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Syntheses and Activities of New C^{10} β-Turn Peptidomimetics

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A program to identify small molecules that mimic or disrupt protein−protein interactions led us to design the peptidomimetics **1**−**3**. Solid-phase syntheses of **1**−**3** were developed. The purities of the crude materials isolated from the resin tend to be highest for the *S*- and *N*-compounds **2** and **3** and better than in the corresponding syntheses of peptidomimetics **A**. The particular dipeptide units incorporated were chosen to correspond with the turn regions of the neurotrophins (e.g., nerve growth factor {NGF} and the neurotrophin factor-3 {NT-3}). Preliminary studies were performed to access the binding of these analogues to Trk receptors and their ability to induce cell survival (just as NGF and NT-3 do). Several active compounds were identified. However, poor water solubilities of some of the other compounds preclude reliable testing. Consequently, solid-phase modifications to the synthetic procedures were investigated to provide access to the derivatives **12**−**14** in which the aromatic nitro group is replaced by amine, guanidine, or sulfonamide functionalities. The latter are more acceptable pharmacophores than nitro groups and also tend to increase the water solubilities of the peptidomimetics.

In the design and testing of peptidomimetics for binding target protein receptors, rigidity is a two-edged sword.[1−3] At one extreme, compounds that are too flexible may pay such high entropic penalties on binding that the process becomes energetically unfavorable. Conversely, severe conformational constraints can prevent molecules from adapting to the binding site at all. A reasonable approach to finding compounds that bind proteins is therefore to prepare and test molecules with the appropriate amino acid side chains and moderate conformational constraints and then to increase the conformational focus in subsequent libraries via incremental changes that reduce flexibilities. Studies of the conformations of the peptidomimetics in solution may be used to guide this process. It is possible that the favored conformation of an active peptidomimetic in solution is not the bound conformation. However, the likelihood of this decreases with the rigidity of the mimic, and hence it is possible to interpret solution-state conformational studies with increasing levels of certainty as the experiments hone in on less flexible molecules.

Previous work from these laboratories have focused on β-turn[4] mimetics **A**, prepared via solid-phase, S_NAr macrocyclization reactions.[5−8] It is now evident that compounds **A** can disrupt some protein−protein interactions. For instance, a small library of mimics **A** was prepared based on the $i + 1$ and $i + 2$ residues of the turn regions of the nerve growth factor (NGF). The NGF turn regions are thought to be hot spots for the interaction of this protein with its high affinity receptor, TrkA.[9,10] Some members of the library were active in several pharmacological tests designed to identify this interaction, including direct and competitive binding to TrkA, studies based on the ability of NGF (or NGF mimics) to promote cell survival in media free of growth hormones, phosphorylation of intracellular tyrosine residues in the TrkA receptor on activation, upregulation of choline acetyl transferase, and induction of neuronal cell growth. Data on one of the lead compounds, **D3**, has been described in full,[11] and several other interesting leads have been identified, implying that the combination of peptidic and nonpeptidic fragments in structures **A** might be especially favorable for mimicry of hot spots in protein−protein interactions that involve β-turn $i+1$ and $i + 2$ regions.

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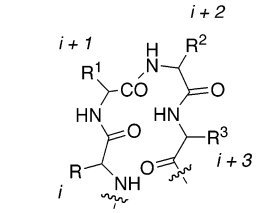
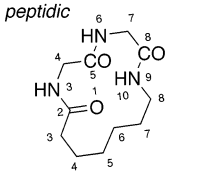
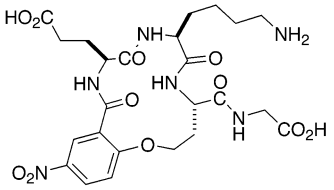


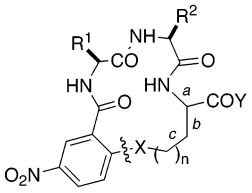
β–turn or C^{10} structure



14-membered ring having a "ring-fused" C^{10} arrangement



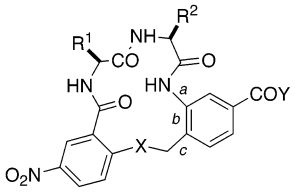
D3



A

n = 0, X = O, S
n = 1, X = NH, O
n = 2, 3, X = NH
Y = OH or NH_2

*movement about bonds **a**, **b**, and **c** not synchronized*



1 X = O, Y = NH_2
2 X = S, Y = OH
3 X = NH, Y = NH_2

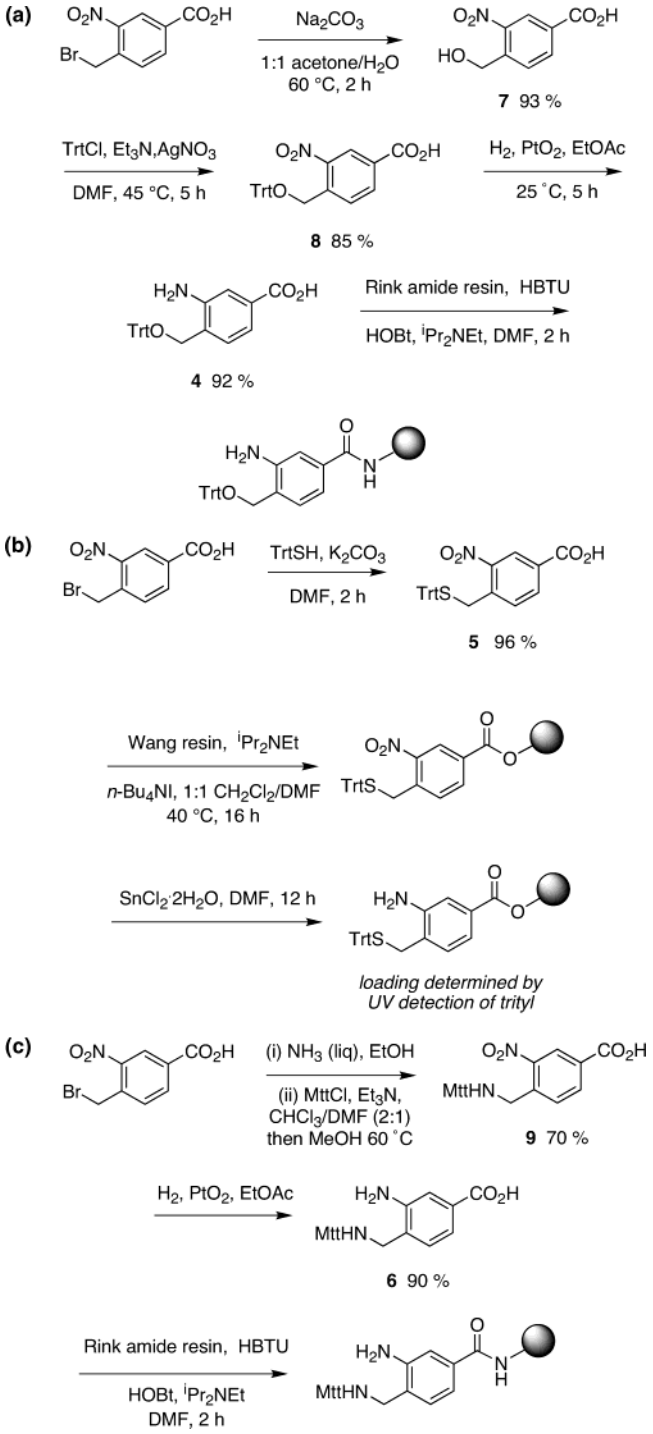
*movement about **a**, **b**, and **c** must be synchronized*

The next step of our work on NGF and related growth hormones was to produce more rigid turn analogues that retain the critical structural features of the original design including the amino acids corresponding to the $i+1$ and $i+2$ sites in the target turns. To attempt this, we designed systems of the general type **1**−**3** shown above. In these compounds, the $a-c$ bonds are essentially coplanar and can only move in concert, whereas the corresponding dihedral angles in **A** can flex more independently. Consequently, mimics **1**−**3** should be more rigid than compounds **A**. The key challenges were to develop solid-phase syntheses of these materials that gave crude products with high purities, to explore their conformations, and to test for biological activities similar to that shown by compounds **A**.

Results and Discussion

Syntheses of Supported Templates. Scheme 1 describes syntheses of templates **4**, **5**, and **6** that were pivotal to the solid-phase synthesis of the desired peptidomimetics. The oxygen template **4** was prepared (Scheme 1a) via hydrolysis of the commercially available benzyl bromide to give alcohol **7**,[12] which was then tritylated to **8**.[13] This trityl ether does not withstand reduction of the nitro group with $SnCl_2$ in DMF (protons are generated in this reaction), but Adams' catalyst

SCHEME 1. Preparation of the (a) O-Containing, (b) S-Containing, and (c) N-Containing Templates



mediated hydrogenation of the nitro group to the amine **4** without significant hydrogenolysis of the *O*-protecting group.[14] Amino acid **4** could be activated and coupled to resins functionalized with the Rink linker.[15] The fact that this reaction is not complicated by reactions at the free amine group[16] simplified the template synthesis but

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implied that conditions to effect subsequent couplings to the supported arylamine might require some investigation (see the next section below).

The preferred protecting group for thiols in our solid-phase synthesis would have been Mmt (monomethoxytrityl),[17] but the corresponding thiol {$(4\text{-MeOC}_6H_4)Ph_2$-CSH} is expensive. Consequently, triphenylmethyl thiol (TrtSH) was used to prepare the sulfur-containing template **5** (Scheme 1b). Trityl groups are unmasked from sulfur using 3% trifluoroacetic acid. These conditions are a little harsh for the Rink amide linker, so the syntheses presented here used the Wang ester linker to give carboxylic acids on cleavage. Syntheses of *carboxamides* via Rink resin would simply involve using the more expensive thiol. In addition to cost factors, however, the more stable Trt protecting group has the advantage that, unlike TrtO in the synthesis of the first template, the TrtS group is stable to on-resin tin(2+) chloride reduction of the nitro group.[18−20] Experimentally, Rink resin has the advantage that complete coupling of the first amino acid is facile and is easily monitored using the Kaiser test.[21] For Wang resin, the coupling is not quite as efficient and cannot be followed as easily. Consequently, the loading of the sulfur template was checked using a UV assay monitoring liberation of the trityl group (see Supporting Information).

The nitrogen-containing template was prepared in Mtt-protected form {Mtt is 4-methyltrityl, $(4\text{-MeC}_6H_4)Ph_2C-$} as indicated in Scheme 1c, via a known benzylic amine.[22,23] Use of Mtt is compatible with Rink resin. A solution-phase hydrogenation was used to form the amine **6**.

Solid-Phase Syntheses of Macrocycles. Coupling of the first amino acids to the supported templates was the most difficult step in the macrocycle syntheses depicted in Scheme 2. The PyBrop reagent (bromo-tris-pyrrolidino-phosphonium hexafluorophosphate)[17,24] with di-*iso*-propylethylamine has been used to couple unreactive amines to acids,[25] but in the current study this particular reagent system only gave satisfactory results for a few amino acids. After optimization of the reaction conditions (Table 1), it was found that PyBrop/2,6-lutidine was a superior system; 15 equiv of base was used here because loss of the trityl-based protecting groups occurred if less was added. As far as we are aware, this coupling agent/base combination is relatively unexplored.[26−28] This set of reagents was superior to any others that were tested in this work, both with respect to number of equivalents of the amino acids/PyBrop required and epimerization. The rest of the steps for the preparation of the macrocycle are similar to those outlined previously for the peptidomimetics **A**. These are standard amino acid coupling steps, removal of the "P" protecting group from "X" (1% TFA in CH_2Cl_2 for OTrt and NHMtt, and 3% TFA for STrt), base-mediated cyclization, and simultaneous removal of the side chain protecting groups and cleavage of the products from the resin.

SCHEME 2. Preparation of the Peptidomimetics 1−3

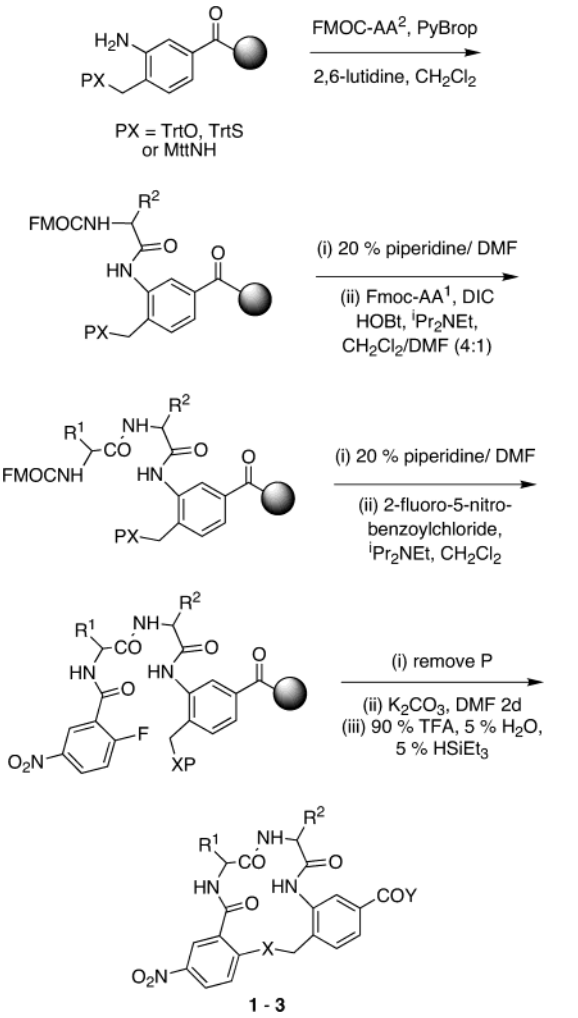

TABLE 1. Conditions for Coupling of First Amino Acids to Supported Template 6

entry	amino acid[3]	PyBrop (equiv)	base (equiv)	time (h)	purity[a] (%)
1	Fmoc-Lys(Boc)-OH	3	$^{i}Pr_2NEt$ (8)	8	57
2	Fmoc-Lys(Boc)-OH	3	2,6-lutidine (8)	8	93
3	Fmoc-Lys(Boc)-OH	3	*N*-methyl-morpholine (8)	8	88
4	Fmoc-Asn(Trt)-OH	3	$^{i}Pr_2NEt$ (8)	8	8
5	Fmoc-Asn(Trt)-OH	3	2,6-lutidine (15)	8	66
6	Fmoc-Asn(Trt)-OH	3	2,6-lutidine (15)	20	79
7	Fmoc-Asn(Trt)-OH[b]	5	2,6-lutidine (15)	8	73
8	Fmoc-Asn(Trt)-OH[b]	5	2,6-lutidine (15)	20	86
9	Fmoc-Gly-OH	3	2,6-lutidine (15)	8	95
10	Fmoc-Gly-OH	3	2,6-lutidine (15)	20	95

[a] Three equivalents unless otherwise indicated. [b] Five equivalents.

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TABLE 2. Yield and Purity Data for Compounds 1−3[a]

compd	amino acids $i+1$	amino acids $i+2$	X/Y	purity[a] (%)	yield (%)
1a	Ile	Lys	O/NH_2	96/97	80
1b	Gly	Lys	O/NH_2	62/81	66
1c	Glu	Lys	O/NH_2	86/98	43
1d	Ile	Arg	O/NH_2	78/92	53
1e	Thr	Gly	O/NH_2	38/49	54
1f	Ile	Asn	O/NH_2	94/98	67
1g	Asn	Asn	O/NH_2	88/96	60
1h	Lys	Gly	O/NH_2	70/87	71
1i	Arg	Gly	O/NH_2	74/92	74
1j	Lys	Thr	O/NH_2	76/92	69
1k	Glu	Asn	O/NH_2	56/90	71
2a	Ile	Lys	S/OH	95/100	47
2b	Gly	Lys	S/OH	97/100	56
2c	Glu	Lys	S/OH	97/100	57
2d	Ile	Arg	S/OH	87/91	50
2e	Thr	Gly	S/OH	94/100	55
2f	Ile	Asn	S/OH	98/100	52
2g	Asn	Asn	S/OH	100/100	67
2h	Lys	Gly	S/OH	99/100	54
2i	Arg	Gly	S/OH	99/100	56
2j	Lys	Thr	S/OH	98/100	52
2k	Glu	Asn	S/OH	85/95	55
3a	Ile	Lys	NH/NH_2	87/94	59
3b	Gly	Lys	NH/NH_2	87/100	44
3c	Glu	Lys	NH/NH_2	90/100	46
3d	Ile	Arg	NH/NH_2	85/93	61
3e	Thr	Gly	NH/NH_2	86/98	61
3f	Arg	Glu	NH/NH_2	71/100	58
3g	Glu	Ser	NH/NH_2	94/100	55
3h	Glu	Thr	NH/NH_2	100/100	64
3i	Pro	Ser	NH/NH_2	97/100	71
3j	Lys	Thr	NH/NH_2	88/100	65
3k	Pro	Gln	NH/NH_2	98/100	82
3l	Lys	Gln	NH/NH_2	100/100	98
3m	Gln	Thr	NH/NH_2	96/100	62
3n	Lys	Ser	NH/NH_2	95/99	94
3o	Gln	Ala	NH/NH_2	97/100	88
3p	Thr	Asp	NH/NH_2	98/100	51

[a] The resin was Wang (1.1 mmol/g) for **1a**−**k**, Rink amide MBHA (0.55 mmol/g) for **2a**−**k**, Rink (0.6 mmol/g) for **3a**−**h**, and Rapp-HG200RAM (0.62 mmol/g) for **3i**−**o**. [b] Purity as assessed by HPLC of crude product, monitored UV absorption at 254 nm. and using an evaporative light scattering detector (Sedex)

Experiments were run in the early stages of this project to ascertain the effects (if any) of resin on the cyclization reactions. In the event, the support made relatively little difference on the outcome of the reaction. These data are summarized in Supporting Information.

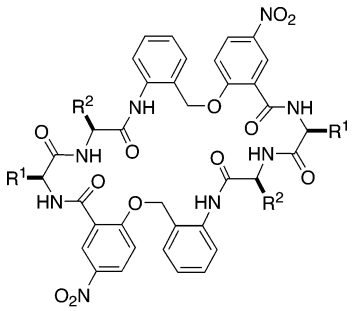
Table 2 summarizes yield and purity data for the macrocycles produced in this work. Throughout, UV and evaporative light scattering (ELS) detection were used to monitor the purities. The percentage purities indicated via UV and ELS detection can differ by as much as 44%, but in most cases they are much closer. UV detection over-represents compounds with larger extinction coefficients at the detection wavelength. ELS has a higher threshold of detection, so it tends to miss trace impurities. In this particular study, we believe that ELS, being based on particle mass, is likely to be more representative of the actual purity, especially since significant impurities probably have molecular masses similar to the product.

Table 2 shows that the oxygen compounds **1** were formed in the lowest purities in the series (49−98% based on ELS, average 84.1%) where 7 of the 11 compounds had purities above 85% (a typical quality control "cut-off value" above which a crude sample might be submitted for a bioassay). In some cases, the major impurity was isolated, subjected to MALDI-MS analysis, and shown to have a molecular mass of twice the value expected for the product. This apparently corresponds to the dimeric structure **10** (MS evidence alone). Similar head-to-tail dimers have been observed in other work from these laboratories, and they tend to be the major byproducts when there is some stereoelectronic impediment to the desired macrocyclization. In this work, however, dimers such as **10** were only detected for the macrocyclic ethers **1**. No dimers were observed in the syntheses of compounds **2** and **3**, even at resin loadings as high as 0.6 mmol/g (conditions that accentuate dimer formation when *O*-nucleophiles are used in the ring closure step). This is in contrast to systems **A** wherein head-to-tail dimers can form at higher resin loadings even if *N*- and *S*-nucleophiles are used. The macrocyclic thiols and amines were formed in significantly higher purities without any appreciable head-to-tail dimer formation. Compounds **2** had average purities of 98.7% (ELS, over 11 samples), and compounds **3** had average purities of 99.0% (ELS, over 16 samples). For **2** and **3** together, the minimum purity observed was 91%.

TABLE 3. Comparison of Purities for Compounds A and 1−3

entry	amino acids $i+1$	amino acids $i+2$	X	purity (%) **A**	purity (%) **1**, **2**, or **3**
1	Ile	Lys	O	80	96
2	Glu	Lys	O	89	86
3	Ile	Asn	O	61	94
4	Ile	Lys	S	87	95
5	Gly	Lys	S	91	97
6	Glu	Lys	S	85	97
7	Asn	Asn	S	65	100
8	Ile	Arg	S	82	87
9	Ile	Lys	NH	86	87
10	Glu	Lys	NH	90	90

[a] Purity assessed by HPLC with UV detection at 254 nm.



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The purity trends outlined above are consistent with those previously observed for compounds **A** where the purity of the products is less if oxygen nucleophiles are involved in the cyclization than for *N*- or *S*-nucleophiles. However, products **1**−**3** tend to be formed in higher purities. Table 3 shows data selected from this study for syntheses of some of the compounds **1**−**3**, presented with other data from these laboratories involving syntheses of systems **A**. Higher purities are obtained for **1**−**3** in most cases.

Conformational Analyses of Compounds 1c, 2c, and 3c. The approach used in this study is the same one

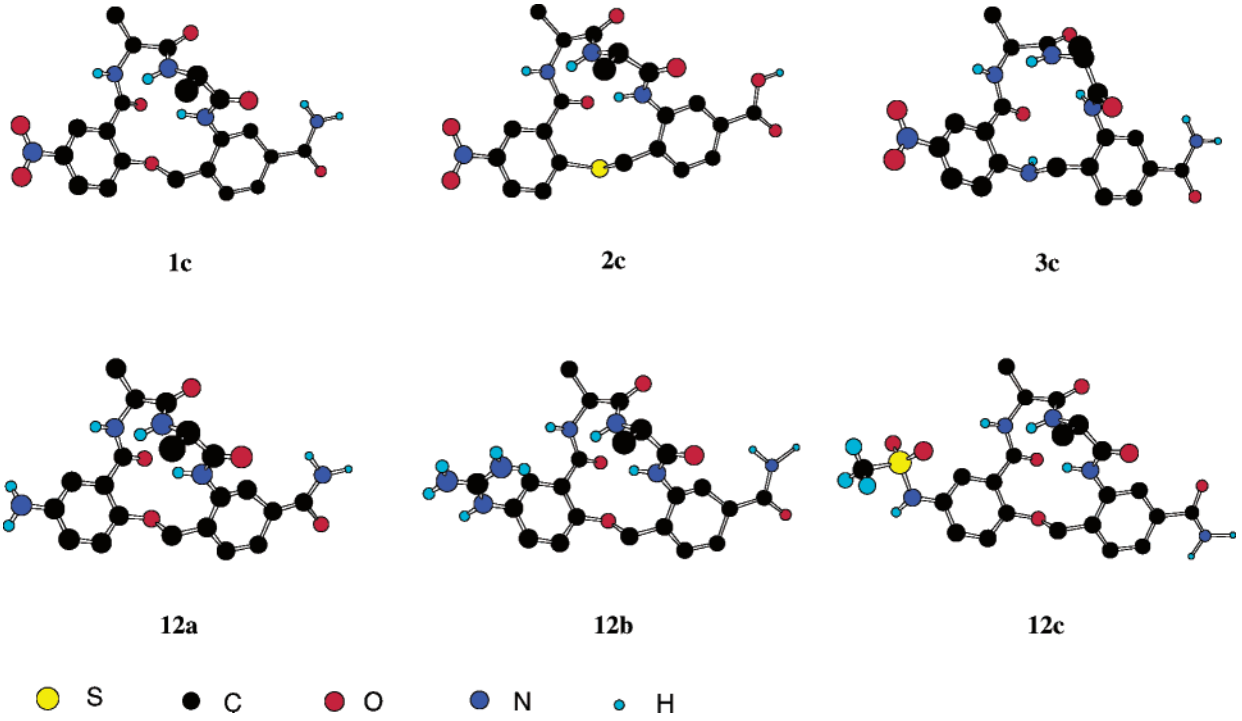
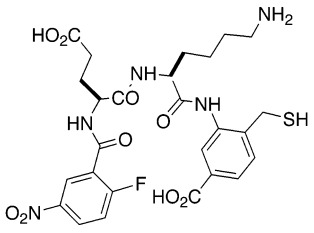


FIGURE 1. Simulated low energy conformers for compound **1c**, **2c**, **3c,** and **12a**−**c**.

used in these laboratories for conformational analyses of other peptidomimetics, including compound **A**.[29] Just as in the previous work, DMSO was used as the solvent so that the data obtained could be compared with that from previous studies and because some of the compounds were insoluble in aqueous media. Details of the approach used are given in the supplementary. The linear precursor peptide **11** was isolated and analyzed to check a feature of the data relating to NH coefficients.



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Overall the data obtained from the combined NMR, CD, and molecular simulations studies of the compounds are consistent with the following conclusions. The ether **1c** and the thioether **2c** populate type I-like turn conformations (Figure 1). CD (in MeOH/H_2O) and QMD data for compound **3c** are indicative of type I-like turn conformations, but this assertion is not supported by the NMR data. It is possible that the strong H-bond acceptor characteristics of DMSO enable it to interact with the arylamine functionality of **3c** in a way that disrupts the turn conformation and that was not evident in the calculations since explicit solvent molecules were not used. Similar interactions would not be so prevalent in MeOH/H_2O during the CD studies. This would explain why the CD and molecular simulations experiments indicate **3c** has a turn conformation but the NMR data does not. An alternative explanation for the atypical conformational behavior of the amine could be that the aniline nitrogen is more planar than the corresponding O or S atoms as a result of conjugation with the nitro group (we thank a referee for suggesting this). Overall, we conclude that **1c**, **2c**, and **3c** can adopt conformations that approximate β-turns, though in the case of the amine **3c** this bias is not so pronounced as for the corresponding ether and thioether.

Preliminary Biological Screens. Two assays were used to evaluate the activities of these compounds: a competitive fluorescence activated cell sorting (FACS) assay to detect binding to cells that overexpress the Trk receptors and a cell survival assay to identify compounds that have neurotrophin-related biological properties. Early in this work, peptidomimetic **3n** was identified as a very interesting compound. It was tested in these and several other assays and found to be active. These data have now been communicated; **3n** is the first reported small molecule mimic of NT-3. It synergizes with this neurotrophin via binding to the TrkC receptor to induce intracellular phosphorylation and, subsequently, protects the cells from apoptotic cell death.[30] The subsequent discussion focuses on the other compounds in this series, since full details of these activities for **3n** have already been given.

The compounds from Table 2 were tested for binding selectivity toward cells expressing TrkC or TrkA receptors in the FACS assay. Such FACS assays are not feasible using labeled natural neurotrophins as the competing ligand. Fortunately, a set of monoclonal antibodies (mAb's) that bind to the domain 5 of the

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TABLE 4. Compounds Inhibit the Binding of mAb's[a]

		mAb binding (% maximal)	
	competitor	2B7·TrkC	5C3·TrkA
1	none	100 ± 0	100 ± 0
2	NTF (0.3 nM)	84 ± 8* (NT-3)	92 ± 9 (NGF)
3	NTF (1 nM)	59 ± 7* (NT-3)	81 ± 7* (NGF)
4	NTF (2 nM)	32 ± 7* (NT-3)	68 ± 6* (NGF)
5	**2b**	92 ± 14	73 ± 8*
6	**2d**	97 ± 6	75 ± 11*
7	**3a**	79 ± 8*	83 ± 7*
8	**3b**	82 ± 7*	92 ± 9
9	**3d**	84 ± 9	83 ± 3*
10	**3e**	94 ± 6	84 ± 5*
11	**3j**	115 ± 16	83 ± 5*
12	**3l**	80 ± 4*	67 ± 8*
13	**3m**	84 ± 15	86 ± 7*
14	**3o**	95 ± 11	85 ± 7*
15	**12b**	79 ± 11*	84 ± 20

[a] The maximal binding of mAb's 2B7 or 5C3 is shown, relative to no inhibition (100%). Data ± standard deviation for competitor compounds (at 50 μM) or for the corresponding neurotrophins (NTF) with statistically significant inhibition are indicated with asterisk, $n = 3-5$; $p \leq 0.05$.

receptors is available from pervious work in these laboratories. Domain 5 of the Trk receptors is thought to be crucial for ligand binding and receptor activation.[31−33] The binding sites of the anti-TrkA mAb 5C3 and the anti-TrkC mAb 2B7 partially overlap with the binding sites of NGF and NT-3, respectively, and the data shown in Table 4, entries 2−4 is consistent with this. These mAb's are therefore useful surrogates for the natural neurotrophins. Simultaneous assays using TrkA and TrkC receptors were used to indicate selectivity. Selective competition of mAb·receptor interactions by a small molecule peptidomimetic (added at 50 μM concentration) suggests that the compound binds to that receptor, likely binding at the domain 5 of the receptor where the mAb binds. Only data for the compounds with statistically significant activities for either receptor are shown in Table 4.

Compounds **3a** and **3l** competed with both mAb 2B7·TrkC and mAb 5C3·TrkA interactions, suggesting that they may bind to both receptors. Compound **3l** at 100 μM competed with 5C3·TrkA interactions to the same degree as 2 nM NGF and also competed 2B7·TrkC interactions to the same degree as 0.3 nM NT-3. Because of solubility issues, higher concentrations of peptidomimetics could not be tested to generate a full inhibitory dose response curve. However, it seems as if peptidomimetic **3l** has a K_i 4−5 logs higher than those of the neurotrophins.

Peptidomimetics **2b**, **2d**, **3d**, **3e**, **3j**, and **3o** inhibited mAb 5C3·TrkA interactions but not mAb 2B7·TrkC interactions; these data suggest that those particular compounds bind selectively to TrkA. Conversely, **3b** (entry 8) appears to have more affinity toward TrkC than TrkA.

All of the other compounds assayed did not have a significant effect in this binding competition assay (data not shown). Some were too insoluble in water/DMSO to be assayed accurately, even though the FACS assay tolerates higher levels of DMSO than most other in vitro screens for neurotrophin/Trk interactions. Others may perhaps have bound the receptor, but not at the Trk-d5 domain where the mAbs used in this assay dock. Conversely, the fact that a particular compound binds does not provide information about biological activity. Consequently, a cell survival assay was used to test the biological effects of the mimics.

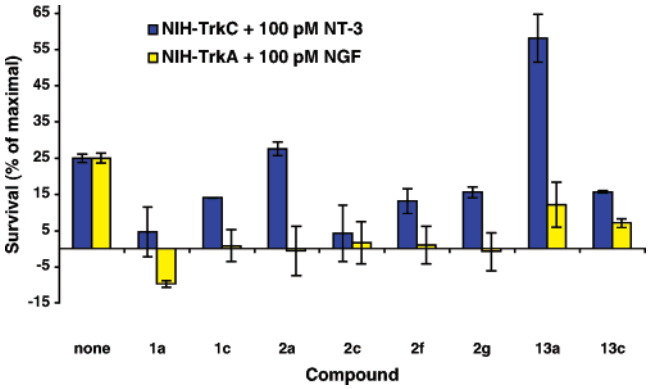


FIGURE 2. Trophic survival assays. The compounds were tested with cells in the presence of limiting concentrations of the corresponding neurotrophin (100 pM NT-3 for NIH-TrkC and 100 pM NGF for NIH-TrkA) that afford ∼25% cell survival. Data are standardized to maximal trophic survival afforded by optimal (2 nM) neurotrophins = 100%. Statistically reproducible survival of less than ∼25% indicates antagonism, whereas higher than ∼25% survival indicates potentiation of agonism. Average ± standard deviation from 3 to 4 independent assays, each assay $n = 6$. Only the compounds active in this assay are shown; all others tested were statistically inactive.

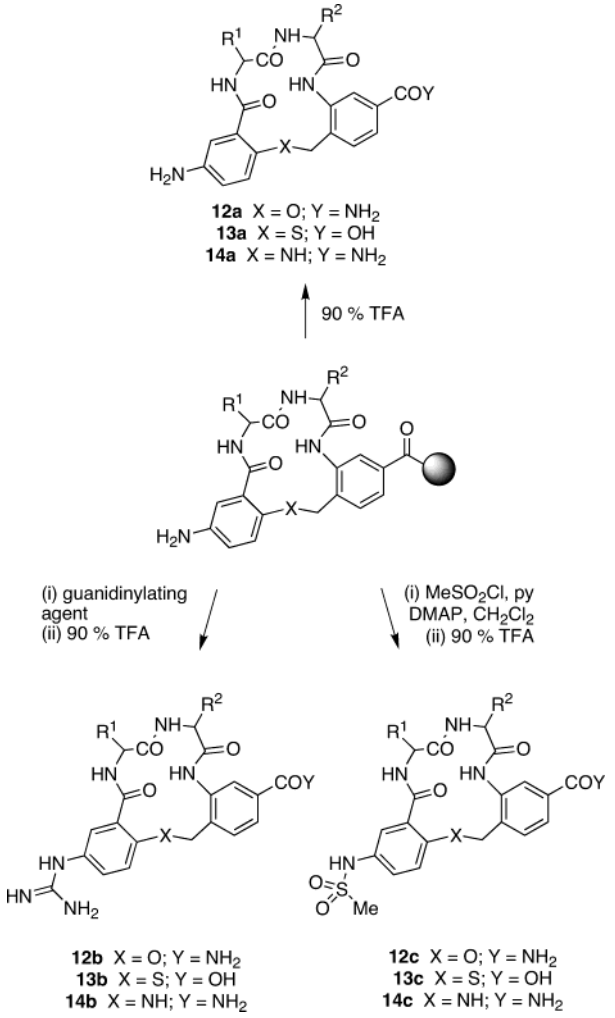
Survival assays feature cells in culture in serum free media (SFM) that would ordinarily go into apoptotic death under these conditions due to lack of growth factors. The cells can be rescued by trophic factors acting at selective receptors, e.g., NGF·TrkA or NT-3·TrkC.

Compounds that enhance the survival afforded by a trophic factor in these assays have agonist-like properties, whereas nontoxic compounds that prevent the survival afforded by a trophic factor are antagonistic. Only the compounds that are at least partially soluble in physiological buffers (e.g., DMSO final concentration less than 5%) could be tested in the cell survival assays. Unfortunately, solubility difficulties were encountered in this assay for all of the compounds listed in Table 4 except **3a**, and that compound did not elicit a positive response in cell survival assays.

Of the compounds that were soluble enough to be tested, **1a** and **2c** prevented the trophic rescue of NIH-TrkA cells by NGF, and NIH-TrkC cells by NT-3 (Figure 2). This antagonism was selective for TrkA and TrkC receptors because compounds **1a** and **2c** had no effect on the trophic rescue of these cells by β-fibroblast growth factor (data not shown). Peptidomimetics **1c**, **2a**, **2f**, **2g**, and **13c** (vide infra) antagonized TrkA·NGF but had no statistically significant effect on TrkC·NT-3. Hence their antagonistic response was TrkA-selective. Like previously reported compound **3n**[30] the compounds shown in Table 2 had no detectable intrinsic biological activity when tested in the absence of neurotrophins (data not shown).

(31) Urfer, R.; Tsoulfas, P.; O'Connell, L.; Shelton, D. L.; Parada, L. F.; Presta, L. G. *EMBO* **1995**, *14*, 2795.
(32) Perez, P.; Coll, P. M.; Hempstead, B. L.; Martin-Zanca, D.; Chao, M. V. *Mol. Cell. Neurosci.* **1995**, *6*, 97.
(33) Urfer, R.; Tsoulfas, P.; O'Connell, L.; Hongo, J.-A.; Zhao, W.; Presta, L. G. *J. Biol. Chem.* **1998**, *273*, 5829.

SCHEME 3. Strategies To Increase the Water Solubilities of the Peptidomimetics

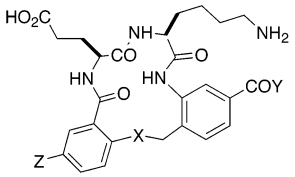


Reactions To Add Pharmacophores and Increase Water Solubilities. The data presented above indicates that compounds in this series can be active in screens for which no small molecule leads have previously been reported. However, the testing also revealed that the water solubilities of the peptidomimetics are generally borderline and in some cases too low to allow for accurate assays of activity. In any case, nitroaryl compounds are not normally regarded as ideal for pharmaceutical development. For these reasons, ways to convert the nitro group to other, more hydrophilic functionalities were examined.

The nitro functionalities of compounds **1**−**3** were reduced to the corresponding arylamines ($SnCl_2 \cdot 2H_2O$, DMF) on the resin. The resulting supported amines were cleaved from the resin to give **12a**−**14a** (Scheme 3) or derivatized to introduce other potential pharmacophores. For instance, reactions with guanidinylating agents (O and N, *N*,*N*′-bis-BOC-1-guanylpyrazole {BOCNHC(pyrazole)=NBOC}; S, *N*,*N*′-bis-BOC-thiourea {BOCNHC(S)-NHBOC} and *N*-methyl-2-chloropyridinium chloride)[34] gave the guanidines **12b**−**14b**, and reaction with methanesulfonyl chloride gave the corresponding sulfonamides **12c**−**14c**. All of these reactions were attempted for three dipeptide sequences in the starting materials **1**−**3**, and products were formed with very high purities except in two cases (Table 5). For the guanidine **13b** two uncharacterized byproducts formed in significant amounts. Guanylation of **14a** proceeded cleanly even though there are two N atoms in the substrate that could potentially react. The corresponding sulfonylation reaction of **14a** was not so straightforward, however, and we were unable to obtain high crude purities for the desired product.

(34) Yong, Y. F.; Kowalski, J. A.; Lipton, M. A. *J. Org. Chem.* **1997**, *62*, 1540.

TABLE 5. Yield and Purity Data for Compounds 12−14[a]



12 X = O, Y = NH_2
13 X = S, Y = OH
14 X = NH, Y = NH_2

compd	X/Y	Z	purity[b] (%)	yield (%)
12a	O/NH_2	NH_2	93/98	56
12b	O/NH_2	NHC(=NH)NH_2	95/100	60
12c	O/NH_2	$NHSO_2Me$	88/96	79
13a	S/OH	NH_2	74/100	64
13b	S/OH	NHC(=NH)NH_2	50/66	60
13c	S/OH	$NHSO_2Me$	95/100	49
14a	NH/NH_2	NH_2	90/93	55
14b	NH/NH_2	NHC(=NH)NH_2	85/88	88
14c	NH/NH_2	$NHSO_2Me$	43/46	*c*

[a] The resin was Wang (1.1 mmol/g) for **1a**−**k**, Rink amide MBHA (0.55 mmol/g) for **2a**−**k**, Rink (0.6 mmol/g) for **3a**−**h**, and Rapp-HG200RAM (0.62 mmol/g) for **3i**−**o**. [b] Purity as assessed by HPLC of crude product, monitored UV absorption at 254 nm, and using an evaporative light scattering detector (Sedex). [b] Yield not determined because of low purity.

Qualitatively, it was observed that the solubilities of compounds **1**−**3** vary according to the R^1 and R^2 side chains. Where these are lypophilic the compounds tend to be insoluble or sparingly soluble in water, and this is unacceptable for bioassays. However, derivatization of these materials as described above gives amine, guanidine, or sulfonamide derivatives that are appreciably more water-soluble.

Conformational analyses of compounds **12a**−**c** were performed to probe the effects of modification of the nitro functionality of **1**−**3**. The calculated low energy conformers for **12a**−**c** are shown in Figure 1d−f, and full data are given in Supporting Information. Salient details from these QMD studies and NMR analyses of **12a**−**c** reveals that their conformations are similar to the parent ether **2** (both in DMSO). The CD spectra of the derivatized compounds (Figure 1b) are similar to the ether **2** in the 190−210 nm region; they only differ in the part of spectra above 210 nm wherein ellipticities due to the aromatic rings prevail. These observations imply that substitution of the nitro group with amine, guanidine, or methanesulfonamide functionalities does not significantly perturb the preferred conformations of these molecules. This implies that structure−activity relationships (SARs) within this series of molecules could be studied without complications arising from conformational differences.Proper evaluation of **12**−**14** as neurotrophin analogues is in progress, but preliminary results are given. Table

TABLE 6. Dose−Response Studies for Compound 13a

		% maximal survival (relative to 2 nM NTF)[a]			
		NIH-TrkA cells in SFM		NIH-TrkC cells in SFM	
	μM **13a**	untreated	+ 0.1 nM NGF	untreated	+ 0.1 nM NT-3
1	0	0 ± 0	**25 ± 1**	0 ± 0	**25 ± 2**
2	0.4	1 ± 2	25 ± 2	**9 ± 2**	**32 ± 2***
3	2	0 ± 1	26 ± 1	**10 ± 1**	**35 ± 3***
4	10	−1 ± 1	25 ± 1	**9 ± 1**	**32 ± 1***

[a] Trophic survival assays of NIH-TrkA and NIH-TrkC in the absence (SFM) or presence of limiting concentrations of the corresponding neurotrophin (100 pM NT-3 for NIH-TrkC and 100 pM NGF for NIH-TrkA) that afford ~25% cell survival. Data are standardized to maximal trophic survival afforded by optimal (2 nM) neurotrophins = 100%. Averaged ± standard deviation, n = 6. Bold indicates survival above no compound, untreated cells. Asterisks indicate survival above suboptimal neurotrophin control.

4 shows that peptidomimetic **12b** may have some binding to TrkA receptors in FACS scans, because it inhibits 5C3·TrkA interactions. Figure 2 shows that compound **12b** had no activity in biological assays; but compound **13a** is a partial agonist of TrkC, i.e., at 50 μM it enhances the trophic activity of NT-3 in cell survival assays from 25% to ~50% protection. Survival assays of dose−response curves for compound **13a** showed that at 0.4 μM it has some intrinsic trophic activity for NIH-TrkC cells in the absence of NT-3 (Table 6), and also continues to potentiate suboptimal concentrations of NT-3. At concentrations lower than 50 μM, compound **13a** had no antagonism of TrkA·NGF function.

Conclusions

Compounds **1**−**3** represent a potentially useful peptidomimetic design. They can be formed in higher purities than the first generation compounds **A** via efficient and straightforward solid-phase syntheses. Moreover, compared with the routes to the first generation mimics **A**, head-to-tail dimers are only a minor impurity (if they are seen at all), underscoring the assertion that the desired ring-closure reaction is more facile as a result of the rigidity of the acyclic precursor to the second generation systems. Being intrinsically more rigid than structures **A**, these second generation turn mimics provide a means to introduce small structural changes to alter the preferred conformations of these molecules in solution. The preferred conformations of **1c** and **2c** are not ideal turns, but there is also no guarantee that the ideal conformation for bioactivity will be a perfect turn either.

Overall, it is evident from these studies that incremental structural changes can have pronounced effects on conformation. The structural change from **A** to **1**, **2**, or **3** is much smaller than the steps usually taken on the pathway from peptidomimetic to constrained peptidomimetic to small molecule.

Numerous biological assays are being performed on compounds **1**−**3**. These data and complete correlations between structures, conformations, and activities will be reported when those biological investigations are complete. Nevertheless, our preliminary data indicate receptor selectivity and biological activity at TrkA and TrkC for some of the compounds.

Several other characteristics of compounds **1**−**3** are potentially beneficial with respect to the objectives we have outlined. Their bioavailabilities[35−38] may be increased since they have one less natural amino acid than compounds **A** (making them more resistant to proteolytic degradation). Moreover, the extra aromatic ring in molecules **1**−**3** is likely to make them more easily crystallized for X-ray diffraction studies and helps assignment of chemical shifts due to anisotropy effects.

Finally, we outline a conclusion that may be pertinent to many studies aimed at design of small molecules to mimic or disrupt protein−protein interactions. In this particular area, structure, conformation, activity relationships (SCARs) may be more appropriate than simpler correlations between structure and activity (SARs).

Experimental Section

NMR Conformation Studies. NMR spectra were recorded on a Varian UnityPlus 500 spectrometer (500 MHz). The concentrations of the samples were approximately 5 mM in DMSO-d_6 throughout. One-dimensional (1D) ^{1}H NMR spectra were recorded with a spectral width of 8000 Hz, 16 transients, and a 3-s acquisition time. Vicinal coupling constants were measured from 1D spectra at 25 °C. Assignments of ^{1}H NMR resonances in DMSO were performed using sequential connectivities. Temperature coefficients of the amide protons were measured via several 1D experiments in the temperature range 20−50 °C adjusted in 5 °C increments with an equilibration time of more than 10 min after successive temperature steps. Coupling constants are given in hertz.

Two-dimensional (2D) NMR spectra were recorded at 25 °C with a spectral width of 8000 Hz. Through-bond connectivities were elucidated by COSY and DQF-COSY spectra, which were recorded with 512 t_1 increments and 16 scans per t_1 increment, with 2 K data points at t_2. Through-space interactions were identified by ROESY spectra. ROESY experiments were performed using mixing times of 100, 200, 300, and 400 ms. The intensities of the ROESY cross-peaks were assigned as S (strong), M (medium), and W (weak) from the magnitude of their volume integrals.

CD Studies. CD measurements were obtained on an Aviv (model 62 DS) spectrometer. For these experiments the cyclic peptidomimetics were dissolved in H_2O/MeOH (80:20 v/v) (c = 0.1 mg/mL, 0.1-cm path length). The CD spectra were recorded at 25 °C.

Molecular Simulations. CHARMm (version 23.2, rev. 96.0501) was used for the molecular simulations performed in this work. Explicit atom representations were used throughout the study. The residue topology files (RTF) for all the peptidomimetics were built using Quanta97 (version 97.0711, Molecular Simulations Inc.). Quenched molecular dynamics simulations for the compounds (compounds **1**−**3**) were performed using the CHARMm standard parameters. All molecules were modeled in a dielectric continuum of 45 (simulating DMSO). Thus, the starting conformer was minimized using 1000 steps of Steepest Descents (SD) and 3000 steps of the Adopted-Basis Newton Raphson method (ABNR), respectively, until an RMS energy derivative of ≤0.01 kcal mol^{-1}A^{-1} was obtained. The minimized structure was then subjected to heating, equilibration, and dynamics simulation. Throughout, the equations of motion were integrated using the Verlet algorithm with a time step 1 fs, and SHAKE was used to constrain all bond lengths containing polar hydrogens. Each peptidomimetic was heated to 1000 K over 10 ps and equili-

(35) Navia, M. A.; Chaturvedi, P. R. *Drug Discovery Today* **1996**, *1*, 179.
(36) Chan, O. H.; Stewart, B. H. *Drug Discovery Today* **1996**, *1*, 461.
(37) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
(38) Walters, W. P.; Ajay, Murcko, M. A. *Curr. Opin. Chem. Biol.* **1999**, *3*, 384.

brated for another 10 ps at 1000 K, and then molecular dynamics runs were performed for a total time of 600 ps with trajectories saved every 1 ps. The resulting 600 structures were thoroughly minimized again, using 1000 steps of SD followed by 3000 steps of ABNR. After a suitable cutoff (5.0 kcal/mol for compound **1**, 7.9 kcal/mol for compound **2**, 3.5 kcal/mol for compound **3**), the lowest energy structures were selected for further analysis.

The Quanta97 package was again used to display and to classify the selected structures into conformational groups. The best clustering was obtained using the grouping method based on calculation of RMS deviation for a subset of atoms; in this study these were the ring backbone atoms. Thus, appropriate threshold values (0.85 for compound **1**, 0.68 for compound **2**, 0.82 for compound **3**) were selected to obtain families with reasonable homogeneity. The lowest energy structure from each family was considered as a favored conformer and thus selected as a representative of the family as a whole. Interproton distances and dihedral angles from the lowest energy structures were calculated for comparisons with the ROE and NMR data.

4-(Hydroxymethyl)-3-nitrobenzoic Acid 7. A mixture of 4-(bromomethyl)-3-nitrobenzoic acid (3.0 g, 10.95 mmol) and sodium carbonate (4.6 g, 43.8 mmol) in 50 mL of 1:1 acetone/water was heated under reflux for 16 h. The mixture was cooled to room temperature, acidified to pH 2 with 1 M HCl, extracted with ethyl acetate (50 mL × 3), dried over $MgSO_4$, and concentrated in vacuo to yield 2.0 g (93%) of brown solid **7**. The ^{1}H NMR of the crude material in DMSO-d_6 is identical to that reported.[39]

Trityl Ether 8. 4-(Hydroxymethyl)-3-nitrobenzoic acid **7** (2.0 g, 10.14 mmol), triphenylmethyl chloride (3.4 g, 12.17 mmol), silver nitrate (2.1 g, 12.17 mmol), *N*,*N*-dimethylformamide (30 mL), and diisopropylethylamine (3.1 mL, 22.3 mmol) were added to a 100-mL round-bottom flask, and the mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure, and the crude residue was purified by flash chromatography on silica gel with an eluent of 30% ethyl acetate and 1% acetic acid in hexane to yield 3.79 g (85%) of yellow solid **8**: mp 217−220 °C; R_f 0.47 (1:2:0.1 ethyl acetate/hexane/AcOH); υ_{max} 3000 (b), 1709, 1542, 1345; ^{1}H NMR (300 MHz, acetone-d_6) δ 8.75 (d, J = 2, 1H), 8.42 (dd, J = 6 and 2, 1H), 8.33 (d, J = 6, 1H), 7.54−7.57 (m, 6H), 7.33−7.41 (m, 9H), 4.77 (s, 2H); ^{13}C NMR (75 MHz, acetone-d_6) δ 165.2, 144.0, 139.9, 134.4, 129.5, 128.8, 128.3, 127.8, 127.6, 127.0, 125.6, 91.6, 63.3; MS (MALDI+) calcd for $[C_{27}H_{21}NO_5Na]^+$ 462, found 462.

Amino Acid 4. A solution of **8** (1.87 g, 4.25 mmol) and PtO_2 (50 mg, 0.21 mmol) in 3:1 ethyl acetate/methanol (40 mL) was stirred vigorously under H_2 (1 atm) for 16 h. The reaction mixture was filtered through Celite and concentrated in vacuo to yield 1.60 g (92%) of yellow solid **4**: mp 180−182 °C; R_f 0.43 (30% ethyl acetate in hexane); υ_{max} 3476, 3360, 3000 (b), 1695, 1629; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55−7.59 (m, 6H), 7.30−7.45 (m, 12H), 4.17 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 145.7, 143.7, 129.1, 128.5, 128.0, 127.2, 119.7, 116.9, 87.3, 64.7; HRMS (FAB+) calcd for $[C_{27}H_{23}NO_3H]^+$ 410.17562, found 410.17464.

Trityl Thioether 5. To triphenylmethanethiol (6.1 g, 22 mmol) and potassium carbonate (5.5 g, 38 mmol) in a 100-mL round-bottom flask at 0 °C was added 4-(bromomethyl)-3-nitrobenzoic acid (5.2 g, 20 mmol) in *N*,*N*-dimethylformamide (40 mL), and the mixture was stirred at room temperature for 16 h. The reaction mixture was acidified to pH 2 using 1 M HCl and extracted with dichloromethane (50 mL × 3). The combined organic layers were dried over $MgSO_4$ and concentrated to yield a yellow oil. The crude residue was purified by flash chromatography on silica gel with an eluent of gradient 50% ethyl acetate in hexane to 1% acetic acid in ethyl acetate, to yield 8.7 g (96%) of yellow powder, **5**: mp 213−215 °C; R_f 0.67 (1% AcOH in ethyl acetate); υ_{max} 3000 (b), 1702, 1542, 1353; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (d, J = 2, 1H), 8.02 (dd, J = 8 and 2, 1H), 7.44−7.47 (m, 6H), 7.25−7.34 (m, 9H), 6.90 (d, J = 8, 1H), 3.83 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 144.3, 139.6, 133.9, 133.1, 129.9, 128.3, 128.0, 127.2, 126.8, 68.4, 34.1; HRMS (FAB+) calcd for $[C_{27}H_{21}NO_4SNa]^+$ 478.1089, found 478.10799.

Mtt-Protected Amino Acid 9. A solution of liquid ammonia (15 mL) in ethanol (15 mL) was added into a stirred solution of 4-(bromomethyl)-3-nitrobenzoic acid (5.0 g) in ethanol (10 mL) at −78 °C in a pressure bottle, and the mixture was stirred for 5 h at 25 °C. After allowing the NH_3 to evaporate, the remaining solution was concentrated in vacuo. The solid was suspended in a small amount of water and filtered. The product was dried in vacuo over P_2O_5 to give 4-(aminomethyl)-3-nitrobenzoic acid,[22] 3.11 g (82%) as an off-white solid. This material was used in the next step without further purification.

4-(Aminomethyl)-3-nitrobenzoic acid (4 g, 20.3 mmol) was added in one portion into a solution of 4-methyltriphenylmethyl chloride (12 g, 40 mmol) in $CHCl_3$/DMF (60 mL, 2:1) and vigorously stirred at 25 °C for 30 min. Then triethylamine (11 mL, 81 mmol) was added dropwise, and the mixture was stirred for 1 h. After addition of methanol (50 mL), the reaction mixture was stirred at 60 °C for 30 min and concentrated in vacuo. The residue was purified via flash chromatography with 25−50% EtOAc/hexane to give 8.72 g of compound **9** as pale yellow oil (95%): R_f 0.21 (60% EtOAc/hexane); υ_{max} 3367 (b), 3011 (b), 2916, 1709, 1622, 1535, 1396, 1353; ^{1}H NMR (300 MHz, acetone-d_6) δ 8.52 (d, J = 1.2 Hz, 1H), 8.39−8.41 (m, 2H), 7.58−7.62 (m, 4H), 7.48 (m, 2H), 7.32−7.37 (m, 4H), 7.21 (t, J = 1.2 Hz, 1H), 7.25 (m, 1H), 7.16 (dd, J = 20.1, 0.9 Hz, 2H), 3.73 (s, 2H), 2.32 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 164.7, 149.0, 146.1, 143.0, 141.6, 136.2, 134.0, 131.4, 128.8, 128.7, 128.1, 125.4, 71.1, 45.4, 20.3; MS (FAB+) calcd for $[C_{28}H_{24}N_2O_4Na]^+$ 475, found 475.

Amino Acid 6. To a solution of 4 g (8.85 mmol) of **9** in EtOAc (100 mL) was added PtO_2 (0.4 g, 0.885 mmol). The mixture was stirred vigorously under H_2 for 5 h. The reaction mixture was filtered through Celite and concentrated to dryness. The residue was purified via flash chromatography with 25−50% EtOAc/hexane to give 3.37 g (90%) of the product **6** as pale yellow oil: R_f 0.39 (60% EtOAc/hexane); υ_{max} 3433 (b), 3316 (b), 3018 (b), 2916, 1709, 1215; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.60−7.63 (m, 1H), 7.47 (s, 1H), 7.50 (s, 1H), 7.43 (m, 1H), 7.35−7.38 (m, 5H), 7.33 (m, 2H), 7.25−7.27 (m, 2H), 7.22 (m, 1H), 7.19 (bs, 1H), 7.16 (m, 1H), 3.40 (s, 2H), 2.33 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 166.8, 146.5, 146.4, 142.5, 136.0, 130.2, 130.0, 128.9, 128.8, 128.7, 128.0, 126.6, 118.7, 116.2, 71.1, 45.1, 20.4; HRMS (FAB) calcd for $[C_{28}H_{26}N_2O_2H]^+$ 423.20725, found 423.20685.

Procedure for Coupling Compound 5 to Polystyrene-Wang Resin. Polystyrene resin functionalized with a 4-hydroxybenzyl linker (1.2 g, 1.1 mmol/g, 1.32 mmol), template **5** (842 mg, 1.85 mmol, tetrabutylammonium iodide (244 mg, 0.66 mmol), and *N*,*N*-diisopropylethylamine (805 μL, 4.62 μL) in 8 mL of 4:1 dichloromethane/*N*,*N*-dimethylformamide were gently stirred at 4 °C for 24 h. The resin was filtered, washed, and treated with 10 equiv of $SnCl_2 \cdot 2H_2O$ in DMF for 1 day. After washing and drying, the loading of the resin was determined to be 0.5 mmol/g by UV analysis (410 nm) of the trityl content of a known amount of resin in 10% trifluoroacetic acid in dichloromethane. Subsequent couplings of amino acids were performed following the general procedure given for peptidomimetics **1**−**3** below.

Procedure for Coupling Compounds 4 and 6 to Polystyrene-Rink Resin. Rink resin (0.15 g, 0.6 mmol/g) was swelled in CH_2Cl_2 (10 mL/g) in a fritted syringe for 30 min. The FMOC group on the resin was removed by treating the resin with 20% piperidine in DMF (2 × 1.5 mL, 10 min and then 15 min). The resin was washed, after which templates **4**

(39) Nicolas, E.; Clemente, J.; Ferrer, T.; Albericio, F.; Giralt, E. *Tetrahedron* **1997**, *53*, 3179.

or **6** (3 equiv), HBTU (3 equiv), HOBt (3 equiv), and DIEA (5 equiv) in DMF (1.5 mL) were added. After 2 h of gentle shaking, a ninhydrin test on a small sample of beads gave a negative result. The reaction mixture was drained, and the resin was subjected to the above washing cycle.

General Experimental Procedure for Preparation of the Macrocyclic Peptidomimetics 1−3. Compound 3c. The resin containing template **6** was treated with FMOC-Lys-(BOC)-OH (4 equiv), PyBrop (4.8 equiv), and 2,6-lutidine (15 equiv) in CH_2Cl_2 (1.5 mL) for 20 h. After washing and Fmoc deprotection, the resin was then treated with FMOC-Glu(O^t-Bu)-OH (3 equiv), DIC (3 equiv), HOBt (3 equiv), and DIEA (5 equiv) in CH_2Cl_2/DMF (4:1, 1.5 mL) for 2 h. The washing cycle and Fmoc deprotection were repeated, and 2-fluoro-5-nitrobenzoic acid moiety was introduced by treating the resin with 2-fluoro-5-nitrobenzoyl chloride (3 equiv) and DIEA (3 equiv) in CH_2Cl_2 (1.5 mL) for 40 min. The side chain protecting group (Mtt) of the template was removed by treatment with 1% TFA and 5% TIS in CH_2Cl_2 (7 × 2 min, or until color disappeared). After the resin was washed and the macrocyclization step was carried out by treating the supported peptide with K_2CO_3 (10 equiv) in DMF at 25 °C. After gentle shaking for 2 d, the peptide-resin was washed then dried in vacuo for 4 h. The peptide was cleaved from the resin by treatment with a mixture of 90% TFA, 5% TIS, and 5% H_2O for 2 h. The cleavage solution was separated from the resin by filtration. After most of the cleavage cocktail (about 90%) was evaporated in vacuo, the crude peptide was triturated using anhydrous ethyl ether, dissolved in H_2O, and then lyophilized to give the crude product. Preparative HPLC (Beckman System, 5−90% B in 40 min) was carried out to provide a yellowish powder of **3c** (18 mg, 46%) obtained as an ammonium salt (in general, all the products were in protonated form.). **Data for 3c:** ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.1 (bs, 1H), 9.62 (s, 1H), 8.51 (d, J = 7.0, 1H), 8.26 (m, 2H), 8.05 (m, 2H), 7.94 (s, 1H), 7.66 (m, 5H), 7.57 (d, J = 7.0, 1H), 7.37 (s, 1H), 6.77 (d, J = 9.5, 1H), 4.92 (dd, J = 7.5, 15.7, 1H), 4.45 (dd, J = 4.5, 16, 1H), 4.31(m, 1H), 4.11 (m, 1H), 2.81 (bm, 2H), 1.96 (m, 1H), 1.88 (m, 1H), 1.78 (m, 1H), 1.63 (m, 4H), 1.33(m, 2 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.6, 173.3, 169.8, 168.0, 151.8, 137.0, 135.9, 134.5, 132.5, 131.9, 128.1, 125.2, 124.5, 124.0, 119.1, 111.5, 60.1, 57.5, 54.6, 45.2, 31.8, 31.1, 28.5, 27.7, 25.8, 23.2. Analytical HPLC: homogeneous single peak, t_R = 13.1 min (8−70% B in 30 min); MALDI MS calcd for $C_{26}H_{31}N_7O_8$ (M + H$^+$) 570.23, found 570.12.

Data for 1a: 24 mg, 80%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.65 (d, J = 7.0, 1H), 8.62 (d, J = 9.0, 1H), 8.39 (s, J = 1.5, 1H), 8.33 (dd, J = 8.5, 2.5, 1H), 8.25 (d, J = 2.5, 1H), 7.99 (s, 1H), 7.72 (bs, 3H), 7.68 (d, J = 9.0, 1H), 7.60 (d, J = 9.0, 1.5, 1H), 7.40 (s, 1H), 5.64 (d, J = 12.5, 1H), 5.32 (d, J = 12.5, 1H), 4.39 (m, 1H), 4.20 (m, 1H), 2.75 (m, 2H), 1.90 (m, 2H), 1.73 (m, 1H), 1.55 (m, 3H), 1.38 (m, 2H), 1.22 (m, 1H), 0.82−0.94 (m, 6H), Analytical HPLC: homogeneous single peak, t_R = 14.50 min (8−70% B in 30 min); MALDI MS calcd for $C_{27}H_{34}N_6O_7$ (M + H$^+$) 555.26, found 555.26.

Data for 1b: 18 mg, 66%; analytical HPLC: homogeneous single peak, t_R = 10.08 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{26}N_6O_7$ (M + H$^+$) 499.19, found 499.23.

Data for 1c: 46 mg, 43%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.2 (bs, 1H), 8.73 (d, J = 7.0, 1H), 8.70 (s, 1H), 8.53 (d, J = 8.5, 1H), 8.41 (dd, J = 3.0, 9.0, 1H), 8.37 (d, J = 3.0, 1H), 8.33 (d, J = 3.0, 1H), 8.00 (s, 1H), 7.68 (d, J = 9.0, 1H), 7.66 (s, 1H), 7.66 (bs, 3H), 7.64 (d, J = 3.0, 1H), 7.40 (s, 1H), 5.67 (d, J = 12.0, 1H), 5.23 (d, J = 12.0, 1H), 4.48 (m, 1H), 4.28 (m, 1H), 2.77 (m, 2H), 2.48 (m, 2H), 2.00 (m, 2H), 1.93 (m, 1H), 1.70 (m, 1H), 1.55 (m, 2H), 1.35 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.6, 173.0, 171.2, 168.1, 165.8, 160.8, 141.1, 138.0, 136.2, 131.3, 129.7, 127.8, 127.3, 124.7, 124.0, 123.7, 114.4, 69.48, 56.0, 53.2, 31.2, 30.9, 26.9, 26.6, 23.1. Analytical HPLC: homogeneous single peak, t_R = 15.00 min (8−70% B in 30 min); MALDI MS calcd for $C_{26}H_{30}N_6O_9$ (M + H$^+$) 571.22, found 571.17.

Data for 1d: 17 mg, 53%. Analytical HPLC: homogeneous single peak, t_R = 15.00 min (8−70% B in 30 min); MALDI MS calcd for $C_{27}H_{34}N_8O_7$ (M + H$^+$) 583.26, found 583.27.

Data for 1e: 14 mg, 54%. Analytical HPLC: homogeneous single peak, t_R = 10.63 min (8−70% B in 30 min); MALDI MS calcd for $C_{21}H_{21}N_5O_8$ (M + H$^+$) 494.13, found 494.11.

Data for 1f: 20 mg, 67%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.82 (d, J = 8.0, 1H), 8.75 (s, 1H), 8.33 (dd, J = 9.0, 3.0, 1H), 8.24 (d, J = 8.5, 1H), 8.23 (s, J = 1.5, 1H), 8.22 (d, J = 3.0, 1H), 8.00 (s, 1H), 7.69 (d, J = 9.0, 1H), 7.64 (d, J = 9.0, 1H), 7.63 (dd, J = 9.0, 1.5, 1H), 7.46 (s, 1H), 7.41 (s, 1H), 6.98 (s, 1H), 5.63 (d, J = 12.0, 1H), 5.30 (d, J = 12.0, 1H), 4.79 (m, 1H), 4.13 (m, 1H), 2.69 (dd, J = 16.0, 6.5, 1H), 2.56 (dd, J = 16.0, 6.5, 1H), 1.80 (m, 1H), 1.52 (m, 1H), 1.18 (m, 1H), 0.85−0.90 (m, 6H). Analytical HPLC: homogeneous single peak, t_R = 14.95 min (8−70% B in 30 min); MALDI MS calcd for $C_{25}H_{28}N_6O_8$ (M + H$^+$) 541.20, found 541.19

Data for 1g: 18 mg, 60%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05 (d, J = 8.0, 1H), 8.75 (s, 1H), 8.38 (dd, J = 9.0, 2.5, 1H), 8.34 (d, J = 8.5, 1H), 8.22 (s, 1H), 8.14 (d, J = 3.0, 1H), 8.01 (s, 1H), 7.66 (d, J = 9.0, 1H), 7.59−7.65 (m, 2H), 7.46 (s, 1H), 7.41−7.44 (b, 3H), 7.01 (s, 1H), 6.95 (s, 1H), 5.62 (d, J = 12.0, 1H), 5.21 (d, J = 12.0, 1H), 4.70 (m, 1H), 4.59 (m, 1H), 2.53−2.75 (m, 4H). Analytical HPLC: homogeneous single peak, t_R = 8.92 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{23}N_7O_9$ (M + Na$^+$) 564.15, found 564.19.

Data for 1h: 3.1 mg, 71%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.96 (d, J = 7.0, 1H), 8.60 (d, J = 9.0, 1H), 8.60 (s, 1H), 8.33 (dd, J = 9.0, 3.0, 1H), 8.32 (s, J = 2.0, 1H), 8.22 (d, J = 3.0, 1H), 8.00 (s, 1H), 7.70 (d, J = 8.0, 1H), 7.69 (bs, 3H), 7.62 (m, 2H), 7.41 (s, 1H), 5.53 (d, J = 12.0, 1H), 5.30 (d, J = 12.0, 1H), 4.33 (m, 1H), 3.93 (m, 1H), 3.71 (m, 1H), 2.81 (m, 2H), 1.72 (m, 2H), 1.55 (m, 2H), 1.38 (m, 2H). Analytical HPLC: homogeneous single peak, t_R = 9.15 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{26}N_6O_7$ (M + H$^+$) 499.19, found 499.14.

Data for 1i: 3.1 mg, 74%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.10 (b, 1H), 8.79 (b, 1H), 8.64 (s, 1H), 8.35 (dd, J = 9.0, 3.0, 1H), 8.33 (s, 1H), 8.22 (d, J = 3.0, 1H), 7.99 (s, 1H), 7.95 (bs, 1H), 7.70 (d, J = 8.0, 1H), 7.62 (m, 2H), 7.41 (s, 1H), 7.00−7.40 (m, 3H), 5.53 (d, J = 12.0, 1H), 5.32 (d, J = 12.0, 1H), 4.38 (m, 1H), 3.84 (m, 2H), 3.13 (m, 2H), 1.60−1.76 (m, 3H). Analytical HPLC: homogeneous single peak, t_R = 9.89 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{26}N_8O_7$ (M + H$^+$) 527.20, found 527.16.

Data for 1j: 3.1 mg, 69%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.98 (d, J = 7.5, 1H), 8.63 (s, 1H), 8.43 (dd, J = 3, 9, 1H), 8.40 (d, J = 3.0, 1H), 8.31 (d, J = 8.5, 1H), 8.28 (d, J = 3.0, 1H), 8.02 (s, 1H), 7.59−7.71 (m, 6H), 7.43 (s, 1H), 5.67 (d, J = 12.0, 1H), 5.16 (d, J = 12.0, 1H), 4.47 (m, 1H), 4.28 (m, 2H), 2.80 (m, 2H), 1.77 (m, 2H), 1.56 (m, 2H), 1.45 (m, 2H), 1.06 (m, 3H). Analytical HPLC: homogeneous single peak, t_R = 9.36 min (8−70% B in 30 min); MALDI MS calcd for $C_{25}H_{30}N_6O_8$ (M + H$^+$) 543.22, found 543.16.

Data for 1k: 26 mg, 71%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.2 (bs, 1H), 8.99 (d, J = 7.0, 1H), 8.75 (s, 1H), 8.38 (m, 2H), 8.30 (d, J = 3.0, 1H), 8.21 (s, 1H), 8.01 (s, 1H), 7.59−7.68 (m, 3H), 7.46 (s, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 5.64 (d, J = 12.0, 1H), 5.17 (d, J = 12.0, 1H), 4.74 (m, 1H), 4.23 (m, 1H), 2.67 (dd, J = 16.0, 6.5, 1H), 2.55 (dd, J = 16.0, 6.5, 1H), 2.40 (m, 2H), 1.89 (m, 2H). Analytical HPLC: homogeneous single peak, t_R = 10.67 min (8−70% B in 30 min); MALDI MS calcd for $C_{24}H_{24}N_6O_{10}$ (M + H$^+$) 557.16, found 557.09.

Data for 2a: 5.0 mg, 47%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (bs, 1H), 9.13 (s, 1H), 8.92 (d, J = 10.0, 1H), 8.52 (d, J = 8.5, 1H), 8.50 (d, J = 2.5, 1H), 8.26 (dd, J = 2.5, 8.5, 1H), 8.10 (d, J = 2.5, 1H), 8.07 (d, J = 8.5, 1H), 7.68 (m, 5H), 4.70 (d, J = 11.5, 1H), 4.48 (d, J = 11.5, 1H), 4.43 (m, 1H), 4.38 (m, 1H), 2.75 (m, 2H), 1.85 (m, 2H), 1.65 (m, 1H), 1.53 (m, 3H), 1.33 (m, 3H), 0.84−0.93 (m, 6H). Analytical HPLC: homogeneous single peak, t_R = 17.40 min (8−70% B in 30 min); MALDI MS calcd for $C_{27}H_{33}N_5O_7S$ (M + H$^+$) 572.22, found 572.24.

Data for 2b: 5.5 mg, 56%. Analytical HPLC: homogeneous single peak, t_R = 14.11 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{25}N_5O_7S$ (M + H^+) 516.16, found 516.16.

Data for 2c: 43 mg, 57%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.6 (bs, 2H), 9.10 (s, 1H), 8.86 (d, J = 8.0, 1H), 8.54 (d, J = 1.5, 1H), 8.42 (d, J = 9.0, 1H), 8.33 (dd, J = 2.5, 8.5, 1H), 8.31 (d, J = 2.5, 1H), 8.13 (d, J = 8.5, 1H), 7.71 (dd, J = 1.5, 8.0, 1H), 7.68 (m, 3H), 7.67 (d, J = 8.0, 1H), 4.77 (d, J = 10.5, 1H), 4.53 (m, 1H), 4.46 (d, J = 10.5, 1H), 4.45 (m, 1H), 2.75 (m, 2H), 2.54 (m, 2H), 2.04 (m, 1H), 1.95 (m, 1H), 1.87 (m, 1H), 1.64 (m, 1H), 1.54 (m, 2H), 1.30 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.9, 172.0, 170.0, 166.8, 166.4, 145.3, 143.3, 139.6, 137.0, 132.0, 131.8, 131.0, 130.9, 130.3, 125.5, 124.4, 123.8, 121.2, 54.2, 53.7, 36.2, 31.0, 30.6, 26.9, 26.4, 22.2. Analytical HPLC: homogeneous single peak, t_R = 14.83 min (8−70% B in 30 min); MALDI MS calcd for $C_{26}H_{29}N_5O_9S$ (M + H^+) 588.18, found 588.24.

Data for 2d: 5.0 mg, 50%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (bs, 1H), 9.18 (s, 1H), 8.84 (d, J = 9.5, 1H), 8.45 (d, J = 8.5, 1H), 8.46 (s, 1H), 8.25 (dd, J = 2.5, 9.0, 1H), 8.09 (d, J = 2.5, 1H), 8.07 (d, J = 8.5, 1H), 7.68 (m, 5H), 7.57 (bs, 1H), 6.90−7.30 (b, 3H), 4.65 (d, J = 11.5, 1H), 4.50 (d, J = 11.5, 1H), 4.39 (m, 1H), 4.32 (m, 1H), 3.11 (m, 2H), 1.86 (m, 2H), 1.65 (m, 1H), 1.52 (m, 3H), 1.22 (m, 1H), 0.81−0.94 (m, 6H). Analytical HPLC: homogeneous single peak, t_R = 18.10 min (8−70% B in 30 min); MALDI MS calcd for $C_{27}H_{33}N_7O_7S$ (M + H^+) 600.22, found 600.17.

Data for 2e: 5.5 mg, 55%. Analytical HPLC: homogeneous single peak, t_R = 14.84 min (8−70% B in 30 min); MALDI MS calcd for $C_{21}H_{20}N_4O_8S$ (M + H^+) 511.09, found 511.09.

Data for 2f: 5.0 mg, 52%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (bs, 1H), 9.12 (s, 1H), 8.81 (d, J = 9.0, 1H), 8.39 (s, 1H), 8.50 (d, J = 2.5, 1H), 8.26 (dd, J = 2.5, 9.0, 1H), 8.21 (d, J = 8.5, 1H), 8.11 (d, J = 2.5, 1H), 8.09 (d, J = 9.0, 1H), 7.66 (s, 2H), 7.42 (s, 1H), 6.91 (s, 1H), 4.73 (m, 1H), 4.65 (d, J = 11.5, 1H), 4.43 (d, J = 11.5, 1H), 4.28 (m, 1H), 2.64 (dd, J = 15.5, 6.0, 1H), 2.55 (dd, J = 15.5, 6.0, 1H), 1.82 (m, 1H), 1.51 (m, 1H), 1.22 (m, 1H), 0.76−0.90 (m, 6H). Analytical HPLC: homogeneous single peak, t_R = 17.82 min (8−70% B in 30 min); MALDI MS calcd for $C_{25}H_{27}N_5O_8S$ (M + Na^+) 580.15, found 580.16.

Data for 2g: 5.5 mg, 67%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (bs, 1H), 9.15 (s, 1H), 8.90 (d, J = 8.5, 1H), 8.35 (s, 1H), 8.30 (d, J = 2.5, 1H), 8.25 (d, J = 8.4, 1H), 8.16 (s, 1H), 8.11 (d, J = 2.5, 1H), 7.67 (d, J = 8.0, 1H), 7.62 (d, J = 8.0, 1H), 7.46 (s, 1H), 7.39 (s, 1H), 7.00 (s, 1H), 6.89 (s, 1H), 4.70−4.80 (m, 2H), 4.70 (d, J = 11.0, 1H), 4.39 (d, J = 11.0, 1H), 2.53−2.69 (m, 4H). Analytical HPLC: homogeneous single peak, t_R = 13.00 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{22}N_6O_9S$ (M + Na^+) 581.11, found 581.11.

Data for 2h: 1.8 mg, 54%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (bs, 1H), 9.07 (d, J = 9.5, 1H), 8.95 (s, 1H), 8.74 (d, J = 8.5, 1H), 8.57 (d, J = 1.5, 1H), 8.25 (dd, J = 8.5, 2.5, 1H), 8.21 (d, J = 2.5, 1H), 8.05 (d, J = 8.5, 1H), 7.65 (m, 3H), 7.63 (dd, J = 8.5, 1.5, 1H), 7.59 (d, J = 8.5, 1H), 4.64 (d, J = 11.5, 1H), 4.47 (m, 1H), 4.43 (d, J = 11.5, 1H), 4.03 (dd, J = 15.5, 8.0, 1H), 3.74 (dd, J = 15.5, 6.0, 1H), 2.83 (m, 2H), 1.76 (m, 2H), 1.57 (m, 2H), 1.44 (m, 2H). Analytical HPLC: homogeneous single peak, t_R = 13.42 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{25}N_5O_7S$ (M + H^+) 516.16, found 516.16.

Data for 2i: 1.9 mg, 56%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (bs, 1H), 9.25 (b, 1H), 8.98 (s, 1H), 8.86 (b, 1H), 8.56 (s, 1H), 8.23 (d, J = 8.5, 1H), 8.18 (s, 1H), 8.06 (d, J = 8.5, 1H), 7.74 (b, 1H), 7.62 (d, J = 8.5, 1H), 7.59 (d, J = 8.5, 1H), 7.00−7.30 (b, 3H), 4.62 (d, J = 12.0, 1H), 4.48 (d, J = 12.0, 1H), 4.45 (m, 1H), 4.02 (dd, J = 15.5, 8.0, 1H), 3.74 (dd, J = 15.5, 6.0, 1H), 3.15 (m, 2H), 1.71 (m, 3H). Analytical HPLC: homogeneous single peak, t_R = 13.94 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{25}N_7O_7S$ (M + H^+) 544.16, found 544.17.

Data for 2j: 1.8 mg, 52%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (bs, 1H), 9.31 (d, J = 9.5, 1H), 8.78 (s, 1H), 8.66 (s, 1H), 8.31 (b, 1H), 8.28 (dd, J = 8.5, 2.5, 1H), 8.24 (d, J = 2.5, 1H), 8.06 (d, J = 8.5, 1H), 7.72 (bs, 3H), 7.65 (m, 2H), 4.86 (d, J = 11.0, 1H), 4.47 (m, 1H), 4.39 (m, 1H), 4.30 (m, 1H), 4.21 (d, J = 11.0, 1H), 2.83 (m, 2H), 1.78 (m, 2H), 1.59 (m, 2H), 1.51 (m, 2H), 1.09 (m, 3H). Analytical HPLC: homogeneous single peak, t_R = 13.54 min (8−70% B in 30 min); MALDI MS calcd for $C_{25}H_{29}N_5O_8S$ (M + H^+) 560.18, found 560.15.

Data for 2k: 1.8 mg, 55%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.6 (bs, 2H), 9.15 (s, 1H), 8.92 (d, J = 8.5, 1H), 8.38 (s, 1H), 8.29 (d, J = 9.0, 1H), 8.28 (s, 1H), 8.20 (d, J = 8.5, 1H), 8.10 (d, J = 9.0, 1H), 7.67 (dd, J = 1.5, 7.5, 1H), 7.62 (d, J = 7.5, 1H), 7.42 (s, 1H), 6.94 (m, 1H), 4.75 (m, 1H), 4.73 (d, J = 11.0, 1H), 4.40 (m, 1H), 4.38 (d, J = 11.0, 1H), 2.61 (d, J = 6.0, 2H), 2.40 (m, 2H), 1.89 (m, 2H). Analytical HPLC: homogeneous single peak, t_R = 14.28 min (8−70% B in 30 min); MALDI MS calcd for $C_{24}H_{23}N_5O_{10}S$ (M + Na^+) 596.11, found 596.07.

Data for 3a: 26 mg, 59%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.45 (d, J = 8.1, 1H), 8.23 (d, J = 1.5, 1H), 8.14 (d, J = 2.7, 1H), 8.05 (dd, J = 2.7, 9.3, 1H), 7.96 (bs, 1H), 7.90 (bt, 1H), 7.60−7.76 (m, 4H), 7.39 (m, 2H), 7.31 (bm, 1H), 6.77 (d, J = 9.6, 1H), 4.92 (m, 1H), 4.46 (m, 1H), 4.35 (m, 1H), 3.90 (m, 2H), 2.81 (m, 2H), 1.82 (m, 2H), 1.64 (m, 2H), 1.35 (m, 2H), 0.80−1.25 (m, 8H). Analytical HPLC: homogeneous single peak, t_R = 16.6 min (8−70% B in 30 min); MALDI MS calcd for $C_{27}H_{35}N_7O_6$ (M + Na^+) 576.25, found 576.17.

Data for 3b: 13 mg, 44%. Analytical HPLC: homogeneous single peak, t_R = 12.4 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{27}N_7O_6$ (M + H^+) 498.20, found 498.14.

Data for 3d: 23 mg, 61%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.1 (bs, 1H), 8.95 (s, 1H), 8.81 (d, J = 8.4, 1H), 8.74 (bt, 1H), 8.45−8.60 (m, 3H), 8.08 (m, 1H), 7.54−7.80 (m, 3H), 7.30 (d, J = 8.1, 1H), 4.83 (s, 2H), 4.50−4.65 (m, 1H), 4.20−4.35 (m, 3H), 1.17 (d, J = 6.3, 3H). Analytical HPLC: 86%, t_R = 11.2 min (8−70% B in 30 min); MALDI MS calcd for $C_{21}H_{22}N_6O_7$ (M + H^+) 471.16, found 471.10.

Data for 3e: 23 mg, 61%. Analytical HPLC: homogeneous single peak, t_R = 11.2 min (8−70% B in 30 min); MALDI MS calcd for $C_{21}H_{22}N_6O_7$ (M + Na^+) 495.15, found 495.06.

Data for 3f: 21 mg, 58%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.1 (bs, 1H), 9.52 (s, 1H), 8.80 (bs, 1H), 8.22 (b, 1H), 8.13 (m, 2H), 8.00 (dd, J = 2.7, 9.3, 1H), 7.94 (m, 1H), 7.80 (b, 1H), 7.65 (s, 2H), 7.36 (s, 2H), 7.18 (b, 3H), 6.76 (d, J = 9.6, 1H), 4.30−4.45 (m, 3H), 4.12 (m, 1H), 3.12 (b, 2H), 2.25 (m, 2H), 1.92 (m, 2H), 1.75 (m, 2H), 1.67 (m, 2H). Analytical HPLC: 71%, t_R = 12.4 min (8−70% B in 30 min); MALDI MS calcd for $C_{26}H_{31}N_9O_9$ (M + H^+) 598.24, found 598.18.

Data for 3g: 17 mg, 55%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.2 (bs, 1H), 9.62 (s, 1H), 8.52 (d, J = 7.2, 1H), 8.28 (m, 2H), 8.06 (m, 2H), 7.96 (s, 1H), 7.65 (m, H), 7.48 (d, J = 7.2, 1H), 7.38 (s, 1H), 6.78 (d, J = 9.6, 1H), 5.00 (m, 2H), 4.36−4.48 (m, 2H), 3.59 (m, 2H), 2.46 (m, 2H), 1.92 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.5, 173.5, 169.8, 169.2, 168.0, 151.8, 137.1. 135.9, 134.6, 132.4, 131.7, 128.1, 125.5, 124.5, 123.9, 119.1, 111.5, 60.9, 57.5, 56.5, 45.3, 31.1, 25.8. Analytical HPLC: homogeneous single peak, t_R = 12.9 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{24}N_6O_9$ (M + H^+) 529.16, found 529.05.

Data for 3h: 18 mg, 64%. Analytical HPLC: homogeneous single peak, t_R = 13.0 min (8−70% B in 30 min); MALDI MS calcd for $C_{24}H_{26}N_6O_9$ (M + H^+) 543.17, found 543.08.

Data for 3i: 24 mg, 80%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.43 (s, 1H), 8.57 (s, 1H), 8.19 (d, J = 2.7, 1H), 8.12 (d, J = 7.2, 1H), 8.00 (dd, J = 2.4, 9.0, 1H), 7.96 (s, 1H), 7.63 (bs, 2H), 7.44 (d, J = 4.8, 1H), 7.36 (bs, 1H), 6.52 (d, J = 9.3, 1H), 4.75 (m, 1H), 4.30−4.54 (m, 3H), 3.70−3.87 (m, 4H), 1.70−2.03 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.1, 168.5, 168.3, 166.8, 150.2, 137.8, 135.2, 130.5, 129.3, 127.3, 123.8, 123.7, 123.3, 120.1, 111.7, 63.5, 61.1, 59.1, 47.4, 47.2, 32.3, 22.8. Analytical HPLC: homogeneous single peak, t_R = 11.9 min (8−70% B in 30 min); MALDI MS calcd for $C_{28}H_{24}N_6O_7$ (M + H^+) 497.17, found 497.08.

Data for 3j: 21 mg, 65%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.18 (s, 1H), 8.88 (d, J = 7.8, 1H), 8.58 (d, J = 2.7, 1H), 8.22 (d, J = 2.7, 1H), 8.10−8.15 (m, 2H), 7.98 (m, 1H), 7.65−7.81 (m, 5H), 7.40−7.48 (m, 2H), 6.83 (d, J = 9.3, 1H), 4.78−4.90 (m, H), 4.43 (m, 1H), 4.30 (m, 2H), 4.15−4.22 (m, 2H), 2.84 (m, 2H), 1.78 (m, 2H), 1.61 (m, 4H), 1.10 (d, J = 6.3, 3H). Analytical HPLC: homogeneous single peak, t_R = 11.4 min (8−70% B in 30 min); MALDI MS calcd for $C_{25}H_{31}N_7O_7$ (M + H^+) 542.23, found 542.13.

Data for 3k: 21 mg, 71%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.30 (s, 1H), 8.60 (s, 1H), 8.30 (d, J = 7.8, 1H), 8.15 (d, J = 2.7, 1H), 7.96 (m, 2H), 7.64 (m, 2H), 7.37−7.45 (m, 3H), 6.89 (bs, 1H), 6.51 (d, J = 9.3, 1H), 4.80 (m, 2H), 4.56 (m, 1H), 4.18 (m, 1H), 3.80 (m, 2H), 1.90−2.20 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.5, 173.5, 169.8, 169.2, 168.0, 151.8, 137.1, 135.9, 134.6, 132.4, 131.7, 128.1, 125.5, 124.5, 123.9, 119.1, 111.5, 60.9, 57.5, 56.5, 45.3, 31.1, 25.8. Analytical HPLC: homogeneous single peak, t_R = 11.8 min (8−70% B in 30 min); MALDI MS calcd for $C_{25}H_{27}N_7O_7$ (M + Na^+) 560.19, found 560.07.

Data for 3l: 34 mg, 98%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.66 (s, 1H), 8.57 (d, J = 6.9, 1H), 8.24 (d, J = 1.2, 1H), 8.20 (d, J = 2.7, 1H), 8.06 (dd, J = 2.7, 9.3, 1H), 7.98 (bm, 1H), 7.60−7.80 (m, 5H), 7.28−7.50 (m, 4H), 6.77 (m, 2H), 4.90 (m, 1H), 4.30−4.50 (m, 2H), 4.08 (m, 1H), 2.83 (m, 2H), 2.11 (m, 2H), 1.91 (m, 2H), 1.76 (m, 2H), 1.40−1.65 (m, 4H). Analytical HPLC: homogeneous single peak, t_R = 11.3 min (8−70% B in 30 min); MALDI MS calcd for $C_{26}H_{32}N_8O_7$ (M + H^+) 569.25, found 569.15.

Data for 3m: 20 mg, 62%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.11 (s, 1H), 8.95 (d, J = 7.2, 1H), 8.59 (d, J = 2.7, 1H), 8.27 (d, J = 2.7, 1H), 8.10−8.15 (m, 2H), 7.97 (bs, 1H), 7.64−7.80 (m, 3H), 7.55 (d, J = 8.4, 1H), 7.42 (bs, 2H), 6.83 (d, J = 9.3, 1H), 5.05 (d, J = 4.8, 1H), 4.84 (m, 1H), 4.43 (m, 1H), 4.20 (m, 1H), 2.34 (m, 2H), 2.00 (m, 2H), 1.10 (d, J = 6.3, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.2, 174.1, 169.9, 169.5, 167.9, 153.6, 151.8, 137.2, 136.6, 134.8, 132.9, 131.6, 128.1, 124.4, 124.2, 112.0, 67.4, 60.0, 57.7, 55.6, 32.3, 26.6, 20.8. Analytical HPLC: 96%, t_R = 11.7 min (8−70% B in 30 min); MALDI MS calcd for $C_{24}H_{27}N_7O_9$ (M + Na^+) 564.18, found 564.07.

Data for 3n: 30 mg, 94%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.64 (s, 1H), 8.50 (d, J = 7.5, 1H), 8.23 (d, J = 1.2, 1H), 8.17 (d, J = 2.4 Hz, 1H), 8.06 (dd, J = 2.7, 9.3, 1H), 7.99 (t, J = 6.3, 1H), 7.95 (s, 1H), 7.66 (m, 5H), 7.36 (s, 1H). 7.30 (d, J = 7.2, 1H), 6.77 (d, J = 9.3, 1H), 4.93−5.02 (m, 2H), 4.34−4.47 (m, 2H), 4.05 (m, 1H), 3.57 (m, 2H), 2.81 (b, 2H), 1.30−1.80 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.8, 169.9, 169.4, 168.0, 151.7, 137.0, 135.9, 134.6, 132.5, 131.8, 128.2, 125.6, 124.4, 124.0, 119.2, 111.5, 61.0, 57.8, 56.5, 45.2, 29.9, 27.2, 23.4. Analytical HPLC: homogeneous single peak, t_R = 11.4 min (8−70% B in 30 min); MALDI MS calcd for $C_{24}H_{29}N_7O_7$ (M + H^+) 528.21, found 528.15.

Data for 3o: 27 mg, 88%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.42 (s, 1H), 8.55 (d, J = 7.0, 1H), 8.42 (dd, J = 1.5, 1H), 8.22 (d, J = 3, 1H), 8.06 (dd, J = 2.5, 9.5, 1H), 7.96 (t, J = 4.5, 1H), 7.92 (s, 1H), 7.66−7.69 (m, 2H), 7.61 (dd, J = 1.5, 8.0, 1H), 7.38 (s, 1H), 7.33 (s, 1H), 6.85 (s, 1H), 6.77 (d, J = 9.5, 1H), 4.93 (m, 1H), 4.45 (m, 1H), 4.31(m, 2H), 4.09 (m, 1H), 2.30 (m, 2H), 1.92 (m, 2H), 1.25 (d, J = 6.5, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.2, 173.1,170.5, 169.5, 168.0, 151.6, 137.4, 136.1, 134.7, 131.8, 131.6, 129.7, 129.0, 127.9, 126.9, 124.1, 124.0, 123.6, 120.0, 111.6, 57.5, 50.3, 45.4, 36.4, 32.2, 26.3, 17.7. Analytical HPLC: homogeneous single peak, t_R = 12.2 min (8−70% B in 30 min); MALDI MS calcd for $C_{28}H_{25}N_7O_7$ (M + H^+) 512.18, found 512.14.

Data for 3p: 16 mg, 51%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.59 (s, 1H), 8.53 (d, J = 1.8, 1H), 8.23 (d, J = 2.7, 1H), 8.19 (d, J = 7.8, 1H), 8.04 (dd, J = 2.7, 9.3, 1H), 7.93 (bs, 2H), 7), 7.81 (d, J = 7.5, 1H), 7.68 (d, J = 7.8, 1H), 7.60 (dd, J = 1.8, 7.8, 1H), 7.35 (bs, 1H), 6.76 (d, J = 9.6, 1H), 5.23 (d, J = 4.5, 1H), 4.95−5.04 (m, 1H), 4.06−4.73 (m, 1H), 4.43−4.52 (m, 2H), 3.88−4.00 (m, 3H), 1.10 (d, J = 6.3, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.7, 171.8, 169.6, 168.9, 168.1, 151.4, 137.6, 135.9, 134.7, 131.8, 130.8, 127.7, 123.7, 123.1, 120.7, 111.3, 65.7, 64.6, 51.4, 45.2, 35.8, 21.1. Analytical HPLC: homogeneous single peak, t_R = 13.2 min (8−70% B in 30 min); MALDI MS calcd for $C_{23}H_{24}N_6O_9$ (M + H^+) 529.16, found 529.06.

General Procedure for Preparation of Derivatized Macrocyclic Peptidomimetics 12a−14a. Resin containing side chain protected **1c** or **2c** or **3c** was swelled in CH_2Cl_2 in a fritted syringe for 30 min and treated with 10 equiv of $SnCl_2 \cdot 2H_2O$ in DMF for 1 day. After washing, the peptide was cleaved from the resin by treatment with a mixture of 90% TFA, 5% TIS, and 5% H_2O for 2 h. The cleavage solution was separated from the resin by filtration. After most of the cleavage cocktail (about 90%) was evaporated in vacuo, the crude peptide was triturated using anhydrous ethyl ether, dissolved in H_2O, and then lyophilized to give the crude product.

Data for 12a: 9.5 mg, 41%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.2 (bs, 1H), 8.79 (s, 1H), 8.47 (d, J = 8.5, 1H), 8.44 (d, J = 8.0, 1H), 8.37 (s, 1H), 7.95 (s, 1H), 7.69 (bs, 3H), 7.61 (s, 2H), 7.32 (s, 1H), 7.31 (d, J = 8.5, 1H), 7.06 (s, 1H), 7.04 (d, J = 8.5, 1H), 5.36 (d, J = 12.0, 1H), 5.07 (d, J = 12.0, 1H), 4.40 (m, 1H), 4.29 (m, 1H), 2.76 (m, 2H), 2.36 (m, 2H), 1.96 (m, 2H), 1.91 (m, 1H), 1.67 (m, 1H), 1.52 (m, 2H), 1.31 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.4, 173.0, 171.0, 168.2, 166.9, 159.5, 159.1, 151.6, 137.7, 135.8, 131.1, 130.3, 127.7, 123.7, 123.1, 120.5, 116.0, 69.3, 55.7, 53.3, 30.9, 26.8, 26.4, 23.1. Analytical HPLC: homogeneous single peak, t_R = 8.20 min (2−40% B in 30 min); MALDI MS calcd for $C_{26}H_{32}N_6O_7$ (M + H^+) 541.24, found 541.26.

Data for 13a: 12.5 mg, 55%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (bs, 2H), 9.01 (s, 1H), 8.50 (d, J = 1.5, 1H), 8.49 (d, J = 8.0, 1H), 8.23 (d, J = 8.5, 1H), 7.66 (dd, J = 1.5, 8.0, 1H), 7.66 (m, 3H), 7.54 (d, J = 8.0, 1H), 7.43 (d, J = 8.5, 1H), 6.63 (dd, J = 2.5, 8.5, 1H), 6.60 (d, J = 2.5, 1H), 4.45 (m, 1H), 4.39 (d, J = 10.5, 1H), 4.32 (m, 1H), 4.06 (d, J = 10.5, 1H), 2.76 (m, 2H), 2.38 (m, 2H), 1.97 (m, 2H), 1.90 (m, 1H), 1.65 (m, 1H), 1.53 (m, 2H), 1.33 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.5, 173.3, 170.8, 170.0, 167.5, 149.9, 143.7, 137.2, 137.0, 133.0, 132.1, 131.2, 126.4, 115.7, 115.4, 112.5, 71.1, 55.2, 53.9, 31.2, 31.1, 27.3, 27.2, 23.0. Analytical HPLC: homogeneous single peak, t_R = 15.51 min (2−40% B in 30 min); MALDI MS calcd for $C_{26}H_{31}N_5O_7S$ (M + H^+) 558.20, found 558.11.

Data for 14a: 8.2 mg, 55%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.1 (bs, 1H), 9.60 (bs, 1H), 8.29 (m, 2H), 7.92 (m, 2H), 7.49−7.82 (m, 5), 7.34 (m, 2H), 7.16 (m, 1H), 6.99 (m, 1H), 6.80 (m, 1H), 4.65 (m, 1H), 4.36 (m, 1H), 4.26 (m, 1H), 4.14 (m, 1H), 2.40 (m, 2H), 1.92 (m, 2H), 1.84 (m, 1H), 1.62 (m, 1H), 1.58 (m, 2H), 1.32 (m, 2H). Analytical HPLC: homogeneous single peak, t_R = 11.3 min (2−40% B in 30 min); MALDI MS calcd for $C_{26}H_{33}N_7O_6$ (M + H^+) 540.25, found 540.30.

General Procedure for Preparation of Derivatized Macrocyclic Peptidomimetics 12b−14b. Resin containing side chain protected **12a** or **13a** or **14a** was swelled in CH_2Cl_2 in a fritted syringe for 30 min and treated with 10 equiv of *N*,*N*′-bis-BOC-1-guanylpyrazole in DMF (for **12a** and **14 a**) or 10 equiv of *N*,*N*′-bis-BOC-thiourea and 10 equiv of *N*-methyl-2-chloropyridinium chloride in CH_2Cl_2 (for **13a**) for 1 day. After washing, the peptide was cleaved from the resin by treatment with a mixture of 90% TFA, 5% TIS, and 5% H_2O for 2 h. The cleavage solution was separated from the resin by filtration. After most of the cleavage cocktail (about 90%) was evaporated in vacuo, the crude peptide was triturated using anhydrous ethyl ether, dissolved in H_2O, and then lyophilized to give the crude product.

Data for 12b: 9.6 mg, 40%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.2 (bs, 1H), 9.76 (s, 1H), 8.76 (s, 1H), 8.64 (d, J = 9.5, 1H), 8.59 (d, J = 7.5, 1H), 8.36 (d, J = 1.5, 1H), 7.96 (s, 1H), 7.68 (bs, 3H), 7.64 (d, J = 8.0, 1H), 7.61 (dd, J = 8.0, 1.5, 1H), 7.46 (d, J = 9.0, 1H), 7.42 (bs, 4H), 7.38 (s, 1H), 7.35 (d, J = 3.0, 1H), 7.30 (dd, J = 9.0, 3.0, 1H), 5.46 (d, J = 12.0, 1H), 5.14 (d, J = 12.0, 1H), 4.41 (m, 1H), 4.32 (m, 1H), 2.76 (m, 2H), 2.37 (m, 2H), 1.97 (m, 2H), 1.92 (m, 1H), 1.68 (m, 1H), 1.52 (m,

2H), 1.31 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.4, 173.0, 171.0, 168.2, 166.5, 156.6, 153.9, 137.8, 135.9, 131.2, 130.1, 129.0, 127.3, 126.6, 123.8, 123.3, 115.3, 68.7, 55.6, 53.3, 30.9, 30.8, 26.8, 26.5, 23.1. Analytical HPLC: homogeneous single peak, t_R = 11.37 min (2−40% B in 30 min); MALDI MS calcd for $C_{27}H_{34}N_8O_7$ (M + H$^+$) 583.26, found 583.18.

Data for 13b: 3.2 mg, 22%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (bs, 2H), 9.94 (s, 1H), 9.05 (s, 1H), 8.68 (d, J = 8.5, 1H), 8.48 (d, J = 1.5, 1H), 8.15 (d, J = 8.0, 1H), 7.89 (d, J = 9.0, 1H), 7.68 (dd, J = 1.5, 8.0, 1H), 7.64 (m, 3H), 7.61 (d, J = 8.0, 1H), 7.56 (bs, 4H), 7.43 (d, J = 8.0, 1H), 7.34 (s, 1H), 4.55 (d, J = 10.5, 1H), 4.39−4.48 (m, 2H), 4.27 (d, J = 10.5, 1H), 2.76 (m, 2H), 2.38 (m, 2H), 1.97 (m, 2H), 1.90 (m, 1H), 1.65 (m, 1H), 1.53 (m, 2H), 1.33 (m, 2H). Analytical HPLC: homogeneous single peak, t_R = 15.78 min (2−40% B in 30 min); MALDI MS calcd for $C_{27}H_{33}N_7O_7S$ (M + H$^+$) 600.22, found 600.16.

Data for 14b: 14 mg, 88%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.1 (s, 1H), 9.52 (s, 1H), 9.40 (s, 1H), 8.27 (d, J = 7.5 Hz, 1H), 7.90 (s, 1H), 7.50−7.75 (m, 6H), 7.33(s, 1H), 7.10−7.25 (m, 5H), 7.05 (dd, 1.2, 5.4 Hz, 1H), 6.75 (bs, 1H), 6.73 (d, J = 9.5 Hz, 1H), 4.72−4.76 (m, 1H), 4.25−4.32 (m, 2H), 4.12−4.17 (m, 1H), 2.80 (m, 2H), 2.41 (m, 2H), 1.90 (m, 2H), 1.80 (m, 1H), 1.68 (m, 1H), 1.57 (m, 2H), 1.32 (m, 2 H). Analytical HPLC: homogeneous single peak, t_R = 12.8 min (2−40% B in 30 min); MALDI MS calcd for $C_{27}H_{35}N_9O_6$ (M + H$^+$) 582.27, found 582.35.

General Procedure for Preparation of Derivatized Macrocyclic Peptidomimetics 12c−14c. Resin containing side-chain protected **12a** or **13a** or **14a** was swelled in CH_2Cl_2 in a fritted syringe for 30 min and treated with 10 equiv of methanesulfonyl chloride, 10 equiv of pyridine, and 1 equiv of 4-(dimethylamino)pyridine in CH_2Cl_2 for 1 day. After washing, the peptide was cleaved from the resin by treatment with a mixture of 90% TFA, 5% TIS, and 5% H_2O for 2 h. The cleavage solution was separated from the resin by filtration. After most of the cleavage cocktail (about 90%) was evaporated in vacuo, the crude peptide was triturated using anhydrous ethyl ether, dissolved in H_2O, and then lyophilized to give the crude product.

Data for 12c: 16.9 mg, 45%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.2 (bs, 1H), 9.57 (s, 1H), 8.78 (s, 1H), 8.46 (d, J = 7.5, 1H), 8.44 (d, J = 7.5, 1H), 8.36 (d, J = 1.5, 1H), 7.96 (s, 1H), 7.67 (bs, 3H), 7.62 (d, J = 8.0, 1H), 7.60 (dd, J = 8.0, 1.5, 1H), 7.40 (d, J = 9.0, 1H), 7.39 (s, 1H), 7.27 (d, J = 2.5, 1H), 7.27 (dd, J = 9.0, 2.5, 1H), 5.41 (d, J = 12.0, 1H), 5.12 (d, J = 12.0, 1H), 4.41 (m, 1H), 4.29 (m, 1H), 2.94 (s, 3H), 2.76 (m, 2H), 2.37 (m, 2H), 1.95 (m, 2H), 1.90 (m, 1H), 1.69 (m, 1H), 1.51 (m, 2H), 1.31 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.5, 173.0, 171.0, 168.2, 167.0, 152.2, 137.7, 135.8, 132.1, 131.0, 130.3, 127.5, 124.6, 123.8, 123.2, 122.1, 115.3, 68.9, 55.7, 53.2, 30.9, 30.8, 26.8, 26.4, 23.0. Analytical HPLC: homogeneous single peak, t_R = 14.66 min (2−40% B in 30 min); MALDI MS calcd for $C_{27}H_{34}N_6O_9S$ (M + H$^+$) 619.22, found 619.14.

Data for 13c: 9.1 mg, 59%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (bs, 2H), 10.07 (s, 1H), 9.00 (s, 1H), 8.72 (d, J = 8.0, 1H), 8.51 (d, J = 1.5, 1H), 8.36 (d, J = 8.5, 1H), 7.82 (d, J = 8.5, 1H), 7.68 (dd, J = 1.5, 8.0, 1H), 7.64 (m, 3H), 7.59 (d, J = 8.0, 1H), 7.30 (dd, J = 2.0, 8.5, 1H), 7.23 (d, J = 2.0, 1H), 4.49 (d, J = 10.5, 1H), 4.48 (m, 1H), 4.37 (m, 1H), 4.29 (d, J = 10.5, 1H), 3.08 (s, 3H), 2.77 (m, 2H), 2.38 (m, 2H), 1.97 (m, 2H), 1.90 (m, 1H), 1.65 (m, 1H), 1.53 (m, 2H), 1.33 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.8, 172.4, 170.0, 168.0, 166.8, 142.0, 138.2, 136.5, 135.3, 131.6, 130.8, 126.1, 125.6, 123.8, 120.3, 117.2, 64.9, 54.3, 53.4, 30.6, 30.4, 26.7, 26.5, 22.2, 15.2. Analytical HPLC: homogeneous single peak, t_R = 20.42 min (2−40% B in 30 min); MALDI MS calcd for $C_{27}H_{33}N_5O_9S_2$ (M + H$^+$) 636.18, found 636.18.

Data for 14c: 5 mg, 23%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.2 (s, 1H), 10.1 (s, 1H), 9.76 (s, 1H), 8.56 (d, J = 9.0 Hz, 1H), 8.35 (s, 1H), 7.95 (s, 1H), 7.85 (d, J = 9.0 Hz, 1H), 7.60−7.73(m, 6H), 7.33−7.38 (m, 4H), 5.15 (d, J = 18 Hz, 1H), 4.41−4.46 (m, 2H), 3.83 (d, J = 17.5 Hz, 1H), 3.07 (s, 3H), 2.80 (m, 2H), 2.37 (m, 2H), 1.95 (m, 2H), 1.72 (m, 1H), 1.57 (m, 2H), 1.50 (m, 1H), 1.32 (m, 2 H). Analytical HPLC: homogeneous single peak, t_R = 12.4 min (2−40% B in 30 min); MALDI MS calcd for $C_{27}H_{35}N_7O_8S$ (M + H$^+$) 618.23, found 618.20.

Cell Lines. Wild-type NIH3T3 cells do not express any of the Trk receptors. These cells were stably transfected with *TrkA* cDNA (NIH-TrkA cells) or *TrkC* cDNA (NIH-TrkC cells). Surface expression of each of the transfected receptors was routinely controlled in all cells by quantitative FACScan assays (Becton Dickinson, CA) (data not shown) using anti-TrkA mAb 5C3 and anti-TrkC mAb 2B7.

FACScan Binding Assays. Cells (2×10^5) in FACScan binding buffer (PBS, 0.5% BSA, and 0.1% NaN_3) were immunostained as described[30,40,41] using saturating anti-TrkA mAb 5C3, or anti-TrkC mAb 2B7, or control nonbinding IgGs (each at ∼7 nM). Data were acquired on a FACScan, and mean channel fluorescence of bell-shaped histograms was analyzed using the LYSIS II program. Data were averaged relative to positive control (immunostaining with no competitor), n = 3 ± sd.

Cell Survival Assays. Five thousand cells/well in protein-free media (PFHM-II, GIBCO, Toronto) containing 0.2% bovine serum albumin (BSA) (Crystalline fraction V, Sigma, St. Louis, MO) were seeded in 96-well plates (Falcon, Mississauga, Ontario). The cultures were untreated or treated with the indicated test or control ligands. Cell viability was quantitated using the MTT assay after 56−72 h of culture, as described.[42] Percent protection was standardized from optical density (OD) readings relative to optimal neurotrophin (1 nM) = 100% (the OD of untreated cells were subtracted).

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Supporting Information Available: ROE connectivities, tables of chemical shift data, temperature coefficient plots, calculated molecular parameters from the QMD studies, tabulated comparisons of ROE cross-peak intensities with simulated distances, tabulated comparisons of calculated and actual coupling constants, and ChemBats3D plots of the lowest energy conformer from each family generated in the QMD studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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