A novel dipeptidomimetic containing a cyclic threonine†

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An efficient and simple two-step procedure for the formation of hydroxy-Freidinger lactams is presented. The methodology allows assembly of the cyclic threonine motif (cThr) in solution and on solid support during conventional peptide synthesis.

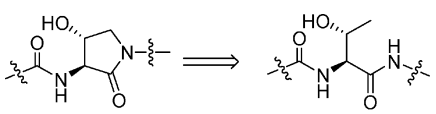
Recent years have witnessed a considerable growth in the number of peptide pharmaceuticals available, which currently cover a broad range of therapeutic indications.[1,2] Some of these peptides contain conformational restrictions through mainly cyclization[3] or introduction of backbone constraints,[4–6] which can overcome problems such as poor metabolic stability and low oral availability.[4–6] These constrained peptides have the potential of enhanced interaction with the receptor and better pharmacokinetic properties than their corresponding peptides.[7] Furthermore, these compounds very often adopt preferred conformations such as turns, which may enhance their binding to a biological target.[8] Ideally, these con formational constraints should maintain the appropriate topological arrangement of functional groups that are essential for recognition, while removing some of the unfavorable characteristics of peptides.[9,10] In this regard, the Freidinger lactam,[4,5,8] which consists basically of an alkyl side-chain bridge to the amide nitrogen of the $n-1$ amino acid, has been widely used for the preparation of a large number of peptidomimetics with biological activity.

Thr and Ser moieties play a key role in several biologically relevant processes. The hydroxyl is involved in biocatalysis as it is located in the active pockets of a variety of enzymes, popular examples are proteases,[11,12] or it acts as an acceptor for covalent modifications as for Ser/Thr directed kinases.[13] The carbohydrate part of O-type glycopeptides and glycoproteins is linked mostly to a Thr, or a Ser residue respectively.[14] Thr and Ser participate in the regulation of biological events through phosphorylation during signal cascades that control activation or deactivation of proteins.[15,16] Furthermore, Thr and Ser are components of a variety of biologically active peptides.

So far, several non-proteogenic amino acids have been introduced into peptides as Thr-mimetics. However, most feature major structural differences in steric and electronic properties when compared to the natural analogs. For instance, these compounds contain an additional α-substituent which result in a tertiary α-carbon like in 1-amino-2-hydroxy-cyclohexane carboxylic acid derivatives.[17–20] Apart from this, Thr has been used for the assembly of several cyclic systems into the peptidic backbone,[21–23] however, in all of these examples the hydroxyl either vanishes or remains part of the heterocycle formed. Since our strategy for finding biologically active peptidomimetics is based on nature-like structures,[24] the simple β-hydroxy-γ-lactam **1**[25] was considered as a cyclic Thr (**2**) motif (cThr) (Fig. 1).[26]

To the best of our knowledge, except for the synthesis of β-substituted α-amino-β-hydroxy-γ-lactam by Giese's group,[27,28] no efficient method has been reported for the introduction of this structure into peptides. Despite displaying a conventional Freidinger lactam containing a β-hydroxy functionality and its obvious structural relation to Thr, **1** has never been considered as a peptidomimetic element. Thus, its synthesis and assembly into peptides respectively, represent a challenge.

Our synthetic strategy is based on the addition of an amino group to epoxide **3**,[29,30] which can be obtained as a mixture of the *threo*- and *erythro*-isomers in a ratio of 4/1 from Met in five steps. Initial attempts to carry out the epoxide opening reaction at reflux in the presence of $LiClO_4$[31] always proceeded incompletely and gave maximum yields of 33%. Finally, performing the reaction in a microwave reactor showed acceptable efficiency for this conversion. Moreover, from a screening of commonly used activators[32] for additions to epoxides only $LiClO_4$ and $Ca(OTf)_2$[33,34] showed the formation of the desired product as shown by TLC. In contrast, no product formation was detected with $Yb(OTf)_3$, $Ti(OiPr)_4$, $BF_3{\cdot}OEt_2$ or $Sc(OTf)_3$. On the basis of these findings, the reaction conditions were optimized using $Ca(OTf)_2$ to obtain excellent yields (76–81%) of the secondary amines **4** with distinct amino acid esters (Scheme 1). Ring closure was subsequently achieved by heating at reflux in toluene to give the desired lactam. Dipeptidomimetics **5a–d** were isolated in yields of 76–98% from the corresponding amino acid esters, whereas the *threo*-isomers were separated by performing conventional column chromatography in mixtures of hexane–EtOAc.



1 2

Fig. 1 Cyclic hydroxylactam **1** as a natural-like Thr-mimetic.

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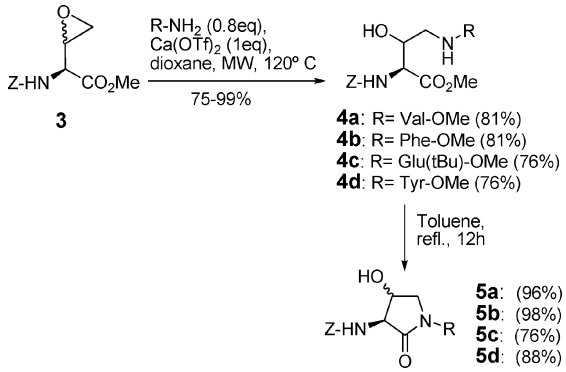
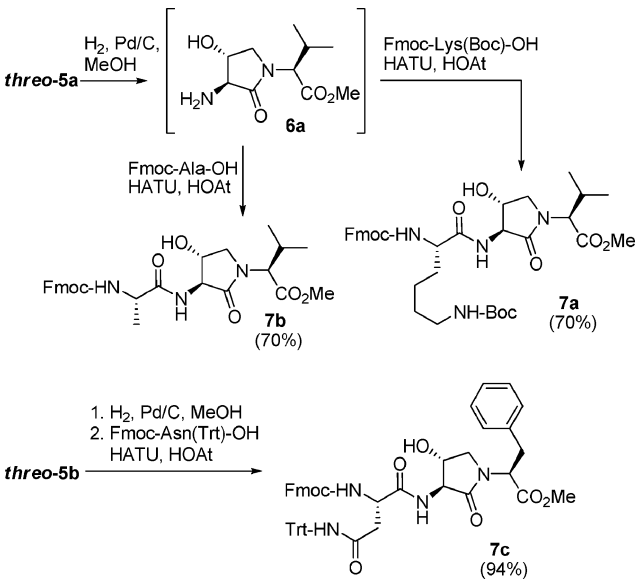


Scheme 1 Two-step sequence for the formation of cThr **5**.

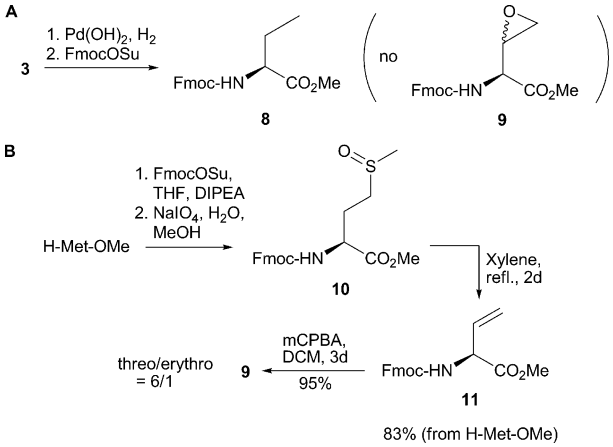
The successful assembly of the hydroxylactam into longer peptides was further demonstrated, by coupling of an additional amino acid to ***threo*-5a**, and ***threo*-5b** respectively (Scheme 2). First of all, the Z group was cleaved by hydrogenolysis on Pd/C, then the peptide bond formation was achieved using HATU/HOAt[35] as coupling reagents to give tripeptidomimetics **7a–c**.

After finding efficient conditions for the assembly of cThr in solution, we sought to develop a methodology to carry out the epoxide opening reaction on solid support as a type of "coupling sequence" during peptide synthesis. Since the Z group is not appropriate as temporary protection on solid phase, we synthesized the Fmoc protected epoxide **9** (Scheme 3A). However, following a procedure employing $Pd(OH)_2$ as catalyst with **3**,[36] which was used for the removal of the Z group of α-substituted derivatives of **3** without affecting the epoxide, only amino butyric acid **8** was detected after treatment with FmocOSu.

Therefore, we followed a synthetic route starting from H-Met-OMe in analogy to the procedure described for **3** (Scheme 3B). After introducing the Fmoc group, the sulfur was oxidized by $NaIO_4$ to give **10**. Subsequently, pyrolysis to



Scheme 2 Tripeptidomimetics **7a–c** obtained from ***threo*-5a** and ***threo*-5b**.



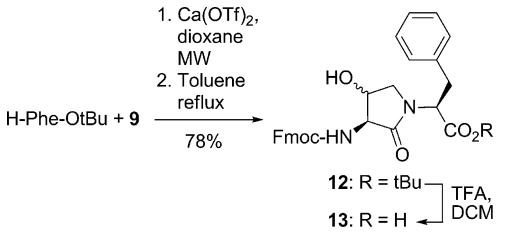
Scheme 3 Synthesis of Fmoc protected epoxide **9**.

obtain Fmoc protected vinyl glycine **11** was carried out by heating at reflux in xylene. Epoxidation with mCPBA finally led to **9** in a diastereomeric ratio of 6/1 (*threo*/*erythro*). The optimized synthetic pathway gave access to the Fmoc protected epoxide building block **9** in an excellent overall yield of 80% on a multigram scale.

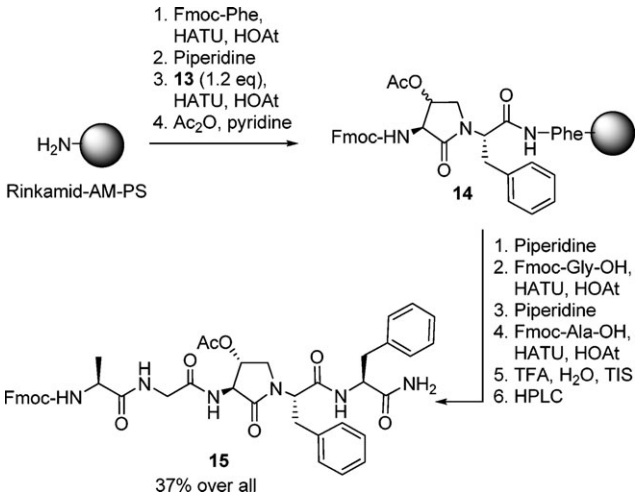
In the attempt to develop a methodology for the construction of the cyclic threonine building block on solid-phase, the mixture of epoxides **9** was used for the addition reaction of the amino group of Phe bound to Rink amide-aminomethyl-polystyrene (AM-PS) resin. However, it was not possible to achieve a complete conversion, even when using 10 equiv. of epoxide. The maximum loading determined after the reaction was 0.25 mmol g^{-1} which corresponds to a 40% yield. Given the low solubility of $Ca(OTf)_2$ in dioxane, we hypothesized that employing a cosolvent would improve the outcome of the reaction. However, addition of 5–10% of either DMF or DMSO to obtain a clear solution did not enhance the reaction. We consider that these findings indicate that the mechanism for the reaction of an epoxide with an amine in the presence of $Ca(OTf)_2$ is based on a heterogeneous activation. Neither did the attempts to carry out $LiClO_4$-mediated epoxide-opening on solid support lead to an improvement.

Therefore, we turned to the dipeptide mimetic building block, which was previously synthesized in solution, for the assembly of the hydroxylactam. Using H-Phe-OtBu and **9** the Fmoc protected tBu ester **12** was obtained following the procedure described above (Scheme 4).

In contrast to the Z-protected derivatives the two diastereoisomers of **12** were not separable by column chromatography as a result of no observable difference in R_f-values. Therefore, **12** was used as mixture for peptide coupling. Acidic hydrolysis



Scheme 4 Synthesis of dipeptide mimetic building block **13**.



Scheme 5 Solid-phase synthesis of pentapeptide mimetic **15** employing **13**.

of the tBu ester with TFA in DCM led to acid **13** which was directly activated without purification and coupled to resin bound Phe (Scheme 5). Subsequently, for the capping of free amino groups, the resin was incubated with Ac_2O in pyridine which acetylated the β-hydroxyl as well (**14**). Following a standard Fmoc procedure the peptide sequence was elongated by Gly and Ala. The Fmoc-protected pentapeptide mimetic **15** was obtained by cleavage with TFA containing 2.5% of each TIS and water and finally isolated by RP-HPLC in a 37% overall yield.

Pentapeptide mimetic **15** was fully characterized by NMR spectroscopy (see ESI†). Further ROESY experiments showed several NOE contacts from Phe^5 to the hydroxylactam. These observations indicate that the C-terminal part of **15** exhibits a turn-type conformation that is stabilized by the lactam ring.

In conclusion, with the objective to develop a synthetic access to a nature-like constrained Thr motif, we developed an efficient synthesis for β-hydroxy-γ-lactam from easily obtainable amino acid epoxides **3** and **9**. The epoxide opening reaction with an amino acid amine as the key-step was optimized to good yields employing a microwave reactor and $Ca(OTf)_2$ as activator. The methodology presented here allowed the assembly of this motif in solution and its subsequent incorporation as a dipeptide building block on solid support as demonstrated by the syntheses of several examples. Structural analysis of pentapeptide mimetic **15** by NMR experiments gives strong evidence of the occurrence of a preferred conformation. Ongoing research includes the biological evaluation as well as detailed structural analysis of peptides containing the cThr motif.

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