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PREPARATION OF NEUROTENSIN ANALOGS WITH A NOVEL PRO-TYR REPLACEMENT

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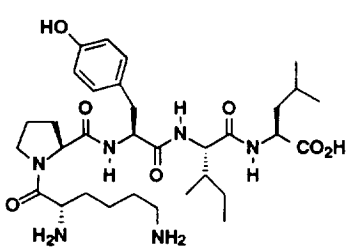
Abstract. Synthesis of novel Neurotensin (NT) 9-13 analogs is described. The Pro-Tyr sequence of NT (9-13) was replaced with a 1-*m*-aminophenyl-1-benzyl urea template, substituting two amino acids with a simple substituted urea linkage.

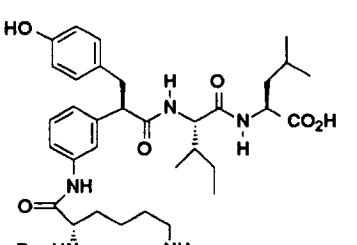
Introduction. Neurotensin (NT)[1] is a 13 amino acid peptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) found distributed throughout the central and peripheral nervous system. Several biological properties are attributed to NT including analgesic,[2] psychotropic,[3] and hypothermic[4] actions. NT is more potent than morphine when administered by i.c.v. route.[5] In addition, NT has been linked with the biology of schizophrenia where depressed levels of NT in the cerebrospinal fluid are found.[6] As a result, a selective NT agonist may have therapeutic benefits in treating schizophrenia and pain.

Several groups have reported smaller fragments of NT (1-13) with similar activity. The minimal native sequence needed for full biological activity is the C-terminal hexapeptide (Arg-Arg-Pro-Tyr-Ile-Leu), NT (8-13). Several studies show similar activity with the NT (8-13) fragment when compared to the complete sequence NT (1-13).[7] In addition, it is possible to replace the Arg-Arg N-terminal fragment in NT (8-13) with Lys without a significant loss of binding affinity.[8] Other studies have explored the replacement of amino acids from the NT (8-13) fragment with simple heterocycles to increase metabolic stability and bioavailability.[9]

This report describes the preparation of novel NT analogs using the NT (9-13) Lys-Pro-Tyr-Ile-Leu sequence as a starting point . Our efforts centered on the Pro-Tyr section of NT (9-13). By replacing the Pro-Tyr fragment with a simpler entity, we could increase the compound's metabolic stability, improve bioavailability, and possibly modulate its agonist or antagonist properties.



NT (9-13)



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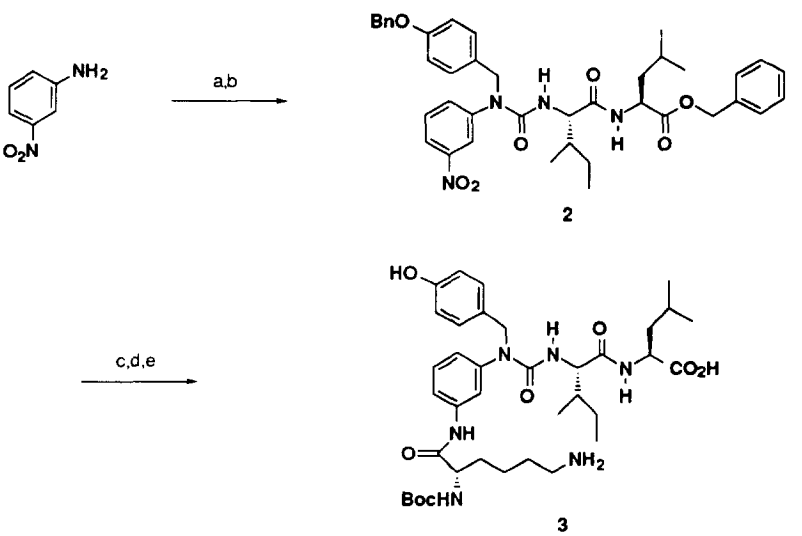
Modeling studies performed on NT (9-13) showed the Pro-Tyr fragment would be a good replacement target.[10] A simple *m*-disubstituted benzene induces a similar type of bend as seen in the native peptide while aligning the other residues in a desirable orientation relative to NT (9-13). Introduction of a *p*-hydroxybenyl substituent would mimic the Tyr side chain. A recent report [11] showed compound **1** had good biological activity in the NT binding assay, and was shown to have agonistic properties in a functional assay. We hoped to improve the activity of **1** and further simplify the molecule by replacing the carbon linker of the aniline ring with a nitrogen atom. Additional modeling studies indicated the urea linkage would slightly alter the desired bend angle, but would retain the necessary binding elements in a similar orientation compared to **1**. Forming a urea linkage would also remove a chiral center and simplify the synthesis.

Scheme 1[a]

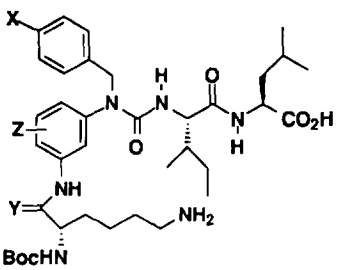


[a]**Reagents and conditions**: a) *p*-Benzyloxybenzaldehyde, $Na(CN)BH_3$, MeOH, 0 °C (90%); b) diphosgene, THF, 0 °C, then H_2NIleLeuOBn, reflux (85%); c) $SnCl_2$, EtOH, reflux (90%); d) Boc(Z)Lys, DCC, CH_2Cl_2, 25 °C (90%); e) H_2, 10% Pd/C (95%).

Chemistry. A representative approach to these analogs is shown in Scheme 1. Reductive amination of *p*-benzyloxybenzaldehyde with *m*-nitroaniline introduced the Tyr-like fragment in good yield. Activation of this secondary amine with diphosgene and coupling with the dipeptide fragment H-Ile-LeuOBn gave compound **2** in 77% overall yield. The nitro group was reduced using $SnCl_2$. The resulting amine was coupled to the Lys portion of NT (9-13) using a standard dicyclohexylcarbodiimide (DCC) promoted coupling reaction. At this point it was also possible to introduce reduced amide bond surrogates. Substituting the DCC coupling reaction with a reductive amination, using Boc(Z)Lys aldehyde, led to the reduced amide bond surrogate **5**. Finally, catalytic hydrogenation

removed the three protecting groups (benzyl ether, benzyl ester, and Cbz) in one pot to give the desired compound **3**. The Boc protecting group was left intact on these compounds. Previous studies showed increased hydrolytic stability and blood-brain-barrier penetration with NT (9-13) analogs carrying a large lipophilic N-terminal protecting group.[12]

Table 1



compound	X	Y	Z	K_i (μM)
1				0.029
3	OH	O	H	1.25
4	OH	O	2-fluoro	2.20
5	OH	H, H	H	0.73
6	F	O	H	0.89
7	OH	O	4-fluoro	>10

Results and Discussion. Compounds were evaluated in a NT binding assay[13] and the results are shown in Table 1. Although the compounds do not bind with similar affinity to the NT receptor as our model compound **1**, they still show submicromolar activity. Replacing a sp^3 carbon with a sp^2 hybridized nitrogen effects the angle of the bend we originally tried to mimic. The bend angle, induced by the urea linkage, mostly effected the Lys residue's orientation when compared to compound **1**. The reduced amide bond surrogate **5** shows a slight increase in affinity. The resulting increased mobility of the Lys moiety, allows it to adopt a more favorable conformation and increase binding afinity. Attempts at gaining additional hydrogen bond interactions, using fluorine substitution on the aromatic ring, reduced the binding affinity. It is also possible to replace the Tyr phenolic hydroxyl group with a fluorine atom with positive results. Compound **6** shows a slight increase in binding relative to compound **3**. This may ultimately improve the pharmacokinetic profile of these compounds. By replacing an undesirable metabolic handle (substituting F for OH), we may decrease the clearance rate of subsequent analogs and increase bioavailability.

Conclusion. We presented a series of nuerotensin analogs with a novel Pro-Tyr replacement having submicromolar binding affinities. This study shows the ability to replace two amino acids in the NT (9-13) sequence with a simple 1-*m*-aminophenyl-1-benzyl urea template and retain binding affinity. The synthetic scheme is straightforward and high yielding starting from simple commercially available materials. It also allows for generating a wide variety of compounds by varying the amino acids in the subsequent coupling reactions. Additional analogs need to be made to fully exploit this finding. The preparation and evaluation of such compounds is in progress and will be reported in due course.

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