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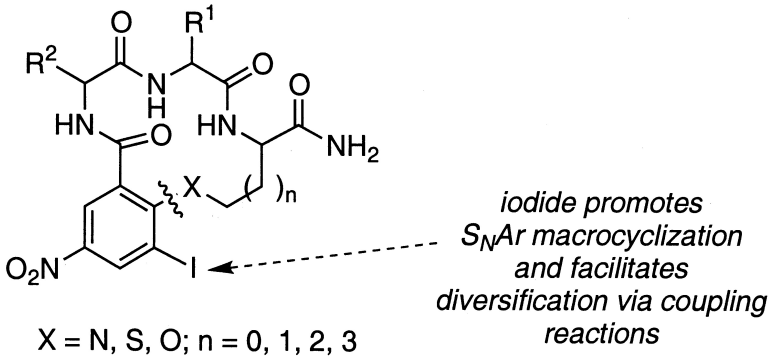


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# *Articles*

## Facile Macrocyclizations to β-Turn Mimics with Diverse Structural, Physical, and Conformational Properties

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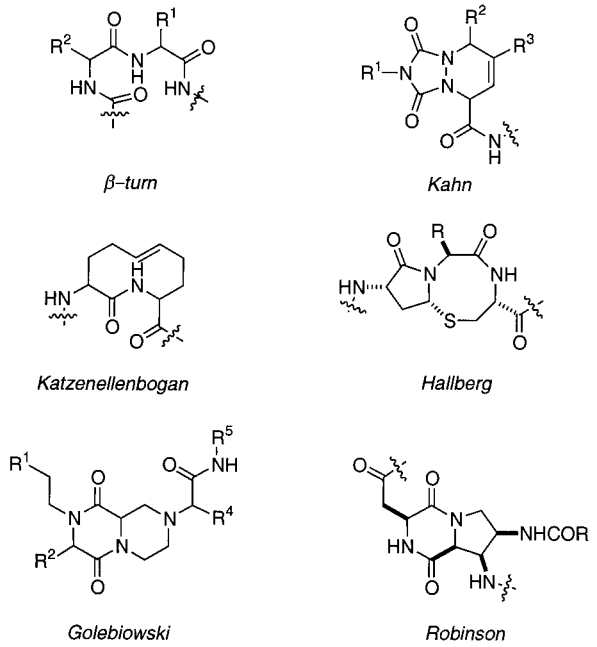
On-resin $S_NAr$ reactions were performed to prepare the macrocyclic β-turn mimics **1a**−**n** (Scheme 1 and Table 1). These reactions occurred more efficiently than completely analogous macrocyclization reactions that do not involve an iodinated aromatic electrophile. The synthesis was also modified to allow introduction of an alkyne via a solid-phase Sonogashira reaction (giving compound **2**, Scheme 2) and an aryne via a solid-phase Suzuki reaction (giving compound **3**, Scheme 2). Conformational analyses of three illustrative compounds, i.e., **1i**, **2**, and **3**, were performed using a combination of NMR, circular dichroism, and computer-aided molecular simulation methods. Overall, the preferred conformations of all three molecules tended to be type-I-like β-turns, but for compound **3** interaction of the electron cloud of the aryl substituent with the oxygen lone pairs seems to cause differences in the preferred orientation of the turn frameworks. This study illustrates how iodinated electrophiles can be used in solid-phase $S_NAr$ reactions to increase the molecular and conformational diversity in a library.

Protein−protein interactions[1] occur via critical points of contact, "hot-spots",[2] that may involve different elements of secondary structure on the component proteins.[3] One structural motif that can be involved is the β-turn. It follows that small molecules designed to resemble β-turn conformations[4] have the potential to mimic or disrupt protein−protein interactions. Much effort has been devoted to syntheses of fused ring and spirocyclic heterocycles that have structural similarities to β-turn conformations. There are too many designs, in fact, to mention all of them here, but the area has been reviewed in 1997[5] and a selection of the other structures that have been reported since is shown in Chart 1.[6]

**Chart 1**




Despite the effort invested in this area, β-turn mimics of the type depicted in Chart 1 and related structures have not provided many useful leads for synthetic compounds that influence protein−protein interactions.[7] There are several reasons for this. Some of the compounds were not designed with pharmaceutical applications in mind, and their structures are inappropriate for that reason. Others simply do not resemble β-turn conformations closely enough.[8,9] A small subset of the compounds have frameworks that overlay well onto β-turn conformations but do not project their substituents in the correct orientations. In other cases the substituents that can be incorporated do not include interesting pharmacophores; syntheses of β-turn mimics via new synthetic schemes that do not permit easy introduction of chemically reactive side chains (e.g., the guanidine of Arg, the indole of Trp, the imidazole of His, and the thiol of Cys) are of mostly academic interest. Amino acid side chains define most of the interactions involved at the interface between two proteins, and it is a significant shortcoming if a variety of diverse ones cannot be included. Some designs may be too rigid to allow the side chain substituents to mold into an appropriate conformation to facilitate the interaction or may be too flexible for docking to be energetically favorable.[10] Finally, these molecules may be too small to

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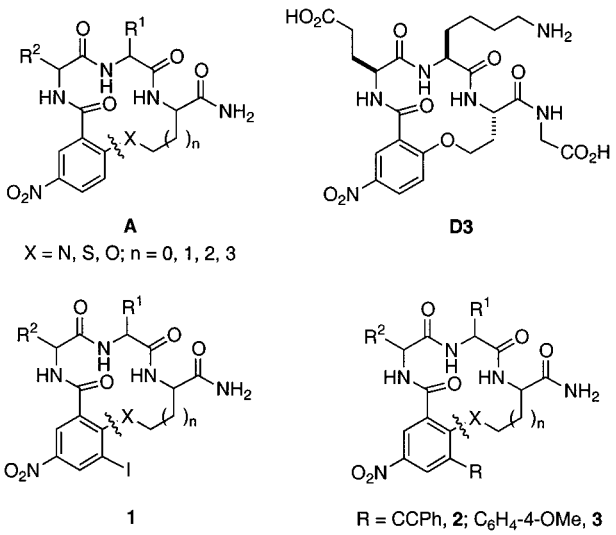
**Chart 2**




effectively influence protein−protein interactions involving hot spots separated from each other by more than a few angstroms.

It is clear that development of synthetic molecules to disrupt or mimic protein−protein interactions is not a simple process. Screening of huge libraries of randomly generated small molecules can give leads, but the size of the libraries involved tends to be huge. Moreover, the assays required to screen for protein−protein interactions are such that a one-compound-per-well format is almost obligatory, and obtaining sufficient quantities of such large numbers of compounds in this format is extremely difficult.[11] For this reason, creation of smaller libraries of compounds focused on $\beta$-turn mimics might be a promising approach. However, data from the fused- and spirocyclic-ring systems described above are discouraging; hence, alternative designs are highly desirable.

Recent contributions from this group have described solid-phase macrocyclization reactions to produce compounds of the type **A** (Chart 2) via $S_NAr$ macrocyclization reactions[12] on a solid phase.[13−16] The products are somewhat flexible, but computer-aided molecular simulations indicate they are significantly more rigid than some medium ring size putative $\beta$-turn mimics produced via other solid-phase syntheses.[17] Conformational analyses of several members of the series of compounds **A** indicate they preferentially adopt type I $\beta$-turn-like conformations.[14] Compounds **A** are prepared from amino acids, and their side chains can be chosen to correspond to those in the $\beta$-turns in target proteins that may be involved in the interaction with another protein component. All of the protein amino acids can be used, so in this respect there are no restrictions on the side chains that can be introduced. In fact, we have prepared a series of compounds with side chains that correspond to turn regions of the nerve growth factor (NGF). These turn regions are thought to be involved in the interaction of NGF with its high affinity transmembrane tyrosine kinase receptor, TrkA.[18,19] Significantly, pharmacological screens of a relatively small number of compounds (less than 30) led to the discovery of a compound **D3** (Chart 2) that interacts with TrkA (binding assays), initiates intracellular tyrosine phosphorylation (Western Blot assays), enhances cell survival of cells transfected with TrkA and suspended in a medium with suboptimal concentrations of NGF and no other growth factors, and causes growth and proliferation of brain cells in vitro.[19] To the best of our knowledge, this is the only report of a small molecule that potentiates the effect of NGF via interactions with TrkA.[20]

Several parameters are important in syntheses of libraries of compounds that include structures like **A**. First, solid-phase routes are highly desirable because these are most conveniently performed in parallel. Second, the purity of the deprotected product formed after cleavage from the resin is more important than the yield if the crude material is to be taken directly into a first-pass biological assay without purification. Third, it is important to be able to diversify the structures with respect to their substituents, solubilities, and molecular shapes. This is because the desired biological activities will be obtained only via fortuitous selection of appropriate structural, physical, and conformational properties of the product.

The work described in this manuscript features compounds **1** (Chart 2) prepared on a solid phase. The only difference between structures **A** and **1** is inclusion of the aryl iodide functionality, but this has some important consequences. This iodide makes the aromatic nucleus more electrophilic (Pauling electronegativities, H 2.20, I 2.66) and sterically encumbered {van der Waals's radii (Å), H 1.2, I 2.15}. This idea is consistent with studies performed in the 1950s in which iodination of aromatic rings was shown to accelerate $S_NAr$ reactions.[21] We hypothesized that the former effect would outweigh the latter, enabling the pivotal $S_NAr$ reaction to proceed more efficiently, giving enhanced purities. The presence of the iodine atom on the aromatic ring in compounds **1** in place of the hydrogen in systems **A** might influence the conformational preferences of these molecules, and it would certainly change their solubility characteristics. Moreover, the aryl iodide functionality could be used as a reactive center for a variety of solid-phase reactions to give products like **2** and **3** (Chart 2) with different structural, physical, and conformational properties. Evidence in support of these assertions is presented below.

## Results and Discussion

**Solid-Phase Macrocyclization Reactions.** Tripeptide units were constructed on a solid phase functionalized with the Rink handle, using the conventional FMOC approach,[22] then capped with 2-fluoro-3-iodo-5-nitrobenzoyl chloride **5**. The latter compound was prepared from the corresponding benzoic acid, which in turn was made by iodination of commercially available 2-fluoro-5-nitrobenzoic acid ($I_2$, fuming $H_2SO_4$, 3 h, 150 °C). The side chain substituents for groups $R^1$ and $R^2$ were the $^t$Bu-esters/Boc-carbamates typically used in the FMOC approach. More care was required, however, for selection of the X protecting group. Experimentation has led us to conclude that mildly acid-sensitive protecting groups are ideal for this, in particular, ones that can be cleaved without removing the $R^1/R^2$ protecting groups or liberating the whole peptide from the resin via cleavage of the Rink handle. Consequently, the protecting group used for Lys and Orn (Table 1, compounds **1a**−**1f**) at the first

**Table 1.** Synthesis Information for the Turn Analogues **1**

| | **1**[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | yields (%) | | **A** yields (%) | |
| | $R^2$ | $R^1$ | X | *n* | ring size | HPLC[b] | isolated[c] | HPLC[b] | isolated[c] |
| a | $(CH_2)_2CO_2H$ | $(CH_2)_4NH_2$ | NH | 3 | 16 | 97 | 68 | 83 | 52 |
| b | $CH_2(OH)CH_3$ | H | NH | 3 | 16 | 95 | 73 | *d* | |
| c | $(CH_2)_4NH_2$ | $CH_2(OH)CH_3$ | NH | 3 | 16 | 89 | 56 | | |
| d | $(CH_2)_2CO_2H$ | $(CH_2)_4NH_2$ | NH | 2 | 15 | 95 | 71 | 93 | 56 |
| e | $(CH_2)_2CO_2H$ | $CH_2CONH_2$ | NH | 2 | 15 | 98 | 65 | | |
| f | $(CH_2)_4NH_2$ | $CH_2(OH)CH_3$ | NH | 2 | 15 | 95 | 69 | | |
| g | $(CH_2)_2CO_2H$ | $(CH_2)_4NH_2$ | NH | 1 | 14 | 88 | 52 | 90 | 30 |
| h | $(CH_2)_2CO_2H$ | $CH_2CONH_2$ | NH | 1 | 14 | 98 | 59 | | |
| i | $(CH_2)_2CO_2H$ | $(CH_2)_4NH_2$ | O | 1 | 14 | 93 | 64 | 89 | 43 |
| j | $CH_2OH$ | $CH_2CH(CH_3)_2$ | O | 1 | 14 | 99 | 59 | 94 | 65 |
| k | $CH_2CH(CH_3)_2$ | $(CH_2)_2CO_2H$ | O | 1 | 14 | 97 | 60 | | |
| l | $(CH_2)_2CO_2H$ | $(CH_2)_4NH_2$ | S | 0 | 13 | 89 | 59 | 75 | 33 |
| m | $CH(CH_3)CH_2CH_3$ | $(CH_2)_4NH_2$ | S | 0 | 13 | 90 | 49 | 67 | 23 |
| n | $(CH_2)_4NH_2$ | H | S | 0 | 13 | 90 | 63 | 85 | 38 |

[a] The resin was Rink amide MBHA (0.54 mmol/g) for X = NH, S, and it was TentaGel S RAM (0.30 mmol/g) for X = O. [b] Percentage areas of peaks corresponding to the desired product relative to all other peaks in the HPLC trace (average of values monitored by UV at 215 and 254 nm). [c] Yields were calculated on the basis of the resin loads and the mass obtained after preparative HPLC separation. [d] The original study of the non-iodinated compounds did not cover all the sequences corresponding to this study.

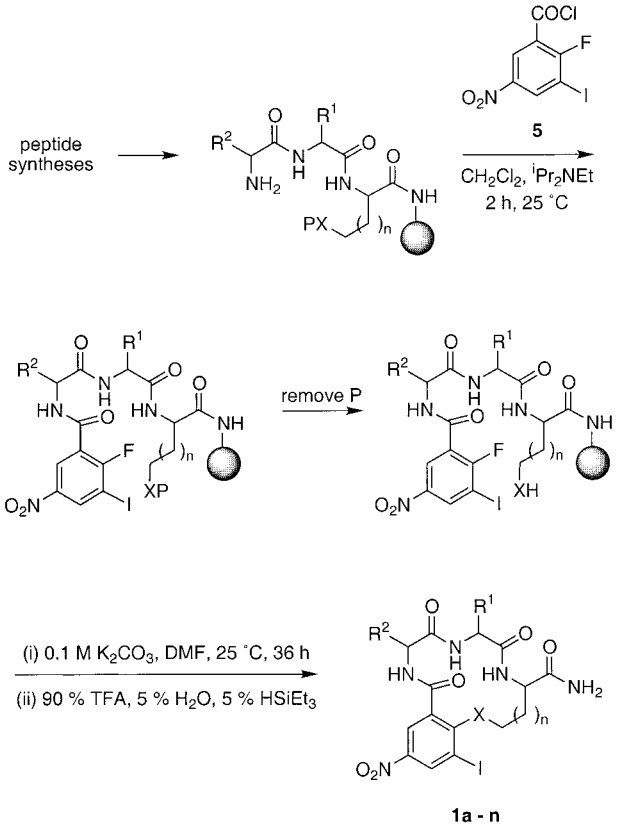
position was (4-methylphenyl)diphenylmethyl {i.e., (4-$MeC_6H_4)Ph_2C$ = Mtt}. To obtain the 14-membered-ring nitrogen heterocycles (Table 1, **1g**−**1h**), Gln was loaded at the first position, degraded via an on-resin Hofmann degradation ($PhI(O_2CCF_3)_2$, $H_2O$, DMF, 16 h), and then protected with MttCl ($CH_2Cl_2$, $^iPr_2NEt$, 2 h). The homoserine derivatives **1i**−**1k** were formed using the trityl-protected amino acids. Finally, cysteine at the first position, to form the sulfur heterocycles **1l**−**1n**, was protected using 4-methoxypheny(diphenyl)methyl {i.e., (4-$MeOC_6H_4)Ph_2C$ = Mmt}. The synthetic route is outlined in Scheme 1.

There is no simple correlation between the composition of the resin used and the yield and purity data obtained. Exploratory experiments (data not shown) indicated Rink amide MBHA resin was superior to TentaGel derivatives for formation of the N- and S-macrocycles. However, TentaGel S RAM resin was slightly better overall for formation of the O-heterocycles. It is hard to understand the reasons for these differences, but we speculate that the rigidity of the Rink MBHA resins retards competitive undesired macrocyclizations (e.g., formation of dimers) for the more reactive N- and S-nucleophiles. Conversely, the less reactive O-nucleophiles are best anchored on a relatively flexible resin like TentaGel if the desired reaction is to occur.

Table 1 summarizes the HPLC purity and isolated yield data for the 14 compounds **1a**−**n** and compares these data with that reported previously by us for compounds **A**. In almost every case, the purities observed for the iodine-substituted compounds **1** were greater than those previously recorded for the noniodinated compounds **A**.[15] For the eight compounds having identical side chains for both compound **1** and **A**, the average purity for compounds **1** was 93% (average yield 61%) whereas that for compounds **A** was 85% (average yield 43%).

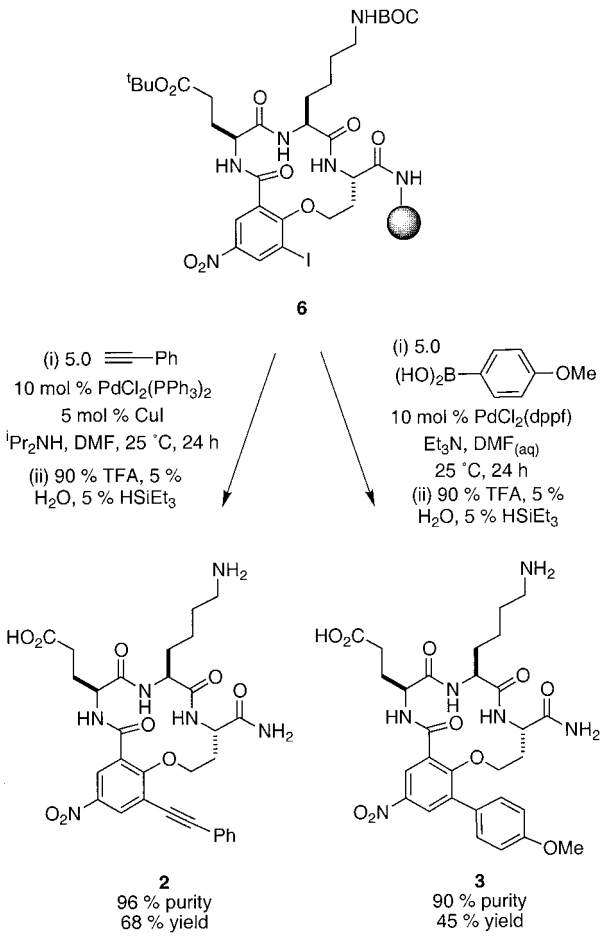
**On-Resin Functionalization of the Aryl Iodides.** Scheme 2 illustrates how the macrocyclization products can be derivatized via Sonogashira and Suzuki coupling reactions, prior to deprotection and cleavage from the resin. These coupling reactions must have proceeded with high efficiencies because the overall purities of the final products in these syntheses are high. Both these reactions proceeded smoothly, and no epimerized impurities were detected. These reaction types clearly provide a means to introduce groups to alter the physical properties of these molecules, and/or provide other pharmacophores.

**Scheme 1**




**Conformational Analyses of Compounds 1i, 2, and 3.** These particular compounds were selected for conformational analyses because they have structures closely related to the

**Scheme 2**




lead compound **D3**. The following paragraph describes the procedures used for the analyses.

Proton NMR signals were assigned using a combination of correlation spectroscopy (COSY) and rotating-frame Overhauser enhancement spectroscopy (ROESY) (DMSO solvent). Coupling constant information was extracted from the 1D spectra, and variations of chemical shifts with temperature, i.e., temperature coefficients, were recorded for the N*H* protons. Close contacts were monitored via ROESY spectroscopy. In parallel studies the molecules were simulated using the quenched molecular dynamics technique to give 600 minimized structures. Cutoff values were decided from consideration of the distribution of energies for the 599 structures relative to the lowest energy one overall. Conformers below the energy cutoff were grouped into families by considering the root-mean-square (rms) deviation of the main chain atoms. The fit of the simulated structures with the physical data was assessed in terms of bond parameters of the lowest energy conformer in each family, the number of structures in each family, and the energy of the lowest energy conformer in each family relative to the lowest energy conformer overall in the simulation. Full data are tabulated in the Supporting Information.

Analysis of compound **1i** indicated it has a conformational preference for a type I $\beta$-turn, though we regard the differences between type I and type III turns to be almost insignificant when considering small molecules such as these that have appreciable flexibility. Figure 1 shows the lowest

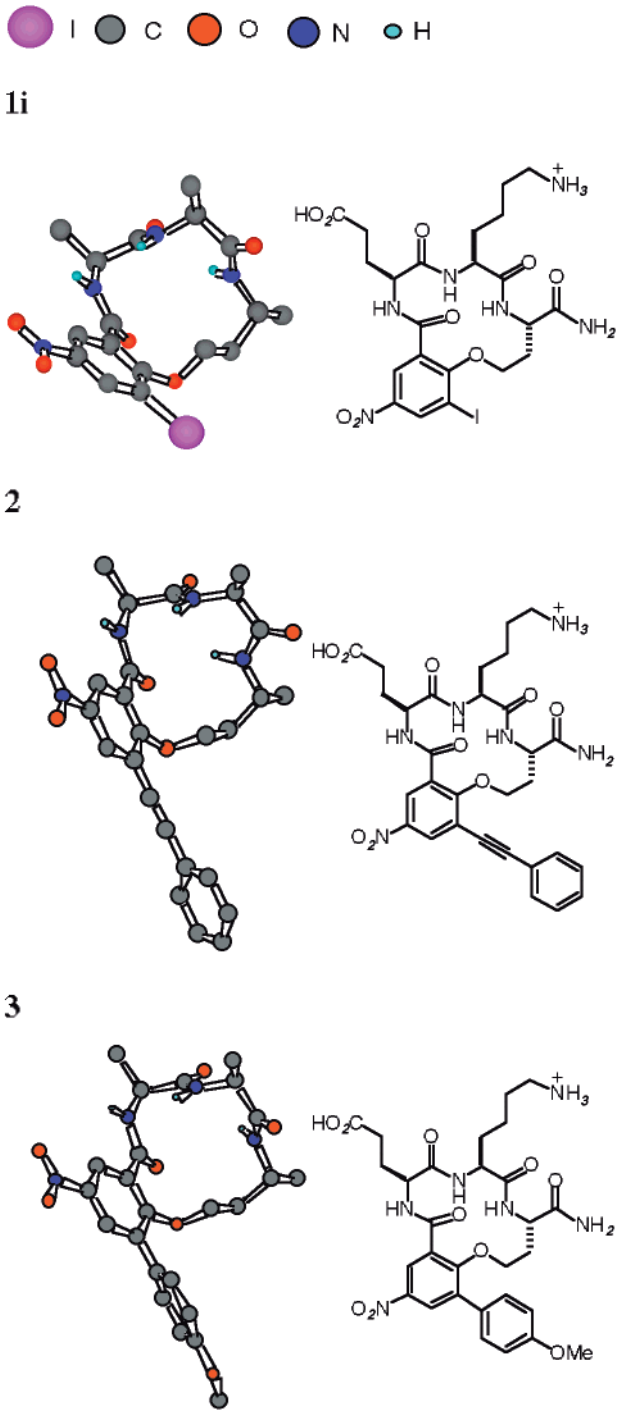



**Figure 1.** Simulated low-energy conformers for compounds **1i**, **2**, and **3**.

energy structure overall from the simulations, which is also a part of the most populated family, and fits the NMR data well. The simulated Hse N*H*−benzoic acid C*O* distance was 3.49 Å, which is acceptable for a type I turn. A low-temperature coefficient (−1.39 ppb/K) for the Hse N*H* was observed, and this is indicative of a hydrogen-bonded/solvent-shielded proton like those involved in C$^{10}$ $\beta$-turn conformations. In fact, similar low-temperature coefficients for the N*H* Hse protons were observed for all three compounds in the series. Overall, the conformational preferences of this compound seem very similar to the analogous non-iodinated compound that has been analyzed in a similar way.[16]

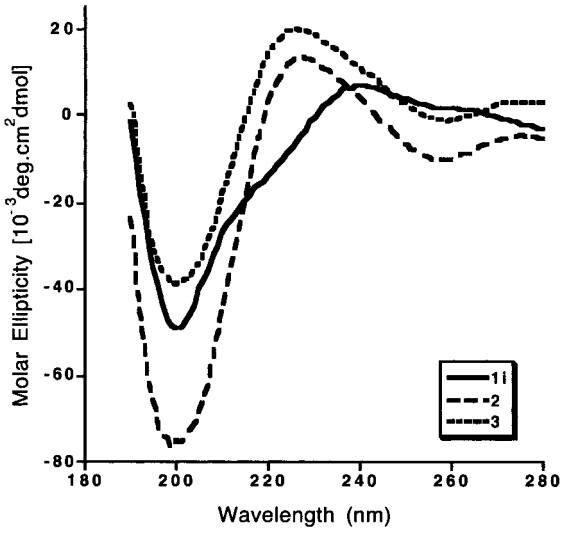



**Figure 2.** CD spectra in 20% MeOH/$H_2O$.

Compound **2** has a conformational bias similar to that of **1i**, while the fit of the simulated bond angles in the low-energy conformer to an ideal type I turn was even better than that for **1i**. In **2**, the simulated Hse N*H*−benzoic acid C*O* distance was 2.46 Å.

The largest conformational deviation from a type I turn was observed when the iodine atom of **1i** is substituted with the 4-methoxyphenyl group as in compound **3**. In this case the preferred conformer identified in the simulations (Figure 1) deviates from a type I situation and is most accurately compared with a type VIII turn. The benzoic acid C*O* and the Hse N*H* are separated by 3.22 Å.

The origin of the conformational differences among structures **1i**, **2**, and **3** is evident from a comparison of the simulated low-energy conformers shown in Figure 1. For compound **3**, interaction of the 4-methoxyphenyl group with the $OCH_2CH_2$ unit of the Hse residue tends to force the latter toward the center of the macrocycle. Similar electronic interactions are not as serious for the iodinated compound **1i** and are least evident for the alkyne **2**.

Though there are conformational differences among **1i**, **2**, and **3**, NMR experiments and molecular simulations indicate that all three compounds can access turn conformations. This assertion is supported by circular dichroism (CD) studies (Figure 2). All have minimum ellipticities at around 200 nm, characteristic of type I turns. At higher wavelengths it is the spectra of compound **1i** that differ from the other two, but this could just be an effect of the different aromatic characters of the three molecules being associated with variable absorptions in this region.

## Conclusions

The iodine substituent on the fluoronitrobenzoic acid fragment of these compounds leads to more efficient $S_NAr$ reactions for this class of compounds than the corresponding non-iodinated compounds, presumably by promoting the rate of the desired macrocyclization relative to competing reactions. That same iodine can then be used to functionalize the products, on the resin, via Sonogashira and Suzuki couplings and presumably via other organometallic catalysis. The coupled products can have conformational biases that differ from the iodinated macrocycles. These coupling reactions also provide a means to introduce additional pharmacophores and to adjust the solubilities of the products.

Other groups have prepared on-resin macrocyclization products via $S_NAr$ reactions.[12,23] The use of iodinated compounds as described here provides another avenue into structurally diverse materials in this series.

## Experimental Section

**General Methods.** All the α-amino acids used had the L-configuration except where otherwise indicated. All chemicals were obtained from commercial suppliers and used without further purification. Di-isopropylcarbodiimide (DIC), *N*-hydroxybenzotriazole (HOBt), di-isopropylethylamine (DIEA), *N*-methylmorpholine (NMM), TFA, $CH_2Cl_2$, DMF, thionyl chloride, bis(trifluoroacetoxy)iodobenzene (IBTFA), piperidine, and tri-isopropylsilane (TIS) were purchased from Aldrich. 2-Fluoro-3-iodo-5-nitrobenzoyl chloride was obtained by refluxing 2-fluoro-3-iodo-5-nitrobenzoic acid in thionyl chloride for 4 h. Rink amide MBHA resin was obtained from NovaBiochem. TentaGel S RAM FMOC resin was purchased from Advanced ChemTech. All α-amino acids were obtained either from Advanced ChemTech or from Chem-Impex.

Reverse-phase high-performance liquid chromatography (HPLC) was carried out on Vydac C-18 columns of the following dimensions: 25 cm × 0.46 cm for analysis; 25 cm × 2.2 cm for preparative work. All HPLC experiments were performed using gradient conditions. Eluents used were solvent A ($H_2O$ with 0.1% TFA) and solvent B ($CH_3CN$ with 0.1% TFA). Flow rates used were 1.0 mL/min for analytical and 6 mL/min for preparative HPLC.

Spectra were recorded on a Varian instrument at 500 or 300 MHz for $^1H$ NMR and at 75 MHz for $^{13}C$ NMR. NMR chemical shifts are expressed in ppm relative to internal solvent peaks, and coupling constants were measured in hertz.

Peptidomimetics in the following Experimental Section are given abbreviated names based on their constituent amino acids. One-letter coding is used for the amino acids in the dipeptide fragment, and three-letter abbreviations are used for the amino acids that constitute part of the template. Less common abbreviations used include Orn = ornithine, Hse = homoserine, and Dbu = 2,4-diaminobutyric acid.

**2-Fluoro-3-iodo-5-nitrobenzoic Acid**. 2-Fluoro-5-nitrobenzoic acid (5 g, 27 mmol), iodine (3.4 g, 13.5 mmol), and fuming sulfuric acid (25 mL, 20%) were mixed in a 100 mL round flask equipped with a reflux condenser. The resulting solution was stirred at 150 °C for 3 h. After cooling to room temperature, the mixture was poured into separatory funnel with ice and water (100 mL) with occasional shaking and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with water (3 × 100 mL), saturated $NaHCO_3$ (3 × 100 mL), and brine (3 × 100 mL), dried over sodium sulfate, and evaporated under reduced pressure to give 8.2 g (98%) of a white solid, mp 184.5−186.5 °C. $^1H$ NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 8.75 (dd, $J$ = 4.8, 3.0, 1H), 8.49 (dd, $J$ = 6.0, 2.7). $^{13}C$ NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 165.3, 162.3 (d, $J$ = 19.1),

144.2 (d, $J = 3.5$), 137.9 (d, $J = 5.0$), 27.4 (d, $J = 3.0$), 120.5 (d, $J = 5.5$), 86.3 (d, $J = 30.5$). Analytical HPLC: homogeneous single peak, retention time = 19.6 min (7−80% B in 30 min). MALDI MS: calcd for $C_7H_2INO_3$ ($M^+ - OH$) 293.9, found 294.0.

**General Experimental Procedure for the Preparation of the Peptidomimetics. Synthesis of Compound 1a**. Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was swelled in DMF (10 mL/g) in a fritted polystyrene syringe for 30 min, then rinsed with DMF (2 × 10 mL/g, for each washing cycle throughout). The FMOC protecting group on the Rink handle was removed by treating the resin with 20% piperidine in DMF (2 × 15 min). After the resin was rinsed with DMF (3×), MeOH (3×), and $CH_2Cl_2$ (3×), FMOC-Lys-(Mtt)-OH (3 equiv), DIC (4 equiv), HOBt (4 equiv), and NMM (5 equiv) were added in 3 mL of $CH_2Cl_2$/DMF (v/v, 4:1). 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 rinsed with DMF (4×). The above deprotection/coupling cycles were repeated to introduce FMOC-Lys(BOC)-OH and FMOC-Glu($O^tBu$)-OH consecutively. The 2-fluoro-3-iodo-5-nitrobenzoic acid moiety was introduced to the N-terminus of the tripeptide−resin by treating with 2-fluoro-3-nitro-5-nitrobenzoyl chloride (3 equiv) and $^iPr_2NEt$ (5 equiv) in 3 mL of $CH_2Cl_2$ for 2 h. The side chain protecting group (Mtt, 4-methyltrityl) of Lys was removed by treatment with 1% TFA and 5% TIS in $CH_2Cl_2$ (5 × 5 min). Afterward, the resin was rinsed with $CH_2Cl_2$ (3×), MeOH (3×), and DMF (3×) and the macrocyclization step was carried out by treating the supported peptide with 5 mL of 0.1 M $K_2CO_3$in DMF at 25 °C with gentle shaking for 36 h. The peptide−resin was washed with DMF (2×), $H_2O$ (3×), DMF (3×), $H_2O$ (2×), MeOH (3×), and $CH_2Cl_2$ (3×) and then dried in vacuo for 4 h. The peptide was cleaved from the resin by treatment with a 5 mL mixture of 90% TFA, 5% TIS, and 5% $H_2O$ for 4 h. The cleavage solution was separated from the resin by filtration. After most of the cleavage cocktail was evaporated by passing $N_2$, the crude peptide was precipitated using anhydrous ethyl ether, dissolved in $H_2O$, and then lyophilized to give the crude product. Preparative HPLC (SIS System, 8−70% B in 30 min) was carried out to provide a yellowish powder (22.9 mg, 68%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 12.23 (b, 1H), 8.98 (d, $J = 8.1$, 1H), 8.57 (d, $J = 2.4$, 1H), 8.26 (d, $J = 2.4$, 1H), 7.94 (d, $J = 8.4$, 1H), 7.80 (b, 3H), 7.66 (d, $J = 8.7$, 1H), 7.34 (s, 1H), 7.05 (s, 1H), 6.82 (s, 1H), 4.43−4.34 (m, 1H), 4.32−4.22 (m, 2H), 3.36−3.26 (m, 1H), 3.22−3.08 (m, 1H), 2.80−2.68 (m, 1H), 2.42−2.30 (m, 1H), 2.10−1.88 (m, 2H), 1.74−1.62 (m, 2H), 1.60−1.42 (m, 6H), 1.38−1.18 (m, 4H), 1.08−0.96 (m, 1H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 173.7, 173.3, 170.9, 170.8, 166.8, 162.3, 152.3, 137.3, 136.3, 125.7, 118.6, 83.5, 54.1, 51.9, 51.3, 46.5, 35.8, 34.3, 31.4, 30.8, 30.5, 30.0, 28.3, 27.3, 26.6, 22.3, 21.7. Analytical HPLC: homogeneous single peak, retention time = 17.1 min (7−80% B in 30 min). MALDI MS: calcd for $C_{24}H_{35}IN_7O_8$ ($MH^+$) 676.16, found 676.12.

**Synthesis of 1b.** Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a yellowish powder (21.0 mg, 73%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 8.71 (d, $J = 8.1$, 1H), 8.51 (d, $J = 3.0$, 1H), 8.49 (d, $J = 6.0$, 1H), 8.16 (d, $J = 3$, 1H), 7.34 (s, 1H), 7.13 (d, $J = 9.3$, 1H), 7.05 (s, 1H), 6.27 (s, 1H), 4.48 (dd, $J = 8.4$, 4.8, 1H), 4.21−4.13 (m, 1H), 4.07−3.98 (m, 1H), 3.72−3.59 (m, 2H), 3.41−3.28 (m, 1H), 3.20−3.05 (m, 1H), 1.72−1.59 (m, 1H), 1.58−1.37 (m, 3H), 1.30−1.15 (m, 2H), 1.14(d, $J = 6.3$, 3H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 173.3, 170.4, 168.8, 167.3, 151.5, 136.3, 136.2, 125.9, 119.6, 84.3, 66.5, 59.8, 51.8, 45.4, 45.5, 31.3, 28.1, 21.9, 20.3. Analytical HPLC: homogeneous single peak, retention time = 15.7 min (8−70% B in 30 min). MALDI MS: calcd for $C_{19}H_{25}IN_6O_7$ ($M + Na^+$) 599.07, found 599.05.

**Synthesis of 1c.** Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a yellowish powder (18.1 mg, 56%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 9.18 (d, $J = 8.7$, 1H), 8.58 (d, $J = 3.0$, 1H), 8.20 (d, $J = 2.4$, 1H), 7.75 (d, $J = 9.0$, 1H), 7.73 (b, 3H), 7.53 (d, $J = 8.7$, 1H), 7.26 (s, 1H), 7.06 (s, 1H), 6.63 (s, 1H), 4.78 (b, 1H), 4.40−4.24 (m, 3H), 3.98−3.88 (m, 1H), 3.34−3.20 (m, 1H), 3.10−2.98 (m, 1H), 2.86−1.74 (m, 2H), 1.86−1.77 (m, 2H), 1.74−1.40 (m, 8H), 1.38−1.24 (m, 1H), 1.02 (d, $J = 0.6.6$, 3H), 1.06−0.90 (m, 1H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 173.2, 171.3, 169.9, 166.6, 151.9, 137.1, 136.1, 125.6, 118.4, 84.1, 65.8, 57.4, 54.8, 50.9, 46.4, 31.2, 30.9, 28.1, 26.4, 22.7, 21.4, 19.9. Analytical HPLC: homogeneous single peak, retention time = 15.2 min (8−70% B in 30 min). MALDI MS: calcd for $C_{22}H_{34}IN_7O_7$ ($M + Na^+$) 670.14, found 670.14.

**Synthesis of 1d.** Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a yellowish powder (23.5 mg, 71%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 8.99 (d, $J = 7.2$, 1H), 8.58 (d, $J = 2.7$, 1H), 8.32 (d, $J = 3.0$, 1H), 8.18 (d, $J = 8.4$, 1H), 7.80 (b, 3H), 7.34 (s, 1H), 7.27 (d, $J = 8.7$, 1H), 7.05 (s, 1H), 6.58 (s, 1H), 4.29 (dd, $J = 14.3$, 8.0, 1H), 4.22−4.12 (m, 2H), 3.40−3.18 (m, 2H), 2.82−2.72 (m, 2H), 2.46−2.38 (m, 2H), 2.08−1.92 (m, 2H), 1.86−1.72 (m, 2H), 1.66−1.44 (m, 6H), 1.32−1.18 (m, 2H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 173.8, 173.1, 171.5, 170.6, 167.2, 153.7, 137.3, 137.1, 125.4, 120.4, 86.1, 54.6, 52.4, 52.0, 47.4, 34.3, 30.6, 29.4, 29.2, 27.6, 26.6, 22.4. Analytical HPLC: homogeneous single peak, retention time = 16.7 min (5−60% B in 30 min). MALDI MS: calcd for $C_{23}H_{33}IN_7O_8$ ($MH^+$) 662.14, found 662.07.

**Synthesis of 1e.** Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a yellowish powder (21.0 mg, 65%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 8.98 (d, $J = 6.9$, 1H), 8.57 (d, $J = 3.0$, 1H), 8.48 (d, $J = 7.8$, 1H), 8.26 (d, $J = 2.7$, 1H), 7.34 (d, $J = 8.7$, 2H), 7.19 (d, $J = 8.7$, 1H), 7.09 (s, 1H),

6.85 (s, 1H), 6.32(b, 1H), 4.39(dd, $J$ = 14.2, 6.8, 1H), 4.27 (t, $J$ = 7.5 1H), 4.19(t, $J$ = 10.2, 1H), 3.33(b, 1H), 3.12(b, 1H), 2.80−2.53(m, 1H), 2.50−2.45(m, 3H), 2.00−1.83(m, 2H), 1.75−1.45 (m, 4H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 173.7, 172.9, 172.2, 171.4, 169.9, 167.0, 153.3, 137.2, 136.8, 125.4, 120.4, 85.9, 54.2, 52.2, 50.0, 47.5, 35.5, 30.4, 28.9, 27.4, 26.2. Analytical HPLC: homogeneous single peak, retention time = 14.6 min (8−70% B in 30 min). MALDI MS: calcd for $C_{21}H_{26}IN_7O_9$ ($M + Na^+$) 670.07, found 670.03.

**Synthesis of 1f.** Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a yellowish powder (21.9 mg, 69%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 9.24 (d, $J$ = 7.8, 1H), 8.61 (d, $J$ = 3.0, 1H), 8.34 (d, $J$ = 2.7, 1H), 7.75 (b, 3H), 7.60 (d, $J$ = 8.1, 1H), 7.31 (d, $J$ = 8.7 1H), 7.30 (s, 1H), 7.08 (s, 1H), 6.79 (s, 1H), 4.26−4.14 (m, 3H), 4.12−4.07 (m, 1H), 3.58−3.40 (m, 2H), 2.86−2.74 (m, 2H), 1.89−1.74 (m, 3H), 1.66−1.44 (m, 7H), 1.00 (d, $J$ = 6.6, 3H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 172.9, 172.2, 169.8, 167.4, 154.1, 137.5, 137.4, 125.5, 119.9, 86.3, 65.1, 57.3, 55.7, 52.0, 47.2, 30.5, 29.3,27.4, 26.4, 22.8, 20.1. Analytical HPLC: homogeneous single peak, retention time = 13.8 min (8−70% B in 30 min). MALDI MS: calcd for $C_{22}H_{32}IN_7O_7$ ($M + Na^+$) 656.13, found 656.08.

**Synthesis of 1g.** Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was used to prepare this compound. The 1,3-diaminobutyric acid was generated through a Hofmann rearrangement reaction on the resin after coupling of the first amino acid FMOC-glutamine. To the resin were added IBTFA (10 equiv) and $CH_3CN/H_2O$ (v/v, 4:1), 16 h at 25 °C. After the resin was rinsed with $CH_2Cl_2$ (3×), $CH_3CN$ (3×), MeOH (3×), and DMF (3×), The amino side chain of Dbu was protected with Mtt-Cl (3 equiv) and $^iPr_2NEt$ (5 equiv) in $CH_2Cl_2$ for 2 h. The subsequent steps were as described in the general procedure illustrated for compound **1a**. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a yellowish powder (16.8 mg, 52%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 12.30 (b, 1H), 9.17 (d, $J$ = 5.4, 1H), 9.00 (d, $J$ = 6.6, 1H), 8.50 (d, $J$ = 2.4, 1H), 8.05 (d, $J$ = 3.0, 1H), 7.76 (b, 3H), 7.34 (s, 1H), 7.03 (s, 2H), 6.42−6.34 (m, 1H), 4.17 (dd, $J$ = 13.2, 7.5, 1H), 3.88−3.80 (m, 1H), 3.76−3.68 (m, 1H), 3.24−2.98 (m, 2H), 2.80−2.72 (m, 2H), 2.44−2.25 (m, 2H), 2.32−1.66 (m, 6H), 1.62−1.44 (m, 2H), 1.36−1.20 (m, 2H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 173.6, 171.9, 170.7, 168.4, 149.3, 136.0, 135.2, 127.4, 116.6, 85.9, 54.6, 53.9, 50.9, 43.0, 38.2, 31.5, 30.3, 27.3, 26.7, 25.6, 23.1. Analytical HPLC: homogeneous single peak, retention time = 13.8 min (8−70% B in 30 min). MALDI MS: calcd for $C_{22}H_{30}IN_7O_8$ ($M + Na^+$) 648.12, found 648.10.

**Synthesis of 1h.** Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was used to prepare this compound according to the above procedure. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a white powder (18.7 mg, 59%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$. 12.10 (b, 1H), 9.19 (d, $J$ = 6.6, 2H), 8.49 (d, $J$ = 2.7, 1H), 8.02 (d, $J$ = 2.4, 1H), 7.40 (s, 2H), 7.03 (d, $J$ = 6.3, 1H), 7.02 (s, 1H), 6.83 (s, 1H), 6.53−6.49 (m, 1H), 4.30−4.12 (m, 2H), 3.81−3.72 (m, 1H), 3.24−3.01 (m, 2H), 2.85 (dd, $J$ = 3.9, 15.6, 1H), 2.60−2.48 (m, 1H), 2.46−2.24 (m, 2H), 2.32−1.99 (m, 1H), 1.98−1.74 (m, 3H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 173.7, 172.5, 172.2, 170.4, 168.4, 149.1, 136.1, 134.9, 127.4, 116.3, 85.8, 54.2, 51.5, 51.2, 42.9, 34.9, 31.5, 30.1, 25.5. Analytical HPLC: homogeneous single peak, retention time = 13.7 min (8−70% B in 30 min). MALDI MS: calcd for $C_{20}H_{25}IN_7O_9$ ($MH^+$) 634.07, found 634.02.

**Synthesis of 1i.** TentaGel S RAM resin (0.05 mmol, 0.3 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a white powder (20.7 mg, 64%). $^1$H NMR (500 MHz, DMSO-$d_6$, 25 °C): $\delta$ 12.21 (b, 1H), 8.91 (d, $J$ = 6.0, 1H), 8.71 (d, $J$ = 3.0, 1H), 8.37 (d, $J$ = 8.0, 1H), 8.36 (d, $J$ = 3.0, 1H), 7.66 (s, 1H), 7.42 (d, $J$ = 8.5, 1H), 7.28 (s, 1H), 6.99 (s, 1H), 4.36−4.31 (m, 1H), 4.22−4.16 (dd, $J$ = 8.7, 3.9, 1H), 4.13−4.07 (m, 3H), 2.78−2.70 (m, 2H), 2.43−2.36 (m, 2H), 2.28−2.12 (m, 2H), 2.02−1.88 (m, 2H), 1.82−1.73 (m, 1H), 1.62−1.46 (m, 3H), 1.36−1.22(m, 2H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 174.2, 173.1, 172.7, 171.5, 165.8, 161.4, 143.6, 136.1, 130.5, 124.9, 94.5, 72.9, 56.2, 53.2, 51.1, 31.7, 30.8, 29.4, 26.9, 26.2, 23.0. Analytical HPLC: homogeneous single peak, retention time = 13.5 min (8−70% B in 30 min). MALDI MS: calcd for $C_{22}H_{30}IN_6O_9$ ($MH^+$) 649.11, found 649.11.

**Synthesis of 1j.** TentaGel S RAM resin (0.05 mmol, 0.3 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a white powder (17.4 mg, 59%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 8.76 (d, $J$ = 6.6, 1H), 8.71 (d, $J$ = 3.0, 1H), 8.37 (d, $J$ = 3.0, 1H), 7.16 (d, $J$ = 8.1, 1H), 7.41 (d, $J$ = 8.4, 1H), 7.29 (s, 1H), 7.03 (s, 1H), 5.12 (b, 1H), 4.36−4.29 (m, 1H), 4.28−4.20 (m, 2H), 4.08−4.04 (m, 1H), 3.72−3.66 (m, 1H), 2.24−2.16 (m, 2H), 1.58−1.46 (m, 3H), 0.85 (d, $J$ = 6.3, 3H), 0.81 (d, $J$ = 6.3, 3H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 172.7, 171.5, 170.6, 165.4, 161.1, 143.1, 135.6, 130.2, 124.3, 94.2, 72.4, 60.4. 59.0, 51.3, 50.6, 38.4, 24.1, 23.1, 21.3. Analytical HPLC: homogeneous single peak, retention time = 16.7 min (8−70% B in 30 min). MALDI MS: calcd for $C_{20}H_{26}IN_5O_8$ ($M + Na^+$) 614.07, found 614.07.

**Synthesis of 1k.** TentaGel S RAM resin (0.05 mmol, 0.3 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a white powder (19.0 mg, 60%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 12.09 (b, 1H), 8.85 (d, $J$ = 6.3, 1H), 8.69 (d, $J$ = 3.0, 1H), 8.41 (d, $J$ = 7.8, 1H), 8.28 (d, $J$ = 3.0, 1H), 7.38 (d, $J$ = 9.0, 1H), 7.27 (s, 1H), 7.06 (s, 1H), 4.36−4.27 (m, 1H), 4.26−4.19 (m, 1H), 4.12−4.02 (m, 3H), 2.28−2.16 (m, 4H), 2.10−1.98 (m, 1H), 1.85−1.58 (m, 3H), 1.56−1.45 (m, 1H), 0.95 (d, $J$ = 6.6, 1H), 0.91(d, $J$ = 6.3,

3H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 173.9, 72.7, 172.6, 170.7, 165.1, 160.8, 142.9, 135.5, 129.5, 124.5, 93.9, 72.1, 54.4, 52.5, 50.3, 31.2, 30.2, 24.7, 24.4, 22.3, 22.2. Analytical HPLC: homogeneous single peak, retention time = 18.4 min (8−70% B in 30 min). MALDI MS: calcd for $C_{22}H_{28}IN_5O_9$ ($M + Na^+$) 656.08, found 656.08.

**Synthesis of 1l.** Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a white powder (19.2 mg, 59%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 9.12 (d, $J$ = 6.0, 1H), 8.68 (d, $J$ = 2.4, 1H), 8.39 (d, $J$ = 8.1, 1H), 8.24 (d, $J$ = 2.1, 1H), 7.73 (s, 3H), 7.57 (s, 1H), 7.26−7.20 (m, 2H), 4.38−4.20 (m, 2H), 4.14−1.05 (m, 1H), 3.36−3.27 (m, 2H), 3.16 (t, $J$ = 10.5, 1H), 2.79−2.72 (m, 2H), 2.39−2.31 (m, 2H), 1.98−1.83 (m, 2H), 1.76−1.66 (m, 1H), 1.60−1.46 (m, 2H), 1.32−1.22 (m, 2H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 173.7, 171.0, 170.9, 170.8, 167.3, 147.2, 144.9, 143.6, 133.8, 121.6, 112.4, 54.9, 52.7, 51.1, 38.7, 30.2, 28.0, 26.7, 25.6, 22.7.

Analytical HPLC: homogeneous single peak, retention time = 15.6 min (8−70% B in 30 min). MALDI MS: calcd for $C_{21}H_{28}IN_6O_8S$ ($MH^+$) 651.07, found 651.02.

**Synthesis of 1m.** Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a white powder (15.5 mg, 49%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 9.08 (d, $J$ = 5.0, 1H), 8.68 (d, $J$ = 3.0, 1H), 8.61 (d, $J$ = 7.0, 1H), 8.12 (d, $J$ = 3.0, 1H), 7.90 (s, 3H), 7.58 (s, 1H), 7.27 (d, $J$ = 7.5, 1H), 7.26 (s, 1H), 4.35−4.11 (m, 1H), 4.12−4.04 (m, 1H), 3.96−3.86 (m, 1H), 3.34−3.26 (m, 1H), 3.22−3.12 (m, 1H), 2.80−2.72 (m, 1H), 1.98−1.86 (m, 1H), 1.86−1.74 (m, 2H), 1.68−1.48 (m, 3H), 1.37−1.16 (m, 3H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 171.1, 170.8, 170.5, 167.4, 147.2, 145.2, 143.4, 133.6, 121.1, 112.2, 59.4, 53.5, 51.1, 38.7, 34.4, 34.3, 27.6, 26.7, 25.0, 22.9, 15.2, 10.5. Analytical HPLC: homogeneous single peak, retention time = 16.4 min (8−70% B in 30 min). MALDI MS: calcd for $C_{22}H_{31}IN_6O_6S$ ($M + Na^+$) 657.09, found 657.12.

**Synthesis of 1n.** Rink amide MBHA resin (0.05 mmol, 0.54 mmol/g) was used to prepare this compound. After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a white powder (18.2 mg, 63%). $^1$H NMR (300 MHz, DMSO-$d_6$, 25 °C): $\delta$ 9.37 (d, $J$ = 6.6, 1H), 9.14−9.08 (m, 1H), 8.69 (d, $J$ = 2.7, 1H), 8.16 (d, $J$ = 2.7, 1H), 7.77 (s, 3H), 7.56 (s, 1H), 7.52 (d, $J$ = 6.9, 1H), 7.21 (s, 1H), 4.34 (dd, $J$ = 14.7, 7.5, 1H), 4.19−4.10 (m, 1H), 3.91 (dd, $J$ = 16.5, 7.5, 1H), 3.56−3.45 (m, 2H), 3.02−2.92 (m, 1H), 2.84−2.76 (m, 2H), 1.74−1.65 (m, 2H), 1.61−1.52 (m, 2H), 1.42−1.26 (m, 2H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 171.1, 171.0, 169.2, 168.1, 147.4, 145.2, 143.4, 133.6, 121.1, 113.1, 54.2, 51.5, 43.1, 38.6, 34.3, 28.9, 26.8, 22.2. Analytical HPLC: homogeneous single peak, retention time = 14.2 min (8−70% B in 30 min). MALDI MS: calcd for $C_{18}H_{24}IN_6O_6S$ ($MH^+$) 579.05, found 579.02.

**Synthesis of 2.** TentaGel S RAM resin (0.05 mmol, 0.3 mmol/g) was used to prepare this compound. General procedure was applied to the synthesis of the cyclic peptide. After the macrocyclization step, the peptide−resin was washed with DMF (2×), $H_2O$ (3×), DMF (3×), $H_2O$ (2×), MeOH (3×), and $CH_2Cl_2$ (3×) and then dried in vacuo for 4 h. To the resin phenyl acetylene (26 mg, 5 equiv), $PdCl_2(PPh_3)_2$ {3.5 mg, 10 mol %} and CuI (0.5 mg, 5 mol %) were added in $^iPr_2NH$/DMF (v/v, 1:4). After shaking overnight at room temperature, the reaction mixture was drained and the resin was rinsed with DMF (4×), $H_2O$ (3×), DMF (3×), $H_2O$ (2×), MeOH (3×), and $CH_2Cl_2$ (3×) and 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 4 h. The cleavage solution was separated from the resin by filtration. After most of the cleavage cocktail was evaporated by passing $N_2$, the crude peptide was precipitated using anhydrous ethyl ether, dissolved in $H_2O$, and then lyophilized to give the crude product. Preparative HPLC (SIS system, 8−70% B in 30 min) was carried out to provide a yellow powder (22.9 mg, 68%). After the peptide was cleaved from the resin, the crude material was subjected to preparative HPLC separation and lyophilization to give a white powder (21.2 mg, 68%). $^1$H NMR (500 MHz, DMSO-$d_6$, 25 °C): $\delta$ 11.95 (b, 1H), 9.13 (d, $J$ = 4.5, 1H), 8.46 (d, $J$ = 3.0, 1H), 8.38 (d, $J$ = 8.0, 1H), 8.31 (d, $J$ = 3.0, 1H), 7.69 (s, 3H), 7.62−7.57 (m, 2H), 7.46−7.42 (m, 2H), 7.38 (d, $J$ = 7.5, 1H), 7.23 (s, 1H), 6.86 (s, 1H), 4.66−4.60 (m, 1H), 4.33−4.26 (m, 2H), 4.16−4.07 (m, 2H), 2.73−2.66 (m, 2H), 2.39 (t, $J$ = 8.0, 2H), 2.34−2.28 (m, 1H), 2.12−2.06 (m, 1H), 2.02−1.86 (m, 2H), 1.82−1.75 (m, 1H), 1.58−1.42 (m, 3H), 1.32−1.18 (m, 2H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 173.9, 172.9, 172.5, 172.2, 166.0, 162.5, 142.2, 131.6, 130.9, 129.9, 129.8, 128.9, 124.1, 121.3, 117.7, 96.5, 83.3, 73.8, 69.8, 56.3, 52.5, 30.9, 30.3, 29.3, 26.4, 25.7, 22.7. Analytical HPLC: homogeneous single peak, retention time = 19.4 min (8−70% B in 30 min). MALDI MS: calcd for $C_{30}H_{35}N_6O_9$ ($MH^+$) 623.24, found 623.24.

**Synthesis of 3.** TentaGel S RAM resin (0.05 mmol, 0.3 mmol/g) was used to prepare this compound. General procedure was applied to the synthesis of the cyclic peptide. After macrocyclization step, the peptide−resin was washed with DMF (2×), $H_2O$ (3×), DMF (3×), $H_2O$ (2×), MeOH (3×), and $CH_2Cl_2$ (3×) and then dried in vacuo for 4 h. To the resin 4-methoxyphenyl boronic acid (38 mg, 5 equiv), $PdCl_2$(DPPF) (4.1 mg, 10 mol %) and triethylamine (0.07 mL, 10 equiv) were added in $H_2O$/DMF (v/v, 1:4). After shaking overnight at 40 °C, the reaction mixture was cooled to room temperature and the resin was rinsed with DMF (4×), $H_2O$ (3×), DMF (3×), $H_2O$ (2×), MeOH (3×), and $CH_2Cl_2$ (3×) and 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 4 h. The cleavage solution was separated from the resin by filtration. After most of the cleavage cocktail was evaporated by passing $N_2$, the crude peptide was precipitated using anhydrous ethyl ether, dissolved in $H_2O$, and then lyophilized to give the crude product. After the peptide was cleaved from the resin, the

crude material was subjected to preparative HPLC separation and lyophilization to give a white powder (14.1 mg, 45%). $^1$H NMR (500 MHz, DMSO-$d_6$, 25 °C): $\delta$ 12.22 (b, 1H), 9.23 (d, $J$ = 5.0, 1H), 8.48 (d, $J$ = 8.0, 1H), 8.29 (d, $J$ = 3.0, 1H), 8.22 (d, $J$ = 3.0, 1H), 7.58 (d, $J$ = 8.5, 1H), 7.47 (d, $J$ = 8.0, 1H), 7.22 (s, 1H), 7.45 (d, $J$ = 8.5, 1H), 6.85 (s, 1H), 4.21−4.10 (m, 3H), 3.82−3.78 (m, 1H), 3.62−3.56 (m, 1H), 2.78−2.72 (m, 1H), 2.44−2.38 (m, 2H), 2.07−1.92 (m, 3H), 1.84−1.72 (m, 2H), 1.68−1.60 (m, 1H), 1.58−1.46 (m, 3H), 1.40−1.26 (m, 2H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): $\delta$ 174.1, 172.7, 171.1, 166.5, 159.5, 158.9, 142.6, 135.3, 131.3, 129.8, 127.5, 127.2, 122.4, 114.3, 71.9, 69.8, 56.3, 55.2, 52.8, 51.6, 40.4, 30.8, 30.6, 29.1, 26.5, 25.7, 22.6. Analytical HPLC: homogeneous single peak, retention time = 16.6 min (8−70% B in 30 min). MALDI MS: calcd for $C_{29}H_{37}N_6O_{10}$ ($MH^+$) 629.25, found 629.23.

**NMR Studies.** NMR spectra were recorded on a Varian UnityPlus 500 spectrometer. 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 2.5 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 25−50 °C adjusted in 5 °C increments with an equilibration time of more than 5 min after successive temperature steps.

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 through-space interactions were identified by ROESY spectra, recorded in 512 $t_1$ increments and 32 scans per $t_1$ increment, with 2K data points at $t_2$. ROESY experiments were performed using mixing times of 100, 300, 500 ms; normally a mixing time of 300 ms was superior. 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, Molecular Simulations, Inc.) was used for the molecular simulations performed in this work (compounds **1i**, **2**, **3**). Explicit atom representations were used throughout the study. The residue topology files (RTF) for all the peptidomimetics were built using QUANTA97 (Molecular Simulations, Inc.).

Quenched molecular dynamics simulations were performed using the CHARMm standard parameters. All four molecules were modeled as neutral compounds in a dielectric continuum of 45 (simulating DMSO). Thus, the starting conformers were minimized using 1000 steps of steepest descent (SD) and 3000 steps of the adopted basis Newton−Raphson method (ABNR). The minimized structures were then subjected to heating, equilibration, and dynamics simulation. Throughout, the equations of motions were integrated using the Verlet algorithm with a time step of 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 equilibrated for another 10 ps at 1000 K. 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 using 1000 steps of SD followed by 3000 steps of ABNR until an rms energy derivative of $\leq$0.01 kcal mol$^{-1}$ Å$^{-1}$ was obtained. Structures with energies less than 3.0 kcal mol$^{-1}$ relative to the global minimum were selected for further analysis.

The QUANTA97 package was again used to display, overlay, and classify the selected structures into conformational groups. The best clustering was obtained using a grouping method based on calculation of rms deviation of a subset of atoms; in this study these were the ring backbone atoms. Thus, threshold cutoff values between 0.60 and 0.75 Å were selected to obtain families with reasonable homogeneity. The lowest energy from each family was considered as a typical representative of the family as a whole. Additionally, a second approach was also used to obtain a representation of each family. In this alternative protocol, the coordinates of all the heavy atoms in each family were averaged in Cartesian space. The protons were rebuilt on those heavy atoms using standard geometries for each atom type, and then the resulting structures were minimized using 50−100 steps of SD to smooth the bond lengths and angles.

Finally, interproton distances and dihedral angles from both the lowest energy and the averaged structure were calculated for comparisons with the ROE data.

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**Supporting Information Available.** Tables of 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 Chem3D 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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