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Peptide−Heterocycle Hybrid Molecules: Solid-Phase Synthesis of a 400-Member Library of N-Terminal 2-Iminohydantoin Peptides

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A method for the heterocyclic modification of the N-terminus of a peptide is described. Reaction of the N-terminal amino group of solid-supported peptides with arylisothiocyanates generates a thiourea intermediate, as in the first step of Edman degradation. Treatment of the resin-supported peptide−thioureas with Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide) generates an electrophilic carbodiimide functionality, which undergoes rapid intramolecular trapping by the adjacent amide group to give a 2-iminohydantoin ring at the N-terminus of the peptide. The dehydrothiolation step in the presence of Mukaiyama's reagent prevents Edman degradation from occurring, in essence leading to a "diversion" of the Edman degradation. Cleavage from the resin then releases the hybrid molecules incorporating a 2-iminohydantoin ring conjugated onto a peptidic fragment. A 400-member library of the iminohydantoin−peptide hybrids was synthesized using this approach, starting from a chlorotrityl resin-supported tripeptides.

Introduction

Peptides are one of the most important classes of bioactive molecules because of their ability to bind to biological macromolecules including proteins and nucleic acids.[1] They often serve as lead molecules for the development of enzyme inhibitors and receptor ligands. In most cases, it is desirable to develop small molecule peptidomimetics, rather than using the peptides themselves as drug candidates because of poor bioavailability and other ADMET properties of peptides. Such small molecule peptidomimetics act by mimicking the 3-dimensional structure of the peptides or peptide sequence recognized by the enzymes or receptors. While such approaches are often challenging, there are many reports of the successful application of this strategy to the discovery of enzyme inhibitors, particularly when X-ray crystallographic or NMR data of the enzymes or their complexes can be used as a guide to rational design.[2] Peptides are also important lead molecules for the discovery of receptor antagonists and agonists.[3] Again, it is desirable to develop small molecule peptidomimetics for this task. However, this is usually significantly more challenging because of the greater conformational flexibility of receptors, such as GPCR's, the lack of reliable structural data for the receptors or their complexes, and the consequent uncertainty of the receptor−ligand models. Even today, computational methods struggle with the challenges inherent in the de novo design of small molecule ligands for receptors. The rational design of completely nonpeptidic molecules, such as morphine, which is known to mimic the peptide β-endorphin, thus, remains one of the great challenges for the medicinal chemist. Initial small-molecule leads for receptors are thus more commonly discovered through high-throughput screening campaigns or through the modification of existing small molecules. Finally, peptides are also capable of interfering with protein−protein interactions.[4] The binding in such systems is often weaker, and the flatter and larger surfaces employed in protein−protein interactions has rendered the identification of small peptidomimetic molecules for the disruption of protein−protein interactions, whether by a rational or high-throughput screening approach, one of the greatest challenges for biological molecular recognition.

Considerable attention has thus been focused on strategies for the development of small-molecule peptidomimetics.[5] There are many approaches that have been taken to achieve this goal, including the use of peptoids and privileged substructures.[6] In addition, modification of peptides is a widely used strategy as, for example, in 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), and cyclic peptides.[7] The introduction of small molecule fragments into a peptide chain, such as aromatic rings or heterocycles,[8] represents a further example of this strategy. These small molecule fragments can be placed in the interior of the peptide chain,[9] at the C-terminus,[10] or at the N-terminus.[11] The presence of the heterocyclic moiety or other small molecule fragment introduces conformational constraints, which can either mimic or provide unusual secondary peptide structures. Our goal is the development of heterocycle−peptide hybrid molecular libraries as a strategy for the discovery of peptidomimetic molecules. We envision this approach being particularly useful for the identification of receptor ligands and for molecules that disrupt protein−protein interactions. Once such a heterocycle−peptide conjugate molecule with desirable properties has been identified, in principle, further replacement of the peptidic portion of

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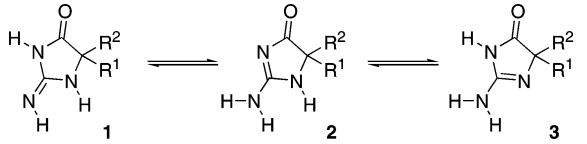
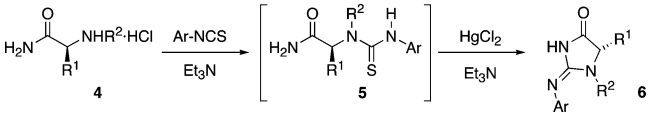


Figure 1. Tautomeric forms of 2-iminohydantoins.

Scheme 1. Solution Phase Synthesis of 2-Iminohydantoins



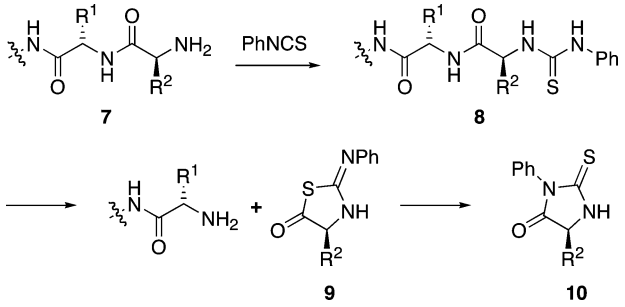
the molecules would provide a route to either minimally peptidic or entirely nonpeptidic small molecules. We now report on the solid-phase synthesis of one such library, created through the conjugation of an iminohydantoin to the N-terminus of a peptide.

Results and Discussion

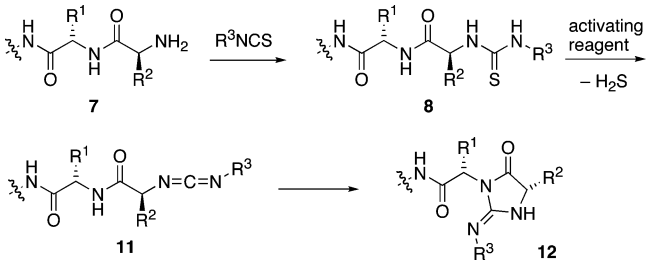
2-Iminohydantoins or 2-iminoimidazolin-4-ones contain amide and guanidine functionalities within a five-membered ring structure, and they can exist in different tautomeric forms, such as **1**, **2**, and **3** (Figure 1). They are known bioactive compounds[12] and are similar to imidazoles[13] and hydantoins,[14] which have a range of biological activities. One simple natural product that contains the 2-iminoimidazolin-4-one ring is creatine. There are several reports on the synthesis of 2-iminoimidazolin-4-ones, both in solution and on a solid support.[15−18] Our group has recently reported a parallel solution-phase synthesis of 2-iminohydantoins (Scheme 1).[16] This approach used the reaction of α-amino acid amides, **4**, with arylisothiocyanates to give an intermediate thiourea **5**. Activation of **5** as an electrophilic intermediate was achieved by treatment with $HgCl_2$, or Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide), which was followed by cyclization to generate the product 2-iminoimidazolin-4-ones, **6**. The activation step involves initial complexation of the thiourea by Hg(II) and, in the case of **5** (R^2 = H), breakdown to an electrophilic carbodiimide. Houghten had earlier reported a similar solid-phase synthesis approach for **6**, starting from single amino acids attached to *p*-methylbenzhydrylamine (MBHA) resin, using Mukaiyama's salt to achieve activation.[17] Several groups have also reported solid-phase synthesis approaches, involving conversion of the N-terminal amino group of a polymer-supported amino acid to a guanidine, followed by cyclization through nucleophilic attack onto the adjacent ester or amide carbonyl group with simultaneous cleavage of the product from the resin support.[18]

The intermediacy of thiourea **5** led us to question if a similar approach could be used to divert the Edman degradation reaction and create more complex peptide−iminohydantoin conjugates. The Edman degradation reaction is widely used for the sequencing of polypeptides and proteins through the controlled removal of the N-terminal amino acids.[19] In the Edman degradation reaction, the N-terminal amino group of a peptide **7** reacts with phenylisothiocyanate to generate a thiourea, **8**, which is analogous to **5**. Intramolecular nucleophilic addition of the thiocarbonyl group of **8** onto the adjacent amide carbonyl generates a phenylthiohydantoin, **10**, via a thiazolinone intermediate **9** (Scheme 2).

Scheme 2. Edman Degradation of Peptides

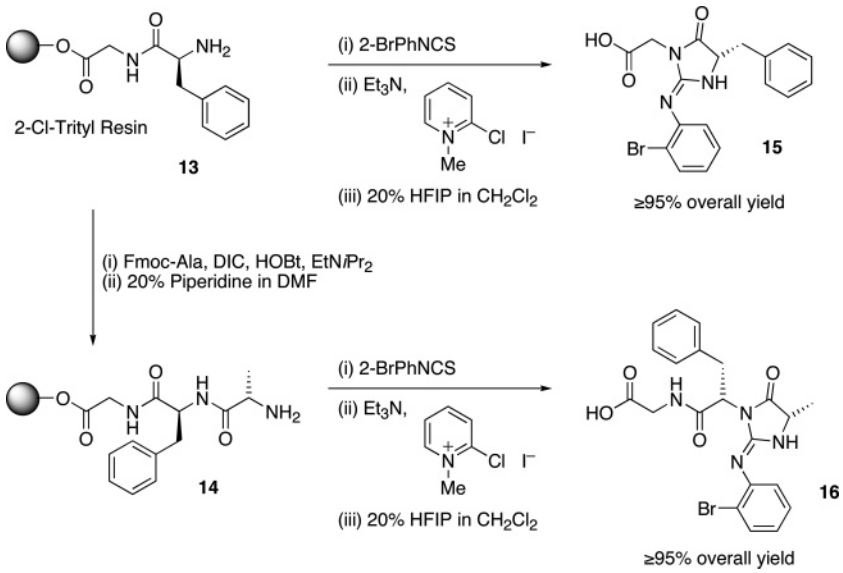


Scheme 3. Diverted Edman Degradation for the Synthesis of Aryl-Substituted 2-Iminohydantoin Peptide Hybrids



By analogy with the solution-phase results (Scheme 1) and the earlier precedent using the simpler systems outlined above,[16−18] conversion of the nucleophilic thiourea group of **8** to the carbodiimide group in **11** through a dehydrothiolation reaction achieves polarity reversal[20] and prevents the Edman degradation reaction from occurring. Instead, the electrophilic carbodiimide group can be trapped intramolecularly by the adjacent weakly nucleophilic amide functionality to generate a 2-iminohydantoin (2-iminoimidazolinone) **12** (Scheme 3). We envisioned developing this diverted Edman degradation chemistry using a solid-phase synthesis approach.[21] Beyond the well-known advantages of product purification that are possible with solid-phase synthesis, we also envisioned the use of a non-mercury-based dehydrothiolation reagent. 2-Chlorotrityl polystyrene resin was chosen as the solid support because of the ease of handling and cleavage of the product from the resin.[22] The bulky chlorotrityl group also prevents formation of diketopiperazines during the initial peptide formation.[23] The ease of product cleavage also permits the monitoring of every step in the synthesis, simply by monitoring by TLC. Standard Fmoc peptide synthesis techniques and the use of DIC/HOBt couplings were used for the synthesis of the chlorotrityl resin supported peptide precursors. The initial feasibility study used the resin-bound dipeptide **13** and tripeptide **14** precursors (Scheme 4). Reaction of dipeptide **13** with 2-bromophenyl isothiocyanate gave the corresponding resin-bound thiourea which on dehydrothiolation with Mukaiyama's reagent (3 equiv) and intramolecular cyclization yields the resin-bound 2-iminohydantoin. Cleavage from the resin occurred under mild conditions with a mixture of hexafluoro-2-propanol (HFIP) and CH_2Cl_2, to give **15** in a ≥95% overall yield and 93% purity, as determined by HPLC analysis. Reaction using $HgCl_2$ (3 equiv) and Et_3N (10 equiv) for 12 h, rather than Mukaiyama's reagent, gave similar results. However, the

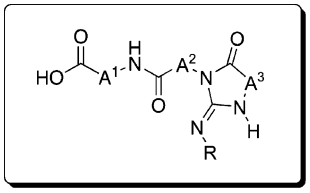
Scheme 4. Solid-Phase Synthesis of Aryl-Substituted 2-Iminohydantoins



final product in the case of $HgCl_2$ required filtration through Celite to remove the mercury byproducts. Similar reaction of tripeptide **14** with 2-bromophenyl isothiocyanate followed by Mukaiyama's reagent (5 equiv) promoted cyclization and cleavage from the resin gave **16** in a ≥95% overall yield and with a purity of 91%. The same process was also achieved using $HgCl_2$ (5 equiv) and Et_3N (10 equiv) for 12 h to give **16** in a ≥95% yield and a purity of 93%, following filtration through Celite. Reaction of the dipeptide or tripeptide with 2-bromophenyl isothiocyanate gave complete conversion to the desired thioureas in less than 10 min as determined by LCMS analysis. It is essential that the intermediate thioureas be rapidly converted to the carbodiimides, to minimize Edman degradation of the thiourea. Thus, if the thioureas were left in basic DMF conditions for longer than 20 min the overall reaction yield and the final product purity suffered.

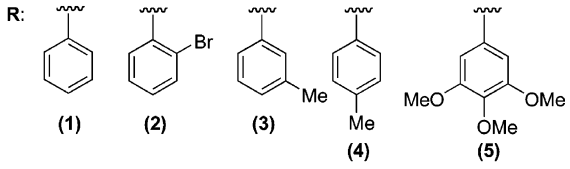
Library Synthesis. As an evaluation of whether the methodology was suitable for a combinatorial synthesis approach, an initial 400-member library of peptide−iminohydantoins was chosen as the synthetic target. Our aim was to synthesize a library of discrete molecules, rather than mixtures, and to synthesize each library member in 10−30 mg quantities. The Mukaiyama's reagent protocol was chosen for the dehydrothiolation/iminohydantoin formation. The library was constructed using chlorotrityl resin as the support and with standard Fmoc coupling protocols. This combination of factors led us to adopt Houghten's "tea-bag" technology for the construction of the library.[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 between room temperature and 120 °C. The library was constructed using 5 different amino acids for the C-terminal position, 4 different amino acids for each of the second and third positions of the peptide, and 5 different arylisothiocyanates. The selection of the building blocks and the encoding scheme are shown in Figure 2.

Reaction of the first Fmoc-protected amino acid with the 2-chlorotrityl chloride resin was conducted in dry CH_2Cl_2 in the presence of excess Hünig's base to give the product bound to the resin through an ester linkage (Scheme 5). To ensure complete consumption of 2-chlorotrityl chloride, unreacted chloride groups were capped by treatment with methanol at the end of the reaction. The resin was dried and the loading was determined by UV spectrometric analysis of the Fmoc−piperidine adduct. A cleavage cocktail of a 20% piperidine solution in DMF was used for the removal of the N-terminal Fmoc-protecting group to give **17**. The sequential attachment of two further amino acids was



5 x 4 x 4 x 5 = 400

A^1: Pro (**1**), Gly (**2**), Ala (**3**), Leu (**4**), Phe (**5**)
A^2: Gly (**A**), Ala (**B**), Leu (**C**), Phe (**D**)
A^3: Gly (**A**), Ala (**B**), Leu (**C**), Phe (**D**)



Example of encoding:

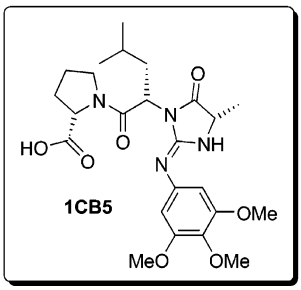


Figure 2. Building block selection and encoding scheme of the library of aryl-substituted 2-iminohydantoin tripeptide hybrids, **21**.

Scheme 5. Synthesis of a 400-Member Library of Aryl-Substituted 2-Iminohydantoin Tripeptide Hybrids

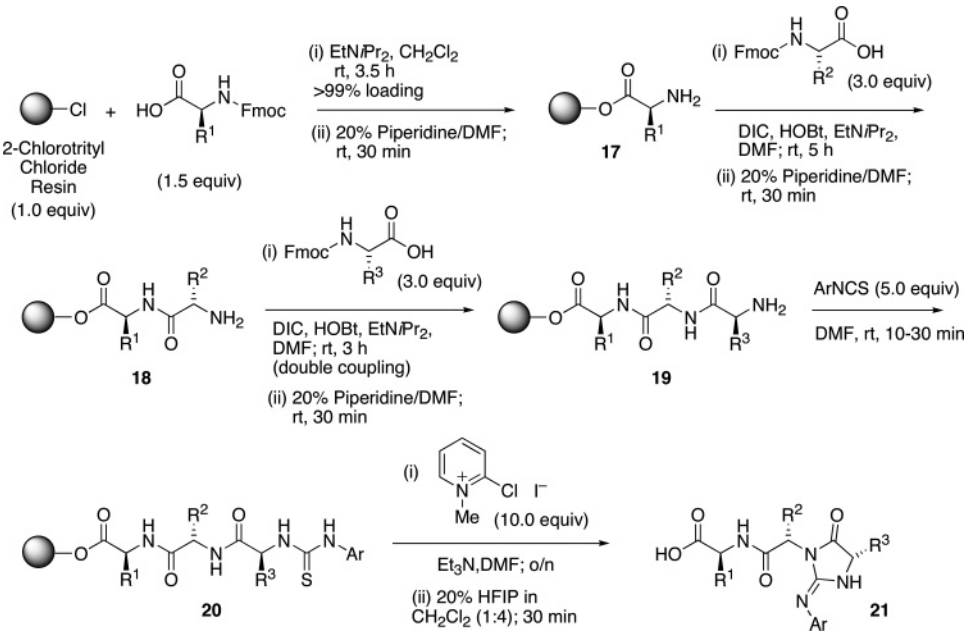


Table 1. Purity Assessment of the 400-Member Library of Aryl-Substituted 2-Iminohydantoin Tripeptide Hybrid Molecules[21]

	sublibrary code	average purity (%)
1	1XXX	85.7
2	2XXX	88.7
3	3XXX	90.5
4	4XXX	89.8
5	5XXX	89.7
6	XAXX	88.9
7	XBXX	88.3
8	XCXX	88.8
9	XDXX	89.5
10	XXAX	87.9
11	XXBX	90.1
12	XXCX	89.4
13	XXDX	88.1
14	XXX1	89.5
15	XXX2	92.5
16	XXX3	84.6
17	XXX4	89.6
18	XXX5	88.2

achieved using DIC/HOBt coupling conditions with the Fmoc-protected amino acids, to give the tripeptide **19**. Tripeptide **19** was then coupled with the arylisothiocyanates (5 equiv) in DMF over 10−30 min to give thiourea **20**. Conversion to the iminohydantoin via the carbodiimide intermediate was achieved using Mukaiyama's reagent (10 equiv) in the presence of excess triethylamine in DMF overnight. The iminohydantoin products **21** were then released from the resin by treatment with 20% hexafluoro-2-propanol in CH_2Cl_2. An average purity of 89% for the iminohydantoin−peptide hybrid **21** 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. The greatest variation in sublibrary purities resulted from the choice of the isothiocyanates.

Conclusion

In conclusion, a library of synthetic hybrid molecules incorporating a 2-iminohydantoin ring conjugated onto a peptidic fragment has been synthesized. The synthesis uses commercially available building blocks and a solid-phase strategy. The synthesis is initiated by the formation of a solid-supported tripeptide, which then undergoes reaction with an arylisothiocyanate to give a thiourea intermediate. Immediate dehydrothiolation with Mukaiyama's reagent prevents Edman degradation from occurring and generates an electrophilic carbodiimide that reacts with the adjacent amide group to give a 2-iminohydantoin. The products were obtained in good yields and purities. Overall, this provides a method for the heterocyclic modification of the N-terminus of a peptide, with the simultaneous incorporation of an element of diversity originating from the isothiocyanate. 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-chlorotrityl chloride 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 supplied 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 a G1310A isopump and a ZORBAX SB-C18 column (cartridge 4.6 × 15 mm, particle diameter 3.5 μm, pH range 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, then 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.

Synthesis of [4(*S*)-Benzyl-2-(2-bromophenylimino)-5-oxoimidazolidin-1-yl]-acetic acid (15). Phe−Gly−trityl resin was prepared using standard Fmoc-based peptide coupling conditions.[25] Phe−Gly−trityl resin (200 mg, 0.175 mmol) was suspended in dry CH_2Cl_2 (4 mL) for 15 min in a peptide synthesis vessel to swell the resin. After the CH_2Cl_2 was drained, the resin was suspended in DMF (4 mL). 2-Bromophenyl isothiocyanate (71 μL, 0.525 mmol) was added to the vessel, and the reaction mixture was shaken for 15 min. After the solution was filtered off, the resin was washed with DMF (3 × 4 mL) and CH_2Cl_2 (3 × 4 mL). Reaction conversion could be monitored at this stage by TLC analysis following cleavage of a small portion of the desired 2-chlorotrityl resin-supported product (<1 mg) using HFIP/CH_2Cl_2 (1:4, 50 μL) for 10 min. The TLC analysis (45:5:1 EtOAc/MeOH/AcOH) showed the disappearance of the starting material (R_f = 0.0, stainable as a purple spot by ninhydrin) and the appearance of a new spot (the thiourea, R_f = 0.2 visible under a UV_{254} lamp). Mukaiyama's reagent (134 mg, 0.525 mmol) and Et_3N (0.24 mL, 1.75 mmol) were added to the intermediate resin-supported thiourea. The reaction vessel was shaken, and the reaction conversion was again monitored by TLC analysis following cleavage of a small portion of the resin-supported product (<1 mg) using HFIP/CH_2Cl_2 (1:4, 50 μL) for 10 min. The TLC analysis (45:5:1 EtOAc/MeOH/AcOH) showed the disappearance of the thiourea (R_f = 0.2) and the appearance of a new spot (the iminohydantoin, R_f = 0.3 visible under a UV_{254} lamp). The reaction was complete after a total of 12 h of shaking. The resin-supported iminohydantoin was then filtered and washed with DMF (3 × 4 mL) and CH_2Cl_2 (3 × 4 mL). The resin-supported iminohydantoin was then suspended in a mixture of HFIP/CH_2Cl_2 (1:4, 4 mL) and shaken for 20 min. The solution was collected in a round-bottom flask, and the resin was washed with CH_2Cl_2 (3 × 20 mL). The solvent was removed in vacuo to give the product, **15**, as a yellowish solid (68.9 mg, in 98% yield based upon conversion from Fmoc−Gly−2-chlorotrityl chloride resin). TLC (9:1:0.2 EtOAc/MeOH/AcOH): R_f = 0.3. HPLC analysis: retention time of 8.91 min (purity of 93%). IR (film): 3427, 3062, 2939, 2527, 1730, 1682, 1581, 1455, 1138, 1026, 737, 702 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.53 (d, J = 7.0 Hz, 1H), 7.11−7.32 (m, 6H), 6.90−7.03 (m, 2H), 5.29 (s, 1H), 4.27−4.47 (m, 3H), 3.23 (dd, J = 14.0, 4.0 Hz, 1H), 2.84−2.97 (m, 1H).^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.4, 170.2, 150.6, 143.0, 135.4, 133.5, 129.5, 129.0, 128.7, 127.5, 126.1, 124.8, 118.3, 59.7, 41.2, 38.8. MS (ESI, MH^+): 402.0, 404.0. MS (EI): *m*/*z* 404 (20), 403 (97, M^+), 402 (23), 401 (100, M^+), 322 (43), 312 (35), 310 (37), 294 (37), 292 (14), 278 (34), 266 (14), 248 (10), 211 (36), 209 (34), 185 (47), 91 (52). HRMS (EI) calculated for (M^+) $C_{18}H_{16}BrN_3O_3$: 401.0375. Observed: 401.0370.

Coupling of Fmoc−Ala to Phe−Gly−Trityl Resin (14). Hünig's base (0.68 mL, 3.93 mmol), Fmoc−Ala (0.46 mg, 1.47 mmol), HOBt (0.20 g, 1.47 mmol), and DIC (0.23 mL, 1.47 mmol) were added to a suspension of the Phe−Gly−trityl resin (539 mg, 0.49 mmol) in DMF (6 mL). The reaction was shaken for 4 h. Reaction conversion was monitored by TLC analysis following cleavage of a small portion of the desired 2-chlorotrityl resin-supported product (<1 mg) using HFIP/CH_2Cl_2 (1:4, 50 μL) for 10 min. TLC analysis (45:5:1 EtOAc/MeOH/AcOH) showed the disappearance of the starting material (R_f = 0.0, stainable as a purple spot by ninhydrin) and the appearance of a new spot (the thiourea, R_f = 0.3 visible under a UV_{254} lamp). After the solution was filtered off, the resin was washed with DMF (3 × 6 mL) and CH_2Cl_2 (3 × 6 mL). The reaction yielded the product resin (702 mg, ≥ 99%; the anticipated mass for a quantitative yield was 710 mg).

Cleavage of the Fmoc-Protecting Group from Fmoc−Ala−Phe−Gly−Trityl Resin (14). Fmoc−Ala−Phe−Gly−trityl resin **14** (100 mg, 0.070 mmol) was suspended in 20% (v/v) piperidine in DMF (4 mL) in a peptide synthesis vessel and shaken for 30 min. Reaction conversion was monitored by TLC analysis following cleavage of a small portion of the resin-supported product (<1 mg) using HFIP/CH_2Cl_2 (1:4, 50 μL) for 10 min. The TLC analysis (45:5:1 EtOAc/MeOH/AcOH) showed the disappearance of Fmoc−Ala−Phe−Gly (R_f = 0.3, visible under a UV_{254} lamp) and the appearance of a new spot (Ala−Phe−Gly, R_f = 0.0, stainable as a purple spot by ninhydrin). After the solution was filtered off, the resin was washed with DMF (3 × 4 mL).

Synthesis of {2(*S*)-[2-(2-Bromophenylimino)-4(*S*)-methyl-5-oxoimidazolidin-1-yl]-3-phenylpropionylamino}-acetic Acid (16). Ala−Phe−Gly−trityl resin **14** (0.070 mmol) was suspended in DMF (4 mL) in a manual peptide synthesis vessel. 2-Bromophenyl isothiocyanate (47 μL, 0.35 mmol) was added to the vessel, and the reaction was shaken for 10 min. After the solution was filtered off, the resin was washed with DMF (1 × 4 mL). Reaction conversion could be monitored at this stage by TLC analysis following cleavage of a small portion of the desired 2-chlorotrityl resin-supported product (<1 mg) using HFIP/CH_2Cl_2 (1:4, 50 μL) for 10 min. The TLC analysis (45:5:1 EtOAc/MeOH/AcOH) showed the disappearance of the starting material (R_f = 0.0, stainable as a purple spot by ninhydrin) and the appearance of a new spot (the thiourea, R_f = 0.3 visible under a UV_{254} lamp). Mukaiyama's reagent (90 mg, 0.35 mmol) and Et_3N (0.10 mL, 0.70 mmol) were immediately added to the intermediate thiourea-supported resin. The reaction vessel was shaken, and reaction conversion was monitored by TLC analysis following cleavage of a small portion of the resin-supported product (<1 mg) using HFIP/CH_2Cl_2 (1:4, 50 μL) for 10 min. TLC analysis (45:5:1 EtOAc/MeOH/AcOH) showed the disappearance of the thiourea (R_f = 0.3) and the appearance of a new spot (the iminohydantoin, R_f = 0.4 visible under a UV_{254} lamp). The reaction was complete after a total of 12 h of shaking. The resin-supported iminohydantoin was then filtered and washed with DMF (3 × 4 mL) and CH_2Cl_2 (3 × 4 mL). The resin-supported iminohydantoin was then suspended in a mixture of HFIP/CH_2Cl_2 (1:4, 4 mL) and shaken for 20 min. The solution was collected in a round-bottom flask, and the resin was washed with CH_2Cl_2 (3 × 10 mL). The solvent was removed in vacuo to give the product, **16**, as a yellowish solid (33.5 mg, quantitative yield based upon conversion from Fmoc−Gly−2-chlorotrityl chloride resin).

TLC (9:1:0.2 EtOAc/MeOH/AcOH): $R_f = 0.2$. HPLC analysis: retention time of 8.11 min (purity of 91%). IR (film): 3337, 3061, 2927, 2855, 1745, 1682, 1583, 1471, 1429, 1320, 909, 735 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz, rotamers): δ 8.76 (br s, 0.8H), 8.03 (br s, 0.2H), 7.52−7.62 (m, 1H), 7.14−7.33 (m, 6H), 6.88−7.08 (m, 2H), 5.23−5.34 (m, 1H), 3.73−4.32 (m, 4H), 3.58−3.49 (m, 1H), 1.20 (d, $J = 7.0$ Hz, 0.6H), 0.93 (d, $J = 7.0$ Hz, 3H), 1.45 (s, 9H), 1.22−1.74 (m, 2.4H). ^{13}C NMR ($CDCl_3$, 75 MHz, rotamers): δ 174.0, 172.5, 170.4, 169.8, 150.7, 150.4, 144.4, 136.7, 136.5, 133.6, 133.5, 129.4, 128.8, 128.7, 127.1, 127.1, 125.5, 125.4, 123.6, 123.5, 118.2, 117.9, 57.5, 57.0, 53.6, 53.5, 42.5, 42.3, 34.9, 34.2, 18.5, 18.3. MS (EI): *m*/*z* 474 (20, M^+), 472 (20, M^+), 403 (12), 401 (14), 393 (15), 372 (26), 370 (27), 278 (12), 270 (64), 269 (53), 268 (67), 267 (48), 225 (19), 223 (20), 219 (13), 189 (14), 188 (100), 182 (10), 131 (20), 103 (16), 91 (34). HRMS (EI) calculated for (M^+) $C_{21}H_{21}BrN_4O_4$: 472.0746. Observed: 472.0743.

General Procedure for the Loading of Fmoc−A_1−OH on the 2-Chlorotrityl Chloride Resin. 2-Chlorotrityl chloride resin was packed into 400 polypropylene mesh tea-bags (50 mg/tea-bag, resin substitution 1.14 mmol Cl^-/g). The tea-bags were labeled and sealed using a manual sealer. They were then split into batches of 80, and each batch was placed into separate 600 mL polypropylene vessels and suspended in dry CH_2Cl_2 (600 mL) for 15 min to swell the resin. After the CH_2Cl_2 was drained, each batch of 80 tea-bags was added to a vessel containing a prestirred solution of each Fmoc−A_1−OH (3 equiv) with Hünig's base (5 equiv) in dry CH_2Cl_2 (500 mL). The reaction mixtures were shaken on a rotary shaker for 3 h at room temperature. Reagent grade MeOH (50 mL) was then added, and the bottles shaken for 15 min to end-cap any remaining traces of unreacted 2-chlorotrityl groups on the resin. The tea-bags were washed with equal volumes of CH_2Cl_2 (3 × 500 mL), DMF (3 × 500 mL), CH_2Cl_2 (3 × 500 mL), and Et_2O (2 × 500 mL); then they were dried in vacuo until no mass change was observed. Substitution measurement was done on small aliquots of resin from randomly selected tea-bags using UV spectrometric analysis of the Fmoc−piperidine adduct.[26] An average substitution of 1.13 mmol/g was obtained.

General Procedure for Cleavage of the Fmoc-Protecting Group from Fmoc−A^1−Trityl Resin. The 400 polypropylene tea-bags with Fmoc−A^1−trityl resin were sorted according to the first label into 4 batches. Each batch of 100 tea-bags was placed into a 1.0 L polypropylene bottle and suspended in dry CH_2Cl_2 (600 mL) for 20 min to swell the resin. After the CH_2Cl_2 was drained, a solution of 20% (v/v) piperidine in DMF (600 mL) was added, and each bottle was shaken for 30 min. After the solution was drained, the tea-bags were washed thoroughly with DMF (4 × 700 mL) and CH_2Cl_2 (4 × 700 mL).

General Procedure for Coupling of Fmoc−A^2−OH to A^1−trityl Resin and Fmoc−A^3−OH to A^2A^1−trityl Resin. Batches of 100 tea-bags were sorted according to the second label into 4 batches. Each batch with A^1−trityl resin (100 tea-bags, 0.57 mmol, 1 equiv) was placed into 1.0 L polypropylene bottle and suspended in DMF (600 mL), followed by the addition of Hünig's base (5 equiv), HOBt (3 equiv), DIC (3 equiv), and Fmoc−A^2−OH (3 equiv). The reaction mixtures were agitated for 4 h. After the solution was drained, the tea-bags were washed with DMF (3 × 700 mL) and CH_2Cl_2 (4 × 700 mL), and then sorted into new batches of 100 tea-bags each according to their third label. The same procedures as outlined above were used for the removal of Fmoc-protecting group and the coupling of Fmoc−A^3−OH.

General Procedure for the Formation of Thiourea−Polypeptide−Trityl Resin. The tea-bags were combined into 5 batches of 80 according to their 4th label. After the removal of the Fmoc-protecting group from the N-terminal of the final amino acid residue, each batch was placed into a 500 mL polypropylene bottle and suspended in DMF (250 mL). The corresponding aryl isothiocyanates (5 equiv) were added to each reaction vessel, and the reaction mixtures were shaken for 20 min at room temperature. After the solution was drained, the tea-bags were washed with DMF (3 × 350 mL) and CH_2Cl_2 (3 × 350 mL). The thiourea−polypeptide−trityl resin was immediately carried into the next step.

General Procedure for the Formation of Aryl-Substituted 2-Iminohydantoin−Tripeptide−Trityl Resin. The batches of tea-bags of thiourea−polypeptide−trityl resin (80 tea-bags, 1 equiv) were placed into 400 mL polypropylene bottles. DMF (200 mL), triethylamine (10 equiv), and Mukaiyama's reagent (10 equiv) were then added. The reaction mixtures were agitated overnight at room temperature. The resultant bright orange solutions were drained, and the tea-bags with resin were thoroughly washed with DMF (5 × 250 mL), CH_2Cl_2 (5 × 250 mL), DMF (3 × 250 mL), CH_2Cl_2 (5 × 250 mL), and Et_2O (3 × 250 mL) and then dried in vacuo.

Cleavage Procedure to Give the Tripeptide-2-Iminohydantoin Hybrids. Each tea-bag was placed into separate labeled 20 mL scintillation vials of known weight. The products 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 vials were shaken for 30 min at room temperature. The tea-bags were removed and washed with CH_2Cl_2 (3 × 2 mL), and the organic filtrates were combined and concentrated using a Genevac HT-4X. Examples of randomly selected tripeptide-2-iminohydantoin hybrids were characterized by IR, ^1H NMR, and ^{13}C NMR.

1-[2-(4-Isobutyl-5-oxo-2-m-tolyliminoimidazolin-1-yl)-acetyl]-pyrrolidine-2-carboxylic Acid (1AC3). Yield: 18.5 mg (94%). Yellow amorphous solid. IR (film): ν 3418, 2959, 1660, 1652, 1455, 1394, 130, 1197, 1025, 995, 920, 766, 624 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 7.32−7.40 (br s, 1H), 7.13 (t, $J = 7.5$ Hz, 1H), 6.77 (d, $J = 7.5$ Hz, 1H), 6.62−6.76 (br s, 1H), 4.14−4.40 (m, 2H), 3.98−4.04 (br s, 1H), 3.36−3.68 (m, 3H), 2.25 (s, 3H), 2.11−2.16 (m, 1H), 1.83−1.96 (m, 4H), 1.43−1.53 (m, 2H), 0.60−1.02 (br s, 6H). ^{13}C NMR (DMSO-d_6, 500 MHz): δ 173.7, 165.2, 164.7, 149.6, 138.4, 129.2, 123.6, 123.2, 120.0, 115.2, 59.3, 46.2, 41.9, 40.9, 31.4, 29.2, 24.9, 24.3, 23.6, 22.6, 21.6. HPLC analysis: retention time of 7.00 min (purity of 95%). MS (EI): *m*/*z* 401.2 (MH^+).

1-{2-[2-(2-Bromophenylimino)-4-isobutyl-5-oxoimidazolidin-1-yl]-acetyl}-pyrrolidine-2-carboxylic Acid (1AC2). Yield: 20.4 mg (86%). Pale yellow amorphous solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.8−13.0 (br s, 1H), 7.53−7.55 (m, 2H), 7.26 (td, J = 8.0, 0.5 Hz, 1H), 6.88−6.96 (m, 2H), 4.17−4.41 (m, 2H), 4.04−4.07 (m, 1H), 3.34−3.69 (m, 3H), 2.08−2.20 (m, 1H), 1.72−1.97 (m, 4H), 1.42−1.57 (m, 2H), 0.86−0.88 (m, 6H). HPLC analysis: retention time of 7.39 min (purity of 96%). MS (EI): *m*/*z* 464.1, 466.1 (MH^+).

1-{2-[2-(2-Bromophenylimino)-5-oxoimidazolidin-1-yl]-3-phenylpropionyl}-pyrrolidine-2-carboxylic Acid (1DA2). Yield: 23.6 mg (93%). Amorphous yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.8−13.0 (br s, 1H), 7.54 (td, J = 8.0, 1.5 Hz, 1H), 7.14−7.29 (m, 6H), 6.87−6.92 (m, 2H), 6.61 (d, J = 8.0 Hz, 1H), 4.23 (d, J = 9.0 Hz, 2H), 3.24−3.86 (m, 6H), 1.71−2.10 (m, 4H). HPLC analysis: retention time of 8.69 min (purity of 96%). MS (EI) *m*/*z* 498.1, 500.1 (MH^+).

{2-[2-(2-Bromophenylimino)-5-oxoimidazolidin-1-yl]-acetylamino}-acetic Acid (2AA2). Yield: 17.0 mg (90%). IR (film): ν 3455, 2925, 2854, 1647, 1459, 1270, 1149, 1026, 768 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.37 (br s, 1H), 7.55 (d, J = 8.0 Hz, 1H), 7.31 (s, 1H), 7.26 (t, J = 7.5 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 6.90 (t, J = 7.5 Hz, 1H), 4.20 (s, 2H), 3.93 (s, 2H), 3.75 (d, J = 5.5 Hz, 2H).^{13}C NMR (DMSO-d_6, 400 MHz): δ 173.7, 165.2, 164.7, 149.6, 138.4, 129.2, 123.6, 123.2, 120.0, 59.3, 46.2, 41.9, 40.9, 31.4, 29.2, 24.9, 24.3, 23.6, 22.6, 21.6. HPLC analysis: retention time of 1.91 min (purity of 97%). MS (EI): *m*/*z* 368.0, 370.0 (MH^+).

[2-(4-Isobutyl-5-oxo-2-p-tolyliminoimidazolidin-1-yl)-acetylamino]-acetic Acid (2AC4). Yield: 18.1 mg (99%). Off-white amorphous solid. IR (film): ν 3442, 2959, 1668, 1513, 1456, 1392, 1266, 1124, 1024, 930, 823, 706 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.31 (s, 1H), 7.66−7.74 (m, 1H), 7.29 (s, 1H), 7.06 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 7.5 Hz, 2H), 4.15 (s, 2H), 3.99−4.02 (m, 1H), 3.75 (d, J = 5.5 Hz, 2H), 2.09 (d, J = 1.5 Hz, 3H), 1.80−1.86 (m, 1H), 1.42−1.54 (m, 2H), 0.86−0.90 (m, 6H). HPLC analysis: retention time of 6.92 min (purity of 97%). MS (EI): *m*/*z* 361.2 (MH^+).

{2-[4-Benzyl-2-(2-bromophenylimino)-5-oxoimidazolidin-1-yl]-acetylamino}-acetic Acid (2AD2). Yield: 22.0 mg (94%). Yellow amorphous solid. IR (film): ν 3419, 2926, 1682, 1582, 1455, 1365, 1232, 1139, 1026, 832, 757, 702, 668 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.08 (s, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.48 (s, 1H), 7.16−7.28 (m, 5H), 6.90 (t, J = 7.5 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 4.38 (s, 1H), 4.03 (s, 2H), 3.69 (d, J = 5.0 Hz, 2H), 2.93−3.02 (m, 2H), 1.04−1.07 (m, 1H). HPLC analysis: retention time of 7.07 min (purity of 96%). MS (EI): *m*/*z* 458.1, 460.1 (MH^+).

{2-[2-(2-Bromophenylimino)-4-methyl-5-oxoimidazolidin-1-yl]-propionylamino}-acetic Acid (2BB2). Yield: 19.1 mg (94%). IR (film): ν 3419, 2077, 1668, 1583, 1469, 1434, 1321, 1223, 1141, 1042, 1025 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.12 (s, 1H), 7.54−7.60 (m, 1H), 7.44 (s, 0.35H), 7.39 (s, 0.65H), 7.24−7.27 (m, 1H), 6.98−7.02 (m, 1H), 6.90−6.97 (m, 1H), 3.99−4.04 (m, 1H), 3.75−3.79 (m, 1H), 3.58−3.68 (m, 2H), 1.55−1.58 (m, 3H), 1.23 (d, J = 6.5 Hz, 3H). HPLC analysis: retention time of 5.83 min (purity of 99%). MS (EI): *m*/*z* 396.1, 398.1 (MH^+).

{2-[2-(2-Bromophenylimino)-4-methyl-5-oxoimidazolidin-1-yl]-4-methylpentanoylamino}-acetic Acid (2CB2). Yield: 20.7 mg (92%). Bright yellow amorphous solid. IR (film): ν 3356, 1660, 1470, 1429, 1385, 1322, 1215, 1047, 1025, 997, 828, 767 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.21 (m, 0.65H), 8.15 (m, 0.35H), 7.54−7.58 (m, 1H), 7.43 (s, 0.4H), 7.37 (s, 0.6H), 7.24−7.30 (m, 1H), 7.27 (d, J = 1.5 Hz, 1H), 6.89−6.94 (m, 1H), 4.82 (dd, J = 12.0, 4.5 Hz, 0.3H), 4.64 (dd, J = 12.0, 4.5 Hz, 0.7H), 4.00−4.09 (m, 1H), 3.64−3.82 (m, 3H), 1.70−1.78 (m, 1H), 1.46−1.56 (m, 1H), 1.23 (dd, J = 5.0, 3.0 Hz, 3H), 0.90−0.97 (m, 6H). HPLC analysis: retention time of 8.40 min (purity of 95%). MS (EI): *m*/*z* 438.1, 440.1 (MH^+).

2-{2-[2-(2-Bromophenylimino)-4-isobutyl-5-oxoimidazolidin-1-yl]-acetylamino}-propionic Acid (3AC2). Yield: 21.8 mg (97%). Yellow amorphous solid. IR (film): ν 3353, 2958, 1667, 1651, 1582, 1538, 1454, 1368, 1232, 1150, 1044, 1026, 832, 738, 668 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.33 (d, J = 5.5 Hz, 1H), 7.54 (d, J = 7.5 Hz, 1H), 7.26 (t, J = 7.5 Hz, 1H), 6.96 (d, J = 7.5 Hz, 1H), 6.90 (t, J = 7.5 Hz, 1H), 4.12−4.18 (m, 3H), 4.02−4.06 (m, 1H), 1.78−1.84 (m, 1H), 1.43−1.54 (m, 2H), 1.26 (d, J = 7.0 Hz, 3H), 0.86 (t, J = 6.0 Hz, 6H). HPLC analysis: retention time of 7.21 min (purity of 98%). MS (EI): *m*/*z* 438.1, 440.1 (MH^+).

2-{2-[2-(2-Bromophenylimino)-4-methyl-5-oxoimidazolidin-1-yl]-propionylamino}-propionic Acid (3BB2). Yield: 20.0 mg (99%). Pale yellow amorphous solid. IR (film): ν 3406, 1652, 1434, 1320, 1222, 1151, 1045, 1025, 992, 829, 755 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.09 (t, J = 7.5 Hz, 1H), 7.55−7.58 (m, 1H), 7.40 (s, 0.4H), 7.34 (s, 0.6H), 7.26−7.30 (m, 1H), 6.89−7.01 (m, 2H), 4.21−4.25 (m, 2H), 4.21−4.25 (m, 2H), 3.96−4.04 (m, 1H), 1.52−1.58 (m, 3H), 1.22−1.28 (m, 6H). ^{13}C NMR (DMSO-d_6, 500 MHz): δ 175.1, 174.7, 168.8, 149.1, 147.1, 133.1, 128.8, 124.1, 124.0, 118.1, 53.5, 49.2, 48.3, 18.1, 17.8, 15.0. HPLC analysis: retention time of 6.02 min (purity of 97%). MS (EI): *m*/*z* 410.2, 412.2 (MH^+).

2-{2-[4-Benzyl-2-(2-bromophenylimino)-5-oxoimidazolidin-1-yl]-4-methylpentanoylamino}-propionic Acid (3CD2). Yield: 25.3 mg (94%). Yellow solid. IR (film): ν 3418, 3062, 2958, 2870, 1674, 1582, 1538, 1470, 1455, 1428, 1385, 1317, 1214, 1158, 1114, 1026, 1004, 826, 751, 701, 665 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.13 (s, 0.7H), 7.91 (s, 0.3H), 7.52−7.60 (m, 1H), 7.12−7.42 (m, 7H), 6.82−6.96 (m, 2H), 4.16−4.42 (m, 2H), 2.88−2.95 (m, 4H), 2.07−2.14 (m, 1H), 1.11−1.63 (m, 7H), 0.61−0.86 (m, 2H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 173.7, 171.0, 168.9, 147.3, 135.6, 133.0, 130.8, 128.8, 128.4, 127.0, 124.2, 95.5, 58.8, 48.2, 36.4, 24.1, 21.6, 17.8. HPLC analysis: retention time of 9.83 min (purity of 90%). MS (EI): *m*/*z* 528.2, 530.2 (MH^+).

2-{2-[2-(2-Bromophenylimino)-4-methyl-5-oxoimidazolidin-1-yl]-3-phenylpropionylamino}-propionic Acid (3DB2). Yield: 23.9 mg (96%). Off-white amorphous solid. IR (film): ν 3418, 1668, 1583, 1557, 1538, 1470, 1455, 1428, 1319, 1216, 1144, 1044, 1026, 993, 917, 898, 836,

748, 702, 665 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.27 (d, J = 7.0 Hz, 1H), 7.58 (d, J = 8.0 Hz, 0.8H), 7.53 (d, J = 8.0 Hz, 0.2H), 7.14−7.32 (m, 6H), 6.86−6.98 (m, 1.8H), 6.69 (d, J = 8.0 Hz, 0.2H), 5.06 (d, J = 8.5 Hz, 0.2H), 4.84 (d, J = 8.5 Hz, 0.8H), 4.27−4.34 (m, 1H), 3.78−3.88 (m, 1H), 3.54−3.70 (m, 1H), 3.34−3.42 (m, 1H), 1.241.30 (m, 4H), 1.01(d, J = 6.5 Hz, 0.8H), 0.84 (d, J = 6.5 Hz, 2.2H). HPLC analysis: retention time of 8.70 min (purity of 98%). MS (EI): m/z 486.1, 488.1 (MH^+).

4-Methyl-2-[2-(5-oxo-2-p-tolyliminoimidazolidin-1-yl)-acetylamino]-pentanoic Acid (4AA4). Yield: 16.9 mg (92%). Yellow amorphous solid. IR (film): ν 3406, 2958, 1674, 1610, 1510, 1455, 1356, 1238, 1141, 1048, 1025, 1000, 824, 764, 668 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.33 (d, J = 8.0 Hz, 1H), 7.05 (d, J = 8.0 Hz, 3H), 6.78 (br s, 2H), 4.15 (s, 2H), 3.88 (s, 2H), 2.24 (s, 3H), 1.46−1.70 (m, 4H), 0.80−0.92 (m, 6H). HPLC analysis: retention time of 6.95 min (purity of 90%). MS (EI): m/z 361.2 (MH^+).

4-Methyl-2-[2-(4-methyl-5-oxo-2-p-tolyliminoimidazolidin-1-yl)-acetylamino]-pentanoic Acid (4AB4). Yield: 18.5 mg (97%). Yellow amorphous solid. IR (film): ν 3418, 1778, 1668, 1557, 1514, 1455, 1393, 1238, 1150, 1049, 1047, 1025, 997, 828, 767 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.33−8.36 (m, 1H), 7.20−7.28 (m, 1H), 7.06 (d, J = 8.0 Hz, 2H), 6.74−6.80 (m, 2H), 4.14 (s, 2H), 4.00−4.08 (m, 1H), 2.24 (s, 3H), 1.69−1.70 (m, 1H), 1.48−1.56 (m, 2H), 1.23 (d, J = 7.0 Hz, 3H), 0.82−0.90 (m, 6H). ^{13}C NMR (DMSO-d_6, 500 MHz): δ 174.5, 166.6, 152.5, 144.0, 131.1, 129.9, 122.7, 90.2, 62.3, 50.9, 40.8, 24.7, 23.4, 21.9, 20.9, 18.2. HPLC analysis: retention time of 7.26 min (purity of 96%). MS (EI): m/z 375.2 (MH^+).

2-{2-[2-(2-Bromophenylimino)-4-isobutyl-5-oxoimidazolidin-1-yl]-acetylamino}-4-methylpentanoic Acid (4AC2). Yield: 24.0 mg (99%). Pale yellow amorphous solid. IR (film): ν 3417, 2960, 1660, 1652, 1584, 1557, 1470, 1455, 1368, 1235, 1151, 1047, 1025, 995, 828, 767 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.30 (t, J = 7.5 Hz, 1H), 7.48−7.58 (m, 2H), 7.24−7.30 (m, 1H), 6.88−6.98 (m, 2H), 4.24−4.32 (m, 1H), 4.18 (s, 2H), 4.04−4.08 (m, 1H), 1.78−1.88 (m, 1H), 1.42−1.68 (m, 5H), 0.80−0.92 (m, 12H). HPLC analysis: retention time of 8.84 min (purity of 96%). MS (EI): m/z 480.2, 482.2 (MH^+).

2-{2-[2-(2-Bromophenylimino)-5-oxoimidazolidin-1-yl]-propionylamino}-4-methylpentanoic Acid (4BA2). Yield: 19.2 mg (86%). Yellow amorphous solid. IR (film): ν 3508, 1667, 1584, 1470, 1448, 1310, 1219, 1157, 1046, 1025, 991, 749 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.13 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.24−7.30 (m, 2H), 6.98 (d, J = 8.0 Hz, 1H), 6.89−6.91 (m, 1H), 4.74−4.80 (m, 1H), 4.22−4.28 (m, 1H), 3.84 (d, J = 6.0 Hz, 2H), 1.42−1.66 (m, 6H), 0.70−0.82 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 174.7, 172.1, 169.2, 150.4, 146.8, 133.0, 128.6, 124.1, 123.8, 118.3, 51.1, 49.4, 47.6, 24.4, 23.5, 21.7, 15.0. HPLC analysis: retention time of 7.98 min (purity of 98%). MS (EI): m/z 438.1, 440.1 (MH^+).

2-{2-[2-(2-Bromophenylimino)-4-methyl-5-oxoimidazolidin-1-yl]-propionylamino}-4-methylpentanoic Acid (4BB2). Yield: 22.3 mg (97%). Bright yellow amorphous solid. IR (film): ν 3349, 2959, 2872, 1668, 1583, 1557, 1470, 1428, 1372, 1321, 1263, 1213, 1156, 1044, 1025, 994, 829, 756 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.04−8.12 (m, 1H), 7.54−7.59 (m, 1H), 7.43 (s, 0.4H), 7.38 (s, 0.6H), 7.26−7.31 (m, 1H), 6.97−7.02 (m, 1H), 6.88−6.94 (m, 1H), 4.73−4.83 (m, 1H), 4.16−4.28 (m, 1H), 3.94−4.04 (m, 1H), 1.44−1.64 (m, 5H), 1.18−1.26 (m, 4H), 0.73−0.89 (m, 6H). HPLC analysis: retention time of 8.28 min (purity of 98%). MS (EI): m/z 452.1, 454.1 (MH^+).

2-{2-[2-(2-Bromophenylimino)-4-isobutyl-5-oxoimidazolidin-1-yl]-propionylamino}-4-methylpentanoic Acid (4BC2). Yield: 21.9 mg (87%). Yellow solid. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.03−8.07 (m, 1H), 7.54−7.58 (m, 1H), 7.50 (d, J = 1.5 Hz, 0.4H), 7.40 (d, J = 1.5 Hz, 0.6H), 7.26−7.31 (m, 1H), 6.88−7.02 (m, 2H), 4.72−4.85 (m, 1H), 4.16−4.36 (m, 1H), 3.93−4.02 (m, 1H), 1.78−1.86 (m, 1H), 1.42−1.66 (m, 8H), 0.74−0.92 (m, 12H). HPLC analysis: retention time of 9.63 min (purity of 98%). MS (EI): m/z 494.2, 496.2 (MH^+).

4-Methyl-2-[4-methyl-2-(4-methyl-5-oxo-2-m-tolyl-iminoimidazolidin-1-yl)-pentanoylamino]-pentanoic Acid. (4CB3) Yield: 20.0 mg (94%). Pale yellow amorphous solid. IR (film): ν 3384, 2959, 2872, 1668, 1558, 1488, 1470, 1417, 1386, 1369, 1320, 1210, 1151, 1088, 1047, 1025, 996, 828, 767, 691 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 12.40 (br s, 1H), 8.17 (d, J = 7.5 Hz, 0.9H), 8.11 (d, J = 7.5 Hz, 0.1H), 7.08−7.17 (m, 2H), 6.77−6.78 (m, 1H), 6.63−6.69 (m, 2H), 4.78 (dd, J = 11.5, 4.0 Hz, 0.4H), 4.70 (dd, J = 11.5, 4.0 Hz, 0.6H), 4.18−4.38 (m, 1H), 3.96−4.07 (m, 1H), 2.22−2.26 (m, 4H), 1.75−1.84 (m, 1H), 1.42−1.68 (m, 4H), 1.21 (d, J = 7.0 Hz, 3H), 0.76−0.96 (m, 12H). HPLC analysis: retention time of 8.74 min (purity of 93%). MS (EI): m/z 431.2 (MH^+).

4-Methyl-2-[4-methyl-2-(4-methyl-5-oxo-2-p-tolyl-iminoimidazolidin-1-yl)-pentanoylamino]-pentanoic Acid (4CB4). Yield: 21.0 mg (96%). Yellow amorphous solid. IR (film): ν 3260, 2960, 1668, 1508, 1423, 1320, 1212, 1182, 1102, 1047, 1025, 995, 828, 767 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.19 (d, J = 7.5 Hz, 1H), 7.18−7.32 (m, 1H), 7.04−7.10 (m, 2H), 6.73−6.79 (m, 2H), 4.79 (dd, J = 11.5, 4.0 Hz, 0.45H), 4.71 (dd, J = 11.5, 4.0 Hz, 0.55H), 3.93−4.33 (m, 2H), 2.35 (s, 0.8H), 2.25 (s, 2.2H), 1.72−1.84 (m, 1H), 1.14−1.66 (m, 8H), 0.76−0.95 (m, 12H). HPLC analysis: retention time of 8.82 min (purity of 94%). MS (EI): m/z 431.2 (MH^+).

2-[2-(4-Isobutyl-5-oxo-2-m-tolyliminoimidazolidin-1-yl)-4-methylpentanoylamino]-4-methylpentanoic Acid (4CC3). Yield: 22.5 mg (96%). Yellow amorphous solid. IR (film): ν 3478, 2958, 2870, 1667, 1492, 1469, 1420, 1386, 1368, 1314, 1206, 1152, 1090, 1048, 1025, 996, 895, 872, 828, 767, 691 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 12.04 (br s, 1H), 8.14−8.16 (m, 1H), 7.06−7.33 (m, 2H), 6.64−6.78 (m, 2H), 4.68−4.82 (m, 1H), 4.24−4.38 (m, 1H), 3.90−4.04 (m, 1H), 2.25 (s, 3H), 1.40−1.84 (m, 9H), 0.75−0.95 (m, 18H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 175.2, 174.7, 169.4, 155.2, 148.2, 138.3, 129.7, 123.4, 120.1, 119.6, 55.9, 54.1, 53.3, 51.1, 50.7, 24.4, 23.7, 23.5, 22.5, 21.5. HPLC analysis: retention time of 9.64 min (purity of 86%). MS (EI): m/z 473.3 (MH^+).

2-[2-(4-Isobutyl-5-oxo-2-p-tolyliminoimidazolidin-1-yl)-4-methylpentanoylamino]-4-methylpentanoic Acid (4CC4). Yield: 21.7 mg (90%). Pale yellow amorphous solid. IR (film): ν 3332, 2959, 2872, 1667, 1509, 1469, 1420, 1387, 1369, 1313, 1214, 1182, 1102, 1047, 1025, 994, 828, 768 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.18 (d, $J = 7.5$ Hz, 1H), 7.04−7.34 (m, 3H), 6.73−6.80 (m, 2H), 4.68−4.82 (m, 1H), 3.94−4.32 (m, 2H), 2.35 (s, 0.7H), 2.25 (s, 2.3H), 1.40−1.92 (m, 9H), 0.76−0.94 (m, 18H). HPLC analysis: retention time of 9.77 min (purity of 97%). MS (EI): *m*/*z* 473.3 (MH^+).

2-{2-[4-Isobutyl-5-oxo-2-(3,4,5-trimethoxyphenylimino)-imidazolidin-1-yl]-4-methyl-pentanoylamino}-4-methyl-pentanoic Acid (4CC5). Yield: 24.3 mg (87%). Yellow solid. IR (film): ν 3353, 2957, 2871, 1674, 1593, 1505, 1469, 1455, 1416, 1231, 1128, 1050, 1026, 1006, 824, 762, 654 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.17−8.22 (m, 2H), 7.50 (s, 0.45H), 7.45 (s, 0.55), 4.67−4.79 (m, 1H), 4.25−4.33 (m, 1H), 3.94−4.06 (m, 1H), 3.76 (s, 0.8H), 3.72 (s, 5.2H), 3.61 (s, 3H), 2.24−2.32 (m, 1H), 1.40−1.84 (m, 9H), 0.78−0.98 (m, 18H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 175.1, 174.7, 169.4, 153.5, 150.4, 149.7, 144.2, 133.2, 100.2, 60.5, 60.3, 57.2, 56.1, 55.9, 54.8, 53.6, 51.2, 50.7, 24.6, 23.7, 23.5, 22.6, 21.7. HPLC analysis: retention time of 9.47 min (purity of 95%). MS (EI): *m*/*z* 549.3 (MH^+).

2-{2-[2-(2-Bromophenylimino)-5-oxoimidazolidin-1-yl]-propionylamino}-3-phenylpropionic Acid (5BA2). Yield: 23.8 mg (99%). Bright yellow amorphous solid. IR (film): ν 3417, 1668, 1651, 1583, 1556, 1470, 1454, 1310, 1218, 1129, 1026, 844, 738, 702 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.07 (d, $J = 7.0$ Hz, 1H), 7.53−7.57 (m, 1H), 7.12−7.28 (m, 7H), 6.86−6.94 (m, 2H), 4.78 (q, $J = 7.5$ Hz, 1H), 4.34−4.42 (m, 1H), 3.83 (q, $J = 11.5$ Hz, 2H), 2.92−3.08 (m, 2H), 1.56 (d, $J = 7.5$ Hz, 3H). HPLC analysis: retention time of 8.17 min (purity of 92%). MS (EI): *m*/*z* 472.1, 474.1 (MH^+).

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Supporting Information Available. HPLC data, ^1H 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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