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How do Disulfide, Thioacetal and Lanthionine Bridges Influence the Conformations of Macrocyclic Peptides?

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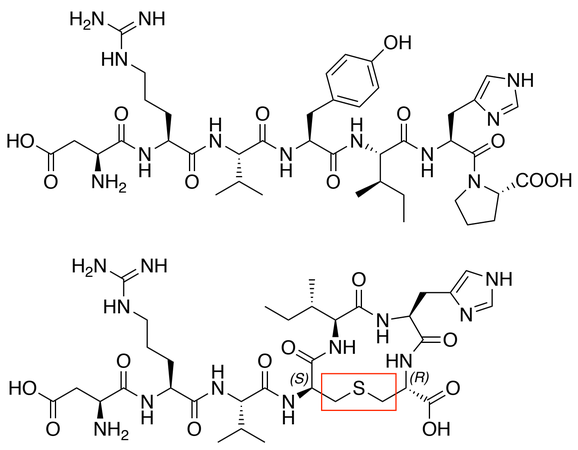
Understanding the effects of different side-chain bridges on the conformations of cyclic peptides

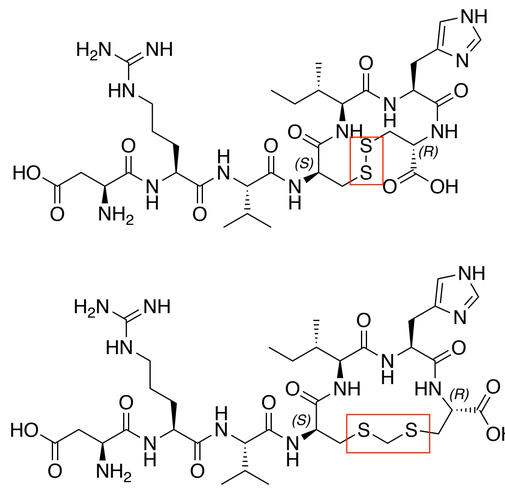


nAng(1-7):
potent GPCR agonist
rapidly inactivated *in vivo*

cAng(1-7) **1**
potent GPCR agonist
protease resistant
active via oral and pulmonary delivery[1]

Thioether (lanthionine) bridge
13 membered ring

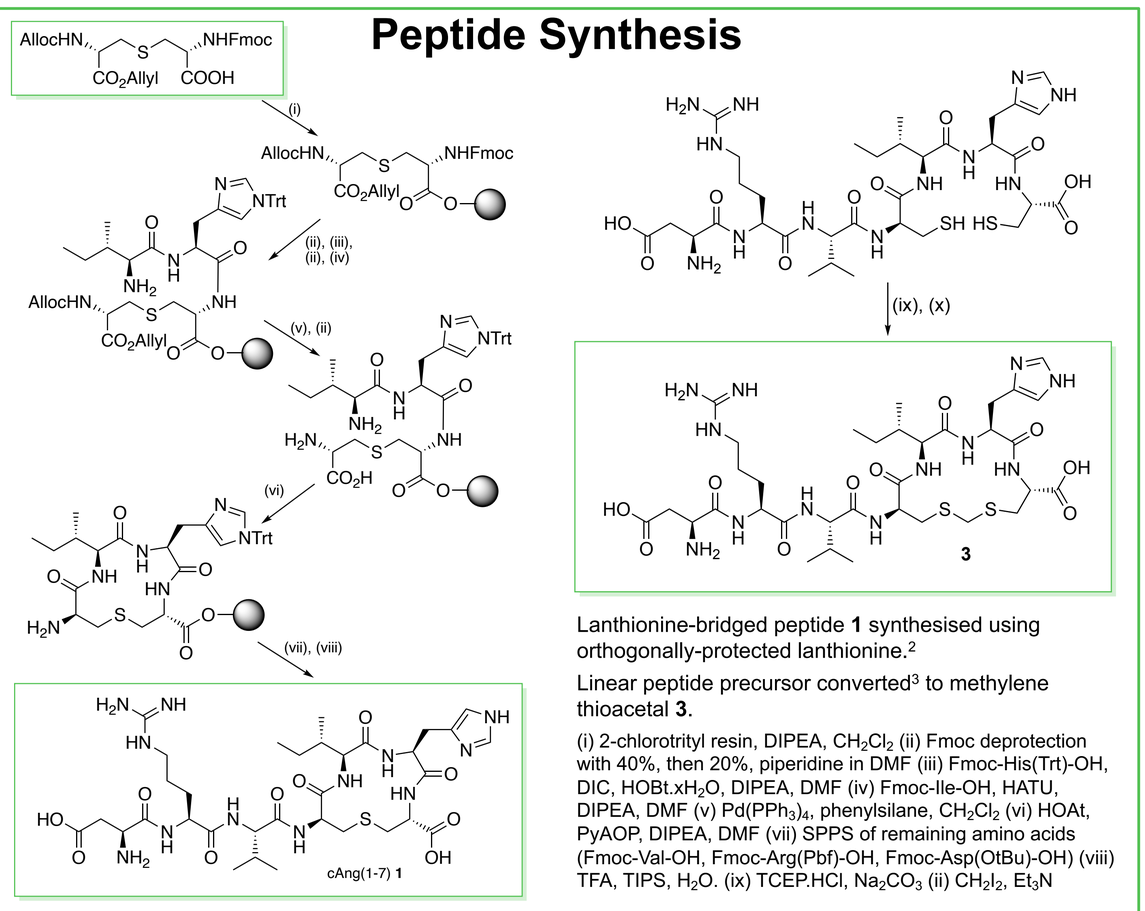
What are the structural effects of the lanthionine bridge? How do these compare with disulfide and methylene thioacetal bridges?



peptide **2**
disulfide bridge
14 membered ring

peptide **3**
methylene thioacetal bridge
15 membered ring

Peptide Synthesis

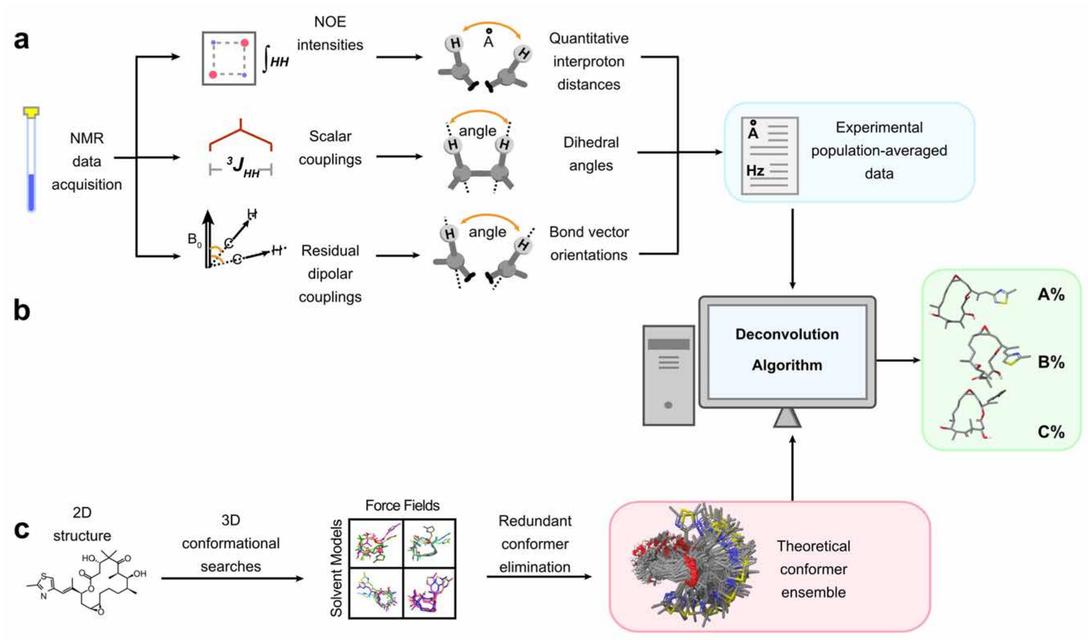


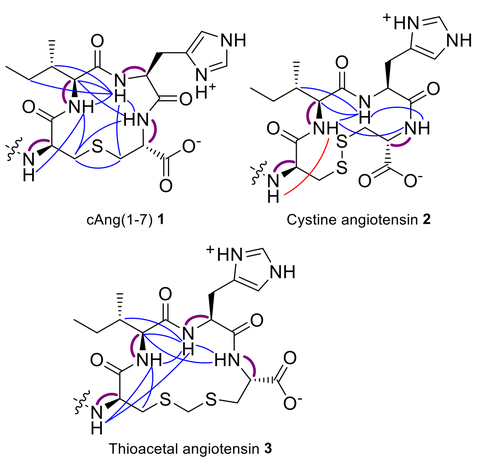
Lanthionine-bridged peptide **1** synthesised using orthogonally-protected lanthionine.[2]

Linear peptide precursor converted[3] to methylene thioacetal **3**.

(i) 2-chlorotrityl resin, DIPEA, CH_2Cl_2 (ii) Fmoc deprotection with 40%, then 20%, piperidine in DMF (iii) Fmoc-His(Trt)-OH, DIC, HOBt.xH_2O, DIPEA, DMF (iv) Fmoc-Ile-OH, HATU, DIPEA, DMF (v) $Pd(PPh_3)_4$, phenylsilane, CH_2Cl_2 (vi) HOAt, PyAOP, DIPEA, DMF (vii) SPPS of remaining amino acids (Fmoc-Val-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asp(OtBu)-OH) (viii) TFA, TIPS, H_2O. (ix) TCEP.HCl, Na_2CO_3 (ii) CH_2I_2, Et_3N

NAMFIS





cAng(1-7) **1**

Cystine angiotensin **2**

Thioacetal angiotensin **3**

NAMFIS uses NOE-derived interproton distances and $^3J_{HH}$ scalar coupling constants to find the best fit solution between a population weighted combination of theoretical conformations and experimentally determined distances and coupling constants.[4]

NMR experiments carried out in 9:1 H_2O:D_2O mixtures.

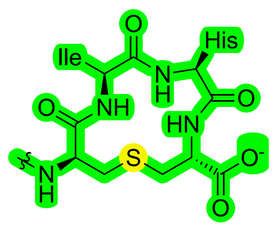
Solvent suppression obscured and altered the integrals of some α protons: for these, interproton distances were instead determined in D_2O with EASY ROESY without solvent suppression.

MCMM conformational searches were performed using MacroModel as implemented within the Schrödinger suite[5] to create the theoretical input ensembles.

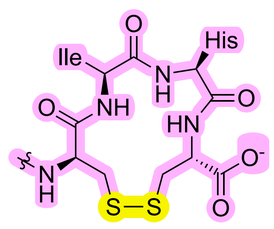
Inter-residue interproton distances (blue lines) and scalar couplings (purple lines) used in the NAMFIS analyses are shown.

The red line indicates an interproton distance that when removed during validation causes an 18% change in the solution ensemble of peptide **2**.

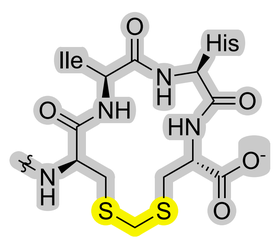
Results



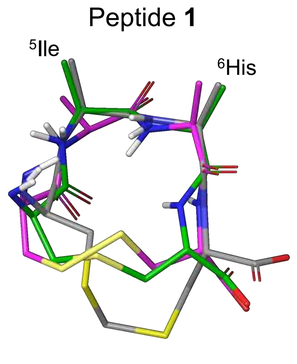
Peptide **1**



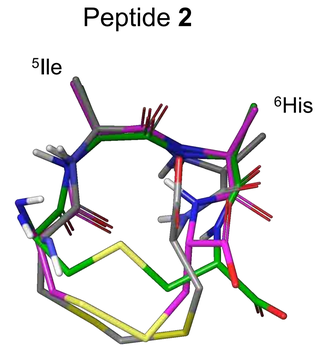
Peptide **2**



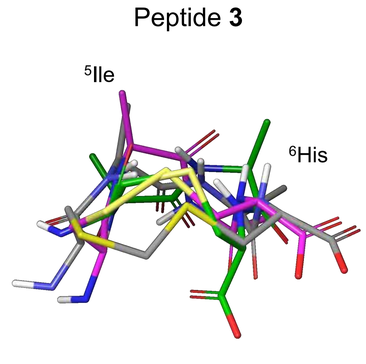
Peptide **3**



Conformer A1
Peptide **1** : 51%
Peptide **2** : 19%
Peptide **3** : 26%
RMSD 0.57 – 0.58 Å



Conformer A2
Peptide **1** : 35%
Peptide **2** : 61%
Peptide **3** : 31%
RMSD 0.57 – 0.77 Å



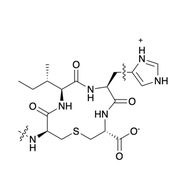
Conformers B1-3
Peptide **1** : 8%
Peptide **2** : 7%
Peptide **3** : 8%
RMSD 1.12 – 1.73 Å

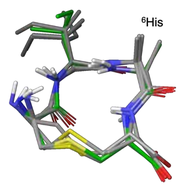
For each of the peptides, two conformational families, **A** and **B**, are observed.

In **A** the ^{4}Xaa NH is oriented towards the macrocyclic R groups.

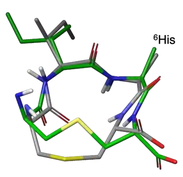
Larger rings have more sub-conformers.

The flexible side-chains of ^{5}Ile and ^{6}His, and the methylene thioacetal, cannot be accurately determined.

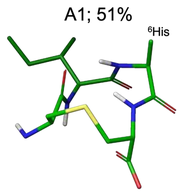




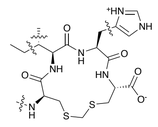
A1; 51%

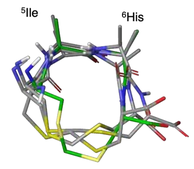


A2; 35%

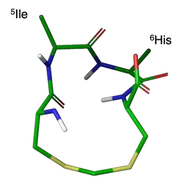


B2; 8%

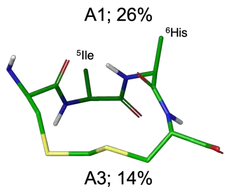




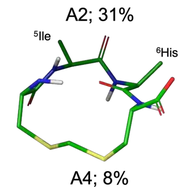
A1; 26%



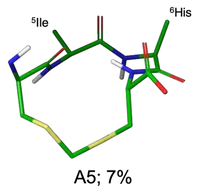
A2; 31%



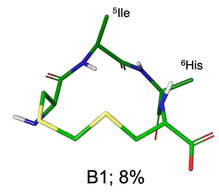
A3; 14%



A4; 8%



A5; 7%



B1; 8%

Conclusions

- Changing the length of the bridge appears to have almost no effect on the conformational families seen[6]
- The molar fraction of each conformation changes between analogues (by up to 35%)
- The three conformations are more equally distributed in the thioacetal, likely due to increased flexibility
- Receptor binding conformation is unknown at present
- Will this apply to other cyclic peptides?

(1) L. D. Kluskens *et al., J. Pharmacol. Exp. Ther.* **2009**, *328*, 849
(2) R. Dickman *et al., J. Org. Chem.* **2019**, *84*, 11493
(3) C. M. B. K. Kourra, N. Cramer, *Chem. Sci.* **2016**, *7*, 7007
(4) L. H. E. Wieske, S. Peintner, M. Erdélyi, *Nature Rev. Chem.* **2023**, *7*, 511
(5) F. Mohamadi *et al., J. Comput. Chem.* **1990**, *11*, 440
(6) W. T. P. Darling *et al., Chem. – Eur. J.* **2024**, *30*, e202401654

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