CHEMISTRY
A EUROPEAN JOURNAL

COMMUNICATION

DOI: 10.1002/chem.201204546

Synthesis of a 6,6-Spiroketal Amino Acid and Its Incorporation into a Peptide Turn Sequence Using Solid-Phase Peptide Synthesis

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Reverse turns are a secondary structure element found in proteins[1] and are known to be involved in the molecular recognition of various biological processes including protein–protein[2] and protein–DNA interactions[3] and protein folding.[4] A variety of turn structures exist, which can be broadly categorised into several classes.[5] Due to the biological significance of this secondary structure, various conformationally constrained structures have been developed to mimic turn motifs. For example, benzodiazepinones **1**,[6] various spirocyclic lactams, such as **2**,[7] (*S*)-aminobicyclo-[2.2.2]octane-2-carboxylic acid (ABOC; **3**),[8] azetidine **4**,[9] triazole **5**,[10] and azabicycloalkane amino acids, such as **6**[11] have all been reported to induce turn structures (Figure 1).

Spiroketals are cyclic motifs found in many natural products that exhibit a wide range of biological activities.[12] The conformationally locked structure of an anomerically stabilised spiroketal is able to project substituents in a well-defined orientation. Notably, Ley et al.[13] replaced the *N*-acetylglucosamine-galactose disaccharide core of sialyl Lewis X with a spiroketal to generate a sialyl Lewis X mimetic, which exhibited anti-inflammatory activity by preventing binding of sialyl Lewis X to E-selectin. Another example was reported by Smith et al.[14] who identified several conformers of the AB spiroketal rings in the natural macrolide (+)-spongistatin that were locked in a turn-like structure.

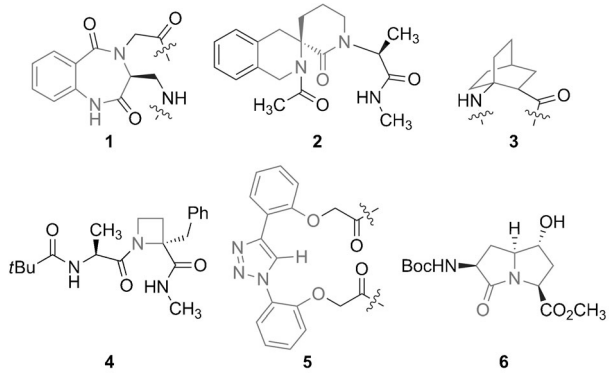

Figure 1. Synthetic reverse turn mimics or turn inducers.

The synthesis of spiroketals on solid support has been reported in natural product synthesis[15] and for the preparation of natural-product-inspired spiroketal libraries.[16] However, very few examples have been reported of spiroketals being incorporated into peptides by solid-phase peptide synthesis (SPPS). A recent example by Wipf et al.[17] entailed the use of Fmoc-SPPS to construct spiroketal–steroid–RGD hybrids that exhibited antagonistic activity for the CD11b/CD18 integrin.

Inspired by these studies, we decided to assess the ability of a spiroketal scaffold to induce turn-like structures by its incorporation into a suitable peptide sequence. For example, spiroketal **7** is a relatively rigid scaffold that contains anchor groups to which the N and C termini of a peptide chain can be attached. Initial modelling studies suggested that the spiroketal might mimic the $i+1$ and $i+2$ positions of a β-turn. This building block might in particular influence the conformations available to cyclic peptides that are already constrained by backbone cyclisation. We set out to test this idea by incorporating this spiroketal template into the cyclic peptide **8** (Figure 2). The related β-hairpin-shaped peptide **9** is a known inhibitor of the p53–HDM2 protein–protein interaction, and has been well-studied structurally, both in solution and when bound to the human double minute 2 (HDM2) protein.[18]

The key building block **7** was prepared as described in Scheme 1. (*R*)-Glycidol (**10**) was initially converted to lactone **11** and then to alkene **12** in eight steps.[19] Sharpless dihydroxylation[20] of alkene **12** gave an inseparable mixture of diols **13a** and **13b** in 74% yield (**13a**/**13b**, 6:1 d.r.).[21] Treatment of the diastereomerically enriched mixture of diols **13a** and **13b** with CSA afforded a mixture of spiroketals containing the major spiroketal **14** that was isolated in 69% yield, and an inseparable mixture of minor isomers.[22] The primary alcohol was converted to azido-spiroketal **15** in good yield (81% over two steps). A nOe interaction be-

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201204546.

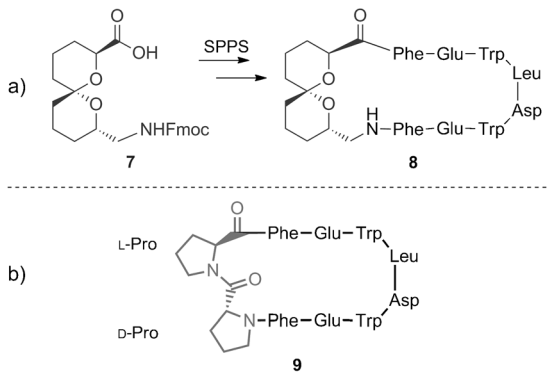
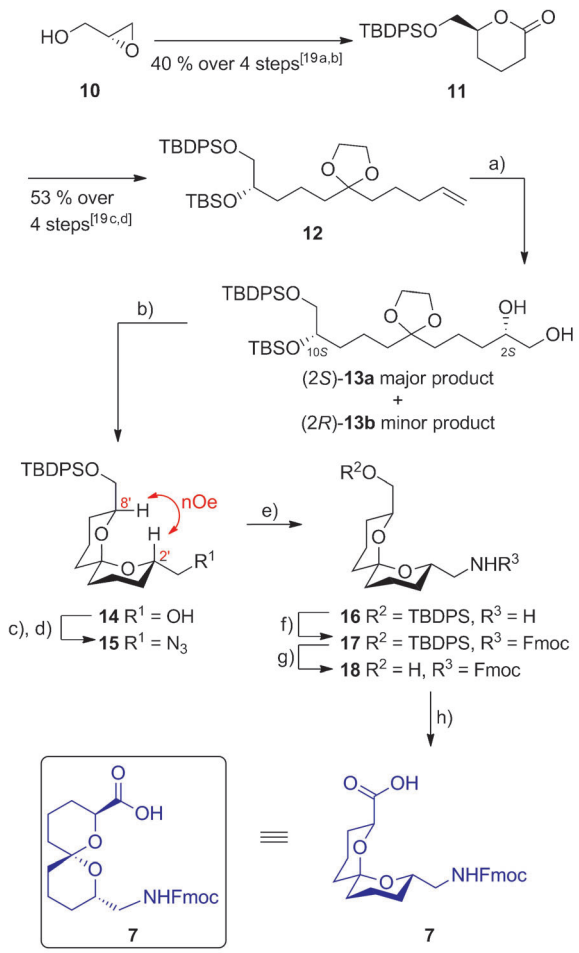


Figure 2. a) Proposed synthesis of cyclic spiroketal peptide **8** by SPPS. b) Cyclic β-hairpin peptide **9** synthesised by Fasan et al.[18]

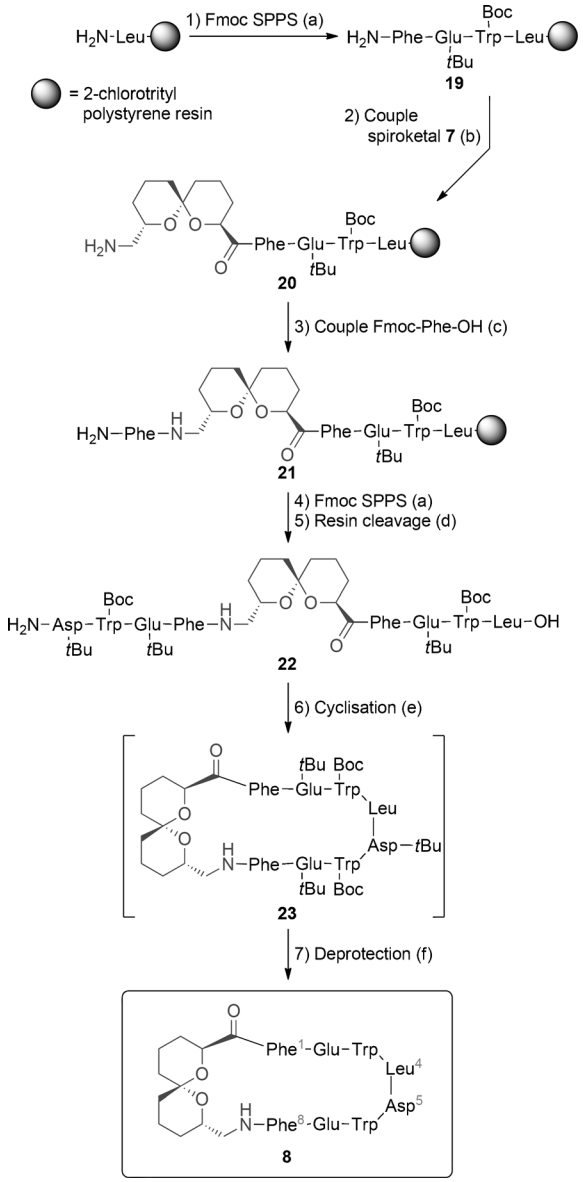


Scheme 1. Synthesis of spiroketal amino acid **7**. a) $(DHQ)_2AQN$, OsO_4, $K_3Fe(CN)_6$, K_2CO_3, $NaHCO_3$, $CH_3SO_2NH_2$, *t*BuOH, H_2O, 0 °C, 74 % (**13a**/**13b**, 6:1 d.r.); b) CSA, aq. EtOH, RT, 69 %; c) TsCl, NEt_3, DMAP, CH_2Cl_2, 0 °C to RT; d) NaN_3, DMF, 80 °C, 81 % over two steps; e) PPh_3, aq. THF, 89 %; f) Fmoc-OSu, DIPEA, CH_2Cl_2/1,4-dioxane (1:1), 0 °C to RT, 90 %; g) BF_3·OEt_2, 4-methoxysalicylaldehyde, CH_2Cl_2, RT, 63 %; h) TEMPO, $PhI(OAc)_2$, CH_3CN/aq. NaH_2PO_4·$2H_2O$ (3:1), 64 %.

tween 2′-H and 8′-H established that azido-spiroketal **15** adopts the expected bis-anomerically stabilised conformation. Staudinger reduction of the azide group proceeded in high yield to afford aminospiroketal **16**.

Fmoc protection followed by acidic cleavage of the silyl protecting group using BF_3·OEt_2 and 4-methoxysalicylaldehyde gave Fmoc-alcohol **18**.[23] Finally, TEMPO oxidation in an acetonitrile-aqueous phosphate buffer furnished the desired spiroketal amino acid **7**.[24]

With the key spiroketal amino acid **7** in hand, the synthesis of cyclic spiroketal peptide **8** was undertaken using Fmoc-SPPS (Scheme 2). Employing standard conditions, the



Scheme 2. Synthesis of cyclic spiroketal peptide **8**. a) i. Fmoc-amino acid, HBTU, NMM, DMF; ii. DMF wash; iii. 20 % piperidine/DMF; iv. DMF wash; b) i. spiroketal amino acid **7**, HOAt, HATU, DIPEA, DMF, RT, two cycles; ii. DMF wash; iii. 20 % piperidine/DMF; iv. DMF wash; c) i. Fmoc-Phe-OH, HOAt, PyBoP, DIPEA, DMF, RT; ii. DMF wash; iii. 20 % piperidine/DMF; iv. DMF wash; d) 20 % $AcOH/CH_2Cl_2$, then 80 % $AcOH/CH_2Cl_2$; e) HOAt, HATU, DIPEA, RT, CH_2Cl_2/DMF (9:1); f) 95 % TFA/H_2O.

linear peptide chain was constructed on 2-chlorotrityl resin preloaded with leucine using automated Fmoc-SPPS conditions. However, the valuable Fmoc-spiroketal amino acid **7** was coupled using a manual procedure employing HATU, HOAt and DIPEA. The second Phe residue was also incorporated using a manual procedure employing the phosphonium coupling reagent, PyBoP, as initial attempts to couple the second Phe residue using HBTU resulted in formation of an N-terminal tetramethyl guanidine by-product.

Upon completion of the peptide sequence, the resin was carefully treated with 20% $AcOH/CH_2Cl_2$, followed by 80% $AcOH/CH_2Cl_2$ to release the linear protected peptide **22** (Scheme 2). A dilute solution (1 mg mL^{-1}) of this material was then treated with HATU to induce the macrocyclisation, the progress of which was monitored by observing the consumption of the linear peptide **22** by analytical RP-HPLC.[25] Upon completion of the reaction, the solvent was removed in vacuo and the crude protected cyclic peptide **23** was immediately treated with 95% TFA in H_2O to cleave the side chain protecting groups (Scheme 2). Subsequent purification by preparative RP-HPLC afforded cyclic spiroketal peptide **8** with no epimerisation of Leu^4 C_α observed by NMR spectroscopy or HPLC analysis (>96% purity, $[M+H]^+ = 1364.4$, Figure 3).

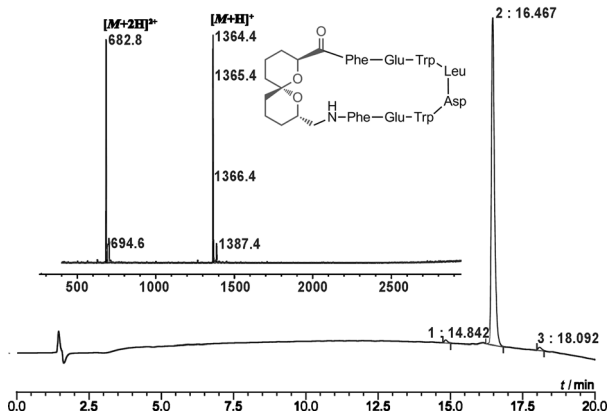


Figure 3. HPLC and MS spectra of purified cyclic spiroketal peptide **8** using a Gemini C18 column (5 μm, 4.6×150 mm), 1–81% CH_3CN/H_2O over 20 min, 1.5 mL min^{-1}.

The solution structure of spiroketal peptide **8** was investigated by ^{1}H NMR spectroscopy in [D$_6$]DMSO, due to its limited solubility in water. At room temperature, the spectrum showed broadening of the NH resonances although sharp signals were observed in the aromatic region (δ_H = 6.7–6.8 ppm). Reducing the peptide concentration had little effect on the chemical shifts or shape of the signals, however, raising the temperature to 355 K in [D$_6$]DMSO caused sharpening of the NH resonances. These findings are consistent with relatively slow exchange process(es) on the NMR time scale at room temperature. In 2D ROESY plots, with a mixing time of 250 ms, most of the observed rOes were intraresidue or sequential, with only a few weak long range rOes being observed (see the Supporting Information for a full list). Notably, however, an important strong H_α–H_α rOe was observed between Trp^3 and Phe^8.

Based on rOe-derived distance restraints, average solution structures were calculated for peptide **8** by restrained molecular dynamics in torsion angle space using the simulated annealing protocol in the program DYANA,[26] with the spiroketal in the bis-anomerically stabilised conformation. The resulting calculated average structures superimpose with a backbone rmsd of approximately 1.2 Å (Figure 4 and Table 1), and clearly reflect a significant backbone flexibility,

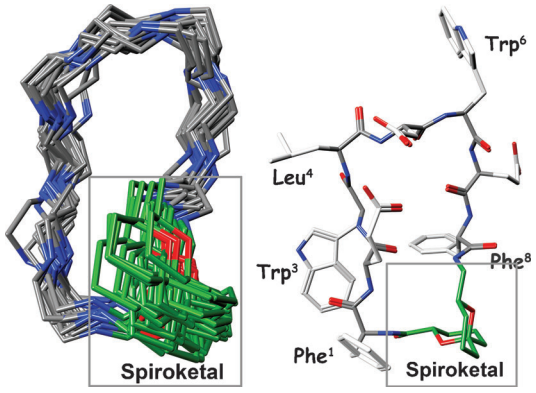


Figure 4. Left: backbone representation and superimposition of the 20 DYANA NMR structures for **8**. All backbone heavy atoms were used for the superimposition. Right: one typical structure is shown. MOLMOL[27] and PyMOL[28] were used for structure analysis and visualisation.

with large torsion angle fluctuations (Table 2). Nevertheless, the backbone conformations show similarities and adopt distorted hairpin-like structures. Interestingly, the spiroketal motif itself does not mimic the central $i+1/i+2$ region of a β-turn. Rather, the calculated structures all contain the spiroketal in a β-turn-like structure, with the spiroketal occupying positions i and $i+1$, and Phe^1 and Glu^2 at positions $i+2$ and $i+3$, respectively. Thus, the spiroketal does not induce a full 180° turn in the peptide chain, but rather induces an approximate 90° turn in the backbone conformation. This is illustrated in Figure 5, showing the turn-like region

Table 1. Summary of experimental distance restraints used and resulting statistics for the final 20 NMR structures calculated for peptide **8**, as superimposed and shown in Figure 4.

Experimental distance restraints	Calcd
number of NOE upper-distance limits	65
intraresidue	21
sequential	35
medium- and long-range	9
residual target function value [Å^2]	0.32±0.08
mean rmsd values [Å]	
all backbone atoms	1.17±0.40
all heavy atoms	2.61±0.80
residual NOE violations	
number >0.2 Å	5
maximum [Å]	0.25

Table 2. Average backbone torsion angles and their standard deviations found in the final 20 NMR structures shown in Figure 4.[a]

Residue	Dihedral	Average	S.D.[a]
Phe[1]	ϕ	−54.9	±99.2
	ψ	−26.0	±63.1
Glu[2]	ϕ	−106.7	±64.0
	ψ	165.4	±54.3
Trp[3]	ϕ	−135.8	±40.1
	ψ	136.6	±48.3
Leu[4]	ϕ	−175.8	±64.6
	ψ	−66.3	±92.6
Asp[5]	ϕ	−73.7	±81.9
	ψ	157.6	±23.4
Trp[6]	ϕ	−2.6	±57.8
	ψ	76.1	±67.5
Glu[7]	ϕ	44.9	±92.5
	ψ	170.0	±18.2
Phe[8]	ϕ	−74.7	±34.4
	ψ	130.4	±33.1
spiro[9][b]	ϕ	−105.1	±34.6
	ψ	−153.7	±40.5

[a] Standard deviations. [b] For the spiroketal, ϕ/ψ correspond to the dihedrals following N and preceding the CO termini, respectively.

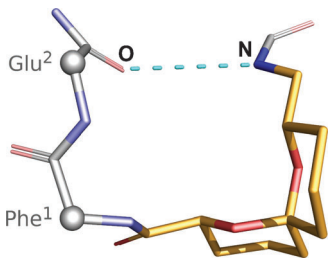


Figure 5. The spiroketal template can occupy the i and $i+2$ positions of a β-turn in the cyclic peptide **8** (dotted line = predicted hydrogen bond).

including the spiroketal, found in the NMR structures. As a result, the backbone as well as the side chain orientation of the residues are influenced in a novel and unusual manner by the spiroketal template (Figure 4). In the NMR structures, the side chains of Trp[3] and Phe[8] are adjacent and point to the same side of the macrocycle, whereas Trp[6] and Phe[1] are significantly separated and located in opposite turn regions.

It is also apparent that the spiroketal-containing cyclic peptide **8** adopts a quite different backbone conformation compared to that seen earlier with the cyclic peptide **9**, containing a D-Pro-L-Pro template, which adopts a β-hairpin conformation in both aqueous and mixed water/methanol solution. A related analogue of **9** containing 6-chlorotryptophan in place of Trp[6] was also shown by crystallography to adopt a β-hairpin structure when bound to HDM2.[18]

Finally, the ability of the cyclic peptide **8** to bind to HDM2 was tested under conditions reported earlier for peptide **9**.[20b] However, we could detect no interaction of **8** with HDM2 in the micromolar range, most likely since the energetically important side chains are not being displayed in an appropriate manner by the cyclic backbone scaffold.

In conclusion, the synthesis and incorporation of the novel spiroketal amino acid **7** into the cyclic spiroketal peptide **8** has been achieved using Fmoc-SPPS. The data obtained from NMR structure calculations indicate that the spiroketal template **7** has an unexpected and unprecedented influence on the backbone conformation of peptide **8**. The results provide synthetic access to this interesting new template **7**, which may find many applications in the design of other novel turn-containing peptidomimetics.

Acknowledgements

The University of Auckland is acknowledged for the award of a Doctoral Scholarship (J.T.B.K.). The authors thank Dr. Chris Squire for his assistance in generating the visual models of the peptide structures.

Keywords: amino acids · peptidomimetics · reverse turns · solid-phase peptide synthesis · spiroketals

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[21] The d.r. was determined by chiral HPLC. HPLC conditions: Chiralpak IC normal phase (0.46 cm × 25 cm); mobile phase, 3 % *i*PrOH in *n*-hexane; flow-rate, 0.5 mL min^{-1}; t_R: 34.3 min (2*R*,10*S*, minor product **13b**), 35.5 min (2*S*,10*S*, major product **13a**).

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Received: December 20, 2012
Published online: February 19, 2013

