Published in final edited form as:
Org Lett. 2012 September 21; 14(18): 4946–4949. doi:10.1021/ol302298k.

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

Zhiguo Yu[†], **Sanja Vodanovic-Jankovic**[⊥], **Michael Kron**[⊥], and **Ben Shen**[†,‡,§]

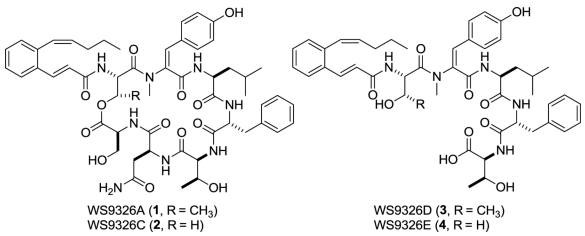
[†] Department of Chemistry, the Scripps Research Institute, Jupiter, FL 33458, United State

[‡]Department of Molecular Therapeutics, the Scripps Research Institute, Jupiter, FL 33458, United State

[§] Natural Products Library Initiative at the Scripps Research Institute, the Scripps Research Institute, Jupiter, FL 33458, United State

[⊥]Department of Pathology, Biotechnology and Bioengineering Center, and Department of Medicine, Medical College of Wisconsin, Milwaukee, WI, 53226, United States.

Abstract



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

Lymphatic filariasis (LF), one of the World Health Organization's (WHO) top ten 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 antifilarial 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 antiparasite agents with new, alternative modes of action.[3]

Correspondence to: Michael Kron; Ben Shen.

mkron@mcw.edu; shenb@scripps.edu.

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.

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 WHO for antiparasite drug discovery.[4] In particular, 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 throughout screening campaign, targeting the *Bm*AsnRS. 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 *Bm*AsnRS 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 *Bm*AsnRS inhibitor, efficiently kills the adult *B. malayi* parasite, representing another lead scaffold for antifilarial drug discovery.

The crude extract from a 9.6-L fermentation culture of *Streptomyces* sp. 9078 was subjected to sequential chromatography over SiO_2 and Sephadex LH-20 columns, followed by semi-preparative HPLC over a C-18 column. Natural product isolation was guided by bioassay for inhibitory activity against the recombinant *Bm*AsnRS, 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 ^{1}H and ^{13}C 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 unambigously confirmed by extensive 1D and 2D NMR analysis of **1** and **1a** (Tables S1 and S2), as well as comparison to the spectropic data reported previously.[7]

The molecular formula of **2** was determined to be $C_{53}H_{66}N_8O_{13}$ 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** ($C_{54}H_{68}N_8O_{13}$) by the absence of a CH_2 unit. The ^{1}H and ^{13}C NMR spectra of **2** indicated that **2** exists as a mixture of two conformers in solution. A similar conformational mixture was known for **1**, the triacetyl derivative of which (**1a**) however afforded a single conformer.[7] Thus, **2** was simiarly converted into its triacetyl derivative (**2a**), the ^{1}H and ^{13}C NMR spectra of which in $CDCl_3$ confirmed it as a single conformer. The structure of **2** was then established by careful comparison of the ^{1}H and ^{13}C 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 ^{1}Thr residue in **1a** was substituted by a ^{1}Ser residue in **2a** (Figure 1). This conclusion was further supported by key correlations observed in gHMBC, COSY, and NOSEY 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 $C_{47}H_{59}N_5O_{10}$ by HRESIMS, affording an $[M + H]^+$ ion at *m/z* 854.4357 (calculated $[M + H]^+$ ion at *m/z* 854.4340). Analysis of the ^{1}H and ^{13}C 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 was assigned in the order of –
NH-^{1}Thr-$^{2}\Delta$MeTyr-^{3}Leu-^{4}(*D*)Phe-^{5}*allo*Thr-CO_2H 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 ^{1}H and ^{13}C 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}-^{1}Thr [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 ^{1}Thr 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 $C_{46}H_{57}N_5O_{10}$ 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 CH_2 unit. The structure of **4** was established by direct comparison of the ^{1}H 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 ^{1}Thr residue in **3** was substituted by a ^{1}Ser residue in **4** (Figure 1). This conclusion was further supported by key correlations observed in gHMBC, COSY, and NOSEY 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 compound was reevaluated for their inhibitory activity against *Bm*AsnRS 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 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 *Bm*AsnRS 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 hours and can efficiently kill the adult worms within 10 days at concentration 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 hours 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 *Bm*AsnRS, we now report that **3**, a new congener of **1**, is a novel *Bm*AsnRS 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 *Bm*AsnRS inhibitors for antifilarial drug discovery. 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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.

References

1. a WHO. Lymphatic Filariasis: Reasons for Hope. Dzenowagis, J., editor. World Health Organization; Geneva: 1997. b Ridley RG, Kita K. Expert Opin. Drug Discovery. 2007; 2(Supl. 1):S1.
2. Woods DJ, Lauret C, Geary TG. Expert Opin. Drug Discovery. 2007; 2:S25–S33.
3. a Geary TG. Trends Parasitol. 2005; 21:530–532. [PubMed: 16126457] b Molyneux DH, Bradley M, Hoerauf A, Kyelem D, Taylor MJ. Trends Parasitol. 2003; 19:516–522. [PubMed: 14580963] c Pritchard RK. Expert Opin Drug Discovery. 2007; 2:S41–S52.d Fox LM, Furness BW, Haser JK, Desire D, Brissau J-M, Milord M-D, Lafontant J, Lammie PJ, Beach M. J. Am. J. Trop. Med. Hyg. 2005; 73:115–121.e Kron MA, Marquard K, Hartlein M, Price S, Lederman R. FEBS Lett. 1995; 374:122–124. [PubMed: 7589498]
4. Kron MA, Ramirez BL, Ramirez Y. Expert Opin. Drug Discovery. 2007; 2:S1–S8.
5. a Merritt EA, Arakaki TL, Gillespie JR, Larson ET, Kelley A, Mueller N, Napuli AJ, Kim J, Zhang L, Verlinde CL, Fan E, Zucker F, Buckner F, Van Voorhis WC, Hol WG. J. Mol. Biol. 2010; 397:481–94. [PubMed: 20132829] b Crepin T, Peterson F, Hartlein M, Kron MA, Page MG. Curr. Drug Discovery Technol. 2012; 8:66–75.c Kron MA, Petridis M, Milev Y, Leykam J, Härtlein M. Mol. Biochem. Parasitol. 2003; 129:33–39. [PubMed: 12798504]
6. Yu Z, Vodanovic-Jankovic S, Ledeboer N, Huang S, Rajski SR, Kron M, Shen B. Org. Lett. 2011; 13:2034–2037. [PubMed: 21405052]
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. [PubMed: 1381343] 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. [PubMed: 1381344] c Shigematsu N, Hayashi K, Kayakiri N, Takase S, Hashimoto M, Tanaka H. J. Org. Chem. 1993; 58:170–175.
8. Danel F, Caspers P, Nuoffer C, Hartlein M, Kron MA, Page MG. Curr. Drug Discovery Technol. 2010; 8:66–75.
9. Comley JCW, Res MJ, Turner CH, Jenkins DC. Int. J. Parastol. 1989; 19:77–83.
10. Newman DJ, Cragg GM. J. Nat. Prod. 2012; 75:311–335. [PubMed: 22316239]

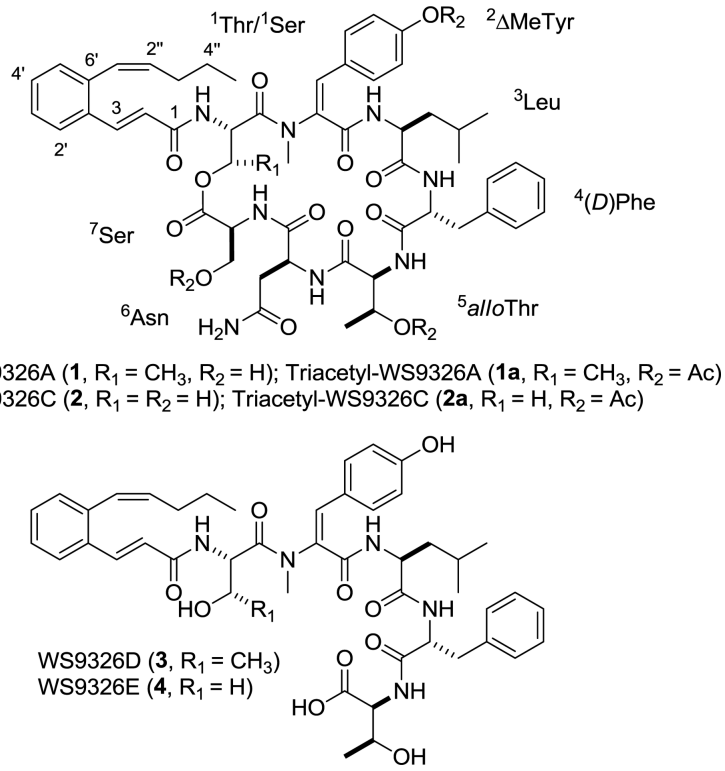


Figure 1.

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

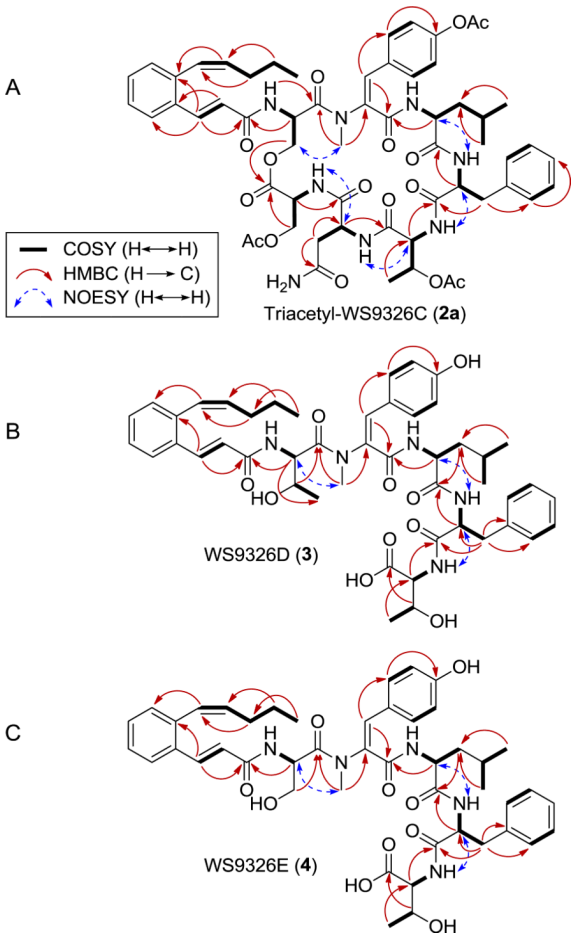


Figure 2.

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

Table 1

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

		2a		3		4	
position[b]		δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
Acyl	1		166.1, s		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[e]	129.7, d	7.20, 1H[f]	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
		4.68, 1H, m				3.80, 1H, m	
	γ			1.05, 3H, brs	20.0, q		
	CO		169.5, s		170.9, s		170.9, s
^{2}ΔMeTyr	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[f]	130.7, d

		2a		3		4	
position[b]		**δ_H (*J* in Hz)**	**δ_C**	**δ_H (*J* in Hz)**	**δ_C**	**δ_H (*J* in Hz)**	**δ_C**
	3,5	7.05, 2H[d]	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
	CO (Ac)		169.6, s				
	Me (Ac)	2.30, 3H, s	21.1, q				
[3]Leu	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
		1.64, 1H, m					
	γ	1.00, 1H, m	24.2, d	1.15, 1H, m	23.9, d	0.85, 1H, m	24.0, d
	δ	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
		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
[4]Phe	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[e]	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[e]	127.8, d	7.22, 2H[f]	129.0, d
	4	7.05, 1H[d]	126.9, d	7.15, 1H, t (6.51)	126.0, d	7.14, 1H, t (7.28)	126.9, d
	CO		173.4, s		170.9, s		170.9, s
[5]*allo* Thr	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				
	Me (Ac)	1.79, 3H, s	20.7, q				

		2a		3		4	
position[b]		δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
[6]Asn	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				
		2.81, 1H, m					
	γCO		173.1, s				
	CO		173.4, s				
[7]Ser	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[7]

[c]Overlapping signals

[d]Overlapping signals

[e]Overlapping signals

[f]Overlapping signals