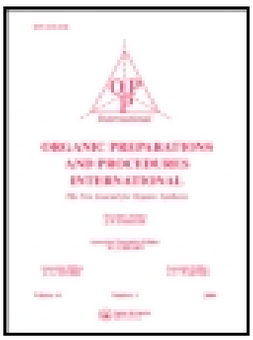

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LARGE SCALE PREPARATION OF CELL PERMEABLE, NON-PHOSPHATE-CONTAINING GRB2 SH2 DOMAIN INHIBITORS

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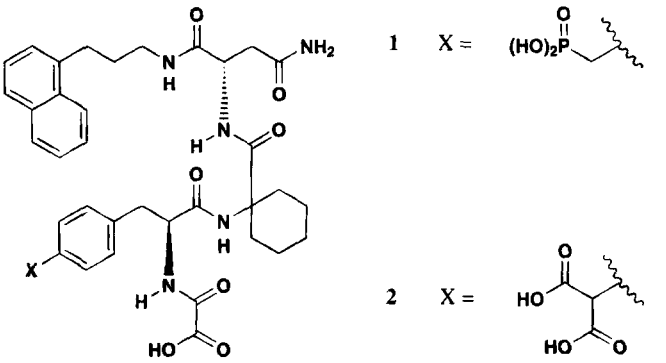
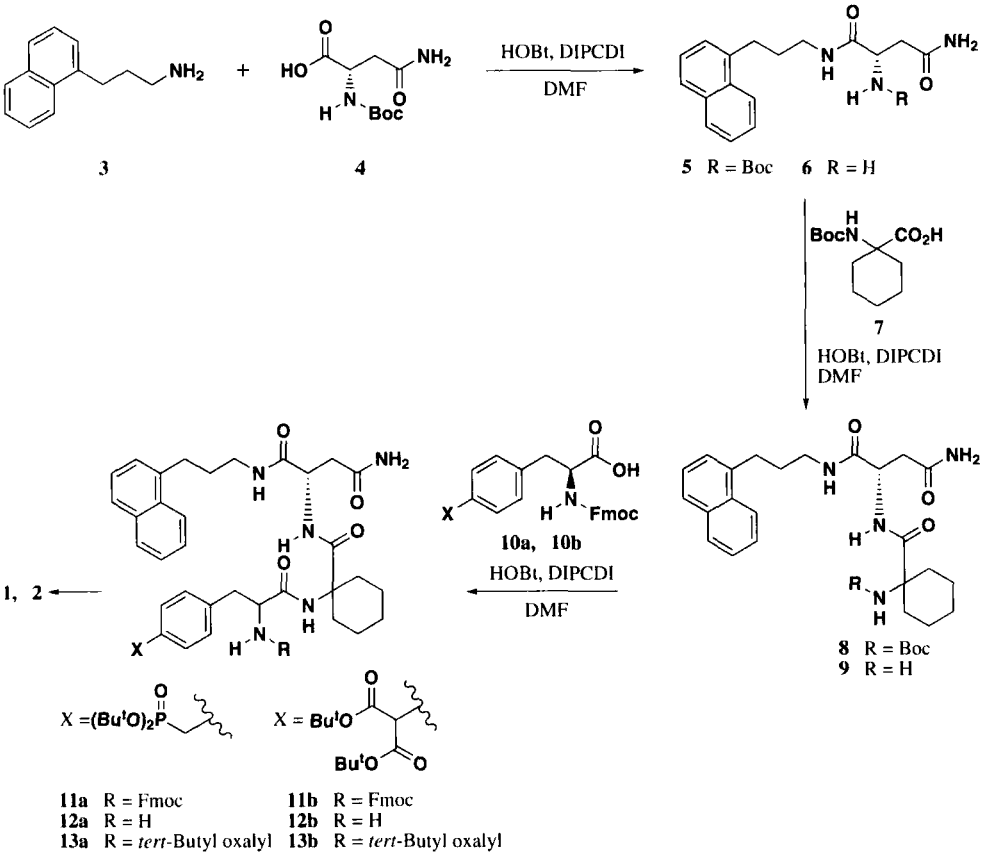
Inhibitors of cellular signal transduction are emerging as important new therapeutics for several diseases including cancers[1] and diabetes.[2] Antagonists of aberrant protein-tyrosine kinase-dependent signalling are particularly interesting,[3] with analogues **1**[4] and **2**[5] representing two noteworthy examples which have been reported recently to potently inhibit Grb2 SH2 domain binding both in extracellular assays and in whole cell preparations. The central roles played by Grb2 SH2 domains in a variety of cancers, including breast cancer and leukemias, renders **1** and **2** as useful pharmacological tools and of potential value as therapeutic agents. Previously both **1** and **2** have been prepared only on milligram scales. However, since significantly larger quantities would be desirable, herein is reported their synthesis on multi-hundred milligram scales by techniques which should be applicable to the scale-up of related signal transduction inhibitors. Of particular note is the application of radial compression technology to achieve final product HPLC purification on a nearly 1 g scale.



Preparation of both **1** and **2** relied on the synthesis of common intermediate **9**, which was obtained by an approximate 6-fold scale up of previously reported methodology (Scheme 1).[4] Coupling of **9** with appropriate pTyr mimetic, (N^{α}-Fmoc-L-Pmp(OBu^{t})$_2$-OH[6] (**10a**) for synthesis of **1** or N^{α}-Fmoc-4-(di-*tert*-butoxycarbonyl-methyl)-L-phenylalanine[7] (**10b**) for synthesis of **2** provided the N^{α}-Fmoc protected intermediates **11a** and **11b**, respectively. Piperidine-mediated removal of N^{α}-Fmoc groups, followed by acylation with Bu^{t}O$_2$CCOCl gave the globally protected penultimate compounds **13a** and **13b** respectively, which were deblocked to the final products **1** and **2** by treatment with TFA. Of particular note was the HPLC purification of **1** on nearly a 1 g scale, which provided a quantitative yield of over 700 mg of this valuable compound. Interestingly, final product **2**

was obtained as over 500 mg in 97% purity, which may suffice for some purposes without the need for HPLC purification. The ability to prepare these agents on the scale reported herein, should greatly facilitate their use in a variety of signal transduction experiments, particularly studies requiring larger quantities of material than had previously been available.



Scheme 1

EXPERIMENTAL SECTION

Fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. ^{1}H NMR data were obtained on Bruker AC250 (250 MHz) and are reported in ppm relative to TMS and referenced to the solvent in which they were run. Solvent was removed by rotary evaporation under reduced pressure and silica gel chromatography was performed using Merck silica gel 60 with a particle size of 40 - 63 μ. Anhydrous solvents were obtained commercially and used without further drying. Preparative HPLC were conducted using a Waters Prep LC4000 system having photodiode array detection.

3-(Naphthy-1-yl)propanamide-*N*-(*tert*-butoxycarbonyl)-L-asparagine (5).- To a solution of Boc-L-Asn-OH (**4**) (2.68 g, 11.5 mmol) and 1-hydroxybenzotriazole hydrate (HOBt) (1.76 g, 11.5 mmol)

in DMF (18 mL) was added 1,3-diisopropylcarbodiimide (DIPCDI) (1.82 mL, 11.5 mmol) and the mixture was stirred at room temperature (30 minutes). To this was then added 3-(naphth-1-yl)propylamine (**3**)[8] (2.13 g, 11.5 mmol) in DMF (5 mL) and stirring continued (4 h). The mixture was poured into ice water (100 mL) and precipitated solid was collected by filtration, washed well with H_2O and dried, yielding **5** as a white solid (4.52 g, 98% yield).[4] ^{1}H NMR (DMSO): δ 8.12 (1H, d, *J* = 7.3 Hz), 7.94 (2H, m), 7.80 (1H, d, *J* = 7.3 Hz), 7.43~7.62 (4H, m), 7.32 (1H, s), 6.93 (2H, m), 4.26 (1H, dt, *J* = 7.1, 6.9 Hz), 3.20 (2H, dt, *J* = 6.3, 6.1 Hz), 3.07 (2H, t, *J* = 7.5 Hz), 2.45 (2H, m), 1.83 (2H, tt, *J* = 7.3, 7.1 Hz), 1.41 (9H, s).

N^{α}-Boc-Protected Dipeptide 8.- A solution of **5** (2.52 g, 6.31 mmol) in CH_2Cl_2 (34 mL) with trifluoroacetic acid (TFA) (6 mL) and triethylsilane (TES) (800 μL) was stirred at room temperature (overnight) then solvent was removed under vacuum. Residue was taken to dryness from ether (2 x 40 mL) then precipitated from a mixture of ether (20 mL) and hexane (10 mL). Solvent was removed by decantation and residue was dried *in vacuo* to provide **6**•TFA as a grey solid (2.08 g, 80% yield). This was dissolved in DMF (10 mL) with diisopropylethylamine (DIEA) (1.76 mL, 10 mmol) and this was added to the active ester solution formed by reacting N^{α}-Boc-1-aminocylohexanecarboxylic acid (**7**) (1.53 g, 6.3 mmol), HOBt hydrate (964 mg, 6.3 mmol) and DIPCDI (1.0 mL, 6.3 mmol) in DMF (10 mL) (30 minutes at room temperature). After coupling (4 h), the mixture was poured into ice water (150 mL), extracted with EtOAc (3 x 150 mL) and washed sequentially with saturated aqueous $NaHCO_3$, NH_4Cl and brine, then dried (Na_2SO_4) and taken to dryness. Residue was purified by silica gel chromatography [EtOAc:$CHCl_3$ (1:10) to MeOH:$CHCl_3$ (1:10)] to provide **8** as a white foam (2.38 g, 72% yield).[4] ^{1}H NMR (DMSO): δ 8.08 (2H, m), 7.90 (1H, m), 7.75 (1H, m), 7.62 (1H, m), 7.50 (2H, m), 7.39 (3H, m), 7.27 (1H, s), 6.92 (1H, s), 4.38 (1H, m), 3.45 (1H, m), 3.24 (1H, m), 3.03 (2H, t, *J* = 6.7 Hz), 2.73 (1H, m), 2.44 (1H, dd, *J* = 5.1, 16.1 Hz), 1.69~2.05 (6H, m), 1.40~1.65 (6H, m), 1.34 (9H, s).

N^{α}-Fmoc-Protected Tripeptide 11a.- A solution of N^{α}-Boc-protected dipeptide **8** (2.38 g, 4.55 mmol) in CH_2Cl_2 (20 mL) with TFA (4 mL) and TES (800 μL) was stirred at room temperature (overnight) then concentrated under vacuum. Residue was treated with ether (20 mL) and precipitated solid was collected by centrifugation and washed with ether (20 mL) to provide after drying in vacuo, **9**•TFA as a white solid (2.40 g, 100% yield). To a portion of **9**•TFA (861 mg, 1.6 mmol) in DMF (3 mL) with DIEA (558 μL, 3.2 mmol) was added an active ester solution formed by stirring N^{α}-Fmoc-L-Pmp($PO_3Bu^t_2$)-OH[6] **10a** (952 mg, 1.60 mmol), HOBt hydrate (245 mg, 1.60 mmol) and DIPCDI (254 μL, 1.60 mmol) in DMF (4 mL) (4 h). Solvent was removed under high vacuum and residue was purified by silica gel chromatography [EtOAc:$CHCl_3$ (1:10) to MeOH:$CHCl_3$ (1:20)] to provide globally protected tripeptide **11a** as a white foam (1.39 g, 87% yield).[4] ^{1}H NMR ($CDCl_3$): δ 8.03 (1H, m), 7.93 (1H, d, J = 6.8 Hz), 7.83~7.64 (4H, m), 7.60~7.24 (11H, m), 7.18 (2H, dd, J = 1.9, 7.6 Hz), 7.05 (2H, d, J = 7.8 Hz), 6.65 (1H, m), 4.73 (1H, m), 4.35 (3H, m), 4.13 (1H, m), 3.37 (2H, m), 3.14~2.80 (7H, m), 2.75~2.50 (1H, m), 2.13~1.35 (12H, m), 1.42 (9H, s), 1.41 (9H, s).

N^{α}-Fmoc-Protected Tripeptide 11b.- To solution of **9** (500 mg, 1.18 mmol) (obtained by $NaHCO_3$ neutralization of **9**•TFA) in DMF (5 mL) was added an active ester solution formed by reacting N^{α}-Fmoc-4-(di-*tert*-butoxycarbonyl-methyl)-L-phenylalanine[7] (**10b**) (710 mg, 1.18 mmol), HOBt hydrate (159 mg, 1.18 mmol) and DIPCDI (188 µL, 1.20 mmol) in DMF (10 mL) at room temperature (10 minutes) and the combined reaction mixture was then stirred at room temperature (overnight). Solvent was removed under high vacuum and residue was purified by silica gel chromatography ($CHCl_3$:EtOAc:MeOH) to provide **11b** as white foam (1.15 g, 97% yield).[5] ^1H NMR ($CDCl_3$): δ 8.01 (2H, m), 7.81~7.71 (6H, m), 7.50~6.95 (12H, m), 6.58 (1H, s), 5.56 (3H, m), 4.69 (1H, m), 4.55~4.40 (1H, m), 4.40 (1H, s), 4.31 (2H, d, J = 6.84 Hz), 4.09 (1H, m), 3.34 (1H, m), 3.12~2.88 (5 H, m), 2.63 (1H, dd, J = 4.4, 15.1 Hz), 2.05~1.11 (12H, m), 1.46 (18H, s). FABMS ($^+$VE) *m/z* 1008 [MH^+].

N^{α}-Oxalyl(OBu^t) Tripeptide 13a.- A solution of globally protected tripeptide **11a** (1.39 g, 1.39 mmol) in acetonitrile (15 mL) with piperidine (445 µL, 4.17 mmol) was stirred at room temperature (1 h), then solvent was removed under high vacuum and residue was purified by silica gel chromatography [MeOH:$CHCl_3$ (1:20)]. L-Pmp-containing product was eluted first (**12a**, 587 mg) followed by D-Pmp-containing diastereomer[9] (198 mg). To an ice-cold solution of L-Pmp-containing **12a** (587 mg, 0.75 mmol) in DMF (3 mL) with DIEA (398 µL, 2.26 mmol) was added Bu^tO_2CCOCl (188 µL, 1.51 mmol) and the mixture was stirred at room temperature (30 minutes). Solvent was removed under high vacuum and residue was purified by silica gel chromatography [MeOH:$CHCl_3$ (1:20)] to provide **13a** as a foam (595 mg, 87% yield based on L-Pmp).[4] ^1H NMR (DMSO): δ 8.18 (1H, s), 8.08 (2H, m), 7.90 (1H, m), 7.75 (1H, m), 7.64 (1H, m), 7.50 (2H, m), 7.41 (3H, m), 7.01 ~ 7.12 (4H, m), 6.92 (1H, s), 4.36 (1H, m), 3.51 (1H, m), 2.90 ~ 3.27 (5H, m, partially covered by water peak), 2.94 (2H, d, J = 21.3 Hz), 2.50~2.75 (3H, m, partially covered by DMSO), 1.35 (18H, s), 1.10~2.00 (12H, m).

N^{α}-Oxalyl(OBu^t) Tripeptide 13b.- A solution of **11b** (1.38 g, 1.37 mmol) in acetonitrile (20 mL) was treated as described above for the preparation of **13a** and crude product was purified by silica gel chromatography ($CHCl_3$-EtOAc-MeOH mixture) to provide **13b** as white foam (1.04 g, 83% yield).[5] ^1H NMR ($CDCl_3$): δ 8.02 (2H, m), 7.83~7.21 (13H, m), 6.69 (1H, s), 6.38 (1H, s), 4.67 (2H, m), 4.40 (1H, s), 3.43~2.88 (7 H, m), 2.56 (1H, dd, J = 4.88, 14.89 Hz), 2.05~0.94 (12H, m), 1.464 (18H, s), 1.457 (9H, s). FABMS ($^+$VE) *m/z* 914 [MH^+].

Final Product 1.- A solution of **13a** (968 mg, 1.07 mmol) in CH_2Cl_2 (10 mL) with TFA (10 mL) and TES (513 µL, 3.21 mmol) was stirred at room temperature (3 h) then concentrated in vacuo. Residue was treated with cold ether and the resulting precipitate was collected by centrifugation, washed with ether (3 x 10 mL) and dried to provide crude **1** as a white solid (900 mg). This was purified by HPLC [Prep NovaPak HR C_{18} 6µ radial compression cartridge (200 mm x 40 mm dia.); flow rate = 50 mL/min.; linear gradient from 5% B to 60% B over 25 minutes; solvent A, 0.1% aqueous TFA; solvent B, 0.1% TFA in acetonitrile: product was collected from 17.0 to 18.6 minutes] to provide pure **1** as a white solid (788 mg, quantitative). Analytical HPLC [NovaPak HR C_{18} column (300 mm x 7.8 mm dia.); flow rate = 2 mL/min.; linear gradient from 5% B to 60% B over 0 - 25 minutes then 60%

B to 100% B over 25 - 30 minutes: product elutes at t = 22.5 minutes] indicated a single peak.[4] ^{1}H NMR (DMSO): δ 8.77 (1H, d, *J* = 8.1 Hz), 8.32 (1H, s), 8.08 (1H, m), 7.97 (1H, d, *J* = 8.1 Hz), 7.91 (1H, m), 7.75 (1H, m), 7.50 (3H, m), 7.39 (3H, m), 7.14 (4H, m), 6.91 (1H, s), 4.68 (1H, m), 4.37 (1H, m), 3.16 (3H, m), 3.03 (3H, m), 2.89 (2H, d, *J* = 21.2 Hz), 2.68 (1H, dd, *J* = 6.3, 14.6 Hz), 2.53 (1H, dd, *J* = 4.9, 14.6 Hz), 1.15 ~2.05 (12H, m).

Final Product 2.- A solution of **13b** (651 mg, 0.712 mmol) in TFA:H_2O:TES (9.25 mL:0.5 mL:0.25 mL), was stirred at room temperature (1 h), then solvent was removed in vacuo. Residue was treated with cold ether and the resulting precipitate was collected by centrifugation, washed with ether (2x) and dried to provide crude **2** as a light pink solid. This was dissolved in H_2O (60 mL) containing a small amount of acetonitrile, and lyophilized to directly afford **2** as a white solid (523 mg, 99% yield). Analytical HPLC [PrepNova-Pak HRC$_{18}$ column (7.8 mm dia x 300 mm); flow rate = 2 mL/min.; linear gradient from 35% B to 95% B over 30 minutes: product elutes at t = 19.2 minutes] indicated 97% purity.[5] ^{1}H NMR (DMSO) δ 8.82 (1H, s), 8.08 (1H, m), 8.00 (1H, d, J = 7.33 Hz), 7.89 (1H, m), 7.74 (1H, m), 7.53~7.20 (10H, m), 6.92 (1H, s), 4.73 (1H, m), 4.60 (1H, s), 4.36 (1H, m), 3.25~2.93 (6H, m), 2.69 (1H, dd, J = 6.75, 15.4 Hz), 2.52 (1H, m), 2.08~1.12 (12H, m). FABMS (-VE), m/z 744 [M-H], 700 [M-H-CO_2].

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