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One-pot, two-step synthesis of unnatural α-amino acids involving the exhaustive aerobic oxidation of 1,2-diols

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Herein, we report the nor-AZADO-catalyzed exhaustive aerobic oxidations of 1,2-diols to α-keto acids. Combining oxidation with transamination using DL-2-phenylglycine led to the synthesis of free α-amino acids (AAs) in one pot. This method enables the rapid and flexible preparation of a variety of valuable unnatural AAs, such as fluorescent AAs, photoactivatable AAs, and other functional AAs for bioorthogonal reactions.

The incorporation of functional α-amino acids (AAs) to peptides is a powerful strategy for probing or modulating their biological properties.[1–4] More specifically, fluorescent AAs can be used to visualize intracellular processes involving peptides,[2,3] and photoactivatable AAs enable the crosslinking of ligands and interacting proteins.[4] AAs bearing azides and alkynes (for click chemistry), alkenes (for olefin cross metathesis), and aryl halides (for Pd-catalyzed cross coupling) enable the introduction of various functional groups into peptides in flasks as well as in living cells by bioorthogonal chemistry.[5] Such functional AAs are usually synthesized by one of the following two synthetic strategies: (1) the modification of a natural AA or commercially available AA[3,4] (Fig. 1a); and (2) the alkylation of a glycine equivalent[6] (Fig. 1b). However, the structures of the AAs accessible by the former strategy are limited by the availability of AAs as starting materials. In terms of the latter strategy, an alkyl halide with a carbon-halogen bond adjacent to an unsaturated bond is generally used as an alkylating reagent owing to the efficiency of S_N2 reaction. These limitations are obstacles to obtaining more-suitable functional AAs according to the intended use. Hence, a de novo synthetic method that enables the preparation of a wide range of unnatural AAs will strengthen research using functional AAs.

We previously developed a three-step method to directly synthesize free AAs,[7] which involves the chemoselective oxidation of 1,2-diols to α-hydroxy acids,[8] the chemoselective oxidation of α-hydroxy acids to α-keto acids,[9] and transamination of α-keto acids with DL-2-phenylglycine to AAs.

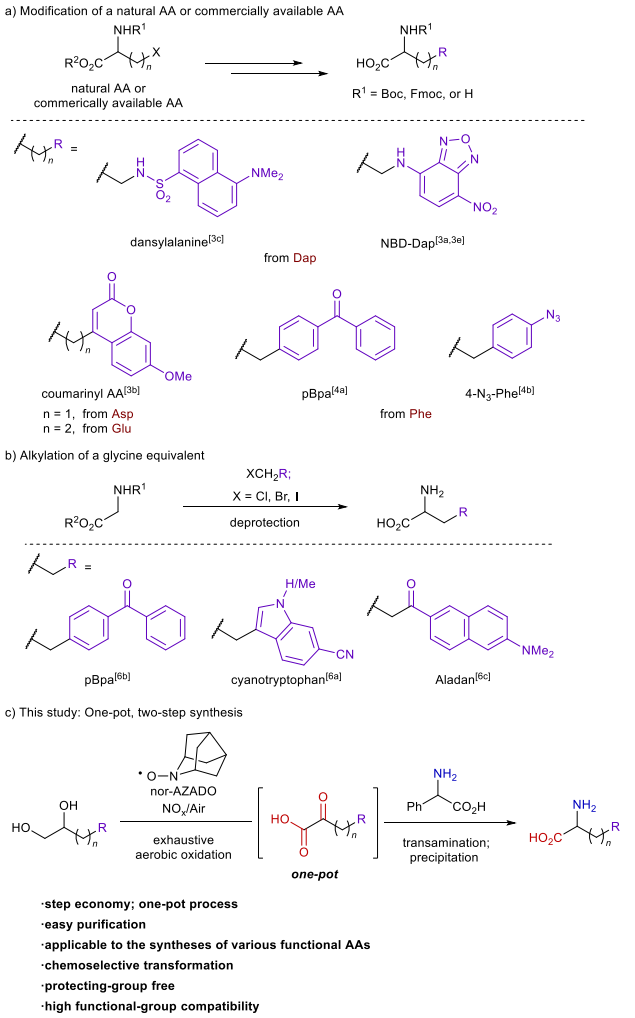

Fig. 1 The conventional synthetic methods of AAs and this study. NBD = 7-nitrobenz-2-oxa-1,3-diazol-4-yl, Dap = 2,3-diaminopropionic acid.

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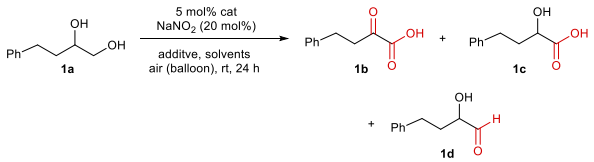
Unfortunately, we were unable to apply this method to the synthesis of a fluorescent coumarinyl AA in our preliminary study. Owing to the poor solubility of the corresponding 1,2-diol in toluene, the first-step reaction does not efficiently proceed. The undesired oxidative C-C bond cleavage easily occurred during the oxidation of a 1,2-diol to the corresponding α-keto acid.[10] To suppress the side reaction, the first step uses two-phase conditions involving hydrophobic toluene and phosphate buffer. Therefore, the physical properties of 1,2-diols and α-hydroxy acids are critical for reaction efficiency. However, the preliminary result suggests that control through phase separation is not applicable to the synthesis of fluorescent and other functional AAs, because their relatively large side chains strongly influence the physical properties of the corresponding 1,2-diols and α-hydroxy acids. Hence, a substantially improved oxidation method is required in order to prepare such functional AAs.

Inspired by the previous finding that the undesired oxidative cleavage of a labile α-keto acid does not proceed under nitroxyl radical-catalyzed aerobic oxidation conditions,[9] we envisaged that the development of a nitroxyl radical-catalyzed aerobic oxidation protocol that converts 1,2-diols to the corresponding α-keto acids would enable the synthesis of fluorescent and other functional AAs. Such an aerobic oxidation protocol would also enable the step-economical synthesis of AAs. On the other hand, a highly efficient aerobic oxidation method capable of triply oxidizing a single molecule is required for the desired transformation. To develop the desired reaction, the use of a highly efficient nitroxyl radical catalyst bearing an α-hydrogen, such as 2-azaadamantane *N*-oxyl (AZADO) and 9-azabicyclo[3.3.1]nonane *N*-oxyl (ABNO) is needed.[11-12] On the other hand, because these catalysts have low chemoselectivities for primary alcohols over secondary alcohols,[13] the non-chemoselective oxidation of a 1,2-diol will generate several intermediates, such as the corresponding α-hydroxyaldehyde, α-hydroxyketone, α-hydroxy acid, α-ketoaldehyde, and the hydrates of these aldehydes. Therefore, a method that exhaustively oxidizes 1,2-diols to the corresponding α-keto acids irrespective of the intermediate species formed is required. Herein, we established an exhaustive aerobic oxidation method. Moreover, by combining oxidation with transamination, we developed a one-pot, two-step method for the synthesis of α-amino acids that provides facile access to a variety of functional AAs (Fig. 1c). This method is also distinguishable from conventional synthetic methods in that functional AAs can be synthesized from the corresponding 1,2-diols without any protecting groups, and the AA moiety is constructed onto the carbon chain of the starting material without increasing or decreasing the length of the carbon chain.

At the outset, we examined the aerobic oxidation of 1,2-diol **1a** in MeCN and pH 3.9 acetate buffer using commercially available AZADOL as the catalyst (entry 1, Table 1) (See the Supporting Information for details). The desired α-keto acid **1b** was produced in 37% yield together with small amounts of α-hydroxy acid **1c**, α-hydroxyaldehyde **1d**, and several unidentified byproducts after 24 h. The unidentified byproducts are presumably partially oxidized intermediates. The use of AZADO instead of AZADOL improved the yield of **1b** to 68%, although the reason for this improvement is unclear (entry 2). The reaction in pH 6.8 or 2.1 phosphate buffer and MeCN did not proceed efficiently (entries 3 and 4), suggesting that weakly acidic conditions are optimal. The TEMPO-catalyzed reaction resulted in the recovery of a large amount of **1a**, whereas the DMN-AZADO-catalyzed reaction afforded the desired product **1b** in moderate yield (entries 5 and 6).[13] We eventually found that nor-AZADO efficiently catalyzed the desired exhaustive oxidation (entries 7 and 8).[14] Although small amounts of **1c**, **1d**, and **1a** remained in the presence of 5 mol% nor-AZADO, the reaction was complete within 12 h in the presence of 10 mol% nor-AZADO. The desired oxidation proceeded efficiently in MeCN and H_2O in the presence of acetic acid (2 equiv) as well as in MeCN and pH 3.9 acetate buffer (entry 9). The reaction conditions of entry 9 were optimal for the synthesis of AAs, because the use of the acetate buffer sometimes results in the AA precipitate being contaminated with AcONa (vide infra).

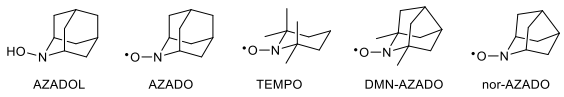
With the exhaustive oxidation of 1,2-diols to the corresponding α-keto acids established, the one-pot conversion to AAs employing transamination with DL-2-phenylglycine was examined in order to maximize operational simplicity (Scheme 1, see the Supporting Information for details).[7] After 1,2-diol **1a**

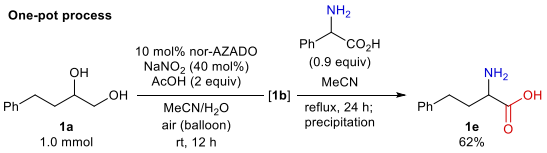
Table 1 Optimization of the exhaustive aerobic oxidations of 1,2-diols to α-keto acids.



entry	cat, additive, and solvents[a]	yield [%][b,c]			
		1b	**1c**	**1d**	**1a**
1	AZADOL MeCN/pH 3.9 acetate buffer	37	6	3	14
2	AZADO MeCN/pH 3.9 acetate buffer	68	3	1	3
3	AZADO MeCN/pH 6.8 phosphate buffer	n.d.	n.d.	1	96
4	AZADO MeCN/pH 2.1 phosphate buffer	35	3	2	13
5[c]	TEMPO MeCN/pH 3.9 acetate buffer	n.d.	n.d.	6	70
6[c]	DMN-AZADO MeCN/pH 3.9 acetate buffer	48	12	trace	trace
7	nor-AZADO MeCN/pH 3.9 acetate buffer	77	3	1	3
8[d,e]	nor-AZADO MeCN/pH 3.9 acetate buffer	82	n.d.	n.d.	n.d.
9[d,e]	nor-AZADO, AcOH (2 equiv) MeCN/H_2O	87	n.d.	n.d.	n.d.

[a] v/v = 1/1. [b] NMR yields. [c] n.d. = not detected. [d] Cat (10 mol%) and $NaNO_2$ (40 mol%) were used. [e] Reaction time is 12 h.





Scheme 1 One-pot synthesis of AA **1e**.

had been exhaustively oxidized to α-keto acid **1b**, DL-2-phenylglycine (0.9 equiv) and additional MeCN were added to the reaction mixture prior to any workup operation. After refluxing the reaction mixture for 24 h and the following addition of diethyl ether, the precipitate was simply collected by filtration. The desired AA **1e** was obtained in 62% isolated yield with high purity. In this manner, we established a one-pot, two-step method for the synthesis of AAs from 1,2-diols.

To investigate the scope of the one-pot, two-step AA-synthesis method, we examined the synthesis of several AAs (Fig. 2). Simple AA **2e** bearing an alkyl chain was prepared in 69% yield., and the transamination of α-keto acid **3b** was selectively promoted with the phenylketone moiety intact to afford **3e** in 68% yield. The exhaustive oxidations of 1,2-diols **4a**–**6a** to the corresponding α-keto acids **4b**–**6b** also proceeded effectively, and AAs **4e**–**6e** were obtained in high yields following transamination. We note that AAs **4e**-**6e** cannot be synthesized by the previously reported three-step method. The initial chemoselective oxidations of diols **4a**–**6a** to the corresponding α-hydroxy acids were unsuccessful because of their low solubilities in toluene or highly hydrophilic properties.[8] These results suggest that aerobic oxidation is less influenced by the physical properties of 1,2-diols and α-hydroxy acids than the previous stepwise oxidation protocol. Fluorophilic AA **7e** bearing the perfluoroalkyl side chain was also synthesized, albeit in moderate yield (17%). Exhaustive oxidations of triols **8a** and **9a** proceeded with the oxidation of the side-chain alcohols to afford AAs **8e** (bearing a carboxyl group) and **9e** (bearing a ketone moiety). These results suggest that this novel method, based on exhaustive aerobic oxidation, is robust.

We next examined the synthesis of functional AAs. The coumarin and pyrene moieties were found to be compatible with the reaction conditions, affording the fluorescent AAs **10e** and **11e**[15] in 51% and 49% yields, respectively. Photoactivatable AAs **12e** and **13e**, bearing a benzophenone moiety and 4-azido-2,3,5,6-tetrafluorophenyl group were effectively synthesized under light-shielded conditions in 58% and 70% yields, respectively. Notably, the relatively hydrophilic side chain of **10** or the hydrophobic side chains of **11**–**13** did not detrimentally affect the reaction efficiency. An alkyl azide and alkyne required for the Huisgen reaction, an alkene required for cross-olefin metathesis, and an aryl halide required for Pd-catalyzed cross coupling were also compatible, affording AAs **14e**–**17e** in high yields (55–71%), and highlighting the mildness of the reaction conditions. These functional AAs are used to modify peptides by bioorthogonal chemistry. During the synthesis of **16e**, aerobic oxidation in MeCN and pH 3.9 acetate buffer instead of MeCN/H_2O/AcOH (2 equiv) led to contamination of the AA precipitate with AcONa, affording a 5:1 mixture of the desired AA and AcONa.

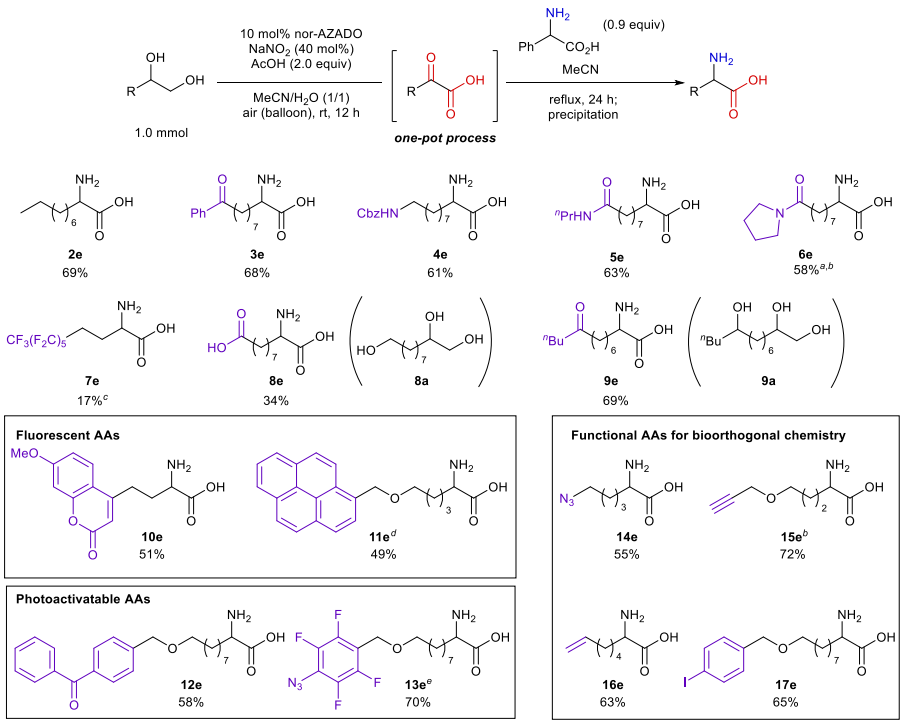
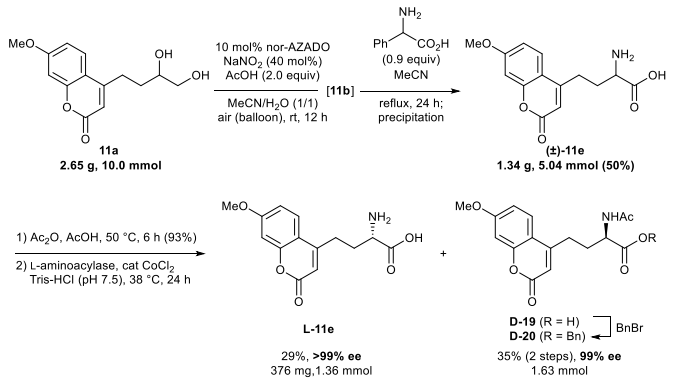


Fig. 2 Substrate scope of the one-pot AA synthesis protocol. [a] An inseparable mixture with 8% of *N*-iminyl amino acid. [b] Purified by ion-exchange chromatography. [c] Purified by filtration and silica gel column chromatography. [d] DL-2-Phenylglycine (0.8 equiv) was used. [e] The reaction was carried out in 0.50 mmol scale.



Scheme 2 Large-scale synthesis of **11e** and its subsequent chemoenzymatic resolution.

Finally, to demonstrate the further utility of this synthetic method, we prepared fluorescent AA (±)-**11e** on the gram scale and chemoenzymatically resolved its two enantiomers (Scheme 2). We confirmed that diol **11a** is easily prepared on over 10 g scale in three steps from commercially available materials.[3b] **11a** (2.65 g, 10 mmol) was subjected to the one-pot, two-step synthetic protocol to afford **(±)-11e** (1.34 g, 5.0 mmol) in 50% yield. Following acetylation, kinetic resolution using L-aminoacylase (EC 3.5.1.14, acylase H, from *Aspergillus melleus*) proceeded with almost complete selectivity to afford optically active **L-11e** in 29% isolated yield and >99% ee. The recovered acetate **D-19** was isolated as benzyl ester **D-20** in 35% yield and 99% ee.

In conclusion, we developed a practical one-pot, two-step method for the synthesis of AAs that involves exhaustive aerobic oxidation and transamination. Precipitation in the final step is the only purification operation required to obtain AAs with high purities in most cases. Since aerobic oxidation of 1,2-diols to the corresponding α-keto acids proceeds under mono-phasic conditions using MeCN and H_2O as the solvents, the hydrophilic/hydrophobic nature of the side chain does not influence the efficiency of the reaction. Owing to its robustness, this method facilitates the preparation of functional AAs such as fluorescent AAs, photoactivatable AAs, and a variety of other AAs. These results suggest that this synthetic method is useful for the preparation of valuable unnatural AAs in chemical biology research.

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Conflicts of interest

There are no conflicts to declare.

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15 Oxalacetic acid was added after transamination to remove unreacted DL-2-phenylglyicine, which produced alanine soluble in the solvents.

