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Two-Step Hantzsch Based Macrocyclization Approach for the Synthesis of Thiazole-Containing Cyclopeptides[§]

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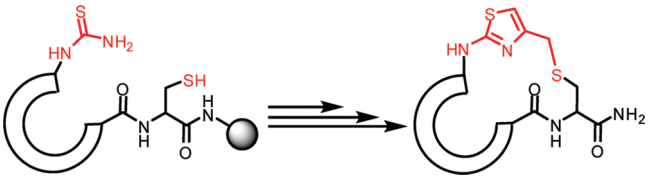

Macrocyclization via an efficient high-yielding solid-phase intramolecular thioalkylation reaction is described. The reaction of S-nucleophiles with newly generated N-terminal 4-chloromethyl thiazoles led to the desired macrocyclization products **5** in high purities and good overall yields.

Conformational constraint by cyclization is a common approach used to restrict the flexibility of peptides and therefore is a valuable approach to study topographical requirements of receptors.[1] Cyclization of peptides can provide potent and selective ligands for receptors when appropriate conformational constraints are incorporated.[1] Furthermore cyclic peptides are often more stable to peptidases, and therefore they can have improved pharmacokinetic profiles and serve as promising lead compounds for further development.[2] Macrocycles are known for their broad range of activities including antitumor activities and antibiotic activities such as the structurally complex vancomycin family.[3] Reported approaches on the solid-phase synthesis of macrocyclic compounds include intramolecular nucleophilic substitutions,[1a,4] intramolecular amide formations,[1c,5] disulfide formations,[6] intramolecular Suzuki reactions,[7] ring-closing metathesis reactions,[8] and S_NAr displacement reactions.[9] Of particular interest, thioalkylation reactions offer a facile and versatile approach to the synthesis of cyclic peptides.[4,10] Examples of described macrocyclizations via thioalkylation include the reaction of the thiol group of a C-terminal cysteine with N-terminal acetyl bromide or N-terminal benzyl bromide.[4,10] A conceptually different approach, wherein thioalkylation proceeds via Michael addition of a thiolate anion to an α,β-unsaturated ester, has been reported for the synthesis of cyclic thioether dipeptides.[11]

Herein, we describe an innovative thioalkylation approach toward the generation of macrocyclic peptides following the intramolecular nucleophilic substitution (S_N2) of N-terminus 4-chloro methyl thiazole peptides with the thiol group of cysteine. The final products are not entirely peptidic, and the described newly generated macrocyclic compounds contain the thiazole ring, a pharmacophore present in many natural and synthetic products with a wide range of pharmacological activities that can be well illustrated by the large numbers of naturally occurring thiazole-containing macrocyclic compounds[12] and drugs in the market containing this function group.[13]

[§] Dedicated to Dr. Richard Houghten for his 65th birthday.

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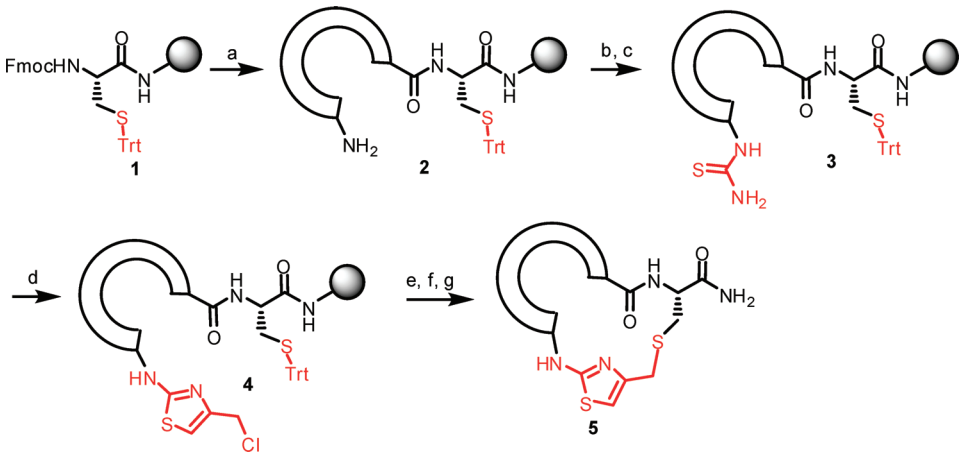
SCHEME 1[a]



[a]Reagents and conditions: (a) solid-phase peptide synthesis using Fmoc chemistry; (b) FmocNCS (6 equiv) in DMF (0.3 M), rt, overnight; (c) 20% piperidine/DMF; (d) 1,3-dichloroacetone (10 equiv) in DMF (0.3 M), 70 °C, overnight; (e) TFA/$(Bu^t)_3SiH$/DCM (5:5:90), 30 min; (f) Cs_2CO_3 in DMF overnight; (g) HF/anisole, 0 °C, 90 min.

We have shown the feasibility of the proposed approach by the parallel synthesis of different thiazole-containing macrocyclic peptides. Starting from resin-bound orthogonally protected Fmoc-Cys-(Trt)-OH **1**, the thiomethyl thiazolyl macrocyclic peptidomimetics **5** were synthesized following stepwise Fmoc deprotection[14] and standard repetitive Fmoc-amino-acid couplings yielding the linear tripeptide **2**. The resulting N-terminal free amine was treated with Fmoc-isothiocyanate. Following Fmoc deprotection, the thioureas were treated with 1,3-dichloroacetone to afford following Hantzsch's cyclocondensation[15] the resulting resin-bound chloro methyl thiazolyl peptide **4**. The Trt group was deprotected in the presence of 5% TFA in DCM, and the resin was treated with a solution of Cs_2CO_3 in DMF to undergo an S_N2 intramolecular cyclization. The resin was cleaved with HF/anisole, and the desired thiazolyl thioether cyclic peptides **5** were obtained in good yield and high purity. An Ellman test for free thiols was performed on all samples. No free thiol was present in the crude material. The identity of the final products was confirmed by LC−MS and NMR spectroscopy.

Many reagents and techniques have been developed to facilitate the synthesis of cyclic peptides, for which the yield-limiting step is generally the cyclization reaction. Particularly, the cyclization of tetra-, penta-, and hexapeptides in the all-L configuration can be problematic, especially in the absence

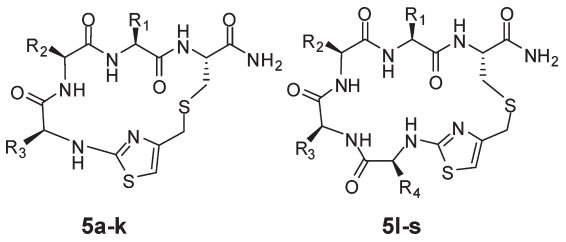


FIGURE 1

of β-turn-promoting structures such as glycine, proline, or a D-amino acid.[16] Using the approach outlined in Scheme 1, we first tested our approach by performing the parallel synthesis of various thiazole-containing cyclic tetrapeptides and pentapeptides from all L-amino acids (Figure 1).

We selected different amino acids for each of the position of diversities R_1, R_2, and R_3 for the synthesis of 11 tetrapeptides (**5a**−**5k**). Thus, we choose Phe, Tyr, Ser, and Val for the position R_1, Phe, Tyr, and Pro for the position R_2 and Tyr, Pro, and Lys for the position R_3. Similarly, we selected three amino acids for each of the position of diversities R_1 (Tyr, Phe, Asp), R_3 (Tyr, Pro, Arg), and two amino acids for each of the position of diversities R_2 (Tyr, Gly) and R_4 (Tyr, Pro) for the synthesis of nine pentapeptides (**5l**−**5t**). As shown in Table 1, high purities were obtained for all compounds. In all cases, the intramolecular thioalkylation reaction led to the desired cyclic monomers with negligible traces of dimerization. The NMR data show a clear singlet at 6.4 ppm that is specific to the proton on C-5 of the aminothiazole ring. The presented methodology is not limited to cyclic tetrapeptides and pentapeptides. It was successfully used for the synthesis of cyclic hexapeptides **5u**−**5v** and heptapeptide **5w**.

We have presented a new method for macrocyclization reaction via thioalkylation. The reaction of S-nucleophiles with newly generated N-terminal 4-chloromethyl thiazoles led to the desired macrocyclization products in high purities and good overall yields. The presented approach can be extended toward the synthesis of macrocyclic libraries where

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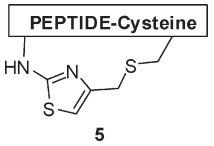
TABLE 1. Synthesis of Thiazole-Containing Cyclic Tetrapeptides and Pentapeptides



5

entry	peptide	purity[a] (%)	yield[b]
5a	Tyr-Phe-Phe	88	45
5b	Pro-Phe-Phe	85	38
5c	Tyr-Tyr-Phe	88	43
5d	Pro-Tyr-Phe	89	41
5e	Tyr-Phe-Tyr	87	40
5f	Pro-Phe-Tyr	76	29
5g	Tyr-Tyr-Tyr	83	38
5h	Pro-Tyr-Tyr	89	46
5i	Tyr-Pro-Ser	85	47
5j	Tyr-Tyr-Ser	86	43
5k	Lys-Tyr-Val	82	40
5l	Tyr-Tyr-Tyr-Phe	86	38
5m	Pro-Tyr-Tyr-Phe	90	39
5n	Tyr-Pro-Tyr-Phe	80	31
5o	Pro-Pro-Tyr-Phe	83	37
5p	Tyr-Tyr-Tyr-Tyr	82	31
5q	Pro-Tyr-Tyr-Tyr	85	35
5r	Tyr-Pro-Tyr-Tyr	80	34
5s	Pro-Pro-Tyr-Tyr	78	35
5t	Tyr-Arg-Gly-Asp	84	43
5u	Arg-G ly-Asp-Tyr-Tyr	82	40
5v	Phe-Tyr-Val-Ser-Ala	80	37
5w	Phe-Ala-Pro-Tyr-Ser-Phe	82	39

[a]Purity of crude products. The products were run on a Vydac column with a gradient of 5% to 95% formic acid in ACN in 7 min. The purity was estimated on analytical traces at $\lambda = 214$ and 254 nm. [b]The yields are based on the weight of the purified products and are relative to the initial loading of the resin. (The purity of the purified compounds is higher than 95% for all compounds).

the cysteine residue can be placed anywhere in the peptide sequence, allowing for extension of the peptide beyond the cyclic link. As part of our drug discovery program, we are in the process of preparing a variety of thiazole-containing macrocyclic libraries. The synthesis and the screening results will be reported elsewhere.

Experimental Section

Synthesis of Resin-Bound Cysteine. A 100 mg sample of *p*-methylbenzhydrylamine hydrochloride (MBHA·HCl) resin (CHEMIMPEX INTERNATIONAL 1.15 mequiv/g, 100−200 mesh, 1% DVB) was contained within a sealed polypropylene mesh bag. Twenty-three bags (23 × 100 mg resin, 2.64 mmol) were put in a polyethylene bottle. Following the neutralization of resin with 500 mL of 5% diisopropylethylamine (DIEA) in dichloromethane (DCM), L-Fmoc-Cys(Trt)-OH (3 equiv, 4.64 g, 7.93 mmol) was coupled using the conventional reagents hydroxybenzotriazole (HOBt, 1.07 g, 7.93 mmol) and diisopropylcarbodiimide (DIC, 1.16 mL, 7.93 mmol) in 300 mL of anhydrous DMF overnight at room temperature. Completion of the coupling was monitored by the ninhydrin test.

General Procedure for the Solid-Phase Synthesis of Resin-Bound Linear Cyclic Peptide 5a. One bag of resin **1** (100 mg, 0.115 mmol) was put into a small polyethylene bottle, and the Fmoc group was deprotected with 15 mL of 20% piperidine in DMF (2 × 10 min). The resin was then washed with 15 mL of DMF (3×) and 15 mL of DCM (3×). L-Fmoc-Phe-OH (6 equiv, 0.267 g, 11.04 mmol) was coupled in the presence of hydroxybenzotriazole (HOBt, 6 equiv, 0.094 g, 11.04 mmol) and diisopropylcarbodiimide (DIC, 6 equiv, 0.101 mL, 11.04 mmol) in 15 mL of anhydrous DMF for 2 h at room temperature. The resin-bound dipeptide was washed with DMF (3×) and DCM (3×). Completion of the coupling was monitored by the ninhydrin test. The Fmoc group was deprotected with 15 mL of 20% piperidine in DMF (2×10 min) and followed by the coupling of L-Fmoc-Phe-OH (6 equiv, 0.267 g, 11.04 mmol) using the same reaction conditions. The Fmoc group was deprotected, and the resin-bound tripeptide was coupled to L-Fmoc-Tyr(^{t}Bu)-OH in the same conditions to yield following Fmoc deprotection the corresponding resin-bound protected linear peptide **2a**.

The resulting N-terminal free amine of resin-bound linear peptide **2a** was treated with Fmoc-isothiocyanate (6 equiv, 0.193 g, 11.04 mmol) in 15 mL of anhydrous DMF overnight at room temperature. Following Fmoc deprotection with a solution of 20% piperidine in DMF, the resin-bound N-terminal thiourea was treated with 1,3-dichloroacetone (10 equiv, 0.145 g, 18.4 mmol) in anhydrous DMF overnight at 70 °C to afford following Hantzsch's cyclocondensation the resulting resin-bound chloro methyl thiazolyl peptide **4a**. The Trt group was deprotected in the presence of TFA/(Bu^t)$_3$SiH/DCM (5:5:90) for 30 min. The resin was washed with DCM (5×) and DIEA/DCM (5:95) and was treated overnight with a solution of Cs_2CO_3 (10 equiv, 0.325 g) in 15 mL of DMF at room temperature to undergo an S_N2 intramolecular thioalkylation. The resin was cleaved with HF/anisole for 90 min at 0 °C, and the desired thiazolyl thioether cyclic peptides **5a** was obtained following extraction with 95% acetic acid in water and lyophilization as a white powder (61.9 mg). The cyclic peptides **5a** was purified by preparative reverse-phase HPLC.

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Supporting Information Available: Experimental details and analytical data including NMR data and spectra; HPLC chromatograms and mass spectra for all reported cyclic peptides; HPLC and LC−MS of additional cyclic tetrapeptides and pentapeptides not reported in the paper. This material is available free of charge via the Internet at http://pubs.acs.org.