Utilization of a β-Aminophosphotyrosyl Mimetic in the Design and Synthesis of Macrocyclic Grb2 SH2 Domain-Binding Peptides

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Grb2 SH2 domains are protein-docking modules that exert important functions in both normal and pathogenic signal transduction processes. Development of synthetic Grb2 SH2 domain binding ligands is being pursued by several groups as potential new therapies for a variety of diseases, including certain cancers. In these efforts, macrocyclization has been successfully utilized to take advantage of preferential recognition by Grb2 SH2 domains of ligands in β-bend conformations. Recent examples of this approach include olefin-metathesis-derived macrocycles that employ ring closure at the β-position of key pTyr-mimicking residues. In the current study, a novel phosphatase-stable β-amino-pTyr mimetic designated "Pmp_β" was utilized to prepare variants of previously reported olefin-metathesis-derived macrocycles. An initial set of simplified cyclic peptides lacking key naphthyl side chain functionality was first synthesized to determine optimum ring size, with results indicating that a four-unit ring-closing segment was appropriate. On the basis of these findings, macrolactamization was undertaken with a more highly functionalized, naphthyl-containing γ-amino acid analogue. The resulting cyclic β-amino peptide is the first of a new class of pTyr-mimetic-containing ligands that may have utility in the development of antagonists of both Grb2 SH2 domains and other pTyr-dependent signaling systems.

The growth factor receptor-bound protein 2 (Grb2) is a noncatalytic adapter module comprising one SH2 domain and two SH3 domains and that serves functions in a variety of signal transduction networks, including the mitogenically important Ras pathway.[1] Recent promising results using kinase inhibitors as anticancer agents[2] highlight the potential therapeutic utility of down-regulating signaling pathways, which include Grb2-dependent processes involved with diseases such as breast and kidney cancers.[3] On the basis of the fact that Grb2 SH2 domains preferentially associate with peptide and protein ligands bearing "pTyr-Xxx-Asn" sequences in β-bend conformations,[4] an objective in the development of Grb2-dependent signaling inhibitors has been the creation of pTyr-mimetic-containing ligands with preinduced "bend geometries".[5] For such purposes, a pTyr + 1 α-aminocyclohexanecarboxylic acid (Ac_6c) residue has demonstrated particular utlility.[6] One example of an Ac_6c-containing tripeptide mimetic is shown by compound **1** (Figure 1).[7] Recently, olefin-metathesis-induced macrocyclizations have provided ring-closed analogues such as **2** that contain additional conformational constraint.[8] These macrocycles can exhibit enhanced Grb2 SH2 domain binding affinity relative to open-chain parents exemplified by **1**.[9] Encouraged by these results, further work was undertaken to explore methods of ring closure other than metathesis approaches.

Macrocycles of type **2** are characterized by ring closure at the pTyr mimetic β-position. This is distinct from traditional cyclic peptides[10] or macrocycles where ring closure is achieved through functionality originating from amino acid α-positions.[8] Maintenance of a similar arrangement without reliance on olefin metathesis chemistries could theoretically be achieved through amide-based ring closure involving β-amino-containing pTyr mimetics. We have recently reported the design and synthesis of such a requisite β-amino analogue (Pmp_β)[11] as an isomeric variant of phosphonomethylphenylalanine (Pmp),[12] which is a widely used phosphatase-stable pTyr mimetic that can exhibit good SH2 domain binding affinity.[13,14] In the first Grb2 SH2 domain directed application of Pmp_β, tripeptide mimetic **3** was synthesized wherein the aromatic functionality that would normally occupy the C-terminus[15] (refer to structures **1** and **2**) had been translocated to the β-position of the Pmp_β residue.[11] The poor binding affinity of **3** (∼600 μM)[16] indicated that the aryl-containing β-amido functionality originating from Pmp_β might be unsuitable in an open-chain configuration. However, it remained to be seen whether ring closure to structures such as **4** might result in reinstatement of Grb2 SH2 domain binding affinity. Of particular note, this genre of ring-closed compounds would represent amido variants of metathesis-derived macrocycles discussed above. Accordingly, reported herein is the design and synthesis of novel peptidomimetics that explore the utility of such β-amido ring-closed macrocycles.

Chemistry

Prior to the synthesis of peptides of type **4**, it was noted that the geometries of β-amido-containing ring-closing segments might differ from those of olefin-metathesis-derived macrocycles such as **2**. An initial study

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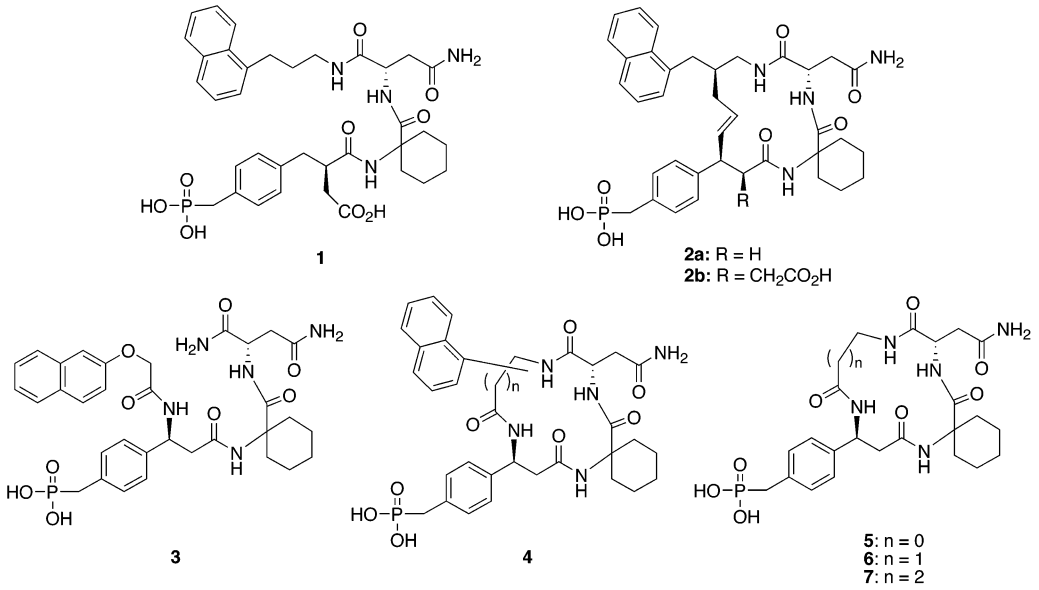


Figure 1. Examples of Grb2 SH2 domain binding compounds.

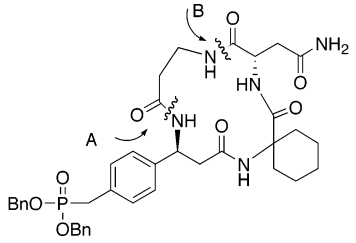


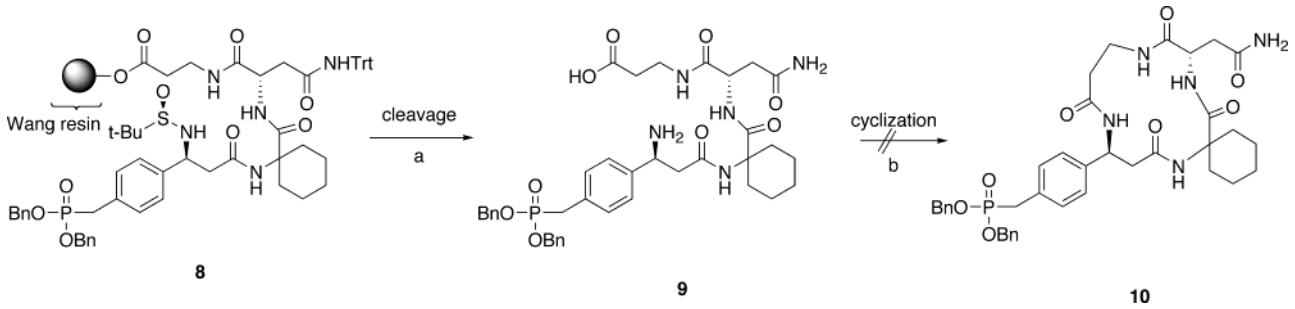
Figure 2. Two possible approaches toward ring closure using a β-amino-pTyr mimetic.

was therefore undertaken to prepare a series of simplified cyclic β-amido-containing peptides (**5**−**7**) that lacked the naphthyl functionality. The intent of this exploratory work was to determine the optimum ring size prior to undertaking a synthetically more demanding fully elaborated naphthyl-containing variant. Preliminary efforts to effect ring closure were directed at condensation of the C-terminal carboxy-containing functionality onto the Pmp$_\beta$ β-amino group (Figure 2, ring closure "A"). Illustrated in Scheme 1 is the attempted preparation of cyclic peptide **6** by this approach. Synthesis of the required protected tripeptide sequence H-Pmp$_\beta$-(OBn)$_2$-Ac$_6$c-Asn-β-Ala-OH (**9**) was achieved by Fmoc-based solid-phase peptide synthesis on Wang resin,[17,18] followed by acidic cleavage from the resin. The linear precursor (**9**) for this route contained orthogonally protected Pmp$_\beta$ (**12**) that was prepared from methyl ester **11**, as previously described (Scheme 2).[11,19] Desired cyclization of the resulting carboxy-terminal peptide **9** did not occur in the presence of either *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene)-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU)[20,21] or diphenylphosphorylazide (DPPA)[22] under conditions of high dilution (5×10^{-4} M). Failure of "route A" raised questions about the suitability of the β-amino center for ring-closing amide bond formation. Therefore, an alternative approach was envisioned whereby acylation of the β-amino group with protected amino-containing residues would proceed prior to ring closure ("route B", Figure 2). An advantage of this protocol is the convergent manner in which penultimate ring-open intermediates **17**−**20** could be derived from a common precursor (**16**, Scheme 3). Synthesis of **17**−**20** necessitated preparation of Fmoc-protected Pmp$_\beta$ residue **13** in order to allow β-amino deprotection/acylation while maintaining C-terminal ^{t}Bu protection (Scheme 3). Building block **13** was readily obtained from common intermediate **11** by acid-catalyzed cleavage of the *N*-(*tert*-butyl)sulfinyl group followed by saponification and in situ Fmoc protection (82% yield, Scheme 2).

Prior to preparing intermediates **18**−**20**, a preliminary ring-closing study was undertaken using tetrapeptide **17**, which can be obtained using commercially available *N*-Fmoc-L-β-phenylalanine (Scheme 3). Tripeptide **15** was prepared by coupling H-Ac$_6$c-Asn(O^{t}Bu) (**14**) with *N*-Fmoc-L-β-phenylalanine in the presence of diisopropylcarbodiimide (DIPCDI) and 1-hydroxybenzotriazole (HOBt). Fmoc deprotection of **15** followed by HOBt active ester coupling with *N*-Boc-β-alanine provided Boc-protected tetrapeptide. Conversion of **15** to **17** was achieved by concomitant TFA-mediated removal of N- and C-terminal protecting groups. In situ activation of **17** using pentafluorophenyl diphenylphosphinate (FDPP) in the presence of *N*-methylmorpholine (NMM)[23] proceeded smoothly to provide the desired macrocycle **21** in 80% yield. It should be noted that use of *O*-[7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) with *N*, *N*-diisopropylethylamine (DIPEA)[24] gave a mixture of **21** and its dehydration product.

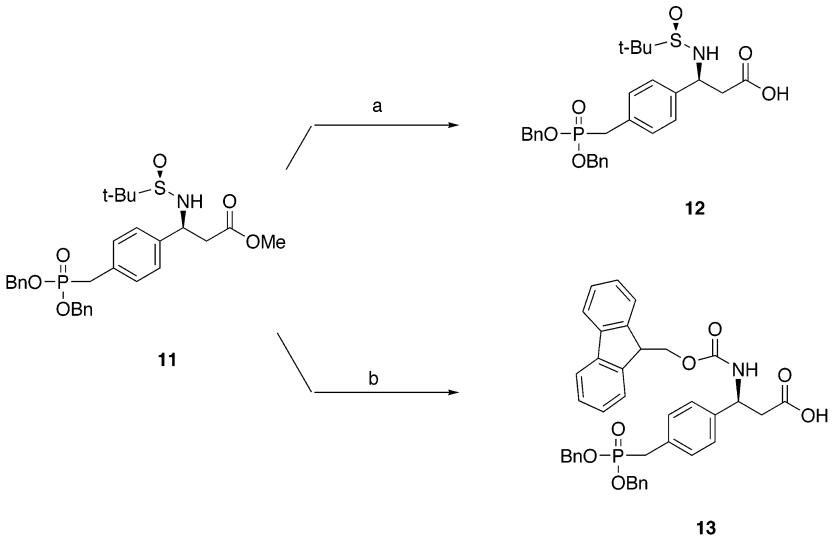
Following a similar strategy, phosphonate-protected linear precursors **18**−**20** were obtained in a convergent fashion from tripeptide **16**, which was prepared by the reaction of *N*-Fmoc-Pmp$_\beta$(OBn)$_2$-OH (**13**) with H-Ac$_6$c-Asn(O^{t}Bu) (**14**) in the presence of 1-hydroxy-7-azabenzotriazole (HOAt) and ethyl(dimethylamino)propylcarbodiimide hydrochloride (EDC·HCl).[7,25] Ring closure of **18**, **19**, and **20** using FDPP under dilute conditions (1×10^{-4} M in DMF at room temperature) provided macrocycles **22**, **10**, and **23**, respectively, in 21−64% yield. The low macrolactamization of **18** (21%) may reflect unfavorable ring strain in the resulting product (**22**).

Scheme 1[a]



[a] Reagents and conditions: (a) TFA/H_2O/Et_3SiH (95:2.5:2.5); (b) HBTU, HOBt, PIPEA, DMF (1 day), or DPPA, NMM, DMF (3 days).

Scheme 2[a]



[a] Reagents and conditions: (a) LiOH, MeOH, H_2O; (b) (i) 4 N HCl/dioxane, MeOH, (ii) LiOH, MeOH, H_2O, then CO_2, Fmoc-OSu, dioxane.

Subsequent hydrogenolytic removal of phosphonate benzyl protection gave target compounds **5**, **6**, and **7**, respectively. The linear des-β-amino peptide **27** was prepared by deprotection of **26**, which was obtained by coupling H-Ac_6C-Asn-NH_2 **24** and 4-phosphonomethyl-caffeic acid analogue **25**[8] under standard conditions (Scheme 4).

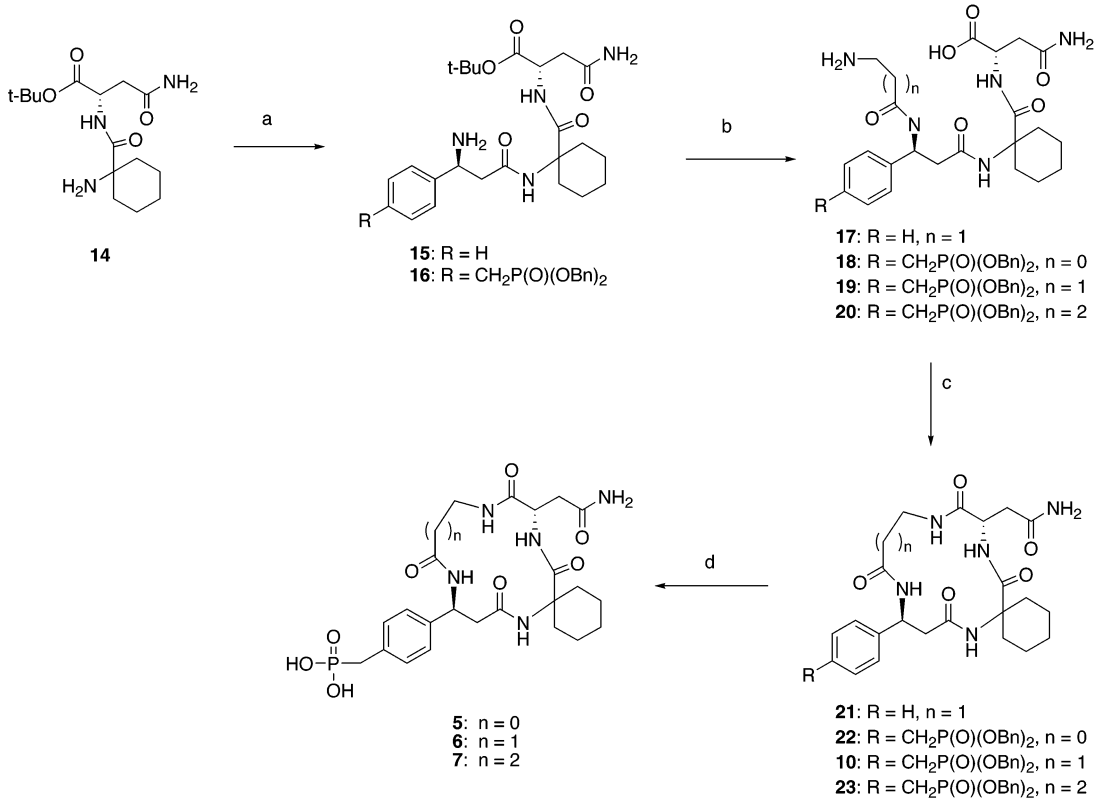
For the synthesis of the more fully elaborated macrocycle **36** bearing naphthyl substitution, the novel amino acid building block **33** was required (Scheme 5). Allyl-containing propylamino derivative **30** was envisioned as a chiral synthon that could provide **33** following oxidation and γ-lactam formation. Amine **30** was synthesized from **29**, which had previously been prepared from acylated Evans' oxazolidinone **28** by protection in the presence of Boc anhydride and TEA.[8] Oxidative cyclization of **30** to lactam **32** proceeded in a two-step fashion by initial osmylation/$NaIO_4$ oxidation to lactol **31**, followed by further oxidation using pyridinium chlorochromate (PCC). Hydrolysis of lactam **32** using 1 N LiOH provided the desired Boc-protected naphthymethyl-γ-amino acid (*N*-Boc-NM-GABA) **33** in 86% yield. Coupling of the requisite β-amino acid **33** with tripeptide **16** in the presence of HOAt yielded open-chain **34** (Scheme 6) in 39% yield after treatment with TFA. Macrocyclization of **34** using FDPP provided benzyl-protected **35**, which gave final product **36** in 26% yield from **34** following hydrogenolytic debenzylation.

Results and Discussion

Although the stereoselective synthesis of β-amino acids[26−29] and their use for induction of turn-geometries[30−33] have found increasing importance in a variety of contexts, the potential utility of these agents in the development of SH2 domain directed ligands has yet to be adequately explored. The recent convergence of two findings (the high Grb2 SH2 domain binding potency of macrocylic tetrapeptide mimetics of type **2** and the availability of a new phosphatase-stable β-amino-pTyr mimetic (Pmp$_\beta$, **12**)) has allowed the design and synthesis of **36** as the first member of a new class of cyclic Grb2 SH2 domain binding ligand. Previous studies with Grb2 SH2 domain−ligand binding ligands had shown the importance of hydrophobic interactions in a region proximal to the C-terminal pTyr + 2 Asn residue.[34,35] This indicated that the design of β-macrocycles should include suitably positioned hydrophobic functionality for interaction with this hydrophobic patch.

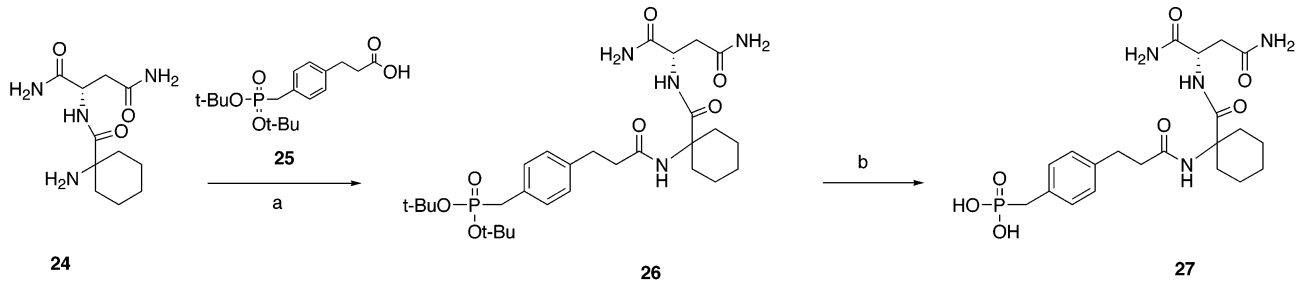
In choosing a ring-closing segment that would serve as an appropriate framework from which to append a desired hydrophobic group, it was important to preserve the bend geometry found in the parent "pTyr-Xxx-Asn" sequence.[4] Therefore, an initial set of analogues **5**−**7** were prepared to investigate effects of ring-closing length on Grb2 SH2 domain binding potency. Analogue **27**, which lacks functionalization at the pTyr mimetic α-position, was chosen as a control for this study in order to reduce synthetic complexity. This simplified

Scheme 3[a]



[a] Reagents and conditions: (a) (i) *N*-Fmoc-L-β-phe-OH or **13**, HOBt, DIPCDI, DMF, (ii) piperidine, DMF; (b) (i) *N*-Boc-β-Ala-OH for **17** and **19**, Boc-Gly-OH for **18**, or Boc-4-Abu-OH for **20**, HOBt, DIPCDI, DMF, (ii) TFA, CH_2Cl_2; (c) FDPP, NMM, DMF; (d) 10% Pd/C, H_2, 40 psi.

Scheme 4[a]

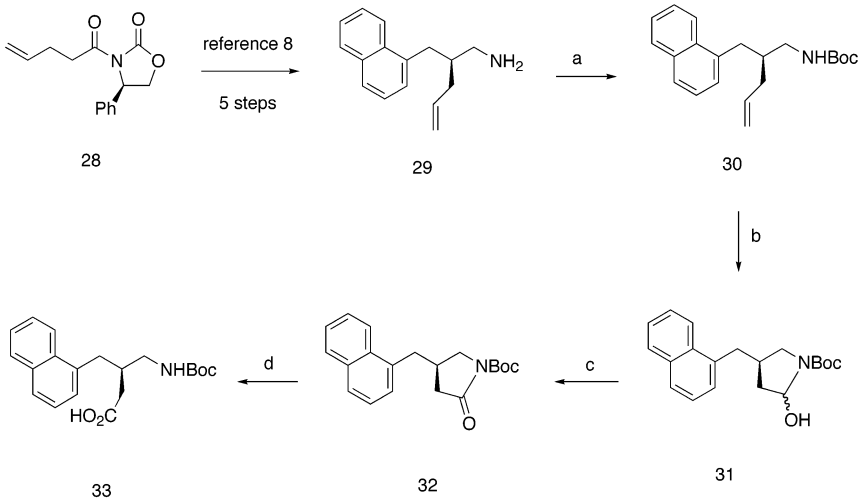


[a] Reagents and conditions: (a) (i) **25**, HOAt, EDC·HCl, DMF, (ii) piperidine, DMF; (b) TFA/H_2O/Et_3Si (95:2.5:2.5).

pTyr mimetic has shown utility in earlier studies that lead to the development of macrocycle **2**.[8] Evaluation of Grb2 SH2 domain binding affinity was performed using a previously reported enzyme linked immunosorbent assay (ELISA) based technique.[36] As shown in Table 1, peptides **5** and **6** containing two- and three-unit ring-closing segments, respectively, were inactive. Alternatively, ring closure with a four-unit segment (peptide **7**) provided affinity equivalent to that of the parent **27**. Since the bend-inducing Ac_6c residue already promotes a turn conformation in open-chain **27**, it was uncertain whether macrocyclization would significantly enhance potency further through bend induction. Indeed, the intent of this initial study was to ascertain ring-closing length that would not *disrupt* proper bend geometry, and within this context, peptide **7** appeared to present an acceptable length. Having established a satisfactory ring-closing length, introduction of naphthyl functionality was achieved next by synthesis of macrocycle **36**, which maintains the same ring-closing size as presented in peptide **7**. A resultant approximate 6-fold enhancement in binding affinity (IC_{50} = 7.9 μM for **36** compared to IC_{50} = 48 μM for **7**) was consistent with previous studies showing the potential benefits of hydrophobic interactions in the region occupied by the naphthyl ring.

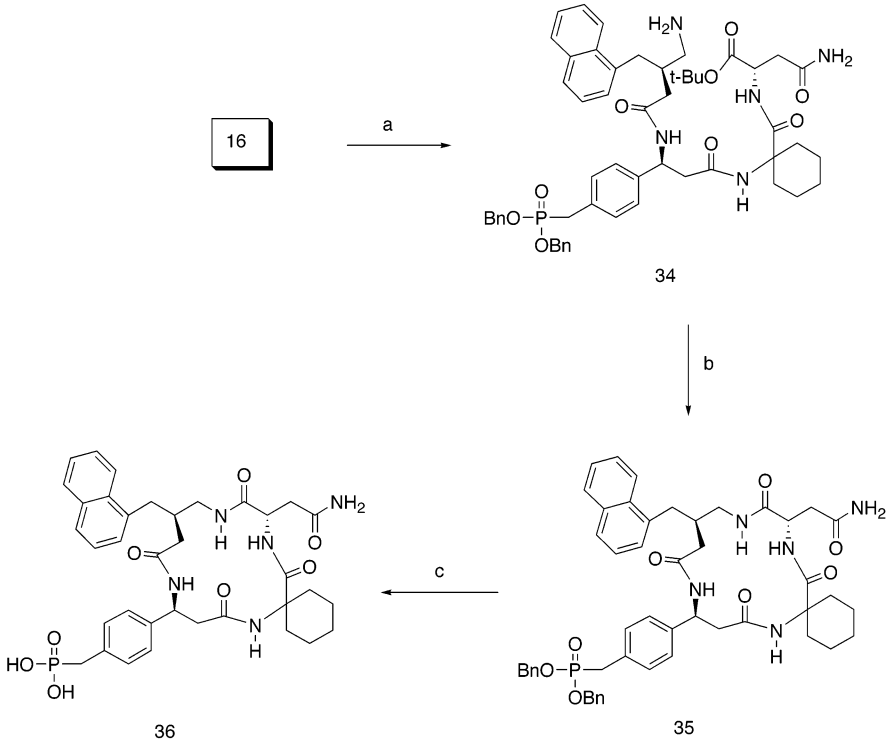
It was also apparent by the approximate 2 orders of magnitude increase in potency in comparing **3** with **36** that appending the naphthyl group onto a ring-closing segment offered significant binding interactions not found in the ring-open parent analogue **3**. However, it seemed that macrocyclization via an amide spacer is less advantageous than via the olefin spacer (IC_{50} = 7.9 μM for **36** versus IC_{50} = 0.026 μM for **2a**). These results are supported by molecular modeling studies. Figure 3 shows the binding of **2a** (panel A) and **36** (panel B) to the Grb2 SH2 domain as generated by molecular mechanics calculations. These panels graphically high-

Scheme 5[a]



[a] Reagents and conditions: (a) $(Boc)_2O$, TEA, MeOH; (b) (i) catalyst OsO_4, NMO, THF/H_2O (1:1), (ii) $NaIO_4$, THF/H_2O (10:1); (c) PCC, molecular sieves, CH_2Cl_2; (d) LiOH, THF.

Scheme 6[a]



[a] Reagents and conditions: (a) (i) **33**, HOAt, EDC·HCl, DMF, (ii) TFA, CH_2Cl_2; (b) FDPP, NMM, DMF; (c) 10% Pd/C, MeOH, H_2 (40 psi).

light potential interactions of ligands with the hydrophobic region. Of note, interactions of **36** with the hydrophobic patch (side chains of Leu β′D1, Lys βD6, and Phe βE3) are less optimal than those of **2a**. This is exemplified by the naphthyl functionality of **36**, which is farther from the side chain of Phe βE3 compared with **2a**. (Estimated distances are shown in panels A and B.) Furthermore, the ring-closing olefin segment of **2a** (pink in panel B) can potentially afford additional favorable interactions with the Lys βD6 side chain that are not possible with the amide spacer found in **36**. A visual comparison of conformational differences, particularly in the naphthyl portions of the ligands, that result from alterations in ring-closing segments is shown in the overlay of **2a** and **36** (Figure 3C). The modest absolute

Table 1. Inhibition Data Obtained in Extracellular ELISA-Based Grb2 SH2 Domain Binding Assays

compound	IC_{50} [a] ± SD (μM)
2a	0.026 ± 0.002
5	≫600
6	≫600
7	47.9 ± 10.2
27	52.5 ± 5.3
36	7.9 ± 0.9

[a] Values were obtained as described in the Experimental Section.

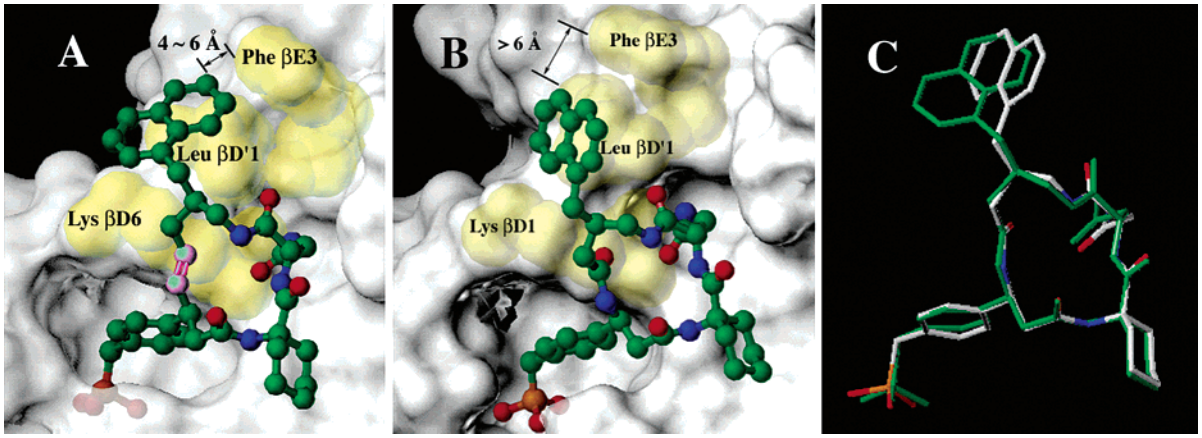


Figure 3. Docking of **2a** (A) and **36** (B) to the Grb2 SH2 domain as determined in the Experimental Section. Panel C shows the overlay of energy-minimized conformations of **2a** (green) and **36** (colored by atom). The ligands were extracted from energy-minimized protein−ligand complexes.

affinity of analogue **36** is also consistent with its lack of functionality at the pTyr mimetic α-position, since functionality at this position is critical for optimum binding interactions in the SH2 domain pTyr-binding pocket.[5] Introduction of such an α-functionality is the objective of ongoing work.

The potential therapeutic value of the Grb2 SH2 domain binding ligands is dependent on their ability to function in whole-cell systems. For analogue **2a**, which represents the olefin-containing homologue of *β*-amino macrocycle **36**, it had been shown previously that despite high affinity in extracellular Grb2 SH2 domain binding assays, markedly reduced potencies were observed in whole-cell assays that examined intracellular binding of Grb2 and growth inhibition.[37] A more recent study has shown that inclusion of carboxylic acid functionality at the pTyr mimetic α-position (compound **2b**) can significantly improve potencies in such whole-cell assays. In the current study, it was therefore expected that without the α-carboxlic acid functionality compound **2a** would exhibit poor inhibitory potencies in cell-based assays.[9] Indeed, side-by-side comparisons of **2a** with **36** showed that neither exhibited significant inhibition of Grb2 SH2 domain binding to growth factor activated erbB-2 in whole cells and that there was no appreciable inhibition of growth of MDA-MB-453 breast cancer cells.[16]

In conclusion, the current study presents the first Grb2 SH2 domain binding ligands derived from carboxamido-based macrocyclization at the pTyr mimetic *β*-position. This class of compound joins recently reported olefin-metathesis-derived macrocycles such as **2** as new Grb2 SH2 domain binding motifs that may provide leads for further development. Results of the work presented herein may also find application in the design of analogues directed at other signal transduction targets that bind ligands in bend conformations, including phosphotyrosine-binding (PTB) domains.[38]

Experimental Section

Biological Procedures. Inhibition of Grb2 SH2 domain binding in extracellular assays using ELISA techniques was performed as previously reported.[36]

Molecular Modeling. Molecular modeling studies were carried out using MacroModel 8.0 (Schrödinger, L.L.C.) and Sybyl 6.8 (Tripops, Inc.) on a Silicon Graphics Octane 2 workstation. Construction of protein−ligand complexes was based on an X-ray structure of the Grb2 SH2 domain complexed with a hexapeptide inhibitor (PDB entry 1TZE). A model of the binding site was constructed consisting of residues between His 58 and Glu 152. Ligands were manually docked into the active site based on the X-ray structure and conformational minima obtained from random conformational analysis. Minimizations were performed with MacroModel using MMFF94 force field (Merck molecular force field)[39] with a continuum solvation model (a generalized Born surface area approach[40]). The PRCG (Polak−Ribier conjugate gradient) method was used with convergence criteria set to Gradient. The ligand as well as protein residues within 5 Å were allowed to move freely during energy minimization, while residues at a distance between 5 and 10 Å were constrained by a parabolic force constant of 50 kJ/Å and residues beyond 10 Å were frozen.

General Synthetic Procedures. Melting points were determined on a Mel-Temp II melting apparatus and are uncorrected. ^{1}H NMR were obtained on a Varian 400 MHz spectrometer, and chemical shifts (δ) are given relative to tetramethylsilane. Fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA. Amino acid analyses were carried out at Peptide Technologies Inc., Gaithersburg, MD. Solvent was removed by rotary evaporation under reduced pressure, and silica gel column chromatography was performed using ICN SiliTech 32-63 D (60 Å). Anhydrous solvents were obtained commercially and used without further drying. HPLC was conducted using a Waters Corp. Prep LC4000 system having photodiodide array detection. Binary solvent systems were as indicated, where solvent system A was 0.1% aqueous TFA and solvent system B was 0.1% TFA in acetonitrile, with a YMC J'sphere ODS-H80 column (4 μm particle size, 8 nm pore size), 20 mm diameter × 250 mm (flow rate of 10 mL/min), being used for preparative work and a YMC J'sphere ODS-H80 column (4 μm particle size, 8 nm pore size), 4.6 mm diameter × 250 (flow rate of 1 mL/min), being used for analytical work.

***N*-Fmoc-Pmp$_\beta$-OH (13).** To a solution of **11**[11] (684.6 mg, 1.228 mmol) in MeOH (2.5 mL) was added 4 N HCl in dioxane (1 mL) at 0 °C, and the resulting mixture was stirred at room temperature (15 min). Volatiles were removed in vacuo, and the resulting amine salt in dioxane/water (1:1, 6 mL) was treated at room temperature with 1 N LiOH (6.1 mL, 6.1 mmol, 20 min). Then CO_2 was introduced for several seconds until the pH was adjusted to 8−8.5 (pH paper). Fmoc *N*-hydroxysuccinimide ester (Fmoc-OSu) (496 mg, 1.47 mmol) in dioxane was added, and the reaction mixture was stirred at room temperature (2 h), diluted with cold 5% citric acid (10 mL), and subjected to an extractive workup (CH_2Cl_2) to provide the crude product. Purification by silica gel column chromatography (CH_2Cl_2/MeOH, 7:1) gave **13** as a white foam (665

mg, 82% yield). ^{1}H NMR (DMSO-d_6): δ 7.41−7.17 (22H, m, aromatic H), 4.91 (5H, m, OCH_2Ph, $C_\beta H$), 4.29−4.10 (3H, m, aliphatic (Fmoc)), 3.72−3.47 (2H, m, CH_2CO_2), 3.29 (2H, d, J = 21.6 Hz, $CH_2P(O)$). FABMS ($^-$Ve) m/z: 660 ($M - H)^-$. Anal. ($C_{39}H_{36}NO_7P\cdot0.1H_2O$) C, H, N.

H-Ac$_6$c-Asn-(O^{t}Bu) (14). To a mixture of H-Asn-(O^{t}Bu)·HCl (1.78 g, 7.92 mmol) and diisopropylethylamine (DIPEA) (1.38 mL, 7.92 mmol) in DMF (20 mL) was added a preformed HOBt active ester solution, which was generated from the reaction of *N*-Fmoc-1-aminocyclohexanecarboxylic acid (*N*-Fmoc-Ac$_6$c-OH)[41] (2.92 g, 8.0 mmol), HOBt (1.13 g, 8.4 mmol), and DIPCDI (1.38 mL, 8.8 mmol) in DMF (20 mL) (10 min). The mixture was stirred overnight, then DMF was removed in vacuo, and the residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH, 15:1) to give *N*-Fmoc-Ac$_6$c-Asn-(O^{t}Bu) (3.86 g, 91% yield). ^{1}H NMR (DMSO-d_6): δ 7.95−7.31 (11H, m, aromatic H, NH, NH_2), 6.86 (1H, s, NH), 4.39 (1H, m, $C_\alpha H$ (Asn)), 4.22 (3H, aliphatic (Fmoc)), 1.43 (2H, m, CH_2C(O)N), 1.17−1.99 (10H, m, cyclohexyl), 1.1 (9H, s, ^{t}Bu). FABMS ($^+$Ve) m/z: 536 (MH^+). To a solution of Fmoc-protected dipeptide (3.8 g, 7.09 mmol) in DMF (60 mL) was added piperidine (3.5 mL), and the resulting solution was stirred at room temperature (1 h). Solvent was removed, and the residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH from 100:1 to 7:1) to give **14** as a colorless oil (596 mg, 92% yield). ^{1}H NMR (CD_3OD): δ 4.57 (1H, dd, J = 5.2 and 6.2 Hz, $C_\alpha H$ (Asn)), 2.80 (1H, dd, J = 6.0 and 16 Hz, CH_2C(O)N), 2.69 (1H, dd, J = 5.2 and 16 Hz, CH_2C(O)N), 1.34−1.90 (10H, m, cyclohexyl), 1.46 (9H, s, ^{t}Bu). FABMS ($^+$Ve) m/z: 314 (MH^+).

H-β-Phe-Ac$_6$c-Asn-(O^{t}Bu) (15). To a solution of **14** (143 mg, 0.448 mmol) in DMF (0.5 mL) was added a preformed active ester solution that was generated by the reaction of *N*-Fmoc-L-β-Phe-OH[42] (175.5 mg, 0.453 mmol), HOBt (64 mg, 0.475 mmol), and DIPCDI (0.08 mL, 0.498 mol) in DMF (1.5 mL) (10 min), and the resulting mixture was stirred overnight. DMF was removed in vacuo and the residue was purified by silica gel flash chromatography (hexanes/EtOAc/1 M NH_3/MeOH, 10:5:1) to give *N*-Fmoc-β-Phe-Ac$_6$c-Asn-(O^{t}Bu) (239 mg, 78% yield) as a white solid. Mp 167−169 °C. ^{1}H NMR (DMSO-d_6): δ 7.22−7.89 (17H, m, aromatic H, NH, NH_2), 6.88 (1H, s, NH), 4.94 (1H, m, $C_\beta H$ (Pmp_β)), 4.33 (1H, m, $C_\alpha H$ (Asn)), 4.18−4.34 (3H, m, aliphatic (Fmoc)), 2.54−2.68 (2H, m, CH_2 (Pmp_β)), 2.44 (d, 2H, J = 5.6 Hz, CH_2C(O)N), 1.28−1.92 (10H, m, cyclohexyl), 1.34 (9H, s, ^{t}Bu). FABMS ($^+$Ve) m/z: 683 (MH^+). To a solution of Fmoc-protected tripeptide (235 mg, 0.344 mmol) in CH_3CN/DMF (2:1, 6 mL) was added piperidine (0.14 mL), and resulting solution was stirred at room temperature for 2 h. Solvent was removed, and the residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH from 15:1 to 7:1) to give **15** as a colorless oil (127 mg, 80% yield). ^{1}H NMR (CD_3OD): δ 7.25−7.39 (5H, m, Ph), 4.48 (1H, t, J = 5.2 Hz, $C_\beta H$ (Pmp_β)), 4.34 (1H, dd, J = 4.8 and 9 Hz, $C_\alpha H$ (Asn)), 2.62−2.75 (4H, m, 2H, m, CH_2 (Pmp_β), CH_2C(O)N), 1.23−2.10 (10H, m, cyclohexyl), 1.42 (9H, s, ^{t}Bu). FABMS ($^+$Ve) m/z: 461 (MH^+).

H-β-Ala-β-Phe-Ac$_6$c-Asn-OH·TFA (17). To a solution of **15** (94 mg, 0.204 mmol) in DMF (0.5 mL) was added the preformed active ester solution that was generated by the reaction of *N*-Boc-β-Ala-OH[38] (38.9 mg, 0.206 mmol), HOBt (29 mg, 0.216 mmol), and DIPCDI (0.035 mL, 0.226 mol) in DMF (1 mL) (10 min), and the resulting mixture was stirred at room temperature (overnight). DMF was removed in vacuo and the residue was purified by silica gel flash chromatography (hexanes/EtOAc/1 M NH_3/MeOH, 4:2:1) to give *N*-Boc-β-Ala-β-Phe-Ac$_6$c-Asn-(O^{t}Bu) as a foam (127 mg, 98% yield). ^{1}H NMR (CD_3OD): δ 7.22−7.38 (5H, m, Ph), 5.33 (1H, m, $C_\beta H$ (β-Phe)), 4.49 (1H, m, $C_\alpha H$ (Asn)), 2.72−2.79 (4H, m, $C_\alpha H_2$ (β-Ala), $C_\alpha H_2$ (β-Phe)), 2.75 (1H, dd, J = 5.2 and 15.8 Hz, C*H*HC(O)N), 2.64 (1H, dd, J = 4.8 and 16 Hz, C*H*HC(O)N), 2.41 (2H, m, $C_\alpha H_2$ (β-Ala)), 2.03 (2H, m, $C_\beta H_2$ (β-Ala)), 1.23−2.04 (10H, m, cyclohexyl), 1.45 (9H, s, ^{t}Bu), 1.42 (9H, s, ^{t}Bu). FABMS ($^+$Ve) m/z: 631 (MH^+). To a solution of Boc-protected tetrapeptide (119 mg, 0.188 mmol) in CH_2Cl_2 (2 mL) was added TFA (2.5 mL), and the resulting mixture was stirred at room temperature (2 h). Volatiles were removed and the resulting residue was re-evaporated from toluene and then triturated with ether and centrifuged to provide **17**·TFA in quantitative yield as a white solid (100 mg). ^{1}H NMR (D_2O): δ 7.40−7.49 (5H, m, Ph), 5.26 (1H, t, J = 7.6 Hz, $C_\beta H$ (Pmp_β)), 4.63 (1H, t, J = 6 Hz, $C_\alpha H$ (Asn)), 3.25 (2H, t, J = 6.8 Hz, $C_\beta H_2$ (β-Ala)), 2.90 (2H, d, J = 6.4 Hz, CH_2C(O)N), 2.65−2.82 (4H, m, CH_2 (Pmp_β), $C_\alpha H_2$ (β-Ala)), 0.99−1.96 (10H, m, cyclohexyl). FABMS ($^+$Ve) m/z: 476 (MH^+).

Cyclo[β-Ala-β-Phe-Ac$_6$c-Asn] (21). To amine salt **17** (25 mg, 0.053 mmol) in DMF (53 mL) was added *N*-methylmorpholine (NMM) (0.06 mL, 0.53 mmol) and pentafluorophenyl diphenylphosphinate (FDPP) (40.4 mg, 0.105 mmol), and the resulting solution was stirred at room temperature for 24 h. Solvent was removed and the resulting residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH from 9:1 to 7:1) to give **6** as a white solid (19 mg, 80% yield). Mp >240 °C (dec). ^{1}H NMR (CD_3OD): δ 7.22−7.39 (5H, m, Ph), 5.49 (1H, dd, J = 3.2 and 12.4 Hz, $C_\beta H$ (Pmp_β)), 4.64 (1H, t, J = 4.4 Hz, $C_\alpha H$ (Asn)), 4.05 (1H, m, $C_\beta H$ (β-Ala)), 2.98−301 (3H, m, $C_\beta H_2$ (β-Ala), $C_\alpha H$ (β-Phe), C*H*HC(O)N), 2.85 (1H, dd, J = 3.6 and 17.8 Hz, $C_\alpha H$ (Pmp_β)), 2.54 (1H, dd, J = 4 and 16.4 Hz, C*H*HC(O)N), 2.08−2.22 (2H, m, $C_\beta H_2$ (β-Ala)), 1.28−1.96 (10H, m, cyclohexyl). FABMS ($^+$Ve) m/z: 458 (MH^+). Anal.($C_{39}H_{36}NO_7P\cdot0.5MeOH\cdot0.5H_2O$) C, H, N.

H-Pmp$_\beta$-Ac$_6$c-Asn-(O^{t}Bu) (16). To a solution of **14** (127 mg, 0.405 mmol), *N*-Fmoc-Pmp$_\beta$-OH (**13**) (268 mg, 0.405 mmol), and HOAt (108 mg, 0.794 mmol) in DMF (10 mL) was added a solution of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide·HCl (EDC·HCl) (113 mg, 0.591 mol) in DMF (10 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 24 h. After removal of volatiles, the residue was dissolved in DMF and treated with piperidine (0.2 mL) for 2 h. The reaction mixture was concentrated to dryness and purified by silica gel flash chromatography (CH_2Cl_2/MeOH from 30:1 to 7:1) to give **16** as a white solid (246 mg, 83% yield). Mp 202−204 °C. ^{1}H NMR (CD_3OD): δ 7.25−7.37 (14H, m, aromatic H), 4.95 (m, 4H, CH_2Ph), 4.55 (1H, m, $C_\beta H$ (Pmp_β)), 4.31 (1H, dd, J = 5.6 and 8.6 Hz, $C_\alpha H$ (Asn)), 3.28 (2H, d, J = 23.6 Hz, $CH_2P(O)$), 2.59−2.83 (4H, m, $C_\alpha H_2$ (Pmp_β), CH_2C(O)-NH), 2.19−1.16 (19H, m, cyclohexyl, ^{t}Bu). FABMS ($^+$Ve) m/z: 735 (MH^+). Anal. ($C_{39}H_{51}N_4O_8P\cdot1MeOH\cdot0.5H_2O$) C, H, N.

H-Gly-Pmp$_\beta$-Ac$_6$c-Asn-OH·TFA (18). To a solution of **16** (152 mg, 0.207 mmol) in DMF (1 mL) was added a preformed active ester solution generated by the reaction of *N*-Boc-Gly-OH (40 mg, 0.228 mmol), HOBt (34 mg, 0.251 mmol), and DIPCDI (0.04 mL, 0.262 mol) in DMF (2 mL) (10 min), and the resulting mixture was stirred overnight. DMF was removed in vacuo and the residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH from 30:1 to 20:1) to give *N*-Boc-Gly-Pmp$_\beta$-Ac$_6$c-Asn-(O^{t}Bu) as a foam (99 mg, 85% yield). ^{1}H NMR (DMSO-d_6): δ 8.27 (1H, d, J = 8.4 Hz, NH), 7.72 (1H, s, NH), 7.67 (1H, d, J = 8 Hz, NH), 7.17−7.38 (15H, m, aromatic H, NH), 6.90 (2H, m, NH_2), 5.18 (1H, m, $C_\beta H$ (Pmp_β)), 4.95 (4H, m, CH_2Ph), 4.33 (1H, m, $C_\alpha H$ (Asn)), 3.53 (2H, d, J = 6 Hz, CH_2 (Gly)), 3.30 (2H, d, J = 21.6, $CH_2P(O)$), 2.52−2.69 (2H, m, CH_2C(O)), 2.43 (2H, dd, J = 2 and 5.4 Hz, $C_\alpha H_2$ (Pmp_β)), 1.91 (2H, m, cyclohexyl), 1.52 (2H, m, cyclohexyl), 1.37 (9H, s, ^{t}Bu), 1.36 (9H, s, ^{t}Bu), 1.27−1.04 (5H, m, cyclohexyl). FABMS ($^+$Ve) m/z: 735 (MH^+). To a solution of Boc-protected tetrapeptide (101 mg, 0.122 mmol) in CH_2Cl_2 (2 mL) was added TFA (4 mL), and the resulting mixture was stirred at room temperature for 2 h. Volatiles were removed and the resulting residue was coevaporated with toluene, treated with ether, and centrifuged to provide **18**·TFA as a white solid (84 mg, 93% yield). ^{1}H NMR (DMSO-d_6): δ 8.79 (1H, d, J = 8 Hz, NH), 7.87 (1H, s, NH), 7.63 (1H, d, J = 8 Hz, NH), 7.15−7.37 (16H, m, aromatic H, NH_2), 6.86 (2H, m, NH_2), 5.22 (1H, m, $C_\beta H$ (Pmp_β)), 4.95−4.98 (4H, m, CH_2Ph), 4.85 (1H, d, J = 7.2, $C_\alpha H$ (Asn)), 4.37 (2H, m, CH_2 (Gly)), 2.74 (1H, dd, J = 7.2 and 14.6 Hz, CH_2C(O)N), 2.58 (1H, dd, J = 7.2 and 14.6 Hz, CH_2C(O)N), 2.46−2.54 (2H, m, $C_\alpha H_2$ (Pmp_β)), 1.83−2.20 (3H, m, cyclohexyl), 0.95−1.55 (m, 7H, cyclohexyl). FABMS ($^+$Ve) m/z: 736 (MH^+).

H-β-Ala-Pmpβ-Ac6c-Asn-OH·TFA (19). To a solution of **16** (149 mg, 0.203 mmol) in DMF (1 mL) was added a preformed active ester solution formed by the reaction of *N*-Boc-β-Ala-OH (38.8 mg, 0.205 mmol), HOBt (30.5 mg, 0.225 mmol), and DIPCDI (0.035 mL, 0.225 mol) in DMF (1.5 mL) (10 min), and the resulting mixture was stirred overnight. DMF was removed in vacuo and the resulting residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH from 18:1 to 12:1) to give *N*-Boc-β-Ala-Pmpβ-Ac6c-Asn-(O^{t}Bu) as a foam (181 mg, 98% yield). ^{1}H NMR (CD_3OD): δ 7.21−7.35 (14H, m, aromatic H), 5.32 (1H, t, J = 6.8 Hz, C_βH (Pmpβ)), 4.89−4.95 (4H, m, CH_2Ph), 4.48 (1H, t, J = 4.8 Hz, C_αH (Asn)), 3.25 (2H, d, J = 23.9 Hz, CH_2P(O)), 2.60−2.85 (6H, m), 2.38 (2H, m, CH_2C(O)N), 1.93−2.06 (2H, m, cyclohexyl), 1.18−1.73 (26H, m, ^{t}Bu, cyclohexyl). FABMS ($^+$Ve) *m*/*z*: 906 (MH^+). To a solution of Boc-protected tetrapeptide (112 mg, 0.123 mmol) in CH_2Cl_2 (2 mL) was added TFA (3 mL), and the resulting mixture was stirred at room temperature for 2 h. Volatiles were removed, and the resulting residue was coevaporated from toluene, treated with ether, and centrifuged to give **19**·TFA as a white solid (91 mg, 99% yield). ^{1}H NMR (DMSO-d_6): δ 8.79 (1H, d, J = 8 Hz, NH), 7.87 (1H, s, NH), 7.63 (1H, d, J = 8 Hz, NH), 7.15−7.37 (16H, m, aromatic H, NH), 6.86 (2H, m, NH_2), 5.22 (1H, m, C_βH (Pmpβ)), 4.95−4.98 (4H, m, CH_2Ph), 4.84 (1H, m, C_αH (Asn)), 4.37 (2H, m, $C_\beta H_2$ (β-Ala)), 2.68−2.98 (8H, m, CH_2P(O), CH_2C(O)N, $C_\alpha H_2$ (β-Ala), $C_\alpha H_2$ (Pmpβ)), 1.83−2.20 (3H, m, cyclohexyl), 0.95−1.55 (7H, m, cyclohexyl). FABMS ($^+$Ve) *m*/*z*: 750 (MH^+).

H-Abu-Pmpβ-Ac6c-Asn-OH·TFA (20). To a solution of **16** (127 mg, 0.173 mmol) in DMF (1 mL) was added a preformed active ester solution formed by the reaction of *N*-Boc-4-aminobutyric acid (*N*-Boc-Abu-OH) (35.6 mg, 0.175 mmol), HOBt (26 mg, 0.19 mmol), and DIPCDI (0.33 mL, 0.21 mol) in DMF (1.5 mL) (10 min), and the resulting mixture was stirred at room temperature overnight. DMF was removed in vacuo, and the residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH from 20:1 to 15:1) to give *N*-Boc-Abu-Pmpβ-Ac6c-Asn-(O^{t}Bu) as a white solid (128 mg, 84% yield). Mp 199−200 °C. ^{1}H NMR (DMSO-d_6): δ 8.25 (1H, d, J = 8.4 Hz, NH), 7.72 (1H, s, NH), 7.68 (1H, d, J = 8 Hz, NH), 7.20−7.36 (15H, m, aromatic H, NH), 6.78 (1H, t, J = 5.2 Hz, NH), 5.20 (1H, dd, J = 8.4 and 15 Hz, C_βH (Pmpβ)), 4.96 (4H, m, 2 CH_2Ph), 4.34 (1H, m, C_αH (Asn)), 3.31 (2H, d, J = 21.6 Hz, CH_2P(O)), 2.87 (2H, dd, J = 6.4 and 12.8 Hz, $C_\gamma H_2$ (Abu)), 2.66 (2H, dd, J = 6.4 and 13.8 Hz, CH_2C(O)N), 2.43 (2H, d, J = 5.6 Hz, $C_\alpha H_2$ (Abu)), 2.06 (2H, t, J = 8 Hz, $C_\alpha H_2$ (Pmpβ)), 1.99 (1H, m, C_βH (Abu)), 1.89 (1H, m, C_βH (Abu)), 1.05−1.59 (28H, m, ^{t}Bu, cyclohexyl). FABMS ($^+$Ve) *m*/*z*: 920 (MH^+). To a solution of Boc-protected tetrapeptide (119 mg, 0.129 mmol) in CH_2Cl_2 (2 mL) was added TFA (4 mL), and the resulting mixture was stirred at room temperature (2.5 h). Volatiles were removed, and the resulting residue was coevaporated with toluene. Then the residue was treated with ether and centrifuged to give **20**·TFA as a white solid (98 mg, 99% yield). ^{1}H NMR (DMSO-d_6): δ 8.39 (1H, d, J = 8.4 Hz, NH), 7.18−7.38 (14H, m, aromatic H), 6.89 (1H, s, NH), 5.21 (1H, m, C_βH (Pmpβ)), 4.95 (4H, m, CH_2Ph), 4.88 (1H, d, J = 7.2 Hz, C_αH (Asn)), 4.39 (2H, m, $C_\gamma H_2$ (Abu)), 2.66−2.97 (4H, m, $C_\alpha H_2$ (Pmpβ), CH_2C(O)N), 2.42−2.51 (2H, m, $C_\alpha H_2$ (Abu)), 2.00 (H, m, $C_\beta H_2$ (Abu)), 1.32−1.88 (10H, m, cyclohexyl). FABMS ($^+$Ve) *m*/*z*: 764 (MH^+).

Cyclo[Gly-Pmpβ(OBn)2-Ac6c-Asn] (22). Following a procedure similar to that reported above for the synthesis of **21**, reaction of amine salt **18** (87.5 mg, 0.119 mmol), NMM (0.13 mL, 1.19 mmol), and FDPP (91.4 mg, 0.238 mmol) in DMF (119 mL) provided benzyl-protected cyclic peptide **22** (18 mg, 21% yield). H NMR (CD_3OD): δ 7.15−7.32 (14H, m, aromatic H), 5.15 (1H, t, J = 5.6 Hz, C_βH (Pmpβ)), 4.89 (4H, m, CH_2Ph), 4.73 (1H, m, C_αH (Asn)), 3.62 (2H, m, CH_2 (Gly)), 3.22 (2H, d, J = 21.6 Hz, CH_2P(O)), 2.72−2.87 (3H, m, CH_2C(O)N, $C_\beta H_2$ (Pmpβ)), 2.56 (1H, dd, J = 6.4 and 15 Hz, CH_2C(O)N), 2.05 (1H, m, cyclohexyl), 1.81−1.89 (2H, m, cyclohexyl), 1.22−1.53 (6H, m, cyclohexyl), 0.86 (1H, m, cyclohexyl). FABMS ($^+$Ve) *m*/*z*: 718 (MH^+). HR-FABMS calcd for $C_{37}H_{44}N_5O_8PCs$: 850.1982 (MCs). Found: 850.1980.

Cyclo[β-Ala-Pmpβ(OBn)2-Ac6c-Asn] (10). Following a procedure similar to that reported above for the synthesis of **21**, reaction of amine salt **19** (39 mg, 0.052 mmol), NMM (0.058 mL, 0.52 mmol), and FDPP (40 mg, 0.10 mmol) in DMF (52 mL) provided benzyl-protected cyclic peptide **10** (24 mg, 63% yield). ^{1}H NMR (CD_3OD): δ 7.22−7.34 (14H, m, aromatic H), 5.23 (1H, m, C_βH (Pmpβ)), 4.94 (4H, m, CH_2Ph), 4.76 (1H, dd, J = 4.8 and 7.4 Hz, C_αH (Asn)), 3.64−3.54 (2H, m, C_βH (β-Ala)), 3.28 (2H, d, J = 21.6 Hz, CH_2P(O)), 2.86 (3H, m, C*H*HC(O)N, $C_\alpha H_2$ (Pmpβ)), 2.77 (1H, dd, C*H*HC(O)N), 2.35 (1H, m, C_αH (β-Ala)), 2.23 (1H, m, C_αH (β-Ala)), 1.86−2.03 (3H, m, cyclohexyl), 1.31−1.66 (7H, m, cyclohexyl). FABMS ($^+$Ve) *m*/*z*: 732 (MH^+). Anal. ($C_{38}H_{46}N_5O_8P·0.6H_2O·1CH_3OH$) C, H, N.

Cyclo[Abu-Pmpβ(OBn)2-Ac6c-Asn] (23). Following a procedure similar to that reported above for the synthesis of **21**, reaction of amine salt **20** (98 mg, 0.128 mmol), NMM (0.14 mL, 1.28 mmol), and FDPP (98.4 mg, 0.256 mmol) in DMF (128 mL) provided benzyl-protected cyclic peptide **23** (40 mg, 42% yield). ^{1}H NMR (CD_3OD): δ 7.19−7.32 (14H, m, aromatic H), 5.29 (1H, d, J = 10.8 Hz, C_βH (Pmpβ)), 4.90 (4H, m, CH_2Ph), 4.60 (1H, dd, J = 4.4 and 9.2 Hz, C_αH (Asn)), 3.48 (1H, m, C_γH (Abu)), 3.04 (1H, m, C_γH (Abu)), 2.69−2.87 (3H, m, CH_2C(O)N, $C_\alpha H_2$ (Pmpβ)), 2.57 (1H, dd, J = 12.8 and 14 Hz, CH_2C(O)N), 2.27 (1H, m, C_αH (Abu), 2.14 (1H, m, C_αH (Abu)), 1.86−1.98 (4H, m, $C_\alpha H_2$ (Abu), cyclohexyl), 1.67−1.84 (2H, m, cyclohexyl), 1.58 (4H, m, cyclohexyl), 1.41 (1H, m, cyclohexyl), 1.29 (1H, m, cyclohexyl). FABMS ($^+$Ve) *m*/*z*: 746 (MH^+). Anal. ($C_{39}H_{48}N_5O_8P·2H_2O$) C, H, N.

Cyclo[Gly-Pmpβ-Ac6c-Asn] (5). A solution of benzyl-protected cyclic peptide **22** (32.8 mg, 0.045 mmol) in MeOH (10 mL) was hydrogenated over 10% Pd/C (30 mg) at 40 psi of H_2 in a Parr shaker (overnight). The mixture was filtered through Celite, the pad was washed with aqueous MeOH, and the combined filtrate was concentrated to a white solid, which was purified on reverse-phase C_{18} silica gel (H_2O/MeOH, 9:1) and lyophilized to give **5** as a white solid (12.4 mg, 51% yield). Mp 262−265 °C. Purity >99% (as determined by reverse-phase HPLC, gradient elution from 0% to 90% of solvent B over 30 min, t_R = 13.44 min). ^{1}H NMR (D_2O): δ 7.32 (4H, m, phenyl), 5.15 (1H, t, J 5.6, C_βH (Pmpβ)), 4.88 (1H, t, J = 8 Hz, C_αH (Asn)), 3.95 (1H, d, J = 15.6 Hz, C_αH (Gly)), 3.84 (1H, d, J = 15.6 Hz, C_αH (Gly)), 2.94−3.05 (3H, m, CH_2P(O) and CHC(O)N), 2.81−2.90 (2H, m, $C_\alpha H_2$ (Pmpβ)), 2.73 (1H, dd, J = 7.6 and 15.4 Hz, CHC(O)N), 2.12 (1H, m, cyclohexyl), 1.90 (3H, m, cyclohexyl), 1.57 (4H, m, cyclohexyl), 1.47 (1H, m, cyclohexyl), 1.35 (1H, m, cyclohexyl). FABMS ($^-$Ve) *m*/*z*: 536 (M − H). HR-FABMS calcd for $C_{23}H_{31}N_5O_8P$: 536.1944 (M − H). Found: 536.1910.

Cyclo[β-Ala-Pmpβ-Ac6c-Asn] (6). Hydrogenolytic debenzylation of protected cyclic peptide **10** as described above for the synthesis of **5** provided final product **6** as a white solid (10.2 mg, 59% yield). Mp >275 °C (dec). Purity 95% (as determined by reverse-phase HPLC, gradient elution from 0% to 90% of solvent B over 20 min, t_R = 5.31 min). ^{1}H NMR (D_2O): δ 7.35 (4H, m, phenyl), 5.29 (1H, dd, J = 3.6 and 12.2 Hz, C_βH (Pmpβ)), 4.82 (1H, m, C_αH (Asn)), 3.64 (1H, m, C_βH (β-Ala)), 3.52 (1H, m, C_βH (β-Ala)), 3.22 (2H, d, J = 21.6 Hz, CH_2P(O)), 2.94 (2H, m, $C_\alpha H_2$ (Pmpβ)), 2.74 (2H, m, CH_2C(O)N), 2.43 (2H, t, J = 5.6 Hz, C_αH (β-Ala)), 2.02 (1H, m, cyclohexyl), 1.88 (3H, m, cyclohexyl), 1.29−1.56 (6H, m, cyclohexyl). FABMS (−Ve) *m*/*z*: 550 (M − H). HR-FABMS calcd for $C_{24}H_{33}N_5O_8P$: 550.2067 (M − H). Found: 550.2056.

Cyclo[Abu-Pmpβ-Ac6c-Asn] (7). Hydrogenolytic debenzylation of protected cyclic peptide **23** (38.3 mg, 0.051 mmol) as described above for the synthesis of **5** provided final product **7** as a white solid (15.6 mg, 54% yield). Mp >282 °C (dec). Purity >99% (as determined by reverse-phase HPLC, gradient elution from 0% to 90% of solvent B over 30 min, t_R = 11.35 min). ^{1}H NMR (D_2O): δ 7.17 (4H, s, phenyl), 5.14 (1H, dd, J = 2.4 and 12.4 Hz, C_βH (Pmpβ)), 4.45 (1H, dd, J = 4.4 and 10.4 Hz, C_αH (Asn)), 3.36 (1H, m, C_γH (Abu)), 2.94 (1H, m,

$C_\gamma H$ (Abu)), 2.82−2.87 (3H, m, $CH_2P(O)$, CHC(O)N), 2.75 (1H, dd, J = 2.4 and 14.6 Hz, $C_\alpha H$ (Pmp$_\beta$)), 2.60 (1H, dd, J = 10.4 and 15.6 Hz, CHC(O)N), 2.52 (1H, dd, J = 12.8 and 14.4 Hz, $C_\alpha H$ (Pmp$_\beta$)), 2.09−2.22 (2H, m, $C_\alpha H$ (Abu)), 1.31−1.82 (12H, m, $C_\beta H$ (Abu, cyclohexyl). FABMS ($^-$Ve) *m*/*z*: 564 (M − H), 79. HR-FABMS calcd for $C_{25}H_{35}N_5O_8P$: 564.2249 (M − H). Found: 564.2223.

Desamino-Pmp(O^{t}Bu)-Ac$_6$c-Asn-NH$_2$ (26). To a solution of **24** (113 mg, 0.443 mmol) and desamino-Pmp(O^{t}Bu)-OH (**25**)[7] (162 mg, 0.456 mmol) in DMF (4 mL) was added HOAt (0.5−0.7 M in DMF, 1.2 mL) followed by a solution of EDC·HCl (110 mg, 0.576 mol) in DMF (4 mL) at 0 °C. Then the mixture was warmed to room temperature and stirred at room temperature for 24 h. The reaction mixture was evaporated to dryness and purified by silica gel flash chromatography (CH_2Cl_2/MeOH from 20:1 to 9:1) to provide **26** as a white solid (224 mg, 85% yield). Mp 117−119 °C. ^{1}H NMR ($CDCl_3$): δ 8.15 (1H, d, J = 8 Hz, NH (Asn)), 7.43 (1H, s, NH (Ac_2C), 7.27 (2H, m, C_γ(O)-NH_2 (Asn)), 7.05−7.11 (4H, m, phenyl), 6.46 (2H, br d, J = 19.6 Hz, $CONH_2$ (Asn)), 4.64 (1H, m, $C_\alpha H$ (Asn)), 2.94 (2H, d, J = 2.94 Hz, $CH_2P(O)$), 2.84 (3H, m, $C_\beta H_2C(O)N$ (Asn), $C_\beta H_2$ (desamino-Pmp)), 2.63 (1H, m, $C_\beta H_2C(O)N$ (Asn), 2.56 (2H, m, $C_\alpha H_2$ (Pmp)), 1.13−2.04 (19H, m, cyclohexyl, ^{t}Bu). FABMS ($^+$Ve) *m*/*z*: 595 (MH^+). HR-FABMS calcd for $C_{29}H_{47}N_4O_7P$: 595.3261 (MH^+). Found: 595.3231.

Desamino-Pmp-Ac$_6$c-Asn-NH$_2$ (27). Treatment of ^{t}Bu-protected phosphonate **26** (48 mg, 0.081 mmol) with a solution of TFA (3.7 mL) and triethylsilane (TES) (0.05 mL) in H_2O (0.1 mL) at room temperature (1 h) with subsequent removal of solvent and purification by reverse-phase HPLC (gradient elution from 0% to 95% of solvent B over 35 min, t_R = 21.49 min) provided **27** as a white solid (25 mg, 65% yield). Mp 142−144 °C. Purity 96% (as determined by reverse-phase HPLC, gradient elution from 0% to 90% of solvent B over 30 min, t_R = 29.29 min). ^{1}H NMR (D_2O): δ 7.27 (4H, m, phenyl), 4.63 (1H, dd, J = 5.2 and 9.4 Hz, $C_\alpha H$ (Asn)), 3.16 (2H, d, J = 21.2 Hz, $CH_2P(O)$), 2.94 (2H, t, J = 6.8 Hz, $C_\beta H$ (desamino-Pmp)), 2.86 (1H, dd, J = 4.8 and 7.8 Hz, CHC(O)), 2.70 (3H, m, $C_\alpha H_2$ (desamino-Pmp, CHC(O)N), 2.11−1.87 (10H, m, cyclohexyl). FABMS ($^-$Ve) *m*/*z*: 481 (M − H). HR-FABMS calcd for $C_{21}H_{31}N_4O_7P$: 481.1873 (M − H). Found: 481.1852.

(2*S*)-2-(Naphthalen-1-ylmethylpent-4-enyl)carbamic Acid *tert*-Butyl Ester (30). To a solution of **29**[8] (1.3 g, 5.8 mmol) and triethylamine (2.4 mL, 17.3 mmol) in MeOH (12 mL) was added Boc anhydride (2 mL, 8.67 mmmol) at 0 °C. Then the mixture was warmed to room temperature and stirred at room temperature overnight. Volatiles were removed, and the residue was dissolved in EtOAc and washed with H_2O. The organic layer was dried (Na_2SO_4), filtered, concentrated to dryness, and then purified by silica gel flash chromatography (petroleum ether/EtOAc from 30:1 to 15:1) to provide **30** as a colorless oil (1.76 g, 94% yield). ^{1}H NMR ($CDCl_3$): δ 7.32−7.95 (7H, m, aromatic-H), 5.84 (1H, m, 4-H), 5.09−5.13 (2H, m, 5-H), 3.23 (1H, m, 1-H), 3.14−2.99 (3H, m, 1-H, 2′-H), 2.18−2.06 (3H, m, 2-H, 3-H), 1.47 (9H, s, ^{t}Bu). FABMS ($^+$Ve) *m*/*z*: 326 (MH^+). Anal. ($C_{21}H_{27}NO_2$) C, H, N.

(3*S*)-*N*-Boc-4-(naphthylmethyl)pyrrolidine-2-one (32). To a solution of **30** (440 mg, 1.35 mmol) in 50% aqueous THF (20 mL) was added 4-methymorpholine *N*-oxide (NMO, 190 mg, 1.62 mmol)) and OsO_4 (4% in H_2O, 0.5 mL) at 0 °C, and the resulting mixture was stirred vigorously at room temperature for 6 h. Additional NMO (190 mg) was added, and stirring was continued overnight. The mixture was poured into 1 M $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was concentrated and purified on silica gel flash chromatography (hexanes/EtOAc from 4:1 to 1:2) to give diol intermediate (367 mg, 76% crude yield) and the recovered starting material **30** (81 mg, 18% yield). To a solution of the diol intermediate (350 mg, 0.97 mmol) in THF (20 mL) was added a suspension of $NaIO_4$ (287.5 mg, 1.62 mmol) in H_2O (2 mL) at 0 °C, and the mixture was stirred for 3.5 h, poured into ice/water, and extracted with EtOAc. The organic layer was concentrated and purified on silica gel flash chromatography (hexanes/EtOAc, 4:1) to give lactol **31** as a colorless oil (246 mg, 77% yield from diol intermediate), which was used without further purification. [No detectable aldehyde proton was apparent in the ^{1}H NMR spectrum.] To a mixture of **31** (245 mg, 0.75 mmol) and molecular sieves (4 Å, 300 mg) in CH_2Cl_2 at room temperature was added pyridinium chlorochromate (PCC, 323 mg), and the mixture was stirred at room temperature (overnight). To this was added Celite and ether. The resulting mixture was filtered through a silica gel pad, and the pad was washed with ether (4 × 100 mL). The combined filtrate was concentrated and purified by silica gel flash chromatography (hexanes/EtOAc from 4:1 to 3:1) to yield lactam **32** as a yellow oil (184 mg, 76% yield). ^{1}H NMR ($CDCl_3$): δ 7.28−7.97 (7H, m, aromatic-H), 3.78 (1H, dd, J = 7.6 and 11.5 Hz, 5-H), 3.54 (1H, dd, J = 6.4 and 11 Hz, 5-H), 3.20 (2H, d, J = 7.6 Hz, 4′-H), 2.79 (1H, m, 4-H), 2.61 (1H, dd, J = 8 and 17.4 Hz, 3-H), 2.39 (1H, dd, J = 7.6 and 17.4 Hz, 3-H), 1.51 (9H, s, ^{t}Bu). FABMS ($^+$Ve) *m*/*z*: 326 (MH^+). Anal. ($C_{20}H_{23}NO_3$) C, H, N.

(3*R*)-3-(*tert*-Butoxycarbonylaminomethyl)-4-naphthalen-1-ylbutyric Acid (*N*-Boc-NM-GABA) (33). To a solution of **32** (153 mg, 0.474 mmol) in THF (2.5 mL) was added 1 M LiOH (1.9 mL, 1.9 mmol), and the resulting solution was stirred at room temperature (2 h). THF was evaporated, and the residue was washed with ether (2 × 10 mL). Then the aqueous layer was acidified with 3 N HCl and extracted with ether (3 × 10 mL). The combined organic layer was dried (Na_2SO_4), filtered, and evaporated to provide **33** as a white foam (140 mg, 86% yield). ^{1}H NMR ($CDCl_3$): δ 7.31−8.07 (7H, m, aromatic-H), 3.24 (2H, br s, 3′-H), 3.54 (2H, m, 4-H), 2.55 (1H, m, 3-H), 2.44 (2H, br s, 2-H), 1.43 (9H, s, ^{t}Bu). FABMS ($^+$Ve) *m*/*z*: 344 (MH^+). FABMS ($^-$Ve) *m*/*z*: 342 (M − H). Anal. ($C_{20}H_{25}NO_4$·0.2H_2O) C, H, N.

Tetrapeptide·TFA (34). To a solution of **16** (260 mg, 0.230 mmol), **33** (122 mg, 0.354 mmol), and HOAt (0.5−0.7 M in DMF, 1.4 mL) in DMF (8 mL) was added a solution of EDC·HCl (99 mg, 0.517 mmol) in DMF (2 mL) at 0 °C. The reaction mixture was warmed to room temperature and then stirred for 22 h. After removal of volatiles, the residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH from 40:1 to 20:1) to provide intermediate Boc-protected tetrapeptide as a white solid (96 mg, 39% yield), which was used without further purification. Mp 247−249 °C. ^{1}H NMR ($CDCl_3$): δ 7.11−7.79 (21H, m, aromatic H), 5.38 (1H, br s, $C_\beta H$ (Pmp$_\beta$)), 4.82−4.88 (4H, m, CH_2Ph), 4.59 (1H, m, $C_\alpha H$ (Asn)), 3.04−3.17 (4H, m, $CH_2P(O)$, $C_\gamma H_2$ (NM-GABA)), 2.90 (1H, m, $C_\beta H$ (NM-GABA)), 2.66−2.80 (5H, m, $CH_2C(O)N$, $C_\alpha H_2$ (Pmp$_\beta$), C*H*-naphthyl), 2.56−2.36 (3H, m, C*H*-naphthyl, $C_\alpha H_2$ (NM-GABA)), 1.92 (2H, m, cyclohexyl), 1.62−1.71 (2H, m, cyclohexyl), 1.18−1.48 (24H, m, cyclohexyl, ^{t}Bu). FABMS ($^+$Ve) *m*/*z*: 1060 (MH^+). To a solution of Boc-protected tetrapeptide (89 mg, 0.084 mmol) in CH_2Cl_2 (2 mL) was added TFA (3 mL), and the resulting mixture was stirred at room temperature (2 h). Volatiles were removed, the resulting residue was coevaporated with toluene, and then the residue was treated with ether and centrifuged to provide **34**·TFA as a white solid (80 mg, quantitative). ^{1}H NMR (DMSO-d_6): δ 8.57 (1H, d, J = 8.4 Hz, NH), 7.15−8.11 (24H, m, aromatic H, NH × 4), 6.90 (2H, d, NH), 5.27 (1H, m, $C_\beta H$ (Pmp$_\beta$)), 4.91 (4H, dd, J = 2.8 and 4.8 Hz, CH_2Ph), 4.84 (1H, d, J = 7.2 Hz, $C_\alpha H$ (Asn)), 4.39 (2H, m, $C_\gamma H_2$ (NM-GABA)), 3.02 (3H, m, $C_\beta H$ (NM-GABA), $CH_2C(O)N$), 2.88 (2H, m, $C_\alpha H_2$ (Pmp$_\beta$)), 2.72 (2H, m, CH_2-naphthyl), 2.21 (2H, m, $C_\alpha H_2$ (NM-GABA)), 1.32−1.52 (10H, m). FABMS ($^+$Ve) *m*/*z*: 904 (MH^+).

Benzyl-Protected Cyclopeptide (35). Following a procedure similar to that reported above for the synthesis of **21**, reaction of amine salt **34** (76 mg, 0.084 mmol), NMM (0.092 mL, 0.84 mmol), and FDPP (64 mg, 0.168 mmol) in DMF (82 mL) provided benzyl-protected cyclic peptide **35** (32 mg, 48% yield). ^{1}H NMR (CD_3OD): δ 7.16−8.40 (21H, m, aromatic H), 5.31 (1H, d, J = 12 Hz, $C_\beta H$ (Pmp$_\beta$)), 4.88 (4H, m, CH_2Ph), 4.65 (1H, m, $C_\alpha H$ (Asn)), 3.78 (1H, dd, J = 12.8 and 6.8 Hz, $C_\gamma H$ (NM-GABA)), 3.34−3.37 (1H, m, $C_\beta H$ (NM-GABA)), 3.22 (2H, d, J = 21.6 Hz, $CH_2P(O)$), 3.03 (1H, m, $C_\gamma H$ (NM-GABA), 2.49−2.91 (6H, m, $CH_2C(O)N$, CH_2-naphthyl, $C_\alpha H$ (Pmp$_\beta$)), 1.99−2.15 (3H, m, $C_\alpha H$ (NM-GABA), cyclohexyl), 1.29−1.85

(9H, m, cyclohexyl). FABMS ($^+$Ve) *m*/*z*: 886 (MH^+). HR-FABMS calcd for $C_{50}H_{56}N_5O_8$PCs: 1018.2921 (MCs). Found: 1018.2962.

Cyclopeptide (36). Hydrogenolytic debenzylation of protected cyclic peptide **35** as described above for the synthesis of **5** provided final product **36** as a white solid (16 mg, 62% yield). Mp >255 °C (dec). Purity >99% (as determined by reverse-phase HPLC, gradient elution from 0% to 90% of solvent B over 35 min, t_R = 10.01 min). ^{1}H NMR (DMSO-d_6): δ 8.37 (1H, d, J = 8 Hz, NH), 8.33 (1H, s, NH), 8.07 (1H, d, J = 8.8 Hz, NH), 7.91 (1H, d, J = 7.6 Hz, NH), 7.78 (1H, dd, J = 2.4 and 7 Hz, NH), 7.10−7.56 (11H, m, aromatic-H), 6.85 (1H, s, NH), 6.52 (1H, s, NH), 5.13 (1H, m, C_βH (Pmp$_\beta$)), 4.24 (1H, m, C_αH (Asn)), 3.08−3.18 (3H, m, $C_\gamma H_2$ (NM-GABA), C_βH (NM-GABA)), 2.89 (2H, d, J = 20.8 Hz, CH_2P(O)), 2.75 (6H, m, CH_2C(O)N, $C_\beta H_2$ (Pmp$_\beta$), CH_2-naphthyl), 1.92−2.10 (2H, m, $C_\alpha H_2$ (NM-GABA)), 1.16−1.82 (10H, m, cyclohexyl). FABMS ($^-$Ve) *m*/*z*: 704 (M − H). HR-FABMS calcd for $C_{36}H_{44}N_5O_8$-PCs: 838.1982 (MCs). Found: 838.2020.

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