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A Highly Efficient, Asymmetric Synthesis of Benzothiadiazine-Substituted Tetramic Acids: Potent Inhibitors of Hepatitis C Virus RNA-Dependent RNA Polymerase

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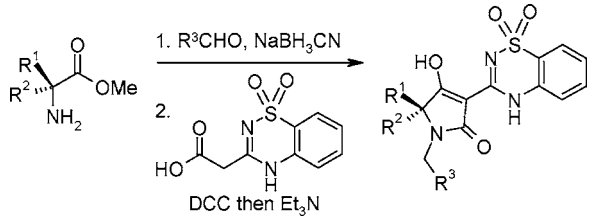
ABSTRACT



An efficient two-pot, asymmetric synthesis of benzothiadiazine-substituted tetramic acids is reported. Starting from commercially available α-amino acids or esters, reductive amination followed by a novel one-pot amide bond formation/Dieckmann cyclization provided the desired products in high yield and optical purity. An analogous solid-phase approach to the same targets is also presented. These compounds were found to be potent inhibitors of hepatitis C virus RNA-dependent RNA polymerase.

Hepatitis C Virus (HCV) was first characterized in 1989 as the major cause of non-A and non-B hepatitis infections.[1] Currently, it is estimated that HCV infects over 170 million people worldwide and is the leading cause of chronic liver disease and liver transplants.[2] The HCV RNA-dependent RNA polymerase (RdRp), NS5B, is essential for viral replication and growth.[3] It has also been structurally characterized,[4] and there are no known mammalian RdRps. For these reasons, it represents an excellent target for the development of anti-HCV therapeutic agents.[5]

High-throughput screening of the GlaxoSmithKline proprietary compound collection resulted in the discovery of 1-butyl-3-(1,1-dioxido-2*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinone (**1**) as a potent HCV polymerase inhibitor.[6] The biochemical characterization and preliminary investigations into the structure−activity relationships (SAR) of compound **1** have been reported elsewhere.[7] Concurrent with these investigations, efforts were undertaken to replace structural elements of the original benzothiadiazinylquinolinone lead in order to alter the physical properties of the

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(1) Choo, Q. L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359−362.
(2) Lauer, G. M.; Walker, B. D. *New Engl. J. Med.* **2001**, *345*, 41−52.
(3) (a) Behrens, S. E.; Tomie, L.; de Francesco, R. *EMBO J.* **1999**, *15*, 12−22. (b) de Francesco, R.; Behrens, S. E.; Tomei, L.; Altamura, S.; Jiricny, J. *Methods Enzymol.* **1996**, *275*, 58−67.
(4) (a) Ago, H.; Adachi, T.; Yoshida, A.; Yamamoto, M.; Habuka, N.; Yatsunami, K.; Miyano, M. *Struct. Fold. Des.* **1999**, *7*, 1417−1426. (b) Bressanelli, S.; Tomei, L.; Roussel, A.; Incitti, I.; Vitale, R. L.; Mathieu, M.; de Francesco, R.; Rey, F. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13034−13039. (c) Lesburg, C. A.; Cable, M. B.; Ferrari, E.; Hong, Z.; Mannarino, A. F.; Weber, P. C. *Nat. Struct. Biol.* **1999**, *6*, 937−943.
(5) For a recent review of known allosteric inhibitors of hepatitis C virus RNA-dependent RNA polymerase, see: Condon, S. M.; LaPorte, M. G.; Herbertz, T. *Curr. Med. Chem. Anti-Infective Agents* **2005**, *4*, 99−110.

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inhibitors and explore the three-dimensional space of the inhibitor binding site. While several modifications led to inactive compounds, replacement of the quinolinone portion of the inhibitor with a tetramic acid was investigated due to its conservation of what was considered to be an essential hydrogen bonding network along the interior of the molecule.[7]

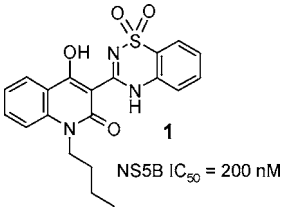
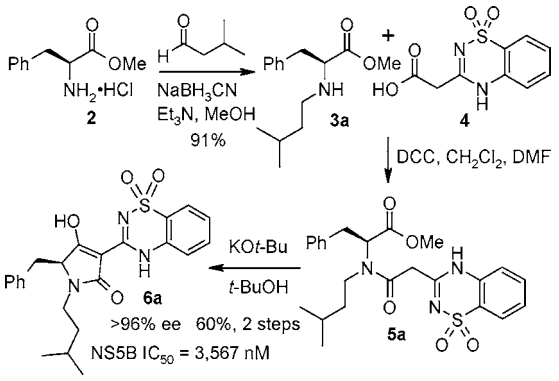


Figure 1. Benzothiadiazinylquinolinone screening lead.

One of the most prominently employed methodologies in the synthesis of tetramic acids is the Dieckmann cyclization.[8] Unfortunately, under standard conditions (NaOMe, MeOH, reflux),[9] racemic products are often obtained. However, investigations by Ley and co-workers demonstrated that such cyclizations can be performed with potassium *t*-butoxide in *tert*-butyl alcohol at ambient temperature without racemization.[10]

Initial investigations into benzothiadiazine-substituted tetramic acids began with the reductive amination[11] of (*S*)-phenylalanine methyl ester followed by acylation with benzothiadiazine acid **4**[12] (Scheme 1). Cyclization under the

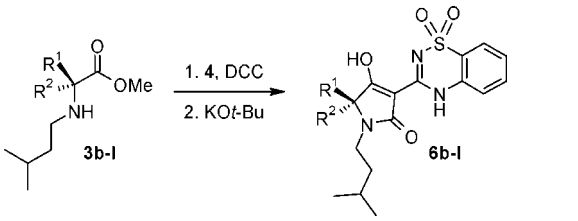
Scheme 1. First Generation Approach to Benzothiadiazine-Substituted Tetramic Acids



Ley conditions provided tetramic acid **6a** in good overall yield and high optical purity.[13] Analogue **6a** demonstrated modest but encouraging activity against NS5B (~3.5 μM), prompting further investigation.[14]

The above synthetic sequence was employed utilizing a variety of α-amino acids in order to rapidly investigate the SAR of this novel inhibitor template (Table 1). Certain trends

Table 1. SAR of Benzothiadiazine-Substituted Tetramic Acids



compound	R^1	R^2	% yield[a]	IC_{50} (nM)	$[\alpha]_D$[b]
6b	H	H	71	2059	
6c	Me	H	69	1436	+23.8°
6d	Me	Me	85	5068	
6e	$-CH_2CH_2-$		83	3468	
6f	Ph	H	77	314	+127.8°
6g	*i*-Pr	H	65	208	−47.5°
6h	H	*i*-Pr	69	6394	+45.0°
6i	*i*-Pr	Me	65	247	−10.5°
6j	*i*-Bu	H	58	546	−43.8°
6k	*c*-Hex	H	87	19	−16.6°
6l	*t*-Bu	H	51	19	−142.1°

[a] Overall yield for two steps. [b] c = 1.0, CH_2Cl_2.

became readily apparent, leading to highly potent inhibitors of NS5B. First, the *S* configuration was greatly preferred over the *R* configuration (e.g., **6g** vs **6h**). Second, inhibitor potency improved with an increase in steric bulk proximal to the stereocenter (R^1 = *t*-Bu ~ *c*-Hex > *i*-Pr > Ph > Me > H). Third, spirocyclic derivatives offered no obvious potency advantage (e.g., **6e**). Importantly, addition of a small substituent to the R^2 position of inhibitor **6g** provided a compound of roughly equal potency (**6i**, R^2 = Me), obviating the risk of racemization.

Given the success of the above solution-phase synthesis of benzothiadiazine-substituted tetramic acids, an analogous solid-phase approach was investigated employing Wang resin

(6) Dhanak, D.; Duffy, K. J.; Johnston, V. K.; Lin-Goerke, J.; Darcy, M. G.; Shaw, A. N.; Gu, B.; Silverman, C.; Gates, A. T.; Nonnemacher, M. R.; Earnshaw, D. L.; Casper, D. J.; Kaura, A.; Baker, A.; Greenwood, C.; Gutshall, L. L.; Maley, D.; DelVecchio, A.; Macarron, R.; Hofmann, G. A.; Alnoah, Z.; Cheng, H. Y.; Chan, G.; Khandekar, S.; Keenan, R. M.; Sarisky, R. T. *J. Biol. Chem.* **2002**, *277*, 38322−38327.

(7) Tedesco, R.; Shaw, A. N.; Bambal, R.; Chai, D.; Concha, N. O.; Darcy, M. G.; Dhanak, D.; Fitch, D. M.; Gates, A.; Gerhardt, W. G.; Halegoua, D. L.; Han, C.; Hofmann, G. A.; Johnston, V. K.; Kaura, A.; Liu, N.; Keenan, R. M.; Lin-Goerke, J.; Sarisky, R. T.; Wiggall, K. J.; Zimmerman, M. N.; Duffy, K. J. *J. Med. Chem.* submitted.

(8) For a review of tetramic acids, see: Royles, B. J. L. *Chem. Rev.* **1995**, *95*, 1981−2001.

(9) Lacey, R. N. *J. Chem. Soc.* **1954**, 850−854.

(10) Ley, S. V.; Smith, S. C.; Woodward, P. R. *Tetrahedron* **1992**, *48*, 1145−1174.

(11) Ramanjulu, J. M.; Joullié, M. M. *Synth. Commun.* **1996**, *26*, 1379−1384.

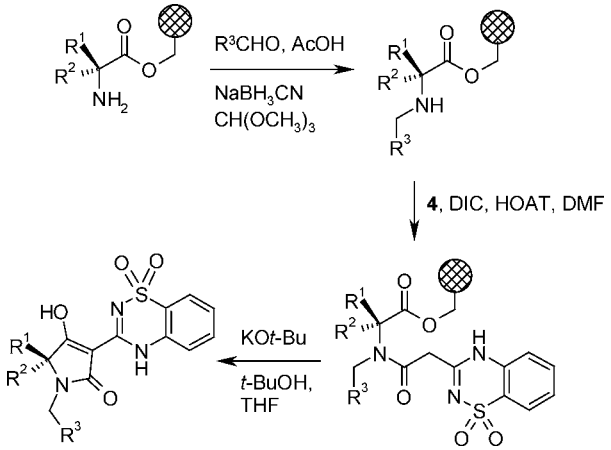
(12) Acid **4** was prepared by hydrolysis (see Supporting Information for details) of the known ethyl ester: Kovalenko, S. N.; Chernykh, V. P.; Shkarlat, A. E.; Ukrainets, I. V.; Gridasov, V. I.; Rudnev, S. A. *Chem. Heterocycl. Compd. (Engl. Transl.)* **1998**, *34*, 791−795.

(13) Optical purity was determined by chiral HPLC analysis (Chiralpak AD column, 0.1% trifluoroacetic acid in ethanol, 1 mL/min, T_r (*S*) = 13.8 min, T_r (*R*) = 25.9 min). Analysis of a sample of **6a** stored for >1 month at ambient temperature showed no sign of racemization.

(14) A scintillation−proximity assay (SPA) using N-terminal-truncated Δ21-NS5B was employed for the determination of IC_{50} values. See refs 6 and 7 for details.

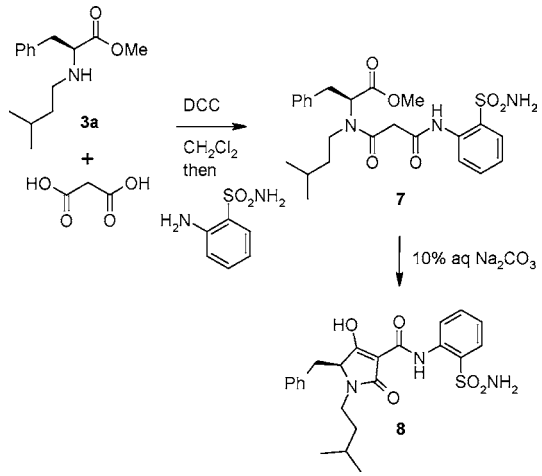
(Scheme 2).[15] Following significant optimization, two separate 96-membered arrays (12 $R^1 \times 8\ R^3$ and 4 $R^1 \times 24\ R^3$)

Scheme 2. Solid-Phase Synthesis of Benzothiadiazine-Substituted Tetramic Acids



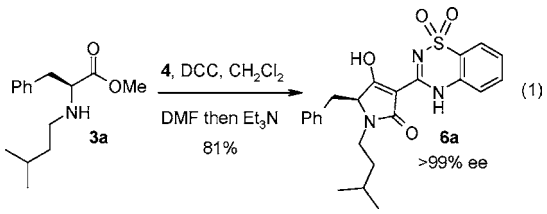
were prepared,[16] allowing for rapid parallel exploration of two different positions on the inhibitor template.[17] Analysis of the SAR obtained from the first array revealed that *N*-benzyl substitution (R^3 = Ph) provided equivalent potency to *N*-isoamyl substitution. Subsequently, the second array demonstrated that further substitution of the *N*-benzyl moiety resulted in attenuated activity. Additionally, *N*-3,3-dimethylbutyl substitution was often found to be superior to other substituents.

Scheme 3. Attempted Synthesis Optimization



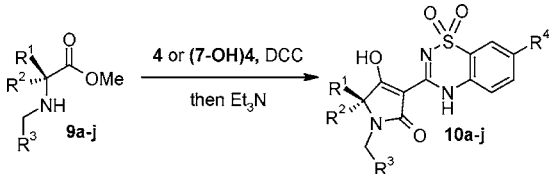
An attempt to further optimize the synthetic sequence through a more convergent approach is shown in Scheme 3. Under unoptimized conditions, α-amino ester **3a** and 2-aminobenzenesulfonamide were iteratively coupled to malonic acid in the presence of DCC. Subjecting malonamide derivative **7** to the mildly basic conditions employed in the synthesis of benzothiadiazine acid **4**[12] resulted exclusively in cyclization to tetramic acid derivative **8**. While limited further attempts to cyclodehydrate **8** to provide the benzothiadiazine were unsuccessful, the formation of **8** under such mild conditions prompted a reinvestigation of the conditions for the Dieckmann cyclization.

Acylation of α-amino ester **3a** with benzothiadiazine acid **4** under the standard conditions followed by in situ addition of triethylamine resulted in clean, rapid cyclization to tetramic acid derivative **6a** (eq 1).[18] This very mild one-pot amide bond formation/Dieckmann cyclization provided optically pure product[13] in high yield and obviated the need for an aqueous workup, allowing for an operationally simplified procedure.



The mild conditions employed in the one-pot amide bond formation/Dieckmann cyclization allowed a variety of functionality to be incorporated into the inhibitor template (Table 2). Phenols, aromatic and saturated heterocycles, and sterically hindered substrates all provided the desired derivatives in high yield.

Table 2. One-Pot Amide Bond Formation/Dieckmann Cyclization



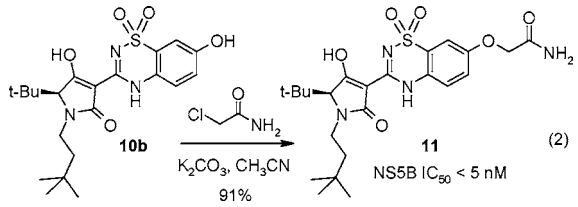
compound	R^1	R^2	R^3	R^4	% yield
10a	*t*-Bu	H	CH_2*t*-Bu	H	85
10b	*t*-Bu	H	CH_2*t*-Bu	OH	83
10c	*i*-Pr	Me	CH_2*i*-Pr	OH	71
10d	$-(CH_2)_2NBoc(CH_2)_2-$		CH_2*i*-Pr	H	79
10e	MePyrrole[a]	H	Ph	H	88
10f	2-Pyr[a]	H	Ph	H	72
10g	3-Pyr[a]	H	Ph	H	81
10h	4-Pyr[a]	H	Ph	H	79
10i	2-Pyr[a]	H	Ph	OH	92
10j	2-Pyr[a]	Me	Ph	OH	93

[a] Racemic started material was employed.

Further modification of the resultant benzothiadiazine-substituted tetramic acids allowed for the incorporation of

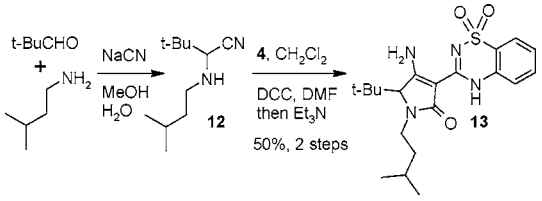
(15) For prior solid-phase approaches to the synthesis of tetramic acids, see: (a) Matthews, J.; Rivero, R. A. *J. Org. Chem.* **1998**, *63*, 4808−4810. (b) Romoff, T. T.; Ma, L.; Wang, Y.; Campbell, D. A. *Synlett* **1998**, 1341−1342. (c) Kulkarni, B. A.; Ganesan, A. *Tetrahedron Lett.* **1998**, *39*, 4369−4372.

key functionality, discovered in the course of optimizing the original benzothiadiazinylquinolinone screening lead,[19] to augment inhibitor binding to NS5B. For example, alkylation of phenol **10b** with chloroacetamide (eq 2) provided oxyacetamide **11** in excellent yield. Excitingly, compound **11** inhibited the HCV polymerase, NS5B, with an *IC_{50} value below the 5 nM limit of detection in our biochemical assay.*



An attempt was made to extend the one-pot amide bond formation/Dieckmann cyclization methodology to the amino variant of the tetramic acid in order to modulate the physical properties of the inhibitors (Scheme 4). A Strecker reaction involving pivalaldehyde and isoamylamine provided α-amino nitrile **12**.[20] Acylation with benzothiadiazine acid **4** under the standard conditions followed by in situ addition of triethylamine provided the desired aminotetramic acid **13**. Although the rate of cyclization was significantly retarded relative to that of the analogous tetramic acid **6l**,[21] a good overall yield was obtained for the two-pot sequence. Interestingly, aminotetramic acid derivative **13** was found to be inactive against NS5B.

Scheme 4. Synthesis of Amino-Substituted Analogue



In conclusion, we have described a novel series of benzothiadiazine-substituted tetramic acids as potent inhibitors of hepatitis C virus RNA-dependent RNA polymerase. Rapid optimization of the original lead structure resulted in the identification of a compound that inhibited the HCV polymerase, NS5B, with an IC_{50} value below the 5 nM limit of detection in our biochemical assay. During the course of these investigations, an efficient one-pot amide bond formation/Dieckmann cyclization method was developed, providing the desired products in high yield and optical purity.

Acknowledgment. Dr. Robert T. Sarisky and Juili Lin-Goerke are gratefully acknowledged for the generation of the IC_{50} data. The authors wish to thank Dr. Arun C. Kaura for the chiral HPLC analysis of compound **6a**.

Supporting Information Available: Experimental procedures and characterization data for all new compounds synthesized. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Details of the solid-phase approach will be reported in a separate publication: Evans, K. A.; Chai, D.; Graybill, T. L.; Sarisky, R. T.; Lin-Goerke, J.; Johnson, V. K.; Burton, G.; Rivero, R. A. Manuscript in preparation.

(17) For certain analogues, a subsequent deprotection step was required. See ref 16 and Supporting Information for details.

(18) Cyclization was generally complete after 1.5 h at ambient temperature.

(19) Details of the optimization of the benzothiadiazine portion of inhibitor **1** will be reported in due course.

(20) For a review of asymmetric variants of the Strecker reaction, see: Gröger, H. *Chem. Rev.* **2003**, *103*, 2795−2827.

(21) Cyclization was incomplete after stirring overnight at ambient temperature with an 18% yield of the uncyclized intermediate obtained in addition to the desire product **13**.