Ring-Opening and Ring-Closing Reactions of a Shikimic Acid-Derived Substrate Leading to Diverse Small Molecules

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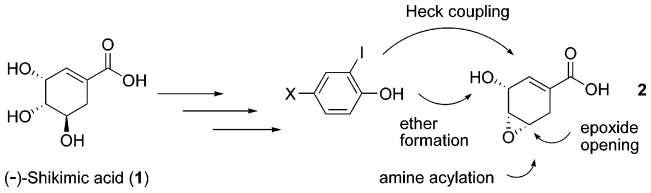
An epoxide derived from (−)-shikimic acid was attached to a solid support and used to synthesize over 5000 diverse small molecules. Key transformations include a Lewis acid-catalyzed epoxide opening with amines and an intramolecular Heck reaction with aryl iodides. Compounds derived from this pathway were printed onto small-molecule microarrays and screened for binding to proteins. Compounds that bound to Aurora A kinase were characterized using surface plasmon resonance.

Introduction

Modern methods for small-molecule synthesis are enabling the examination of a far greater array of chemical diversity in biological processes than previously possible.[1] When high yielding and selective chemical transformations are performed on functionalized solid supports, thousands of stereochemically and skeletally diverse compounds can be synthesized in only 3−4 chemical steps. To provide a broad assessment of the properties of these compounds in biological settings, we have focused on the use of high-throughput cell-based[2] and small-molecule microarray-based assays.[3]

Shikimic acid can be a useful starting material for such syntheses because of its three contiguous chiral centers and activated double bond. This naturally occurring small molecule has been used to synthesize many biologically active natural products[4] and several potent non-natural compounds such as neuraminidase inhibitors, for example, oseltamivir[5] (Tamiflu). Shikimic acid-based libraries have been synthesized and tested as glycomimetics.[6] Previously, a large collection of compounds was prepared using *trans*-4,5-epoxy-3-hydroxy-2-cyclohexenoic acid, both enantiomers of which are derived from (−)-shikimic acid.[7] Only a few compounds were generated via a Lewis acid-catalyzed epoxide opening by amines, followed by N-acylation; this method to increase diversity could not be used because of the nature of the linker used in the previous library synthesis.

Here, we use a different solid-phase resin that permits epoxide opening by amines and subsequent acylation of the resulting ring-opened product. Skeletal diversity is then introduced by an intramolecular Heck reaction that yields tricyclic dihydrobenzofurans, a structure found in a number of biologically active natural products. This strategy was recently used in the total synthesis of galanthamine,[8] a reversible inhibitor of acetylcholinesterase used for the treatment of Alzheimer's disease.[9]



Results and Discussion

(−)-Shikimic acid (**1**) was first esterified;[7] then the trans diol was converted to an epoxide,[10] and the ester was hydrolyzed to give carboxylic acid **2**. Both enantiomeric trans stereoisomers of **2** are accessible from **1**.[7,11] Polystyrene resin (500−600 μm macrobeads) functionalized with a diisopropyl 4-methoxyphenyl silyl group was activated under the recommended conditions[12] and coupled to *N*-Fmoc ethanolamine (Scheme 1). Deprotection with piperidine/DMF gave the amino-modified resin to which **2** was coupled under standard conditions.

A solid-phase Mitsunobu reaction was first tested using 2-iodophenol at low temperatures.[13] After it sat overnight at −20 °C, the reaction mixture was rinsed, and one bead of resin was treated with HF to cleave the silyl linker for analysis. Using HPLC/MS, we detected the product as a single entity with no starting alcohol. Opening of the solid phase-supported epoxide with amines was tested with varying amounts of benzylamine in a number of solvents (THF, CH_3CN, DMF, dioxane, CH_2Cl_2) and Lewis acids ($MgBr_2 \cdot OEt_2$, $LiClO_4$, $Yb(OTf)_3$, LiOTf) at various temperatures. This survey revealed that 20 equiv of the amine with 1.1

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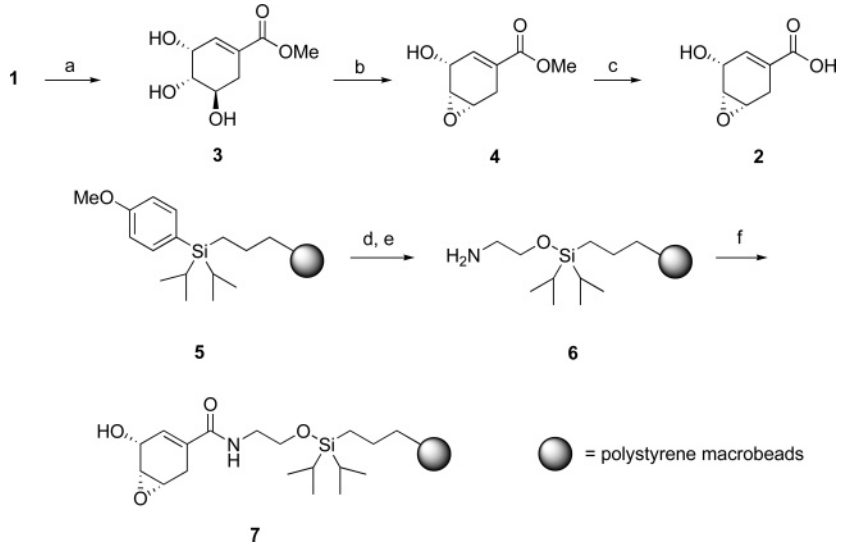
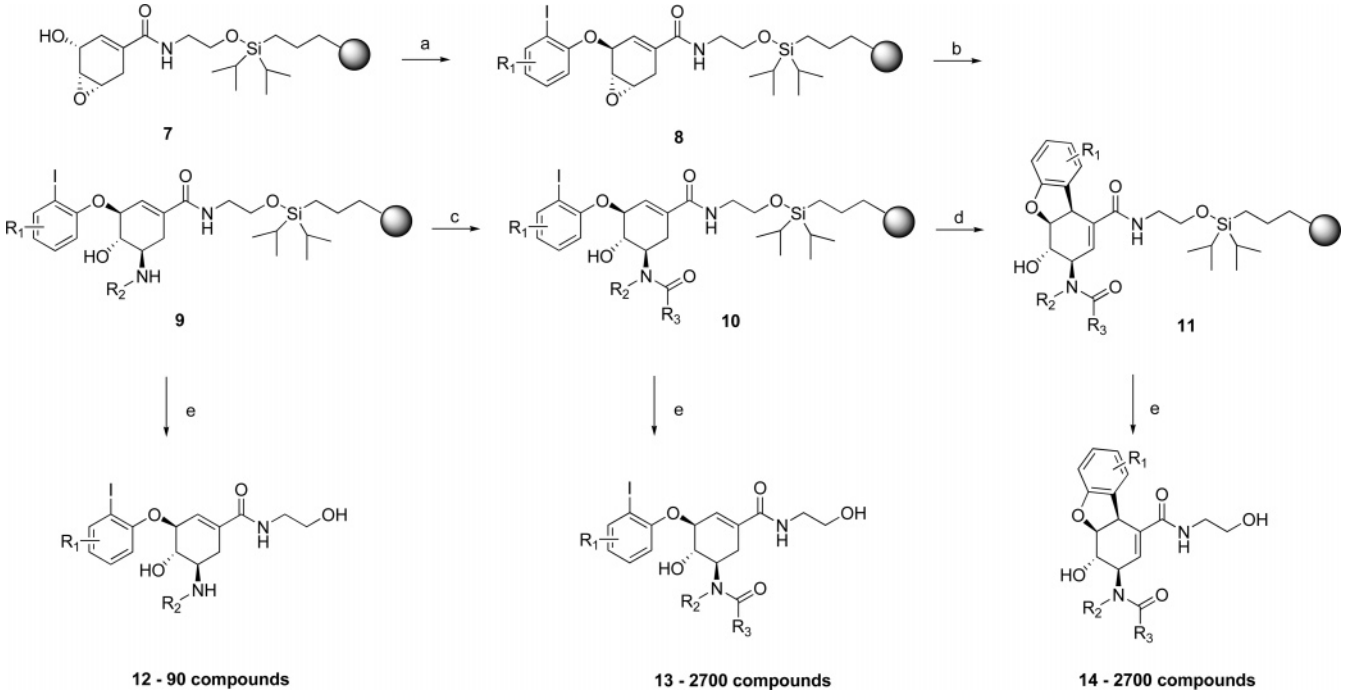
Scheme 1. Synthesis and Solid-Phase Attachment of a Derivative of Shikimic Acid[a]



[a] Conditions: (a) MeOH, Amberlite-IR120, reflux; (b) PPh_3, DEAD/THF; (c) LiOH, THF/H_2O; (d) (1) triflic acid/CH_2Cl_2, (2) *N*-Fmoc-ethanolamine, 2,6-lutidine/CH_2Cl_2; (e) piperidine/DMF; (f) **2**, PyBOP, DIPEA/NMP.

Scheme 2. Shikimic Acid-Based Library Synthesis[a]



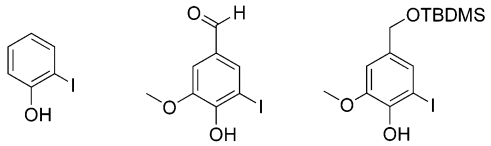
[a] Conditions: (a) ArOH, PPh_3, DEAD (10 equiv each)/THF, −78 to −20 °C; (b) amine (20 equiv), $MgBr_2$·OEt_2 (1.1 equiv), CH_3CN, 60 °C; (c) carboxylic acid, PyBrOP, DIPEA (10 equiv each)/NMP; (d) $Pd(OAc)_2$, dcpe, DIPEA, Bu_4NOAc/dioxane, 45−50 °C; (e) HF·pyridine/pyridine/THF (5:10:85), then TMSOEt.

equiv of $MgBr$·OEt_2 in CH_3CN was optimal. HPLC and NMR (500 MHz) analyses following cleavage indicated the presence of a single amine product; previous studies with shikimic acid-based systems showed that $MgBr_2$·OEt_2-catalyzed epoxide opening by amines is highly regioselective.[5] Acylation was tested using propanoic acid with various coupling agents, for example, DCC, EDAC, PyBOP, and PyBrOP. Finally, Heck cyclization was tested using various Pd sources ($Pd(PPh_3)_4$, $PdCl_2(PPh_3)_2$, $Pd(OAc)_2$), ligands (dppp, dcpe, dcpp), and bases (DIPEA, K_2CO_3) in different solvents (DMF, *N*,*N*-dimethylacetamide, dioxane). The use of 1,2-(dicyclohexylphosphino)ethane (dcpe), which has been used for similar cyclizations,[14] with palladium acetate in dioxane at 80 °C for 24 h proved optimal and yielded single tricyclic products. Later it was discovered that the chemical tags used as a means of encoding the reaction history were cleaved from the resin at this temperature.[15,16] The addition of tetrabutylammonium acetate led to equally clean reaction mixtures in less time (12 h) and at lower temperature (45−50 °C), such that the chemical tags were retained.[17]

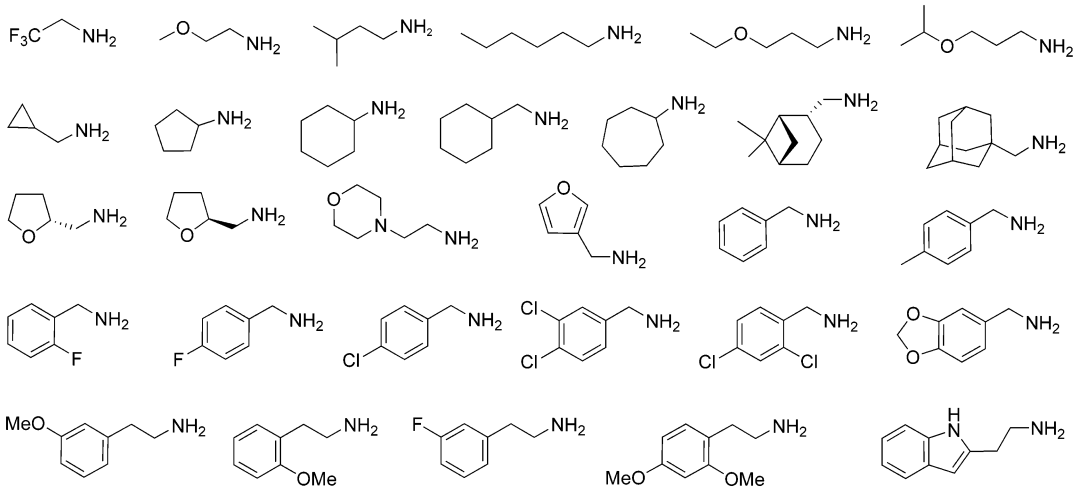
A library synthesis (Scheme 2) was achieved using the one-bead/one-stock solution procedure[12] with chemical tagging of the resin being performed for each reaction using a variation of Still's procedure.[15,16] The reactions were all performed with shaking overnight followed by extensive washings. Products were cleaved from the resin after each

Table 1. Library Building Blocks

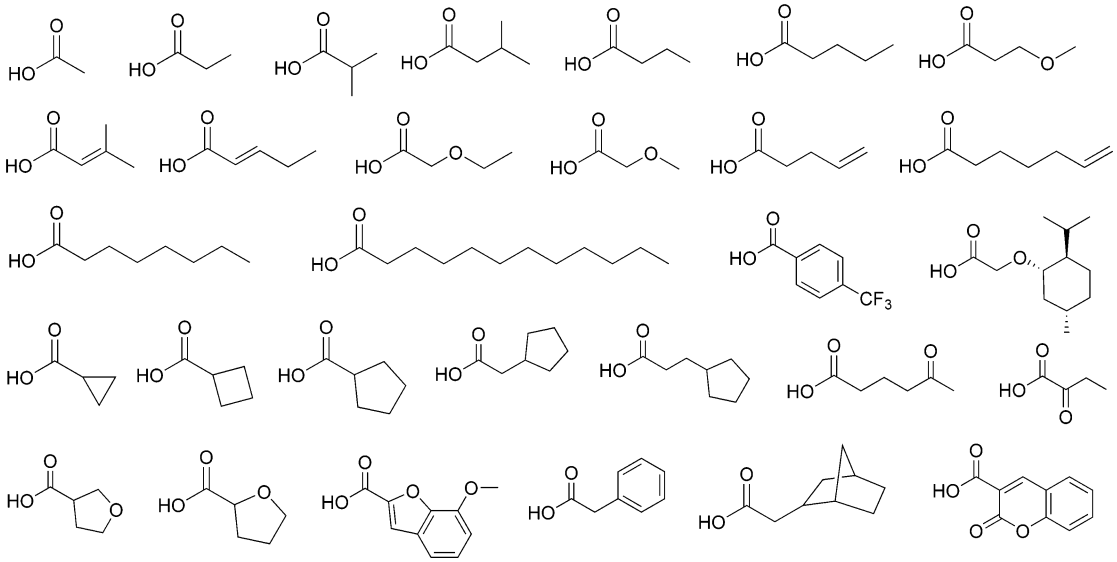
Phenols



Amines

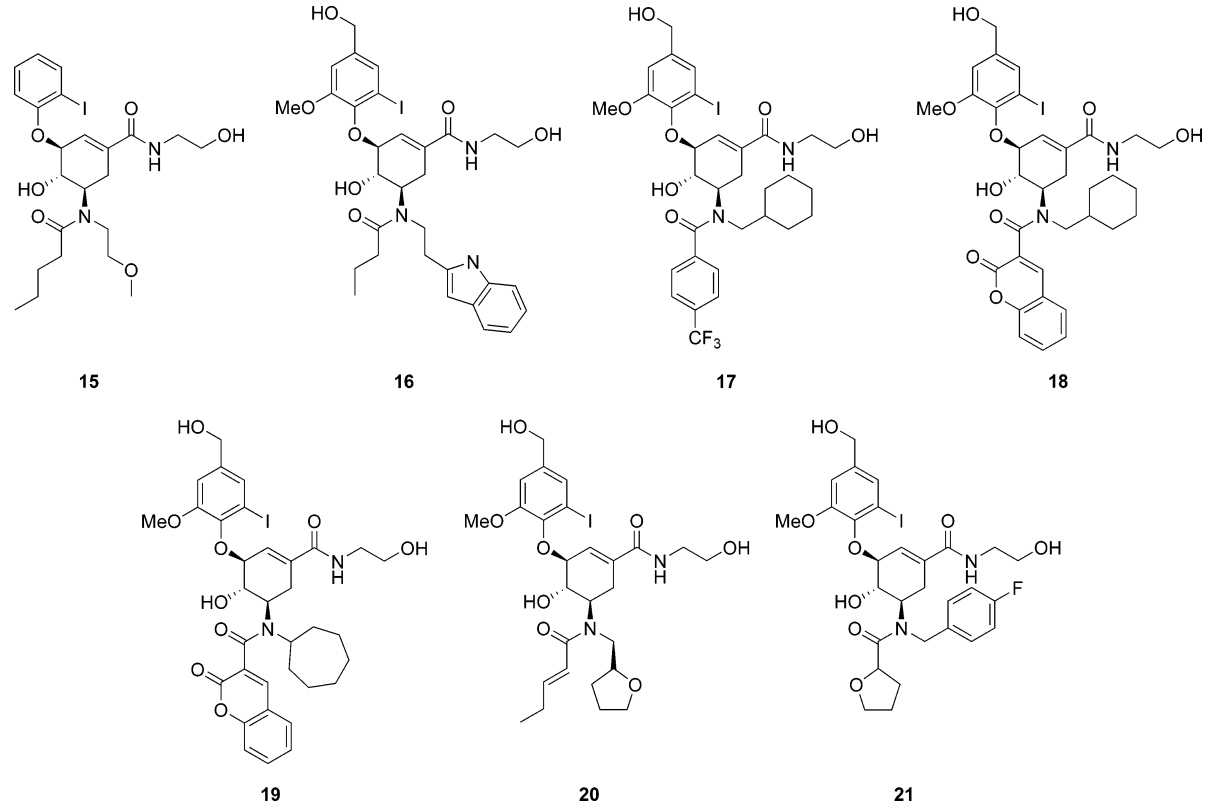


Carboxylic acids

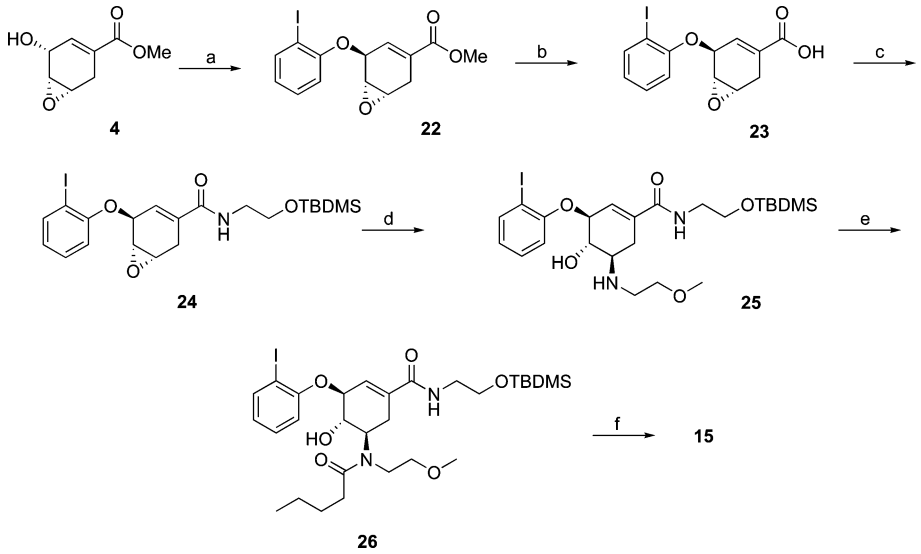


step with HF·pyridine for 3 h followed by treatment with ethoxytriethylsilane. The Mitsunobu reactions were achieved with 2-iodophenol, 4-(*tert*-butyldimethylsilyloxy)methyl-6-methoxy-2-iodophenol, and 4-formyl-6-methoxy-2-iodophenol,[18] the last two phenols having handles for chemical transformation after library realization.[1] HPLC and NMR analyses after cleavage indicated that the desired product was formed in each case, generally as a predominantly single entity. Each of the three products was then chemically tagged, and the products were combined, mixed thoroughly and divided into 30 even portions. An additional tagging was then performed to track 30 structurally diverse amines which were used in the library synthesis to open the resin-bound epoxide (Table 1). Thirty beads (out of 90) were cleaved, decoded, and analyzed by HPLC/MS; no starting epoxides were detected and the product amines were generally >90% pure. After mixing and dividing, the resins were further functionalized with 30 carboxylic acids (Table 1) followed by tagging. Thirty bead portions were analyzed; no starting amines were detected and the products were typically >90% pure. The collection of beads was divided in half at this point, one-half was subjected to the Heck reaction conditions. The Heck cyclization gave less pure products; analysis of a set of 20 products indicated that purities ranged mostly between 70 and 90%. The structures of the side products could not be determined. One expected side product, the reduced, non-cyclized des-iodo compound, was not detected in the crude reaction mixture; authentic material was synthesized by

Table 2. Aurora A SMM Positives



Scheme 3. Synthesis of Compound **15**[a]



[a] Conditions: (a) 2-iodophenol, DEAD, PPh_3/THF, −78 to −20 °C (90%); (b) LiOH·H_2O, H_2O/THF (85%); (c) $TBDMSOCH_2CH_2NH_2$, EDAC, HOBt, DIPEA/CH_2Cl_2 (80%); (d) 2-methoxyethylamine, $MgBr_2$·OEt_2/CH_3CN, 60 °C, (88%); (e) pentanoyl chloride, DIPEA/CH_2Cl_2 (90%); (f) HF·pyridine/pyridine/THF (5:10:85) 3 h, then TMSOEt, 0.5 h (86%).

running the entire reaction sequence replacing 2-iodo phenol with phenol.

The overall process should theoretically produce a total of 5493 (3 + 90 + 2700 + 2700) distinct compounds. Enough resin (3.3 g, ∼5 beads/mg, ∼16 500 beads) was used to make three copies of each product, some of which are used for quality control in the library synthesis. Prior to the Heck reaction, little bead breakage was observed. The Heck reaction did result in bead breakage so that only about 70% of the beads remained intact after this reaction. A total of 6336 wells were filled with compound for multipurpose screening.

Stock solutions corresponding to 1408 members of the resulting collection of small-molecule products were printed onto isocyanate-functionalized glass slides to prepare small-molecule microarrays (SMMs).[3] The SMMs were screened for binding to a number of proteins; hits were detected in various assays for amine, amide, and Heck products. Among the screens producing positives was one that used the anticancer target Aurora A kinase. The aurora kinase family

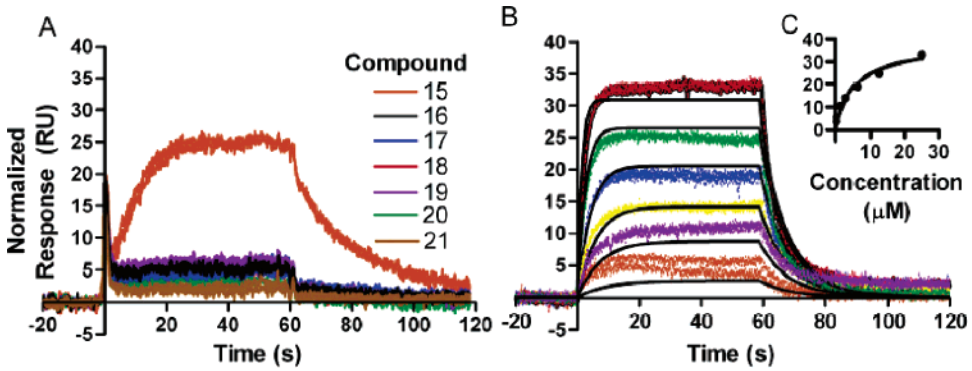


Figure 1. (A) Compounds **15**−**21** at 5 μM were tested for binding to Aurora A kinase by surface plasmon resonance. Compound **15** was further characterized ($n = 4$) by measuring binding in a dilution series (391 nM to 25 μM) and calculating the affinity by (B) kinetic and (C) equilibrium analyses. Kinetic $k_{on} = 1.2 \times 10^{-4}$ M^{-1} s^{-1}, $k_{off} = 6.8 \times 10^{-2}$ s^{-1}, $K_D = 5.3 \pm 1$ μM; equilibrium $K_D = 6.0 \pm 1$ μM.

is involved in regulating multiple steps in mitosis, including centrosome duplication, formation of bipolar mitotic spindles, and chromosome alignment on the mitotic spindle.[19] Amplification and overexpression of Aurora A kinase has been observed in several types of human cancers, including cancers of the colon, breast, pancreas, ovaries, and stomach, among others.[19] High-level Aurora A expression in human head and neck squamous cell carcinoma is a negative predictor for various clinical parameters including disease stage, presence of local and distant metastases, as well as overall patient survival.[20] Several small-molecule inhibitors of Aurora A kinase are in clinical trials and have been shown to inhibit cell-cycle progression and induce apoptosis in multiple human tumor types in vitro and in animal models.[21]

Seven positives from the SMM assay (**15**−**21**, Table 2) were resynthesized either in solution (with a TBDMS group as a surrogate for the resin, Scheme 3) or on solid phase; 20 mg of functionalized resin (**8**) typically produced 5−10 mg of purified product. Compounds **15**−**21** were analyzed for binding to Aurora A kinase in a secondary binding assay using surface plasmon resonance (SPR, Figure 1A).[22] Compounds **16**−**21** each bound to Aurora A weakly with affinities greater than 40 μM. Compound **15** had a K_D value of 5.3 ± 1 μM ($n = 4$) by kinetic analysis and a K_D value of 6.0 ± 1 μM by equilibrium analysis ($n = 4$) (Figure 1B and C). The activity of **15** was assessed in a cell-free Aurora A kinase enzymatic activity assay.[23] Although the binding assay demonstrated that **15** possessed good binding affinity, this Aurora A-binding compound did not inhibit kinase activity. Compound **15** is being used as a starting point for the synthesis of derivatives that exhibit better binding affinity for Aurora A without inhibiting the protein's enzymatic activity. Such molecules may be useful for diagnostic purposes, to detect cells in clinical specimens that overexpress Aurora A.

Conclusion

A multifunctional building block derived from shikimic acid was attached to a solid-phase resin as the starting point for the synthesis of a library using epoxide opening by amines, acylation, and intramolecular Heck reactions. Compound **15**, which was produced by this synthetic route, was shown to bind Aurora A as determined by SPR. This process, solid-phase synthesis and small molecule microarrays, followed by further chemistry and secondary biological testing, may prove useful to study the effect of diverse small molecules against different protein targets.

Experimental Procedures

General Procedures. All reactions were run under inert atmosphere. Starting materials and reagents were purchased from commercial suppliers and used without further purification with the exception of 2,6-lutidine, which was distilled from CaH_2 under nitrogen before use, and CH_2Cl_2 and THF, which were passed through two activated alumina columns to remove impurities. Brominated polystyrene macrobeads were purchased from Polymer Labs. The 500−600 μm macrobeads were prepared, and the loading levels were determined according to a published procedure.[12]

Purification of reaction products was carried out by flash chromatography using E. Merck silica gel 60 (230−400 mesh). Analytical thin layer chromatography was performed on E. Merck 0.25 mm silica gel 60-F plates. Visualization was accomplished with UV light and aqueous cerium ammonium molybdate (CAM) solution, followed by heating. Analytical LC/MS chromatography was performed on Waters Alliance 2690 HPLC system with a Waters Symmetry C18 column (3.5 μm, 4.6 × 100 mm) with a gradient of 20−80% CH_3CN in water with constant 0.1% formic acid, with UV detection at 214 and 280 nm and a Micromass LCZ (ESI) spectrometer. ^{1}H NMR spectra were recorded on a Varian 500 MHz spectrometer; the shifts are reported in parts per million and are referenced to residual protons in the NMR solvent. Data are reported as shift, splitting (s = singlet, d = doublet, t = triplet, m = multiplet; br = broad), coupling constant in hertz, and intergration. ^{13}C NMR spectra were recorded at 125 MHz on a Varian spectrometer; ^{13}C shifts are reported in parts per million and are referenced to carbon resonances in the NMR solvent.

Small-scale solid-phase reactions (5−10 mg resin) were performed in 1 mL fritted polyacrylamide Bio-Spin chromatography columns (Bio-Rad Laboratories, Hercules, CA; 732-6008) or Wheaton glass vials, fitted with Teflon-coated caps, with gentle mixing provided by Thermoline Vari-Mix shaker or a Vortex Genie-2 vortexer. Larger-scale solid-phase reactions (>500 mg resin) were performed in 10−50 mL Amersham columns or silanized 50 or 100 mL fritted glass tubes equipped for vacuum filtration and N_2 bubbling. The

tubes were silanized by treatment with 20% $(CH_3)_2Cl_2Si$ in CH_2Cl_2 for 15 min and MeOH for 15 min, followed by oven heating at 120 °C for 2 h. Resin samples were washed as indicated, and the solvent was removed under argon flow or in vacuum on a Vac-Man laboratory vacuum manifold (Promega, Madison, WI; A7231) fitted with nylon 3-way stopcocks (Biorad 732−8107). Resin samples in glass vials were filtered through 10 mL Amersham columns and were washed on the Vac-Man vacuum manifold. The following standard wash procedures were used for rinsing: Method A 3 × THF, 3 × DMF, 3 × *i*PrOH, 3 × DMF, 3 × THF; Method B 3 × THF, 3 × THF/H_2O (9:1), 3 × DMF, 3 × *i*PrOH, 3 × DMF, 3 × THF.

All compounds were cleaved from macrobeads using the following standard procedure. A freshly prepared solution of 85:10:5 THF/pyridine/HF·pyridine was added to resin in a polypropylene Eppendorf tube. The resulting mixture was then agitated at room temperature for 3 h before it was quenched with TMSOEt (10/1, v/v, TMSOEt/HF·pyridine) The resulting mixture was agitated at room temperature for 30 min; then it was transferred to an Eppendorf vial and evaporated on a Speedvac. Resin was washed twice with THF, and the wash solutions were combined, concentrated, and analyzed by HPLC and NMR.

Loading of *N*-Fmoc-ethanolamine onto Silicon-Functionalized Polystyrene Macrobeads and Deprotection (6). Dry 3-[diisopropyl(*p*-methoxyphenyl)silyl]propyl-functionalized macrobeads[12] (**5**, 400 mg, 1.41 mmol Si/g, 0.56 mmol) in a 20 mL polypropylene tube were allowed to swell in anhydrous CH_2Cl_2 (15 mL) for 30 min under an argon atmosphere. A 4% (v/v) solution of CF_3SO_3H in anhydrous CH_2Cl_2 (0.25 mL, 3.4 mmol) was added to the mixture by syringe, and the reaction tube was shaken periodically. The beads turned orange upon acid treatment and were allowed to stand for 20 min. After filtration under positive argon pressure, the orange beads were washed with anhydrous CH_2Cl_2 (2 × 15 mL) and then resuspended in CH_2Cl_2 (1 mL). Freshly distilled 2,6-lutidine (0.26 mL, 2.2 mmol) was added resulting in bead discoloration, followed by addition of a CH_2Cl_2/DMF (2:1) solution of *N*-Fmoc-ethanolamine (476 mg, 1.68 mmol). The reaction mixture was gently agitated for 12 h. The beads were drained and rinsed (method A). The resin was air-dried for 2 h and then placed under vacuum for 12 h. The beads were allowed to swell in anhydrous DMF for 30 min before the addition of a solution of 20% piperidine in DMF (10 mL), and the reaction tube was agitated for 5 h before the beads were drained and rinsed with method A. The loading level was determined spectrophotometrically as in ref 12. The resin was air-dried for 2 h and then placed under vacuum for 12 h to remove solvent.

Solid-Phase Amide Coupling (7). Resin **6** (15 mg, 1.17 mmol Si/g, 18 μmol) was allowed to swell for 30 min in 5 mL of anhydrous NMP before the addition of PyBOP (14 mg, 26 μmol), DIPEA (9.2 μL, 53 μmol), and acid **2** (4.1 mg, 26 μmol), and the reaction mixture was gently agitated for 24 h. The beads were drained and rinsed using method A. The resin was then air-dried for 2 h and placed under vacuum for 12 h. Cleavage produced 3.2 mg of (1*S*,5*R*,6*R*)-5-hydroxy-*N*-(2-hydroxyethyl)-7-oxa-bicyclo[4.1.0]hept-3-ene-3-carboxamide as white foam (91%). ^{1}H NMR (500 MHz, CD_3OD): δ 6.28−6.27 (m, 1H), 4.58−4.57 (m, 1H), 3.61 (t, *J* = 6.0 Hz, 2H), 3.52−3.49 (m, 1H), 3.48−3.46 (m, 1H), 3.37 (t, *J* = 6.0 Hz, 2H), 2.91 (d, *J* = 19.0 Hz, 1H), 2.50 (dd, *J* = 19.0, 2.5 Hz, 1H). ESI-MS (+): *m*/*z* 200 $(M + H)^+$.

Solid-Phase Ether Formation (8). Resin **7** (15 mg, 18 μmol), 2-iodophenol (39 mg, 180 μmol), PPh_3 (46 mg, 180 μmol), and THF (0.67 mL) were briefly vortexed prior to cooling to −78 °C and the addition of DEAD (27 μL, 180 μmol). The reaction mixture was maintained for 0.5 h at −78 °C and then at −20 °C for 12 h with occasional agitation. Filtration, washing (method A), and cleavage produced 6.2 mg of (1*S*,5*S*,6*S*)-*N*-(2-hydroxyethyl)-5-(2-iodophenoxy)-7-oxa-bicyclo[4.1.0]hept-3-ene-3-carboxamide (94% yield). ^{1}H NMR (500 MHz, CD_3OD): δ 7.81 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.39 (td, *J* = 7.5, 1.5 Hz, 1H), 7.22 (dd, *J* = 7.5, 1.5 Hz, 1H), 6.81 (td, *J* = 7.5, 1.5 Hz, 1H), 6.53 (m, 1H), 5.29 (m, 1H), 3.63 (t, *J* = 5.5 Hz, 2H), 3.56 (m, 1H), 3.48 (m, 1H), 3.37 (t, *J* = 5.5 Hz, 2H), 2.92 (dd, *J* = 20.0, 1.5 Hz, 1H), 2.85 (dd, *J* = 20.0, 2.0 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 169.11, 156.93, 139.82, 132.76, 129.83, 124.64, 123.80, 114.90, 87.41, 70.82, 60.31, 51.01, 50.44, 42.11, 24.44. ESI-MS (+): *m*/*z* 402 $(M + H)^+$.

Solid-Phase Epoxide Opening (9). Resin **8** (20 mg, 23 μmol) was suspended in anhydrous CH_3CN (800 μL). After 15 min, $MgBr_2$·OEt_2 (7.2 mg, 28 μmol) and *N*-benzylamine (51 μL, 470 μmol) were added, and the reaction mixture was heated at 55−60 °C for 24 h. The beads were drained and rinsed with method B. Cleavage of a single bead produced 5-benzylamino-4-hydroxy-3-(2-iodophenoxy)-cyclohex-1-enecarboxylic acid (2-hydroxyethyl)amide. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.79 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.24−7.38 (m, 6H), 7.00 (dd, *J* = 8.0, 1.0 Hz, 1H), 6.77 (td, *J* = 8.0, 1.0 Hz, 1H), 6.38−6.40 (br m, 1H), 6.28 (br m, 1H), 4.82−4.84 (br m, 1H), 4.03 (d, *J* = 12.5 Hz, 1H), 3.94 (dd, *J* = 10.5, 7.5 Hz, 1H), 3.74−3.82 (m, 4H), 3.51−3.48 (m, 2H), 3.05 (dd, *J* = 16.5, 5.0 Hz, 1H), 2.93−2.98 (m, 1H), 2.20−2.22 (m, 1H). ESI-MS (+): *m*/*z* 509 $(M + H)^+$.

Solid-Phase Amide Formation (10). Resin **9** (20 mg, ca. 23 μmol) was suspended in anhydrous NMP (800 μL) for 15 min. Acetic acid (1.3 μL, 230 μmol), and PyBrOP (109 mg, 230 μmol) were added, followed by the addition of DIPEA (82 μL, 47 μmol). The reaction mixture was shaken for 24 h. The beads were drained and washed using method B. Cleavage of a single bead gave 5-(*N*-acetyl-*N*-benzylamino)-4-hydroxy-3-(2-iodophenoxy)-cyclohex-1-enecarboxylic acid (2-hydroxyethyl)amide. The LC/MS was consistent with clean desired product. The NMR spectrum was complicated because of the presence of rotamers. ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O): δ 7.92−7.95 (m, 1H), 7.78 (m, 1H), 7.13−7.39 (m, 7H), 6.73−6.78 (m, 1H), 6.25 (br s, 1H), 5.15 (br s, 0.5H), 4.95 (br s, 0.5H), 4.87 (d, *J* = 16.5 Hz, 0.5H), 4.60−4.80 (br s, 0.5 H), 4.43−4.60 (br, 0.5H), 4.06−4.15 (m, 1H), 3.92−3.99 (m, 1H), 3.31−3.36 (m, 2H), 3.04−3.10 (m, 2H), 2.49 (s, 1.5 H), 2.10−2.40 (m, 3H), 2.18 (s, 1.5 H). ESI-MS (+): *m*/*z* 573 $(M + Na)^+$.

Solid-Phase Heck Cyclization (11). Resin **10** (20 mg, ca. 23 μmol) was suspended in degassed dioxane (800 μL) for

15 min before the addition of $Pd(OAc)_2$ (5.3 mg, 23 μmol), 1,2-bis(dicyclohexylphosphino)ethane (dcpe, 9.9 mg, 23 μmol), Bu_4NOAc (7.1 mg, 23 μmol), and DIPEA (82 μL, 470 μmol). The reaction mixture was heated at 45−50 °C for 12 h. The beads were drained and rinsed with method B, after they were washed with NaCN solution (7/3 THF/1 M $NaCN_{(aq)}$, 10 mL × 3); cleavage from the resin resulted in 6.2 mg of the expected fused-ring product (75% yield from resin **8**). The LC/MS showed the desired product with purity of 85%, and the NMR spectrum was complicated by the presence of rotamers. ^{1}H NMR (500 MHz, CD_3OD): δ 7.26−7.60 (m, 4H), 7.04−7.18 (m, 4H), 6.68−6.78 (m, 2H), 6.24 (s, 0.5H), 6.18 (s, 0.5H), 4.66−4.82 (m, 3H), 4.42−4.58 (m, 2H), 4.04−4.10 (m, 0.5H), 3.60−3.70 (m, 2.5H), 3.38−3.44 (m, 2H), 2.15 (s, 1.5H), 2.12 (s, 1.5H). ESI-MS (+): *m*/*z* 445 $(M + Na)^+$.

(1*S*,5*R*,6*R*)-5-hydroxy-7-oxa-bicyclo[4.1.0]hept-3-ene-3-carboxylic Acid (2). A solution of (1*S*,5*R*,6*R*)-methyl 5-hydroxy-7-oxa-bicyclo[4.1.0]hept-3-ene-3-carboxylate[10] (**4**, 0.50 g, 2.9 mmol) in 10 mL of THF was cooled to −20 °C before the slow addition of $LiOH·H_2O$ (0.14 g, 3.5 mmol) in 2 mL of water. The reaction mixture was stirred at 0 °C for 7 h. The pH of the reaction mixture was adjusted to ∼5 by adding acidic resin Amberlite IRC-86 before filtration and evaporation to provide white solid product **2** (0.26 g, 57%). ^{1}H NMR (CD_3OD): δ 6.54−6.56 (m, 1H), 4.53−4.54 (m, 1H), 3.43−3.45 (m, 1H), 3.40−3.42 (m, 1H), 2.89 (d, J = 19.5 Hz, 1H), 2.41 (dd, J = 19.5, 2.5 Hz, 1H). ^{13}C NMR (CD_3OD): δ 175.23, 133.78, 131.79, 66.98, 56.22, 53.12, 26.79. ESI-MS (+): *m*/*z* 157 $(M+H)^+$.

(1*S*,5*S*,6*S*)-Methyl 5-(2-iodophenoxy)-7-oxa-bicyclo-[4.1.0]hept-3-ene-3-carboxylate (22). A solution of **4**[10] (3.62 g, 21.3 mmol), 2-iodophenol (5.63 g, 25.6 mmol), and PPh_3 (8.73 g, 33.3 mol) in THF (200 mL) was cooled to −78 °C, and DEAD (4.04 mL, 25.6 mmol) was added slowly via syringe. The reaction mixture was stirred 1 h at −78 °C and then kept at −20 °C overnight. The reaction mixture was concentrated and purified by column chromatography (hexane/Et_2O from 5/3 to 3/1) producing **22** as a semisolid (7.15 g, 90%). ^{1}H NMR ($CDCl_3$): δ 7.82 (dd, J = 8.0, 1.5 Hz, 1H), 7.33 (dt, J = 8.0, 1.5 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.92 (m, 1H), 6.79 (dt, J = 8.0, 1.5 Hz, 1H), 5.19 (s, 1H), 3.78 (s, 3H), 3.55 (d, J = 3.0 Hz, 1H), 3.54 (s, 1H), 3.01 (dd, J = 20.0, 1.5 Hz, 1H), 2.83 (dd, J = 20.0, 3.0 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 166.72, 156.51, 140.11, 130.57, 129.94, 128.91, 124.38, 114.93, 88.49, 70.98, 52.44, 51.13, 50.92, 24.61. ESI-MS: (+) *m*/*z* 371 $(M - H)^+$.

(1*S*,5*S*,6*S*)-5-(2-Iodophenoxy)-7-oxa-bicyclo[4.1.0]hept-3-ene-3-carboxylic Acid (23). A solution of **22** (6.11 g, 16.4 mmol) in 50 mL of THF was cooled to 0 °C before the slow addition of $LiOH·H_2O$ (0.83 g, 20 mmol) in 10 mL of H_2O. The reaction mixture was warmed to room temperature and stirred for 5 h. The pH of the reaction mixture was adjusted to ∼5 by adding Amberlite IRC-86 acidic resin before filtration and evaporation to provide 4.98 g of **23** as a white solid (85%). ^{1}H NMR (CD_3OD): δ 7.79 (dd, J = 8.0, 1.5 Hz, 1H), 7.37 (dt, J = 8.5, 1.0 Hz, 1H), 7.19 (d, J = 8.5 Hz, 1H), 6.88 (d, J = 1.5 Hz, 1H), 6.80 (dt, J = 8.0, 1.0 Hz, 1H), 5.27 (d, J = 1.5 Hz, 1H), 3.54 (s, 1H), 3.47 (d, J = 1.0 Hz, 1H), 2.93 (dd, J = 20.0, 1.5 Hz, 1H), 2.76 (ddd, J = 20.0, 1.5, 4.0 Hz, 1H). ^{13}C NMR (CD_3OD): δ 169.54, 158.08, 141.09, 131.58, 131.04, 130.58, 125.17, 116.38, 88.87, 72.10, 52.21, 51.83, 25.59. ESI-MS (+): *m*/*z* 359 $(M + H)^+$.

(1*S*,5*S*,6*S*)-*N*-(2-(*tert*-Butyldimethylsilyloxy)ethyl)-5-(2-iodophenoxy)-7-oxa-bicyclo[4.1.0]hept-3-ene-3-carboxamide (24). Compound **23** (5.87 g, 16.4 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC, 3.45 g, 18.0 mmol), 1-hydroxybenzotriazole (HOBt, 2.43 g, 18.0 mmol), and CH_2Cl_2 (150 mL) were added to an oven-dried flask, and the mixture was cooled to 0 °C. 2-(*tert*-Butyldimethylsilyloxy)ethylamine[24] (4.31 g, 24.6 mmol) was added, and the mixture was stirred overnight at room temperature. The reaction was filtered, and the filtrate was washed twice with brine, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography (EtOAc/hexane, 3/7) to afford **24** (6.72 g, 80%). ^{1}H NMR ($CDCl_3$): δ 7.80 (d, J = 8.0 Hz, 1H), 7.31 (dt, J = 8.0, 1.5 Hz, 1H), 7.01 (d, J = 8.0 Hz, 1H), 6.79 (dt, J = 8.0, 1.5 Hz, 1 H), 6.35 (br, 1H), 6.20 (br, 1H), 5.15 (br, 1H), 3.70 (t, J = 4.5 Hz, 2H), 3.54 (s, 1H), 3.52 (s, 1H), 3.41−3.44 (m, 2H), 2.98 (d, J = 19.5 Hz, 1H), 2.85 (dd, J = 19.5, 2.0 Hz, 1H); 0.88 (s, 9H), 0.05 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 167.13, 156.27, 139.75, 132.98, 129.62, 123.96, 123.53, 114.84, 88.25, 70.79, 61.42, 50.91, 50.55, 41.76, 25.83, 24.53, 18.12, −5.36. ESI-MS (+): *m*/*z* 516 $(M + H)^+$.

(3*S*,4*S*,5*R*)-*N*-(2-(*tert*-Butyldimethylsilyloxy)ethyl)-4-hydroxy-3-(2-iodophenoxy)-5-(2-methoxyethylamino)cyclohex-1-enecarboxamide (25). 2-Methoxyethylamine (40 μL, 0.47 mmol) was added to a suspension of **24** (0.12 g, 0.23 mmol) and $MgBr_2·OEt_2$ (0.66 g, 0.25 mmol) in CH_3CN (2 mL), and the reaction mixture was heated at 60 °C for 8 h. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2 before it was rinsed with aqueous NH_4Cl and brine. After it was dried (Na_2SO_4), filtered, and concentrated, the residue was purified by column chromatography (CH_2Cl_2 and CH_2Cl_2/MeOH, 60/1) to afford **25** (0.12 g, 88%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.73 (dd, J = 8.0, 1.5 Hz, 1H), 7.25 (dt, J = 8.0, 1.5 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 6.71 (dt, J = 8.0, 1.5 Hz, 1H), 6.30 (br, 1H), 6.22 (t, J = 5.0 Hz, 1H), 4.80−4.82 (m, 1H), 3.85 (dd, J = 10.5, 8.0 Hz, 1H), 3.65 (t, J = 5.0 Hz, 2H), 3.46−3.49 (m, 2H), 3.35−3.38 (m, 2H), 3.31 (s, 3H), 2.80−2.94 (m, 3H), 2.70−2.74 (m, 1H), 0.82 (s, 9H), 0.01 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 167.00, 156.93, 139.72, 135.03, 129.70, 127.83, 123.60, 115.00, 88.48, 81.16, 73.68, 72.14, 61.68, 58.91, 56.86, 46.21, 41.93, 31.04, 26.00, 18.33, −5.19, −5.20. ESI-MS (+): *m*/*z* 591 $(M + H)^+$.

(3*S*,4*S*,5*R*)-*N*-(2-(*tert*-Butyldimethylsilyloxy)ethyl)-4-hydroxy-3-(2-iodophenoxy)-5-(*N*-(2-methoxyethyl)pentanamido)cyclohex-1-enecarboxamide (26). DIPEA (16 μL, 89 μmol) was added to a solution of **25** (35 mg, 59 μmol) in 2 mL of CH_2Cl_2, followed by the addition of pentanoyl chloride (86 μL, 71 μmol). The reaction mixture was stirred overnight; then more CH_2Cl_2 was added before the mixture was rinsed with aqueous NH_4Cl and brine. The organic layer was dried (Na_2SO_4), filtered, concentrated, and purified by

column chromatography (CH_2Cl_2 and CH_2Cl_2/MeOH, 100/0.5) to afford **26** (36 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 7.78 (dd, J = 8.0, 1.5 Hz, 1H), 7.31 (dt, J = 8.0, 1.5 Hz, 1H), 7.14 (dd, J = 8.0, 1.0 Hz, 1H), 6.77 (dt, J = 8.0, 1.0 Hz, 1H), 6.33 (br s, 1H), 6.20 (t, J = 5.5 Hz, 1H), 5.23 (d, J = 3.0 Hz, 1H), 4.92−4.94 (m, 1H), 4.04−4.11 (m, 2H), 3.93−3.98 (m, 1H), 3.77 (d, J = 14.0 Hz, 1H), 3.67−3.71 (m, 2H), 3.40−3.47 (m, 3H), 3.39 (s, 3H), 3.09−3.14 (m, 1H), 2.61 (dd, J = 17.5, 5.5 Hz, 1H), 2.43−2.52 (m, 2H), 2.30−2.39 (m, 1H), 1.61−1.67 (m, 2H), 1.34−1.39 (m, 2H), 0.94 (t, J = 6.5 Hz, 3H), 0.86 (s, 9H), 0.05 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 174.95, 166.60, 157.71, 139.67, 134.66, 129.81, 128.43, 124.08, 116.38, 89.14, 81.62, 70.83, 70.48, 61.72, 59.10, 58.21, 42.37, 42.02, 33.82, 29.03, 27.32, 26.07, 22.65, 18.42, 14.15, −5.11, −5.13. ESI-MS (+): m/z 675 $(M + H)^+$.

(3*S*,4*S*,5*R*)-4-Hydroxy-*N*-(2-hydroxyethyl)-3-(2-iodophenoxy)-5-(*N*-(2-methoxyethyl)pentanamido)cyclohex-1-enecarboxamide (15). In a Teflon vial, compound **26** (15 mg) was dissolved in THF (1.25 mL) and pyridine (0.150 mL). HF·pyridine (75 μL) was added, and the mixture was shaken 3 h before the addition of TMSOEt (0.75 mL); it was then shaken for an additional 0.5 h. The reaction mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH, 95/5) to afford **15** (11 mg, 86%). ^{1}H NMR ($CDCl_3$): δ 7.79 (dd, J = 8.0, 1.5 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.78 (dt, J = 8.0, 1.5 Hz, 1H), 6.37−6.40 (br, 1H), 6.26 (t, J = 5.5 Hz, 1H), 5.20 (d, J = 3.0 Hz, 1H), 4.90−4.94 (br, 1H), 4.04−4.14 (m, 2H), 3.93−3.98 (m, 1H), 3.74−3.80 (m, 3H), 3.42−3.52 (m, 4H), 3.39 (s, 3H), 3.09−3.14 (m, 1H), 2.62 (dd, J = 17.5, 5.5 Hz, 1H), 2.46−2.52 (m, 2H), 2.30−2.38 (m, 1H), 1.58−1.68 (m, 2H), 1.34−1.40 (m, 2H), 0.93 (t, J = 7.5 Hz, 3H). ESI-MS (+): m/z 561 $(M + H)^+$.

Small-Molecule Microarray Assays. Recombinant Aurora A was expressed in *Escherichia* coli Rosetta2 (DE3) cells (Novagen) as a C-terminal 6xHis-tagged protein and was purified using Ni-NTA agarose affinity chromatography according to the standard protocols. Small-molecule microarrays were incubated in triplicate with 400 μL of a 10 μg/mL solution of purified Aurora A-6xHis in TBST buffer for 30 min at room temperature. The arrays were washed three times in TBST (1 min for each wash) on an orbital platform shaker. Arrays were then incubated with 300 μL of a 0.2 μg/mL solution of Cy5-labeled anti-5xHis antibody (Qiagen) in TBST for 30 min at room temperature. The probed arrays were washed three times in TBST (5 min for each wash), followed by doubly distilled water for 3 min on an orbital platform shaker. Arrays were dried by centrifugation and scanned for fluorescence at 635 nm using a Genepix 4000B microarray scanner.

Surface Plasmon Resonance Experiments. The Aurora A kinase surface plasmon resonance assay was conducted on a Biacore S51 instrument using Biacore CM5 sensor chips. Ethanolamine, EDC, NHS, and P-20 surfactant were all obtained from Biacore, Inc. Aurora A was directly immobilized through primary amines using standard EDC/NHS chemistry. The sensor surface was conditioned using alternating injection of 10 mM glycine pH 2.2 and 50 mM NaOH. The surface was then activated with 1:1 4 M EDC/1 M NHS for 10 min. Aurora A diluted to 15 μg/mL in 10 mM acetate pH 5.5 was exposed to the activated sensor. The surface was quenched by a 7 min injection of 1 M ethanolamine. Protein activity was assessed by observing the binding of staurosporine. Small-molecule binding assays were conducted in 23 mM Tris buffer pH 7.4 with 137 mM NaCl, 3 mM KCl, 0.005% P-20 surfactant, and DMSO or DMF cosolvent. The cosolvent was varied from 2 to 5%, and 5 mM $MgCl_2$ was used in some assays; however neither had an effect on compound **15** affinity. Sensor data was analyzed using the Scrubber 2 software (BioLogic Software Pty Ltd). Data was double-reference subtracted and corrected for variation in solvent concentration. Binding affinity was calculated using kinetic and equilibrium analyses. A least-squares fit of a Langmuir 1:1 binding model was implemented for kinetic analysis.

Sensor Chip Preparation. A Biacore sensor chip was prepared by direct immobilization of the Aurora A protein. Aurora A was thawed before use, divided into 5 μL aliquots, and stored at −20 °C. Freshly thawed Aurora A was used for each assay. The running buffer used during immobilization was PBS, pH 7.4 with 0.005% P-20 surfactant. Binding assays were performed at 25 °C. Aurora A was diluted to 23 μg/mL in 10 mM acetate buffer (pH 5.5) and immobilized on the sensor surface on *Spot 1* using standard EDC/NHS chemistry.[19] Between 2900 and 3900 response units (RU) of protein was immobilized in each assay. An anti-RGSHis antibody (Qiagen) was immobilized at a level of ∼3000 RU on *Spot 2* and used as a reference surface.

Assay Parameters. Compounds **15**−**21** were diluted from 5 mM stocks in DMF to the appropriate concentration in buffer with the same solvent concentration as the running buffer (2%). Compounds were injected at a flow rate of 30 μL/min into the flow cell for 60 s followed by 90 s of buffer with no compound. Compound **15** was further characterized by measuring the binding at concentrations from 391 nM to 25 μM in half dilution. A flow rate of 90 μL/minute was used in the characterization of compound **15**.

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Supporting Information Available. Product analysis of 30 solid-phase acylation reactions and 20 solid-phase Heck

reactions and synthetic procedures and characterizations for compounds **16**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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