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Synthesis of symmetrical dimeric *N*,*N*′-linked peptides on solid support by olefin metathesis

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Abstract

A method has been developed for the synthesis of dimeric ligands of biological relevance on solid support using olefin metathesis as a key step. With the ruthenium catalyst used, the size of the peptide fragment did not influence the reaction. If the double bond involved was separated by at least ~2 methylene groups from an amide group, products of good purity could be recovered. © 2000 Elsevier Science Ltd. All rights reserved.

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Ligand induced receptor dimerization is a growing field of interest.[1] Also, some pharmacologically interesting targets, such as the HIV protease, are composed of two or more similar or identical subunits. We are therefore trying to develop methods for the synthesis of dimers of biologically relevant ligands, covalently linked by variable spacers. Although an elegant solution-phase approach towards dimeric peptides with olefin metathesis as a key step was reported by Boger and co-workers,[2–4] a solid-phase procedure using a similar key step has to our knowledge not been described yet. A solid-phase approach would be desirable for the synthesis of single-compound libraries using automated synthesizers. Herein we report our first experiments towards the dimerization of peptides on solid-phase using a ruthenium catalyst.[5–7]

As model compound four different peptides composed of one to four amino acids were synthesized on polystyrene.[8] The terminal amino groups of the peptides were acylated with five different ω-alkenoic acids. The synthesis of the peptides is summarized in Scheme 1. All amide bonds were formed under standard conditions with *N*,*N*′-diisopropylcarbodiimide (DIC), diisopropylethylamine (DIPEA), and 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HODhbt) in *N*-methyl-pyrrolidone (NMP). Removal of the 9-fluorenylmethyl carbamate (Fmoc) protecting group was performed with 50% piperidine in NMP.

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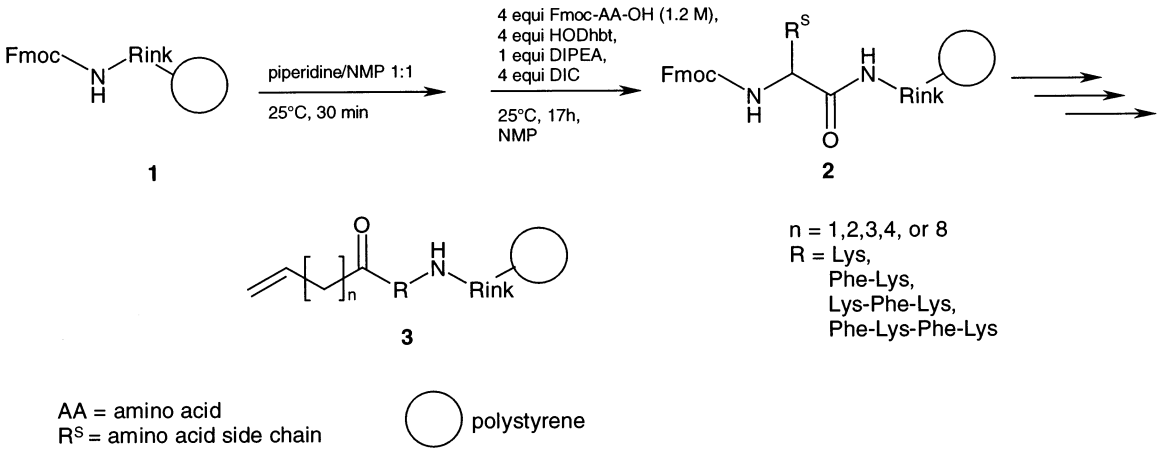
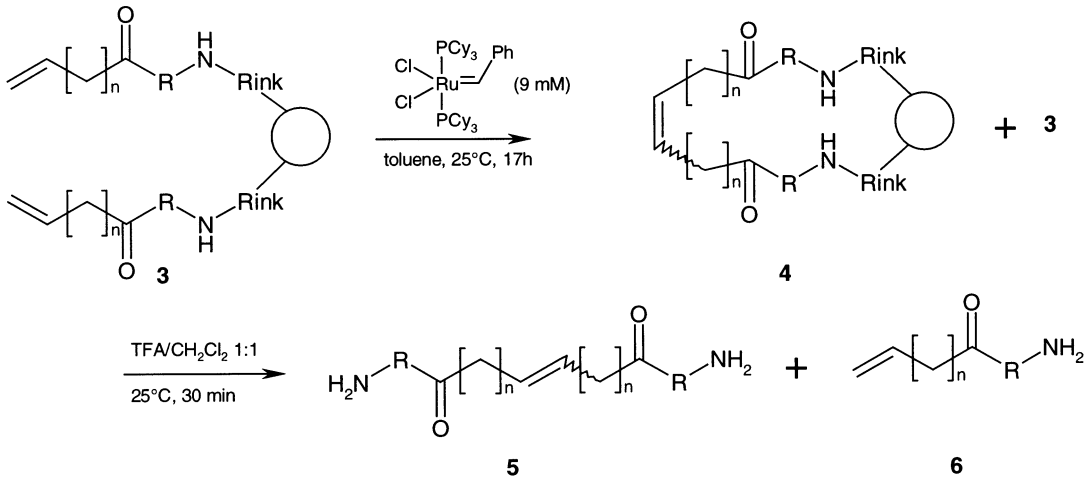


Scheme 1.

The olefin metathesis was performed by treating the support-bound alkenoyl peptides **3** twice at 25°C for 17 h with 15 mol% benzylidene-bis(tricyclohexylphosphine)dichloro ruthenium in toluene (Scheme 2). The products were cleaved from the resin with trifluoroacetic acid (TFA)/dichloromethane 1:1 for 30 min.



Scheme 2.

All samples were analysed by LC-MS. Unfortunately the monomeric peptoids **6** could not be separated from the dimeric peptoids **5** by HPLC. The results obtained are summarized in Table 1.

Some of the peptoids were also analysed by 400 and 600 MHz NMR to determine the E/Z ratio and to confirm their structures. The E/Z ratios were determined by measuring the integral ratio of the separated ^{1}H NMR signals attributable to the olefinic protons in the E- and Z-isomers. The proton signals were assigned by HSQC,[9] HSQC–TOCSY[9] and HMBC[10] experiments using the characteristic ^{13}C chemical shifts for the allylic methylene groups as diagnostic probes for the geometry around the double bond (Scheme 3).[11,12]

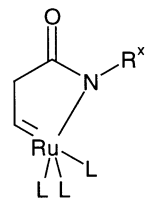
Table 1
Purities of crude, dimeric peptoids **5**

R	n 1	2	3	4	8
Lys	0%[c]	Purity 99%[a]	Purity 98%[a]	Purity 100%[a] *E*/*Z* 2.3/1.0[b]	Purity 96%[a] *E*/*Z* 3.3/1.0[b]
Phe-Lys	0%[c]	–[d]	Purity 99%[a]	Purity 100%[a] *E*/*Z* 1.9/1.0[b]	Purity 97%[a] *E*/*Z* 2.7/1.0[b]
Lys-Phe-Lys	0%[c]	–[d]	–[d]	Purity 97%[a]	Purity 98%[a] *E*/*Z* 2.7/1.0[b]
Phe-Lys-Phe-Lys	0%[c]	–[d]	Purity 93%[a] *E*/*Z* 2.0/1.0[b]	Purity 93%[a] *E*/*Z* 1.9/1.0[b]	Purity 94%[a] *E*/*Z* 2.1/1.0[b]

[a] Determined by HPLC/evaporative light scattering, only product **5** was detected by MS.
[b] Determined by ^{13}C NMR. See main text.
[c] Only monomeric product **6** was detected.
[d] Products **5** and **6** could not be separated by HPLC, both products were detected by MS.

Scheme 3.

The purities of the crude products of the desired dimeric peptoids **5** appear to be independent of the peptide fragment R, meaning that the required length allowing cross linking is already reached with the smallest peptide (one amino acid) and pentenoic acid. On the other hand, two or more methylene groups are required between the double bond and the carbonyl group for metathesis to proceed smoothly. The complete failure to obtain the dimeric product **5** with 3-butenoic acid derived peptoids is not due to the inability to reach a second alkene on solid support, since the longer peptides should compensate for the shortness of the butenoic acid. Also, the proximity of the carbonyl group should not be a problem, in view of the fact that there is precedence for an intramolecular metathesis with a 3-butenoic acid amide.[13] The difference to this reported example is the presence of an amide proton in our circumstances. A possible explanation could therefore be a stabilizing effect of the amide on the intermediate ruthenium complex (Scheme 4).



Scheme 4.

Several examples of the ring-closing olefin metathesis reaction on solid support have been published.[14–16] The reaction presented here is essentially also a ring-closing metathesis with a very large ring size, but the products are not cyclic, since the ring is opened during the process of releasing the peptoid from the resin.

Typical procedure for the olefin metathesis reaction: To 100 mg resin loaded with an *N*-alkenoyl substituted oligopeptide (loading $\leq$ 0.6 mmol/g) was added 14.8 mg benzylidene-bis(tricyclohexylphosphine)dichlororuthenium in 2 mL toluene. The resin was shaken overnight and then washed five times with 3 mL CH_2Cl_2. The step above was repeated once. The product was cleaved from the resin with 2 mL TFA/CH_2Cl_2 1:1 for 30 min.

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