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ChemComm
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Syntheses of second generation, 14-membered ring β-turn mimics†

Hong Boon Lee, Mookda Pattarawarapan, Sudipta Roy and Kevin Burgess*
Department of Chemistry, PO Box 30012, Texas A & M University, College Station, TX 77842-3012, USA.
E-mail: burgess@tamu.edu; Fax: (+1)979 845 8839; Tel: (+1)979 845 4345

Received (in Cambridge, MA, USA) 23rd April 2003, Accepted 22nd May 2003
First published as an Advance Article on the web 18th June 2003

Efficient solid phase syntheses of the constrained β-turn peptidomimetics 1–3 were devised, and the conformational properties of three representative compounds in DMSO were determined.

β-Turn structures[1] are often involved in protein–protein interactions.[2] Our approach to mimicking turns at protein hot-spots is to fuse dipeptides in a ring with an organic fragment.[3] Within this broad class of structures, several type **A** compounds (Fig. 1) have interesting biological activities as mimics of the neurotrophins.[4] A logical next step in this research was then to devise similar molecules that have less flexibility and peptidic character, but which may retain conformational biases to β-turn orientations in the dipeptide fragment. Compounds **1–3** were identified as substances that could have these attributes. These molecules must be less flexible than structures **A** because the bonds *a–c* are forced to be co-planar. Furthermore, the third amino acids in structures **A** (*i.e.* homoserine, homocysteine, or 2,4-diaminobutyric acid, corresponding to X = O, S, or NH) are expensive and do not contribute side-chain pharmacophores. Replacement of these residues using synthetically accessible templates like **4–6** (Fig. 2) is therefore highly desirable. This communication presents efficient solid phase syntheses of **1–3**, and NMR, CD, and modelling data to explore whether or not these compounds can populate β-turn-like conformations.

The templates **4–6** were obtained from 3-nitro-4-bromomethylbenzoic acid *via* synthetic sequences that involved displacement of the benzylic bromide, tritylation, and reduction of the nitro group. Coupling of these templates to the resin was relatively facile, but attaching the amino acids to the supported aniline intermediates **7** was relatively problematic. Eventually, it was found that PyBrop–2,6-lutidine (15 eq.) was effective if 15 equivalents of base were used to prevent loss of the trityl-based protecting groups.‡ As far as we are aware, this coupling agent–base combination is relatively unexplored. This set of reagents was superior to any others that were tested in this work, both with respect to number of equivalents of the amino acids–PyBrop required, and lack of epimerization in the product. Subsequent couplings in the sequence shown in Scheme 1 were relatively routine. Removal of the P protecting group from X (1% TFA in CH_2Cl_2 for OTrt and NHMtt, and 3% TFA for

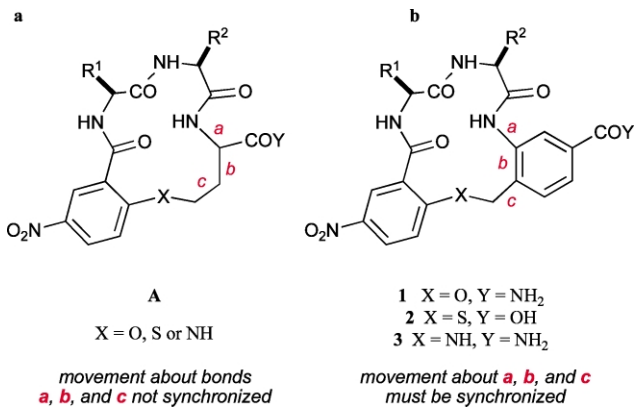


Fig. 1 a First generation mimics, and **b** target compounds in this work.

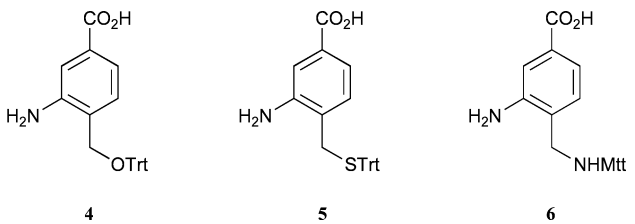
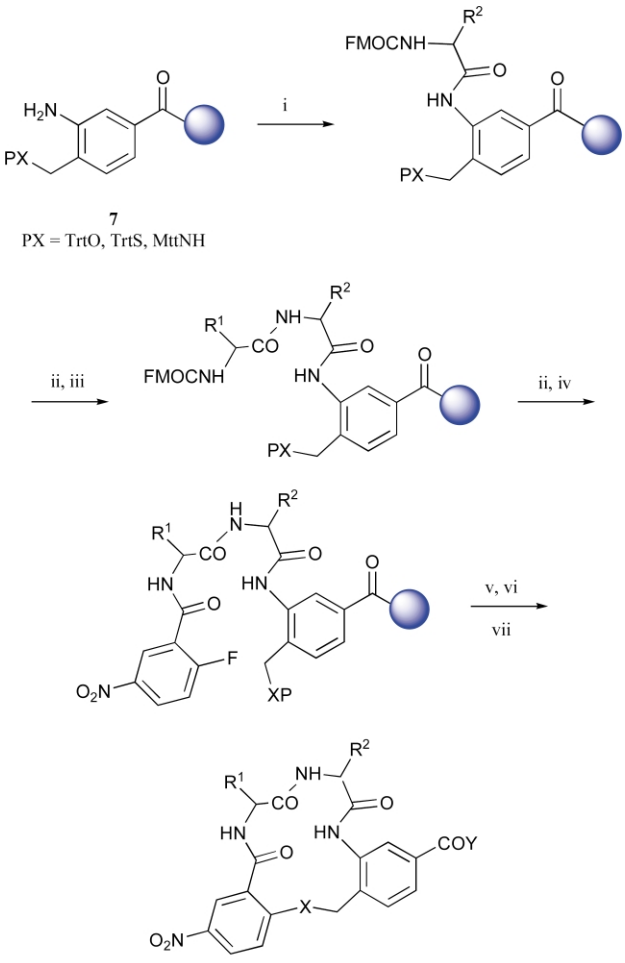


Fig. 2 Templates used in the syntheses.

† Electronic supplementary information (ESI) available: Conformational analyses of **1b, 2b** and **3b**. See http://www.rsc.org/suppdata/cc/b3/b304454h/



Scheme 1 Solid phase syntheses of **1–3**. (i) FMOC–AA[2], PyBrop, 15 eq. 2,6-lutidine, CH_2Cl_2; (ii) 20% piperidine–DMF; (iii) FMOC–AA[1], DIC, HOBt, $^iPr_2NEt$, CH_2Cl_2–DMF (4 : 1); (iv) 2-fluoro-5-nitrobenzoyl chloride, $^iPr_2NEt$, CH_2Cl_2; (v) remove P (see text); (vi) K_2CO_3, DMF, 2 d (vii) 90% TFA, 5% H_2O, 5% HSi^iPr_3. Rink linker was used for making compounds **1** and **3**, and Wang linker for compound **2**.

DOI: 10.1039/b304454h

STrt), base mediated cyclization, and simultaneous removal of the side-chain protection and of the material from the resin gave the desired products.

Approximately 60 different compounds were prepared *via* the route shown in Scheme 1. These incorporated various amino acid side-chains. Table 1 gives typical purity and yield data obtained for some illustrative compounds.

Conformational analyses of the peptidomimetics in DMSO were performed to test if they could access β-turn conformations. Proton NMR assignments were made *via* COSY and ROESY spectra. Coupling constants were deduced from the 1D spectra, and variation of N*H* chemical shifts with temperature, *i.e.* temperature coefficients, were deduced. ROESY spectra were used to deduce close proton contacts.[5] These data were compared with molecular simulations *via* the quenched molecular dynamics technique[6,7] without using any constraints from the experimental data; they are therefore not biased towards the anticipated result.

Fig. 3 shows low energy conformations of compounds **1b** and **2b** which match the physical data obtained. The ether **1b** and the thioether **2b** populate type-I-like turn conformations. This assertion is based on several observations. First, the temperature coefficients for the arylN*H* protons are relatively low (0 ppb K^{-1} for **1c** and −0.35 ppb K^{-1} for **2b**), much lower than the coefficients for the other amide protons in these molecules (−3.26 to −3.72 ppb K^{-1}). Low temperature coefficients are indicative of H-bonding and/or solvent shielded protons. The corresponding protons in **1b** and **2b** are expected to be both H-bonded and solvent shielded if the molecule adopts a β-turn conformation. The N*H* coupling constants for compounds **1b** and **2b** were within 3.0 Hz of values calculated for an ideal type-I β-turn. Moreover, significant ROE connectivities between the $i + 1$ and $i + 2$ N*H*, and between the $i + 2$ NH and {pseudo} $i + 3$ N*H* protons were observed, just as expected for a type-I-like turn conformations.

Table 1 Purity and yield data for compounds **1–3**.

Compound	Amino acids AA^{i+1}	Amino acids AA^{i+2}	X,Y	Purity[a] (%) UV,sedex	Yield (%)
1a	Ile	Lys	O,NH_2	96,97	80
1b	Glu	Lys	O,NH_2	86,98	43
1c	Glu	Asn	O,NH_2	56,90	71
2a	Ile	Lys	S,OH	95,100	47
2b	Glu	Lys	S,OH	97,100	57
2c	Glu	Asn	S,OH	85,95	55
3a	Ile	Lys	NH,NH_2	87,94	59
3b	Glu	Lys	NH,NH_2	90,100	46
3c	Lys	Thr	NH,NH_2	88,100	65

[a] Purity as assessed by HPLC of crude product, monitored UV absorption at 254 nm and using an evaporative light scattering detector (Sedex).

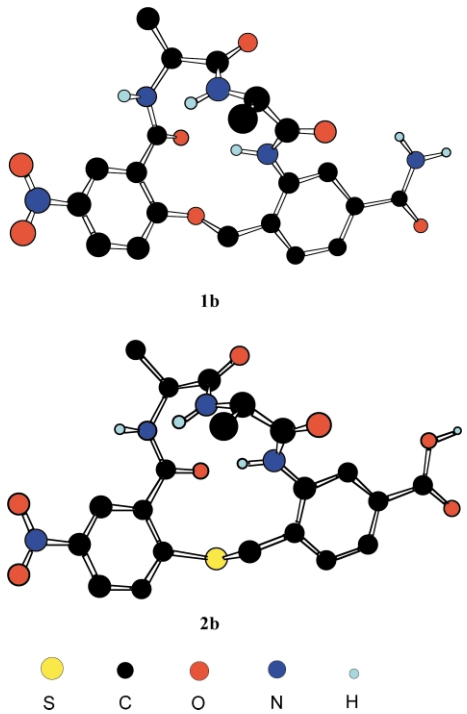


Fig. 3 Simulated low energy conformers of **1b** and **2b**.

Simulations indicate that compound **3b** can adopt type I-like turn conformations, but this is not supported by the NMR data. No evidence is available at this time to explain why this cyclic amine should be less likely to rest in β-turn structures, though an H-bond donor interaction of the amine NH proton with the DMSO solvent is a possibility that does not exist for the ether and thioether compounds. Further evidence for this assertion comes from CD studies. Spectra of compounds **1b–3b** (not shown) in 20% MeOH–H_2O were similar and at least consistent with the types of spectra associated with larger molecules containing type-I turn conformations.[8] This indicates that in a more aqueous environment the compounds may all have similar structures that are similar to β-turns. Unfortunately, NMR studies of the compounds in H_2O–D_2O mixtures were not possible due to solubility problems.

In conclusion, **1–3** are less peptidic compounds than our original turn design **A**. They are accessible in high purites (actually higher than compounds **A**) after a multi-step solid phase syntheses. The ether **1b** and the thioether **2b** can adopt β-turn conformations, but that state is less prevalent for the amine **3b** in DMSO. Limited water solubility of the products can be a limitation for with respect to biological testing, but the compounds are formed so cleanly that further chemistry to convert the nitro functionality into more hydrophilic groups is practical. Full data on the syntheses and conformational analyses of these and more water soluble derivatives, will be reported soon along with some biological activities of these compounds.

We thank Argonaut Technologies, Rapp Polymere, and Chiron Mimetopes for supplying supports for studies on the resin effects. Financial support for this work was provided by The NIH (CA82642), The Texas Technology Program, The Robert A. Welch Foundation, and The Welcome Trust {for a fellowship to HBL (059980)}.

Notes and references

‡ PyBrop = bromo-tris-pyrrolidino-phosphonium hexafluorophosphate; Trt = trityl; Mtt = 4-methylphenyldiphenylmethyl.

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