Tetrahedron 65 (2009) 9673–9679

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Preparation of orthogonally protected (2*S*,3*R*)-2-amino-3-methyl-4-phosphonobutyric acid (Pmab) as a phosphatase-stable phosphothreonine mimetic and its use in the synthesis of polo-box domain-binding peptides

Fa Liu [a,*], Jung-Eun Park [b], Kyung S. Lee [b], Terrence R. Burke, Jr. [a,*]

[a] *Laboratory of Medicinal Chemistry, Center for Cancer Research, National Cancer Institute, National Institutes of Health, NCI-Frederick, PO Box B, Bldg. 376 Boyles St., Frederick, MD 2170, USA*
[b] *Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA*

ARTICLE INFO

Article history:
Received 20 July 2009
Received in revised form
22 September 2009
Accepted 23 September 2009
Available online 26 September 2009

ABSTRACT

Reported herein is the first stereoselective synthesis of (2*S*,3*R*)-4-[bis-(*tert*-butyloxy)phosphinyl]-2-[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino-3-methylbutanoic acid [(*N*-Fmoc, *O*,*O*-(bis-(*tert*-butyl))-Pmab), **4**] as a hydrolytically-stable phosphothreonine mimetic bearing orthogonal protection compatible with standard solid-phase protocols. The synthetic approach used employs Evans' oxazolidinone for chiral induction. Also presented is the application of **4** in the solid-phase synthesis of polo-like kinase 1 (Plk1) polo box domain (PBD)-binding peptides. These Pmab-containing peptides retain PBD binding efficacy similar to a parent pThr containing peptide. Reagent **4** should be a highly useful reagent for the preparation of signal transduction-directed peptides.

Published by Elsevier Ltd.

1. Introduction

Phosphorylation of proteins facilitates critical protein–protein binding interactions that may result in signal propagation or modulation of enzyme activity.[1–4] Changes in normal post-translational modification of proteins through phosphorylation of tyrosine, serine and threonine residues is a central paradigm in oncogenic transformation.[5–7] In light of this, development of kinase-directed signal transduction inhibitors is a promising approach toward new anticancer therapeutics.[8–10] Synthetic phosphopeptides based on shortened sequences derived from phosphoproteins, can retain significant binding affinities and they can serve as competitive antagonists of cognate protein–protein interactions. In this fashion they can provide initial starting points for the design of peptidomimetic-based therapeutics. Typically, a key component of the recognition provided by phosphoamino acids is derived from the phosphoryl group itself.[11] However the hydrolytic lability of phosphoryl esters to phosphatases limits the use of phosphopeptides in cellular contexts. Development of hydrolytically-stable mimetics, in which the labile phosphoryl ester oxygen has been replaced non-hydrolyzable methylene or difluoromethylene groups, offers one approach to circumvent this limitation. Peptides containing metabolically stable analogues have proven to be useful biological tools that may serve as potential leads for further therapeutic design.[12–17]

Although a significant body of literature exists concerning the development and application of phosphotyrosyl (pTyr) mimetics,[18] fewer examples can be found dealing with mimetics of phosphothreonine (pThr, **1**, Fig. 1). Stereoselective synthesis of the pThr mimetic (2*S*,3*R*)-2-amino-3-methyl-4-phosphonobutanoic acid (Pmab, **2**) has been reported using Schollkopf's bislactim ether. This has provided derivatized Pmab bearing *O*,*O*-(bis-allyl) protection of the phosphonic acid group along with *N*-Fmoc protection.[19] Synthesis of the corresponding 4,4-difluoro analogue (F_2Pmab, **3**) bearing *O*,*O*-(bis-ethyl) phosphonic acid and *N*-Boc protection groups, has been approached using both (*R*)-isopropylideneglycerol as a chiral synthon[20] and Oppolzer's sultam chiral auxiliary.[21] To date, there have been no stereoselective syntheses reported of Pmab bearing orthogonal *O*,*O*-[bis-(*tert*-butyl)] phosphonic acid and *N*-Fmoc groups. This protection scheme would allow facile use in standard solid-phase protocols on acid-labile resins. Therefore, we report herein the first synthesis of (2*S*,3*R*)-4-[bis-(*tert*-butyloxy)phosphinyl]-2-[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino-3-methylbutanoic acid [(*N*-Fmoc, *O*,*O*-(bis-(*tert*-butyl))-Pmab), **4**]

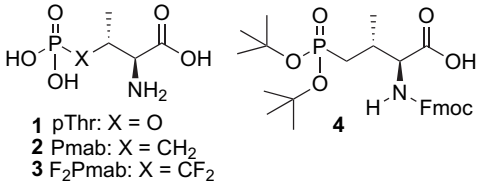


Figure 1. Structures of pThr and pThr mimetics discussed in the text.

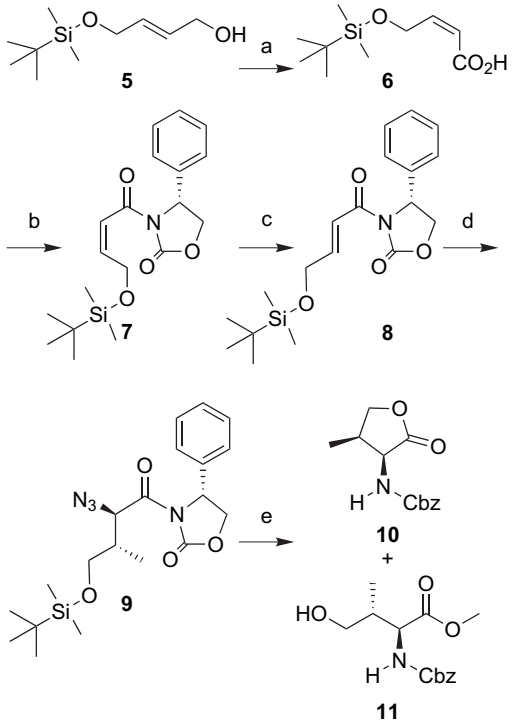
* Corresponding authors. Tel.: +301 846 5906; fax: +301 846 6033.
E-mail addresses: liuf@ncifcrf.gov (F. Liu), tburke@helix.nih.gov (T.R. Burke, Jr.).

0040-4020/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.tet.2009.09.093

by a route using Evans' oxazolidinone for chiral induction. We also present the application of this reagent in the solid-phase synthesis of a biologically active peptide.

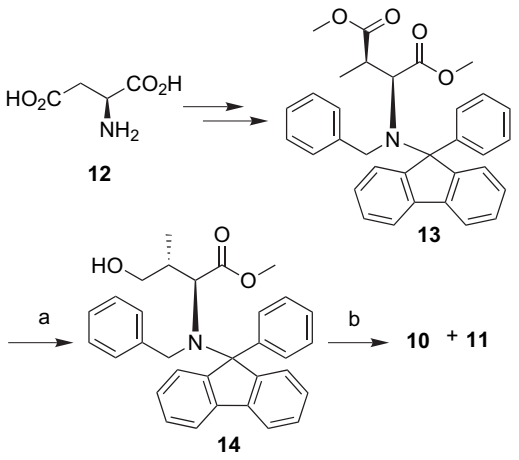
1.1. Chemistry

Stereoselective synthesis of orthogonally-protected Pmab (**4**) began with the Swern oxidation of *tert*-butyldimethylsilyl (TBDMS) mono-protected (2*E*)-2-butene-1,4-diol **5**[22] followed by sodium chlorite oxidation. This provided acid **6** with *Z*-double bond geometry (previously reported as the *E*-isomer[23]) (Scheme 1). Acid **6** was coupled with Evan's chiral auxiliary, (4*R*)-4-phenyl-2-oxazolidinone[24] and the *Z*-double bond geometry was isomerized by treatment with tri-*n*-butyl phosphine in THF to give the desired *E*-isomer (**7**). Both α and β stereogenic centers of **9** were constructed by a tandem sequence consisting of an asymmetric Cu(I)-catalyzed 1,4-Michael addition of methylmagnesium chloride followed by electrophilic α-bromination.[25] The crude (2*R*)-bromide was then converted to the corresponding (2*S*)-azide by nucleophilic SN2 replacement using sodium azide. A single (2*S*,3*R*)-diastereomer (**9**) was obtained by column chromatographic purification and crystallization. Assignment of absolute stereochemistries was based on well-established literature precedence.[25–27] Removal of the TBDMS group by treatment with catalytic *p*-toluenesulfonic acid was followed by cyclization to release Evan's auxiliary group and provide the five-membered lactone. The azide was reduced by hydrogenation in a mixture of AcOH and MeOH and protected in situ to provide the lactone **10** as well as the ring-open alcohol **11** in a 1:4 ratio. Lactone **10** was further converted to **11** (Scheme 1).



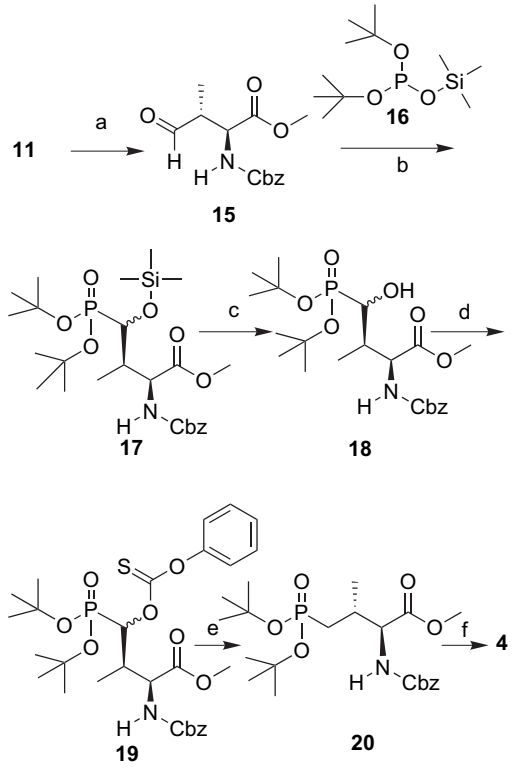
Scheme 1. Reagents and conditions: (a) 1. Oxalyl chloride, DMSO, DCM, −78 °C, 2 h; 2. $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, *tert*-butanol/H_2O, rt, overnight, 97% for 2 steps. (b) 1. Trimethylacetyl chloride, triethylamine, THF, −78 °C–0 °C, 20 min; 2. (*R*)-(+)-Phenyl-2-oxazolidione lithium salt, THF, −78 °C–0 °C, overnight, 100%. (c) Tributylphosphine, THF, rt, 1 h, 84%. (d) 1. Methylmagnesium chloride, copper(I) bromide dimethyl sulfide, dimethyl sulfide/THF, −78 °C–−40 °C, 2 h; 2. NBS, −78 °C, 1.5 h; 3. NaN_3, DMF, 0 °C, 2 h, 79% for 2 steps. (e) 1. *p*-Toluenesulfonic acid monohydrate, MeOH, rt, 6 h; 2. 1 atm H_2, 10% Pd·C (10%), MeOH/AcOH, rt, overnight; 3. Benzyl chloroformate, $NaHCO_3$, THF/H_2O, 0 °C, 4 h, 49% for **11** over 3 steps.

It is of note that alcohol **11** can also be prepared from L-aspartic acid through the known bis-methyl ester **13**.[28] Selectively reduction of the γ-carboxyl of **13** using DIBAL provided the alcohol **14** (Scheme 2). Key to this reaction was the use of substrate concentrations less than 0.03 M. Similar to above, N-deprotection of **14** by hydrogenation in a mixture of AcOH and MeOH and subsequent Cbz protection gave the lactone **10** and the alcohol **11** in a 1:4 ratio.



Scheme 2. Reagents and conditions: (a) DIBAL, THF, −40 °C to 0 °C, 4 h, 61%. (b) 1. 1 atm H_2, 10% Pd·C (10%), MeOH/AcOH, rt, overnight; 2. Benzyl chloroformate, $NaHCO_3$, THF/H_2O, 0 °C, 4 h.

Swern oxidation of alcohol **11** gave the corresponding aldehyde (**15**). This aldehyde was subjected to a phospho-Mukaiyama aldol reaction with freshly-prepared di-*tert*-butyltrimethylsilyl phosphite (**17**) to yield the aldehyde **17** (Scheme 3).[29,30] Subsequent treatment with citric acid gave the free alcohol (**18**), which was derivatized as the phenylthiocarbonate **19** and subjected to Barton–

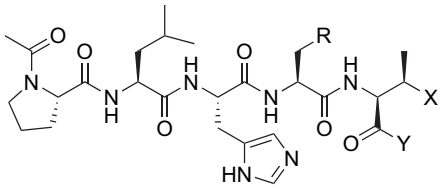


Scheme 3. Reagents and conditions: (a) Oxalyl chloride, DMSO, DCM, −78 °C, 2 h. (b) **16**, DCM, rt, 3 h. (c) citric acid, MeOH/H_2O, rt, overnight, 88% over 3 steps. (d) *O*-phenylchlorothionoformate, DMAP (cat.) and *N*,*N*-diisopropylethylamine, DCM, rt, overnight. (e) Tributyltin hydride, AIBN, toluene, 100 °C, 20 min, 58% over 2 steps. (f) 1. LiOH, THF/H_2O, rt, overnight; 2. 1 atm H_2, 10% Pd·C (10%), MeOH, rt, overnight; 3. FmocOSu, $NaHCO_3$, dioxane/H_2O, rt, overnight, 100% over 3 steps.

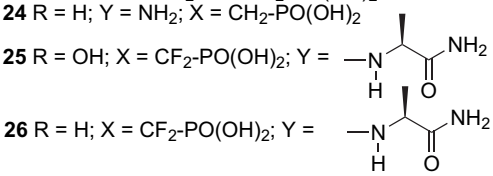
McCombie deoxygenation to yield **20**.[31] Hydrolysis of the methyl ester, then hydrogenation and re-protection using Fmoc-OSu provided the orthogonally protected Pmab derivative **4**.

1.2. Application of reagent 4 to the synthesis of polo box domain-binding peptides

The polo-like kinase 1 (Plk1) functions as an important mitotic regulator that phosphorylates serine and threonine residues.[32] Its over-expression in a number of cancers[33] and its association with poor prognosis have made it a potential anticancer therapeutic target.[34,35] A main focus of Plk1 inhibitor development has been directed at the kinase catalytic domain.[36–43] However, Plk1 contains modular C-terminal 'Polo-box domains' (PBDs) that bind specific phosphoserine and phosphothreonine-containing sequences to provide critical localization of Plk1.[32,44,45] Competitive PBD binding antagonists could serve as inhibitors of Plk1 function that are distinct from kinase-directed agents.[44] A starting point for the development of PBD-binding antagonists is given by short pThr-containing peptides modeled on consensus binding sequences derived from the p-Thr78 region (p-T78) of the PBD-binding protein, PBIP1.[46–48] By examining various PBD-binding phosphopeptides, it has recently been shown that a 5-mer phosphopeptide 'PLHSpT' (**21**, Fig. 2) specifically interacts with the Plk1 PBD with high affinity (K_d=0.45 μM).[49] In order to provide phosphatase-stable peptides for in vivo studies, F_2Pmab (**3**) was also incorporated into a 6-mer T78 peptide, 'PLHSTA', to give the corresponding peptide **25**.[49] (Note: The 6-mer sequence 'PLHS-F_2Pmab-A' (**25**) was synthesized due to inefficient synthesis of the 5-mer sequence, 'PLHS-F_2Pmab'). It was found that **25** showed much weaker PBD-binding affinity than the respective p-T78 peptide, 'PLHSpTA', and it exhibited significant toxicity in cell-based experiments. The toxicity can potentially be attributed to the highly acidic CF_2PO_3H moiety. Therefore, using solid-phase techniques and standard Fmoc-based protocols, we employed reagent **4** to synthesize the Pmab-containing peptides **23** and **24** (Fig. 2).



21 R = OH; Y = NH_2; X = OH
22 R = OH; Y = NH_2; X = O-$PO(OH)_2$
23 R = OH; Y = NH_2; X = CH_2-$PO(OH)_2$
24 R = H; Y = NH_2; X = CH_2-$PO(OH)_2$



25 R = OH; X = CF_2-$PO(OH)_2$; Y =

26 R = H; X = CF_2-$PO(OH)_2$; Y =

Figure 2. Structures of synthetic peptides used in the study.

To examine the ability of Pmab- and F_2Pmab-containing peptides to inhibit PBD-dependent interactions, Plk1 PBD-binding inhibition assays were conducted in the presence of various concentrations of synthetic peptides. It was found that 'PLHS–Pmab' (**23**) inhibits the interaction of the Plk1 PBD with an immobilized p-T78 peptide as effectively as the wild-type peptide, 'PLHSpT' (**22**, Fig. 3A). In contrast, the peptide, 'PLHS-F_2Pmab-A' (**25**, Fig. 2), inhibits the interaction at a somewhat reduced level (data not shown). Replacement of the critical (pThr-1) Ser residue with an alanine (equivalent to S77A mutation) is known to significantly attenuate PBD binding affinity.[49] The non-phosphorylated control peptide 'PLHST' (**21**, Fig. 2) and the S77A mutants of the Pmab- and the F_2Pmab-containing peptides (**24** and **26**, respectively, Fig. 2), did not inhibit PBD binding even at 1000-fold higher molar concentrations (Fig. 3A and data not shown).

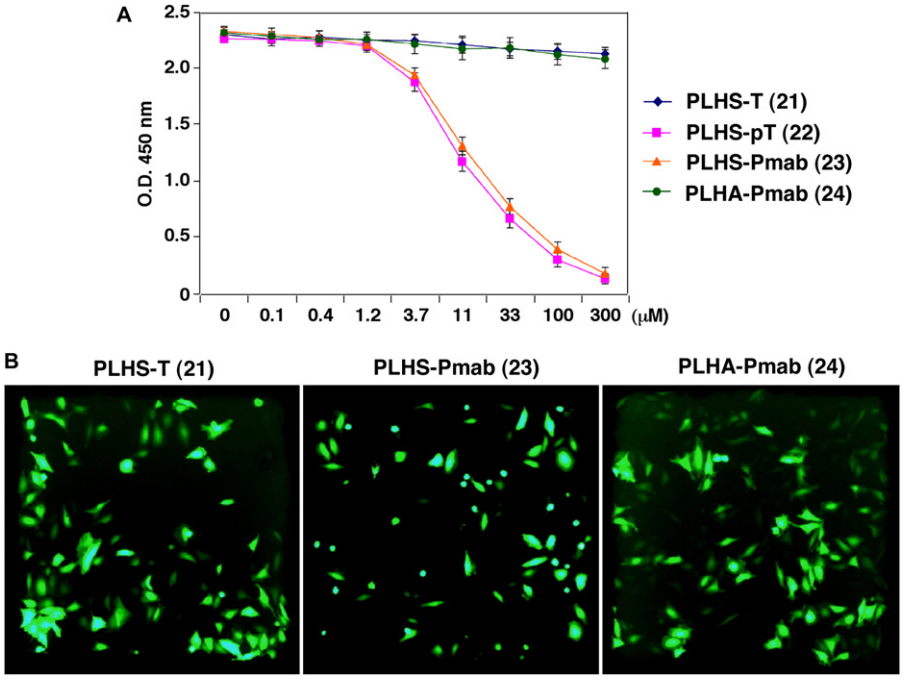


Figure 3. Measurement of the ability of synthetic peptides **21**–**24** to inhibit PBD-dependent interactions. (A) PBD-binding inhibition assays were carried out in the presence of different concentrations of the indicated inhibitory peptides.[49] The level of the remaining interaction between a p-T78 peptide and full-length Plk1 was quantified by optical density (O.D.) at 450 nm (error bars represent standard deviation). (B) Representative images of green fluorescence in EGFP plasmid-containing HeLa cells following microinjection with PLHS–Pmab (**23**) or the PBD-binding defective peptides, PLHST (**21**) and PLHA–Pmab (**24**), are shown (procedure described in Experimental Section). Note induction of mitotically-arrested, rounded-up, morphologies associated with the PBD-binding competent PLHS–Pmab.

Evidence suggests that the PBD plays critical roles in the proper sub-cellular localization and mitotic functions of Plk1. Disruption of PBD-dependent Plk1 functions by expressing a dominant-negative form of PBD results in a mitotic arrest that ultimately leads to apoptotic cell death.[50] To investigate the effects of inhibiting Plk1 PBD interactions peptides **21**, **23** and **24** were introduced into HeLa cells. In order to overcome poor membrane permeability of the negatively charged Pmab-containing peptides, microinjection was employed. The Pmab-containing peptide (**23**), but not the non-phosphorylated peptide **21** or the respective S77A mutant (**24**), induced mitotically arrested, rounded-up, morphology in approxi-mately 50% of the microinjected, green fluorescent protein (GFP)-positive population (Fig. 3B). These results demonstrate that inhibition of PBD function by the Pmab-containing p-T78 mimetic peptide is sufficient to interfere with the mitotic functions of Plk1.

2. Conclusions

Although a significant body of literature exists concerning the development and application of pTyr mimetics, fewer examples can be found dealing with mimetics of pThr. Presented herein is the first stereoselective synthesis of the hydrolytically-stable phosphothreonine mimetic Pmab (**4**), bearing (*O*,*O*)-bis-*tert*-butyl protection of the phosphonic acid group along with *N*-Fmoc derivatization. This orthogonal protection scheme allows facile use in standard solid-phase protocols on acid-labile resins, where side chain protecting groups can be removed during TFA-mediated resin cleavage. Our synthetic approach to Pmab utilizes Evans' oxazolidinone for chiral induction. We also present the application of **4** in the solid-phase synthesis of biologically active peptides directed against the Plk1 PBD. Here we show that Pmab-containing peptides retain PBD binding efficacy similar to a parent pThr-containing peptide, while retaining the ability to inhibit PBD-dependent interactions in whole cells. In summary, reagent **4** should be a highly useful reagent for the preparation of signal transduction-directed peptides.

3. Experimental procedures

3.1. General

All experiments involving moisture-sensitive compounds were conducted under dry conditions (positive argon pressure) using standard syringe, cannula, and septa apparatus. Solvents: All solvents were purchased anhydrous (Aldrich) and used directly. HPLC-grade hexanes, EtOAc, CH_2Cl_2, and MeOH were used in chromatography. TLC: analytical TLC was performed on Analtech precoated plates (Uniplate, silica gel GHLF, 250 μm) containing a fluorescence indicator; NMR spectra were recorded using a Varian Inova 400 MHz spectrometer. The coupling constants are reported in Hertz, and the peak shifts are reported in the δ (ppm) scale. Low resolution mass spectra (ESI) was measured with Agilent 1200 LC/MSD-SL system, and high resolution mass spectra (ESI or APCI) was measured by UCR Mass Spectrometry Facility, Department of Chemistry, University of California, 3401 Watkins Dr., Riverside CA 92521. Optical rotations were measured on a Jasco P-1010 polarimeter at 589 nm. IR spectra were obtained neat with a Jasco FTIR/615 spectrometer.

3.2. ELISA-based PBD-binding inhibition assays

An ELISA-based PBD-binding inhibition assay was carried out using an immobilized p-T78 peptide and cellular lysates expressing HA-EGFP-Plk1 as described in Ref. [49] Results are shown in Figure 3A.

3.3. Cell microinjection and confocal microscopy

Similar to procedures described in reference 49, HeLa cells were arrested at the G1/S boundary by double thymidine treatment and released into fresh medium. Six hours after release, the cells were microinjected with a mixture of 3 mM of peptides **21**, **23** or **24** and 30 ng/μL of pEGFP-C1 vector and the cells were then photographed 15 h after G1/S release. Co-injected EGFP plasmid provided a convenient marker to identify the microinjected cells. Results are shown in Figure 3B.

*3.3.1. Synthetic (E)-4-[(tert-butyldimethylsilyl)oxy]-2-buten-1-ol (**5**).* To a solution of (2*E*)-2-butene-1,4-diol (8.22 mL, 0.10 mol) and imidazole (8.50 g, 0.125 mol) in DMF (50 mL) at 0 °C, was added *tert*-butyldimethylsilyl chloride (7.50 g, 0.050 mol) in several portions over 10 min. The resulting mixture was warmed to room temperature and stirred (2 h), then poured into H_2O (200 mL) and extracted with EtOAc (2×150 mL). The organic layer was washed (brine), dried (Na_2SO_4) and purified by silica gel column chromatography (hexanes:EtOAc) to yield **5** as a colorless oil (9.0 g, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60–5.50 (m, 2H), 4.18 (m, 2H), 4.09 (m, 2H), 2.76 (br, 1H), 0.83 (s, 9H), 0.01 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.3, 135.4, 64.8, 63.8, 31.1, 23.6.

*3.3.2. Z-4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-2-butenoic acid (**6**).* To a solution of oxalyl chloride (3.55 mL, 40.8 mmol) in CH_2Cl_2 (100 mL) at −78 °C, was added a solution of DMSO (5.80 mL, 81.7 mmol) in CH_2Cl_2 (40 mL) and the mixture was stirred (15 min). Alcohol **5** (5.50 g, 27.2 mmol) in dry CH_2Cl_2 (40 mL) was added over 5 min, then the mixture was stirred at −75 °C (2 h) then triethylamine (31 mL, 0.22 mol) was added. The mixture was warmed room temperature and saturated NH_4Cl (50 mL) was added and the mixture was extracted with Et_2O (2×100 mL). The combined organic layers were washed (brine), dried (Na_2SO_4) and evaporated to yield the intermediate aldehyde as a pale yellow liquid. Without purification, to a mixture of the aldehyde, potassium phosphate monobasic (5.55 g, 40.8 mmol) and 2-methyl-2-butene (14.4 mL, 136 mmol) in *tert*-butanol (150 mL) and H_2O (30 mL) at 0 °C, was added sodium chlorite (9.23 g, 81.6 mmol, 80% technical grade) in several portions over 10 min. The mixture was warmed to room temperature slowly and stirred (night). After cooling to 0 °C, a solution of sodium bisulfate (31.8 g, 0.30 mol) in H_2O (100 mL) was added slowly and the mixture was stirred (30 min) and extracted with EtOAc (2×150 mL). The combined organic layer was washed (brine), dried (Na_2SO_4) and purified by silica gel column chromatography (hexanes:EtOAc) to yield acid **6** as a colorless oil (5.70 g, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.40 (dt, J=11.6, 4.6 Hz, 1H), 5.68 (dt, J=12.0, 2.6 Hz, 1H), 4.65 (dd, J=4.6, 2.4 Hz, 2H), 0.83 (s, 9H), 0.00 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 159.7, 123.0, 67.1, 31.0, 23.0, 0.00. APCI (−VE) *m*/*z*: 215.2 $(M-H)^-$. HR-ESI MS ca.lcd for $C_{10}H_{19}O_3Si$ $(M-H)^-$: 215.1109, found: 215.1103.

*3.3.3. (4R)-3-[(2Z)-[4-[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1-oxo-2-buten-1-yl]-4-phenyl-2-oxazolidinone (**7**).* To a solution of acid **6** (6.0 g, 28.2 mmol) in THF (40 mL) at −78 °C, was added triethylamine (4.00 mL, 28.2 mmol) followed by trimethylacetyl chloride (3.46 mL, 28.2 mmol) drop-wise. The mixture was warmed to 0 °C over 20 min, then the anhydride mixture was cooled to −78 °C. Separately, to a solution of (*R*)-(+)-phenyl-2-oxazolidione (Aldrich) (4.60 g, 28.2 mmol) in THF (40 mL) at −78 °C was carefully added *n*-BuLi (2.50 M in THF, 11.3 mL, 28.2 mmol) and the mixture was stirred (30 min) then transferred to the anhydride solution at −78 °C. 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 (hexanes:EtOAc) to yield **7** as

a colorless oil (10.2 g, 100% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.20 (m, 5H), 7.10 (dt, J=11.6, 2.6 Hz, 1H), 6.50 (dt, J=12.0, 4.6 Hz, 1H), 5.44 (dd, J=8.8, 4.0 Hz, 1H), 4.68–4.59 (m, 3H), 4.22 (dd, J=8.8, 4.0 Hz, 1H), 0.85 (s, 9H), 0.00 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 160.6, 158.8, 144.3, 134.5, 134.0, 131.0, 122.0, 75.2, 67.9, 62.8, 31.1, 23.4, 0.00. ESI (+VE) m/z: 384.1 $(M+Na)^+$. HR-ESI calcd for $C_{19}H_{28}NO_4Si$ $(M+Na)^+$: 362.1782, found: 362.1789.

*3.3.4. (4R)-3-[(2E)-[4-[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1-oxo-2-buten-1-yl]-4-phenyl-2-oxazolidinone (**8**).* To a solution of **7** (5.00 g, 13.9 mmol) in anhydrous THF (70 mL) at room temperature was added tributylphosphine (0.34 mL, 1.39 mmol). The resulting solution was stirred at room temperature (60 min), then diluted with EtOAc (200 mL), washed (H_2O and brine), dried (Na_2SO_4), and purified by silica gel column chromatography (hexanes:EtOAc) to yield **8** as a white solid (4.20 g, 84% yield). $[\alpha]^{20}_D$ −54.5 (*c* 1.40, $CHCl_3$). Mp 79–81 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (dt, J=15.2, 2.4 Hz, 1H), 7.30–7.21 (m, 5H), 7.02 (dt, J=15.2, 3.4 Hz, 1H), 5.39 (dd, J=8.6, 3.8 Hz, 1H), 4.60 (t, J=8.8 Hz, 1H), 4.28 (dd, J=3.4, 2.2 Hz, 2H), 4.17 (dd, J=8.8, 4.0 Hz, 1H), 0.85 (s, 9H), 0.00 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 159.0, 155.4, 144.5, 134.6, 134.1, 131.4, 124.1, 75.3, 68.1, 63.2, 31.3, 23.8, 0.00. IR (KBr) $\nu_{max:}$ 2927, 2855, 1759, 1693, 1324, 1201, 1104, 951, 834, 715 cm^{-1}. ESI (+VE) m/z: 384.1 $(M+Na)^+$. HR-ESI calcd for $C_{19}H_{28}NO_4Si$ $(M+Na)^+$: 362.1782, found: 362.1790.

*3.3.5. (4R)-3-[(2S,3R)-[2-Azido-4[(1,1-dimethylethyl)dimethylsilyl]oxy]-3-methyl-1-oxo-butyl]-4-phenyl-2-oxazolidinone (**9**).* To a solution of copper (I) bromide dimethyl sulfide complex (2.56 g, 12.45 mmol) in dimethyl sulfide (20 mL) and THF (30 mL) at −78 °C was added a solution of methylmagnesium chloride (3.0 M in THF, 5.50 mL, 16.4 mmol). The suspension was stirred at −78 °C (20 min), then warmed to 0 °C (20 min) and cooled to −78 °C. The mixture was then transferred to a pre-cooled (−78 °C) solution of **8** (1.80 g, 4.98 mmol) in THF (16.0 mL) and CH_2Cl_2 (8.0 mL) using a cannula. The resulting mixture was kept at −78 °C (60 min) then warmed to −40 °C (60 min) and cooled again to −78 °C. To the mixture was added a pre-cooled (−78 °C) solution of *N*-bromosuccinimide (4.45 g, 25.0 mmol) in THF (50 mL) and the mixture was stirred at −78 °C (90 min). The reaction was quenched by addition of saturated $NaHSO_3$ (50 mL), extracted with EtOAc (100 mL×2). The combined organic phase was washed (H_2O and brine), dried (Na_2SO_4), and purified by silica gel column chromatography (hexanes:EtOAc) to yield the requisite α-bromo-containing intermediate as a white solid (1.93 g). To a solution of the α-bromo compound (1.93 g) in DMF (25 mL) at 0 °C, was added sodium azide (1.00 g, 15.4 mmol) and the mixture was stirred (2 h). The mixture was diluted with EtOAc (150 mL), washed (H_2O and brine), dried (Na_2SO_4), and purified by silica gel column chromatography (hexanes:EtOAc) then crystallized (EtOAc:petroleum ether) to yield azide **9** as a white solid (1.65 g, 79% yield). $[\alpha]^{20}_D$ −73.0 (*c* 1.10, $CHCl_3$). Mp 80–82 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.30 (m, 5H), 5.49 (dd, J=8.8, 4.0 Hz, 1H), 5.17 (d, J=8.8 Hz, 1H), 4.75 (t, J=9.0 Hz, 1H), 4.34 (dd, J=8.8, 4.0 Hz, 1H), 3.65 (dd, J=10.2, 5.4 Hz, 1H), 3.48 (dd, J=10.2, 3.4 Hz, 1H), 2.14 (m, 1H), 0.89 (s, 9H), 0.83 (d, J=6.8 Hz, 3H), 0.03 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 158.7, 143.9, 134.8, 134.6, 131.9, 75.7, 69.3, 66.7, 63.4, 43.5, 31.4, 23.8, 19.4, 0.00. IR (KBr) $\nu_{max:}$ 2930, 2359, 2106, 1786, 1710, 1206, 1097, 833, 778 cm^{-1}. ESI (+VE) m/z: 441.1$(M+Na)^+$. HR-ESI MS calcd for $C_{20}H_{31}N_4O_4Si$ $(M+H)^+$: 419.2109, found: 419.2114.

*3.3.6. [(3S,4R)-Tetrahydro-4-methyl-2-oxo-3-furanyl]-carbamic acid phenylmethyl ester (10) and (2S,3R)-4-hydroxy-N-(phenylmethoxycarbonyl)-L-valine methyl ester (**11**).* To a solution of **9** (600 mg, 1.44 mmol) in MeOH (20 mL) at room temperature was added *p*-toluenesulfonic acid monohydrate (14 mg, 0.07 mmol). The solution was stirred at room temperature (6 h), then diluted with EtOAc (150 mL), washed (H_2O and brine), dried (Na_2SO_4), and purified by silica gel column chromatography (hexanes:EtOAc) to yield the intermediate azide-containing lactone as a colorless liquid (270 mg, containing a small amount EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.36 (dd, J=8.8, 6.4 Hz, 1H), 4.26 (d, J=7.2 Hz, 1H), 4.02 (dd, J=9.2, 4.0 Hz, 1H), 2.75 (m, 1H), 1.13 (d, J=7.2 Hz, 3H). A suspension of this lactone and Pd·C (10%, 60 mg) in MeOH (9.0 mL) and acetic acid (1.0 mL) was stirred under H_2 (1 atmosphere) at room temperature (overnight). The catalyst was removed by filtration though a Celite pad under argon and the filtrate was concentrated. The residue was re-dissolved in THF (10.0 mL) containing H_2O (10 mL) and then cooled to 0 °C. To this was added benzyl chloroformate (0.32 mL, 2.25 mmol) and $NaHCO_3$ (840 mg, 10.0 mmol) and the mixture was stirred (4 h). The mixture was diluted with EtOAc (150 mL), washed (H_2O and brine), dried (Na_2SO_4), and purified by silica gel column chromatography (hexanes:EtOAc) to yield **10** as a white crystalline solid (90 mg, 25% yield over 3 steps) and **11** as a viscous colorless oil (250 mg, 49% yield over 3 steps). For (**10**): mp 125–127 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.30 (m, 5H), 5.33 (m, 1H), 5.10 (s, 2H), 4.53 (t, J=6.8 Hz, 1H), 4.35 (dd, J=9.2, 5.2 Hz, 1H), 4.05 (d, J=9.2 Hz, 1H), 2.92 (m, 1H), 0.95 (d, J=7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 156.1, 135.9, 128.5, 128.3, 128.1, 72.4, 67.3, 54.5, 34.1, 12.7. ESI (+VE) m/z: 272.1 $(M+Na)^+$. HR-ESI calcd for $C_{13}H_{16}NO_4$ $(M+H)^+$: 250.1074, found: 250.1081.

For (**11**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.28 (m, 5H), 5.98 (d, J=8.4 Hz, 1H), 5.04 (s, 2H), 4.34 (m, 1H), 3.65 (s, 3H), 3.54 (dd, J=11.2, 4.4 Hz, 1H), 3.44 (dd, J=11.2, 6.0 Hz, 1H), 2.92 (s, 1H), 2.14 (m, 1H), 0.92 (d, J=7.2 Hz, 3H). ESI (+VE) m/z: 304.2 $(M+Na)^+$. HR-ESI MS calcd for $C_{14}H_{20}NO_5$ $(M+H)^+$: 282.1336, found: 282.1343.

*3.3.7. (2S,3R)-4-Hydroxy-N-(9-phenylfluoren-9-yl)-N-benzyl-L-valine methyl ester (**14**).* To a solution of **13** (4.00 g, 7.91 mmol) in anhydrous THF (260 mL) at −40 °C, was added DIBAL (1.0 M in hexanes, 19.8 mL, 19.8 mmol). The mixture was stirred for 4 h (−40 °C to 0 °C) before cooled down to −78 °C, quenched by acetone (10 mL), warmed to rt, stirred with 1 N KH_2PO_4 (500 mL) and sodium potassium tartrate (30.0 g) overnight, filtered through the Celite. The filtrate was extracted with EtOAc, washed (H_2O and brine), dried (Na_2SO_4), and purified by silica gel column chromatography (hexanes:EtOAc) to yield alcohol **14** as a white wax (2.30 g, 61% yield, quantitative yield based on recovered starting material)and recycled **13** as a white wax (1.60 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76–7.60 (m, 8H), 7.35–7.20 (m, 10H), 4.70 (AB, J_{AB}=13.6 Hz, 1H), 4.38 (AB, J_{AB}=13.6 Hz, 1H), 3.84 (dd, J=10.8, 3.6 Hz, 1H), 3.33 (dd, J=10.8, 6.4 Hz, 1H), 3.04 (d, J=8.4 Hz, 1H), 2.93 (s, 3H), 1.40 (m, 1H), 0.34 (d, J=6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 148.3, 144.8, 144.0, 142.0, 141.3, 139.7, 129.7, 128.6, 128.4, 128.0, 127.7, 127.3, 127.2, 127.1, 127.0, 125.3, 120.2, 80.3, 65.5, 63.3, 50.6, 36.3, 14.2. ESI (+VE) m/z: 478.2 $(M+H)^+$. HR-ESI MS calcd for $C_{32}H_{32}NO_3$ $(M+H)^+$: 478.2377, found: 478.2385.

*3.3.8. (2S,3R)-4-[Di-(tert-butyl)-oxyphosphinyl]-(4-hydroxy-N-phenylmethoxycarbonyl)-L-valine methyl ester (**18**).* To a solution of oxalyl chloride (0.96 mL, 10.1 mmol) in CH_2Cl_2 (40 mL) at −78 °C, was added a solution of DMSO (1.60 mL, 20.2 mmol) in CH_2Cl_2 (5 mL) and the mixture was stirred (15 min). To this was added alcohol **11** (0.63 g, 2.24 mmol) in dry CH_2Cl_2 (5 mL) over 5 min and the mixture was stirred at −75 °C (2 h). triethylamine (8.40 mL, 53.8 mmol) was added and the mixture was warmed to room temperature. To this was added saturated NH_4Cl (50 mL) and the mixture was extracted with Et_2O (100 mL×2) and the combined organic phase was washed (brine), dried (Na_2SO_4), and purified by silica gel column chromatography (hexanes:EtOAc). Aldehyde **15** was obtained as a viscous colorless oil (450 mg, 96% yield based on recovered starting material) along with starting alcohol **11** (160 mg). To a solution of di-*tert*-butyl phosphite (0.30 mL, 1.50 mmol) and triethylamine (0.21 mL, 1.50 mmol) in CH_2Cl_2 (5 mL) at 0 °C, was added chlorotrimethylsilane (0.19 mL, 1.50 mmol) and the mixture was stirred (5 min) and then

transfer to a solution of aldehyde **15** (300 mg, 1.08 mmol) in CH_2Cl_2 (5 mL) at room temperature and the mixture was stirred (3 h). The mixture was diluted with EtOAc (150 mL), washed (brine), dried (Na_2SO_4) and concentrated. The resulting crude silyl-protected **17** was re-dissolved in MeOH (10 mL), to this was added H_2O (1.0 mL) and citric acid (200 mg) and the mixture was stirred at room temperature (overnight). The mixture was diluted with EtOAc (200 mL), washed (saturated $NaHCO_3$ and brine), dried (Na_2SO_4) and purified by silica gel column chromatography (hexanes:EtOAc) to yield **18** as a white wax epimeric at the γ-carbon (450 mg, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.29 (m, 5H), 6.30 (d, *J*=8.0 Hz, 0.7H), 5.30 (m, 0.3H), 5.10–5.05 (m, 2H), 4.30 (m, 0.7H), 4.09 (m, 0.3H), 3.75–3.55 (m, 4H), 2.51 (m, 0.7H), 1.51–1.40 (m, 18H), 1.15–1.00 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 156.7, 136.4, 128.4, 128.0, 70.5, 68.8, 67.3, 66.8, 59.8, 53.9, 53.1, 52.2, 36.6, 35.4, 30.3, 24.1, 14.7, 11.5, 9.4. ESI (+VE) *m*/*z*: 496.2 $(M+Na)^+$. HR-ESI MS calcd for $C_{22}H_{36}NO_8NaP$ $(M+Na)^+$: 496.2071, found: 496.2065.

*3.3.9. (2S,3R)-4-[Di-(tert-butyl)-oxyphosphinyl]-N-(phenylmethoxycarbonyl)-L-valine methyl ester (**20**).* A solution of alcohol **18** (250 mg, 0.53 mmol), *O*-phenylchlorothionoformate (215 μL, 1.60 mmol), 4-(dimethylamino) pyridine (DMAP) (15 mg, 0.20 equiv) and *N*,*N*-diisopropylethylamine (363 μL, 2.10 mmol) in anhydrous CH_2Cl_2 (8.0 mL) was stirred at room temperature (overnight). The mixture was diluted with EtOAc (100 mL), washed (satd $NaHCO_3$ and brine), dried (Na_2SO_4) and purified by silica gel column chromatography (hexanes:EtOAc) to give the intermediate thiocarbonate **19** as a pale brown wax (225 mg). Crude **19** was dissolved in toluene (10 mL) and to this was added tributyltin hydride (0.42 mL, 1.59 mmol) and azobisisobutyronitrile (AIBN) (one spatula tip). The mixture was maintained at 100 °C (20 min), then cooled to room temperature and concentrated under vacuum. The residue was purified by silica gel column chromatography (hexanes:EtOAc) to give **20** as viscous colorless oil (140 mg, 58% yield for 2 steps). $[\alpha]^{20}_D$+2.4 (*c* 0.85, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.20 (m, 5H), 5.80 (d, *J*=8.4 Hz, 1H), 5.07 (AB, J_{AB}=12.4 Hz, 1H), 5.02 (AB, J_{AB}=12.4 Hz, 1H), 4.23 (m, 1H), 3.67 (s, 3H), 2.33 (m, 1H), 1.69–1.10 (m, 20H), 1.05 (d, *J*=6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 156.2, 136.3, 128.4, 128.1, 82.1, 66.9, 59.4, 52.2, 32.2, 30.3, 29.6, 27.8, 26.8, 17.5, 13.5. IR (KBr) ν_{max}: 2976, 1720, 1535, 1322, 1252, 975 cm^{-1}. ESI (+VE) *m*/*z*: 480.3 $(M+Na)^+$. HR-ESI MS calcd for $C_{22}H_{36}NO_7NaP$ $(M+Na)^+$: 480.2122, found: 480.2126.

*3.3.10. (2S,3R)-4-[Di-(tert-butyl)-oxyphosphinyl]-N-[(9H-fluoren-9-ylmethoxy)carbonyl]-L-valine (**4**).* To a solution of **20** (140 mg, 0.31 mmol) in THF (3.0 mL) and H_2O (3.0 mL) at 0 °C, was added $LiOH\cdot H_2O$ (26 mg, 0.62 mmol) and the mixture was stirred room temperature (overnight). The THF was removed by rotary evaporation and the residual aqueous phase was neutralized by addition of saturated aqueous NH_4Cl (20 mL) and extracted with EtOAc (3×50 mL). The combined organic extract was washed with H_2O (50 mL), brine (50 mL), dried (Na_2SO_4) and concentrated. The resulting residue was dissolved in MeOH (20 mL) and hydrogenated (1 atmosphere H_2) over 10% Pd·C (40 mg) at room temperature (overnight). The catalyst was removed by filtration and the filtrate was concentrated. The resulting residue was dissolved in dioxane (5.0 mL) and H_2O (5.0 mL) and 9-fluorenylmethyl–succinimidyl carbonate Fmoc–OSu (173 mg, 0.465 mmol) and $NaHCO_3$ (62 mg, 0.62 mmol) were added and the mixture was stirred at room temperature (overnight). The reaction mixture was neutralized by addition of saturated NH_4Cl (20 mL) and extracted with EtOAc (3×50 mL). The combined EtOAc layer was washed with H_2O (50 mL), brine (50 mL), dried (Na_2SO_4) and purified by silica gel column chromatography (CH_2Cl_2:MeOH) to yield **4** as a white wax (166 mg, quantitative yield over 3 steps). $[\alpha]^{20}_D$+16.5 (*c* 0.65, $CHCl_3$). ^{1}H NMR (400 MHz, DMS)-d_6 δ 7.88 (d, *J*=7.6 Hz, 2H), 7.70 (d, *J*=7.2 Hz, 2H), 7.41 (t, *J*=7.4 Hz, 2H), 7.31 (t, *J*=7.6 Hz, 2H), 4.30–4.19 (m, 4H), 3.84 (m, 1H), 2.31 (m, 1H), 1.80–1.55 (m, 2H), 1.42 (s, 18H), 0.96 (d, *J*=6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 143.9, 140.7, 127.6, 127.0, 125.1, 120.0, 80.7, 65.4, 60.5, 46.7, 31.5, 30.0, 16.9. ESI (+VE) *m*/*z*: 554.2 $(M+Na)^+$. HR-ESI MS calcd for $C_{28}H_{38}NO_7NaP$ $(M+Na)^+$: 554.2278, found: 554.2277.

3.4. Peptide synthesis 21–24

Fmoc-Thr(PO(OBzl)OH)-OH and other Fmoc protected amino acids were purchased from Novabiochem. Peptides were synthesize on NovaSyn®TGR resin (Novabiochem, cat. no. 01-64-0060) using standard Fmoc solid-phase protocols in *N*-Methyl-2-pyrrolidone (NMP). 1-*O*-Benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (5.0 equiv), hydroxybenzotriazole (HOBT) (5.0 equiv) and *N*,*N*-Diisopropylethylamine (DIPEA) (10.0 equiv) were used as coupling reagents. The N-terminal was acetylated by 1-Acetylimidazole. The final resin was washed with *N*,*N*-dimethylformamide (DMF), methanol, dichloromethane and ether then dried under vacuum (overnight). Peptides were cleaved from resin (200 mg) by treatment with 5 mL of trifluoroacetic acid:triisbutylsilane:H_2O (90:5:5) (4 h). The resin was filtered off and the filtrate was concentrated under vacuum, then precipitated with ether and the precipitate washed with ether. The resulting solid was dissolved in 50% aqueous acetonitrile 5 mL and purified by reverse phase preparative HPLC using a Phenomenex C_{18} column (21 mm dia×250 mm, cat. no: 00G-4436-P0) with a linear gradient from 0% aqueous acetonitrile (0.1% trifluoroacetic acid) to 50% acetonitrile (0.1% trifluoroacetic acid) over 35 min at a flow rate of 10.0 mL/min. Peptide **21**: ESI (+VE) *m*/*z*: 595.3 $(M+H)^+$. Peptide **22**: ESI (+VE) *m*/*z*: 675.3 $(M+H)^+$. Peptide **23**: ESI (+VE) *m*/*z*: 673.3 $(M+H)^+$. Peptide **24**: ESI (+VE) *m*/*z*: 657.3 $(M+H)^+$. Analytical HPLC [By using Phenomenex C_{18} column (4.60 mm dia×250 mm, cat. no: 00G-4435-E0) with a linear gradient from 5% aqueous acetonitrile (0.1% trifluoroacetic acid) to 100% acetonitrile (0.1% trifluoroacetic acid) over 25 min at a flow rate of 1.0 mL/min indicated the purity of peptide **21**: 100%, peptide **22**: 100%, peptide **23**: 87%, peptide **24**: 83%.

Acknowledgements

This Work was supported in part by the Intramural Research Program of the NIH, Center for Cancer Research, NCI-Frederick and the National Cancer Institute, National Institutes of Health. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

References and notes

1. Pawson, T. *Cell* **2004**, *116*, 191.
2. Smerdon, S. J.; Yaffe, M. B. *Handbook Cell Signaling*; Elsevier: San Diego, CA, 2004; Vol. 1, p. 505.
3. Ladbury, J. E. *Protein Rev.* **2005**, *3*, 165.
4. Mayer, B. J. *Methods Mol. Biol.* **2006**, *332*, 79.
5. Hubbard, S. R.; Till, J. H. *Annu. Rev. Biochem.* **2000**, *69*, 373.
6. Blume-Jensen, P.; Hunter, T. (London, UK) *Nature* **2001**, *411*, 355.
7. Tsatsanis, C.; Spandidos, D. A. *Ann. N.Y. Acad. Sci.* **2004**, *1028*, 168.
8. Adjei, A. A.; Hidalgo, M. *J. Clin. Oncol.* **2005**, *23*, 5386.
9. Krause, D. S.; Van Etten, R. A. *N. Engl. J. Med.* **2005**, *353*, 172.
10. Bianco, R.; Melisi, D.; Ciardiello, F.; Tortora, G. *Eur. J. Cancer* **2006**, *42*, 290.
11. Yaffe, M. B. *Nat. Rev. Mol. Cell. Biol.* **2002**, *3*, 177.
12. Brockman, J. A.; Scherer, D. C.; McKinsey, T. A.; Hall, S. M.; Qi, X.; Lee, W. Y.; Ballard, D. W. *Mol. Cell. Biol.* **1995**, *15*, 2809.
13. McKinsey, T. A.; Chu, Z. L.; Ballard, D. W. *J. Biol. Chem.* **1997**, *272*, 22377.
14. Bex, F.; Murphy, K.; Wattiez, R.; Burny, A.; Gaynor, R. B. *J. Virol.* **1999**, *73*, 738.
15. Zheng, W.; Zhang, Z.; Ganguly, S.; Weller Joan, L.; Klein David, C.; Cole Philip, A. *Nat. Struct. Biol.* **2003**, *10*, 1054.
16. Zheng, W.; Schwarzer, D.; Lebeau, A.; Weller Joan, L.; Klein David, C.; Cole Philip. *J. Biol. Chem.* **2005**, *280*, 10462.
17. Foss Frank, W., Jr.; Snyder Ashley, H.; Davis Michael, D.; Rouse, M.; Okusa Mark, D.; Lynch Kevin, R.; Macdonald Timothy, L. *Bioorg. Med. Chem.* **2007**, *15*, 663.

18. Burke Terrence, R., Jr.; Lee, K. *Acc. Chem. Res.* **2003**, *36*, 426.
19. Ruiz, M.; Ojea, V.; Shapiro, G.; Weber, H.-P.; Pombo-Villar, E. *Tetrahedron Lett.* **1994**, *35*, 4551.
20. Berkowitz, D. B.; Eggen, M.; Shen, Q.; Shoemaker, R. K. *J. Org. Chem.* **1996**, *61*, 4666.
21. Otaka, A.; Mitsuyama, E.; Kinoshita, T.; Tamamura, H.; Fujii, N. *J. Org. Chem.* **2000**, *65*, 4888.
22. Ziegler, F. E.; Lim, H. *J. Org. Chem.* **1984**, *49*, 3278.
23. Tilley, S. D.; Reber, K. P.; Sorensen, E. J. *Org. Lett.* **2009**, *11*, 701.
24. Evans, D. A.; Chapman, K. T.; Bisaha, J. *J. Am. Chem. Soc.* **1988**, *110*, 1238.
25. Liu, F.; Zha, H. Y.; Yao, Z. J. *J. Org. Chem.* **2003**, *68*, 6679.
26. Nicolas, E.; Russell, K. C.; Knollenberg, J.; Hruby, V. J. *J. Org. Chem.* **1993**, *58*, 7565–7571.
27. Lung, F.-D.; Li, G.; Lou, B.-S.; Hruby, V. J. *Synth. Commun.* **1995**, *25*, 57–61.
28. Humphrey, J. M.; Bridges, R. J.; Hart, J. A.; Chamberlin, A. R. *J. Org. Chem.* **1994**, *59*, 2467.
29. Evans, D. A.; Hurst, K. M.; Takacs, J. M. *J. Am. Chem. Soc.* **1978**, *100*, 3467.
30. Wiemann, A.; Frank, R.; Tegge, W. *Tetrahedron* **2000**, *56*, 1331.
31. Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574.
32. Lowery, D. M.; Lim, D.; Yaffe, M. B. *Oncogene* **2005**, *24*, 248.
33. Eckerdt, F.; Yuan, J.; Strebhardt, K. *Oncogene* **2005**, *24*, 267.
34. Goh, K. C.; Wang, H.; Yu, N.; Zhou, Y.; Zheng, Y.; Lim, Z.; Sangthongpitag, K.; Fang, L.; Du, M.; Wang, X.; Jefferson, A. B.; Rose, J.; Shamoon, B.; Reinhard, C.; Carte, B.; Entzeroth, M.; Ni, B.; Taylor, M. L.; Stuenkel, W. *Drug Dev. Res.* **2004**, *62*, 349.
35. Strebhardt, K.; Ullrich, A. *Nat. Rev. Cancer* **2006**, *6*, 321.
36. McInnes, C.; Mezna, M.; Fischer, P. M. *Curr. Top. Med. Chem.* **2005**, *5*, 181.
37. Strebhardt, K. *ACS Chem. Biol.* **2006**, *1*, 683.
38. Peters, U.; Cherian, J.; Kim, J. H.; Kwok, B. H.; Kapoor, T. M. *Nat. Chem. Biol.* **2006**, *2*, 618.
39. Lansing, T. J.; McConnell, R. T.; Duckett, D. R.; Spehar, G. M.; Knick, V. B.; Hassler, D. F.; Noro, N.; Furuta, M.; Emmitte, K. A.; Gilmer, T. M.; Mook, R. A.; Cheung, M. *Mol. Cancer Ther.* **2007**, *6*, 450.
40. Santamaria, A.; Neef, R.; Eberspacher, U.; Eis, K.; Husemann, M.; Mumberg, D.; Prechtl, S.; Schulze, V.; Siemeister, G.; Wortmann, L.; Barr, F. A.; Nigg, E. A. *Mol. Biol. Cell* **2007**, *18*, 4024.
41. Hanan, E. J.; Fucini, R. V.; Romanowski, M. J.; Elling, R. A.; Lew, W.; Purkey, H. E.; VanderPorten, E. C.; Yang, W. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5186.
42. Fucini, R. V.; Hanan, E. J.; Romanowski, M. J.; Elling, R. A.; Lew, W.; Barr, K. J.; Zhu, J.; Yoburn, J. C.; Liu, Y.; Fahr, B. T.; Fan, J.; Lu, Y.; Pham, P.; Choong, I. C.; VanderPorten, E. C.; Bui, M.; Purkey, H. E.; Evanchik, M. J.; Yang, W. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5648.
43. Emmitte, K. A.; Adjebang, G. M.; Andrews, C. W.; Badiang-Alberti, J. G.; Bambal, R.; Chamberlain, S. D.; Davis-Ward, R. G.; Dickson, H. D.; Hassler, D. F.; Hornberger, K. R.; Jackson, J. R.; Kuntz, K. W.; Lansing, T. J.; Mook, R. A.; Nailor, K. E.; Pobanz, M. A.; Smith, S. C.; Sung, C.-M.; Cheung, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1694.
44. Sillje, H. H. W.; Nigg, E. A. (Washington, DC, United States) *Science* **2003**, *299*, 1190.
45. van de Weerdt, B. C. M.; Littler, D. R.; Klompmaker, R.; Huseinovic, A.; Fish, A.; Perrakis, A.; Medema, R. H. *Biochim. Biophys. Acta, Mol. Cell. Res.* **2008**, *1783*, 1015.
46. Reindl, W.; Yuan, J.; Kraemer, A.; Strebhardt, K.; Berg, T. (Cambridge, MA, U.S.) *Chem. Biol.* **2008**, *15*, 459.
47. Cheng, K.-Y.; Lowe, E. D.; Sinclair, J.; Nigg, E. A.; Johnson, L. N. *EMBO J.* **2003**, *22*, 5757.
48. Elia, A. E. H.; Rellos, P.; Haire, L. F.; Chao, J. W.; Ivins, F. J.; Hoepker, K.; Mohammad, D.; Cantley, L. C.; Smerdon, S. J.; Yaffe, M. B. (Cambridge, MA, United States) *Cell* **2003**, *115*, 83.
49. Yun, S.-M.; Moulaei, T.; Lim, D.; Bang, J. K.; Shenoy, S. R.; Park, J.-E.; Liu, F.; Kang, Y. H.; Liao, C.; Soung, N.-K.; Lee, S.; Yoon, D.-Y.; Lim, Y.; Lee, D.-H.; Otaka, A.; Appella, E.; McMahon, J. B.; Nicklaus, M. C.; Burke, T. R., Jr.; Yaffe, M. B.; Wlodawer, A.; Lee, K. S. *Nat. Struct. Biol.* **2009**, *16*, 876.
50. Hanisch, A.; Wehner, A.; Nigg, E. A.; Sillje, H. H. *Mol. Biol. Cell* **2006**, *17*, 448.