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SYNTHESIS OF SOMATOSTATIN ANALOGUES INCORPORATING NUCLEO AMINO ACIDS

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Abstract: Somatostatin analogues incorporating nucleo amino acids have been chemically synthesised by both solution phase coupling of protected fragments and solid phase methods. Methodology is presented enabling the preparation of these previously unknown reduced size somatostatin analogues. The observation of high biological activity with modified receptor binding affinities and levels of inhibition of growth hormone release from cultured pituitary cells is discussed.

Somatostatin,[1] Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH, a tetradecapeptide isolated from ovine hypothalamic extracts, has been shown to inhibit the release of growth hormone (GH) in primary cultures of enzymatically dispersed rat anterior pituitary cells. It is also highly active in patients, lowering plasma levels of GH in acromegalics,[2] and as such is of potential therapeutical importance in clinical treatment of acromegaly and gastroenteropancreatic tumours.[3] However, it was evident that the therapeutic utility of somatostatin was limited because of its short biological half life. A process of rational design led to the potent, selective, minimal-sequence long-acting octapeptide somatostatin analogue (D)-Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Throl, SMS 201-995,[4] known as octreotide. Moreover, this breakthrough illustrated that it is possible to exceed the activity of the natural hormone with a synthetic analogue having a duration of action meeting therapeutic requirements. Nevertheless, the pluripotent inhibitory activity of somatostatin against a number of endocrine hormones such as GH, insulin, glucagon and prolactin suggested that the design of analogues exhibiting modified biological activities would be advantageous.

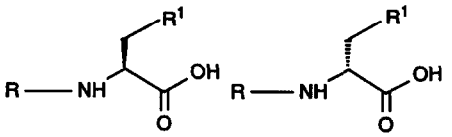
The incorporation of structural features from different classes of biomolecules into biologically active peptides has been one such approach towards this goal.[5] Recently we introduced special hydrogen bonding amino acids, termed nucleo amino acids,[6,7,8] into the peptide backbone of octreotide. Several reasons led us to undertake the synthesis of these analogues. The incorporation of novel hydrogen bonding amino acids would potentially open a route to previously unknown potent small-ring somatostatin analogues. These hydrogen bonding amino acids would undoubtably bring about altered amino acid side chain orientations. This novel array of side chain orientations could in turn give rise to a modified ligand-receptor-bound conformation with associated modification of biological activity. Indeed, the consequence of incorporating these novel hydrogen bonding amino acids at important sensitive positions in octreotide could be the discovery of surprising new structure activity relationships. Furthermore, since at least five SRIF receptor subtypes have been identified,[9] the associated longer range goal of synthesising functional antagonists or subtype specific analogues of octreotide was considered to be of great interest. A highly potent SRIF receptor antagonist would be invaluable in elucidating the physiological processes regulated by somatostatin.[10]

Two strategies were adopted in the synthesis of these octreotide analogues. The first strategy involved solution coupling of protected peptide fragments. In the second approach, solid phase synthesis using Nα-Boc protection was carried out. Prior to investigation of these strategies, synthesis of the nucleo amino acids was carried out.

The nucleo amino acids were synthesised from Boc-serine β-lactone according to Vederas,[11,12] with the Boc-serine β-lactone ring being opened by the nucleo base in the presence of diazabicycloundecane or K_2CO_3 to give the Nα-Boc protected nucleo amino acid in optically pure form. In the case of the adenine amino acid, **L-1**, the adenine exocyclic 6-amino group could be protected by treatment with carboxybenzyl-ethylimidizolium chloride, according to Rapoport,[13,14] although this was not routinely required for synthesis of octapeptides.

Nucleo Amino Acid

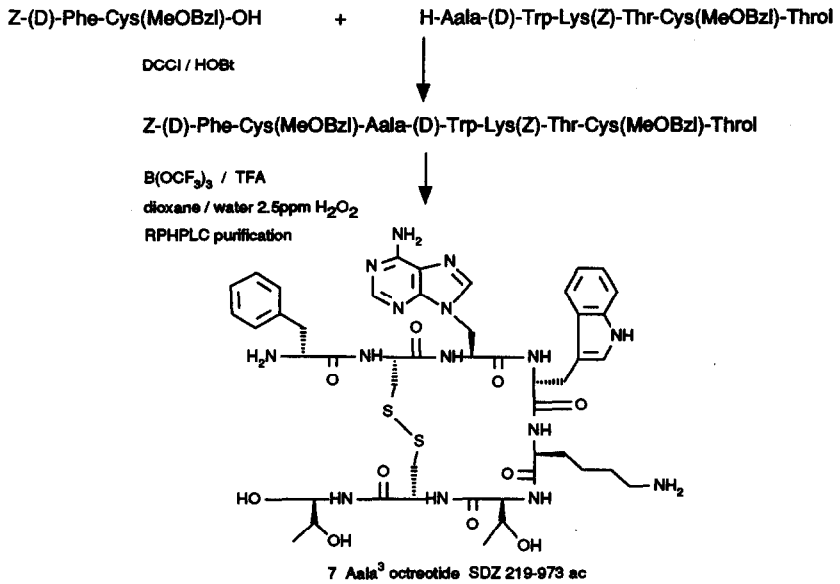
L-1 R^1=Adenine, R=H, L-Aala
D-2 R^1=Adenine, R=H, D-Aala
L-3 R^1=Uracil, R=H, L-Uala
L-4 R^1=Thymine, R=H, L-Tala



L-1,3,4 D-2

Solution coupling of protected fragments was the first strategy employed. Considering **7**, the octreotide analogue with nucleo amino acid **L-1** incorporated in the 3 position as a representative example, this involved synthesis of the protected peptide fragment up to the nucleo amino acid with Nα-Fmoc protection on the threoninol acetal resin. The Nα-Boc protected nucleo amino acid was then coupled and the peptide resin was cleaved with TFA. This fragment was then coupled in solution with the N-terminal dipeptide, deprotected and cyclised as shown in scheme 1.

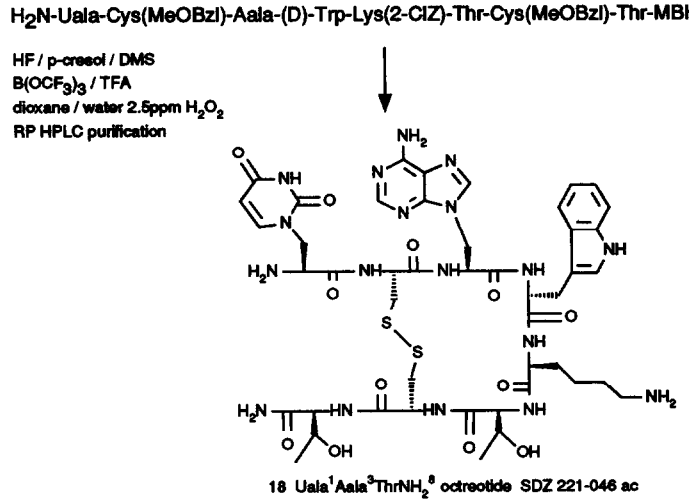
Scheme 1



7 Aala[3] octreotide SDZ 219-973 ac

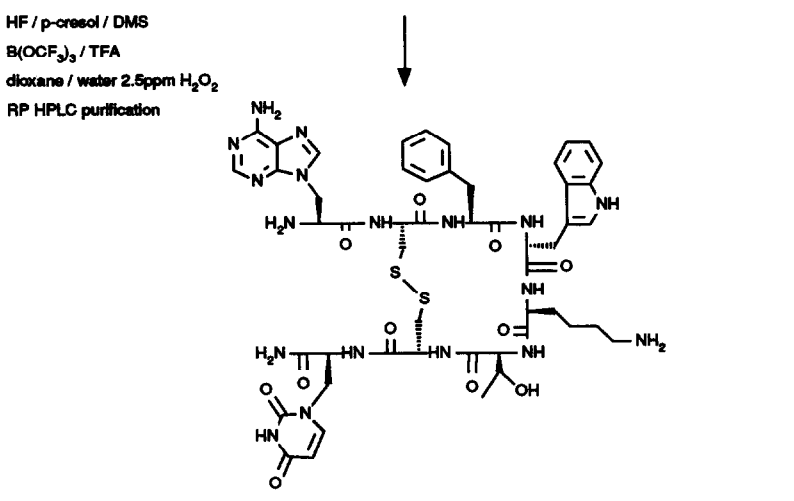
It was considered, however, that a solid phase approach would have advantages in terms of flexibility and in preparation of a series of analogues. This was accomplished using Merrifield's solid phase approach[15,16,17] coupling the Nα-Boc protected amino acids using diisopropylcarbodiimide and hydroxybenzotriazole in dimethylformamide. As an example, 18 Uala^{1}Aala^3 ThrNH$_2$^{8} octreotide was synthesised as shown in scheme 2. The peptide resin was deprotected using HF / p-cresol / DMS under standard conditions. Removal of the MeOBzl cysteine protecting group was carried out with B(OCF$_3$)$_3$ / TFA and the peptide was oxidised to give the crude product. Purification was by reversed phase HPLC using Merck LiChrospher 100 RP-18 (5mm) columns and gradient elution. The synthesis of 19, Aala^{1}UalaNH$_2$^{8} octreotide, was carried out analogously as shown in scheme 3.

Scheme 2



18 Uala^{1}Aala^{3}ThrNH$_2$^{8} octreotide SDZ 221-046 ac

Scheme 3



19 Aala^{1}UalaNH$_2$^{8} octreotide SDZ 221-047 ac

These two approaches enabled us to synthesise the following analogues. The Throl[8] analogues were synthesised by coupling of the protected peptide fragments, and the Thr-NH_2[8] by the solid phase method using N-α Boc protection.

Radioligand binding studies to SRIF receptors on rat brain cortex membranes were performed using SDZ[^{125}I]-204-090, the radioactively iodinated Tyr[3]-analogue of octreotide, as radioligand. This analogue has been shown to specifically label SRIF receptors.[18] The inhibition of growth hormone (GH) release in vitro was determined using cultured anterior pituitary cells from adult male rats, with the amount of growth hormone released being determined by RIA.[19] Binding curves were calculated from triplicate determinations using the computer fitting program of De Lean.[20]

A systematic scan of positions in octreotide was initiated with the adenine nucleo amino acid L-1. Results are shown in Table 1, and in graphical form in Figure 1. Figure 1 summarises pKi values of binding and pIC_{50} values for the inhibition of GH release for the octreotide analogues described. The bar graph represents pKi values relative to octreotide for binding and pIC_{50} values relative to octreotide for inhibition of GH. Replacement of D-Phe[1] in octreotide with Aala[1] resulted in decreased binding affinity to cortex of 1.12 pKi units accompanied by a greater decrease in inhibition of GH in vitro by 1.60 pIC_{50} units. This tendency was increased when Phe[3] of octreotide was replaced with Aala[3]. In this case, binding affinity to cortex decreased by 0.64 pKi units with the inhibition of GH in vitro decreasing by 1.64 pIC_{50} units. Replacement of Thr[6] with Aala[6] exhibited a pattern resembling the replacement at position 3. These results are shown in Fig 1.1. Replacement of D-Trp[4] of octreotide was carried out with L-1 and D-2, as shown in Fig 1.2, in order to compare the altered biological effects of the enantiomeric nucleo amino acids. However, although the tendency to decrease inhibition of GH in vitro was greater than the decrease in binding affinity, the binding affinity itself was too weak to attract greater interest.

Table 1 Peptide	Substitutions of octreotide with Aala	Displacement of radioligand [I^{125}]204-090 pKi	Inhib'n of GH *in vitro* pIC_{50}
5 201-995ac octreotide		9.34	8.88
6 219-646ac Aala[1]		8.22	7.28
7 219-973ac Aala[3]		8.70	7.24
8 220-775ac Aala[4]		6.10	<5.00
9 220-776ac D-Aala[4]		7.15	<6.00
10 220-961ac Aala[6]		8.52	7.49

Incorporation of L-3, the uracil nucleo amino acid, was carried out in selected positions. In positions 1, 4 and 6 this resulted in decreases of binding affinity too large to warrant further interest. However, incorporation of this building block in position 3 showed a more interesting tendency. In this case the binding affinity decreased by 0.64 pKi units, whereas the inhibition of GH in vitro showed a slight tendency to increase. These results are summarised in Table 2 and Figure 1.3.

Table 2 Peptide	Substitutions of octreotide with Uala	Displacement of radioligand [I^{125}]204-090 pKi	Inhib'n of GH *in vitro* pIC_{50}
11 220-232ac Uala[1]		8.10	7.27
12 221-048ac Uala[3]ThrNH_2[8]		8.70	9.00
13 220-793ac Uala[4]		7.40	<6.00
14 221-414ac Uala[6]ThrNH_2[8]		7.30	<7.00

FIGURE 1

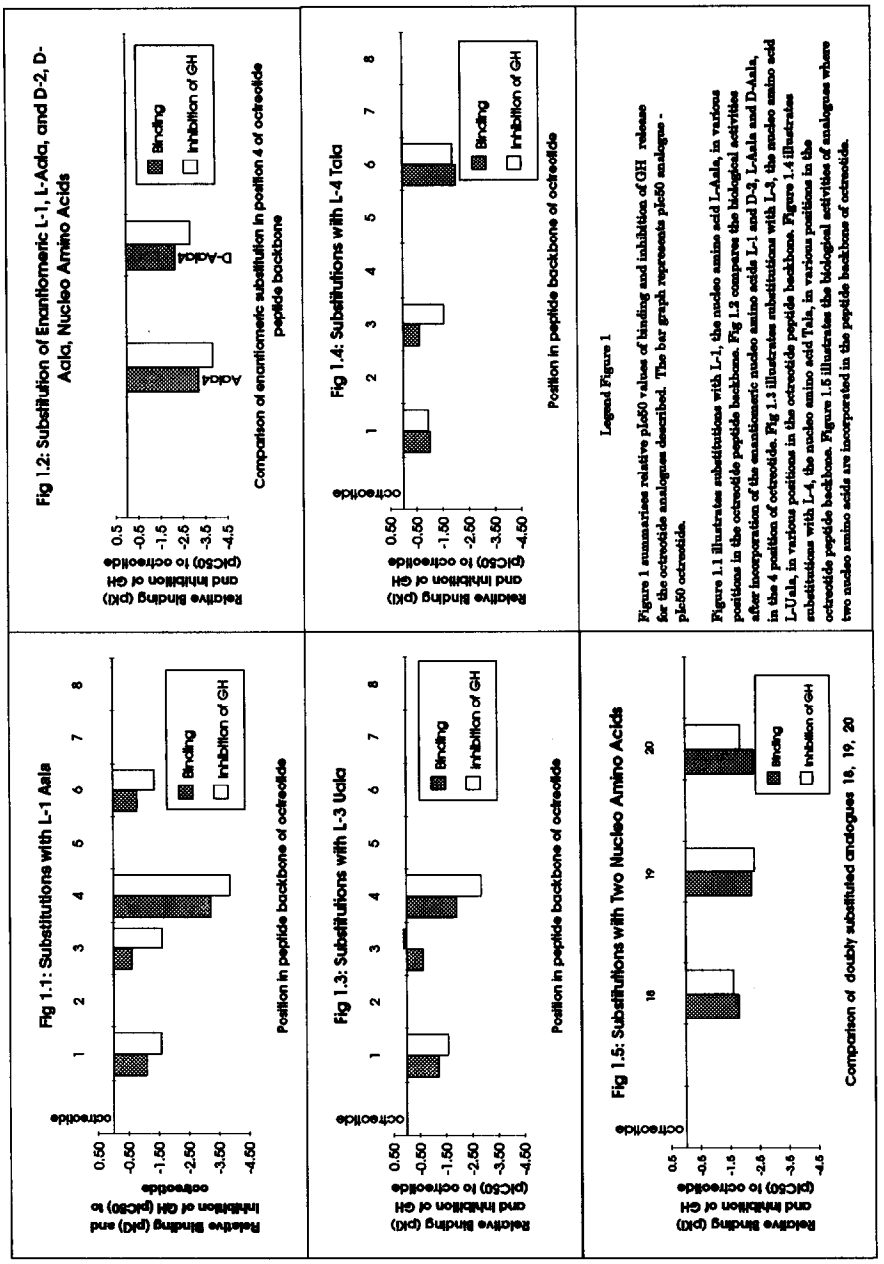


Fig 1.1: Substitutions with L-1 Aala
Fig 1.2: Substitution of Enantiomeric L-1, L-Ala, and D-2, D-Ala, Nucleo Amino Acids
Fig 1.3: Substitutions with L-3 Ucla
Fig 1.4: Substitutions with L-4 Tala
Fig 1.5: Substitutions with Two Nucleo Amino Acids
Legend Figure 1
Binding
Inhibition of GH
Position in peptide backbone of octreotide
Comparison of enantiomeric substitution in position 4 of octreotide peptide backbone
Comparison of doubly substituted analogues 18, 19, 20

Selected substitutions at positions 1, 3 and 6 were carried out with L-4, the thymine nucleo amino acid. These results are shown in Table 3, and illustrated in Fig 1.4. The substitution of Phe^3 of octreotide with L-4 was the most interesting substitution. In this case binding affinity was only reduced by 0.64 pKi units but was accompanied by a reduction in inhibition of GH in vitro of 1.56 pIC_{50} units.

The conformational influence of incorporating nucleo amino acids in the octreotide peptide backbone was analysed by molecular modelling. This was accomplished using the Insight and Discover software packages (Biosym Technologies Inc., San Diego, CA 92121). Structures were optimised using the Discover CVFF force field and the conjugate gradient method. In particular **16**, SDZ 220-774 ac $Tala^3$ octreotide, was an interesting choice for these studies in view of the modified biological activity observed. Molecular modelling studies showed the thymine nucleus in **16** to be oriented coplanar with the peptide backbone, perpendicular to the indole nucleus. The carbonyl hydrogen bonding acceptor functions characteristic of thymine consequently face outwards towards the receptor, with the thymine 5-methyl group directed towards the indole. Modified ligand-receptor-bound conformation arising from this novel incorporation of L-4 with the characteristic thymine hydrogen bonding acceptor functions brings about a reduction in inhibition of GH proportionately greater than the decrease in binding affinity.

Table 3

Peptide	Substitutions of octreotide with Tala	Displacement of radioligand [I^{125}]204-090 pKi	Inhib'n of GH *in vitro* pIC_{50}
15 220-375ac	$Tala^1$	8.30	7.92
16 220-774ac	$Tala^3$	8.70	7.32
17 221-069ac	$Tala^6$	7.30	6.96

As an extension of this program, analogues incorporating two nucleo amino acids were investigated. **19**, SDZ 221-047 ac $Aala^1Uala^8$ octreotide, showed a tendency to display a reduction in inhibition of GH greater than the accompanied reduction in binding affinity. In contrast **18**, 221-046 ac $Uala^1Aala^3ThrNH_2{}^8$ octreotide, and **20**, 221-394 ac, $Uala^3Aala^6ThrNH_2$ octreotide, showed the opposite tendency, that is a larger decrease in binding affinity accompanied by a lesser decrease in inhibition of GH. These results are illustrated in Table 4 and Fig 1.5, where the X-axis represents the three analogues described and the Y-axis shows the pKi values relative to octreotide for binding affinity and the pIC_{50} values relative to octreotide for inhibition of GH release.

Table 4

Peptide	Substitutions of octreotide with two nucleo amino acids	Displacement of radioligand [I^{125}]204-090 pKi	Inhib'n of GH *in vitro* pIC_{50}
18 221-046ac	$Uala^1Aala^3ThrNH_2{}^8$	7.52	7.25
19 221-047ac	$Aala^1UalaNH_2{}^8$	7.09	6.52
20 221-394ac	$Uala^3Aala^6ThrNH_2{}^8$	<7.00	<7.00

In conclusion, chemical incorporation of novel hydrogen bonding amino acids was accomplished, enabling the preparation of previously unknown small ring somatostatin analogues. Modified biological activities in respect of binding and inhibition of growth hormone were observed. In particular, interesting tendencies involving differing effects on binding affinity and inhibition of growth hormone in vitro were identified. Indeed, position 3 of octreotide was determined to be the most interesting position in terms of the alteration of binding affinity relative to the associated inhibition of GH secretion. Further study of these interesting differences in binding and in GH inhibition would involve pharmacological characterisation of the SRIF receptor subtype selectivity of these novel analogues. It remains our goal to achieve magnification of these effects to furnish analogues exhibiting functional antagonism or subtype specificity.[21]

Experimental Section

Synthesis of 7 Aala^3 octreotide SDZ 219-973 ac:

The peptide was synthesised by a combined solid phase and fragment coupling approach. The desired Boc-Aala^3-(D)-Trp^4-Lys(Z)^5-Thr^6-Cys(MeOBzl)^7-Throl^8-Acetal-anchor Resin was synthesised on the solid phase by sequential coupling of the Fmoc amino acids using DCCI / HOBt, followed by coupling of Boc-Aala-OH.[7,15] The peptide fragment resin was cleaved with TFA / dichloromethane, and coupled in solution with the dipeptide Z-(D)-Phe^1-Cys(MeOBzl)^2-OH using DCCI / HOBt. Removal of the protecting groups was carried out with BTFA in TFA, and oxidation was carried out in dioxane / water solution with 2.5ppm hydrogen peroxide to give the crude product. Purification was by Reversed phase HPLC using Merck LiChrospher 100 RP-18 (5mm) columns and gradient elution (solvent A: acetonitrile / water / phosphoric acid / tetramethylammonium hydroxide 10/90/0.1/0.1 (v/v/v/v), solvent B: acetonitrile / water / phosphoric acid / tetramethylammonium hydroxide 140/60/0.8/2 (v/v/v/v)) with detection wavelength at 205nm. RPHPLC 99%, 6.31 mins., 10 - 50% B in 20 min. Amino acid analysis Phe 1.0 (1.0), Cys 2.0 (1.1), Aala - (1.0), Trp 0.2 (1.0), Lys 0.9, (1.0), Thr 0.8 (1.0), Throl - (1.0); Factor 1.33; FAB M.S 1076 (MH^+), 1044, 737, 666, 645, 615, 583, 553, 539; ^{1}H NMR illustrated in Figure 2, 600MHz d^{6}DMSO δ; 3.52(D-Phe^1 α-CH, m 1H), 2.56, 3.05(D-Phe^1 β-CH_2, dd J=15,7Hz 2H), 4.95(Cys^2 α-CH, 1H), 2.84(Cys^2 β-CH_2, m 2H), 3.95(Aala^3 α-CH, m 1H), 4.35, 4.45(Aala^3 β-CH_2, m 2H), 6.75(Aala^3 6-NH_2, br s 1.5H), 7.82(Aala^3 H-C8', s 1H), 8.12(Aala^3 H-C2', s 1H), 8.50(D-Trp^4 NH, br s 0.7H), 4.13(D-Trp^4 α-CH, m 1H), 2.70, 3.00(D-Trp^4 β-CH_2, m 2H), 8.25(Lys^5 NH, 0.5H), 3.98(Lys^5 α-CH, m 1H), 1.67(Lys^5 β-CH_2, m 2H), 0.78(Lys^5 γ-CH_2, m 2H), 2.52(Lys^5 ϵ-CH_2, m 2H), 7.69(Thr^6 NH, br s 0.5H), 4.47(Thr^6 α-CH, m 1H), 3.99(Thr^6 β-CH_2, dd J=7,7Hz 2H), 1.07(Thr^6 γ-CH_2, m 2H), 8.42(Cys^7 NH, br s 0.7H), 5.12(Cys^7 α-CH, m 1H), 2.85, 2.92(Cys^7 β-CH_2, m 2H), 7.54(Thr^8(ol) NH, br s 0.7H), 3.67(Thr^8(ol) α-CH, m 1H), 3.90(Thr^8(ol), β-CH_2, m 2H), 1.04(Thr^8(ol) γ-CH_2, d 2H), 3.40, 3.54(Thr^8(ol) CH_2OH, overlapping H_2O 0.7H).

Synthesis of 19 Uala^{1}Aala^{3}ThrNH_2^8 octreotide SDZ 221-046 ac:

The peptide was synthesised by solid phase methodology on the MBHA resin using Boc tactic. The Boc protected amino acids were coupled sequentially and deprotected with 95:5 TFA:H_2O to give the desired H_2N-Uala^1-Cys(MeOBzl)^2-Aala^3-(D)-Trp^4-Lys(2Cl-Z)^5-Thr^6-Cys(MeOBzl)^7-Thr ^{8}-MBHA peptide resin. Cleavage of all side chain protecting groups as well as peptide from the resin was achieved with liquid HF (approximately 15 ml) with the addition of p-cresol / dimethyl sulphide. Oxidation was carried out in dioxane / water solution with 2.5ppm hydrogen peroxide to give the crude product. Purification was by Reversed phase HPLC using Merck LiChrospher 100 RP-18 (5mm) columns and gradient elution (solvent A: acetonitrile / water / phosphoric acid / tetramethylammonium hydroxide 10/90/0.1/0.1 (v/v/v/v), solvent B acetonitrile / water / phosphoric acid / tetramethylammonium hydroxide 140/60/0.8/2 (v/v/v/v)) with detection wavelength at 205nm. RPHPLC 93.9%, 2.53 mins, 0 - 40% B in 20 min. Amino acid analysis Uala - (1.0), Cys 0.4 (2.0), Aala - (1.0),Trp - (1.0),Lys 1.0 (1.0), Thr 1.9 (2.0); Factor 1.64; FAB M.S 1123 (MH^+), 1080, 1061, 1022, 1006, 963, 910, 866, 818, 713, 676,614, 551; ^{1}H NMR , illustrated in Figure 2, 600MHz d^{6}DMSO δ; 4.45(Uala^1 α-CH, br dd 1H), 3.97, 4.15(Uala^1 β-CH_2, overlapping m 2H), 5.51(Uala^1 H-C3", 1H, d J=7Hz), 7.52(Uala^1 H-C2", 1H, d J=7Hz), 5.13(Cys^2 α-CH, m 1H), 2.84(Cys^2 β-CH_2, br m 2H), 4.40(Aala^3 α-CH, dd J=7,7Hz 1H), 3.20(Aala^3 β-CH_2, dd J=15,7Hz 2H), 6.90(Aala^3 6-NH_2, br s 1.4H), 7.90(Aala^3 H-C8', s 1H), 8.15(Aala^3 H-C2', s 1H), 8.70(D-Trp^4 NH, br s 0.6H), 4.23(D-Trp^4 α-CH, overlapping m 1H), 2.70, 3.00(D-Trp^{4}β-CH_2, m 2H), 8.51(Lys^5 NH,br s 0.8H), 3.98(Lys^5 α-CH, m 1H), 1.67(Lys^5 β-CH_2, m 2H), 0.78(Lys^5 γ-CH_2, m 2H), 2.52(Lys^5 ϵ-CH_2, m 2H), 7.69(Thr^6 NH, br s 0.8H), 4.47(Thr^6 α-CH, dd J=7,7Hz 1H), 3.99(Thr^6 β-CH_2, overlapping m 2H), 1.07(Thr^6 γ-CH_2, d 2H), 8.42(Cys^7 NH, br s 0.6H), 5.12(Cys^7 α-CH, m 1H), 2.85, 2.92(Cys^7 β-CH_2, m 2H), 7.74(ThrNH_2^8 NH, br s 0.8H), 3.67(ThrNH_2^8 α-CH, m 1H), 3.90(ThrNH_2^8 β-CH_2, dd 2H), 1.04(ThrNH_2^8 γ-CH_2, d 2H), 7.15, 7.25(ThrNH_2^8 $CONH_2$,br s, br s 1.5H).

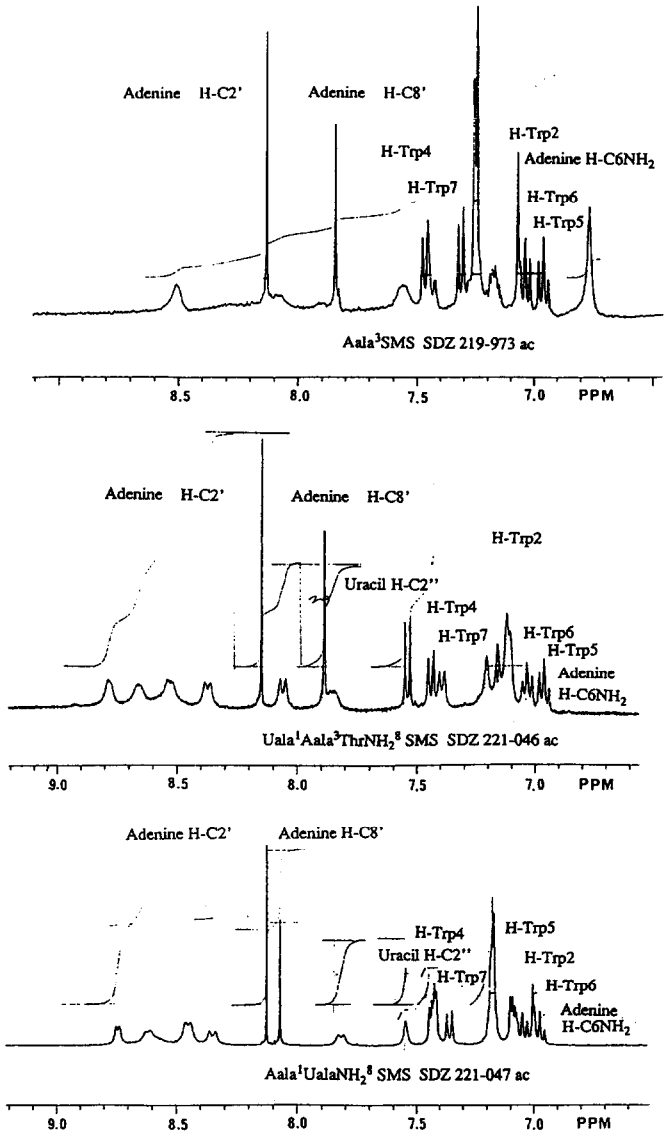


Figure 2: Selected ^{1}H NMR data δ d^{6}DMSO between 6.5 PPM and 9.0 PPM.

Legend: The proton signals of the nucleo amino acids incorporated are clearly visible. In the case of Aala^3 octreotide SDZ 219-973 ac, the adenine H-C8' is observed at 7.82 ppm with the adenine H-C2' being observed at 8.12 ppm, 0.3 ppm upfield. The proton spectrum of Uala^{1}Aala^{3}ThrNH$_2$^{8} octreotide SDZ 221-046 ac shows the adenine H-C8' at 7.90 ppm and the adenine H-C2' at 8.15 ppm, 0.25 ppm upfield. The uracil H-C2'' is observed as a doublet centered at 7.52 ppm. However, in the case of Aala^{1}UalaNH$_2$^{8} octreotide SDZ 221-047 ac, the adenine H-C8' and H-C2' resonances exhibit chemical shifts much closer to each other. More particularly, the adenine H-C8' resonance is observed at 8.07 ppm and the adenine H-C2' at 8.14 ppm, only 0.07 ppm upfield. The uracil H-C2'' is observed overlapping the H-Trp^4 resonance at 7.43 ppm. "SMS" = octreotide.

Synthesis of 20 Aala[1]UalaNH$_2$[8] octreotide SDZ 221-047 ac:

The peptide was synthesised by solid phase methodology on the MBHA resin using Boc tactic. The Boc protected amino acids were coupled sequentially and deprotected with 95:5 TFA:H_2O to give the desired H_2N-Aala[1]-Cys(MeOBzl)[2]-Phe[3]-(D)-Trp[4]-Lys(2Cl-Z)[5]-Thr[6]-Cys(MeOBzl)[7]- Uala[8]-MBHA peptide resin. Cleavage of all side chain protecting groups as well as peptide from the resin was achieved with liquid HF (approximately 15 ml) with the addition of p-cresol / dimethyl sulphide. Oxidation was carried out in dioxane / water solution with 2.5ppm hydrogen peroxide to give the crude product. Purification was by Reversed phase HPLC using Merck LiChrospher 100 RP-18 (5mm) columns and gradient elution (solvent A: acetonitrile / water / phosphoric acid / tetramethylammonium hydroxide 10/90/0.1/0.1 (v/v/v/v), solvent B acetonitrile / water / phosphoric acid / tetramethylammonium hydroxide 140/60/0.8/2 (v/v/v/v)) with detection wavelength at 205nm. RPHPLC 98%, 4.79 mins., 0 - 40% B in 20 min. Amino acid analysis Aala - (1.0), Cys 0.3 (2.0), Uala - (1.0), Trp - (1.0), Lys 0.9 (1.0), Thr 0.9 (2.0); Factor 1.3; FAB M.S 1169 (MH^+), 1137, 1039, 1014, 989, 965, 946,585, 468, 427, 399, 369, 315; ^{1}H NMR, illustrated in Figure 2, 600MHz d^{6}DMSO δ; 3.72(Aala[1] α-CH, m 1H), 4.25, 4.45(Aala[1] β-CH_2, dd J=15,7Hz 2H), 6.95(Aala[3] 6-NH_2, br s 1.5H), 8.07(Aala[1] H-C8', s 1H), 8.14(Aala[1] H-C2', s 1H), 5.13(Cys[2] α-CH, m 1H), 2.84(Cys[2] β-CH_2, m 2H), 8.49(Phe[3] NH, br s 0.7H), 4.63(Phe[3] α-CH, overlapping m 1H), 2.87(Phe[3] β-CH_2, m 2H), 8.70(D-Trp[4] NH,br s 0.5H), 4.23(D-Trp[4] α-CH_2 m 1H), 2.70, 3.00(D-Trp[4] β-CH_2, m 2H), 8.51(Lys[5] NH, br s 0.6H), 3.98(Lys[5] α-CH, m 1H), 1.67(Lys[5] β-CH_2, m 2H), 0.78(Lys[5] γ-CH_2, m 2H), 2.52(Lys[5] ϵ-CH_2, m 2H), 7.69(Thr[6] NH, br s 0.6H), 4.47(Thr[6] α-CH, dd J=7,7Hz 1H), 3.99(Thr[6] β-CH_2, m 2H), 1.07(Thr[6] γ-CH_2, d 2H), 8.42(Cys[7] NH, br s 0.5H), 5.12(Cys[7] α-CH, m 1H), 2.85, 2.92(Cys[7] β-CH_2, overlapping m 2H), 3.92(UalaNH$_2$[8] α-CH, br m 1H), 3.67, 4.25(UalaNH$_2$[8] β-CH_2, m 2H), 5.40(UalaNH$_2$[8] H-C3", 1H, d J=7Hz), 7.43(UalaNH$_2$[8] H-C2", 1H overlapping m), 7.55(UalaNH$_2$[8] $CONH_2$, br d 1.3H).

Radioligand Binding Studies[18]

Binding studies on rat brain cortex membranes were performed using SDZ [I^{125}]-204-090, the radioactively iodinated Tyr[3]-analogue of octreotide as radioligand. Briefly, cerebral cortex was homogenized in 10 mM HEPES buffer, pH 7.6. Cortex membranes corresponding to 50 mg protein/tube were incubated for 30 minutes with 20,000 cpm of the radioligand and increasing concentrations of the test analogue. Incubation was stopped by rapid filtration through Whatman GF/C filters. Binding curves were calculated from triplicate determinations using the computer fitting program of De Lean.[17]

Inhibition of Growth Hormone Release *in vitro*[19]

Anterior pituitary glands from adult male rats were cut into small pieces and dispersed using 0.1% trypsin in 20mM HEPES buffer. The dispersed cells were cultured for four days in MEM (Gibco) supplementd with 5% fetal calf serum, 5% horse serum, 1 mM $NaHCO_3$, 2.5 nM dexamethasone, 2.5 mg/ml insulin and 20 U/ml Pen/Strep. On the day of the experiment the attached cells were washed two times with Krebs-Ringer medium buffered with 20 mM HEPES and supplemented with 5 mM glucose and 0.2% BSA. Subsequently the cells were incubated for four hours with test compounds at concentrations from 10^{-10} - 10^{-6} M in the presence of $3x10^{-10}$ M growth hormone releasing factor. The total amount of growth hormone released into the medium was measured by RIA.

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