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An Access to Aza-Freidinger Lactams and *E*-Locked Analogs

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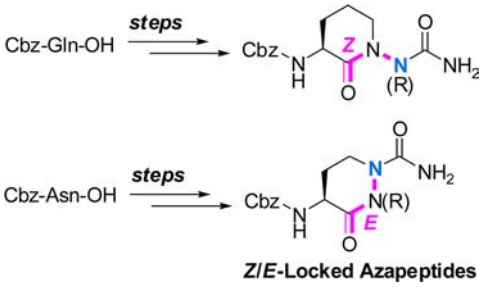
ABSTRACT



Freidinger lactams, possessing a peptide bond configuration locked to *Z*, are important key elements of conformationally restricted peptidomimetics. In the present work, the $C_\alpha H_{i+1}$ unit has been replaced by N, leading to novel aza-Freidinger lactams. A synthesis to corresponding building blocks and their *E*-locked analogs is introduced. The versatile buildings blocks reported here are expected to serve as useful elements in peptide synthesis.

Peptidomimetics are of great interest for drug development. This is due to the opportunity to improve selectivity and potency and to overcome the disadvantages of natural peptides, such as proteolytic susceptibility and poor bioavailability. Azapeptides, peptides in which the $C_\alpha H$ unit of at least one amino acid is replaced by nitrogen,[1] emerged as particularly important structures among peptidomimetics.[2]

Freidinger lactams (**1**) possess a linkage from $C_\alpha H_i$ to N_{i+1} and thus a configuration of the peptide bond restricted to *Z* (Figure 1).[3] Incorporation of Freidinger lactams is valued as a major contribution to the concept of conformational constraint in peptides.[4] Examples of constrained peptidomimetics related to Freidinger lactams include bi- to tetracyclic lactams,[5] pyrrolinones,[6] and imidazolinones.[7] Ring-closing metathesis[8] and Mitsunobu cyclization[9] allow access to similar peptide lactams.

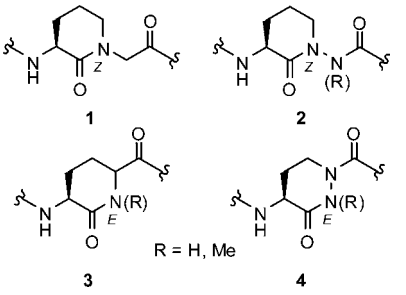


Figure 1. (aza-)Freidinger lactams **1**, **2** with *Z*-locked peptide bond and *E*-locked (aza)peptides **3**, **4**.

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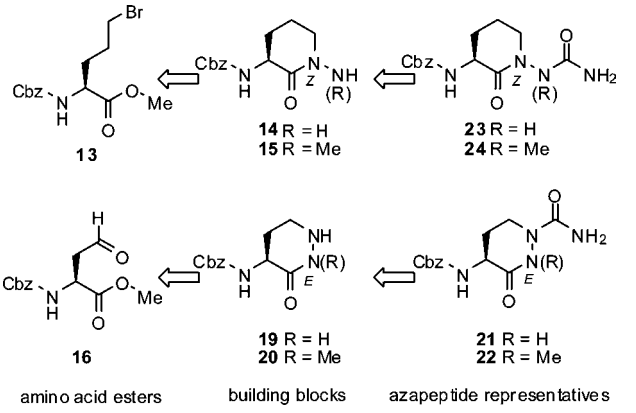
The formal exchange of $C_\alpha H_{i+1}$ by N results in *Z*-configured aza-Freidinger lactams (**2**). A linkage from $C_\alpha H_i$ to $C_\alpha H_{i+1}$ provides the 5-amino-6-oxopiperidine-2-carboxylic acid (Apc) derivatives **3** with a constrained *E* configuration,[10] and an analogous exchange of $C_\alpha H_{i+1}$ by N yields the corresponding azapeptides of type **4**.

As recently demonstrated, methylation of the hydrazine fragment in model azapeptides leads to the *E* configuration of the respective CO−N bonds.[11] Inspired by this finding and by the importance of structural motifs **1** and **3**, we envisaged six-membered rings by installing an alkyl bridge from $C_\alpha H_i$ to both N atoms in order to lock the peptide bond into either a *Z* configuration in aza-Freidinger lactams **2** or an *E* configuration in **4**. Moreover, for both cases, *N*-methylation was implemented as an important feature of synthetic and natural peptides.[12] To establish this approach, Cbz-protected azadipeptide amides have been employed as model compounds. As an example to demonstrate the biological activity of *Z*- or *E*-locked azapeptides, derived azadipeptide nitriles have been designed and evaluated against a panel of pathologically relevant cysteine cathepsins.

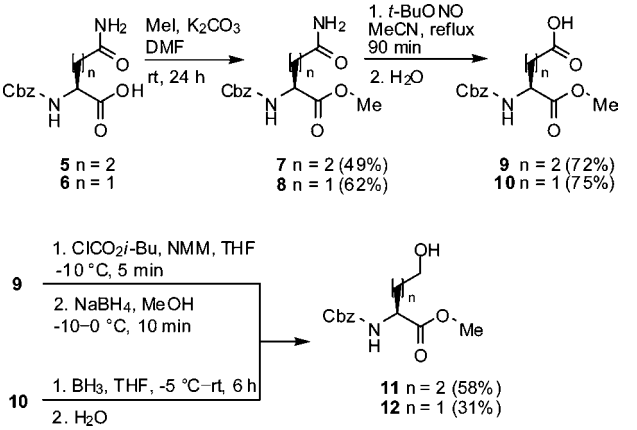
The initial aim of this work was to find a synthetic access to the building blocks **14** and **15** for aza-Freidinger lactams with a peptide bond configuration restricted to *Z* as well as the *E*-analogous building blocks **19** and **20** (Scheme 1). Key cyclizations should be achieved by reacting Cbz-5-bromo-norvaline methyl ester (**13**) or methyl 2-(benzyloxycarbonylamino)-4-oxobutanoate (**16**) with hydrazine or methyl hydrazine, respectively.

Starting from Cbz-Gln-OH (**5**) or Cbz-Asn-OH (**6**), alkylation under basic conditions[13] smoothly provided methyl esters Cbz-Gln-OMe (**7**) or Cbz-Asn-OMe (**8**) (Scheme 2). The primary amide function in **7** or **8** was converted with *t*-BuONO and hydrolyzed to give Cbz-Glu-OMe (**9**) or Cbz-Asp-OMe (**10**) with unprotected side chains.[14] Selective reduction of the carboxylic acid to the corresponding alcohol function in Cbz-5-hydroxynorvaline methyl ester (**11**) was achieved by transforming **9** into the mixed anhydride with $ClCO_2i$-Bu and subsequent addition of $NaBH_4$.[15] To obtain Cbz-homoSer-OMe (**12**), reduction of **10** with BH_3−THF[16] was advantageous with respect to yield. To produce Cbz-5-bromo-norvaline-methyl ester (**13**), required for building blocks **14** and **15**, alcohol **11** was subjected to bromination under Appel conditions (Scheme 3). Next, **13** was reacted with hydrazine hydrate and underwent 6-*exo*-*trig* cyclization. Gratifyingly, the *Z*-locked building block **14** was isolated in excellent yield and purity. The (mono)methylation of hydrazides has rarely been investigated.[17] However, a recently described two-step protocol,[18] involving reductive alkylation with

Scheme 1. Amino Acid-Derived *Z*- or *E*-Locked Building Blocks for Azapeptides



Scheme 2. Synthesis of Side Chain Alcohols **11** and **12**



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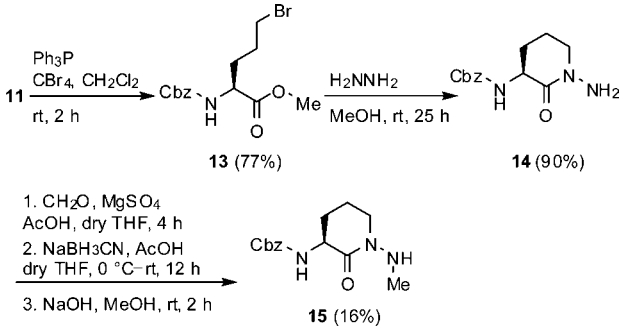
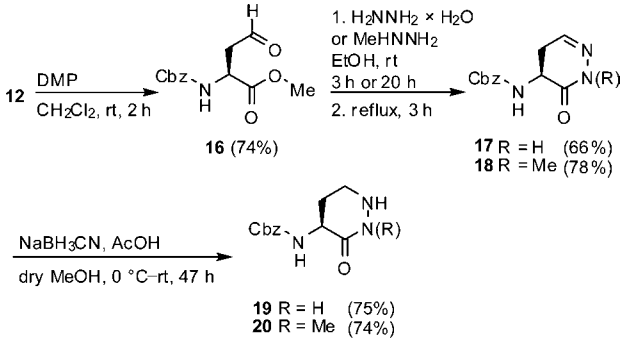
formaldehyde and $NaBH_3CN$, was successfully applied to give the monoalkylated building block **15**.[19]

Next, we focused on the building blocks **19** and **20**, bearing a CO−N bond restricted to *E*. Aldehyde **16** was prepared from Cbz-homoSer-OMe (**12**) via Dess-Martin oxidation (Scheme 4). Installing an aldehyde function in

Scheme 3. Preparation of *Z*-Locked Building Blocks **14** and **15**



Scheme 4. Synthesis of *E*-Locked Building Blocks **19** and **20**



16, instead of the alkyl halide moiety in **13**, should facilitate *both* nitrogens of (methyl) hydrazine in being incorporated into the heterocycle. A similar procedure was reported but was only executed for an *N*-unmethylated product.[20] Aldehyde **16** was subjected to reaction with hydrazine hydrate or methyl hydrazine, respectively, to yield, indeed, dihydro-3(2*H*)-pyridazinones **17** and **18**. With respect to the synthesis of **18**, it was expected that the more nucleophilic, methylated nitrogen of methyl hydrazine attacks the aldehyde rather than the ester carbon in a reversible, nonproductive step. The generation of **18**, however, is driven by hydrazone formation involving the NH_2 group of methyl hydrazine, followed by a 6-*exo*-*trig* cyclization, thus directing the substituted nitrogen to the ester. Selective C−N double bond reduction with $NaBH_3CN$ afforded the *E*-locked building blocks **19** and **20** in good yields. With the four building blocks in hand, the constrained azadipeptide amides **21**−**24** should be synthesized in the course of *N*-carbamoylation reactions. There are no reports on systematic $CONH_2$ group introductions to hydrazides in the literature. To produce the *E*-locked azadipeptide amides **21** and **22**, triphosgene[21] was employed as a carbonyl donor (Scheme 5). To prevent the formation of symmetrical carbonohydrazides, the building block **19** or **20** was added slowly to 1 equiv of triphosgene, corresponding to 3 equiv of phosgene.[22] Gaseous NH_3 was then introduced to transform the chloroformylated intermediates to the *E*-locked azapeptides **21** and **22**. However, when applying a triphosgene/NH_3 protocol to *Z*-locked building block **14**, a mixture of several compounds, including the symmetrical carbonohydrazide as the major product, was formed according to LC/MS, and the desired aza-Freidinger lactam **23** was not isolated. A successful carbamoylation in this case was achieved with trimethylsilyl isocyanate. The $NH_{(2)}$ function of **14** or **15**, respectively, reacted to *N*-TMS carbamoyl derivatives, which were hydrolyzed to give the *Z*-locked aza-Freidinger lactams **23** and **24** (Scheme 5).[23]

After showing that the configuration of the peptide bond in azapeptides can be restricted to *Z* or *E*, respectively, we were eager to see, if, in principle, the locked building blocks are suitable nuclei for the design of bioactive compounds. To choose one application, a nitrile function, a well-known electrophilic warhead to interact with cysteine proteases,[24] was installed. For this purpose, methylated building blocks **15** and **20** were reacted with cyanogen bromide to obtain configurationally constrained azadipeptide nitriles **25** and **26** (Table 1). Peptide nitriles interact with the active site thiol of cysteine proteases to form a reversible thioimidate complex. **25** and **26** were evaluated against a panel of pathologically relevant cysteine cathepsins K, S, B, L, and F. Although their activity was only moderate, both nitriles exhibited different inhibition profiles. In general, proteases preferentially bind ligands in an extended conformation.[25]

Scheme 5. Carbamoylation to *Z*/*E*-Locked Azadipeptides **21**−**24** via Triphosgene/NH_3- or TMSNCO-Assisted Reactions

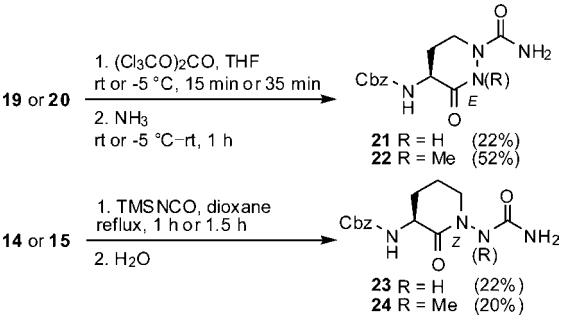


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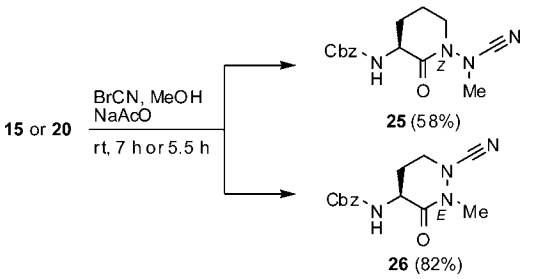
Table 1. Preparation of *Z*- or *E*-Locked Azadipeptide Nitriles **25**, **26**, and K_i Values



		$K_i \pm$ SE (μM)				
compd	*Z*/*E*	cat. K	cat. S	cat. B	cat. L	cat. F
25	*Z*	0.162 ± 0.018	7.77 ± 0.72	0.29 ± 0.01	5.15 ± 0.39	3.77 ± 0.29
26	*E*	8.67 ± 1.82	283 ± 89	1.79 ± 0.06	3.87 ± 0.25	46.5 ± 5.8

Such a conformation is preformed in the azadipeptide nitrile **25**, but not in **26**. Actually, **25** was more potent than **26** toward cathepsins K, S, B, and F. In particular, a more than 30-fold stronger inhibition of cathepsins K and S by **25** was observed. Thus, using the example of cysteine cathepsins, it was shown that the incorporation of the building blocks **15** and **20** into bioactive peptides resulted in a remarkable impact on protein−ligand interaction.

In conclusion, a synthetic access to aza-Freidinger lactams and *E*-locked analogs (**21**−**24**) was developed. The possibility of constraining the peptide bond in azapeptides into the *Z* or *E* configuration was demonstrated for the first time. Cysteine protease inhibition was chosen to prove that stable *Z*/*E* prearrangements of the peptide bond in azapeptides represent an attractive approach for the design of bioactive compounds. The corresponding building blocks (**14**, **15**, **19**, and **20**) are accessible with moderate effort.[26] The key cyclization steps to obtain these deprotectable compounds proceeded in good to excellent yields. They are expected to be useful elements for incorporation into various peptides and to be sufficiently stable toward acidic or basic conditions that are required for protecting group removal. Moreover, the preparation of the corresponding Boc-protected analogs of **14**, **15**, **19**, and **20** would allow for an application in solid-phase synthesis when reacted with resin-bound peptide isocyanates.[27] Such constrained peptide mimetics may serve as valuable motifs to elucidate bioactive conformations required for the binding of azapeptides to different targets or to improve their pharmacokinetic properties.

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Supporting Information Available. Detailed synthetic procedures, analytical data, NMR spectra, description of enzymatic assays and representative kinetic plots, chiral HPLC analyses of **25**, **26**, molecular plot of **24**, an X-ray crystallographic file in CIF format, electronic structure calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) For X-ray crystallographic analysis of **24**, see SI and CCDC 896854. Compound **24** occurred as a mixture of diastereomers in NMR spectra due to slow rotation around the N−N bond; see ref 11. However, DFT calculations gave a value of $\Delta G^{\ddagger}_{298\ K}$ = 83 kJ mol^{-1} for the barrier of rotation, indicating that slow rotation only appears on the NMR time scale and **24** exists as a single compound; see SI.

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(26) For a rough comparison, the preparation of Freidinger lactams **1** (Figure 1) is synthetically less demanding than that of **2**, whereas the routes to **3** and **4** require similar effort. In contrast to **3**, aza analogs **4** lack the second chiral center.

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The authors declare no competing financial interest.