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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, excema, and food allergies to potentially life threatening asthma and anaphylactic shock are increasing worldwide.[1] 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.[2−4] Disruption of these interactions represents one possible therapeutic strategy for treatment of these allergic disorders.[5−10] We have previously described a 21-residue disulfide-constrained cyclic peptide, **1**, 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 FcϵRI with an IC_{50} of ∼12 μM (Scheme 1).[11]

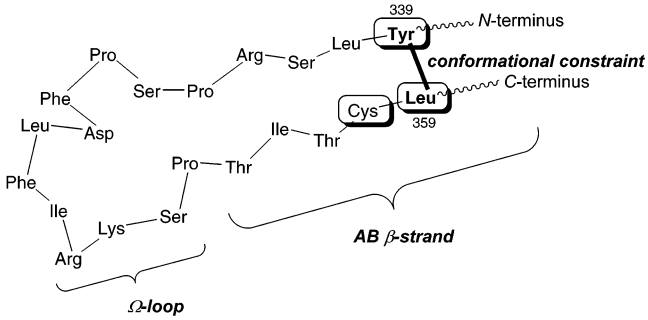

FIGURE 1. Schematic representation of the antiparallel A−B β-strand and Ω-loop regions in the Cϵ3 domain of hIgE. Boxed residues Tyr339, Cys358, and Leu359 are those mutated in disulfide-constrained cyclic peptide **1** [i.e., Tyr339Cys, Cys358Ser, and Leu359Cys, Scheme 1).

This antagonist was designed to crudely mimic an exposed Ω-loop[12,13] in hIgE terminating the antiparallel A−B β-strand in the Cϵ3 domain which had been implicated as a binding "hot spot" [14] for the interactions with both receptors (Figure 1).[15]

Although ^{1}H NMR studies indicated that, in aqueous solution, cyclic peptide **1** 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-

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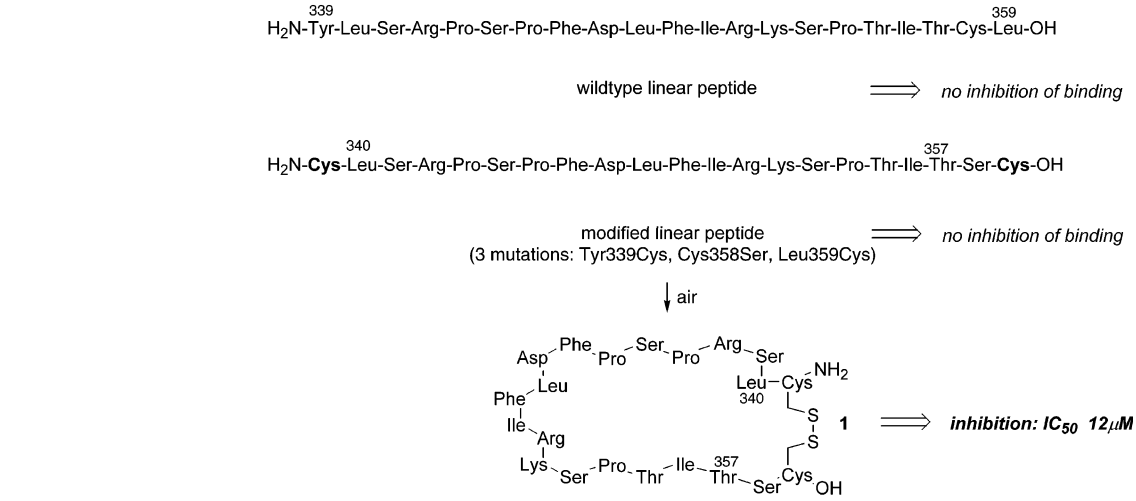
SCHEME 1. Structures and Binding Characteristics of the Wild-Type and Mutated Linear Peptides Comprising the Tyr339−Leu359 Epitope of hIgE and of the Disulfide-Constrained Cyclic Peptide 1[a]



[a] For details of the binding assay see Helm et al., ref 15.

gram to prepare more conformationally rigid analogues of this lead structure.[16]

Conformationally restricted mimetics of peptide ligands often exhibit enhanced specificity, affinity, and oral activity against a given receptor.[17] Such peptidomimetics range in sophistication from peptides incorporating minor structural modifications to increase their rigidity and decrease their proteolytic susceptibility, through scaffold-constrained peptide loops (e.g., containing β-turn mimetics),[18] to totally nonpeptidic compounds that mimic the key structural elements of the peptide.[19,20]

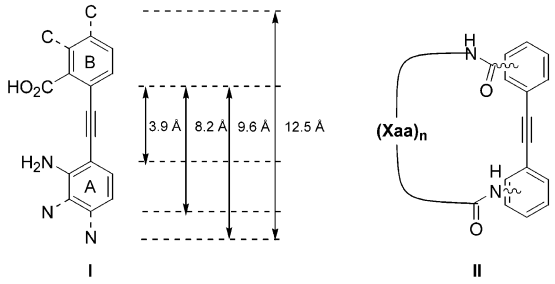


FIGURE 2. General structure of tolan amino acids **I**, with approximate dimensions, and schematic representation of generalized peptidomimetic **II** incorporating such a constraining element.

Our plan was first to replace the flexible and labile disulfide constraint present in compound **1** 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 scaffold that would be amenable to solid-phase parallel synthesis of small libraries of peptidomimetics for screening. To this end we were attracted to the use of diphenylacetylene (tolan) amino acids **I** as scaffolds (Figure 2).

We envisaged that generic chemistry could be developed to allow access to a series of these tolans in which the amine and carboxylate moieties could occupy *o*-, *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 Li[22] have described a synthesis of the di-*o*-tolan amino acid and its use as a β-turn mimetic.

(15) When we initiated this investigation in 1997, there was no experimental high-resolution structural information for hIgE and our modeling used a structure of hIgE (Brookhaven PDB code 2IGE) derived from sequence homology with an X-ray structure of human IgG1−Fc [Brookhaven PDB code 1FC1, 2.9 Å resolution (Padlan, E. A.; Davies, D. R. *Mol. Immunol.* **1986**, *23*, 1063−1075)]. The evidence that the Leu340−Thr357 A−B Ω-loop targeted in this study constituted a binding hot spot for the "high-affinity" hIgE−FcεRI interaction came from studies with truncated IgE-derived peptides (summarized by Helm et al. in ref 11). However, site-directed mutagenesis studies reported in 1998 failed to identify specific residues in the A−B loop responsible for this binding (Sayers, I.; Cain, S. A.; Swan, J. R. M.; Pickett, M. A.; Watt, P. J.; Holgate, S. T.; Padlan, E. A.; Schuck, P.; Helm, B. A. *Biochemistry* **1998**, *37*, 16152−16164). Moreover, in 2000 an X-ray structure of human IgE bound to the extracellular α-subunit of the FcεRI receptor was disclosed [Brookhaven PDB code 1F6A, 3.5 Å resolution (Garman, S. C.; Wurzburg, B. A.; Tarchevskaya, S. S.; Kinet, J.-P.; Jardetzky, T. S. *Nature* **2000**, *406* (July 20), 259−266)]. In this structure the A−B loop region of hIgE did not form part of the contact surface with FcεRI. A *direct* role for the A−B loop in the FcεRI interaction therefore appears doubtful at the present time, and studies are ongoing in our laboratories to gain further understanding of the dynamics of the binding at this protein−protein interface. However, a direct role for the Leu340−Thr357 A−B Ω-loop in the binding of hIgE to its "low-affinity" receptor FcεRII/CD23 (Sayers, I.; Helm, B. A. *Clin. Exp. Allergy* **1999**, *29*, 585−594) is more clear-cut. Indeed, we have recently obtained clear evidence for the involvement of Lys352 in this interaction (Sayers, I.; Housden, J. A.; et al. Manuscript in preparation). Since this interaction has a role in maintaining and modulating the level of mediator release initiated by hIgE, the use of peptidomimetics such as **22** as the basis of novel vaccines is an attractive prospect (cf. the following two references: Shakib, F.; Hooi, D. S. W.; Smith, S. J.; Furmonaviciene, R.; Sewell, H. F. *Clin. Exp. Allergy* **2000**, *30*, 1041−1046. Robinson, J. A. *Synlett* **1999**, 429−441).

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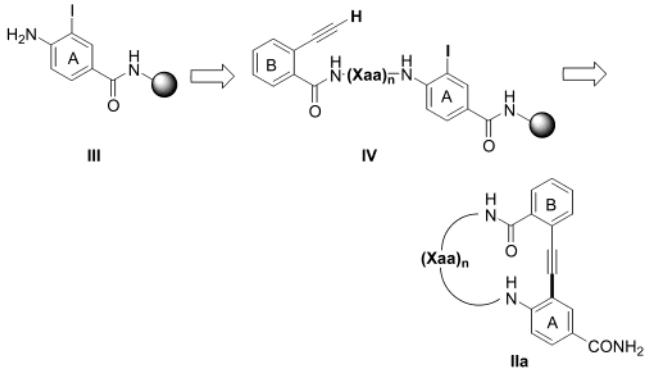
SCHEME 2. Overview of Our Strategy for the Solid-Phase Synthesis via Sonogashira Macrocyclization of Peptidomimetic IIa Incorporating a Di-*o*-tolan Amino Acid Constraining Element



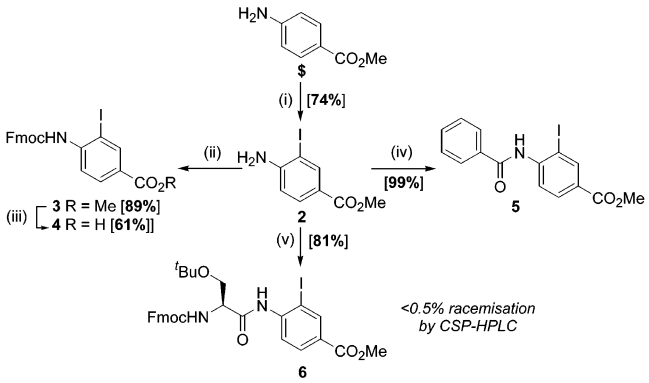
Here we present a novel method for the solid-phase synthesis of cyclic tolan amino acid-constrained peptides exemplified by the synthesis of a direct analogue of cyclic peptide **1** in which the disulfide bridge has been replaced by a di-*o*-tolan amino acid. The method features an "on-resin" Sonogashira macrocyclization. Although the Heck reaction has been used previously for macrocyclization,[23−26] 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 linker[28] 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] Conseqently, we decided to perform exploratory studies in solution.

SCHEME 3[a]



[a] Reagents and conditions: (i) ICl, HOAc; (ii) FmocCl, pyridine, CH_2Cl_2; (iii) TFA, concd HCl; (iv) BzCl, AgCN, CH_2Cl_2, MeCN; (v) (*S*)-Fmoc-Ser(*t*-Bu)-Cl, AgCN, CH_2Cl_2.

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 AcOH[38] was particularly convenient. This reaction proceeded smoothly on a preparative scale to give methyl 4-amino-3-iodobenzoate (**2**) in 74% yield. Protection of the aniline nitrogen of iodobenzoate **2** with 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl) gave methyl ester **3** in 89% yield. Subsequent hydrolysis using HCl−TFA[39] 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, starting materials being returned in all cases.[40] Benzoyl

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(30) A 4-amino-3-iodobenzoate, linked via an amide bond to Rink amide AM resin (cf. ring-A-containing unit **III**, Scheme 2) has been prepared previously for the solid-phase synthesis of 2,3-disubstituted indoles via a Pd(0)-mediated heteroannulation of nonterminal alkynes (see Zhang et al., ref 31). An analogous resin having an ester linkage to TentaGel-S resin has also been employed for the solid-phase synthesis of 2-substituted indoles via a Pd(0)/Cu(I)-mediated "one-pot" Sonogashira/5-*endo*-dig coupling/cyclization protocol (see Fagnola et al., ref 33).
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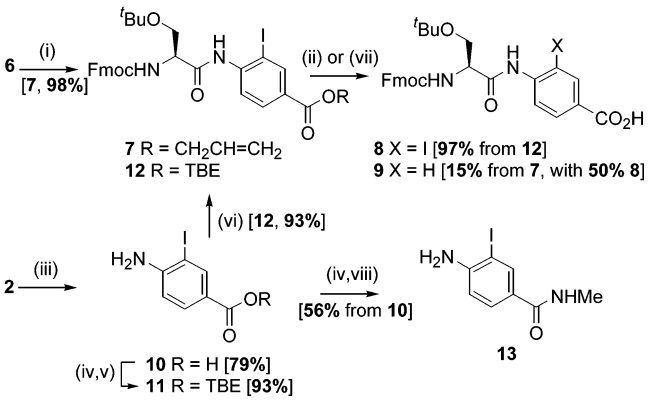
(40) Since Kemp and Li (ref 22) have reported that they employed "standard peptide coupling reactions" to attach N-blocked amino acids to the aniline nitrogen of the di-*o*-tolan amino acid methyl ester, the failure of the aniline nitrogen to couple in our system can probably be attributed to the influence of the adjacent iodine atom.

SCHEME 4[a]



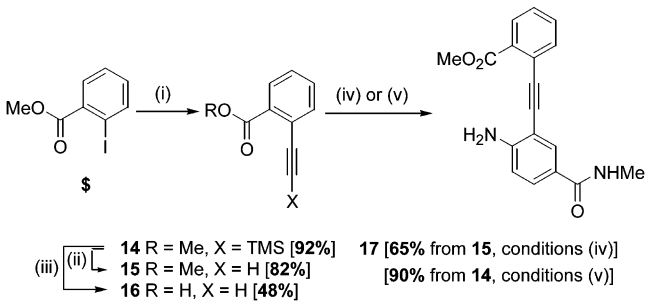
[a] Reagents and conditions: (i) CH_2=$CHCH_2OH$, Ti(O-*i*-Pr)$_4$; (ii) $RhCl(PPh_3)_3$, EtOH, H_2O; (iii) NaOH, MeOH; (iv) $SOCl_2$, DMF, CH_2Cl_2; (v) 2,2,2-TBE-OH, *i*-Pr_2EtN, DMAP, CH_2Cl_2; (vi) (*S*)-Fmoc-Ser(*t*-Bu)-Cl, AgCN, CH_2Cl_2; (vii) Zn powder, HOAc; (viii) $MeNH_2$·HCl, Et_3N, CH_2Cl_2.

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

Although Carpino[43] and others[41,42] 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 polyethylene 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 CH_2Cl_2−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(O*i*-Pr)$_4$ gave allyl ester **7** quantitatively (Scheme 4).[44] Conditions for deallylation in the presence of the aryl iodide were

SCHEME 5[a]



[a] Reagents and conditions: (i) 2-TMS-acetylene, PPh_3, CuI, Et_3N, $Pd(PPh_3)_2Cl_2$, THF; (ii) KF·H_2O, MeOH; (iii) 2 M NaOH, MeOH; (iv) **13**, PPh_3, CuI, Et_3N, $Pd(PPh_3)_2Cl_2$, THF; (v) **13**, PPh_3, CuI, piperidine, K_2CO_3, $Pd(PPh_3)_2Cl_2$, MeOH, THF.

required; $Pd(PPh_3)_4$-catalyzed conditions[45−47] failed to exhibit any chemoselectivity, whereas $Rh(PPh_3)_3Cl$-catalyzed conditions[48] furnished a mixture of the desired acid **8** and the deiodinated acid **9** in 50% and 15% yields, respectively (Scheme 4). Since these products were difficult to separate, and we were unable to delineate conditions under which deoiodination could be completely suppressed, we abandoned allyl ester protection in favor of 2,2,2-tribromoethyl (TBE) ester protection.[49] The TBE ester could not be introduced by transesterification of methyl ester **6**, so it was prepared from acid **10** via conversion to the acid chloride, reaction with TBE alcohol to give TBE ester **11** (93% yield), and then AgCN-mediated coupling with (*S*)-Fmoc-Ser(*t*-Bu)-Cl to give serine amide **12** in 93% yield. Deprotection of the TBE ester using powdered Zn in AcOH gave the desired acid **8** smoothly in 97% yield (Scheme 4).

For the preparation of the ring-B-containing unit (see **IV**, Scheme 2), we required access to 2-ethynylbenzoic acid (**16**). Thus, Sonogashira coupling of methyl 2-iodobenzoate with 2-(trimethylsilyl)acetylene [2-(TMS)acetylene] using $Pd(PPh_3)_2Cl_2$, CuI, and Et_3N in THF[50] gave the diprotected derivative **14** in 92% yield. Treatment of this ester with KF in MeOH effected desilylation to give ester **15** in 82% yield. Alternatively, treatment of ester **14** with NaOH in MeOH gave the desired acid **16** as a colorless oil in 48% yield after rapid flash chromatography over SiO_2 (Scheme 5).

With both fragments in hand we turned our attention to the key Sonogashira reaction. Initial attempts to couple iodoanilines **11** and **12** with 2-ethynylbenzoate **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

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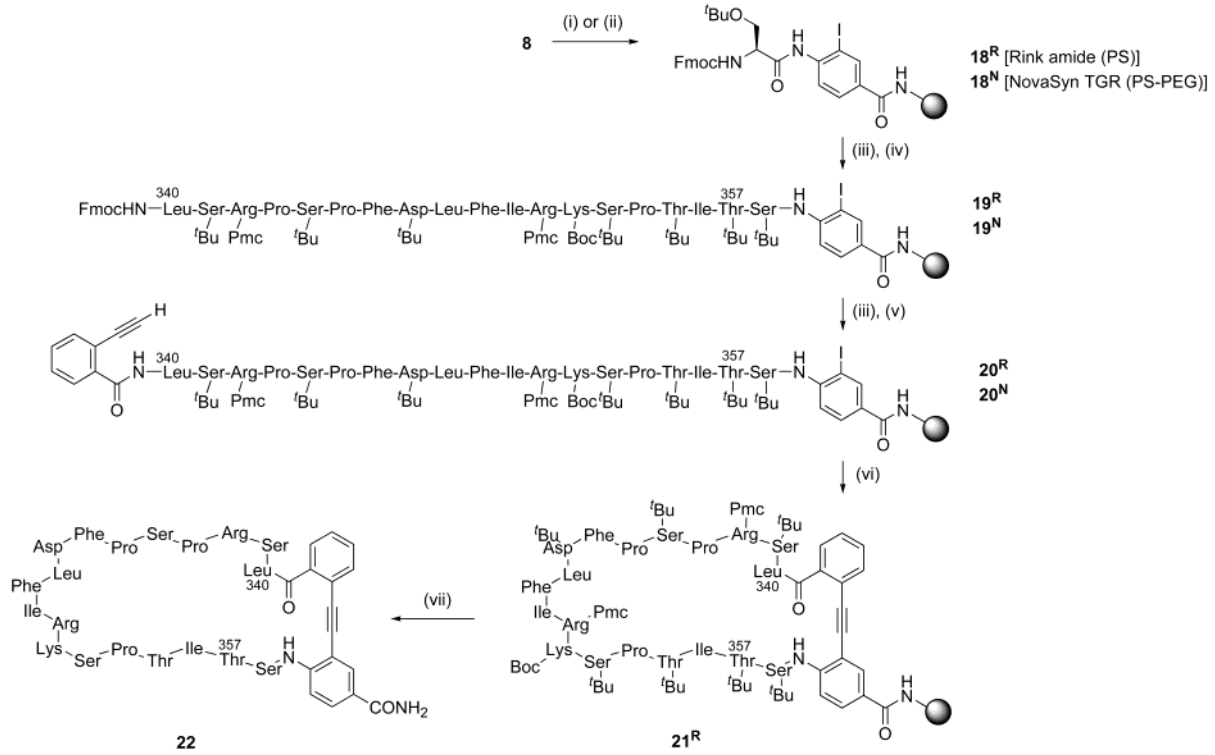
SCHEME 6[a]



[a] Reagents and conditions: (i) Rink amide resin (0.58 mmol g^{-1}), PyBOP, *i*-Pr_2EtN, CH_2Cl_2; (ii) NovaSynTGR resin (0.23 mmol g^{-1}), PyBOP, *i*-Pr_2EtN, CH_2Cl_2; (iii) piperidine, DMF; (iv) automated, sequential coupling and deprotection of Fmoc-Pfp/Dhbt amino acid esters [Ser(*t*-Bu), Thr(*t*-Bu), Ile, Pro, Lys(Boc), Arg(Pmc), Phe, Leu, Asp(*t*-Bu)]; (v) **16**, PyBOP, *i*-Pr_2EtN, CH_2Cl_2; (vi) PPh_3, CuI, Et_3N, $Pd(PPh_3)_2Cl_2$, DMF; (vii) TFA−H_2O−*i*-Pr_3SiH (95:4:1 v/v/v).

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,[51] 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).[29] Thus, benzoate **8** was coupled to Rink PS and NovaSynTGR resins using benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) to give functionalized resins $\mathbf{18}^R$ and $\mathbf{18}^N$, respectively (Scheme 6).

The syntheses of the linear peptides $\mathbf{19}^R$ and $\mathbf{19}^N$ having the IgE Cϵ3 sequence 358−340[52] 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.[53] Following Fmoc deptotection of the N-terminal Leu residue, **16** was coupled manually using PyBOP to give resins $\mathbf{20}^R$ and $\mathbf{20}^N$. 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 condtions optimized for the coupling of iodobenzamide **13** with ethynylbenzoate **15**, we were pleased to note that cyclization occurred using the Rink resin $\mathbf{20}^R$. 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 $Pd(PPh_3)_4$, $Pd_2(dba)_3$, 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 $\mathbf{20}^N$. 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]

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

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and other[63] 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.[29] 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., $Pd(PPh_3)_2Cl_2$, CuI, and THF, $Pd(OAc)_2$, CuI, $P(C_6H_4$-*o*-$SO_3Na)_3$, and H_2O−MeCN (1:1), and $Pd(OAc)_2$, $P(C_6H_4$-*o*-$SO_3Na)_3$, and H_2O−MeCN (1:15)].[64,65]

Conclusions

We have demonstrated that Sonogashira coupling can be used to cyclize resin-bound peptide derivative **20**^{R} 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 responses.[15] 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).[66] These inhibition studies and the results of surface plasmon resonance SPR studies of the binding of peptidomimetic **22**, and related peptidomimetics, with recombinent 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 and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise indicated. Reagents were used as obtained from commercial sources or purified according to known procedures.[67] Flash chromatography (FC) was carried out using Kieselgel 60 F_{254} (230−400 mesh) silica gel. Only distilled solvents were used as eluents. Thin-layer chromatography (TLC) was performed on DC-Alufolien plates precoated with silica gel 60 F_{254} which were visualized either by quenching of ultraviolet fluorescence ($\lambda_{max} = 254$ nm) or by charring with aqueous $KMnO_4$ in 5% K_2CO_3. All reaction solvents were distilled before use and stored over activated 4 Å molecular sieves, unless otherwise indicated. Anhydrous CH_2Cl_2, Et_3N, piperidine, and *i*-Pr_2EtN were obtained by distillation from CaH_2. Anhydrous THF and Et_2O were obtained by distillation, immediately before use, from sodium/benzophenone ketyl under an atmosphere of nitrogen. Anhydrous DMF was obtained by distillation from $MgSO_4$ 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±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)-ODhbt, Fmoc-L-Thr(*t*-Bu)-ODhbt, Fmoc-L-Ile-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-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluorobarate (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, Pro337, 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 deprotection were effected by treatment with a mixture of TFA−PhOH−H_2O−PhSMe−1,2-ethanedithiol (EDT) (20:0.7:0.5:0.5:0.25 v/w/v/v/v) for 30 min. Following filtration, the filtrate was concentrated in vacuo, precipitated from cold Et_2O (4×), collected by filtration, and lyophilized from 70% MeCN−H_2O (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−H_2O (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 $C_{105}H_{171}N_{28}O_{30}S_2$ (MH^+) 2368.2, found 2368.

The pH of an aqueous solution of this crude peptide (0.1 mg mL^{-1}) was adjusted to 8.5 using NH_4HCO_3, and the resulting solution was stirred in air for 90 h at 4 °C. The 90 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 $C_{105}H_{169}N_{28}O_{30}S_2$ (MH^+) 2366.2, found 2367.

4-Amino-3-iodobenzoic Acid Methyl Ester (2).[38] A solution of ICl (11.0 g, 67.7 mmol) in AcOH (500 mL) was added

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(65) Genet, J. P.; Savignac, M. *J. Organomet. Chem.* **1999**, *576*, 305−317.
(66) The fact that the IC_{50} exhibited by tolan-constrained peptide **22** does not appear to exceed that of disulfide-constrained cyclic peptide **1** is not surprising given the limited degree of conformational control that the tolan amino acid could be expected to exert over such an extended peptide loop.

(67) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, 1988.

dropwise over a period of 15 min to a stirred solution of methyl 4-aminobenzoate (10.0 g, 66.0 mmol) in AcOH (500 mL) and the resulting solution stirred at rt for a further 1 h. Evaporation of the solvent left a brown solid which was suspended in $H_2O-CH_2Cl_2$ (1:1, 500 mL), and solid $NaHCO_3$ was added until a clear solution was obtained. The organic layer was then separated and further washed with saturated $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness in vacuo to leave a brown oil which solidified on standing. Purification by FC (EtOAc/hexane, 1:4) gave iodobenzoate **2** as a brown solid (13.6 g, 74%): mp 89−90 °C [lit.[38] mp 85−87 °C (EtOAc)]; ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 4.58 (br s, 1H), 6.68 (d, J = 8.4 Hz, 1H), 7.80 (dd, J = 8.4, 1.9 Hz, 1H), 8.38 (d, J = 1.9 Hz, 1H).

4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-iodobenzoic Acid Methyl Ester (3). To a solution of iodobenzoate **2** (250 mg, 0.9 mmol) and pyridine (88.0 μL, 1.2 equiv) in CH_2Cl_2 (5 mL) at 0 °C was added Fmoc-Cl (258 mg, 1.1 equiv). 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. CH_2Cl_2 (10 mL) was added and the solution washed with 1 M $NaHSO_3$ (2 × 10 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to leave a white solid which was recrystallized (CH_2Cl_2/petrol) to give iodobenzoate **3** as a white solid (400 mg, 89%): mp 183−185 °C; ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3H), 4.33 (t, J = 6.2 Hz, 1H), 4.51 (d, J = 6.2 Hz, 2H), 7.25−7.47 (m, 5H), 7.63 (d, J = 7.5 Hz, 2H), 7.78 (d, J = 7.5 Hz, 2H), 7.98 (dd, J = 9.3, 3.75 Hz, 1H), 8.13 (br d, J = 9.3 Hz, 1H), 8.46 (d, J = 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 47.0, 52.3, 67.8, 87.6, 118.8, 120.2, 125.0, 126.4, 127.2, 128.0, 130.9, 140.4, 141.4, 142.1, 143.5, 152.8, 165.2; MS (EI+) m/z 499 (M^+, 10), 178 (100); HRMS (EI+) m/z calcd for $C_{23}H_{18}INO_4$ (M^+) 499.0281, found 499.0270.

4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-iodobenzoic Acid (4). A solution of iodobenzoate **3** (200 mg, 0.4 mmol) in TFA (9 mL) and concd HCl (5 mL) was heated at 80 °C for 36 h. H_2O (25 mL) was added and the precipitate collected via filtration. The solid was triturated with CH_2Cl_2 (10 mL) and collected by filtration to give iodobenzoic acid **4** as a pale yellow solid (119 mg, 61%): mp 174−176 °C; ^{1}H NMR (DMSO) δ 4.31 (t, J = 7 Hz, 1H), 4.43 (d, J = 7 Hz, 2H), 7.25−7.48 (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); ^{13}C NMR (DMSO) 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, 143.9, 144.1, 154.2, 166.1; MS (EI+) m/z 485 (M^+, 5), 178 (100); HRMS (EI+) m/z calcd for $C_{22}H_{16}INO_4$ (M^+) 485.0124, found 485.0131.

4-Benzoylamino-3-iodobenzoic Acid Methyl Ester (5). To a solution of iodobenzoate **2** (50.0 mg, 0.18 mmol) in CH_2Cl_2 (3 mL) were added BzCl (32.0 μL, 2.0 equiv) and a suspension of AgCN (24.0 mg, 1.0 equiv) in MeCN (3 mL). The suspension was stirred for 48 h and filtered through Celite. The filtrate was partitioned between CH_2Cl_2 (10 mL) and 5% $NaHCO_3$ (10 mL). The organic layer was further washed with 1 M citric acid (10 mL) and brine (10 mL), dried ($MgSO_4$), and concentrated in vacuo to leave a pale brown solid. Purification by FC (EtOAc/hexane, 1:10) gave iodobenzoate **5** as a white solid (68.0 mg, 99%): mp 144−146 °C; ^{1}H NMR ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) δ 52.3, 88.8, 120.0, 127.2, 129.1, 131.0, 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 $C_{15}H_{13}INO_3$ (MH^+) 381.9940, found 381.9955.

(*S*)-4-[3-*tert*-Butoxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)propionylamino]-3-iodobenzoic Acid Methyl Ester (6). To a solution of iodobenzoate **2** (73.0 mg, 0.26 mmol) in CH_2Cl_2 (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 48 h and filtered through Celite. The filtrate was concentrated in vacuo to leave an off-white solid. Purification by FC (EtOAc/hexane, 1:4) gave iodobenzoate **6** as a white solid (138 mg, 81%, >99.5% ee by HPLC; see the Supporting Information): mp 121−122 °C; ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 9H), 3.55 (dd, J = 9.3, 6.2 Hz, 1H), 3.9 (s, 3H), 3.94 (br m, 1H), 4.25 (t, J = 6.2 Hz, 1H), 4.45−4.6 (br m, 3H), 5.8 (br s, 1H), 7.25−7.45 (br m, 4H), 7.6 (br s, 2H), 7.77 (d, J = 6.8 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); ^{13}C NMR ($CDCl_3$) 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 $C_{30}H_{32}IN_2O_6$ (MH^+) 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.

(*S*)-4-[3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]-3-iodobenzoic Acid Allyl Ester (7). Methyl ester **6** (50.0 mg, 0.80 mmol) and Ti(O*i*-Pr)$_4$ (443 mg, 2.0 equiv) were dissolved in allyl alcohol (5 mL), and the resulting solution was sealed in a pressure tube under nitrogen. The solution was heated at 120 °C for 1 h, allowed to cool to rt, and quenched by the addition of saturated NH_4Cl (5 mL). CH_2Cl_2 (10 mL) was added and the precipitate removed by filtration through Celite. The organic phase was separated, dried (Na_2SO_4), and concentrated in vacuo to leave an oily residue, which was purified by FC (EtOAc/hexane, 1:4) to give allyl ester **7** as a colorless solid (51.0 mg, 98%): mp 71−73 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 3.55 (dd, J = 7.5, 5.6, 1H), 3.96 (br d, J = 6.2 Hz, 1H), 4.25 (t, J = 6.3 Hz, 1H), 4.40−4.55 (m, 3H), 4.81 (m, 2H), 5.25−5.45 (m, 2H), 5.78 (br s, 1H), 5.95−6.06 (m, 1H), 7.26−7.48 (m, 4H), 7.63 (br d, J = 6.8 Hz, 2H), 7.78 (br d, J = 7.5 Hz, 2H), 8.05 (br d, J = 8.1 Hz, 1H), 8.37−8.43 (m, 1H), 8.46 (d, J = 1.2 Hz, 1H), 8.70 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.9, 27.5, 47.1, 61.5, 65.2, 65.8, 67.4, 68.8, 74.4, 88.7, 118.6, 120.1, 125.0, 127.2, 127.6, 127.8, 130.8, 132.0, 140.3, 141.4, 141.8, 143.6, 164.3, 169.2; MS (FAB+) m/z 669 (MH^+, 100); HRMS (FAB+) m/z calcd for $C_{32}H_{33}IN_2O_6I$ (M^+) 668.1383, found 668.1320.

(*S*)-4-[3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]-3-iodobenzoic Acid (8). Method 1. Allyl ester **7** (380 mg, 0.56 mmol) was dissolved in a mixture of EtOH and H_2O (10 mL, 9:1). To the solution was added Wilkinson's catalyst [$RhCl(PPh_3)_3$, 60.0 mg, 11 mol %], and the solution was heated at reflux for 2 h with rapid stirring. Upon cooling, the solution was concentrated in vacuo and the residue purified by FC (EtOAc/hexane/AcOH, 4:1:0.01) to give iodobenzoic acid **8** as a white solid (179 mg, 50%) and (*S*)-4-[3-*tert*-butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]benzoic acid (**9**) as a pale brown solid (42.0 mg, 15%).

Data for 8: mp 124−126 °C; ^{1}H NMR ($CDCl_3$) δ 1.15 (s, 9H), 3.50 (dd, J = 7.5, 6.2 Hz, 1H), 3.92 (br d, 1H), 4.17 (t, J = 6.2 Hz, 1H), 4.40−4.51 (m, 3H), 5.85 (br s, 1H), 7.19−7.40 (m, 4H), 7.55 (br s, 2H), 7.69 (br d, J = 6.5 Hz, 2H), 7.99 (br d, J = 7.5 Hz, 1H), 8.36 (d, J = 7.5 Hz, 1H), 8.42 (d, J = 1.9 Hz, 1H), 8.64 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 27.5, 47.1, 57.2, 61.5, 69.3, 74.6, 88.6, 120.1, 125.0, 126.3, 127.1, 127.8, 131.4, 140.9, 141.4, 142.3, 143.6, 156.2, 169.2; MS (EI+) m/z 628 (M^+, 15), 178 (100); HRMS (EI+) m/z calcd for $C_{29}H_{29}IN_2O_6$ (M^+) 628.1070, found 628.1058.

Data for 9: mp 113−115 °C; ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 9H), 3.48 (t, J = 8.5 Hz, 1H), 3.92 (br s, 1H), 4.24 (t, J = 6.7 Hz, 1H), 4.35−4.55 (m, 3H), 5.88 (br d, J = 4.6 Hz, 1H), 7.24−7.43 (m, 4H), 7.60 (br s, 4H), 7.76 (d, J = 7.3 Hz, 2H), 8.09 (d, J = 7.3 Hz, 2H), 9.00 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 27.5, 47.1, 62.3, 118.9, 120.1, 125.0, 127.1, 127.8, 131.6, 141.3, 142.3, 143.7, 156.2, 168.9, 170.6; MS (FAB+) m/z 503 (MH^+, 10), 179 (100); HRMS (FAB+) m/z calcd for $C_{29}H_{31}N_2O_6$ (MH^+) 503.2182, found 503.2196.

4-Amino-3-iodobenzoic acid (10).[68] To a solution of methyl ester **2** (2.0 g, 7.2 mmol) in MeOH (6 mL) was added 2 M NaOH (12 mL). The solution became turbid and was stirred for 24 h, during after which time the solution became clear. The mixture was concentrated in vacuo, the residue was suspended in H_2O (25 mL), and 1 M HCl was added until a pH of 4 was reached. The aqueous phase was extracted with EtOAc (2 × 10 mL), and the combined organic washings were dried ($MgSO_4$) and concentrated in vacuo to give acid **10** as a pale brown solid (1.5 g, 79%): mp 201−203 °C [lit.[68] mp 193−194 °C (EtOH−H_2O)]; ^{1}H NMR (d_6-DMSO) δ 6.00 (br s, 2H), 6.58 (d, J = 7.5 Hz, 1H), 7.66 (dd, J = 7.5, 1.2 Hz, 1H), 8.18 (d, J = 1.2 Hz, 1H), 12.32 (br s, 1H).

4-Amino-3-iodobenzoic Acid 2,2,2-Tribromoethyl Ester (11). A solution of iodobenzoic acid **10** (1.40 g, 5.32 mmol) in $SOCl_2$ (8.0 mL, excess) was heated to reflux for 1.5 h. The solution was then concentrated in vacuo and the residue dissolved in toluene (15 mL) and then reconcentrated in vacuo to give the crude acid chloride as a yellow oil. This oil was added to a solution of 2,2,2-TBE-OH (1.58 g, 1.05 equiv), *i*-Pr_2EtN (1.12 mL, 1.50 equiv), and DMAP (20.0 mg, catalytic) in CH_2Cl_2 (10 mL) and the resulting solution stirred for 1 h. The reaction mixture was then diluted with CH_2Cl_2 (50 mL) and washed with 5% $NaHCO_3$ (30 mL) and brine (2 × 30 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to leave a brown solid. Purification by FC (EtOAc/hexane, 1:4) gave TBE ester **11** as a pale brown solid (2.61 g, 93%): mp 126−127 °C; ^{1}H NMR ($CDCl_3$) δ 4.52 (br s, 2H), 5.11 (s, 2H), 6.75 (d, J = 6.9 Hz, 1H), 7.95 (dd, J = 6.9, 1.2 Hz, 1H), 8.43 (d, J = 1.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 36.4, 77.0, 113.2, 119.5, 131.8, 141.7, 151.5, 163.4; MS (EI+) *m*/*z* 525, 527, 529, 531 (M^+, 15, 50, 50, 15), 246 (100); HRMS (CI+) *m*/*z* calcd for $C_9H_8{}^{79}Br_3INO_2$ (MH^+) 525.7150, found 525.7140.

(*S*)-4-[3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]-3-iodobenzoic Acid 2,2,2-Tribromoethyl Ester (12). To a solution of (*S*)-Fmoc-Ser(*t*-Bu)-OH (140 mg, 0.42 mmol) in CH_2Cl_2 (4 mL) were added $SOCl_2$ (55.0 μL, 2.0 equiv) and DMF (10 μL, catalytic). The solution was stirred for 3 h and then concentrated in vacuo to give the crude acid chloride as an oil. This oil was added to a solution of aminobenzoate **11** (200 mg, 0.38 mmol) in CH_2Cl_2 (5 mL) before AgCN (51.0 mg, 1.0 equiv) was added with stirring. The resulting suspension was stirred for 48 h and then filtered through a pad of Celite. The filtrate was concentrated in vacuo and the residue purified by FC (EtOAc/hexane, 1:4) to give iodobenzoate **12** as a colorless solid (315 mg, 93%): mp 88−90 °C; ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 9H), 3.58 (dd, J = 7.9, 2.2 Hz, 1H), 3.95 (br d, J = 7.9 Hz, 1H), 4.25 (t, J = 5.6 Hz, 1H), 4.40−4.57 (m, 3H), 5.15 (s, 2H), 5.75 (br d, J = 4.5 Hz, 1H), 7.20−7.48 (m, 4H), 7.61 (br s, 2H), 7.74 (d, J = 9.0 Hz, 2H), 8.13 (dd, J = 9.0, 1.2 Hz, 1H), 8.45 (d, J = 10.1 Hz, 1H), 8.54 (d, J = 1.2 Hz, 1H), 8.68 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 27.5, 35.6, 47.1, 57.2, 61.5, 67.4, 74.4, 77.1, 88.8, 120.1, 120.3, 124.9, 125.8, 127.1, 127.8, 131.3, 140.8, 141.4, 142.5, 143.6, 157.0, 162.8, 169.3; MS (FAB+) *m*/*z* 891, 893, 895, 897 (MH^+, 30, 100, 100, 30); HRMS (ES+) *m*/*z* calcd for $C_{31}H_{31}{}^{79}Br_3IN_2O_6$ (MH^+) 890.8777, found 890.8741.

(*S*)-4-[3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]-3-iodobenzoic Acid (8). Method 2. 2,2,2-Tribromoethyl ester **12** (100 mg, 0.11 mmol) was dissolved in AcOH (5 mL), and Zn powder (200 mg, excess) was added. The suspension was stirred for 0.5 h, filtered through Celite, and concentrated in vacuo. The residue was purified by FC (EtOAc/hexane/AcOH, 4:1:0.01) to give acid **8** as a white solid (68.0 mg, 97%). The spectroscopic data are given above.

4-Amino-3-iodo-*N*-methylbenzamide (13). The acid chloride of iodobenzoic acid **10** was prepared as detailed in the preparation of compound **11**. To a solution of this crude acid chloride (750 mg, 2.80 mmol) in CH_2Cl_2 (10 mL) were added $MeNH_2$·HCl (250 mg, 1.75 equiv) and Et_3N (0.90 mL, 2.30 equiv). The resulting solution was stirred for 0.5 h, diluted with EtOAc (25 mL), and washed with 5% $NaHCO_3$ (50 mL). The organic layer was then washed with brine (2 × 25 mL), dried ($MgSO_4$), and concentrated in vacuo. Purification by FC (EtOAc/hexane, 4:6) gave methylbenzamide **13** as a white solid (432 mg, 56%): mp 64−67 °C; ^{1}H NMR ($CDCl_3$) δ 2.90 (2 s, 3H), 4.46 (br s, 2H), 6.35 (br s, 1H), 6.63 (d, J = 6.9 Hz, 1H), 7.52 (dd, J = 7.5, 1.8 Hz, 1H), 8.05 (d, J = 1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 26.9, 113.5, 118.7, 125.6, 128.4, 138.2, 149.6, 166.7; MS (ES+) *m*/*z* 299 (MNa^+, 100), 277 (MH^+, 50); HRMS (ES+) *m*/*z* calcd for $C_8H_{10}IN_2O$ (MH^+) 276.9838, found 276.9839.

2-Trimethylsilanylethynylbenzoic Acid Methyl Ester (14).[69] To a solution of methyl 2-iodobenzoate (1.0 g, 4.65 mmol), 2-TMS-acetylene (986 μL, 1.5 equiv), PPh_3 (30.0 mg, 2.5 mol %), CuI (22.0 mg, 2.5 mol %), and Et_3N (978 μL, 1.5 equiv) in THF (10 mL) was added $Pd(PPh_3)_2Cl_2$ (163 mg, 5 mol %) with stirring. The solution was stirred at rt for 18 h, concentrated in vacuo, and then purified by FC (EtOAc/hexane, 1:100) to give benzoate **14** as a colorless oil (993 mg, 92%): ^{1}H NMR ($CDCl_3$) δ 0.25 (s, 9H), 3.85 (s, 3H), 7.33 (td, J = 7.7, 1.5 Hz, 1H), 7.41 (td, J = 7.6, 1.5 Hz, 1H), 7.55 (ddd, J = 7.6, 1.5, 0.5 Hz, 1H), 7.87 (ddd, J = 7.6, 1.5, 0.6 Hz, 1H).

2-Ethynylbenzoic Acid Methyl Ester (15).[70] To a solution of **14** (500 mg, 2.1 mmol) in MeOH (10 mL) was added KF·H_2O (1.0 g, 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 ($MgSO_4$), and concentrated in vacuo to leave a brown oil. Purification by FC (EtOAc/hexane, 1:50) gave ethynylbenzoate **15** as a colorless oil (281 mg, 82%): ^{1}H NMR ($CDCl_3$) δ 3.40 (s, 1H), 3.96 (s, 3H), 7.34−7.49 (m, 2H), 7.57−7.64 (m, 1H), 7.88−7.96 (m, 1H).

2-Ethynylbenzoic acid (16).[71] 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 ($MgSO_4$) 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%): ^{1}H NMR ($CDCl_3$) δ 3.46 (s, 1H), 7.41−7.61 (m, 2H), 7.68 (dd, J = 7.5, 1.2 Hz, 1H), 8.10 (dd, J = 6.2, 1.2 Hz, 1H).

2-(2-Amino-5-methylcarbamoylphenylethynyl)benzoic Acid Methyl Ester (17). Method 1. To a solution of iodobenzamide **13** (175 mg, 0.63 mmol), ethynylbenzoate **15** (100 mg, 0.63 mmol), PPh_3 (16.0 mg, 10 mol %), CuI (12.0 mg, 10 mol %), and Et_3N (131 μL, 1.5 equiv) in THF (5.0 mL) was added $Pd(PPh_3)_2Cl_2$ (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 ($MgSO_4$), concentrated in vacuo, and then purified by FC (EtOAc/hexane, 7:3) to give tolan **17** as a pale yellow solid (132 mg, 65%): mp 60−62 °C; ^{1}H NMR ($CDCl_3$) δ 2.99 (2 s, 3H), 3.93 (s, 3H), 4.40 (br s, 2H), 6.15 (br s, 1H), 6.68 (d, J = 8.5 Hz, 1H), 7.34−7.43 (m, 1H), 7.50−7.68 (m, 3H), 7.88 (d, J = 1.2 Hz, 1H), 8.01−8.09 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 26.8, 52.4, 91.7, 94.0, 106.5, 113.4, 122.9, 124.3, 127.6, 129.3, 130.0, 130.6, 131.1, 132.2, 133.7, 152.3, 165.2, 167.6; MS (EI+) *m*/*z* 308 (M^+, 40), 277 (100); HRMS (EI+) *m*/*z* calcd for $C_{18}H_{16}N_2O_3$ (M^+) 308.1161, found 308.1165.

2-(2-Amino-5-methylcarbamoylphenylethynyl)benzoic Acid Methyl Ester (17). Method 2. A solution of iodobenzamide **13** (100 mg, 0.38 mmol), ethynylbenzoate **14**

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(177 mg, 2.0 equiv), PPh_3 (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 K_2CO_3 (116 mg, 2.2 equiv) and $Pd(PPh_3)_2Cl_2$ (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).[51] After 15 h, saturated NH_4Cl and Et_2O (100 mL, 1:1) were added, and the aqueous layer was discarded. The organic layer was dried ($MgSO_4$) 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, 90%). The spectroscopic data are given above.

Resin 18^R. A portion of Fmoc-protected Rink amide 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 CH_2Cl_2 (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*-Pr_2EtN (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), CH_2Cl_2 (3 × 25 mL), MeOH (3 × 25 mL), and Et_2O (3 × 25 mL). The resin was then resuspended in DMF (8 mL) and Ac_2O (2.0 mL, excess) and *i*-Pr_2EtN (2.0 mL, excess) added to cap off any remaining unreacted amino groups. 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), CH_2Cl_2 (3 × 25 mL), MeOH (3 × 25 mL), and Et_2O (3 × 25 mL). The resin was then dried under high vacuum to give **18^R** as a pale orange resin [825 mg, 0.34 mmol g^{-1} (by Fmoc test)].

Resin 19^R. A portion of resin **18^R** (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 subjected to automated coupling cycles to build up the linear peptide sequence using the method described above for the preparation of cyclic peptide **1**. The resin was then dried under high vacuum to give **19^R** as an orange resin [320 mg, 0.12 mmol g^{-1} (by Fmoc test)]. Analytical cleavage/side-chain deprotection of 10 mg of this resin with TFA−PhOH−H_2O (95:2.5:2.5 v/w/v) for 2 h, then precipitation from Et_2O, and lyophilization from 70% MeCN−H_2O (v/v) with 0.1% TFA (v/v) gave a crude sample of the Fmoc-terminally-protected peptide: MS (ES+) *m*/*z* calcd for $C_{121}H_{176}IN_{28}O_{30}$ (MH^+) 2628.2, found 2628.

Resin 20^R. A portion of resin **19^R** (300 mg, 0.12 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 CH_2Cl_2 (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*-Pr_2EtN (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), CH_2Cl_2 (3 × 15 mL), MeOH (3 × 15 mL), and Et_2O (3 × 15 mL). The resin was then dried under high vacuum to give **20^R** 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* calcd for $C_{115}H_{170}IN_{28}O_{29}$ (MH^+) 2534.2, found 2534; analytical reversed-phase HPLC, gradient 0−70% MeCN−H_2O (v/v) with 0.1% TFA (v/v) over 60 min, R_f 14.5 min.

Resin 21^R and Tolan-Constrained Cyclic Peptide 22. A suspension of resin **20^R** (25.0 mg, assumed ∼0.1 mmol g^{-1}), PPh_3 (1.0 mg), CuI (1.0 mg), and Et_3N (5.0 μL) in THF (1 mL) was purged with nitrogen for 15 min. $Pd(PPh_3)_2Cl_2$ (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), CH_2Cl_2 (3 × 5 mL), MeOH (3 × 5 mL), and Et_2O (3 × 5 mL). The resin was then dried under high vacuum to give **21^R** as an orange resin (20.1 mg). This resin sample was treated with TFA−H_2O−*i*-PrSiH (95:4:1 v/v/v) for 2 h. Following filtration, the filtrate was concentrated in vacuo, precipitated from cold Et_2O, and lyophilized from 70% MeCN−H_2O (v/v) with 0.1% TFA (v/v). The resulting solid (3.8 mg) was purified by analytical reversed-phase HPLC, gradient 0−70% MeCN−H_2O (v/v) with 0.1% TFA (v/v) over 60 min, to give *peptidomimetic* **22** as a white solid (0.7 mg, ∼15%), R_f 12.4 min: MS (ES+) *m*/*z* calcd for $C_{115}H_{169}N_{28}O_{29}$ (MH^+) 2406.2, found 2406.2.

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Supporting Information Available: ^1H and ^{13}C 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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