared from a later run by recrystallizing twice from dry acetonitrile, mp 248-249° dec; ir: ν 2112 (NCS), 1643 (C=N), 1266 (C-O-C); pmr (250 MHz, dimethyl sulfoxide-d_6): δ 2.28 (2H, m, $CH_2CH_2CH_2$), 2.70 (2H, t, J = 8.13 Hz, $ArCH_2$), 3.86 (3H, s, OCH_3), 4.67 (2H, t, J = 7.05 Hz, $PyrNCH_2$), 7.05 (2H, d, J = 8.60 Hz, ArH *ortho* to OCH_3), 7.31 (2H, d, J = 8.35 Hz, ArH *ortho* to NCS), 7.38 (2H, d, J = 8.35 Hz, ArH *meta* to NCS), 7.97 (2H, d, J = 8.60 Hz, ArH *meta* to OCH_3), 8.12 (1H, s, oxazole H), 8.64 (2H, d, J = 6.38 Hz, ArH *meta* to PyrN), 9.17 ppm (2H, d, J = 6.43 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{25}H_{22}BrN_3O_2S$: C, 59.04; H, 4.36; N, 8.30. Found: C, 59.09; H, 4.47; N, 8.26.

1-[3-{1-(2,5-Dioxopyrrolidinyl)oxycarbonyl}phenylmethyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Bromide Monohydrate (Stain **7-I**).

A solution of 1.50 g (5.95 mmoles) of Dye **I** and 3.72 g (11.90 mmoles) of Linker **7** in 60 ml of ethyl acetate was stirred at room temperature for 11 days during which time a yellow precipitate formed. Ethyl acetate (50 ml) was added to the mixture, which was stirred for 10 minutes, filtered, washed with 100 ml of ethyl acetate, and dried (1.5 hours/70°), resulting in 2.03 g

(60%) of yellow solid, mp 243-244.5° dec. An analytical sample was prepared by recrystallizing a portion of the sample from acetonitrile at -20°, then recrystallization from dimethylformamide. The solid was collected by vacuum filtration, washed with ethyl acetate, and dried (80°/6 hours), mp 240° dec; ir: ν 1768, 1736 (C=O), 1640 (C=N), 1256 (C-O-C); pmr (250 MHz, (deuteriochloroform/dimethyl sulfoxide-d_6): δ 2.94 (4H, s, succinimidyl H), 3.89 (3H, s, OCH_3), 6.36 (2H, s, CH_2), 7.02 (2H, d, J = 8.75 Hz, ArH *ortho* to OCH_3), 7.64 (1H, m, ArH *meta* to carbonyl), 7.66 (1H, s, oxazole H), 7.74 (2H, d, J = 8.70 Hz, ArH *meta* to OCH_3), 8.17 (1H, d, J = 7.75 Hz, ArH *para* to carbonyl), 8.33 (1H, d, J = 7.93 Hz, ArH *para* to CH_2), 8.41 (1H, s, ArH *ortho* to CH_2 and carbonyl), 8.48 (2H, d, J = 6.55 Hz, ArH *meta* to PyrN), 9.76 ppm (2H, d, J = 6.58 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{27}H_{22}BrN_3O_6 \cdot H_2O$: C, 55.68; H, 4.15; N, 7.21. Found: C, 55.57, 55.51; H, 4.03, 3.79; N, 7.27, 7.21.

1-[2-(2,5-Dioxo-1*H*-pyrrol-1-yl)ethyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Methanesulfonate Hemihydrate (Stain **8-I**, Figure 14).

A solution of 1.25 g (3.97 mmoles) of Dye **I** and 1.50 g (6.78 mmoles) of Linker **8** in 25 ml of dry toluene was boiled under reflux for three days, during which time an orange preciptate formed. The mixture was filtered and dried, resulting in 1.55 g (66% crude) of solid. Approximately 1 g of the solid was recrystallized from 15 ml of dimethylformamide, filtered, washed with 15 ml of acetonitrile, and dried in an Abderhalden pistol (4 hours/100°/mechanical pump), resulting in 0.24 g of amorphous orange solid, mp 254-257° dec. with decomposition beginning at 250°; ir: ν 1722 (C=O), 1641 (C=N), 1251 (C-O-C), 1190 (SO_3); pmr (60 MHz, dimethyl sulfoxide-d_6, 8%): δ 2.37 (3H, s, CH_3SO_3), 3.80 (3H, s, OCH_3), 4.07 (2H, m, $PyrNCH_2CH_2$), 4.86 (2H, m, $PyrNCH_2$), 7.12 (2H, s, maleimidyl H), 7.21 (2H, d, J = 10 Hz, ArH *ortho* to OCH_3), 8.07 (2H, d, J = 9 Hz, ArH *meta* to OCH_3), 8.18 (1H, s, oxazole H), 8.75 (2H, d, J = 7 Hz, ArH *meta* to PyrN), 9.42 ppm (2H, d, J = 6 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{22}H_{21}N_3O_7S \cdot 1/2\ H_2O$: C, 54.99; H, 4.62; N, 8.75. Found: C, 55.04, 55.09; H, 4.60, 4.55; N, 9.07, 9.08.

1-[2-(2,5-Dioxo-1*H*-pyrrol-1-yl)ethyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Trifluoromethanesulfonate (Stain **9-I**).

A solution of 1.55 g (6.15 mmoles) of Dye **I**, 1.71 g (6.26 mmoles) of Linker **9** in 50 ml of diethyl ether was stirred overnight at room temperature. The precipitate that formed was filtered, washed with 50 ml of diethyl ether followed by 30 ml of ethyl acetate, and dried (60°/6 hours) to give 3.12 g (97%) of orange solid, mp 207-211°. An analytical sample was prepared by recrystallizing 0.50 g from 10 ml of acetonitrile. The solution was filtered hot, washed with ethyl acetate and dried (3 hours/90°) to give 0.30 g of red solid, mp 213-215.5°; ir: ν 1709 (C=O), 1641 (C=N), 1276 (C-O-C), 1249 (SO_3); pmr (60 MHz, dimethyl sulfoxide-d_6, 10%): δ 3.92 (3H, s, OCH_3), 4.14 (2H, m, $PyrNCH_2CH_2$), 4.88 (2H, m, $PyrNCH_2$), 7.15 (2H, s, maleimidyl H), 7.24 (2H, d, J = 8 Hz, ArH *ortho* to OCH_3), 8.08 (2H, d, J = 8 Hz, ArH *meta* to OCH_3), 8.18 (1H, s, oxazole H), 8.75 (2H, d, J = 5 Hz, ArH *meta* to PyrN), 9.37 ppm (2H, d, J = 5 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{22}H_{18}F_3N_3O_7S$: C, 50.29; H, 3.45; N, 8.00. Found: C, 50.21; H, 3.40; N, 8.05.

1-(Oxiranylmethyl)-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Trifluoromethanesulfonate (Stain **10-I**, Figure 15).

A solution of 2.10 g (8.33 mmoles) of Dye **I** and 1.72 g (8.33 mmoles) of Linker **10** in 25 ml of dry diethyl ether was stirred overnight at room temperature. The yellow solid that formed was collected by vacuum filtration, washed with diethyl ether, and dried (75°/6 hours). Isolated 3.78 g (99%) of solid, mp 145.5-148.5°; ir: ν 1643 (C=N), 1256 (C-O-C), 1227, 1181 (SO_3); pmr (60 MHz, dimethyl sulfoxide-d_6, 12%): δ 2.78 (1H, m, H_a or H_b), 3.02 (1H, m, H_a or H_b), 3.68 (1H, m, H_c), 3.90 (3H, s, H_f), 4.65 (1H, dd, H_d or H_e), 5.20 (1H, dd, H_d or H_e), 7.12 (2H, d, J = 9 Hz, H_g), 7.97 (2H, d, J = 9Hz, H_h), 8.12 (1H, s, H_i), 8.70 (2H, d, J = 7 Hz, H_j), 9.17 ppm (2H, d, J = 7 Hz, H_k).

Anal. Calcd. for $C_{19}H_{17}F_3N_2O_6S$: C, 49.78; H, 3.74; N, 6.11. Found: C, 49.83; H, 3.74; N, 6.10.

1-{3-[(Thiocarbonyl)amino]phenylmethyl}-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Bromide (Stain **1-II**).

A solution of 2.00 g (7.19 mmoles) of Dye **II** and 2.00 g (8.77 mmoles) of Linker **1** in 50 ml of dry toluene was boiled under reflux for 3 days, during which time a yellow precipitate formed. The mixture was cooled, filtered, and the solid washed with 15 ml of toluene and dried (90°/16 hours), resulting in 3.65 g (100%) of yellow solid, mp 244-249° dec. An analytical sample was prepared by recrystallizing 0.50 g from 110 ml of acetonitrile. The solution was filtered hot, concentrated by boiling to 60 ml, cooled, filtered, and the solid washed with a few ml of acetonitrile followed by 15-20 ml of ethyl acetate. After drying (90°/2 hours), 0.37 g of yellow plates were recovered, mp 249-252° dec; ir: ν 2120 (NCS), 1640 (C=N), 1250 (C-O-C); pmr (250 MHz, deuteriochloroform): δ 2.06 (2H, m, $ArCH_2CH_2$), 2.86 (2H, t, J = 5.98 Hz, $ArCH_2$), 4.27 (2H, t, J = 4.45 Hz, OCH_2), 6.36 (2H, s, $PyrNCH_2$), 6.87 (1H, d, J = 9.15 Hz, ArH *ortho* to OCH_2), 7.18 (1H, d, J = 7.38 Hz, ArH *para* to $PyrNCH_2$), 7.37 (1H, m, ArH *meta* to NCS), 7.54 (1H, s, ArH *ortho* to $PyrNCH_2$ and NCS), 7.73 (1H, d, J = 7.65 Hz, ArH *para* to NCS), 7.80 (2H, m, ArH *meta* to OCH_2), 8.13 (2H, d, J = 6.88 Hz, ArH *meta* to PyrN), 8.13 (1H, s, oxazole H), 9.57 ppm (2H, d, J = 7.08 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{25}H_{20}BrN_3O_2S$: C, 59.29; H, 3.98; N, 8.30. Found: C, 59.24; H, 3.94; N, 8.32.

1-{2-[(Thiocarbonyl)amino]ethyl}-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Bromide (Stain **3-II**).

The procedure was the same as that used for Stain **1-II**, using 2.00 g (7.19 mmoles) of Dye **II**, 3.00 g (18.1 mmoles) of Linker **3** in 50 ml of dry toluene. After 3 days of reflux recovered 2.48 g (78%) of orange solid, mp 241-245° dec, softening at 237°. An analytical sample was prepared by recrystallizing 0.50 g from 300 ml of acetonitrile. The solution was filtered hot and concentrated by boiling until a precipitate started to form. The cooled

solution was filtered and washed with a few ml of acetonitrile followed by 15-20 ml of ethyl acetate. After drying (90°/2 hours), 0.36 g of orange needles were recovered, mp 247-249° dec; ir: ν 2115 (NCS), 1636 (C=N), 1267 (C-O-C); pmr (60 MHz, dimethyl sulfoxide-d_6, 10%): δ 2.02 (2H, m, $ArCH_2CH_2$), 2.89 (2H, t, J = 5 Hz, $ArCH_2$), 4.36 (4H, m, OCH_2 and CH_2NCS), 4.99 (2H, m, $PyrNCH_2$), 6.95 (1H, d, J = 9 Hz, ArH *ortho* to OCH_2), 8.00 (2H, m, ArH *meta* to OCH_2), 8.63 (2H, d, J = 7 Hz, ArH *meta* to PyrN), 8.71 (1H, s, oxazole H), 9.28 ppm (2H, d, J = 6 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{20}H_{18}BrN_3O_2S$: C, 54.06; H, 4.08; N, 9.46. Found: C, 54.04; H, 4.10; N, 9.46.

1-{3-[1-(2,5-Dioxopyrrolidinyl)oxycarbonyl]phenylmethyl}-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Bromide (Stain **7-II**, Figure 13).

The procedure was the same as that used for Stain **7-I**, using 1.50 g (5.39 mmoles) of Dye **II** and 3.37 g (10.8 mmoles) of Linker **7** in 50 ml of ethyl acetate. After stirring 10 days, obtained 2.40 g (75%) of solid, mp 200-230° softening 195°. An analytical sample was prepared by recrystallizing 1.02 g from 400 ml of acetonitrile. The solution was filtered hot, concentrated to 100 ml by boiling, cooled, filtered, and washed with a few ml of acetonitrile followed by diethyl ether to give 0.71 g of solid, mp 265-266.5° dec, softening at 264°; ir: ν 1774, 1736 (C=O), 1636 (C=N), 1271 (C-O-C); pmr Bruker (250 MHz, (deuteriochloroform/dimethyl sulfoxide-d_6): δ 2.07 (2H, m, $ArCH_2CH_2$), 2.89 (2H, t, J = 6.20 Hz, $ArCH_2$), 2.95 (4H, s, succinimidyl H), 4.29 (2H, t, J = 5.10 Hz, OCH_2), 6.07 (2H, s, $PyrNCH_2$), 6.90 (1H, d, J = 9.18 Hz, ArH *ortho* to OCH_2), 7.66 (1H, m, ArH *meta* to carbonyl), 7.88 (2H, m, ArH *meta* to OCH_2), 8.09 (1H, d, J = 7.55 Hz, ArH *para* to carbonyl), 8.19 (1H, d, J = 7.58, ArH *para* to $PyrNCH_2$), 8.30 (2H, d, J = 6.68 Hz, ArH *meta* to PyrN), 8.39 (1H, s, oxazole H), 8.41 (1H, s, ArH *ortho* to $PyrNCH_2$ and carbonyl), 9.40 ppm (2H, d, J = 6.68 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{29}H_{24}BrN_3O_6$: C, 58.99; H, 4.10; N, 7.12. Found: C, 58.41; H, 4.09; N, 7.23.

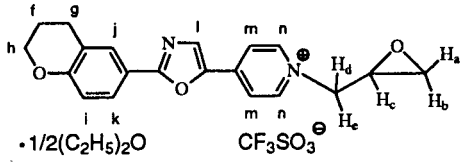
1-[2-(2,5-Dioxo-1*H*-pyrrol-1-yl)ethyl]-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Trifluoromethanesulfonate (Stain **9-II**).

The procedure was the same as that used for Stain **9-I**, using 1.50 g (5.43 mmoles) of Dye **II** and 1.49 g (5.45 mmoles) of Linker **9** in 50 ml of dry diethyl ether. After stirring overnight, 2.88 g (97%) of bright yellow solid was recovered, mp 233-240°. An analytical sample was prepared by recrystallizing 2.0 g of solid from 20 ml of acetonitrile. The solution was filtered hot, cooled, filtered, washed with 5-10 ml of cold acetonitrile, and dried (4 hours/90°) to give 0.86 g of yellow solid, mp 251-253°; ir: ν 1714 (C=O), 1641 (C=N), 1264, 1153 (SO_3); pmr (250 MHz, (deuteriochloroform/dimethyl sulfoxide-d_6): δ 2.08 (2H, m, $ArCH_2CH_2$), 2.89 (2H, m, $ArCH_2$), 4.15 (2H, m, $PyrNCH_2CH_2$), 4.29 (2H, m, OCH_2), 4.86 (2H, m, $PyrNCH_2$), 6.74 (2H, s, maleimidyl H), 6.91 (1H, d, J = 9.23 Hz, ArH *ortho* OCH_2), 7.88 (2H, m, ArH *meta* to OCH_2), 8.13 (2H, d, J = 6.45 Hz, ArH *meta* to PyrN), 8.19 (1H, s, oxazole H), 9.00 ppm (2H, d, J = 6.43 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{24}H_{20}F_3N_3O_7S$: C, 52.27; H, 3.66; N, 7.62. Found: C, 51.87; H, 3.63; N, 7.56.

1-(Oxiranylmethyl)-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Trifluoromethanesulfonate Hemietherate (Stain **10-II**, Figure 15).

The procedure was the same as that used for Stain **10-I**, using 2.10 g (7.55 mmoles) of Dye **II** and 1.56 g (7.55 mmoles) of Linker **10** in 25 ml of dry diethyl ether. After filtration and drying, 3.91 g of bright yellow solid was isolated, mp 153-156°. Drying at 100° for several more hours resulted in 3.78 g (96%), mp 152.5-155°; ir: ν 1638 (C=N), 1263 (SO_3); pmr (250 MHz, deuteriochloroform): δ 1.21 (3H, t, J = 6.85 Hz, OCH_2CH_3), 2.06 (2H, m, H_f), 2.76 (1H, dd, H_a or H_b), 2.87 (2H, t, J = 6.25 Hz, H_g), 3.03 (1H, dd, H_a or H_b), 3.48 (2H, m, J = 7.03 Hz, OCH_2CH_3), 3.57 (1H, m, H_c), 4.28 (2H, t, J = 5.08 Hz, H_h), 4.40 (1H, dd, H_d or H_e), 5.30 (1H, dd, H_d or H_e), 6.89 (1H, d, J = 9.23 Hz, H_i), 7.85 (2H, m, H_j and H_k), 8.08 (1H, s, H_l), 8.09 (2H, d, J = 7.28 Hz, H_m), 8.88 ppm (2H, d, J = 6.88 Hz, H_n).



Anal. Calcd. for $C_{21}H_{19}F_3N_2O_6S$•1/2 $(C_2H_5)_2O$: C, 52.97; H, 4.64; N, 5.37. Found: C, 52.65; H, 4.52; N, 5.35.

1-[3-{3-Propyl[(thiocarbonyl)diimino]}phenylmethyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Bromide (**1-I-Pr**).

To a small covered beaker equipped with magnetic stirring, were added 5 ml of acetone and 0.35 g (0.73 mmole) of Stain **1-I** and an excess (~0.5 ml) of 1-propylamine. There was a change in the appearance of the mixture as soon as the 1-propylamine was added. The mixture was stirred for 3 hours, filtered, and washed with acetone. After drying 0.370 g (95%) of an orange solid was obtained, mp 217-220° dec. A portion was recrystallized from 1-butanol (filtered hot) and washed with cyclohexane to yield fine orange needles to provide an analytical sample, mp 218.5-220.5° dec; ir: ν 3246 (NH), 1637 (C=N), 1264 (C-O-C); pmr (80 MHz, Unisol™): δ 0.97 (3H, t, J = 7.33 Hz, CH_2CH_3), 1.60 (2H, m, CH_2CH_3), 3.48 (2H, m, $NHCH_2$), 3.89 (3H, s, OCH_3), 5.97 (2H, s, $PyrNCH_2$), 7.03 (2H, d, J = 8.74 Hz, ArH *ortho* to OCH_3), 7.26 (2H, m, ArH *meta* and *para* to $PyrNCH_2$), 7.34 (1H, s, ArH *ortho* to NH and $PyrNCH_2$), 7.49 (1H, m, ArH *para* to NH), 7.73 (1H, s, oxazole H), 7.78 (2H, d, J = 8.63 Hz, ArH *meta* to OCH_3), 8.06 (1H, s, $NHCH_2$), 8.50 (2H, d, J = 6.42 Hz, ArH *meta* to PyrN), 9.39 (2H, d, J = 6.65 Hz, ArH *ortho* to PyrN), 9.76 ppm (1H, s, ArNH).

Anal. Calcd. for $C_{26}H_{27}BrN_4O_2S$: C, 57.89; H, 5.04; N, 10.39. Found: C, 57.88; H, 5.07; N, 10.20.

The same reaction was run with acetonitrile as solvent, yield was 86%, mp 219-222° dec (crude).

1-[4-{3-Propyl[(thiocarbonyl)diimino]}phenylmethyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Bromide (**2-I-Pr**).

The procedure was the same as that used for **1-I-Pr**, using 0.485 (1.01 mmoles) of Stain **2-I** and ~0.5 ml (excess) 1-propylamine in 4 ml of acetone. The mixture was stirred three hours, filtered, and washed with *t*-butyl methyl ether. After drying, 0.536 g (98%) of orange solid was isolated, mp 197-200° dec. A portion was recrystallized from 1-butanol (filtered hot) and

washed with 1-butanol followed by *t*-butyl methyl ether, to provide an analytical sample of fine needles, mp 192-194° dec; ir: ν 3256 (NH), 1636 (C=N), 1266 (C-O-C); pmr (250 MHz, dimethyl sulfoxide-d_6): δ 0.88 (3H, t, J = 7.33 Hz, CH_2CH_3), 1.52 (2H, m, CH_2CH_3), ~3.39 ($NHCH_2$, obscured by deuterated solvent peak), 3.85 (3H, s, OCH_3), 5.84 (2H, s, $PyrNCH_2$), 7.14 (2H, d, J = 8.80 Hz, ArH *ortho* to OCH_3), 7.51 (2H, d, J = 8.40 Hz, ArH *ortho* to NH), 7.57 (2H, d, J = 8.32 Hz, ArH *meta* to NH), 7.95 (3H, d, J = 8.63 Hz, ArH *meta* to OCH_3 and $NHCH_2$), 8.11 (1H, s, oxazole H), 8.66 (2H, d, J = 6.58 Hz, ArH *meta* to PyrN), 9.29 (2H, d, J = 6.35 Hz, ArH *ortho* to PyrN), 9.69 ppm (1H, s, br, ArNH).

Anal. Calcd. for $C_{26}H_{27}BrN_4O_2S$: C, 57.89; H, 5.04; N, 10.39. Found: C, 57.97; H, 5.00; N, 10.08.

1-[2-{3-Propyl[(thiocarbonyl)diimino]}ethyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Bromide (**3-I-Pr**, Table 2).

The procedure was the same as that for Derivative **1-I-Pr**, using 0.35 g (0.84 mmole) of Stain **3-I** and ~ 0.5 ml (excess) of 1-propylamine in 5 ml of acetone. There was an immediate change in the appearance of the mixture upon the addition of the 1-propylamine. The reaction was stirred 2 hours, filtered, washed with acetone, and dried to obtain 0.370 g (93%) of an orange solid, mp 185-187° dec. A portion was recrystallized from 1-butanol and washed with cyclohexane, mp 197-198° dec, to provide an analytical sample; ir: ν 3220 (NH), 1642 (C=N), 1262 (C-O-C); pmr (80 MHz, Unisol™): δ 0.91 (3H, t, J = 6.86 Hz, $NCH_2CH_2CH_3$), 1.49 (2H, m, $NCH_2CH_2CH_3$), 3.39 (2H, m, $NCH_2CH_2CH_3$), 3.89 (3H, s, OCH_3), 4.28 (2H, m, $PyrNCH_2CH_2$), 4.95 (2H, m, $PyrNCH_2$), 7.04 (2H, d, J = 8.74 Hz, ArH *ortho* to OCH_3), 7.60 (1H, s, br, $NHCH_2CH_2CH_3$), 7.73 (1H, s, oxazole H), 7.78 (2H, d, J = 8.99 Hz, ArH *meta* to OCH_3), 8.04 (1H, s, br, $PyrNCH_2CH_2NH$), 8.46 (2H, d, J = 6.54 Hz, ArH *meta* to PyrN), 9.21 ppm (2H, d, J = 6.88 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{21}H_{25}BrN_4O_2S$: C, 52.83; H, 5.28; N, 11.74. Found: C, 52.54; H, 5.24; N, 11.41.

1-{2-[4-{3-Propyl[(thiocarbonyl)diimino]}phenoxy]ethyl}-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium 4-Toluenesulfonate Hemihydrate (**5-I-Pr**, Figure 12).

The procedure was the same as that for **1-I-Pr**, using 0.260 g (0.432 mmole) of Stain **5-I** and ~0.5 ml (excess) of 1-propylamine in 3 ml of acetone. The mixture was stirred at room temperature for 7 hours then treated with 5 ml of *t*-butyl methyl ether. The yellow solid was filtered, washed with 10 ml of *t*-butyl methyl ether, and dried (80°/2 hours) to yield 0.266 g (93%). Recrystallization from alcohols resulted in oils. A portion was recrystallized from acetone. Initially, no solid formed, but when the solution was reheated, a precipitate began to develop. The solid was collected by vacuum filtration, mp 175.5-177° dec; ir: ν 3250 (NH), 1638 (C=N), 1268 (C-O-C), 1233, 1181 (SO_3); pmr (250 MHz, deuteriochloroform): δ 0.86 (3H, t, J = 7.35 Hz, CH_2CH_3), 1.56 (2H, m, CH_2CH_3), 2.30 (3H, s, $ArCH_3$), 3.47 (2H, m, $NHCH_2$), 3.88 (3H, s, OCH_3), 4.30 (2H, m, OCH_2), 5.04 (2H, m, $PyrNCH_2$), 6.62 (2H, d, J = 8.23 Hz, ArH *ortho* to OCH_2), 7.00 (2H, d, J = 8.88 Hz, ArH *ortho* to OCH_3), 7.14 (3H, d, J = 8.08 Hz, ArH *ortho* to OCH_2 and $NHCH_2$), 7.23 (2H, d, J = 9.05 Hz, ArH *ortho* to CH_3), 7.57 (1H, s, oxazole H), 7.71 (2H, d, J = 8.78 Hz, ArH *meta* to OCH_3), 7.77 (2H, d, J = 8.13 Hz, ArH *ortho* to SO_3), 8.31 (2H, d, J = 6.28 Hz, ArH *meta* to PyrN), 8.75 (1H, s, br, ArNH), 9.18 ppm (2H, d, J = 6.98 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{34}H_{36}N_4O_6S_2 \cdot 1/2H_2O$: C, 60.97; H, 5.57; N, 8.36. Found: C, 60.59, 60.75; H, 5.37, 5.37; N, 8.32, 8.29.

1-{3-[4-{3-Propyl[(thiocarbonyl)diimino]}phenyl]propyl}-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Bromide (**6-I-Pr**).

The procedure was the same as that for **1-I-Pr**, using 0.250 g (0.689 mmole) of Stain **6-I** and ~0.5 ml (excess) of 1-propylamine in 2.5 ml of acetonitrile. The mixture was stirred for 2 hours at room temperature, filtered, washed with 5 ml of *t*-butyl methyl ether and dried (50°/overnight) to give 0.268 g (96%) of yellow solid, mp 210-213° dec. A portion was recrystallized from acetone, filtered hot, to provide an analytical sample of small spherical pellets, mp 213-215° dec; ir: ν 3249 (NH), 1639 (C=N), 1262 (C-O-C); pmr (80 MHz, Unisol™): δ 0.95 (3H, t, J = 7.03 Hz, CH_2CH_3), 1.57 (2H, m, CH_2CH_3), 2.40 (2H, m, $PyrNCH_2CH_2$), 2.78 (2H, m, $ArCH_2$), 3.47 (2H, m, $NHCH_2$), 3.89 (3H, s, OCH_3), 4.80 (2H, m, $PyrNCH_2$), 7.04 (2H, d, J = 8.17 Hz, ArH *ortho* to OCH_3), 7.14 (2H, d, J = 7.43 Hz, ArH *ortho* to NH), 7.44 (3H, d, J = 7.62 Hz, ArH *meta* to NH and $NHCH_2$), 7.74 (1H, s, oxazole H), 7.79 (2H, d, J = 9.18 Hz, ArH *meta* to OCH_3), 8.49 (2H, d, J = 6.40 Hz, ArH *meta* to PyrN), 9.34 ppm (3H, m, ArH *ortho* to PyrN and ArNH).

Anal. Calcd. for $C_{28}H_{31}BrN_4O_2S$: C, 59.26; H, 5.51; N, 9.87. Found: C, 59.38; H, 5.54; N, 9.55.

1-[3-(*N*-Propylcarbamoyl)phenylmethyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Bromide (**7-I-Pr**).

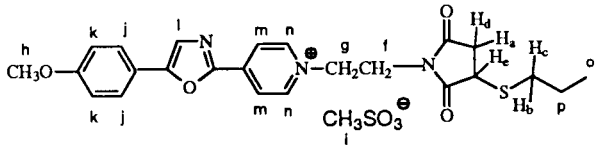
The procedure was the same as that for **1-I-Pr**, using 0.30 g (0.53 mmole) of Stain **7-I** and ~0.3 ml (excess) of 1-propylamine in 3 ml of acetone. The mixture was stirred for 2 hours at room temperature, filtered, washed with 10 ml of acetone, and dried to obtain 0.26 g (96%) of yellow solid, mp 175-195°, which was recrystallized from 5 ml of 2-propanol; yellow plates formed, which were collected, washed with a few ml of 2-propanol followed by 25 ml of *t*-butyl methyl ether, and dried (60°/overnight), resulting in 0.18 g (67%), mp 224.5-227° dec, softening at 222°. An analytical sample was prepared by another recrystallization from 2-propanol, mp 225.5-228° dec; ir: ν 3314 (NH), 1640 (C=O, C=N), 1264 (C-O-C); pmr (60 MHz, dimethyl sulfoxide-d_6): δ 0.89 (3H, t, J = 7 Hz, CH_2CH_3), 1.50 (2H, m, CH_2CH_3), 3.17 (2H, m, $NHCH_2$), 3.85 (3H, s, OCH_3), 6.06 (2H, s, $PyrNCH_2$), 7.09 (2H, d, J = 8 Hz, ArH *ortho* to OCH_3), 8.06 (7H, m, ArH *meta* and *para* to carbonyl, *para* to $PyrNCH_2$, *meta* to OCH_3, NH, and oxazole H), 8.67 (3H, d, J = 6 Hz, ArH *meta* to PyrN and *ortho* to $PyrNCH_2$ and carbonyl), 9.46 ppm (2H, d, J = 6 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{26}H_{26}BrN_3O_3$: C, 61.42; H, 5.15; N, 8.26. Found: C, 60.89; H, 5.21; N, 8.09.

1-{2-[3-Propylthio-1-(2,5-dioxopyrrolidinyl)]ethyl}-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Methanesulfonate (**8-I-Pr**, Figure 14).

To a small covered beaker, equipped with magnetic stirring, were added 0.61 g (1.29 mmoles) of Stain **8-I**, ~0.5 ml (excess) of 1-propanethiol, and 5 ml of 1:1:: acetonitrile:water. The mixture was stirred at room temperature for 5 days, at which time it was poured into 400 ml of diethyl ether, causing the formation of a precipitate. After stirring for 0.5 hour, the liquid layer was decanted from the solid. The solid was dissolved in

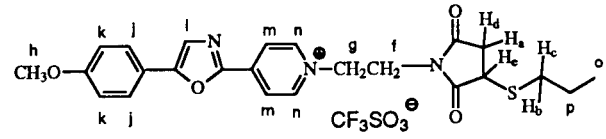
acetonitrile/absolute ethanol and concentrated with rotary evaporation. An additional 30 ml of absolute ethanol was added and the sample concentrated once more, resulting in 0.68 g (96%) of solid. The solid was recrystallized from 15 ml of 2-propanol. An oil developed as the solution cooled; the solution was decanted from the oil and allowed to crystallize at room temperature. Orange rosettes formed, along with some amorphous orange solid. All the solid was collected by vacuum filtration and dried (60°/16 hours) to give 0.46 g (65%) of orange solid, mp 138-145°, softening at 130°. An analytical sample was prepared by another recrystallization from 2-propanol. Upon crystallization rosettes formed on top of an orange semi-solid present at the bottom of the flask. The crystals were gently scraped from the semi-solid, filtered, washed with a few ml of 2-propanol, and dried (80°/3.5 hours), mp 139-145°; ir: ν 1704 (C=O), 1642 (C=N), 1264 (C-O-C), 1192 (SO_3); pmr (250 MHz, deuteriochloroform): δ 0.90 (3H, t, J = 7.35 Hz, H_o), 1.55 (2H, m, H_p), 2.42 (1H, dd, H_a), 2.57 (1H, m, H_b or H_c), 2.69 (1H, m, H_b or H_c), 2.80 (3H, s, H_i), 3.45 (1H, dd, H_d), 3.86 (3H, s, H_h), 3.98 (1H, dd, H_e), 4.21 (2H, m, H_f), 5.11 (2H, m, H_g), 6.97 (2H, d, J = 8.83 Hz, H_k), 7.51 (1H, s, H_l), 7.64 (2H, d, J = 8.70 Hz, H_j), 8.42 (2H, d, J = 6.03 Hz, H_m), 9.46 ppm (2H, d, J = 6.10 Hz, H_n). (hplc) Analysis using an Alltech 4.6 x 250 mm, 5 micron, C18 Econosphere column, with methanol/dimethylformamide/trifluoroacetic acid (97.5:2.5:0.1) as the mobile phase, detection by absorbance at 400 nm showed the sample to be 98% pure, R_t (flow rate = 2 ml/minute) = 3.74 minutes.



Anal. Calcd. for $C_{25}H_{29}N_3O_7S_2$: C, 54.83; H, 5.34; N, 7.67. Found: C, 54.42; H, 5.24; N, 7.49.

1-{2-[3-Propylthio-1-(2,5-dioxopyrrolidinyl)]ethyl}-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Trifluoromethanesulfonate (**9-I-Pr**).

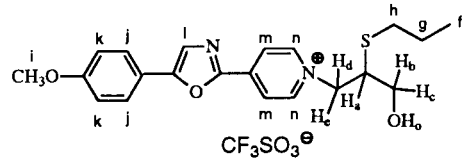
To a small covered beaker, equipped with magnetic stirring, were added 0.50 g (0.95 mmole) of Stain **9-I**, 0.5 ml (excess) of 1-propanethiol, and 10 ml of 9:1::acetonitrile:water. The mixture was stirred at room temperature for 24 hours, poured into 150 ml of diethyl ether; the suspension was stirred for 15 minutes and filtered to give 0.49 g (86%) after drying (80°/3 hours). A pmr spectrum still showed a significant amount of starting material to be present. The solid was reprocessed (0.46 g) using 30 ml of 2:1::acetonitrile:water and 1 ml of 1-propanethiol by stirring overnight at room temperature. The mixture was poured into 300 ml of diethyl ether and stirred. A granular solid developed, which was collected by vacuum filtration and dried to give 0.39 g (68%) of solid, mp 125-135°, softening at 120°. A portion was recrystallized from 2-propanol, filtered, washed with diethyl ether, dried, mp 129-132°, softening at 120°. Two different crystalline forms were present. One initially formed as an oil upon cooling and solidified as cooling continued. The other was flocculent. The combined solids were recrystallized a second time from 2-propanol to give 0.26 g (46%), mp 127-132°. An analytical sample was prepared by two more recrystallizations from 2-propanol. The solution was decanted while hot from the oil that had formed during solution and placed at -20° to crystallize. The solid was collected with vacuum filtration, washed with a few ml of 2-propanol, and dried (70°/16 hours), mp 128.5-131° softening at 126.5°; ir: ν 1708 (C=O), 1642 (C=N), 1251 (SO_3); pmr (250 MHz, deuteriochloroform): δ 0.91 (3H, t, J = 7.35 Hz, H_o), 1.56 (2H, m, H_p), 2.43 (1H, dd, H_a), 2.56 (1H, m, H_b or H_c), 2.69 (1H, m, H_b or H_c), 3.36 (1H, dd, H_d), 3.87 (3H, s, H_h), 3.90 (1H, dd, H_e), 4.19 (2H, m, H_f), 4.97 (2H, m, H_g), 6.99 (2H, d, J = 8.87 Hz, H_k), 7.55 (1H, s, H_l), 7.68 (2H, d, J = 8.78 Hz, H_j), 8.43 (2H, d, J = 6.83 Hz, H_m), 9.10 ppm (2H, d, J = 6.83 Hz, H_n).



Anal. Calcd. for $C_{25}H_{26}F_3N_3O_7S_2$: C, 49.91; H, 4.36; N, 6.98. Found: C, 49.60; H, 4.24; N, 6.92.

1-(2-Propylthio-3-hydroxypropyl)-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Trifluoromethanesulfonate Hemihydrate (**10-I-Pr**, Figure 15).

To a small covered beaker, equipped with magnetic stirring, were added 0.30 g (0.66 mmole) of Stain **10-I**, ~0.5 ml (excess) of triethylamine, ~0.5 ml (excess) of 1-propanethiol, and 3 ml of acetonitrile. The mixture was stirred for 3 days at room temperature, and poured into 150 ml of diethyl ether, causing the formation of an oil. The ether layer was decanted and the oil placed at -20°, where it quickly solidified and was dried (65°/16 hours). The solid was recrystalllized from 50 ml of water with hot filtration, resulting in the formation of yellow/orange needles, which were collected on a Büchner funnel, washed with water, and dried (80°/4 hours) to give 0.20 g (57%) of solid, mp 112.5-116°. An analytical sample was prepared by a second recrystallization from 2-propanol. The solution required seeding for crystallization to occur. The solid was filtered, washed with 2-propanol, and dried (80°/1 hour), mp 113-116°; ir: ν 3436 (OH), 1641 (C=N), 1267 (C-O-C), 1252 (SO_3); pmr (major isomer, 250 MHz, deuteriochloroform): δ 0.87 (3H, t, J = 7.28 Hz, H_f), 1.45 (2H, m, H_g), 2.0 (1H, br, H_o) 2.46 (2H, t, J = 7.28 Hz, H_h), 3.42 (1H, m, H_a), 3.47 (1H, dd, H_b or H_c), 3.87 (3H, s, H_i), 4.02 (1H, dd, H_b or H_c), 4.69 (1H, dd, H_d or H_e), 5.13 (1H, dd, H_d or H_e), 7.00 (2H, d, J = 8.90 Hz, H_k), 7.58 (1H, s, H_l), 7.70 (2H, d, J = 8.85 Hz, H_j), 8.45 (2H, d, J = 6.78 Hz, H_m), 8.99 ppm (2H, d, J = 6.98 Hz, H_n).

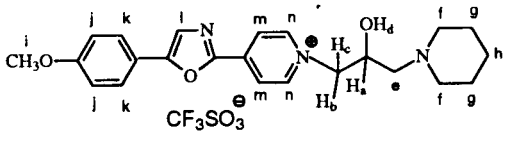


Anal. Calcd. for $C_{22}H_{25}F_3N_2O_6S_2 \cdot 1/2H_2O$: C, 48.61; H, 4.82; N, 5.15. Found: C, 48.80; H, 4.82; N, 5.17.

Attempted preparation of:

1-[2-Hydroxy-3-(azacyclohex-1-yl)propyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium Trifluoromethanesulfonate (**10-I-Pip**).

A solution of 0.097 g (1.14 mmoles) of piperidine in 10 ml of methylene chloride was added over a 7 to 8 hour period to a solution of 0.520 g (1.14 mmoles) of Stain **10-I** in 10 ml of methylene chloride. The mixture was stirred overnight and the product was precipitated from the the reaction mixture by pouring it into 400 ml of diethyl ether, resulting in the formation of both a gummy and crystalline solid. Most of the crystalline orange solid was present as either a suspension or had collected on the sides of the beaker. This was separated from the gummy material primarily present in the bottom of the beaker by vacuum filtration. The solid was extracted from a Soxhlet extractor with diethyl ether, removing a blue fluorescent impurity, most likely the free base. The solvent was than changed to methylene chloride/diethyl ether to extract the product. The product was precipitated by addition of ether, and filtered to give 0.1 g (16%) of orange solid, mp 125-133° shrinks 115°; ir: ν 3431 (OH), 1638 (C=N), 1252, 1162 (SO_3); pmr (250 MHz, deuteriochloroform): δ 1.47 (2H, m, H_h), 1.60 (4H, m, H_g), 2.53 (6H, m, H_f and H_e), 3.87 (3H, s, H_i), 4.2 (1H, s, br, H_d), 4.31 (1H, m, H_a), 4.56 (1H, dd, H_b or H_c), 4.95 (1H, dd, H_b or H_c), 6.99 (2H, d, J = 8.85 Hz, H_j), 7.56 (1H, s, H_l), 7.69 (2H, d, J = 8.78 Hz, H_k), 8.41 (2H, d, J = 6.80 Hz, Hm), 9.01 ppm (2H, d, J = 6.78 Hz, Hn).



Anal. Calcd. for $C_{24}H_{28}F_3N_3O_6S$: C, 53.03; H, 5.19; N, 7.73. Found: C, 50.90; H, 4.70; N, 7.19.

Further attempts at purification did not result in better assays.

1-[3-{3-Propyl[(thiocarbonyl)diimino]}phenylmethyl]-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Bromide (**1-II-Pr**).

The procedure was the same as that for **1-I-Pr**, using 0.50 g (0.99 mmole) of Stain **1-II** and ~0.5 ml (excess) of 1-propylamine in 9 ml of acetonitrile. The solution was stirred at room temperature for 3 hours. The solid was collected by vacuum filtration, washed with diethyl ether, and dried (70°/16 hours). Recovered 0.51 g (91%) of yellow solid, mp 180-183.5° softening at 177°. The solid was dissolved in 400 ml of hot 2-propanol, filtered hot, and concentrated to ~150 ml by boiling until a floculent precipitate began to develop. The solution was cooled to room temperature, the solid collected by vacuum filtration, washed with a few ml of 2-propanol, followed by diethyl ether, and dried (room temperature/6 hours) to give 0.36 g (64 %) of solid, mp 184.5-186.5° softening at 181°. An analytical sample was prepared by recrystallization from 20 ml of absolute ethanol. Only a film formed when the solution cooled to room temperature. The solution was decanted and cooled to -20°. Only a small amount of solid formed. The solid was filtered and dried, mp ~170-180°. A heavier solid had developed in the filtrate after filtration. This solid was collected with vacuum filtration and dried, mp 178-181°. This solid was dissolved in 200 ml of 2-propanol, concentrated to ~20 ml with boiling, and cooled to room temperature. The solid that developed upon cooling was collected with vacuum filtration, washed wtih a few ml of 2-propanol, and dried (90°/7 hours), mp 175.5-178°; ir: ν 3359 (NH), 1636 (C=N), 1265 (C-O-C); pmr (250 MHz, deuteriochloroform): δ 0.90 (3H, t, J = 7.45 Hz, CH_2CH_3), 1.59 (2H, m, CH_2CH_3), 2.03 (2H, m, $ArCH_2CH_2$), 2.83 (2H, t, $ArCH_2$), 3.46 (2H, m, $NHCH_2$), 4.24 (2H, t, J = 5.28 Hz, OCH_2), 5.86 (2H, s, $PyrNCH_2$), 6.83 (1H, d, J = 9.20 Hz, ArH *ortho* to OCH_2), 7.11 (2H, m, ArH *meta* and *para* to $PyrNCH_2$), 7.62 (1H, s, ArH *ortho* to PyrN and NH), 7.68 (1H, m, ArH *para* to NH), 7.76 (2H, m, ArH *meta* to OCH_2), 8.03 (3H, d, J = 6.75 Hz, ArH *meta* to PyrN and $NHCH_2$), 8.14 (1H, s, oxazole H), 9.08 (2H, d, J = 6.58 Hz, ArH *ortho* to PyrN), 10.01 ppm (1H, s, ArNH).

Anal. Calcd. for $C_{28}H_{29}BrN_4O_2S$: C, 59.47; H, 5.17; N, 9.91. Found: C, 59.11; H, 5.06; N, 9.78.

1-[2-{3-Propyl[(thiocarbonyl)diimino]}ethyl]-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Bromide (**3-II-Pr**).

The procedure was the same as that for **1-I-Pr**, using 0.30 g (0.68 mmole) of Stain **3-II** and ~0.5 ml (excess) of 1-propylamine in 3 ml of acetonitrile. The mixture was stirred for 3 hours at room temperature, filtered, and dried to obtain 0.33 g (97%) of orange solid, mp 198-200°. The solid was recrystallized from 15 ml of absolute ethanol, filtered hot, cooled, filtered, washed with 1-2 ml of absolute ethanol, and dried (80°/16 hours) to give 0.26 g (76%) of solid, mp 198-199°, softening at 197°; ir: ν 3278, 3214 (NH), 1635 (C=N), 1264 (C-O-C); pmr (250 MHz, deuteriochloroform): δ 0.91 (3H, t, J = 7.18 Hz, CH_2CH_3), 1.54 (2H, m, CH_2CH_3), 2.07 (2H, m, $ArCH_2CH_2$), 2.88 (2H, t, J = 6.10 Hz, $ArCH_2$), 3.41 (2H, m, $NHCH_2CH_2CH_3$), 4.29 (2H, t, J = 5.28 Hz, OCH_2), 4.36 (2H, m, $PyrNCH_2CH_2$), 4.92 (2H, s, br, $PyrNCH_2$), 6.91 (1H, d, J = 9.25 Hz, ArH *ortho* to OCH_2), 7.52 (1H, s, br, $NHCH_2CH_2CH_3$), 7.85 (2H, m, ArH *meta* to OCH_2), 8.04 (2H, d, J = 6.30 Hz, ArH *meta* to PyrN), 8.08 (1H, s, oxazole H), 8.57 (1H, s, br, $PyrNCH_2CH_2NH$), 9.10 ppm (2H, d, J = 6.55 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{23}H_{27}BrN_4O_2S$: C, 54.87; H, 5.41; N, 11.13. Found: C, 54.57; H, 5.24; N, 11.02.

1-[3-(*N*-Propylcarbamoyl)phenylmethyl]-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Bromide Hemihydrate (**7-II-Pr**, Figure 13).

The procedure was the same as that for **1-I-Pr**, using 0.50 g (0.85 mmole) of Stain **7-II** and ~0.5 ml (excess) of 1-propylamine in 5 ml of acetone. Shortly after the addition of 1-propylamine a brown oil formed. The mixture was stirred overnight at room temperature. The mixture was concentrated with rotary evaporation, dissolved in ethanol and precipitated by the addition of diethyl ether. The precipitate was collected by vacuum filtration and dried resulting in 0.33 g (73%) of solid, mp 125-140°. The solid was recrystallized with filtration when hot from 60 ml of water, cooling to 4°, to give needles, mp 102-107°; with water given off at 115-120°. The solid was next dissolved in a few ml of absolute ethanol, filtered hot, concentrated to 2 ml by boiling, and allowed to crystallize at room temperature. The solid was collected with vacuum filtration, and dried (90°/overnight), mp 162-170°, shrinks at 153°; ir: ν 3288 (NH), 1650 (C=O), 1643 (C=N), 1274 (C-O-C); pmr (250 MHz, deuteriochloroform): δ 0.89 (3H, t, J = 7.45 Hz, CH_2CH_3), 1.63

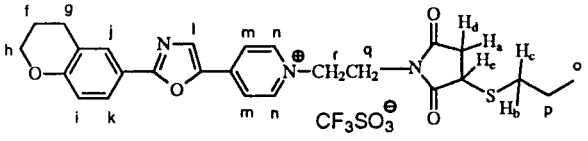
(2H, m, CH_2CH_3), 2.00 (2H, m, $ArCH_2CH_2$), 2.78 (2H, t, J = 6.10 Hz, $ArCH_2CH_2$), 3.32 (2H, m, $NHCH_2$), 4.21 (2H, t, J = 6.10 Hz, OCH_2), 5.98 (2H, s, $PyrNCH_2$), 6.79 (1H, d, J = 8.35 Hz, ArH *ortho* to OCH_2), 7.30 (1H, m, ArH *meta* to carbonyl), 7.67 (3H, m, ArH *meta* to OCH_2 and N*H*), 7.84 (1H, d, J = 7.73 Hz, ArH *para* to carbonyl), 8.07 (3H, m, ArH *meta* to PyrN and ArH *para* to $PyrNCH_2$), 8.12 (1H, s, oxazole H), 8.40 (1H, s, ArH *ortho* to $PyrNCH_2$ and carbonyl), 9.40 (2H, d, J = 6.55 Hz, ArH *ortho* to PyrN).

Anal. Calcd. for $C_{28}H_{28}BrN_3O_3 \cdot 1/2\ H_2O$: C, 61.88; H, 5.38; N, 7.73. Found: C, 61.38; H, 5.21; N, 7.62.

In another run, using acetonitrile as the solvent, 0.47 g (> 100%) of crude product was obtained, mp 145-165°.

1-{2-[3-Propylthio-1-(2,5-dioxopyrrolidinyl)]ethyl}-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Trifluoromethanesulfonate (**9-II-Pr**).

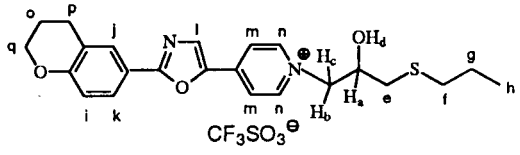
The procedure was the same as that for **8-I-Pr**, using 0.50 g (0.91 mmole) of Stain **9-II** and ~0.5 ml (excess) of 1-propanethiol in 5 ml of 1:1::acetonitrile:water. The mixture was stirred overnight at room temperature, resulting in the formation of two layers. The layers were separated, the organic layer was poured into 300 ml of diethyl ether, causing a yellow precipitate to form. The aqueous layer was washed with 5 ml of ethyl acetate. The ethyl acetate was also poured into diethyl ether. The precipitates were collected and dried (70°/16 hours). The solid was recrystallized from 45 ml of 2-propanol with filtration hot (Norit). Only a small amount of solid had formed at room temperature, so the solution was cooled to -20°, the liquid was decanted away from the solid, and the solid dried (70°/16 hours). During drying, the solid melted and then resolidified, to give 0.40 g (70%) of a yellow flocculent solid, mp ~80-95°; ir: ν 1708 (C=O), 1639 (C=N), 1265, 1154 (SO_3); pmr (250 MHz, deuteriochloroform): δ 0.91 (3H, t, J = 7.35 Hz, H_o), 1.56 (2H, m, H_p), 2.04 (2H, m, H_f), 2.45 (1H, dd, H_a), 2.58 (1H, m, H_b or H_c), 2.72 (1H, m, H_b or H_c), 2.84 (2H, m, H_g), 3.36 (1H, dd, H_e), 3.91 (1H, dd, H_d), 4.16 (2H, m, H_q), 4.25 (2H, t, J = 4.75 Hz, H_h), 4.87 (2H, m, H_r), 6.85 (1H, d, J = 9.18 Hz, H_i), 7.80 (2H, m, H_j and H_k), 8.06 (1H, s, H_l), 8.07 (2H, d, J = 5.30 Hz, H_m), 8.93 ppm (2H, d, J = 6.83 Hz, H_n). (hplc) Analysis using an Alltech 4.6 x 250 mm, 5 micron, C18 Econosphere column, with methanol/dimethylformamide/trifluoroacetic acid (97.5:2.5:0.1) as the mobile phase, detection by absorbance at 385 nm showed the sample to be 98% pure, R_t (flow rate = 2 ml/minute) = 4.39 minutes.



Anal. Calcd. for $C_{27}H_{28}F_3N_3O_7S_2$: C, 51.67; H, 4.50; N, 6.69. Found: C, 51.59; H, 4.37; N, 6.72.

1-(2-Hydroxy-3-propylthiopropyl)-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl}pyridinium Trifluoromethanesulfonate (**10-II-Pr**, Figure 15).

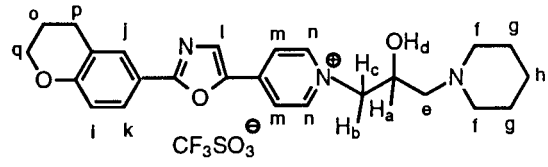
The procedure was the same as that for **10-I-Pr**, using 0.35 g (0.76 mmole) of Stain **10-II**, ~0.5 ml (excess) of triethylamine, and ~0.5 ml (excess) of 1-propanethiol in 3 ml of acetonitrile. The mixture was stirred overnight at room temperature, was poured into 150 ml of diethyl ether, causing the formation of a yellow precipitate, and cooled to -20°. The solid was collected by vacuum filtration, and dried (65°/16 hours) to give 0.31 g (76%), which was recrystallized from 2-propanol, filtered, and washed with a few ml of 2-propanol to give 0.15 g (37%), after drying (80°/4hours), mp 159-161°. An analytical sample was prepared by recrystallizing 0.15 g of solid from 4 ml of 2-propanol to obtain 0.10 g of solid, mp 160-161°; ir: ν 3417 (OH), 1639 (C=N), 1266, 1159 (SO_3); pmr (250 MHz, deuteriochloroform): δ 0.96 (3H, t, J = 7.35 Hz, H_h), 1.60 (2H, m, H_g), 1.9 (1H, s, br, H_d), 2.04 (2H, m, H_o), 2.56 (2H, t, J = 7.38 Hz, H_f), 2.73 (2H, d, J = 6.48 Hz, H_e), 2.84 (2H, t, J = 6.23 Hz, H_p), 4.20 (1H, m, H_a), 4.25 (2H, t, J = 5.00 Hz, H_q), 4.50 (1H, dd, H_b or H_c), 4.86 (1H, dd, H_b or H_c), 6.85 (1H, d, J = 9.23 Hz, H_i), 7.79 (2H, m, H_j and H_k), 8.04 (1H, s, H_l), 8.06 (2H, d, J = 6.79 Hz, H_m), 8.78 ppm (2H, d, J = 6.85 Hz, H_n).



Anal. Calcd. for $C_{24}H_{27}F_3N_2O_6S_2$: C, 51.42; H, 4.85; N, 5.00. Found: C, 51.44; H, 4.96; N, 5.01.

1-[2-Hydroxy-3-(azacyclohex-1-yl)propyl]-4-{2-[6-(3,4-dihydro-2*H*-1-benzopyranyl)]-5-oxazolyl} pyridinium Trifluoromethanesulfonate (**10-II-Pip**, Figure 17).

The procedure was the same as that for **10-I-Pr**, using 0.41 g (0.85 mmole) of Stain **10-II** and ~0.3 ml (excess) of piperidine in 3 ml of acetonitrile. The mixture was stirred at room temperature overnight. The solution was poured into 150 ml of diethyl ether, causing a precipitate to form. The mixture was stirred for an additional half hour and the solid collected with vacuum filtration. After drying (60°), 0.40 g (83%) of solid was obtained. The solid was recrystallized from 15 ml of 2-propanol, filtered hot, cooled, filtered, and washed with a few ml of 2-propanol. After drying (80°/5 hours), recovered 0.33 g (69%) of yellow solid, mp 171.5-173°, softening at 170°; ir: ν 3446 (OH), 1636 (C=N), 1259, 1159 (SO_3); pmr (250 MHz, deuteriochloroform): δ 1.43 (2H, m, H_h), 1.54 (4H, m, H_g), 2.07 (2H, m, H_o), 2.38 (6H, m, H_f and H_e), 2.85 (2H, t, J = 6.28 Hz, H_p), 4.25 (3H, m, H_a and H_q), 4.45 (1H, dd, H_b or H_c), 4.80 (1H, dd, H_b or H_c), 6.86 (1H, d, J = 9.18 Hz, H_i), 7.82 (2H, m, H_j and H_k), 8.05 (1H, s, H_l), 8.06 (2H, d, J = 6.93 Hz, H_m), 9.04 ppm (2H, d, J = 7.00 Hz, H_n), (H_d not observed).



Anal. Calcd. for $C_{26}H_{30}F_3N_3O_6S$: C, 54.82; H, 5.31; N, 7.38. Found: C, 54.91; H, 5.46; N, 7.33.

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