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Synthesis of cyclic peptides via *O–N*-acyl migration

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ABSTRACT

We describe here a novel and convenient synthesis of head-to-tail cyclic peptide avoiding racemization. Linear depsipeptides including a serine residue as the key element for ester bond formation and acyl transfer were synthesized on 2-chlorotrityl chloride resin. After cleavage from the resin, intramolecular head-to-tail cyclization was performed in solution by C-terminal activation of urethane protected *O*-acyl serine residue. After removal of the N^{α}-serine protecting group, the final step consisted in *O–N*-acyl migration reaction on the 'switch' or 'click' element to restore native cyclic peptides.

1. Introduction

Despite their involvement in several physiological and physiopathological processes, few peptides have been successfully developed as therapeutic agents, mainly due to their poor bioavailability and their lack of stability against enzymatic degradation. However cyclic peptides are of great pharmacological interest in view of their improved chemical and biochemical stability. Moreover, the conformational restrictions induced by cyclization significantly improve their activity and/or selectivity towards biological targets. Due to the interest in cyclization to overcome the limitations of linear peptides, many efforts have been devoted to develop efficient methods for the synthesis of cyclic peptides.[1–3] From a strategic point of view, in most cases head-to-tail cyclization requires activation of a non-urethane-protected amino acid, leading to epimerization. To minimize this problem the choice of coupling reagent needs to be carefully examined.[1] On-resin cyclization strategies were developed using as examples, Kaiser oxime,[4] thioester[5] resins, only compatible with Boc-chemistry, and 'safety catch' linker.[6,7] Some of them are described to be free from racemization.

We propose here an alternative route for epimerization-free cyclization of serine-containing peptides. Our study focused on the serine as a key element to generate a depsipeptide and to promote racemization-free head-to-tail cyclization. The *O–N*-acyl migration was used to restore the native peptide. This strategy was the subject of extensive studies and applications, mainly for the synthesis of peptides containing difficult sequence such as the amyloid beta peptide and prodrug design but also for 'racemization-free' segment condensation.[8–13] The presence of the hydroxylated side chain of the serine was used to build up the peptide sequence via an *O*-acyl isopeptide bond. The depsipeptide with a C-terminal urethane-protected serine residue was then employed for the cyclization. In order to restore native cyclic peptides, the last step of the synthesis consisted in an *O–N*-acyl intramolecular transfer on the serine, called 'switch' or 'click' element (Scheme 1).[8,10]

In this study, four model depsipeptides (Table 1) were synthesized on solid support, and the cyclization step was performed in solution followed by the *O–N*-acyl transfer. The influence of the cycle size for the intramolecular migration was investigated. The synthesis of difficult and non-favorable cyclic tetrapeptide by using the acyl-transfer reaction strategy as an induction element was included in this study. Effectively, cyclic depsitetrapeptides with less severe conformational constraints could be templates for the very difficult formation of cyclic tetrapeptides.

2. Results

The linear depsipeptides **6**–**9** were synthesized on solid support (Scheme 1). Boc-Ser-OH was anchored on a 2-chlorotrityl chloride resin.

The second amino acid, Fmoc-Ala-OH was added by acylation on the serine side chain using DIC/DMAP as coupling reagent.

Both reactions were performed at room temperature and under microwave irradiation, and the latter condition allowed a drastic reaction time decrease (from 24 h to 40 min). The extent of epimerization in the synthesis of Ser-containing depsipeptides is reported to be very low.[14] However as it could be a limitation to develop a racemization-free cyclization strategy, this point was

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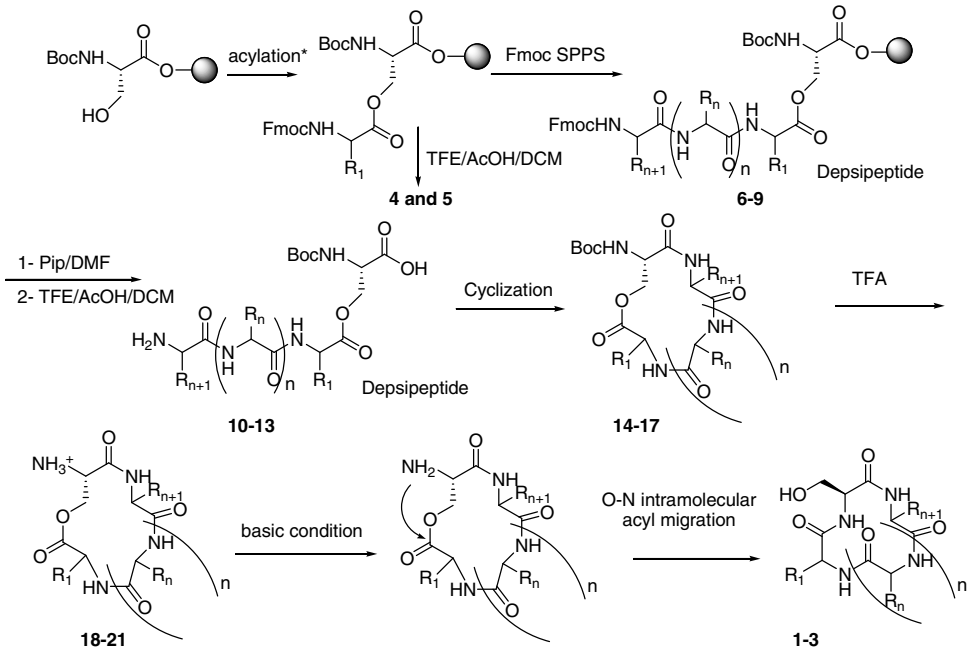
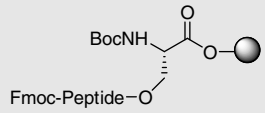
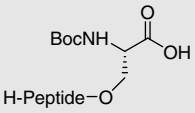
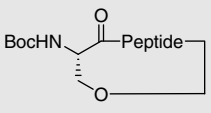
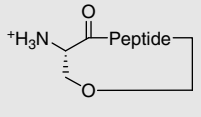
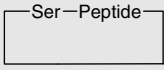


Scheme 1. General conditions for cyclic peptide synthesis. Resin: 2-chlorotrityl chloride; loading: Boc-Ser-OH (5 equiv), DIEA (10 equiv), DCM 30 min microwave 32 °C; acylation: Fmoc-AA1-OH (6 equiv/ DIC 3 equiv), DMAP (0.3 equiv), DMF, 2 × 5 min microwave 70 °C; **SPPS**: deprotection: Pip/DMF (20/80); coupling: Fmoc-AA-OH (3 equiv), DIC (3 equiv)/HOBt (3 equiv), DMF; cleavage: TFE/AcOH/DCM (20/10/70); cyclization: $NaHCO_3$ (5 equiv), BOP (1 equiv), DMF; *O–N*-Acyl shift: aqueous phosphate buffer pH 7.4 or DMF/Pip (80/20) solution. *: Acylation with Fmoc-Ala-OH and Fmoc-D-Ala-OH gives, respectively, after TFE/AcOH/DCM cleavage compounds **4** and **5**.

Table 1
Depsi- and peptide sequences

Peptide sequence					
Leu-Phe-Ala	**6**	**10**	**14**	**18**	**No transfer**
Phe-Leu-Phe-Ala	**7**	**11**	**15**	**19**	**1**
Ala-Phe-Leu-Phe-Ala	**8**	**12**	**16**	**20**	**2**
Leu-Ala-Phe-Leu-Phe-Ala	**9**	**13**	**17**	**21**	**3**

analyzed by synthesizing D-alanine-containing peptides. After TFE/AcOH/DCM treatment of a small sample of each batch of resin, the resulting compounds **4** and **5** were analyzed on chiral RP-HPLC for determining the loss of configuration. Examination of the spectra showed that the acylation at room temperature and under microwave occurs with less than 1% epimerization (Fig. 1).

Subsequent amino acids were incorporated by using DIC/HOBt coupling strategy. After Pip/DMF treatment, the linear depsipeptides **6**–**9** were cleaved from the solid support with a mixture of TFE/AcOH/DCM to keep the N-terminal Boc protection. The depsipeptides **10**–**13** were cyclized in solution by activation of the carboxylic acid function of the N-protected serine residue using BOP, $NaHCO_3$, DMF, and diluted conditions to favor intramolecular coupling (compounds **14**–**17**). After removing the Boc-protecting group, the cyclic depsipeptides (**18**–**21**) were subjected to the *O*–*N*-acyl migration reaction. They were dissolved and stirred in aqueous phosphate buffer at pH 7.4, and the *O*–*N* intramolecular acyl migration for each compound was followed by RP-HPLC and LC/MS analyses. Under these conditions after several days in the phosphate buffer (pH 7.4) at room temperature or at 80 °C or under microwave irradiation, no transfer could be observed on the depsitetrapeptide **18**, certainly due to the important cycle constraint of the final structure. Only a side reaction corresponding to ester bond hydrolysis occurred after 72 h.

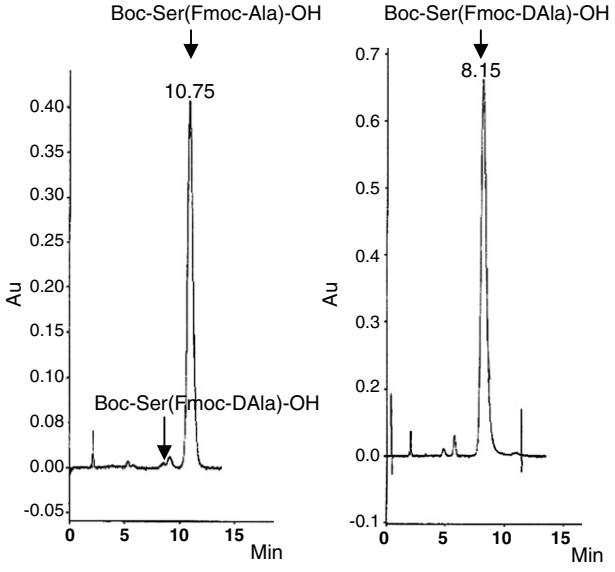


Figure 1. HPLC chromatogram (chiralcel OD-RH column; detection at 254 nm; elution under isocratic conditions: 55% B during 30 min at a flow rate of 1 mL/min. Solvent A = H_2O/0.1% TFA. Solvent B = CH_3CN/0.1% TFA) of the crude Boc(L)Ser(Fmoc-Ala)-OH (**4**) and the crude Boc(L)Ser(Fmoc-D-Ala)-OH (**5**).

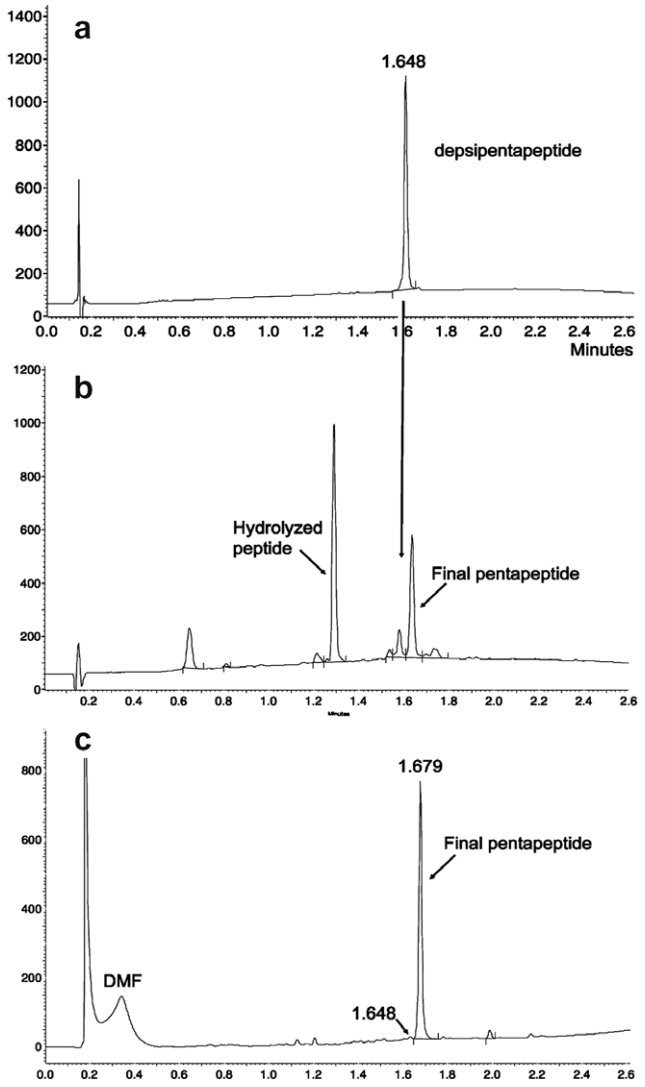


Figure 2. RP-HPLC chromatogram at 214 nm (a) of the cyclic depsipentapeptide; (b) after 72 h in phosphate buffer at room temperature; (c) after 3 h under organic conditions (DMF/piperidine).

After 72 h in phosphate buffer at room temperature, the depsipentapeptide **19** was completely consumed. However, both *O–N* acyl migration and undesired hydrolysis of the ester bond occurred (Fig. 2b). When using organic conditions DMF/Pip (80/20), no side reaction was observed and we also avoided the problem of low solubility of the starting material in phosphate buffer. In such conditions, the cyclic pentapeptide **1** was quantitatively obtained after 3 h (Fig. 2c).

It is worth noting that no hydrolysis was observed when *O–N*-acyl migration switch was applied to the linear depsipentapeptide (data not shown), clearly suggesting that hydrolysis was mainly promoted by a transition state cycle constraint.

For cyclic depsipeptides with higher cycle sizes (depsihexapeptide **20** and depsiheptapeptide **21**) the intramolecular transposition reaction was quantitative in phosphate buffer at room temperature after 12 h affording compounds **2** and **3**.

In conclusion, the *O–N* intramolecular acyl migration was successfully applied to the non-racemizing synthesis of cyclic peptides containing serine residue. We demonstrated the influence of the cycle size on *O–N*-acyl intramolecular transfer and the existence of a hydrolysis side reaction when the cycle constraints become significant. However, this side reaction can be totally suppressed under organic conditions. The strategy using more conformationally favorable cyclic depsitetrapeptide intermediates did not allow the difficult formation of cyclic tetrapeptides. Finally, the efficiency of this methodology will be applied to threonine-containing peptides and to the design of a new strategy for the synthesis of cyclic peptides on solid support.

Supplementary data

Experimental procedures, HPLC and mass spectrometry analyses. ^{1}H and ^{13}C NMR data of compounds **19** and **1**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.05.049.

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