Peptide-Heterocycle Hybrid Molecules: Solid-Phase-Supported Synthesis of Substituted *N*-Terminal 5-Aminotetrazole Peptides via Electrocyclization of Peptidic Imidoylazides

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A method for the synthesis of polypeptides modified with a tetrazole ring at the *N*-terminus is described. Reaction of the *N*-terminal amino group of solid-supported peptides with arylisothiocyanates generates thiourea intermediates, which upon treatment with Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide) generate electrophilic carbodiimide functionality. Trapping by the azide anion and electrocyclization of the intermediate imidoylazide generates an aryl-substituted 5-aminotetrazole at the *N*-terminus of the peptide. To prevent competitive cyclization of a neighboring amide N−H into the carbodiimide, there should not be a free N−H at the [X-1] position relative to the activated carbodiimide. Protection of the N−H group at this position or incorporation of a secondary amino acid is thus required for optimal tetrazole formation. Cleavage from the resin releases the hybrid molecules incorporating a 5-aminotetrazole ring conjugated onto a peptidic fragment.

Introduction

Peptidomimicry is a well-established concept in medicinal chemistry. Whether peptidomimetic small molecules elicit the same biological response as the corresponding natural peptides, as in agonists, inhibit, or interfere with the function of the peptide, as for enzyme inhibitors or anatagonists, such compounds are of considerable interest because they can serve as leads for the development of clinically useful compounds. There are a range of approaches that have been used for the creation of small molecules that mimic peptides of biological relevance.[1] These include the use of isosteric replacement strategies for peptide bonds, side-chain modifications, constrained mimics for amino acids or dipeptides, transition state mimics (e.g., hydroxyethylene isosteres), cyclic peptides, peptoids, and so-called privileged substructures.[2] Another strategy is the fusion of a small-molecule fragment with a peptide motif to create hybrid molecules. Such fragments include aromatic rings or heterocycles,[3] which may be positioned in the interior of the peptide chain,[4] at the *C*-terminus,[5] or at the *N*-terminus.[6] The small-molecule fragments may confer stabilized or unusual three-dimensional structures on these hybrid molecules, leading to improvements in ligand binding or inhibition, relative to the peptide. Alternatively, these hybrid molecules may display improved ADMET properties.

One of the goals of our research program is the development of a synthetic toolbox suitable for the creation of such hybrid molecules, particularly those comprising heterocyclic fragments. For example, we have recently disclosed a method for the creation of *N*-terminal 2-iminohydantoin peptide hybrids.[7] Such peptide-heterocycle molecules may have biological properties suitable for use as biological tools or serve as lead molecules in medicinal chemistry programs. Moreover, further replacement of the peptidic portion of peptide-heterocycle molecules would provide a route to either minimally or entirely non-peptidic small molecules. We now report a strategy for the synthesis of *N*-terminal 5-aminotetrazole peptide hybrid molecules based on a protocol for the synthesis of aminotetrazoles from thioureas. Tetrazoles are well established not only as important heterocycles in medicinally and biologically active compounds,[8] both as carboxylate isosteres,[9] but also as important structural elements.[10] For example, disubstituted tetrazoles have been incorporated into biologically active peptides such as bradykinin.[11] The importance of tetrazoles in drug discovery provided us with a further incentive to investigate structurally novel *N*-terminal aminotetrazole peptide hybrid molecules.[12]

Results and Discussion

Previous results from our laboratory have outlined a general solution-phase synthesis of substituted 5-aminotetrazoles from thioureas.[13] Thus, nucleophilic addition of primary or secondary amines **1** to isothiocyanates **2** lead to thioureas **3**. Treatment of **3** with sodium azide in the presence of a Hg(II) salt leads to intermediate imidoylazides **4** which undergo rapid electrocyclization to the 5-aminotetrazoles **5** (Scheme 1).[14] This solution-phase protocol provides a convenient route to mono-, di-, and trisubstituted 5-aminotetrazoles **5** and is also suitable for the synthesis of amino acid-derived 5-aminotetrazoles. In the case of the monosubstituted derivatives **5** (i.e., R^1 and $R^3 \neq H$, $R^2 = H$), derived from primary amines **1**, it is likely that carbodiimides are involved as intermediates.

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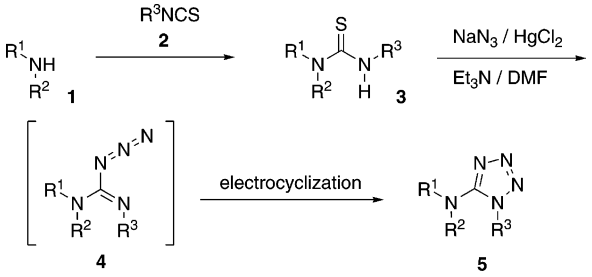
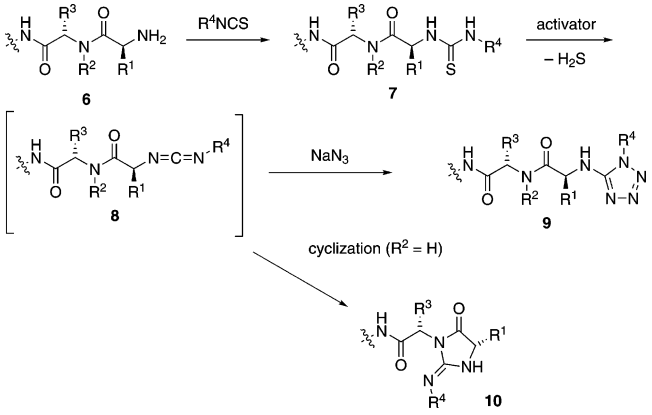
Scheme 1. Formation of Substituted 5-Aminotetrazoles **5** from Thioureas **3**



Scheme 2. Formation of Peptidic *N*-Terminal 5-Aminotetrazoles **9** and Iminohydantoins **10**

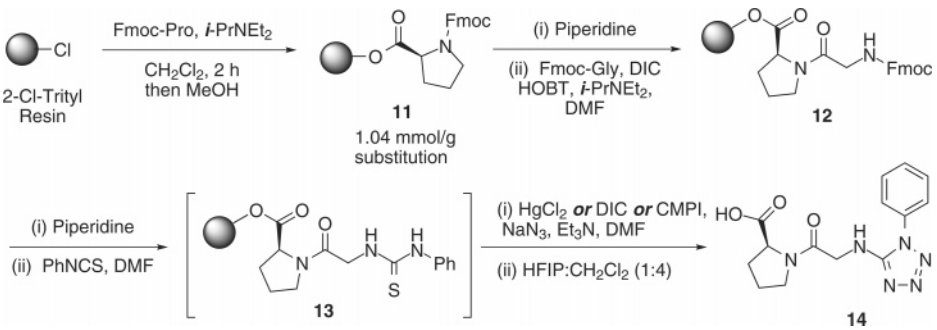


Given the success of this reaction protocol for the synthesis of amino acid-derived 5-aminotetrazoles, we became interested in its application to the formation of peptide-aminotetrazole hybrid molecules using a polymer-supported strategy. Thus, we envisioned an approach in which a peptide **6**, on treatment with isothiocyanate, would generate the peptidic thiourea **7** (Scheme 2). Elimination of H_2S from **7** would then give the peptidic carbodiimide **8**. Nucleophilic trapping of **8** by azide anion and subsequent electrocyclization would then give the *N*-terminal peptidic tetrazole **9**. We have previously demonstrated that in situ-generated peptidic carbodiimides **8** ($R^2 = H$) undergo cyclization through nucleophilic attack of the neighboring amide group to give *N*-terminal iminohydantoin peptide hybrid molecules **10**. The formation of **10** via this approach is equivalent to a diversion of the classic Edman degradation reaction,[15] which proceeds via intramolecular cyclization of peptidic thiourea **7**. The successful synthesis of the *N*-terminal peptidic tetrazole **9** would therefore require that nucleophilic attack by azide anion on **8** occur more rapidly than cyclization to **10**. Although the azide anion is an excellent nucleophile, substitution of the neighboring amide nitrogen (i.e., $R^2 \neq H$) would prevent cyclization to **10**. Accordingly, for the present study, we elected to employ only amide substituted peptides **6** ($R^2 \neq H$), that is, substrates incorporating secondary amino acid in the [X-1] position relative to the thiourea functionality. We were particularly interested in the incorporation of a proline-rich peptide sequence into our library as it could potentially target signal transduction pathways mediated by SH3 and WW domains.[16] In principle, the use of a primary amino acid at the [X-1] position relative to the carbodiimide/thiourea functionality would be possible with an appropriate orthogonal protecting group,[17] such as, for example, allyloxycarbonyl[18] or 2-hydroxybenzyl groups.[19]

The 2-chlorotrityl resin was chosen as the polymer support using standard Fmoc-coupling protocols.[20] 2-Chlorotrityl chloride resin can be used as a support for the simple attachment of the first amino acid without special activation of resin. This support is also convenient because the final compounds may be readily cleaved with hexafluoroisopropanol (HFIP) in a short period of time. The facile cleavage conditions also allow reaction conversion to be evaluated by TLC (or LCMS) by cleavage of a small portion of the resin. Finally, the use of 2-chlorotrityl resin also minimizes the formation of diketopiperazines which can occur after attachment and cleavage of the second amino acid using polymer supports such as Merrifield and Wang resins.[21]

An initial evaluation of the approach was undertaken for the synthesis of the dipeptide-aminotetrazole hybrid molecule **14** (Scheme 3). The reaction of the 2-chlorotrityl resin with Fmoc-proline in the presence of Hünig's base and capping of the unreacted trityl chloride resin with MeOH provided resin-bound Fmoc-proline **11** with 1.04 mmol/g substitution. Loading of **11** was determined by UV spectrometric analysis of the Fmoc-piperidine adduct. Removal of the Fmoc group and coupling to Fmoc-glycine gave the resin-supported Fmoc-protected dipeptide **12** with complete conversion as shown by TLC analysis. Peptide coupling was achieved using standard diisopropylcarbodiimide/hydroxybenzotriazole coupling conditions. Deprotection of **12** with piperidine, followed by treatment with phenylisothiocyanate gave the resin bound thiourea **13**, which was immediately treated with a dehydrothiolation agent in the presence of sodium azide and triethylamine. Finally, cleavage from the resin by treatment with hexafluoroisopropanol (HFIP) for 10 min afforded the dipeptide-aminotetrazole hybrid molecule **14**. Three different dehydrothiolation conditions were evaluated. In the case of $HgCl_2$, as originally employed in our solution-phase protocol,

Scheme 3. Solid-Supported Synthesis of Dipeptide 5-Aminotetrazole Hybrid **14**



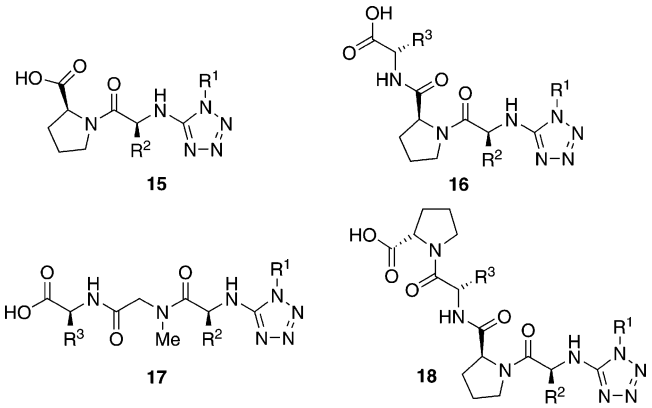


Figure 1. Library of dipeptide, tripeptide, and tetrapeptide derived 5-aminotetrazole hybrid molecules **15**−**18**.

product **14** was isolated, after a simple filtration through Celite, in 97% isolated yield and ≥98% purity (as determined by HPLC analysis). It should be noted that Yu and Houghten have previously adapted the solution-phase $HgCl_2$ protocol to a solid-supported synthesis of substituted 5-aminotetrazoles.[22] The use of diisopropylcarbodiimide (DIC) gave the product with ≥95% purity but with variable yields. Finally, the use of Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide or CMPI) reproducibly afforded the product **14** in ≥95% isolated yield and with purities of ≥90%. In addition, Mukaiyama's reagent had also worked well for the synthesis of **10**. Thus, although our solution-phase 5-aminotetrazole synthesis had employed $HgCl_2$ as the dehydrothiolation reagent, Mukaiyama's reagent was chosen as the reagent of choice for the remaining studies because it avoids the possibility of product contamination by toxic mercury residues.[23] Moreover, the peptide-aminotetrazole hybrid products are readily isolated in excellent yields and high purities by simply evaporating the solvents after HFIP-promoted cleavage from the resin.

Library Synthesis. Our aim was to construct a small library of peptide-aminotetrazole hybrid molecules, synthesized as discrete molecules, rather than mixtures. Houghten's "tea-bag" technology was chosen for library construction because of its operational simplicity and convenience for multistep synthesis.[24] It is a convenient and simple way to produce libraries of any desired size, whether as discrete compounds or as mixtures, using a split and mix approach. The resin beads are placed inside sealed and labeled polypropylene mesh pockets or tea-bags, which are then suitable for multistep synthesis over a wide range of reaction conditions. To evaluate the robustness of the method, our aim was to synthesize each library member in 50−100 mg quantities. Sets of 30 dipeptide-aminotetrazole hybrid molecules **15**, 30 tripeptide-aminotetrazole hybrid molecules **16** and **17**, and 128 tetrapeptide-aminotetrazole hybrid molecules **18** were synthesized (Figure 1). The combined library of **15**−**18** was constructed from eleven different isothiocyanates and nine different Fmoc-protected amino acids. The isothiocyanates used had phenyl, *o*-, *m*-, and *p*-tolyl, 2-chlorophenyl, 2- and 3-bromophenyl, 2-iodophenyl, 3,4,5-trimethoxyphenyl, 4-bromo-2-trifluoromethylphenyl, and 4-nitrophenyl substituents (R^1). The product molecules are encoded using the numbers 1−11 respectively for the R^1 substituents. The Fmoc-protected amino acids used were derived from glycine, alanine, leucine, phenylalanine, methionine, tryptophan, tyrosine, proline, and sarcosine. In the case of tyrosine, the phenolic group was protected as the *t*-butyl ether. The product molecules are encoded using the standard one-letter amino acid codes[25] and are ordered from the *N*- to *C*-termini. Thus, for example the compound **14** (i.e., Ph-aminotetrazole-Gly-Pro-OH) is equivalent to **15-*1GP***.

2-Chlorotrityl resin was used as the polymer support. Attachment of the first Fmoc-protected amino acid to the 2-chlorotritylchloride resin was conducted using excess Hünig's base to give the product *N*-Fmoc-protected amino acids bound to the resin through an ester linkage. Unreacted chloride groups were capped by treatment with methanol at the end of the reaction, and loading was determined by UV spectrometric analysis of the Fmoc-piperidine adduct. Deprotection of the Fmoc group was accomplished using 20% piperidine in DMF. Standard Fmoc-based peptide synthesis protocols were employed for the attachment of further amino acids. Thus, iterative cycles of DIC/HOBt coupling with Fmoc-protected amino acids, followed by Fmoc cleavage using a 20% piperidine solution in DMF, were used to give the corresponding resin-bound polypeptides. The aminotetrazole formation was conducted by coupling of the free amino termini of the resin-supported peptide with the arylisothiocyanates (5 equiv) in DMF over 10−30 min. Treatment of the resultant intermediate thioureas with Mukaiyama's reagent (5 equiv) in the presence of excess triethylamine in DMF, followed by reaction of the carbodiimide intermediates with excess sodium azide overnight, affords the resin-supported aminotetrazole peptide hybrids. It is essential that the intermediate thioureas be rapidly converted to the carbodiimides, to minimize the possibility of competing Edman degradation. Depending upon the composition of the resin-supported aminotetrazole peptide hybrids, different cleavage cocktails were used to give the products **15**−**18**. Most of the compounds were cleaved using a HFIP/CH_2Cl_2 (1:4 v/v) cleavage cocktail for 30 min. The resin-bound compounds containing Tyr(^{t}Bu) residues were subjected to the cleavage cocktail of TFA/EDT/Anisole (95/2.5/2.5 v/v) for 35 min. The resin-bound compounds containing both Met and Tyr(^{t}Bu) residues were cleaved using a TFA/TIS (95:5 v/v) mixture for 30 min. The cleaved products were processed in parallel by filtration through a short plug of silica gel using MeOH, followed by concentration in a centrifugal evaporator and drying in vacuo. Further purification procedures were not applied. The products of the 188-compound library were obtained in high yields and good to excellent purities. An average purity of 91% for the 5-aminotetrazole peptide hybrids **15**−**18** was determined by LCMS analysis. A small sample of the library was also independently analyzed by ^{1}H and ^{13}C NMR to confirm identity and purity. A more detailed analysis of library purity according to sublibraries is given in Table 1 in the Supporting Information. Compounds containing tyrosine residues were

observed to have slightly lower purities, presumably because of the modification of cleavage/deprotection conditions required. Compounds containing tryptophan residues also have slightly lower purities. The choice of isothiocyanate has little influence on yield and purity outcome. An average purity of 95% was observed for the subset of dipeptide 5-aminotetrazole hybrids (30 compounds), 90% average purity in tripeptide 5-aminotetrazole subset (30 compounds), and 89% in tetrapeptide 5-aminotetrazole subset (128 compounds). A potential concern in peptide synthesis is the possibility of epimerization of the side-chains on the amino acid residues. We examined the possibility of epimerization by independently synthesizing a series of control di- tri-, and tetrapeptide containing tetrazole hybrids containing both L- and D-phenylalanine moieties. HPLC analysis of these compounds (e.g., Ph-aminotetrazole-(L *or* D)-Phe-Pro-Ala-OH) showed discrete resolved peaks for each pair of diastereomeric compounds using a Zorbax SB-C18 column. In every case examined, no detectable epimerization had occurred.

Conclusion

N-Terminal peptidic-aminotetrazole hybrid molecules can be readily synthesized using a polymer-supported approach. The key aminotetrazole-forming step occurs via reaction of the *N*-terminal amino group with an arylisothiocyanate to give a thiourea intermediate, which upon dehydrothiolation gives a peptidic carbodiimide. Subsequent nucleophilic trapping by sodium azide and electrocyclization then gives the 5-aminotetrazole group. The use of Mukaiyama's reagent for the dehydrothiolation step avoids the use of toxic Hg(II) salts, as employed in a previously disclosed solution-phase synthesis of 5-aminotetrazoles. The products were obtained in good yields and purities with minimal purification, and epimerization was not observed. Furthermore, the use of a solid-phase strategy and the commercially availability of the building blocks renders this approach suitable for the synthesis of both libraries and more targeted or complex examples of peptidic aminotetrazoles. Further studies on the generation of peptide-heterocycle hybrid molecules and their biological evaluation will be reported in due course.

Experimental Section

All amino acids and the 2-chlorotritylchloride resin were purchased from Calbiochem (NovaBiochem, San Diego, CA). Isothiocyanates, piperidine, and solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI). All chemicals were used as such without any further purification. ^{1}H and ^{13}C NMR spectra were obtained using 300, 400, and 500 MHz Varian Unity spectrometers. All HPLC analyses were performed using a Hewlett-Packard series 1100 HPLC operating with G1310A isopump and ZORBAX SB-C18 column (cartridge 4.6 × 15 mm, particle diameter 3.5 μm, pH range of 1−6). A solvent gradient of 90:10 H_2O/MeOH (with 0.1% of TFA) to 10:90 H_2O/MeOH over a period of 10 min, followed by 5 min at 10:90 H_2O/MeOH, was used. The HP pump delivered solvents to the source at a flow rate of 0.75 mL/min with a sample injection of 10 μL. The retention time (R_t) for the expected (major) product was recorded. Fmoc-protected sarcosine was prepared according to published method from unprotected sarcosine and Fmoc-chloride.[26]

General Procedure for the Loading of Fmoc-Pro-OH or (Fmoc-Ala-OH) onto the 2-Chlorotritylchloride Resin. Hünig's base (5 equiv) was added at room temperature to a suspension of Fmoc-Pro-OH (1.5 equiv) in dry CH_2Cl_2 (15 mL per mmol of resin), and the mixture was stirred for 5 min before addition of 2-chlorotritylchloride resin (1 equiv, based on the resin substitution). The suspension was stirred for 3 h, and reagent grade MeOH (1.5 mL per mmol) was added; the mixture was stirred for 15 min to end-cap the remaining traces of 2-chlorotrityl groups on the resin. The resin was filtered and washed with equal volumes of CH_2Cl_2 (3 × 10 mL/mmol), DMF (3 × 10 mL/mmol), CH_2Cl_2 (3 × 10 mL/mmol), and Et_2O (2 × 10 mL/mmol); then it was dried in vacuo until no further mass change was observed. The mass of the substituted resins **11/19** corresponded to a quantitative substitution by mass balance. Substitution measurement by UV spectrometric analysis of the Fmoc-piperidine adduct[27] gave an average substitution of 1.37 mmol/g for the Pro-loaded resin (initial substitution of chlorotrityl chloride resin 1.4 mmol Cl/g). The substituted resin was then packed into labeled polypropylene mesh tea-bags (140 mg each) and sealed using a manual sealer.

Typical Procedure for Cleavage of Fmoc Protecting Group from Resin 11/19 to give 20. Polypropylene tea-bags with Fmoc-Pro-trityl or Fmoc-Ala-trityl resin (1 equiv) were placed into a 250 mL polypropylene bottle and suspended in dry CH_2Cl_2 (15 mL/mmol) for 20 min to swell the resin. After the CH_2Cl_2 was drained, a solution of 20% (v/v) piperidine in DMF (15 mL/mmol) was added, and the bottle was agitated for 30 min on a rotary shaker. After the solution was drained, the tea-bags were washed thoroughly with DMF (4 × 10 mL/mmol) and CH_2Cl_2 (4 × 10 mL/mmol) to afford either Pro-trityl or Ala-trityl resin **20**. Then the tea-bags were subjected to the next synthetic step.

Typical Procedure for Iterative Coupling of Fmoc-Protected Amino Acids to 20 (or 21) and Subsequent Fmoc Removal to give Peptide-trityl Resin 21. Tea-bags with Pro-trityl or Ala-trityl resin **20** or peptide-trityl resin **21** (1 equiv) were placed into a 250 mL polypropylene bottle and suspended in DMF (15 mL/mmol). Hünig's base (8 equiv), HOBT (3 equiv), and Fmoc-A^n-OH (3 equiv) were then added, and the reaction mixture was agitated for 4 h using a rotary shaker. If required, reaction progress could be monitored by cleavage of a small amount of the resin (~1 mg) in HFIP/CH_2Cl_2 (1:4, 50 μL) for 10 min, followed by TLC or LCMS analysis. After the solution was drained, the resin was washed with DMF (3 × 10 mL/mmol) and CH_2Cl_2 (4 × 10 mL/mmol). If not taken directly to the next step, the tea-bags with resin were allowed to air-dry, followed by drying in vacuo using a vacuum pump. Otherwise, immediately after the coupling step, removal of the Fmoc group was then accomplished as for the formation of **20** to yield **21**. Subsequent couplings and Fmoc protecting group removal were accomplished using the same protocol in an iterative manner.

Typical Procedure for the Formation of 5-Aminotetrazole-polypeptide-trityl Resin 22 from 21. The polypetide-trityl resin-containing tea-bags (1 equiv) were suspended in DMF (15 mL/mmol), and aryl isothiocyanate (5 equiv) was added. The polypropylene reaction vessel was then shaken for 20 min by rotary shaker. After the solution was drained, the tea-bags with resin were washed with DMF (3 × 10 mL/mmol) and CH_2Cl_2 (3 × 10 mL/mmol) to give the intermediate trityl resin-supported thioureas, which were immediately used in the next step. The freshly formed trityl resin-supported thiourea tea-bags (1 equiv) were placed into DMF (15 mL/mmol). Triethylamine (10 equiv) and Mukaiyama's reagent (5 equiv) were then added, and the reaction mixture was agitated for 3 h by rotary shaker. Sodium azide (10 equiv) was then added, and the reaction vessel was shaken overnight. The resulting dark orange solution was drained, and the resin was thoroughly washed with DMF (5 × 10 mL/mmol), CH_2Cl_2 (5 × 10 mL/mmol), DMF (3 × 10 mL/mmol), CH_2Cl_2 (5 × 10 mL/mmol), and Et_2O (3 × 10 mL/mmol) and dried in vacuo using vacuum pump to give the 5-aminotetrazole-polypeptide-trityl resin **22**. Again if required, reaction progress could be monitored at any stage by cleavage of a small amount of the resin (~1 mg) in HFIP/CH_2Cl_2 (1:4, 50 μL) for 10 min, followed by TLC or LCMS analysis.

Cleavage Procedures. Each tea-bag was placed into separate 20 mL scintillation vials of known weight. Three different cleavage cocktails were used:

(a) Most of the compounds **22** were cleaved using a HFIP/CH_2Cl_2 (1:4 v/v) cocktail. Five milliliters of the cleavage cocktail was added to each tea-bag, and the vial was agitated by rotary shaker for 30 min.

(b) Resin-bound compounds **22** containing Tyr(^{t}Bu) residues were cleaved using a TFA/EDT/Anisole (95/2.5/2.5 v/v) cocktail. Five milliliters of the cleavage cocktail was added to each tea-bag, and the vial was agitated by rotary shaker for 35 min.

(c) Resin-bound compounds **22** containing both Met and Tyr(^{t}Bu) residues were cleaved using a TFA/TIS (95:5 v/v) cocktail. Five milliliters of the cleavage cocktail was added to each tea-bag, and the vial was agitated by rotary shaker for 30 min.

Following cleavage, the tea-bag with resin was removed from the vial and washed with CH_2Cl_2 (3 × 2 mL); all the organic filtrates were combined and concentrated. The resulting bright-yellow residues were dissolved in MeOH (1 mL) and filtered through a short plug of silica gel using additional 10 mL of MeOH. The filtrate was concentrated using a Genevac HT-4X and dried in vacuo to give the products **15**−**18** which were analyzed and used without further purification. Selected examples of di-, tri-, and tetrapeptide-containing aryl-substituted 5-aminotetrazoles **15**−**18** were characterized by IR, ^{1}H NMR, ^{13}C NMR, and MS.

1-Phenyl-1H-tetrazol-5-ylamino-Gly-Pro-OH (14 or 15-*1GP*). Yield: 39 mg (89%). IR (film): ν 3445, 2966, 2908, 1647, 1634, 1557, 1455, 1403, 1317, 1247, 761, 695 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz, rotamers): δ 7.53−7.65 (m, 4H), 6.88−6.96 (m, 1H), 3.97−4.24 (m, 3H), 3.29−3.56 (m, 2H), 1.67−2.12 (m, 4H). ^{13}C NMR (CD_3OD, 75 MHz): δ 176.1, 170.0, 169.5, 156.7, 134.7, 131.3, 131.1, 125.7, 61.1, 47.4, 46.8, 32.7, 30.3, 25.8, 23.5. MS (ESI, M + H$^+$): 317.1. HRMS (ESI) Calcd for (M + H$^+$) $C_{14}H_{16}N_6O_3$: 317.1356. Observed: 317.1356.

1-*o*-Tolyl-1H-tetrazol-5-ylamino-Leu-Pro-OH (15-*2LP*). Yield: 49 mg (91%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (s, 1H), 7.28−7.54 (m, 4H), 4.93−4.99 (m, 1H), 4.74−4.82 (m, 1H), 4.44−4.56 (m, 2H), 3.96−4.02 (m, 1H), 3.60−3.68 (m, 1H), 2.08−2.22 (m, 5H), 1.76−1.86 (m, 1H) 1.54−1.60 (m, 2H), 0.88−1.08 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.2, 172.7, 155.6, 136.2, 131.9, 131.4, 127.6, 127.1, 59.5, 54.3, 47.2, 41.3, 28.4, 24.9, 24.7, 23.3, 21.6, 17.5. MS (ESI, M + H$^+$): 387.2.

1-(2-Bromophenyl)-1H-tetrazol-5-ylamino-Leu-Pro-OH (15-*6LP*). Yield: 61 mg (97%). mp: 114−120 °C (uncorrected). IR (film): ν 3251, 2957, 2871, 1715, 1644, 1606, 1449, 1192, 1087, 764, 646 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (s, 1H), 7.81−7.82 (m, 1H), 7.46−7.58 (m, 3H), 4.92−4.98 (m, 1H), 4.78−7.86 (m, 1H), 4.52−4.57 (m, 1H), 4.42−4.51 (m, 1H), 3.97−4.03 (m, 1H), 3.62−3.68 (m, 1H), 2.08−2.28 (m, 4H), 1.52−1.66 (m, 2H), 0.92−1.06 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.1, 155.3, 134.4, 132.6, 129.6, 129.2, 121.6, 59.7, 54.3, 47.4, 41.6, 28.0, 25.0, 24.7, 23.4, 21.7. HRMS (ESI) Calcd for (M + H$^+$) $C_{18}H_{24}BrN_6O_3$: 451.1088. Observed: 451.1087.

1-(3,4,5-Trimethoxyphenyl)-1H-tetrazol-5-ylamino-Leu-Pro-OH (15-*9LP*). Yield: 63.7 mg (95%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.79 (s, 2H), 5.30−5.36 (m, 1H), 4.78−4.86 (m, 1H), 4.48−4.52 (m, 1H), 3.92−3.98 (m, 11H), 3.62−3.68 (m, 2H), 2.12−2.22 (m, 2H), 1.86−1.72 (m, 1H), 1.56−1.74 (m, 3H), 0.98−1.10 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.5, 171.0, 154.9, 154.3, 154.2, 128.5, 101.5, 61.0, 59.8, 56.5, 54.6, 47.3, 41.3, 28.1, 24.9, 23.4, 21.7. MS (ESI, M + H$^+$): 463.1.

1-Phenyl-1H-tetrazol-5-ylamino-Phe-Pro-Ala-OH (16-*1FPA*). Yield: 65 mg (97%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.10−7.90 (m, 11H), 4.60−4.72 (m, 1H), 4.28−4.38 (m, 1H), 3.96−4.12 (m, 2H), 2.68−2.82 (m, 1H), 1.74−2.14 (m, 4H), 1.58−1.72 (m, 2H), 1.15−1.40 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 176.4, 172.3, 171.7, 154.3, 135.5, 133.2, 129.9, 129.5, 129.4, 128.7, 128.6, 127.6, 123.5, 61.2, 57.6, 48.4, 47.3, 38.2, 29.1, 24.2, 17.1. MS (ESI, M + H$^+$): 478.2.

1-Phenyl-1H-tetrazol-5-ylamino-(D)Phe-Pro-Ala-OH (16-*1(D)FPA*). Yield: 62 mg (93%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.10−7.94 (m, 11H), 4.59−4.71 (m, 1H), 4.26−4.38 (m, 1H), 3.94−4.08 (m, 2H), 2.66−2.78 (m, 1H), 1.78−2.10 (m, 4H), 1.58−1.68 (m, 2H), 1.19−1.32 (m, 4H). MS (ESI, M + H$^+$): 478.2.

1-Phenyl-1H-tetrazol-5-ylamino-Gly-Pro-Ala-OH (16-*1GPA*). Yield: 52 mg (95%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.30−7.58 (m, 5H), 4.39 (br s, 1H), 4.13−4.30 (m, 3H), 3.47−3.65 (m, 2H) 1.88−2.24 (m, 5H), 1.36−1.38 (m, 3H). MS (ESI, M + H$^+$): 388.2.

1-(2-Bromophenyl)-1H-tetrazol-5-ylamino-Phe-Pro-Ala-OH (16-*6GPA*). Yield: 76 mg (98%). mp: 193−200 °C (uncorrected). IR (film): ν 3382, 2924, 1594, 1455, 1094, 757, 700 cm^{-1}. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.82−7.88

(m, 1H), 7.52−7.60 (m, 2H), 7.16−7.38 (m, 6H), 4.76−4.82 (m, 1H), 4.42−4.48 (m, 1H), 4.14−4.22 (m, 1H), 3.90−4.00 (m, 1H), 3.62−3.72 (m, 1H), 3.26−3.36 (m, 1H), 2.96−3.02 (m, 1H), 2.00−2.24 (m, 3H), 1.30−1.48 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 178.8, 171.6, 171.2, 155.2, 137.1, 133.8, 132.4, 131.9, 129.2, 128.8, 128.1, 126.4, 121.6, 78.2, 60.7, 57.4, 50.5, 36.9, 29.0, 24.7, 17.7. HRMS (ESI) Calcd for (M + H$^+$) $C_{24}H_{27}BrN_7O_4$: 556.1315. Observed: 556.1302.

1-Phenyl-1H-tetrazol-5-ylamino-Leu-Sar-Ala-OH (17-*1LSarA*). Yield: 57 mg (98%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.48−7.6.0 (m, 5H), 5.43 (d, 1H, J = 8.2 Hz), 4.80−4.86 (m, 1H), 4.26−4.38 (m, 2H), 3.78 (d, 1H, J = 16.5 Hz), 3.24 (s, 3H), 1.76−1.84 (m, 1H), 1.58−1.66 (m, 1H), 1.46−1.54 (m, 2H), 1.32 (d, 3H, J = 7.1 Hz), 0.98 (m, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 177.1, 174.1, 168.5, 154.8, 132.9, 130.3, 130.1, 124.0, 53.2, 51.9, 49.2, 40.8, 24.9, 23.2, 21.3, 17.5. MS (ESI, M + H$^+$): 418.2.

1-Phenyl-1H-tetrazol-5-ylamino-Leu-Pro-Gly-Pro-OH (18-*1LPGP*). Yield: 72 mg (98%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38−7.60 (m, 5H), 4.62−4.74 (m, 1H), 4.32−4.48 (m, 2H), 3.88−4.14 (m, 2H), 3.04−3.54 (m, 6H), 1.47−2.23 (m, 11H), 0.93−1.04 (m, 6H). MS (ESI, M + H$^+$): 527.2.

1-Phenyl-1H-tetrazol-5-ylamino-Phe-Pro-Phe-Pro-OH (18-*1FPFP*). Yield: 87 mg (96%). mp: 128−132 °C (uncorrected). IR (film): ν 3410, 3060, 2954, 1722, 1644, 1499, 1453, 1343, 1183, 1101, 918, 760, 734, 700 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.14−7.48 (m, 16H), 4.50−5.00 (m, 5H), 2.80−3.70 (m, 8H), 1.48−2.18 (m, 8H). HRMS (ESI) Calcd for (M + H$^+$) $C_{35}H_{38}N_8O_5$: 651.3073. Observed: 651.3064.

1-Phenyl-1H-tetrazol-5-ylamino-Met-Pro-Gly-Pro-OH (18-*1MPGP*). Yield: 75 mg (96%). mp: 135−140 °C (uncorrected). IR (film): ν 33833, 2921, 1716, 1644, 1455, 1409, 1197, 1097, 1020, 764, 735, 697 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.42−7.60 (m, 5H), 4.80−4.90 (m, 1H), 4.36−4.44 (m, 1H), 3.68−4.30 (m, 8H), 2.62−2.68 (m, 2H), 1.80−2.14 (m, 14H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 177.6, 176.2, 170.8, 167.6, 154.8, 133.5, 131.5, 129.9, 129.6, 124.2, 123.6, 61.1, 60.4, 55.1, 47.8, 46.4, 42.0, 31.2, 30.5, 29.4, 25.3, 24.7, 15.7. HRMS (ESI) Calcd for (M + H$^+$) $C_{23}H_{32}N_8O_5S$: 545.2276. Observed: 545.2289.

1-m-Tolyl-1H-tetrazol-5-ylamino-Phe-Pro-Leu-Pro-OH (18-*3FPLP*). Yield: 85 mg (96%). mp: 120−122 °C (uncorrected). IR (film): ν 3403, 2957, 2874, 1716, 1644, 1454, 1285, 1182, 1101, 893, 787, 735, 701 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.06 (d, 1H, J = 7.7 Hz), 7.25−7.55 (m, 8H), 4.44−4.64 (m, 3H), 4.25−4.26 (m, 1H), 3.44−3.88 (m, 5H), 3.04−3.12 (m, 2H), 2.43 (s, 3H), 1.24−2.16 (m, 10H), 1.08 (d, 6H, J = 4.9 Hz). HRMS (ESI) Calcd for (M + H$^+$) $C_{33}H_{43}N_8O_5$: 631.3370. Observed: 631.3350.

1-*m*-Tolyl-1H-tetrazol-5-ylamino-Trp-Pro-Gly-Pro-OH (18-*3WPGP*). Yield: 78 mg (91%). mp: 165−170 °C (uncorrected). IR (film): ν 3390, 2924, 1652, 1455, 1100, 788, 742, 696 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.22 (s, 1H), 7.03−7.83 (m, 9H), 3.15−4.75 (m, 12H), 2.36 (s, 3H), 1.23−2.04 (m, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 178.4, 172.2, 167.6, 154.3, 140.2, 136.2, 133.1, 130.1, 129.6, 127.4, 125.6, 124.5, 121.7, 120.3, 119.5, 118.1, 111.8, 108.0, 61.5, 60.3, 56.0, 47.6, 46.3, 41.9, 29.7, 28.9, 27.7, 24.8, 24.6, 21.2. HRMS (ESI) Calcd for (M + H$^+$) $C_{31}H_{36}N_9O_5$: 614.2833. Observed: 614.2833.

1-*p*-Tolyl-1H-tetrazol-5-ylamino-Tyr-Pro-Tyr-Pro-OH (18-*4YPYP*). Yield: 89 mg (91%). mp: 154−158 °C (uncorrected). IR (film): ν 3418, 2923, 1652, 1634, 1614, 1385, 1233, 1156, 820, 701 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz, rotamers): δ 9.23 (d, 1H, J = 18.5 Hz), 8.02 (d, 0.1H, J = 7.9 Hz), 7.72 (d, 0.9H, J = 7.9 Hz), 6.59−7.53 (m, 12H), 4.21−4.57 (m, 3H), 2.64−3.76 (m, 9H), 2.47 (s, 3H), 1.14−2.25 (m, 8H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.8, 171.5, 170.9, 169.9, 156.4, 156.3, 155.1, 139.7, 131.0, 130.8, 130.7, 128.6, 128.0, 124.6, 115.5, 115.5, 115.4, 60.0, 59.2, 58.4, 53.7, 46.9, 46.6, 42.2, 33.0, 31.3, 29.1, 28.1, 25.0, 21.2. HRMS (ESI) Calcd for (M + H$^+$) $C_{36}H_{41}N_8O_7$: 697.3121. Observed: 697.3092.

1-*p*-Tolyl-1H-tetrazol-5-ylamino-Met-Pro-Trp-Pro-OH (18-*4MPWP*). Yield: 87 mg (91%). mp: 145−150 °C (uncorrected). IR (film): ν 3333, 2921, 1644, 1520, 1455, 1182, 1098, 821, 740 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz, rotamers): δ 10.88 (s, 0.6H), 10.85 (s, 0.4H), 8.08 (d, 0.67H, J = 7.7 Hz), 7.84 (d, 0.33H, J = 7.7 Hz), 6.90−7.53 (m, 9H), 4.22−4.52 (m, 3H), 2.85−3.79 (m, 10H), 2.53−2.67 (m, 3H), 2.40 (s, 3H), 1.23−2.15 (m, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.8, 171.7, 171.0, 154.6, 140.2, 136.0, 130.9, 130.7, 130.5, 127.8, 123.8, 121.6, 119.3, 118.2, 111.4, 61.3, 60.3, 54.8, 51.7, 47.5, 31.3, 31.0, 30.2, 29.7, 29.4, 28.6, 25.0, 21.2, 15.8. HRMS (ESI) Calcd for (M + H$^+$) $C_{34}H_{42}N_9O_5S$: 688.3041. Observed: 688.3024.

1-*p*-Tolyl-1H-tetrazol-5-ylamino-Trp-Pro-Met-Pro-OH (18-*4WPMP*). Yield: 94 mg (94%). mp: 150−155 °C (uncorrected). IR (film): ν 3306, 2921, 1682, 1652, 1455, 1182, 1100, 820, 737, 701, 685 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz, rotamers): δ 10.96 (s, 0.3H), 10.91 (s, 0.7H), 8.15 (d, 1H, J = 7.7 Hz), 7.56 (d, 1H, J = 7.9 Hz), 7.23−7.40 (m, 6H), 6.96−7.08 (m, 3H), 3.14−4.69 (m, 11H), 2.51−2.56 (m, 2H), 2.41 (s, 3H), 1.23−2.09 (m, 11H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.1, 172.0, 171.7, 170.3, 154.2, 139.7, 136.1, 130.9, 130.5, 130.3, 127.8, 125.0, 124.5, 124.1, 123.5, 121.8, 119.6, 118.3, 111.5, 61.0, 60.4, 56.3, 49.8, 47.6, 47.0, 31.4, 30.1, 29.8, 29.1, 27.3, 25.2, 24.8, 21.2, 15.5. HRMS (ESI) Calcd for (M + H$^+$) $C_{34}H_{42}N_9O_5S$: 688.3053. Observed: 688.3024.

1-(3-Bromophenyl)-1H-tetrazol-5-ylamino-Leu-Pro-Gly-Pro-OH (18-*7LPGP*). Yield: 80 mg (94%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84−8.2 (br s, 1H), 7.44−7.78 (m, 4H), 4.68−4.72 (m, 1H), 4.22−4.30 (m, 1H), 3.36−4.08 (m, 7H), 1.52−2.22 (m, 10H), 1.36−1.40 (m, 1H), 1.24−1.30 (m, 1H), 1.00 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.0, 172.1, 167.9, 163.1, 154.8, 134.3, 132.8, 131.5, 127.0, 123.4, 122.1, 60.4, 60.1, 54.9, 47.4, 46.3, 41.8, 40.6, 36.8, 28.8, 25.2, 25.0, 24.6, 23.2, 21.2. MS (ESI, M + H$^+$): 607.2.

1-(2-Iodophenyl)-1H-tetrazol-5-ylamino-Leu-Pro-Gly-Pro-OH (18-*8LPGP*). Yield: 88 mg (97%). mp: 135−140 °C (uncorrected). IR (film): ν 3418, 2957, 2873, 1644, 1455, 1334, 1266, 1182, 1086, 1016, 875, 765, 735 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.98−8.03 (m, 1H), 7.76 (br s, 1H),

7.52−7.57 (m, 1H), 7.41−7.43 (m, 1H), 7.23−7.29 (m, 1H), 4.52−5.01 (m, 5H), 3.87−4.23 (m, 3H), 3.38−3.63 (m, 2H), 1.59−2.13 (m, 9H), 1.49−1.54 (m, 2H), 0.89−1.00 (m, 6H). HRMS (ESI) Calcd for ($M + H^+$) $C_{25}H_{34}N_8O_5I$: 653.1719. Observed: 653.1691.

1-(4-Nitrophenyl)-1H-tetrazol-5-ylamino-Leu-Pro-Gly-Pro-OH (18-*11LPGP*). Yield: 76 mg (95%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.45 (d, 2H, $J = 6.4$ Hz), 8.23 (d, 1H, $J = 8.8$ Hz), 8.00 (s, 1H), 7.86 (d, 2H, $J = 7.2$ Hz), 3.38−4.82 (m, 9H), 1.28−2.22 (m, 11H), 0.88−1.06 (m, 6H). MS (ESI, $M + H^+$): 572.1.

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Supporting Information Available. LCMS analysis and ^{1}H and ^{13}C NMR spectra of randomly selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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