

# The synthesis of bimane constrained peptides and their fluorescent and structural properties



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Aimee Jade Horsfall

31/10/2016



## Abstract

Aberrant protein-protein interactions often result in disease, and as such, effective protein-protein interaction inhibitors are needed to mitigate the disease state. These interaction interfaces often involve secondary structural motifs, for example, an  $\alpha$ -helix or  $\beta$ -sheet. Small molecule drugs are not well suited to inhibit protein-protein interactions however constrained peptides, have shown to have great therapeutic potential.<sup>1-17</sup> Short peptides display little secondary structure in aqueous solution and as such, peptide sequences derived from a protein-protein interaction interface for use as a protein-protein interaction inhibitor, must be constrained into the native secondary structure. This can be achieved by installing a linker between the side-chains of two appropriately spaced amino-acids in the sequence. Many different linker chemistries have been designed and implemented with good biological results. However, these constrained peptide therapeutics are still restricted by traditional small-molecule drug hurdles including cell permeability, protease degradation and the ability to visualise and track a molecule intracellularly. Linkers such as the all-hydrocarbon metathesis linker have shown great promise in reducing protease degradation and increasing cell permeability,<sup>3,7,18-21</sup> however a fluorescent tag is still necessary to visualise a drug candidate. Here, a bimeane linker is proposed as a new peptide linker to help overcome these limitations. Dibromobimeane is reacted with thiol-containing amino-acid side chains to introduce a new fluorescent constraint in a series of model peptides. The reaction conditions with dibromobimeane are optimised in solution to reveal that a buffered system is required for the cyclisation to occur efficiently. Optimal reaction conditions, determined by monitoring the increase of the fluorescent product, were 0.5 mg/ml peptide in 10 mM PBS with one equivalent of dibromobimeane. The reaction was shown to be facile and versatile; in this thesis an array of peptides with varied sequence length, constraint length and amino-acid composition were cyclised under the same conditions, all reaching reaction completion in under 30 minutes. Additionally, these same conditions were applied successfully to react monobromobimeane with series of short peptides. Cyclisation on reaction with

dibromobimane, was also demonstrated on-resin with similar efficiency. The fluorescent properties of the resultant peptides were then explored to reveal that pH does not affect the observed fluorescence however a longer peptide length resulted in greater fluorescence intensity. Furthermore, acyclic mono-bimane-functionalised peptides displayed lower fluorescence intensity than the bimane-cyclised counterparts. The fluorescence of the bimane cyclised peptide could be detected as low as 10 nM on a plate reader, which is expected to further improve on a more sensitive instrument. The secondary structure of a series of tri- and penta-peptides were investigated through CD and NMR techniques. It was deduced that the bimane linker can induce  $\beta$ -strand like structure in an  $i-i+2$  constrained peptide; in contrast an  $i-i+4$  constrained pentapeptide with homocysteine in the 1 and 5 positions results in a  $3_{10}$  helical like structure.  $\beta$ -alanine containing analogues of these peptides were also synthesised and showed minimal structure.

This work outlines the synthesis of macrocyclic peptides containing a peptide constraint, in the form of a fluorescent bimane, both in solution and on-resin to produce cyclised peptides. The fluorescent properties of the resultant peptides have been shown to be biologically compatible with great fluorescence sensitivity. Furthermore, different secondary structure can be introduced by simply alterations of the constraint length from  $i-i+2$  to  $i-i+4$ . This work provides a foundation on which to design new fluorescent bimane-cyclised peptide-based protein-protein interaction inhibitors.







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## Abbreviations

**AAB:** Ammonium acetate buffer; **Ac<sub>2</sub>O:** Acetic anhydride; **ACN:** Acetonitrile; **dBb:** Dibromobimane; **CD:** Circular Dichroism; **DCM:** Dichloromethane; **DIPEA:** *N,N*-Diisopropylethylamine; **DMF:** *N,N'*-Dimethylformamide; **DMSO:** Dimethylsulfoxide; **DODT:** 2,2'-(Ethylenedioxy)diethanethiol; **Fmoc:** 9-Fluorenylmethoxycarbonyl; **HATU:** 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; **HCy:** Homocysteine; **HOBT:** 1-Hydroxybenzotriazole hydrate; **HPLC:** High Performance Liquid Chromatography; **HRMS:** High Resolution Mass Spectrometry; **LCMS:** Liquid Chromatography Mass Spectrometry; **mBB:** Monobromobimane; **Mmt:** 4-Methoxytrityl; **NMR:** Nuclear Magnetic Resonance; **Pbf:** 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; **PBS:** Phosphate buffered saline; **PPI:** Protein-protein interaction; **PyBOP** : (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; **RP-HPLC:** Reverse-Phase High Performance Liquid Chromatography; **SPPS:** Solid-phase peptide synthesis; **TFA:** Trifluoroacetic acid; **TFE:** 2,2,2-Trifluoroethanol; **TIPS:** Triisopropylsilane; **TNBS:** 2,4,6-Trinitrobenzenesulfonic acid; **Trt:** Trityl.



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