Synthetic Route Optimization of PF-00868554, An HCV Polymerase Inhibitor in Clinical Evaluation

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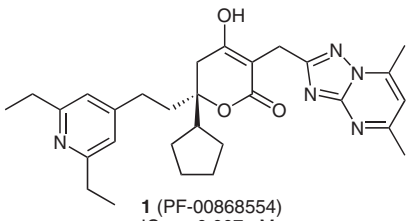
Abstract: This paper describes the optimization efforts to establish an enabling synthesis to provide multigram quantity of PF-00868554, an HCV polymerase inhibitor currently in phase II clinical evaluations.

Key words: HCV polymerase inhibitor, Heck reaction, dihydropyrone, pyridine synthesis, borane pyridine

Hepatitis C virus (HCV) is a blood-borne virus that infects over 170 million people worldwide and is the leading cause of liver transplantation in the USA.[1] Currently there is no vaccine for HCV nor is there any specific antiviral agent directed at HCV available on the market. The standard treatment for the disease, a combination of pegylated-interferon α with ribavirin, is only effective in ca. 50% of genotype 1 patients (the most prevalent genotype in North America and Europe) and suffers from serious side effects.[2] Therefore, development of a safer and more effective HCV therapy will address an urgent unmet medical need.



1 (PF-00868554)
IC_{50} = 0.007 μM
EC_{50} = 0.041 μM
CC_{50} > 320 μM

Figure 1 Molecular structure and profile of PF-00868554

In the past decade, particularly after the introduction of the cell-based subgenomic replicon assay,[3] there has been tremendous progress in the discovery and development of novel anti-HCV therapy targeting essential viral enzymes. Both HCV NS3-4A protease and NS5B polymerase inhibitors have entered human clinical trials and achieved proof-of-concept (POC). In our previous communications, we have reported the identification and optimization of a series of dihydropyrone-based HCV polymerase inhibitors.[4–6] Among them, PF-00868554 (**1**, Figure 1) achieved great potency in both biochemical and cell-based replicon assays, as well as favorable pharmacokinetic profiles in preclinical animal evaluations. Herein, we report our efforts in synthetic optimization that enabled the preparation of multigram quantities of this compound in support of further safety evaluations.[4]

The original synthesis of **1** (Scheme 1) started with formation of the requisite 4-bromo-2,6-diethylpyridine (**7**). A nickel-catalyzed cross coupling between 2,6-dichloropyridine (**2**) and ethyl magnesium bromide in diethyl ether gave 2,6-diethylpyridine (**3**) in 93% yield. Oxidation of the resulting 2,6-diethylpyridine (**3**) with *m*-chloroperoxybenzoic acid (MCPBA) in chloroform formed *N*-oxide **4**, which was subjected to nitration conditions to give the 4-nitropyridine *N*-oxide **5** in good overall yields. Bromination with acyl bromide in acetic acid gave bromo intermediate **6**, which upon treatment with phosphorous tribromide converted the pyridine *N*-oxide into the desired pyridine **7** in good yield. The dihydropyrone core was introduced through a palladium-catalyzed Sonogashira coupling[7] between bromide **7** and 6-(2-cyclopentyl-2-hydroxybut-3-ynyl)-2,2-dimethyl-4*H*-1,3-dioxin-4-one (**8**),[8] which gave acetylene **9** in 60% yield. $Pd(OH)_2$-catalyzed hydrogenation of the resulting alkyne yielded intermediate **10**, which underwent K_2CO_3-mediated cyclization in methanol at 50 °C to provide dihydropyrone **11**. Incorporation of aldehyde **12**[8] through a borane–dimethylamine-mediated reductive coupling gave the fully elaborated racemic dihydropyrone **13**, which after chiral separation produced the desired enantiomer **1**.

While the original synthesis worked well for the purpose of generating structure–activity relationship (SAR) by allowing introduction of diversity at both sides of the dihydropyrone core, the route posed several challenges to produce multigram quantities of material. Those challenges included the late-stage introduction of a chiral center, low yields in key steps (particularly step ix, introduction of the triazolopyrimidine piece), and potentially dangerous synthetic intermediates (electron-deficient *N*-oxides) on larger scale.

From a synthetic efficiency point of view, it was highly desirable to introduce the chirality of the target molecule early in the synthesis as compared to relying on chiral separation at the final step. Previously, we reported preparation of enantiomerically pure (3*S*)-3-cyclopentyl-3-hydroxypent-4-enoic acid (**14**) through a classical resolu-

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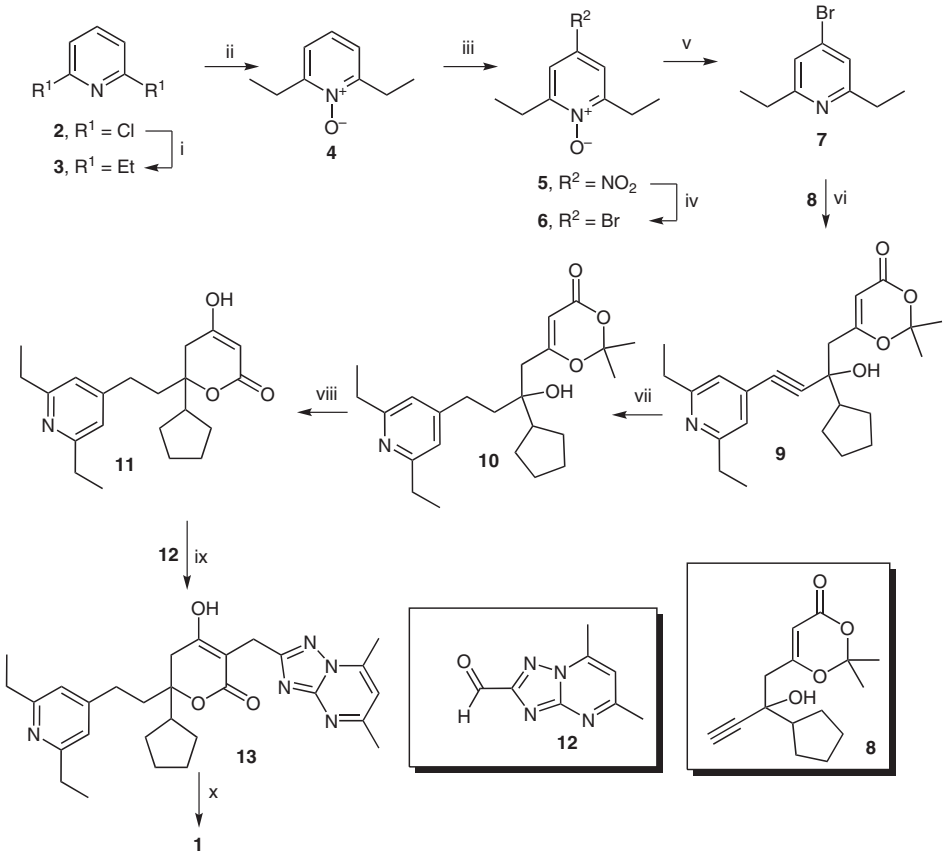
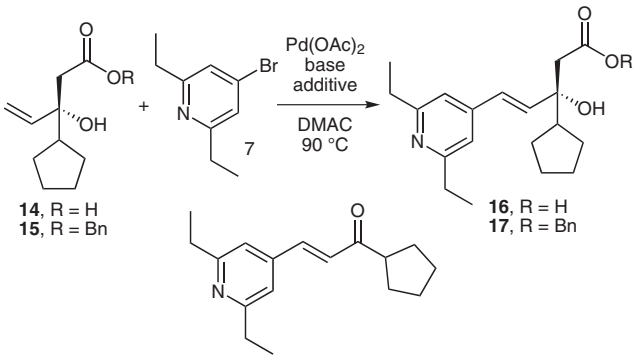


Scheme 1 Original synthesis of **1** (PF-00868554). *Reagents and conditions*: (i) $NiCl_2$Dppp, EtMgBr, Et_2O, 93%; (ii) MCPBA, $CHCl_3$, 94%; HNO_3, H_2SO_4, 65%; (iv) AcOH, AcBr, 40%; (v) PBr_3, CH_2Cl_2, 64%; (vi) **8**, $Pd(Ph_3P)_2Cl_2$, CuI, DIPEA, DMF, 90 °C, 60%; (vii) $Pd(OH)_2$/C, EtOH, 90%; (viii) K_2CO_3, MeOH, 50 °C, 46%; (ix) **12**, BH_3·Me_2NH, MeOH, 25%; (x) chiral separation, 25%.

tion with chiral amines, which offered a stable intermediate suitable for a Heck coupling[9,10] to replace the original Sonogashira reaction. Initial reaction between optically pure carboxylic acid **14** and bromo pyridine **7** resulted in no reaction (entry 1, Table 1). After conversion of acid **14** into the corresponding benzyl ester **15** by conventional methods (CDI, benzyl alcohol, 75% yield), the Heck coupling did proceed in dimethylacetamide (DMAC) at 90 °C with $Pd(OAc)_2$ as catalyst to yield the desired allylic alcohol **17**, along with a significant amount of ketone **18** (entry 2, **17/18** = 2:1), presumably formed through a retro-aldol reaction. To minimize this undesirable side reaction, a number of additives and bases were screened and the results are summarized in Table 1. From initial experiments, it was noticed that while addition of lithium salts, such as LiCl and LiOAc, accelerated the Heck coupling, it also produced the retro-aldol product in significant quantity (entries 2, 4). Replacement of the lithium salt with NaOAc completely suppressed the retro-aldol reaction, although it also slowed down the Heck coupling dramatically (entries 3, 5). We rationalized that while the chloride anion enhanced the reaction rate by stabilizing the palladium intermediate, the lithium ion served as a Lewis acid, chelating both the hydroxy group and ester carbonyl via a six-membered ring, which could lead to a lithium ion catalyzed retro-aldol reaction to give the product **18**. When LiCl was replaced with tetrabutylammonium chloride (Bu_4NCl) as shown in entries 6–8, the desired Heck product **17** was formed in high yields without detection of retro-aldol product **18**. While Bu_4NCl was important in increasing the reaction rate in the Heck coupling (conversion rate 40% vs. 95%, entry 5 and 6), additional NaOAc had little impact (entry 6 vs. 7). Generally hindered amine bases accelerated the Heck coupling (entry 2 vs. entry 4) and *N*-methyl dicyclohexylamine and Hünig's base provided the best results (entries 7 and 8).

In the initial synthesis of PF-00868554 (**1**), introduction of the triazolopyrimidine moiety was achieved through coupling between dihydropyrone **19** and aldehyde **12** in the presence of borane–dimethylamine complex in methanol at room temperature (Scheme 2). The reaction provided a mixture of desired product **1** and one major side product that was identified as olefin **21** (30%) which required HPLC purification to separate. Attempts to convert olefin **21** into **1** under a variety of reductive conditions were unsuccessful. It was hypothesized that the reaction proceeds through an aldol condensation to form hydroxy ketone **20**. Subsequently, two independent mechanistic pathways were possible: the elimination pathway led to olefin **21**, and the reduction pathway provided the desired product **1**. In order to slow down the competing elimination mechanism, we employed less basic amines and found replacing dimethylamine–borane complex with pyridine borane, along with lowering the reaction temper-

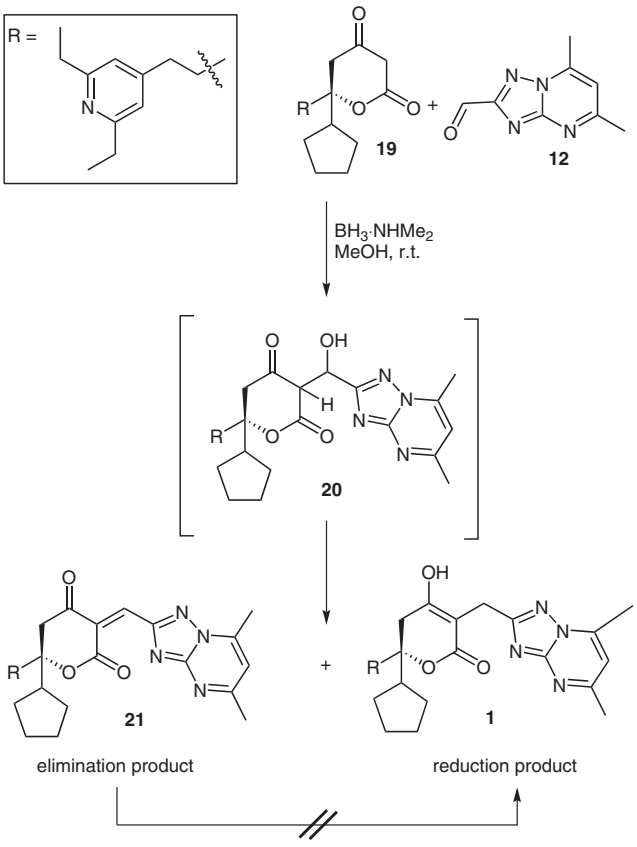
Table 1 Optimization of Heck Coupling



Entry	Substrate	Base	Additive	Results
1	**14**	Et_3N	LiCl, LiOAc	no reaction
2	**15**	Et_3N	LiCl, LiOAc	2 h, low conversion, **17**/**18** (2:1)
3	**15**	Et_3N	NaOAc	0.5 h, 10% conversion, only **17** formed
4	**15**	*i*-Pr_2EtN	LiCl, LiOAc	2 h, 80% conversion, **17**/**18** (1:1)
5	**15**	dicyclohexNMe	NaOAc	12 h, 40% conversion, only **17** formed
6	**15**	dicyclohexNMe	NaOAc, Bu_4NCl	12 h, >95% conversion, only **17** formed
7	**15**	dicyclohexNMe	Bu_4NCl	12 h, >95% conversion, only **17** formed
8	**15**	*i*-Pr_2EtN	Bu_4NCl	7 h, >95% conversion, only **17** formed

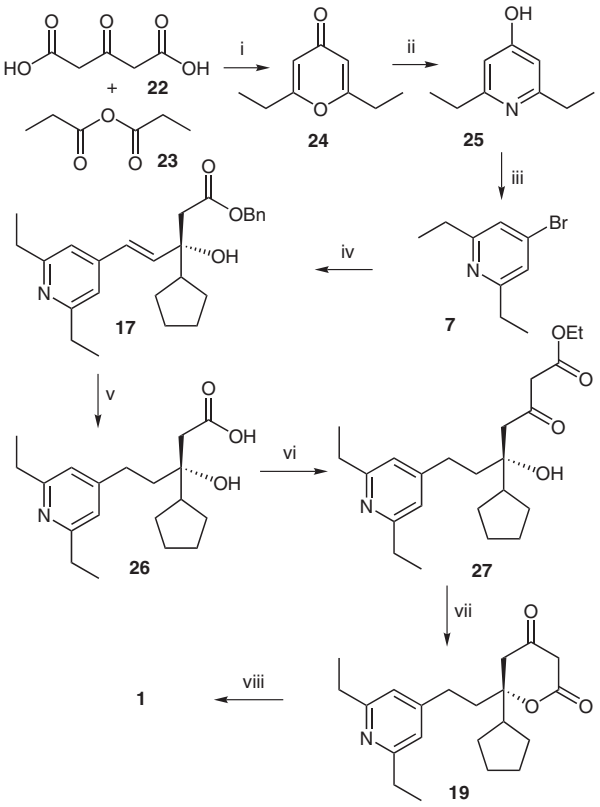
ature to 0 °C, effectively suppressed the elimination product and provided PF-00868554 (**1**) in 75% yield with high purity.

Scheme 3 shows the optimized synthesis of PF-00868554 (**1**). Since *N*-oxides are generally high energy intermediates that can potentially cause safety issues on large-scale synthesis, we prepared the key intermediate pyridine **7** through an alternative route previously reported in the literature.[11] Condensation between acetonedicarboxylic acid (**22**) and propanoic anhydride (**23**) provided pyrone **24** which was heated with ammonium hydroxide to give pyridone **25**. Bromination with phosphorous oxybromide provided the Heck coupling partner **7** in 88% yield. The coupling between pyridine **7** and chiral alkene **15** was carried out using the optimized conditions[12] of $Pd(OAc)_2$, Bu_4NCl, and dicyclohexylmethyl amine in DMAC at 90 °C (entry 7, Table 1). After aqueous workup, intermediate **17** was used directly in the next step without further purification. Subsequent hydrogenation of olefin **17** reduced the carbon–carbon double bond and removed the benzyl ester group in a single step. The resulting acid **26** was precipitated as a dicyclohexylamine salt in 65% yield over the two steps. Treatment of acid **26** with carbodiimidazole (CDI) and ethyl magnesium malonate, followed by acid-mediated decarboxylation yielded β-keto ester **27**, which under K_2CO_3-mediated cyclization conditions afforded the penultimate intermediate **19** in 80% overall yield. Under the optimal conditions established for the reductive



Scheme 2 The final reductive coupling

coupling,[13] dihydropyrone **19** reacted with aldehyde **12** in the presence of pyridine–borane complex in methanol at 0 °C to provide crude compound **1**. Because the carbon-linked dihydropyrone **1** is mildly acidic, it can be effectively extracted into a basic aqueous solution. After washing the aqueous layers with diethyl ether and acidification with AcOH, the crude product was extracted into EtOAc and further crystallized to provide the desired product in 75% yield with over 95% purity.



Scheme 3 Optimized synthesis of PF-00868544. *Reagents and conditions*: (i) 1. H_2SO_4, 100 °C; 2. Na_2SO_4, 100 °C; 3. HCl, 100 °C; (ii) NH_4OH, 50 °C, 80% over 4 steps; (iii) $POBr_3$, DMF, 120 °C, 88%; (iv) **15**, $Pd(OAc)_2$, Bu_4NCl, dicyclohexylmethylamine, DMAC, 90 °C; (v) H_2, Pd/C, EtOH, 65% over 2 steps; (vi) CDI, ethyl magnesium malonate, 1 N HCl, MTBE; (vii) K_2CO_3, MeOH, 80% over 2 steps; (viii) **12**, pyridine·BH_3, MeOH, 0 °C, 75%.

In conclusion, an optimized synthetic route to prepare gram quantities of PF-00868554 (**1**) has been developed. By using a precedented pyridine synthesis through a pyrone intermediate, the use of a potentially dangerous high-energy *N*-oxide **5** was eliminated, and the overall yield of pyridine **7** was improved from 14% to 70%. The late-stage chiral separation was replaced by an early introduction of an optically pure Heck coupling partner **15**. Substitution of LiCl with Bu_4NCl effectively suppressed the formation of the undesired retro-aldol side product in the Heck reaction and provided olefin **17** in high yields. Finally, utilization of milder base (pyridine–borane) under lower reaction temperature successfully improved the reductive condensation between dihydropyrone **19** and aldehyde **12** to 75% yield. The new synthetic sequence led to preparation of PF-00868554 (**1**) in a 27% overall yield without the requirement of column purification, which has provided multigram quantities of material which supported preclinical studies.

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(12) **Experimental Conditions of Optimized Heck Coupling and Product Characterization**
To a solution of benzyl ester **15** (1.30 g, 4.74 mmol) in DMAC (11 mL) in a two-neck round-bottomed flask was added 4-bromo-2,6-diethylpyridine (**7**, 1.22 g, 5.69 mmol), followed by TBACl (1.30 g, 4.74 mmol), and $Pd(OAc)_2$. The resulting solution was degassed by house vacuum followed by argon back filling (3×). Dicyclohexylmethylamine (2.0 mL, 9.50 mmol) was added, and the reaction vessel was lowered into a pre-heated oil bath at 90 °C. The reaction was stirred at this temperature under argon until all benzyl ester **15** was consumed (about 5 h). The solution was cooled to r.t., diluted with H_2O (60 mL) and then extracted with MTBE (2 × 50 mL). The combined organic layers were washed with H_2O (2 × 50 mL), sat. $NaHCO_3$ (50 mL), 5% aq AcOH (50 mL), then brine (sat., 50 mL). The yellow solution was dried ($MgSO_4$), filtered, and concentrated to provide a crude amber oil (2.1 g). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.26–1.32 (m, 6 H), 1.48–1.64 (m, 8 H), 2.71–2.74 (m, 2 H), 2.75–2.84 (m, 5 H), 5.02–5.07 (m, 1 H), 5.10–5.12 (m, 1 H), 6.35–6.42 (m, 1 H), 6.51–6.59 (m, 1 H), 6.89 (s, 2 H), 7.22–7.26 (m, 5 H). MS (APCI): *m/z* = 408.20 $[M + H]^+$.

(13) **Experimental Conditions for Scheme 3, Step viii**
To a solution of pyrone **19** (710 mg, 2.07 mmol) in anhyd MeOH (10 mL) at –25 °C was added borane–pyridine complex (ca. 8 M solution, 0.5 mL, 4.13 mmol). To this aldehyde (1.3 equiv, 2.69 mmol, 475 mg) was added, and the reaction was slowly warmed to 0 °C. The cloudy mixture was stirred for 3 h during which time it became homogeneous. H_2O (1 mL) was added to quench the remaining borane. The reaction was stirred for an additional 30 min before it was concentrated to about 1/10 volume and diluted in 10% MeOH–EtOAc. The mixture was extracted with 1 N HCl (2×), and the combined aqueous layers were carefully neutralized to pH 7 with sat. $NaHCO_3$. The aqueous layer was extracted with 10% MeOH–EtOAc (3×), and the resulting organic extract showed a single peak with mass corresponding to the product. The organic layer was washed with brine and dried ($MgSO_4$), filtered, and concentrated to a white solid (789 mg, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.23 (t, *J* = 7.63 Hz, 6 H), 1.48–1.59 (m, 4 H), 1.59–1.75 (m, 4 H), 1.96–2.09 (m, 4 H), 2.38 (d, *J* = 8.48 Hz, 1 H), 2.63–2.67 (m, 4 H), 2.69–2.80 (m, 9 H), 4.06–4.15 (m, 2 H), 6.76 (s, 2 H), 6.83 (s, 1 H). MS (APCI): *m/z* = 504.2 $[M + H]^+$.