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Alternative One-Pot Synthesis of (Trifluoromethyl)phenyldiazirines from Tosyloxime Derivatives: Application for New Synthesis of Optically Pure Diazirinylphenylalanines for Photoaffinity Labeling

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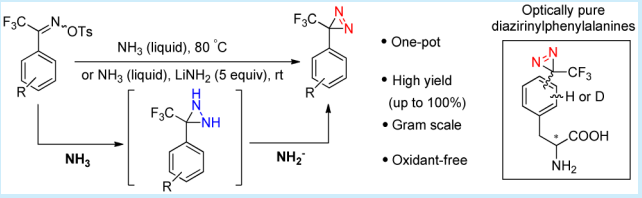
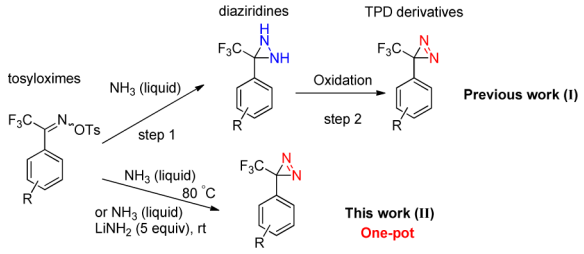
Supporting Information

ABSTRACT: Alternative one-pot synthesis of 3-(trifluoromethyl)-3-phenyldiazirine derivatives from corresponding tosyloximes is developed. The deprotonation of intermediate diaziridine by NH_2^- is a new approach for construction of diazirine. Moreover, a novel synthesis of optically pure (trifluoromethyl)diazirinylphenylalanine derivatives was attempted involving these methods.



3-(Trifluoromethyl)-3-phenyldiazirine (TPD)[1] has emerged as the most effective photoreactive group in photoaffinity labeling[2] because of its relatively small size, long activation wavelength (approximately 360 nm), thermal and chemical stability, low rate of rearrangement, and high reactivity of its intermediate.[3] Nevertheless, compared to the other commonly used photoreactive groups in photoaffinity labeling (aryl azides[2b] and benzophenones[4]), the tedious synthetic procedure of TPD derivatives is a major consideration for researchers. In the past decades, the formation of diaziridines from tosyloxime derivatives is the crucial step to further construct TPD (Scheme 1, I). Generally, liquid NH_3 was used to construct diaziridines from tosyloximes (Scheme 1, I, step 1). Despite its wide use, long reaction time and low yields were reported.[5] To further construct TPD derivatives by oxidation[6] (Scheme 1, I, step 2), the inevitable isolation of diaziridines, preparation of fresh oxidant,[6b] and post-treatment after oxidation were also time-consuming and yield-diminishing. In light of the importance of TPD derivatives, a convenient method to prepare TPD derivatives from tosyloximes can not only decrease the cost and waste but also minimize the synthetic cycles of TPD-containing probes in photoaffinity labeling.

Scheme 1. Construction of TPD Derivatives from the Corresponding Tosyloximes



Optically pure (trifluoromethyl)diazirinylphenylalanine ((Tmd)Phe) has been used as important building block for investigation of peptides, proteins, and other biomacromolecule.[7] One widely used method to prepare optically pure (Tmd)Phe derivatives involved the concatenation of halogenated TPD and α-amino acids followed by the enzymatic resolution of *N*-acylamino acids[7e,8] or the use of amino acid oxidase to afford the relative configuration.[9] Asymmetric syntheses were achieved through treatment of halogenated TPD with the chiral Ni complex[10] or cinchonidine-based asymmetric catalyst.[7b] By combination of *p*-diodobenzene and L-serine, Fillion et al. prepared Fmoc-L-(4-Tmd)Phe with a 14% overall yield via 10 steps.[7c] However, although the preparation were successful, all of the above-mentioned methods generally could not circumvent tedious enzymatic procedure, low yields, complicated preparation of chiral complex, or a limited configuration of the desired products. Herein, for the first time, we developed a one-pot synthesis of TPD derivatives from the corresponding tosyloximes (Scheme 1, II). For further application, direct construction of the Tmd group on optically pure phenylalanines for preparation of (Tmd)Phe derivatives was first reported.

During the synthesis of diaziridines with liquid NH_3, we found that the corresponding TPD derivatives were sometimes generated in small amounts, possibly derived from diaziridines. To confirm our hypothesis, initial studies were performed using

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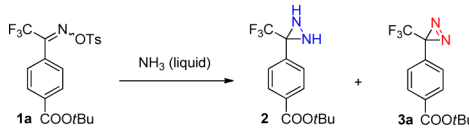
tosyloxime **1a** as the model substrate. It was found that formation of TPD **3a**, at room temperature, was dependent on reaction time to a great extent (Table 1, entries 1–3).

Table 1. Optimization of the Reaction Conditions[a]



entry	solvent	temp (°C)	additive (equiv)	time (h)	yield **2**/**3a**[b] (%)
1	Et_2O	rt		1	100/0
2	Et_2O	rt		12	82/18
3	Et_2O	rt		96	3/97
4[c]	Et_2O	60		8	0/100
5[c]	**Et_2O**	**80**		**4**	**0/100 (97)**
6[c]	CH_2Cl_2	80		4.5	0/100
7[c]	THF	80		5	0/100
8[c]		80		4	0/100
9[d]		80		4	100/0
10[c]	Et_2O	80	NH_4Cl (5)	4	84/16
11	Et_2O	−78	$LiNH_2$ (5)	12	100/0
12	Et_2O	0	$LiNH_2$ (5)	10	0/100
13	**Et_2O**	**rt**	**$LiNH_2$ (5)**	**4**	**0/100 (96)**
14	Et_2O	rt	$NaNH_2$ (5)	1	
15	Et_2O	rt	NaH (5)	1	
16	Et_2O	rt	$LiNH_2$ (10)	4	0/100
17	Et_2O	rt	$LiNH_2$ (2)	8	80/20
18	CH_2Cl_2	rt	$LiNH_2$ (5)	7	0/100
19	THF	rt	$LiNH_2$ (5)	6	0/100
20		rt	$LiNH_2$ (5)	4.5	0/100

[a]Reaction conditions: **1a** (0.3 mmol), solvent (0.5 mL), liquid NH_3 (5 mL) in a sealed tube. [b]Yields were determined by ^1H NMR spectroscopy. Isolated yield of **3a** in parentheses. [c]Liquid NH_3 (10 mL) was used. [d]**1a** was directly treated with gaseous NH_3 at 80 °C in a sealed tube.

Temperature optimization indicated that 80 °C was clearly ideal for the reaction (Table 1, entries 4 and 5). However, reaction at 100 °C resulted in decomposition of the diazirine ring within 1 h (Figure S1, Supporting Information). No decomposition of diazirine and high isolated yield of **3a** (Table 1, entry 5) indicated the viability of this strategy. Other solvents also worked well, although reactions did not improve (Table 1, entries 6 and 7). An attempt to perform the reaction in the absence of solvent was successful (Table 1, entry 8), which was beneficial for the tosyloximes with low solubility in common organic solvents. To confirm the species responsible for the formation of **3a**, tosyloxime **1a** was directly treated with gaseous NH_3 at 80 °C (Table 1, entry 9). Diaziridine **2** was detected as the sole product without **3a**, indicating liquid NH_3 was essential for the formation of **3a**. On the basis of these results, we postulated that the NH_2^- species generated from liquid NH_3[11] may be responsible for the formation of TPD **3a**, despite liquid NH_3 having a low self-ionization constant (pK_a = 27.6 at 25 °C).[12] A control experiment with NH_4Cl as an ion counter for inhibiting the self-ionization of liquid NH_3 also confirmed this hypothesis (Table 1, entry 10). Inspired by these results, a series of experiments with alkali amide as NH_2^- supplier in liquid NH_3 were carried out at low temperature. Initially, lithium amide was tested, but the reaction at −78 °C afforded **2** in 100% yield without **3a** after 12 h (Table 1, entry 11). To our delight, reaction at 0 °C provided **3a** in 100% yield within 10 h (Table 1, entry 12). The reaction was completed within 4 h at room temperature (Table 1, entry 13). Sodium amide and sodium hydride afforded to no desired product due to the ammonolysis of ester (Table 1, entries 14 and 15), but diazirine ring was formed. Further investigation indicated that both reagents were suitable for synthesis of TPD without substituent (Table S1, Supporting Information). Compared to sodium amide, lithium amide displays low solubility in liquid NH_3,[13] which has a wide application scope due to its milder conditions. The highly isolated yield of **3a** (96%, Table 1, entry 13) also proved its viability. The amount of lithium amide should be set as 5 equiv to maintain the efficiency (Table 1, entries 16 and 17). Solvent optimization was carried out, although the efficiency was not improved (Table 1, entries 18–20). Because of the similar efficiency and various advantages, we outlined the alternative strategies for one-pot construction of TPD from tosyloxime: one was accomplished with liquid NH_3 at 80 °C without any additive (Table 1, entry 5, method A), and the other involved lithium amide in liquid NH_3 at room temperature (Table 1, entry 13, method B).

To clearly present the conversion of the substrates, we carried out a kinetic investigation of the model reaction by method A (Figure 1). As shown in the kinetic curve plot,

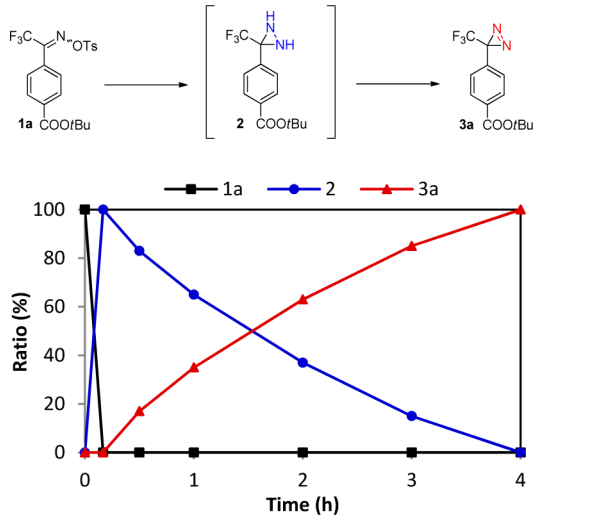


Figure 1. Kinetic investigation of one-pot synthesis of **3a**. Reaction conditions: **1a** (0.3 mmol), Et_2O (0.5 mL), liquid NH_3 (10 mL) in a sealed tube at 80 °C. Ratio were determined by ^1H NMR spectroscopy (for details, see Figure S2, Supporting Information).

tosyloxime **1a** decreased rapidly and diaziridine **2** was quickly generated within 10 min. TPD **3a** formed gradually with the consumption of diaziridine **2**. After 4 h, diaziridine **2** was completely converted to TPD **3a**. These results also indicated that diaziridine **2** was the intermediate for formation of **3a** in this reaction.

To highlight the viability of these strategies, gram-scale reactions were carried out, respectively (Table 2, entries 1 and 2). As expected, TPD **3a** was obtained in high yield by these methods. With the optimal conditions in hand, the scope of the reaction was explored with commonly used TPD derivatives for postfunctional synthesis in photoaffinity labeling.[14] Both of the methods readily produced the desired products regardless of the electronic properties of the substituents (Table 2, entries 3–16). Substrates with both electron-deficient and -rich

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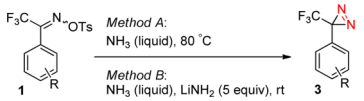
Table 2. One-Pot Synthesis of TPD Derivatives[a]



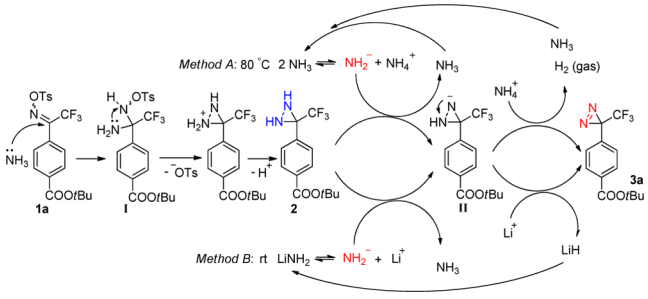
entry	R	method	yield (%), time (h)
1[b]	(**1a**) *p*-COO*t*Bu	A	(**3a**) 90 (5)
2[b]	(**1a**) *p*-COO*t*Bu	B	(**3a**) 88 (6)
3	(**1b**) H	A	(**3b**) 97 (7)
4	(**1b**) H	B	(**3b**) 99 (6)
5	(**1c**) *p*-CH_3	A	(**3c**) 98 (11)
6	(**1c**) *p*-CH_3	B	(**3c**) 97 (12)
7	(**1d**) *m*-CH_3	A	(**3d**) 99 (6)
8	(**1d**) *m*-CH_3	B	(**3d**) 100 (8)
9	(**1e**) *p*-OCH_3	A	(**3e**) 94 (13)
10	(**1e**) *p*-OCH_3	B	(**3e**) 92 (16)
11	(**1f**) *m*-OCH_3	A	(**3f**) 96 (10)
12	(**1f**) *m*-OCH_3	B	(**3f**) 99 (9)
13	(**1g**) *m*-NO_2	A	(**3g**) 99 (1)
14	(**1g**) *m*-NO_2	B	(**3g**) 98 (1)
15	(**1h**) *m*-NHBoc	A	(**3h**) 93 (10)
16	(**1h**) *m*-NHBoc	B	(**3h**) 91 (9)

[a]Reaction conditions: **1** (0.3 mmol), Et_2O (0.5 mL), isolated yields, NH_3 (liquid): 10 mL for method A, 5 mL for method B. [b]**1a** (1.0 g) was used.

substituents afforded to the desired products in high yields, and the former generally led to completion in shorter time. The nitro group also survived under these reactions (Table 2, entries 13 and 14). It should be noted that, for these two methods, byproducts could be readily removed by washing with water and a normal purification procedure such as silica gel column chromatography is not necessary, which averts the material-consuming and yield-diminishing purification procedure.

A plausible mechanism is outlined in Scheme 2. Initially, tosyloxime **1a** is attacked by NH_3 molecule to form the

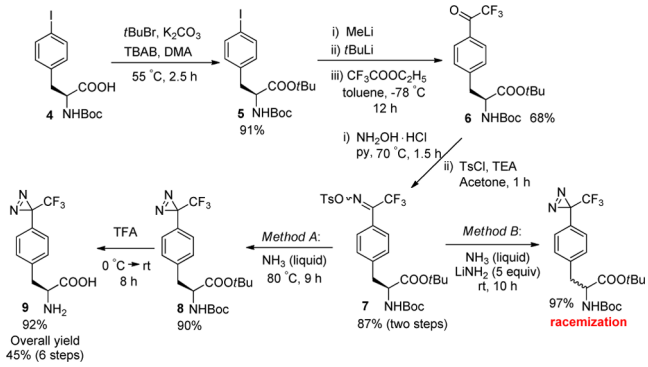
Scheme 2. Plausible Mechanism



intermediate *gem*-diamine **I**.[15] With the removal of tosyl group and proton, diaziridine **2** is formed. At a high temperature (method A), deprotonation occurs at the diaziridine ring, forming the intermediate **II** in the presence of NH_2^- derived from self-ionization of liquid NH_3. The electron pair over nitrogen can attack the other one to release TPD **3a** along with hydrogen and NH_3. When lithium amide is added to the reaction (method B), deprotonation of **2** was mainly triggered by the NH_2^- derived from lithium amide. The generated lithium hydride can further react with liquid NH_3 to form lithium amide.[16]

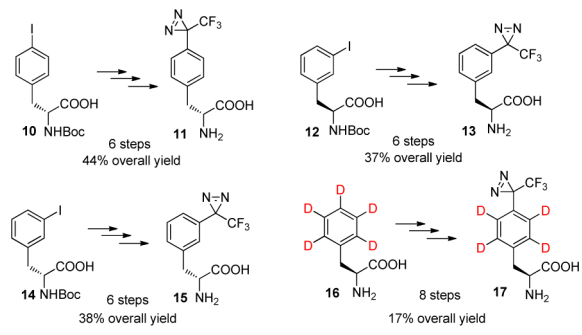
Using these newly developed methods, a novel strategy for preparation of optically pure (Tmd)Phe derivatives was attempted. Commercially available Boc-L-Phe(4-I)-OH **4** was treated with *tert*-butyl bromide to form Boc-L-Phe(4-I)-O-*t*-Bu **5** (Scheme 3). Lithiation of Boc-L-Phe(4-I)-O-*t*-Bu **5** with *t*-

Scheme 3. Synthesis of Optically Pure L-(4-Tmd)Phe



BuLi (2.5 equiv) and MeLi (1.3 equiv) followed by treatment with ethyl trifluoroacetate afforded **6**. However, use of a 1.5 equiv of *t*-BuLi or less led to the deiodination of **5**. Following further oximation and tosylation, tosyloxime **7** was obtained. One-pot synthesis of **8** was conducted via methods A and B, respectively. It was found that method A afforded optically pure product in 90% yield but method B resulted in the racemization of phenylalanine. The possible reason could be that, for method B, deprotonation of the amino group at the aliphatic chain may result in proton transfer at the adjacent position to form carbanion. Further deprotection of **8** with trifluoroacetic acid afforded L-(4-Tmd)Phe **9** in 92% yield. Thus, using method A, the overall yield of L-(4-Tmd)Phe was calculated as 45% via six steps. The synthesis of L-(4-Tmd)Phe was significantly shortened in comparison to previous methods (9 steps in 36% yield from trifluoroacetophenone,[7b] 10 steps in 14% yield from *p*-diodobenzene and L-serine for Fmoc-L-(4-Tmd)Phe,[7c] and 12 steps in 47% yield from 4-bromobenzyl alcohol[10a]). Inspired by this result, D-(4-Tmd)Phe **11**, L-(3-Tmd)Phe **13**, and D-(3-Tmd)Phe **15** were all prepared in moderate yields (Scheme 4). The use of isotope-labeled compounds for mass analysis is an effective technique to identify the photolabeled components in photoaffinity labeling.[17] Previously, we reported the preparation of deuterated L-(4-Tmd)Phe by H/D exchange with TfOD,[18] but the deuteration efficiency was up to 70%.[19] Using our new method, we successfully prepared the highly

Scheme 4. Comprehensive Synthesis of Optically Pure (Tmd)Phe Derivatives by Method A



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deuterated L-(4-Tmd)Phe **17** from deuterated L-Phe **16**. HPLC spectra indicated that all of the (Tmd)Phe derivatives (**9**, **11**, **13**, **15**, and **17**) retained their optical purity (Figure S3, Supporting Information). The photoreactive properties of the L-(Tmd)Phe derivatives (**9**, **13**, and **17**) were further investigated in the presence of CH_3OH and CD_3OD under UV irradiation (Figure S4, Supporting Information). The half-lives ($t_{1/2}$) were calculated as 1.3, 0.6, and 1.3 min for **9**, **13**, and **17**, respectively, indicating their effective photoreactivity. Then, the reaction mixtures were subjected to MS analysis for investigation of the photoreaction products (Figure S5, Supporting Information).

In conclusion, we developed a novel approach to synthesize TPD derivatives from the corresponding tosyloximes in a one-pot reaction. NH_2^- species generated from liquid NH_3 or lithium amide contribute to the formation of TPD derivatives. These one-pot methods will significantly shorten the synthetic route of TPD derivatives in photoaffinity labeling. To conveniently prepare optically pure (Tmd)Phe derivatives, these one-pot methods were applied. The result indicated that liquid NH_3 at 80 °C was feasible while reaction with lithium amide in liquid NH_3 led to the racemization of phenylalanine.

■ ASSOCIATED CONTENT

Supporting Information

General information, experimental procedures, characterization, and NMR, HPLC, UV, and mass spectra of corresponding products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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