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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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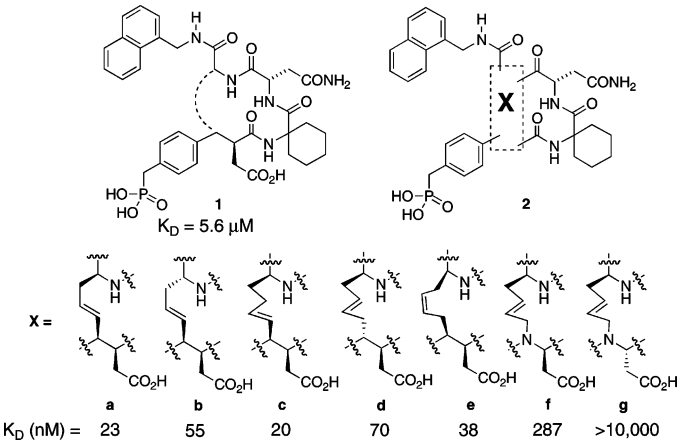

Ring-closing metathesis (RCM) was employed to join carboxy-terminal alkenyl glycine side chains together with vinyl- and allyl-functionality appended to the β-methylene of amino-terminal phosphotyrosyl (pTyr) mimetics. This required the synthesis of a variety of new pTyr mimetics, including a novel aza-containing 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 macrocyle-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 enhancement[1−5] 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 tar-

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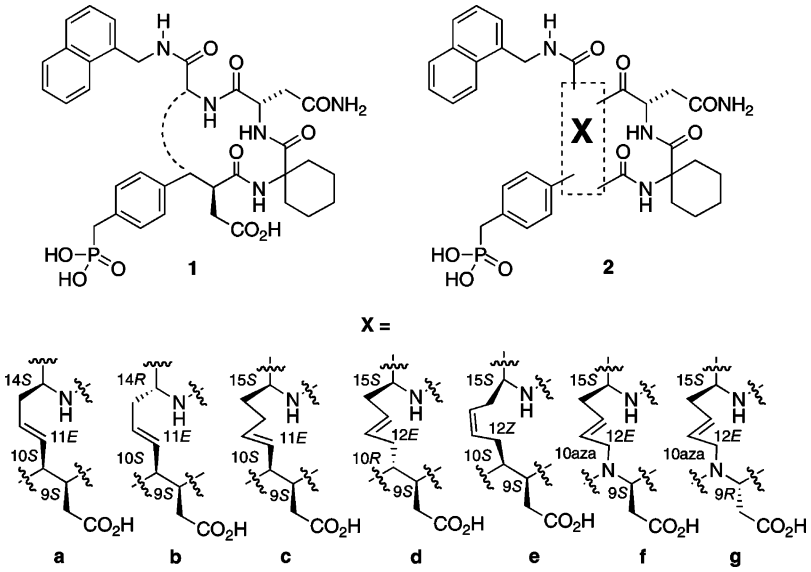

FIGURE 1. Structure of open-chain **1** and macrocyclic variants **2**.

gets.[7] These domains are characterized by their preferential recognition of "pTyr-Xxx-Asn-Yyy" sequences in type I β 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 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** ($K_D = 5.6\ \mu M$)[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** ($K_D = 23$ nM) and **2b** ($K_D = 55$ 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**−**g** 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 rederivatization 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**.

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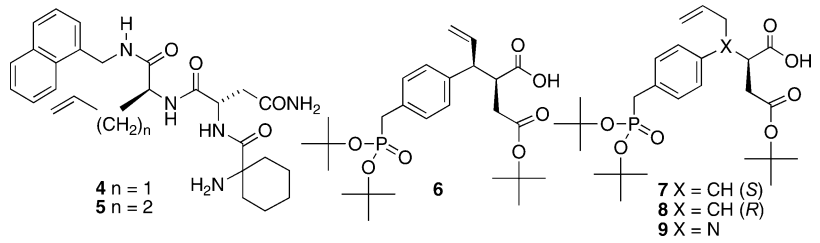
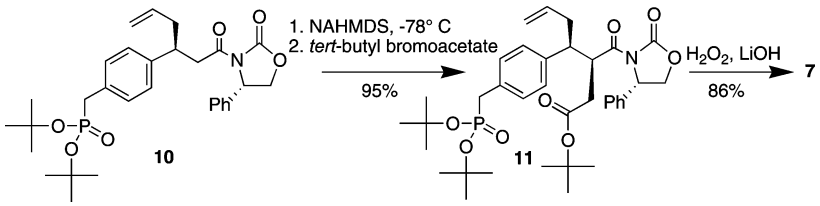
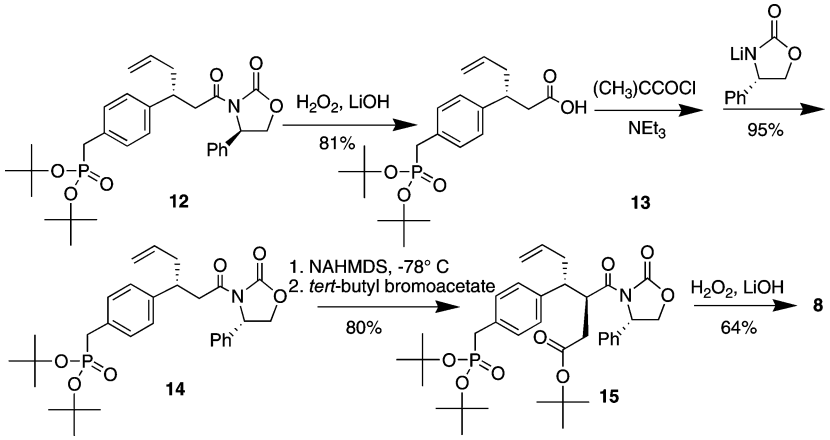


FIGURE 2. Synthetic building blocks for the synthesis of macrocycles **2**.

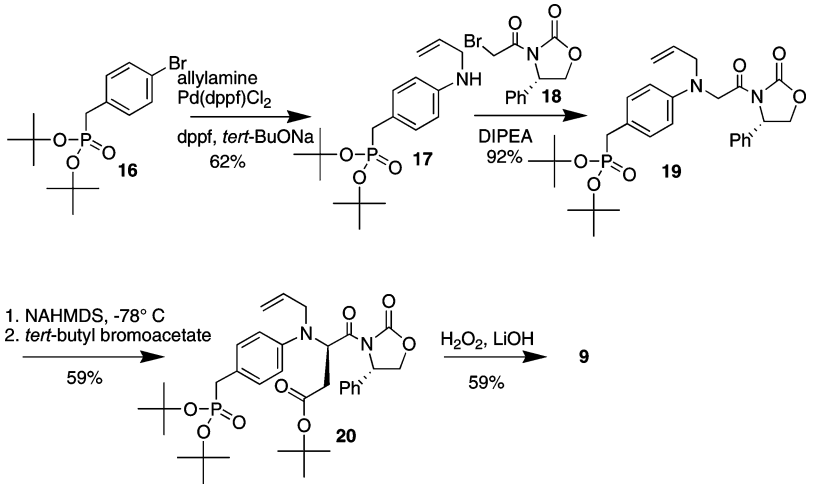
SCHEME 1



SCHEME 2



SCHEME 3



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

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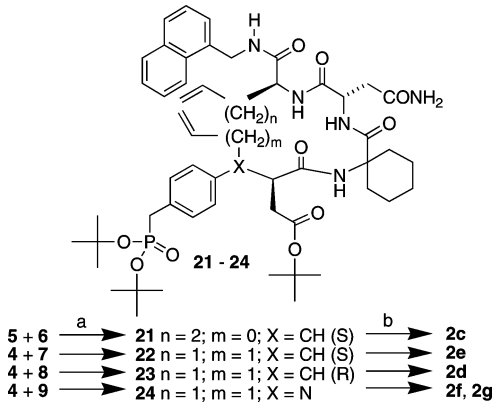
SCHEME 4 [a]



[a] (a) HOAt, EDCI, DIPEA, DMF, 50 °C, 36 h; yields: **21**, 21%; **22**, 45%; **23**, 38%; **24**, 65%. (b) (1) [$(PCy_3)(Im(Mes)_2)Ru{=}CHPh$], CH_2Cl_2, reflux, 48 h; (2) TFA, H_2O and $HSiEt_3$, 1 h; yields: **2c**, 12%; **2e**, 50%; **2d**, 30%; **2f** and **2g**, 56% combined (5:1 ratio).

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). Because of the sterically crowded environment of the *N*-terminal Ac_6c 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 CH_2Cl_2 using commercially available (Aldrich) Grubbs second-generation catalyst [$(PCy_3)(Im(Mes)_2)$-$Ru{=}CHPh$].[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**−**g**) (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 stereochemisties 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 β-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 nonepimerized **2f**. The stereochemistry of **2g** was confirmed by synthesis using the racemic form of pTyr mimetic **9**.

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TABLE 1. Grb2 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[d,f]
2b	55 ± 1.0[b]	**2f**	287 ± 64[c,f]
2c	20 ± 5[c,e]	**2g**	>10000[c,e]
2d	70 ± 24[d,f]		

[a] Performed using surface plasmon resonance as decribed 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.

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 αA2 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 macrocyle-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.

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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 proetins were expressed and purified (Protein Expression Laboratory and The Protein Chemistry Laboratory, SAIC-Frederick). For the T100 using biotin-capture surfaces, neutravidin (Pierce catalog 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 H_2O 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 to 1500 nM and serially injected at 25 °C at a flow rate of 30 μL/min for 2 min. 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]

(β*S*,4*S*) β-[(1*S*)-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 min) and 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 NH_4Cl, exacted with EtOAc (150 mL), dried (Na_2SO_4), and purified by silica gel column chromatography (hexane:EtOAc) to yield **11** as a white waxy solid (2.30 g, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (100 MHz, $CDCl_3$) δ 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 cacld for $C_{36}H_{50}NNaO_8P$ $(M + Na)^+$: 678.3171, Found: 678.3161.

(2*S*)-[(1*S*)-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 H_2O (5 mL) at 0 °C was added H_2O_2 (50% in H_2O, 0.70 mL, 12.4 mmol) dropwise, followed by LiOH·H_2O (192 mg, 4.58 mmol). The mixture was stirred at 0 °C (2 h) and then at room temperature (2 h), and then excess H_2O_2 was quenched by addition of saturated aqueous Na_2SO_3, THF was removed by rotary evaporation, and the residue was acidified to pH 3−4 and exacted with EtOAc (2 × 100 mL). The combined organic layer was washed (H_2O), dried (Na_2SO_4), and purified by silica gel column chromatography (CH_2Cl_2:MeOH) to yield **7** as a white waxy solid (1.00 g, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (100 MHz, $CDCl_3$) δ 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 cacld for $C_{27}H_{43}NaO_7P$ $(M + Na)^+$: 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 H_2O (2.0 mL) at 0 °C was added dropwise H_2O_2 (50% in H_2O, 0.23 mL, 4.07 mmol) followed by LiOH·H_2O (64 mg, 1.52 mmol). The mixture was stirred at 0 °C (2 h) and then at rt (2 h), excess H_2O_2 was quenched by addition of saturated aqueous Na_2SO_3, THF was removed by rotary evaporation, and the residue was acidified to pH 3−4 and exacted with EtOAc (2 × 50 mL). The combined organic layer was washed (H_2O), dried (Na_2SO_4) and purified by silica gel column (CH_2Cl_2:MeOH) to yield **13** as a white waxy solid (250 mg, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (100 MHz, $CDCl_3$) 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 cacld for $C_{21}H_{33}NaO_5P$ $(M + Na)^+$: 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 min and then cooled to −78 °C again. Separately, to a solution of (*S*)-(+)-phenyl-2-oxazolidione (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 min) 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 (H_2O and brine), dried (Na_2SO_4), and purified by silica gel column chromatography (hexane:EtOAc) to yield **14** as a white solid (1.45 g, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (100 MHz, $CDCl_3$) 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 cacld for $C_{30}H_{40}NNaO_6P$ $(M + Na)^+$: 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. ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.29, 170.78, 153.62, 139.43, 139.39, 138.80, 136.12, 132.15, 132.05, 130.16,

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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 cacld for $C_{36}H_{50}NNaO_8P$ $(M + Na)^+$: 678.3171, Found: 678.3181.

(2*S*)-[(1*R*)-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. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (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 $(M + Na)^+$. HR-ESI/APCI cacld for $C_{27}H_{43}NaO_7P$ $(M + Na)^+$: 533.2644, Found: 533.2635.

[4-[(3-Propenyl)amino]phenyl]methyl]-phoshonic Acid Bis-(*tert*-butyl) Ester (17). To THF (30 mL) degassed under argon (5 min) were 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), $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (400 mg, 0.50 mmol), and NaO^t-Bu (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 × 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). ^{1}H 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 cacld for $C_{18}H_{30}NO_3P$ (M^+): 339.1963, Found: 339.1961.

[[[[[[2-Oxo-2-[(4*S*)-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), (4*S*)-3-(bromoacetyl)-4-phenyl-2-oxazolidinone (**18**)[19] (1.32 g, 4.64 mmol), and NEt(*i*-Pr)$_2$ (1.20 mL, 6.65 mmol) in DMF (20 mL) was heated at 60 °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). ^{1}H 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 cacld for $C_{29}H_{39}N_2O_6P$ (M^+): 542.2546, Found: 542.2535.

(*βR*,4*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. ^{1}H 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, $CDCl_3$) δ 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. FAB-MS (+VE) *m/z*: 656 M^+. HR-FABMS cacld for $C_{35}H_{49}N_2O_8P$ (M^+): 656.3227, Found: 656.3216.

(*βR*) *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. ^{1}H NMR (400 MHz, $CDCl_3$) δ 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 $(M + H)^+$. HR-FAB cacld for $C_{26}H_{43}NO_7P$ $(M + H)^+$: 512.2777, Found: 512.2775.

1-[[(2*S*,3*S*)-3-[4-[[Bis(*tert*-butyloxy)phosphinyl]methyl]phenyl]-2-[2-(*tert*-butyloxy)-2-oxoethyl]-1-oxo-4-pentenyl]amino]cyclohexanecarbonyl-L-asparagin yl-4,5-didehydro-*N*-(1-naphthalenylmethyl)-L-homoallylglycinamide (21). To a solution of **6** (160 mg, 0.321 mmol) in DMF (1.0 mL) were added HOAt (64 mg, 0.472 mmol), EDCI (121 mg, 0.63 mmol), and NEt(*i*-Pr)$_2$ (0.27 mL, 1.57 mmol), and the mixture was stirred at room temperature (15 min). To this was added a solution of tripeptide **5** (80 mg, 0.157 mmol) in DMF (1.0 mL), and then the mixture was heated (50 °C) and stirred (36 h). The mixture was cooled to room temperature, diluted with H_2O (20 mL), extracted with and EtOAc (3 × 50 mL), and 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). ^{1}H 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* $(M + H)^+$: 986.7.

1-[[(2S,3*S*)-3-[4-[[Bis(*tert*-butyloxy)phosphinyl]methyl]phenyl]-2-[2-(*tert*-butyloxy)-2-oxoethyl]-1-oxo-5-hexenyl]amino]cyclohexanecarbonyl-L-asparaginyl-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. ^{1}H 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 $(M + H)^+$. HR-ESI/ APCI cacld for $C_{54}H_{77}N_5O_{10}P$ $(M + H)^+$: 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 l-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. ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (100 MHz, $CDCl_3$) 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 cacld for $C_{54}H_{76}N_5NaO_{10}P$ $(M + Na)^+$: 1008.5227. Found: 1008.5209.

1-[(*βR*)-*β*-[[[4-[[Bis(*tert*-butyl)phosphinyl]methyl]phenyl]-3-propenyl]amino]-*γ*-(*tert*-but yl)butandioyl]amino]cyclohexanecarbonyl-L-asparaginyl-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. ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (100 MHz, $CDCl_3$) 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 cacld for $C_{53}H_{75}N_6NaO_{10}P$ $(M + Na)^+$: 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]eicos-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 min), Grubbs second generation catalyst [$((PCy_3)(Im(Mes)_2)$-Ru=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 (dichoromethane: MeOH) to provide intermediated *tert*-butyl-protected product as a brown material. This was treated with a mixture of CF_3CO_2H (3.7 mL), triethylsilane (0.10 mL), and H_2O (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 C_{18} column (21 mm diam × 250 mm; cat. no. 00G-4436-P0) using a linear gradient from solvent A, 0% aqueous acetonitrile (0.1% CF_3CO_2H) to solvent B, 100% acetonitrile (0.1% CF_3CO_2H) over 25 min at a flow rate of 10.0 mL/min (detection at 225 nm), retention time: 23.1 min. Lyophilization provided the macrocyclic product **2c** as a white powder (3 mg, 12% yield from **21**). ^{1}H 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 cacld for $C_{40}H_{48}N_5NaO_{10}P$ $(M + Na)^+$: 812.3031, Found: 812.3010.

(9*S*,10*R*,12*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]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% to 100% over 35 min; retention time: 26.5 min) yielded the macrocyclic final product **2d** as a white powder (6 mg, 30% yield from **23**). ^{1}H NMR (400 MHz, acetone-d_6 with one drop D_2O) 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 cacld for $C_{40}H_{48}N_5NaO_{10}P$ $(M + Na)^+$: 812.3036, Found: 812.3011.

(9*S*,10*S*,12*Z*,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]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% to 70% over 30 min; retention time: 27.5 min) yielded the macrocyclic final product **2e** as a white powder (16 mg, 50% yield from **22**). ^{1}H 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 H, 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 cacld for $C_{40}H_{47}N_5O_{10}P$ $(M - H)^-$: 788.3060, Found: 788.3082.

(9*S*,12*E*,15*S*,18*S*)-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 (9*R*,12*E*,15*S*,18*S*)-18-(2-Amino-2-oxoethyl)-15-[[(1-naphthalenylmethyl)amino]carbonyl]-8,17,20-trioxo-10-[4-(phosphonomethyl)phenyl]-7,10,16,19-tetraaza spiro[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 min) and **2g** (3 mg, retention time: 25.4 min) For **2f**: ^{1}H NMR (400 MHz, acetone-d_6, with one drop D_2O) 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 $(M + Na)^+$. HR-MALDI cacld for $C_{39}H_{47}N_6NaO_{10}P$ $(M + Na)^+$: 813.2984. Found: 813.3011. For **2g**: ^{1}H NMR (400 MHz, 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 $(M + Na)^+$. HR-MALDI cacld for $C_{39}H_{47}N_6NaO_{10}P$ $(M + Na)^+$: 813.2984, Found: 813.2988.

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Supporting Information Available: Synthetic procedures for compounds **4** and **5**, ^{1}H NMR spectra of compounds **7**−**9**, **11**, **13**−**15**, **17**, **19**−**24**, and **2c**−**g** and ^{13}C NMR spectra of compounds **5**, **7**−**9**, **11**, **13**−**15**, **17**, **19**, **20**, **22**−**24**, and **2e**−**g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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