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Cyclizing Pentapeptides: Mechanism and Application of Dehydrophenylalanine as a Traceless Turn-Inducer

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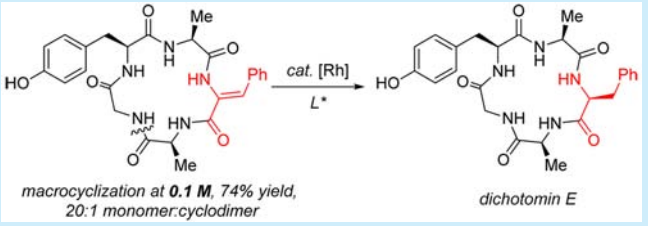
Supporting Information

ABSTRACT: Dehydrophenylalanine is used as a traceless turn-inducer in the total synthesis of dichotomin E. Macrocyclization of the monomer is achieved in high yields and selectivity over cyclodimerization under conditions 100 times more concentrated than previously achieved. The enamide facilitates ring closing, and Rh-catalyzed hydrogenation of the unsaturated cyclic peptide results in selective formation of the natural product or its epimer, depending on our choice of phosphine ligand. NMR analysis and molecular modeling revealed that the linear peptide adopts a left-handed α-turn that preorganizes the N- and C-termini toward macrocyclization.



Naturally occurring cyclic peptides have inspired the invention of strategies[1] for organic synthesis and therapeutics for use as antibiotics[2] and immunosuppressants.[3] In comparison with their linear counterparts, these cyclic structures show enhanced metabolic stability,[4] conformational rigidity,[5] and potential to mimic protein−protein interactions.[6] While significant progress has been made in the construction of relatively large cyclic peptides, the construction of smaller peptides (i.e., those containing five or fewer amino acids) remains a challenge.[7] In addition, cyclizing peptides at high concentrations on an industrial scale is important, and thus, a turn-inducer is desirable to ensure an efficient and economically feasible process.[8] Specific amino acids (e.g., proline, pseudoproline, D-amino acids, and *N*-methylated amino acids) have been identified as turn-inducers that can be incorporated into a linear precursor to facilitate macrocyclizations.[9] Ring closing of small peptides without such turn-inducers is plagued by competitive dimerization and epimerization.[10] Toward a more general solution to this challenge, we propose the use of dehydroamino acids as *traceless* turn-inducers.

Dehydroamino acids modulate backbone conformations and produce folded structures.[11] The impact of dehydrophenylalanine on the conformation of small peptides has been studied extensively over the past decade.[12] For example, Singh has shown that dehydrophenylalanines can induce β-turns in a linear tetrapeptide on the basis of X-ray crystallography studies (Figure 1a).[13] The ability of dehydroamino acids to impart folded conformations has yet to be exploited to achieve efficient ring closings in order to gain access to various cyclic peptides. We envisioned that this unsaturated moiety could serve as a versatile functional handle for further elaboration in the late-stage preparation of natural product derivatives.[14] Moreover, these unsaturated derivatives could serve as analogues in structure−activity relationship (SAR) studies or serve as potential epitope mimetics.[15,16] Here we report the first use of dehydrophenylalanine as a traceless turn-inducer via its application in the synthesis of dichotomin E (**1**).

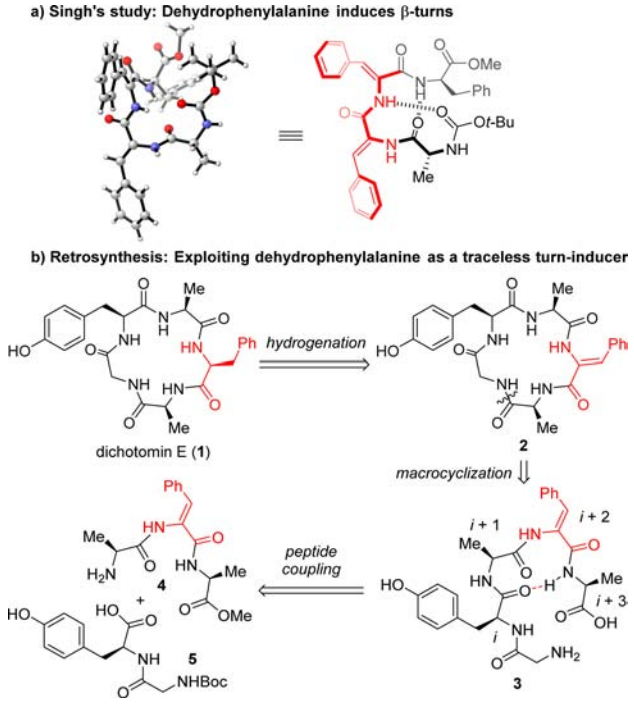


Figure 1. (a) Crystal structure of a tetrapeptide containing dehydrophenylalanine. (b) Proposed strategy using dehydrophenylalanine as a traceless turn-inducer.

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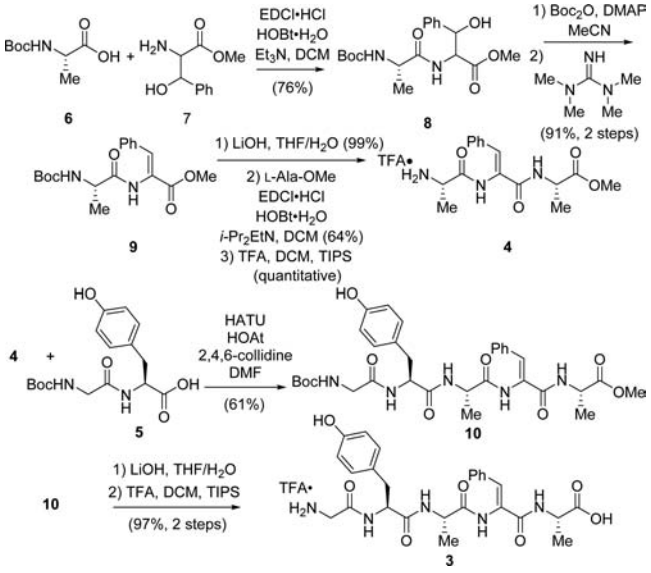
Isolated from the chickweed plant, *Stellaria dichotoma*, **1** is a cyclic peptide containing five amino acids with cell growth inhibitory activity against leukemia cells.[17] Our retrosynthetic analysis for construction of this small cyclic peptide is summarized in Figure 1b. First, we imagined that the natural product could be obtained from cyclic peptide **2**, containing a (*Z*)-dehydrophenylalanine,[18] by catalytic hydrogenation. In contrast to the incorporation of other turn-inducers, the dehydrophenylalanine can be easily unveiled to the L- or D-amino acid. Next, we chose to disconnect the glycine−alanine peptide bond to reveal the linear and unsaturated peptide **3**. We chose this disconnection to help favor an effective macrocyclization by placing the dehydrophenylalanine at the $i+2$ position, where it was previously reported to induce a β-turn.[19] A similar disconnection was used in the previous synthesis of **1** by Tam.[20] In general, macrocyclizations are more favorable using glycine because it is a relatively unhindered nucleophile.[21]

With this retrosynthetic analysis in mind, we prepared unsaturated pentapeptide **3** (Scheme 1). Boc-L-alanine (**6**) was

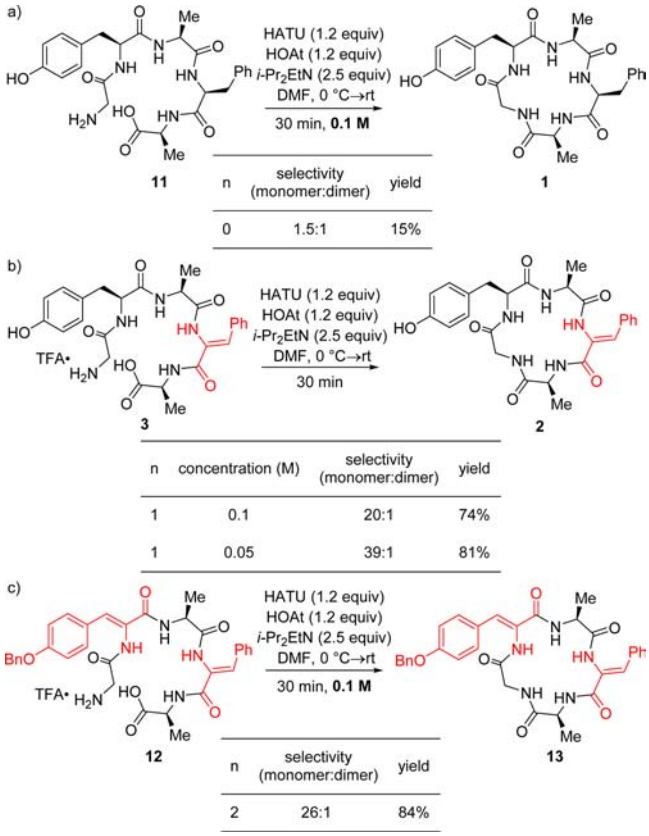
Scheme 1. Synthesis of Unsaturated Pentapeptide 3



coupled to DL-(β-OH)-Phe-OMe (**7**) to afford dipeptide **8** in 76% yield. Treatment of **8** with Boc anhydride and tetramethylguanidine afforded unsaturated dipeptide **9** in 91% yield.[22] Subjecting **9** to hydrolysis, peptide coupling, and deprotection gave tripeptide **4** in 63% yield. **4** was then coupled to dipeptide **5** in 61% yield to afford pentapeptide **10**. After hydrolysis and deprotection of **10**, unsaturated pentapeptide **3** was obtained in 97% yield. For comparison, we also prepared saturated linear peptide **11** in 64% yield using solid-phase peptide synthesis (SPPS) (see the Supporting Information (SI)).

When saturated linear pentapeptide **11** was subjected to macrocyclization at 0.1 M, only a 15% yield of **1** was obtained, with 1.5:1 selectivity for the desired monomer over the cyclodimer (Scheme 2a). In stark contrast, treatment of unsaturated pentapeptide **3** under the same conditions resulted in the formation of cyclic pentapeptide **2** in 74% yield, and the selectivity improved to 20:1 for the monomer versus the cyclodimer. Subsequently, cyclic pentapeptide **2** was isolated in 81% yield with 39:1 selectivity for the desired monomer over cyclodimer at 0.05 M. In comparison to Tam's method, where a silver-ion-assisted orthogonal cyclization at 0.001 M concen-

Scheme 2. Effect of Dehydroamino Acid on Macrocyclization (n = Number of Dehydroamino Acid Residues)[a,b]

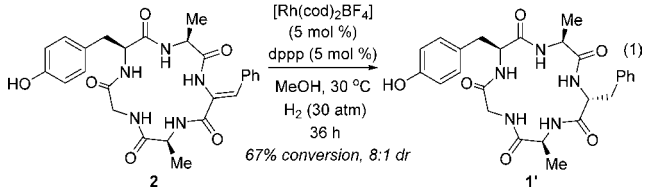


[a]Isolated yields are shown. [b]Selectivity was determined by HPLC.

tration afforded the macrolactam in 87% yield, our approach circumvents the need for high dilution by using *100 times* less solvent in the macrocyclization.

Next, we prepared pentapeptide **12** bearing two dehydroamino acids (see the SI) and subjected this linear precursor to ring closing. Under the same cyclization conditions at 0.1 M concentration, cyclic pentapeptide **13** was isolated in 84% yield with improved 26:1 selectivity for the monomer over the cyclodimer (cf. Scheme 2b,c).[23] Together, these results demonstrate that dehydrophenylalanines act as turn-inducers that greatly favor macrocyclization even at high concentrations.

With unsaturated cyclic peptides **2** and **13** in hand, we applied hydrogenation to install the final stereocenters. Hydrogenation of cyclic peptide **2** using $Rh(cod)_2BF_4$ and the achiral dppp ligand resulted in the formation of an 8:1 mixture favoring epimer **1′** of dichotomin E (eq 1). To overcome the inherent substrate bias, we



turned to asymmetric hydrogenation, which is commonly used in the synthesis of medicines in industry.[24] Liu and Zhang[25] previously reported the asymmetric hydrogenation of enamides using Rh(I) with Duanphos as the ligand to afford the corresponding amide with 99% *ee*. By using 5 mol % $Rh(cod)_2BF_4$

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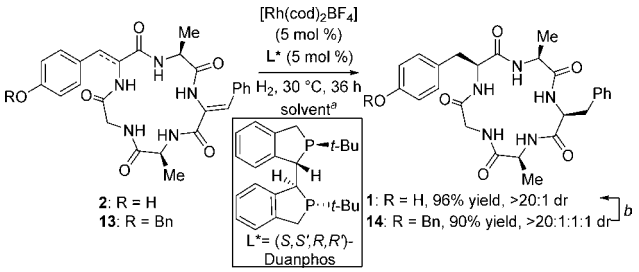
Scheme 3. Asymmetric Hydrogenation Affords Dichotomin E



[a]For **2**, MeOH was used. For **13**, THF was used. [b]Pd/C (20 mol %), H_2, 28 h, 30°, 99%.

and 5 mol % (*S*,*S*′,*R*,*R*′)-Duanphos in THF under 30 atm hydrogen, we were able to hydrogenate peptide **2** and obtain dichotomin E (**1**) in 96% yield with >95:5 dr (Scheme 3). It is worthy of note that reduction of cyclic peptide **2** using (*R*,*R*′,*S*,*S*′)-Duanphos affords the epimer **1**′ in 82% yield with >95:5 dr. Cyclic peptide **13** bearing two enamides can also be transformed to dichotomin E by tandem asymmetric reduction followed by debenzylation (Scheme 3).

To better understand the mechanism of macrocyclization, we performed CD spectroscopy experiments on pentapeptides **11**, **3**, and **2** in MeOH (298 K) to investigate the presence of secondary structure (Figure 2).[26] Uncyclized dehydropeptide **3** showed

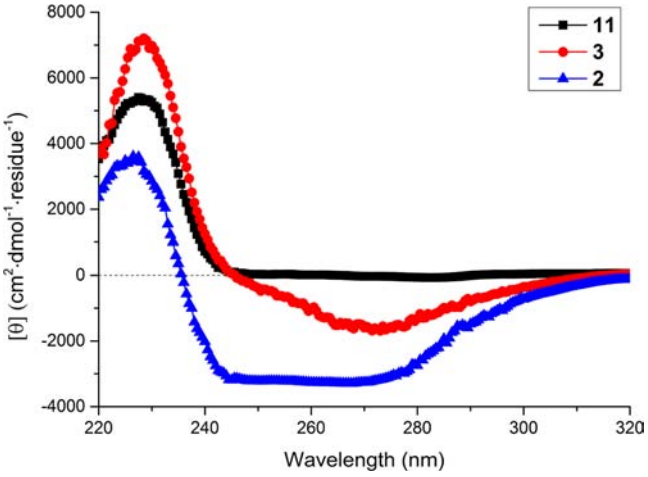
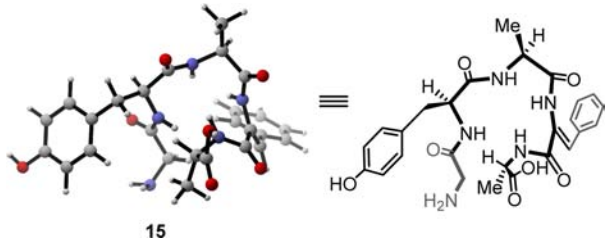


Figure 2. CD spectra of pentapeptides **11**, **3**, and **2**.

absorption patterns consistent with a cyclized structure, similar to cyclic dehydropeptide **2**. In contrast, uncyclized, saturated pentapeptide **11** showed no absorption patterns indicative of any secondary structural motif. The CD spectrum supports the pronounced effect of the presence of dehydrophenylalanine on the secondary structure of uncyclized dehydropeptide **3**, which helps to facilitate macrocyclization.

We used solution-state NMR spectroscopy and molecular modeling to elucidate the structure of unsaturated pentapeptide **3**. The ^3J couplings for the Tyr residue and the two Ala residues were obtained from 2D *J*-resolved ^{1}H NMR spectra.[27] These couplings were used to calculate H_NH_α ϕ dihedral angles via the Karplus relation.[28] Using these dihedral angle and 2D NOESY restraints, we performed molecular modeling studies with Maestro[29] to obtain 20 low-energy conformations that were consistent with our experimental observations (see the SI). These calculations support the lowest-energy structure **15** containing a



15

Figure 3. Minimized-energy conformation **15** of unsaturated peptide **3** consistent with NMR analysis.

left-handed α-turn, which is preorganized toward macrocyclization (Figure 3). Intramolecular H-bonding was also investigated using the temperature coefficients of the N−H chemical shifts ($\Delta\delta/\Delta T$), which can be used as an indicator of intramolecular H-bonding as opposed to H-bonds to solvent.[30,31] A value of −0.0039 ppm/K was obtained for the internal alanine N−H in dehydropeptide **3**, in contrast to the value of −0.0052 ppm/K observed for saturated pentapeptide **11** (see the SI). This difference suggests that there is dynamic intramolecular H-bonding in dehydropeptide **3** but not in saturated pentapeptide **11**, consistent with the ensemble of structures predicted by the molecular modeling. Together, these results demonstrate that a single dehydrophenylalanine residue can induce a left-handed α-turn.

When we replaced the phenyl substituent with a cyclohexyl substituent in **3**, cyclic monomer formation was observed in a promising yet less efficient 54% yield by ^{1}H NMR analysis (see the SI). This result suggests that the steric impact of the substituent influences the cyclization. In view of the higher-yielding macrocyclizations we observed in Scheme 2, conjugation between the phenyl substituent and the alkene helps promote ring closing by increasing the steric impact of the phenyl group. Weiss, Lawrence, and co-workers used dehydrophenylalanine as a β-breaker to study insulin and showed that extended conjugation of the aromatic π electrons with the neighboring C═C and C═O electrons enforces near-planarity.[32] The near-planar conformation of dehydrophenylalanine results in a greater steric interaction between the phenyl group and the adjacent amide group, as shown in **15**, which ultimately restricts the ϕ angle of the dehydroamino acid.[33] This restriction, through the increased $A_{1,3}$ strain, biases the N- and C-termini toward cyclization. Interestingly, a peptide containing three consecutive dehydroalanine units has been shown to adopt an extended conformation in which all of the amide groups show near-planarity.[34] This example suggests that the steric interactions of the group at the β-carbon of the dehydroamino acid are correlated to its ability to induce a turn.

In conclusion, we have demonstrated dehydrophenylalanine as an effective and traceless turn-inducer in the synthesis of dichotomin E. NMR analysis revealed that unsaturated pentapeptide **3** adopts a cyclic, preorganized structure. The enamide serves as a turn-inducer to facilitate ring closing without the need for high dilution. Moreover, it is a convenient functional handle for the late-stage construction of natural products and their derivatives. In SPPS, the overall yield is typically exceptional because each step in this linear approach is driven by exploiting excess reagents.[35] Combined with the need for dilute solvent conditions, the amount of waste generated in this traditional approach to cyclic peptide construction is significant. Our approach aims for a more efficient synthesis of cyclic peptides, especially on a large scale, while SPPS enables the rapid synthesis

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of peptide libraries on a small scale. Future studies in our laboratory will be focused on better understanding (1) the scope and limitations of dehydroamino acids as turn-inducers for macrocyclization[36] and (2) the mechanism of tandem hydrogenations in cyclic enamides. We expect that our simple yet effective strategy for ring closing will be of use to chemists interested in accessing cyclic pentapeptides for use as biological probes and therapeutics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03308.

Procedures and additional data (PDF)

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§D.N.L. and J.R. contributed equally.

Notes

The authors declare no competing financial interest.

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(36) Our method is currently limited to the synthesis of cyclic pentapeptides. When we cyclized the linear tetrapeptide (HO-Ala-ΔPhe-Ala-Gly-NH_2) at 0.01 M, we observed the formation of the cyclooctamer via LC−MS analysis.