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A real time reaction monitoring using fluorescent dansyl group as a solid-phase leaving group

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Abstract—A real time monitoring method to monitor the progress of the solid-phase reaction has been developed. The substitution reaction on the solid-phase was clearly monitored as a change in the color depth using the fluorescent dansyl group as the leaving group. This methodology is applicable to both parallel and split combinatorial synthesis.

Organic reactions on a solid support have become very important for constructing a small organic molecule library. Though many types of reactions on solid supports have been extensively developed, relatively limited numbers of monitoring methods of the solid-phase reaction are known.[1]

Several methods such as the Kaiser test,[2] bromophenol blue test,[3] picric acid test[4] and other colorimetric assays on a solid support have been developed mainly for detecting solid supported amines.[5] A few methods have been reported to detect other functional groups such as hydroxyl group,[6] carbonyl group,[7] carboxyl group,[6a,8] thiols,[6b,c,9] some protective groups.[10,11] We now report development of a very simple and rapid real time method for monitoring the progress of the solid-phase substitution reaction using stained leaving group.

If leaving groups or protective groups are stained, the substitution or deprotection process would be clearly monitored as a change in the color depth without using any spectroscopic apparatus, which guarantee rapid analysis for parallel syntheses and also one bead analysis for split synthesis. First, we investigated commercially available dyes. The essential properties of the 'dye' should be: (1) deep color other than yellow in order to distinguish the dye color from the color of the resin itself or remaining impurity on the resin, (2) chemically stable, (3) soluble in standard solvents in order to be easily washed off from the resin after the reaction, (4) small molecule so as not to disturb the reactions on the solid support by its bulkiness, and (5) having a functional group to convert or attach leaving group or protecting group. Unfortunately, we were not able to find an appropriate dye with all the properties shown above, but we found the dansyl (5-dimethylamino-1-naphthalenesulfonyl) group to be promising because of its chemical stability, bright fluorescent color upon irradiation, relatively small molecular weight compared to normal dye molecules and its commercial availability as dansyl chloride (**1**) (Fig. 1).

Though dansyl chloride is well known as a reagent for the fluorescent labeling of amines, amino acids, proteins, and phenols, the dansyl group has not been extensively investigated as a leaving group, nevertheless, it has a structural similarity to the tosyl group.[12,13] If the dansyl group could be used in place of the tosyl or mesyl group, many kinds of substitution reactions would be easily monitored on a solid support. Therefore, we initially investigated the reactivity of the dansyl group in the solution phase by comparing it with the reactivity of the tosyl group. The sulfonylation with dansyl chloride and tosyl chloride is shown in Scheme 1. The dansylation of a primary alcohol **2** is slightly

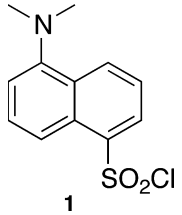

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Figure 1. Dansyl chloride.

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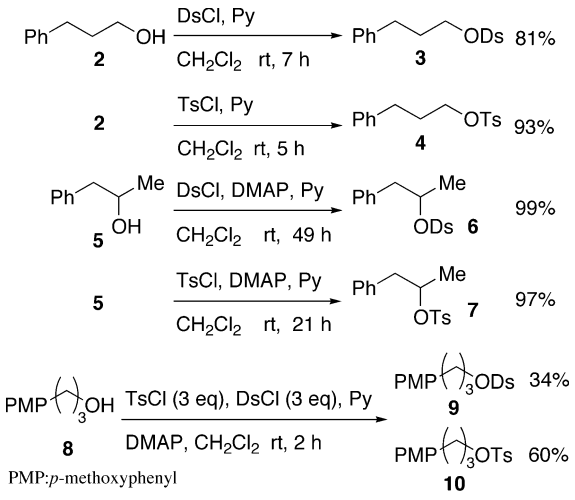


Scheme 1. Dansylation and tosylation of various alcohols.

Table 1. Substitution reactions of dansylates and tosylates

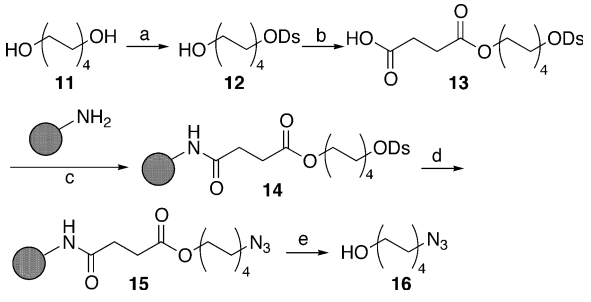
Substrate	Condition[a]	Time	Product	Yield (%)
3	A	2.5 h	3-Iodopropyl-benzene	87
4	A	1.5 h	3-Iodopropyl-benzene	94
3	B	1.5 h	3-Azidopropyl-benzene	87
4	B	1.0 h	3-Azidopropyl-benzene	90
3	C	4.0 h	4-Phenylbutyro-nitrile	91
4	C	3.5 h	4-Phenylbutyro-nitrile	92
3	D	16 h	Propylbenzene	83[b]
4	D	16 h	Propylbenzene	80[b]
3	E	30 min	Heptylbenzene	92
4	E	30 min	Heptylbenzene	92
6	A	2.5 days	2-Iodopropyl-benzene	77
7	A	3.5 days	2-Iodopropyl-benzene	82

[a] A: NaI, acetone, reflux. B: NaN_3, DMF, reflux. C: NaCN, DMSO, 90°C. D: $LiBH_4$, THF, reflux. E: Bu_2CuLi, ether, −20°C.
[b] Without any further purification.

slower than the tosylation. The dansylation of a secondary alcohol **5** needs more than twice as much time as the tosylation. Competitive sulfonylation of *p*-methoxyphenylpropanol (**8**) with dansyl chloride (3 equiv.) and tosyl chloride (3 equiv.) gave 34% of the dansylate **9** and 60% of the tosylate **10**.

Table 1 shows the results of various nucleophilic substitution reactions of the dansylates and tosylates. The iodination, azidation and cyanation of the primary dansylate **3** were slightly slower than those of the tosylate **4**. The reduction and alkylation of **3** had comparable rates to **4**. The iodination of the secondary dansylate **6** was faster than that of **7**. In all cases, the yields are comparable to each other. From these reactivity results, it is clear that the dansyl group can be used in place of the tosyl group.

We then examined the substitution reaction of the dansylated substrate on a solid support (Scheme 2). The mono dansylated 1,8-octanediol **12** was prepared from the diol **11** and 1.1 equiv. of dansyl chloride in 45% yield. **12** Was then attached to the succinic acid linker by treating with succinic anhydride to give the acid **13** in 88% yield. The acid **13** was loaded onto Tenta Gel with DIC. The completion of the reaction was confirmed by the standard Kaiser test.[2] An intense light green yellow fluorescence was observed for the resulting dansylated resin **14** upon irradiating with 365 nm UV light. The dansylate **14** was treated with sodium azide in DMF at 70°C for the azidation. At first, we tried the direct observation of the reaction progress for maximum convenience. The solution began to immediately show a light blue fluorescence and the fluorescence of the beads became weak and finally was hidden by the intense background fluorescence of the solution upon irradiation. Though it was clear that the azidation proceeded and was nearly completed, it was difficult to judge the exact completion of the reaction. To precisely monitor the progress of the reaction, a small portion of the resin was taken out from the reaction vessel and placed into a vial. The resin in a vial was washed with DMF (2×) and CH_2Cl_2 (2×) and then observed under 365 nm UV light. As shown in Figure 2, the strong fluorescence gradually became weak in 7 min. Only a very weak fluorescence was observed in 10 min. No fluorescence was observed in 20 min. Thus, the progress of the reaction was monitored as the transition of fluorescence as initially expected. To confirm the completion of the reaction, the azide was cleaved from resin with K_2CO_3 in methanol at room temperature. The crude solution contains only one compound based on the TLC analysis. The solution was neutralized with Dowex 50W-X2 and purified by silica gel chromatography to give pure 8-azidooctan-1-ol (**16**) in 87% based on the initial loading amount of **13** on the Tenta Gel.



Scheme 2. Azidation of dansylated substrate on the resin. *Reagents and conditions*: (a) DsCl (1.1 equiv.), Py, CH_2Cl_2, 45%; (b) succinic anhydride, Py, DMAP, CH_2Cl_2, 88%; (c) DIC, CH_2Cl_2; (d) NaN_3, DMF, 70°C, 1 h; (e) K_2CO_3, MeOH, 87% (two steps).

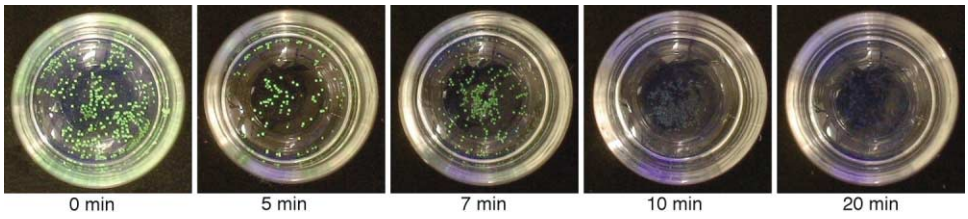


Figure 2. Monitoring azidation reaction by fluorescence.

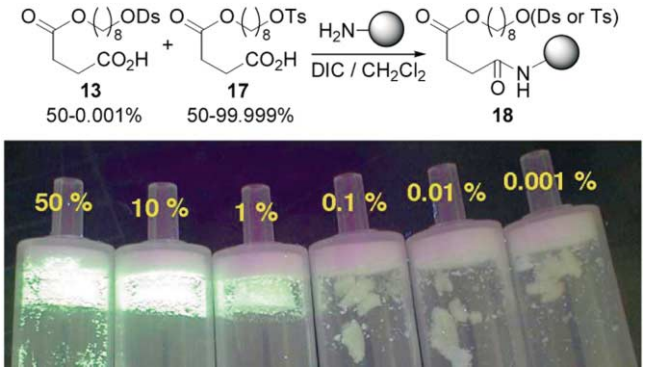


Figure 3. Substitution rate and fluorescence intensity.

It is important to quantitatively establish the sensitivity of this method. Tosylated acid **17** was prepared as described for the dansylated acid **13**. From 50% to 0.001% of **13** were mixed with the acid **17** and each mixture was loaded onto the Tenta Gel with DIC to afford partially dansylated resins, of which the fluorescences were observed upon irradiation as shown in Figure 3. While it was hard to observe the fluorescence at the 0.01% loading, a weak but easily detectable fluorescence was observed at the 0.1% loading. This indicates that the substitution reaction of the dansylated resin is monitored up to at least 99.9% completion. Since the initial loading of the resin used here was 0.23 mmol/g, as little as 0.2 μmol/g dansyl group could be detected. This indicates that this method is more than ten times more sensitive than the Kaiser test of which the sensitivity was reported to be 5 μmol/g.[2a]

In conclusion, we have developed a new type of colorimetric assay[5] that can monitor the progress of the solid-phase *substitution reaction* using the dansyl group as the leaving group.

Acknowledgements

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References

1. Cin, M. D.; Davalli, S.; Marchioro, C.; Passarini, M.; Perini, O.; Provera, S.; Zaramella, A. *Farmaco* **2002**, *57*, 497.
2. (a) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595; (b) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147.
3. Hancock, W. S.; Battersby, J. E. *Anal. Biochem.* **1976**, *71*, 260.
4. Gisin, B. F. *Anal. Chim. Acta* **1972**, *58*, 248.
5. Kay, C.; Lorthioir, O. E.; Parr, N. J.; Congreve, M.; McKeown, S. C.; Scicinski, J. J.; Ley, S. V. *Biotechnol. Bioeng.* **2001**, *71*, 110.
6. (a) Yan, B.; Liu, L.; Astor, C. A.; Tang, Q. *Anal. Chem.* **1999**, *71*, 4564; (b) Attardi, M. E.; Falchi, A.; Taddei, M. *Tetrahedron Lett.* **2000**, *41*, 7395; (c) Attardi, M. E.; Taddei, M. *Tetrahedron Lett.* **2001**, *42*, 2927; (d) Reddy, M. P.; Voelker, P. J. *Int. J. Pept. Protein Res.* **1988**, *31*, 345; (e) Kuisle, O.; Lolo, M.; Quiñoa, E.; Riguera, R. *Tetrahedron* **1999**, *55*, 14807.
7. (a) Yan, B.; Li, W. *J. Org. Chem.* **1997**, *62*, 9354; (b) Vazquez, J.; Albericio, F. *Tetrahedron Lett.* **2001**, *42*, 6691; (c) Cournoyer, J. J.; Kshirsagar, T.; Fantauzzi, P. P.; Figliozzi, G. M.; Makdessian, T.; Yan, B. *J. Comb. Chem.* **2002**, *4*, 120.
8. Attardi, M. E.; Porcu, G.; Taddei, M. *Tetrahedron Lett.* **2000**, *41*, 7391.
9. (a) Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11580; (b) Badyal, J. P.; Cameron, A. M.; Cameron, N. R.; Coe, D. M.; Cox, R.; Davis, B. G.; Oates, L. J.; Oye, G.; Steel, P. G. *Tetrahedron Lett.* **2001**, *42*, 8531.
10. (a) Atherton, E.; Sheppard, R. C. *Solid Phase Peptide Synthesis*: *A Practical Approach*; IRL Press: Oxford, 1989; (b) Carpino, L. A.; Ismail, M.; Truran, G. A.; Mansour, E. M. E.; Iguchi, S.; Ionescu, D.; El-Faham, A.; Riemer, C.; Warrass, R. *J. Org. Chem.* **1999**, *64*, 4324; (c) Fukuyama, T.; Jow, C.-K.; Chueng, M. *Tetrahedron Lett.* **1995**, *36*, 6373; (d) Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1998**, *120*, 2690; (e) Manabe, S; Ito, Y. *J. Am. Chem. Soc.* **2002**, *124*, 12638.
11. Dansyl derivative has been used as solid-phase hydroxy protective group: Bergmann, F.; Pfleiderer, W. *Helv. Chim. Acta* **1994**, *77*, 988 and references cited therein.
12. Solvolysis in aqueous media has been reported: Lawrence, C.; Bell, I. M.; Abell, C.; Leeper, F. J. *Isr. J. Chem.* **1996**, *36*, 161.
13. Solution phase Heck reaction was monitored: Stambuli, J. P.; Stauffer, S. R.; Shaughnessy, K. H.; Hartwig, J. F. *J. Am. Chem. Soc.* **2001**, *123*, 2677.