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Development of potent macrocyclic inhibitors of genotype 3a HCV NS3/4A protease

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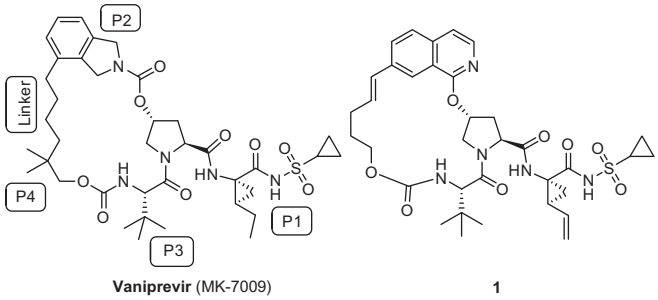
ABSTRACT

A series of macrocyclic compounds containing 2-substituted-quinoline moieties have been discovered and shown to exhibit excellent HCV NS3/4a genotype 3a and genotype 1b R155K mutant activity while maintaining the high rat liver exposure. Cyclization of the 2-substituted quinoline substituent led to a series of tricyclic P2 compounds which also display superb gt3a potency.

Hepatitis C is an infection caused by the hepatitis C virus (HCV) that affects 130–170 million people worldwide and is the leading indication for liver transplantation.[1,2] Prior to 2011, the standard treatment for HCV was co-dosing with Pegylated IFN-α and Ribavirin for up to one year, and along with producing severe side-effects, this regimen is only moderately effective.[3,4] The development of antivirals which would inhibit key steps in the viral replication process has been a major focus in the efforts to improve this treatment. One such target that is critical for viral replication is the serine protease HCV NS3/4A.[5,6] Boehringer–Ingelheim initially established clinical proof of concept for inhibition of HCV NS3/4a protease with the macrocycle BILN-2061,[7] and more recently both Merck (SCH-503034, boceprevir[9]) and Vertex (VX-950, telaprevir[8]) have gained FDA approval for α-keto-amide HCV NS3/4A inhibitors used in combination with the previous standard of care.

While a number of research programs have identified other HCV NS3/4a protease inhibitors[10] based upon the P1-P3 macrocyclic system present in BILN-2061, our program has focused on an alternative series of HCV NS3/4A protease inhibitors utilizing a different macrocyclization strategy[11] with a linker from P2 to P4 (Fig. 1), exemplified by vaniprevir,[12,13] MK-1220,[14] and MK-5172.[15,16] As we have previously published, prior to the development of vaniprevir, optimization for genotype 1b potency initially led to compound **1**[11] (Fig. 1), which has picomolar potency (1b K_i = 0.04 nM), good cellular activity (1b replicon IC_{50} = 4.5 nM)[11] and excellent liver exposure in rat following a 5 mg/kg oral dose ($[liver]_{4h}$ = 18,600 nM). Obtaining high and sustained liver exposure may be critical to the success of any anti-HCV compound because the primary HCV



Vaniprevir (MK-7009) **1**

Figure 1. Merck developed P2–P4 macrocyclic HCV protease NS3/4a inhibitors.

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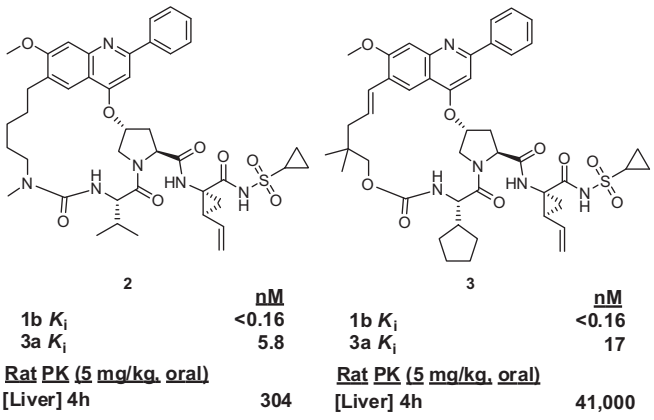
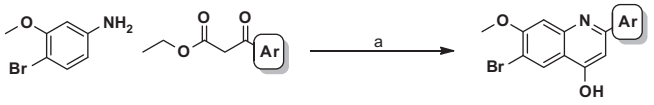


Figure 2. Genotype 3 leads with improved potency.



Scheme 1. Reagents and conditions: (a) polyphosphoric acid, heat.

replication site is in the liver,[17] and optimizing for high liver concentrations in preclinical species became the focus of our preliminary pharmacokinetic studies.

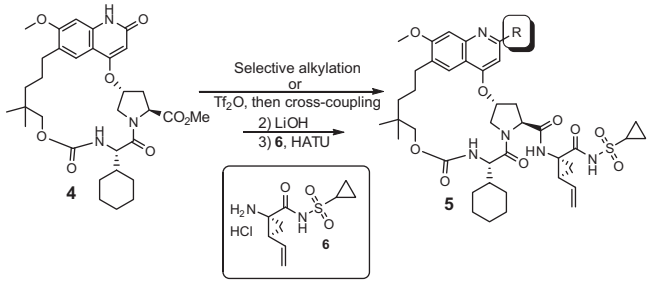
As an initial assessment of liver exposure, our standard practice was to measure the concentration of compound present in rat liver 4 h after a 5 mg/kg oral dose. HCV has a positive-strand RNA genome with significant genetic heterogeneity and can be grouped into 6 major genotypes (gt). The major genotype in the United States is genotype 1, with genotypes 2 and 3 representing sizeable populations as well. While **1** and vaniprevir are quite potent against gt 1, they both are less active against gt 3a (**1** gt3a K_i = 475 nM; vaniprevir gt 3a K_i = 54 nM). This modest potency motivated our efforts towards the identification of potential second-generation compounds with improved gt 3a activity and which maintain excellent cellular (replicon with 10% FBS/50% NHS) potency and high liver exposure in preclinical species. We recently published the strategy which led to the identification of MK-5172.[15,16] Herein, we disclose the discovery of a different series of compounds with excellent potency against both genotype 1b and 3a.

In the context of our quinoline/isoquinoline (e.g., **1**) P2–P4 macrocycles we noticed that 2-phenylquinolines tended to produce inhibitors with improved genotype 3a potency relative to benchmark compound **1**. For example, **2** and **3** have significantly improved genotype 3a potency (K_i = 6-17 nM) relative to **1**, as well as good liver exposure in the case of carbamate **3** (Fig. 2).

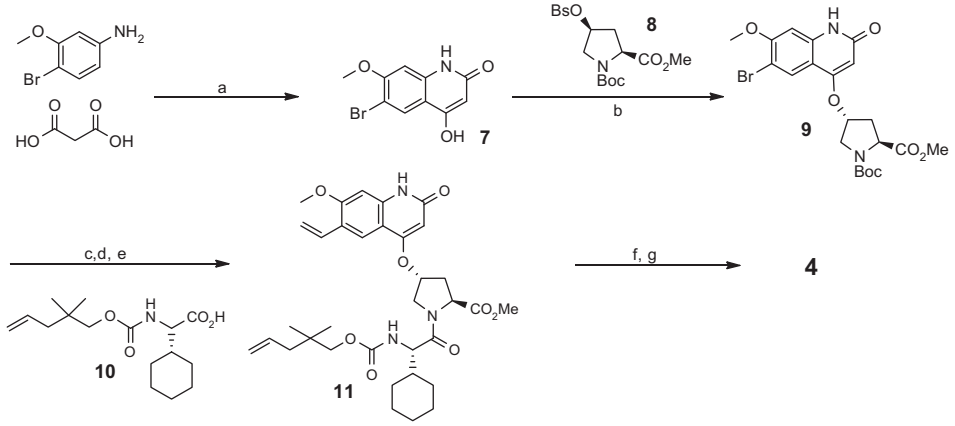
Since a major difference between these compounds and **1** is the presence of a phenyl ring on the P2-heterocycle, we decided to examine the effect of various substituents at this position.

In order to fully explore the SAR at the 2-position, we needed to generate a large number of analogs, and using the classic Conrad–Limpach quinoline synthesis[18] used to prepare **2** and **3**, the substituent in the 2-position is installed in the first step (Scheme 1). Utilization of this synthesis would require an additional 9 steps to complete each analog, We hoped to avoid this inefficiency by developing a more versatile route which introduced the 2-substituent near the end. We envisioned that **4** could serve as a common intermediate to introduce a variety of groups in the 2-position (Scheme 2). These intermediates could then be transformed into the desired compounds in two straightforward steps. For the initial SAR studies, the carbamate linker was chosen based upon the high liver exposure obtained with compound **3** (Fig. 2).

In practice, we began with a regioselective hydroxyquinolone formation,[19] followed by chemoselective alkylation[20] at the 4-position with brosylate **8**[21] to give **4** as the only isolable mono-alkylated product (Scheme 3). Removal of the Boc group, HATU coupling with **10**,[22] and vinylation with Molander's vinyl trifluoroborate reagent[23] led to intermediate **11**. Ring-closing metathesis[24] using the Zhan 1B catalyst[25] then produced the desired macrocycle in good yield. Hydrogenation of the styrene double bond yielded key intermediate **4** in 7 steps with an overall yield of 26%.

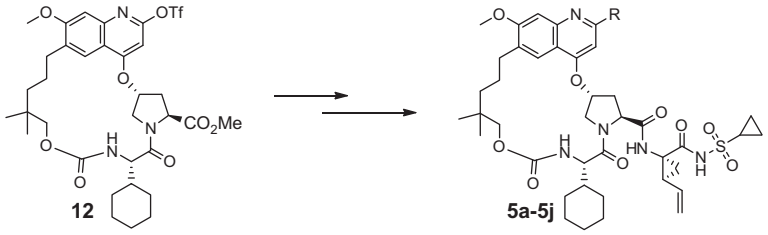


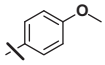
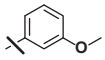
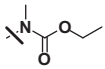
Scheme 2. Strategy for rapid synthesis of 2-substitutedquinoline analogs.



Scheme 3. Reagents and conditions: (a) 1 equiv $POCl_3$, 105 °C, 1 h, neat (66%); (b) NMP, Cs_2CO_3 (53%); (c) EtOH, Et_3N, 5% Pd(dppf)Cl_2, (CH_2=CH_2BF_3)K, reflux (99%); (d) HCl/dioxane; (e) HATU, DMF, DIEA (82% over two steps); (f) 10% Zhan 1b, DCE, 45 °C (99%); (g) 5% Pd/C, H_2, EtOH (92%).

Table 1
2-Aryl-, heteroaryl-, and aminoquinoline SAR



#	R	1b Ki (nM)[a]	3a Ki (nM)[a]	1b replicon IC_{50} (nM)[b]		$[liver]_{4h}$ (μM)[c]
				10% FBS	50% NHS	
5a		0.05	21	3.6	28	9.9
5b		0.07	18	3.0	25	6.1
5c		0.07	78	6.0	19	6.7
5d		0.09	62	7.0	31	6.3
5e		0.18	29	11	110	–
5f		–	55	27	92	0.2
5g		0.09	5.4	1.6	90	<0.005
5h		0.03	4.3	5.0	27	42
5i		0.07	7.5	5.0	26	0.1
5j		0.12	7.6	39	1200	–

[a] NS3/4a protease time-resolved fluorescence assay.
[b] Cell-based replicon assay.
[c] 5 mg/kg dosed orally in PEG400 ($n = 2$, rat), liver levels after 4 h.

In order to access products via cross-coupling and nucleophilic substitution reactions, we first converted **4** to the corresponding quinoline-2-triflate (**12**), and then carried out Suzuki reactions[26] (see Supplementary data) with a variety of boronic acids (Table 1). These were then readily converted into products **5a**–**5f** using standard conditions. As is readily apparent from table 1, substitution at the ortho-, meta-, and para-positions (**5a**–**5e**) led to either no improvement or a reduction in gt 3a potency, although similar rat liver concentrations and replicon potency was generally maintained. Changing the simple aryl ring to a heterocycle (**5f**) also did not lead to improved gt 3a potency, and instead resulted in the loss of rat liver exposure.

The triflate **12** can also be readily converted into N-substituted analogs via simple displacement reactions (Table 1, **5g**–**5j**).[27] The resulting 2-aminoquinolines generally display excellent gt 3a potency, but poor rat liver exposure follow oral dosing. Alkylamine analogs (**5g**, **5i**, **5j**) possessed single digit nanomolar activity versus gt 3a, but all led to <100 nM rat liver concentrations.

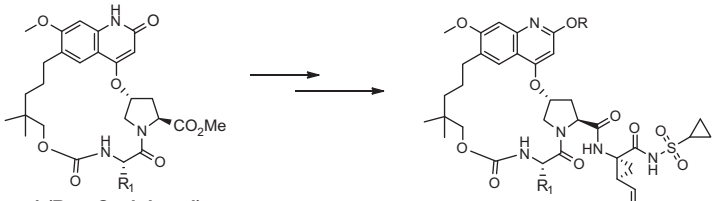
Capping **5g** with ethylchloroformate leads to N-methyl-ethylcarbamate **5h**, which displays a promising profile of 4 nM gt 3a potency, good gt 1 replicon activity and rat liver exposure (42 μM) at 4 h.

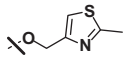
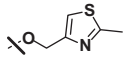
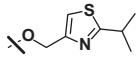
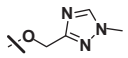
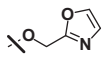
The excellent potency of amino analogs prompted us to examine ether derivatives as well. Unfortunately, neither phenols nor simple alcohols participated in triflate-displacement reactions or palladium-catalyzed C–O cross-couplings. Therefore, we started with quinolone **4** and utilized a series of oxygen-selective alkylations to access a range of 2-alkoxyquinolines (see Supplementary data for details). Simple alkyl ethers such as methyl and ethyl are readily available through use of Meerwein-type reagents in the absence of base which leads to nearly exclusive O-alkylation.[28] More functionalized ethers can be selectively accessed through the Mitsunobu reaction[29] or alkylation of alkyl halides in the presence of silver salts.[30]

As Table 2 illustrates, a variety of 2-alkoxyquinolines possess excellent genotype 3a potency. Methoxy- and ethoxyquinolines (**5l**, **5m**) were particularly potent, however, in contrast to the amines, the corresponding ethers generally exhibited excellent cellular potency and rat liver exposure. Increasing the size or complexity of the alkyl group did not generally lead to any further improvement (**5n**). However, methylthiazole analog **5o** does have slightly improved gt 3a potency (3 nM) and also offers an improved gt 1 replicon activity with 50% NHS compared to ethyl analog **5m**, as well as improved rat liver exposure compared to methyl analog **5l**.

Given this excellent profile, we decided to explore other heterocyclic ether analogs, this time with cyclopentyl glycine in P3 (Table 2, **14a**–**14e**). One can see the generally potency enhancing

Table 2
2-Alkoxyquinoline SAR



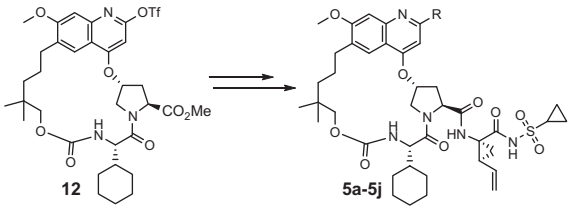
#	OR	1b Ki (nM)[a]	3a Ki (nM)[a]	1b replicon IC_{50} (nM)[b]		[liver]$_{4h}$(μM)[c]
				10% FBS	50% NHS	
5k		0.07	23	85	460	0.07
5l		0.04	4.1	4	19	5.4
5m		0.09	7.6	6	43	40
5n		0.05	15	5	34	4.6
5o		0.1	3.1	3.5	16	19
14a		0.09	1.6	5	26	0.8
14b		<0.02	2	4	26	2.7
14c		0.03	1.0	36	170	0.03
14d		<0.02	1.1	4	16	2.6
14e		0.03	2.2	2	11	35

[a] NS3/4a protease time-resolved fluorescence assay.
[b] Cell-based replicon assay.
[c] 5 mg/kg dosed orally in PEG400 (n = 2, rat), liver levels after 4 h.

effect of changing to cyclopentyl glycine by comparing **5o**–**14a**. Other related heterocyclic analogs (**14b**–**14d**) have excellent genotype 3a potency, however they uniformly show low to moderate liver exposure in rat following oral dosing, and generally have poor to moderate replicon potency in the presence of 50% NHS. The preferred group within this sub-series appears to be oxazole **14d** which has 1 nM gt 3a potency, good replicon activity, and moderate rat liver level (2.6 μM). Interestingly, returning to the simple ethyl ether derivative with the P3 cyclopentyl group led to a compound with the overall optimum profile within the 2-alkoxyquinoline series. **14e** has excellent gt 1b (0.03 nM) and gt 3a potency (2 nM) as well as gt 1b replicon activity (2 nM (10% FBS); 11 nM (50% NHS)) and rat liver exposure (35 μM).

In an effort to further explore the space occupied by 2-amino and -ethoxy groups, an effort was undertaken to tie back the substituent into another ring, thus forming a tricyclic P2 group. The versatility of the triflate intermediate **12** was again exploited through simple conversion to imidazo-tricycle **15a** (Scheme 4).

As Table 3 illustrates, compared to **14e**, P2 tricyclic analog **15a** possesses similar gt 3a potency (1.8 nM) however, unfortunately it has relatively poor replicon potency and rat liver exposure. Removal of the C7 methoxy group (**15b**) reduces the gt 3a potency slightly (5.2 nM) and this compound also suffers from poor replicon potency and rat liver exposure. However, given the promising potency of the imidazo-tricycles, we synthesized the related isoquinoline-tricycle **15c** to further explore P2-tricycle SAR (see Supplementary data). Gratifyingly, **15c** displayed excellent gt 3a potency (1.7 nM), and, in contrast to **15a**, also had good replicon

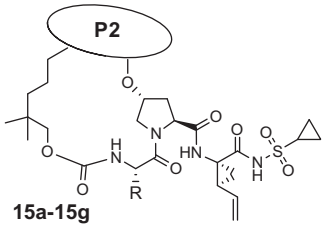


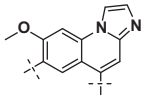
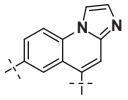
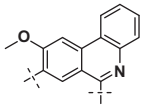
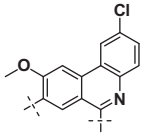
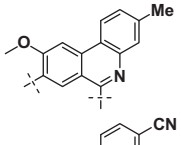
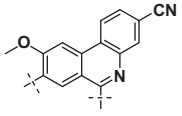
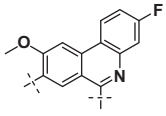
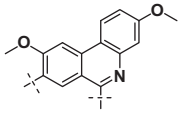
Scheme 4. Strategy for synthesis of tricyclic P2.

activity (3 nM) and rat liver exposure (27 μM). The effects of substituents on the top ring of the tricycle were then explored, and while substitution at the 2-position was not well tolerated (**15h**) in terms of gt 3a potency, substitution at the 3-position was generally somewhat beneficial. C3-methyl analog **15d** provided similar gt 3a potency and rat liver exposure, but was 2-fold more potent in the 50% NHS replicon assay. Cyano (**15e**), fluoro (**15f**), and methoxy (**15g**) groups all boosted gt 3a potency by ~2-fold to sub-nanomolar regions, while methoxy analog **15g** also provided a slight increase in rat liver exposure compared to unsubstituted **15c**.

In summary, optimization for genotype 3a potency and rapid screening for rat liver exposure[31] (following oral dosing) in the P2-quinoline series of the P2–P4 macrocyclic HCV NS3/4a protease inhibitors has led to the discovery of several key structural features; notably the genotype 3a potency enhancing effects of various 2-amino and 2-alkoxy quinoline groups, as well as two different types of tricyclic analogs. Within these sub-series, it appears

Table 3
Tricyclic P2



#	P2	R[d]	1b Ki (nM)[a]	3a Ki (nM)[a]	1b replicon IC_{50} (nM)[b]		$[liver]_{4h}$ (μM)[c]
					10% FBS	50% NHS	
15a		Cyh	0.04	1.8	43	140	0.8
15b		Cyh	0.08	5.2	33	180	0.7
15c		Cyp	<0.02	1.7	3	29	27
15h		Cyp	<0.02	36	7	31	–
15d		Cyp	<0.02	1.9	4	15	16
15e		Cyp	<0.02	0.8	4	13	7.5
15f		Cyp	<0.02	0.9	9	43	22
15g		Cyp	<0.02	0.8	4	27	35

[a] NS3/4a protease time-resolved fluorescence assay.
[b] Cell-based replicon assay.
[c] 5 mg/kg dosed orally in PEG400 ($n = 2$, rat), liver levels after 4 h.
[d] Cyh = cyclohexyl; Cyp = cyclopentyl.

Table 4
Compound comparison and gt 1mutant profile

#	1b Ki (nM)[a]	3a Ki (nM)[a]	1b replicon IC_{50} (nM)[b]		$[liver]_{4h}$(μM)[c]	gt 1b Ki (nM)[a]	
			10% FBS	50% NHS		R155K	A156T
5h	0.03	4.3	5	27	42	3.3	87
5o	0.1	3.1	3.5	16	19	2.1	88
14e	0.03	2.2	2	11	35	1.3	41
15g	<0.02	0.8	4	27	35	0.4	16

[a] NS3/4a protease time-resolved fluorescence assay.
[b] Cell-based replicon assay.
[c] 5 mg/kg dosed orally in PEG400 ($n = 2$), liver levels after 4 h.

the compounds which offer the best balance between gt 3a potency, gt 1b replicon activity, and rat liver exposure following oral dosing are amino-carbamate **5h**, quinolinoxymethyl-thiazole analog **5o,** ethoxy derivative **14e**, and 3-methoxy substituted isoquinoline tricycle **15g**. As is shown in Table 4, in addition to excellent gt 1b/3a potency, these analogs also possess excellent potency versus the gt 1b mutant R155K,[32] but lose some activity against gt 1b A156T. Improving potency against this variant will be the subject of a subsequent publication.[33]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.106.

References and notes

1. WHO. Hepatitis C-Global prevalence (update) Wkly. Epidemiol. Reci 1999, 74, 425.
2. Liang, T. J.; Heller, T. *Gastroenterology* **2004**, *127*, S62.
3. Hadziyannis, S. J.; Sette, H.; Morgan, T. R., et al *Ann. Intern. Med.* **2004**, *140*, 346.
4. Fried, M. W. *Hepatology* **2002**, *36*, S237.
5. Moradpour, D.; Penin, F.; Rice, C. M. *Nat. Rev. Microbiol.* **2007**, *5*, 453.
6. Chen, K. X.; Njoroge, F. G. *Curr. Opin. Invest. Drugs* **2009**, *10*, 821.
7. Lamarre, D.; Anderson, P. C.; Bailey, M., et al *Nature* **2003**, *426*, 186.
8. Perni, R. B.; Almquist, S. J.; Byrn, R. A., et al *Antimicrob. Agents Chemother.* **2006**, *50*, 899.
9. Malcolm, B. A.; Liu, R.; Lahser, F., et al *Antimicrob. Agents Chemother.* **2006**, *1013*, 50.
10. For examples of other P1-P3 macrocycles see TMC435350: (a) Lin, T.-I.; Lenz, O.; Fanning, G., et al *Antimicrob. Agents Chemother.* **2009**, *53*, 1377; (b) Raboisson, P.; Herman, De K.; Rosenquist, A.; Nilsson, M.; Salvador-Oden, L., et al *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4853. and ITMN-191 (R7227); (c) Seiwert, S. D.; Andrews, S. W.; Jiang, Y., et al *Antimicrob. Agents Chemother.* **2008**, *52*, 4432.
11. Liverton, N. J.; Holloway, M. K.; McCauley, J. A., et al *J. Am. Chem. Soc.* **2008**, *130*, 4607.
12. McCauley, J. A.; McIntyre, C. J.; Rudd, M. T., et al *J. Med. Chem.* **2010**, *53*, 2443.
13. Liverton, N. J.; Carroll, S. S.; DiMuzio, J. M., et al *Antimicrob. Agents Chemother.* **2010**, *54*, 305.
14. Rudd, M. T.; McCauley, J. A.; Butcher, J. W., et al *ACS Med. Chem. Lett.* **2011**, *2*, 207.
15. Harper, S.; McCauley, J. A.; Rudd, M. T., et al *ACS Med. Chem. Lett.* **2012**, *3*, 332.
16. Summa, V.; Ludmerer, S. W.; McCauley, J. A., et al *Antimicrob. Agents Chemother.* **2012**, *56*, 4161.
17. Lindenbach, B. D.; Rice, C. M. *Nature* **2005**, *436*, 933.
18. Reitsema, R. H. *Chem. Rev.* **1948**, *43*, 47.
19. Knierzinger, A.; Wolfbeis, O. S. *J. Heterocycl. Chem.* **1980**, *17*, 225.
20. Tagawa, Y.; Kawaoka, T.; Goto, Y. *J. Heterocycl. Chem.* **1997**, *34*, 1677.
21. Arasappan, A.; Chen, K. X.; Njoroge, F. G.; Parekh, T. N.; Girijavallabhan, V. *J. Org. Chem.* **2002**, *67*, 3923.
22. Holloway, M. K.; Liverton, N. J.; Ludmerer, S. W.; McCauley, J. A.; Olsen, D. B.; Rudd, M. T.; Vacca, J. P. (Merck & Co., Inc., USA). PCT Int. Appl. 2006, WO 2006119061.
23. Molander, G. A.; Ellis, N. *Acc. Chem. Res.* **2007**, *40*, 275.
24. Lee, C. W.; Grubbs, R. H. *J. Org. Chem.* **2001**, *66*, 7155.
25. Available from Strem Chemicals, catalog # 44–0082.
26. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
27. Cacchi, S.; Carangio, A.; Fabrizi, G.; Moro, L.; Pace, P. *Syn. Lett.* **1997**, *12*, 1400.
28. Beak, P.; Covington, J. B.; Smith, S. G.; White, J. M.; Zeigler, J. M. *J. Org. Chem.* **1980**, *45*, 1354.
29. Hughes, D. L. *Organic Reactions* **1992**, *42*, 335.
30. Gonzales, R.; Ramos, M. T.; de la Cuesta, E.; Avendano, C. *Heterocycles* **1993**, *36*, 315.
31. While liver exposure in other species was not routinely assessed, the predictive nature of this rat liver screening paradigm for discovering compounds which provide liver exposure in additional species within the P2–P4 macrocyclic space has been demonstrated several times (see references for: vaniprevir,[9,10] MK-1220,[14] and MK-5172[15,16])
32. The R155K mutant arises clinically in gt 1a infected patients, but we screened against the gt 1b mutant such that the same background was utilized in relation to the main screening genotype 1 subtype. In cases where we have data for both gt 1a and 1b R155K, the K_i values are similar.
33. Rudd, M. T.; McIntyre, C. J.; Romano, J. J., et al *Bioorg. Med. Chem. Lett.* **2012**. accepted.