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HCV NS5A replication complex inhibitors. Part 5: Discovery of potent and pan-genotypic glycinamide cap derivatives

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ABSTRACT

The isoquinolinamide series of HCV NS5A inhibitors exemplified by compounds **2b** and **2c** provided the first dual genotype-1a/1b (GT-1a/1b) inhibitor class that demonstrated a significant improvement in potency toward GT-1a replicons compared to that of the initial program lead, stilbene **2a**. Structure–activity relationship (SAR) studies that uncovered an alternate phenylglycine-based cap series that exhibit further improvements in virology profile, along with some insights into the pharmacophoric elements associated with the GT-1a potency, are described.

Hepatitis C virus (HCV) infection is responsible for causing serious liver diseases, including cirrhosis, hepatocellular carcinoma and liver failure. It is estimated that HCV has infected over 180 million people worldwide and, according to a recent survey conducted in the United States, about 50% of these individuals might be unaware of their infection.[1] The significant heterogeneity of the viral genome—six genotypes and over 100 subtypes have been identified thus far—coupled with its high mutation rate have hampered the development of effective therapies.[2] A combination of pegylated interferon-α (PEG-IFN-α) and ribavirin (RBV), which has served as the standard of care for HCV for the past decade, has many drawbacks, including poor tolerability and non-optimal efficacy toward GT-1, the most dominant genotype across the world.[3] In May 2011, two first-generation protease inhibitors that enhanced the efficacy of the PEG-IFN-α/RBV regimen from ~45% to ~70% in treatment-naïve GT-1 patients, while decreasing the therapy duration by up to 50%, were approved.[4] Despite this marked progress, however, the new triple combinations exhibit less than optimal efficacy in treatment-experienced subjects and are associated with more side effects than the original PEG-IFN-α/RBV regimen. The development of more effective, tolerable and interferon-free therapies to cure HCV infection that rely upon direct-acting antiviral agents is an ongoing enterprise, with promising initial results. These regimens have examined combinations of inhibitors of NS3 protease, NS5B polymerase and NS5A.[5]

Clinical validation of the HCV NS5A protein as a viable target for antiviral therapy was established with trials of daclatasvir (**1**).[6] We have previously described early aspects of the medicinal chemistry effort that generated the NS5A inhibitor lead, stilbene **2a**, and the preliminary SAR studies that delineated some of the chemotype's pharmacophoric elements, including the identification of an isoquinoline class of derivatives that significantly enhanced its inhibitory potency toward a GT-1a replicon (compare isoquinolinamide **2c** vs phenyl acetamide **2a**).[7] Although this improvement in GT-1a inhibitory potency marked an important milestone for the program, the polyaromatic makeup of the isoquinolinamide cap series—as reflected by the low percent composition of sp^3-carbons for **2c** (22%) compared to that of marketed drugs (47%)—was of some concern, providing an impetus for additional structural exploration.[8] Herein, we report the SAR investigation that uncovered a phenylglycine-based cap series exhibiting high GT-1a and -1b inhibitory potency in in vitro replicon assays, a key development in the optimization campaign that culminated in the discovery of daclatasvir (**1**).

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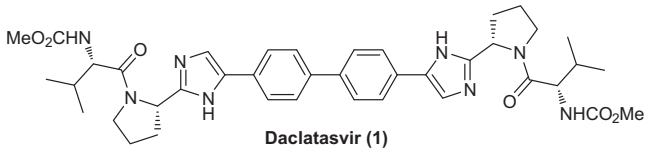


Daclatasvir (1)

Homodimeric cap analogues were prepared and screened for inhibitory activity in GT-1a and -1b replicons and for cellular toxicity and target specificity in GT-1b and bovine viral diarrhea virus (BVDV) replicons, respectively. On a selective basis, target specificity was also assessed for some compounds in a GT-1b L31V/Y93H double mutant resistant replicon, henceforth referred to as GT-1b LV/YH resistant replicon.[9]

The design concept envisioned amides represented by the generic structure **A** in Figure 1 as a deannelated isostere of the isoquinolinamide **2b**, which facilitated further SAR evolution by variation of the benzylic substituent. The first analogue prepared in this exercise, ketoamide **3a**, exhibited a GT-1a inhibitory potency that was comparable with that of **2b** (see Table 2). Replacing the benzylic carbonyl moiety of **3a** with small functional groups had neutral to detrimental impact on GT-1a inhibitory potency (see **3b**–**3d**). In comparing the GT-1a/-1b activities of the diastereomeric pairs **3b.1** verses **3b.2** or **3d.1** verses **3d.2**, the (*R*)-stereoisomer appears to correlate with better GT-1a and/or GT-1b inhibitory potency, although the magnitude of the effect was not consistently compelling (e.g., the GT-1a EC_{50}s of **3b.1** and **3b.2** were within the margins of error of the assay, and the enhanced GT-1a potency of **3d.1** over that of its diastereomer **3d.2** was accompanied with some increase in non-specific signal in the BVDV assay).[10] Saturation or homologation of the mandelamide cap generally resulted in reduced potency, albeit it is noteworthy that in both cases the (*R*)-stereoisomer was relatively more potent in the GT-1b replicon (compare **3e.1** vs **3e.2** and **3f.1** vs **3f.2**). A marked improvement in GT-1a potency was secured with the introduction of a methyl group at the benzylic position of the mandelamide (EC_{50} of **3g.2** = 84 nM), which was also accompanied by single digit picomolar inhibitory potency in the GT-1b replicon. Interestingly, contrary to the initial SAR trend, it was the (*S*)-stereoisomer that was the more active analogue toward the GT-1a subtype for the methyl carbinol. In general, assessment of target specificity for the analogues discussed thus far indicated favorable properties. For example, **3g.2** had a reduced activity in the GT-1b LV/YH resistant replicon (EC_{50} = 620 nM), minimal activity in the BVDV assay, and an excellent selectivity index.

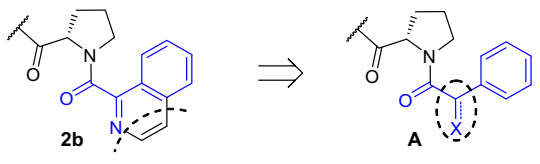
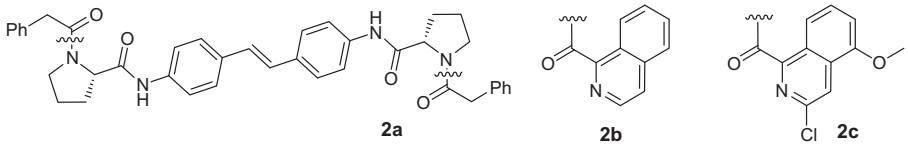


Figure 1. Deannelation design strategy.

It was not initially clear to us how the benzylic substituents were influencing replicon potency. Although it was tempting to hypothesize that the hydroxyl group of the mandelamide moiety might be involved in some form of electrostatic interaction with the proximal carbonyl group and, thus, perhaps populating a preferred conformational state, rationalization of the stereochemical disparity between the relatively more active diastereomers (*S*)-**3g.2** and (*R*)-**3b.1** was not feasible.[11] Alternatively, the similar GT-1a activities between alcohols **3b.1**/**3b.2** and methyl analogue **3d.1** in the GT-1a replicon indicated that a hydrogen-bond interaction between the benzylic region and the NS5A protein was unlikely to be the sole driving factor for the observed inhibitory effects.[7b] To gain additional insight into these SAR findings, the single crystal X-ray structures of bromides **4a**, (*S*)-**4b**, and (*R*)-**4c**, which represent the monomeric structural fragments of **2a**, (*S*)-**3g.2**, and (*R*)-**3b.1**, respectively, were determined. Although fully cognizant of the limitations that such an approach may have in illuminating aspects of a dynamic process, interestingly this exercise revealed a similar dihedral angle between the cap carbonyl moiety and the methyl group of (*S*)-**4b** (4–22°) or the alcohol group (*R*)-**4c** (4–16°), resulting in similar topological dispositions for their respective phenyl groups and yet clearly different from that of **4a**, the substructure of the least GT-1a active analogue of the set (i.e., compound **2a**) (see Fig. 2).[12,13]

At this juncture, it became apparent that the alkene linker of the stilbene chemotype was susceptible to *trans–cis* isomerization when compounds were stored as DMSO solutions under standard fluorescent lighting.[7b] In order to obviate the complications that such isomerization could create in building reliable SAR, the corresponding alkyne-linked template was considered. Surveying a select set of caps uncovered in the stilbene series on an alkyne-linked core revealed a similar stereochemical preference, accompanied with comparable or slightly decreased GT-1a inhibitory potency (compare **3g** vs **3h** in Table 2; data for remaining subset is not shown). Thus, the alkyne template was adopted for the next phase of the study, the objective of which was to enhance GT-1a potency further by examining glycine-based caps. For the majority of the cases, both symmetrical diastereomeric analogues (i.e., analogues with identical cap stereoconfigurations) were prepared and assayed in a fashion similar to the SAR investigation illustrated in Table 2. For each pair of symmetrical diastereomers tested, one was consistently more active than its alternate isomer toward GT-1a and/or GT-1b replicons in all of the cases where such a comparison could be made. In addition, at least for the arylglycinamide subset where the absolute stereochemistry of the cap was known, the (*R*)-isomer exhibited the better inhibitory potency (e.g., compare **5b.1** vs **5b.2** in Table 3).

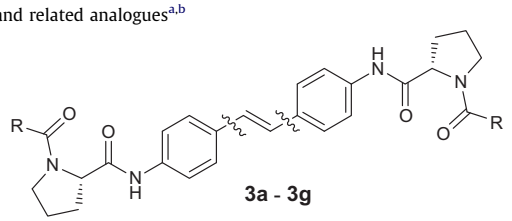
Table 1
Activity and specificity of selected early leads[a]

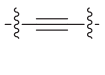


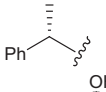
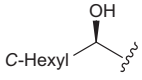
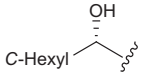
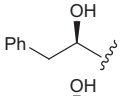
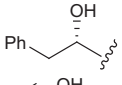
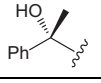
GT-1a EC_{50} (nM)	$>10^4$	390	19
GT-1b EC_{50} (nM)	0.086	22	7.1
GT-1b CC_{50} (nM)	$>10^4$	$>10^4$	$>10^4$
BVDV EC_{50} (nM)	$>10^4$	$>10^4$	$>10^4$

[a] Data represent mean values of at least two experiments with a maximum of threefold variation.

Table 2
Activity and specificity of mandelamides and related analogues[a,b]

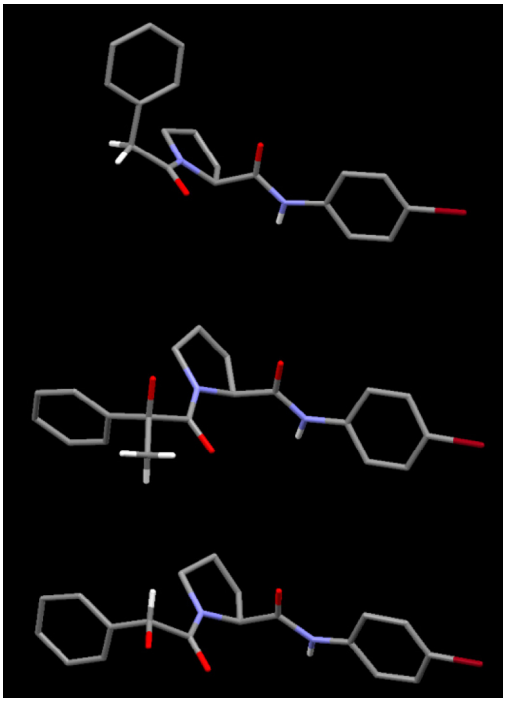


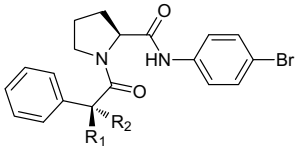


Compd	R	Replicon activity		GT-1b CC_{50} (nM)	BVDV EC_{50} (nM)
		GT-1a EC_{50} (nM)	GT-1b EC_{50} (nM)		
3a		447	7.0, <4.6	$>10^4$	$>10^4$
3b.1		640	0.022	$>10^4$	$>10^4$
3b.2		1020	3.8, < 4.6	$>10^4$	5110, $>10^4$
3c.1		1290	<4.6	$>10^4$	$>10^4$
3c.2		1270	<4.6	$>10^4$	$>10^4$
3d.1[c]		772	<4.6	$>10^4$	2500
3d.2[c]		$>10^4$	5.0	$>10^4$	$>10^4$
3e.1		4650	<4.6	$>10^4$	$>10^4$
3e.2		970	63	$>10^4$	6740
3f.1		1860	5.2, <4.6	$>10^4$	7760
3f.2[d]		2320	229	980, $>10^4$	6390
3g.1		6850	<4.6	$>10^4$	NT[e]
3g.2		84	0.006	$>10^4$	$>10^4$
3h.1		6510	<4.6	8380	$>10^4$
3h.2		284	<4.6	$>10^4$	$>10^4$

[a] Data represent mean values of at least two experiments with a maximum of threefold variation.
[b] EC_{50} in GT-1b LV/YH resistant replicon: **3b.1** (2030 nM), **3g.2** (620 nM).
[c] The inhibitory activities of **3d.1** and **3d.2** in GT-1b replicon were disclosed previously in Ref. 7b.
[d] Sample was not retested to check on the difference in CC_{50} value between the two experiments.
[e] NT, not tested.

The parental phenylglycine analogue **5a.1** exhibited a GT-1a inhibitory activity that was comparable to that of the mandelamide **3b.1**, and introduction of a simple dimethyl group (as in **5b.1**) enhanced the GT-1a inhibitory potency by nearly a 100-fold. Further structural refinement of this region afforded an additional ~10-fold potency gain toward the GT-1a replicon, resulting in a low single digit nanomolar EC_{50} for compounds **5f**, **5g.1** and **5i.1**. Although the benzylic position initially appeared to exhibit reasonable tolerance for structural diversity, there was an unexpected reduction in GT-1a inhibitory potency for the relatively less basic morpholine analogue **5h** (see Table 4 for measured pK_a values for a selected set of analogues). Equally noteworthy was the fact that analogues containing less basic piperazine appendages, such as **5j** and **5k**, also exhibited decreased activity toward the GT-1a





4a (R_1 = H, R_2 = H)
4b (R_1 = OH, R_2 = CH_3)
4c (R_1 = H, R_2 = OH)

Figure. 2. The X-ray structures of prolinamides **4a–c**.

replicon when compared with the parental analogue **5i.1**. Although it is possible that the decreased potency of these latter two analogues could be due to unfavorable interactions between their acetyl (**5j**) or oxa (**5k**) functional groups and the NS5A protein, the reduced potency associated with morpholine **5h** could not be rationalized similarly considering the relatively high inhibitory potency exhibited by a range of functionally diverse analogues, including dimethylamine **5b.1**, hydroxypiperidine **5g.1** and piperazine **5i.1**. In light of these observations, we postulate that caps with benzylic substituents that are protonated at physiological pH could be involved in a productive H-bond interaction with the NS5A protein, particularly for the GT-1a subtype and, hence, modifications that attenuate their basicity would negatively impact such an interaction, translating into a weaker inhibitory effect. It should be noted, however, that the ability of a compound to engage in this type of H-bond interaction does not necessarily guarantee a desirable outcome as reflected in the weaker potency of pyrrolidine **5d**, which is actually more basic than the more potent piperidine **5g.1**. Moreover, although the alcohol group of mandelamide **3g.2** might be involved in a similar type of H-bond interaction with the NS5A protein, in this scenario the vectorial disposition of its phenyl group would likely be different from that of the potent phenylglycine isomers in light of their differing stereoconfigurations.

An attempt to replicate the favorable effect that the introduction of an α-methyl group had on the activity of mandelamide **3g.2** in the phenylglycinamide series proved detrimental to both GT-1a activity and HCV specificity (compare **5b.1** vs **5l.1**). In addition, homologation of the benzyl moiety of **5b.1** to afford **5m.1** resulted in over a 100-fold potency loss toward the GT-1a replicon.

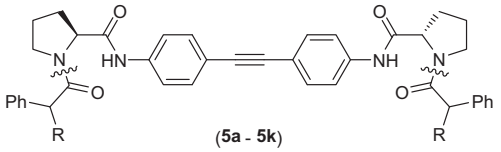
Some basic appendages are associated with relatively weak cytotoxicity in the replicon assay and this may have contributed to the observed weak BVDV inhibitory activities.[14] Considering that potent analogues have been prepared in this series with minimal cytotoxic signals (e.g., **5g.1**: GT-1a EC_{50} = 0.82 nM, GT-1b <0.13 nM, and CC_{50} >10 μM), it is unlikely that generic cytotoxicity is making a meaningful contribution to the observed antiviral activity. Furthermore, the potency of piperidine **5g.1** is >90-fold weaker toward the GT-1b LV/YH resistant mutant than the GT-1b wild type strain (EC_{50} = 12 nM vs <0.13 nM), which is consistent with a mode of action targeting the NS5A protein.

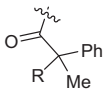
Concurrent with the above SAR surveys, non-basic phenylglycine cap derivatives with H-bond donating capability were also investigated and a diverse set of analogues were identified that exhibited sub-100 nM GT-1a EC_{50}s and picomolar GT-1b EC_{50}s, as illustrated in Table 5. The stereochemical preference was determined for the pair of compound **6a.1** and **6a.2** and, in line with the basic phenylglycine series discussed above, the (*R*)-stereoisomer was the more active. Despite the similar stereochemical requirement, however, there appeared to be a decreased steric tolerance around the benzylic region for this series, as indicated by the ~9-fold GT-1a potency difference between Boc analogue **6a.1** and its methyl carbamate variant **6e**. It is noteworthy that methylation of the acetamide group in **6g**, to afford **6h.1** or **6h.2**, resulted in a significant erosion in both GT-1a and GT-1b inhibitory activities, highlighting the likely role of the NH moiety of the cap in enabling favorable interactions with the NS5A protein. This observation is in concordance with the deleterious effect that tempering the basicity of the phenylglycine-based caps had on GT-1a activity. Although the non-basic glycinamide derivatives illustrated in Table 5 generally had weaker GT-1a potency than the basic family compiled in Table 3—the pK_a of a subset of which is noted in Table 4—they did exhibit an improved cytotoxicity profile.[14]

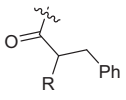
Pharmacokinetic assessment of the selected set of compounds **3g.2**, **5i.1** and **6g** in a 4 h rat screen indicated poor systemic exposure after oral dosing (see Table 6). Since all three analogues demonstrated very good stability in rat liver microsomes (100% remaining after 10 min of incubation) and that two of them had moderate to low clearance after IV dosing, it is believed that the lack of oral exposure most likely reflects low absorption due to poor intestinal permeability.[15]

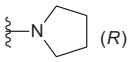
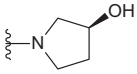
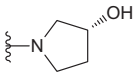
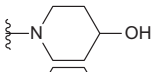
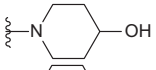
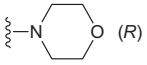
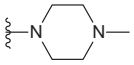
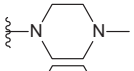
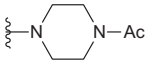
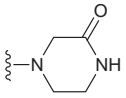
We reported previously that even though the isoquinolinamide cap enhanced the GT-1a potency of the stilbene chemotype, the outcome with other strains was mixed, with enhanced potency for some genotypes (GT-2a NIH) and reduced potency for others (GT-1b & GT-4a, compare **2c** vs **2a** in Tables 1 and 7).[7c] On the other hand, compound **5g.1** exhibited an excellent pan-genotypic inhibitory effect that was significantly improved over that of the prototype **2c** (see Table 7). In addition, **5g.1** showed encouraging potency toward the GT-1b LV/YH resistant replicon (EC_{50} = 12 nM), representing a >800-fold improvement over isoquinolinamide **2b** (EC_{50} >10 μM).[16,17] The virological profile of **5g.1** was critical to the program because it demonstrated that potent and pan-genotypic antiviral activity accompanied by favorable potency toward resistant phenotypes, at least for GT-1b, could be achieved in replicons by appropriate modification of the peripheral regions of the lead chemotype. Beside the virology gain, the phenylglycinamide series attained an improved sp3-carbon percent composition compared to the original isoquinolinamide series (e.g., 40% for **5g.1** vs 22% for **2c**).[8] Finally, while certain aspects of the SAR were discrete and that GT-1a activity was more sensitive than GT-1b activity to the structural and stereochemical factors considered, it became apparent that the locus in the NS5A protein where these

Table 3
Activity and specificity of phenylglycinamide analogues[a,b]


(**5a** - **5k**)


(**5l**)


(**5m**)

Compd	R (stereochemistry)	Replicon activity		GT-1b CC_{50} (nM)	BVDV EC_{50} (nM)
		GT-1a EC_{50} (nM)	GT-1b EC_{50} (nM)		
5a.1	 (*R*)	910	<4.6	9800, >10^4	3760
5a.2	 (*S*)	>9400	<4.6; 13	>10^4	>10^4
5b.1	 (*R*)	<4.6; 13	<4.6	5390	1900
5b.2	 (*S*)	628	<4.6	2300	1200
5c		7.9	<0.13; 0.13	4750	2790
5d		19	<4.6	3170	1380
5e		3.0	0.5	>10^4	>10^4
5f		1.4	0.094	>10^4	3280
5g.1[c]		0.82	<0.13	>10^4	6980
5g.2		600	<4.6, 9.8	5000	2970
5h		125	<4.6	>10^4	>10^4
5i.1		<4.6	0.152	6020	>3330
5i.2		2700	<4.6; 4.4	2840	1360
5j		37	0.42	>10^4	>10^4
5k		388	<4.6	>10^4	NT[d]
5l.1		100	<4.6	3110	697
5l.2		1080	<4.6	2130	769
5m.1	 (*R*)	1290	<4.6	>10^4	1730
5m.2	 (*S*)	3240	500	5820	1490

[a] Except for **5d** and **5h**, both symmetrical diastereomers were prepared for all of the indicated analogues and that for a subset of the analogues (**5c**, **5e**, **5f**, **5j** and **5k**) only the data for the more active stereoisomer are included. Where known, the stereochemistry of the benzylic center is noted in parentheses. Compounds **5g.2**, **5i.2** and **5l.2** are the alternate symmetrical diastereomers of **5g.1**, **5i.1** and **5l.1**, respectively.
[b] Data represent mean values of at least two experiments with a maximum of threefold variation.
[c] EC_{50} in GT-1b LV/YH resistant replicon = 12 nM.
[d] NT, not tested.

Table 4
pK_a of conjugate acid[a]

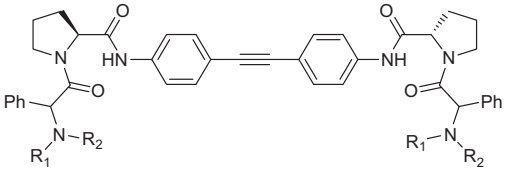
Compd	**5d**	**5g.1**	**5h**	**5k**
pK_a-1	8.6	7.6	5.7	3.4
pK_a-2	8.1	6.8	4.6	2.5

[a] pK_a was measured in pH range 2–11 using potentiometric titration in varying MeOH/H_2O mixture.

inhibitors interact accommodates a wider range of functionalities, an early and encouraging indication of the chemotype's potential for further optimization.

Compounds were prepared as single stereoisomers in one of two ways.[18] In the first approach, pyrrolidines **8x** or **8y**, readily synthesized from the commercially-available anilines **7x** and **7y**, respectively, were coupled with the single stereoisomers of the cap acid precursors to afford final products, as summarized in Scheme 1. Deprotection of carbamate **6a** afforded amine **5a**, which was elaborated further under standard acylation, sulfonylation or reductive amination protocols to provide additional analogues.[19] Compound **5m** was prepared from *N*-Boc-phenylalanine by adoption of the procedure outlined for **5b**. When the requisite acid precursors were not readily available in optically pure form,

Table 5
Activity and specificity of non-basic phenylglycinamide analogues[a,b,c]



Compd	R_1NR_2	Replicon activity	
		GT-1a EC_{50} (nM)	GT-1b EC_{50} (nM)
6a.1	(*R*) BocNH	245	0.002, <0.046
6a.2	(*S*) BocNH	>10^4	<4.6
6b	(*R*) *i*-PrOCONH	137	0.28
6c	(*R*) *n*-PrOCONH	88	0.16
6d	(*R*) EtOCONH	114	0.018
6e[d]	(*R*) MeOCONH	26	0.013
6f	(*R*) *i*-PrCONH	70	0.002, <0.046
6g	(*R*) AcNH	24	0.005, <0.046
6h.1[e]	AcNMe	2450	57
6h.2[e]	AcNMe	>5000	632
6i	(*R*) $MeSO_2NH$	71	<4.6

[a] Where known, the stereoconfiguration of the benzylic center is noted in parentheses.
[b] Data represent mean values of at least two experiments with a maximum of threefold variation.
[c] GT-1b CC_{50} and BVDV EC_{50} >10 μM.
[d] EC_{50} in GT-1b LV/YH resistant replicon = 382 nM.
[e] **6h.1** and **6h.2** are symmetrical diastereomers.

Table 6
Data from 4 h rat PK screen[a]

Compd	**3g.2**	**5i.1**	**6g**
0–4 h AUC (nM h)-PO	166	<LLQ[b]	93
0–4 h AUC (nM h)-IV	5690	ND[c]	2110
CL (mL/min kg)-IV	5.1	ND[c]	13.3

[a] PO/IV dose: 5/2 mg/kg; plasma was sampled 5 times (0.17, 0.5, 1, 2 and 4 h); $n = 2$
[b] Below level of quantitation.
[c] ND, not determined (both rats died).

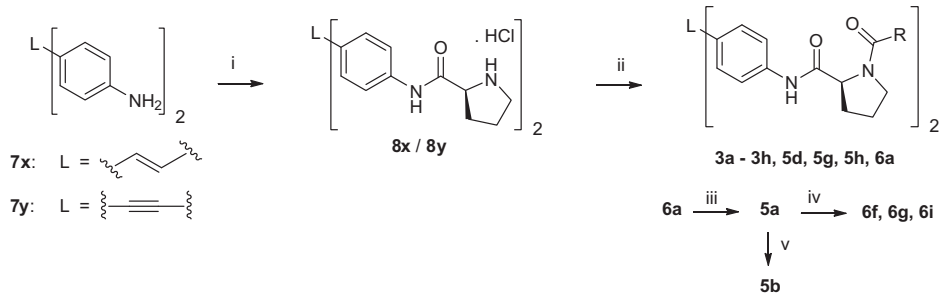
Table 7
EC_{50} (nM) toward a panel of genotype strains[20]

Compd	**2a**	**2c**	**5g.1**
G-2a NIH	260	14	0.083
G-3a	0.30	3.2	0.037
G-3aYH	>3000	74.9	5.4
G-4a	0.16	>10,000	0.042
G-5a	<0.23	3.0	0.017

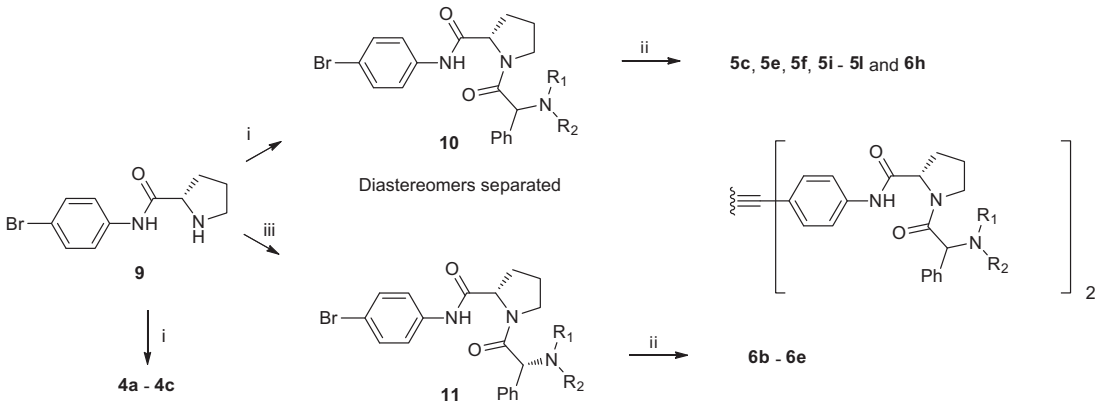
[a] Data represent mean values of at least two experiments with a maximum of threefold variation.

a second approach was implemented where the diastereomeric mixture exemplified by bromide **10** was prepared from the racemic acid, separated by reverse phase preparative HPLC methodology and homocoupled to the alkyne linker under Stille conditions to afford the two symmetrical diastereomers of the final products (see Scheme 2). Compounds **6b–6e** were prepared from the appropriately derivatized (*R*)-phenylglycine under the homocoupling conditions shown in Scheme 2.[20] The acid precursors utilized in both routes were readily obtained either from commercial sources or by adoption of well-established literature protocols, as exemplified in Scheme 3. Assessment of a select set of the monomeric synthetic precursors noted in Scheme 2 in the replicon assay indicated significantly reduced antiviral activity. For example, of the two diastereomeric bromides **10** that were precursors to piperazine **5i.1** and **5i.2**, one exhibited GT-1a/-1b EC_{50}s of >10 μM/0.45 μM while the second had no meaningful activity toward either genotypes, EC_{50}s >10 μM. It is noteworthy that the precursor that exhibited measurable activity toward the GT-1b replicon is the one that afforded piperazine **5i.1**, the more potent of the diastereomeric pair, suggesting discriminatory interaction between the monomeric-pharmacophore element and the NS5A protein.

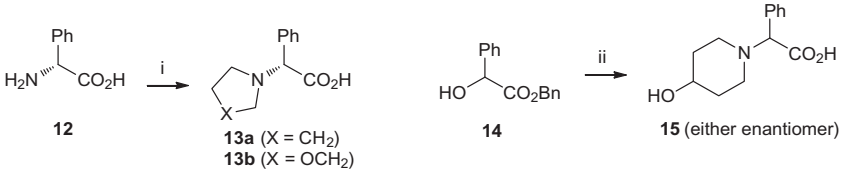
In summary, an SAR campaign that was implemented to address the potential structural liabilities of an early GT-1a/-1b inhibiting lead culminated in the discovery of a new class of phenylglycine cap derivatives that exhibited potent inhibitory activity toward both GT-1a and GT-1b HCV replicons, with EC_{50} reaching single digit picomolar for GT-1b and subnanomolar for GT-1a. In general, these compounds were relatively more active toward GT-1b than GT-1a replicons and that the GT-1a inhibitory activity was considerably more sensitive to the structural modifications examined. Whereas significant steric tolerance was observed for the benzylic position of the glycinamide series, securing high inhibitory potency particularly toward GT-1a required a benzylic substituent with H-bonding capability and (*R*)-stereoconfiguration. Supportive evidence for this pharmacophore hypothesis was garnered, in part, from directional correlation that was established between basicity and the GT-1a EC_{50}. Unlike the phenylglycinamide series, the less potent mandelamide series exhibited a nuance in the SAR where stereochemical preference was dependent on whether the substituent at the carbinol carbon was hydrogen or a methyl group. The potent inhibition exhibited by **5g.1** toward a wider panel of HCV genotypes was an early but crucial demonstration that potent and pangenotypic in vitro replicon inhibitory activity could be secured by targeting the NS5A protein with evolved derivatives of the stilbene chemotype. The subsequent phase of the SAR campaign that dealt with the further optimization of these leads toward the discovery of daclatasvir (**1**) will be the subject of future communications.



Scheme 1. Reagents and conditions: (i) (a) Boc-L-proline, EEDQ, CH_2Cl_2; (b) 4 N HCl, dioxane; (ii) RCO_2H, HATU, *i*-Pr_2EtN, DMF; (iii) 25% TFA/ CH_2Cl_2; (iv) standard acylation or sulfonylation conditions;[18] (v) H_2CO, HCO_2H, CH_2Cl_2, Δ.



Scheme 2. Reagents and conditions: (i) RCO_2H, HATU, *i*-Pr_2EtN, DMF; (ii) $Me_3SnC{\equiv}CSnMe_3$, $Pd(Ph_3P)_4$, DMF, 80 °C; (iii) (a) Boc-(*R*)-phenylglycine, HATU, *i*-Pr_2EtN, DMF; (b) 4 N HCl, dioxane; (c) $ClCO_2R$, Et_3N, THF



Scheme 3. Reagents and conditions: (i) 1,4-dibromobutane or 1-bromo-2-(2-bromoethoxy)ethane, Na_2CO_3, EtOH, 100 °C; (ii) (a) *p*-TsCl, Et_3N, DMAP, CH_2Cl_2; (b) 4-hydroxypiperidine, *i*-Pr_2EtN, THF, Δ; (c) enantiomers separated on Chiracel OJ column; (d) H_2, 10% Pd/C, MeOH. Note: close monitoring is required to minimize the cleavage of the benzylic C–N bond.

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9. ELISA, FRET and luciferase assays were used to assess inhibitory activities toward GT-1a, GT-1b and BVDV replicons, respectively, and Alamar blue assay was used to determine cytotoxicity in GT-1b replicon. ELISA and FRET assays afforded similar EC_{50} value for control compounds in GT-1a replicon. For additional details on the replicon assays, see Ref. 7d and those cited therein.
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12. Number of conformers observed: **4a** (1), **4b** (2), and **4c** (8). Cambridge Structural Database deposition numbers are: CCDC 939741-939743.
13. It is noteworthy that **4b** exhibited EC_{50}s of >1.3 μM (G-1a) and 0.38 μM (G-1b), which were significantly weaker than that of the parental dimeric analogue **3g.2**.
14. For discussions on potential off-target liabilities of basic compounds, see: (a) Azzaoui, K.; Hamon, J.; Faller, B.; Whitebread, S.; Jacoby, E.; Bender, A.; Jenkins, J. L.; Urban, L. *ChemMedChem* **2007**, 874; (b) Peters, J.-U.; Schnider, P.; Mattei, P.; Kansy, M. *ChemMedChem* **2009**, *4*, 680; (c) Meanwell, N. A. *Chem. Res. Toxicol.* **2011**, *24*, 1420; (d) Tarcsay, A.; Keseru, G. *J. Med. Chem.* **2013**, *56*, 1789.
15. All the three analogues had Caco-2 cell permeability <15 nm/s. The susceptibility of compounds to PGP-efflux was not determined.
16. The GT-1b LV/YH EC_{50} of **2c** was not determined.
17. The pan-genotype coverage assessment used hybrid replicons in a Con1 (GT-1b) backbone where either the first 110 amino acid of NS5A (for G-3a, G-3aYH, G-4a or G-5a) or the full length NS5A (for G-2a NIH) was replaced by that of the respective genotype.

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19. It is noteworthy that (*R*)-2-acetamido-2-phenylacetic acid was susceptible to epimerization when coupled with pyrrolidine **8y** under HATU/DIEA activation, an observation with a literature precedent: Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; John Wiley & Sons, Inc: NY, New York, 1999. p 503. Thus, **6g** was prepared from amine **5a** through acylation..
20. Although compounds **6b**–**6e** could also be accessed readily from amine **5a**, they were prepared via the homocoupling route in order to assess the replicon inhibitory activities of some of their monomeric synthetic precursors.