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Solid phase synthesis of cyclic peptides by oxidative cyclative cleavage of an aryl hydrazide linker—synthesis of stylostatin 1

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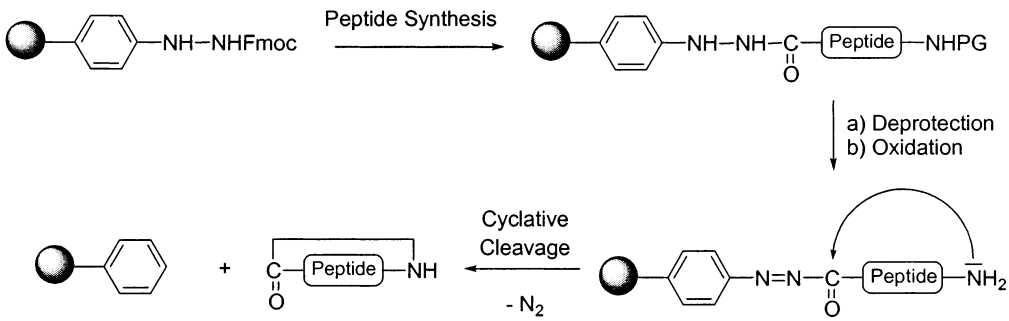
Abstract—The synthesis of cyclic peptides using an oxidation labile aryl hydrazide linker is reported. After oxidation with NBS release from the resin is triggered via an intramolecular cyclative cleavage which proceeds without racemization.

1. Introduction

Cyclic peptides are widely applied in numerous areas of biomedical research to study the conformational requirements of peptide and protein recognition and as diagnostically and therapeutically useful tools.[1] For instance, the peptide cyclization approach has provided conformationally constrained analogs with suitable properties for investigating ligand–receptor interactions and structure–activity relationships, and cyclic peptides have served as models for the design of bioavailable drugs.[2] In addition, they often have increased metabolic stability over their linear counterparts. Among the synthetic methodologies available for the synthesis of cyclic peptides, solid phase mediated cyclizations have distinct advantages in that they reduce the number of isolation/purification steps required by conventional solution synthesis.[2c] Several methods for on-resin cyclization have been established mostly using two types of linking strategies. One is the on-resin cyclization of peptides anchored via their backbone or a side chain functional group.[3] The other is the cyclization–cleavage approach, in which the C-terminus of the linear peptide is attached to an electrophilic linker that is labile to free amines.[4] The published methods for the latter strategy use either Kaiser oxime resin[4a] or thioester resin,[4b] which are only compatible to Boc-chemistry due to the lability of the ester bond towards nucleophiles. Alternatively, a sulfonamide 'safety-catch' linker[4c] is employed for the head to tail cyclization–cleavage process. The sulfonamide linker is stable to nucleophilic attack until it is activated by cyanomethylation of the sulfonamide.

While these methods have allowed for the successful synthesis of numerous cyclic peptides they leave substantial room for the development of alternative linker groups which, for instance, are compatible with a wide range of synthetic methods and can be activated under alternative conditions. The purpose of this paper is to report on the use of the aryl hydrazide linker[5] for the



Scheme 1. Principle of cyclative cleavage employing the oxidation labile aryl hydrazide linker.

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solid phase synthesis of cyclic peptides via the cyclization–cleavage approach (Scheme 1). This oxidation labile linker was originally introduced by Wieland et al.[5b] but its successful application for the synthesis of cyclic peptides could not be achieved. We have recently shown that aryl hydrazides can be used as efficient linkers for the traceless synthesis of a variety of compound classes and that they are compatible with various types of synthetic transformations and reagents.[6]

The principle of the approach described herein is to employ an acid- and base-stable hydrazide linker, i.e. the commercially available 4-Fmoc-hydrazinobenzoyl AM resin,[7] for peptide assembly, e.g. via the Boc-strategy. After N-terminal deprotection the hydrazide is then oxidized to give an acyl diazene which subsequently is attacked by the liberated amine resulting in cyclization and release from the solid support (Scheme 1).

To investigate whether efficient solid phase peptide cyclization via this approach can be achieved, cyclic model peptide **4** was selected as initial target (Scheme 2). This compound has been synthesized in solution before and has proven to be a demanding and difficult case.[8]

From commercially available 2-Fmoc-hydrazinobenzoyl HM resin (loading 0.67 mmol·g^{-1}) the Fmoc group was cleaved with 20% piperidine in DMF and then the peptide sequence was built up using the standard Boc protocol (see e.g. Ref. 9). In order to prevent acylation of the hydrazide linker itself during the capping step, the sterically demanding pivalic acid anhydride was chosen as capping reagent. After deprotection of the N-terminal alanine the linker group was oxidized to the acyldiazene by treatment with NBS–pyridine. Upon treatment with NEt_3 the N-terminal ammonium function is deprotonated and the nucleophilic amino group attacks the activated carbonyl group resulting in cyclization and release of the cyclic peptide into solution. HPLC analysis of the crude product obtained after oxidation and cyclization revealed that the desired peptide was obtained directly with high purity (97%; see Fig. 1). Desired cyclic peptide **4** was isolated in an overall yield of 19%. Gratifyingly, formation of dimers and oligomers was not observed at all. This result compares very favorably with the results recorded in a solution phase synthesis of **4**. Thus, Schmidt et al.[8a] recorded an overall yield of 8% for the synthesis of the cyclic Phe-Leu-Pro-Ala-Ala via the pentafluorophenyl ester approach and Meutermans et al.[8b] synthesized cyclic peptide **4** in an overall yield of 7% via a multistep ring construction strategy.

In order to demonstrate that naturally occurring, biologically relevant cyclopeptides can be synthesized via this strategy we embarked on a synthesis of stylostatin 1 a cell growth inhibitory cycloheptapeptide isolated from the marine organism *Stylotella aurantium* (Fig. 2).[3a,9] Assembly of the peptide chain was achieved via the method described above. For the final cyclization nucleophilic attack of leucine to alanine as the first resin-anchored amino acid was chosen (Fig. 2, cyclization site a). After oxidation with NBS and subsequent treatment with triethylamine the desired cyclic peptide was isolated in 7% overall yield. Once more dimer formation was not observed. Stylostatin 1 also served

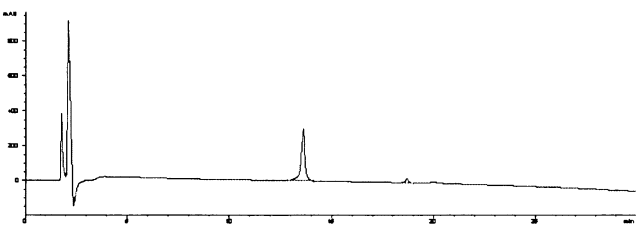
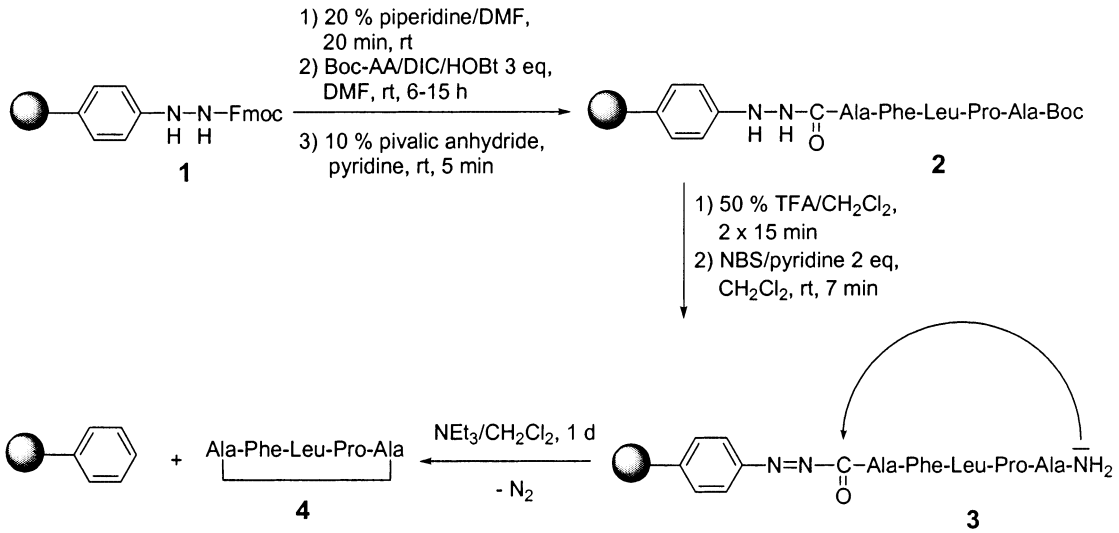


Figure 1. HPLC analysis of crude cyclic peptide **4** released from the solid support after oxidation and cyclization. Analysis was performed employing a C18 PPN HPLC column (Macherey–Nagel) as stationary phase and a linear acetonitrile/H_2O gradient (10–90%; 1% trifluoroacetic acid in 20 min) for elution. The product was detected at $\lambda = 215$ nm.



Scheme 2. Peptide synthesis and cyclative cleavage with the aryl hydrazide linker.

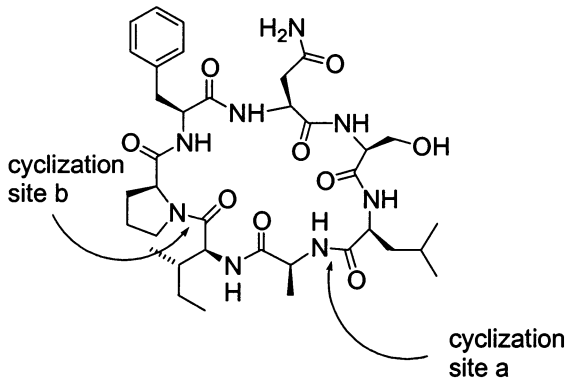


Figure 2. Structure of Stylostatin 1.

as a model to investigate the sequence dependence of cyclization via the hydrazide linker. To this end, a cyclization via attachment and activation of proline at the solid support and nucleophilic attack by isoleucine was investigated (Fig. 2, cyclization site b). In the course of this final attack of the sterically demanding Ile to the cyclic, *N*-alkylated amino acid Pro the desired cyclic peptide was finally obtained in less than 1% overall yield.

Thus, although the hydrazide linker displays very favorable properties in cyclization at less demanding sequences, not surprisingly it has limitations if steric bulk is built up on both sides of the cyclization site.

Finally, two additional possible limitations of the method were addressed. On the one hand the possibility of partial racemization of the C-terminal amino acid after oxidative conversion to the acyldiazene was investigated. To this end (L)-Fmoc-Ala was attached to the hydrazine-modified solid support and after oxidation of the hydrazide the formed acyldiazene was trapped by addition of L-phenylalanine methyl ester to yield Fmoc-Ala-Phe-OMe. Analysis of the resulting compound and comparison with the authentic (L,L)- and (D,L)-peptides by chiral HPLC unambiguously proved that epimerization had not occurred. On the other hand the very surprising and gratifying finding was addressed that no dimer formation was observed in the cyclization step (see above). In order to determine whether this is due to steric bulk in the vicinity of the cyclization site, cyclopeptide formation from the pentapeptide Gly-Val-Pro-Leu-Gly was studied. In this peptide neither the activated C-terminal nor the nucleophilic N-terminal amino acid carry a sterically demanding side chain. Indeed, upon oxidation with NBS and subsequent treatment with NEt_3 both the cyclic monomer and the cyclic dimer were isolated in a ratio of ca. 3:2. In conclusion we have shown that the hydrazide linker provides an efficient alternative for the synthesis of cyclopeptides via the cyclization–cleavage approach. It is compatible with a wide variety of transformations and allows formation and release of cyclic peptides from the solid support under mild oxidative conditions.

2. Cyclization experiments

Deprotection: After completion of the linear peptide synthesis, the resin (200 mg) was treated twice (15 min each) with 5 ml of a 1:1 mixture of trifluoroacetic acid and dichloromethane. The resin was drained and washed with dichloromethane (2×), a 3% solution of triethylamine in dichloromethane and again dichloromethane (2×).
Oxidation and cyclization: the resin was treated with a solution of *N*-bromosuccinimide and pyridine (2 equiv. each) in 3 ml dichloromethane for 7 min, drained and washed three times with dichloromethane. The resin was treated with a solution of 5 equiv. of triethylamine in dicholormethane for 2 days, drained and washed five times with dichloromethane. The filtrates obtained after the oxidation and the cyclization steps were combined and evaporated to dryness. The products were purified by preparative HPLC (C18 PPN column; Macherey–Nagel) as stationary phase and a linear acetonitrile/H_2O gradient (10–90%); 1% trifluoroacetic acid) for elution. The product was detected at $\lambda = 215$ nm. The spectroscopic data determined for the products are in agreement with literature values.

Acknowledgements

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