Viral Specificity in Cell Culture

The antiviral selectivity of BMS-790052 was measured by evaluating its activity against a variety of RNA and DNA viruses, including the closely related pestivirus bovine viral diarrhea virus (BVDV). As shown in Table S1, BMS-790052 was inactive against all ten viruses tested (selectivity ≥1,000,000-fold), suggesting that BMS-790052 is highly selective for HCV.

Table S1: Antiviral Selectivity of BMS-790052

Replicon / Virus	Cell line	BMS-790052 EC_{50} (μM)
HCV Replicon (1b)	Huh-7	0.000009
BVDV Replicon	Huh-7	9
BVDV	MDBK	12
HIV	MT-2	>20
HSV-1, HSV-2	Vero	>11
Influenza	MDBK	>36
CPIV	Vero	26
Human Rhinovirus	MRC-5	>29
Coxsackie virus	MRC-5	>29
Poliovirus	MRC-5	>29
Human Coronavirus	MRC-5	>29

Cytotoxicity in Cell Culture

Cytotoxicity of BMS-790052 was determined in several cell lines derived from various tissue origins. Cells were incubated with compound for up to five days and viability determined by Alamar blue or MTS staining. CC_{50} values were ≥17 μM in all cell lines tested (Table S2).

Table S2: Cytotoxicity of BMS-790052 toward Cells from Various Tissues

Cell line	Tissue Origin	BMS-790052 CC_{50} (μM)
Huh-7	Liver	17
Vero	Kidney	26
MDBK	Kidney	36
MRC-5	Lung fibroblasts	29
MT-2	T lymphocytes	90

Inhibitor Combination Studies

Resistance to antiviral therapy has become a major issue in the management of patients with chronic viral infections. In the case of HCV, the turnover rate is rapid and the virus produces quasi-species, which increases the chance for resistance to emerge. To achieve sustained viral responses in a clinical setting, it will be critical to utilize combination therapies, especially those inhibiting distinct HCV viral targets.

Since clinical drug resistance often develops in viral infections following single-agent therapies, there is a need to assess the additive, antagonistic, or synergistic properties of combination therapies. We used the HCV replicon system to assess the potential use of BMS-790052 in combination therapy with Intron A and inhibitors that target other HCV proteins. Intron A (recombinant IFN-α), an NS3 protease inhibitor, a palm site allosteric inhibitor of the NS5B polymerase and a nucleoside inhibitor of NS5B (4-amino-1-((2*R*,3*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)-3-methyltetrahydro-2-furanyl)-2(1*H*)-pyrimidinone (NM-107)) were tested in combination with BMS-790052.

For the experiments shown in Tables S3-S7, BMS-790052 and other inhibitors were tested as monotherapies and in combination. The potential cytotoxicities of the combined agents were also analyzed in parallel and none found to reach CC_{50} values. The degree of antagonism, additivity, or synergy was determined over a range of drug concentrations, and combination response curves were fit to assess the antiviral effects of the drug treatment combinations. The concentration ratios were analyzed using the method of Chou[24]. The tables report the estimated EC_{50} values for the compounds tested, as well as the combination indices (CI).

Activity of BMS-790052 in Combination with IFN-α

Table S3 summarizes data from the combination of BMS-790052 with Intron A. EC_{50} values for each monotherapy are also presented. In three experiments, BMS-790052 in combination with Intron A yielded synergistic effects at the 50%, 75%, and 90% effective doses. Additivity was observed at the 1:2.5 ratio of Intron A to BMS-790052 at the 50%, 75% and 90% effective doses in experiment 3, and only at the 50% effective dose in experiment 2.

Table S3: Activity of BMS-790052 in Combination with IFN-α

Expt	BMS-790052 EC_{50}, nM	IFN-α EC_{50}, units per mL	Ratio, IFN-α to BMS-790052	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	0.003	216	1:1	0.73 (0.69, 0.77)	0.63 (0.57, 0.68)	0.58 (0.50, 0.66)	Synergy
			2.5:1	0.67 (0.62, 0.72)	0.53 (0.48, 0.59)	0.47 (0.40, 0.54)	Synergy
			1:2.5	0.76 (0.72, 0.80)	0.66 (0.61, 0.72)	0.60 (0.53, 0.68)	Synergy
2	0.003	210	1:1	0.73 (0.68, 0.79)	0.63 (0.57, 0.70)	0.58 (0.49, 0.68)	Synergy
			2.5:1	0.69 (0.62, 0.75)	0.64 (0.56, 0.72)	0.65 (0.52, 0.78)	Synergy
			1:2.5	1.00 (0.92, 1.08)	0.88 (0.79, 0.97)	0.81 (0.68, 0.94)	Synergy/ Additivity
3	0.002	171	1:1	0.72 (0.65, 0.80)	0.73 (0.62, 0.83)	0.77 (0.59, 0.94)	Synergy
			2.5:1	0.88 (0.79, 0.97)	0.75 (0.64, 0.85)	0.67 (0.52, 0.82)	Synergy
			1:2.5	1.11 (0.99, 1.23)	1.06 (0.90, 1.21)	1.04 (0.80, 1.27)	Additivity

Triple Combination Using BMS-790052 with IFN-α and Ribavirin

BMS-790052 was tested in triple drug combination experiments with IFN-α and ribavirin (standard of care), as summarized in Table S4. Additive effects were observed at all effective doses using the triple combination.

Table S4: Triple Combination Using BMS-790052 with IFN-α and Ribavirin

Expt	BMS-790052 EC_{50}, nM	IFN-α EC_{50}, units per ml	Ribavirin EC_{50}, μM	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	0.005	1.4	22.8	1.00 (0.90, 1.11)	0.97 (0.83, 1.11)	0.97 (0.75, 1.19)	Additivity
2	0.004	6.5	49.2	0.99 (0.84, 1.13)	0.86 (0.68, 1.03)	0.76 (0.52, 1.00)	Additivity

Combination of BMS-790052 with a NS3 Inhibitor

The effects of BMS-790052 in combination with the HCV NS3 inhibitor (2*R*,6*S*,13a*S*,14a*R*,16a*S*,*Z*)-6-(tert-butoxycarbonylamino)-14a-(cyclopropylsulfonylcarbamoyl)-5,16-dioxo-1,2,3,5,6,7,8,9,10,11,13a,14,14a,15,16,16a-hexadecahydrocyclopropa[*e*]pyrrolo[1,2-*a*][1,4]diazacyclopentadecin-2-yl 4-fluoroisoindoline-2-carboxylate (ITMN-191)[25] are summarized in Table S5. The EC_{50} for BMS-790052 is higher in these experiments since these combinations were performed in the genotype 1a replicon cells. The results of two experiments indicate mixed additivity/synergy.

Table S5: Combination of BMS-790052 with ITMN-191, a NS3 Protease Inhibitor

Expt	BMS-790052 EC_{50}, nM	ITMN-191 EC_{50}, nM	Ratio, BMS-790052 to NS3 Inhibitor	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	0.031	5.56	1:1	1.15 (0.96, 1.34)	0.99 (0.75, 1.23)	0.85 (0.52, 1.17)	Additivity
			1:2.5	0.94 (0.77, 1.11)	088 (0.66, 1.10)	0.83 (0.51, 1.15)	Additivity
			2.5:1	1.12 (0.89, 1.35)	1.10 (0.80, 1.41)	1.09 (0.62, 1.56)	Additivity
2	0.033	7.41	1:1	1.05 (0.96, 1.14)	1.03 (0.90, 1.16)	1.02 (0.82, 1.23)	Additivity
			1:2.5	0.86 (0.78, 0.95)	0.90 (0.78, 1.03)	0.96 (0.76, 1.16)	Synergy/ Additivity
			2.5:1	0.91 (0.83, 0.99)	0.93 (0.82, 0.1.04)	0.98 (0.80, 1.15)	Synergy/ Additivity

Combination of BMS-790052 with a Palm Site II NS5B Inhibitor

2-(4-fluorophenyl)-5-isopropoxy-N-methyl-6-(methylsulfonamido)benzofuran-3-carboxamide, an allosteric NS5B inhibitor targeting a palm site II on the NS5B polymerase[26], was tested in combination with BMS-790052. The overall results of all three experiments indicate mixed additivity/synergy (Table S6).

Table S6: Combination of BMS-790052 with a Site II NS5B Inhibitor

Expt	BMS-790052 EC_{50}, nM	Palm Site II NS5B Inhib EC_{50}, μM	Ratio, NS5B inhibitor to BMS-790052	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	0.002	0.117	1:1	1.06 (0.97, 1.16)	0.90 (0.78, 1.02)	0.77 (0.60, 0.93)	Synergy/ Additivity
			2.5:1	0.66 (0.59, 0.72)	0.60 (0.52, 0.69)	0.56 (0.44, 0.67)	Synergy
			1:2.5	1.09 (0.99, 1.20)	0.96 (0.84, 1.09)	0.84 (0.67, 1.01)	Additivity
2	0.003	0.137	1:1	1.00 (0.90, 1.09)	0.93 (0.80, 1.06)	0.88 (0.69, 1.06)	Additivity
			2.5:1	0.93 (0.86, 1.00)	0.87 (0.78, 0.97)	0.82 (0.68, 0.97)	Synergy/ Additivity
			1:2.5	0.89 (0.81, 0.97)	0.89 (0.78, 1.00)	0.89 (0.72, 1.05)	Synergy/ Additivity
3	0.002	0.199	1:1	0.93 (0.85, 1.01)	0.88 (0.77, 0.99)	0.84 (0.68, 0.99)	Synergy/ Additivity
			2.5:1	0.94 (0.86, 1.02)	0.85 (0.75, 0.95)	0.78 (0.63, 0.92)	Synergy/ Additivity
			1:2.5	1.00 (0.92, 1.08)	0.95 (0.84, 1.06)	0.90 (0.74, 1.06)	Additivity

Combination of BMS-790052 with a NS5B Nucleoside Inhibitor

BMS-790052 was also tested in combination with 4-amino-1-((2*R*,3*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)-3-methyltetrahydro-2-furanyl)-2(1*H*)-pyrimidinone (NM-107), a NS5B nucleoside analog[27]. In three experiments, synergy/additivity was observed at all three ratios and effective levels (Table S7). The results of all three experiments indicate mixed synergy/additivity.

Table S7: Combination of BMS-790052 with NM-107, a NS5B Nucleoside Inhibitor

Expt	BMS-790052 EC_{50}, nM	NM-107 EC_{50}, μM	Ratio, NS5B inhibitor to BMS-790052	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	0.004	1.00	1:1	0.67 (0.61, 0.73)	0.80 (0.70, 0.89)	0.96 (0.78, 1.13)	Synergy/ Additivity
			2.5:1	0.63 (0.58, 0.68)	0.70 (0.62, 0.79)	0.80 (0.65, 0.95)	Synergy
			1:2.5	0.78 (0.72, 0.85)	0.89 (0.79, 1.00)	1.03 (0.84, 1.21)	Synergy/ Additivity
2	0.003	1.33	1:1	0.99 (0.92, 1.06)	0.92 (0.84, 1.01)	0.86 (0.73, 0.99)	Synergy/ Additivity
			2.5:1	0.95 (0.89, 1.02)	0.89 (0.80, 0.97)	0.83 (0.70, 0.95)	Synergy/ Additivity
			1:2.5	0.97 (0.90, 1.04)	0.93 (0.84, 1.02)	0.90 (0.76, 1.04)	Additivity
3	0.004	0.96	1:1	0.49 (0.44, 0.55)	0.60 (0.50, 0.70)	0.74 (0.56, 0.93)	Synergy
			2.5:1	0.57 (0.51, 0.63)	0.66 (0.56, 0.76)	0.79 (0.61, 0.98)	Synergy
			1:2.5	0.48 (0.43, 0.53)	0.62 (0.53, 0.71)	0.80 (0.61, 0.98)	Synergy

These results demonstrate that combination treatment of replicon cells with BMS-790052 and IFN-α +/- ribavirin, or inhibitors targeting the HCV NS3 protease or NS5B polymerase, yield additive to synergistic antiviral effects. Importantly, neither antagonism of antiviral activity, nor enhancement of cytotoxicity was observed with any of these combinations. The ability to use BMS-790052 in combination therapy can provide major advantages over single drug therapy for the treatment of HCV.

Inhibition of Replicon Replication in Different Cell Types

The HCV genome displays very restricted tissue and host tropism. To date, efficient replication of genotype 1a and 1b replicons with cell culture adaptive mutations is mainly observed in the human hepatoma cell line Huh-7. To ensure that the inhibition of HCV replication by NS5A inhibitors is not an artifact of the Huh-7 cell line, the potency of BMS-790052 was evaluated in transient replicon assays using a genotype 2a (JFH-1) replicon, whose replication does not require adaptive mutations, in Huh-7, HeLa and HEK 293T cells. An HCV NS3 protease inhibitor was used as a positive control. As shown in Table S8, the potency of BMS-790052 is similar in all 3 cell types with EC_{50} values ranging from 3.5 to 20 pM, suggesting that the function(s) of NS5A inhibited by BMS-790052 are highly conserved in different cell environments.

Table S8: Potency of BMS-790052 on JFH-1 Replicon in Different Cell Types

Cell Types	HeLa	HEK 293T	Huh.7
BMS-790052, EC_{50} (pM)	3.7 ± 3.3	3.5 ± 1.9	20 ± 5.7
HCV NS3 protease inhibitor, EC_{50} (μM)	0.14 ± 0.05	0.09 ± 0.05	0.04 ± 0.02

Cyclosporin A (CsA) is equally potent against HCV genotype 1a wild type replicon and replicons containing NS5A substitutions associated with BMS-790052 resistance.

To date, all substitutions causing resistance to BMS-790052 and other BMS NS5A inhibitors have been mapped to domain I of the N-terminal 100 amino acids of NS5A. Cyclosporin A (CsA) and related inhibitors can inhibit HCV replication *in vitro* and *in vivo*[15]. It has been reported that substitutions causing resistance to CsA map to HCV NS5B polymerase and to NS5A domains II and III[15].

To determine if there is cross-resistance between NS5A inhibitors and CsA, we tested the inhibition by CsA of the replication of HCV genotype 1a wild-type replicon cells and replicon cells containing amino acid substitutions associated with resistance to BMS-790052. Replicon cell lines were generated. The HCV genotype 1a mutant replicons show various levels of resistance to BMS-790052 but they are equally sensitive to CsA compared to the wild type 1a replicon (Table S9). Significant variations in cytotoxicity in different replicon cell lines may be due to different expression levels of cellular factor(s) which have been reported to be present in individual cell lines and may modulate CsA toxicity[28].

Table S9. HCV inhibitory effect of BMS-790052 and CsA on wild-type and resistant replicon cell lines

1a replicon [a]	BMS-790052 EC_{50} (μM) [b]	CsA EC_{50} ± SD (μM) [c]	CsA CC_{50} ± SD (μM) [c]
WT 1a	0.0000049	0.23 ± 0.02	3.03 ± 0.96
L31V	0.021	0.31 ± 0.07	>20
M28T	0.0041	0.29 ± 0.02	>20
Q30H	0.0097	0.27 ± 0.02	>20
Q30R	0.0062	0.29 ± 0.05	>20
Y93H	0.029	0.32 ± 0.02	5.19 ± 2.12

[a] WT = wild type. The HCV NS5A mutant is referred to by the amino acid number with observed change from wild-type listed last.

[b] Transient assays.

[c] Stable cell lines assays.

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CLINICAL STUDIES

Clinical Study Population

Subjects selected for this study included men and women, aged 18 to 49 years, inclusive, who were chronically infected with HCV genotype 1 and were:

- treatment naive subjects; or
- treatment non-responders, defined as subjects who received the current standard of care (interferon and/or ribavirin) and continued to have a detectable HCV RNA level (including relapsers) or subjects who did not attain a 2 $\log_{10}$ decline in HCV RNA levels at 12 weeks and stopped treatment; or
- treatment-intolerant subjects, defined as subjects who were unable to tolerate the toxicities associated with interferon and/or ribavirin; and
- had not received another NS5A replication co-factor inhibitor; and
- not co-infected with HIV, HBV, or HCV other than genotype 1.

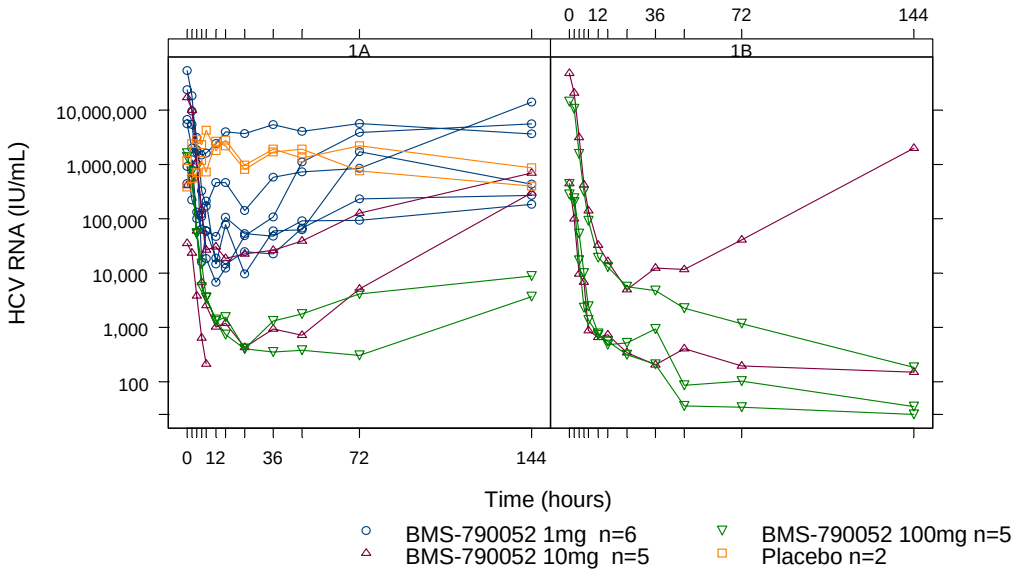
Eligible subjects also had a HCV RNA viral load of $\geq 10^5$ IU/mL, a documented FibroSure™ score of $\leq$ 0.59 and aspartate aminotransferase (AST) to platelet ratio index (APRI) $\leq$ 2, and a body mass index (BMI) of 18 to 35 kg/m^2, inclusive. All subjects signed the informed consent form, and were screened and determined to be eligible based on medical history, physical examination and measurements, vital signs, a 12-lead ECG, and clinical laboratory measurements including serology.

BMS-790052 was dosed in a vehicle contained 33.3% Simple Syrup and 66.6% 0.1 M citrate buffer. Placebo recipients received the vehicle.

Table S10. Individual HCV RNA and Change from Baseline at Specific Timepoints

			Pre-Dose	Post-Dose	
Treatment	**Subject**	**Genotype Subtype**	**Baseline HCV RNA at 0 hours (log_{10} IU/mL)**	**Change in HCV RNA at 24 hours (log_{10} IU/mL)**	**Change in HCV RNA at 144 hours (log_{10} IU/mL)**
1 mg	1-17	1a	7.37	-2.22	-0.23
	1-20	1a	6.75	-0.18	-0.19
	3-16	1a	5.96	-1.57	-0.53
	10-6	1a	6.83	-2.14	-0.08
	10-10	1a	5.65	-1.67	-0.01
	10-28	1a	7.73	-3.00	-2.46
10 mg	1-43	1a	4.55	unavailable	unavailable
	1-44	1a	7.24	-2.88	-1.39
	3-23	1a	5.61	-2.98	-0.13
	1-42	1b	5.65	-3.12	-3.47
	7-36	1b	7.68	-3.99	-1.38
100 mg	3-69	1a	6.14	-3.53	-2.57
	10-53	1a	6.20	-3.60	-2.26
	3-83	1b	5.45	-2.73	-3.90
	10-8	1b	5.64	-3.14	-4.24
	10-68	1b	7.15	-3.40	-4.89
Placebo	3-31	1a	6.07	-0.09	-0.14
	10-63	1a	5.58	0.32	0.01

Figure S1: Individual HCV RNA Over Time by Genotype Subtype



Single Ascending Dose Study in HCV Infected Subjects: *In vivo* Variant Analysis

Viral RNA was isolated from patient serum with a QIA Amp MiniElute Viral Vaccum kit (Qiagen, Inc. Valencia, CA). First strand cDNA was synthesized from random hexamer primers with a SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen Corporation, Carslbad, CA). The NS5A coding region was amplified with genotype specific primers selected from those listed in Table S11.

Table S11. Primers employed for PCR amplification of NS5A

Forward	**GENOTYPE 1a**	
name	**Primer Sequence (5'-3')**	**Position**
BF199	GACCAACTGGCAGAAACTCGAGG	5594
UP6003	TCGCCTGGAGCCCTTGTAGT	6003
UP6014	CCTTGTAGTCGGTGTGGTCTGCGC	6014
BF399-1a	GCGCCGGCACGTTGGCCCGGGCGAGGGGGCAGTGCAATGGATGAACCGG	6046
1AUP6070A	AGGGGGCAGTGCAATGGATGAACC	6070
1a-US1	GAACCATGTTTCCCCCACGCACTACG	6119
BF400-1a	TCCCCCACGCACTACGTGCCGGAGAGCGATGCAGCCGCCCGCGTCAC	6129
UP6161	AGCCGCCCGCGTCACTGCCATACT	6161
BF205	CAGCAGCCTCACTGTAACCCAGCTC	6185
MP9	AAGCTCGGAGTGTACCACTCCATG	6233
MP11	AGCCAGCTGTCCGCTCCATCTCTCAAGGC	6951
Reverse		
BF206	GTAGTCATACCCGATACGTAGTGG	6661
BF200-1a	CCACAGGAGGTTAGCCTCTATGAG	7040
BF402-1a	GCAGTCTGTCAAATGTGACTTTCTTCTGCCTTTGGCAAGCAC	7773
DQ30	CGTCCTGGTAATGGCTGTCCAGAAC	7801
BF401-1a	GCTTTGACCTCCTTGAGCACGTCCTGGTAATGGCTGTCCAGAAC	7821
LOW8091	CGGGGAACACGATGAGACGAG	8091
LOW8202	CCCGCTGTCCTGGTGAGTATTGG	8202
DQ31	CGAGGAATTCAACCCGCTGTCC	8214
LOW8383	GCCCCCAACATAAAGCCTCTCA	8393
LOW8506A	GCTGCCCGGGCCTTGATGTAG	8506
3'UTR-286	CAGTCATGCGGCTCACGGACCTT	9562
Forward	**GENOTYPE 1b**	
BR906	AGCCCGCTCACCACCCAAAATACC	5724
UP5800	GCGCTGCTTCGGCTTTCGTG	5800
UP6004	TGGTCGTCGGGGTCGTGT	6004
DQ29	GGCTGATAGCGTTCGCTTCGCG	6082
BR907	CGTGGCTAAGAGATGTTTGGG	6246
BR908	CTGTGGCGGGTGGCTGCTG	6573
BR909	GCGGCAGGAGATGGGCGGGAACAT	7028
BR910	ACGGAGGAAGAGGACGGTTGT	7310
Reverse		
BF19	CCACTACGTGACGGGCATGAC	6646

BF20	GGTTGGCCTCGATGAGGTCAGCG	7020
BF21	GCCGAAGGTCTTTGTGGCGAGC	7388
BF22	TCCTCCGCAGCGCATGGCGTG	7639
LOW7817	TGTGGACGCCTTCGCCTTCATCTC	7817
LOW7995	TGGTGTCAATTGGTGTCTCAGTGT	7995
LOW8052	GCTTGCGGCCTCCTTTCTCTGG	8052
LOW8232	CCATAGGGCTTTTCTTTGATTTC	8232

Genotypic analysis was performed by population sequencing (providing that HCV RNA levels were >1,000 IU/mL) to determine the emergence of viral variants following the administration of single doses of BMS-790052. Total RNA was isolated and viral sequences amplified from serum samples collected from HCV-infected subjects in the three cohorts (1, 10 and 100 mg of BMS-790052).

Population sequencing results from samples derived at baseline (T0), 24 (T24) and 144 (T144) hours post-dose are summarized in Tables S12-S14. The RNA changes at these timepoints relative to baseline are presented in the Table S10. To determine the relative sensitivity of detecting sequence variations, reconstitution experiments were performed with mixtures of wild-type and resistance-containing DNA (Y93H). Mixtures of wild-type and Y93H (tyrosine residue 93 changed to histidine) DNA at ratios of 100:0, 95:5, 90:10, 80:20 and 60:40 were sequenced. The experiment revealed that 20% of the Y93H mutant could readily be detected in the wild-type population (results not shown).

Table S12. Genotypic Analysis of Cohorts Treated with 1 mg BMS-790052

Subject #	Genotype	Time	M28 (1a)	Q30 (1a)	L31	Y93
1-20	1a	T0			M (~100%)[a]	
		T24				
		T144				
1-17	1a	T0	no variants detected			
		T24				
		T144				
3-16	1a	T0	no variants detected			
		T24				
		T144				
10-6	1a	T0	no variants detected			
		T24				
		T144				
10-10	1a	T0	no variants detected			
		T24				
		T144				
10-28	1a	T0	no variants detected			
		T24	Further analysis is needed[b]			
		T144	no variants detected			

[a] Parentheses indicate the % of resistance estimated from population sequencing chromatograms
[b] Variable substitutions observed from different primers

Table S13. Genotypic Analysis of Cohorts Treated with 10 mg BMS-790052

Subject #	Genotype	Time	M28 (1a)	Q30 (1a)	L31	Y93
1-44	1a	T0	no variants detected			
		T24	T (~50%)	R (<20%)[a]		
		T144	T (~10-20%)			
3-23	1a	T0	no variants detected			
		T24	n.a.[b]			
		T144		R (~20%)		
7-36	1b	T0	no variants detected			
		T24				H (~50%)
		T144				H (~80%)
1-42	1b	T0	100% R30Q, Q is wild type for 1a			
		T24	n.a.			
		T144				
1-43		Withdrew from the study				

[a] Parentheses indicates the % of resistance estimated from population sequencing chromatograms
[b] n.a.: not analyzed - HCV RNA <1,000 IU/mL

Table S14. Genotypic Analysis of Cohorts Treated with 100 mg BMS-790052

Subject #	Genotype	Time	M28 (1a)	Q30 (1a)	L31	Y93
3-69	1a	T0	no variants detected			
		T24	n.a.[b]			
		T144		H (~20%)[a]	M (~30%)	
10-53	1a	T0	no variants detected			
		T24	n.a.			
		T144	T (~30%)			
3-83	1b	T0	no variants detected			
		T24	n.a.			
		T144				
10-68	1b	T0	no variants detected			
		T24				
		T144	n.a.			
10-8	1b	T0	no variants detected			
		T24	n.a.			
		T144				

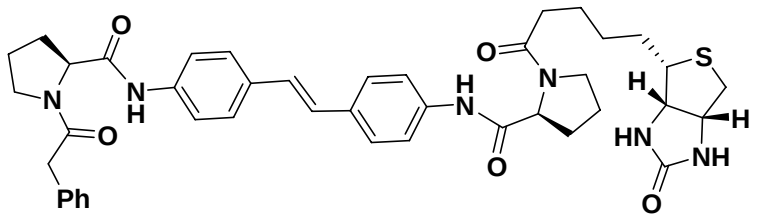
[a] Parentheses indicate the % of resistance estimated from population sequencing chromatograms

[b] n.a.: not analyzed - HCV RNA <1,000 IU/mL

CHEMISTRY EXPERIMENTAL

General note: All solvents were obtained from commercial sources and used without further drying and/or purification. Starting materials were either purchased from commercial sources or prepared according to the noted literature protocols. ^{1}H-NMR spectra were recorded using Bruker 300, 400 or 500 MHz instruments and ^{13}C-NMR spectra were recorded using a Bruker 125 MHz instrument. Chemical shifts were assigned in ppm relative to respective solvent signals (e.g., signal of residual $CHCl_3$ present in $CDCl_3$ for ^{1}H-NMR). The following abbreviations are used to describe signal splitting patterns: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br).

(2*S*)-1-(5-((3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoyl)-*N*-(4-((*E*)-2-(4-((((2*S*)-1-(phenylacetyl)-2-pyrrolidinyl)carbonyl)amino)phenyl)vinyl)phenyl)-2-pyrrolidinecarboxamide (1)

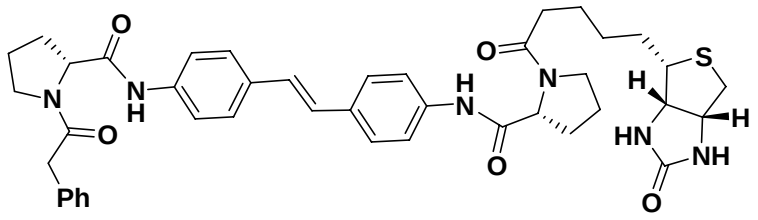


Ethyl 2-ethoxyquinoline-1(2*H*)-carboxylate (1.29 g, 5.23 mmol) was added in one portion to a stirred solution of 4,4'-((*E*)-1,2-ethenediyl)dianiline (0.50 g, 2.38 mmol), Boc-L-proline (0.51 g, 2.38 mmol) and 1-(phenylacetyl)-L-proline (prepared according to the procedure described by T.A. Gudasheva *et al*., *Eur. J. Med. Chem.*, **31**, 151-157 (1996)) (0.55 g, 2.38 mmol) in dry CH_2Cl_2 (30 mL) and stirring was continued at room temperature for 2 hr to afford a statistical mixture of three products. The mixture was treated with CF_3CO_2H (5 mL), stirred for 1 hr at -10 °C before adding 4N HCl solution in dioxane (10 mL) and stirring was continued for an additional 2 hr. The mixture was concentrated *in vacuo*, the residue dissolved in a 1:1 mixture of DMSO and MeOH and purified using reverse phase HPLC (Phenomenex Luna C18; H_2O/MeOH/CF_3CO_2H). The desired fractions were concentrated and the residue taken up in CH_2Cl_2 and washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated *in vacuo* to afford (2*S*)-1-(phenylacetyl)-*N*-(4-((*E*)-2-(4-(((2*S*)-2-pyrrolidinylcarbonyl)amino) phenyl)vinyl)phenyl)-2-pyrrolidinecarboxamide as an off-white solid (440 mg, 35%). ^{1}H-NMR (500 MHz, DMSO-d_6, δ = 2.5 ppm) δ 10.25, 10.02 and 9.97 (3s, 2H), 7.67-7.65 (m, 2H), 7.62-7.58 (m, 2H), 7.55-7.50 (m, 4H), 7.34-7.18 (m, 5H), 7.11-7.09 (m, 2H), 4.66-4.64 and 4.46-4.44 (m, 1H), 3.73-3.39 (series of m, 5H), 3.14-3.00 (br m, 1H), 2.93-2.86 (m, 2H), 2.37-2.00 (m, 3H),

1.94-1.73 (m, 3H), 1.68-1.63 (m, 2H). HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{32}H_{35}N_4O_3$, 523.2709; found, 523.2696.

HATU (128 mg, 0.34 mmol) was added in one portion to a stirred solution of (2*S*)-1-(phenylacetyl)-*N*-(4-((*E*)-2-(4-(((2*S*)-2-pyrrolidinylcarbonyl)amino) phenyl)vinyl)phenyl)-2-pyrrolidinecarboxamide, (+)-biotin (82 mg, 0.34 mmol) and *i*-Pr_2NEt (80 µL, 0.46 mmol) in DMF (3 mL) and the mixture stirred at room temperature for 1 hr. The mixture was concentrated, the residue dissolved in MeOH and subjected to purification by reverse phase HPLC (Phenomenex Luna column S5; H_2O/MeOH/CF_3CO_2H) to afford compound **1** as a light tan solid (114 mg, 47% yield) and an impure batch which was purified under alternative reverse phase HPLC conditions (Waters-SunFire column S5; CH_3CN/H_2O/CF_3CO_2H) to afford an additional sample of **1** as a white solid (44 mg, 19% yield). ^{1}H-NMR (500 MHz, DMSO-d_6, δ = 2.5 ppm): δ 10.29, 10.23, 10.06, 10.04 (4s, 2H), 7.68-7.60 (m, 4H), 7.53-7.48 (m, 4H), 7.33-7.17 (m, 5H), 7.12-7.10 (m, 2H), 6.43 (br m, 1.7H), 4.69-4.07 (series of m, 4H), 3.75-3.37 (series of m, 6H), 3.15-2.99 (m, 1H), 2.87-2.76 (m, 1H), 2.64-2.54 (m, 1H), 2.39-2.25 (m, 2H), 2.20-2.09 (m, 2H), 2.07-1.95 (m, 2H), 1.94-1.78 (m, 4H), 1.70-1.62 (m, 1H), 1.60-1.44 (m, 3H), 1.42-1.20 (m, 2H). HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{42}H_{49}N_6O_5S$: 749.3480, found, 749.3482. The two samples were 97.4-97.7% pure when analyzed under the following reverse phase HPLC conditions: column: Waters Acquity BEH C18, 1.7 µM; 2.1 x 150 mm; temp. = 35°C; flow rate: 0.35 mL/min; wavelength: 338 nM; solvent-A: H_2O with 0.05% CF_3CO_2H, and solvent-B: CH_3CN with 0.05% CF_3CO_2H.

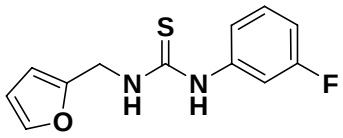
(2*R*)-1-(5-((3aS,4S,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)pentanoyl)-*N*-(4-((*E*)-2-(4-((((2*R*)-1-(phenylacetyl)-2-pyrrolidinyl)carbonyl)amino)phenyl)vinyl)phenyl)-2-pyrrolidinecarboxamide (2)



Compound **2** was prepared as an off-white solid according to the procedures described for the diastereomer **1** by employing the appropriate starting materials. ^{1}H-NMR (500 MHz, DMSO-d_6, δ = 2.5 ppm): δ 10.27, 10.19, 10.05, 10.03 (4s, 2H), 7.65-7.59 (m, 4H), 7.56-7.51 (m, 4H), 7.35-7.20 (m, 5H), 7.13-7.10 (m, 2H), 6.43 and 6.37 (br m, 2H), 4.52-4.01 (series of m, 4H), 3.75-3.39 (series of m, 6H), 3.13-3.00 (m, 1H), 2.85-2.74 (m, 1H), 2.65-2.54 (m, 1H), 2.37-2.30 (m, 2H), 2.22-2.10 (m, 2H), 2.08-1.96 (m, 2H), 1.94-1.76 (m, 4H), 1.67-1.27 (series of m, 6H). HRMS

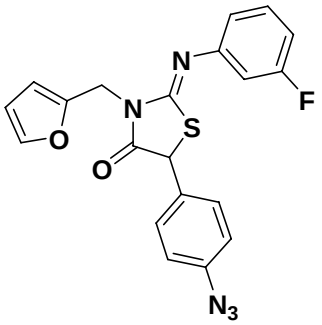
(*m*/*z*): $[M+H]^+$ calcd for $C_{42}H_{49}N_6O_5S$, 749.3480; found, 749.3484. The sample was 95.6% pure when analyzed under the following reverse phase HPLC conditions: column: Waters Acquity BEH C18, 1.7 μM; 2.1 x 150 mm; temp. = 35°C; flow rate: 0.35 mL/min; wavelength: 340 nM; solvent-A: 30 mM $(NH_4)_2CO_3$ in H_2O, pH = 10.0, and solvent-B: CH_3CN.

1-(3-Fluorophenyl)-3-furan-2-ylmethyl-thiourea (3)



Furfurylamine (6.34 g, 65 mmol) in CH_2Cl_2 (25 mL) was added dropwise to a solution of 3-fluorophenyl isothiocyanate (10.0 g, 65 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred at room temperature for 20 hours, concentrated, and subjected to chromatography on silica gel using 30-45% EtOAc/hexanes as eluant to afford thiourea **3** as an oil which solidified on standing (15.4 g, 94%). Trituration with hexanes afforded a sample with m.p. = 79-80 ^oC. ^1H-NMR (300 MHz, $CDCl_3$) δ 7.98 (br s, 1H), 7.41-7.34 (m, 2H), 7.00-6.93 (m, 3H), 6.34-6.29 (m, 3H), 4.86 (d, *J* = 5.1 Hz, 2H); MS (*m/z*): $[M+H]^+$ = 251.0.

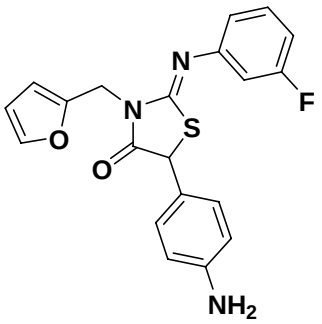
(2*Z*)-5-(4-Azidophenyl)-2-((3-fluorophenyl)imino)-3-(2-furylmethyl)-1,3-thiazolidin-4-one (4)



Ethyl 2-(4-azidophenyl)-2-bromoacetate (prepared according to G. Campiani *et al.*, *J. Med. Chem*, **39**, 3435-3450 (1996)) (800 mg, 2.83 mmol) and thiourea **3** (706 mg, 2.83 mmol) were dissolved in absolute EtOH (70 mL), NaOAc (3.0 g, 36.6 mmol) was added, and the mixture heated at 70°C for 4.5 hr. After being cooled, the solvent was partially removed by rotary evaporation (1/3 volume) before being diluted with EtOAc (2 vol). After washing with H_2O, the aqueous layer was extracted with Et_2O, the combined organic layer was washed with brine and concentrated. The crude product was subjected to chromatography on silica gel, eluting with 10% EtOAc/hexanes, to afford azide **4** as a yellow oil (700 mg, 64%). ^1H-NMR (300 MHz, $CDCl_3$)

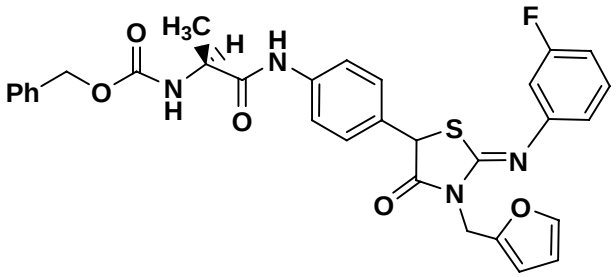
δ 7.38-7.37 (m, 1H), 7.33-7.27 (m, 3H), 7.02-6.98 (m, 2H), 6.87-6.77 (m, 2H), 6.74 (dt, *J* = 9.9 Hz, *J'* = 2.2 Hz, 1H), 6.40 (d, *J* = 3.3 Hz, 1H), 6.35-6.33 (m, 1H), 5.15 (s, 1H), 5.12-5.00 (m, 2H); MS (*m/z*): $[M+H]^+$ = 408.0.

(2*Z*)-5-(4-Aminophenyl)-2-((3-fluorophenyl)imino)-3-(2-furylmethyl)-1,3-thiazolidin-4-one (5)



Tin (II) chloride dihydrate (1.53 g, 6.78 mmol) was added to a solution of azide **4** (913 mg, 2.24 mmol) in EtOAc (200 mL) and the reaction mixture heated at 60°C for 2 hr. After being cooled, a precipitate was removed by filtration and washed with EtOAc. The filtrate was washed with 20% saturated $NaHCO_3$ solution and the aqueous phase extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated to afford aniline **5** as an amber residue (831 mg, 97%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.37-7.36 (m, 1H), 7.31-7.23 (m, 1H), 7.09-7.05 (m, 2H), 6.85-6.76 (m, 2H), 6.72 (dt, *J* = 9.9 Hz, *J'* = 2.2 Hz, 1H), 6.39 (d, J = 2.9 Hz, 1H), 6.65 -6.61 (m, 2H), 6.34-6.32 (m, 1H), 5.11-4.99 (m, 3H); MS (*m/z*): $[M+H]^+$ = 382.1.

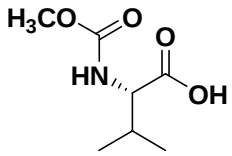
Benzyl ((1*S*)-2-((4-((2*Z*)-2-((3-fluorophenyl)imino)-3-(2-furylmethyl)-4-oxo-1,3-thiazolidin-5-yl)phenyl)amino)-1-methyl-2-oxoethyl)carbamate (BMS-858)



A solution of carbobenzyloxy-L-alanine (480 mg, 2.16 mmol) and oxalyl chloride (1.0 mL of 2M in CH_2Cl_2, 2.0 mmol) in CH_2Cl_2 (15 mL) was stirred under an atmosphere of N_2 for 30 min. The resulting acid chloride was added dropwise to a solution of aniline **5** (327 mg, 0.86 mmol)

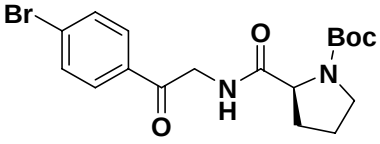
and 4-methylmorpholine (0.25 ml, 2.16 mmol) in CH_2Cl_2 (30 mL), and stirred for 1 hr. The mixture was poured onto H_2O, diluted with CH_2Cl_2, and the organic phase washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Purification by flash chromatography (silica gel; 15% EtOAc/CH_2Cl_2 as eluant) afforded BMS-858 as a foam (231 mg, 46%). ^{1}H-NMR (500 MHz, CD_3CN) δ 8.65 (br s, 1H), 7.58 (d, J = 8.2 Hz, 2H), 7.48 (s, 1H), 7.44-7.25 (m, 8H), 6.92 (dt, J = 8.6 Hz, J' = 2.4 Hz, 1H), 6.84 (d, J = 7.9 Hz, 1H), 6.78 (td, J = 10.4 Hz, J' = 2.4 Hz, 1H), 6.42 (m, 2H), 6.09 (br s, 1H), 5.32 (s, 1H), 5.11 (br m, 2H), 5.02 (s, 1H), 4.25 (m, 1H), 1.39 (d, J = 7.0 Hz, 3H); ^{13}C-NMR (125 MHz, CD_3CN) δ 173.1, 171.9, 163.6 (J_{CF} = 244.9 Hz), 156.5, 154.5, 150.4 (J_{CF} = 9.4 Hz), 149.8, 142.9, 139.4, 137.5, 131.6, 131.4, 131.3, 129.4, 128.9, 128.3 (J_{CF} = 22.1 Hz), 120.5, 117.4 (J_{CF} = 2.2 Hz), 111.5 (J_{CF} = 21.3 Hz), 111.1, 109.1, 108.7 (J_{CF} = 22.3 Hz), 66.7, 51.9, 51.5, 39.8, 17.8; IR (film, NaCl) 3321.0 (broad NH) 1724.6, 1685.1, 1638.1, 1602.4, 1535.1, 1515.6 cm^{-1}. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{28}FN_4O_5S$, 587.1759; found, 587.1754.

_N_-(Methoxycarbonyl)-L-valine (6)



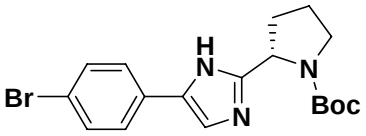
Na_2CO_3 (1.83 g, 17.2 mmol) was added to a solution of L-valine (3.9 g, 33.29 mmol) in NaOH solution (33 mL of 1M/H_2O, 33 mmol) and the mixture cooled with an ice-water bath. Methyl chloroformate (2.8 mL, 36.1 mmol) was added drop-wise over 15 min, the cooling bath was removed and the reaction mixture stirred at ambient temperature for 3.25 hr. The reaction mixture was washed with Et_2O (50 mL, 3x), the aqueous phase was cooled in an ice-water bath and acidified with concentrated HCl until pH = 1-2. The mixture was extracted with CH_2Cl_2 (3 x 50 mL) and the combined organic phase dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford crude acid **6** as a white solid (6.0 g), used without further purification. ^{1}H-NMR for the dominant rotamer (500 MHz, DMSO-d_6, δ = 2.5 ppm): δ 12.54 (s, 1H), 7.33 (d, J = 8.6 Hz, 1H), 3.84 (dd, J = 8.4 Hz, J' = 6.0 Hz, 6.0, 1H), 3.54 (s, 3H), 2.03 (m, 1H), 0.87 (m, 6H). HRMS (*m/z*): $[M+H]^+$ calcd for $C_7H_{14}NO_4$, 176.0923; found, 176.0922.

_N_-(2-(4-Bromophenyl)-2-oxoethyl)-1-(*tert*-butoxycarbonyl)-L-prolinamide (7)



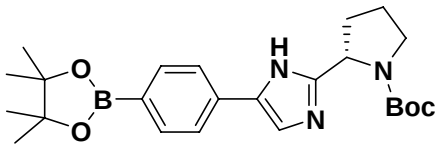
N,N-Diisopropylethylamine (18.0 mL, 103 mmol) was added dropwise over 15 min to a heterogeneous mixture of *N*-Boc-L-proline (7.14 g, 33.17 mmol), HATU (13.33 g, 35.04 mmol), the HCl salt of 2-amino-1-(4-bromophenyl)ethanone (8.13 g, 32.44 mmol) and DMF (105 mL) and the mixture stirred at ambient temperature for 55 min. Most of the volatile component was removed *in vacuo* and the resulting residue was partitioned between EtOAc (300 mL) and H_2O (200 mL). The organic layer was washed with H_2O (200 mL) and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. A silica gel mesh was prepared from the residue and submitted to flash chromatography (silica gel; 50-60% EtOAc/hexanes as eluant) to provide ketoamide **7** as a white solid (12.8 g; 96% yield). ^{1}H-NMR (400 MHz, DMSO-d_6, δ = 2.5 ppm): δ 8.25-8.14 (m, 1H), 7.92 (br d, *J* = 8.0 Hz, 2H), 7.75 (br d, *J* = 8.6 Hz, 2H), 4.61 (dd, *J* = 18.3 Hz, *J'* = 5.7 Hz, 1H), 4.53 (dd, *J* = 18.1 Hz, *J'* = 5.6 Hz, 1H), 4.22-4.12 (m, 1H), 3.43-3.35 (m, 1H), 3.30-3.23 (m, 1H), 2.18-2.20 (m, 1H), 1.90-1.70 (m, 3H), 1.40/1.34 (two app br s, 9H). LC/MS: $[M+Na]^+$ = 433.09.

tert-Butyl (2S)-2-(4-(4-bromophenyl)-1*H*-imidazol-2-yl)-1-pyrrolidinecarboxylate (8)



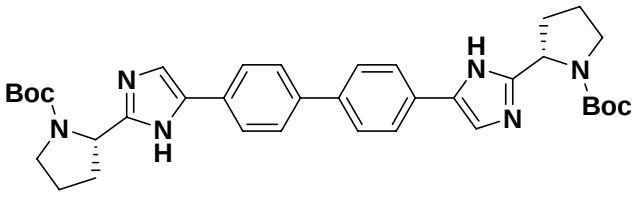
A mixture of ketoamide **7** (12.8 g, 31.12 mmol) and NH_4OAc (12.0 g, 155.7 mmol) in xylenes (155 mL) was heated in a sealed tube at 140 °C for 2 hr. The volatile component was removed *in vacuo*, the residue partitioned carefully between EtOAc and H_2O, and saturated $NaHCO_3$ solution added carefully with stirring until the pH of the aqueous phase was slightly basic. The layers were separated, the aqueous layer was extracted with EtOAc and the combined organic phase washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting material was recrystallized from EtOAc/hexanes to provide two crops of imidazole **8** as a dense light yellow solid (5.85 g). The mother liquor was concentrated *in vacuo* and subjected to a flash chromatography (silica gel; 30% EtOAc/hexanes as eluant) to provide an additional 2.23 g of imidazole **8** (66% combined yield). ^{1}H-NMR (400 MHz, DMSO-d_6, δ = 2.5 ppm): δ 12.17/11.92/11.86 (m, 1H), 7.72-7.46/7.28 (m, 5H), 4.86-4.70 (m, 1H), 3.52 (app br s, 1H), 3.36 (m, 1H), 2.30-1.75 (m, 4H), 1.40/1.15 (app br s, 9H). HRMS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{23}BrN_3O_2$, 392.0974; found, 392.0959. The optical purities of the two samples of imidazole **8** were assessed under the following chiral HPLC conditions: column (Chiralpak AD, 10 µm, 4.6 x 50 mm); solvent (2% EtOH/heptane, isocratic); flow rate (1 mL/min); wavelength (either 220 nm or 254 nm); relative retention time [2.83 min for (*R*)-isomer, 5.34 min for (*S*)-isomer]. Result: ee >99% for the combined crops; ee = 96.7% for the sample obtained from flash chromatography.

(*S*)-*tert*-Butyl 2-(5-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate (9)



$Pd(Ph_3P)_4$ (469 mg, 0.406 mmol) was added to a pressure tube containing a mixture of bromide **8** (4.01 g, 10.22 mmol), bis(pinacolato)diboron (5.42 g, 21.35 mmol), KOAc (2.57 g, 26.21 mmol) and 1,4-dioxane (80 mL). The reaction flask was purged with N_2, capped and heated in an oil bath at 80 °C for 16.5 hr. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The crude material was partitioned carefully between CH_2Cl_2 (150 mL) and an aqueous medium comprising 50 mL H_2O and 10 mL saturated $NaHCO_3$ solution. The aqueous layer was extracted with CH_2Cl_2, the combined organic phase dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting material was purified using flash chromatography (the sample was loaded with the eluting solvent: 20-35% EtOAc/CH_2Cl_2) to provide boronate **9**, contaminated with pinacol, as a dense, off-white solid (3.93 g); the relative mole ratio of boronate **9** to pinacol was ~10:1 according to ^{1}H-NMR analysis and the sample was submitted to the next step without further purification. ^{1}H-NMR (400 MHz, DMSO-d_6, δ = 2.5 ppm): δ 12.22/11.94/ 11.87 (m, 1H), 7.79-7.50/ 7.34-7.27 (m, 5H), 4.86-4.70 (m, 1H), 3.52 (app br s, 1H), 3.36 (m, 1H), 2.27-1.77 (m, 4H), 1.45-1.10 (m, 21H). LC/MS: $[M+H]^+$ = 440.23.

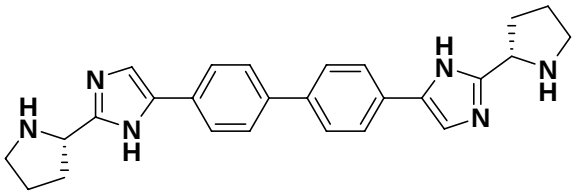
***tert*-Butyl (2*S*)-2-(4-(4'-(2-((2*S*)-1-(*tert*-butoxycarbonyl)-2-pyrrolidinyl)-1*H*-imidazol-5-yl)-4-biphenylyl)-1*H*-imidazol-2-yl)-1-pyrrolidinecarboxylate (10)**



$Pd(Ph_3P)_4$ (59.9 mg, 0.0518 mmol) was added to a mixture of bromide **8** (576.1 mg, 1.469 mmol), boronate **9** (621.8 mg, 1.415 mmol), $NaHCO_3$ (400.4 mg, 4.766 mmol) in 1,2-dimethoxyethane (12 mL) and H_2O (4 mL). The reaction mixture was flushed with N_2 and heated with an oil bath at 80 °C for 5.75 hr before the volatile component was removed *in vacuo*. The residue was partitioned between 20% MeOH/$CHCl_3$ (60 mL) and H_2O (30 mL), and the aqueous

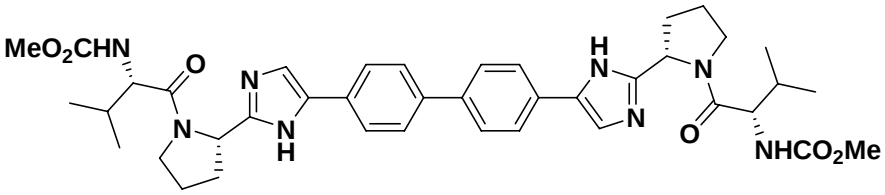
phase extracted with 20% MeOH/$CHCl_3$ (30 mL). The combined organic phase was washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. A silica gel mesh was prepared from the resulting crude material and submitted to flash chromatography using EtOAc as eluant to afford biphenyl **10**, contaminated with a small amount of Ph_3PO, as an off-white solid (563 mg, ~61% yield). ^{1}H-NMR (400 MHz, DMSO-d_6, δ = 2.5 ppm): δ 12.21-12-16/11.95-11.78 (m, 2H), 7.85-7.48/ 7.32-7.25 (m, 10H), 4.90-4.71 (m, 2H), 3.60-3.32 (m, 4H), 2.30-1.79 (m, 8H), 1.41 (br s, 7.5H), 1.16 (m, 10.5H). HRMS (*m/z*): $[M+H]^+$ calcd for $C_{36}H_{45}N_6O_4$, 625.3502; found, 625.3502.

5,5'-(4,4'-Biphenyldiyl)bis(2-((2*S*)-2-pyrrolidinyl)-1*H*-imidazole) (11)



A mixture of biphenyl **10** (560 mg, 0.871 mmol) and 25% CF_3CO_2H/CH_2Cl_2 (9.0 mL) was stirred at room temperature for 3.2 hr. The volatile component was removed *in vacuo*, and the resulting material was free-based using an MCX column (MeOH wash; 2.0 M NH_3/MeOH elution) to afford pyrrolidine **11** as a dull yellow solid (340 mg, 92% yield). ^{1}H-NMR (400 MHz, DMSO-d_6, δ = 2.5 ppm): δ 11.83 (br s, 2H), 7.80 (d, *J* = 8.1 Hz, 4H), 7.66 (d, *J* = 8.3 Hz, 4H), 7.46 (br s, 2H), 4.16 (app t, *J* = 7.2 Hz, 2H), 2.99-2.69 (m, 6H), 2.09-2.00 (m, 2H), 1.94-1.66 (m, 6H). HRMS (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{29}N_6$, 425.2454; found, 425.2448.

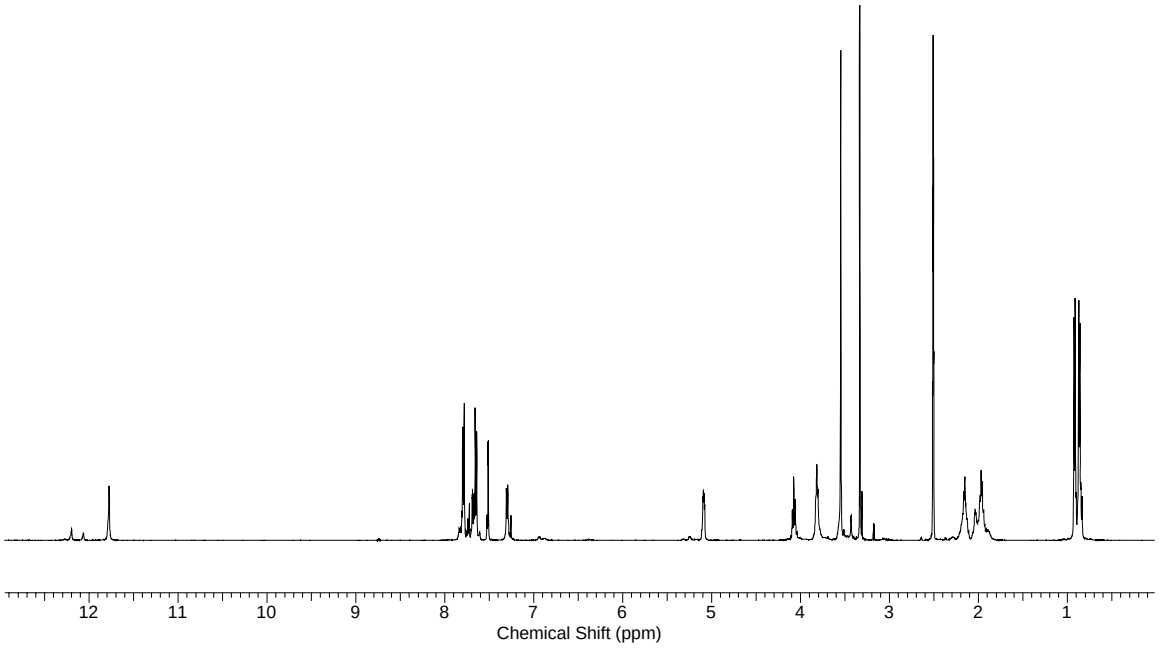
Methyl ((1*S*)-1-(((2*S*)-2-(5-(4'-(2-((2*S*)-1-((2*S*)-2-((methoxycarbonyl)amino)-3-methylbutanoyl)-2-pyrrolidinyl)-1*H*-imidazol-5-yl)-4-biphenylyl)-1*H*-imidazol-2-yl)-1-pyrrolidinyl)carbonyl)-2-methylpropyl)carbamate (BMS 790052)



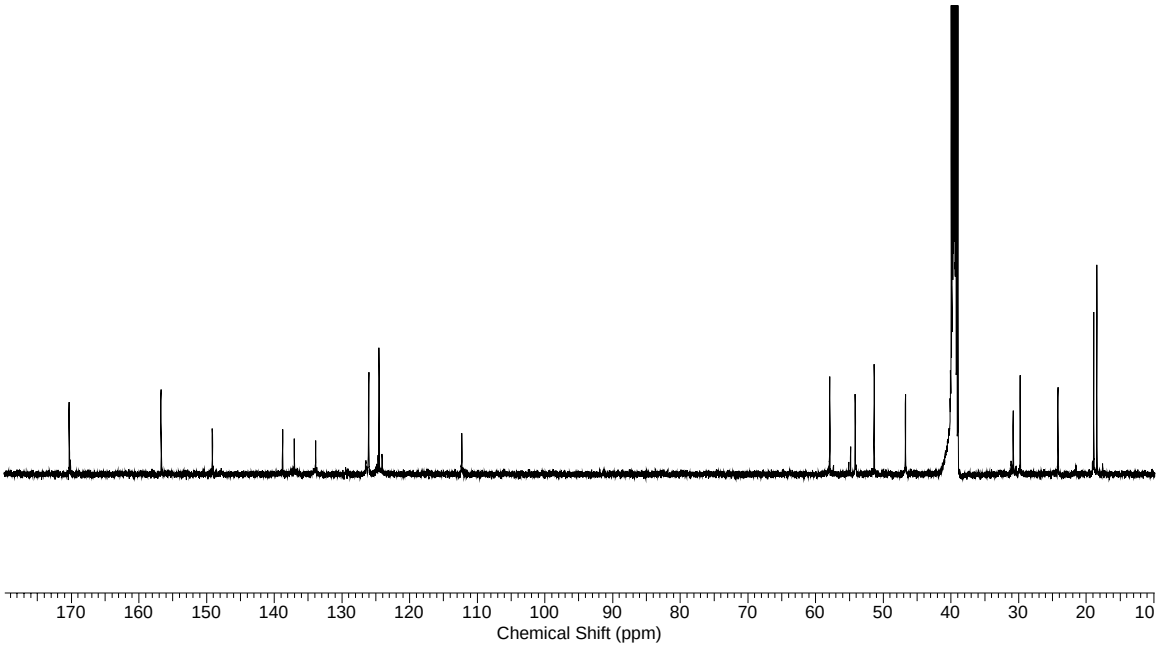
HATU (1.81 g, 4.76 mmol) was added in batches over 4 min to a solution of pyrrolidine **11** (1.00 g, 2.35 mmol), acid **6** (0.946 g, 5.40 mmol) and *i*-Pr_2EtN (1.30 mL, 7.46 mmol) in DMF (25 mL) and the reaction mixture stirred at room temperature for 75 min. The volatile component

was removed *in vacuo,* the residue passed through an MCX column (12 g; MeOH wash; 2.0 N NH_3/MeOH elution) and the resulting material purified by flash chromatography (0-5% MeOH/CH_2Cl_2 as eluant) followed by a reverse phase HPLC (Phenomenex-Luna S10; MeOH/ CF_3CO_2H/H_2O) to afford the CF_3CO_2H salt of BMS-790052 as a white foam. Partitioning the sample between CH_2Cl_2 (100 mL) and $NaHCO_3$ solution (prepared from 0.6 g of $NaHCO_3$ and 10 mL water), drying the organic layer ($MgSO_4$) followed by removal of volatile component *in vacuo* afforded the free-base of BMS-790052 as a white foam (0.88 g, 51% yield). For the free-base form: ^{1}H-NMR (500 MHz, DMSO-d_6, δ = 2.51 ppm): δ 12.20/12.07/11.78 (three br s, 2H), 7.84-7.51 (m, 10H), 7.34-7.26 (m, 1.76H), 6.94-6.88 (m, 0.24H), 5.53-5.24 (m, 0.22H), 5.11-5.08 (m, 1.78H), 4.11-4.00 (br m, 2H), 3.87-3.70 (m, 3.58H), 3.8-3.43 (m, 6.42H), 2.32-1.85 (m, 10H), 0.93-0.84 (m, 12H). ^{13}C-NMR for the dominant rotamer (125.8 MHz, DMSO-d_6, δ = 39.5 ppm): δ 170.30, 156.71, 149.16, 138.77, 137.05, 133.89, 126.04, 124.52, 112.29, 57.91, 54.16, 51.35, 46.73, 30.80, 29.76, 24.17, 18.90, 18.43. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{40}H_{51}N_8O_6$, 739.3932; found, 739.3942. The sample was 99.9% pure when analyzed under the following reverse phase HPLC conditions (column: Acquity UPLC/BEH C18/1.7 µM/100 x 2.1 mm; temp. = 30°C; flow rate: 0.50 mL/min; wavelength: 315 nM; solvent-A: 25 mM NH_4OAc/H_2O @ pH = 5, and solvent-B: CH_3CN).

^{1}H NMR for BMS-790052 (free base form)



^{13}C NMR for BMS-790052 (free base form)


170
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Chemical Shift (ppm)