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Structural Examination of Ring-Closing Metathesis-Derived 15-Member Macrocycles as Grb2 SH2 Domain-Binding Tetrapeptide Mimetics

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Abstract

Ring-closing metathesis (RCM) was employed to join carboxy-terminal alkenyl glycine side chains together with vinyl- and allyl-functionality appended to the β -methylene of amino-terminal phosphotyrosyl (pTyr) mimetics. This required the synthesis of a variety of new pTyr mimetics, including a novel aza-containing analogue. Many of the resulting 15-member macrocyclic tetrapeptide mimetics exhibited low nanomolar Grb2 SH2 domain-binding affinities in spite of the fact that differing ring junction stereochemistries and geometries of the RCM-derived double bond were employed. The finding that significant latitude exists in the structural requirements for ring closure, may facilitate the development of therapeutically-relevant macrocycle-based Grb2 SH2 domain-binding antagonists. The synthetic approaches used in this study may also find application to peptide mimetics directed at other biological targets.

Introduction

Development of high affinity protein-binding ligands ultimately entails the complimentary alignment of functional groups between the binding molecule and the target protein. Restricting ligand conformational flexibility as one traditional approach toward affinity enhancement¹⁻⁵ has been based on the rationale that increased free energy may result from reducing entropy penalties incurred during binding.⁶ Grb2 SH2 domains are docking modules found in a variety of cellular signaling pathways that represent potentially important therapeutic targets.⁷ These domains are characterized by their preferential recognition of “pTyr-Xxx-Asn-Yyy” sequences in type I beta turns.⁸ Accordingly, the design of Grb2 SH2 domain-binding inhibitors has focused on variation of the individual amino acid residues within the short recognition sequence as well as on induction of turn-geometries within the peptide secondary structure.⁹ This has offered an important target for application of novel chemistries to the design of new peptide mimetics. In our own program to develop Grb2 SH2 domain binding antagonists, a central theme has been global restriction of overall peptide conformation through ring-closing olefin metathesis (RCM) macrocyclization. A unique feature of our approach has been the joining of

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Supporting Information **Available**: Synthetic procedures for compounds **4** and **5**, ¹H NMR spectra of compounds **7 - 9**, **11**, **13 - 15**, **17**, **19 - 24** and **2c - 2g** and ¹³C NMR spectra of compounds **5**, **7 - 9**, **11**, **13 - 15**, **17**, **19**, **2022 - 24** and **2e - 2g**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

C-terminal alkenyl subsituents onto vinyl- and allyl-functionality appended to the β -methylene of *N*-terminal pTyr mimetics.¹⁰⁻¹³

The open-chain peptide **1** ($K_D = 5.6 \mu\text{M}$)¹⁴ represents a low micromolar affinity starting point for exploring the application of RCM macrocyclization within a unified family of Grb2 SH2 domain-binding inhibitors (Figure 1). Earlier we reported **2a** ($K_D = 23 \text{ nM}$) and **2b** ($K_D = 55 \text{ nM}$) as the first members of this genre of analogues.¹⁴ Compounds **2a** and **2b** share a common 14-member macrocyclic core in which the respective RCM-derived ring-closing segments consist of an 11*E*-propenyl chain joined in (10*S*)-configuration to the lower pTyr mimetic residue with upper ring junctions configured as either (14*S*) (for **2a**) or (14*R*) (for **2b**) (segments X, Figure 1).¹⁴ Macrocycles **2a** and **2b** present a novel template for the design of β -bend peptide mimetics. However, to date there has been little elucidation of structural requirements in the ring-closing segment needed for high affinity binding. To further explore the effects of structural variation in this region, analogues were designed to enlarge the macrocycle ring size (**2c**); reverse the stereochemistry of the C10 ring junction (**2d**), utilize *Z*-geometry at the alkenyl bond (**2e**); replace the C10 ring junction with an aza-center (**2f**) and reverse the stereochemistry of the C9 carboxymethyl subsituent (**2g**).

Results and Discussion

Synthesis of target macrocycles **2c** -**2g** required preparation of the building blocks shown in Figure 2. The upper ring junction-forming (1-naphthyl)methylamido tripeptide **4** was obtained by solid-phase techniques using commercially-available (*S*)-*N*-Fmoc-allylglycine as previously reported.¹⁴ The tripeptide **5** was obtained in similar fashion using (*S*)-*N*-Fmoc-(3-butenyl)glycine (**3**),¹⁵ which was prepared from the corresponding *N*-Boc *O*-(*tert*-butyl) ester derivative.¹⁶ The lower ring junction-forming components consisted of pTyr mimetics **6** – **9**. Enantioselective synthesis of the vinyl-containing **6** was achieved using oxazolidinone chiral auxiliary induction as previously reported.¹¹

Preparation of the 2-propenyl-containing **7** proceeded from the known **10** through **11** in a fashion similar to that used to synthesize **6** (Scheme 1).¹¹ Construction of the epimeric pTyr mimetic **8**, having (*R*)-stereochemistry at the site of 2-propenyl attachment, was achieved starting with the enantiomer of **10** (compound **12**) and involved hydrolysis of the initial (*R*)-phenyloxazolidinone to yield the free acid (**13**) followed by re-derivatization to **14** with the (*S*)-phenyloxazolidinone (Scheme 2). Alkylation in the desired (*S*)-configuration was achieved to yield **15**, which was converted to the free acid **8**.

Synthesis of the aza-containing pTyr mimetic **9** began with the Pd-catalyzed coupling of allylamine with the known **16**¹⁷ to yield the secondary amine **17** (Scheme 3).¹⁸ This was subjected to a second alkylation using the α -bromoacetylated Evans oxazolidinone **18**¹⁹ to give the chiral intermediate **19**. Stereoselective (*R*)-introduction of the carboxymethyl ester using *tert*-butyl α -bromoacetate and NaHMDS in THF (-78°C , then room temperature) gave **20**, which was hydrolyzed to the desired aza-containing **9**.

Coupling of the upper ring junction-forming tripeptides (**4** or **5**) with the appropriate protected pTyr mimetics (**6** – **9**) gave the open-chain metathesis substrates **21** – **24** (Scheme 4). Due to the sterically crowded environment of the *N*-terminal Ac₆C residue, satisfactory coupling yields required the use of active ester methodology [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide•HCl (EDC•HCl) and 1-hydroxy-7-azabenzotriazole (HOAt)] in DMF at 50°C for 36 h.^{11,14} Ring-closing metatheses of the *tert*-butyl-protected peptides **21** – **24** were conducted in refluxing CH₂Cl₂ using commercially available (Aldrich) Grubbs second-generation catalyst [(PCy₃)(Im(Mes)₂)Ru=CHPh].²⁰ The crude metathesis products were directly deprotected (aqueous TFA in the presence of triethylsilane) prior to HPLC purification

to yield the final products (**2c** – **2g**) (Scheme 4). The lower yields of final products **2c** (12%) and **2d** (30%) relative to **2e** (50%) and **2f,g** (56%) may reflect the sensitivity of RCM reactions to environmental factors resulting from differences in stereochemistries and substrate geometries. The *E*-geometry was obtained in all cases except for **2e**, where only the *Z*-geometry was isolated. This may reflect a different relative spatial positioning of the two terminal double bonds on the beta-turn scaffold of the RCM precursor **22**. Also of note, treatment of the crude aza-containing RCM product **24** under acidic conditions resulted in partial epimerization at the pTyr mimetic α -position to yield the (*R*)-containing **2g** in a 1 : 5 ratio relative to the non-epimerized **2f**. The stereochemistry of **2g** was confirmed by synthesis using the racemic form of pTyr mimetic **9**.

Conclusions

Surface plasmon resonance was used to determine K_D values for the direct association of ligands in solution to chip-bound Grb2 SH2 domain protein. We had previously shown that for the 14-member parent macrocycle reversal of the upper ring junction from (*S*) to (*R*) configuration was well tolerated [(14*S*)-**2a**, $K_D = 22.7 \pm 0.5$ nM; (14*R*)-**2b**, $K_D = 55 \pm 1.0$ nM, Table 1].¹⁴ In the current study we found that adding a methylene spacer to the ring-closing segment of **2a** had no effect on binding affinity (**2c**, $K_D = 20 \pm 5$ nM). Analogue **2c** presents a 15-member ring size, which is the same as related high affinity RCM-derived macrocycles that lack an amino acid residue at the pTyr mimetic + 3 position.^{11,21} Reversal of the lower ring junction stereochemistry in **2c** from (10*S*) to (10*R*) resulted in only a modest loss of binding affinity (**2d**, $K_D = 70 \pm 24$). When considered with previous data,¹⁴ this indicates a relative stereochemical insensitivity at either the upper or lower ring junctions for binding to Grb2 SH2 domains. In contrast, introduction of an aza-center at the lower ring junction resulted in an order-of-magnitude loss of binding affinity (**2f**, $K_D = 287 \pm 64$ nM). This may have been due to unfavorable interaction of the Grb2 Arg67 residue with the cation resulting from 10-aza protonation. A further dramatic loss of affinity occurred by reversal of the pTyr mimetic carboxymethyl configuration from (9*S*) to (9*R*) (**2g**, $K_D > 10,000$ nM). This may be attributed to loss of important interactions between the α A2 Arg67 residue and the pTyr mimetic carboxylic acid.²² While **2f** and **2g** contained highly unfavorable alterations, changing the position and geometry of the metathesis-derived double bond in **2c** from 11*E* to 12*Z* was well tolerated (**2e**, $K_D = 38 \pm 15$ nM).

Although much work has been done examining structural variation of individual amino acid residues in relation to Grb2 SH2 domain-binding affinity, much less has been done to delineate effects of global conformational constraint.⁹ The current study has demonstrated that within a family of RCM-derived macrocycles typified by **2**, considerable diversity in size, composition and ring junction stereochemistry is compatible with high affinity Grb2 SH2 domain binding. In contrast, the stereochemical requirements at the pTyr mimetic α -position appear to be more demanding. The finding that significant latitude exists in the structural requirements for ring closure, may facilitate the development of therapeutically-relevant macrocycle-based Grb2 SH2 domain-binding antagonists. Additionally, the synthetic approaches used in this study may find application to peptide mimetics directed at other biological targets.

Experimental Section

Biosensor Analysis

As indicated in Table 1, Grb2 SH2 domain-binding experiments were performed either on Biacore S51 or Biacore T100 instruments (Biacore Inc., Piscataway NJ). Procedures for analysis on the S51 have been reported.¹⁴ All biotinylated Grb2 SH2 domain proteins were expressed and purified (Protein Expression Laboratory and The Protein Chemistry Laboratory, SAIC - Frederick). For the T100 using biotin-capture surfaces, neutravidin (Pierce catalogue

number 31000) dissolved in 10 mM sodium acetate, pH 4.5 was immobilized onto carboxymethyl 5' dextran surface (CM5 sensor chip, Biacore Inc.) by amine coupling using the immobilization wizard supplied by the Biacore T100 software with a 5000 RU target on each of four flow cells. Lyophilized biotinylated Grb2 SH2 domain protein was reconstituted in 50% DMSO in H₂O to make a stock solution of 1 mg/mL and stored at -80 °C. A 20 µg aliquot of this solution was used for immobilization by diluting in 1XPBS (phosphate buffered Saline, pH 7.4), which was also used as the running buffer for Grb2 SH2 domain capture. A manual method for building a capture surface was used with a flow rate of 20 µL/min and a capture target of 2500 RU's for flow cells 2 and 4. Synthetic inhibitors were serially diluted in running buffer to concentrations ranging from 1.25 nM – 1500 nM and serially injected at 25 °C at a flow rate of 30 µL/min for 2 minutes. Each concentration was followed by two blank buffer injections and every injection was performed in duplicate within each experiment. In order to subtract any background noise from each data set, all samples were also run over neutravidin reference surfaces to allow “double referencing.” Data were fit to a simple 1:1 interaction model, using the global data analysis program CLAMP.²³

Synthetic

(βS,4S) β-[(1S)-1-[4-[[Bis(*tert*-butyl)phosphinyl]methyl]phenyl]-3-butenyl]-γ,2-dioxo-4-phenyl-3-oxazolidinebutanoic Acid *tert*-Butyl Ester (**11**)

To a solution of **10**¹¹ (2.00 g, 3.70 mmol) in THF (50 mL) at -78 °C, was added NaHMDS (1.0 M in THF, 4.50 mL, 4.50 mmol) and the mixture was stirred (30 minutes) then *tert*-butyl bromoacetate (0.82 mL, 5.55 mmol) was added. The reaction mixture was slowly warmed to room temperature and stirred (overnight). The reaction was quenched by the addition of saturated aqueous NH₄Cl, extracted with EtOAc (150 mL), dried (Na₂SO₄) and purified by silica gel column chromatography (hexane : EtOAc) to yield **11** as a white waxy solid (2.30 g, 95% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.30 – 7.15 (m, 9 H), 5.48 (m, 1 H), 4.95 (dd, *J* = 17.2, 2.0 Hz, 1 H), 4.86 – 4.81 (m, 2 H), 4.62 (m, 1 H), 4.08 (t, *J* = 8.4 Hz, 1 H), 3.95 (dd, *J* = 9.0, 2.6 Hz, 1 H), 3.02 (d, *J* = 21.6 Hz, 2 H), 2.88 – 2.77 (m, 2 H), 2.60 (dd, *J* = 16.8, 3.6 Hz, 1 H), 2.52 (t, *J* = 6.8 Hz, 2 H), 1.45 (s, 18 H), 1.28 (s, 9 H). ¹³C NMR (100 MHz, CDCl₃) 174.35, 170.80, 152.98, 138.98, 138.80, 138.76, 135.69, 132.09, 131.99, 129.69, 129.63, 128.62, 128.31, 128.28, 127.92, 125.50, 116.50, 82.13, 82.05, 81.96, 69.71, 57.66, 47.87, 44.48, 38.31, 36.89, 35.74, 35.41, 30.31, 30.28, 30.24, 27.81. ESI-MS (+VE) *m/z*: 678.8 (M + Na)⁺. HR-ESI/APCI calcd for C₃₆H₅₀NNaO₈P (M + Na)⁺ : 678.3171, Found: 678.3161.

(2S) [(1S)-1-[4-[[Bis(*tert*-butyl)phosphinyl]methyl]phenyl]-3-butenyl]-butanedioic Acid Mono (*tert*-butyl) Ester (**7**)

To a solution of compound **11** (1.50 g, 2.29 mmol) in a solution of THF (15 mL) and H₂O (5 mL) at 0 °C was added H₂O₂ (50% in H₂O, 0.70 mL, 12.4 mmol) dropwise, followed by LiOH•H₂O (192 mg, 4.58 mmol). The mixture was stirred at 0 °C (2 h), then at room temperature (2 h) and then excess H₂O₂ was quenched by addition of saturated aqueous Na₂SO₃, THF was removed by rotary evaporation and the residue was acidified to pH 3 – 4 and extracted with EtOAc (2 × 100 mL). The combined organic layer was washed (H₂O), dried (Na₂SO₄) and purified by silica gel column chromatography (CH₂Cl₂ : MeOH) to yield **7** as a white waxy solid (1.00 g, 86% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.15 – 7.06 (m, 4 H), 5.56 (m, 1 H), 4.93 (dd, *J* = 17.2, 1.6 Hz, 1 H), 4.83 (dd, *J* = 10.4, 2.0 Hz, 1 H), 3.08 (m, 1 H), 3.03 – 2.91 (m, 3 H), 2.56 – 2.49 (m, 3 H), 2.22 (dd, *J* = 16.8, 4.4 Hz, 1 H), 1.35 (m, 27 H). ¹³C NMR (100 MHz, CDCl₃) 176.54, 171.27, 138.97, 136.20, 131.64, 129.88, 129.12, 128.30, 125.97, 116.52, 82.59, 82.52, 80.51, 46.60, 37.80, 36.38, 35.74, 34.66, 30.18, 27.94. ESI-MS (+VE) *m/z*: 533.8 (M + Na)⁺. HR-ESI/APCI calcd for C₂₇H₄₃NaO₇P (M + Na)⁺: 533.2644, Found: 533.2655.

(β R)-4-[[Bis(*tert*-butyloxy)phosphinyl]methyl]- β -2-propenyl-benzenepropanoic Acid (13**)**

To a solution of **12** (prepared as described for its enantiomer using commercially-available (Aldrich) (*R*)-4-phenyl-2-oxazolidinone)¹¹ (0.420 g, 0.78 mmol) in a mixture solution of THF (6.0 mL) and H₂O (2.0 mL) at 0 °C was added dropwise H₂O₂ (50% in H₂O, 0.23 mL, 4.07 mmol) followed by LiOH•H₂O (64 mg, 1.52 mmol). The mixture was stirred at 0 °C (2 h) then at rt (2 h) and then excess H₂O₂ was quenched by addition of saturated aqueous Na₂SO₃, THF was removed by rotary evaporation and the residue was acidified to pH 3–4 and extracted with EtOAc (2 × 50 mL). The combined organic layer was washed (H₂O), dried (Na₂SO₄) and purified by silica gel column (CH₂Cl₂ : MeOH) to yield **13** as a white waxy solid (250 mg, 81% yield). ¹H NMR (400 MHz, CDCl₃) δ 10.0 (brs, 1 H), 7.25–7.10 (m, 4 H), 5.58 (m, 1 H), 4.92–4.85 (m, 2 H), 3.15 (m, 1 H), 2.88 (d, *J* = 21.6 Hz, 2 H), 2.63 (dd, *J* = 15.2, 6.8 Hz, 1 H), 2.50 (dd, *J* = 15.6, 8.4 Hz, 1 H), 2.40–2.25 (m, 2 H), 1.35 (s, 18 H). ¹³C NMR (100 MHz, CDCl₃) δ 176.09, 141.77, 135.88, 131.16, 131.06, 129.89, 129.82, 127.28, 116.72, 82.66, 41.25, 40.66, 37.74, 36.32, 30.19. ESI-MS (+VE) *m/z*: 419.0 (M + Na)⁺. HR-ESI/APCI calcd for C₂₁H₃₃NaO₅P (M + Na)⁺: 419.1963, Found: 419.1969.

[[4-[(1*R*)-2-[2-Oxo-2-[(4*S*)-2-oxo-4-phenyl-3-oxazolidinyl]ethyl]-3-butenyl]phenyl]methyl]-phosphonic Acid Bis(*tert*-butyl) Ester (14**)**

To a solution of **13** (1.15 g, 2.90 mmol) in THF (40 mL) at –78 °C, was added triethylamine (0.49 mL, 3.48 mmol) followed by trimethylacetic chloride (0.39 mL, 3.19 mmol) dropwise. The mixture was warmed to 0 °C over 20 minutes, then cooled to –78 °C again. Separately, to a solution of (S)-(+)-phenyl-2-oxazolidinone (Aldrich) (568 mg, 3.48 mmol) in THF (40 mL) at –78 °C was carefully added *n*-BuLi (1.60 M in THF, 2.20 mL, 3.48 mmol) and the mixture was stirred (30 minutes) then transferred to anhydride solution. The final reaction mixture was warmed to room temperature and stirred (overnight). The mixture was diluted with EtOAc (200 mL), washed (H₂O and brine), dried (Na₂SO₄), and purified by silica gel column chromatography (hexane : EtOAc) to yield **14** as a white solid (1.45 g, 95% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.23–7.21 (m, 3 H), 7.11 (dd, *J* = 8.0, 2.0 Hz, 2 H), 7.03 (d, *J* = 8.0 Hz, 2 H), 7.01–6.99 (m, 2 H), 5.55 (m, 1 H), 5.30 (dd, *J* = 8.6, 3.8 Hz, 1 H), 4.89–4.83 (m, 2 H), 4.57 (t, *J* = 8.8 Hz, 1 H), 4.11 (dd, *J* = 9.0, 3.8 Hz, 1 H), 3.36 (dd, *J* = 14.6, 6.2 Hz, 1 H), 3.25–3.10 (m, 2 H), 2.95 (d, *J* = 21.6 Hz, 2 H), 2.29 (t, *J* = 6.8 Hz, 2 H), 1.35 (s, 18 H). ¹³C NMR (100 MHz, CDCl₃) δ 171.43, 153.58, 141.37, 138.74, 135.95, 131.51, 131.41, 129.90, 129.84, 129.03, 128.38, 127.40, 125.53, 116.61, 82.06, 81.97, 69.80, 57.52, 41.02, 40.86, 40.57, 38.19, 36.77, 30.25. ESI-MS (+VE) *m/z*: 564.0 (M + Na)⁺. HR-ESI/APCI calcd for C₃₀H₄₀NNaO₆P (M + Na)⁺: 564.2490, Found: 564.2495.

(β S,4*S*) β -[(1*R*)-1-[4-[[Bis(*tert*-butyl)phosphinyl]methyl]phenyl]-3-butenyl]- γ ,2-dioxo-4-phenyl-3-oxazolidinebutanoic Acid *tert*-Butyl Ester (15**)**

Treatment of **14** as described above for the preparation of **11** provided **15** as a white waxy solid in 80% yield. ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.31 (m, 2 H), 7.28–7.21 (m, 3 H), 7.18 (dd, *J* = 8.0, 2.4 Hz, 2 H), 7.06 (d, *J* = 8.0 Hz, 2 H), 5.45–5.35 (m, 2 H), 4.82 (dd, *J* = 17.0, 1.4 Hz, 1 H), 4.75 (dd, *J* = 10.2, 1.8 Hz, 1 H), 4.69 (m, 1 H), 4.62 (t, *J* = 8.8 Hz, 1 H), 4.17 (q, *J* = 8.4 Hz, 1 H), 2.96 (d, *J* = 21.2 Hz, 2 H), 2.71 (td, *J* = 10.4, 3.6 Hz, 1 H), 2.48 (dd, *J* = 21.4, 11.8 Hz, 1 H), 2.46 (m, 1 H), 2.35 (m, 1 H), 2.01 (m, 1 H), 1.34 (s, 9 H), 1.33 (s, 9 H), 1.16 (s, 9 H). ¹³C NMR (100 MHz, CDCl₃) δ 175.29, 170.78, 153.62, 139.43, 139.39, 138.80, 136.12, 132.15, 132.05, 130.16, 130.10, 128.79, 128.13, 125.93, 116.20, 82.01, 81.91, 80.33, 69.69, 58.19, 48.51, 43.54, 38.19, 37.74, 37.21, 36.77, 30.24, 30.21, 27.86. ESI-MS (+VE) *m/z*: 678.0 (M + Na)⁺. HR-ESI/APCI calcd for C₃₆H₅₀NNaO₈P (M + Na)⁺: 678.3171, Found: 678.3181.

(2S) [(1R)-1-[4-[[Bis(*tert*-butyl)phosphinyl]methyl]phenyl]-3-butenyl]-butanedioic Acid Mono (*tert*-butyl) Ester (8)

Treatment of **15** as described above for the preparation of **7** provided **8** as a white waxy solid in 64% yield. ^1H NMR (400 MHz, CDCl_3) δ 16 (dd, $J = 8.2, 2.6$ Hz, 2 H), 7.00 (d, $J = 8.0$ Hz, 2 H), 5.44 (m, 1 H), 4.84 (d, $J = 17.2$ Hz, 1 H), 4.76 (d, $J = 10.4$ Hz, 1 H), 3.00 (d, $J = 21.6$ Hz, 2 H), 2.94 (m, 1 H), 2.86 (m, 1 H), 2.48 (m, 1 H), 2.41 (m, 1 H), 2.36 (dd, $J = 16.4, 11.2$ Hz, 1 H), 2.02 (dd, $J = 16.6, 3.0$ Hz, 1 H), 1.33 (s, 9 H), 1.32 (s, 9 H), 1.30 (s, 9 H). ^{13}C NMR (100 MHz, CDCl_3) δ 139.11, 135.82, 131.77, 131.67, 130.10, 130.05, 128.09, 116.49, 82.55, 82.46, 80.55, 47.26, 47.10, 38.17, 37.91, 36.48, 35.97, 30.21, 30.18, 27.90. ESI-MS (+VE) m/z : 533.8 ($\text{M} + \text{Na}$) $^+$. HR-ESI/APCI calcd for $\text{C}_{27}\text{H}_{43}\text{NaO}_7\text{P}$ ($\text{M} + \text{Na}$) $^+$: 533.2644, Found: 533.2635.

[4-[(3-Propenyl)amino]phenyl]methyl]-phosphonic Acid Bis(*tert*-butyl) Ester (17)

To THF (30 mL) degassed under argon (5 minutes) was added [(4-bromophenyl)methyl]-phosphonic acid bis(*tert*-butyl) ester **16** 11 (3.64 g, 10.0 mmol), allylamine (1.50 mL, 20.0 mmol), 1,1-bis(diphenylphosphino)ferrocene (dppf) (620 mg, 1.0 mmol), $\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{CH}_2\text{Cl}_2$ (400 mg, 0.50 mmol) and NaOtBu (2.00 g, 20.0 mmol) and the mixture was stirred at reflux (overnight). The mixture was poured into H_2O (100 mL), extracted with EtOAc (3 $\times$ 50 mL), the combined organic layer was washed (brine), dried (Na_2SO_4) and purified by silica gel column chromatography (hexane: EtOAc) to yield **17** as colorless crystals (2.10 g, 62% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.08 (d, $J = 8.4, 2.4$ Hz, 2 H), 6.57 (d, $J = 8.4$ Hz, 2 H), 5.95 (m, 1 H), 5.29 (dd, $J = 17.2, 1.6$ Hz, 1 H), 5.15 (dd, $J = 17.8, 1.4$ Hz, 1 H), 3.78 (brs, 1 H), 3.76 (d, $J = 5.6$ Hz, 2 H), 2.93 (d, $J = 20.8$ Hz, 2 H), 1.42 (s, 18 H). ^{13}C NMR (100 MHz, CDCl_3) δ 146.53, 135.43, 130.57, 130.51, 122.04, 121.94, 116.07, 112.90, 112.87, 81.71, 81.62, 46.67, 37.55, 36.12, 30.31, 30.28. FAB-MS (+VE) m/z : 339 M^+ . HR-FABMS calcd for $\text{C}_{18}\text{H}_{30}\text{NO}_3\text{P}$ (M^+): 339.1963, Found: 339.1961.

[[[[[2-Oxo-2-[(4S)-2-oxo-4-phenyl-3-oxazolidinyl]ethyl]3-propenyl]amino]phenyl]methyl]-phosphonic Acid Bis(*tert*-butyl) Ester (19)

A solution of **17** (1.50 g, 4.43 mmol), (4S)-3-(bromoacetyl)-4-phenyl-2-oxazolidinone (**18**) 19 (1.32 g, 4.64 mmol) and $\text{NEt}(i\text{-Pr})_2$ (1.20 mL, 6.65 mmol) in DMF (20 mL) was heated at 60 $^\circ\text{C}$ (overnight). The reaction mixture was diluted by the addition of EtOAc (100 mL), washed (H_2O and brine), dried (Na_2SO_4) and purified by silica gel column chromatography (hexane: EtOAc) to yield **19** as a white waxy solid (2.20 g, 92% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.29 – 7.27 (m, 5 H), 7.00 (dd, $J = 8.8, 2.4$ Hz, 2 H), 6.45 (d, $J = 8.4$ Hz, 2 H), 5.80 (m, 1 H), 5.42 (dd, $J = 8.8, 4.0$ Hz, 1 H), 5.13 (dd, $J = 17.2, 1.6$ Hz, 1 H), 5.10 (dd, $J = 11.2, 1.6$ Hz, 1 H), 4.75 (d, $J = 17.6$ Hz, 1 H), 4.72 (d, $J = 20.4$ Hz, 1 H), 4.58 (d, $J = 19.2$ Hz, 1 H), 4.33 (dd, $J = 9.0, 3.8$ Hz, 1 H), 3.93 (dd, $J = 16.8, 5.2$ Hz, 1 H), 3.84 (dd, $J = 16.8, 5.2$ Hz, 1 H), 2.89 (d, $J = 20.8$ Hz, 2 H), 1.40 (s, 18 H). ^{13}C NMR (100 MHz, CDCl_3) δ 170.03, 154.04, 146.73, 138.63, 133.98, 130.40, 130.33, 129.13, 128.77, 125.91, 121.82, 121.72, 116.51, 112.36, 81.79, 81.74, 81.70, 81.65, 70.78, 57.43, 54.14, 54.05, 37.39, 35.95, 30.31, 30.28. FAB-MS (+VE) m/z : 542 M^+ . HR-FAB calcd for $\text{C}_{29}\text{H}_{39}\text{N}_2\text{O}_6\text{P}$ (M^+): 542.2546, Found: 542.2535.

($\beta\text{R},4\text{S}$) β -[[[4-[[Bis(*tert*-butyl)phosphinyl]methyl]phenyl]3-propenyl]amino]- γ ,2-dioxo-4-phenyl-3-oxazolidinebutanoic Acid *tert*-Butyl Ester (20)

Treatment of **19** as described above for the preparation of **11** provided **20** as a pale yellow waxy solid in 59% yield. ^1H NMR (400 MHz, CDCl_3) δ 7.34 – 7.22 (m, 5 H), 7.07 (dd, $J = 8.8, 2.4$ Hz, 2 H), 6.75 (dd, $J = 8.4$ Hz, 2 H), 6.14 (dd, $J = 9.2, 5.6$ Hz, 1 H), 5.76 (m, 1 H), 5.22 (dd, $J = 8.4, 2.8$ Hz, 1 H), 5.10 (dd, $J = 17.0, 1.4$ Hz, 1 H), 5.03 (dd, $J = 10.4, 1.4$ Hz, 1 H), 4.51 (t, $J = 8.6$ Hz, 1 H), 4.18 (ddd, $J = 8.8, 2.8, 1.2$ Hz, 1 H), 3.87 (dd, $J = 18.2, 4.6$ Hz, 1 H), 3.77 (dd, $J = 18.0, 2.0$ Hz, 1 H), 2.90 (d, $J = 20.4$ Hz, 2 H), 2.86 (dd, $J = 16.2, 9.2$ Hz, 1 H), 2.49

(dd, $J = 16.2, 5.6$ Hz, 1 H), 1.38 (s, 18 H), 1.23 (s, 9 H). ^{13}C NMR (100 MHz, CDCl_3) 170.91, 169.64, 152.74, 146.11, 146.08, 138.68, 135.38, 130.39, 130.32, 128.95, 128.42, 125.69, 122.84, 122.74, 115.10, 114.15, 81.83, 81.77, 81.74, 81.68, 80.92, 70.06, 67.81, 57.78, 56.13, 49.26, 37.45, 36.02, 33.64, 30.24, 27.74, 25.51. FABMS (+VE) m/z : 656 M^+ . HR-FABMS calcd for $\text{C}_{35}\text{H}_{49}\text{N}_2\text{O}_8\text{P}$ (M^+): 656.3227, Found: 656.3216.

(β R) β -[[[4-[[Bis(*tert*-butyl)phosphinyl]methyl]phenyl]3-propenyl]amino]-butandioic Acid Mono(*tert*-butyl) Ester (9)

Treatment of **20** as described above for the preparation of **7** provided **9** as a pale yellow waxy solid in 59% yield. ^1H NMR (400 MHz, CDCl_3) 7.05 (dd, $J = 8.8, 2.0$ Hz, 2 H), 6.74 (d, $J = 8.4$ Hz, 2 H), 5.84 (m, 1 H), 5.20 (d, $J = 17.2$, 1 H), 5.11 (d, $J = 10.4$, 1 H), 4.83 (t, $J = 7.0$ Hz, 1 H), 3.95 (dd, $J = 17.6, 4.8$ Hz, 1 H), 3.88 (dd, $J = 17.4, 4.2$ Hz, 1 H), 3.01 (dd, $J = 16.4, 6.4$ Hz, 1 H), 2.93 (d, $J = 21.2$ Hz, 2 H), 2.66 (dd, $J = 16.2, 7.8$ Hz, 1 H), 1.40 (s, 9 H), 1.39 (s, 9 H), 1.38 (s, 9 H). ^{13}C NMR (100 MHz, CDCl_3) δ 174.15, 170.42, 146.90, 146.86, 135.44, 130.37, 122.15, 122.05, 116.26, 114.85, 82.70, 82.60, 80.93, 59.35, 51.73, 37.10, 36.42, 35.66, 30.24, 30.21, 27.94. FAB-MS (+VE) m/z : 512 ($\text{M} + \text{H}$) $^+$. HR-FAB calcd for $\text{C}_{26}\text{H}_{43}\text{NO}_7\text{P}$ ($\text{M} + \text{H}$) $^+$: 512.2777, Found: 512.2775.

1-[[[(2S,3S)-3-[4-[[Bis(*tert*-butyloxy)phosphinyl]methyl]phenyl]-2-[2-(*tert*-butyloxy)-2-oxoethyl]-1-oxo-4-pentenyl]amino]cyclohexanecarbonyl-L-asparaginy]l-4,5-didehydro-N-(1-naphthalenylmethyl)-L-homoallylglycinamide (21)

To a solution of **6** (160 mg, 0.321 mmol) in DMF (1.0 mL) was added HOAt (64 mg, 0.472 mmol), EDCI (121 mg, 0.63 mmol) and $\text{NEt}(i\text{-Pr})_2$ (0.27 mL, 1.57 mmol) and the mixture was stirred at room temperature (15 minutes). To this was added a solution of tripeptide **5** (80 mg, 0.157 mmol) in DMF (1.0 mL), then the mixture was heated (50 $^\circ\text{C}$) and stirred (36 h). The mixture was cooled to room temperature, diluted with H_2O (20 mL), extracted with EtOAc (3 $\times$ 50 mL), the combined organic phase was washed (brine), dried (Na_2SO_4) and purified by silica gel column chromatography (dichloromethane: MeOH) to afford **21** as a pale yellow solid (33 mg, 21% yield). ^1H NMR (CDCl_3) δ 8.00 (m, 1 H), 7.80 (m, 1 H), 7.70 (m, 1 H), 7.60 (m, 1 H), 7.50 – 7.30 (m, 5 H), 7.25 – 7.00 (m, 7 H), 6.34 (brs, 1 H), 5.80 (m, 1 H), 5.60 (m, 1 H), 5.03 – 4.80 (m, 6 H), 4.35 (m, 1 H), 4.25 (m, 1 H), 3.50 (m, 1 H), 3.00 – 2.85 (m, 3 H), 2.70 – 2.40 (m, 4 H), 2.20 – 1.90 (m, 4 H), 1.50 – 1.20 (m, 37 H). FAB-MS (+VE) m/z ($\text{M} + \text{H}$) $^+$: 986.7.

1-[[[(2S,3S)-3-[4-[[Bis(*tert*-butyloxy)phosphinyl]methyl]phenyl]-2-[2-(*tert*-butyloxy)-2-oxoethyl]-1-oxo-5-hexenyl]amino]cyclohexanecarbonyl-L-asparaginy]l-4,5-didehydro-N-(1-naphthalenylmethyl)-L-norvalinamide (22)

Coupling pTyr mimetic **7** with tripeptide **4** as described above for the preparation of **21** provided **22** as a colorless solid in 45% yield. ^1H NMR (400 MHz, CDCl_3) δ 8.02 (d, $J = 8.0$ Hz, 1 H), 7.81 (d, $J = 7.6$ Hz, 1 H), 7.72 (d, $J = 8.0$ Hz, 2 H), 7.56 – 7.36 (m, 7 H), 7.20 (dd, $J = 8.0, 1.6$ Hz, 2 H), 7.08 (d, $J = 8.0$ Hz, 2 H), 6.77 (s, 1 H), 6.43 (s, 1 H), 5.80 (m, 1 H), 5.44 (m, 1 H), 5.14 – 4.77 (m, 6 H), 4.46 (m, 1 H), 4.32 (q, $J = 6.0$ Hz, 1 H), 3.00 – 2.92 (m, 4 H), 2.80 (m, 1 H), 2.70 – 2.55 (m, 3 H), 2.50 – 2.40 (m, 3 H), 2.25 (m, 1 H), 1.60 – 1.30 (m, 34 H), 1.20 (m, 1 H), 1.05 (m, 1 H), 0.90 (m, 1 H). ^{13}C NMR (100 MHz, CDCl_3) δ 174.88, 173.97, 172.94, 172.63, 171.10, 139.81, 135.40, 134.31, 133.80, 133.62, 131.37, 130.14, 130.07, 128.43, 128.16, 127.78, 126.07, 125.75, 125.57, 125.35, 123.64, 117.45, 116.83, 82.21, 82.07, 81.57, 59.56, 53.98, 51.90, 47.09, 45.44, 41.15, 38.03, 36.41, 35.54, 35.29, 34.56, 30.28, 27.97, 24.78, 20.88. ESI-MS (+VE) m/z : 986.2 ($\text{M} + \text{H}$) $^+$. HR-ESI/APCI calcd for $\text{C}_{54}\text{H}_{77}\text{N}_5\text{O}_{10}\text{P}$ ($\text{M} + \text{H}$) $^+$: 986.5408, Found: 986.5399.

1-[[[(2*R*,3*S*)-3-[4-[[Bis(*tert*-butyloxy)phosphinyl]methyl]phenyl]-2-[2-(*tert*-butyloxy)-2-oxoethyl]-1-oxo-5-hexenyl]amino]cyclohexanecarbonyl-*L*-asparaginy]-4,5-didehydro-*N*-(1-naphthalenylmethyl)-*L*-norvalinamide (23)

Coupling pTyr mimetic **8** with tripeptide **4** as described above for the preparation of **21** provided **23** as a colorless solid in 38% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.03 (d, *J* = 8.4 Hz, 1 H), 7.96 (d, *J* = 5.6 Hz, 1 H), 7.86 (d, *J* = 8.4 Hz, 1 H), 7.81 (d, *J* = 8.0 Hz, 1 H), 7.72 (d, *J* = 7.6 Hz, 1 H), 7.51 – 7.36 (m, 5 H), 7.27 – 7.20 (m, 4 H), 6.97 (d, *J* = 7.6 Hz, 2 H), 6.58 (brs, 1 H), 5.81 (m, 1 H), 5.71 (m, 1 H), 5.42 – 5.34 (m, 2 H), 5.18 – 5.00 (m, 3 H), 4.86 – 4.77 (m, 2 H), 4.47 (m, 1 H), 3.00 (d, *J* = 21.2 Hz, 2 H), 2.89 – 2.83 (m, 2 H), 2.79 – 2.71 (m, 2 H), 2.70 – 2.62 (m, 3 H), 2.52 (m, 1 H), 2.37 (m, 1 H), 2.19 (m, 1 H), 1.95 – 1.80 (m, 3 H), 1.75 – 1.50 (m, 5 H), 1.50 – 1.10 (m, 29 H). ¹³C NMR (100 MHz, CDCl₃) δ 175.16, 174.91, 172.84, 171.09, 170.96, 138.94, 135.62, 134.39, 133.79, 133.63, 133.58, 131.40, 130.29, 130.22, 128.64, 128.40, 128.08, 127.73, 126.82, 126.71, 126.04, 125.71, 125.54, 125.34, 123.66, 117.48, 116.65, 82.06, 81.97, 81.49, 59.24, 53.98, 52.11, 48.23, 46.20, 41.25, 38.66, 38.16, 36.74, 36.05, 35.80, 35.38, 30.25, 27.91, 21.29, 21.04, 20.71. ESI-MS (+VE) *m/z*: 986.4 (M+H)⁺. HR-ESI/APCI calcd for C₅₄H₇₆N₅NaO₁₀P (M + Na)⁺: 1008.5227. Found: 1008.5209.

1-[(β*R*) β-[[[4-[[Bis(*tert*-butyl)phosphinyl]methyl]phenyl]3-propenyl]amino]-γ-(*tert*-butyl)butandiol]amino]cyclohexanecarbonyl-*L*-asparaginy]-4,5-didehydro-*N*-(1-naphthalenylmethyl)-*L*-norvalinamide (24)

Coupling pTyr mimetic **9** with tripeptide **4** as described above for the preparation of **21** provided **24** as a white waxy solid in 65% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.04 (d, *J* = 8.0 Hz, 1 H), 7.82 (m, 2 H), 7.74 (d, *J* = 8.0 Hz, 1 H), 7.68 (d, *J* = 8.4 Hz, 1 H), 7.50 – 7.36 (m, 5 H), 7.19 (s, 1 H), 7.17 (dd, *J* = 8.8, 2.4 Hz, 2 H), 6.87 (s, 1 H), 6.74 (d, *J* = 8.8 Hz, 2 H), 5.90 (m, 1 H), 5.72 (m, 1 H), 5.48 (s, 1 H), 5.24 – 5.07 (m, 4 H), 5.01 (dd, *J* = 10.0, 0.8 Hz, 1 H), 4.75 – 4.69 (m, 2 H), 4.50 (m, 1 H), 4.41 (dd, *J* = 11.2, 5.6 Hz, 1 H), 4.05 (dd, *J* = 17.4, 4.4 Hz, 1 H), 3.95 (dd, *J* = 17.4, 4.4 Hz, 1 H), 2.98 – 2.80 (m, 4 H), 2.76 (dd, *J* = 15.2, 6.0 Hz, 1 H), 2.69 (dd, *J* = 17.2, 7.2 Hz, 1 H), 2.55 (dd, *J* = 15.2, 5.6 Hz, 1 H), 2.50 (m, 1 H), 1.60 – 1.45 (m, 5 H), 1.42 (s, 18 H), 1.38 – 1.25 (m, 11 H), 1.06 (m, 2 H), 0.95 (m, 1 H). ¹³C NMR (100 MHz, CDCl₃) δ 174.72, 172.64, 170.93, 170.79, 145.95, 134.68, 134.43, 133.83, 133.63, 131.52, 130.85, 130.78, 128.32, 127.75, 126.09, 126.02, 125.52, 125.36, 123.82, 117.37, 117.01, 114.91, 82.13, 82.03, 81.97, 81.87, 81.41, 61.55, 59.33, 53.71, 52.02, 50.38, 41.28, 37.46, 36.03, 35.40, 35.12, 34.91, 34.04, 30.30, 28.14, 27.88, 24.64, 21.05, 20.98. MALDI-MS (+VE) *m/z*: 1010 (M + Na)⁺. HR-MALDI calcd for C₅₃H₇₅N₆NaO₁₀P (M + Na)⁺: 1009.5180. Found: 1009.5159.

(9*S*,10*S*,11*E*,15*S*,18*S*)-18-(2-Amino-2-oxoethyl)-15-[[[(1-naphthalenylmethyl)amino]carbonyl]-8,17,20-trioxo-10-[4-(phosphonomethyl)phenyl]-7,16,19-triazaspiro[5.14]icosa-11-ene-9-acetic Acid (2c)

A solution of tetrapeptide **21** (30 mg, 0.0304 mmol) in anhydrous dichloromethane (30 mL) was degassed under argon (5 minutes) then Grubbs 2nd generation catalyst [(PCy₃)(Im)(Mes)₂Ru=CHPh]²⁰ (13 mg, 0.0152 mmol) was added and the mixture was refluxed (2 days). The mixture was concentrated and purified by silica gel column chromatography (dichloromethane: MeOH) to provide intermediated *tert*-butyl-protected product as a brown material. This was treated with a mixture of CF₃CO₂H (3.7 mL), triethylsilane (0.10 mL) and H₂O (0.20 mL) at room temperature (1 h). The solvent was removed and the residue was purified by reverse phase preparative HPLC on a Phenomenex C₁₈ column (21 mm dia × 250 mm; cat. No. 00G-4436-P0) using a linear gradient from solvent A, 0% aqueous acetonitrile (0.1% CF₃CO₂H) to solvent B, 100% acetonitrile (0.1% CF₃CO₂H) over 25 minutes at a flow rate of 10.0 mL/minute (detection at 225 nm), retention time: 23.1 minutes. Lyophilization provided the macrocyclic product **2c** as a white powder (3 mg, 12% yield from **21**). ¹H NMR

(DMSO- d_6) δ 8.56 (s, 1 H), 8.33 (d, J = 7.2 Hz, 1 H), 8.10 – 8.06 (m, 2 H), 7.93 (m, 1 H), 7.80 (d, J = 8.4 Hz, 1 H), 7.60 (m, 1 H), 7.55 – 7.52 (m, 3 H), 7.48 (d, J = 6.8 Hz, 1 H), 7.40 (m, 1 H), 7.30 – 7.17 (m, 5 H), 7.76 – 7.74 (m, 2 H), 4.83 (dd, J = 15.8, 5.8 Hz, 1 H), 4.74 (dd, J = 15.6, 7.5 Hz, 1 H), 4.45 (m, 1 H), 4.32 (dd, J = 12.0, 5.0 Hz, 1 H), 4.09 (m, 1 H), 3.30 (d, J = 11.6 Hz, 1 H), 2.93 (d, J = 21.2 Hz, 1 H), 2.80 (dd, J = 15.4, 5.0 Hz, 1 H), 2.67 (dd, J = 16.2, 12.2 Hz, 1 H), 2.42 (dd, J = 15.6, 5.2 Hz, 1 H), 2.30 (m, 1 H), 2.00 – 1.85 (m, 5 H), 1.83 – 1.65 (m, 3 H), 1.57 – 1.35 (m, 5 H), 1.15 (m, 1 H). FAB-MS (–VE) m/z (M – H) $^-$: 788.6. HR-MALDI calcd for C₄₀H₄₈N₅NaO₁₀P (M + Na) $^+$: 812.3031, Found: 812.3010.

(9S,10R,12E,15S,18S)-18-(2-Amino-2-oxoethyl)-15-[[[(1-naphthalenylmethyl)amino]carbonyl]-8,17,20-trioxo-10-[4-(phosphonomethyl)phenyl]-7,16,19-triazaspiro[5.14]eicos-12-ene-9-acetic Acid (2d)

Ring-closing metathesis of **23** followed by deprotection and HPLC purification as reported above for the conversion of **21** to **2c** (linear gradient solvent B: 0% – 100% over 35 minutes; retention time: 26.5 minutes) yielded the macrocyclic final product **2d** as a white powder (6 mg, 30% yield from **23**). ^1H NMR (400 MHz, acetone- d_6 with one drop D₂O) δ 8.06 (d, J = 8.8 Hz, 1 H), 7.92 (s, 1 H), 7.85 (d, J = 7.6 Hz, 1 H), 7.75 (d, J = 8.4 Hz, 1 H), 7.54 – 7.38 (m, 4 H), 7.20 (d, J = 7.6 Hz, 2 H), 7.12 (d, J = 8.0 Hz, 2 H), 5.37 (dd, J = 17.4, 9.4 Hz, 1 H), 5.20 (m, 1 H), 4.88 (AB, J_{AB} = 15.6 Hz, 1 H), 4.78 (AB, J_{AB} = 15.6 Hz, 1 H), 4.64 (m, 1 H), 4.32 (m, 1 H), 3.11 (m, 1 H), 3.03 (d, J = 21.6 Hz, 2 H), 2.96 – 2.87 (m, 2 H), 2.79 (dd, J = 15.8, 6.2 Hz, 1 H), 2.59 (dd, J = 15.4, 5.0 Hz, 1 H), 2.51 (m, 1 H), 2.43 (dd, J = 14.0, 7.6 Hz, 1 H), 2.26 (dd, J = 16.6, 9.8 Hz, 2 H), 2.10 (m, 1 H), 1.88 – 1.77 (m, 2 H), 1.59 – 1.40 (m, 5 H), 1.26 – 1.12 (m, 3 H). ESI-MS (+VE) m/z : 812 (M + Na) $^+$. HR-ESI/APCI calcd for C₄₀H₄₈N₅NaO₁₀P (M + Na) $^+$: 812.3036, Found: 812.3011.

(9S,10S,12Z,15S,18S)-18-(2-Amino-2-oxoethyl)-15-[[[(1-naphthalenylmethyl)amino]carbonyl]-8,17,20-trioxo-10-[4-(phosphonomethyl)phenyl]-7,16,19-triazaspiro[5.14]eicos-12-ene-9-acetic Acid (2e)

Ring-closing metathesis of **22** followed by deprotection and HPLC purification as reported above for the conversion of **21** to **2c** (linear gradient solvent B: 0% – 70% over 30 minutes; retention time: 27.5 minutes) yielded the macrocyclic final product **2e** as a white powder (16 mg, 50% yield from **22**). ^1H NMR (400 MHz, DMSO- d_6) δ 8.36 (s, 1 H), 8.24 (d, J = 7.2 Hz, 1 H), 8.07 (d, J = 8.0 Hz, 1 H), 8.03 (t, J = 6.0 Hz, 1 H), 7.94 (dd, J = 8.0, 1.2 Hz, 1 H), 7.83 (d, J = 8.0 Hz, 1 H), 7.59 – 7.43 (m, 7 H), 7.22 (dd, J = 8.4, 2.0 Hz, 2 H), 7.00 (s, 1 H), 6.94 (d, J = 9.2 Hz, 1 H), 5.21 (t, J = 11.6 Hz, 1 H), 5.12 (td, J = 10.8, 3.2 Hz, 1 H), 4.76 (t, J = 6.0 Hz, 2 H), 4.38 – 4.30 (m, 2 H), 3.33 (t, J = 11.6 Hz, 1 H), 3.06 (d, J = 11.6 Hz, 1 H), 2.94 (d, J = 21.2 Hz, 2 H), 2.76 – 2.60 (m, 3 H), 2.40 (dd, J = 15.6, 4.8 Hz, 1 H), 2.23 (m, 2 H), 2.14 (m, 2 H), 1.76 – 1.67 (m, 3 H), 1.49 – 1.30 (m, 6 H), 1.13 (m, 1 H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 176.00, 175.90, 173.60, 172.80, 171.56, 171.42, 141.02, 134.62, 133.63, 131.16, 128.93, 127.92, 125.72, 123.75, 59.22, 53.00, 51.00, 47.00, 35.00. ESI-MS (–VE) m/z : 758.2 (M – H) $^-$. HR-ESI/APCI calcd for C₄₀H₄₇N₅O₁₀P (M – H) $^-$: 788.3060, Found: 788.3082.

(9S,12E,15S,18S)-18-(2-Amino-2-oxoethyl)-15-[[[(1-naphthalenylmethyl)amino]carbonyl]-8,17,20-trioxo-10-[4-(phosphonomethyl)phenyl]-7,10,16,19-tetraazaspiro[5.14]eicos-12-ene-9-acetic Acid (2f) and 9R,12E,15S,18S)-18-(2-Amino-2-oxoethyl)-15-[[[(1-naphthalenylmethyl)amino]carbonyl]-8,17,20-trioxo-10-[4-(phosphonomethyl)phenyl]-7,10,16,19-tetraazaspiro[5.14]eicos-12-ene-9-acetic Acid (2g)

Ring-closing metathesis of **24** followed by deprotection and HPLC purification as reported above for the conversion of **21** to **2c** yielded macrocyclic final products as white powders in 56% combined yield from **24**: **2f** (15 mg, retention time: 26.6 minutes) and **2g** (3 mg, retention time: 25.4 minutes) For **2f**: ^1H NMR (400 MHz, acetone- d_6 , with one drop D₂O) δ 8.12 (d, J

= 7.2 Hz, 2 H), 7.91 (d, J = 8.0 Hz, 1 H), 7.83 (d, J = 8.4 Hz, 1 H), 7.67 – 7.52 (m, 5 H), 7.46 (t, J = 7.6 Hz, 1 H), 7.41 (t, J = 9.6 Hz, 1 H), 7.14 (dd, J = 8.8, 2.0 Hz, 2 H), 6.82 (d, J = 8.8 Hz, 2 H), 5.62 (m, 1 H), 5.49 (ddd, J = 15.8, 9.2, 4.8 Hz, 1 H), 5.18 (dd, J = 14.8, 7.2 Hz, 1 H), 4.90 (t, J = 7.0 Hz, 1 H), 4.72 (m, 1 H), 4.60 (dd, J = 15.0, 3.8 Hz, 1 H), 4.33 (m, 1 H), 3.92 (d, J = 17.2 Hz, 1 H), 3.82 (dd, J = 16.2, 7.4 Hz, 1 H), 2.97 (d, J = 20.8 Hz, 2 H), 2.85 (d, J = 13.6 Hz, 1 H), 2.63 (dd, J = 15.6, 4.4 Hz, 1 H), 2.52 (d, J = 7.6 Hz, 2 H), 2.46 (dd, J = 15.6, 8.0 Hz, 1 H), 2.07 (m, 1 H), 1.88 (m, 1 H), 1.65 (m, 1 H), 1.56 – 1.40 (m, 5 H), 1.30 (m, 1 H), 1.18 – 1.09 (m, 2 H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.35, 172.17, 171.51, 171.17, 170.80, 146.76, 134.56, 133.60, 131.20, 128.78, 127.88, 125.99, 123.89, 113.82, 59.06, 51.64, 46.14, 35.28, 34.14. MALDI-MS (+VE) m/z : 813 ($\text{M} + \text{Na}$) $^+$. HR-MALDI calcd for $\text{C}_{39}\text{H}_{47}\text{N}_6\text{NaO}_{10}\text{P}$ ($\text{M} + \text{Na}$) $^+$: 813.2984. Found: 813.3011. For **2g**: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.32 (t, J = 5.8 Hz, 1 H), 8.18 (s, 1 H), 7.94 (m, 1 H), 7.86 (m, 1 H), 7.81 (d, J = 8.4 Hz, 1 H), 7.75 (dd, J = 6.0, 3.2 Hz, 1 H), 7.49 – 7.44 (m, 2 H), 7.38 – 7.33 (m, 3 H), 7.20 (d, J = 7.6 Hz, 1 H), 6.97 (dd, J = 8.8, 2.0 Hz, 2 H), 6.86 (s, 1 H), 6.58 (d, J = 8.8 Hz, 2 H), 5.49 (dt, J = 15.6, 5.6 Hz, 1 H), 5.32 (m, 1 H), 4.77 (t, J = 7.6 Hz, 1 H), 4.70 (dd, J = 15.6, 6.0 Hz, 1 H), 4.56 (dd, J = 15.2, 5.2 Hz, 1 H), 4.46 (dd, J = 14.0, 6.0 Hz, 1 H), 4.18 (m, 1 H), 3.83 (m, 1 H), 3.72 (dd, J = 17.6, 6.0 Hz, 1 H), 2.93 (dd, J = 16.0, 6.8 Hz, 1 H), 2.73 (d, J = 20.8 Hz, 2 H), 2.58 – 2.50 (m, 2 H), 2.37 (dd, J = 15.8, 6.2 Hz, 1 H), 2.06 (m, 2 H), 1.82 (m, 1 H), 1.67 (m, 1 H), 1.55 – 1.33 (m, 6 H), 1.16 – 1.08 (m, 2 H). ^{13}C NMR (100 MHz, CDCl_3) δ 174.32, 172.90, 172.71, 171.97, 171.38, 171.09, 146.70, 134.74, 133.62, 131.15, 128.91, 127.75, 126.04, 125.34, 123.81, 122.33, 112.66, 59.78, 50.03. MALDI-MS (+VE) m/z : 813 ($\text{M} + \text{Na}$) $^+$. HR-MALDI calcd for $\text{C}_{39}\text{H}_{47}\text{N}_6\text{NaO}_{10}\text{P}$ ($\text{M} + \text{Na}$) $^+$: 813.2984. Found: 813.2988.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

pTyr, phosphotyrosyl; Ac₆c, aminocyclohexanecarboxylic acid; EDC•HCl, [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide•HCl]; HOAt, 1-hydroxy-7-azabenzotriazole.

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Figure 1.
Structure of open-chain 1 and macrocyclic variants 2.

Figure 2.
Synthetic building blocks for the synthesis of macrocycles 2.

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

(a) 1, HOAt, EDCl, DIPEA, DMF, 50 °C, 36 h; yields: **21**, 21%; **22**, 45%; **23**, 38%; **24**, 65%.

(b) [1] [(PCy₃)(Im(Mes)₂)Ru=CHPh], CH₂Cl₂, reflux, 48 h; [2] TFA, H₂O and HSiEt₃, 1 h; yields: **2c**, 12%; **2e**, 50%; **2d**, 30%; **2f** and **2g**, 56% combined (5 : 1 ratio).

Table 1Grb2 SH2 Domain-Binding Affinities of Synthetic Macrocycles.^a

Compound	K _D (nM) ± sd	Compound	K _D (nM) ± sd
2a	22.7 ± 0.5 ^b	2e	38 ± 15, ^{df}
2b	55 ± 1.0 ^b	2f	287 ± 64, ^{cf}
2c	20 ± 5, ^{ce}	2g	>10,000, ^{ce}
2d	70 ± 24, ^{df}		

^a Performed using surface plasmon resonance as described in the Experimental Section.^b Values previously reported in reference ¹⁴.^c Amine-coupled surface.^d Biotin-capture surface.^e Biacore S51 instrument.^f Biacore T100 instrument.