Tetrahedron Letters 49 (2008) 4116–4118

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: http://www.elsevier.com/locate/tetlet

Progress toward the synthesis of piperazimycin A: exploration of the synthesis of γ-hydroxy and γ-chloropiperazic acids

J. Phillip Kennedy, John T. Brogan, Craig W. Lindsley *

Department of Chemistry and Pharmacology, Vanderbilt University, Vanderbilt Medical Center, Vanderbilt Program in Drug Discovery, Vanderbilt Institute of Chemical Biology, Nashville, TN 37232, USA

ARTICLE INFO

Article history:
Received 4 March 2008
Revised 22 April 2008
Accepted 23 April 2008
Available online 27 April 2008

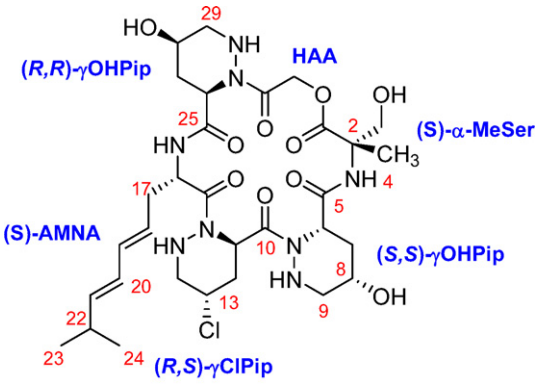
ABSTRACT

Employing Hamada's chemistry with MAOS optimization of several steps, an expedient route to key (3*S*,5*S*)- and (3*R*,5*R*)-γ-hydroxy and (3*R*,5*S*)-γ-chloropiperazic acids, was developed *en route* to a total synthesis of piperazimycin A.

Fenical and co-workers recently reported on the isolation of piperazimycin A **1** from the fermentation broth of a *Streptomyces* sp., cultivated from marine sediments near the island of Guam.[1] A cyclic hexadepsipeptide, **1**, is composed of rare amino acids such as hydroxyacetic acid (HAA), α-methylserine, a novel (*S*)-2-amino-8-methyl-4,6-nonadecadienoic acid (AMNA), two γ-hydroxypiperazic acids (*S*,*S*)-γOHPip1 and (*R*,*R*)-γOHPip2 and one γ-chloropiperazic acid (*R*,*S*)-γClPip (Fig. 1).[1] Piperazimycin A (**1**) proved to be a potent cancer cell cytotoxin which exhibited in vitro cytotoxicity toward multiple tumor cell lines with a mean GI_{50} of 100 nM.[1] Based on its novel molecular architecture, the diversity of non-proteogenic amino acid building blocks, and its potent cytotoxicity, we embarked on a total synthesis campaign aimed at delivering piperazimycin A (**1**) in sufficient quantities to elucidate the molecular target(s) responsible for the biological activity. In this Letter, we describe our progress towards and optimization of the key γ-functionalized piperazic acids.

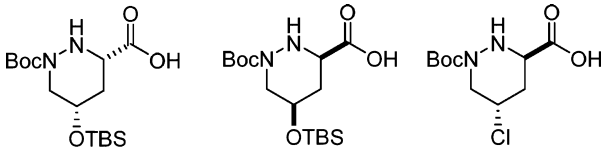
In order to complete a total synthesis of **1**, we first had to synthesize the requisite γ-functionalized piperazic acids **2**–**4** (Fig. 2). Upon examination of the literature, we were surprised that there are very few syntheic routes to these unnatural amino acids, especially with mono-N-protection.[2–8] A single report by Hamada and co-workers demonstrated that the mono-N-Boc analogs **2** and **3** could be accessed, which we desired for the sequence of peptide couplings en route to **1**.[2] In accord with a report from Hale et al.,[7] we anticipated that deprotection of the TBS group of **3** and exposure to PPh_3 and $MeCN–CCl_4$ would install the chlorine with inversion providing the corresponding γ-ClPip **4**; however, this step had never been performed on a mono-N-protected piperazic acid.[3,7]

Hamada's protocol begins with (*R*)-4-chloro-3-hydroxybutanoic acid ethyl ester **5** which is silylated and reduced with DIBAL-H in hexanes to deliver aldehyde **6** in 75% yield for the two steps (Scheme 1).[2] Exposure of **6** to hydrazine hydrate in EtOH for 16 h afforded the cyclic hydrazone **7**, which was then mono-N-benzylated to provide **8** in 89% yield over 2 steps. A Lewis acid-promoted Strecker reaction with stoichiometric $Zn(OTf)_2$ results in an 81:19 (**9**:**10**) ratio of diastereomers in favor of the cis adduct **9**, which



1, Piperazimycin A

Figure 1. Structure of piperazimycin A (**1**).

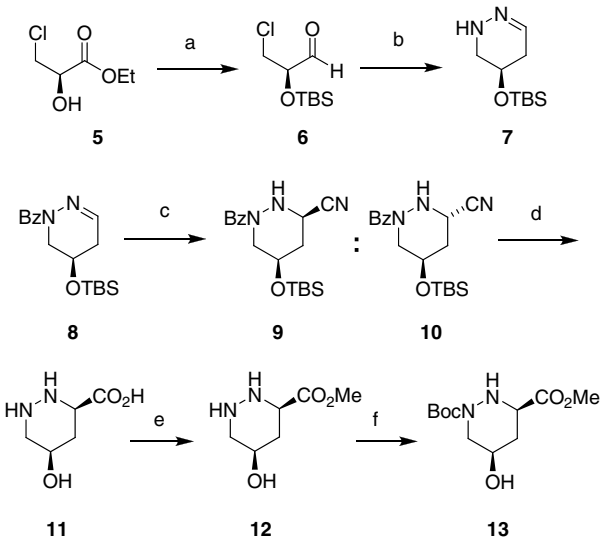


2, (*S*,*S*)-γOTBSPip1 **3**, (*R*,*R*)-γOTBSPip2 **4**, (*R*,*S*)-γClPip

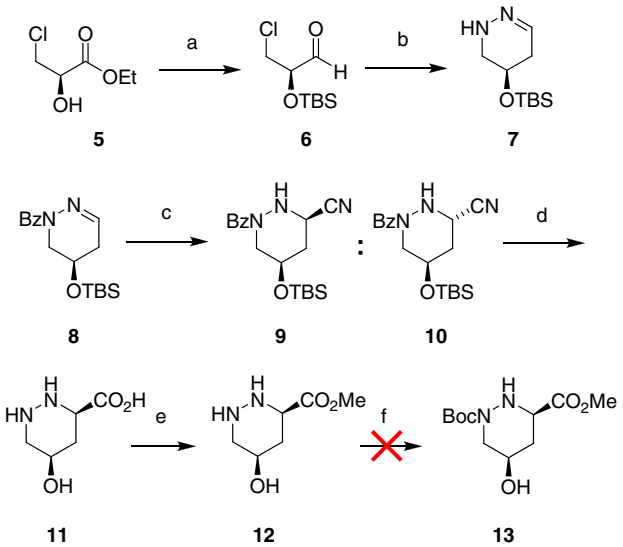
Figure 2. γ-Functionalized piperazic acids target molecules **2**–**4**.

* Corresponding author. Tel.: +1 615 322 8700; fax: +1 615 343 6532.
E-mail address: craig.lindsley@vanderbilt.edu (C. W. Lindsley).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.04.132



Scheme 1. Reagents and conditions: (a) (i) TBSCl, imid., DMF, 0 °C, 98%, (ii) DIBAL-H, hexanes, −78 °C, 77%; (b) NH_2NH_2, EtOH, 0 °C to rt, 16 h; (c) BzCl, pyr, 89%, 2 (b–c) steps; (c) TMSCN, $Zn(OTf)_2$ (1.0 equiv), HOAc (1.0 equiv), 81:19 (**9**:**10**), 70%; (d) 6 N HCl, reflux, 12 h (under argon); (e) cat. TsOH, MeOH, reflux, 19 h; (f) BOC_2O, 1:1 diox–H_2O, 46 h, 59% over 3 (d–e) steps.



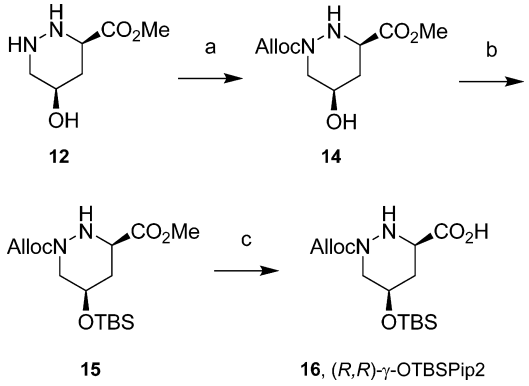
Scheme 2. Reagents and conditions: (a) (i) TBSCl, imid., DMF, 0 °C, 98%, (ii) DIBAL-H, toluene, −78 °C, 91%; (b) NH_2NH_2, EtOH, mw, 120 °C, 20 min; (c) BzCl, pyr, MW, 120 °C, 20 min 90%, 2 (b–c) steps; (c) TMSCN, $Zn(OTf)_2$ (1.0 equiv), HOAc (1.0 equiv), 81:19 (**9**:**10**), 57%:13%; (d) 6 N HCl, mw, 120 °C, 10 min; (e) cat. TsOH, MeOH, MW, 120 °C, 30 min; 75% over 2 steps (d–e); (f) BOC_2O, 1:1 diox–H_2O, 46 h, 5–25%.

can be easily separated by column chromatography. Deprotection and hydrolysis with 6 N HCl at reflux for 12 h under argon led to **11** which is then esterified to **12** with catalytic TsOH in refluxing methanol for 19 h. Finally, **12** is selectively mono-Boc protected at the *N*1 position by treatment with BOC_2O in 1:1 dioxane–water for 46 h to deliver **13**, two steps away from (*R*,*R*)-γOHPip2 (**4**).[2] Moreover, the anlaogous precursor to (*S*,*S*)-γOHPip1 (**3**) could also be accessed via this route by starting the sequence with (*S*)-4-chloro-3-hydroxybutanoic acid ethyl ester in place of **5**. While this was an attractive route, we felt there was room to optimize as many steps required long reaction times (12–46 h).[2]

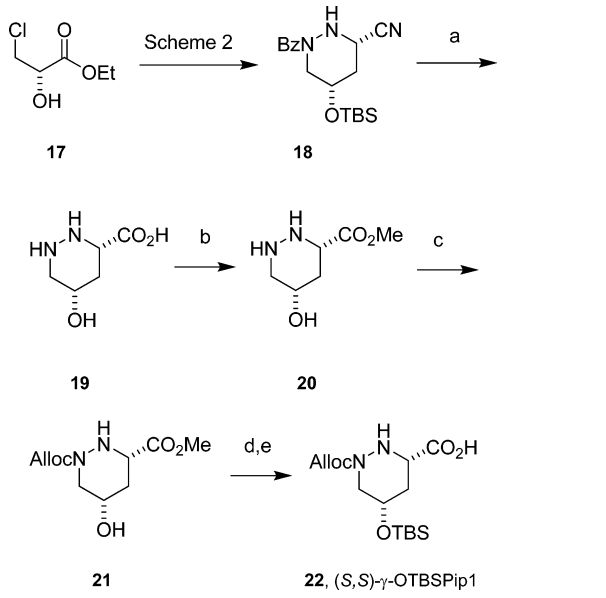
Our modified Hamada protocol employed microwave-assisted organic synthesis (MAOS) to dramatically reduce reaction times for four key steps.[9,10] In our hands, (*R*)-4-chloro-3-hydroxybutanoic acid ethyl ester **5** is silylated and reduced with DIBAL-H in *toluene*, in place of hexanes, to deliver aldehyde **6** in an improved yield of 91% for the two steps (Scheme 2). An MAOS condensation of **6** with hydrazine provides the cyclic hydrazone **7** in only 20 min (vs 16 h), which is then mono-N-benzylated under microwave irradiation (120 °C, 20 min) to provide **8** in 90% yield over 2 steps. The key Strecker reaction was performed as prescribed and delivered an 81:19 ratio of **9**:**10**, which were easily separated by flash column chromatography to afford a 57% yield of the cis diastereomer **9**. Deprotection and saponification with 6 N HCl in the microwave (120 °C, 10 min) delivers **11** which is then subjected to another MAOS reaction (120 °C, 30 min) with cat. TsOH in MeOH to provide ester **12** in 75% yield for the two steps. At this point, the modified reaction protocol reduced total reaction time for the synthesis dramatically and provided improvements in the overall yield on a mutli-gram scale. Specifically, the reaction times for the synthesis of **7**, **8**, **11,** and **12** were reduced from 49 h to only 80 min (an ~40-fold reduction) by virtue of microwave irradiation.

However, we were unable to access the mono-N-Boc derivative **13** in reasonable yield. While we could prepare **13** on an ~25 mg scale in yields ranging from 5% to 25%, the reaction failed on a larger, synthetically useful scale en route to a total synthesis of piperazimycin A (**1**). Revisiting Hamada's paper indicated that they only prepared **13** on a <30 mg scale.[2] We explored alternative protocols (MAOS and conventional) and alternative reagents for formation of the *t*-butyl carbamate $(BOC)_2O$ and various conditions, BOC-ON, etc. . ., but all attempts provided very little conversion to **13**. Danishevsky and co-workers previously demonstrated that the analogous Teoc derivative of **13** could be prepared on a large scale, which also worked well in our hands, but we required an alternative protecting group orthogonal to fluoride deprotection.[4–6] Other simple aliphatic carbamates (methyl, ethyl) worked equally well, but deprotection conditions proved too harsh.

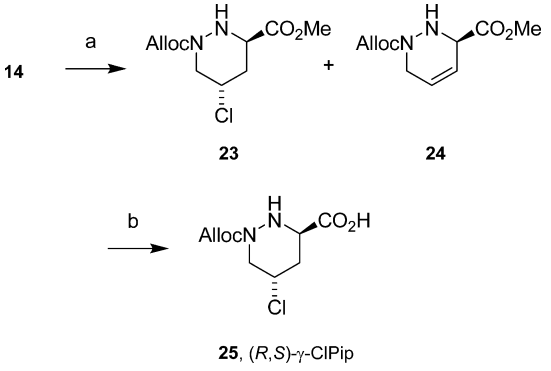
After many unsuccessful attempts, we developed a high, yielding, and scalable route to an Alloc-protected congener of **13** as our modified MAOS-route to **12** worked well on multi-gram scale, and at this point, we did not further explore selective *N*1 protection. As shown in Scheme 3, exposure of **12** to Alloc-Cl in pyridine under microwave irradiation for 10 min at 120 °C provides the previously undescribed *N*-Alloc congener (*R*,*R*)-**14** in 90% yield. Another MAOS mediated silyation provided **15** in 81% yield, followed by a saponification step to deliver the key *N*-Alloc-(*R*,*R*)-γ-OTBSPip2 **16** in quantitative yield. Following Scheme 2, but substituting the (*R*)-4-chloro-3-hydroxybutanoic acid ethyl ester **5** with the corresponding (*S*)-enantiomer **17** provides **18** in equivalent yield and diastereomeric ratio as **9** (Scheme 4). Deprotection and hydrolysis



Scheme 3. Reagents and conditions: (a) Alloc-Cl, TEA, pyr, MW, 120 °C, 10 min 90%; (b) TBS–OTf, 2,4,6-collidine, MW, 120 °C, 10 min, 81%; (c) LiOH, aq THF, 99%.



Scheme 4. Reagents and conditions: (a) 6 N HCl, mw, 120 °C, 10 min; (b) cat. TsOH, MeOH, MW, 120 °C, 30 min; (c) Alloc-Cl, TEA, pyr, MW, 120 °C, 10 min 74% over three steps (a–c); (d) (i) TBS-OTf, 2,4,6-collidine, MW, 120 °C, 10 min; (ii) LiOH, aq THF, 82% over 2 steps.



Scheme 5. Reagents and conditions: (a) PPh_3, MeCN–CCl_4 (1:1), rt, 4 h, 40% (**23**), 20% (**24**); (b) LiOH, aq THF, 0 °C, 89%.

with 6 N HCl in the microwave (120 °C, 10 min) delivers **19** which is then subjected to another MAOS reaction (120 °C, 30 min) with cat. TsOH in MeOH to provide ester **20**. Finally, **20** is selectively mono-Alloc protected at the *N*1 position by treatment with Alloc-Cl in pyridine at 0 degrees, then heated under microwave irradiation for 10 min at 120 °C providing the previously undescribed *N*-Alloc congener (*S*,*S*)-**21** in 75% yield for the 3 steps. Another MAOS mediated silyation provided **22** in 83% yield, followed by a saponification step to deliver the key *N*-Alloc-(*S*,*S*)-γ-OTBSPip1 **22** in quantitative yield.

With two of the three requisite piperazic acids in hand, effort now focused on preparing the remaining *N*-Alloc analog of target molecule (*R*,*S*)-γ-ClPip **4**.[11] As shown in Scheme 5, the application of the Hale protocol[7] (PPh_3, MeCN–CCl_4) employing (*R*,*R*)-**14** provided (*R*,*S*)-**23** in 40% isolated yield along with a 20% yield of the elimination product **24**. We were pleased to see that the first application of the Hale protocol[7] with a mono-N-protected substrate provided an equivalent yield to the di-N-protected piperazic acids without greater propensity for elimination to form **24**. With **23** in hand, mild saponification conditions delivered previously unknown *N*-Alloc-(*R*,*S*)-γ-ClPip **25** in 89% yield (unoptimized).

In summary, we have modified Hamada's original approach and developed an accelerated, high yielding protocol, taking advantage of the power of MAOS, for the synthesis of γ-functionalized piperazic acids. We have also demonstrated that selective mono-Boc protection at the *N*1 position of piperazic acids is a poor reaction that proceeds only on small scale. Importantly, we have developed a scalable MAOS protocol for selective Alloc protection at the *N*1 position of functionalized γ-piperazic acids. Following this new synthetic route, we have prepared previously unknown *N*-Alloc-(*S*,*S*)-γ-OTBSPip1 **22**, *N*-Alloc-(*R*,*R*)-γ-OTBSPip2 **16**, and *N*-Alloc-(*R*,*S*)-γ-ClPip **25** and are now poised to initiate the first total synthesis of piperazimycin (A) **1**. Further refinements and the total synthesis of **1** will be reported in due course.

Acknowledgments

The authors thank the Department of Pharmacology at Vanderbilt and the Vanderbilt Institute of Chemical Biology (VICB) for support of this research. J.P.K. thanks the VICB for a generous predoctoral fellowship.

References and notes

1. Miller, E. D.; Kauffma, C. A.; Jensen, P. R.; Fenical, W. *J. Org. Chem.* **2007**, *72*, 323–330.
2. Makino, K.; Jiang, H.; Suzuki, T.; Hamada, Y. *Tetrahedron: Asymmetry* **2006**, *17*, 1644–1649.
3. Ushiyama, R.; Yonezawa, Y.; Shin, C.-g. *Chem. Lett.* **2001**, *11*, 1172–1173.
4. Kamenecka, T. M.; Danishefsky, S. J. *Chem. A. Eur J.* **2001**, *7*, 41–63.
5. Depew, K. M.; Kamenecka, T. M.; Danishefsky, S. J. *Tetrahedron Lett.* **2000**, *41*, 289–292.
6. Kamenecka, T. M.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2995–2998.
7. Hale, K. J.; Jogiya, N.; Manaviazar, S. *Tetrahedron Lett.* **1998**, *39*, 7163–7166.
8. Hassall, C. H.; Ramachandran, K. L. *Heterocycles* **1977**, *7*, 119–122.
9. Shipe, W. D.; Yang, F.; Zhao, Z.; Wolkenberg, S. E.; Nolt, M. B.; Lindsley, C. W. *Heterocycles* **2006**, *70*, 665–689.
10. Daniels, R. N.; Kim, K.; Lewis, J. A.; Lebois, E. P.; Muchalski, H.; Lindsley, C. W. *Tetrahedron Lett.* **2008**, *49*, 305–310.
11. Representative experimental for the synthesis of (3*R*,5*S*)-1-(allooxycarbonyl)-5-chloropiperazine-3-carboxylic acid (*N*-Alloc-(*R*,*S*)-γ-ClPip) **26**: Into a 5 mL microwave reaction vessel was placed **12** (116 mg, 0.72 mmol), pyridine (4 mL) and cooled to 0 °C. Allyl chloroformate (154 μL, 1.45 mmol) was slowly added via syringe. The microwave vial was then sealed and irradiated for 10 min at 120 °C. After cooling, the reaction mixture was transferred to a separatory funnel, extracted into EtOAc and washed with 100 mL satd $NaHCO_3$. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude product was purified via silica gel chromatography with EtOAc–hexanes (1:3 to 1:2 gradient) to afford pure **14** (130 mg, 74%) as a light brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.94 (m, 1H), 5.33 (d, J = 17.2 Hz, 1H), 5.25 (d, J = 10.4 Hz, 1H), 4.65 (d, J = 6.0 Hz, 2H), 4.07 (m, 1H), 3.87 (m, 1H), 3.78 (s, 3H), 3.15 (m, 1H), 2.36 (m, 1H), 1.76 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.4, 155.4, 132.7, 118.4, 77.5, 67.1, 64.7, 57.1, 52.8, 51.4; LCMS, single peak, 1.41 min, *m*/*e*, 245.1 (M+1). In a 25 mL a round-bottomed flask was placed **14** (50 mg, 0.21 mmol) and it was dissolved in 5 mL of 1:1 MeCN:CCl_4 and cooled to 0 °C. Triphenylphosphine (81 mg, 0.31 mmol) was then added in one portion. The reaction was allowed to slowly reach room temperature and stirred overnight. Once complete by TLC, the reaction was concentrated and purified via silica gel chromatography with EtOAc–hexanes (1:1) to afford pure **24** (54 mg, 40%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.91 (m, 1H), 5.31 (d, J = 17.2 Hz, 1H), 5.21 (d, J = 10.4 Hz, 1H), 4.65 (d, J = 7.6 Hz, 2H), 4.33 (m, 1H), 4.02 (m, 2H), 3.73 (s, 3H), 3.69 (m, 1H), 2.21 (m, 2H), 1.76; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171, 155.7, 132.6, 118.2, 71.8, 67, 53.8, 52.6, 50.9, 35.8; LCMS, single peak, 2.43 min, *m*/*e*, 263.1 (M+1). In 25 mL a round-bottomed flask was placed **24** (65 mg, 0.25 mmol) and dissolved in 5 mL of THF and cooled to 0 °C. One-hundred forty microliters of 2 M LiOH was slowly added via pipette for 20 min. The reaction was then acidified to pH 3 with 2 M HCl, and extracted two times with EtOAc. The organic layers were combined and washed with brine, dried over $MgSO_4$ and concentrated to provide a light yellow oil (**26**) of analytical purity (55 mg, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.92 (m, 1H), 5.32 (d, J = 17.2 Hz, 1H), 5.25 (d, J = 10.4 Hz, 1H), 4.65 (m, 2H), 4.2 (m, 1H), 4.02 (m, 1H), 3.9 (m, 1H), 3.75 (m, 1H), 2.4 (m, 1H), 2.2 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173, 155.5, 132.6, 118.9, 77.5, 72.1, 67.6, 52.1, 51.2, 35.3; LCMS, single peak, 2.11 min, *m*/*e*, 249.1 (M+1).