Identification and Specificity Studies of Small-Molecule Ligands for SH3 Protein Domains

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The Src Homology 3 (SH3) domains are small protein−protein interaction domains that bind proline-rich sequences and mediate a wide range of cell-signaling and other important biological processes. Since deregulated signaling pathways form the basis of many human diseases, the SH3 domains have been attractive targets for novel therapeutics. High-affinity ligands for SH3 domains have been designed; however, these have all been peptide-based and no examples of entirely nonpeptide SH3 ligands have previously been reported. Using the mouse Tec Kinase SH3 domain as a model system for structure-based ligand design, we have identified several simple heterocyclic compounds that selectively bind to the Tec SH3 domain. Using a combination of nuclear magnetic resonance chemical shift perturbation, structure−activity relationships, and site-directed mutagenesis, the binding of these compounds at the proline-rich peptide-binding site has been characterized. The most potent of these, 2-aminoquinoline, bound with K_d = 125 μM and was able to compete for binding with a proline-rich peptide. Synthesis of 6-substitued-2-aminoquinolines resulted in ligands with up to 6-fold improved affinity over 2-aminoquinoline and enhanced specificity for the Tec SH3 domain. Therefore, 2-aminoquinolines may potentially be useful for the development of high affinity small molecule ligands for SH3 domains.

Introduction

Src Homology 3 (SH3) domains are small, highly conserved protein−protein interaction domains found within a wide range of proteins throughout eukaryotes. The function of the domain is to mediate binding of specific proline-rich sequences on partner proteins, thereby allowing the build-up of complex signaling networks within the cell. These networks are involved in a wide range of biological processes including controlling cell proliferation, apoptosis, cytoskeletal rearrangements and other signal transduction pathways.[1,2] More than 400 examples of the SH3 sequence motif are encoded in the human genome with many proteins containing more than one copy of the SH3 domain in their sequence.

The 3D structure of a large number of SH3 domains, some in complex with ligands, have been determined. The canonical SH3 domain consists of two perpendicular β-sheets that form a β-barrel. The peptide-binding site can be subdivided into two regions: the hydrophobic grooves that accommodate the proline core of the ligand (PxxP) and the specificity pocket that binds the charged residues at the end of the ligand sequence.[3] An unusual consequence of the left-handed 3_{10} helix geometry of proline-rich sequences is that the ligand can potentially lie across this binding site in either N−C or C−N orientations.[3] The relative positions of the positively charged amino acids within the ligand sequence dictate which orientation is allowed through interactions with the specificity pocket. In Class I ligands (R/KxxPxxP) the charged residues are at the N-terminus of the consensus and contain an extra residue preceding the proline motif. Class II ligands (PxxPxR/K), however, have the charged residues at the C-terminus of the consensus. These ligand−protein interactions are generally weak, with biologically relevant partners having measured affinities in the range 1−100 μM.[4]

As key regulators of a large number of intracellular cell signaling processes, SH3 domains are an important class of potential therapeutic target. Despite significant interest in designing small molecule inhibitors for SH3 domains, little work has been published in this area, and the only novel ligands reported have been largely composed of the traditional proline-rich peptide sequences. One approach has been to replace one or more of the core proline residues within a proline-rich peptide sequence with nonnatural N-substituted amino acids. This resulted in significantly higher affinity ligands (K_d as low 30 nM) that showed up to 3 orders of magnitude selectivity across a limited set of SH3 domains.[5,6] In these examples, the N-substituents were able to make hydrophobic contacts not normally satisfied by the smaller proline ring, thereby explaining the significant improvements in affinity over the corresponding peptide ligand. An alternative strategy has been to attach nonpeptide moieties at the termini of consensus proline-rich sequences. These moieties were able to replace either the peptide residues that targeted the specificity pocket, and/or the residues (including proline) that targeted the 'terminal' PxxP binding grooves of the

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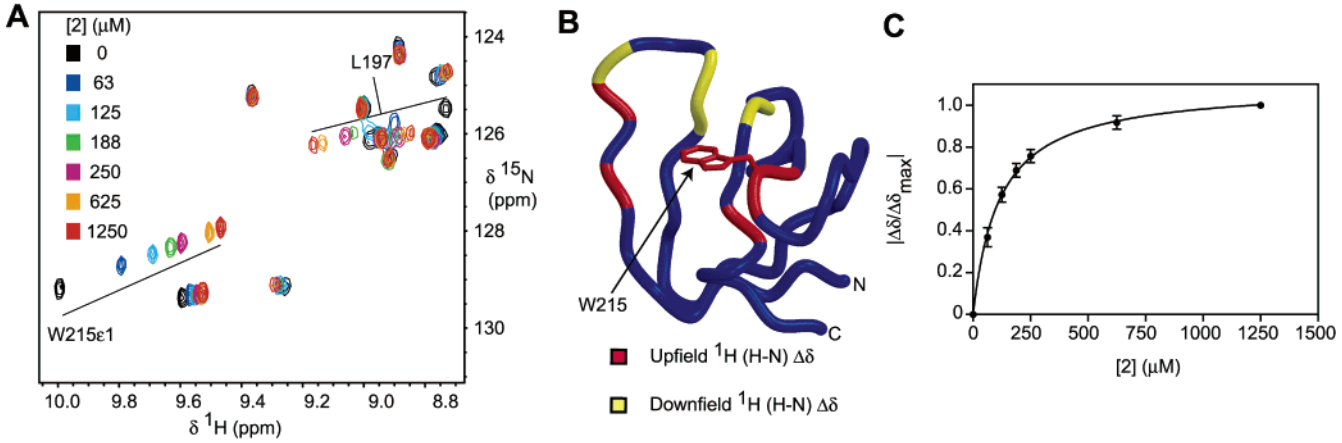


Figure 1. Binding of **2** to the Tec SH3 domain using NMR spectroscopy. (A) A region of overlaid NMR [^{1}H,^{15}N]-HSQC spectra of ^{15}N-labeled Tec SH3 protein in the presence of increasing concentrations of **2**. (B) Chemical shift mapping of backbone or side-chain (H−N) resonances where the δ ^{1}H had changed by at least 0.1 ppm at or near saturation binding of **2**. (C) Binding isotherm represented by normalized chemical shift changes for residues involved in binding of **2**.

Table 1. Ligand Binding Results and pK_as for First Series of Compounds Tested for Tec SH3 Binding

ligand	K_d (μM)[a]	EC_{50} (μM)[b]	pK_a	ligand	K_d (μM)[a]	EC_{50} (μM)[b]	pK_a
1	800 ± 170	-	4.82[19]	**8**	no binding	-	-
2	125 ± 24	160 ± 36	7.34[20]	**9**	no binding	-	-
3	650 ± 90	-	7.62[21]	**10**	no binding	-	4.32[22]
4	no binding	-	3.54[23]	**11**	380 ± 40	-	-
5	>4000	-	6.86[20]	**12**	no binding	-	-
6	-	215 ± 90	-	**13**	-	no competition	-
7	no binding	-	-				

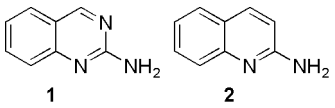
[a] Quoted values are average ± standard deviation over residues where ^{1}H (H−N) chemical shifts changes of protein were 0.1 ppm or greater, at or near saturation binding of ligand. [b] Quoted values are mean ± standard deviation over three replicate experiments.

peptide binding surface.[7,8] The key to this approach is that the peptide portion provides the anchor and orients the molecule in the binding site, and the large surface area buried by the nonpeptide component aids in binding affinity. While both of the above strategies produced molecules with improved affinity and specificity over native peptide ligands, the resulting ligands are too large and peptide like to be useful lead molecules for therapeutic purposes. More recently, small molecule compounds have been reported that inhibit proline-rich peptide/SH3 interactions but the mechanism of this inhibition is thought to be mediated by binding of the small molecules to the proline-rich peptide.[9,10]

We have used the mouse Tec IV Kinase SH3 domain as a model system for structure-based ligand design. Tec is the prototypic member of a family of intracellular tyrosine kinases that include Btk, Itk, Txk and Bmx.[11,12] These enzymes are expressed mainly in hematopoietic tissues and are thought to play important roles in growth and differentiation processes in immune cells such as monocytes and B and T lymphocytes. These proteins are composed of multiple domains with the activity of the C-terminal catalytic domain modulated by the more N-terminal pleckstrin homology, zinc-binding Btk motif, SH3 and SH2 domains. These regulatory domains also provide subcellular localization and substrate specificity functions for the protein. The region between the Btk motif and the SH3 domain of Tec contains proline-rich sequences, that play regulatory roles through both inter- and intramolecular interactions with the SH3 domain.[13]

We have used a structure-based design approach to target the Tec SH3 ligand-binding surface and focused initially on the residues in the specificity pocket. Here, we report the first identification and SAR studies of selective, entirely nonpeptide SH3 ligands.

Discovery of Small Molecule Ligands for the Tec SH3 Domain. Using the solution structure of the mouse Tec IV SH3 domain,[13,14] in silico screens of small molecule fragments were performed using LUDI.[15] From these studies, 2-aminoquinazoline **1** was predicted to bind the Tec SH3 domain at conserved residues in the specificity pocket of the proline-rich peptide binding site. 2-Aminoquinoline **2**, structurally related to **1**, was also selected for initial binding experiments.



The binding of both these compounds to the Tec SH3 domain was demonstrated by NMR chemical shift perturbation experiments using [^{1}H,^{15}N]-heteronuclear single quantum coherence (HSQC)[16] spectra with ^{15}N-labeled SH3 protein (Figure 1A). For all protein ^{1}H and ^{15}N (H−N) chemical shifts that were altered upon ligand binding, only one signal was observed, the population weighted average resonance between bound and unbound states, indicating a fast exchange process (Figure 1A). Mapping the chemical shift changes induced by **1** or **2** onto the SH3-fold (see for **2** in Figure 1B) suggested that both ligands were binding in the same tightly focused region on the surface, consistent with the site specified by the LUDI ligand design. Residues whose ^{1}H (H−N) chemical shifts were altered by at least 0.1 ppm at or near saturation binding were used as monitors for calculating equilibrium binding dissociation constants (K_d) (Figure 1C). Ligand **1** bound the SH3

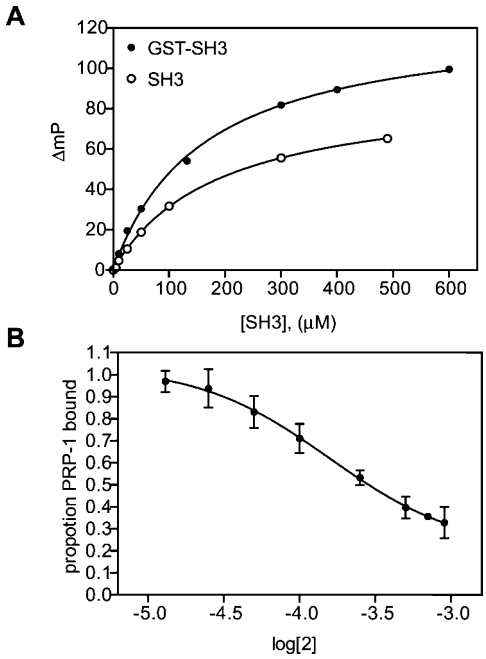
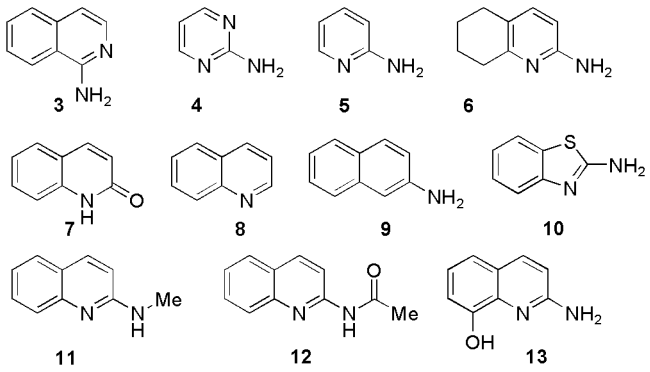


Figure 2. Fluorescence Polarization studies of Tec SH3 domain with fluorescently labeled proline-rich peptide [fluorescein-βA-RRPPPPIPPE-CO_2H (**PRP-1**)]. (A) Equilibrium binding isotherms of SH3 and GST-SH3 fusion proteins to **PRP-1**. (B) Isotherm for competition of **PRP-1** by **2** from the TEC GST-SH3 fusion protein.

domain with K_d = 800 μM; however, **2** bound with a 6-fold improvement in affinity (K_d = 125 μM) (Table 1).

To investigate whether **2** was able to compete for SH3 binding with a proline-rich peptide, a fluorescence polarization (FP) peptide displacement assay[17,18] was established. A proline-rich peptide derived from a sequence that has previously been used in binding studies with the Tec SH3 domain[13] was synthesized with fluorescein attached to the N-terminus via a β-alanine linkage (fluorescein-βA-RRPPPPIPPE-CO_2H, here after referred to as **PRP-1**). Using FP, equilibrium binding dissociation constants (K_d) for **PRP-1** were determined by measuring the change in millipolarization units (ΔmP) with increasing concentrations of the SH3 and the GST-SH3 fusion proteins (Figure 2A). **PRP-1** bound to both the Tec SH3 and the Tec GST-SH3 fusion proteins with similar affinity (K_d SH3 = 190 $\pm$ 6 μM, K_d GST-SH3 = 160 $\pm$ 10 μM). This indicates that the presence of the GST-fusion partner has no influence on the binding of **PRP-1** to the SH3 domain. In addition to removing the need to cleave and separate the GST-fusion partner, using a molecule with a larger molecular mass for an FP assay provides the additional advantage of increased signal-to-noise and therefore enhanced sensitivity. Hence, for all remaining FP experiments the GST-SH3 fusion protein was used. The ligand concentration required for 50% displacement of **PRP-1** (EC_{50}) from the SH3 domain for **2** was determined by maintaining a constant concentration of **PRP-1** and GST-SH3 protein, but varying the concentration of **2**. By then plotting the normalized ΔmP (proportion **PRP-1** bound) against the concentration of **2** (Figure 2B), the EC_{50} is calculated following nonlinear regression analysis. Compound **2** was able to compete with **PRP-1** for SH3 binding with an EC_{50} of 160 $\pm$ 35 μM (Figure 2B, Table 1), providing confirmation of overlap between the binding sites of **2** and **PRP-1**.

Development of Model for 2-Aminoquinoline/SH3 Domain Binding. A further 11 compounds (**3**−**13**) with structural similarities to **1** and **2** were also tested for binding using either the NMR or the FP assay methods described above, and a further four ligands were identified (Table 1). The isomer of **2**, 1-aminoisoquinoline **3**, bound the SH3 domain with similar affinity (K_d = 650 μM) to **1**. The single ring equivalent of **1**, 2-aminopyrimidine **4**, did not bind the SH3 domain; however, the single ring equivalent of **2**, 2-aminopyridine **5**, bound weakly (K_d > 4000 μM). 2-Amino-5,6,7,8-tetrahydroquinoline **6**, in which the second ring is in the reduced form bound the SH3 domain with similar affinity (EC_{50} = 215 μM) to **2**. Quinolin-2(1*H*)-one **7**, in which the functionality at the 2-position differs to **1** and **2**, was unable to bind the SH3 domain. Furthermore, if the 2-amino functionality was removed as for quinoline **8**, or the ring nitrogen was removed as in 2-aminonaphthalene **9**, or if the ring shape was changed as in 2-aminobenzothiazole **10**, binding was also abolished. A 3-fold reduction in binding affinity resulted from amino-*N*-methylation of **2** (**11**, K_d = 380 μM); however, when the basicity of 2-aminoquinoline was substantially altered by amino-*N*-acetylation (**12**) binding was again abolished. Addition of a hydroxyl group at the 8-position of the quinoline ring, as in 2-amino-8-hydroxyquinoline **13**, was also detrimental to binding.



The largest change in NMR ^{1}H (H−N) chemical shift observed for the protein on binding of all of the ligands, with the exception of **1**, was the indole side-chain (H−N) signal of tryptophan (W215ϵ1), a highly conserved residue in the proline-rich peptide binding site of SH3 domains. This upfield ^{1}H (H−N) shift ($\Delta\delta$ = − 0.53 ppm for **2**) (Figure 1A) suggests the aromatic π cloud of the ligands shields the W215ϵ1 proton, and the two ring systems are therefore involved in π−π stacking. However, given that a range of other aromatic compounds were unable to bind to the SH3 domain, π−π stacking alone is not sufficient for binding to occur. Consultation of pK_a values available in the literature (Table 1) indicates that apart from **1**, all the ligands are substantially protonated under the experimental conditions (pH $\sim$ 6.5 for NMR chemical shift perturbation experiments, pH 7.3 for FP peptide competition assay). The similarity in structure, yet large differences in K_ds and pK_as of **1** and **2** (Table 1), suggests that protonation of the ligand may be important for binding. Inspection of the region adjacent to the W215 residue indicates there is an

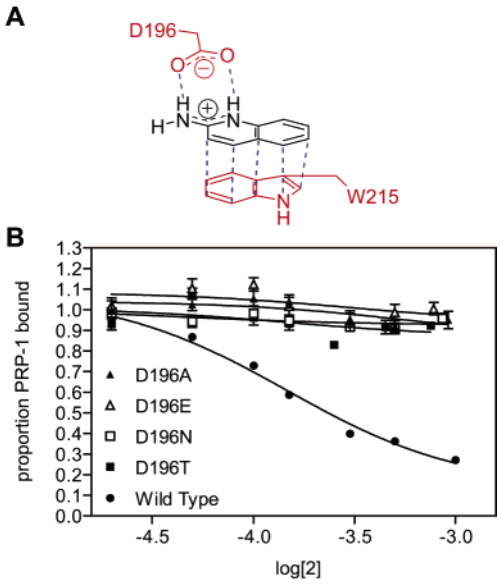


Figure 3. (A) Proposed model for binding of **2** with the Tec SH3 domain. (B) Binding isotherms for competition of **PRP-1** by **2** from wild-type and mutant Tec GST-SH3 proteins.

aspartic acid residue (D196) that is sufficiently close for additional interactions with ligands that may bind the SH3 domain in this location. Specifically, this residue may form a hydrogen bond or a salt bridge with positively charged ligands that can contribute to $\pi-\pi$ stacking in this site. Our results indicate that only mono or bicyclic heteroaromatic systems with a pyridyl type nitrogen atom ortho to an amino group (**1**-**3**, **5**, **6** and **11**) are able to bind the Tec SH3 domain. Therefore a preliminary model for the mechanism of binding can be developed, involving combined ligand/W215 side-chain $\pi-\pi$ stacking, and a salt-bridge between the amino group of the ligand and the proximal D196 side-chain (Figure 3A).

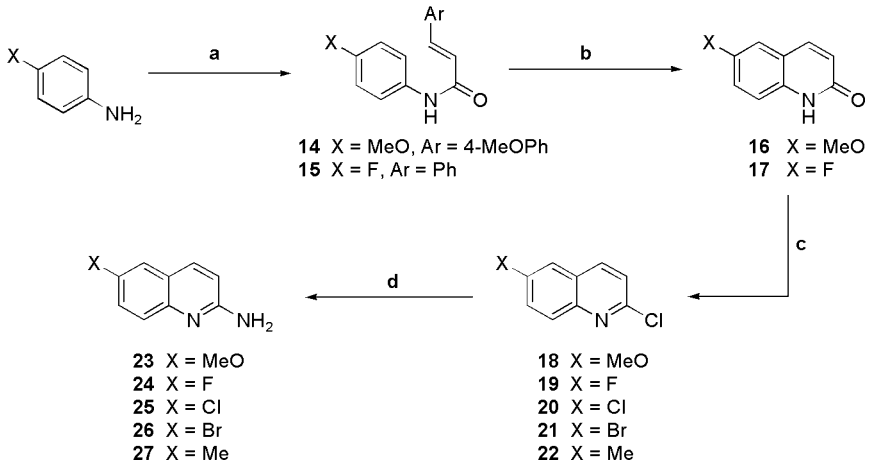
Using this model, further explanations for some of the observations can be offered. In the case of **1**, because there is no significant protonation of the ligand, the electrostatic interaction with D196 is weaker than for **2**, and this results in the lower affinity observed for **1**. Ligands **3** and **5** are also significantly protonated under the experimental conditions; however, **3** has similar affinity for the SH3 domain to **1**, (K_ds: **1**; 800 μM, **3**; 650 μM, Table 1), and **5** has even lower affinity (K_d > 4000 μM). These results may be explained by considering the differences in the ring systems of **3** and **5**. Ligand **3** has the positions of the amino group and the ring nitrogen atoms swapped relative to **2**, which results in the ring systems of **3** and W215 not overlapping as effectively as for **2**, assuming that the relative orientation of the atoms involved in salt-bridge formation remains the same. In the case of **5**, the hydrophobic contact area available for $\pi-\pi$ stacking is considerably reduced due to the absence of the additional ring, again resulting in lowered affinity. Similarly, loss of ligand protonation in conjunction with loss of the second ring as for **4** abolishes binding. If the additional ring is in a reduced form as for **6**, then there is not a significant impact on binding affinity, suggesting that bicyclic systems are preferred, but aromaticity of both of the rings is not essential. Using these arguments, **10** may have been expected to bind with similar affinity to **1**, but clearly the smaller size and/or the different electronic character of this heterocycle results in loss of binding. Some support for the importance of the D196 residue of the SH3 domain for ligand binding is provided by considering the results obtained for **11** and **12**. Substitution on the amino group is tolerated but results in 3-fold reduced affinity in the case of **11** where the substituent is a methyl group, suggesting that a stronger interaction is made with a primary amine. However, if the basicity of the amino group is substantially altered by substitution with an acetyl group as for **12**, binding is abolished altogether, providing additional evidence for the importance of protonation in the binding process. The observation that **13** does not bind the SH3 domain provides some evidence for the orientation of the quinoline ring in the binding process. Specifically, according to the model, the 8-position on the quinoline ring faces into the protein surface, and substituents here were predicted to not be tolerated on steric grounds.

Of all the compounds tested at this stage, **2** was the highest affinity SH3 ligand. This indicates that ligand binding is optimal when the amino group is at the 2-position of a six-membered bicyclic system and is unsubstituted and there is substantial protonation on the pyridyl nitrogen. Since the ligand binding observed is in fast exchange on the NMR time scale, there is inadequate time for intermolecular nuclear Overhauser effect (NOE) build-up and transfer between the ligand and SH3 domain. This makes the complex unsuitable for 3D structure determination by NMR methods. [^{1}H,^{15}N]-HSQC experiments were attempted at lower temperatures; however, the binding remained in fast exchange (data not shown).

To test the role of D196 in binding of **2** to the Tec SH3 protein, a series of mutants (D196A, D196E, D196N and D196T) were prepared as GST fusion proteins. These proteins bound **PRP-1** with reduced affinity (data not shown); however, **2** was unable to displace **PRP-1** from any of these mutant proteins (Figure 3B). This indicates that a negatively charged polar group is required at the D196 position and that neutral polar groups (D196N, D196T), groups of different size (D196A, D196E, D196T) or completely nonpolar groups (D196A) are detrimental to binding. These results provide additional evidence for the importance of D196 for binding of **2** and support for the proposed ligand-binding model in the absence of a 3D structure of the complex. No changes in either ^{1}H or ^{15}N (H−N) NMR chemical shift were observed when [^{1}H,^{15}N]-HSQC experiments were performed on the D196A SH3 protein in the presence of **2**, confirming single site binding of this ligand (data not shown).

Discovery of 2-Aminoquinoline Derivatives with Improved Affinity. Using the established 2-aminoquinoline/SH3 binding model (Figure 3A), 2-aminoquinoline derivatives with substitution at the 6-position of the quinoline ring were predicted to make contacts with regions adjacent to the W215 residue of the Tec SH3 protein as illustrated in Figure 4. This region contains both hydrophilic (N211, D212, H214) and hydrophobic (L213) surface residues. To investigate the types of contacts that could be made between the ligand and the protein in this region, simple substituents (e.g.,

Scheme 1. Method for Synthesis of 2-Aminoquinolines **23**−**27**[a]



[a] (a) Ar−CH=CHCOCl/pyridine/DMAP/CH_2Cl_2; (b) **14** P_2O_5/H_3PO_4/110 °C; **15** $AlCl_3$/melt. (c) $POCl_3$/60−105 °C; (d) $AcNH_2/K_2CO_3$/~200 °C.

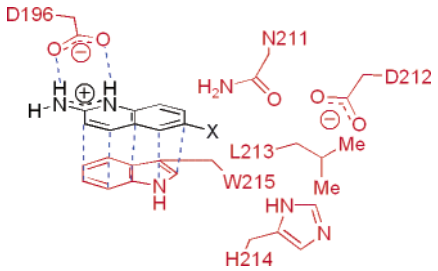


Figure 4. Schematic representation of 2-aminoquinoline/Tec SH3 ligand binding model and residues predicted to make possible contacts with 2-aminoquinoline derivatives with substituents at the 6-position of the quinoline ring.

methyl, halogen, oxygen, etc.) at the 6-position were initially sought.

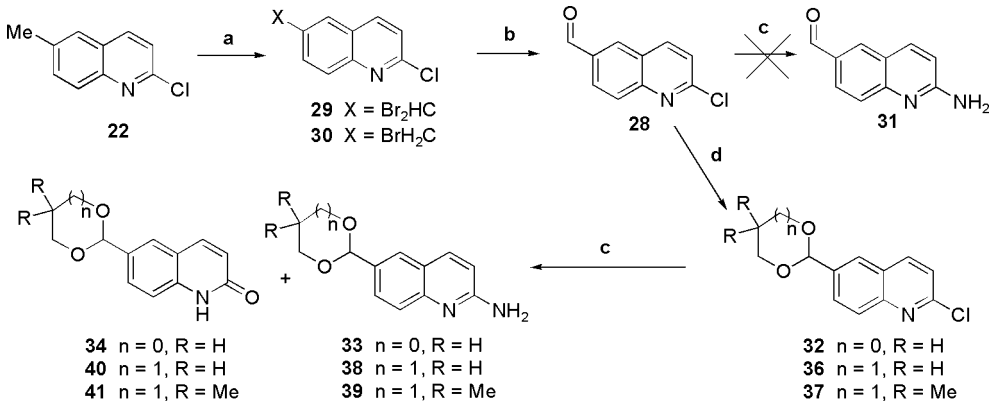
Chemistry. 2-Aminoquinolines with simple substituents at the 6-position (e.g. halogen, methyl) can easily be prepared from 4-substituted anilines by conversion to *N*-arylcinnamanilides, followed by cyclization to 6-substituted-quinolin-2(1*H*)-ones with either aluminum chloride[24,25] or polyphosphoric acid,[26] followed by treatment with phosphoryl chloride[25,27] to obtain 2-chloroquinolines. Treatment of 2-chloroquinolines with acetamide near reflux temperature gives 2-aminoquinolines.[28] For example, cinnamanilides **14** and **15** were prepared by treatment of the 4-substituted anilines with the appropriate cinnamoyl chlorides under basic conditions as shown in Scheme 1. Amide **14** was converted to 6-methoxyquinolin-2(1*H*)-one **16** by treatment with polyphosphoric acid according to the method of Johnston,[26] as it has been reported that treatment of *N*-4-methoxycinnamanilide with aluminum chloride results in formation of the demethylated cinnamanilide as the only product.[25] Amide **15** was converted to 6-fluoroquinolin-2(1*H*)-one **17** by treatment with aluminum chloride as a melt according to the method of Chambard.[24] The quinolin-2(1*H*)-ones **16** and **17** were converted to 2-chloroquinolines **18** and **19** by treatment with an excess of phosphoryl chloride at either 60 °C overnight or for 1 h at reflux temperature. The remaining 2-chloroquinolines **20**,[25] **21**[25] and **22**[24,27] were prepared according to literature methods.

2-Chloroquinolines **18**−**22** were converted to 2-aminoquinolines **23**−**27** in low to reasonable yields (29−54%) using the method of Kóródi[28] (Scheme 1), and as expected, the ^{1}H NMR spectra of the crude isolates indicated that small amounts (~5−10%) of the corresponding 6-substituted-quinolin-2(1*H*)-ones were also formed. The purified amines were readily obtained following silica gel chromatography.

To synthesize 2-aminoquinolines with more complex functionality, it was envisaged that 2-chloro-6-formylquinoline **28** (Scheme 2) might be a versatile intermediate from which a range of new compounds may be prepared. Benzylic oxidation was therefore achieved by treatment of 2-chloro-6-methylquinoline **22** with 2 equiv of NBS in the presence of a catalytic amount of benzoyl peroxide and heating at reflux in benzene, an adaption of the method of Newman[29] (Scheme 2). Benzene was found to be a suitable alternative solvent to carbon tetrachloride, the traditional solvent for this reaction. The crude product from this reaction was purified by chromatography with silica gel, and the major isolate was an approximately 6:1 mixture of 2-chloro-6-dibromomethylquinoline **29** and 6-bromomethyl-2-chloroquinoline **30** (Scheme 2), as judged by ^{1}H NMR. An additional 5% of pure **30** was also isolated. The mixture of **29** and **30** was then treated with an excess of hexamethylenetetramine in aqueous ethanol at reflux temperature, again an adaption of the method of Newman,[29] and following workup pure 2-chloro-6-formylquinoline **28** was isolated in 52% overall yield from **22**. An attempt was made to convert **28** to 2-amino-6-formylquinoline **31** using the amination conditions of Kóródi;[28] however, only low recovery of an insoluble material was observed (Scheme 2).

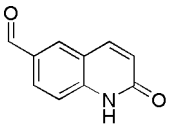
Aldehyde **28** was therefore protected as a dioxolane acetal **32** in good yield (77%) by treatment with an excess of ethylene glycol in benzene with a catalytic amount of *p*-toluenesulfonic acid at reflux temperature (Scheme 2). Acetal **32** was stable to the amination conditions of Kóródi,[28] and pure 6-(1,3-dioxolan-2-yl-)quinolin-2-ylamine **33** could be isolated following the usual work up and chromatography system. A small amount of the 6-(1,3-dioxolan-2-yl-)quinolin-2(1*H*)-one **34** byproduct (Scheme 2) was also isolated; however, some conversion of this acetal to 6-formylquinolin-2(1*H*)-one **35** was evident by ^{1}H NMR, most likely a result of traces of hydrochloric acid present in the deuterated

Scheme 2. Strategy for Synthesis of 2-Aminoquinolines with More Complex Functionality[a]



[a] (a) NBS/$(PhCO_2)_2$/benzene/Δ; (b) hexamethylenetetraamine/EtOH/H_2O/Δ; (c) $AcNH_2/K_2CO_3$/∼200 °C; (d) $HOCH_2CR_2(CH_2)_nOH$/*p*-TosOH/benzene/Δ.

chloroform. Prior to any attempts to convert **33** into 2-amino-6-formylquinoline **31**, **33** was tested for binding to the Tec SH3 domain, and an unexpected improvement in binding was found (see below). Therefore, two additional acetals **36** and **37** were synthesized by varying the diol used in the acetal formation reaction (Scheme 2), and both of these were similarly converted to 2-aminoquinolines **38** and **39** for ligand binding studies. The quinolin-2(1*H*)-one byproducts **40** and **41** were also isolated from these amination reactions.



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6-Substituted-2-aminoquinolines/Tec SH3 Binding Studies. All of the 6-substituted 2-aminoquinolines prepared were tested for binding to the Tec SH3 domain using either the fluorescence polarization (FP) peptide displacement assay and/or the NMR chemical shift perturbation assay described in the earlier sections. 2-Aminoquinolines **23**−**27**, **33** and **38** were tested using the FP method. Of these, **23** and **25**−**27** all competed with **PRP-1** for binding to the SH3 domain with approximately two to 3-fold improved affinity relative to 2-aminoquinoline **2** (EC_{50} ca. 60−75 μM for **23** and **25**−**27**; EC_{50} = 160 μM for **2**) (Figure 5A, Table 2). However, 2-amino-6-fluoroquinoline **24** competed for binding with similar affinity (EC_{50} = 150 μM) to **2** (Table 2). 2-Aminoquinolines **33** and **38** were able to compete with the proline-rich peptide for SH3 binding with a further improvement in EC_{50} relative to **23** and **25**−**27** and an approximately five to 6-fold improvement in affinity relative to **2** (EC_{50} ca. 30 μM for **33** and **38**) (Figure 5A, Table 2).

To complement the results obtained from fluorescence polarization peptide displacement experiments, **27**, **33** and **38** were also tested for binding to the Tec SH3 domain using the NMR chemical shift perturbation method described previously with uniformly labeled ^{15}N protein. In addition **39** was tested using this method, as it was poorly soluble in the phosphate-buffered saline system used in the FP method. The NMR chemical shift perturbation method involves a 10% DMSO buffer system that assists with aqueous solubility of hydrophobic compounds. FP peptide binding experiments using a 10% DMSO buffer system led to an approximately 4-fold decrease in affinity of **PRP-1** for the SH3 domain (data not shown), making this approach less

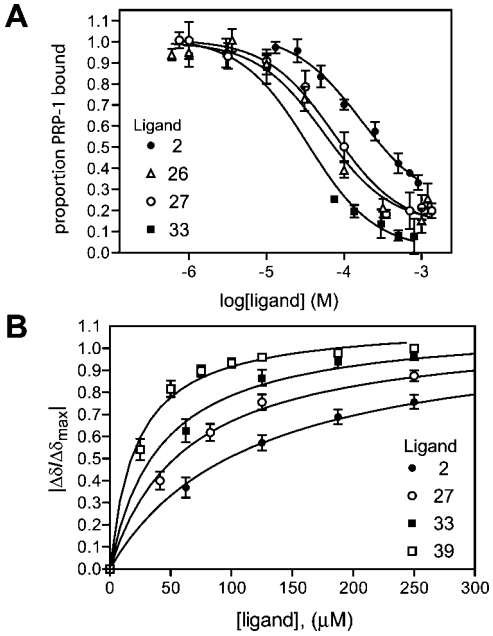

Figure 5. 6-Substituted-2-aminoquinolines/Tec SH3 binding studies. (A) Overlays of isotherms obtained from independent experiments for competition of fluorescent proline-rich peptide **PRP-1** by **2**, **26**, **27** and **33** from Tec GST-SH3 protein using the fluorescence polarization assay. (B) Overlays of isotherms obtained from independent experiments, represented by normalized chemical shift changes for residues involved in binding of **2**, **27**, **33**, and **39** to Tec SH3 domain as determined by NMR chemical shift perturbation experiments.

Table 2. Summary of Tec SH3 Binding Studies with 6-Substituted 2-Aminoquinolines

ligand	EC_{50} (μM)[a]	K_d (μM)[b]	ligand	EC_{50} (μM)[a]	K_d (μM)[b]
2	160 ± 36	125 ± 24	**27**	75 ± 15	61 ± 6
23	63 ± 22	-	**33**	34 ± 5	40 ± 8
24	150 ± 36	-	**38**	26 ± 6	52 ± 16
25	76 ± 8	-	**39**	-	22 ± 5
26	58 ± 15	-			

[a] Quoted values are mean ± standard deviation over three replicate experiments. [b] Quoted values are mean ± standard deviation over residues where ^1H (H−N) chemical shift changes of protein exceeded 0.1 ppm at or near saturation binding of ligand.

Table 3. Specificity of 2-Aminoquinolines for the Selected SH3 Domains As Determined by FP Proline-Rich Peptide Displacement Experiments

ligand	Tec[b] EC_{50} (μM)[a]	Nck[b] EC_{50} (μM)[a]	Hck[c] EC_{50} (μM)[a]	Fyn[b] EC_{50} (μM)[a]
2	160 ± 35	150 ± 30	>1000	no competition
27	75 ± 15	90 ± 12	-	-
33	34 ± 5	300 ± 30	-	-
38	26 ± 6	>500	-	-

[a] Quoted values are mean ± standard deviation over three replicate experiments. [b] **PRP-1** displacement. [c] **PRP-2** displacement.

efficient for determining EC_{50} values for ligands poorly soluble in buffer alone. Importantly, for NMR experiments involving **33**, **38** and **39** the ^{1}H (H−N) chemical shifts for the side chain of W215 and the backbones for other residues close to the ligand binding site exhibited an intermediate instead of fast exchange process at early stages of the titrations, as evidenced by either complete loss of or substantial drops in the intensity of the H−N cross-peaks at ligand concentrations less than 1 molar equiv of the protein (see Supporting Information). For ease of comparison, only residues that exhibited the fast exchange process were used for calculation of equilibrium binding constants. There was a small decrease in the intensity of the W215 side chain H−N cross-peak for the experiments involving **27** at 0.33 molar equiv of ligand (data not shown); however, the fast exchange assumption was retained in this case. **27** bound the Tec SH3 domain with equilibrium binding dissociation constant (K_d) of 61 μM (Figure 5B, Table 2) while **33**, **38** and **39** bound with K_ds of 40, 52, and 22 μM, respectively (Figure 5B, Table 2). These results suggest that **33** and **38** bind with similar affinity, but improved affinity relative to **27**; however, **39** binds with a further improvement in affinity relative to **33** and **38**.

All of the presented structure activity information for the 6-substituted-2-aminoquinolines suggests that a new lipophilic contact is made between the substituents on the quinoline ring and the protein. When a simple lipophilic group is placed in the 6-position (e.g., MeO, Cl, Br, Me as in **23** and **25**−**27**), a two to 3-fold improvement in affinity is obtained; however, if a smaller lipophilic group is present (e.g., F or H as for **24** and **2**), this contact is not made. These results also suggest that electronegative substituents at the 6-position have little or no effect on the ligand binding process according to the ligand binding model (Figure 3A). When more complex and larger (cyclic-acetal) groups are placed at the 6-position of the quinoline ring (**33**, **38** and **39**), a further improvement in affinity is obtained, and this interaction is optimal in the case of **39**, that has extra methyl groups that may make additional contacts with the protein surface. Currently, **39** is the highest affinity Tec SH3 domain ligand we have identified (K_d = 22 μM) and has 6-fold improved affinity relative to unsubstituted 2-aminoquinoline **2**. However, ligand **39** remains unsuitable for ligand/protein structure determination using NMR methods, as slow exchange ligands are required to allow adequate time to transfer magnetization from the ligand to the protein to perform nuclear Overhauser experiments for structure calculations.

Although the highest affinity ligands (**33**, **38** and **39**) identified in these studies provide useful SAR information and are good starting points for developing ligands with improved potency, the acetal functionality is not 'druglike' in character. Specifically, acetals hydrolyze to carbonyl compounds under acidic aqueous conditions. To determine whether a substantial amount of hydrolysis of these acetals was occurring during the protein binding experiments, the rate of hydrolysis of **33** to form aldehyde **31** was investigated using NMR spectroscopy. A dilute sample of **33** was made in a 10% DMSO/phosphate buffer system identical to the conditions used for the NMR K_d determinations. Similarly a sample that mimicked the conditions of the FP assay system was also prepared. ^{1}H NMR experiments were recorded over a time course of several hours and the relative percentage of **31** present was calculated by comparing the relative integrations of the ^{1}H signals representing the acetal and the aldehyde protons. In the case of the 10% DMSO sample, only 3−4% of **31** was present after 4.5 h and about 5% of **31** was present after 8 h (see Supporting Information). Given that a typical series of experiments for determining the K_d of a ligand by NMR is of the order of 8 h, this amount of hydrolysis is not expected to have a significant impact on the accuracy of the K_d determinations for **33**, **38** and **39**. In the case of the sample that mimicked the FP conditions, approximately 7% **31** was present after 4 h, and after 10 h this had risen to 9%. It is likely that the slightly higher amount of **31** present at the earlier times in this case is a result of the heating required to assist dissolving the ligands in the aqueous medium. A typical FP experiment takes of the order of 3 h between commencing to dissolve a ligand and recording measurements. Therefore this level of aldehyde present in the sample is again unlikely to substantially influence the accuracy of the EC_{50} values determined. Furthermore, it is expected that the rates of acetal hydrolysis for ligands **38** and **39** would be even lower due to the increased stability associated with dioxane acetals relative to dioxolane acetals; hence, even less influence of **31** is expected in determining the binding constants for these ligands.

Specificity Studies of 2-Aminoquinolines with Other SH3 Domains. Comparison of the mouse Tec IV SH3 domain with sequences from a range of human SH3 domains indicates that W215 and D196, two residues that we have identified as important for binding of **2** to the Tec SH3 domain, are highly conserved (Figure 6). These residues both play key roles in the binding of **PRP-1** to the Tec SH3 domain.[13] The tryptophan residue corresponding to W215 is almost completely conserved throughout all SH3 domain sequences (data not shown) consistent with its role as part of the hydrophobic surface that contacts one of the core proline residues within the PxxP ligand sequence. Greater variety is found at the position corresponding to D196, however, with other charged or hydrophilic residues including glutamate, threonine, asparagine and glutamine as common substitutes.

Given that we have shown the essential nature of D196 in Tec SH3 binding of **2**, it was of interest to determine whether **2** or its derivatives could bind other SH3 domains. Hck and Fyn are two members of the Src family of tyrosine kinase enzymes that have both of the conserved aspartate and tryptophan residues, while the

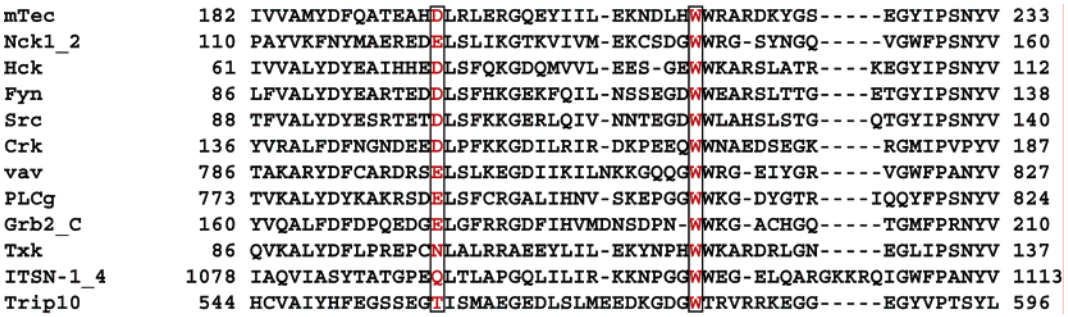

Figure 6. Sequence Alignments of mouse Tec (denoted mTec) SH3 domain with selected human SH3 domains, indicating the degrees of conservation of tryptophan (W215) and aspartatic acid (D196) residues of mTec, both important for binding of 2-aminoquinolines (residues of interest are shown in red, and boxed).

second of three SH3 domains from the Nck-1 protein [Nck-1(2)] has a glutamate and tryptophan at these positions.

The ability of **2** to displace fluorescently labeled proline-rich peptides was tested using the FP ligand-competition assay. **PRP-1** was used for experiments involving the Fyn and Nck-1(2) SH3 domains; however, a different fluorescent proline-rich peptide [fluorescein-βA-STPRPLPPLPTTR$-CO_2H$ (**PRP-2**)] was obtained for testing of the Hck SH3 domain. Ligand **2** binds Tec and Nck-1(2) domains with similar EC_{50} values (160 μM versus 150 μM) but **2** binds to the Hck SH3 domain poorly (EC_{50} > 1000 μM) and undetectably to the Fyn SH3 domain (see Table 3). This shows that in the correct context glutamate side chains can be tolerated at position 196, as in Nck-1(2); however, in the wrong context aspartate and tryptophan (as in Hck and Fyn) are not sufficient to facilitate **2** binding. These results also indicate that even with a simple heterocycle such as **2**, specificity between SH3 domains can be achieved.

To investigate whether the 6-substituted 2-aminoquinolines provide additional specificity, **27**, **33** and **38** were tested against the Nck-1(2) SH3 domain. Ligand **27** was able to compete for binding with **PRP-1** with similar affinity for the Nck-1(2) SH3 domain as it does for the Tec SH3 domain (EC_{50} = 90 μM for Nck and 75 μM for Tec); however, **33** and **38** with the much bulkier substituents were only able to compete weakly for the Nck-1(2) SH3 domain (EC_{50} = 300 μM and > 500 μM, respectively). When combined with the improved affinity of **33** and **38** for the Tec SH3 domain, it can been seen that approximately 10 to 20-fold selectivity between the Tec and Nck-1(2) SH3 domains can be achieved.

Conclusions

We have used computational methods to identify a lead compound, 2-aminoquinazoline **1** that binds weakly (K_d = 800 μM) to the Tec SH3 domain as illustrated by NMR chemical shift perturbation experiments using [^{1}H,^{15}N]-HSQC spectra. The structurally similar 2-aminoquinoline **2** bound with 6-fold improved affinity (K_d = 125 μM). SAR information in conjunction with site-directed mutagenesis studies has assisted in characterizing the ligand binding event and indicates that two residues (W215 and D196) in the Tec SH3 sequence that form part of the proline-rich peptide binding site are important for binding of these small molecules to occur. Furthermore, **2** is able to compete with a proline-rich peptide for SH3 binding as shown by a fluorescence polarization peptide displacement assay. By synthesizing both known and new 6-substituted-2-aminoquinolines, ligands with improved affinity relative to **2** have been identified. The highest affinity ligand 6-(5,5-dimethyl-1,3-dioxan-2-yl)quinolin-2-ylamine **39** bound the Tec SH3 domain with a K_d of 22 μM, a 6-fold improvement from **2** and a 36-fold improvement from **1**. Although ligand **39** has some non 'druglike' characteristics, the SAR information that is provided by this and the similar ligands **33** and **38** warrants further investigation of alternative functional groups with more 'druglike' character that may replace the acetal moiety yet retain or further improve the binding affinity. Additional functionality in the 6-position and/or other positions on the quinoline ring still awaits investigation.

To the best of our knowledge, this is the first report of entirely nonpeptide ligands for an SH3 domain supported by direct structural data. Furthermore, our preliminary specificity studies have indicated that **2** binds weakly to the Hck SH3 domain and binds with similar affinity to the Nck SH3 domain as it does for Tec. Ligand **2** was unable to bind to the Fyn SH3 domain. However, **33** and **38**, two of the higher affinity ligands for the Tec SH3 domain, bind weakly to the Nck SH3 domain. These results provide encouragement for the use of **2** as a scaffold for the development of high affinity ligands for a range of SH3 domains with engineered specificity.

Experimental Section

Testing of Compounds for Binding to the Tec SH3 Domain Using NMR Spectroscopy. Uniformly ^{15}N-labeled Tec SH3 domain was prepared according to developed methods.[14,30] Samples of the Tec SH3 domain for ligand binding experiments were made at 125 μM in 10 mM Na_2HPO_4, 10% v/v D_2O, 10% v/v d_6-DMSO, 0.01% w/v NaN_3 to a total volume of 600 μL, pH 6.6. NMR spectra were recorded on a Varian INOVA 600 Spectrometer (3 RF channels), using a 5 mm ^{1}H-{^{13}C/^{15}N} inverse triple resonance PFG probe fitted with *z*-axis gradients. Sensitivity-enhanced [^{1}H,^{15}N]-heteronuclear single quantum coherence (HSQC)[16] spectra were recorded at 25 °C with spectral widths of 8000 and 2000 Hz in F1 and F2, respectively, with 64 t_1 increments. The Fourier transformed data resulted in final matrix sizes of 1024 × 512 data points for F1 and F2, respectively.

Stock solutions of compounds to be tested were dissolved in d_6-DMSO at concentrations of ~0.5−1 M. Spectra were first recorded for the protein in the absence of any compounds, before compounds were titrated into the sample at concentrations varying between 0.2 and 1 mol equiv of the protein in 2 μL of d_6-DMSO. The pH was adjusted by the addition of small amounts of HCl or NaOH if necessary, and a new spectrum was recorded. This process was repeated with a range of ligand concentrations until no or only very small changes in protein

^{1}H (H−N) chemical shifts were observed (typical range of concentrations were 0.5, 1, 2, or 5 mol equiv of the protein, or greater for the lower affinity ligands). If no changes in chemical shift were observed when 10 mol equiv of compound were present, then the compound was deemed to not bind to the protein.

Processed NMR spectra were analyzed using SPARKY[31] centered at 4.72 and 120 ppm for F1 and F2, respectively, and chemical shifts for amino acids involved in binding of ligands over the range of concentrations were collected. For all residues whose δ ^{1}H (H−N) was altered upon ligand binding, $\Delta\delta = \delta_{PL} - \delta_{P0}$ values were calculated where $\delta_{PL} = \delta$ ^{1}H for the protein in the presence of ligand L at a given [L], and $\delta_{P0} = \delta$ ^{1}H for the unbound protein. The $\Delta\delta$ values for residues where $|\Delta\delta_{max}| \geq 0.1$ ppm (where $\Delta\delta_{max}$ is the maximum change in chemical shift observed for a particular residue of the protein) were plotted against ligand concentration from which nonlinear regression analyses were performed using GraphPad Prism[32] with a one-site hyperbola binding model and the default settings. The equilibrium binding dissociation constants (K_d) obtained for all the residues were then averaged, to give the overall K_d as the mean ± standard deviation over all residues where $|\Delta\delta_{max}| \geq 0.1$ ppm. For ease of comparison between ligands, the normalized shift $|\Delta\delta/\Delta\delta_{max}|$ was calculated for all residues where $|\Delta\delta_{max}| \geq 0.1$ ppm. The $|\Delta\delta/\Delta\delta_{max}|$ values over the range of ligand concentrations were then averaged and plotted against ligand concentration, with the error bars representing the standard deviation between the residues. Chemical shift mapping of residues involved in binding was performed using INSIGHT-II (Molecular Simulations Incorporated).

Fluorescence Polarization (FP) Peptide-Displacement Assay. Unlabeled Tec SH3 and GST-SH3 fusion proteins were prepared by a similar procedure to that indicated for SH3 NMR samples. To generate mutant SH3 proteins, plasmid DNA from the pGEX4T-2 expression vector was isolated from *E. coli* strain DH5α via a midi prep, and the aspartic acid (D196) residue was mutated (D196A, D196E, D196N, D196T) by site directed mutagenesis of the isolated DNA using newly synthesized primers. Nck, Hck and Fyn GST-SH3 constructs were prepared by inserting cDNAs representing the appropriate sequences into the pGEX4T-2 vector, and the correct sequences were confirmed by DNA sequencing. Samples of either SH3 or GST-SH3 proteins used for fluorescence polarization studies were prepared at concentrations of ∼1 mM in 12 mM Na_2HPO_4, 150 mM NaCl at pH 7.3 (phosphate buffer).

Fluorescence polarization[17,18] experiments were carried out with a BMG Laboratories PolarStar Galaxy Plate Reader, using black BMG 96-well plates, preblocked with 1% w/v casein for 2 h at 37 °C. The plate reader was set in polarization mode with 485 and 520 nm excitation and emission filters, respectively, and millipolarization units (mP) were calculated, where P is defined as; $P = (Int_{||} - Int_{\perp})/(Int_{||} + Int_{\perp})$ where $Int_{||}$ = intensity of emission in the plane parallel to excitation (channel 1), and $Int_{\perp}$ = intensity of emission in plane perpendicular to excitation (channel 2).[17] The gain was adjusted for channel 1 and 2 using 100 nM fluorescein in phosphate buffer (100 μL), such that an mP value of 35 was obtained.

Peptide binding experiments were performed in triplicate (five cycles for each replicate) with Fluorescein-β-A-RRPPP-PIPPE-CO_2H (**PRP-1**) maintained at 100 nM, and [SH3] or [GST-SH3] protein varied between 0 and 600 μM, with total well volumes of 100 μL. Millipolarization units (mP) were calculated as the average of the replicates over five cycles, and $\Delta mP = mP_{PT} - mP_T$ was calculated where mP_{PT} is the mP for the protein/**PRP-1** system at a given concentration of protein, and mP_T is the mP for free **PRP-1**. The derived ΔmP values were plotted as a function of protein concentration from which K_d values were determined using GraphPad Prism[32] with a one-site hyperbola binding model and the default settings. The reported values are the K_d ± standard error as determined in the Prism calculations.

PRP-1 displacement assays were performed in triplicate (five cycles for each replicate) with **PRP-1** maintained at 100 nM, and [GST-SH3] maintained at ∼50−130 μM (200 μM for mutant proteins). Stocks solutions of ligands were prepared in phosphate buffer at concentrations of 1−10 mM (heating required) prior to dilution. Concentrations of ligands were varied between 0 and 1500 μM, in the presence of the protein/**PRP-1** system (100 μL total). Changes in millipolarization, $\Delta mP = mP_L - mP_T$ were calculated for each replicate where mP_L is the mP for the protein/**PRP-1**/ligand system at a given ligand concentration, and mP_T is the mP for free **PRP-1.** Proportion of proline-rich peptide bound terms were calculated for each replicate with proportion bound = $\Delta mP_L/\Delta mP_0$, where $\Delta mP_L = \Delta mP$ for the system at a given ligand concentration, and $\Delta mP_0 = \Delta mP$ for the system when [ligand] = 0. Proportion bound was then plotted as a function of log[ligand] for each replicate, from which EC_{50} values were determined using GraphPad Prism[32] with a one-site competition binding model and the default settings. The stated EC_{50} values represent the mean ± standard deviation of the calculated EC_{50} values for the three replicate experiments. For ease of comparison between ligands, the proportion bound terms obtained for each replicate were averaged and plotted against log[ligand] with error bars representing the standard deviation between replicates.

The peptide competition experiments involving the Hck SH3 domain were performed using essentially the same procedure as described above using Fluorescein-βA-STPRPLPPLPTTR−CO_2H (**PRP-2**).

Chemistry. General. All solvents were distilled, dried and stored according to standard procedures.[33] Melting points were determined using a Kofler hot-stage apparatus equipped with a Reichart microscope, and values are uncorrected. Infrared spectra were recorded on an ATI Mattson Genesis FTIR spectrometer either as Nujol mulls or as liquid films between sodium chloride plates. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian Gemini-2000 spectrometer (^{1}H: 200.13 MHz, ^{13}C: 50.32 or ^{1}H: 300.13 MHz, ^{13}C: 74.47 MHz), or a Varian INOVA 600 spectrometer (^{1}H: 599.842 MHz, ^{13}C: 150.842 MHz). The Varian INOVA 600 spectrometer was fitted with a ^{1}H{^{13}C/^{15}N} inverse triple resonance PFG probe with *z*-axis gradients. Spectra were recorded as solutions in $CDCl_3$ [tetramethylsilane ($\delta_H = 0.0$) or $CDCl_3$ ($\delta_C = 77.7$) as internal standards], d_6-acetone [$\delta_H = 2.05$, $\delta_C = 29.9$ as internal standards], or d_6-DMSO [$\delta_H = 2.50$, $\delta_C = 39.5$ as internal standards]. Chemical shift values are given on the δ scale quoted in parts per million, followed by the integration, multiplicity, coupling constant *J* and assignment. The following abbreviations have been used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. ^{13}C signals for new compounds were assigned from heteronuclear multiple quantum correlation (HMQC), and heteronuclear multiple bond correlation (HMBC) experiments. Flash chromatography was performed using Scharlau Silica Gel 60, 230−400 mesh. Thin-layer chromatography (TLC) was performed on aluminum backed silica gel 60 plates (Merck) and were visualized under UV light (254 nm) or by staining with a $KMnO_4/K_2CO_3$ solution. Electron impact (EI) mass spectra were recorded using a ZAB 2HF mass spectrometer. High-resolution mass spectrometry was performed at Monash University (Victoria, Australia) or the University of Tasmania (Tasmania, Australia). Elemental analyses were performed at the University of Otago, (Dunedin, New Zealand).

Sources of Compounds. Compounds **2**−**5**, **7**−**10** and **13** were all obtained from commercial sources. Compounds **1,**[34] **6,**[35] **11,**[36] **12,**[37] **20**,[25] **21**[25] and **22**[24,27] were synthesized according to literature methods.

(2*E*)-*N*,3-Bis(4-methoxyphenyl)acrylamide 14. A solution of 4-methoxycinnamoyl chloride[38] (7.53 g, 38.3 mmol) in dichloromethane (25 mL) was added to a stirring mixture of pyridine (3.20 mL, 40.2 mmol) and DMAP (0.473 g, 3.87 mmol) in dichloromethane (20 mL) at 0 °C under a nitrogen atmosphere. The mixture was stirred for 15 min before a solution of 4-methoxyaniline (4.75 g, 38.6 mmol) in dichloromethane (20 mL) was added over 10 min. After stirring for 15 min at 0 °C, the mixture was allowed to warm to room temperature then stirred for a further 90 min. The mixture was diluted

with dichloromethane (65 mL), washed with 5% aqueous hydrochloric acid (3 × 100 mL), water (3 × 100 mL) and brine (100 mL) and dried (Na_2SO_4), and the solvent was removed to give the title compound **14** (8.98 g, 83%) as an off-white powder, mp 182−184 °C. Anal. ($C_{17}H_{17}NO_3$) C, H, N. HRMS (EI): $C_{17}H_{17}NO$ ($M^{+\bullet}$) calcd 283.1208, found 283.1213. IR (Nujol) υ/cm^{-1}: 3268 (NH), 1651 (C═O), 1610, 1602 (C═C). ^{1}H NMR (300 MHz, d_6-DMSO) δ: 3.73 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 6.41 (1H, d, J = 15.6 Hz, ═CH), 6.82−6.94 (4H, m, ArH), 7.44−7.54 (4H, m, ArH), 7.69 (1H, d, J = 15.4 Hz, ═CH), 10.01 (1H, s, NH). ^{13}C NMR (75 MHz, d_6-DMSO) δ: 55.1 (OCH_3), 55.2 (OCH_3), 113.8 (C3/C5), 114.4 (C3′/C5′), 119.8 (═CHCO), 120.5 (C2′/C6′), 127.3 (C1), 129.2 (C2/C6), 132.5 (═CHAr), 139.3 (C1′), 155.1 (C4), 160.4 (C4′), 163.3 (C═O). m/z (EI): 283 ($M^{+\bullet}$, 22%), 161 ($M^{+\bullet} - CH_3OC_6H_4NH$, 100), 133 ($M^{+\bullet} - CH_3OC_6H_4NHC{=}O$, 44), 123 ($M^{+\bullet} - CH_3OC_6H_4C{=}C{=}C{=}O$, 58).

(2*E*)-*N*-(4-Fluorophenyl)-3-phenylacrylamide 15. A solution of cinnamoyl chloride (3.0 g, 18.0 mmol) in dichloromethane (10 mL) was added to a stirring mixture of pyridine (1.46 mL, 18.00 mmol) and DMAP (0.22 g, 1.80 mmol) in dichloromethane (10 mL) at 0 °C under an inert atmosphere. The mixture was stirred for 15 min before a solution of 4-fluoroaniline (2.0 g, 18.0 mmol) in dichloromethane (10 mL) was added over 10 min. After being stirred for a further 15 min, the mixture was allowed to warm to room temperature. The precipitate formed was collected, washed with cold CH_2Cl_2 and dried to afford the title compound **15** (3.64 g, 84%) as fine white needles, mp 155−156 °C (lit. mp[26] 156 °C). IR (Nujol) υ/cm^{-1}: 3332, 3063, 1662, 1651, 1626, 1512; ^{1}H NMR (200 MHz, $CDCl_3$/d_6-DMSO) δ: 6.77 (1H, d, J = 15.6 Hz, ═CH), 6.96−7.05 (2H, m, H3/H5), 7.35−7.67 (8H, m, ArH & NH), 7.74 (1H, d, J = 15.6 Hz, ═CH). ^{13}C NMR (50 MHz, $CDCl_3$/d_6-DMSO) δ: 114.6 (d, $^2J_{CF}$ = 22.0 Hz, C3/C5), 120.9 (d, $^3J_{CF}$ =7.6 Hz, C2/C6), 121.4 (═CHCO), 127.1 (C2′/C6′), 128.3 (C3′/C5′), 129.0 (C4′), 134.5 (C1′), 134.8 (d, $^4J_{CF}$ = 3.0 Hz, C1), 140.2 (═CHAr), 158.2 (d, $^1J_{CF}$ = 240.9 Hz, C4), 163.7 (C═O).

6-Methoxyquinolin-2(1*H*)-one 16. A mixture of phosphoric acid (8.56 g, 85.6 mmol) and phosphorus pentoxide (4.59 g, 32.3 mmol) was heated with stirring at 80 °C until the mixture became homogeneous (∼2 h). Amide **14** (0.493 g, 1.74 mmol) was added, and the mixture was then heated at 110 °C for a further 2 h. After cooling to room-temperature, water (20 mL) was added then the mixture was extracted with chloroform (3 × 30 mL). The combined extracts were washed with water (2 × 75 mL) and dried, and then the solvent was removed to give the title compound **16** (0.211 g, 69%), mp 205−206 °C (lit. mp[39] 207−208 °C; lit. mp[40] 216−219 °C). IR (Nujol) υ/cm^{-1}: 3185, 1668, 1611. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.86 (3H, s, OCH_3), 6.73 (1H, d, J = 9.5 Hz, H3), 6.99 (1H, d, J = 2.4 Hz, H5), 7.16 (1H, dd, J = 2.4, 9.3 Hz, H7), 7.39 (1H, d, J = 9.3 Hz, H8), 7.77 (1H, d, J = 9.5 Hz, H4), 12.59 (1H, br s, NH). m/z (EI): 175 ($M^{+\bullet}$, 45%), 160 (42), 121 (87), 105 (43), 77 (58), 69 (78), 57 (83), 43 (100).

6-Fluoroquinolin-2(1*H*)-one 17. An intimate mixture of the amide **15** (3.52 g, 14.59 mmol) and aluminum chloride (5.84 g, 43.78 mmol) was heated rapidly to melting then heated at 100 °C for 1 h. After cooling to room temperature, iced water was added and the resultant precipitate was washed with water and 5% aqueous hydrochloric acid to give 6-fluoroquinolin-2(1*H*)-one **17** (2.07 g, 87%) which was used without further purification, mp 270−275 °C. HRMS (ESI): $C_9H_6FNNaO^+$ ($M + Na^+$) calcd 186.0331, found 186.0327. IR (Nujol) υ/cm^{-1}: 3159, 1681, 1661, 1615. ^{1}H NMR (200 MHz, $CDCl_3$/d_6-DMSO) δ: 6.78 (1H, d, J = 9.6 Hz, H3), 7.26−7.41 (3H, m, H5/H7/H8), 7.84 (1H, d, J = 9.6 Hz, H4), 11.61 (1H, br s, NH).^{13}C NMR (50 MHz, $CDCl_3$/d_6-DMSO) δ: 112.2 (d, $^2J_{CF}$ = 22.4 Hz, C5), 117.2 (d, $^3J_{CF}$ = 7.9 Hz, C8), 118.2 (d, $^2J_{CF}$ = 23.9 Hz, C7), 120.0 (d, $^3J_{CF}$ = 9.2 Hz, C4a), 122.6 (C3), 135.2 (C8a), 139.5 (d, $^4J_{CF}$ = 3.5 Hz, C4), 157.2 (d, $^1J_{CF}$ = 238.7 Hz, C6), 162.2 (C2). m/z (EI): 163 ($M^{+\bullet}$, 100%), 135 (50), 108 (31), 107 (30).

2-Chloro-6-methoxyquinoline 18. A mixture of quinolinone **16** (0.211 g, 0.88 mmol) and phosphorus oxychloride (2.0 mL, 21.8 mmol) was stirred at 60 °C overnight. The mixture was then poured onto ice and extracted with dichloromethane (3 × 30 mL). The combined extracts were washed with water (2 × 100 mL) and dried (Na_2SO_4), and the solvent was removed. The residue was chromatographed on silica gel using dichloromethane as eluant to provide the title compound **18** as small colorless crystals (0.106 g, 63%), mp 105−107 °C (lit. mp[41] 106−107 °C). IR (Nujol) υ/cm^{-1}: 1621, 1583. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.93 (3H, s, OCH_3), 7.07 (1H, d, J = 2.8 Hz, H5), 7.33 (1H, d, J = 8.6 Hz, H3), 7.38 (1H, dd, J = 2.8, 9.2 Hz, H7), 7.92 (1H, d, J = 9.2 Hz, H8), 7.99 (1H, d, J = 8.6 Hz, H4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 56.2 (CH_3O), 105 9 (C5), 123.1 (C3), 123.7 (C7), 128.5 (C4a), 130.5 (C8), 138.3 (C4), 144.6 (C8a), 148.6 (C2), 158.7 (C6).

2-Chloro-6-fluoroquinoline 19. The quinolinone **17** (1.5 g, 9.19 mmol) was suspended in phosphorus oxychloride (8.6 mL, 91.94 mmol) and heated at reflux for 1 h. After cooling, the excess phosphorus oxychloride was removed by distillation and then iced water was added. The precipitate formed was washed with water and dried to give the title compound **19** (1.25 g, 75%) as a pale brown solid. A small sample was purified by recrystallization from hexane to give small white needles, mp 100−104 °C. HRMS (ESI): $C_9H_6{}^{35}ClFN^+$ ($M + H^+$) calcd 182.0173, found 182.0168. IR (Nujol) υ/cm^{-1}: 1626, 1593. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.46 (1H, d, $^3J_{3,4}$ = 8.6 Hz, H3), 7.49 (1H, dd, $^3J_{5,F}$ = 8.6 Hz, $^4J_{5,7}$ = 2.7 Hz, H5), 7.56 (1H, ddd, $^3J_{7,8}$ = 9.3 Hz, $^3J_{7,F}$ = 8.3 Hz, $^4J_{5,7}$ = 2.7 Hz, H7), 8.07 (1H, ddd, $^3J_{7,8}$ = 9.3 Hz, $^4J_{8,F}$ = 5.3 Hz, $^5J_{4,8}$ = 0.6 Hz, H8), 8.11 (1H, dd, $^3J_{3,4}$ = 8.6 Hz, $^5J_{4,8}$ = 0.6 Hz, H4). ^{13}C NMR (50 MHz, $CDCl_3$/d_6-DMSO) δ: 112.7 (d, $^2J_{CF}$ = 22.1 Hz, C5), 120.3 (d, $^2J_{CF}$ = 23.1 Hz, C7), 122.9 (C3), 127.2 (d, $^3J_{CF}$ = 7.6 Hz, C4a), 129.9 (d, $^3J_{CF}$ = 7.5 Hz, C8), 136.4 (d, $^4J_{CF}$ = 3.4 Hz, C4), 144.8 (C8a), 154.9 (C2), 157.2 (d, $^1J_{CF}$ = 239.3 Hz, C6). m/z (EI): 183 ($M^{+\bullet}$ + 2, 32%), 181 ($M^{+\bullet}$, 100), 146 (93), 126 (19).

2-Chloro-6-formylquinoline 28. A mixture of recrystallized 2-chloro-6-methylquinoline **22** (3.18 g, 17.9 mmol), *N*-bromosuccinimide (6.37 g, 35.8 mmol), and benzoyl peroxide (0.433 g, 1.79 mmol) was heated at reflux in benzene (20 mL) for 4 h. After cooling, the benzene was removed under reduced pressure, and the residue was dissolved in dichloromethane (120 mL) and washed with 10% sodium bicarbonate (3 × 120 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed. The crude product (5.75 g) was chromatographed over silica gel using 4:1 dichloromethane/hexane as solvent to afford 3.85 g of a white solid that consisted of approximately a 6:1 mixture of 2-chloro-6-dibromomethylquinoline **29** (R_f 0.58) and 6-bromomethyl-2-chloroquinoline **30** (R_f 0.48) as judged by ^{1}H NMR. In addition, pure 6-bromomethyl-2-chloroquinoline **30** (0.234 g, 5%) was also isolated.

29: HRMS (ESI): $C_{10}H_7{}^{79}Br_2{}^{35}ClN^+$ ($M + H^+$) calcd 333.8634, found 333.8627. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.85 (1H, s, $CHBr_2$), 7.49 (1H, d, J = 8.6 Hz, H3), 7.94 (1H, d, J = 1.9 Hz, H5), 8.08 (1H, dd, J = 1.9, 9.0 Hz, H7), 8.11 (1H, d, J = 9.0 Hz, H8), 8.16 (1H, d, J = 8.6 Hz, H4). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 40.5 ($CHBr_2$), 124.1 (C3), 125.0 (C5), 126.5 (C4a), 130.2 (C8), 130.4 (C7), 138.8 (C4), 140.9 (C6), 148.7 (C8a), 152.8 (C2). m/z (EI): 338, ($M^{+\bullet}$ [$^{81}Br_2{}^{37}Cl$] − H, 15%), 337 ($M^{+\bullet}$ [$^{81}Br_2{}^{35}Cl$], 2), 336 ($M^{+\bullet}$ [$^{81}Br_2{}^{35}Cl$] − H, 15), 335 ($M^{+\bullet}$ [$^{81}Br^{79}Br^{35}Cl$], 4), 334 ($M^{+\bullet}$ [$^{81}Br^{79}Br^{35}Cl$] − H, 20), 333 ($M^{+\bullet}$ [$^{79}Br_2{}^{35}Cl$], 1), 258 ($M^{+\bullet}$ - Br, 41), 256 ($M^{+\bullet}$ - Br, 100), 254 ($M^{+\bullet}$ - Br, 80).

30: mp 140−150 °C. HRMS (ESI): $C_{10}H_8{}^{79}Br^{35}ClN^+$ ($M + H^+$) calcd 255.9529, found 255.9522. IR (Nujol) υ/cm^{-1}: 1625, 1586, 1499. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.65 (2H, s, CH_2Br), 7.4 (1H, d, J = 8.4 Hz, H3), 7.76 (1H, dd, J = 1.8, 8.7 Hz, H7), 7.81 (1H, d, J = 1.8 Hz, H5), 8.01 (1H, d, J = 8.7 Hz, H8), 8.07 (1H, d, J = 8.4 Hz, H4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 33.3 (CH_2Br), 123.6 (C3), 127.3 (C4a), 128 (C5), 130 (C8), 132.2 (C7), 137.2 (C6), 139.4 (C4), 148.1 (C8a), 152 (C2). m/z (EI): 259 ($M^{+\bullet}$ [$^{81}Br^{37}Cl$], 20%), 258 ($M^{+\bullet}$ [$^{81}Br^{35}Cl$] − H, 80), 257 ($M^{+\bullet}$ [$^{81}Br^{35}Cl$], 22), 256 ($M^{+\bullet}$ [$^{81}Br^{35}Cl$] − H, 93), 255 ($M^{+\bullet}$ [$^{79}Br^{35}Cl$], 14), 254 ($M^{+\bullet}$ [$^{79}Br^{35}Cl$] − H, 23), 178 ($M^{+\bullet}$ - Br, 32), 176 ($M^{+\bullet}$ - Br, 100).

The 6:1 mixture of **29** and **30** prepared above (3.85 g) and hexamethylenetetramine (4.42 g, 0.0316 mol) were heated at

reflux in 50% aqueous ethanol (20 mL) for 1 h at which point thin-layer chromatography indicated that all of the starting material had been consumed. After cooling, the mixture was diluted with water (10 mL), concentrated hydrochloric acid (2.1 mL) was carefully added over 5 min and the mixture was brought to reflux for a further 30 min. After cooling, the mixture was added to brine (40 mL), and extracted with dichloromethane (4 × 80 mL). The combined organic extracts were then washed with brine (250 mL) and dried (Na_2SO_4), and then the solvent was removed to provide 2-chloro-6-formylquinoline **28** as a white solid (1.77 g, 52% from **22**). This material was used for further synthesis without purification; however, a small amount was chromatographed over silica gel using 49:1 dichloromethane/ethyl acetate as solvent to obtain an analytical sample (mp 165−167 °C). Anal. ($C_{10}H_6ClNO$) C, H, N. IR (Nujol) υ/cm^{-1}: 1697, 1624, 1583. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.5 (1H, d, J = 8.7 Hz, H3), 8.13 (1H, d, J = 8.7 Hz, H8), 8.22 (1H, dd, J = 1.8, 8.7 Hz, H7), 8.27 (1H, d, J = 8.7 Hz, H4), 8.35 (1H, d, J = 1.8 Hz, H5), 10.19 (1H, s, CHO). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 124.4 (C3), 127 (C4a), 128.9 (C8), 130.6 (C7), 133.3 (C5), 135.3 (C6), 140.6 (C4), 151.3 (C8a), 154.4 (C2), 191.7 (CHO). m/z (EI): 193 (%), 192 ($M^{+\cdot}$ [^{37}Cl] − H, 45), 191 ($M^{+\bullet}$ [^{35}Cl], 100), 190 ($M^{+\bullet}$ [^{35}Cl] − H, 100), 164 ($M^{+\bullet}$ [^{37}Cl] − H − CO, 45), 162 ($M^{+\bullet}$ [^{35}Cl] −H − CO, 45), 127 (63).

General Procedure for Acetal Formation. 2-Chloro-6-formylquinoline **28**, the diol (1.1−2 equiv.) and *p*-toluenesulfonic acid monohydrate (0.05−0.1 equiv.) were heated at reflux in benzene (∼4 mL/mmol aldehyde) using a Dean Stark apparatus until thin-layer chromatography indicated that all the starting material had been consumed. After cooling, the benzene was removed under reduced pressure, the residue was taken up into dichloromethane, washed twice with water and once with brine and dried (Na_2SO_4) and the solvent was removed, to afford a product that was sufficiently pure for subsequent use. Small amounts were purified using silica gel chromatography to obtain analytically pure samples.

2-Chloro-6-(1,3-dioxolan-2-yl)quinoline 32. 2-Chloro-6-formylquinoline **28** (1.25 g, 6.56 mmol) was treated with ethylene glycol (0.814 g, 13 mmol) and *p*-toluenesulfonic acid monohydrate (0.062 g, 0.33 mmol) in 25 mL of benzene for 4.5 h as described above. After workup, the title compound **32** was isolated as a white solid (1.2 g, 77%). An analytical sample was obtained after chromatography with 49:1 dichloromethane/ethyl acetate as solvent, mp 99−102 °C. Anal. ($C_{12}H_{10}ClNO_2$) C, H, N. IR (Nujol) υ/cm^{-1}: 1587, 1497. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.07−4.23 (4H, m, H4′/H5′), 5.99 (1H, s, H2′), 7.41 (1H, d, J = 8.4 Hz, H3), 7.85 (1H, dd, J = 1.7, 8.7 Hz, H7), 7.94 (1H, d, J = 1.7 Hz, H5), 8.05 (1H, d, J = 8.7 Hz, H8), 8.13 (1H, d, J = 8.4 Hz, H4). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 66.2 (C4′/C5′), 103.8 (C2′), 123.4 (C3), 126.2 (C5), 127.0 (C4a), 129.4 (C8), 129.6 (C7), 137.6 (C6), 139.8 (C4), 148.9 (C8a), 151.9 (C2). m/z (EI): 237 ($M^{+\bullet}$ [^{37}Cl], 26%), 236 ($M^{+\bullet}$ [^{37}Cl] − H, 34), 235 ($M^{+\bullet}$ [^{35}Cl], 68), 234 ($M^{+\bullet}$ [^{35}Cl] − H, 67), 192 (37), 190 (100).

2-Chloro-6-(1,3-dioxan-2-yl)quinoline 36. 2-Chloro-6-formylquinoline **28** (1.65 g, 8.65 mmol) was treated with 1,3-propanediol (0.724 g, 9.52 mmol) and *p*-toluenesulfonic acid monohydrate (0.083 g, 0.43 mmol) in 25 mL of benzene for 5 h as described above. After workup, the title compound was isolated as a white solid (1.68 g, 78%). An analytical sample was obtained after chromatography with 24:1 dichloromethane/ethyl acetate as solvent, mp 111−113 °C. Anal. ($C_{13}H_{12}ClNO_2$) C, H, N. IR (Nujol) υ/cm^{-1}: 1582, 1568, 1502, 1456. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.51 (1H, dtt, J = 1.4, 2.6, 13.6 Hz, H5′$_{eq}$), 2.27 (1H, dtt, J = 5.0, 12.4, 13.6 Hz, H5′$_{ax}$), 4.02−4.11 (2H, m, H4′/H6′), 4.30−4.36 (2H, m, H4′/H6′), 5.67 (1H, s, H2′), 7.39 (1H, d, J = 8.6 Hz, H3), 7.86 (1H, dd, J = 1.7, 8.7 Hz, H7), 7.97 (1H, d, J = 1.7 Hz, H5), 8.03 (1H, d, J = 8.7 Hz, H8), 8.11 (1H, d, J = 8.6 Hz, H4). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 26.4 (C5′), 68.2 (2 x C4′/C6′), 101.4 (C2′), 123.3 (C3), 125.6 (C5), 127.1 (C4a), 129.2 (C8), 129.5 (C7), 138.2 (C6), 140.1 (C4), 148.5 (C8a), 151.7 (C2). m/z (EI): 251 ($M^{+\cdot}$ [^{37}Cl], 30%), 250 ($M^{+\bullet}$ [^{37}Cl] − H, 30), 249 ($M^{+\bullet}$ [^{35}Cl], 85), 248 ($M^{+\bullet}$ [^{35}Cl] − H, 50), 192 (50), 190 (100).

2-Chloro-6-(5,5-dimethyl-1,3-dioxan-2-yl)quinoline 37. 2-Chloro-6-formylquinoline **28** (0.350 g, 1.83 mmol) was treated with 2,2-dimethyl-1,3-propanediol (0.209 g, 2.0 mmol) and *p*-toluenesulfonic acid monohydrate (0.017 g, 0.09 mmol) in 5 mL of benzene for 4 h as described above. After workup, the title compound **37** was isolated as a white solid (0.395 g, 78%). An analytical sample was obtained after chromatography with dichloromethane as solvent, mp 135−140 °C. Anal. ($C_{13}H_{12}ClNO_2$) C, H, N. IR (Nujol) υ/cm^{-1}: 1584, 1501, 1458. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.83 (3H, s, CH_3), 1.31 (3H, s, CH_3), 3.71 (2H, d, J = 11.0 Hz, H4′/H6′), 3.82 (2H, d, J = 11.0 Hz, H4′/H6′), 5.55 (1H, s, H2′), 7.38 (1H, d, J = 8.6 Hz, H3), 7.88 (1H, d, J = 1.8, 8.7 Hz, H7), 7.97 (1H. br s, H5), 8.03 (1H, d, J = 8.7 Hz, H8), 8.12 (1H, d, J = 8.6 Hz, H4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.5 (CH_3), 23.7 (CH_3), 31.0 (C5′), 78.4 (2 x C4′/C6′), 101.6 (C2′), 123.2 (C3), 125.8 (C5), 127.1 (C4a), 129.3 (C8), 129.5 (C7), 138.0 (C6), 140.0 (C4), 148.6 (C8a), 151.7 (C2). m/z (EI): 279 ($M^{+\bullet}$ [^{37}Cl], 10%), 278 ($M^{+\bullet}$ [^{37}Cl] − H, 10), 277 ($M^{+\bullet}$ [^{35}Cl], 30), 276 ($M^{+\bullet}$ [^{35}Cl] − H, 12), 192 (50), 190 (100).

General Procedure for Amination of 2-Chloroquinolines.[28] The 2-chloroquinoline (1 equiv) was treated with acetamide (20 equiv) and potassium carbonate (5 equiv) at ∼200 °C until thin-layer chromatography (9:1 dichloromethane/ethanol) indicated the reaction was complete (1−2 h). After cooling, water was added to the residue, and the aqueous layer was extracted three times with chloroform. The combined organic extracts were washed with brine and dried (Na_2SO_4) and the solvent was removed. Unless otherwise indicated, the residues were chromatographed over silica gel using 9:1 dichloromethane/ethanol as eluant to afford the pure 2-aminoquinolines. In the case of amines **33**, **38** and **39**, the accompanying quinolin-2(1*H*)-ones were also isolated.

2-Amino-6-methoxyquinoline 23. 2-Chloro-6-methoxyquinoline **18** (0.215 g, 1.11 mmol) was treated with acetamide (1.32 g, 22.3 mmol) and potassium carbonate (0.724 g, 5.24 mmol) as described above. After workup and chromatography (9:1 dichloromethane/methanol), the title compound **23** was isolated as a pale brown solid (0.0589 g, 31%), mp 175−182 °C (lit. mp[42] 178−180 °C.) IR (Nujol) υ/cm^{-1}: 3447, 3301, 1643, 1603. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.88 (3H, s, OCH_3), 5.25 (2H, br s, NH_2), 6.79 (1H, d, J = 9.0 Hz, H3), 6.99 (1H, d, J = 3.0 Hz, H5), 7.25 (1H, dd, J = 3.0, 9.0 Hz, H7), 7.62 (1H, d, J = 9.0 Hz, H8), 7.84 (1H, d, J = 9.0 Hz, H4).^{13}C NMR (75 MHz, d_6-acetone) δ: 55.8 (CH_3O), 107 7 (C5), 113.4 (C3), 121.8 (C7), 124.5 (C4a), 126.6 (C8), 138.3 (C4), 142.3 (C8a), 156.1 (C2), 157.3 (C6). m/z (EI): 174 ($M^{+\bullet}$, 100%), 159 (85), 131 (59).

2-Amino-6-fluoroquinoline 24. 2-Chloro-6-fluoroquinoline **19** (0.181 g, 0.99 mmol) was treated with acetamide (1.36 g, 23.05 mmol) and potassium carbonate (0.69 g, 5.0 mmol) as described above. After workup and chromatography, the title compound **24** (0.054 g, 33%) was isolated as a yellow powder, mp 141−148 °C. HRMS (LSIMS FAB): $C_9H_8FN_2$ ($M + H^+$) calcd 163.0672, found 163.0668. IR (Nujol) υ/cm^{-1}: 3413, 1670, 1608. ^{1}H NMR (600 MHz, d_6-acetone) δ: 5.85 (br s, 2H, NH_2), 6.91 (1H, d, $^3J_{3,4}$ = 8.9 Hz, H3), 7.31 (1H, dt, $^4J_{5,7}$ = 3.0 Hz, $^3J_{7,8}$ = $^3J_{7,F}$ = 9.0 Hz, H7), 7.36 (1H, dd, $^4J_{5,7}$ = 3.0 Hz, $^3J_{5,F}$ = 9.3 Hz, H5), 7.55 (1H, ddd, $^3J_{7,8}$ = 9.0, $^4J_{8,F}$ = 5.3 Hz, $^5J_{4,8}$ = 0.6 Hz, H8), 7.91 (1H, dd, $^3J_{3,4}$ = 8.9, $^5J_{4,8}$ = 0.6 Hz, H4). ^{13}C NMR (150 MHz, d_6-acetone) δ: 111.23 (d, $^2J_{CF}$ = 22.4 Hz, C5), 113.65 (d, $^5J_{CF}$ = 5.6 Hz, C3), 118.55 (d, $^2J_{CF}$ = 25.0 Hz, C7), 123.97 (d, $^3J_{CF}$ = 9.9 Hz, C4a), 128.41 (d, $^3J_{CF}$ = 8.8 Hz, C8), 137.03 (d, $^4J_{CF}$ = 4.3 Hz, C4), 145.81 (d, $^4J_{CF}$ = 1.1 Hz, C8a), 158.20 (d, $^1J_{CF}$ = 238.9 Hz, C6), 158.39 (s, C2). m/z (EI): 162 ($M^{+\bullet}$, 100%), 135 ($M^{+\bullet}$ − HCN, 32).

2-Amino-6-chloroquinoline 25. 2,6-Dichloroquinoline **20** (0.24 g, 1.21 mmol), was treated with acetamide (1.43 g, 24.24 mmol) and potassium carbonate (0.84 g, 6.06 mmol) as described above. Following workup and chromatography, the title compound **25** (0.12 g, 54%) was afforded as a pale yellow solid, mp 146−150 °C (lit. mp[43] 151.5−153 °C). IR (Nujol) υ/cm^{-1}: 3449, 1652, 1608. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.03 (2H, br s, NH), 6.78 (1H, d, J = 9.0 Hz, H3), 7.46 (1H, dd, J =

2.2, 8.8 Hz, H7), 7.50 (1H, d, J = 8.8 Hz, H8), 7.58 (1H, d, J = 2.2 Hz, H5), 7.78 (1H, d, J = 9.0 Hz, H4). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 112.9 (C3), 124.3 (C4a), 126.5 (C5), 127.2 (C8), 128.2 (C6), 130.7 (C7), 137.5 (C4), 145.7 (8a), 157.2 (C2). m/z (EI): 180 ($M^{+\bullet}$ [^{37}Cl], 33%), 178 ($M^{+\bullet}$ [^{35}Cl], 100), 153 ($M^{+\bullet}$ [^{37}Cl] − HCN, 10), 151 ($M^{+\bullet}$ [^{35}Cl] − HCN, 29).

2-Amino-6-bromoquinoline 26. 6-Bromo-2-chloroquinoline **21** (0.55 g, 2.27 mmol) was treated with acetamide (2.54 g, 43.05 mmol) and potassium carbonate (1.44 g, 10.31 mmol) as described above. Following workup and chromatography, the title compound **26** (0.143 g, 29%) was isolated as an off white solid, mp 141−146 °C. HRMS (LSIMS FAB): $C_9H_8{}^{79}BrN_2$ ($M + H^+$) calcd, 222.9871; found 222.9881. IR (Nujol) υ/cm^{-1}: 3472, 3301, 1644, 1610. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 4.89 (2H, br s, NH_2), 6.73 (1H, d, J = 8.4 Hz, H3), 7.52 (1H, d, J = 9.0 Hz, H8), 7.61 (1H, dd, J = 2.4, 9.0 Hz, H7), 7.75 (1H, d, J = 2.4 Hz, H5), 7.78 (1H, d, J = 8.4 Hz, H4). ^{13}C NMR ($CDCl_3$, 150 Hz) δ: 112.5 (C3), 115.6 (C6), 124.7 (C4a), 127.5 (C4), 129.5 (C8), 133.0 (C5), 137.1 (C7), 146.1 (C8a), 157.0 (C2). m/z (EI): 224 ($M^{+\bullet}$ [^{81}Br], 92%), 222 ($M^{+\bullet}$ [^{79}Br], 100), 197 ($M^{+\bullet}$ [^{81}Br] − HCN, 14), 195 ($M^{+\bullet}$ [^{79}Br] − HCN, 14), 143($M^{+\bullet}$ − Br, 8).

2-Amino-6-methylquinoline 27. 2-Chloro-6-methylquinoline **22** (0.6 g, 3.38 mmol) was treated with acetamide (4.0 g, 67.6 mmol) and potassium carbonate (2.33 g, 16.9 mmol) as described above. Following work up and chromatography, the title compound **27** (0.288 g, 54%) was isolated as a white fluffy solid, mp 137−145 °C. (lit. mp[42] 147−149 °C). IR (Nujol) υ/cm^{-1}: 3453, 3149, 1625, 1610, 1563. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (3H, s, CH_3), 5.04 (2H, br s, NH_2), 6.72 (1H, d, J = 9.0 Hz, H3), 7.4 (2H, m, H5/H7), 7.57 (1H, d, J = 9.0 Hz, H8), 7.81 (1H, d, J = 9.0 Hz, H4). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.8 (CH_3), 112.3 (C3), 124.3 (C4a), 126.5 (C8), 127.3 (C5), 132.4 (C7), 132.8 (C6), 138.2 (C4), 146.6 (C8a), 157.1 (C2). m/z (EI): 158 ($M^{+\bullet}$, 100%), 157 ($M^{+\bullet}$ − H, 50), 130 (20).

6-(1,3-Dioxolan-2-yl)quinolin-2-amine 33. 2-Chloro-6-(1,3-dioxolan-2-yl)quinoline **32** (0.275 g, 1.17 mmol) was treated with acetamide (1.38 g, 23.4 mmol) and potassium carbonate (0.806 g, 5.83 mmol) as described above. Following workup and chromatography, the title compound **33** (0.114 g, 46%) (R_f 0.20) was isolated as a pale yellow solid (mp 156−162 °C). In addition a yellow solid (0.035 g) (R_f 0.51) was isolated, and ^{1}H NMR in $CDCl_3$ revealed this material consisted of approximately a 4:1 mixture of 6-(1,3-dioxolan-2-yl)quinolin-2(1*H*)-one **34** and 6-formylquinolin-2(1*H*)-one **35**.

33: Anal. ($C_{12}H_{12}N_2O_2$) C, H, N. IR (Nujol) υ/cm^{-1}: 3419, 3124, 1654, 1621, 1610, 1566. ^{1}H NMR (300 MHz, d_6-acetone) δ: 3.94−4.16 (4H, m, H4′/H5′), 5.80 (1H, s, H2′), 6.00 (2H, br s, NH_2), 6.86 (1H, d, J = 9.0 Hz, H3), 7.52 (1H, d, J = 8.4 Hz, H8), 7.57 (1H, dd, J = 1.7, 8.4 Hz, H7), 7.70 (1H, d, J = 1.7 Hz, H5), 7.92 (1H, d, J = 9.0 Hz, H4). ^{13}C NMR (75 MHz, d_6-acetone) δ: 66.0 (C4′/C5′), 104.6 (C2′), 113.2 (C3), 123.5 (C4a), 126.5 (C8), 126.8 (C5), 128.4 (C7), 132.9 (C6), 138.4 (C4), 149.4 (C8a), 159.4 (C2). m/z (EI): 216 ($M^{+\bullet}$, 40%), 215 ($M^{+\bullet}$ − H, 20), 171 (38), 44 (100).

34: HRMS (ESI): $C_{12}H_{11}NNaO_3{}^+$ ($M + Na^+$) calcd 240.0637, found 240.0630. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.01−4.21 (4H, m, H4′/H5′), 5.87 (1H, s, H2′), 6.75 (1H, d, J = 9.5 Hz, H3), 7.49 (1H, d, J = 8.4 Hz, H8), 7.64 (1H, dd, J = 1.8, 8.4 Hz, H7), 7.71 (1H, d, J = 1.8 Hz, H5), 7.85 (1H, d, J = 9.5 Hz, H4), 12.58 (1H, br s, NH). m/z (EI): 217 ($M^{+\bullet}$, 5%), 216 ($M^{+\bullet}$ − H, 5), 172 (100).

35: HRMS (ESI): $C_{10}H_7NNaO_2{}^+$ ($M + Na^+$) calcd 196.0374, found 196.0366. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.80 (1H, d, J = 9.6 Hz, H3), 7.63 (1H, d, J = 8.6 Hz, H8), 7.92 (1H, d, J = 9.6 Hz, H4), 8.03 (1H, dd, J = 1.7, 8.6 Hz, H7), 8.09 (1H, d, J = 1.7 Hz, H5), 10.02 (1H, s, CHO), 13.00 (1H, br s, NH). m/z (EI): 173 ($M^{+\bullet}$, 100%), 172 ($M^{+\bullet}$ − H, 91), 144 ($M^{+\bullet}$ − H − CO, 19).

6-(1,3-Dioxan-2-yl)quinolin-2-amine 38. 2-Chloro-6-(1,3-dioxan-2-yl)quinoline **36** (0.485 g, 1.94 mmol) was treated with acetamide (2.295 g, 38.8 mmol) and potassium carbonate (1.34 g, 9.7 mmol) for 1 h as described above. Following workup and chromatography, the title compound **38** (0.278 g, 62%) (R_f 0.21) was isolated as a pale yellow solid (mp 155−170 °C). In addition 6-(1,3-dioxan-2-yl)-quinolin-2(1*H*)-one **40** (0.035 g, 8%) (R_f 0.50) was isolated (mp 230−240 °C).

38: Anal. ($C_{13}H_{14}N_2O_2$) H, N; C: calcd, 67.81; found, 67.20. HRMS (ESI): $C_{13}H_{15}N_2O_2$ ($M + H^+$) calcd 231.1134, found 231.1130. IR (Nujol) υ/cm^{-1}: 3441, 3100, 1649, 1624, 1609, 1570. ^{1}H NMR (300 MHz, d_6-acetone) δ: 1.47 (1H, dtt, J = 1.4, 2.6, 13.4 Hz, H5′$_{eq}$), 2.07 (1H, dtt, J = 5.0, 12.3, 13.4 Hz, H5′$_{ax}$), 3.96−4.05 (2H, m, H4′/H6′), 4.16−4.22 (2H, m, H4′/H6′), 5.59 (1H, s, H2′), 5.88 (2H, br s, NH_2), 6.85 (1H, d, J = 8.7 Hz, H3), 7.48 (1H, d, J = 8.4 Hz, H8), 7.56 (1H, dd, J = 1.9, 8.4 Hz, H7), 7.68 (1H, d, J = 1.9 Hz, H5), 7.91 (1H, d, J = 8.7 Hz, H4). ^{13}C NMR (75 MHz, d_6-acetone) δ: 26.8 (C5′), 67.8 (C4′/C6′), 102.3 (C2′), 113.1 (C3), 123.5 (C4a), 125.9 (C8), 126.4 (C5), 128.2 (C7), 140.0 (C6), 138.3 (C4), 149.4 (C8a), 159.4 (C2). m/z (EI): 230 ($M^{+\bullet}$, 95%), 229 ($M^{+\bullet}$ − H, 35), 171 (100).

40: HRMS (ESI): $C_{13}H_{13}NNaO_3{}^+$ ($M + Na^+$) calcd 254.0793; found 254.0787. IR (Nujol) υ/cm^{-1}: 3311, 3150, 1662, 1610, 1568. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.48 (1H, dtt, J = 1.4, 2.6, 13.5 Hz, H5′$_{eq}$), 2.25 (1H, dtt, J = 5.0, 12.3, 13.5 Hz, H5′$_{ax}$), 3.95−4.09 (2H, m, H4′/H6′), 4.25−4.34 (2H, m, H4′/H6′), 5.57 (1H, s, H2′), 6.71 (1H, d, J = 9.3 Hz, H3), 7.39 (1H, d, J = 8.6 Hz, H8), 7.63 (1H, dd, J = 1.8, 8.6 Hz, H7), 7.72 (1H, d, J = 1.8 Hz, H5), 7.81 (1H, d, J = 9.3 Hz, H4), 12.06 (1H, br s, NH). ^{13}C NMR (150 MHz, $CDCl_3$/d_6-acetone) δ: 26.3 (C5′), 67.9 (C4′/C6′), 101.4 (C2′), 116.4 (C8), 120.0 (C4a), 122.2 (C3), 126.0 (C5), 129.2 (C7), 134.0 (C6), 139.2 (C8a), 141.6 (C4), 164.8 (C2). m/z (EI): 231 ($M^{+\bullet}$, 20%), 230 ($M^{+\bullet}$ − H, 20), 172 (30), 43 (100).

6-(5,5-Dimethyl-1,3-dioxan-2-yl)quinolin-2-amine 39. 2-Chloro-6-(5,5-dimethyl-1,3-dioxan-2-yl)quinoline **37** (0.18 g, 0.65 mmol) was treated with acetamide (0.765 g, 13 mmol) and potassium carbonate (0.448 g, 3.25 mmol) for 1 h as described above. Following workup and chromatography, the title compound **39** (0.108 g, 65%) (R_f 0.29) was isolated as a white fluffy solid (mp 192−202 °C). In addition 6-(5,5-dimethyl-1,3-dioxan-2-yl)quinolin-2(1*H*)-one **41** (0.012 g, 7%) (R_f 0.58) was isolated as a white solid (mp 242−246 °C).

39: Anal. ($C_{15}H_{18}N_2O_2$) C, H, N. HRMS (ESI): $C_{15}H_{19}N_2O_2{}^+$ ($M + H^+$) calcd 259.1447, found 259.1442. IR (Nujol) υ/cm^{-1}: 3452, 3120, 1649, 1623, 1610, 1568. ^{1}H NMR (300 MHz, d_6-acetone) δ: 0.8 (3H, s, CH_3), 1.26 (3H, s, CH_3), 3.67−3.75 (4H, m, H4′/H6′), 5.49 (1H, s, H2′), 5.98 (2H, br s, NH_2), 6.86 (1H, d, J = 8.8 Hz, H3), 7.51 (1H, d, J = 8.7 Hz, H8), 7.61 (1H, dd, J = 2.0, 8.7 Hz, H7), 7.71 (1H, d, J = 2.0 Hz, H5), 7.93 (1H, d, J = 8.8 Hz, H4). ^{13}C NMR (50 MHz, d_6-acetone) δ: 22.0 (CH_3), 23.4 (CH_3), 30.8 (C5′), 78.1 (C4′/C6′), 102.5 (C2′), 113.1 (C3), 123.5 (C4a), 126.1 (C8), 126.4 (C5), 128.3 (C7), 133.7 (C6), 138.4 (C4), 149.4 (C8a), 159.4 (C2). m/z (EI): 258 ($M^{+\bullet}$,18%), 257 ($M^{+\bullet}$ − H, 8), 171 (95), 41 (100).

41: HRMS (ESI): $C_{15}H_{17}NNaO_3{}^+$ ($M + Na^+$) calcd 282.1106, found 282.1103. IR (Nujol) υ/cm^{-1}: 3435, 1668, 1610, 1568. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.82 (3H, s, CH_3), 1.31 (3H, s, CH_3), 3.69 (2H, d, J = 11.0 Hz, H4′/H6′), 3.80 (2H, d, J = 11.0 Hz, H4′/H6′), 5.46 (1H, s, H2′), 6.74 (1H, d, J = 9.6 Hz, H3), 7.46 (1H, d, J = 8.6 Hz, H8), 7.66 (1H, dd, J = 1.7, 8.6 Hz, H7), 7.75 (1H, d, J = 1.7 Hz, H5), 7.85 (1H, d, J = 9.6 Hz, H4), 12.54 (1H, br s, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.6 (CH_3), 23.8 (CH_3), 30.9 (C5′), 78.4 (2 x C4′/C6′), 101.7 (C2′), 116.7 (C8), 120.3 (C4a), 122.3 (C3), 126.2 (C5), 129.5 (C7), 134.0 (C6), 139.3 (C8a), 141.8 (C4), 165.2 (C2). m/z (EI): 259 ($M^{+\bullet}$, 70%), 258 ($M^{+\bullet}$ − H, 45), 173 (100), 172 (65).

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Supporting Information Available: Elemental analyses for compounds **14, 28, 32, 33, 36**−**38** and **39**, [^{1}H,^{15}N]-HSQC NMR spectra for selected ligand binding experiments, examples of ligand binding data-analyses for both NMR and FP assays, and graphical results of stability experiments for ligand **33**. This material is available free of charge via the Internet at http://pubs.acs.org.

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