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DIOL DERIVATIVE OF (3-TRIFLUOROMETHYL)PHENYLDIAZIRINE FOR POST-LABELING OF PHOTOCROSSLINK

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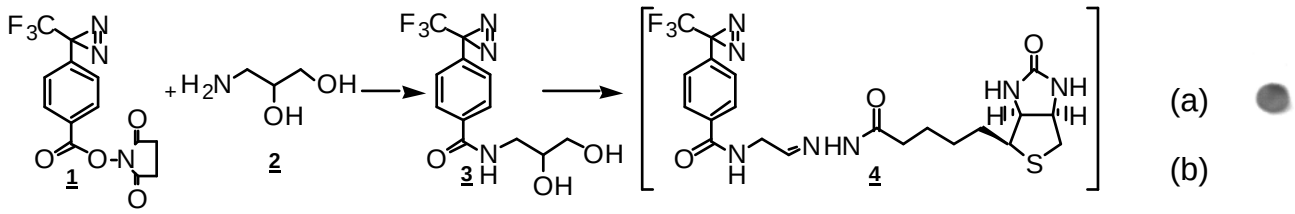
Abstract – 3-Trifluoromethylphenyldiazirinylated diol derivative was utilized to introduce an aldehyde by periodate oxidation, followed by the formation of a Schiff base with biotin hydrazide on a PVDF membrane for post-labeling of photocrosslinked proteins. The biotin hydrazide was able to post-label the 2.5 x 10^{-13} mole of closslinked component for chemiluminescent visualization.

Photoaffinity labeling with the carbene generating precursor, 3-phenyl-3-trifluoromethyldiazirine, has become increasingly important as a photophor.[1] The introduction of tags after photolabeling is one of the rational approaches for the detection of affinity labeled targets, because the process includes minimal chemical modifications of the ligand skeleton to avoid the reduction of ligand activity due to pre-installed unnatural detection tags.[2] A substituent group for specific chemical reaction in the aqueous phase should be performed for this purpose. Staudinger[2c] and click chemistry[2d] have reported on as possible methods. Diol is well known as a precursor of aldehyde with periodate oxidation. Photolabeling reagents containing a sugar moiety are easily to applied for oxidations.[2a] However, not all ligands contain a sugar moiety, so photoreactive diol compounds are one of the useful groups of reagents for this purpose. A few diazirinyl diol compounds have been reported on and some of them utilize the periodate oxidation reaction to generate formyl groups,[3] but the introduction of key diol functionality was rather laborious. We describe the simple synthesis for installing the diol within diazirinyl photophore and the post-labeling of photocrosslinked component on a PVDF membrane for rapid detection.

This paper is dedicated to the memory of the Emeritus Professor K. Koga of Tokyo University.

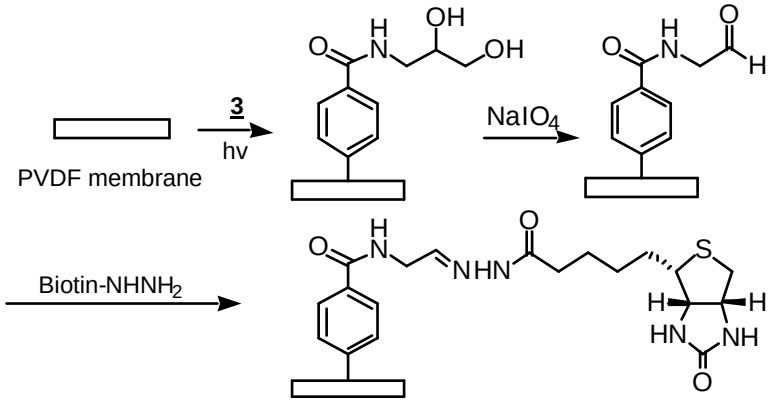
Diazirinyl diol compound (**3**) was synthesized from diazirinylcarboxylic acid *N*-hydroxy succinimide ester (1)[4] and 3-amino-1,2-propanediol (**2**).



Scheme 1. Synthesis of diazirinyl diol (**3**) and Schiff base formation at the nano mole level in solution. (a) and (b) are chemiluminescence detections of photoimmobilized Schiff base **4** with and without biotin hydrazide, respectively.

Periodate oxidation followed by Schiff base formation of **3** at the nano mole level in solution was performed. Periodate oxidized **3** was reacted with biotinyl hydrazide, then the reaction mixture was blotted on polyvinylidene difluoride (PVDF) membrane. The membrane was irradiated with black light to immobilize the diazirinyl compound, then subjected to chemiluminescence detection with Streptavidin-horseradish peroxidase conjugate. A chemiluminescence signal was only observed on treatment with biotinhydrazide (Scheme 1 (a)).

As a typical experiment of photocrosslinking, the PVDF membrane was immersed in the methanolic solution of compound (**3**), followed by irradiation with a black light at a 1 cm distance for 40 min. The irradiated membrane was washed with methanol, then equilibrated with water.[5] Sodium periodate was added to the aqueous solution to perform periodate oxidation.



Scheme 2. Photocrosslinking of compound (**3**) on the PVDF membrane and post-function with biotin hydrazide.

After drying the membrane, biotin-hydrazide was spotted as a dot on the activated membrane. The dotted samples were incubated at 50 °C for 3 h. The membrane was subjected to chemiluminescence detection with a Streptavidin-biotin specific interaction. Biotin hydrazide was immobilized on the photoactivated membrane via Schiff base formation and the immobilized biotin was detected at 0.25 x

10^{-13} mol on the PVDF membrane by chemiluminescence. Biotin did not produce chemiluminescence signals under the same conditions, even at 10^{-10}mol (data not shown).

These results indicate that the periodate oxidation of diol followed by Schiff base formation is sensitive at the sub-picomole level and is one of the highly sensitive methods for postfunctional photocrosslinking.

(a) (b) (c) (d)

Scheme 3. Chemiluminescence detection of the post-functionalized PVDF membrane with biotin hydrazide. Amounts of blotted biotin-hydrazide were 1, 0.5, 0.25 and 0.125 pmol for (a) to (d), respectively.

EXPERIMENTAL

^{1}H NMR, IR and FAB-MS spectra were obtained using a JEOL JNM-ECA500, JASCO FT-IR 420 and JMS AX-500 spectrometer, respectively. All solvents were of reagent grade and distilled using the appropriate methods. The PVDF membrane (Immobilon P) was purchased from Millipore.

***N*-(2,3-Dihydroxypropyl)-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzamide (3).** Compound (**1**) (0.0491 g, 0.15 mmol) was dissolved in CH_3CN (1.5 mL). 3-Amino-1,2-propanediol (**2**) in methanol (0.254 M, 1.5 mL, 0.38 mmol) was added to the CH_3CN solution. The reaction mixture was stirred at rt for 2 h, and then concentrated. The residue was partitioned between ethyl acetate and 1N HCl. The organic layer was washed with saturated NaCl, dried over $MgSO_4$, filtrated and concentrated. The residue was subjected to silica column chromatography ($CHCl_3$: MeOH = 6 : 1) to afford a pale orange oil (0.0390 g, 86%). FAB-MS *m/z*: 304 (MH^+), ^{1}H-NMR ($CDCl_3$) 7.70 (2H, d, J = 6.6 Hz), 7.07 (2H, d, J = 6.6 Hz), 6.67 (1H, s), 3.90 - 3.80 (1H, m), 3.55 - 3.30 (4H, m), ^{13}C-NMR ($CDCl_3$) 168.04, 135.02, 132.61, 127.75, 126.63, 122.02, (q, J_{CF} = 275.5 Hz), 70.77, 63.69, 42.66, 28.42 (q, $^2J_{CF}$ = 40.8 Hz), IR (neat) 3340 (broad), 2935, 1644, *Anal*. Calcd for $C_{12}H_{12}N_3O_3F_3$: C, 47.53; H, 3.99; N, 13.86. Found: C, 47.80; H, 4.02; N, 14.03.

Schiff base formation of 3 with biotin hydrazide at the nano mole scale in a solution phase *via* periodate oxidation. The methanolic solution of **3** (40 μM, 50 μL, 2 nmol) and aqueous solution of $NaIO_4$ (300 μM, 10 μL, 3 nmol) were incubated at rt for 1 h. Biotin hydrazide in DMSO (200 μM, 10 μL, 2 nmol) was then added and the reaction mixture was incubated at 50 °C for 2 h. Part of the reaction mixture was blotted on a PVDF membrane and dried. The membrane was irradiated with black light (15W) for 20 min, washed with methanol, then subjected to chemiluminescence detection in an identical manner as described previously.[6]

Surface modification of the PVDF membrane with compound (3) and immobilized biotin hydrazide. The PVDF membrane (9 cm square) was wetted with 1 mM methanolic solution of **3**, dried, then irradiated with black light (15W) for 20 min on each side. The irradiated membrane was washed with MeOH three times, then equilibrated with water. The membrane was soaked in 0.2 M $NaIO_4$ aqueous solution at rt for 1 h, well washed with DW, then dried on a heating block. Biotin hydrazide in methanol was blotted on the membrane. The blotted membrane was heated at 50 °C for 3 h, washed with MeOH and water, then subjected to chemiluminescence detection.

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REFERENCES

1. a) Y. Hatanaka, H. Nakayama, and Y. Kanaoka, *Rev. Heteroatom Chem.*, 1996, **14**, 213. b) J. Brunner, *Annu. Rev. Biochem.*, 1993, **62**, 483. c) A. K. Gillingham, F. Koumanov, M. Hashimoto, and G. D. Holman, 'Membrane Transport : A Practical Approach', ed. by S. A. Baldwin, Oxford University Press, Oxford, 2000, pp. 193-207.
2. a) T. Nagase, E. Nakata, S. Shinkai, and I. Hamachi, *Chem. Eur. J.*, 2003, **9**, 3660. b) T. Nagase, S. Shinkai, and I. Hamachi, *Chem. Commun.*, 2001, 229. c) T. Hosoya, T. Hiramatsu, T. Ikemoto, H. Aoyama, T. Ohmae , M. Endo, and M. Suzuki, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1289. d) T. Hosoya, T. Hiramatsu, T. Ikemoto, M. Nakanishi, H. Aoyama, A. Hosoya, T. Iwata, K. Maruyama, M. Endo, and M. Suzuki, *Org. Biomol. Chem.*, 2004, **2**, 637.
3. a) D. E. Bochkariov, and A. A. Kogon, *Anal. Biochem.*,1992, **204**, 90., b) A. A. Kogon, D. E. Bochkarev, B. P. Baskunov, and A. V. Cheprakov, *Liebigs Ann. Chem.*, 1992, 879. c) U. Kempin, Y. Kanaoka, and Y. Hatanaka, *Heterocycles*, 1998, 49, 465. d) M. Hashimoto, Y. Hatanaka, J. Yang, J. Dhesi, and G. D. Holman, *Carbohydr. Res.*, 2001, **331**, 119.
4. M. Nassal, *Liebigs Ann. Chem.*, 1983, 1510.
5. Y. Hatanaka, M. Hashimoto, and Y. Kanaoka, *J. Am. Chem. Soc.*, 1998, **120**, 453.
6. Y. Hatanaka, M. Hashimoto, S. Nishihara, H. Narimatsu, and Y. Kanaoka, *Carbohydr. Res.*, 1996, **294**, 95.