Direct Synthesis of Free α-Amino Acids by Telescoping Three-Step Process from 1,2-Diols

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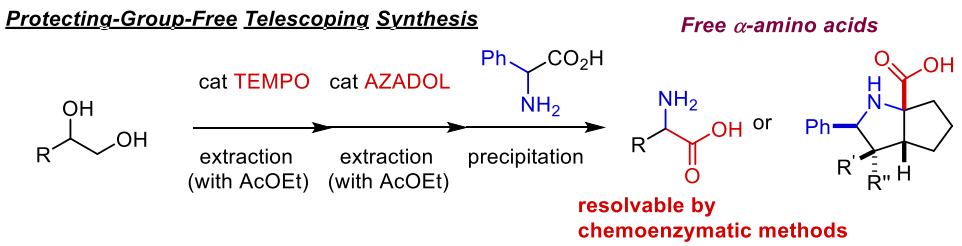
Supporting Information Placeholder

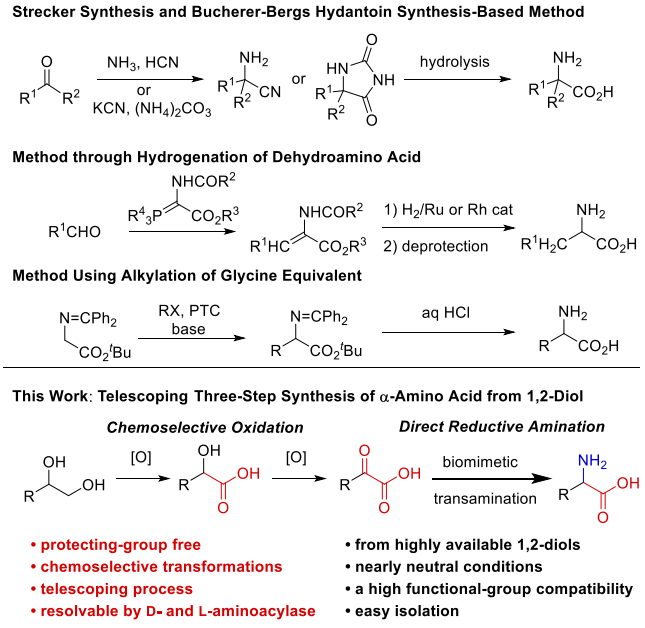


ABSTRACT: A practical telescoping three-step process for the syntheses of α-amino acids from the corresponding 1,2-diols has been developed. This process enables the direct synthesis of free α-amino acids without any protection/deprotection step. This method was also effective for the preparation of a ^{15}N-labelled α-amino acid. 1,2-Diols bearing α,β-unsaturated ester moieties afforded bicyclic α-amino acids through intramolecular [3+2] cycloadditions. A preliminary study suggests that the resultant α-amino acids are resolvable by aminoacylases with almost complete selectivity.

Amino acids, peptides, and proteins play essential roles in animal life and are also of interest as medicinal agents or materials. Recent advances in peptide-based drugs have led to more than 25 peptide drugs being approved in the United States, Europe, and Japan since 2000.[1] The incorporation of unnatural amino acids in peptides is an important strategy for controlling their physical and biological properties, and their introduction into functional molecules as luminescent markers or affinity tags is also worthwhile.[2,3] Hence, an effective method for the synthesis of unnatural amino acids is highly sought after.

The Strecker synthesis and the related method using Bucherer-Bergs hydantoin synthesis are the most common methods for the preparation of α-amino acids (Scheme 1).[4,5] However, these methods require a toxic cyanide reagent, and strong acidic or basic conditions for the subsequent hydrolysis of the nitrile or the hydantoin. Yet another synthetic method is based on the formation of dehydroamino acids and their subsequent hydrogenation. Asymmetric hydrogenation using Rh or Ru catalysts with chiral phosphine ligands facilitates the asymmetric syntheses of α-amino acids.[6] More recently, methods that alkylate glycine equivalents in the presence of a phase transfer catalysts (PTC) have been reported.[7] The utilities of these methods have been amply demonstrated through the industrial syntheses of α-amino acids. In addition to these established methods, other strategies for the syntheses of α-amino acids also exist.[8] However, substrate scope of α-amino acid syntheses still needs to be improved. The most formidable obstacle to the synthesis of an α-amino acid is its amphoteric nature, which commonly controlled by the introduction of protecting groups that mask the carboxylic acid and/or the amino group. This strategy inevitably decreases synthetic efficiency and requires the judicious choice of pro-

Scheme 1. Traditional α-Amino Acid Synthesis and This Work

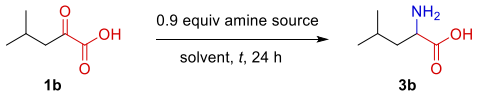


tecting group, which can be tedious. In particular, the final deprotection step is often problematic, since it is required to proceed in the presence of the carboxylic acid or the amino group. Hence, a method to directly synthesize a free α-amino acid is highly desirable to avoid this formidable final deprotection step.

During the course of our previous efforts to develop nitroxyl-radical-catalyzed oxidations, we developed methods for the chemoselective oxidation of 1,2-diols to α-hydroxy acids, and the chemoselective oxidation of α-hydroxy acids to α-keto acids.[9-11] α-Keto acids are readily prepared from easily available 1,2-diols[12] by employing these two oxidation methods. Based on this knowledge, we surmised that the direct transformation of an α-keto acid into the corresponding α-amino acid would lead to a novel method to synthesize free α-amino acids. Since the planned method circumvents the need for final-step hydrolysis and hydrogenation for deprotection, it is likely to demonstrate broad functional-group compatibility. To achieve the desired transformation, a chemoselective reductive amination of the ketone carbonyl in the presence of the adjacent carboxyl group is required. Therefore, we turned our attention to the transamination of an α-keto acid with an α-amino acid, which is a well-known biochemical reaction catalyzed by transaminase using vitamin B_6 as a co-enzyme involved in the metabolism and biosynthesis of α-amino acids.[13] Biomimetic transaminations using vitamin B_6 analogs have been described previously.[14] Zhao and co-workers used the precisely designed pyridoxamine catalysts for the enantioselective transamination of α-keto acids with 2,2-diphenylglycine.[15] While these are seminal studies, the reported catalyst-preparation methods are untenable for practical applications. Herein, we report a practical process for α-amino acid synthesis involving the direct transformation of α-keto acids into α-amino acids. The general method for direct syntheses of free α-amino acids is demonstrated for the first time.

At the outset, we found that the transamination of 2-oxo-4-phenylbutanoic acid (**1a**) with 2,2-diphenylglycine (**2**) proceeded successfully in refluxing THF/H_2O in the absence of any catalyst (Table S1). It was also found that the reaction of **1a** with 0.9 equiv of **2** afforded the desired product **3a** in high purity by simple filtration of the precipitate after the addition of Et_2O to the reaction mixture. As this protocol did not require any laborious purification techniques such as high-performance liquid chromatography (HPLC) or ion-exchange chromatography, it was applied in further investigations. Unfortunately, the reaction of 4-methyl-2-oxovaleric acid (**1b**) with **2** did not proceed at all, presumably because of the steric hindrance associated with its γ-branched structure (Table 1, entry 1). We found that the reaction with DL-2-phenylglycine (**4**) as the amine source instead of **2** proceeded to afford the desired product **3b** in 46% yield together with 7% of unreacted **4** (entry 2).[16] The reaction conducted at a higher temperature (100 °C) using a sealed tube led to a decrease in the yield of **3b** (entry 3), whereas the reaction in refluxing MeCN/H_2O afforded **3b** in 68% yield in high purity (entry 4), as confirmed by NMR spectroscopy and elemental analysis. The reaction in H_2O,[16] and the reaction using glycine instead of **4**, failed (entries 5,6). We then evaluated the scope of the transamination using readily available α-keto acids under the conditions in entry 4 (Table S2). It suggests that this transamination methodology has broad scope.

Table 1. Optimizing the Reaction Conditions.

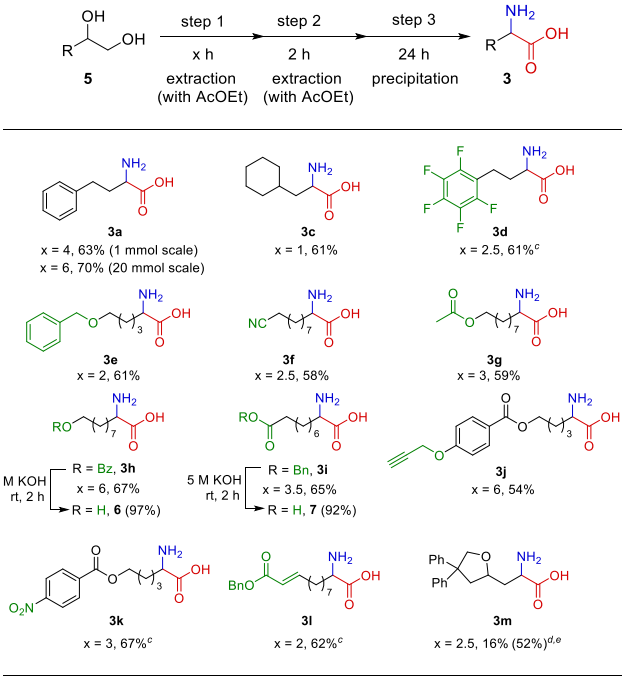


entry	amine source	solvent	*t* (°C)	yield (%)[a]
1	2,2-diphenylglycine (**2**)	THF/H_2O	reflux	0
2	DL-2-phenylglycine (**4**)	THF/H_2O	reflux	46 (7)
3	DL-2-phenylglycine (**4**)	THF/H_2O	100	25
4	DL-2-phenylglycine (**4**)	MeCN/H_2O	reflux	68 (trace)
5	DL-2-phenylglycine (**4**)	H_2O	reflux	7 (53)
6	glycine	MeCN/H_2O	reflux	4 (trace)

[a]Numbers in parentheses are yields of recovered **4** in an inseparable mixture with **3b**.

With an effective methodology for the transamination in hand, we examined the three-step syntheses of α-amino acids from the corresponding 1,2-diols, combining the transamination with the two chemoselective oxidations from the 1,2-diol to the α-keto acid (Table 2). After validating the three-step synthesis by a step-by-step protocol, we successfully established a practical telescoping protocol. The purification procedures of the first and second steps involved extraction with AcOEt after the addition of sodium phosphate buffer (pH = 2.1). The resultant product was used in the next step without any further purification. The final purification step involved precipitation as described above. It is worth noting that the telescoping protocol avoids daunting purifications of the polar products using silica-gel chromatography or HPLC. α-Amino acid synthesis using the three-step telescoping procedure afforded the desired free α-amino acid **3a** in 63% total yield based on 1,2-diol **5a**; the average yield per step was ~85%. Knowing the optimal protocol, we examined the scope of the telescoping three-step synthetic method (Table 2). A broad range of unnatural α-amino acids **3a**-**3l**, including those bearing a variety of functional groups, such as benzyl-ether, ester, cyano, nitro, alkene, and alkyne moieties were synthesized. Ester and cyano groups are reactive under the typical hydrolysis conditions used in the Strecker synthesis. Moreover, benzyl-ether, nitro, alkene, and alkyne groups are typically reactive under the hydrogenolysis conditions often used for the removal of the Cbz and benzyl groups of protected α-amino acids. Our results definitely suggest the advantage of the direct synthesis of free α-amino acids. In the case of the synthesis of **3m**, non-negligible amount of the one-carbon shorter carboxylic acid was produced in the first step.[9c] Although **3m** was then obtained in 52% yield (determined by NMR) as a mixture with the carboxylic acid, its isolated yield was 16% because of the difficulty of the separation. Although the yield of **3m** was low, nine α-amino acids (**3e**-**3m**) out of twelve were new unnatural α-amino acids, which demonstrates the versatility of this method for the synthesis of unnatural α-amino acids. Especially, uncommon α-amino acids bearing ester side chains were synthesizable by this method. We also examined the hydrolyses of **3h** and **3i**, which efficiently proceeded on treatment with 5 M KOH to afford serine and glutamic acid homologues **6** and **7** in high yields (97% and 92%, respectively). Moreover, to demonstrate that this synthetic method is amenable to scale-up, 3.3 g (20 mmol) of **5a** was used to synthesize **3a**; the scaled-up reaction

Table 2. Scope of the Telescoping Three-Step Synthesis of Unnatural α-Amino Acids[a,b]

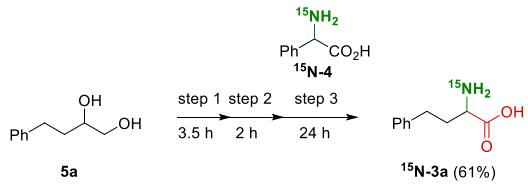


[a]Reaction Conditions: Step 1: 5 mol% TEMPO, 5-10 mol% NaOCl, 3 equiv $NaClO_2$ toluene/phosphate buffer (pH 6.8), rt. Step 2: 5 mol% AZADOL, 20 mol% $NaNO_2$, Air (balloon). Step 3: 0.8 equiv **4**, MeCN/H_2O reflux. [b]Percentage figures are isolated yields based on the amount of 1,2-diols **5** over the three steps. [c]Reaction temperature in step 1 is 50 °C. [d]Ion-exchange chromatography was used for the purification. [e]Percentage figure in parenthesis is NMR yield.

protocol proceeded efficiently without any detriment to afford α-amino acid **3a** in a slightly improved yield (70%).

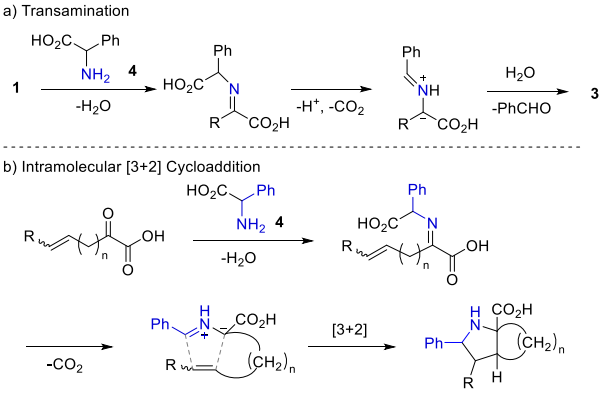
Because the N atom is introduced in the final step of this protocol, this method proved to be effective for the preparation of a ^{15}N-labelled α-amino acid. The incorporation of a ^{15}N-labelled α-amino acid is an important technique for NMR and mass spectrometric analyses of peptide and protein structures.[17,18] In spite of the importance, reports on the preparation of ^{15}N-labelled amino acid by chemical synthesis are rather limited. As an example, we synthesized ^{15}N-labelled **^{15}N-3a** (Scheme 2)**.** ^{15}N-labelled DL-2-phenylglycine (**^{15}N-4**) was prepared from benzaldehyde using readily available ammonium chloride-^{15}N. **^{15}N-3a** was obtained in 61% yield in high purity.

Scheme 2. Synthesis of ^{15}N-Labelled α-Amino Acid 3a. (The procedures used in steps 1-3 are as indicated in Table 2).



In the transamination of **1** to **3**, DL-2-phenylglycine (**4**) acts as the amine source**:** the azomethine ylide generated by the decarboxylation of the imine intermediate is hydrolyzed to form the α-amino acid and benzaldehyde (Scheme 3a). We were next interested in the intramolecular trapping of the azomethine ylide intermediate employing 1,3-dipolar cycloaddition without hydrolysis (Scheme 3b).[19] If the intramolecular reaction proceeds, it will form novel bicyclic α-amino acids that contain the benzylamino moiety derived from **4**. We prepared 1,2-diol **5n** bearing an (*E*)-α,β-unsaturated ester side chain as the dipolarophile and subjected it to the three-step protocol (Scheme 4). Fortunately, the desired reaction proceeded to afford bicyclic α-amino acid **8** as a single diastereomer in 65% yield. Its ^{1}H/^{13}C NMR, 2D NMR, and mass spectra supported the bicyclic structure. The structure of **8** was also unambiguously determined by X-ray crystallographic analysis of α-amino ester **9**, which was prepared by a similar synthetic method from the corresponding 1,2-diol **5n'** bearing an *p*-bromobenzyl ester side chain and subsequent methylation. The stereochemistry of **8** suggested that W-shaped ylide **10** underwent the cycloaddition.[20] The synthesis from (*Z*)-α,β-unsaturated ester **5o** afforded the epimeric bicyclic α-amino acid **11** as a single diastereomer. It is worth noting that there are few reports of the cycloaddition of azomethine ylide generated from an α-keto acid and an α-amino acid.[19b]

Scheme 3. Transamination and Intramolecular [3+2] Cycloaddition of Azomethine Ylide.



Scheme 4. Synthesis of Bicyclic α-Amino Acids (The procedures used in steps 1-3 are as indicated in Table 2).

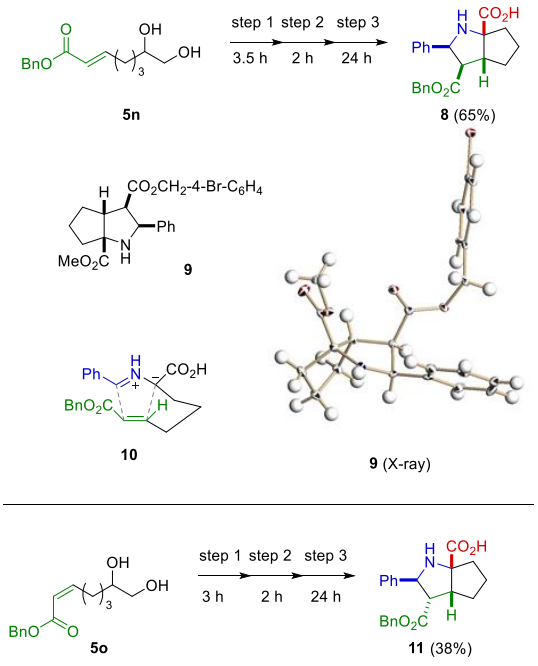
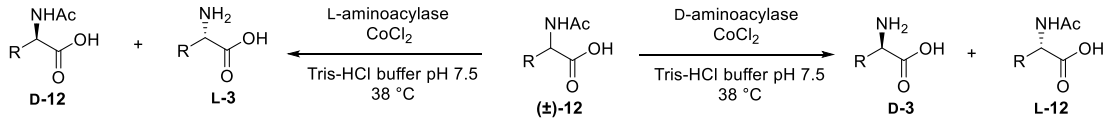


Table 3. Chemoenzymatic Kinetic Resolution of α-Amino Acids.



D-12[a,b]	L-3[a,b]	time (h)	(±)-12	R	time (h)	D-3[a,b]	L-12[a,b]
51%, >99% ee	39%, >99% ee	1	**12a**	Ph	1	41%, >99% ee	50%, >99% ee
51%, >99% ee	40%, >99% ee	2	**12d**	2,3,5,6-tetrafluorophenyl	1	44%, 99% ee	50%, >99% ee
48%, >99% ee	38%, >90% ee[d]	5	**12f**[c]	NC-(CH2)8-	24	45%, >90% ee[d]	50%, 99% ee
51%, >99% ee	39%, >99% ee	3	**12h**	Ph-C(=O)O-(CH2)8-	24	37%, 99% ee	51%, >99% ee
50%, >99% ee	41%, 99% ee	5	**12i**	BnO-C(=O)-(CH2)7-	24	43%, >99% ee	51%, 98% ee

[a] Enantiomeric excess was determined by chiral HPLC. [b] Isolated yield. [c] Phosphate buffer (1 M, pH 7.5) was used. [d]Ion-exchange chromatography (Dowex 50W-8) was used for the purification.

Finally, the chemoenzymatic kinetic resolution of α-amino acids was examined (Table 3).[21] We selected homophenylalanine [**(±)-12a**], and the four α-amino acids **(±)-12d**, **(±)-12f**, **(±)-12h**, and **(±)-12i** bearing functional groups that are distinguishable from those of natural α-amino acids, namely; pentafluorophenyl, cyano, and ester. L-Aminoacylase (EC 3.5.1.14, acylase H, from *Aspergillus melleus*) and D-aminoacylase (EC 3.5.1.81, from *E. coli*) were employed in the resolution experiments, and the reactions were performed in tris-HCl buffer (1 M, pH 7.5) or phosphate buffer (1 M, pH 7.5) at 38 °C in the presence of a catalytic amount of $CoCl_2$. At the end of the reaction, the deacetylated free α-amino acids were precipitated using Et_2O and isolated by filtration. Ion-exchange chromatography was used to purify **L-** and **D-3f**, owing to its high solubility. Interestingly, we found that both aminoacylases catalyzed deacetylation with almost complete enantioselectivity.

In summary, we developed a novel three-step synthetic method of α-amino acids from 1,2-diols. The first and second steps involve the chemoselective oxidations of a 1,2-diol to the α-keto acid, which is prone to the oxidative C-C bond cleavage. The third transamination facilitates the chemoselective reductive amination of α-keto acids. It requires neither hydrolysis nor hydrogenolysis for deprotection, which facilitates the syntheses of α-amino acids bearing functional groups. As demonstrated by the synthesis of **^{15}N-3a**, this method is also effective for the selective synthesis of ^{15}N-labelled α-amino acids. In addition, L- and D-aminoacylase-catalyzed resolution proved highly effective for the preparation of optically active α-amino acids. This method will undoubtedly accelerate α-amino-acid, peptide, and protein drug discovery as it is also a useful strategy for the synthesis of unnatural α-amino acids.

ASSOCIATED CONTENT

Supporting Information.

Tables S1 and S2, Experimental details, characterization of new compounds, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

This work was partially supported by JSPS KAKENHI (No. JP16K08162), the Platform Project for Supporting Drug Discovery and Life Science Research (BINDS, No. JP18am0101099) from AMED, the Research Foundation for Pharmaceutical Sciences, and Grant for Basic Science Research Projects from The Sumitomo Foundation. We are grateful to Amano enzyme Inc. for the gift of acylase H "Amano", and thank Dr. Y. Kojima (Amano Enzyme Inc,) and Y. Sato (Amano Enzyme Inc.) for insightful discussion. We also thank Dr. K. Oyama (RCMS, Nagoya University) for elemental analysis.

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