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Trifluoromethylated proline surrogates as part of 'Pro-Pro' turn-inducing templates for the design of β-hairpin mimetics

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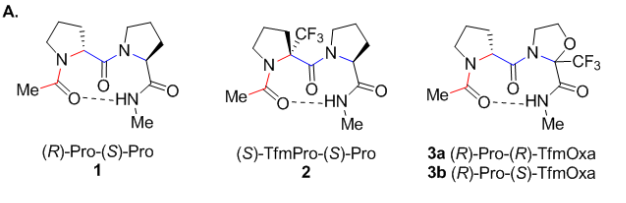
Proline is often found as a turn inducer in peptides or proteins. Exploitation of its propensity to induce structuration led to the development of a d-Pro-Pro(pP) 1 segment as a 'templating' unit, frequently used in the design of β-hairpin peptidomimetics.[1] Following this well-established strategy, our lab recently published the design of a series of cyclic peptides mimicking the β-hairpin structure of the CDR3 loop of Nb80.[2] Although one of the reported structures could adopt a conformation featuring a high overlap with the backbone structure of Nb80 CDR3 loop (MD Cα RMSD = 1.4 Å), NMR analysis revealed several sets of resonances arising from the existence of several conformers, and complex NOE patterns and secondary chemical shifts indicated random coil conformations for all of the residues. Those results illustrate well the limitations of the diproline template: it is known that the conformational stability of the type II' β-turn adopted by d-Pro-Pro(and herein required for a stable β-hairpin)is highly compromised by the cis-trans isomerization of the prolyl amide bond in larger ring systems. Use of constrained prolines, such as CF_3-pseudoprolines, demonstrated the possibility of stabilizing the backbone geometry by favouring the cis or inversely the trans population of a series of pseudo tetrapeptide sequences.[3]

In view of finding alternative templates with a stronger capacity to fix the β-hairpin conformation,we investigated different fluorinated analogues of the well-established pP segment as a β-turn promoter.

An in silico conformational study was performed on a set of 12 variants of the pP sequence, incorporating α-trifluoromethyl-proline (TfmPro) and α-trifluoromethyl-oxazolidine (TfmOxa),[4] and capped with acetyl (Ac) and N-methyl amide (NHMe) end groups, providing therefore the required H-bond donor and acceptor for a type II' β-turn(Figure 1A). For each peptide, all rotameric combinations were generated with Open Babel software, using the MMFF94 force field, and followed by a geometry optimization of the lowest energy trans and cis conformers. Out of the investigated combinations only TfmPro-Pro2 and d-Pro-(R)-TfmOxa 3a exhibited a strongly stabilized β-turn conformation, relative to the 'parent' pP (Figure 1, panel B). For those two dipeptides, the rotational barrier energy between the trans-cis and trans-trans conformers was indeed increased by 3.1 to 4.6 kcal/mol, compared to the d-Pro-Pro template and still maintained H-bond formation (Figure 1C). It is also noteworthy that the favourable effect of (R)-TfmOxa was totally lost for diastereoisomer 3b bearing (S)-TfmOxa. Considering synthetic accessibility, d-Pro-TfmOxa was selected for the experimental incorporation into the model and the cyclic Nb80 CDR3 loop peptidomimetics.



B. Energies relative to the lowest energy *trans-trans* conformer (calculated with the MMFF94 force field, OpenBabel®, in kcal/mol)
H-bond: lowest energy conformer with a H-bond between *O*-acetyl and *N*-methyl-NH

	D-Pro-Pro	(*S*)-TfmPro-Pro	D-Pro-(*R*)-TfmOxa	D-Pro-(*S*)-TfmOxa
trans-trans	0	0	0	0
trans-cis	3.6	6.7	8.2	2.4
cis-trans	2.2	3.8	5.6	3.0
cis-cis	7	12.2	15.2	8.7
H-bond	0.3	0	0	2.3

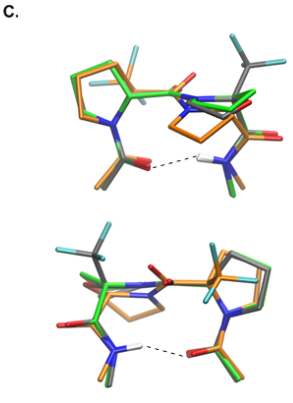
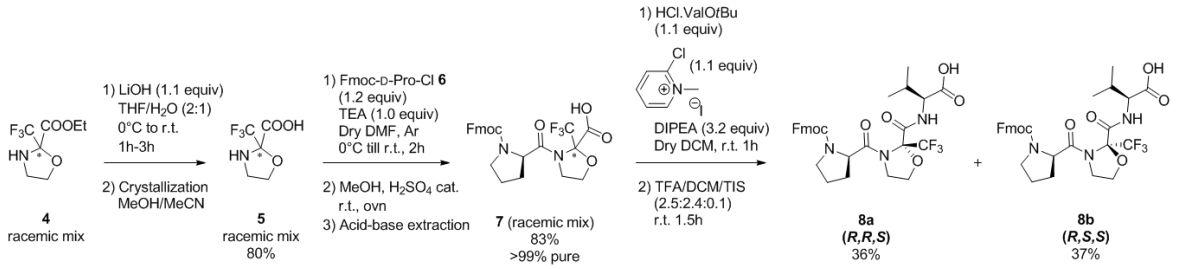


Figure 1: A. Diproline templates. B. Table of the calculated lowest energy conformers. C. Overlay between 1 (green), 2 (orange) and 3a (grey) in front (panel top) and back view (panel bottom).

A strong deactivation of the secondary amine's nucleophilicity and the steric hindrance generated by the CF3 group in TfmOxa prevent any direct application in SPPS. We therefore chose to 'cap' the TfmOxa with the adequate amino acids, by synthesizing in solution the tripeptideFmoc-d-Pro-TfmOxa-Val-OH 8 for incorporation *via* SPPS. Through a reported procedure,[4] a racemic mixture of the ethyl ester of TfmOxa was first saponified and then engaged in a coupling with the bench stable Fmoc-d-Pro-Cl 6. To our knowledge, such an activation is the only method reported to date, permitting coupling with amines of α-trifluoromethylated amino acids. Eventually, the desired dipeptide 7 could be isolated and purified in good yield once an intermediate esterification and extraction of the unreacted Fmoc-d-Pro-Cl 6 was performed. Then, access to the tripeptide by C-terminal coupling of TfmOxa turned out to be challenging: preliminary synthetic screening efforts with standard coupling reagents performed on H-TfmOxa-OHall failed in giving the targeted amide bond. This led us to investigate the Mukaiyama reagent. Due to their poor solubility in most organic solvents, pyridinium iodide compounds are not preferred reagents in peptide synthesis. It proved however to be highly efficient in TfmOxa's C-terminal coupling. After a thorough optimization study, examining stoichiometry, time and temperature conditions, the two diastereoisomers of Fmoc-d-Pro-TfmOxa-Val-OtBu were obtained in good yield, and ultimately in situ deprotected and separated by chromatography to give 8a and 8b (Scheme 1). Crystallization and X-Ray analysis were eventually carried out to attribute the stereochemistry of each diastereoisomers.



Scheme 1: Synthetic pathways toward tripeptides bearing TfmOxa

Next, efforts were dedicated to the incorporation of the Fmoc-d-Pro-TfmOxa-Val-OH fragments 8a and 8b into the Nb80 CDR3 loop sequence. Interestingly, coupling of both tripeptides and subsequent peptide elongation on 2-chlorotrityl resin and cleavage went smoothly in standard conditions. However, due to low solubility of the fully protected linear peptides in CH_2Cl_2, head-to-tail cyclisation required the use of trifluoroethanol (TFE) as a co-solvent. Associated TFE ester formation could not be fully prevented, but it did not hamper the successful formation and isolation of the cyclic peptidomimetics 9a-(R,R) and 9b-(R,S) (Figure 2).

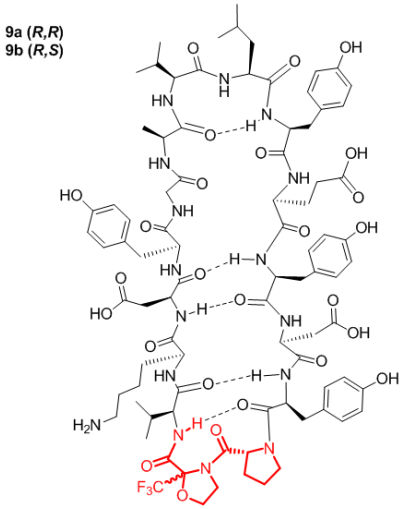


Figure 2: CDR3 Nb80 Cyclic peptidomimetics 9a & b bearing TfmOxa

Conformational NMR analysis of both mimetics 9a-(R,R) and 9b-(R,S) was first initiated in a mixture of water/acetonitrile 80:20 (this percentage allowed full solubility of the mimetics). In such conditions, no clear structuration could be identified. However, an assignment of the signals, achieved unambiguously up to 90%,

showed for each compound the existence of only one conformer; an improvement knowing that the d-Pro-Pro bearing cyclic peptidomimetic exhibited 4 conformers and multiple overlapping resonances between major and minor conformers. These early findings demonstrate the beta-hairpin stabilizing properties of the trifluoromethylated diproline analogues. The influence of NMR solvents and temperature changes are currently inspected. In particular, focus on the trans conformation of the prolyl amide bonds and the existence of intra-strand H-bond are examined to further verify the hypothetical beta-turn hairpin inducing feature of d-Pro-TfmOxa template.

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