A microwave enhanced cross-metathesis approach to peptidomimetics†

Thomas Morris,[a] David Sandham[b] and Stephen Caddick*[a]

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Functionalization of amino acid C- and N-termini with appropriate olefinic moieties allows for the generation of a peptidomimetic *via* a stereoselective cross-metathesis.

The amide bonds in a polypeptide chain are capable of hydrogen-bonding, a phenomenon that plays a major role in protein folding. Furthermore, amide functionalities that are not involved in folding can be available for protein–protein interactions, and such interactions underpin many biological processes. The ability to modify a protein so that it lacks an amide bond, and thus the potential for hydrogen-bonding, at a particular site would therefore be a valuable tool for probing both protein structure and function. Recent work by Kelly *et al.*[1] substitutes an amide bond for an *E*-alkene moiety, introduced *via* a Wittig reaction. This was then used to show that the removal of H-bonding ability in a single site of Aβ peptide inhibited the formation of fibrils or protofibrils in amyloidogenesis. Their approach is attractive because it removes the hydrogen-bonding ability whilst retaining some of the geometrical constraints present in the amide linkage.

Olefin cross-metathesis is a process which yields a C–C double bond and has a very high functional-group tolerance, and is, therefore, worthy of consideration in the context of making modified peptides selectively in order to perturb their ability to form hydrogen bonds.

There have been examples of olefin metathesis applied to peptide chemistry,[2] and cross-metathesis has been used to functionalize amino acid side chains.[3] However, we aim to use cross-metathesis in order to incorporate more fundamental changes into peptide structures. Specifically, we are interested in using cross-metathesis to create peptidomimetics in which the main chain is substantially modified. The inherent directionality of the native peptide will be maintained by the selectivity of cross-metathesis. Self cross-metathesis could not achieve this (by definition), and statistical cross-metathesis cannot without substantial wastage.

Here we present a protocol for the modification and linkage of single protected amino-acids *via* stereoselective olefin cross-metathesis using Grubbs' second generation catalyst;[4] yielding a linker with a length equivalent to that of two amino-acids yet lacking the central amide bond (Fig. 1). This novel use of cross-metathesis thus has potential for use in the synthesis of modified proteins, and in developing a greater understanding of both protein folding and protein–protein interactions. This methodology

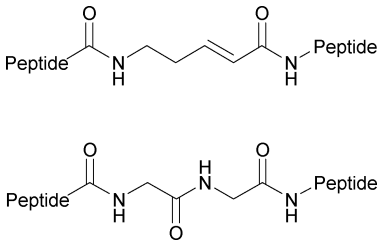
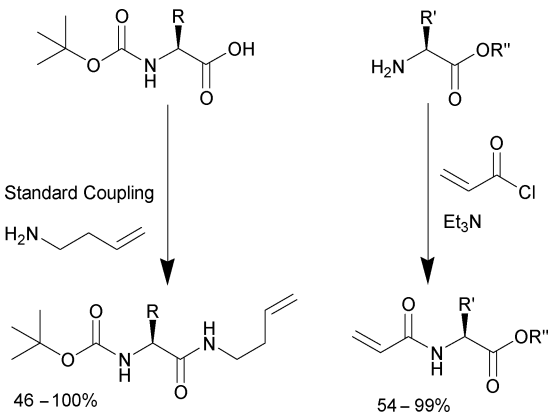


Fig. 1 Linker unit and two amino-acids, for comparison.

could also have implications in convergent peptide synthesis as a novel form of ligation. Indeed, we additionally present the linkage of a dipeptide to a tripeptide *via* our methodology, supporting this idea. To the best of our knowledge, cross-metathesis has not been performed on peptides of this size.

The approaches used to modify the C- and N-termini of the peptides were chosen for their simplicity in yielding the correct classes of olefins for successful cross-metathesis (Scheme 1).[5]



Scheme 1 Functionalization of amino acids.

Metathesis reactions utilized Grubbs' second generation catalyst (**3**), chosen for its stability to air (Scheme 2).[4] Metathesis reactions were carried out using microwave irradiation, which has been reported to greatly increase the rate of cross-metathesis reactions.[6] The synthesis of **4d** (AA_1 = Tyr(Bzl); AA_2 = PheOEt) was used to optimise the reaction (Table 1).

The best yields were obtained using 18 mol% **3**, DCM as the solvent, a small excess of **1** : **2**, typically 1.1–1.3 equivalents, and by heating in the microwave at 300 W for two 30 min periods. The reaction mixture was degassed with argon after the first 30 minutes to drive off any dissolved ethene. Addition of the catalyst in two portions decreased the yield, as did the use of an excess of the electron-deficient olefin **2**. It is worth noting that we found the Hoveyda–Grubbs catalyst to be far less active in this particular cross-metathesis than the Grubbs second generation.

[a]*University College London, Department of Chemistry, Christopher Ingold Laboratories, 20 Gordon Street, London, UK WC1H 0AJ. E-mail: s.caddick@ucl.ac.uk; Fax: +44 (0)20 7679 7463; Tel: +44 (0)20 7679 4694*
[b]*Novartis Institutes for Biomedical Research, Horsham Research Centre, Wimblehurst Road, Horsham, West Sussex, UK RH12 5AB. E-mail: david.sandham@novartis.com*

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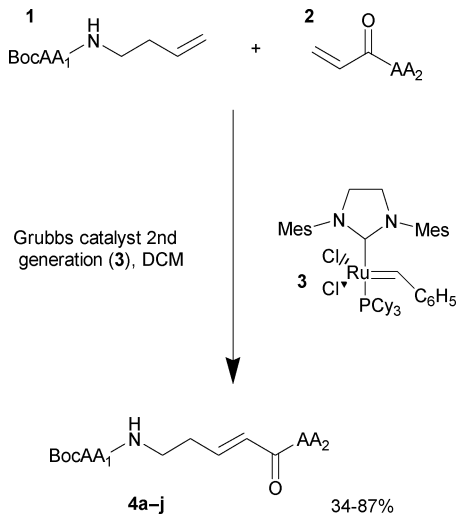
Table 1 Synthesis of **4d**—results of optimisation

1 (equiv.)	**2** (equiv.)	**3** (mol%)	Temperature/°C	Yield (%)
1	1.3	19	90	57[a]
1	1.1	18	80	55
1	1.3	18	90	66
1.2	1	26	90	61[b]
1	1.3	18	90	36[c]
1	2.1	20	90	52
1.2	1	18	90	58[d]
1	1.3	18	100	82
1	1.3	18	110	59

[a] Reaction involved 4 irradiations of 15 min each with no degassing. [b] 60 min irradiation with no degassing. [c] Hoyveda–Grubbs catalyst used in place of **5**. [d] Catalyst added in 2 × 9 μmol portions.



Scheme 2 Cross-metathesis of functionalized amino acids.

Results of the optimized conditions were applied to various other combinations of peptides as shown in Table 2.

Although cross-metathesis is often not stereoselective,[7] we were pleased to observe that, in the present case, products were obtained as a single isomer, exhibiting a vicinal coupling of 15 Hz between olefinic protons, confirming it to be a *trans* bond. This was supported by NOESY analysis of the cross-metathesis product, **4f**. In terms of peptidomimetics this is ideal, as it installs a structural motif with a conformation closer to that of the native peptide than in the case of the *cis* isomer.

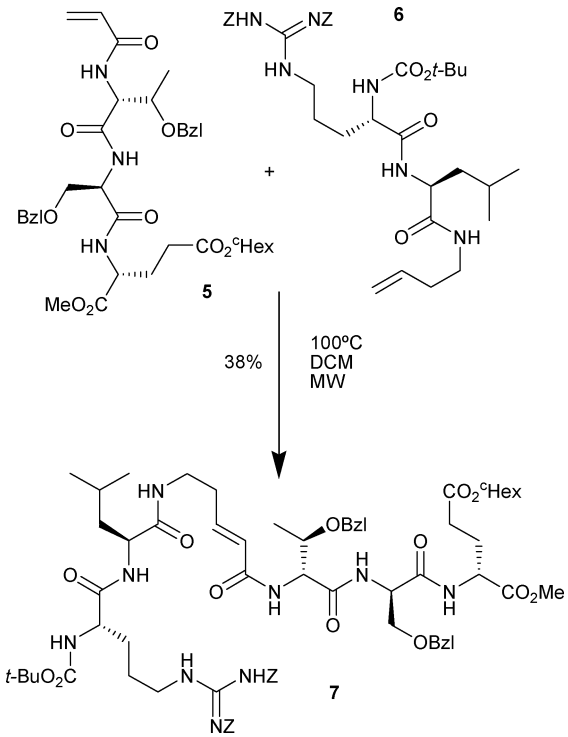
Table 2 Isolated yields of cross-metathesis products

Product	AA_1	AA_2	CM yield (%)[a]
4a	Phe	PheOEt	86[b]
4b	His(Tos)	PheOEt	41[c]
4c	Cys(4-MeBzl)	PheOEt	51
4d	Tyr(Bzl)	PheOEt	82
4e	Ser(Bzl)	PheOEt	73
4f	Arg(diZ)	PheOEt	70[d]
4g	Phe	ValOEt	34
4h	Leu	Ser(Bzl)OMe	75[e]
4i	Leu	Thr(Bzl)OMe	61
4j	Ser(Bzl)	ProOMe	50

[a] Utilized optimized conditions unless otherwise indicated. 1.3 equivalents **2** used for synthesis of **4a**–**d**; 1.1 for **4e**–**i**; 1.2 for **4j**. [b] MW reaction carried out at 80 °C, for 1 h, with no degassing. [c] MW reaction carried out at 90 °C, for 30 mins, with no degassing. 3 drops of DMF in addition to DCM. [d] Obtained by refluxing in DCM for 96 h; 43% obtained using microwave conditions. [e] Unwanted diastereomer (14% approx.) present in product, resulting from susceptibility of serine to racemisation.[8]

Once the general applicability of this technique was established, we were keen to examine the applicability of the method to larger peptides. DCM was used as a solvent, as before, and although we have not yet explored other solvents which may be significant in future applications, we note the use of DCM in solubilizing large fully protected peptide segments in the synthesis of thioesters for native chemical ligation.[9]

The functionalized tripeptide, **5**, was synthesized using standard peptide coupling procedures in solution to produce the protected tripeptide (52%). Removal of the Boc group was achieved using trifluoroacetic acid and the acryloylation of the free amine (78%), was achieved as previously described. **6** was prepared using standard peptide coupling procedures in solution (33%). **5** and **6** were then subjected to the optimised microwave conditions, as described previously, yielding **7** (Scheme 3). We determined the HPLC yield to be 67% using ELSD (evaporative light scattering detection). The product mixture was purified directly by flash column chromatography giving an isolated yield of 38%. Further material was obtained indicating the higher level of conversion, but this material could not be further purified.



Scheme 3 Application of the methodology to a larger peptide.

Interestingly, although the *trans* isomer was the major product, in this case we did observe the *cis* isomer as a minor component, (*trans* : *cis*, 8 : 1).

In summary, we have shown that two single, protected amino-acids can be functionalized with olefinic groups respectively at the N and C termini, and subsequently undergo cross-metathesis with high product selectivity. Furthermore, we have shown the

applicability of this methodology to significantly larger peptides. The high level of *trans* selectivity, coupled with the ease of synthesis of the precursors, suggest this reaction could have a number of applications in chemical biology.

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