The Ansacarbamitocins: Polar Ansamitocin Derivatives

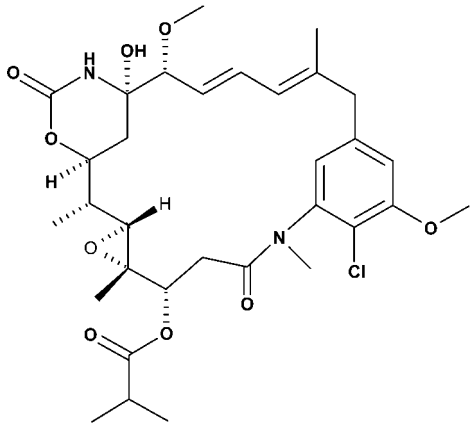
Carl E. Snipes, Dennis O. Duebelbeis, Monica Olson, Donald R. Hahn, William H. Dent, III, Jeffrey R. Gilbert, Todd L. Werk, George E. Davis, Rebecca Lee-Lu, and Paul R. Graupner*

Dow AgroSciences, 9330 Zionsville Road, Indianapolis, Indiana 46268

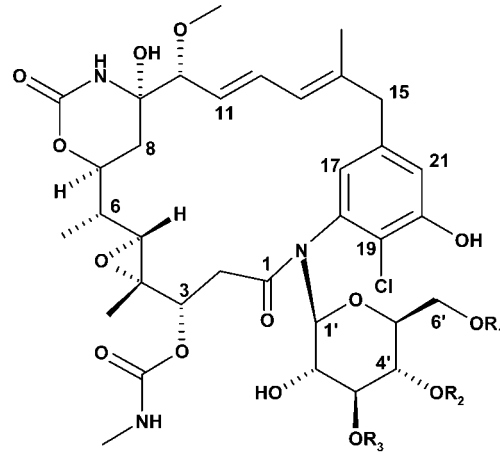
Received June 8, 2007

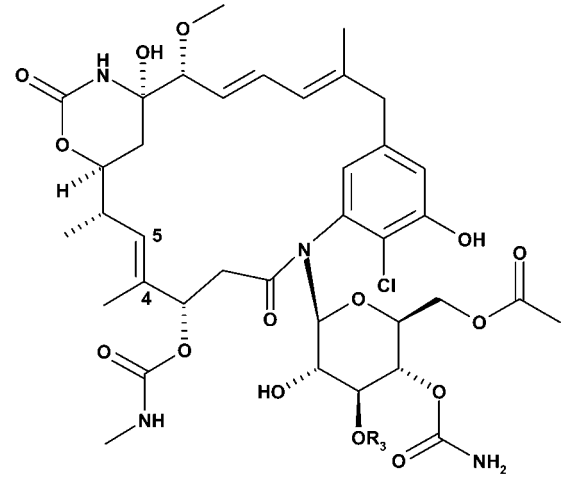
The ansacarbamitocins are a new family of maytansinoids that are unusually substituted with a glucose subunit and two carbamate functional groups and exhibit modest activity against some agricultural fungal disease organisms. Ansacarbamitocins A–F (**1**–**6**) all consist of the same macrocyclic core as the ansamitocins, with variation occurring on the glucose unit, while ansacarbamitocins A1 and B1 (**7**, **8**) additionally lack the epoxide group on C-4 and C-5.

The maytansinoids are a family of potent cytotoxic agents discovered originally from an Ethiopian shrub.[1] Although the maytansines were originally plant-derived, their structure was strongly indicative of microbial origin, and soon the subset ansamitocins was found in actinomycetes. The antimitotic activity of these compounds has been shown to be due to their binding to tubulin,[2] which is also a mechanism of action against fungi. Indeed in these and other laboratories, ansamitocins P3 (**9**) and P4 have been shown to be potent antifungal agents. Although their potential as anticancer agents has yet to be fulfilled, their deleterious action on mammalian cells precludes their potential as agrochemical agents. Because of this, any program initiated to find agrochemically relevant natural products from actinomycetes needs to have a method to dereplicate these compounds. Fortunately, the ansamitocins—a family of macrocyclic lactams linking two nonadjacent positions on an aromatic ring—have a fairly distinctive UV spectrum, and this may be used together with the chlorine isotope pattern in the mass spectrum to detect these compounds early in active extracts using bioassay-directed LC-MS dereplication techniques.



9 Ansamitocin P3





1 R1 = Ac, R2 = $CONH_2$, R3 = Me
2 R1 = Ac, R2 = $CONH_2$, R3 = H
3 R1 = H, R2 = $CONH_2$, R3 = Me
4 R1 = H, R2 = $CONH_2$, R3 = H
5 R1 = R2 = H, R3 = Me
6 R1 = R2 = R3 = H

7 R3 = Me
8 R3 = H

Results and Discussion

Actinomycete strain CP2808 (deposited as NRRL B-24544) was isolated from a soil sample collected in central Indiana, extracts of which showed activity in our fungicidal screens. The organism was characterized as an *Amycolatopsis* species, which is from the family Pseudonocardiaceae, the same family as the ansamitocin-producing strain *Actinosynnema pretiosum*.[3] Bioassay-directed LC-UV-MS dereplication of the active extracts indicated the presence of ansamitocin-like actives by UV spectral matching; however the molecular weights were substantially higher than, for example, ansamitocin P3 and the LC retention times (reversed phase) indicated that the CP2808 compounds were also somewhat more polar than ansamitocins. A small sample of the main component was isolated (MW 840), and NMR indicated that the compound was related to the ansamitocins with some notable changes; both the aromatic methoxy group and the *N*-methyl group were absent, there was a new *N*-methyl group at 2.77 ppm, and a number of extra protons on oxygenated carbon atoms were present, indicating

* Corresponding author. E-mail: prgraupner@dow.com. Phone: (317) 337 3496. Fax: (317) 337-3546.

10.1021/np070275t CCC: $37.00
Published on Web 09/25/2007

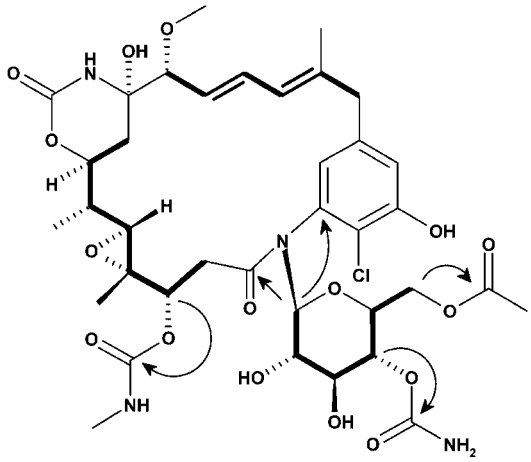


Figure 1. Spin systems detected by COSY (thick bonds) and distinguishing HMBC correlations (arrows) for ansacarbamitocin B (**2**).

a possible glycoside derivative. A search against published maytansinoid structures indicated that these were likely new compounds with significant structural variations, leading to different physical properties from those ansamitocin molecules previously screened. Therefore a large batch was fermented and subjected to formal isolation and structural characterization.

LC-UV-MS analysis of the aqueous EtOH extract from the fermentation of the *Amycolatopsis* sp. revealed a large number of compounds that all had identical UV spectra and molecular weights in the range 750–868 amu, the most abundant peak having MW 840 amu. The most obvious physical property difference between the ansacarbamitocins and **9** was that of solubility. The ansacarbamitocins were not soluble in $CHCl_3$, whereas they were soluble in DMSO, but the spectra suffered from some broad resonances. Therefore all of the following NMR experiments were undertaken in MeOH-d_4, in which the molecules exhibited excellent NMR characteristics.

HRMS analysis of the most abundant compound ansacarbamitocin B (**2**) indicated that this molecule had two more nitrogen atoms than found in the ansamitocins; thus the $[M + H]^+$ ion detected by +ESI implied $M^+ = 840.2853$ with $C_{37}H_{49}N_4O_{16}Cl$ requiring 840.2832. The structure was solved by NMR using COSY, TOCSY, HSQC, and HMBC spectra. Structural similarities to **9** were immediately recognizable, with two elongated spin-systems being readily identified (C-2 to C-8 and C-10 to C-15; Figure 1). Apart from some minor discrepancies with chemical shifts, these spin systems matched those previously recorded for **9**.[4] H-3 was shifted upfield from 4.8 to 4.4 ppm, but still appeared to be acyl substituted, as this was still downfield with respect to the chemical shift for the free alcohol, and there was a possible cross-peak to a carbonyl carbon at 159 ppm detected in HMBC experiments (see below); however there was no obvious sign of an ester group substituted here. Only one methoxy group was apparent, HMBC revealing that this was the 10-OMe; therefore the aromatic methoxy group present in **9** was missing from these molecules. There was an *N*-methyl group, although this was at a different chemical shift than the *N*-methyl found in **9**. HMBC confirmed the different nature of this methyl group, but only revealing one correlation to a carbon atom, a carbonyl at ~159 ppm, rather than the two correlations seen for the *N*-methyl group in **9**. One other methyl group was detected, which was assigned as an *O*-acetyl group. One new major spin-system was assigned as being consistent with a carbohydrate group. All vicinal coupling constants for this ring were >6 Hz, indicating a β-D-glucose subunit. Interestingly, the carbon chemical shift for the anomeric carbon was ~82 ppm, suggesting N-substitution rather than the more usual O-substitution. Cross-peaks from this anomeric proton were observed to C-1 and C-18, indicating that the sugar was unusually substituted on the amide nitrogen in place of the missing methyl group. For this sugar, the proton resonances were somewhat downfield for 4′-H and 6′-H, suggesting O-substitution. The HMBC revealed a cross-peak from 6′-H to the *O*-acetyl carbonyl carbon. There was a cross-peak from 4′-H to a carbon at 159 ppm, but it was difficult to be certain, as 4′-H had a very similar proton chemical shift to 3-H, which, as described above, may correlate to a carbon at 159 ppm.

With the bulk of the molecule characterized, it remained to add the other functional groups. The only proton resonance yet to be assigned was that from the *N*-methyl group. Assuming a molecular formula of $C_{37}H_{49}N_4O_{16}Cl$ and accounting for the glycoside-containing macrocycle left $C_3H_6N_2O_2$ unaccounted for. The cross-peak from the methyl group to a carbonyl at 159 ppm suggested a methylated carbamoyl group, leaving a nonsubstituted carbamoyl group (two carbon resonances at 158–159 ppm were therefore assigned as carbamate carbon atoms). As there were a number of possible positions for these two groups, cross-peaks from macrocyclic protons were imperative in order to ascertain their positions. A cross-peak from H-3 *or* H-4′ to one of the carbonyls had been assigned, but none to the other carbonyl. An HMBC experiment optimized for 4 Hz also revealed this cross-peak, but now there was some asymmetry to it, suggesting that *both* H-3 and H-4′ correlated to the two different carbonyls. HMBC experiments run at higher resolution in F1 revealed that H-3 correlated to the same carbonyl as the *N*-methyl group, thus leaving C-4′ substituted with the primary carbamoyl group. Confirmation of these assignments came from other members of this group of chemistry, where H-3 and H-4′ had slightly different chemical shifts and where factors were found without the primary carbamoyl group, for which H-4′ moved upfield by 1.2 ppm (see below). The ^1H and ^{13}C NMR data for ansacarbamitocin B are shown in Table 1. The unsubstituted phenol group was confirmed by methylation of a small sample of **6** with K_2CO_3 and methyl iodide. Small changes were seen in the NMR spectrum of the product, the biggest changes to H-21 and H-17, with the expected methyl resonance at 3.98 ppm, correlating in an HMBC experiment to C-20 at 156 ppm.

Most of the other ansacarbamitocins showed variation in the substitution pattern of the glucose unit (^1H NMR data shown in Table 2). Ansacarbamitocin A (**1**, MW 855) had an additional methoxy group at 3.6 ppm, along with a slight upfield shift of H-3′; HMBC confirmed methylation at the 3′ hydroxy group. This methoxy substituent was also seen in ansacarbamitocin C (**3**, MW 813). The *O*-acetyl group was not visible here, and both the H-6′ protons were shifted upfield by ca. 0.5 ppm; the *O*-acetyl group and the methoxy group were both absent in ansacarbamitocin D (**4**, MW 799). In ansacarbamitocin E (**5**, MW 770), the *O*-acetyl group at C-6′ was absent, although the 3′-methoxy group was present. H-4′ was shifted upfield by 1.2 ppm when compared to **2**. The mass of 770 amu indicated that the primary carbamate group was not present in this molecule, and thus its position at C-4′ was confirmed in other compounds. Finally, the most polar ansacarbamitocin F (**6**, MW 756) was identified as an unsubstituted glucose derivative.

Additional minor derivatives were found in which the epoxide group of the macrocycle was replaced by a double bond. Two of these were isolated and characterized; ansacarbamitocin B1 (**8**, MW 825) was equivalent to **2** except that COSY spectra revealed that H-3 and H-5 had shifted downfield to 5.2 and 5.3 ppm, respectively, resonating as broad doublets, and that the 4-methyl group was broad, resonating at 1.3 ppm (versus 0.8 ppm in **2**), consistent with olefinic substitution. HSQC also revealed that C-5 was olefinic, and HMBC experiments showed that the 4-methyl group correlated to two olefinic carbon atoms instead of two oxygenated atoms. The same detail was evident in ansacarbamitocin A1 (**7**, MW 839) with the addition of the 3′-OMe group on the glucose.

Table 1. NMR Data for Ansacarbamitocin B (**2**) in MeOH

position	δ_H (*J* in Hz)	δ_C
1		173.4
2	2.48, dd (13.3, 12.6)	34.8
	2.17, dd (13.3, 3.1)	
3	4.39, dd (12.2, 3.2)	79.5
3-carbam		158.0
NMe	2.77, s	28.1
4		62.1
4-Me	0.78, s	11.9
5	2.90, d (9.4)	69.2
6	1.53, m	39.5
6-Me	1.23, d (6.4)	14.9
7	4.26, ddd (12.4, 10.3, 2.0)	76.3
8	1.66, dd (14.3, 2.1)	37.5
	1.44, dd (13.5, 12.4)	
9		82.0
10	3.54, d (8.9)	90.4
10-OMe	3.36, s	57.1
11	5.47, dd (15.9, 9.3)	129.0
12	6.59, dd (15.6, 11.1)	134.6
13	6.34, d (11.3)	125.6
14		141.7
14-Me	1.74, s	16.0
15	3.58, d (12.7)	47.0
	3.15, d (12.7)	
16		141.2
17	7.07, d (1.9)	125.7
18		137.1
19		121.7
20		155.6
21	6.99, d (1.9)	119.2
22		155.4
1′	5.81, d (9.3)	84.2
2′	3.20, t (9.2)	72.2
3′	3.67, t (9.4)	76.8
4′	4.38, t (9.9)	73.1
4′-carbam		159.0
5′	3.71, ddd (10.1, 5.4, 2.7)	75.5
6′	4.16, dd (12.2, 2.9)	64.3
	4.02, dd (11.8, 5.4)	
Ac C=O		172.8
Ac	2.01, s	20.8

Table 2. ^{1}H NMR Data (δ_H) of the Ansacarbamitocins in MeOD

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
2	2.49	2.48	2.48	2.52	2.48	2.5	2.31	2.32
2	2.18	2.17	2.17	2.16	2.18	2.16	2.25	2.26
3	4.40	4.39	4.40	4.39	4.40	4.38	5.21	5.31
5	2.89	2.90	2.90	2.90	2.90	2.9	5.19	5.19
6	1.52	1.53	1.52	1.53	1.52	1.53	2.51	2.52
7	4.26	4.26	4.26	4.26	4.26	4.26	4.08	4.08
8	1.65	1.66	1.65	1.66	1.65	1.66	1.57	1.57
8	1.44	1.44	1.43	1.44	1.42	1.43	1.30	1.30
10	3.54	3.54	3.54	3.54	3.54	3.54	3.58	3.58
11	5.47	5.47	5.47	5.47	5.46	5.47	5.39	5.39
12	6.59	6.59	6.59	6.59	6.59	6.58	6.54	6.53
13	6.31	6.34	6.31	6.32	6.32	6.33	6.00	6.01
15	3.59	3.58	3.60	3.57	3.57	3.56	3.45	3.46
	3.16	3.15	3.16	3.13	3.17	3.12	3.21	3.21
17	7.00	7.07	7.05	7.03	7.06	7.04	6.97	7.01
21	6.98	6.99	6.99	6.96	6.97	6.95	6.97	6.96
NMe	2.78	2.77	2.79	2.77	2.79	2.78	2.72	2.72
4-Me	0.78	0.78	0.79	0.78	0.76	0.77	1.31	1.31
6-Me	1.22	1.23	1.22	1.23	1.22	1.23	1.08	1.08
14-Me	1.74	1.74	1.75	1.75	1.74	1.75	1.86	1.87
10-OMe	3.37	3.36	3.37	3.37	3.38	3.36	3.36	3.38
1′	5.82	5.81	5.78	5.76	5.74	5.73	5.78	5.75
2′	3.21	3.20	3.24	3.25	3.22	3.18	3.18	3.16
3′	3.41	3.67	3.41	3.50	3.20	3.48	3.38	3.62
3′-OMe	3.59		3.59		3.57		3.58	
4′	4.42	4.38	4.32	4.29	3.12	3.06	4.41	4.37
5′	3.71	3.71	3.52	3.67	3.38	3.39	3.70	3.69
6′	4.14	4.16	3.61	3.64	3.80	3.82	4.14	4.15
6′	4.00	4.02	3.47	3.58	3.55	3.58	3.98	4.00
6′Ac	2.01	2.01					2.00	2.01

Recently a paper appeared that described the production of a novel *N*-glycoside of the ansamitocins from a cultivation of *Actinosynnema pretiosum*.[5] The structure of the novel ansamitocinoside P-2 was given as the unsubstituted glycoside **10**. With our discovery of the ansacarbamitocins, this compound gave us an opportunity to test our structural assignment, considering the number of changes to the classical ansamitocins that we had. One problem became immediately apparent, and that was the proton resonance of the glycoside proton H-4′. In our work, this proton resonated at 4.38 ppm in **2** where this position was substituted with the carbamate group, and at 3.06 in the unsubstituted compound. The published value for ansamitocinoside P-2 at 4.25 ppm is therefore strongly indicative of acyl substitution at C-4′, similar to a carbamate. Closely checking the ^{13}C NMR assignments in this work, a few discrepancies were found downfield in the aromatic region. C-19 was assigned at 155 ppm, which is very low field for a chlorinated aromatic resonance; in **2** and **9** this resonance is at 121 and 120 ppm, respectively. A resonance at 123 ppm was assigned to C-16, for which 142 ppm was found in our studies. In this work, the resonance at 155 ppm was assigned to C-20, the cyclic carbamate found in all these molecules, but for ansamitocinoside, this was assigned to 159 ppm, which in the ansacarbamitocins was assigned to the 4′-carbamoyl resonance. Assigning 155 ppm to C-20 therefore leaves the resonance at 159 ppm unassigned, and therefore suggests that this could be assigned to the substituent at C-4′, similar to the carbamate found in this work. These findings lead us to propose that the published structure of ansamitocinoside P2 needs to be reviewed and may be instead the carbamoylated derivative **11**. We suggest that the FABMS data may well be linked to a fragment ion, but in all our studies we were unable to produce a spectrum in which the mass ion was not present using ESI methods, FAB being unavailable to us; however MS-MS experiments revealed that the carbamoyl group was readily labile, so it may be possible that the mass ion was not elucidated in the FAB spectrum described.

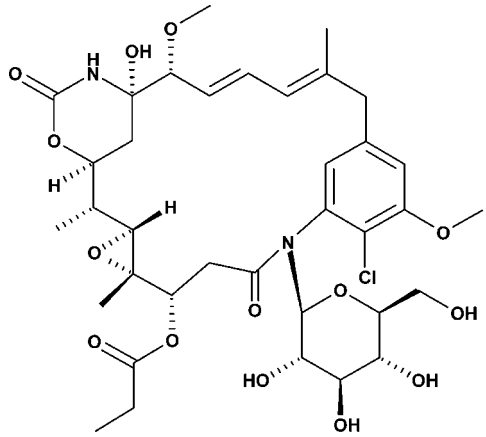
Biological Data. Ansacarbamitocins were discovered from *in vitro* antifungal screening assays using *Septoria tritici* and *Septoria nodurum*. While there was adequate activity during LC analysis to associate the activity of the broth with ansacarbamitocins, when tested as purified compounds, they were not active *in vitro* at 25 μg/mL. However, *in vivo* testing on plant diseases in the greenhouse produced modest activity against *Septoria tritici*, *Erisiphe graminis*, and *Puccinia recondita*.

Summary. The ansacarbamitocins represent a new subgroup of the maytansinoid class of antibiotics, with substantially different solubility profiles due to the large number of exchangeable protons from carbamates and alcohols. Initial indications show that their fungicidal activity is not as good as that for the better-known ansamitocin derivatives.

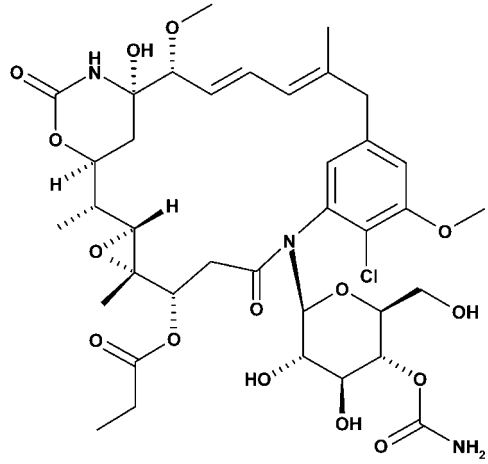
Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. All NMR spectra were recorded on a Bruker DRX600 spectrometer operating at 600.13 MHz (^{1}H) and 150.62 MHz (^{13}C). Samples were dissolved in MeOH-d_4. If necessary, the residual HOD resonance was suppressed by presaturation. LC/MS was performed on a Micromass Platform single-quadrupole mass spectrometer in both positive electrospray (+ESI) and negative electrospray (–ESI) modes. Accurate LC-MS and LC-MS-MS experiments were conducted on a Micromass hybrid quadrupole time-of-flight (Q-Tof) instrument. Values for M^+ where given were calculated from all adducts measured in +ve or −ve modes and averaged to give the values shown.

Biological Material. Strain CP2808 was isolated from soil and determined to be a novel isolate of *Amycolatopsis* by partial sequence of the 16S rRNA gene. The partial DNA sequence of the 16S rRNA gene has been submitted to GenBank under the accession number EF051628. The designation as *Amycolatopsis* was confirmed by



10



11

morphologic observation and sensitivity to *Amycolatopsis oerientalis* bacteriophage (data not shown). Fermentation of CP2808 was carried out with 350 mL of GAPY broth (glucose 1%, soluble starch 2%, yeast extract 0.5%, bacto peptone 0.5%, and $CaCO_3$ 1%)[6] in 2800 mL Fernbach flasks (Pyrex Bellco Glass, Inc.; No. 4420). Each flask was inoculated with at least 4.7 mL of mature vegetative culture grown in ½ NZ Amine broth (glucose 0.5%, soluble starch 1%, yeast extract 0.25%, NZ Amine 0.25%, and $CaCO_3$ 0.05%). Fermentation was incubated for at least 8 days without shaking at room temperature (~22 °C). *Amycolatopsis* sp. CP2808 has been deposited with the National Center for Agricultural Utilization Research, Peoria, IL, under the number NRRL B-24544.

Extraction and Isolation. CP2808 fermentations were extracted by addition of 1 equiv of EtOH (350 mL) to the whole broth. After 2 h, the extract was centrifuged and filter-sterilized, resulting in an aqueous EtOH extract. The 50% EtOH extract (500 mL) was diluted to 10% EtOH with Millipore Milli-Q H_2O and pumped through a Mitsubishi Diaion HP20 SPE (40 × 150 mm) cartridge from Biotage. After rinsing the cartridge with Milli-Q water (100 mL), it was eluted with 80% and 100% MeOH in Milli-Q water (250 mL each). Each eluate was evaporated to dryness, resuspended in MeOH (5 mL), applied to 10 g of silica gel (Merck LiChroprep Si 60), and dried under vacuum. The silica gel was packed dry into an SPE cartridge and eluted with a step gradient of 0% to 20% MeOH in EtOAc. Fractions were analyzed by HPLC (Agilent 1050 pump and UV-DAD, 4.6 × 250 mm Phenomenex Synergi Hydro-RP column eluted with 20% CH_3CN in 0.02 M NH_4OAc over 2 min followed by a gradient to 30% CH_3CN from 2 min to 15 min, and then held at 30% CH_3CN for a further 5 min). The fractions that had been eluted from silica with 4% to 10% MeOH contained the ansacarbamitocins. These fractions were evaporated to dryness, redissolved in small volumes of MeOH, and separated by semipreparative HPLC using an Agilent 1100 LC-MSD instrument.

Ansacarbamitocin A (1): yield (from 3 L) 11 mg; beige solid; ^{1}H NMR (MeOH-d_4) see Table 2.

Ansacarbamitocin B (2): 12 mg; colorless crystals (from EtOAc/*n*-hexane); mp 195 °C (dec); $[\alpha]^{25}_D$ −8.8 (*c* –0.17, MeOH); ^{1}H and ^{13}C NMR (MeOH-d_4) see Table 1; ESIMS *m/z*, M^+ = 840.2853, $C_{37}H_{49}N_4O_{16}Cl$ requires 840.2832.

Ansacarbamitocin C (3): 3 mg; beige solid; ^{1}H NMR (MeOH-d_4) see Table 2.

Ansacarbamitocin D (4): 2 mg; beige solid; ^{1}H NMR (MeOH-d_4) see Table 2; HRESIMS *m/z*, M^+ = 798.2740, $C_{35}H_{47}N_4O_{15}Cl$ requires 798.2726.

Ansacarbamitocin E (5): 3 mg; beige solid; ^{1}H NMR (MeOH-d_4) see Table 2.

Ansacarbamitocin F (6): 3 mg; beige solid; ^{1}H NMR (MeOH-d_4) see Table 2; HRESIMS *m/z*, M^+ = 755.2685, $C_{34}H_{46}N_3O_{14}Cl$ requires 755.2668.

Ansacarbamitocin A1 (7): 3 mg; beige solid; ^{1}H NMR (MeOH-d_4) see Table 2.

Ansacarbamitocin B1 (8): 3 mg; beige solid; ^{1}H NMR (MeOH-d_4) see Table 2.

Methylation of Ansacarbamitocin B. To a stirred solution of ansacarbomitocin B (5.7 mg) in 1 mL of acetone was added K_2CO_3 (5 equiv) followed by MeI (10 equiv). The reaction was allowed to stir at room temperature for 4 h. HPLC analysis revealed some starting material remained. An additional 5 equiv of K_2CO_3 and 10 equiv of MeI were added, and the reaction was stirred for an additional 2 h at room temperature. The reaction was filtered, and the solids were washed with 10 mL of EtOAc. The solution was concentrated to afford a residue, which was purified via reversed-phase chromatography. The conditions were 4.7 mL/min 30% MeCN in 0.01 M NH_4OAc pH 6, with gradient to 34% MeCN from 0 min to 40 min on a 10 × 250 mm Phenomenex Synergi Hydro-C18, 10 μm particles. The methylated product (14.2 min) was isolated as a white solid (2.1 mg; 36%): ^{1}H NMR (600 MHz, CD_3OD) δ 7.17 (d, J = 1.5 Hz, 1 H), 7.16 (d, J = 1.5 Hz, 1 H), 6.59 (dd, J = 15.4, 11.3 Hz, 1 H), 6.35 (d, J = 11.1 Hz, 1 H), 5.81 (d, J = 9.7 Hz, 1 H), 5.48 (dd, J = 15.3, 9.4 Hz, 1 H), 4.39 (dd, J = 11.9, 2.9 Hz, 1 H), 4.35 (dd, J = 9.6, 9.6 Hz, 1 H), 4.26 (ddd, J = 12.1, 10.5, 1.6 Hz, 1 H), 4.14 (dd, J = 12.0, 2.8 Hz, 1 H), 4.01 (dd, J = 6.1, 6.1 Hz, 1 H), 3.99 (s, 3 H, arom OMe), 3.70 (ddd, J = 10.0, 5.5, 2.8 Hz, 1 H), 3.66 (dd, J = 9.2, 9.2 Hz, 1 H), 3.64 (d, J = 4.2 Hz, 1 H), 3.55 (d, J = 9.0 Hz, 1 H), 3.37 (s, 3 H), 3.28 (d, J = 11.8 Hz, 1 H), 3.16 (dd, J = 9.3, 9.3 Hz, 1 H), 2.90 (d, J = 9.6 Hz, 1 H), 2.78 (s, 3 H), 2.44 (dd, J = 13.0, 12.0 Hz, 1 H), 2.16 (dd, J = 13.0, 3.2 Hz, 1 H), 2.00 (s, 3 H), 1.76 (s, 3 H), 1.65 (dd, J = 13.8, 1.8 Hz, 1 H), 1.53 (m, 1 H), 1.44 (dd, J = 13.8, 12.2 Hz, 1 H), 1.23 (d, J = 6.4 Hz, 3 H), 0.76 (s, 3 H).

Biological Testing. The samples were evaluated for fungicidal activity by application as 1-day protectant (1DP) treatments for control of barley spot blotch (*Helminthosporium sativum*), wheat powdery mildew (*Erysiphe graminis* f. sp. *tritici*), wheat glume blotch (*Septoria nodorum*), wheat brown rust (*Puccinia triticina*), and rice blast (*Pyricularia oryzae*).

Acknowledgment. The authors would like to acknowledge J. Lira and M. Grayson for their assistance in this study.

Supporting Information Available: NMR spectra (proton and HSQC) for all ansacarbamitocins. This material is available free of charge via the internet at http://pubs.acs.org

References and Notes

(1) Cassidy, J. M.; Chan, K. K.; Floss, H. G.; Leistner, E. *Chem. Pharm. Bull.* **2004**, *52*, 1.
(2) Ikeyama, S.; Takeuchi, M. *Biochem. Pharmacol.* **1981**, *30*, 2421.
(3) Hatano, K.; Higashide, E.; Yoneda, M. *Agric. Biol. Chem.* **1984**, *48*, 1889.
(4) Asai, M.; Mizuta, E.; Izawa, M.; Haibara, K.; Kishi, T. *Tetrahedron* **1979**, *35*, 1079.
(5) Lu, C.; Bai, L.; Shen, Y. *J. Antibiot.* **2004**, *57*, 348.
(6) Daniel, D.; Tiraby, G. *J. Antibiot.* **1983**, *36*, 181.

NP070275T