Brief Articles

Design and Synthesis of 4-(α-Hydroxymalonyl)phenylalanine as a New Phosphotyrosyl Mimetic and Its Use in Growth Factor Receptor Bound 2 Src-Homology 2 (Grb2 SH2) Domain-Binding Peptides

Sang-Uk Kang,[†] Karen M. Worthy,[‡] Lakshman K. Bindu,[‡] Manchao Zhang,[§] Dajun Yang,[§] Robert J. Fisher,[‡] and Terrence R. Burke, Jr.*,[†]

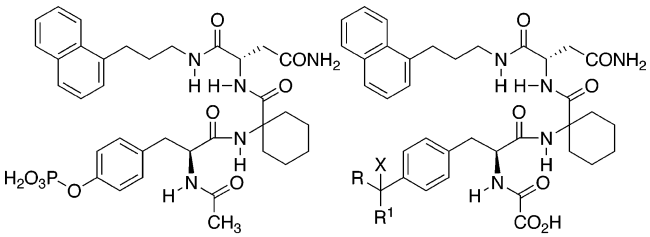
Laboratory of Medicinal Chemistry, CCR, National Cancer Institute, National Institutes of Health, Frederick, Maryland 21702, Protein Chemistry Laboratory, SAIC—Frederick, Frederick, Maryland 21702, and University of Michigan Medical School, Ann Arbor, Michigan 48109

Received February 17, 2005

A new phosphotyrosyl mimetic 4-(α-hydroxymalonyl)phenylalanine and its incorporation into a Grb2 SH2 domain-binding tripeptide are presented. In whole-cell studies using malonyl ethyl ester prodrug derivatives, it was observed that the 4-(α-hydroxymalonyl)phenylalanyl-containing peptide exhibited greater efficacy than the nonhydroxylated 4-(malonyl)phenylalanyl-containing congener in blocking the association of Grb2 with activated erbB-2 tyrosine kinase. These results are consistent with de-esterification and at least partial intracellular decarboxylation.

Introduction

Phosphotyrosyl (pTyr) mimetics are important components of binding antagonists directed against the growth factor receptor bound 2 (Grb2)[1] SH2 domain.[2] Compound **1** represents a pTyr-containing tripeptide that was originally reported by Novartis Corp. as exhibiting high-affinity Grb2 domain-binding affinity (Figure 1).[3] The related structure **2** was developed in which the phosphoryl group has been replaced by a malonyl moiety.[4,5] However, a drawback of the malonyl-based phosphoryl mimetic is its susceptibility to chemical decarboxylation leading to the less potent analogue **3**.[6] Indeed, under slightly acidic conditions appreciable conversion of **2** to **3** is observed over the course of 24 h.[7] On the basis of work in a $p56^{lck}$ SH2 domain-binding system that showed conversion of a 4-(carboxymethyl)-phenylalanyl pTyr mimetic to 4-(carboxy(α-hydroxymethyl))phenylalanyl residue resulting in a doubling of affinity,[8] it was felt that the hydroxyl-containing **4** could potentially exhibit higher affinity than **3** in a Grb2 SH2 domain-binding system. Furthermore, it was hoped that the binding affinity of 4-(α-hydroxymalonyl)phenylalanyl-containing **5**, which would yield **4** following decarboxylation, would be equivalent to or greater than 4-(malonyl)phenylalanyl-containing **2**. The current study was undertaken to prepare **5** and **4** and to compare their affinities with **2** and **3**. Additionally, ethyl esters **6** and **7** were prepared as potential prodrugs of **2** and **5** to examine efficacy in a whole-cell system.



1

2 R = R^1 = CO_2H; X = H
3 R = CO_2H; R^1 = X = H
4 R = CO_2H; R^1 = H; X = OH
5 R = R' = CO_2H; X = OH
6 R = R^1 = CO_2Et; X = H
7 R = R^1 = CO_2Et; X = OH

Figure 1. Structures of Grb2 SH2 domain-directed analogues discussed in the text.

Synthesis

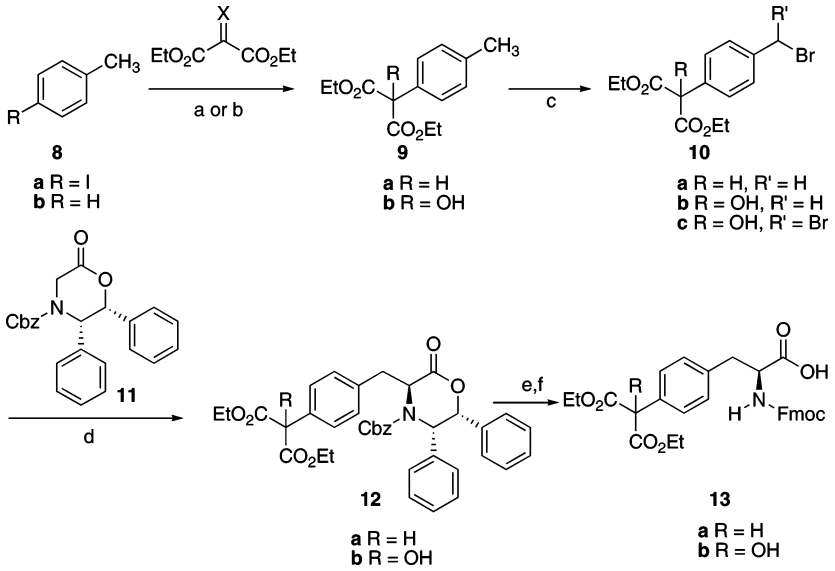
Of compounds **2**−**7** that are required for the current study, **2** and **3** have been previously reported[4−6] while analogues **4**−**7** are new. Because **5** can be prepared from **7** by treatment with aqueous LiOH and because **4** can be derived from **5** by decarboxylation in DMSO, analogues **6** and **7** represent pivotal objectives to the overall study. Key to the syntheses of **6** and **7** are the *N*-Fmoc-protected pTyr mimetics **13a** and **13b**, respectively. These were prepared by nearly identical routes from intermediates **9a** and **9b**, which were derived from 4-iodotoluene (**8a**) by reaction of diethyl malonate and sodium hydride in the presence of CuBr (for **9a**) or from alkylation of toluene (**8b**) with diethyl ketomalonate and $SnCl_4$ (for **9b**) (Scheme 1).[9]

Benzylic bromination of **9a** to **10a** was readily achieved in 78% yield; however, similar bromination of **9b** gave an inseparable mixture of monobrominated (**10b**) and dibrominated product **10c** in a 10:3 ratio. Alkylation of

* To whom correspondence should be addressed. Phone: (301) 846-5906. Fax: (301) 846-6033. E-mail: tburke@helix.nih.gov.
[†] National Cancer Institute.
[‡] SAIC—Frederick.
[§] University of Michigan Medical School.

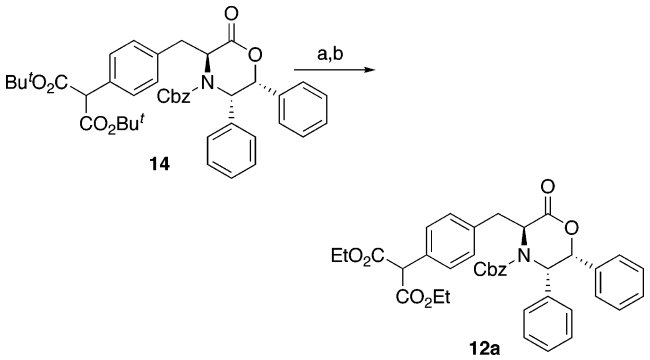
10.1021/jm050154v CCC: $30.25
Published on Web 07/12/2005

Scheme 1[a]



[a] Reagents and conditions: (a) [for R = I, X = H, H] CuBr, NaH, HMPA, dioxane; (b) [for R = H, X = O] $SnCl_4$; (c) NBS, AIBN, [for R = H] CH_2Cl_2, reflux, [for R = OH] CCl_4; (d) LHMDS, HMPA, THF, −78 °C to room temperature; (e) H_2, Pd−C, MeOH; (f) Fmoc-OSu, $NaHCO_3$, H_2O, dioxane.

Scheme 2[a]



[a] Reagents and conditions: (a) TFA, $SiEt_3$; (b) EtOH, H_2SO_4, 80 °C.

this mixture with Williams lactone **11**[10] allowed ready separation of the desired **12b** in 30% overall yield from **9b**. Similar alkylation of **10a** gave the lactone adduct **12a** in extremely low yield (14%). Since the reaction proceeds in higher yield when the malonyl moiety bears bis-^{t}Bu ester protection,[5] the known ^{t}Bu-protected **14** was prepared and converted to desired diethyl ester **12a** (Scheme 2). Finally, hydrogentation of lactones **12a** and **12b** gave the corresponding free amino acids, which were converted to the protected pTyr mimetics **13a** and **13b**, respectively, by treatment with Fmoc-OSu. Synthesis of final products **6** and **7** was by known methods.[11]

Grb2 SH2 Domain-Binding Affinities in Extracellular Assays

Determination of Grb2 SH2 domain-binding affinities by surface plasmon resonance (SPR) was accomplished using Biacore instruments as previously described.[11] Recombinant Grb2 SH2 domain protein was coupled to sensor chips, and steady-state equilibrium constants were determined for binding of ligands in solution to the chip-bound protein (Table 1). Reference compound **1** provided a K_{eq} of 0.240 nM, which was somewhat higher than what had previously been reported using an ELISA assay (IC_{50} = 0.047 μM).[3] The binding affinity of parent malonyl-containing **2** (K_{eq} = 0.149 μM) was also higher than what had previously been reported by ELISA techniques (IC_{50} = 0.07 μM).[4] The corresponding decarboxylation product **3** exhibited less affinity in the current assay (K_{eq} = 6.11 μM) than previously reported using an ELISA assay (IC_{50} = 1 μM)[4] or using a Biacore instrument, in which inhibitor in solution competed with chip-bound pTyr-containing peptide for binding to Grb2 SH2 domain protein in solution (IC_{50} = 0.6 μM).[6] Introduction of a hydroxyl group onto the malonyl methylene of **2** resulted in a nearly 2-fold reduction in binding affinity (**5**, K_{eq} = 0.270 μM). However the corresponding decarboxylation product (**4**, K_{eq} = 1.54 μM) was approximately 4-fold more potent than the congener **3**, which lacked the hydroxyl group.

Table 1. SPR-Derived Steady-State Affinity Constants for Binding to Grb2 SH2 Domain Protein

compd	K_{eq} (μM)
1	0.240[a,b]
2	0.149[a,c]
3	6.11[d,e]
4	1.54
5	0.270

[a] Complex kinetics were observed. [b] ELISA IC_{50} value previously reported as 0.047 μM in ref 3. [c] ELISA IC_{50} value previously reported as 0.07 μM in ref 4. [d] ELISA IC_{50} value previously reported as 1 μM in ref 4. [e] SPR-derived IC_{50} value previously reported as 0.6 μM in ref 6.

Inhibition by Ester Prodrugs 6 and 7 of Grb2 Binding to p185^{erbB-2} in MDA-MB-453 Breast Cancer Cells in Culture

By use of previously reported procedures,[4] prodrugs **6** and **7** were examined in cultured MDA-MB-453 breast cancer cells overnight. It was found that nonhydroxylated **6** is less potent than the hydroxyl-containing **7** in blocking the intracellular association of Grb2 with the p185^{erbB-2} tyrosine kinase (Figure 2). These results are the reverse of what would be expected on the basis of

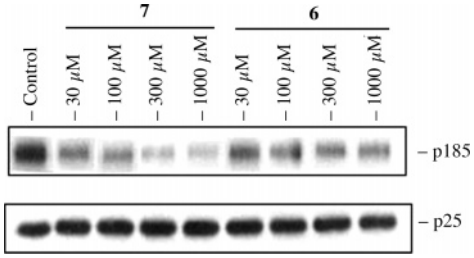


Figure 2. SDS gels showing the inhibition of binding of $p185^{erbB-2}$ to Grb2 (p25) by analogues **5** and **2** in whole cells. Experiments were conducted as described in ref 4. The p185 bands were obtained by immunopreciptating with Grb2 antibodies and immunoblotting for $p185^{erbB-2}$. The intensity of the p25 bands, which were obtained by immunopreciptating with Grb2 antibodies and immunoblotting for Grb2, indicates uniform expression of Grb2 at each concentration of inhibitor.

the Grb2 SH2 domain-binding potencies of the free malonyl-containing species **2** and **5**, respectively. One possible explanation for the observed results is that at least a component of the active intracellular species is the monocarboxy analogues **3** and **4**, which could arise through de-esterification and intracellular decarboxylation.

Conclusion

The current study presents a new α-hydroxymalonyl-containing phosphotyrosyl mimetic and its incorporation into a Grb2 SH2 domain-binding peptide. It was rationalized that introduction of a hydroxyl group into the malonyl methylene could potentially enhance the binding affinity of the decarboxylation product. Indeed, for the decarboxylation products, a 4-fold enhancement of binding affinity was observed for α-hydroxyl-containing **4** relative to its nonhydroxylated counterpart **3**. It was also hoped that the parent α-hydroxymalonyl-containing species (**5**) would exhibit binding affinity equal to or greater than its nonhydroxylated counterpart (**2**). However, it was found that **2** displayed an approximate 2-fold higher affinity than **5**. Nonetheless, in whole-cell studies using ethyl ester prodrug derivatives of **2** and **5**, it was observed that the hydroylated species exhibited higher efficacy than the nonhydroxylated species in blocking the association of Grb2 with activated erbB-2 tyrosine kinase.

Experimental Section

Diethyl 2-(4-Methylphenyl)propane-1,3-dioate (9a). To a stirred suspension of NaH (2.2 g, 55 mmol) in 200 mL of dioxane at 0 °C was slowly added diethyl malonate (7.6 mL, 50 mmol). The mixture was stirred at room temperature (30 min). Then CuBr (8.6 g, 60 mmol) and 4-iodotoluene (16.4 g, 75 mmol) were added, and the mixture was heated to 95−98 °C (1 h). Then HMPA (5 mL) was added and stirring continued (9 h). Inorganics were removed by filtration, volatiles were evaporated under reduced pressure, and residue was purified by silica gel flash chromatography to provide **9a** as a colorless oil (4.38 g, 35% yield). ^{1}H NMR ($CDCl_3$) δ 7.28 (2 H, d, J = 8.2 Hz), 7.16 (2 H, d, J = 8.0 Hz), 4.57 (1 H, s), 4.15−4.27 (4 H, m), 2.34 (3 H, s), 1.26 (6 H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ 185.7, 168.5, 138.2, 130.1, 129.5, 129.3, 61.9, 57.8, 21.3, 14.2. FAB-MS (+VE) *m/z* 251 (MH^+). Anal. ($C_{14}H_{18}O_4$) C, H, O.

Diethyl 2-Hydroxy-2-(4-methylphenyl)propane-1,3-dioate (9b). To a solution of diethyl ketomalonate (15.4 mL, 100 mmol) in toluene (53.3 mL, 500 mmol) at 0 °C was added $SnCl_4$ (14 mL, 120 mmol) over 20 min, and the mixture was stirred at 0 °C (10 min). Then the ice bath was removed, and stirring continued at room temperature (2 h). The mixture was poured into ice-cold dilute aqueous HCl, extracted with $CHCl_3$, and dried ($MgSO_4$). Residue was purified by silica gel flash chromatography to give **9b** as a colorless oil (99% yield). ^{1}H NMR ($CDCl_3$) δ 7.53 (2 H, d, J = 8.2 Hz), 7.18 (2 H, d, J = 8.6 Hz), 4.30 (4 H, m), 2.35 (3 H, s), 1.29 (6 H, t, J = 7.22 Hz). ^{13}C NMR ($CDCl_3$) δ 170.2, 138.6, 133.2, 128.9, 126.7, 80.1, 63.1, 21.3, 14.1. FAB-MS (+VE) *m/z* 267 (MH^+). Anal. ($C_{14}H_{18}O_5$) C, H, O.

Diethyl 2-[4-(Bromomethyl)phenyl]propane-1,3-dioate (10a). To a stirred solution of **9a** (3.0 g, 12.0 mmol) in CH_2Cl_2 (100 mL) were added *N*-bromosuccinimide (NBS) (2.2 g, 12.2 mmol) and AIBN (197 mg, 1.2 mmol), and the mixture was stirred at 85 °C (1 h). Additional AIBN (100 mg, 0.6 mmol) was added, and stirring continued at 85 °C (1 h). The mixture was cooled to room temperature, volatiles were removed by rotary evaporation, and the residue was purified by silica gel flash to give **10a** as a colorless oil (3 g, 78% yield based on a 5% yield of recovered **9a**). ^{1}H NMR ($CDCl_3$) δ 7.39 (4 H, s), 4.61 (1 H, s), 4.48 (2 H, s), 4.15−4.28 (4 H, m), 2.34 (3 H, s), 1.26 (6 H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ 185.7, 168.1, 137.9, 133.2, 129.9, 129.5, 62.1, 57.8, 33.1, 14.2. FAB-MS (+VE) *m/z* 329 (MH^+). Anal. ($C_{14}H_{17}BrO_4 \cdot 0.1H_2O$) C, H.

Diethyl 2-[4-({(3*S*,5*S*,6*R*)-2-Oxo-5,6-diphenyl-4-[benzyloxycarbonyl]morpholin-3-yl}methyl)phenyl]propane-1,3-dioate (12a). To a stirred solution of Williams lactone **11** (3.6 g, 9.36 mmol) in anhydrous THF (200 mL) with HMPA (10 mL) at −78 °C was added 1 M LHMDS in hexanes (9.36 mL, 9.36 mmol), and the mixture was stirred at −78 °C (30 min). To this was transferred via cannula a precooled solution of **10a** (2.8 g, 8.5 mmol) in THF (50 mL). Then cooling was removed, and the mixture was stirred at room temperature (1 h). The mixture was poured into cold aqueous NH_4Cl solution and extracted (EtOAc), the combined extracts were dried ($MgSO_4$), and solvent was removed by rotary evaporation. Purification by silica gel flash chromatography gave **12a** as a colorless oil (750 mg, 14% yield) (see below for spectral data).

Synthesis of 12a from 14. To a stirred solution of **14** (346 mg, 0.5 mmol) in CH_2Cl_2 (8 mL) at 0 °C was added $SiEt_3$ (1 mL) and TFA (2 mL). Then the mixture was stirred at room temperature (2 h). Solvent was removed by rotary evaporation, and residue was placed under high vacuum. Then the residue was dissolved in EtOH (5 mL), concentrated H_2SO_4 (2 drops) was added, and the mixture was refluxed (2 h). The mixture was cooled to 0 °C, acid was neutralized by addition of saturated aqueous $NaHCO_3$, and the mixture was extacted with EtOAc. The combined organic phase was purified by silica gel flash chromatography to give **12a** (76 mg, 24% yield) as a colorless oil along with decarboxylated material (130 mg, 46% yield). ^{1}H NMR ($CDCl_3$) (two conformers were observed in a ratio of 1:0.5): major conformer, δ 7.41 (2 H, d, J = 8.0 Hz), 7.24 (2 H, d, J = 8.0 Hz), 7.04−7.21 (10 H, m, overlapping), 6.46−6.81 (5 H, m, overlapping), 5.34 (1H, dd, J = 2.7 and 6.1 Hz, −NCHCOO−), 4.96−5.11 (2 H, m, $-OCH_2Ph$, overlapping), 4.80 (1 H, d, J = 3.1 Hz, −CHPhOOC−), 4.60 (1 H, s, $CH(CO_2Et)_2$), 4.10−4.28 (5 H, m, −NCHPh−, $(OCH_2CH_3) \times 2$, overlapping), 3.75(1 H, dd, J = 6.1 and 13.7 Hz, one of $-CH_2$-CH(N)COO−), 3.39 (1 H, dd, J = 2.9 and 13.7 Hz, the other of $-CH_2CH(N)COO-$, overlapping), 1.26 (3 H, m, OCH_2CH_3, overlapping), 1.15−1.19 (3 H, m, OCH_2CH_3, overlapping); minor conformer, δ 7.34 (2H, d, J = 8.0 Hz), 7.04−7.21 (12 H, m, overlapping), 6.46−6.81 (5 H, m, overlapping), 5.24 (1 H, dd, J = 3.1 and 6.4 Hz, −NCHCOO−), 4.96−5.11 (3 H, m, $-OCH_2Ph$, CHPhOOC−, overlapping), 4.58 (1 H, s $CH(CO_2Et)_2$), 4.36 (1 H, d, J = 3.1 Hz, NCHPh−,), 4.10−4.30 (4 H, m, $(OCH_2CH_3) \times 2$, overlapping), 3.54 (1 H, dd, J = 7.0 and 13.9 Hz, one of $-CH_2CH(N)COO-$), 3.34 (1 H, dd, J = 3.3 and 13.9 Hz, $CH_2CH(N)COO-$), 1.26 (3 H, m, OCH_2CH_3, overlapping), 1.15−1.19 (3 H, m, OCH_2CH_3, overlapping). FAB-MS (+VE) *m/z* 636 (MH^+). Anal. ($C_{38}H_{37}NO_8 \cdot 0.2H_2O$) C, H, N.

Diethyl 2-[4-({(3*S*,5*S*,6*R*)-2-Oxo-5,6-diphenyl-4-[benzyloxycarbonyl]morpholin-3-yl}methyl)phenyl]-2-hydroxypropane-1,3-dioate (12b). A mixture of **9b** (2.66 g, 10 mmol),

NBS (1.87 g, 10.5 mmol), and AIBN (164 mg, 1 mmol) in CCl_4 (50 mL) was stirred at reflux (1 h). Then the mixture was cooled to room temperature and evaporated to dryness and the residue was purified by silica gel flash chromatography to yield **10a** contaminated with dibrominated byproduct. To a stirred solution of Williams lactone **11** (5 g, 13 mmol) in anhydrous THF (120 mL) with HMPA (15 mL) at −78 °C was added 1 M LHMDS in THF (15 mL, 15 mmol). The mixture was stirred at −78 °C and then transferred via cannula to a stirred solution of crude **10a** in THF (40 mL) at −78 °C. Cooling was removed, and the mixture was stirred at room temperature (16 h) and then poured into cold aqueous NH_4Cl solution. The mixture was extracted (EtOAc), and the combined organic phase was dried ($MgSO_4$) and reduced in volume to give starting Williams lactone **11** as a solid, which was removed by filtration. The filtrate was taken to dryness and purified by silica gel flash chromatography to give **12b** as a white solid (30% yield over two steps). Mp 53 °C. ^{1}H NMR ($CDCl_3$) (two conformers were observed in a ratio of 1:0.5): major conformer, δ 7.69 (2 H, d, J = 8.4 Hz), 7.36−7.45 (2 H, m, overlapping), 7.04−7.28 (10 H, m, overlapping), 6.46−6.81 (5 H, m, overlapping), 5.35 (1 H, m, −NCHCOO−), 5.03 (2 H, m, $-OCH_2Ph$, overlapping), 4.79 (1 H, d, J = 3.1 Hz, −CHPhOOC−), 4.07 (1H, d, J = 2.9 Hz, −NCHPh−), 3.93−4.40 (4 H, m, (OCH_2CH_3) × 2, overlapping), 3.77 (1 H, dd, J = 6.1 and 13.7 Hz, one of $-CH_2CH(N)COO-$), 3.35−3.44 (1 H, m, $-CH_2CH(N)COO-$, overlapping), 1.22−1.32 (3 H, m, OCH_2CH_3, overlapping), 1.06−1.12 (3 H, m, OCH_2CH_3, overlapping); minor conformer, δ 7.62 (2 H, d, J = 8.4 Hz), 7.36−7.45 (2 H, m, overlapping), 7.04−7.28 (10 H, m, overlapping), 6.46−6.81 (5 H, m, overlapping), 5.27 (1 H, m, −NCHCOO−), 5.03 (2 H, m, $-OCH_2Ph$, overlapping), 4.95 (1 H, d, J = 2.9 Hz, −CHPhOOC−), 3.93−4.40 (5 H, m, −NCHPh−, (OCH_2CH_3) × 2, overlapping), 3.57 (1 H, dd, J = 6.4 and 13.7 Hz, one of $-CH_2CH(N)COO-$), 3.35−3.44 (1 H, m, $-CH_2CH(N)COO-$, overlapping), 1.22−1.32 (3 H, m, OCH_2CH_3, overlapping), 1.06−1.12 (3 H, m, OCH_2CH_3, overlapping). FAB-MS (+VE) *m/z* 652 (MH^+).

(2*S*)-3-{4-[Bis(ethoxycarbonyl)methyl]phenyl}-2-[(fluoren-9-ylmethoxy)carbonylamino]propanoic Acid (13a). A solution of **12a** (636 mg, 1.0 mmol) in MeOH (10 mL) was hydrogenated over 10% Pd−C (200 mg) using a hydrogen-filled balloon (17 h). Catalyst was removed by filtration through Celite 545, and the filtrate was evaporated to provide the intermediate free amino acid. To this was added dioxane (8 mL) and H_2O (8 mL) along with $NaHCO_3$ (252 mg, 3.00 mmol) and Fmoc-Osu (725 mg, 2.15 mmol), and the mixture was stirred at room temperature (1.5 h). The mixture was diluted with $CHCl_3$, and ice was added. Then dilute HCl was added to attain pH 3. The mixture was extracted well with $CHCl_3$, and the combined organic phase was dried ($MgSO_4$), concentrated, and purified by silica gel flash chromatography to afford **13a** as a solid (284 mg, 52% from **12a**). Mp 137−138 °C. ^{1}H NMR (DMSO-d_6) δ 7.88 (2 H, d, J = 7.4 Hz), 7.71 (1 H, d, J = 8.4 Hz), 7.66 (2H, t, J = 8.2 Hz), 7.38−7.43 (2 H, m), 7.25−7.33 (6 H, m), 4.87 (1 H, s), 4.02−4.25 (8 H, m), 3.08 (1 H, dd, J = 4.4, 14.0 Hz), 2.88 (1 H, dd, J = 10.8, 14.0 Hz), 1.11−1.17 (6 H, m). FAB-MS (+VE) *m/z* 546 (MH^+). Anal. ($C_{38}H_{37}NO_8$) C, H, N.

(2*S*)-3-{4-[Bis(ethoxycarbonyl)hydroxymethyl]phenyl}-2-[(fluoren-9-ylmethoxy)carbonylamino]propanoic Acid (13b). A suspension of **12b** (1.4 g, 2.15 mmol) in MeOH (20 mL) was hydrogenated over 10% Pd−C (280 mg) using a hydrogen-filled balloon (19 h). Catalyst was removed by filtration through Celite 545, and the filtrate was evaporated to provide the intermediate free amino acid. To this was added dioxane (10 mL) and H_2O (10 mL) along with $NaHCO_3$ (541 mg, 6.44 mmol) and Fmoc-Osu (725 mg, 2.15 mmol), and the mixture was stirred at room temperature (20 h). The mixture was diluted with $CHCl_3$, and ice was added followed by dilute HCl to attain pH 3. The mixture was extracted well with $CHCl_3$, and the combined organic phase was dried ($MgSO_4$), concentrated, and purified by silica gel flash chromatography to afford **13b** as a white solid (95% yield from **12b**). Mp 80−82 °C. ^{1}H NMR (DMSO-d_6) δ 7.88 (2 H, d, J = 7.4 Hz), 7.66 (2 H, t, J = 7.2 Hz), 7.28−7.42 (5 H, m), 7.17 (2 H, d, J = 8.4 Hz), 7.07 (1 H, s), 4.26(1 H, m), 4.14 (6 H, m), 3.94 (1 H, m), 3.08 (1 H, dd, J = 3.9, 13.3 Hz), 2.91 (1 H, dd, J = 7.4, 14.2 Hz), 1.15 (6 H, m). FAB-MS (−VE) *m/z* 560 (M − H).

References

(1) Lowenstein, E. J.; Daly, R. J.; Batzer, A. G.; Li, W.; Margolis, B.; Lammers, R.; Ullrich, A.; Skolnik, E. Y.; Barsagi, D.; Schlessinger, J. The SH2 and SH3 domain-containing protein Grb2 links receptor tyrosine kinases to Ras signaling. *Cell* **1992**, *70*, 431−442.

(2) Fretz, H.; Furet, P.; Garcia-Echeverria, C.; Rahuel, J.; Schoepfer, J. Structure-based design of compounds inhibiting Grb2-SH2 mediated protein−protein interactions in signal transduction pathways. *Curr. Pharm. Des.* **2000**, *6*, 1777−1796.

(3) Furet, P.; Gay, B.; Caravatti, G.; Garcia-Echeverria, C.; Rahuel, J.; Schoepfer, J.; Fretz, H. Structure-based design and synthesis of high affinity tripeptide ligands of the Grb2-SH2 domain. *J. Med. Chem.* **1998**, *41*, 3442−3449.

(4) Gao, Y.; Luo, J.; Yao, Z.-J.; Guo, R.; Zou, H.; Kelley, J.; Voigt, J. H.; Yang, D.; Burke, T. R., Jr. Inhibition of Grb2 SH2 domain binding by non-phosphate containing ligands. 2. 4-(2-Malonyl)-phenylalanine as a potent phosphotyrosyl mimetic. *J. Med. Chem.* **2000**, *43*, 911−920.

(5) Gao, Y.; Burke, T. R., Jr. Stereoselective preparation of L-4-(2′-malonyl)phenylalanine suitably protected for Fmoc-based synthesis of potent signal transduction inhibitory ligands. *Synlett* **2000**, 134−136.

(6) Burke, T. R., Jr.; Luo, J.; Yao, Z.-J.; Gao, Y.; Milne, G. W. A.; Guo, R.; Voigt, J. H.; King, C. R.; Yang, D. Monocarboxylic phosphotyrosyl mimetics in the design of Grb2 SH2 domain inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 347−352.

(7) Unreported results.

(8) Beaulieu, P. L.; Cameron, D. R.; Ferland, J. M.; Gauthier, J.; Ghiro, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; Llinas-Brunet, M.; Betageri, R.; Cardozo, M.; Hickey, E. R.; Ingraham, R.; Jakes, S.; Kabcenell, A.; Kirrane, T.; Lukas, S.; Patel, U.; Proudfoot, J.; Sharma, R.; Tong, L.; Moss, N. Ligands for the tyrosine kinase $p56^{lck}$ SH2 domain: Discovery of potent dipeptide derivatives with monocharged, nonhydrolyzable phosphate replacements. *J. Med. Chem.* **1999**, *42*, 1757−1766.

(9) Ghosh, S.; Pardo, S. N.; Salomon, R. G. Ester enolates from a-acetoxy esters. Synthesis of arylmalonic and a-arylalkanoic esters from aryl nucleophiles and α-keto esters. *J. Org. Chem.* **1982**, *47*, 4692−702.

(10) Williams, R. M.; Im, M. N. Asymmetric synthesis of monosubstituted and α,α-disubstituted α-amino acids via diastereoselective glycine enolate alkylations. *J. Am. Chem. Soc.* **1991**, *113*, 9276−86.

(11) Yao, Z. J.; King, C. R.; Cao, T.; Kelley, J.; Milne, G. W. A.; Voigt, J. H.; Burke, T. R. Potent inhibition of Grb2 SH2 domain binding by non-phosphate-containing ligands. *J. Med. Chem.* **1999**, *42*, 25−35.

(12) Oishi, S.; Karki, R. G.; Shi, Z.-D.; Worthy, K. M.; Bindu, L.; Chertov, O.; Esposito, D.; Frank, P.; Gillette, W. K.; Maderia, M. A.; Hartley, J.; Nicklaus, M. C.; Barchi, J. J., Jr.; Fisher, R. J.; Burke, T. R., Jr. Evaluation of macrocyclic Grb2 SH2 domain-binding peptide mimetics prepared by ring-closing metathesis of C-terminal allylglycines with an N-terminal β-vinyl-substituted phosphotyrosyl mimetic. *Bioorg. Med. Chem.* **2005**, *13*, 2431−2438.

JM050154V