Discovery of Pyrano[3,4-*b*]indoles as Potent and Selective HCV NS5B Polymerase Inhibitors

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A novel series of HCV NS5B RNA-dependent RNA polymerase inhibitors containing a pyrano-[3,4-*b*]indole scaffold is described leading to the discovery of compound **16**, a highly potent and selective inhibitor that is active in the replicon system.

Introduction

Virally encoded polymerases are essential enzymes as targets for therapeutic intervention. Inhibitors of polymerases of human cytomegalovirus, human immunodeficiency virus (HIV), human herpes simplex virus type I, and hepatitis B virus are currently in clinical use.[1−4] Hepatitis C virus (HCV) is emerging as one of the most significant infections in humans. Current estimates of approximately 2.7−4 million people of the U.S. population and 150 million worldwide as HCV carriers represent a significant medical problem with negative economic implications.[5] The current approved treatments, interferon monotherapy or interferon and ribavirin combination therapy, are effective in 10−40% of the patients. Considerable side effects are associated with these regimens, causing up to 20% of the patients to discontinue the therapy.[6] As a result, there is an unmet need for developing a safe and effective antiviral agent.

The HCV genome encodes the RNA-dependent RNA polymerase NS5B as a 65 KDa protein essential for viral replication. The activities of this enzyme have been extensively characterized in vitro.[7] Recently, a cell-culture model system in Huh7 cells containing a subgenomic replicon capable of supporting HCV replication has been developed.[8] The availability of these in vitro systems makes it possible to screen for inhibitors that might have clinical utility for treatment of diseases caused by HCV.

Both base-modified nucleoside analogues such as β-D-*N*4-hydroxycytidine,[9] sugar-modified 2′-alkyl nucleosides analogues,[10] and 3′-deoxyribonucleotides[11] have been reported to block HCV replication in replicon systems. In analogy to HIV, various nonnucleosides from different chemical classes were recently reported[12] (Figure 1). The diketo acid inhibitor **1** is reported to interact with the metal ions present in the enzyme active site, whereas benzimidazole inhibitors **3** and *N*,*N*-disubstituted phenylalanine inhibitors **4** are reported to display allosteric interactions.

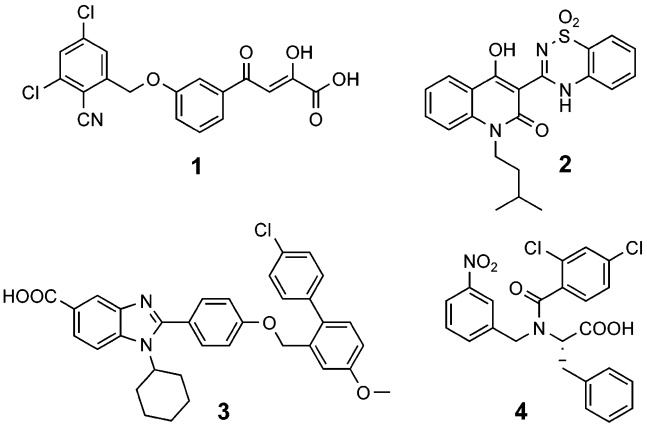

Figure 1. HCV NS5B polymerase inhibitors.

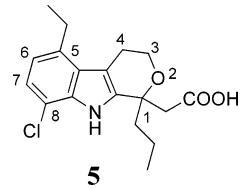

Figure 2. Pyranoindole lead **5** for HCV NS5B polymerase.

Our effort toward identifying a HCV polymerase inhibitor started with the high throughput screening (HTS) of the various compound libraries. The HTS consisted of an in vitro nucleotide incorporation assay in which a functionally active recombinant NS5B enzyme and a hairpin-like primed RNA substrate were used. A positive control consisted of the enzyme in the absence of compound, and a negative control consisted of the enzyme in the presence of basilen blue, a dye shown to inhibit NS5B polymerase activity in this assay. The effort culminated in the identification of pyranoindole **5** (Figure 2), which had an IC_{50} of 3.0 μM. It was found to be selective against human polymerase β (IC_{50} > 100 μM), calf thymus polymerase α (IC_{50} > 100 μM), helicase (IC_{50} > 75 μM), as well as HIV reverse transcriptase (IC_{50} > 100 μM). Compound **5** was not cytotoxic in rapidly dividing and stationary Vero and Huh7 cells as measured by a standard MTS metabolic assay. On the basis of these results preliminary SAR studies and biological characterization of this novel lead were

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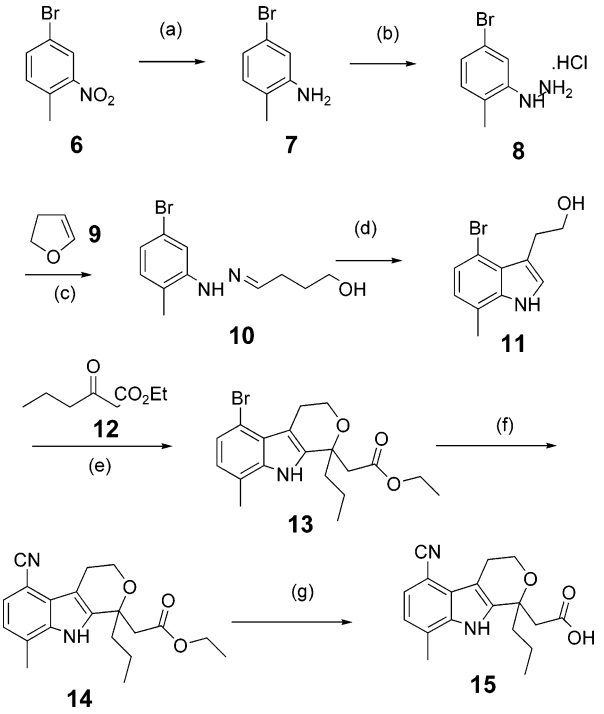
Scheme 1. Synthesis of Pyranoindole Derivatives[a]



[a] Reagents and conditions: (a) Fe, NH_4Cl, 100 °C, 3 h, 92%; (b) $NaNO_2$/HCl, $SnCl_2$, 0−10 °C, 75%; (c) **9**, THF−H_2O; (d) $ZnCl_2$, ethylene glycol, 25% from **8**; (e) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 93%; (f) CuCN, NMP, microwave, 88%; (g) 1 N NaOH, 98%.

undertaken. Herein, we report on the synthesis, chemical resolution, and biological activity of pyrano[3,4-*b*]-indoles as promising inhibitors of HCV NS5B enzyme.

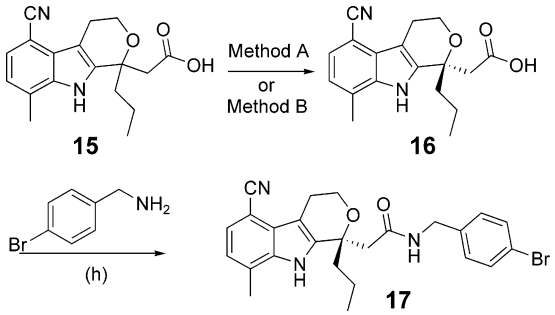
Chemistry

The pyranoindole analogues were synthesized as shown in Scheme 1 starting from the appropriately substituted nitrobenzenes, such as **6**. Reduction to the aniline **7** followed by diazotization using sodium nitrite and tin chloride reduction resulted in hydrazine **8**. Reaction of the hydrazine with 2,3-dihydrofuran **9** gave the alcohol **10**, which underwent cyclization upon treatment with zinc chloride to give the tryptophol **11**. Condensation of the tryptophol with β-keto esters **12** gave the pyranoindole **13**. The bromo compound was converted to the nitrile **14** by treating with CuCN. Hydrolysis of the ester gave the acid **15**. The enantiomers were separated by a chiral HPLC method or by resolution using cinchonine to form the salt, which was separated and hydrolyzed to give pure enantiomers (>99% enantiomeric excess) (Scheme 2). The free acid was converted to the 4-bromobenzyl amide derivative **17** and was used for structural confirmation by X-ray crystallography.

Results and Discussions

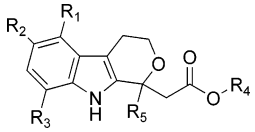
Attempts to understand the SAR of the lead molecule started with the acid moiety. Conversion to ester or amide derivatives rendered the molecules inactive as NS5B inhibitors, indicating the importance of the free acid. Replacement of the *n*-propyl group in the C1-position of the pyran unit with an ethyl group **18** decreased the NS5B potency significantly. On the basis of this preliminary structure−activity information, it was decided to focus on the aromatic substitution

Scheme 2. Resolution of Pyrano[3,4-*b*]indole Enantiomers[a]



[a] Reagents and conditions: method A, (1) cinchonine, 2-butanone/water; (2) 1 N HCl/EtOAc; method B, HPLC, CHIRALPACK-AD (250 × 20 mm) column, 10% isopropyl alcohol in heptane (0.1% TFA) eluant; (h) EDCI, HOBT, DIEA, DMF, rt, 88%.

Table 1. HCV NS5B Polymerase Inhibitory Activity of Pyranoindole Analogues



compd	R_1	R_2	R_3	R_4	R_5	IC_{50} (μM)
5	Et	H	Cl	H	propyl	3.0
18	Et	H	Cl	H	ethyl	>30
19	Cl	H	Cl	H	propyl	0.27
20	Cl	H	H	H	Propyl	6.0
14	CN	H	Me	Et	propyl	>30
15	CN	H	Me	H	propyl	0.57
21	CN	H	F	H	propyl	1.0
22	CN	F	Me	H	propyl	0.12

pattern to improve the potency. It was found that replacing the ethyl group on C5 with chlorine to give the 5,8-dichloro compound **19** increased the enzymatic potency significantly. Of the other substituents placed on the C-5 position, a cyano group was well-tolerated. A substituent on the C-8 position was found to be essential for activity, since compound **20** showed a significant loss of potency in comparison to **19**. A small substituent like methyl in **15** or fluoro in **21** was sufficient to regain the activity. The trisubstituted aromatic pattern led to a slight improvement in the potency, as shown by compound **22** (Table 1).

Since the hit identified from HTS was racemic, it was of importance to establish if there is a preference for binding one enantiomer over the other and, if found to be the case, to identify the absolute stereochemistry of the active enantiomer. Toward this end, acid **15** was separated by chiral HPLC method, and the fast-eluting active enantiomer was converted to the amide derivative **17**. X-ray crystallography of the amide identified the eutomer to be the *R* isomer.

The chiral separation protocol using a CHIRALPACK-AD column was utilized to resolve compounds **5**, **19**, **15**, **21**, and **22** for further biological evaluation. The enantiomeric excess (ee) was in the range of 86−100%. For each pair of enantiomers, greater activity of 7−20-fold was found with the *R* isomer (Table 2). In the case of compounds **25** and **26**, good inhibitory activity was found for the enantiomers; however, these compounds were less desirable than others, due to their activity in the MTS assay.

Table 2. HCV NS5B Polymerase Inhibitory Activity of Separated Enantiomers

compd	R_1	R_2	R_3	R_5	stereo	IC_{50} (μM)	MTS (μM)
23	Et	H	Cl	propyl	R	2.0	>50
24	Et	H	Cl	propyl	S	>15	>50
25	Cl	H	Cl	propyl	R	0.06	33
26	Cl	H	Cl	propyl	S	1.1	49
16	CN	H	Me	propyl	R	0.33	>50
27	CN	H	Me	propyl	S	2.4	>50
28	CN	H	F	propyl	R	0.44	>50
29	CN	H	F	propyl	S	5.2	>50
30	CN	F	Me	propyl	R	0.08	>50
31	CN	F	Me	propyl	S	0.63	>50

On the basis of these findings, compound **16** was selected for further characterization and advancement. By employing fluorescence spectroscopy techniques, the apparent K_D of 0.8 μM was determined from the changes in the tryptophan fluorescence intensity at the emission wavelength of 340 nm in comparison to the NS5B−inhibitor complex excitation wavelength of 295 nm. From these experiments the stoichiometry of binding was found to be in a 1:1 ratio. The details of binding of **16** to an allosteric site of NS5B[12a] will be reported elsewhere.

Compound **16** displayed broad inhibitory activities against the NS5B enzyme derived from HCV genotypes 1a, 1b, and 3a with IC_{50} values ranging from 0.3 to 1.4 μM for 90% of the genotypes. It showed no inhibitory activity against a panel of human polymerases, including mitochondrial DNA polymerase γ, and other unrelated viral polymerases up to 80 μM, demonstrating its specificity for the HCV polymerase. Moreover, a single administration to Huh7 cells containing the HCV subgenomic replicon for 3 days resulted in a dose-dependent reduction of the steady-state levels of viral RNA and protein (EC_{50} = 4.8 μM for HCV RNA).

It is interesting to note that Mobilio et al. reported analgesic activity for certain *cis*-1,4-disubstituted tetrahydropyrano[3,4-*b*]indoles residing in *S*-stereoisomers.[13] Compound **16** is devoid of analgesic activity.

Conclusions

In summary, we have identified a novel series of 1,3,4,9-tetrahydropyrano[3,4-*b*]indole derivatives as potent selective inhibitors of HCV NS5B RNA dependent RNA polymerase.

Experimental Section

General Procedures. Melting points were determined in an open capillary tubes on a Meltemp melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined with a Bruker DPX-300 spectrometer at 300 MHz. Chemical shifts are reported in parts per million (δ) relative to residual chloroform (7.26 ppm), TMS (0 ppm), or dimethyl sulfoxide (2.49 ppm) as an internal reference with coupling constants (J) reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Electrospray (ES) mass spectra were recorded in positive or negative mode on a Micromass Platform spectrometer. Electron impact and high-resolution mass spectra were obtained on a Finnegan MAT-90 spectrometer. Combustion analyses were obtained using a Perkin-Elmer Series II 2400 CHNS/O analyzer. The combustion analysis was conducted on the free base. Chromatographic purifications were performed by flash chromatography using Baker 40-μm silica gel. Thin-layer chromatography (TLC) was performed on Analtech silica gel GHLF 250 M prescored plates. The terms "concentrated" and "evaporated" refer to removal of solvents using a rotary evaporator at water aspirator pressure with a bath temperature equal to or less than 60 °C. Unless otherwise noted, reagents were obtained from commercial sources and were used without further purification

5-Bromo-2-methylaniline (7). A mixture of Fe powder (9.31 g, 167 mmol) and NH_4Cl (2.48 g, 46.3 mmol) in water (50 mL) was refluxed for 30 min. To this hot mixture was added 4-bromo-2-nitrotoluene (**6**) (10 g, 46.3 mmol) slowly and then the reaction mixture was refluxed for 48 h. The mixture was cooled to room temperature and extracted with ethyl acetate (3 × 100 mL). The organic solution was washed with water (3 × 200 mL) and brine (200 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (silica, 15% EtOAc in hexanes) to give 7.9 g (92%) of the title compound as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.88 (m, 1H), 6.81 (m, 2H), 3.63 (bs, 2H), 2.09 (s, 3H). Anal. Calcd for C_7H_8BrN·HCl: C, 37.79; H, 4.08; N, 6.29. Found: C, 37.91; H, 3.92; N, 6.22.

5-Bromo-2-methylphenylhydrazine Hydrochloride (8). To a suspension of 5-bromo-2-methylaniline (**7**) (4.80 g, 25.8 mmol) in concentrated HCl (16 mL) was added dropwise a solution of sodium nitrite (1.96 g, 28.4 mmol) in water (10 mL) over 30 min at 0 °C. To the mixture was added dropwise a solution of $SnCl_2·2H_2O$ (17.46 g, 77.4 mmol) in concentrated HCl (15 mL) over 50 min. After stirring for 1 h at 0 °C, the reaction mixture was basified with 50% NaOH (30 mL). The mixture was further diluted with water (20 mL) and treated with another portion of 50% NaOH (10 mL) and then crushed ice (100 g). The reaction mixture was extracted with ether (3 × 100 mL), and the combined organic phases were washed with brine, dried over Na_2SO_4, and filtered. The filtrate was acidified by adding an anhydrous solution of HCl in ether (1 N in ether, 31 mL, 31 mmol). The precipitate was collected and dried under reduced pressure to give 4.57 g (75%) of the title compound as a white amorphous solid. ^{1}H NMR (300 MHz, DMSO): δ 10.31 (bs, 3H), 8.11 (bs, 1H), 7.12 (s, 1H), 7.06 (m, 2H), 2.14 (s, 3H). Anal. Calcd for $C_7H_9BrN_2$·HCl: C, 35.40; H, 4.24; N, 11.79. Found: C, 35.40; H, 4.16; N, 11.78.

4-Bromo-7-methyltryptophol (11). To a solution of 5-bromo-2-methylphenylhydrazine hydrochloride (**8**) (4.57 g, 19.2 mmol) in 30% aqueous THF (100 mL) at 0 °C was added dropwise a solution of 2,3-dihydrofuran (**9**) (1.60 mL, 21.2 mmol) in THF (10 mL). After stirring for 2 h at 0 °C and 12 h at room temperature, the reaction mixture was diluted with ether (100 mL). The organic solution was washed with saturated $NaHCO_3$ (2 × 100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated. The residue (**10**) was dissolved in ethylene glycol (30 mL), treated with $ZnCl_2$ (5.76 g, 42.2 mmol), and heated at 170 °C for 4 h. The reaction mixture was cooled to room temperature and 6 N HCl (100 mL) was added. The mixture was extracted with ether (3 × 100 mL) and washed with water (200 mL) and brine (200 mL). The organic solution was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (silica, 40% EtOAc in hexanes) to give 1.22 g (25%) of the title compound as a light brown oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (bs, 1H), 7.18 (d, J = 7.65 Hz, 1H), 7.08 (d, J = 2.16 Hz, 1H), 6.81 (d, J = 7.65 Hz, 1H), 3.95 (t, J = 6.42 Hz, 2H), 3.27 (t, J = 6.42 Hz, 2H), 2.40 (s, 3H), 1.69 (bs, 1H). Anal. Calcd for $C_{11}H_{12}$-BrNO: C, 51.99; H, 4.76; N, 5.51. Found: C, 51.74; H, 4.92; N, 5.28.

5-Bromo-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indole-1-acetic Acid Ethyl Ester (13). To a solution of 4-bromo-7-methyltryptophol (**11**) (1.12 g, 4.41 mmol) and ethyl butyryl acetate (**12**) (0.71 mL, 4.41 mmol) in CH_2Cl_2 (20 mL) was added BF_3·OEt_2 (0.56 mL, 4.41 mmol) dropwise at room temperature. The solution was stirred for 2 h and then washed with saturated aqueous $NaHCO_3$ (15 mL) and brine (15 mL). The organic phase was dried (Na_2SO_4) and filtered through a pad of silica gel. The filter cake was washed with additional CH_2Cl_2 and the combined organic layer was evaporated to provide 1.62 g (93%) of the title compound as a white

solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.33 (bs, 1H), 7.11 (d, J = 7.65 Hz, 1H), 6.76 (d, J = 7.65 Hz, 1H), 4.19 (m, 2H), 4.03 (m, 1H), 3.90 (m, 1H), 3.15 (m, 2H), 3.03 (d, J = 16.6 Hz, 1H), 2.89 (d, J = 16.6 Hz, 1H), 2.43 (s, 3H), 2.08 (m, 1H), 1.96 (m, 1H), 1.38 (m, 1H), 1.27 (t, J = 7.14 Hz, 3H), 1.18 (m, 1H), 0.87 (t, J = 7.29 Hz, 3H). Anal. Calcd for $C_{19}H_{24}BrNO_3$: C, 57.88; H, 6.14; N, 3.55. Found: C, 57.63; H, 5.91; N, 3.74

5-Cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indole-1-acetic Acid Ethyl Ester (14). 5-Bromo-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid ethyl ester (**13**) (1.27 g, 3.22 mmol) and CuCN (0.433 g, 4.83 mmol) were dissolved in *N*-methyl-2-pyrrolidinone (15 mL) and the solution was divided into four microwave reaction vessels (3.75 mL of each). The reaction vessels were heated in a microwave at 220 °C for 15 min. The reaction mixtures were combined and then diluted with water (30 mL). The crude mixture was extracted with EtOAc (3 × 50 mL). The combined organic phase was washed with brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography (silica, 20% EtOAc in hexanes) to give 0.959 g (88%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.75 (bs, 1H), 7.33 (d, J = 7.52 Hz, 1H), 6.93 (d, J = 7.52 Hz, 1H), 4.21 (m, 2H), 4.11 (m, 1H), 4.03 (m, 1H), 3.08 (t, J = 5.52, 2H), 2.99 (d, J = 4.17 Hz, 2H), 2.57 (s, 3H), 2.06 (m, 2H), 1.42 (m, 1H), 1.26 (t, J = 7.16 Hz, 3H), 1.18 (m, 1H), 0.88 (t, J = 7.32 Hz, 3H). Anal. Calcd for $C_{20}H_{24}N_2O_3$: C, 70.57; H, 7.11; N, 8.23. Found: C, 70.27; H, 6.92; N, 8.18.

5-Cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indole-1-acetic Acid (15). To a solution of 5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid ethyl ester (**14**) (0.959 g, 2.82 mmol) in THF/MeOH (7 mL/15 mL) was added 1 N NaOH (5.64 mL, 5.64 mmol). The reaction mixture was stirred at ambient temperature overnight. Most of the THF/MeOH was removed under reduced pressure and the resulting mixture was acidified with 1 N HCl. The mixture was extracted with EtOAc (3 × 30 mL). The combined organic phase was washed with brine (60 mL), dried over Na_2SO_4, and concentrated to provide 0.868 g (99%) of the title compound as a white solid. ^{1}H NMR (300 MHz, acetone-d_6): δ 10.37 (bs, 1H), 7.35 (d, J = 7.50 Hz, 1H), 7.03 (d, J = 7.50 Hz, 1H), 4.05 (m, 2H), 3.08−2.91 (m, 4H), 2.54 (s, 3H), 2.09 (m, 2H), 1.45 (m, 1H), 1.03 (m, 1H), 0.84 (t, J = 7.26 Hz, 3H). Anal. Calcd for $C_{18}H_{20}N_2O_3$: C, 69.21; H, 6.45; N, 8.97. Found: C, 68.97; H, 6.84; N, 8.80.

[(*R*)-5-Cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic Acid (16). Method A. [(*R*)-5-Cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid was obtained by resolution with cinchonine according to the following procedure. (±)-5-Cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid (6.4 g, 20.5 mmol) and cinchonine (5.9 g, 20.0 mmol) were dissolved in a mixture of 2-butanone (125 mL) and water (5 mL) with heating. The clear solution was stirred and allowed to cool to room temperature overnight. The resulting solid was isolated, washed with 10 mL of 2-butanone, and dried to give 2.4 g (20% yield, >98% ee). The mother liquor was concentrated and dissolved again in a mixture of 2-butanone (100 mL) and water (1.5 mL) with heating. The solution was stirred and allowed to cool to room temperature overnight. The resulting solid was isolated, washed with 10 mL of 2-butanone, and dried to give a second crop of salt (2.3 g, 18% yield, >98% ee). The two crops (total 4.7 g) were combined and treated with 50 mL of 1 N HCl and 100 mL of ethyl acetate. The ethyl acetate layer was washed with 1 N HCl (30 mL) and water (50 mL). The aqueous layers were combined and extracted with ethyl acetate (50 mL). This ethyl acetate layer was washed with water (50 mL). The combined ethyl acetate layers were dried over sodium sulfate, filtered, and concentrated in vacuo to give 2.25 g. This material was triturated with 10 mL of ethyl acetate and the precipitate was collected, rinsed with 5 mL of ethyl acetate, and dried to give 1.27 g (>98% ee). The mother liquor was concentrated to a volume of 5 mL and the precipitate was collected, rinsed with 2 mL of ethyl acetate, and dried. A second crop of 0.4 g was obtained (>99% ee). The mother liqour was concentrated and gave a third crop of 0.5 g (>99% ee).

Method B. Alternatively, preparative HPLC using CHIRALPACK-AD (250 × 20 mm) and 10% isopropyl alcohol in heptane (0.1% TFA) as eluant gave *R*- and *S*-enantiomers of 5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic acid as white solids. HRMS (ESI): $[M + H]^+$ calcd for $C_{18}H_{21}N_2O_3$ 313.1547, found 313.1545 (*R*-enantiomer) and 313.1547 (*S*-enantiomer). Chiral HPLC: HP 1100 with spiderlink CHIRALPACK-AD, 250 × 4.6 mm, isopropyl alcohol/heptane containing 0.1% TFA (10:90), 1.0 mL/min, DAD 215 nm; t_R = 6.98 min (*R*-enantiomer), 9.37 min (*S*-enantiomer). Mp: 225−227 °C. $[\alpha]^{25}_D = -11 \pm 1°$ (c = 1.1%, acetone). ^{1}H NMR (300 MHz, acetone-d_6): δ 10.37 (bs, 1H), 7.35 (d, J = 7.50 Hz, 1H), 7.03 (d, J = 7.50 Hz, 1H), 4.05 (m, 2H), 3.08−2.91 (m, 4H), 2.54 (s, 3H), 2.09 (m, 2H), 1.45 (m, 1H), 1.03 (m, 1H), 0.84 (t, J = 7.26 Hz, 3H). MS (ESI): *m/z* 311.2. Anal. Calcd for $C_{18}H_{20}N_2O_3$: C, 69.21; H, 6.45; N, 8.97. Found: C, 69.19; H, 6.32; N, 8.90.

[(*S*)-5-Cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic Acid (27). Mp: 224−226 °C. $[\alpha]^{25}_D = +11 \pm 1°$ (c = 1%, acetone). ^{1}H NMR (300 MHz, acetone-d_6): δ 10.37 (bs, 1H), 7.35 (d, J = 7.50 Hz, 1H), 7.03 (d, J = 7.50 Hz, 1H), 4.05 (m, 2H), 3.08−2.91 (m, 4H), 2.54 (s, 3H), 2.09 (m, 2H), 1.45 (m, 1H), 1.03 (m, 1H), 0.84 (t, J = 7.26 Hz, 3H). MS (ESI): *m/z* 311.2. Anal. Calcd for $C_{18}H_{20}N_2O_3$: C, 69.21; H, 6.45; N, 8.97. Found: C, 68.94; H, 6.42; N, 9.28.

1-(*R*)-*N*-(4-Bromobenzyl)-2-(5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl)acetamide (17). To a solution of 1-(*R*)-5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid (**16**) (20.0 mg, 0.064 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 15.0 mg, 0.077 mmol), and 1-hydroxybenzotriazole (10.4 mg, 0.077 mmol) in DMF (4 mL) was added *N*,*N*-diisopropylethylamine (67 μL, 0.384 mmol) followed by 4-bromobenzylamine hydrochloride (17.1 mg, 0.077 mmol) at room temperature. The reaction mixture was stirred for 20 h at ambient temperature. Water (5 mL) was added to the mixture and the resulting mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic phase was washed with brine (20 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography (silica, 40% EtOAc in hexanes) to give 27 mg (88%) of the title compound as a white solid. The solid was crystallized from ethyl acetate for X-ray crystallography. Mp: 173−175 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.15 (bs, 1H), 7.33 (m, 3H), 6.97 (m, 2H), 6.88 (m, 1H), 4.42 (dd, J = 11.2, 4.6 Hz, 1H), 4.29 (dd, J = 11.2, 4.6 Hz, 1H), 4.03 (m, 2H), 3.11−2.95 (m, 4H), 2.24 (s, 3H), 2.07 (m, 1H), 1.91 (m, 1H), 1.35 (m, 2H), 0.89 (t, J = 5.4 Hz, 3H). HRMS (ESI): $[M + H]^+$ calcd for $C_{25}H_{27}BrN_3O_2$ 480.1281, found 480.1285.

The following compounds were prepared by following the above procedure.

(8-Chloro-5-ethyl-1-propyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indol-1-yl)acetic Acid (5). Mp: 152−154 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 10.91 (s, 1H), 7.01 (d, J = 8.0 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H), 3.98−3.84 (m, 2H), 2.96 (d, J = 13.6 Hz, 2H), 2.94−2.82 (m, 2H), 2.78 (d, J = 13.6 Hz, 2H), 2.10−1.93 (m, 2H), 1.33−1.19 (m, 1H), 1.21 (t, J = 7.6 Hz, 3H), 0.81−0.74 (m, 4H). HRMS: calcd for $C_{18}H_{22}ClNO_3$ − H, 334.12154, found (ESI−, $[M - H]^-$), 334.12124.

8-Chloro-1,5-diethyl-1,3,4,9-tetrahydro-pyrano[3,4-*b*]-indole-1-acetic Acid (18). Mp: 148−150 °C. ^{1}H NMR (400 MHz, DMSO): δ 12.0 (s, 1H), 10.9 (s, 1H), 7.05 (d, J = 8 Hz, 1H), 6.8 (d, J = 8 Hz, 1H), 4.0 (m, 2H), 3.02−3.0 (m, 2H), 2.7−2.95 (m, 4H), 2.05 (m, 2H), 1.4 (t, J = 7.4 Hz 3H), 0.5 (t, J = 7.4 Hz 3H). HRMS: calcd for $C_{17}H_{20}ClNO_3$ + H, 322.12045, found (ESI−FTMS, [M + H]), 322.12023.

(5,8-Dichloro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indol-1-yl)acetic Acid (19). Mp: 166−167 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.12 (bs, 1H), 7.03 (d, J = 8.26 Hz, 1H), 6.96 (d, J = 8.26 Hz, 1H), 4.04 (m, 2H), 3.14 (m, 2H), 3.06 (m, 2H), 2.03 (m, 2H), 1.42 (m, 1H), 1.21 (m, 1H), 0.89 (t, J = 7.34 Hz,

3H). HRMS: calcd for $C_{16}H_{17}Cl_2NO_3$ + H, 342.06582, found (ESI−FTMS, [M + H]), 342.06567.

5-Chloro-1,3,4,9-tetrahydro-1-propylpyrano[3,4-*b*]indole-1-acetic Acid (20). ^{1}H NMR (400 MHz, DMSO): δ 12.0 (s, 1H), 11.0 (s, 1H), 7.2 (d, J = 8 Hz, 1H), 6.9−7.0 (m, 2H), 4.0 (m, 2H), 3.2−2.7 (m, 3H), 2.05 (m, 2H), 1.4 (m, 1H), 0.7 (m, 4H). MS: *m/z* 307. Anal. Calcd for $C_{16}H_{18}ClNO_3$: C, 62.44; H, 5.89; N, 4.55. Found: C, 62.43; H, 5.62; N, 4.39.

(5-Cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indol-1-yl)acetic Acid (21). Mp: 228−230 °C.^{1}H NMR (300 MHz, DMSO): δ 12.02 (bs, 1H), 11.33 (bs, 1H), 7.00 (d, J = 9.00 Hz, 1H), 3.96 (m, 2H), 2.95 (d, J = 10.3 Hz, 1H), 2.83 (t, J = 3.9 Hz, 1H), 2.72 (d, J = 10.3 Hz, 1H), 2.54 (s, 3H), 1.99 (m, 2H), 1.28 (m, 1H), 0.85 (m, 1H), 0.79 (t, J = 5.41 Hz, 3H). MS (ESI): *m/z* 315.1. Anal. Calcd for $C_{17}H_{17}FN_2O_3$: C, 64.55; H, 5.42; N, 8.86. Found: C, 64.61; H, 5.27; N, 8.86.

(5-Cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl)acetic Acid (22). Mp: 249−251 °C. ^{1}H NMR (300 MHz, DMSO): δ 12.02 (bs, 1H), 11.33 (bs, 1H), 7.00 (d, J = 9.00 Hz, 1H), 3.96 (m, 2H), 2.95 (d, J = 10.3 Hz, 1H), 2.83 (t, J = 3.9 Hz, 1H), 2.72 (d, J = 10.3 Hz, 1H), 2.54 (s, 3H), 1.99 (m, 2H), 1.28 (m, 1H), 0.85 (m, 1H), 0.79 (t, J = 5.41 Hz, 3H). MS (ESI): *m/z* 329.2. Anal. Calcd for $C_{18}H_{19}FN_2O_3$: C, 65.44; H, 5.80; N, 8.48. Found: C, 65.43; H, 5.86; N, 8.44.

[(1*R*)-8-Chloro-5-ethyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic Acid (23). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.99 (s, 1H), 10.91 (s, 1H), 7.01 (d, J = 8.0 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H), 3.98−3.84 (m, 2H), 2.96 (d, J = 13.6 Hz, 2H), 2.94−2.82 (m, 2H), 2.78 (d, J = 13.6 Hz, 2H), 2.10−1.93 (m, 2H), 1.33−1.19 (m, 1H), 1.21 (t, J = 7.6 Hz, 3H), 0.81−0.74 (m, 4H). HRMS: calcd for $C_{18}H_{22}ClNO_3$ + H, 336.1361, found (ESI−FTMS, [M + H]), 336.13619.

(1*S*)-8-Chloro-5-ethyl-1-propyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indol-1-yl]acetic Acid (24). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.99 (s, 1H), 10.91 (s, 1H), 7.01 (d, J = 8.0 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H), 3.98−3.84 (m, 2H), 2.96 (d, J = 13.6 Hz, 2H), 2.94−2.82 (m, 2H), 2.78 (d, J = 13.6 Hz, 2H), 2.10−1.93 (m, 2H), 1.33−1.19 (m, 1H), 1.21 (t, J = 7.6 Hz, 3H), 0.81−0.74 (m, 4H). HRMS: calcd for $C_{18}H_{22}ClNO_3$ − H, 334.12154, found (ESI−, [M − H]$^-$), 334.12143.

[(1*R*)-5,8-Dichloro-1-propyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indol-1-yl]acetic Acid (25). Mp: 57 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.12 (bs, 1H), 7.03 (d, J = 8.26 Hz, 1H), 6.96 (d, J = 8.26 Hz, 1H), 4.04 (m, 2H), 3.14 (m, 2H), 3.06 (m, 2H), 2.03 (m, 2H), 1.42 (m, 1H), 1.21 (m, 1H), 0.89 (t, J = 7.34 Hz, 3H). HRMS: calcd for $C_{16}H_{17}Cl_2NO_3$ + H, 342.06582, found (ESI−FTMS, [M + H]), 342.06637.

[(1*S*)-5,8-Dichloro-1-propyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indol-1-yl]acetic Acid (26). Mp: 58 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.12 (bs, 1H), 7.03 (d, J = 8.26 Hz, 1H), 6.96 (d, J = 8.26 Hz, 1H), 4.04 (m, 2H), 3.14 (m, 2H), 3.06(m, 2H), 2.03 (m, 2H), 1.42 (m, 1H), 1.21 (m, 1H), 0.89 (t, J = 7.34 Hz, 3H). HRMS: calcd for $C_{16}H_{17}Cl_2NO_3$ + H, 342.06582, found (ESI−FTMS, [M + H]), 342.06576.

[(1*R*)-5-Cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic Acid (28). Mp: 163−165 °C.^{1}H NMR (300 MHz, DMSO): δ 12.02 (bs, 1H), 11.33 (bs, 1H), 7.00 (d, J = 9.00 Hz, 1H), 3.96 (m, 2H), 2.95 (d, J = 10.3 Hz, 1H), 2.83 (t, J = 3.9 Hz, 1H), 2.72 (d, J = 10.3 Hz, 1H), 2.54 (s, 3H), 1.99 (m, 2H), 1.28 (m, 1H), 0.85 (m, 1H), 0.79 (t, J = 5.41 Hz, 3H). MS (ESI): *m/z* 315.5. Anal. Calcd for $C_{17}H_{17}FN_2O_3$: C, 64.55; H, 5.42; N, 8.86. Found: C, 64.78; H, 5.81; N, 8.70.

[(1*S*)-5-Cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic Acid (29). Mp: 163−166 °C.^{1}H NMR (300 MHz, DMSO): δ 12.02 (bs, 1H), 11.33 (bs, 1H), 7.00 (d, J = 9.00 Hz, 1H), 3.96 (m, 2H), 2.95 (d, J = 10.3 Hz, 1H), 2.83 (t, J = 3.9 Hz, 1H), 2.72 (d, J = 10.3 Hz, 1H), 2.54 (s, 3H), 1.99 (m, 2H), 1.28 (m, 1H), 0.85 (m, 1H), 0.79 (t, J = 5.41 Hz, 3H). HRMS: calcd for $C_{17}H_{17}FN_2O_3$ + H, 317.12960, found (ESI−FTMS, [M + H]), 317.12936.

[(1*R*)-5-Cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic Acid (30). Mp: 237−238 °C. $[\alpha]^{25}_D = -7 \pm 1°$ (c = 1%, acetone). ^{1}H NMR (300 MHz, DMSO): δ 12.02 (bs, 1H), 11.33 (bs, 1H), 7.00 (d, J = 9.00 Hz, 1H), 3.96 (m, 2H), 2.95 (d, J = 10.3 Hz, 1H), 2.83 (t, J = 3.9 Hz, 1H), 2.72 (d, J = 10.3 Hz, 1H), 2.54 (s, 3H), 1.99 (m, 2H), 1.28 (m, 1H), 0.85 (m, 1H), 0.79 (t, J = 5.41 Hz, 3H). MS (ESI) *m/z* 329.1. Anal. Calcd for $C_{18}H_{19}FN_2O_3 \cdot 0.25H_2O$: C, 64.56; H, 5.87; N, 8.37. Found: C, 64.61; H, 5.93; N, 8.38.

[(1*S*)-5-Cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic Acid (31). Mp: 241−242 °C. $[\alpha]^{25}_D = +19 \pm 1°$ (c = 1%, acetone). ^{1}H NMR (300 MHz, DMSO): δ 12.02 (bs, 1H), 11.33 (bs, 1H), 7.00 (d, J = 9.00 Hz, 1H), 3.96 (m, 2H), 2.95 (d, J = 10.3 Hz, 1H), 2.83 (t, J = 3.9 Hz, 1H), 2.72 (d, J = 10.3 Hz, 1H), 2.54 (s, 3H), 1.99 (m, 2H), 1.28 (m, 1H), 0.85 (m, 1H), 0.79 (t, J = 5.41 Hz, 3H). HRMS: calcd for $C_{18}H_{19}FN_2O_3$ + H, 331.14580, found (ESI+, [M + H]$^+$), 331.14515. Anal. Calcd for $C_{18}H_{19}FN_2O_3$: C, 65.44; H, 5.80; N, 8.48. Found: C, 65.45; H, 5.75; N, 8.47.

Supporting Information Available: Analysis data for the compounds synthesized. This material is available free of charge via the Internet at http://pubs.acs.org.

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