Supporting Information

Total Synthesis of Novel 6-Substituted Lavendamycin Antitumor Agents

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Experimental Section

General Methods

Solvents: 1,4-dioxane, xylene, and anisole were dried and distilled over sodium. Ethanol and chloroform were dried and distilled over CaH_2 . DMF was dried over molecular sieve. All other solvents were reagent grade and used as such.

Melting Ranges: were recorded with a Thomas-Hoover capillary melting point apparatus and are in celcius.

Infrared Spectra (IR): were recorded on a Perkin-Elmer FT-IR spectrometer 1000. All solid sample spectra were recorded on their KBr pellets.

Nuclear Magnetic Resonance Spectra (NMR): were recorded on a JEOL Eclipse 400 spectrometer using CDCl_3 or DMSO-d_6 purchased from Cambridge Isotope Laboratories.

Low and High Resolution Mass Spectra: EI, CI and FAB mass spectra were obtained at Indiana University Mass Spectrometry Laboratory, Bloomington, IN.

Thin-Layer Chromatography (TLC): Eastman silica gel and alumina strips with fluorescent indicator were used.

Aziridine was prepared according to the procedure described by: Allen, C. F. H.; Spangler, F. W.; Webster, E. R. *Organic Syntheses Collective Volume IV* **1963**, 433 and stored in refrigerator over KOH pellets.

7-Amino-2-formyl-6-methoxyquinoline-5,8-dione (25)

In a dry 25 mL round-bottomed two-necked flask, equipped with a magnetic bar, water cooled reflux condenser under argon, 7-amino-6-methoxy-2-methylquinoline-5,8-dione (**24**, 100 mg, 0.46 mmol), selenium dioxide (91.7 mg, 0.83 mmol), 7 mL dried and distilled 1,4-dioxane, and 0.08 mL of water were stirred at reflux in an oil bath for 9 h. Another 0.2 equivalent of selenium dioxide (10.2 mg, 0.092 mmol) and water (0.001) was added to the mixture and stirred and refluxed for 3 more hours. The reaction was monitored by TLC. The mixture was hot filtered and washed with hot chloroform. The filtrate was rotaevaporated to dryness. The dried compound was dissolved in 100 mL of chloroform and washed with saturated sodium bicarbonate solution (3 × 15 mL) and then cold water (15 mL). The solution was dried over magnesium sulfate and rotaevaporated to dryness and then further dried under a vacuum pump. The dark red solid product weighed 54.6 mg (51%): ^1H NMR (CDCl_3) δ 10.25 (1H, s), 8.53 (1H, d, $J = 8.0$ Hz), 8.21 (1H, d, $J = 8.0$ Hz), 5.30 (2H, s), 4.10 (3H, s); IR (KBr) ν_{max} 3401, 3309, 1710, 1685, 1615, 1557, 1440, 1398, 1350, 1225, 1071, 1011, 919, 873, 744, 514 cm^{-1} ; EIMS m/e (relative intensity) 232.1 (M^+ , 100.0), 220.1 (26.4), 219.1 (84.2), 218.1 (50.5), 205.1 (37.5), 204.1 (23.6), 191.1 (33.4), 189.1 (30.9), 106.1 (22.1); Anal. calcd. for $\text{C}_{11}\text{H}_8\text{N}_2\text{O}_4$; C, 56.90; H, 3.47; N, 12.06 found C, 56.73; H, 3.71; N, 11.88.

7-Amino-6-chloro-2-formylquinoline-5,8-dione (31)

In a dry 100 mL round-bottomed two-necked flask, equipped with a magnetic bar, water cooled reflux condenser under argon, 7-amino-6-chloro-2-methylquinoline-5,8-dione (**30**, 133.8 mg, 0.6 mmol), selenium dioxide (106.6 mg), 20 mL dried and distilled 1,4-dioxane, and 0.09 mL of water were stirred at reflux in an oil bath for 23 h. The reaction was monitored by TLC. The mixture was hot filtered and the solid was washed with hot chloroform. The filtrate was rotaevaporated to dryness. The dried material was dissolved in 480 mL of chloroform and washed with saturated sodium chloride solution (4 × 60 mL). The solution was dried over magnesium sulfate and rotaevaporated to dryness and then further dried under a vacuum pump. The product

weighed 105.2 mg (74%): ^1H NMR (CDCl_3) δ 10.30 (1H, s), 8.67 (1H, d, $J = 8.1$ Hz), 8.28 (1H, d, $J = 8.1$ Hz), 5.60 (2H, br s); IR (KBr) ν_{max} 3434, 3292, 3057, 2924, 1712, 1698, 1633, 1602, 1551, 1381, 1350, 1326, 1257, 1075, 831, 742 cm^{-1} ; EIMS m/e 238.0, 236.0 (M^+), 222.0, 210.0, 208.0, 201.0, 187.0, 171.0.

6-Methoxydemethylavendamycin methyl ester (6)

In a dry 250 mL 3-necked round-bottomed flask equipped with a Dean-Stark trap under an argon flow, 7-amino-2-formyl-6-methoxyquinoline-5,8-dione (**25**, 48 mg, 0.2 mmol) and tryptophan methyl ester (44 mg, 0.2 mmol) were dissolved in dry anisole (120 mL). The reaction mixture was heated to reflux gradually over 3 h and then refluxed for another 8 h. The reaction mixture was cooled and rotaevaporated to dryness. The solid was washed with acetone (20 mL), filtered, and air dried. The dark orange brown solid weighed 38.4 mg (45%): mp. >280 °C; ^1H NMR (CDCl_3) δ 11.94 (1H, br s), 9.11 (1H, d, $J = 8.3$ Hz), 8.98 (1H, s), 8.53 (1H, d, $J = 8.3$ Hz), 8.23 (1H, d, $J = 7.4$ Hz), 7.78 (1H, d, $J = 8.1$ Hz), 7.65 (1H, m), 7.38 (1H, m), 5.20 (2H, br s), 4.12 (3H, s), 4.09 (3H, s); IR (KBr) ν_{max} 3444, 3335, 1712, 1680, 1615, 1557, 1489, 1435, 1400, 1346, 1264, 1232, 1121, 1075, 1017, 921, 735 cm^{-1} ; FABMS m/e (relative intensity) 431 ($\text{M}+1$, 10), 309 (70), 275 (20), 195 (22), 155 (73), 135 (59), 119 (100); HRMS calcd. for $\text{C}_{23}\text{H}_{19}\text{N}_4\text{O}_5$ 431.135 ($\text{M}+3$) found 431.135.

6-Methoxylavendamycin methyl ester (7)

In a dry 100 mL 3-necked round-bottomed flask equipped with a Dean-Stark trap under an argon flow, 7-amino-2-formyl-6-methoxyquinoline-5,8-dione (**25**, 23 mg, 0.1 mmol) and β -methyltryptophan methyl ester (23 mg, 0.1 mmol) were dissolved in dry anisole (60 mL). The reaction mixture was heated to reflux gradually over 3 h and then refluxed for another 7 h. The reaction mixture was cooled and rotaevaporated to dryness. The solid was washed with acetone (3 mL), filtered, and air dried. The orange brown solid weighed 18 mg (41%): mp. >280 °C; ^1H NMR (CDCl_3) δ 11.99 (1H, br s), 9.03 (1H, d, $J = 8.3$ Hz), 8.49 (1H, d, $J = 8.3$ Hz), 8.35 (1H, d, $J = 7.8$ Hz), 7.80 (1H, d, $J = 8.0$ Hz), 7.64 (1H, m), 7.38 (1H, m), 5.18 (2H, br s), 4.11 (3H, s), 4.06 (3H, s), 3.20 (3H, s); IR (KBr) ν_{max} 3325, 1701, 1616, 1489, 1437, 1400, 1337, 1276, 1234, 1079, 918, 733 cm^{-1} ; EIMS m/e (relative intensity) 442.13 (M^+ , 90.1), 374.01 (100), 316.98 (22.4), 201.00 (22.2), 151.00 (58.6); HRMS calcd. for $\text{C}_{24}\text{H}_{18}\text{N}_4\text{O}_5$ 442.127 found 442.127.

6-Methoxydemethylavendamycin amide (**8**)

In a dry 250 mL 3-necked round-bottomed flask equipped with a Dean-Stark trap under an argon flow, 7-amino-2-formyl-6-methoxyquinoline-5,8-dione (**25**, 46.5 mg, 0.2 mmol) and tryptophanamide (42 mg, 0.2 mmol) were dissolved in dry anisole (120 mL). The reaction mixture was heated to reflux gradually over 3 h and then refluxed for another 9 h. The solution was cooled and rotaevaporated to dryness. The solid was washed with acetone (20 mL), filtered, and air dried. The tan solid weighed 27 mg (32%): ^1H NMR ($\text{DMSO}-d_6$) δ 11.99 (1H, br s), 9.41 (1H, d, $J = 8.1$ Hz), 9.07 (1H, s), 8.60 (1H, br s), 8.53 (1H, d, $J = 8.1$ Hz), 8.47 (1H, d, $J = 8.0$ Hz), 7.78 (1H, d, $J = 8.0$ Hz), 7.74 – 7.70 (1H, m), 7.67 (1H, br s), 7.44 – 7.40 (1H, m), 7.14 (2H, br s), 3.89 (3H, s); EIMS m/e (relative intensity) 413.2 (M^+ , 100), 369.2 (13), 353.1 (12), 325.1 (19), 270.1 (10), 242.1 (15), 130.1 (36); HRMS calcd. for $\text{C}_{22}\text{H}_{15}\text{N}_5\text{O}_4$ 413.116 found 413.112.

6-Chlorolavendamycin methyl ester (9)

In a dry 500 mL 3-necked round-bottomed flask equipped with a magnetic stirring bar, and a Dean-Stark trap under an argon flow, 7-amino-6-chloro-2-formylquinoline-5,8-dione (**31**, 118.5 mg, 0.5 mmol) was placed with β -methyltryptophan methyl ester (115 mg, 0.5 mmol) in 300 mL of dry anisole. The solution was stirred and heated slowly to 130 °C over 3 h. The reaction mixture was refluxed for one hour. The mixture was then cooled down to room temperature under argon. The solvent was rotaevaporated to dryness and then the solid was washed with acetone (20 mL). The resulting solid was vacuum filtered and dried under a vacuum pump. The product weighed 131.9 mg (59%): ^1H NMR (CDCl_3) δ 11.89 (1H, br s), 9.10 (1H, d, $J = 8.4$ Hz), 8.61 (1H, d, $J = 8.4$ Hz), 8.38 (1H, d, $J = 7.7$ Hz), 7.80 (1H, d, $J = 8.0$ Hz), 7.67 (1H, m), 7.41 (1H, m), 5.70 (2H, br s), 4.08 (3H, s), 3.22 (3H, s); CIMS m/e (relative intensity) 446.1 (M^+ , 100), 416.0 (10.3), 386.0 (64.4), 351.1 (22.0), 113.0 (9.0); HRMS calcd. for $\text{C}_{23}\text{H}_{15}\text{N}_4\text{O}_4\text{Cl}$ 446.081 found 446.078.

6-Chlorodemethylavendamycin ethyl ester (10)

In a dry 500 mL 3-necked round-bottomed flask equipped with a magnetic stirring bar, and a Dean-Stark trap under an argon flow, 7-amino-6-chloro-2-formylquinoline-5,8-dione (**31**, 47.4 mg, 0.2 mmol) was placed with tryptophan ethyl ester (46.4 mg, 0.2 mmol) in 120 mL of dry anisole. The solution was stirred and

heated slowly to 130 °C over 3 h. The reaction mixture was refluxed for one hour. The mixture was then cooled down to room temperature under argon. The solvent was rotaevaporated to dryness and then the solid was washed with acetone (20 mL). The resulting solid was vacuum filtered and dried under a vacuum pump. The product weighed 47 mg (49%): ^1H NMR (CDCl_3) δ 11.86 (1H, br s), 9.22 (1H, d, J = 8.3 Hz), 8.99 (1H, s), 8.67 (1H, d, J = 8.3 Hz), 8.27 (1H, d, J = 7.8 Hz), 7.77 (1H, d, J = 7.3 Hz), 7.68 (1H, m), 7.42 (1H, m), 5.60 (2H, br s), 4.57 (2H, q, J = 7.2 Hz), 1.57 (3H, t, J = 7.2 Hz); IR (KBr) ν_{max} 3463, 3312, 2976, 1708, 1611, 1367, 1330, 1229, 826, 740 cm^{-1} ; FABMS m/e (relative intensity) 447 (M^+ , 5.2), 391 (36.7), 371 (37.4), 307 (19.9), 289 (14.5), 167 (11.7), 154 (100), 149 (45.5), 136 (68.6), 129 (31.1), 106 (24.3); HRMS calcd. for $\text{C}_{23}\text{H}_{15}\text{N}_4\text{O}_4\text{Cl}$ 447.086 ($M+1$) found 447.086.

6-Chlorodemethylavendamyacin pyrrolidine amide (11)

In a dry 250 mL 3-necked round-bottomed flask equipped with a magnetic stirring bar, and a Dean-Stark trap under an argon flow, 7-amino-6-chloro-2-formylquinoline-5,8-dione (**31**, 35.5 mg, 0.15 mmol) was placed with tryptophan pyrrolidine amide (38.6 mg, 0.15 mmol) in 60 mL of dry anisole. The solution was stirred and heated slowly to 110 °C over 3.5 h. The reaction mixture was refluxed for 30 minutes. The mixture was then cooled down to room temperature under argon. The solvent was rotaevaporated to dryness and then the solid was washed with petroleum ether (10 mL). The resulting solid was vacuum filtered and dried under a vacuum pump. Flash column chromatography (silica gel 40, 6 in., d = 1.8 cm, 2% MeOH in CH_2Cl_2 as eluent) of the crude product afforded 23.7 mg of an orange solid (36%): ^1H NMR ($\text{DMSO}-d_6$) δ 11.82 (1H, br s), 8.86 (1H, d, J = 8.4 Hz), 8.84 (1H, s), 8.55 (1H, d, J = 8.4 Hz), 8.48 (1H, d, J = 7.7 Hz), 7.74 (1H, d, J = 8.4 Hz), 7.70 (1H, m), 7.39 (1H, m), 3.97 (2H, m), 3.64 (2H, m), 1.95 (4H, m), EIMS m/e (relative intensity) 471.1 (M^+ , 33), 376.1 (33), 374.1 (100), 236 (36), 181 (26), 169 (29), 119 (32), 97 (21).

6-Chlorodemethylavendamycin isoamyl ester (12)

In a dry 500 mL 3-necked round-bottomed flask equipped with a magnetic stirring bar, and a Dean-Stark trap under an argon flow, 7-amino-6-chloro-2-formylquinoline-5,8-dione (**31**, 47.4 mg, 0.2 mmol) was placed with tryptophan isoamyl ester (56 mg, 0.2 mmol) in 120 mL of dry anisole. The solution was stirred and heated slowly to 130 °C over 3 h. The reaction mixture was refluxed for one hour. The mixture was then cooled down to room temperature under argon. The solvent was rotaevaporated to dryness and then the solid was washed with acetone (20 mL). The resulting solid was vacuum filtered and dried under a vacuum pump. The product weighed 54.5 mg (56%): ^1H NMR (CDCl_3) δ 11.91 (1H, br s), 9.20 (1H, d, J = 8.2 Hz), 9.00 (1H, s), 8.65 (1H, d, J = 8.2 Hz), 8.25 (1H, d, J = 8.1 Hz), 7.81 – 7.60 (2H, m), 7.45 (1H, m), 5.70 (2H, br s), 4.50 (2H, t, J = 6.9 Hz), 1.90 (2H, m), 1.26 (1H, m), 1.08 (6H, d, J = 8.1 Hz); IR (KBr) ν_{max} 3462, 3337, 2957, 1696, 1614, 1473, 1330, 1262, 1224, 828, 738 cm^{-1} ; EIMS m/e (relative intensity) 488 (M^+ , 40.8), 454 (24.1), 446 (25.1), 376 (35.7), 375 (29.9), 374 (96.0), 340 (46.4), 199 (100.0), 184 (35.7), 91 (27.2), 69 (32.1), 57 (37.7); HRMS calcd. for $\text{C}_{26}\text{H}_{21}\text{N}_4\text{O}_4\text{Cl}$ 488.125 found 488.125.

6-Chlorodemethylavendamycin octyl ester (13)

In a dry 500 mL 3-necked round-bottomed flask equipped with a magnetic stirring bar, and a Dean-Stark trap under an argon flow, 7-amino-6-chloro-2-formylquinoline-5,8-dione (**31**, 47.4 mg, 0.2 mmol) was placed with tryptophan octyl

ester (63.2 mg, 0.2 mmol) in 120 mL of dry anisole. The solution was stirred and heated slowly to 130 °C over 3 h. The reaction mixture was refluxed for 2 hours. The mixture was then cooled down to room temperature under argon. The solvent was rotaevaporated to dryness and then the solid was washed with acetone (20 mL). The resulting solid was vacuum filtered and dried under a vacuum pump. The product weighed 27.3 mg (26%): ^1H NMR (CDCl_3) δ 11.82 (1H, br s), 9.18 (1H, d, J = 8.3 Hz), 8.96 (1H, s), 8.63 (1H, d, J = 8.3 Hz), 8.25 (1H, d, J = 7.8 Hz), 7.75 (1H, d, J = 7.4 Hz), 7.67 (1H, m), 7.39 (1H, m), 5.60 (2H, br s), 4.48 (2H, t, J = 6.9 Hz), 1.90 (2H, m), 1.27 (10H, m), 0.88 (3H, t, J = 3.8 Hz); IR (KBr) ν_{max} 3470, 3332, 2925, 1725, 1611, 1472, 1329, 1228, 830, 739 cm^{-1} ; EIMS m/e (relative intensity) 532 ($M+2$, 3.1), 446 (29.2), 265 (22.0), 250 (27.7), 226 (23.5), 212 (22.6), 199 (100.0), 184 (40.3), 149 (33.8), 97 (24.0), 91 (30.6), 83 (32.8), 69 (50.2); HRMS calcd. for $\text{C}_{29}\text{H}_{27}\text{N}_4\text{O}_4\text{Cl}$ 530.172 found 530.171.

6-Chlorodemethylavendamycin amide (14)

In a dry 500 mL 3-necked round-bottomed flask equipped with a magnetic stirring bar, and a Dean-Stark trap under an argon flow, 7-amino-6-chloro-2-formylquinoline-5,8-dione (**31**, 47.4 mg, 0.2 mmol) was placed with tryptophanamide (40.8 mg, 0.2 mmol) in 120 mL of dry anisole. The solution was stirred and heated slowly to 130 °C over 3 h. The reaction mixture was refluxed for 13 h. The mixture was then cooled down to room temperature under argon. The solvent was rotaevaporated to dryness and then the solid was washed with acetone (20 mL). The resulting solid was vacuum filtered and dried under a vacuum pump. The product weighed 44.4 mg (53%): ^1H NMR ($\text{DMSO}-d_6$) δ 11.95 (1H, br s), 10.21 (2H, s), 9.44 (1H, d, J = 8.4 Hz), 9.07 (1H, s), 8.52 (1H, d, J = 8.4 Hz), 8.49 (1H, d, J = 8.4 Hz), 7.90 (2H, br s), 7.79 – 7.67 (2H, m), 7.43 (1H, m); IR (KBr) ν_{max} 3450, 3326, 1609, 1391, 1337, 1232, 830, 739 cm^{-1} ; FABMS m/e (relative intensity) 420 ($M+3$, 1.6), 385 (9.9), 309 (9.1), 233 (35.7), 195 (11.6), 159 (14.4), 157 (100.0), 155 (33.5), 135 (32.2), 118 (74.0); HRMS calcd. for $\text{C}_{21}\text{H}_{15}\text{N}_5\text{O}_3\text{Cl}$ 420.086 ($M+3$) found 420.086.

7-*N*-Acetyl-6-aziridinolavendamycin methyl ester (15)

To a stirred solution of 7-acetylavendamycin methyl ester (**3**, 50 mg, 0.11 mmol) in dry chloroform (30 ml) was added aziridine (0.7 mL, 580 mg, 13.5 mmol) and the resulting orange solution was allowed to stir at room temperature for 24 h. The reaction mixture was rotaevaporated to dryness. The solid was washed with ether (10 mL) and further dried under a vacuum pump to afford 43 mg (79 %) of the product as a yellowish orange powder: mp. 250 °C (Dec.); ¹H NMR (DMSO-*d*₆) δ 12.11 (1H, s), 9.57 (1H, s), 8.82 (1H, d, *J* = 7.3 Hz), 8.57 (1H, d, *J* = 7.3 Hz), 8.42 (1H, d, *J* = 7.9 Hz), 7.83 (1H, d, *J* = 7.9 Hz), 7.75 – 7.68 (1H, m), 7.47 – 7.40 (1H, m), 3.98 (3H, s), 3.10 (3H, s), 2.39 (4H, s), 2.17 (3H, s); IR (KBr) ν_{max} 3341, 3244, 3000, 2955, 1708, 1664, 1581, 1494, 1368, 1335, 1299, 1272, 1076, 736 cm⁻¹; EIMS *m/e* (relative intensity) 495.15 (M⁺, 100.0), 421.1 (28.2), 393.1 (87.2), 378.1 (25.8), 282.1 (10.2), 196.6 (10.2), 125.1 (10.8), 111.1 (14.4), 83.1 (19.7); HRMS calcd. for C₂₇H₂₁N₅O₅ 495.158 found 495.154.

7-*N*-Acetyl-6-pyrrolidinolavendamycin methyl ester (16)

To a stirred solution of 7-acetylavendamycin methyl ester (**3**, 50 mg, 0.11 mmol) in dry chloroform (30 ml) was added pyrrolidine (0.2 mL, 170 mg, 2.4 mmol) and the resulting red-brown solution was allowed to stir at room temperature for 2 h.

Then the reaction mixture was rotaevaporated to dryness. The solid was washed with ether (10 mL) and further dried under a vacuum pump to afford 51 mg (88 %) of the product as a reddish brown powder: mp. 210 °C (Dec.); ^1H NMR (DMSO- d_6) δ 12.36 (1H, s), 9.33 (1H, s), 8.78 (1H, d, J = 8.2 Hz), 8.50 (1H, d, J = 8.2 Hz), 8.45 (1H, d, J = 7.9 Hz), 7.82 (1H, d, J = 7.9 Hz), 7.76 – 7.70 (1H, m), 7.48 – 7.41 (1H, m), 3.98 (3H, s), 3.78 – 3.58 (4H, br m), 3.11 (3H, s), 2.11 (3H, s), 1.95 – 1.75 (4H, br m); IR (KBr) ν_{max} 3437, 3326, 2948, 1712, 1664, 1590, 1553, 1398, 1331, 1294, 1276, 1073, 744 cm^{-1} ; FABMS m/e (relative intensity) 546.2 (M+Na, 15.4), 527.3 (14.2), 507.3 (25.2), 506.3 (100.0), 413.5 (29.0), 391.5 (30.5), 232.0 (31.0), 176.0 (50.0), 154.0 (99.5); HRMS calcd. for $\text{C}_{29}\text{H}_{25}\text{N}_5\text{O}_5\text{Na}$ (M+Na) 523.180 found 546.175.

7-*N*-Acetyl-6-aziridinodemethylavendamycin amide (17)

To a stirred solution of 7-acetyldemethylavendamycin amide (**5**, 50 mg, 0.12 mmol) in dry chloroform (25 mL) and dry ethanol (25 mL) was added aziridine (0.7 mL, 580 mg, 13.5 mmol) and the resulting reddish brown solution was allowed to stir at room temperature for 48 h. Then the reaction mixture was rotaevaporated to dryness. The solid was washed with ether (10 mL) and further dried under a vacuum pump to afford 52 mg (93 %) of the product as a reddish brown powder: mp > 260 °C; ^1H NMR (DMSO- d_6) δ 12.05 (1H, s), 9.57 (1H, s), 9.45 (1H, d, J = 8.2 Hz), 9.07 (1H, s), 8.62 (1H, s), 8.55 – 8.49 (2H, m), 7.83 (1H, d, J = 8.2 Hz), 7.74 – 7.69 (1H, m), 7.68 (1H, s), 7.44 – 7.38 (1H, m), 2.39 (4H, s), 2.17 (3H, s); IR (KBr) ν_{max} 3429, 3326, 1675, 1653, 1577, 1494, 1472, 1387, 1368, 1303, 1231, 736 cm^{-1} ; ESMS m/e (relative intensity) 468 (M+2, 2.50), 467 (100), 441 (60), 318 (30), 296 (43); HRMS calcd. for $\text{C}_{25}\text{H}_{19}\text{N}_6\text{O}_4$ (M+1) 467.150 found 467.148.

7-*N*-Acetyl-6-pyrrolidinodemethylavendamycin amide (18)

To a stirred solution of 7-acetyldemethylavendamycin amide (**5**, 50 mg, 0.12 mmol) in DMF (10 mL) was added pyrrolidine (0.3 mL, 255 mg, 3.6 mmol) and the resulting dark brown solution was allowed to stir at room temperature for 2 h. Then the reaction mixture was rotaevaporated to dryness. The solid was washed with THF (10 mL) and further dried under a vacuum pump to afford 50 mg (86%) of the product as a reddish brown powder: mp 245 °C (Dec.); ¹H NMR (DMSO-d₆) δ 12.25 (1H, s), 9.40 – 9.30 (2H, m), 9.06 (1H, s), 8.61 (1H, s), 8.52 (1H, d, J = 7.7 Hz), 8.43 (1H, d, J = 8.2 Hz), 7.78 (1H, d, J = 7.9 Hz), 7.73 – 7.69 (1H, m), 7.67 (1H, s), 7.43 – 7.38 (1H, m), 3.78 – 3.60 (4H, br m), 2.11 (3H, s), 1.98 – 1.75 (4H, br m); IR (KBr) ν_{max} 3452, 3296, 2970, 2926, 1675, 1623, 1590, 1533, 1390, 1301, 1235, 747 cm⁻¹; FABMS m/e (relative intensity) 495.2 (M+1, 9.9), 484.4 (26.2), 483.4 (100.0); HRMS calcd. for C₂₇H₂₃N₆O₄ 495.182 (M+1) found 495.178.

Tryptophan isoamyl ester

Isoamyl alcohol was dried over anhydrous cupric sulfate for 24 h and then distilled under argon. Tryptophan (1.43 g, 7 mmol) was placed in a 100 mL round-bottomed flask along with 60 mL of the dried isoamyl alcohol and 10 mL of HCl/ether solution. The solution was refluxed in an oil bath for 22 h. The mixture was then rotaevaporated to dryness. A portion of the resulting tryptophan isoamyl ester hydrochloride (723 mg, 2.84 mmol) was suspended in 36 mL ethyl acetate. To this stirred suspension, a 14% solution of ammonium hydroxide (~ 3 mL) was added until

the aqueous layer was at pH = 8. The aqueous layer was separated and the organic layer was washed with a saturated sodium chloride solution (3 × 2 mL) and water (2 mL) and dried over magnesium sulfate. The solution was filtered and rotaevaporated to dryness. The thick liquid was further dried under a vacuum pump at 50 – 60 °C for two days. The overall product weighed 1.6 g (83%): mp 50 °C (Dec.); ¹H NMR (CDCl₃) δ 8.08 (1H, br s), 7.59 (1H, d, J = 7.3 Hz), 7.33 (1H, d, J = 7.6 Hz), 7.20 (1H, m), 7.10 (1H, m), 7.00 (1H, s), 4.10 (2H, t, J = 6.7 Hz), 3.81 (1H, m), 3.22 – 2.99 (2H, m), 1.60 (4H, m), 1.50 (1H, m), 0.90 (6H, d, J = 6.3 Hz); IR (KBr) ν_{max} 3353, 3058, 2957, 2928, 2870, 1727, 1670, 1620, 1519, 1458, 1436, 1341, 1234, 1204, 1098, 1010, 943, 743 cm⁻¹; CIMS m/e (relative intensity) 275.2 (M+1, 20), 274.2 (28), 257.1 (37), 159.1 (10), 131 (22), 130.1 (100); HRMS calcd. for C₁₆H₂₂N₂O₂ 274.168 found 274.168.