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Efficient and Regiospecific Syntheses of Peptides with Piperazic and Dehydropiperazic Acids via a Multicomponent Reaction

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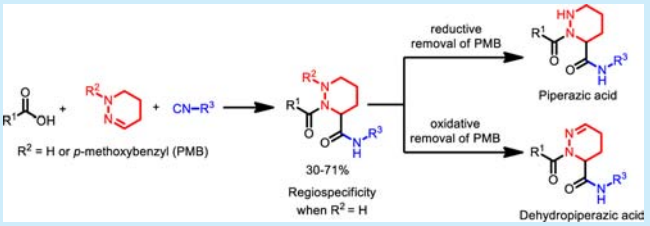
Supporting Information

ABSTRACT: Peptides containing N2-acyl piperazic or 1,6-dehydropiperazic acids can be formed efficiently via a novel multicomponent reaction of 1,4,5,6-tetrahydropyridazines, isocyanides, and carboxylic acids. Remarkably, the reaction's induced intramolecularity can enable the regiospecific formation of products with N2-acyl piperazic acid, which counters the intrinsic and troublesome propensity for piperazic acids to react at N1 in acylations. The utility of the methodology is demonstrated in the synthesis of the bicyclic core of the interleukin-1β converting enzyme inhibitor, Pralnacasan.



Numerous peptide natural products are constituted by nonproteinogenic amino acids.[1] The presence of these amino acids often imparts nonribosomal peptides with properties that are critical for their biological activities and/or stabilities.[1a] The peculiar structures and reactivities of noncoded amino acids can make the chemical syntheses of peptides containing them especially challenging. Among these unusual constituents of peptides, a heterocyclic amino acid with a N−N bond known as piperazic acid is particularly notable (Figure 1). Congeners such as 1,6-dehydropiperazic acid, 5-hydroxy piperazic acid, and 5-chloropiperazic acid have also been observed in peptide natural products (Figures 1 and 2).[2]

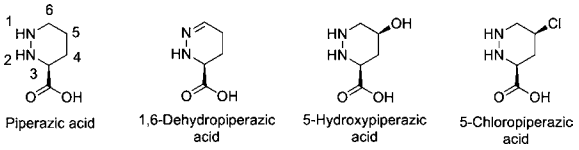


Figure 1. Piperazic acid and its congeners.

The piperazic acids can be found in many peptide antibiotics that have antitumor, antibacterial, antifungal, and antiviral activities.[2] The conformationally constrained amino acid can also be found in synthetic compounds, such as the antihypertensive drug, Cilazapril,[3] and Vertex Pharmaceuticals' rationally designed inhibitor of the interleukin-1β converting enzyme, Pralnacasan (Figure 2).[4] In both of these molecules, piperazic acid is part of a rigid, bicyclic core structure. Some structural diversity of molecules containing the piperazic acids is illustrated in Figure 2.

The unique structures and medicinal potential of molecules containing the piperazic acids have made them popular targets in target-oriented synthesis.[2] Whereas peptides are typically synthesized in a straightforward fashion by condensation of commercially available amino acids using a familiar set of

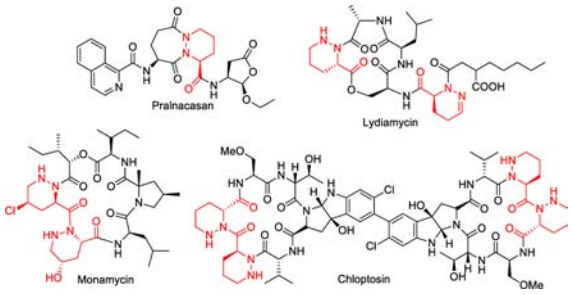


Figure 2. Molecules containing the piperazic acids.

coupling reagents, syntheses of those containing the piperazic acids require special considerations and efforts.[2,5−8] One issue is that these amino acids are not widely available for purchase and must be synthesized via multistep routes.[9] Examples of these routes include a Diels−Alder cycloaddition of a diene and an azodicarboxylate ester,[10] a tandem α-aminoalkylation/olefination sequence promoted by an organocatalyst,[5] or sequential stereoselective electrophilic hydrazination and nucleophilic cyclization.[6] Importantly, the piperazic acids have been prepared enantioselectively and used in peptide synthesis.[5−8,11a]

The intrinsic reactivity of the piperazic acids presents challenges in peptide synthesis. For example, in addition to the tendency of its esters to slowly oxidize and polymerize under ambient conditions,[8] piperazic acid preferentially undergoes acylation at N1.[11b] Likewise, dehydropiperazic acid is difficult to acylate at N2.[11a] These reactivity patterns are problematic because most known piperazic acid containing peptides have amide bonds exclusively at N2 (Figure 2). Studies by Ciufolini et al. indicate that the carbonyl group of these amino acids exerts a stereoelectronic effect that attenuates the nucleophilicity of

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N2.[12] This problem can be solved by reacting N1-carbamate protected piperazic acid with highly reactive acyl chlorides, but the yields of those reactions are modest at best.[2] Alternatively, the stereoelectronic effect can be circumvented via the use of a reduced form of piperazic acid as the acylation substrate.[2] N2-acylations of the amino alcohols can be effected with activated carboxylic acids other than acyl chlorides and have higher yields, but the resulting products must be oxidized to generate the desired piperazic acid moieties. The piperazic acids' reactivity issues can be avoided altogether by effecting heterocycle formation after regiospecific acylation of α-hydrazino esters.[2,13] As is necessary, the dehydropiperazic acid formed in the cyclization can be reduced to the saturated amino acid.[9a]

In contemplating a new strategy for achieving regioselective syntheses of peptides with N2-acyl piperazic acids, we envisaged an intramolecular acyl-transfer via a mixed anhydride intermediate. Indeed, leveraging intramolecularity is a well-known strategy in chemical synthesis.[14] Further consideration of this approach led to recognition of an analogy to the intramolecular acyl transfer in the Joullié–Ugi reaction.[15] In this multicomponent reaction, peptides containing N-acylated proline, or pipecolate residues are formed via the one-pot condensation of a cyclic imine with an isocyanide and a carboxylic acid. Its mechanism involves a series of reversible steps terminated by an irreversible Mumm rearrangement of an *O*-acylimidate, which is the intramolecular acyl transfer of interest. Beyond its favorable intramolecularity, we deemed the multicomponent reaction as a viable means to effect the desired transformation because its *O*-acylimidate intermediate has comparable reactivity with the acyl chlorides that are required for N2 acylation of piperazic acid. In analogy to the Joullié–Ugi reaction, we predicted that peptides with N2-acyl piperazic acid moieties could be formed regioselectively by condensation of an isocyanide, a carboxylic acid, and the cyclic hydrazone, 1,4,5,6-tetrahydropyridazine (THP) (Figure 3). A series of experiments were carried out to assess the viability of this new, hypothetical isocyanide multicomponent reaction (IMCR).[16]

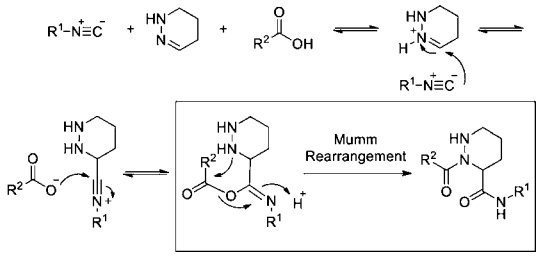
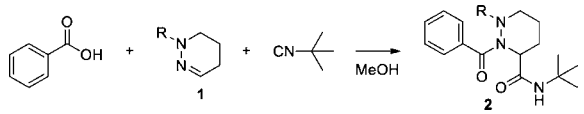
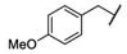
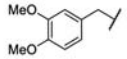
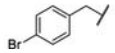


Figure 3. Proposed mechanism of the IMCR with THP.

In initial experiments, we reacted THP with benzoic acid and *tert*-butyl isocyanide in methanol. The expected N2-benzoyl piperazamide product was isolated as a racemate in 17% yield after a reaction time of 48 h (Table 1, entry 1). Notably, the apparent absence of N1-benzoyl piperazamide in the reaction indicated that the desired regiospecificity was achieved. Reaction parameters such as time, temperature, solvent, and stoichiometry were varied systematically to gain insights into the reaction and to improve its yield. We first investigated the parameter of reaction time and found that the yield improved with longer reaction times (30% after 5 days). The slow rate of the reaction led us to revisit our findings that Lewis acidic metal catalysts can promote IMCRs.[17] Thus, we carried out the reaction in the

Table 1. Optimization of IMCR with THP[a]



entry	THP	R	product	yield[b]
1	1a	H	2a	17%
2	1b		2b	NR[c]
3	1c		2c	38%
4	1d		2d	36%
5	1e		2e	45%
6	1f		2f	36%
7	1g		2g	19%

[a]Reactions were performed at rt for 48 h with equimolar substrates. [b]Isolated yields. [c]NR = No reaction.

presence of catalytic quantities (1, 10, or 25 mol %) of scandium triflate, but were disappointed to find that its inclusion did not improve the yields. Next, we assessed the effect of different solvents on reactivity. The rates and yields of IMCRs are known to be influenced by the solvent.[16a] The yield of the reaction in dichloromethane was equivalent to that observed in methanol. However, for reasons that are not obvious, no product formation could be detected by thin layer chromatography (TLC) when the reaction was performed in tetrahydrofuran. We then investigated the effect of temperature on yields, as heating is known to enhance some IMCRs.[18] Disappointingly, both conventional and microwave heating reduced product formation. These observations could be explained by reports that tetrahydropyridazines exhibit some chemical lability.[19] Since we suspected that THP could decompose during the reaction, we performed experiments wherein the reactant stoichiometry was varied. Interestingly, neither reactions with THP in a 4-fold excess over the other two reactants nor those in which the isocyanide and carboxylic acids were used in a 3-fold excess over the THP had higher yields than a reaction with equimolar reactants.

While we found that THP was a viable substrate in a regiospecific IMCR, the low yields led us to predict that THP could be weakly reactive and/or prone to side reactions. We proposed that both potential problems could be addressed by using N1-substituted THPs as substrates in IMCRs. In principle, substituents at N1 would both attenuate the atom's nucleophilicity as protecting groups and influence the reactivity of the THPs' hydrazone moiety via inductive effects.

In a preliminary examination of the substituent effects, we prepared a N1-acetyl THP (**1b**). We found that it did not react with benzoic acid and *tert*-butyl isocyanide, even after a 96 h reaction time. By comparing the outcome of this reaction (with an electron-withdrawing substituent on the THP) to one wherein unsubstituted THP was the substrate (Table 1, entries

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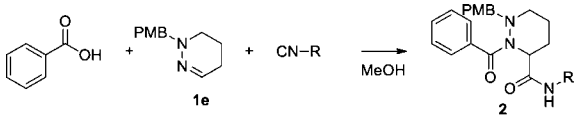
1 and 2), one could argue that the deactivating acetyl group on N1 further diminishes the nucleophilicity of N2 in the acylimidate intermediate[12] and thus precludes the key *O*-to-*N* acyl transfer (Figure 3). These observations are especially interesting in light of reports that acyclic *N*-acyl hydrazones are viable substrates in IMCRs.[20] In any case, we prepared THPs functionalized at N1 with an allyl moiety (**1c**) or benzyl moieties with either electron-donating or -withdrawing groups (**1d**–**g**) to further explore the influence of substituent effects.[20] They were then used as substrates in IMCRs with benzoic acid and *tert*-butyl isocyanide (Table 1, entries 3–7). The reactions with N1-substituted THPs had much higher yields and generated fewer side products than the reaction with unsubstituted THP (as assessed by TLC). The lowest yield was observed in the reaction wherein the THP substrate had an electron-deficient 4-bromobenzyl substituent. In contrast, reactions with electron-donating methoxybenzyl substituents on the THPs had nearly 3-fold higher yields compared to the reaction with unsubstituted THP (Table 1, entries 3–5). The most promising of these activating/protecting groups was the *p*-methoxybenzyl (PMB), as a THP substituted with it (**1e**) was the substrate in the highest yielding IMCR (45% after 48 h). Importantly, the PMB group can be removed from amines easily under conditions in which the peptide product is likely to be stable.[21] Unfortunately, we found that PMB-THPs with hydroxy substituents on the heterocycle were poor substrates in IMCRs (data not shown).

Although the presence of the PMB group on the THP precludes the advantageous regiospecificity of the IMCR, its positive effect on the reaction yield and the possibilities of its removal from the reaction product warranted further studies. Accordingly, we set out to determine the compatibility of the PMB-THP in reactions with various carboxylic acids and isocyanides. We were gratified to find that these reactions yielded the expected products in moderate to good yields (Tables 2 and 3). Conveniently, reactions of PMB-THP with

Table 2. Isocyanide Scope of the Optimized IMCR[a]

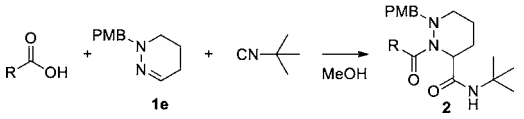


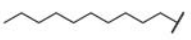
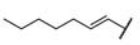
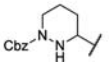
entry	R	product	yield[b]
1		2c	45%
2		2h	51%
3		2i	45%
4		2j	46%
5		2k	41%[c]

[a]Reactions were performed at rt for 48 h with equimolar substrates. [b]Isolated yields. [c]~1:1 mixture of diastereomers.

either an isocyanoacetate (Table 2, entry 5) or N-Boc-protected amino acids (Table 3, entries 4–5) yielded dipeptide products. It is particularly noteworthy that the reaction of racemic Cbz-N1-protected piperazic acid (Table 3, entry 6), PMB-THP, and *tert*-butyl isocyanide yielded a diastereomeric mixture of dipeptide products with contiguous piperazic acid residues in 34% yield because this connectivity is observed in many peptide anti-

Table 3. Carboxylic Acid Scope of the Optimized IMCR[a]



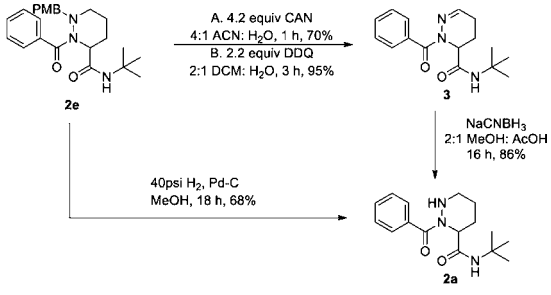
entry	R	product	yield[b]
1		2l	43%
2		2m	36%
3		2n	49%
4		2o	71%
5		2p	45%[c]
6		2q	34%

[a]Reactions were performed at rt for 48 h with equimolar substrates. [b]Isolated yields. [c]~1:1 mixture of diastereomers.

biotics.[2] To demonstrate the capacity of this IMCR to form a formal tripeptide, we also performed a reaction using PMB-THP, *N*-Boc-glycine, and methyl, 2-isobutyl-α-isocyanoacetate (derived from *N*-formyl leucine methyl ester). The expected tripeptide product was isolated in 45% yield (**2r**, see Supporting Information). These results indicate that the IMCR reaction is useful in the efficient syntheses of peptides. Despite the ease with which they are set up and their efficiencies, a drawback is that the reactions with chiral substrates are not stereoselective and yield diastereomeric products in ~1:1 ratios. However, the diastereomers can be separated by silica gel chromatography.

The aforementioned IMCRs yield peptides containing a piperazic acid moiety with a PMB-group on N1. We predicted that its removal from the peptide product under reductive conditions would yield a piperazic acid moiety and under oxidative conditions would yield a dehydropiperazic acid moiety.[21] We found that the PMB group can be removed from N1 of the IMCR product oxidatively by utilizing either ceric ammonium nitrate (CAN) or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to produce dehydropiperazic acid (Scheme 1). If desired, the unsaturated heterocycle can be transformed to piperazic acid using $NaCNBH_3$ as a reductant. Otherwise, the piperazic acid residue can be formed directly from the IMCR product via reductive cleavage of the PMB group using

Scheme 1. Formation of Piperazic Acids via PMB Removal



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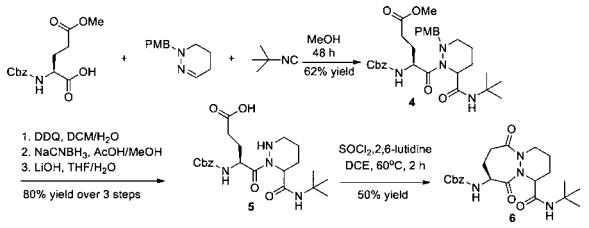
catalytic hydrogenation (Scheme 1). Through judicious selection of conditions for removal of the PMB group, it is possible to prepare peptides with the piperazic acid moiety in the desired oxidation state.

To demonstrate this novel IMCR's utility, we used it to efficiently synthesize the bicyclic core structure of Pralnacasan (Figure 1). This interleukin 1β-converting enzyme inhibitor was rationally designed and synthesized by Vertex Pharmaceuticals for use as a potential anti-inflammatory drug.[4] The first and key transformation in the synthetic route was condensation of N-Cbz-Glu(OMe)-OH, PMB-THP, and *tert*-butyl isocyanide (Scheme 2). Following removal of the IMCR product's PMB

Scheme 2. Synthesis of the Pralnacasan Bicyclic Core



protecting group via a tandem oxidation/reduction sequence and unmasking of its glutamic acid side chain by saponification, we used thionyl chloride to promote the desired ring closure as described in the patent literature.[22]

In summary, we have developed a novel IMCR that can be used to efficiently prepare peptides with a N2-acyl piperazic acid residue or its oxidized congener in only two steps. We have shown the IMCR can tolerate a variety of carboxylic acid and isocyanide substrates, including those that can be used for the direct formation of peptides with tandem piperazic acid constituents. Although the reaction is not stereoselective, we found that the diastereomeric products can be separated easily. This work is a practical and conceptual advance in peptide synthesis because it capitalizes on induced intramolecularity to circumvent challenges associated with the peculiar reactivities of piperazic and dehydropiperazic acids. Application of the methodology in the syntheses of natural products containing piperazic acids will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Procedures and spectroscopic data for all compounds. 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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