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An efficient, asymmetric solid-phase synthesis of benzothiadiazine-substituted tetramic acids: Potent inhibitors of the hepatitis C virus RNA-dependent RNA polymerase

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Abstract—An efficient, asymmetric solid-phase synthesis of benzothiadiazine-substituted tetramic acids is reported. Starting from commercially available chiral Fmoc-protected α-amino acids loaded onto Wang resin, Fmoc removal, reductive amination followed by amide bond formation, and base-catalyzed cyclization with simultaneous cleavage from the resin provided the desired products. Compounds described are potent inhibitors of the hepatitis C virus RNA-dependent RNA polymerase.
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Worldwide, 170 million people are estimated to be infected chronically with hepatitis C virus (HCV). HCV infection is responsible for 40–60% of all chronic liver disease and 30% of all liver transplants.[1] The inhibition of HCV RNA-dependent RNA polymerase (RdRp), NS5B, is predicted to cure HCV infection with minimal selectivity risk as there are no known human RdRps. In addition, allosteric inhibitors of the NS5B polymerase have been reported and are now entering clinical trials, in hopes of offering improvement in efficacy and tolerability over the current gold standard treatment of interferon with ribavirin.[2] The thiadiazine **2** was known to us as a potent inhibitor of HCV polymerase (NS5B IC_{50} = 200 nM, cellular replicon IC_{50} = 269 nM).[3] In order to explore alternate templates within the thiadiazine series, the tetramic acid core was designed as a mimic for the quinolinone left-hand side of compound **2**. Compound **1a** was thus prepared and although it showed weak NS5B activity, this result suggested that a tetramic acid moiety could indeed serve as a mimic of the quinolinone moiety in compound **2** (Fig. 1).

An efficient parallel array synthesis was sought to optimize this lead (**1a**) and improve the potency.[4] There are several reported methods to prepare tetramic acid derivatives both in solution[5] and solid-phase,[6] but none of these reports contain detailed studies of optical purity of the products prepared via solid-phase. Though an in-house solution-phase asymmetric synthesis of tetramic acid thiadiazines **1** had been demonstrated,[7] we elected to extend this solution-phase asymmetric approach to the solid-phase by using Wang resin[8] (Scheme 1) in order to fully explore the SAR of this tetramic acid lead as efficiently as possible. The final base-promoted Dieckmann cyclization provides the desired tetramic acid ring

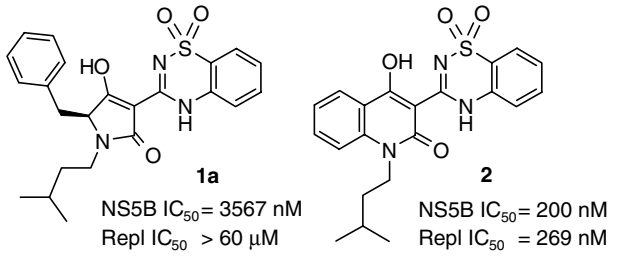

Figure 1. Potent inhibitor of HCV polymerase (**2**) and the alternative inhibitor template lead (**1a**).

Keywords: Hepatitis C; HCV polymerase inhibitors; Solid-phase synthesis.
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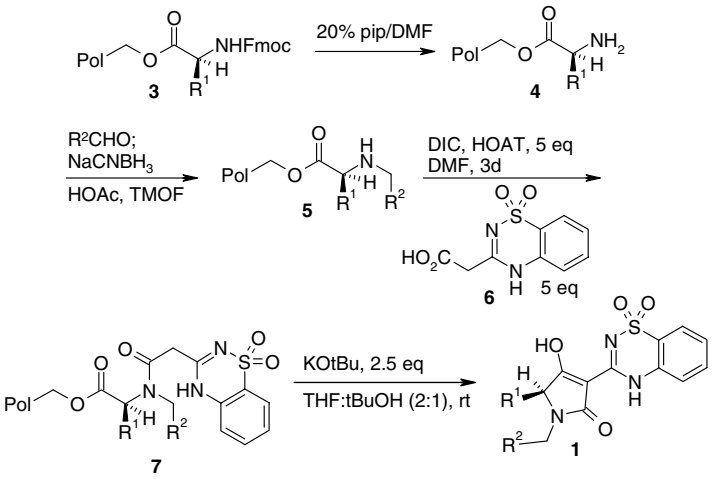
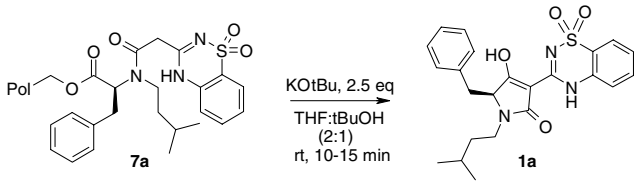


Scheme 1. Asymmetric solid-phase synthesis of benzothiadiazine-substituted tetramic acids.

with convenient simultaneous cleavage from the resin. Our objective was to employ the solid-phase route to rapidly identify the SAR at the N-1 (R^2) and C-2 (R^1) positions of the tetramic acid core.

Solid-phase syntheses often capitalize on the use of excess reagents in solution, sometimes 10–20 equiv, to drive reactions to completion, thereby removing the necessity of purification. A critical challenge was to minimize the equivalents of the custom thiadiazine monomer **6** needed in the acylation step. In addition, it was necessary to optimize conditions for complete cyclization from resin and to determine the enantiomeric excess (ee) of the final product. After some study, it was determined that only 5 equiv of the custom acid **6**,[9] 5 equiv 1-hydroxy-7-azabenzotriazole (HOAT), and 5 equiv diisopropyl carbodiimide (DIC) in DMF for three days at rt were required for the complete acylation.

For the final step, potassium *tert*-butoxide (KO-*t*-Bu) was initially selected because it had been reported to cause minimal racemization in the synthesis of 3-acyl tetramic acids in solution.[10] Using resin-bound **7a**, the cyclization and concomitant cleavage from the resin occurred readily at room temperature and was complete within 15 min.[11] No unreacted starting material was found on the resin after the reaction.



Lastly, the enantiomeric purity of a representative final product needed to be determined. All preparations used enantiomerically pure Fmoc-protected L-amino acids. Many pre-loaded on resin are commercially available and were used where possible. For Fmoc amino acids which were not readily available on Wang resin, we loaded them onto Wang using the reagent combination 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-tetrazole (MSNT)/1-methylimidazole (MeIm) which is reported to minimize racemization.[12]

Racemization can also occur during the Dieckmann cyclization step. The chiral HPLC results shown in Table 1[13] indicate that this on-resin cyclization and cleavage occurs with retention of configuration if either potassium *tert*-butoxide or triethylamine is employed. Although the tetramic acid (**1a**) did not appear to racemize when stored as a solid, it should be noted that deterioration of ee was seen when it was stored over time in solution or was purified by reverse-phase HPLC purification (0.1% TFA). For example, the enantiomeric excess of **1a** dropped from 96% to 84% after one month in ethanol solution. After HPLC purification, a sample of **1a** deteriorated to 23% ee. It was postulated that the acidity of the tetramic acid methine proton would control the rate of acid-promoted racemization via protonation of the ring carbonyl. This acidity would be expected to be similar for the various substitutions at R^2, with more hindered groups being only marginally less acidic. The compound **1a** was thus selected as a suitable model for epimerization studies.[14] A representative procedure for preparation of these tetramic acids is described.[15]

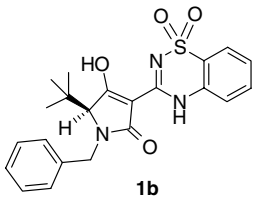
The design elements used in planning the first-generation array of 96 compounds ($12R^1 \times 8R^2$) featured a combination of structural diversity, predicted physical

Table 1. Enantiomeric purity of compound **1a** formed from **7a**[a]

Reaction	Solvent	Base	Equiv	% ee of crude product
1	THF/*t*-BuOH (2:1)	KO-*t*-Bu	2.5	84
2[b]	THF/*t*-BuOH (2:1)	KO-*t*-Bu	2.5	92
3	CH_2Cl_2	TEA	5	95
4[b]	CH_2Cl_2	TEA	5	95

[a] Reactions were halted after 15 min.
[b] Reactions 2 and 4 were quenched with acetic acid, which had little effect on the final enantiomeric purity.

properties (C log *P* and molecular weight), and limited SAR knowledge based on the lead template **1**. Initial results from analogues generated via a solution-phase asymmetric synthesis demonstrated that the *S* configuration was greatly preferred over the *R* configuration, and that R^1 alkyl substitution was preferred in the order *t*-Bu > *i*-Pr > Me > H. The building blocks that composed the array at R^1 included commercially available Fmoc-protected amino acids that possessed branched alkyl, heteroalkyl, cycloalkyl, aryl, and benzyl side chains. Any *tert*-butyl- and Boc-protecting groups on R^1 were removed under acidic conditions (50% TFA/CH_2Cl_2) after the release of the product from the resin. The diverse building blocks that composed the array at R^2 included aryl, heteroaryl, alkyl, cycloalkyl, and branched alkyl aldehydes. The compounds were prepared using polypropylene tubes in conjunction with the Mettler-Toledo Bohdan MiniBlock™ synthesis system. However, this solid-phase synthesis would also be amenable to a variety of synthetic platforms including IRORI, the Robbins Flex-Chem™ System, and the Argonaut Quest™ 210.

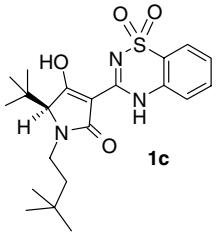


1b

NS5B IC_{50} = 22 nM

Repl IC_{50} = 13 μM

Figure 2. Inhibitory activity for compound **1b** from first lead optimization array.



1c

NS5B IC_{50} = 1.7 nM

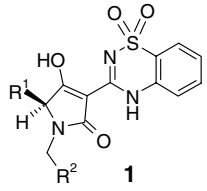
Figure 3. Inhibitory activity for compound **1c** from second lead optimization array.

After protecting group removal and purification, 73 tetramic acid analogues (76% success rate) provided first-generation SAR around sites R^1 and R^2. Analysis of the SAR achieved from this first-generation array revealed primarily that N-benzyl substitution (R^2 = phenyl, **1b**, Fig. 2) provided not only equivalent potency to the N-isoamyl substitution (**1d**, R^2 = $CH_2CH(CH_3)_2$), but also provided improved cellular activity (Replicon) over compound **1a**.

A second-generation array of 96 compounds ($4R^1 \times 24R^2$) was then prepared to probe a variety of substitutions on the benzyl ring (R^2) as well as isosteric replacements at this position. Unfortunately, the 83 compounds prepared (87% success rate) demonstrated that many substitutions on the *N*-benzyl group, as well as replacements of the benzyl group with heteroaryl groups, afforded decreased in vitro potency. However, replacement of the benzyl group (**1b**, Fig. 2) with the 3,3-dimethylbutyl group (**1c**, Fig. 3) was found to be superior (Table 2).

In summary, we report an efficient synthesis of benzothiadiazine-substituted tetramic acids in high enantiomeric purity. Starting from commercially available chiral Fmoc-protected α-amino acids (on resin or loaded onto Wang resin using the MSNT protocol), Fmoc removal, reductive amination followed by amide bond formation,

Table 2. Selected inhibitory activity from initial lead optimization arrays for compound **1** with various R groups



1

Compounds	R^1	R^2	Polymerase IC_{50} (nM)	Repl (μM)
1a	CH_2Ph	$CH_2CH(CH_3)_2$	3567	>60
1b	*t*-Bu	Ph	22	13
1c	*t*-Bu	$CH_2C(CH_3)_3$	1.7	
1d	*t*-Bu	$CH_2CH(CH_3)_2$	18	8
1e	*t*-Bu	4-FPh	59	
1f	*t*-Bu	CH_2-cyclopropyl	530	
1g	Cyclohexyl	$CH_2C(CH_3)_3$	486	
1h	4-FPh	$CH_2C(CH_3)_3$	>748	
1i	CH_2(3-pyridyl)	4-FPh	2466	
1j	*t*-Bu	2-Pyridyl	>100,000	
1k	$CH(CH_3)CH_2CH_3$	Ph	88	37
1m	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	147	159

then base-catalyzed cyclization with simultaneous cleavage from the resin provided the desired products in high optical purity. This solid-phase approach allowed for rapid generation of analogues and SAR. Compound **1c** (Table 2, IC_{50} = 1.7 nM) was the most potent inhibitor of the hepatitis C virus RNA-dependent RNA polymerase found using this approach. Full details of the biological results and additional lead optimization of this tetramic acid series will be reported in a separate communication.

Acknowledgments

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References and notes

1. WHO 1999; CDC/NIH 2002.
2. Condon, S. M.; LaPorte, M. G.; Herbertz, T. *Curr. Med. Chem.-Anti-infective Agents* **2005**, *4*, 99.
3. (a) Darcy, M. G.; Dhanak, D.; Duffy, K. J.; Fitch, D. M.; Sarisky, R. T.; Shaw, A. N.; Tedesco, R.; Zimmerman, M. N. Novel anti-infectives. WO0359356A2, 2003.; (b) Chai, D.; Darcy, M. G.; Dhanak, D.; Duffy, K. J.; Erickson, G. A.; Fitch, D. M.; Gates, A. T.; Johnston, V. K.; Sarisky, R. T.; Sharp, M. J.; Shaw, A. N.; Tedesco, R.; Wiggall, K. J.; Zimmerman, M. N. Novel anti-infectives. WO0298424A1, 2002.
4. In vitro data reported herein were obtained using the Δ21 enzyme.
5. (a) Fustero, S.; de la Torre, M. G.; Sanz-Cervera, J. F.; de Arellano, C. R.; Piera, J.; Simon, A. *Org. Lett.* **2002**, *4*, 3651; For a review on tetramic acids, see: (b) Royles, B. J. L. *Chem. Rev.* **1995**, *95*, 1981.
6. (a) Matthews, J.; Rivero, R. A. *J. Org. Chem.* **1998**, *63*, 4808, and literature cited therein; Synthesis of 3-acyl tetramic acids: (b) Romoff, T. T.; Ma, L.; Wang, Y.; Campbell, D. A. *Synlett* **1998**, 1341.
7. Fitch, D. F.; Evans, K. A.; Chai, D.; Duffy, K. J. *Org. Lett.* **2005**, *7*, 5521.
8. Commercially available resins pre-loaded with L-amino acids were obtained from Polymer Labs (www.polymerlabs.com), typically 0.86 mmol/g, >99% optical purity. Amino acids can also be loaded onto Wang using standard techniques such as described in Sieber, P. *Tetrahedron Lett.* **1987**, *28*, 6147.
9. Fitch, D. M. Anti-infectives. WO2004058150, 2004.
10. Ley, S. V.; Smith, S. C.; Woodward, P. R. *Tetrahedron* **1992**, *48*, 1145.
11. Reaction progress was monitored using LCMS comparing the UV214 absorbance of the product in solution and a constant internal standard versus time. The residual resin was also cleaved under acidic conditions (trifluoroacetic acid in dichloromethane) to check for any remaining starting material.
12. Blankemeyer-Menge, B.; Nimtz, M.; Frank, R. *Tetrahedron Lett.* **1990**, *31*, 1701, MSNT can be obtained from Novabiochem (www.novabiochem.com).
13. A chiral HPLC method was used to determine the enantiomeric excess (Chiralpak AD column with 0.1% TFA in 100% ethanol at 1 mL/min).
14. All products were subjected to final purification via reverse-phase HPLC before biological analysis. Due to the presence of TFA, products suffered some loss of enantiopurity under the acidic reverse-phase HPLC conditions. Compounds of interest could be resynthesized and repurified using alternative conditions, but the SAR trends discovered for this series remained unchanged.
15. Duffy, Kevin J.; Evans, K. A.; Fitch, D. M.; Gates, A. T.; Johnston, V. K.; Sarisky, R. T. Novel anti-infectives. WO2003099801, 2003. All compounds met with satisfactory LC–MS analysis.