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Simple method for the introduction of iodo-label on (3-trifluoromethyl) phenyldiazirine for photoaffinity labeling

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Abstract—A simple and mild method was developed for the introduction of iodo-label on (3-trifluoromethyl) phenyldiazirine (TPD) aromatic ring in the presence of three membered diazirine ring. An iodination protocol, I_2–BTI in CH_3CN, was found to be effective even though affinity ligands are pre-installed.

Photoaffinity labeling is a powerful method in the study of biological structures and functions.[1–3] It is suitable for the analysis of biological interactions because it is based on the affinity of the ligand moiety. Various photophores, such as phenyldiazirine, arylazide, and benzophenone, have been used. Comparative irradiation studies of these three photophors in living cells suggest that a carbene precursor, (3-trifluoromethyl) phenyldiazirine (TPD), is the most promising, especially concerning photolabeling of living cells.[4] This is because other photophors promote cell death by long time irradiation in attempts to generate active species for cross-links. However, synthesis of TPD analogues is hampered when trying to repeat constructions of the TPD photophor for each derivative. Approaches for the direct modification of diazirine photophor bypassed the time consuming diazirine ring construction steps.[5–7] Iodinated TPD derivatives are widely used for photoaffinity labeling. One method is where iodinated compounds are utilized as a tag to detect labeled components by radioisotope.[8] The other one is where iodinated compounds are used as precursors for chemoselective hydrogenolysis to introduce hydrogen isotopes.[9,10] Furthermore, it was reported that iodine introduction sometimes caused a higher affinity for membrane protein than unsubstituted TPD compound.[11] The iodination routine, however, has still remained as inefficient. There are several reports on the preparation of iodinated diazirinyl compounds. Iodination with Chloramine T and NaI could be applied when the compounds contained OH[12] or isothiocyanate,[13] which promote very strong activation for electrophilic substitution on the benzene ring. However, this method does not apply to alkoxy TPD, because the alkoxy group is less activated for electrophilic substitution than the hydroxyl group. Several other iodination methods were examined for alkoxy TPD analogues: (1) Iodination was performed before construction of the diazirinyl three-membered ring. The method should involve repeated constructions of a diazirine moiety (over five steps) for each derivative;[14,15] (2) Iodine was exchanged for a pre-installed aryl-tin group on TPD with NaI in the presence of peracetic acid[16] or Chloramine T.[17] This required additional steps to introduce the alkyl-tin group on TPD, and that the yield of iodination is not sufficient (~60%) for the applications; (3) Direct iodination was performed with $Tl(OCOCH_3)_3$–NaI[18] for alkoxy TPD analogues. This method is capable of introducing iodine without pre-installed substituent groups, but thallic compounds were well known as being very toxic. The last two methods require a long reaction time for the iodination. Therefore, the direct iodination of TPD still remains to be improved with mild and effective conditions. Combination of molecular iodine (I_2) and bis(trifluoroacetyl)phenyl iodinate (BTI) in CH_3CN was reported as one of the electrophilic iodination reagents for aromatics under mild conditions.[19–23] In this report, we will describe selective and mild iodination of alkoxy TPD analogues in which affinity ligands have also already been installed.

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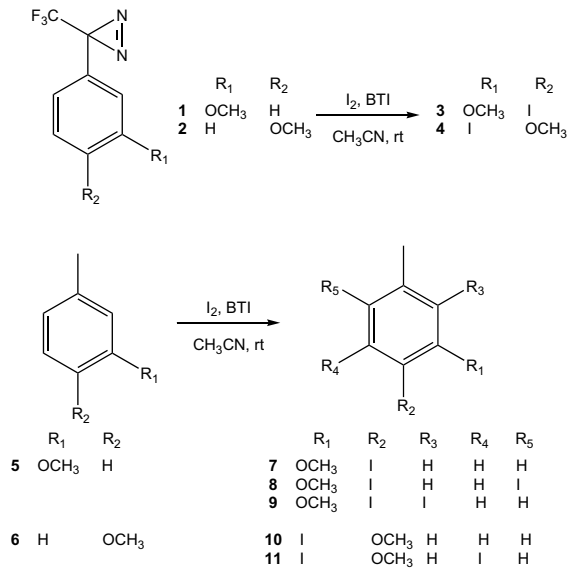
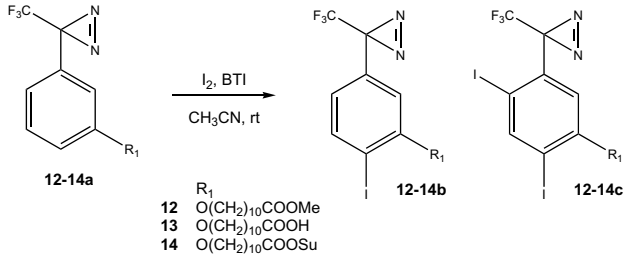
Iodinations of 3- and 4-methoxy TPD (**1**, **2**) were not efficient with 0.6 equiv of I_2 and 1.2 equiv of BTI, which is consistent with a previous report. Large amounts of both reagents, 2.4 equiv of I_2 and 4.8 equiv of BTI, were needed for moderate yields (86% and 74% for **3** and **4**, respectively) and no decomposition of the diazirinyl ring was observed.[24] The iodinated position of 3-methoxy diazirine **1** was defined via the NOESY spectrum from the methoxy group. Excess reagents seem to prompt poly-iodinations for the aromatics. Comparative studies of iodination for 3- and 4-methoxytoluene (**5** and **6**) were carried out (Scheme 1), and the results are summarized in Table 1. With the same conditions as in the previous report, 0.6 equiv of I_2 and 1.2 equiv of BTI produced mono-iodinated products **7** and **10**, but the excess reagents promoted further iodination to mono-iodinated compounds (**8**, **9**, **11**, entries 4–6 and 10–12). These results indicated that the (3-trifluoromethyl) diazirinyl group deactivated the electrophilic aromatic substitution reactivity of the benzene ring compared with alkane substituents. This deactivation power by the (3-trifluoromethyl) diazirinyl moiety was also observed in Friedel–Crafts reaction for TPD analogues.[25,26]



Scheme 1.

The alkoxy moiety will be useful for the introduction of ligand skeletons in TPD. We elucidated the mild direct iodination methods for photoreactive diazirinyl analogues, which already contained a ligand moiety. Photoaffinity fatty acid analogues are useful reagents to investigate related biomolecules, because many fatty acids are recognized by membrane proteins, where it is difficult to utilize other functional analysis methods. Previous studies have indicated that phenoxy TPD fatty acid analogues[12,27] are very useful. Several researchers attempted the synthesis of iodinated fatty acid TPD analogues, but they performed iodination before construction of the (3-trifluoromethyl) diazirinyl ring or used pre-installed iodinated precursors. The I_2–BTI method was applied for several alkoxy TPD fatty acid derivatives (Scheme 2). The results are summarized in Table 2.

The diazirinyl fatty acid methyl ester (**12a**) is easily mono-iodinated with I_2 (1.2 equiv) and BTI (2.4 equiv) in a moderate yield (73%, entry 1). Larger amounts of both reagents (two times) improved the yield (90%, entry 2). Excess reagents did not promote further iodination of the mono-iodinated compound (**12b**). The carboxylic acid analogue (**13a**) showed optimal iodination with 1.2 equiv of I_2 and 2.4 equiv of BTI (84%, entry 3) for mono-iodination. A larger excess of reagents caused further iodination, but the di-iodinated compound was less than 25% (entries 4 and 5). The results indicated that the acidity of the reaction mixture caused secondary iodination. The succinimide ester (**14a**), which is one of the highly reactive groups for the condensation of amino groups, was also iodinated with 1.2 equiv of I_2 and 2.4 equiv of BTI (86%, entry 7) without hydrolysis.



Scheme 2.

Table 1. Iodination of alkoxyphenyl compounds with various amounts of I_2 and BTI

Entry	Substrate[a]	I_2 (equiv)	BTI (equiv)	Mono-iodinated (%)	Di-iodinated (%)
1	**1**	0.6	1.2	29 (**3**)	n.d.
2	**1**	1.2	2.4	77 (**3**)	n.d.
3	**1**	2.4	4.8	86 (**3**)	n.d.
4	**5**	0.6	1.2	69 (**7**)	26, 5
5	**5**	1.2	2.4	n.d.	75 (**8**), 15 (**9**)
6	**5**	2.4	4.8	n.d.	79 (**8**), 13 (**9**)
7	**2**	0.6	1.2	45 (**4**)	n.d.
8	**2**	1.2	2.4	70 (**4**)	n.d.
9	**2**	2.4	4.8	74 (**4**)	n.d.
10	**6**	0.6	1.2	80 (**10**)	n.d
11	**6**	1.2	2.4	33 (**10**)	42 (**11**)
12	**6**	2.4	4.8	n.d.	81 (**11**)

[a] Each substrates are used 0.2 mmol.

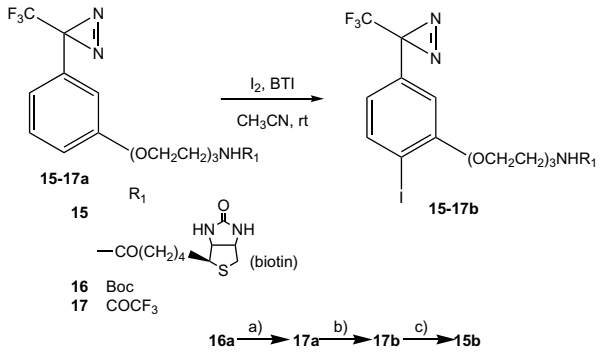
Table 2. Iodination of diazirinyl fatty acid analogues with various amounts of I_2 and BTI

Entry	Substrate[a]	I_2 (equiv)	BTI (equiv)	Mono-iodinated (%)	Di-iodinated (%)
1	**12a**	1.2	2.4	73 (**12b**)	n.d.
2	**12a**	2.4	4.8	90 (**12b**)	n.d.
3	**13a**	1.2	2.4	84 (**13b**)	n.d.
4	**13a**	2.4	4.8	68 (**13b**)	25 (**13c**)
5	**13a**	6.0	12.0	70 (**13b**)	24 (**13c**)
6	**14a**	1.2	2.4	12 (**12b**)	n.d.
7	**14a**	2.4	4.8	86 (**12b**)	n.d.

[a] Each substrate 0.2 mmol.

We have demonstrated that photoaffinity labeling in combination with avidin–biotin systems (photoaffinity biotinylation)[28–32] is very useful for the manipulation of photolabeled biomolecules. The iodinated diazirinyl biotin analogue could be useful for photoaffinity biotinylation. Results for the direct iodination of biotinylated diazirine **15a** to **15b** were not sufficient, because the biotin moiety was not stable under the conditions. The Boc protected analogue **16a** was iodinated with a 23% yield for **16b**, but de-Boc reaction of iodinated and uniodinated intermediates occurred because trifluoroacetic acid was contaminated in BTI and liberated during the reaction. Trifluoroacetyl derivative **17a** was stable under the iodination conditions (1.2 equiv of I_2 and 2.4 equiv BTI) with a moderate yield (67%). The iodinated compound **17b** was de-trifluoroacetyl and biotinylated, affording the iodinated diazirinyl biotin analogue **15b** (Scheme 3).

These results indicate that the iodination of TPD containing photoligands with I_2–BTI in CH_3CN could be widely used without photophor decomposition in moderate yields under milder conditions than previous ones. It is very useful that direct iodination was achieved even though the compounds contained a ligand moiety. The iodinated compounds can be effectively utilized to elucidate biofunctional analysis with photoaffinity labeling. The results may promote the use of diazirine based photoaffinity labeling for functional analysis.



Scheme 3. Reagents and conditions: (a) (1) TFA–CH_2Cl_2, rt, (2) $(CF_3CO)_2O$, rt, 76%; (b) I_2, BTI, CH_3CN, rt, 67%; (c) (1) 1 N NaOH, (2) biotin-OSu, 0.1 M $NaHCO_3$–DMF, rt, 78%.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.03.068.

References and notes

1. Hatanaka, Y.; Nakayama, H.; Kanaoka, Y. *Rev. Heteroatom Chem.* **1996**, *14*, 213.
2. Hatanaka, Y.; Sadakane, Y. *Curr. Top. Med. Chem.* **2002**, 271.
3. Tomohiro, T.; Hashimoto, M.; Hatanaka, Y. *Chem. Records* **2005**, *5*, 385.
4. Gillingham, A. K.; Koumanov, F.; Hashimoto, M.; Holman, G. D. Detection and analysis of glucose transporters using photolabelling techniques. In *Membrane Transport: A Practical Approach*; Baldwin, S. A., Ed.; Oxford University Press: Oxford, 2000; p 193.
5. Hashimoto, M.; Nabeta, K.; Hatanaka, Y. *Heterocycles* **2003**, *59*, 395.
6. Hashimoto, M.; Hatanaka, Y. *Chem. Pharm. Bull.* **2004**, *52*, 1385.
7. Hashimoto, M.; Komano, T.; Nabeta, K.; Hatanaka, Y. *Chem. Pharm. Bull.* **2005**, *53*, 140.
8. Brunner, J.; Semenza, G. *Biochemistry* **1981**, *20*, 7174.
9. Ambroise, Y.; Miskowski, C.; Djega-Mariadassou, G.; Rousseau, B. *J. Org.Chem.* **2000**, *65*, 7183.
10. Ambroise, Y.; Pillon, F.; Miskowski, C.; Valleix, A.; Rousseau, B. *Eur. J. Org. Chem.* **2001**, *65*, 3961.
11. Hashimoto, M.; Yang, J.; Hatanaka, Y.; Sadakane, Y.; Nakagomi, K.; Holman, G. D. *Chem. Pharm. Bull.* **2002**, *50*, 1004.
12. Hashimoto, M.; Nabeta, K.; Murakami, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1531.
13. Dolder, M.; Michel, H.; Sigrist, H. *J. Protein Chem.* **1990**, *9*, 407.
14. Li, G.; Bittman, R. *Tetrahedron Lett.* **2000**, *41*, 6737.
15. Tyagi, N. K.; Kinne, R. K. H. *Anal. Biochem.* **2003**, *323*, 74.
16. Weber, T.; Brunner, J. *J. Am. Chem. Soc.* **1995**, *117*, 3084.
17. Panasiewicz, M.; Mieczkowski, J.; Domek, H.; Pacuszka, T. *Anal. Biochem.* **2005**, *340*, 373.

18. Hatanaka, Y.; Hashimoto, M.; Kurihara, H.; Nakayama, H.; Kanaoka, Y. *J. Org. Chem.* **1994**, *59*, 383.
19. Merkushev, E. B.; Simakhina, N. D.; Koveshnikova, G. M. *Synthesis* **1980**, 486.
20. D'Auria, M.; Mauriello, G. *Tetrahedron Lett.* **1995**, *36*, 4883.
21. Togo, H.; Nogami, G.; Yokoyama, M. *Synlett* **1998**, 534.
22. Benhida, R.; Blanchard, P.; Fourrey, J.-L. *Tetrahedron Lett.* **1998**, *39*, 6849.
23. Panunzi, B.; Rotiroti, L.; Tingoli, M. *Tetrahedron Lett.* **2003**, *44*, 8753.
24. Typical procedure: The aromatics (0.18 mmol) and I_2 were dissolved in anhydrous CH_3CN (2.5 ml). BTI in anhydrous CH_3CN was added to the above solution at room temperature. The reaction mixture was stirred at room temperature for 2 h in the dark and neutralized with 0.1 M NaOH, then partitioned between diethyl ether and H_2O. The organic layer was washed with saturated NaCl, dried over $MgSO_4$, filtrated, and concentrated. The residue was subjected to silica column chromatography to afford the iodinated compounds.
25. Hashimoto, M.; Kanaoka, Y.; Hatanaka, Y. *Heterocycles* **1997**, *46*, 119.
26. Kempin, U.; Kanaoka, Y.; Hatanaka, Y. *Heterocycles* **1998**, *49*, 465.
27. Hashimoto, M.; Hatanaka, Y.; Nabeta, K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 89.
28. Hatanaka, Y.; Hashimoto, M.; Kanaoka, Y. *J. Am. Chem. Soc.* **1998**, *120*, 453.
29. Hashimoto, M.; Yang, J.; Holman, G. D. *ChemBioChem* **2001**, *2*, 52.
30. Hashimoto, M.; Okamoto, S.; Nabeta, K.; Hatanaka, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2447.
31. Hashimoto, M.; Hatanaka, Y. *Heterocycles* **2005**, *66*, 531.
32. Hashimoto, M.; Hatanaka, Y. *Anal. Biochem.* **2006**, *348*, 154.