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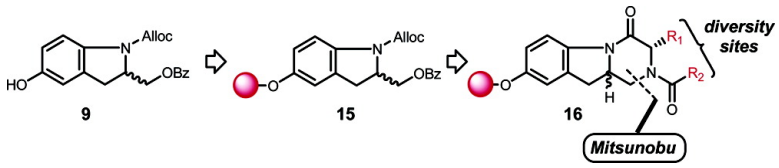

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A Solid Phase Library Synthesis of Hydroxyindoline-Derived Tricyclic Derivatives by Mitsunobu Approach

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Hydroxyindoline-derived scaffold, **9**, was synthesized with the goal of generating a library of indoline-based natural product-like tricyclic derivatives to be utilized as small-molecule chemical probes. The tricyclic ring was obtained by a Mitsunobu reaction of the *N*-nosyl amino acid conjugate with the primary hydroxyl group. The solid-phase synthesis was achieved by immobilizing scaffold **9** onto the solid support giving a compound, **15**. This was then subjected to a series of reactions on solid phase, including the Mitsunobu reaction, leading to the desired indoline-derived tricyclic derivative. The final product has two diversity sites: (i) amino acid as the first diversity and (ii) amidation of the secondary amine for the second diversity. These two diversity sites were utilized in the library generation by IRORI split-and-mix approach.

Introduction

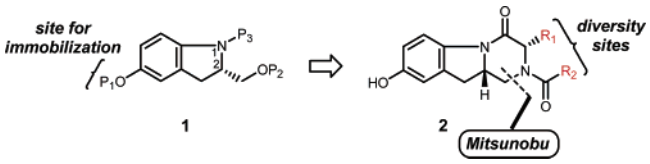
In recent years, interest in the generation of small-molecule libraries that are inspired by bioactive natural products has grown significantly.[1] This is largely due to the fact that over the years, natural products have proven to be quite valuable for providing small molecules that can be used in understanding protein functions.[2] The rapid rise in chemical genomics-based research that relies heavily on the use of small-molecule chemical probes has generated a growing need for natural-product-guided libraries.[3] Of particular interest is the design of natural-product-like chiral scaffolds in which one could explore the 3-dimensional space around the scaffold by having several sites to explore stereocontrolled diversity-oriented reactions.[4]

There are several natural products known in nature that possess the indole and indoline scaffold, and a number of these derivatives exhibit a wide range of biological activities.[5] Because of the broad applications of indole and indoline-based bioactive natural products, we recently initiated a program aimed at developing solid-phase synthesis leading to a variety of complex polycyclic derivatives.[6]

With our continued efforts toward the use of chiral hydroxyindolinol **1** as a scaffold, herein we report a Mitsunobu reaction-based strategy for the synthesis of hydroxyindoline-derived tricyclic derivatives **2** (Scheme 1) by solution- and solid-phase synthesis. The 10-step solid-phase synthesis was then successfully utilized in the library generation of 16 and 100 derivatives in yields ranging from 20 to 35% using the IRORI[7] split-and-mix-type approach.

Mitsunobu Approach to Indoline-Derived Tricyclic Derivatives. Shown in Scheme 1 is the Mitsunobu-based approach toward the potential synthesis of indolinol-derived tricyclic derivatives. The plan is to use the orthogonally protected hydroxyindolinol derivative **1** in which the phenolic hydroxyl group could be utilized as a site for immobilization for solid-phase synthesis. Upon deprotection of the protected amino group of the indolinol moiety, this could then be coupled with a Fmoc-protected amino acid (introduction of the first diversity site, R_1). Following the Fmoc removal, the free amine could be protected as the *o*-nosyl derivative, which could also be easily accomplished on solid phase. The key step in our plan is the intramolecular Mitsunobu reaction of the nosylated amine with the primary hydroxyl group, which is available after deprotection. The mild and the neutral reaction conditions for the Mitsunobu reaction is the main attraction of this approach for the synthesis of the tricyclic derivatives on solid phase. There are several examples in the literature in which this reaction has been successfully applied on solid phase.[8] Another interesting feature is the ease of the *o*-nosyl removal. This will generate the free amine that could then further be subjected to amide coupling with various carboxylic acids, thereby giving the second diversity site (R_2). After the completion of solid-phase synthesis, one could easily cleave the product from the solid support to obtain the corresponding free hydroxyl-based tricyclic derivatives.

Scheme 1. A Mitsunobu Approach to the Library Synthesis of Indoline-Derived Tricyclic Derivatives



Results and Discussion

Solution Phase Synthesis of Indoline-Derived Tricyclic Derivatives 8 from Indolinol 3 (Scheme 2). The orthogonally protected hydroxyindolinol **3** was synthesized.[6] Following the *N*-Alloc removal, the free amine **4** was subjected to amino acid coupling (HOBt, DIC) giving the coupled

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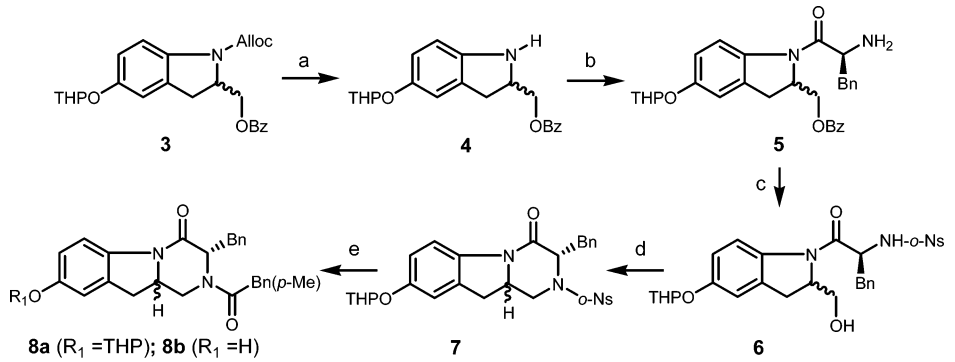
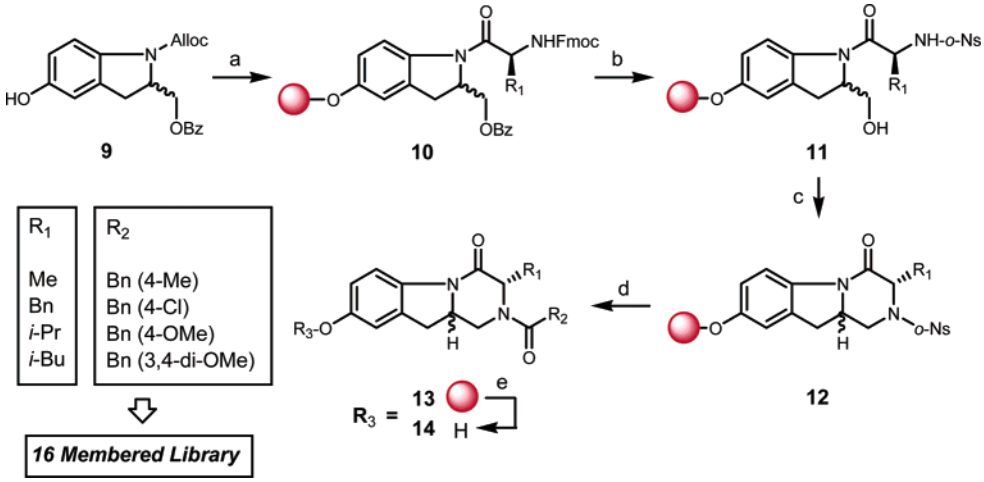
Scheme 2. Solution Synthesis of Indoline-Derived Tricyclic Derivative **8** by a Mitsunobu Approach



(a) (i) $Pd(PPh_3)_4$, *N*-methyl morpholine, CH_2Cl_2, 95%. (b) (i) Fmoc-Phe-OH, HOBt, DIC, DMF, room temp; (ii) 20% piperidine, CH_2Cl_2, 85% for two steps. (c) (i) 2-Nitrobenzensulfonyl chloride, DIPEA, CH_2Cl_2, 91%; (ii) NaOMe, MeOH, 70%. (d) EtOOC−N=N−COOEt, Ph_3P, THF, room temp, 92%. (e) (i) K_2CO_3, PhSH, room temp, 79%; (ii) *p*-tolyl acetic acid, HOBt, DIC, DMF, 92%; (iii) PPTS, EtOH, 50 °C, (85%).

Scheme 3. A Split-and-Mix-Type, Solid-Phase Library Synthesis of Idoline-Derived Tricyclic Derivatives by Mitsunobu Approach

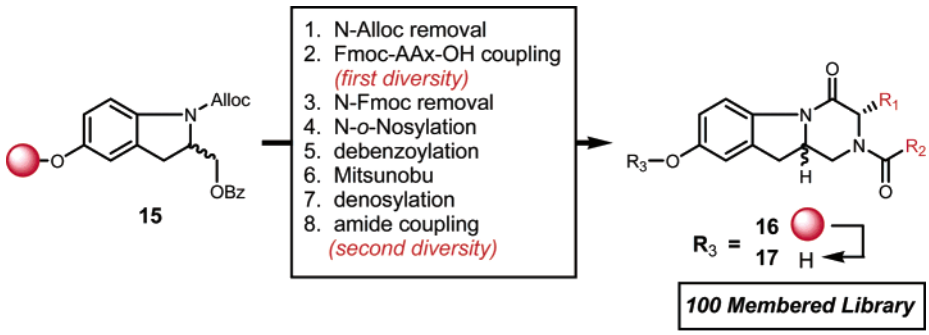


(a) (i) Bromo Wang resin, Cs_2CO_3, NaI, DMF; (ii) $Pd(PPh_3)_4$, *N*-methyl morpholine, CH_2Cl_2; (iii) Fmoc-AA(R1)-OH, HOBt, DIC, DIPEA, DMF. (b) (i) 20% piperidine, DMF; (ii) 2-nitrobenzensulfonyl chloride, CH_2Cl_2; (iii) NaOMe, MeOH/THF. (c) EtOOC−N=N−COOEt, Ph_3P, THF, room temp. (d) (i) PhSH, DBU, DMF; (ii) HOBt, DIC, R_2CO_2H, DMF. (e) 10% TFA, CH_2Cl_2.

product in a high yield. After the Fmoc removal (85% for 2 steps), the free amine **5** was then protected as an *o*-nosyl derivative (91% yield). To obtain the free hydroxyl required for the intramolecular Mitsunobu reaction, it was then subjected to debenzoylation (NaOMe, MeOH) giving product **6**. Having a free hydroxyl group in compound **6**, the stage was now set to study the crucial, intramolecular Mitsunobu reaction (EtOOC−N=N−COOEt, Ph_3P). The Mitsunobu reaction went smoothly, giving the desired cyclic product in 92% yield. To complete the model solution synthesis, compound **7** was subjected to *o*-nosyl removal (K_2CO_3, PhSH) giving the free amine that could easily be coupled with various carboxylic acids to introduce the second diversity. The free amine was coupled with the *p*-tolyl acetic acid under standard amide coupling reaction conditions (HOBt, DIC) to give compound **8a**. Finally, the deprotection of the −OTHP ether was achieved to obtain compound **8b** in 85% yield.

Solid-Phase Synthesis of Indoline-Derived Tricyclic Derivatives by Mitsunobu Approach. The manual solid-phase synthesis of indoline-derived tricyclic derivative **14** and the small library synthesis (16 compounds) by a IRORI split-and-mix-type approach is shown in Scheme 3. Compound **9** was anchored onto the solid support using (bromomethyl)phenoxymethyl polystyrene (loading 1.3 mmol/g). The resin in DMF was mixed with free phenol derivative **9**, Cs_2CO_3, and sodium iodide. The mixture was bubbled vigorously under nitrogen for 24 h and then filtered. The resin was washed with DMF, H_2O, CH_3OH, and CH_2Cl_2 (3× with each solvent) and then dried under vacuum, giving the indoline scaffold anchored onto the solid support (loading 87% after cleavage from the support with 10% TFA). The *N*-Alloc was removed by treating the resin with $Pd(PPh_3)_4$ in CH_2Cl_2 in the presence of acetic acid and *N*-methylmorpholine. The free amine derivative was then coupled with Fmoc-protected phenylalanine under standard amide coupling reaction conditions (DIC, HOBt). This introduced the first diversity site in an overall 80% yield for three steps after cleavage from the resin. Following the Fmoc removal, the free amine was further protected as an *o*-nosyl derivative on reaction with *o*-nitrobenzenesulfonyl chloride, a requirement for exploring the intramolecular Mitsunobu reaction. The free primary hydroxyl group was generated by debenzoylation (NaOMe, MeOH/THF) giving compound **11** (overall 65% yield in six steps after cleavage from the support). This was then subjected to Mitsunobu reaction conditions (EtOOC−N=N−COOEt, Ph_3P). We were pleased to note that the intramolecular cyclization went as smoothly

Scheme 4. 100-Compound Library Synthesis of Indoline-Based Tricyclic Derivatives by IRORI Split-and-Mix-Type Approach



Amino acids for diversity, R_1

Fmoc-Asp(*O*-*t*Bu)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(*N*-Boc)-OH, Fmoc-Nle-OH, Fmoc-Orn(*N*-Boc)-OH, Fmoc-Phe-OH, Fmoc-Thr(*O*-*t*Bu)-OH, Fmoc-Tyr(*O*-*t*Bu)-OH, Fmoc-Val-OH

Carboxylic acids for diversity, R_2

4-Biphenylacetic acid, 4-Chlorophenylacetic acid, (3,4-Dimethoxyphenyl)acetic acid, Indole-3-acetic acid, 4-Methoxyphenylacetic acid, 3,4-(Methylenedioxy)phenyl-acetic acid, [1R-(1α,2β,3α)]-(+)-3-Methyl-2-(nitromethyl)-5-oxocyclopentaneacetic acid, 4-Nitrophenylacetic acid, Phenylacetic acid, and p-Tolylacetic acid

on solid phase as observed earlier in solution synthesis. To complete the test synthesis on solid phase, the *o*-nosyl group was removed without any difficulties, and the free amine was then coupled with *p*-tolyl acetic acid under standard amide coupling conditions, giving compound **13**. Cleavage from the support on treatment with 10% TFA in CH_2Cl_2 provided the desired indoline-derived tricyclic derivative in an overall 35% yield for 10 steps. Thus, we have shown that it is possible to utilize a Mitsunobu approach to obtain the indoline-derived tricyclic compound in 10 steps on solid phase while introducing two sites of diversity for the library generation.

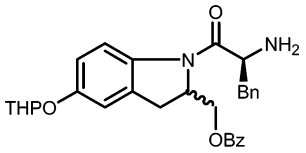
A Library Synthesis of Indoline-Derived Tricyclic Derivatives by IRORI Split-and-Mix-Type Approach. Following the success with manual solid-phase synthesis, we then synthesized a small library (16 compounds, see Scheme 3) using an IRORI split-and-mix-type approach. This was further repeated for the library synthesis of 100 compounds shown in Scheme 4. The synthesis steps were very similar to those used in the manual solid-phase synthesis; however, the semiporous nature of the MicroKans containing the resin demanded longer reaction times. The library synthesis was reasonably successful, because 78 of the 100 target compounds were generated.

To summarize, we have shown that the idoline-derived tricyclic derivatives having two diversity sites could be obtained in solution and on solid phase by an intramolecular Mitsunobu approach. The ease of this reaction on solid phase makes this strategy highly practical in library synthesis, and this was shown by the synthesis of a modestly sized library with IRORI's split-and-mix-type approach. Further, work is in progress to explore the use of these indoline-derived tricyclic derivatives as small-molecule probes to study cellular processes. For example, in one case, the library is being tested in the search for small-molecule inhibitors of eukaryotic protein translation synthesis, and the findings will be reported as they become available.[9]

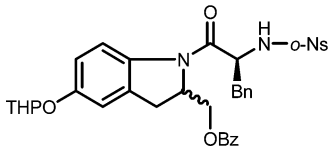
Experimental Section

General Methods. The materials were obtained from commercial suppliers and used without purification. THF and CH_2Cl_2 were distilled under N_2 over sodium/benzophenone and CaH_2, respectively. All NMR experiments were recorded on an AC-Brüker instrument (400 MHz). Unless otherwise noted, proton and carbon chemical shifts are reported in parts per million using residual $CHCl_3$ as an internal standard at 7.26 and 77.0 ppm, respectively. Analysis by mass spectrometry was performed on a VG Quattro I (Micromass) mass spectrometer equipped with a pneumatically assisted electrospray ionization source operating in positive mode. The enantiomeric excess was determined by chiral HPLC using a Hewlett-Packard (Agilent) 1090 LC equipped with a diode array detector and Chiracel-OD column. The HPLC spectra were recorded on a Gilson Combinatorial Chromatography System with 215 Liquid Handler/Injector and equipped with a Vydac C-18 monomeric column and a diode array detector. The split-and-mix-like combinatorial chemistry was achieved by use of the IRORI Technology (Accutag-100 Combinatorial Chemistry System, Accucleave-96 Cleavage Station, MicroKan Reactor Pk96, Radio frequency tag Pk500).

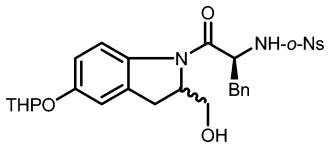
Solution-Phase Synthesis. Compound 5. To a solution of amine **4** (106 mg, 0.3 mmol), Fmoc-Phe-OH (116 mg, 0.3 mmol), and HOBt (44.8 mg, 0.33 mmol) in anhydrous DMF (2 mL) was added DIC (56 μL, 0.36 mmol). The resulting mixture was stirred at room temperature overnight, and DMF was then evaporated under high vacuum. The residue was dissolved in ethyl acetate (10 mL), washed sequentially with saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4. The evaporation of the solvent gave the crude product that was directly submitted for the Fmoc removal without purification. The crude product was dissolved in DMF (8 mL), piperidine (2.0 mL) was added, and the resulting solution was stirred at room temperature for 1 h. Following the solvent evaporation, the residue was purified by silica gel chromatography (50% ethyl acetate in hexanes to 10% methanol in chloroform) to provide product **5** (128 mg) in 85% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.12−8.00 (2H, m), 7.90−7.80 (2H, m), 7.70−7.54 (2H, m), 7.42 (2H, m), 7.35−7.20 (2H, m), 6.97−6.83 (3H, m), 5.32 (1H, m), 4.32 (1H, m), 4.20−4.03 (2H, m), 3.96−3.80 (2H, m), 3.60 (1H, m), 2.98 (3H, m), 2.88 (2H, s), 2.60 (1H, s), 2.00 (1H, m), 1.85 (2H, m), 1.77−1.55 (3H, m); MS (ES$^+$) m/z = 501.4 (M + 1).



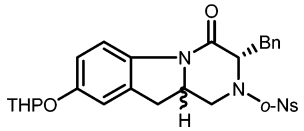
To a solution of the primary amine 5 (128 mg, 0.256 mmol) in dichloromethane (3 mL) were added diisopropyl ethylamine (67 μL, 0.38 mmol) and 2-nitrobenzenesulfonyl chloride (68 mg, 0.31 mmol). The reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by adding aqueous $NaHCO_3$ and extracted with dichloromethane. The combined organic layer was washed with saturated sodium chloride solution, dried over sodium sulfate, filtered, and then concentrated. Purification by column chromatography (33% ethyl acetate in hexanes) afforded 160 mg of the product (91%) as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (1H, m), 7.95 (1H, m), 7.72 (2H, m), 7.58 (3H, m), 7.40 (3H, m), 7.20 (4H, m), 6.83 (2H, m), 6.65 (1H, m), 5.35 (2H, m), 4.88 (1H, m), 4.52 (1H, m), 4.05 (1H, m), 3.89 (3H, m), 3.61 (2H, m), 3.18 (1H, m), 3.10 (1H, m), 2.68 (1H, m), 2.52 (1H, m), 2.00 (1H, m), 1.86 (2H, m), 1.75−1.60 (3H, m); MS (ES$^+$) m/z = 686.3 (M + 1).



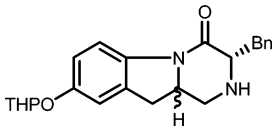
Compound 6. To a solution of the above derivative (124 mg, 0.18 mmol) in methanol (2 mL) was added sodium methoxide (0.5 M in methanol, 0.72 mL, 0.36 mmol). The reaction mixture was stirred at room temperature for 12 h. After completion, the reaction mixture was neutralized and then concentrated. Purification by column chromatography (50% ethyl acetate in hexanes) gave product **6** (74 mg) in 70% yield as a mixture of two diastereomers. ^{1}H NMR (CDCl3, 400 MHz) δ 8.05 (1H, d, J = 7.3 Hz), 7.90 (1H, d, J = 7.9 Hz), 7.77−7.60 (3H, m), 7.23−7.11 (3H, m), 6.94 (1H, m), 6.82 (2H, m), 6.59 (1H, m), 5.35 (1H, m), 4.91 (1H, m), 3.92 (1H, m), 3.80 (1H, m), 3.70−3.51 (2H, m), 3.41 (1H, m), 3.32 (1H, m), 3.08 (2H, m), 2.52 (2H, m), 2.07 (1H, m), 1.86 (2H, m), 1.67 (3H, m); MS (ES$^+$) m/z = 582.3 (M + 1).



Compound 7. To a solution of **6** (74 mg, 0.127 mmol) in THF (5 mL) were added triphenylphosphine (40 mg, 0.152 mmol) and diethyl azodicarboxylate (12 μL, 0.152 mmol). The reaction mixture was stirred at room temperature for 2 h. After completion, the reaction mixture was concentrated. Purification by column chromatography (33% ethyl acetate in hexanes) gave 66 mg of product **7** (92% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (1H, d, J = 8.3 Hz), 7.81 (1H, d, J = 7.8 Hz), 7.64 (2H, m), 7.55 (1H, m), 7.12 (2H, m), 7.07 (3H, m), 6.94 (2H, m), 5.37 (1H, s), 4.86 (1H, s), 4.28 (2H, m), 3.90 (1H, t, J = 9.8 Hz), 3.61 (1H, d, J = 10.3 Hz), 3.32 (2H, m), 2.98 (1H, m), 2.88 (1H, t, J = 12.5 Hz), 2.65 (1H, dd, J = 10.6, 24.4 Hz), 2.01 (1H, m), 1.87 (2H, m), 1.56−1.77 (3H, m). MS (ES$^+$) m/z = 564.3 (M + 1).

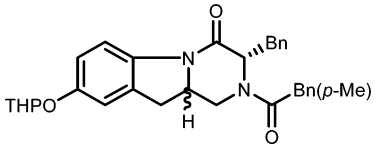


Compound 7a. To a solution of **7** (66 mg, 0.117 mmol) in DMF (4.7 mL), were added K_2CO_3 (65 mg, 0.468 mmol) and thiophenol (24 μL, 0.234 mmol). The reaction mixture was stirred at room temperature for 12 h. After completion, the reaction mixture was filtered and concentrated. Purification by column chromatography (50% ethyl acetate in hexanes to ethyl acetate) gave 35 mg of free amine in 79% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (1H, d, J = 8.6 Hz), 7.36 (2H, m), 7.28 (3H, m), 6.98 (2H, m), 5.39 (1H, t, J = 3.1 Hz), 4.13 (1H, m), 3.93 (1H, t, J = 9.7 Hz), 3.88 (1H, dd, J = 3.4, 10.7 Hz), 3.62 (1H, m), 3.34 (1H, dd, J = 3.4, 13.9 Hz), 3.26 (1H, dd, J = 4.0, 12.4 Hz), 3.02 (3H, m), 2.88 (1H, m), 2.02 (1H, m), 1.88 (2H, m), 1.57−1.77 (3H, m); MS (ES$^+$) m/z = 379.2 (M + 1).

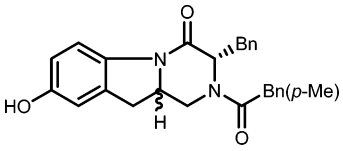


Compound 8a. To a solution of free amine (20 mg, 0.053 mmol) in anhydrous DMF (1 mL) were added *p*-tolyl acetic acid (7.95 mg, 0.053 mmol), HOBt (8.64 mg, 0.063 mmol), and DIC (9.9 μL, 0.063 mmol). The resulting mixture was stirred at room-temperature overnight, and then DMF was

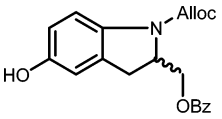
evaporated under high vacuum. The residue was dissolved in ethyl acetate (10 mL), washed with aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4. Purification by column chromatography (50% ethyl acetate in hexanes) afforded 24 mg (92%) of product **8a** as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (2H, m), 7.36 (4H, m), 7.27 (4H, m), 7.18−7.04 (8H, m), 6.95 (4H, m), 6.83 (2H, d, J = 7.9 Hz), 5.39 (2H, m), 5.12 (1H, dd, J = 3.3, 13.3 Hz), 4.67 (1H, dd, J = 3.2, 9.6 Hz), 4.20 (1H, m), 3.90 (2H, m), 3.80−3.65 (2H, m), 3.61 (2H, m), 3.53 (1H, dd, J = 4.3, 13.6 Hz), 3.42 (1H, dd, J = 3.3, 13.7 Hz), 3.30 (1H, dd, J = 5.8, 13.6 Hz), 3.17 (1H, dd, J = 9.7, 13.7 Hz), 3.10−3.00 (3H, m), 2.90−2.78 (4H, m), 2.70 (1H, m), 2.46 (1H, m), 2.36 (3H, s), 2.29 (5H, s); MS (ES$^+$) m/z = 511.4 (M + 1).



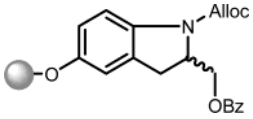
Compound 8b. To a solution of 8a (24 mg, 0.049 mmol) in ethanol (2 mL) was added PPTS (6 mg, 10 mol %). The reaction mixture was stirred at 55 °C for 3 h. After completion, the reaction mixture was concentrated. Purification by column chromatography (50% ethyl acetate in hexanes) gave 17.0 mg of product **8b** in 85% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (1H, d, J = 8.7 Hz), 7.93 (1H, d, J = 8.6 Hz), 7.40−7.32 (4H, m), 7.26 (2H, m), 7.19−7.12 (4H, m), 7.10−7.02 (6H, m), 6.84−6.63 (6H, m), 5.39 (1H, t, J = 5.0 Hz), 5.10 (1H, dd, J = 3.6, 13.3 Hz), 4.68 (1H, dd, J = 2.9, 9.6 Hz), 4.18 (1H, m), 3.90 (1H, dd, J = 3.3, 13.7 Hz), 3.80−3.65 (3H, m), 3.52 (1H, dd, J = 4.4, 13.7 Hz), 3.42 (1H, dd, J = 3.3, 13.8 Hz), 3.30 (1H, dd, J = 5.7, 13.7 Hz), 3.17 (1H, dd, J = 9.8, 13.7 Hz), 3.03 (3H, m), 2.85 (3H, m), 2.70 (1H, dd, J = 7.8, 15.3 Hz), 2.45 (1H, dd, J = 11.2, 15.3 Hz), 2.36 (3H, s), 2.29 (3H, s); MS (ES$^+$) m/z = 427.3 (M + 1).



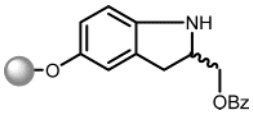
Compound 9. To a solution of **3** (1.152 g, 2.64 mmol) in ethanol (21 mL) was added PPTS (67 mg, 10mol %). The reaction mixture was stirred at 55 °C for 3 h. After completion, the reaction mixture was concentrated. Purification by column chromatography (33% ethyl acetate in hexanes) gave 0.91 g of the product **9** in 97% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (2H, m), 7.69 (1H, m), 7.51 (1H, t, J = 7.4 Hz), 7.35 (2H, m), 6.71 (2H, m), 5.98 (1H, br. s), 5.58 (1H, s), 5.35 (1H, d, J = 16.5 Hz), 5.25 (1H, d, J = 10.0 Hz), 4.89 (1H, m), 4.75 (1H, m), 4.64 (1H, m), 4.50 (1H, m), 4.39 (1H, dd, J = 4.1, 11.0 Hz), 3.40 (1H, dd, J = 9.8, 16.3 Hz), 2.90 (1H, d, J = 16.3 Hz); MS (ES$^+$) m/z = 354.2 (M + 1).



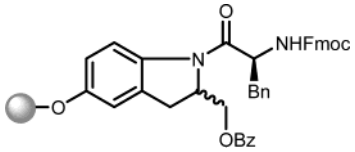
Solid-Phase Synthesis. Compound 9a. To a suspension of 4-bromomethyl phenoxymethyl polystyrene (1.11 g, 1.33 mmol) in DMF (15 mL) was added compound **9** (0.94 g, 2.66 mmol), followed by Cs_2CO_3 (0.868 g, 2.66 mmol) and sodium iodide (0.399 g, 2.66 mmol). The mixture was stirred gently for 24 h and then filtered. The resin was washed with DMF (3×), H_2O (3×), CH_3OH (3×), and CH_2Cl_2 (3×) and then dried under vacuum to give 1.18 g of compound **10** (87% loading after cleavage from the support).



Compound 9b. To a suspension of compound **9a** (90 mg, 0.108 mmol) in CH_2Cl_2 (11.1 mL) was added acetic acid (0.6 mL), 4-methylmorpholine (0.3 mL), and $Pd(PPh_3)_4$ (0.499 g, 0.432 mmol). The resulting mixture was stirred at room temperature for 3 h and then filtered. The resin was washed with DMF (3×), CH_3OH (3×), and CH_2Cl_2 (3×) and then dried under vacuum to give 89.5 mg of compound **9b**.



Compound 10. To a suspension of compound **9b** (80 mg, 0.096 mmol) in DMF (3 mL) were added Fmoc-Phe-OH (74.4 mg, 0.192 mmol), HOBt (28.4 mg, 0.21 mmol), and DIC (36 μL, 0.23 mmol). The mixture was stirred gently overnight and then filtered. The resin was washed with DMF (3×), CH_3OH (3×), and CH_2Cl_2 (3×) and then dried under vacuum to give 80.8 mg of compound **10** (overall 80% yield in three steps after cleavage from the support).

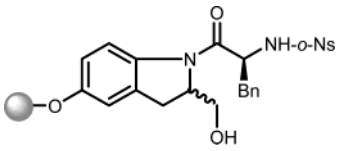


Compound 10a. To a suspension of resin **10** (70 mg, 0.084 mmol) in DMF (3 mL) was added piperidine (0.6 mL). The mixture was stirred gently for 1 h and then filtered. The resin was washed with DMF (3×), CH_3OH (3×), and CH_2Cl_2 (3×) and then dried under vacuum to give 70.2 mg of compound **10a**.

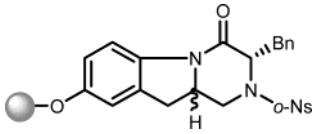
Compound 10b. To a suspension of resin **10a** (70.2 mg, 0.084 mmol) in CH_2Cl_2 (3 mL) were added diisopropylethylamine (58 μL, 0.336 mmol) and 2-nitrobenzenesulfonyl chloride (37 mg, 0.168 mmol). The mixture was stirred gently overnight and then filtered. The resin was washed with DMF

(3×), CH_3OH (3×), and CH_2Cl_2 (3×) and then dried under vacuum to give 70.7 mg of compound **10b**.

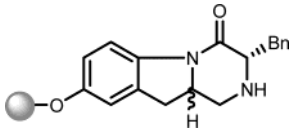
Compound 11. To a suspension of resin **10b** (60 mg, 0.072 mmol) in THF (2 mL) was added sodium methoxide (0.5 M in methanol, 0.72 mL, 0.36 mmol). The mixture was stirred gently overnight and then filtered. The resin was washed with DMF (3×), H_2O (3×), CH_3OH (3×), and CH_2Cl_2 (3×) and then dried under vacuum to give 59.7 mg of resin **11** (overall 65% yield in six steps after cleavage from the support).



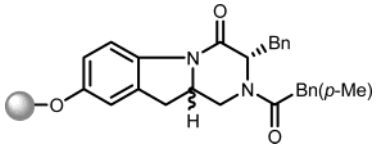
Compound 12. To a suspension of resin **11** (50 mg, 0.06 mmol) in THF (2 mL) were added PPh_3 (62.9 mg, 0.24 mmol) and diethyl azodicarboxylate (38 μL, 0.24 mmol). The mixture was stirred gently overnight and then filtered. The resin was washed with DMF (3×), CH_3OH (3×), and CH_2Cl_2 (3×) and then dried under vacuum to give 50.2 mg of resin **12** (overall 55% yield in seven steps after cleavage from the support).



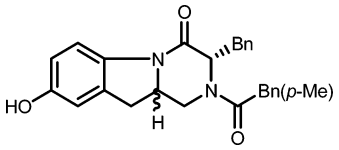
Compound 12a. To a suspension of resin **12** (40 mg, 0.048 mmol) in DMF (3 mL) were added thiophenol (0.154 mL, 1.5 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.448 mL, 3.0 mmol). The mixture was stirred gently overnight and then filtered. The resin was washed with DMF (3×), CH_3OH (3×), and CH_2Cl_2 (3×) and dried under vacuum to give 39.8 mg of resin **12a**.



Compound 13. To a suspension of resin **12a** (30 mg, 0.036 mmol) in DMF (2 mL) were added *p*-tolylacetic acid (13.5 mg, 0.09 mmol), HOBt (12.2 mg, 0.09 mmol), and DIC (17 μL, 0.108 mmol). The mixture was stirred gently overnight and then filtered. The resin was washed with DMF (3×), CH_3OH (3×), and CH_2Cl_2 (3×) and then dried under vacuum to give 30.3 mg of resin 13.



Compound 14. Resin **13** (20 mg, 0.024 mmol) was stirred in 10% TFA in CH_2Cl_2 (2 mL) for 1 h and then filtered. After removal of solvents, the residue was dried under vacuum, followed by purification to give product **14** (35% overall yield for 10 steps after cleavage from the support).



Library Synthesis (100 Compounds) by IRORI Split &-and-Mix-Type Approach. The preloaded resin was weighed into 104 MicroKans, at an average of 29.8 mg of resin per MicroKan. With a resin loading of 0.691 mmol/g, that averaged out to 0.0206 mmol per MicroKan. An RF tag was added to 100 of the MicroKans; 4 were left without RF tags for synthesis monitoring purposes, and then the MicroKans were sealed.

The MicroKan washing procedure starts by immersing the MicroKans in a clean solvent. They are gently stirred for 5−10 min, and then the solvent is removed by filtration with a fritted Buchner funnel. The MicroKans are then transferred to a hand-operated centrifugal rotating solvent removal device (salad spinner) that is used to remove the solvent remaining inside the MicroKans. This washing sequence is repeated many times with different solvents.

Reaction 1 (Alloc Removal). The 104 MicroKans (2.143 mmol total) were placed in a 500-mL round-bottom flask equipped with a magnetic stir bar. They were immersed in a solution of acetic acid (7.5 mL), 4-methylmorpholine (3.75 mL), and CH_2Cl_2 (138.75 mL). Vacuum was applied to remove the air bubbles from the MicroKans. The tetrakis-(triphenylphosphine) palladium(0) (4.9657 g, 4.286 mmol) was added, and the mixture was allowed to stir gently overnight (~20 h). The MicroKans were filtered and washed with CH_2Cl_2 (4×), DMF (3×), methanol (3×), and finally, CH_2Cl_2 (3×). The MicroKans were dried under high vacuum overnight. One MicroKan was removed, and the resin was subjected to cleavage conditions (5 mL of 10% TFA in CH_2Cl_2). The compound was checked by MS. (ES^+) $m/z = 270.1$ (M + 1).

Reaction 2 (Amino Acid Coupling, First Diversity Point). The 100 MicroKans were sorted into 10 50-mL reaction vessels using the IRORI AccuTag Synthesis Manager to plan and track the synthesis. The MicroKans were scanned into the computer with the AccuTag 100 Scanning Station. The computer directed which vessel each MicroKan should be placed into. The reaction solutions consisted of a 0.15 M concentration of amino acid, DIC, and HOBT in 20 mL of DMF with the following order: Fmoc-Asp(*O*-*t*Bu)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(*N*-Boc)-OH, Fmoc-Nle-OH, Fmoc-Orn(*N*-Boc)-OH, Fmoc-Phe-OH, Fmoc-Thr(*O*-*t*Bu)-OH, Fmoc-Tyr(*O*-*t*Bu)-OH, and Fmoc-Val-OH. An additional three Kans were added to vessel 3 (Fmoc-Leu-OH) for monitoring purposes, and the solution was adjusted to 0.15 M of reagents in 25 mL of DMF. The 10 reactions were slowly stirred for 56 h. They were then filtered and combined for washing [DMF (3×), methanol (3×), and finally, CH_2Cl_2 (3×)]. The MicroKans, resorted into 10 reactions vessels, were resubjected to coupling conditions

for another 24 h. The Kans were again filtered, combined, washed, and allowed to dry under high vacuum prior to the next step.

Reaction 3 (Fmoc Removal). The 103 MicroKans were immersed in a solution of 20% piperidine in DMF (200 mL), and the mixture was gently stirred for 1 h. The MicroKans were filtered and then treated with 200 mL of 20% piperidine in DMF for an additional 1 h. The MicroKans were filtered and washed with DMF (4×), methanol (3×), and CH_2Cl_2 (3×). The MicroKans were dried under high vacuum.

Reaction 4 (Nosylation). The 103 MicroKans (2.121 mmol total) were immersed in 180 mL of CH_2Cl_2. 2-Nitrobenzenesulfonyl chloride (3.9892 g, 18.0 mmol) and *N*,*N*-diisopropylethylamine (6.27 mL, 36.0 mmol) were added, and the mixture was allowed to stir for 56 h. The MicroKans were filtered and washed with CH_2Cl_2 (4×), DMF (3×), methanol (3×), and finally, CH_2Cl_2 (3×). The compound in one spare Kan was cleaved (10% TFA in CH_2Cl_2) and was checked by MS. (ES^+) $m/z = 568.1$ (M + 1). Since the nosylation reaction was incomplete, the reaction was repeated for another 48 h.

Reaction 5 (Debenzoylation). The 102 MicroKans (2.1012 mmol total) were immersed in dry THF (180 mL). A 0.5 M solution of sodium methoxide in methanol (21 mL, 10.5 mmol) was added and the reaction was stirred for 2 h. The MicroKans were then filtered and washed with DMF (3×), water (3×), methanol (3×), and finally, CH_2Cl_2 (3×). One of the spare MicroKans was cleaved and checked by MS. (ES^+) $m/z = 464.2$ (M + 1).

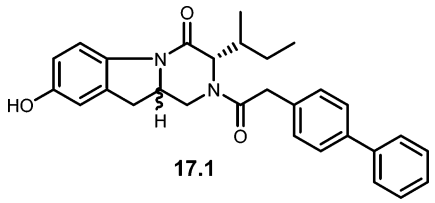
Reaction 6 (Intramolecular Mitsunobu Reaction). The 101 MicroKans (2.080 mmol total) were immersed in dry THF (180 mL). Triphenyl phosphine (2.7278 g, 10.4 mmol) and diethyl azodicarboxylate (1.64 mL, 10.4 mmol) were sequentially added, and the reaction was stirred for 40 h. The MicroKans were then filtered and washed with DMF (4×), methanol (3×), and finally, CH_2Cl_2 (3×). The reaction was then repeated once, with the reaction stirring for 64 h. The final spare MicroKan was subjected to cleavage conditions (10% TFA in CH_2Cl_2) and checked by MS. (ES^+) $m/z = 446.2$ (M + 1).

Reaction 7 (Nosyl Removal). Thiophenol (6.6 mL, 63.83 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (19.1 mL, 127.66 mmol) were added to 100 MicroKans (2.059 mmol total) immersed in dry DMF (180 mL). The reaction was gently stirred for 24 h. The MicroKans were filtered, washed with DMF (5×), methanol (3×), and finally, CH_2Cl_2 (3×) and dried under high vacuum.

Reaction 8 (Acid Coupling, Second Diversity Point). The 100 MicroKans were sorted into 10 50-mL reaction vessels using the IRORI AccuTag Synthesis Manager and AccuTag 100 Scanning Station. The coupling reaction solutions consisted of a 0.15 M concentration of amino acid, DIC, and HOBT in 20 mL of DMF with the following order: 4-biphenylacetic acid, 4-chlorophenylacetic acid, (3,4-dimethoxyphenyl)acetic acid, indole-3-acetic acid, 4-methoxyphenylacetic acid, 3,4-(methylenedioxy)phenylacetic acid, [1R-(1α,2*β*,3α)]-(+)-3-methyl-2-(nitromethyl)-5-oxocyclopentaneacetic acid, 4-nitrophenylacetic acid, phenylacetic acid, and *p*-tolylacetic acid. The 10 reactions were slowly stirred for 64 h. They were then filtered and combined for washing [DMF (5×), methanol (3×), and finally, CH_2Cl_2 (3×)]. The Kans, resorted into 10 reactions vessels, were resubjected to coupling conditions for 40 h. The Kans were again filtered, combined, washed, and allowed to dry under high vacuum prior to the final step.

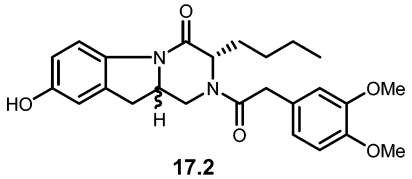
Reaction 9 (Sorting and Cleavage). The 100 MicroKans were sorted into preassigned locations in two 96-well microreactor carriers using the IRORI AccuTag Synthesis Manager and AccuTag 100 Scanning Station. The carriers were placed into AccuCleave-96 cleaving stations, and 3 mL of 10% TFA in CH_2Cl_2 was added to each well. The cleaving stations were vibrated for 1.5 h, and then the contents of the wells were filtered into preweighed vials. An additional 3 mL of 10% TFA in CH_2Cl_2 was added to each well, and the cleavage stations were vibrated again for another 1.5 h. The contents of the wells were again emptied into the same vials. The vial racks were transferred to a speed-vac, and the solvent was removed. The contents of the vials were transferred to microtiter plates after their weights were recorded. Mass spec analysis showed that 78 of the 100 compounds were successfully synthesized. Few compounds from the library were subjected to purification by HPLC and well characterized by MS and NMR.

Compound 17.1 (Mixture of Two Diastereomers). ^1H NMR (400 MHz, acetone-d_6): δ 0.72−1.10 (m, 6H), 1.15−1.24 (bs, 2H), 2.82−3.17 (m, 4h), 3.50−3.68 (m, 2H), 3.92−4.06 (m, 1H), 4.10−4.25 (m, 1H), 4.60−4.69 (m, 1H), 6.63−6.81 (m, 2H), 7.39−7.50 (m, 6H), 7.54−7.69 (m, 4H), 7.75−7.88 (m, 1H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{29}H_{30}N_2O_3$: 455.5 (MH^+).



17.1

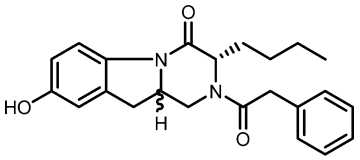
Compound 17.2 (Mixture of Two Diastereomers). ^1H NMR (400 MHz, acetone-d_6): δ 0.80−0.99 (m, 3H), 1.12−1.48 (m, 6H), 1.86 (bs, 2H), 3.44−3.73 (m, 2H), 3.78 (bs, 6H), 4.02−4.33 (m, 2H), 4.50−4.60 (m, 1H), 4.97−5.12 (m, 1H), 6.62−7.00 (m, 4H), 7.48 (bs, 1H), 7.67−7.87 (m, 1H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{25}H_{30}N_2O_5$: 439.3 (MH^+).



17.2

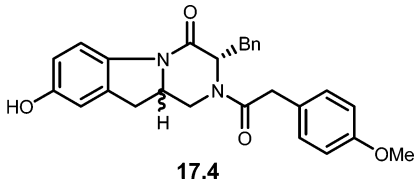
Compound 17.3 (Mixture of Two Diastereomers). ^1H NMR (400 MHz, acetone-d_6): δ 0.72−1.02 (m, 3H), 1.05−1.66 (m, 6H), 1.86 (bs, 2H), 3.28−4.35 (m, 4H), 4.50−4.60 (m, 1H), 4.97−5.12 (m, 1H), 5.68 (s, 1H), 6.63−6.82 (m, 2H), 7.20−7.41 (m, 5H), 7.76−7.85 (m, 1H); LRMS

(electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{23}H_{26}N_2O_3$: 379.2 (MH^+).



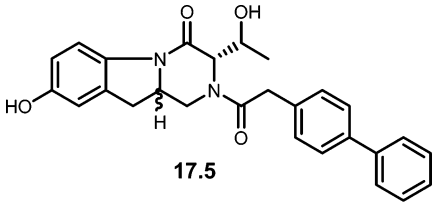
17.3

Compound 17.4 (Mixture of Two Diastereomers). ^{1}H NMR (400 MHz, acetone-d_6): δ 2.49−2.67 (m, 1H), 2.85−2.95 (m, 1H), 3.25−65 (m, 4H), 3.80 (s, 3H), 3.88−4.45 (m, 3H), 4.60−5.30 (m, 1H), 5.81 (s, 1H), 6.64−6.99 (m, 4H), 7.03−7.42 (m, 6H), 7.76−7.95 (m, 2H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{27}H_{26}N_2O_4$: 443.2 (MH^+).



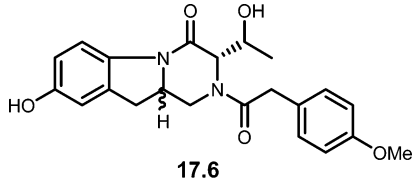
17.4

Compound 17.5 (Mixture of Two Diastereomers). ^{1}H NMR (400 MHz, acetone-d_6): δ 1.08 and 1.18 (ds, J = 6.2 and 6.6 Hz, 3H), 3.20−3.82 (m, 5H), 3.93−4.41 (m, 3H), 4.50−4.67 (m, 1H), 5.01 (bs, 1H), 5.63 (s, 1H), 6.62−6.82 (m, 2H), 7.33−7.50 (m, 6H), 7.58−7.69 (m, 3H), 7.80−7.85 (m, 1H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{27}H_{26}N_2O_4$: 443.2 (MH^+).



17.5

Compound 17.6 (Mixture of Two Diastereomers). ^{1}H NMR (400 MHz, acetone-d_6): δ 1.08 and 1.16 (ds, J = 6.2 and 6.5 Hz, 3H), 3.27−3.73 (m, 3H), 3.78 (s, 3H), 3.82−4.23 (m, 4H), 4.46−4.63 (m, 2H), 4.98 (bs, 1H), 5.63 (s, 1H), 6.61−6.94 (m, 4H), 7.17−7.39 (m, 2H), 7.77−7.84 (m, 1H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{22}H_{24}N_2O_5$: 397.2 (MH^+).



17.6

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References and Notes

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