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Synthesis and Binding Potencies of Cyclohexapeptide Somatostatin Analogs Containing Naphthylalanine and Arylalkyl Peptoid Residues

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Abstract: We report the synthesis, binding affinities to the recombinant human somatostatin receptors, and structure-activity relationship studies of compounds related to the cyclic hexapeptide, *c*-[Pro^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}], L-363,301 (the numbering in the sequence refers to the position of the residues in native somatostatin). The Pro residue in this compound is replaced with the arylalkyl peptoid residues Nphe (*N*-benzylglycine), (S)βMeNphe [(S)-N-[(α-methyl)benzyl]glycine] or (*R*)βMeNphe [(*R*)-*N*-[(α-methyl)-benzyl]glycine] and L-1-naphthylalanine is incorporated into either position 7 or 11 of the parent compound. The synthesis and binding data of the Nnal^6 ([*N*-naphthylmethyl]glycine) analog of L-363,301 is also reported. The incorporation of the Nnal residue into position 6 of L-363,301 resulted in an analog with weaker binding affinities to all hsst receptors but enhanced selectivity towards the hsst2 receptor compared with the parent compound. The other compounds bind effectively to the hsst2 receptor but show some variations in the binding to the hsst3 and hsst5 receptors resulting in different ratios of binding affinities to the hsst5 and hsst2 or hsst3 and hsst2, respectively. The incorporation of the Nphe residue into position 6 and the Nal residue into position 7 of L-363,301 led to a compound which binds potently to the hsst2 and has increased selectivity towards this receptor (weaker binding to hsst3 and hsst5 receptors) compared with the parent compound. The analogs with β-methyl chiral substitutions in the aromatic peptoid side chain and Nal in position 7 or 11 bind effectively to the hsst2 and hsst5 receptors. They exhibit similar ratios of binding affinities to the hsst5 and hsst2 receptors as observed for L-363,301. There are however minor differences in binding to the hsst3 receptor among these analogs. These studies allow us to investigate the influence of additional hydrophobic groups on the binding activity to the isolated human somatostatin receptors and the results are important for the design of other somatostatin analogs. Copyright © 1999 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: synthesis; somatostatin analogs; peptoids; hsst receptors; binding activities

Abbreviations: DPPA, diphenylphosphoryl azide; EDC, 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide; GH, growth hormone; hsst receptor, recombinant human somatostatin receptor; Nphe, *N*-benzylglycine; (*R*)βMeNphe, (*R*)-*N*-(α-methyl)benzylglycine; (S)βMeNphe, (S)-*N*-(α-methyl)benzylglycine; Nnal, *N*-(naphthylmethyl)glycine; 4-EM, 4-ethylmorpholine; DIEA, diisopropylethylamine; DCM, dichloromethane.

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Somatostatin, a tetradecapeptide hormone released by the hypothalamus is a potent inhibitor of the release of several hormones (i.e. glucagon, growth hormone, insulin, gastrin) and regulates many other biological activities [1,2]. Somatostatin induces its biological effects by interacting with a family of structurally related receptors. Five human somatostatin receptors have been cloned and are referred to as hsst1–5 receptors [3] (the nomenclature suggested by Hoyer *et al.* is used) [4]. The

receptors hsst2 and hsst5 have been reported to mediate antiproliferative effects of somatostatin on tumor cell growth [5].

The wide range of physiological roles of somatostatin against a number of endocrine hormones and its very short biological half-life have led to substantial efforts to synthesize peptide analogs in search of molecules exhibiting potent and selective biological activity and longer duration of action than native somatostatin. In particular, the studies of Veber and coworkers resulted in the discovery of the cyclic hexapeptide analog *c*-[Pro^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}] [6], or L-363,301. This molecule shows higher biological activity than native somatostatin in inhibiting the release of growth hormone, insulin, and glucagon. From NMR studies, Veber proposed a type II′ β-turn about the tetrapeptide sequence Phe^7-D-Trp^8-Lys^9-Thr^{10}, which is considered to be the biologically active region. The Phe^{11}-Pro^6 dipeptide is contained within a type VI β-turn which includes a *cis* peptide bond between the Phe^{11} and Pro residues. This dipeptide unit, the so-called bridging region, is important for maintaining the proper orientation of the tetrapeptide portion and contains a component of ligand-receptor interaction *via* the phenyl ring [7].

The contribution of the dipeptide Phe^{11}-Pro^6 to the binding affinities of somatostatin analogs has been investigated extensively [8]. Veber *et al.* synthesized cyclic hexapeptides in which Pro^6 is replaced with other amino acids such as L-(NMe)Ala and D-(NMe)Ala [9].

Extensive structure-activity relationship studies of somatostatin analogs related to L-363,301 in which the side chain torsions were constrained as a result of C$^\beta$-methylation have revealed the features of a side chain topology for analogs that bind well to somatostatin receptors [10].

Recently, we have reported the synthesis and SAR studies of a first generation of peptoid analogs of L-363,301 [11]. These compounds with the general structure *c*-[Nxaa^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}] (Nxaa = Nphe, (*R*)βMeNphe and (*S*)βMeNphe) exhibit potent and selective binding to the hsst2 receptor and effectively inhibit the release of GH while they have no affect on the release of insulin *in vivo*.

The selectivity in *in vivo* and *in vitro* studies of the **Nphe^6**, (***R***)-**βMeNphe^6**, (**S**)-**βMeNphe^6** analogs of L-363,301 suggests the importance of an additional aromatic group at the bridging region. It can be assumed that an aromatic stacking in position 11, 6, and 7 of the cyclic hexapeptides might play a role in stabilizing a particular bioactive conformation of these peptides. Indeed, the conformational analysis by ^{1}H-NMR and computer simulations has shown that there is a hydrophobic stacking of the aromatic residues in positions 6 and 11 which we believe to be important for the selectivity towards the hsst2 receptor [11].

We now report the synthesis and binding affinities to the five recombinant human somatostatin receptors of two series of peptoid analogs of L-363,301. For the first series of compounds we used the analog *c*-[Nphe^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}] (**Nphe^6** compound) as a starting compound to investigate the influence of additional bulky aromatic groups on the binding affinities to the hsst receptors. We envisioned that the incorporation of the Nnal residue ([*N*-1-naphthylmethyl]alanine) into position 6 would lead to a conformationally more restricted analog with an altered binding profile. Furthermore, we incorporated L-1-naphthylalanine residues into the **Nphe^6** compound in order to probe the effects of the bulky aromatic residues on binding to the hsst receptors. The structures of the compounds *c*-[Nphe^6-Nal^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}] (**Nphe^6-Nal^7** analog), *c*-[Nnal^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}] (**Nnal^6** analog) and *c*-[Nphe^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Nal^{11}] (**Nal^{11}-Nphe^6** analog) are shown in Figure 1.

Starting with the β-methylated peptoid analogs **(S)-** and **(*R*)-β-MeNphe^6** of L-363,301, we synthesized a second series of compounds containing L-1-naphthylalanine in either position 7 or 11. This led to the compounds *c*-[(*R*)-βMeNphe^6-Nal^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}] (**(*R*)-βMeNphe^6-Nal^7** analog), *c*-[(S)-βMeNphe^6-Nal^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}] (**(S)-βMeNphe^6-Nal^7** analog), *c*-[(*R*)-βMeNphe^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Nal^{11}] (**Nal^{11}-(*R*)-βMeNphe^6** analog), and *c*-[(S)-βMeNphe^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Nal^{11}] (**Nal^{11}-(S)-βMeNphe^6** analog) (Figure 2).

These studies are part of an effort to investigate the role of the bridging region using hydrophobic interactions to develop a more complete understanding of the structural features which promote strong and specific somatostatin receptor–ligand interactions. This family of compounds coupled with their effects on biological activity provides insight into the structural requirements for binding to the isolated receptors.

The conformational analysis of our peptoid analogs using ^{1}H-NMR and computer simulations is described in the accompanying paper [12].

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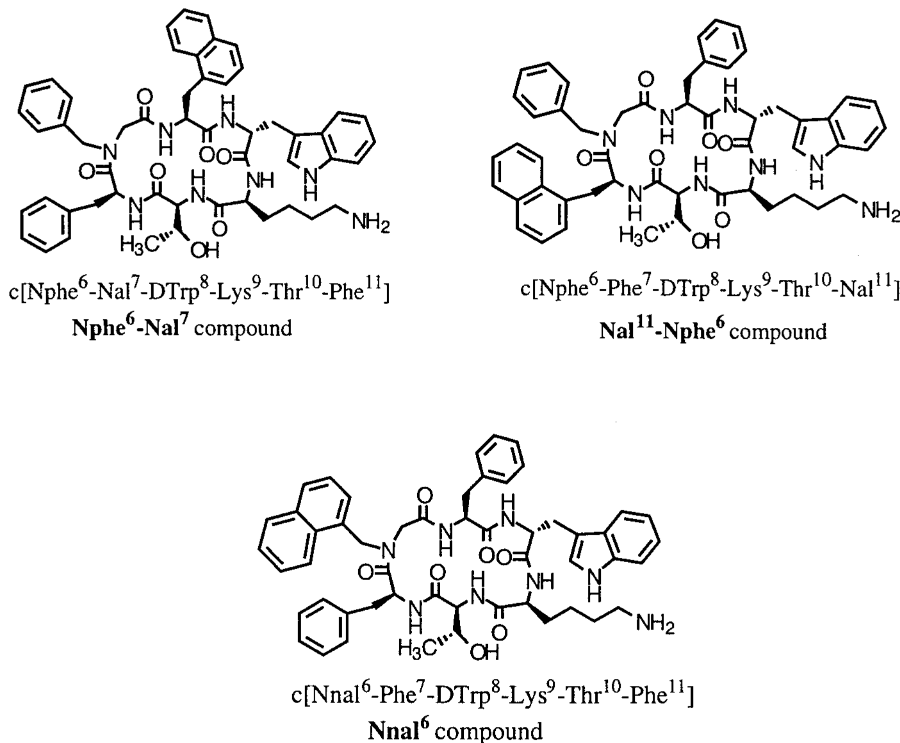

Figure 1 The naphthylalanine-peptoid analogs of L-363,301.

Synthesis

The synthesis of the peptoid residues which involves the alkylation of benzyl, (*R*)-α-methylbenzyl, (S)-α-methylbenzyl or 1-naphthylmethyl amines with either ethyl bromoacetate or benzyl bromoacetate resulted in *N*-benzylglycine, (*R*)- or (S)-*N*[(α-methyl)benzyl]glycine, and *N*-(naphthyl methyl)-glycine respectively [13,14]. The syntheses of our analogs were designed around a reaction between the tripeptide Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-OH [15] and a second tripeptide H-Xaa-Nxbb-Xcc-OBzl (Xaa = Phe or Nal, Nxbb = Nphe or Nnal, (*R*) or (*S*)βMeNphe, Xcc = Phe or Nal) fragments incorporating the bridging region. Ethyl[(*N*,*N*-dimethyl-amino)propyl]carbodiimide (EDC) was used as coupling agent in the presence of HOAt to prepare the protected tripeptides.

For the synthesis of **Nphe⁶-Nal⁷**, **(*R*)-βMeNphe⁶-Nal⁷**, and **(S)-βMeNphe⁶-Nal⁷** analogs, the dipeptides Boc-Phe-Nxaa-OH (Nxaa = Nphe, (*R*)βMeNphe, or (*S*)βMeNphe) were synthesized as described previously [11]. These dipeptides were allowed to react with TosOH·H-Nal-OBzl in the presence of EDC/HOAt to give the tripeptides Boc-Phe-Nxaa-Nal-OBzl (Nxaa = Nphe, (*R*)βMeNphe, or (*S*)βMeNphe) (Scheme 1). For the synthesis of **Nal¹¹-Nphe⁶**, **Nal¹¹-(*R*)-βMeNphe⁶**, and **Nal¹¹-(S)-βMeNphe⁶** analogs, Boc-Nal was allowed to react with the Nxaa-OEt peptoid residues (Nxaa = Nphe, (*R*)βMeNphe, or (*S*)βMeNphe) in the presence of EDC/HOAt and the dipeptides Boc-Nal-Nxaa-OEt were obtained in good yields (Scheme 2). After treatment of the dipeptides Boc-Nal-Nxaa-OEt with LiOH, the Boc-Nal-Nxaa-OH free acids were allowed to react with TosOH·H-Phe-OBzl in the presence of EDC/HOBt to give the tripeptides Boc-Nal-Nxaa-Phe-OBzl.

The tripeptides H-Nal-Nxaa-Phe-OBzl or H-Phe-Nxaa-Nal-OBzl (Nxaa = Nphe, (*R*)βMeNphe or (*S*)βMeNphe) were condensed with the tripeptide Cbz-D-TrpLys(Boc)-Thr(*t*Bu)-OH to give the protected hexapeptides Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-Nal-Nxaa-Phe-OBzl or Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-Phe-Nxaa-Nal-OBzl. Catalytic hydrogenolysis of the terminal protecting groups was carried out and DPPA was used for the cyclization of the terminally deprotected hexapeptides to yield *c*-[D-Trp⁸-Lys⁹-(Boc)-Thr¹⁰(*t*Bu)-Phe¹¹-Nxaa⁶-Nal⁷] or *c*-[D-Trp⁸-Lys⁹(Boc)-Thr¹⁰(*t*Bu)-Nal¹¹-Nxaa⁶-Phe⁷]. Treatment of the protected cyclic hexapeptides with TFA in the presence of scavengers allowed for the simulta-

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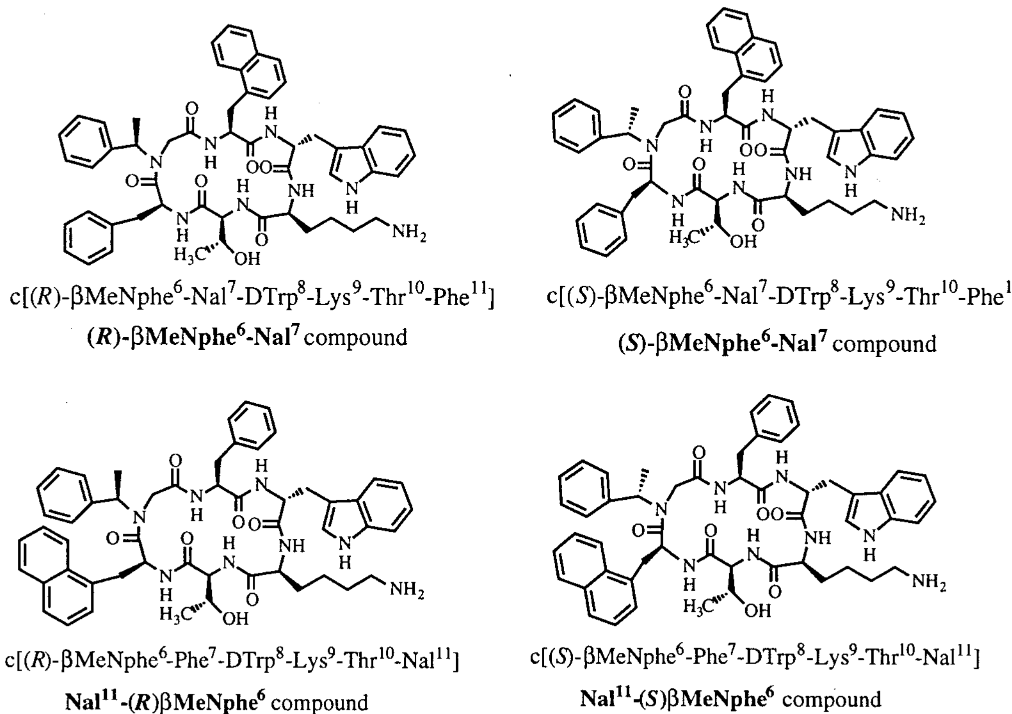
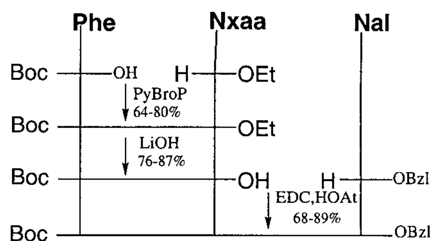


Figure 2 The β-methyl peptoid analogs of L-363,301.

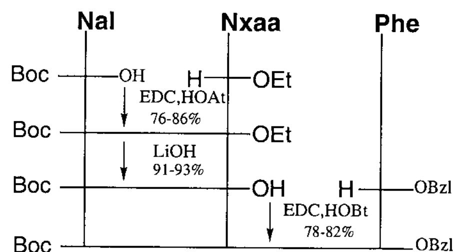
neous deprotection of the *tert*-butylcarbonyl and *tert*-butyl ether side chain protecting groups. Following purification by RP-HPLC using aqueous acetonitrile as eluent, the target compounds were obtained. Detailed synthesis and characterization of all new compounds are given in the Experimental Section.

Biological Results

The somatostatin analogs were tested *in vitro* for their specific binding to the five human somatostatin receptors expressed in CHO cell lines. The binding activities of the **Nphe⁶**, the **Nphe⁶-Nal⁷** and the **Nal¹¹-Nphe⁶** are given in Table 1 and are compared with those for L-363,301 and the **Nphe⁶** compound. The incorporation of the peptoid residue Nnal into position 6 of L-363,301 resulted in a compound with lower binding affinities to all receptors but increased selectivity towards the hsst2 receptor (7-fold compared with L-363,301 and 2-fold compared with the **Nphe⁶** compound). Incorporation of L-naphthylalanine into position 7 or position 11 led to analogs with slightly increased binding affinity to the hsst2 compared with L-363,301 and the **Nphe⁶** analog. There are differences in the binding to the hsst3 and hsst5 receptors between the two analogs **Nphe⁶-Nal⁷** and **Nal¹¹-Nphe⁶**. The



Scheme 1 Synthesis of Boc-Phe-Nxaa-Nal-OBzl where Nxaa = Nphe, (R)βMeNphe or (S)βMeNphe.



Scheme 2 Synthesis of Boc-Nal-Nxaa-Phe-OBzl where Nxaa = Nphe, (R)βMeNphe or (S)βMeNphe.

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Table 1 *In Vitro* Inhibition of Radioligand Binding to Human Recombinant Receptors: K_i (nM) ± SEM[a]

Compound	hsst1	hsst2	hsst3	hsst4	hsst5	hsst5/hsst2	hsst3/hsst2
c-[Phe^{11}-Pro^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	5.10 ± 0.76	129 ± 51	>1000	20.3 ± 10.5	4.0	25.2
c-[Phe^{11}-Nphe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	6.98 ± 0.83	253 ± 57	>1000	100.7 ± 45.5	14.4	36.2
c-[Phe^{11}-Nnal^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	32.2 ± 2.51	>1000	>1000	830 ± 137	27.1	>31
c-[Phe^{11}-Nphe^{6}-Nal^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	3.57 ± 0.2	204 ± 21.7	>1000	54.2 ± 14.4	15.2	57.1
c-[Nal^{11}-Nphe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	3.73 ± 0.23	88.8 ± 2.4	>1000	24.9 ± 4.4	6.7	23.8

[a] Binding assays were carried out with cell membranes from CHO-K1 cells.

Table 2 *In Vitro* Inhibition of Radioligand Binding to Human Recombinant Receptors: K_i (nM) ± SEM[a]

Compound	hsst1	hsst2	hsst3	hsst4	hsst5	hsst5/hsst2	hsst3/hsst2
c-[Phe^{11}-Pro^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	5.10 ± 0.76	129 ± 51	>1000	20.3 ± 10.5	4.0	25.2
c-[Phe^{11}-(*R*)βMeNphe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	2.33 ± 0.41	425 ± 100	>1000	33.5 ± 12.5	14.4	182.4
c-[Phe^{11}-(S)βMeNphe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	29.5 ± 2.49	797 ± 125	987 ± 13	87.0 ± 22.6	2.9	27.0
c-[Phe^{11}-(*R*)βMeNphe^{6}-Nal^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	7.14 ± 0.16	380 ± 73	>1000	10.3 ± 3.7	1.4	53.2
c-[Phe^{11}-(S)βMeNphe^{6}-Nal^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	3.15 ± 0.23	198 ± 37	>1000	20.3 ± 10.3	6.4	62.8
c-[Nal^{11}-(*R*)βMeNphe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	2.77 ± 0.28	125 ± 7.5	>1000	10.7 ± 3.1	3.9	45.1
c-[Nal^{11}-(*S*)βMeNphe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}]	>1000	7.25 ± 2.01	267 ± 58	>1000	27.6 ± 8.5	3.8	37

[a] Binding assays were carried out with cell membranes from CHO-K1 cells.

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Nal^{11}-Nphe^{6} analog binds more effectively to both the hsst3 and hsst5 receptors compared with the **Nphe^{6}-Nal^{7}** compound. This results in lower hsst5/hsst2 and hsst3/hsst2 ratios for the **Nal^{11}-Nphe^{6}** analog (lower selectivity) compared with the **Nphe^{6}-Nal^{7}** compound. The ratios for the **Nal^{11}-Nphe^{6}** analog as presented in Table 1 are very similar to those found for L-363,301. The **Nphe^{6}-Nal^{7}** compound is more selective than the **Nphe^{6}** compound and L-363,301 with regard to binding to the hsst2 receptor.

The binding affinities for the β-methylated analogs **(*R*)-βMeNphe^{6}-Nal^{7}, (S)-βMeNphe^{6}-Nal^{7}, Nal^{11}-(*R*)-βMeNphe^{6}**, and **Nal^{11}-(S)-βMeNphe^{6}** are presented in Table 2 and compared with those of L-363,301, the **(S)-βMeNphe^{6}** and **(*R*)-βMeNphe^{6}** analogs. There are two important aspects regarding these data: the reduced binding affinities to the hsst2 and hsst3 receptors found for the **(S)-βMeNphe^{6}** are enhanced by the presence of the Nal residue in position 7 or 11 and this leads to very similar binding data for the **(S)-βMeNphe^{6}-Nal^{7}** and the **Nal^{11}-(S)-βMeNphe^{6}** compounds compared with those observed for L-363,301. The selectivity towards the hsst2 receptor found for the **(*R*)-βMeNphe^{6}** analog is reduced in the analogs with Nal in position 7 or 11. This is obvious from the hsst5/hsst2 and hsst3/hsst2 ratios as given in Table 2. In fact all four analogs show very similar hsst5/hsst2 and hsst3/hsst2 ratios despite minor differences in the binding affinities. It is also worth noting that the binding affinities to the hsst2 receptors of the **(*R*)-βMeNphe^{6}-Nal^{7}** and **Nal^{11}-(S)-βMeNphe^{6}** compounds and those for the **(S)-βMeNphe^{6}-Nal^{7}** and **Nal^{11}-(*R*)-βMeNphe^{6}** compounds are identical indicating that the presence of the Nal residue in either position 11 or 7 together with the different orientations of the aromatic side chain within the peptoid residue in position 6 leads to a very similar topology which influences the binding to the hsst2 receptor. To a lesser extent this can also be observed for the hsst3 receptor. The hsst5 receptor on the other hand seems to be primarily influenced by the sterochemistry within the peptoid residue since the Nal containing analogs with the same configuration within the peptoid residue show identical binding affinities. Overall the effects observed in the **(*R*)-βMeNphe^{6}** and **(S)-βMeNphe^{6}** analogs which had Phe residues in positions 7 and 11 have been reduced by incorporating Nal residues into either position 11 or 7. Significantly, the selectivity for the hsst2 receptor of the **(*R*)-βMeNphe^{6}** analog was lost by incorporation of the bulky Nal residues. These data suggest that the concurrent presence of the Nal residue in either positions 11 or 7 and a β-methylated peptoid residue leads to conformations with a less defined topochemical array.

General Considerations

The incorporation of arylalkyl peptoid residues into positions 6 of L-363,301 which we have reported previously gave rise to hsst2 selective analogs of somatostatin. In particular the **(*R*)-βMeNphe^{6}** analog showed increased *in vitro* activity towards the hsst2 receptor. These peptoid analogs exhibit selective inhibition of the release of growth hormone *in vivo* while they have no effect on the release of insulin. Based upon these results we envisioned that additional restrictions of the accessible side chain orientations in and around the bridging region could further enhance the selectivity of these compounds. We believed that the incorporation of Nal into position 7 or 11 of our peptoid analogs could enhance potency and/or selectivity of these analogs. However, the replacement of Phe^{7} or Phe^{11} with the larger aromatic Nal residue has reduced the selectivity observed in the first generation of peptoid analogs of L-363,301. Conformational analysis of these analogs has shown that the presence of Nal in position 7 or 11 does not lead to hydrophobic stacking of the side chains of the aromatic residues in positions 6 and 11 as observed in the **(*R*)-βMeNphe^{6}** analog but instead leads to an enhancement of flexibility of the side chains and the backbone within the bridging region. Our results show that the incorporation of several constrained residues into the bridging region of L-363,301 can overcome the improvements in selectivity achieved by the incorporation of a single side chain constrained residue. Nevertheless, our analogs bind effectively to the hsst2 and hsst5 receptors and the presence of unusual peptoid residues and the Nal residue might improve the bioavailability of our analogs compared with L-363,301. The incorporation of the Nnal residue in position 6 resulted in an analog which exhibits reduced binding affinity to all hsst receptors but has the best selectivity to the hsst2 receptor in this series of compounds. These structure-activity relationship studies will be useful for the design of other somatostatin analogs.

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EXPERIMENTAL PART

General

Fast atom bombardment mass spectra and electrospray ionization mass spectra were obtained from the University of California, Riverside and the Scripps Research Institute. Analysis by ^{1}H-NMR spectroscopy was carried out on a 360 MHz spectrometer, employing a TechMag pulse programmer and an Oxford Instruments superconducting magnet or on a 500 MHz Bruker AMX 500 instrument. High pressure liquid chromatography was run on Waters HPLC systems, equipped with two Waters model M45 solvent pumps, a Kratos Spectroflow 757 absorbance detector and a Hewlett-Packard 3396 Series II integrator or equipped with two Water model 510 Pumps, a Waters model 715 ULTRA WISP automated injector, a Waters 996 PDA (photodiode array) multi wavelength absorbance detector and a NEC model 484/33 computer system with Millipore Millenium HPLC control software. Dichloromethane and dimethylforamide were dried over 4 Å molecular sieves and stored under nitrogen. Diisopropylethylamine was distilled prior to use. Boc amino acids were purchased from Bachem California. The syntheses of Boc-Phe-Nphe-OH, Boc-Phe(*R*)βMeNphe-OH, Boc-Phe-(*S*)βMeNphe-OH and Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-OH was reported previously [11].

Receptor Binding Assays

Stable Expression of hsst Receptor Subtypes. The complete coding sequences of genomic fragments of the hsst1–4 receptor genes and a cDNA clone for the hsst5 were subcloned into mammalian expression vector pCMV. Clonal cell lines stably expressing the hsstl-5 receptors were obtained by transfection into CHO-K1 cells (ATCC) using the calcium phosphate co-precipitation method [16]. The plasmid pRSV-neo (ATCC) was included as a selectable marker. Clonal cell lines were selected in RPMI 1640 media containing 0.5 mg/mL of G418 (Gibco), ring cloned, and expanded into culture.

Radioligand Binding Assays. Membranes for *in vitro* receptor binding assays were obtained by homogenizing (Polytron setting 6, 15 s) the CHO-K1 cells, expressing the hsst receptor subtypes, in ice-cold 50 mM Tris–HCl and centrifuging twice at 39000 × g (10 min), with an intermediate resuspension in fresh buffer. The final pellets were resuspended in 10 mM Tris–HCI for assay. For the hsst1, 3, 4, 5 assays, aliquots of the membrane preparations were incubated (30 min/37°C) with 0.05 nM [^{125}I-Tyr^{11}]SRIF-14 in 50 mM HEPES (pH 7.4) containing BSA (10 mg/mL), $MgCl_2$ (5 mM), Trasylol (200 KIU/mL), bacitracin (0.02 mg/mL), and phenylmethylsuphonyl fluoride (0.02 mg/mL). The final assay volume was 0.3 mL. For the hsst2 assay, [^{125}I]MK-678 (0.05 nM) was employed as the radioligand and the incubation time was 90 min/25°C. The incubations were terminated by rapid filtration through GF/C filters (pre-soaked in 0.3% polyethylene imine) using a Brandel filtration manifold. Each tube and filter were then washed three times with 5-mL aliquots of ice-cold buffer. Specific binding was defined as the total radioligand bound minus that bound in the presence of 1000 nM SRIF-14 (hsst1, 3, 4, 5) or 1000 nM MK678 for hsst2.

Synthesis. **Nal-OBzl tosylate (1).** (1-Naphthyl)-alanine (5.38 g, 25 mmol), and p-toluenesulfonic acid(4.8 g, 25.5 mmol) were added to a mixture of benzyl alcohol (10 mL) and toluene (25 mL). The mixture was heated to reflux and the water formed in the reaction trapped in a Dean-Stark receiver. After 6 h, the mixture was allowed to cool to room temperature, was diluted with ether (50 mL) and cooled in an ice water bath for 2 h. The precipitate was filtered and recrystallized with MeOH/ether to give 8.9 g slightly yellow solid (75% yield). ^{1}H-NMR (360 MHz, DMSO-d_6) δ 8.57 (br, s, 2H, NH), 7.9 – 6.81 (m, 12H, Nal and OBzl H arom), 4.83 (dd, 2H, OCH_2), 4.61 (m, 1H, CH^{α}), 3.91 – 2.73 (m, 2H, CH_2^{β}).

Boc-Phe-Nphe-Nal-OBzl (2). The dipeptide Boc-Phe-Nphe-OH (1.28 g, 3 mmol), TosOH·H-Nal-OBzl (**1**) (1.43 g, 3 mmol) and HOAt (0.57 g, 4.2 mmol) were dissolved in 10 mL DCM and the mixture was cooled to 0°C. EDC·HCl (0.92 g, 4.8 mmol) was added, followed by 4-EM (0.38 mL, 3 mmol). The reaction was stirred overnight at room temperature. The reaction solvent was removed under reduced pressure and the residue was dissolved in EtOAc. The organic layer was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, and H_2O. After drying over $MgSO_4$, the solvent was removed under reduced pressure. The crude product was chromatographed on silica gel (hexane/EtOAc: 3/2) to give **2** as a white solid 2.06 g (98%). TLC $R_f = 0.47$ (hexane/EtOAc: 1/1); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 8.12 – 6.83 (m, 22H, Phe, Nal, Nphe, and OBzl H arom.), 5.20 – 2.72 (m, 14H, two NH, OCH_2, Phe, Nal, Nphe CH^{α}, and Phe, Nal, Nphe CH_2^{β}), 1.38, 1.32 (s, s, 9H, Boc CH_3 c/t). FAB-MS: calculated M^+ 699; observed: m/z 700 $(M+H)^+$, 722 $(M+Na)^+$.

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Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-Phe-Nphe-Nal-OBzl (3). A solution of the tripeptide **2** (1.05 g, 1.5 mmol) in 20 mL of CH_2Cl_2 was treated with 20 mL of TFA. After 1 h the solvents were removed under reduced pressure, and toluene (3 × 10 mL) was sequentially added and removed under reduced pressure to give a white solid. To the cooled (0°C) solution of the amine salt, the tripeptide Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-OH (1.08 g, 1.5 mmol) and HOAt (0.33 g, 2.4 mmol) in 5.0 mL of DMF was added EDC·HCl (0.46 g, 2.4 mmol) followed by 4-EM (0.19 mL, 1.5 mmol). The mixture was allowed to warm to 20°C and was stirred at room temperature overnight. After removal of DMF under reduced pressure, the residue was suspended in EtOAc which was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, H_2O and dried ($MgSO_4$). Purification on silica gel column chromatography gave **3** as a white solid 1.04 g (53% yield). TLC $R_f = 0.43$ ($CHCl_3$/MeOH: 90/10); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.80 (br, s, 1H, indole NH), 8.87 – 6.61 (m, 38H, Cbz, Trp, Phe, Nal, Nphe, OBzl arom. and six NH), 5.11 – 3.35 (m, 14H, two OCH_2, seven CH^{α}, Thr CH^{β}, and Nphe CH_2^{β}), 3.10 – 2.82 (m, 8H, Phe, Nal, Trp CH_2^{β}, and Lys CH_2^{ε}), 1.53 – 0.89 (m, 6H, three Lys CH_2), 1.33 (s, 9H, Boc CH_3), 1.03 (s, 9H, *t*Bu CH_3), 0.97, 0.90 (d, d, $J = 10.4$ Hz, 3H, Thr CH_3^{δ} c/t). FAB-MS: calculated M^+ 1304; observed m/z 1305 $(M+H)^+$, 1327 $(M+Na)^+$.

***c*-[Nphe^6-Nal^7-D-Trp^8-Lys^9(Boc)-Thr^10(*t*Bu)-Phe^11] (4).** To a solution of hexapeptide **3** (0.90 g, 0.69 mmol), in 100 mL of 20% AcOH in MeOH was added 10% Pd/C (90 mg). Hydrogen gas was introduced at atmospheric pressure at 20°C and the mixture was allowed to react overnight. The catalyst was removed by filtration and the solvent removed under reduced pressure. Methylene chloride (20 mL) and 4 N HCl in dioxane (0.2 mL) were sequentially added and removed under reduced pressure. The sample was dried under reduced pressure to give 0.62 g (80%) of a white solid which was used directly without further purification. A solution of the deprotected linear hexapeptide (0.60 mg, 0.54 mmol) in 500 mL of freshly distilled DMF was chilled to −10°C. To this solution DPPA (0.32 mL, 1.61 mmol) and K_2HPO_4 (0.93 g, 5.37 mmol) were added. After 20 min at −10°C, the reaction was allowed to stir at 4°C for 2 days. The solvent was removed under reduced pressure. Purification was carried out by silica gel chromatography ($CHCl_3$/MeOH/AcOH: 98/1/1) to give the cyclic peptide **4** as a yellow powder 368 mg (64% yield). TLC $R_f = 0.38$ ($CHCl_3$/MeOH: 90/10); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.17, 10.14 (s, s, 1H, indole NH c/t), 8.41–6.69 (m, 28H, Trp, Phe, Nal, Nphe arom. and six NH), 5.40 – 3.63 (m, 10H, seven CH^{α}, Thr CH^{β}, and Nphe CH_2^{β}), 3.58 – 2.20 (m, 8H, Trp, Phe, Nal CH_2^{β}, and Lys CH_2^{ε}), 1.65 – 0.80 (m, 6H, three Lys CH_2), 1.33 (s, 9H, Boc CH_3), 1.19, 1.15 (s, s, 9H, *t*BuCH_3 c/t), 1.06 (d, $J = 7.2$ Hz, 3H, Thr CH_3^{γ}). FAB-MS: calculated M^+ 1063; observed: m/z 1064 $(M+H)^+$, 1086 $(M+Na^+)$.

***c*-[Nphe^6-Nal^7-D-Trp^8-Lys^9-Thr^10-Phe^11] or Nphe^6-Nal^7 analog.** To a solution of cyclic peptide **4** (100 mg, 0.094 mmol) in 14 mL CH_2Cl_2 anisole (10% v/v, 2.8 mL) and ethanedithiol (5% v/v, 1.4 mL) were added. The mixture was chilled (0°C) and treated with TFA (14 mL) for 1 h at 0°C and 2 h at 20°C. The solvent was removed under reduced pressure and toluene (3 × 10 mL) was added and removed under reduced pressure. The residue was crystallized in cold (0°C) ether, isolated by filtration and purified by RP-HPLC to give 46 mg (64%); EMS: calculated M^+ 906; observed: positive m/z 907 $(M+H)^+$, 929 $(M+Na)^+$; negative m/z 905 $(M-H^+)$, 941 $(M+Cl)^-$; 1019 $(M+TFA)^-$. The NMR data for this compound are presented in Table 3.

Boc-Phe-(S)*β*MeNphe-Nal-OBzl (5). Coupling of Boc-Phe-(S)*β*MeNphe-OH (1.28 g, 3.0 mmol) with TosOH·H-Nal-OBzl (**1**) (1.43 g, 3 mmol) using HOAt (0.57 g, 4.2 mmol), EDC·HCl (0.92 g, 4.8 mmol), and 4-EM (0.38 mL, 3 mmol) as described for compound **2** furnished compound **5** as a white solid 1.89 g (88% yield). TLC $R_f = 0.48$ (EtOAc/hexane: 1/1); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 8.27 – 6.82 (m, 24 H, Phe, Nal, (*S*)*β*MeNphe, OBzl H arom. and two NH), 5.92, 5.83 (m, m, 1H, CH^{α} c/t), 5.12, 5.10 (s, s, OCH_2 c/t), 5.33 – 4.08 (m 3H, Phe and Nal CH^{α}, (*S*)*β*MeNphe CH^{β} c/t), 3.98 – 2.70 (m, 4H, Phe and Nal CH_2^{β}), 1.43, 1.41 (s, s, 9H, Boc CH_3 c/t), 1.06, 0.82 (d, d, $J = 7.9$ Hz, 3H, (*S*)*β*MeNphe CH_3 c/t). FAB-MS: calculated: M^+ 713; observed: m/z 714 $(M+H)^+$, 736 $(M+Na)^+$.

Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-Phe-(S)*β*MeNphe-Nal-OBzl (6). A solution of the tripeptide **5** (1.07 g, 1.05 mmol) in 10 mL of DCM was treated with 10 mL of TFA. After 1 h the solvent was removed under reduced pressure and toluene was added (3 × 10 mL) and removed to give a white solid. Coupling between the deprotected tripeptide and the tripeptide Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-OH (1.08 g, 1.5 mmol) using HOAt (0.33 g, 2.4 mmol), EDC·HCl (0.46 g, 2.4 mmol), and 4-EM (0.19 mL, 1.5 mmol) in DMF as described for compound **3** furnished compound **6** as an off-white solid 1.25 g (63% yield). TLC $R_f = 0.6$ ($CHCl_3$/MeOH: 90/10); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.79 (br, s, 1H, indole NH), 8.80 – 6.70 (m, 38H, Cbz, Trp, Phe, Nal, (*S*)*β*MeNphe, Obzl arom. and six

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Table 3 Chemical Shifts of *c*-[Phe[11]-Nphe[6]-Nal[7]-D-Trp[8]-Lys[9]-Thr[10]] (1), *c*-[Nal[11]-Nphe[6]-Phe[7]-D-Trp[8]-Lys[9]-Thr[10]] (2), *c*-[Phe[11]-Nnal[6]-Phe[7]-D-Trp[8]-Lys[9]-Thr[10]] (3) (δ in ppm relative to internal DMSO-d_6)

	Phe[11]-Nphe[6]-Nal[7]		Nal[11]-Nphe[6]-Phe[7]		Phe[11]-Nnal-Phe[7]	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*
Xaa[11]						
HN	8.69	8.10	8.75	8.05	8.68	8.18
Hα	3.91	3.36	5.02	3.61	4.92	4.30
Hβ	2.99/2.95	2.79	2.38/2.31	3.31	2.97/2.93	2.92/2.79
Arom. H	7.22	6.92	7.32, 7.38, 7.80	7.27, 7.45, 7.89	7.19	6.83
	7.18	7.22	7.90, 7.52, 7.53	7.86, 7.21, 7.26	7.06	7.13
	7.2	7.29	8.09	7.48	7.31	7.16
Nxbb[6]						
Hα	4.34/2.84	4.68/3.21	3.68/2.50	4.65/3.06	4.31/2.98	4.71/3.11
Hβ	4.84/3.30	5.07/4.29	4.87/3.25	4.92/4.18	5.28/3.98	5.46/4.79
β-Me	–	–	–	–	6.60, 7.26	6.82, 7.32, 7.83
Arom. H	6.65	6.97	6.55	6.84	7.78	7.97, 7.55, 7.53
	7.17	7.27	7.10	7.19	7.91, 7.51	7.94
	7.27	7.3	7.18	7.24	7.53, 7.87	
Xcc[7]						
HN	8.10	7.47	7.70	7.41	7.96	7.64
Hα	4.72	4.06	4.55	4.48	4.58	4.50
Hβ	3.29/–	3.59/3.11	2.77/2.72	2.92/2.78	2.72/2.64	2.88/2.60
Arom. H	7.11, 7.23, 7.78	7.18, 7.24, 7.77	6.86	6.99	6.92	7.03
	7.49, 7.88, 7.50	7.49, 7.92, 7.53	7.02	7.09	7.06	7.13
	8.22	8.40	7.09	7.1	7.32	7.16
D-Trp						
HN	8.41	8.50	8.49	8.52	8.44	8.12
Hα	4.38	4.06	4.36	4.11	4.38	4.35
Hβ	2.88/2.44	2.88/2.50	2.96/2.67	2.98/2.74	2.97/2.65	3.07/2.83
H2	6.90	6.83	7.01	6.99	6.99	7.03
H4	7.44	7.38	7.51	7.48	7.49	7.50
H5	6.99	6.98	6.99	6.99	6.97	6.98
H6	7.09	7.05	7.04	7.04	7.03	7.05
H7	7.31	7.31	7.32	7.32	7.30	7.32
H1	10.71	10.68	10.76	10.76	10.75	10.78
Lys						
HN	8.51	8.35	8.57	8.34	8.56	8.38
Hα	3.78	3.92	3.87	4.00	3.84	4.04
Hβ	1.65/1.36	1.72/1.28	1.69/1.38	1.75/1.31	1.67/1.40	1.75/1.36
Hγ	0.83	0.72	0.93	0.86	0.93	0.87
Hδ	1.33	1.27	1.40	1.35	1.35	1.34
Hε	2.56	2.55	2.59	2.57	2.57	2.58
NH2	7.56	7.55	7.62	7.60	7.62	7.61
Thr						
HN	7.12	7.82	7.15	7.77	7.12	7.70
Hα	4.33	4.36	4.38	4.24	4.33	4.25
Hβ	3.92	3.85	3.91	3.79	3.91	3.83
Hγ	1.07	1.21	1.06	1.12	1.09	1.09
OH	4.96	5.45	5.04	5.40	5.03	5.38

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NH), 5.66 – 3.60(m, 13H, two OCH_2, seven CH^{α}, Thr CH^{β}, and (S)βMeNphe CH^{β}), 3.31 – 2.81 (m, 8H, Phe, Nal, Trp CH_2^{β}, and Lys CH_2^{ε}), 1.57 – 0.93 (m, 6H, three Lys CH_2), 1.32 (s, 9H, Boc CH_3), 1.10, 1.05 (s, 9H, *t*Bu CH_3 c/t), 1.20 – 0.94 (m, 6H, Thr CH_3^{δ} c/t and (S)βMeNphe CH_3^{δ} c/t). FAB-MS: calculated: M^+ 1318; observed: *m/z* 1319 $(M+H)^+$, 1341 $(M+Na)^+$.

***c*-[(S)βMeNphe⁶-Nal⁷-D-Trp⁸-Lys⁹(Boc)-Thr¹⁰-(*t*Bu)-Phe¹¹] (7).** Deprotection of the linear hexapeptide **6** (0.66 g, 0.5 mmol) as described for **4** yielded 466 mg pinkish solid. 400 mg (0.35 mmol) of the deprotected compound was cyclized with DPPA (0.21 mL, 1.05 mmol) and K_2HPO_4 (0.61 g, 3.5 mmol) to furnish 193 mg yellow solid (**7**) (51% yield). TLC $R_f = 0.55$ ($CHCl_3$/MeOH: 9/1); ^1H-NMR (360 MHz, DMSO-d_6) δ 10.72, 10.66(s, s, 1H, indole NH c/t), 8.55 – 6.85 (m, 28H, Trp, Phe, Nal, (S)βMeNphe arom. and six NH), 5.70 (m, 1H, CH^{α}), 4.85 – 3.54 (m, 8H, six CH^{α}, Thr CH^{β}, and (S)βMeNphe CH^{β}), 3.12 – 2.31 (m, 8H, Trp, Phe, Nal CH_2^{β}, and Lys CH_2^{ε}), 1.72–0.80 (m, 6H, three Lys CH_2), 1.36 (s, 9H, Boc CH_3), 1.17 (s, 9H, *t*Bu CH_3), 1.08 – 1.00 (m, 6H, Thr CH_3^{δ} c/t and (S)βMeNphe CH_3^{δ} c/t). FAB-MS: calculated: M^+ 1077; observed: *m/z* 1078 $(M+H)^+$, 1100 $(M+Na^+)$.

***c*-[(S)βMeNphe⁶-Nal⁷-D-Trp⁸-Lys⁹-Thr¹⁰-Phe¹¹** or **(S)βMeNphe⁶-Nal⁷** analog. Deprotection of cyclic peptide (**7**) (100 mg, 0.093 mmol) in 14 mL CH_2Cl_2 with anisole (10% v/v, 2.8 mL), ethanedithiol (5% v/v, 1.4 mL), and TFA (14 mL) as described for the Nphe⁶-Nal⁷ analog gave 48 mg of the **(S)βMeNphe⁶-Nal⁷** analog. (56%); FAB-MS: calculated: M^+ 920; observed: 921 $(M+H)^+$, 943 $(M+Na)^+$. The NMR data for this compound are presented in Table 4.

Boc-Phe-(*R*)βMeNphe-Nal-OBzl (8). Coupling of Boc-Phe-(*R*)βMeNphe-OH (1.28 g, 3.0 mmol) with TosOH·H-Nal-OBzl (**1**) (1.43 g, 3 mmol) using HOAt (0.57 g, 4.2 mmol), EDC·HCl (0.92 g, 4.8 mmol), and 4-EM (0.38 mL, 3 mmol) as described for compound **2** furnished **8** as a white solid 1.45 g (68% yield). TLC $R_f = 0.31$ (EtOAc/hexane: 4/6); ^1H-NMR (360 MHz, $CDCl_3$) δ 8.19 – 6.74 (m, 24H, Phe, Nal, (*R*)βMeNphe, OBzl H arom. and two NH), 5.74, 5.26 (m, m, 1H, CH^{α} c/t), 5.04, 5.02 (s, s, OCH_2 c/t), 4.94 – 4.26 (m 3H, Phe and Nal CH^{α}, (*R*)βMeNphe CH^{β} c/t), 3.83 – 2.62 (m, 4H, Phe and Nal CH_2^{β}), 1.35, 1.31 (s, s, 9H, Boc CH_3 c/t), 0.97, 0.73 (d, d, $J = 7.2$ Hz, 3H, (*R*)βMeNphe CH_3 c/t) FAB-MS: calculated: M^+ 713; observed: *m/z* 714 $(M+H)^+$, 736 $(M+Na)^+$.

Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-Phe-(*R*)βMeNphe-Nal-OBzl (9). A solution of the tripeptide **8** (0.54 g, 0.75 mmol) in 10 mL of DCM was treated with 10 mL of TFA. After 1 h the solvent was removed under reduced pressure and toluene was added (3 × 10 mL) and removed to give a white solid. Coupling between deprotected tripeptide and the tripeptide Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-OH (0.54g, 0.75 mmol) using HOAt (0.16 g, 1.2 mmol), EDC·HCl (0.23 g, 1.2 mmol), and 4-EM (0.10 mL, 0.75 mmol) in DMF as described for compound **3** furnished compound **9** as an off-white solid 6.1 g (62% yield). $R_f = 0.61$ ($CHCl_3$/MeOH: 90/10); ^1H-NMR (360 MHz, DMSO-d_6) δ 10.80 (s, 1H, indole NH), 8.85 – 6.71 (m, 38H, Cbz, Trp, Phe, Nal, (*R*)βMeNphe, OBzl arom. and six NH), 5.68 – 4.14 (m, 13H, two OCH_2, seven CH^{α}, Thr CH^{β}, and (*R*)βMeNphe CH^{β}), 3.03 – 2.79 (m, 8H, Phe, Nal, Trp CH_2^{β}, and Lys CH_2^{ε}), 1.50 – 0.86 (m, 6H, three Lys CH_2), 1.33 (s, 9H, Boc CH_3), 1.04 (s, 9H, *t*Bu CH_3), 1.17, 1.16 (d, d, $J = 5.8$ Hz, 3H, Thr CH_3^{δ} c/t), 0.92, 0.83 (d, d, $J = 8.2$ Hz, 3H, (*R*)βMeNphe CH_3 c/t). FAB-MS: calculated: M^+ 1318; observed: *m/z* 1341 $(M+Na)^+$.

***c*-[(*R*)βMeNphe⁶-Nal⁷-D-Trp⁸-Lys⁹(Boc)-Thr¹⁰-(*t*Bu)-Phe¹¹] (10).** Deprotection of the linear hexapeptide **9** (0.55 g, 0.42 mmol) as described for compound **4** gave 398 mg of the deprotected hexapeptide as a pinkish solid. 350 mg of this product (0.31 mmol) was cyclized with DPPA (0.19 mL, 0.88 mmol) and K_2HPO_4 (0.53 g, 3.5 mmol) to furnish 189 mg of **10** as a yellow solid (57% yield). $R_f = 0.55$ ($CHCl_3$/MeOH: 90/10); ^1H-NMR (360 MHz, DMSO-d_6) δ 10.72, 10.66 (s, s, 1H, indole NH c/t), 8.65 – 6.84 (m, 28H, Trp, Phe, Nal, (*R*)βMeNphe arom. and six NH), 5.85 – 3.58 (m, 9H, seven CH^{α}, Thr CH^{β}, and (*R*)βMeNphe CH^{β}), 3.21 – 2.17 (m, 8H, Trp, Phe, Nal CH_2^{β}, and Lys CH_2^{ε}), 1.67 – 0.78 (m, 6H, three Lys CH_2), 1.33 (s, 9H, Boc CH_3), 1.17, 1.15 (s, s, 9H, *t*Bu CH_3 c/t), 1.08 – 1.00 (m, 6H, Thr CH_3^{δ} c/t and (*R*)βMeNphe CH_3^{δ} c/t). FAB-MS: calculated: M^+ 1077; observed: *m/z* 1078 $(M+H)^+$, 1100 $(M+Na^+)$.

***c*-[(*R*)βMeNphe⁶-Nal⁷-D-Trp⁸-Lys⁹-Thr¹⁰-Phe¹¹]** or **(*R*)βMeNphe⁶-Nal⁷** analog. Deprotection of the cyclic peptide **10** (100 mg, 0.093 mmol) in 14 mL CH_2Cl_2 with anisole (10% v/v, 2.8 mL), ethanedithiol (5% v/v, 1.4 mL), and TFA (14 mL) as described for the **Nphe⁶-Nal⁷** compound gave 41 mg (46% yield) of the **(*R*)βMeNphe⁶-Nal⁷** analog; FAB-MS: calculated: M^+ 920; observed: 921 $(M+H)^+$, 943 $(M+Na)^+$. The NMR data for this compound are presented in Table 4.

Boc-Nal-OH (11). A solution of 1-naphthylalanine (2.15 g, 10 mmol) in a mixture of dioxane (20 mL), water (10 mL), and 1 N NaOH (10 mL) was stirred and cooled in an ice-water bath. Di-*tert*-butyl pyrocar-

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Table 4 Proton Assignments of *c*-[Nal^{11}-(*R*)-β-MeNPhe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}] **1**, *c*-[Nal^{11}-(*S*)-β-MeNPhe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}] **2**, *c*-[Phe^{11}-(*R*)-β-MeNPhe^{6}-Nal^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}] **3** and *c*-[Phe^{1}-(*S*)-β-MeNPhe^{6}-Nal^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}] **4**[a]

Xaa^{11}-Nxbb^{6}-Xcc^{7}	Phe^{11}-(*R*)βNMePhe^{6}-Nal		Phe^{11}-(*S*)βNMePhe^{6}-Nal		Na^{11}-(*R*)βNMePhe^{6}-Phe^{7}		Nal^{11}-(*S*)NMephe^{6}-Phe^{7}	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
Xaa^{11}								
HN	8.40	8.23	8.46	8.15	8.53	8.22	8.52	8.21
Hα	4.45	4.63	4.45	4.82	4.69	4.73	4.67	4.97
Hβ	2.97/2.82	2.84	2.89	3.10/2.99	3.42/3.36	3.44/3.31	3.47/3.31	3.54
H2, H3, H4	7.18	7.04	7.16	7.29	7.39, 7.27, 7.54	7.32, 7.03, 7.54	7.20, 7.78, 7.22	7.39, 7.86, 7.46
H5, H6, H7, H8	7.13	7.19	7.20	7.22	7.94, 7.50	7.58, 7.29, 7.32,	7.95, 7.53, 7.56	7.92, 7.52, 7.58
	7.2	7.2	7.3	7.3	7.53, 8.07	7.83	8.08	8.15
Nxbb^{6}								
Hα	346/3.21	4.33/3.15	3.51/3.05	4.07/3.02	3.15/3.06	4.32/3.02	2.92/2.71	3.98/2.89
Hβ	5.31	5.51	5.65	5.61	5.22	5.32	5.55	5.24
β-Me	1.24	1.47	1.20	0.95	1.14	1.37	1.14	0.53
H2,6	7.28	6.96	7.09	7.53	7.22	6.79	6.89	7.34
H3,5	7.3	7.26	7.29	7.38	7.2	7.17	7.27	7.21
H4	7.3	7.2	7.3	7.3	7.2	7.2	7.07	7.17
Xcc^{7}								
HN	7.40	7.51	8.01	6.97	7.22	7.46	7.73	6.92
Hα	4.47	4.69	4.63	4.17	4.31	4.55	4.40	4.02
Hβ	2.78	3.45/3.21	3.49/3.26	3.31/3.00	2.28/2.24	2.85	2.81/2.76	2.69
H2,6	6.91, 7.08, 7.6	7.22, 7.03, 7.62	7.28, 7.04, 7.71	7.25, 7.22, 7.76	6.65	7.06	6.75	6.89
H3,5	7.79, 7.39, 7.41	7.93, 7.48	7.85, 7.45	7.84, 7.48, 7.42	6.92	7.28	7.00	7.03
H4	8.04	7.52, 8.38	7.39, 8.20	8.27	7.03	7.2	7.07	7.06
D-Trp								
HN	8.38	8.47	8.41	8.38	8.40	8.53	8.42	8.34
Hα	4.38	4.13	4.45	4.03	4.35	4.14	4.43	4.09
Hβ	2.71/2.18	2.91/2.53	2.76/2.31	2.96/2.63	2.84/2.45	2.98/2.74	2.94/2.58	3.03/2.81
H2	6.95	6.86	6.92	6.88	7.02	6.97	7.00	7.04
H4	7.37	7.38	7.30	7.32	7.42	7.41	7.49	7.51
H5	6.98	6.94	6.99	7.01	6.96	6.96	7.01	7.01
H6	7.05	7.02	7.04	7.07	7.00	7.01	7.06	7.08
H7	7.28	7.29	7.39	7.39	7.29	7.31	7.32	7.32
H1	10.74	10.70	10.72	10.70	10.75	10.72	10.77	10.79
Lys								
HN	8.50	8.32	8.50	8.31	8.56	8.32	8.57	8.34
Hα	3.70	3.94	3.71	3.99	3.80	4.00	3.87	4.08
Hβ	1.56/1.27	1.69/1.28	1.58/1.36	1.71/1.26	1.63/1.38	1.71/1.31	1.68/1.42	1.73/1.31
Hγ	0.73	0.72	0.81	0.69	0.88	0.85	0.95	0.83
Hδ	1.32	1.31	1.31	1.30	1.29	1.35	1.37	1.37
Hε	2.56	2.56	2.58	2.54	2.56	2.54	2.64	2.57
NH2	7.54	7.54	7.61	7.57	7.60	7.60	7.63	7.59
Thr								
HN	7.06	7.72	7.08	7.88	7.11	7.74	7.16	7.79
Hα	4.17	4.38	4.20	4.50	4.24	4.32	4.20	4.48
Hβ	3.98	3.87	3.96	3.89	3.99	3.85	3.99	3.90
Hγ	0.97	1.19	1.01	1.21	1.02	1.15	1.04	1.11
OH	–	–	4.86	5.54	4.89	5.35	–	–

[a] Chemical shifts are reported in ppm relative to internal DMSO-d_6.

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bonate (2.4 g, 11 mmol) was added and stirring was continued at room temperature for 2 h. The solution was concentrated *in vacuo* and extracted with ether (3 × 10 mL). The aqueous layer was cooled in an ice bath and acidified with saturated $NaHSO_4$ to pH 2–3. The aqueous phase was extracted with EtOAc (3 × 15 mL). The organic layers were pooled, washed with water and brine, dried over Na_2SO_4, and evaporated to give **11** as a white solid 3.15 g (100% yield). ^{1}H-NMR (360 MHz, $CDCl_3$) δ 12.75 (br, 1H, OH), 8.08 – 7.42 (m, 7H, Nnal H arom.), 7.26 (d, $J = 7.9$ Hz, 1H, NH), 4.19 (m, 1H, CH^{α}), 3.59 – 3.15(m, 2H, CH_2^{β}), 1.25(s, 9H, $BocCH_3$).

Boc-Nal-Nphe-OEt (12). To a cooled (0°C) solution of Nphe-OEt (0.48 g, 2.5 mmol), Boc-Nal-OH (**11**) (0.79 g, 2.5 mmol) and HOAt (0.54 g, 4 mmol) in 10 mL DCM, was added EDC·HCl (0.76 g, 4 mmol). The mixture was allowed to warm to 20°C and was stirred overnight. The solvent was removed under reduced pressure and the residue was suspended in EtOAc. The organic layer was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, H_2O and dried over $MgSO_4$. The solvent was removed under reduced pressure to give 1.01 g (82%) of **12** as an off-white solid. TLC $R_f = 0.48$ (hexane/EtOAc: 5/5); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 8.22 – 6.71 (m, 12H, Nal and Nphe H arom.), 5.43, 5.37 (d, d, $J = 6.8$ Hz, 1H, NH c/t), 5.17 – 3.26 (m, 9H, OCH_2 c/t, three CH^{α} c/t, Nal and Nphe CH_2^{β}), 1.38, 1.36 (s, s, 9H, Boc CH_3 c/t), 1.22, 1.12(t, t, $J = 7.2$ Hz, 3H, OEt CH_3 c/t).

Boc-Nal-Nphe-OH (13). Ethyl ester **12** (0.89 g, 1.82 mmol) was dissolved in 18 mL THF and the solution was chilled to 0°C. LiOH (0.38 g, 9.09 mmol) in 36 mL of H_2O/MeOH (1/4) was added dropwise over 1 h. The mixture was stirred at 0°C for 1 h and at room temperature for 2 h. The solvents were removed under reduced pressure and the residue was dissolved in saturated $NaHCO_3$. The aqueous layer was extracted with ether, acidified with saturated $NaHSO_4$ to a pH of 2–3, and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with saturated NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to give **13** as a white solid 0.78 g (93%) which was used directly without further purification. TLC $R_f = 0.54$ ($CHCl_3$/MeOH/AcOH: 90/10/1).

Boc-Nal-Nphe-Phe-OBzl (14). The dipeptide **13** (0.71 g, 1.54 mmol), TosOH·H-PheOBzl (0.66 g, 1.54 mmol) and HOBt (0.38 g, 2.46 mmol) were dissolved in 10 mL DCM and the mixture cooled to 0°C. EDC·HCl (0.47 g, 2.46 mmol) was added, followed by DIEA (0.28 mL, 1.54 mmol). The reaction was stirred overnight at room temperature. The reaction solvent was removed under reduced pressure and the residue was dissolved in EtOAc. The organic layer was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, and H_2O. After drying over MgSO4, the solvent was removed under reduced pressure. The crude product was chromatographed on silica gel (hexane/EtOAc: 7/3) to give **14** as a white solid 0.88 g (82%). TLC $R_f = 0.50$ (hexane/EtOAc: 1/1); ^{1}H-NMR (360 MHz, CDCl3) δ 7.93 – 6.73 (m, 23H, Phe, Nal, Nphe, OBzl H arom., and NH), 6.01, 5.43 (d, d, $J = 10.4$ Hz, 1H, NH c/t), 5.21 – 4.17(m, 6H, four CH^{α}, and OCH_2), 3.61 – 2.70 (m, 6H, Phe, Nal, and Nphe CH_2^{β}), 1.42, 1.35 (s, s, 9H, Boc CH_3 c/t).

Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-Nal-Nphe-Phe-OBzl (15). A solution of the tripeptide **14** (0.49 g, 0.7 mmol) in 10 mL of CH_2Cl_2 was treated with 10 mL of TFA. After 1 h the solvent was removed under reduced pressure, and toluene (3 × 10 mL) was sequentially added and removed under reduced pressure to give a white solid. To the cooled (0°C) solution of the amine salt, the tripeptide Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-OH (0.51 g, 0.7 mmol) and HOAt (0.18 g, 1.12 mmol) in 7.0 mL of DMF was added EDC·HCl (0.21 g, 1.12 mmol) followed by 4-EM (88 μL, 0.7 mmol). The mixture was allowed to warm to 20°C and was stirred at room temperature overnight. After removal of DMF under reduced pressure, the residue was suspended in EtOAc and was washed with saturated $NaHCO_3$, H_2O, 1 N NaHSO4, H_2O and dried over $MgSO_4$. The white solid (0.65 g (72%)) obtained after removal of the solvent was used directly for the next step. $R_f = 0.47$ ($CHCl_3$/MeOH/AcOH: 90/10/1); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.79 (br, s, 1H, indole NH), 8.66 – 6.72 (m, 38H, Cbz, Trp, Phe, Nal, Nphe, OBzl arom. and six NH), 5.07 – 3.46 (m, 14H, two OCH_2, seven CH^{α}, Thr CH^{β}, and Nphe CH_2^{β}), 3.11 – 2.78 (m, 8H, Phe, Nal, Trp CH_2^{β}, and Lys CH_2^{ε}), 1.67 – 0.89 (m, 6H, three Lys CH_2), 1.32 (s, 9H, Boc CH_3), 1.04 (s, 9H, *t*Bu CH_3), 0.90, 0.80 (d, d, $J = 5.4$ Hz, 3H, Thr CH_3^{δ} c/t). EMS: calculated M^+ 1305; observed: m/z: positive 1306 $(M + H)^+$, 1328 $(M + Na)^+$; negative 1340 $(M + Cl)^-$, 1419 $(M + TFA)^-$.

***c*-[Nphe^6-Phe^7-D-Trp^8-Lys^9(Boc)-Thr^{10}(*t*Bu)-Nal^{11}] (16).** To a solution of the deprotected hexapeptide **15** (0.45 g, 0.345 mmol) in 50 mL of 20% AcOH in MeOH was added 10% Pd/C (50 mg). Hydrogen gas was introduced at atmospheric pressure at 20°C and the mixture was allowed to react overnight. The catalyst was removed by filtration and the solvent was removed under reduced pressure. DCM (20 mL) and 4 N HCl in dioxane (0.2 mL) were sequentially added and removed under reduced pressure. The sample was dried under reduced pressure to give

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0.36 g (93%) of a white solid which was used without further purification.

A solution of the deprotected linear hexapeptide (224 mg, 0.2 mmol) in 400 mL of freshly distilled DMF was cooled to -10°C. To this solution DPPA (0.12 mL, 0.6 mmol) and K_2HPO_4 (0.35 g, 2 mmol) were added. After 20 min at -10°C, the reaction was allowed to stir at 4°C for 2 days. The solvent was removed under reduced pressure. Purification was carried out by silica gel chromatography ($CHCl_3$/MeOH/AcOH: 98/1/1) to give cyclic peptide **16** as a yellow powder 126 mg (59%). TLC $R_f = 0.57$ ($CHCl_3$/MeOH: 90/10); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.74 (s, 1H, indole NH), 8.63 – 6.63 (m, 28H, Trp, Phe, Nal, Nphe arom. and six NH), 5.37 – 3.52 (m, 10H, seven CH^{α}, Thr CH^{β}, and Nphe CH_2^{β}), 3.07 – 2.45 (m, 8H, Trp, Phe, Nal CH_2^{β}, and Lys CH_2^{ε}), 1.65 – 0.93 (m, 6H, three Lys CH_2), 1.36, 1,34 (s, s, 9H, Boc CH_3 c/t), 1.20, 1.14 (s, s, 9H, *t*Bu CH_3 c/t), 1.03 (d, $J = 8.6$ Hz, 3H, Thr CH_3^{γ}). FAB-MS: calculated M^+ 1063; observed: m/z 1086 $(M + Na^+)$, 1107 $(M - H + 2Na)^+$.

***c*-[Nphe⁶-Phe⁷-D-Trp⁸-Lys⁹-Thr¹⁰-Nal¹¹]** or **Nal¹¹-Nphe⁶** analog. To a solution of cyclic peptide **16** (100 mg, 0.094 mmol) in 14 mL CH_2Cl_2 anisole (10% v/v, 2.8 mL) and ethanedithiol (5% v/v, 1.4 mL) were added. The mixture was cooled to 0°C and treated with TFA (14 mL) for 1 h at 0°C and 2 h at 20°C. The solvents were removed under reduced pressure and toluene (3 × 10 mL) was added and removed under reduced pressure. The residue was crystallized in cold (0°C) ether, isolated by filtration and purified by RP-HPLC to give the **Nal¹¹-Nphe⁶** analog as a white powder 40 mg (47% yield); FAB-MS: calculated M^+ 906; observed: m/z 907 $(M + H)^+$; 929 $(M + Na)^+$; 945 $(M + K)^+$. The NMR data for this compound are presented in Table 3.

Boc-Nal-(*R*)*β*MeNphe-OEt (17). To a cooled (0°C) solution of (*R*)*β*MeNphe-OEt (0.52 g, 2.5 mmol), Boc-Nal-OH (0.79 g, 2.5 mmol) and HOAt (0.54 g, 4 mmol) in 10 mL DCM, was added EDC·HCl (0.76 g, 4 mmol). The mixture was allowed to warm to 20°C and was stirred overnight. The solvent was removed under reduced pressure and the residue was suspended in EtOAc. The organic layer was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, H_2O and dried over $MgSO_4$. The solvent was removed under reduced pressure to give 0.96 g (76%) of **17** as an off-white solid. TLC $R_f = 0.54$ (hexane/EtOAc: 1/1); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 8.23 – 6.98 (m, 12H, Nal and (*R*)*β*MeNphe H arom.), 6.35, 5.98 (d, d, $J = 9$Hz, 1H, NH c/t), 5.49, 5.42 (m, m, 1H, CH^{α} c/t), 5.02 – 3.01 (m, 7H, OCH_2, two CH^{α}, Nal CH_2^{β}, (*R*)*β*MeNphe CH^{β}), 1.51 – 0.99 (m, 6H, OEt CH_3 c/t, (*R*)*β*MeNphe CH_3 c/t), 1.40, 1.37 (s, s, 9H, Boc CH_3 c/t).

Boc-Nal-(*R*)*β*MeNphe-OH (18). The ethyl ester **17** (0.92 g, 1.82 mmol) was dissolved in 18 mL THF and the solution cooled to 0°C. LiOH (0.38 g, 9.09 mmol) in 36 mL of H_2O/MeOH (1/4) was added dropwise over 1 h. The mixture was stirred at 0°C for 1 h and at room temperature for 2 h. The solvents were removed under reduced pressure and the residue was dissolved in saturated $NaHCO_3$. The aqueous layer was extracted with ether, acidified with saturated $NaHSO_4$ to a pH of 2–3, and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with saturated NaCl, dried over $MgSO_4$, and the solvent was evaporated *in vacuo* to give **18** as a white solid 0.80 g (93%) which was used directly without further purification. TLC $R_f = 0.40$ ($CHCl_3$/MeOH/AcOH: 90/10/1).

Boc-Nal-(*R*)*β*MeNphe-Phe-OBzl (19). The dipeptide **18** (0.73 g, 1.54 mmol), TosOH·H-Phe-OBzl (0.66 g, 1.54 mmol) and HOBt (0.38 g, 2.46 mmol) were dissolved in 10 mL DCM and the mixture was cooled to 0°C. EDC·HCl (0.47 g, 2.46 mmol) was added, followed by DIEA (0.28 mL, 1.54 mmol). The reaction was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc. The organic layer was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, and H_2O. After drying over $MgSO_4$, the solvent was removed under reduced pressure. The crude product was chromatographed on silica gel (hexane/EtOAc: 7/3) to give **19** as a white solid 0.90 g (82%): TLC $R_f = 0.50$ (hexane/EtOAc: 1/l); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 8.12 – 6.97 (m, 23H, Phe, Nal and (*R*)*β*MeNphe and OBzl H arom. and NH), 6.78, 6.49 (d, d, $J = 9$Hz, 1H, NH c/t), 5.81 – 4.42 (m, 6H, OCH_2, four CH^{α}), 3.47 – 2.79 (m, 5H, Nal, Phe, and (*R*)*β*MeNphe CH^{β}), 1.43, 1.13 (d, d, $J = 7.6$ Hz, 3H, (*R*)*β*MeNphe CH_3 c/t) 1.38 (s, 9H, Boc CH_3).

Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-Nal-(*R*)*β*MeNphe-Phe-OBzl (20). A solution of the tripeptide **19** (0.50 g, 0.7 mmol) in 10 mL of CH_2Cl_2 was treated with 10 mL of TFA. After 1 h the solvents were removed under reduced pressure, and toluene (3 × 10 mL) was sequentially added and removed under reduced pressure to give a white solid. The cooled (0°C) solution of the amine salt, the tripeptide Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-OH (0.51 g, 0.7 mmol) and HOAt (0.18 g, 1.12 mmol) in 7.0 mL of DMF was added EDC·HCl (0.21 g, 1.12 mmol) followed by 4-EM (88 μL,0.7 mmol). The mixture was allowed to warm to 20°C and was stirred at room temperature overnight. After removal of DMF under reduced pressure, the residue was suspended in EtOAc and washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, H_2O and dried over

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$MgSO_4$. The white solid obtained after removal of the solvent 0.69 g (75%) was used directly for the next step. TLC $R_f = 0.61$ ($CHCl_3$/MeOH/AcOH: 90/10/1); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.80 (s, 1H, indole NH), 8.73 – 6.72 (m, 38H, Cbz, Trp, Phe, Nal, (*R*)βMeNphe, OBzl arom. and six NH), 5.76 – 3.39 (m, 13H, two OCH_2, seven CH^{α}, Thr CH^{β}, and (*R*)βMeNphe CH^{β}), 3.20 – 2.78 (m, 8H, Phe, Nal, Trp CH_2^{β}, and Lys CH_2^{ε}), 1.63 – 0.86 (m, 6H, three Lys CH_2), 1.33 (s, 9H, Boc CH_3), 1.05, 1.02 (s, s, 9H, *t*Bu CH_3 c/t), 0.96 (m, 3H, Thr CH_3^{δ} c/t), 0.85, 0.70 (d, d, $J = 8.2$ Hz, 3H, (*R*)βMeNphe CH_3 c/t). EMS: calculated M^+ 1319; observed: m/z: positive 1320 $(M+H)^+$, 1342 $(M+Na)^+$; negative 1354 $(M+Cl)^-$.

***c*-[(*R*)βMeNphe^6-Phe^7-D-Trp^8-Lys^9(Boc)-Thr^{10}-(*t*Bu)-Nal^{11} (21).** To a solution of hexapeptide **20** (0.45 g, 0.341 mmol), in 50 mL of 20% AcOH in MeOH was added 10% Pd/C (50 mg). Hydrogen gas was introduced at atmospheric pressure at 20°C and the mixture was allowed to react overnight. The catalyst was removed by filtration and the solvent was removed under reduced pressure. Methylene chloride (20 mL) and 4 N HCl in dioxane (0.2 mL) were sequentially added and removed under reduced pressure. The sample was dried under reduced pressure to give 0.37 g (95%) of a white solid which was used directly. A solution of the deprotected linear hexapeptide (226 mg, 0.2 mmol) in 400 mL of freshly distilled DMF was cooled to − 10°C. To this solution DPPA (0.12 mL, 0.6 mmol) and K_2HPO_4 (0.35 g, 2 mmol) were added. After 20 min at − 10°C, the reaction was allowed to stir at 4°C for 2 days. The solvent was removed under reduced pressure. Purification was carried out by silica gel chromatography ($CHCl_3$/MeOH/AcOH: 98/1/1) to give cyclic peptide **21** as a yellow powder 112 mg (50% yield). TLC $R_f = 0.50$ ($CHCl_3$/MeOH: 90/10); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.75, 10.63 (s, s, 1H, indole NH c/t), 8.60 – 6.63 (m, 28H, Trp, Phe, Nal, (*R*)βMeNphe arom. and six NH), 5.43 – 3.45 (m, 9H, seven CH^{α}, Thr CH^{β}, and(*R*)βMeNphe CH^{β}), 3.12 – 2.72 (m, 8H, Trp, Phe, Nal CH_2^{β}, and Lys CH_2^{ε}), 1.71 – 0.82 (m, 6H, three Lys CH_2), 1.47 – 0.99 (m, 6H, Thr CH_3^{δ} c/t and (*R*)βMeNphe CH_3^{δ} c/t), 1.36, 1.33 (s, s, 9H, Boc CH_3 c/t), 1.19, 1.11 (s, s, 9H, *t*Bu CH_3 c/t). FAB-MS m/z 1078 $(M+H)^+$, 1100 $(M+Na^+)$, 1121 $(M-H+2Na)^+$.

***c*-[(*R*)βMeNphe^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Nal^{11}]** or **Nal^{11}-(*R*)βMeNphe^6** analog. To a solution of cyclic peptide **21** (100 mg, 0.093 mmol) in 14 mL CH_2Cl_2 anisole (10% v/v, 2.8 mL) and ethanedithiol (5% v/v, 1.4 mL) were added. The mixture was chilled (0°C) and treated with TFA (14 mL) for 1 h at 0°C and 2 h at 20°C. The solvents were removed under reduced pressure and toluene (3 × 10 mL) was added and removed under reduced pressure. The residue was crystallized in cold (0°C) ether, isolated by filtration and purified by RP-HPLC to give the Nal^{11}-(*R*)βMeNphe^6 analog as a white powder (43 mg, 50% yield). FAB-MS m/z 921 $(M+H)^+$, 943 $(M+Na)^+$. The NMR data for this compound are presented in Table 4.

Nal-(S)βMeNphe-OEt (22). To a cooled (0°C) solution of (*S*)βMeNphe-OEt (0.52 g, 2.5 mmol), Boc-Nal-OH (0.79 g, 2.5 mmol) and HOAt (0.54 g, 4 mmol) in 10 mL DCM, was added EDC·HCl (0.76 g, 4 mmol). The mixture was allowed to warm to 20°C and was stirred overnight. The solvent was removed under reduced pressure and the residue was suspended in EtOAc. The organic layer was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, H_2O and dried over $MgSO_4$. The solvent was removed under reduced pressure to give 1.08 g (86%) of **22** as an off-white solid. TLC $R_f = 0.54$ (hexane/EtOAc: 1/1); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 8.30 – 7.03 (m, 12H, Nal and (*S*)βMeNphe H arom.), 6.65 (d, $J = 7.9$ Hz, 1H, NH), 5.78 – 2.88 (m, 8H, three CH^{α}, Nal CH_2^{β}, (*S*)βMeNphe CH^{β}, and OCH_2), 1.41, 1.39 (s, s, 9H, Boc CH_3 c/t), 1.31 – 0.62 (m, 6H, OEt CH_3 c/t, (S)βMeNphe CH_3 c/t).

Boc-Nal-(S)βMeNphe-OH (23). The ethyl ester **22** (0.92 g, 1.82 mmol) was dissolved in 18 mL THF and the solution was cooled to 0°C. LiOH (0.38 g, 9.09 mmol) in 36 mL of H_2O/MeOH (1/4) was added dropwise over 1 h. The mixture was stirred at 0°C for 1 h and at room temperature for 2 h. The solvents were removed under reduced pressure and the residue was dissolved in saturated $NaHCO_3$. The aqueous layer was extracted with ether, acidified with saturated $NaHSO_4$ to a pH of 2–3, and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with saturated NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to give **23** as a white solid 0.78 g (91%) which was used directly without further purification. TLC $R_f = 0.34$ ($CHCl_3$/MeOH/AcOH: 90/10/1).

Boc-Nal-(S)βMeNphe-Phe-OBzl (24). The dipeptide **23** (0.73 g, 1.54 mmol), TosOH·H-Phe-OBzl (0.66 g, 1.54 mmol) and HOBt (0.38 g, 2.46 mmol) were dissolved in 10 mL DCM and the mixture was cooled to 0°C. EDC·HCl (0.47 g, 2.46 mmol) was added, followed by DIEA (0.28 mL, 1.54 mmol). The reaction was stirred overnight at room temperature. The reaction solvent was removed under reduced pressure and the residue was dissolved in EtOAc. The organic layer was washed with saturated

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$NaHCO_3$, H_2O, 1 N $NaHSO_4$, and H_2O. After drying over $MgSO_4$, the solvent was removed under reduced pressure. The crude product was chromatographed on silica gel (hexane/EtOAc: 7/3) to give **24** as a white solid 0.86 g (78%). TLC $R_f = 0.45$ (hexane/EtOAc: 1/1); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 8.27 – 6.66 (m, 23H, Phe, Nal and (*S*)βMeNphe, OBzl Harom. and NH), 5.79–4.65 (m, 7H, NH, OCH_2, and four CH^{α}), 3.81 – 2.84 (m, 5H, Nal, Phe, and (*S*)βMeNphe CH^{β}), 1.43, 1.41 (s, s, 9H, Boc CH_3 c/t), 0.92, 0.56 (d, d, $J = 5.4$ Hz, 3H, (*S*)βMeNphe CH_3 c/t).

Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-Nal-(S)βMeNphe-Phe-OBzl (25). A solution of the tripeptide **24** (0.50 g, 0.7 mmol) in 10 mL of CH_2Cl_2 was treated with 10 mL of TFA. After 1 h the solvent was removed under reduced pressure, and toluene (3 × 10 mL) was sequentially added and removed under reduced pressure to give a white solid. To the cooled (0°C) solution of the amine salt, the tripeptide Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu) OH (0.51 g, 0.7 mmol) and HOAt (0.18 g, 1.12 mmol) in 7.0 mL of DMF was added EDC·HCl (0.21 g, 1.12 mmol) followed by 4-EM (88 μL, 0.7 mmol). The mixture was allowed to warm to 20°C and was stirred at room temperature overnight. After removal of DMF under reduced pressure, the residue was suspended in EtOAc which was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, H_2O and dried over $MgSO_4$. The white solid obtained after removal of the solvent (0.72 g (78%)) was used directly for the next step. TLC $R_f = 0.60$ ($CHCl_3$/MeOH/AcOH: 90/10/1); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.80 (br, s, 1H, indole NH), 8.29 – 6.74 (m, 38H, Cbz, Trp, Phe, Nal, (S)βMeNphe, OBzl arom. and six NH), 5.69 – 3.44 (m, 13H, two OCH_2, seven CH^{α}, Thr CH^{β}, and (S)βMeNphe CH^{β}), 3.13 – 2.81 (m, 8H, Phe, Nal, Trp CH_2^{β}, and Lys CH_2^{ε}), 1.78, 1.63 (d, d, $J = 9.0$ Hz, 3H, (S)βMeNphe CH_3 c/t), 1.63 – 0.86 (m, 6H, three Lys CH_2), 1.33, 1.31 (s, s, 9H, Boc CH_3 c/t), 1.12 1.03 (s, s, 9H, *t*Bu CH_3 c/t), 0.90 (m, 3H, Thr CH_3^{δ} c/t). EMS: calculated M^+: 1319; observed: *m*/*z* 1342 $(M + Na)^+$.

***c*-[(S)βMeNphe^6-Phe^7-D-Trp^8-Lys^9(Boc)-Thr^{10}-(*t*Bu)-Nal^{11}] (26).** To a solution of hexapeptide **25** (0.45 g, 0.341 mmol), in 50 mL of 20% AcOH in MeOH was added 10% Pd/C (50 mg). Hydrogen gas was introduced at atmospheric pressure at 20°C and the mixture was allowed to react overnight. The catalyst was removed by filtration and the solvent was removed under reduced pressure. Methylene chloride (20 mL) and 4 N HCl in dioxane (0.2 mL) were sequentially added and removed under reduced pressure. The sample was dried under reduced pressure to give 0.32 g (82% yield) of a white solid which was used directly for the next reaction.

A solution of this compound (226 mg, 0.2 mmol) in 400 mL of freshly distilled DMF was cooled to −10°C. To this solution DPPA (0.12 mL, 0.6 mmol) and K_2HPO_4 (0.35 g, 2 mmol) were added. After 20 min at −10°C, the reaction was allowed to stir at 4°C for 2 days. The solvent was removed under reduced pressure. Purification was carried out by silica gel chromatography ($CHCl_3$/MeOH/AcOH: 98/1/1) to give cyclic peptide **26** as a yellow powder 145 mg (67% yield). TLC $R_f = 0.50$ ($CHCl_3$/MeOH: 90/10); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.83, 10.76 (s, s, 1H, indole NH c/t), 8.74 – 6.66 (m, 28H, Trp, Phe, Nal, (*S*)βMeNphe arom. and six NH), 6.30 – 3.77 (m, 9H, seven CH^{α}, Thr CH^{β}, and (*S*)βMeNphe CH^{β}), 3.17 – 2.57 (m, 8H, Trp, Phe, Nal CH_2^{β}, and Lys CH_2^{ε}), 1.71 – 0.82 (m, 6H, three Lys CH_2), 1.35, 1.33 (s, s, 9H, Boc CH_3 c/t), 1.20 – 1.03 (m, 6H, ThrCH_3^{δ} c/t and (*S*)βMeNphe CH_3^{δ} c/t), 1.18, 1.17 (s, s, 9H, *t*Bu CH_3 c/t). FAB-MS *m*/*z* 1078 $(M + H)^+$, 1100 $(M + Na^+)$, 1121 $(M - H + 2Na)^+$.

***c*-[(S)βMeNphe^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Nal^{11}] or Nal^{11}-(S)βMeNphe^6** analog. To a solution of cyclic peptide **26** (100 mg, 0.093 mmol) in 14 mL CH_2Cl_2 anisole (10% v/v, 2.8 mL) and ethanedithiol (5% v/v, 1.4 mL) were added. The mixture was chilled (0°C) and treated with TFA (14 mL) for 1 h at 0°C and 2 h at 20°C. The solvents were removed under reduced pressure and toluene (3 × 10 mL) was added and removed under reduced pressure. The residue was crystallized in cold (0°C) ether, isolated by filtration and purified by RP-HPLC to give 35 mg of the Nal^{11}-(S)βMeNphe^6 analog (41% yield). FAB-MS *m*/*z* 921 $(M + H)^+$, 943 $(M + Na)^+$; HR-FAB-MS calculated for $C_{53}H_{61}N_8O_7$ $(M + H)^+$ 921.4663, found 921.4628. The NMR data for this compound are presented in Table 4.

Nnal-OBzl (27). To a cooled (0°C) solution of 1-napthyl amine (6.28 g, 40 mmol) in 150 mL THF, benzyl bromoacetate (4.58 g, 20 mmol) was added over 1 h. The mixture was stirred overnight at room temperature. The precipitate was removed by filtration. THF was removed *in vacuo* from the filtrate to give a yellow oil which was suspended in EtOAc. The organic layer was washed with 1 N HCl and saturated NaCl and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was obtained as a colorless oil after silica gel chromatography (hexane/EtOAc: 4/1). Yield: 5.38 g (88%). TLC $R_f = 0.21$ (hexane/EtOAc: 75/25); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 8.21 – 7.24 (m, 12H, Nnal and OBzl H arom.), 5.13 (s, 2H, OCH_2), 4.22 (s, 2H, CH_2^{β}), 3.56 (s, 2H, two CH^{α}), 1.96 (br, s, 1H, NH).

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Boc-Phe-Nnal-OBzl (28). To a cooled (0°C) solution of Nnal-OBzl (**27**) (2.03 g, 6.67 mmol), Boc-Phe-OH (2.12 g, 8.0 mmol) and HOBt (1.22 g, 8.0 mmol) in 15 mL DCM, was added EDC·HCl (1.52 g, 8.0 mmol). The mixture was allowed to warm to 20°C and was stirred overnight. The solvent was removed under reduced pressure and the residue was suspended in EtOAc. The organic layer was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, H_2O and dried over $MgSO_4$. The solvent was removed under reduced pressure. Column chromatography on silica gel (hexane/EtOAc: 4/1) gave 2.8 g (76% yield) of **28** as a white solid. TLC $R_f = 0.30$ (hexane/EtOAc: 3/1); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 7.98 – 6.95 (m, 17H, Phe, Nnal, and OBzl H arom.), 5.26 – 4.61 (m 6H, NH, three CH^α, OCH_2), 4.18 – 3.76 (m, 2H, Nnal CH_2^β), 3.16 – 2.95 (m, 2H, Phe CH_2^β), 1.31, 1.33 (s, s, 9H, Boc CH_3 c/t).

Boc-Phe-Nnal-OH (29). To a solution of dipeptide **28** (2.81 g, 5.1 mmol) in 20 mL MeOH was added 10% Pd/C (0.3 g). Hydrogen gas was introduced at atmospheric pressure at 20°C and the mixture was allowed to react for 3 h. The catalyst was removed by filtration and the solvent removed under reduced pressure. The deprotected **29** was obtained as a white solid 2.31 g (98% yield) and used directly without further purlfication. TLC $R_f = 0.26$ ($CHCl_3$/MeOH/AcOEt/AcOH: 94/3/3/1).

Boc-Phe-Nnal-Phe-OBzl (30). The dipeptide **29** (2.31 g, 5 mmol), TosOH·H-Phe-OBzl (2.14 g, 5 mmol) and HOBt (0.92 g, 6 mmol) were dissolved in 5 mL DCM and the mixture was cooled to 0°C. EDC·HCl (1.15 g, 6 mmol) was added, followed by DIEA (0.89 mL, 5 mmol). The reaction was stirred overnight at room temperature. The reaction solvent was removed under reduced pressure and the residue was dissolved in EtOAc. The organic layer was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, and H_2O. After drying over $MgSO_4$, the solvent was removed under reduced pressure. The crude product was chromatographed on silica gel (hexane/EtOAc: 7/3) to give **30** as a white solid (3.02 g (87%)). TLC $R_f = 0.44$ (hexane/EtOAc: 5/5); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 8.16 – 6.88 (m, 22H, Phe^1, Phe^2, Nnal, and OBzl arom.), 5.31 – 5.0 (m, 4H, two NH and OCH_2), 4.90 – 3.71 (m, 4H, CH^α), 3.36 – 2.81(m, 6H, Phe^1, Phe^2, and Nnal CH^β), 1.35, 1.30 (s, s, 9H, Boc CH_3 c/t). FAB-MS: calculated M^+ 699; observed: m/z 700 $(M + H)^+$.

Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-Phe-Nnal-Phe-OBzl (31). A solution of the tripeptide **30** (1.0 g, 1.43 mmol) in 10 mL of CH_2Cl_2 was treated with 10 mL of TFA. After 1 h the solvents were removed under reduced pressure, and toluene (3 × 10 mL) was sequentially added and removed under reduced pressure to give a white solid. The cooled (0°C) solution of the amine salt, the tripeptide Cbz-D-Trp-Lys(Boc)-Thr(*t*Bu)-OH (1.16 g, 1.6 mmol) and HOAt (0.35 g, 2.56 mmol) in 5.0 mL of DMF was added EDC·HCl (0.49 g, 2.56 mmol) followed by 4-EM (0.20 mL, 1.6 mmol). The mixture was allowed to warm to 20°C and was stirred at room temperature overnight. After removal of DMF under reduced pressure, the residue was suspended in EtOAc which was washed with saturated $NaHCO_3$, H_2O, 1 N $NaHSO_4$, H_2O and dried over $MgSO_4$. The yellow-white solid obtained after removal of the solvent 1.28 g (73%) was used directly for the next step. TLC $R_f = 0.56$ ($CHCl_3$/MeOH: 90/10); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.79 (s, 1H, indole NH), 8.69 – 6.69 (m, 38H, Cbz, Trp, Phe^1, Phe^2, Nnal, OBzl arom. and six NH), 5.19 – 3.62 (m, 14H, 7 CH^α, two OCH_2, Thr CH^β, and Nnal CH_2^β), 3.11 – 2.80 (m, 8H, Trp, Phe^1, Phe^2 CH_2^β, and Lys CH_2^ε), 1.60 – 0.9 (m, 6H, three Lys CH_2), 1.33 (s, 9H, Boc CH_3), 1.08, 1.02 (s, s, 9H, *t*Bu CH_3 c/t), 0.97, 0.90 (d, d, $J = 7.6$ Hz, 3H, Thr CH_3^γ). FAB-MS: calculated: M^+ 1304; m/z 1327 $(M + Na)^+$.

***c* - [Nnal^6 - Phe^7 - D - Trp^8 - Lys^9(Boc) - Thr^{10}(*t*Bu)-Phe^{11}] (32).** To a solution of hexapeptide **31** (0.60 g, 0.46 mmol), in 100 mL of 20% AcOH in MeOH was added 10% Pd/C (60 mg). Hydrogen gas was introduced at atmospheric pressure at 20°C and the mixture was allowed to react for 5 h. The catalyst was removed by filtration and the solvent removed under reduced pressure. Methylene chloride (20 mL) and 4 N HCl in dioxane (0.2 mL) were sequentially added and removed under reduced pressure. The sample was dried under reduced pressure to give 0.46 g (88% yield) of a white solid which was used directly. TLC $R_f = 0.33$ ($CHCl_3$/MeOH/AcOH: 90/10/1).

A solution of this compound (250 mg, 0.22mmol) in 400 mL of freshly distilled DMF was chilled to −10°C. To this solution DPPA (133 μL, 0.66 mmol) and K_2HPO_4 (0.39 g, 2.23 mmol) were added. After 20 min at −20°C, the reaction was allowed to stir at 4°C for 3 days. The solvent was removed under reduced pressure. Purification was carried out by silica gel chromatography ($CHCl_3$/MeOH/AcOH: 98/1/1) to give 125 mg (54% yield) cyclic peptide **31** as a yellow powder. TLC $R_f = 0.51$ ($CHCl_3$/MeOH: 90/10); ^{1}H-NMR (360 MHz, DMSO-d_6) δ 10.78, 10.75 (s, s, 1H, indole NH c/t), 8.60 – 6.71 (m, 28H, Trp, Phe^1, Phe^2, Nnal arom. and six NH), 5.96 – 3.63 (m, 10H, seven CH^α, Thr CH^β, and Nnal CH_2^β), 3.22 – 2.60 (m, 8H, Trp, Phe^1, Phe^2 CH_2^β, and Lys CH_2^ε), 1.68 – 0.85 (m, 6H, three Lys CH_2), 1.35 (s, 9H, Boc CH_3), 1.19, 1.12

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(s, s, 9H, *t*Bu CH_3 c/t), 1.05 (d, $J = 7.2$ Hz, 3H, Thr CH_3^{γ}. FAB-MS: calculated: M^+ 1062; observed: m/z 1195 $(M + Cs)^+$, 1327 $(M - H^+ + 2Cs^+)$.

***c*-[Nnal⁶-Phe⁷-D-Trp⁸-Lys⁹-Thr¹⁰-Phe¹¹** or **Nnal⁶** analog. To a solution of the cyclic peptide **32** (100 mg, 0.094 mmol) in 13.5 mL CH_2Cl_2 anisole (10% v/v, 2.7 mL) and ethanedithiol (5% v/v, 1.35 mL) were added. The mixture was chilled (0°C) and treated with TFA (13.5 mL) for 1 h at 0°C and 2 h at 20°C. The solvents were removed under reduced pressure and toluene (3 × 10 mL) was added and removed under reduced pressure. The residue was crystallized in cold (0°C) ether, isolated by filtration and purified by RP-HPLC to give 45 mg (53% yield). EMS: calculated: M^+ 906; observed: m/z: positive 907 $(M + H)^+$, 929 $(M + Na)^+$, negative 905 $(M - H+)$. The NMR data for this compound are presented in Table 3.

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