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Investigating the scope of pseudoproline assisted peptide cyclization

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ABSTRACT

The cyclization of linear peptides from six to nine amino acids in length and containing between two and four pseudoproline turn inducers derived from serine or threonine was investigated to determine the effect of peptide length, amino acid composition and spacing between the pseudoproline residues on macrocyclization yield.

1. Introduction

Cyclic peptides are of significant interest across fields ranging from medicinal chemistry to materials science. They exhibit a broad range of biological activity and have improved proteolytic stability relative to their linear counterparts. The conformational constraints of their macrocyclic structure, when chosen correctly, can provide selectivity and potency in binding to a therapeutically relevant target. However, the head-to-tail cyclization of a linear peptide precursor is frequently a slow and low-yielding reaction, because it requires the linear peptide to adopt an entropically unfavoured conformation before the product is formed. Common side reactions include epimerisation of the *C*-terminal amino acid and the formation of linear and cyclic oligomers.[1] This has lead to the search for new methods to facilitate peptide cyclisation.[1,2] The incorporation of pseudoprolines (derived from serine (Ser), threonine (Thr) or cysteine (Cys) residues) bearing *gem*-dimethyl substituents ($\Psi^{Me,Me}$pro) into a linear peptide results in a curved peptide shape that favours macrocyclization as a result of the *cis*-amide conformations that are predominant N-terminal to the $\Psi^{Me,Me}$pro residues.[3] While $\Psi^{Me,Me}$pros were originally employed as tools to prevent aggregation and improve yields in solid phase peptide synthesis,[4] for which they have found extensive use,[5] there are now a number of examples in which $\Psi^{Me,Me}$pro derivatives have been employed to successfully facilitate the head-to-tail cyclisation of peptides.[6–11] We have previously reported the use of $\Psi^{Me,Me}$pro derived from Thr and Ser as removable turn inducers to aid the cyclization of a number of model tetra- and hexa-peptides.[6,7] In these highly sterically hindered model peptides, we found that the incorporation of more than one $\Psi^{Me,Me}$pro residue increased cyclisation yields and that positioning a $\Psi^{Me,Me}$pro residue at the *C*-terminal position prevented undesired epimerisation. In addition we have shown that $\Psi^{Me,Me}$pro turn inducers derived from Thr or Cys can increase the rate of reaction and result in improved cyclisation yields for the natural products mahafacyclin B[10] and cyclogossine,[11] respectively. Our interest in exploring the scope of $\Psi^{Me,Me}$pro-assisted peptide cyclisation lead to the current studies, in which we have altered (i) the length of the linear peptide precursors, (ii) the spacer amino acids, (iii) the type of $\Psi^{Me,Me}$pro (from Ser or Thr) and (iv) the spacing between the $\Psi^{Me,Me}$pro residues, to explore the effect that each of these has on the macrocyclization reaction.

2. Results and discussion

Given that our initial studies had found that the cyclisation of a linear hexapeptide with Thr derived $\Psi^{Me,Me}$pro residues placed at every second amino acid (linear precursor **1**) proceeded in near quantitative yield,[6] we first designed a range of hexapeptide precursors. Linear peptide precursors (Fig. 1) incorporating $\Psi^{Me,Me}$pro residues derived from Thr (**1**, **2**), or Ser (**3**, **4**) at every second amino acid were prepared, together with a second set of linear hexapeptides in which the $\Psi^{Me,Me}$pro residues were spaced further apart (linear precursors **5**–**7**) in order to probe the effect of $\Psi^{Me,Me}$pro position on the cyclisation reactions. Similarly, a pair of linear octapeptides **8** and **9**, in which the $\Psi^{Me,Me}$pro residues were placed at every second amino acid, together with octapeptide analogue **10** and **11** in which the $\Psi^{Me,Me}$pro residues were placed at every fourth amino acid position were designed, as was a pair of linear nonapeptides **12** and **13**, in which the $\Psi^{Me,Me}$pro residues were placed

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1: H_2N-Val-Thr($\Psi^{Me,Me}$)-Val-Thr($\Psi^{Me,Me}$)-Val-Thr($\Psi^{Me,Me}$)-OH
2: H_2N-Phe-Thr($\Psi^{Me,Me}$)-Phe-Thr($\Psi^{Me,Me}$)-Phe-Thr($\Psi^{Me,Me}$)-OH
3: H_2N-Val-Ser($\Psi^{Me,Me}$)-Val-Ser($\Psi^{Me,Me}$)-Val-Ser($\Psi^{Me,Me}$)-OH
4: H_2N-Phe-Ser($\Psi^{Me,Me}$)-Phe-Ser($\Psi^{Me,Me}$)-Phe-Ser($\Psi^{Me,Me}$)-OH
5: H_2N-Phe-Phe-Thr($\Psi^{Me,Me}$)-Phe-Phe-Thr($\Psi^{Me,Me}$)-OH
6: H_2N-Val-Phe-Thr($\Psi^{Me,Me}$)-Val-Phe-Thr($\Psi^{Me,Me}$)-OH
7: H_2N-Phe-Val-Thr($\Psi^{Me,Me}$)-Phe-Val-Thr($\Psi^{Me,Me}$)-OH
8: H_2N-Val-Thr($\Psi^{Me,Me}$)-Val-Thr($\Psi^{Me,Me}$)-Val-Thr($\Psi^{Me,Me}$)-Val-Thr($\Psi^{Me,Me}$)-OH
9: H_2N-Phe-Thr($\Psi^{Me,Me}$)-Phe-Thr($\Psi^{Me,Me}$)-Phe-Thr($\Psi^{Me,Me}$)-Phe-Thr($\Psi^{Me,Me}$)-OH
10: H_2N-Val-Val-Val-Thr($\Psi^{Me,Me}$)-Val-Val-Val-Thr($\Psi^{Me,Me}$)-OH
11: H_2N-Phe-Phe-Phe-Thr($\Psi^{Me,Me}$)-Phe-Phe-Phe-Thr($\Psi^{Me,Me}$)-OH
12: H_2N-Phe-Phe-Thr($\Psi^{Me,Me}$)-Phe-Phe-Thr($\Psi^{Me,Me}$)-Phe-Phe-Thr($\Psi^{Me,Me}$)-OH
13: H_2N-Phe-Val-Thr($\Psi^{Me,Me}$)-Phe-Val-Thr($\Psi^{Me,Me}$)-Phe-Val-Thr($\Psi^{Me,Me}$)OH

Fig. 1. Linear peptide precursors **1**–**13**.

at every third amino acid. In all cases a $\Psi^{Me,Me}$pro residue was positioned at the *C*-terminus of the linear precursor to avoid issues with epimerisation of this amino acid during the cyclisation reaction. Valine (Val) and phenylalanine (Phe) were chosen as the spacer amino acids, with Val providing a β-branched and hence sterically challenging spacer residue, whereas Phe is also a sterically demanding residue, but with the bulk further away from the peptide backbone.

The synthesis of the required linear peptides **1**–**13** was achieved by standard solid phase peptide synthesis (SPPS), using Fmoc/HBTU chemistry on a PS3 automated peptide synthesizer. The $\Psi^{Me,Me}$pros were incorporated as the preformed dipeptides already containing the modified Ser or Thr residues. The 2-chlorotrityl chloride resin was chosen as the solid support to enable cleavage of the peptides from the solid phase using a mildly acidic mixture of hexafluoroisopropanol, trifluoroethanol and dichloromethane (1:2:7 v/v/v), while leaving the $\Psi^{Me,Me}$pro groups intact.

We have previously found pentafluorophenyl diphenylphosphinate (FDPP) to give consistently high cyclisation yields,[6,7] thus the linear peptides **1**–**13** were treated with FDPP and *N*,*N*-diisopropylethylamine (DIPEA) in DMF at a concentration of 5 mM for three days, and the corresponding cyclic products **14**–**24** (Fig. 2) were obtained after purification by HPLC (Table 1). As expected, cyclisation of hexapeptides **1**–**4**, each of which contains three $\Psi^{Me,Me}$pro residues, gave excellent yields (90–99%) of the cyclic peptide products **14**–**17**, suggesting that for a hexapeptide, three $\Psi^{Me,Me}$pro turn inducers provide an ideal conformation for cyclisation and indicating that the type of $\Psi^{Me,Me}$pro and the nature of the 'spacer' amino acid has little effect on the cyclisation yields. Therefore, Thr derived $\Psi^{Me,Me}$pro residues were employed in the remaining reactions.

To further probe the effect of the spacing between $\Psi^{Me,Me}$pro residues on cyclisation, the hexapeptides **5**–**7**, each of which contains only two $\Psi^{Me,Me}$pro residues, were cyclised. The substantially lower cyclisation yields obtained for the formation of cyclic hexapeptides **18**–**20** (20–35%) suggest that, for sterically congested hexapeptides, spacing the two $\Psi^{Me,Me}$pro residues three amino acids apart is not sufficient to provide a favourable conformation for cyclisation to occur, whereas the closer spacing and incorporation of a third $\Psi^{Me,Me}$pro residue as in linear precursors **1**–**4**, provides the appropriate peptide backbone conformation required for efficient cyclisation.

Given that the results above, together with our previous studies on tetrapeptide cyclisation suggested that spacing the $\Psi^{Me,Me}$pro residues at every second amino acid resulted in optimised cyclisation yields, we next examined the cyclisation of linear

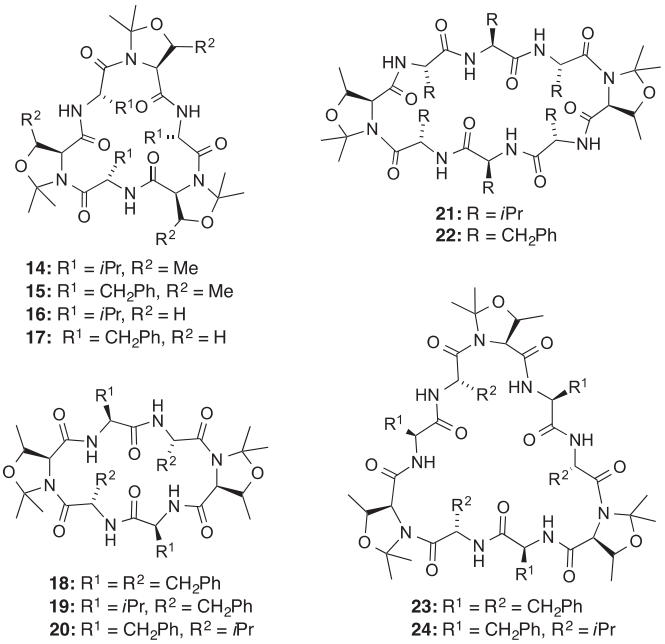

Fig. 2. Structures of the cyclic peptides **14**–**24**.

Table 1
Cyclisation yields for the linear peptides

Linear peptide precursor	Cyclic peptide product	Yield (after RP-HPLC purification)
1	**14**	99%
2	**15**	92%
3	**16**	95%
4	**17**	90%
5	**18**	25%
6	**19**	35%
7	**20**	20%
8	—	—
9	—	—
10	**21**	30%
11	**22**	65%
12	**23**	77%
13	**24**	65%

octapeptides **8** and **9**, each of which contains four $\Psi^{Me,Me}$pro residues. Using our standard cyclisation conditions (FDPP, DMF, DIPEA, 0.005 M) we were unable to obtain any of the corresponding cyclic peptide products. Depsite attempts using alternate coupling reagents (HBTU, HATU, PyBOP, EDC), solvents (CH_3CN) and concentrations (0.001 M), we were unable to obtain any cyclised peptide from these precursors and in most cases, mixtures of starting material and linear oligomers were observed by LC–MS. We postulated that this might be the result of adding too much 'turn' to the linear peptide backbones such that they adopted a helical conformation. This is supported by our previous observation of an NOE between the α-protons of the *N*-and *C*-terminal residues of hexapeptide **1**, indicating that these are in close proximity and suggesting that lengthening the peptide backbone might result in an overlap of the terminal amino acids.[2] Unfortunately, attempts to elucidate whether or not this overlap occurs in **8** and **9** using 2D-NMR techniques were complicated by the presence of multiple slowly interconverting conformers of these linear peptides. We therefore prepared the linear octapeptides **11** and **12**, each containing only two $\Psi^{Me,Me}$pro residues spaced at every fourth amino acid, to reduce the total 'turn' of the peptide backbones. Pleasingly, when treated under our standard cyclisation conditions, **11** and **12**