

New WS9326A Congeners from *Streptomyces* sp. 9078 Inhibiting *Brugia malayi* Asparaginyl-tRNA Synthetase

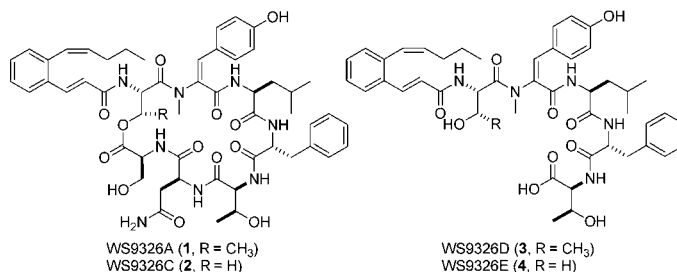
Zhiguo Yu,[†] Sanja Vodanovic-Jankovic,^{||} Michael Kron,^{*,||} and Ben Shen^{*,†,‡,§}

Department of Chemistry, Department of Molecular Therapeutics, and Natural Products Library Initiative at the Scripps Research Institute, the Scripps Research Institute, Jupiter, Florida 33458, United States, Department of Pathology, Biotechnology and Bioengineering Center, and Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, United States

mkron@mcw.edu; shenb@scripps.edu

Received August 17, 2012

ABSTRACT



Lymphatic filariasis is caused by the *Brugia malayi* parasite. Three new congeners of the decapeptide WS9326A (1), WS9326C (2), WS9326D (3), and WS9326E (4), were isolated from *Streptomyces* sp. 9078 by using a *B. malayi* asparaginyl-tRNA synthetase (*BmAsnRS*) inhibition assay. WS9326D specifically inhibits the *BmAsnRS*, kills the adult *B. malayi* parasite, and does not exhibit significant general cytotoxicity to human hepatic cells, representing a new lead scaffold for antiparasitic drug discovery.

Lymphatic filariasis (LF), one of the World Health Organization's (WHO) top 10 neglected tropical diseases, is caused by the filarial nematode parasite *Brugia malayi*. LF affects more than 200 million people worldwide.¹ Thus a top priority of the WHO is to search for new antihelminthic drugs that kill adult worms but exhibit fewer side effects than currently available medications such as albendazole and ivermectin. Because so few effective antiparasitic drugs exist, the same drugs have been commonly used to treat both human and animal helminth diseases for more than three decades.² Consequently, drug resistance has

emerged clinically around the world, underscoring the clear need to identify antiparasitic agents with new, alternative modes of action.³

Aminoacyl-tRNA synthetases (AARS) are a family of enzymes that play a key role in protein synthesis and thus AARS are one of the new molecular targets embraced by the WHO for antiparasitic drug discovery.⁴ In particular,

(3) (a) Geary, T. G. *Trends Parasitol.* **2005**, *21*, 530–532. (b) Molyneux, D. H.; Bradley, M.; Hoerauf, A.; Kyelem, D.; Taylor, M. J. *Trends Parasitol.* **2003**, *19*, 516–522. (c) Pritchard, R. K. *Expert Opin. Drug Discovery* **2007**, *2*, S41–S52. (d) Fox, L. M.; Furness, B. W.; Haser, J. K.; Desire, D.; Brissau, J.-M.; Milord, M.-D.; Lafontant, J.; Lammie, P. J.; Beach, M. J. *Am. J. Trop. Med. Hyg.* **2005**, *73*, 115–121. (e) Kron, M. A.; Marquard, K.; Hartlein, M.; Price, S.; Lederman, R. *FEBS Lett.* **1995**, *374*, 122–124.

(4) Kron, M. A.; Ramirez, B. L.; Ramirez, Y. *Expert Opin. Drug Discovery* **2007**, *2*, S1–S8.

(5) (a) Merritt, E. A.; Arakaki, T. L.; Gillespie, J. R.; Larson, E. T.; Kelley, A.; Mueller, N.; Napuli, A. J.; Kim, J.; Zhang, L.; Verlinde, C. L.; Fan, E.; Zucker, F.; Buckner, F.; Van Voorhis, W. C.; Hol, W. G. *J. Mol. Biol.* **2010**, *397*, 481–494. (b) Crepin, T.; Peterson, F.; Hartlein, M.; Kron, M. A.; Page, M. G. *Curr. Drug Discovery Technol.* **2012**, *8*, 66–75. (c) Kron, M. A.; Petridis, M.; Milev, Y.; Leykam, J.; Hartlein, M. *Mol. Biochem. Parasitol.* **2003**, *129*, 33–39.

[†] Department of Chemistry, the Scripps Research Institute.

^{*} Department of Molecular Therapeutics, the Scripps Research Institute.

[§] Natural Products Library Initiative at the Scripps Research Institute, the Scripps Research Institute.

^{||} Medical College of Wisconsin.

(1) (a) WHO: *Lymphatic Filariasis: Reasons for Hope*; Dzenowagis, J., Ed.; World Health Organization: Geneva, 1997. (b) Ridley, R. G.; Kita, K. *Expert Opin. Drug Discovery* **2007**, *2* (Supl. 1), S1.

(2) Woods, D. J.; Lauret, C.; Geary, T. G. *Expert Opin. Drug Discovery* **2007**, *2*, S25–S33.

the asparaginyl-tRNA synthetase (AsnRS) in *B. malayi* (i) is highly expressed in all stages of the parasite life cycle, (ii) is biochemically and structurally well characterized, and (iii) shows significant structural differences in comparison to human and other eukaryotic AARS.⁵

In an effort to discover new antifilarial drug leads, we recently completed a high throughput screening campaign, targeting the *BmAsnRS*. Of the ~73 000 extracts from a collection of 36 720 microbial strains screened, we identified 177 active strains. Previously, we reported the discovery of the tirandamycins (TAMs) from *Streptomyces* sp. 17944 and showed that TAM B was a potent and specific *BmAsnRS* inhibitor that efficiently killed the adult *B. malayi* parasite.⁶ We now report bioassay-guided fractionation of another active strain, *Streptomyces* sp. 9078, leading to the discovery of three new congeners of the known depsipeptide WS9326A (**1**),⁷ WS9326C (**2**), WS9326D (**3**), and WS9326E (**4**) (Figure 1). Importantly, **3**, a moderate *BmAsnRS* inhibitor, efficiently kills the adult *B. malayi* parasite, representing another lead scaffold for antifilarial drug discovery.

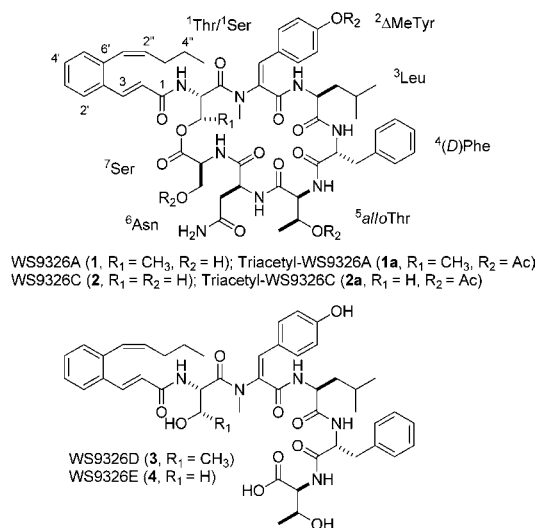


Figure 1. Structures of depsipeptide WS9326A (**1**)⁷ and the three new congeners, WS9326C (**2**), WS9326D (**3**), and WS9326E (**4**), from *Streptomyces* sp. 9078.

The crude extract from a 9.6-L fermentation culture of *Streptomyces* sp. 9078 was subjected to sequential chromatography over SiO₂ and Sephadex LH-20 columns, followed by semipreparative HPLC over a C-18 column. Natural product isolation was guided by bioassay for inhibitory activity against the recombinant *BmAsnRS*,

affording pure **1** (13.1 mg), **2** (5.2 mg), **3** (3.9 mg), and **4** (3.2 mg) as white powders, respectively [see Supporting Information (SI)]. Analysis of high resolution ESI-MS (HRESIMS) data and ¹H and ¹³C NMR spectra of **1** and its triacetyl derivative **1a** suggested **1** to be WS9326A, a depsipeptide that has been previously isolated from *Streptomyces violaceoniger* no. 9326.⁷ The identity of **1** was unambiguously confirmed by extensive 1D and 2D NMR analysis of **1** and **1a** (Tables S1 and S2), as well as comparison to the spectroscopic data reported previously.⁷

The molecular formula of **2** was determined to be C₅₃H₆₆N₈O₁₃ by HRESIMS, affording an [M + H]⁺ ion at *m/z* 1023.4842 (calculated [M + H]⁺ ion at *m/z* 1023.4827) and indicating that **2** differs from **1** (C₅₄H₆₈N₈O₁₃) by the absence of a CH₂ unit. The ¹H and ¹³C NMR spectra of **2** indicated that **2** exists as a mixture of two conformers in solution. A similar conformational mixture was known for **1**; however its triacetyl derivative (**1a**) afforded a single conformer.⁷ Thus, **2** was similarly converted into its triacetyl derivative (**2a**), with ¹H and ¹³C NMR spectra confirming it, in CDCl₃, as a single conformer. The structure of **2** was then established by careful comparison of the ¹H and ¹³C NMR data between **2a** and **1a** (Tables 1, S1, and S2). The absence of a doublet methyl signal [δ_C 17.1 and δ_H 1.40 (3H, d, 6.30 Hz)] and a methine signal [δ_C 71.0 and δ_H 5.47 (1H, m)] with the concomitant presence of one new methylene signal [δ_C 63.8 and δ_H 4.43 (1H, m), 4.68 (1H, m)] led to the conclusion that the ¹Thr residue in **1a** was substituted by a ¹Ser residue in **2a** (Figure 1). This conclusion was further supported by key correlations observed in gHMBC, COSY, and NOESY experiments of **2a** (Figure 2A). Since the absolute stereochemistry of **1** was known,⁷ **2**, named as WS9326C, was assigned the same stereochemistry as that of **1** on the basis of its biosynthetic origin (Figure 1).

The molecular formula of **3** was determined to be C₄₇H₅₉N₅O₁₀ by HRESIMS, affording an [M + H]⁺ ion at *m/z* 854.4357 (calculated [M + H]⁺ ion at *m/z* 854.4340). Analysis of the ¹H and ¹³C NMR spectra indicated that **3** is a linear lipopeptide consisted of five amino acids. The structures of the five amino acids were determined by 1D and 2D NMR, the sequence of which assigned in the order -NH-¹Thr-²ΔMeTyr-³Leu-⁴(D)Phe-⁵alloThr-CO₂H with the assistance of gHMBC and NOESY (Table 1 and Figures 1 and 2B). The identity of the 3-[2-(1(Z)-pentenyl)phenyl]-2(E)-propenoyl moiety was readily evident upon analysis of its ¹H and ¹³C NMR spectra (Table 1) and in comparison with the same moiety in **1** and **2** (Tables S1 and S2). The regiochemistry of the acyl moiety was assigned on the basis of key gHMBC correlations between H α -¹Thr [δ_H 5.01 (1H, m)] and C-1 [δ_C 165.3 (s)] of the acyl unit of **3**, establishing that the acyl moiety is attached to α -C of ¹Thr via an amide linkage (Figure 2B). Thus **3**, named as WS9326D, could be envisaged as a biosynthetic intermediate of **1**, thereby sharing the same absolute stereochemistry (Figure 1).

The molecular formula of **4** was determined to be C₄₆H₅₇N₅O₁₀ by HRESIMS, yielding an [M + H]⁺ ion at *m/z* 840.4194 (calculated [M + H]⁺ ion at *m/z* 840.4183) and differing from **3** by the absence of a CH₂ unit. The structure of **4** was established by direct comparison of the

(6) Yu, Z.; Vodanovic-Jankovic, S.; Ledebner, N.; Huang, S.; Rajski, S. R.; Kron, M.; Shen, B. *Org. Lett.* **2011**, *13*, 2034–2037.

(7) (a) Hayashi, K.; Hashimoto, M.; Shigematsu, N.; Nishicawa, M.; Ezaki, M.; Yamashita, M.; Kiyoto, S.; Okuhara, M.; Kohsaks, M.; Imanaka, H. *J. Antibiot.* **1992**, *45*, 1055–1063. (b) Hashimoto, M.; Hayashi, K.; Murai, M.; Fujii, T.; Nishikawa, M.; Kiyoto, S.; Okuhara, M.; Kohsaka, M.; Imanaka, H. *J. Antibiot.* **1992**, *45*, 1064–1070. (c) Shigematsu, N.; Hayashi, K.; Kayakiri, N.; Takase, S.; Hashimoto, M.; Tanaka, H. *J. Org. Chem.* **1993**, *58*, 170–175.

Table 1. Summary of ^1H (700 MHz) and ^{13}C (175 MHz) NMR Data for **2a** in CDCl_3 and **3** and **4** in $\text{DMSO}-d_6^a$

| position ^b | 2a | | 3 | | 4 | | |
|-----------------------------|---------------------------------------|---------------------------|---------------------------------------|-----------------------|---------------------------------------|-----------------------|---------------|
| | δ_{H} (<i>J</i> in Hz) | δ_{C} | δ_{H} (<i>J</i> in Hz) | δ_{C} | δ_{H} (<i>J</i> in Hz) | δ_{C} | |
| Acyl | 1 | | | 165.3, s | | 165.5, s | |
| | 2 | 6.98, 1H, d (16.0) | 122.2, d | 6.88, 1H, d (15.7) | 123.3, d | 6.71, 1H, d (15.7) | 123.3, d |
| | 3 | 7.91, 1H, d (16.0) | 140.0, d | 7.54, 1H, d (15.7) | 137.1, d | 7.55, 1H, d, 15.7) | 137.0, d |
| | 1' | | 133.6, s | | 138.0, s | | 138.0, s |
| | 2' | 7.56, 1H, d (7.80) | 126.3, d | 7.62, 1H, d (6.86) | 125.9, d | 7.58, 1H, d (7.63) | 125.9, d |
| | 3' | 7.18, 1H, t (7.60) | 127.0, d | 7.36, 1H, t (7.00) | 129.0, d | 7.37, 1H, t (7.21) | 129.4, d |
| | 4' | 7.29, 1H ^c | 128.9, d | 7.33, 1H, t (6.72) | 127.4, d | 7.33, 1H, t (7.35) | 127.8, d |
| | 5' | 7.20, 1H, d (7.80) | 129.9, d | 7.19, 1H ^c | 129.7, d | 7.20, 1H ^c | 130.5, d |
| | 6' | | 138.4, s | | 137.0, s | | 137.0, s |
| | 1'' | 6.55, 1H, d (11.4) | 126.9, d | 6.53, 1H, d (11.5) | 126.9, d | 6.51, 1H, d (10.8) | 127.4, d |
| | 2'' | 5.80, 1H, dt (11.5, 7.40) | 134.9, d | 5.79, 1H, m | 134.1, d | 5.78, 1H, m | 134.1, d |
| | 3'' | 2.00, 2H, m | 30.5, t | 1.97, 2H, m | 30.0, t | 1.98, 2H, m | 29.0, t |
| | 4'' | 1.35, 2H, m | 22.7, t | 1.36, 2H, m | 22.1, t | 1.36, 2H, m | 22.9, t |
| | 5'' | 0.80, 3H, t (7.50) | 13.8, q | 0.90, 3H, t (7.33) | 13.6, q | 0.81, 3H, t (7.28) | 13.7, q |
| | ¹ Ser/ ¹ Thr | NH | 7.65, 1H, d (7.80) | | 8.58, 1H, brs | | 8.64, 1H, brs |
| α | | 5.45, 1H, m | 50.9, d | 5.01, 1H, m | 52.9, d | 5.05, 1H, m | 51.3, d |
| β | | 4.43, 1H, m | 63.8, t | 4.11, 1H, m | 66.6, d | 3.56, 1H, m | 61.3, t |
| ² Δ MeTyr | γ | | | 1.05, 3H, brs | | 2.00, q | |
| | CO | | 169.5, s | | 170.9, s | | 170.9, s |
| | NMe | 3.57, 3H, s | 40.4, q | 2.88, 3H, s | 34.0, q | 2.86, 3H, s | 33.4, q |
| | α | | 138.8, s | | 130.7, s | | 129.4, s |
| | β | 6.84, 1H, s | 128.2, d | 6.60, 1H, s | 131.6, d | 6.70, 1H, s | 130.7, d |
| | 1 | | 131.0, s | | 123.5, s | | 123.3, s |
| | 2,6 | 7.29, 2H ^c | 129.9, d | 7.28, 2H, d (7.63) | 129.3, d | 7.19, 2H ^c | 130.7, d |
| | 3,5 | 7.05, 2H ^c | 121.9, d | 6.67, 2H, d (7.07) | 115.1, d | 6.67, 2H, d (7.98) | 115.2, d |
| | 4 | | 150.9, s | | 158.4, s | | 158.6, s |
| | CO | | 166.0, s | | 164.9, s | | 165.3, s |
| ³ Leu | CO (Ac) | | 169.6, s | | | | |
| | Me (Ac) | 2.30, 3H, s | 21.1, q | | | | |
| | NH | 8.08, 1H, d (8.20) | | 7.28, 1H, brs | | 7.35, 1H, d (9.35) | |
| | α | 4.45, 1H, m | 51.1, d | 4.35, 1H, m | 51.5, d | 4.39, 1H, m | 51.5, d |
| | β | 1.28, 1H, m | 38.0, t | 1.20, 2H, m | 40.0, t | 1.20, 2H, m | 39.2, t |
| | γ | 1.64, 1H, m | | | | | |
| | δ | 1.00, 1H, m | 24.2, d | 1.15, 1H, m | 23.9, d | 0.85, 1H, m | 24.0, d |
| | | 0.71, 3H, d (6.60) | 23.0, q | 0.70, 3H, brs | 21.7, q | 0.73, 3H, d (6.20) | 22.1, q |
| ⁴ Phe | | 0.79, 3H, d (7.10) | 21.5, q | 0.70, 3H, brs | 22.7, q | 0.73, 3H, d (6.20) | 22.5, q |
| | CO | | 170.9, s | | 172.4, s | | 173.2, s |
| | NH | 7.94, 1H, d (8.10) | | 8.49, 1H, brs | | 8.44, 1H, brs | |
| | α | 4.70, 1H, m | 54.9, d | 4.72, 1H, m | 53.9, d | 4.74, 1H, m | 53.8, d |
| | β | 2.79, 1H, m | 36.7, t | 2.71, 1H, m | 38.0, t | 2.72, 1H, m | 37.4, t |
| | | 3.13, 1H, dd (13.8, 8.20) | | 3.05, 1H, m | | 3.05, 1H, m | |
| | 1 | | 137.2, s | | 137.1, c | | 137.0, s |
| | 2,6 | 7.12, 2H, d (7.50) | 129.2, d | 7.20, 2H ^c | 130.7, d | 7.30, 2H, d (7.14) | 129.7, d |
| | 3,5 | 6.99, 2H, t (7.70) | 128.4, d | 7.22, 2H ^c | 127.8, d | 7.22, 2H ^c | 129.0, d |
| | 4 | 7.05, 1H ^c | 126.9, d | 7.15, 1H, t (6.51) | 126.0, d | 7.14, 1H, t (7.28) | 126.9, d |
| ⁵ alloThr | CO | | 173.4, s | | 170.9, s | | 170.9, s |
| | NH | 7.28, 1H, d (8.40) | | 8.10, 1H, brs | | 8.07, 1H, brd (6.23) | |
| | α | 4.93, 1H, dd (8.10, 3.60) | 56.0, d | 4.04, 1H, m | 58.5, d | 4.02, 1H, m | 58.8, d |
| | β | 5.28, 1H, m | 69.1, d | 3.65, 1H, m | 67.0, d | 3.68, 1H, m | 67.6, d |
| | γ | 1.18, 3H, d (6.60) | 15.1, q | 0.98, 3H, brs | 20.0, q | 0.97, 3H, brs | 20.1, q |
| | CO | | 168.1, s | | 170.4, s | | 170.5, s |
| | CO (Ac) | | 170.5, s | | | | |
| ⁶ Asn | Me (Ac) | 1.79, 3H, s | 20.7, q | | | | |
| | NH | 7.65, 1H, d (7.80) | | | | | |
| | α | 4.86, 1H, td (9.00, 4.40) | 49.3, d | | | | |
| | β | 2.63, 1H, dd (15.2, 4.30) | 36.0, t | | | | |
| ⁷ Ser | | 2.81, 1H, m | | | | | |
| | γ CO | | 173.1, s | | | | |
| | CO | | 173.4, s | | | | |
| | NH | 7.19, 1H, d (7.21) | | | | | |
| | α | 4.26, 1H, dd (11.3, 5.30) | 52.7, d | | | | |
| | β | 4.42, 1H, m | 62.4, t | | | | |
| | | 4.58, 1H, dd (13.2, 7.70) | | | | | |
| | CO | | 170.2, s | | | | |
| | CO (Ac) | | 170.9, s | | | | |
| | Me (Ac) | 2.06, 3H, s | 20.8, q | | | | |

^a Assignments were based on COSY, HSQC, HMBC, and NOSEY experiments. ^b Numbering followed literature precedence. ^c Overlapping signals.

^1H and ^{13}C NMR data between **4** and **3** (Table 1). The absence of a methyl signal [δ_{C} 20.0 and δ_{H} 1.05 (3H)] and a methine signal [δ_{C} 66.6 and δ_{H} 4.11 (1H, m)] with the concomitant presence of one new methylene signal [δ_{C} 61.3 and δ_{H} 3.56 (1H, m), 3.80 (1H, m)] revealed that the ^1Thr residue in **3** was substituted by a ^1Ser residue in **4** (Figure 1). This conclusion was further supported by key correlations observed in gHMBC, COSY, and NOESY experiments of **4** (Figure 2C). Hence, **4**, named as WS9326E, could be viewed as a biosynthetic intermediate of **2** with the same absolute stereochemistry (Figure 1).

Each of the purified compounds was reevaluated for their inhibitory activity against *BmAsnRS* using the recently reported nonradioactive assay.⁸ This pretransfer editing assay exploits L-aspartate β -hydroxamate, a novel asparagine substrate mimic, to drive the enzymatic activity of *AsnRS* and maximize the production of inorganic phosphate that can be measured by its reaction with malachite green (SI). While **1** and **2** showed little activity at 100 μM , moderate inhibition against *BmAsnRS* was observed for **3** and **4** with apparent IC_{50}s estimated to be 50 μM (for **3**) and 75 μM (for **4**), respectively (Figure S1). We next tested **3** for its ability to kill adult *B. malayi* worms in vitro following the published procedure.⁶ Live adult *B. malayi* worms were maintained in 6-well plates, and **3**, varying from 10 nM to 50 μM , was added to selected wells with both albendazole (100 μM), a known LF drug,³ and TAM B, the new LF drug lead we have discovered previously from *S. sp.* 17944,⁶ as positive controls (SI). Remarkably, **3** kills the adult *B. malayi* worms rapidly within 24 h and can efficiently kill the adult worms within 10 days at concentrations as low as 10 nM (Figure S2); worm death was unambiguously confirmed from simple paralysis by the modified MTT assay^{6,9} (Figure S3, SI). Finally, **3** was evaluated using human HepG2 cells for general cytotoxicity, which was defined as > 50% cell death at 24 h as measured by the MTT assay for mitochondrial activity,⁹ and **3** was found to be nontoxic at concentration as high as 100 μM (Figure S4, SI).

Natural products remain the best sources of drugs and drug leads; however, natural products are underrepresented unfortunately in all small molecule libraries currently available.¹⁰ Depsipeptide **1** was first isolated as a tachykinin antagonist from *S. violaceoniger* no. 9326 two decades ago.⁷ Guided by the innovative high throughput screening targeting *BmAsnRS*, we now report that **3**, a new congener of **1**, is a novel *BmAsnRS* inhibitor, efficiently kills the adult *B. malayi* parasite, and does not exhibit significant general cytotoxicity to human hepatic cells. Therefore, **3** represents another new lead scaffold for antifilarial drug discovery. These findings, together with our early report of TAM B as a promising antifilarial drug lead,⁶ underscore the great promise of our strategy in screening microbial natural products as *BmAsnRS* inhibitors for antifilarial drug discovery.

(8) Danel, F.; Caspers, P.; Nuoffer, C.; Hartlein, M.; Kron, M. A.; Page, M. G. *Curr. Drug Discovery Technol.* **2010**, *8*, 66–75.

(9) Comley, J. C. W.; Res, M. J.; Turner, C. H.; Jenkins, D. C. *Int. J. Parasitol.* **1989**, *19*, 77–83.

(10) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2012**, *75*, 311–335.

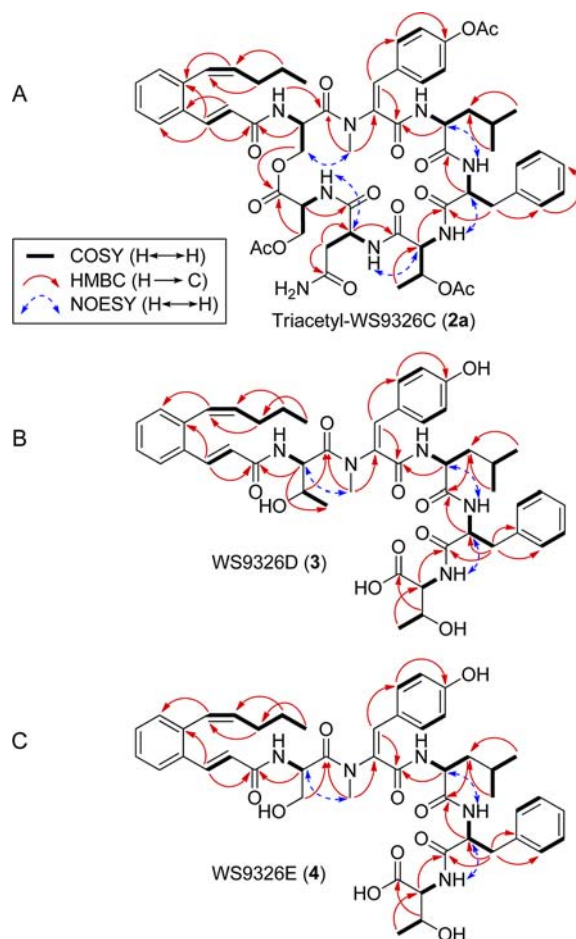


Figure 2. Key COSY, HMBC, and NOESY correlations supporting the structures of (A) WS9326C (**2**) and triacetyl-WS9326C (**2a**), (B) WS9326D (**3**), and (C) WS9326E (**4**).

The fact that these leads can be produced in sufficient quantities by scale up microbial fermentation and that their biosynthetic machinery could be subjected to combinatorial biosynthetic strategies for titer improvement and structural diversity should greatly facilitate follow-up mechanistic and preclinical studies, thereby realistically developing these promising leads into potential clinical drugs.

Acknowledgment. We thank the Filariasis Research Reagent Resource (FR3), Division of Microbiology and Infectious Diseases, NIAID, NIH for providing adult *B. malayi*. This work was supported in part by NIH Grants A1053877 (M.K.) and GM086184 (B.S.) as well as the Natural Products Library Initiative at TSRI.

Supporting Information Available. Detailed experimental procedures, biological evaluations of **3** (Figures S1 to S4), and assorted 1D and 2D NMR spectra for **1**, **1a**, **2**, **2a**, **3**, and **4** (Tables S1, S2 and Figures S5 to S28). This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.