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Pseudo-prolines (ΨPro): direct insertion of ΨPro systems into cysteine containing peptides

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Abstract—The direct conversion of cysteine (Cys) containing peptides into conformationally constrained pseudo-proline (ΨPro) derivatives by intraresidual *N*,*S*-acetalisation has been achieved. This post-synthetic modification represents a versatile tool in structure–activity studies of bioactive peptides as exemplified for the immunosuppressive cyclosporine A (CsA) analogue [D-Cys]^{8}CsA.

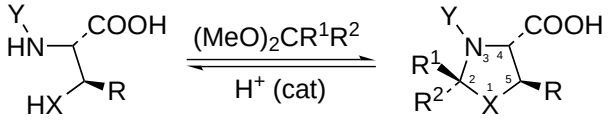
The pseudo-proline (ΨPro) concept introduced some years ago as a solubilizing protection technique in peptide synthesis[1] has recently been extended as a versatile tool in structure–activity relationship studies of bioactive peptides,[2] as well as in molecular recognition.[3] According to this approach, serine, threonine or cysteine derivatives are converted into the corresponding five-membered ring systems (oxazolidines or thiazolidines, ΨPro) by intraresidual *N*,*O*- or *N*,*S*-acetalisation (Scheme 1).[4]

For use in peptide synthesis, the incorporation of ΨPro systems is effectively achieved by transforming the *N*-protected dipeptide derivatives (Y = *N*-protected Xaa_{i-1}, Xaa_i = Ser or Thr) to the corresponding ΨPro-containing building blocks and subsequent coupling to the growing peptide chain. The direct insertion of ΨPro systems into peptides of higher structural complexity has been recently achieved for Thr- or Ser-containing peptides.[5] Due to substantial differences in the formation and physico-chemical properties of thiazolidines and oxazolidines, the incorporation of Cys-derived ΨPro systems into native peptides was only feasible so far by total chemical synthesis via its *N*-unprotected derivative Cys($\Psi^{R1,R2}$pro) in stepwise peptide synthesis (Y = H, Scheme 1). With the aim of extending the ΨPro concept as a tool in biomolecular recognition studies, we elaborate here the direct insertion of ΨPro systems into cysteine containing peptides.

As prototype reaction[6] C2-mono- and disubstituted ΨPro systems were inserted into the *N*-protected dipeptide ester Fmoc-Ala-CysOMe (**I**) by intraresidual acetalisation applying catalytic amounts of *para*-toluenesulfonic acid (PTSA, Scheme 2).

After optimisation, the target compounds **IIa** and **IIb** were obtained in 73 and 84% yields, respectively. The two diastereoisomers of **IIb** (ratio 40:60), resulting from the formation of a new chiral center at the C-2 position, could be separated by chromatography on silica gel.

For probing the general application of the established procedure, we applied this post-synthetic modification as a tool in our ongoing structure–activity studies of cyclosporine A (CsA).[5,7] To this end, the immunosuppressive CsA analogue D-Cys^{8}-CsA^{8} (**III**) was used as a structurally complex template for the direct insertion of ΨPro systems (Scheme 3).



Scheme 1. Conversion of Ser, Thr or Cys (Xaa_i) derivatives into the corresponding ΨPro-containing building blocks (see text).

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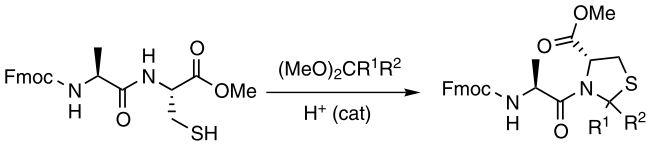
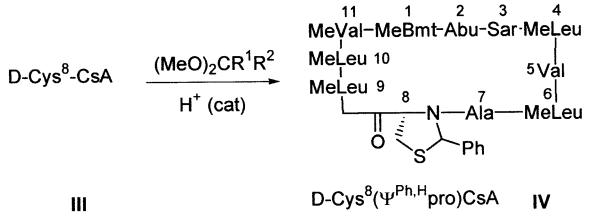


I IIa,b

Scheme 2. Direct insertion of ΨPro-systems into cysteine dipeptide derivatives: IIa: $R^1=R^2=CH_3$; IIb: $R^1=Ph$, $R^2=H$.



III D-Cys8(ΨPh,Hpro)CsA IV

Scheme 3. Direct insertion of ΨPro-systems into the Cys-containing cyclosporine analogue III.

In applying similar reaction conditions,[6] the novel CsA analogue **IV** was obtained in acceptable yield (19%). This product was identified by electrospray mass spectroscopy (m/z 1323.4 $[M+H]^+$) and NMR. In contrast to CsA, ^1H NMR spectra in DMSO and $CDCl_3$ show numerous conformations, indicating that the direct insertion of a ΨPro system results in a highly constrained cyclic analogue of distinct conformational preference. Detailed structure–function studies of the target compound **IV** and other cysteine-containing bioactive peptides are in progress.

Acknowledgements

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6. Typical procedure: **Fmoc-Ala-Cys($\Psi^{Me,Me}$pro)-OMe (IIa)**: A solution of Fmoc-Ala-Cys-OMe (0.14 mmol; 62 mg), *p*-toluene sulfonic acid (0.04 mmol; 10 mg) and 2,2-dimethoxypropane (7 ml) in 7 ml of tetrahydrofuran was refluxed during 90 min. After concentration under reduced pressure, the crude product was purified by chromatography on silica gel ($R_f=0.47$ ethyl acetate/hexane, 5/5) to yield 48 mg (73%) of the title compound as a white solid. ^1H NMR $CDCl_3$ (400 MHz): 7.78 (d, $J=7.5$, 2H), 7.62 (d, $J=7.5$, 2H), 7.42 (t, $J=7.5$, 2H), 7.33 (t, $J=7.5$, 2H), 7.21 (d, $J=8.1$, 1H), 5.51 (d, $J=7.0$, 1H), 4.91 (dd, $J=5.1$, 4.2, 1H), 4.42 (d, $J=6.8$, 2H), 4.33 (m, 1H), 4.25 (t, $J=7.0$, 1H), 3.78 (s, 3H), 3.08 (dd, $J=14.2$, 4.2, 1H), 3.03 (dd, $J=14.2$, 5.1, 1H), 1.55 (s, 3H), 1.50 (s, 3H), 1.46 (d, $J=6.6$, 3H).
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8. The synthesis of this analogue was achieved according to Eberle, M. K.; Nuninger, F. *J. Org. Chem.* **1993**, *58*, 673–677.