

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

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A family of previously reported ring-closing metathesis (RCM)-derived macrocycles that exhibit potent Grb2 SH2 domain-binding affinity is characterized by stereoselectively-introduced upper ring junctions that bear bicyclic aryl substituents. However, the synthetic complexity of these macrocycles presents a potential limit to their therapeutic application. Therefore, the current study was undertaken to simplify these macrocycles through the use of achiral 4-pentenylamides as ring-forming components. A series of macrocycles (**5a–f**) was prepared bearing both open and cyclic constructs at the upper ring junction. The Grb2 SH2 domain-binding affinities of these macrocycles varied, with higher affinities being obtained with cyclo-substituents. The most potent analogue (**5d**) contained a cyclohexyl group and exhibited Grb2 SH2 domain-binding affinity ( $K_D = 1.3$  nM) that was nearly equal to the parent macrocycle (**2**), which bore a stereoselectively-introduced naphthylmethyl substituent at the upper ring junction ( $K_D = 0.9$  nM). The results of this study advance design considerations that should facilitate the development of Grb2 SH2 domain-binding antagonists.

## Introduction

Inhibition of protein-tyrosine kinase (PTK)-dependent signaling has emerged as an important new approach toward anticancer therapeutics.<sup>1,2</sup> Most clinically effective small molecule PTK inhibitors interact at the kinase catalytic cleft, where they function as ATP-competitive agents. Although PTK inhibitors generally exhibit low collateral cytotoxicity, the cardiotoxicity recently reported following administration of the c-Abl PTK inhibitor, imatinib mesylate (Gleevec)<sup>3</sup> highlights a need for alternate approaches toward PTK down regulation.

Src homology 2 (SH2) domain binding interactions are key components of PTK pathways that function downstream of the kinase step and afford potentially complimentary targets for blocking PTK signaling.<sup>4</sup> Development of SH2 domain binding inhibitors, including inhibitors of the growth factor receptor bound protein 2 (Grb2) SH2 domain, has been undertaken by several groups.<sup>5,6</sup> Grb2 is a non-catalytic docking module that plays critical roles in several cancers, including erbB-2 dependent breast cancers and c-Met dependent renal carcinomas.<sup>7</sup> The design of Grb2 SH2 domain signaling inhibitors has been assisted by the preferential binding of native “pTyr-Xxx-Asn-Yyy” ligands in type-I beta turn conformations.<sup>8</sup> A group at Novartis developed

peptide mimetics exemplified by **1** that are characterized by a C-terminal (3-bicyclicarylpropyl)amide<sup>9</sup> and a bend-inducing pTyr + 1 Ac<sub>6</sub>c (1-aminocyclohexanecarboxylic acid) residue (Fig. 1).<sup>10</sup>

We had previously prepared macrocycle **2** as a conformationally constrained variant of **1** using ring-closing metathesis (RCM) chemistries (Fig. 1).<sup>11,12</sup> Macrocycles based on **2** represent a new family of highly potent Grb2 SH2 domain-binding antagonists that can provide single digit nanomolar binding constants even when mono-anionic phosphoryl mimetics are employed.<sup>13</sup> In designing **2** we hypothesized that introduction of the ring-closing propenyl segment into **1** could enhance binding affinity in part due to rotational restriction at the upper ring junction. However, when macrocycle **3** was prepared bearing both *R* and *S* stereochemistries at the upper ring junction, little difference was observed in the binding affinities (Fig. 1).<sup>14</sup> The related RCM-derived macrocycle **4** also showed a surprising lack of dependence of binding affinity on upper ring junction stereochemistry.<sup>15,16</sup> Therefore, a study was undertaken to prepare a series of macrocycles **5a–5f** lacking a stereogenic center at the upper ring junction and to examine their Grb2 SH2 domain binding affinities.

## Results and discussion

### Synthesis

A key feature of macrocycles **5a–5f** is the facile synthesis of achiral 4-penten-1-amines (**9a–9f**) that serve as the upper ring junction components during RCM ring closure (Scheme 1). With the exception of 4-penten-1-amine (**9a**), which was derived from 4-penten-1-ol,<sup>17</sup> amines **9b–9f** were prepared by the method of Bender and Widenhoefer, namely by allylation of the nitriles **6b–6f** to yield the corresponding known 1-(2-propenyl)carbonitriles (**8b–8f**) followed by reduction.<sup>18</sup> For analogue **9d**, commercially available cyclohexanecarbonitrile (**6d**) was first halogenated to the

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† Electronic supplementary information (ESI) available: Procedures for the whole cell Grb2 binding studies shown in Fig. 2 as well as synthetic experimental details for compounds **9c**, **9e**, **9f**, **10b**, **10c**, **10e**, **10f**, **13b**, **13c**, **13d**, **13e**, **13f**, **5b**, **5c**, **5d**, **5e** and **5f**. See DOI: 10.1039/b611887a

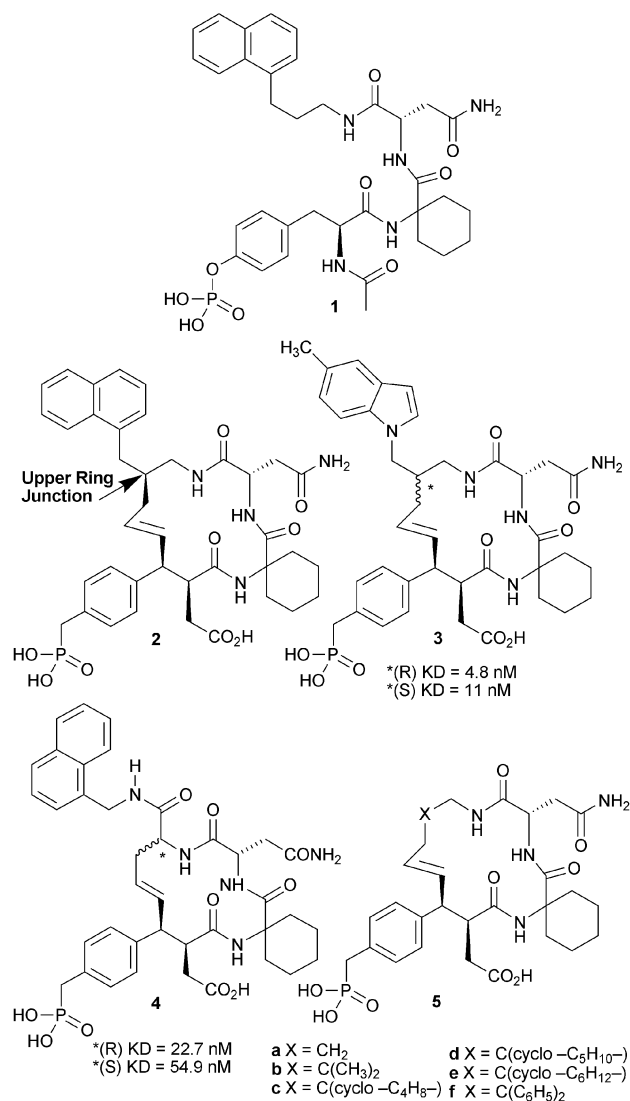


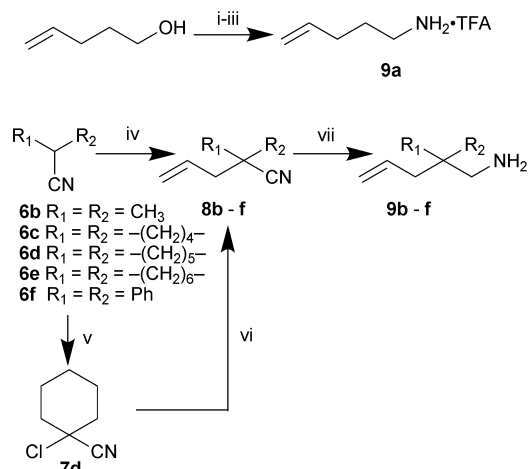
Fig. 1 Structures of Grb2 SH2 domain-binding inhibitors.

known 1-chloro derivative (**7d**) by the method of Fleming *et al.*, then metalated (*n*-butyl lithium) and reacted with allyl bromide to yield the intermediate **8d**.<sup>19</sup>

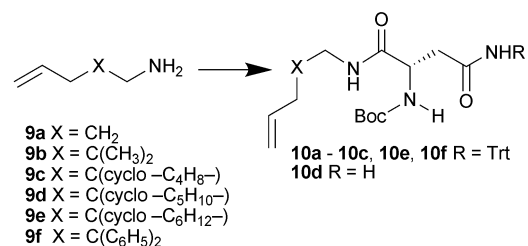
Initial attempts to couple the cyclohexyl-containing amine **9d** with *N*<sup>α</sup>-Boc-Asn-OH using HOBT-EDCI active ester methodology<sup>20</sup> gave only a low yield of the desired asparagine amide **10d**. A similar effort at coupling the diphenyl-containing amine **9f** resulted in no product. Alternatively, the mixed anhydride (*i*-BuOC(O)Cl, 4-methylmorpholine) coupling of amines **9a–9c** and **9e**, **9f** with *N*<sup>α</sup>-Boc-Asn(Trt)-OH gave the desired asparagine amides **10** in high yields (Scheme 2).

Previous syntheses of macrocycles exemplified by **2** and **3** have involved the low yield coupling of the protected β-vinyl-containing pTyr mimetic **12** with the dipeptides **11**, obtained by condensing the appropriate asparagine alkenylamides with *N*-protected 1-aminocyclohexanecarboxylic acid (Scheme 3).<sup>12–14,21–23</sup> The poor yields of this key step can be attributed to steric interactions between the highly hindered **12** and the dipeptide **11**.

In order to overcome the low yields encountered in the condensation of **12** with the dipeptides **11**, coupling of **12** with the



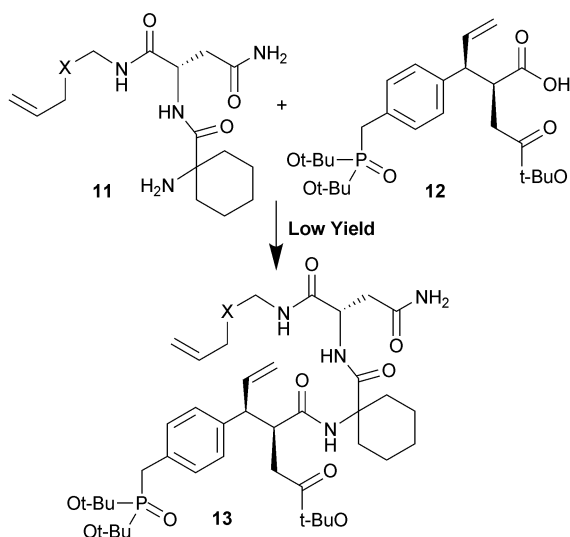
**Scheme 1** Reagents and conditions: (i) CH<sub>3</sub>SO<sub>2</sub>Cl, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, room temp, 3 h; then NaN<sub>3</sub>, DMF–H<sub>2</sub>O, 50 °C, overnight; (ii) LiAlH<sub>4</sub>, Et<sub>2</sub>O, 0 °C, 1 h then (Boc)<sub>2</sub>O, Et<sub>2</sub>O–H<sub>2</sub>O, room temp, overnight; (iii) CF<sub>3</sub>CO<sub>2</sub>H, Et<sub>3</sub>SiH, CH<sub>2</sub>Cl<sub>2</sub>, room temp, 2 h (52% yield over three steps); (iv) LiN(*i*-Pr)<sub>2</sub>, THF, –78 °C, 45 minutes, then allyl bromide, warmed to room temp overnight; for **8f**; NaH, DMF 0° to room temp, overnight (65% yield for **8b**; 84% yield for **8c**; 91% yield for **8e**); (v) PCl<sub>5</sub>, pyridine, CHCl<sub>3</sub>, reflux overnight (95% yield); (vi) allyl bromide, *n*-BuLi, THF, –78 °C, 2 h (76% yield); (vii) LiAlH<sub>4</sub>, Et<sub>2</sub>O for **9b**, **9d**, **9f**; THF for **9c**, **9e**, 0 °C, 1 h (82% yield for **9b**; 92% yield for **9c**; 92% yield for **9e**; quantitative over two steps for **9f**).



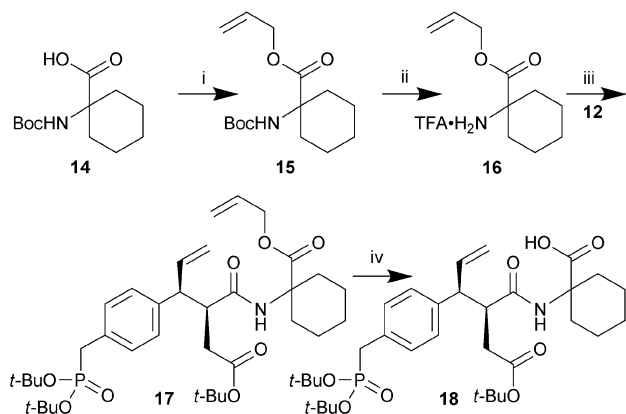
**Scheme 2** Reagents and conditions: For **10a–10c**, **10e** and **10f**, *N*<sup>α</sup>-Boc-L-Asn(Trt), 4-methylmorpholine, *i*-BuOC(O)Cl, DMF, 0 °C then room temp, overnight (30% yield for **10a**; 82% yield for **10b**; 92% yield for **10c**; 92% yield for **10e**; quantitative for **10f**). For **10d**, *N*<sup>α</sup>-Boc-L-Asn, EDCI, HOBT, NEt(*i*-Pr)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, room temp, overnight (11% yield over two steps from **8d**).

sterically less crowded monomeric 1-aminocyclohexanecarboxylic acid allyl ester (**16**) was undertaken (Scheme 4). Preparation of **16** was achieved in two steps from commercially-available *N*-Boc-1-aminocyclohexanecarboxylic acid (**14**) by initial *O*-allylation to **15** (50% yield) followed by TFA cleavage of the *N*-Boc group. Without purification, crude **16** was directly reacted with **12** using HOAt-EDCI active ester coupling<sup>24</sup> in the presence of NEt(*i*-Pr)<sub>2</sub> to provide desired product **17** in 90% yield. Cleavage of the *O*-allyl ester [Pd(PPh<sub>3</sub>)<sub>4</sub>, morpholine] gave the dipeptide equivalent **18** in 91% yield suitably protected for further coupling.

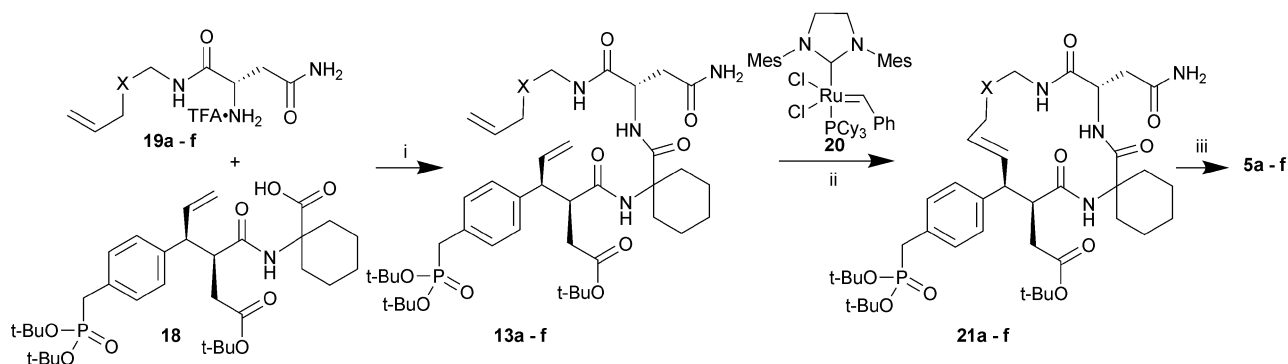
Treatment of the *N*-Boc protected asparagine alkenylamides **10a–10f** with TFA gave the corresponding TFA salts (**19a–19f**), which were directly subjected to HOAt-EDCI active ester coupling with pseudodipeptide **18** in the presence of NEt(*i*-Pr)<sub>2</sub> (Scheme 5). In this fashion the open-chain metathesis precursors **13a–13f** were uniformly obtained in good to high yields.



**Scheme 3** Previous low yield route to metathesis precursors **13**.



**Scheme 4** Reagents and conditions: (i) Allyl bromide,  $\text{Na}_2\text{CO}_3$ , DMF, room temperature, 2 days (50% yield); (ii)  $\text{CF}_3\text{CO}_2\text{H}$ ,  $\text{Et}_3\text{Si}$ ,  $\text{CH}_2\text{Cl}_2$ , room temp, 2 h; (iii) HOAt, EDCI,  $\text{NEt}(i\text{-Pr})_2$ , DMF, room temp, 2 days (90% yield over two steps); (iv)  $\text{Pd}(\text{PPh}_3)_4$ , morpholine, THF, room temp, 30 min (91% yield).



**Scheme 5** Reagents and conditions: (i) HOAt, EDCI,  $\text{NEt}(i\text{-Pr})_2$ , DMF, room temp, 2 days (for **13a** 91% yield over two steps from **10a**; for **13b** 90% yield over two steps from **10b**; for **13c** quantitative yield over two steps from **10c**; for **13d** 60% yield over two steps from **10d**; for **13e** quantitative yield over two steps from **10e**; for **13f** 85% yield over two steps from **10f**); (ii)  $\text{C}_2\text{H}_4\text{Cl}_2$ , reflux, 2 days; (iii)  $\text{CF}_3\text{CO}_2\text{H}$ ,  $\text{Et}_3\text{Si}$ ,  $\text{CH}_2\text{Cl}_2$ , room temp, 2 h (for **5a** 39% yield over two steps from **13a**; for **5b** 11% yield over two steps from **13b**; for **5c** 7% yield over two steps from **13c**; for **5d** 17% yield over two steps from **13d**; for **5e** 6% yield over two steps from **13e**; for **5f** 4% yield over two steps from **13f**).

**Table 1** Grb2 SH2 domain-binding affinities determined by surface plasmon resonance

Compound no.	$K_D/\text{nM}^a$
<b>2</b>	0.9
<b>5a</b>	320
<b>5b</b>	360
<b>5c</b>	24
<b>5d</b>	1.3
<b>5e</b>	39
<b>5f</b>	72

<sup>a</sup> Steady state values determined on Biacore 2000 and S51 instruments using amine coupled surfaces as described in references 15 and 34.

Metathesis ring closure to the intermediate protected macrocycles **21** using Grubbs 2<sup>nd</sup> generation catalyst  $[(\text{PCy}_3)(\text{Im}(\text{Mes})_2)\text{Ru}=\text{CHPh}]$  **20**<sup>25</sup> failed when conducted in refluxing dichloromethane (bp 40 °C). However, increasing the reaction temperature through the use of refluxing 1,2-dichloroethane (bp 83 °C) provided the desired protected ring-closed intermediates **21a–21f**, which were passed down a silica gel column. The brown products were deprotected using TFA and purified by reverse phase HPLC to yield the final products **5a–5f** as white solids. Yields of HPLC-purified products were disappointing, ranging from 4% (for **5f**) to 39% (for **5a**).

#### Grb2 SH2 domain-binding affinities

Grb2 SH2 domain binding affinities of final macrocycles **5a–5f** were determined by surface plasmon resonance (SPR) using Biacore 2000 and S51 instruments. While SPR-determination of SH2 domain binding constants has often been indirect through the measurement of an inhibitor's ability to compete with sensor-bound reference pTyr-containing peptide for binding to Grb2 SH2 domain protein in solution,<sup>26–28</sup> our current protocol directly measured the binding of macrocycles **5a–5f** to biotinylated Grb2 SH2 domain protein immobilized onto the sensor chip. Immobilization of protein was achieved either by amine coupling or by streptavidin capturing of the biotin functionality. Binding affinities determined from steady state  $K_D$  values using amine-coupled surfaces (Table 1) were generally poorer by up to an order of magnitude or more than

the corresponding values obtained from biotin capture surfaces (data not shown). The reasons for these differences are not known.

The binding affinity of the parent macrocycle **2**, which bears a stereoselectively-introduced naphthylmethyl substituent at the upper ring junction, was found to be  $K_D = 0.9$  nM, in close agreement with previous determinations.<sup>12</sup> The affinities of macrocycles **5a** ( $K_D = 320$  nM) and **5b** ( $K_D = 360$  nM) that contained either unsubstituted or bis-methyl substituted upper ring junctions were significantly less than other members of the series. The highest affinities were obtained with macrocycles having cyclopentyl and cyclohexyl substituents at the upper ring junctions (**5c**,  $K_D = 24$  nM and **5d**,  $K_D = 1.3$  nM, respectively). Increasing the substituent ring size to seven members resulted in a decrease in affinity (**5e**,  $K_D = 39$  nM). Macrocycle **5f** having a bis-phenyl-substituted ring junction showed a further loss of affinity ( $K_D = 72$  nM), yet retained greater affinity than the unsubstituted and bis-methyl-containing macrocycles **5a** and **5b**, respectively.

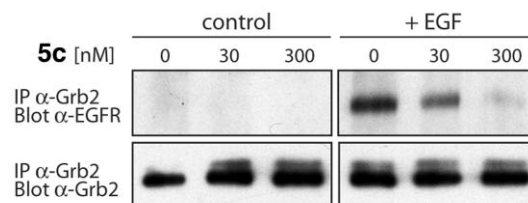
In open-chain **1** the naphthyl ring system has been hypothesized to interact with a hydrophobic patch on the Grb2 SH2 domain protein formed by the side chains of Lys $\beta$ D6 and Leu  $\beta$ D1 and bounded by Phe  $\beta$ E3.<sup>9</sup> Structure–activity studies on the related phosphopeptides “Ac-pTyr-Ile-Asn-NH-R” showed an approximate 10-fold loss of binding affinity when R = 3-(1-naphthyl)propyl was replaced by the less hydrophobic 3-phenylpropyl, isopropyl or isobutyl groups.<sup>9</sup> The structural importance of substituents occupying the naphthyl ring position in **1** has also been shown by affinity enhancements achieved through the introduction of oxygen functionality onto the naphthyl ring<sup>29</sup> or by replacement of the naphthyl group with indole ring systems.<sup>30</sup>

Molecular modeling had previously indicated that the naphthyl ring system in **2** should interact with the hydrophobic patch formed by the side chains of Lys $\beta$ D6 and Leu  $\beta$ D1.<sup>21</sup> A recent X-ray crystal structure of a similar naphthyl-containing macrocycle bound to Grb2 SH2 domain protein showed that the naphthyl ring lay primarily over the Leu  $\beta$ D1 region with the macrocycle ring-closing propenyl bridge interacting with the methylenes of the Lys $\beta$ D6 side chain.<sup>31</sup> Because macrocycles **5a–5f** lack the naphthylmethyl substituent of parent **2**, reduced interactions with the hydrophobic patch would be expected relative to **2**, although hydrophobic interactions with the Lys $\beta$ D6 side chain would be retained. Therefore, note was taken of the fact that cyclohexyl-containing **5d** retained nearly all the affinity of the parent **2**, which contained a stereoselectively introduced naphthylmethyl group at the upper ring junction.

The unsubstituted **5a** and bis-methyl-substituted **5b** exhibited significantly reduced affinity. This could arise from greater flexibility of their macrocycle-forming pentenyl segments and reduced hydrophobic interactions with the Leu  $\beta$ D1 region. The greater affinities of the cyclopentyl (**5c**) and cyclohexyl (**5d**)-containing macrocycles may be attributed to a combination of reduced conformational flexibility of their pentenyl amide chains and greater hydrophobic interactions with the Leu  $\beta$ D1 region. In spite of its greater lipophilic character, the cycloheptyl-containing **5e** may bind with less affinity due to increased conformational flexibility.

It was also of interest to examine the ability of a representative macrocycle to inhibit the binding of Grb2 to cognate protein in whole cells. For this purpose, **5c** was incubated at different concentrations with A431 breast cancer cells in the presence

and absence of epidermal growth factor. A431 cells over-express the epidermal growth factor receptor (EGFR) PTK. Following incubation with inhibitor the cells were washed, lysed and binding of Grb2 to EGFR measured as described in the Electronic Supplementary Information (ESI).† As shown in Fig. 2, at 300 nM concentration macrocycle **5c** was able to achieve a nearly complete blockade of intracellular binding of Grb2 to EGFR.



**Fig. 2** Concentration-dependent inhibition by **5c** of binding of Grb2 to epidermal growth factor receptor (EGFR) tyrosine kinase in intact A431 cells as described in the Electronic Supplementary Information.† Cells were treated with the indicated concentrations of **5c**, then left untreated (control) or briefly stimulated with EGF (+EGF). Cells were lysed and the amount of EGFR bound to Grb2 determined (upper panels) as well as the amount of total Grb2 (lower panels).

## Conclusions

Macrocycles exemplified by **2** exhibit promising Grb2 SH2 domain inhibitory profiles. However, their synthetic complexity presents potential limitations to their therapeutic application. The goal of the current study was to employ achiral 4-pentenylamides as ring-forming components in the RCM formation of a new family of macrocycles that do not require stereoselective construction of the upper ring junction. In the course of this work it was found that the low synthetic yields associated in the original synthesis with the key coupling of the pTyr mimetic portion with an Ac<sub>6</sub>c-Asn-alkenylamide dipeptide unit could be overcome by preconstruction of a pseudo-dipeptide formed from the pTyr mimetic and the Ac<sub>6</sub>c residue and then coupling this with the Asn-alkenylamide residue. Unfortunately, yields of RCM macrocyclization for this series were disappointingly low. The Grb2 SH2 domain-binding affinities of these macrocycles varied, depending on the substituents at the upper ring-forming junction. Higher affinities were obtained with cycloalkyl substituents, with the most potent analogue having a cyclohexyl group. It was significant that binding affinity nearly equivalent to the parent **2** could be obtained without employing defined chirality at the upper ring junction or using bicyclic aryl substituents. The results of this study contribute significantly to a greater understanding of design considerations that may be useful for the development of Grb2 SH2 domain-binding antagonists.

## Experimental

### 2,2-Dimethyl-4-penten-1-amine (**9b**)

To a suspension of LiAlH<sub>4</sub> (5.60 g, 147 mmol) in ether (100 mL) at 0 °C was added dropwise nitrile **8b** (4.0 g, 36.7 mmol) and the reaction mixture was warmed to room temperature and stirred overnight. To the mixture was cautiously added H<sub>2</sub>O (25 mL) and the mixture was vigorously stirred until white. It was dried



(Na<sub>2</sub>SO<sub>4</sub>) and solvent removed to provide previously reported<sup>32</sup> **9b** as a colorless oil (3.20 g, 77% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.80 (m, 1 H), 5.03 (m, 2 H), 2.45 (s, 2 H), 1.97 (m, 2 H), 1.55 (m, 2 H), 0.86 (s, 6 H).

#### *N*<sup>α</sup>-Boc-*N*-(triphenylmethyl)-L-asparagine (4-pentenyl)amide (**10a**)

To a solution of *N*<sup>α</sup>-Boc-L-Asn(Trt)-OH (950 mg, 2.00 mmol) in DMF (10 mL) at 0 °C with 4-methylmorpholine (1.00 mL, 9.10 mmol) was added isobutylformate (0.32 mL, 2.45 mmol) and the mixture was stirred at 0 °C (10 minutes) then amine **9a** (478 mg, 2.40 mmol) was added and the reaction mixture was warmed to room temperature and stirred overnight. The mixture was diluted with ethyl acetate (150 mL), washed with H<sub>2</sub>O (2 × 50 mL), brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and solvent removed. Purification by silica gel column chromatography (hexane and ethyl acetate) afforded **10a** as a white solid (325 mg, 30% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.32–7.15 (m, 15 H), 6.98 (br s, 1 H), 6.70 (br s, 1 H), 6.18 (br s, 1 H), 5.77 (m, 1 H), 5.00 (m, 2 H), 4.42 (m, 1 H), 3.21 (m, 2 H), 3.08 (dd, *J* = 15.0, 3.4 Hz, 1 H), 2.53 (dd, *J* = 15.0, 2.2 Hz, 1 H), 2.05 (q, *J* = 7.2 Hz, 2 H), 1.55 (m, 2 H), 1.43 (s, 9 H). FAB-MS (+VE) *m/z* 542.4 (M + H)<sup>+</sup>. HR-FABMS calcd for C<sub>33</sub>H<sub>40</sub>N<sub>3</sub>O<sub>4</sub> (M + H)<sup>+</sup>: 542.3019. Found: 542.3009.

#### *N*<sup>α</sup>-Boc-*N*-(triphenylmethyl)-L-asparagine 1-(2-propenyl)-cyclohexanemethanamide (**10d**)

To a solution of **7d** (3.80 g, 25.5 mmol) in dry ether (200 mL) at 0 °C, was cautiously added LiAlH<sub>4</sub> (2.0 g, 52.6 mmol) and the mixture was warmed to room temperature and stirred overnight. The reaction mixture was cooled to 0 °C and quenched by the cautious addition of H<sub>2</sub>O (5.0 mL). The mixture was stirred vigorously until the color became white, then it was dried (Na<sub>2</sub>SO<sub>4</sub>) and solvent evaporated to yield crude amine **9d** as a colorless oil (3.20 g). An aliquot (0.75 g, 5.0 mmol) was added to a pre-formed active ester solution formed by reacting at room temperature for 15 minutes *N*<sup>α</sup>-Boc-L-Asn-OH (1.16 g, 5.0 mmol), HOBT (0.70 g, 5.0 mmol), EDCI (1.00 g, 5.0 mmol) and NEt(*i*-Pr)<sub>2</sub> (1.75 mL, 10 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (25 mL). The resulting reaction mixture was stirred at room temperature overnight. The mixture was diluted with ethyl acetate (200 mL) and washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and solvent evaporated. Purification by silica gel column chromatography (hexane and ethyl acetate) afforded **10d** as a colorless solid (200 mg, 11% yield from **7d**). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.94 (s, 1 H), 6.29 (s, 1 H), 6.20 (d, *J* = 6.4 Hz, 1 H), 5.84 (m, 1 H), 5.64 (s, 1 H), 5.10 (m, 2 H), 4.45 (m, 1 H), 3.17 (m, 2 H), 2.94 (dd, *J* = 15.2, 3.6 Hz, 1 H), 2.55 (dd, *J* = 15.4, 6.2 Hz, 1 H), 2.03 (m, 2 H), 1.50–1.20 (m, 19 H). FAB-MS (+VE) *m/z*: 368.4 (M + H)<sup>+</sup>. HR-FABMS calcd for C<sub>19</sub>H<sub>34</sub>N<sub>3</sub>O<sub>4</sub> (M + H)<sup>+</sup>: 368.2549. Found: 368.2550.

#### *N*<sup>α</sup>-Boc-α-aminocyclohexanecarboxylic acid 2-propenyl ester (**15**)

A mixture of *N*<sup>α</sup>-Boc-1-aminocyclohexanecarboxylic acid<sup>33</sup> (1.70 g, 7.00 mmol), allyl bromide (0.89 mL, 10.5 mmol) and Na<sub>2</sub>CO<sub>3</sub> (881 mg, 10.5 mmol) in DMF (20 mL) was stirred at room temperature (2 days). The reaction mixture was diluted with ethyl acetate (150 mL), washed with H<sub>2</sub>O (2 × 50 mL) and brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and solvent evaporated. Purification by silica gel column chromatography (hexane and ethyl acetate) afforded **15** as

a colorless oil (1.00 g, 50% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.92 (m, 1 H), 5.25 (m, 1 H), 5.20 (m, 1 H), 4.72 (br s, 1 H), 4.61 (m, 2 H), 2.00 (m, 2 H), 1.84 (m, 2 H), 1.65–1.53 (m, 3 H), 1.53–1.43 (m, 11 H), 1.30 (m, 1 H). FAB-MS (+VE) *m/z*: 284.2 (M + H)<sup>+</sup>. HR-FAB (M + H)<sup>+</sup>: 284.1873, Calc: 284.1862.

#### Dipeptide allyl ester **17**

Treatment of *N*<sup>α</sup>-Boc-protected **15** (384 mg, 1.35 mmol) with CF<sub>3</sub>CO<sub>2</sub>H (1.56 mL) and triethylsilane (0.64 mL) in CH<sub>2</sub>Cl<sub>2</sub> (3.00 mL) at room temperature (2 h) and removal of volatiles provided the free amine as its CF<sub>3</sub>CO<sub>2</sub>H salt (**16**). This was dissolved in DMF (2.0 mL) and added to a pre-formed active ester solution obtained by reacting pTyr mimetic **12**<sup>12</sup> (450 mg, 0.90 mmol) in DMF (4.0 mL) along with HOAt (183 mg, 1.35 mmol), EDCI (258 mg, 1.35 mmol) and NEt(*i*-Pr)<sub>2</sub> (0.78 mL, 4.50 mmol) with stirring at room temperature (15 minutes). The combined reaction mixture was stirred at room temperature (2 days). The mixture was diluted with ethyl acetate (150 mL), washed with H<sub>2</sub>O (2 × 50 mL) and brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and solvent evaporated. Purification by silica gel column chromatography (hexane and ethyl acetate) provided **17** as a white solid (540 mg, 90% combined yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.22–7.10 (m, 4 H), 5.94 (s, 1 H), 5.83–5.77 (m, 2 H), 5.25–5.00 (m, 4 H), 4.46 (m, 2 H), 3.52 (m, 1 H), 3.00 (m, 1 H), 2.92 (d, *J* = 21.2 Hz, 2 H), 2.52 (m, 2 H), 1.70–1.20 (m, 36 H), 0.85 (m, 1 H). FAB-MS (+VE) *m/z*: 662.4 (M + H)<sup>+</sup>. HR-FABMS calcd for C<sub>36</sub>H<sub>56</sub>NNaO<sub>4</sub>P (M + Na)<sup>+</sup>: 684.3641. Found: 684.3644.

#### Dipeptide acid **18**

To a solution of dipeptide allyl ester **17** (540 mg, 0.82 mmol) in anhydrous THF (30 mL) which had been degassed under argon for 5 minutes was added Pd(PPh<sub>3</sub>)<sub>4</sub> (93 mg, 0.080 mmol) and morpholine (0.70 mL, 8.00 mmol). The mixture stirred at room temperature (30 minutes) then 0.1 M HCl (100 mL) was added, THF was removed *in vacuo* and the remaining residue was extracted with ethyl acetate (3 × 50 mL). The combined ethyl acetate extracts were washed with brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and purified by silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub> and methanol) to afford **18** as a white solid (460 mg in 91% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.21–7.12 (m, 4 H), 5.91 (m, 1 H), 5.75 (s, 1 H), 5.15 (m, 2 H), 3.49 (m, 1 H), 3.10–2.90 (m, 3 H), 2.72–2.55 (m, 2 H), 1.70 (m, 2 H), 1.60–1.25 (m, 33 H), 1.15 (m, 2 H). FAB-MS (+VE) *m/z*: 644 (M + Na)<sup>+</sup>. HR-FABMS calcd for C<sub>33</sub>H<sub>52</sub>NNaO<sub>8</sub>P (M + Na)<sup>+</sup>: 644.3328. Found: 644.3359.

#### Metathesis precursor **13a**

*N*<sup>α</sup>-Boc-protected asparagine amide **10a** (94 mg, 0.174 mmol) was treated with CF<sub>3</sub>CO<sub>2</sub>H (1.60 mL) and triethylsilane (0.30 mL) in CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) at room temperature (4 h), then volatiles were removed *in vacuo* and the residue was placed in a vacuum (30 minutes) to yield the CF<sub>3</sub>CO<sub>2</sub>H amine salt **19a**. This was dissolved in DMF (2.0 mL) and added to a pre-formed active ester solution prepared by stirring dipeptide acid **18** (70 mg, 0.116 mmol) in DMF (2.0 mL) with HOAT (19 mg, 0.140 mmol), EDCI (28 mg, 0.140 mmol) and NEt(*i*-Pr)<sub>2</sub> (91 μL, 0.522 mmol) at room temperature (15 minutes). The resulting reaction mixture was stirred at room temperature (2 days) then diluted with ethyl

acetate (150 mL), washed with H<sub>2</sub>O (2 × 50 mL) and brine (50 mL) and dried (Na<sub>2</sub>SO<sub>4</sub>). Purification by silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub> and methanol) provided **13a** (85 mg, 91% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.35 (t, *J* = 6.0 Hz, 1 H), 7.24 (dd, *J* = 8.4, 2.0 Hz, 2 H), 7.19 (d, *J* = 8.4 Hz, 2 H), 7.11 (d, *J* = 8.4 Hz, 1 H), 6.78 (br s, 1 H), 6.28 (s, 1 H), 5.89–5.76 (m, 2 H), 5.56 (br s, 1 H), 5.12–4.97 (m, 4 H), 4.49 (dd, *J* = 13.4, 7.0 Hz, 1 H), 3.46 (t, *J* = 9.4 Hz, 1 H), 3.25 (m, 1 H), 3.18 (m, 1 H), 3.07 (m, 1 H), 3.00 (dd, *J* = 21.4, 3.4 Hz, 2 H), 2.79 (m, 2 H), 2.64 (m, 2 H), 2.14–2.08 (m, 4 H), 1.78–1.57 (m, 5 H), 1.52–1.27 (m, 29 H), 1.13 (m, 2 H), 0.60 (m, 1 H). FAB-MS (+VE) *m/z*: 803.5 (M + H)<sup>+</sup>. HR-FABMS calcd for C<sub>42</sub>H<sub>67</sub>N<sub>4</sub>NaO<sub>9</sub>P (M + Na)<sup>+</sup>: 825.4543. Found: 825.4568.

### Macrocyclic final product 5a

A solution of tripeptide **13a** (50 mg, 0.063 mmol) in 1,2-dichloroethane (20 mL) was degassed under argon (5 minutes) then Grubbs 2<sup>nd</sup> generation catalyst [(PCy<sub>3</sub>)(Im(Mes)<sub>2</sub>)Ru=CHPh]**20**<sup>24</sup> (26 mg, 0.032 mmol) was added and the mixture was refluxed (2 days). The mixture was concentrated and purified by silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub> and methanol) to provide the brown crude product **21a**. This was treated with a mixture of CF<sub>3</sub>CO<sub>2</sub>H (9.0 mL), triethylsilane (0.50 mL) and H<sub>2</sub>O (0.50 mL) at room temperature (2 h). The solvent was removed and the residue was purified by reverse phase preparative HPLC using a Phenomenex C<sub>18</sub> column (21 mm diameter × 250 mm, cat. no: 00G-4436-P0) using a linear gradient from 0% aqueous acetonitrile (0.1% CF<sub>3</sub>CO<sub>2</sub>H) to 100% acetonitrile (0.1% CF<sub>3</sub>CO<sub>2</sub>H) over 35 minutes at a flow rate of 10.0 mL min<sup>-1</sup> (detection at 225 nm). Lyophilization provided the macrocyclic final product **5a** as a white solid (15 mg, 39% yield from **13a**). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.50 (s, 1 H), 8.32 (d, *J* = 8.0 Hz, 1 H), 7.57 (br s, 1 H), 7.31 (d, *J* = 8.4 Hz, 2 H), 7.20–7.13 (m, 4 H), 5.78 (dd, *J* = 15.0, 10.0 Hz, 1 H), 5.56 (m, 1 H), 4.26 (m, 1 H), 4.09 (d, *J* = 8.4 Hz, 1 H), 3.55 (m, 1 H), 3.28 (d, *J* = 11.6 Hz, 1 H), 2.93 (d, *J* = 21.2 Hz, 2 H), 2.83 (dd, *J* = 15.8, 5.0 Hz, 1 H), 2.76 (m, 1 H), 2.53 (m, 1 H), 2.33 (dd, *J* = 15.2, 4.8 Hz, 1 H), 2.20–1.95 (m, 4 H), 1.90–1.70 (m, 3 H), 1.60–1.40 (m, 7 H), 1.20 (m, 1 H). FAB-MS (–VE) *m/z*: 605.2 (M – H)<sup>–</sup>. HR-FABMS calcd for C<sub>28</sub>H<sub>40</sub>N<sub>4</sub>O<sub>9</sub>P (M + H)<sup>+</sup>: 607.2533. Found: 607.2558.

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