Facile solid-phase synthesis of head-side chain cyclothiodepsipeptides through a cyclative cleavage from MeDbz-resin

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ABSTRACT

Head to side-chain cyclothiodepsipeptides were conveniently prepared through a cyclative cleavage using the MeDbz linker. Briefly, the peptide sequence was elongated on a MeDbz-Gly-ChemMatrix resin and reacted with 4-nitrophenyl chloroformate, followed by treatment with DIEA to render an activated cyclic N-acyl-N0-methylurea-resin. Removal of the Cys protecting group and further treatment with DIEA allowed the formation of the thiolactone with the concomitant release of the cyclic peptide from the resin.

Introduction

Recent years have witnessed a significant increase in the relevance of peptides in the pharmaceutical arena. Current data indicate that approximately 100 peptides are on the market and, more importantly, over 400 of these molecules are in clinical trials. Furthermore, the peptide therapeutic market is forecasted to experience a 10% annual growth in value, reaching approximately $47 billion by 2025.

This increase in peptide business has been possible due to the implementation of the Solid-Phase Peptide Synthesis (SPPS) strategy for both research and production purposes. One of the benefits of SPPS is that it can be applied for the preparation of mg to a multi kg scales. The use of mild chemistry involving the fluorenlymethyloxycarbonyl (Fmoc) group for the protection of the Nα-amino function, which is removed with 20% piperidine-DMF, and side-chain protecting groups and resins labile to trifluoroacetic acid (TFA) has allowed the automatization of this strategy. This can be considered a democratization of the use of peptides. In the past, only laboratories with well-trained chemists were able to synthesize peptides for research activities. Nowadays, a broad range of laboratories can prepare them. A further benefit of SPPS is that chemistry performed while the peptide remains anchored to the solid support often outperforms that undertaken in solution, thereby facilitating the preparation of complex peptides. This is often the case of cyclic peptides. The pseudo-dilution phenomenon associated with the solid-phase allows cyclization steps in solid-phase to occur with less formation of cyclooligomers—the main side products in this kind of reactions—than in solution. A special case is when the cyclization takes place concomitantly with the cleavage, namely Cyclative Cleavage. This case in point has commonly been illustrated by the preparation of diketopiperazines (DKPs), which are the smallest cyclic peptides. The cyclative cleavage of DKP is favored for the formation of the highly stable six-member ring. In fact, DKP often forms spontaneously after removal of the protecting group of the second amino acid and is consequently considered a side-reaction that jeopardizes the synthesis of lineal peptides. However, cyclative cleavage has been also used for other less facile cyclic peptides.

Cyclodepsipeptides are common in nature. Prominent examples include valinomycin, which was discovered in the 1960s, a head to tail peptide in which amide and ester bonds are alternated. More recently, a large number of head to side chain cyclodepsipeptides, mostly of marine origin, have been found to show biological activity of interest. In this case, a lactone is formed...
between the C-terminal carboxylic acid and the hydroxyl function on a Ser/Thr or other hydroxyl trifunctional amino acid.

In contrast, cyclodepsithiopeptides are less encountered in nature. Thiocoraline is a dimeric bicyclic peptide that contains two thioester moieties formed between the two C-terminal carboxylic acids and the two thiols of the Cys placed at the N-terminal. In addition, a disulfide bridge between two NMe-Cys in the middle of the molecule conforms the bicyclic structure.14 A new family of head to side chain cyclodepsithiopeptides has recently attracted the attention of the research community because these molecules are associated with increased resistance of bacteria to antibiotics.15

These quorum-sensing molecules are a family of cyclothiodepsipeptides termed auto-inducing peptides (AIPs). They all comprise seven to nine amino acids and contain a thiolactone macrocycle involving the C-terminal carboxylic acid and the thiol side-chain of Cys.

To date, the synthesis of these peptides has been achieved by two strategies. In both, the formation of the thiolactone takes place in solution. In the first one, the protected peptide is synthesized in solid-phase with a chlorotrityl chloride (CTC) resin, while Cys is protected with the methoxytrityl (Mmt) group, which is removed at the same time the protected peptide is cleaved from the resin. Cyclization then takes place in the presence of coupling reagents and final global deprotection with TFA affords the target peptide.16

The second strategy takes advantage of the same tools as those used for Native Chemical Ligation (NCL).17 Thus, the total unprotected peptide, which has a thioester at the C-terminal, is prepared in solid-phase and is purified to remove the excess of thiols. The cyclization then takes place smoothly in solution to directly afford the desired peptide.15b,c

Here we describe a convenient method in which the peptide is built on a solid-phase support, the protecting groups are removed, and a cyclative cleavage takes place to render the target peptide. For this purpose, we used the Fmoc-MeDbz (3) or 3-(fmoc-amino)-4-(methylamino) benzoic acid, coming from Me-diaminobenzoic acid) linker developed by Blanco-Canosa and Dawson 3-(fmoc-amino).18 This linker is anchored directly to a Gly-ChemMatrix resin, without an extra cleavable linker. After removal of the Fmoc group, peptide elongation takes place smoothly using diisopropylcarbodiimide (DIC) and Oxymapure as coupling cocktail. Once the peptide has been elongated, the peptide resin is reacted with p-nitrophenyl chloroformate in methylene chloride (DMC) (twice), followed by cyclization with 0.5 M diisopropylthelylamine (DIEA) in N,N-dimethylformamide (DMF) to render N-acyl-N'-methyl-benzimidazolinone (MeNbz) resin, which is an activated N-acylurea (N-acyl-N'-methylurea). In the original method for NCL,16 the peptide resin was subjected to TFA treatment, which removed the protecting group and cleaved N-acyl-N'-methylurea from the resin, because the Fmoc-Dbz was directly incorporated to a Rink-resin. After purification, this peptide was allowed to react with an N-terminal Cys unprotected peptide in the presence of thiols to perform the ligation. In this case, the treatment with TFA removed only the side-chain protecting group(s), thereby allowing the peptide to remain on the resin. Finally, the peptide was released from the resin by treatment with DIEA, which facilitated the nucleophile attack of the thiol of the Cys on the activated N-acyl-N'-methylurea moiety to render the thiolactone (Scheme 1).

To validate this strategy, two cyclothiodepsipeptides with 19 and 22 member rings, respectively, were synthesized (Fig. 1).

In both cases, the purity of the crude peptides was excellent (see SI). Given that only the cyclic peptide is cleaved from the resin if the peptide does not cycle, the corresponding lineal peptide remains in the resin and thus does not affect the purity of the final product. However, the presence of DIEA in the crude required to carry out a purification.