Ring-Closing Metathesis of C-Terminal Allylglycine Residues with an N-Terminal β-Vinyl-Substituted Phosphotyrosyl Mimetic as an Approach to Novel Grb2 SH2 Domain-Binding Macrocycles

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Ring-closing metathesis (RCM) of peptides often requires insertion of allylglycines at the intended sites of ring juncture, which can result in the displacement of residues that are needed for biological activity. This type of side-chain deletion can be avoided by appending β-vinyl substituents onto the parent residues at the intended sites of ring juncture, thereby effectively converting them into functionalized allylglycine equivalents. Such an approach has been previously applied in modified form to growth-factor receptor bound 2 (Grb2) SH2 domain-binding peptides by using an N-terminal β-vinyl-functionalized phosphotyrosyl mimetic and C-terminal 2-allyl-3-aryl-1-propanamides that lacked the α-carboxyl portion of allylglycine residues. These C-terminal moieties involved lengthy synthesis and once prepared, required an individual total synthesis of each final macrocycle. Work reported herein significantly enhances the versatility of the original approach through the use of C-terminal allylglycine amides that can be prepared from commercially available L- and D-allylglycines and suitable amines. This methodology could be generally useful where macrocylization is desired with maintenance of functionality at a site of ring juncture.

Introduction

Growth-factor receptor-bound 2 (Grb2)[1,2] proteins are noncatalytic docking modules that consist of one SH2 domain and two SH3 domains that participate in oncogenic signaling by receptor protein tyrosine kinases, and include the breast cancer-related erbB-2.[3,4] Because inhibition of Grb2-dependent signaling could attenuate cancerous growth, binding antagonists of SH2[5,6] and SH3 domains[7,8] are being developed by several groups. Compound **1** depicts a potential inhibitor directed against the Grb2 SH2 domain (Scheme 1). The structure of **1** is based on preferential binding of the "pTyr-Xxx-Asn-Yyy" sequences,[9,10] wherein critical recognition components are provided by the pTyr phenylphosphate and Asn side chains. Additionally, lipophilic functionality located C-terminally to the Asn residue ("R^{2}" in Scheme 1) has been included for interaction with a region of the protein formed by LeuβD1 and LysβD6 side chains of the Grb2 SH2 domain protein.[11] Because the binding of peptide ligands to Grb2 SH2 domains occurs in β-bend conformations,[10] attempts at affinity enhancement through induction of conformational constraint have been examined by using a variety of cyclization strategies, including disulfide-bond formation.[12,13] Of the several current approaches toward the synthesis of cyclic β-turn mimetics,[14] ring-closing metathesis (RCM) has become an important emerging technique.[15,16] In order to introduce terminal alkene units that are needed for RCM macrocyclization, allylglycine residues are often inserted into the linear parent peptide at the sites of intended ring closure. However, this can result in the displacement of biologically essential side-chain functionalities in the open-chain parent. For example, traditional RCM ring closure between the *i* and *i*+3 residues of structure **1** would require insertion of allylglycines that would eliminate the critical pTyr phenylphosphate moiety (**2**). Alternatively, the addition of a β-vinyl group onto the pTyr mimetic would transform it into a functionalized allylglycine variant that could undergo RCM ring closure with retention of phenylphosphate functionality at the site of ring juncture (**3**).[17]

We previously proposed such an approach to Grb2 SH2 domain-binding antagonists.[17] However, our earlier work was limited by its use of C-terminal 2-allyl-3-aryl-1-propanamides that lacked the α-carboxyl portion of true allylglycine residues (**4**, Scheme 2). This significantly limited the ability to explore structural variation, since the C-terminal moieties themselves involved lengthy synthesis and, once prepared, their use required an individual total synthesis of the entire macrocycle.[18–21] Presented herein is an alternative application of this idea that utilizes C-terminal allylglycine amides (**5**) to circumvent these deficiencies. In this new approach, L- and D-allylglycine and amines that are required for terminal amide construc-

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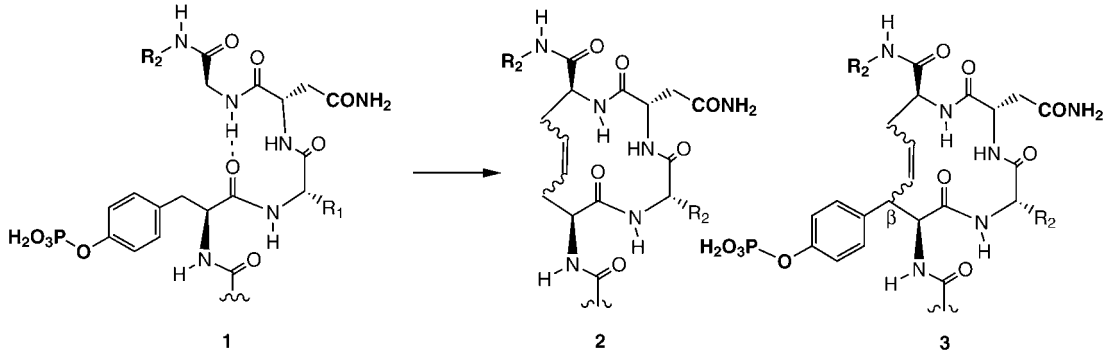
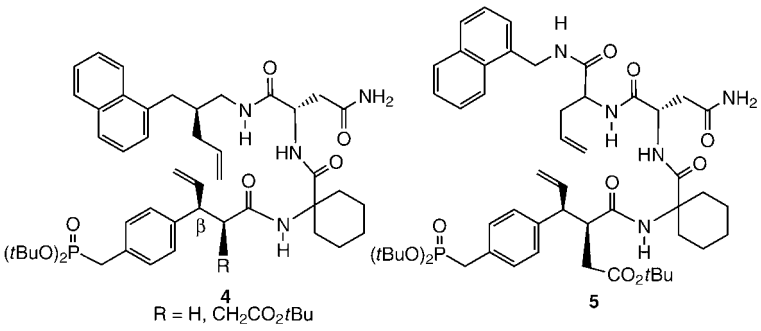


1 2 3

Scheme 1. *RCM macrocyclization of a prototypical Grb2 SH2 domain-binding inhibitor (**1**) without (**2**) and with (**3**) a β-functionalized allylglycine residue at the pTyr mimetic location. Bold indicates important structural features needing preservation.*



4
R = H, CH_2CO_2tBu

5

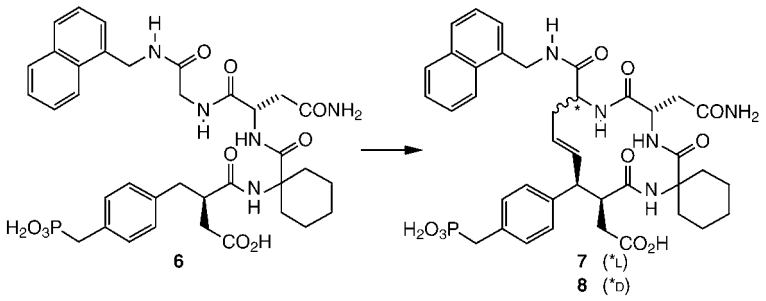
Scheme 2. *Comparison of the previously reported RCM approach that uses a β-vinyl pTyr mimetic (**4**) with the proposed new use (**5**).*

tion are commercially available, thereby facilitating synthetic access. More importantly, the use of C-terminal allylglycine amides potentially allows the preparation of multiple analogues through the use of solid-phase chemistries.

Results and Discussion

Synthetic approach

Compounds **7** and **8** (Scheme 3) were chosen as targets that could allow investigation of synthetic approaches toward RCM ring-closure of C-terminal allylglycines[15,22–25] onto an N-terminal β-vinyl-containing residue within the context of a Grb2 SH2 domain-binding system. Preparation of the corresponding open-chain compound **6** was deemed important for examination of the biological consequences of this kind of macrocyclization. By using solution chemistries, the L- and D-enantiomers of *N*-Boc-allylglycine were condensed in high yield with (1-naphthyl)methylamine to give the corresponding amides **9a** and **9b**, respectively (Scheme 4). These were deblocked by using aqueous TFA and, without purification, they were treated with *N*-Fmoc-L-asparagine by using 1-hydroxybenzotriazole (HOBt) active ester coupling to give the resulting epimeric dipeptides (**10a**) and (**10b**), which were sparingly soluble in DMF. For the L-allylglycine-containing **10a**, coupling to *N*-Fmoc-1-aminocyclohexane carboxylic acid (*N*-Fmoc-Ac_6c-OH) was done in two steps that consisted of an initial piperidine-mediated deblock with purification of the intermediate free amine (**11a**) followed by HOBt active ester coupling with *N*-Fmoc-Ac_6c-OH to yield tripeptide **12a** (78% yield from **10a**). The epimeric *N*-Fmoc dipeptide **10b** was deblocked by using Et_2NH in DMF and, without purification, it was directly treated with *N*-Boc-Ac_6c-OH by using 1-hydroxy-7-azabenzotriazole (HOAt) active ester coupling, to give the *N*-Boc-tripeptide **12b** (28% yield from **10b**).

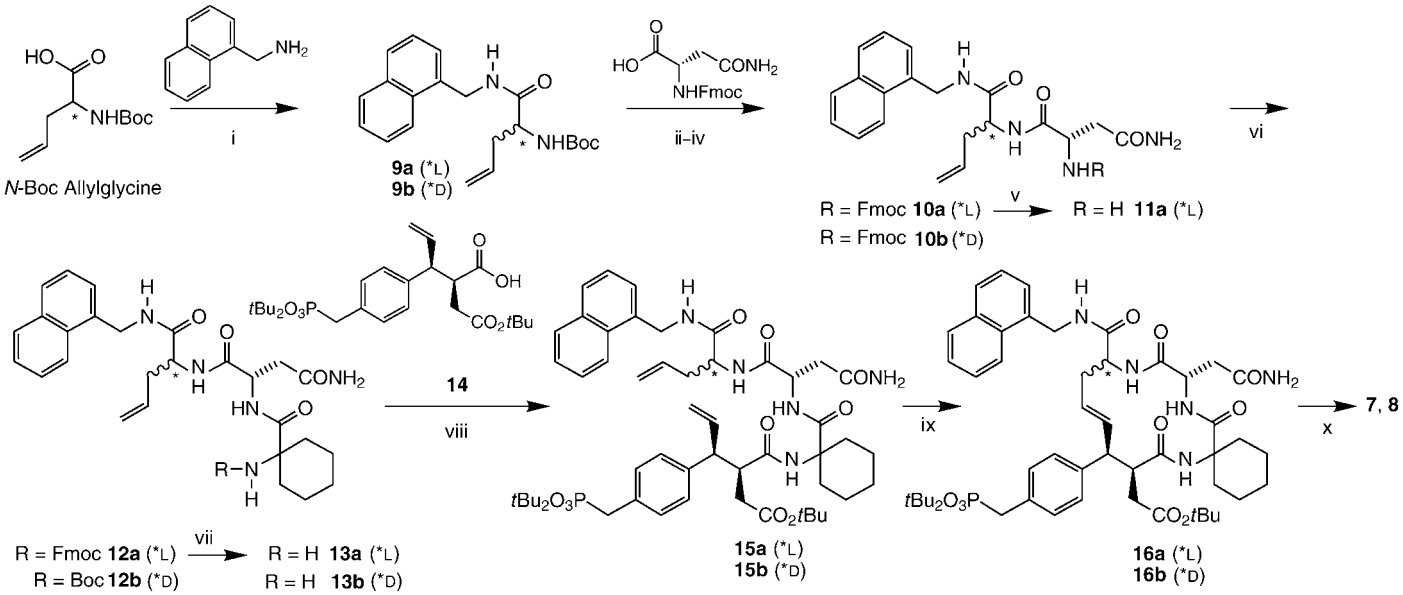


6

7 (*L)
8 (*D)

Scheme 3. *Structures of final targets.*

The conversion of **12a** and **12b** to the RCM substrates **15a** and **15b** required condensation of the sterically crowded N-terminal Ac_6c residues with the hindered pTyr mimetic **14**. It had previously been observed that HOAt active ester coupling in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl (EDCI·HCl) could achieve satisfactory yields if conducted for prolonged reaction times at elevated temperatures.[19] Accordingly, *N*-Fmoc-protected tripeptide **12a** was piperidine-deblocked, and the resulting free amine **13a** was purified prior to HOAt/EDCI active ester coupling with **14** (12 h at 50 °C) to yield the desired product **15a** (12% yield). *N*-Boc-protected tripeptide **12b** was deblocked to give amine **13b**, followed by direct coupling with **14** (at 50 °C for 24 h); this yielded the product **15b** (45% yield). Consistent with previous work,[19–21,26,27] ring-closing metathesis of **15a** and **15b** at 45 °C by using

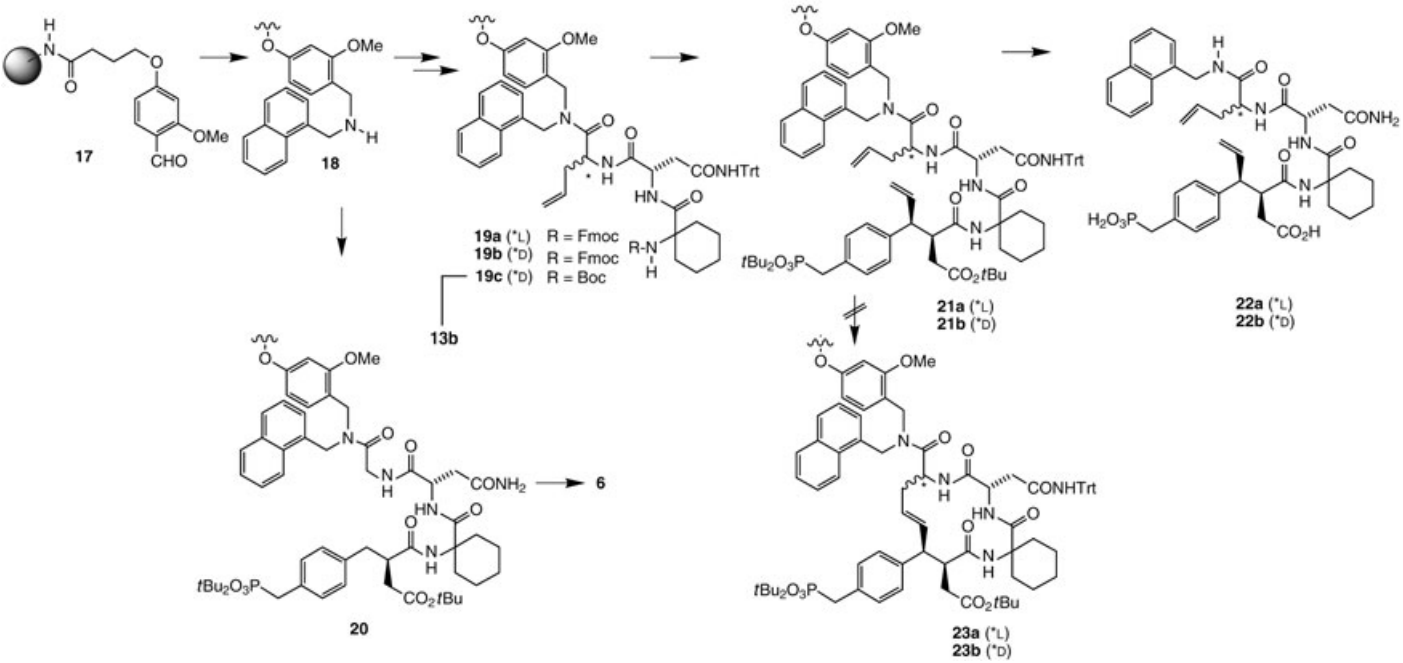


Scheme 4. *Reagents: i) HOBt, DIPCDI, DMF; ii) TFA; iii) aqueous $NaHCO_3$; iv) HOBt, DIPCDI, DMF; v) 20% piperidine in DMF; vi)* N*-Fmoc-Ac_6c-OH, HOBt, DIPCDI, DMF for* ***11a****; Et_2N/DMF then Boc-Ac_6c-OH, HOAT, EDCI, DMF for* ***10b****; vii) 20% piperidine in DMF; viii) HOAt, EDCI·HCl, DMF for* ***12a****; TFA-anisol (10:1) for* ***12b****; ix) [(PCy_3)(Im(Mes)$_2$}Ru=CHPh], CH_2Cl_2; x) TFA, H_2O.*

commercially available (Aldrich) Grubbs second-generation catalyst [(PCy_3)(Im(Mes)$_2$}Ru=CHPh],[28] yielded macrocycles **16a** (73% yield) and **16b** (52% yield), respectively. The ring-forming double bonds of both compounds were shown by vinylic ^{1}H NMR coupling constants to be exclusively of the *E* geometry.

Solid-phase chemistries

Solid-phase chemistries could allow the rapid synthesis of multiple analogues without tedious purification of intermediates. However, difficulties in the solution coupling of the sterically hindered **14** raised questions regarding the feasibility of a solid-phase approach. Reductive amination (1-naphthylamine, $NaBH(OAc)_3$) of acid-labile 4-(4-formyl-3-methoxyphenoxy)-butyryl-NovaGel HL resin (**17**),[24] yielded the naphthylmethyl amino resin **18** (Scheme 5). This was elaborated along three pathways. In one pathway, coupling of an initial *N*-Fmoc Gly residue was accomplished by using HOAt and HATU (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexaflorophosphate) in the presence of diisopropylethylamine (DIEA). This was fol-



Scheme 5. *Solid-phase synthesis of open-chain* ***5*** *and RCM intermediates* ***13b****,* ***22a*** *and* ***22b****.*

lowed by the sequential coupling of *N*-Fmoc Asn(Trt)-OH (HOBt active ester), *N*-Fmoc Ac_6c-OH (HOAt active ester), and the des-vinyl form of pTyr mimetic **14**[28] (HOAt active ester, 50 °C, 2 days). Coupling of this last step went to completion as indicated by a negative Kaiser test. Cleavage of the resulting resin **20** (95% aqueous TFA) provided open-chain **6** in 46% overall yield (Scheme 5).

Elaboration of resin **18** along the next two pathways differed in the initial coupling of either *N*-Fmoc L-allylglycine or *N*-Fmoc D-allylglycine (resins **19a** and **19b**, respectively). Coupling of Asn and Ac_6c residues, followed by pTyr mimetic **14**, yielded resins **21a** and **21b**, respectively. HPLC analysis of small samples of cleaved resin provided single major peaks that gave MALDI mass spectra consistent with the anticipated peptides **22a** and **22b**. This indicated that the potentially troublesome coupling of pTyr mimetic **14** had been achieved satisfactorily. However, attempts with on-resin ring-closure by using second-generation Grubbs catalyst (CH_2Cl_2, 24 °C, 2 days) failed to provide the desired ring-closed products **23a** and **23b**. Although the target macrocycles **7** and **8** could not be obtained directly on solid support, the ability to synthesize advanced intermediates such as **13b**, **22a**, and **22b** was shown. The ready access to advanced intermediates through solid-phase chemistries should greatly extend the usefulness of the approach.

Effects of macrocyclization on Grb2 SH2 domain binding affinity

The potential value of RCM macrocyclization between allylglycines and a β-vinyl-containing residue is to allow retention of critical functionality at one ring juncture site. In the present case, in which ring closure is intended to mimic the bend conformation of a Grb2 SH2 domain-bound peptide, the overall effectiveness of the approach is reflected by potency enhancement of the macrocycles **7** and **8** as compared to the open-chain parent **6**. It should be noted that the unmodified peptide **6** was chosen for biological reference rather than the free acid forms of metathesis precursors **15a** and **15b**, since the purpose of macrocyclization was the induction of conformational constraint of **6** and the ring-forming propylene bridges in **7** and **8** are considered to be components of the macrocyclization process. By using plasmon-resonance techniques, steady-state K_D values were obtained for direct binding of peptides **6**, **7**, and **8** to chip-bound Grb2 SH2 domain protein. Relative to open-chain **6** ($K_D = 5610 \pm 75.0$ nM), macrocycles **7** ($K_D = 22.7 \pm 0.455$ nM), and **8** ($K_D = 54.9 \pm 0.945$ nM) provided potency enhancements of two orders of magnitude (Table 1). The preference for the L-configuration at the epimeric allylglycine is real and within the sensitivity of the assay to detect. However, the difference in potency between **7** and **8** is sufficiently small to be of questionable significance.

Table 1. *Grb2 SH2 steady-state binding constants.*

Gr2 SH2 Domain	Compound **6**	Compound **7**	Compound **8**
steady-state K_D [nM]	5610 ± 75.0	22.7 ± 0.455	54.9 ± 0.945

Conclusion

The significant potency enhancements obtained in the current study by using RCM macrocyclizations between allylglycines and a β-functionalized allylglycine equivalent are in contrast to more modest effects produced by disulfide-bond-dependent ring-closure (approximately tenfold potency enhancements)[12,13] or by RCM reactions that do not make use of β-functionalized allylglycine equivalents (potency unchanged).[25] The particular effectiveness in the present case might be due to the binding requirement for phenylphosphate functionality at the site of one ring juncture. The methodology may be of general applicability to RCM macrocyclizations, in which critical functionality must be maintained at a ring-juncture site. As such, this represents a new approach for the synthesis of biologically active macrocycles by RCM chemistries.

Experimental Section

General synthetic: Reactions were carried out under argon. Anhydrous solvents were purchased from Aldrich Chemical Corporation and used without further drying. Combustion analyses were obtained from Atlantic Microlab, Inc. (GA, USA). ^{1}H NMR spectra were obtained by using a Varian 400 MHz spectrometer and are reported in ppm relative to TMS and referenced to the solvent in which they were run. Fast-atom-bombardment mass spectra (FABMS) were acquired with a VG analytical 7070E mass spectrometer. HPLC separations were conducted by using a Waters Prep LC4000 system with photodiode array detection and a J-sphere ODS-H80 column (20×250 mm) with a solvent system consisting of 0.1% aqueous TFA (*v/v*, solvent A) or 0.1% TFA in MeCN (*v/v*, solvent B).

***N*-Boc-L-allylGly-(1-naphthyl)methyl amide (9a)**: An ice-cold solution of *N*-Boc L-allylglycine·dicyclohexylamine salt (1.61 g, 4.05 mmol; Fluka) in 0.1 N HCl (100 mL) was extracted with ice-cold EtOAc (3×50 mL), and the combined extracts were dried (Na_2SO_4) and taken to dryness to yield the free amine as a syrup (898 mg). HOBt (601 mg, 4.46 mmol) and 1,3-diisopropylcarbodiimide (DIPCDI; 696 μL, 4.46 mmol) were added to a solution of this syrup in anhydrous DMF (9 mL), and, after 5 min, (1-naphthyl)methylamine (653 μL, 4.46 mmol) was added. The resulting mixture was stirred at RT overnight. The mixture was taken to dryness under high vacuum at 50 °C, and the resulting syrup was dissolved in EtOAc and purified by silica gel flash chromatogaphy (hexanes in EtOAc) to yield product **9a** as cream-colored crystals (1.15 g, 80% yield). ^{1}H NMR ($CDCl_3$): $\delta = 1.31$ (s, 9H), 2.44–2.53 (m, 2H), 4.08–4.16 (m, 1H), 4.81–4.94 (m, 2H), 5.04–5.12 (m, 2H), 5.64–5.78 (m, 1H), 6.32–6.38 (br m, 1H), 7.39 (dd, $J = 7.0$, 10.7 Hz, 1H), 7.46 (dd, $J = 1.5$, 6.8 Hz, 1H), 7.49 (dd, $J = 1.3$, 2.1 Hz, 1H), 7.59 (dd, $J = 1.6$, 7.8 Hz, 1H), 7.77–7.80 (br m, 1H), 7.83–7.86 (br m, 1H), 7.94 (br d, $J = 8.0$ Hz, 1H); FAMBS: *m/z*: 355.2 $[M+H]^+$.

***N*-Boc-D-allylGly-(1-naphthyl)methyl amide (9b)**: Treatment of *N*-Boc-D-allylglycine·dicyclohexylamine salt (PepTech; 2.72 g, 6.86 mmol) in a manner similar to that used to prepare **9a** provided product **9b** as a light yellow solid in quantitative yield. ^{1}H NMR: see data provided for enantiomeric **9a**; FABMS: *m/z*: 355.1 $[M+H]^+$.

***N*-Fmoc-L-Asn-L-allylGly-(1-naphthyl)methyl amide (10a)**: A solution of **9a** (254 mg, 1.00 mmol) in TFA/H_2O (9:1, 10 mL) was stirred at RT for 1 h, TFA was then removed by rotary evaporation under reduced pressure, and the residue was partitioned between

saturated $NaHCO_3$ in brine (25 mL) and EtOAc (2×25 mL). The organic extract was dried (Na_2SO_4), evaporated to an oil (203 mg), and taken up in DMF (1 mL). To this was added an HOBt active ester solution prepared by treating *N*-Fmoc-L-asparagine (357 mg, 1.10 mmol; Novabiochem) with HOBt (148 mg, 1.10 mmol) and DIPCIDI (172 μL, 1.10 mmol) in DMF (3 mL) for 15 min. The resulting clear solution was stirred at RT to rapidly yield a thick white suspension. The suspension was diluted with DMF (4 mL) and agitated at RT overnight. The solvent was removed at 50 °C under high vacuum, and the residue was thoroughly triturated with MeOH (10 mL), collected by filtration, and washed well with MeOH to yield product **10a** as a white solid (424 mg, 72% yield). ^{1}H NMR ([D$_6$]DMSO): δ = 2.24–2.56 (m, 4H), 4.14–4.20 (m, 3H), 4.24–4.36 (m, 2H), 4.66 (d, J = 6.9 Hz, 2H), 4.89 (br d, J = 10.4 Hz, 1H), 4.98 (br d, J = 17.2 Hz, 1H), 5.61–5.72 (m, 1H), 6.90 (br s, 1H), 7.24–7.40 (m, 8H), 7.44–7.50 (m, 2H), 7.57 (d, J = 8.0 Hz, 1H), 7.64 (d, J = 5.8 Hz, 2H), 7.78–7.79 (m, 1H), 7.84 (d, J = 7.5 Hz, 2H), 7.87–7.90 (m, 1H), 8.00 (br t, J = 6.1 Hz, 2H), 8.46 (br t, J = 5.6 Hz, 1H); FABMS: *m/z*: 591.3 $[M+H]^+$.

***N*-Fmoc-L-Asn-D-allylGly-(1-naphthyl)methyl amide (10b)**: Treatment of **9b** (1.64 g, 6.45 mmol) in a manner similar to that used to prepare **10a** from **9a** provided product **10b** as a white foam in quantitative yield. H NMR ([D$_6$]DMSO): δ = 2.24–2.43 (m, 4H), 4.06–4.14 (m, 3H), 4.25–4.34 (m, 2H), 4.65 (d, J = 5.8 Hz, 2H), 4.90 (br d, J = 9.6 Hz, 1H), 4.98 (br d, J = 17.5 Hz, 1H), 5.58–5.70 (m, 1H), 6.89 (br s, 1H), 7.24 (dd, J = 7.4, 15.0 Hz, 2H), 7.30–7.38 (m, 5H), 7.44–7.48 (m, 2H), 7.56 (br d, J = 7.6 Hz, 1H), 7.60 (br t, J = 6.5 Hz, 2H), 7.75 (br d, J = 8.0 Hz, 1H), 7.82–7.88 (m, 3H), 7.96–7.99 (m, 1H), 8.05 (d, J = 8.4 8.4 Hz, 1H); FABMS: *m/z*: 591.3 $[M+H]^+$.

***H*-L-Asn-L-allylGly-(1-naphthyl)methyl amide (11a)**: Piperidine (15 equiv) was added to a suspension of **10a** (1.11 g, 1.88 mmol) in DMF (10 mL), and the mixture was stirred at RT for 2 h. Solvent and piperidine were removed under high vacuum to yield a white solid, which was dissolved in MeOH/EtOAc and purified by silica gel flash chromatography (MeOH/EtOAc, from 5 to 25% MeOH) to yield free amine **11a** as a gel that became a white solid after prolonged exposure to vacuum (540 mg, 78% yield). ^{1}H NMR ([D$_6$]DMSO): δ = 2.12–2.46 (m, 4H), 3.44 (dd, J = 5.7, 8.6 Hz, 1H), 4.30 (br s, 1H), 4.63–4.53 (m, 2H), 4.95 (dd, J = 2.1, 10.1 Hz, 1H), 5.01 (dd, J = 2.1, 17.2 Hz, 1H), 5.61–5.72 (m, 1H), 6.82 (br s, 1H), 7.34–7.44 (m, 3H), 7.46–7.52 (m, 3H), 7.79 (br d, J = 7.6H, 1H), 7.87–7.92 (m, 1H), 7.98–8.02 (m, 1H), 8.11 (br s, 1H), 8.52 (t, J = 5.9 Hz, 1H); FABMS: *m/z*: 369.2 $[M+H]^+$.

***N*-Fmoc-Ac$_6$c-L-Asn-L-allylGly-(1-naphthyl)methyl amide (12a)**: A HOBt active ester solution, prepared by treating *N*-Fmoc-Ac$_6$c-OH (420 mg, 1.10 mmol; Advanced ChemTech) with HOBt (148 mg, 1.10 mmol) and DIPCIDI (172 μL, 1.10 mmol) in DMF (3 mL) for 10 min, was added to a solution of amine **10a** (368 mg, 1.00 mmol) in DMF (3 mL), and the resulting solution was stirred at RT overnight. The reaction mixture was taken to dryness under high vacuum and purified by silica gel flash chromatography (MeOH/EtOAc, from 0 to 20% MeOH) to give product **12a** as a white foam in quantitative yield. ^{1}H NMR ($CDCl_3$): δ = 1.10–1.82 (m, 10H), 2.38–2.56 (m, 2H), 2.73–2.79 (m, 2H), 4.02–4.23 (m, 3H), 4.35–4.45 (m, 2H), 4.62 (dd, J = 4.7, 14.8 Hz, 1H), 4.87 (dd, J = 1.7, 10.3 Hz, 1H), 4.94–5.01 (m, 2H), 5.58–5.75 (m, 1H), 6.82 (s, 1H), 7.18–7.39 (m, 9H), 7.40–7.42 (m, 1H), 7.46–7.48 (m, 1H), 7.61–7.64 (m, 2H), 7.68–7.74 (m, 3H), 7.92 (d, J = 8.6 Hz, 1H), 7.96 (s, 1H), 8.27 (d, J = 5.7 Hz, 1H); FABMS: *m/z*: 716.2 $[M+H]^+$.

***N*-Boc-Ac$_6$c-L-Asn-D-allylGly-(1-naphthyl)methyl amide (12b)**: Protected peptide **10b** (300 mg, 0.507 mmol) was treated with 20% Et_2NH in DMF (5 mL) for 30 min at RT. Concentration under reduced pressure gave the corresponding crude amine. *N*-Boc-Ac$_6$c-OH (135 mg, 0.558 mmol) and EDCI·HCl (116 mg, 0.609 mmol) were added to a stirred solution of the amine in dry DMF (1 mL) and HOAt in DMF (0.5 M, 1.11 mL, 0.558 mmol), and the mixture was stirred for 12 h at RT. The mixture was extracted with EtOAc, and the extract was washed successively with 5% citric acid solution, brine, 5% aquesous $NaHCO_3$, and brine, and dried over Na_2SO_4. Concentration followed by flash chromatography over silica gel by using CH_2Cl_2/MeOH (95:5) gave the product (**12b**) as a colorless solid (84 mg, 28% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.25 (m, 2H), 1.40 (s, 9H), 1.63 (m, 6H), 1.84 (m, 1H), 2.03 (m, 1H), 2.39 (dd, J = 16.5, 4.2 Hz, 1H), 2.54 (ddd, J = 14.6, 10.7, 7.7 Hz, 1H), 2.88 (m, 1H), 2.94 (dd, J = 16.5, 4.9 Hz, 1H), 4.32 (br, 1H), 4.45 (m, 1H), 4.58 (m, 2H), 4.90 (s, 1H), 5.05 (m, 2H), 5.15 (dd, J = 16.9, 1.6 Hz, 1H), 5.35 (br, 1H), 5.79 (m, 1H), 7.16 (m, 1H), 7.41 (m, 2H), 7.49 (m, 2H), 7.68 (d, J = 8.8 Hz, 1H), 7.74 (m, 2H), 7.81 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 8.6 Hz, 1H); FABMS: *m/z*: 594 $[M+H]^+$.

***H*-Ac$_6$c-L-Asn-L-allylGly-(1-naphthyl)methyl amide (13a)**: Piperidine (1.0 mL, 10 mmol) was added to a solution of **12a** (738 mg, 1.00 mmol) in DMF (5 mL), and the resulting solution was stirred at RT for 40 min. The solvent was then removed under high vacuum at 50 °C, and the residue was purified by silica gel flash chromatography (MeOH/EtOAc, from 0 to 20% MeOH) to give an oil. Lyophilization from dioxane provided product **13a** as a white solid (343 mg, 84% yield). FABMS: *m/z*: 494.3 $[M+H]^+$.

***H*-Ac$_6$c-L-Asn-D-Gly-(1-naphthyl)methyl amide (13b)**: Preparation of peptide **13b** with solid-phase methods is outlined below.

((2*R*,3*R*)-3-(4-Di-*tert*-butyloxyphosphonomethyl)phenyl-2-(*tert*-butyloxycarbonylmethyl)pent-4-enyl)-Ac$_6$c-L-Asn-L-allylGly-(1-naphthyl)methyl amide (15a): Protected pTyr mimetic **14**[19] (77 mg, 0.155 mmol) and EDCI·HCl (32 mg, 0.170 mmol) were added to a stirred solution of **13a** (70 mg, 0.141 mmol) in dry DMF (0.30 mL) and HOAt in DMF (0.5 M, 0.311 mL, 0.155 mmol), and the mixture was stirred for 12 h at 50 °C. The mixture was extracted with EtOAc, and the extract was washed successively with saturated citric acid solution, brine, 5% aqueous $NaHCO_3$ solution, and brine, and dried over Na_2SO_4. Concentration of the mixture was followed by flash chromatography over silica gel by using CH_2Cl_2/MeOH (95:5) provided **15a** as a colorless solid (16 mg, 12% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.72–1.62 (m, 37H), 2.47 (m, 2H), 2.62 (m, 3H), 2.82 (m, 1H), 2.96 (d, J = 21.5 Hz, 2H), 3.02 (m, 1H), 3.51 (m, 1H), 4.31 (m, 1H), 4.44 (m, 1H), 4.83 (dd, J = 15.0, 5.3 Hz, 1H), 4.97 (dd, J = 15.0, 6.0 Hz, 1H), 5.05 (m, 3H), 5.15 (dd, J = 17.1, 1.6 Hz, 1H), 5.25 (br, 1H), 5.69 (m, 1H), 5.81 (m, 1H), 6.22 (s, 1H), 6.71 (br, 1H), 7.11–7.25 (m, 5H), 7.35–7.55 (m, 5H), 7.59 (d, J = 8.3 Hz, 1H), 7.72 (d, J = 8.1 Hz, 1H), 7.81 (d, J = 9.2 Hz, 1H), 8.04 (d, J = 8.1 Hz, 1H); FABMS: *m/z*: 972 $[M+H]^+$.

((2*R*,3*R*)-3-(4-Di-*tert*-butyloxyphosphonomethyl)phenyl-2-(*tert*-butyloxycarbonylmethyl)pent-4-enyl)-Ac$_6$c-L-Asn-D-allylGly-(1-naphthyl)methyl amide (15b): Protected peptide **12b** (70 mg, 0.117 mmol) was treated with TFA/anisole (10:1, 5.5 mL) for 2 h at RT. Concentrating the mixture under reduced pressure gave the corresponding amine as its TFA salt. Protected pTyr mimetic **14** (64 mg, 0.129 mmol), EDCI·HCl (27 mg, 0.141 mmol), and *i*Pr$_2$NEt (0.040 mL, 0.234 mmol) were added to a stirred solution of the amine in dry DMF (0.300 mL) and HOAt in DMF (0.5 M, 0.259 mL, 0.129 mmol) at 0 °C, and agitation was continued for 24 h at 50 °C. The mixture was extracted with EtOAc, and the extract was washed successively with saturated citric acid solution, brine, saturated aqueous $NaHCO_3$ solution, and brine, and dried over Na_2SO_4.

Concentration of the mixture was followed by flash chromatography over silica gel by using CH_2Cl_2/MeOH (95:5) and provided **15b** as a colorless solid (52 mg, 45% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78–1.66 (m, 37H), 2.51–2.73 (m, 4H), 2.80 (m, 1H), 2.88–3.05 (m, 4H), 3.32 (t, J = 10.0 Hz, 1H), 4.11 (m, 1H), 4.52 (m, 1H), 4.77 (dd, J = 15.5, 5.1 Hz, 1H), 4.99–5.23 (m, 6H), 5.70–5.99 (m, 4H), 7.06 (m, 2H), 7.20 (m, 2H), 7.37 (m, 1H), 7.43–7.61 (m, 6H), 7.72 (d, J = 8.1 Hz, 1H), 7.82 (m, 1H), 8.04 (d, J = 8.3 Hz, 1H); FABMS: *m/z*: 972 $[M+H]^+$.

Cyclo(((2*R*,3*R*)-3-(4-di-*tert*-butyloxyphosphonomethyl)phenyl-2-(*tert*-butyloxycarbonylmethyl)pent-4-enyl)-Ac$_6$c-L-Asn-L-allylGly)-(1-naphthyl)methyl amide (16a): Second-generation Grubbs RCM catalyst [1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene)-(tricyclohexylhosphine) ruthenium] [(PCy_3)-{Im(Mes)$_2$}Ru=CHPh][29] (6.5 mg, 0.0077 mmol; Aldrich) in CH_2Cl_2 (1.3 mL) was added to a solution of **15a** (15 mg, 0.015 mmol) in CH_2Cl_2 (3.7 mL) under argon. The reaction mixture was stirred at 45°C for 24 h. The crude reaction mixture was evaporated in vacuo, and the residue was purified with silica gel chromatography by using CH_2Cl_2/MeOH (10:1) to provide **16a** as colorless powder (11 mg, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.16–1.58 (m, 30H), 1.61 (m, 1H), 1.72 (m, 1H), 1.89 (m, 4H), 2.07 (m, 1H), 2.35 (m, 1H), 2.46–2.73 (m, 4H), 2.99 (m, 3H), 3.57 (m, 1H), 3.88 (m, 1H), 4.83 (m, 2H), 4.90 (m, 1H), 5.13 (m, 2H), 5.83 (dd, J = 15.3, 7.6 Hz, 1H), 6.24 (br, 1H), 7.08 (m, 1H), 7.11–7.25 (m, 4H), 7.34–7.56 (m, 4H), 7.68 (m, 1H), 7.73 (d, J = 8.3 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.98 (d, J = 8.8 Hz, 1H), 8.03 (d, J = 8.3 Hz, 1H); FABMS: *m/z*: 944 $[M+H]^+$.

Cyclo(((2*R*,3*R*)-3-(4-di-*tert*-butyloxyphosphonomethyl)phenyl-2-(*tert*-butyloxycarbonylmethyl)pent-4-enyl)-Ac$_6$c-L-Asn-D-allylGly)-(1-naphthyl)methyl amide (16b): [(PCy_3){Im(Mes)$_2$}Ru=CHPh] (27 mg, 0.032 mmol) in CH_2Cl_2 (8 mL) was added to a solution of **15b** (55 mg, 0.057 mmol) in CH_2Cl_2 (25 mL) under argon, and the reaction mixture was stirred at 45°C for 48 h. The crude reaction mixture was then evaporated in vacuo, and the residue was purified by silica gel flash chromatography with CH_2Cl_2/MeOH (15:1) to provide **16b** as a yellow oil (28 mg, 52% yield). ^{1}H NMR ($CDCl_3$): δ = 8.04 (m, 1H), 7.87 (d, 1H, J = 8.5 Hz), 7.83 (m, 1H), 7.75 (d, J = 8.3 Hz, 1H), 7.54–7.40 (m, 6H), 7.24 (dd, J = 2.4, 8.3 Hz, 2H), 7.14 (d, J = 7.9 Hz, 2H), 6.81 (d, J = 7.5 Hz, 1H), 5.98 (s, 1H), 5.81 (dd, J = 9.1, 15.5 Hz, 1H), 5.72 (s, 1H), 5.40 (m, 1H), 5.02 (m, 1H), 4.79 (dd, J = 5.0, 14.9 Hz, 1H), 4.57 (m, 1H), 4.48 (m, 1H), 3.54 (m, 1H), 3.06 (m, 1H), 3.02 (d, 2H, J = 21.5 Hz), 2.97 (m, 1H), 2.88 (dd, J = 5.6, 15.9 Hz, 1H), 2.63 (dd, J = 4.1, 16.1 Hz, 1H), 2.43 (dd, J = 11.2, 17.2 Hz, 1H), 2.23 (m, 1H), 1.95 (dd, J = 3.0, 17.2 Hz, 1H), 1.86–1.44 (m, 6H), 1.42 (s, 9H), 1.40 (s, 9H), 1.36 (s, 9H), 1.31–1.20 (m, 4H); FABMS: *m/z*: 944 $[M+H]^+$.

Cyclo(((2*R*,3*R*)-3-(4-dihydroxyphosphonomethyl)phenyl-2-(hydroxycarbonylmethyl)pent-4-enyl)-Ac$_6$c-L-Asn-L-allylGly)-1-naphthyl)methyl amide (7): Protected peptide **16a** (10 mg, 0.010 mmol) was treated with TFA/H_2O (95:5, 2 mL) for 2 h at RT. Concentration of the mixture was followed by preparative HPLC purification (linear gradient 30 to 40% solution B in solution A, over 30 min) provided **7** as a colorless powder (4.6 mg, 56% yield). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.23 (m, 1H), 1.48 (m, 5H), 1.78 (m, 4H), 2.06 (m, 1H), 2.35 (m, 1H), 2.42–2.72 (m, 4H), 2.92 (d, J = 21.2 Hz, 2H), 3.21 (m, 1H), 4.02 (m, 1H), 4.34 (m, 1H), 4.58 (m, 1H), 4.72 (dd, J = 15.4, 5.2 Hz, 1H), 4.81 (dd, J = 15.6, 5.6 Hz, 1H), 5.34 (m, 1H), 5.84 (dd, J = 15.2, 8.0 Hz, 1H), 6.86 (br, 1H), 7.06 (d, J = 8.0 Hz, 1H), 7.18–7.29 (m, 4H), 7.36 (br, 1H), 7.42–7.60 (m, 4H) 7.70 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 7.2 Hz, 1H), 7.94 (d, J = 7.2 Hz, 1H), 8.06 (d, J = 8.5 Hz, 1H), 8.16 (s, 1H), 8.31 (t, J = 5.4 Hz, 1H); FABMS: *m/z*: 774 $[M-H]^-$.

Cyclo(((2*R*,3*R*)-3-(4-dihydroxyphosphonomethyl)phenyl-2-(hydroxycarbonylmethyl)pent-4-enyl)-Ac$_6$c-L-Asn-D-allylGly)-(1-naphthyl)methyl amide (8): A solution of **16b** (22 mg, 0.023 mmol) in a mixture of TFA/triethylsilane/H_2O (3.7:0.1:0.2, *v/v/v*, 4.0 mL) was stirred at RT for 1 h. The solvent was evaporated in vacuo, and the residue was purified by HPLC by using a linear gradient (5% to 95% B over 25 min) to provide product **8** as a white solid (8.4 mg, 46% yield). ^{1}H NMR ([D$_6$]DMSO): δ = 8.22 (m, 1H), 8.15 (s, 1H), 8.09 (m, 1H), 8.00 (m, 1H), 7.84 (dd, J = 2.6, 6.7 Hz, 1H), 7.72 (d, J = 6.4 Hz, 1H), 7.46–7.36 (m, 4H), 7.31 (s, 1H), 7.12–7.06 (m, 5H), 6.83 (s, 1H), 5.80 (dd, J = 9.2, 15.9 Hz, 1H), 5.27 (m, 1H), 4.72–4.64 (m, 2H), 4.56 (m, 1H), 3.91 (m, 1H), 3.81 (m, 1H), 3.16 (m, 1H), 2.69 (d, J = 20.8 Hz, 2H), 2.60–2.50 (m, 3H), 2.25 (m, 1H), 1.84 (m, 1H), 1.78–1.16 (m, 10H); FABMS: *m/z*: 775 $[M]^+$, 776 $[M+H]^+$.

Conversion of 8 to its trisodium salt: Compound **8** (5.2 mg, 0.0067 mmol) was dissolved in a solution of acetonitrile/H_2O (1:1, *v/v*, 1.0 mL), and a solution of $NaHCO_3$ (0.334 mL, 0.0201 mmol) was added. The resulting solution was lyophilized to provide the trisodium salt of **8** as a white solid (5.6 mg, 100% yield).

General procedure for reductive amination on resin. 1-Naphthylmethylamine (0.044 mL, 0.30 mmol) and $NaBH(OAc)_3$ (64 mg, 0.30 mmol) were added to a suspension of 4-(4-formyl)-3-methoxyphenoxy)butyryl-NovaGel HL resin (**17**; 55 mg, 0.030 mmol; Novabiochem, Inc.) in dry 1,2-dichloroethane/trimethyl orthoformate (2:1, 1.2 mL), and agitation was continued for 12 h at RT. The resin was washed successively with DMF, 10% *i*Pr$_2$NEt/DMF, and DMF to provide *N*-(1-naphthyl)methylamino-modified resin **18**.

General procedure for the solid-phase synthesis of protected peptides on resin: Protected-peptide resins were manually constructed by Fmoc-based solid-phase peptide synthesis. Trityl was employed for Asn side-chain protection. Fmoc deprotection was achieved by 20% piperidine in DMF (2 mL, 1 min; 1 mL, 20 min). Fmoc amino acids were coupled by treatment with Fmoc amino acid (5 equiv) and coupling reagents [HATU/HOAt for Gly and AllylGly for 6 h; DIPCDI/HOBt for Asn(Trt) for 2 h; DIPCDI/HOAt for Ac$_6$c for 6 h] in DMF. pTyr mimetic **14** was coupled by using DIPCDI/HOAt in DMF for 2 days at 50°C.

((2R)-3-(4-Dihydroxyphosphonomethyl)phenyl-2-(hydroxycarbonylmethyl)propionyl)-Ac$_6$c-L-Asn-Gly-(1-naphthyl)methyl amide (6): Protected peptide resin **20** (87 mg, 0.030 mmol), which resulted from elaboration of modified resin **18** by using the appropriate amino acids as described under general procedures for solid-phase synthesis, was treated with TFA/H_2O (95:5, 10 mL) for 2 h at RT. After the removal of resin by filtration, the filtrate was concentrated and purified by preparative HPLC (linear gradient from 30 to 40% solution B in solution A, over 30 min) to provide **6** as a colorless powder (10 mg, 45% yield based on resin substitution). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.00–1.29 (m, 2H), 1.29–1.63 (m, 6H), 1.82 (m, 2H), 2.04 (dd, J = 16.6, 3.9 Hz, 1H), 2.43–2.65 (m, 4H), 2.90 (d, J = 21.3 Hz, 2H), 2.96 (m, 1H), 3.11 (m, 1H), 3.68 (dd, J = 16.9, 5.7 Hz, 1H), 3.83 (dd, J = 16.9, 6.4 Hz, 1H), 4.34 (dt, J = 7.4, 5.7 Hz, 2H), 4.73 (m, 2H), 6.91 (br, 1H), 7.09–7.19 (m, 4H), 7.42 (m, 3H), 7.54 (m, 2H), 7.73 (d, J = 7.4 Hz, 1H), 7.81 (m, 1H), 7.90–8.00 (m, 2H), 8.06 (m, 1H), 8.11 (t, J = 5.7 Hz, 1H), 8.28 (s, 1H); FABMS: *m/z*: 736 $[M-H]^-$.

***H*-Ac$_6$c-L-Asn-D-allylGly-(1-naphthyl)methyl amide (13b)**: Protected-peptide resin **19c** (429 mg, 0.15 mmol), which resulted from

elaboration of modified resin **18** by using the appropriate amino acids as described under general procedures for solid-phase synthesis, was treated with TFA/H_2O (95:5, 10 mL) for 2 h at RT. After filtration, the filtrate was concentrated and neutralized with saturated $NaHCO_3$ solution. The whole mixture was extracted with EtOAc, and the extract was washed with brine and dried over Na_2SO_4. Concentration of the mixture followed by silica gel flash chromatography with EtOAc/MeOH (8:2) provided **13b** (74 mg, quant) as a colorless solid. ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 1.13$ (m, 1H), 1.27 (m, 2H), 1.36–1.57 (m, 5H), 1.66 (m, 2H), 2.34 (m, 1H), 2.40–2.58 (m, 3H), 4.31 (m, 1H), 4.45 (m, 1H), 4.74 (d, $J =$ 5.8 Hz, 2H), 4.98 (d, $J = 10.2$ Hz, 1H), 5.04 (d, $J = 17.2$, 1.6 Hz, 1H), 5.69 (m, 1H), 6.95 (br, 1H), 7.45 (m, 3H), 7.54 (m, 2H), 7.84 (dd, $J =$ 7.0, 2.3 Hz, 1H), 7.92 (m, 2H), 8.06 (m, 1H), 8.48 (m, 2H); FABMS: m/z: 494 $[M+H]^+$.

Biosensor analysis: Binding experiments were performed on a Biacore S51 instrument (Biacore Inc., NJ, USA). All biotinylated Grb2 SH2 domain proteins (biotinylated Grb2) were expressed and purified (Protein Expression Laboratory and The Protein Chemistry Laboratory, SAIC, Frederick, USA). The biotinylated Grb2 was immobilized onto carboxymethyl 5′ dextran surface (CM5 sensor chip, Biacore Inc.) by amine coupling. The lyophilized biotinylated Grb2 was reconstituted in 50% DMSO in H_2O to make a stock solution of 1 mg mL^{-1} and stored at −80 °C. A 1:12.5 dilution of biotin–Grb2 was used for immobilization, by dilution in acetate buffer, pH 5.0, with 5% DMSO. 1XPBS (phosphate buffered saline, pH 7.4) was used as the running buffer.

An immobilization wizard was used to optimize the immobilization target. For biotinylated Grb2, 2500–5000 resonance units (RU) of protein were captured on the CM5 sensor chip. Small molecules were serially diluted in running buffer to concentrations 1.25–1500 nM as indicated in each sensorgram and injected at 25 °C at a flow rate of 30 μL min^{-1} for 2 min. Varying concentrations of small molecules were injected in increasing concentrations, and every injection was performed in duplicate within each experiment. In order to subtract background noise from each data set, all samples were also run over an unmodified reference surface and random injections of running buffer were performed throughout every experiment ("double referencing"). Data were fitted to a simple 1:1 interaction model by using the global data analysis program CLAMP.[30]

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