Cyclisation/cleavage of macrocycles by ring-closing metathesis on solid support—conformational studies

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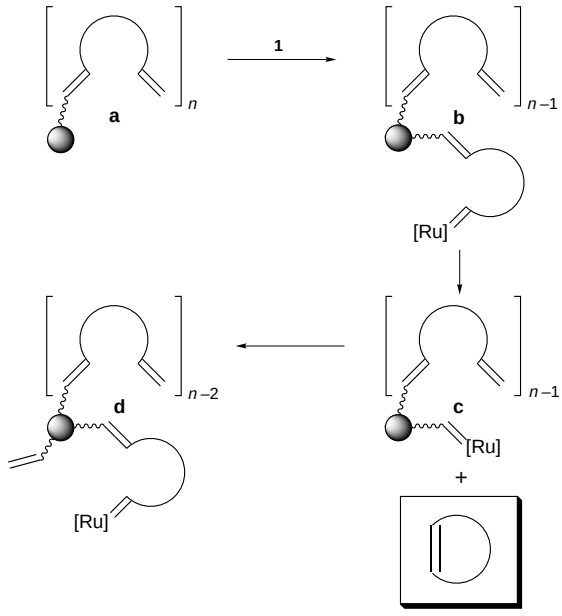
Grubbs' ruthenium metathesis catalyst 1 was used to investigate the catalytic cyclisation/cleavage of tetrapeptide-derived macrocycles from a solid support; the rate dependence on substrate conformation has been studied.

Recent developments in the field of alkene metathesis[1] were initiated by the introduction of catalysts[2] with unprecedented activities even in the presence of polar functional groups. In particular, ring-closing metathesis (RCM) has emerged as a powerful tool for the synthesis of different ring systems from five-membered rings to macrocycles.[3,4] Usually, cyclisations to large rings are performed in diluted solutions to supress competing acyclic diene metathesis (ADMET). RCM is also applicable to polymer-supported substrates.[5] In addition to ring closure, metathesis catalysts have been employed to facilitate catalytic binding[6] of alkenes to polystyrene-based resins as well as their catalytic cleavage.[7] A most interesting approach combines both RCM and the liberation of the cyclic alkene.[8]

Herein, we report investigations into the cyclisation/cleavage of macrocycles from solid support. Metatheses were performed using the well-established Grubbs' ruthenium initiator $Cl_2(PCy_3)_2Ru{=}CHPh$ **1** (Cy = cyclohexyl).[2] According to our concept (Scheme 1), **1** is expected to selectively attack a terminal double bond for steric reasons. This initial step (**a** → **b**) is followed by a RCM resulting in the liberation of one equivalent of the macrocycle and in the formation of polymer-bound metal–carbene complex **c**. Assuming sufficient flexibility, a transfer of the metal complex on the polymer surface (**c** → **d**) should take place, thus, enabling further catalytic cleavage cycles.[9] Byproducts resulting from quasi-intermolecular crossed metatheses of terminal double bonds remain bound to the resin during the entire process.

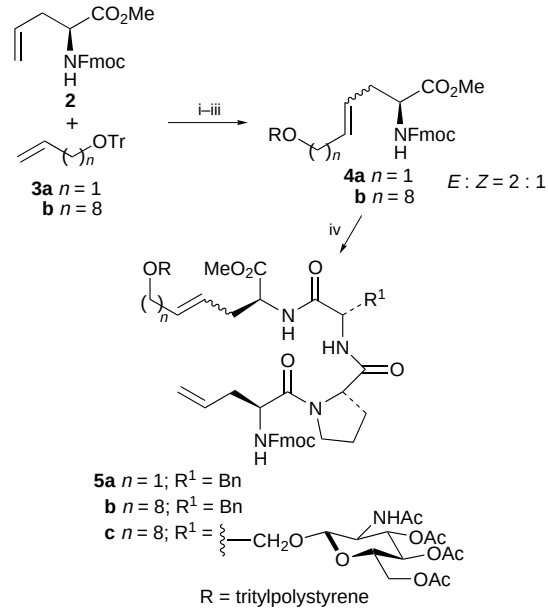


Scheme 1

We choose to perform our studies on peptide structures, since the sequence-dependence of their conformations is empirically well described.[10] Chlorotritylpolystyrene was used as the solid support, since solid-phase-bound reaction byproducts are easily monitored after cleaving the acid-labile trityl ether bond. Polymer-supported dialkenic tetrapeptides were obtained by standard Fmoc peptide chemistry using L-Fmoc-amino acids.

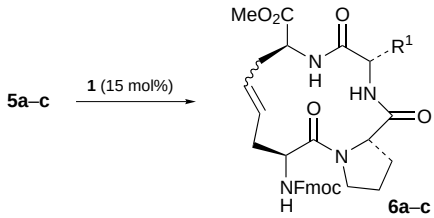
Under catalysis of **1** the side chain of Fmoc-allylglycine methyl ester **2** was selectively cross-coupled with *O*-trityl-protected hydroxy alkenes **3a,b** of different chain length.† To date, few comparable examples for synthetic applications of catalytic crossed metatheses have been described.[11] After deprotection, metathesis products were reacted with chlorotritylpolystyrene resin to give resin-linked, Fmoc-protected *C*-allylglycines **4** serving as starting materials for the solid phase synthesis of dialkenic tetrapeptides **5a–c** (Scheme 2). The terminal double bond was introduced by coupling Fmoc-*C*-allylglycine as the N-terminal amino acid. All resins described herein contained approximately 0.5 mmol g^{-1} of tetrapeptide. The tetrapeptide motif of **5a** and **5b**, respectively, is closely related to disulfide bridge-stabilized β-turns found in redox-active proteins[12] and is, therefore, well suited for cyclisation as demonstrated by Miller *et al.* recently.[13] Results for ruthenium-catalysed cyclisations are given in Table 1.‡

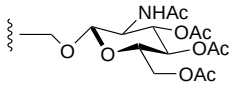
Remarkably, **5b** cyclised smoothly despite the high loading of 0.5 mmol g^{-1}. Comparison with the conversion of **5a** reveals that the extent of cyclisation/cleavage depends strongly on spacer length and, thus, mobility of the polymer-supported intermediates. Subsequent work was therefore carried out using the long spacer. Cyclisation of **5c** to cyclic glycopeptide **6c** with



Scheme 2 *Reagents and conditions*: i, **1** (4 mol%); ii, TFA; iii, RCl; iv, piperidine–DMF, Fmoc-amino acid, PyBOP (iteratively) (PyBOP = benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate)

Table 1 Results of cyclisation/cleavage of acyclic **5** to macrocycles **6**



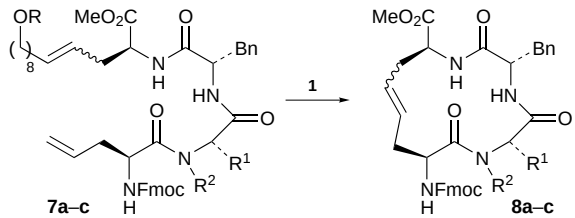
Product	R^1	*n*	Yield (%)	Purity (%)
6a	Bn	1	30	65
6b	Bn	8	70	85
6c		8	30	85

good product purity demonstrates that highly functionalized dienes are also tolerated by our reaction system.

Since cleavage rates were expected to depend on the conformations of the acyclic precursors, we attempted to correlate the relative cyclisation/cleavage rates of different precursor dienes with their probability to form β-turn-like structures. Rate experiments are easily performed, since most byproducts formed during the metathesis reaction remain bound to the polymer. In **5a**–**c** a β-turn-like structure is induced by the proline residue. Consequently, the proline in **5b** was exchanged by alanine, sarcosine and glycine, respectively, all representing minimal structural analogues of proline. The resulting polymer-supported tetrapeptides **7a**–**c** are less likely to exhibit β-turn-like structures and are, therefore, expected to cyclise significantly slower than **5b** itself. To test this assumption, **5b**, **7a**, **7b** or **7c** were mixed with an equal amount of **5c** serving as an internal standard and reacted with **1**.§ The amounts of cyclic tetrapeptides formed relative to **6c** were determined by HPLC analysis. Relative rates are given in Table 2.

As expected, **7a**–**c** all exhibit significantly decreased cleavage rates as compared to **5b**. The higher rate of sarcosine-containing **7a** compared to **7c** may be explained by the *N*-methyl group expanding the conformational space by abolishing the partial double bond character of the X–Gly peptide bond. To characterize the reaction system polymer-bound byproducts formed during the cyclisation of **5b** (very fast) and **7c** (very slow) were analysed after acidic cleavage from the trityl resin. In case of **5b**, 25% of Δ^9-octadecene-1,18-diol, resulting from dimerisation of the spacer after cleavage of the macrocycle, as well as negligible amounts of acyclic products were isolated. No starting material could be detected. In the case of **9**, besides open-chain byproducts (36%), 40% of the starting material also remained bound to the resin after cyclisation.¶ This latter finding suggests that intermolecular reaction of two terminal double bonds on the resin surface proceeds significantly slower than the cyclisation/cleavage.

Table 2 Relative cyclisation/cleavage rates of polymer-supported tetrapeptides with different residues in the (*i* + 1)-position (R = tritylpolystyrene)



Substrate	R^1	R^2	Cyclisation rate relative to **5c**
5b			1.36
7a	H	Me	0.15
7b	Me	H	0.11
7c	H	H	< 0.02

In summary, polymer-bound substrates cyclise rapidly when exhibiting favourable conformations. Macrocycles cleaved at lower rates are obtained in good purity, since byproducts remain bound to the polymer.

Footnotes and References

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† Selective cross-metathesis: **2** (0.6 g, 17 mmol) was combined with **3** (34 mmol) in CH_2Cl_2 (10 ml) and reacted with **1** (50 mg, 0.062 mmol) at reflux for 12 h with exclusion of moisture and air. Cross-metathesis products were purified by flash chromatography and isolated in 75% yield each.

‡ Cyclisation/cleavage: polymer (200 mg) was suspended in CH_2Cl_2 (1.5 ml) and refluxed after addition of **1** (9 mg, 0.011 mmol). After 17 h the resin was filtered off and washed twice with CH_2Cl_2. The contents of the filtrate were analysed by analytical HPLC. A SEDEX 55 (ERC, Germany) Evaporative-Light-Scattering Detector (ELSD) was employed to ensure comparable mass responses for different analytes. Cyclic products were purified by semi-preparative HPLC and characterized by ^{1}H NMR spectroscopy and HRMS. They were formed as *E*/*Z* mixtures, as indicated by HPLC and ^{1}H NMR spectroscopy.

§ Competition experiments: **5c** and the diene to be tested (**5b**, **7a**, **b** or **c**) (30 mg of each) were suspended in CH_2Cl_2 and heated to reflux for 2 h after addition of **1** (2.5 mg, 0.003 mmol). Product mixtures were filtered and dried. The relative amounts of cyclic products formed were determined by HPLC. For product characterisation, cyclisation/cleavage of the tested diene was performed on a preparative scale using the procedure given above.

¶ Acyclic byproducts were unambigiously identified as cross-metathesis products between the double bonds of two tetrapeptide molecules or tetrapeptide and spacer arm.

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