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Expedited Articles

Synthesis of Potent Cyclic Hexapeptide NK-1 Antagonists. Use of a Minilibrary in Transforming a Peptidal Somatostatin Receptor Ligand into an NK-1 Receptor Ligand via a Polyvalent Peptidomimetic

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The endogenous peptides somatostatin (SRIF) and substance P comprise very different structures. Although both bind G-protein-coupled receptors, the SRIF receptors (SSTR 1−5) recognize SRIF and related peptides which retain its β-turn such as the potent cyclic hexapeptide SRIF agonist L-363,301 (**6a**), but not substance P. Conversely the NK-1 receptor binds substance P but not the above ligands. In contrast, the β-D-glucosides **1** and **2**, designed to mimic the β-turn of **6a**, bind both receptors. This observation led us to attempt the conversion of **6a** into the first potent, selective cyclic hexapeptide ligand for the NK-1 receptor. To this end, we combined design with a minilibrary approach. The goal was accomplished with surprising ease, leading to the NK-1 receptor antagonist **9** (IC_{50} 2.0 ± 0.4 nM). This demonstrates that peptidomimetics, incorporating in this case the promiscuous β-D-glucose scaffold, can provide valuable clues about receptor similarities not revealed by their endogenous ligands. In addition, this work suggests that the use of libraries and rational design need not be mutually exclusive approaches to lead discovery.

In 1990 we introduced β-D-glucose as a peptidomimetic scaffold, devoid of an amide backbone.[1] Compounds **1** and **2** (Figure 1) were shown to inhibit completely the binding of [^{125}I]CGP 23996 to somatostatin receptors on membranes from cerebral cortex, pituitary, and AtT-20 cells with approximate IC_{50}s of 5 μM. Compound **2** was found to inhibit GRH-induced growth hormone release in a functional assay, showing it to be a somatostatin agonist.[1b,d] These two β-D-glycosides also bound weakly to the cloned human β_2-adrenergic receptor[1a,d] and were shown to be antagonists with an IC_{50} of 3 μM in a functional assay.[1b]

To our knowledge this work[1a] represented the second demonstration that it is possible to effect the wholesale replacement of the amide backbone with retention of biological activity, provided that side chains required for binding are attached to the scaffold. The first implementation of the underlying idea, using a bicyclooctane framework, designed by Bélanger and Dufresne,[2] had been initially overlooked by us and by others. Olson *et al.*[3] employed cyclohexane as the scaffold at about the same time we introduced β-D-glucose for this purpose. A theoretical paper by Farmer[4] had proposed the use of cyclohexane in 1980. In addition to introducing a carbohydrate scaffold as a β-turn mimetic, we have also successfully applied the concept in the design and synthesis of a pyrrolinone-based scaffold to mimic β-strands and β-pleated sheets.[5]

1 X = OBn, R' = H
2 X = R' = H
3 X = OBn, R' = Ac

L-363,301 (**6a**) R^1 = H, R^2 = OH, R^3,R^4 = -$(CH_2)_3$-
MK 678 (**6b**) R^1 = OH, R^2 = R^3 = R^4 = Me

Figure 1. SRIF and NK-1 receptor ligands.

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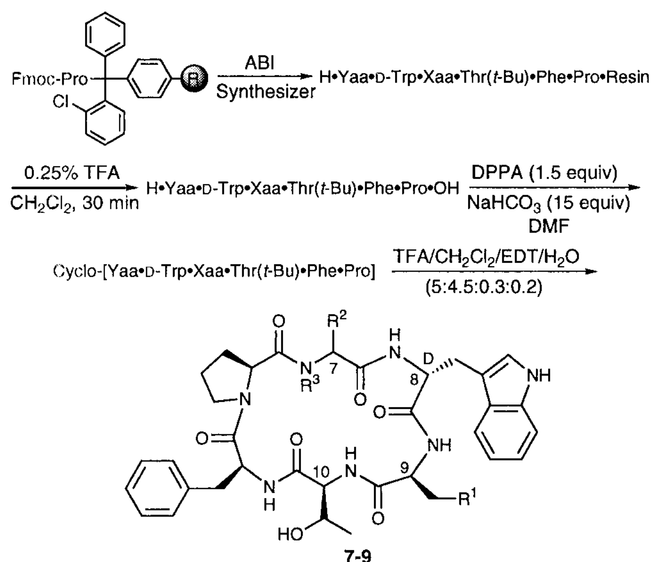
Scheme 1



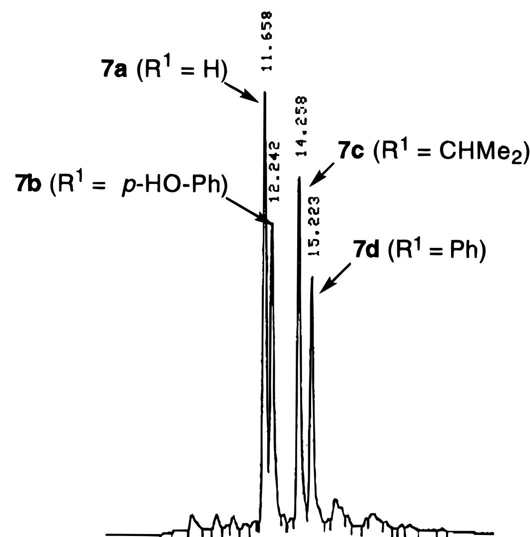


Figure 2. RP-HPLC trace of the crude mixture of the four cyclic hexapeptides **7a**–**d**.

Subsequently,[1b] we reported that **1** and **2** unexpectedly display high affinity for a substance P receptor (NK-1) with IC_{50}s of 120 and 180 nM, respectively. The N-acetylated derivative **3** bound the NK-1 receptor with an IC_{50} of about 60 nM.[6] To our knowledge, these three compounds were the first nonpeptidal peptidomimetics containing indole substituents which were reported to bind the NK-1 receptor.[7] Several laboratories have subsequently reported[8,9] the same observation. The structure–activity relationships of **1**–**5** differed at the three receptors, showing that the binding is specific. For example, the potent NK-1 receptor antagonist **3** did not bind to either the β_2-adrenergic receptor or the somatostatin receptor, and the replacement of the 2-benzyl group of **2** by methylimidazole giving **5** enhanced affinity for the somatostatin receptor but blocked binding to the NK-1 receptor.

It has been suggested[10] that sugar-based scaffolds to which appropriate side chains have been attached cannot possess high binding affinities because they have too many degrees of freedom. Our results with compounds **1**–**3** at the NK-1 receptor cast some doubt on this proposition.

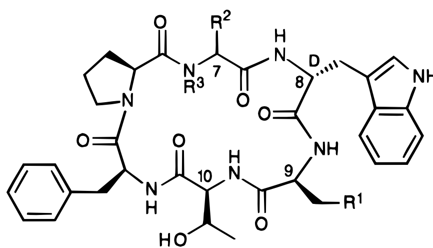
That the peptidomimetics **1** and **2** can bind both the somatostatin and the NK-1 receptors led us to propose that **1** and **2** interact within the transmembrane binding domain for small molecules that is thought to be a common feature of G-protein-coupled receptors.[11] The polyvalency of **1** and **2** suggested, moreover, that the topology of the binding sites of the SRIF and NK-1 receptors must have some significant similarities not revealed by the endogenous ligands SRIF and substance P.

To test this hypothesis, we sought to design an analog of L-363,301 (**6a**)[12] with high affinity for the NK-1 receptor.[13] Because of the presence of three phenyl groups in the non-peptide NK-1 antagonists CP-96,345[14] and RP 67581,[15] we chose to replace the lysine side chain of **6a** with hydrophobic amino acids. The synthesis of the linear hexapeptides was carried out on solid support as shown in Scheme 1,[16,17] where four different amino acids (Ala, Tyr, Leu, Phe) were simultaneously introduced as Lys replacements. The resulting mixture of four linear hexapeptides was removed from the resin, cyclized, and deprotected to afford a mixture of the four expected cyclic hexapeptides in a combined yield of 82%. (See Figure 2.) These four hexapeptides were isolated in pure form by reverse-phase HPLC and the resulting entities characterized by 500 MHz NMR spectroscopy. The success of the minilibrary method depended on obtaining a high yield both in the preparation of the partially protected linear peptides and in the subsequent cyclizations. The 2-chlorotrityl resin proved to be the method of choice as it provided the cyclization precursors in high yield and excellent purity. The cyclization protocol (1.5 equiv of DPPA, 15 equiv of $NaHCO_3$, DMF), which has become a standard in our laboratory for the preparation of a variety of cyclic peptides, proved again to be a reliable method.

Compounds **7a**–**c** had only very weak affinity for the NK-1 receptor (>1 μM),[18] but **7d** had an IC_{50} of 95 ± 35 nM (Table 1). That this "library" of just four compounds would afford three essentially inactive peptides and one possessing good affinity is, we believe, testimony to the power of the library concept to test rapidly a hypothesis in *lead discovery*. Had we synthesized separately just compounds **7a**–**c**, we might have concluded that hydrophobic substituents, aliphatic or aromatic, are not promising replacements for lysine. Interestingly, treatment of **7b** with methyl iodide/potassium carbonate afforded the corresponding methyl ether **7e** with an IC_{50} for the NK-1 receptor of 59 ± 22 nM, suggesting that the methoxyl group may act as a hydrogen bond acceptor.[19] The *p*-F-Bn analog **7f**, synthesized as one component of a second library, had an IC_{50} of 28 ± 14 nM, consistent with this interpretation.

For lead *optimization* we arbitrarily chose to replace Phe 7 by nine amino acids retaining Phe in position 9 (SRIF numbering). In the case of this larger library, the Houghton method[16] (adjusting for the reactivity disparity among individual amino acids by modulation of the molar ratio) proved unsatisfactory. The description by Carpino[20] of the more powerful coupling reagent HATU led us to employ this reagent using only 1 equiv of an equimolar mixture of the nine amino acids. This modification provided a mixture of the nine desired linear peptides. After cyclization, deprotection, isolation, and characterization of the pure entities, the most

Table 1. IC_{50}s of Compounds **7**–**9** at the NK-1 receptor[18]



compd	Xaa-9	R¹	Yaa-7	R²	R³	IC_{50} ± mean SD (nM)
6a			see Figure 1			>1 μM
6b			see Figure 1			>1 μM
7a	Ala	H	Phe	CH_2Ph	H	4093 ± 869
7b	Tyr	*p*-HO-Ph	Phe	CH_2Ph	H	1045 ± 230
7c	Leu	$CHMe_2$	Phe	CH_2Ph	H	2732 ± 221
7d	Phe	Ph	Phe	CH_2Ph	H	95 ± 35
7e	*p*-MeO-Phe	*p*-MeO-Ph	Phe	CH_2Ph	H	59 ± 22
7f	*p*-F-Phe	*p*-F-Ph	Phe	CH_2Ph	H	28 ± 14
8a	Phe	Ph	Ser	CH_2OH	H	596 ± 49
8b	Phe	Ph	Asp	CH_2CO_2H	H	1389 ± 780
8c	Phe	Ph	D-Pro	$-(CH_2)_3-$		51% at 2 μM
8d	Phe	Ph	Ala	MeH	H	549 ± 171
8e	Phe	Ph	Trp	CH_2Ind	H	35 ± 16
8f	Phe	Ph	D-Phe	CH_2Ph	H	496 ± 94
8g	Phe	Ph	D-HomoPhe	CH_2CH_2Ph	H	85 ± 10
8h	Phe	Ph	Cha	CH_2Chx	H	198 ± 33
8i	Phe	Ph	Nal	CH_2-α-Naphth	H	15 ± 4
9	*p*-F-Phe	*p*-F-Phe	Nal	CH_2-α-Naphth	H	2.0 ± 0.4

potent analog, the β-naphthylalanine derivative **8i**, had an affinity of 15 ± 4 nM. Incorporating the optimal substituents in both the 7 and 9 positions afforded **9** which had an IC_{50} of 2.0 ± 0.4 nM.

Compound **9** does not stimulate inositol phosphate synthesis in CHO cells expressing the human NK-1 receptor at concentrations up to 3 μM. In contrast, increasing concentrations of **9** shift the dose–response curve for substance P-stimulated inositol phosphate synthesis to the right without decreasing the maximal stimulation achieved. Schild analysis of these data gives a K_b of 3.5 nM and a linear plot with a slope of 1.07, consistent with competitive antagonism of substance P by **9** (Figure 3). Compound **9** did not inhibit binding of the relevant ligands of the somatostatin, neurokinin-2, or neurokinin-3 receptors, indicating that **9** is a selective antagonist of the NK-1 receptor.

Compound **9** has 20-fold reduced affinity for a mutant of the NK-1 receptor in which histidine 197 in transmembrane domain helix 5 is replaced with alanine, but it has normal affinity for a mutant of the NK-1 receptor in which histidine 265 in transmembrane domain helix 6 has been replaced with alanine. Histidines 197 and 265 are important components of the binding sites of various classes of nonpeptidyl NK-1 antagonists including our β-D-glucosides.[22] Our data suggest that **9** interacts with His 197 but not His 265 and localizes the binding site of this compound to the same transmembrane region as the other structurally dissimilar NK-1 antagonists. A similar binding site has been postulated for the SRIF receptor by Fitzpatrick and Vandlen,[23] on the basis of the fact that some of the structural determinates for the binding of MK-678 (**6b**, an analog of L-363,301) are also localized near the second and third extracellular loops between the transmembrane helices V and VI of the SSTR2 receptor. More recently, Kaupmann *et al.*[24] employed site-directed mutagenesis to show that transmembrane domains V, VI, and VII determine the selectivity of SMS 201-995 for the SSTR2 somatostatin receptor. Thus, the cyclic hexapeptides appear to be interacting at similar sites on the two G-protein-linked receptors.

It is interesting that cyclic hexapeptide **6a** and sugar **2** are *agonists* of the somatostatin receptor, whereas both **2** and cyclic hexapeptide **9** are *antagonists* of the NK-1 receptor. This observation may suggest that the compounds bind differently to the two receptors, such that binding of **1** or **2** to the somatostatin receptor prompts a conformational change that results in receptor activation, whereas binding to the NK-1 receptor does not trigger such a change. Alternatively, the difference may reside in the receptors themselves: the resting state of the somatostatin receptor may be closer to the activated state,[25] and therefore be intrinsically more readily activated than that of the NK-1 receptor. According to this hypothesis, compounds would have to cause only a minimal conformational change to function as agonists of the somatostatin receptor, whereas antagonists would have to cause a more substantial change; the converse would be true for the NK-1 receptor. This interpretation would explain why synthetic ligands have been likely to be agonists of the somatostatin receptor, but antagonists of the NK-1 receptor. A more detailed analysis of the relative enthalpy and entropy contributions will be required to determine whether this hypothesis is correct.

The structures of the linear undecapeptide substance P and the cyclic tetradecapeptide somatostatin are very different, making important similarities in the binding sites of their respective receptors unobvious. However, the remarkably facile transformation of a potent peptide ligand of the somatostatin receptor *via* **1** and **2** into a highly potent peptide ligand for the NK-1 receptor, illustrated schematically in Figure 4, supports our

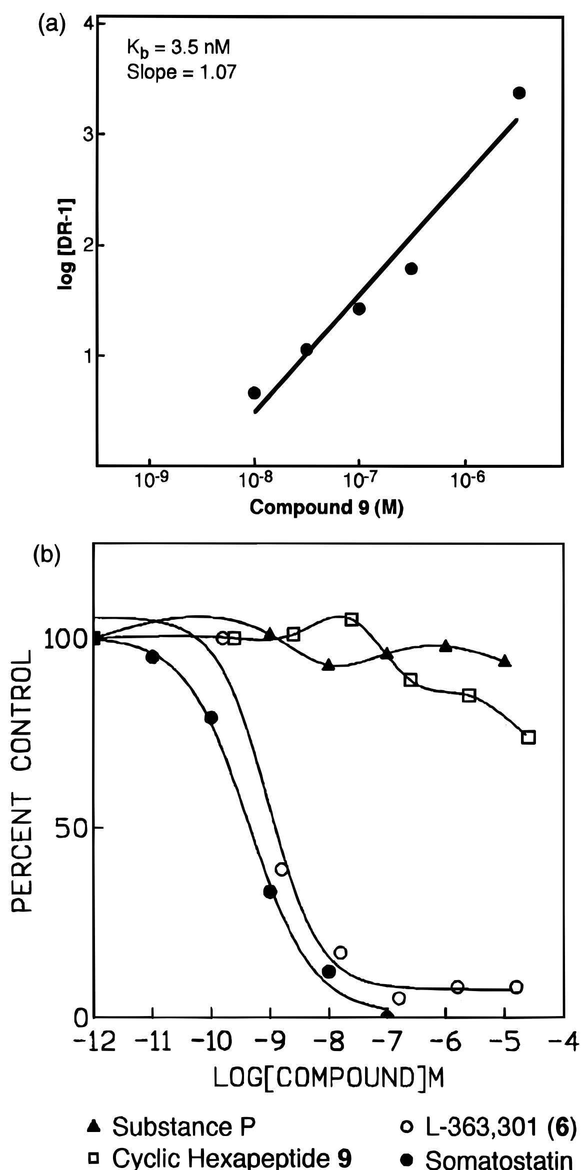


Figure 3. (a) Schild analysis of the inhibition of SP-stimulated inositol phosphate synthesis by compound **9**. (b) Representative dose response curves of the inhibition of the binding of 100 pM [^{125}I]Tyr^{11}-SRIF-14 to AtT-20 cell membranes.[21]

conclusion that a common binding site within the transmembrane domain of G-protein-coupled receptors can be utilized to fashion potent agonists and antagonists, respectively, of these receptors. It is worth mentioning that peptidomimetics of similar chemical structures are known to bind the CCK-A[26] and gastrin (CCK-B) receptors,[27] and that peptidomimetics of similar structure can bind to both oxytocin and vasopressin receptors.[28] However, because the structures of CCK/gastrin or of oxytocin/vasopressin have much in common, the similarity between the respective receptors is suggested by the endogenous ligands.[29] This is in contrast to the disparaties between the structures of substance P and somatostatin. In this context it is relevant that Hruby and his collaborators[30] designed and synthesized conformationally restricted cyclic octapeptides containing the tetrapeptide sequence Tyr-D-Trp-Lys-Thr which resembles the β-turn of somatostatin. These octapeptides were ligands for the μ-opioid receptor. Thus D-Phe-cyclo-(Cys-Tyr-D-Trp-Lys-Thr-Pen)-Thr-NH_2 was a μ-opioid receptor antagonist with an IC_{50} of 3.7 nM. These accomplishments differ from the work described herein in the size of the peptides and the number of atoms in the ring, but most importantly in the fact that it had been known that SRIF itself binds to the opiate receptor,[31] albeit weakly, and that somatostatin therefore served as the point of departure for the studies by the Hruby group.

Our results also demonstrate the power of the library method even on a miniscale to discover leads and then to optimize structure−activity relationships. Further, our results show that cyclic hexapeptides and sugars like the benzodiazapines,[26,27] the so-called tricyclics,[32] and the steroid nucleus,[33] may be viewed as promiscuous platforms. Finally, the results show that the use of libraries and rational design need not be mutually exclusive approaches to lead discovery. Experiments are now in progress to determine whether incorporation of the *p*-F-Phe and/or α-naphthyl side chains, that enhanced affinity of the cyclic hexapeptides for the NK-1 receptor, will further enhance the affinity of the β-D-glucosides for this receptor.

Experimental Section[34]

H-Phe-Thr(*t*-Bu)-Xaa-D-Trp-Phe-Pro-2-chlorotrityl Resin. Assembly of multiple peptides on a single solid support was carried out using an Applied Biosystems, Inc. Model 431A automated peptide synthesizer. Methylene chloride washing was avoided throughout the synthesis to prevent acid-catalyzed cleavage of the peptide from the resin by small amounts of HCl that can be present in that solvent. *N*-α-Fmoc amino acids, from Bachem, Inc., with appropriately protected side chains were employed throughout. Starting from 0.25 mmol of Fmoc-L-Pro-2-chlorotrityl polystyrene resin (0.44 g, 0.57 mmol/g), the H-Phe-Thr(*t*-Bu)-Xaa-D-Trp-Phe-Pro-2-chlorotrityl resin was assembled according to standard procedure[35]

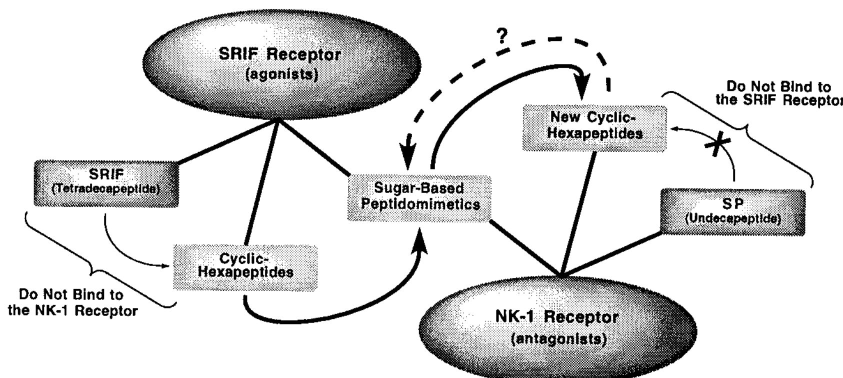


Figure 4. Insight gained about receptor relationships via the synthesis of peptidomimetic ligands.

with some modification. For the coupling at the mixture positions, a 4 molar excess (1.0 mmol) of the individual amino acids was used along with HBTU at room temperature for 2.0 h. A total combined 1.0 mmol of Fmoc-Xaa-OH [Xaa: Ala (0.13 mmol), Leu (0.28 mmol), Phe (0.12 mmol), Tyr(*t*-Bu) (0.48 mmol)] was used with molar ratio adjusted to compensate for the reactivity difference according to Houghten's procedure. Thus, incorporated in order, were Fmoc-Phe-OH, Fmoc-Thr-(*t*-Bu)-OH, Fmoc-Xaa-OH [Xaa: Ala, Leu, Phe, Tyr(*t*-Bu)], Fmoc-D-Trp-OH, Fmoc-Phe-OH. After each coupling, a Kaiser test was performed to monitor the coupling reaction, and if necessary a second coupling reaction was performed. The *N*-α-Fmoc group was removed at the end of the synthesis. After the completion of the solid phase syntheses, the resin was dried under vacuum to afford 686.0 mg of peptide resin.

Cyclo-(Phe-Thr-Xaa-D-Trp-Phe-Pro) (7a–d: Xaa = Ala, Leu, Phe, Tyr). The above peptide resin H-Phe-D-Trp-Xaa-Thr(*t*-Bu)-Phe-Pro-resin [665 mg; Xaa: Ala, Leu, Phe, Tyr(*t*-Bu)] was treated with TFA/CH_2Cl_2 [0.25% (v/v), 15 mL] at room temperature. After 30 min, the slurry was filtered and washed with 0.25% TFA/CH_2Cl_2 solution. The filtrate was concentrated, and the resulting residue was tritrated with ice-cold dry diethyl ether. The solid was collected by filtration and washed with ether to provide the crude product (247 mg) as a white powder, which was subjected to cyclization without further purification.

To a suspension of H-Phe-D-Trp-Xaa-Thr(*t*-Bu)-Phe-Pro-OH [200 mg; Xaa: Ala, Leu, Phe, Tyr(*t*-Bu)] and $NaHCO_3$ (solid, 251 mg) in dry DMF (33 mL) was added DPPA (65 μL) dropwise at 0 °C. The reaction mixture was stirred at 4 °C and monitored by analytical RP-HPLC. After 21 h at 4 °C, the reaction mixture was filtered and washed with DMF, and the combined filtrates were concentrated. The residue was redissolved in 50% MeCN/water (v/v) and lyophilized to afford the cyclized product (212.0 mg) as a white powder.

Half of the above material (106 mg) was dissolved in a mixture of CH_2Cl_2 (3.4 mL), ETD (225 μL), and H_2O (150 μL), and to this solution was added dropwise TFA (3.75 mL) at room temperature. After stirring at room temperature for 50 min, the reaction mixture was concentrated to half of its orginial volume and azeotroped with dry benzene (3 × 10 mL). The resulting residue was precipitated with dry ether, filtered, and washed extensively with ether and purified by RP-HPLC [C18 Dynamax 300 Å (21.4 × 250 mm) column; gradient, 35–25′–95% buffer B; flow rate, 12 mL/min] to afford pure compounds **7a** (23 mg), **7b** (14 mg), **7c** (13 mg), and **7d** (14.4 mg) in a combined yield of 82% from Fmoc-Pro-2-chlorotrityl polystyrene resin.

Cyclo-(Phe-D-Trp-Ala-Thr-Phe-Pro) (7a): $[\alpha]^{25}_D$ −78.5° (*c* 0.475, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 0.82–0.85 (m, 1 H), 0.90–0.96 (m, 1 H), 1.12 (d, *J* = 7.34 Hz, 3 H), 1.16 (d, *J* = 6.34 Hz, 3 H), 1.41–1.42 (m, 1 H), 1.74–1.78 (m, 1 H), 2.88–2.96 (m, 3 H), 3.05–3.13 (m, 4 H), 3.20–3.26 (m, 2 H), 3.63–3.64 (m, 1 H), 3.91–3.94 (m, 1 H), 4.11–4.13 (m, 1 H), 4.37–4.40 (m, 2 H), 4.42–4.50 (m, 1 H), 7.02–7.09 (m, 4 H), 7.11–7.19 (m, 4 H), 7.23–7.35 (m, 7 H), 7.58 (d, *J* = 7.84 Hz, 1 H); high-resolution mass spectrum (FAB) *m/z* 772.3405 [(M + Na)$^+$; calcd for $C_{41}H_{47}N_7O_7$ 772.3434].

Cyclo-(Phe-D-Trp-Tyr-Thr-Phe-Pro) (7b): $[\alpha]^{25}_D$ −71.5° (*c* 0.26, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 0.80–0.90 (m, 1 H), 1.0–1.13 (m, 1 H), 1.12 (d, *J* = 6.08 Hz, 3 H), 1.44–1.48 (m, 1 H), 1.74–1.75 (m, 1 H), 2.64–2.67 (m, 1 H), 2.77–2.88 (m, 2 H), 2.91–3.08 (m, 6 H), 3.12–3.16 (m, 1 H), 3.22–3.26 (m, 1 H), 3.63–3.65 (m, 1 H), 4.09–4.10 (m, 1 H), 4.15–4.22 (m, 1 H), 4.32–4.38 (m, 2 H), 4.47–4.49 (m, 1 H), 6.59 (d, *J* = 7.80 Hz, 2 H), 6.65 (d, *J* = 8.10 Hz, 2 H), 6.92 (bs, 1 H), 7.01 (d, *J* = 5.00 Hz, 2 H), 7.07 (t, *J* = 7.40 Hz, 1 H), 7.15 (s, 3 H), 7.22 (d, *J* = 7.05 Hz, 2 H), 7.27–7.32 (m, 4 H), 7.39 (d, *J* = 8.34 Hz, 1 H), 7.51 (d, *J* = 7.64 Hz, 1 H); high-resolution mass spectrum (FAB) *m/z* 864.3713 [(M + Na)$^+$; calcd for $C_{47}H_{51}N_7O_8$ 864.3696].

Cyclo-(Phe-D-Trp-Leu-Thr-Phe-Pro) (7c): $[\alpha]^{25}_D$ −122° (*c* 0.28, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 0.56 (d, *J* = 6.35 Hz, 3 H), 0.66 (d, *J* = 6.44 Hz, 3 H), 0.75–0.76 (m, 1 H), 0.88–0.99 (m, 1 H), 1.01–1.09 (m, 1 H), 1.16 (d, *J* = 6.37 Hz, 3 H), 1.30–1.42 (m, 2 H), 1.47–1.53 (m, 1 H), 1.79–1.96 (m, 1 H), 2.85 (dd, *J* = 5.12, 13.5 Hz, 1 H), 2.90–3.11 (m, 5 H), 3.17–3.21 (m, 1 H), 3.26–3.30 (m, 1 H), 3.65 (d, *J* = 7.78 Hz, 1 H), 3.87–3.92 (m, 1 H), 4.11–4.16 (m, 1 H), 4.37–4.41 (m, 2 H), 4.60–4.67 (m, 1 H), 4.72–4.75 (m, 1 H), 7.00–7.36 (m, 15 H), 7.58 (d, *J* = 7.78 Hz, 1 H); high-resolution mass spectrum (FAB) *m/z* 814.3903 [(M + Na)$^+$; calcd for $C_{44}H_{53}N_7O_7$ 814.3904].

Cyclo-(Phe-D-Trp-Phe-Thr-Phe-Pro) (7d): $[\alpha]^{25}_D$ −67.1° (*c* 0.31, MeOH); ^{1}H NMR (500 MHz, CD_3OD, 315 K) δ 0.78–0.86 (m, 1 H), 0.96–1.05 (m, 1 H), 1.13 (d, *J* = 6.39 Hz, 3 H), 1.43–1.47 (m, 1 H), 1.75 (dd, *J* = 6.36, 12.26 Hz, 1 H), 2.79 (dd, *J* = 6.31, 13.87 Hz, 1 H), 2.85 (d, *J* = 6.93 Hz, 2 H), 2.86–2.95 (m, 3 H), 3.02 (dd, *J* = 6.62, 13.55 Hz, 1 H), 3.07 (dd, *J* = 5.59, 12.78 Hz, 1 H), 3.11–3.14 (m, 1 H), 3.19–3.25 (m, 1 H), 3.63 (d, *J* = 7.76 Hz, 1 H), 4.07–4.12 (m, 1 H), 4.26–4.29 (m, 1 H), 4.38–4.41 (m, 2 H), 4.48 (dd, *J* = 6.44, 8.99 Hz, 1 H), 4.64–4.67 (m, 1 H), 6.85–6.86 (m, 2 H), 6.89 (s, 1 H), 6.98–7.00 (m, 2 H), 7.03–7.06 (m, 1 H), 7.12–7.15 (m, 7 H), 7.22–7.23 (m, 2 H), 7.25–7.28 (m, 1 H), 7.30–7.36 (m, 3 H), 7.49 (d, *J* = 7.88 Hz, 1 H); high-resolution mass spectrum (FAB) *m/z* 848.3731 [(M + Na)$^+$; calcd for $C_{47}H_{51}N_7O_7$ 848.3747].

Cyclo-(Phe-D-Trp-*p*-MeO-Phe-Thr-Phe-Pro) (7e). To a suspension of **6c** (3.3 mg) and K_2CO_3 (1.6 mg) in DMF (0.1 mL) was added MeI (3.6 μL). After 1.0 h, the reaction mixture was filtered, dissolved in 50% MeCN/water, and lyophilized to afford a solid which was directly purified by RP-HPLC to afford **7e** (2.9 mg, 85%) as a white solid: ^{1}H NMR (500 MHz, CD_3OD) δ 0.79–0.84 (m, 1 H), 0.95–1.05 (m, 1 H), 1.14 (d, *J* = 6.41 Hz, 3 H), 1.40–1.46 (m, 1 H), 1.76 (dd, *J* = 5.96, 12.15 Hz, 1 H), 2.71 (dd, *J* = 4.9, 14.03 Hz, 1 H), 2.78–2.84 (m, 2 H), 2.87–2.98 (m, 3 H), 3.01–3.14 (m, 3 H), 3.20–3.26 (m, 1 H), 3.64 (d, *J* = 7.95 Hz, 1 H), 3.75 (s, 3 H), 4.07–4.09 (m, 1 H), 4.11–4.22 (m, 1 H), 4.38–4.40 (m, 2 H), 4.41–4.48 (m, 1 H), 4.64–4.67 (m, 1 H), 6.6 (d, *J* = 8.71 Hz, 2 H), 6.72 (d, *J* = 8.63 Hz, 2 H), 6.95 (s, 1 H), 7.01–7.08 (m, 3 H), 7.13–7.18 (m, 4 H), 7.23 (d, *J* = 6.97 Hz, 2 H), 7.26–7.38 (m, 4 H), 7.51 (d, *J* = 7.85 Hz, 1 H), 7.99 (d, *J* = 6.81 Hz, 1 H), 8.19 (d, *J* = 6.89 Hz, 1 H), 8.39 (bs, 1 H); high-resolution mass spectrum (FAB) *m/z* 878.3831 [(M + Na)$^+$; calcd for $C_{48}H_{53}N_7O_8$ 878.3853].

Cyclo-(Phe-D-Trp-Xaa-Thr-Phe-Pro) (7f–j: Xaa = *p*-F-Phe, Homo-Phe, Cha, Trp, D-Phe). Cyclic hexapeptides **7f–j** were prepared using the procedure described above for the synthesis of **7a–d**, except that a modified coupling protocol was used at the mixture position. In this case, a total combined 1 equiv (0.25 mmol) of an equimolar Fmoc-Xaa-OH mixture was used per 1 equiv of peptide resin (0.25 mmol) in the reaction along with HATU as the coupling reagent. The final crude mixture was separated with RP-HPLC [C18 Dynamax 300 Å (21.4 × 250 mm) column; gradient, 35–25′–95% B; flow rate, 12 mL/min] to afford **7f–h** as pure compounds and a mixture of **7i** and **7j**.

Cyclo-(Phe-D-Trp-*p*-F-Phe-Thr-Phe-Pro) (7f): $[\alpha]^{25}_D$ −74.4° (*c* 0.53, MeCN); ^{1}H NMR (500 MHz, CD_3OD) δ 0.85–0.91 (m, 1 H), 1.04–1.08 (m, 1 H), 1.14 (d, *J* = 6.38 Hz, 3 H), 1.44–1.47 (m, 1 H), 1.75–1.79 (m, 1 H), 2.77–2.85 (m, 3 H), 2.90–2.99 (m, 3 H), 3.02–3.09 (m, 2 H), 3.12–3.15 (m, 1 H), 3.21–3.27 (m, 1 H), 3.33–3.37 (m, 1 H), 3.64 (d, *J* = 7.68 Hz, 1 H), 4.09–4.13 (m, 1 H), 4.24 (dd, *J* = 5.35, 8.25 Hz, 1 H), 4.37–4.41 (m, 2 H), 4.49 (dd, *J* = 5.94, 9.50 Hz, 1 H), 6.79 (s, 2 H), 6.80 (d, *J* = 1.85 Hz, 2 H), 6.93 (s, 1 H), 7.04–7.10 (m, 4 H), 7.13–7.20 (m, 4 H), 7.22–7.24 (m, 2 H), 7.29–7.38 (m, 4 H), 7.50 (d, *J* = 7.88 Hz, 1 H); high-resolution mass spectrum (FAB) *m/z* 866.3631 [(M + Na)$^+$; calcd for $C_{47}H_{50}FN_7O_7$ 866.3654].

Cyclo-(Phe-D-Trp-Homo-Phe-Thr-Phe-Pro) (7g): $[\alpha]^{25}_D$ −75.7° (*c* 0.6, MeCN); high-resolution mass spectrum (FAB) *m/z* 862.3918 [(M + Na)$^+$; calcd for $C_{48}H_{53}N_7O_7$ 862.3904].

Cyclo-(Phe-D-Trp-Cha-Thr-Phe-Pro) (7h): $[\alpha]^{25}_D$ −80.1° (*c* 0.85, MeCN); high-resolution mass spectrum (FAB) *m/z* 854.4211 [(M + Na)$^+$; calcd for $C_{47}H_{57}N_7O_7$ 854.4217].

Cyclo-(Yaa-D-Trp-Phe-Thr-Phe-Pro) (8a–i: Yaa = Ser, Ala, Asp, D-Pro, D-Homo-Phe, Cha, Trp, D-Phe, Nal). Peptides **8a–i** were prepared using the same procedure described for the synthesis of **7f–k**. The final crude mixture was separated by RP-HPLC (C18 Dynamax 300 Å (21.4 × 250 mm) column; gradient 35–25′–95% buffer B; flow rate, 12 mL/

min) to afford **8a**–**h** and a mixture of **8i**–**j** which was further resolved using a C8 Vydac column (10 × 250 mm) [gradient, 35–35′–70% buffer B; flow rate, 6 mL/min] to afford pure **8i** and **8j**.

Cyclo-(Ser-D-Trp-Phe-Thr-Phe-Pro) (8a): $[\alpha]^{25}_D$ −46.0° (*c* 0.31, MeCN); ^{1}H NMR (500 MHz, CD_3OD) δ 0.89–0.94 (m, 1 H), 1.11 (d, *J* = 6.42 Hz, 3 H), 1.56–1.63 (m, 2 H), 1.90 (dd, *J* = 6.2, 12.31 Hz, 1 H), 2.71 (dd, *J* = 4.98, 14.31 Hz, 1 H), 2.86 (dd, *J* = 8.53, 14.29 Hz, 1 H), 2.92–2.99 (m, 1 H), 3.05–3.12 (m, 3 H), 3.34–3.43 (m, 2 H), 3.65–3.74 (m, 3 H), 4.06–4.07 (m, 1 H), 4.29 (dd, *J* = 4.94, 8.41 Hz, 1 H), 4.37–4.40 (m, 1 H), 4.44–4.48 (m, 2 H), 4.57 (t, *J* = 7.75 Hz, 1 H), 6.77–6.79 (m, 2 H), 6.98 (s, 1 H), 7.02–7.05 (m, 1 H), 7.10–7.16 (m, 4 H), 7.24–7.38 (m, 6 H), 7.54 (d, *J* = 7.91 Hz, 1 H); high-resolution mass spectrum (FAB) *m/z* 788.3398 [(M + Na)$^+$; calcd for $C_{41}H_{47}N_7O_8$ 788.3384].

Cyclo-(Asp-D-Trp-Phe-Thr-Phe-Pro) (8b): $[\alpha]^{25}_D$ −54.2° (*c* 0.26, MeCN); ^{1}H NMR (500 MHz, CD_3OD) δ 0.83–0.87 (m, 1 H), 1.13 (d, *J* = 6.33 Hz, 3 H), 1.54–1.58 (m, 2 H), 1.94 (dd, *J* = 6.07, 12.33 Hz, 1 H), 2.64 (dd, *J* = 7.35, 15.93 Hz, 1 H), 2.77 (dd, *J* = 5.94, 16.00 Hz, 1 H), 2.81–2.88 (m, 2 H), 2.94 (t, *J* = 11.67 Hz, 1 H), 3.06 (d, *J* = 7.44 Hz, 2 H), 3.11 (dd, *J* = 5.13, 12.55 Hz, 1 H), 3.32–3.39 (m, 2 H), 3.71 (d, *J* = 7.61 Hz, 1 H), 4.05–4.09 (m, 1 H), 4.23–4.27 (m, 1 H), 4.38 (d, *J* = 4.62 Hz, 1 H), 4.43 (dd, *J* = 4.87, 10.82 Hz, 1 H), 4.52–4.55 (m, 1 H), 4.75–4.81 (m, 1 H), 6.85–6.86 (m, 2 H), 6.96 (s, 1 H), 7.01–7.04 (m, 1 H), 7.11–7.15 (m, 4 H), 7.26–7.31 (m, 3 H), 7.33–7.37 (m, 3 H), 7.51 (d, *J* = 7.92 Hz, 1 H), 7.96 (d, *J* = 6.08 Hz, 1 H), 8.02 (d, *J* = 6.10 Hz, 1 H), 8.08 (d, *J* = 7.90 Hz, 1 H), 8.35 (s, 1 H); high-resolution mass spectrum (FAB) *m/z* 816.3310 [(M + Na)$^+$; calcd for $C_{42}H_{47}N_7O_9$ 816.3333].

Cyclo-(D-Pro-D-Trp-Phe-Thr-Phe-Pro) (8c): $[\alpha]^{25}_D$ +28.7° (*c* 0.32, MeCN); high-resolution mass spectrum (FAB) *m/z* 798.3565 [(M + Na)$^+$; calcd for $C_{43}H_{49}N_7O_7$ 798.3591].

Cyclo-(Ala-D-Trp-Phe-Thr-Phe-Pro) (8d): $[\alpha]^{25}_D$ −65.6° (*c* 0.56, MeCN); ^{1}H NMR (500 MHz, CD_3OD) δ 1.02–1.10 (m, 1 H), 1.10 (d, *J* = 6.40 Hz, 3 H), 1.18 (d, *J* = 6.71 Hz, 3 H), 1.53–1.65 (m, 1 H), 1.62–1.70 (m, 1 H), 1.88 (dd, *J* = 5.95, 12.03 Hz, 1 H), 2.78 (dd, *J* = 4.75, 14.16 Hz, 1 H), 2.85–2.96 (m, 2 H), 3.00–3.07 (m, 3 H), 3.35–3.44 (m, 1 H), 3.66 (dd, *J* = 7.98 Hz, 1 H), 4.10–4.12 (m, 1 H), 4.33–4.40 (m, 3 H), 4.56–4.75 (m, 3 H), 6.84–6.86 (m, 2 H), 6.98 (s, 1 H), 7.02–7.05 (m, 1 H), 7.10–7.14 (m, 1 H), 7.15–7.19 (m, 2 H), 7.22 (d, *J* = 7.0 Hz, 2 H), 7.27–7.36 (m, 5 H), 7.51 (d, *J* = 7.87 Hz, 1 H), 7.79 (d, *J* = 5.61 Hz, 1 H), 7.99 (d, *J* = 7.10 Hz, 1 H), 8.18 (d, *J* = 3.60 Hz, 1 H), 8.34 (d, *J* = 7.68 Hz, 1 H); high-resolution mass spectrum (FAB) *m/z* 772.3437 [(M + Na)$^+$; calcd for $C_{41}H_{47}N_7O_7$ 772.3435].

Cyclo-(Trp-D-Trp-Phe-Thr-Phe-Pro) (8e): $[\alpha]^{25}_D$ −56.4° (*c* 0.56, MeCN); ^{1}H NMR (500 MHz, CD_3OD) δ 0.85–0.89 (m, 2 H), 1.15 (d, *J* = 6.35 Hz, 3 H), 1.34–1.40 (m, 1 H), 1.69–1.73 (m, 1 H), 2.59 (dd, *J* = 5.45, 13.80 Hz, 1 H), 2.71 (dd, *J* = 4.89, 14.37 Hz, 1 H), 2.83–2.88 (m, 2 H), 2.90–2.95 (m, 2 H), 3.05–3.08 (dd, *J* = 5.69, 12.69 Hz, 1 H), 3.12–3.21 (m, 3 H), 3.35 (d, *J* = 1.0 Hz, 1 H), 3.61 (d, *J* = 6.80 Hz, 1 H), 4.09–4.13 (m, 1 H), 4.23 (dd, *J* = 4.89, 8.32 Hz, 1 H), 4.36–4.39 (m, 2 H), 4.43 (dd, *J* = 5.61, 9.81 Hz, 1 H), 6.76 (d, *J* = 7.21 Hz, 2 H), 6.83 (s, 1 H), 6.96–6.99 (m, 2 H), 7.02–7.08 (m, 2 H), 7.09–7.10 (m, 1 H), 7.12–7.18 (m, 4 H), 7.21–7.23 (m, 2 H), 7.25–7.28 (m, 1 H), 7.29–7.33 (m, 3 H), 7.35–7.38 (m, 1 H), 7.44 (d, *J* = 7.77 Hz, 1 H), 7.53 (d, *J* = 7.58 Hz, 1 H); high-resolution mass spectrum (FAB) *m/z* 887.3879 [(M + Na)$^+$; calcd for $C_{49}H_{52}N_8O_7$ 887.3857].

Cyclo-(D-Phe-D-Trp-Phe-Thr-Phe-Pro) (8f): high-resolution mass spectrum (FAB) *m/z* 848.3721 [(M + Na)$^+$; calcd for $C_{47}H_{51}N_7O_7$ 848.3748].

Cyclo-(D-Homo-Phe-D-Trp-Phe-Thr-Phe-Pro) (8g): high resolution mass spectrum (FAB) *m/z* 862.3911 [(M + Na)$^+$; calcd for $C_{48}H_{53}N_7O_7$ 862.3904].

Cyclo-(Cha-D-Trp-Phe-Thr-Phe-Pro) (8h): $[\alpha]^{25}_D$ −63.2° (*c* 0.53, MeCN); ^{1}H NMR (500 MHz, CD_3OD) δ 0.78–0.90 (m, 3 H), 0.98–1.12 (m, 5 H), 1.14 (d, *J* = 6.39 Hz, 3 H), 1.42–1.53 (m, 2 H), 1.56–1.62 (m, 5 H), 1.63–1.71 (m, 1 H), 1.91 (dd, *J* = 6.33, 12.49 Hz, 1 H), 2.86–2.88 (m, 2 H), 2.92–2.96 (m, 1 H), 2.98–3.02 (m, 2 H), 3.09 (dd, *J* = 5.36, 12.69 Hz, 1 H), 3.36–3.39 (m, 2 H), 3.73 (d, *J* = 7.71 Hz, 1 H), 4.06–4.08 (m, 1 H), 4.30 (dd, *J* = 5.45, 8.49 Hz, 1 H), 4.40 (d, *J* = 4.49 Hz, 1 H), 4.43–4.51 (m, 2 H), 4.55 (t, *J* = 7.78 Hz, 1 H), 6.91–6.92 (m, 2 H), 6.98 (s, 1 H), 7.02–7.05 (m, 1 H), 7.10–7.15 (m, 1 H), 7.15–7.17 (m, 3 H), 7.25–7.26 (m, 2 H), 7.28–7.30 (m, 1 H), 7.35 (t, *J* = 8.12 Hz, 3 H), 7.52 (d, *J* = 7.90 Hz, 1 H); high-resolution mass spectrum (FAB) *m/z* 854.4203 [(M + Na)$^+$; calcd for $C_{47}H_{57}N_7O_7$ 854.4218].

Cyclo-(Nal-D-Trp-Phe-Thr-Phe-Pro) (8i): $[\alpha]^{25}_D$ −52.4° (*c* 0.73, MeCN); ^{1}H NMR (500 MHz, CD_3OD) δ 0.82–0.90 (m, 2 H), 1.16 (d, *J* = 6.30 Hz, 3 H), 1.38–1.41 (m, 1 H), 1.72–1.74 (m, 1 H), 2.50 (dd, *J* = 5.35, 13.6 Hz, 1 H), 2.72–2.90 (m, 3 H), 2.93–2.99 (m, 2 H), 3.05–3.09 (dd, *J* = 5.57, 12.8 Hz, 1 H), 3.16–3.22 (m, 1 H), 3.42 (dd, *J* = 7.48, 13.65 Hz, 1 H), 3.52 (dd, *J* = 7.98, 13.88 Hz, 1 H), 3.62 (d, *J* = 6.99 Hz, 1 H), 4.12–4.16 (m, 1 H), 4.22 (dd, *J* = 4.63, 8.03 Hz, 1 H), 4.35–4.40 (m, 2 H), 4.43 (dd, *J* = 5.57, 9.58 Hz, 1 H), 4.81 (t, *J* = 7.49 Hz, 1 H), 6.78 (t, *J* = 5.22 Hz, 3 H), 7.05 (t, *J* = 7.85 Hz, 1 H), 7.09–7.15 (m, 4 H), 7.22 (d, *J* = 7.53 Hz, 2 H), 7.27 (d, *J* = 6.67 Hz, 2 H), 7.31 (t, *J* = 6.40 Hz, 3 H), 7.34–7.37 (m, 1 H), 7.43 (d, *J* = 7.86 Hz, 1 H), 7.45–7.52 (m, 2 H), 7.73 (d, *J* = 8.11 Hz, 1 H), 7.83 (d, *J* = 7.98 Hz, 1 H), 8.22 (8.36, 1 H); high-resolution mass spectrum (FAB) *m/z* 898.3926 [(M + Na)$^+$; calcd for $C_{51}H_{53}N_7O_7$ 898.3904].

Cyclo-(Nal-D-Trp-*p*-F-Phe-Thr-Phe-Pro) (9). Compound **9** was synthesized using a procedure similar to that described for **7a**–**d**. The final product was purified by using RP-HPLC [C18 Dynamax 300 Å (21.4 × 250 mm) column; gradient, 55–25′–95% buffer B; flow rate, 9.9 mL/min] to afford **9** (119.0 mg, 70% yield) as a white powder: $[\alpha]^{25}_D$ −64.2° (*c* 0.73 MeCN); ^{1}H NMR [CD_3OD MHz, 500 (315 K)] δ 0.77–0.80 (m, 1 H), 0.87–0.88 (m, 1 H), 1.17 (d, *J* = 6.39 Hz, 3 H), 1.37–1.40 (m, 1 H), 1.74 (dd, *J* = 5.52, 11.62 Hz, 1 H), 2.46 (dd, *J* = 5.11, 13.74 Hz, 1 H), 2.71 (dd, *J* = 4.90, 14.42 Hz, 1 H), 2.80 (dd, *J* = 9.94, 14.17 Hz, 2 H), 2.92 (t, *J* = 11.1 Hz, 1 H), 2.98 (t, *J* = 9.93 Hz, 1 H), 3.05 (dd, *J* = 5.53, 12.74 Hz, 1 H), 3.15–3.19 (m, 1 H), 3.43 (dd, *J* = 7.6, 13.6 Hz, 1 H), 3.53 (dd, *J* = 7.70, 13.63 Hz, 1 H), 3.61 (d, *J* = 7.44 Hz, 1 H), 4.11–4.16 (m, 2 H), 4.36 (dd, *J* = 5.50, 10.91 Hz, 1 H), 4.39 (d, *J* = 4.82 Hz, 1 H), 4.43 (dd, *J* = 5.19, 10.37 Hz, 1 H), 4.83 (t, *J* = 7.6 Hz, 1 H), 6.69–6.72 (m, 2 H), 6.74–6.78 (m, 2 H), 6.82 (s, 1 H), 7.05 (t, *J* = 7.84 Hz, 1 H), 7.14 (t, *J* = 7.88 Hz, 1 H), 7.20–7.22 (m, 2 H), 7.25–7.37 (m, 7 H), 7.42 (d, *J* = 7.91 Hz, 1 H), 7.46–7.49 (m, 1 H), 7.51–7.54 (m, 1 H), 7.73 (d, *J* = 7.99 Hz, 1 H), 7.84 (d, *J* = 8.07 Hz, 1 H), 8.24 (d, *J* = 8.38 Hz, 1 H); high-resolution mass spectrum (FAB) *m/z* 916.3810 [(M + Na)$^+$; calcd for $C_{51}H_{52}FN_7O_7$ 916.3810].

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Supporting Information Available: Complete spectral data for **7a**–**h**, **8a**–**i**, and **9** including ^{13}C NMR data for **7a**–**d**,**f**–**h**, **8e**,**h**,**i**, and **9** (8 pages). Ordering information is given on any current masthead page.

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(34) **Materials and Methods.** Unless otherwise noted, all solvents and reagents were obtained from commercial sources and used without further purification. Analytic reverse-phase HPLC was carried out employing an LKB system (2152 LC controller, 2150 HPLC pump, 2141 variable wavelength monitor) on a C18 Dynamax 300 Å (0.46−25 cm) column; semi or preparative reverse-phase HPLC separations were achieved using a Ranin solvent delivery system equipped with a dynamax detector (model UV-D) utilizing either C18 Dynamax 300 Å (21.4 × 250 mm) column or C8 Vydac column (10 × 250 mm). The mobile phase consisted of 0.1% TFA in water (buffer A) and 0.1% TFA in acetonitrile (buffer B). The FAB-mass spectra were obtained on a ZAB-E VG analytical spectrometer. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AM500 spectrometer. Chemical shifts are reported in δ values relative to tetramethylsilane for proton and solvent for carbon spectra. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter.

(35) Applied Biosystem Inc. Publication No. 35, Fast Moc 0.25 and 0.10 mmol on the Model 431A.

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