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Synthesis and evaluation of a (3*R*, 6*S*, 9*S*)-2-oxo-1-azabicyclo[4.3.0]nonane scaffold as a mimic of Xaa-*trans*Pro in poly-L-proline type II helix conformation.

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We describe the development of a small-molecule mimic of Xaa*trans*-Pro dipeptide in poly-L-proline type II helix conformation, based upon a (3*R*, 6*S*, 9*S*)-2-oxo-1-azabicyclo[4.3.0]nonane core structure. Stereoselective synthesis of the mimic from L-pyroglutamic acid is achieved in twelve linear steps and 9.9% yield. Configurational and conformational analyses are conducted using a combination of ^{1}H NMR spectroscopy, X-ray crystallography and circular dichroism spectroscopy; and evaluation of the mimic as a promising surrogate dipeptide, in a protein-protein interaction between the SH3 domain of human Fyn kinase (Fyn SH3) and peptidomimetics of its biological ligand, are conducted by ^{1}H-^{15}N HSQC NMR titration experiments.

Introduction

The poly-L-proline type II (PPII) helix is an element of secondary structure characterised by a left-handed helix of three residues per turn, a helical pitch of 9.3Å, and torsional angles with distribution maxima of ϕ = -75° and ψ = 145°. The major role played by the PPII helix in protein structure and function has been widely reviewed[1] and, in particular, the intercession of PPII-helical peptides in protein-protein interactions between proline-rich regions and their recognition domains[2] has highlighted the potential of small-molecule peptidomimetics of PPII conformation[3] as tools for the study of the interaction of the PPII helix with its recognition domains, or as structural motifs for possible therapeutic intervention in protein-protein interactions.

To date, only Kühne and Schmalz have combined rational design, synthesis and conformational analysis of a small molecule PPII-mimetic scaffold with measurement of the binding of a peptide ligand incorporating the mimetic in a protein-protein interaction characterised by PPII conformation in that ligand.[3m] We sought to similarly apply a systematic approach to the development of a new conformational mimetic based upon a (3*R*, 6*S*, 9*S*)-2-oxo-1-azabicyclo[4.3.0]nonane core structure **1**. The candidate structure was identified as a potential Xaa-*trans*-Pro dipeptide surrogate *via* screening of a series of azabicyclo[X.Y.0]alkane/-ene candidates for optimal constraint in their energy-minimised conformation, of dihedral angles corresponding to ω, ϕ and ψ to those which define PPII conformation (Figure 1).

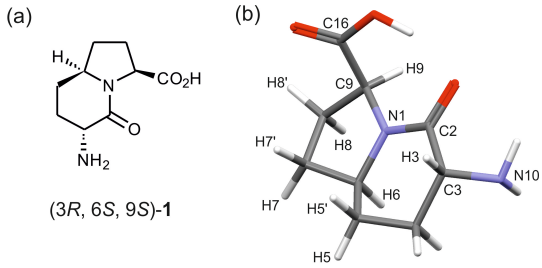


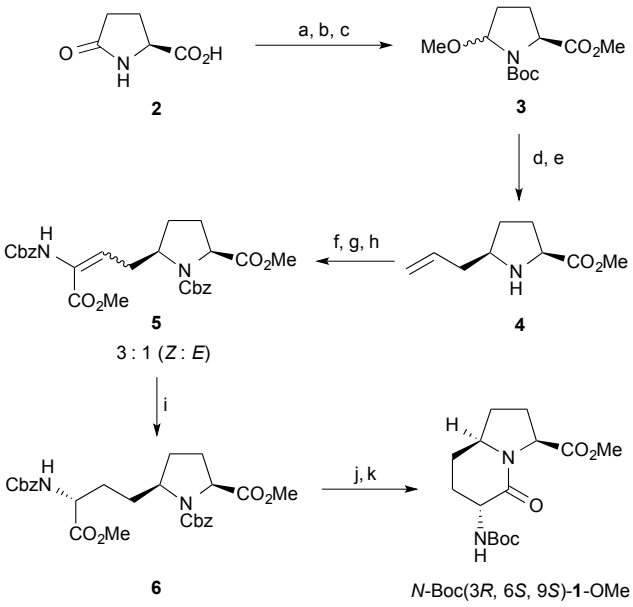
Figure 1 (a) Xaa-*trans*-Pro mimic candidate (3*R*, 6*S*, 9*S*)-**1**. (b) Optimised geometry for (3*R*, 6*S*, 9*S*)-**1** was obtained by DFT using B3LYP/6-31G(d,p).[7] Partial atom labelling is shown for clarity. Calculated dihedral angles correspond to ω, ϕ and ψ of Xaa-*trans*-Pro in PPII conformation as indicated in square brackets.

The DFT-optimised structure of (3*R*, 6*S*, 9*S*)-**1** predicts dihedral angles of $\omega = 174.6°$, $\phi = -68.5°$ and $\psi = 144.3°$; each well-centred within known permitted deviation for these PPII conformation descriptors.[1b] Diagnostic ^3J coupling constants for the ring-junction proton H6, of $^3J_{H6\text{-}H5} \approx {}^3J_{H6\text{-}H7} = 2.8 - 4.3$ Hz and $^3J_{H6\text{-}H5'} \approx {}^3J_{H6\text{-}H7'} = 9.8 - 10.2$ Hz are predicted[4] if the conformation of (3*R*, 6*S*, 9*S*)-**1** in solution is to prove consistent with the calculated structure.[5] Dipeptide surrogates based upon the diastereomeric (3*S*,6*S*,9*S*)-2-oxo-1-azabicyclo[4.3.0]nonane structure have been widely explored, and recently reviewed, as mimics of β-turn conformation.[6]

Results and Discussion

Preparation of **1** was carried out by consolidation of established procedures for synthesis of a 5-substituted L-proline methyl ester derivative **6**,[8] as precursor to the (3*R*, 6*S*, 9*S*)-2-oxo-1-azabicyclo[4.3.0]nonane core motif *via* hydrogenolysis and lactam cyclisation[9] (Scheme 1). Hence, conversion of enantiopure L-pyroglutamic acid (**2**) to methyl (2*S*)-1-(*tert*-butoxycarbonyl)-pyroglutamate, followed by reduction with L-selectride and subsequent conversion to the hemiaminal ether, allowed the multigram preparation of **3**. Treatment of the *N*-acyliminium ion derived from **3** with allyltrimethylsilane at low temperature[8c] gave the products of C5-allylation as an inseparable 3:1 *cis*:*trans* mixture. *N*-deprotection then resulted in clean separation of the (2*S*, 5*R*) *cis*-isomer **4**.[10] Relative stereochemistry in **4** was confirmed by nOe correlation between protons at the 2- and 5-position stereogenic centres. Introduction of benzyloxy carbamate *N*-protection and ozonolysis followed by (*Z*)-selective olefination,[11] then afforded **5**. Rhodium-catalysed asymmetric hydrogenation of the mixture of isomers **5**, using Rh(I)COD-(*R*,*R*)-Et-DuPHOS,[12] furnished a single diastereomer **6** (>90% *de*) in which the expected convergence of the geometrically impure substrate to *R*-configuration at the newly created stereogenic centre in **6** is verified by extrapolation of 3*R*-configuration in **1** (*vide infra*). Finally, treatment of **6** with Boc_2O, followed by hydrogenolysis, led to Xaa-*trans*-Pro dipeptide mimic **1**, the product of intramolecular δ-lactamisation, with suitable *N*-Boc and *C*-methyl ester terminus protection for incorporation into standard peptide coupling protocols.

The relative configuration of **1** was confirmed by a combination of nOe experiments and crystallographic analysis. We sought to distinguish *N*-Boc(3*R*, 6*S*, 9*S*)-**1**-OMe from its 3*S*-epimer[13] by the respective absence (3*R*-isomer) and presence (3*S*-isomer) of an nOe correlation of H3 with both H6 and H9. Whilst these expected nOes were indeed present in the spectra of *N*-Boc(3*S*, 6*S*, 9*S*)-**1**-OMe, overlap of the ring-junction proton H6 with the methyl group resonance occurred in the ^{1}H spectrum of *N*-Boc(3*R*, 6*S*, 9*S*)-**1**-OMe such that irradiation of H6 was impossible. We therefore confirmed the apparent lack of nOe between H3 and H6 in the desired 3*R*-epimer by direct hydrogenolysis / δ-lactamisation of **6**, to provide *N*-H(3*R*, 6*S*, 9*S*)-**1**-OMe in which there is no overlap in the ^{1}H NMR spectrum. No nOe correlation of H3 with either H6 or H9 is observed for *N*-H(3*R*, 6*S*, 9*S*)-**1**-OMe (Figure 2). The relative configuration of the 2-oxo-1-azabicyclo[4.3.0]nonane product was further determined by single crystal diffraction† and (3*R*, 6*S*, 9*S*) absolute configuration inferred from that of **2**. ^3J coupling constants for resonances arising from H3, H6 and H9 in the ^{1}H NMR spectrum of *N*-H(3*R*, 6*S*, 9*S*)-**1**-OMe, and dihedral angles ω, ϕ and ψ determined from the solid-phase



Scheme 1 Synthesis of *N*-Boc(3*R*, 6*S*, 9*S*)-**1**-OMe.

Reagents and Conditions: a) MeOH, Dowex®, Δ, 16 h, 96%; b) Boc_2O, DMAP, CH_3CN, 0 °C to room temp., 82%; c) L-selectride, THF, -78 °C then MeOH, *p*-TsOH (0.1 equiv.); 52% over two steps; d) $BF_3{\cdot}Et_2O$, $Me_3SiCH_2CH{=}CH_2$, -78 °C, 77%; e) TFA, CH_2Cl_2, 55%; f) NaH, benzyl chloroformate, 88%; g) O_3, -78 °C then PPh_3, 96%; h) $(MeO)_2P(O)CH(NHCbz)CO_2Me$, ^{tert}BuOK, -78 °C to room temp., 5 h, 79%; i) Rh(I)COD-(*R*,*R*)-Et-DuPHOS, H_2 (100 psi), 24 h, 90%; j) Boc_2O, DMAP, CH_3CN, room temp., 97%; k) H_2, Pd/C, MeOH, 16 h, 98%.

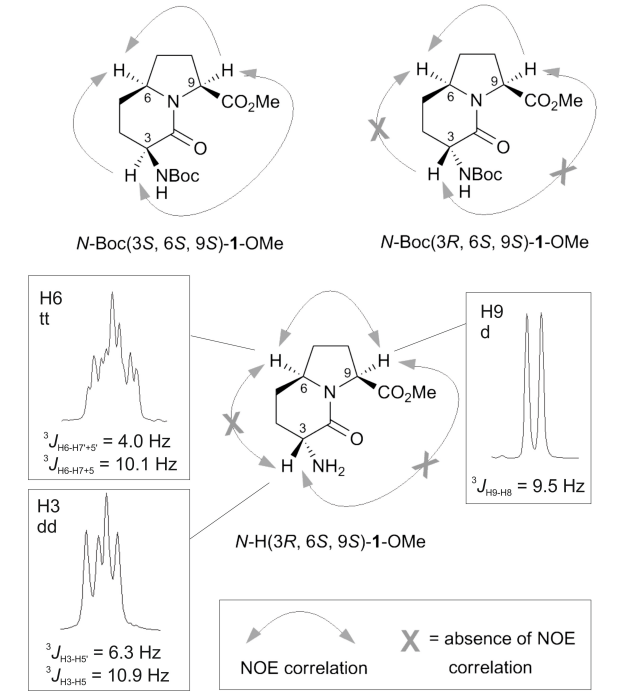


Figure 2 1H NMR (400 MHz, $CDCl_3$) features, diagnostic of conformation and relative configuration in 2-oxo-1-azabicyclo[4.3.0]nonane mimetics of Xaa-*trans*-Pro.

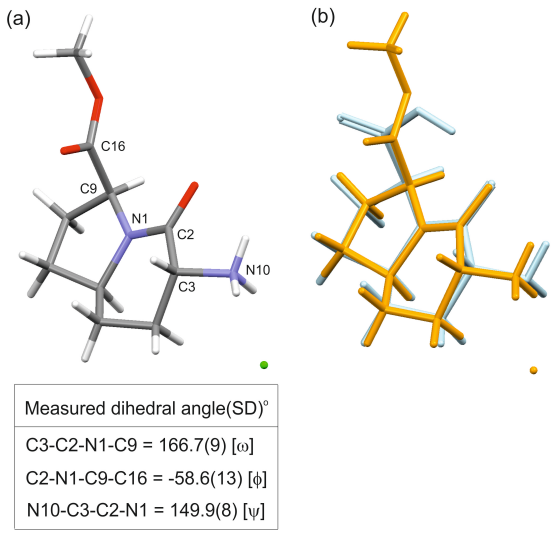


Figure 3 (a) X-Ray crystal structure of (3*R*, 6*S*, 9*S*)-**1**-OMe·HCl and measured dihedral angles ω, ϕ and ψ. Solvent excluded, partial atom labelling for clarity; (b) Overlay of (3*R*, 6*S*, 9*S*)-**1**-OMe·HCl (orange) and DFT-optimised minimum energy conformer of (3*R*, 6*S*, 9*S*)-**1** (cyan).

structure (Figure 3) are consistent with those expected for the intended (PPII-mimetic) conformation predicted by DFT. Moreover, the ϕ and ψ pair fall within the boundary of a region of the Ramachandran plot defining PPII conformation by the clustering of PPII helices determined through surveys of protein structure.[1b]

Conformational studies upon short-chain oligomers of (3*R*, 6*S*, 9*S*)-**1** were next undertaken. The dimer (**9**), trimer (**10**) and tetramer (**11**) represent 4-, 6- and 8-residue peptide mimics respectively, and were prepared using standard procedures of solution-phase peptide coupling (Scheme 2). We define a second set of dihedral angles (') in **9-11**, which correspond to ω', ϕ' and ψ' of the peptidomimetic, but are exocyclic to those of the core (3*R*, 6*S*, 9*S*)-2-oxo-1-azabicyclo[4.3.0]nonane motif and thus unconstrained. Indeed, in the solid-phase conformation of **9** only ω' and ϕ' approximate the torsional angles of an extended PPII conformation, whilst ψ' does not (Figure 4).

Circular dichroism (CD) analysis of **9-11** was undertaken in methanol and in water and, in each case, gave spectra which

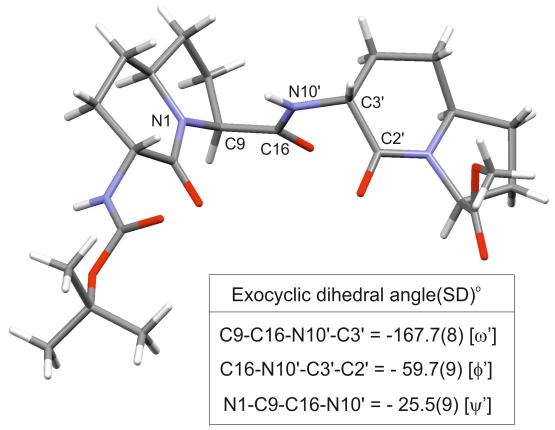
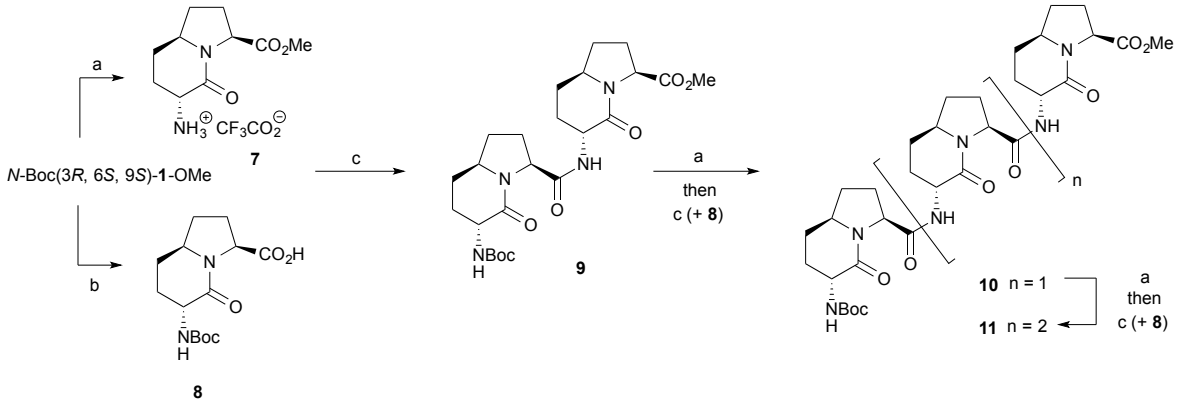


Figure 4 X-Ray crystal structure of 9 and measured unconstrained dihedral angles w', f' and y'. Solvent excluded, partial numbering for clarity.

exhibited a strong negative maximum at 208 nm in methanol and at 202 nm in water ($\pi\rightarrow\pi^*$), and a weak positive maximum at 226-8 nm in methanol and 224 nm in water ($O_{i-1}\bullet\bullet\bullet C_i'=O_i$, $n\rightarrow\pi^*$) which we attribute to PPII conformation[14] within those sections of the peptidomimetic backbone which are ordered as a result of the conformationally locked subset of ω, ϕ and ψ (Figure 5). Interestingly, monomer *N*-Boc(3*R*, 6*S*, 9*S*)-**1**-OMe does not display clear PPII conformational order, and a decrease in the relative band strength ρ ($\theta_{max}/\theta_{min}$)[15] indicative of increasing conformational stability is then observed with increasing chain length **9→11** in both solvents, arising predominantly from an increase in intensity of the $\pi\rightarrow\pi^*$ CD signal. Mean molar ellipticity was found to be independent of sample concentration,[16] suggesting that there is no intermolecular stabilisation of an induced PPII conformation by backbone solvation, which is described to stabilise the PPII helix in pure polypeptides.[17] Rather, a favourable $n\rightarrow\pi^*$ interaction may stabilise *trans*-conformation in the exocyclic amide bond(s) of **9-11** leading to an overall cooperative stabilisation of helical comformation, related to chain length, *via* an induced dihedral angle ϕ' close to that describing the PPII helix (and consistent with that observed in the solid state, Figure 4).[14b, 18]



Scheme 2 Solution-phase synthesis of oligomers **9-11**.

Reagents and Conditions: a) CF_3CO_2H (20% v/v in CH_2Cl_2), room temp., 16 h; b) 1M LiOH, THF, room temp., 1 h; c) EDC, HOBt, $^iPr_2EtN$, DMF, 0 °C to room temp.

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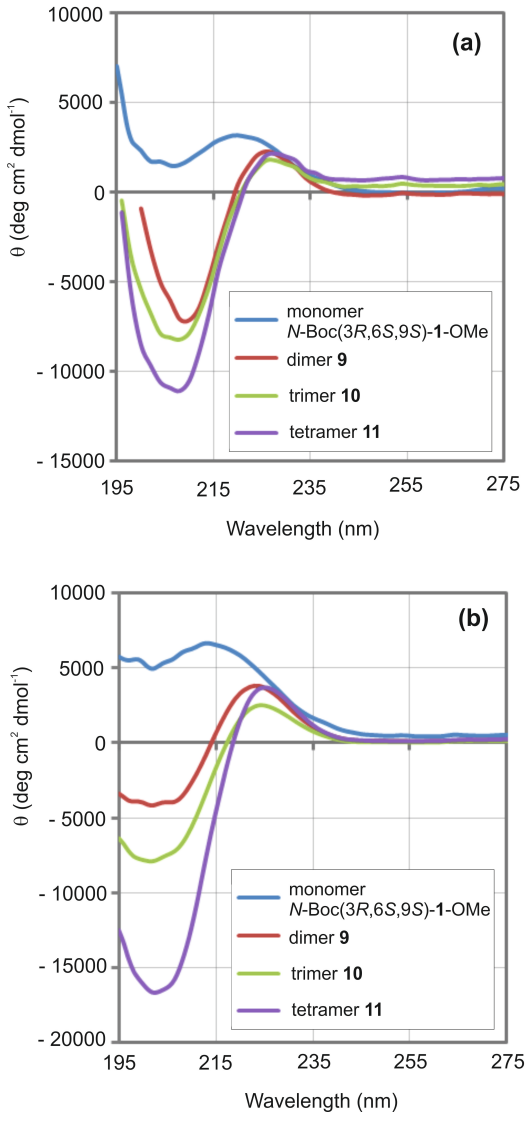
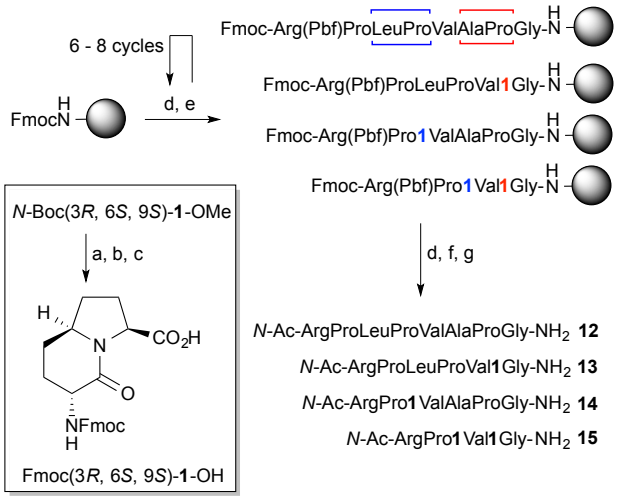


Figure 5 Circular dichroism spectra in (a) MeOH and (b) water. Spectra were recorded at 23 °C and concentrations of *N*-Boc(3*R*, 6*S*, 9*S*)-**1**-OMe = 0.5 mM, **9** = 0.5 mM, **10** = 0.25 mM, **11** = 0.125 mM; and are normalised for concentration and to give θ = mean molar ellipticity per amide bond. Relative band strengths ρ ($\theta_{max}/\theta_{min}$) are as follows; **9**: ρ_{MeOH} = 0.33 and ρ_{water} = 0.91; **10**: ρ_{MeOH} = 0.22 and ρ_{water} = 0.31; **11**: ρ_{MeOH} = 0.19 and ρ_{water} = 0.22.

Interpretation of CD spectra in order to identify conformational order is not necessarily straightforward however,[19] and it is important to emphasise that there is here insufficient evidence to conclude that an induced helical conformation in the exocyclic regions of **9-11** exists in solution. Indeed, in the ^{1}H NMR spectra of **9-11**, $^3J_{HN\alpha}$ coupling constants between 5.6 Hz (**9**, 400MHz, $CDCl_3$) and 7.6 Hz (**10**, **11**, 400MHz, CD_3CN) correspond to ϕ' = -71° and -87° respectively,[20] which are distributed around the median values of $^3J_{HN\alpha}$ = 6.5 Hz, ϕ = -71° characteristic of PPII conformation;[21] but an αN(i, i+1) nOe correlation between H9 and NH10' (which would be indicative that ψ' occupies the area of Ramachandran space diagnostic of PPII helicity)[21b] is not observed for **9**, **10** or **11**.

We next sought to explore the potential of the (3*R*, 6*S*, 9*S*)-2-oxo-1-azabicyclo[4.3.0]nonane scaffold as an Xaa-*trans*-Pro mimic in peptidomimetics. Well-documented structural and interaction studies[2e] exist for the proline-rich, 14 amino acid peptide PPRPLPVAPGSSKT which adopts a PPII conformation upon complex formation with its biological target. The peptide corresponds to residues 91-104 of the p85α regulatory subunit of human phosphoinositide 3-kinase and is the binding motif involved in the protein's interaction with the SH3 domain of human Fyn kinase (Fyn SH3).[22] In the complex, key intermolecular nOe contacts are observed for Arg3, Leu5, Pro6, Ala8 and Pro9 of PPRPLPVAPGSSKT, and we therefore selected the shortened peptide RPLPVAPG as a surrogate to test the effects of incorporation of the Xaa-*trans*Pro dipeptide mimic on the modified peptide's interaction with Fyn SH3 using NMR. The *C*-terminus glycine residue is retained to aid solubility.

Mimetic substitution of the dipeptide regions LeuPro and/or AlaPro of RPLPVAPG defines 3 potential PPII mimics **13-15**. *N*-Ac-RPLPVAPG-NH_2 (**12**) and peptidomimetic analogues **13**–**15** were prepared using conventional Fmoc solid-phase synthesis, supported on Rink amide resin (Scheme 3).



Scheme 3 Solid-phase synthesis of *N*-Ac-RPLPVAPG-NH_2 (**12**) and analogues **13**–**15**.

Reagents and Conditions: a)1M LiOH, THF, room temp., 1 h; b) CF_3CO_2H (20% v/v in CH_2Cl_2), room temp.; c) Fmoc-OSu, Na_2CO_3 (sat. aq. solution), dioxane, room temp.; d) piperidine (20% v/v in DMF), 20-30 min; e) FmocAA-OH (AA-OH = Gly-OH, Pro-OH, Ala-OH, Val-OH, Leu-OH or (3*R*, 6*S*, 9*S*)-**1**-OH), HOBt, DIC, ^{i}Pr$_2$NEt, DMF, room temp., 5 h, or FmocArg(Pbf)-OH, HBTU, ^{i}Pr$_2$NEt, DMF, room temp., 5 h; f) Ac_2O (50% v/v in pyridine), room temp., 45 min; g) CF_3CO_2H/^{i}Pr$_3$SiH/H_2O (96:2:2 v/v), room temp., 2 h.

The expected binding model for a Fyn SH3-(*N*-Ac-RPLPVAPG-NH_2) complex was extrapolated from that reported for Fyn SH3-(PPRPLPVAPGSSKT)[2e] in which intermolecular nOe crosspeaks in the solution-phase NMR structure indicate that residues Trp119, Asn136, Tyr137 and Tyr91 of the Fyn SH3 domain are involved in interaction with the peptide ligand (Figure 6).

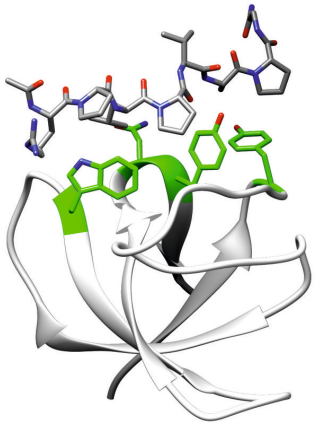


Figure 6 Simulated binding of *N*-Ac-RPLPVAPG-NH_2 (**12**) in PPII conformation by Fyn SH3 domain, extrapolated from Morton *et al.*.[2e] Residues displaying reported nOe crosspeaks with PPRPLPVAPGSSKT are shown in green: Trp119, Asn136, Tyr137 and Tyr91 from left→right.

We obtained NMR assignments of backbone resonances for $^{13}C/^{15}N$ Fyn SH3 using standard triple resonance measurement and sequential assignment techniques, and ^{1}H-^{15}N HSQC spectra were then recorded upon titration of each ligand (**12**-**15**) in various ratios against the protein in pH 6 potassium phosphate buffer solution with co-solvents 5% *v*/*v* D_2O and 10% *v*/*v* DMSO-d_6 to aid ligand solubility. Binding of *N*-Ac-RPLPVAPG-NH_2 (**12**) was confirmed as a suitable surrogate for the protein-protein interaction between p85α and Fyn SH3 since chemical shift perturbation ($\Delta\delta_{HN}$ 0.2 – 0.8 ppm) of backbone NH resonances corresponding to involvement of Trp119, Asn136, Tyr137 and Tyr91 (of Fyn SH3) in the binding event were observed. Binding curves† were determined for all resonances in the region $\Delta\delta_{HN}$ >0.2 ppm by plotting fractional shift ($\Delta\delta/\Delta\delta_{max}$) against [**12**] and an average dissociation constant of K_d = 41 μM was calculated, in good agreement with that reported for binding of the 14mer homologue PPRPLPVAPGSSKT (K_d = 50 μM) by Morton *et al.*.[2e] Location of the binding site in the expected region of Fyn SH3 is depicted in Figure 7(a). Pleasingly, substitution of the AlaPro dipeptide sequence by the mimetic (3*R*, 6*S*, 9*S*)-**1**, did not markedly affect the binding orientation of resulting peptidomimetic ligand **13** [Fig. 7(b)], although weaker association (K_d = 300 μM averaged across nine resonances with $\Delta\delta_{HN}$ >0.17 ppm) took place, presumably as a result of the loss of key contacts with Trp119 and Tyr91 which have been characterised for the alanine residue of PPRPLPVAPGSSKT. It is worth noting that key contacts between the leucine residue of ligand **13**, and SH3 residues Gly117, Asp118 and Tyr137 appear to be maintained. The leucine side-chain is absent from ligands **14** and **15**, following peptidomimetic replacement of LeuPro with the backbone mimic (3*R*, 6*S*, 9*S*)-**1**, a defined interaction between ligands **14** or **15** and Fyn SH3 was not measurable upon NMR titration. Small chemical shift

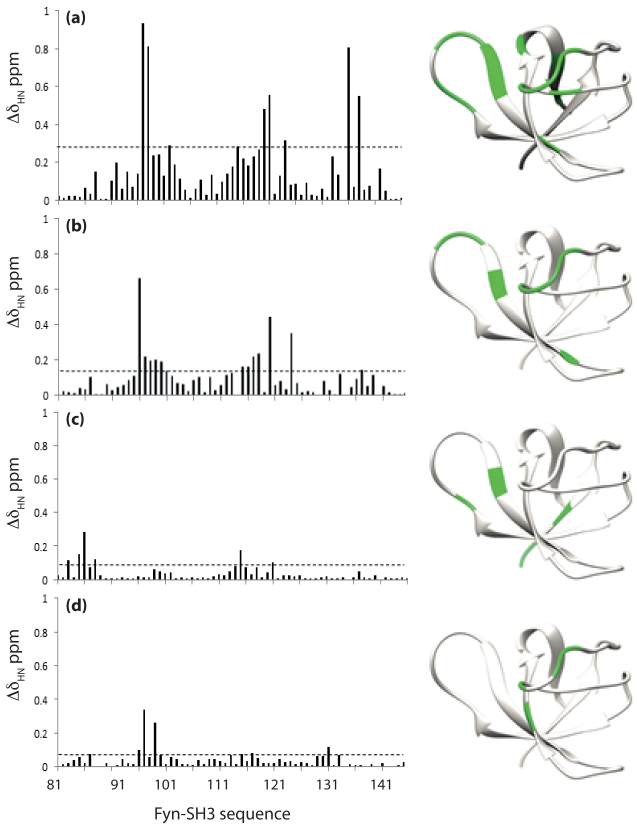


Figure 7 Histograms of ^{1}H-^{15}N chemical shift change for residues of Fyn SH3 upon binding of (a) **12** (b) **13** (c) **14** and (d) **15**. Residues with no amide proton (proline) are not shown. In each case, residues showing $\Delta\delta_{HN}$ > 0.2 ppm for binding of **12**, $\Delta\delta_{HN}$ > 0.17 ppm for binding of **13**, $\Delta\delta_{HN}$ > 0.1 ppm for binding of **14**, and $\Delta\delta_{HN}$ > 0.075 ppm for binding of **15**, are highlighted in green on the known X-ray structure of free human Fyn SH3.[23]

perturbations, disparately located around the protein, were seen in each case [Fig. 7(b) and (c)] and suggest only weak and non-specific association.

Conclusions

In summary, we have described the rational design, stereoselective synthesis and conformational analysis of a (3*R*, 6*S*, 9*S*)-2-oxo-1-azabicyclo[4.3.0]nonane scaffold, (3*R*, 6*S*, 9*S*)-**1**, as a promising small-molecule dipeptide mimic in poly-L-proline type II helix conformation. Peptidomimetic ligands featuring Xaa-*trans*Pro sequence replacement by the mimic demonstrate a general loss of affinity and selectivity for targeted binding to Fyn SH3 with increasing peptidomimetic content, corresponding to loss of side-chain functionality totalling four of the five residues involved in specific components of the protein-protein interaction between Fyn SH3 and the 'parent' peptide ligand. However, in maintaining important features of the ligand-protein complex **13**-Fyn SH3 following transposition of Ala-*trans*Pro by (3*R*, 6*S*, 9*S*)-**1** we suggest that (3*R*, 6*S*, 9*S*)-**1** is a promising core 'backbone' conformational mimetic of regions of *trans*Pro-, Ala- and Gly-rich primary sequence adopting PPII secondary structure. Our continuing work in this area, towards useful pseudo-peptide compounds, will involve the development of a second

generation of (3*R*, 6*S*, 9*S*)-2-oxo-1-azabicyclo[4.3.0]nonane scaffolds, suitably functionalised at C3 with substituents for mimicry of the L-amino acid side-chain.

Methods

Protein preparation

The gene for the Fyn SH3 domain (residues 73-145) encoded with an N-terminal GST tag in the pGEX-2T plasmid was a kind gift from Xavier Morelli and Stéphane Betzi (Cancer Research Center of Marseille). The plasmid was transformed into *E. coli* cell line BL21-CodonPlus(DE3)-RIPL (Agilent Technologies) and expressed and purified according to methods adapted from Campbell *et al*.[†, 2e]

NMR Spectroscopy

All samples were prepared in potassium phosphate buffer (10 mM, pH 6.0), 5% D_2O and 10% DMSO-d_6. NMR spectra of $^{13}C/^{15}N$ labelled Fyn SH3 were collected at 25 °C on a 600 MHz Varian INOVA spectrometer equipped with a cold probe (Varian). Resonance assignments of Fyn SH3 for backbone ^{15}N and HN nuclei were obtained by 2D ^{1}H-^{15}N–HSQC, HNCA, HNCACB and CBCA(CO)NH spectra incorporating water flip-back and gradient enhancement[24] on a 1 mM protein sample. Assignments for peptide-bound Fyn SH3 were deduced by tracking peak movements in titration data. All peptide interaction studies were carried out on samples containing 5% DMSO to aid mimetic solubility. No change was observed in the K_d of the native peptide (**12**) for Fyn SH3 under these conditions.

The peptide binding site was obtained by recording a series of nine 2D ^{1}H-^{15}N–HSQC spectra at peptide/protein ratios of 0:1, 0.25:1, 0.5:1, 0.75:1, 1:1, 2:1, 4:1, 8:1, 12:1 and 20:1 with the protein at 0.1 mM. K_d values were obtained for residues in the fast-exchange regime by fitting the fractional shift

$$\Delta\delta/\Delta\delta_{max} = \frac{(K_d+[L]+[P])-\sqrt{(K_d+[L]+[P])^2-4[P][L]}}{2[P]}$$

where $\Delta\delta/\Delta\delta_{max}$ is the fractional shift and [L] and [P] are the ligand (peptide or mimetic) and protein (Fyn SH3) concentrations respectively. The backbone chemical shift perturbation upon peptide addition was calculated by weighting ^{1}HN and ^{15}N chemical shift differences as follows:

$$\Delta\delta_{H,N} = \sqrt{[(\Delta\delta_H)^2 + \frac{1}{6}(\Delta\delta_N)^2]}$$

where $\Delta\delta_H$ and $\Delta\delta_N$ are the differences between the HN and ^{15}N chemical shifts, respectively, of the fully saturated and free protein.

All NMR data were processed with NMRPipe,[25] using mild resolution enhancement and linear prediction in the heteronuclear dimension, before being subjected to Fourier transformation. The resulting spectra were analysed with CcpNmr Analysis software.[26]

Circular Dichroism Spectroscopy

CD measurements were performed at 25 °C with a Jasco J720 spectrophotometer at a spectral bandwith of 2 nm with a time constant of 2 sec (scan speed 100 nm min^{-1}) and a step resolution of 1 nm. Spectra were recorded as an average of 16 scans. For each spectrum, the background was subtracted prior to smoothing and processing. Machine data units of millidegrees ellipticity were normalised as a function of the concentration of solutions and the number of amide bonds by conversion to mean residue molar ellipticity (θ) using the following equation (where n is the number of peptide bonds and Ellipticity is the raw data from the instrument).

$$\theta\ (\mathrm{deg.cm^2.dmol^{-1}}) = \frac{\mathrm{Ellipticity\ (mdeg).10^6}}{\mathrm{Pathlength\ (mm).[C](\mu M).}n}$$

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† Electronic Supplementary Information (ESI) available: Experimental details, ^{1}H and ^{13}C NMR spectra for preparation and characterisation of all new compounds. Fyn SH3 expression and purification, NMR assignment of Fyn SH3 and binding data for titration against **12**–**15**. Structural data. Crystallographic data for the structures in this manuscript have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC 1014671. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.. See DOI: 10.1039/b000000x/

1 (a) A. A. Adzhubei and M. J. E. Sternberg, *J. Mol. Biol*., 1993, **229**, 472; (b) A. A. Adzhubei, M. J. E. Sternberg and A. A. Makarov, *J. Mol. Biol*., 2013, **425**, 2100 and references therein; (c) R. Berisio, S. Loguercio, A. De Simone, A. Zagari and L. Vitagliano, *Protein Peptide Lett*., 2006, **13**, 847; (d) Y. Mansiaux, A. P. Joseph, J. C. Gelly and A. G. de Brevern, *Plos One*, 2011, **6**; (e) B. J. Stapley and T. P. Creamer, *Protein Sci*., 1999, **8**, 587; (f) R. Chebrek, S. Leonard, A. G. de Brevern and J. C. Gelly, *Database-Oxford*, 2014. DOI 10.1093/database/bau102.
2 (a) S. B. Feng, J. K. Chen, H. T. Yu, J. A. Simon and S. L. Schreiber, *Science*, 1994, **266**, 1241; (b) C. Freund, H.-G. Schmalz, J. Sticht and R. Kuhne, in *Handbook of Experimental Pharmacology 186*, eds. E.

Klussmann and J. Scott, Springer-Verlag, Berlin Heidelberg, 2008, pp. 407-429; (c) W. A. Lim, F. M. Richards and R. O. Fox, *Nature*, 1994, **372**, 375; (d) J. M. Martin-Garcia, I. Luque, J. Ruiz-Sanz and A. Camara-Artigas, *Acta Crystallogr*., Sect. *D: Biol*. *Crystallogr*., 2012, **68**, 1030; (e) C. J. Morton, D. J. R. Pugh, E. L. J. Brown, J. D. Kahmann, D. A. C. Renzoni and I. D. Campbell, *Structure*, 1996, **4**, 705; (f) A. Musacchio, M. Saraste and M. Wilmanns, *Nat*. *Struct*. *Biol*., 1994, **1**, 546; (g) J. T. Nguyen, C. W. Turck, F. E. Cohen, R. N. Zuckermann and W. A. Lim, *Science*, 1998, **282**, 2088; (h) A. R. Viguera, J. L. R. Arrondo, A. Musacchio, M. Saraste and L. Serrano, *Biochemistry*, 1994, **33**, 10925; (i) H. T. Yu, J. K. Chen, S. B. Feng, D. C. Dalgarno, A. W. Brauer and S. L. Schreiber, *Cell*, 1994, **76**, 933.

3 (a) N. G. Bandur, K. Harms and U. Koert, *Synlett*, 2005, 773; (b) F. Bernardi, M. Garavelli, M. Scatizzi, C. Tomasini, V. Trigari, M. Crisma, F. Formaggio, C. Peggion and C. Toniolo, *Chem*. *-Eur*. *J*., 2002, **8**, 2516; (c) S. Bhagwanth, R. K. Mishra and R. L. Johnson, *Beilstein J*. *Org*. *Chem*., 2013, **9**, 204; (d) G. Chaubet, T. Coursindel, X. Morelli, S. Betzi, P. Roche, Y. Guari, A. Lebrun, L. Toupet, Y. Collette, I. Parrot and J. Martinez, *Org*. *Biomol*. *Chem*., 2013, **11**, 4719; (e) S. Flemer, A. Wurthmann, A. Mamai and J. S. Madalengoitia, *J*. *Org*. *Chem*., 2008, **73**, 7593; (f) A. Mamai, N. E. Hughes, A. Wurthmann and J. S. Madalengoitia, *J*. *Org*. *Chem*., 2001, **66**, 6483; (g) A. Mamai, R. Zhang, A. Natarajan and J. S. Madalengoitia, *J*. *Org*. *Chem*., 2001, **66**, 455; (h) B. Raghavan, K. J. Skoblenick, S. Bhagwanth, N. Argintaru, R. K. Mishra and R. L. Johnson, *J*. *Med*. *Chem*., 2009, **52**, 2043; (i) R. V. Somu and R. L. Johnson, *J*. *Org*. *Chem*., 2005, **70**, 5954; (j) C. Tomasini, V. Trigari, S. Lucarini, F. Bernardi, M. Garavelli, C. Peggion, F. Formaggio and C. Toniolo, *Eur*. *J*. *Org*. *Chem*., 2003, 259; (k) P. Tremmel and A. Geyer, *J*. *Am*. *Chem*. *Soc*., 2002, **124**, 8548; (l) D. J. Witter, S. J. Famiglietti, J. C. Cambier and A. L. Castelhano, *Bioorg*. *Med*. *Chem*. *Lett*., 1998, **8**, 3137; (m) J. Zaminer, C. Brockmann, P. Huy, R. Opitz, C. Reuter, M. Beyermann, C. Freund, M. Muller, H. Oschkinat, R. Kuhne and H. G. Schmalz, *Angew*. *Chem*. *Int*. *Edit*., 2010, **49**, 7111; (n) R. Zhang, F. Brownewell and J. S. Madalengoitia, *J*. *Am*. *Chem*. *Soc*., 1998, **120**, 3894; (o) R. Zhang, C. K. Nickl, A. Mamai, S. Flemer, A. Natarajan, W. R. Dostmann and J. S. Madalengoitia, *J*. *Pept*. *Res*., 2005, **66**, 151; (p) C. Martin, B. Legrand, A. Lebrun, D. Berthomieu, J. Martinez and F. Cavelier, *Chem*. *-Eur*. *J*., 2014, **20**, 14240; (q) C. Reuter, M. Kleczka, S. de Mazancourt, J. M. Neudorfl, R. Kuhne and H. G. Schmalz, *Eur*. *J*. *Org*. *Chem*., 2014, **2014**, 2664; (r) A. Soicke, C. Reuter, M. Winter, J. M. Neudorfl, N. Schlorer, R. Kuhne and H. G. Schmalz, *Eur*. *J*. *Org*. *Chem*., 2014, 6467.

4 A. Navarro-Vazquez, J. C. Cobas, F. J. Sardina, J. Casanueva and E. Diez, *J*. *Chem*. *Inf*. *Comp*. *Sci*., 2004, **44**, 1680.

5 Encouraging precedent for the solution-phase conformation of (3*S*, 6*S*, 9*S*)-**1** is found in the work of Scolastico and co-workers [Reference 7(a)] who report ^{3}J = 4.2 Hz, 11.2 Hz for the ring-junction proton of a (3*S*, 6*S*, 9*S*)-analogue (bearing *N*-Boc, and C-terminus *tert*-butyl ester groups) despite unresolved multiplicity of the resonance (200 MHz, $CDCl_3$).

6 (a) A. Khashper and W. D. Lubell, *Org. Biomol. Chem.*, 2014, **12**, 5052; and references therein. (b) A related (6*R*)-carboxylate substituted indolizidin-2-one has been reported, bearing (3*R*)-NHFmoc substitution as a handle for solid-phase synthesis: D. Gramberg, C. Weber, R. Beeli, J. Inglis, C. Bruns and J. A. Robinson, *Helv*. *Chim*. *Acta*, 1995, **78**, 1588.

7 (a) A. D. Becke, *J*. *Chem*. *Phys*., 1993, **98**, 5648; (b) C. T. Lee, W. T. Yang and R. G. Parr, *Phys*. *Rev*. *[Sect.] B*, 1988, **37**, 785; (c) Y. Shao, L. F. Molnar, Y. Jung, J. Kussmann, C. Ochsenfeld, S. T. Brown, A. T. B. Gilbert, L. V. Slipchenko, S. V. Levchenko, D. P. O'Neill, R. A. DiStasio, R. C. Lochan, T. Wang, G. J. O. Beran, N. A. Besley, J. M. Herbert, C. Y. Lin, T. Van Voorhis, S. H. Chien, A. Sodt, R. P. Steele, V. A. Rassolov, P. E. Maslen, P. P. Korambath, R. D. Adamson, B. Austin, J. Baker, E. F. C. Byrd, H. Dachsel, R. J. Doerksen, A. Dreuw, B. D. Dunietz, A. D. Dutoi, T. R. Furlani, S. R. Gwaltney, A. Heyden, S. Hirata, C. P. Hsu, G. Kedziora, R. Z. Khalliulin, P. Klunzinger, A. M. Lee, M. S. Lee, W. Liang, I. Lotan, N. Nair, B. Peters, E. I. Proynov, P. A. Pieniazek, Y. M. Rhee, J. Ritchie, E. Rosta, C. D. Sherrill, A. C. Simmonett, J. E. Subotnik, H. L. Woodcock, W. Zhang, A. T. Bell, A. K. Chakraborty, D. M. Chipman, F. J. Keil, A. Warshel, W. J. Hehre, H. F. Schaefer, J. Kong, A. I. Krylov, P. M. W. Gill and M. Head-Gordon, *Phys*. *Chem*. *Chem*. *Phys*., 2006, **8**, 3172.

8 (a) M. Angiolini, S. Araneo, L. Belvisi, E. Cesarotti, A. Checchia, L. Crippa, L. Manzoni and C. Scolastico, *Eur*. *J*. *Org*. *Chem*., 2000, 2571; (b) J. Mulzer, F. Schulzchen and J. W. Bats, *Tetrahedron*, 2000, **56**, 4289; (c) W. Wang, J. Q. Yang, J. F. Ying, C. Y. Xiong, J. Y. Zhang, C. Z. Cai and V. J. Hruby, *J*. *Org*. *Chem*., 2002, **67**, 6353.

9 (a) H. G. Lombart and W. D. Lubell, *J*. *Org*. *Chem*., 1994, **59**, 6147; (b) *J*. *Org*. *Chem*., 1996, **61**, 9437.

10 L. Colombo, M. Di Giacomo, V. Vinci, M. Colombo, L. Manzoni and C. Scolastico, *Tetrahedron*, 2003, **59**, 4501.

11 U. Schmidt, A. Lieberknecht and J. Wild, *Synthesis-Stuttgart*, 1984, 53.

12 M. J. Burk, *Acc*. *Chem*. *Res*., 2000, **33**, 363.

13 *N*-Boc(3*S*, 6*S*, 9*S*)-**1**-OMe was obtained upon *N*-Boc protection of **5** followed by treatment with H_2 over Pd/C in MeOH, to effect stereo-random olefin hydrogenation with concurrent Cbz-removal and lactamisation. The resulting (2 : 1) mixture of *N*-Boc(3*R*, 6*S*, 9*S*)-**1**-OMe : *N*-Boc(3*S*, 6*S*, 9*S*)-**1**-OMe was separable by column chromatography (SiO_2 eluted with (98 : 2 CH_2Cl_2 : MeOH).

14 (a) L. E. Bretscher, C. L. Jenkins, K. M. Taylor, M. L. DeRider and R. T. Raines, *J*. *Am*. *Chem*. *Soc*., 2001, **123**, 777; (b) M. P. Hinderaker and R. T. Raines, *Protein Sci*., 2003, **12**, 1188; (c) N. W. Owens, J. Stetefeld, E. Lattova and F. Schweizer, *J*. *Am*. *Chem*. *Soc*., 2010, **132**, 5036; (d) E. S. Pysh, *Biopolymers*, 1974, **13**, 1563; (e) E. W. Ronish and S. Krimm, *Biopolymers*, 1974, **13**, 1635; (f) J. L. S. Lopes, A. J. Miles, L. Whitmore and B. A. Wallace, *Protein Sci*., 2014, **23**, 1765.

15 S. K. Brahmachari, M. Bansal, V. S. Ananthanarayanan and V. Sasisekharan, *Macromolecules*, 1979, **12**, 23.

16 Dimer **9** exhibited λ_{max} = 226 nm, λ_{min} = 208 nm and ρ ($\theta_{max}/\theta_{min}$) = 0.33, at both 0.125 mM and 0.5 mM in methanol. Trimer **10** exhibited λ_{max} = 227 nm, λ_{min} = 208 nm and ρ ($\theta_{max}/\theta_{min}$) = 0.22, at both 0.125 mM and 0.25 mM in methanol.

17 T. P. Creamer and M. N. Campbell, *Adv*. *Protein Chem*., 2002, **62**, 263.

18 C. W. Wu, T. J. Sanborn, R. N. Zuckermann and A. E. Barron, *J*. *Am*. *Chem*. *Soc*., 2001, **123**, 2958.

19 R. W. Woody, *Advances in Biophysical Chemistry*, 1992, **2**, 37-79.
20 Torsional angles ϕ' were calculated according to the Karplus equation $^3J_{HN\alpha} = 6.4\ \cos^2(\phi - 60°) - 1.4\ \cos(\phi - 60°) + 1.9$ using the Sweet J calculator; G. Balacco, *J. Chem. Inf. Comp. Sci.*, 1996, **36**, 885.
21 (a) S. L. Lam and V. L. Hsu, *Biopolymers*, 2003, **69**, 270; (b) K. Ma, L. S. Kan and K. Wang, *Biochemistry*, 2001, **40**, 3427.
22 Fyn SH3 has similarly been used to establish the reduced binding affinity of a peptidomimetic of *N*-Ac-RALPPLP-NH_2 incorporating a tricyclic diproline mimic (Reference 3m) compared to that of the parent ligand.
23 M. E. M. Noble, A. Musacchio, M. Saraste, S. A. Courtneidge and R. K. Wirenga, *EMBO J.*, 1993, **12**, 2617.
24 (a) L. E. Kay, *Prog. Biophys. Mol. Biol.*, 1995, **63**, 277; (b) M. Sattler, J. Schleucher and C. Griesinger, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1999, **34**, 93.
25 F. Delaglio, S. Grzesiek, G. W. Vuister, G. Zhu, J. Pfeifer and A. Bax, *J. Biomol. NMR*, 1995, **6**, 277.
26 W. F. Vranken, W. Boucher, T. J. Stevens, R. H. Fogh, A. Pajon, P. Llinas, E. L. Ulrich, J. L. Markley, J. Ionides and E. D. Laue, *Proteins*, 2005, **59**, 687.