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Synthesis and Incorporation into Cyclic Peptides of Tolan Amino Acids and Their Hydrogenated Congeners: Construction of an Array of A−B-loop Mimetics of the Cε3 Domain of Human IgE

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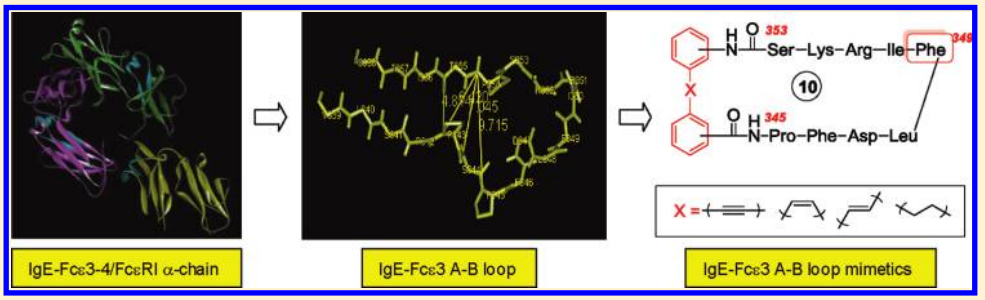
Supporting Information



ABSTRACT: The disruption of the human immunolobulin E−high affinity receptor I (IgE−FcεRI) protein−protein interaction (PPI) is a validated strategy for the development of anti asthma therapeutics. Here, we describe the synthesis of an array of conformationally constrained cyclic peptides based on an epitope of the A−B loop within the Cε3 domain of IgE. The peptides contain various tolan (i.e., 1,2-biarylethyne) amino acids and their fully and partially hydrogenated congeners as conformational constraints. Modest antagonist activity (IC_{50} ~660 μM) is displayed by the peptide containing a 2,2′-tolan, which is the one predicted by molecular modeling to best mimic the conformation of the native A−B loop epitope in IgE.

INTRODUCTION

Incidence of asthma and related allergic manifestations is increasing in the Western world.[1] The symptoms associated with these conditions are invariably debilitating, and the resulting financial burden on national healthcare systems[2] has focused attention on the development of improved therapeutic strategies to combat these afflictions.[3,4] One strategy that offers the prospect of a preventative rather than ameliorative intervention is to administer molecules that can block the interaction of human immunolobulin E (IgE) with its high affinity receptor FcεRI; a protein−protein interaction (PPI) that is central to the allergic signal transduction cascade.[5−17] Proof of principle that this strategy can be effective, and does not suffer from unacceptable side effects, comes from the successful clinical use of the monoclonal antibody omalizumab (Xolair), which is indicated for severe persistent asthma and operates by sequestering IgE in the blood and preventing binding to FcεRI.[18,19] However, its high cost and non oral mode of delivery has fueled interest in the development of alternative antagonists of this PPI.[20] The most well-studied class of compounds that has been evaluated for this purpose is peptides,[9,13−15,21] initially as tools for mapping the binding interface,[22−34] as synthetic vaccines,[33,34] as biochemical tools,[35−43] and as affinity purification aids,[44−54] and more recently as potential leads for therapeutic development.[55−71] Linear and disulfide-bond constrained loop peptides have been described, and several patents[72−74] have been filed relating to the use of epitope sequences of both the protein partners (i.e., the Cε3 domain of IgE and the extracellular α-chain of FcεRI) in potential peptide-based therapeutics.

Previously, we have described the solid-phase synthesis of a cyclic peptide containing a 19-residue epitope (Leu-340 to Cys-358) found in the A−B loop of the Cε3 domain of human IgE via an on-resin Sonogashira macrocyclization which concomitantly installed a 2,2′-tolan (i.e., 1,2-biarylethyne) amino acid conformational constraint to mimic the Tyr-339/Leu-359 loop entry/exit residues (see Figure 1).[75] This compound, like its

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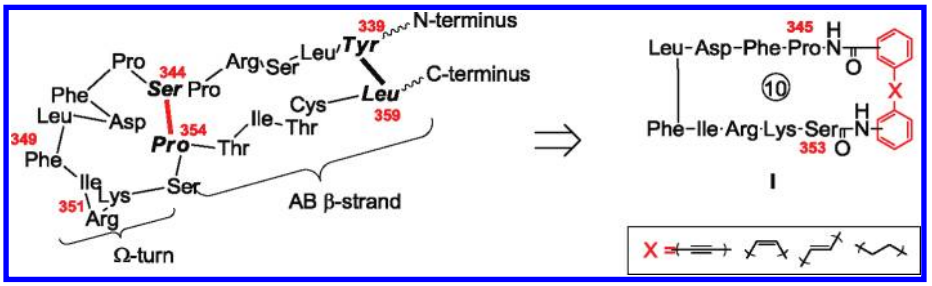

Figure 1. Schematic representation of the antiparallel A−B β-strand/Ω-turn region of the Cε3 domain of IgE showing the site of our previous 2,2′-tolan amino acid constraint (Tyr-339/Leu-359) and the proposed site of introduction of this and related constraints in this work (Ser-344/Pro-354).

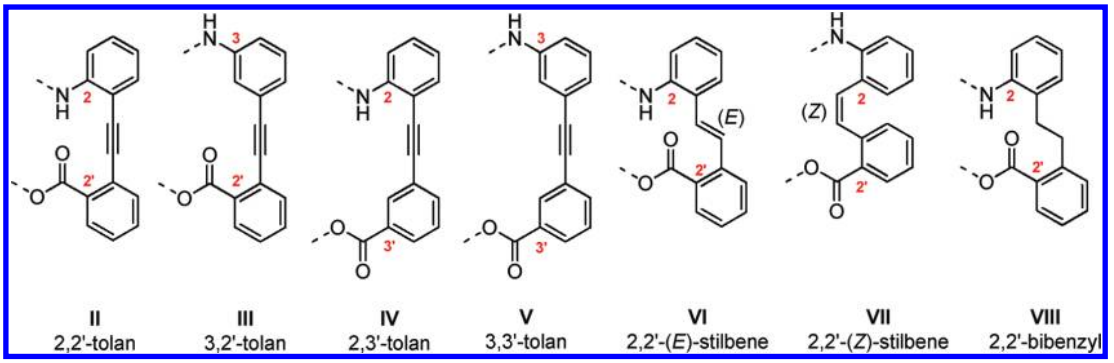

Figure 2. Amino acid conformational constraints employed in this sudy.

disulfide-constrained "parent" peptide, showed inhibition of IgE-triggered 5-hydroxytryptamine secretion in a rat basophilic leukemia cell line transfected with human FcεRI α-chain with an IC_{50} of ∼12 μM.[15] The Cε3 A−B loop epitope of human IgE is not part of the extensive contact surface between IgE and FcεRI as revealed in the X-ray crystal structure of a complex between human IgE−Fc and the extracellular, soluble α-chain of FcεRI (Brookhaven PDB code 1F6A, 3.5 Å resolution).[76−78] Consequently, we were motivated to prepare further constrained analogues of this peptide to aid elucidation of the mechanism of action of these mimetics. In particular, we were keen to reduce the number of amino acid residues contained within the loop as this was anticipated to increase conformational rigidity and enhance proteolytic stability. It was envisaged to achieve this by removing the residues within the original sequence which comprise an occluded β-strand leading to the surface exposed Ω-turn; the only individual residues that have been demonstrated to be important for activity are Phe-349 and Arg-351 (as determined by Phe-349-Ala and Arg-351-Lys site-directed mutagenesis on native human IgE) which are located within the Ω-turn region.[66] Consequently, the plan was to prepare a small array of cyclic peptides containing the 9-residue Pro-345 to Ser-353 epitope in which the Ser-344/Pro-354 loop entry/exit residues would be mimicked by a set of tolan amino acid conformational constraints and their fully and partially hydrogenated congeners. We envisioned that the synthesis and biological evaluation of this array of compounds as potential antagonists of the IgE−FcεRI PPI using an enzyme-linked immunosorbent assay (ELISA) and comparison with appropriate controls might provide important insight into the conformational requirements for activity and a lead peptidomimetic suitable for NMR conformation studies and cocrystallization experiments with the soluble FcεRI α-chain protein (Figure 1).

The four-tolan and three-tolan-derived amino acid constraints (**II**−**VIII**) prepared in this work were designed as a generic suite of linkages that could be introduced as cyclizing elements for the construction of peptidomimetics for many peptide epitopes having a loop conformation in their native protein environment (Figure 2).[75]

The 2,2′-tolan unit **II** is an established conformational constraint introduced by Kemp,[79,80] which can induce a β-turn-like structure via a 10-membered H-bonded ring,[81] but the 2,3′-, 3,2′-, and 3,3′-regioisomers **III**−**V** as well as the partially reduced 2,2′-(*E*)- and 2,2′-(*Z*)-congeners **VI** and **VII** and the fully reduced 2,2′-bibenzyl analogue **VIII** have not previously been described. Related suites of biphenyl[82] and 2-butynyl/2-butenyl/butyl[83] amino acid families have been described, the latter as stable replacements for disulfide linkages in protein stapling applications.

Inspection of the A−B loop region of IgE protein within the X-ray crystal structure of the IgE−FcεRI complex reveals that the Ser-344 carbonyl carbon and Pro-354 amine atoms are separated by ∼8.0 Å and deliver/receive the intervening loop residues with a vectorial disposition that can be mimicked closely with the 2,2′-tolan amino acid **II** (Figure 3).[75]

Here, we describe the synthesis of this A−B loop mimetic peptide containing the 2,2′-tolan amino acid **II** and also analogous looped peptides containing the same 9-residue A-B loop epitope but constrained by each of the other amino acids **III**−**VIII** inserted at the same position as well as the screening of these and two control peptidomimetics as potential antagonists of the IgE−FcεRI PPI using ELISA.

RESULTS AND DISCUSSION

The general approach we envisaged for the construction of our library of A−B-loop mimics is shown below (Scheme 1).

It was expected that the linear resin-bound peptide **IX** would be constructed via standard[84,85] automated solid-phase peptide synthesis. The various tolans and hydrogenated analogues **X** would then be coupled to give the linear solid-supported peptide **XI**. Finally, cleavage from the resin and macro-

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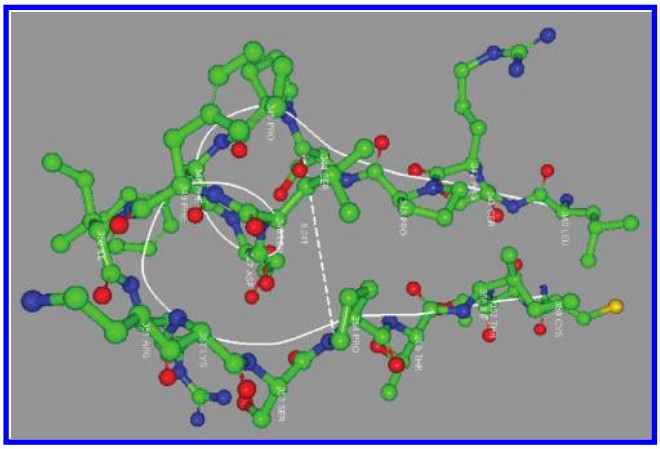


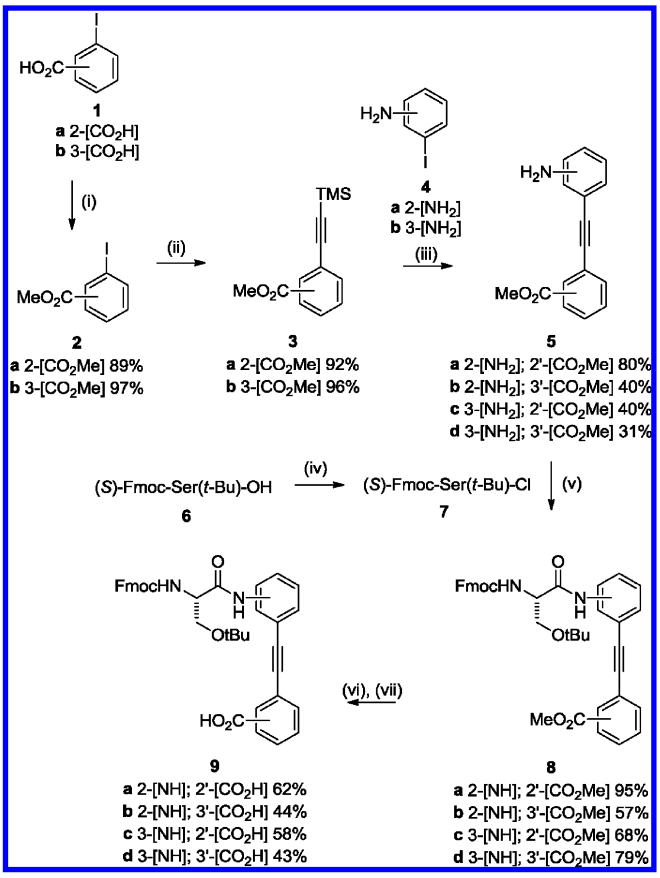
Figure 3. Portion of the X-ray crystal structure of IgE–Fc (Brookhaven PDB code 1F6A) showing the A–B loop and the position for synthetic insertion of tolan and related conformational constraints.

cyclization would yield the target peptidomimetics **I**. This strategy was selected in preference to our previous on-resin Sonogashira cyclization strategy[75] because despite extensive experimentation we were unable to obtain comparable yields using this latter approach.

We began with the preparation of the tolan intermediates and related analogues. At the outset, we were concerned that the restricted flexibility of the tolan moiety might conformationally restrict opportunities for cyclization and that the low nucleophilicity of the aniline nitrogen would also render cyclization via intramolecular peptide coupling challenging.[86] To improve the cyclization potential of our peptides, we therefore decided to attach the first amino acid (Ser-344) to the tolan prior to cyclization (Scheme 2).

To this end, 2- and 3-iodobenzoic acids (**1a** and **1b**) were first esterified under acidic conditions. The resulting methyl esters **2a** and **2b** were treated with TMS-acetylene under Pd/Cu catalyzed Sonogashira coupling conditions to afford alkynes **3a** and **3b**. Each of these was coupled separately to 2- and 3-iodoanilines (**4a** and **4b**) via a second Sonogashira coupling to give tolan amino esters **5a–d** after an in situ TMS-deprotection. The order of addition of reagents for the Sonogashira couplings was found to be crucial and yields were also improved by the addition of further portions of palladium and copper catalysts after 12 h. These optimized conditions were only employed for the construction of tolan **5a**

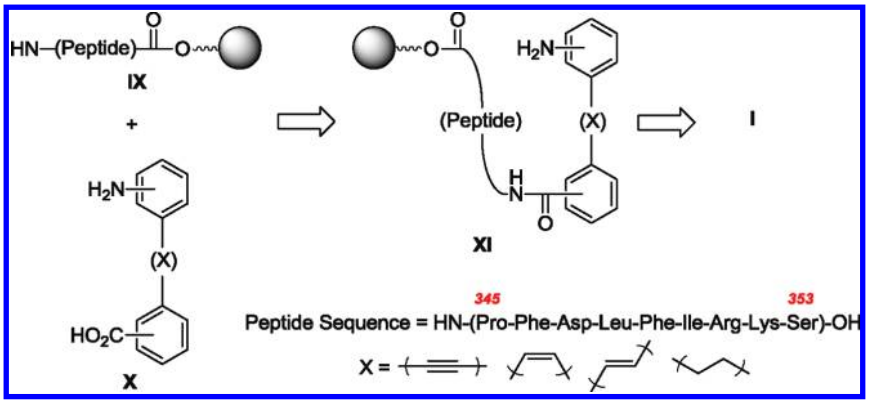
Scheme 2. Tolan Synthesis[a]



[a]Reagents and conditions: (i) H_2SO_4, MeOH; (ii) 2-TMS-acetylene, PPh_3, $Pd(PPh_3)_2Cl_2$, Et_3N, CuI, THF; (iii) iodoaniline, PPh_3, $Pd(PPh_3)_2Cl_2$, piperidine, CuI, THF, K_2CO_3, MeOH; (iv) oxalyl chloride, cat. DMF, CH_2Cl_2; (v) AgCN, CH_2Cl_2; (vi) $LiAlH_4$, THF; (vii) 1 M Jones' reagent, acetone.

and this is reflected in the improved isolated yield of **5a** (80% from **1a**) compared to the related compounds **5b–d** (31–40%). Moreover, the two Sonogashira coupling steps and intermediate TMS-deprotection could be performed in a single operation in a manner similar to that described by Doye and co-workers.[87] Thus, intitial Pd/Cu-catalyzed Sonogashira coupling of 2-iodoaniline (**4a**) with TMS-acetylene followed by treatment with KOH to affect TMS-deprotection, and Pd/Cu catalyzed Sonogashira coupling of the crude product with 2-

Scheme 1. Overview of Synthetic Approach to Peptidomimetics



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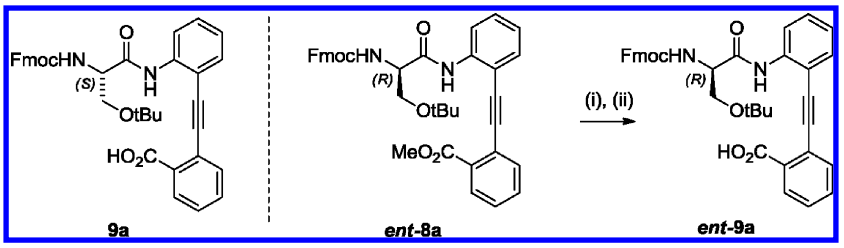
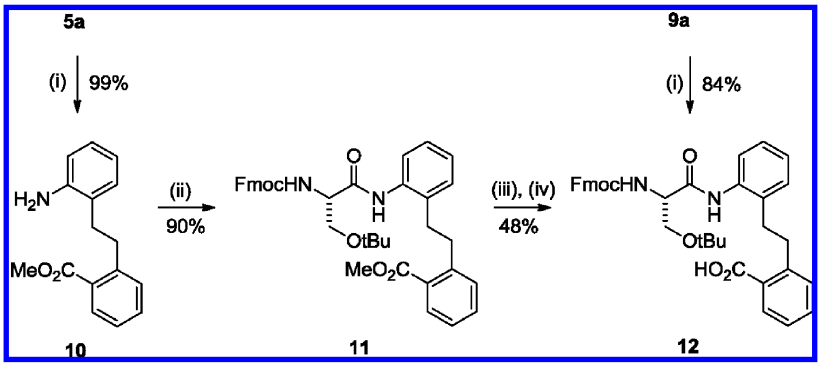
Scheme 3. Enantiomeric Dipeptides *ent*-8a and *ent*-9a for Verifying Stereochemical Integrity[a]



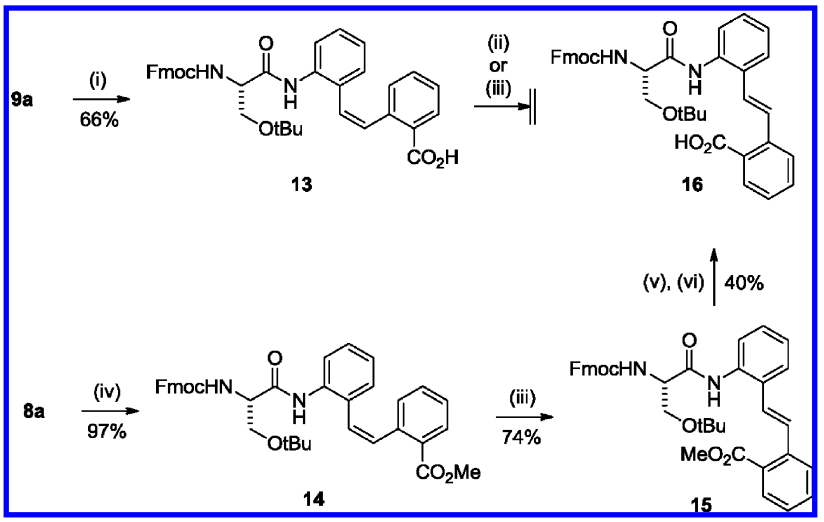
[a]Reagents and conditions: (i) $LiAlH_4$, THF; (ii) 1 M Jones' reagent, acetone.

Scheme 4. Synthesis of Fully Saturated 2,2′-Tolan Analogue[a]



[a]Reagents and conditions: (i) Pd/C, H_2, EtOAc; (ii) **7**, AgCN, CH_2Cl_2; (iii) $LiAlH_4$, THF; (iv) 1 M Jones' reagent, acetone.

Scheme 5. Synthesis of Partially Hydrogenated 2,2′-Tolan Analogues[a]



[a]Reagents and conditions: (i) Lindlar's catalyst, H_2, EtOAc; (ii) I_2, $CHCl_3$; (iii) $Pd(CN)_2Cl_2$, silica gel, $CHCl_3$; (iv) Lindlar's catalyst, $PhCO_2H$, H_2, EtOAc; (v) $LiAlH_4$, THF; (vi) 1 M Jones' reagent, acetone.

iodobenzoic acid methyl ester (**2a**) gave tolan amino ester **5a** directly in excellent yield (85%).

Treatment of commercially available (*S*)-Fmoc-Ser(*t*-Bu)-OH (**6**) with oxalyl chloride and catalytic DMF afforded acid chloride **7**, which was in turn coupled to each tolan amino ester **5a**–**d** to yield the corresponding amides **8a**–**d**. Not surprisingly, initial attempts to saponify the methyl ester **8a** while retaining the Fmoc-protecting group failed.[88] Pleasingly, however, a two-step procedure involving reduction of the methyl ester with $LiAlH_4$, followed by oxidation of the resultant alcohol using Jones' reagent, afforded the serine-containing tolan acids **9a**–**d** ready for peptide incorporation.

In the light of the need to convert (*S*)-Fmoc-Ser(*t*-Bu)−OH (>99.5% ee) to its acid chloride and subsequently expose the resulting dipeptide to $LiAlH_4$, we considered that it would be prudent to verify the stereochemical integrity of the resulting Ser-containg tolans **9a**–**d**. Therefore, following an analogous synthetic sequence, (*R*)-Fmoc-Ser(*t*-Bu)-OH (>97% ee, ***ent*-6**) was coupled with 2,2′-tolan methyl ester **5a** to construct the antipodal dipeptides ***ent*-8a** and ***ent*-9a** (Scheme 3).

Chiral HPLC analysis showed no detectable erosion in stereochemistry of dipeptide **9a** (>99.8% ee) or its unnatural enantiomer ***ent*-9a** (>97.2% ee) when compared to the starting commercial amino acids. Reassured as to the stereochemical integrity of our synthetic approach, we continued with the

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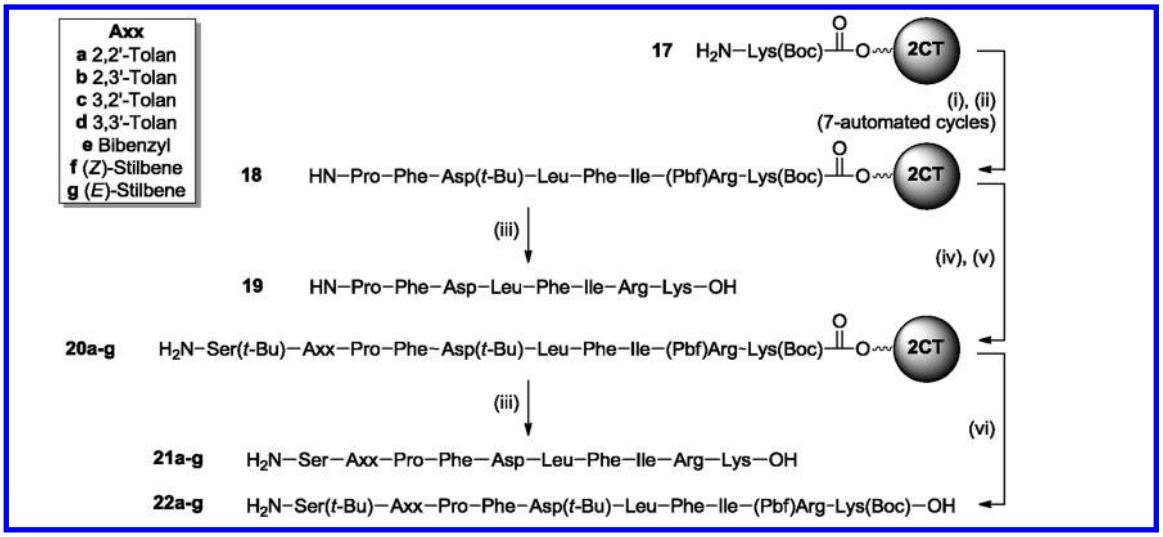
Scheme 6. Solid-Phase Synthesis of Linear Peptides[a]



[a]Reagents and conditions: (i) protected amino acid, HOBt/HBTU, *i*-Pr_2EtN, DMF; (ii) 20% piperidine/DMF; (iii) TFA/*i*-Pr_3SiH/H_2O (95:2.5:2.5); (iv) PyBOP, tolan or hydrogenated analogue **9a–d/12/13/16**, *i*-Pr_2EtN, DMF; (v) 20% piperidine/DMF; (vi) 1% TFA/CH_2Cl_2.

construction of the remaining tolan analogues. Starting with the fully saturated analogue of the 2,2′-tolan (Scheme 4).

Tolan amino ester **5a** was hydrogenated to provide bibenzyl amino ester **10** in near quantitative yield. Commercially available (*S*)-Fmoc-Ser(*t*-Bu)-OH (**6**) was again converted to its acid chloride **7** and then coupled to aniline **10** to afford serine-containing dipeptide **11** in excellent yield. Treatment of ester **11** with $LiAlH_4$, followed by oxidation using Jones' reagent, afforded carboxylic acid **12** ready for incorporation into a peptide. Alternatively, bibenzyl dipeptide **12** could be accessed directly by hydrogenating the previously prepared tolan dipeptide acid **9a** in similar overall yield. Stereoselective partial hydrogenation of this dipeptide derivative was also envisaged to give access to the (*E*) and (*Z*) stilbene-containing congeners (Scheme 5).

Tolan **9a** was partially hydrogenated to (*Z*)-stilbene **13** in modest yield, using Lindlar's catalyst (Scheme 4). Unfortunately, attempts to isomerize to (*E*)-stilbene **13** using either iodine[89] or catalytic $Pd(CN)_2Cl_2$[90] failed. Increasing the palladium catalyst loading (to 50 mol %) and reaction time (to 48 h) led to the formation of an unwanted byproduct, presumed to be the lactone resulting from cyclization of the carboxylic acid onto the tolan triple bond.[91] To avoid this unwanted transformation, it was decided to perform the isomerization on the corresponding methyl ester **8a**. Surprisingly, the use of Lindlar's catalyst under an atmosphere of dihydrogen failed to hydrogenate tolan **8a**. It was found however, that the addition of benzoic acid allowed for almost quantitative conversion to (*Z*)-stilbene methyl ester **14**. To our delight, isomerization of this substrate using catalytic $Pd(CN)_2Cl_2$ afforded (*E*)-stilbene methyl ester **15** cleanly. The usual procedure of reduction using $LiAlH_4$, followed by oxidation with Jones' reagent finally provided carboxylic acid **16** ready for peptide incorporation.

With the completion of the Ser-containing tolan dipeptides **9a–d** and hydrogenated analogues **12**, **13** and **16**, work began on the synthesis of the A–B epitope peptide (Scheme 6).

All peptides were constructed on the solid phase using 2-chlorotrityl (2CT) resin preloaded with the first amino acid (Lys). This resin is cleaved under mildly acidic conditions, allowing the final cyclization step to be performed in solution. An on-resin cyclization method, which employed a sulfamylbutyryl safety-catch linker, was also investigated.[92] However, a comparison of cost, product yields, and ease of handling favored the use of 2CT resin.[93−95]

Automated solid-phase peptide synthesis[96] was employed to construct resin-bound linear peptide **18** from commercially available H_2N-Lys(Boc)-2CT resin **17**. An iterative process involving amino acid coupling (using HOBt/HBTU) followed by Fmoc-deprotection was used to construct the desired sequence in seven cycles. At this stage, a small quantity of resin-bound peptide **18** was treated with concentrated TFA to provide linear peptide **19**, the identity of which was confirmed by MALDI-MS (MH^+ *m*/*z* 1036). The remaining resin-bound peptide **18** was coupled (using PyBOP) as separate aliquots to each of the Ser-containing tolan dipeptides **9a–d** or hydrogenated analogues **12**, **13**, and **16** to yield resin-bound peptides **20a–g**. For analytical purposes, a small quantity of each of these resin-bound peptides was treated with concentrated TFA to provide linear peptides **21a–g**, the identity of which was confirmed by MALDI-MS. The remaining resin-bound peptides **20a–g** were treated with 1% TFA/CH_2Cl_2 for just 2 min to reveal linear peptides **22a–g** with side-chain protecting groups intact.

We were pleased to note that addition of PyBOP to a DMF solution of linear peptide **22a** and *i*-Pr_2EtN afforded the desired cyclization product **23a**, albeit in only 6% yield after side-chain removal. Spurred on by this result, we investigated a variety of conditions in an attempt to improve this key step including increased dilution, slow addition of reagents, and the use of different coupling agents. Our best cyclization results were achieved by slow addition (0.5 mL h^{-1}) of a DMF solution of linear peptides **22a–g** into a DMF solution of HATU (Scheme 7).

Additional coupling agent (6 equiv total) was added portionwise over the course of the 32 h reactions. Side-chain deprotection was then achieved by treatment with neat TFA (and scavengers) for 3 h to afford our target peptidomimetics **23a–g** in fair yields.

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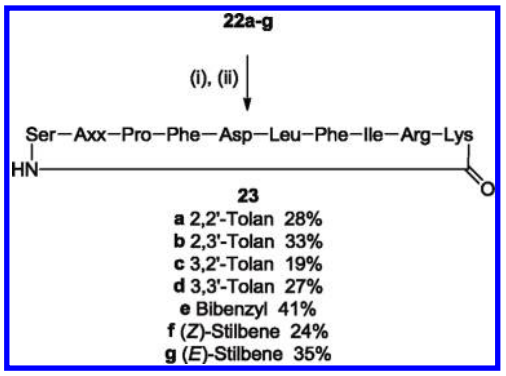
Scheme 7. Solution-Phase Peptide Cyclization[a]



[a]Reagents and conditions: (i) HATU, *i*-Pr_2EtN, DMF; (ii) TFA/*i*-Pr_3SiH/H_2O (95:2.5:2.5).

Two peptidomimetics containing the 2,2′-tolan constraint but alternative peptides sequences were also prepared as controls. The first, cyclic peptide **24**, was a scrambled analogue of **23a** which was designed using GenScript's scrambling tool,[97] keeping the positions of the Ser residue at the N-terminus of the 2,2′-tolan amino acid and the Lys residue at the C-terminus constant. The second, cyclic peptide **25,** was a Phe-349-Ala mutant of peptidomimetic **23a**, designed to correspond to the Phe-349-Ala mutant of human IgE reported by Presta et al.[66] which displayed just 10% binding to FcεRI α-chain cf. wildtype[98](Figure 4).

The ability of the seven A–B loop peptidomimetics analogues **23a–g** and the two control peptidomimetics **24** and **25** to inhibit the IgE–FcεRI PPI was assessed using an ELISA we have described previously (Table 1).[20,99]

The ELISA binding assay was performed as described in the Supporting Information. All the titrations were performed twice in duplicate. The IC_{50} values were obtained using GraFit[100] and calculated as described in the Supporting Information.

Rather disappointingly, none of the peptidomimetics displayed comparably potent antagonism to the parent 19-mer disulfide- and 2,2′-tolan-constrained peptides (IC_{50}~12 μM) from which they were designed. Indeed, the only member of the array that displayed submillimolar activity was the 2,2′-tolan peptidomimetic **23a** (IC_{50} ~660 μM, entry 1). The data does, however, indicate that, as expected, the nature of the conformational constraint strongly influences the ability of the A–B loop epitope peptide to act as an antagonist of the IgE–FcεRI PPI: the IC_{50} values for the other peptidomimetics (**23b–g**, entries 2–7) are at least ~6 fold less potent. The level of activity diminishes significantly for the 2,3′- and the 3,2′-tolan-constrained peptides **23b** and **23c** (IC_{50} ~5.3 and ~3.7 mM, respectively, entries 2 and 3) and the 3,3′-tolan-, 2,2′-bibenzyl-, and both 2,2-stilbene-constrained peptides display no discernible inhibition. That the 2,2′-tolan peptidomimetic **23a** shows the greatest level of activity is consistent with our hypothesis that the 2,2′-tolan constraint **II** is best able to span the separation between the Ser-344 N- and the Pro-345 C-

Table 1. Evaluation of the Binding Affinity of Peptidomimetics 23a–g, 24, and 25 Using IgE-FceRIα ELISA

entry	compd	constraint (Axx)	sequence	IC_{50} ± std error[d] (μM)
1	**23a**	2,2′-tolan	A–B loop[a]	660 ± 70
2	**23b**	2,3′-tolan	A–B loop[a]	5300 ± 1800[e]
3	**23c**	3,2′-tolan	A–B loop[a]	3700 ± 600[e]
4	**23d**	3,3′-tolan	A–B loop[a]	no inhibition[f]
5	**23e**	2,2′-bibenzyl	A–B loop[a]	no inhibition[f]
6	**23f**	2,2′-(*Z*)-stilbene	A–B loop[a]	no inhibition[f]
7	**23g**	2,2′-(*E*)-stilbene	A–B loop[a]	no inhibition[f]
8	**24**	2,2′-tolan	scrambled[b]	2500 ± 150
9	**25**	2,2′-tolan	Phe-349-Ala mutant[c]	no inhibition[f]

[a]Cyclic --Ser-Axx-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--. [b]Cyclic --Ser-Axx-Phe-Arg-Pro-Phe-Ile-Leu-Asp-Lys--. [c]Cyclic --Ser-Axx-Pro-Phe-Asp-Leu-Ala-Ile-Arg-Lys--. [d]For details for the ELISA protocol, see the Supporting Information. [e]Inhibition less than 50% at highest concentration tested and so the IC_{50} value is only indicative of potency. [f]Inhibition less than 20% at highest concentration tested.

termini of the A–B peptide epitope so as to present the intervening residues in an active conformation. This correlates with our molecular modeling based on the conformation of this epitope as found within the crystal structure for IgE (cf. Figure 3), although the drop in activity of this truncated sequence relative to the parent 19-mer peptidomimetic suggests that the conformation presented and/or its ability to flex appropriately during a dynamic binding event is suboptimal. The absence of measurable activity for the 2,2′-tolan-constrained peptide analogue containing the Phe-349-Ala mutation (peptidomimetic **25**, entry 9) is consistent with published mutagenesis experiments on IgE itself vide infra and is indicative that the interaction is sequence dependent. The weak inhibition observed for the 2,2′-tolan-constrained peptide analogue containing the scrambled A–B loop sequence (peptidomimetic **24**, IC_{50} ~2.5 mM, entry 8) is difficult to interpret in the absence of additional structural data but could conceivably be attributed to this sequence, by chance, displaying some key residues in an appropriate manner for productive interaction; e.g., both Phe residues are only slightly displaced relative to the wildtype sequence.

CONCLUSIONS

We have prepared a range of Ser-containing tolan dipeptides and their hydrogenated analogues **9a–g** which were incorporated into an array of cyclic peptidomimetics **23a–g**. The use of solid-phase peptide techniques ensures that our approach is amenable to automation and hence incorporation into larger libraries. In principle, incorporation into virtually any peptide sequence is possible using the strategy outlined herein. It is also anticipated that the hydrogenation of tolan regioisomers, other

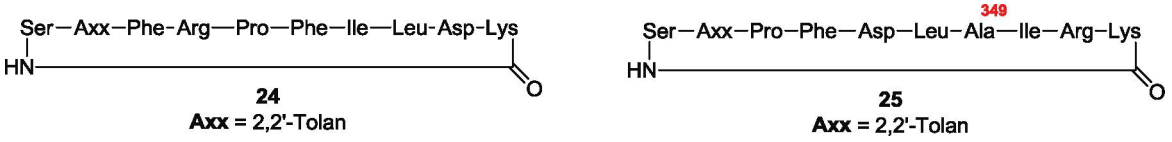


Figure 4. Structures of control peptidomimetics: **24** (scrambled) and **25** (Phe-349-Ala mutant).

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than those demonstrated, would be possible using the protocols described. These tolans and their congeners have been designed to be deployed as, e.g., redox-inert, conformation-restricted replacements for Cys-Cys disulfide links in peptidomimetics or as hydrocarbon "staples" for protein secondary structural motifs.[101]

Although the level of binding displayed by the 2,2′-tolan-constrained peptidomimetic **23a**, which was designed to present the IgE A−B loop epitope Pro-345 to Ser-353 in a close-to-native conformation, was significantly lower (IC_{50} ~660 μM) than the parent 2,2′-tolan-constrained 19-mer peptidomimetic that we employed as a starting point (IC_{50} ~12 μM) it is hoped that the reduced conformational flexibility of the compound may aid cocrystal formation with one of the protein partners of this PPI in order to throw light on the mode of action of these noninterface mimetics. Further experiments toward this end are underway in our laboratories.

EXPERIMENTAL SECTION

2-Iodobenzoic Acid Methyl Ester (2a). Commercially available 2-iodobenzoic acid (**1a**, 12.98 g, 52.33 mmol) was dissolved in methanol (300 mL), and then concentrated H_2SO_4 (30.0 mL) was added cautiously and the resulting solution refluxed under an atmosphere of nitrogen for 2.5 h and allowed to cool to rt. The reaction mixture was diluted with diethyl ether (300 mL) and then washed with H_2O (2 × 300 mL), saturated $NaHCO_3$ solution (300 mL), and brine (300 mL). The organic layer was dried (Na2SO4) and concentrated in vacuo to afford **2a** as a pale yellow oil (12.17 g, 89%): IR (neat) 2950, 1730, 1431, 1291, 1253, and 739 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.91 (s, 3H), 7.13 (td, J = 7.7 and 1.7 Hz, 1H), 7.38 (td, J = 7.7 and 1.1 Hz, 1H), 7.78 (dd, J = 7.8 and 1.6 Hz, 1H), 7.97 (dd, J = 8.1 and 0.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.5, 94.1, 127.9, 130.9, 132.7, 135.1, 141.3, 167.0; MS (EI+) m/z 262 (M^+, 97), 231 (100), 203 (44); HRMS (EI+) m/z calcd for $C_8H_7O_2I$ (M^+) 261.9491, found 261.9492.

3-Iodobenzoic Acid Methyl Ester (2b). Commercially available 3-iodobenzoic acid (**1b**, 4.00 g, 16.13 mmol) was dissolved in methanol (100 mL), concentrated H_2SO_4 (10.0 mL) was added cautiously, and the resulting solution refluxed under an atmosphere of nitrogen for 2.5 h and allowed to cool to rt. The reaction mixture was then diluted with diethyl ether (100 mL) and washed with H_2O (2 × 100 mL), saturated $NaHCO_3$ solution (100 mL), and then brine (100 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to afford **2b** as a white solid (4.11 g, 97%): mp 54−56 °C (lit.[102] mp 54−55 °C); IR (Nujol mull) 2923, 1731, 1436, 1259, 1116, and 744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.89 (s, 3H), 7.16 (t, J = 7.8 Hz, 1H), 7.86 (m, 1H), 7.98 (m, 1H), 8.35 (t, J = 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.4, 93.8, 128.8, 130.1, 132.0, 138.5, 141.8, 165.6; MS (EI+) m/z 262 (M^+, 100), 231 (89), 203 (44); HRMS (EI+) m/z calcd for $C_8H_7O_2I$ (M^+) 261.9491, found 261.9503.

2-Trimethylsilanylethynylbenzoic Acid Methyl Ester (3a). Compound **2a** (11.00 g, 41.98 mmol), 2-TMS-acetylene (4.50 g, 45.82 mmol), PPh_3 (280 mg, 1.07 mmol), $Pd(PPh_3)_2Cl_2$ (1.50 g, 2.14 mmol), and Et_3N (35.0 mL, 252 mmol) were dissolved in THF (150 mL). The reaction mixture was degassed and stirred under an atmosphere of nitrogen for 20 min before the addition of CuI (170 mg, 0.89 mmol). The reaction was then stirred at rt for 12 h, after which time additional $Pd(PPh_3)_2Cl_2$ (300 mg, 0.43 mmol) and CuI (40 mg, 0.21 mmol) were added and stirring continued for 6 h. The reaction mixture was diluted with diethyl ether (200 mL) and then washed with 0.1 M HCl solution (2 × 200 mL), H_2O (200 mL), and brine (200 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo and the resulting residue purified by FC (0−25% v/v, EtOAc/hexane) to afford **3a** as a pale yellow oil (9.01 g, 92%): IR (neat) 2957, 2159, 1730, 1297, 1252, and 1081 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.25 (s, 9H), 3.90 (s, 3H), 7.34 (td, J = 7.6 and 1.3 Hz, 1H), 7.42 (td, J = 7.6 and 1.4 Hz, 1H), 7.56 (dd, J = 7.7 and 1.0 Hz, 1H), 7.88 (dd, J = 7.8 and 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.1, 52.0, 99.7, 103.3, 123.2, 128.2, 130.3, 131.5, 132.5, 134.5, 166.9; MS (EI+) m/z 232 (M^+, 19), 217 (54), 187 (100); HRMS (EI+) m/z calcd for $C_{13}H_{16}O_2Si$ (M^+) 232.0920, found 232.0916.

3-Trimethylsilanylethynylbenzoic Acid Methyl Ester (3b). Compound **2b** (4.00 g, 15.3 mmol), 2-TMS-acetylene (1.64 g, 16.7 mmol), PPh_3 (102 mg, 0.389 mmol), $Pd(PPh_3)_2Cl_2$ (545 mg, 0.776 mmol), and Et_3N (13.0 mL, 93.5 mmol) were dissolved in THF (50 mL). The reaction mixture was degassed and stirred under an atmosphere of nitrogen for 20 min before the addition of CuI (62 mg, 0.326 mmol). The reaction was then stirred at rt for 12 h, after which time additional $Pd(PPh_3)_2Cl_2$ (110 mg, 0.157 mmol) and CuI (15 mg, 0.079 mmol) were added and stirring continued for 6 h. The reaction mixture was diluted with diethyl ether (100 mL) and then washed with 0.1 M HCl solution (2 × 100 mL), H_2O (100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo and the resulting residue purified by FC (10% v/v, EtOAc/hexane) to afford **3b** as an orange oil (3.41 g, 96%): IR (neat) 2957, 2159, 1729, 1292, 1104, and 848 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.23 (s, 9H), 3.90 (s, 3H), 7.36 (t, J = 7.8 Hz, 1H), 7.61 (dt, J = 7.5 and 1.2 Hz, 1H), 7.95 (dt, J = 7.8 and 1.3 Hz, 1H), 8.11 (t, J = 1.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.1, 52.3, 95.4, 103.9, 123.6, 128.4, 129.4, 130.3, 133.1, 136.0, 166.4; MS (EI+) m/z 232 (M^+, 30), 217 (100); HRMS (EI+) m/z calcd for $C_{13}H_{16}O_2Si$ (M^+) 232.0920, found 232.0917.

2-(2-Aminophenylethynyl)benzoic Acid Methyl Ester (5a). *Method 1.* Compound **3a** (9.01 g, 38.78 mmol), 2-iodoaniline (**4a**, 9.34 g, 42.64 mmol), PPh_3 (2.00 g, 7.62 mmol), $Pd(PPh_3)_2Cl_2$ (2.70 g, 3.85 mmol), and piperidine (56.0 mL, 566 mmol) were dissolved in THF (500 mL). The reaction mixture was degassed and stirred under an atmosphere of nitrogen for 20 min before the addition of CuI (1.50 g, 7.88 mmol) and K_2CO_3 (6.40 g, 46.31 mmol). The reaction mixture was refluxed, MeOH (50 mL) was added dropwise, and heating was continued for 12 h. Additional $Pd(PPh_3)_2Cl_2$ (270 mg, 0.385 mmol) and CuI (150 mg, 0.788 mmol) were then added, and stirring was continued for 6 h at reflux. The reaction mixture was cooled to rt, diluted with ethyl acetate (500 mL), and then washed with 0.1 M HCl solution (2 × 500 mL), H_2O (500 mL), and brine (500 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo and the resulting residue purified by FC (0−20% v/v, EtOAc/hexane) to afford **5a** as a bright yellow solid (7.77 g, 80%): mp 77−78 °C; IR (neat) 3471, 3363, 2207, 1718, 1255, and 1081 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.91 (s, 3H), 5.02 (br s, 2H), 6.66 (m, 1H), 6.70 (d, J = 8.1 Hz, 1H), 7.13 (m, 1H), 7.39−7.30 (m, 2H), 7.49 (td, J = 7.6 and 1.2 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 8.01 (dd, J = 7.9 and 1.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.3, 92.6, 93.7, 107.2, 114.1, 117.1, 124.7, 127.4, 130.2, 130.3, 130.6, 132.1, 133.7, 149.8, 166.3; MS (ES+) m/z 252, 253 (MH^+, 100, 19); HRMS (ES+) m/z calcd for $C_{16}H_{14}NO_2$ (MH^+) 252.1025, found 252.1032.

Method 2. A solution of 2-iodoaniline (**4a**, 200 mg, 0.91 mmol), $Pd(PPh_3)_2Cl_2$ (13 mg, 0.019 mmol), PPh_3 (10 mg, 0.038 mmol), and CuI (3.6 mg, 0.019 mmol) in toluene (1.5 mL) was evacuated and purged with N_2 repeatedly (×3). i-Pr_2NH (0.31 mL, 2.21 mmol) was then added and the mixture evacuated and purged with N_2 repeatedly (×3) and then stirred for 20 min at rt before the addition of TMS-acetylene (0.26 mL, 1.84 mmol) under a flow of N_2. The reaction mixture was stirred for 18 h at rt, and a solution of KOH (0.25 g, 0.49 mmol) in H_2O/MeOH (1:2, 0.6 mL) was added. The reaction mixture was stirred for 2 h at rt and then quenched with a saturated solution of NH_4Cl (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with H_2O (10 mL) and brine (10 mL), dried ($MgSO_4$), and filtered, and the solvents were removed in vacuo to give the crude coupling product. A solution of methyl 2-iodobenzoate (**2a**, 0.28 mL, 1.91 mmol), $Pd(PPh_3)_2Cl_2$ (13 mg, 0.019 mmol), PPh_3 (10 mg, 0.038 mmol), and CuI (3.6 mg, 0.019 mmol) in toluene (0.5 mL) was evacuated and purged with N_2 repeatedly (×3). i-Pr_2NH (0.31 mL, 2.21 mmol) was then added and the mixture

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evacuated and purged with N_2 repeatedly (×3) and then stirred for 20 min at rt before the addition of the crude coupling product in toluene (1.5 mL) under a flow of N_2. The reaction mixture was stirred for 23 h at rt and then quenched with a saturated solution of NH_4Cl (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with H_2O (10 mL) and brine (10 mL), dried ($MgSO_4$), and filtered, and the solvents were removed in vacuo. Purification of the crude product by FC (10% v/v, EtOAc/hexane) gave the *title compound* as a yellow oil (196 mg, 85%).

3-(2-Aminophenylethynyl)benzoic Acid Methyl Ester (5b). Compound **3b** (1.70 g, 7.32 mmol), 2-iodoaniline (**4a**, 1.77 g, 8.08 mmol), PPh_3 (380 mg, 1.45 mmol), $Pd(PPh_3)_2Cl_2$ (510 mg, 0.727 mmol), and piperidine (10.5 mL, 106 mmol) were dissolved in THF (100 mL). The reaction mixture was degassed and stirred under an atmosphere of nitrogen for 20 min before the addition of CuI (285 mg, 1.50 mmol) and K_2CO_3 (1.21 g, 8.75 mmol). The reaction mixture was refluxed, MeOH (9.0 mL) was added dropwise, and heating was continued for 12 h. Additional $Pd(PPh_3)_2Cl_2$ (51 mg, 0.073 mmol) and CuI (29 mg, 0.15 mmol) were then added, and stirring was continued for 6 h at reflux. The reaction mixture was cooled to rt, diluted with CH_2Cl_2 (150 mL), and then washed with 0.1 M HCl solution (2 × 100 mL), H_2O (100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the resulting residue was purified by FC (20−33% v/v, EtOAc/hexane) to afford **5b** as a yellow oil (744 mg, 40%): IR (neat) 3375, 2198, 1721, 1616, 1261, and 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 3H), 4.28 (br s, 2H), 6.74−6.67 (m, 2H), 7.14 (td, J = 8.0 and 1.4 Hz, 1H), 7.35 (dd, J = 8.1 and 1.2 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.68 (m, 1H), 7.97 (d, J = 7.9 Hz, 1H), 8.18 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.4, 87.0, 93.7, 107.4, 114.4, 118.0, 123.8, 128.6, 129.1, 130.1, 130.5, 132.3, 132.5, 135.5, 147.9, 166.5; MS (ES+) m/z 252, 253 (MH^+, 100, 21); HRMS (ES+) m/z calcd for $C_{16}H_{14}NO_2$ (MH^+) 252.1025, found 252.1025.

2-(3-Aminophenylethynyl)benzoic Acid Methyl Ester (5c). Compound **3a** (2.60 g, 11.19 mmol), 3-iodoaniline (**4b**, 2.70 g, 12.33 mmol), PPh_3 (580 mg, 2.21 mmol), $Pd(PPh_3)_2Cl_2$ (780 mg, 1.11 mmol), and piperidine (16.0 mL, 162 mmol) were dissolved in THF (150 mL). The reaction mixture was degassed and stirred under an atmosphere of nitrogen for 20 min before the addition of CuI (433 mg, 2.27 mmol) and K_2CO_3 (1.85 g, 13.39 mmol). The reaction mixture was refluxed, MeOH (14.0 mL) was added dropwise, and heating was continued for 12 h. Additional $Pd(PPh_3)_2Cl_2$ (78 mg, 0.11 mmol) and CuI (43 mg, 0.23 mmol) were then added, and stirring was continued for 6 h at reflux. The reaction mixture was cooled to rt, diluted with CH_2Cl_2 (200 mL), then washed with 0.1 M HCl solution (2 × 200 mL), H_2O (200 mL) and brine (200 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo and the resulting residue purified by FC (10−50% v/v, EtOAc/hexane) to afford **5c** as an orange oil (1.12 g, 40%): IR (neat) 3371, 1720, 1598, 1297, 1253, and 1079 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.69 (br s, 2H), 3.94 (s, 3H), 6.65 (m, 1H), 6.88 (m, 1H), 6.96 (d, J = 7.7 Hz, 1H), 7.12 (t, J = 7.8 Hz, 1H), 7.35 (td, J = 7.7 and 1.2 Hz, 1H), 7.46 (td, J = 7.6 and 1.3 Hz, 1H), 7.61 (dd, J = 7.8 and 0.7 Hz, 1H), 7.94 (dd, J = 7.9 and 1.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.2, 87.6, 94.7, 115.6, 117.9, 122.2, 123.8, 124.0, 127.8, 129.3, 130.4, 131.7, 131.9, 134.0, 146.3, 166.8; MS (ES+) m/z 252, 253 (MH^+, 100, 19); HRMS (ES+) m/z calcd for $C_{16}H_{14}NO_2$ (MH^+) 252.1025, found 252.1020.

3-(3-Aminophenylethynyl)benzoic Acid Methyl Ester (5d). Compound **3b** (1.70 g, 7.32 mmol), 3-iodoaniline (**4b**, 1.77 g, 8.08 mmol), PPh_3 (381 mg, 1.45 mmol), $Pd(PPh_3)_2Cl_2$ (510 mg, 0.727 mmol), and piperidine (10.5 mL, 106 mmol) were dissolved in THF (100 mL). The reaction mixture was degassed and stirred under an atmosphere of nitrogen for 20 min before the addition of CuI (285 mg, 1.50 mmol) and K_2CO_3 (1.21 g, 8.75 mmol). The reaction mixture was refluxed, MeOH (9.0 mL) was added dropwise, and heating was continued for 12 h. Additional $Pd(PPh_3)_2Cl_2$ (52 mg, 0.074 mmol) and CuI (28 mg, 0.147 mmol) were then added, and stirring was continued for 6 h at reflux. The reaction mixture was cooled to rt, diluted with CH_2Cl_2 (150 mL), and then washed with 0.1 M HCl solution (2 × 100 mL), H_2O (100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo and the resulting residue purified by FC (10−40% v/v, EtOAc/hexane) to afford **5d** as a yellow solid (572 mg, 31%): mp 92−95 °C; IR (Nujol mull) 3466, 2924, 1712, 1457, 1293, and 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.75 (br s, 2H), 3.96 (s, 3H), 6.70 (dd, J = 7.9 and 2.0 Hz, 1H), 6.89 (m, 1H), 8.22 (m, 1H), 6.97 (d, J = 7.6 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.71 (m, 1H), 8.01 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.3, 87.7, 90.6, 115.6, 117.8, 122.1, 123.5, 123.9, 128.5, 129.1, 129.4, 130.4, 132.8, 135.7, 146.4, 166.5; MS (EI+) m/z 251, 252 (M^+, 100, 17); HRMS (ES+) m/z calcd for $C_{16}H_{14}NO_2$ (MH^+) 252.1025, found 252.1037.

2-{2-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenylethynyl}-benzoic Acid Methyl Ester (8a). To a stirred solution of (*S*)-Fmoc-Ser(^{t}Bu)-OH (**6**, 3.52 g, 9.18 mmol) and oxalyl chloride (1.35 mL, 15.95 mmol) in CH_2Cl_2 (100 mL) was added DMF (5 drops) at 0 °C under an atmosphere of nitrogen. After 20 min, the reaction mixture was allowed to warm to rt, and stirring was continued for 1 h. The reaction mixture was then concentrated in vacuo to afford (*S*)-Fmoc-Ser(^{t}Bu)-Cl (**7**) as a yellow oil which was used immediately without further purification.

To a stirred solution of the above prepared (*S*)-Fmoc-Ser(^{t}Bu)-Cl (**7**) and **5a** (1.92 g, 7.66 mmol) in CH_2Cl_2 (100 mL) was added AgCN (1.36 g, 10.12 mmol) under an atmosphere of nitrogen. Stirring was continued at rt with the exclusion of light for 2 h. The reaction mixture was then filtered and concentrated in vacuo, and the resulting residue purified by FC (20% v/v, EtOAc/hexane) to afford **8a** as a white foam (4.49 g, 95%): mp 135−138 °C; IR (neat) 3320, 1696, 1528, 1450, 1271, and 1081 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.06 (s, 9H), 3.64 (dd, J = 9.1 and 5.2 Hz, 1H), 3.84 (dd, J = 9.0 and 4.1 Hz, 1H), 4.01 (s, 3H), 4.18 (t, J = 7.1 Hz, 1H), 4.33 (d, J = 7.0 Hz, 2H), 5.08 (m, 1H), 6.20 (d, J = 8.1 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 7.30−7.24 (m, 2H), 7.41−7.30 (m, 4H), 7.55−7.51 (m, 2H), 7.62−7.56 (m, 2H), 7.68 (d, J = 7.6 Hz, 1H), 7.72 (d, J = 7.5 Hz, 2H), 8.07 (d, J = 7.6 Hz, 1H), 8.55 (d, J = 8.2 Hz, 1H), 9.45 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3, 47.2, 53.1, 55.7, 63.1, 66.9, 73.3, 90.7, 95.1, 112.2, 119.8, 119.9, 123.4, 124.2, 125.2, 127.0, 127.6, 128.2, 129.9, 130.1, 130.8, 132.1, 132.4, 134.0, 140.2, 141.3, 143.9, 156.0, 166.3, 169.7; MS (ES+) m/z 639, 640, 641 (MNa^+, 100, 42, 10), 617 (MH^+, 13); HRMS (ES+) m/z calcd for $C_{38}H_{37}N_2O_6$ (MH^+) 617.2652, found 617.2659.

3-{2-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenylethynyl}-benzoic Acid Methyl Ester (8b). To a stirred solution of (*S*)-Fmoc-Ser(^{t}Bu)-OH (**6**, 861 mg, 2.25 mmol) and oxalyl chloride (395 μL, 4.53 mmol) in CH_2Cl_2 (30 mL) was added DMF (2 drops) at 0 °C under an atmosphere of nitrogen. After 10 min, the reaction mixture was allowed to warm to rt, and stirring was continued for 1 h. The reaction mixture was then concentrated in vacuo to afford (*S*)-Fmoc-Ser(^{t}Bu)-Cl (**7**) as a yellow oil which was used immediately without further purification.

To a stirred solution of the above prepared (*S*)-Fmoc-Ser(^{t}Bu)-Cl (**7**) and **5b** (583 mg, 2.32 mmol) in CH_2Cl_2 (50 mL) was added AgCN (324 mg, 2.42 mmol) under an atmosphere of nitrogen. Stirring was continued at rt with the exclusion of light for 2 h. The reaction mixture was then filtered and concentrated in vacuo and the resulting residue purified by FC (10−50% v/v, EtOAc/hexane) to afford **8b** as a white solid (810 mg, 57%): mp 158−160 °C; IR (neat) 3367, 1725, 1524, 1449, 1261, and 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.11 (s, 9H), 3.53 (dd, J = 8.8 and 6.7 Hz, 1H), 3.86 (s, 3H), 3.91 (m, 1H), 4.10 (m, 1H), 4.28 (m, 1H), 4.49−4.34 (m, 2H), 5.84 (s, 1H), 7.11 (t, J = 7.5 Hz, 1H), 7.22 (br s, 2H), 7.43−7.28 (m, 4H), 7.56−7.45 (m, 3H), 7.70 (dd, J = 7.6 and 1.0 Hz, 3H), 7.96 (d, J = 7.7 Hz, 1H), 8.21 (s, 1H), 8.40 (d, J = 8.0 Hz, 1H), 8.89 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.5, 47.2, 52.5, 56.3, 61.9, 67.4, 74.4, 85.5, 95.5, 112.6, 120.2, 123.1, 124.1, 125.1, 125.2, 127.2, 127.9, 128.8, 129.9, 130.1, 130.7, 132.2, 133.0, 135.8, 138.7, 141.4, 143.8, 143.8, 156.4, 166.4, 168.8; MS (ES+) m/z 639, 640, 641 (MNa^+, 100, 45, 11), 617, 618 (MH^+, 34, 14); HRMS (ES+) m/z calcd for $C_{38}H_{37}N_2O_6$ (MH^+) 617.2652, found 617.2667.

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2-{3-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenylethynyl}-benzoic Acid Methyl Ester (8c). To a stirred solution of (*S*)-Fmoc-Ser(^{t}Bu)-OH (**6**, 820 mg, 2.14 mmol) and oxalyl chloride (375 μL, 4.30 mmol) in CH_2Cl_2 (30 mL) was added DMF (2 drops) at 0 °C under an atmosphere of nitrogen. After 10 min, the reaction mixture was allowed to warm to rt, and stirring was continued for 1 h. The reaction mixture was then concentrated in vacuo to afford (*S*)-Fmoc-Ser(^{t}Bu)-Cl (**7**) as a yellow oil which was used immediately without further purification.

To a stirred solution of the above prepared (*S*)-Fmoc-Ser(^{t}Bu)-Cl (**7**) and **5c** (555 mg, 2.21 mmol) in CH_2Cl_2 (50 mL) was added AgCN (308 mg, 2.30 mmol) under an atmosphere of nitrogen. Stirring was continued at rt with the exclusion of light for 2 h. The reaction mixture was then filtered and concentrated in vacuo and the resulting residue purified by FC (10−40% v/v, EtOAc/hexane) to afford **8c** as an off-white foam (929 mg, 68%). The product was obtained as a 5:1 mixture of rotamers as observed by ^{1}H NMR spectroscopy: mp 137−138 °C; IR (neat) 3317, 1715, 1547, 1490, 1253, and 1080 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (s, 9H), 3.45 (m, 1H), 3.92 (m, 1H), 3.95 (s, 3H), 4.23 (t, *J* = 6.9 Hz, 1H), 4.35 (br s, 1H), 4.49−4.38 (m, 2H), 5.84 (br s, 1H), 7.41−7.26 (m, 7H), 7.53−7.45 (m, 2H), 7.65−7.57 (m, 3H), 7.79−7.69 (m, 3H), 7.97 (d, *J* = 7.9 Hz, 1H), 8.91 and 8.74 (each br s, minor and major, total 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.7, 47.3, 52.4, 55.0, 61.9, 67.4, 75.1, 88.8, 94.0, 120.1, 120.2, 122.8, 123.7, 124.4, 125.3, 127.3, 128.0, 128.2, 129.4, 130.7, 131.9, 132.1, 134.2, 137.8, 141.5, 144.0, 156.3, 166.9, 168.7; MS (ES+) *m*/*z* 639, 640 (MNa^+, 100, 42), 617, 618 (MH^+, 39, 16); HRMS (ES+) *m*/*z* calcd for $C_{38}H_{37}N_2O_6$ (MH^+) 617.2652, found 617.2646.

3-{3-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenylethynyl}-benzoic Acid Methyl Ester (8d). To a stirred solution of (*S*)-Fmoc-Ser(^{t}Bu)−OH (**6**, 680 mg, 1.77 mmol) and oxalyl chloride (310 μL, 3.55 mmol) in CH_2Cl_2 (25 mL) was added DMF (1 drop) at 0 °C under an atmosphere of nitrogen. After 10 min, the reaction mixture was allowed to warm to rt, and stirring was continued for 1 h. The reaction mixture was then concentrated in vacuo to afford (*S*)-Fmoc-Ser(^{t}Bu)-Cl (**7**) as a yellow oil which was used immediately without further purification.

To a stirred solution of the above prepared (*S*)-Fmoc-Ser(^{t}Bu)-Cl (**7**) and **5d** (460 mg, 1.83 mmol) in CH_2Cl_2 (30 mL) was added AgCN (255 mg, 1.90 mmol) under an atmosphere of nitrogen. Stirring was continued at rt with the exclusion of light for 2 h. The reaction mixture was then filtered and concentrated in vacuo and the resulting residue purified by FC (10−40% v/v, EtOAc/hexane) to afford **8d** as an off-white foam (892 mg, 79%): mp 179−182 °C; IR (neat) 3317, 1723, 1263, 910, and 739 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (s, 9H), 3.46 (m, 1H), 3.91 (m, 1H), 3.92 (s, 3H), 4.23 (t, *J* = 6.9 Hz, 1H), 4.36 (br s, 1H), 4.47−4.40 (m, 2H), 5.85 (br s, 1H), 7.33−7.26 (m, 4H), 7.41−7.36 (m, 2H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.46 (dt, *J* = 7.3 and 2.1 Hz, 1H), 7.60 (d, *J* = 7.2 Hz, 2H), 7.68 (m, 1H), 7.78−7.71 (m, 3H), 7.99 (m, 1H), 8.20 (t, *J* = 1.3 Hz, 1H), 8.75 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.7, 47.3, 52.5, 55.0, 60.6, 61.9, 67.4, 75.07, 88.8, 90.0, 120.1, 120.2, 122.8, 123.7, 123.9, 125.3, 127.3, 127.9, 128.0, 128.7, 129.4, 129.5, 130.7, 133.0, 135.9, 137.8, 141.5, 143.9, 156.3, 166.6, 168.7; MS (ES+) *m*/*z* 639, 640 (MNa^+, 100, 48), 617, 618 (MH^+, 73, 30); HRMS (ES+) *m*/*z* calcd for $C_{38}H_{37}N_2O_6$ (MH^+) 617.2652, found 617.2643.

2-{2-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenylethynyl}-benzoic Acid (9a). To an ice-cold solution of **8a** (2.24 g, 3.63 mmol) in THF (120 mL) was added $LiAlH_4$ (413 mg, 10.88 mmol) and stirring continued under an atmosphere of nitrogen for 20 min. The reaction mixture was then diluted with diethyl ether (200 mL) and cautiously quenched with a saturated solution of Rochelle's salt (100 mL). The organic phase was then separated, washed with H_2O (2 × 200 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was dissolved in acetone (120 mL), a solution of 1 M Jones' reagent in acetone (12.0 mL) was added dropwise, and stirring was continued at rt for 2 h. The reaction mixture was diluted with H_2O (200 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo, and the resulting residue was purified by FC (10−50% v/v, EtOAc/hexane) to afford **9a** as an off-white foam (1.35 g, 62%, >99.8% ee by HPLC): mp 90−93 °C; $[\alpha]_D^{20}$ +10.2 (*c* 1.01, $CHCl_3$); HPLC [Chiralpak AS-H, isocratic TFA/*i*PrOH/hexane (0.5:9.5:90), 40 °C, 0.25 mL/min] R_t = 19.8 min; IR (neat) 3303, 1688, 1526, 1450, 1261, and 757 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (s, 9H), 3.62 (dd, *J* = 9.1 and 6.7 Hz, 1H), 3.76 (dd, *J* = 9.2 and 5.6 Hz, 1H), 4.19 (t, *J* = 7.0 Hz, 1H), 4.44−4.31 (m, 2H), 5.30 (m, 1H), 5.93 (d, *J* = 9.0 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 7.28 (t, *J* = 7.3 Hz, 2H), 7.36 (dd, *J* = 13.8 and 6.8 Hz, 4H), 7.51 (t, *J* = 7.2 Hz, 2H), 7.60−7.54 (m, 2H), 7.63 (d, *J* = 7.7 Hz, 1H), 7.73 (d, *J* = 7.5 Hz, 2H), 8.09 (d, *J* = 7.8 Hz, 1H), 8.52 (d, *J* = 8.3 Hz, 1H), 9.62 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3, 47.1, 55.4, 60.4, 63.9, 65.9, 67.5, 74.4, 90.3, 95.6, 112.5, 119.7, 120.0, 123.6, 123.9, 125.1, 127.1, 127.8, 128.2, 130.0, 131.1, 132.0, 132.2, 133.4, 140.3, 141.3, 143.7, 143.8, 156.8, 168.1, 169.3; MS (ES+) *m*/*z* 625, 626, (MNa^+, 100, 45), 603, 604 (MH^+, 90, 36); HRMS (ES+) *m*/*z* calcd for $C_{37}H_{35}N_2O_6$ (MH^+) 603.2495, found 603.2510.

3-{2-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenylethynyl}-benzoic Acid (9b). To an ice-cold solution of **8b** (780 mg, 1.62 mmol) in THF (40 mL) was added $LiAlH_4$ (145 mg, 3.82 mmol) and stirring continued under an atmosphere of nitrogen for 20 min. The reaction mixture was then diluted with diethyl ether (100 mL) and cautiously quenched with a saturated solution of Rochelle's salt (50 mL). The organic phase was then separated, washed with H_2O (2 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was dissolved in acetone (40 mL), a solution of 1 M Jones' reagent in acetone (4.10 mL) was added dropwise, and stirring was continued at rt for 2 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo, and the resulting residue was purified by FC (50% v/v, EtOAc/hexane) to afford **9b** as an off-white foam (335 mg, 44%): mp 121−124 °C; $[\alpha]_D^{27}$ +4.3 (*c* 1.10, EtOAc); IR (neat) 3303, 1685, 1525, 1260, 1078, and 738 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (s, 9H), 3.56 (t, *J* = 7.6 Hz, 1H), 3.90 (m, 1H), 4.11 (t, *J* = 7.1 Hz, 1H), 4.29 (m, 1H), 4.41 (m, 1H), 4.49 (m, 1H), 5.91 (br s, 1H), 7.11 (t, *J* = 7.5 Hz, 1H), 7.25−7.15 (m, 2H), 7.31 (t, *J* = 7.3 Hz, 2H), 7.42−7.35 (m, 2H), 7.55−7.46 (m, 3H), 7.68 (d, *J* = 7.3 Hz, 2H), 7.73 (d, *J* = 7.4 Hz, 1H), 7.97 (d, *J* = 7.5 Hz, 1H), 8.24 (br s, 1H), 8.41 (m, 1H), 8.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.5, 47.3, 56.2, 61.9, 66.1, 67.5, 74.5, 95.3, 112.6, 120.2, 123.2, 124.1, 125.2, 127.2, 127.9, 128.9, 130.2, 130.4, 132.3, 133.5, 136.5, 138.7, 141.5, 143.8, 156.5, 169.0, 170.3; MS (ES+) *m*/*z* 625, 626, (MNa^+, 100, 42), 603, 604 (MH^+, 97, 42); HRMS (ES+) *m*/*z* calcd for $C_{37}H_{35}N_2O_6$ (MH^+) 603.2495, found 603.2518.

2-{3-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenylethynyl}-benzoic Acid (9c). To an ice-cold solution of **8c** (892 mg, 1.45 mmol) in THF (50 mL) was added $LiAlH_4$ (166 mg, 4.37 mmol) and stirring continued under an atmosphere of nitrogen for 20 min. The reaction mixture was then diluted with diethyl ether (100 mL) and cautiously quenched with a saturated solution of Rochelle's salt (50 mL). The organic phase was then separated, washed with H_2O (2 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was dissolved in acetone (50 mL), a solution of 1 M Jones' reagent in acetone (4.70 mL) was added dropwise, and stirring was continued at rt for 2 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo, and the resulting residue was purified by FC (50−75% v/v, EtOAc/hexane) to afford **9c** as an off-white foam (505 mg, 58%): mp 102−103 °C; $[\alpha]_D^{27}$ −8.6 (*c* 0.92, $CHCl_3$); IR (neat) 3410, 1683, 1557, 1238, 1079, and 737 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (s, 9H), 3.46 (t, *J* = 8.5 Hz, 1H), 3.85 (m, 1H), 4.19 (t, *J* = 6.9 Hz, 1H), 4.48−4.32 (m, 3H), 6.00 (m, 1H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.30−7.24 (m, 3H), 7.41−7.33 (m, 3H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.50

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(t, J = 7.6 Hz 1H), 7.58 (d, J = 5.9 Hz, 2H), 7.63 (d, J = 7.6 Hz, 1H), 7.75−7.68 (m, 3H), 8.08 (d, J = 7.9 Hz, 1H), 8.79 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.6, 47.3, 55.1, 62.0, 67.5, 75.1, 88.6, 95.0, 120.2, 120.3, 122.9, 124.1, 124.3, 125.3, 127.3, 128.0, 128.3, 129.3, 131.1, 131.5, 132.6, 134.4, 137.7, 141.5, 144.0, 156.5, 168.9, 170.3; MS (ES+) m/z 625, 626, (MNa^+, 100, 42), 603, 604 (MH^+, 57, 23); HRMS (ES+) m/z calcd for $C_{37}H_{35}N_2O_6$ (MH^+) 603.2495, found 603.2501.

3-{3-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenylethynyl}-benzoic Acid (9d). To an ice-cold solution of **8d** (860 mg, 1.40 mmol) in THF (50 mL) was added $LiAlH_4$ (160 mg, 4.22 mmol) and stirring continued under an atmosphere of nitrogen for 20 min. The reaction mixture was then diluted with diethyl ether (100 mL) and cautiously quenched with a saturated solution of Rochelle's salt (50 mL). The organic phase was then separated, washed with H_2O (2 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was dissolved in acetone (50 mL), a solution of 1 M Jones' reagent in acetone (4.50 mL) was added dropwise, and stirring was continued at rt for 2 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo, and the resulting residue was purified by FC (50−75% v/v, EtOAc/hexane) to afford **9d** as a pale yellow solid (360 mg, 43%): mp 158−161 °C; $[\alpha]_D^{27}$ −14.1 (c 1.00 EtOAc); IR (Nujol mull) 3283, 1695, 1661, 1463, 1082, and 740 cm^{-1}; ^{1}H NMR (400 MHz, d_6-Acetone) δ 1.18 (s, 9H), 3.69 (dd, J = 9.1 and 6.1 Hz, 1H), 3.80 (dd, J = 9.1 and 4.9 Hz, 1H), 4.26 (t, J = 7.0 Hz, 1H), 4.46−4.34 (m, 3H), 7.35−7.28 (m, 3H), 7.44−7.35 (m, 3H), 7.58 (t, J = 7.8 Hz, 1H), 7.76−7.65 (m, 3H), 7.80 (d, J = 7.7 Hz, 1H), 7.85 (d, J = 7.5 Hz, 2H), 8.02 (br s, 1H), 8.06 (d, J = 7.8 Hz, 1H), 8.19 (m, 1H); ^{13}C NMR (100 MHz, d_6-Acetone) δ 28.1, 48.4, 57.5, 63.3, 67.8, 74.6, 89.3, 91.2, 121.3, 123.5, 124.4, 124.8, 126.6, 128.2, 128.4, 129.0, 130.3, 130.5, 130.8, 132.5, 133.7, 136.9, 140.4, 142.5, 145.4, 145.5, 157.4, 167.3, 170.4; MS (ES+) m/z 625, 626, (MNa^+, 52, 22), 603, 604 (MH^+, 100, 42); HRMS (ES+) m/z calcd for $C_{37}H_{35}N_2O_6$ (MH^+) 603.2495, found 603.2503.

2-{2-[(*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenylethynyl}-benzoic Acid Methyl Ester (*ent*-8a). To a stirred solution of (*R*)-Fmoc-Ser(^{t}Bu)-OH (***ent*-6**, 533 mg, 1.39 mmol) and oxalyl chloride (210 μL, 2.41 mmol) in CH_2Cl_2 (25 mL) was added DMF (1 drop) at 0 °C under an atmosphere of nitrogen. After 10 min the reaction mixture was allowed to warm to rt and stirring was continued for 1 h. The reaction mixture was then concentrated in vacuo to afford (*R*)-Fmoc-Ser(^{t}Bu)-Cl (***ent*-7**) as a yellow oil which was used immediately without further purification.

To a stirred solution of the above prepared (*R*)-Fmoc-Ser(^{t}Bu)-Cl (***ent*-7**) and 2,2′-tolan methyl ester **5a** (315 mg, 1.25 mmol) in CH_2Cl_2 (25 mL) was added AgCN (185 mg, 1.57 mmol) under an atmosphere of nitrogen. Stirring was continued at rt with the exclusion of light for 2 h. The reaction mixture was then filtered and concentrated in vacuo and the resulting residue purified by FC (20% v/v, EtOAc/hexane) to afford dipeptide methyl ester ***ent*-8a** as a white foam (686 mg, 89%): mp 133−136 °C; IR (neat) 3320, 1701, 1527, 1450, 1271, and 1081 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (s, 9H), 3.64 (dd, J = 9.1 and 5.1 Hz, 1H), 3.84 (dd, J = 9.1 and 4.1 Hz, 1H), 4.01 (s, 3H), 4.17 (t, J = 7.1 Hz, 1H), 4.32 (d, J = 7.1 Hz, 2H), 5.08 (m, 1H), 6.20 (d, J = 8.2 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 7.29−7.25 (m, 2H), 7.41−7.31 (m, 4H), 7.55−7.51 (m, 2H), 7.61−7.56 (m, 2H), 7.68 (d, J = 7.7 Hz, 1H), 7.72 (d, J = 7.5 Hz, 2H), 8.07 (d, J = 7.8 Hz, 1H), 8.54 (d, J = 8.3 Hz, 1H), 9.45 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3, 47.2, 53.1, 55.7, 63.1, 66.9, 73.3, 90.7, 95.1, 169.7, 112.2, 119.8, 119.9, 123.4, 124.2, 125.2, 125.2, 127.0, 127.7, 128.2, 129.9, 130.1, 130.8, 132.1, 132.4, 134.0, 140.2, 141.3, 143.9, 144.0, 156.0, 166.3; MS (ES+) m/z 639, 640, 641 (MNa^+, 100, 44, 8), 617 (MH^+, 13); HRMS (ES+) m/z calcd for $C_{38}H_{37}N_2O_6$ (MH^+) 617.2652, found 617.2658.

2-{2-[(*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenylethynyl}-benzoic Acid (*ent*-9a). To an ice-cold solution of ***ent*-8a** (424 mg, 0.688 mmol) in THF (30 mL) was added $LiAlH_4$ (80 mg, 2.11 mmol) and stirring continued under an atmosphere of nitrogen for 20 min. The reaction mixture was then diluted with diethyl ether (60 mL) and cautiously quenched with a saturated solution of Rochelle's salt (30 mL). The organic phase was then separated, washed with H_2O (2 × 60 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was dissolved in acetone (30 mL), a solution of 1 M Jones' reagent in acetone (2.10 mL) was added dropwise, and stirring was continued at rt for 2 h. The reaction mixture was diluted with H_2O (60 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo, and the resulting residue was purified by FC (10−50% v/v, EtOAc/hexane) to afford ***ent*-9a** as a white foam (315 mg, 76%, >97.2% ee by HPLC): mp 88−91 °C; $[\alpha]_D^{20}$ −10.5 (c 1.11, $CHCl_3$); HPLC [Chiralpak AS-H, isocratic TFA/iPrOH/hexane (0.5:9.5:90), 40 °C, 0.25 mL/min] t_R = 23.9 min; IR (neat) 3303, 1687, 1526, 1450, 1260, and 1078 cm^{-1}; ^{1}H NMR (400 MHz, CDCl3) δ 1.14 (s, 9H), 3.58 (dd, J = 9.1 and 7.3 Hz, 1H), 3.77 (dd, J = 9.3 and 5.2 Hz, 1H), 4.20 (t, J = 7.1 Hz, 1H), 4.44−4.33 (m, 2H), 5.34 (m, 1H), 5.95 (d, J = 9.1 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 7.28 (t, J = 7.3 Hz, 2H), 7.40−7.34 (m, 4H), 7.59−7.50 (m, 4H), 7.65 (d, J = 7.2 Hz, 1H), 7.74 (d, J = 7.5 Hz, 2H), 8.13 (d, J = 7.8 Hz, 1H), 8.54 (d, J = 8.4 Hz, 1H), 9.68 (s, 1H); ^{13}C NMR NMR (100 MHz, $CDCl_3$) δ 27.2, 46.9, 55.2, 63.9, 65.8, 67.3, 74.4, 90.2, 95.4, 112.3, 119.5, 119.9, 123.5, 123.9, 125.0, 127.0, 127.6, 128.1, 129.9, 130.5, 131.1, 131.9, 132.1, 133.4, 140.2, 141.2, 143.5, 143.6, 156.7, 167.8, 169.1; MS (ES+) m/z 625, 626, (MNa^+, 100, 43), 603, 604 (MH^+, 65, 27); HRMS (ES+) m/z calcd for $C_{37}H_{35}N_2O_6$ (MH^+) 603.2495, found 603.2484.

2-[2-(2-Aminophenyl)ethyl]benzoic Acid Methyl Ester (10). To a solution of **5a** (500 mg, 1.99 mmol) in ethyl acetate (50 mL) was added 10% palladium on charcoal (50 mg). The mixture was degassed, flushed with dihydrogen, and stirred at rt for 16 h under an atmosphere of dihydrogen. The reaction mixture was then filtered and concentrated in vacuo to afford bibenzyl **10** as an off-white solid (505 mg, 99%): mp 51−53 °C; IR (neat) 3383, 1715, 1497, 1271, 1082, and 748 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.85−2.74 (m, 2H), 3.22−3.13 (m, 2H), 3.90 (s, 3H), 4.27 (br s, 2H), 6.75−6.66 (m, 2H), 7.12−7.00 (m, 2H), 7.35−7.26 (m, 2H), 7.47 (td, J = 7.5 and 1.3 Hz, 1H), 7.96 (dd, J = 7.9 and 1.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.0, 35.3, 52.2, 115.5, 118.2, 125.9, 126.3, 127.3, 128.7, 130.2, 131.0, 131.6, 132.5, 144.4, 145.1, 167.9; MS (ES+) m/z 256, 257 (MH^+, 100, 20), 224 (51); HRMS (ES+) m/z calcd for $C_{16}H_{18}NO_2$ (MH^+) 256.1338, found 256.1350.

2-(2-{2-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenyl}ethyl)-benzoic Acid Methyl Ester (11). To a stirred solution of (*S*)-Fmoc-Ser(^{t}Bu)-OH (**6**, 370 mg, 0.965 mmol) and oxalyl chloride (170 μL, 1.95 mmol) in CH_2Cl_2 (20 mL) was added DMF (1 drop) at 0 °C under an atmosphere of nitrogen. After 10 min, the reaction mixture was allowed to warm to rt and stirring was continued for 1 h. The reaction mixture was then concentrated *in vacuo* to afford (*S*)-Fmoc-Ser(^{t}Bu)-Cl (**7**) as a yellow oil which was used immediately without further purification.

To a stirred solution of the above prepared (*S*)-Fmoc-Ser(^{t}Bu)-Cl (**7**) and aniline **10** (250 mg, 0.979 mmol) in CH_2Cl_2 (25 mL) was added AgCN (140 mg, 1.05 mmol) under an atmosphere of nitrogen. Stirring was continued at rt with the exclusion of light for 2 h. The reaction mixture was then filtered and concentrated in vacuo and the resulting residue purified by FC (25−50% v/v, EtOAc/hexane) to afford bibenzyl dipeptide **11** as a white solid (540 mg, 90%): mp 137−139 °C; IR (neat) 3356, 1692, 1534, 1450, 1252, and 1079 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (s, 9H), 2.88 (dd, J = 9.4 and 8.0 Hz, 2H), 3.21−3.01 (m, 2H), 3.57 (m, 1H), 3.85 (dd, J = 8.8 and 3.9 Hz, 1H), 3.90 (s, 3H), 4.22 (t, J = 6.8 Hz, 1H), 4.40 (dd, J = 10.4 and 6.8 Hz, 1H), 4.48 (m, 1H), 4.85 (s, 1H), 6.37 (d, J = 8.1 Hz, 1H), 7.11 (t, J = 7.3 Hz, 1H), 7.39−7.20 (m, 8H), 7.49 (t, J = 7.4 Hz, 1H), 7.62−7.57 (m, J = 4.0, 7.1, 2H), 7.68 (d, J = 7.4 Hz, 1H), 7.72 (d, J = 7.5 Hz, 1H), 8.02 (d, J = 7.7 Hz, 1H), 8.08 (d, J = 7.9 Hz, 1H), 8.84 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.5, 34.9, 37.2, 47.4, 52.9, 55.8, 63.0, 66.9, 73.7, 120.1, 123.2, 125.1, 125.2, 126.7, 127.2, 127.8, 128.2, 130.3,

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131.5, 131.9, 132.2, 133.2, 135.8, 141.5, 141.5, 144.0, 144.1, 144.5, 156.2, 168.1, 169.7; MS (ES+) *m/z* 643, 644 (MNa^+, 97, 49), 621, 622 (MH^+, 100, 42); HRMS (ES+) *m/z* calcd for $C_{38}H_{41}N_2O_6$ (MH^+) 621.2965, found 621.2952.

2-(2-{2-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenyl}ethyl)-benzoic Acid (12). To an ice-cold solution of ester **11** (510 mg, 0.823 mmol) in THF (30 mL) was added $LiAlH_4$ (94 mg, 2.48 mmol) and stirring continued under an atmosphere of nitrogen for 20 min. The reaction mixture was then diluted with diethyl ether (50 mL) and cautiously quenched with a saturated solution of Rochelle's salt (50 mL). The organic phase was then separated, washed with H_2O (2 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was dissolved in acetone (30 mL), a solution of 1 M Jones' reagent in acetone (2.50 mL) was added dropwise, and stirring was continued at rt for 2 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo, and the resulting residue was purified by FC (100% CH_2Cl_2, then 50% v/v, EtOAc/hexane) to afford acid **12** as a white solid (241 mg, 48%).

Alternatively, dipeptide tolan acid **9a** (50 mg, 0.083 mmol) was dissolved in ethyl acetate (20 mL) and 10% palladium on charcoal (5 mg) was added. The mixture was degassed, flushed with dihydrogen, and stirred at rt for 16 h under an atmosphere of dihydrogen. The reaction mixture was then filtered and concentrated in vacuo to afford acid **12** as a white solid (42 mg, 84%): mp 139−141 °C; $[\alpha]_D^{26}$ +10.2 (*c* 1.01, $CHCl_3$); IR (neat) 3333, 1686, 1540, 1452, 1247, and 737 cm^{-1}; ^1H NMR (400 MHz, d_6-Acetone) δ 1.11 (s, 9H), 2.94 (t, *J* = 8.4 Hz, 2H), 3.27−3.17 (m, 2H), 3.71 (dd, *J* = 9.0 and 5.9 Hz, 1H), 3.84 (dd, *J* = 9.0 and 4.8 Hz, 1H), 4.23 (t, *J* = 7.0 Hz, 1H), 4.36 (d, *J* = 6.7 Hz, 2H), 4.78 (m, 1H), 7.08 (t, *J* = 7.3 Hz, 1H), 7.21 (t, *J* = 7.5 Hz, 1H), 7.40−7.24 (m, 7H), 7.44 (d, *J* = 7.2 Hz, 1H), 7.49 (t, *J* = 7.2 Hz, 1H), 7.70 (t, *J* = 6.7 Hz, 2H), 7.82 (d, *J* = 7.5 Hz, 2H), 8.10−8.02 (m, 2H); ^{13}C NMR (100 MHz, d_6-Acetone) δ 28.0, 35.3, 37.5, 48.3, 55.3, 57.0, 63.7, 67.9, 74.3, 121.1, 124.3, 125.8, 126.5, 127.6, 127.7, 128.3, 128.9, 130.1, 131.2, 132.5, 132.9, 133.8, 137.4, 142.4, 145.2, 145.3, 157.5, 170.3, 170.4; MS (ES+) *m/z* 629, 630 (MNa^+, 58, 28), 607, 608 (MH^+, 100, 47); HRMS (ES+) *m/z* calcd for $C_{37}H_{39}N_2O_6$ (MH^+) 607.2808, found 607.2800.

2-((*Z*)-2-{2-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenyl}vinyl)-benzoic Acid (13). To a solution of tolan acid **9a** (100 mg, 0.166 mmol) in ethyl acetate (25 mL) was added Lindlar's catalyst (10 mg, 5% Pd loading). The mixture was degassed, flushed with dihydrogen, and stirred at rt for 16 h under an atmosphere of dihydrogen. The reaction mixture was then filtered and concentrated in vacuo and the resulting residue purified by FC (50−75% v/v, EtOAc/hexane) to afford (*Z*)-stilbene dipeptide acid **13** as a white solid (66 mg, 66%): mp 147−150 °C; $[\alpha]_D^{27}$ +10.5 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD) δ 1.21 (s, 9H), 3.66 (m, 1H), 3.72 (m, 1H), 4.23 (t, *J* = 6.7 Hz, 1H), 4.39 (m, 1H), 4.42 (d, *J* = 6.7 Hz, 2H), 6.63 (d, *J* = 12.0 Hz, 1H), 6.95−6.87 (m, 2H), 7.31−7.07 (m, 8H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.69−7.63 (m, *J* = 5.2 Hz, 2H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.92 (d, *J* = 7.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.6, 47.3, 55.5, 62.4, 67.6, 74.8, 120.2, 121.8, 124.4, 125.3, 127.3, 127.6, 128.0, 128.1, 130.3, 131.2, 132.4, 134.9, 135.3, 139.1, 141.5, 144.0, 156.6, 168.7, 171.4; MS (ES+) *m/z* 627, 628 (MNa^+, 100, 42), 605, 606 (MH^+, 94, 39); HRMS (ES+) *m/z* calcd for $C_{37}H_{37}N_2O_6$ (MH^+) 605.2652, found 605.2652.

2-((*Z*)-2-{2-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenyl}vinyl)-benzoic Acid Methyl Ester (14). To a solution of tolan dipeptide methyl ester **8a** (987 mg, 1.60 mmol) and benzoic acid (290 mg, 2.37 mmol) in ethyl acetate (100 mL) was added Lindlar's catalyst (100 mg, 5% Pd loading). The mixture was degassed, flushed with dihydrogen, and stirred at rt for 16 h under an atmosphere of dihydrogen. The reaction mixture was then filtered and concentrated in vacuo to afford (*Z*)-stilbene dipeptide methyl ester **14** as a white foam (953 mg, 97%). The product was obtained as a 5:1 mixture of rotamers as observed by ^1H NMR spectroscopy: mp 53−56 °C; ^1H NMR (400 MHz, CDCl3) δ 1.22 (s, 9H), 3.48 (m, 1H), 3.84 (dd, *J* = 8.7 and 3.7 Hz, 1H), 3.88 (s, 3H), 4.23 (t, *J* = 7.1 Hz, 1H), 4.33 (m, 1H), 4.40 (d, *J* = 7.2 Hz, 2H), 5.90 (br d, *J* = 4.9 Hz, 1H), 6.62 (d, *J* = 11.9 Hz, 1H), 6.89 (t, *J* = 7.4 Hz, 1H), 6.95 (d, *J* = 6.5 Hz, 1H), 7.03 (m, 1H), 7.32−7.14 (m, 7H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.1 Hz, 2H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.87 (m, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 8.83 and 8.43 (each br s, minor and major, total 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.5, 27.7, 47.3, 52.4, 55.6, 62.2, 67.3, 74.7, 120.2, 122.0, 124.5, 125.3, 125.6, 127.3, 127.6, 127.9, 128.2, 129.4, 130.4, 130.6, 131.1, 132.1, 133.9, 135.3, 138.4, 141.5, 143.9, 144.1, 156.2, 167.9, 168.8; MS (ES+) *m/z* 641, 642 (MNa^+, 100, 42); HRMS (ES+) *m/z* calcd for $C_{38}H_{39}N_2O_6$ (MH^+) 619.2808, found 619.2802.

2-((*E*)-2-{2-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenyl}vinyl)-benzoic Acid Methyl Ester (15). To a solution of (*Z*)-stilbene dipeptide methyl ester **14** (875 mg, 1.42 mmol) in $CHCl_3$ (10 mL) was added silica gel (200 mg) and $Pd(MeCN)_2Cl_2$ (40 mg, 0.154 mmol). The reaction mixture was degassed and then stirred at rt under an atmosphere of nitrogen for 16 h. The mixture was filtered and concentrated in vacuo and the resulting residue purified by FC (10−50% v/v, EtOAc/hexane) to afford (*E*)-stilbene dipeptide methyl ester **15** as a white solid (646 mg, 74%): mp 151−153 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 1.09 (s, 9H), 3.53 (m, 1H), 3.88 (s, 3H), 3.94 (m, 1H), 4.16 (t, *J* = 7.0 Hz, 1H), 4.36 (d, *J* = 7.3 Hz, 2H), 4.47 (m, 1H), 6.00 (br s, 1H), 6.99 (d, *J* = 16.0 Hz, 1H), 7.39−7.16 (m, 9H), 7.44 (t, *J* = 7.4 Hz, 1H), 7.54 (d, *J* = 7.4 Hz, 2H), 7.58 (d, *J* = 7.2 Hz, 1H), 7.65 (d, *J* = 6.4 Hz, 1H), 7.72 (d, *J* = 7.5 Hz, 2H), 7.93−7.81 (m, 3H), 8.56 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.5, 47.3, 52.4, 55.5, 62.1, 67.3, 74.7, 76.9, 77.2, 77.6, 120.2, 124.1, 125.2, 125.3, 125.8, 126.6, 127.2, 127.8, 127.8, 127.9, 128.6, 130.4, 130.8, 131.8, 132.5, 134.6, 139.5, 141.5, 143.9, 156.4, 167.8, 169.1; MS (ES+) *m/z* 641, 642 (MNa^+, 100, 42); HRMS (ES+) *m/z* calcd for $C_{38}H_{39}N_2O_6$ (MH^+) 619.2808, found 619.2816.

2-((*E*)-2-{2-[(*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]phenyl}vinyl)-benzoic Acid (16). To an ice-cold solution of (*E*)-stilbene dipeptide methyl ester **15** (530 mg, 0.857 mmol) in THF (30 mL) was added $LiAlH_4$ (100 mg, 2.64 mmol) and stirring continued under an atmosphere of nitrogen for 20 min. The reaction mixture was then diluted with diethyl ether (50 mL) and cautiously quenched with a saturated solution of Rochelle's salt (50 mL). The organic phase was then separated, washed with H_2O (2 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was dissolved in acetone (30 mL), a solution of 1 M Jones' reagent in acetone (3.00 mL) was added dropwise, and stirring was continued at rt for 2 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo, and the resulting residue was purified by FC (50% v/v, EtOAc/hexane) to afford (*E*)-stilbene dipeptide acid **16** as an off-white solid (207 mg, 40%): mp 159−162 °C; $[\alpha]_D^{27}$ −4.4 (*c* 0.40, EtOAc); ^1H NMR (400 MHz, CD_3OD) δ 1.17 (s, 9H), 3.70 (m, 1H), 3.77 (dd, *J* = 9.0 and 5.1 Hz, 1H), 4.18 (t, *J* = 6.8 Hz, 1H), 4.32 (m, 1H), 4.45−4.36 (m, *J* = 8.4, 2H), 7.17 (d, *J* = 16.0 Hz, 1H), 7.30−7.20 (m, 5H), 7.34 (t, *J* = 7.4 Hz, 4H), 7.42 (m, 1H), 7.61 (d, *J* = 7.3 Hz, 2H), 7.81−7.70 (m, 4H), 7.87 (d, *J* = 7.5 Hz, 1H), 7.93 (d, *J* = 16.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 27.9, 55.0, 57.8, 63.1, 68.2, 75.0, 121.1, 126.3, 127.5, 127.7, 127.9, 128.3, 128.5, 128.7, 128.9, 129.2, 131.2, 131.8, 133.2, 134.5, 135.9, 140.4, 142.7, 145.3, 158.6, 171.4, 172.5; MS (ES+) *m/z* 627, 628 (MNa^+, 100, 39), 605, 606 (MH^+, 82, 33); HRMS (ES+) *m/z* calcd for $C_{37}H_{37}N_2O_6$ (MH^+) 605.2652, found 605.2650.

Resin-Bound Peptide 18 and Linear Peptide 19. Commercially available L-Lys(Boc)-2-chlorotrityl resin (**17**, 240 mg, nominal loading level 0.83 mmol g^{-1}) was swelled in DMF (4 mL) at rt for 1 h. The resin was then subjected automated coupling/deprotection cycles to build up the linear peptide sequence required. The amino acid derivatives employed were Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Ile-OH, Fmoc-L-Phe-OH, Fmoc-L-Leu-OH, Fmoc-L-Asp(^{t}Bu)-OH, and Fmoc-L-Pro-OH. All residues were double coupled using standard

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HOBt/HBTU (5 equiv) coupling cycles at rt for 45 min. Fmoc deprotection was achieved via treatment with 20% piperidine/DMF at rt for 15 min. The resulting resin-bound peptide was then filtered, washed with DMF (3 × 5 mL), CH_2Cl_2 (3 × 5 mL), MeOH (3 × 5 mL), diethyl ether (3 × 5 mL) and dried to afford resin-bound peptide **18** as an orange resin (420 mg, 0.66 mmol g^{-1} by Fmoc test). For analytical purposes, a portion of the resin (3 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was then decanted, concentrated (~100 μL) under a stream of nitrogen, and diluted with diethyl ether (1.5 mL). The resulting precipitate was collected by centrifugation and washed with diethyl ether (3 × 1.5 mL) to afford crude peptide **19** as a white solid. Analysis by reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 11.1 min, purity >95%; MS (MALDI-TOF+) *m/z* 1036, 1037 (MH^+, 100, 63).

Resin-Bound Peptide 20a and Linear Peptides 21a and 22a. 2,2′-Tolan dipeptide **9a** (360 mg, 0.600 mmol), PyBOP (315 mg, 0.605 mmol), and *i*-Pr_2EtN (210 μL, 1.20 mmol) were dissolved in DMF (12 mL) and allowed to stand at rt for 10 min. The solution was then transferred to resin-bound peptide **18** (180 mg, 0.66 mmol g^{-1}) and the suspension shaken at rt for 16 h. The resulting resin was filtered, washed with DMF (3 × 10 mL), and then treated with 20% piperidine/DMF (12 mL) and shaken at rt for 3 h. The resin was again filtered and washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL) to afford resin-bound peptide **20a**. For analytical purposes, a portion of the resin (3 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was then decanted, concentrated (~100 μL) under a stream of nitrogen, and diluted with diethyl ether (1.5 mL). The resulting precipitate was collected by centrifugation and washed with diethyl ether (3 × 1.5 mL) to afford crude peptide **21a** as a white solid. Analysis by reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 12.1 min, purity >95%; MS (MALDI-TOF+) *m/z* 1342, 1343, 1344 (MH^+, 100, 90, 42).

The remaining resin-bound peptide **20a** was suspended in 1% TFA/CH_2Cl_2 (12 mL), shaken at rt for 2 min, and then filtered (repeated five times). The combined filtrate was neutralized with 10% pyridine/MeOH (18 mL) and concentrated under reduced pressure to approximately 5 mL. Water was then added, and the resulting precipitate was collected by centrifugation, washed with H_2O (3 × 10 mL), and lyophilized to afford crude peptide **22a** as a white solid (200 mg): MS (ES+) *m/z* 1806, 1807, 1808, 1809 (MH^+, 70, 79, 46, 20), 903, 904, 904, 905 (MH^+/2, 91, 100, 61, 29).

Resin-Bound Peptide 20b and Linear Peptides 21b and 22b. 2,3′-Tolan dipeptide **9b** (180 mg, 0.299 mmol), PyBOP (156 mg, 0.300 mmol), and *i*-Pr_2EtN (105 μL, 0.601 mmol) were dissolved in DMF (12 mL) and allowed to stand at rt for 10 min. The solution was then transferred to resin-bound peptide **18** (180 mg, 0.66 mmol g^{-1}) and the suspension shaken at rt for 16 h. The resulting resin was filtered, washed with DMF (3 × 10 mL), and then treated with 20% piperidine/DMF (12 mL) and shaken at rt for 3 h. The resin was again filtered and washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL) to afford resin-bound peptide **20b**. For analytical purposes, a portion of the resin (3 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was then decanted, concentrated (~100 μL) under a stream of nitrogen, and diluted with diethyl ether (1.5 mL). The resulting precipitate was collected by centrifugation and washed with diethyl ether (3 × 1.5 mL) to afford crude peptide **21b** as a white solid. Analysis by reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 12.2 min, purity >95%; MS (MALDI-TOF+) *m/z* 1342 (MH^+, 100).

The remaining resin-bound peptide **20b** was suspended in 1% TFA/CH_2Cl_2 (12 mL), shaken at rt for 2 min, then filtered (repeated five times). The combined filtrate were neutralized with 10% pyridine/MeOH (18 mL) and concentrated under reduced pressure to approximately 5 mL. Water was then added and the resulting precipitate was collected by centrifugation, washed with H_2O (3 × 10 mL) and lyophilized to afford crude peptide **22b** as a white solid (122 mg): MS (ES+) *m/z* 1806, 1807 (MH^+, 13, 7), 903, 904, 904, 905 (MH^+/2, 93, 100, 64, 29).

Resin-Bound Peptide 20c and Linear Peptides 21c and 22c. 3,2′-Tolan dipeptide **9c** (240 mg, 0.398 mmol), PyBOP (208 mg, 0.400 mmol), and *i*-Pr_2EtN (140 μL, 0.802 mmol) were dissolved in DMF (12 mL) and allowed to stand at rt for 10 min. The solution was then transferred to resin-bound peptide **18** (180 mg, 0.66 mmol g^{-1}) and the suspension shaken at rt for 16 h. The resulting resin was filtered, washed with DMF (3 × 10 mL), and then treated with 20% piperidine/DMF (12 mL) and shaken at rt for 3 h. The resin was again filtered and washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL) to afford resin-bound peptide **20c**. For analytical purposes, a portion of the resin (3 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was then decanted, concentrated (~100 μL), under a stream of nitrogen and diluted with diethyl ether (1.5 mL). The resulting precipitate was collected by centrifugation and washed with diethyl ether (3 × 1.5 mL) to afford crude peptide **21c** as a white solid. Analysis by reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 13.7 min, purity >95%; MS (MALDI-TOF+) *m/z* 1342 (MH^+, 100).

The remaining resin-bound peptide **20c** was suspended in 1% TFA/CH_2Cl_2 (12 mL), shaken at rt for 2 min, then filtered (repeated five times). The combined filtrate were neutralized with 10% pyridine/MeOH (18 mL) and concentrated under reduced pressure to approximately 5 mL. Water was then added and the resulting precipitate was collected by centrifugation, washed with H_2O (3 × 10 mL), and lyophilized to afford crude peptide **22c** as a white solid (88 mg): MS (ES+) *m/z* 1806, 1807 (MH^+, 26, 22), 903, 904, 904, 905 (MH^+/2, 92, 100, 61, 29).

Resin-Bound Peptide 20d and Linear Peptides 21d and 22d. 3,3′-Tolan dipeptide **9d** (180 mg, 0.299 mmol), PyBOP (156 mg, 0.300 mmol), and *i*-Pr_2EtN (105 μL, 0.601 mmol) were dissolved in DMF (12 mL) and allowed to stand at rt for 10 min. The solution was then transferred to resin-bound peptide **18** (180 mg, 0.66 mmol g^{-1}) and the suspension shaken at rt for 16 h. The resulting resin was filtered, washed with DMF (3 × 10 mL), then treated with 20% piperidine/DMF (12 mL) and shaken at rt for 3 h. The resin was again filtered and washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL) to afford resin-bound peptide **20d**. For analytical purposes, a portion of the resin (3 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was then decanted, concentrated (~100 μL) under a stream of nitrogen and diluted with diethyl ether (1.5 mL). The resulting precipitate was collected by centrifugation and washed with diethyl ether (3 × 1.5 mL) to afford crude peptide **21d** as a white solid. Analysis by reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 13.3 min, purity >95%; MS (MALDI-TOF+) *m/z* 1342 (MH^+, 100).

The remaining resin-bound peptide **20d** was suspended in 1% TFA/CH_2Cl_2 (12 mL), shaken at rt for 2 min, then filtered (repeated five times). The combined filtrate were neutralized with 10% pyridine/MeOH (18 mL) and concentrated under reduced pressure to approximately 5 mL. Water was then added and the resulting precipitate was collected by centrifugation, washed with H_2O (3 × 10 mL) and lyophilized to afford crude peptide **22d** as a white solid (119 mg): MS (ES+) *m/z* 1806, 1807 (MH^+, 19, 17), 903, 904, 904, 905 (MH^+/2, 49, 53, 34, 13), 282 (100).

Resin-Bound Peptide 20e and Linear Peptides 21e and 22e. 2,2′-Bibenzyl dipeptide **12** (162 mg, 0.267 mmol), PyBOP (140 mg, 0.269 mmol) and *i*-Pr_2EtN (92 μL, 0.527 mmol) were dissolved in DMF (12 mL) and allowed to stand at rt for 10 min. The solution was then transferred to resin-bound peptide **18** (180 mg, 0.66 mmol g^{-1}) and the suspension shaken at rt for 16 h. The resulting resin was filtered, washed with DMF (3 × 10 mL), and then treated with 20% piperidine/DMF (12 mL) and shaken at rt for 3 h. The resin was again filtered and washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL) to afford resin-bound peptide **20e**. For analytical purposes, a portion of the resin (3 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 3 h. The

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acidic solution was then decanted, concentrated (~100 μL) under a stream of nitrogen and diluted with diethyl ether (1.5 mL). The resulting precipitate was collected by centrifugation and washed with diethyl ether (3 × 1.5 mL) to afford crude peptide **21e** as a white solid. Analysis by reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 11.9 min, purity >95%; MS (MALDI-TOF+) m/z 1346 (MH^+, 100).

The remaining resin-bound peptide **20e** was suspended in 1% TFA/CH_2Cl_2 (12 mL), shaken at rt for 2 min, then filtered (repeated 5 times). The combined filtrate were neutralized with 10% pyridine/MeOH (18 mL) and concentrated under reduced pressure to approximately 5 mL. Water was then added and the resulting precipitate was collected by centrifugation, washed with H_2O (3 × 10 mL), and lyophilized to afford crude peptide **22e** as a white solid (110 mg): MS (ES+) m/z 905, 906, 907, 907 (MH^+/2, 79, 90, 55, 25), 338 (100).

Resin-Bound Peptide 20f and Linear Peptides 21f and 22f. 2,2′-(*Z*)-Stilbene dipeptide **13** (98 mg, 0.162 mmol), PyBOP (80 mg, 0.154 mmol), and *i*-Pr_2EtN (53 μL, 0.303 mmol) were dissolved in DMF (6 mL) and allowed to stand at rt for 10 min. The solution was then transferred to resin-bound peptide **18** (90 mg, 0.66 mmol g^{-1}) and the suspension shaken at rt for 16 h. The resulting resin was filtered, washed with DMF (3 × 10 mL), and then treated with 20% piperidine/DMF (8 mL) and shaken at rt for 3 h. The resin was again filtered and washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL) to afford resin-bound peptide **20f**. For analytical purposes, a portion of the resin (3 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was then decanted, concentrated (~100 μL) under a stream of nitrogen and diluted with diethyl ether (1.5 mL). The resulting precipitate was collected by centrifugation and washed with diethyl ether (3 × 1.5 mL) to afford crude peptide **21f** as a white solid. Analysis by reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 12.2 min, purity >95%; MS (MALDI-TOF+) m/z 1344 (MH^+, 100).

The remaining resin-bound peptide **20f** was suspended in 1% TFA/CH_2Cl_2 (12 mL), shaken at rt for 2 min, then filtered (repeated 5 times). The combined filtrate were neutralized with 10% pyridine/MeOH (18 mL) and concentrated under reduced pressure to approximately 5 mL. Water was then added and the resulting precipitate was collected by centrifugation, washed with H_2O (3 × 10 mL) and lyophilized to afford crude peptide **22f** as a white solid (76 mg): MS (ES+) m/z 1808, 1809, 1810, 1811 (MH^+, 24, 27, 19, 5), 905, 906, 906 (MH^+/2, 17, 12, 5), 208 (100).

Resin-Bound Peptide 20g and Linear Peptides 21g and 22g. 2,2′-(*E*)-Stilbene dipeptide **16** (100 mg, 0.165 mmol), PyBOP (80 mg, 0.154 mmol), and *i*-Pr_2EtN (53 μL, 0.303 mmol) were dissolved in DMF (6 mL) and allowed to stand at rt for 10 min. The solution was then transferred to resin-bound peptide **18** (90 mg, 0.66 mmol g^{-1}) and the suspension shaken at rt for 16 h. The resulting resin was filtered, washed with DMF (3 × 10 mL), then treated with 20% piperidine/DMF (8 mL) and shaken at rt for 3 h. The resin was again filtered and washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL) to afford resin-bound peptide **20g**. For analytical purposes, a portion of the resin (3 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was then decanted, concentrated (~100 μL) under a stream of nitrogen and diluted with diethyl ether (1.5 mL). The resulting precipitate was collected by centrifugation and washed with diethyl ether (3 × 1.5 mL) to afford crude peptide **21g** as a white solid. Analysis by reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 12.1 min, purity >95%; MS (MALDI-TOF+) m/z 1344 (MH^+, 100).

The remaining resin-bound peptide **20g** was suspended in 1% TFA/CH_2Cl_2 (12 mL), shaken at rt for 2 min, and then filtered (repeated five times). The combined filtrate were neutralized with 10% pyridine/MeOH (18 mL) and concentrated under reduced pressure to approximately 5 mL. Water was then added and the resulting precipitate was collected by centrifugation, washed with H_2O (3 × 10 mL), and lyophilized to afford crude peptide **22g** as a white solid (59 mg): MS (ES+) m/z 1808, 1809, 1810 (MH^+, 23, 32, 12), 905, 905, 906, 906 (MH^+/2, 96, 100, 71, 34).

Cyclic Peptide 23a. 2,2′-Tolan peptide **22a** (140 mg, 0.078 mmol) and *i*-Pr_2EtN (150 μL, 0.859 mmol) were dissolved in DMF (10 mL). The resulting solution was added via syringe pump (0.5 mL h^{-1}) into a stirred solution of HATU (45 mg, 0.118 mmol) in DMF (10 mL). Stirring was continued at rt under an atmosphere on nitrogen for 32 h. Additional HATU (3 × 45 mg, 0.355 mmol) was added at 6 h intervals. The reaction mixture was then concentrated (~2 mL) under a stream of nitrogen and then diluted with H_2O (30 mL). The resulting precipitate was collected by centrifugation, washed with H_2O (3 × 30 mL), and lyophilized to afford a white solid (153 mg). The solid was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 5 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was concentrated (~1 mL) under a stream of nitrogen then diluted with diethyl ether (10 mL). The resulting precipitate was collected by centrifugation, washed with diethyl ether (3 × 10 mL), and dried. Purification by preparative reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v) afforded cyclic peptide **23a** as a white solid after lyophilization (38 mg, 28%): t_R 13.7 min; MS (ES+) m/z 1324, 1325 (MH^+, 32, 26), 662, 663, 663 (MH^+/2, 100, 82, 34).

Cyclic Peptide 23b. 2,3′-Tolan peptide **22b** (120 mg, 0.066 mmol) and *i*-Pr_2EtN (130 μL, 0.744 mmol) were dissolved in DMF (10 mL). The resulting solution was added via syringe pump (0.5 mL h^{-1}) into a stirred solution of HATU (39 mg, 0.103 mmol) in DMF (10 mL). Stirring was continued at rt under an atmosphere on nitrogen for 32 h. Additional HATU (3 × 39 mg, 0.308 mmol) was added at 6 h intervals. The reaction mixture was then concentrated (~2 mL) under a stream of nitrogen and then diluted with H_2O (30 mL). The resulting precipitate was collected by centrifugation, washed with H_2O (3 × 30 mL), and lyophilized to afford a white solid (96 mg). The solid was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 5 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was concentrated (~1 mL) under a stream of nitrogen and then diluted with diethyl ether (10 mL). The resulting precipitate was collected by centrifugation, washed with diethyl ether (3 × 10 mL) and dried. Purification by preparative reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v) afforded cyclic peptide **23b** as a white solid after lyophilization (29 mg, 33%): t_R 13.4 min; MS (ES+) m/z 1324, 1325 (MH^+, 25, 20), 662, 663, 663 (MH^+/2, 100, 83, 37).

Cyclic Peptide 23c. 3,2′-Tolan peptide **22c** (86 mg, 0.048 mmol) and *i*-Pr_2EtN (92 μL, 0.527 mmol) were dissolved in DMF (10 mL). The resulting solution was added via syringe pump (0.5 mL h^{-1}) into a stirred solution of HATU (28 mg, 0.074 mmol) in DMF (10 mL). Stirring was continued at rt under an atmosphere on nitrogen for 32 h. Additional HATU (3 × 28 mg, 0.221 mmol) was added at 6 h intervals. The reaction mixture was then concentrated (~2 mL) under a stream of nitrogen then diluted with H_2O (30 mL). The resulting precipitate was collected by centrifugation, washed with H_2O (3 × 30 mL), and lyophilized to afford a white solid (64 mg). The solid was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 5 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was concentrated (~1 mL) under a stream of nitrogen then diluted with diethyl ether (10 mL). The resulting precipitate was collected by centrifugation, washed with diethyl ether (3 × 10 mL), and dried. Purification by preparative reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v) afforded cyclic peptide **23c** as a white solid after lyophilization (12 mg, 19%): t_R 13.9 min; MS (ES+) m/z 1324, 1325 (MH^+, 47, 38), 662, 663, 663 (MH^+/2, 100, 83).

Cyclic Peptide 23d. 3,3′-Tolan peptide **22d** (110 mg, 0.061 mmol) and *i*-Pr_2EtN (117 μL, 0.670 mmol) were dissolved in DMF (10 mL). The resulting solution was added via syringe pump (0.5 mL h^{-1}) into a stirred solution of HATU (35 mg, 0.092 mmol) in DMF (10 mL). Stirring was continued at rt under an atmosphere on nitrogen for 32 h. Additional HATU (3 × 35 mg, 0.276 mmol) was added at 6 h intervals. The reaction mixture was then concentrated (~2 mL) under a stream of nitrogen then diluted with H_2O (30 mL). The resulting precipitate was collected by centrifugation, washed with

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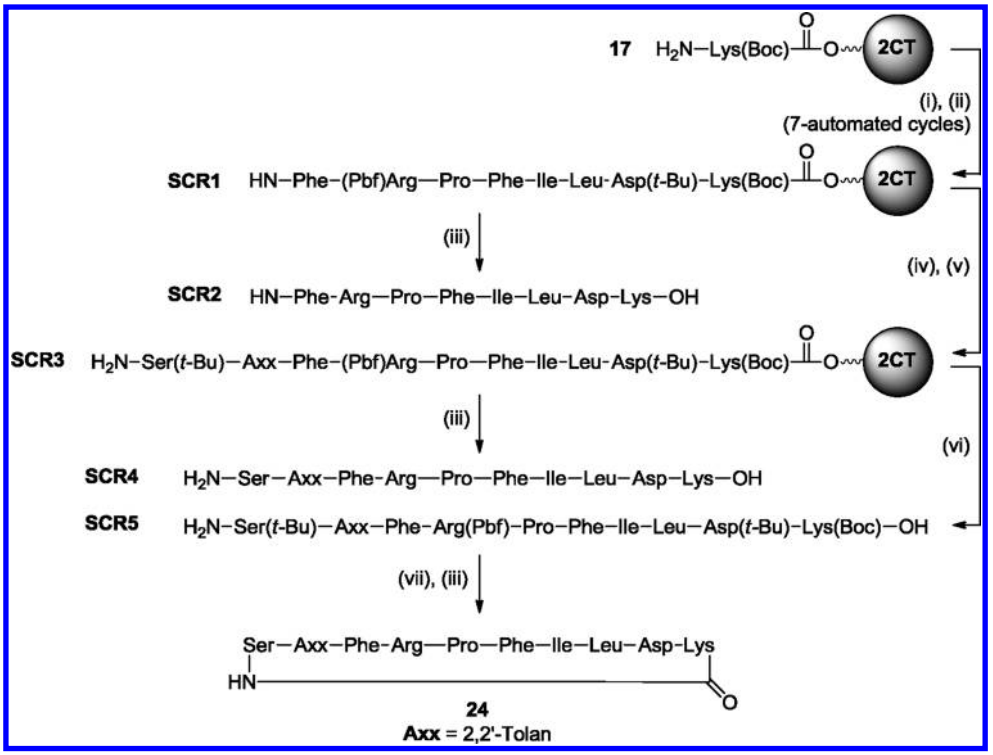
Scheme 8. Solid-Phase Synthesis and Solution-Phase Cyclization of Scrambled Control Peptidomimetic 24[a]



[a]Reagents and conditions: (i) protected amino acid, HOBt/HBTU, *i*-Pr_2EtN, DMF; (ii) 20% piperidine/DMF; (iii) TFA/*i*-Pr_3SiH/H_2O (95:2.5:2.5); (iv) PyBOP, tolan **9a**, *i*-Pr_2EtN, DMF; (v) 20% piperidine/DMF; (vi) 1% TFA/CH_2Cl_2; (vii) HATU, *i*-Pr_2EtN, DMF.

H_2O (3 × 30 mL), and lyophilized to afford a white solid (96 mg). The solid was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 5 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was concentrated (~1 mL) under a stream of nitrogen and then diluted with diethyl ether (10 mL). The resulting precipitate was collected by centrifugation, washed with diethyl ether (3 × 10 mL), and dried. Purification by preparative reversed-phase HPLC (gradient 2–98% MeCN/H_2O v/v with 0.1% TFA v/v) afforded cyclic peptide **23d** as a white solid after lyophilization (22 mg, 27%): t_R 14.5 min; MS (ES+) *m/z* 1324, 1325, 1326 (MH^+, 65, 55, 22), 662, 663, 663 (MH^+/2, 68, 56, 25), 282 (100).

Cyclic Peptide 23e. 2,2′-Bibenzyl peptide **22e** (100 mg, 0.055 mmol) and *i*-Pr_2EtN (106 μL, 0.607 mmol) were dissolved in DMF (10 mL). The resulting solution was added via syringe pump (0.5 mL h^{-1}) into a stirred solution of HATU (32 mg, 0.084 mmol) in DMF (10 mL). Stirring was continued at rt under an atmosphere on nitrogen for 32 h. Additional HATU (3 × 32 mg, 0.252 mmol) was added at 6 h intervals. The reaction mixture was then concentrated (~2 mL) under a stream of nitrogen and then diluted with H_2O (30 mL). The resulting precipitate was collected by centrifugation, washed with H_2O (3 × 30 mL), and lyophilized to afford a white solid (108 mg). The solid was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 5 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was concentrated (~1 mL) under a stream of nitrogen then diluted with diethyl ether (10 mL). The resulting precipitate was collected by centrifugation, washed with diethyl ether (3 × 10 mL), and dried. Purification by preparative reversed-phase HPLC (gradient 2–98% MeCN/H_2O v/v with 0.1% TFA v/v) afforded cyclic peptide **23e** as a white solid after lyophilization (30 mg, 41%): t_R 13.4 min; MS (ES+) *m/z* 1328, 1329, 1330 (MH^+, 55, 42, 18), 664, 665, 665 (MH^+/2, 78, 64, 27), 282 (100).

Cyclic Peptide 23f. 2,2′-(*Z*)-Stilbene peptide **22f** (76 mg, 0.042 mmol) and *i*-Pr_2EtN (85 μL, 0.487 mmol) were dissolved in DMF (8 mL). The resulting solution was added via syringe pump (0.5 mL h^{-1}) into a stirred solution of HATU (25 mg, 0.066 mmol) in DMF (8 mL). Stirring was continued at rt under an atmosphere on nitrogen for 32 h. Additional HATU (3 × 25 mg, 0.197 mmol) was added at 6 h intervals. The reaction mixture was then concentrated (~2 mL) under a stream of nitrogen and diluted with H_2O (20 mL). The resulting precipitate was collected by centrifugation, washed with H_2O (3 × 20 mL), and lyophilized to afford a white solid (58 mg). The solid was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 4 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was concentrated (~1 mL) under a stream of nitrogen then diluted with diethyl ether (10 mL). The resulting precipitate was collected by centrifugation, washed with diethyl ether (3 × 10 mL), and dried. Purification by preparative reversed-phase HPLC (gradient 2–98% MeCN/H_2O v/v with 0.1% TFA v/v) afforded cyclic peptide **23f** as a white solid after lyophilization (13 mg, 24%): t_R 13.2 min; MS (ES+) *m/z* 1326 (MH^+, 7), 663, 664, 664 (MH^+/2, 100, 79, 38).

Cyclic Peptide 23g. 2,2′-(*E*)-Stilbene peptide **22g** (58 mg, 0.032 mmol) and *i*-Pr_2EtN (70 μL, 0.401 mmol) were dissolved in DMF (7 mL). The resulting solution was added via syringe pump (0.5 mL h^{-1}) into a stirred solution of HATU (20 mg, 0.053 mmol) in DMF (7 mL). Stirring was continued at rt under an atmosphere on nitrogen for 32 h. Additional HATU (3 × 20 mg, 0.158 mmol) was added at 6 h intervals. The reaction mixture was then concentrated (~2 mL) under a stream of nitrogen then diluted with H_2O (20 mL). The resulting precipitate was collected by centrifugation, washed with H_2O (3 × 20 mL) and lyophilized to afford a white solid (52 mg). The solid was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 4 mL) and allowed to stand with occasional swirling at rt for 3 h. The acidic solution was concentrated (~1 mL) under a stream of nitrogen and then diluted with diethyl ether (10 mL). The resulting precipitate was collected by centrifugation, washed with diethyl ether (3 × 10 mL), and dried. Purification by preparative reversed-phase HPLC (gradient 2–98% MeCN/H_2O v/v with 0.1% TFA v/v) afforded cyclic peptide **23g** as a white solid after lyophilization (15 mg, 35%): t_R 13.1 min; MS (ES+) *m/z* 1326 (MH^+, 5), 663, 664, 664, 665 (MH^+/2, 100, 86, 43, 16).

Synthetic Procedures for Preparation of Control Peptidomimetics (Scheme 8). Resin-Bound Peptide SCR1 and Linear Peptide SCR2. Commercially available L-Lys(Boc)-2-chlorotrityl resin (**17**, 200 mg, nominal loading level 1.0 mmol g^{-1}) was swelled

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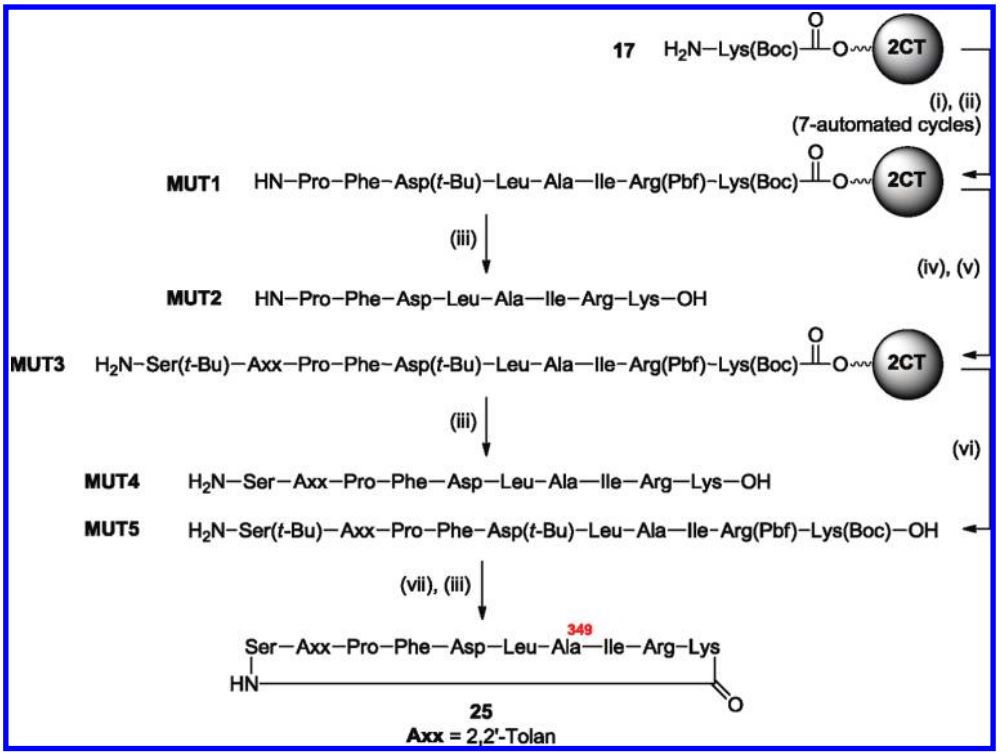
Scheme 9. Solid-Phase Synthesis and Solution-Phase Cyclization of Phe-349-Ala Mutant Control Peptidomimetic 25[a]



[a]Reagents and conditions: (i) protected amino acid, HOBt/HBTU, *i*-Pr_2EtN, DMF; (ii) 20% piperidine/DMF; (iii) TFA/*i*-Pr_3SiH/H_2O (95:2.5:2.5); (iv) PyBOP, tolan **9a**, *i*-Pr_2EtN, DMF; (v) 20% piperidine/DMF; (vi) 1% TFA/CH_2Cl_2; (vii) HATU, *i*-Pr_2EtN, DMF.

in DMF (4 mL) at rt for 1 h. The resin was then subjected automated coupling/deprotection cycles to build up the linear peptide sequence required. The amino acid derivatives employed were Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Ile-OH, Fmoc-L-Phe-OH, Fmoc-L-Leu-OH, Fmoc-L-Asp(^{t}Bu)-OH, and Fmoc-L-Pro-OH. All residues were double coupled using standard HOBt/HBTU (5 equiv) coupling cycles at rt for 35 min. Fmoc deprotection was achieved *via* treatment with 20% piperidine/DMF at rt for 15 min. The resulting resin-bound peptide was then filtered, washed with DMF (3 × 5 mL), CH_2Cl_2 (3 × 10 mL), MeOH (3 × 10 mL), diethyl ether (3 × 10 mL) and dried to afford resin-bound peptide **SCR1** as an orange resin. For analytical purposes, a portion of the resin (2 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 1 h. The acidic solution was then decanted and concentrated under a stream of nitrogen to afford crude peptide **SCR2** as an off-white solid. Analysis by reversed-phase HPLC (gradient 5–98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 3.98 min; MS (ES+) *m*/*z* 1036 (MH^+).

Linear Peptides SCR4 and SCR5. 2,2′-Tolan dipeptide **9a** (420 mg, 0.700 mmol), PyBOP (375 mg, 0.720 mmol), and *i*-Pr_2EtN (260 μL, 1.49 mmol) were dissolved in DMF (12 mL) and allowed to stand at rt for 10 min. The solution was then transferred to resin-bound peptide **SCR1** and the suspension shaken at rt for 22 h. The resulting resin was filtered, washed with DMF (3 × 20 mL), treated with 20% piperidine/DMF (20 mL), and shaken at rt for 4 h. The resin was again filtered and washed with DMF (4 × 20 mL) and CH_2Cl_2 (4 × 20 mL) to afford resin-bound peptide **SCR3**. For analytical purposes, a portion of the resin (2 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 1 h. The acidic solution was then decanted and concentrated under a stream of nitrogen to afford crude peptide **SCR4** as an off-white solid. Analysis by reversed-phase HPLC (gradient 5–98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 11.32 min; MS (ES+) *m*/*z* 1342 (MH^+).

The remaining resin-bound peptide **SCR3** was suspended in 1% TFA/CH_2Cl_2 (14 mL), shaken at rt for 2 min, and then filtered (repeated five times). The combined filtrates were neutralized with 10% pyridine/MeOH (5 mL) and concentrated under reduced pressure to approximately 5–6 mL. Water (25 mL) was then added, and the resulting precipitate was collected by centrifugation, washed with H_2O (3 × 15 mL) and lyophilized to afford crude peptide **SCR5** as a white solid (293 mg): MS (ES+) *m*/*z* 1806, 1807, 1808, 1809 (MH^+, 43, 46, 29, 13), 903, 904, 904, 905 (MH^+/2, 85, 100, 65, 30).

Cyclic Peptide 24. 2,2′-Tolan peptide **SCR5** (200 mg, 0.11 mmol) and *i*-Pr_2EtN (70 μL, 0.40 mmol) were dissolved in DMF (15 mL). The resulting solution was added via syringe pump (0.5 mL h^{-1}) into a stirred solution of HATU (65 mg, 0.17 mmol) and *i*-Pr_2EtN (150 μL, 0.86 mmol) in DMF (15 mL). Stirring was continued at rt under an atmosphere on nitrogen for 45 h. Additional HATU (3 × 65 mg, 0.51 mmol) was added at 8 h intervals. The reaction mixture was then quenched with H_2O (2 mL), concentrated (~2.5–3 mL) under a stream of nitrogen, and diluted with H_2O (35 mL). The resulting precipitate was collected by centrifugation, washed with H_2O (3 × 40 mL), and lyophilized to afford a cream colored solid (172 mg). The solid was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 7.5 mL) and allowed to stand with occasional swirling at rt for 4 h. The acidic solution was concentrated (~1.5 mL) under a stream of nitrogen then diluted with diethyl ether (15 mL). The resulting precipitate was collected by centrifugation, washed with diethyl ether (3 × 15 mL), and dried. Purification by preparative reversed-phase HPLC (gradient 2–98% MeCN/H_2O v/v with 0.1% TFA v/v) afforded cyclic peptide **24** as a white solid after lyophilization (8 mg, 6%) (the yield of this peptide was compromised by its precipitation on the HPLC column): t_R 12.25 min; MS (ES+) *m*/*z* 1324, 1325, 1326 (MH^+, 45, 38, 15), 662, 663, 663, 664 (MH^+/2, 100, 83, 36, 13).

Resin-bound Peptide MUT1 and Linear Peptide MUT2 (Scheme 9). Commercially available L-Lys(Boc)-2-chlorotrityl resin (**17**, 200 mg, nominal loading level 1.0 mmol g^{-1}) was swelled in DMF (4 mL) at rt for 1 h. The resin was then subjected automated coupling/deprotection cycles to build up the linear peptide sequence required. The amino acid derivatives employed were Fmoc-L-Ala-OH, Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Ile-OH, Fmoc-L-Phe-OH, Fmoc-L-Leu-

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OH, Fmoc-L-Asp(^{t}Bu)-OH, and Fmoc-L-Pro-OH. All residues were double coupled using standard HOBt/HBTU (5 equiv) coupling cycles at rt for 35 min. Fmoc deprotection was achieved via treatment with 20% piperidine/DMF at rt for 15 min. The resulting resin-bound peptide was then filtered, washed with DMF (3 × 5 mL), CH_2Cl_2 (3 × 10 mL), MeOH (3 × 10 mL), and diethyl ether (3 × 10 mL), and dried to afford resin-bound peptide **MUT1** as an orange resin. For analytical purposes, a portion of the resin (2 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 1 h. The acidic solution was then decanted and diluted with *tert*-butylmethyl ether (1.0 mL). The resulting precipitate was collected by centrifugation and washed with *tert*-butylmethyl ether (3 × 1.5 mL) to afford crude peptide **MUT2** as an off-white solid. Analysis by reversed-phase HPLC (gradient 5−98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 9.76 min; MS (ES+) m/z 960, 961 (MH^+, 100, 53).

Linear Peptides MUT4 and MUT5. 2,2′-Tolan dipeptide **9a** (420 mg, 0.700 mmol), PyBOP (375 mg, 0.720 mmol), and *i*-Pr_2EtN (260 μL, 1.49 mmol) were dissolved in DMF (12 mL) and allowed to stand at rt for 10 min. The solution was then transferred to resin-bound peptide **MUT1** and the suspension shaken at rt for 22 h. The resulting resin was filtered, washed with DMF (3 × 20 mL), treated with 20% piperidine/DMF (20 mL), and shaken at rt for 4 h. The resin was again filtered, washed with DMF (4 × 20 mL) and CH_2Cl_2 (4 × 20 mL) to afford resin-bound peptide **MUT3**. For analytical purposes, a portion of the resin (2 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 1 mL) and allowed to stand with occasional swirling at rt for 1 h. The acidic solution was then decanted and concentrated under a stream of nitrogen to afford crude peptide **MUT4** as an off-white solid. Analysis by reversed-phase HPLC (gradient 5−98% MeCN/H_2O v/v with 0.1% TFA v/v), t_R 10.74 min; MS (ES+) m/z 1266 (MH^+).

The remaining resin-bound peptide **MUT3** was suspended in 1% TFA/CH_2Cl_2 (14 mL), shaken at rt for 2 min, and then filtered (repeated five times). The combined filtrate were neutralized with 10% pyridine/MeOH (5 mL) and concentrated under reduced pressure to approximately 5−6 mL. Water (25 mL) was then added and the resulting precipitate was collected by centrifugation, washed with H_2O (3 × 15 mL) and lyophilized to afford crude peptide **MUT5** as a white solid (283 mg): MS (ES+) m/z 1730, 1731, 1732 (MH^+, 49, 53, 29), 865, 866, 866, 867 ($MH^+/2$, 94, 100, 61, 28).

Cyclic Peptide 25. 2,2′-Tolan peptide **MUT5** (190 mg, 0.11 mmol) and *i*-Pr_2EtN (70 μL, 0.40 mmol) were dissolved in DMF (15 mL). The resulting solution was added via syringe pump (0.5 mL h^{-1}) into a stirred solution of HATU (65 mg, 0.17 mmol) and *i*-Pr_2EtN (150 μL, 0.859 mmol) in DMF (15 mL). Stirring was continued at rt under an atmosphere on nitrogen for 45 h. Additional HATU (3 × 65 mg, 0.51 mmol) was added at 8 h intervals. The reaction mixture was then quenched with H_2O (2 mL) and concentrated (∼2.5−3 mL) under a stream of nitrogen then diluted with H_2O (35 mL). The resulting precipitate was collected by centrifugation, washed with H_2O (3 × 40 mL) and lyophilized to afford a cream colored solid (180 mg). The solid was treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 7.5 mL) and allowed to stand with occasional swirling at rt for 4 h. The acidic solution was concentrated (∼1.5 mL) under a stream of nitrogen then diluted with diethyl ether (15 mL). The resulting precipitate was collected by centrifugation, washed with diethyl ether (3 × 15 mL), and dried to afford a cream-colored solid (153 mg). Purification of a smaller sample (45 mg) by preparative reversed-phase HPLC (gradient 2−98% MeCN/H_2O v/v with 0.1% TFA v/v) afforded cyclic peptide **25** as a white solid after lyophilization (15 mg, 38%): t_R 11.02 min; MS (ES+) m/z 1248, 1249 (MH^+, 26, 19), 624, 625, 625, 626 ($MH^+/2$, 100, 75, 31, 9).

■ ASSOCIATED CONTENT

Supporting Information

General experimental directions, ^1H and ^{13}C NMR spectra for compounds **2a**−**16**, CSP-HPLC chromatograms for **9a** and *ent*-**9a**, HPLC chromatograms for all peptides, and ELISA protocol and plots for Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(98) Note: Presta et al. use the Kabat numbering system for IgE residues (Kabat, E. A.; Wu, T. T.; Reid-Miller, M.; Perry, H.; Gottesman, K.S. *Sequences of Proteins of Immunological Interest*; Department of Health and Human Services, Public Health Service, NIH: Washington, DC, 1987; pp 313−331), whereaswe use the Bennich numbering system (Dorrington, K. J.; Bennich, H. H. *Immun. Rev.* **1978**, *41*, 3−25); their Phe-381 corresponds to our Phe-349. Additionally,the importance of Phe-349 for the interaction between hIgE and FcεRIhas been disputed; see: (a) Henry, A. J.; Cook, J. P. D.; McDonnell, J. M.; Mackay, G. A.; Shi, J.; Sutton, B. J.; Gould, H. J. *Biochemistry* **1997**, *36*, 15568−15578. (b) Cook, J. P. D; Henry, A. J.; McDonnell, J. M.; Owens, R. J.; Sutton, B. J.; Gould, H. J. *Biochemistry* **1997**, *36*, 15579−15588. (c) 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.
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■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on March 7, 2012, with errors in Table 1. The corrected version was reposted on March 8, 2012.

Synthesis and Incorporation Into Cyclic Peptides of Tolan Amino Acids and their Hydrogenated Congeners: Construction of an Array of A-B-loop Mimetics of the Cε3 Domain of Human IgE

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General Directions……...**2**

^{1}H and ^{13}C NMR Spectra for all New Small Molecules (**2a**-**16**).....**3-27**

Chiral HPLC Chromatograms for 2,2'tolan dipeptides **9a** and ***ent*-9a**……..……..**28-29**

HPLC Chromatograms for all New Peptides.....**30-41**

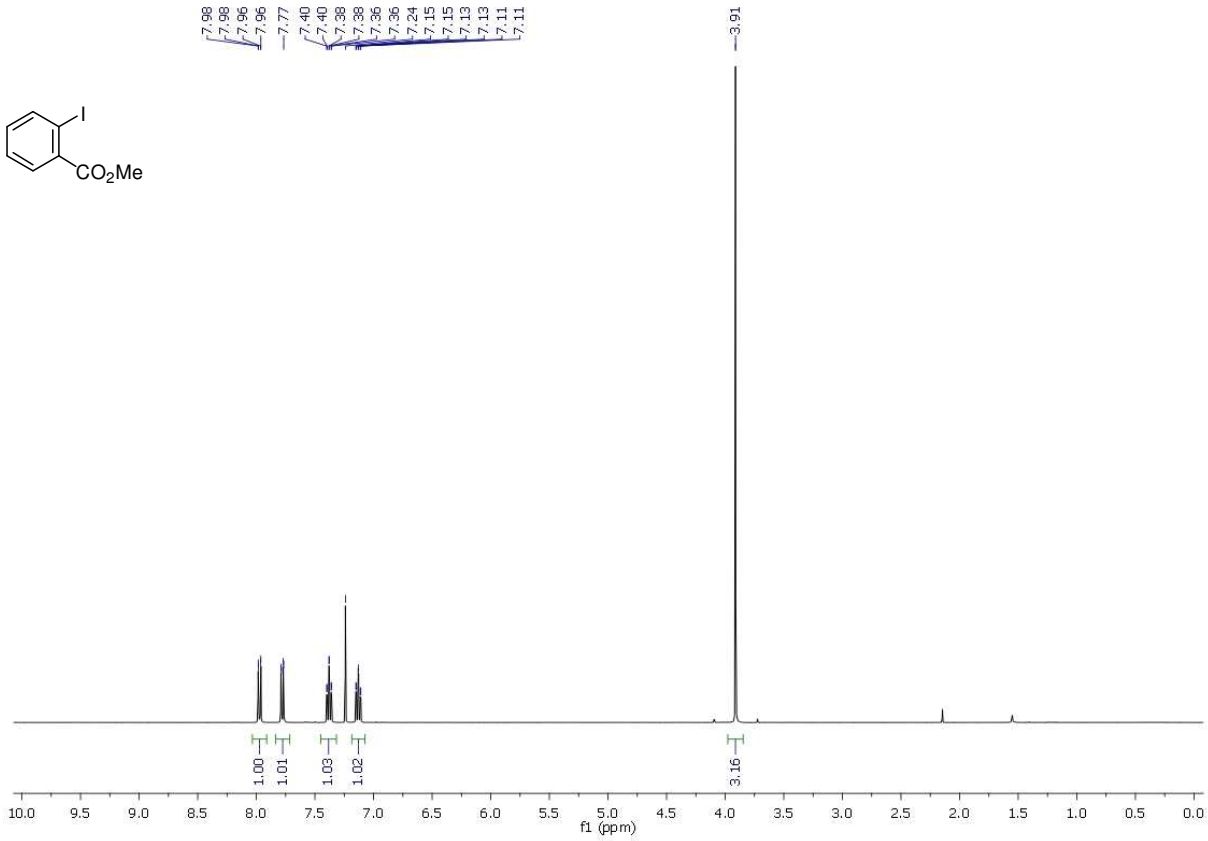
ELISA protocol and Plots……**42-48**

General directions

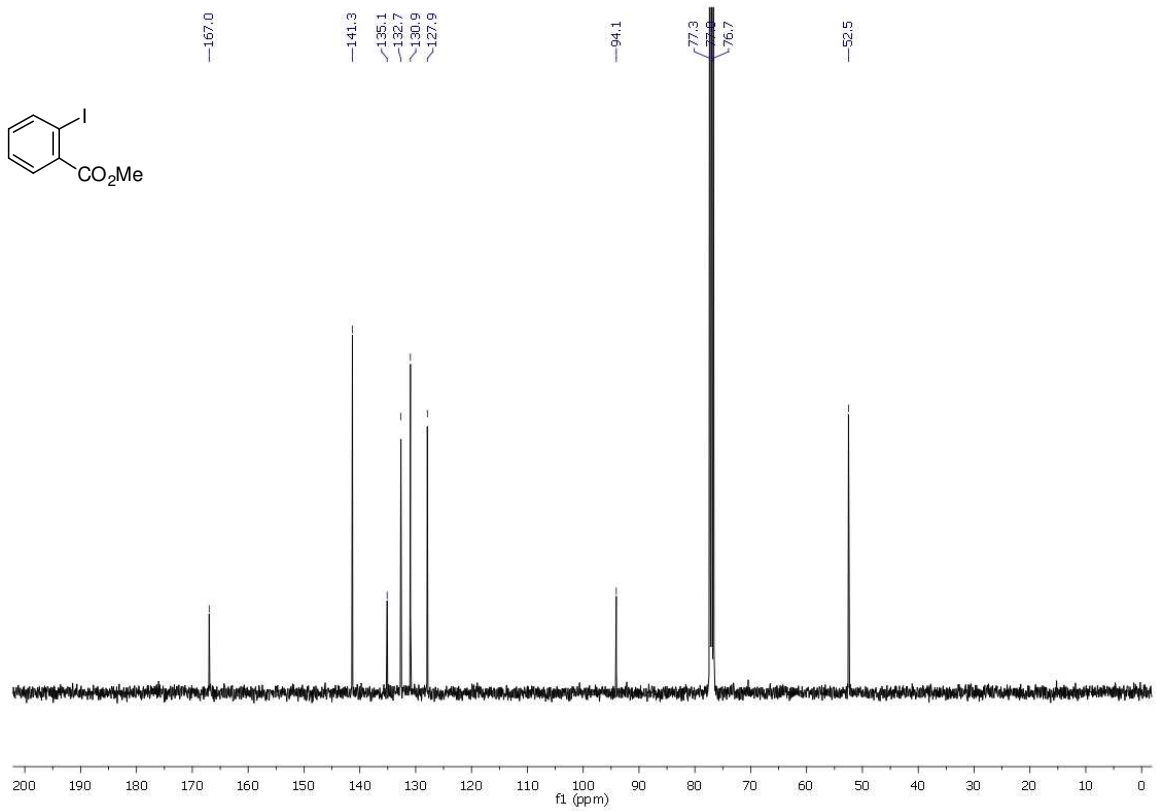
Solvents and reagents: Solvents were distilled as follows: THF and Et_2O over Na-benzophenone ketyl, toluene over Na, CH_2Cl_2 and DMF over CaH_2; HPLC grade EtOAc and petrol were used as commercially supplied. Reagents were used as commercially supplied unless otherwise stated and handled in accordance with COSHH regulations. **Chromatography:** Flash chromatography (FC) was carried out on Silica gel according to the method described by Still.[1] Alumina was grade 1 basic. TLC was performed on aluminium backed silica gel plates which were developed with UV fluorescence (254 nm and 365 nm) and $KMnO_4$(aq)/Δ. **FTIR spectra:** These were recorded on a Fourier transform spectrometer. Only selected absorbances (ν_{max}) are reported. **^{1}H NMR spectra:** These were recorded at 400 MHz or at 500 MHz. Chemical shifts (δ_H) are given in parts per million (ppm) as referenced to the appropriate residual solvent peak. Coupling constants are reported to the nearest 0.1 Hz. Broad signals are assigned as br. **^{13}C NMR spectra:** These were recorded at 100 MHz or at 125 MHz. Chemical shifts (δ_C) are given in parts per million (ppm) as referenced to $CHCl_3$. **Mass Spectra:** Low-resolution and high-resolution mass spectra (*m/z*) of non-peptidic molecules were recorded on a Micromass AutoSpec Premier [This instument is a magnetic secto mass spectrometer and has EI, CI and LCIMS (FAB) ion sources] or Micromass LCT Premier [This instrument has an ESI ion source, coupled to a time-of-flight (ToF) analyser] spectrometers, with intensities quoted as percentages of the base peak, with molecular ions and major peaks being reported. Values are valid to ±5 ppm. Mass identification for peptides and their derivatives was by Matrix-Assisted Laser Desorption/Ionisation Time of Flight (MALDI-TOF) was performed using α-cyano-4-hydroxycinnamic acid as matrix. **HPLC:** Preparative purification of peptides and analytical reverse-phase HPLC was performed on a semi-preparative reverse-phase HPLC system equipped with a UV/Vis detector. Analytical samples were eluted with 2-98% MeCN in H_2O over 30 min. The peptide bond absorption was detected at 223 nm. **Melting points:** Analyses were carried out using a hot stage apparatus and are uncorrected.

NMR Data for all New Compounds

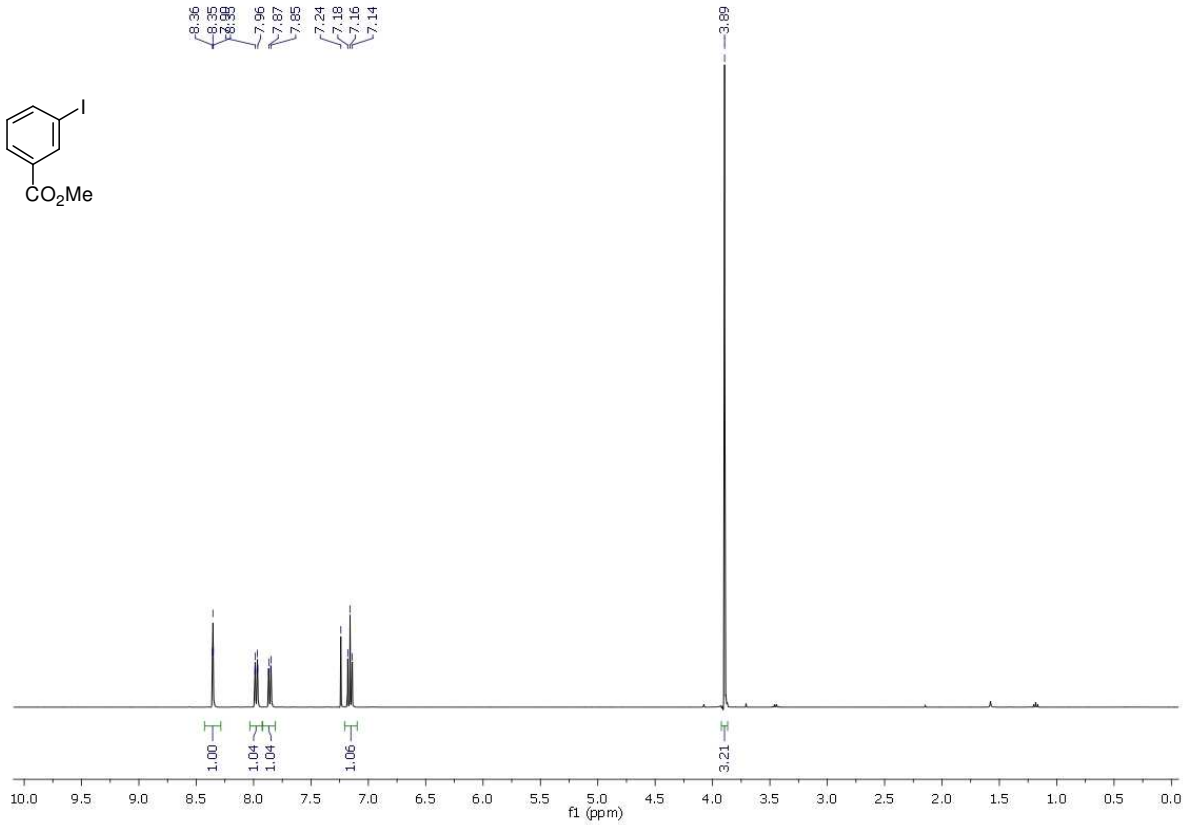
^{1}H NMR (400 MHz, $CDCl_3$): **2a**



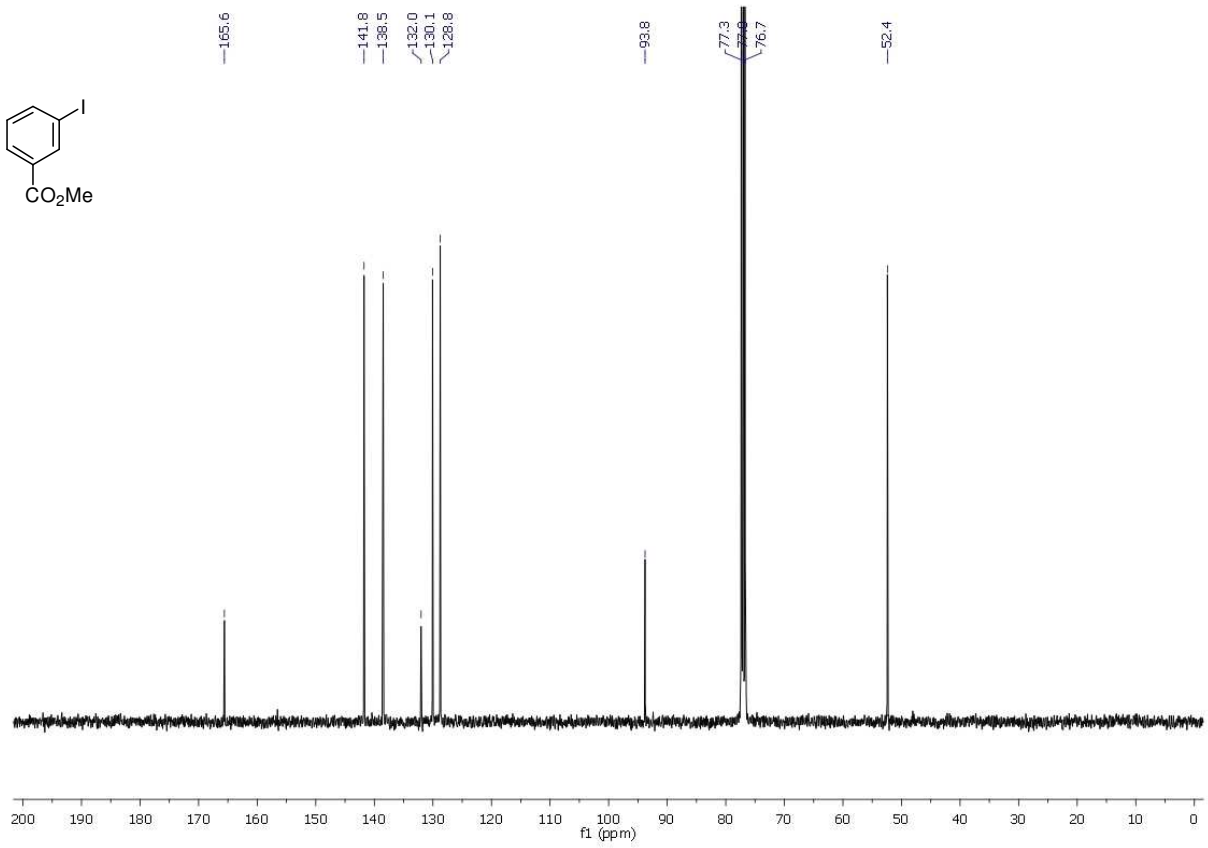
^{13}C NMR (100 MHz, $CDCl_3$): **2a**



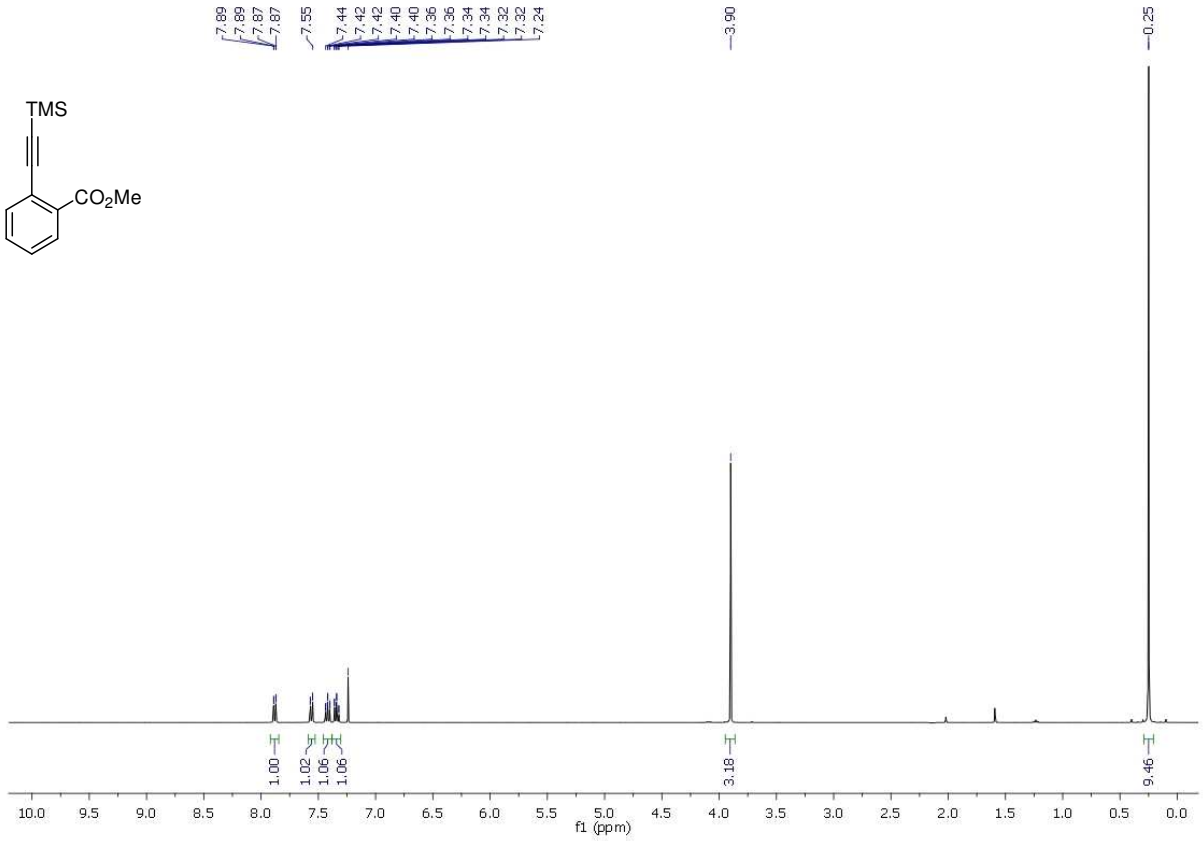
^{1}H NMR (400 MHz, $CDCl_3$): **2b**



^{13}C NMR (100 MHz, $CDCl_3$): **2b**



^{1}H NMR (400 MHz, $CDCl_3$): **3a**



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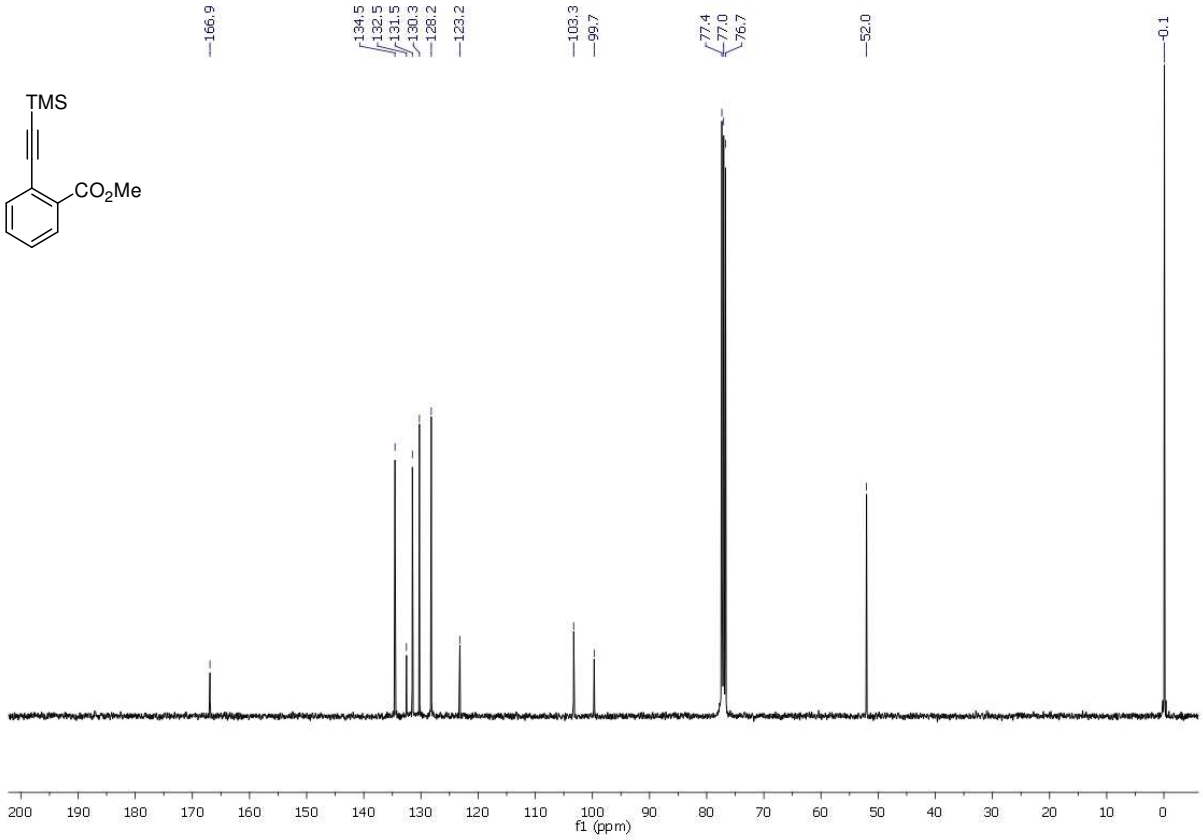
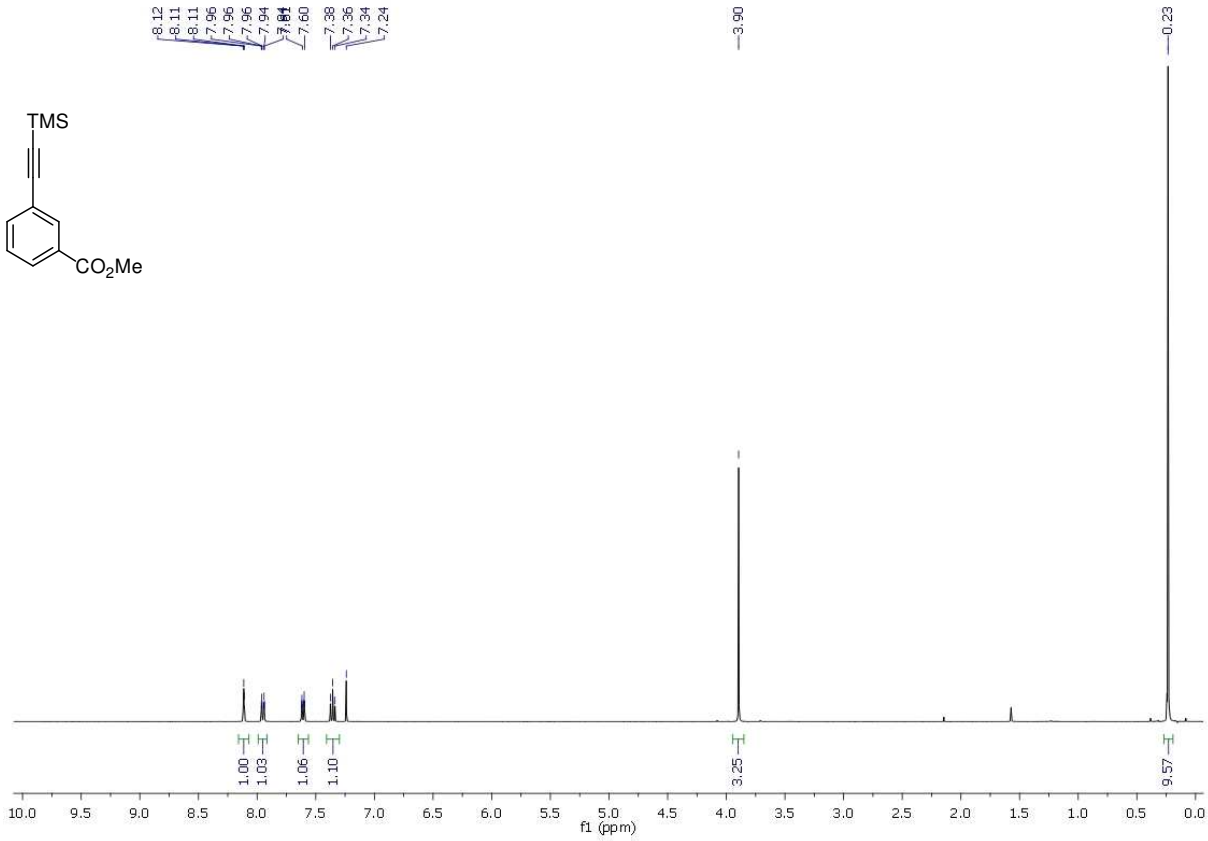


^{1}H NMR (400 MHz, $CDCl_3$): **3b**



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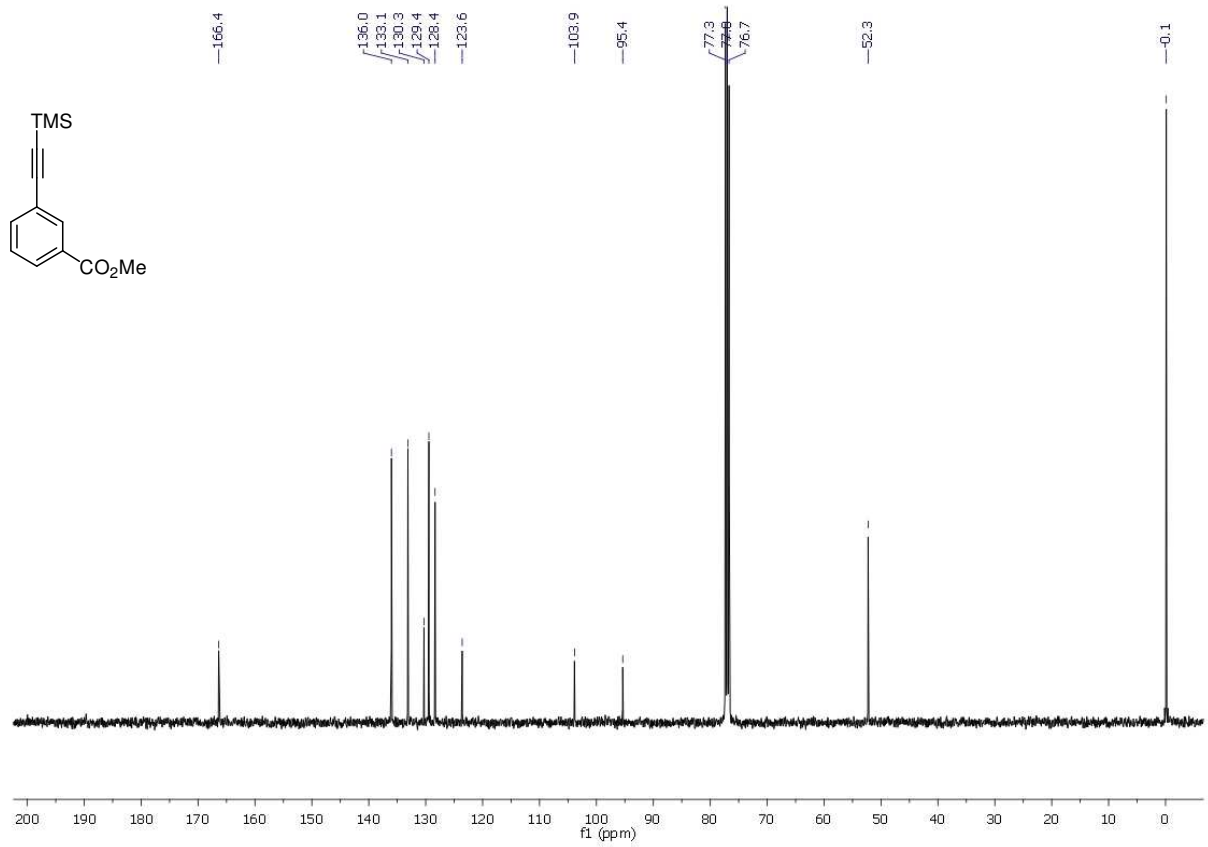
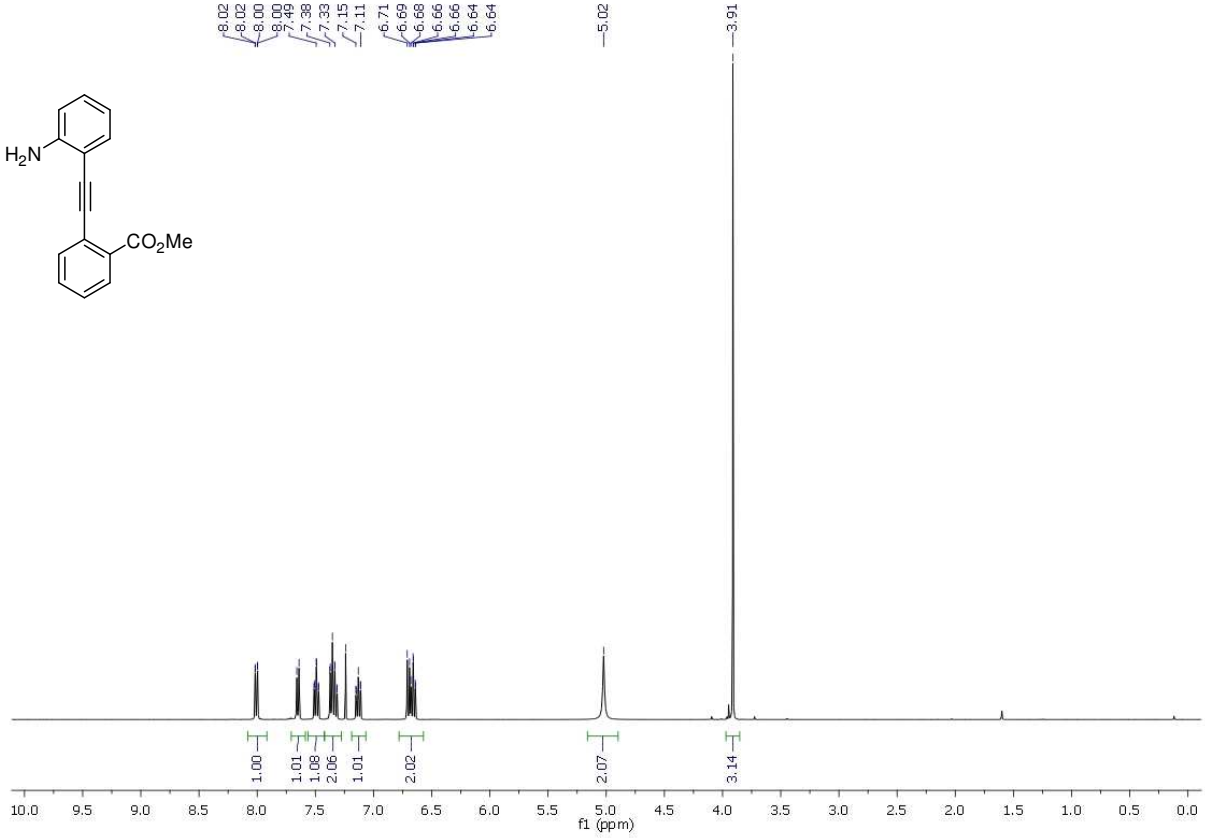
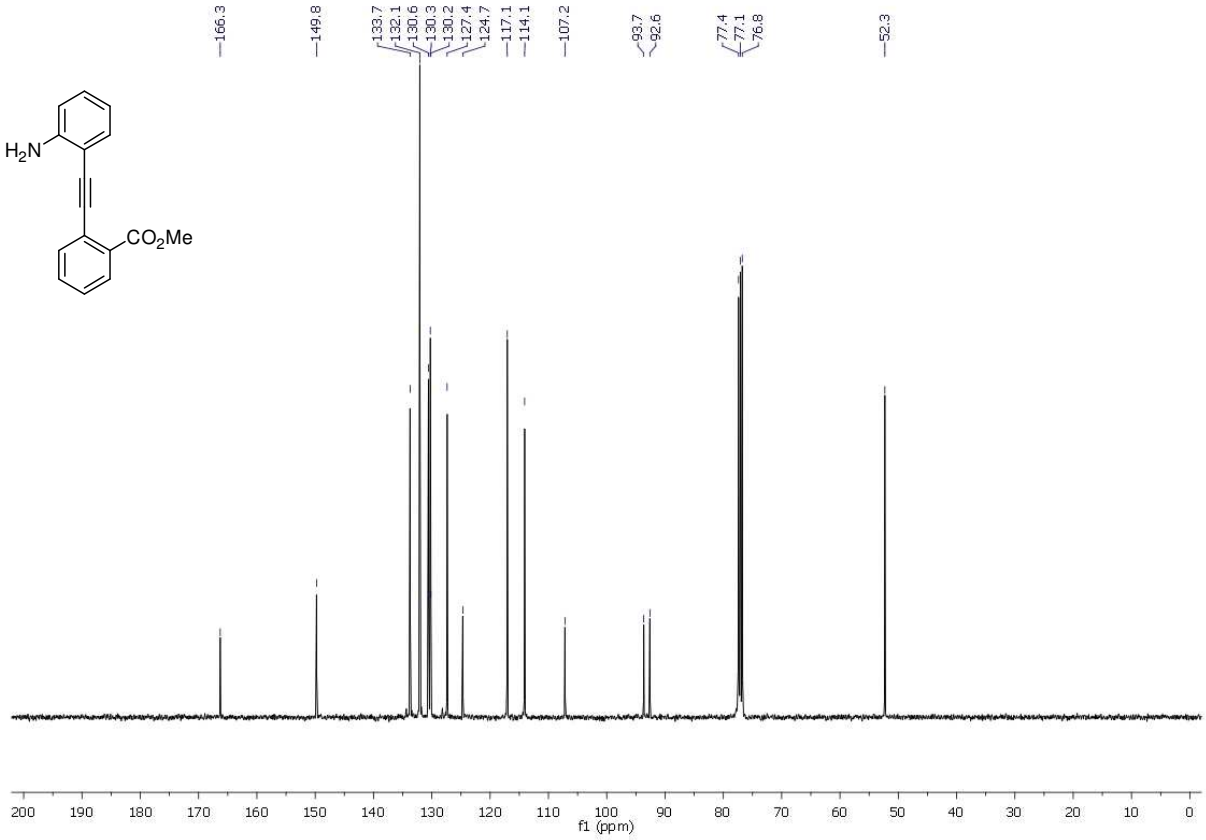


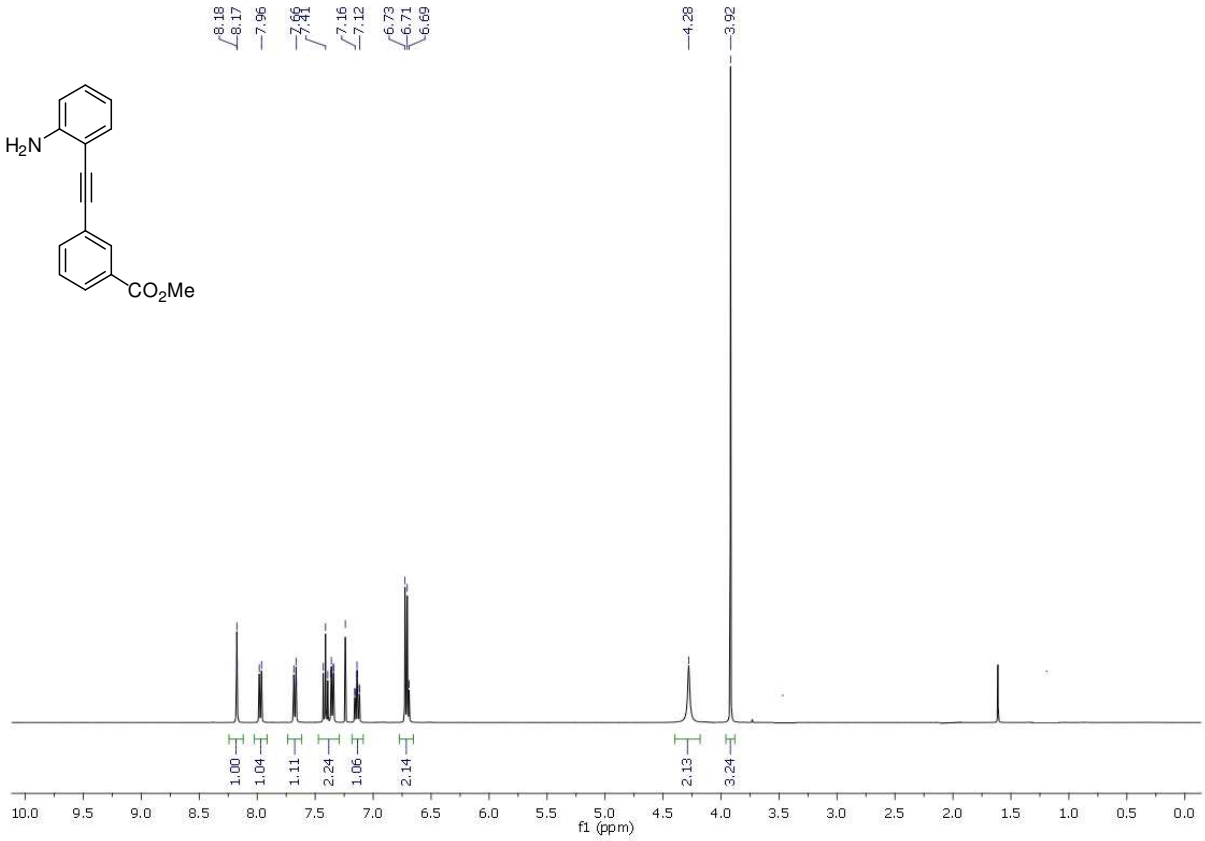
^{1}H NMR (400 MHz, $CDCl_3$): **5a**



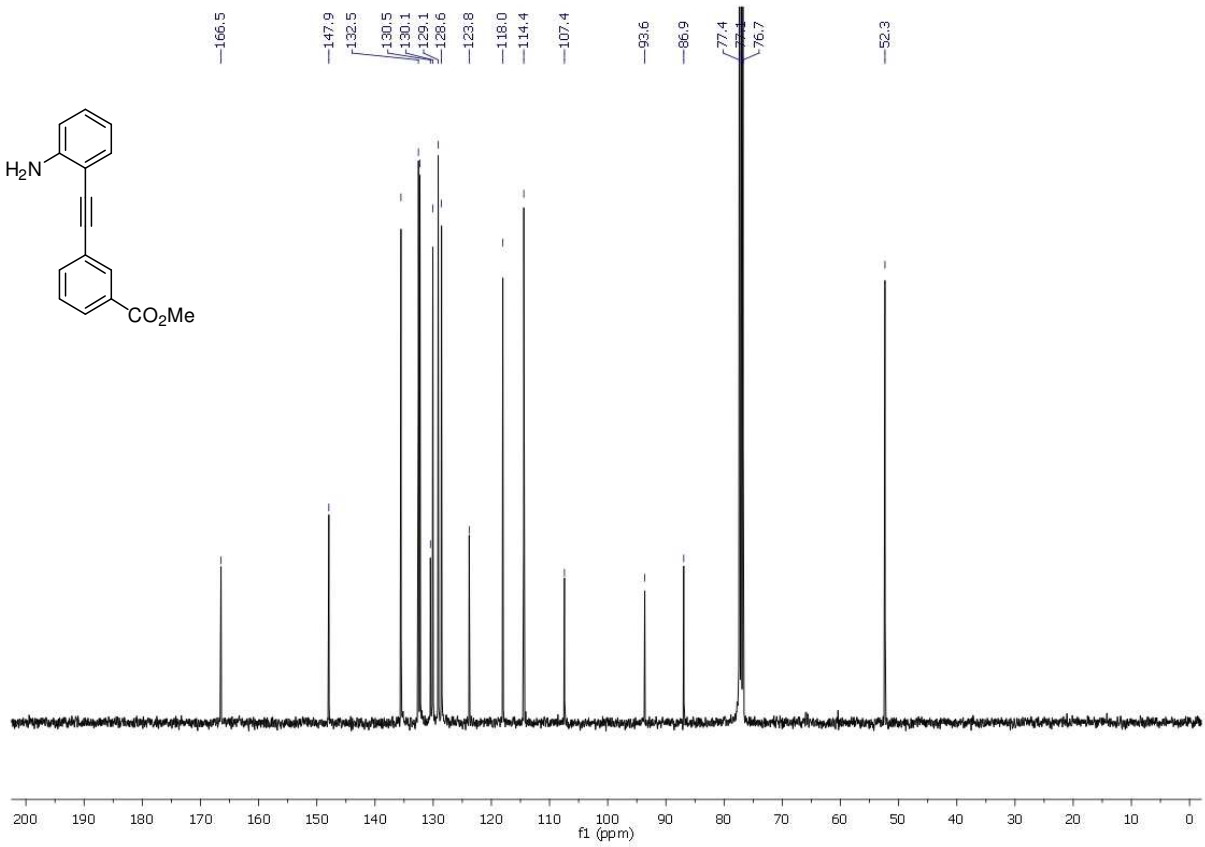
^{13}C NMR (100 MHz, $CDCl_3$): **5a**



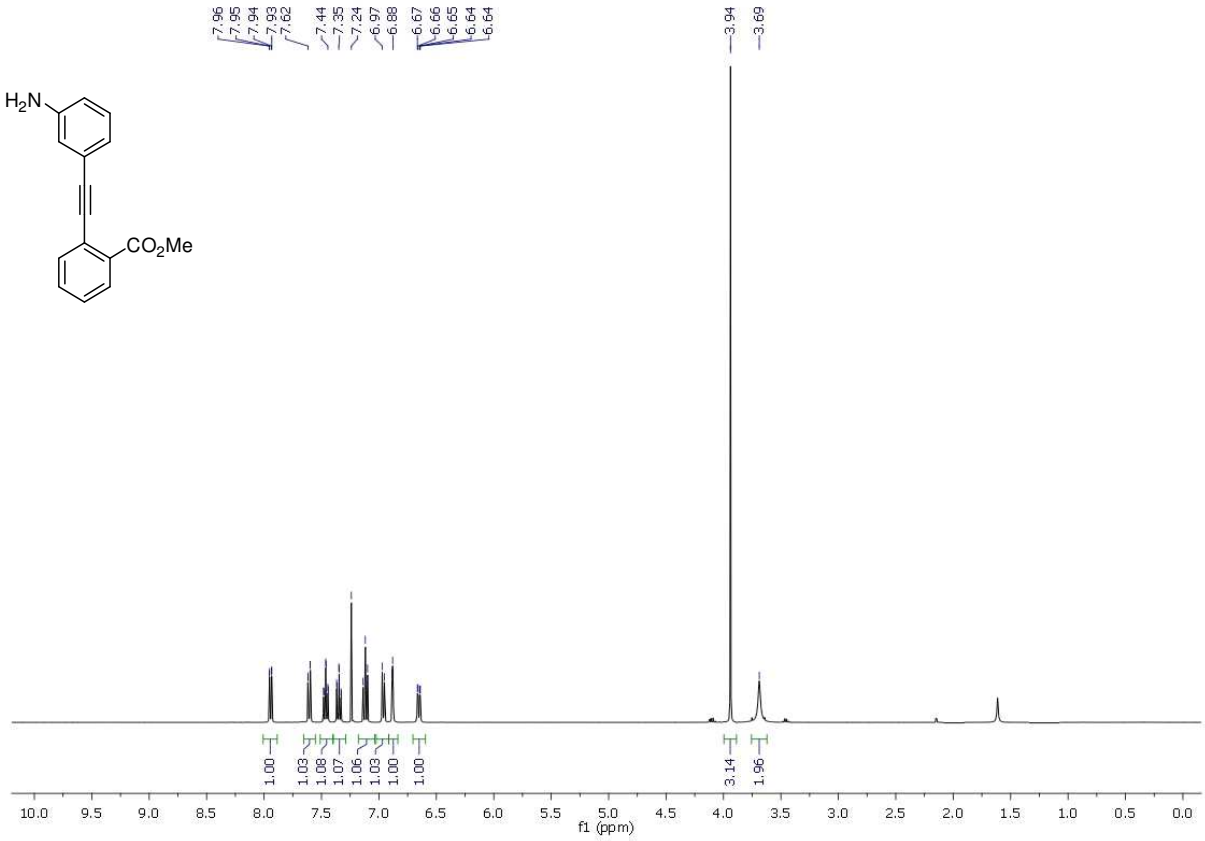
^{1}H NMR (400 MHz, $CDCl_3$): **5b**



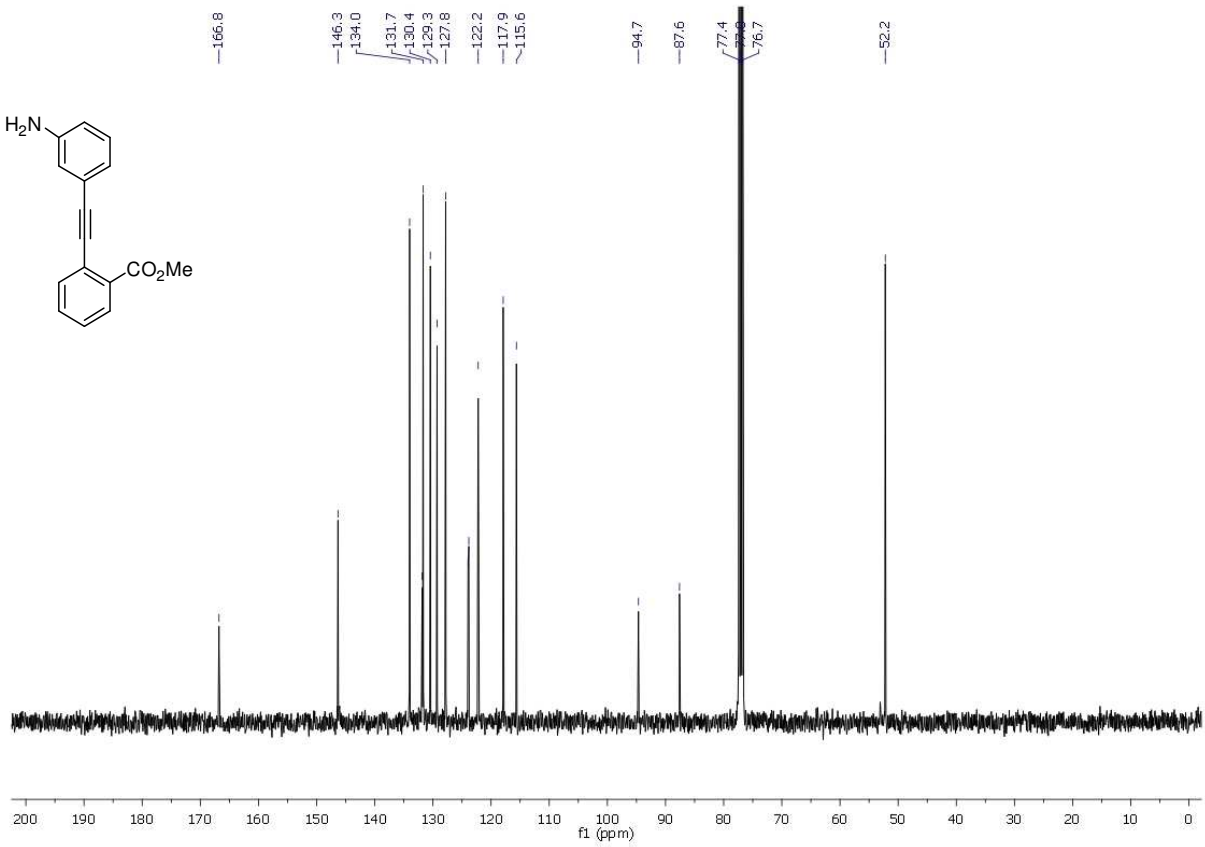
^{13}C NMR (100 MHz, $CDCl_3$): **5b**



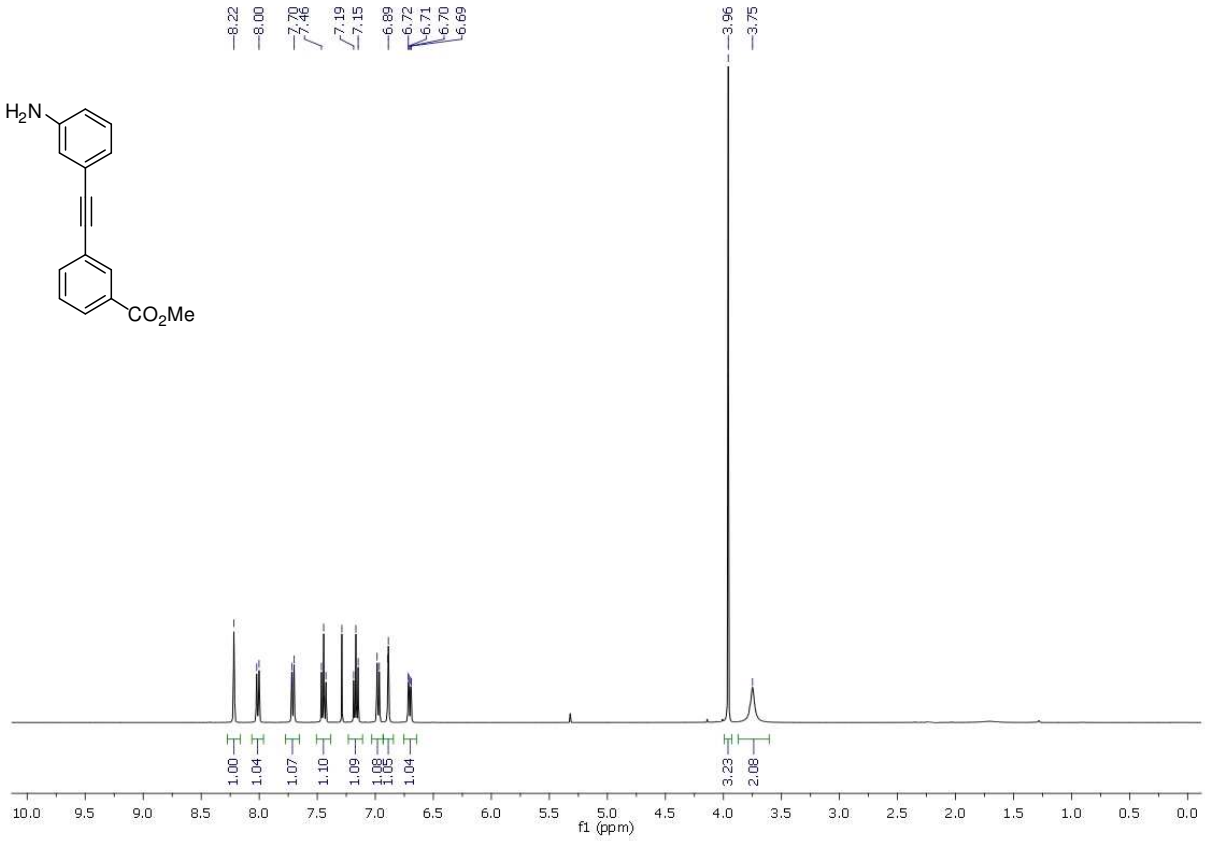
^{1}H NMR (400 MHz, $CDCl_3$): **5c**



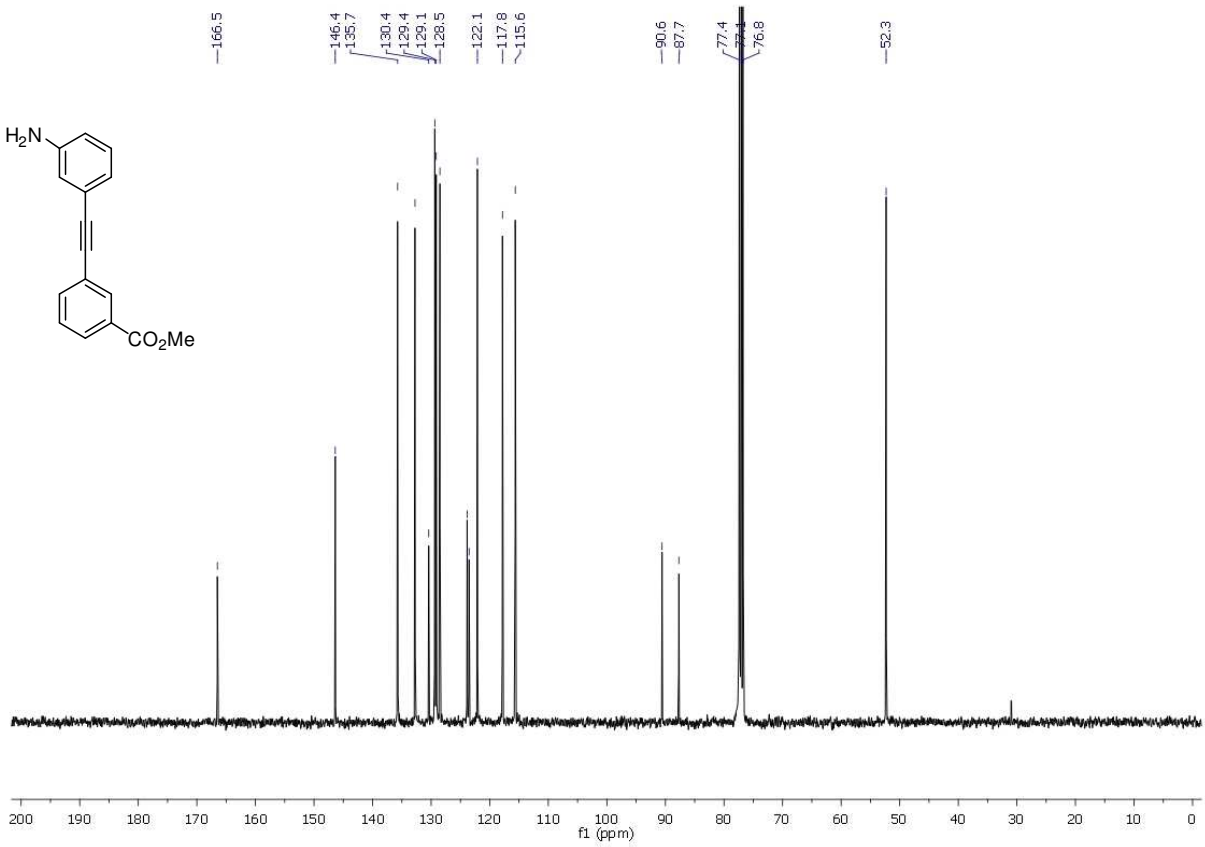
^{13}C NMR (100 MHz, $CDCl_3$): **5c**



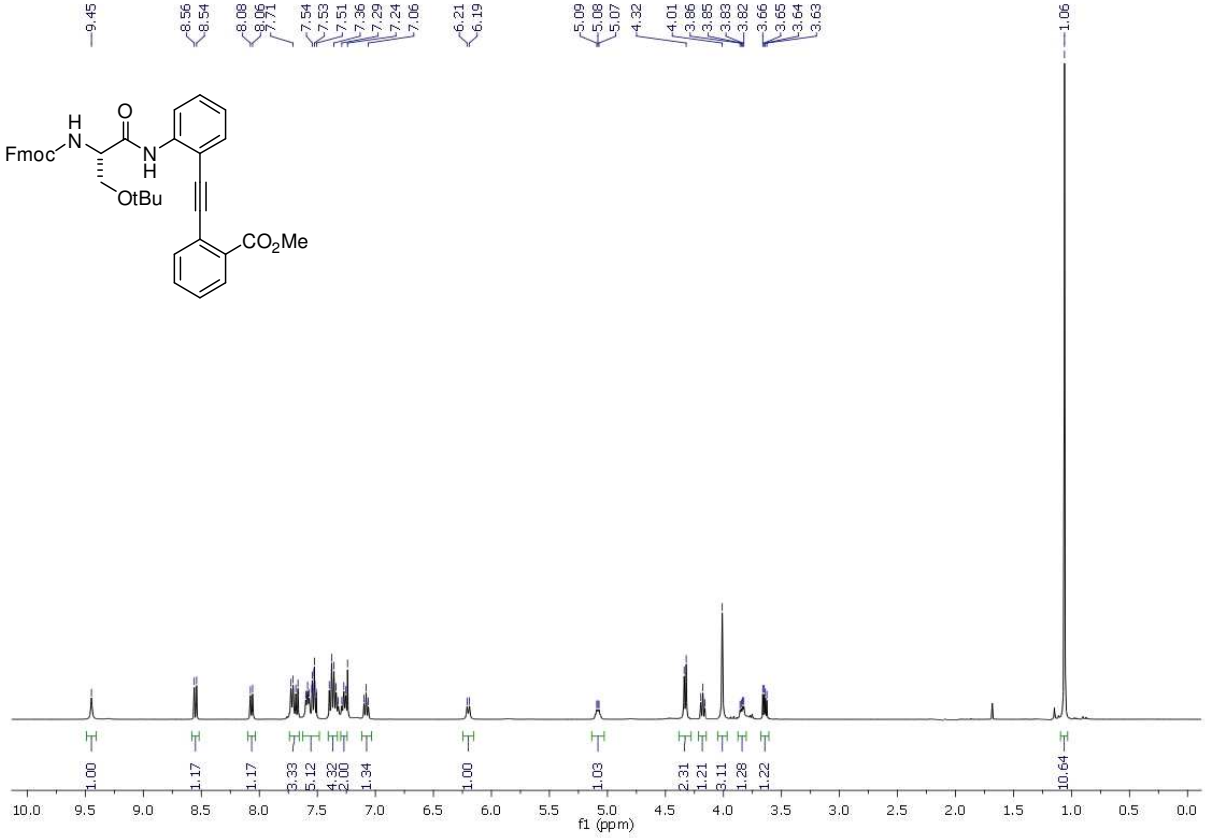
^{1}H NMR (400 MHz, $CDCl_3$): **5d**



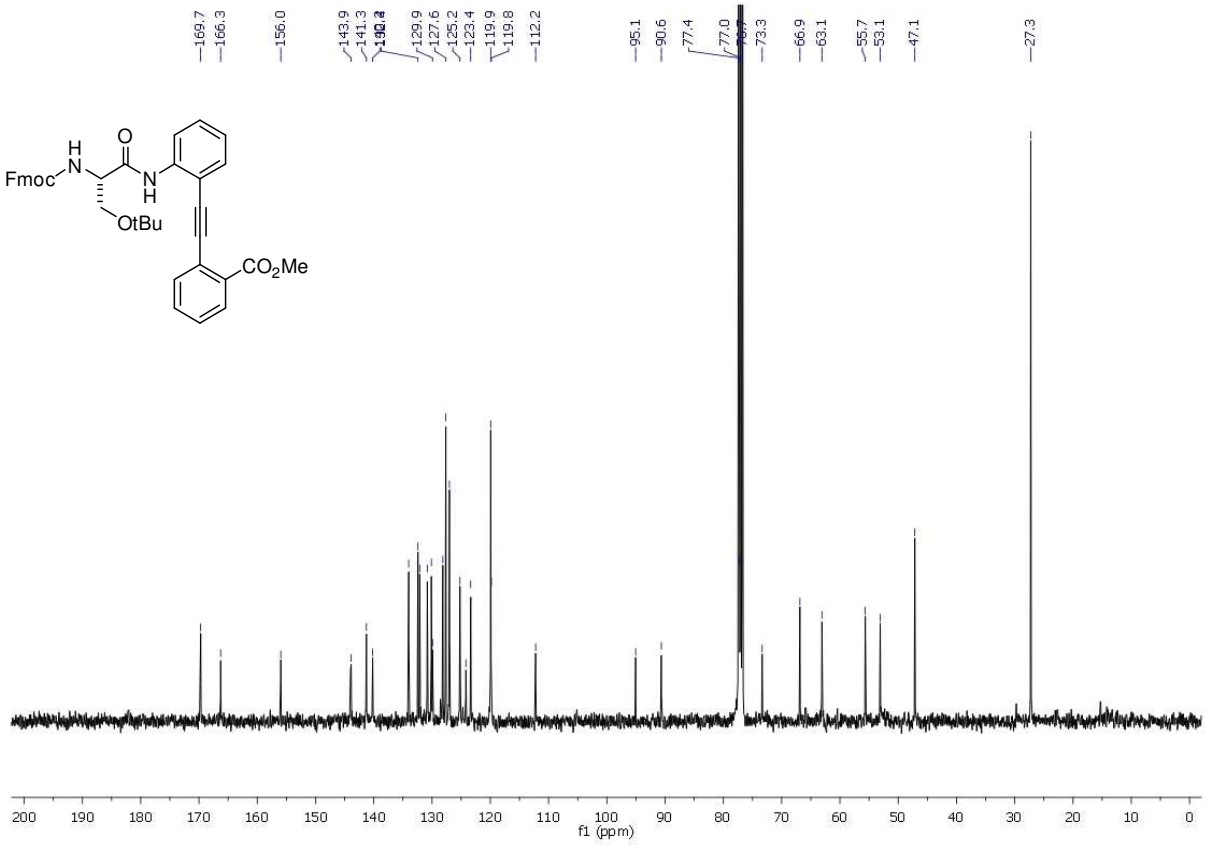
^{13}C NMR (100 MHz, $CDCl_3$): **5d**



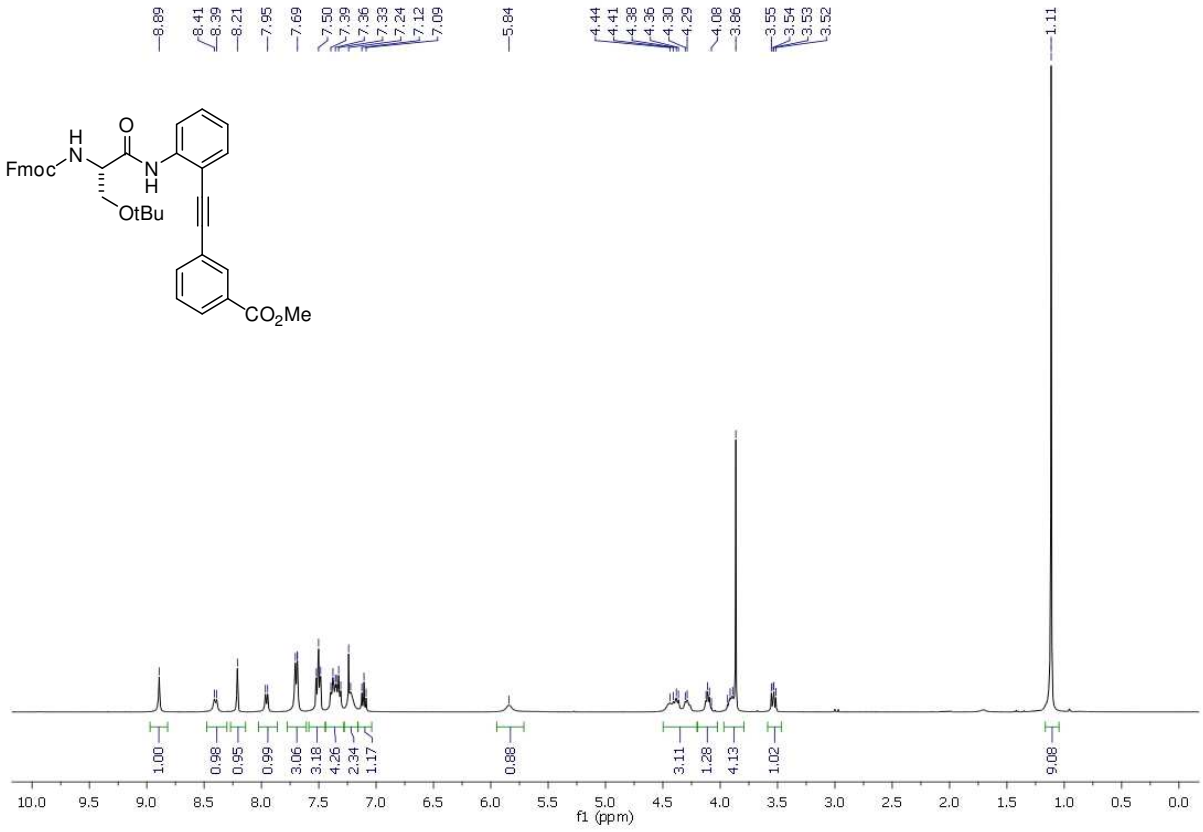
^{1}H NMR (400 MHz, $CDCl_3$): **8a**



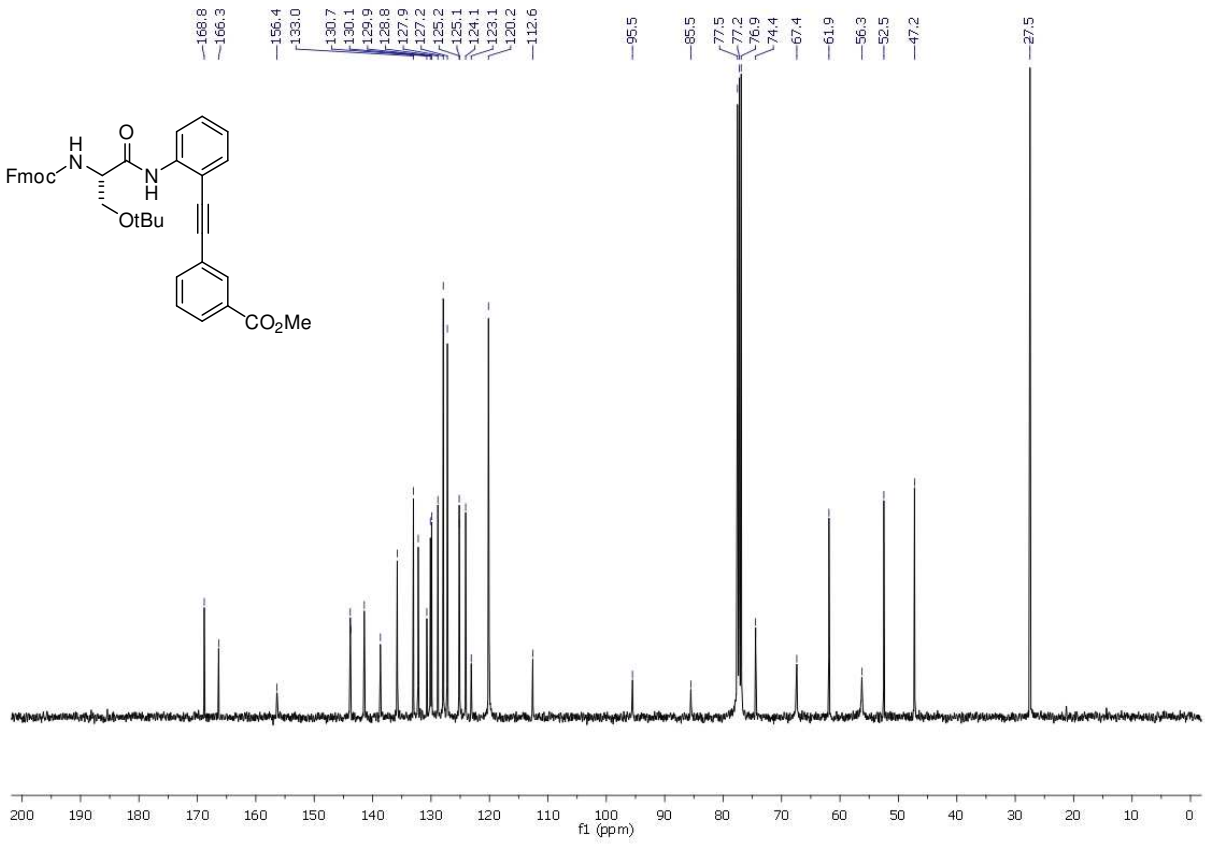
^{13}C NMR (100 MHz, $CDCl_3$): **8a**



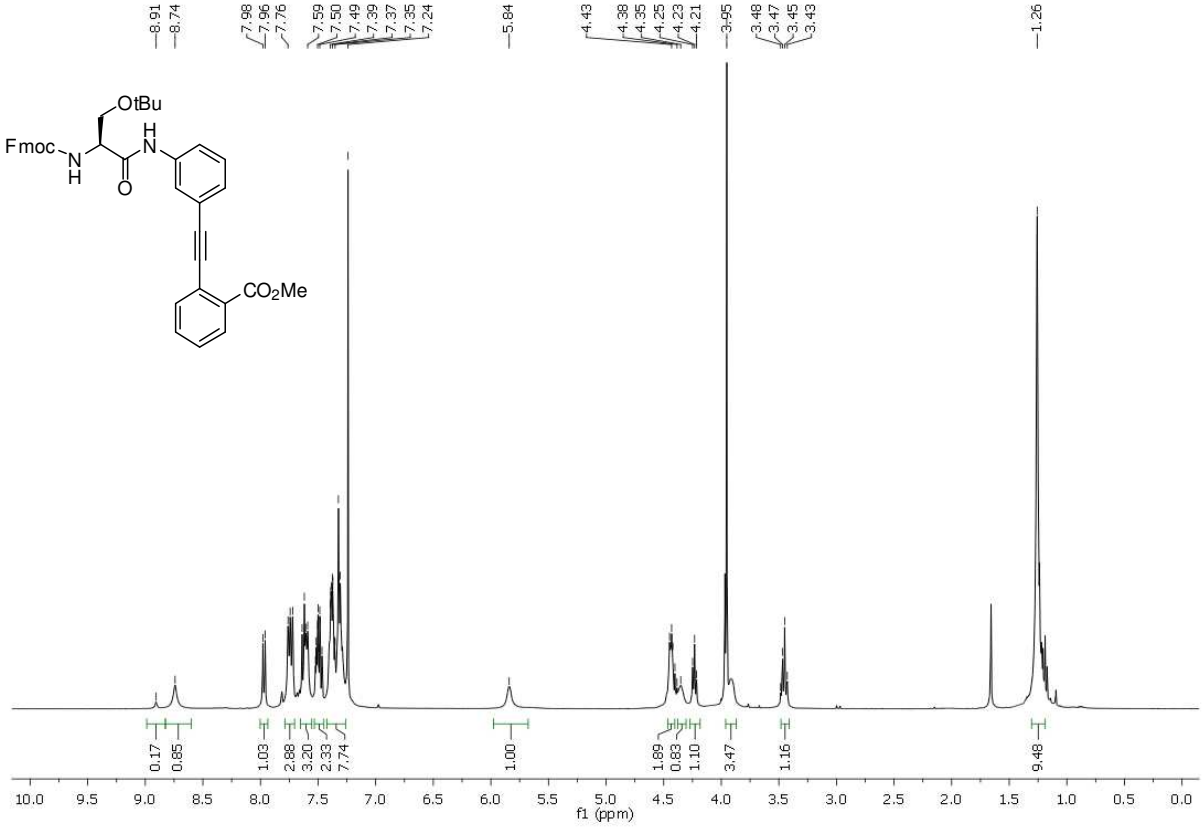
^{1}H NMR (400 MHz, $CDCl_3$): **8b**



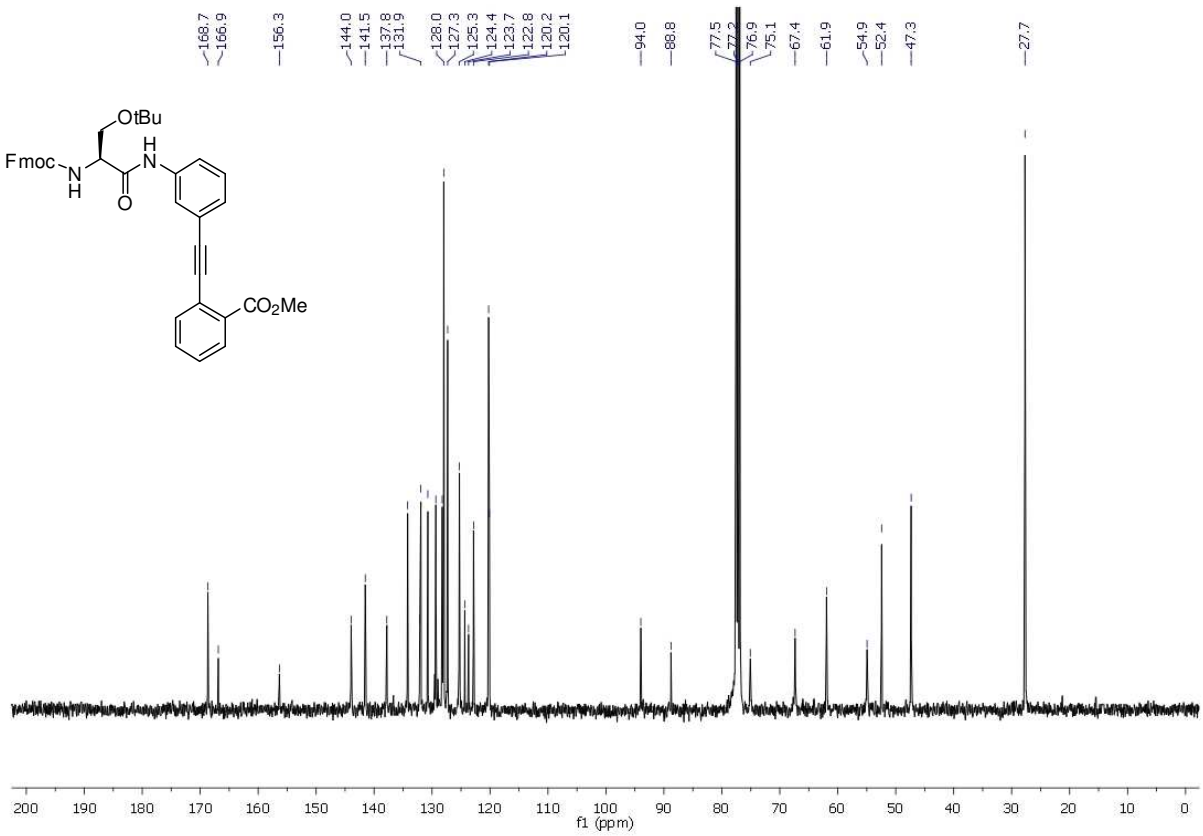
^{13}C NMR (100 MHz, $CDCl_3$): **8b**



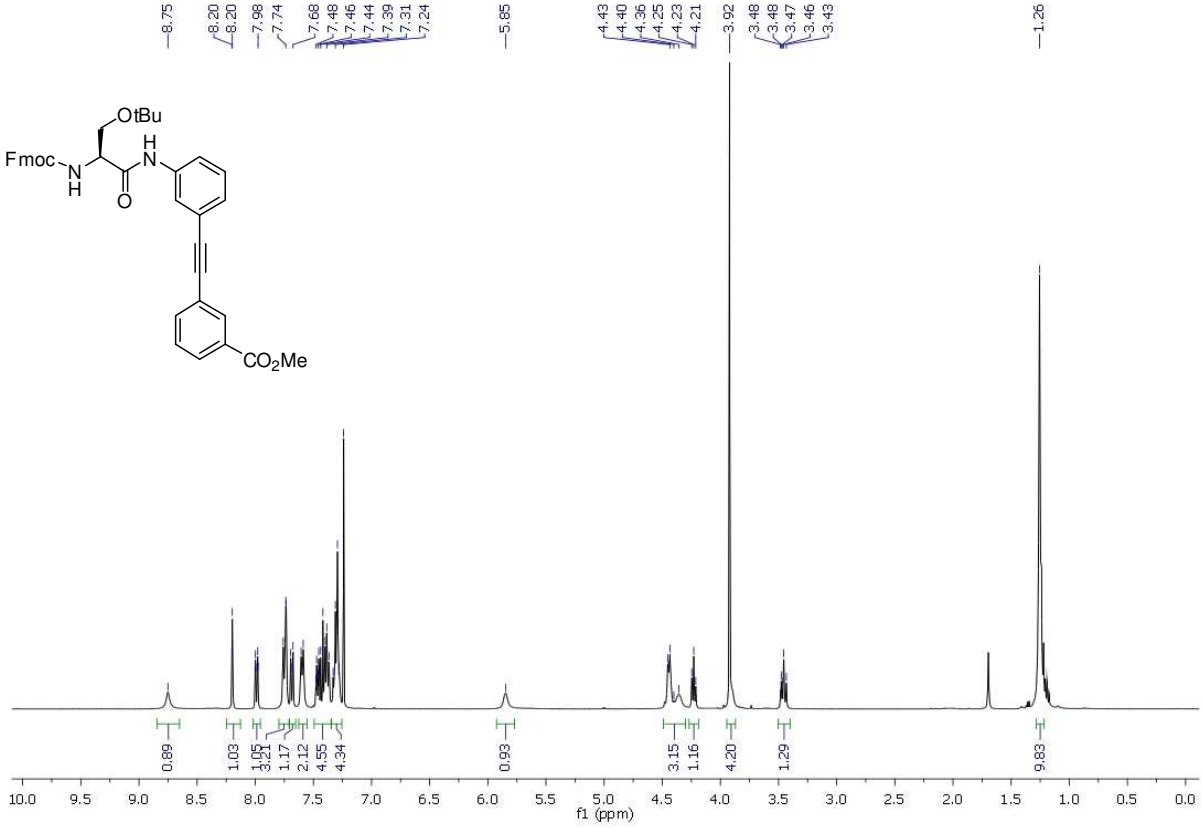
^{1}H NMR (400 MHz, $CDCl_3$): **8c**



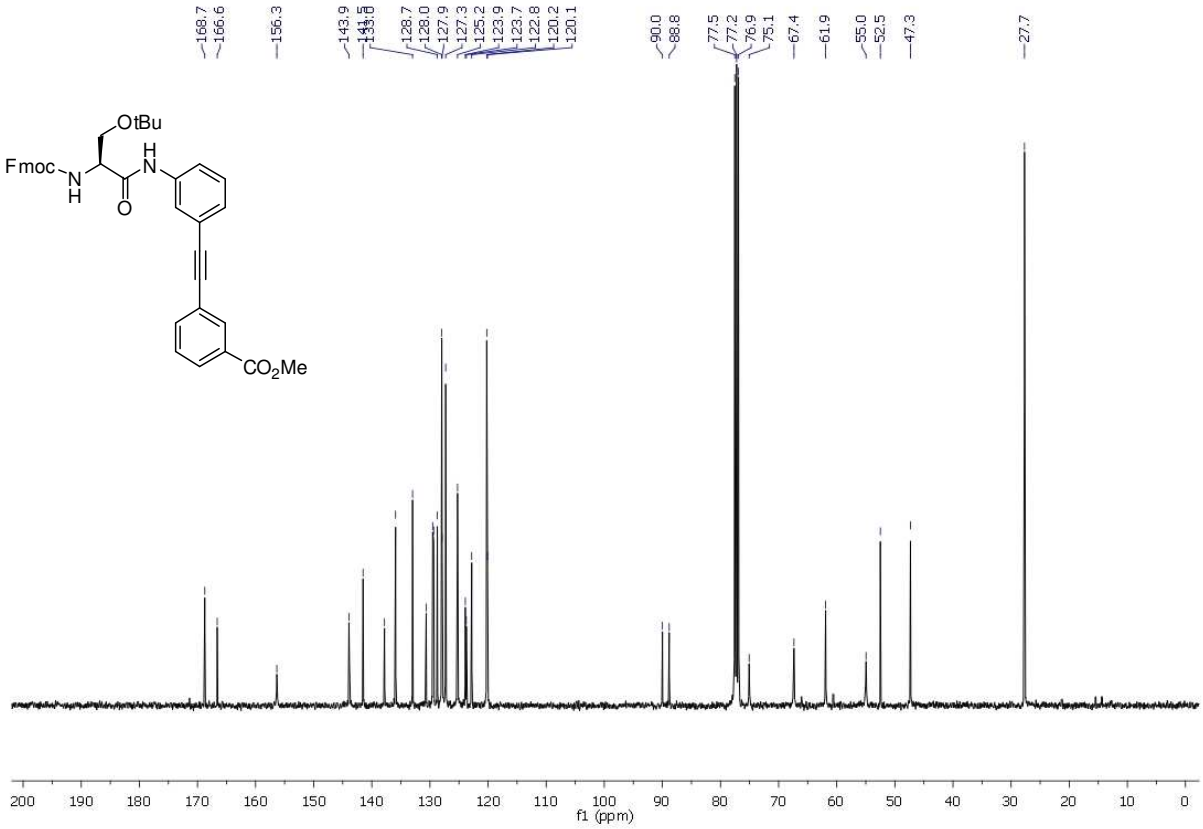
^{13}C NMR (100 MHz, $CDCl_3$): **8c**



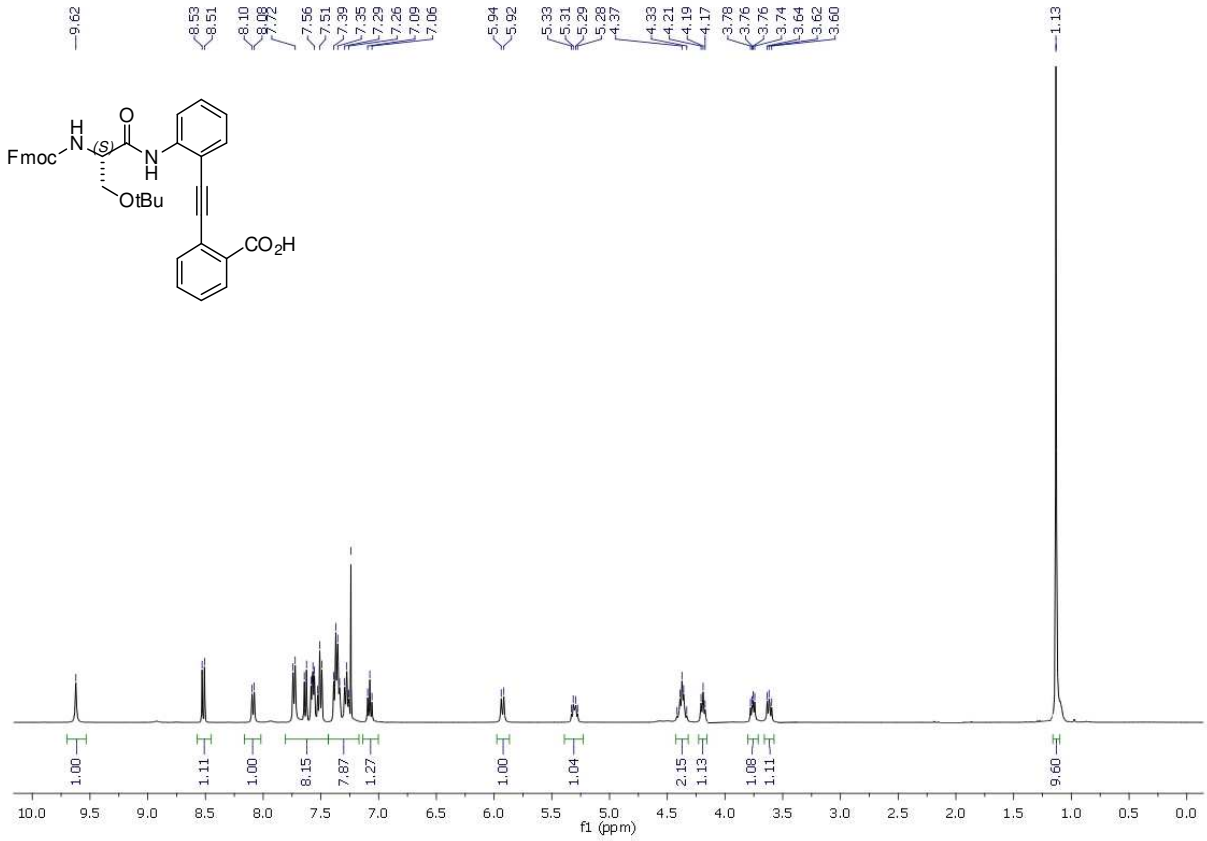
^{1}H NMR (400 MHz, $CDCl_3$): **8d**



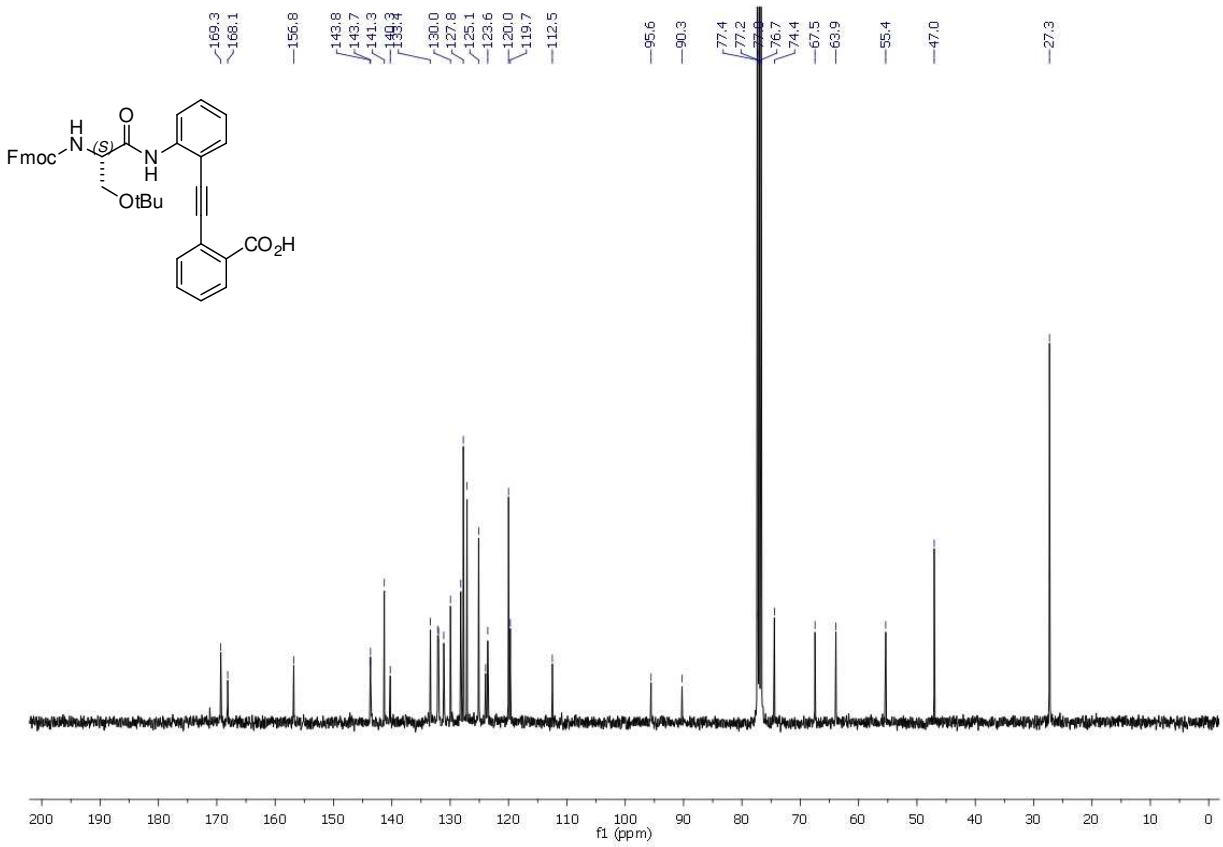
^{13}C NMR (100 MHz, $CDCl_3$): **8d**



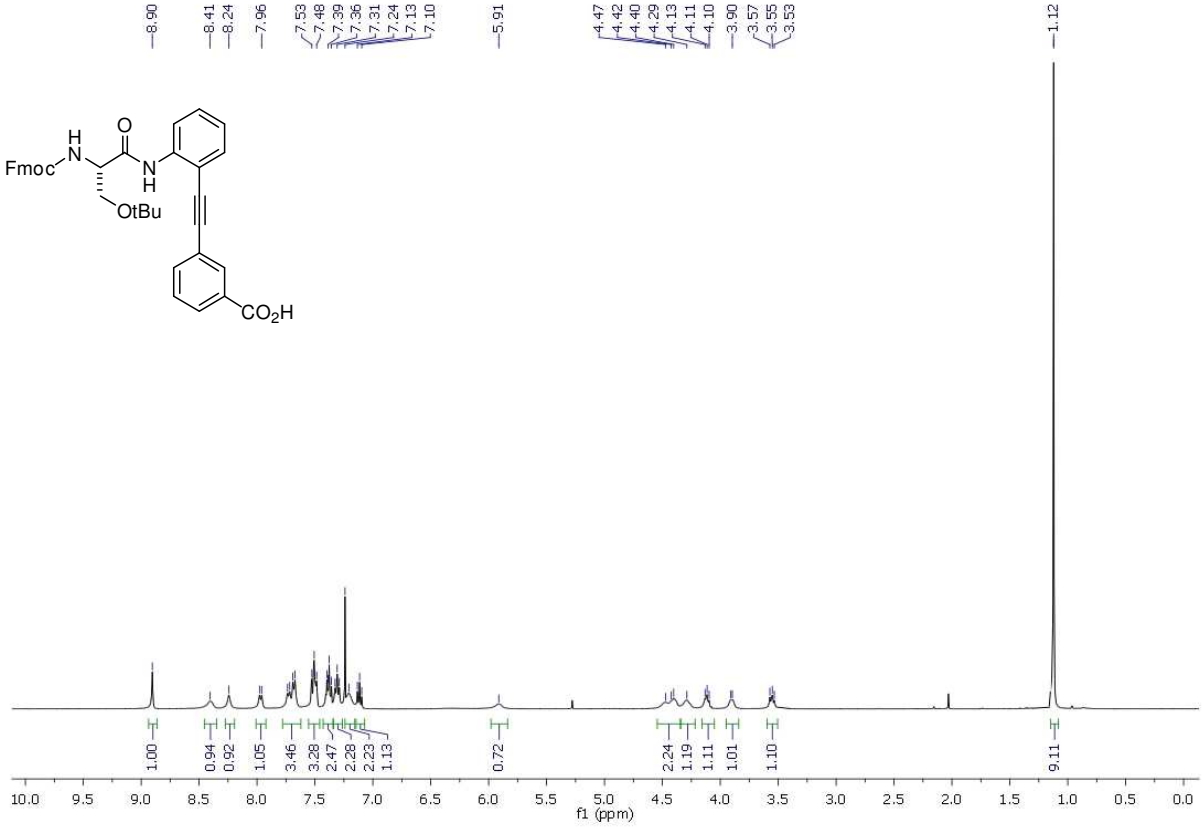
^{1}H NMR (400 MHz, $CDCl_3$): **9a**



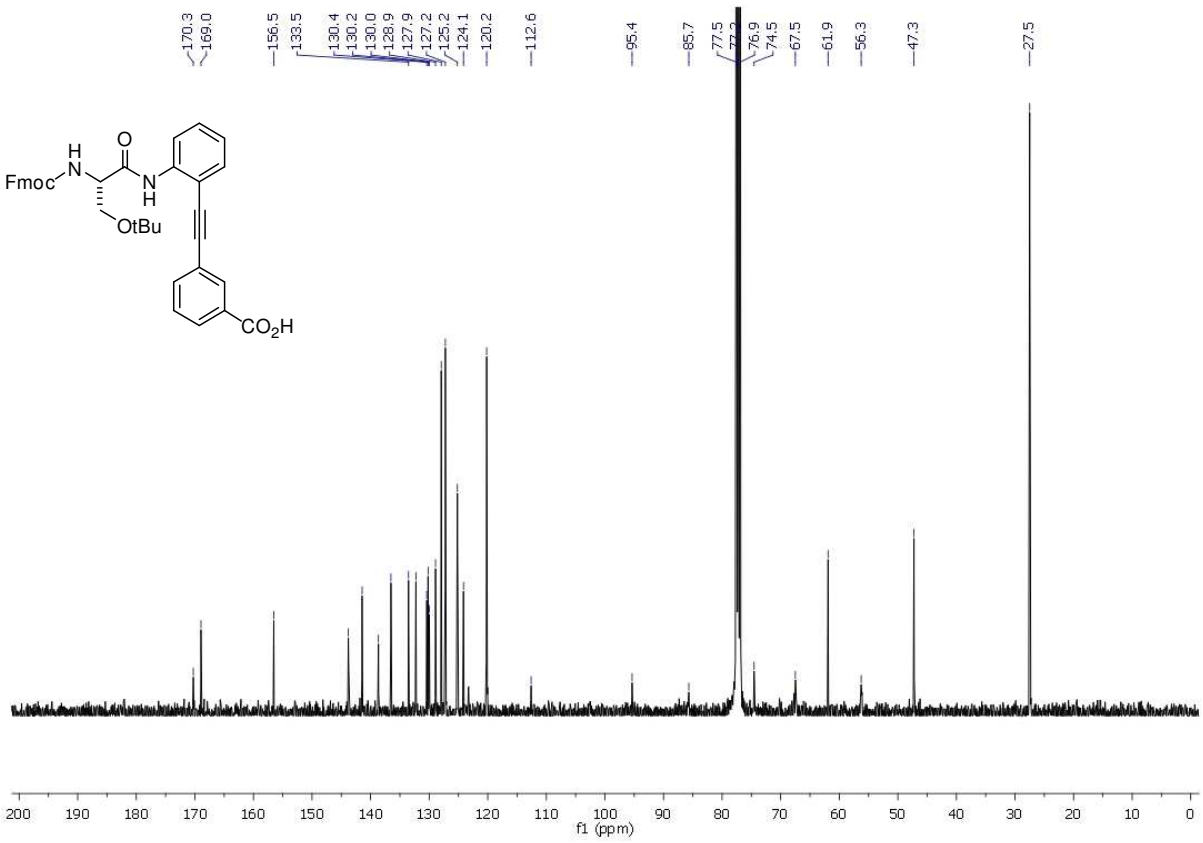
^{13}C NMR (100 MHz, $CDCl_3$): **9a**



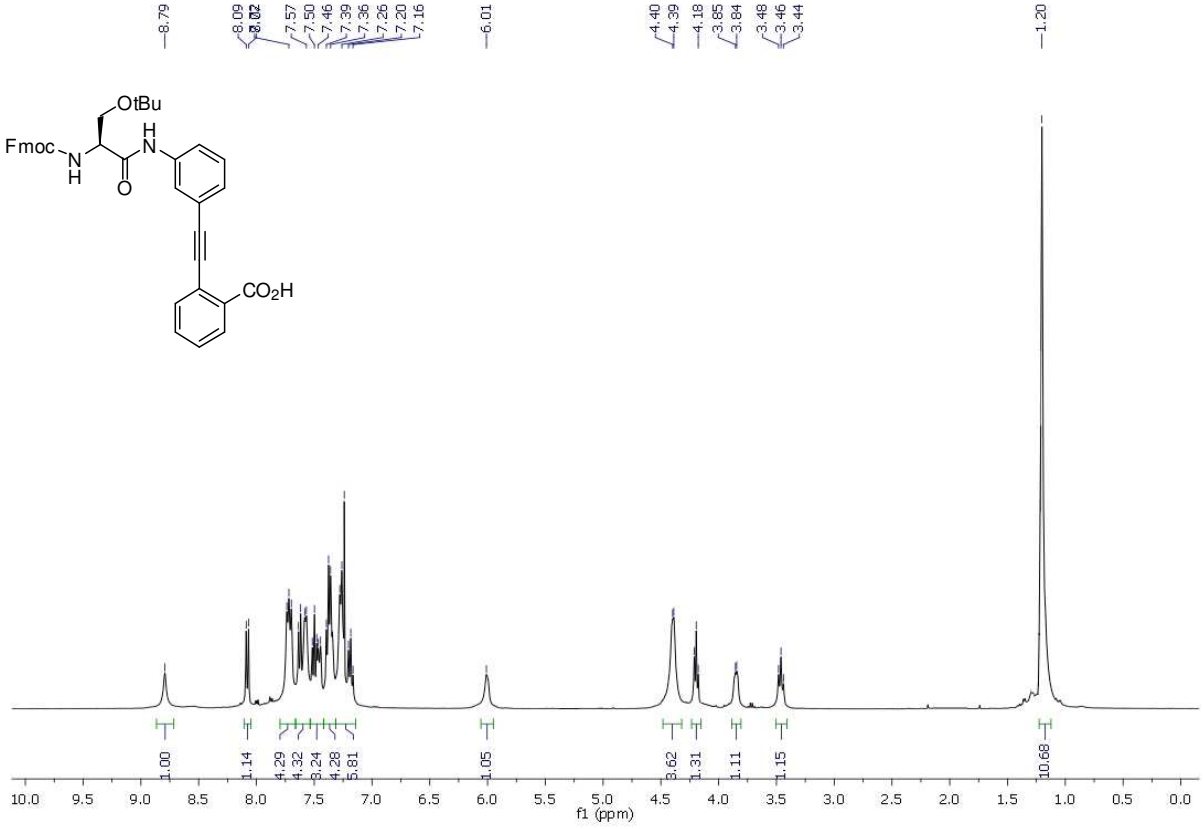
^{1}H NMR (400 MHz, $CDCl_3$): **9b**



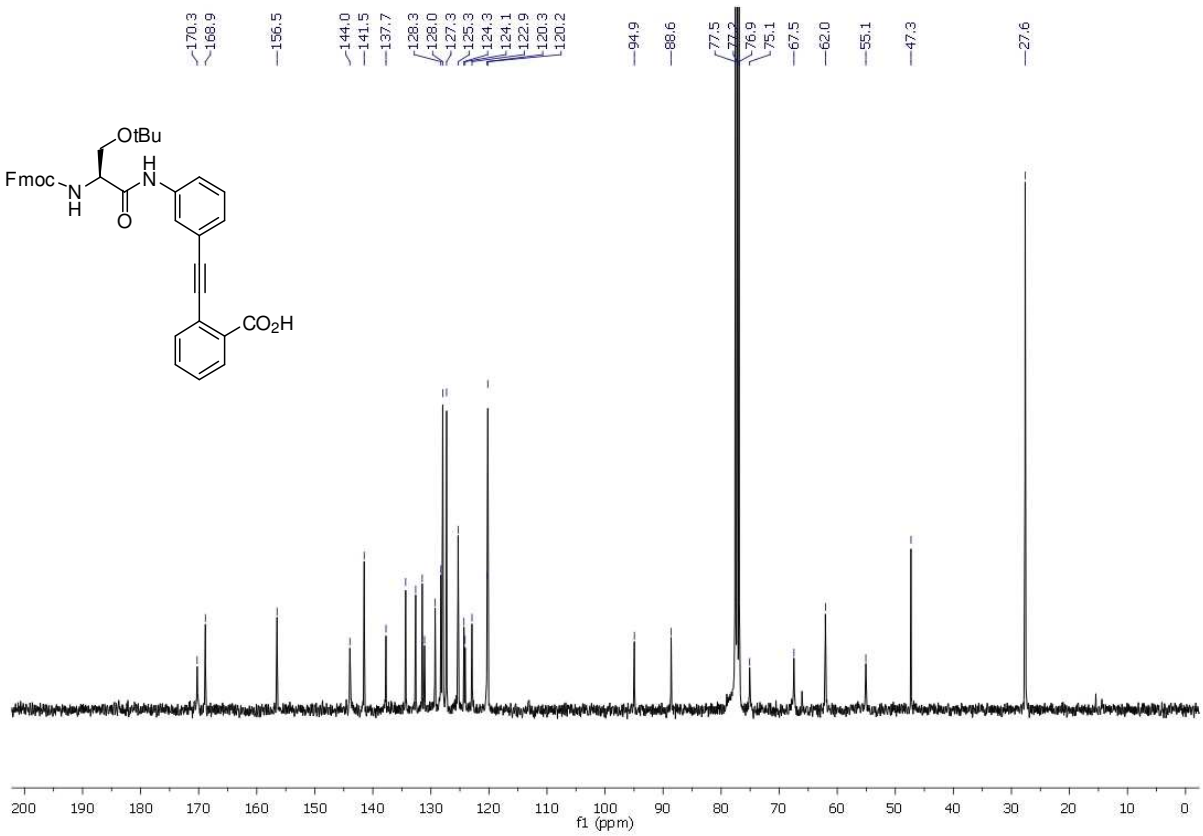
^{13}C NMR (100 MHz, $CDCl_3$): **9b**



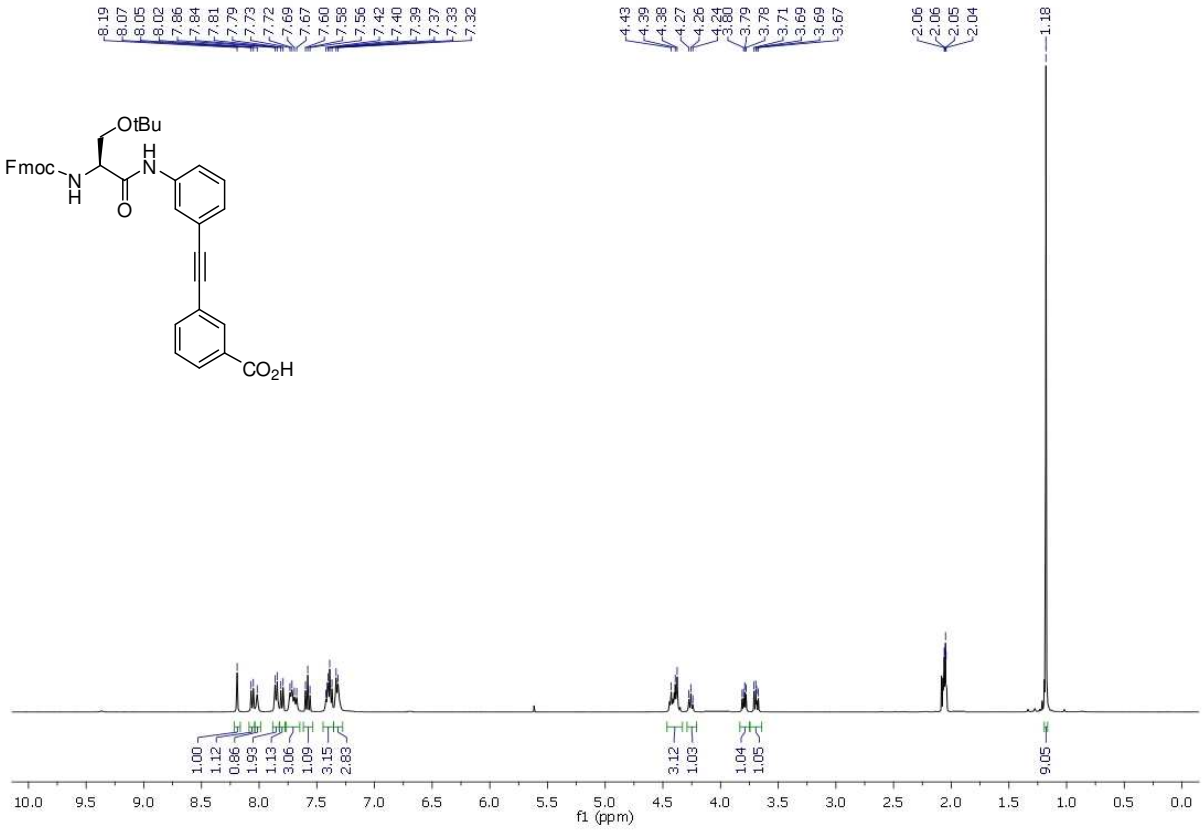
^{1}H NMR (400 MHz, $CDCl_3$): **9c**



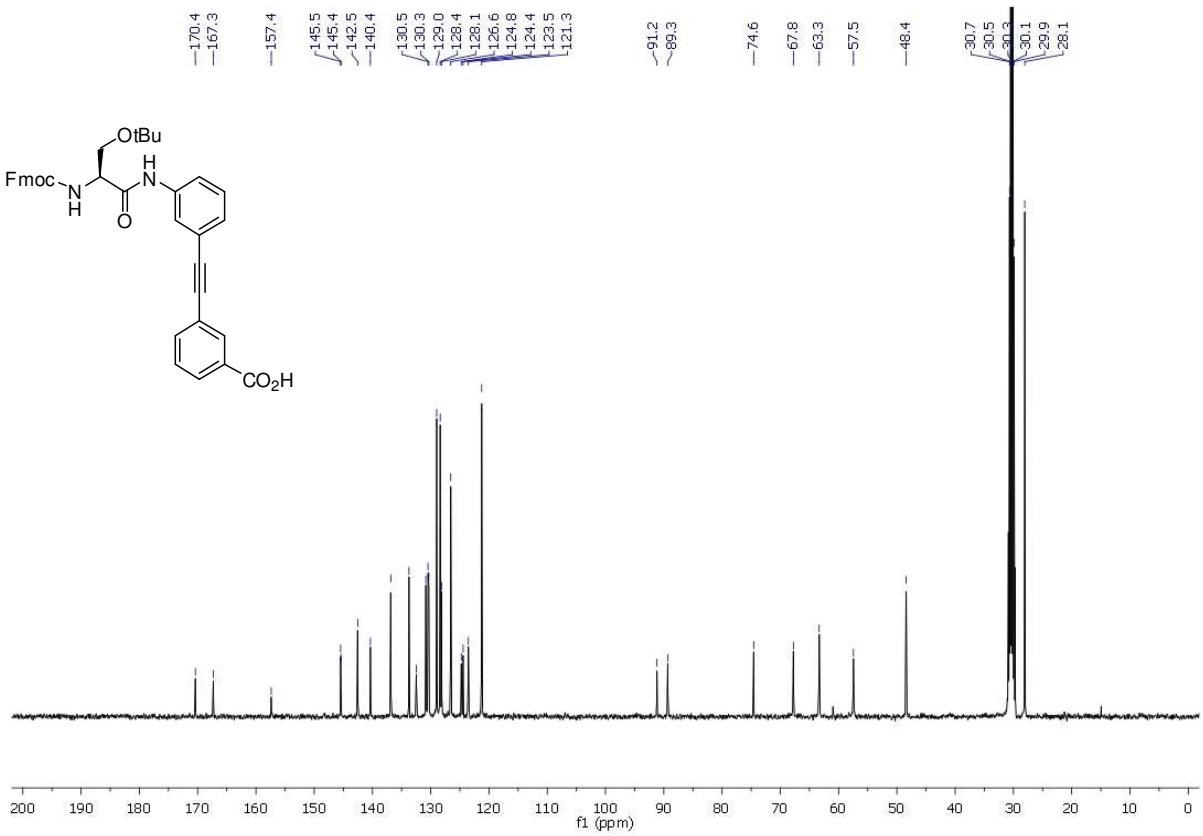
^{13}C NMR (100 MHz, $CDCl_3$): **9c**



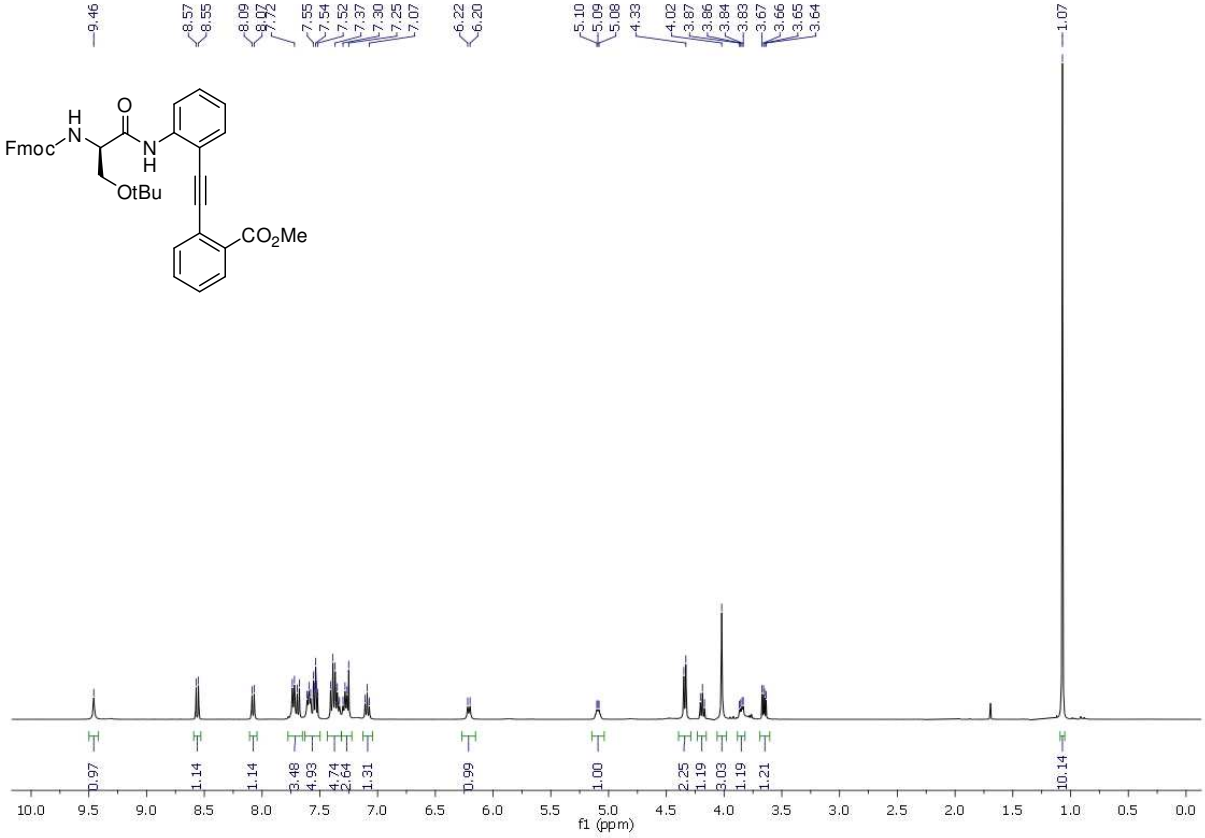
^{1}H NMR (400 MHz, d_6-acetone): **9d**



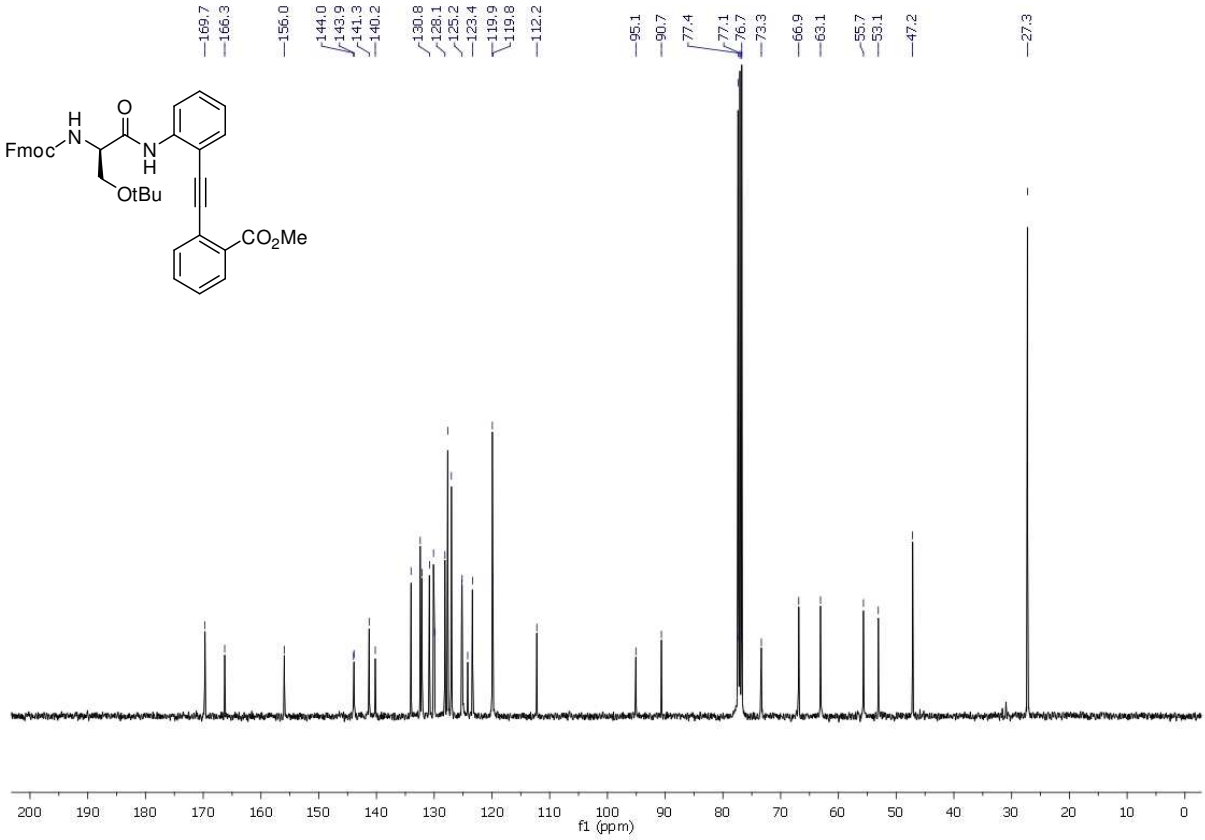
^{13}C NMR (100 MHz, d_6-acetone): **9d**



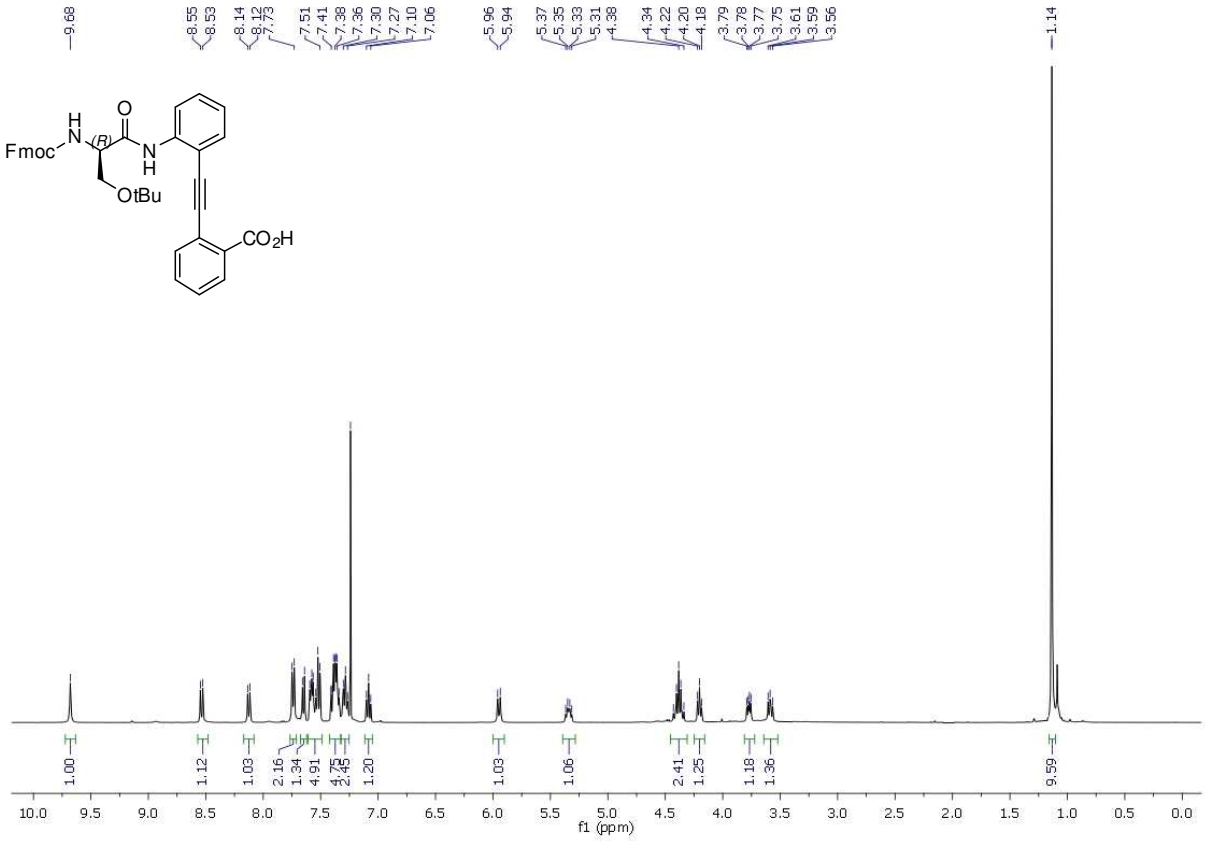
^{1}H NMR (400 MHz, $CDCl_3$): ***ent*-8a**



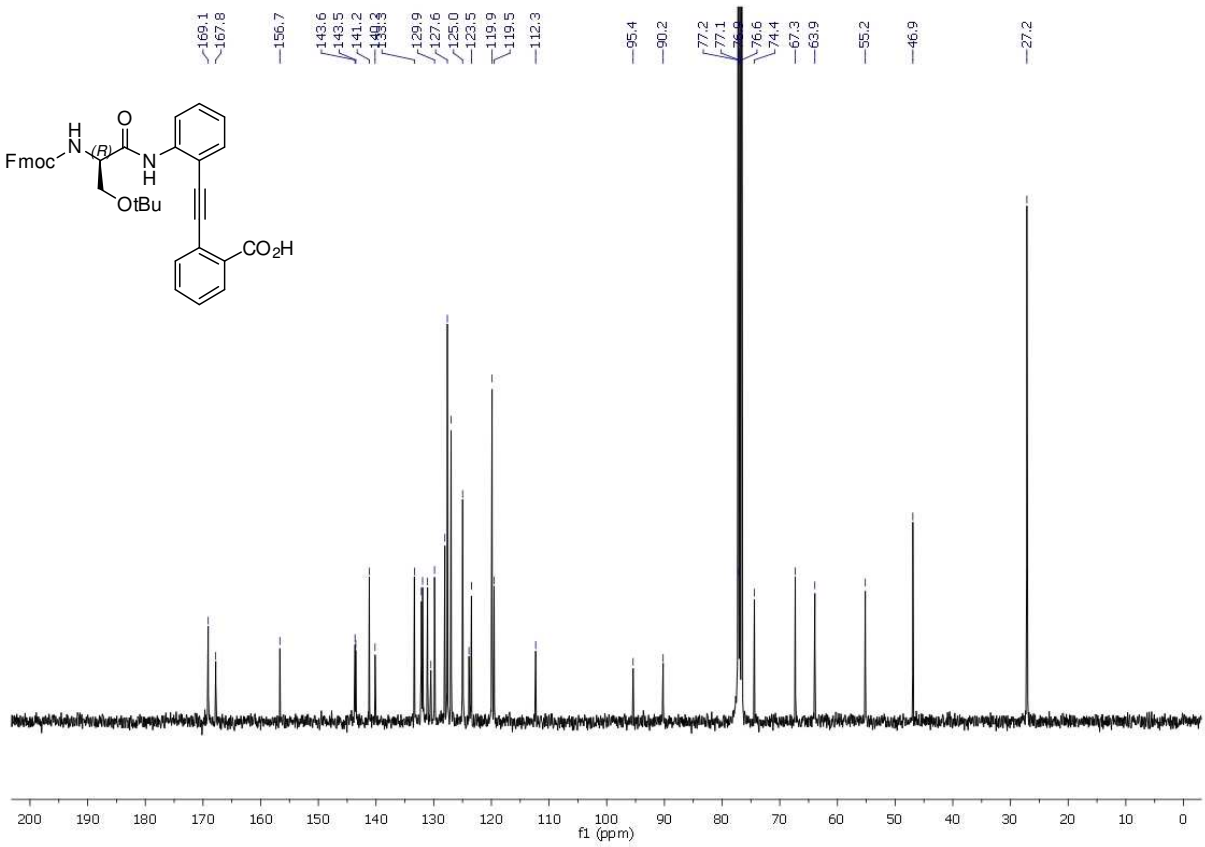
^{13}C NMR (100 MHz, $CDCl_3$): ***ent*-8a**



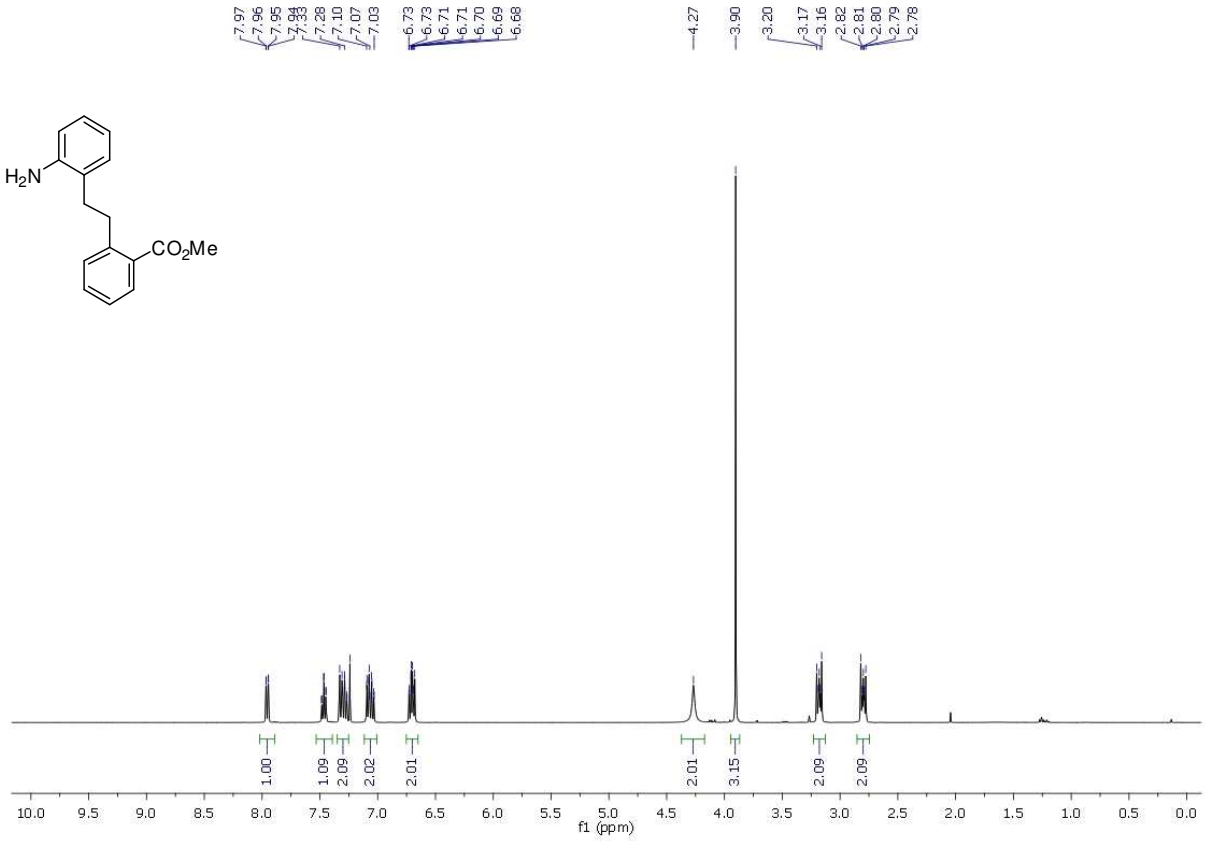
^{1}H NMR (400 MHz, $CDCl_3$): ***ent*-9a**



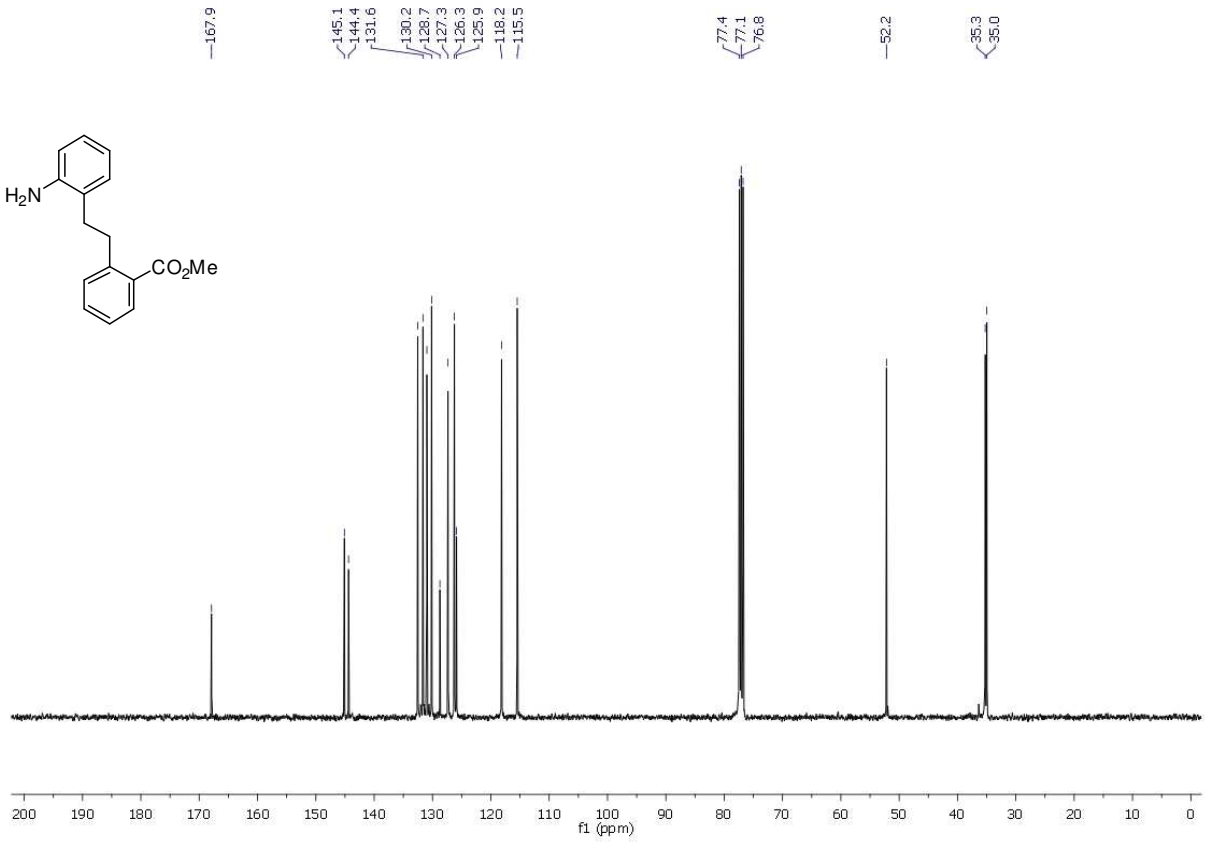
^{13}C NMR (100 MHz, $CDCl_3$): ***ent*-9a**



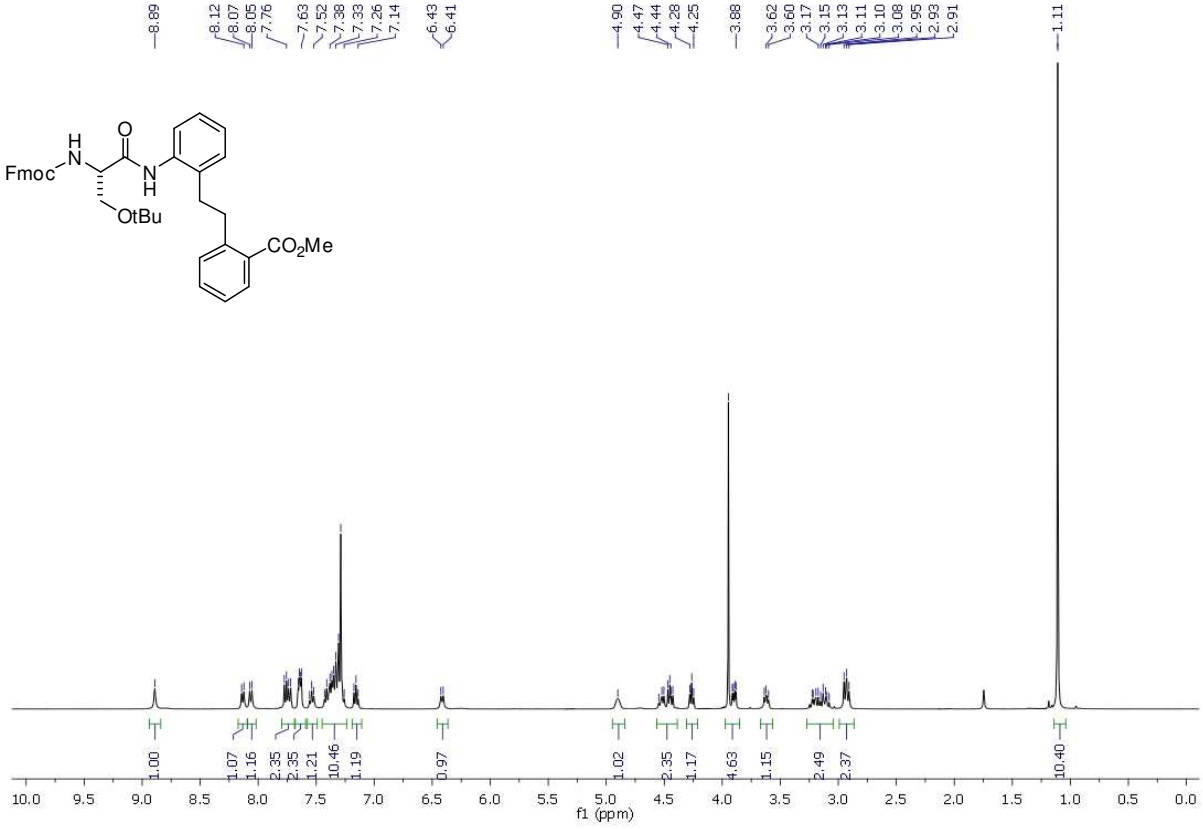
^{1}H NMR (400 MHz, $CDCl_3$): **10**



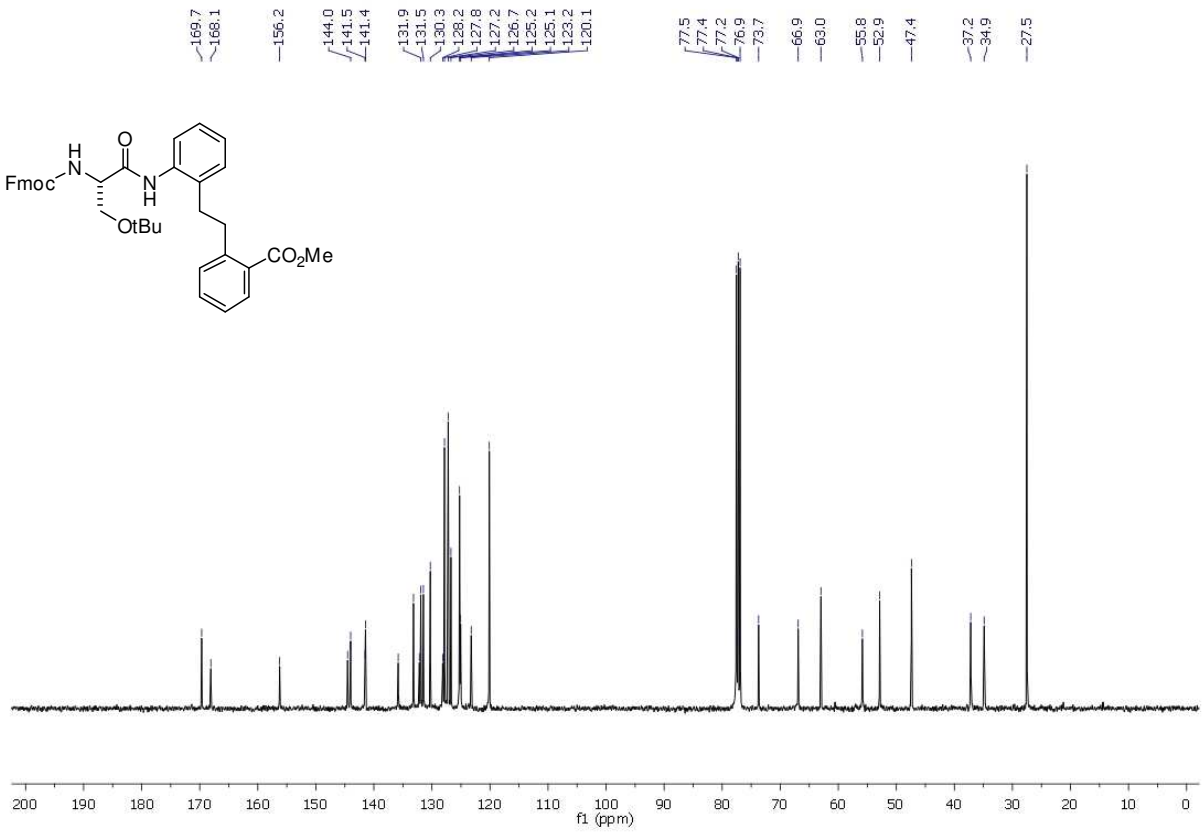
^{13}C NMR (100 MHz, $CDCl_3$): **10**



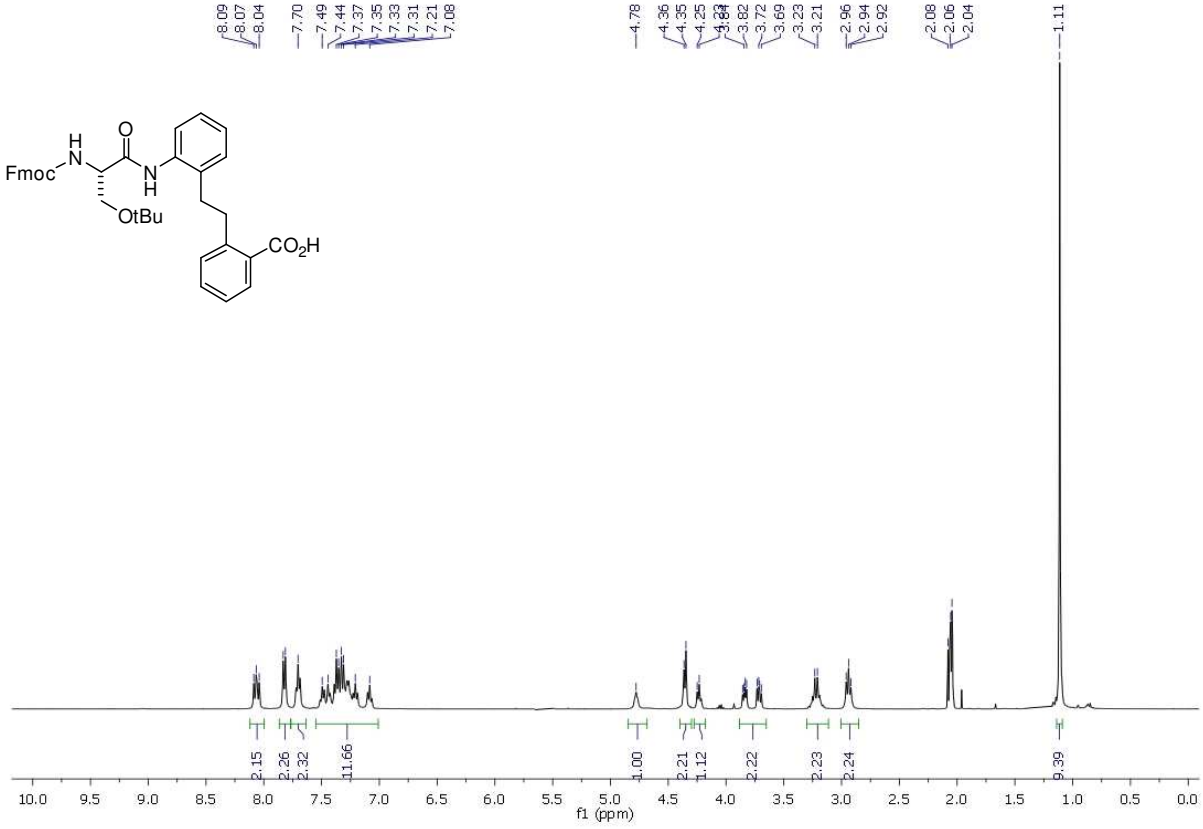
^{1}H NMR (400 MHz, $CDCl_3$): **11**



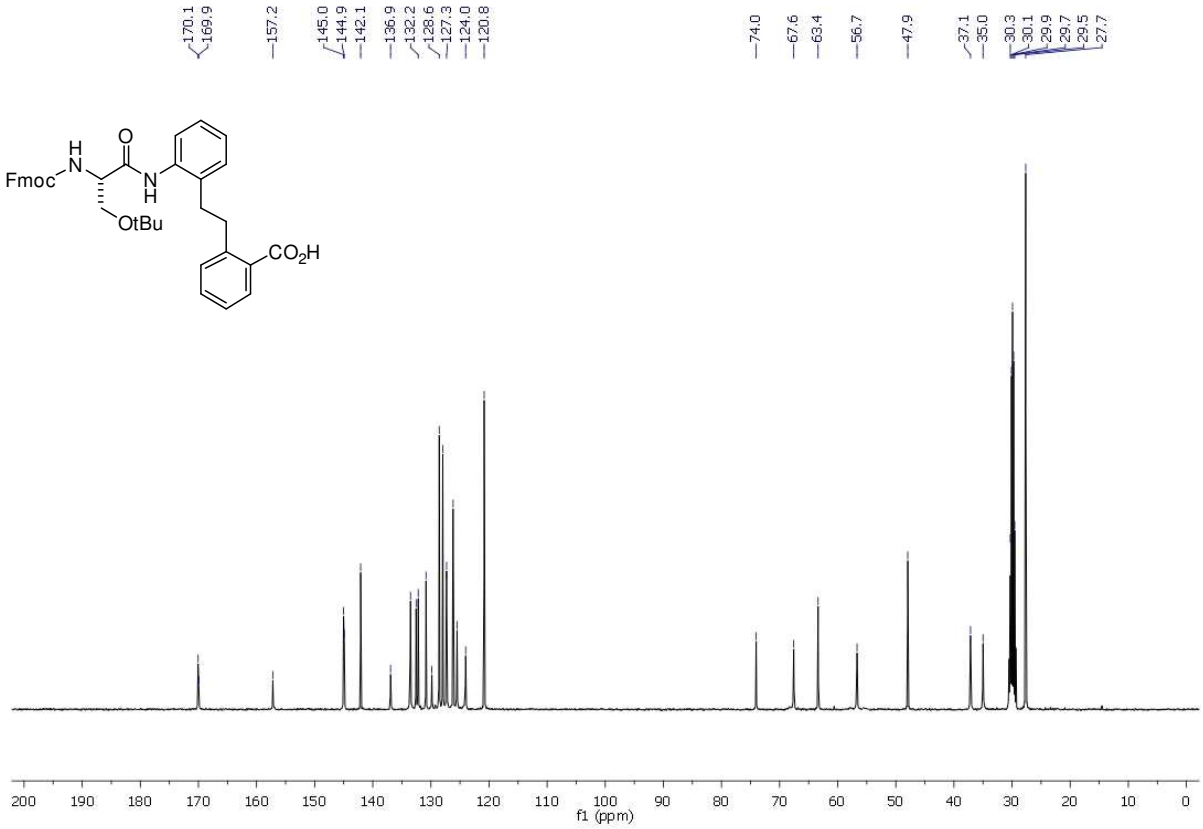
^{13}C NMR (100 MHz, $CDCl_3$): **11**



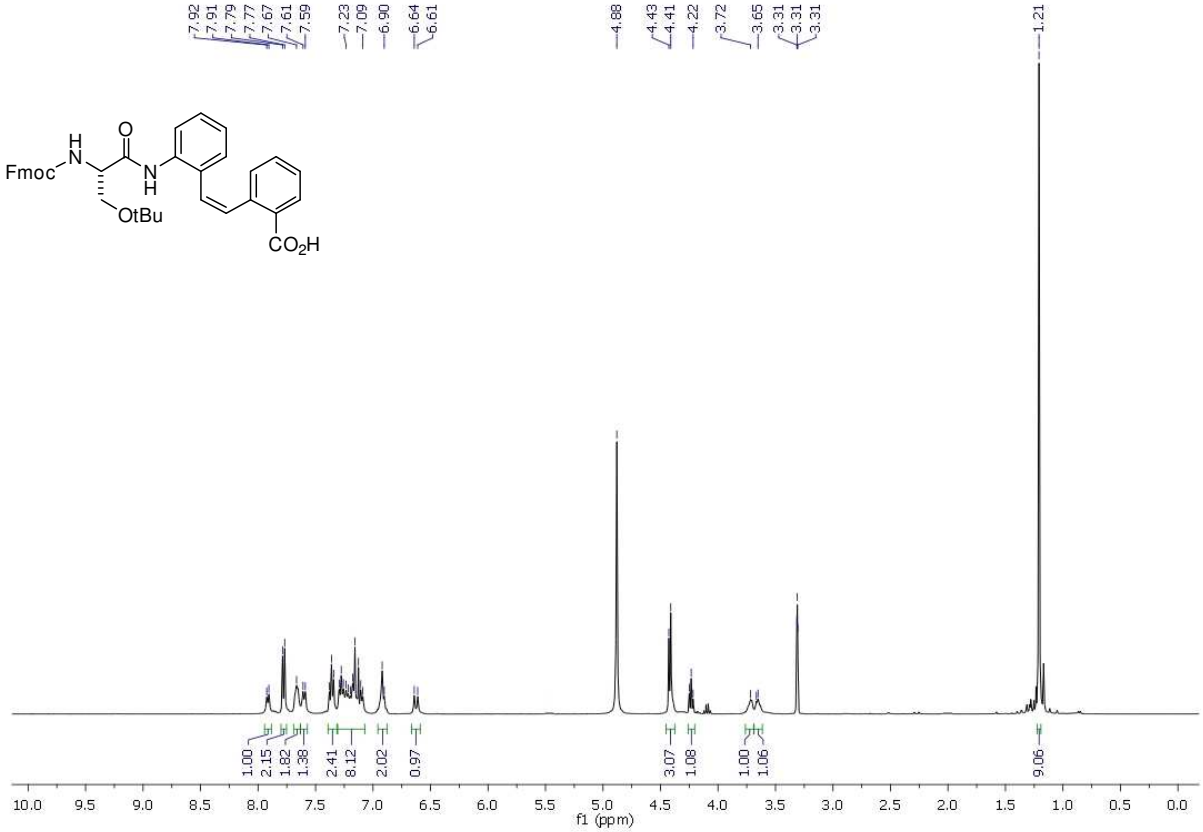
^{1}H NMR (400 MHz, d_6-acetone): **12**



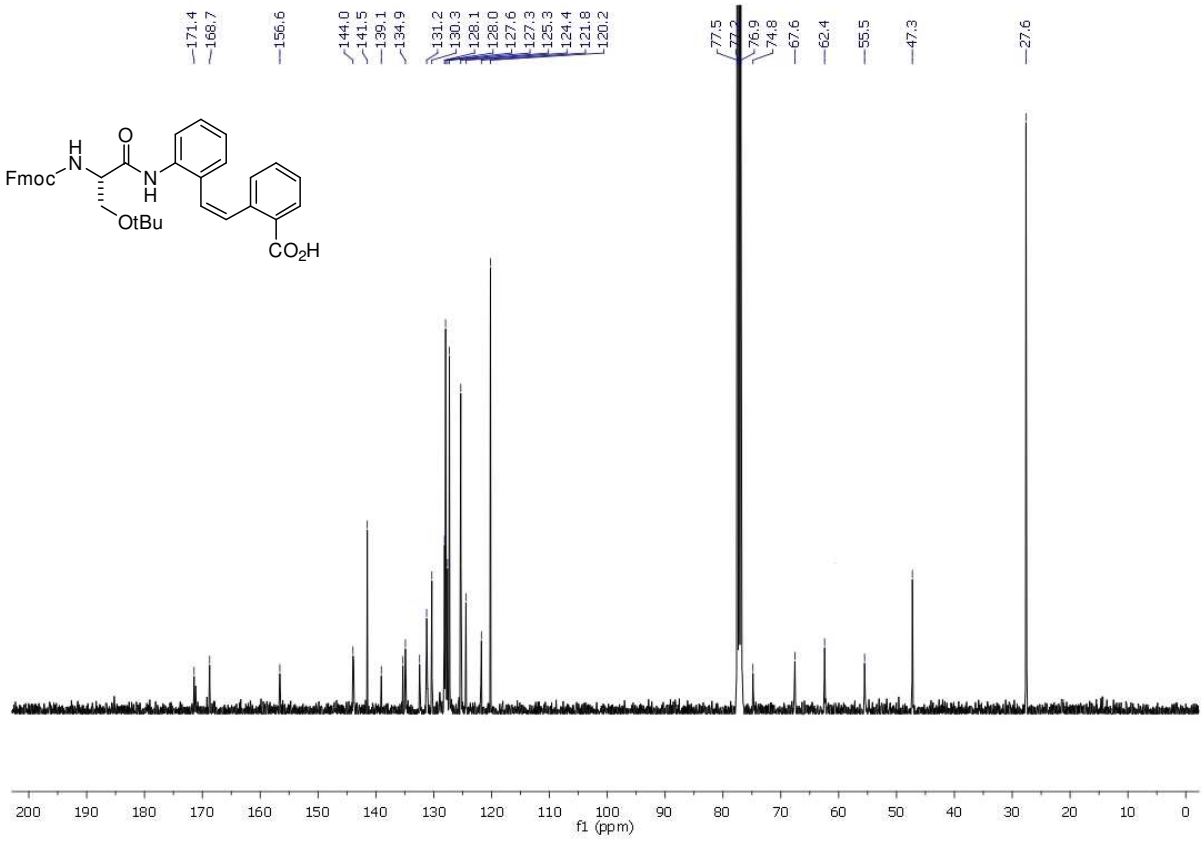
^{13}C NMR (100 MHz, d_6-acetone): **12**



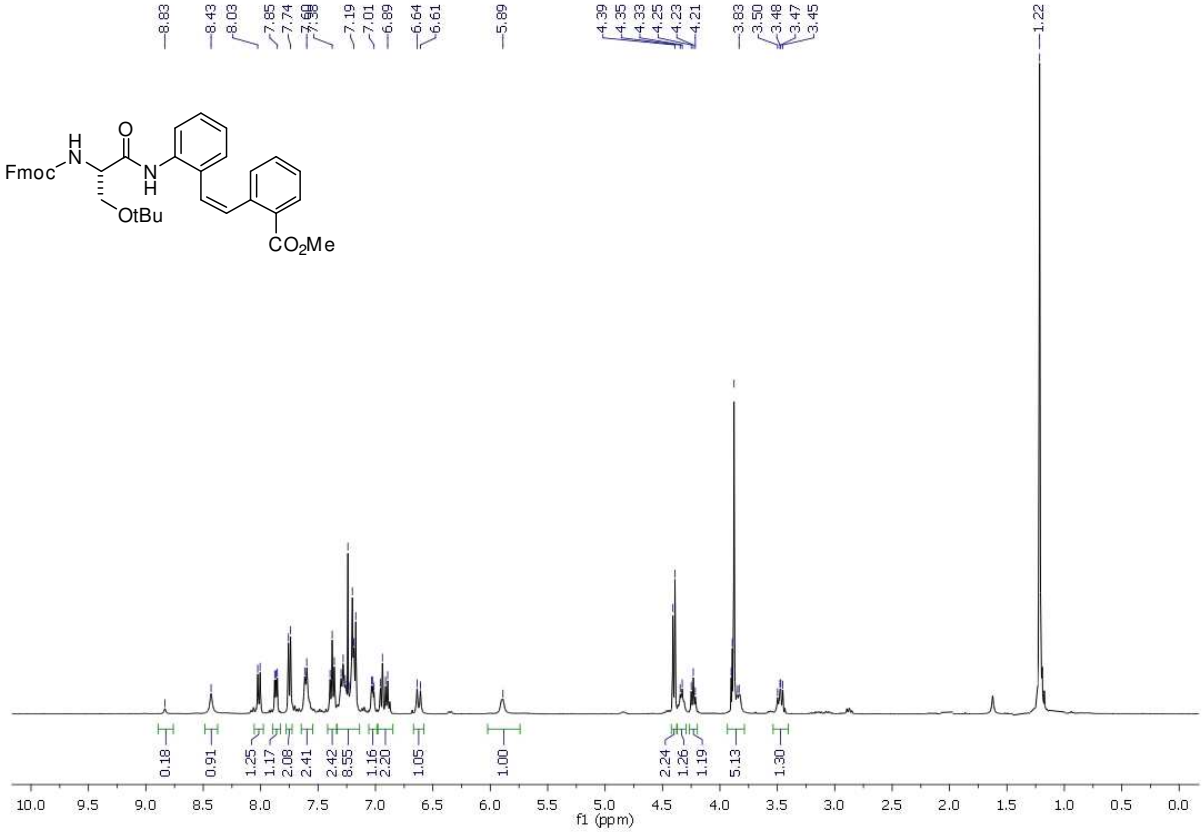
^{1}H NMR (400 MHz, CD_3OD): **13**



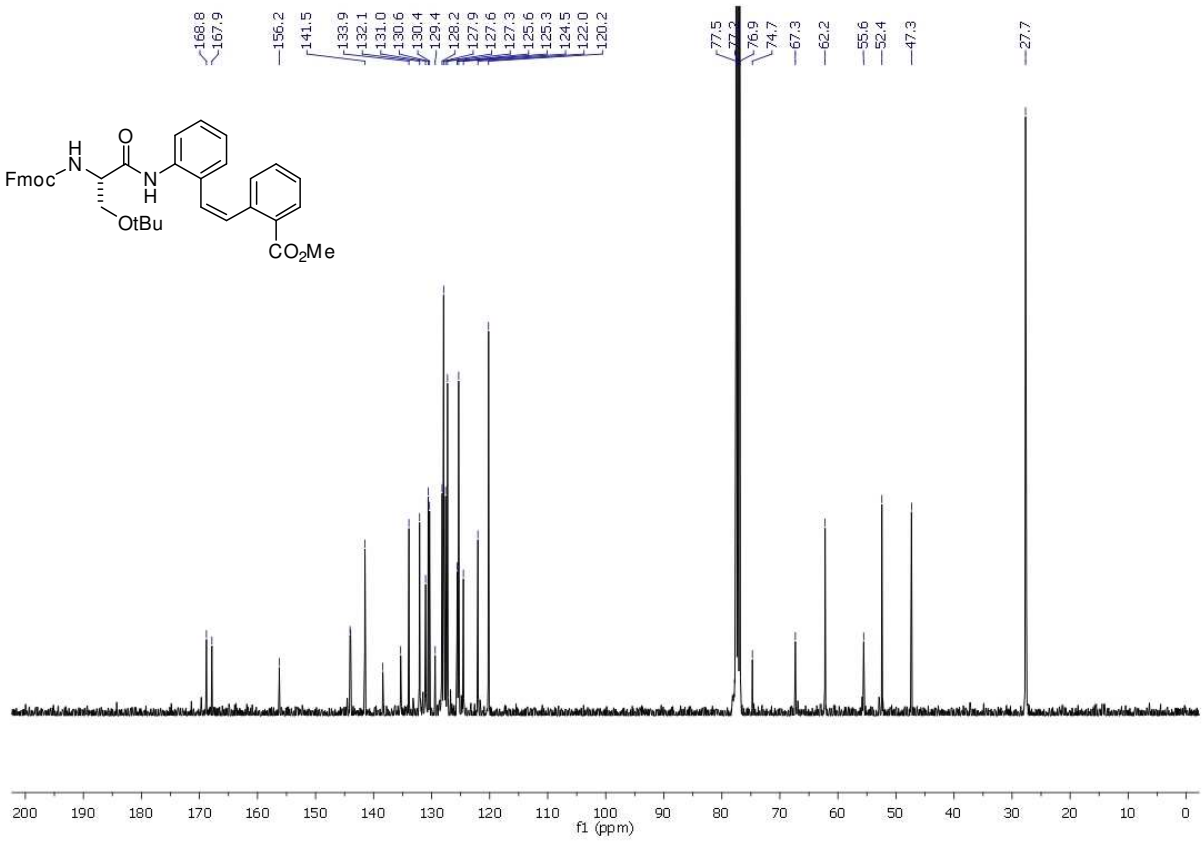
^{13}C NMR (100 MHz, $CDCl_3$): **13**



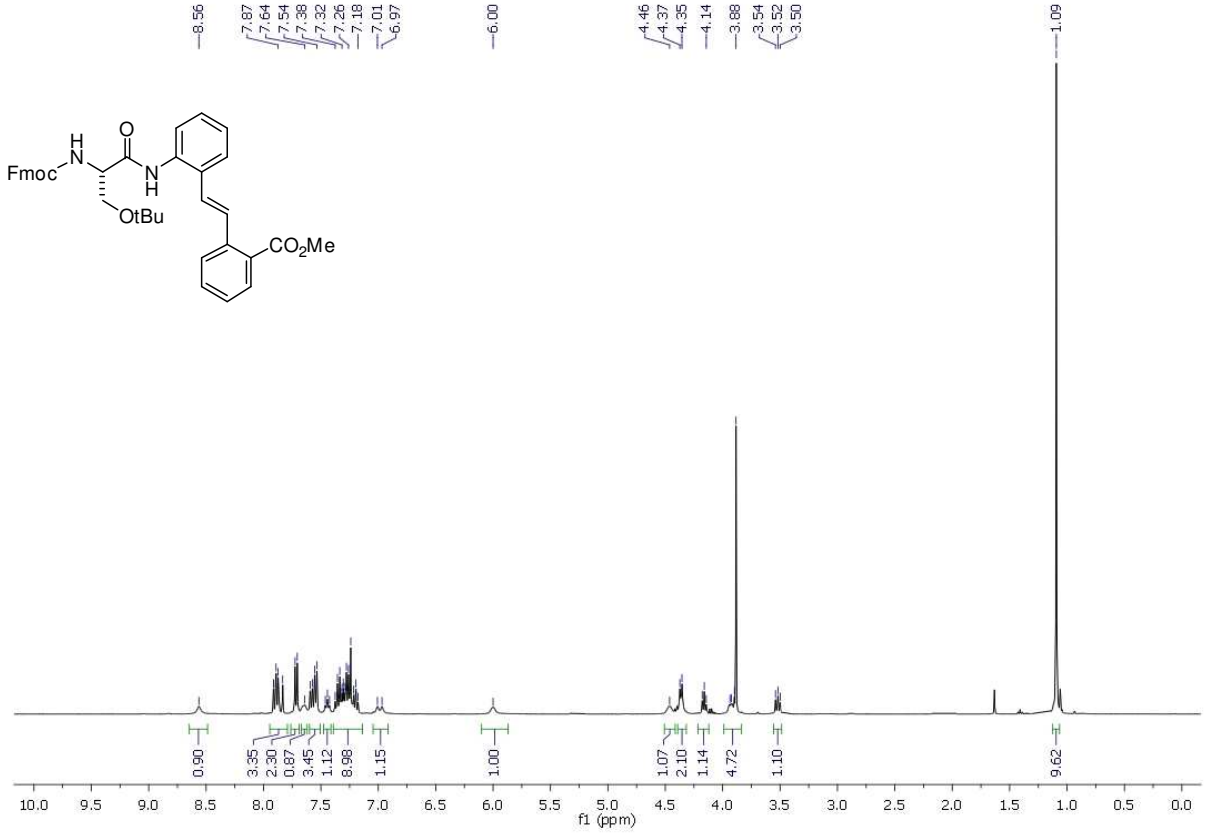
^{1}H NMR (400 MHz, $CDCl_3$): **14**



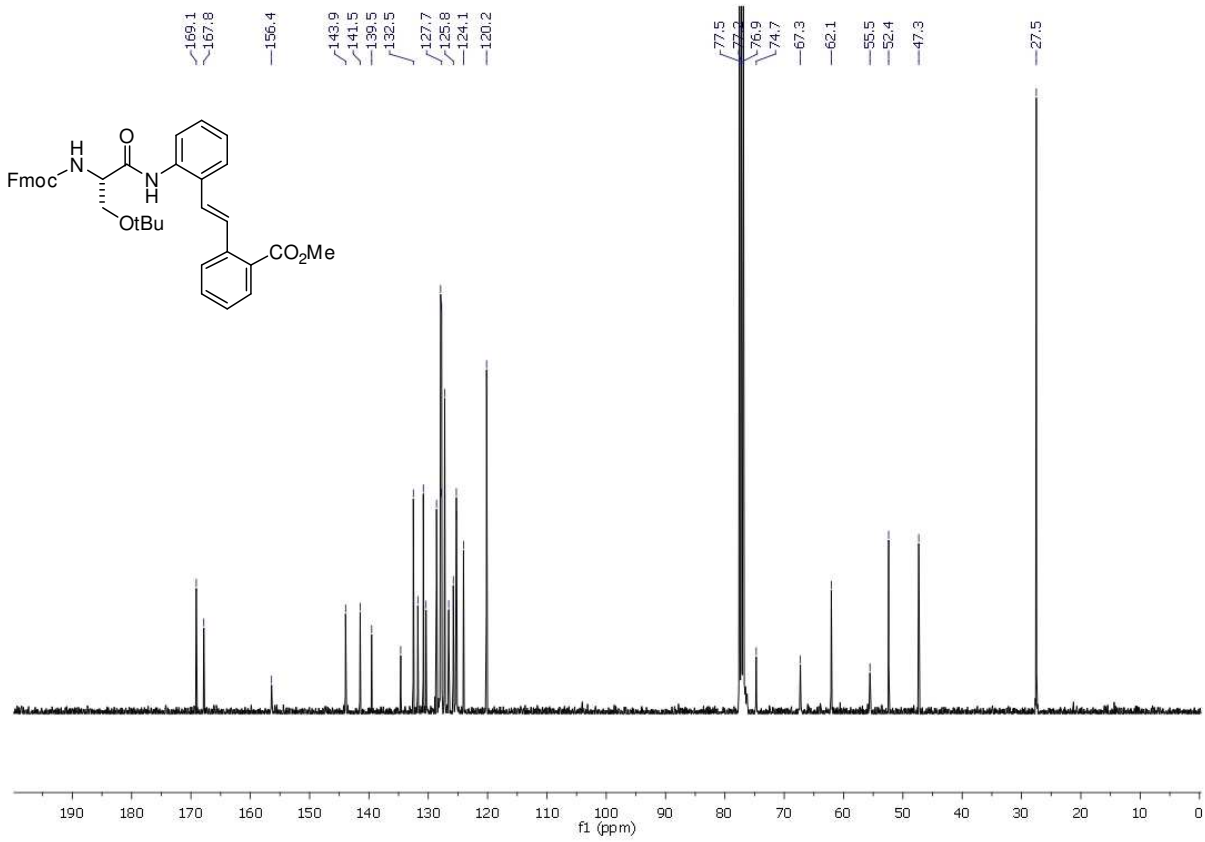
^{13}C NMR (100 MHz, $CDCl_3$): **14**



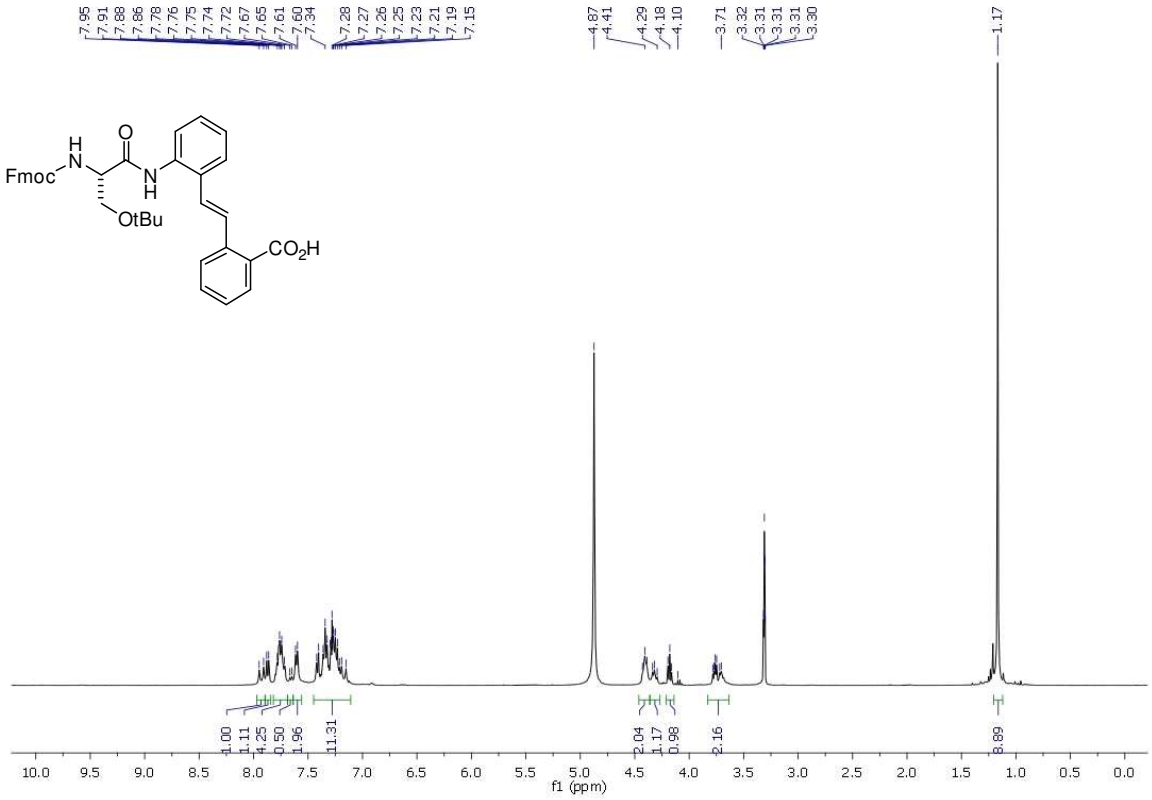
^{1}H NMR (400 MHz, $CDCl_3$): **15**



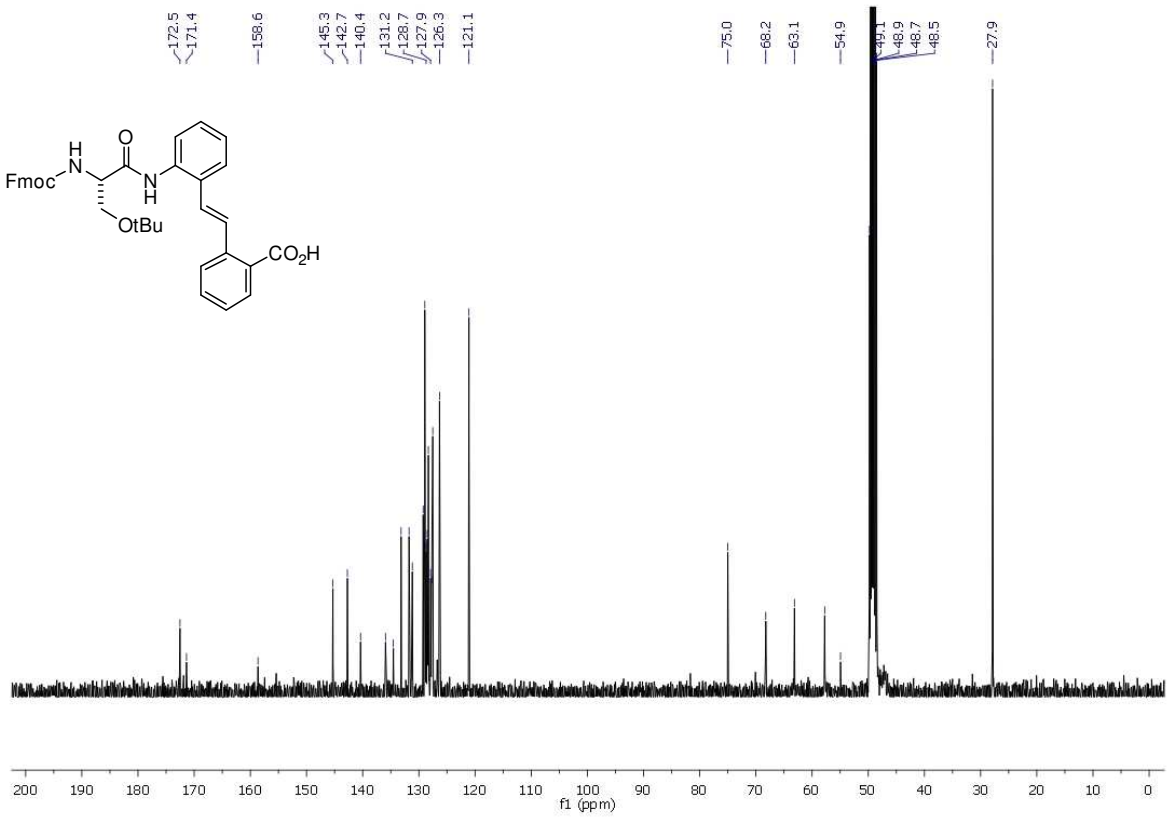
^{13}C NMR (100 MHz, $CDCl_3$): **15**



^{1}H NMR (400 MHz, CD_3OD): **16**



^{13}C NMR (100 MHz, CD_3OD): **16**

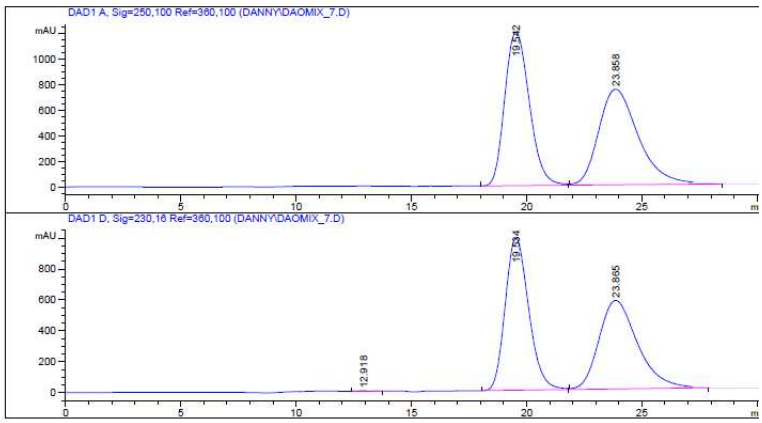


Chiral HPLC Chromatograms for 2,2'tolan dipeptides 9a and *ent*-9a

The enantiomeric purity of compounds **9a** and ***ent*-9a** were evaluated by Chiral Stationary Phse (CSP)-High Performance Liquid Chromatography (HPLC) using a Diacel Chiralpak AS-H column (25 cm × 0.46 cm i.d.) maintained at 40 ^{o}C while eluting with TFA/IPA/hexane 0.5:9.5:90 at 0.25 mL/min. Detection was by a diode arrary UV detector at 250 nm referenced to 360 nm: L-enantiomer [=(*S*)]: 99.9% ee (R_t = 19.5 min); D-enantiomer [=(*R*)]: 97.2% ee (R_t = 23.8 min).

Racemic **9a**:

```
Sample Info     : DA0049 Fmoc-LD-Ser(OtBu)-2,2'-Tolan-COOH_Analytical; Ch
                  iralcel AS-H column; Column No. ASH0CE-KJ018; 0.46 cmI.
                  D. X 25 cmL; Temp 40oC; Flow rate: 0.25/min; Detector:
                  230 and 250.0 nm; Mobile Phase: 0.5%TFA/9.5%IPA/90% n-H
                  exane; Injection: 3 microlitre, Sample conc.: 10 mg in
                  1.5 ml of 90% n-Hexane/10%IPA/
```




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=250,100 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  19.542 BB    1.0944 8.87625e4  1197.96545  49.8255
   2  23.858 BB    1.4947 8.93841e4   745.54352  50.1745

Totals :                  1.78147e5  1943.50897
```

(*S*)-enantiomer **9a**:

```
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Last changed     : 2/29/08 8:01:28 PM by Danny
                   (modified after loading)
Analysis Method  : D:\HPCHEM\1\METHODS\DANNY1.M
Last changed     : 2/29/08 9:22:30 PM by Danny
                   (modified after loading)
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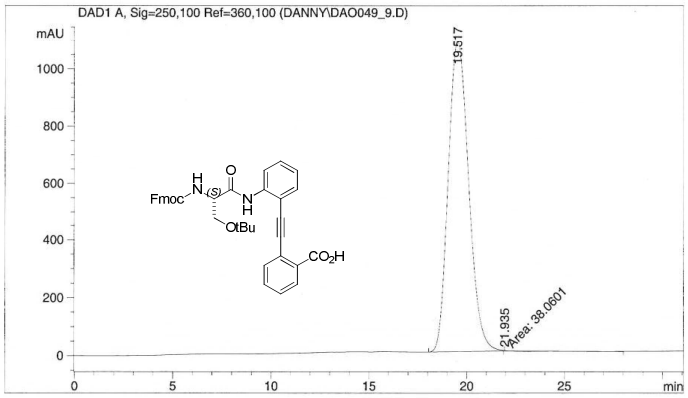


```
Signal 1: DAD1 A, Sig=250,100 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  19.517 PB    1.1509 7.85156e4  1081.57300  99.9515
   2  21.935 MM    0.2728   38.06005    1.63679   0.0485

Totals :                  7.85537e4  1083.20979

 Results obtained with enhanced integrator!
=================================================================
                        *** End of Report ***
```

(*R*)-enantiomer ***ent*-9a**:

```
                                                        Inj Volume : 1 µl
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                   (modified after loading)
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Last changed     : 2/29/08 9:22:30 PM by Danny
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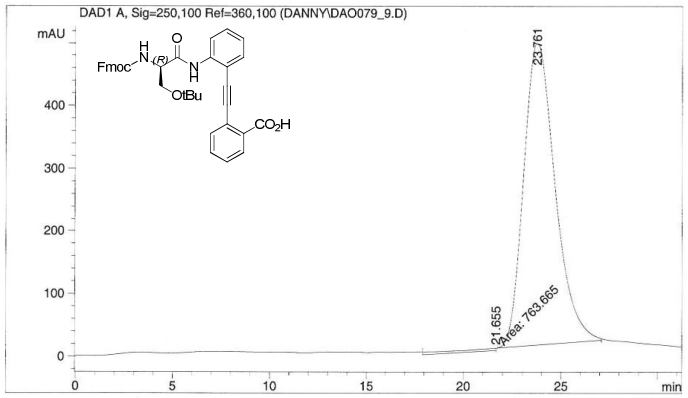


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Signal 1: DAD1 A, Sig=250,100 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  21.655 MM    3.4818  763.66479    3.65553   1.3902
   2  23.761 BB    1.6918 5.41682e4   482.50528  98.6098

Totals :                  5.49319e4   486.16081

 Results obtained with enhanced integrator!
=================================================================
                        *** End of Report ***
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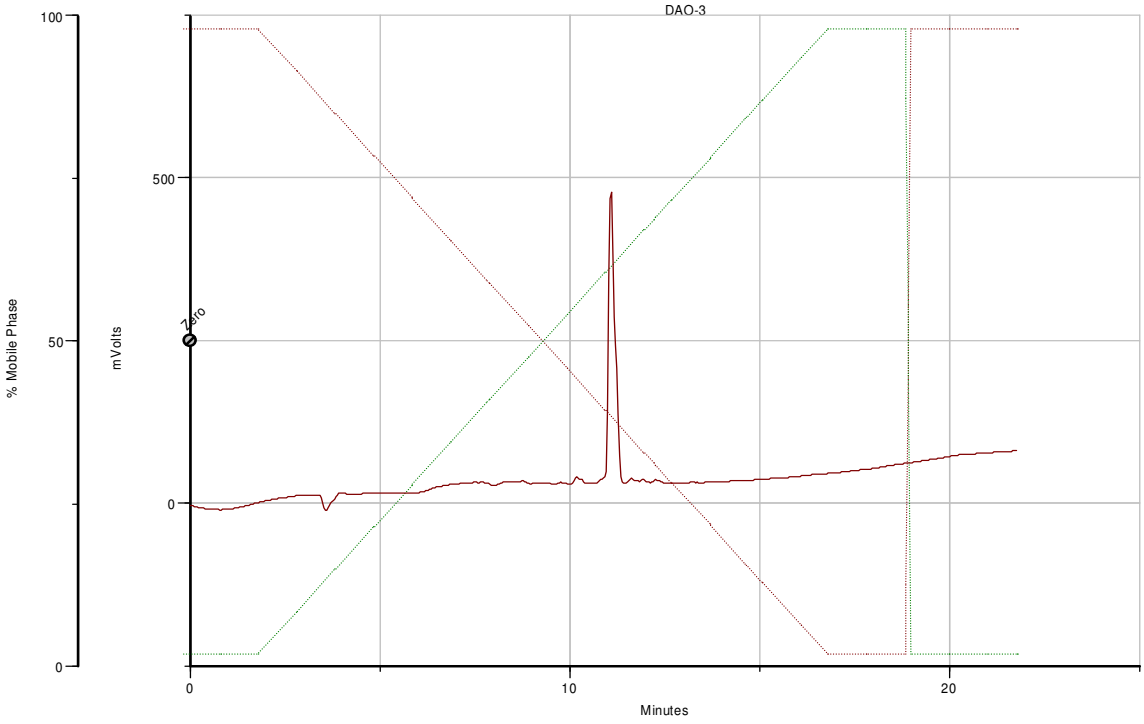
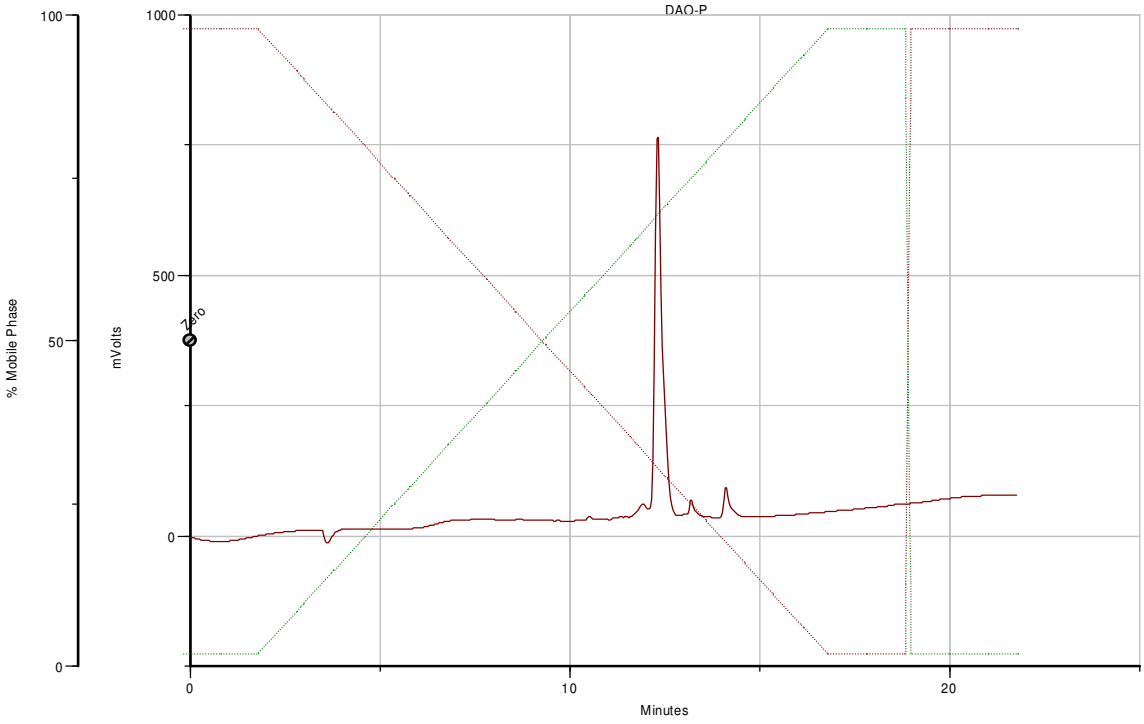
HPLC traces for selected peptidic compounds

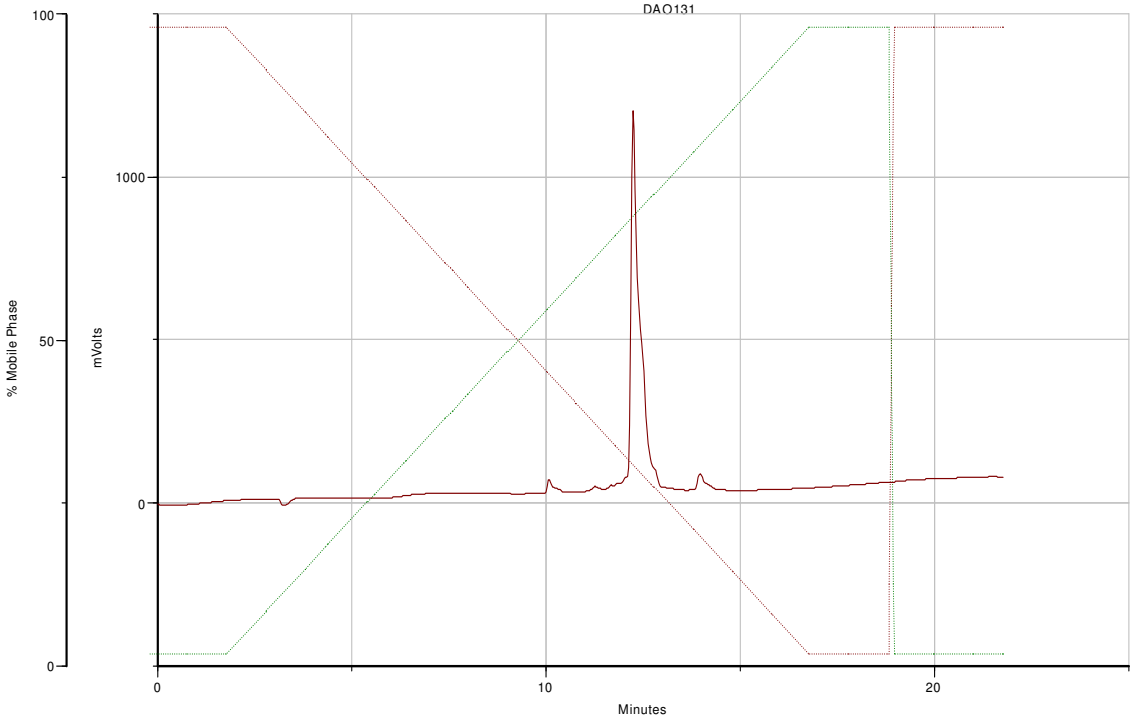
Linear HN-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys-OH (crude) **19**



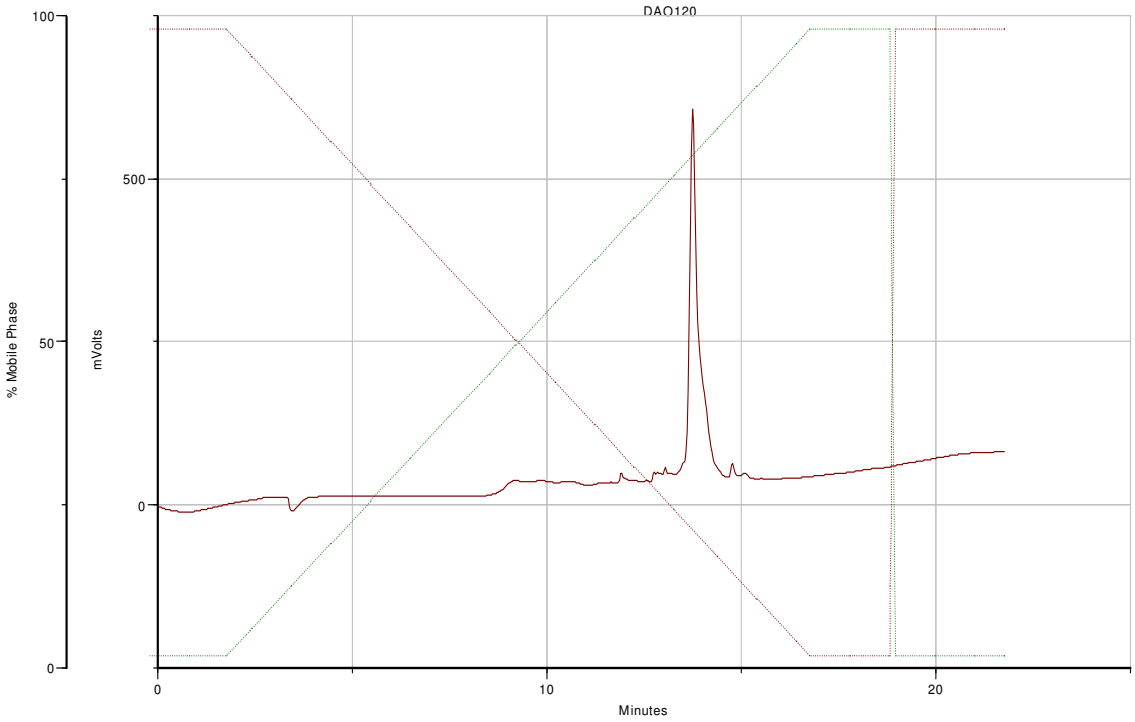
Linear H_2N-Ser-[2,2'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys-OH (crude) **21a**



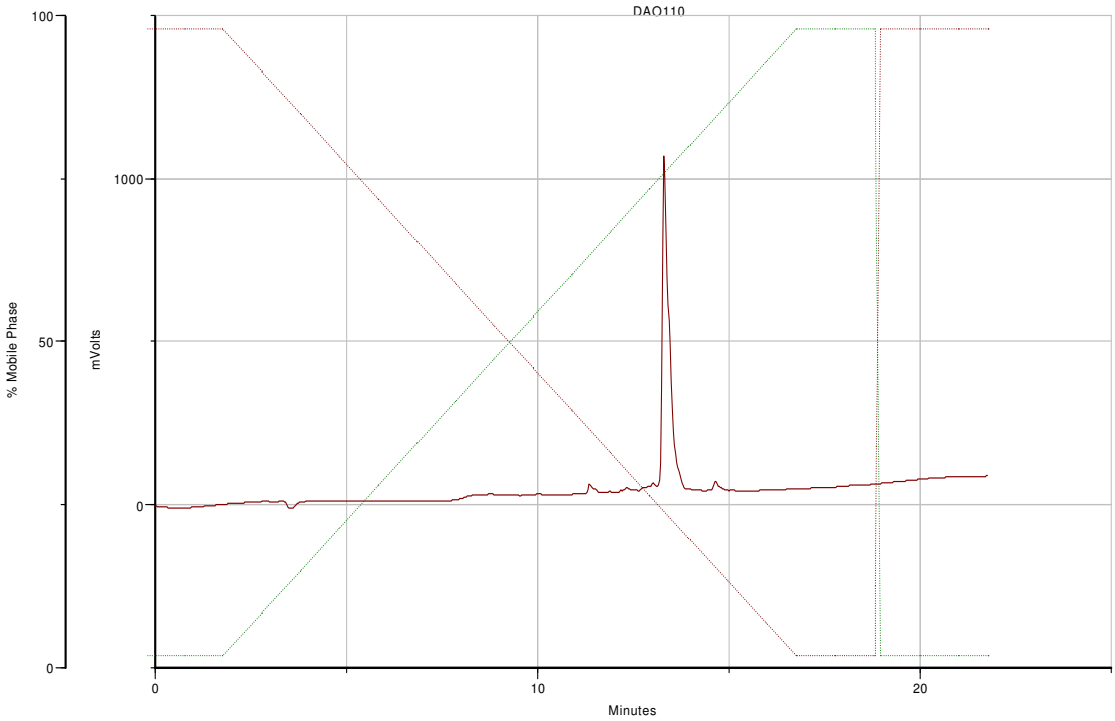
Linear H_2N-Ser-[2,3'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys-OH (crude) **21b**



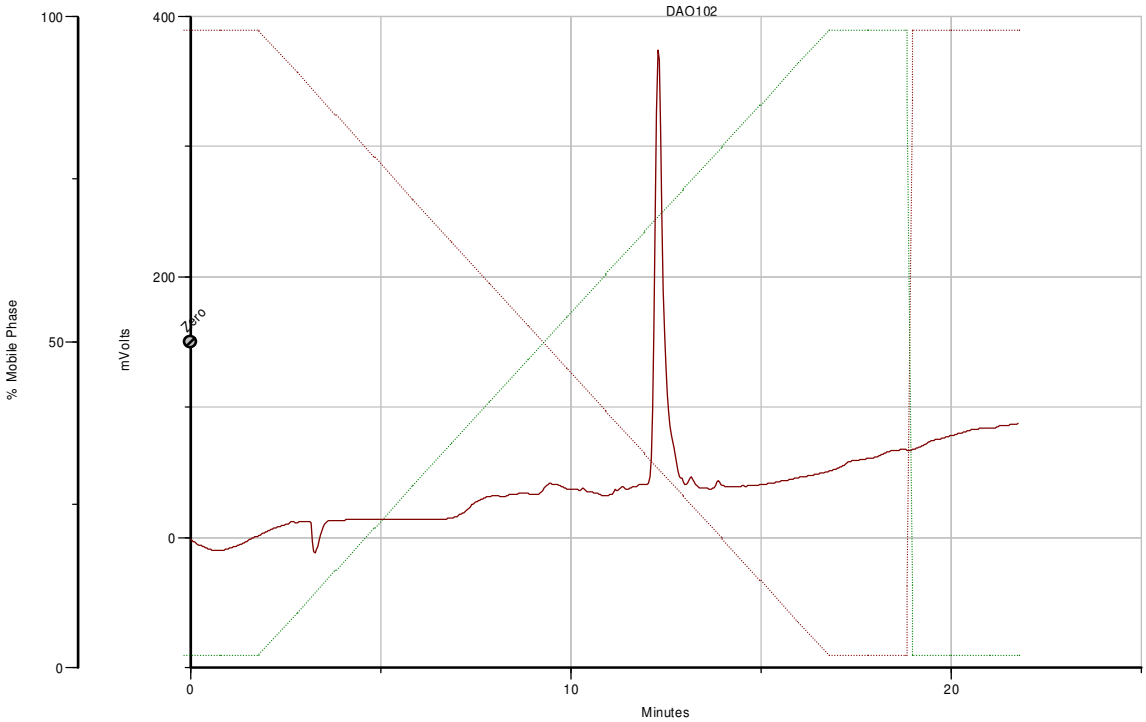
Linear H_2N-Ser-[3,2'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys-OH (crude) **21c**



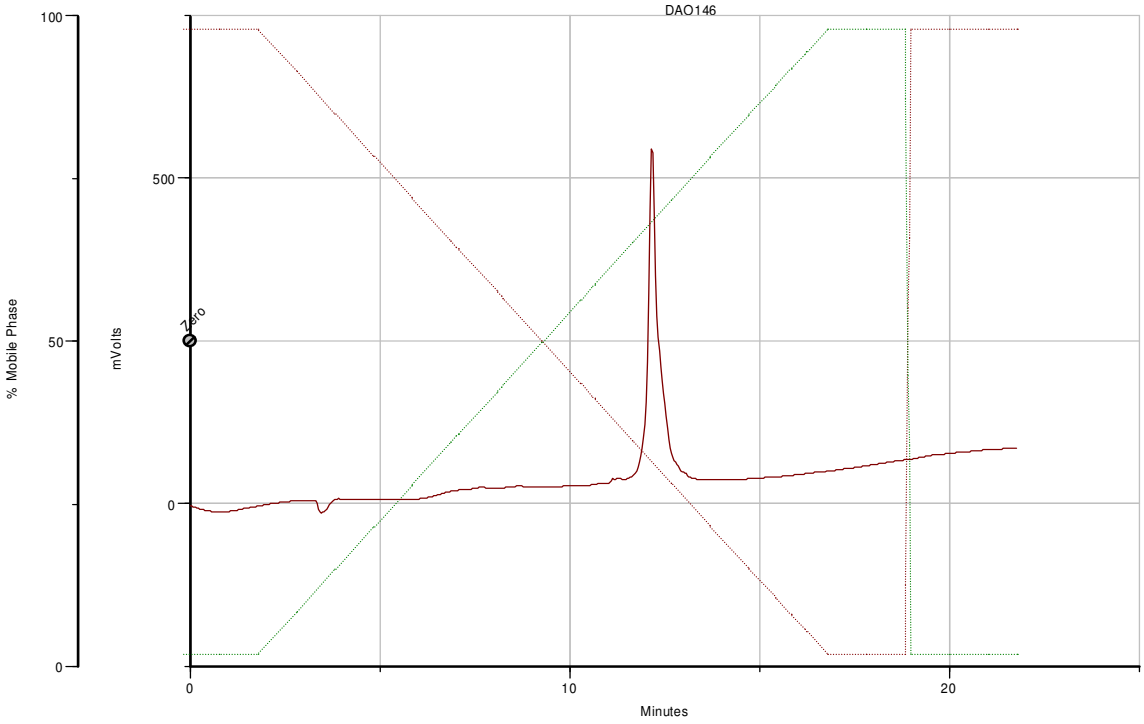
Linear H_2N-Ser-[3,3'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys-OH (crude) **21d**



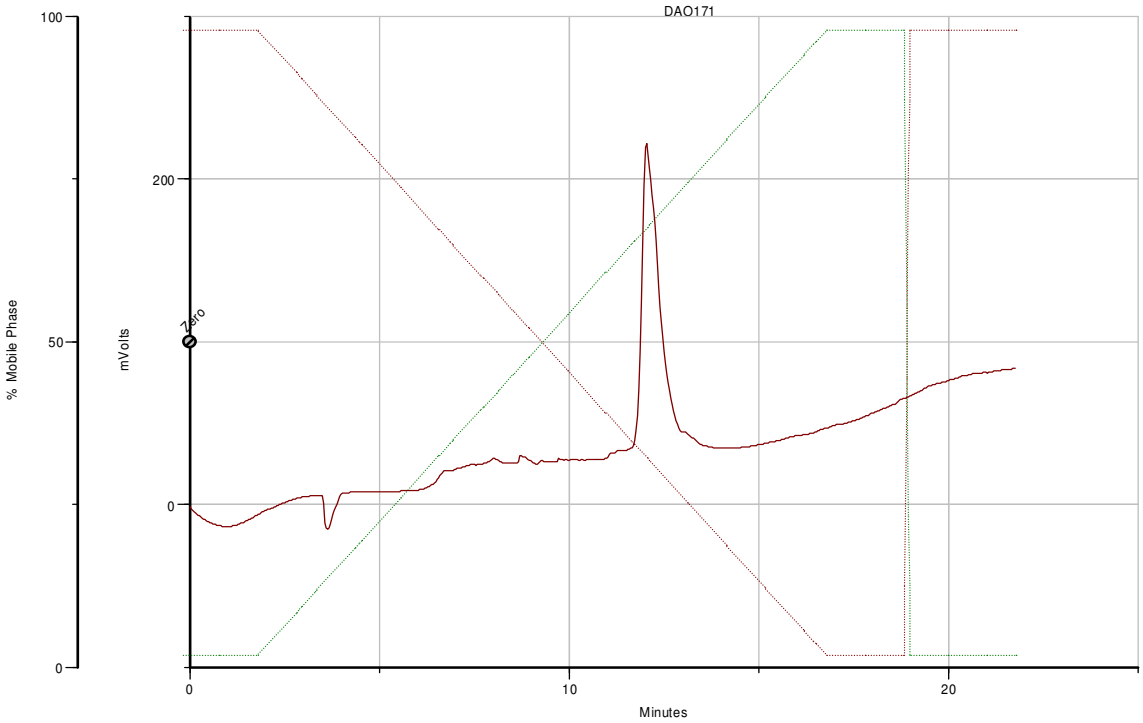
Linear H_2N-Ser-[2,2'Bibenzyl]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys-OH (crude) **21e**



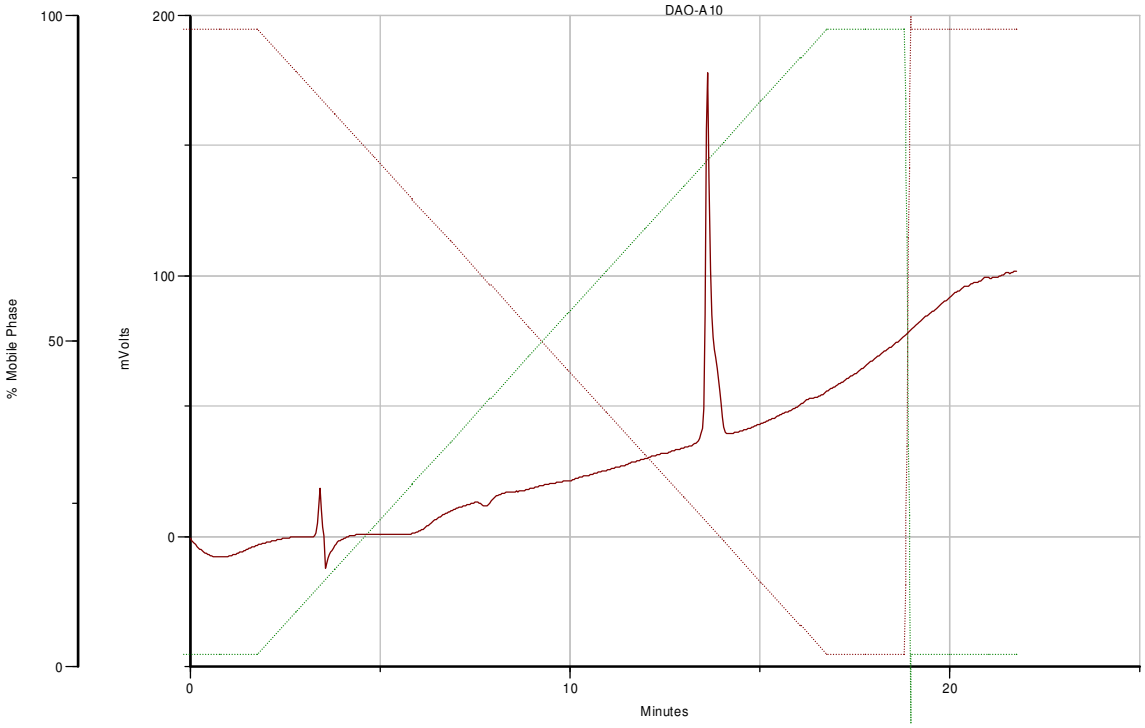
Linear H_2N-Ser-[2,2'-(*Z*)-Stilbene]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys-OH (crude) **21f**



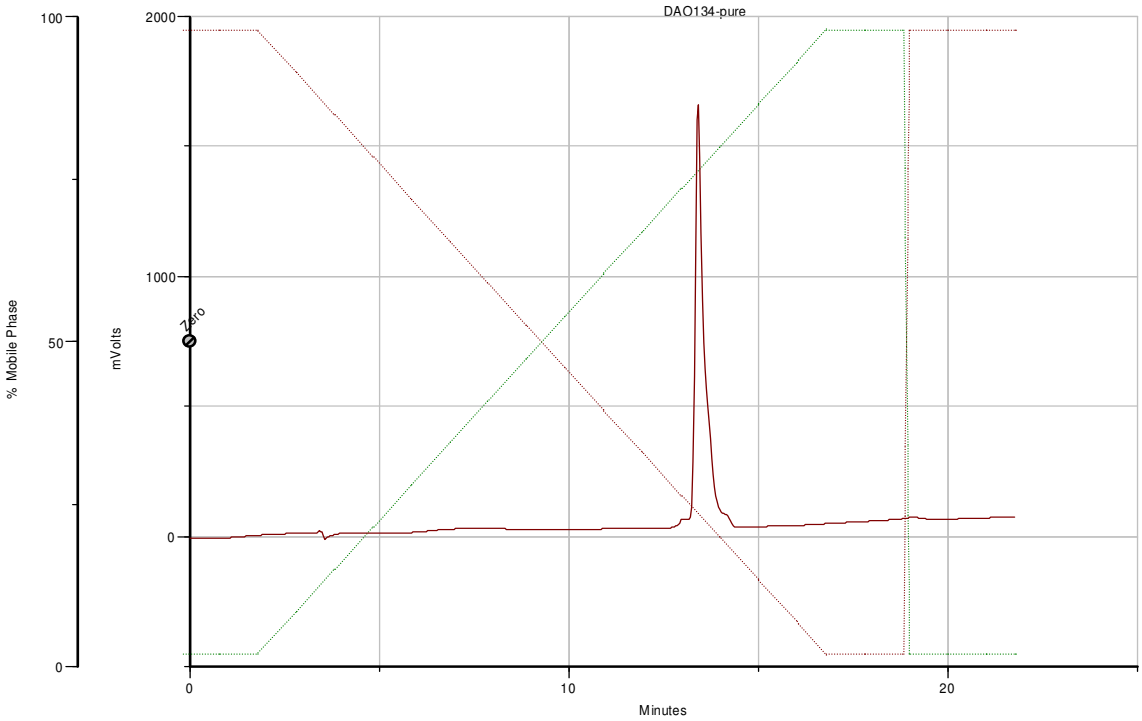
Linear H_2N-Ser-[2,2'-(*E*)-Stilbene]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys-OH (crude) **21g**



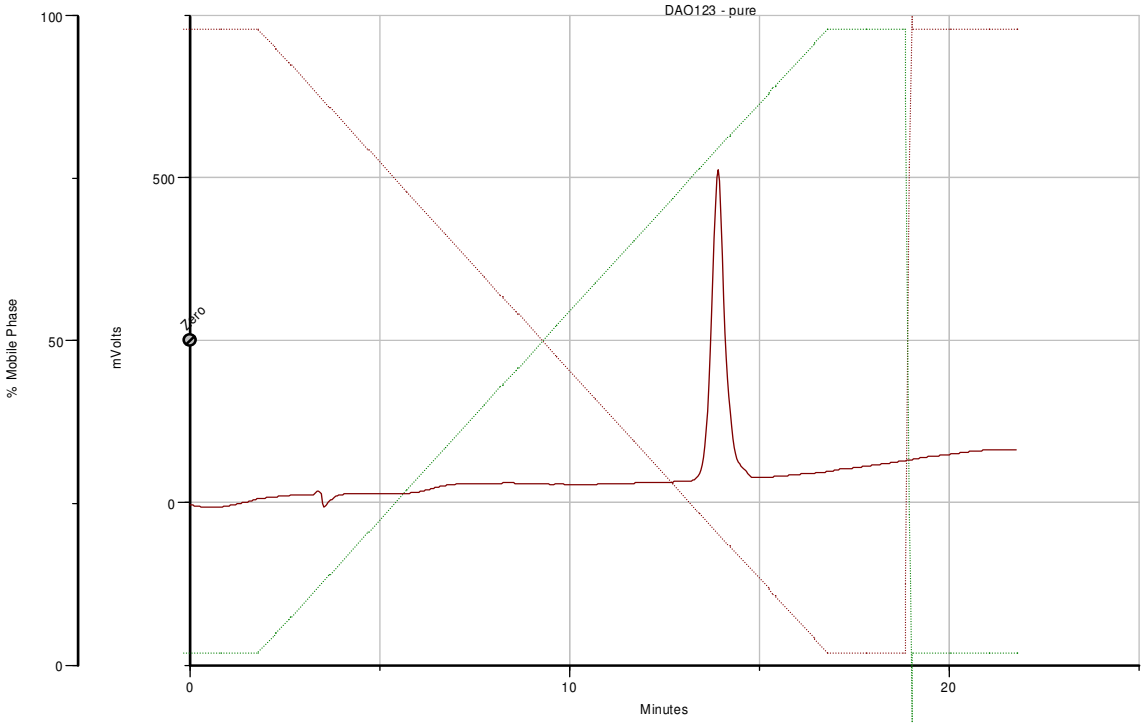
Cyclic ---Ser-[2,2'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23a**



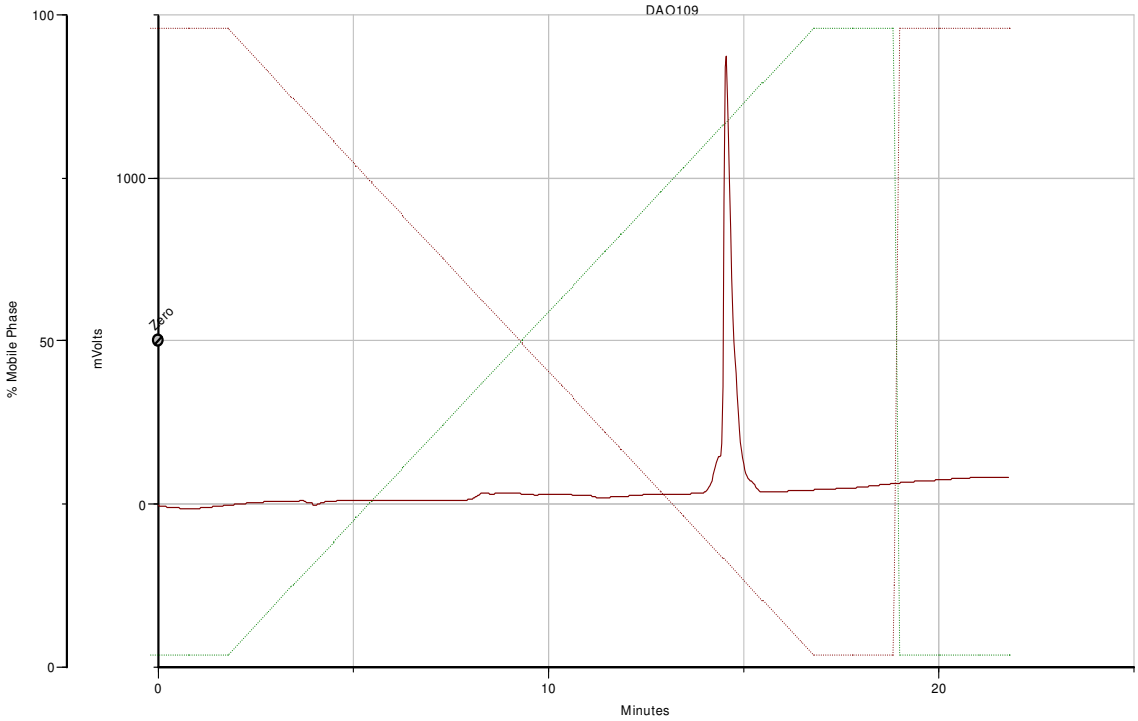
Cyclic ---Ser-[2,3'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23b**



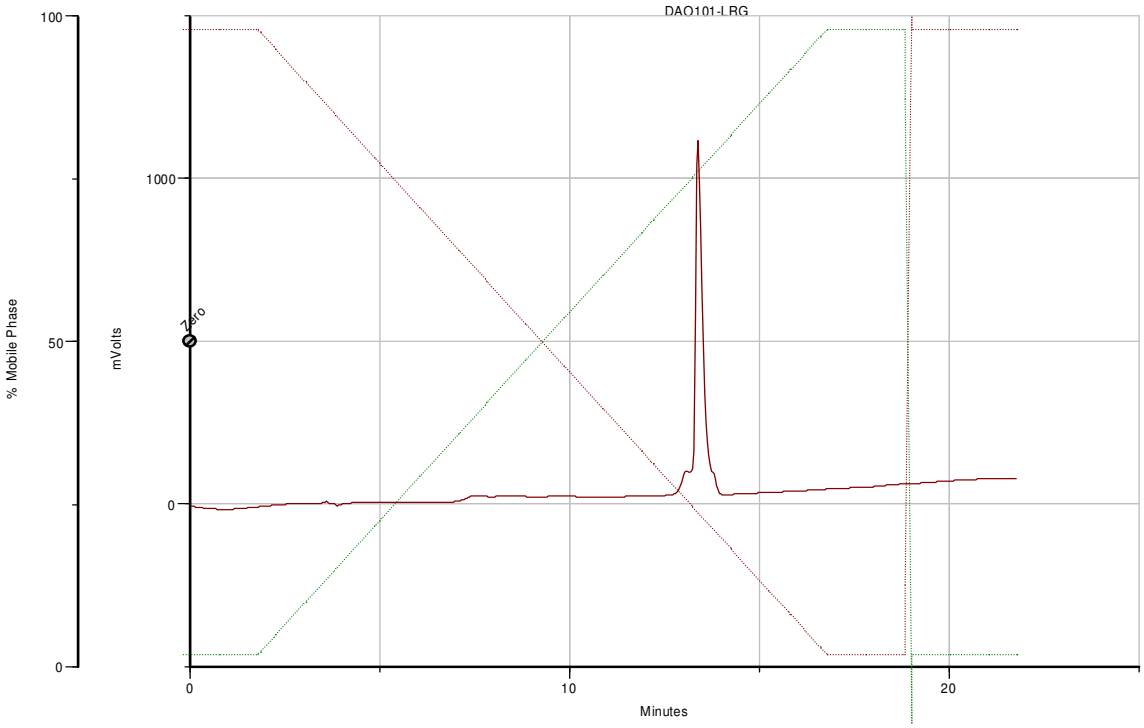
Cyclic ---Ser-[3,2'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23c**



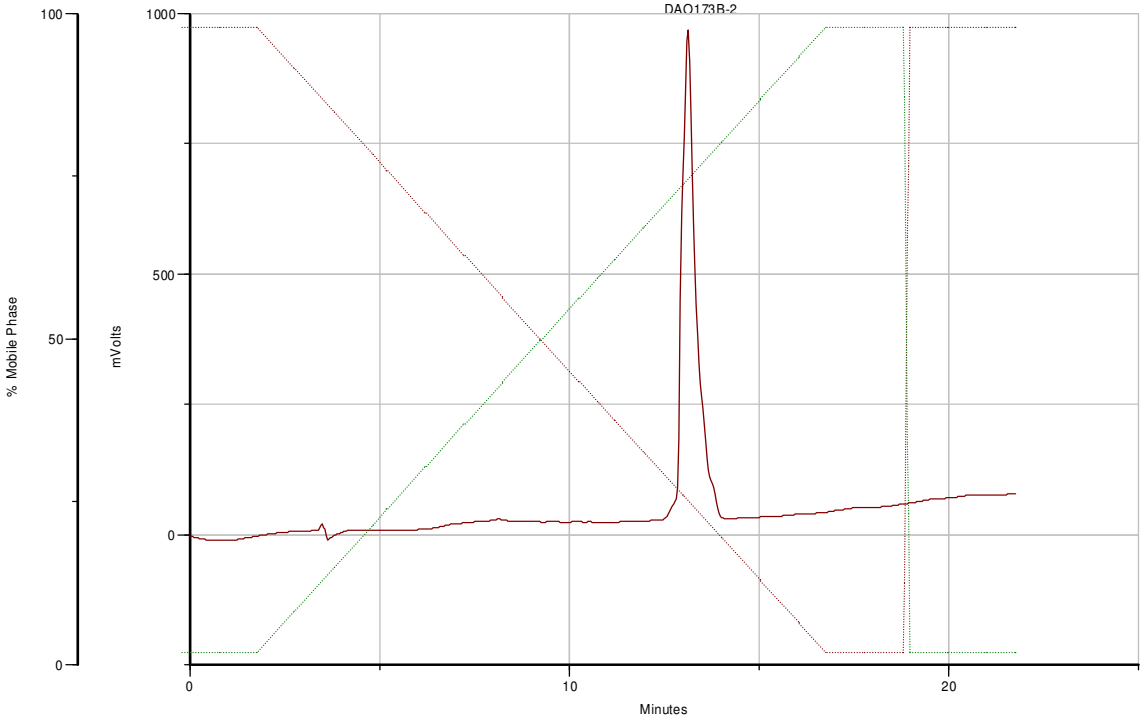
Cyclic ---Ser-[3,3'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23d**



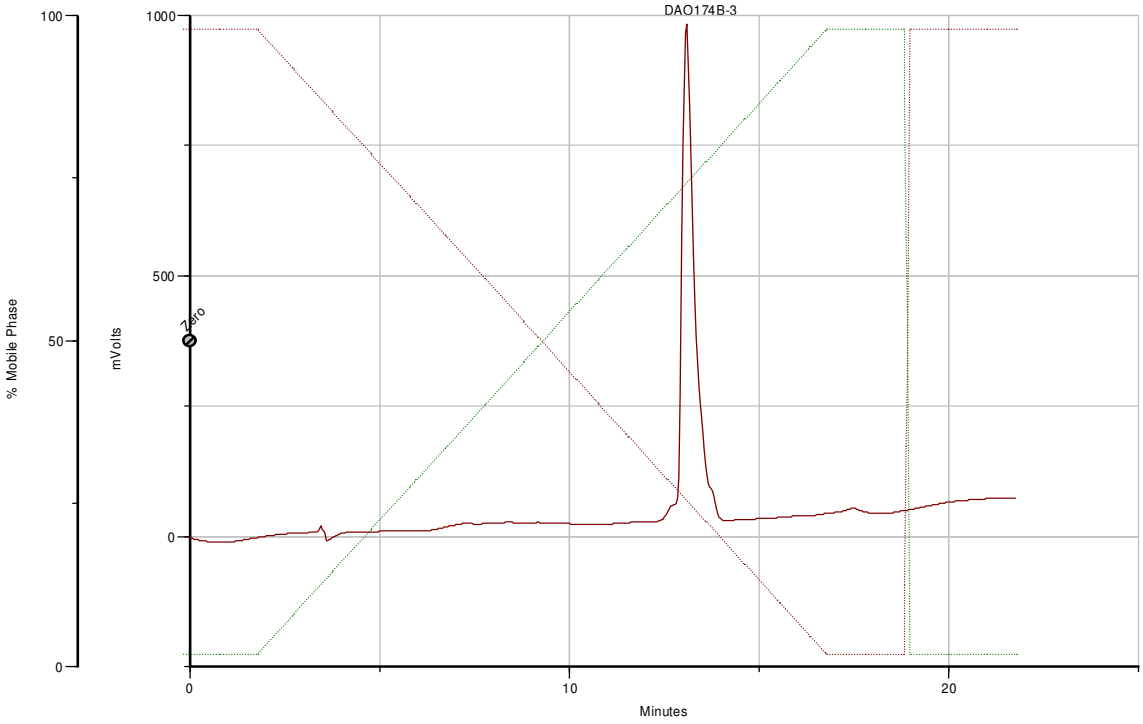
Cyclic ---Ser-[2,2'-Bibenzyl]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23e**



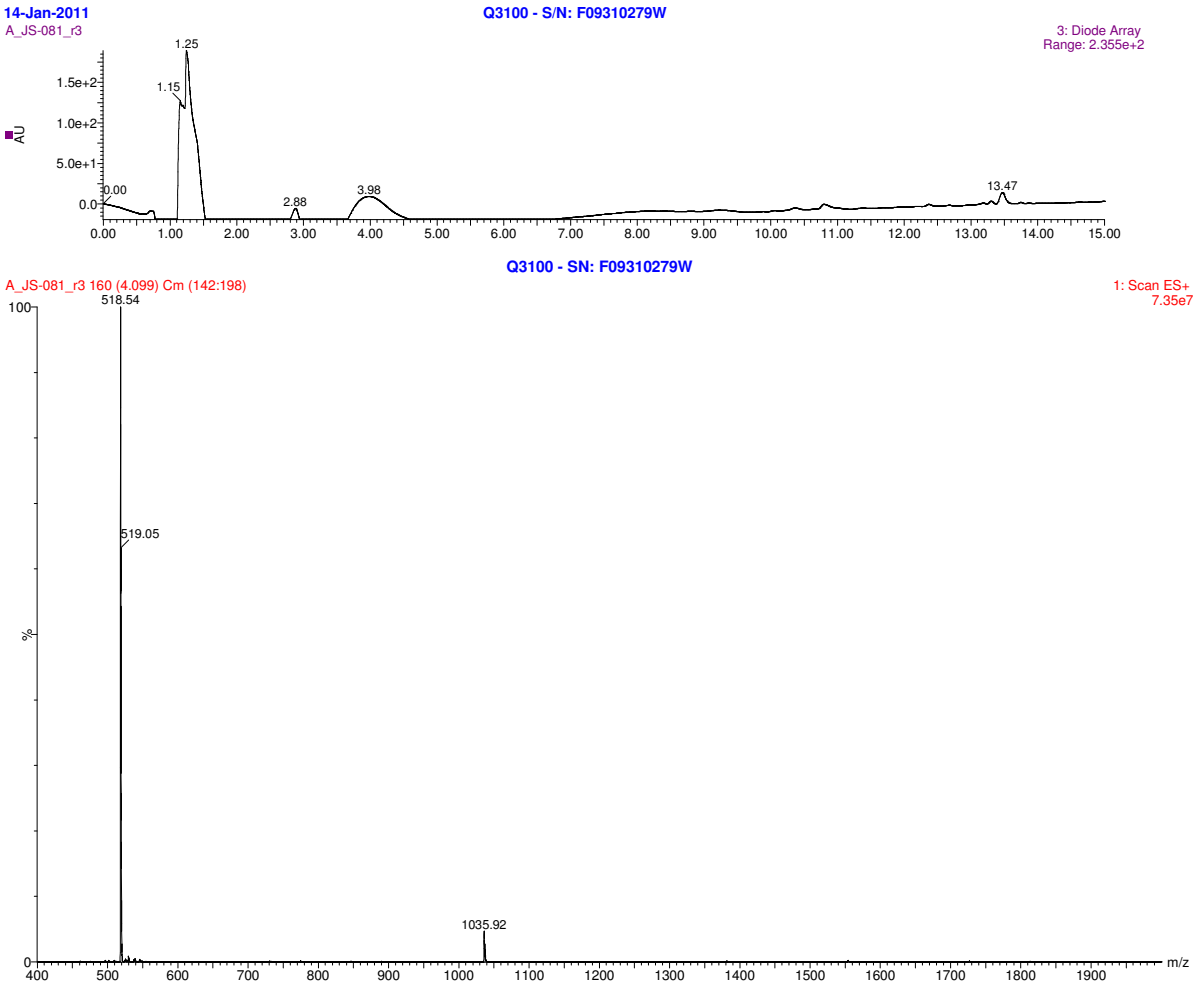
Cyclic ---Ser-[2,2'-(*Z*)-Stilbene]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23f**



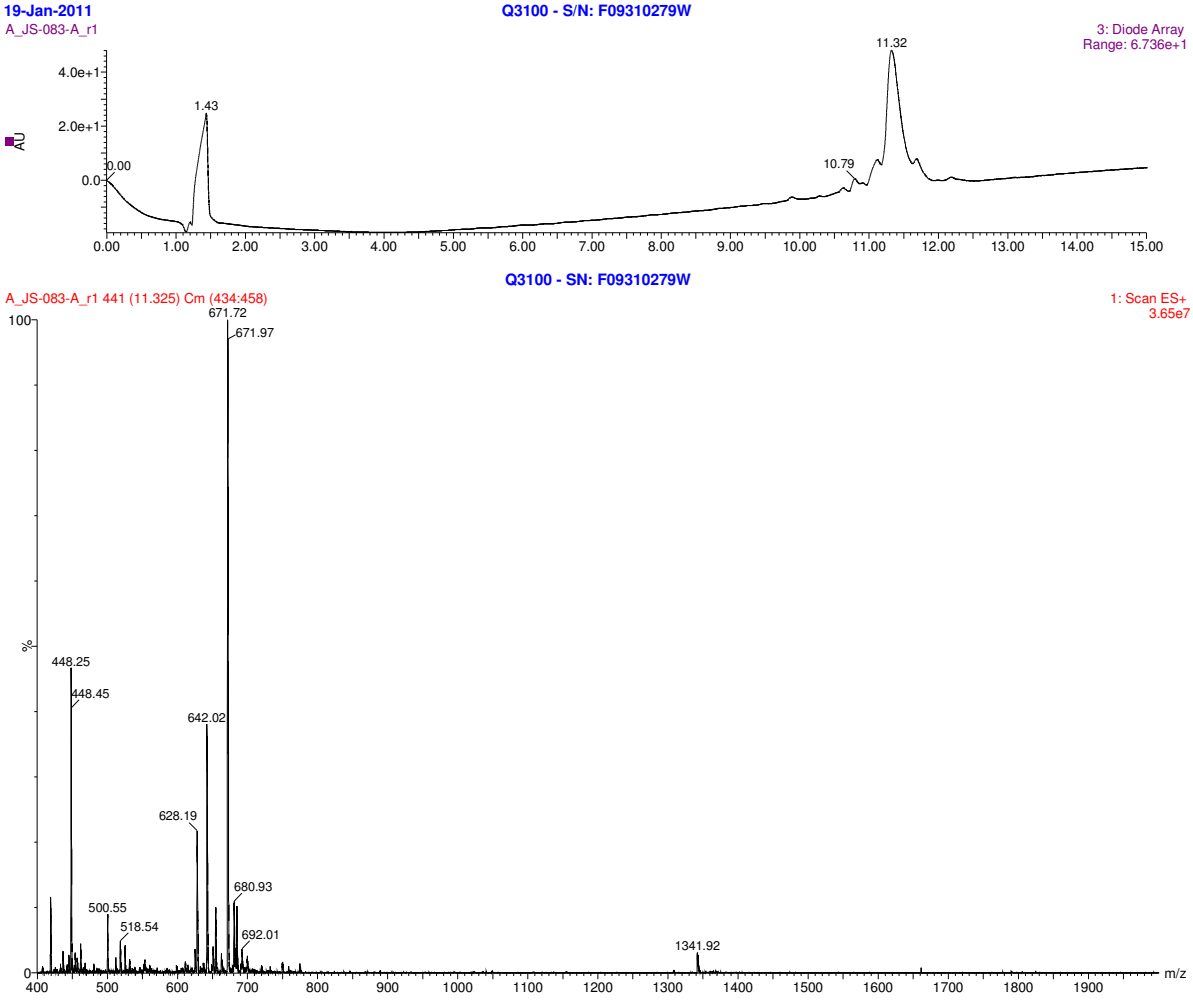
Cyclic ---Ser-[2,2'-(*E*)-Stilbene]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23g**



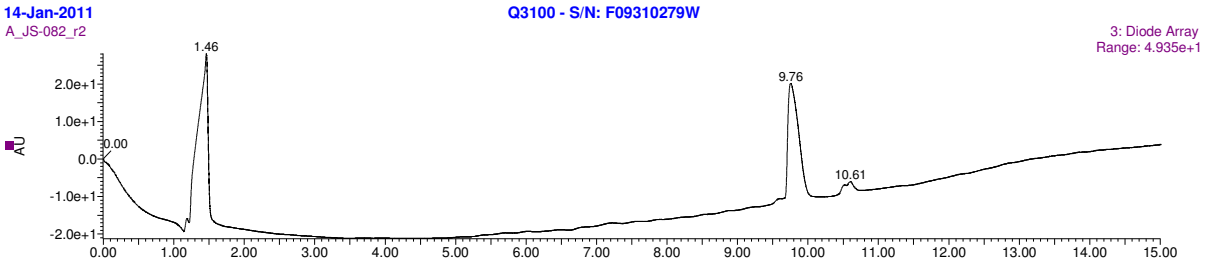
Linear HN- Phe-Arg-Pro-Phe-Ile-Leu-Asp-Lys-OH (crude) **SCR2**

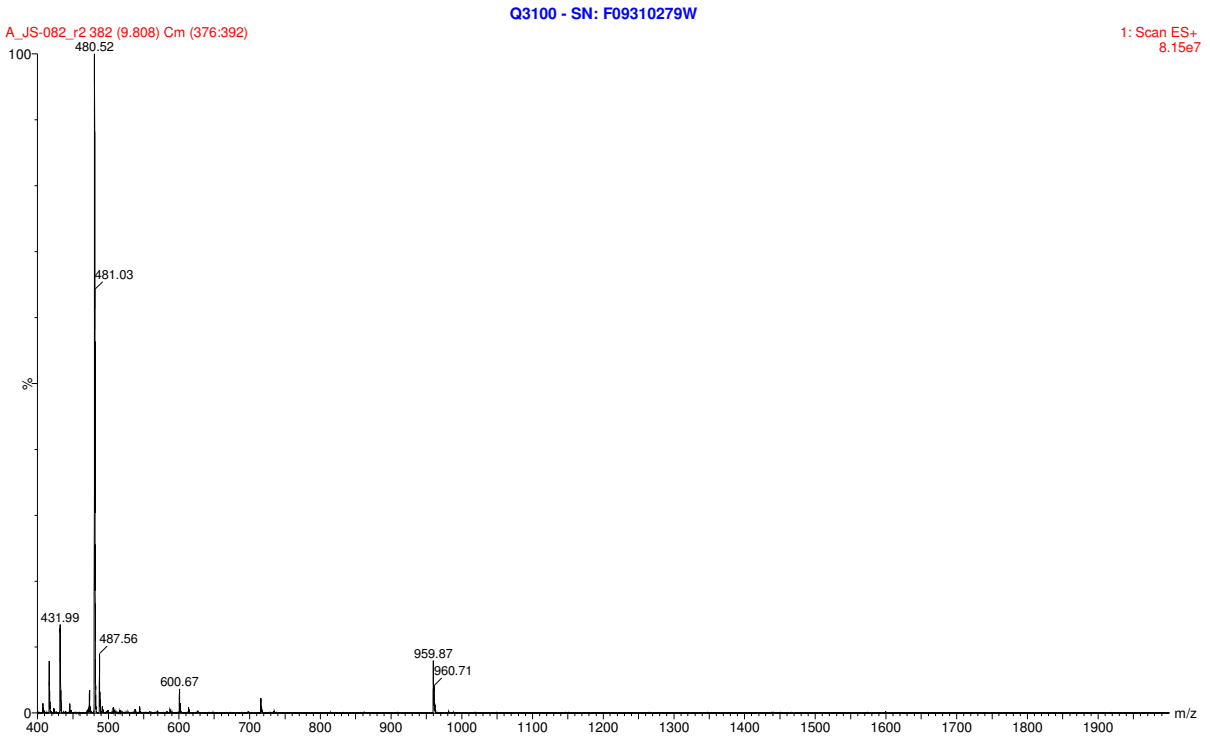


Linear H_2N-Ser-[2,2'-Tolan]-Phe-Arg-Pro-Phe-Ile-Leu-Asp-Lys-OH (crude) **SCR4**

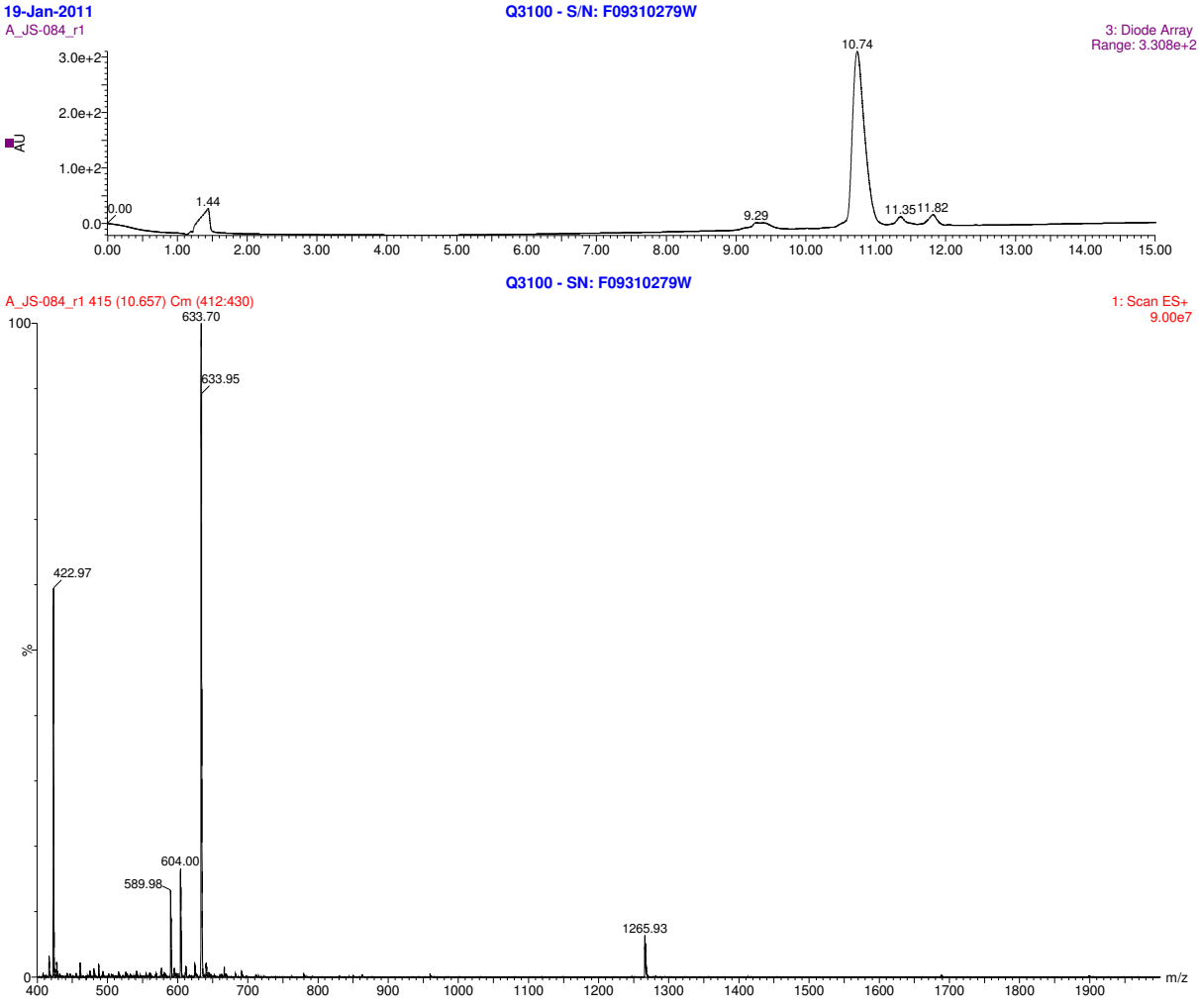


Linear HN-Pro-Phe-Asp-Leu-Ala-Ile-Arg-Lys-OH (crude) **MUT2**

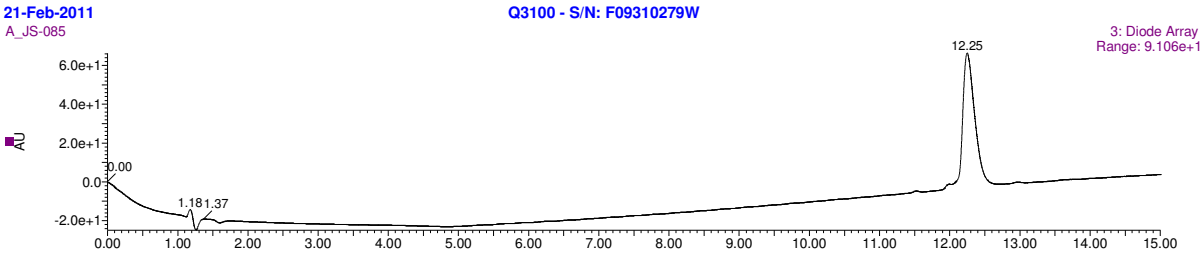




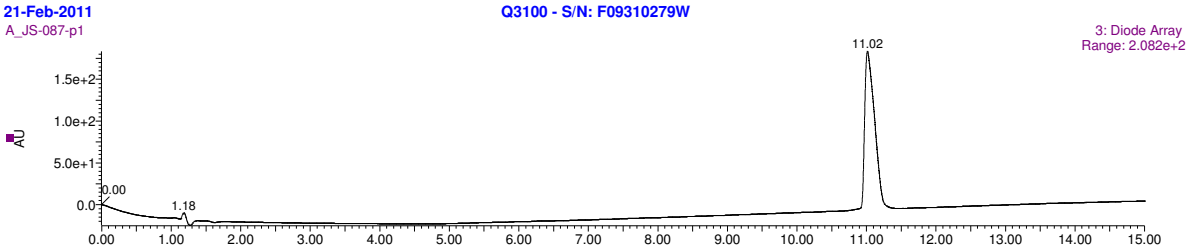
Linear H_2N-Ser-[2,2'-Tolan]-Pro-Phe-Asp-Leu-Ala-Ile-Arg-Lys-OH (crude) **MUT4**

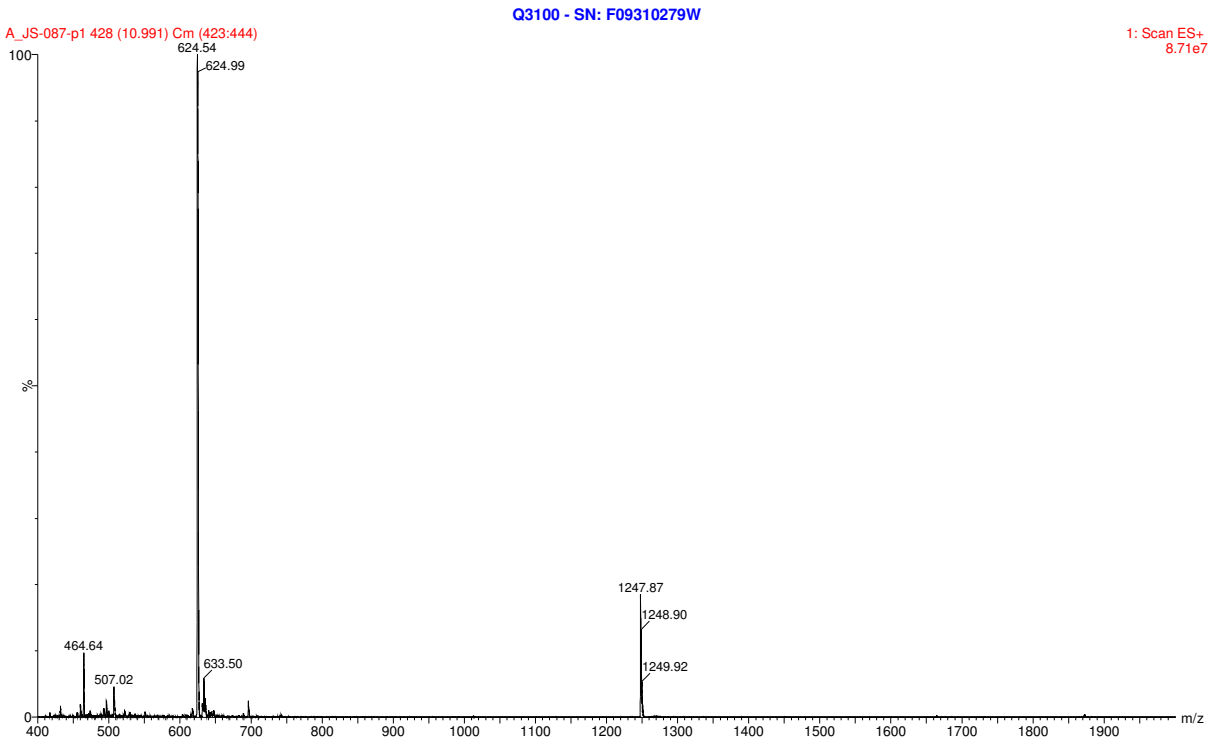


Cyclic ---Ser-[2,2'-Tolan]-Phe-Arg-Pro-Phe-Ile-Leu-Asp-Lys--- (crude) **24**



Cyclic ---Ser-[2,2'-Tolan]-Pro-Phe-Asp-Leu-Ala-Ile-Arg-Lys--- (crude) **25**




Q3100 - SN: F09310279W
A_JS-087-p1 428 (10.991) Cm (423:444)
1: Scan ES+
8.71e7
100
%
0
624.54
624.99
464.64
507.02
633.50
1247.87
1248.90
1249.92
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
m/z

IgE Receptor Binding Enzyme-linked Immunosorbent Assay (ELISA)

Compounds were evaluated in a receptor binding ELISA assay using IgE-Fc (Cε2-4) protein, a chimeric construct of the α-subunit of FcεRI fused to the Fc region of IgG4 [referred to below as AG (alphagamma) receptor protein], Biotinylated anti-IgE (Vector Laboratories), Streptavidin-Horse Radish Peroxidase conjugate (Biosource International, Inc.) and *o*-phenylenediamine dihydrochloride (OPD, Sigma-Aldrich) in P96 Maxisorp NUNC 439454 Immunoplates (Nalge Europe Ltd.) named ELISA plate.

The protocol was as follows: The ELISA plate was coated with 100 µL/well of an 2 µg mL^{-1} AG receptor protein solution made up in aqueous carbonate buffer (0.045 M $NaHCO_3$ + 0.018 M Na_2CO_3, pH 9.6) then was incubated overnight at 4 °C. After 3 washing steps with 300 µL/well of PBS, 0.1% Tween 20 v/v for 5 min, to remove residual AG protein, the unbound sites were blocked by adding 300 µL/well of PBS, 2 % Bovine Serum Albumin (BSA) v/v. The plate was then incubated for 1 h at 37 °C. Controls and peptidomimetic solutions (*i.e.* compounds **23a-g**, **24** and **25** were prepared on a classic clear 96-wells plate (NUNC 262162) separately from the ELISA plate. Each well has a total volume of 110 µL and a final DMSO concentration of 5% v/v. The antagonists were tested in duplicate from 5 mM to 11.4 µM with 1.5-fold dilution or 2.5 mM to 39.1 µM with 2-fold dilution. Six different controls named Fc, D, RB, AG, P and W were prepared in triplicate for each plate as indicated in the table below. Rose Bengal (Sigma-Aldrich) was used as a control antagonist as well as alphagamma. In all the wells except in the P and W controls, were added 20 µL of an IgE Fc solution (0.0137 ng/µL in PBS) in order to have a total volume of 110 µL. The plate was covered and left at room temperature for a 1 h pre-incubation step.

Controls for 1 well volumes in µL	IgE Fc 0.0137 ng/µL in PBS	DMSO	Rose Bengal 10 mM DMSO	Alphagamma 1.15 mg/mL in PBS	PBS	MilliQ water
Fc	20	-	-	-	90	-
RB	20	5.39	0.11	-	84.5	-
AG	20	5.5	-	1	83.5	-
D	20	5.5	-	-	84.5	-
P	-	5.5	-	-	104.5	-
W	-	5.5	-	-	-	104.5

The blocked ELISA plate was washed with PBS-Tween 20 (0.1% v/v, 3 × 300 µL/well, 2-3 min) then 100 µL/well were transferred from the pre-incubated plate to the ELISA plate keeping to the same template. The ELISA plate was then incubated for 1 h at 37 °C to allow the binding of IgE to its receptor. The plate was washed with PBS-Tween 20 (0.1% v/v, 3 ×

300 µL/well) with a first washing step of 30 min, then 5 min for the 2 others washing steps in order to ensure the removal of the compounds. Bound IgE-Fc was detected by adding 100 µL/well of biotinylated anti-IgE (1/5000 dilution in PBS, 1% v/v BSA), then the plate was incubated for 1 h at 37 °C. After 3 washing steps with PBS-Tween 20 (0.1% v/v, 300 µL/well, 5 min), 100 µL/well of streptavidin-HRP (1/5000 dilution in PBS, 1% v/v BSA) were added. The plate was incubated 1 h at 37 °C, and then washed with PBS (3 × 300 µL/well, 5 min). The HRP substrate solution was prepared by dissolving 1 tablet of OPD in 9 mL of water and 1 mL of 10X peroxide buffer, then 50 µL/well were added to the plate. After 4 min incubation at room temperature in the dark, the enzyme reaction was stopped by addition of 50 µL/well of 3M HCl. Absorbance detection of the plate was then performed at 492 nm using an ELISA plate reader (Titertek Multiskan).

All the titrations were performed twice in duplicate. All data were plotted and analysed using GraFit programme (Erithacus Software Ltd., Horley, UK). For each well, the percent of inhibition was calculated using the following equation (1):

$$\% \text{ activity} = 100*(OD_{492\ nm} - \mu c-) / (\mu c+ - \mu c-) \quad (1)$$

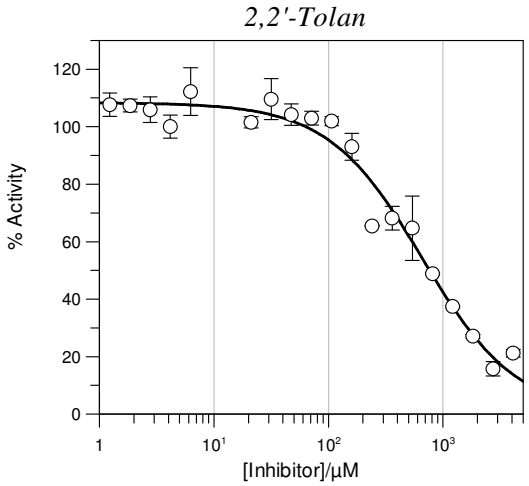
in which $OD_{492\ nm}$ is the absorbance measured at 492 nm; µc- is the average of the negative control P (100% inhibition); µc+ is the average of the positive control D (0% inhibition).

To obtain the inhibition curves, the logarithms of each compounds concentration were plotted *versus* the average inhibition percentages obtained for each concentration. The data were fitted using the sigmoidal equation (2):

$$y = \{Range/[1+(x/IC_{50})^{s}]\}+Background \quad (2)$$

where Range = fitted uninhibited value minus the Background, s = slope factor; this equation assumes that y falls with increasing x.

Cyclic ---Ser-[2,2'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23a**

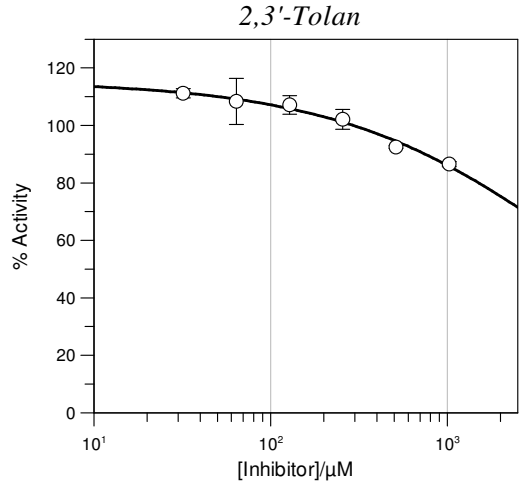


Reduced Chi²: 35.1703

Parameter	Value	Std. Error
Y Range	108.3849	2.4573
IC 50	659.3038	67.3222
Slope factor	1.0570	0.1054

This antagonist was tested in duplicate from 5 mM to 11.4 µM with 1.5-fold dilution.

Cyclic ---Ser-[2,3'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23b**

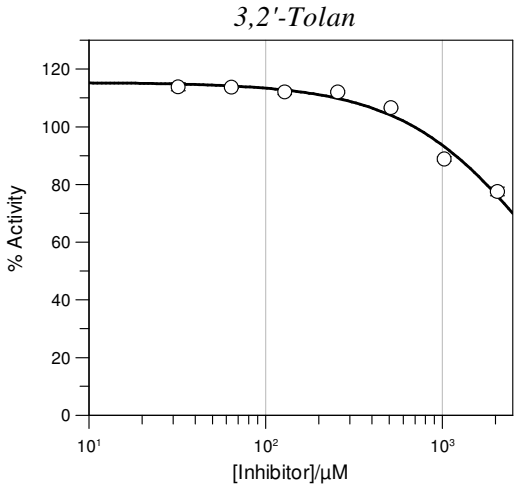


Reduced Chi²: 2.6683

Parameter	Value	Std. Error
Y Range	115.4961	3.9587
IC 50	5307.8969	1787.7662
Slope factor	0.6438	0.1722

This antagonist was tested in duplicate from 1.0 mM to 39.1 µM with 2-fold dilution.

Cyclic ---Ser-[3,2'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23c**

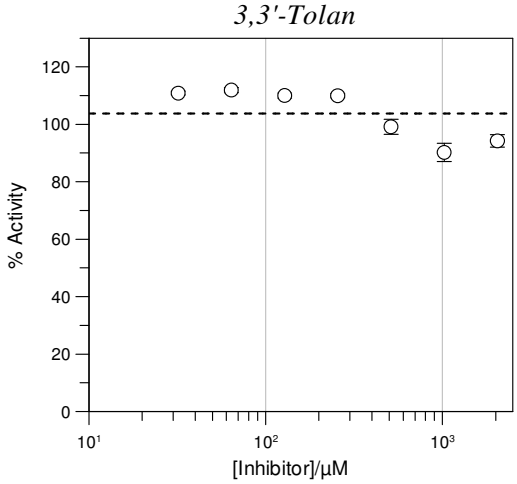


Reduced Chi²: 8.5977

Parameter	Value	Std. Error
Y Range	115.3926	2.3345
IC 50	3683.5801	606.4306
Slope factor	1.1187	0.2259

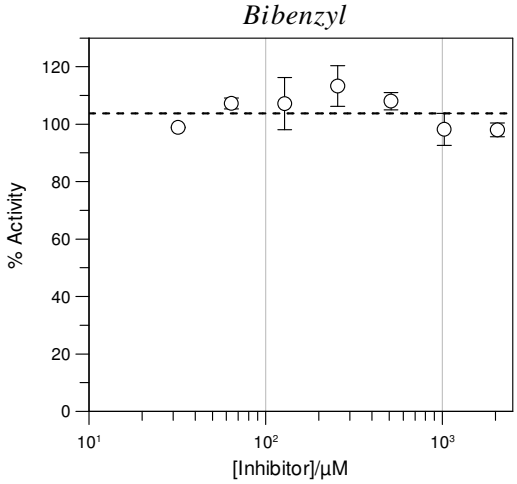
This antagonist was tested in duplicate from 2.5 mM to 39.1 µM with 2-fold dilution.

Cyclic ---Ser-[3,3'-Tolan]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23d**



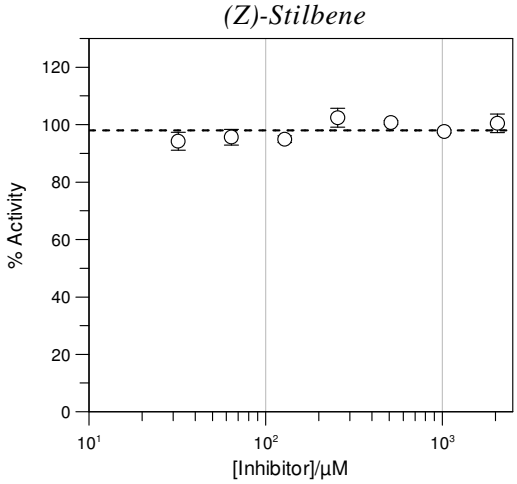
This antagonist was tested in duplicate from 2.5 mM to 39.1 µM with 2-fold dilution.

Cyclic ---Ser-[2,2'-Bibenzyl]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23e**



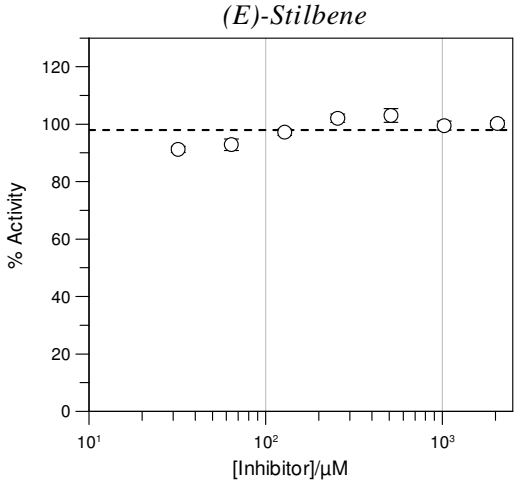
This antagonist was tested in duplicate from 2.5 mM to 39.1 µM with 2-fold dilution.

Cyclic ---Ser-[2,2'-(*Z*)-Stilbene]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23f**



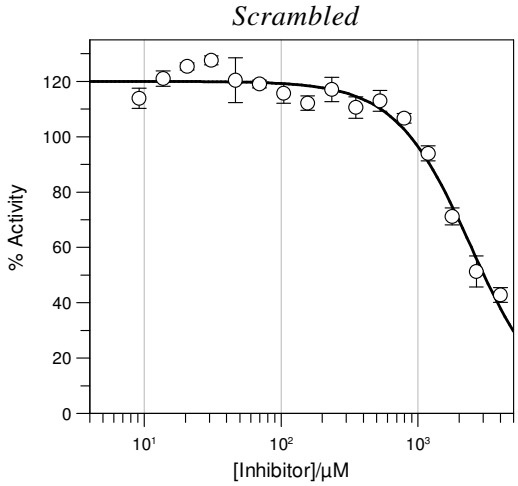
This antagonist was tested in duplicate from 2.5 mM to 39.1 µM with 2-fold dilution.

Cyclic ---Ser-[2,2'-(*E*)-Stilbene]-Pro-Phe-Asp-Leu-Phe-Ile-Arg-Lys--- (crude) **23g**



This antagonist was tested in duplicate from 2.5 mM to 39.1 µM with 2-fold dilution.

Cyclic ---Ser-[2,2'-Tolan]-Phe-Arg-Pro-Phe-Ile-Leu-Asp-Lys--- (crude) **24**

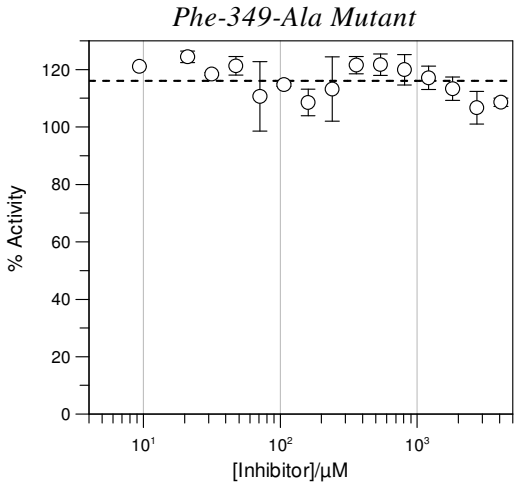


Reduced Chi²: 22.1228

Parameter	Value	Std. Error
Y Range	120.0163	1.6768
IC 50	2457.9887	149.5992
Slope factor	1.5705	0.1654

This antagonist was tested in duplicate from 5 mM to 11.4 µM with 1.5-fold dilution.

Cyclic ---Ser-[2,2'-Tolan]-Pro-Phe-Asp-Leu-Ala-Ile-Arg-Lys--- (crude) **25**



This antagonist was tested in duplicate from 5 mM to 11.4 µM with 1.5-fold dilution.