

New Oxazole-Based Peptidomimetics: Useful Building Blocks for the Synthesis of Orthogonally Protected Macrocyclic Scaffolds

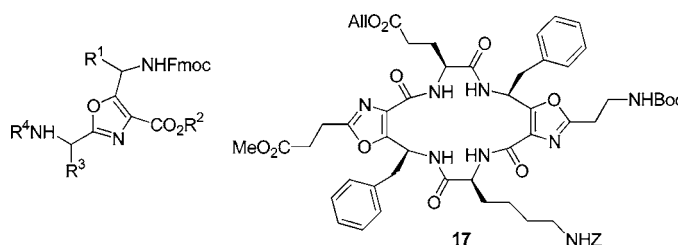
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ABSTRACT



The synthesis of a new family of densely functionalized oxazole-containing amino acids is described. These building blocks were employed for preparing macrocycles containing Lys and Glu residues by a combination of solid- and solution-phase synthesis. The resulting structures are presented as orthogonally protected scaffolds for supramolecular chemistry.

The design and development of new amino acids and peptidomimetics has attracted considerable attention due to the pharmacological limitations of bioactive peptides.¹ In connection with our interest in the synthesis of unnatural amino acids and their application as peptidomimetic structural templates,² we considered oxazole-containing amino acids as suitable building blocks for the preparation of systems with well-defined secondary structure. Amino acids containing five-membered heterocyclic rings resulting from intramolecular condensation of Cys, Thr, or Ser side chains are usually found in naturally occurring cyclic peptides isolated from marine organisms.³ Analogous compounds have already been employed as precursors of peptidomimet-

ics⁴ and macromolecular scaffolds⁵ and as building blocks for combinatorial chemistry.⁶ Here we report the synthesis of new cyclic peptides containing highly functionalized and orthogonally protected oxazole amino acids, designed to be incorporated into peptide backbones through *N*-terminal extension at position 5 of the heterocyclic core. Potential applications of these macrocyclic scaffolds are numerous, for example, as building blocks for the synthesis of branched peptides or as templates for the preparation of multimeric ligands with use of polyvalency, that is, compounds which can bind simultaneously to several receptors.⁷ Furthermore,

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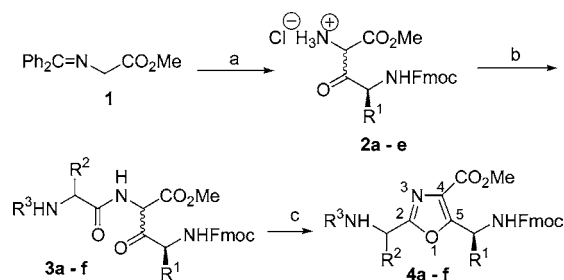
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the resulting structures are new examples of potentially Regioselectively Addressable Functionalized Templates (RAFTs),⁸ which have demonstrated their usefulness as scaffolds for Template-Assembled Synthetic Proteins (TASP).⁹

Our approach for the synthesis of oxazole amino acids was inspired by the protocol previously reported by Singh et al.¹⁰ for the preparation of α -amino β -keto esters by acylation of the anion derived from *N*-(diphenylmethylene)-glycine methyl ester (Scheme 1). We envisioned the use of

Scheme 1. Synthesis of Oxazoles **4a–f**^a



^a Reagents and conditions: (a) ^tBuOK, THF, -78 °C, 30 min, then Fmoc-aa-Cl; HCl 3 N, -78 °C to rt; (b) *N*-protected aa, ^tBuOCOCl, NMM, THF, -20 °C; (c) Ph₃P, I₂, Et₃N, THF, 0 °C.

Fmoc-protected amino acid chlorides as acylating agents. Our choice of the Fmoc protecting group was based on its extensive use in solution- and solid-phase peptide synthesis and the ease of formation of stable amino acid chlorides. Thus, treatment of imine **1**¹¹ with potassium *tert*-butoxide at low temperature and acylation of the resulting anion with Fmoc protected amino acid chlorides¹² derived from Gly, Ala, Phe, Glu, or Lys, followed by in situ hydrolysis of the resulting Schiff bases gave the α -amino β -keto esters **2** as stable hydrochloride salts in good yields (68–82%). Subsequent coupling with *L*-Ala, Phe, Asp, or *D*-Ser with mixed anhydride activation via isobutylchloroformate afforded intermediates **3** (57–73%).

Construction of the oxazole ring was performed with Ph₃P in the presence of I₂ and Et₃N¹³ to obtain the cyclized products **4** (Table 1) in good yields (63–85%).

Table 1. Synthesized Oxazoles **4**

	R ¹	R ²	R ³	yield, %
4a	H	Me	Boc	67
4b	H	CH ₂ O ^t Bu	Z	68
4c	Me	CH ₂ O ^t Bu	Z	63
4d	Bn	CH ₂ CO ₂ Bn	Boc	85
4e	(CH ₂) ₂ CO ₂ Bn	Bn	Boc	71
4f	(CH ₂) ₄ NHZ	Bn	Boc	84

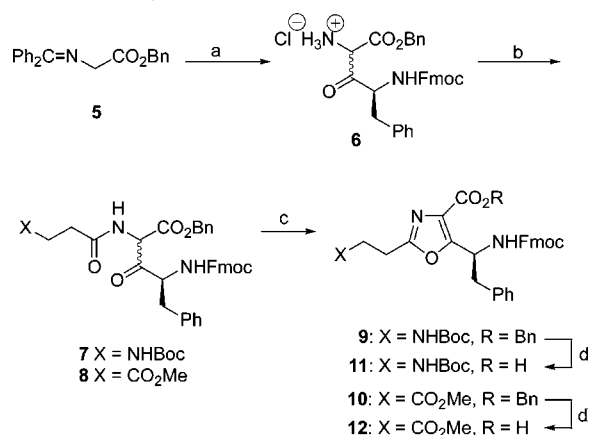
The synthetic protocol developed for preparing compounds **4** provides a highly versatile and flexible method for

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derivatization of positions 2 and 5 of the oxazole core, allowing the introduction of diverse functional groups orthogonally protected. Furthermore, the enantioconservative nature of the synthesis enables a total control over the configuration of the stereogenic centers present in these positions.

For the preparation of macrocyclic peptides containing oxazole amino acids we designed compounds **11** and **12** as building blocks, which were prepared in a similar fashion as described for **4** (Scheme 2).

Scheme 2. Synthesis of Oxazole Amino Acids **11** and **12**^a



^a Reagents and conditions: (a) ^tBuOK, THF, -78 °C, 30 min, then Fmoc-Phe-Cl; HCl 3 N, -78 °C to rt; (b) BocNH(CH₂)₂CO₂H for **7** or MeO₂C(CH₂)₂CO₂H for **8**; ^tBuOCOCl, NMM, THF, -20 °C; (c) Ph₃P, I₂, Et₃N, THF, 0 °C; (d) H₂, Pd/C, MeOH, 1 h.

To avoid difficulties associated with the methyl ester basic hydrolysis at position 4 with the presence of the base-labile Fmoc group, we decided to use benzyl ester as the protecting group. Thus, Schiff base **5** derived from diphenylimine and glycine benzyl ester⁶ was treated with ^tBuOK. Slow addition of the resulting anion into a cooled solution of Fmoc-Phe-Cl in THF, followed by acidic hydrolysis of the imine afforded keto ester **6** (80%). Condensation of **6** with Boc- β -alanine and monomethyl succinate resulted in compounds **7** (75%) and **8** (71%), respectively. Oxazoles **9** and **10** were obtained by intramolecular cyclization as described above, with excellent yields (81% for **9**, 83% for **10**). Benzyl ester deprotection under catalytic hydrogenation afforded acids **11** and **12**.

Before attempting the synthesis of the cyclic peptides containing building blocks **11** and **12**, we prepared the model

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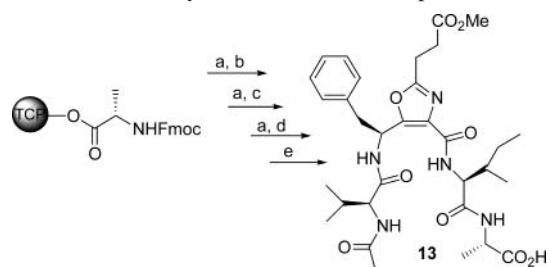
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compound **13** to evaluate the influence of these oxazole amino acids on the conformation of small peptides. We chose Ala, Ile, and Val as they display characteristic spin patterns in NMR. The synthesis of compound **13**, which contains oxazole amino acid **12**, was performed by standard solid-phase peptide techniques applying the Fmoc strategy, using chlorotriptyl chloride (CTC) resin, HATU¹⁴ and HOAt,¹⁵ or TBTU and HOBt as coupling reagents, DIPEA as a base, and DMF as solvent (Scheme 3). Cleavage from the resin

Scheme 3. Synthesis of Linear Compound **13**^a



^a Reagents and conditions: (a) 1:4 piperidine/DMF, 2 × 10 min, rt; (b) Fmoc-Ile-OH (3 equiv), TBTU (3 equiv), HOBt (3 equiv), DIPEA (7.8 equiv), DMF, 2 h, rt; (c) **12** (1.3 equiv), HATU (1.3 equiv), HOAt (1.3 equiv), DIPEA (3.4 equiv), DMF, 12 h, rt; (d) Ac-Val-OH (3 equiv), TBTU (3 equiv), HOBt (3 equiv), DIPEA (7.8 equiv), DMF, 2 h, rt; (e) 1:4 HFIP/CH₂Cl₂, 2 × 45 min.

with 20% hexafluoro-2-propanol (HFIP) in DCM and subsequent purification by preparative HPLC afforded the target compound **13** in 71% yield.

Solution ¹H NMR studies on compound **13** in CDCl₃ and DMSO-*d*₆ confirmed that the presence of the oxazole ring in the peptide backbone induces severe conformational restrictions. The chemical shifts for Ala NH (δ 8.55), Val NH (δ 7.86), and Phe NH (δ 8.69) in DMSO-*d*₆ are temperature dependent ($\Delta\delta/T$ 4.8, 5.0, and 4.4 ppb/K, respectively). By contrast, the Ile NH resonance (δ 7.53, $\Delta\delta/T$ 0.2 ppb/K) is practically temperature independent.¹⁶ Furthermore, the chemical shift of this proton is almost independent of the solvent employed, showing a quite similar resonance when CDCl₃ is used as solvent (δ 7.67), indicating its participation in an intramolecular hydrogen bond. In addition, ROE data support the presence of an intramolecular (Ile)NH \cdots OC(Val) bond, with a strong correlation between Ile NH and Phe C α H. All these data indicate that the oxazole amino acid introduced into the peptide backbone acts as a turn mimetic, adopting an unusual nine-member-ring H-bond stabilized arrangement instead of the ten-membered H-bond stabilized structure found in typical β -turns.¹⁷ As turns are

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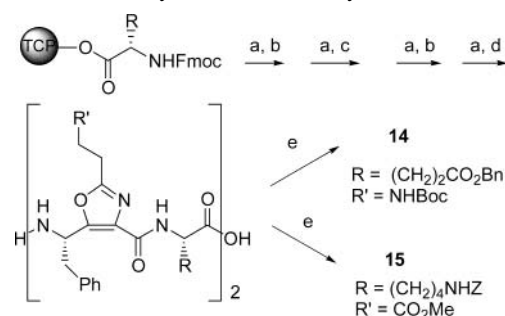
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important recognition elements, the flexible synthesis of the here described compounds would be used for new design of bioactive compounds.

Synthesis of linear oligomers of **11** and **12** alternating with L forms of Glu or Lys was performed in a similar fashion as described for **13**, employing solid-phase peptide techniques (Scheme 4). Oligomers were cleaved from the resin with

Scheme 4. Synthesis of Macrocycles **14** and **15**^a



^a Reagents and conditions: (a) 1:4 piperidine/DMF, 2 × 10 min, rt; (b) **11** (1.5 equiv) for **14** or **12** (1.5 equiv) for **15**, HATU (1.5 equiv), HOAt (1.5 equiv), DIPEA (3.9 equiv), DMF, 15 h, rt; (c) Fmoc-Glu(OBn)-OH (2 equiv) for **14** or Fmoc-Lys(Z)-OH (2 equiv) for **15**, HATU (2 equiv), HOAt (2 equiv), DIPEA (5.2 equiv), DMF, 3 h, rt; (d) 1:4 HFIP/CH₂Cl₂, 2 × 45 min; (e) DPPA (3 equiv), NaHCO₃ (5 equiv), DMF (1 mM), 12 h, rt, preparative HPLC purification.

20% hexafluoro-2-propanol in DCM.

End-to-end cyclizations (Scheme 4) were carried out under high dilution conditions (1 mM), using diphenylphosphorazidate (DPPA) with sodium bicarbonate¹⁸ as the solid base in DMF, to afford cyclopeptides **14** (57%) and **15** (68%) after HPLC purification (Figure 1).

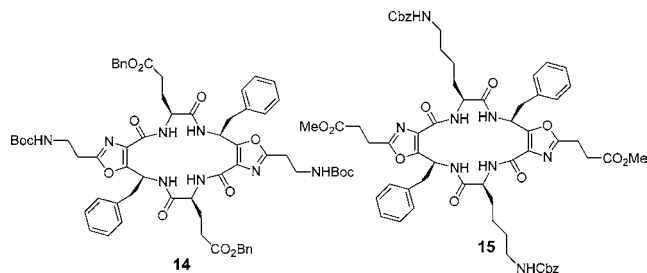
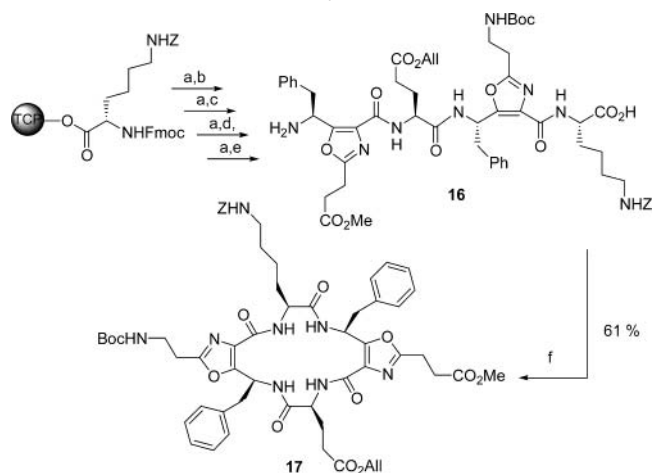


Figure 1. Structure of macrocycles **14** and **15**.

We also prepared cyclopeptide **17** by combining oxazole building blocks **11** and **12** with Lys and Glu residues conveniently protected (Scheme 5).

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Scheme 5. Synthesis of the Orthogonally Protected Macrocycle **17**^a



^a Reagents and conditions: (a) 1:4 piperidine/DMF, 2 × 10 min, rt; (b) **11** (1.3 equiv), HATU (1.3 equiv), HOAt (1.3 equiv), DIPEA (3.4 equiv), DMF, 12 h, rt; (c) Fmoc-Glu(OAllyl)-OH (2 equiv), HATU (2 equiv), HOAt (2 equiv), DIPEA (5.2 equiv), DMF, 3 h, rt; (d) **12** (1.3 equiv), HATU (1.3 equiv), HOAt (1.3 equiv), DIPEA (3.4 equiv), DMF, 12 h, rt; (e) 1:4 HFIP/CH₂Cl₂, 2 × 45 min; (f) DPPA (3 equiv), NaHCO₃ (5 equiv), DMF (1 mM), 12 h, rt.

Cyclization of the linear oligomer **16** employing similar conditions as for **14** and **15** (DPPA with NaHCO₃ in DMF) afforded the cyclic module **17** in 61% yield after HPLC purification.

Compound **17** presents a high level of orthogonality with two amino groups protected as Boc and Z derivatives and two acid functionalities protected as methyl and allyl esters. Furthermore, due to the severe conformational restrictions

imposed by the presence of two oxazoles and four peptidic bonds, we think that cyclopeptides **14**, **15**, and **17** are probably nearly planar and are expected to be quite rigid. Thus, owing to the homochirality of the amino acid residues employed, these templates direct their side chains to the same face of the structure. Substitution of the benzyl groups with other suitable protected side chains, simply by employing the corresponding amino acid chloride in the synthesis of the oxazole amino acid used as building block, can provide additional anchoring points.

In summary, we have developed a convenient route for the synthesis of new highly functionalized oxazole-containing amino acids, which have been used to prepare three prototypes of cyclic peptides with several orthogonally protected side chains, oriented to well-defined directions. Applications of these macrocyclic scaffolds are numerous, for example, as RAFTs to prepare systems which mimic structural motifs present in proteins, as building blocks for the synthesis of branched peptides, or as templates for the preparation of multimeric ligands with use of polyvalency. The synthesis of new cyclopeptides incorporating oxazole-based building blocks and their structural studies by NMR and computational methods is currently under investigation.

Acknowledgment. The authors thank Dr. J. Furrer for help in NMR experiments and B. Cordes for technical assistance. Financial support by the DFG is acknowledged.

Supporting Information Available: Characterization and detailed descriptions of the synthesis of key compounds; ¹H and ROESY spectra of **13**; variable-temperature experimental spectra of **13**; ¹H and selected 2D spectra of compounds **14**, **15**, and **17**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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