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SYNTHESIS OF 'HEAD-TO-TAIL' CYCLIZED PEPTIDES ON SOLID SUPPORT BY FMOC CHEMISTRY

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Abstract: Two cyclic peptides were synthesized directly on solid support by 'head-to-tail' cyclizations. Key features are side chain attachment of an Asp residue to an amide or a hydroxy linker and orthogonal protection of the α-carboxyl function of this amino acid as allyl ester. Cyclization was performed with TBTU as coupling reagent. Depending on the attachment the cyclic peptides contain either an Asp or an Asn residue. The method is also applicable to Glx-containing cyclic peptides.

Cyclic peptides are known to exhibit constrained flexibility when compared to their free, linear forms and at the same time their resistance to enzymatic degradation is increased.

In the past, most cyclic peptides have been prepared by classical solution chemistry under conditions of high dilution. The 'head to tail' cyclization of peptides while still attached to a solid support offers several advantages as compared to solution methods. Ring formation should proceed with higher efficiency since there is minimal risk of oligomerization and reagents applied to the cyclization reaction can easily be removed by filtration.

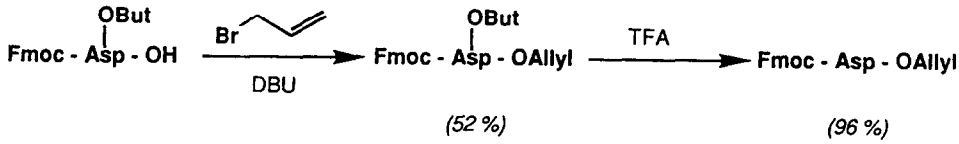
There have appeared a few examples of cyclizations on solid support where the Boc-strategy of synthesis was used [1,2]. In combination with Boc chemistry the Kaiser oxim resin allows cyclization with concomitant loss of the cyclized peptide from the resin [3].

Little attention has so far been drawn to solid phase cyclization using Fmoc chemistry. Recently a cyclization in which a glutamic acid side chain attached via a hydroxy linker to a solid support was reported [4]. The α-carboxyl group was protected as its 2,4-dimethoxy benzyl (Dmb) ester. After completion of peptide synthesis the Dmb group was removed with 1 % TFA which was followed by removal of the Fmoc-group and cyclization using either BOP [5] or DIPCDI/HOBt in DMF. A disadvantage of this strategy is that cleavage of the Dmb ester function proceeds with concomitant loss of the trityl protecting group on the side chains of His and Cys residues [4].

Here we present our results on the synthesis of two cyclic hexapeptides applying a similar approach, however using orthogonal allyl protection for the α-carboxyl function of Asp. This protecting group can be specifically cleaved with $Pd^0[P(C_6H_5)_3]_4$ under neutral conditions without affecting other protecting groups [6,7].

Furthermore, an amide [8,9] or a hydroxy linker was used to attach the Asp side chain to the solid support. This yielded after cleavage of the peptide from the support the corresponding desired Asn or Asp residue in the cyclic peptide sequence.

The required building block Fmoc-Asp-OAllyl was synthesized according to *Scheme 1.*



Scheme 1

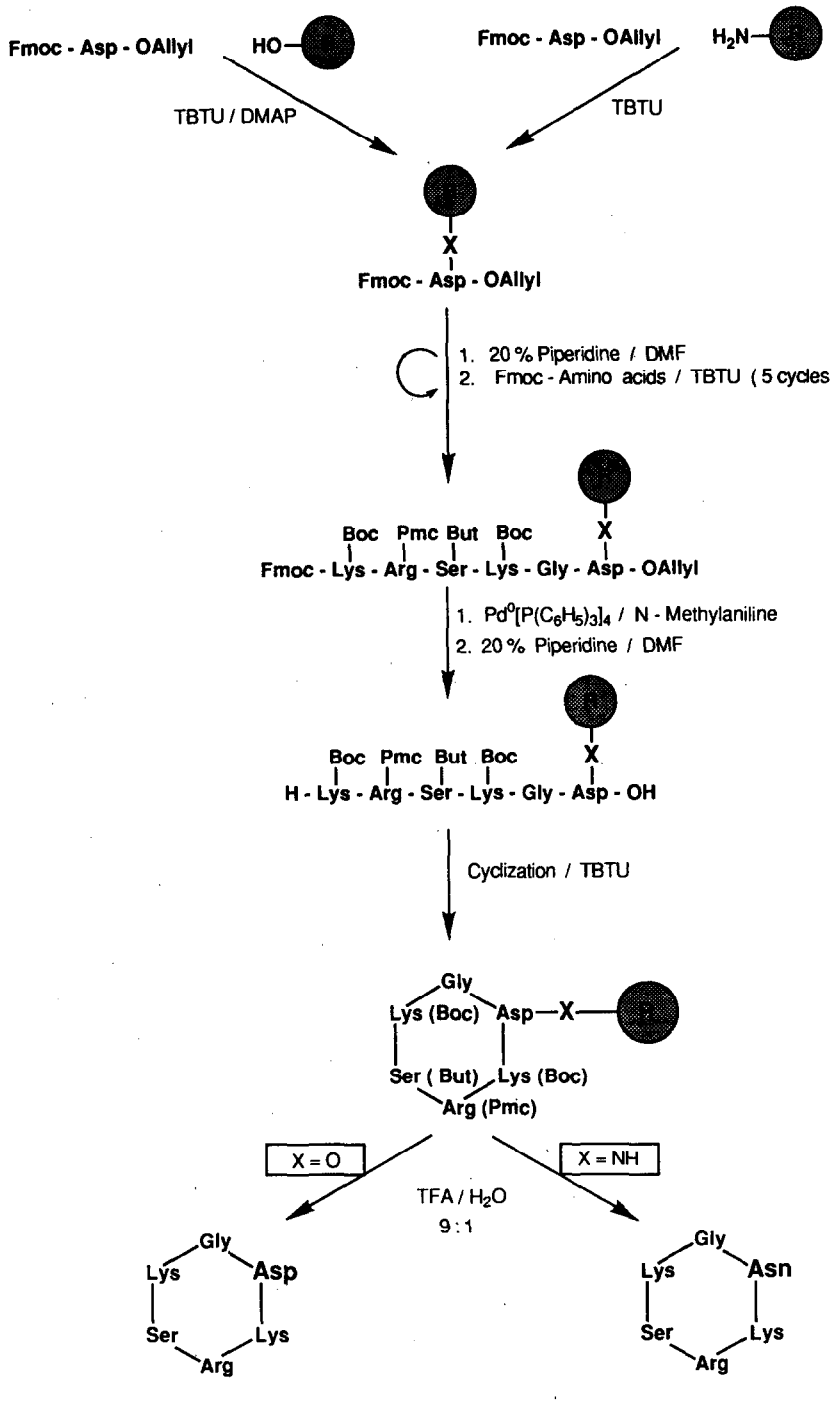
Thus, reaction of Fmoc-Asp(OBut)-OH with allyl bromide in the presence of DBU yielded the corresponding allyl ester. TFA cleavage of the tert. butyl ester of the side chain resulted in the desired Fmoc-Asp-OAllyl as a crystalline compound [10].

Synthesis of the cyclic peptides was performed according to *Scheme 2* [11]. Fmoc-Asp-OAllyl was coupled to the amide linker by activation with TBTU [12] or esterified to the hydroxylinker with TBTU/DMAP. Further acylations to the final linear peptide were performed by standard TBTU coupling cycles. After the last cycle the allyl protecting group was removed with $Pd^0[P(C_6H_5)_3]_4$ in THF/DMSO/0.5 M HCl (2:2:1) and N-methylaniline as the nucleophile [13], which was followed by cleavage of the Fmoc group. Prior to cyclization, part of the peptide-resin was completely deprotected to generate the linear peptide sequences for comparison.

The cyclization on the support was also achieved by TBTU activation. Deprotection and release from the support were performed with a mixture of TFA/H_2O (9:1). RP-HPLC analysis of crude product indicated an almost quantitative cyclization on the solid support.

The crude material was purified by RP-HPLC or Sephadex G 25 chromatography yielding the desired cyclic peptides. Purity was assessed by analytical RP-HPLC and capillary zone electrophoresis. FAB-MS confirmed the molecular weight calculated for the cyclic peptides.

In summary, we have shown that Asp side-chain attachment in combination with its allyl protection at the α-carboxyl function is ideally suited for 'head to tail' cyclizations of peptides on solid support. The same strategy can be applied to the synthesis of Glx-containing cyclic peptides. Currently we are extending the approach to other amino acids bearing suitable side chain functionalities for the attachment to a solid support.


Fmoc - Asp - OAllyl
HO—
TBTU / DMAP
Fmoc - Asp - OAllyl
H2N—
TBTU
X
Fmoc - Asp - OAllyl
1. 20 % Piperidine / DMF
2. Fmoc - Amino acids / TBTU (5 cycles)
Boc Pmc But Boc X
Fmoc - Lys - Arg - Ser - Lys - Gly - Asp - OAllyl
1. Pd°[P(C6H5)3]4 / N - Methylaniline
2. 20 % Piperidine / DMF
Boc Pmc But Boc X
H - Lys - Arg - Ser - Lys - Gly - Asp - OH
Cyclization / TBTU
Gly
Lys (Boc)
Asp—X
Ser (But)
Lys (Boc)
Arg (Pmc)
X = O
X = NH
TFA / H2O
9 : 1
Gly
Lys
Asp
Ser
Lys
Arg
Gly
Lys
Asn
Ser
Lys
Arg
= Solid Support

Scheme 2

Acknowledgements

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References and Notes

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[10] Preparation of **1**: Fmoc-Asp(OBut)-OH (120 mmol; 49.4g) was reacted with allyl bromide (120 mmol; 14.5g) in DMF in the presence of DBU (120 mmol; 18.24g) for 12 h. Fmoc-Asp(OBut)-OAllyl was obtained in a yield of 52 % after crystall. from ether/hexane. Mp. 82-83 °C; $[\alpha]_D^{20}$= -19.0 (c: 1.0 in DMF). Treatment of 50 mmol Fmoc-Asp(OBut)-OAllyl with TFA yielded after crystall. from ether/hexane 19 g of Fmoc-AspO-Allyl (96 %) . M.p. 93 °C; $[\alpha]_D^{20}$= -24.5 (c: 1.0 in DMF). The corresponding Fmoc-Glu-OAllyl was prepared by the same pathway. M.p.: 116-117 °C; $[\alpha]_D^{20}$ = -20.5 (c: 1.0 in DMF).

[11] Cyclic peptides were synthesized on a Labortec Synthesizer SP 650 (Bachem) using a polystyrene resin modified with the amide linker or p-hydroxymethyl-phenoxy resin. Attachment to the support and elongations were performed with 1.5 eqivalents each using TBTU as condensing reagent. The allyl and the Fmoc group were removed from the peptide resin and cyclization was performed with 1.5 equivalents of TBTU in the presence of 1.5 equivalents of DIPEA for 3 h. After deprotection and purification homogeneous cyclized peptides were obtained. Analysis by FAB-MS showed the expected molecular ions.

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[14] Abbreviations: Boc: tert. Butyloxycarbonyl; BOP: Benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate; DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene; DIPCDI: 1,3-Diisopropylcarbodiimide; DMAP: N,N-Dimethylaminopyridine; Fmoc: 9-Fluorenylmethoxycarbonyl; FAB-MS: Fast-Atom Bombardment Mass-Spectrometry; HOBt: 1-hydroxybenzotriazole; TBTU: 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA: Trifluoroacetic acid; RP-HPLC: Reversed Phase High Performance Liquid Chromatography. Asx: Aspartic acid or Asparagine; Glx: Glutamic acid or Glutamine.

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