Letters

Synthesis of a 5-Methylindolyl-Containing Macrocycle That Displays Ultrapotent Grb2 SH2 Domain-Binding Affinity

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Abstract: The growth factor receptor-bound protein 2 (Grb2) is an SH2 domain-containing docking module that represents an attractive target for anticancer therapeutic intervention. Here, a ring-closing metathesis approach is utilized to synthesize a 5-methylindolyl-containing tetrapeptide mimetic (**6**) that exhibits unprecedented in vitro Grb2 SH2 domain-binding affinity (K_d = 93 pM). Key to the preparation of **6** is the enantioselective synthesis of (2*S*)-2-(3-(5-methylindolyl)methyl)pent-4-enylamine (**12**) as one of two ring-closing segments.

Induction of conformational constraint through macrocyclization is a potentially useful technique for enhancement of biological potency.[1,2] This is exemplified by the recent macrocyclization of a Grb2 SH2 domain-binding tetrapeptide mimetic using ring-closing metathesis (RCM) on a simplified phosphatase-stable variant of the high-affinity Grb2 SH2 domain-binding tetrapeptide mimetic **1**[3] that lacked a substituent at the pTyr mimetic α-position (**2**, Figure 1).[4] Although analogues such as **3**[5] containing carboxylic acid functionality at the pTyr mimetic α-position had been shown to exhibit enhanced Grb2 SH2 domain-binding potency in whole cell systems, for reasons of synthetic accessibility the first RCM-derived macrocycle (**4**) lacked such important α-functionality. Even though it was structurally simplified, **4** exhibited good Grb2 SH2 domain-binding affinity in extracellular ELISA-based assays (IC_{50} = 20 nM). However, disappointing potency was observed in whole cells ($IC_{50} \geq 10\ \mu$M).[6] More recently, the fully elaborated macrocycle **5** bearing an α-carboxymethyl group was prepared and found to have high Grb2 SH2 domain-binding potency in both extracellular (IC_{50} = 2 nM) and whole cell assays ($IC_{50} \leq 1\ \mu$M).[7] This was significant, since the issue of potency in whole cell systems is central to SH2 domain-signaling inhibitor development because of the unique requirement for high SH2 domain-binding affinity of anionic phosphate-mimicking functionality[8,9] and the limitations, which this typically presents for cell membrane transit. Inhibitor **5** could be termed a "second generation" analogue that results from modification of the lower pTyr-mimicking portion of the ring-closing olefin segment (Figure 1). To further extend this genre of RCM macrocyclization, it was of interest to modify functionality residing on the upper ring-closing olefin segment. Accordingly, efforts were undertaken to replace the naphthyl ring of **5** with a 5-methylindolyl group. As reported herein, the resulting macrocycle (**6**) has been found to exhibit unprecedented Grb2 SH2 domain-binding potency.

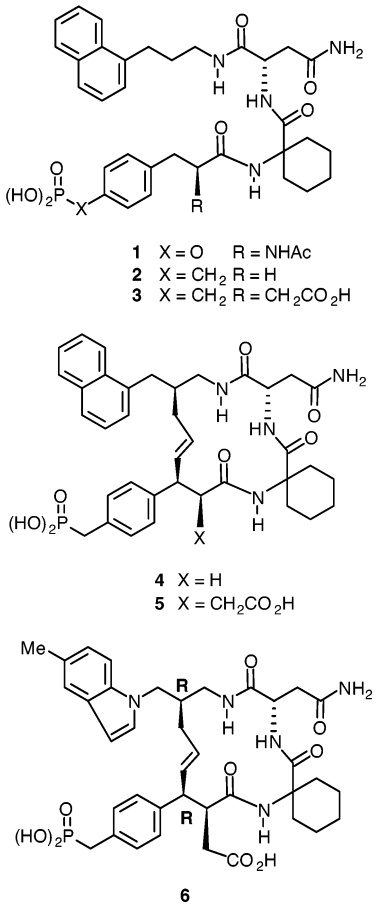

Figure 1. Structures of synthetic Gr2 SH2 domain-binding analogues.

Synthesis. Key to the construction of title compound **6** was the enantioselective synthesis of methylindolyl-containing pentenylamine **12** bearing the *R*-configuration (Scheme 1).[19] Stereochemistries at both upper and lower macrocycle ring junctures were determined previously as being most appropriate for binding to the Grb2 SH2 domain.[6,7] Starting from 3-(5-methylindolyl)propanoic acid (**7**),[11] chiral induction was achieved by coupling with commercially available (*S*)-4-phenyl-1,3-oxazolidin-2-one[12] using trimethylacetyl chloride mixed anhydride coupling in the presence of *n*-BuLi, (92% yield) to yield oxazolidine **8**. This was subjected to α-alkylation using lithium hexamethyldisilylamide (LHMDS) and 3-iodopropene to provide **9** (69% de, 61% yield; Scheme

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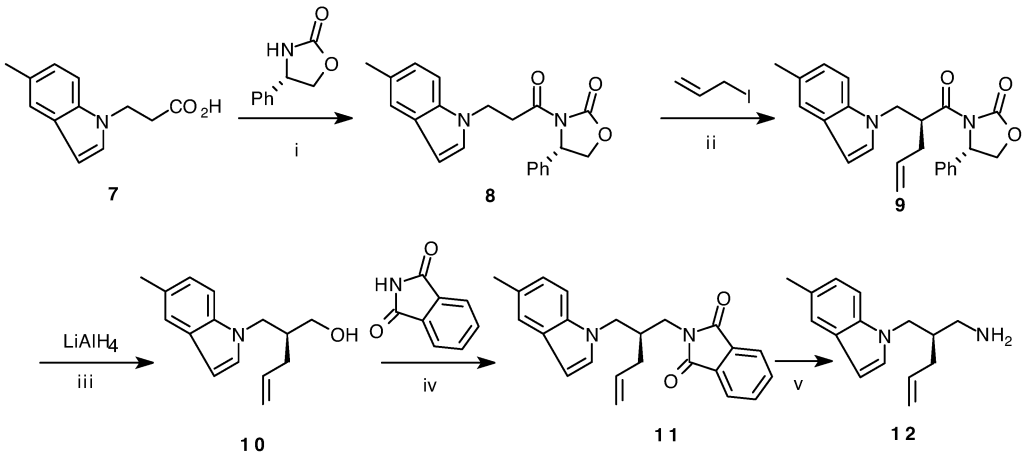
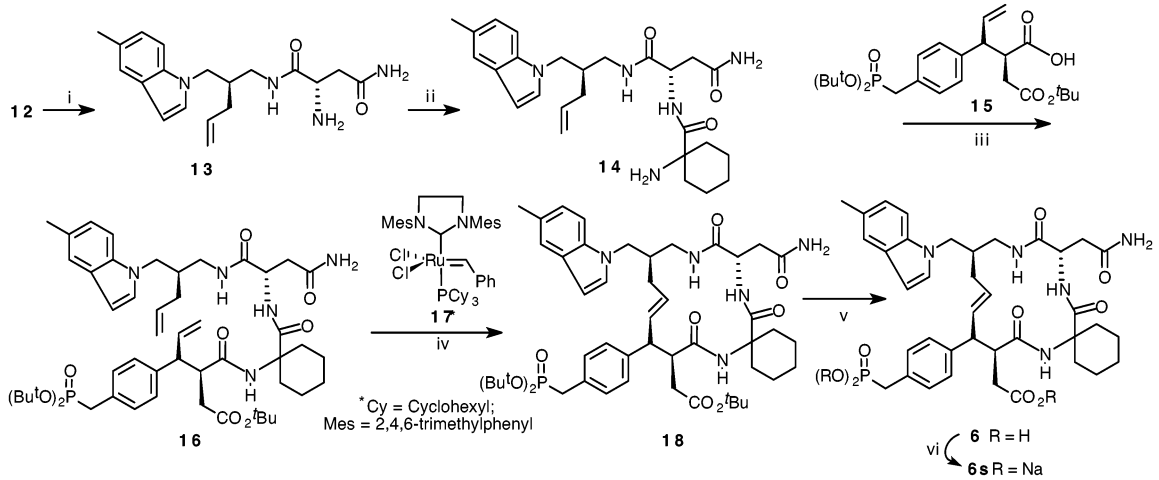
Scheme 1[a]



[a] Reagents and conditions: (i) Me_3CCOCl, *N*-methylmorpholine, BuLi, THF, −78 °C, 2 h, (87% yield); (ii) LHMDS, THF, −78 °C, 2 h, (69% de in 61% yield); (iii) $LiAlH_4$, THF, −78 °C, 1 h, then 0 °C, 3 h, (92% yield); (iv) DIAD, Ph_3P, THF, room temp, 12 h, (88% yield); (v) $N_2H_4{\cdot}H_2O$, EtOH, H_2O, reflux, 3 h, (84% yield).

Scheme 2[a]



[a] Reagents and conditions: (i) (a) Boc-Asn-OH, DIPCDI, HOBt, DMF, room temp, 12 h; (b) $HCl_{(aq)}$ (2 N), CH_3CN, room temp, 12 h (75% yield); (ii) (a) *N*-Fmoc Ac_6c, EDCI·HCl, HOBt, DMF, room temp, 12 h (83% yield); (b) piperidine, CH_3CN, room temp, 2 h (86% yield); (iii) **15**,[7] EDCI·HCl, HOAt, DMF, 50 °C, 24 h (56% yield); (iv) **17**, CH_2Cl_2, reflux, 48 h (70% yield); (v) TFA−$HS(CH_2)_2SH$−H_2O, room temp, 1 h (35% yield); (vi) aqueous $NaHCO_{3(aq)}$ (quantitative).

1). The unexpectedly low chiral induction was attributed to potential chelation interference by the indolyl nitrogen atom. The undesired (3*S*)-diastereomer could be removed by careful column flash chromatography. This approach was the inverse of that used to prepare the corresponding naphthyl-containing analogue, where alkylation of 4-pentenoyl-derivatized (*R*)-4-phenyl-1,3-oxazolidin-2-one was achieved using a 1-(bromomethyl)-naphthalene group.[6] Lithium aluminum hydride reduction of **9** at −78 °C afforded a mixture of alcohol and aldehyde. However, warming to 0 °C provided complete reduction, yielding **10** as the sole product. The synthesis of **12** was completed in a two-step process by Mitsunobu-mediated phthalimide coupling with **10** that yielded **11**, which was subjected to hydrazine reflux (74% combined yield).

With the upper 5-methylindolyl-containing ring-closing segment (**12**) in hand, synthesis of metathesis precursor **16** was accomplished next (Scheme 2). Unexpectedly, coupling of **12** with *N*-Fmoc Asn-OH failed, even with the use of 1-hydroxy-7-azabenzotriazole (HOAt). However, coupling of *N*-Boc Asn-OH with diisopropylcarbodiimide (DIPCDI) in the presence of 1-hydroxybenzotriazole (HOBt) proceeded successfully. Subsequent attempted Boc deprotection using TFA in organic solvent resulted in the generation of highly colored decomposition byproducts. Use of aqueous 2 N HCl in acetonitrile cleanly provided the free amine **13** in 72% yield from **12**. Coupling of **13** with commercially available *N*-Fmoc 1-aminocyclohexanecarboxylic acid (*N*-Fmoc Ac_6c) followed by piperidine-mediated N-deprotection gave **14** in good yield. Condensation of **14** with known **15**[7] proved troublesome. This was attributed to steric interaction of the Ac_6c ring with the bulky α-(CH_2-$CO_2{}^t$Bu) group. However, use of active ester coupling (HOAt and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI·HCl)) gave satisfactory yields of **16** (56%) if conducted at elevated temperatures (50 °C). Finally, metathesis ring closure was achieved using second generation Grubbs' catalyst [((PCy$_3$)(Im-(Mes)$_2$)Ru=CHPh) (**17**))][12,13] to yield protected macrocycle **18** (70% yield) exclusively as the trans isomer (vinylic coupling constant J = 15.0 Hz). Global removal of ^{t}Bu ester protecting groups using TFA in the presence

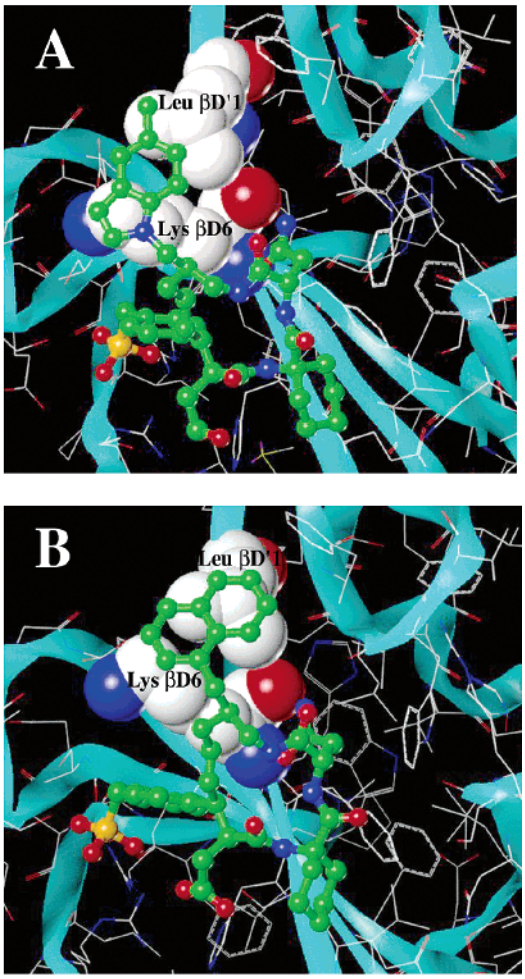


Figure 2. Molecular mechanics docking of macrocycles **6** (A) and **5** (B) into the Grb2 SH2 domain highlighting potential interactions of ligands (ball-and-stick rendering) with a hydrophobic patch of the protein (CPK rendering).

of 1,2-ethanedithiol gave HPLC-purified **6**, which was converted to its trisodium salt **6s**.

Grb2 SH2 Domain Binding Interactions. To approximate potential binding interactions of **6** with the Grb2 SH2 domain, molecular modeling studies were carried out using MacroModel 8.0 (Schroding, L.L.C.) and Sybyl 6.9 (Tripos, Inc.) on a Silicon Graphics Octane 2 workstation based on the previously reported X-ray structure of a phosphohexapeptide bound to the Grb2 SH2 domain (PDB entry 1TZE[14]). Low-energy binding modes were compared for naphthyl-containing **5** and 5-methylindolyl-containing **6** (Figure 2). Both the indolyl (**6**) and the naphthyl (**5**) rings are involved in hydrophobic interactions with the side chains of residues Lys βD6 and Leu βD′1.[3,15] However, the 5-methyl substituent of **6** participated in more extended contacts with the hydrophobic area of the protein without observable steric clashes. It has also been previously reported that electron enrichment of aromatic ring systems by electron-donating substituents, such as methyl or hydroxyl, result in enhanced CH/π interactions.[15] On the basis of these modeling results, the indolyl analogue **6** was expected to exhibit higher affinity than the corresponding naphthyl analogue **5**.

Affinity of macrocycle **6** for the Grb2 SH2 domain protein was measured using surface plasmon resonance

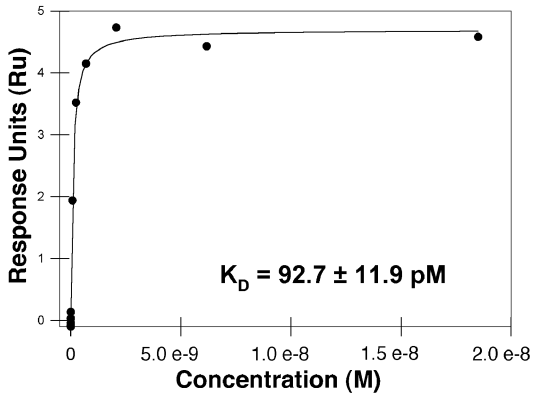


Figure 3. SPR-derived steady-state binding of **6s** to Grb2 SH2 domain protein immobilized by amine coupling to a carboxymethylated dextran-coated sensor chip.

(SPR) performed on Biacore 2000 and Biacore 3000 instruments (Biacore, Inc., Piscataway, NJ). Previously reported SH2 domain SPR studies have provided indirect determinations of K_d values by measuring the ability of test ligands to compete with sensor chip-bound reference pTyr-peptide for binding to Grb2 SH2 domain protein in solution.[16,17] The current protocol examined direct binding of **6s** to Grb2 SH2 domain protein immobilized to the sensor chip. Binding affinity determined in this fashion has consistently provided K_d values in the range ~60−90 pM, with a typical value of $K_d = 75 \pm 16$ pM ($n = 6$) having been reported recently.[18] For this previously reported set of experiments, the K_d value was calculated as the ratio of K_{off}/K_{on}, where a value of $K_{on} = 4.7 \times 10^8$ s^{-1} appeared to indicate that binding was diffusion-limited. In the current study, the very high binding affinity was supported by steady-state analysis of the rate at which binding saturation was achieved. This provided a K_d value of 92.7 ± 11.9 pM (Figure 3). This is significantly more potent than the naphthyl-containing congener **5** ($K_d = 0.91$ nM[18]), and to our knowledge, it is the highest affinity yet reported for a synthetic Grb2 SH2 domain-binding ligand.

A characteristic of the previously reported naphthyl-containing macrocycle **5** was its ability to exhibit high potency in whole cell assays without the need for prodrug protection or use of carrier peptide motifs.[7] In similar whole cell assays, 5-methylindolyl-containing **6** behaves in similar fashion, only with significantly higher potency. Recent studies have shown that **6** is able to block the association of Grb2 with cytoplasmic erbB-2 with an apparent IC_{50} value of ≤10 nM and to inhibit the growth-factor-promoted growth of MDA-MB-453 breast cancer cells with an IC_{50} value of 0.6 μM.[18]

Conclusions. Using ring-closing metathesis, synthesis of 5-methylindolyl-containing macrocyclic tetrapeptide mimetic **6** has been achieved. Macrocycle **6** exhibits a low-picomolar Grb2 SH2 domain-binding affinity in extracellular assays while exerting blockade of Grb2 association with cognate intracellular proteins in whole cell assays at low nanomolar concentrations. This is achieved without the use of prodrug protection or carrier peptide motifs. The ability of **6** to elicit antimitogenic effects in growth-factor-driven breast cancer cells at noncytotoxic submicromolar concentrations may indi-

cate the potential therapeutic utility of this class of signal-transduction-altering agent.

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Supporting Information Available: Synthetic procedures and spectral characterization for compounds **6**, **6s**, **8**−**14**, **16**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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