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# Fluorescence as a Means for Kinetic Studies. II. Preparation and Reactions of Bifunctional Fluorescent Reagents as Kinetic Probes

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**Synopsis.** 1,5-Naphthalenedisulfonyl dichloride was found to be useful as a bifunctional fluorescent probe for kinetic studies. Its reaction with butylamine in chloroform proceeds stepwise. The first reaction produces a nonfluorescent monosulfonamide and the second slow process forms a fluorescent disulfamide. Their rate constants were obtained. Another bifonunctional fluorescent probe was also examined briefly.

Bifunctional reagents have been used extensively in coupling reactions for the network formation of polymers *etc.* The coupling reactions of a bifunctional reagent, X-X, with functional groups in polymers, Y-Ⓟ, consist of two elementary steps, *viz.*, X-X+Y-Ⓟ→X-Ⓟ ($k_2^1$) and Ⓟ-X+Y-Ⓟ→Ⓟ-Ⓟ ($k_2^2$). The latter step is a polymer-polymer reaction and may differ from a corresponding polymer-small molecule reaction. However, kinetic studies on the latter step, separately from the former, have been rarely reported because of its experimental difficulties.[1,2] When appropriate bifunctional fluorescent reagents are prepared, they will become useful "kinetic probes" to analyse the coupling reactions for following reasons: (i) The coupling reaction can be traced by fluorometry in very dilute concentration region ($10^{-5}$ to $10^{-7}$ M) and (ii) if only the final reaction product is fluorescent, the reaction of the second functional group could be traced independently. Fluorescent reagents used widely in biochemistry are usually effective only in aqueous media.[3] But in our previous paper,[4] some monofunctional fluorescent reagents for amine were shown to react with substrates quantitatively by simple bimolecular reaction in organic solvents.

In this note, the reaction of some bifunctional fluorescent reagents with butylamine are investigated in chloroform as a simple model reaction and it will be shown that the reagents meet the requirments stated above and they are useful as bifunctional reagents for the coupling reaction of amino groups.

## Experimental

*Materials.* 1,5-Naphthalenedisulfonyl dichloride (NDSCl) was prepared by the reaction of disodium 1,5-naphthalenedisulfonate with phosphorus pentachloride (72% yield).[5] 7-Dimethylamino-2,4-naphthalenedisulfonyl dichloride (DNDSCl) was prepared from 7-amino-2,4-naphthalenedisulfonic acid *via* trimethylammonium formation (the method of Cope *et al.*:[6] 76% yield), and dimethylamination (a similar method to that of Hünig *et al.*:[7] 83% yield), followed by chlorination (60% yield). The orange extract by chloroform was recrystallized from diethyl ether to give red needles which melted at 124—125 °C (68% yield). The structure was ascertained by NMR and IR. NMR ($CDCl_3$): $\delta$ 3.2 (N-$C\underline{H}_3$), $\delta$ 7.6—9.2 (Ar$\underline{H}$). IR: 1170 and 1370 cm$^{-1}$ ($\underline{SO_2}Cl$), 2900 cm$^{-1}$ (N-$\underline{C}H_3$). Butylamine ($n$-$BuNH_2$) and 5-dimethylamino-1-naphthalenesulfonyl chloride (DNSCl), both from Tokyo Kasei, and chloroform of "Dotite Spectrosol" grade were used as received.

*Apparatus.* NMR and infrared spectra were obtained with a 100-MHz JEOL MH-100 and a JASCO IR-G spectrometer, respectively. A Toyo Soda GPC chromatograph (HCL-802UR; G2000H8×2) was used with THF at 40 °C. The fluorometry for kinetic studies was performed without degassing with a JASCO FP-550 spectrofluorometer. Fluorescence was excited at 290 nm (350 nm) and observed in the range of 320—350 nm (475—500 mn) for NDSCl(DNSCl and DNDSCl).

## Results and Discussion

As we have reported already,[4] DNSCl is unstable and converted to fluorescent products at room temperature in polar solvents such as methanol, pyridine, and DMF, while it is stable in less polar solvents such as THF, ethyl acetate, and chloroform. In organic solvents, hydrogen chloride formed reacts imediately with amine. The total reaction of NDSCl with amines is expressed as

$$ClO_2S\text{-}R\text{-}SO_2Cl + 2H_2NR' \xrightarrow{k_2^1} ClO_2S\text{-}R\text{-}SO_2NHR' + H_3N^+R'Cl^- \tag{1}$$

and

$$ClO_2S\text{-}R\text{-}SO_2NHR' + 2H_2NR' \xrightarrow{k_2^2} R'NHO_2S\text{-}R\text{-}SO_2NHR' + H_3N^+R'Cl^-. \tag{2}$$

As a bifunctional fluorescent kinetic probe, NDSCl has to satisfy the following requirements: (i) Only the disulfonamide is fluorescent and (ii) the reaction of the second sulfonyl chloride with amine is the rate determining step.

Fluorescence of each of the GPC fraction of the mixture of NDSCl, the monosulfonamide, and the disulfonamide was checked and it was confirmed that only the disulfonamide is fluorescent. Its fluorescence spectrum is shown in Fig. 1 together with its absorption spectrum.

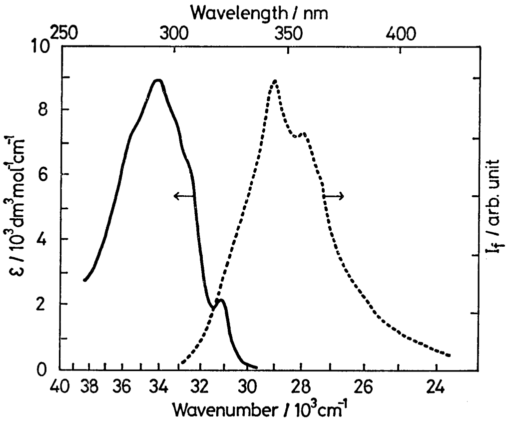

Fig. 1. Absorption and fluorescence spectra of the final reaction product of NDSCl with $n$-$BuNH_2$ in chloroform,

TABLE 1. REACTION CONDITIONS AND $k_2^1$ FOR COMPETITIVE REACTIONS OF NDSCl AND DNSCl WITH $n$-BuNH$_2$

| | | | $t_1$ | | $t_2$ | |
|---|---|---|---|---|---|---|
| $\frac{[n\text{-BuNH}_2]_0}{10^{-5}\ \text{M}}$ | $\frac{[\text{DNSCl}]_0}{10^{-5}\ \text{M}}$ | $\frac{[\text{NDSCl}]_0}{10^{-5}\ \text{M}}$ | $\frac{[\text{DNSCl}]}{[\text{DNSCl}]_0}\times100$ | $\frac{k_2^1}{\text{M}^{-1}\ \text{s}^{-1}}$ | $\frac{[\text{DNSCl}]}{[\text{DNSCl}]_0}\times100$ | $\frac{k_2^1}{\text{M}^{-1}\ \text{s}^{-1}}$ |
| 1.01 | 0.99 | 1.00 | 9.32 | 0.56 | 9.83 | 0.53 |
| 0.99 | 0.98 | — | 28.2 | | 30.3 | |
| 0.20 | 0.99 | 0.20 | 3.78 | 0.59 | 3.90 | 0.59 |
| 0.20 | 0.99 | — | 5.66 | | 5.90 | |

While the chloroform solution of NDSCl (*ca.* $10^{-5}$ M) and $n$-BuNH$_2$ in equimolar amount or excess to the sulfonyl group becomes highly fluorescent in a few hours at room temperature, the reaction mixture with $n$-BuNH$_2$ in half equimolar amount to the sulfonyl chloride group or less is almost nonfluorescent even after a few days. This preliminary test clearly shows that the reaction of the first sulfonyl chloride group is much faster than that of the second one.

The second-order rate constants, $k_2^1$ and $k_2^2$, were estimated from the pseudo first-order rate constants for the reaction of NDSCl with large excess of $n$-BuNH$_2$ $k_1=k_2^1[n\text{-BuNH}_2]$ and $k_2=k_2^2[n\text{-BuNH}_2]$. The concentration of the final product, [BuNHO$_2$S-R-SO$_2$NHBu], at a certain time $t$ can be obtained as,

$$\frac{[\text{BuNHO}_2\text{S-R-SO}_2\text{NHBu}]}{[\text{ClO}_2\text{S-R-SO}_2\text{Cl}]_0} = 1-\frac{k_2}{k_2-k_1}\cdot\exp(-k_1t)-\frac{k_1}{k_1-k_2}\cdot\exp(-k_2t). \quad (3)$$

For $k_1>k_2$ and large $t$, the last term can be disregarded and Eq. 3 is converted to

$$\ln\left(1-\frac{[\text{BuNHO}_2\text{S-R-SO}_2\text{NHBu}]}{[\text{ClO}_2\text{S-R-SO}_2\text{Cl}]_0}\right)=\ln\left(\frac{k_1}{k_1-k_2}\right)-k_2t. \quad (4)$$

Figure 2 shows the pseudo first-order plot of the reaction of NDSCl with large excess of $n$-BuNH$_2$ in chloroform at 40 °C. The slope and the intercept of the extraporation of the linear line at the later stage of the reaction give $k_2$ (hence $k_2^2$) and the ratio $k_1/k_2$ (hence $k_2^1$). They are: $k_2^1=5.0\times10^{-1}$ M$^{-1}$ s$^{-1}$ and $k_2^2=4.8\times10^{-2}$ M$^{-1}$ s$^{-1}$.

The values of $k_2^1$ of the reaction was obtained also by the analysis of the three competitive reactions, *i.e.*, the reactions (1), (2), and the reaction of DNSCl with $n$-BuNH$_2$,

$$\text{DN-SO}_2\text{Cl}+2\text{H}_2\text{N-Bu}\xrightarrow{k_2^3}\text{DN-SO}_2\text{NHBu}+\text{H}_3\text{N}^+\text{BuCl}^-. \quad (5)$$

For the reactions between DNSCl and $n$-BuNH$_2$ with and without NDSCl in chloroform at 40 °C, the concentration of the reaction product from DNSCl at a certain time was obtained by fluorometry. With the values of $k_2^2$ and $k_2^3$ ($4.8\times10^{-2}$ and $3.3\times10^{-2}$ 5) M$^{-1}$ s$^{-1}$ respectively), computer simulations of the competitive and non competitive reactions were performed to obtain the optimum value of $k_2^1$ which satisfies a couple of values of [DNSCl]/[DNSCl]$_0$ at a certain time. Table 1 shows that four values of $k_2^1$, obtained independently for two different concentrations of NDSCl and two different moments, are in fair agreement with each other. The mean value of $k_2^1$, 0.57 M$^{-1}$ s$^{-1}$, is in accord with the value from the pseudo first-order reaction described above. These results confirm that the reactivity of the first sulfonyl chloride group in NDSCl is about 10 times higher than that of the second one and the rate determining step is the reaction of the second sulfonyl chloride group.

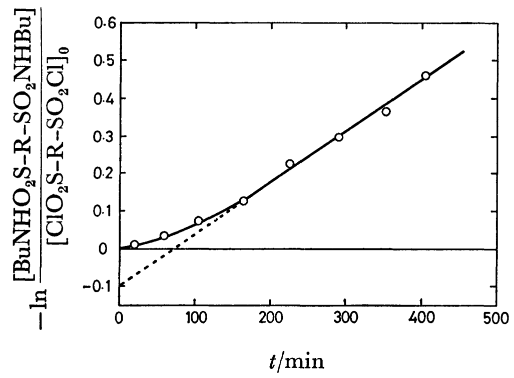

Fig. 2. Pseudo first-order plot of the reaction of NDSCl with $n$-BuNH$_2$ in chloroform at 40 °C: [NDSCl]$_0$=$1.13\times10^{-5}$ M and [$n$-BuNH$_2$]$_0$/[NDSCl]$_0$=42.

As another bifunctional fluorescent amine reagent, DNDSCl was synthesized and its reaction with $n$-BuNH$_2$ was studied briefly. The preliminary test similar to NDSCl indicates that the second reaction of DNDSCl with amine can be detected by the fluorometry which is characterized by an excitation wavelength reached at visible region (absorption maxima: 280 and 390 nm). DNDSCl may, therefore, be more useful than NDSCl as a general fluorescent probe for studies in many fields, because even the presence of most chromophores (benzene derivatives, indole rings, *etc.*) does not interfere the fluorescence measurements with the excitation wavelength.

In conclusion, NDSCl and probably DNDSCl as well are proved to be useful as bifunctional fluorescent probes to analyse the coupling reaction of amino groups kinetically. Its application will be published in near future.8)

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