Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 6087–6093

TETRAHEDRON

Synthesis of a phosphotyrosyl analogue having χ_1, χ_2 and ϕ angles constrained to values observed for an SH2 domain-bound phosphotyrosyl residue

Xiang-Zhu Wang,[a] Zhu-Jun Yao,[a,†] Hongpeng Liu,[b] Manchao Zhang,[b] Dajun Yang,[b] Clifford George[c] and Terrence R. Burke, Jr.[a,*]

[a]*Laboratory of Medicinal Chemistry, Center for Cancer Research, National Cancer Institute, National Institutes of Health, NCI-Frederick, P.O. Box B, Bldg 376 Boyles St., Frederick, MD 21702-1201, USA*
[b]*Department of Hematology/Oncology, University of Michigan Medical School, Ann Arbor, MI 48109, USA*
[c]*Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC 20375, USA*

Received 1 May 2003; revised 11 June 2003; accepted 19 June 2003

Abstract—Src homology 2 (SH2) domains provide connectivity in protein-tyrosine kinase (PTK)-dependent signaling through their high affinity association with phosphotyrosyl (pTyr)-containing peptide sequences. Because recognition of pTyr residues is central to SH2 domain-binding affinity, design of pTyr-mimicking residues has been one component of SH2 domain signaling antagonist development. Reported herein is the synthesis of (±)-(*rel*-1*R*,2*R*,5*S*)-3-acetyl-1,2,3,4,5,6-hexahydro-8-*O*-phosphoryl-1,5-methano-3-benzazocine-2-carboxylic acid methyl ester (**3c**) as a monomeric pTyr-mimicking analogue that constrains three torsion angles (χ_1=168°; χ_2=−85°; ϕ_1=−113°) to values approximating those observed for a pTyr residue bound to the Grb2 SH2 domain (χ_1=182°; χ_2=−89°; ϕ_1=−132°). Compound **3c** differs from our previously reported analogue, (±)-(*rel*-1*R*,2*R*,5*S*)-3-acetyl-1,2,3,4,5,6-hexahydro-1-methyl-1,5-methano-3-benzazocin-8-ol, in lacking a methyl substituent at the bridgehead 1-position. Molecular modeling studies had indicated that this methyl group could potentially hinder SH2 domain binding. Synthesis of the desmethyl derivative was achieved by formation of the methanobenzazocine ring system using an intramolecular electrophilic cyclization that proceeds through an activated acyliminium intermediate. Importantly, the correct relative (2*R*) stereochemistry at the 'α-carboxyl'-bearing carbon is obtained through base-catalyzed equilibration of a (2*S*/2*R*) diastereomeric mixture that results from intramolecular ring closure. Comparison of Grb2 SH2 domain-binding affinity of **3c** (IC_{50}=1167 μM) with conformationally flexible phosphorylated (±)-*N*-acetyl-tyrosine methyl ester (**15**; IC_{50}=1469 μM) revealed no apparent enhancement in affinity. This apparent ineffectiveness of 'local conformational constraint' on SH2 domain-binding affinity of the monomeric pTyr mimetic is consistent with previous reports obtained by conformationally constraining pTyr-mimicking residues that were contained within peptide platforms. Although not providing high binding affinity in its current form, the novel 1,5-methano-3-benzazocine ring system may afford a novel platform for further elaboration and development of small molecule SH2 domain signaling antagonists.

1. Introduction[1]

Aberrations in protein-tyrosine-kinase (PTK)-dependent signaling are associated with proliferative disorders, including certain cancers.[2,3] Accordingly, recent studies showing the promise of kinase inhibitors have begun to validate PTK signaling blockade as an efficacious anticancer therapeutic approach.[4–7] Src homology 2 (SH2) domains are a family of homologous non-catalytic protein modules that serve downstream roles in PTK-dependent signaling by binding phosphotyrosyl (pTyr)-containing proteins in sequence-dependent fashions.[8] Agents that compete with cognate proteins for binding to select SH2 domains could disrupt PTK-dependent signaling and thereby represent alternatives to kinase inhibitors as novel therapeutics.[4,9,10] The structural basis of SH2 domain recognition of pTyr-containing ligands involves interactions of pTyr residues in well-formed pockets that contain one or two highly conserved arginine residues.[11,12] The central role of the pTyr residue (**1**) in the overall binding process[12,13] has made design of pTyr mimetics an important component of SH2 domain-directed antagonist development.[14–16] One objective of these efforts has been reduction of binding entropy penalties by introduction of conformational constraint into the pTyr-mimicking residue.[17–20] Accordingly,

Keywords: phosphotyrosyl analogue; protein-tyrosine-kinase; SH2 domains.
* Corresponding author. Tel.: +1-301-846-5906; fax: +1-301-846-6033; e-mail: tburke@helix.nih.gov
† Present address: Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, People's Republic of China.

0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0040-4020(03)00978-5

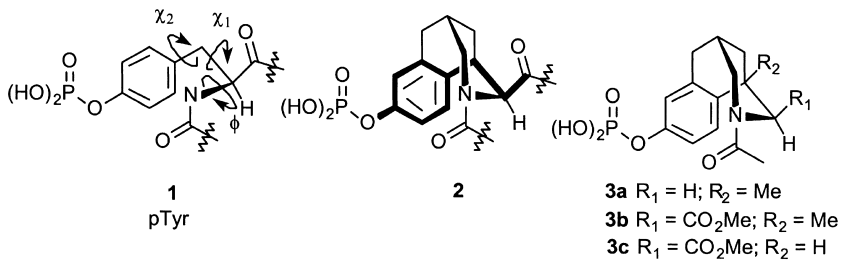
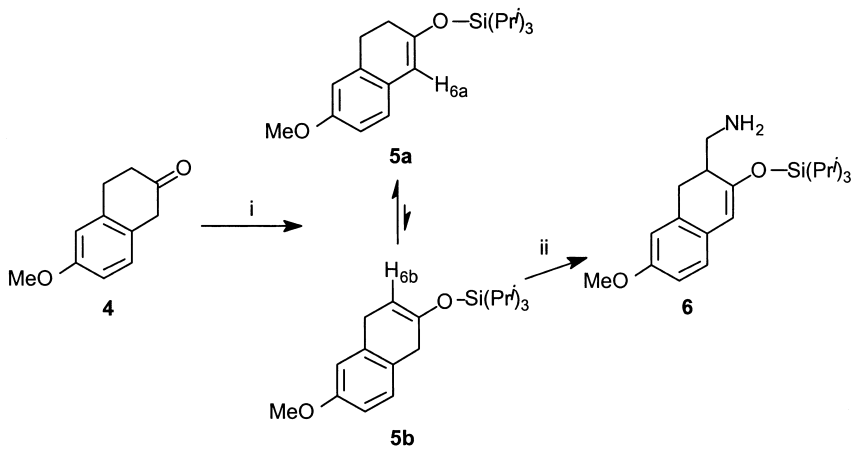


Figure 1. Structure of pTyr (**1**) showing locations of torsion angles as well as conformationally constrained pTyr mimetics. Structure **2** highlights the pTyr substructure within the constrained analogues.

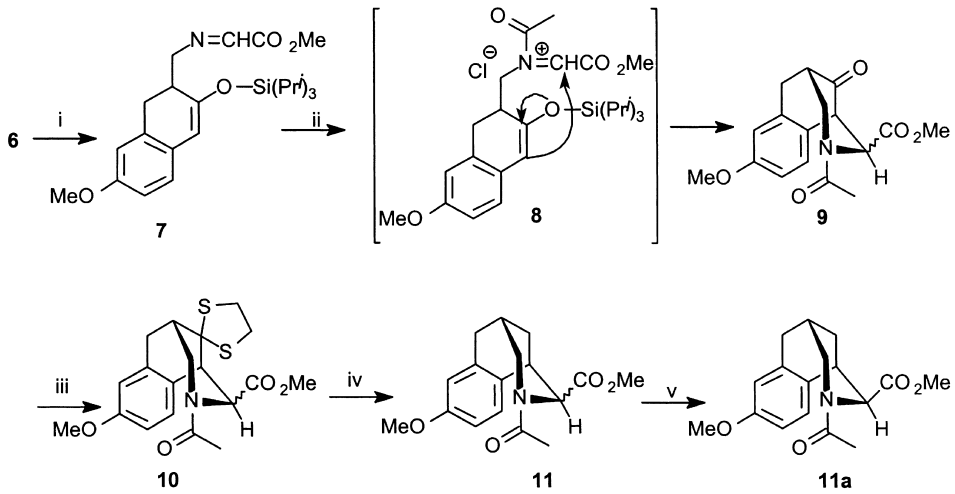
we had previously proposed the tricyclic methanobenzazocino—**2** as containing within its framework a tyrosyl substructure having χ_1, χ_2 and ϕ angles constrained to values observed for an SH2 domain-bound pTyr residue (Fig. 1).[21] For reasons of synthetic simplicity, the structurally abbreviated compound **3a**, lacking an α-carboxyl equivalent and having an undesired 1-methyl substituent, was initially prepared using a Mannich condensation approach.[21] Subsequently, the more highly functionalized **3b**, containing an α-carboxyl equivalent was prepared in its unphosphorylated form. Unfortunately, as with the earlier synthesis, this analogue also retained the unwanted 1-methyl substituent.[22] In both syntheses, incorporation of the 1-methyl group was required to block unwanted alkylation at this reactive position. However, molecular modeling studies had indicated that this methyl group could potentially hinder SH2 domain binding, and it was highly desirable to derive synthetic approaches that could circumvent its use. For this purpose we have devised an alternate synthetic route that obviates the unwanted 1-methyl group. Reported herein is the culmination of this work, wherein the fully elaborated, carboxyl-containing **3c** is prepared by a novel approach that eliminates retention of the unwanted 1-methyl group. Included are the synthesis of **3c** in its phosphorylated form, verification of structure by X-ray crystallography and evaluation of Grb2 SH2 domain-binding potency.

2. Results and discussion

Mannich-type reactions have been extensively utilized for the synthesis of nitrogen-containing heterocycles, natural products and drug-like biomimetics.[23–27] The conformationally constrained tricyclic mimetic **2** can be viewed as a pTyr residue in which the amino acid α-nitrogen is methano-bridged to a tetrahydronaphthalenol ring system. Following introduction of a methyl group at the 1-position of 6-methoxy-2-tetolone (**4**), previous approaches to this tricyclic ring system have utilized either Mannich reactions,[21] or intramolecular 1,4-Michael-type reaction of a carboxamido group onto an exocyclic double bond.[22] This ultimately has resulted in retention of an unwanted 1-methyl substituent in the final products (**3a** and **3b**, respectively). In order to eliminate this methyl substituent, tetralone **4** was subjected to an initial aminomethylation of silyl enol ethers **5a** and **5b**,[28] which had been obtained from **4** as an inseparable mixture (Scheme 1). The ratio of **5a** to **5b** was determined by integration of vinylic ^{1}H NMR signals at 5.66 ppm and 5.05 ppm, respectively. Depending on reaction conditions, the thermodynamically favored **5a** could be obtained as a single regioisomer if passed through basic aluminum oxide. However passage through silica gel resulted in significant generation of **5b** (**5a**:**5b**≈1:1). Subjecting a mixture of **5a** and **5b** to aminomethylation provided the desired primary amine **6** as a single



Scheme 1. *Reagents and Conditions*: (i) NEt_3, TIPSiOTf, benzene, rt, 1 h (>90% yield). (If purified through basic Al_2O_3, only **5a** is observed to ^{1}H NMR, however if purified through silica gel, **5a**:**5b**=52:48.); (ii) $Me_3SiCH_2N_3$, $AlCl_3$, CH_2Cl_2.



Scheme 2. *Reagents and Conditions*: (i) $OHCCO_2Me$, $MgSO_4$; (ii) AcCl, toluene (68% yield over 3 steps); (iii) $HSCH_2CH_2SH$, $BF_3{\cdot}Et_2O$, CH_2Cl_2 (80% yield); (iv) Raney nickel, EtOH (90% yield); (v) NH_3, MeOH.

regioisomer. The reaction occurred in high yield in spite of the fact that formation of **6** proceeded from **5b**, which was a minor component in the starting reaction mixture. This implied that during aminomethylation a reversible equilibrium between **5a** and **5b** was maintained with depletion of **5b** occurring through transformation to product **6**.

Aminomethylation product **6** was reacted with methyl glyoxalate in the presence of magnesium sulfate to form imine **7** (Scheme 2). This was acetylated without purification to form an activated acyliminium intermediate (**8**) that underwent intramolecular electrophilic cyclization to yield the key tricyclic ketone **9** in 68% overall yield from **6**.[29] Of note, the 2-carbomethoxy group in **9** was obtained as an *endo*/*exo*-diastereomeric mixture. Reductive deoxygenation of the keto group of **9** could be achieved in a two-step manner by initial thioketalization to spiro bisdithiane **10**, followed by desulfurization to **11** using Raney Nickel in refluxing EtOH. Although undesired *endo* (2*S*)-2-carbomethoxy-containing material could be removed chromatographically at this point, it was found that treatment of **11** with mild base (2 M methanolic ammonia) resulted in epimerization at the 2-position to yield the desired *exo* isomer (2*R*)-**11a** as a single product. Remarkably, formation of carboxamide side product was not detected under these conditions.

Selective demethylation of **11a** to **12** without de-esterification could be achieved in good yield using BBr_3. Finally, phosphorylation to final product **3c** was accomplished in two steps by initial conversion to protected phosphoester **13** followed by acidic cleavage of *tert*-butyl-groups (Scheme 3).

Subjecting **3c** to X-ray crystallographic analysis confirmed its structure and provided a measure of χ_1, χ_2 and ϕ torsion angles (Fig. 2C). Comparison of these values (χ_1=168°; χ_2=−85°; ϕ=−113°) with the corresponding torsion angles of a pTyr residue bound to the Grb2 SH2 domain as part of a larger peptide ligand[30] (Fig. 2A: χ_1=182°; χ_2=−89°; ϕ=−132°) showed remarkable congruence that can be appreciated visually through overlay of the two structures (Fig. 2B).

The Grb2 SH2 domain-binding affinity of **3c** was examined using an ELISA-based assay as previously described.[31] For comparison, unconstrained phosphorylated *N*-acetyl-tyrosine methyl ester was also examined in parallel in both racemic and (L)-forms (**15** and **16**, respectively). Consistent with earlier findings,[21] affinities for monomeric pTyr analogues were markedly reduced as compared with values obtained when pTyr residues are contained within peptide or peptide mimetic display platforms (Table 1).[13] Of primary interest however, were potential relative increases in affinity resulting from reduction of binding entropy penalties incurred through conformational constraint. Here it was observed by comparison of IC_{50} values obtained for **3c** relative to **15** and **16**, that all three analogues were approximately equipotent within statistical error. Although this contrasts with marked binding enhancement achieved through 'global' conformational constraint of Grb2 SH2 domain-binding molecules,[32–34] the apparent ineffectiveness of 'local conformational constraint' of the pTyr residue in enhancing SH2 domain-binding affinity has also been observed with conformationally constrained pTyr-mimicking residues contained within peptide platforms.[18,20,35] In one study the ineffectiveness of local conformational constraint in enhancing overall peptide binding was in spite of successful realization of an intended decrease in

Scheme 3. *Reagents and Conditions*: (i) BBr_3, CH_2Cl_2 (85% yield); (ii) $(Bu^tO)_2P(NPr^i)_2$, tetrazole, Bu^tOOH (47% yield); (iii) CF_3COOH, THF (92% yield).

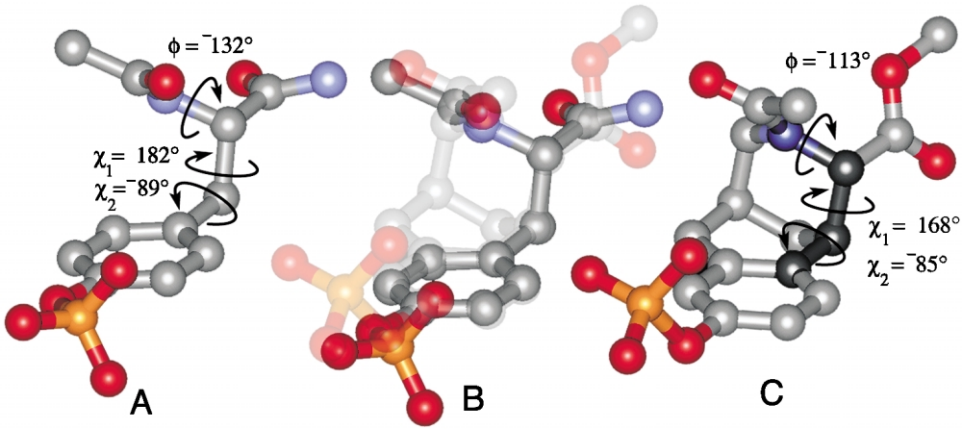
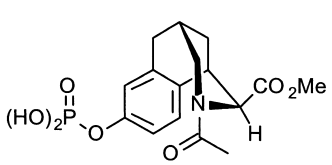
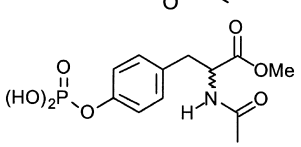
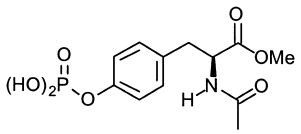


Figure 2. (A) Grb2 SH2 domain-bound pTyr residue derived from data presented in Ref. 30 showing relevant torsion angles; (B) Overlay of A and C; (C) X-ray crystal structure of constrained pTyr mimetic **3c** showing relevant torsion angles.

entropy penalties.[35] This failure of entropic advantage to be reflected in total binding free energy was attributed to an offsetting reduction in binding enthalpy, which is an example of 'entropy–enthalpy compensation'[36,37] that has been observed in other peptide–SH2 domain binding interactions.[38] Although a detailed calorimetric binding analysis is beyond the scope of the current work, it can be speculated that some component of entropy–enthalpy compensation may underlie the absence of conformationally induced binding enhancement of **3c** relative to **15** and **16**.

In conclusion, reported herein is a novel and direct synthesis of a new amino acid analogue that is remarkable in simultaneously constraining three torsion angles to values observed for a Grb2 SH2 domain-bound pTyr residue. An important aspect of the current synthetic approach is its ability to prepare this new constrained pTyr mimetic in the absence of a potentially undesirable methyl substituent at the bridgehead 1-position. Although only modest binding affinity was achieved through such conformational constraint, the tricyclic methano-3-benzazocine structure may potentially afford an anchoring platform from which to append additional recognition functionality that may lead to more potent small molecule, non-peptidic SH2 domain signaling antagonists.

Table 1. Extracellular ELISA-derived Grb2 SH2 domain binding affinities of synthetic residues

No.	Structure	IC_{50} (μM)
3c		1167±465
15		1469±326
16		1419±28

Assays were conducted as described in Ref. 35.

3. Experimental

3.1. Single-crystal X-ray diffraction analysis of compound 3c

$C_{16}H_{20}NO_7P$, M_w=369.30, monoclinic space group $P2_1/c$, a=7.614(1), b=23.416(1), c=9.785(1) Å, β=98.122(1)°, V=1727.16(15) Å^3, Z=4, ρ_{calc}=1.420 mg mm^{-3}, λ(Cu Kα)=1.54184 Å, μ=1.786 mm^{-1}, $F(000)$=776, T=22°C. A 0.18×0.15×0.02 mm^3 crystal was used for data collection on an automated Bruker 6K CCD equipped with a Gobel mirrors monochromator. Lattice parameters were determined from 2856 centered reflections within $7.55 \leq 2\theta \leq 113.27°$. The data collection range of hkl was: $-7 \leq h \leq 8$, $-25 \leq k \leq 24$, $-10 \leq l \leq 10$, with $[(\sin\theta/\lambda)]_{max}$=0.55. A set of 7593 reflections was collected using ω scans. There were 2322 unique reflections, and 1440 were observed with $I>2\sigma(I)$. The structure was solved and refined with a full-matrix least squares[39,40] on F^2. The refinement varied 223 parameters: atom coordinates and anisotropic thermal parameters for all non-H atoms. H atoms were included using a riding model [coordinate shifts of C applied to attached H atoms, C–H distances set to 0.96 Å, H angles idealized, U_{iso}(H) were set to 1.2 U_{eq}(C) hydroxyl hydrogen coordinates were refined. Final residuals were $R1$=0.054 for $I>2\sigma(I)$ and $wR2$=0.159 for all data with final difference Fourier excursions of 0.27 and −0.25 eÅ^{-3}

3.2. General synthetic

Reactions were carried out under argon in oven-dried glassware using standard gas-tight syringes, cannulas and septa. Anhydrous solvents were purchased from Aldrich Chemical Corporation and used without further drying. Melting points were measured using a MEL-TEMP II apparatus and are uncorrected. Elemental analyses were

obtained from Atlantic Microlab, Inc., Norcross, Ga. ^{1}H NMR data were recorded on a Varian 400 MHz spectrometer and are reported in ppm relative to TMS and referenced to the solvent in which they were run. Fast atom bombardment mass spectra (FABMS) were acquired with a VG analytical 7070E mass spectrometer under the control of a VG 2035 data system. Where indicated, Preparative HPLC purification was conducted with a Waters Prep LC4000 system using photodiode array detection and binary solvent systems as indicated, where A=0.1% aqueous TFA and B=0.1% TFA in acetonitrile and an Advantage C_{18} (5μ) column (preparative size, 20 mm dia.×250 mm long with a flow rate of 10 mL/min).

3.2.1. [(3,4-Dihydro-6-methoxy-2-naphthalenyl)oxy]-tris(1-methylethyl)-silane (5a) and [(1,4-dihydro-6-methoxy-2-naphthalenyl)oxy]tris(1-methylethyl)-silane (5b). A solution of 6-methoxy-2-teralone (**4**) (1.86 g, 10.0 mmol) in anhydrous benzene (30 mL) under argon at room temperature was treated with triethylamine (1.52 g, 15.0 mmol) and triisopropylsilyl trifluomethanesulfonate (3.37 g, 11.0 mmol) and the mixture was stirred at room temperature (1 h). The reaction was quenched by addition of saturated aqueous $NaHCO_3$ and the organic layer was collected and the aqueous phase was extracted with EtOAc (twice) and the combined organic layers were washed with brine and dried ($MgSO_4$). Solvent was removed under vacuum and residue was purified through basic aluminum oxide to provide **5a** as an oil (3.24 g, 98% yield). [Note: Purification through silica gel provided a mixture of **5a** and **5b** in an approximate 1:1 ratio.]. ^{1}H NMR ($CDCl_3$) **5a**: δ 6.68 (d, 1H, J=7.8 Hz), 6.66 (m, 2H), 5.66 (s, 1H), 3.78 (s, 3H), 2.88 (t, 2H, J=8.2 Hz), 2.38 (t, 2H, J=8.2 Hz), 1.24 (m, 3H), 1.12 (d, 18H, J=7.0 Hz). **5b**: δ 7.03 (d, 1H, J=8.3 Hz), 6.73 (dd, 1H, J=2.7 and 8.5 Hz), 6.64 (dd, 1H, J=2.7 and 8.5 Hz), 5.02 (m, 1H), 3.79 (s, 3H), 3.44 (m, 2H), 3.36 (m, 2H), 1.24 (m, 3H), 1.12 (d, 18H, J=7.0 Hz); IR (neat) 2938, 2865, 1642, 1498, 1257, 1175, 1119, 882 cm^{-1}; FABMS (*m/z*) 333 (M+H)$^+$. Anal. Calcd ($C_{20}H_{32}O_2Si$): C, 72.23; H, 9.70. Found: C, 72.47; H, 9.82.

3.2.2. 1,2-Dihydro-7-methoxy-3-[[tris(1-methylethyl)silyl]oxy]-2-naphthalenemethanamine (6). To a suspension of aluminum chloride (1.46 g, 11.4 mmol) in anhydrous CH_2Cl_2 (25 mL) at −78°C under argon was added $TMSCH_2N_3$ (1.32 g, 12.4 mmol) under argon. The mixture was stirred at −78°C (30 min), then at 0°C (1 h) and then the mixture was warmed to room temperature and stirred (1 h) to provide a clear solution. The solution was re-cooled to −78°C and a mixture of silyl enol ethers **5a/5b** (3.04 g, 9.14 mmol) in CH_2Cl_2 (10 mL) was added dropwise, then the mixture was warmed to room temperature and stirred overnight. The reaction was quenched by cautious addition of 2N NaOH (2×30 mL) at 0°C. The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2 and the combined organic layer was washed with brine and dried ($MgSO_4$). Solvent was removed under reduced pressure to provide crude **6** as a light brown solid (3.39 g). This was used directly without purification due to instability. ^{1}H NMR ($CDCl_3$) δ 6.76 (d, 1H, J=8.2 Hz), 6.62 (m, 2H), 5.61 (s, 1H), 3.78 (s, 3H), 3.02 (dd, 1H, J=7.2 and 16.0 Hz), 2.83 (m, 2H), 2.72 (dd, 1H, J=7.0 and 13.1 Hz), 2.35 (m, 1H), 1.60 (br, 2H), 1.21 (m, 3H), 1.07 (18H); IR (neat): 3455, 2945, 2866, 1636, 1498, 1173, 882 cm^{-1}; FABMS (*m/z*) 362 (M+H)$^+$.

3.2.3. (±)-(*rel*-1*R*,2*R*/*S*,5*S*)-3-Acetyl-1,2,3,4,5,6-hexahydro-8-methoxy-11-oxo-1,5-methano-3-benzazocine-2-carboxylic acid methyl ester (9). A mixture of crude **6** (3.39 g, 9.37 mmol) and methyl glyoxalate (924 mg, 10.5 mmol) in anydrous CH_2Cl_2 (25 mL) was stirred with $MgSO_4$ at room temperature (1.5 h). Solid was removed by filtration, the filter pad was washed with CH_2Cl_2 and the combined filtrate was concentrated under vacuum to afford crude **7** as an oil (4.13 g). This was dissolved in anhydrous toluene (20 mL), cooled to −78°C and acetyl choride (12.5 mmol) was added under argon. The mixture was stirred at 70°C (10 h), then solvent was removed under vacuum and residue was purified by silica gel flash chromatography to provide tricyclic **9** (2.07 g, 68% yield over 3 steps). ^{1}H NMR ($CDCl_3$) δ 6.97 (m, 1H), 6.74 (m, 1H), 6.63 (m, 1H), 5.18 (m, 1H), 4.20 (m, 1H), 3.97 (dd, 1H, J=1.7 and 7.8 Hz), 3.82 (m, 1H), 3.78 (s, 3H), 3.63 (s, 3H), 3.27 (m, 1H), 3.11 (d, 2H, J=15.2 Hz), 2.17 (s, 3H); IR (neat): 742, 1646, 1219 cm^{-1}; FABMS (*m/z*) 318 (M+H)$^+$. Anal. Calcd ($C_{17}H_{19}NO_5\cdot H_2O$): C, 60.89; H, 5.71; N, 4.17. Found: C, 61.11; H, 5.72; N, 3.99.

3.2.4. (±)-(*rel*-1′*R*,2′*R*/*S*,5′*S*)-3′-Acetyl-1′,2′,3′,4′,5′,6′-hexahydro-8′-methoxy-spiro[1,3-dithiolane-2,11′-[1,5]methano[3]benzazocine]-2′-carboxylic acid methyl ester (10). To a solution of **9** (1.41 g, 4.41 mmol) and ethane-1,2-dithiol (4.85 mmol) in anhydrous CH_2Cl_2 (10 mL) was slowly added $BF_3\cdot Et_2O$ (6.60 mmol) under argon, then the mixture was stirred at room temperature (18 h). The reaction was quenched by addition of dilute aqueous NaOH and the mixture was washed with H_2O and brine and dried ($MgSO_4$). Purification by silica gel flash chromatography afforded **10** as a foam (1.27 g, 73% yield). ^{1}H NMR ($CDCl_3$) δ 7.0 (d, 1H, J=8.4 Hz), 6.68 (d, 1H, J=8.4 Hz), 6.60 (s, 1H), 4.98 (m, 1H), 3.97 (m, 1H), 3.77 (s, 3H), 3.61 (m, 1H), 3.50 (m, 1H), 3.48 (s, 3H), 3.37 (m, 3H), 3.19 (m, 2H), 2.80 (m, 1H), 2.66 (m, 1H), 2.08 (m, 3H); IR (neat): 1742, 1652 cm^{-1}; FABMS (*m/z*) 394 (M+H)$^+$. Anal. Calcd ($C_{19}H_{23}NO_4S_2$): C, 57.99; H, 5.89; N, 3.56. Found: C, 57.78; H, 6.12; N, 3.42.

3.2.5. (±)-(*rel*-1*R*,2*R*/*S*,5*S*)-3-Acetyl-1,2,3,4,5,6-hexahydro-8-methoxy-1,5-methano-3-benzazocine-2-carboxylic acid methyl ester (11). A solution of **10** (1.37 g, 3.48 mmol) in absolute EtOH (20 mL) was refluxed under argon with two spoonfuls of activated Raney nickel (24 h). The reaction mixture was cooled to room temperature and carefully filtered through celite. The filter pad was washed well with EtOH and the combined filtrate was taken to dryness under vacuum and the residue was purified by silica gel flash chromatography to afford **11** as a foam (997 mg, 95% yield). ^{1}H NMR ($CDCl_3$) (2*S*)-**11**: δ 6.98 (d, H, J=8.4 Hz), 6.64 (m, 2H), 4.87 (d, 1H, J=7.2 Hz), 3.91 (m, 1H), 3.75 (s, 3H), 3.58 (s, 3H), 3.55 (m, 1H), 3.22 (m, 1H), 2.91 (m, 1H), 2.60 (m 2H), 2.05 (s, 2.4H), 1.78 (m, 2H), 1.63 (s, 0.6H). (2*R*)-**11**: δ 7.0 (m, 1H), 6.62 (m, 1H), 6.57 (m, 1H), 5.08 (s, 0.6H), 4.57 (d, 0.4H, J=13.7 Hz), 4.20 (s, 0.4H), 3.90 (m, 7.4H), 3.43 (m, 1H), 3.07 (m, 0.7H), 2.98 (m, 1.H), 2.70 (m, 1H), 2.17 (m, 1H), 1.98 (s, 1.8H), 1.90 (m, 0.5H), 1.78 (m, 1.5H), 1.46 (s, 1.2H); IR(neat): 2933,

1736, 1650, 1423, 1233 cm^{-1}; FABMS (*m/z*) 304 $(M+H)^+$. Anal. Calcd ($C_{17}H_{21}NO_4 \cdot 0.5H_2O$): C, 65.37; H, 6.78; N, 4.48. Found: C, 65.38; H, 7.05; N, 4.30.

3.2.6. (±)-(*rel*-1*R*,2*R*,5*S*)-3-Acetyl-1,2,3,4,5,6-hexahydro-8-methoxy-1,5-methano-3-benzazocine-2-carboxylic acid methyl ester (11a). To a solution of **11** (1.35 g, 4.45 mmol) in MeOH (5 mL) was added 2 M NH_3 in MeOH (5 mL) and the mixture was stirred at room temperature (24 h). Removal of solvent under vacuum provided **11a** in quantitative yield. Confirmation of the relative stereochemistries was achieved by single crystal X-ray crystallography as described previously in Section 3 and as shown in Fig. 2C.

3.2.7. (±)-(*rel*-1*R*,2*R*,5*S*)-3-Acetyl-1,2,3,4,5,6-hexahydro-8-hydroxy-1,5-methano-3-benzazocine-2-carboxylic acid methyl ester (12). A solution of **11a** (715 mg, 2.36 mmol) in anhydrous CH_2Cl_2 (6 mL) at −78°C was treated with BBr_3, 1.0 M in ether (4.6 mmol) (1 h) and then warmed to 0°C and stirred (3 h). Excess BBr_3 was destroyed by addition of MeOH, then solvent was removed under vacuum and the residue was purified by silica gel flash chromatography to provide **12** as a solid (584 mg, 86% yield): mp 117.5–119.0°C. ^1H NMR ($CDCl_3$) [Note: *N*-Acetyl rotomers, observed in an approximate 4:6 ratio, resulted in partial proton counts for certain peaks.] δ 6.92 (t, 1H, *J*=8.1 Hz), 6.53 (dd, 0.4H, *J*=2.4 and 8.3 Hz), 6.45 (d, 0.6H, *J*=2.4 Hz), 6.40 (dd, 0.6H, *J*=2.4 and 8.3 Hz), 6.34 (d, 0.4H, *J*=2.4 Hz), 5.18 (d, 0.6H, *J*=2.4 Hz), 4.68 (dd, 0.4H, *J*=1.5 and 13.7 Hz), 4.20 (s, 0.4H), 3.76 (d, 0.6H, *J*=13.7 Hz), 3.77 (s, 1H), 3.73 (s, 2H), 3.67 (m, 1H), 3.44 (m, 1H), 3.0 (m, 1H), 2.68 (m, 1H), 2.14 (m, 1H), 1.96 (s, 2H), 1.90 (m, 0.6H), 1.81 (m, 1.4H), 1.47 (s, 1H), 1.20 (s, 1H); IR (neat): 3212, 1738, 1614, 1434, 1207 cm^{-1}; FABMS (*m/z*) 290 $(M+H)^+$. Anal. Calcd ($C_{16}H_{19}NO_4$): C, 66.42; H, 6.62; N, 4.84. Found: C, 66.29; H, 6.77; N, 4.80.

3.2.8. (±)-(*rel*-1*R*,2*R*,5*S*)-3-Acetyl-1,2,3,4,5,6-hexahydro-8-(di-(*tert*-butyl)phosphoryloxy)-1,5-methano-3-benzazocine-2-carboxylic acid methyl ester (13). To a solution of **12** (130 mg, 0.45 mmol) in anhydrous THF (3 mL) was added tetrazole (97 mg, 1.35 mmol) and di-(*tert*-butyl) diisopropylphosphoramidite at room temperature under argon and the mixture was stirred (overnight). The mixture was cooled to 0°C and a solution of 14% *tert*-butyl hydroperoxide was added slowly and the mixture was stirred (30 min), then the reaction was quenched by addition of saturated aqueous Na_2SO_3. The mixture was extracted (CH_2Cl_2) and the combined organic extracts were washed with H_2O and brine and dried ($MgSO_4$). Concentration under vacuum provided a residue, which was purified by silica gel flash chromatography to afford **13** as an oil (103 mg, 47% yield). ^1H NMR ($CDCl_3$) δ 7.11 (m, 1H), 6.97 (m, 2H), 5.11 (d, 0.6H, *J*=2.2 Hz), 4.64 (d, 0.4H, *J*=13.7 Hz), 4.36 (s, 0.4H), 3.82 (s, 1H), 3.80 (d, 0.6H, *J*=13.8 Hz), 3.79 (s, 3H), 3.73 (m, 0.6H), 3.72 (s, 1H), 3.54 (d, 1H, *J*=15.1 Hz), 3.14 −3.02 (m, 1H), 2.77 (t, 1H, 21.2 Hz), 2.23 (m, 1H), 2.01 (s, 2H), 1.94 (m, 1H), 1.85 (m, 1H), 1.57 (s, 1H), 1.55 (s, 18H); IR (neat): 2979, 2928, 1740, 1652, 991 cm^{-1}; FABMS (*m/z*) 482 $(M+H)^+$. Anal. Calcd ($C_{24}H_{36}NO_7P$): C, 59.86; H, 7.54; N, 2.91. Found: C, 59.88; H, 7.82; N, 2.85.

3.2.9. (±)-(*rel*-1*R*,2*R*,5*S*)-3-Acetyl-1,2,3,4,5,6-hexahydro-1,5-methano-8-phosphoryloxy-3-benzazocine-2-carboxylic acid methyl ester (3c). To a solution of **13** (97 mg, 0.2 mmol) in anhydrous CH_2Cl_2 (4 mL) at room temperature under argon was added a solution of CF_3CO_2H (1.0 mL) in H_2O (0.2 mL) and the mixture was stirred (1 h). Solvent was removed under vacuum and residue was purified by preparative HPLC (Advantage J-sphere ODS-H80 column; flow=10 mL/min; gradient elution 10–25% B over 20 minutes; retention time=24.1 min) to provide **3c** as a white solid (75 mg, 92% yield); m.p 232°C (dec.). ^1H NMR ($CDCl_3$) δ 7.16 (m, 1H), 6.93 (m, 2H), 5.02 (s, 0.6H), 4.51 (s, 0.4H), 4.50 (d, 0.4H, *J*=9.8 Hz), 3.86 (0.6H), 3.80 (s, 1H), 3.77 (s, 2H), 3.58 (m, 2H), 3.28 (m, 2H), 3.07 (m, 1H), 2.76 (m, 1H), 2.20 (m, 1H), 1.98 (s, 2H), 1.86 (m, 2H), 1.67 (s, 1H); IR (neat): 2947, 1737, 1490, 962 cm^{-1}; FABMS (*m/z*) 368 $(M-H)^-$. Anal. Calcd ($C_{16}H_{20}NO_7P \cdot 0.2CF_3CO_2H$): C, 50.23; H, 5.19; N, 3.57. Found: C, 50.35; H, 5.17; N, 3.60.

Acknowledgements

Appreciation is expressed to Dr James Kelley of the LMC for mass spectral analysis and to the Komen Foundation for Breast Cancer for financial support (to D. Y.). Work was supported in part (for C. G.) by the Office of Naval Research.

References

1. A preliminary account of this work has been presented:Yao, Z. J.; Barchi, J. J.; Burke, T. R., Jr. *Peptides for the Millenium: Proceedings of the 16th American Peptide Symposium*; Fields, G. B., Tam, J. P., Barany, G., Eds.; Kluwer: Dordrecht, 2000; pp 575–578.
2. Tsatsanis, C.; Spandidos, D. A. *Int. J. Mol. Med.* **2000**, *5*, 583–590.
3. Renhowe, P. A. *Annual Reports on Medicinal Chemistry*; Doherty, A. M., Ed.; Academic: London, UK, 2001; Vol. 36, pp 109–118.
4. Morin, M. J. *Oncogene* **2000**, *19*, 6574–6583.
5. Levitzki, A. *Top. Curr. Chem.* **2000**, *211*, 1–15.
6. Sawyers, C. L. *Curr. Opin. Genet. Dev.* **2002**, *12*, 111–115.
7. Traxler, P.; Bold, G.; Buchdunger, E.; Caravatti, G.; Furet, P.; Manley, P.; O'Reilly, T.; Wood, J.; Zimmermann, J. *Med. Res. Rev.* **2001**, *21*, 499–512.
8. Pawson, T.; Gish, G. D.; Nash, P. *Trends Cell Biol.* **2001**, *11*, 504–511.
9. Garcia-Echeverria, C. *Curr. Med. Chem.* **2001**, *8*, 1589–1604.
10. Sawyer, T. K.; Bohacek, R. S.; Dalgarno, D. C.; Eyermann, C. J.; Kawahata, N.; Metcalf, C. A., III; Shakespeare, W. C.; Sundaramoorthi, R.; Wang, Y.; Yang, M. G. *Mini-Rev. Med. Chem.* **2002**, *2*, 475–489.
11. Eck, M. J.; Shoelson, S. E.; Harrison, S. C. *Nature* **1993**, *362*, 87–91.
12. Bradshaw, J. M.; Grucza, R. A.; Ladbury, J. E.; Waksman, G. *Biochemistry* **1998**, *37*, 9083–9090.
13. Bradshaw, J. M.; Mitaxov, V.; Waksman, G. *J. Mol. Biol.* **1999**, *293*, 971–985.

14. Burke, T. R., Jr.; Yao, Z.-J.; Smyth, M. S.; Ye, B. *Curr. Pharm. Des.* **1997**, *3*, 291–304.
15. Burke, T. R., Jr.; Yao, Z.-J.; Liu, D.-G.; Voigt, J.; Gao, Y. *Biopolymers* **2001**, *60*, 32–44.
16. Burke, T. R., Jr.; Lee, K. *Acc. Chem. Res.* **2003**, *36*, 426–433.
17. Liu, W. Q.; Carreaux, F.; Meudal, H.; Roques, B. P.; Garbay-Jaureguiberry, C. *Tetrahedron* **1996**, *52*, 4411–4422.
18. Davidson, J. P.; Martin, S. F. *Tetrahedron Lett.* **2000**, *41*, 9459–9464.
19. Plake, H. R.; Sundberg, T. B.; Woodward, A. R.; Martin, S. F. *Tetrahedron Lett.* **2003**, *44*, 1571–1574.
20. Liu, D-G.; Wang, X-Z.; Gao, Y.; Li, B.; Yang, D.; Burke, T. R., Jr. *Tetrahedron* **2002**, *58*, 10423–10428.
21. Burke, T. R., Jr.; Barchi, J. J., Jr.; George, C.; Wolf, G.; Shoelson, S. E.; Yan, X. *J. Med. Chem.* **1995**, *38*, 1386–1396.
22. Ye, B.; Yao, Z.-J.; Burke, T. R., Jr. *J. Org. Chem.* **1997**, *62*, 5428–5431.
23. Arend, M.; Westermann, B.; Risch, N. *Angew. Chem. Int. Ed.* **1998**, *37*, 1044–1070.
24. Muller, R.; Rottele, H.; Henke, H.; Waldmann, H. *Chem. Eur. J.* **2000**, *6*, 2032–2043.
25. Martin, S. F. *Acc. Chem. Res.* **2002**, *35*, 895–904.
26. List, B. *Tetrahedron* **2002**, *58*, 5573–5590.
27. Padwa, A.; Heidelbaugh, T. M.; Kuethe, J. T.; McClure, M. S.; Wang, Q. *J. Org. Chem.* **2002**, *67*, 5928–5937.
28. Tanino, K.; Takahashi, M.; Murayama, K.; Kuwajima, I. *J. Org. Chem.* **1992**, *57*, 7009–7010.
29. Takahashi, M.; Tanino, K.; Kuwajima, I. *Chem. Lett.* **1993**, 1655–1658.
30. Rahuel, J.; Gay, B.; Erdmann, D.; Strauss, A.; Garcia-Echeverria, C.; Furet, P.; Caravatti, G.; Fretz, H.; Schoepfer, J.; Grutter, M. G. *Nature Struct. Biol.* **1996**, *3*, 586–589.
31. Wei, C-Q.; Li, B.; Guo, R.; Yang, D.; Burke, T. R., Jr. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2781–2784.
32. Gao, Y.; Voigt, J.; Wu, J. X.; Yang, D.; Burke, T. R., Jr. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1889–1892.
33. Wei, C-Q.; Gao, Y.; Lee, K.; Guo, R.; Li, B.; Zhang, M.; Yang, D.; Burke, T. R., Jr. *J. Med. Chem.* **2003**, *46*, 244–254.
34. Ettmayer, P.; France, D.; Gounarides, J.; Jarosinski, M.; Martin, M-S.; Rondeau, J. M.; Sabio, M.; Topiol, S.; Weidmann, B.; Zurini, M.; Bair, K. W. *J. Med. Chem.* **1999**, *42*, 971–980.
35. Davidson, J. P.; Lubman, O.; Rose, T.; Waksman, G.; Martin, S. F. *J. Am. Chem. Soc.* **2002**, *124*, 205–215.
36. Liu, L.; Guo, Q-X. *Chem. Rev.* **2001**, *101*, 673–695.
37. Sharp, K. *Protein Sci.* **2001**, *10*, 661–667.
38. Dekker, F. J.; de Mol, N. J.; Bultinck, P.; Kemmink, J.; Hilbers, H. W.; Liskamp, R. M. *J. Bioorg. Med. Chem.* **2003**, *11*, 941–949.
39. SMART and SAINT data collection and reduction software for the SMART system. Bruker-AXS, Madison, Wisconsin, USA, 1995.
40. Sheldrick, G. M. *SHELXTL Version 5.1*. Bruker Analytical X-ray Instruments: Madison, 1997.