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Via Ugi reactions to conformationally fixed cyclic peptides†

Christina Hebach and Uli Kazmaier

Institut für Organische Chemie, Universität des Saarlandes, D-66123, Saarbrücken, Germany. E-mail: u.kazmaier@mx.uni-saarland.de; Fax: +49 681 3022409; Tel: +49 681 3023409

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A simple approach to several cyclopeptidmimetics containing an *N*-alkylated amino acid was found *via* a multicomponent reaction followed by a ring-closing metathesis starting from readily available precursors. The combinatorial technique has the advantage that different polar, hydrophilic or hydrophobic moieties can be placed at any position in the cycles and unnatural amino acids can also be incorporated.

Peptides play an important role as neurotransmitters, neuromodulators and hormones having a strong influence on a variety of physiological processes *via* signal transduction.[1,2] From a pharmaceutical point of view, endogene peptides are not relevant because of their scarce bioavailability and their metabolic lability.[3] Additionally, the different peptide conformers could interact unspecifically with receptors because of their conformational flexibility.[4] The trend in recent years therefore is going towards the synthesis of peptidomimetics with increased metabolic stability.

Interactions between adhesion receptors on cell surfaces, termed integrins, and specific cyclic peptides have a determining influence on many diseases like cancer, thrombosis or wound healing.[5] Many integrins interact by recognising a specific peptide. Usually only between three and eight amino acids are involved in this process, while the others stabilise the bioactive conformation *via* loop and turn structures.[6] An effective limitation of the conformations could be artificially introduced by turn-inducing elements such as proline, (*R*)-amino acids, *N*-alkylated amino acids and cyclisations. In general this results in an increased stability toward proteases.

Two fundamentally different protocols are suitable for the cyclisation of peptides (Fig. 1). The so-called backbone-to-backbone cyclisation is a ring closure from the *N*-terminal end of the peptide to the *C*-terminus. For example, Kessler *et al.* have investigated synthetic cyclic penta- and hexapeptides with an RGD sequence (Arg-Gly-Asp).[7] Sewald *et al.* and also Kieffer *et al.* have developed methodologies for the synthesis of specific cyclic peptides;[8,9] the functional groups in the side chains can also be used. Recently, Miller and Grubbs described the synthesis of a dicarba analogue of disulfide-stabilised tetrapeptides, glutaredoxin, a group of redoxactive proteins.[10] This synthesis is based on the replacement of the critical disulfide moiety by a non-cleavable C–C double bond. Tethered allyl glycines can be dimerised *via* ring closing metatheses. Liskamp *et al.* have published an investigation on the cyclisation of *N*-allylated peptides.[11]

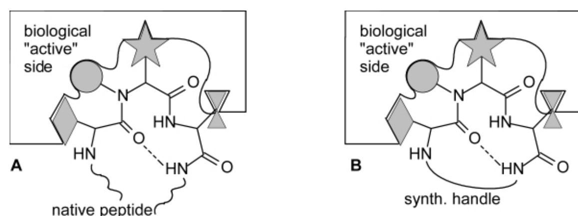
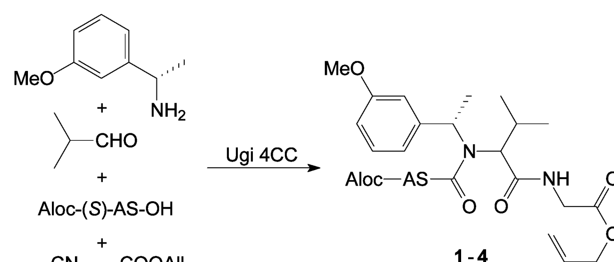


Fig. 1 From peptides to cyclic peptidomimetics.

† Dedicated to Professor Dr D. Seebach on the occasion of his 65th birthday.

Herein we describe a new approach towards cyclic peptidomimetics. The Ugi reaction with *N*-terminal protected aloc-amino acids and allyl isocyanoacetate — a masked glycine moiety — generates allylic esters of tripeptides in high yields (Scheme 1). A good selectivity was optained by using (*S*)-2,2-(*m*-methoxyphenyl)ethylamine as the chiral component. The use of chiral amines to induce diastereoselectivity is, in principle, known,[12] and we have observed a strong dependence of this reaction on temperature: the diastereoselectivity in our system increased up to 95:5 if the reaction was carried out at −30 °C (Table 1). The yield of the four-component reaction strongly depends on the chosen solvent.[13] Excellent results could be obtained in trifluoroethanol with a half-molar concentration.‡ This protocol was also suitable for different acid compounds. Thus, it was possible to incorporate proline and unnatural amino acids such as *N*-methylvaline (Scheme 1, **3**, **4**).

At the stage of the open-chained peptides a structure elucidation of the newly generated amino acid was not possible. High pressure liquid chromatography shows in all cases that the major diastereomers have longer retention times than the minor products on silica. Unfortunately, the open-chained peptide esters **1–4** are highly viscous oils and do not crystallise at all. For that reason we converted the main diastereomers of the linear peptide ester into the rigid cyclic structures **5–7** *via* a ring closing metathesis (Scheme 2). Initial attempts were carried out with peptide **1**, but metathesis with 5 mol% of Grubbs catalyst gave the desired products in only 5–10% yields. With a turnover



Scheme 1 The Ugi reaction.

Table 1 The Ugi reaction

Entry	Aloc-(*S*)-AS	Peptide	Yield	dr[a]
1	Aloc-Val	**1**	77%	28:72[b]
2	Aloc-Ala	**2**	96%	25:75[b]
3	Aloc-Ala	**2**	91%	5:95[c]
4	Aloc-Pro	**3**	89%	[b,d]
5	Aloc-*N*-MeVal	**4**	72%	25:75[b,d]

[a] dr = diastereomeric ratio (*S*/*S*/*R*)/(*S*/*S*/*S*) determined by HPLC of the crude product. [b] Reaction conditions: 0 °C → RT, 1.5 days. [c] Reaction conditions: −30 °C, 7 days. [d] Diastereomeric ratio determined from the isolated product.

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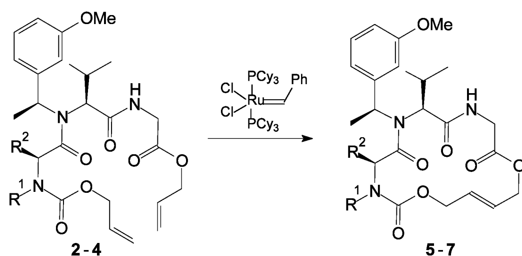
number of one or two, the catalyst was probably inactivated by the substrate under these conditions.

Fürstner and Langemann have studied the influence of polar groups in the molecule and the distance of this moiety from the terminal C–C double bond.[14] The position of the carbonyl group plays a significant role. In some cases stable chelated complexes between the substrate and the catalyst inactivate the latter. In our case, these interactions are in principle possible, but under optimised reaction conditions the yield of the corresponding 16-membered cyclic peptides could be increased to about 30–50% (Scheme 2).§



Scheme 2 Ring closing metathesis.

A significant dependence of the linear precursor on the *N*-terminal amino acid was observed (Table 2). The reaction times as well as the yields vary under standard conditions. The conversion of the alanine peptide **2** (entry 1) was not complete, and starting material was recovered during workup. The best results were obtained for proline derivative **3** (entry 2). To make sure that alkylated peptides are more suited, an *N*-methylated valine was incorporated at the *N*-terminal position (**4,** entry 3). The conversion achieved with this peptide under the conditions mentioned above was very slow, but heating to 60 °C gave acceptable results. The higher yields observed in entries 2 and 3 support the proposal that a negligible energy difference concerning the *cisoid* and *transoid* amide bond of the two rotamers increases the possibility of a preorientation of both ends.

Table 2 Metathesis of linear peptides **2–4**

Entry	Peptide	Cycle	R^1	R^2	Yield
1	**2**	**5**	H	Methyl	27%[a] (37%)[b]
2	**3**	**6**	$-CH_2CH_2CH_2-$		37%[a]
3	**4**	**7**	CH_3	*iso*propyl	33%[a] (51%)[b]

[a] Isolated yield. [b] Corrected yield.

The products turned out to be crystalline, and so the configuration could be determined by X-ray crystallography.¶ Relative to the known chiral centers of the amine and the acid component, the (*S*)-configured amino acids were incorporated preferentially, so that the main diastereomers of the Ugi reaction have the (*S*/*S*/*S*)-configuration. The selective formation of the *E*-configured double bond is very surprising, and only in the case of cyclic peptide **7** a small amount of the *Z*-product could be observed (*Z*/*E*: 97/3). According to the literature the catalyst should produce mixtures of *E*- and *Z*-configured isomers. The X-ray structure showed the lack of tension, as even the amide bonds and the ester moiety are *transoid* (Fig. 2).

In conclusion we have shown that linear natural and unnatural tripeptide allylic esters can be obtained by an Ugi reaction in one step with excellent yields and good selectivities. Subsequent ring-closing metathesis means that this sequence is a very flexible approach to cyclic peptides. The ring closure works in a stereoselective manner, and in almost every case only the *E*-configured olefin is formed. This strategy is a useful alternative to the standard sequential method of peptide coupling.

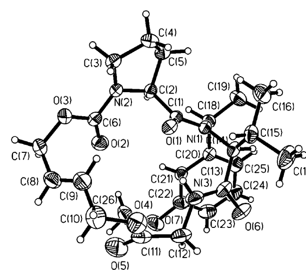


Fig. 2 X-Ray structure of cyclopeptide **6**.

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Notes and references

‡ General procedure for the Ugi reaction: 755 mg (5 mmol) of the amine was dissolved in 5 ml of trifluoroethanol, then 0.46 ml (5 mmol) of isobutyraldehyde was added slowly at 0°C. After stirring for 15 min the Aloc-amino acid (5 mmol) and the allyl isocyanoacetate (5 mmol) were added. After two days at room temperature the solvent was removed *in vacuo*, ethyl acetate (30 ml) was added and the mixture was washed three times with saturated $NaHCO_3$ (20 ml each) and 1 M $KHSO_4$ (20 ml each). The combined organic layer was dried (Na_2SO_4), evaporated and the crude product was purified by flash chromatography (silicagel) to give a viscous oil.

§ General procedure for the ring closing metatheses: 20 mg (0.025 mmol) of Grubbs catalyst dissolved in 4 ml of dry toluene was added over a period of one hour to a stirred solution of the corresponding peptide ester (0.5 mmol) in 46 ml of dry toluene. The reaction mixture was degassed during the reaction with nitrogen and stirred at room temperature overnight. The solvent was concentrated to 20 ml and 10 ml of hydrogen peroxide (1% aq. sol.) was added and stirred vigorously for one hour. After separation of the aqueous layer, the organic layer was washed twice with brine. The solvent was evaporated and the residue was purified by flash chromatography (silica gel, petroleum ether–ethyl acetate) or crystallised (chloroform, hexane).

¶ *Crystal data* for **6**: $C_{26}H_{35}N_3O_7$, M = 501.57, monoclinic, a = 10.131(2), b = 20.904(4), c = 12.459(2) Å, $\alpha = \gamma = 90°$, β = 92.53(3)°, space group $P2(1)$, V = 2636.0(8) Å^3, Z = 4, D = 1.264 Mg m^{-3}, μ(Mo-Kα) = 0.092 mm^{-1}, F(000) = 1072, 16604 reflections collected, 8015 independent [R(int) = 0.0593], final R indices [$I > 2\sigma(I)$]: R_1 = 0.0359, wR_2 = 0.0845. CCDC 197619. See http://www.rsc.org/suppdata/cc/b2/b210952b/ for crystallographic data in CIF or other electronic format.

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