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Synthesis of benzimidazoles from *o*-phenylenediamines with α -keto acids *via* amino acid catalysis

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A green and simple method for the synthesis of benzimidazoles by amino acid catalysis of *o*-phenylenediamines with α -keto acids has been developed. The reaction proceeded smoothly in water under air and metal catalyst-free conditions to obtain 2-substituted benzimidazoles. Furthermore, the method was performed in an open system at room temperature, making the experimental operation very simple and greatly enhancing the accessibility of the method.

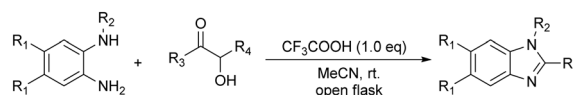
Benzimidazole is an aromatic heterobicyclic compound containing a benzene ring fused to a pentacyclic 1,3-diazole.¹ The substituents on the peripheral positions of its skeleton exhibit a wide range of biological activities, making it a “master key” in drug synthesis.² Benzimidazole derivatives with biological activities have been applied to gastric antacid, antiviral agents, anticoagulants, antihypertensive agents, antiallergic agents,³ antioxidant agents,⁴ and anticancer medications,^{5,6} and for anti-inflammatory⁷ and anthelmintic activity.⁸ Additionally, researchers have developed various benzimidazoles with anti-protozoal and antibacterial activities,⁹ which have facilitated the development of many important pharmaceuticals.¹⁰ Benzimidazoles are also excellent fungicides,^{11–13} some of which show 13 times higher activity than norfloxacin in inhibiting *Staphylococcus aureus*.¹⁴ Furthermore, they also have great potential in the development of antiviral agents targeting herpes simplex virus (HSV).¹⁵ In recent years, benzimidazole derivatives have also been employed as high-performance, sustainable electrode materials for supercapacitors,¹⁶ as well as in chemosensors for the detection of toxic ions.¹⁷

The versatile applications of benzimidazoles have sparked a surge of interest among synthetic chemists, leading to the proliferation of modified synthetic methodologies in recent years.^{18–20} In addition to the commonly used iron,^{21,22} copper,²³ silver,²⁴ and palladium catalyzed^{25,26} methods for synthesizing benzimidazoles, photochemical^{27–29} and electrochemical^{30,31} approaches have also been explored. Some groups have even used novel materials.^{32–39} Some reports have introduced metal free methods.^{40,41} Recently, Biswas and co-workers demonstrated the synthesis of benzimidazole under visible light irradiation in the presence of 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine.⁴²

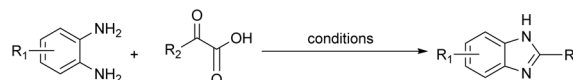
Besides, the acid catalyzed method has also been developed by other researchers.^{43–45} Our group has previously developed an acid-promoted approach.⁴⁶ Although this method carried out under ambient conditions in an open flask, it still required 1.0 equivalent of acid, and *o*-phenylenediamine necessitated a protecting group. Therefore, we wanted to explore a new method of synthesizing benzimidazole without protecting group in an open flask.

In our previous studies, we discovered that α -keto acids reacted with *o*-phenylenediamines to afford benzimidazoles, and some methods suggested that amino acids exhibit catalytic activity.^{47,48} Consequently, we explored the use of amino acids as catalysts (Scheme 1). Initially, the reaction between *o*-phenylenediamine (**1a**) and pyruvic acid (**2a**) was conducted in water at room temperature. Disappointingly, only 3-methylquinoxaline-2(1*H*)-one (**4a**) was obtained, which was obviously not the desired product (Table 1, entry 1). Similar results have also been reported in previous reports.⁴⁹ Subsequently, each of the 20 standard amino acids was individually screened as an additive. For brevity, only those yielding 2-methyl-1*H*-benzo[*d*]imidazole (**3a**) were reported herein. Fortunately, the addition of L-Lysine and L-arginine afforded **3a** (Table 1, entries 2,3), while the

Previous our work:



This work:



Scheme 1 Methods for the synthesis of benzimidazoles.

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Table 1 Optimization of the reaction conditions^a

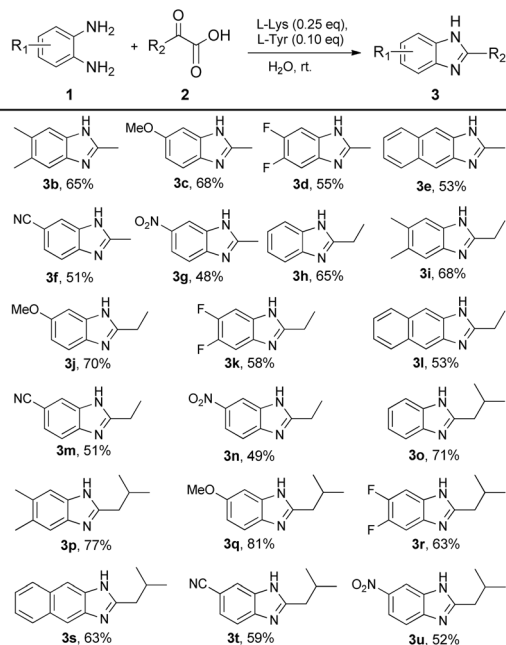
Entry	Additive	Solvent	T (°C)	Yield (%) / 3a	Yield (%) / 4a
1	—	H ₂ O	25	—	91
2	L-lys (0.25 eq)	H ₂ O	25	47	12
3	L-Arg (0.25 eq)	H ₂ O	25	45	12
4	D-Lys (0.25 eq)	H ₂ O	25	48	12
5	D-Arg (0.25 eq)	H ₂ O	25	46	12
6	(±)-Lys (0.25 eq)	H ₂ O	25	48	12
7	(±)-Arg (0.25 eq)	H ₂ O	25	45	11
8	L-Lys (0.50 eq)	H ₂ O	25	38	7
9	L-Arg (0.50 eq)	H ₂ O	25	36	7
10	L-Lys (0.06eq), L-Arg (0.06eq)	H ₂ O	25	46	12
11	L-Lys (0.25eq), L-Tyr (0.10eq)	H ₂ O	25	53	7
12	L-Arg (0.25eq), L-Tyr (0.10eq)	H ₂ O	25	51	7
13	L-Lys (0.25eq), L-Phe (0.10eq)	H ₂ O	25	41	5
14	L-Lys (0.25eq), L-Tyr (0.10eq)	MeCN	25	13	31
15	L-Lys (0.25eq), L-Tyr (0.10eq)	DMF	25	13	27
16	L-Lys (0.25eq), L-Tyr (0.10eq)	EtOH	25	21	13
17	L-Lys (0.25eq), L-Tyr (0.10eq)	THF	25	—	65
18	L-Lys (0.25eq), L-Tyr (0.10eq)	DMSO	25	—	63
19	L-Lys (0.25eq), L-Tyr (0.10eq)	DCM	25	—	51
20	L-Lys (0.25eq), L-Tyr (0.10eq)	H ₂ O	0	52	8
21	L-Lys (0.25eq), L-Tyr (0.10eq)	H ₂ O	37	53	7
22	L-Lys (0.25eq), L-Tyr (0.10eq)	H ₂ O	50	61	7
23	L-Lys (0.25eq), L-Tyr (0.10eq)	H ₂ O	60	59	7
24	L-Lys (0.25eq), L-Tyr (0.10eq)	H ₂ O	80	35	21
25	—	H ₂ O	50	—	89
26 ^b	L-Lys (0.25eq), L-Tyr (0.10eq)	H ₂ O	25	65	7
27 ^c	L-Lys (0.25eq), L-Tyr (0.10eq)	H ₂ O	25	69	7
28 ^c	L-Lys (0.25eq), L-Tyr (0.10eq)	H ₂ O	50	71	1
29	L-Lys (0.25eq), L-Tyr (0.10eq), TEMPO (1.00 eq)	H ₂ O	25	63	8
30	L-Lys (0.25eq), L-Tyr (0.10eq), pH = 4	H ₂ O	25	—	80
31	L-Lys (0.25eq), L-Tyr (0.10eq), pH = 6	H ₂ O	25	—	83
32	L-Lys (0.25eq), L-Tyr (0.10eq), pH = 8	H ₂ O	25	51	8
33	L-Lys (0.25eq), L-Tyr (0.10eq), pH = 10	H ₂ O	25	53	8
34	[0.1 M] PBS buffer, pH = 8	H ₂ O	25	43	4
35	[0.1 M] PBS buffer, pH = 10	H ₂ O	25	48	5
36	NaOH (1.2 eq)	H ₂ O	25	35	12
37	<i>t</i> -BuOK (1.2 eq)	EtOH	25	45	11

^a Reaction conditions: substrate **1a** (0.5 mmol), **1b** (0.6 mmol), amino acids, water (15.0 mL) in an open flask. ^b Pyruvic acid was divided into two batches. ^c Pyruvic acid was divided into four batches.

remaining 18 amino acids failed (see SI for details). It was evident that a significant amount of **4a** was produced. Next, D-lysine and D-arginine were used for experiments respectively, and the yield of **3a** did not enhance significantly (Table 1, entries 4, 5), even if (±)-lysine and (±)-arginine were added (Table 1, entries 6, 7). This suggested that the amino acid configuration did not play a decisive role in improving the yield. With the increase of the amount of amino acids added, the yield of **3a** decreased, mainly because **1a** was not completely consumed (Table 1, entries 8, 9). Subsequently, the test of adding two different amino acids was detected. The addition of L-lysine and L-arginine did not improve the yield of **3a** (Table 1, entry 10), whereas the combination of L-lysine/L-Arg with L-

tyrosine made it (Table 1, entries 11, 12). A reaction was conducted by adding L-phenylalanine with similar structure to L-tyrosine, but no increase in the yield was detected (Table 1, entry 13). Solvent screening was then performed: MeCN, DMF, and EtOH provided **3a**, but with a lower yield (Table 1, entries 14–16). THF, DMSO or DCM were used as solvents, the target product was not obtained (Table 1, entries 17–19). Considering that decarboxylation might be involved in the reaction, temperature optimization was performed. The yield almost unchanged at low temperature and slowed down the reaction (Table 1, entry 20). As the temperature increased, the reaction time also shortened, and the yield was improved at 50 °C (Table 1, entries 21, 22). However, when the temperature rose to 60 °C,





Scheme 2 Substrate scope of *o*-phenylenediamines and α -keto acids. Reaction conditions: **1a** (0.5 mmol), **2a** (0.6 mmol), L-Lys (0.125 mmol), L-Tyr (0.05 mmol), H₂O (15.0 mL) in an open flask at room temperature.

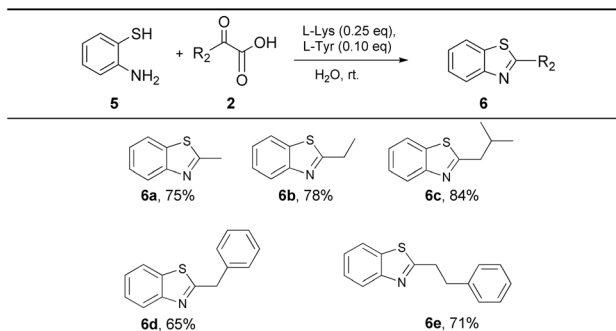
the yield slightly decreased (Table 1, entry 23), and then the temperature reached 80 °C, the yield showed a significant decrease (Table 1, entry 24). To verify the effectiveness of amino acids in the reaction at high temperatures, a control experiment was conducted at 50 °C without adding amino acids. As a result, **3a** was not obtained but **4a** (Table 1, entry 25). Considering the instability of pyruvic acid, it was divided into two and four batches. It was obvious that the effect of batch addition was better than that of single addition (Table 1, entries 26–28). In order to investigate whether the reaction involved free radicals, 2,2,6,6-tetramethyl-1-piperidinoxy free radical (TEMPO) was added, and the result showed that TEMPO had almost no effect (Table 1, entry 29). Because the pH value affected the chemical properties of amino acids, we adjusted the pH value with HCl solution and NaOH solution. No **3a** was found in the acidic system, but the yield of **3a** increased with the increase of pH value (Table 1, entries 30–33). We hypothesized that **3a** was

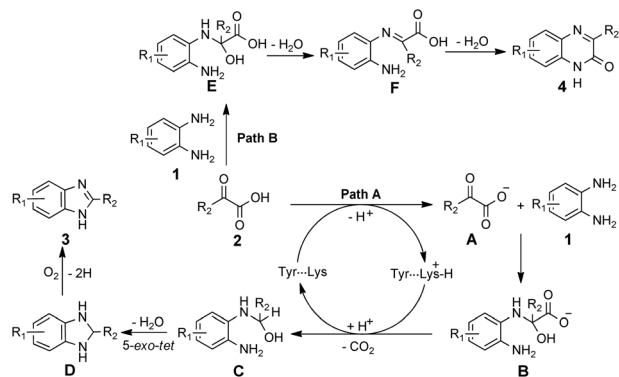
obtained in the presence of base. Further experiments with different bases showed that **3a** was obtained when the amount of base was added to 1.2 equivalents. The buffer solution increased the yield of **3a** in the absence of amino acids. This suggested that the addition of Tyr to form the buffer enhanced the reaction conditions, thereby improving the yield of **3a** (Table 1, entries 34, 35). Water as solvent in the presence of NaOH, **3a** was obtained at low yield (Table 1, entry 36), while ethanol as solvent in the presence of *t*-BuOK, the yield was slightly improved (Table 1, entry 37). Unfortunately, many substrates did not afford the target product in the presence of base, or the yield was very low, so this method was abandoned. Considering the convenience of the experimental operation, we concluded that the optimized reaction conditions as follows: *o*-phenylenediamine (1.0 eq), lysine (0.25 eq), tyrosine (0.1 eq) and α -keto acid (1.2 eq) in water and in an open flask at room temperature.

Under the optimized reaction conditions, we explored the substrate scope of α -keto acids and *o*-phenylenediamines to assess the practicality of the reaction (Scheme 2). Electron-rich 4,5-dimethyl- and 4-methoxy substituted *o*-phenylene-diamines afforded the target products with excellent yields (**3b**, **3c**). **3d** gave the desired product, indicating the tolerance of halogen groups. Moreover, 2,3-diaminonaphthalene also obtained the corresponding product **3e**. It was noteworthy that *o*-phenylenediamines bearing electron withdrawing substituents such as cyano and nitro group afforded **3f** and **3g** with moderate yields. Subsequently, the scope of α -keto acids was further investigated. Using 2-ketobutyric acid or 4-methyl-2-oxovanoic acid, the yield was better than pyruvic acid (**3h–3u**). Obviously, the yields of benzimidazoles were significantly improved with the growth of alkyl chain of α -keto acids. Phenylpyruvic acid was adopted, but the target product was not isolated.

In order to explore the practicability of this method in the synthesis of other heterocyclic compounds (Table 2). 2-Amino-phenol and 2-aminothiophenol reacted with α -keto acid respectively. Unfortunately, benzoxazoles were not obtained under this reaction condition, but benzothiazoles were easily afforded (**6a–6e**). Benzothiazoles were obtained without the addition of amino acids, which was confirmed in previous reports.³⁰ Under the optimized conditions, the yields of benzothiazoles were appropriately increased by using water as solvent in an open flask at room temperature.

Table 2 Further applications in the synthesis of heterocyclic compounds





Scheme 3 Proposed reaction mechanism.

Based on the above results and previous report,⁵¹ a possible reaction mechanism was proposed (Scheme 3). Lysine reacted with the α -keto acid (2) to afford α -oxocarboxylate A and Tyr-Lys⁺-H. Subsequently, Condensation of *o*-phenylene-diamine (1) with A to obtain hemiaminal B. The decarboxylation of B was then facilitated by the abstraction of H⁺ from Lys⁺-H to afford the tetrahedral intermediate C and Lys.⁵² Benzimidazole D was formed by the favored 5-*exo-tet* cyclization.⁵³ Finally, the dehydrogenation of D in air obtained the desired benzimidazole 3 (Path A). On the other hand, *o*-phenylenediamine (1) condensed with α -keto acid (2) to form hemiamines E. Subsequent two steps of dehydration, 4 was obtained (Path B).

Conclusions

In summary, we have developed a method for the synthesis of benzimidazoles in an open flask at room temperature without organic solvents and metal catalysts. The method is efficient and environment-friendly. Such a simple operation further improves the practical performance. Our laboratory is currently further exploring the synthetic applications of benzimidazoles and developing more sustainable and environmentally conscious methods for organic synthesis.

Author contributions

Z. Luo: writing – editing, resources, project administration, funding acquisition, and synthesis of part products. M. Huang: synthesis of most products; X. Wang: investigations on the mechanism; X. Wang and Z. Hu: investigations on the applications; Z. Liang and T. Huang: writing – review.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data that support the findings of this study are available in the supplementary information (SI). Supplementary information: Experimental procedures, characterization data, and

copies of NMR spectra for all compounds. See DOI: <https://doi.org/10.1039/d5ra06371j>.

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Notes and references

- 1 L. M. Aroua, F. M. Alminderej, H. R. Almuhaylan, A. H. Alosaimi, F. Medini, H. A. Mohammed, S. A. Almahmoud, R. A. Khan and N. H. Mekni, *RSC Adv.*, 2025, **15**, 7571–7608.
- 2 H. Ahmad, I. Anjum, H. Usman, A. Mobashar, A. Shabbir, Y. A. B. Jordan, A. Metouekel, M. Daelbait and M. Bourhia, *Open Life Sci.*, 2025, **20**, 20251083–20251097.
- 3 B. Narasimhan, D. Sharma and P. Kumar, *Med. Chem. Res.*, 2012, **21**, 269–283.
- 4 P. Ahmadfilab, M. Pordel, S. Kheirkhahnia and S. Ziaei, *J. Mol. Struct.*, 2025, **1342**, 142646–142688.
- 5 S. Tahlan, S. Kumar, S. Kakkar and B. Narasimhan, *BMC Chem.*, 2019, **13**, 66–81.
- 6 B. Farag, M. E. A. Zaki, D. A. Elsayed and S. M. Gomha, *RSC Adv.*, 2025, **15**, 18593–18647.
- 7 A. F. Mohammed, S. G. Abdel-Moty, M. A. Hussein and A. M. Abdel-Alim, *Arch. Pharm. Res.*, 2013, **36**, 1465–1479.
- 8 N. T. Chung, V. C. Dung and D. X. Duc, *RSC Adv.*, 2023, **13**, 32734–32771.
- 9 S. Tahlan, S. Kumar and B. Narasimhan, *BMC Chem.*, 2019, **13**, 101–121.
- 10 B. Pathare and T. Bansode, *Results Chem.*, 2021, **3**, 100200–100208.
- 11 S. Tahlan, S. Kumar and B. Narasimhan, *BMC Chem.*, 2019, **13**, 18–34.
- 12 S. Bai, M. Zhang, S. Tang, M. Li, R. Wu, S. Wan, L. Chen, X. Wei and F. Li, *Molecules*, 2024, **29**, 1218–1231.
- 13 N. Singh, A. Pandurangan, K. Rana, P. Anand, A. Ahamad and A. K. Tiwari, *Int. Curr. Pharmaceut. J.*, 2012, **1**, 110–118.
- 14 S. Li, C. Zeng, S. Huang, N. Ahmad, X. Peng, J. Meng and C. Zhou, *J. Agric. Food Chem.*, 2025, **73**, 13985–13997.
- 15 A. O. Arnautova, I. A. Aleksakhina, E. A. Zorina, M. Y. Berzina, I. V. Fateev, B. Z. Eletskaia, K. V. Antonov, O. S. Smirnova, A. S. Paramonov, A. L. Kayushin, V. L. Andronova, G. A. Galegov, M. A. Kostromina, E. A. Zayats, I. L. Karpenko, S. K. Kotovskaya, V. N. Charushin, R. S. Esipov, A. I. Miroshnikov and I. D. Konstantinova, *Biomolecules*, 2025, **15**, 922–941.
- 16 P. Divya, A. A. J. Ranchani, H. Ahmad, V. S. J. Reeda, T. Radwan and D. Haridas, *Sci. Rep.*, 2025, **15**, 20483–20499.



- 17 D. Udhayakumari, *J. Mol. Struct.*, 2025, **1342**, 142767–142783.
- 18 A. A. Ibrahim, E. G. Said, A. M. AboulMagd, N. H. Amin and H. M. Abdel-Rahman, *RSC Adv.*, 2025, **15**, 22097–22127.
- 19 T. Sakaguchi, R. Nishio, H. Awata, J. Tan, R. Nakata, H. Sato, Y. Matsumura, O. Shimomura and A. Ohtaka, *J. Org. Chem.*, 2025, **90**, 11372–11377.
- 20 V. A. S. Pardeshi, N. S. Chundawat, S. I. Pathan, P. Sukhwat, T. P. S. Chundawat and G. P. Singh, *Synth. Commun.*, 2020, **51**, 485–513.
- 21 N. Anagha, M. Neetha and G. Anilkumar, *J. Organomet. Chem.*, 2022, **958**, 122174–122188.
- 22 Z. Ma, B. Zhang, Z. He, T. Xu, Y. Cheng, Y. Cui and Z. Chen, *ACS Catal.*, 2025, **15**, 11875–11885.
- 23 M. P. Athira, R. Arun and H. Suja, *Org. Biomol. Chem.*, 2025, **23**, 5635–5642.
- 24 X. Xu, W. Xu, J. Wu, J. He and H. Xu, *Org. Biomol. Chem.*, 2016, **14**, 9970–9973.
- 25 L. D. Luca and A. Porcheddu, *Eur. J. Org. Chem.*, 2011, 5791–5795.
- 26 M. Pizzetti, E. D. Luca, E. Petricci, A. Porcheddu and M. Taddei, *Adv. Synth. Catal.*, 2012, **354**, 2453–2464.
- 27 T. d. Santos, C. S. Buettner, D. B. Yildiz, M. Mamone, A. Ruffoni and D. Leonori, *Angew. Chem., Int. Ed.*, 2025, **64**, e202423804.
- 28 Z. Li, H. Song, R. Guo, M. Zuo, C. Hou, S. Sun, X. He, Z. Sun and W. Chu, *Green Chem.*, 2019, **21**, 3602–3605.
- 29 J. Liu, Q. Liu, H. Yi, C. Qin, R. Bai, X. Qi, Y. Lan and A. Lei, *Angew. Chem., Int. Ed.*, 2014, **53**, 502–506.
- 30 J. Zou, J. Xu, L. Li, Z. Yang and C. Zhou, *J. Org. Chem.*, 2025, **90**, 7793–7800.
- 31 J. Saetan, N. Purahong, K. La-ongthong, N. Hassa, N. Chotsaeng, C. Kuhakarn and J. Meesin, *Org. Biomol. Chem.*, 2025, **23**, 4226–4231.
- 32 H. Liu, S. Wu and Z. Li, *J. Catal.*, 2025, **447**, 116119–116125.
- 33 Z. Asdollahpour, R. Baharfar and S. Asghari, *Sci. Rep.*, 2025, **15**, 11384–11404.
- 34 Y. Shiraiishi, Y. Sugano, S. Tanaka and T. Hirai, *Angew. Chem., Int. Ed.*, 2010, **49**, 1656–1660.
- 35 S. Sharma, A. S. Yaminia and P. Das, *Adv. Synth. Catal.*, 2019, **361**, 67–72.
- 36 K. Das, A. Mondal and D. Srimani, *J. Org. Chem.*, 2018, **83**, 9553–9560.
- 37 H. Liu, S. Wu and Z. Li, *J. Catal.*, 2025, **447**, 116119–116126.
- 38 M. Jafarpour, A. Rezaeifard, N. Pourmorteza, N. Fouladshakan and P. Jamshidinajafabadi, *Sci. Rep.*, 2025, **15**, 22479–22493.
- 39 D. Gonzalez-Vera, E. Leal-Villarroel, T. M. Bustamante, J. N. Díaz de Leon, L. Camarena, M. E. Domine, C. C. Torres and C. H. Campos, *Catal. Today*, 2025, **459**, 115416–115427.
- 40 P. J. Wanjari, N. Saha, G. Dubey and P. V. Bharatam, *Tetrahedron*, 2023, **130**, 133143–133155.
- 41 R. Dubey and N. S. H. N. Moorthy, *Chem. Pharm. Bull.*, 2007, **55**, 115–117.
- 42 S. Samanta, S. Das and P. Biswas, *J. Org. Chem.*, 2013, **78**, 11184–11193.
- 43 T. Sakaguchi, R. Nishio, H. Awata, J. Tan, R. Nakata, H. Sato, Y. Matsumura, O. Shimomura and A. Ohtaka, *J. Org. Chem.*, 2025, **90**, 11372–11377.
- 44 A. V. Aksenov, A. N. Smirnov, N. A. Aksenov, A. S. Bijieva, I. V. Aksenovaa and M. Rubin, *Org. Biomol. Chem.*, 2015, **13**, 4289–4295.
- 45 M. S. Mayo, X. Yu, X. Zhou, X. Feng, Y. Yamamoto and M. Bao, *Org. Lett.*, 2014, **16**, 764–767.
- 46 Z. Luo, M. Wang, G. Jiang, X. Wang, L. Zhao, Z. Hu, H. Li and Q. Ji, *RSC Adv.*, 2024, **14**, 35386–35390.
- 47 W. Notz and B. List, *J. Am. Chem. Soc.*, 2000, **122**, 7386–7387.
- 48 B. List, P. Pojarliev and C. Castello, *Org. Lett.*, 2001, **3**, 573–575.
- 49 R. Mahesh, A. K. Dhar, T. S. TVNV, S. Thirunavukkarasu and T. Devadoss, *Chin. Chem. Lett.*, 2011, **22**, 389–392.
- 50 I. I. Padilla-Martínez, A. Cruz, E. V. García-Báez, J. E. Mendieta-Wejebe and M. C. Rosales-Hernández, *Int. J. Mol. Sci.*, 2025, **26**, 5901–5944.
- 51 Z. A. Siddiqui, S. Lambud, A. Bhadke, R. Kumar, N. Prajesh, N. Sekar and S. More, *Chem. Phys.*, 2023, **565**, 111735–111742.
- 52 H. Wang and J. Huang, *Adv. Synth. Catal.*, 2016, **358**, 1975–1981.
- 53 Y. Lee, Y. Cho, S. Lee, J. Bin, J. Yang, G. Chae and C. Cheon, *Tetrahedron*, 2015, **71**, 532–538.

