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Synthesis of All-L Cyclic Tetrapeptides Using Pseudoprolines as Removable Turn Inducers

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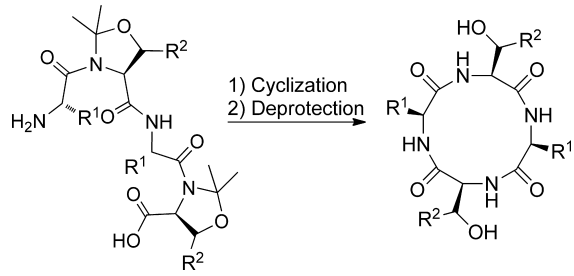
ABSTRACT



Cyclic tetrapeptides have generated great interest because of their broad-ranging biological properties. In order to synthesize these highly strained 12-membered cyclic compounds, a cyclization strategy using pseudoprolines as removable turn inducers has been developed. The pseudoproline derivatives induce a *cisoid* amide bond in the linear peptide backbone which facilitates cyclization. After cyclization, the turn inducers can be readily removed to afford cyclic tetrapeptides containing serine or threonine residues.

The significant biological activity displayed by cyclic peptides, together with their potential as biomaterials,[1] makes these compounds of great interest. In particular, cyclic tetrapeptides are considered to be naturally occurring "privileged structures"[2] as they are able to present substituents in a spatially well-defined manner and display a variety of biological activities, including histone deacetylase inhibition,[3] tyrosinase inhibition,[4] antimitogenic activity,[5] antitumor activity,[6] and antimalarial acivity.[7] However, the synthesis of head-to-tail cyclic tetrapeptides is generally difficult as a result of their highly constrained 12-membered ring structure.[8] Cyclization of a linear precursor frequently yields the cyclic dimer (a cyclic octapeptide), rather than the required cyclic monomer,[9] indicating that the primary hurdle to cyclization is bringing the *N*- and *C*-termini of the peptide into close spatial proximity. This can be attributed to the strong π-character of peptide bonds, which tend to adopt a *transoid* conformation, resulting in an extended linear peptide conformation.

The synthesis of all-L cyclic tetrapeptides is particularly challenging; almost all of the cyclic tetrapeptides synthesized to date contain either a mixture of L- and D-amino acids,

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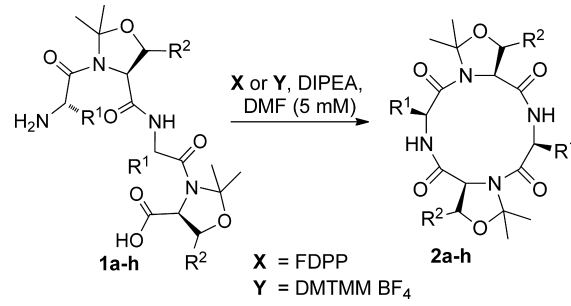
unnatural amino acids or at least one Pro residue.[9−12] While there have been several reported syntheses of all-L cyclic tetrapeptides containing two Pro residues,[4,13] most of these have since been identified to yield the cyclic octapeptides,[10] and the most recent example provided the all-L cyclic tetrapeptide cyclo(Leu-Pro-Leu-Pro) in only 5% yield after optimization of the cyclization conditions.[9] Recently, the use of a photolabile ring-contraction auxiliary to synthesize a small library of all-L cyclic tetrapeptides, with yields ranging from 4 to 29%, has been reported.[14,15] However, while this two-step cyclization/ring contraction procedure provides all-L cyclic tetrapeptides from linear precursors in which Gly is the *C*-terminal amino acid, when a chiral center is present at the *C*-terminus significant epimerization is observed during the cyclization step.

We have recently reported the use of pseudoprolines ($\Psi^{Me,Me'}$pro) derived from threonine residues as removable turn-inducers to facilitate head-to-tail peptide cyclization.[16,17] The incorporation of these oxazolidine derivatives into a linear peptide favors *cis*-amide conformations at the amide bonds *N*-terminal to the $\Psi^{R,R'}$pro residues.[18−20] This facilitates cyclization by favoring a conformer in which the *N*- and *C*-termini of the peptide are spatially proximate and was found to provide significantly increased head-to-tail cyclization yields of hexa- and heptapeptides when compared to those obtained for analogous peptides with standard Ser/Thr protecting groups. After cyclization the $\Psi^{Me,Me'}$pro "protecting" groups were readily removed by treatment with acid to yield cyclic peptides devoid of turn inducers.[16,17] To further demonstrate the utility of this methodology, we now report the synthesis of a small library of all-L cyclic tetrapeptides that do not contain turn-inducers, facilitated by the use of both serine and threonine derived $\Psi^{Me,Me'}$pro residues.

During our previous studies on the cyclization of hexapeptides containing alternating Val and Thr residues, we observed that cyclization yields were significantly improved if more than one $\Psi^{Me,Me'}$pro turn-inducer was incorporated into the linear precursor and that the positioning of a $\Psi^{Me,Me'}$pro residue as the *C*-terminal amino acid prevented epimerization from occurring during the cyclization reaction.[16] We therefore chose the linear tetrapeptides **1a**−**h** (Scheme 1), which each incorporate two $\Psi^{Me,Me'}$pro residues to investigate the utility of this methodology for the synthesis

Scheme 1. Cyclization of Linear Tetrapeptides **1a**−**h**



of all-L cyclic tetrapeptides. Both Ser (**1a**−**d**) and Thr (**1e**−**h**) derived $\Psi^{Me,Me'}$pro residues were incorporated into the linear peptides, alternating with "spacer" amino acids (Phe, Ile, Leu, and Val), and in all cases, a $\Psi^{Me,Me'}$pro residue was positioned at the *C*-terminus of the linear precursor.

The required linear tetrapeptides **1a**−**h** were prepared using standard Fmoc solid-phase peptide synthesis (SPPS) (HBTU/DIPEA couplings) by loading and coupling of the commercially available dipeptides containing the modified Ser or Thr residues. The 2-chlorotrityl chloride resin was used as the solid support to enable cleavage of the linear peptides from the solid phase, without removal of the side chain protecting groups; optimized conditions involved treatment with a mixture of hexafluoro-2-propanol, trifluoroethanol, and dichloromethane (1:2:7 v/v/v).

The ^{1}H and ^{13}C NMR spectra of the linear peptides **1a**−**d** and **1f**−**h** exhibit predominantly a single set of resonances (>90% peak intensity relative to other resonances as estimated by integration of the ^{1}H spectra), indicating that these tetrapeptides predominantly adopt a single conformation in CD_3CN solution. In the case of **1e**, a major and minor set of resonances in a 7:3 ratio are clearly observed in both the ^{1}H and ^{13}C NMR spectra. To identify the structure of the major tetrapeptide conformers, compound **1c** was investigated using a combination of 1D and 2D NMR experiments. The major conformer was determined to be that in which the amide bonds preceding both the $\Psi^{Me,Me'}$pro residues adopt a *cisoid* conformation on the basis of the typical cross-peaks observed by 2D NMR ROESY and NOESY experiments (i.e., $\alpha H_{i-1}-\alpha H_i$ crosspeaks) (Figure 1) that reflect the spatial

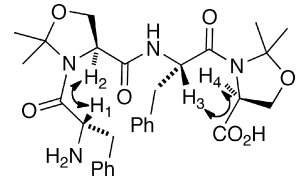


Figure 1. Structure of **1c** with the observed NOEs indicated by double-headed arrows.

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proximity of the αH_{i-1} and αH_i protons in the *cisoid* form.[21] The lack of any observable NOE cross peak between αH_2 and αH_3 suggests that the remaining amide bond adopts a *transoid* configuration.

Further evidence that this "*cis,trans,cis*" conformer is the major one adopted by the linear peptides **1a**−**h** was obtained upon derivitization of **1g** to provide the Cbz and Me ester-protected tetrapeptide **3** (Figure 2). As was observed for

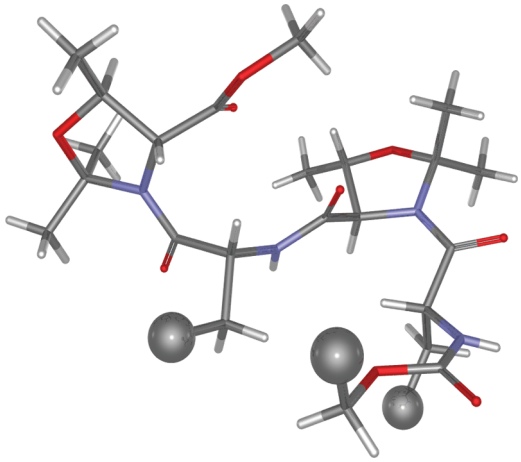

Figure 2. Perspective drawing of the crystal strucutre of **3**. Three phenyl rings are represented with gray balls for clarity.

1a−**h**, the ^{1}H and ^{13}C NMR spectra of **3** exhibit predominantly a single set of resonances, indicating that **3** adopts a single conformation in DMSO-d_6 solution. A single-crystal X-ray diffraction structure determination of **3** indicated that in the solid state the amide bonds also adopt an alternate "*cis,trans,cis*" conformation with torsional angles of 4.0(4)°, −178(3)°, and 7.1(5)°, respectively, from the *N*- to *C*-terminus.

Cyclization of **1a**−**h** was initially performed using pentafluorophenyl diphenylphosphinate (FDPP) as the coupling reagent, as we have previously found this to give consistently higher cyclization yields than other standard coupling reagents (e.g., HATU, PyBOP). Thus, treatment of **1a**−**h** with FDPP and *N*,*N*-diisopropylethylamine (DIPEA) under high dilution (0.005 M) in DMF for 3 days gave the corresponding cyclic products **2a**−**h** (Scheme 1) in 24−47% yield (Table 1). In all cases, the linear precursors containing Ser-derived $\Psi^{Me,Me'}$pro residues (**1a**−**d**) cyclized in higher yield than the analogous compounds containing Thr-derived $\Psi^{Me,Me'}$pro residues (**1e**−**h**), suggesting that the extra steric bulk imparted by the additional methyl group at the β-position of the threonine derivatives hinders the cyclization. However, for both the Ser- and Thr-containing compounds, the cyclization yields are pleasingly high when compared to those previously reported for the synthesis of cyclic tetrapeptides.[9,13] In particular, the cyclization yields for compounds **1b** (38%) and **1f** (27%) can be directly compared to that reported previously for H_2N-Leu-Pro-Leu-Pro-OH (5%),[9] indicating that both the Ser- and Thr-derived $\Psi^{Me,Me'}$pro residues are more effective turn inducers than Pro itself. Notably, in contrast to reported cyclizations of Pro containing tetrapeptides, no products of cyclodimerization were observed in any of the reactions performed in this study. In addition, analysis of the ^{1}H and ^{13}C NMR spectra of cyclic peptides **2a**−**f** and **2h** indicates that all of these compounds have C_2 symmetry, confirming that the cyclization reactions occur with no epimerization of the *C*-terminal amino acid. While compound **2g** does not display the expected C_2 symmetry, further reactions on this compound indicate that this is due to the presence of a single asymmetric conformer in solution rather than to loss of stereochemical integrity during the cyclization reaction (see below).

Table 1. Cyclization Yields

R^1, R^2	substrate	product	yield with X[a] (%)	yield with Y[a] (%)
R^1 = Ile, R^2 = H	**1a**	**2a**	47	73
R^1 = Leu, R^2 = H	**1b**	**2b**	38	66
R^1 = Phe, R^2 = H	**1c**	**2c**	45	60
R^1 = Val, R^2 = H	**1d**	**2d**	40	60
R^1 = Ile, R^2 = Me	**1e**	**2e**	24	20
R^1 = Leu, R^2 = Me	**1f**	**2f**	27	31
R^1 = Phe, R^2 = Me	**1g**	**2g**	40	57
R^1 = Val, R^2 = Me	**1h**	**2h**	35	20

[a] Yields reported are of RP-HPLC-purified products. X = FDPP; Y = DMTMM BF_4.

In attempts to further improve the cyclization yields, a second series of cyclization experiments were performed using the same linear tetrapeptides but replacing FDPP with the newer coupling reagent 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM BF_4). This reagent has recently been found to provide good coupling results (high yields, low epimerization) for a wide range of amino acid and peptide substrates, including fragment coupling and cyclization procedures.[22] Treatment of **1a**−**h** with DMTMM BF_4, under the same conditions as described for FDPP above, gave the corresponding cyclic tetrapeptides **2a**−**h** in 20−73% yield (Table 1). Using this method the yields for **2e**−**h**, containing Thr-derived $\Psi^{Me,Me'}$pro residues, were similar to those obtained using FDPP (20−57% using DMTMM BF_4, 24−40% using FDPP). However, significant enhancements in cyclization yield were observed for **2a**−**d** (60−73% using DMTMM BF_4, 40−47% using FDPP) containing Ser-derived $\Psi^{Me,Me'}$pro residues.

Removal of the pseudoproline "protecting" groups from the tetrapeptides **2a**−**h** was achieved upon treatment with a standard peptide cleavage cocktail solution of TFA/triiso-

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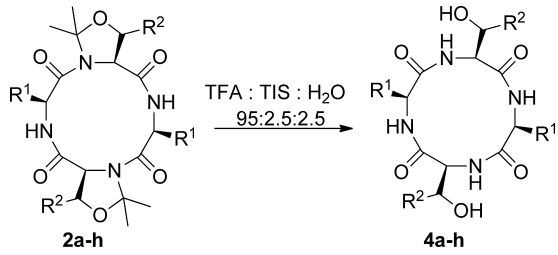
propylsilane (TIS)/water (95:2.5:2.5 v/v/v). The deprotection reaction is slow (4 d), but analysis of the reaction mixtures by LCMS indicated that after this time, complete conversion to the Ser- and Thr-containing cyclic peptides **4a**−**h** occurred. Cyclic peptides **4a**−**h** were obtained in moderate to high yield after purification by RP-HPLC (Scheme 2). The

Scheme 2. Removal of the Pseudoproline "Protecting" Groups



large variation in yields (30% to quant, Table 2) for the deprotection reaction is attributable to the low solubility of some of these compounds under the conditions required for RP-HPLC.

In all cases, the ^{1}H and ^{13}C NMR spectra of the deprotected cyclic tetrapeptides **4a**−**h** indicate that the compounds have C_2 symmetry, confirming that the two-step cyclization, deprotection sequence proceeds without epimerization, providing cyclic tetrapeptides devoid of turn-inducers.

In conclusion, despite their potential in therapeutic and materials applications the use of cyclic tetrapeptides has been limited, largely due to the synthetic difficulties enountered when preparing such highly strained 12-membered cyclic structures. The present study reports the synthesis of a number of serine and threonine containing all-L cyclic

Table 2. Deprotection Yields

R^1, R^2	substrate	product	yield[a] (%)
R^1 = Ile, R^2 = H	**2a**	**4a**	80
R^1 = Leu, R^2 = H	**2b**	**4b**	79
R^1 = Phe, R^2 = H	**2c**	**4c**	quant
R^1 = Val, R^2 = H	**2d**	**4d**	39
R^1 = Ile, R^2 = Me	**2e**	**4e**	30
R^1 = Leu, R^2 = Me	**2f**	**4f**	quant
R^1 = Phe, R^2 = Me	**2g**	**4g**	53
R^1 = Val, R^2 = Me	**2h**	**4h**	50

[a] Yields reported are of RP-HPLC-purified products.

tetrapeptides in overall yields of up to 60% from the linear precursor. This was achieved by employing $\Psi^{Me,Me'}$pro "protecting" groups to induce linear tetrapeptide conformations amenable to cyclization. Deprotection of the $\Psi^{Me,Me'}$pro groups was carried out under standard conditions to afford cyclic tetrapeptides devoid of turn inducers. Research is currently underway to extend this methodology to allow the synthesis of a wider range of cyclic peptides, including those that lack Ser and Thr residues.

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Supporting Information Available: Experimental procedures and spectral data for all compounds. X-ray crystallographic data for compound **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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