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SYNTHESIS OF PARTIALLY NON-PEPTIDIC NEUROTENSIN MIMETICS

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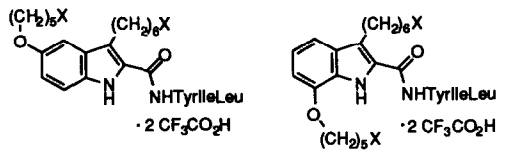
Abstract. The synthesis of partially non-peptidic mimetics for NT(8-13) is described. The sequence $Arg^8Arg^9Pro^{10}$ of NT(8-13) was replaced by an appropriately substituted indole-2-carboxylate as the non-peptidic equivalent.

INTRODUCTION

Neurotensin (NT)[1] is a tridecapeptide (pGlu^{1}Leu^{2}Tyr^{3}Glu^{4}Asn^{5}Lys^{6}Pro^{7}Arg^{8}Arg^{9}Pro^{10}Tyr^{11}-Ile^{12}Leu^{13}) that is found distributed throughout the central nervous system (CNS) and in the peripheral nervous system. NT is associated with many physiological functions,[1] including the production of hypotension[1a] and reduction of pain sensation.[2] As an analgesic, NT has proven to be more potent than morphine when it is administered directly into the CNS.[2] More interestingly, NT has been associated with the pathophysiology of schizophrenia.[1b,3] Treatment of schizophrenic patients with antipsychotic drugs causes the normally depressed NT levels in the cerebrospinal fluid of these patients to return to normal levels. It has been suggested that there may be a possible role for selective NT agonists as therapeutic agents in the treatment of schizophrenia[3a] and in the alleviation of acute and chronic pain.[2a]

Given the important role of NT in biology, we began a program[4] aimed ultimately at developing a full NT peptidomimetic based on the *C*-terminal hexapeptide ($Arg^8Arg^9Pro^{10}Tyr^{11}Ile^{12}Leu^{13}$), NT(8-13). Various studies[1c,2] have shown that NT(8-13) elicits biological activity with a similar potency to that shown by NT(1-13). These mimetics should be organic molecules with improved stability and hydrophobicity which may additionally possess improved blood brain barrier permeability, selectivity, affinity, and degrees of agonism or antagonism. We have recently reported the synthesis[4a] and the biological activity[4b] of partially nonpeptidic NT mimetics **1** and **2**[4b] and their diamino analogs **3** and **4**[4a] (Figure 1). We wish now to disclose the synthesis of additional NT mimetics and preliminary results regarding their receptor binding.

Figure 1



1: X = NHC(=NH)NH_2 **2**: X = NHC(=NH)NH_2
3: X = NH_2 **4**: X = NH_2

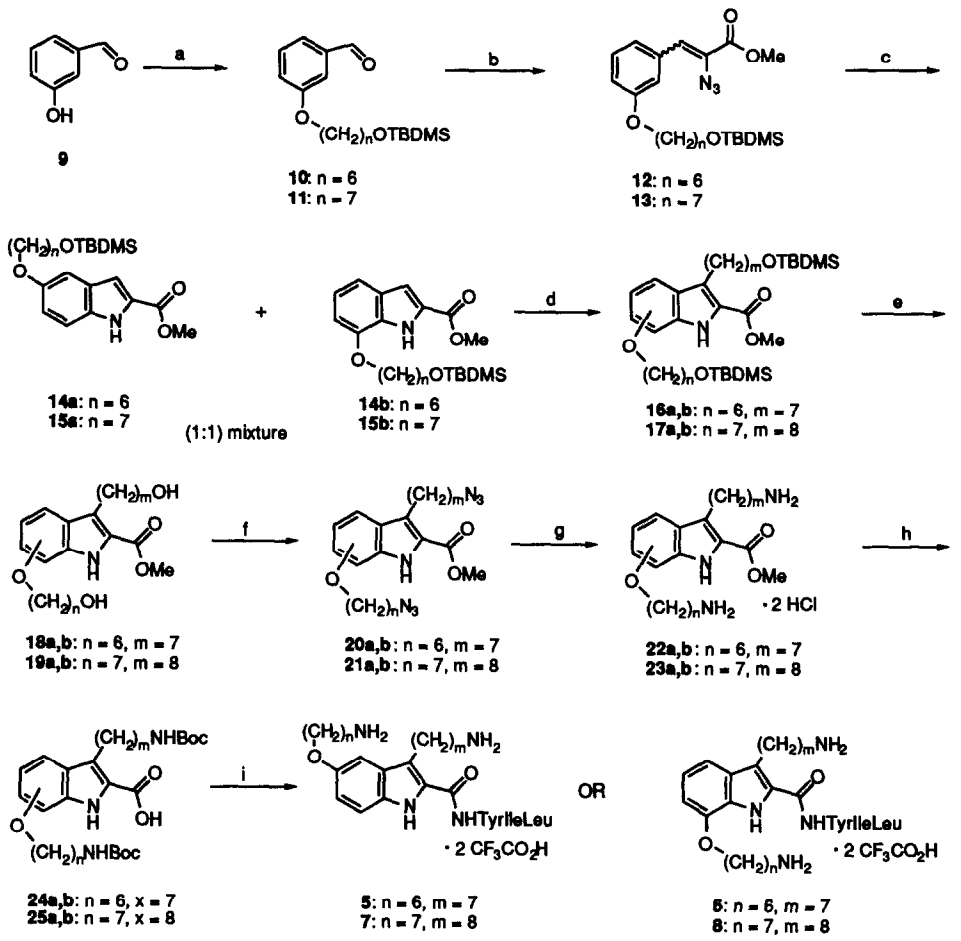
In the design of these partially non-peptidic mimetics, it was our plan to first optimize the nonpeptidic equivalent of the tripeptide fragment $Arg^8Arg^9Pro^{10}$ of NT(8-13) and later to search for equivalents of the last three amino acids. For the former fragment, a range of dimensions was calculated with the aid of computer modeling[4c] of which a subset was translated synthetically into two structures (**1** and **2**) containing indole-2-carboxylates substituted with guanidine containing appendages at C-3/C-5 and C-3/C-7, respectively.

Mimetics **1** and **2** were tested for their ability to compete for [^{3}H]NT binding and for their action on intracellular cGMP production or on PI turnover in N1E-115 neuroblastoma cells.[4a,b] The equilibrium dissociation constants (K_d) for **1** and **2** were measured to be 3.3 μM and 1.9 μM, respectively. Functionally, **1** and **2** antagonized NT stimulated production of cGMP. Interestingly, mimetic **2** displayed a dualistic pharmacological profile depending on its concentration.[4b] Mimetic **2** behaves as a full agonist stimulating cGMP production at higher doses in the 10-100 μM range with an EC_{50} of 19 μM. We have proposed that mimetic **2** binds to a higher affinity site when acting as an antagonist and to a different, low affinity site when acting as an agonist. Hoping that the replacement of the guanidino by amino groups might decrease the hydrophilicity of these mimetics without significant loss in binding, as found when Arg^8 and Arg^9 in NT(8-13) are replaced by Lys,[5] we also prepared the diamino analogs **3** and **4** of mimetics **1** and **2**. They were also found to bind to the NT receptor of N1E-115 cells but with decreased potency, possibly as a consequence of shortening the chains at C-3 and C-5 or C-7 by two atoms. To compensate for this effect, mimetics **5** and **6** as well as **7** and **8** (Scheme 1) containing one or two extra carbon atoms on each side chain were synthesized and evaluated.

SYNTHESIS

Mimetics **5** to **8** were prepared by a protocol similar to that employed in the synthesis of **3** and **4**. The synthesis of mimetics **5** and **6** (Scheme 1)[6] is described as an example. Alkylation of *m*-hydroxybenzaldehyde (**9**) with 1-iodo-6-(*tert*-butyldimethylsilyloxy)hexane gave the alkoxybenzaldehyde **10** in 90-95% yield. Reaction of **10** with 4 equivalents of methyl azidoacetate[7] in the presence of NaOMe in MeOH at -10 °C gave the azidoacrylate **12** which on refluxing in toluene for 4 h led via a thermally induced nitrene insertion reaction[7] to a mixture of the two indoles **14a** and **14b** in a combined yield of ~50 %. These indoles were separated on silica gel and subjected individually to further modifications. Alkylation at the indole 3-position with 1-iodo-6-(*tert*-butyldimethylsilyloxy)heptane in refluxing CH_3CN in the presence of anhydrous K_2CO_3 gave **16a** and **16b**, respectively, in 75-80% yield. Treatment of these intermediates with tetrabutylammonium fluoride generated diols **18a** and **18b**, respectively, which were then converted to their respective diazides **20a** and **20b** by the sequence of mesylation followed by reaction with NaN_3 in DMF in combined yields of 80-85%. Catalytic hydrogenation (10% Pd/C) of these azides at atmospheric pressure in the presence of concentrated HCl yielded the diamine hydrochlorides **22a** and **22b**, respectively, in 90-95% yield. Their reaction with Boc_2O followed by ester saponification gave the acids **24a** and **24b** in 80% overall yield. HOBt/DCC mediated coupling of **24a** to the tripeptide H_2NTyrIleLeu bound to Wang resin[8] followed by treatment with a trifluoroacetic acid cocktail and purification by reverse phase HPLC provided mimetic **5**. The same procedure involving carboxylic acid **24b** gave mimetic **6**. Both mimetics exhibited correct LSI (liquid secondary ion)-HRMS data. Mimetics **7** and **8** were prepared similarly starting from indoles **15a** and **15b**, respectively.

Scheme 1[a]

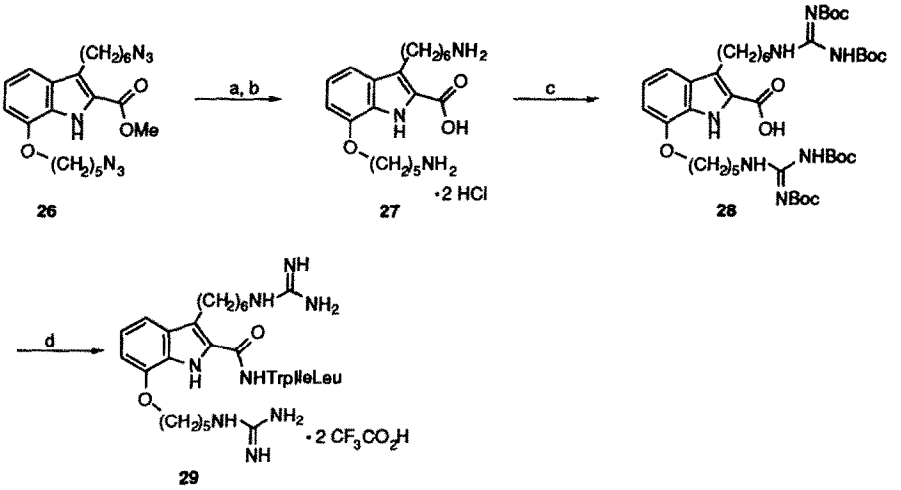


[a]Key: a) NaH, $I(CH_2)_nOTBDMS$, DMF; b) $N_3CH_2CO_2Me$, NaOMe, MeOH, -10 °C; c) $PhCH_3$, reflux; d) $I(CH_2)_mOTBDMS$, K_2CO_3, CH_3CN, reflux; e) TBAF, THF; f) 1. CH_3SO_2Cl, Et_3N, CH_2Cl_2; 2. NaN_3, DMF; g) 10% Pd/C, H_2, MeOH, HCl; h) 1. Boc_2O, Et_3N, CH_2Cl_2; 2. 2N KOH, MeOH, THF, rt; i) 1. H_2NTyr-Ile-Leu-CO_2-resin, HOBt, DCC, *N*-methylpyrrolidone; 2. Cleaving cocktail (10 mL TFA/0.25 mL ethanedithiol/0.5 mL thioanisole/0.5 mL H_2O), then HPLC purification.

We also prepared mimetic **29**, an analog of **2** in which Tyr was substituted with a Trp residue. This is our first attempt at the eventual replacement of the remaining amino acids NT(11-13). It has been shown that Tyr^{11} in NT(8-13) can be replaced by Trp without loss of activity.[9] The synthesis of **29** (Scheme 2) began with the saponification of the methyl ester in diazide **26**[4a] with 2N KOH followed by catalytic hydrogenation

using 10% Pd/C to give diamino acid **27** as the bishydrochloride salt in 80-85% yield over two steps. Treatment of **27** with *N,N'*-bis(*tert*-butyloxycarbonyl)-*S*-methylisothiourea in the presence of a methanolic solution of Triton-B[10] in DMSO gave the protected bisguanidine **28** in 82-85% yield. Acid **28** was then coupled to the tripeptide H_2NTrpIleLeuCO_2-Wang resin[8] using the HOBt/DCC conditions. The resin-bound coupled product was subsequently cleaved by treatment with the trifluoroacetic acid cocktail to give a crude mixture that was purified by reverse phase HPLC to give mimetic **29** which exhibited correct LSI-HRMS data.

Scheme 2[a]



[a]**Key**: a) 2N KOH, MeOH-THF; b) H_2, 10% Pd/C, MeOH, HCl; c) (CH_3S)(BocNH)C=NBoc, methanolic Triton-B, DMSO; d) 1. H_2NTrp-Ile-Leu-CO_2-resin, HOBt, DCC, *N*-methylpyrrolidone; 2. Cleaving cocktail (10 mL TFA/0.25 mL ethanedithiol/0.5 mL thioanisole/0.5 mL H_2O), then HPLC purification.

BIOLOGICAL RESULTS

Membranal preparations from both the rat and human NT receptor expressed in CHO-K1 cells[11a,b] revealed K_d's in the μM range for mimetics **2** to **8** and **29**.[12,13] The preliminary screening of these compounds allows us to group these mimetics according to their potency at the NT receptor. At the cloned rat NT receptor, mimetics **2**, **4**, and **29** were found to be equipotent with K_d's in the 0.3 - 0.7 μM range,[13] while **3** exhibited a K_d of about 40 μM. Mimetic **4** was also tested for its effect on PI turnover in these intact cells. An EC_{50} of ~ 1.3 μM was derived with some indication that this may be a partial NT agonist. The remaining mimetics were not examined at the rat NT receptor. These were further ranked according to their binding affinity at the human NT receptor with a final mimetic concentration of 50 μM. The rank order of binding affinity is as follows: (**29**) = (**2**) = (**4**) > (**3**) = (**6**) > (**5**) > (**7**) = (**8**). The effect of these mimetics on second messenger (PI or cGMP) turnover on the human NT receptor was not determined.

From these results, it is clear that increasing the lengths of the carbon chains that append the amino groups to the indole ring decreases rather than increases the binding potency at the NT receptor. Interestingly, the C-7 substituted indoles **4** and **6** bind more strongly to the NT receptor than their C-5 substituted counterparts **3** and **5**, respectively. This difference is less apparent between **7** and **8** where the chains have been increased by two carbon atoms. It is possible that these compounds are near the maximum limit of the chain length acceptable for mimetic-receptor interaction. Mimetic **29**, containing the Trp substitution, binds to NT receptors with similar affinity as the Tyr containing mimetic **2**. This result may prove useful for the design of the second generation of NT mimetics.

In conclusion, we have prepared partially non-peptidic mimetics for NT(8-13) that bind to NT receptors with K_d's in the μM range. This study shows that the tripeptide $Arg^8Arg^9Pro^{10}$ of NT(8-13) can be replaced by non-peptidic equivalents without abolishing biological activity. Although we failed to improve upon the binding affinity of mimetics **1** and **2**,[4a,b,13] the results presented herein show a trend that by increasing the lengths of the chains containing the amino groups on the indole template the binding potency decreases. Our future work will include the preparation of mimetics containing shorter side chains on the indole nucleus or of mimetics containing smaller molecular templates as $Arg^8Arg^9Pro^{10}$ equivalents.

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12. **Biological assays**: The rat and human neurotensin receptors were cloned in Chinese hamster ovary (CHO-K1) cell lines according to ref. 11a,b, harvested, and used in binding and functional assays as previously described in ref. 4b. Membrane preparation: harvested cells were homogenized in ice-cold 50 mM Tris-HCl (pH 7.4). The homogenate was spun at 38,000 g for 10 min, and the pellets were resuspended in fresh Tris-HCl buffer and spun again at 38,000 g. The final pellets were resuspended in Tris-HCl buffer containing 1 mM EDTA, 0.1% (wt/vol) bovine serum albumin, and 0.20 mM bacitracin. The protein concentration of the membrane preparation was estimated by the method of Lowry *et al.* (Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265), using bovine serum albumin as a standard. Radioligand binding assays were done on a Beckman Biomek 1000 workstation outfitted with a side arm loader (Cusack, B. M.; Richelson, E. *J. Rec. Res.* **1993**, *13*, 123.). Cellular membranes were diluted to provide 30 μg and 10 μg of protein per assay tube, respectively. Membranal preparations were incubated with 2 μM [^{3}H]neurotensin (DuPont NEN, Boston, Massachusetts) and varying concentrations of unlabeled NT, and the respective mimetics. Nonspecific binding was determined with 1 μM unlabeled NT in an assay tube with a total volume of 1 mL. Incubation was carried out at 4 °C for 30 min. The assay was routinely terminated by the addition of cold 0.9% NaCl (4 x 1.5 mL) followed by rapid filtration through a GF/B filter strip which had been pretreated with 0.2% polyethylenimine. The details of the binding assay, PI turnover, and the analysis of the data are as reported in ref. 4b.
13. We consistently find that crude membranal preparations of NT receptors exhibit a higher affinity than the same NT receptors in the intact cell line. Therefore, we cannot directly compare the K_d's obtained for this study with our previous findings.[4a,b]

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