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Total Synthesis of the Cytotoxic Enehydrazide Natural Products Hydrazidomycins A and B by a Carbazate Addition/Peterson Olefination Approach

Ramsay E. Beveridge and Robert A. Batey*

Davenport Research Laboratories, Department of Chemistry, University of Toronto, 80 St. George Street, Toronto ON, Canada

rbatey@chem.utoronto.ca

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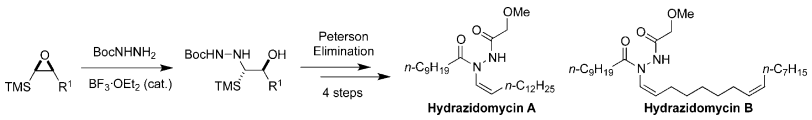
ABSTRACT



The first total syntheses of two natural antitumor enehydrazide compounds (hydrazidomycins A and B) and a related positional isomer of hydrazidomycin B (elaiomycin B) have been accomplished in a rapid and stereocontrolled fashion using a Peterson elimination approach. A regioselective silyl epoxide ring opening reaction with Boc-carbazate followed by base-mediated Peterson siloxide elimination stereospecifically installed the key *Z*-enehydrazide functionality. The use of Boc-carbazate allowed for the differential functionalization of the hydrazide nitrogens.

In contrast to their common incorporation in synthetic pharmaceutical molecules,[1] hydrazine and hydrazide functional groups rarely occur in natural products.[2−4] Although unusual, natural products with hydrazine functionality can exhibit impressive biological activities, as demonstrated by the potent broad spectrum antibiotic (+)-negamycin[3] and the piperazic acid containing[4] cytotoxic agents chloptosin, himastatin, and piperazimycin. In addition, a number of unusual enehydrazide compounds have recently been isolated from *Streptomyces* species including hydrazidomycins A−C,[5] elaiomycins B and C,[6] and geralcin B and C[7] **1**−**6** (Figure 1). These compounds constitute a new family of N−N bond containing biologically active natural products, which include cytotoxic properties. In particular, hydrazidomycin A displays an average *in vitro* IC_{50} cytotoxicity of 370 nM across a panel of 12 human cancer cell lines with specific values approaching clinical relevance (e.g., prostate PC-3M = 105 nM, stomach GXF 251L = 144 nM, colon CXF 269L = 222 nM).[5]

The unprecedented Z-enehydrazide structure of the hydrazidomycins and the nanomolar anticancer activity of **1** prompted us to investigate this new enehydrazide antineoplastic pharmacophore and target these compounds for synthesis. We now outline a method for the synthesis of previously unknown Z-enehydrazides utilizing

(1) For reviews on hydrazines and derivatives, see: (a) Schmidt, E. W., Ed. *Hydrazine and Its Derivatives: Preparation, Properties, Applications*, 2nd ed.; John Wiley & Sons: New York, 2001. (b) Rademacher, P. *Sci. Synth.* **2009**, *40b*, 1133–1210. (c) Rollas, S.; Kucukguzel, S. G. *Molecules* **2007**, *12*, 1910–1939. (d) Hassan, A. A.; Shawky, A. M. *J. Heterocycl. Chem.* **2010**, *47*, 745–763. (e) Ragnarsson, U. *Chem. Soc. Rev.* **2001**, *30*, 205–213.

(2) Reviews on N−N bond containing natural products, see: (a) Blair, L. M.; Sperry, J. *J. Nat. Prod.* **2013**, *76*, 794–812. (b) LaRue, T. A. *Lloydia* **1977**, *40*, 307–321.

(3) (+)-Negamycin: (a) Isolation: Hamada, M.; Takeuchi, T.; Kondo, S.; Ikeda, Y.; Naganawa, H.; Maeda, K.; Okami, Y.; Umezawa, H. *J. Antibiot.* **1970**, *23*, 170–171. Select recent syntheses: (b) Nishiguchi, S.; Sydnes, M. O.; Taguchi, A.; Regnier, T.; Kajimoto, T.; Node, M.; Yamazaki, Y.; Yakushiji, F.; Kiso, Y.; Hayashi, Y. *Tetrahedron* **2010**, *66*, 314–320. (c) Hayashi, Y.; Regnier, T.; Nishiguchi, S.; Sydnes, M. O.; Hashimoto, D.; Hasegawa, J.; Katoh, T.; Kajimoto, T.; Shiozuka, M.; Matsuda, R.; Node, M.; Kiso, Y. *Chem. Commun.* **2008**, 2379–2381.

(4) For a recent review, see: Oelke, A. J.; France, D. J.; Hofmann, T.; Wuitschik, G.; Ley, S. V. *Nat. Prod. Rep.* **2011**, *28*, 1445–1471.

(5) Ueberschaar, N.; Le Sage Tchize Ndejouong, B.; Ding, L.; Maier, A.; Fiebig, H.-H.; Hertweck, C. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5839–5841.

(6) (a) Helaly, S. E.; Pesic, A.; Fiedler, H.-P.; Sussmuth, R. D. *Org. Lett.* **2011**, *13*, 1052–1055. (b) Kim, B.-Y.; Willbold, S.; Kulik, A.; Helaly, S. E.; Zinecker, H.; Wiese, J.; Imhoff, J. F.; Goodfellow, M.; Sussmuth, R. D.; Fiedler, H.-P. *J. Antibiot.* **2011**, *64*, 595–597.

(7) (a) Geralcins A and B: Le Goff, G.; Martin, M.-T.; Servy, C.; Cortial, S.; Lopes, P.; Bialecki, A.; Smadja, J.; Ouazzani, J. *J. Nat. Prod.* **2012**, *75*, 915–919. (b) Geralcins, C-E; Le Goff, G.; Martin, M.-T.; Iorga, B. I.; Adelin, E.; Servy, C.; Cortial, S.; Ouazzani, J. *J. Nat. Prod.* **2013**, *76*, 142–149.

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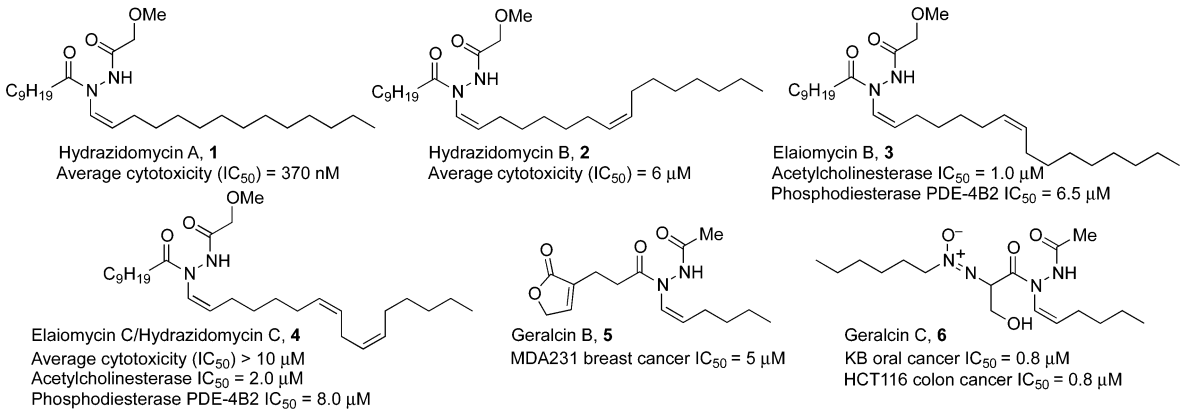
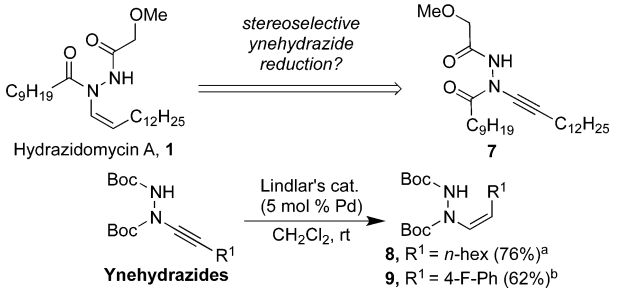


Figure 1. Biologically active enehydrazide natural products **1**–**6**.

a Peterson olefination reaction sequence, and its application to the first stereocontrolled synthesis of the most biologically relevant members **1**–**3** of this unusual family of natural products.

The main synthetic challenges presented by structures **1**–**3** include the central *cis*-enehydrazide moiety, differentiation of the N-atoms by two different acyl groups, and positional/geometric control of the additional *cis*-alkene in **2** and **3**. Surprisingly, in comparison to the structurally similar and highly studied enamides,[8,9] there are very few reports concerning enehydrazide synthesis[10] and to the best of our knowledge there are no examples of stereocontrolled *cis*-enehydrazide generation. In addition, while synthetic strategies to access enamides should in principle be applicable to target molecules **1**–**3**, the synthesis of highly substituted hydrazide derivatives possessing three or more distinct functional groups is not trivial.[11] Consequently, the combined task of developing a route to access the synthetically unexplored core Z-enehydrazide functionality with the correct N–N bond substituent distribution posed unique synthetic challenges. This was for instance encountered in our initial synthetic strategy based on the application of our recent work on related ynehydrazide functional groups,[12] via a syn-stereoselective reduction of an appropriately functionalized alkynyl hydrazide. While model reactions on simple ynehydrazides provided proof-of-concept for the selective synthesis of *Z*-enehydrazides via Lindlar reduction (Scheme 1), synthesis of the multisubstituted ynehydrazide precursor **7** required for the synthesis of **1** proved difficult.[13] For example, reaction of the lithium acetylide of tetradecyne with DBAD (di-*tert*-butyl-azodicarboxylate) and *in situ* capping with methoxyacetyl chloride furnished ynehydrazide **10** (Scheme 2). Selective conversion of the N-Boc protected alkynyl-linked group to the required decanoyl amide was not possible, due to the acid lability of the ynehydrazide component. Nevertheless, the structural analogs **11** and **13** were readily accessible from **10**, demonstrating the utility of an ynehydrazide-based strategy for the synthesis of congeners of **1** to test for cytotoxic activity.

Scheme 1. Stereoselective Ynehydrazide Reduction Based Disconnection to Hydrazidomycin A



[a] ≥10:1 *Z*/*E* as determined by ^{1}H NMR analysis. [b] 6:1 *Z*/*E* as determined by ^{1}H NMR analysis.

(8) For reviews on enamide synthesis, see: (a) Dehli, J. R.; Legros, J.; Bolm, C. *Chem. Commun.* **2005**, 973–986. (b) Tracey, M. R.; Hsung, R. P.; Antoline, J.; Kurtz, K. C. M.; Shen, L.; Slafer, B. W.; Zhang, Y. *Sci. Synth.* **2005**, *21*, 387–475.

(9) Selected recent examples: (a) Murugan, K.; Huang, D.-W.; Chien, Y.-T.; Liu, S.-T. *Tetrahedron* **2013**, *69*, 268–273. (b) Panda, N.; Mothkuri, R. *J. Org. Chem.* **2012**, *77*, 9407–9412. (c) Genovino, J.; Lagu, B.; Wang, Y.; Toure, B. B. *Chem. Commun.* **2012**, 6735–6737. (d) Pasqua, A. E.; Crawford, J. J.; Long, D.-L.; Marquez, R. *J. Org. Chem.* **2012**, *77*, 2149–2158. (e) Buba, A. E.; Arndt, M.; Gooßen, L. J. *J. Organomet. Chem.* **2011**, *696*, 170–178. (f) Goossen, L. J.; Blanchot, M.; Arndt, M.; Salih, K. S. M. *Synlett* **2010**, 1685–1687. (g) Bolshan, Y.; Batey, R. A. *Angew. Chem., Int. Ed.* **2008**, *47*, 2109–2112.

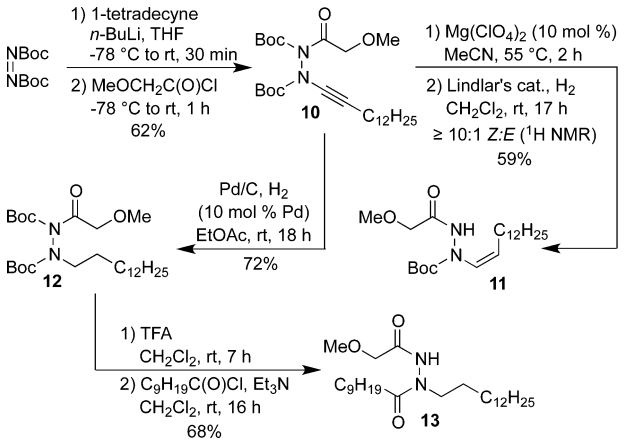
(10) (a) Lerche, H.; Wanninger, G.; Severin, T. *Synthesis* **1982**, 1111–1112. (b) Barluenga, J.; Moriel, P.; Aznar, F.; Valdes, C. *Org. Lett.* **2007**, *9*, 275–278. (c) Rodriguez Rivero, M.; Buchwald, S. L. *Org. Lett.* **2007**, *9*, 973–976. (d) Dubois, M.; Deniau, E.; Couture, A.; Granclandon, P. *Tetrahedron* **2012**, *68*, 7140–7147. (e) Ahlbrecht, H.; Henk, H. *Chem. Ber.* **1975**, *108*, 1659–1681.

(11) (a) Brosse, N.; Pinto, M.-F.; Jamart-Gregoire, B. *J. Org. Chem.* **2000**, *65*, 4370–4374. (b) Brosse, N.; Pinto, M.-F.; Jamart-Gregoire, B. *Eur. J. Org. Chem.* **2003**, 4757–4764. (c) Rasmussen, L. K. *J. Org. Chem.* **2006**, *71*, 3627–3629. (d) Pinto, M.-F.; Brosse, N.; Jamart-Gregoire, B. *Synth. Commun.* **2002**, *32*, 3603–3610. (e) Tsubrik, O.; Maeorg, U. *Org. Lett.* **2001**, *3*, 2297–2299. (f) Tsubrik, O.; Sillard, R.; Maeorg, U. *Synthesis* **2006**, 843–846. (g) Maeorg, U.; Ragnarsson, U. *Tetrahedron Lett.* **1998**, *39*, 681–684.

(12) Beveridge, R. E.; Batey, R. A. *Org. Lett.* **2012**, *14*, 540–543.

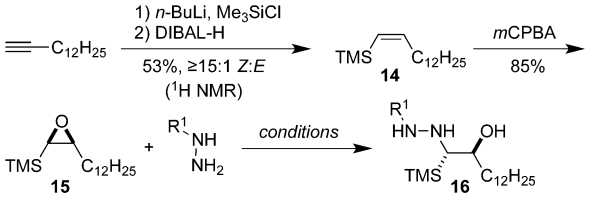
(13) Further details of hydrazidomycin synthetic studies including unsuccessful approaches will be published elsewhere in a full article.

Scheme 2. Ynehydrazide Based Approach to Hydrazidomycin A Analogs



The problems encountered above prompted the investigation of alternative strategies for the stereocontrolled formation of enehydrazides. A Peterson elimination[14] based approach was attractive due to the possibility of achieving highly stereocontrolled formation of either *Z*- or *E*-enehydrazides by appropriate substrate choice or elimination conditions.[15,16] The successful realization of this synthetic plan required development of regio- and stereoselective access to previously unknown hydrazine functionalized vicinal silanol derivatives. In this regard, prior work on Z-enamides[15,16] suggested that the key *anti*-β-silyl-β-hydrazidoalcohols, needed for a base mediated Peterson elimination to Z-enehydrazides, might be accessible by silyl-directed ring opening of a *cis*-silyl epoxide with a hydrazine nucleophile. Although sodium azide is known to regioselectively ring-open silyl epoxides, there are very few examples of such a reaction with other less nucleophilic heteroatom nucleophiles[15d,e] and none using hydrazines. Attempts to develop a new silyl epoxide hydrazine ring-opening reaction using either hydrazine or $MeOCH_2CONHNH_2$ with either NH_4Cl[15a,b,16] or $BF_3\cdot OEt_2$[15d,e] catalysis were unsuccessful (Table 1). However, the reaction of an excess of Boc-carbazate with **15** at 45 °C under $BF_3\cdot OEt_2$ catalysis (10 mol %) allowed for the regioselective and stereospecific opening of the silyl epoxide to give **16**.

Table 1. Hydrazide Ring Opening of *syn*-Silyl Epoxide **15** to **16**



R^1	conditions[a]	yield
H[b]	NH_4Cl (2 equiv), $MeOH/H_2O$, 16 h, 65 °C	–[c]
Boc	NH_4Cl (2 equiv), $MeOH/H_2O$, 16 h, 65 °C	–[c]
H[b]	$BF_3\cdot OEt_2$ (10 mol %), THF, 16 h, rt	–[d]
$MeOCH_2C(O)$	$BF_3\cdot OEt_2$ (10 mol %), THF, 16 h, 45 °C	–[c,e]
Boc	$BF_3\cdot OEt_2$ (10 mol %), THF, 16 h, rt	30%
Boc[f]	$BF_3\cdot OEt_2$ (10 mol %), THF, 16 h, 45 °C	75%

[a] 2.0 equiv of R^1NHNH_2 used. [b] Hydrazine monohydrate used. [c] Near-quantitative recovery of **15**. [d] Decomposition of **15**. [e] Decomposition of hydrazide. [f] 4.0 equiv of R^1NHNH_2 used.

As planned, the use of Boc-carbazate in this ring-opening reaction provides suitably differentiated hydrazine nitrogens for subsequent site selective functionalization. As a result, acylation of hydrazide derivative **16** provided trisubstituted hydrazide intermediate **17** which was then successfully converted to the natural product target **1** in only three additional steps (Scheme 3). For convenience, and to limit silica gel exposure of the potentially labile *Z*-enehydrazide moiety, a telescoped three-step protocol proved optimal, utilizing a KO*t*Bu mediated Peterson elimination followed immediately by Boc-carbamate acylation and catalytic Mg(II) imide-Boc deprotection.[17] The total synthesis of hydrazidomycin A was thus achieved with complete control of *Z*-olefin geometry, in an overall yield of 16% over eight steps.

An attractive aspect of the Peterson based strategy to enehydrazides is the stereospecific and stereodivergent nature of acid or base mediated silanol elimination.[15e] Thus, a complementary acid mediated silanol elimination sequence of **17** conveniently furnished the corresponding isomeric *trans*-hydrazidomycin A analog **18** in 50% yield over three steps (Scheme 4). Comparison of olefin coupling constants for **1** (J = 9.0 Hz) with **18** (J = 14.0 Hz) confirms the *cis*-stereochemical assignment for **1**.

Adaptation of this Peterson olefination sequence toward the total synthesis of **2** and **3** was then undertaken using a similar alkyne hydroalumination based route to access the required hydrazino silanol precursors (Scheme 5). Reaction of **19** and **20** with a slight excess of DIBAL-H resulted in mixtures of the expected *Z*-alkenyl TBS protected alcohols and the corresponding O-deprotected products[18]

(14) For reviews on Peterson eliminations, see: (a) Ager, D. J. *Org. React*. **1990**, *38*, 1–223. (b) Ager, D. J. *Synthesis* **1984**, 384–398. (c) Barrett, A. G. M.; Hill, J. M.; Wallace, E. M.; Flygare, J. A. *Synlett* **1991**, 764–770. (d) Kawashima, T.; Okazaki, R. *Synlett* **1996**, 600–608. (e) van Staden, L. F.; Gravestock, D.; Ager, D. J. *Chem. Soc. Rev*. **2002**, *31*, 195–200. (f) Ager, D. J. *Sci. Synth*. **2010**, *47a*, 85–104.

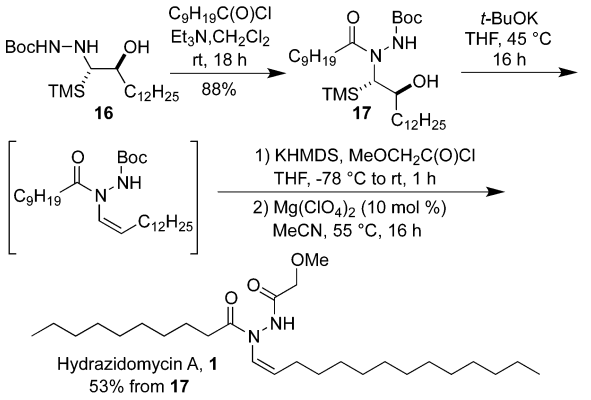
(15) For examples of Peterson elimination approaches to enamides, see: (a) Palomo, C.; Aizpurua, J. M.; Legido, M. *Tetrahedron Lett*. **1992**, *33*, 3903–3906. (b) Furstner, A.; Brehm, C.; Cancho-Grande, Y. *Org. Lett*. **2001**, *3*, 3955–3957. (c) Cuevas, J. C.; Patil, P.; Snieckus, V. *Tetrahedron Lett*. **1989**, *30*, 5841–5844. (d) Hudrlick, P. F.; Hudrlick, A. M.; Rona, R. J.; Misra, R. N.; Withers, G. P. *J. Am. Chem. Soc*. **1977**, *99*, 1993–1996. (e) Hudrlik, P. F.; Hudrlik, A. M.; Kulkarni, A. K. *Tetrahedron Lett*. **1985**, *26*, 139–142.

(16) For a related Peterson elimination approach to the Z-enamide containing crocacin natural products, see: (a) Chakraborty, T. K.; Laxman, P. *Tetrahedron Lett*. **2003**, *44*, 4989–4992. (b) Chakraborty, T. K.; Laxman, P. *Tetrahedron Lett*. **2002**, *43*, 2645–2648.

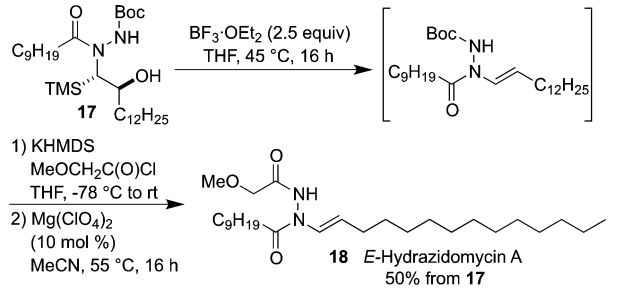
(17) Stafford, J. A.; Brackeen, M. F.; Karanewsky, D. S.; Valvano, N. L. *Tetrahedron Lett*. **1993**, *34*, 7873–7876.

(18) DIBAL-H is known to occasionally deprotect silyl ethers; see for example: (a) Corey, E. J.; Jones, G. B. *J. Org. Chem*. **1992**, *57*, 1028–1029. (b) Kuranaga, T.; Ishihara, S.; Ohtani, N.; Satake, M.; Tachibana, K. *Tetrahedron Lett*. **2010**, *51*, 6345–6348.

Scheme 3. Peterson Olefination Route to Hydrazidomycin A



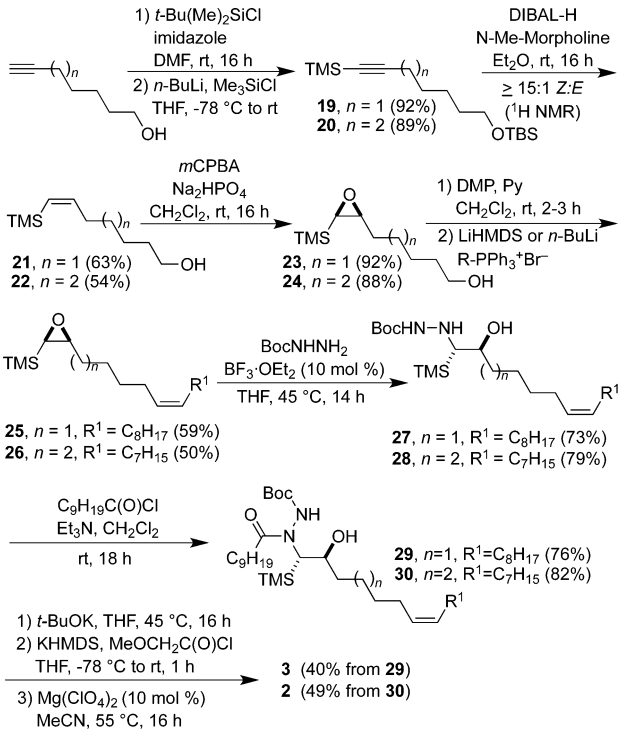
Scheme 4. Synthesis of an *E*-Hydrazidomycin A Analog



21 and **22**. This observation led to the development of a direct one-pot conversion of **19**/**20** into **21**/**22** using an excess amount of DIBAL-H to accomplish the highly stereoselective hydroalumination and TBS group deprotection. After epoxidation, the internal *Z*-olefins of these two enehydrazide natural products were then successfully installed using an oxidation/Wittig olefination sequence to provide **25** and **26**, followed by Boc-carbazate epoxide ring opening to afford the key enehydrazide precursors **27** and **28**. The observation of a single set of olefin carbons in the ^{13}C NMR of **25**–**28** combined with alkene ^{1}H NMR vicinal coupling constants of ~5.5–6.0 Hz support a *cis*-alkene stereochemical assignment.

Completion of the total syntheses of hydrazidomycin B and elaiomycin B from **27** and **28** was accomplished in four steps in an analogous manner to the hydrazidomycin A synthesis. Thus, compounds **2** and **3** were prepared in 7.3% and 6.7% overall yields respectively in a stereocontrolled fashion over 11 steps.

Scheme 5. Synthesis of Hydrazidomycin B and Elaiomycin B



In conclusion, a Lewis acid catalyzed Boc-carbazate silyl epoxide ring-opening/Peterson olefination based strategy for the highly stereoselective formation of both *Z*- and *E*-enehydrazides has been developed and applied toward the first total syntheses of members of a novel family of cytotoxic enehydrazides and analogs. Further studies on this approach and biological activity studies will be reported in due course.

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Supporting Information Available. Full experimental details and characterization data for all compounds including ^{1}H and ^{13}C NMR, and comparison of spectral data of **1**–**3** with reported data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.