

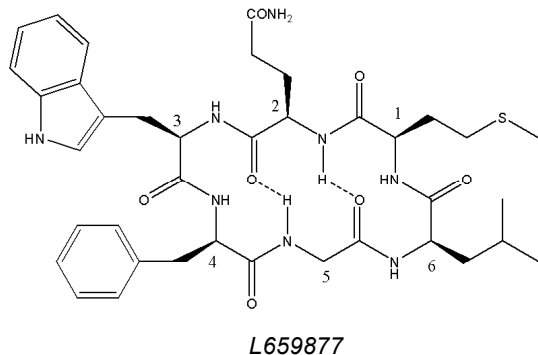
## 7-Azabicyclo[2.2.1]heptane-2-carboxylic acids as peptidomimetic conformational constraints. NK2 antagonists as an example.

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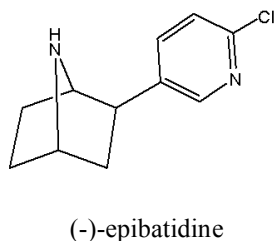
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### Introduction

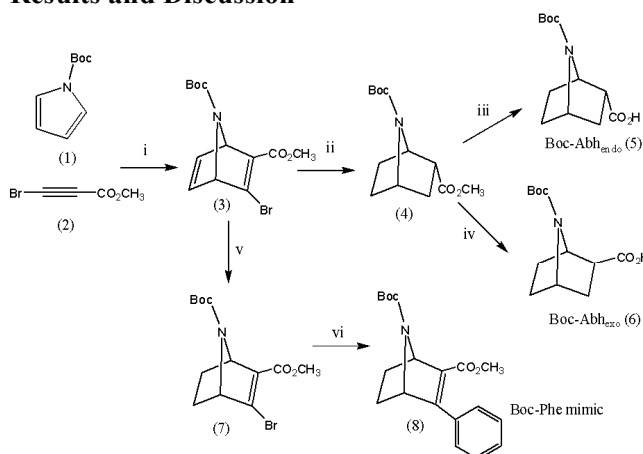
L659877 is a moderately potent, selective NK2 antagonist. It was the lead compound in the development of nepadutant, a bicyclic glycopeptide currently under clinical evaluation for irritable bowel syndrome. Based upon the x-ray structure of another bicyclic analogue, MEN10627, the bioactive conformation has been postulated to contain two reverse turns as shown (Fig. 1). As such it represents an interesting benchmark against which to examine alternate approaches to peptidomimetic design and the use of conformationally defined scaffolds in particular. In our review of the conformational data, we speculated that placing a constraining residue at position 6 of L659877 might stabilize the bioactive conformation. Successful restriction of peptide conformation can lead to increased agonistic or antagonistic potency, increased target selectivity, prolonged biological activity and increased resistance to enzymatic degradation



We have been examining the 7-azabicyclo[2.2.1]heptane structure as a scaffolding template; found in the alkaloid epibatidine, it offers a relatively rigid template structure and the potential for generating chirality driven conformational diversity.



### Results and Discussion

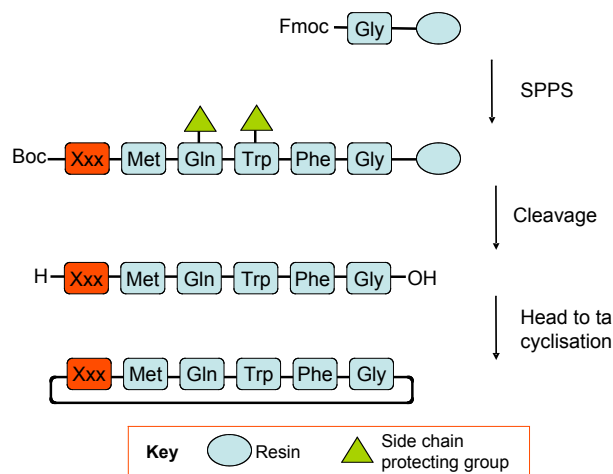


Reagents and Conditions: i, 90°C, 30h; ii, H<sub>2</sub>, Pd/C, RT, 4h; iii, LiOH(aq), THF; iv, NaOCH<sub>3</sub>, CH<sub>3</sub>OH then H<sub>2</sub>O; v, NiBr, vii, PhBO<sub>2</sub>H, Pd(OAc)<sub>2</sub>.

Scheme 1 Synthesis of Boc-protected 7-azabicyclo[2.2.1]heptanoates

The syntheses of these residues have been achieved using concise and versatile routes adapted from the literature, that also allow for the incorporation of side chain mimetics. (Fig. 1) Starting from N-Boc-pyrrole, **1**, the bicyclic nucleus is obtained by a Diels-Alder reaction followed by reduction to remove the double bond and the bromo-substituent, yielding predominantly the endo ester, **4**. Ester hydrolysis gives the Boc-protected endo acid, **5**, while a one pot epimerisation and hydrolysis gives the exo form of the acid, **6**. The method can also be adapted to yield substituted forms such as the phenylalanine mimetic, **8**. Selective reduction of **2** with Nickel Boride followed by Suzuki coupling provides the protected ester.

We have synthesized the cyclic NK2 antagonist L659877 and Abh-containing analogues via the head to tail cyclisation of the linear peptides using BOP reagent in DMF (Scheme 1). Inclusion of the variable amino acid as the amino terminus of the linear chain allows the use of an Fmoc-based strategy and batch synthesis of the resin bound pentapeptide. In addition to Leu, and the Abh isomers, L-Pro- and D-Pro-containing peptides were prepared. Intramolecular cyclisation was found to be sequence dependent, with poor cyclisation yields obtained in the case of Abh<sub>endo</sub> in particular.



Scheme 2 Synthetic route to L659877 and analogues

The  $Abh_{exo}$  and  $Abh_{endo}$  isomers exist as pairs of enantiomers around the bridgehead nitrogen. While these enantiomers have not been resolved in synthesis, as shown in Figure 2, the stereoisomers of the  $Abh_{exo}$ -containing peptides are resolvable on an analytical scale. To date, just one (L659 B) has been purified to homogeneity in sufficient quantities for assay. The absolute stereochemistry has yet to be determined. The  $Abh_{endo}$  containing peptides either do not resolve or only a single diastereomer undergoes cyclisation.

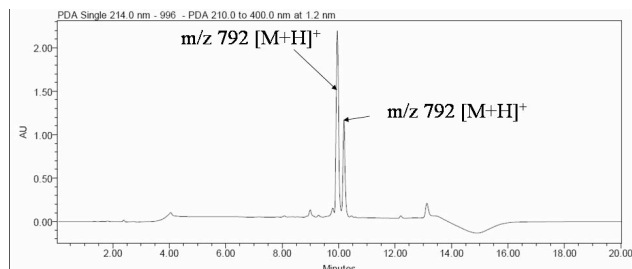


Figure 1 Analytic RP-HPLC chromatogram of cyclo( $Abh_{exo}$ -Met-Gln-Trp-Phe-Gly)

L659877 and analogues were tested in a functional organ bath assay of NK2 function. In brief, sections of mouse bladder tissue were treated with the Neurokinin A in the presence or absence of test compounds, and the shift in the dose response curve evaluated. As shown in Figure 3, all compounds except  $Abh_{endo}$  show a significant shift in the curve, in some cases the shift is typical of competitive antagonism, in others (eg. D-Pro) non-competitive phenomena are observed. These potent inhibitors are currently being further analysed in more extensive assays, to determine their antagonist profile.

### Conclusion

An effective means of generating conformational restricted peptides to study peptide SAR requires the development of scaffold libraries that can produce an array of shapes. The azabicycloalkanes achieve this and moreover can be adapted to introduce "side-chain" like

groups. Incorporation of these amino acids into cyclic L659877 analogues yielded compounds with enhanced antagonist affinity, suggesting stabilisation of the bioactive conformers. Future work to provide for enantioselective synthesis will allow for the full exploration of the potential of these scaffolding moieties.

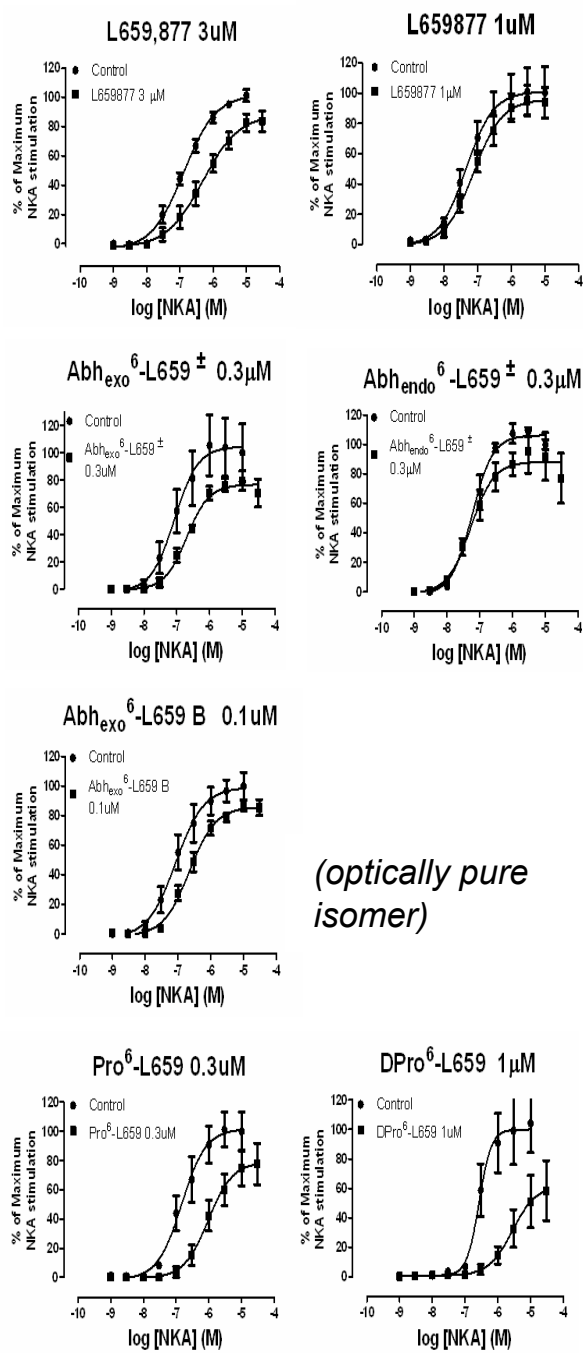


Figure 2 Antagonism of NKA-induced stimulation of mouse bladder sections by L659877 and analogues.

### References

- Thompson, PE; Steer, DH, Aguilar, M.-I., Hearn, MTW (1998) *Bio-org. Med. Chem. Letters* **8**, 2699-2705
- Johansen, A. (2003) *Current Topics Med. Chem.* **3**, 1436-1445.