Novel Lavendamycin Analogues as Potent HIV-Reverse Transcriptase Inhibitors: Synthesis and Evaluation of Anti-Reverse Transcriptase Activity of Amide and Ester Analogues of Lavendamycin

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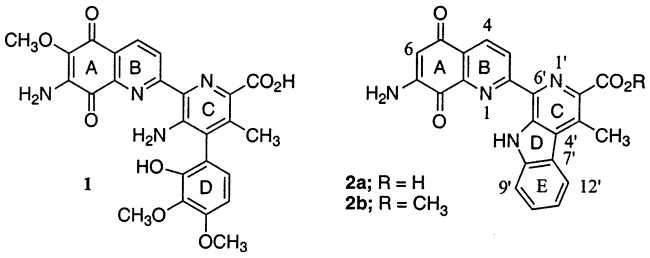
Novel lavendamycins including two water soluble derivatives were synthesized via short and efficient methods. Pictet−Spengler condensation of 7-*N*-acylamino-2-formylquinoline-5,8-diones with tryptophans produced lavendamycin esters or amides **11**−**17**. Lavendamycins **18**−**21** were obtained, respectively, by further transformations of **13**−**15** and **17.** Several lavendamycins were found to be potent HIV reverse transcriptase inhibitors with very low toxicity in vitro and in vivo. Several compounds also acted either additively or synergistically to inhibit enzyme activity together with AZT-triphosphate.

Introduction

Human immunodeficiency virus (HIV) infection, with its clinical progression to AIDS, is one of the leading causes of morbidity and mortality in the world.[1] Although highly active anti-retroviral therapy (HAART) using a combination of anti-retrovirals has been highly effective in suppressing HIV load and decreasing mortality of these infections, the emergence of drug resistance among HIV and inherent toxicity of most of the anti-retroviral therapies makes the continued search for novel anti-HIV drugs imperative.[2] Indeed, it is now estimated that over 30% of patients are failing combination therapy. Such failure is partly due to the development of drug resistance and drug toxicity but also to the lack of patient compliance due to demanding drug dosing. [2a,3] Currently approved HIV drugs include six nucleoside HIV reverse transcriptase (HIV-RT) inhibitors, zidovudine (AZT), didanosine(ddI), stuvudine (d4T), lamivudine, (3TC), zalcitabine (ddC), and abacavir (ABC); three nonnucleoside RT inhibitors, nevirapinne, delavirdine, and efavirenz; and five protease inhibitors, squinavir, indinavir, ritonavir, nelfinavir, amprenovir, and lopiavir.[3] Currently HAART using different combinations of these drugs is the preferred treatment for AIDS patients.[2a,4] Nonnucleoside HIV-1 reverse transcriptase inhibitors (NNRTI) are becoming increasingly important additions to this combination retroviral therapy, and these compounds tend to have good antiviral potency, high specificity, and low toxicity.[5] It has been suggested that NNRTI's used in combination regimens with protease inhibitors (PI) may be used in the future either as first-line or second-line or salvage therapy in patients who need to change anti-retroviral treatments.[6] Furthermore, anti-HIV combination strategies that demonstrate favorable drug interactions (e.g., synergy) may allow the use of individual agents below their toxic concentrations, provide more complete viral suppression, and limit the emergence of drug-resistant HIV mutants.

Earlier work has shown that two structurally related antibiotics produced by *Streptomyces*, streptonigrin (**1**) and lavendamycin (**2a**), have significant biological activity.[7] Streptonigrin and several of its derivatives, particularly its alkyl esters, were shown to have potent anti-reverse transcriptase activity against HIV-RT.[8] Unfortunately both the streptonigrins and the parent lavendamycin were found to be highly toxic. Until recently, evaluation of the therapeutic potential of synthetic lavendamycin analogues were precluded due to the lack of efficient synthetic methods. Indeed, initial reported total syntheses of lavendamycin methyl ester involved a large number of steps with low overall yields of 0.5−2%.[9] In 1993 and 1996, we reported short and efficient methods for the preparation of lavendamycin methyl ester in excellent overall yields of 33−40%.[10] We now describe the total synthesis of a number of novel demethyllavendamycin esters, amides as well as two water-soluble derivatives via short and practical methods. This is the first report to describe the synthesis of these lavendamycins possessing the full pentacyclic structure of the system and its C-7 amino and C-2′ acid functions and their derivatives. In addition, efficient methods are introduced to synthesize water-soluble lavendamycins essential for meaningful in vivo studies and potential chemotherapeutic application**.** This is also the first report showing the high degree of activity that these novel nonnucleoside lavendamycin agents have in inhibiting HIV-RT. Fortunately these inhibitors also



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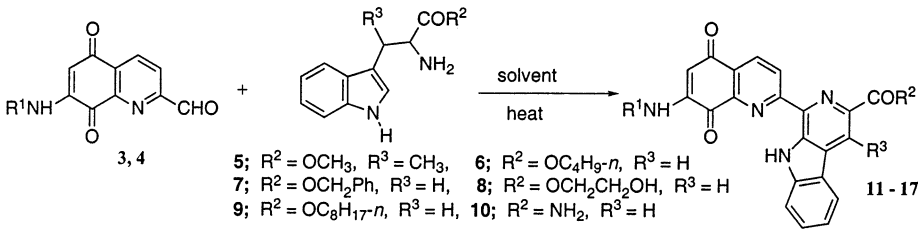
Scheme 1



Scheme 2

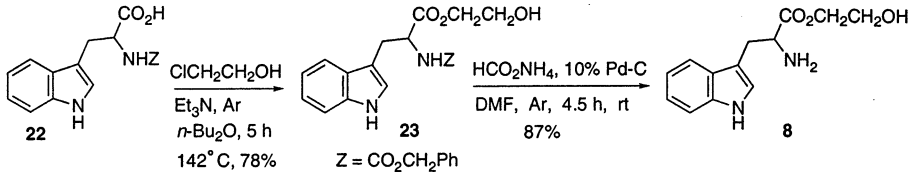
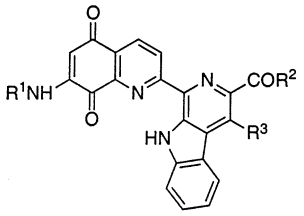


Table 1. Structures of Lavendamycins, Reaction Conditions, and Yields



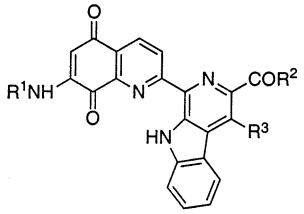
no.	R^1	R^2	R^3	% yield	solvent	h (°C)
11	CH_3CO	OCH_3	CH_3	79	xylene	19 (reflux)
12	*n*-C_3H_7CO	OC_4H_9-*n*	H	54	anisole	3.5 (reflux)
13	CH_3CO	OCH_2Ph	H	72	anisole	18 (152 °C)
14	CH_3CO	OCH_2CH_2OH	H	87−94	DMF/anisole	2 (reflux)
15	CH_3CO	OC_8H_{17}-*n*	H	48	xylene	3 (reflux)
16	CH_3CO	NH_2	H	62	anisole	14 (reflux)
17	*n*-C_3H_7CO	NH_2	H	63	anisole	13 (reflux)

have low toxicity both in vitro and in vivo. In addition to being inhibitory alone, several of the analogues also display additive or synergistic activity against HIV RT together with AZT-triphosphate. The synthesis of these lavendamycin analogues is briefly described, and the biological evaluation of these compounds presented.

Results and Discussion

Synthetic Chemistry. As shown in Scheme 1, Pictet−Spengler condensation of 7-*N*-acylamino-2-formylquinoline-5,8-diones **3** or **4** with *β*-methyltryptophan methyl ester (**5**) or trytophan butyl, benzyl, 1,2-ethanediol, octyl, esters (**6**−**9**), or tryptophan amide (**10**) yielded the corresponding lavendamycin derivatives **11**−**17**, respectively, in yields of 79, 54, 72, 94, 48, 62, and 63% **.** In a typical procedure, aldehydes **3** or **4** (0.1 mmol) were mixed with the corresponding tryptophan derivatives in 60 mL of dry anisole or xylene under argon, and while being magnetically stirred, the mixture was gradually heated to reflux over a period of 3 h. The resulting clear solution was refluxed until TLC showed the absence of the starting materials. The mixture was concentrated or evaporated to dryness. The products were either precipitated from the concentrated solutions[10a] or purified by washing with acetone followed by ether. The structures of the resulting lavendamycins, the reaction conditions, and the yields are shown in Table 1. Carboxylic acid **18** was prepared in 62% yield

Table 2. Lavendamycins Derived from **13**−**15**, **17**



no.	R^1	R^2	R^3
18	CH_3CO	OH	H
19	CH_3CO	$OCH_2CH_2OPO_3H_2$	H
20	H	OC_8H_{17}-*n*	H
21	H	NH_2	H

by the hydrogenolysis of **13** in the presence of Pd/black in CH_2Cl_2-MeOH−THF mixture. The free amino lavendamycins **20** and **21** were obtained by the acid hydrolysis of the corresponding **15** and **17,** respectively, in 64 and 96.5% yields, according to our reported method for the conversion of **11** to lavendamycin methyl ester (**2b**).[10a] Table 2 shows the structures of compounds **18**−**21**.

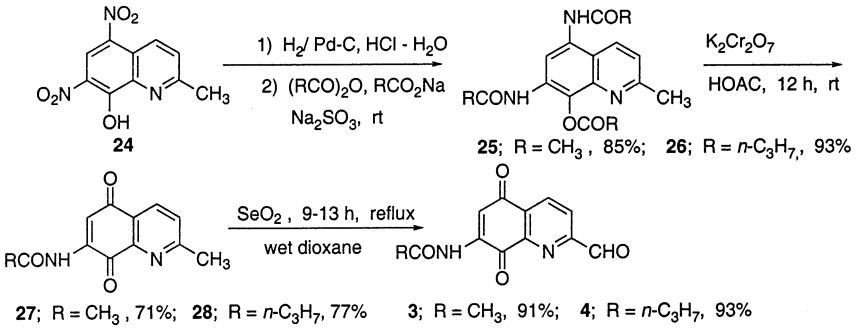
Tryptophan **5** was prepared according to our own procedure. [11] Tryptophans **6**, **7**, **9**, and **10** were obtained by the neutralization of the commercially available salts with ammonium hydroxide. The procedure for the preparation of 1,2-ethanediol ester **8** is shown in Scheme 2.

Aldehyde **3** was prepared according to our previously reported method[10a,12] as shown in Scheme 3. A similar procedure was used for the preparation of 7-butyramido-2-formylquinoline-5,8-dione (**4**).

Water soluble dihydrogen phosphate **19** was prepared by the Mitsunobu method (Scheme 4).[13]

Anti-HIV Reverse Transcriptase and In Vitro and In Vivo Toxicity Studies. The lavendamycin analogues, **11**, **12**, **16**, **18**−**21**, were tested for their ability to inhibit the HIV reverse transcriptase using a modified assay of Okada et al.[14] Briefly, the analogues were initially dissolved in either DMSO or HEPES buffer (pH 7.2) and then diluted to the appropriate concentration before addition to the assay mixtures. Varying concentrations of either AZT-TP or the analogues or both were added to wells of a 24-well microtiter plate containing 1 unit /mL HIV-RT, ^{3}HTTP, the template primer [poly(rA)-oligo $(dT)_{12-18}$], and appropriate

Scheme 3



Scheme 4

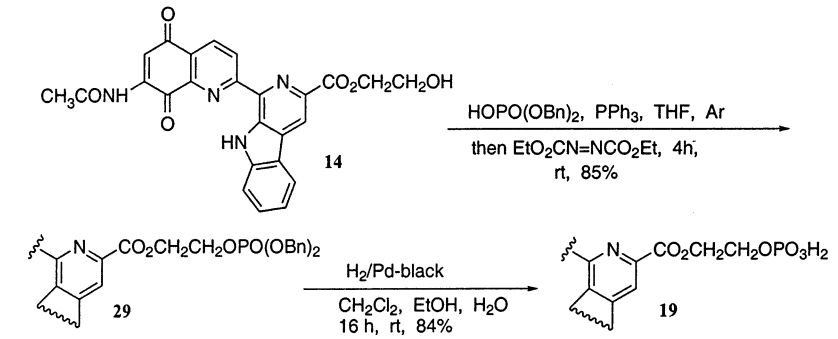


Table 3. Inhibitory Activity of Lavendamycin Analogues on HIV-RT and Cellular Cytotoxicity

compd	mean IC_{50} (μM)[a] ± SEM (no. of expts)	mean CC_{50} (μM)[b] (normal mouse spleen) ± SEM (no. of expts)	mean CC_{50} (μM)[c] (human cell lines) ± SEM (no. of expts)
11	15.1 ± 5.9 (3)	62 ± 5 (4)	31 ± 2 (4)
12	5.8 ± 1.5 (4)	81 ± 17 (4)	50 ± 15 (4)
16	12.7 ± 5.2 (6)	50 ± 7 (4)	95 ± 45 (4)
18	13.3 ± 4.4 (3)	47 ± 8.5 (4)	81 ± 35 (4)
19	3.0 ± 0.5 (3)	29 ± 5 (4)	27 ± 3 (4)
20	7.1 ± 2.7 (5)	48 ± 10 (4)	47 ± 14 (4)
21	20.5 ± 5 (3)	15 ± 2 (4)	17 ± 3 (4)

[a] Mean concentration of analogue at which 50% inhibition of HIV RT activity toward 1 unit of enzyme occurred. [b] Mean concentration of analogue at which normal mouse spleen stimulated with concanavalin A was inhibited by 50%. [c] Mean concentration of analogue at which human lymphocytic cell lines H-9 and CEM was inhibited by 50%.

salts and buffers. Several control wells without any inhibitors were also included on each microtiter plate. The reactions were allowed to proceed for 1 h at 37 °C. The amount of radioactivity incorporated under each experimental condition was compared to that found in the control wells and the amount of inhibition of the HIV-RT was calculated for each analogue and, for certain experiments, the inhibition found with each combination of analogue and AZT-TP. A more detailed description of reaction conditions are found in the Experimental Section. The concentration of drug which inhibited 50% of the enzyme activity (IC_{50})was determined by using the computer software program Calcusyn, which plots the dose−effect curves from the inhibition data and calculates the IC_{50} for each drug. The results of this screen clearly show that several of these analogues have significant anti-HIV RT activity at levels considerably below the level which is cytotoxic as measured either with murine lymphocytes or human lymphocytic cell lines H-9 and CEM (Table 3). Analogues **12**, **19**, and **20** were the most effective HIV-RT inhibitors with IC_{50}'s below 8 μM. One of the two more water soluble analogues, namely the dihydogen phosphate **19**, had the greatest activity with 15 μM inhibiting the HIV-RT >92% (data not shown) with an IC_{50} of 3 μM. With the exception of compound **21**, the concentrations at which 50% cytotoxicity (CC_{50}'s) in tissue culture were observed for the analogues were considerably higher than the IC_{50}'s toward both normal mouse splenocytes and human lymphocytic cell lines. Furthermore, the levels of cytotoxicity for both human and murine cells was comparable in most cases, which allows for further testing in both murine and human systems. At 10 and 20 μM concentrations, all the analogues had a reversible inhibitory effect on normal Con A-stimulated murine spleen cells, i.e., when the drugs were removed from the cultures after 12 h, the growth resumed at the same level as unexposed control cells. This suggests that at the levels tested, the drugs are cytostatic rather than cytocidal (data not shown). As the human cells studied are lines permissive for HIV replication and commonly used to examine the effectiveness of potential anti-HIV therapeutic agents, these compounds may be readily evaluated for anti-HIV activity in culture.[15] Furthermore, we found that BALB/c mice both survived and tolerated the highly active anti-HIV RT analogue **19** with little weight loss when given either 50 mg/kg as one ip injection or 75 mg/kg given ip in two injections within a 24 h period. In other studies, we have found that several of the lavendamycin analogues have remarkably low animal toxicity. For example, the National Cancer Institute found that in mice the maximum tolerated dose of **21** was 400 mg/kg and **15** was tolerated in SCID mice at 300 mg/kg/ day for 10 days with no animal death or loss of weight (data not shown). Interestingly, lavendamycin and its analogues appear to be much less toxic in experimental animals than the related streptonigrin and its analogues.[7b,16] Although the maximum tolerated dose of the naturally occurring parent lavendamycin in mice has been re-

ported to be 12.8 mg/kg, streptonigrin, which also has anti-HIV RT activity, is lethal to mice at doses as low as 0.4 mg/kg. The substantially lower level of animal toxicity of lavendamycins as compared to stretonigrin, another quinolinedione, may be due to the presence of the β-carboline moiety in the lavendamycins. Other quinonoid agents such as mitomycin C and adriamycin are toxic at levels far below those we have observed with the lavendamycins. In one reported tumor treatment trial in mice, the maximum tolerated dose of mitomycin C was less than 2 mg/kg when given for five consecutive days.[17] Likewise, adriamycin (doxorubicin) reportedly has a maximally tolerated dose below 10 mg/kg when given to mice once a week for three weeks.[18]

Combined Activity of Analogues with Azidothymidine Triphosphate. As combined drug therapy is an essential feature of AIDS treatment today, it is critical to assess the effect that any new drug may have on the activity of other mainstay anti-HIV treatments such as AZT-TP. Thus, we examined in a checkerboard fashion the combined effect of the lavendamycin analogues with AZT-triphosphate (AZT-TP) over a range of concentrations for both drugs. The results of this evaluation are presented in Table 4 and represent two separate trials for each of the analogues **11**, **12**, **16**, **18**−**21**. The results were analyzed by the method of Chou and Talalay[19] based on the median-effect principle and represented by combination indices (CI) determined for each of the two drug combinations. This method involves the plotting of dose−effect curves for each agent and for each of the different combinations of the agents. The slope of the median-effect plot, which signifies the shape of the dose−effect curve, and the *x* intercept of the plot, which signifies the potency of each compound alone and each combination, were then used for a computerized calculation of a combination index. Concentrations of AZT-TP were used which were both slightly below or slightly above the IC_{50} (0.012 μM) and concentrations of the lavendamycins were well below the CC_{50} of each of the analogues. Many combinations of the lavendamycins with AZT-TP were found to be either synergistic (CI $\leq$ 0.7) or moderately or slightly synergistic (CI $\geq$ 0.7$\leq$ 0.9), particularly at the lower concentrations of the analogues. Additive effects were also seen for many of combinations and some antagonism for some combinations, particularly at the higher concentrations of both drugs (e.g. **18** at 1.5 μM together with AZT-TP at 0.02 μM). The greatest amount of synergism over a wide range of concentrations was observed with compounds **18**−**20.** Interestingly, the increased water solubility of compounds **18** and **19** may play a role in the increased synergism with AZT -TP (see Experimental Section for solubilization of analogues). Clearly, this entire series of lavendamycin analogues have the ability to inhibit the HIV-RT together with AZT in either additive or synergistic manner. To derive the dose reduction index of several of these compounds, the method of Chou and Talalay was again employed using the classic isobologram technique. This method also involves the plotting of dose−effect curves for each agent as well as for multiply diluted fixed-ratio combinations of the agents. The inhibitory capacity of **12**, **18**−**20** with AZT-TP were evaluated by this method, and the determination of CI's at 50% and 70% effect level as well as the dose reduction

Table 4. Combination Indices Determined for Lavendamycin Analogues (μM) Performed by Checkerboard Analysis

AZT (μM)		**11**				**12**				**16**				**18**				**19**				**20**			
		0.25	0.5	0.75	1.5	0.25	0.5	0.75	1.5	0.25	0.5	0.75	1.5	0.25	0.5	0.75	1.5	0.75	1.5	3	6	0.25	0.5	0.75	1.5
0.007	trial 1	**0.57**	*0.81*	1.30	1.40	*0.82*	*0.88*	1.60	1.30	*0.82*	**0.54**	1.50	0.66	**0.39**	**0.40**	**0.66**	1.00	**0.47**	**0.49**	**0.49**	**0.32**	**0.65**	**0.62**	**0.68**	*0.83*
	trial 2	**0.48**	*0.71*	1.00	1.23	*0.83*	*0.71*	1.30	1.20	*0.80*	**0.65**	1.61	0.67	**0.37**	**0.42**	ND	0.94	**0.68**	*0.81*	*0.79*	**0.60**	**0.61**	**0.61**	**0.66**	*0.83*
0.01	trial 1	0.94	*0.84*	1.20	1.40	**0.58**	*0.76*	*0.86*	*0.81*	*0.85*	*0.87*	1.0	1.0	**0.44**	**0.51**	**0.59**	**0.90**	**0.64**	**0.69**	**0.28**	0.28	*0.77*	**0.60**	**0.64**	**0.70**
	trial 2	*0.89*	*0.76*	1.08	1.12	**0.58**	*0.76*	0.99	0.91	1.18	**0.70**	1.20	1.0	**0.45**	**0.50**	**0.57**	*0.82*	1.04	**0.69**	**0.56**	*0.75*	*0.77*	**0.61**	**0.63**	**0.69**
0.02	trial 1	0.90	*0.72*	0.94	1.17	**0.59**	*0.85*	1.00	1.10	**0.67**	**0.58**	1.10	1.00	**0.59**	0.97	0.95	2.00	ND	ND	ND	ND	0.97	*0.86*	**0.61**	**0.64**
	trial 2	**0.68**	*0.84*	*0.84*	1.08	**0.50**	0.93	0.92	0.96	*0.75*	**0.60**	1.10	1.00	**0.60**	0.98	0.97	2.20	ND	ND	ND	ND	1.00	*0.88*	**0.64**	**0.63**

[a] In two separate trials six different lavendamycin analogues were combined in differing ratios with AZT-triphosphate and added to HIV-RT enzyme assays in triplicate. The combination index (CI) of each drug combination was analyzed by the method of Chou and Talalay.[19a] CI values <1, 1, and >1 indicate synergism, additive effects, and antagonism. Values <0.7 are considered to be synergistic and are shown as bold. Values >0.71 to <0.9 are considered to be moderately or slightly synergistic and are shown in italics.

Table 5. Two Drug Combination of AZT and Lavendamycin Analogues at Constant Molar Ratios

	molar ratio AZT:analogue	CI at HIV-RT inhibition of:[a] 50%	70%	dose reduction index[b] (50%) AZT-TP	analogue
12	1:600[c]	0.45 ± 0.06	0.32 ± 0.08	3.0	8.2
	1:857[d]	0.39 ± 0.06	0.32 ± 0.09	3.9	7.3
18	1:600	1.03 ± 0.29	1.35 ± 0.64	1.5	2.6
	1:857	1.14 ± 0.49	1.7 ± 1.49	1.7	2.0
19	1:600	<0.1 ± 0.10	<0.1 ± 0.16	>100	>100
	1:857	<0.1 ± 0.15	0.21 ± 0.18	>40	>15
20	1:214[e]	0.46 ± 0.11	0.56 ± 0.22	4.08	4.63
	1:600	0.49 ± 0.17	0.40 ± 0.09	2.4	13.7

[a] Combination index at the effect level of 50% and 70% as calculated by the method of Chou and Talalay using a computer software program on an IBM PC.[19] Additive effect (CI = 1), synergism (CI < 1), or antagonism (CI > 1) [b] Dose reduction index is a measure of how much (how many times less) the dose of each drug in a synergistic combination may be reduced at the 50% effect level compared with the doses of each drug alone.[19b] [c] At least four different combinations of the analogues and AZT-TP were tested for HIV−RT inhibition whereby the ratio of the two drugs was kept constant at 1:600 but the concentrations increased from 0.005 μM AZT-TP and 3 μM analogue to 0.04 μM AZT-TP and 24 μM analogue. [d] At least four different combinations of the analogues and AZT-TP were tested for HIV-RT inhibition whereby the ratio of the two drugs was kept constant at 1:857, but the concentrations increased from 0.0035 μM AZT-TP and 3 μM analogue to 0.28 μM AZT-TP and 24 μM analogue. [e] Four different combinations of analogues were tested whereby the ratio of the two drugs was kept constant at 1:214, but the concentrations increased from 0.0035 μM AZT-TP and 0.75 μM to 0.028 μM AZT-TP and 6 μM analogue.

index (DRI) at 50% inhibition is shown in Table 5. The dose reduction index is a measure of how much the dose of each drug in a synergistic combination may be reduced at a given effect level compared with the doses of each drug alone.[19] The combination of AZT-TP and each of the four analogues **12**, **18**, **19**, and **20** lead to considerable dose reduction with the highly soluble analogue **19** showing both the highest level of synergism at the 50th and 70th percent inhibition level and greatest dose reduction index in this in vitro system.

In summary, a new family of biologically active lavendamycins including water soluble derivatives were synthesized through short and practical methods. Several of these lavendamycins have low toxicity, potent anti-reverse transcriptase activity themselves, and are synergistically inhibitory in combination with AZT-TP. In particular, the water soluble analogue **19** had the greatest inhibitory activity both alone and with AZT-TP and was remarkably nontoxic in animals. It may be that these compounds, particularly those with greater water solubility, will also show similar activity toward the replication of HIV in tissue culture and these or other derivatives may become useful adjuncts to our nonnucleoside anti-HIV arsenal.

Experimental Section

HIV-RT Assay. Adapted from Okada et al.[14] Except for analogue **19**, the lavendamycin analogues were initially dissolved in DMSO at a concentration of 1 mg/mL. Occasionally mild sonication and heat were used to dissolve the less soluble compounds. As **19** had greater water solubility, it was initially dissolved in 2.5 mM HEPES buffer (pH 7.2) at a concentration of 0.05 mg/mL. Compound **18** also had higher solubility readily dissolving in DMSO at 1 mg/mL without any heating or sonication. The dissolved analogues were then serially diluted in distilled water to achieve concentrations in the final reaction mixture ranging from 0.5 to 15 μM. In addition to the lavendamycin analogues, the final reaction mixture contained 100 mM Tris-HCl (pH 8.0), 5mM $MgCl_2$, 5 mM dithiothreitol, 60 mM NaCl, 0.2 mM [^{3}H] thymidine triphosphate (5 μCi well), 4 μg/mL poly (rA)-oligo(dT)$_{12-18}$ (Amersham Pharmacia Biotech, Piscataway, NJ), and 1 unit /ml of HIV-RT (Worthington Biochemical Corp., Freehold, NJ) in a total volume of 300 μL. For each assay, the reaction mixture containing 8×buffer, double distilled H_2O, ^{3}HTTP, template primer, and HIV-RT was premixed in a 15 mL sterile tube in quantities required for the particular experiment. After addition of the enzyme, the reaction mixture was mixed and kept on ice. The inhibitors, either AZT (Moravek Biochemicals, Brea, CA), analogues, or both were added to appropriate wells in a 24-well microtiter plate and ddH_2O was added to each well to bring the volume of all test wells to 100 μL. Two hundred microliters of the chilled reaction mixture was then added to each of the test wells, mixed, and incubated for 1 h at 37 °C. At least 9 control wells with no inhibitors were included on each test plate. After stopping the reactions with 25 μL of 0.1 M EDTA, triplicate samples of 15 μL from each test well were spotted on DE81 ion exchange filter paper squares (Whatman Paper, Maidstone, England). After drying, each filter was washed for 10 min three times with 5% TCA−NaH_2PO_4, three times with 0.6 M NaCl−0.06 mM Na citrate, one time with ddH_2O, and one time with 95% ethanol. After drying, the individual filter papers were added to 5 mL Scintiverse E (Fisher Scientific, Chicago, IL) scintillation fluid and the amounts of 3H-TTP uptake measured as counts per minute on a Beckman liquid scintillation counter. Mean uptake obtained with triplicate wells containing inhibitors were compared with the mean uptake found in at least three sets of triplicate control wells containing enzyme plus substrate, and percent inhibition for each experimental condition was calculated. The results were then analyzed using Calcusyn, a windows software program for dose effect analysis designed by T.-C.Chou and M. P. Hayball and published by Biosoft, UK.

Cytotoxicity Assays. Normal murine spleen cells were washed in HBSS, cultured at 2.5 × 10^6 or 5 × 10^6 cells per mL in RPMI 1640 (with Hepes) complete medium with 2mM-L-glutamine, 1 mM sodium pyruvate, 0.05 mM β-mercaptoethanol, penicillin, streptomycin, and amphotericin B with 10% fetal calf serum and concanavalin A (ConA) at 2.5 μg/mL in 96-well microtiter plate. A range of diluted analogues were added to triplicate culture wells, and the cells were incubated at 37 °C in 5% CO_2. Wells containing cells, media, and ConA were used as controls. At 48 h postincubation, 20 μL of the vital dye MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) at 5 mg/mL was added to each well, and the cultures were reincubated for 4−6 h. Following centrifugation, the supernatants were decanted, and the stained cells were resuspended in 100 μL of 0.4% HCl acidified 2-propanol. The optical densities of each well were read at 600 nm using a Cambridge Technologies series 700 microplate reader. The human lymphocytic cell lines CCRF−CEM (ATCC: HTB 176) and H-9 (ATCC: CCL 119) were subcultured from recently cultured cells grown in complete RPMI 1640 medium as described above but without the β-mercaptoethanol and ConA at 6.25 × 10^5 and 2 × 10^6 per mL in a 96-well culture plate. These cell numbers represented log phase and early stationary phase of the freshly cultured cells. As these cell lines rapidly reproduce without mitogenic stimulation, Con A was not used. A range of analogues or media alone was added in triplicate to the cultures and the cultures incubated, pulsed with MTT, and harvested, and their growth was estimated as described above for the murine cultures. The CC_{50} or the concentration of drug which inhibited cell growth as estimated by MTT formazan production to 50% of that of untreated control cells was determined by linear interpolation for each lavendamycin analogue. As the results from the cytotoxicity assays of the two human cell lines were not significantly different, the data was pooled and analyzed together.

In Vivo Toxicity. Analogue **19** was first solubilized in 10% dry DMSO, diluted in water, and given to the mice intraperi-

toneally either in one dose or in two doses per day. Mice were weighed and observed for 3 days following treatment.

Chemistry. General Methods. Chemical reagents and solvents were purchased from Aldrich, Sigma, and Fisher Chemical companies and used as received unless otherwise noted. Dioxane, xylene, anisole, and THF were distilled from sodium/benzophenone. DMF was dried and distilled over calcium hydride. Ammonium formate was dried in a vacuum desiccator over calcium sulfate. Analytical TLC was performed on 0.1 mm Eastman Kodak and Baker silica gel 60 F_{254} plates. Baker silica gel (particle size 4.0 μm) was used for flash column chromatography. NMR spectra were recorded on Varian Gemini 200 and JEOL 400 spectrophotometers and calibrated by using the residual undeuterated solvent as internal standard. Chemical shifts (δ) are in ppm, and coupling constants (J) are in Hz. Infrared spectra were recorded on a Perkin-Elmer 1000 series FT-IR spectrometer. Low resolution mass spectra and HRMS were recorded on Kratos MS 80, and the relative peak intensities are given in parentheses. Elemental analyses were performed by the Midwest Microlabs. Ltd. Melting points are uncorrected. Each new compound showed a single TLC spot (SiO_2, unless indicated otherwise), was pure as shown by NMR, and gave excellent elemental analyses or HRMS data.

7-Acetamido-2-formylquinoline-5,8-dione (3). This is a known aldehyde and was prepared from dinitro **24** according to our previously reported method (see Scheme 3).[10b]

7-Butyramido-2-formylquinoline-5,8-dione (4). In a 25 mL round-bottomed flask equipped with a magnetic bar, a condenser, and an argon filled balloon, 7-butyramido-2-methylquinoline-5,8-dione (516 mg, 2 mmol), selenium dioxide (255 mg, 2.3 mmol), 12 mL of dried distilled 1,4-dioxane, and 0.25 mL of water were stirred and slowly heated to reflux over 2 h. The reaction mixture was refluxed for 13 h, the black selenium metal was allowed to settle, and the supernatant was pipetted off and filtered. Dioxane (10 mL) was added to the solid residue, stirred, and refluxed for 5 min. The entire mixture was filtered, and the selenium was washed with dichloromethane (10 mL). To the combined filtrates was added 50 mL dichloromethane, and they were washed with 3% sodium bicarbonate solution (2 × 50 mL), dried ($MgSO_4$), and evaporated in vacuo to give 356 mg, (65%) of a pale yellow solid. The product was recrystallized from ethyl acetate: mp 208−210°C; R_f = 0.39 (1/1 EtOAc/CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 1.02 (t, 3H, J = 7.4), 1.70−1.89 (m, 2H), 2.52 (t, 2H, J = 7.4), 8.06 (s,1H), 8.31 (d, 1H, J = 8.0), 8.39 (br s, 1H), 8.61 (d, 1H, J = 8.0), 10.28 (s,1H); EIMS, *m/z*, 272 (54), 202 (36), 175 (9); Analysis for $C_{14}H_{12}N_2O_4$ calculated C, 61.76; H, 4.44; N, 10.29; found C, 61.31; H, 4.36; N, 9.94.

7-Butyramido-2-methylquinoline-5,8-dione (28). In a 500 mL round-bottomed flask equipped with a magnetic bar, 5,7-dibutyramido-8-Butyroxy-2-methylquinoline (**26**, 3.29 g, 8.25 mmol) was suspended in 122 mL of glacial acetic acid. A solution of potassium dichromate (8.8 g, 30 mmol) in 115 mL of water was added and stirred at room temperature. Dichloromethane (70 mL) was added to promote solution, and the resulting two-phase mixture was stirred overnight. The organic layer was separated, and the aqueous portion was extracted with dichloromethane (12 × 50 mL). The combined extracts were washed with 200 mL of 3% sodium bicarbonate solution, dried ($MgSO_4$), evaporated, and dried under vacuum to give an orange yellow solid (1.65 g, 77%). The product was recrystallized from ethyl acetate: mp 188−189 °C; R_f = 0.54 (1/1 EtOAc/CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ1.00 (t, 3H, J = 7.4), 1.69−1.82 (m, 2H), 2.48 (t, 2H, J = 7.4), 2.74 (s, 3H), 7.53 (d, J = 8.0), 7.90 (s, 1H), 8.28 (d, 1H, J = 8.0), 8.36 (br s, 1H); EIMS, *m/z*, 258 (81), 215 (7), 188 (100), 161 (66); Analysis for $C_{14}H_{14}N_2O_3$ calculated C, 65.11; H, 5.46; N, 10.85; found C, 65.22; H, 5.51; N, 10.90.

5,7-Dibutyramido-8-butyroxy-2-methylquinoline (26). In a 500 mL heavy-walled hydrogenation bottle, 5.00 g (0.2 mmol) of finely powdered 8-hydroxy-2-methyl-5,7-dinitroquinoline[10a] and 5% Pd/C (1.5 g) were suspended in 100 mL of water and 12 mL of concentrated hydrochloric acid. In a Parr Hydrogenator, this mixture was shaken under 30 psi of hydrogen for 15 h. The catalyst was removed, and the dark red solution containing the dihydrochloride salt of 5,7-diamino-8-hydroxy-2-methylquinoline was placed in a 250 mL round-bottomed flask equipped with a magnetic bar. To the stirred solution, were added as quickly as possible, sodium sulfite (12 g), sodium acetate (16 g), and butyric anhydride (65 mL). The thick whitish solid that continued to form over a 3-h period was filtered, washed with water, and dried under vacuum (7.4 g, 93%). Attempts to recrystallized **26** from methanol−water caused it to hydrolyze to the corresponding 5,7-dibutyramido-8-hydroxyquinoline-5,8-dione. However, NMR showed the crude **26** to be relatively pure and was used as such for the preparation of **28.** Analytical data for **26**: mp 195−200 °C; R_f = 0.26 (0.1/5 MeOH/EtOAc); ^{1}H NMR (DMSO-d_6) δ 0.92 (t, 3H, J = 8.0), 0.96 (t, 3H, J = 8.0), 1.08 (t, 3H, J = 8.0), 1.52−1.72 (m, 4H), 2.58 (s, 3H), 2.70 (t, 2H, J = 8.0), 7.37 (d,1H, J = 8.8), 8.21 (d, 1H, J = 8.8), 8.24 (s, 1H), 9.65 (br s, 1H), 9.94 (br s, 1H).

5,7-Dibutyramido-8-hydroxy-2-methylquinoline. This compound was obtained by dissolving **26** in a minimum amount of hot methanol−water (1/1).[10b] Pale white crystals were filtered and dried: mp 200−208 °C (dec); R_f = 0.32 (0.1/5 MeOH/EtOAc); ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3H, J = 7.0), 0.96 (t, 3H, J = 7.0), 2.3−2.55 (m, 4H), 2.7 (s, 3H), 7.3 (d, 1H, J = 8.8), 8.03 (3, 1H), 7.37 (d, 1H, J = 8.8), 9.45 (br s, 1H), 9.63 (br s, 1H); EIMS, *m/z*, 329 (95), 286 (20), 259(98), 241 (17), 216 (6), 188 (100); HRMS, *m/z* calculated for $C_{18}H_{23}N_3O_3$ 329.173942, found 329.173043; Analysis calculated C, 65.63; H, 7.04; N 12.76; found C, 65.80; H, 7.05; N, 12.75.

Tryptophan β-Hydroxyethyl Ester (8). In a 250 mL two-necked round-bottomed flask was dissolved compound **23** (2.088 g, 5.4 mmol) in 100 mL dry DMF under an argon balloon. Dry ammonium formate (1.124 g, 17.82 mmol) and 964 mg of 10% Pd/C were added, and the mixture was stirred for 4.5 h at room temperature. The reaction mixture was filtered through a layer of Celite, and the filter cake was washed with 3 × 8 mL of EtOAc. The light orange solution was evaporated in vacuo and dried under a vacuum pump at 50−60 °C for two or more days until no trace of DMF was observed in the product NMR. The product was a gel and weighed 1.164 g (87%); R_f = 0.25 (0.25/5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 2.95 (dd, 1H, J = 14.1, 6.2), 3.04 (dd, 1H, J = 14.1, 6.0), 3.60−3.70 (m, 2H), 3.63 (dd, 1H, J = 6.0, 6.2), 3.90−4.10 (m, 2H), 6.97 (dd, 1H,J = 8.1, 7.0), 7.05 (dd, 1H, J = 8.1, 7.0), 7.14 (s, 1H), 7.33 (d, 1H, J = 8.1), 7.51 (d, 1H, J = 8.1), 7.95 (s, 1H),10.86 (br s, 1H); HRMS(EI) calculated for $C_{13}H_{16}N_2O_3$ 248.1161, found 248.1158.

***N*-(Benzyloxycarbonyl)tryptophan β-Hydroxyethyl Ester (23).** To partially dissolved CBZ-tryptophan (**22**, 1.02 g, 3 mmol) in 18 mL of *n*-butyl ether in a 50 mL three-necked round-bottomed flask under argon were added 2-chloroethanol (483 mg, 6 mmol) and triethylamine (0.4 mL, 291 mg, 2.9 mmol) via a syringe. The reaction mixture was refluxed overnight for 5 h. The two-layered mixture was evaporated in vacuo, and the residue was dissolved in 12 mL of EtOAc and placed in the refrigerator overnight. The white solid material was filtered and rinsed with EtOAc, and the filtrate was washed with 5 mL of a 5% solution of sodium bicarbonate followed by water until the pH of the aqueous layer reached 7 (5 × 3 mL). The organic solution was dried ($MgSO_4$), filtered, and evaporated in vacuo and then dried on a pump at 40−50 °C to give a dark orange gel (880.4 mg, 78%); R_f = 0.44 (1/5 EtOH/EtOAc, Al_2O_3); ^{1}H NMR ($CDCl_3$) δ 3.20−3.40 (m, 2H), 3.55−3.70 (m, 2H), 4.00−4.25 (m, 2H), 4.60−4.80 (m, 1H), 5.00−5.15 (m, 2H), 5.25−5.45 (m, 2H), 7.02 (s, 1H), 7.05−7.15 (m, 1H), 7.17−7.25 (m, 1H), 7.25−7.4 (m, 5H), 7.50−7.60 (m, 1H), 8.09 (br s, 1H). HRMS(EI) calculated for $C_{21}H_{22}N_2O_5$ 382.1528, found 382.1523.

7-*N*-Acetyldemethyllavendamycin β-Hydroxyethyl Ester (14). In a 500 mL three-necked round-bottomed flask equipped with a Dean−Stark trap, a condenser, a dropping funnel, and a magnetic bar, 7-acetamido-2-formylquinoline-5,8 -dione (**3**, 146.4 mg, 0.6 mmol) in 250 mL dry anisole was

stirred and heated under argon to 80 °C over 30 min. Nearly all of aldehyde **3** was solubilized. To this mixture was dropwise added a solution of tryptophan β-hydroxyethyl ester (**8**,198.64 mg, 0.8 mmol) in 12 mL of dry DMF over 40 min at 95 °C, and the temperature was slowly raised to 160 °C over 4.5 h and then heated at this temperature for an additional 1 h.

The reaction mixture was allowed to cool for 10 min and then filtered to remove a small amount of colloidal material. The filtrate was evaporated in vacuo to dryness, and then 15 mL acetone was added and allowed to stand at room-temperature overnight. The solid material was filtered and washed with acetone followed by ether to give 177 mg (94%) of yellow orange solid **14**: mp 270.5−271 (dec); $R_f = 0.69$ (0.2/5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6)**:** δ 2.32 (s, 3H), 3.80−3.90 (m, 2H) 4.40−4.50 (m, 2H), 7.45 (t, 1H, $J = 7.3$), 7.72 (d, 1H,$J = 7.3$), 7.70−7.75 (m, 1H), 7.83 (s, 1H), 8.53 (d, 1H, $J = 8.0$),, 8.61 (d, 1H, $J = 8.4$), 8.98 (d, 1H, $J = 8.4$) 10.31 (s, 1H), 12.01 (br s, 1H); HRMS(EI) calculated for $C_{25}H_{18}N_4O_6$ 470.1226, found 470.12406.

Dibenzyl Phosphate 29. To a stirred mixture of **14** (135.7 mg, 0.289 mmol), dibenzyl hydrogen phosphate (241.2 mg, 0.87 mmol), and triphenyphosphine (228.2 mg, 0.87 mmol) in 8 mL dry THF, under Ar, was dropwise added a solution of diethyl azodicarboxylate (151.52 mg, 0.87 mmol) in 2 mL dry THF via a syringe over a course of 12 min. The mixture was stirred for 4 h at room temperature, and the resulting solid was filtered off. The filtrate was evaporated to dryness, and the solid was washed with ethyl ether to give 179 mg (85%) of orange solid **29**. Silica gel chromatography (1% MeOH in CH_2Cl_2) gave 142 mg of pure **29**: mp 183.5−184°C; $R_f = 0.46$ (EtOAc); ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 4.45 (br s, 2H), 4.64 (br s, 2H), 5.07 (s, 2H), 5.12 (s, 2H) 7.20−7.34 (m, 10H), 7.40 (dd, 1H, $J = 8.0$, 7.3), 7.68 (dd, 1H, $J = 8.0$, 7.3), 7.76 (d, 1H, $J = 8.0$), 8.01 (s, 1H), 8.20 (d, 1H, $J = 8.0$), 8.45 (s, 1H), 8.48 (d, 1H, $J = 8.3$), 8.99 (s, 1H), 9.18 (d, 1H, $J = 8.3$), 11.85 (br s, 1H); ^{31}P NMR ($CDCl_3$): δ 1.69; HRMS(EI) calculated for $C_{39}H_{34}N_4O_9P$ (M+3) 733.2065, found 733.2065.

Dihydrogen Phosphate 19. To a solution of **29** (38. 8 mg, 0.053 mmol) in 7.4 mL of a mixture of CH_2Cl_2/EtOH/H_2O (5/2/0.4) was added 14.3 mg of palladium black, and the mixture was stirred under a balloon full of hydrogen for 16 h. The balloon was removed, the mixture was filtered, the filter cake was thoroughly washed with 22 mL of a 1/1 mixture of EtOH/CH_2Cl_2, and then the filtrate was stirred in the presence of air for 2 h. The resulting filtrate was evaporated in vacuo to dryness to yield 19.6 mg (84%) of pure orange brown **19**: mp 179 °C (dec); Water solubility (0.05 mg/1 mL HEPES buffer solution); $R_f = 0.54$ (Eastman Kodak SiO_2, 8/3/0.5 MeOH/H_2O/EtOAc); ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 3H), 4.27 (br s, 2H), 4.57 (br s 2H), 7.41 (dd, 1H, $J = 8.0$, 7.6), 7.64 (d, 1H, $J = 8.0$), 7.71 (dd, 1H, $J = 8.0$, 7.6), 7.79 (s, 1H), 8.47 (d, 1H, $J = 7.7$), 8.53 (1H, d, $J = 8.0$), 8.91 (d, 1H $J = 8.0$), 9.08 (s, 1H), 1026 (s, 1H), 11.89 (br s, 1H); ^{31}P NMR ($CDCl_3$); δ − 0. 02. HRMS(FAB) calculated for $C_{25}H_{19}N_4O_9P$ 550.0089, found 550.0912.

7-*N*-*n*-Butyryldemethyllavendamycin *n*-Butyl Ester (12). Tryptophan *n*-butyl ester (**6**, 78.1 mg, 0.3 mmol) was dissolved in 180 mL of dry anisole. To this solution under an argon balloon was added 7-*n*-butyramido-2-formylquinoline-5,8-dione (81.6 mg, 0.3 mmol), and the mixture was stirred and heated to reflux over a course of 3 h. The reflux was continued for another 3.5 h, and the solution was evaporated in vacuo to dryness. The residue was dissolved in acetone and allowed to stir at room temperature in the presence of air for 24 h. The yellow-orange product was filtered and dried under vacuum (82.3 mg, 54%): mp 220.8−221 °C (dec); $R_f = 0.54$ (0.5/5 EtOAc/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.82 (t, 3H, $J = 7.3$), 0.95 (t, 3H, $J = 7.5$), 1.42−1.6 (m, 4H), 1.75−1.86 (m, 4H), 4.56 (t, 2H, $J = 6.6$), 7.15−7.35 (m, 2H), 7.56 (d, 1H, $J = 8.1$), 7.85 (d, 1H,$J = 8.1$), 7.89 (br s, 1H), 8.14 (d, 1H, $J = 8.1$), 8.22 (s, 1H), 9.15 (d, 1H, $J = 8.1$), 9.19 (s, 1H), 11.96 (br s, 1H); EIMS, *m*/*z*, 510 (M^+, 69), 446 (12), 417 (11.5), 410 (100), 394 (36), 340 (39), 217 (13); HRMS calculated for $C_{29}H_{26}N_4O_5$ 510.1903, found 510.1886.

7-*N*-Acetyldemethyllavendamycin Benzyl Ester (13). In a 500 mL three-necked round-bottomed flask under argon was dissolved 7-acetamido-2-formylquinoline-5,8-dione (**3**, 146.4 mg, 0.6 mmol) in 300 mL of dry anisole and heated to 100 °C. To this mixture was dropwise added tryptophan benzyl ester (**7**, 176.4 mg, 0.6 mmol) over 30 min. The yellow lemon solution was gradually heated to 152 °C over 2 h and kept at this temperature for 18 h. To convert any generated hyroquinone intermediate to the desired product, a flow of oxygen gas was passed through the lemon yellow hot solution for 20 min. The resulting golden yellow mixture was filtered hot to remove some impurities, evaporated in vacuo, and then left overnight in a vessel open to the air. The small amount of the brownish solid was removed, and the solution was concentrated to near dryness. Acetone (10 mL) was added, and the resulting solid material was filtered and washed with a small portion of acetone and then petroleum ether to give a pure golden crystalline solid (222 mg. 72%): mp 174.6−175 °C; $R_f = 0.68$ (0.1/5 MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 5.55 (s, 2H), 7.35−7.48 (m, 5H), 7.58−7.62 (m, 2H), 7.64−7.7 (m, 1H), 7.72−7.78 (m, 1H), 8.00 (s, 1H), 8.23 (d, 1H, $J = 8.0$), 8.43 (br s, 1H), 8.56 (d, 1H, $J = 8.4$), 8.98 (s, 1H), 9.21 (d, 1H, $J = 8.4$), 11.83 (br s, 1H); HRMS(EI) calculated for $C_{30}H_{20}N_4O_5$ 516.14347, found 516.14340.

7-*N*-Acetyldemethyllavendamycin (18). To a stirred mixture of benzyl ester **13** (125.8 mg, 0.24 mmol) in dichloromethane (186 mL), methanol (14 mL), water (0.01 mL) was added 42 mg of palladium black, and the mixture was hydrogenated under a hydrogen filled balloon for 20 h. Palladium was removed, and the solution was stirred under an oxygen-filled balloon for 96 h and then evaporated in vacuo. The solid was washed with acetone followed by ether and then dried under vacuum to give 94.2 mg (92%) of brown orange **13**: mp > 270 °C; Water solubility (0.05 mg/1 mL HEPES buffer solution; $R_f = 0.34$ (1/2 MeOH/CH_3CN); ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H), 7.43 (t, 1H, $J = 7.7$), 7.66−7.88 (m, 2H), 7.82 (s, 1H), 8.54 (d, 1H, $J = 7.7$), 8.58 (d, 1H, $J = 8.4$), 9.18 (d, 1H, $J = 8.4$), 10.28 (s, 1H), 11.99 (br s, 1H); HRMS-(EI) calculated for $C_{23}H_{14}N_4O_5$ 426.0964, found 426.0959.

7-*N*-Acetyldemethyllavendamycin *n*-Octyl Ester (15). This compound was prepared according to a method similar to that of **17.** The final product was obtained by the evaporation of the reaction mixture in vacuo, and the brown solid was washed with a mixture of ethyl acetate−hexane to give the orange solid **15** in 48%: mp 210.5−211 °C; $R_f = 0.41$ (0.06/5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 0.86 (t, 3H, $J = 7.3$), 1.25−1.6 (m, 10H), 1.83 (m, 2H), 2.32 (s, 3H), 4.41 (t, 2H, $J = 6.6$), 7.44 (dd, 1H, $J = 8.0$, 7.3), 7.65−7.8 (m, 2H), 7.82 (s, 1H), 8.54 (d, 1H, $J = 8.0$), 8.57 (d, 1H, $J = 8.2$), 8.93 (d, 1H, $J = 8.2$), 9.09 (s, 1H), 10.29 (s, 1H), 11.96 (br s, 1H); EIMS, *m*/*z*, 538 (M^+, 55), 382 (100), 366 (5), 340 (16); HRMS(EI) calculated for $C_{31}H_{30}N_4O_5$ 538.2216, found 538.2220.

Demethyllavendamycim *n*-Octyl Ester (20). 7-*N*-Acetyldemethyllavendamycin *n*-octyl ester (**15**, 76.3 mg, 0.142 mmol) was added to 7 mL of a 70% solution of sulfuric acid and under an argon balloon was stirred and heated at 60 °C for 45 min. The reaction mixture was carefully basified with a saturated solution of sodium carbonate to pH = 8 and then extracted with chloroform (4 × 45 mL). The combined extracts were washed with water (2 × 20 mL), dried ($MgSO_4$), and concentrated to a small volume. The solution was cooled in the refrigerator, and the red solid was filtered off. Silica gel flash chromatography using chloroform and then methanol−chloroform (1.6 mL/100 mL) as the solvent system gave the pure orange red product **20** (45 mg, 64%): mp 175−176 °C (dec); $R_f = 0.12$ (0.06/5 MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H, $J = 7.3$), 1.25−1.40 (m, 10H), 1.93 (m, 2H), 4.59 (t, 2H, $J = 6.9$), 7.10−7.30 (m, 1H), 7.35−7.43 (m, 1H), 7.51 (d, 1H, $J = 8.0$), 7.74 (br s,1H), 7.82 (d, 1H, $J = 8.0$), 8.11 (d, 1H, $J = 8.0$), 8.15 (s, 1H), 9.10 (d, 1H, $J = 8.0$), 9.16 (s, 1H), 11.84 (br s, 1H); HRMS(EI) calculated for $C_{29}H_{28}N_4O_4$ 496.2110, found 496.2099

7-*N*-Acetyldemethyllavendamycin Amide (16). This compound was prepared according to a method similar to the

procedure used for the synthesis of **17**. The final product was obtained as a lemon yellow solid in 62% yield by cooling the reaction mixture in the refrigerator, filtration of the solid, and washing with hexane: mp > 280 °C (dec); R_f = 0.65 (0.2/5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H), 7.41 (d, 1H, J = 7.5, 7.0), 7.6−7.75 (m, 3H), 7.82 (s, 1H), 8.48 (dd, 1H, J = 8.4), 8.52 (d, 1H,J = 8.0), 8.61 (br s, 1H), 9.07 (s, 1H), 10.29 (s, 1H), 11.95 (br s, 1H); HRMS(EI) calculated for $C_{23}H_{15}N_5O_4$ 425.1124, found 425.1115.

7-*N*-*n*-Butyramidodemethyllavendamycin Amide (17). To a stirred solution of tryptophan amide (**10**, 243.6 mg, 1.2 mmol) in 480 mL of dry anisole under argon balloon was added 7-*N*-butyramido-2-formylquinoline-5,8-dione (**4**, 326.4 mg,1.2 mmol), and the mixture was heated to reflux over a 3-h period. The reaction mixture was refluxed for 13 h and then allowed to stand at room-temperature overnight. The yellow solid was filtered off and washed with a small volume of acetone followed by ether to give 343.3 mg (63%) of pure **17:** mp 244 °C (dec); R_f = 0.58 (0.2/5 MeOHCH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 0.95 (t, 3H, J = 7.3), 1.64 (quintet**,** 2H, J = 7.3), 2.64 (t, 2H, J = 7.3), 7.41 (dd, 1H, J = 8.2, 7.9), 7.70−7.76 (m, 2H), 7.83 (s, 1H), 8.47 (d, 1H, 8.2), 8.51 (d, 1H, J = 7.9), 9.05 (s, 1H), 9.45 (d, 1H, 8.2),10.18 (s, 1H), 11.92 (br s, 1H). HRMS(EI) calculated for $C_{25}H_{20}N_5O_4$ (M + 1) 454.15153, found 454.15129.

Demethyllavendamycin Amide (21). 7-*N*-*n*-Butyryldemethyllavendamycin amide (**17**, 147.8 mg, 0.32 mmol) was placed in a 50 mL two-necked round-bottomed flask under argon balloon. A 70% solution of sulfuric acid (7.5 mL) was dropwise added, and the homogeneous mixture was stirred and heated at 60 °C in an oil bath for 6 h. The dark red solution was cooled to 0 °C and then added to 75 mL of ice−water. The mixture was carefully basified with a saturated solution of sodium carbonate to about pH = 8. The solution was evaporated in vacuo to dryness and then water (75 mL) was added and stirred. The orange red crystals were washed with water and dried under vacuum (115.4 mg, 96.5%): 154.2 °C (dec); R_f = 0.45 (0.2/5 MeOH/CH_2Cl_2); ^{1} H NMR (DMSO-d_6) δ 5.96 (s, 1H), 7.41 (t, 1H, J = 7.3), 7.60−7.74 (m, 2H), 7.79 (d, 1H, J = 8.0), 8.46 (d, 1H, J = 8.0), 8.52 (d, 1H, J = 7.3), 8.60 (s, 1H), 9.06 (s, 1H), 9.44 (d, 1H, J = 8.0), 12.00 (br s, 1H); HRMS(EI) calculated for $C_{21}H_{13}N_5O_3$ 383.1018, found 383.1026.

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Supporting Information Available: NMR spectra for compounds **26**, **8**, **23**, **14**, **29**, **19**, **12**, **13**, **18**, **15**, **20**, **16**, **17**, and **21** are available free of charge via the Internet at http://pubs.acs.org.

References

(1) Piot, P.; Bartos, M.; Ghys, P. D.; Walker, N.; Schwartlander, B. The Global Impact of HIV/AIDS. *Nature* **2001**, *410*, 968−973.

(2) Richman, D. D. HIV Chemotherapy. *Nature.* **2001**, *410*, 995−1001. (b) Menendez-Arias, L. Targeting HIV: Antiretroviral Therapy and Development of Drug Resistance. *Trends Pharmacol. Sci*. **2002**, *23*, 381−388.

(3) Robinson, B. S.; Riccardi, K. A.; Gong, Y.-F.; Guo, Q.; Stock, D. A.; Blair, W. S.; Terry, B. J.; Deminie, C. A.; Djang, F.; Colonno, R. J.; Lin, P.-F. BMS-232632, A Highly Potent Human Immunodeficiency Virus Protease Inhibitor that Can Be Used in Combination with Other Available Antiretroviral Agents. *Antimicrob. Agents Chemother.* **2000**, *44*, 2093−2099.

(4) (a) van Praag, R. M.; Wit, F. W.; Jurriaans, S.; de Wolf, F.; Prins, J. M.; Lange, J. M. Improved Long-Term Suppression of HIV-1 Replication with a Triple-Class Multidrug Regimen Compared with Standard of Care Antiretroviral Therapy. *AIDS* **2002**, *16*, 719−725. (b) Havlir, D. V.; Lange, J. M. New Antiretrovirals and New Combinations. *AIDS* **1998**, *12 suppl. A*, s165−174.

(5) (a) Campiani, G.; Ramunno, A. Maga, G.; Nacci, V.; Fattorusso, C.; Catalanotti, B.; Morelli, E.; Novellino, E. Nonnucleoside HIV Reverse Transcriptase (RT) Inhibitors: Past, Present, and Future Perspectives. *Curr. Pharm. Des.* **2002**, *8*, 615−657. (b) Romero, D. L.; Busso, M.; Tan, C.-K.; Reusser, F.; Palmer, J. R.; Poppe. S. M.; Aristoff, P. A.; Downey, K. M.; So, A. G.; Resnick, L.; Tarpley, W. G. Non-Nucleoside Reverse Transcriptase Inhibtors That Potently and Specifically Block Human Immunodeficiency Virus Type 1 Replication. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8806−8810. (c) De Clerq, E. The Role of Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs) in the Therapy of HIV-1 Infection. *Antiviral Res*. **1998**, *38*, 153−179.

(6) Joly V.; Descamps, D.; Yeni, P. NNRTI Plus PI Combinations in the Perspective of Nucleoside-Sparing or Nucleoside-Failing Antiretroviral Regimens. *AIDS Rev*. **2002**, *4*, 128−139.

(7) (a) Take, Y.; Kubo, T.; Takemori, E.; Inouye, Y.; Nakamura, S.; Nishimura, T.; Suzuki, H.; Yamaguchi, H. Biological Properties of Streptonigrin Derivatives III. In Vitro and In Vivo Antiviral and Antitumor Activities. *J. Antibiot*. **1989**, *42*, 968−976(b) Balitz, D. M.; Bush, J. A.; Bradner, W. T.; Doyle, T.W.; O'Herron, F. A.; Nettleton, D. E. Isolation of Lavendamycin, a New Antibiotic from *Streptomyces lavendulae*. *J. Antibiot*. **1982**, *35*, 259−265.

(8) Take, Y.; Inouye, Y.; Nakamura, S. Allaudeen, H. S.; Kubo, A. Comparative Studies of the Inhibitory Properties of Antibiotics on Human Immunodeficiency Virus and Avian Myeloblastosis Virus Reverse Transcriptases and Cellular DNA Polymerases. *J. Antibiot*. **1988**, *42*, 107−115.

(9) (a) Boger, D. L.; Mitscher, Y. M.; Drake, S. D.; Kitos, P. A.; Thompson, S. C. Streptonigrin and Lavendamycin Partial Structures. Probes for the Minimum, Potent Pharmacophore of Streptonigrin, Lavendamycin, and Synthetic Quinoline-5,8-diones. *J. Med. Chem*. **1987**, *30*, 1918−1928. (b) Kende, A. S.; Ebetino, F. H. The Regiospecific Total Synthesis of Lavendamycin Methyl Ester. *Tetrahedron Lett*. **1984**, *25*, 923−926.

(10) (a) Behforouz, M.; Gu, Z.; Cai, W.; Horn, M. A.; Ahmadian, M. A Highly Concise Synthesis of Lavendamycin Methyl Ester. *J Org. Chem*. **1993**, *58*, 7089−7091. (b) Behforouz, M.; Haddad, J.; Cai, W.; Arnold, M. B.; Mohammadi, F.; Sousa, A. C.; Horn, M. A. Highly Efficient and Practical Syntheses of Lavendamycin Methyl Ester and Related Novel Quinolinediones. *J. Org. Chem*. **1996**, *61*, 6552−6555.

(11) Behforouz, M.; Zarrinmayeh, H.; Ogle, M. E.; Riehle, T. J.; Bell, F. W. β-Carbolines Derived from β-Methyltryptophan and a Stereoselective Synthesis of (2RS, 3SR)-β-Methyltryptophan Methyl Ester. *J. Heterocycl. Chem*. **1988**, *25*, 1627−1632.

(12) Behforouz, M.; Haddad, J.; Cai, W.; Gu, Z. Chemistry of Quinoline-5,8-diones. *J. Org. Chem*. **1998**, *63*, 343−346.

(13) Mitsunobu, O.; Kato, K.; Kimura, J. Selective Phosphorylation of 5-Hydroxy Groups of Thymidine and Uridine. *J. Am. Chem. Soc*. **1969**, *91*, 6510−6511.

(14) Okada, H.; Mukai, H.; Inouye, Y.; Nakamura, S. Biological Properties of Streptonigrin Derivatives II. Inhibition of Reverse Transcriptase Activity. *J. Antibiot*. **1986**, *39*, 306−308.

(15) (a) Coates, A. V.; Cammack, N.; Jenkinson, H. J.; Jowett, A. J.; Jowett, M. I.; Pearson, B. A.; Penn, C. R.; Rouse, P. L.; Viner, K. C.; Cameron, J. M. (−)-2′-Deoxy-3′-Thiacytidine Is A Potent, Highly Selective Inhibitor of Human Immunodeficiency Virus Type 1 and Type 2 Replication *In Vitro. Antimicrob. Agents Chemother.* **1992**, *36*, 733−739. (b) Bilello, J.; Bauer, G.; Dudley, M**.** Effect of 2′-3′-dihydro-3′-deoxythymidine in an In Vitro Hollow Fiber Pharmacodynamic Model System Correlates with Results of Dose-Ranging Clinical Studies. *Antimicrob. Agents Chemother*. **1994**, *38*, 1386-1391.

(16) (a) Reilly, H. C.; Sugiura, K. An Antitumor Spectrum of Stretonigrin. *Antibiot. Chemother*. **1961**, *11*, 174−177. (b) Olesen, J. J.; Calderella, L. A.; Mjos, K. J.; Reith, A. R.; Thie, R. S.; Toplin, I. Effects of Streptonigrin on Experimental Tumors. *Antibiot. Chemother*. **1961**, *11*, 158−164.

(17) Kim, J. Y.; Su, T.-L.; Chou, T.-C.; Koehler, B.; Scarborough, A.; Ouerfelli, O.; Watanabe, K. A. Cyclopent[*a*] anthraquinones as DNA −Intercalating Agents with Covalent Bond Formation Potential; Synthesis and Biological Activity. *J. Med. Chem*. **1996**, *39*, 2812−2818

(18) Casazza, A. M.; Savi, G.; Pratesi, G.; Di Marco, A. Antitumor Activity in Mice of 4′- Deoxydoxorubicin in Comparison with Doxorubicin. *Eur J. Cancer Clin. Oncol.* **1983**, *19*, 411−418.

(19) (a) Chou, T. C.; Talalay, P. Quantitative Analysis of Dose−Effect Relationships: The Combined Effects of Multiple Drugs or Enzyme Inhibitors. *Adv. Enzyme Regul*. **1984**, *22*, 27−55. (b) Chou J.; Chou, T. C. Dose BEffect Analysis with Microcomputers: Quantitation of ED50, LD50, Synergism, Antagonism, Low-Dose Risk, Receptor−Ligand Binding and Enzyme Kinetics. In: A computer Software for IBM-PC and Manual. Cambridge, UK: Elsevier-Biosoft, 1987.

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