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Inverse γ-Turn-Inspired Peptide: Synthesis and Analysis of Segetalin A Indole Hemiaminal

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Substitution of a peptide bond for an imine transforms the irreversible macrocyclization of peptides into a reversible process. The inherent cyclization tendency of a linear peptide is then analyzable through the equilibrium between the aldehyde and the imine by virtue of the higher reactivity of the corresponding linear peptide aldehyde. The tryptophan side chain of segetalin A aldehyde forms a 12-membered cyclic indole hemiaminal instead of the 18-membered macrocyclic imine expected. Herein, we analyzed this uncommon hemiaminal that shows that the biosynthesis of cyclic peptides is not necessarily based on linear precursor peptides with a high inherent macrolactamization tendency.

Introduction

Indole derivatives have received much attention in medical chemistry and natural products synthesis.[1–3] A host of natural products contain the indole core in the form of alkaloids and tryptophan derivatives, which creates new challenges for synthetic chemistry and offers new features and surprising reactivity.[4] Indole hemiaminals, which have already proved their antitumor properties in the inhibition of tubuline polymerization, are also interesting as structural motifs.[5,6] In peptide drugs, the indole skeleton is represented by the side chain of tryptophan and is based on the slight reactivity of the indole NH group; this functional group will not react during conventional peptide synthesis. The conditions required to acylate the aromatic nitrogen atom are quite harsh and are not achieved during peptide macrocyclization.[7]

In many cases, macrocyclization in peptide natural product synthesis is the limiting step, and in biosynthetic routes, this cyclization is often supported by complex enzyme machineries.[8] The substitution of a nonhydrolyzable amide bond [CONH] through a pH-dependent hydrolyzable isosteric imine Ψ[CH=N] or iminium Ψ[CH=NH$^+$] bond in a macrocyclic peptide has already been established by our group for the cyclopeptide tyrocidine A and the nostocyclopeptides.[9,10] Through this substitution, the irreversibility of the macrocyclization will be converted into a reversible process, which can answer the question whether the linear or cyclic peptide is preferred. Additionally, in the cases of tyrocidine A and the nostocyclopeptides, thermodynamic analysis of the cyclization process was possible.[9,10] In both cases, the expected macrocyclic ring with the correct ring size was formed, although many side-chain functionalities caused side reactions. Therefore, in the case of segetalin A we also expected a clean reaction to the macrocyclic peptide. Substitution of the amide bond can take place at any amino acid and leads to cyclic imines; in the case of proline, the functionality formed is an enamine. Owing to fast racemization of aldehydes, nonracemizable glycine is often chosen as an aldehyde moiety. Another advantage of this reaction is the use of water or phosphate buffer as the solvent. This enables NMR-spectroscopy-based analysis of the reaction, even at very low concentrations. Furthermore, the aqueous solution promotes intramolecular interactions, such as hydrogen bonding and the hydrophobic effect. On the basis of this concept, we analyzed several cyclic peptides with regard to their inherent cyclization tendency and studied linear peptide aldehyde **1** of the corresponding natural product segetalin A (Figure 1). Despite the low reactivity of the indole NH group, as described already, the reversible cyclization of **1** should lead to 18-membered macrocyclic imine **2**. However, against all expectations, we identified 12-membered indole hemiaminal **3**, which was exclusively formed. Thus, the lower reactivity of the indole NH

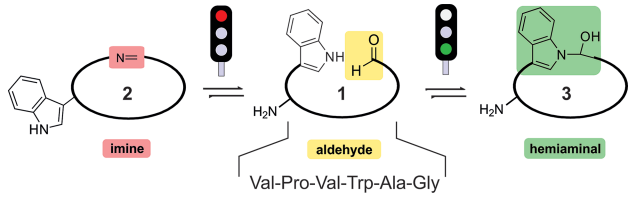

Figure 1. The linear peptide aldehyde (yellow, **1**) can react with the N-terminal amine (red, **2**) or with the indole NH (green, **3**). The competition reaction between both functionalities is monitored by ^{1}H NMR spectroscopy. In this case, we could only detect a new signal for the addition of the indole NH group.

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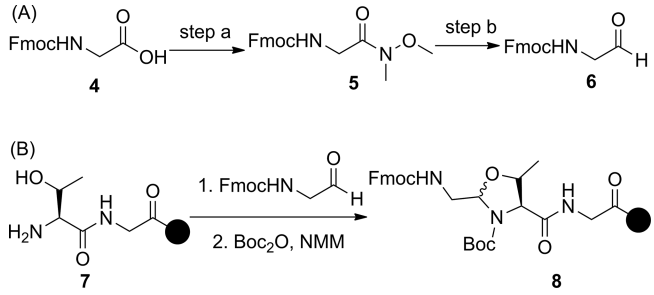
group is compensated by the high reactivity of the aldehyde. In this work, we analyzed the reversible reaction and the resulting structural motif by ^{1}H NMR spectroscopy.

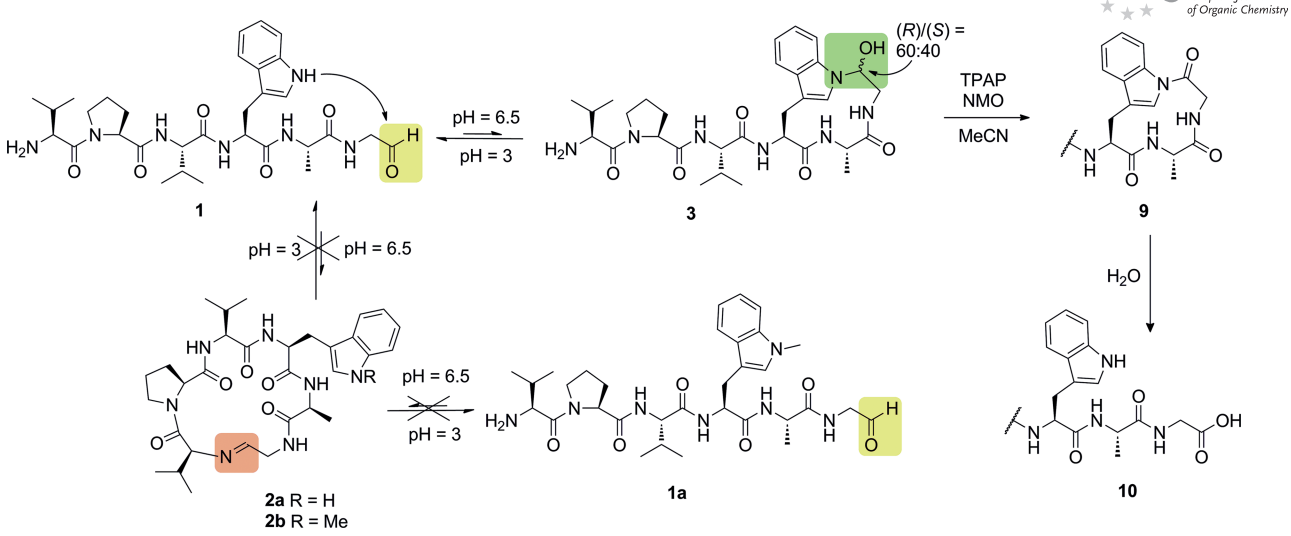
Results and Discussion

To perform the NMR experiments, we synthesized peptide aldehyde **1**, a linearized derivative of segetalin A cyclo-[–Val–Pro–Val–Trp–Ala–Gly]. The synthesis route started with 9-fluorenylmethoxycarbonyl (Fmoc) protected glycine **4**, which was transformed into the corresponding Weinreb amide **5** (Scheme 1). After selective lithium aluminum hydride (LAH) reduction, aldehyde **6** formed an oxazolidine, which was directly *tert*-butoxycarbonyl (Boc) protected. The corresponding amino alcohol is unprotected threonine **7**, and it was covalently linked through glycine as a spacer to a NovaSyn TG resin. The loaded resin **8** was then used in a conventional Fmoc-based solid-phase peptide synthesis (SPPS). After cleavage with $AcOH/H_2O/CH_2Cl_2$/MeOH (10:5:63:22), the peptide aldehyde was purified by semipreparative HPLC. The cyclic hexapeptide segetalin A was isolated from the seeds of *Vaccaria segetalis* (Caryophyllaceae), and it shows potent estrogen-like activity.[11] The structure of native segetalin A has been well studied by crystal-structure analysis and in solution by NMR spectroscopy.[12,13] In the solid state, segetalin A is characterized by two β turns, one type I (Trp^4 and Ala^5) and one type VI (Val^1 and Pro^2) with a *cis*-Pro bond.[12] The structure is fixed by two *trans*-annular hydrogen bonds formed between Gly^6 and Val^3, which results in an overall antiparallel β-sheet structure. In solution, the CO–Val^3 group of the cyclic hexapeptide forms two hydrogen bonds to NH–Gly^6 and NH–Val^1. That changes the type II turn into a type I turn.[12] It is known that it adopts a rigid main conformation without the formation of a second set of NMR signals for the *trans*-Pro rotamer.[13] The ^{1}H NMR spectrum of the linear peptide aldehyde **1** shows instead only the *trans* conformer. The configuration of the Val–*trans*-Pro rotamer was assigned on the basis of the chemical shift of the β- and γ-CH_2 groups (δ = 29.0 and 24.5 ppm, respectively) of the proline by HSQC.[14] In contrast to most peptides containing Pro, no other signal set for the *cis*-Pro rotamer was observed in the ^{1}H NMR spectrum of **1**, **1a**, or **3**. Additionally, no exchange signals were visible for a secondary set of NMR signals in the homonuclear 2D NMR spectra (TOCSY and ROESY, Figure 3). The stretched conformation was, furthermore, confirmed through the relatively weak NH^i–Hα^i ROEs and intense contacts of Hα^i–NH^{i+1} across the whole peptide chain of **1**. In this context, we analyzed the influence of the *i*–1 and *i*+1 amino acids to proline by synthesizing tripeptides. Determination of the *cis*/*trans* ratio by ^{1}H NMR spectroscopy showed that β-branched amino acids lead to a higher amount of *trans*-Pro. The valine residues in both positions in segetalin A could explain the *trans* configuration (for more details, see the Supporting Information). With this information in hand, it was exciting to analyze the cyclization tendency of linear peptide aldehyde **1**.



Scheme 1. Synthesis of Fmoc-glycine aldehyde loaded TG resin **8**. The black dot symbolizes the TG resin. (A) Reagents and conditions: (a) *N*,*O*-dimethylhydroxylamine (2.6 equiv.), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexauorophosphate (HBTU, 1.1 equiv.), hydroxybenzotriazole (HOBt, 1.1 equiv.), *N*,*N*-diisopropylethylamine (DIPEA, 2.6 equiv.), DMF, r.t., 4 h. (b) LAH (2.5 equiv.), THF, 0 °C, 20 min. (B) Reagents and conditions: THF, 4 h, 50 °C.

Under standard cyclization conditions, it cannot be distinguished whether a macrocyclic ring has a constrained conformation or a low-energy conformation stabilized by intramolecular hydrogen bonds. So, if aldehyde **1** is subjected to reversible imine cyclization, neither the structure of 12-membered ring **3** nor the type of covalent bond – an indole hemiaminal – is comparable to known peptides (Figure 1 and Scheme 2). Surprisingly, peptide aldehyde **1** does not show any tendency to form the anticipated 18-membered ring **2a**, although segetalin A is known as a cyclic natural product. All molecular species involved can be differentiated by NMR spectroscopy and can, thus, be classified by their pH and temperature dependency. Achiral glycine could be used as the perfect ring-closing position for the cyclization of segetalin A aldehyde **1**. If **1** is dissolved in aqueous buffer at pH = 3 (Figure 2), the conformation adopted is almost exclusively open chain. In the ^{1}H NMR spectrum, the resonance for the NH protons is found at δ = 0.78 ppm; this is quite low relative to the same signal for the cyclic peptide (δ = 1.61 ppm).[12,13] Increasing the pH value stepwise to 6.5 should lead to head-to-tail cyclization of the macrocyclic 18-membered imine. Indeed, the ring that is formed could not be assigned to the expected macrocycle **2a**. Instead, the addition of the indole NH group to the glycine aldehyde, which forms indole hemiaminal **3**, is identified in the HMBC spectrum through a long-range correlation (Figure 3a). The observation of this cross peak is due to the antiperiplanar arrangement of the bonds, as shown in the energy-minimized 3D structure (Figure 4). The side chain of Trp is flexible enough to approach the C-terminus and to cyclize the peptide through the indole NH group. In the HMBC spectrum, the cross peak caused by $^3J_{C,H}$ coupling between $H_{aminal}{}^R$–Gly and C_{7a}–Trp can be recognized. The corresponding HMBC and ROESY spectra are displayed in Figure 3. The covalent structure of the postulated hemiaminal **3** is proven by this coupling and the subsequent ROE signals. The low cyclization tendency of segetalin A aldehyde is, therefore, explained by the preferred stretched conformation of the N-terminal amino acids. The maximum amount of the formed



Scheme 2. Reversible cyclization of segetalin A aldehyde in aqueous phosphate buffer. Peptide **1** cyclizes reversibly at pH = 6.5 to 12-membered hemiaminal **3** with a diastereomeric ratio of 60:40 at the stereocenter of the hemiaminal instead of forming 18-membered *cis*-Pro cyclic imine **2a**. Oxidation of hemiaminal **3** with tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO) in acetonitrile yields cyclic amide **9**, which hydrolyzes quickly to linear segetalin A (**10**). Owing to methylation, peptide **1a** cannot form the indole hemiaminal and also shows no head-to-tail cyclization to **2b**.

cyclic peptide is 30% at pH = 6.5 and a temperature of 320 K in a *R*/*S* stereoisomer ratio of 60:40 (Scheme 2). The selective *N*-methylation of the indole NH group suppresses cyclization of the side chain, which, therefore, should lead to the formation of the macrocyclic ring. The corresponding amino acid was synthesized in two steps, and the synthesis started with Boc-protected tryptophan **11**. The indole NH group was deprotonated by using *t*BuOK in THF and was methylated by using MeI. After aqueous workup, the methylated amino acid was directly deprotected with trifluoroacetic acid (TFA)/CH_2Cl_2 (3:1), and the corresponding TFA salt **12** was obtained in 95% yield.

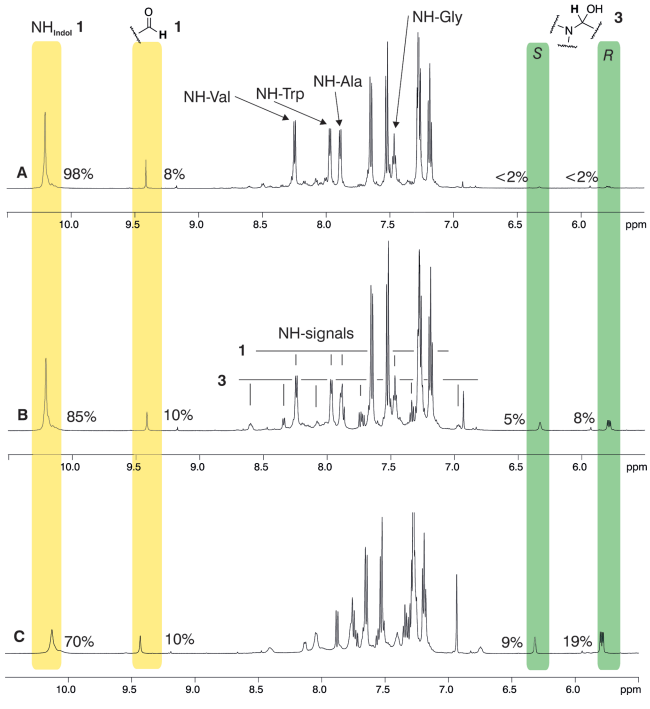


Figure 2. Excerpts from the WATERGATE ^{1}H NMR spectra of **1** and **3** at different temperatures and pH values (600 MHz; H_2O/D_2O, 5:1; H_3PO_4/KH_2PO_4). (A) The indole NH group and the aldehyde signal between δ = 9 and 10.5 ppm (highlighted in yellow) at 300 K and pH = 3 are characteristic for open-chain peptide **1**. (B) At pH = 6.5 and 300 K, a new signal set for cyclic hemiaminal **3** is detectable, and the corresponding signals are highlighted in green. The ratio between linear and cyclic under these conditions is about 85:15. (C) We raised the temperature to 320 K at pH = 6.5 to increase the ratio up to 70:30.

The building block for the SPPS was Fmoc-protected, and **13** was afforded in an overall yield of 67% (Scheme 3). The peptide synthesis of the methylated derivative was accomplished in analogy to the synthesis of peptide aldehyde **1**. The blocked indole nitrogen atom cannot react, and the formation of the 12-membered hemiaminal is, therefore, suppressed. Instead, we expected the formation of the 18-membered cyclic imine, which is the only possible product. Upon performing the NMR experiment as described previously, we could not detect any new NMR signals; consequently, the linear peptide aldehyde also does not form any cyclic product or oligomers, only the hydrate. This also confirmed the already described stretched conformation of the Val–*trans*-Pro rotamer of **1**.

Owing to their importance as model systems with a minimum antiparallel β-sheet structure, cyclic hexapeptides are among the most intensively studied macrocyclic rings.[15,16] An explanation for the formation of hemiaminal **3** could be the structure motif itself, which shows surprising analogy to an inverse γ turn. The definition of a γ turn includes a hydrogen bond between the CO group of the *i*th amino acid and the (*i*+2)th amino acid.[17] Most of the time, classic γ turns can be found at the ends of β hairpins, whereas the inverse γ turn can be involved in the folding of β strands as intermediates.[18,19]

The proposed structure shown in Figure 4 is based on ROE signals that are assigned in the ROESY spectra start-

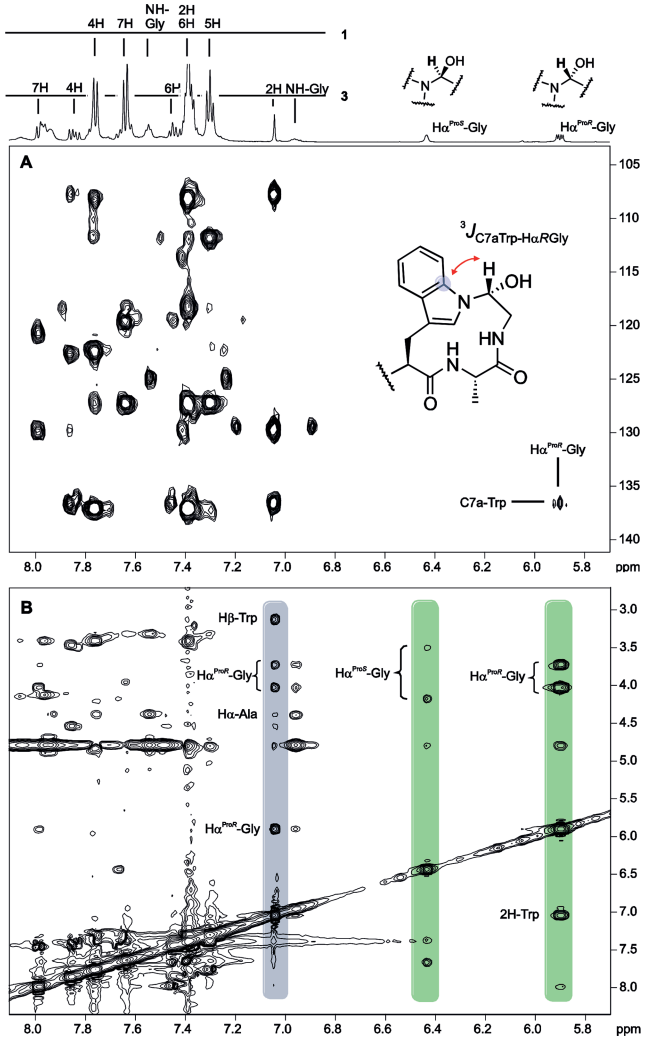


Figure 3. Excerpts from HMBC and ROESY spectra of **1/3** (600 MHz; 310 K; H_2O/D_2O, 5:1; H_3PO_4/KH_2PO_4; pH = 6.5). (a) The $^3J_{C,H}$ correlation between C_{7a}–Trp and $H_{aminal}{}^R$–Gly in the HMBC spectrum is clearly separated from the cross peaks within the indole ring. The arrangement of the two antiperiplanar atoms leads to a large coupling, which causes an intensive cross peak. No cross peak is observed for the *gauche* arrangement in the diastereomeric ring with $H_{aminal}{}^S$–Gly. (b) In the ROESY spectrum shown, the cross peaks of $H_{aminal}{}^R$–Gly and the indole-2H are highlighted by bars.

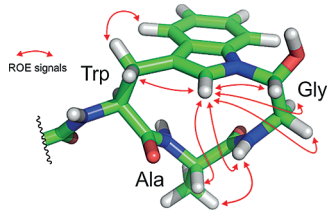
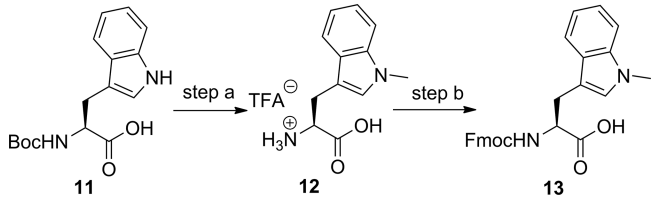


Figure 4. Energy-minimized conformation of the 12-membered ring of hemiaminal **3**. The NOE contacts from the rotating frame NOESY spectrum are displayed by arrows (ROESY: Figure 3b).

ing from the indole-2H of the *R* stereoisomer. The corresponding signals are blue, and the ROE signals of $H_{aminal}{}^R$–Gly and diastereomeric $H_{aminal}{}^S$–Gly are highlighted by



Scheme 3. Synthesis of Fmoc-protected, methylated tryptophan building block **13**. Reagents and conditions: (a) (1) *t*BuOK (2.0 equiv.), MeI (1.05 equiv.), DMF/THF (1:1), 0 °C, 2 h; (2) TFA/CH_2Cl_2 (3:1), 0 °C, 3 h. (b) Fmoc-succinimide (1.50 equiv.), 10% aq. Na_2CO_3, acetone, r.t., 16 h.

bars. The distinction between the two possible planar chiral stereoisomers is made through the local network of ROEs, which is formed by the 2H of the indole side chain (arrows in Figure 4). The ROE signals of Hα–Trp could not be identified, because the chemical shift is located next to water suppression. The ROE signals of indole-2H to $H\beta^{Pro S}$–Trp, Hα–Gly, Hα–Ala, $H_{aminal}{}^R$–Gly, and NH–Gly are assigned. Detailed information of the ROE signals is given in the Supporting Information. Accordingly, hemiaminal **3** displayed in Figure 4 shows close similarity to a γ_i loop structural motif, in which Ala takes the central *i*+1 position.

Trp–NH possesses a large temperature dependency (–10 ppb K^{-1}) of the chemical shift, which is typical for solvent-oriented NH groups. The temperature gradient of Ala–NH (–6 ppb K^{-1}) is significantly lower, which can be explained by a lower solvent accessibility. The value for Gly–NH is –3 ppb K^{-1}, which is indicative of coordination with Trp–CO, and this is typical for a hydrogen bond. With a value of ϕ = –102°, the ϕ torsion angle (CO^i–N^{i+1}–$C\alpha^{i+1}$–CO^{i+1}) of Ala differs by about –20° from the idealized value of a γ_i loop. However, this is largely compensated by the opposite deviation of the ψ torsion angle (N^{i+1}–$C\alpha^{i+1}$–CO^{i+1}–N^{i+2}) by about +20° (ψ = +93°). The two larger numeric values, relative to the idealized value of a γ_i loop, lead to an overall straighter conformation of the γ_i loop, without detracting from the characteristic intramolecular hydrogen bond Gly–NH–Trp–CO. The 12-membered ring was oxidized to promote and analyze hemiaminal formation more precisely. The oxidation of indole hemiaminal **3** to amide **9** was realized by the method of Scheidt and Maki involving catalytic dehydrogenative coupling of indoles and primary alcohols.[7] There, the resulting hemiaminal, which was also generated in organic solvents, was oxidized to the corresponding amide **9** by using TPAP and NMO as co-oxidants (Scheme 2). Here, the reaction was performed in acetonitrile at room temperature to yield crude product **9** of the oxidized hemiaminal, which could only be detected by ESI-MS: *m*/*z* = 674.3276 [M + Na^+] (calcd. *m*/*z* = 674.3273).

The oxidation was performed with *N*-acylated segetalin A aldehyde. The reason for the low yield and difficult isolation of the corresponding amide is the high reactivity of 12-membered cyclic acylindoles. Such motifs were already described by Katrizky et al. as active acylating esters in a similar system.[20] It was found that these 12-membered rings occur as intermediates in inter- and intramolecular

acylation reactions and are, therefore, prone to hydrolysis. This could also be observed by ESI-MS: m/z = 692.3377 [M + Na^+] (calcd. m/z = 692.3378).

Conclusions

We synthesized a linear peptide aldehyde precursor of the cyclic peptide segetalin A and analyzed its cyclization as a reversible process in water. In previous examples, the reversible cyclization of a peptide aldehyde led to the expected macrocyclic ring despite the presence of reactive side chains. In this work, the peptide aldehyde surprisingly showed no tendency for macrocyclic ring closure in the equilibrium; instead, we observed the formation of a 12-membered cyclic hemiaminal. The indole-based structure of this unexpected, inverse γ-turn-like structure motif was elucidated by 2D NMR spectroscopy. The high reactivity of the aldehyde shows the great potential of this type of reaction and provides new perspectives for studies regarding the inherent cyclization tendency of natural products with a peptide backbone. Furthermore, this approach enables the analysis of topologically challenging knotted peptides, such as microcin J25 and capistruin, which can also be synthesized as peptide aldehydes.

Experimental Section

General Information: The reactions were performed under nitrogen at ambient pressure. Thin-layer chromatography was performed on silica gel 60 F_{254} (Merck KGaA), and detection was performed by fluorescence quenching under UV light (λ = 254 nm) or by staining with ninhydrin solution followed by heating to 500 °C. Flash chromatography was performed on silica gel 60 (0.040–0.063 mm) from Merck KGaA. Semipreparative HPLC was performed with a Dionex HPLC system with a diode array detector by using an ACE HPLC C18 RP, 7.75 × 150 mm, and analytical HPLC was performed with the same system by using an Intersil ODS-4, C18 RP, 3.0 × 150 mm. The NMR spectra were recorded with Bruker AV 600 spectrometers. Chemical shifts (δ) are given in ppm and are referenced to the solvent signal. The coupling constants are ^3J couplings unless otherwise indicated. All mass spectra were recorded with a Finnigan LTQ-FT spectrometer. The SPPS was performed on an H–Thr–Gly–NovaSyn TG resin from Merck KGaA.

***N*$^{\alpha}$-Fmoc-Gly-*N*,*O*-dimethylhydroxamic Acid (5):** HBTU (2.62 g, 6.92 mmol, 1.1 equiv.), HOBt·H_2O (1.06 g, 6.92 mmol, 1.1 equiv.), DIPEA (2.78 mL, 16.4 mmol, 2.6 equiv.), and *N*,*O*-dimethylhydroxylamine hydrochloride (1.60 g, 16.4 mmol, 2.6 equiv.) were added at room temperature to a solution of Fmoc-Gly-OH (**S1**; 1.87 mg, 6.29 mmol, 1.0 equiv.) in DMF (20 mL). After stirring for 4 h, the mixture was diluted with EtOAc (200 mL) and then washed with 5% aq. $NaHCO_3$ (3×), 10% aq. citric acid (2×), and brine (3×). The organic phase was dried with $MgSO_4$, filtered, and concentrated in vacuo to afford Weinreb amide **5** in quantitative yield (2.12 g, 6.24 mmol) as a pale yellow waxy solid. R_f = 0.62 (EtOAc/toluene, 7:1). ^{1}H NMR (300 MHz, [D_6]DMSO, 300 K): δ = 7.90 (d, J_{HH} = 7.3 Hz, 2 H, Fmoc-$CH_{arom.}$), 7.72 (d, J_{HH} = 7.7 Hz, 2 H, Fmoc-$CH_{arom.}$), 7.48 (t, $J_{NH/\alpha H}$ = 6.1 Hz, 1 H, NH), 7.31–7.44 (m, 4 H, Fmoc-$CH_{arom.}$), 4.20–4.30 (m, 3 H, Fmoc-CH_2, Fmoc-CH), 3.91 (d, $J_{\alpha H/NH}$ = 5.8 Hz, 2 H, αH), 3.68 (s, 3 H, OCH_3), 3.09 (s, 3 H, NCH_3) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO, 300 K): δ = 156.6 (Fmoc-*C*O), 140.7, 143.8 (each Fmoc-$C_{arom.,quart.}$), 120.1, 125.2, 127.0, 127.6 (each Fmoc-$CH_{arom.}$), 65.7 (Fmoc-CH_2), 61.1 (OCH_3), 46.6 (Fmoc-*C*H), 41.1 (αCH), 32.1 (NCH_3) ppm. HRMS (ESI): calcd. for $C_{19}H_{20}N_2O_4Na^+$ 363.1315; found 363.1313.

***N*$^{\alpha}$-Fmoc-Gly-H (6):** A solution of *N*$^{\alpha}$-Fmoc-Gly *N*,*O*-dimethylhydroxamic acid (**5**; 1.10 g, 3.23 mmol, 1.0 equiv.) in THF_{abs} (10 mL) was cooled to 0 °C under nitrogen. $LiAlH_4$ (306 mg, 8.08 mmol, 2.5 equiv.) was added in small portions under vigorous stirring over 15 min. After complete addition, the mixture was stirred at 0 °C for 5 min. It was then carefully diluted with EtOAc (10 mL) and 10% aq. citric acid (10 mL) and stirred at room temperature for another 15 min. The phases were separated, and the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with 5% aq. $NaHCO_3$, H_2O, and 2 N aq. HCl. After washing with brine (2×) and drying with $MgSO_4$, the solution was filtered and concentrated in vacuo. The crude product was purified by flash chromatography (petroleum ether/EtOAc, 2:1) to yield aldehyde **6** (435 mg, 1.55 mmol, 48%) as a crystalline colorless solid. R_f = 0.32 (petroleum ether/EtOAc, 2:1). ^{1}H MR (300 MHz, [D_6]DMSO, 300 K): δ = 9.46 (s, 1 H, CHO), 7.90 (d, J_{HH} = 7.4 Hz, 2 H, Fmoc-$CH_{arom.}$), 7.72 (d, $J_{\alpha H/NH}$ = 7.2 Hz, 2 H, Fmoc-$CH_{arom.}$), 7.31–7.44 (m, 4 H, Fmoc-$CH_{arom.}$), 4.22–4.35 (m, 3 H, Fmoc-CH_2, Fmoc-CH), 3.84 (m, 2 H, αH) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO, 300 K): δ = 200.2 (CHO), 156.6 (Fmoc-*C*O), 143.8–140.7, (each Fmoc-$C_{arom.,quart.}$), 120.1, 125.1, 127.0, 127.6 (each Fmoc-$CH_{arom.}$), 65.7 (Fmoc-CH_2), 59.7 (αCH), 46.6 (Fmoc-*C*H) ppm. HRMS (ESI): calcd. for $C_{17}H_{15}NO_4Na^+$ 304.0944; found 304.0944.

H-L-Trp-(NMe)-OH (12): A solution of Boc-L-Trp-OH (**11**; 300 mg, 986 μmol, 1.0 equiv.) in DMF/THF (1:1, 10 mL) was cooled to 0 °C under nitrogen. 1 N *t*BuOK in THF (2.00 mL, 2.00 mmol, 1.05 equiv.) was added at 0 °C. The mixture was stirred for 10 min, and MeI (64.5 μL, 1.03 mmol, 1.03 equiv.) was added in one portion. After stirring at room temperature for 3 h, the reaction was quenched with 2 N aq. HCl, the mixture extracted with EtOAc (3 × 10 mL) and washed with 10% aq. citric acid and H_2O. After washing with brine (2×) and drying with $MgSO_4$, the solution was filtered and concentrated in vacuo. The crude product [R_f = 0.31 (EtOAc)] was dissolved in TFA/CH_2Cl_2 (3:1; 4 mL) at 0 °C and stirred for 4 h. The mixture was coevaporated with toluene and chloroform to afford the TFA salt of **S5** (310 mg, 933 μmol, 95%). ^{1}H NMR (300 MHz, [D_6]DMSO, 300 K): δ = 3.02 (dd, $^2J_{HH}$ = 14.5 Hz, J_{HH} = 9.4 Hz, 1 H, βH$_{diast.}$), 3.15–3.21 (m, 1 H, βH$_{diast.}$), 3.70 (s, 1 H, NCH_3), 4.19–4.23 (m, 1 H, αH), 7.14 (s, 1 H, 2-H_{indol}), 7.24–7.73 (m, 4 H, CH_{indole}), 7.88 (d, $J_{NH/\alpha H}$ = 7.6 Hz, 3 H, NH), 12.68 (br. s, 1 H, COOH) ppm.

Fmoc-L-Trp-(NMe)-OH (13): Fmoc-OSu (365 mg, 1.08 mmol, 1.2 equiv.) was added to a solution of H-L-Trp-(NMe)-OH as the TFA salt (**12**; 300 mg, 903 μmol, 1.0 equiv.) in acetone/10% aq. Na_2CO_3 (1:1; 10 mL). After stirring for 16 h, the mixture was diluted with H_2O (10 mL) and extracted with Et_2O (3 × 10 mL). The aqueous phase was diluted with 2 N aq. HCl to pH = 2 and extracted with EtOAc (3 × 10 mL). After washing with brine (2×) and drying with $MgSO_4$, the solution was filtered and concentrated in vacuo. The crude product was purified by flash chromatography (EtOAc) to yield the methylated tryptophan **S6** (280 mg, 635 μmol, 70%) as a colorless solid. R_f = 0.26 (EtOAc). ^{1}H NMR (300 MHz, [D_6]DMSO, 300 K): δ = 3.02 (dd, $^2J_{HH}$ = 14.6 Hz, J_{HH} = 9.6 Hz, 1 H, βH$_{diast.}$), 3.17–3.27 (m, 1 H, βH$_{diast.}$), 3.70 (s, 1 H, NCH_3), 4.01–4.29 (m, 4 H, αH, Fmoc-CH, Fmoc-CH_2), 6.95–7.89 (m, 14 H, Fmoc-$CH_{arom.}$, indole-$CH_{arom.}$, NH), 12.65 (br. s, 1 H, COOH)

ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 300 K): δ = 26.8 (βCH_2), 32.3 (NCH_3), 46.6 (Fmoc-*C*H), 55.0 (αCH), 65.6 (Fmoc-*C*H$_2$), 109.6, 118.5, 120.0, 121.0, 125.3, 127.0, 128.0, 140.7, 143.8 (Fmoc-*C*H$_{arom.}$, Fmoc-*C*$_{arom.,quart.}$, indole-CH$_{arom,}$ indole-C$_{arom.}$), 156.0 (Trp-CO), 173.6 (Fmoc-CO) ppm. HRMS (ESI): calcd. for $C_{27}H_{24}N_2O_4H^+$ 439.1652; found 439.1668.

General Protocol for Loading of H-Thr-Gly-NovaSyn TG Resin: (1) Swelling of the resin in 1% AcOH in CH_2Cl_2/MeOH (1:1) for 30 min; (2) addition of Fmoc-protected amino acid aldehyde (5.0 equiv.) in CH_2Cl_2; (3) 4 h incubation at r.t.; (4) washing with CH_2Cl_2, DMF, and THF (3× each); (5) 3 h incubation with Boc_2O (5.0 equiv.) and *N*-methylmorpholine (NMM, 5.0 equiv.) in THF at 50 °C; (6) washing with CH_2Cl_2, DMF, and THF (3× each).

General Protocol for Manual Synthesis of Peptide Aldehydes: (1) Swelling of the amino acid aldehyde loaded resin in DMF for 30 min; (2) Fmoc deprotection: 10 min and 20 min incubation with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)/piperidine/DMF (2:2:96); (3) washing with THF, *i*PrOH, and DMF (3× each); (4) chain elongation: 50 min incubation with the Fmoc-protected amino acid (3.0 equiv.), HBTU (3.0 equiv.), HOBt (1.0 equiv.), and DIPEA (6.0 equiv.) in DMF; (5) washing with THF, *i*PrOH, and DMF (3× each); repeat steps (2)–(5) until assembly of target sequence; (6) Fmoc deprotection: 10 min and 20 min incubation with DBU/piperidine/DMF (2:2:96); (7) removal of Boc and side-chain protecting groups: 10 min incubation with 100% TFA (2×); (8) washing with CH_2Cl_2 (2×); (9) cleavage of the peptide aldehyde: 30 min incubation with AcOH/H_2O/CH_2Cl_2/MeOH (10:5:63:21).

General Protocol for the Purification of the Peptide Aldehydes: The peptide solutions, which were obtained from cleavage, were concentrated in vacuo. The peptides were precipitated from dry Et_2O at –30 to –40 °C and then lyophilized after centrifugation. Segetalin A aldehyde (**1**) was characterized by HPLC and ESI-MS; the purity without further purification was approximately 90%. The peptide was directly analyzed by NMR spectroscopy.

Segetalin A^1-CHO (1): Yield: 87% (20 mg, 29.8 μmol) pale yellow solid. HPLC [r.t.; 15–65% CH_3CN in 0.05% TFA/H_2O, in 20 min (gradient)]: t_R = 11.54 min. MS (ESI): *m*/*z* = 612.5 [M + H]$^+$. HRMS: calcd. for $C_{31}H_{46}N_7O_6$ [M + H]$^+$ 612.3504; found 612.3508.

Segetalin A^1 Aldehyde (1a): The peptide was characterized by HPLC and ESI-MS, and the purity without further purification was approximately 70%. The peptide was purified by semipreparative HPLC to a purity of approximately 97%. After purification, the peptide was analyzed by NMR spectroscopy. The ESI MS data showed mainly methyl acetal [Seg A-CH(OMe)$_2$]. Yield: 77% (18 mg, 26.3 μmol), colorless solid. HPLC [r.t.; 15–65% CH_3CN in 0.05% TFA/H_2O, in 20 min (gradient)]: t_R = 13.09 min. MS (ESI): *m*/*z* = 658.4 [M + MeOH + H]$^+$. HRMS: calcd. for $C_{33}H_{52}N_7O_7$ [M + MeOH + H]$^+$ 658.3923; found 658.3918.

Cyclic Amide 9: TPAP (0.5 mg, 1.50 μmol, 0.1 equiv.), NMO (5.3 mg, 46.0 μmol, 3.0 equiv.), DIPEA (2.7 μL, 15.1 μmol, 1.0 equiv.), and molecular sieves (4 Å; 5 beads) were added to a solution of *N*-acylated segetalin A aldehyde as TFA salt (**1**; 10 mg, 15.1 μmol, 1.0 equiv.) in acetonitrile (0.15 mL). After stirring for 16 h, the mixture was purified by HPLC. The product and the hydrolyzed amide were verified by mass spectrometry only. MS (ESI): *m*/*z* = 652.34 [M + H]$^+$. HRMS: calcd. for $C_{33}H_{46}N_7O_7$ [M + H]$^+$ 652.3453; found 652.3452.

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