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Synthesis of Piperazinones and their Application in Constrained Mimetics of the Growth Hormone Secretagogue NN703

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Abstract: The focus of this paper is the chemistry of 2-piperazinones and the use of this building block to restrict the conformational freedom of the growth hormone secretagogue NN703 (currently in clinical development). We exploit classical methods for 2-piperazinone synthesis as well as some novel approaches such as a Boronic Mannich reaction. Finally, we report on the ability of the constrained target compounds to release growth hormone *in vitro*.

Keywords: *peptide mimetics; piperazinone; boron and compounds, amino acids.*

Introduction: The novel orally active growth hormone secretagogue[1] NN703[2] is currently undergoing clinical trials. It is a linear and very flexible molecule and its flexibility can be a problem in modelling studies. Recently we published a modelling study[2] of the overlap between NN703 and the less flexible MK677. We desired more diversely constrained molecules in order to gain more information about the binding conformation of NN703 *via* molecular modelling. This prompted us to undertake the task of synthesising constrained versions of NN703. We chose to incorporate piperazinones as amino acid mimics as this would reduce the conformational flexibility and could lead to improved receptor binding due to reduced entropy. In particular, we were interested in making N-terminal, central or C-terminal constraints as indicated in Figure 1. This approach suggested the use of methods for piperazinone synthesis that were either uncommon or unknown in the literature in order to accomplish the synthesis of constrained analogues of NN703.

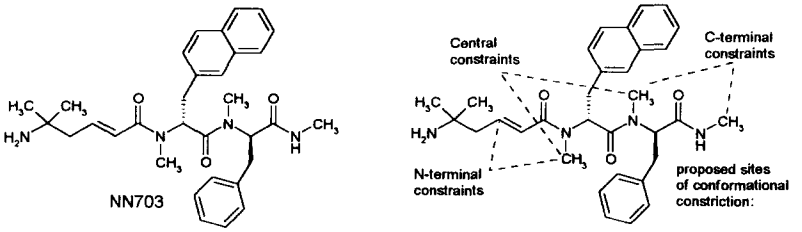

Fig. 1

Our first objective[3] was the incorporation of (3*R*)-3-benzyl-2-piperazinone as a constrained mimetic of the C-terminal *D*-phenylalanine group of NN703 (Fig. 2). For this purpose we started from Boc-glycinal **1** (in our hands best prepared

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freshly by the method of Dueholm et al[4]) and performed a reductive alkylation with *D*-phenylalanine methyl ester to yield **2** (50-60% along with a few percent bis-alkylation).

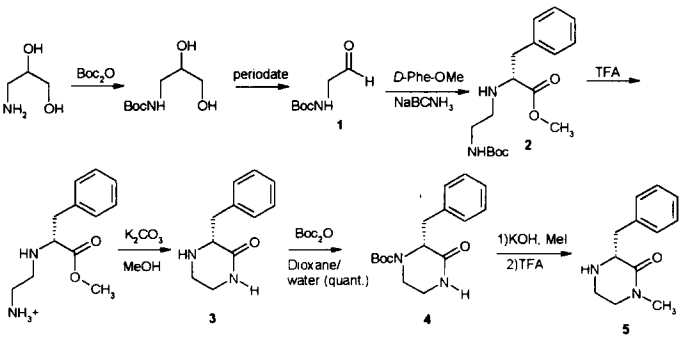

Fig. 2

After deprotection of **2** with TFA we attempted acidic as well as basic cyclisation and obtained the best results with potassium carbonate in methanol at room temperature (75-82 % yield of **3**). Such piperazinones[5] participated readily in EDAC/HOAt mediated peptide couplings analogous[2] to NN703. However, for the purpose of alkylation of the amide nitrogen of the piperazinone we performed a Boc protection and investigated a number of alkylation methods. Using 1 eq LDA/THF/alkyl bromide gave poor results, whereas DMSO/4 eq KOH/alkylhalide was satisfactory (55-60% yield) for the conversion of **4** to **5**. (However, for S_nAr substrates like 2-fluoronitrobenzene our method of choice is TBAF in refluxing THF). This method provided *N*-alkylated piperazinones without detectable racemisation. Drawbacks of this method are the number of steps involved and the fact that only a relatively limited number of amino acids are commercially available. Attracted by the inherent simplicity we turned to Petasis' amino acid protocol[6] to assess its potential for synthesising piperazinones (Fig 3). This method provides amino acids in just one step by a three-component Boronic Mannich reaction based on simply mixing an aryl or alkenyl boronic acid, an amine and an aldehyde at room temperature in methanol or methylene chloride as shown in fig. 3.

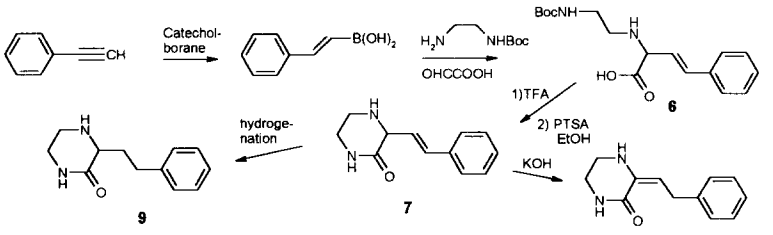

Fig. 3

We found that the reaction proceeds well when glyoxylic acid is used as the aldehyde component generating the aminoethyl derivative **6** from a protected diamine in 88% yield by direct precipitation from the reaction mixture. It was possible to cyclise **6** after removal of the Boc group by refluxing in EtOH with catalytic PTSA (64%). Care should be taken during work-up as compound **7** readily isomerized to the dehydroamino acid **8** if conditions were too basic. (We are pursuing asymmetric hydrogenation of **8** as a way of solving the enantioselectivity problem).

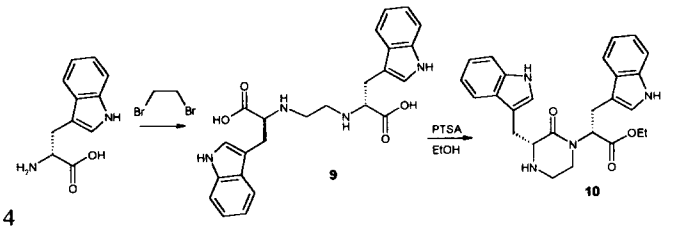

Fig.4

The piperazinone moiety was introduced in the central part of the molecule (Fig.4) by Yamashita's method[7] which is an extremely simple method for generating a constrained mimetic of a dipeptide composed of two identical amino acids. We chose *D*-trypthophan as it is known that the indole unit is tolerated at both positions in derivatives of NN703. Compound **10** was generated in just two steps (21% and 69% yields respectively) from D-tryptophan *via* ethylene-bridged compound **9** and could readily be elongated by ordinary peptide couplings. It remains to find an easy method for generating such compounds from two different amino acids.

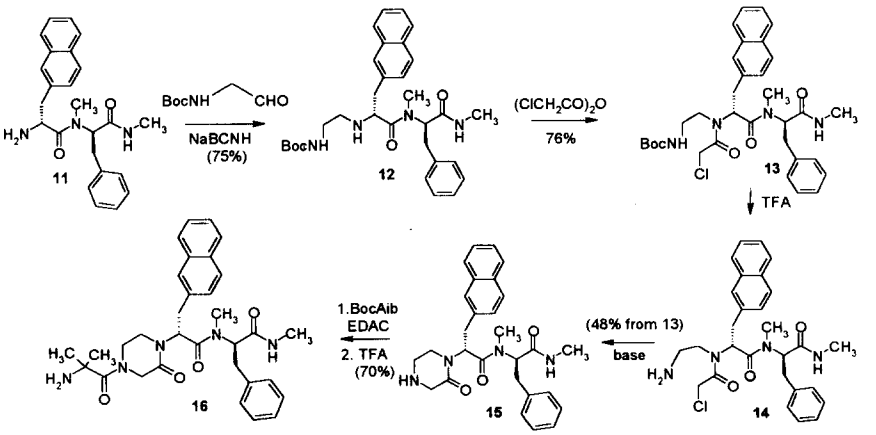

Fig. 5

Finally, we introduced constraints in the *N*-terminal region of NN703 by substituting the alkenyl spacer with a piperazinone ring (Fig 5). Such very rigid N-terminals were readily prepared by a reductive alkylation of Boc-glycinal on the dipeptide **11** to give **12** which was then acylated with chloroacetic anhydride to afford **13**. After removal of the Boc protecting group with TFA cyclisation of **14** was achieved by raising the pH to neutral. The piperazinone **15** could then be elongated by ordinary peptide couplings to the NN703 analogue **16**. Yields in this sequence were good.

The analogues of NN703 that have been prepared by the methods above were biologically evaluated with respect to GH-release in an assay[8] based on rat pituitary cells. The EC_{50}-values found in this assay are given in the compilation of final products in figure 6. These values should be compared to NN703 which in the same setup was found to be 18 nM. It is clear that most of the novel analogues are less potent than NN703 and consequently our work was not very helpful in modelling studies. This is particularly true for those constraints that are incorporated in the central or N-terminal part of NN703. However, there is one important exception as the C-terminally constrained derivative NNC26-0737 (fig 6) turned out to be equipotent or even slightly more potent than NN703. This result has been expanded into a new series of GH-secretagogues with interesting biological properties[5]. But

already from the results presented here it is clear that the piperazinone is sensitive to substitution as shown by the reduced potency of **17** and **18** and that a one carbon chain from the piperazinone to the phenyl is better than a two carbon chain in contrast to previous findings[9].

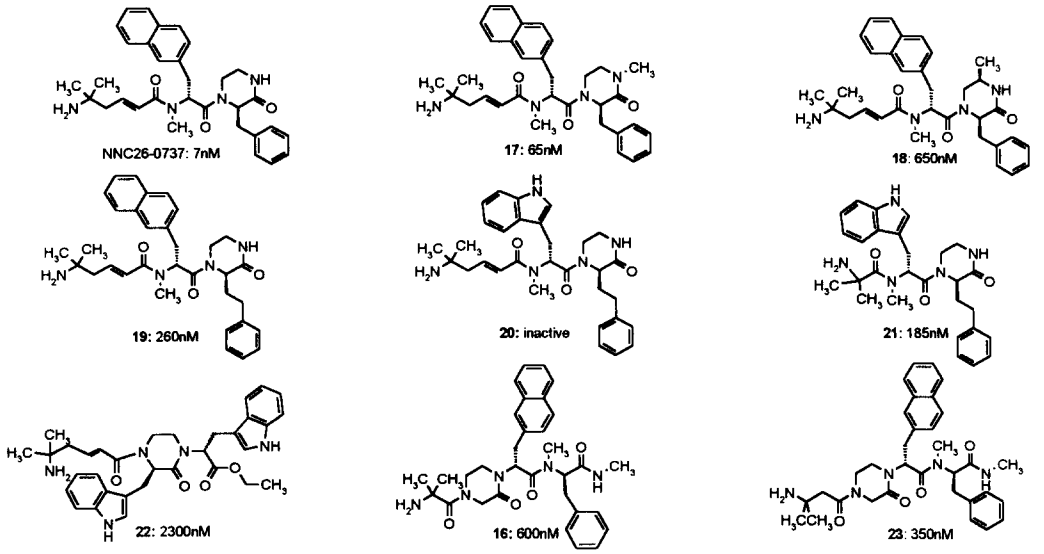

Fig. 6

In summary we have developed methodology which may be useful in the determination of the active conformation of peptides *via* incorporation of piperazinones and we have provided examples of the use of such chemistry to incorporate constraints in three different positions of the growth hormone secretagogue NN703. A C-terminally constrained analogue was found to be as active as NN703.

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