

PAPER

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[View Journal](#) | [View Issue](#)Cite this: *Org. Biomol. Chem.*, 2024, **22**, 6999Ammonium carboxylates in the ammonia-Ugi reaction: one-pot synthesis of α,α -disubstituted amino acid derivatives including unnatural dipeptides†Keisuke Tomohara,^{a,b} Satoru Kusaba,^c Mana Masui,^b Tatsuya Uchida,^{a,c,d} Hisanori Nambu^b and Takeru Nose^{a,c}

Despite the remarkable developments of the Ugi reaction and its variants, the use of ammonia in the Ugi reaction has long been recognized as impractical and unsuccessful. Indeed, the ammonia-Ugi reaction often requires harsh reaction conditions, such as heating and microwave irradiation, and competes with the Passerini reaction, thereby resulting in low yields. This study describes a robust and practical ammonia-Ugi reaction protocol. Using originally prepared ammonium carboxylates in trifluoroethanol, the ammonia-Ugi reaction proceeded at room temperature in high yields and showed a broad substrate scope, thus synthesizing a variety of α,α -disubstituted amino acid derivatives, including unnatural dipeptides. The reaction required no condensing agents and proceeded without racemization of the chiral stereocenter of α -amino acids. Furthermore, using this protocol, we quickly synthesized a novel dipeptide, D-Leu-Aic-NH-CH₂Ph(*p*-F), which exhibited a potent inhibitory activity against α -chymotrypsin with a *K*_i value of 0.091 μ M.

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Introduction

Peptides have attracted global interest as new modalities of drug carriers,¹ beyond Lipinski's rule of 5 (bRo5) medicines,² and biomaterials.³ Unnatural α,α -disubstituted amino acid residues have played an important role in providing peptides with biostability and conformational stability, which in turn enhances the bioavailability, target specificity, and binding activity of peptides for *in vivo* applications.⁴ Such potential has resulted in a high demand for peptides containing unnatural α,α -disubstituted amino acid residues. Conventional peptide synthesis relies on the sequential coupling of protected amino acids on solid supports, typically using large amounts of condensing agents. During this

process, introducing sterically hindered α,α -disubstituted amino acids into peptides usually requires harsh conditions, such as heating and microwave irradiation, and also uses excessive amounts of condensing reagents and *N*-protected α,α -disubstituted amino acids,⁵ the latter of which are prepared by time- and step-consuming processes including protection and deprotection steps, typically from glycine-derived Schiff bases (Fig. 1a).⁶

The Ugi reaction is a classical four-component coupling reaction involving an aldehyde or a ketone, an isocyanide, a carboxylic acid, and a primary amine to give an α -acylamino amide (α -amino acid derivative) in a single synthetic operation.⁷ A series of Ugi variants,⁸ including disrupted-Ugi⁹ and on-resin Ugi reactions,¹⁰ have been developed, and recently a catalytic asymmetric Ugi reaction¹¹ and an Ugi reaction using natural product extracts as substrates¹² have been introduced, and thus the Ugi reaction has now been recognized as one of the well-established methods for diversity-oriented synthesis.¹³ In contrast to the great expansion of the Ugi variants, the use of ammonia in the classical Ugi reaction (the ammonia-Ugi reaction) has long been recognized to be impractical and unsuccessful, although this reaction directly provides unnatural peptides containing α,α -disubstituted amino acids (Fig. 1b). Mechanistically, the ammonia-Ugi reaction begins with the reversible formation of an *N*-unsubstituted imine intermediate. Herein, the relatively weak nucleophilicity of

^aFaculty of Arts and Science, Kyushu University, 744 Motoooka, Nishi-ku, Fukuoka, 819-0395, Japan^bFaculty and Graduate School of Pharmaceutical Science, Kyoto Pharmaceutical University, 1 Misasagishichono-cho, Yamashina-ku, Kyoto 607-8412, Japan. E-mail: tomohara47@mb.kyoto-phu.ac.jp^cGraduate School of Science, Kyushu University, 744 Motoooka, Nishi-ku, Fukuoka, 819-0395, Japan. E-mail: nose@artsci.kyushu-u.ac.jp^dInternational Institute for Carbon-Neutral Energy Research, Kyushu University, 744 Motoooka, Nishi-ku, Fukuoka, 819-0395, Japan†Electronic supplementary information (ESI) available: General experimental information; synthetic details; NMR spectra; HPLC charts. See DOI: <https://doi.org/10.1039/d4ob00924j>

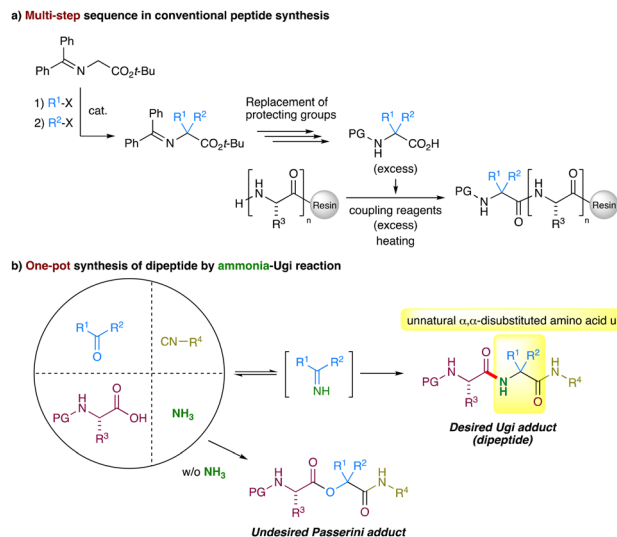


Fig. 1 Synthetic methods of peptides containing unnatural α,α -disubstituted amino acid residues. (a) Conventional multi-step synthetic method and (b) one-pot synthesis of dipeptides by the ammonia-Ugi reaction.

ammonia, compared with primary amines, hampers the imine formation. This situation, in turn, can lead to the undesired Passerini reaction (the three-component coupling reaction of an aldehyde or ketone, an isocyanide, and a carboxylic acid).^{14,15} Thus, the ammonia-Ugi reaction has often required harsh conditions such as heating,¹⁶ microwave irradiation at high temperatures,¹⁷ or high dilution¹⁸ and has suffered from low yields.^{16a,b,18a,19} This situation becomes more serious when a ketone is used as the carbonyl component.²⁰ Indeed, previous reports on the practical ammonia-Ugi reaction are limited where aldehydes are used as the carbonyl component.²¹ Overall, the applications of the ammonia-Ugi reaction toward the synthesis of unnatural α,α -disubstituted amino acid derivatives²² and peptides containing α,α -disubstituted amino acids²³ have been sparingly reported.

Here, we came across a small number of successful reports on the ammonia-Ugi reaction, where the ammonia-Ugi adducts were obtained in moderate to high yields under mild reaction conditions by using commercially available ammonium formate, acetate, or benzoate as sources of both ammonia and carboxylic acids.^{22–24} Despite such unique potential of ammonium carboxylates, commercially unavailable ammonium carboxylates have never been applied to the ammonia-Ugi reaction and thus the effects of ammonium carboxylates remain unclear. In this context, this study systematically investigated their effects on the ammonia-Ugi reaction through establishing a method for preparing a variety of ammonium carboxylates, including the ammonium salts of α -amino acids. Using these unique reagents, a variety of α,α -disubstituted amino acid derivatives, including unnatural dipeptides, were successfully synthesized in high yields in a practical and robust manner.

Results and discussion

First, the ammonia-Ugi reaction conditions were examined using model substrates, including cyclopentanone, benzyl isocyanide, acetic acid, and ammonia, to synthesize the 1-amino-cyclopentane-1-carboxylic acid (Ac₅C) derivative **1a** (Table 1). Previously, inorganic ammonium salts have often been used as ammonia sources;^{14,25} therefore, we initially tested typical inorganic ammonium salts in the ammonia-Ugi reaction at room temperature in trifluoroethanol (TFE)/H₂O (1 : 1) (entries 1–4). However, these reactions resulted in a low conversion of the substrates and gave both the desired ammonia-Ugi adduct Ac-Ac₅C-NH-Bn (**1a**) and the undesired Passerini adduct (α -acyloxy amide) **2a** in low yields (up to 19% yields). Among these inorganic ammonium salts, the salts of strong acids delivered **2a** preferentially and gave no or trace amounts of the ammonia-Ugi adduct **1a** (entries 1 and 2). In contrast, those of weak acids preferentially gave **1a** over **2a** (entries 3 and 4). Furthermore, the combination of NH₄Cl and AcONa also resulted in the preferential production of **1a** (entry 5). These results, although low yields, imply that the dissociated ammonium ions would prompt imine formation, and concomitantly the carboxylate ions would keep ketones away from the undesirable Passerini pathway, thus providing **1a** preferentially. The use of aqueous NH₃ greatly improved the isolated yield of **1a** (55%); however, these conditions still yielded an appreciable amount of **2a** (18% yield, entry 6). Finally, the use of ammonium acetate in TFE (0.5 M) at room temperature dramatically enhanced the conversion of the starting materials and concomitantly minimized the formation of the Passerini adduct **2a** (6% yield), thus providing the desired Ugi adduct **1a** in an excellent yield (90%, entry 7). As expected, the polar

Table 1 Optimization of the ammonia-Ugi reaction conditions

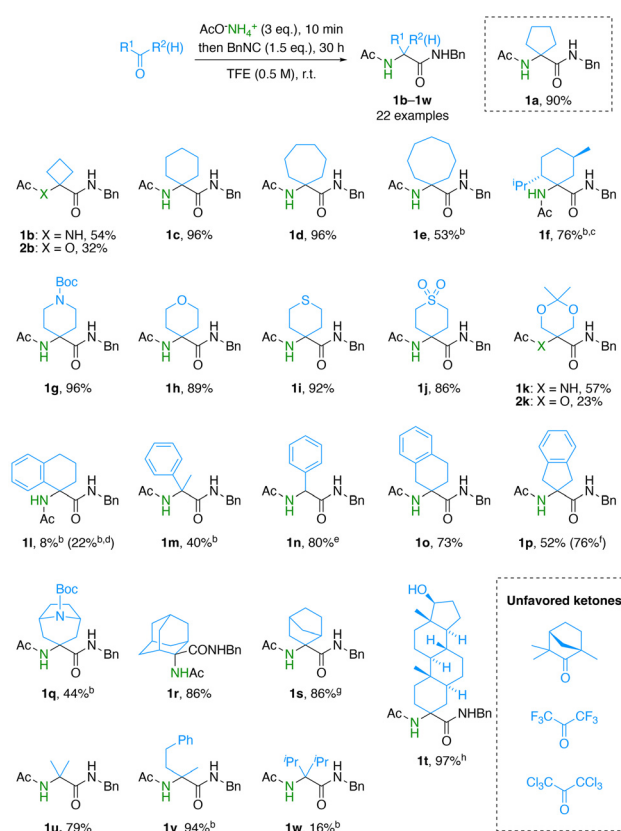
				Yield ^a (%)
Entry	NH ₃ source	AcOH source	Solvent	1a 2a
1	(NH ₄) ₂ SO ₄	AcOH	TFE/H ₂ O (1 : 1) ^b	— ^c 19
2	NH ₄ Cl	AcOH	TFE/H ₂ O (1 : 1) ^b	1 ^c 19
3	NH ₄ HCO ₃	AcOH	TFE/H ₂ O (1 : 1) ^b	17 ^c 7
4	(NH ₄) ₂ CO ₃	AcOH	TFE/H ₂ O (1 : 1) ^b	19 ^c 8
5	NH ₄ Cl	AcONa	TFE/H ₂ O (1 : 1) ^b	22 ^c 9
6	NH ₃ aq.	AcOH	TFE	55 18
7	AcONH ₄		TFE	90 6
8	AcONH ₄		MeOH	68 —
9	AcONH ₄		HFIP	53 25
10	AcONH ₄		Dry TFE ^d	89 4
11	AcONH ₄		TFE ^e	95 3
12	AcONH ₄		TFE ^f	92 4

^a Isolated yield. ^b v/v. ^c Starting materials remained. ^d Under a N₂ atmosphere. ^e In the presence of Na₂SO₄ (100 mg). ^f At 60 °C, 3 hours.



solvent methanol completely suppressed the Passerini competition; however, the nucleophilicity of methanol caused several side reactions and the reaction mixture became complicated, thereby delivering **1a** in only a moderate yield (68%, entry 8). Other alcoholic solvents, ethanol, 2-propanol, and *tert*-butyl alcohol, also resulted in incomplete conversion of the starting materials because ammonium acetate was often insoluble in these alcohols (Table S1, entries 1–3†). Although the addition of water (up to 10%, v/v) made the reaction mixture homogeneous, it did not improve the isolated yields of **1a** (Table S1, entries 4–6†). In contrast, 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) dissolved ammonium acetate well, but accelerated the Passerini reaction (Table 1, entry 9). The use of dry TFE under a N₂ atmosphere had no impact on the isolated yield of **1a** (entry 10), while the addition of Na₂SO₄ slightly increased the isolated yield of **1a** (entry 11). By increasing the reaction temperature to 60 °C, the reaction time could be dramatically reduced from 30 h to 3 h with no loss of the isolated yield of **1a** (entry 12). Overall, the systematic screening of a set of reagents and solvents confirmed that the use of ammonium acetate in TFE is optimal and practical for the ammonia-Ugi reaction.

Having confirmed the potential of ammonium acetate as a key reagent for the ammonia-Ugi reaction, we then explored the scope of ketones in the synthesis of various α,α -disubstituted amino acid derivatives (Scheme 1). The six- and seven-membered ketones cyclohexanone and cycloheptanone were compatible with the reaction conditions to give the corresponding Ugi adducts **1c** and **1d** in excellent yields (96%), while cyclobutanone afforded the Ugi adduct **1b** in a moderate yield (54%) probably because the conformationally strained four-membered ring hampered the formation of the imine intermediate. Instead, this reaction yielded a considerable amount of the Passerini adduct **2b** (32%). Cyclooctanone also resulted in a low yield (53%), and the starting materials were mostly recovered. The sterically congested (–)-menthone tolerated the present reaction conditions to give **1f** in 76% yield in an approximately 2 : 1 diastereomeric ratio (dr). The major diastereomer of **1f** has a configuration of (1*R*), which was determined by a NOESY experiment (Fig. S1†). A series of cyclohexanone analogs containing *N*-Boc, O, S, and SO₂ at the γ -position were well tolerated under the present reaction conditions providing the corresponding Ugi adducts **1g–1j** in excellent isolated yields (86%–96%). An acetal moiety was found to be partially tolerated under the present reaction conditions to give **1k** in 57% yield together with the Passerini adduct **2k** (23%). Aromatic ketones resulted in low yields (**1l** and **1m**) due to a slow conversion, while benzaldehyde exhibited a high reactivity, even at 4 °C, producing **1n** in 80% yield. The ketones containing an acidic hydrogen, β -tetralone and 2-indanone, gave **1o** and **1p** in moderate yields (73% and 52%, respectively). The yield of **1p** could be increased to 76% by using benzyl isocyanide in excess at low temperature (4 °C) in the presence of Na₂SO₄. The bicyclic *N*-Boc-nortropinone provided **1q** in a moderate yield (44%), and furthermore both 2-adamantanone and 2-norbornanone were well compatible with the present



Scheme 1 Scope of ketones in the ammonia-Ugi reaction.^a ^a Isolated yield. ^b Starting materials remained. ^c dr approx. 2 : 1. ^d 14 d. ^e 3 h. ^f At 4 °C in the presence of Na₂SO₄ (100 mg). ^g dr approx. 1.5 : 1. ^h dr > 20 : 1.

reaction conditions to give **1r** and **1s** in high yields (86%). The hydroxy group of steroidal stanolone had no detrimental effect, affording the unusual amino acid derivative **1t** in 97% yield. Acyclic ketones, *i.e.*, acetone and 4-phenyl-2-butanone, were tolerated under these reaction conditions (**1u** and **1v**). In contrast, the highly bulky diisopropyl ketone resulted in a low yield (**1w**, 16%) because of the incomplete conversion, and (+)-fenchone and hexafluoroacetone remained unreacted. The use of hexachloroacetone resulted in the isolation of the corresponding imine intermediate, which remained unreactive toward the further nucleophilic addition of isocyanide. Unless otherwise mentioned, all reactions could suppress the formation of the competing Passerini adducts to less than 5% isolated yields. Overall, these screenings clearly illustrated the generality, functional group tolerance, and utility of the developed ammonia-Ugi reaction for the one-pot synthesis of unnatural α,α -disubstituted amino acid derivatives.

We next investigated how the acidity of carboxylic acids affects the outcome of the ammonia-Ugi reaction, compared with the classical Ugi reaction using benzylamine (Table 2).²⁶ Both Ugi and ammonia-Ugi reactions were performed at room temperature in TFE (0.5 M). In the Ugi reaction, upon increasing the acidity of acids, the isolated yields of the Ugi adducts



Table 2 Scope of acids in the ammonia-Ugi reaction and Ugi reaction

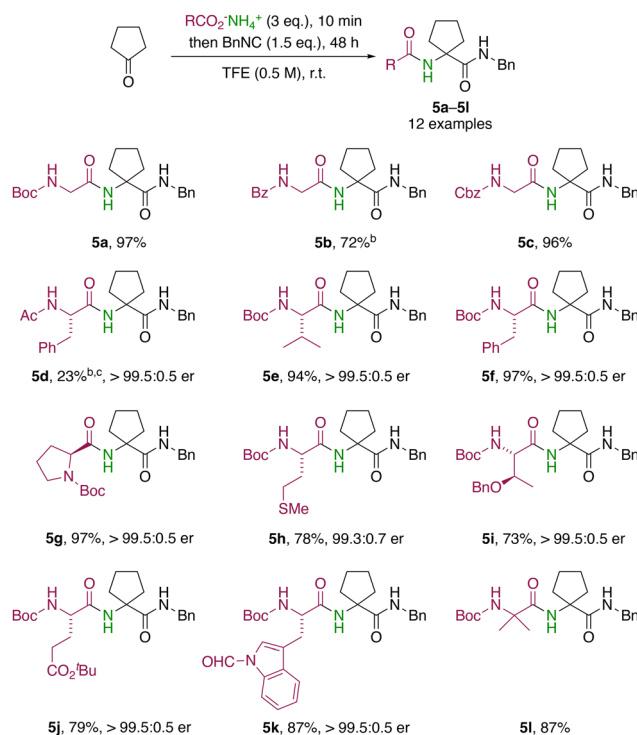
Ugi				
Ammonia-Ugi				
Yield ^a (%)				
Entry	Acid	pK _a of acid	3 ^b	4 ^c
1	Pivalic acid	5.03	3a	92
2	Acetic acid	4.76	3b	88
3	Benzoic acid	4.20	3c	83
4	Formic acid	3.77	3d	83
5	<i>p</i> -Nitrobenzoic acid	3.41	3e	73
6	Chloroacetic acid	2.86	3f	63
7	<i>o</i> -Nitrobenzoic acid	2.16	3g	55
8	Trifluoroacetic acid	−0.25	3h	6

^a Isolated yield. ^b Reaction protocol: a solution of cyclopentanone (0.5 mmol) and benzylamine (1.5 mmol) in TFE was stirred at room temperature for 10 min, and then carboxylic acid (1.5 mmol) and benzyl isocyanide (0.75 mmol) were successively added. ^c Reaction protocol: a solution of cyclopentanone (0.5 mmol) and ammonium carboxylate (1.5 mmol) was stirred at room temperature for 10 min, and then benzyl isocyanide (0.75 mmol) was added. ^d Quoted from Table 1, entry 6. ^e Starting materials remained. ^f Isolated yield of the corresponding Passerini adduct.

3a–3g gradually decreased from 92% to 55%, and finally, the Ugi adduct **3h** derived from trifluoroacetic acid (TFA) was obtained only in a trace amount (5% yield). Thus, the Ugi reaction was found to be susceptible to acidity. In contrast, the present ammonia-Ugi reaction conditions could tolerate a variety of acids with pK_a values in the range of 2.16 to 5.03 to give the ammonia-Ugi adducts **4a–4g** in moderate to high yields (42%–92%). The relatively low yields of **4c** and **4e** (73% and 42%, respectively) were attributed to the fact that the corresponding ammonium benzoates were poorly soluble in TFE. To our delight, TFA was well compatible with the ammonia-Ugi reaction conditions to afford **4h** in a moderate yield (53%). Here, ammonium acetate, benzoate, and formate were commercially available and the other ammonium carboxylates could be easily prepared as pure crystals by adding aqueous ammonia to a solution of the carboxylic acid in acetone, acetonitrile, or THF at 4 °C, followed by filtration (Table S2†). Thus, volatile acids, such as formic acid and TFA, could be handled accurately and easily as solid ammonium carboxylates in the ammonia-Ugi reaction. Taken together, in sharp contrast to the classical Ugi reaction, the present ammonia-Ugi reaction conditions allowed the use of a broad range of acids providing a variety of *N*-acyl α,α-disubstituted amino acid derivatives.

To further explore the utility of the ammonia-Ugi reaction, a variety of dipeptides containing the unnatural Ac₅c residue

were synthesized using a set of commercially available *N*-protected α-amino acids as coupling partners (Scheme 2). The ammonium salts of the *N*-protected α-amino acids could be carefully prepared by adding aqueous ammonia to a solution of *N*-protected α-amino acid in acetone or acetonitrile at 4 °C, followed by filtration (Table S2†). Typical nitrogen-protecting groups of amino acids, such as Boc, Bz, and Cbz, were well compatible with the present reaction conditions to afford the corresponding dipeptides PG-Gly-Ac₅c-NH-Bn **5a–5c** in high to excellent yields (72%–97%). In contrast, Ac-Phe-ONH₄ was unsuitable because it was insoluble in TFE even at 0.1 M, resulting in a low yield of **5d** (23%). Similar results were obtained using Fmoc-Gly-ONH₄ (data not shown). Representative Boc-protected α-amino acids (Val, Phe, Pro, and Met) were successfully used to produce the corresponding dipeptides **5e–5h** in high yields (78%–97%). A series of side-chain-protecting groups, such as benzyl ether, *tert*-butyl ester, and formyl amide, were all compatible with the present conditions to afford the dipeptides **5i–5k** in high yields (73%–87%). Importantly, chiral HPLC analysis confirmed that no racemization of the chiral stereocenter of the α-amino acids had occurred under the present reaction conditions (Fig. S130–138†). Furthermore, when the reaction temperature was set at 60 °C, **5f** could be obtained within 4 hours in a high yield (78%) without the loss of stereochemical integrity (>99.5 : 0.5 er). Notably, the sterically hindered 2-aminoisobutyric acid (Aib) was smoothly converted into the dipeptide Boc-Aib-Ac₅c-NH-Bn (**5l**), containing two contiguous

**Scheme 2** Scope of amino acids in the ammonia-Ugi reaction.^a

^a Isolated yield. ^b Starting materials remained. ^c 0.1 M.



α,α -disubstituted amino acid residues, in 87% yield. Thus, the present reaction conditions enabled the one-pot synthesis of dipeptides containing unnatural α,α -disubstituted amino acid motifs.

To gain mechanistic insight into the role of the ammonium carboxylates, two control experiments were performed (Table 3). Compared with the optimal conditions (Table 1, entry 7), the procedure of mixing ammonia and ketone before the addition of carboxylic acid considerably reduced the isolated yield of **1a** (55%), mainly because of the production of **2a** (18%) as a by-product (Table 3, entry 1). In turn, the *in situ* formation of AcONH_4 slightly improved the isolated yield of **1a** (76%); however, this procedure still gave the Passerini adduct **2a** (11%) probably due to the incomplete formation of AcONH_4 (entry 2). Eventually, both procedures suffered from competition with the undesired Passerini pathway. These results indicated that the preparation of the ammonium carboxylates outside of the reaction vessel minimized the generation of free carboxylic acids *in situ* and effectively suppressed the undesired Passerini pathway (Fig. 2). Moreover, the ammonium ions are expected to promote the formation of *N*-unsubstituted ketiminium intermediates and guide the reaction toward the otherwise unfavored ammonia-Ugi pathway.

Previously, we have reported the inhibitors of the serine proteinase α -chymotrypsin.²⁷ Among these, the dipeptidic inhibitor $\text{D-Leu-Phe-NH-CH}_2\text{Ph}(p\text{-F})$ exhibited the most potent inhibitory activity.^{27b} Mechanistically, its C-terminal *p*-fluoro-

benzyl group occupies the chymotrypsin S1 site where the fluorine atom forms a hydrogen bonding interaction with Ser189 and the hydrophobic core consisting of the alkyl group of D-Leu and the phenyl group of Phe binds to the S2 site.²⁸ These unique enzyme-inhibitor interactions were essentially stabilized by the π - π stacking interaction between the phenyl group of Phe and the imidazole group of His57.²⁹ Inspired by this inhibitory mechanism, we designed a new dipeptide analog, D-Leu-Aic-NH-R , containing the conformationally blocked residue 2-aminoindane-2-carboxylic acid (Aic), to strengthen the key π - π stacking interaction with the imidazole group of His57. Retrosynthetically, the dipeptide D-Leu-Aic-NH-R is accessible by the ammonia-Ugi reaction using Boc- D-Leu-ONH_4 , 2-indanone, and isocyanide as readily available building blocks (Scheme 3). Using the isocyanides **6a–6e**, the ammonia-Ugi reaction followed by deprotection of the Boc group rapidly delivered a set of dipeptides **7a–7e** as pure HCl salts after recrystallization in synthetically useful yields, again without the loss of the stereochemical integrity of the D-Leu residue (Fig. S134–138†). Thus, the present ammonia-Ugi reaction conditions were found to exhibit a broad scope of isocyanides. Then, the inhibitory activity of these dipeptides against α -chymotrypsin was investigated by using acetyl-tyrosine ethyl ester as the substrate and analyzed by non-linear fitting to the Michaelis-Menten equation (Table 4). Consistent with our previous results,^{27b} the dipeptides **7a–7c**, lacking the C-terminal benzyl group, exhibited no inhibitory activity even at 100 μM (entries 1–3), while dipeptide **7d**, containing a C-terminal benzylamide, inhibited chymotrypsin with a K_i value of 0.78 μM , and was more effective than $\text{D-Leu-Phe-NH-CH}_2\text{Ph}$ (K_i , 3.1 μM) (entries 4 vs. 6). Fortunately, the compound **7e** exhibited the most potent inhibitory activity with a K_i value of 0.091 μM , which was approximately 7-fold higher than that of the original inhibitor $\text{D-Leu-Phe-NH-CH}_2\text{Ph}(p\text{-F})$ (K_i , 0.63 μM) (entries 5 vs. 7). These findings indicated that the conformationally rigid bicyclic system of the Aic residue³⁰ contributed to strengthening the π - π interactions with the imidazole ring of the His57 residue, resulting in enhanced inhibitory potency against α -chymotrypsin. Thus, the ammonia-Ugi reaction enabled the rapid synthesis of potent α -chymotrypsin inhibitors from readily available building blocks. This synthetic simplicity and quickness are in sharp contrast to conventional

Table 3 Mechanistic insights into the ammonia-Ugi reaction

Entry	Procedure	Yield ^a (%)	
		1a	2a
1	Mixing ammonia and ketone before acid addition ^b	55 ^d	18
2	<i>In situ</i> formation of AcONH_4 ^c	76 ^d	11

^a Isolated yield. ^b Reaction procedure: a solution of cyclopentanone (0.5 mmol) and aqueous ammonia in TFE was stirred at room temperature for 10 min, and then acetic acid and benzyl isocyanide were successively added. ^c Reaction procedure: a solution of aqueous ammonia and acetic acid in TFE was stirred at room temperature for 10 min and then cyclopentanone was added and the mixture was stirred at room temperature for 10 min, followed by the addition of benzyl isocyanide. ^d Starting materials remained.

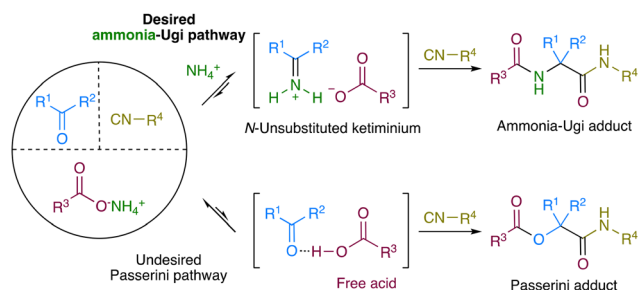
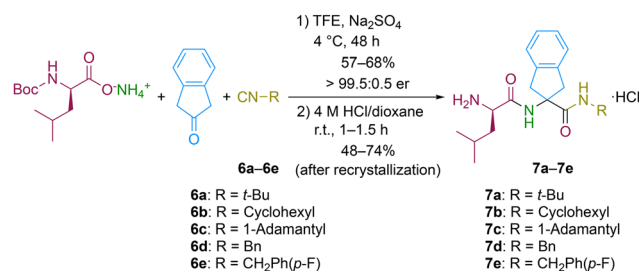


Fig. 2 Effect of ammonium carboxylates on the ammonia-Ugi reaction.



Scheme 3 Synthesis of dipeptidic inhibitors of α -chymotrypsin by the ammonia-Ugi reaction.



Table 4 α -Chymotrypsin inhibitory activity of dipeptide amides

Entry	Dipeptide	K_i^a (μ M)
1	7a	n.i. ^b
2	7b	n.i. ^b
3	7c	n.i. ^b
4	7d	0.78 ± 0.12
5	7e	0.091 ± 0.012
6	D-Leu-Phe-NH-Bn ^c	3.1 ± 0.5
7	D-Leu-Phe-NH-CH ₂ Ph(<i>p</i> -F) ^c	0.63 ± 0.14

^a Calculated by non-linear fitting to the Michaelis-Menten equation.^b No inhibition at 100 μ M. ^c Positive control.

peptide synthesis methods that begin with the preparation of the unnatural amino acid Aic.³¹

Conclusions

In summary, we have developed a robust and practical protocol for the ammonia-Ugi reaction. Using originally prepared ammonium carboxylates in TFE, the ammonia-Ugi reaction proceeded with high yields (up to 97%) at room temperature under air, and unnatural α,α -disubstituted amino acid motifs could be constructed *in situ* from readily available ketones as building blocks. Furthermore, the present ammonia-Ugi reaction was used to synthesize various dipeptides containing α,α -disubstituted amino acid residues without the need for any coupling reagents and, importantly, without racemization of the chiral stereocenter of α -amino acids (99.5:0.5 er). Among them, D-Leu-Aic-NH-CH₂Ph(*p*-F) (**7e**) exhibited a potent inhibitory activity against α -chymotrypsin with a K_i value of 0.091 μ M. Further work will focus on the synthesis of longer peptide chains and the development of a catalytic asymmetric ammonia-Ugi reaction to construct the chiral stereocenters of α,α -disubstituted unnatural amino acids. Extensions in these directions will be reported from our laboratory in due course.

Data availability

The data supporting this article have been included as part of the ESI.†

Author contributions

K.T. conceived this study. K.T., S.K., M.M., and T.N. performed the experiments and analyzed the data. K.T., S.K., T.U., H.N., and T.N. discussed the results. T.N. supervised the project.

Conflicts of interest

There are no conflicts to declare.

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