Solid-Phase Synthesis of an A–B Loop Mimetic of the Cε3 Domain of Human IgE: Macrocyclization by Sonogashira Coupling

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The solid-phase synthesis of a cyclic peptide containing the 21-residue epitope found in the A–B loop of the Cε3 domain of human immunoglobulin E has been carried out. The key macrocyclization step to form the 65-membered ring is achieved in ~15% yield via an "on-resin" Sonogashira coupling reaction which concomitantly installs a diphenylacetylene amino acid conformational constraint within the loop.

Introduction

Human allergic disorders (type I hypersensitivity responses) ranging from hay fever, eczema, and food allergies to potentially life threatening asthma and anaphylactic shock are increasing worldwide. Central to the cascade of events that lead to these clinical allergic manifestations are protein–protein binding events between human immunoglobulin E (hIgE) and its class-specific Fc receptors (FcεRI and FcεRII) on effector cells. Disruption of these interactions represents one possible therapeutic strategy for treatment of these allergic disorders. We have previously described a 21-residue disulfide-constrained cyclic peptide, containing residues Leu340–Thr357 of native hIgE that inhibits hIgE-triggered 5-hydroxytryptamine secretion in a genetically engineered rat basophilic leukemia cell line transfected with the extracellular domain (α-chain) of human FcεRII with an IC₅₀ of ~12 μM (Scheme 1). This antagonist was designed to crudely mimic an exposed Ω-loop in hIgE terminating the antiparallel A–B β-strand in the Cε3 domain which had been implicated as a binding “hot spot” for the interactions with both receptors (Figure 1).

Although 1H NMR studies indicated that, in aqueous solution, cyclic peptide displayed no discernible secondary structure, its ability to inhibit hIgE–FcεRI complex formation did display strong pH dependence. We considered that this could be indicative of a pronounced conformational binding dependence and initiated a pro-

FIGURE 1. Schematic representation of the antiparallel A–B β-strand and Ω-loop regions in the Cε3 domain of hIgE. Boxed residues Tyr339, Cys358, and Leu359 are those mutated in disulfide-constrained cyclic peptide 1 (i.e., Tyr339Cys, Cys358Ser, and Leu359Cys, Scheme 1).

![Image](318x327 to 558x445)

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Constrained peptide loops (e.g., containing decapeptides) decrease their proteolytic susceptibility, through scaffold-structural modifications to increase their rigidity and range in sophistication from peptides incorporating minor peptidomimetics,18 to totally nonpeptidic compounds that mimic the key structural elements of the peptide.19,20

...Conformationally restricted mimetics of peptide ligands...16

...such as the basis of novel vaccines is an attractive prospect...19

...Our plan was first to replace the flexible and labile disulfide constraint present in compound I with a more rigid and robust scaffold and then to explore progressively shorter loop sequences. In the expectation that significant conformational changes would accompany binding, we were looking to develop a semirigid structure that would be amenable to solid-phase parallel synthesis of small libraries of peptides for screening. To this end we were attracted to the use of diphenylacetylene (tolan) amino acids as scaffolds (Figure 2).

We envisaged that generic chemistry could be developed to allow access to a series of these tolan in which the amine and carboxylate moieties could occupy α-, m-, or p-positions in the A and B rings, respectively. These tolan amino acids should be capable of spanning a range of relative separations and orientations encompassing those found between the N- and C-termini of target hIgE epitopes, the appropriate tolan for a given epitope being selected with the aid of molecular modeling. A similar family of biphenyl amino acids has been described by Neustadt et al.21 Moreover, Kemp and Li22 have described a synthesis of the di-tolan amino acid and its use as a β-turn mimic.
Here we present a novel method for the solid-phase synthesis of cyclic tolant amino acid-constrained peptides exemplified by the synthesis of a direct analogue of cyclic peptide 1 in which the disulfide bridge has been replaced by a di-o-tolant amino acid. The method features an “on-resin” Sonogashira macrocyclization. Although the Heck reaction has been used previously for macrocyclization,25–26 this is the first example, to our knowledge, of macrocyclization by Sonogashira coupling.27

Results and Discussion

The approach we envisaged for the synthesis of our peptidomimetic is shown in Scheme 2. First, a 2-iodoaniline moiety would be immobilized to the solid support. The aniline nitrogen of this unit, which would form the A-ring of the tolan, would then be used as the anchor point for building up the peptide. The C-terminus of the peptide would then be capped off with 2-ethynylbenzoic acid, which would form the B-ring of the tolan unit, setting the stage for the on-resin Sonogashira macrocyclization. Finally, cleavage by acid from what was anticipated to be a Rink amide-type linker would provide the macrocyclic peptidomimetic in solution. Although this strategy leaves a primary amide function at the position where the tolan was attached to the solid support, we did not anticipate that this group, which should remain unprotonated at physiological pH, would adversely affect the performance of the peptidomimetic. Furthermore, we reasoned that it would be possible to use a traceless linker28 in a “second-generation” approach.

At the outset, we had two concerns regarding the implementation of this strategy: the expected low reactivity of the aniline nitrogen toward peptide coupling,22 and the expected difficulty of performing Sonogashira coupling with an aryl iodide having an o-amide group.29 Consequently, we decided to perform exploratory studies in solution.

For the preparation of the ring-A-containing unit III (Scheme 2), we required access to iodobenzoic acid derivative 4.30 Of various reagent combinations that have been reported to effect iodination of methyl 4-aminobenzoate,31–37 we found that use of ICl in AcOH38 was particularly convenient. This reaction proceeded smoothly on a preparative scale to give methyl 4-aminobenzoic acid (Fmoc-Cl) gave methyl ester 31 in 89% yield. Subsequent hydrolysis using HCl–TFA39 then gave the required acid 4 (61% yield, Scheme 3).

To examine the reactivity of aniline 2 toward amide formation, we performed exploratory coupling with benzoic acid. All the standard peptide coupling reagents that we examined failed to provide any coupled product,30 particularly convenient. This reaction proceeded smoothly on a preparative scale to give methyl 4-aminobenzoic acid derivative 4.30 Of various reagent combinations that have been reported to effect iodination of methyl 4-aminobenzoate,31–37 we found that use of ICl in AcOH38 was particularly convenient. This reaction proceeded smoothly on a preparative scale to give methyl 4-aminobenzoic acid (Fmoc-Cl) gave methyl ester 3 in 89% yield. Subsequent hydrolysis using HCl–TFA39 then gave the required acid 4 (61% yield, Scheme 3).

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t-serine amide derivative 6 readily coupled to Rink amide-functionalized polystyrene (Scheme 2), we required access to 2-ethynylbenzoic acid (16). Thus, Sonogashira coupling of methyl 2-iodo-benzoate with 2-(trimethylsilyl)acetylene [2-(TMS)acetyl-

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For the preparation of the ring-B-containing unit (see IV, Scheme 2), we required access to 2-ethylbenzoic acid (16). Thus, Sonogashira coupling of methyl 2-iodo-benzoate with 2-(trimethylsilyl)acetylene [2-(TMS)acetyl-

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Unfortunately, we were unable to translate this success in solution to the solid phase. Although acid 4 could be readily coupled to Rink amide-functionalized polystyrene (PS) and Rink amide-functionalized polystyrene glycol (PEG)-grafted PS (NovaSynTGR), and the Fmoc group could be removed quantitatively (piperidine, DMF) from either resin, the resulting resin-bound anilines would not couple with (S)-Fmoc-Ser(t-Bu)-Cl under the AgCN-mediated conditions. This failure can probably be attributed to the heterogeneity of the reaction conditions; AgCN has very low solubility in CH2Cl2; (v) 2,2,2-TBE-OH, AgCN, CH2Cl2; (vi) Zn powder, HOAc; (vii) MeNH2, HCl, Et3N, CH2Cl2.

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With both fragments in hand we turned our attention to the key Sonogashira reaction. Initial attempts to couple iodoanilines 11 and 12 with 2-ethylbenzoate 15 under a range of conditions failed to furnish more than trace amounts (<10%) of the desired tolans and furthermore resulted in very poor recovery of the iodoanilines. Suspecting that the TBE ester was unstable to the coupling conditions, we prepared the methylamide analogue of TBE ester 11 (i.e., 13) from acid 10 (Scheme 4). This resulted in a striking improvement in coupling.

Reagents and conditions: (i) CH2=CHCH2OH, Ti(O-i-Pr)4; (ii) RhCl(PPh3)3Cl, ETOH, H2O; (iii) NaOH, MeOH; (iv) SOCl2, DMF, CH2Cl2; (v) 2,2,2-TBE-OH, i-PrEtN, DMAP, CH2Cl2; (vi) (S)-Fmoc-Ser(t-Bu)-Cl, AgCN, CH2Cl2; (vii) Zn powder, HOAc; (viii) MeNH2, HCl, Et3N, CH2Cl2.

chloride (B2Cl) was also unreactive even in the presence of a variety of additives [e.g., 1-hydroxybenzotriazole (HOBr) or 4-(dimethylamino)pyridine (DMAP)] but was eventually found to couple almost quantitatively, albeit slowly, in the presence of AgCN.41,42

Although Carpino43 and others41,42 have shown that many amino acid chlorides can be coupled with little or no racemization, we thought it would be prudent to verify this for the coupling of aniline 2 with Fmoc-Ser(t-Bu)-Cl, as required for our A–B loop peptidomimetic. Thus, (S)-Fmoc-Ser(t-Bu)-Cl was coupled to aniline 2 to give serine amide derivative 6 in 81% yield. The antipodal serine amide ent-6 was similarly prepared using (R)-Fmoc-Ser(t-Bu)-Cl. Analysis of both amides by chiral stationary-phase (CSP) HPLC (Chiralcel OD) indicated that there was less than 0.5% racemization during coupling (Scheme 3).

Unfortunately, we were unable to translate this success in solution to the solid phase. Although acid 4 could be readily coupled to Rink amide-functionalized polystyrene (PS) and Rink amide-functionalized polystyrene glycol (PEG)-grafted PS (NovaSynTGR), and the Fmoc group could be removed quantitatively (piperidine, DMF) from either resin, the resulting resin-bound anilines would not couple with (S)-Fmoc-Ser(t-Bu)-Cl under the AgCN-mediated conditions. This failure can probably be attributed to the heterogeneity of the reaction conditions; AgCN has very low solubility in CH2Cl2; MeCN. To circumvent this impasse, we opted to attach the "pre-
coupled" serine amide 6 directly to the resin. This necessitated a change of ester protecting group to one orthogonal to both tert-butyl ether and Fmoc groups. Our initial choice was the allyl ester. Thus, transesterification of methyl ester 6 with allyl alcohol and Ti(Oi-Pr)4 gave allyl ester 7 quantitatively (Scheme 4).44 Conditions for deallylation in the presence of the aryl iodide were

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efficiency with ethynylbenzoate 15, giving tolan 17 in 65% yield. Moreover, the mass balance in this reaction could now be accounted for by recovered iodoaniline. By employing TMS-protected ethynylbenzoate 14 as the coupling partner, and employing in situ deprotection, tolan 17 could be obtained in 90% yield (Scheme 5). Since we planned to use Rink amide-type attachment to the resin, these were pleasing developments.

We were now in a position to check out our strategy on the solid phase and opted to investigate two resins in parallel: Rink PS and NovaSynTGR, since significant differences in coupling efficiencies between the two resins have been noted previously for certain solid-phase Sonogashira couplings (vide infra).31 Thus, benzoate 8 was coupled to Rink PS and NovaSynTGR resins using benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) to give functionalized resins 18R and 18N, respectively (Scheme 6).

The syntheses of the linear peptides 19R and 19N having the IgE C3 sequence 358-360 were carried out on an automated peptide synthesizer using the Fmoc-pentafluorophenyl (Pfp)/3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazinyl (Dhbt)-activated ester method. With acid removal of the N-terminal Leu residue, 16 was coupled manually using PyBOP to give resins 20R and 20N. Treatment of analytical samples of both resins with acid effected cleavage from the respective resins with concomitant side-chain deprotection to afford soluble peptide derivatives that were homogeneous by RP-HPLC and had the expected molecular weight by ES-MS (MH+ m/z 2534).

The stage was now set to investigate macrocyclization by Sonogashira coupling. Using the conditions optimized for the coupling of iodobenzamide 13 with ethynylbenzoate 15, we were pleased to note that cyclization occurred using the Rink resin 20R. The cyclized product 22 was purified by RP-HPLC (yield ~15%) and its identity confirmed by high-resolution MALDI-MS (MH+ m/z 2406.2).

We investigated the use of a variety of alternative macrocyclization conditions to try to improve the efficiency of the macrocyclization, including the use of Pd(PPh3)4, Pd2(dba)3, and Pd(OAc)2 as alternative palladium sources. However, despite considerable experimentation, we were unable to improve upon these original solution-optimized conditions. Moreover, we did not observe any cyclization when using NovasynTGR resin 20N. This was somewhat surprising since previous solid-phase Sonogashira coupling reactions have successfully been carried out on PS-based,31,54-59 PEG-based,33,60-62 (51) Schultz, D. A.; Gwaltney, K. P.; Lee, H. J. Org. Chem. 1998, 63, 4034-4038.

(52) The sequence prepared incorporates a Cys358Ser "mutation" as in disulfide-cyclized peptide 1 (Scheme 1 and Figure 1).

and other types of solid supports. In particular, the success of this cyclization on Rink PS and not on NovasynTGR is contrary to the findings of Dyatkin and Rivero, who reported that the latter was superior for the preparation of a library of propargylamines by solid-phase Sonogashira coupling from a resin-bound 3-iodobenzoate. Corroborative evidence for a decisive role for the resin in the cyclization comes from our inability also to obtain any cyclized material via attempted off-resin macrocyclization under a variety of conditions (e.g., Pd(PPh3)4Cl2, Cul, and THF, Pd(OAc)2, Cul, P(C6H4-o-SO3Na)2, and H2O-MeCN (1:1), and Pd(OAc)2, P(C6H4- o-SO3Na)2, and H2O-MeCN (1:1)).

Conclusions

We have demonstrated that Sonogashira coupling can be used to cyclize resin-bound peptide derivative 20 to give peptidomimetic 22, a process that concomitantly incorporates a tolan amino acid conformational constraint within the macrocycle. We are currently exploring this strategy for the synthesis of a focused library of related tolan-constrained peptidomimetics having shorter loop sequences.

Compound 22 was designed as a peptidomimetic of an exposed Ω-loop in hIgE that has been implicated as having a role in the binding of hIgE to both Fc receptors, interactions that play a pivotal role in type I hypersensitivity reactions. Initial studies into the inhibition by peptidomimetic 22 of IgE-triggered 5-hydroxytryptamine secretion in our rat basophilic leukemia cell line transfected with human FcεRI α-chain indicate that it is not a significantly more potent antagonist than disulfide-constrained cyclic peptide 1 (vide supra). These inhibition studies and the results of surface plasmon resonance SPR studies of the binding of peptidomimetic 22, and related peptidomimetics, with recombinate soluble FcεRI α-chain will be reported in full elsewhere.

Experimental Section

General Methods. All synthetic reactions were performed under anhydrous conditions and an atmosphere of nitrogen in oven-dried glassware. Yields refer to chromatographically homogeneous materials, unless otherwise indicated. Reagents were used as obtained from commercial sources or purified according to known procedures.

Flash chromatography (FC) was carried out using a silica gel 60F254 which were visualized either by quenching of ultraviolet fluorescence (λmax = 254 nm) or by charring with aqueous KMNNO4 in 5% K2CO3. All reaction solvents were distilled before use and stored over activated 4 Å molecular sieves, unless otherwise indicated. Anhydrous CH2Cl2, Et3N, piperidine, and i-Pr2EtN were obtained by distillation from CaH2. Anhydrous THF and Et2O were obtained by distillation, immediately before use, from sodium/benzophenone ketyl under an atmosphere of nitrogen. Anhydrous DMF was obtained by distillation from MgSO4 under reduced pressure. Petrol refers to the fraction of light petroleum boiling between 40 and 60 °C. High-resolution mass spectrometry (HRMS) measurements are valid to ±0.5 ppm.

Amino acids, coupling reagents, and resins were obtained commercially. Peptide syntheses were performed on a peptide synthesizer using an Fmoc-Pfp/Dhbt-activated ester strategy.

All HPLC was carried out using an HPLC system with a diode-array UV detector monitoring at 219 nm. Normal-phase analytical CSP HPLC was performed using a Chiralcel OD column (4.6 mm × 25 cm), flow rate 1 mL min−1. Reversed-phase analytical HPLC was performed using a C18, 5 µm column (4.6 mm × 25 cm), flow rate 1 mL min−1. Reversed-phase preparative HPLC was performed using a C18, 10 µm column (2.2 cm × 25 cm), flow rate 5 mL min−1.

Disulfide-Constrained Cyclic Peptide 1. A portion of Fmoc-L-Cys(Tr)-PEG-PS resin (1.1 g, nominal loading level 0.18 mmol g−1) was treated with piperidine–DMF (1:4 v/v) for 30 min to deprotect the terminal Fmoc group. After being washed with DMF, the resin was subjected to automated coupling cycles to build up the linear peptide sequence required. The amino acid derivatives employed were Fmoc-L-Ser(t-Bu)-ODbt, Fmoc-L-Thr(t-Bu)-ODbt, Fmoc-L-Leu-OPfp, Fmoc-L-Pro-OPfp, Fmoc-L-Lys(Boc)-OPfp, Fmoc-L-Arg(Pmc)-OH, Fmoc-L-Phe-OPfp, Fmoc-L-Leu-OPfp, Fmoc-L-Asp(t-Bu)-OPfp, and Fmoc-L-Cys(Tr)-OPfp. A standard 2(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU; 4 equiv)–HOBt (4 equiv)/60 min coupling cycle was employed for the two Fmoc-L-Arg(Pmc)-OH residues, all the other residues were coupled using standard activated ester coupling cycles (30–80 min). Residues Pro359, Phe334, Pro335, Ser336, and Arg338 were double coupled. A portion of the resulting resin (250 mg) was treated with piperidine–DMF (1:4 v/v) for 30 min to deprotect the terminal Fmoc group. Cleavage of the linear peptide from the resin and global side-chain protection were effected by treatment with a mixture of TFA–PhOH–H2O–PhSeMe–1,2-ethanedithiol (EDT) (20:0.7:5.0:0.25:0.25 v/v/w/v/v/v) for 30 min. Following filtration, the filtrate was concentrated in vacuo, precipitated from cold EtO (4×), collected by filtration, and lyophilized from 70% MeCN–H2O (v/v) with 0.1% TFA (v/v) to yield 90 mg of the crude peptide. This was purified by preparative reversed-phase HPLC, gradient 0–70% MeCN–H2O (v/v) with 0.1% TFA (v/v) over 60 min, to give the linear peptide as a white solid (67 mg, 63%): MS (ES+) m/z calcd for C105H171N28O30S2 (MH+) 2368.2, found 2367.3.

The pH of an aqueous solution of this crude peptide (0.1 mg mL−1) was adjusted to 8.5 using NH4HCO3, and the resulting solution was stirred in air at 40 °C for 8 h. The 24 h incubation time was determined empirically by a time course taking aliquots for ES-MS at 24, 48, 72, and 90 h. The residue was lyophilized to give disulfide-constrained cyclic peptide 1 as a white solid (65 mg, 61%): MS (ES+) m/z calcd for C105H191N28O30S2 (MH+) 2368.2, found 2367.3.

4-Amino-3-Iodo benzonic Acid Methyl Ester (2). A solution of ICl (11.0 g, 67.7 mmol) in AcOH (500 mL) was added...
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The solution was stirred at 0 °C for a further 15 min before the ice bath was removed and stirring continued at rt for 1 h. The solution was filtered and concentrated in vacuo to leave a brown oil which was recrystallized (CH2Cl2/petrol) to give iodobenzoate 3 as a white solid (400 mg, 89%); 1H NMR (CDCl3) δ 3.91 (s, 3H), 4.33 (t, J = 6.2 Hz, 1H); 6.72 (d, J = 1.5 Hz, 1H), 7.89 (d, J = 7.6 Hz, 2H); 8.34 (m, 1H), 9.27 (s, 1H); 13C NMR (CDCl3) δ 47.0, 52.3, 67.8, 87.6, 118.7, 128.5, 126.5, 126.7, 128.0, 130.9, 140.4, 141.2, 142.2, 143.1, 152.8, 165.2; MS (EI+) m/z 485 (M+, 10), 178 (100); HRMS (EI+) m/z calcd for C26H19INO4 (M+) 499.0281, found 499.0270.

4-(9-Fluoren-9-ylmethoxycarbonylamino)-3-iodobenzoic Acid Methyl Ester (5). To a solution of iodobenzoate 2 (200 mg, 0.45 mmol) in TFA (9 mL) and concd HCl (5 mL) was heated at 80 °C for 36 h. H2O (25 mL) was added and the precipitate collected by filtration. The solid was triturated with CH2Cl2 (10 mL) and collected by filtration to give iodobenzoic acid 4 as a pale yellow solid (119 mg, 61%); mp 174–176 °C; 1H NMR (DMSO-d6) δ 4.43 (s, 3H), 7.25–7.46 (m, 6H), 7.72 (d, J = 7.3 Hz, 2H), 7.86 (d, J = 1.5 Hz, 1H), 7.89 (d, J = 7.6 Hz, 2H); 8.34 (m, 1H), 9.27 (s, 1H), 13C NMR (DMSO-d6) δ 47.0, 66.8, 95.7, 120.6, 125.8, 126.2, 127.6, 128.2, 129.5, 130.2, 140.4, 141.2, 142.2, 143.9, 144.1, 154.2, 166.1; MS (EI+) m/z 485 (M+, 5), 178 (100); HRMS (EI+) m/z calcd for C26H19INO4 (M+) 485.0124, found 485.0131.

4-Benzoylaminio-3-iodobenzoic Acid Methyl Ester (5). To a solution of iodobenzoate 2 (200 mg, 0.45 mmol) in CH2Cl2 (5 mL) were added BzCl (320 μL, 2.0 equiv) and a suspension of AgCN (240 mg, 1.0 equiv) in MeCN (3 mL). The suspension was stirred for 4 h and filtered through Celite. The filtrate was partitioned between CH2Cl2 (10 mL) and 5% NaHCO3 (10 mL). The organic layer was further washed with 1 M NaHSO4 (2 × 10 mL). The organic layer was dried (MgSO4) and concentrated in vacuo to leave a pale brown solid. Purification by FC (EtOAc/hexane, 1:1) gave iodobenzoate 5 as a white solid (58.0 mg, 99%): mp 144–146 °C; 1H NMR (CDCl3) δ 3.82 (s, 3H), 7.51–7.67 (m, 3H), 7.96–8.03 (m, 2H), 8.08 (dd, J = 8.1, 1.8 Hz, 1H), 8.50 (d, J = 1.8 Hz, 1H), 8.52 (br, s, 1H), 8.14 (d, J = 7.5 Hz, 1H); 13C NMR (CDCl3) δ 52.3, 88.8, 120.0, 127.2, 129.1, 130.7, 132.6, 134.1, 140.2, 142.1, 165.2; MS (ES+) m/z 404 (MNa+, 100), 382 (MH+, 40); HRMS (ES+) m/z calcd for C23H17INO4 (M+) 381.9940, found 381.9955.

(S)-4-[3-tert-Butoxy-2-(9-fluoren-9-ylmethoxycarbonylamino)propionylamino]-3-iodobenzoic Acid Methyl Ester (6). To a solution of iodobenzoate 2 (73.0 mg, 0.26 mmol) in CH2Cl2 (6 mL) were added (S)-Fmoc-Ser(t-Bu)-Cl (96.0 mg, 1.0 equiv) and AgCN (35.0 mg, 1.0 equiv). The suspension was stirred for 4 h and filtered through Celite. The filtrate was concentrated in vacuo to leave an off-white solid. Purification by FC (EtOAc/hexane, 1:1) gave iodobenzoate 6 as a white solid (138 mg, 81%, >99.5% ee by HPLC; see the Supporting Information): mp 121–122 °C; 1H NMR (CDCl3) δ 1.20 (s, 3H), 3.94 (s, 3H), 6.62 (br, s, 1H), 4.25 (t, J = 6.9 Hz, 1H), 4.45–4.6 (br, m, 3H), 5.8 (br, s, 1H), 7.25–7.15 (br, m, 4H), 7.6 (br, s, 2H), 7.77 (d, J = 6.6 Hz, 2H), 8.00 (dd, J = 7.5, 1.9 Hz, 1H), 8.40 (d, J = 7.5 Hz, 1H), 8.45 (d, J = 1.9 Hz, 1H), 8.70 (br, s, 1H); 13C NMR (CDCl3) δ 27.5, 47.1, 52.3, 56.4, 61.5, 67.4, 74.4, 88.7, 120.1, 120.3, 125.0, 127.1, 127.2, 127.8, 130.7, 140.3, 141.4, 141.7, 143.6, 156.1, 165.2, 169.2; MS (ES+) m/z 665 (MNa+, 100), 643 (MH+, 20); HRMS (ES+) m/z calcd for C28H20N2O6 (MNa+) 643.1305, found 643.1290.

The enantiomer of this compound was also prepared in 84% yield and >99.5% ee by HPLC (see the Supporting Information) using the same procedure but employing (R)-Fmoc-Ser(t-Bu)-Cl.
4-Amino-3-iodobenzoic acid (10). To a solution of methyl ester 2 (2.0 g, 7.2 mmol) in MeOH (6 mL) was added 2.0 M HCl (12 mL). The solution became turbid and was stirred for 24 h, during which time the solution became clear. The aqueous phase was extracted with EtOAc (2 × 10 mL), and the combined organic washings were dried (MgSO₄) and concentrated in vacuo to give the crude acid chloride as an oil. This oil was added to a solution of compound 10 (0.50 g, 5.97 mmol) and triethylamine (1.0 mL), and the resulting solution was stirred for 1 h, then concentrated in vacuo. Purification by FC (EtOAc/hexane, 1:100) gave acid chloride (750 mg, 2.80 mmol) in CH₂Cl₂ (10 mL) were added MeOH/H₂O (250 mg, 1.75 equiv) and Et₂N (0.90 mL, 5.20 equiv). The reaction solution was stirred for 0.5 h, then concentrated in vacuo, and washed with EtOAc (25 mL), and washed with 5% NaHCO₃ (50 mL). The organic layer was then washed with brine (2 × 25 mL), dried (MgSO₄), and concentrated in vacuo. Purification by FC (EtOAc/hexane, 4:6) gave methylbenzamide 13 as a white solid (432 mg, 57%).

2-Trimethylsilylbenzylamine Acid Methyl Ester (14). To a solution of methyl 2-iodobenzoate (1.0 g, 4.65 mmol), 2-TRMS-acetylene (986 μL, 1.5 equiv), PPh₃ (30.0 mg, 2.5 mol %), and CuI (22.0 mg, 2.5 mol %) in THF (50 mL) was added Pd(PPh₃)₂Cl₂ (165 mg, 3 mol %) with stirring. The solution was stirred for 1 h, concentrated in vacuo, and then purified by FC (EtOAc/hexane, 1:100) to give benzamide 14 as a colorless oil (299 mg, 92%).

2-Ethynylbenzylamine Acid Methyl Ester (15). To a solution of 14 (500 mg, 2.1 mmol) in MeOH (10 mL) was added KF (4.0 mmol, excess). The suspension was stirred for 36 h, filtered, and partitioned between EtOAc (20 mL) and 0.1 M HCl (20 mL). The organic layer was further washed with brine (2 × 25 mL), dried (MgSO₄), and concentrated in vacuo to leave a brown oil. Purification by FC (EtOAc/hexane, 1:50) gave ethynylbenzamide 15 as a colorless oil (281 mg, 82%).

2-Ethynylbenzylamine acid (16). To a solution of 14 (250 mg, 1.05 mmol) in MeOH (5 mL) was added a 2.0 M NaOH solution (5 mL). The resulting solution was stirred for 2 h, EtOAc (40 mL) was added, and the organic layer was discarded. The aqueous layer was acidified to pH 1 by the addition of 1 M HCl and extracted with EtOAc (2 × 25 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo to leave a pale brown foam. Purification by FC (EtOAc/hexane, 1:4) gave acid 16 as a yellow solid (75.0 mg, 48%).

2-(2-Amino-5-methylcarbamoylphenylethynyl)benzoic Acid Methyl Ester (17). Method 1. To a solution of 10 (175 mg, 0.63 mmol), ethynylbenzamide 15 (100 mg, 0.63 mmol), PPh₃ (16.0 mg, 10 mol %), and Et₂N (131 μL, 1.5 equiv) in THF (5.0 mL) was added Pd(PPh₃)₂Cl₂ (22 mg, 5 mol %). The solution was stirred at rt for 72 h, diluted with EtOAc (25 mL), and washed with 0.1 M HCl (20 mL) and brine (25 mL). The organic layer was dried (MgSO₄), concentrated in vacuo, and then purified by FC (EtOAc/hexane, 7:3) to give 17 as a pale yellow solid (130 mg, 65%).


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(177 mg, 2 equiv), PPh₃ (28.0 mg, 28 mol %), CuI (20.0 mg, 28 mol %), and piperidine (753 µL, 20 equiv) in THF (15 mL) was purged with nitrogen for 15 min. Solid K₂CO₃ (116 mg, 2.2 equiv) and Pd(PPh₃)₂Cl₂ (28 mg, 14 mol %) were added, and the resulting suspension was heated to reflux, during which time MeOH (2 mL) was added dropwise (to desilylate 14 in situ). After 15 h, saturated NH₄Cl and Et₂O (100 mL, 1:1) were added, and the aqueous layer was discarded. The organic layer was dried (MgSO₄) and concentrated in vacuo to leave a brown oil. Purification by FC (EtOAc/hexane, 1:1) gave tolan 17 as a yellow oil (100 mg, 99%). The spectroscopic data are given above.

Resin 18. A portion of Fmoc-protected Rink amid resin (750 mg, nominal loading level 0.58 mmol g⁻¹) was treated with piperidine–DMF (1:4 v/v) for 30 min to deprotect the Fmoc group. After being washed with DMF, the resin was suspended in CH₂Cl₂ (10 mL) and allowed to swell for 5 min before addition of acid 8 (311 mg, 0.49 mmol), PyBOP (300 mg, 0.58 mmol), and i-Pr₂EtN (175 µL, 1.0 mmol). The resulting mixture was allowed to stand for 3 h with occasional swirling before the resin was separated by filtration and washed with DMF (3 × 25 mL), CH₂Cl₂ (3 × 25 mL), MeOH (3 × 25 mL), and Et₂O (3 × 25 mL). The resin was then resuspended in DMF (8 mL) and Ac₂O (2.0 mL, excess) and i-Pr₂EtN (2.0 mL, excess) added to cap off any remaining unprotected amino groups. The resulting mixture was allowed to stand for 2 h with occasional swirling before the resin was separated by filtration and washed with DMF (3 × 25 mL), CH₂Cl₂ (3 × 25 mL), MeOH (3 × 25 mL), and Et₂O (3 × 5 mL). The resin was then dried under high vacuum to give 18 as a pale orange resin (825 mg, 0.34 mmol g⁻¹ (by Fmoc test)).

Resin 19. A portion of resin 18 (220 mg, 0.34 mmol g⁻¹) was treated with piperidine–DMF (1:4 v/v) for 30 min to deprotect the terminal Fmoc group. After being washed with DMF, the resin was suspended in CH₂Cl₂ (5 mL) and allowed to swell for 5 min before addition of acid 16 (58 mg, 0.40 mmol), PyBOP (205 mg, 0.40 mmol), and i-Pr₂EtN (78 µL, 0.45 mmol). The resulting mixture was allowed to stand for 3 h with occasional swirling before the resin was separated by filtration and washed with DMF (3 × 15 mL), CH₂Cl₂ (3 × 15 mL), MeOH (3 × 15 mL), and Et₂O (3 × 15 mL). The resin was then dried under high vacuum to give 20 as an orange resin (260 mg). Analytical cleavage/side-chain deprotection as above gave a crude sample of the Fmoc-terminally-protected peptide: MS (ES⁺) m/z calc for C₁₁₅H₁₆₉N₂₈O₂₉ (MH⁺) 2534.2, found 2534; analytical reversed-phase HPLC, gradient 0–70% MeCN–H₂O (v/v) with 0.1% TFA (v/v) over 60 min, Rₜ 14.5 min.

Resin 21 and Tolan-Constrained Cyclic Peptide 22. A suspension of resin 20 (25.0 mg, assumed ∼0.1 mmol g⁻¹), PPh₃ (1.0 mg), CuI (1.0 mg), and Et₃N (5.0 µL) in THF (1 mL) was purged with nitrogen for 15 min. Pd(PPh₃)₂Cl₂ (1.2 mg) was added, and the resulting suspension was shaken at rt. After 15 h, the resin was separated by filtration and washed with DMF (3 × 5 mL), CH₂Cl₂ (3 × 5 mL), MeOH (3 × 5 mL), and Et₂O (3 × 5 mL). The resin was then dried under high vacuum to give 21 as an orange resin (20.1 mg). This resin sample was treated with TFA–H₂O–i-PrSiH (95:4:1 v/v/v) for 2 h. Following filtration, the filtrate was concentrated in vacuo, precipitated from cold Et₂O, and lyophilized from 70% MeCN–H₂O (v/v) with 0.1% TFA (v/v) over 60 min, to give peptidomimetic 22 as a white solid (0.7 mg, ∼15%), Rₜ 12.4 min: MS (ES⁺) m/z calc for C₁₃₃H₁₇₀N₂₉O₂₉ (MH⁺) 2406.2, found 2406.2.

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Supporting Information Available: ¹H and ¹³C NMR spectra for compounds 3–9, 11–13, and 17 and ES-MS spectra of compounds 1 and 22 and samples cleaved from resins 19 and 20. This material is available free of charge via the Internet at http://pubs.acs.org.

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