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TWO NOVEL AMINO ACID DERIVATIVES CONTAINING SIDE-CHAIN THIOAMIDES FOR THE SYNTHESIS OF PHOTOACTIVATABLE PEPTIDES

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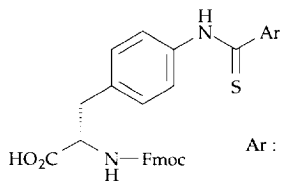
Abstract: The highly efficient preparation of two optically pure phenylalanine analogues containing *p*-N-aryl thiobenzamide moieties are described. These amino acids are readily incorporated into peptides *via* standard solid-phase strategies. © 1997 Elsevier Science Ltd. All rights reserved.

The manner in which signalling molecules in the body bind to membrane-bound receptors is largely unknown. In general, it has not been possible to study ligand-receptor interactions using biophysical approaches because of the difficulties associated with obtaining sufficient material for analysis. Furthermore, crystals of integral membrane proteins suitable for X-ray diffraction are very difficult to obtain. At present, therefore, our knowledge of ligand-receptor complexes is largely based upon computer models incorporating the results of mutagenesis experiments.[1] Many residues in membrane-bound receptors have been identified as being critical for ligand binding but there is very little information available regarding which parts of the ligand are involved in making specific contacts with the receptor. Recently, however, Li and co-workers have demonstrated that peptide ligands incorporating photoactivatable moieties have the potential to provide valuable information about ligand-receptor contacts.[2]

As part of our studies aimed at mapping the binding sites of neuropeptide receptors[3], we wished to develop new photocrosslinking strategies and in particular, to study the feasibility of the thioamide moiety as a ligand-based photolabile unit. Here, we describe the syntheses of two photoactivatable peptide ligands, each containing a novel side-chain incorporating a thiocarbonyl moiety. The thiocarbonyl group has well documented photochemistry[4] and includes hydrogen abstraction from primary alcohol, photoreduction, ($2\pi+2\pi$) photocycloaddition and C-H bond insertion processes. Addtionally thiocarbonyls have been used to photo-link peptides to modified RNA molecules.[5] Thus, ligands such as those described here should prove to be valuable tools for mapping the binding sites of peptide-binding receptors.



Ar : Ph, Bz.

1

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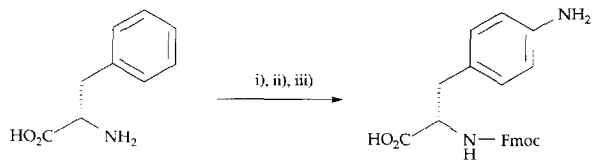
We decided to synthesise the phenylalanine based amino acids **1**, each containing a thioamide moiety as part of the side-chain. As the amino acids **1** were required in optically pure form, efforts were concentrated on manipulating the commercially available L-(+)-phenylalanine **2**. Nitration of the aromatic ring was achieved under standard conditions to give the *p*-nitro substituted system. This was then protected as the N-Fmoc derivative prior to reduction of the nitro group by catalytic hydrogenation (93% yield) to give the *p*-amino phenylalanine derivative **3** (Scheme 1).[6]

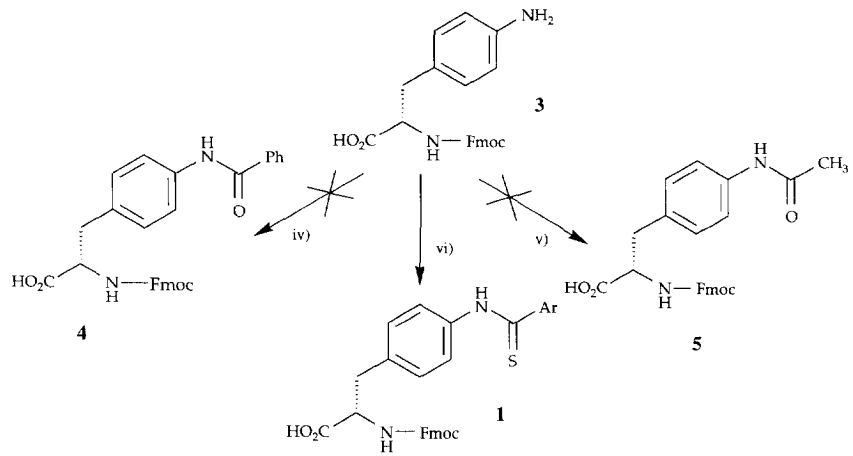


2 **3**

Scheme 1

Reagents: i) H_2SO_4, HNO_3, 50 °C, 1 hr, (80 %); ii) 1.2 eq FmocCl, 10 % Na_2CO_3, dioxane, 0 °C, 3 hrs, (64 %); iii) Pd / C, H_2, EtOH, 1 hr, (93 %).

Our initial strategy of constructing the required thioamides from the amine **3** involved benzoylation and subsequent thiation of the resulting amides. Unfortunately, all attempts to convert **3** into the benzoyl or acetoyl derivatives (**4** and **5**, respectively) under standard procedures were unsuccessful (Scheme 2). However, an alternative method for direct thiobenzoylation of amines under mild conditions has been reported by Kjear.[7] This procedure when applied to amine **3** efficiently gave the desired thioamides **1** in good yields (Scheme 2). Thiobenzoylating reagents **6** were synthesised using the method as described by Kjear and were subsequently reacted with **3** for a total of 6 hrs in neutral aqueous media affording the desired amino acids **1** in 65 % (Ar = Ph) and 60 % (Ar = Bz) yields, respectively,[8] as light- and air-stable solids.



Scheme 2

Reagents: iv) PhCOCl, DIPEA, DMF, 60 °C; v) $(CH_3CO)_2O$, DMF, 50 °C; vi) $ArCSSCH_2CO_2H$ (**6**), 2 eq NaOH, 6 hrs, rt, (Ar = Ph, 65 % and Ar = Bz, 60 %).

To test the applicability of **1** in solid-phase peptide syntheses[9], we prepared analogues of neurokinin A (Figure 1), the natural peptidic agonist of the membrane-bound NK_2 receptor. Neurokinin A was chosen because it has been implicated in a large number of important biological processes.[10] Thus, information regarding interactions between neurokinin A and its receptor may be of great value to the rational design of therapeutic agents that specifically target NK_2 receptors.

Ligand	**Sequence**	**Mass**
Neurokinin A	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-$CONH_2$	1133
Peptide - 1	His-**X**-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-$CONH_2$	1414
Peptide - 2	Tyr-**Y**-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-$CONH_2$	1455

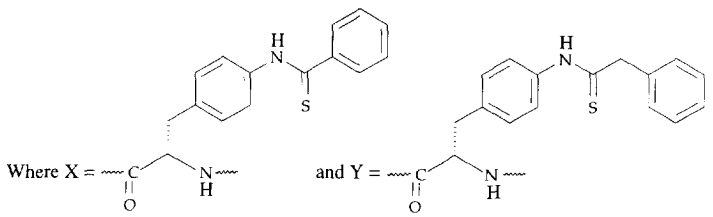


Figure 1

We chose to incorporate the thioamide-containing amino acids in the N-terminal portion of neurokinin A, since substitutions at these positions do not usually abolish high affinity binding to NK_2 receptors.[11] The coupling efficiency of amino acids **1** during peptide synthesis was in excess of 97 %. Following purification of the peptides by reverse-phase HPLC, mass spectral analysis of the major peptide components identified species of the correct molecular weight (Figure 2), thus, reinforcing the presence of the correct peptides containing the intact amino acid analogues. The novel peptide analogs had longer elution time compared to the neurokinin A (order of elution: neurokinin A, peptide-1, peptide-2). To determine the binding affinity of the prepared neurokinin A analogues, *in vitro* binding assays were carried out using intact *Sf*-9 insect larval cells expressing the human NK_2 receptor.[12] Both analogues retained high affinity binding for the NK_2 receptor (Table 1).

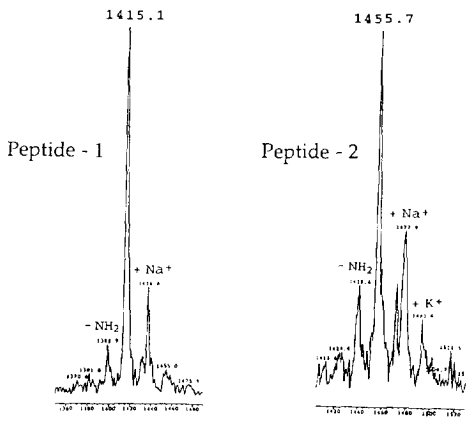


Figure 2. MALDI-TOF spectra of Peptide - 1 and Peptide - 2.

Table 1. Binding affinities of peptides to the human NK_2 receptor expressed in *Sf*-9 insect larval cells.

Ligand	**IC_{50} (nM)***
Neurokinin A	7.70 ± 0.71 (3)
Peptide - 1	7.72 ± 1.57 (3)
Peptide - 2	53.36 ± 5.35 (3)

** Values are quoted as Mean ± SEM for 0.1 nM 2-[^{125}I] iodohistidyl neurokinin A in competition with unlabelled ligand. The number of independent experiments are shown in parentheses.*

In summary, these new, readily available amino acid analogues are efficiently incorporated into peptides using standard solid-phase techniques and in the present case, the resulting peptide derivatives retain high affinity binding. Studies are underway to investigate the photochemical binding behaviour of these novel peptides which should be of value in the identification of direct contacts made between the ligand and the receptor.

Acknowledgements

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References and Notes

Abbreviations used: Fmoc, 9-fluorenylmethoxycarbonyl; DIPEA, N,N-diisopropylethylamine; DMF, dimethyl formamide.

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8. Analytical and spectroscopic data.**1** (Ar=Ph); **m. p.** 145-146 °C; $C_{31}H_{26}N_2O_4S$: Calc. (%) C 71.1, H 5.0, N 5.3, S 6.12; Found C 69.85, H 5.3, N 5.0, S 6.0; υ_{max} /cm^{-1} (neat): 3200-2800 (bm, NH, OH, CH), 1750 (s, C=O), 1650 (s, *sec*-amide), 1520 (w, C-N), 1460 (m), 1240 (w), 860 (s); **^{1}H NMR** (400 MHz, d-6 DMSO) δ: 11.25 (1H, bs, OH), 8.0-7.44 (17H, m, ArH), 4.42-4.39 (3H, m, $CHCH_2$(Fmoc)), 4.38-4.31 (1H, m, H_α), 3.30-3.26 (1H, m, H_β), 3.11-3.05 (1H, m, $H_{\beta'}$); **^{13}C NMR** (100 MHz, d-6 DMSO) δ: 201.14 (C=S), 177.2, 174.2, 159.9, 147.7, 144.6, 142.5, 139.9, 134.1, 132.7, 131.9, 131.3, 131.0, 129.2, 129.2, 127.6, 124.0, 69.6, 63.7, 50.6, 40.1; ***m/z*** (%): 523 (M^+(S35), 0.4), 495 (M^+(S35)-CO·, 0.5), 491 (M^+- S·, 3), 467 (2), 402 (M^+-PhC=S·, 10), 178 (Fmoc$^+$, 99), 41 (100); **UV** (MeOH); λ_{max} ($\log_{10}\varepsilon$): 320 (1031), 290 (1069); $[\alpha]_d$ (c=0.1, THF) +46.5°

   1 (Ar=Bz) **m. p.** 193-195 °C; $C_{32}H_{28}N_2O_4S$: Calc. (%) C 71.6, H 5.2, N 5.2; Found C 71.8, H 5.15, N 4.9.υ_{max} /cm^{-1} (neat): 3200-2800 (bm, NH, OH, CH), 1750 (s, C=O), 1655 (s, *sec*-amide), 1540 (w, C-N), 1480 (m), 1240 (w), 860 (s).**^{1}H NMR** (400 MHz, d-6 DMSO) δ: 12.13 (1H, bs, OH), 7.9-7.21 (17H, m, ArH), 4.43 (1H, m, H_α), 4.32-4.26 (5H, m, $CSCH_2$, $CHCH_2$(Fmoc)), 3.27 (1H, m, H_β), 3.05 (1H, m, $H_{\beta'}$); **^{13}C NMR** (100 MHz, d-6 DMSO) δ: 200.1 (C=S), 175.4, 155.5, 144.1, 140.8, 138.0, 137.8,137.0, 129.4, 128.9, 128.37, 127.7, 127.2, 125.4, 123.7, 122.5, 120.2, 65.4, 56.8, 53.5, 46.8, 40.8; ***m/z*** (%): 537 (M^+(S35), 7), 521 (M^+(S35)-O·, 4.2), 447 (25), 307 (10), 179 (Fmoc$^+$, 100); **UV** (MeOH); λ_{max} ($\log_{10}\varepsilon$): 320 (29), 290 (1060); $[\alpha]_d$ (c=0.1, THF) +52.5°. The optical purity of both amino acids was determined via chiral TLC analysis and was found to be >99 % L- isomer.
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