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Featured Letter

The Discovery of Conformationally Constrained Bicyclic Peptidomimetics as Potent Hepatitis C NS5A Inhibitors

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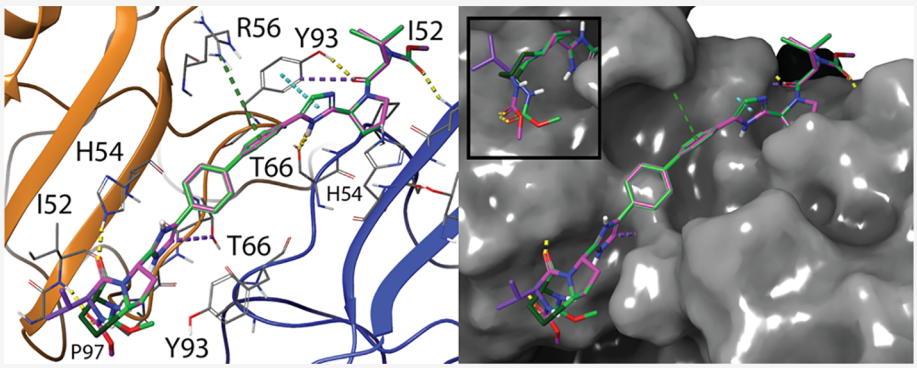

ABSTRACT: HCV NS5A inhibitors are the backbone of directly acting antiviral treatments against the hepatitis C virus (HCV). While these therapies are generally highly curative, they are less effective in some specific HCV patient populations. In the search for broader-acting HCV NS5A inhibitors that address these needs, we explored conformational restrictions imposed by the [7,5]-azabicyclic lactam moiety incorporated into daclatasvir (**1**) and related HCV NS5A inhibitors. Unexpectedly, compound **5** was identified as a potent HCV genotype 1a and 1b inhibitor. Molecular modeling of **5** bound to HCV genotype 1a suggested that the use of the conformationally restricted lactam moiety might have resulted in reorientation of its N-terminal carbamate to expose a new interaction with the NS5A pocket located between amino acids P97 and Y93, which was not easily accessible to **1**. The results also suggest new chemistry directions that exploit the interactions with the P97−Y93 site toward new and potentially improved HCV NS5A inhibitors.

KEYWORDS: *HCV, hepatitis C virus, genotype 1a, genotype 1b, nonstructural protein 5A, HCV inhibitor*

The hepatitis C virus (HCV) is a 9.6 kb positive-strand RNA virus that can cause chronic liver disease, cirrhosis, and hepatocellular carcinoma (primary liver cancer). The World Health Organization estimated that at least 170 million people worldwide were infected with HCV and that in 2016 about 400 000 people died from hepatocellular carcinoma.[1] HCV is a blood-borne virus that is transmitted primarily through unsafe injection practices, transfusion of unscreened blood, and unsafe sexual contacts. The HCV genome contains seven genotypes (1−7) and more than 90 subtypes and encodes 10 proteins, some structural (core, E1 glycoprotein, E2) and some nonstructural (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B).[2,3] The NS5A protein, which is active as a homodimer, consists of three domains, of which domain 1 is the most structured and essential for HCV genome replication.[4] The FDA-approved dual, triple, and quad regimens combine NS5A inhibitors with other directly acting agents (DAAs) and provide high levels of virologic cure in most patients.[5,6] However, these treatments are less effective in patients with cirrhosis, in genotype 1a (gt1a) patients undergoing interferon-free treatment, and in interferon non-responders, thus arguing for the continued need to discover improved pharmaceutical options for these populations.[7−9]

We previously reported the discovery of the clinical NS5A inhibitor GSK2336805 (JNJ-5614845) and the conformationally constrained NS5A inhibitor GSK2818713 (Figure 1).[6,10,11] Conformational constraints can enhance potency and reduce metabolic liability.[10a,12] In a continued effort to explore this approach toward discovery of improved HCV NS5A inhibitors, we decided to incorporate the [7,5]-azabicyclic lactam motif as a dipeptide mimetic into **1** and related compounds and synthesized analogues **2**−**13** (Tables 1−3 and Schemes 1−3).[12,13a−e,14]

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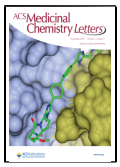


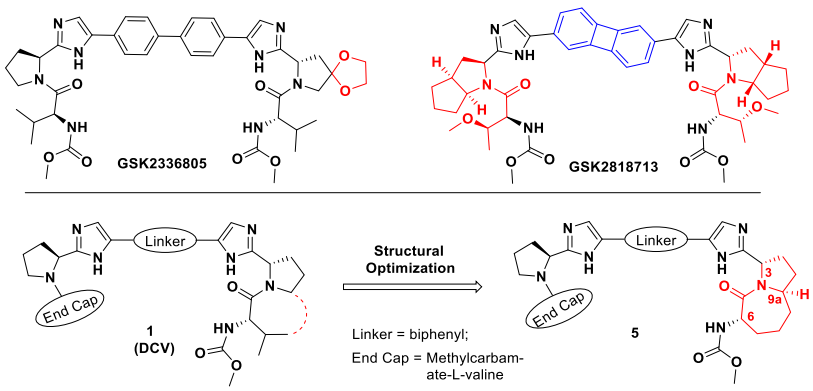
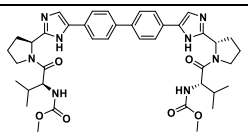
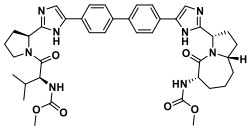
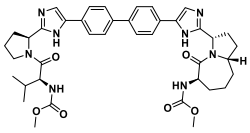
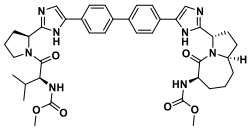
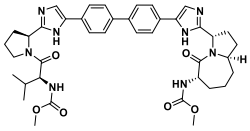


Figure 1. Conversion of **1** into bicyclic **5**.

With fixed position 3*S*, four sets of diastereomers were produced to explore the effect of both *R* and *S* chirality at positions 6 and 9a on the potency in the HCV replicon assay (Figure 1 and Table 1). Compared with daclatasvir (**1**), the potency of **2** (6*S*,9a*S*) decreased slightly against gt1b and significantly (~221-fold) against gt1a. A similar potency profile was observed for **3** (6*R*,9a*S*), but isomer **4** (6*R*,9a*R*) regained gt1a and gt1b potency compared with **2** (~27-fold and ~2-fold, respectively). These gains were further enhanced in inhibitor **5** (6*S*,9a*R*), which was essentially equipotent to **1** against gt1b and only ~6 times less potent against gt1a. The finding that **5** was a subnanomolar HCV inhibitor contrasted with data published elsewhere after our work was completed,[13f] which prompted us to disclose details of our discoveries.

Table 1. SAR of Bicyclic Compounds 2−5

		Huh-luc/neo-ET replicon cells EC_{50} [nM][a]				
Entry	Structure	gt1b	gt1a	3	6	9a
1		0.032	0.079			
2		0.109	17.44	*S*	*S*	*S*
3		0.133	12.48	*S*	*R*	*S*
4		0.059	0.673	*S*	*R*	*R*
5		0.037	0.471	*S*	*S*	*R*

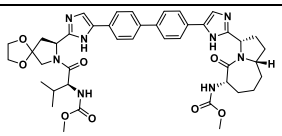
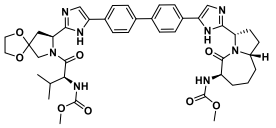
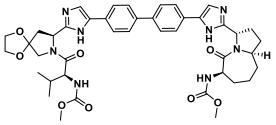
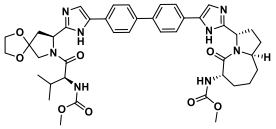
[a]All values are averages of at least three independent experiments.

Another interesting finding is that while *R* chirality at position 9a is required for high gt1a potency, the chirality at position 6 turned out to be much less consequential, which contrasts with the well-established structure−activity relationship (SAR) in the linear (**1**) series, where *S* chirality is required (Table 1). Such divergent SARs can indicate a profound conformational change in the bicyclic series (see Modeling Studies).[15]

The lactam-imposed constraints were further evaluated on the framework of two other NS5A inhibitors. GSK2236805 (gt1b EC_{50} = 0.011 nM and gt1a EC_{50} = 0.010 nM; Figure 1) has a superior potency profile compared with **1** resulting from interactions of the ketal moiety with NS5A.[10a] Compared with GSK2236805, bicyclic inhibitors **6−9** exhibited slight potency loss on gt1b and a significant one on gt1a (Table 2).[10] Similar to the SAR of **2−5**, the 6*R*,9a*R* isomer (**8**) and the 6*S*,9a*R* isomer (**9**) exhibited the best potency against HCV gt1a.

In another very potent series, where the carbamate moiety is replaced with a tertiary amine,[16] the SAR of resulting lactams **10−13** again mirrored the one observed for **2−5**, with **13** (6*S*,9a*R*) being the most potent in this group (Table 3).[11,16] The absolute potencies in this series were lower than for **2−9**, (e.g., **13** was 16-fold less potent than **5** against gt1a).

Table 2. SAR of Bicyclic Compounds 6−9

		Huh-luc/neo-ET replicon cells EC_{50} [nM][a]				
Entry	Structure	gt1b	gt1a	3	6	9a
6		0.046	8.581	*S*	*S*	*S*
7		0.08	14.52	*S*	*R*	*S*
8		0.047	0.707	*S*	*R*	*R*
9		0.064	1.524	*S*	*S*	*R*

[a]All values are averages of at least three independent experiments.

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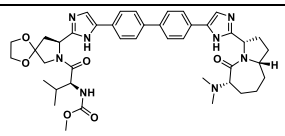
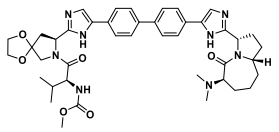
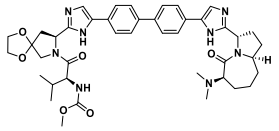
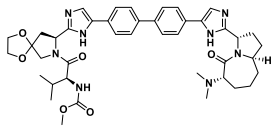
Table 3. SAR of Bicyclic Compounds 10−13

		Huh-luc/neo-ET replicon cells EC_{50} [nM][a]				
Entry	Structure	gt1b	gt1a	3	6	9a
10		0.209	32.165	*S*	*S*	*S*
11		0.217	119.15	*S*	*R*	*S*
12		0.298	10.635	*S*	*R*	*R*
13		0.084	7.707	*S*	*S*	*R*

[a]All values are averages of at least three independent experiments.

Modeling Studies. To rationalize the impact of the bicyclic constraints, we performed molecular modeling studies of analogues. The published structures of NS5A genotypes 1a (PDB ID 4CL1)[17] and 1b (PDB ID 3FQQ)[18] were used to generate models of NS5A in two dimeric states, and their interactions with **1** and **5** were modeled using the docking software Glide.[19−22] The generated low-energy poses, in which **5** had the expected higher (worse) docking score than **1** to gt1a NS5A protein (−6.925 vs −7.737 kcal/mol, respectively), were consistent with the experimental data. The most significant difference between **1** and **5** docked to gt1a appears to be the loss of favorable hydrophobic interactions between the isopropyl of **1** and I52 of NS5A (Figure 2, bottom left of each panel and inset). Another consequence of the new conformational preference in **5** is reorientation of its methyl carbamate group away from the typically utilized solvent-exposed edge of P97 inward toward a hydrophobic cleft located between P97 of one monomer and Y93 of the other. This pocket is not easily accessible and thus is not utilized by the low-energy conformers of **1** (Figure 2, inset). The interaction of carbamate **5** with the new site is unoptimized, and further chemical modifications of **5** should improve its potency. For example, modeling showed that replacement of the carbamate in **5** with phenyl, difluorophenyl, thiophene, and pyrrole amides significantly increased the affinity of such compounds, as shown by their lower (improved) docking scores versus **1** (−8.434, −8.523, −8.166, and −7.989 vs −7.737 kcal/mol, respectively), as a result of optimized interactions of the aromatic groups with P97−Y93 (Supplemental Figure 1). On the other hand, modeling of the same amides replacing the methyl carbamate in **1** indicated that they were not able to interact strongly with the P97−Y93 pocket, as reflected by their higher docking scores vs the same amides in the bicyclic series (−7.024 vs −8.434, −6.883 vs −8.523, −7.544 vs −8.166, and −7.567 vs −7.989 kcal/mol for the phenyl, difluorophenyl, thiophene, and pyrrole amides, respectively; Supplemental Table 1).

Syntheses. *Syntheses of [7,5]-Fused Bicyclic Acids **30−33** and **44−47**.* The [7,5]-fused *cis*-bicyclic acids **30−33** were synthesized from (*S*)-5-oxopyrrolidine-2-carboxylic acid **14** (Scheme 1a).[23,24] Briefly, **14** was converted to its *tert*-butyl ester **15** with ^{t}BuOAc and $HClO_4$, after which the −NH group was Cbz-protected and the amide was reduced with $LiBHEt_3$ to yield alcohol **17** in 62% yield. **17** was then converted into methoxy derivative (*S*)-**18** with TsOH/MeOH. The requisite *cis*-allyl intermediate (*S*,*R*)-**19** was secured by allylation of (*S*)-

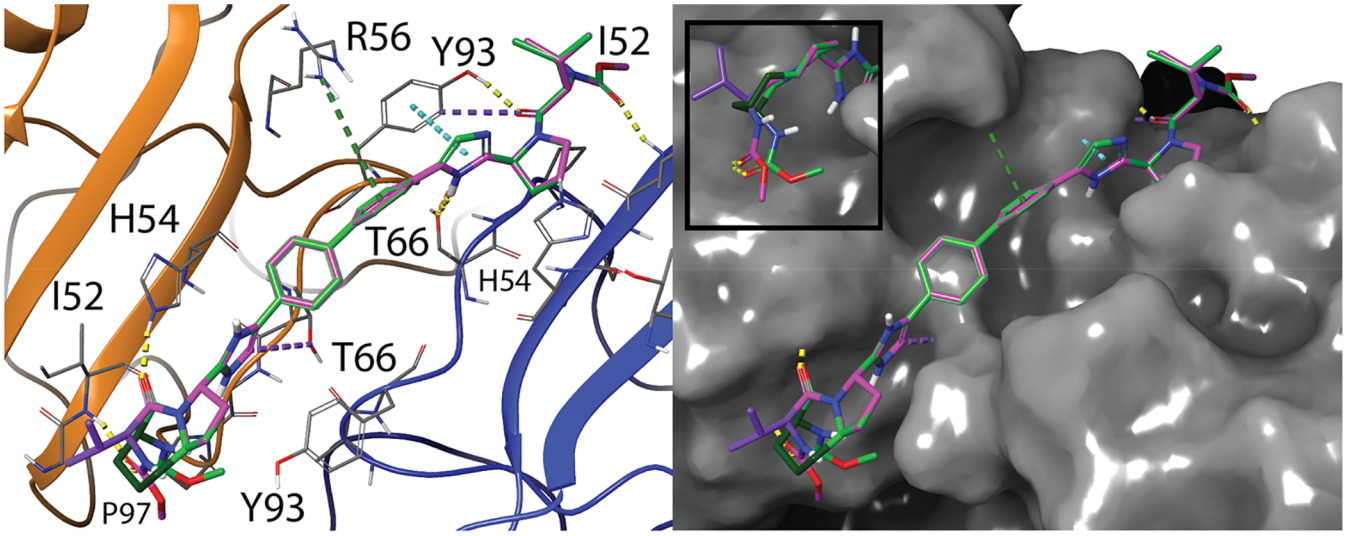


Figure 2. Model of compounds **1** and **5** bound to gt1a. Compounds **1** (magenta) and **5** (green) are modeled to bind at the dimeric interface of gt1a (cartoons and stick, left; surface representation, right). The backbone cartoons are colored by chain. While the common substructures have the same pose and make the same interactions (hydrogen bonds are represented as yellow dashes, aromatic hydrogen-bonding interactions as magenta dashes, $\pi-\pi$ interactions as cyan dashes, and cation−π interactions as green dashes), the bicyclic moiety is modeled to interact differently. The inset features an alternate projection of the bicyclic ring to highlight the loss of interaction between the isopropyl group (darker magenta) and I52 modeled with compound **1** while showing that the bicyclic ring (dark green) constrains the methyl group of the carbamate of compound **5** into a new orientation inward toward the P97−Y93 pocket.

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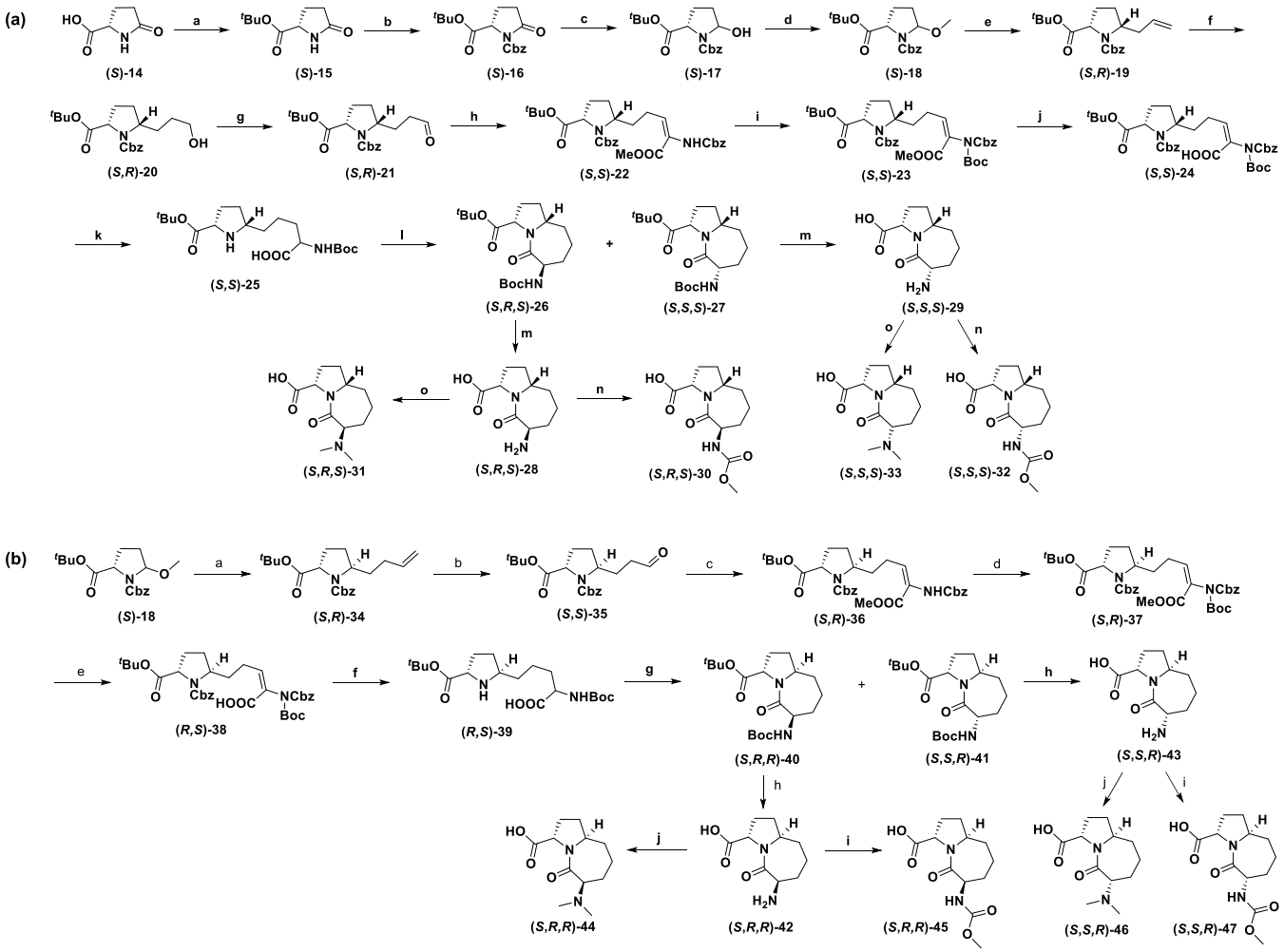
Scheme 1. Syntheses of (a) [7,5]-Fused *cis*-Bicyclic Acids 30−33[a] and (b) [7,5]-Fused *trans*-Bicyclic Acids 44−47[b]



[a]Reagents and conditions: (a) $HClO_4$, ^tBuOAc, rt, 12 h, 50%; (b) (i) NaH, THF, 0 °C, 1 h, (ii) Cbz-Cl, rt, 12 h, 50%; (c) $LiBHEt_3$, THF, −78 °C, 1 h, 63%; (d) TsOH, MeOH, rt, 24 h, 52%; (e) allyltributylstannane, $BF_3{\cdot}OEt_2$, −78 °C to rt, 3 h, 18%; (f) (i) 9-BBN, THF, rt, 12 h, (ii) H_2O_2, 3 N NaOH, rt, 2 h, 81%; (g) PCC, CH_2Cl_2, rt, 2 h, 80%; (h) (i) (±)-benzyloxycarbonyl-α-phosphonoglycine trimethyl ester, ^tBuOK, CH_2Cl_2, −78 °C, 30 min, (ii) **21**, CH_2Cl_2, −78 °C, 4 h, rt, 12 h, 88%; (i) Boc_2O, THF, DMAP, rt, 12 h, 90%; (j) 1 N NaOH, MeOH, rt, 12 h, 66%; (k) Pd/C, MeOH, H_2, rt, 4 h, quant.; (l) EDC, HOBt, Et_3N, DMAP, CH_2Cl_2, rt, 12 h, 37−39%; (m) TFA, CH_2Cl_2, rt, 2 h, quant.; (n) methyl chloroformate, dioxane, 1 N NaOH, rt, 12 h, 85−88%; (o) HCHO, 1 N HCl, Pd/C, MeOH, H_2, rt, 12 h, 64%. [b]Reagents and conditions: (a) (i) 4-bromobut-1-ene, Mg, THF, reflux, 1.5 h, (ii) $CuBr{\cdot}Me_2S$, THF, −78 °C, 2.5 h, 55%; (b) $NaIO_4$, THF, H_2O, OsO_4, rt, 2 h, 83%; (c) (i) (±)-benzyloxycarbonyl-α-phosphonoglycine trimethyl ester, ^tBuOK, CH_2Cl_2, −78 °C, 30 min, (ii) aldehyde, CH_2Cl_2, −78 °C, 4 h, rt, 12 h, 86%; (d) Boc_2O, THF, DMAP, rt, 12 h, 87%; (e) 1 N NaOH, MeOH, rt, 12 h, 80%; (f) Pd/C, MeOH, H_2, rt, 4 h, 93%; (g) EDC, HOBt, Et_3N, DMAP, CH_2Cl_2, rt, 12 h, 23−25%; (h) TFA, CH_2Cl_2, rt, 2 h, quant.; (i) methyl chloroformate, dioxane, 1 N NaOH, rt, 12 h, 43−54%; (j) HCHO, 1 N HCl, Pd/C, MeOH, H_2, rt, 12 h, 60−80%.

18 with allyltributylstannane/$BF_3{\cdot}OEt_2$, and 9-BBN-mediated hydroboration of the allyl moiety in (*S*,*R*)-**19** and subsequent oxidation furnished alcohol **20** in 81% yield. Oxidation of **20** using PCC afforded the key intermediate *cis*-aldehyde **21** in 80% yield, and subsequent Horner−Emmons olefination with (±)-benzyloxycarbonyl-α-phosphonoglycine trimethyl ester furnished **22** in 89% yield. Boc protection of the Cbz carbamate and hydrolysis of **23** afforded acrylic acid **24**. Catalytic hydrogenation of **24** and cyclization of **25** gave a 1:1 mixture of diastereomers, which could be separated to yield the desired pure bicyclic (*S*,*R*,*S*)-**26** and (*S*,*S*,*S*)-**27** (dr > 98%).[24] Deprotection of **26** and **27** to obtain the respective amines **28** and **29** was then followed by their conversion to carbamates **30** and **32**, whereas *N*,*N*-dimethyl-substituted **31** and **33** were obtained by reductive alkylation of **28** and **29**, respectively, with formaldehyde. The overall yields for **30**−**33** were 0.12−0.17% over 14 steps from **14**. The *trans*-bicyclic acid diastereomers **44**−**47** were synthesized in a similar fashion (dr > 98%) (Scheme 1b).[23,24] Key intermediate *trans*-(*S*,*R*)-**34** was obtained by reacting (*S*)-**18** with 4-bromobut-1-ene/Mg and $CuBr{\cdot}Me_2S$, paving way for the syntheses of *trans*-bicyclic (*S*,*R*,*R*)-**44**, (*S*,*R*,*R*)-**45**, (*S*,*S*,*R*)-**46**, and (*S*,*S*,*R*)-**47** in overall yields of 2.5−5.1% over nine steps from **18**.

General Syntheses of Bicyclic Inhibitors ***2*−*5***. Inhibitors **2**−**5** were synthesized in 1.2−5.3% overall yield in eight steps starting from **48**[10a] by coupling of amine **54** with bicyclic acids **32**, **30**, **45**, and **47** (Scheme 1) followed by imidazole cyclization (Scheme 2) as described in our previous work.[10a] Attempts to couple amine **54** with the bicyclic acids required T3P as a coupling agent to secure **55a**−**55d**, which were then subjected to imidazole cyclization to yield targets **2**−**5**.

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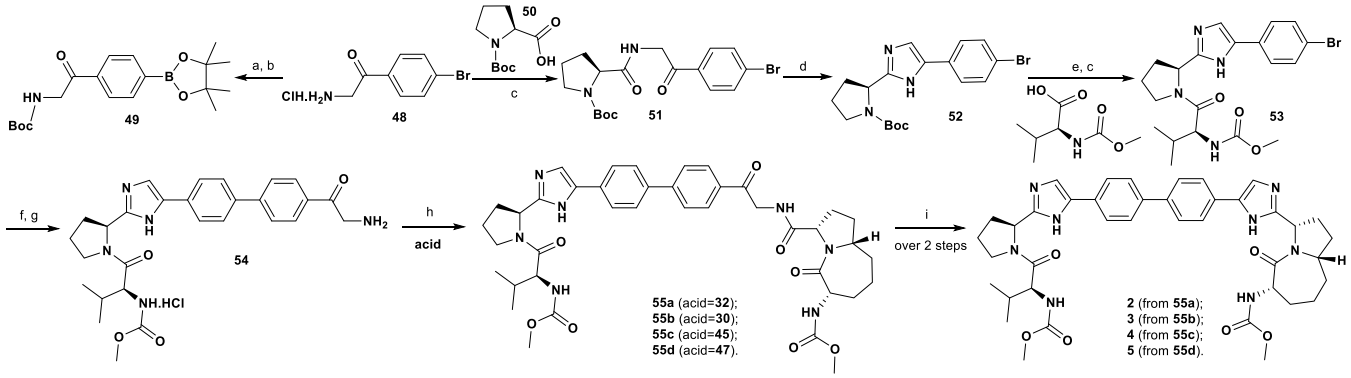
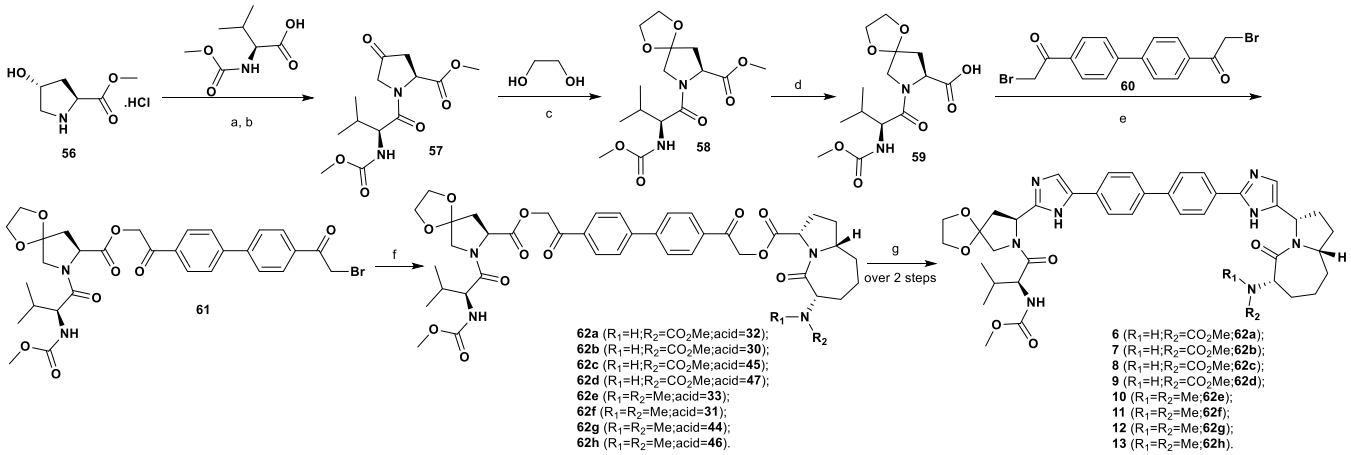
Scheme 2. Synthesis of Bicyclic Inhibitors 2−5[a]



[a]Reagents and conditions: (a) 1,4-dioxane, Et_3N, Boc_2O, 64%; (b) dioxane, $Pd(dppf)Cl_2$, bis(pinacolato)diboron, CH_3COOK, 58%; (c) CH_2Cl_2, HATU, DIEA, 61%; (d) NH_4OAc, dioxane, 110 °C, 48 h or microwave, 160 °C, 20 min, 89%; (e) HCl(aq), 90%; (f) DME, $NaHCO_3$, $Pd(dppf)Cl_2$, 18 h, 80 °C, **49**, 54%; (g) HCl(aq), quant.; (h) T3P, TEA; (i) NH_4OAc, microwave, 140 °C, 30 min, 7−30%.

Scheme 3. Synthesis of Spiroketal Bicyclic Inhibitors 6−13[a]



[a]Reagents and conditions: (a) HATU, Et_3N, 70%; (b) Dess−Martin periodinane, 20%; (c) tosic acid, 44%; (d) LiOH, THF−water−MeOH, 91%; (e) DMF, NaH, rt, 15 min, Et_3N, quant.; (f) acid, Et_3N, ACN, 50 °C, 2 h; (g) NH_4OAc, dioxane, microwave, 145 °C, 40 min, 25−37%.

General Syntheses of Bicyclic Inhibitors 6−13. Compounds **6−13** were synthesized analogously to **2−5** in overall yields of 1.4−2.1% starting from **56** (Scheme 3).[10a,11e] Coupling of intermediate **61** with sterically hindered bicyclic acids (**32**, **30**, **45**, and **47**) required elevated temperatures (50 °C) to furnish the desired amides **62a−h**, and subsequent imidazole cyclization secured **6−13**.

In the search for improved HCV NS5A-based replication inhibitors, we examined conformational restrictions imposed by the [7,5]-azabicyclic lactam in the HCV NS5A inhibitor series and unexpectedly found bicyclic **5** to be nearly equipotent against HCV gt1b and only moderately less potent (∼6×) against gt1a versus daclatasvir (**1**).[13f] Detailed molecular modeling of NS5A gt1a-docked **5** allowed us to postulate the binding mode for **5**, in which the N-terminal substituent in **5** is reoriented toward a new site located between gt1a NS5A P97 and Y93 that is hard for **1** to access. Furthermore, aromatic amides were modeled to develop strong interactions with P97−Y93, suggesting facile ways to potentially improve the inhibitory potency against HCV by these analogues. Bicyclic rings of various sizes and substitutions in conjunction with the postulated amides create a substantial new chemistry space for further systematic explorations. It is tempting to examine whether molecules in this space can bridge the unmet medical needs of patients that are not well served with current NS5A-based therapies, such as resistant or hard-to-treat HCV infections.[9] The results of our explorations in this space will be communicated in due time.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00391.

Synthesis methods and experimental data for compounds, protocol for testing and data analysis of compounds in the HCV replicon assay, modeling methods, Supplemental Figure 1, and Supplemental Table 1 (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

Biography

Wieslaw "Wes" Kazmierski received his Ph.D. in Organic Chemistry from the University of Arizona under the supervision of Professor Victor Hruby for his work on peptide mimetics. He was the founding scientist of the Selectide Corporation, where he codeveloped the One-Bead-One-Peptide concept. He codiscovered multiple clinical HIV and HCV small molecule inhibitors and modulators, including the drug Lexiva during his work at GlaxoSmithKline/ViiV Healthcare, where he was Scientific Leader and GSK Fellow. He is interested in new drug modalities, protein degradation, peptide therapies, antibody−drug conjugates, immunology, cancer research, and anti-infectives. He is currently Vice President of Chemistry at Biohaven Pharmaceuticals.

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