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Synthesis of *N*-Fmoc 3-(4-(di-(*tert*-butyl)phosphonomethyl)-phenyl)pipecolic acid as a conformationally constrained phosphotyrosyl mimetic suitably protected for peptide synthesis☆

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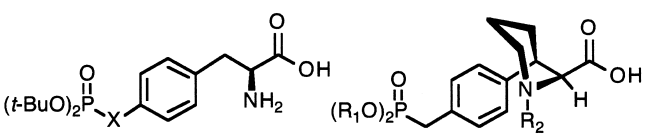
Abstract—Phosphonomethylphenylalanine (Pmp, **2**) has shown wide utility as a hydrolytically stable phosphtyrosyl (pTyr, **1**) mimetic, particularly in Src homology 2 (SH2) domain-binding peptides. (2*S*,3*R*)-3-(4-(phosphonomethyl)phenyl)pipecolic acid (**3**) represents a variant of Pmp having ϕ and χ_1 torsion angles constrained through incorporation into the piperidinyl ring structure contained within pipecolic acid. Reported herein is the enantioselective preparation of **3**, in an orthogonally protected form (**4**) suitable for use in peptide synthesis. Stereochemistries at both the 2- and 3-positions are derived inductively from a single chiral center provided by the commercially available Evans chiral auxiliary, (4*S*)-4-benzyl-1,3-oxazolidin-2-one. Incorporation of **4** into a Grb2 SH2 domain-directed tripeptide (**18**) showed that Grb2 SH2 domain-binding affinity was reduced relative to the parent Pmp-containing tripeptide (**19**). Although conformational constraint did not enhance affinity in this case, novel amino acid analogue **4** may serve as a useful tool for the induction of defined phosphotyrosyl geometry in peptides directed at other signal transduction targets.

1. Introduction

Binding of flexible peptide ligands to target proteins can be negatively affected by entropy terms arising when specific binding geometries must be achieved from random populations of solution conformations. To minimize such penalties and thereby achieve higher affinities, amino acid analogues have been developed, which either restrict peptide backbone or side chain torsion angles.[2,3] Phosphotyrosyl residues (pTyr, **1**) provide critical recognition elements in a variety of cellular signal transduction processes. Accordingly, pTyr mimetics can serve important roles in the development of signaling inhibitors.[4,5] Among pTyr mimetics, phosphonomethyl phenylalanine (Pmp, **2**), has proven useful in the preparation Src of homology 2 (SH2) domain-binding ligands,[6–10] particularly for Grb2 SH2 domain-directed agents, where Pmp exhibits binding affinity nearly equal to parent pTyr.[11] We have previously detailed synthesis of conformationally constrained monomeric pTyr mimetics which approximate binding geometries observed in the X-ray structure of a p56lck SH2 domain-bound pTyr residue.[12] However, little has been reported on the development of conformationally restricted pTyr mimetics for use in peptide-based inhibitors.[13,14] As an entry into this area of investigation, we therefore designed analogue **3** as a variant of Pmp, wherein restriction of χ_1 and ϕ angles is achieved by a 3-carbon bridge between the β-carbon and the α-nitrogen. The resulting 3-(4-phosphonomethylphenyl)pipecolic acid derivative bears the 2*S* α-amino configuration, which is the more potent SH2 domain-binding enantiomer of Pmp.[15,16] Molecular modeling studies of analogue **3** docked in an SH2 domain pTyr-binding pocket mandate 3*R*-stereochemistry. Our utilization of pipecolic acid adds to recent reports where this nucleus has been used for construction of constrained amino acid analogues.[17–19] Herein is reported the synthesis of *N*-Fmoc-protected **4** as a conformationally constrained pTyr mimetic suitably protected for peptide synthesis (Fig. 1).



1 X = O (pTyr) **3** R_1 = H; R_2 = H
2 X = CH_2 (Pmp) **4** R_1 = Bu*t*; R_2 = Fmoc

Figure 1. Structures of pTyr and selected pTyr mimetics.

☆ See ref. 1.

Keywords: tripeptide; inhibitor; mimetic.

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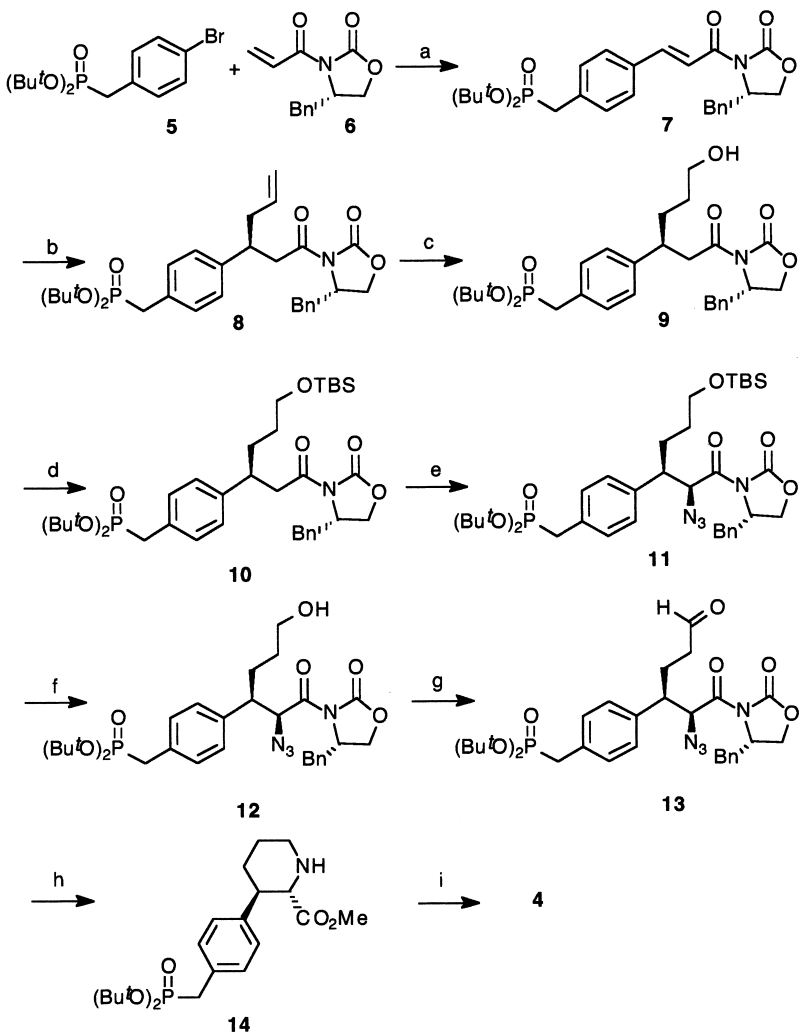
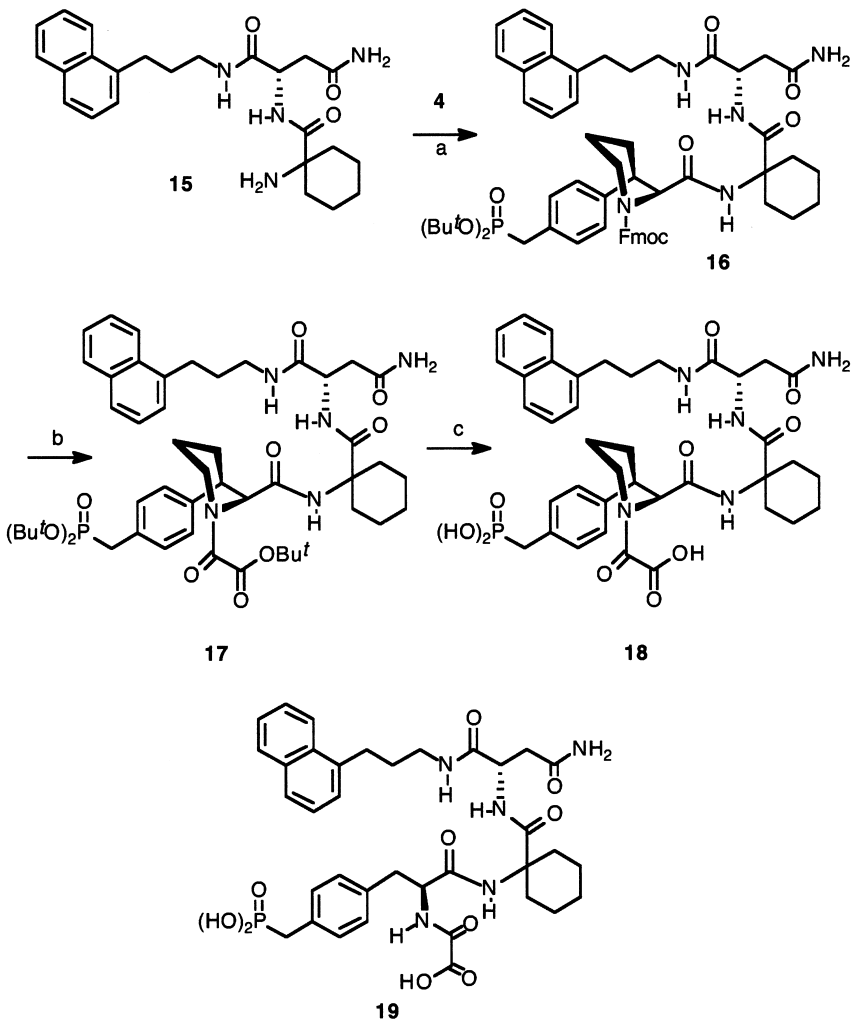


Scheme 1. *Reagents and conditions*: (a) $Pd(OAc)_2$, tri-*o*-tolylphosphine, NEt_3, reflux (90% yield); (b) allylmagnesium bromide, CuBr·SMe_2, THF, −78°C (80% yield); (c) (i) BH_3, THF, 0°C, (ii) $NaBO_3·H_2O$, H_2O (90% yield, two steps); (d) TBDMS-Cl, imidazole, DMF (73% yield); (e) (i) KHMDS, trisyl azide, −78°C; (ii) HOAc, KOAc, 30–35°C (35% yield); (f) HF·pyridine, THF, 0°C (87% yield); (g) oxalyl chloride, DMSO, NEt_3, −78°C–room temperature; (h) H_2/10% Pd·C, MeOH (39% yield, two steps); (i) 1N NaOH, 0°C; CO_2, Fmoc-OSu (47% yield).

2. Results and discussion

Our synthetic approach (Scheme 1) was predicated on our recently reported synthesis of 3-phenylpipecolic acid,[19] wherein introduction of chirality at both the 2- and 3-positions is achieved through stereochemical induction originating from commercially available Evans reagent.[20] As previously reported,[21] cinnamoyl derivative **7** is obtained by Heck reaction of phosphonate **5** with acrylamide **6**, which was prepared from (*S*)-(−)-4-benyl-2-oxazolidinone.[22] Michael addition of allylmagnesium bromide in the presence of copper bromide·dimethyl sulfide complex leads to **8** (80% yield), which was converted to primary alcohol **9** by hydroboration/oxidation[23] (90% yield). Silylation yields **10**, which upon electrophilic Cα asymmetric azidation by the method of Evans,[21,24] yields **11**. Removal of silyl protection through treatment with HF·pyridine[25] (use of tetrabutylammonium fluoride resulted in partial cleavage of the chiral auxiliary) gave free alcohol **12** in 87% yield. Sequential Swern oxidation[26] of **12** to aldehyde **13** was followed by hydrogenation over 10% Pd·C in MeOH (40 psi H_2). Similar to our recent report,[19] this protocol achieved ring closure with concomitant methanolysis of the chiral auxillary to provide **14** directly in 39% yield. Finally, hydrolysis of the methyl ester and in situ Fmoc-amino protection gave desired final product **4** in 47% yield.

Pmp-containing tripeptide **19** (Scheme 2) previously has been shown to have high Grb2 SH2 domain-binding affinity[27] and to exhibit interesting biological effects in whole cells driven by Grb2-dependent signaling pathways.[28] In order to evaluate the potential utility of **4**, peptide **18** was prepared as a variant of **19** bearing conformationally restricted Pmp analogue **3**. Synthesis of **18** was achieved by standard Fmoc-protocols (Scheme 2) similar to that reported for the preparation of parent **19**.[27] The resulting peptide was examined for Grb2 SH2 domain binding potency using an extracellular ELISA-based assay.[29] As shown in Fig. 2, peptide **18** exhibited an approximate 200-fold reduction in binding potency relative to parent **19**. Although enhanced binding affinity was not achieved, the utility of **4** as a new amino acid analogue suitably protected for peptide synthesis has been demonstrated. The extensive variety of contexts in which pTyr residues serve important roles in cellular signal



Scheme 2. *Reagents and conditions*: (a) HOBT, DPCDI, DMF (quantitative); (b) (i) piperidine, acetonitrile; (ii) (*tert*-BuO)COCOCl, N(*i*-Pr)$_2$Et, DMF (77% yield); TFA, SiEt$_3$, CH_2Cl_2 (79% yield).

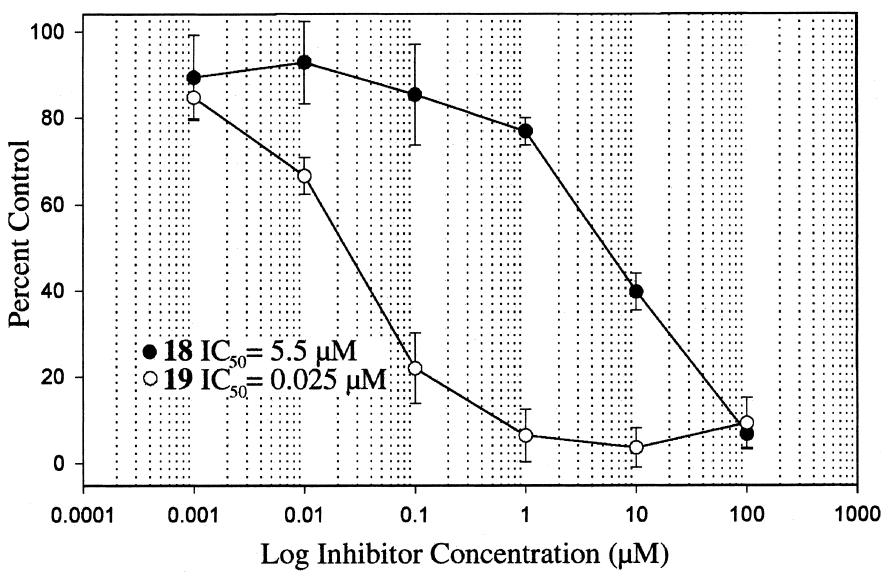


Figure 2. ELISA Grb2 SH2 domain-binding data for compounds **18** and **19**, performed as described in Section 3.

transduction, may render **4** of potential value for the development of pharmacological tools directed at other systems.

3. Experimental

3.1. Evaluation of Grb2 SH2 domain binding using ELISA techniques

A biotinated phosphopeptide encompassing the Grb2 SH2 domain-binding sequence derived from the SHC protein, was bound at 20 ng/mL to 96-well plates, overnight. Nonspecific interactions were inhibited by 5% bovine serum albumin containing TBS. Samples of recombinant purified Grb2 SH2-GST fusion protein were pre-incubated with serial of dilutions of inhibitor peptides prior to addition into each well. After extensive washing with 0.1% bovine serum albumin in TBS, bound Grb2 SH2 domain protein was detected using anti-GST antibodies and goat anti-mouse antibody conjugated to alkaline phosphatase. Quantitation of bound alkaline phosphatase was achieved by a colorimetric reaction employing *para*-nitrophenyl phosphate as substrate.

3.2. Synthesis

3.2.1. (4*S*)-3-((3*S*)-3-(4-((Bis-(*tert*-butyl)phosphono)-methyl)phenyl)hex-5-enoyl)-4-benzyl-1,3-oxazolidin-2-one (8). To a slurry of $CuBr{\cdot}SMe_2$ (4.44 g, 21.6 mmol) in THF (150 mL), was added a solution of allylmagnesium bromide (43.2 mL, 43.2 mmol) at −78°C under argon, and the resulting mixture was stirred at −78°C (1.5 h). A solution of **7**[21] (7.38 g, 14.4 mmol) in THF (150 mL) was added at −78°C, and stirring was continued (2.5 h) then the reaction was quenched by addition of saturated NH_4Cl. The mixture was extracted with EtOAc (3×100 mL) and the combined organic extracts were washed with H_2O and brine, dried (Na_2SO_4), filtered and evaporated to provide crude product. Purification by silica gel flash chromatography (EtOAc–hexane from 1:2 to 1:1) afforded **8** (6.40 g, 80%). Mp 83–84°C; $[\alpha]_D^{20}$=+49.3 (*c* 1.08, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.20 (3H, m), 7.18–7.11 (6H, m), 5.67 (1H, ddt, *J*=17.0, 10.2, 7.0 Hz), 5.03–4.92 (2H, m), 4.44 (1H, m), 4.04–3.96 (2H, m), 3.40–3.28 (2H, m), 3.24–3.16 (2H, m), 2.96 (2H,d, *J*=21.3 Hz), 2.63 (1H, dd, *J*=13.2, 9.8 Hz), 2.41 (2H, t, *J*=7.0 Hz), 1.37 (9H, s), 1.36 (9H, s). IR (neat) 2980, 1782, 1698, 1172 cm^{-1}. FABMS (+VE) *m*/*z* 556 (M+1). Anal. calcd for $C_{31}H_{42}NO_6P$: C 67.01, H 7.62, N 2.52. Found: C 66.71, H 7.73, N 2.48.

3.2.2. (4*S*)-3-((3*S*)-6-Hydroxy-3-(4-((bis-(*tert*-butyl)phosphono)methyl)phenyl) hexanoyl)-4-benzyl-1,3-oxazolidin-2-one (9). To a solution of **8** (3.84 g, 6.91 mmol) in THF (36 mL), was added BH_3 (6.91 mL, 1.0 M in THF, 6.91 mmol) at 0°C under argon, then the mixture was stirred at 0°C (1 h). To the mixture were added H_2O (36 mL) and $NaBO_3{\cdot}H_2O$ (690 mg, 6.91 mmol) and the mixture was stirred at room temperature (3 h). The mixture was diluted with EtOAc and the organic phase was collected and combined with EtOAc extracts (3×40 mL) of the aqueous phase. The combined organic layers were washed with H_2O and brine, dried (Na_2SO_4), filtered and evaporated. Crude product was purified by silica gel flash chromatography (from EtOAc–hexane, 1:1 to MeOH–$CHCl_3$, 3:100) to afford **9** as white solid (3.56 g, 90%). Mp 98–99°C; $[\alpha]_D^{20}$=+35.3 (*c* 0.60, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.14–7.35 (9H, m), 4.48 (1H, m),4.05 (2H, m), 3.60 (2H, t, *J*=6.4 Hz), 3.38 (1H, m), 3.21 (3H, m), 3.0 (2H, d, *J*=21.3 Hz), 2.67 (1H, dd, *J*=13.2, 10.3 Hz), 1.41 (9H, s), 1.40 (9H, s), 1.30–1.85 (4H, m). IR (neat) 2980, 1782, 1698, 1172 cm^{-1}. FABMS (+VE) *m*/*z* 574 (MH^+). Anal. calcd for $C_{31}H_{44}NO_7P{\cdot}0.5H_2O$: C 63.90, H 7.78, N 2.40. Found: C 63.91, H 7.62, N 2.36.

3.2.3. (4*S*)-3-((3*S*)-3-(4-((Bis-(*tert*-butyl)phosphono)-methyl)phenyl)-6-(1,1,2,2-tetramethyl-1-silapropoxy)-hexanoyl)-4-benzyl-1,3-oxazolidin-2-one (10). To a solution of **9** (722 mg, 1.26 mmol) in DMF (5 mL) were added *tert*-butyldimethylsilyl chloride (TBDMS-Cl) (230 mg, 1.51 mmol) and imidazole (214 mg, 3.16 mmol) and the mixture was stirred overnight at room temperature. Solvent was evaporated and the resulting residue was partitioned between EtOAc (30 mL) and brine, dried (Na_2SO_4), filtered and evaporated. Crude product was purified by silica gel flash chromatography (EtOAc–hexane 1:1) to afford **10** as a colorless oil (712 mg, 82%); $[\alpha]_D^{20}$=+37.5 (*c* 0.99, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.13–7.35 (9H, m), 4.45 (1H, m), 3.95–4.08 (2H, m), 3.55 (2H, t, *J*=6.4 Hz), 3.41 (1H, m), 3.11–3.24 (3H, m), 2.99 (2H, d, *J*=21.4 Hz), 2.66 (1H, dd, *J*=13.2, 10.3 Hz), 1.60–1.85 (2H, m), 1.35–1.45 (2H, m), 1.40 (9H, s), 1.39 (9H, s), 0.87 (9H, s), 0.01 (6H, s). IR (neat) 2980, 1782, 1698, 984 cm^{-1}. FABMS (+VE) *m*/*z* 482 (MH^+). Anal. calcd for $C_{37}H_{58}NO_7SiP$: C, 64.60; H, 8.50; N, 2.04. Found: C, 64.88; H, 8.56; N, 2.01.

3.2.4. 1-((4*S*)-2-Oxo-4-benzyl(1,3-oxazolidin-3-yl))-(2*S*,3*R*)-2-azido-3-(4-((bis-(*tert*-butyl)phosphono)-methyl)phenyl)-6-(1,1,2,2-tetramethyl-1-silapropoxy)-hexan-1-one (11). To a solution of KHMDS (3 mL, 0.5 M in toluene, 1.50 mmol) in THF (6 mL), was added a solution of **10** (870 mg, 1.26 mmol) in THF (6 mL) at −78°C under argon and stirring was continued at −78°C (40 min). To this was added a cooled (−78°C) solution of trisyl azide (585 mg, 1.89 mmol) in THF (3 mL). The mixture was stirred at −78°C for 2 min then the reaction was quenched by addition of acetic acid (0.36 mL, 6.3 mmol) and KOAc (1.24 g, 12.6 mmol) and the mixture was stirred at 30–35°C (3 h). The mixture was diluted with EtOAc, washed with saturated $NaHCO_3$, H_2O and brine. The solvent was dried (Na_2SO_4) and evaporated. Crude product was purified by silica gel flash chromatography (EtOAc–hexane from 1:3 to 1:1) to afford **11** as a colorless oil (319 mg, 35% yield); $[\alpha]_D^{20}$=+96.5 (*c* 1.59, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (4H, m), 7.11 (5H, m), 5.24 (1H, d, *J*=10.2 Hz), 3.97 (1H, m), 3.85 (1H, dd, *J*=9.2, 1.9 Hz), 3.58 (1H, m), 3.56 (2H, t, *J*=6.4 Hz), 3.13 (1H, dd, *J*=13.3, 3.0 Hz), 3.05 (1H, t, *J*=10 Hz), 2.93 (2H, d, *J*=21.3 Hz), 2.66 (1H, dd, *J*=13.2, 9.8 Hz), 2.14 (1H, m), 1.82 (1H, m), 1.30–1.43 (2H, m), 1.41 (9H, s), 1.36 (9H, s), 0.88 (9H, s), 0.02 (6H, s). IR (neat) 2931, 2103 ($-N_3$), 1783, 1739, 1699, 1240, 977 cm^{-1}. FABMS (+VE) *m*/*z* 673 ($MH^+-C_4H_8$). Anal. calcd for $C_{37}H_{57}N_4O_7PSi$: C 60.97, H 7.88, N 7.69. Found: C 61.27, H 8.15, N 7.22. HR-FABMS calcd for

$C_{29}H_{42}N_4O_7PSi$ ($MH^+-2[C_4H_8]$): 617.2560. Found: 617.2546.

3.2.5. 1-((4*S*)-2-Oxo-4-benzyl(1,3-oxazolidin-3-yl))-(2*S*,3*R*)-2-azido-6-hydroxy-3-(4-((bis-(*tert*-butyl)phosphono)methyl)phenyl)hexan-1-one (12). To a solution of **11** (60 mg, 0.098 mmol) in THF (3 mL) in a plastic vial was added HF·pyridine (0.1 mL) at 0°C and the mixture was stirred at 0°C (30 min), then at room temperature (3 h). The mixture was cooled to 0°C, diluted with EtOAc and neutralized with saturated $NaHCO_3$ until generation of carbon dioxide ceased. The mixture was extracted with EtOAc (3×15 mL) and evaporated. Crude product was purified by silica gel flash chromatography (from EtOAc–hexane, 1:1 to MeOH–$CHCl_3$, 3:100) to afford **12** as a white solid (44 mg, 87%). Mp 52–55°C; $[\alpha]_D^{23}=+126.8$ (*c* 0.57, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 7.13–7.33 (9H, m), 5.33 (1H, d, *J*=9.8 Hz), 4.03 (1H, m), 3.90 (1H, d, *J*=7.7 Hz), 3.62 (1H, m), 3.60 (2H, t, *J*=6.4 Hz), 2.90–3.21 (3H, m), 2.94 (2H, d, *J*=21.3 Hz), 2.67 (1H, dd, *J*=13.2, 9.8 Hz), 2.17 (1H, m), 1.85 (1H, m), 1.30–1.43 (2H, m), 1.42 (9H, s), 1.37 (9H, s). IR (neat) 2102 ($-N_3$), 1780, 980 cm^{-1}. FABMS (+VE) *m/z* 615 (MH^+). Anal. calcd for $C_{31}H_{43}N_4O_7P$: C 60.57, H 7.05, N 9.11. Found: C 60.21, H 7.16, N 8.91.

3.2.6. Methyl (2*S*,3*R*)-3-(4-((bis-(*tert*-butyl)phosphono)-methyl)phenyl)piperidine-2-carboxylate (14). To a solution of oxalyl chloride (44 μL, 0.50 mmol) in CH_2Cl_2 (1 mL), was added a solution of DMSO (71 μL, 1.00 mmol) in CH_2Cl_2 (1 mL) at −78°C. The mixture was stirred at −78°C (20 min), then a solution of **12** (62 mg, 0.10 mmol) in CH_2Cl_2 (2 mL) was added and the mixture was stirred at −78°C (40 min). To this was added triethylamine (140 μL) and stirring was continued at −78°C (20 min). The mixture was allowed to warm to room temperature, then it was diluted with EtOAc and washed with H_2O, brine and dried over anhydrous Na_2SO_4. Filtration and evaporation gave a residue, which was filtered through a short silica gel column to provide crude aldehyde **13**. This was dissolved in MeOH (10 mL) and hydrogenated at room temperature over 10% Pd·C (30 mg) 40 psi H_2 overnight. The mixture was filtered through celite, evaporated and residue was purified by silica gel flash chromatography (CH_3OH–$CHCl_3$ from 1:100 to 1:6) to afford **14** as a colorless oil (17 mg, 39%); $[\alpha]=+32.4$ (*c* 0.73, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.20 (2H, dd, *J*=8.2, 2.4 Hz), 7.09 (2H, d, *J*=8.2 Hz), 3.52 (1H, d, *J*=10.4 Hz), 3.36 (3H, s), 3.26 (1H, m), 2.97 (2H, d, *J*=21.5 Hz), 2.73–2.80 (2H, m), 2.20 (1H, br), 1.98 (1H, m), 1.64–1.82 (3H, m), 1.41 (9H, s), 1.40 (9H, s) ppm. IR (neat) 3542, 2979, 1735, 1170 cm^{-1}. FABMS (+VE) *m/z* 426 (MH^+). HR-FABMS calcd for $C_{22}H_{37}NO_5P$ (MH^+): 426.2409. Found 426.2381.

3.2.7. (2*S*,3*R*)-1-((Fluoren-9-ylmethyl)oxycarbonyl)-3-(4-((bis-(*tert*-butyl)phosphono) methyl)phenyl)piperidine-2-carboxylic acid (4). To a solution of **14** (17 mg, 0.040 mmol) in a mixture of dioxane (3 mL) and H_2O (2 mL) was added at 0°C, a mixture of 1N NaOH in dioxane (1:1:0.6 mL) and the mixture was stirred at 0°C (2 h). The mixture was buffered by addition of a small quantity of dry ice, then Fmoc-OSu (13.5 mg, 0.040 mmol) was added and the mixture was stirred at room temperature (overnight). The pH was adjusted to 6.5 using 2 M $KHSO_4$ at 0°C, then dioxane was evaporated and the solution was diluted with H_2O. The resulting mixture was acidified to pH 5 using 2 M $KHSO_4$, extracted with EtOAc (3×20 mL) and evaporated to give a residue, which was purified by silica gel flash chromatography (MeOH–$CHCl_3$ from 1:50 to 1:10) to afford title compound **4** as a white solid (12 mg, 47%). Mp 102–104°C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (2H, m), 7.57 (2H, m), 7.20–7.40 (8H, m), 5.29 (3/5H, s), 5.11 (2/5H, s), 4.46 (2H, m), 4.31 (3/5H, m), 4.23 (2/5H, m), 4.07 (1H, m), 3.69 (1H, m), 3.29 (3/5H, m), 3.15 (2/5H, m), 3.07 (1H, s), 3.02 (1H, s), 1.82–1.92 (2H, m), 1.40–1.55 (2H, m), 1.41 (18H, s). FABMS (+VE) *m/z* 634 (MH^+). HR-FABMS calcd for $C_{36}H_{45}NO_7P$ (MH^+): 634.2934. Found: 634.2872.

3.2.8. Tripeptide 16. To a solution of **4** (12 mg, 0.019 mmol) in DMF (1 mL) were added HOBt (3 mg, 0.019 mmol) and 1,3-diisopropylcarbodiimide (3 μL, 0.019 mmol), and the mixture was stirred at room temperature (30 min). Dipeptide **15**[27] (8.1 mg, 0.019 mmol) was added and the mixture was stirred at room temperature (overnight). Solvent was evaporated under high vacuum and residue was purified by silica gel flash chromatography (MeOH–$CHCl_3$ from 1:50 to 1:20) to afford tripeptide **16** as a colorless oil (20 mg, quantitative). ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (1H, d, *J*=7.8 Hz), 7.98 (1H, s), 7.79 (2H, t, *J*=7.8 Hz), 7.74 (2H, d, *J*=7.4 Hz), 7.65 (1H, m), 7.57 (1H, m), 7.53 (2H, d, *J*=7.0 Hz), 7.23–7.45 (8H, m), 7.11 (2H, d, *J*=6.3 Hz), 6.94 (2H, d, *J*=7.4 Hz), 6.27 (1H, s), 5.42 (1H, s), 4.66 (1H, m), 4.46 (1H, m), 4.37 (1H, m), 4.22 (1H, t, *J*=6.6 Hz), 4.14 (1H, m), 3.81 (2H, m), 3.28–3.40 (3H, m), 3.02–3.11 (2H, m), 2.93 (d, *J*=21.5 Hz, 2H), 2.52 (1H, dd, *J*=15.2, 5.0 Hz), 1.90–2.10 (4H, m), 1.75–1.86 (3H, m), 1.66 (1H, m), 1.32–1.56 (8H, m), 1.40 (9H, s), 1.37 (9H, s). FABMS (+VE) *m/z* 1040 (MH^+).

3.2.9. Tripeptide 17. To a solution of **16** (20 mg, 0.019 mmol) in acetonitrile was added piperidine (15 μL, 0.152 mmol), the mixture was stirred at room temperature (4 h) then solvent was evaporated to provide a residue, which was purified by silica gel flash chromatography (from MeOH–$CHCl_3$=1:100 to NH_4OH_{aq}–MeOH–$CHCl_3$=1:5:50) to give Fmoc-deprotected intermediate (14 mg). This was dissolved in DMF (1.5 mL) and to this were added successively diisopropylethylamine (12 μL, 0.068 mmol) and *tert*-butyloxalyl chloride (6.4 μL, 0.051 mmol). The mixture was stirred at room temperature (overnight), then DMF was evaporated under high vacuum and residue was purified by silica gel flash gel chromatography (MeOH–$CHCl_3$ from 1:50 to 1:20) to afford **17** as a colorless oil (14 mg, 77%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (1H, d, *J*=8.1 Hz), 7.81 (1H, d, *J*=7.6 Hz), 7.74 (1H, d, *J*=8.0 Hz), 7.68 (1H, dd, *J*=6.0, 3.3 Hz), 7.54 (1H, t, *J*=5.4 Hz), 7.35–7.49 (3H, m), 7.28 (m, 2H), 7.13 (2H, dd, *J*=8.2, 2.3 Hz), 6.92 (2H, d, *J*=8.0 Hz), 6.74 (1H, s), 6.34 (1H, s), 5.50 (1H, s), 5.07 (1H, d, *J*=4.9 Hz), 4.71 (1H, dt, *J*=8.0, 5.1 Hz), 3.50 (1H, m), 3.35–3.47 (3H, m), 3.25 (1H, td, *J*=13.3, 4.7 Hz), 3.10–3.17 (2H, m), 2.96 (d, *J*=21.4 Hz, 2H), 2.92 (1H, dd, *J*=15.3, 5.1 Hz), 2.55 (1H, dd, *J*=15.3, 5.1 Hz), 1.92–2.18 (5H, m), 1.82–1.92 (2H, m), 1.32–1.70 (8H, m), 1.56 (9H, s), 1.43 (9H, s), 1.40 (9H, s). FABMS (+VE) *m/z* 946 (MH^+).

3.2.10. Tripeptide 18. To a solution of **17** (14 mg, 0.015 mmol) in a mixture of dichloromethane (1.5 mL) and TFA (1.5 mL), was added triethylsilane (7.1 μL, 0.044 mmol) and the mixture was stirred at room temperature (3 h), then solvent was evaporated. The resulting residue was triturated with ether (10 mL) to give a light gray suspension, which was collected by centrifugation. The procedure was repeated two times, then the resulting light gray solid was purified by HPLC [Vydac Protein and Peptide C_{18} column (250 mm×20 mm dia.); flow rate=10 mL/min.; linear gradient from 5% B to 60% B over 25 min then 60% B to 95% B over 5 minutes; solvent A, 0.1% aqueous TFA; solvent B, 0.1% TFA in acetonitrile]. Product was collected from 22.5 to 23.8 min to afford **18** as a white solid (9.1 mg, 79%). Analytical HPLC [Vydac Protein and Peptide C_{18} column (250 mm×10 mm dia.); flow rate=2 mL/min.; linear gradient from 5% B to 60% B over 10 min then 60% B to 70% B over 20 min; solvent A, 0.1% aqueous TFA; solvent B, 0.1% TFA in acetonitrile] indicated a major peak at t=20.7 with a shoulder at 22.5 min (ratio of 80:20). The main peak and the shoulder were separately collected and each provided an identical elution profile upon re-injection. This suggested that the shoulder was potentially a stable conformational isomer of the main peak. Mp 203.5°C (dec). ^{1}H NMR (400 MHz, D_2O) δ 8.18 (1H, d, J=8.8 Hz), 8.00 (1H, d, J=7.3 Hz), 7.87 (1H, d, J=8.1 Hz), 7.48–7.68 (4H, m), 7.22 (2H, d, J=7.3 Hz), 7.03 (2H, d, J=8.1 Hz), 4.70 (1H, d, J=8.8 Hz), 4.61 (1H, m), 3.43–3.49 (2H, m), 3.31–3.38 (2H, m), 3.22 (1H, m), 3.17 (2H, t, J=8.0 Hz), 2.94 (1H, s), 2.89 (1H, s), 2.84 (1H, dd, J=15.4, 5.9 Hz), 2.70 (1H, dd, J=15.4, 8.8 Hz), 1.98–2.07 (2H, m), 1.71–1.91 (4H, m), 1.53–1.65 (4H, m), 1.31–1.47 (4H, m), 1.05–1.12 (2H, m). IR (neat) 3318, 1635, 1534, 1205 cm^{-1}. FABMS (+VE) m/z 776 ($M-H^+$). HR-FABMS calcd for $C_{39}H_{48}N_5O_{10}P$: 776.30626. Found: 776.30866.

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