



Title	Base-Mediated One-Pot Synthesis of Aliphatic Diazirines for Photoaffinity Labeling
Author(s)	Wang, Lei; Tachrim, Zetryana Puteri; Kurokawa, Natsumi; Ohashi, Fumina; Sakihama, Yasuko; Hashidoko, Yasuyuki; Hashimoto, Makoto
Citation	Molecules, 22(8), 1389 https://doi.org/10.3390/molecules22081389
Issue Date	2017-08-22
Doc URL	http://hdl.handle.net/2115/67259
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	article
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	molecules-22-01389-v2.pdf



[Instructions for use](#)

Article

Base-Mediated One-Pot Synthesis of Aliphatic Diazirines for Photoaffinity Labeling

Lei Wang ¹, Zetryana Puteri Tachrim ², Natsumi Kurokawa ², Fumina Ohashi ²,
Yasuko Sakihama ² , Yasuyuki Hashidoko ² and Makoto Hashimoto ^{2,*}

¹ Center for Drug Design, Academic Health Center, University of Minnesota, Minneapolis, MN 55455, USA; lei870610@gmail.com

² Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kita-ku, Sapporo 060-8589, Japan; z317_style@live.com (Z.P.T.); natsumi.k0420@gmail.com (N.K.); fumina28ohsei@gmail.com (F.O.); sakihama@abs.agr.hokudai.ac.jp (Y.S.); yasu-h@abs.agr.hokudai.ac.jp (Y.H.)

* Correspondence: hasimoto@abs.agr.hokudai.ac.jp; Tel./Fax: +81-11-706-3849

Received: 2 August 2017; Accepted: 21 August 2017; Published: 22 August 2017

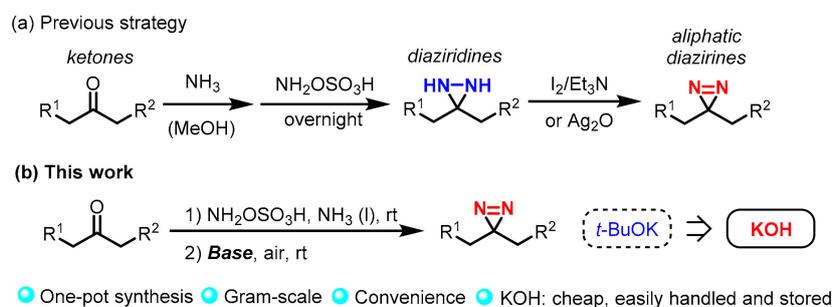
Abstract: Aliphatic diazirines have been widely used as prominent photophores for photoaffinity labeling owing to their relatively small size which can reduce the steric effect on the natural interaction between ligands and proteins. Based on our continuous efforts to develop efficient methods for the synthesis of aliphatic diazirines, we present here a comprehensive study about base-mediated one-pot synthesis of aliphatic diazirines. It was found that potassium hydroxide (KOH) can also promote the construction of aliphatic diazirine with good efficiency. Importantly, KOH is cheaper, highly available, and easily handled and stored compared with the previously used base, potassium *tert*-butoxide (*t*-BuOK). Gram-scale study showed that it owned great advantages in being used for the large-scale production of aliphatic diazirines. This protocol is highly neat and the desired products can be easily isolated and purified. As the first comprehensive study of the base-mediated one-pot synthesis of aliphatic diazirines, this work provided good insight into the preparation and utilization of diazirine-based photoaffinity labeling probes.

Keywords: photoaffinity labeling; one-pot synthesis; aliphatic diazirine; base-mediated reaction

1. Introduction

Photoaffinity labeling [1–5] has emerged as a powerful methodology in the field of biology due to its availability for elucidating the interactions between bioactive molecules and proteins. Various photophores—including phenyl azides, benzophenones, and diazirines—are commonly employed in this field [6–11]. Under UV irradiation, photophores are converted into highly reactive intermediates which can form covalent bonds between ligands and proteins. The stable links in the components allow for further separation and detection. Among these photophores, aliphatic diazirines [12–16] have attracted tremendous attention due to their relatively small size, thus minimizing the steric effect of the natural interaction between ligands and proteins. For the conventional synthesis procedure of aliphatic diazirines [17,18], the ketones precursor was first treated with ammonia for several hours, then it was reacted with hydroxylamine-*O*-sulfonic acid (NH₂OSO₃H) to form diaziridines. After removal of the ammonia, diaziridines were further converted to diazirine in the presence of I₂-TEA or Ag₂O (Scheme 1a). Despite the wide applications, this protocol suffers many issues such as low yield and tedious procedures. To solve these issues, we have recently developed a novel one-pot synthesis of aliphatic diazirines by using *t*-BuOK under air [19]. In spite of its good efficiency, *t*-BuOK is a flammable base and it should be commonly handled and stored under inert gas. Furthermore, its relatively high price also limits the large-scale production of diazirines and the

corresponding labeling probes. Herein, for the first time, we carried out a comprehensive study to screen the bases and other conditions, and it was found that KOH also could be used for one-pot synthesis of aliphatic diazirines with great efficiency (Scheme 1b). Significantly, it is cheaper, easily handled, and stored as well as being highly available in many labs. Gram-scale study confirmed its usability for the large-scale production of aliphatic diazirines. Furthermore, a kinetic study to elaborate the one-pot process was also performed on the basis of NMR analysis.



Scheme 1. Previous (a) and base-mediated one-pot (b) synthesis of aliphatic diazirines.

2. Results and Discussion

2.1. Reaction Optimization

We initiated our studies by using levulinic acid (**1a**) as a model substrate for reaction optimization, which was treated with 1.1 equivalent of $\text{NH}_2\text{OSO}_3\text{H}$ in liquid ammonia at room temperature for 12 h to generate the corresponding diaziridine in situ. Then, base (3.3 equiv.) was gradually added into the mixture and the reaction was further stirred at room temperature under air for 2 h. Lithium amide was first tested and it was able to afford aliphatic diazirine (**2a**) at a 36% yield (Table 1, entry 1). Sodium amide was examined and a slightly higher yield of **2a** was obtained (44%, entry 2). Several metal hydrides were able to mediate the construction of diazirine (**2a**), albeit with moderate yields (entries 3–5). Alkoxides were also utilized to promote the formation of products and the results indicated that a potassium base is preferred to sodium one (entries 6–8). Furthermore, NaOH and KOH [20–25] were employed and it was found that KOH was able to afford **2a** in 67% yield (entries 9 and 10). Although its efficiency was slightly lower compared to *t*-BuOK [19], KOH is easily handled, stored, and highly available in many labs. Its relatively low price makes it feasible for large-scale synthesis of aliphatic diazirines. Moreover, the by-products derived from OH anion can easily be removed in this reaction. Thus, we optimized KOH for the subsequent study. The reaction carried out at either low temperature (entry 11) or in methanolic ammonia (entry 12) caused a low yield of product (**2a**), indicating that both temperature and concentration of ammonia are crucial for the generation of diazirine in this protocol.

2.2. Scope Investigation

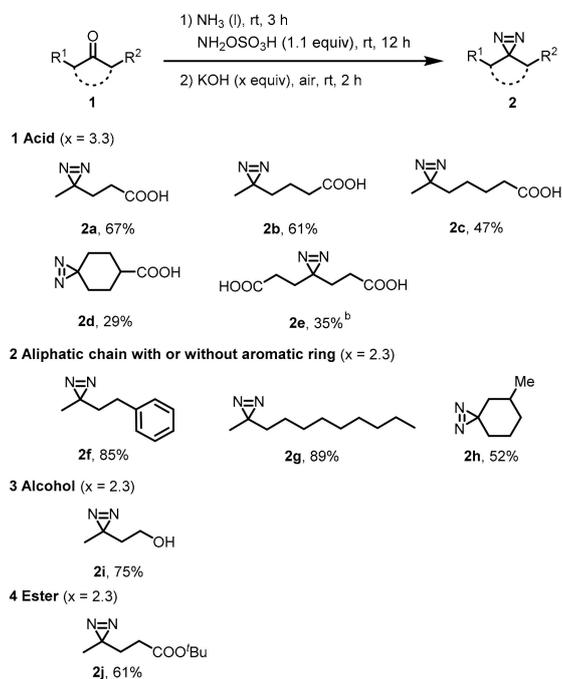
With the optimized condition in hand, the generality of the protocol was evaluated with different ketone procedures. A wide range of substituted ketones—including acid, alcohol, ester, and hexatomic rings—were tolerated under this condition to be converted into the corresponding diazirine products. When monoacid substrates were tested, 3.3 equivalents of KOH were required to create the desired product and the corresponding diazirines were obtained in moderate to good yield (Scheme 2, **2a–d**). A binary acid derivative (**1e**) was subjected to the optimal reaction with 4.3 equivalents of KOH, and the desired product (**2e**) was isolated in 35% yield. Neutral ketones with aliphatic chain or aromatic ring are good substrates (**2f–h**) and up to an 89% yield was given for compound (**2g**). 3-Azibutanol (**2i**), a widely utilized photophore, was readily synthesized with a 75% yield. Compared to the previously synthetic method for **2i**, the KOH-mediated procedure showed a relatively good efficiency. Furthermore, *t*-butyl

alcohol ester was also tolerated in the optimal condition, and the desired product was isolated in 61% yield (**2j**). The spectroscopic data were summarized in Supplementary Materials.

Table 1. Reaction optimization of one-pot synthesis for aliphatic diazirine **2a** ^a.

Entry	Solvent	Base	Temp	Yield (%) ^b
1	NH ₃	LiNH ₂	RT	36
2	NH ₃	NaNH ₂	RT	44
3	NH ₃	NaH	RT	47
4	NH ₃	MgH ₂	RT	20
5	NH ₃	CaH ₂	RT	17
6	NH ₃	EtONa	RT	45
7	NH ₃	MeONa	RT	45
8	NH ₃	MeOK	RT	63
9	NH ₃	NaOH	RT	28
10	NH ₃	KOH	RT	67
11	NH ₃	KOH	0 °C	11
12	NH ₃ /MeOH _c	KOH	RT	5

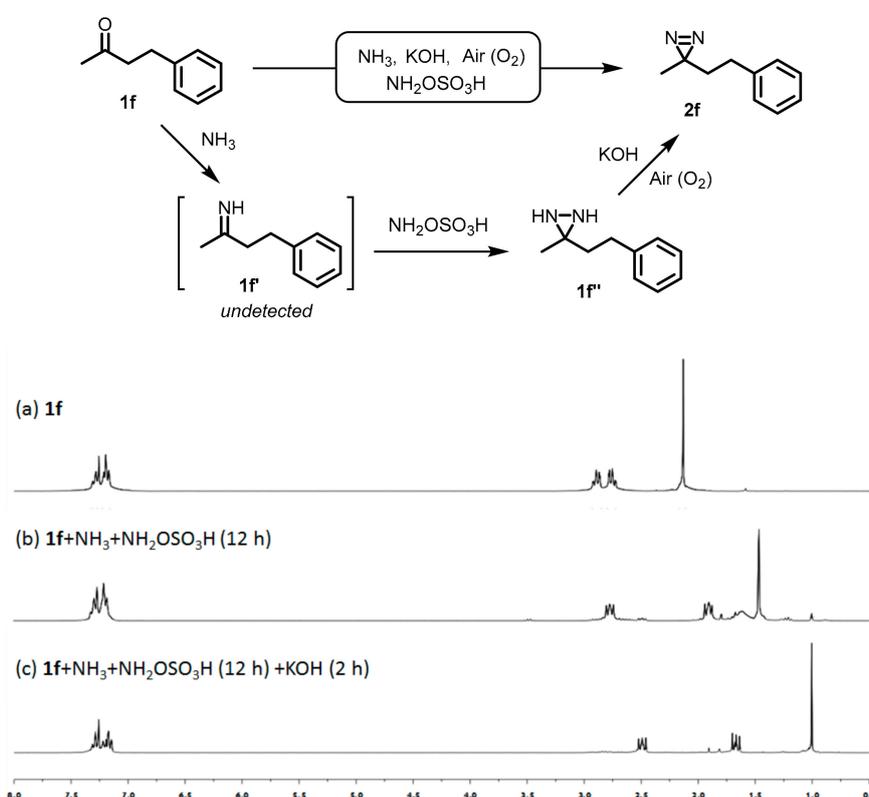
^a Unless otherwise mentioned, **1a** (2 mmol) and NH₂OSO₃H (1.1 equiv.) was added to liquid ammonia (8 mL) at −78 °C and the reaction mixture was stirred at room temperature for 12 h until base (3.3 equiv.) was added. After the addition of base, the reaction was further stirred at room temperature under air for 2 h. ^b Isolated yields were presented. ^c Ammonia gas was bubbled into MeOH (8 mL) at 0 °C for 30 min.



Scheme 2. Scope investigation of one-pot synthesis of aliphatic diazirine with KOH ^a. ^a Unless otherwise mentioned, **1** (2 mmol) and NH₂OSO₃H (1.1 equiv.) was added into liquid ammonia (8 mL) at −78 °C and the reaction mixture was stirred at room temperature for 12 h until base was added. After the addition of base, the reaction was further stirred at room temperature under air for 2 h. ^b 4.3 equivalent of KOH was used.

2.3. Kinetic Study of One-Pot Synthesis of Aliphatic Diazirine

To investigate the one-pot process, we performed a kinetic study based on $^1\text{H-NMR}$ analysis. Aliphatic ketone (**1f**) (Scheme 3a) was initially treated with liquid ammonia at room temperature, while no imine (**1f'**) was observed although the treatment was continued to 12 h. When **1f** was subjected to the reaction with $\text{NH}_2\text{OSO}_3\text{H}$ in liquid ammonia at room temperature for 12 h, the corresponding diaziridine (**1f''**) was detected (Scheme 3b). To be noted, a trace amount of diazirine (**2f**) was generated at this stage which can be detected by $^1\text{H-NMR}$ spectrum. Following with further treatment by KOH for 2 h, the formation of diazirine (**2f**) was dramatically improved (Scheme 3c). Furthermore, no other by-products were distinctly detected in the spectrum during the formation of diaziridine as well as diazirine. These results indicated that diaziridine as the precursor can be directly converted to diazirine in KOH-mediated one-pot reaction. This method is useful with the isolation and purification of the final products being more convenient.

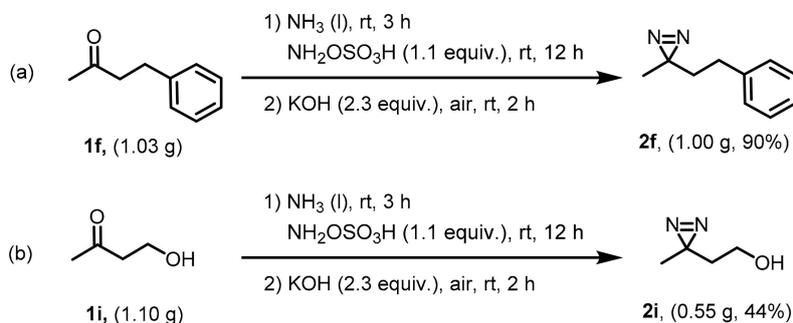


Scheme 3. One-pot synthesis of aliphatic diazirine **2f**. The reaction stages were monitored using $^1\text{H-NMR}$ with CDCl_3 . (a) starting material **1f**; (b) **1f** was treated with $\text{NH}_2\text{OSO}_3\text{H}$ in liquid ammonia for 12 h; (c) The residue was further treated with KOH in liquid ammonia under air for 2 h.

2.4. Gram-Scale Synthesis

To explore the behavior of this protocol for large-scale synthesis of aliphatic diazirines, we designed gram-scale reactions. Compound **1f** (1.03 g) was subjected to the optimized conditions and the desired product (**2f**) can be readily isolated at a 90% yield (Scheme 4a). It undoubtedly confirmed the advantages and applicability of KOH-mediated synthetic strategy for the large-scale synthesis of aliphatic diazirines. 3-Azibutanol (**2i**) is a widely used photophore that labels active molecules in the field of photoaffinity labeling [26–30]. While many syntheses suffered relatively low yields. By using the KOH-mediated one-pot strategy, we can synthesize **2i** in a 75% yield (Scheme 2). It is a great improvement compared with previous synthetic methods as well as the *t*-BuOK-mediated

one. A gram-scale synthesis was carried out and the protocol was able to afford **2i** at a 44% yield from gram-scale **1i** (Scheme 4b), thus indicating the good applicability of KOH-mediated one-pot strategy in large-scale synthesis.



Scheme 4. Gram-scale synthesis of **2f** (a) and **2i** (b).

3. Materials and Methods

3.1. General Procedures

All reactions were carried out in a metal sealed tube (300 mL, 10 MPa, 200 °C). The NMR spectra were measured on JEOL EX-270 (JEOL, Tokyo, Japan). All solvents were of reagent grade and purified using the appropriate methods. HRMS spectra were obtained with a Waters UPLC ESI-TOF mass spectrometer (Waters, Milford, CT, USA).

3.2. Typical Procedures for One-Pot Synthesis of Aliphatic Diazirines from Corresponding Ketones

3.2.1. Acidic Substrates (**1a–e**)

Ketone (2 mmol) was dissolved in liquid NH_3 (8 mL) in a sealed tube at -78 °C. Hydroxylamine-*O*-sulfonic acid (1.1 equiv.) was carefully added at the same temperature. After closing the tube, the reaction mixture was stirred at room temperature for 12 h, before it was cooled at -78 °C and subsequently opened. KOH (3.3 equiv. for **1a–d** and 4.3 equiv. for **1e**) was added to liquid NH_3 mixture and then the tube was sealed. The reaction mixture was further stirred at room temperature under air for 2 h. The ammonia was gradually removed at room temperature in a fume hood. The residue was dissolved with water (30 mL) which was adjust pH to 2 with HCl aq, then extracted twice with Et_2O (30 mL). The organic layer was washed with brine, dried over MgSO_4 , and evaporated. The residue was subjected to silica-gel column chromatography to afford the diazirinyl products.

3.2.2. Neutral Substrates (**1f–j**)

Ketone (2 mmol) was dissolved in liquid NH_3 (8 mL) in a sealed tube at -78 °C. Hydroxylamine-*O*-sulfonic acid (1.1 equiv.) was carefully added at same temperature. After closing the tube, the reaction mixture was stirred at room temperature for 12 h, before it was cooled at -78 °C and subsequently opened. KOH (2.3 equiv.) was added to liquid NH_3 mixture then the tube was sealed. The reaction mixture was stirred at room temperature under air for 2 h. The ammonia was gradually removed at room temperature under a fume hood. The residue was partitioned between water (30 mL) and Et_2O (30 mL). The aqueous layer was extracted twice with Et_2O (30 mL). The combined organic layer was washed by brine, dried over MgSO_4 , and evaporated. The residue was subjected to silica-gel column chromatography to afford the diazirinyl products.

3-(3-Methyl-3H-diazirin-3-yl)propanoic acid (**2a**): Pale yellow oil, $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 11.88 (s, 1H), 2.26 (t, $J = 7.6$ Hz, 2H), 1.73 (t, $J = 7.6$ Hz, 2H), 1.05 (s, 3H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) 178.3, 29.1, 28.2, 24.8, 19.2; HRMS (ESI) m/z [$\text{M} + \text{Na}$] $^+$ calcd. for $\text{C}_5\text{H}_8\text{N}_2\text{O}_2\text{Na}$ 151.0483, found 151.0508.

4-(3-Methyl-3H-diazirin-3-yl)butanoic acid (**2b**): Pale yellow oil, $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 2.35 (t, $J = 7.2$ Hz, 2H), 1.48–1.57 (m, 2H), 1.38–1.44 (m, 2H), 1.02 (s, 3H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) 179.7, 33.4, 33.1, 25.2, 19.5, 19.0; HRMS (ESI) m/z [$\text{M} + \text{Na}$] $^+$ calcd. for $\text{C}_6\text{H}_{10}\text{N}_2\text{O}_2\text{Na}$ 165.0640, found 165.0641.

5-(3-Methyl-3H-diazirin-3-yl)pentanoic acid (**2c**): Pale yellow oil, $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 2.34 (t, $J = 7.4$ Hz, 2H), 1.56–1.67 (m, 2H), 1.35–1.41 (m, 2H), 1.19–1.29 (m, 2H), 1.01 (s, 3H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) 179.9, 33.9, 33.7, 25.5, 24.0, 23.4, 19.6; HRMS (ESI) m/z [$\text{M} + \text{Na}$] $^+$ calcd. for $\text{C}_7\text{H}_{12}\text{N}_2\text{O}_2\text{Na}$ 179.0796, found 179.0778.

1,2-Diazaspiro[2.5]oct-1-ene-6-carboxylic acid (**2d**): Pale yellow solid, $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 2.47–2.57 (m, 1H), 2.02–2.11 (m, 2H), 1.82–1.92 (m, 2H), 1.61–1.73 (m, 2H), 0.87 (dt, $J = 14.1, 3.6$ Hz, 2H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) 181.6, 41.2, 29.8, 27.3, 26.4; HRMS (ESI) m/z [$\text{M} + \text{H}$] $^+$ calcd. for $\text{C}_7\text{H}_{11}\text{N}_2\text{O}_2$ 155.0821, found 155.0809.

3,3'-(3H-Diazirine-3,3-diyl)dipropionic acid (**2e**): Pale yellow solid, $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 2.11 (t, $J = 7.5$ Hz, 4H), 1.72 (t, $J = 7.5$ Hz, 4H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) 176.2, 29.1, 28.9, 28.7; HRMS (ESI) m/z [$\text{M} + \text{Na}$] $^+$ calcd. for $\text{C}_7\text{H}_{10}\text{N}_2\text{O}_4\text{Na}$ 209.0538, found 209.0551.

3-Methyl-3-phenethylidiaziridine (**1f'**): Pale yellow oil, $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 7.27–7.32 (m, 2H), 7.18–7.22 (m, 3H), 2.77 (t, $J = 7.8$ Hz, 2H), 1.91 (t, $J = 7.8$ Hz, 2H), 1.61–1.70 (m, 2H), 1.47 (s, 3H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) δ 141.1, 128.5, 128.2, 126.1, 55.0, 40.4, 31.3, 22.9; HRMS (ESI) m/z [$\text{M} + \text{H}$] $^+$ calcd. for $\text{C}_{10}\text{H}_{15}\text{N}_2$ 163.1235, found 163.1238.

3-Methyl-3-phenethyl-3H-diazirine (**2f**): Pale yellow oil, $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 7.26–7.31 (m, 2H), 7.15–7.22 (m, 3H), 2.46–2.52 (m, 2H), 1.64–1.70 (m, 2H), 1.00 (s, 3H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) 140.9, 128.6, 128.3, 126.2, 36.3, 30.0, 25.6, 19.8; HRMS (ESI) m/z [$\text{M} + \text{H}$] $^+$ calcd. for $\text{C}_{10}\text{H}_{13}\text{N}_2$ 161.1079, found 161.1085.

3-Methyl-3-nonyl-3H-diazirine (**2g**): Pale yellow oil, $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 1.25–1.35 (m, 16H), 0.99 (s, 3H), 0.88 (t, $J = 6.2$ Hz, 3H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) 34.2, 31.8, 29.33, 29.31, 29.2, 29.1, 23.9, 22.5, 19.8, 13.9; HRMS (ESI) m/z [$\text{M} + \text{H}$] $^+$ calcd. for $\text{C}_{11}\text{H}_{23}\text{N}_2$ 183.1861, found 183.1888.

5-Methyl-1,2-diazaspiro[2.5]oct-1-ene (**2h**): Pale yellow oil, $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 1.58–1.81 (m, 5H), 1.36–1.45 (m, 1H), 1.00–1.10 (m, 1H), 0.91 (d, $J = 6.4$ Hz, 3H), 0.50–0.59 (m, 2H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) 39.8, 33.6, 31.0, 30.9, 28.3, 23.5, 21.8; HRMS (ESI) m/z [$\text{M} + \text{H}$] $^+$ calcd. for $\text{C}_7\text{H}_{13}\text{N}_2$ 125.1079, found 125.1084.

2-(3-Methyl-3H-diazirin-3-yl)ethanol (**2i**): Pale yellow oil, $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 3.54 (t, $J = 6.3$ Hz, 2H), 1.65 (t, $J = 6.3$ Hz, 2H), 1.08 (s, 3H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) 57.6, 36.9, 24.1, 20.1; HRMS (ESI) m/z [$\text{M} + \text{H}$] $^+$ calcd. for $\text{C}_4\text{H}_9\text{N}_2\text{O}$ 101.0715, found 101.0738.

Tert-butyl 3-(3-methyl-3H-diazirin-3-yl)propanoate (**2j**): $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ ppm: 2.03 (t, $J = 7.7$ Hz, 2H), 1.59 (t, $J = 7.7$ Hz, 2H), 1.38 (s, 9H), 0.95 (s, 3H); $^{13}\text{C-NMR}$ (68 MHz, CDCl_3) 171.7, 80.6, 30.0, 29.6, 27.9, 25.1, 19.5; HRMS (ESI) m/z [$\text{M} + \text{H}$] $^+$ calcd. for $\text{C}_9\text{H}_{17}\text{N}_2\text{O}_2$ 185.1285, found 185.1302.

4. Conclusions

In conclusion, we reported a comprehensive study about the base-mediated one-pot synthesis of aliphatic diazirines. KOH, a cheap, easily-handled and stored base, was optimized for the one-pot reaction. Many precious diazirines were prepared in moderate to excellent yields. Kinetic study showed that diaziridine as the intermediate can be directly converted to diazirine after the addition

of KOH under air. Gram-scale synthesis indicated its good applicability in the large-scale synthesis of aliphatic diazirines for photoaffinity labeling. The reactions are pretty neat and products can be easily isolated and purified. This protocol will be helpful for effective synthesis of diazirine-based photoaffinity label ligands to elucidate functional analysis of biological activities.

Supplementary Materials: Copies of the ^1H - and ^{13}C -NMR spectra are available online.

Acknowledgments: This research was partially supported by the Ministry of Education, Science, Sports, and Culture Grant-in-Aid for Scientific Research (C), (17K0194007 to MH). Part of this work was performed under the Cooperative Research Program of "Network Joint Research Center for Materials and Devices".

Author Contributions: L.W. and M.H. conceived and designed the experiments; L.W., Z.P.T., N.K., and F.O. performed the experiments; L.W. and M.H. analyzed the data; M.H. contributed reagents/materials/analysis tools; Y.S., Y.H., and M.H. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Brunner, J. New photolabeling and cross-linking methods. *Ann. Rev. Biochem.* **1993**, *62*, 483–514. [[CrossRef](#)] [[PubMed](#)]
2. Ruoho, A.E.; Kiefer, H.; Roeder, P.E.; Singer, S.J. The Mechanism of Photoaffinity Labeling. *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 2567–2571. [[CrossRef](#)] [[PubMed](#)]
3. Hatanaka, Y. Development and leading-edge application of innovative photoaffinity labeling. *Chem. Pharm. Bull.* **2015**, *63*, 1–12. [[CrossRef](#)] [[PubMed](#)]
4. Tomizawa, M.; Casida, J.E. Molecular recognition of neonicotinoid insecticides: The determinants of life and death. *Acc. Chem. Res.* **2009**, *42*, 260–269. [[CrossRef](#)] [[PubMed](#)]
5. Hashimoto, M.; Hatanaka, Y. Recent progress in diazirine-based photoaffinity labeling. *Eur. J. Org. Chem.* **2008**, *2008*, 2513–2523. [[CrossRef](#)]
6. Fleet, G.W.J.; Porter, R.R.; Knowles, J.R. Affinity labeling of antibodies with aryl nitrene as reactive group. *Nature* **1969**, *224*, 511–512. [[CrossRef](#)]
7. Ishida, A.; Wang, L.; Tachrim, Z.P.; Suzuki, T.; Sakihama, Y.; Hashidoko, Y.; Hashimoto, M. Comprehensive synthesis of photoreactive phenylthiourea derivatives for the photoaffinity labeling. *Chemistryselect* **2017**, *2*, 160–164. [[CrossRef](#)]
8. Murai, Y.; Yoshida, T.; Wang, L.; Masuda, K.; Hashidoko, Y.; Monde, K.; Hatanaka, Y.; Hashimoto, M. Efficient synthesis of photoreactive 2-propoxyaniline derivatives as artificial sweeteners. *Synlett* **2016**, *27*, 946–950. [[CrossRef](#)]
9. Wang, L.; Yoshida, T.; Muto, Y.; Murai, Y.; Tachrim, Z.P.; Ishida, A.; Nakagawa, S.; Sakihama, Y.; Hashidoko, Y.; Masuda, K.; et al. Synthesis of diazirine based photoreactive saccharin derivatives for the photoaffinity labeling of gustatory receptors. *Eur. J. Org. Chem.* **2015**, 3129–3134. [[CrossRef](#)]
10. Wang, L.; Hashidoko, Y.; Hashimoto, M. Co-solvent promoted *O*-benzylation with silver(I) oxide: Synthesis of 1'-benzylated sucrose derivatives, mechanistic studies and scope investigation. *J. Org. Chem.* **2016**, *81*, 4464–4474. [[CrossRef](#)] [[PubMed](#)]
11. Wang, L.; Murai, Y.; Yoshida, T.; Ishida, A.; Masuda, K.; Sakihama, Y.; Hashidoko, Y.; Hatanaka, Y.; Hashimoto, M. Alternative one-pot synthesis of (trifluoromethyl)phenyldiazirines from tosyloxime derivatives: Application for new synthesis of optically pure diazirinylphenylalanines for photoaffinity labeling. *Org. Lett.* **2015**, *17*, 616–619. [[CrossRef](#)] [[PubMed](#)]
12. Das, J. Aliphatic diazirines as photoaffinity probes for proteins: Recent developments. *Chem. Rev.* **2011**, *111*, 4405–4417. [[CrossRef](#)] [[PubMed](#)]
13. Thirumurugan, P.; Matosiuk, D.; Jozwiak, K. Click chemistry for drug development and diverse chemical–biology applications. *Chem. Rev.* **2013**, *113*, 4905–4979. [[CrossRef](#)] [[PubMed](#)]
14. Böttcher, T.; Pitscheider, M.; Sieber, S.A. Natural products and their biological targets: Proteomic and metabolomic labeling strategies. *Angew. Chem. Int. Ed.* **2010**, *49*, 2680–2698. [[CrossRef](#)] [[PubMed](#)]
15. Takaoka, Y.; Ojida, A.; Hamachi, I. Protein organic chemistry and applications for labeling and engineering in live-cell systems. *Angew. Chem. Int. Ed.* **2013**, *52*, 4088–4106. [[CrossRef](#)] [[PubMed](#)]

16. Wratil, P.R.; Horstkorte, R.; Reutter, W. Metabolic glycoengineering with *N*-acyl side chain modified mannosamines. *Angew. Chem. Int. Ed.* **2016**, *55*, 9482–9512. [[CrossRef](#)] [[PubMed](#)]
17. Church, R.F.R.; Kende, A.S.; Weiss, M.J. Diazirines. I. Some observations on the scope of the ammonia-hydroxylamine-*O*-sulfonic acid diaziridine synthesis. The preparation of certain steroid diaziridines and diazirines. *J. Am. Chem. Soc.* **1965**, *87*, 2665–2671. [[CrossRef](#)]
18. Church, R.F.R.; Weiss, M.J. Diazirines. II. Synthesis and properties of small functionalized diazidine molecules. Observations on the reaction of a diaziridine with the iodine-iodide ion system. *J. Org. Chem.* **1970**, *35*, 2465–2471. [[CrossRef](#)]
19. Wang, L.; Ishida, A.; Hashidoko, Y.; Hashimoto, M. Dehydrogenation of NH-NH bond triggered by potassium *t*-butoxide in liquid NH₃. *Angew. Chem. Int. Ed.* **2017**, *56*, 870–873. [[CrossRef](#)] [[PubMed](#)]
20. Baars, H.; Beyer, A.; Kohlhepp, S.V.; Bolm, C. Transition-metal-free synthesis of benzimidazoles mediated by KOH/DMSO. *Org. Lett.* **2014**, *16*, 536–539. [[CrossRef](#)] [[PubMed](#)]
21. Xu, J.; Zhuang, R.; Bao, L.; Tang, G.; Zhao, Y. KOH-mediated transition metal-free synthesis of imines from alcohols and amines. *Green Chem.* **2012**, *14*, 2384–2387. [[CrossRef](#)]
22. Beyer, A.; Reucher, C.M.M.; Bolm, C. Potassium hydroxide/dimethyl sulfoxide promoted intramolecular cyclization for the synthesis of benzimidazol-2-ones. *Org. Lett.* **2011**, *13*, 2876–2879. [[CrossRef](#)] [[PubMed](#)]
23. Reddy, V.P.; Kumar, A.V.; Swapna, K.; Rao, K.R. Copper oxide nanoparticle-catalyzed coupling of diaryl diselenide with aryl halides under ligand-free conditions. *Org. Lett.* **2009**, *11*, 951–953. [[CrossRef](#)] [[PubMed](#)]
24. Anderson, K.W.; Ikawa, T.; Tundel, R.E.; Buchwald, S.W. The selective reaction of aryl halides with KOH: Synthesis of phenols, aromatic ethers, and benzofurans. *J. Am. Chem. Soc.* **2006**, *128*, 10694–10695. [[CrossRef](#)] [[PubMed](#)]
25. Kawabata, T.; Moriyama, K.; Kawakami, S.; Tsubaki, K. Powdered KOH in DMSO: An efficient base for asymmetric cyclization via memory of chirality at ambient temperature. *J. Am. Chem. Soc.* **2008**, *130*, 4153–4157. [[CrossRef](#)] [[PubMed](#)]
26. Kambe, T.; Correia, B.E.; Niphakis, M.J.; Cravatt, B.F. Mapping the protein interaction landscape for fully functionalized small-molecule probes in human cells. *J. Am. Chem. Soc.* **2014**, *136*, 10777–10782. [[CrossRef](#)] [[PubMed](#)]
27. Liang, J.; Zhang, L.; Tan, X-L.; Qi, Y-K.; Feng, S.; Deng, H.; Yan, Y.; Zheng, J-S.; Liu, L.; Tian, C-L. Chemical synthesis of diubiquitin-based photoaffinity probes for selectively profiling ubiquitin-binding proteins. *Angew. Chem. Int. Ed.* **2017**, *56*, 2744–2748. [[CrossRef](#)] [[PubMed](#)]
28. Arevalo, E.; Shanmugasundararaj, S.; Wilkemeyer, M.F.; Dou, X.; Chen, S.; Charness, M.E.; Miller, K.W. An alcohol binding site on the neural cell adhesion molecule L1. *Proc. Natl. Acad. Sci. USA.* **2008**, *105*, 371–375. [[CrossRef](#)] [[PubMed](#)]
29. Shigdel, U.K.; Zhang, J.; He, C. Diazirine-based DNA photo-cross-linking probes for the study of protein–DNA interactions. *Angew. Chem. Int. Ed.* **2008**, *47*, 90–93. [[CrossRef](#)] [[PubMed](#)]
30. Morieux, P.; Salomé, C.; Park, K.D.; Stables, J.P.; Kohn, H. The structure-activity relationship of the 3-Oxy site in the anticonvulsant (*R*)-*N*-Benzyl 2-acetamido-3-methoxypropionamide. *J. Med. Chem.* **2010**, *53*, 5716–5726. [[CrossRef](#)] [[PubMed](#)]

Sample Availability: Samples of the compounds are available from the authors.



© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).