ORGANIC LETTERS

2003
Vol. 5, No. 24
4567−4570

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

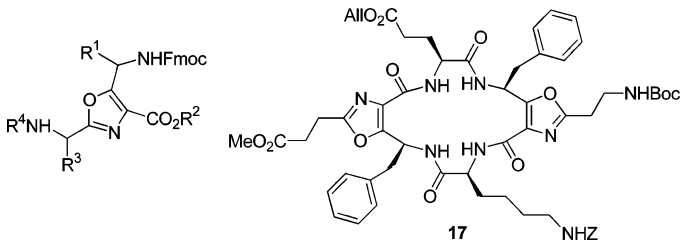
Enrique Mann and Horst Kessler*

Institut für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstrasse 4, D-85747 Garching, Germany

kessler@ch.tum.de

Received September 2, 2003

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 peptidomimetics[4] and macromolecular scaffolds[5] 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 polivalency, that is, compounds which can bind simultaneously to several receptors.[7] Furthermore,

(1) (a) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267. (b) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699−1720. (c) Hruby, V. J.; Li, G.; Haskell-Luevano, C.; Shenderovich, M. *Biopolymers* **1997**, *43*, 219−248.

(2) (a) Locardi, E.; Stöckle, M.; Gruner, S.; Kessler, H. *J. Am. Chem. Soc.* **2001**, *123*, 8189−8196. (b) Gruner, S. A. W.; Truffault, V.; Voll, G.; Locardi, E.; Stöckle, M.; Kessler, H. *Chem. Eur. J.* **2002**, *8*, 4365−4376. (c) Stöckle, M.; Voll, G.; Gunter, R.; Lohof, E.; Locardi, E.; Gruner, S.; Kessler, H. *Org. Lett.* **2002**, *4*, 2501−2504.

(3) (a) Wipf, P.; Miller, C. P. *Chem. Rev.* **1995**, *95*, 2115−2134. (b) Roy, R. S.; Gehring, A. M.; Milne, J. C.; Belshaw, P. J.; Walsh, C. T. *Nat. Prod. Rep.* **1999**, *16*, 249−263.

(4) (a) Plant, A.; Stieber, F.; Scherkenbeck, J.; Lösel, P.; Dyker, H. *Org. Lett.* **2001**, *3*, 3427−3430. (b) Falorni, M.; Giacomelli, G.; Porcheddu, A.; Dettori, G. *Eur. J. Org. Chem.* **2000**, 3217−3222.

(5) (a) Singh, Y.; Stoermer, M. J.; Lucke, A. J.; Glenn, M. P.; Fairlie, D. P. *Org. Lett.* **2002**, *4*, 3367−3370. (b) Somogyi, L.; Haberhauser, G.; Rebek, J., Jr. *Tetrahedron* **2001**, *57*, 1699−1708.

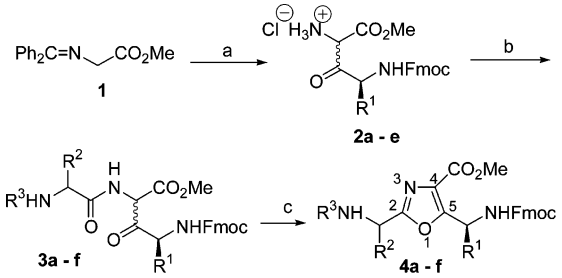
(6) Grabowska, U.; Rizzo, A.; Quibell, M. *J. Comb. Chem.* **2000**, *2*, 475−490.

10.1021/ol035673b CCC: $25.00
Published on Web 10/29/2003

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) ^{t}BuOK, THF, −78 °C, 30 min, then Fmoc-aa-Cl; HCl 3 N, −78 °C to rt; (b) *N*-protected aa, ^{i}BuOCOCl, NMM, THF, −20 °C; (c) Ph_3P, I_2, Et_3N, 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 chlorides[12] 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 Ph_3P in the presence of I_2 and Et_3N[13] to obtain the cyclized products **4** (Table 1) in good yields (63−85%).

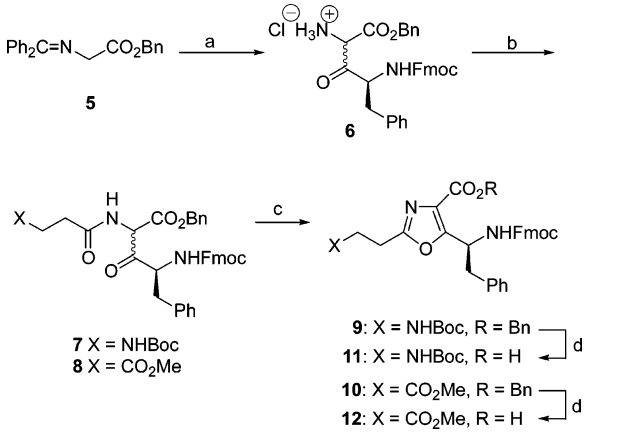
Table 1. Synthesized Oxazoles **4**

	R^1	R^2	R^3	yield, %
4a	H	Me	Boc	67
4b	H	CH_2O^tBu	Z	68
4c	Me	CH_2O^tBu	Z	63
4d	Bn	CH_2CO_2Bn	Boc	85
4e	$(CH_2)_2CO_2Bn$	Bn	Boc	71
4f	$(CH_2)_4NHZ$	Bn	Boc	84

The synthetic protocol developed for preparing compounds **4** provides a highly versatile and flexible method for 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) ^{t}BuOK, THF, −78 °C, 30 min, then Fmoc-Phe-Cl; HCl 3 N, −78 °C to rt; (b) $BocNH(CH_2)_2CO_2H$ for **7** or $MeO_2C(CH_2)_2CO_2H$ for **8**, ^{i}BuOCOCl, NMM, THF, −20 °C; (c) Ph_3P, I_2, Et_3N, THF, 0 °C; (d) H_2, 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 ester[6] was treated with ^{t}BuOK. 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

(7) Thumshirn, G.; Hersel, U.; Goodman, S. L.; Kessler, H. *Chem. Eur. J.* **2003**, *9*, 2717−2725.

(8) Dumy, P.; Eggelston, I. M.; Cervini, S.; Sila, U.; Sun, X.; Mutter, M. *Tetrahedron Lett.* **1995**, *36*, 1255−1258.

(9) (a) Tuchscherer, G. *Tetrahedron Lett.* **1993**, *34*, 8419−8422. (b) Mutter, M.; Tuchscherer, G. *Cell. Mol. Life Sci.* **1997**, *53*, 851−863. (c) Peluso, S.; Rückle, T.; Lehmann, C.; Mutter, M.; Peggion, C.; Crisma, M. *ChemBioChem* **2001**, *2*, 432−437.

(10) Singh, J.; Gordon, T. D.; Earley, W. G.; Morgan, B. A. *Tetrahedron Lett.* **1993**, 34, 211−214.

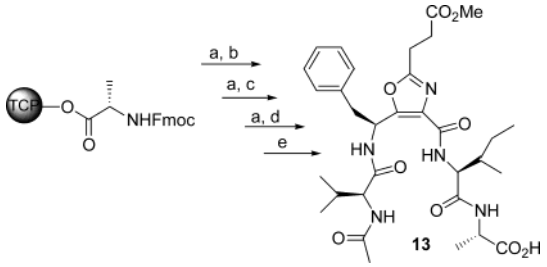
(11) O'Donnell, M. J.; Polt, R. L. *J. Org. Chem.* **1982**, *47*, 2663−2666.

(12) Carpino, L. A.; Cohen, B. J.; Stephens, K. E., Jr.; Sadat-Aalae, Y.; Tien, J.-H.; Landgridge, D. C. *J. Org. Chem.* **1986**, *51*, 3734−3736.

(13) Wipf, P.; Miller, C. P. *J. Org. Chem.* **1993**, *58*, 3604−3606.

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 chlorotrityl chloride (CTC) resin, HATU[14] 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]



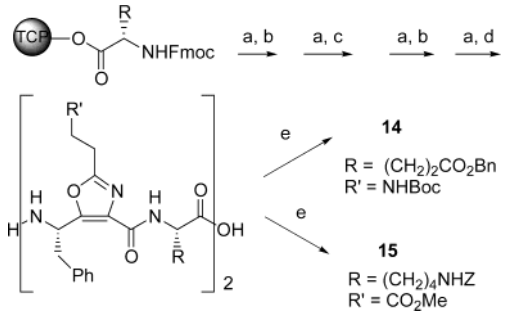
[a] Reagents and conditions: (a) 1:4 piperidine/DMF, 2 × 10 min, rt; (b) Fmoc-Ile-OH (3 equiv),TBTU (3 equiv), HOBt (3 equiv), DIPEA (7.8 equiv), DMF, 2 h, rt; (c) **12** (1.3 equiv), HATU (1.3 equiv), HOAt (1.3 equiv), DIPEA (3.4 equiv), DMF, 12 h, rt; (d) Ac-Val-OH (3 equiv), TBTU (3 equiv), HOBt (3 equiv), DIPEA (7.8 equiv), DMF, 2 h, rt; (e) 1:4 HFIP/CH_2Cl_2, 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 ^{1}H NMR studies on compound **13** in $CDCl_3$ 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 $CDCl_3$ 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···OC(Val) bond, with a strong correlation between Ile NH and Phe CαH. 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 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/CH_2Cl_2, 2 × 45 min; (e) DPPA (3 equiv), $NaHCO_3$ (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 bicarbonate[18] as the solid base in DMF, to afford cyclopeptides **14** (57%) and **15** (68%) after HPLC purification (Figure 1).

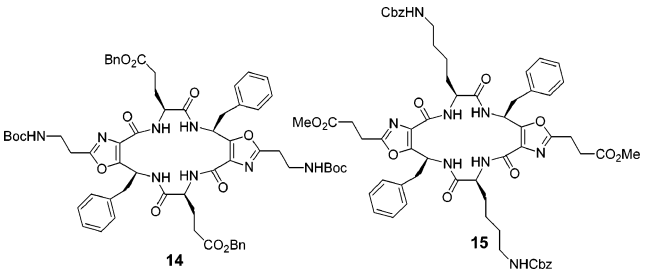


Figure 1. Structure of macrocycles **14** and **15**.

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

(14) (a) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201. (b) Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678−9683.

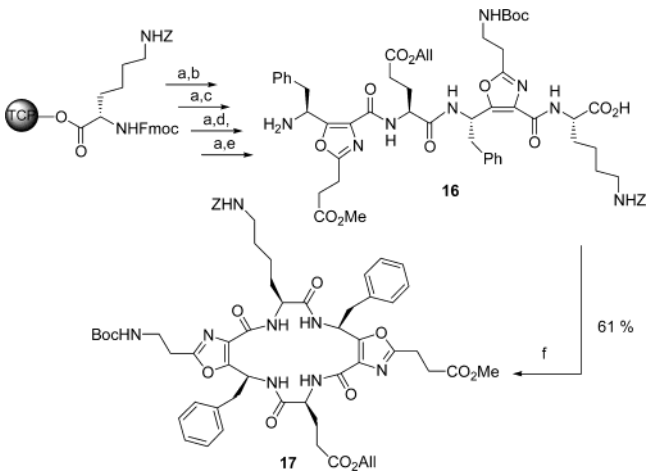
(15) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

(16) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512−523.

(17) For a recent study of similar nine-membered-ring H-bond stabilized β-turn-like structures, see: van Well, R. M.; Marinelli, L.; Altona, C.; Erkelens, K.; Siegal, G.; van Raaij, M.; Llamas-Saiz, A. L.; Kessler, H.; Novellino, E.; Lavecchia, A.; van Boom, J. H.; Overhand, M. *J. Am. Chem. Soc.* **2003**, *125*, 10822−10829.

(18) (a) Shioiri, T.; Ninomiya, K.; Yamada, S.-I. *J. Am. Chem. Soc.* **1972**, *94*, 6203−6205. (b) Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. *J. Org. Chem.* **1979**, *44*, 3101−3105.

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/CH_2Cl_2, 2 × 45 min; (f) DPPA (3 equiv), $NaHCO_3$ (5 equiv), DMF (1 mM), 12 h, rt.

Cyclization of the linear oligomer **16** employing similar conditions as for **14** and **15** (DPPA with $NaHCO_3$ 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 polivalency. 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.

OL035673B