Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Electronic Supplementary Information

Utilization of achiral alkenyl amines for the preparation of high affinity Grb2 SH2 domain-binding macrocycles by ring-closing metathesis

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Grb2 SH2 Domain-Receptor Tyrosine Kinase Binding Assays in Intact Cells

Intact cells were serum-deprived for 16 hours in the presence or absence of Grb2 SH2 domain binding antagonists (**5c** and a positive control). Cells were then treated with growth factor (100 ng/ml) for 10 minutes (EGF, R&D Systems, Minneapolis, MN) and then lysed in cold buffer (pH 7.4) containing non-ionic detergent and protease and phosphatase inhibitors. After incubation with anti-Grb2 (Santa Cruz Biotechnology Inc., Santa Cruz, CA) for 1 hour on ice, immunocomplexs were captured using Protein G-Sepharose (GE Healthcare, Uppsala, Sweden) for 1 hour at 4 °C with rotation. The beads were washed three times with cold lysis buffer, and samples were eluted with SDS sample buffer and resolved by SDS-PAGE before electrophoretic transfer to PVDF membranes (Immobilon P; Millipore, Billerica, MA). Chemiluminescent detection of anti-EGFR (Santa Cruz Biotechnology), or anti-Grb2 (Upstate Biotechnology, Millipore) was performed using ECL (GE Healthcare). Analysis of Grb2-EGFR interaction was performed using the epidermoid carcinoma cell line A431 (American Type Culture Collection, Manassas, VA).

Synthetic

1-(2-Propenyl)-cyclopentanemethanamine (9c). Treatment of nitrile **8c** (5.1 g, 37.7 mmol) as described above for the preparation of **9b** gave **9c** as a colorless oil (4.60 g, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (m, 1 H), 5.05 (m, 2 H), 2.52 (s, 2 H), 2.12 (d, J = 7.2 Hz, 2 H), 1.60 (m, 4 H), 1.40 (m, 4 H), 1.25 (m, 2 H). DEI-MS (+VE) *m/z* 140.1 $(M + H)^+$; HR-DEI $(M + H)^+$: 140.1433, Calc: 140.1439.

1-(2-Propenyl)-cycloheptanemethanamine (9e). Treatment of nitrile **8e** as described above for the preparation of **9b** gave **9e** in 96% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (m, 1 H), 5.05 (m, 2 H), 2.44 (m, 2 H), 2.02 (d, *J* = 7.2 Hz, 2 H), 1.55 – 1.30 (12 H), 1.20 (m, 2 H). DEI-MS (+VE) *m/z* 168.1 $(M + H)^+$; HR-DEI $(M + H)^+$: 168.1749, Calc: 168.1752.

β-Phenyl-β-(2-propenyl)-benzeneethanamine (9f). To a suspension of sodium hydride 95% in oil (0.70 g, 27.7 mmol) in DMF (50 mL), was slowly added a solution of α-phenyl-benzeneacetonitrile **6f**[32] (5.0 g, 25.8 mmol) and the mixture was stirred at room temperature (1 h). The mixture was cooled to 0 °C and allyl bromide (3.50 g, 28.9 mmol) was added then the reaction was brought to room temperature and stirred overnight. The reaction mixture was poured into ice-water, extracted with benzene (2 x100 mL), dried (Na_2SO_4) and solvent evaporated to yield **8f** as a viscous oil. [^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 10 H), 5.70 (m, 1 H), 5.20 (m, 2 H), 3.13 (d, J = 6.8 Hz, 2 H).] The crude **8f** was dissolved in ether (20 mL) and added dropwise to a suspension of $LiAlH_4$ (4.0 g, 103 mmol) in anhydrous ether (50 mL) at 0 °C and the mixture was warmed to room temperature and stirred overnight. The reaction mixture was cooled to 0 °C and quenched by the careful addition of H_2O (10 mL). The mixture was vigorously stirred until white, dried (Na_2SO_4) and solvent evaporated to afford the known **9f** [*J. Org. Chem.* **2006**, *71*, 3980-3983] as a colorless oil (4.00 g, quantitative yield from **6f**).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.16 (m, 10 H), 5.39 (m, 1 H), 5.07 – 4.95 (m, 2 H), 3.32 (s, 2 H), 2.92 (d, *J* = 6.4 Hz, 2 H), 0.88 (brs, 2 H). FAB-MS (+VE) *m/z* 238.2 $(M+H)^+$.

***N*$^\alpha$-Boc-N-(triphenylmethyl)-L-asparagine (2,2-dimethyl-4-pentenyl)amide (10b).** Treatment of **9b** as described above for the preparation of **10a** provided **10b** in 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.20 (m, 15 H), 7.10 (brs, 1 H), 6.83 (brs, 1 H), 6.35 (d, *J* = 6.4 Hz, 1 H), 5.78 (m, 1 H), 5.03 (m, 2 H), 4.08 (m, 1 H), 3.12 – 3.00 (m, 3 H), 2.52 (dd, *J* = 14.8, 5.2 Hz, 1 H), 1.90 (m , 2 H), 1.42 (s, 9 H), 0.83 (s, 6 H). FAB-MS (+VE) *m/z* 570.2 $(M + H)^+$. HR-FABMS calculated for $C_{35}H_{43}N_3NaO_4$ $(M + Na)^+$: 592.3151. Found: 592.3131.

***N*$^\alpha$-Boc-N-(triphenylmethyl)-L-asparagine 1-(2-propenyl) cyclopentanemethanamide (10c).** Treatment of **9c** as described above for the preparation of **10a** provided **10c** in 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.10 (m, 16 H), 6.85 (brs, 1 H), 6.34 (d, *J* = 6.8 Hz, 1 H), 5.77 (m, 1 H), 5.05 (m, 2 H), 4.40 (m, 1 H), 3.18 (dd, *J* = 13.4, 6.6 Hz, 1 H), 3.10 – 3.01 (m, 2 H), 2.51 (dd, *J* = 14.8, 5.6 Hz, 1 H), 2.03 (m, 2 H), 1.65 – 1.55 (m, 4 H), 1.43 – 1.30 (m, 13 H). FAB-MS (+VE) *m/z* 634.4 $(M + K)^+$. HR-FABMS calcd for $C_{37}H_{45}N_3NaO_4$ $(M + Na)^+$: 618.3308. Found: 618.3293.

***N*$^\alpha$-Boc-N-(triphenylmethyl)-L-asparagine 1-(2-propenyl)-cycloheptanemethanamide (10e).** Treatment of **9e** as described above for the preparation of **10a** provided **10e** in 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.15 (m, 16 H), 6.81 (brs, 1 H), 6.32 (d, *J* = 7.2 Hz, 1 H), 5.78 (m, 1 H), 5.05 (m, 2 H),

4.40 (m, 1 H), 3.16 (dd, *J* = 13.4, 7.0 Hz, 1 H), 3.04 (dd, *J* =1 4.8, 4.0 Hz, 1 H), 2.96 (dd, *J* = 13.2, 5.2 Hz, 1 H), 2.50 (dd, *J* = 14.6, 5.0 Hz, 1 H), 1.93 (d, *J* = 7.2 Hz, 2 H), 1.50 – 1.30 (m, 21 H). FAB-MS (+VE) *m/z*: 624.4 $(M + H)^+$; HR-FABMS calcd for $C_{39}H_{50}N_3O_4$ $(M + H)^+$: 624.3801. Found: 624.3777.

N^{α}-Boc-N-(triphenylmethyl)-L-asparagine β-phenyl-β-(2-propenyl)-benzeneethanamide (10f). Treatment of **9f** as described above for the preparation of **10a** provided **10f** in quantitative yield.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.10 (m, 25 H), 6.94 (brs, 1 H), 6.32 (m, 1 H), 5.95 (d, *J* = 7.6 Hz, 1 H), 5.38 (m, 1 H), 4.90 (m, 2 H), 4.30 (m, 1 H), 4.13 (m, 1 H), 3.73 (m, 1 H), 3.00 (dd, *J* = 14.8, 3.6 Hz, 1 H), 2.79 (m, 2 H), 2.44 (dd, *J* = 15.0, 5.0 Hz, 1 H),1.40 (s, 9 H). FAB-MS (+VE) *m/z*: 694.2 $(M + H)^+$. HR-FABMS calcd for $C_{45}H_{47}N_3NaO_4$ $(M + Na)^+$: 716.3464. Found: 716.3492.

Metathesis precursor 13b. Deprotection of N^{α}-Boc-protected asparagine amide **10b** to yield the CF_3CO_2H amine salt **19b** and coupling with dipeptide acid **18** as described above for the preparation of **13a** provided **13b** as a colorless solid in 86% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.17 (m, 6 H), 6.74 (brs, 1 H), 6.16 (s, 1 H), 5.90 – 5.77 (m, 2 H), 5.52 (brs, 1 H), 5.11 – 5.03 (m, 4 H), 4.50 (dd, *J* = 13.6, 6.0 Hz, 1 H), 3.49 (t, *J* = 10.0 Hz, 1 H), 3.12 (dd, *J* = 13.2, 6.8 Hz, 1 H), 3.01 – 2.95 (m, 4 H), 2.78 (d, *J* = 6.4 Hz, 2 H), 2.62 (m, 2 H), 2.00 (d, *J* = 7.6 Hz, 2 H), 1.80 (m, 1 H), 1.70 – 1.59 (m, 3 H), 1.53 – 1.25 (m, 30 H), 1.22 (m, 2 H), 0.88 (s, 6 H), 0.59 (m, 1 H). FAB-MS (+VE) *m/z* 831.6 $(M + H)^+$. HR-FABMS calcd for $C_{44}H_{71}N4NaO_9P$ $(M + Na)^+$: 853.4856. Found: 853.4834.

Metathesis precursor 13c. Deprotection of N^{α}-Boc-protected asparagine amide **10c** to yield the CF_3CO_2H amine salt **19c** and coupling with dipeptide acid **18** as described above for the preparation of **13a** provided **13c** in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 – 7.11 (m, 6 H), 6.73 (brs, 1 H), 6.15 (s, 1 H), 5.88 – 5.72 (m, 2 H), 5.52 (brs, 1 H), 5.04 – 4.96 (m, 4 H), 4.45 (dd, *J* = 13.4, 6.2 Hz, 1 H), 3.43 (t, *J* = 10.0 Hz, 1 H), 3.16 (dd, *J* = 12.8, 6.4 Hz, 1 H), 3.07 – 2.97 (m, 2 H), 2.95 (dd, *J* = 21.6, 2.8 Hz, 2 H), 2.72 (m, 2 H), 2.55 (m, 2 H), 2.05 (d, *J* = 7.2 Hz, 2 H), 1.72 (m, 1 H), 1.65 – 1.45 (m, 6 H), 1.45 – 1.20 (m, 35 H), 1.15 – 1.00 (m, 2 H), 0.52 (m, 1 H). FAB-MS (+VE) *m/z*: 857.6 $(M + H)^+$. HR-FABMS calcd for $C_{46}H_{73}N_4NaO_9P$ $(M + Na)^+$: 879.5013. Found: 879.4986.

Metathesis precursor 13d. Deprotection of N^{α}-Boc-protected asparagine amide **10d** to yield the CF_3CO_2H amine salt **19d** and coupling with dipeptide acid **18** as described above for the preparation of **13a** provided **13d** in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 – 7.16 (m, 5 H), 7.10 (m, 1 H), 6.67 (s, 1 H), 6.00 (s, 1 H), 6.00 - 5.80 (m, 2 H), 5.40 (s, 1 H), 5.12 – 5.05 (m, 4 H), 4.55 (m, 1 H), 3.50 (t, *J* = 14.0 Hz, 1 H), 3.25 (dd, *J* = 13.2, 6.8 Hz, 1 H), 3.10 – 2.95 (m, 4 H), 2.83 – 2.70 (m, 2 H), 2.64 (m, 2 H), 2.08 (m, 2 H), 1.90 – 1.10 (m, 47 H). FAB-MS (+VE) *m/z*: 871.6 $(M + H)^+$. HR-FABMS calcd for $C_{47}H_{75}N_4NaO_9P$ $(M + Na)^+$: 893.5169. Found: 893.5149.

Metathesis precursor 13e. Deprotection of N^{α}-Boc-protected asparagine amide **10e** to yield the CF_3CO_2H amine salt **19e** and coupling with dipeptide acid **18** as described above for the preparation of **13a** provided **13e** in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.16 (m, 6 H) 6.74 (brs, 1 H), 6.07 (s, 1 H), 5.93 (m, 1 H), 5.84 (m, 1 H), 5.46 (brs, 1 H), 5.11 – 5.03 (m, 4 H), 4.53 (dd, *J* = 13.6, 6.4 Hz, 1 H), 3.49 (t, *J* = 7.5 Hz, 1 H), 3.19 (dd, *J* = 13.2, 6.8 Hz, 1 H), 3.05 – 2.95 (m, 4 H), 2.76 (m, 2 H), 2.62 (m, 2 H), 2.04 (m, 2 H), 1.80 (m, 1 H), 1.70 – 1.60 (m, 4 H), 1.56 – 1.27 (m, 41 H), 1.13 (m, 2 H), 0.63 (m, 1 H). FAB-MS (+VE) *m/z*: 885.6 $(M + H)^+$. HR-FABMS calcd for $C_{48}H_{77}N_4NaO_9P$ $(M + Na)^+$: 907.5326. Found: 907.5285.

Metathesis precursor 13f. Deprotection of N^{α}-Boc-protected asparagine amide **10f** to yield the CF_3CO_2H amine salt **19f** and coupling with dipeptide acid **18** as described above for the preparation of **13a** provided **13f** in 85% yield.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.15 (m, 15 H), 6.68 (t, *J* = 5.8 Hz, 1 H), 6.63 (brs, 1 H), 5.87 – 5.78 (m, 2 H), 5.46 (m, 1 H), 5.33 (brs, 1 H), 5.12 – 2.00 (m, 3 H), 4.94 (dd, *J* = 10.4, 2.4 Hz, 1 H), 4.41 (dd, *J* = 13.8, 5.8 Hz, 1 H), 4.05 (dd, *J* = 9.2, 6.4 Hz, 1 H), 3.93 (dd, *J* = 13.0, 5.4 Hz, 1 H), 3.48 (t, *J* = 9.8 Hz, 1 H), 3.00 – 2.89 (m, 5 H), 2.60 – 2.53 (m, 3 H), 2.45 (dd, *J* = 15.6, 5.6 Hz, 1 H), 1.66 – 1.56 (m, 3 H), 1.50 – 1.35 (m, 30 H), 1.29 (m, 1 H), 1.09 (m, 2 H), 0.62 (m, 1 H). FAB-MS (+VE) *m/z*: 955.7 $(M + H)^+$. HR-FABMS calcd for $C_{54}H_{75}N_4NaO_9P$ $(M + Na)^+$: 977.5169. Found: 977.5144.

Macrocyclic final product 5b. Ring-closing metathesis of **13b** to **21b** followed by deprotection and HPLC purification as reported above for the conversion of **13a** to **5a** yielded the macrocyclic final product **5b** as a white powder (4 mg, 11% yield from **13b**).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 – 8.40 (m, 2 H), 7.57 (brs, 1 H), 7.28 (d, *J* = 8.0 Hz, 2 H), 7.20 (dd, *J* = 8.4, 2.0 Hz, 2 H), 7.16 (brs, 1 H), 6.69 (dd, *J* = 8.4, 4.0 Hz, 1 H), 5.80 (dd, *J* = 15.0, 6.2 Hz, 1 H), 5.70 (m, 1 H), 4.28 (m, 1 H), 4.13 (d, *J* = 9.6 Hz, 1 H), 3.44 (dd, *J* = 13.0, 5.0 Hz, 1 H), 3.29 (d, *J* = 12.0 Hz, 1 H), 2.92 (d, *J* = 21.2 Hz, 2 H), 2.81 (dd, *J* = 16.0, 5.2 Hz, 1 H), 2.74 (m, 1 H), 2.49 (m, 1 H), 2.39 (dd, *J* = 16.0, 4.8 Hz, 1 H), 2.07 – 1.90 (m, 4 H), 1.88 – 1.70 (3 H), 1.55 – 1.40 (m, 5 H), 1.20 (m, 1 H), 0.95 (s, 3 H), 0.85 (s, 3 H). FAB-MS (-VE) *m/z*: 633.2 $(M - H)^-$. HR-FABMS calc for $C_{30}H_{43}N_4NaO_9P$ $(M + Na)^+$: 657.2665. Found: 657.2680.

Macrocyclic final product 5c. Ring-closing metathesis of **13c** to **21c** followed by deprotection and HPLC purification as reported above for the conversion of **13a** to **5a** yielded the macrocyclic final product **5c** in 7% yield.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, *J* = 8.0 Hz, 1 H), 8.38 (s, 1 H), 7.57 (s, 1 H), 7.29 (d, *J* = 8.0 Hz, 2 H), 7.20 – 7.17 (m, 3 H), 6.66 (dd, *J* = 8.4, 3.2 Hz, 1 H), 5.80 (dd, *J* = 15.2, 9.6 Hz, 1 H), 5.66 (m, 1 H), 4.26 (m, 1 H), 4.14 (d, *J* = 10.0 Hz, 1 H), 3.56 (dd, *J* = 13.2, 8.4 Hz, 1 H), 3.29 (d, *J* = 12.0 Hz, 1 H), 2.93 (d, *J* = 21.2 Hz, 2 H), 2.80 (dd, *J* = 15.8, 5.0 Hz, 1 H), 2.73 (m, 1 H), 2.54 (dd, *J* = 13.4, 3.0 Hz, 1 H), 2.35 (dd, *J* = 15.6, 5.2 Hz, 1 H), 2.16 (dd, *J* = 14.2, 10.6 Hz, 1 H), 2.07 – 1.95 (m, 3 H), 1.82 – 1.67 (m, 4 H), 1.62 – 1.37 (m, 11 H), 1.18 (m, 2 H). FAB-MS (-VE) *m/z*: 659.2 $(M - H)^-$. HR-FABMS calcd for $C_{32}H_{45}N_4NaO_9P$ $(M + Na)^+$: 683.2822. Found: 683.2807.

Macrocyclic final product 5d. Ring-closing metathesis of **13d** to **21d** followed by deprotection and HPLC purification as reported above for the conversion of **13a** to **5a** yielded the macrocyclic final product **5d** in 17% yield.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (m, 2 H), 7.55 (s, 1 H) 7.30 (AB, J_{AB} = 8.0 Hz, 2 H), 7.19 (AB, J_{AB} = 8.0 Hz, 2 H), 7.16 (s, 1 H), 6.59 (m, 1 H), 5.80 (dd, *J* = 15.2, 9.6 Hz, 1 H), 5.66 (m, 1 H), 4.28 (m, 1 H), 4.13 (m, 1 H), 3.60 (m, 2 H) 3.16 (m, 1 H), 2.90 (d, *J* = 21.2 Hz, 2 H), 2.77 (m, 2 H), 2.36 (dd, *J* = 16.0, 4.8 Hz, 1 H), 2.20 (m, 1 H), 2.07 – 1.94 (m, 2 H), 1.90 – 1.40 (m, 20 H). FAB-MS (-VE) *m/z*: 673.4 $(M-H)^-$. HR-MALDI-MS calcd for $C_{33}H_{47}N_4NaO_9P$ $(M + Na)^+$: 697.2978. Found: 697.2985.

Macrocyclic final product 5e. Ring-closing metathesis of **13e** to **21e** followed by deprotection and HPLC purification as reported above for the conversion of **13a** to **5a** yielded the macrocyclic final product **5e** in 6% yield.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, *J* = 8.0 Hz, 1 H), 8.36 (s, 1 H), 7.56 (s, 1 H), 7.29 (d, *J* = 8.0 Hz, 2 H), 7.20 – 7.16 (m, 3 H), 6.56 (dd, *J* = 8.8, 3.6 Hz, 1 H), 5.81 (dd, *J* = 15.0, 6.2 Hz, 1 H), 5.67 (m, 1 H), 4.26 (m, 1 H), 4.12 (d, *J* = 9.6 Hz, 1 H), 3.52 (m, 1 H), 3.27 (d, *J* = 11.6 Hz, 1 H), 2.93 (d, *J* = 21.2 Hz, 2 H), 2.80 – 2.70 (m, 2 H), 2.52 (dd, *J* = 13.4, 3.4 Hz, 1 H), 2.45 (dd, *J* = 15.6, 4.8 Hz, 1 H), 2.12 – 1.87 (m, 4 H), 1.82 – 1.70 (m, 3 H), 1.60 – 1.20 (m, 18 H). FAB-MS (-VE) *m/z*: 687.2 $(M - H)^-$. HR-FABMS calcd for $C_{34}H_{49}N_4NaO_9P$ $(M + Na)^+$: 711.3135. Found: 711.3114.

Macrocyclic final product 5f. Ring-closing metathesis of **13f** to **21f** followed by deprotection and HPLC purification as reported above for the conversion of **13a** to **5a** yielded the macrocyclic final product **5f** in 4% yield.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1 H), 7.88 (d, *J* = 7.2 Hz, 1 H), 7.28 – 7.08 (m, 15 H), 6.84 (s, 1 H), 6.68 (dd, *J* = 10.2, 2.2 Hz, 1 H), 6.01 (dd, *J* = 15.0, 9.8 Hz, 1 H), 5.70 (m, 1 H), 4.50 (m, 1 H), 4.22 (q, *J* = 6.4 Hz, 1 H), 4.06 (d, *J* = 7.2 Hz, 1 H), 3.40 (m, 1 H), 3.20 (m, 1 H), 2.95 – 2.84 (m, 3 H), 2.70 – 2.64 (m, 2 H), 2.05 (m, 2 H), 2.00 – 1.70 (m, 5 H), 1.50 – 1.35 (m, 5 H), 1.20 (m, 1 H). FAB-MS (-VE) *m/z*: 757.2 $(M - H)^-$. HR-FABMS calcd for $C_{40}H_{47}N_4NaO_9P$ $(M + Na)^+$: 781.2978. Found: 781.2998.