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The Discovery of Pyrano[3,4-*b*]indole-Based Allosteric Inhibitors of HCV NS5B Polymerase with In Vivo Activity

Matthew G. LaPorte,*[a] Randy W. Jackson,[b] Tandy L. Draper,[b] Janet A. Gaboury,[b] Kristin Galie,[b] Torsten Herbertz,[c] Alison R. Hussey,[b] Susan R. Rippin,[b] Christopher A. Benetatos,[d] Srinivas K. Chunduru,[d] Joel S. Christensen,[d] Glen A. Coburn,[d] Christopher J. Rizzo,[d] Gerry Rhodes,[e] John O'Connell,[f] Anita Y. M. Howe,[f] Tarek S. Mansour,[f] Marc S. Collett,[d] Daniel C. Pevear,[d] Dorothy C. Young,[d] Tiejun Gao,[g] D. Lorne J. Tyrrell,[g, h] Norman M. Kneteman,[g, i] Christopher J. Burns,[b] and Stephen M. Condon*[a]

Hepatitis C (HCV) belongs to the Flaviviridae family of positive-sense, single-stranded RNA viruses. The HCV genome encodes a polyprotein, comprised of 3000 amino acid residues, which is processed into both structural and nonstructural proteins.[1] HCV infection is a significant global health issue; the World Health Organization estimates that over 170 million people carry the HCV[2] infection, which can ultimately result in chronic hepatitis, cirrhosis, and hepatocellular carcinoma. These complications are responsible for about 10 000–20 000 deaths annually in the U.S. alone.[3] HCV is the leading cause of advanced liver disease and liver transplantation.[4]

Current therapies for HCV infection rely on the combination of the nonspecific antiviral medication, ribavirin, and interferon-α (IFN).[4] Not only does this treatment regimen cause undesirable side effects such as leucopenia, thrombocytopenia, and hemolytic anemia, but only ~40% of patients achieve a sustained viral response.[4b,5,6]

Recently, several pegylated forms of IFN, including Pegasys and PEG-INTRON, have been approved for HCV treatment, which, in combination with low-dose ribavirin, has resulted in improved viral response rates (>50%) and reduced side effects.[7]

In 2000, Bartenschlager described a cell-based replication model (replicon) that contains all of the enzymes necessary for efficient HCV replication but lacks the structural proteins required for virion packaging, and hence transmission.[1,8] In 2001, a chimeric mouse model (SCID/*Alb-uPA*) was developed to study HCV infection in vivo using human liver explants.[9] The results obtained from this murine infection model have been consistent with observed clinical outcomes.[10] Most recently, a robust cell culture model for HCV infection has been developed, which promises to allow a greater understanding of the early steps in viral transmission.[11] These recent advances in the screening and evaluation of new chemical entities promise to accelerate the discovery and development of anti-HCV treatments. In fact, several inhibitors of HCV NS3/4A protease have advanced into clinical trials as monotherapies, or as combination therapies with pegylated IFNs.[12]

Another structural protein essential for viral replication is NS5B RNA-dependent RNA polymerase (RdRp).[12e,13,14] Allosteric inhibition of reverse transcriptase has proven affective in the treatment of HIV-1; for this reason allosteric inhibition of HCV NS5B is considered a promising strategy and, as such, has received considerable attention from the pharmaceutical industry.[12d,14,15] In 2004, Gopalsamy et al. reported the synthesis and biological evaluation of a series of tetrahydropyrano[3,4-*b*]indole-based allosteric inhibitors of HCV NS5B.[16] This work resulted in the identification and clinical evaluation of HCV-371 (**1**).[17] Pyranoindole **1** selectively inhibits HCV NS5B (IC_{50} = 0.3–1.4 μM for 90% of HCV genotypes) and displays activity in the subgenomic HCV replicon assay (EC_{50} = 4.8 ± 0.5 μM). Acceptable safety and tolerability of **1** were observed in Phase I clinical trials. Disappointingly, compound **1** did not demonstrate significant antiviral activity in a Phase Ib efficacy study. Retrospective evaluation of **1** using the SCID/*Alb-uPA* mouse model also failed to afford a significant antiviral effect.[10]

HCV-371 (**1**) **2**

[a] M. G. LaPorte, S. M. Condon
TetraLogic Pharmaceuticals
343 Phoenixville Pike, Malvern, PA 19355 (USA)
Fax: (+1) 610-889-9994
E-mail: mlaporte@tetralogicpharma.com
scondon@tetralogicpharma.com

[b] R. W. Jackson, T. L. Draper, J. A. Gaboury, K. Galie, A. R. Hussey, S. R. Rippin, C. J. Burns
Department of Medicinal Chemistry, ViroPharma Inc.
397 Eagleview Boulevard, Exton, PA 19341 (USA)

[c] T. Herbertz
Department of Computational Chemistry, ViroPharma Inc.
397 Eagleview Boulevard, Exton, PA 19341 (USA)

[d] C. A. Benetatos, S. K. Chunduru, J. S. Christensen, G. A. Coburn, C. J. Rizzo, M. S. Collett, D. C. Pevear, D. C. Young
Department of Biology, ViroPharma Inc.
397 Eagleview Boulevard, Exton, PA 19341 (USA)

[e] G. Rhodes
Department of Pharmacology, ViroPharma Inc.
397 Eagleview Boulevard, Exton, PA 19341 (USA)

[f] J. O'Connell, A. Y. M. Howe, T. S. Mansour
Department of Infectious Diseases, Wyeth, Pearl River, NY 10965 (USA)

[g] T. Gao, D. L. J. Tyrrell, N. M. Kneteman
KMT Hepatech Inc., Edmonton, Alberta, T6G 2E1 (Canada)

[h] D. L. J. Tyrrell
Department of Medical Microbiology and Infectious Diseases
University of Alberta, Edmonton, Alberta T6G 2B7 (Canada)

[i] N. M. Kneteman
Department of Surgery
University of Alberta, Edmonton, Alberta T6G 2B7 (Canada)

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Herein, we describe the synthesis and in vivo evaluation of analogue **2**, a member of a novel class of pyranoindole-based inhibitors of HCV NS5B polymerase, which are substituted at the C7 position of the heteroaromatic core structure.[18] Our program, directed at the in vivo evaluation of derivative **2** and the exploration of the structure–activity relationships within this class of allosteric, pyranoindole-based HCV NS5B inhibitors, requires a reproducible, gram-scale synthesis of the pyranoindole core.

Retrosynthetically, the 7-hydroxypyranoindole precursor **3** can arise via the Baeyer–Villiger oxidation of aldehyde **4,** which is in turn available in four steps from tryptophol **5** (Figure 1).

Figure 1. Retrosynthetic approach to key intermediate **3**.

As described for the synthesis of HCV-371 (**1**),[16,20] the key tryptophol intermediate **5** would be constructed by condensation of hydrazine **6** with dihydrofuran **7**. Practically, the acid-mediated Fischer reaction of hydrazine **6** and dihydrofuran **7** proved problematic, affording compound **5** in only modest yields together with the dimeric analogue **8,** formed via the abnormal Fischer process, and varying amounts of dihydrofuran- and polyethylene glycol-containing products, which proved difficult to remove (Scheme 1).

Recognizing that the Fischer indolization route was unreliable for the synthesis of large quantities of material, an alternative route to the key 3-hydroxypyranoindole intermediate **3** was designed based on the Lewis acid-catalyzed oxa-Pictet-Spengler reaction of the tryptophol **9** with ethyl 3-oxohexanoate to give the racemic pyranoindole.[23] The highly functionalized tryptophol **9** could be synthesized using the well-documented Pd-mediated reaction of *o*-iodoanilines with alkynes.[21,22] The success of this new route therefore hinged on a reliable, gram-scale synthesis of the *o*-iodoaniline **10** (Figure 2).

Scheme 1. Synthesis of tryptophol **5**: a) **7**, ethylene glycol, H_2O, 100 °C, 3 h, 30–40 % (**5**) and 15 % (**8**).

Figure 2. Revised retrosynthesis of key intermediate **3**.

Phenylamine **12** was synthesized from the commercially available 2,6-dinitrotoluene (**11**). Diazotization and hydrolysis of compound **12** yielded the corresponding phenol in good overall yield, which was subsequently *O*-benzylated to give compound **13** (Scheme 2). Copper(I)-mediated cyanation of the arylbromide **13** followed by reduction of the nitro group using powdered Fe and acetic acid gave aniline **14**. Interestingly, a significant amount of aniline **14** was also isolated from the copper-mediated cyanation reaction. Treatment of compound **14** with benzyltrimethylammonium dichloroiodate in the presence of $CaCO_3$ gave the *o*-iodoaniline **10** exclusively.[24]

Scheme 2. Preparation of iodoaniline **10**: a) 1,3-dibromo-5,5-dimethylhydantoin, H_2SO_4, RT, 1 h, 92 %; b) $(NH_4)_2S$, EtOH, Pyr, H_2O, Δ, 83 %; c) $NaNO_2$, H_2SO_4, H_2O, 72 %; d) NaH, BnBr, DMF, RT, 16 h, 62 %; e) CuCN, NMP, Δ, 43 % (+**14**, 37 %); f) Fe, AcOH, Δ, 2 h, 96 %; g) $Bn(Me)_3NICl_2$, DCE, MeOH, $CaCO_3$, Δ, 7 h, 86 %.

Reaction of *o*-iodoaniline **10** with the TES-protected alkyne **15**, under Larock conditions, provided derivative **9** in a 72 % yield with no detectable amount of the undesired regioisomer (Scheme 3).[21] Although the direct treatment of compound **9** and ethyl 3-oxohexanoate with 1 equivalent of BF_3-etherate afforded the desired pyranoindole **16**, an improvement in the overall yield was observed when compound **9** was pretreated with BF_3-etherate at 0 °C to effect initial desilylation; the subsequent addition of ethyl 3-oxohexanoate at RT then gave the benzylated precursor **16**. The *O*-benzyl and ester protecting

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Scheme 3. Synthesis of pyranoindole **17**: a) $Pd(OAc)_2$, PPh_3, DIPEA, Bu_4NCl, DMF, 85 °C, 3.5 h, 72 %; b) BF_3-etherate, CH_2Cl_2, RT, 1 h then ethyl 3-oxohexanoate, RT, 3 h, 50 %; c) H_2, $Pd(OH)_2$, EtOAc, RT, 1 h, 61 %; d) aq NaOH, EtOH, 20 h, 96 %.

Scheme 5. Preparation of compound **2**: a) BF_3-etherate, CH_2Cl_2, RT, 1 h then ethyl (4*S*)-methyl-3-oxohexanoate, RT, 3 h, 50 %; b) K_2CO_3, *N*-(2-bromoethyl)-pyrazole, RT, 16 h, 60 %; c) NaOH, MeOH, 75 %.

groups were sequentially removed to provide the desired C7-oxypyranoindole (**17**).

The (1*R*)-stereoisomers of this series are known to be active against HCV polymerase.[16] For this reason, the racemic acid **17** was treated with 1 equivalent of (−)-quinine in anhydrous methanol at reflux to give the desired (1*R*)-isomer as an insoluble white quinine salt. The quinine salt was neutralized to the free acid and re-esterified under nonacidic conditions to give methyl ester **18** in a high enantiomeric excess as determined by chiral HPLC analysis. Notably, yields could be improved by recycling the undesired (1*S*)-isomer; concurrent esterification and racemization was achieved by heating with trimethyl orthoacetate in toluene in the presence of methanol and an acid, subsequent ester hydrolysis reformed the racemic acid **17**, which could be recycled effectively.[25] Alkylation of the phenol moiety with *N*-(2-bromoethyl)pyrazole was then followed by saponification of the ester to give the C1-*n*-propyl-C7-substituted pyranoindole **19** (Scheme 4).

The preparation of the C1-*sec*-butyl-C7-substituted pyranoindole **2** proceeded along similar lines (Scheme 5). Treatment of **9** with ethyl (4*S*)-methyl-3-oxohexanoate[26] gave pyranoindole **20** as a ~1:1 mixture of diastereomers. However, fractional recrystallization of the diastereomeric (−)-quinine salt proved ineffective. Inspection of other cinchonine and cinchonidine salts under a variety of solvent conditions was also fruitless. Ultimately, re-esterification afforded the diastereomeric methyl esters, which could be separated using chiral HPLC [CHIRALPAK AS, 250×20 mm, 20 % 2-propanol/heptane] to give **21** in reasonable quantities. *O*-Alkylation and ester saponification then provided the desired *sec*-butyl derivative **2**.

Scheme 4. Preparation of compound **19**: a) (−)-quinine, MeOH, 60 °C, 20 h; b) H_3O^+; c) $(MeO)_3CCH_3$, PhMe, 100 °C, 2 h, 43 %-3 steps; d) K_2CO_3, *N*-(2-bromoethyl)pyrazole, RT, 16 h, 65 %; e) NaOH, MeOH, 75 %.

Having secured a robust synthesis of key tryptophol **9**, and with access to sufficient quantities of chiral esters **18** and **21**, we sought to explore the structure–activity relationship of the heteroaryl moiety using a small set of readily-accessible fragments. Alkylation of either **18** or **21** with a heterocyclic ethylbromide followed by saponification gave compounds **22**–**28**, which were evaluated for their anti-HCV activity. As described in Table 1, simple substitution of the pyrazole nucleus (Entry 2) or replacement with a thiazole (Entry 3) resulted in decreased NS5B inhibition, which translated into reduced activity in the replicon assay.[27] Similarly, other *N*-linked heterocycles (pyrrole, imidazole, triazoles) and *C*-linked pyrazoles demonstrated a reduced ability to inhibit NS5B and/or to penetrate cells (data not shown). Substitution on the alkyl side chain was generally tolerated with a modest preference for the (*S*)-isomers (Entries 4 and 5).

We had previously reported that (*S*)-*sec*-butyl substituent at the C1 position in the C7-unsubstituted series could effect a dramatic increase in cellular activity,[19] therefore this feature was re-examined within the C7-substituted series (Table 1). As anticipated, both *sec*-butyl-containing analogs (Entries 6 and 7) potently inhibited in vitro viral replication. Compound **2** (Entry 6) inhibits NS5B polymerase across the spectrum of HCV genotypes tested (IC_{50} = 0.002–0.026 μM) including genotypes 3a (IC_{50} = 0.013 μM) and 4 (IC_{50} = 0.026 μM, Table 1). Using the in vitro replicon system, compound **2** reduces the steady-state levels of HCV RNA with EC_{50} values of 0.0045 and 0.023 μM for genotypes 1a and genotype 1b, respectively (Table 2).[8a,28–30] Within the effective antiviral concentrations, compound **2** demonstrates no adverse effects on cellular functions (ΔGAPDH RNA, CC_{50} > 20 μM) indicating that the inhibitory effects of compound **2** are specific for HCV. After 16 days, com-

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Table 1. Biological activities of pyranoindoles.

Entry	Compound	R^1	R^2	R^3	IC_{50} BB7Δ21 [μM]	EC_{50} HCV RNA [μM]	CC_{50} GAPDH RNA [μM]
1	**19**	pyrazol-1-yl	H	H	0.005	0.14 ± 0.10	99.2 ± 1.51
2	**22**	3-methylpyrazol-1-yl	H	H	0.030	1.18 ± 0.46	25.2 ± 1.86
3	**23**	thiazol-4-yl	H	H	0.022	0.37 ± 0.25	21.7 ± 14.2
4	**24**	pyrazol-1-yl	(*S*)-Me	H	0.003	0.10 ± 0.05	75.0 ± 28.8
5	**25**	pyrazol-1-yl	(*R*)-Me	H	0.007	0.15 ± 0.08	70.6 ± 18.9
6	**2**	pyrazol-1-yl	H	Me	0.003	0.02 ± 0.01	21.1 ± 9.2
7	**26**	pyrazol-1-yl	(*S*)-Me	Me	0.002 ± 0.0007	0.02 ± 0.01	18.1 ± 17.2

Table 2. Biological profile comparison of HCV-371 (**1**) and compound **2**.

Assay type/secondary activity	Inhibition values[a,b] HCV-371 (**1**)	Compound **2**
HCV NS5B polymerase assay		
HCV 1b, BB7Δ21 His-tagged	IC_{50} = 0.4 ± 0.2 μM	IC_{50} = 0.002 ± 0.0007 μM
HCV 1b, BK Δ21 His-tagged	IC_{50} = 0.3 ± 0.1 μM	IC_{50} = 0.009 ± 0.011 μM
HCV 3a, Δ21 His-tagged	IC_{50} = 1.8 ± 1.3 μM	IC_{50} = 0.013 ± 0.004 μM
HCV 4 Δ21 His-tagged	IC_{50} = 17.8 ± 8.0 μM	IC_{50} = 0.026 ± 0.010 μM
Surrogate cell-based assays		
HCV replicon 1a (HCV RNA)	ND	EC_{50} = 0.0045 ± 0.003 μM
HCV replicon 1b (HCV RNA)	EC_{50} = 4.8 ± 0.5 μM	EC_{50} = 0.023 ± 0.011 μM
L419M replicon (HCV RNA)	EC_{50} = 34.3 ± 8.1 μM	EC_{50} = 0.49 ± 0.007 μM
Serum shift assay (replicon 1a/50% HS[c])	ND	EC_{50} = 0.027 ± 0.012 μM
Serum shift assay (replicon 1b/50% HS[c])	EC_{50} > 10 μM	EC_{50} = 0.39 ± 0.26 μM
Cytotoxicity assay (GAPDH RNA)	CC_{50} > 30 μM	CC_{50} = 21.1 ± 9.2 μM
Secondary activity		
HIV RT	IC_{50} > 30 μM	IC_{50} > 30 μM
Human RNA polymerase II	IC_{50} > 30 μM	IC_{50} > 30 μM
Calf thymus DNA polymerase α	IC_{50} > 30 μM	IC_{50} > 30 μM
RNA interchelation	ND	IC_{50} > 30 μM

[a] ND; not determined. [b] Values represent the mean of at least two independent determinations.
[c] HS; human serum.

pound **2** effected a 4.9 log_{10} reduction in HCV levels when incubated at 2.2 μM with replicon-containing Huh-7 cells (genotype 1b). In the presence of 50% human serum (HS), the HS-adjusted EC_{50} value of compound **2** in the replicon system is 0.027 and 0.39 μM for genotypes 1a and 1b, respectively, despite being highly protein-bound (99% to HSA).

Pharmacokinetic analysis of a single 50 mg kg^{-1} subcutaneous dose of compound **2** in SCID/*Alb-uPA* transgenic mice indicated a C_{max} and C_{8h} of 5169 ± 47 and 976 ± 9 ng mL^{-1}, respectively (Figure 3). To maximize plasma exposure, we opted to dose compound **2** subcutaneously every 8 h (q8 h, sc) for 7 consecutive days in the efficacy study. Compound **2** demon-

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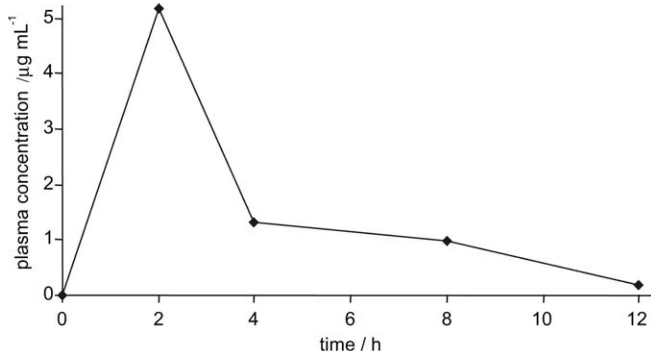

Figure 3. Pharmacokinetic evaluation of compound **2**: plasma exposure of compound **2** in SCID/*Alb-uPA* mice following a single 50 mpk sc dose.

strated significant reductions in plasma HCV RNA levels in the transgenic mouse model of HCV infection during the first three days of treatment, although the HCV viral load increased during the remainder of the dosing schedule (days 4–7, Figure 4).[31,32] Pyranoindole **2** was tolerated well over the

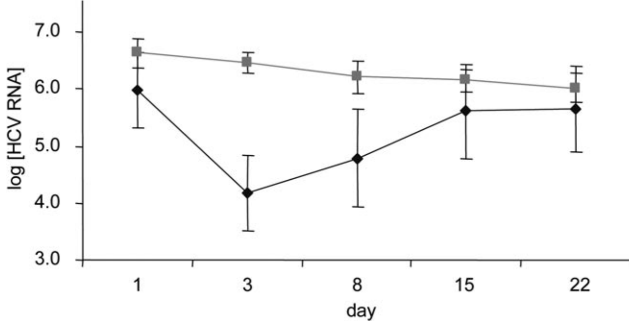

Figure 4. Viral response in HCV-infected chimeric mice: efficacy study of compound **2** in HCV-infected SCID/*Alb-uPA* mice (compound **2**: ◆; control: ■).

course of the treatment and no adverse effects were noted. On day 8 (1 day after treatment ended), the measured HCV RNA levels in the treated animals were on average 1.2 $\log_{10}$ lower than untreated animals ($p = 0.006$), and the HCV titres returned to baseline by day 15 of the study.

In summary, we have described a novel route to a potent family of pyranoindole-based HCV NS5B inhibitors. These inhibitors show a 1000-fold improvement in in vitro potency of the previously reported pyrano[3,4-*b*]indoles, and are the first members of this class to demonstrate in vivo antiviral activity using the chimeric mouse model. This result confirms that allosteric inhibition of HCV NS5B can significantly suppress HCV replication in vivo, and provides support for the further development of allosteric inhibitors of HCV NS5B polymerase.[12d,e,14,33]

Experimental Section

^{1}H NMR spectra were obtained on a Varian 300 MHz spectrometer. MS analyses were performed on a Finnigan LCQ or a Thermo-MSQ Plus that was equipped with a Finnigan Surveyor LC Pump Plus with PDA detector. The solvent system used was MeCN/H_2O with 0.1% AcOH. All reagents were obtained from commercial suppliers. Preparative HPLC was performed on a Varian ProStar instrument using a 215 pump, a UV-Vis 320 detector and a fraction collector 701, using Varian Star software. N-(2-bromoethyl)pyrazole was prepared using literature procedures.[34] All Animal work was performed in accordance to policy.[9–10]

5-Bromo-2-methyl-3-nitro-phenylamine (12): 2,6-Dinitrotoluene (**11**, 1.0 g, 5.5 mmol) was suspended in concd H_2SO_4 (4 mL) and 1,3,-dibromo-5,5-dimethylhydantoin (0.86 g, 3 mmol) was added portionwise to the mixture over 10 min. The reaction was initially exothermic leading to dissolution of the solid before the formation of a precipitate. The heterogeneous mixture was stirred at RT for 1 h before the pale yellow solid was collected and dried in vacuo to give 5-bromo-2-methyl-1,3-dinitro-benzene (1.33 g, 92%); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.13$ (s, 2H), 2.52 ppm (s, 3H); MS (ESI): *m/z* (%): 260, 262 (Br pattern). 5-Bromo-2-methyl-1,3-dinitro-benzene (1.0 g, 3.8 mmol) was dissolved in EtOH (23 mL) and treated with pyridine (1.5 mL, 19.0 mmol) and subsequently heated to reflux for 1 h. H_2O (4 mL) was added to aq $(NH_4)_2S$ (20 wt.%, 3.9 g, 0.057 mol) and this solution was then added to the refluxing mixture via addition funnel over 1 h. After the addition was complete, the reflux was continued for 2 h. The reaction was cooled to RT then poured onto a 1:1 mixture of water and ice (200 mL). The bright yellow precipitate was collected and dried in vacuo to give compound **12** (0.73, 83%); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.29$ (d, $J = 1.7$ Hz, 1H), 6.99 (d, $J = 1.7$ Hz, 1H), 3.97 (br s, 2H), 2.18 ppm (s, 3H); MS (ESI): *m/z* (%): 231, 233 (Br pattern).

1-Benzyloxy-5-bromo-2-methyl-3-nitro-benzene (13): Phenylaniline **12** (5.0 g, 21.5 mmol) was suspended in a solution of concd H_2SO_4 (6.4 mL) and H_2O (21.2 mL). The reaction was cooled (0 °C) and a solution of sodium nitrite (1.66 g, 24.0 mmol) in H_2O (6.4 mL) was added via an addition funnel while maintaining the temperature <10 °C. The reaction was stirred at <10 °C for 1 h before being transferred via a plastic cannula to a solution of concd H_2SO_4 (21.2 mL) in H_2O (14.9 mL) at 130–150 °C. An initial drop in the internal temperature of the reaction was observed (~80 °C) during the addition, heating was continued until the internal temperature returned to ≥ 110 °C. The reaction was cooled, poured on ice (200 mL), and extracted with *tert*-butylmethyl ether (3×100 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo to give 5-bromo-2-methyl-3-nitro-phenol as a dark orange-red solid (3.61 g, 72%); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 10.82$ (s, 1H), 7.53 (d, $J = 1.7$ Hz, 1H), 7.25 (d, $J = 1.7$ Hz, 1H), 2.17 ppm (s, 3H); MS (ESI): *m/z* (%): 231, 233 (Br pattern). A solution of 5-bromo-2-methyl-3-nitro-phenol (6.4 g, 27 mmol) in DMF (10 mL) was cooled to 0 °C and treated with a slurry of NaH (767 mg, 32 mmol) in DMF (10 mL). After 15 min, the solution was treated dropwise with BnBr (3.4 mL, 28 mmol). The reaction mixture was warmed to RT and stirred for 16 h. The reaction was carefully diluted with brine and extracted with Et_2O (3×100 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash chromatography (SiO_2, hexanes/EtOAc, 6:1) gave compound **13** as a tan-orange solid (5.4 g, 62%); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.57$ (s, 1H), 7.42–7.36 (m, 6H), 5.10 (s, 2H), 2.35 ppm (s, 3H); MS (ESI): *m/z* 322.

3-Amino-5-benzyloxy-4-methyl-benzonitrile (14): A solution of compound **13** (11 g, 34 mmol) in NMP (40 mL) was treated with CuCN (18 g, 202 mmol) at RT and heated at 180 °C for 1 h. The solution was then cooled to RT, diluted with water and EtOAc (100 mL) and filtered through a Celite/silica gel pad. The filtrate was extracted with EtOAc, washed with brine (2×100 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash

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chromatography (SiO_2, hexanes/EtOAc, 5:1) followed by trituration (Et_2O/hexanes) provided 3-benzyloxy-4-methyl-5-nitro-benzonitrile as an off-white solid (3.9 g, 43%) and compound **14** as a light tan solid (3 g, 37%); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.69$ (s, 1H), 7.43–7.39 (m, 6H), 5.17 (s, 2H), 2.47 ppm (s, 3H); MS (ESI): *m/z* 268. A solution of 3-benzyloxy-4-methyl-5-nitro-benzonitrile (3.9 g, 14.5 mmol) in EtOH (40 mL) was treated with Fe powder (2.5 g, 45 mmol) followed by AcOH (4.0 mL) at RT and the reaction was heated to reflux for 2 h. The solution was cooled to RT, filtered through a pad of Celite/silica gel and rinsed with EtOAc. The filtrate was concentrated in vacuo, redissolved in EtOAc, washed with saturated $NaHCO_3$ (2×100 mL) and brine (100 mL), dried (Na_2SO_4), filtered and concentrated in vacuo to give compound **14** as a light tan solid (3.3 g, 96%); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta =$ 7.43–7.32 (m, 6H), 6.62 (s, 1H), 5.04 (s, 2H), 3.80 (br s, 2H), 2.11 ppm (s, 3H); MS (ESI): *m/z* 238.

3-Amino-5-benzyloxy-2-iodo-4-methyl-benzonitrile (10): A solution of compound **14** (4.2 g, 22 mmol) in dichloroethane (40 mL) and MeOH (20 mL) was treated with $CaCO_3$ (9 g, 90 mmol) followed by $Bn(Me)_3NICl_2$ (9.2 g, 26 mmol) at RT and heated to reflux for 7 h. The reaction was cooled to RT, filtered through a Celite/silica gel pad and thoroughly rinsed with EtOAc. The filtrate was concentrated then diluted with EtOAc (100 mL). The organic phase was washed with 10% $NaHSO_3$ (2×100 mL) and brine (100 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash chromatography (SiO_2, hexanes/EtOAc, 4:1) followed by trituration (Et_2O/hexanes) gave compound **10** as a light tan solid (5.9 g, 86%); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.41$–7.35 (m, 5H), 6.71 (s, 1H), 5.04 (s, 2H), 4.36 (br s, 2H), 2.20 ppm (s, 3H); MS: *m/z* 364.

6-Benzyloxy-3-(2-hydroxy-ethyl)-7-methyl-2-triethylsilanyl-1*H*-indole-4-carbonitrile (9): A solution of compound **15** (913 mg, 4.9 mmol) in DMF (2 mL) was treated with compound **10** (497 mg, 1.4 mmol) in DMF (2 mL) followed by Bu_4NCl (401 mg, 1.4 mmol), Ph_3P (115 mg, 0.43 mmol), $Pd(OAc)_2$ (90 mg, 0.40 mmol) and DIPEA (1.0 mL, 5.7 mmol) at RT. The reaction was heated at 85 °C for 3.5 h. The reaction was cooled to RT, filtered through a Celite/silica gel pad, which was thoroughly rinsed with EtOAc. The filtrate was extracted with EtOAc, washed with brine (2×100 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was diluted with Et_2O, washed with brine (2×100 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash chromatography (SiO_2, hexanes/EtOAc, 4:1 to 1:1) gave compound **9** as an orange oil that solidified upon standing (422 mg, 72%); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.99$ (s, 1H), 7.47–7.34 (m, 5H), 7.17 (s, 1H), 5.12 (s, 2H), 3.95–3.91 (m, 2H), 3.33–3.28 (m, 2H), 2.47 (s, 3H), 2.12–2.09 (m, 1H), 1.05–0.92 ppm (m, 15H); MS (ESI): *m/z* 419 $[M-H]^-$.

(±)-Ethyl-(7-benzyloxy-5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydro-pyrano[3,4-*b*]indol-1-yl)-acetate (16): A solution of compound **9** (242 mg, 0.58 mmol) in CH_2Cl_2 (4 mL) was treated with BF_3-etherate (0.08 mL, 0.64 mmol) at RT and stirred for 30 min. Additional BF_3-etherate (0.10 mL, 1.1 mmol) was added and the reaction was stirred for a further 30 min before ethyl 3-oxohexanoate (0.12 mL, 0.75 mmol) was added. After 3 h, the reaction was quenched with brine, extracted with EtOAc (3×100 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Trituration (Et_2O/hexanes) gave compound **16** as an off-white solid (130 mg, 50%); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 9.44$ (s, 1H), 7.45–7.33 (m, 5H), 7.10 (2, 1H), 5.11 (s, 2H), 4.29–4.11 (m, 2H), 4.08–4.02 (m, 1H), 3.95–3.79 (m, 1H), 3.06–3.02 (m, 2H), 2.96 (q, $J = 18$ Hz, 2H), 2.43 (s, 3H), 2.12–1.83 (m, 2H), 1.42–1.33 (m, 1H), 1.29 (t, $J = 6.9$ Hz, 3H), 1.23–1.14 (m, 1H), 0.88 ppm (t, $J = 7.2$ Hz, 3H); MS (ESI): *m/z* 445 $[M-H]^-$.

(±)-(5-Cyano-7-hydroxy-8-methyl-1-propyl-1,3,4,9-tetrahydro-pyrano[3,4-*b*]indol-1-yl)-acetic acid (17): A solution of ester **16** (143 mg, 0.32 mmol) in EtOAc (10 mL) and MeOH (10 mL) was treated with $Pd(OH)_2$ (20 mg, 20 wt.% on C) and placed under a H_2 atmosphere using a Parr shaker. After 1 h, the solution was filtered through a Celite plug and the filtrate was concentrated in vacuo. Trituration (Et_2O/hexanes) gave the corresponding phenol as an off-white solid (69 mg, 61%); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 9.45$ (s, 1H), 6.94 (s, 1H), 4.65 (s, 1H), 4.26–4.11 (m, 2H), 4.07–4.00 (m, 1H), 3.94–3.86 (m, 1H), 3.04–3.01 (m, 2H), 2.96 (q, $J = 17$ Hz, 2H), 2.41 (s, 3H), 2.12–2.01 (m, 1H), 1.96–1.86 (m, 1H), 1.43–1.33 (m, 1H), 1.29 (t, $J = 7.5$ Hz, 3H), 1.23–1.17 (m, 1H), 0.88 ppm (t, $J = 7.5$ Hz, 3H). The phenol (3.0 g, 8.43 mmol) was dissolved in EtOH (120 mL) and treated with aq NaOH (10 mL, 2 M). After stirring for 20 h, the reaction was concentrated in vacuo and the residue was redissolved in H_2O (10 mL). The aqueous solution was acidified with HCl (3 M) to pH 2, and the resulting precipitate was collected by filtration and washed with H_2O. The solid was dried in a vacuum oven (40 °C) overnight to afford compound **17** as an off-white solid (2.7 g, 96%); ^{1}H NMR ([D$_6$]DMSO, 300 MHz). $\delta = 11.96$ (s, 1H), 10.71 (s, 1H), 9.35 (s, 1H), 6.93 (s, 1H), 3.95 (m, 2H), 2.93 (d, $J = 13.5$ Hz, 1H), 2.80 (m, 2H), 2.71 (d, $J = 13.5$ Hz, 1H), 2.33 (s, 3H), 1.96 (m, 2H), 1.28 (m, 1H), 0.80 ppm (m, 4H); MS (ESI+): *m/z* 329 $[M+H]^+$.

1*R*-Methy-(5-cyano-7-hydroxy-8-methyl-1-propyl-1,3,4,9-tetrahydro-pyrano[3,4-*b*]indol-1-yl)-acetate (18): A solution of compound **17** (2.7 g, 8.22 mmol) in MeOH (50 mL) was warmed to 60 °C and treated with a solution of (−)-quinine (2.9 g, 8.94 mmol) in MeOH (40 mL). A white precipitate formed as the reaction was stirred at 60 °C for 20 h. After cooling to RT, the solids were isolated by filtration and washed with MeOH to afford (*R*)-**17** as the quinine salt. The salt was partitioned between aq HCl (50 mL, 1 M) and EtOAc (50 mL) and the organic layer was separated, washed with brine, dried ($MgSO_4$) and concentrated in vacuo to afford (*R*)-**17** as a white solid. The solid was redissolved in toluene (30 mL) and trimethyl orthoacetate (60 mL) and the solution was heated at 100 °C for 2 h. The reaction was concentrated in vacuo, and the resulting residue was purified by flash chromatography (SiO_2, hexanes/EtOAc, 7:3) to afford the methyl ester **18** (1.25 g, 46%); HPLC (HP 100 with CHIRALPAK AS, 250×4.6 mm, 2-propanol/heptane (1:4), 1.0 mL min^{-1}, 235 nm UV detection): RT = 6.35 min (*R*-enantiomer), 97% *ee*; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 9.40$ (s, 1H), 6.94 (s, 1H), 4.69 (s, 1H), 4.02 (m, 1H), 3.92 (m, 1H), 3.75 (s, 3H), 3.06–2.94 (m, 2H), 2.41 (s, 3H), 2.04–1.91 (m, 2H), 1.53–1.26 (m, 2H), 0.88 ppm (t, $J = 7.0$ Hz, 3H).

General procedure for alkylation: A solution of phenol in DMF (0.05–0.07 M) was treated with K_2CO_3 (1.2–1.5 equiv) at RT and stirred for 15 min before the addition of a heterocyclic-ethylbromide in DMF (0.5 M, 1–1.2 equiv) and then stirred for a further 16 h at RT. The reaction was quenched with H_2O and extracted with EtOAc. The organic phase was washed with H_2O, brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by flash chromatography (SiO_2, hexanes/EtOAc) gave the desired *O*-alkylated products.

General procedure for saponification: A solution of ester in MeOH (0.01–0.05 M) was treated with NaOH (2 M, 2–5 equiv) at RT and stirred until complete. The organic solvent was removed in vacuo and the aqueous residue was diluted with H_2O and then extracted with EtOAc. The aqueous layer was acidified with HCl (3 M) and re-extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) filtered and concentrated in vacuo to give the desired free acids.

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Derivative **19** (Entry 1, Table 1); MS (ESI−): m/z 421.1 $[M-H]^-$.

Derivative **22** (Entry 2, Table 1); MS (ESI−): m/z 435.1 $[M-H]^-$.

Derivative **23** (Entry 3, Table 1); MS (ESI−): m/z 438 $[M-H]^-$.

Derivative **24** (Entry 4, Table 1); ^{1}H NMR ([D$_6$]DMSO, 300 MHz): $\delta=$ 11.98 (s, 1H), 10.84 (s, 1H), 7.82 (s, 1H), 7.46 (s, 1H), 7.16 (s, 1H), 6.24 (s, 1H), 4.79–4.75 (m, 1H), 4.36–4.21 (m, 2H), 3.98–3.86 (m, 2H), 2.92 (d, $J=13.8$ Hz, 1H), 2.81 (m, 2H), 2.69 (d, $J=13.5$ Hz, 1H), 2.17 (s, 3H), 1.96 (m, 2H), 1.52 (d, $J=6.9$ Hz, 3H), 1.36–1.25 (m, 1H), 0.86–0.82 (m, 1H), 0.79 ppm (t, $J=5.4$ Hz, 3H); MS (ESI−): m/z 435 $[M-H]^-$.

Derivative **25** (Entry 5, Table 1); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=9.16$ (s, 1H), 7.64 (d, $J=1.8$ Hz, 1H), 7.57 (d, $J=2.4$ Hz, 1H), 6.83 (s, 1H), 6.31 (t, $J=2.4$ Hz, 1H), 4.85–4.79 (m, 1H), 4.16 (d, $J=6.3$ Hz, 2H), 4.08–3.96 (m, 2H), 3.09–2.96 (m, 4H), 2.09–2.04 (m, 1H), 1.99 (s, 3H), 1.97–1.89 (m, 1H), 1.67 (d, $J=7.2$ Hz, 3H), 1.43–1.37 (m, 1H), 1.26–1.17 (m, 1H), 0.88 ppm (t, $J=7.5$ Hz, 3H); MS (ESI+): m/z 437.2 $[M+H]^+$.

Derivative **2** (Entry 6, Table 1) ^{1}H NMR ([D$_6$]DMSO, 300 MHz): $\delta=$ 11.89 (br s, 1H), 10.63 (br s, 1H), 7.80 (d, $J=1.7$ Hz, 1H), 7.47 (s, 1H), 7.16 (s, 1H), 6.26 (d, $J=1.7$ Hz, 1H), 4.50 (dd, $J=4.6$, 5.2 Hz, 2H), 4.38 (dd, $J=4.6$, 5.2 Hz, 2H), 3.95 (m, 1H), 2.94–2.73 (m, 4H), 2.24 (s, 3H), 1.98 (m, 1H), 1.24–0.99 (m, 3H), 0.93 (d, $J=7.0$ Hz, 3H), 0.74 ppm (t, $J=7.0$ Hz, 3H).

Derivative **26** (Entry 7, Table 1); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=9.64$ (br s, 1H), 7.60 (s, 1H), 7.56 (s, 1H), 6.82 (s, 1H), 6.30 (s, 1H), 4.79 (m, 1H), 4.19 (m, 2H), 4.12 (m, 1H), 3.92 (m, 1H), 3.13 (s, 2H), 3.02 (m, 2H), 2.17 (m, 1H), 2.02 (s, 3H), 1.69 (d, $J=6.4$ Hz, 3H), 1.05 (d, $J=7.0$ Hz, 3H), 0.77–1.40 (m, 3H), 0.74 ppm (t, $J=7.0$ Hz, 3H); MS (ESI+): m/z 451.3 $[M+H]^+$.

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[19] "Discovery of VP19744: A Pyrano[3,4-*b*]indole-Based Inhibitor of HCV NS5B Polymerase Demonstrating in vivo Antiviral Activity": M. G.

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