Journal of Medicinal Chemistry

Article

Discovery of ABT-267, a pan-genotypic inhibitor of HCV NS5A

David Allen DeGoey, John Randolph, Dachun Liu, John Pratt, Charles Hutchins, Pamela Donner, Chris Krueger, Mark Matulenko, Sachin Patel, Christopher Motter, Lissa Nelson, Ryan Keddy, Michael Tufano, Daniel Caspi, Preethi Krishnan, Neeta Mistry, Gennadiy Koev, Thomas Reisch, Rubina Mondal, Tami Pilot-Matias, Gao Yi, David Beno, Clarence Maring, Akhter Molla, Emily Dumas, Andrew Campbell, Laura Williams, Christine Collins, Rolf Wagner, and Warren Kati

J. Med. Chem., **Just Accepted Manuscript** • Publication Date (Web): 08 Jan 2014

Just Accepted

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Discovery of ABT-267, a pan-genotypic inhibitor of HCV NS5A

David A. DeGoey,[*] John T. Randolph, Dachun Liu, John Pratt, Charles Hutchins, Pamela Donner, A. Chris Krueger, Mark Matulenko, Sachin Patel, Christopher E. Motter,[†] Lissa Nelson, Ryan Keddy,[††] Michael Tufano,[‡] Daniel D. Caspi, Preethi Krishnan, Neeta Mistry, Gennadiy Koev, Thomas J. Reisch, Rubina Mondal,[#] Tami Pilot-Matias, Yi Gao, David W. A. Beno,[¶] Clarence J. Maring, Akhter Molla, Emily Dumas, Andrew Campbell, Laura Williams, Christine Collins, Rolf Wagner, and Warren M. Kati.

Research and Development, AbbVie Inc., North Chicago, IL 60064, USA

KEYWORDS. Hepatitis C, antiviral, NS5A, pyrrolidine, prolinamide.

ABSTRACT. We describe here *N*-phenylpyrrolidine-based inhibitors of HCV NS5A with excellent potency, metabolic stability and pharmacokinetics. Compounds with 2*S*,5*S* stereochemistry at the pyrrolidine ring provided improved genotype 1 (GT1) potency compared to the 2*R*,5*R* analogs. Furthermore, the attachment of substituents at the 4-position of the central *N*-phenyl group resulted in compounds with improved potency. Substitution with *tert*-butyl, as in compound **38** (ABT-267), provided compounds with low-picomolar EC_{50} values and superior pharmacokinetics. It was discovered that compound **38** was a pan-genotypic HCV inhibitor, with an EC_{50} range of 1.7 – 19.3 pM against GT1a, 1b, 2a, 2b, 3a, 4a, and 5a, and 366 pM against GT 6a. Compound **38** decreased HCV RNA up to 3.10 log10 IU/mL during 3-day monotherapy in

treatment-naïve HCV GT1-infected subjects and is currently in Phase 3 clinical trials in combination with an NS3 protease inhibitor with ritonavir (r) (ABT-450/r) and an NS5B non-nucleoside polymerase inhibitor (ABT-333), with and without ribavirin.

Introduction

Once treatable only through lengthy, poorly tolerated and marginally efficacious regimens using pegylated interferon alpha (pegIFN-α) and ribavirin (RBV), chronic hepatitis C virus (HCV) infection has now been cured with high rates in clinical trials using IFN-free combinations of direct-acting antivirals (DAA).[1] HCV is a blood-borne virus, transmitted largely through needle sharing and blood transfusions that occurred prior to 1992 before routine screening was implemented.[2] Left untreated, chronic HCV infection can result in cirrhosis, an increased incidence of hepatocellular carcinoma and ultimately liver failure. Complications due to chronic HCV infection have now become the predominant driver for liver transplantation.[3] The widespread incidence of HCV infections worldwide (estimated at 170-200 million), together with a large number of baby boomers in the US with more difficult to treat HCV genotype 1 (GT1) infections (approximately 2.1 million), have contributed to the urgent need to identify more tolerable and efficacious treatments with oral administration. For more than a decade, HCV treatment consisted of a lengthy (48 weeks) regimen of pegIFN-α and ribavirin (RBV), a therapy associated with significant tolerability issues and poor efficacy (40-50% cure rate in GT1 patients).[4] Recently, the approval of the first DAAs, the NS3/4A protease inhibitors telaprevir or boceprevir, led to a significant improvement in the cure rate (up to 75% in GT1 treatment naïve patients) and their use in combination with pegIFN-α and RBV is the current standard of care.[5,6] While these new regimens provide improved efficacy, tolerability remains suboptimal due to side effects associated with pegIFN-α and RBV as well as gastrointestinal symptoms and

significant anemia associated with telaprevir and boceprevir. Clearly, there is a compelling need for new agents that will bolster the cure rate for patients infected with HCV, while eliminating IFN as a requirement for successful treatment.

The genome of HCV encodes several nonstructural (NS) proteins. While inhibition of the enzymatic activities of NS3/4A protease and NS5B polymerase has been the subject of intense drug discovery efforts, the NS5A protein has no known intrinsic enzymatic activity, although it is essential for viral RNA replication.[7] The discovery of compounds that select mutations in NS5A using the replicon assay established it as a potential drug target, with mutations at Tyr93 conferring resistance to multiple classes of compounds.[8,9] Because adaptive mutations in NS5A that increase replication facilitated the development of the replicon cell culture assay,[7,10] it remained to be demonstrated that compounds active in the replicon assay would exert an antiviral effect in vivo against the native virus. The observation of robust viral load declines in human clinical trials with NS5A inhibitors has now firmly established NS5A as a viable drug target.[11] Recent efforts at AbbVie to study HCV inhibitors yielded potent naphthyridine inhibitors such as **1** that lacked activity against HCV protease and polymerase (Figure 1).[12,13] Efforts to improve potency and metabolic stability culminated in the discovery of compound **2**, which demonstrated a robust viral load decline in an GT1b HCV-infected chimpanzee. [12] As was the case with many of the early NS5A chemical series, however, the compounds demonstrated weaker activity against GT1a compared to GT1b.

Here we describe the structure-activity relationships (SAR) of a novel symmetric series of *N*-phenylpyrrolidine-based inhibitors (Figure 1) leading to the discovery of compound **38** (ABT-267), which is currently undergoing phase 3 clinical trials in combination with an NS3 protease inhibitor with ritonavir (r) (ABT-450/r)[14] and an NS5B non-nucleoside polymerase

inhibitor (ABT-333),[14] with and without RBV. X-ray crystallographic studies of domain I fragments of NS5A have yielded dimeric structures, although the biological relevance of the dimeric forms has not been firmly established.[7,15] NS5A inhibitors with symmetric dimer or dimer-like structures have recently become the subject of intense research due to their potency, presumably from improved interactions with the dimeric form of the NS5A protein.[16-21] For example, BMS-790052 (Figure 1) was inspired from the isolation of dimeric impurities formed as byproducts of the initial lead compound BMS-824 and which were determined to be the active species.[17,18] Other approaches to symmetry-based NS5A inhibitors have also been actively studied.[9,16-21] We explored inhibitors with chiral pyrrolidine-based cores (Figure 1), which projected *N*-aryl functional groups orthogonal to the vector linking the proline amide groups that greatly impacted the potency and pharmacokinetics. We also describe the effect of the pyrrolidine ring stereochemistry on the compound potency and pharmacokinetics.

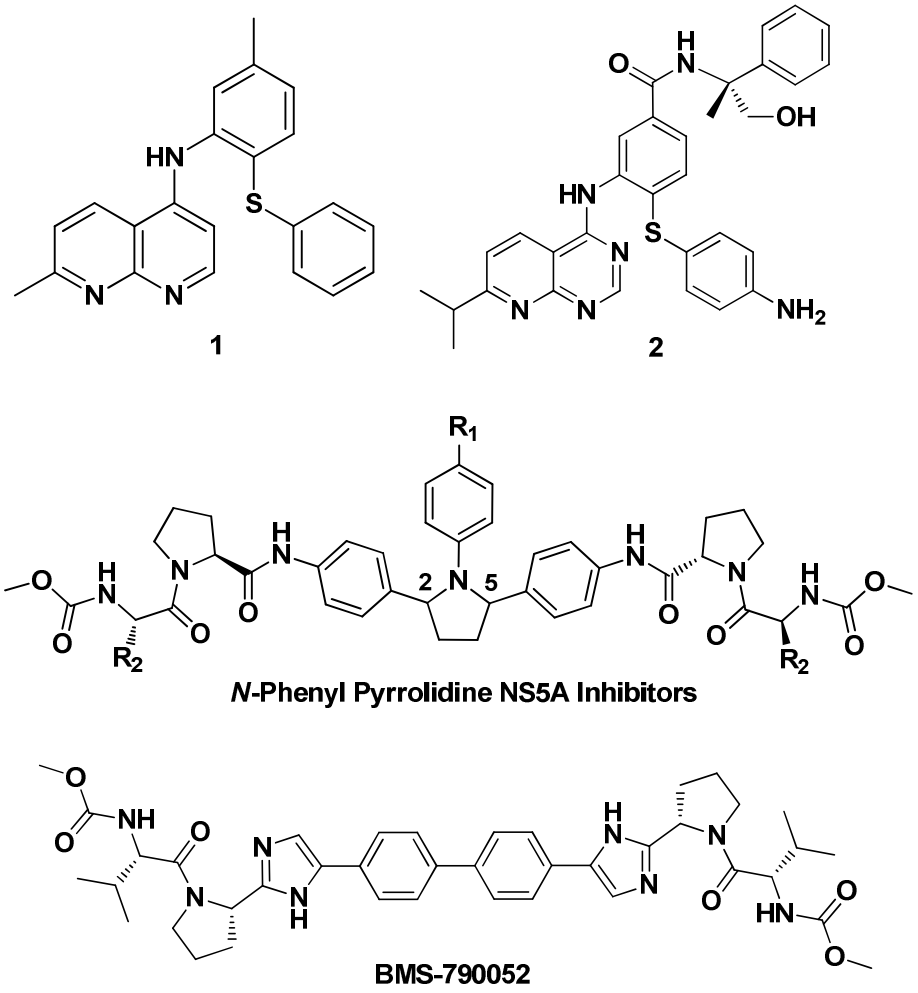


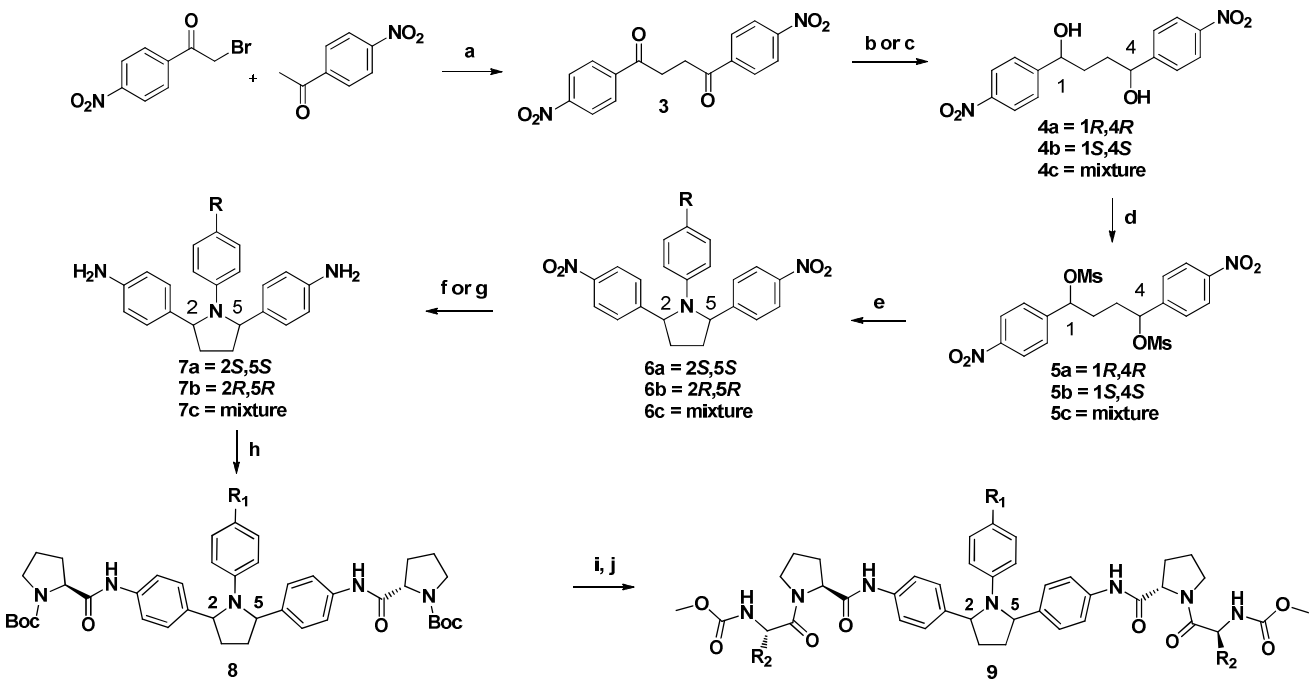
Figure 1. Structures of HCV NS5A inhibitors.

Chemistry

As shown in Scheme 1, compounds **12**-**46** (Table 1) were synthesized starting from the diketone **3**, which was prepared by reaction of 2-bromo-1-(4-nitrophenyl)ethanone with 1-(4-nitrophenyl)ethanone in the presence of zinc(II) chloride.[22] Chiral diols **4a** and **4b** were prepared by asymmetric reduction of **3** using *N*,*N*-diethylaniline borane in combination with (*S*)-(-)-α,α-diphenyl-2-pyrrolidinemethanol or (*R*)-(-)-α,α-diphenyl-2-pyrrolidinemethanol, respectively, in the presence trimethyl borate in > 99% ee.[23] The mixture of diols **4c** was generated using sodium

borohydride. Generation of the dimesylate followed by treatment with the aniline of choice provided pyrrolidines **6a-6c**. Reduction of the nitro groups to give **7a-c** could be affected under a variety of conditions, including heating with iron and ammonium chloride or catalytic hydrogenation. Hydrogenation with Raney nickel or platinum (IV) oxide was best suited for enantiomerically pure compounds **6a** and **6b** in order to minimize stereochemical isomerization. Compound **7c** could be readily separated into *cis*-pyrrolidine and racemic *trans*-pyrrolidine isomers by column chromatography on silica gel. The proline amide side chains were attached by peptide coupling with Boc-Proline to give **8**, followed by deprotection and peptide coupling with methyl carbamate-capped amino acids to give the final products **9**. Final compounds prepared using **6c** were separated into the *trans*-pyrrolidine and *cis*-pyrrolidine analogs by reversed phase HPLC. Further separation of the diastereomeric mixture of *trans*-pyrrolidines was accomplished by chiral chromatography on a Chiralpak AD-H column. Alternatively, diastereomerically pure *trans*-pyrrolidine compounds were synthesized starting from the enantiomerically pure intermediates **6a** and **6b**.

Scheme 1. Synthesis of pyrrolidine-based HCV NS5A inhibitors



[a] Reagents and conditions: (a) $ZnCl_2$, *t*-BuOH, Et_2NH; (b) (*S*)-(-)-α,α-diphenyl-2-pyrrolidinemethanol or (*R*)-(-)-α,α-diphenyl-2-pyrrolidinemethanol, $PhNEt_2 \bullet BH_3$, $B(OMe)_3$; (c) $NaBH_4$; (d) MsCl, $EtN(i\text{-}Pr)_2$; (e) $RC_6H_4NH_2$; (f) Fe, NH_4Cl; (g) PtO_2 or Raney nickel, H_2; (h) Boc-Pro, HATU, $EtN(i\text{-}Pr)_2$; (i) TFA or HCl; (j) $MeOC(O)NHCH(R_2)CO_2H$, HATU or EDCI and HOBT, $EtN(i\text{-}Pr)_2$.

Results and Discussion

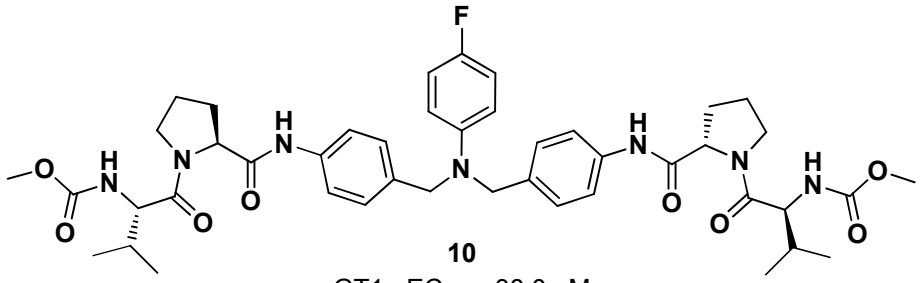
In vitro biological data for these compounds are summarized in Table 1. The compounds were tested in GT1a and GT1b subgenomic replicon assays with and without the addition of 40% human plasma (HP), as binding to human plasma proteins can reduce the pharmacological activity of antiviral compounds via reduction of the free drug levels.[24,25] Thus, the replicon EC_{50} values obtained in the presence of 0% HP more closely represent the intrinsic potencies of the

compounds, whereas values obtained in the presence of 40% HP may be more closely correlated with clinical efficacy.[26,27] As indicated in Table 1, the compounds were tested either as mixtures of the *trans*-pyrrolidine isomers or as the pure 2*S*,5*S* or 2*R*,5*R* diastereomers. Testing the separated *trans*-pyrrolidine isomers **12** and **13** revealed a stereochemical dependence for their replicon potencies. The 2*S*,5*S* isomer **12** was approximately 10-fold more potent than the 2*R*,5*R* isomer **13** against GT1a, while **12** was only 3.5-fold more potent than **13** against GT1b. Comparison of the other pairs of *trans*-pyrrolidine diastereomers in Table 1 revealed a similar trend of increased potency for 2*S*,5*S* isomers against GT1a and GT1b compared to the 2*R*,5*R* isomers. The *cis*-pyrrolidine isomer **14** was significantly less active against GT1a (55-fold) and GT1b (7.5-fold) than the 2*S*,5*S* *trans*-pyrrolidine compound **12**.

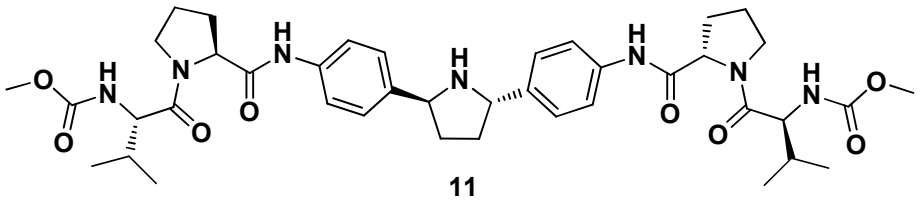
A comparison of the 2*S*,5*S* *trans*-pyrrolidine compound **12** with acyclic analog **10** (Figure 2) revealed the effect of the pyrrolidine ring formation on potency, with **12** demonstrating 96- and 33-fold improved potency against GT1a and GT1b, respectively. Likewise, comparison of **12** with the unsubstituted pyrrolidine analog **11** (Figure 2) revealed that **12** was 639- and 23,500-fold more potent against GT1a and GT1b, respectively.

The addition of 40% HP to the replicon assay resulted in a 17-fold loss of potency on average for the compounds in Table 1 against GT1a and GT1b. Compounds bearing more lipophilic groups generally demonstrated larger shifts than less lipophilic compounds. For example, the *tert*-butyl glycine capped compound **15** (LogD = 5.2) demonstrated 59- and 88-fold shifts in potency with the addition of 40% HP against GT1a and GT1b, respectively, while the valine capped compound **12** (LogD = 4.6) demonstrated 9- and 15-fold shifts against GT1a and GT1b, respectively. This observation is consistent with the findings of others who have noted that increasing lipophilicity (log D) within a series increases plasma protein binding.[28,29] A

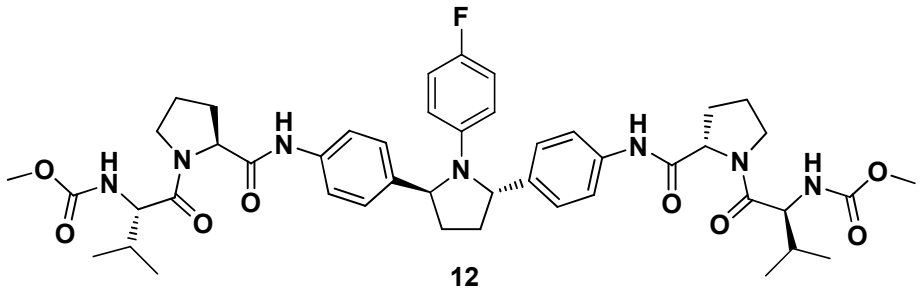
survey of amino acid capping groups was conducted on the 4-fluorophenyl substituted pyrrolidine core (compare compounds **12**-**26**). In addition to valine (**12**, **13**), other lipophilic amino acids were well tolerated, including *tert*-butylglycine (**15**, **16**), isoleucine (**19**), alloisoleucine (**20**) and phenylglycine (**21**), while the 2-aminobutyric acid analog **18** was less potent. The introduction of more polar amino acid capping groups, such as 3-hydroxy-valine compound **22**, was also tolerated. The incorporation of amino acids bearing ether side chains, such as *O*-methyl-threonine (**23** and **24**) and THF-glycine (**25** and **26**), resulted in compounds with more uniform GT1a and GT1b potencies, with replicon potencies in the 1 to 2 nanomolar range in the presence of 40% HP.



10
GT1a EC_{50} = 38.0 nM
GT1b EC_{50} = 0.40 nM



11
GT1a EC_{50} = 253 nM
GT1b EC_{50} = 282 nM



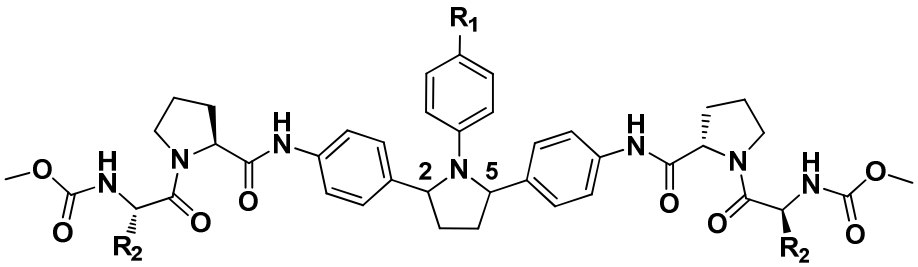
12
GT1a EC_{50} = 0.396 nM
GT1b EC_{50} = 0.012 nM

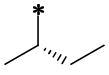
Figure 2. Structures of acyclic analog **10**, unsubstituted analog **11** and *N*-phenyl pyrrolidine **12.**

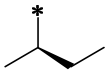
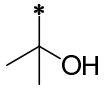
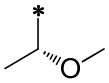
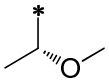
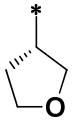
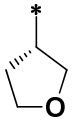
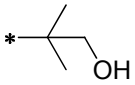
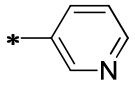
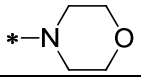
Modification of the 4-position of the central *N*-phenyl ring also proved to have a significant effect on the GT1a and GT1b potencies. While hydrophobic substitutions of the fluoro substituted analog **12** with methyl (**27**), chloro (**28**), bromo (**29**) and methoxy (**30**) provided compounds with similar potency, the more polar phenol **31** was significantly less active. Notably, the trifluoromethyl analog **32** demonstrated picomolar activity against GT1a and GT1b, while retaining subnanomolar potency in the presence of 40% HP. Substitution with

isopropyl (**36**), sometimes considered to be isosteric with the trifluoromethyl group, produced similar potency, while the cyclopropyl analog **37** was slightly less active. With the larger *t*-butyl analog **38**, additional potency improvement in GT1a compared to **32** was observed. Compound **38** demonstrated uniform picomolar GT1a and GT1b activity, including picomolar activity in the presence of 40% HP. Once again, the 2*S*,5*S trans*-pyrrolidine isomer **38** proved to be 10-fold more potent against GT1a and 1.8-fold more potent against GT1b than the 2*R*,5*R* isomer **39**. The *tert*-butyl glycine capped analog **40**, although similarly potent against GT1a and 1b, exhibited a greater potency loss with the addition of 40% HP. Exploration of other large groups with more polarity, such as hydroxylated *tert*-butyl (**42)** and the methyl sulfone (**43)**, resulted in a loss of potency, compared to **38**. While the phenyl substituted compound **44** retained high potency against GT1a and GT1b, the introduction of polar heterocycles such as pyridine (**45**) and morpholine (**46**) resulted in a loss of potency compared to **38**.

Table 1. In vitro activity against HCV GT1a and GT1b in the replicon assay.



Compound				Replicon EC_{50} (nM)			
no.	Configuration	R_1	R_2	GT1a	GT1b	GT1a 40% HP (fold [a])	GT1b 40% HP (fold [a])
12	2*S*,5*S*	F	*i*-Pr	0.396	0.012	3.68 (9)	0.184 (15)
13	2*R*,5*R*	F	*i*-Pr	2.53	0.042	11.1 (4)	0.208 (5)
14	*cis*	F	*i*-Pr	22.0	0.090	81.0 (4)	0.300 (3)
15	2*S*,5*S*	F	*t*-Bu	0.263	0.006	15.5 (59)	0.528 (88)
16	2*R*,5*R*	F	*t*-Bu	1.18	0.008	20.8 (18)	0.162 (20)
17	*cis*	F	*t*-Bu	6.22	0.032	103.0 (17)	0.278 (9)
18	*trans* [b]	F	Et	4.60	0.140	34.0 (7)	1.00 (7)
19	*trans* [b]	F		1.31	0.030	9.79 (7)	0.171 (6)

20	*trans* [b]	F		0.202	0.010	6.56 (32)	0.090 (9)
21	*trans* [b]	F	Ph	1.06	0.002	22.8 (22)	0.073 (37)
22	*trans* [b]	F		0.600	0.050	18.0 (30)	2.60 (52)
23	2*S*,5*S*	F		0.168	0.086	1.40 (8)	0.539 (6)
24	2*R*,5*R*	F		0.794	0.134	1.95 (2)	1.33 (10)
25	2*S*,5*S*	F		0.102	0.054	1.39 (14)	1.27 (24)
26	2*R*,5*R*	F		0.281	0.106	1.92 (7)	0.917 (9)
27	*trans* [b]	Me	*i*-Pr	0.720	0.040	4.36 (6)	0.204 (5)
28	*trans* [b]	Cl	*i*-Pr	0.370	0.030	4.07 (11)	0.300 (10)
29	*trans* [b]	Br	*i*-Pr	0.286	0.0068	4.46 (16)	0.089 (13)
30	*trans* [b]	OMe	*i*-Pr	0.992	0.076	5.92 (6)	0.447 (6)
31	*trans* [b]	OH	*i*-Pr	23.0	6.54	242.0 (11)	59.5 (9)
32	2*S*,5*S*	CF_3	*i*-Pr	0.057	0.0071	0.650 (11)	0.072 (10)
33	2*R*,5*R*	CF_3	*i*-Pr	0.299	0.0062	2.55 (9)	0.077 (12)
34	2*S*,5*S*	CF_3	*t*-Bu	0.099	0.0088	4.14 (42)	0.211 (24)
35	2*R*,5*R*	CF_3	*t*-Bu	0.829	0.011	14.3 (17)	0.184 (17)
36	*trans* [b]	*i*-Pr	*i*-Pr	0.033	0.0079	0.706 (21)	0.086 (11)
37	2*R*,5*R*	*c*-Pr	*i*-Pr	0.164	0.0087	1.68 (10)	0.114 (13)
38	2*S*,5*S*	*t*-Bu	*i*-Pr	0.014	0.005	0.186 (13)	0.056 (11)
39	2*R*,5*R*	*t*-Bu	*i*-Pr	0.138	0.0088	0.986 (7)	0.090 (10)
40	2*S*,5*S*	*t*-Bu	*t*-Bu	0.021	0.0022	1.06 (50)	0.117 (53)
41	2*R*,5*R*	*t*-Bu	*t*-Bu	0.123	0.0084	4.31 (35)	0.344 (41)
42	2*S*,5*S*		*i*-Pr	0.116	0.095	1.47 (13)	0.948 (10)
43	2*R*,5*R*		*t*-Bu	0.659	0.226	27.6 (42)	3.42 (15)
44	*trans* [b]	Ph	*i*-Pr	0.089	0.011	1.69 (19)	0.256 (23)
45	*trans* [b]		*i*-Pr	0.143	0.069	2.57 (18)	0.597 (9)
46	2*R*,5*R*		*t*-Bu	1.24	0.211	11.2 (9)	1.60 (8)

[a] Fold shift in potency upon addition of 40% HP. [b] Tested as a mixture of 2*S*,5*S*+ 2*R*,5*R* isomers.

Based on their in vitro potencies, compounds were selected for pharmacokinetic studies (Table 2). In vitro physicochemical data (including solubility and LogD) and metabolic stability

in human and rat liver microsomes (HLM and RLM, respectively) for the set of compounds appear in Table 3. The compounds generally demonstrated low aqueous solubility (< 10 μM) and compounds with higher observed LogD values demonstrated the lowest solubility (i.e., **40** and **41**). Due to the low aqueous solubility of the compounds, permeability data generated using in vitro assays such as PAMPA proved to be of low utility for predicting oral absorption in vivo. Human and rat microsomal stability were generally similar for the compounds. Oral pharmacokinetic data were obtained using solutions in organic solvents in order to aid in dissolution and absorption of the sparingly soluble compounds. In rat, the *tert*-butyl glycine-capped compound **16** showed a longer half-life and higher oral bioavailability than the corresponding valine-capped compound **13**, consistent with the higher metabolic stability for **16** observed in RLM. Both **13** and **16** showed higher oral bioavailability in dog (F = 62 and 94, respectively) than in rat, while both compounds demonstrated high clearance and low oral bioavailability in monkey. The *O*-methyl threonine compounds **23** and **24** along with the THF-glycine analogs **25** and **26** were of high interest due to their potency in the presence of human plasma. However, the compounds demonstrated short half-lives, high clearance and low oral bioavailability, despite their moderate stability in RLM. The ether oxygen containing compounds **23**-**26** also had higher total polar surface areas (TPSA) of 197.2 compared to the other compounds in Table 2 which had TPSA values of 178.7, and this factor may have also contributed to their lower oral bioavailability. The potent trifluoromethyl-substituted compounds **32** and **33** and the *tert*-butyl-substituted compound **38** demonstrated high stability in RLM and long half-lives in rat (6, 4, and 9 hours, respectively), although they suffered from low oral bioavailability. However, compound **38** demonstrated significantly higher oral bioavailability and exposures in the dog compared to compound **32** and **33**. Unlike compounds **13** and **16**,

compound **38** also demonstrated a reasonable half-life with low clearance and moderate oral bioavailability in monkey. The *tert*-butyl glycine analogs **40** and **41** showed comparable rat PK to the valine analogs **38** and **39**, including low oral bioavailability, likely a result of their high lipophilicity (LogD = 5.5) and low aqueous solubility.

Table 2. Pharmacokinetic parameters for selected compounds. [a]

no.	Species	IV			Oral				
		$t_{1/2}$	Vss	Cl	$t_{1/2}$	T_{max}	C_{max}	AUC	*F*
12	rat	2.6	2.4	0.90	3.0	1.0	0.19	0.75	22
13	rat	3.3	0.5	0.13	2.8	1.3	1.38	6.36	22
	dog	2.0	1.9	1.3	2.2	1.2	0.42	1.28	62
	monkey	0.8	1.5	3.1	0.6	1.5	0.05	0.08	10
15	rat	ND [b]	ND [b]	< 0.39	ND [b]	3.0	0.11	1.85	23
16	rat	7.5	1.0	0.10	7.0	2.3	0.96	10.21	39
	dog	9.1	1.6	0.13	10.3	2.7	1.07	18.26	94
	monkey	4.6	4.1	0.93	5.4	1.7	0.12	0.49	18
23	rat	0.6	1.3	3.4	1.1	0.7	0.07	0.12	14
24	rat	0.4	0.9	2.7	0.9	0.5	0.12	0.17	15
25	rat	0.9	0.5	1.9	0.6	0.4	0.02	0.02	1.5
26	rat	0.8	1.5	4.0	0.5	0.3	0.03	0.02	3.2
32	rat	6.0	8.9	1.2	12.1	2.2	0.03	0.50	16
	dog	6.0	1.3	0.16	5.8	4.7	0.07	0.85	5.5
33	rat	4.2	1.3	0.29	4.9	1.5	0.20	1.36	13
	dog	4.4	1.5	0.29	5.5	1.7	0.05	0.37	4.2
38	rat	9.9	5.9	0.65	15.9	3.7	0.01	0.29	6.2
	dog	7.9	1.8	0.18	7.3	3.3	0.64	8.00	57
	monkey	4.4	1.5	0.38	5.0	3.3	0.29	2.40	35
39	rat	5.5	2.2	0.43	8.6	4.7	0.02	0.22	3.1
40	rat	8.2	2.3	0.17	11.0	5.3	0.09	1.25	7.2 [c]
41	rat	10.2	1.7	0.17	12.4	2.5	0.09	1.39	7.2 [c]

[a] Units: $t_{1/2}$ (h); Cl (L/h/kg); C_{max} (μg/mL); $AUC_{0\text{-}24h}$ (μg*h/mL); *F* (%); Doses: 3 mg/kg IV and oral for rat, 1 mg/kg IV and 2.5 mg/kg oral for dog and monkey; Vehicle: Capmul PG8/Cremophor RH40 (90/10) for oral; DMSO/PEG-400 (10/90) for IV. [b] Unable to calculate from 24 hour study. [c] Vehicle: PEG-400/Tween/Poloxamer (70/20/10).

Table 3. In vitro physicochemical and metabolic stability data.

no.	Solubility [a] (μM)	LogD [b]	RLM (% Remaining [c])	HLM (% Remaining [c])

12	4.1	4.6	49	44
13	6.0	4.6	38	38
15	4.4	5.2	56	52
16	3.9	5.2	69	54
23	9.6	3.0	47	54
24	7.7	3.0	46	48
25	9.7	3.2	42	41
26	8.5	3.2	46	47
32	3.4	4.8	56	52
33	3.2	4.9	58	58
38	2.7	5.4	73	60
39	1.8	5.2	65	NA[d]
40	1.2	5.5	87	78
41	2.2	5.6	83	80

[a] Kinetic solubility determined by chemiluminescent nitrogen detection (CLND) in 10 mM phosphate buffer (pH 7.2). [b] Determined by HPLC method (pH 7.4, 25 °C). [c] Percentage of parent compound remaining after 30 minute incubation. [d] Not available.

As a result of its picomolar potency and good pharmacokinetics, compound **38** was selected for further in vitro virologic evaluation.[30] As shown in Table 4, **38** demonstrated picomolar potency not only against GT1a and GT1b, but also against GT2 through GT6. Replicon resistance selection experiments in GT1 with compound **38** identified variants at positions 28, 30 and 93 as the predominant resistance associated variants, although additional minor variants were also observed (Table 5). In GT1a, variants M28V, L31V, and H58D conferred 58- to 243-fold resistance to **38**. Single variants M28T, Q30R, and Y93C/S conferred 800- to 8965-fold resistance, while Y93H/N conferred >40,000 fold resistance to **38**. In GT1b, the predominant variant selected in vitro with **38** was Y93H, which conferred 77-fold resistance. Double variants R30Q+Y93H and L31M+Y93H conferred 142- to 284-fold resistance, whereas all other double substitutions including Y93H in combination with substitutions at positions 28, 31, or 58 conferred more than 400-fold resistance to **38**. This resistance profile exhibits considerable overlap with BMS-790052, although there are some differences in fold-resistance and variants selected.[31] These observations of highly resistant mutants selected in vitro

underscores the need for combination therapy with DAAs that inhibit viral replication through different mechanisms of action in order to increase the barrier to resistance.

Table 4. Antiviral activity of **38** in HCV replicon cell lines containing NS5A from GT1-6

HCV Replicon Genotype	N[b]	Mean EC_{50} ± Std. Dev. (pM)
1a-H77	9	14.1 ± 6.8
1b-Con1	9	5.0 ± 1.9
2a[c]	6	12.4 ± 2.7
2b[c]	6	4.3 ± 1.2
3a[c]	6	19.3 ± 5.8
4a[c]	6	1.71 ± 0.88
5a[c]	5	4.3 ± 0.9
6a[c]	9	415 ± 97

[a] Assay contains 5% fetal bovine serum. [b] Number of independent replicates. [c] Chimeric 1b-Con1 replicon containing NS5A gene from a treatment-naïve patient isolate of the indicated GT.

Table 5. Selection of Resistant Variants in NS5A by **38** in GT1 Replicon Cell Lines

Genotype	Variant	Fold Resistance[a]	Replication Efficiency
1a	M28T	8965	100%
	M28V	58	87%
	Q30R	800	60%
	H58D	243	66%
	Y93C	1675	24%
	Y93H	41,382	18%
	Y93N	66,739	25%
1b	L28T	661	17%
	L31F	10	127%
	L31V	8	86%
	Y93H	77	73%
	L28M + Y93H	415	104%

R30Q + Y93H	284	60%
L31F + Y93H	10,270	35%
L31M + Y93H	142	11%
L31V + Y93H	12,323	24%

[a] Fold resistance is the ratio of variant EC_{50}/wild-type EC_{50}.

To assess the safety, tolerability, pharmacokinetics and antiviral activity of compound **38**, it was dosed for 3 days in HCV GT1-infected treatment naïve patients, with patients receiving once-daily doses of **38** for three days at doses ranging from 5 mg to 200 mg.[32] On day 3, dose-normalized C_{max} and AUC values were similar across doses. Compound **38** demonstrated C_{max} values ranging from 5.7 to 442 ng/mL and a half-life ranging from 25 to 32 hours across the dose groups. As shown in Figure 3, **38** decreased HCV RNA up to 3.10 log_{10} IU/mL during 3-day monotherapy with a nearly 3 log reduction observed in all dose groups. Compound **38** was generally well tolerated at all doses and there were no serious or severe adverse events, no clinically significant laboratory abnormalities, and no subjects discontinued. Most adverse events were mild and were not dose related.[32]

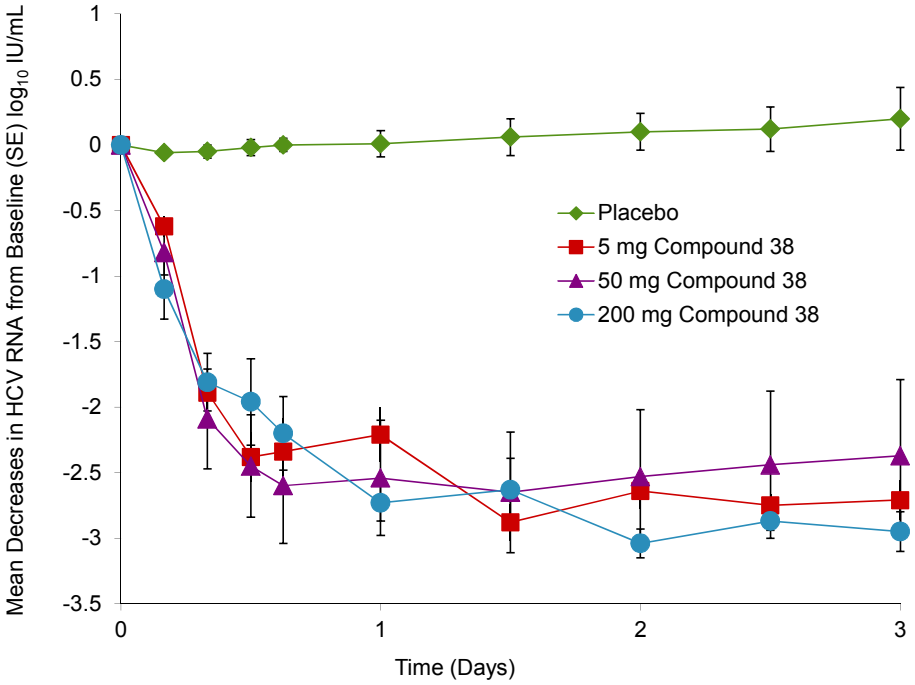


Figure 3. Mean decreases in HCV RNA from baseline during 3-day monotherapy with compound **38** in HCV GT1-infected treatment-naïve subjects.

Conclusions

There is a tremendous unmet medical need to discover new treatments for HCV infection with improved efficacy, tolerability and convenience relative to the current standard of care. The addition of inhibitors of HCV NS5A to the arsenal of DAAs that are available for use in combination therapy holds significant promise for delivering such therapies. We discovered

compound **38**, based on a chiral pyrrolidine core, which demonstrates picomolar potency against GT1a and GT1b. Compound **38** is a pan-genotypic HCV inhibitor, with an EC_{50} range of 1.7 – 19.3 pM against genotypes 1a, 1b, 2a, 2b, 3a, 4a, and 5a, and 366 pM against genotype 6a. Subsequent to its discovery, compound **38** has demonstrated excellent human pharmacokinetics with a long 25 to 32 hour half-life, consistent with once-daily dosing. Compound **38** decreased HCV RNA up to 3.10 log10 IU/mL during 3-day monotherapy in treatment-naïve HCV GT1-infected subjects, and it is currently undergoing phase 3 clinical trials in combination with an NS3 protease inhibitor and an NS5B non-nucleoside polymerase inhibitor, with and without ribavirin.

Experimental Section

Biological Evaluation. The GT1a replicon construct (GenBank accession number NC004102) contained the 5' nontranslated region (NTR) from 1a-H77 followed by a firefly luciferase reporter gene and the neomycin phosphotransferase (Neo) gene, which together comprised the first cistron of the bicistronic replicon construct. This was followed by the EMCV IRES and then the second cistron containing the 1a-H77 NS3-NS5B coding region with adaptive mutations E1202G, K1691R, K2040R, and S2204I, and finally the 1a-H77 3' NTR. The GT1b-Con1 replicon construct (GenBank accession number AJ238799) contained the 5' nontranslated region (NTR) from 1b-Con1 followed by a firefly luciferase reporter gene and the neomycin phosphotransferase (Neo) gene, which together comprised the first cistron of the bicistronic replicon construct. This was followed by the EMCV IRES and the second cistron containing the 1b-Con1 NS3-NS5B coding region with adaptive mutations K1609E, K1846T, and Y3005C, and finally the 1b-Con1 3' NTR. In addition, the 1b-Con1 replicon construct contained a poliovirus IRES between the HCV 5' NTR and the firefly luciferase gene. In order to assess the ability of

compounds to inhibit NS5A from non-GT1 HCV, a 1b-Con1 replicon shuttle vector was constructed that containing restriction sites just upstream of NS5A in the C-terminus of NS4B, and just after NS5A amino acid 214. Six chimeric subgenomic replicon cell lines were generated for evaluation of the activity of compounds. The NS5A regions for generation of chimeric replicons were derived from GT 2a, 2b, 3a, 4a, 5a, and 6a HCV-infected patient sera. RT-PCR was conducted on the viral RNA from each of these patient serum samples to generate a DNA fragment encoding the first 214 amino acids of NS5A. The fragment was ligated into the replicon shuttle vector, and in vitro transcribed into replicon RNA. This RNA was introducing into human hepatoma cells (Huh-7) to create stable replicon cell lines. The inhibitory effect of compounds on HCV replication was determined (in the presence or absence of 40% HP), by measuring decrease in luciferase signal. The percent inhibition of HCV replicon replication was calculated for each compound concentration and the EC_{50} value was calculated using nonlinear regression curve fitting to the 4-parameter logistic equation in the GraphPad Prism 4/5 software.

The methods describing the measurement of the effects of individual amino acid variants on the activity of an inhibitor in HCV replicon cell culture assays were described previously.[33] Briefly, the resistance-associated variants in NS5A were each introduced into GT 1a-H77, GT 1b-Con1, or chimeric replicons. In transient assays, the replicon containing the variant was transfected *via* electroporation into a Huh-7 derived cell line. The percent inhibition of HCV replicon replication as determined by decreased luciferase signal was calculated for each compound concentration, and the EC_{50} values were calculated as described above. Replication capacity was calculated as a percentage of wild-type replication using the following equation, 100x{(variant 4 day luciferase counts/wild-type 4 day luciferase counts)/(variant 4 hr luciferase counts/wild-type 4 hr luciferase counts)}.

Chemistry. General Procedures. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from Aldrich (Milwaukee, WI) and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. NMR spectra were collected on either a Varian Inova 400 MHz or 500 MHz spectrometer as specified with chemical shifts given in ppm (δ) and are referenced to an internal standard of tetramethylsilane (δ 0.00). $^{1}H - {}^{1}H$ couplings are assumed to be first-order and peak multiplicities are reported in the usual manner. All final compounds were purified to >95% purity as determined by two independent HPLC analyses. Reverse phase HPLC purity determinations were performed on a Waters e2695 Separation Module equipped with a Waters 2489 UV-Visible Detector and a YMC ODS-A, 5.5 μm, 120Å, 4.6 X 150 mm column and an XTerra RP18, 5 μm, 4.6 X 150 mm column. Silica gel chromatography purifications were performed using either glass columns packed with silica gel 60 (230-400 mesh) or prepacked silica gel cartridges (various vendors / various automated chromatography systems). Mass spectral (MS) ESI data were determined on a Thermo-Finnigan SSQ7000 spectrometer. Mass spectral (MS) APCI data were determined on either a Thermo-Finnigan Navigator or Thermo-Finnigan MSQ-Plus mass spectrometer. Analytical LCMS was performed on an Agilent Series 1100 HPLC system using a Phenomenex Luna Combi-HTS C8(2) 5 μm 100Å (2.1 mm × 50 mm) column. The system was equipped with an autosampler and coupled to a Finnigan Thermoquest atmospheric pressure chemical ionization (APCI) mass spectrometer.

1,4-Bis(4-nitrophenyl)butane-1,4-dione (3). Anhydrous zinc (II) chloride (2.73 g, 20.0 mmol) was stirred in dry benzene (15 mL) while diethylamine (1.6 mL, 15.0 mmol) and t-butanol (1.4 mL, 15.0 mmol) were added, and the resulting mixture was stirred at room temperature for 90

minutes to give a cloudy solution. To this mixture was added 2-bromo-1-(4-nitrophenyl)ethanone (2.44 g, 10.0 mmol) and 1-(4-nitrophenyl)ethanone (2.48 g, 15.0 mmol), and the resulting mixture was stirred at room temperature for 16 hours. The mixture was poured into water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The resulting residue was triturated with dichloromethane to give an orange solid that was collected by filtration and dried to give the product (2.0 g, 61% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.46 (s, 4H), 8.09 – 8.16 (m, 4H), 8.27 – 8.32 (m, 4H).

1,4-Bis(4-nitrophenyl)butane-1,4-diol (4c). To a solution of **3** (1.0 g, 3.05 mmol) in anhydrous THF (30 mL) at 0 °C was added sodium borohydride (0.357 g, 9.44 mmol). The resulting mixture was stirred at 50 °C for 16 hours. The cooled mixture was poured into water, extracted with ethyl acetate, dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting solid was triturated with dichloromethane to give a tan solid that was collected by filtration and dried to give **4c** (0.82 g, 81% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.52 – 1.75 (m, 4H), 4.62 – 4.77 (m, 2H), 5.47 – 5.55 (m, 2H), 7.51 – 7.60 (m, 4H), 8.15 – 8.22 (m, 4H).

1,4-Bis(4-nitrophenyl)butane-1,4-diyl dimethanesulfonate (5c). To a stirred mixture of **4c** (0.950 g, 2.86 mmol) in anhydrous dichloromethane (30 mL) at 0 °C was added triethylamine (1.2 mL, 8.58 mmol), followed by dropwise addition of methanesulfonyl chloride (0.56 mL, 7.15 mmol). The resulting mixture was stirred at 0 °C for 30 minutes, during which time **4c** slowly went into solution. After stirring an additional 1 hour at 0 °C, saturated aqueous NH_4Cl (4 mL) was added, and the resulting mixture was stirred at room temperature for 20 minutes and then transferred to a separatory funnel and washed with water (2 x 10 mL). The organic layer was separated, dried over Na_2SO_4, and filtered. Hexanes (10 mL) was added to give an orange

precipitate that was collected by filtration and dried to give the product (1.4 g, quantitative) which was used without further purification.

1-(4-Fluorophenyl)-2,5-bis(4-nitrophenyl)pyrrolidine (47). To a solution of **5c** (1.4 g, 2.86 mmol) in anhydrous *N,N*-dimethylformamide (4 mL) was added 4-fluoroaniline (2.0 mL, 20.8 mmol), and the resulting mixture was stirred at 50 °C for 6 hours. The resulting mixture was cooled and partitioned between 1 N aqueous HCl (40 mL) and ethyl acetate (3 x 40 mL), and the combined organic layers were dried over Na_2SO_4. The drying agent was filtered off, and the solvent was evaporated to give a crude product that was purified by column chromatography on silica gel using a solvent gradient of 0-50% ethyl acetate in hexanes to give **47** as a 2.4 to 1 ratio of *trans*- to *cis*-substituted pyrrolidine isomers (0.385 g, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.84 – 2.04 (m, 2H), 2.50 – 2.66 (m, 2H), 4.82 – 4.90 (m, 0.6H), 5.27 – 5.34 (m, 1.4H), 6.14 – 6.21 (m, 1.4H), 6.27 – 6.34 (m, 0.6H), 6.71 – 6.89 (m, 2H), 7.33 – 7.40 (m, 2.8H), 7.63 – 7.69 (m, 1.2H), 8.17 – 8.23 (m, 2.8H), 8.25 – 8.31 (m, 1.2H).

4,4'-(*trans*-1-(4-Fluorophenyl)pyrrolidine-2,5-diyl)dianiline (48a) and **4,4'-(*cis*-1-(4-Fluorophenyl)pyrrolidine-2,5-diyl)dianiline (48b).** To a solution of **47** (0.38 g, 0.93 mmol) in THF (4 mL) and ethanol (4 mL) were added a solution of NH_4Cl (0.15 g, 2.80 mmol) in water (1 mL) and iron powder (0.26 g, 4.66 mmol). The resulting mixture was stirred at 80 °C for 90 minutes. The cooled mixture was filtered through Celite® and concentrated in vacuo. Purification of the crude product by column chromatography on silica gel using a solvent gradient of 0-100% ethyl acetate in hexanes allowed for the separation of the *trans*-pyrrolidine and *cis*-pyrrolidine isomers. The first eluting component was the *trans*-pyrrolidine isomer **48a** (55 mg, 17%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.48 – 1.65 (m, 2H), 2.31 – 2.44 (m, 2H), 4.88 (s, 4H), 4.96 (d, J = 6.7 Hz, 2H), 6.16 – 6.27 (m, 2H), 6.47 (d, J = 8.3 Hz, 4H), 6.71 – 6.78 (m,

2H), 6.82 (d, *J* = 8.3 Hz, 4H). The second eluting component was the *cis*-pyrrolidine isomer **48b** (52 mg, 16%). ^{1}H NMR (400 MHz, DMSO) δ 1.70 – 1.83 (m, 2H), 2.20 – 2.31 (m, 2H), 4.48 (t, *J* = 5.3 Hz, 2H), 4.93 (s, 4H), 6.33 – 6.43 (m, 2H), 6.56 (d, *J* = 8.4 Hz, 4H), 6.83 (t, *J* = 8.9 Hz, 2H), 7.12 (d, *J* = 8.3 Hz, 4H).

(2*S*,2'*S*)-*tert*-Butyl 2,2'-(4,4'-(*trans*-1-(4-fluorophenyl)pyrrolidine-2,5-diyl)bis(4,1-phenylene))bis(azanediyl)bis(oxomethylene)dipyrrolidine-1-carboxylate (49). A mixture of the *trans*-pyrrolidine isomer of **48a** (0.050 g, 0.144 mmol), (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid (0.077 g, 0.360 mmol), Hünig's base (0.075 mL, 0.432 mmol), and HATU (0.137 g, 0.360 mmol) in anhydrous dimethylsulfoxide (1 mL) was stirred at room temperature for 90 minutes. The mixture was partitioned between ethyl acetate and water, and the organic layer was dried over Na_2SO_4. The drying agent was filtered off, and the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel using a solvent gradient of 0-100% ethyl acetate in hexanes to give **49** as a light orange solid (0.107 g, quant.). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.24 – 1.44 (m, 18H), 1.59 – 1.71 (m, 2H), 1.72 – 1.96 (m, 8H), 2.07 – 2.27 (m, 2H), 3.37 – 3.50 (m, 2H), 4.13 – 4.28 (m, 2H), 4.34 (t, *J* = 5.1 Hz, 2H), 5.17 (d, *J* = 4.2 Hz, 2H), 6.15 – 6.28 (m, 2H), 6.78 (t, *J* = 8.9 Hz, 2H), 7.14 (d, *J* = 7.9 Hz, 4H), 7.46 – 7.56 (m, 4H), 9.87 – 9.97 (m, 2H); MS (ESI) *m/z* 742 $(M+H)^+$.

Dimethyl (2*S*,2'*S*)-1,1'-((2*S*,2'*S*)-2,2'-(4,4'-((2*S*,5*S*)-1-(4-fluorophenyl)pyrrolidine-2,5-diyl)bis(4,1-phenylene))bis(azanediyl)bis(oxomethylene)bis(pyrrolidine-2,1-diyl))bis(3-methyl-1-oxobutane-2,1-diyl)dicarbamate (12) and **Dimethyl (2*S*,2'*S*)-1,1'-((2*S*,2'*S*)-2,2'-(4,4'-((2*R*,5*R*)-1-(4-fluorophenyl)pyrrolidine-2,5-diyl)bis(4,1-phenylene))bis(azanediyl)bis(oxomethylene)bis(pyrrolidine-2,1-diyl))bis(3-methyl-1-

oxobutane-2,1-diyl)dicarbamate (13). A solution of **49** (107 mg, 0.148 mmol) in dichloromethane (1 mL) and trifluoroacetic acid (2 mL) was stirred at room temperature for 45 minutes and then concentrated in vacuo. The residue was partitioned between dichloromethane and saturated aqueous $NaHCO_3$, and the organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was dissolved in anhydrous dimethylsulfoxide (1.5 mL), and (*S*)-2-(methoxycarbonylamino)-3-methylbutanoic acid (65 mg, 0.37 mmol), HATU (141 mg, 0.37 mmol) and Hünig's base (0.077 mL, 0.44 mmol) were added. The resulting mixture was stirred at room temperature for 90 minutes, and was partitioned between water and ethyl acetate (3x). The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo, and the crude product was purified by column chromatography on silica gel using a solvent gradient of 0-5% methanol in dichloromethane to give a mixture of *trans*-pyrrolidine isomers (110 mg, 87%). The mixture was separated on a Chiralpak AD-H column eluting with a mixture of 1 part (2:1 isopropanol:ethanol) and 1 part hexanes (0.1% TFA). Compound **12** was the first of 2 stereoisomers to elute. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84 – 0.97 (m, 12H), 1.64 (d, J = 5.6 Hz, 2H), 1.88 (m, 6H), 1.95 – 2.05 (m, 2H), 2.08 – 2.19 (m, 2H), 3.52 (s, 6H), 3.58 – 3.66 (m, 2H), 3.76 – 3.85 (m, 2H), 4.02 (t, J = 8.5 Hz, 2H), 4.42 (dd, J = 8.0, 4.9 Hz, 2H), 5.15 (d, J = 6.5 Hz, 2H), 6.20 (dd, J = 9.2, 4.4 Hz, 2H), 6.78 (t, J = 8.9 Hz, 2H), 7.13 (d, J = 8.5 Hz, 4H), 7.31 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 8.5 Hz, 4H), 9.99 (s, 2H); MS (ESI) *m/z* 856.9 $(M+H)^+$. Compound **13** was the second of 2 stereoisomers to elute. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.82 – 0.97 (m, 12H), 1.65 (d, J = 5.7 Hz, 2H), 1.80 – 2.05 (m, 8H), 2.08 – 2.20 (m, 2H), 3.52 (s, 6H), 3.57 – 3.68 (m, 2H), 3.76 – 3.87 (m, 2H), 4.01 (t, J = 8.5 Hz, 2H), 4.42 (dd, J = 8.0, 4.9 Hz, 2H) 5.16 (d, J = 6.4 Hz, 2H), 6.20 (dd, J = 9.2, 4.5 Hz, 2H), 6.77 (t, J = 9.0 Hz, 2H), 7.12 (d, J =

8.6 Hz, 4H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.5 Hz, 4H), 9.98 (s, 2H). MS (ESI) *m/z* 856.4 $(M+H)^+$.

Dimethyl (2*S*,2'*S*)-1,1'-((2*S*,2'*S*)-2,2'-(4,4'-((2*S*,5*R*)-1-(4-fluorophenyl)pyrrolidine-2,5-diyl)bis(4,1-phenylene))bis(azanediyl)bis(oxomethylene)bis(pyrrolidine-2,1-diyl))bis(3-methyl-1-oxobutane-2,1-diyl)dicarbamate 2,2,2-trifluoroacetate (14). Compound **14** was prepared using the methods described for the synthesis of **12** and **13**, substituting a mixture of both *cis*-pyrrolidine **48a** and *trans*-pyrrolidine of **48b**. Compound **14** was separated from the *trans*-pyrrolidine component by C-18 HPLC using a gradient of 10-100% acetonitrile in water (0.1% TFA), and was the second of 2 isomers to elute. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 (dd, *J* = 6.7, 2.1 Hz, 6H) 1.22 (dd, *J* = 6.8, 2.1 Hz, 6H) 3.87 – 3.97 (m, 2H) 3.84 (s, 6H) 3.99 – 4.09 (m, 2H) 4.42 – 4.52 (m, 2H) 4.67 – 4.78 (m, 2H) 4.87 – 4.97 (m, 2H) 6.23 (d, *J* = 8.4 Hz, 2H) 6.61 – 6.71 (m, 2H) 7.01 (t, *J* = 9.0 Hz, 2H) 7.68 – 7.73 (m, 4H) 7.77 – 7.83 (m, 4H) 8.26 (s, 2H) 9.35 (s, 2H). MS (ESI) *m/z* 856.1 $(M+H)^+$.

1-(4-*tert*-Butylphenyl)-2,5-bis(4-nitrophenyl)pyrrolidine (50). A mixture of **5c** (3.67 g, 7.51 mmol) and 4-*tert*-butylaniline (11.86 mL, 75 mmol) in anhydrous *N,N*-dimethylformamide (40 mL) was stirred at 50 °C for 4 hours. The mixture was partitioned between 1M aqueous HCl and ethyl acetate, and the organic layer was washed with brine and dried over Na_2SO_4. The drying agent was filtered off, and the solution was concentrated in vacuo to give a crude product that was purified by column chromatography on silica gel using a solvent gradient of 5-30% ethyl acetate in hexanes to give **50** as a 3:1 mixture of *trans*-pyrrolidine and *cis*-pyrrolidine isomers (1.21 g, 36%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (s, 3H), 1.15 (s, 6H), 1.69 – 1.79 (m, 0.5H), 1.79 – 1.91 (m, 1.5H), 2.49 – 2.61 (m, 2H), 4.94 (t, *J* = 5.4 Hz, 1.5H), 5.47 (d, *J* = 7.1 Hz, 0.5H), 6.19 (d, *J* = 8.8 Hz, 0.5H), 6.28 (d, *J* = 8.8 Hz, 1.5H), 6.99 (d, *J* = 8.8 Hz, 0.5H), 7.08 (d,

J = 8.8 Hz, 1.5H), 7.53 (d, *J* = 8.7 Hz, 1H), 7.81 (d, *J* = 8.7 Hz, 3H), 8.21 (d, *J* = 8.7 Hz, 1H), 8.27 (d, *J* = 8.7 Hz, 3H).

4,4'-(*trans*-1-(4-*tert*-butylphenyl)pyrrolidine-2,5-diyl)dianiline (51). A mixture of **50** (1.10 g, 2.47 mmol) and platinum (IV) oxide (0.22 g, 0.97 mmol) in 1:1 ethanol:THF (40 mL) was stirred under hydrogen gas at 30 psi for 30 minutes. The resulting mixture was filtered and concentrated in vacuo to give a crude product which was purified by column chromatography on silica gel using a solvent gradient of 20-60% ethyl acetate in hexanes. The desired *trans*-pyrrolidine isomer was the first of 2 stereoisomers to elute, providing **51** (510 mg, 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (s, 9H), 1.47 – 1.62 (m, 2H), 2.32 – 2.44 (m, 2H), 4.86 (s, 4H), 4.96 (d, *J* = 6.7 Hz, 2H), 6.19 (d, *J* = 8.8 Hz, 2H), 6.47 (d, *J* = 8.3 Hz, 4H), 6.82 (d, *J* = 8.3 Hz, 4H), 6.91 (d, *J* = 8.8 Hz, 2H).

(2*S*,2'*S*)-*tert*-Butyl 2,2'-(4,4'-(*trans*-1-(4-*tert*-butylphenyl)pyrrolidine-2,5-diyl)bis(4,1-phenylene)bis(azanediyl)bis(oxomethylene))dipyrrolidine-1-carboxylate (52). A mixture of **51** (250 mg, 0.648 mmol), (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid (307 mg, 1.427 mmol), HATU (542 mg, 1.427 mmol) and Hünig's base (0.453 mL, 2.59 mmol) in dimethylsulfoxide (10 mL) was stirred at room temperature for 1 hour. The mixture was partitioned between ethyl acetate and water, and the organic layer was washed with brine, dried with Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with a solvent gradient of 10-50% ethyl acetate in hexanes to give compound **52** as a colorless solid (0.500 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (s, 9H), 1.28 (m, 11H), 1.39 (m, 7H), 1.58 – 1.69 (m, 2H), 1.71 – 1.95 (m, 8H), 2.06 – 2.25 (m, 2H), 2.42 – 2.48 (m, 2H), 3.35 – 3.47 (m, 2H), 4.12 – 4.28 (m, 2H), 5.16 (d, *J* = 5.4 Hz, 2H),

6.19 (d, *J* = 8.3 Hz, 2H), 6.94 (d, *J* = 8.6 Hz, 2H), 7.08 – 7.22 (m, 4H), 7.50 (t, *J* = 7.8 Hz, 4H), 9.91 (m, 2H); MS (ESI) *m/z* 780.4 $(M+H)^+$.

Dimethyl (2*S*,2'*S*)-1,1'-((2*S*,2'*S*)-2,2'-(4,4'-((2*S*,5*S*)-1-(4-tert-butylphenyl)pyrrolidine-2,5-diyl)bis(4,1-phenylene))bis(azanediyl)bis(oxomethylene)bis(pyrrolidine-2,1-diyl))bis(3-methyl-1-oxobutane-2,1-diyl)dicarbamate (38) and **Dimethyl (2*S*,2'*S*)-1,1'-((2*S*,2'*S*)-2,2'-(4,4'-((2*R*,5*R*)-1-(4-tert-butylphenyl)pyrrolidine-2,5-diyl)bis(4,1-phenylene))bis(azanediyl)bis(oxomethylene)bis(pyrrolidine-2,1-diyl))bis(3-methyl-1-oxobutane-2,1-diyl)dicarbamate (39).** A solution of **52** (498 mg, 0.638 mmol) in trifluoroacetic acid (6 mL) and dichloromethane (4 mL) was stirred at room temperature for 1 hour. The solution was concentrated to dryness, and the residue was partitioned between saturated aqueous $NaHCO_3$ and a 3:1 mixture of chloroform and isopropanol (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. A portion of this material (232 mg, 0.400 mmol) was dissolved in DMF (10 mL) and cooled to 0 °C, followed by addition of (*S*)-2-(methoxycarbonylamino)-3-methylbutanoic acid (210 mg, 1.200 mmol), 1-hydroxybenzotriazole hydrate (184 mg, 1.200 mmol), *N*-methylmorpholine (0.220 mL, 2.000 mmol), and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (230 mg, 1.200 mmol). The resulting mixture was stirred at room temperature for 16 hours. The mixture was partitioned between ethyl acetate and water, and the organic layer was washed with saturated aqueous $NaHCO_3$, brine (2x), and dried with Na_2SO_4. The drying agent was filtered off and the solution was concentrated in vacuo to give a crude product that was purified by column chromatography on silica gel eluting with a solvent gradient of 2-8% methanol in dichloromethane to give a 1:1 mixture of trans-pyrrolidine isomers (290 mg, 96%). The mixture was separated on a Chiralpak AD-H column eluting with a mixture of 1 part (2:1

isopropanol:ethanol) and 2 parts hexanes (0.1% TFA). Compound **38** was the first of 2 stereoisomers to elute (101 mg, 99% ee by chiral HPLC). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88 (d, J = 6.61 Hz, 6H), 0.93 (d, J = 6.72 Hz, 6H), 1.11 (s, 9H), 1.63 (d, J = 5.42 Hz, 2H), 1.80 – 2.04 (m, 8H), 2.09 – 2.19 (m, 2H), 2.44 – 2.47 (m, 2H), 3.52 (s, 6H), 3.59 – 3.66 (m, 2H), 3.77 – 3.84 (m, 2H), 4.02 (t, J = 8.40 Hz, 2H), 4.42 (dd, J = 7.86, 4.83 Hz, 2H), 5.14 (d, J = 6.18 Hz, 2H), 6.17 (d, J = 8.67 Hz, 2H), 6.94 (d, J = 8.78 Hz, 2H), 7.13 (d, J = 8.46 Hz, 4H), 7.31 (d, J = 8.35 Hz, 2H), 7.50 (d, J = 8.35 Hz, 4H), 9.98 (s, 2H); MS (ESI) *m/z* 894.9 $(M+H)^+$. Compound **39** was the second of 2 stereoisomers to elute. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87 (d, J = 6.51 Hz, 6H), 0.92 (d, J = 6.72 Hz, 6H), 1.11 (s, 9H), 1.63 (d, J = 5.53 Hz, 2H), 1.82 – 2.04 (m, 8H), 2.09 – 2.18 (m, 2H), 2.41 – 2.47 (m, 2H), 3.52 (s, 6H), 3.58 – 3.67 (m, 2H), 3.75 – 3.84 (m, 2H), 4.02 (t, J = 7.26 Hz, 2H), 4.43 (dd, J = 7.92, 4.88 Hz, 2H), 5.14 (d, J = 6.18 Hz, 2H), 6.17 (d, J = 8.78 Hz, 2H), 6.94 (d, J = 8.67 Hz, 2H), 7.12 (d, J = 8.46 Hz, 4H), 7.31 (d, J = 8.35 Hz, 2H), 7.49 (d, J = 8.46 Hz, 4H), 9.98 (s, 2H). MS (ESI) *m/z* 895.0 $(M+H)^+$.

(1*R*,4*R*)-1,4-Bis(4-nitrophenyl)butane-1,4-diol (4a). To a stirred mixture of (*S*)-(-)-α,α-diphenyl-2-pyrrolidinemethanol (2.71 g, 10.70 mmol) in anhydrous THF (15 mL) at 23 °C was added trimethyl borate (1.44 g, 13.86 mmol), and the resulting solution was stirred at room temperature for 1 hour. The solution was cooled to 16-19 °C, and *N,N*-diethylaniline borane (21.45 g, 132 mmol) was added dropwise via syringe over 3-5 minutes (caution: vigorous H_2 evolution), while the internal temperature was maintained at 16-19 °C. After 15 minutes, the H_2 evolution had ceased. The resulting borane mixture was cooled to 11°C before being transferred via cannula over 3-5 minutes to a separate flask that contained a stirred slurry of **3** (22.04 g, 63.8 mmol) in anhydrous THF (80 mL). During the transfer period, the internal temperature of the slurry rose to 16 °C. After the addition was complete, the reaction was maintained at room

temperature for an additional 2.5 hours. After reaction completion, the mixture was cooled to 5 °C and methanol (16.7 g, 521 mmol) was added dropwise over 5-10 minutes, while maintaining an internal temperature of <20 °C (note: vigorous H_2 evolution). After the exotherm had ceased (ca. 10 minutes), the reaction was brought to room temperature, and stirring was continued until complete dissolution of the solids had occurred. Ethyl acetate (300 mL) and 1 M HCl (120 mL) were added, and the phases were separated. The organic phase was washed successively with 1 M HCl (2 x120 mL), H_2O (65 mL), and 10% aqueous NaCl (65 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. Crystallization of the product occurred during the concentration. The slurry was warmed to 50 °C, and heptane (250 mL) was added over 15 minutes. The slurry was then brought to room temperature and allowed to stand for 30 minutes. Solids were collected by filtration, and washed with 3:1 heptane:ethyl acetate (75 mL). The orange, crystalline solid was dried at 45 °C for 24 hours to provide **4a** (15.35 g, >99% ee, 61% yield), which was contaminated with 11% of the meso isomer. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 – 1.78 (m, 4H), 4.55 – 4.77 (m, 2H), 5.51 (br s, 2H), 7.55 (d, J = 8.7 Hz, 1H), 8.16 (d, J = 8.7 Hz, 1H).

(1*S*,4*S*)-1,4-Bis(4-nitrophenyl)butane-1,4-diol (4b). Compound **4b** was prepared by the method used for **4a**, substituting (*R*)-(+)-α,α-diphenyl-2-pyrrolidinemethanol for (*S*)-(-)-α,α-diphenyl-2-pyrrolidinemethanol.

(2*R*,5*R*)-1-(4-Cyclopropylphenyl)-2,5-bis(4-nitrophenyl)pyrrolidine (53). To a solution of **4b** (1.50 g, 4.51 mmol) in anhydrous dichloromethane (45 mL) at -20 ºC was added triethylamine (1.89 mL, 13.5 mmol). The cooled mixture was stirred for 10 minutes, and methanesulfonyl chloride (0.88 mL, 11.23 mmol) was added dropwise over a period of 15 minutes. The resulting mixture was stirred at -20 ºC for 30 minutes and then concentrated in vacuo. To the residue was

added 4-cyclopropylaniline (4.80 g, 36.0 mmol) and *N,N*-dimethylformamide (6.43 mL), and the resulting mixture was stirred at 50 ºC for 2 hours. The resulting mixture was diluted with ethyl acetate and washed with 1 M aqueous HCl and brine, and dried over Na_2SO_4. The drying agent was filtered off, and the filtrate was concentrated in vacuo to give the crude product which was purified by column chromatography on silica gel eluting with 10-33% ethyl acetate in hexanes to give **53**, contaminated with ~17% of the *cis*-pyrrolidine isomer (1.25 g, 65%). ^{1}H NMR (400 MHz, DMSO) δ 0.34 – 0.50 (m, 2H), 0.66 – 0.79 (m, 2H), 1.56 – 1.89 (m, 3H), 2.47 – 2.56 (m, 2H), 4.93 (t, *J* = 5.5 Hz, 0.3H), 5.44 (dd, *J* = 17.5, 6.2 Hz, 1.7H), 6.17 (d, *J* = 8.7 Hz, 1.7H), 6.24 (d, *J* = 8.6 Hz, 0.3H), 6.70 (d, *J* = 8.6 Hz, 1.7H), 6.79 (d, *J* = 8.6 Hz, 0.3H), 7.51 (d, *J* = 8.7 Hz, 3.3H), 7.79 (d, *J* = 8.7 Hz, 0.7H), 8.19 (d, *J* = 8.7 Hz, 3.3H), 8.26 (d, *J* = 8.7 Hz, 0.7H).

4,4'-((2*R*,5*R*)-1-(4-Cyclopropylphenyl)pyrrolidine-2,5-diyl)dianiline (54). A mixture of **53** (1.25 g, 2.91 mmol) and platinum (IV) oxide (0.20 g, 0.873 mmol) in 1:1 ethanol:THF (20 mL) was stirred at room temperature under a hydrogen atmosphere at 30 psi for 30 minutes. The mixture was filtered and concentrated in vacuo, and the crude product was purified by column chromatography on silica gel eluting with a solvent gradient of 5-50% ethyl acetate in hexanes (the desired *trans*-pyrrolidine isomer eluted prior to the *cis*-pyrrolidine isomer) to give **54** (567 mg, 53%). 1H NMR (400 MHz, DMSO) δ 0.34 – 0.45 (m, 2H), 0.65 – 0.75 (m, 2H), 1.49 – 1.68 (m, 3H), 2.29 – 2.42 (m, 2H), 4.86 (s, 4H), 4.95 (d, *J* = 6.6 Hz, 2H), 6.16 (d, *J* = 8.7 Hz, 2H), 6.46 (d, *J* = 8.3 Hz, 4H), 6.62 (d, *J* = 8.6 Hz, 2H), 6.80 (d, *J* = 8.3 Hz, 4H); MS (ESI) *m/z* 370.3 $(M+H)^+$.

(2*S*,2'*S*)-*tert*-Butyl 2,2'-(4,4'-((2*R*,5*R*)-1-(4-cyclopropylphenyl)pyrrolidine-2,5-diyl)bis(4,1-phenylene))bis(azanediyl)bis(oxomethylene)dipyrrolidine-1-carboxylate (55). To a solution of **54** (0.20 g, 0.541 mmol), (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid (0.245 g,

1.137 mmol), and HATU (0.453 g, 1.191 mmol) in dimethylsulfoxide (10.83 mL) was added Hünig's base (0.38 mL, 2.17 mmol). The resulting mixture was stirred at room temperature for 30 minutes. The mixture was partitioned between ethyl acetate and water, and the organic layer was washed with brine and dried over Na_2SO_4. The drying agent was filtered off, and the filtrate was concentrated in vacuo to give the crude product which was purified by column chromatography on silica gel eluting with a solvent gradient of 25-75% ethyl acetate in hexanes to give **55** (370 mg, 89%). 1H NMR (400 MHz, DMSO) δ 0.35 – 0.47 (m, 2H), 0.64 – 0.77 (m, 2H), 1.29 (s, 11H), 1.37 (s, 7H), 1.52 – 1.69 (m, 3H), 1.68 – 1.97 (m, 6H), 2.06 – 2.27 (m, 2H), 2.38 – 2.48 (m, 2H), 3.35 – 3.48 (m, 2H), 4.11 – 4.28 (m, 2H), 5.15 (d, *J* = 5.8 Hz, 2H), 6.16 (d, *J* = 7.1 Hz, 2H), 6.65 (d, *J* = 8.6 Hz, 2H), 7.05 – 7.18 (m, 4H), 7.43 – 7.54 (m, 4H), 9.91 (m, 2H); MS (ESI) *m/z* 764.5 $(M+H)^+$.

Dimethyl (2*S*,2'*S*)-1,1'-((2*S*,2'*S*)-2,2'-(4,4'-((2*R*,5*R*)-1-(4-cyclopropylphenyl)pyrrolidine-2,5-diyl)bis(4,1-phenylene))bis(azanediyl)bis(oxomethylene)bis(pyrrolidine-2,1-diyl))bis(3-methyl-1-oxobutane-2,1-diyl)dicarbamate (37). To a solution of **55** (100 mg, 0.131 mmol) in 1,4-dioxane (5 mL) was added 4N HCl in 1,4-dioxane (5 mL, 20 mmol), and the resulting mixture was stirred at room temperature for 2.5 hours and concentrated in vacuo to give a colorless solid. The solid was dissolved in *N,N*-dimethylformamide (3 mL), and (*S*)-2-(methoxycarbonylamino)-3-methylbutanoic acid (52 mg, 0.295 mmol) and 4-methylmorpholine (0.144 mL, 1.310 mmol) were added. The resulting solution was cooled in an ice bath, and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (62.8 mg, 0.328 mmol) and 1-hydroxybenzotriazole hydrate (50 mg, 0.328 mmol) were added. The resulting mixture was stirred at room temperature for 4 hours, and was partitioned between ethyl acetate and water. The organic layer was washed successively with 10% aqueous $NaHCO_3$ and brine, and dried over

Na_2SO_4. The drying agent was filtered off, and the filtrate was concentrated in vacuo to give the crude product which was purified by column chromatography on silica gel eluting with a solvent gradient of 1-3.5% methanol in dichloromethane to give **37** (62 mg, 52%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.36 – 0.46 (m, 2H), 0.63 – 0.77 (m, 2H), 0.87 (d, J = 6.6 Hz, 6H), 0.92 (d, J = 6.7 Hz, 6H), 1.52 – 2.46 (m, 15H), 3.52 (s, 6H), 3.57 – 3.66 (m, 2H), 3.75 – 3.85 (m, 2H), 4.02 (t, J = 8.5 Hz, 2H), 4.42 (dd, J = 8.0, 4.9 Hz, 2H), 5.14 (d, J = 6.4 Hz, 2H), 6.14 (d, J = 8.8 Hz, 2H), 6.65 (d, J = 8.7 Hz, 2H), 7.10 (d, J = 8.6 Hz, 4H), 7.30 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 8.6 Hz, 4H), 9.97 (s, 2H). MS (APCI) *m/z* 878.5 $(M+H)^+$.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 847-937-7205. E-mail: david.degoey@abbvie.com.

Present Addresses

†Division of Research Safety, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

††Sebeka, MN 56477, USA.

‡Department of Chemistry, Northeastern Illinois University, Chicago, IL 60625, USA.

#Global Pharmaceutical Regulatory Affairs, Abbott Laboratories, Abbott Park, IL 60064, USA.

¶Abbott Nutrition R&D, Abbott Nutrition, Columbus, OH 43219, USA.

Disclosures

DD, JR, DL, JP, RW, CH, SP, PD, ACK, LN, MM, DC, PK, NM, GK, TR, TP-M, YG, CJM, AM, ED, AC, LW, CC, and WK are employees of AbbVie. CEM, RK, MT, RM, and DB are former Abbott/AbbVie employees. This study was sponsored by AbbVie. AbbVie contributed to the study design, research and interpretation of data, writing, reviewing and approving the publication.

Acknowledgements

We thank Tammie Jinkerson and Todd Soltwedel for contributions to the chemistry described here. We thank Carolyn Setze for contributions to the clinical study design and Sarah Kopecky-Bromberg for assistance in creating Figure 3. All are current or former AbbVie employees.

ABBREVIATIONS USED:

GT, genotype; RBV, ribavirin; pegIFN, pegylated interferon; DAA, direct-acting antiviral; NS, nonstructural; r, ritonavir; ee, enantiomeric excess; TFA, trifluoroacetic acid; HP, human plasma; SVR, sustained virologic response; PK, pharmacokinetics; PAMPA, parallel artificial membrane permeability assay; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate.

ASSOCIATED CONTENT

Supporting Information

Additional syntheses and analytical information for the tabulated inhibitors. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Shah, N.; Pierce, T.; Kowdley, K. V. Review of direct-acting antiviral agents for the treatment of chronic hepatitis C. *Expert Opin. Investig. Drugs* **2013**, *22*. Published online June 4, 2013. (doi:10.1517/13543784.2013.806482).

(2) For a recent review see: Nature Outlook: Hepatitis C. *Nature* **2011**, *474*, S1–S21.

(3) Roche1, B.; Samuel, D. Hepatitis C virus treatment pre- and post-liver transplantation. *Liver Int.* **2012**, *32,* Supplement s1, 120-128.

(4) Witthöft, Th.; Möller, B.; Wiedmann, K. H.; St. Mauss, St.; Link, R.; Lohmeyer, J.; Lafrenz, M.; Gelbmann, C. M.; Hüppe, D.; Niederau, C.; Alshuth U. Safety, tolerability and efficacy of peginterferon alpha-2a and ribavirin in chronic hepatitis C in clinical practice: the German open safety trial. *Journal of Viral Hepatitis*, **2007**, *14*, 788–796.

(5) Jacobson, I. M.; McHutchison, J. G.; Dusheiko, G.; Di Bisceglie, A. M.; Reddy, K. R.; Bzowej, N. H.; Marcellin, P.; Muir, A. J.; Ferenci, P.; Flisiak, R.; George, J.; Rizzetto, M.; Shouval, D.; Sola, R.; Terg, R. A.; Yoshida, E. M.; Adda, N.; Bengtsson, L.; Sankoh, A. J.; Kieffer, T. L.; George, S.; Kauffman, R. S.; Zeuzem, S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N. Engl. J. Med.* **2011**, *364*, 2405-2416.

(6) Kiser, J. J.; Flexner, C. Direct-acting antiviral agents for hepatitis C virus infection. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 427-449.

(7) Tellinghuisen, T. L.; Marcotrigiano, J.; Rice, C. M. Structure of the zinc-binding domain of an essential component of the hepatitis C virus replicase. *Nature* **2005**, *435*, 374-379.

(8) Schmitz, U.; Tan, S.-L. NS5A - from obscurity to new target for HCV therapy. *Recent Pat. Antiinfect. Drug Discov.* **2008**, *3*, 77-92.

(9) Cordek, D. G.; Bechtel, J. T.; Maynard, A. T.; Kazmierski, W. M.; Cameron, C. E. Targeting the NS5A Protein of HCV: an emerging option. *Drugs Future 2011*, **36**, 691–711.

(10) Bukh, J.; Pietschmann, T.; Lohmann, V.; Krieger, N.; Faulk, K.; Engle, R. E.; Govindarajan, S.; Shapiro, M.; St. Claire, M.; Bartenschlager, R. Mutations that permit efficient replication of hepatitis C virus RNA in Huh-7 cells prevent productive replication in chimpanzees. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14416–14421.

(11) Pawlotsky, J.-M. NS5A inhibitors in the treatment of hepatitis C. *J. Hepatol.* **2013**, *59*, 375–382.

(12) DeGoey, D. A.; Betebenner, D. A.; Grampovnik, D. J.; Liu, D.; Pratt, J. K.; Tufano, M. D.; He, W.; Krishnan, P.; Pilot-Matias, T. J.; Marsh, K. C.; Molla, A; Kempf, D. J.; Maring, C. J. Discovery of pyrido[2,3-d]pyrimidine-based inhibitors of HCV NS5A. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3627-3630.

(13) DeGoey, D. A.; Betebenner, D. A.; Grampovnik, D. J.; Iwasaki, N.; Liu, D.; Pratt, J. K.; Tufano, M. D.; He, W.; Krishnan, P.; Pilot-Matias, T. J.; Marsh, K. C.; Molla, A.; Kempf, D. J.; Maring, C. J. Discovery of novel inhibitors of HCV NS5A and proof of concept study in an HCV-infected chimpanzee. 243rd National Meeting of the American Chemical Society, San Diego, CA, March 25th-29th, **2012**, MEDI 169.

(14) Poordad, F; Lawitz, E.; Kowdley, K. V.; Cohen, D. E.;T Podsadecki, T.; Siggelkow, S.; Heckaman, M.; Larsen, L.; Menon, R.; Koev, G.; Tripathi, R.; Pilot-Matias, T.; Bernstein, B. Exploratory study of oral combination antiviral therapy for hepatitis C. *N. Engl. J. Med.* **2013**, *368*, 45-53.

(15) Lim, P. J.; Chatterji, U.; Cordek, D.; Sharma, S. D.; Garcia-Rivera, J. A.; Cameron, C. E.; Lin, K.; Targett-Adams, P.; Gallay, P. A. Correlation between NS5A Dimerization and Hepatitis C Virus Replication. *J. Biol. Chem.* **2012**, *287*, 30861–30873.

(16) Lemm, J. A.; O'Boyle, D.; Liu, M.; Nower, P.T.; Colonno, R.; Deshpande, M.S.; Snyder, L.B.; Martin, S.W.; St Laurent, D.R.; Serrano-Wu, M.H.; Romine, J.L.; Meanwell, N.A.; Gao, M. Identification of hepatitis C virus NS5A inhibitors. *J. Virol.* **2010**, *84*, 482-491.

(17) Gao, M.; Nettles, R. E.; Belema, M.; Snyder, L. B.; Nguyen, V. N.; Fridell,R. A.; Serrano-Wu, M. H.; Langley, D. R.; Sun, J.-H.; O'Boyle II, D. R.; Lemm, J. A.; Wang, C.; Knipe, J. O.; Chien, C.; Colonno, R. C.; Grasela, D. M.; Meanwell, N. A.; Hamann, L. G. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* **2010**, *465*, 96-102.

(18) Lemm, J. A.; Leet, J. E.; O'Boyle II, D. R.; Romine, J. L.; Huang, X. S.; Schroeder, D. R.; Alberts, J.; Cantone, J. L.; Sun, J.-H.; Nower, P. T.; Martin, S. W.; Serrano-Wu, M. H.; Meanwell, N. A.; Snyder, L. B.; Gao, M. Discovery of potent hepatitis C virus NS5A inhibitors with dimeric structures. *Antimicrob. Agents Chemother.* **2011**, *55*, 3795-3802.

(19) Chang, W.; Mosley, R. T.; Bansal, S.; Keilman, M.; Lam, A. M.; Furman, P. A.; Otto, M. J.; Sofia, M. J. Inhibition of hepatitis C virus NS5A by fluoro-olefin based γ-turn mimetics. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2938-2942.

(20) Shi, J.; Zhou, L.; Amblard, F.; Bobeck, D. R.; Zhang, H.; Liu, P.; Bondada, L.; McBrayer, T. R.; Tharnish, P. M.; Whitaker, T.; Coats, S. J.; Schinazi, R. F. Synthesis and biological evaluation of new potent and selective HCV NS5A inhibitors. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3488-3491.

(21) Zhang, H.; Zhou, L.; Amblard, F.; Shi, J.; Bobeck, D. R.; Tao, S.; McBrayer, T. R.; Tharnish, P. M.; Whitaker, T.; Coats, S. J.; Schinazi, R. F. Synthesis and evaluation of novel potent HCV NS5A inhibitors. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4864-4868.

(22) Nevar, N. M.; Kel'in, A. V.; Kulinkovich, O. G. One step preparation of 1,4-diketones from methyl ketones and α-bromomethyl ketones in the presence of $ZnCl_2$●t-BuOH·Et_2NR as a condensation agent. *Synthesis* **2000**, 1259-1262.

(23) Periasamy, M.; Seenivasaperumal, M.; Rao, V. D. Convenient procedures for the asymmetric reduction of 1,4-diphenylbutane-1,4-dione and synthesis of 2,5-diphenylpyrrolidine derivatives. *Synthesis* **2003**, 2507–2510.

(24) Molla, A.; Vasavanonda, S.; Kumar, G.; Sham, H. L.; Johnson, M.; Grabowski, B.; Denissen, J. F.; Kohlbrenner, W.; Plattner, J. J.; Leonard, J. M.; Norbeck, D. W.; Kempf, D. J. Human serum attenuates the activity of protease inhibitors towards wild-type and mutant human immunodeficiency virus. *Virology* **1998**, *250*, 255–262.

(25) Hickman, D.; Vasavanonda, S.; Nequist, G.; Colletti, L.; Kati, W. M.; Bertz, R.; Hsu, A.; Kempf, D. J. Estimation of serum-free 50-percent inhibitory concentrations for human immunodeficiency virus protease inhibitors lopinavir and ritonavir. *Antimicrob. Agents Chemother.* **2004**, *48*, 2911–2917.

(26) Bilello, J. A.; Drusano, G. L. Relevance of plasma protein binding to antiviral activity and clinical efficacy of inhibitors of human immunodeficiency virus protease. *J. Infect. Dis.* **1996**, *173*, 1524-1525.

(27) Fischl, M. A.; Richman, D. D.; Flexner, C.; Para, M. F.; Haubrich, R.; Karim, A.; Yeramian, P.; Holden-Wiltse, J.; Meehan, P. M. Phase I/II study of the toxicity,

pharmacokinetics, and activity of the HIV protease inhibitor SC-52151. *J. Acquir. Immune Defic. Syndr.* **1997**, *15*, 28-34.

(28) Ermondi, G.; Lorenti, M.; Caron, G. Contribution of ionization and lipophilicity to drug binding to albumin: a preliminary step toward biodistribution prediction. *J. Med. Chem.* **2004**, *47*, 3949-3961.

(29) Gleeson, M. P.; Plasma protein binding affinity and its relationship to molecular structure: an in-silico analysis. *J. Med. Chem*. **2007**, *50*, 101-112.

(30) Krishnan, P.; Beyer, J.; Mistry, N.; Koev, G.; Reisch, T.; Mondal, R.; Liu, D.; Pratt, J.; DeGoey, D.; Wagner, R.; Maring, C.; Kati, W.; Molla, A.; Campbell, A.; Bernstein, B.; Williams, L.; Collins, C.; Pilot-Matias, T. Antiviral activity and resistance profiles for ABT-267, a novel HCV NS5A inhibitor, in vitro and during 3-day monotherapy in HCV genotype-1 (GT1)-infected treatment-naïve subjects. Presented at The 63rd Annual Meeting of the American Association for the Study of Liver Diseases, November 9–13, 2012, Boston, Massachusetts.

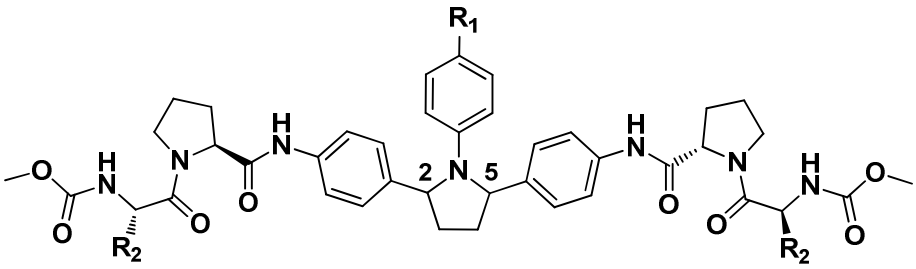
(31) Fridell, R. A.; Qiu, D.; Wang, C.; Valera, L.; Gao, M. Resistance analysis of the hepatitis C virus NS5A inhibitor BMS-790052 in an in vitro replicon system. *Antimicrob. Agents Chemother.* **2010**, *54*, 3641-3650.

(32) Lawitz, E.; Marbury, T.; Campbell, A.; Dumas, E.; Pilot-Matias, T.; Krishnan, P.; Setze, C.; Xie, W.; Podsadecki, T.; Bernstein, B.; Williams, L. Safety and antiviral activity of ABT-267, a novel NS5A inhibitor, during 3-day monotherapy: first study in HCV genotype-1 (GT1)-infected treatment-naïve subjects. Presented at the 47th Annual Meeting of the

European Association for the Study of the Liver (ILC 2012), April 18–22, 2012, Barcelona, Spain.

(33) Mo H.; Lu L.; Pilot-Matias T;. Pithawalla R.; Mondal R.; Masse S.; Dekhtyar T.; Ng T.; Koev G.; Stoll V.; Stewart K. D.; Pratt J.; Donner P.; Rockway T.; Maring C.; Molla A. Mutations conferring resistance to a hepatitis C virus (HCV) RNA-dependent RNA polymerase inhibitor alone or in combination with an HCV serine protease inhibitor in vitro. *Antimicrob. Agents Chemother.* **2005**, *49*, 4305-4314.

Table of Contents Graphic:



N-Phenyl Pyrrolidine NS5A Inhibitors