32-Ascomycinyloxyacetic Acid Derived Immunosuppressants. Independence of Immunophilin Binding and Immunosuppressive Potency

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The potent immunosuppressant ascomycin (**1b**) was selectively alkylated at the C-32 carbinol, thus providing esters and amides of 32-ascomycinyloxyacetic acid (**4**, AOAA). These compounds present structural variation at the FKBP/calcineurin interface. While the native carboxylic acid **4** shows no activity in vitro, esters and simple amides of **4** exhibit potent immunosuppression in the human MLR assay. Moreover, amides show inhibitory activity in the rat popliteal lymph node hyperplasia assay. Surprisingly, FKBP binding was weakened by several orders of magnitude when secondary hydrophobic aryl amides of **4** were tested, while maintaining potent immunosuppressive efficacy in vitro.

Introduction

Transplantation medicine has moved from a field of speculation and obscurity several decades ago to a discipline that now offers organ allografting as a readily approved form of treatment. The acceptance of transplantation as a viable treatment modality would have been impossible without the development of immunosuppressive drugs. Due to their antiproliferative nature, immunosuppressants have been haunted by numerous toxic side effects,[1] which include increased risk of infection, tumor appearance and growth, and myelosuppression. Cyclosporin A (CsA), an immunosuppressant approved for use in kidney transplatation in the early 1980s, accelerated the acceptance of transplantation therapy due to a significantly improved safety profile relative to previously known drugs such as steroids and azathioprine. Nevertheless, despite being approved for kidney transplantion, CsA showed kidney damaging side effects, leaving much room for less toxic therapies. Among the newer immunosuppressants, FK506 (**1a**) has recently been approved for use in liver transplantation, and its structural relative rapamycin (**2**) is in clinical trials. While these compounds provide alternatives to cyclosporin therapy, they still demonstrate toxicity, despite great initial hopes to the contrary.

CsA, FK506 (**1a**), and rapamycin (RAP, **2**) exert their immunosuppressive effects by inhibiting the proliferation of T lymphocytes, albeit by different mechanisms. These effects are produced by the formation of three unique trimolecular protein complexes, with the biochemical fate determined by the last protein being bound. FK506 and RAP bind to a peptidyl-prolyl-isomerase (PPIase) FKBP-12,[2] a necessary event for inhibition of downstream T-cell proliferation, but which by itself is not a sufficient condition.[3] In a parallel sense, CsA binds to cyclophilin (CyP), another PPIase, an event that likewise is necessary but alone inadequate for immunosuppression.[4] It was later discovered that all three agents, **1a**, **2**, and CsA, sandwich between these PPIase's and other protein partners. These drugs apparently behaving as a molecular "glue" thus enable the formation of the aforementioned trimolecular protein complexes. Structural similarities of FK506 and RAP notwithstanding, the RAP/FKBP complex binds to a unique effector protein,[5] while the CsA/Cyp and FK506/FKBP complexes converge upon a common target, calcineurin.[6] The molecular and cellular mechanisms of these agents have recently been reviewed.[7] It is now accepted that the cellular modes of action of these three agents are defined by inhibition of the final protein component.

Significant similarity in the toxicity/biological profile of FK506 and CsA has emerged[8] with reports implicating mechanism based toxicity.[9] It is therefore likely that calcineurin inhibition is the origin of toxicity. Calcineurin is a ubiquitous protein with significant concentrations found in kidney and brain tissue. Among the serious side effects being reported for FK506 and CsA are nephrotoxicity and neurotoxicity.

We therefore defined the global objective of our program as discovering an analogue of FK506 (**1a**) or ascomycin (**1b**), which would retain potent immunosuppressive activity, but at the same time exhibit reduced systemic toxicity. Since **1a** and **1b** are potent immunosuppressants, we consequently realized the need to identify a region of **1a** or **1b** which could tolerate alteration without compromising immunosuppressive potency. Also, since there was no structurally driven rationale to reduce the nephrotoxicity of **1a** or **1b**, the chemistry would also need to provide access to diverse functionality. Given that **1a** (or **1b**) functions as the exquisite sandwiching partner between two proteins, one would expect this exercise to be fraught with difficulty. It was therefore critical to identify the correct region of the drug to be modified. Viewing rapamycin as a distant analogue of FK506 inspired us to pursue changes in the boundary surface of the FKBP/ascomycin complex which was also positioned to interact with calcineurin. This perspective justified the expectation that such changes would alter the mode of action and/or the toxicity of the ensuing analogues.[10] Beneath the global objective of discovering a less toxic immunosuppressant, we then set out to identify compounds which altered the binding characteristics of the target drug with FKBP

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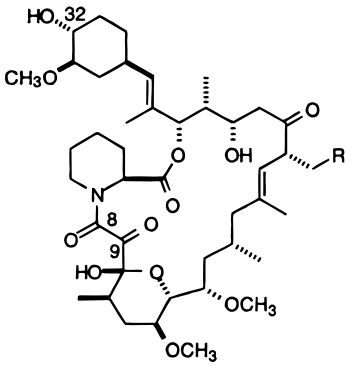
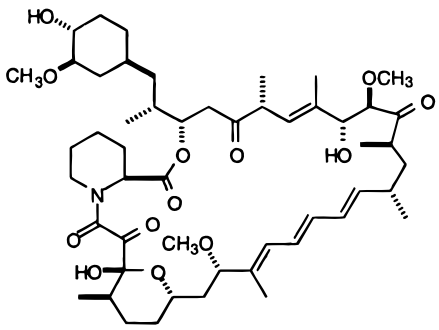
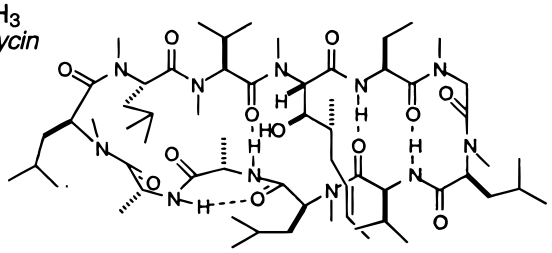


1a: R = -CH=CH$_2$
FK506
1b: R = -CH$_3$
Ascomycin



2
Rapamycin

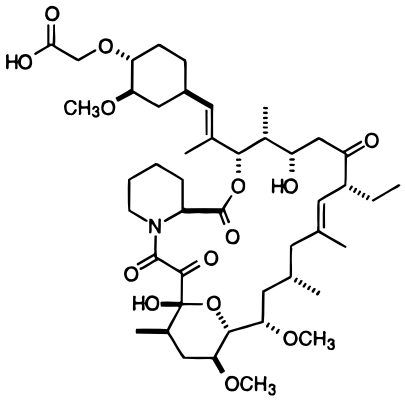


Cyclosporin A

and/or calcineurin. Herein we report modifications to the 32-hydroxyl group of ascomycin (**1b**),[11] which resides at that interface.[12] This moiety is perfectly positioned between both proteins to allow modifications to be made which could affect the stability of the consequent trimolecular complex.

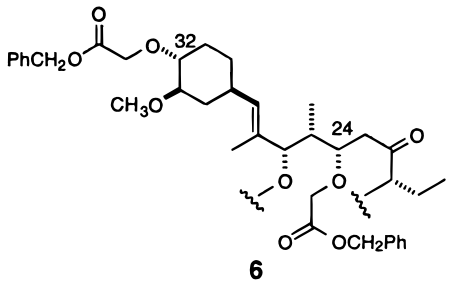
Chemistry

Since there was no clear structural rationale to solve the toxicity problem associated with FK506 (**1a**), reliance upon a serendipitous discovery would be needed. The incorporation of structural diversity at the C-32 position could probe effects on FKBP binding, immunosuppression (in vitro and in vivo), and finally toxicity. One intermediate that would efficiently provide synthetic access to many different analogues is 32-ascomycinyloxyacetic acid **4** (AOAA).



4

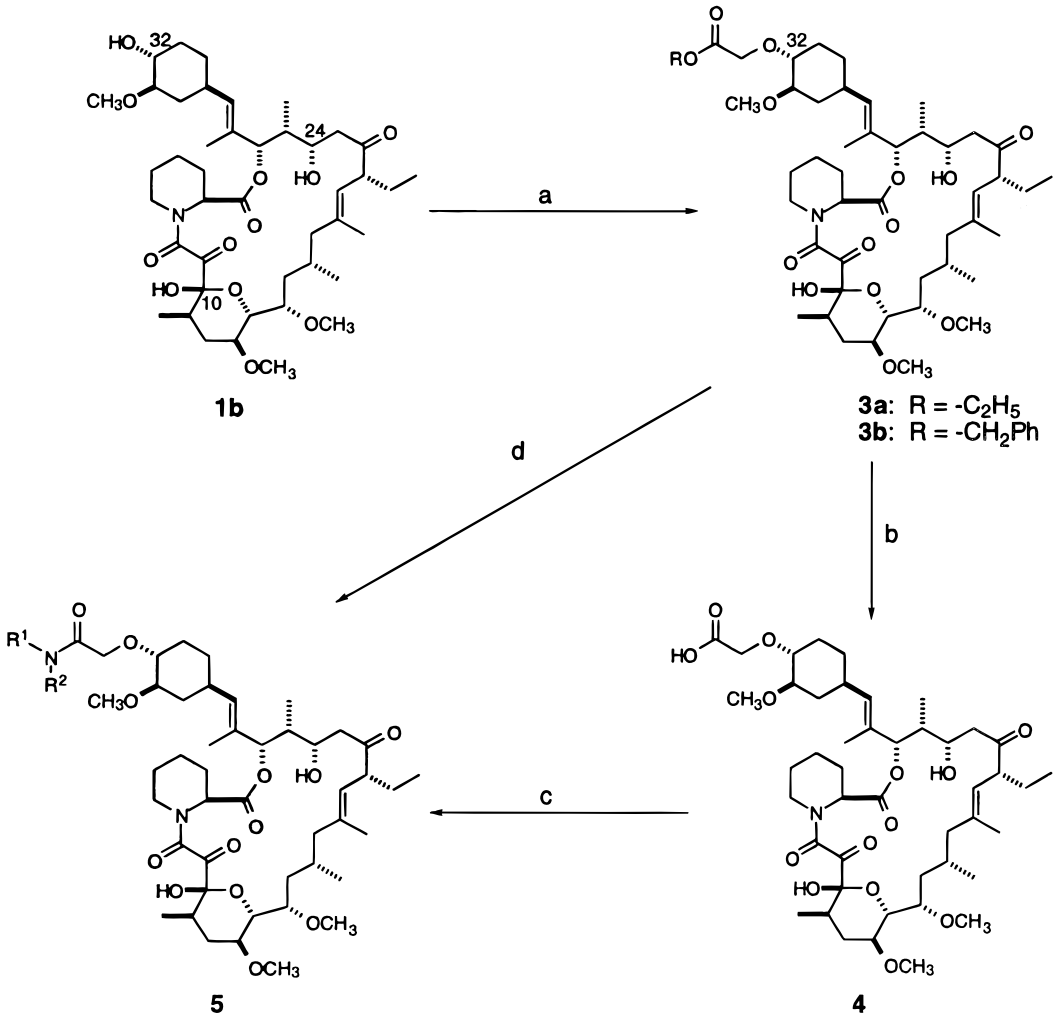
Ascomycin (**1b**) contains three hydroxyl groups, a tertiary alcohol at C-10, and secondary alcohols at C-24 and C-32. Reports by other researchers have shown that the C-32 carbinol of ascomycin or FK-506 can be alkylated selectively. Novartis researchers report the methylation of the C-32 alcohol by treating ascomycin with diazomethane in the presence of Lewis acid.[11a] Of particular interest is the work published by the Merck group which utilized trichloroacetimidate esters to prepare numerous C-32 ethers.[11b,c] On the basis of their investigations, trichloroacetimidate esters of moieties not capable of stabilizing a positive charge, such as a glycolate, would not be expected to provide compounds such as **3a** or **3b** (Scheme 1). We therefore turned our attention to the use of alkyl diazoacetates, which had been foreshadowed by the Novartis work. Ascomycin, stirred in the presence of ethyl diazoacetate and Rh(OAc)$_2$ in refluxing methylene chloride, provided 32-ascomycinyloxyacetic acid ethyl ester **3a** (AOAA ethyl ester) in 50% yield. It has been shown that ascomycin is unstable in the presence of strong bases, such as sodium hydroxide, and therefore synthesis of AOAA (**4**) via simple base hydrolysis was avoided.[13] Moreover, even treatment with mild acids can dehydrate the C-24 β-hydroxy ketone.[14] Since hydrogenolysis of a benzyl ester was anticipated to be uneventful, the synthesis of AOAA benzyl ester (**3b**) was pursued. Alkylation of ascomycin with benzyl diazoacetate at 0 °C gave **3b** in 40% yield. Further analysis of the reaction mixture showed that the bis-alkylated product **6** was formed. No



6

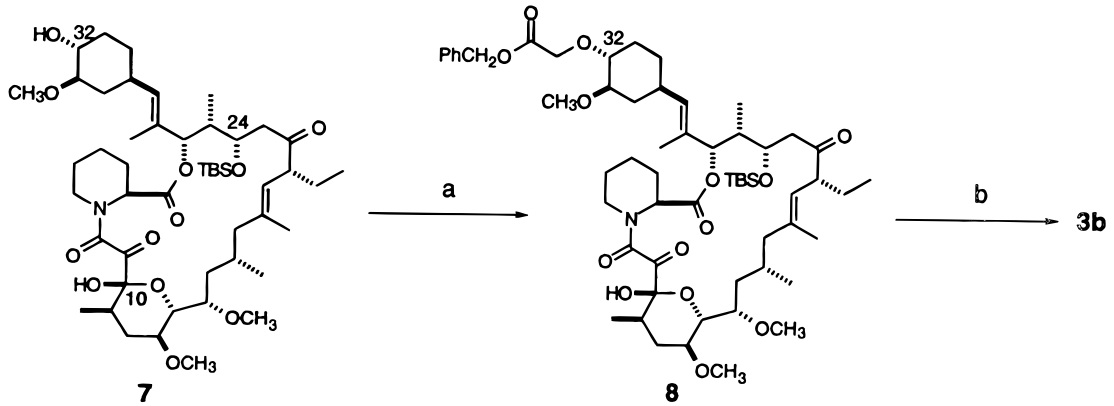
C-24-monoalkylated product was ever detected, dem-

Scheme 1[a]



[a] (a) Method A: RO_2CCHN_2, Rh(II)$(OAc)_2$ (0.02 equiv), CH_2Cl_2; Method B: RO_2CCH_2I, Ag_2O, CH_3CN; (b) (R = CH_2Ph) H_2 (1 atm), 10% Pd(C) cat., EtOH; (c) Method A: EDAC, R^1R^2NH, CH_2Cl_2, 0 °C to room temperature; Method B: ^{i}BuOCOCl, *N*-methylmorpholine, R^1R^2NH, THF, 0 °C to room temperature; (d) $ArNH_2$ (12 equiv), EtMgBr (12 equiv), THF, −78 °C.

Scheme 2[a]

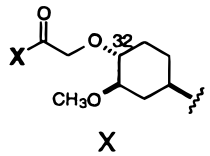


[a] (a) $PhCH_2O_2CCHN_2$, Rh(II)$(OAc)_2$ (cat.), CH_2Cl_2, room temperature; (b) HF (4 equiv), CH_3CN, 0 °C.

onstrating that the 32-carbinol is more reactive than the 24-hydroxyl group.

Therefore, it was necessary to protect the C-24 as its silyl ether **7**, prior to alkylation (Scheme 2).[15] Consequently, the alkylation provided **8** in 70% yield. Removal of the *tert*-butyldimethylsilyl group by brief exposure to hydrogen fluoride at 0 °C provided **3b** in 61% yield. While the overall yield of this procedure did not add a measurable improvement, in practice the chromatographic purification of final product was simplified. Hydrogenolysis of benzyl ester **3b** proceeded cleanly, giving the pivotal carboxylic acid **4**, in 70% yield. Closer examination of this reaction mixture revealed that the C-9 carbonyl was reduced to the carbinol if the reaction was allowed to proceed too long or at higher pressures.

Table 1.



X

compd	X	IC_{50}, nM (n)[a] FKBP binding	IC_{50}, nM (n)[a] MLR inhibition	FKBP IC_{50}/ MLR IC_{50}	rat PLN hyperplasia inhibition ED_{50} mpk/d ip (*n*) (95% range)[b]
1a		2.5 ± 0.2 (36)	0.48 ± 0.09 (56)	5.2	0.1, 5 (0.06−0.16)
1b		2.1 ± 0.2 (32)	0.25 ± 0.05 (50)	8.4	0.3, 10 (0.18−0.54)
2		1.1 ± 0.5 (6)	1.27 ± 0.30 (47)	0.9	2.1
3a	OC_2H_5	9.4 (1)	0.91 ± 0.39 (6)	10.	>3, 1
3b	OCH_2Ph	68. ± 28 (2)	7.62 ± 4.26 (4)	8.9	>3, 1
4	OH	4.0 ± 2.2 (2)	140. ± 79.5 (3)	0.03	>3, 1
9	NH_2	0.6 (1)	0.13 ± 0.10 (3)	4.6	0.3, 2 (0.20−0.50)
10	NHPh	170 ± 110 (7)	0.29 ± 0.16 (9)	590.	1.4, 1
11	$N(CH_3)Ph$	2.9 ± 1.5 (3)	0.26 ± 0.07 (4)	11.	1.9, 2 (1.2−3.2)
12	$NHCH_2Ph$	48. ± 23. (4)	0.79 ± 0.26 (7)	61.	1.2, 2 (0.71−3.11)
13	$N(CH_3)CH_2Ph$	5.6 ± 2.3 (4)	0.09 ± 0.04 (4)	62.	>3, 1
14	$NH(CH_2)_2Ph$	13. ± 0.34 (2)	0.34 ± 0.21 (6)	38.	>3, 1
15	$N(CH_3)(CH_2)_2Ph$	0.4 ± 0.1 (2)	0.64 ± 0.17 (5)	0.63	>3, 3

[a] Data reported as the mean ± SD for *n* determinations as noted. [b] 95% confidence limits for *n* determinations.

Condensations with amines to provide amides **5** were run uneventfully using either EDAC or isobutyl chloroformate as the activating agent. One exception to this sequence resulted from the direct addition of the magnesium bromide salt of 3,4-dichloroaniline to the ester **3b** at low temperature.[16] Treatment of esters with aluminum amides generated by the pretreatment of anilines with trimethylaluminum gave reaction mixtures containing more products than the ammonolyses mediated by the magnesium salts.[17] While more direct, this procedure was explored very late in the development of the aryl amide series and therefore remained less favored than conventional amine acylation through AOAA (**4**).

Upon scaleup, the impractical nature of the current strategy becomes apparent. In addition to the large glassware and unwieldy solvent requirements, the large volume of the benzyl diazoacetate solution needs to be added over 14 h with a syringe pump. Furthermore, the introduction of protection/deprotection steps resulted in a process that was deemed too inefficient for the production of 100 g quantities of **3b**. A more practical synthesis of benzyl ester **3b**, without the need for protecting the C-24 carbinol, resulted from Ag_2O promoted alkylation of ascomycin using benzyl iodoacetate (Scheme 1, method B of step a). This reaction was routinely run on 100−200 g of ascomycin (**1b**).

Results and Discussion

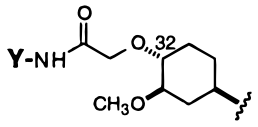
The assays used to evaluate the biological consequences of these structural modifications, the human mixed lymphocyte response (HuMLR), human FKBP binding inhibition, and Brown Norway to Lewis rat popliteal lymph node hyperplasia inhibition assay (PLN), have been described previously.[18] Table 1 illustrates that FK506 (**1a**) and ascomycin (**1b**) are comparable in vitro and in vivo. Although rapamycin (**2**) displays similar FKBP binding and comparable in vitro immunosuppressive potency (HuMLR) with respect to those of **1a** and **1b**, it is less potent in the in vivo model of lymphoproliferation (PLN).

A crude comparison index can be constructed that illustrates the influence of FKBP binding on immunosuppressive potency. Thus, the ratio of the FKBP IC_{50} to HuMLR IC_{50} is 5.2 and 8.4 for FK506 (**1a**) and ascomycin (**1b**), respectively. Therefore, a new compound displaying a ratio between 5.2 and 8.4 can be said to display consistent behavior with respect to **1a** or **1b**. If one compares the FKBP/HuMLR ratios for **1a** (or **1b**) and **2**, a significant difference between FK506 and rapamycin is illustrated, a noteworthy result since divergent mechanisms of immunosuppression have been demonstrated. Esters **3a** and **3b** bind less avidly to FKBP than the preceding compounds; however the proportional decrease of the in vitro immunosuppressive potency (HuMLR) results in FKBP/HuMLR ratios which fall near the expected range.

The first compound displaying aberrant behavior is carboxylic acid **4**, with a ratio of 0.03. Although FKBP affinity is reduced only 2-fold, immunosuppressive potency (HuMLR IC_{50}) is *2−3 orders of magnitude weaker* than **1a** or **1b**. In vivo (PLN assay), **4** gave no measurable immunosuppression at 3 mpk/day. While it is not clear that the inactivity of **4** could be attributed to its inability to penetrate cell membranes or tissue barriers, esters of AOAA (**4**) should transverse these restrictions. Nevertheless, esters **3a** and **3b**, which could conceivably be metabolized to **4**, did not have measurable in vivo activity at the same dose either.

Changing the functional group at "X" (Table 1) to an amide alters the in vivo response significantly for compounds **9**, **10**, **11**, and **12**, while **13**, **14**, and **15** remain inactive at 3 mpk/day in the PLN assay. All of the amides in Table 1 have subnanomolar MLR IC_{50}'s ranging from 0.09 to 0.79 nM. Furthermore, each amide except for **10**, **12**, and perhaps **14** have unremarkable FKBP IC_{50}'s. These three exceptional amides are the

Table 2.



compd	Y	IC_{50}, nM, (n)[a] FKBP binding	IC_{50}, nM, (n)[a] MLR inhibition	FKBP IC_{50}/MLR IC_{50}
1b		2.1 ± 0.2 (32)	0.25 ± 0.05 (50)	8.4
10	Ph	170 ± 110 (7)	0.29 ± 0.16 (9)	590.
16	cyclohexyl	2.0 (1)	0.47 ± 0.15 (4)	4.3
17	2-pyridinyl	2.4 (1)	0.05 ± 0.01 (4)	48.
18	3-pyridinyl	3.3 ± 2.1 (3)	0.10 ± 0.04 (6)	33.
19	4-pyridinyl	3.2 ± 1.8 (3)	0.16 ± 0.05 (4)	20.
20	*p*-(HO)Ph	16. ± 5.5 (5)	0.14 ± 0.04 (11)	110.
21	*p*-(4-morpholino)Ph	19. ± 3.1 (3)	0.58 ± 0.19 (10)	33.
22	3,4-dichloro-Ph	500. ± 80. (3)	0.55 ± 0.36 (4)	910.
23	*p*-(Cl)Ph	180. ± 45. (2)	0.07 ± 0.03 (4)	1400.
24	*p*-(CH_3O)Ph	50. ± 13. (2)	0.04 ± 0.02 (4)	1300.
25	*m*-(CF_3)Ph	470. (1)	0.14 ± 0.05 (4)	3400.
26	*p*-(CF_3)Ph	570. (1)	0.23 ± 0.10 (4)	2700.
27	*m*-(F)Ph	180. (1)	0.16 ± 0.09 (4)	2500.
28	*p*-(F)Ph	46. ± 0.80 (2)	0.03 ± 0.01 (6)	1500.
29	*p*-(CH_3)Ph	71. ± 3.4 (2)	0.02 ± .003 (3)	3600.

[a] Data reported as the mean ± SD for *n* determinations as noted.

first known ascomycin analogues shown to retain subnanomolar immunosuppressive efficacy, while showing marked loss of affinity for the immunophilin. Indeed, the FKBP/HuMLR ratios for **10**, **12**, and **14** are 590, 61, and 38, respectively. These amides likely adopt a predominantly *E*-amide geometry, which must disrupt binding to FKBP. This effect is amplified as the hydrophobic aryl ring gets closer to the amide nitrogen, with the greatest binding disruption occurring with phenyl amide **10**.[19] The option of a *Z*-amide rotamer, provided by tertiary amides **11**, **13**, and **15**, results in profound increases in FKBP affinity.

Disparate behavior, such as has been discovered for the phenyl amide **10**, could enable the discovery of an agent with an altered mechanism of action and safety profile. Toward that end, additional aryl amides were synthesized, and the impact on FKBP binding and immunosuppressive efficacy was determined (Table 2). Without exception, each analogue exhibited a HuMLR IC_{50} at a subnanomolar concentration ranging from 0.02 to 0.58 nM. On the other hand, FKBP binding ranged from 2.0 to 570 nM, and the FKBP/HuMLR ratios ranged from 4.3 to 3600, demonstrating a profound lack of correlation between FKBP binding and in vitro immunosuppressive efficacy.

The amide must satisfy several criteria if dissociation from FKBP is to be observed. These are secondary amide structure and hydrophobic substitution. While no protracted effort has been made to show the requirement for aromaticity, the FKBP affinity of the cyclohexyl amide **16** resembles the FKBP affinity of ascomycin (**1b**) much more than that of the phenyl amide **11**. It is therefore likely that aromaticity is a third requirement for attenuated immunophilin binding.

Pyridyl amides **17**, **18**, and **19** are unremarkable, indicating that aryl rings with hydrogen bonding capabilities display FKBP affinities similar to that of ascomycin. Furthermore, AOAA phenolic amide **20** and AOAA p-(4-morpholino)-phenyl amide **21** show affinities to FKBP that are less than 10-fold weaker than ascomycin (**1b**). In both of these cases, however, a slight boost in immunosuppressive potency is obtained, with the FKBP/HuMLR ratios being 110 and 33, respectively. As hydrophobic substituents are added (**22**−**29**), this immunosuppressive boost is retained in most cases, while FKBP binding is further disrupted, in some cases to nearly micromolar levels. Consequently, these two effects elevate the FKBP/HuMLR ratios from 910 to 3600. Clearly, significant disruption of the FKBP/drug/calcineurin mating surfaces can happen without compromising MLR potency.

Conclusion

Is it possible to create a potent immunosuppressant based on the ascomycin framework that is profoundly less nephrotoxic than FK506 (**1a**)? This remains the critical question that compels researchers in this area to continue searching for such an agent. The fact that rapamycin (**2**) is immunosuppressive, binds FKBP, and yet operates via a novel mechanism suggests that great opportunity awaits the medicinal chemist who is willing to alter the mating surface of the FKBP−drug complex. Indeed, in recent reports Merck researchers have unveiled 32-*O*-(1-hydroxyethylindol-5-yl)ascomycin, a compound that shows approximately a 10-fold decrease in FKBP affinity, while maintaining equivalent in vitro and in vivo potency when compared with FK506 (**1a**). Most interestingly, an improved toxicity profile appears to be emerging from this agent.[20] With the AOAA-derived amides, we have also achieved significant binding disruption at the immunophilin site, while demonstrating surprising immunosuppressive activity in human T-lymphocytes. The impact of these manipulations and others on kidney function will be the subject of future reports.

Experimental Section

General Methods. Unless otherwise specified, proton magnetic resonance spectra were run as chloroform-*d* solutions at 500 MHz. Carbon-13 nuclear magnetic resonance spectra were run as chloroform-*d* solutions at 125 MHz. Chloroform-*d* was used as an internal standard. DEPT (distortionless

enhancement by polarization transfer) was employed to assign carbon multiplicities in some cases. Melting points are uncorrected. Mass spectra, infrared spectra, and the above determinations were performed by the Analytical Research Department, Abbott Laboratories. Elemental analyses were performed either by the Analytical Research Department, Abbott Laboratories, or Robertson Microlit Laboratories, Inc., Madison, NJ.

Analytical thin-layer chromatography was done on 2 × 6 cm Kieselgel 60 F-254 plates precoated with 0.25 mm thick silica gel distributed by E. Merck. Visualization was accomplished with a solution consisting of ammonium molybdate (50 g) and ceric sulfate (20 g) in 10% sulfuric acid (2 L) followed by charring on a hot plate, or with short-wavelength ultraviolet light. Unless otherwise specified, column chromatography was performed on silica gel (Kieselgel 60, 70−230 mesh) from E. Merck. Preparative HPLC purification was performed at ambient temperature on a 20 × 300 mm column packed with YMC 15 μm 60-Å spherical silica gel, eluting at 10−20 mL/min, using a differential refractometer to monitor eluate composition. The term in vacuo refers to solvent removal via a rotary evaporator at 30 mmHg. All final products were lyophylized from benzene. Analytical samples were further dried by final evacuation at 0.1 mmHg for 18 h.

With the exception of amines, solvents and reagents were purchased from Aldrich Chemical Co. and were used without further purification unless otherwise specified. All amines were dried over molecular sieves (4 Å) for at least 24 h prior to use. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl.

32-Ascomycinyloxyacetic Acid Ethyl Ester (3a). A solution of ascomycin (0.5 g, 0.6 mmol) in dichloromethane (10 mL) containing rhodium(II) acetate dimer (3.0 mg, 0.01 mmol, 0.02 equiv) was refluxed while ethyl diazoacetate (66 uL, 0.6 mmol) in dichloromethane (1 mL) was added dropwise. After the addition was complete, the reaction was refluxed for 30 min and additional ethyl diazoacetate (132 uL, 1.26 mmol) in dichloromethane (1.5 mL) was added dropwise with reflux continuing 30 min after complete addition. Solvent was removed in vacuo and the residue purified by HPLC on silica gel eluting with hexane:acetone (3:1). Fractions containing the desired product were pooled, concentrated, dissolved in CCl_4, and concentrated to constant weight under high vacuum to give the desired product (274 mg) as an oil in 50% yield: IR ($CDCl_3$) 3500, 2930, 1742, 1700, 1645, 1452 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 5.34 (d, 0.67H, J = 3.0 Hz), 5.21 (d, 0.33H, J = 3.0 Hz), 5.09−4.98 (mult, 2H), 4.82 (d, 1H, J = 2.5 Hz), 4.61 (d, 1H, J = 6.0 Hz), 4.44 (br d, 0.67H, J = 15.0 Hz), 4.33 (d, 1H, J = 16.0 Hz), 4.29 (d, 1H, J = 16.0 Hz), 4.25 (s, 1H), 4.23 (d, 1H, J = 7.0 Hz), 4.20 (d, 1H, J = 7.0 Hz), 3.98−3.87 (mult, 1.33H), 3.74 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.58 (mult, 1H), 3.50−3.06 (comp, 4.33H), 3.42 (s, 2H), 3.41 (s, 1H), 3.40 (s, 2H), 3.39 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.64 (s, 3H), 1.61 (s, 2H), 1.29 (dd, 3H, J = 7.5, 7.5 Hz), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ 9.4, 11.7, 14.1, 14.2, 15.8, 16.2, 20.5, 21.1, 24.2, 24.6, 26.3, 27.6, 30.3, 30.8, 32.7, 32.9, 33.6, 34.6, 36.4, 39.2, 39.7, 43.1, 48.7, 54.7, 56.3, 56.6, 56.9, 57.2, 60.6, 68.5, 70.1, 72.9, 73.7, 75.2, 77.2, 82.8, 83.6, 97.0, 123.1, 129.6, 132.4, 138.7, 164.7, 169.0, 171.1, 196.1, 213.5; MS (FAB) m/z M + K = 916. Anal. Calcd for $C_{47}H_{75}NO_{14}$·1.0CCl_4: C, 54.70; H, 7.33; N, 1.36. Found: C, 54.42; H, 7.22; N, 1.26.

32-Ascomycinyloxyacetic Acid Benzyl Ester (3b). (a) A mixture of sodium iodide (608 g, 4.0 mol) in acetone (1.6 L) was heated to reflux for 5 min and cooled to room temperature. Benzyl bromoacetate (114 mL, 0.72 mol) was added, and the reaction was stirred for 30 min. The solvent was removed in vacuo, and the resulting slurry was partitioned between water (800 mL) and ethyl acetate (400 mL). The organic layer was washed sequentially with saturated sodium bisulfite (2 × 400 mL) and brine (400 mL). The aqueous portions were extracted with additional ethyl acetate (400 mL), and the combined organics were dried (Na_2SO_4) and concentrated in vacuo to a brown oil. This was passed through a pad of basic alumina (100 mL) and eluted with hexanes (200 mL). Concentration of the eluate provided benzyl iodoacetate as an amber oil (171 g) which was sufficiently pure to use in the next step.

(b) In a three-necked 2 L round-bottom flask equipped with an overhead stirrer, ascomycin **1b** (100 g, 0.13 mol, crystalline material completely dissolved in methylene chloride then concentrated to a dry foam) and benzyl iodoacetate (171 g, 0.62 mol, 4.9 equiv) were mixed together and then dissolved completely in acetonitrile (50 mL).[21] The thick solution was cooled to 0 °C whereupon Ag_2O (119 g, 0.51 mol, 4 equiv) was added portionwise over 15 min (ca. 15 additions).[22] After the addition was complete and the reaction was mixed (5 min after last addition), the ice bath was removed and the reaction allowed to stir at ambient temperature for 7 days. Diethyl ether (400 mL) was added to the reaction mixture, and this was then poured over silica gel (600 mL), mixed and allowed to air-dry overnight. A 3 L coarse fritted Buchner funnel was charged with silica gel (2 L) and the adsorbed silica carefully layered over the dry fresh bed, followed by a filter paper disk. The silica pad was eluted with CH_2Cl_2 (3 L), CH_2Cl_2:CH_3CN (9:1, 4 L), CH_2Cl_2:CH_3CN (6:1, 4 L), CH_2Cl_2:CH_3CN (3:1, 8 L), and acetone (4 L), collecting 1 L fractions throughout. Eluate-containing product was pooled and concentrated to semipure **3b** (72 g), while ascomycin (27 g) was isolated from the acetone eluate. Final purification was achieved by another passage through dry-packed silica gel (2 L) in a Buchner funnel. Elution with CH_2Cl_2 (2 L), hexane:acetone (4:1, 6 L), and hexane:acetone (3:1, 8 L), collecting 1 L fractions throughout, resulted in pure solutions of **3b** which were combined and concentrated to a pale yellow foam (61 g, 65 mmol): IR ($CDCl_3$) 3510, 2930, 1740, 1695, 1642, 1450 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 7.39−7.29 (comp, 5H), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (m, 0.33H), 5.21 (d, 1H, J = 12.0 Hz), 5.18 (d, 1H, J = 12.0 Hz), 5.11−4.98 (mult, 2H), 4.77 (d, 0.67H, J = 2.5 Hz), 4.72 (s, 0.33H), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.39 (d, 1H, J = 16.0 Hz), 4.34 (d, 1H, J = 16.0 Hz), 4.25 (d, 1H, J = 2.5 Hz), 3.98−3.87 (mult, 1.33H), 3.74 (br d, 0.33H, J = 14.5 Hz), 3.69 (d, 0.67H, J = 10.0 Hz), 3.60 (mult, 1H), 3.51−3.14 (comp, 4.33H), 3.42 (s, 2H), 3.41 (s, 3H), 3.40 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.04 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.75 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.66 (s, 1H), 1.65 (s, 2H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ 9.4, 11.7, 14.1, 15.8, 16.2, 20.4, 21.1, 24.1, 24.5, 26.3, 27.6, 30.3, 30.8, 32.7, 32.8, 34.4, 34.5, 36.3, 39.2, 39.6, 43.1, 48.6, 53.4, 54.6, 56.3, 56.6, 57.1, 66.3, 68.5, 70.1, 72.8, 73.6, 75.1, 76.8, 82.7, 83.6, 96.9, 123.0, 128.3, 128.4, 128.5, 129.5, 132.3, 135.6, 138.7, 164.7, 168.9, 171.0, 196.2, 213.4; MS (FAB) m/z M + H − H_2O = 922, M + K = 978. Anal. Calcd for $C_{52}H_{77}NO_{14}$: C, 66.43; H, 8.26; N, 1.49. Found: C, 66.12; H, 8.14; N, 1.41.

32-Ascomycinyloxyacetic Acid (4). A Parr shaker flask was charged with AOAA benzyl ester **3b** (41 g, 44 mmol), 10% Pd/C (4.7 g), and ethanol (1.1 L) and then flushed with nitrogen for 10 min. The reaction vessel was shaken under a hydrogen atmosphere (4 atm) for 15 min. The mixture was filtered, and the catalyst was extracted once with additional ethanol (1 L), whereupon the filtrates were combined and the solvent was removed in vacuo (41 g). A column was packed with dry silica gel (5.2 L, E. Merck Kieselgel 60, 230−400 mesh) and prewetted with CH_2Cl_2:2-propanol (19:1) containing 0.5% CH_3CO_2H. Elution with CH_2Cl_2:2-propanol (19:1, 12 L) containing 0.5% CH_3CO_2H, followed by CH_2Cl_2:2-propanol (9:1, 12 L) containing 0.5% CH_3CO_2H, collecting 1 L fractions throughout, provided recovered **3b** (1.7 g) and AOAA **4** (33 g, 39 mmol) in 88% yield: IR ($CDCl_3$) 3450, 1740 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 5.43 (d, 0.67H, J = 3 Hz), 5.22 (d, 0.33H, J = 3 Hz), 5.03 (mult, 2H), 4.88 (br s, 1H), 4.63 (d, 1H, J = 5.0

Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.30 (d, 1H, J = 17.5 Hz), 4.26 (s, 1H), 3.99 (d, 0.67H, J = 17.5 Hz), 3.98 (d, 0.33H, J = 17.5 Hz), 3.95−3.82 (mult, 1.33H), 3.75 (br d, 0.33H, J = 15.0 Hz), 3.68 (d, 0.67H, J = 10 Hz), 3.59 (mult, 1H), 3.55−2.95 (mult, 5H), 3.50 (s, 3H), 3.40 (s, 2H), 3.39 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 2.79 (dd, 0.67H, J = 15.0, 3.0 Hz), 2.73 (dd, 0.33H, J = 15.0, 3.0 Hz), 2.37−0.83 (comp, 26H), 1.69 (s, 1H), 1.66 (s, 2H), 1.64 (s, 1H), 1.62 (s, 2H), 1.01 (d, 2H, J = 7.5 Hz), 0.98 (d, 1H, J = 7.5 Hz), 0.95 (d, 2H, J = 7.0 Hz), 0.93 (d, 1H, J = 7.0 Hz), 0.89 (d, 2H, J = 7.0 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.0 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 172.1, 169.0, 168.7, 165.8, 164.7, 139.7, 138.8, 133.1, 132.5, 128.7, 127.1, 123.8, 123.0, 98.7, 97.1, 83.5, 82.0, 77.8, 77.2, 76.6, 75.2, 73.7, 73.6, 72.9, 72.2, 70.0, 68.9, 67.6, 57.6, 57.0, 56.6, 56.5, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.4, 43.1, 40.3, 39.7, 39.2, 35.5, 35.2, 34.7, 34.3, 33.7, 33.0, 32.7, 32.5, 30.4, 29.5, 27.5, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 21.1, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.6, 14.2, 14.1, 11.7, 9.8, 9.5; MS (FAB) m/z M + K = 888. Anal. Calcd for $C_{45}H_{71}NO_{14}\cdot H_2O$: C, 62.61; H, 8.80; N, 1.33. Found: C, 62.26; H, 8.47; N, 1.61.

32-Ascomycinyloxyacetamide (9). To a stirred solution of AOAA (**4**) (1.0 g, 1.2 mmol) in dry THF (4 mL) at 0 °C was added *N*-methylmorpholine (130 μL, 1.2 mmol) followed by isobutyl chloroformate (150 μL, 1.2 mmol). After 30 min, concentrated NH_4OH (160 μL, 2.4 mmol) was added, and the reaction was warmed gradually to room temperature, followed by stirring overnight. The mixture was partitioned between EtOAc (150 mL) and 1 N HCl (150 mL). The organic layer was washed with an additional portion of 1 N HCl (150 mL), saturated $NaHCO_3$ solution (2 × 150 mL), and then brine (150 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude material was dissolved in CH_2Cl_2 and then passed through a pad of silica gel (50 g) eluting with CH_2Cl_2 (300 mL), 2:1 hexane:acetone (600 mL), 1:1 hexane:acetone (300 mL), and then finally 1:2 hexane:acetone (600 mL), collecting 20 mL fractions throughout. Eluate containing the desired product was pooled and concentrated in vacuo. Further purification by HPLC eluting with 1:2 hexane:acetone afforded pure **9** (0.61 g, 61% yield): IR (KBr) ν 3425, 2960 (sh), 2920, 2850 (sh), 2820 (sh), 1740, 1670 (sh), 1643 cm^{-1}; ^{1}H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 7.80 (m, 1H), 5.44 (br s, 1H), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.25 (br s, 1H), 4.13 (d, 0.67H, J = 16.5 Hz), 4.12 (d, 0.33H, J = 16.0 Hz), 3.96 (d, 0.67H, J = 17.0 Hz), 3.95 (d, 0.33H, J = 17.5 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.59 (mult, 1H), 3.50−3.12 (comp, 4.33H), 3.45 (s, 3H), 3.44 (s, 2H), 3.39 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.4, 213.4, 196.1, 192.6, 174.0, 169.0, 168.7, 165.8, 164.7, 139.6, 138.7, 132.8, 132.2, 129.2, 129.2, 123.3, 123.1, 98.7, 97.1, 82.3, 82.2, 77.9, 77.2, 76.6, 75.2, 73.7, 73.6, 72.9, 72.3, 70.0, 69.0, 68.0, 57.5, 57.0, 56.8, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.3, 40.4, 39.8, 39.2, 35.8, 35.7, 35.5, 34.6, 34.5, 33.7, 33.0, 32.7, 32.6, 30.5, 29.3, 27.5, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 21.1, 20.8, 20.4, 19.5, 16.2, 16.0, 15.7, 15.8, 14.2, 14.1, 11.7, 9.8, 9.5; mass spectrum (FAB), 887 [M + K]$^+$. Anal. Calcd for $C_{45}H_{72}N_2O_{13}$: C, 63.65; H, 8.54; N, 3.29. Found: C, 63.95; H, 8.71; N, 3.15.

Aniline−(32-Ascomycinyloxyacetic acid)amide (10). Aniline was coupled to AOAA (**4**) on a 1.2 mmol scale according to the method used for synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (150 mL) followed by 3:1 hexane:acetone (100 mL) and then 2:1 hexane:acetone (200 mL). Fractions containing the desired product were pooled and concentrated in vacuo to give 1.03 g of yellow foam which was further purified by HPLC eluting with 5:2 hexane:acetone to provide **10** (0.80 g, 73% yield): IR (KBr) ν 3340, 2940, 2880, 2830, 1740, 1690, 1645 cm^{-1}; ^{1}H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.59 (s, 0.33H), 9.57 (s, 0.67H), 7.62 (d, 2H, J = 8.0 Hz), 7.34 (dd, 2H, J = 8.0, 8.0 Hz), 7.12 (dd, 2H, J = 8.0, 8.0 Hz), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.88 (d, 1H, J = 2.5 Hz), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.25 (d, 0.67H, J = 16.5 Hz), 4.24 (d, 0.33H, J = 16.0 Hz), 4.24 (s, 1H), 4.02 (d, 0.67H, J = 17.0 Hz), 4.01 (d, 0.33H, J = 17.5 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.59 (mult, 1H), 3.50−3.12 (comp, 4.33H), 3.57 (s, 3H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.2, 213.1, 196.1, 192.6, 169.0, 168.9, 168.6, 165.7, 164.6, 139.5, 138.6, 137.8, 132.7, 132.2, 129.1, 129.0, 128.8, 124.0, 123.2, 123.0, 119.6, 98.5, 96.9, 82.1, 82.0, 77.7, 77.2, 76.5, 75.1, 73.5, 72.8, 72.2, 69.9, 68.9, 67.8, 57.4, 56.8, 56.7, 56.5, 56.2, 55.9, 54.8, 54.6, 52.6, 48.6, 48.3, 43.8, 43.4, 43.2, 40.1, 39.7, 39.1, 35.5, 35.5, 35.3, 34.4, 34.4, 33.6, 32.9, 32.6, 32.4, 31.4, 30.3, 28.9, 27.5, 26.2, 26.1, 25.9, 24.4, 24.4, 24.1, 22.5, 21.0, 20.7, 20.3, 19.4, 16.1, 15.9, 15.7, 15.6, 14.1, 14.0, 11.6, 9.7, 9.3; mass spectrum (FAB), 963 [M + K]$^+$. Anal. Calcd for $C_{51}H_{76}N_2O_{13}$: C, 66.21; H, 8.27; N, 3.02. Found: C, 66.99; H, 8.94; N, 2.81.

***N*-Methylaniline−(32-Ascomycinyloxyacetic acid) amide (11).** *N*-Methylaniline was coupled to AOAA (**4**) on a 0.94 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (100 mL) followed by 1:1 hexane:acetone (150 mL). Fractions containing the desired product were pooled and concentrated in vacuo to give 640 mg of yellow foam which was further purified by HPLC eluting with 3:2 hexane:acetone to provide **11** (0.48 g, 54% yield): IR (KBr) ν 3425, 2960 (sh), 2930, 2850, 2820, 1740, 1700, 1643, 1598 cm^{-1}; ^{1}H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 7.42 (m, 2H), 7.35 (m, 1H), 7.21 (m, 2H), 5.23 (d, 0.67H, J = 3.0 Hz), 5.19 (d, 0.33H, J = 3.0 Hz), 5.07−4.97 (mult, 2H), 4.82 (d, 1H, J = 2.5 Hz), 4.60 (d, 1H, J = 6.0 Hz), 4.43 (br d, 0.67H, J = 15.0 Hz), 4.23 (s, 1H), 4.09 (br s, 2H), 3.98−3.87 (mult, 1.33H), 3.73 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.59 (mult, 1H), 3.50−3.09 (comp, 4.33H), 3.40 (s, 2H), 3.39 (s, 1H), 3.36 (s, 1H), 3.35 (s, 2H), 3.30 (s, 2H), 3.28 (s, 1H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 15.5, 7.5 Hz), 2.74 (dd, 0.33H, J = 15.5, 7.5 Hz), 2.63 (s, 0.25H), 2.37−0.74 (comp, 26H), 2.19 (s, 0.25H), 2.18 (s, 2.5H), 1.65 (s, 1H), 1.64 (s, 1H), 1.62 (s, 2H), 1.60 (s, 2H), 1.01 (d, 2H, J = 7.0 Hz), 0.97 (d, 1H, J = 7.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.91 (d, 1H, J = 7.5 Hz), 0.87 (dd, 3H, J = 7.5, 7.5 Hz), 0.86 (d, 2H, J = 6.5 Hz), 0.84 (d, 1H, J = 6.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.6, 169.8, 168.9, 168.6, 165.8, 164.7, 142.9, 139.6, 138.7, 132.2, 131.7, 129.7, 129.6, 129.7, 127.9, 127.1, 123.3, 123.1, 98.6, 97.0, 83.6, 83.6, 82.4, 77.8, 77.2, 76.7, 75.2, 73.7, 73.7, 72.9, 72.3, 70.1, 69.2, 69.1, 57.5, 57.3, 57.2, 56.9, 56.6, 56.3, 56.1, 55.0, 54.7, 52.7, 48.7, 48.5, 43.8, 43.5, 43.1, 40.3, 39.7, 39.2, 37.3, 36.5, 36.4, 35.5, 34.6, 33.6, 32.9, 32.8, 32.6, 30.8, 30.2, 30.2, 29.3, 27.6, 26.4, 26.2, 26.0, 24.6, 24.5, 24.2, 21.1, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.7, 14.2, 14.1, 11.7, 9.8, 9.4; mass spectrum (FAB), 977 [M + K]$^+$. Anal. Calcd for $C_{52}H_{78}N_3O_{13}$: C, 66.50; H, 8.37; N, 2.98. Found: C, 66.31; H, 8.50; N, 2.94.

Benzylamine−(32-Ascomycinyloxyacetic acid)amide (12). Benzylamine was coupled to AOAA (**4**) on a 1.2 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (100 mL) followed by 2:1 hexane:acetone (300 mL). Fractions containing the desired product were pooled and concentrated in vacuo to give 1.1 g of yellow foam which was further purified by HPLC eluting with 3:2 hexane:acetone to give semipure **12** (0.8 g). A final purification by HPLC eluting with 4% IPA/CH_2Cl_2 afforded pure **12** (0.45 g, 41% yield): IR (KBr) ν 3420, 2950, 2920, 2865,

2820, 1740, 1700, 1645 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 8.15 (m, 1H), 7.36−7.25 (m, 5H), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.85 (d, 1H, J = 2.5 Hz), 4.63−4.56 (comp, 2H), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.25 (s, 1H), 4.19 (d, 0.67H, J = 16.5 Hz), 4.18 (d, 0.33H, J = 16.0 Hz), 3.98 (d, 0.67H, J = 17.0 Hz), 3.97 (d, 0.33H, J = 17.5 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.59 (mult, 1H), 3.50−3.12 (comp, 4.33H), 3.40 (s, 2H), 3.39 (s, 1H), 3.35 (s, 1H), 3.32 (s, 2H), 3.12 (s, 2H), 3.10 (s, 1H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.2, 213.1, 196.1, 192.7, 170.6, 168.9, 168.6, 165.7, 164.6, 139.5, 138.6, 138.1, 132.6, 132.1, 129.4, 129.2, 128.4, 128.0, 127.2, 123.3, 123.0, 98.6, 97.0, 82.3, 81.8, 77.8, 77.2, 76.6, 75.2, 73.6, 72.8, 72.2, 69.9, 68.9, 68.1, 57.4, 56.9, 56.5, 56.2, 56.1, 55.9, 54.9, 54.7, 52.6, 48.6, 48.3, 43.8, 43.5, 43.4, 43.1, 40.2, 39.7, 39.1, 35.5, 35.5, 35.4, 34.5, 34.4, 33.6, 33.0, 32.6, 32.5, 30.3, 29.2, 27.5, 26.2, 26.1, 26.0, 24.5, 24.4, 24.1, 21.0, 20.7, 20.3, 19.4, 16.1, 15.9, 15.7, 15.6, 14.1, 13.9, 11.6, 9.7, 9.4; mass spectrum (FAB), 977 $[M + K]^+$. Anal. Calcd for $C_{52}H_{78}N_2O_{13}$: C, 66.50; H, 8.37; N, 2.98. Found: C, 66.74; H, 8.38; N, 2.88.

***N*-Methylbenzylamine−(32-Ascomycinyloxyacetic acid)amide (13).** *N*-Methylbenzylamine was coupled to AOAA (**4**) on a 0.94 mmol scale according to the method used for the synthesis of **9**. The silica plug was eluted with CH_2Cl_2 (100 mL) followed by 1:1 hexane:acetone (150 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 640 mg of yellow foam which was further purified by HPLC eluting with 3:2 hexane:acetone to provide **13** (0.41 g, 45% yield): IR (KBr) ν 3425, 2960 (sh), 2930, 2850, 2820, 1740, 1700, 1643, cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 7.39−7.18 (m, 5H), 5.34 (br, 0.67H), 5.20 (br, 0.33H), 5.09−4.97 (mult, 2H), 4.83 (s, 1H), 4.68−4.35 (comp, 5.67H), 4.23 (s, 1H), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.59 (mult, 1H), 3.50−2.86 (comp, 5H), 3.43 (s, 2H), 3.42 (s, 1H), 3.39 (s, 1H), 3.35 (s, 2H), 3.33 (s, 2H), 3.31 (s, 1H), 2.79 (m, 0.67H), 2.74 (m, 0.33H), 2.63 (s, 0.25H), 2.37−0.74 (comp, 26H), 2.19 (s, 0.25H), 2.18 (s, 2.5H), 1.67 (s, 1H), 1.64 (s, 1H), 1.62 (s, 2H), 1.60 (s, 2H), 1.01 (d, 2H, J = 7.0 Hz), 0.97 (d, 1H, J = 7.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.93 (d, 1H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.87 (d, 2H, J = 6.5 Hz), 0.84 (d, 1H, J = 6.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.6, 170.1, 170.0, 169.0, 168.7, 165.8, 164.7, 139.7, 138.7, 137.1, 136.8, 132.4, 131.8, 129.6, 128.8, 128.8, 128.5, 128.2, 127.5, 127.4, 126.8, 123.3, 123.1, 98.7, 97.0, 83.5, 83.4, 82.4, 77.8, 77.1, 76.7, 75.2, 73.7, 73.7, 72.9, 72.3, 70.3, 70.1, 70.0, 69.2, 57.5, 57.3, 57.2, 57.0, 56.9, 56.6, 56.3, 56.1, 55.0, 54.7, 53.9, 52.7, 52.6, 51.0, 48.7, 48.5, 43.8, 43.5, 43.1, 40.3, 39.7, 39.2, 36.5, 36.3, 36.2, 35.5, 34.6, 34.6, 33.8, 33.6, 33.5, 32.9, 32.7, 32.6, 30.8, 30.1, 30.0, 29.3, 27.6, 26.4, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.7, 14.3, 14.2, 11.7, 9.8, 9.4; mass spectrum (FAB), 991 $[M + K]^+$. Anal. Calcd for $C_{53}H_{80}N_2O_{13}\cdot 0.5H_2O$: C, 66.15; H, 8.48; N, 2.91. Found: C, 66.08; H, 8.24; N, 3.07.

2-Phenethylamine−(32-Ascomycinyloxyacetic acid)-amide (14). 2-Phenethylamine was coupled to AOAA (**4**) on a 0.71 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (100 mL) followed by 1:1 hexane:acetone (125 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 630 mg of yellow foam which was further purified by HPLC eluting with 3:2 hexane:acetone to produce **14** (0.45 g, 67% yield): IR (KBr) ν 3435, 1740, 1700, 1650 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 7.82 (t, 0.33H, J = 6.5 Hz), 7.79 (t, 0.67H, J = 6.5 Hz), 7.30 (dd, 2H, J = 7.5, 7.5 Hz), 7.22 (d, 2H, J = 7.5 Hz), 5.33 (d, 0.67H, J = 3.7 Hz), 5.22 (d, 0.33H, J = 3.7 Hz), 5.03 (mult, 2H), 4.85 (br s, 1H), 4.62 (d, 1H, J = 6.5 Hz), 4.45 (br d, 0.67H, J = 14.0 Hz), 4.26 (s, 1H), 4.11 (d, 1H, J = 17.5 Hz), 4.0−3.86 (mult, 1.33H), 3.93 (d, 0.67H, J = 17.5 Hz), 3.92 (d, 0.33H, J = 17.5 Hz), 3.75 (br d, 0.33H, J = 14.0 Hz), 3.68 (d, 0.67H, J = 10 Hz), 3.67−3.56 (mult, 2H), 3.56−3.42 (mult, 2H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.32 (s, 2H), 3.28 (s, 3H), 3.22 (mult, 1H), 3.10 (mult, 1H), 3.07−2.98 (mult, 2H), 2.92−2.79 (mult, 3H), 2.79 (dd, 0.67H, J = 16.5, 5.0 Hz), 2.73 (dd, 0.33H, J = 17.5, 3.5 Hz), 2.37−1.03 (comp, 26H), 1.68 (s, 1H), 1.64 (s, 3H), 1.61 (s, 2H), 1.10 (d, 2H, J = 6.5 Hz), 0.98 (d, 1H, J = 6.5 Hz), 0.95 (d, 2H, J = 6.5 Hz), 0.93 (d, 1H, J = 6.5 Hz), 0.88 (d, 2H, J = 6.5 Hz), 0.87 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 6.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.6, 170.8, 169.0, 168.7, 165.8, 164.7, 139.6, 139.0, 138.7, 132.7, 132.2, 129.2, 129.2, 128.8, 128.4, 126.3, 123.3, 123.1, 98.6, 97.1, 82.1, 82.1, 77.9, 76.8, 76.6, 75.2, 73.7, 73.6, 72.9, 72.2, 70.0, 69.0, 68.1, 68.0, 57.5, 57.0, 56.7, 56.6, 56.3, 56.0, 54.9, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.2, 40.3, 40.1, 39.8, 39.2, 35.7, 35.6, 35.4, 34.6, 34.5, 33.7, 33.0, 32.7, 32.5, 30.4, 29.2, 27.6, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 21.1, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.7, 14.1, 14.0, 11.7, 9.8, 9.5; mass spectrum (FAB), 953 $(M + H)^+$, 991 $(M + K)^+$. Anal. Calcd for $C_{53}H_{80}N_2O_{13}$: C, 66.78; H, 8.46; N, 2.94. Found: C, 67.13; H, 8.33; N, 3.04.

***N*-Methyl-2-phenethylamine−(32-Ascomycinyloxyacetic acid)amide (15).** *N*-Methylphenethylamine was coupled to AOAA (**4**) on a 4.9 mmol scale according to the method used for the synthesis of **9**. The silica pad (200 g, 230−400 mesh) was eluted with CH_2Cl_2 (800 mL) followed by 1% IPA/CH_2Cl_2 (1 L), 2% IPA/CH_2Cl_2 (1 L), 3% IPA/CH_2Cl_2 (1 L), 4% IPA/CH_2Cl_2 (1 L), and then 5% IPA/CH_2Cl_2 (1 L). Fractions containing the desired product were combined and concentrated in vacuo to give **15** (4.4 g, 88% yield): IR (KBr) ν 3440, 3035, 2940, 2880, 2830, 1740, 1700, 1643 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 7.34−7.16 (comp, 5H), 5.34 (s, 0.67H), 5.20 (s, 0.33H), 5.09−4.97 (mult, 2H), 4.83 (s, 1H), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.36 (d, 0.67H, J = 15.0 Hz), 4.32 (d, 0.33H, J = 15.0 Hz), 4.23 (s, 1H), 4.17 (d, 0.67H, J = 15.0 Hz), 4.10 (d, 0.33H, J = 15.0 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.50−3.12 (comp, 7.33H), 3.44−3.31 (comp, 9H), 3.03 (comp, 0.67H), 2.97 (s, 1.5H), 2.91 (s, 1.5H), 2.87 (dd, 2H, J = 7.5, 7.5 Hz), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.1, 196.0, 192.6, 169.6, 169.4, 168.7, 168.4, 165.6, 164.5, 139.3, 138.8, 138.4, 138.1, 132.0, 131.5, 129.4, 129.2, 128.5, 128.5, 128.4, 128.2, 126.4, 126.0, 123.1, 122.8, 98.4, 96.8, 83.3, 83.2, 82.1, 82.0, 77.5, 77.1, 76.4, 74.9, 73.4, 72.6, 72.0, 69.8, 69.6, 69.3, 68.9, 63.9, 57.2, 57.0, 56.9, 56.8, 56.7, 56.7, 56.3, 56.0, 55.8, 54.7, 54.5, 52.4, 50.5, 49.8, 48.4, 48.1, 43.6, 43.3, 43.0, 39.9, 39.5, 38.9, 36.2, 36.1, 35.2, 34.5, 34.3, 33.4, 33.3, 33.2, 32.7, 32.4, 32.3, 30.5, 29.8, 27.3, 26.0, 26.0, 25.8, 25.1, 24.3, 24.2, 23.9, 20.8, 20.6, 20.2, 19.3, 15.9, 15.7, 15.5, 14.0, 13.8, 11.4, 9.5, 9.2; mass spectrum (FAB), 1005 $[M + K]^+$. Anal. Calcd for $C_{54}H_{82}N_2O_{13}$: C, 67.05; H, 8.54; N, 2.89. Found: C, 67.21; H, 8.37; N, 2.93.

Cyclohexylamine−(32-Ascomycinyloxyacetic acid) amide (16). Cyclohexylamine was coupled to AOAA (**4**) on a 0.54 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (60 mL) followed by 2:1 hexane:acetone (200 mL) and then 1:1 hexane:acetone (100 mL). Fractions containing the desired product were pooled and concentrated in vacuo to give 0.45 g of white foam which was further purified by HPLC eluting with 7:4 hexane:acetone to provide **16** (0.44 g, 86% yield): IR (KBr) ν 3425, 2920, 2850 (sh), 2860 (sh), 2850, 2820, 1740, 1700, 1643 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 7.66 (d, 0.33H, J = 9.0 Hz), 7.63 (d, 0.67H, J = 9.0 Hz), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 14.0 Hz), 4.25 (s,

1H), 4.09 (d, 0.67H, J = 16.0 Hz), 4.09 (d, 0.33H, J = 16.0 Hz), 3.98−3.85 (mult, 2.33H), 3.83−3.71 (comp, 1.33H), 3.68 (d, 0.67H, J = 10.0 Hz), 3.59 (mult, 1H), 3.52−2.97 (comp, 4.33H), 3.45 (s, 3H), 3.40 (s, 2H), 3.39 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 36H), 1.67 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 169.6, 169.0, 168.7, 165.8, 164.7, 139.7, 138.7, 132.7, 132.2, 129.2, 123.3, 123.1, 98.7, 97.1, 82.1, 77.8, 77.2, 76.6, 75.2, 73.7, 73.6, 72.9, 72.3, 70.0, 69.0, 67.9, 67.9, 57.5, 57.0, 56.7, 56.6, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 47.7, 43.9, 43.5, 43.2, 40.4, 39.8, 39.2, 35.9, 35.8, 35.5, 34.5, 33.6, 33.0, 32.7, 32.6, 30.4, 29.2, 27.5, 26.3, 26.2, 26.0, 25.6, 24.5, 24.5, 24.2, 21.1, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.7, 14.2, 14.1, 11.7, 9.8, 9.5; mass spectrum (FAB), 969 $[M + K]^+$, 931 $[M + H]^+$. Anal. Calcd for $C_{51}H_{82}N_2O_{13}$: C, 65.78; H, 8.88; N, 3.01. Found: C, 65.60; H, 8.70; N, 2.93.

2-Aminopyridine−(32-Ascomycinyloxyacetic acid) amide (17). 2-Aminopyridine was coupled to AOAA (**4**) on a 0.94 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (100 mL) followed by 2:1 hexane:acetone (250 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 0.42 g of yellow foam which was further purified by HPLC eluting with 2:1 hexane:acetone to afford **17** (0.15 g, 17% yield): IR (KBr) ν 3425, 3398 (sh), 3250 (sh), 2960 (sh), 2938, 2850, 2820, 1740, 1700, 1643, 1595(sh) cm^{-1}; ^{1}H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 10.04 (s, 1H), 8.33 (d, 1H, J = 3.5 Hz), 8.26 (d, 1H, J = 9.5 Hz), 7.70 (ddd, 1H, J = 7.5, 7.5, 2.0 Hz), 7.04 (ddd, 1H, J = 7.5, 7.5, 5.5 Hz), 5.35 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.87 (s, 1H), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.29 (d, 0.67H, J = 16.0 Hz), 4.28 (d, 0.33H, J = 16.0 Hz), 4.24 (br s, 1H), 4.05 (d, 1H, J = 16.0 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.58 (s, 3H), 3.57 (mult, 1H), 3.50−3.09 (comp, 4.33H), 3.39 (s, 3H), 3.38 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.65 (s, 2H), 1.64 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 169.9, 169.0, 168.7, 165.8, 164.7, 151.4, 148.2, 139.7, 138.7, 138.0, 132.7, 132.2, 129.2, 123.4, 123.1, 119.7, 114.1, 98.7, 97.1, 83.4, 82.2, 77.8, 77.1, 76.6, 75.2, 73.7, 73.6, 72.9, 72.2, 70.0, 69.2, 69.1, 57.5, 57.0, 56.9, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.1, 40.4, 39.8, 39.2, 35.9, 35.7, 34.6, 34.5, 33.7, 32.9, 32.7, 32.6, 30.4, 29.7, 27.5, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.6, 14.3, 14.2, 11.7, 9.8, 9.5; mass spectrum (FAB), 964 $[M + K]^+$. Anal. Calcd for $C_{50}H_{75}N_3O_{13}$: C, 64.84; H, 8.16; N, 4.54. Found: C, 65.10; H, 8.13; N, 4.78.

3-Aminopyridine−(32-Ascomycinyloxyacetic acid) amide (18). 3-Aminopyridine was coupled to AOAA (**4**) on a 0.94 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (60 mL) followed by 1:1 hexane:acetone (150 mL) and then 1:2 hexane:acetone (100 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 0.55 g of yellow foam which was further purified by HPLC eluting with 1:1 hexane:acetone to provide **18** (0.42 g, 48% yield): IR (KBr) ν 3425, 3310 (sh), 2960 (sh), 2930, 2850, 2820, 1740, 1700, 1643, 1595 cm^{-1}; ^{1}H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.82 (s, 1H), 8.62 (m, 1H), 8.37 (dd, 1H, J = 4.5, 2.0 Hz), 8.29 (comp dd, 1H, J = 9.0, 9.0 Hz), 7.29 (m, 1H), 5.39 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.19−4.97 (mult, 2H), 4.89 (s, 1H), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.28 (d, 0.67H, J = 16.0 Hz), 4.27 (d, 0.33H, J = 16.0 Hz), 4.25 (br s, 1H), 4.03 (d, 0.67H, J = 16.0 Hz), 4.02 (d, 0.33H, J = 16.0 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.58 (s, 3H), 3.57 (mult, 1H), 3.50−3.09 (comp, 4.33H), 3.39 (s, 3H), 3.38 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.65 (s, 2H), 1.64 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 170.3, 169.0, 168.7, 165.8, 164.7, 150.7 144.8, 139.7, 138.8, 133.0, 132.5, 129.0, 128.9, 123.3, 123.1, 113.7, 98.7, 97.1, 82.3, 82.2, 77.8, 77.1, 76.6, 75.2, 73.7, 73.6, 72.9, 72.2, 70.0, 68.9, 67.9, 57.5, 57.0, 56.9, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.1, 40.4, 39.8, 39.2, 35.7, 35.6, 35.5, 34.6, 34.5, 33.7, 32.9, 32.7, 32.6, 30.4, 29.0, 27.5, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.6, 14.3, 14.2, 11.7, 9.8, 9.5; mass spectrum (FAB), 964 $[M + K]^+$. Anal. Calcd for $C_{50}H_{75}N_3O_{13}$: C, 64.84; H, 8.16; N, 4.54. Found: C, 64.98; H, 8.22; N, 4.36.

4-Aminopyridine−(32-Ascomycinyloxyacetic acid) amide (19). 4-Aminopyridine was coupled to AOAA (**4**) on a 0.94 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (60 mL) followed by 1:1 hexane:acetone (150 mL) and then 1:2 hexane:acetone (150 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 0.53 g of yellow foam which was further purified by HPLC eluting with 1:1 hexane:acetone to provide **19** (0.21 g, 25% yield): IR (KBr) ν 3425, 3310 (sh), 2960 (sh), 2930, 2850, 2820, 1740, 1700, 1643, 1595 cm^{-1}; ^{1}H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.84 (s, 0.33H), 9.82 (s, 0.67H), 8.53 (d, 2H, J = 6.0 Hz), 7.54 (d, 2H, J = 5.5 Hz), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.87 (s, 1H), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.28 (d, 0.67H, J = 16.0 Hz), 4.27 (d, 0.33H, J = 16.0 Hz), 4.25 (br s, 1H), 4.03 (d, 0.67H, J = 16.0 Hz), 4.02 (d, 0.33H, J = 16.0 Hz), 4.00−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.62−3.08 (comp, 5.33H), 3.58 (s, 3H), 3.39 (s, 3H), 3.35 (s, 1H), 3.31 (s, 2H), 3.01 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.40−0.74 (comp, 26H), 1.70 (s, 1H), 1.67 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 170.3, 169.0, 168.7, 165.8, 164.7, 150.7 144.8, 139.7, 138.8, 133.0, 132.5, 129.0, 128.9, 123.3, 123.1, 113.7, 98.7, 97.1, 82.3, 82.2, 77.8, 77.1, 76.6, 75.2, 73.7, 73.6, 72.9, 72.2, 70.0, 68.9, 67.9, 57.5, 57.0, 56.9, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.1, 40.4, 39.8, 39.2, 35.7, 35.6, 35.5, 34.6, 34.5, 33.7, 32.9, 32.7, 32.6, 30.4, 29.0, 27.5, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.6, 14.3, 14.2, 11.7, 9.8, 9.5; mass spectrum (FAB), 964 $[M + K]^+$. Anal. Calcd for $C_{50}H_{75}N_3O_{13}$: C, 64.84; H, 8.16; N, 4.54. Found: C, 65.15; H, 8.30; N, 4.27.

4-Aminophenol−(32-Ascomycinyloxyacetic acid)amide (20). 4-Aminophenol was coupled to AOAA (**4**) on a 0.94 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (150 mL) followed by 3:1 hexane:acetone (100 mL) and then 2:1 hexane:acetone (200 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 0.58 g tan foam which was further purified by HPLC eluting with 3:2 hexane:acetone to provide **20** (0.29 g, 33% yield): IR (KBr) ν 3425, 3275 2960 (sh), 2938, 2850, 2820, 1740, 1700, 1643 cm^{-1}; ^{1}H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.51 (s, 0.33H), 9.50 (s, 0.67H), 7.48 (d, 2H, J = 9.5 Hz), 6.81 (d, 2H, J = 9.5 Hz), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.94 (br s, 2H), 4.87 (d, 1H, J = 2.5 Hz), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.23 (d, 1H, J = 15.5 Hz), 4.01 (d, 0.67H, J = 17.0 Hz), 4.00 (d, 0.33H, J = 17.0 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5

Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.50−3.09 (comp, 4.33H), 3.54 (s, 3H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.69 (s, 1H), 1.67 (s, 2H), 1.64 (s, 1H), 1.56 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.6, 213.5, 196.1, 192.6, 169.0, 169.0, 168.7, 165.8, 164.7, 152.9, 139.6, 138.8, 132.8, 132.2, 130.7, 129.2, 129.1, 123.3, 123.1, 121.7, 115.6, 98.7, 97.1, 82.2, 82.1, 77.8, 77.3, 76.6, 75.3, 73.7, 73.6, 72.9, 72.3, 70.0, 69.0, 68.0, 57.5, 57.0, 56.8, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.3, 40.4, 39.8, 39.2, 35.7, 35.6, 35.5, 34.6, 34.5, 33.7, 33.0, 32.7, 32.6, 31.6, 30.5, 29.3, 29.1, 27.5, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 22.6, 21.1, 20.8, 20.4, 19.5, 16.2, 16.0, 15.8, 15.7, 14.2, 14.1, 11.7, 9.8, 9.5; mass spectrum (FAB), 979 $[M + K]^+$. Anal. Calcd for $C_{51}H_{76}N_2O_{14}$: C, 65.08; H, 8.13; N, 2.97. Found: C, 64.80; H, 8.10; N, 2.89.

4-Morpholinoaniline−(32-Ascomycinyloxyacetic acid)-amide (21). To a stirring solution of AOAA (**4**) (0.75 g, 0.88 mmol) in CH_2Cl_2 (10 mL) at 0 °C were added DMAP (0.02 g, 0.18 mmol) and 4-morpholinoaniline (0.31 g, 1.76 mmol) followed by 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDAC) (0.20 g, 1.1 mmol). The reaction was stirred overnight at room temperature then partitioned between EtOAc (150 mL) and 1 N HCl (150 mL). The organic layer was washed with an additional portion of 1 N HCl (150 mL), saturated $NaHCO_3$ solution (2 × 150 mL), and then brine (150 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and then passed through a pad of silica gel (20 g) eluting with CH_2Cl_2 (150 mL), 2:1 hexane:acetone (100 mL), 6:4 hexane:acetone (100 mL), and then finally 1:1 hexane:acetone (100 mL), collecting 20 mL fractions throughout. The eluate containing the desired product was pooled and concentrated in vacuo to give 0.64 g of yellow foam. This material was further purified by HPLC eluting with 4:5 CH_2Cl_2:CH_3CN to provide **21** (0.43 g, 48% yield): IR (KBr) ν 3425, 3310 (sh), 2960 (sh), 2930, 2850, 2820, 1740, 1700, 1670 (sh), 1643, 1595 cm^{-1}; ^{1}H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.49 (br, 1H), 7.53 (d, 2H, J = 9.5 Hz), 6.91 (br, 2H), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.86 (d, 1H, J = 1.0 Hz), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.23 (d, 1H, J = 17.0 Hz), 4.21 (s, 1H), 4.01 (d, 0.67H, J = 17.5 Hz), 4.00 (d, 0.33H, J = 17.5 Hz), 3.98−3.82 (mult, 5.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.59 (mult, 1H), 3.60−3.07 (comp, 8.33H), 3.55 (s, 3H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.69 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 169.0, 168.7, 165.8, 164.7, 148.1, 139.7, 138.7, 132.9, 132.3, 130.9, 129.1, 129.0, 123.3, 123.1, 120.9, 116.3, 98.7, 97.1, 82.2, 82.2, 77.8, 77.1, 76.6, 75.2, 73.7, 73.6, 72.9, 72.2, 70.0, 69.0, 68.0, 66.9, 57.5, 57.0, 56.8, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 49.9, 48.7, 48.5, 43.9, 43.5, 43.1, 40.3, 39.8, 39.2, 35.7, 35.6, 35.5, 34.6, 34.5, 33.6, 32.9, 32.7, 32.6, 30.4, 29.1, 27.5, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.7, 14.3, 14.2, 11.7, 9.8, 9.5; mass spectrum (FAB), 1048 $[M + K]^+$, 1009 $[M]^+$. Anal. Calcd for $C_{55}H_{83}N_3O_{14}$: C, 65.39; H, 8.28; N, 4.16. Found: C, 65.67; H, 8.32; N, 3.96.

3,4-Dichloroaniline−(32-Ascomycinyloxyacetic acid)-amide (22). Ethylmagnesium bromide (1 M in THF, 9.6 mL) was cooled to 0 °C, to which was added in a dropwise fashion a solution of 3,4-dichloroaniline (1.6 g, 9.6 mmol) in dry THF (1.5 mL). The mixture was stirred for 15 min and cooled to −78 °C, whereupon a solution of AOAA benzyl ester (**3b**) (0.8 g, 0.8 mmol) in dry THF (2 mL) was added dropwise. After 3 h the mixture was added dropwise to a stirring biphasic solution of 1 N H_3PO_4 (75 mL) and EtOAc (75 mL). The organic layer was washed with saturated $NaHCO_3$ solution (75 mL) and brine (75 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and then passed through a pad of silica gel (50 g) eluting with CH_2Cl_2 (200 mL), 6:1 CH_2Cl_2:CH_3CN (200 mL), 4:1 CH_2Cl_2:CH_3CN (100 mL), and then 1:1 CH_2Cl_2:CH_3CN (100 mL), collecting 20 mL fractions throughout. Eluate containing the desired product was concentrated in vacuo to give 0.5 g of yellowish-brown foam, which was further purified by HPLC eluting with 4:1 CH_2Cl_2:CH_3CN to provide **22** (0.3 g, 42% yield): IR (KBr) ν 3440, 3280, 3100, 2960 (sh), 2920, 2865, 2820, 1740, 1700, 1640, 1580, 1520 cm^{-1}; ^{1}H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.74 (s, 0.33H), 9.73 (s, 0.67H), 7.79 (dd, 1H, J = 3.0, 3.0 Hz), 7.49 (comp, 1H), 7.39 (d, 1H, J = 9.5 Hz), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.88 (d, 1H, J = 2.5 Hz), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.25 (s, 1H), 4.24 (d, 0.67H, J = 16.5 Hz), 4.23 (d, 0.33H, J = 16.0 Hz), 3.99 (d, 0.67H, J = 17.0 Hz), 3.98 (d, 0.33H, J = 17.5 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.59 (mult, 1H), 3.56 (s, 3H), 3.50−3.12 (comp, 4.33H), 3.40 (s, 2H), 3.39 (s, 1H), 3.35 (s, 1H), 3.30 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 169.4, 169.0, 168.7, 165.8, 164.7, 139.7, 138.8, 137.5, 133.0, 132.6, 132.4, 130.5, 128.9, 128.8, 123.3, 123.1, 121.4, 119.0, 98.7, 97.1, 82.3, 82.2, 77.8, 77.1, 76.6, 75.2, 73.7, 73.6, 72.9, 72.3, 70.0, 69.0, 67.8, 57.5, 57.0, 56.8, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.1, 40.4, 39.8, 39.2, 35.6, 35.5, 34.6, 34.5, 33.7, 33.0, 32.7, 32.6, 30.4, 29.0, 27.5, 26.4, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.9, 15.7, 14.2, 14.1, 11.7, 9.8, 9.5; mass spectrum (FAB), 1031 $[M + K]^+$. Anal. Calcd for $C_{51}H_{74}N_2O_{13}Cl_2$: C, 61.62; H, 7.50; N, 2.81. Found: C, 62.02; H, 7.54; N, 2.84.

4-Chloroaniline−(32-Ascomycinyloxyacetic acid) amide (23). 4-Chloroaniline was coupled to AOAA (**4**) on a 0.59 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (100 mL) followed by 1:1 hexane:acetone (250 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 0.49 g of yellow foam which was further purified by HPLC eluting with 5:2 hexane:acetone to provide **23** (0.42 g, 74% yield): IR (KBr) ν 3440, 3300 (sh), 3120, 3060, 2960 (sh), 2930, 2865, 2820, 1740, 1695, 1650, 1595 cm^{-1}; ^{1}H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.66 (s, 0.33H), 9.65 (s, 0.67H), 7.57 (dd, 2H, J = 10.0, 3.0 Hz), 7.30 (dd, 2H, J = 9.5, 3.0 Hz), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.88 (d, 1H, J = 2.5 Hz), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.26 (s, 1H), 4.24 (d, 0.67H, J = 16.5 Hz), 4.23 (d, 0.33H, J = 16.0 Hz), 4.00 (d, 0.67H, J = 17.0 Hz), 3.99 (d, 0.33H, J = 17.5 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.59 (mult, 1H), 3.50−3.12 (comp, 4.33H), 3.55 (s, 3H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.30 (s, 2H), 3.01 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 169.2, 169.0, 168.7, 165.8, 164.7, 139.7, 138.8, 136.6, 133.0, 132.4, 129.1, 128.9, 123.3, 123.1, 121.0, 98.7, 97.1, 82.2, 82.2, 77.8, 77.1, 76.5, 75.2, 73.7, 73.6, 72.9, 72.3, 70.0, 69.0, 67.9, 57.5, 57.0, 56.8, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.1, 40.4, 39.8, 39.2, 35.6, 35.6, 35.5, 34.6, 34.5, 33.7, 33.0, 32.7, 32.6, 30.4, 29.0, 27.5, 26.4, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.9, 15.7, 14.2, 14.1, 11.7, 9.8, 9.5; mass spectrum

(FAB), 997 $[M + K]^+$. Anal. Calcd for $C_{51}H_{75}N_2O_{13}Cl$: C, 63.83; H, 7.87; N, 2.91. Found: C, 63.72; H, 7.75; N, 2.91.

4-Methoxyaniline−(32-Ascomycinyloxyacetic acid) amide (24). 4-Methoxyaniline was coupled to AOAA (**4**) on a 0.59 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (150 mL) followed by 2:1 hexane:acetone (300 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 0.46 g of yellow foam which was further purified by HPLC eluting with 2:1 hexane:acetone to afford **24** (0.27 g, 48% yield): IR (KBr) ν 3440, 3305 (sh), 2960 (sh), 2930, 2865, 2820, 1740, 1695, 1650, 1600 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.51 (s, 0.33H), 9.50 (s, 0.33H), 5.34 (d, 0.67H, $J = 3.0$ Hz), 5.22 (d, 0.33H, $J = 3.0$ Hz), 5.09−4.97 (mult, 2H), 4.88 (d, 1H, $J = 2.5$ Hz), 4.62 (d, 1H, $J = 6.0$ Hz), 4.45 (br d, 0.67H, $J = 15.0$ Hz), 4.25 (s, 1H), 4.23 (d, 0.67H, $J = 16.5$ Hz), 4.22 (d, 0.33H, $J = 16.0$ Hz), 4.00 (d, 0.67H, $J = 17.0$ Hz), 3.99 (d, 0.33H, $J = 17.5$ Hz), 3.98−3.87 (mult, 1.33H), 3.81 (s, 3H), 3.75 (br d, 0.33H, $J = 14.5$ Hz), 3.68 (d, 0.67H, $J = 10.0$ Hz), 3.59 (mult, 1H), 3.55 (s, 3H), 3.50−3.12 (comp, 4.33H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, $J = 17.5$, 4.5 Hz), 2.74 (dd, 0.33H, $J = 17.5$, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, $J = 6.0$ Hz), 0.98 (d, 1H, $J = 6.0$ Hz), 0.95 (d, 2H, $J = 7.5$ Hz), 0.92 (d, 1H, $J = 7.5$ Hz), 0.89 (d, 2H, $J = 7.5$ Hz), 0.88 (dd, 3H, $J = 7.5$, 7.5 Hz), 0.85 (d, 1H, $J = 7.5$ Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 169.0, 168.7, 165.8, 164.7, 156.3, 139.7, 138.7, 132.9, 132.3, 131.2, 129.0, 129.0, 123.3, 123.0, 121.3, 114.1, 98.7, 97.1, 82.2, 82.2, 77.8, 77.1, 76.6, 75.2, 73.7, 73.6, 72.9, 72.2, 70.0, 69.0, 68.0, 57.5, 57.0, 56.9, 56.6, 56.3, 56.0, 55.5, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.1, 40.3, 39.8, 39.2, 35.7, 35.6, 35.5, 34.6, 34.5, 33.6, 32.9, 32.7, 32.6, 30.4, 29.1, 27.5, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.7, 14.2, 14.1, 11.7, 9.8, 9.4; mass spectrum (FAB), 993 $[M + K]^+$. Anal. Calcd for $C_{52}H_{78}N_2O_{14}$: C, 65.38; H, 8.23; N, 2.93. Found: C, 65.28; H, 8.13; N, 2.92.

3-Aminobenzotrifluoride−(32-Ascomycinyloxyacetic acid)amide (25). 3-Aminobenzotrifluoride was coupled to AOAA (**4**) on a 0.59 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (50 mL) followed by 4:1 hexane:acetone (250 mL), 3:1 hexane: acetone (100 mL), and then finally 1:1 hexane:acetone (150 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 0.57 g of yellow foam which was further purified by HPLC eluting with 4:1 CH_2Cl_2: CH_3CN to provide **25** (0.39 g, 67% yield): IR (KBr) ν 3425, 3290, 2920, 2860, 2820, 1740, 1700, 1645, 1620 (sh), 1601, 1550 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 10.84 (m, 1H), 7.94 (dd, 1H, $J = 9.0$, 9.0 Hz), 7.80 (comp, 1H), 7.46 (dd, 1H, $J = 9.0$, 9.0 Hz), 7.37 (d, 1H, $J = 9.0$), 5.34 (d, 0.67H, $J = 3.0$ Hz), 5.22 (d, 0.33H, $J = 3.0$ Hz), 5.09−4.97 (mult, 2H), 4.62 (d, 1H, $J = 5.0$ Hz), 4.45 (br d, 0.67H, $J = 15.0$ Hz), 4.25 (s, 1H), 4.26 (d, 0.67H, $J = 17.5$ Hz), 4.25 (d, 0.33H, $J = 17.5$ Hz), 4.02 (d, 0.67H, $J = 17.5$ Hz), 4.01 (d, 0.33H, $J = 17.5$ Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, $J = 14.5$ Hz), 3.68 (d, 0.67H, $J = 10.0$ Hz), 3.61−3.06 (comp, 5.33H), 3.57 (s, 3H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, $J = 15.5$, 3.0 Hz), 2.74 (dd, 0.33H, $J = 15.5$, 3.0 Hz), 2.37−0.74 (comp, 26H), 1.67 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, $J = 6.0$ Hz), 0.98 (d, 1H, $J = 6.0$ Hz), 0.95 (d, 2H, $J = 7.5$ Hz), 0.92 (d, 1H, $J = 7.5$ Hz), 0.89 (d, 2H, $J = 7.5$ Hz), 0.88 (dd, 3H, $J = 7.5$, 7.5 Hz), 0.85 (d, 1H, $J = 7.5$ Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 169.5, 169.0, 168.7, 165.8, 164.7, 139.7, 138.8, 138.5, 133.0, 132.4, 131.7, 131.4, 131.1, 130.8, 129.5, 128.9, 128.9, 123.3, 123.1, 122.8, 120.6, 116.4, 98.7, 97.1, 82.3, 82.1, 77.8, 77.1, 76.6, 75.2, 73.7, 73.6, 72.9, 72.3, 70.0, 69.0, 67.8, 57.5, 57.0, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.1, 40.3, 39.8, 39.2, 35.6, 35.5, 34.6, 34.5, 33.7, 32.9, 32.7, 32.6, 30.4, 29.0, 27.5, 26.4, 26.2, 26.0, 24.6, 24.6, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.9, 15.7, 14.3, 14.2, 11.7, 9.8, 9.5; mass spectrum (FAB), 1031 $[M + K]^+$. Anal. Calcd for $C_{52}H_{75}N_2O_{13}F_3$: C, 62.89; H, 7.61; N, 2.82. Found: C, 62.49; H, 7.31; N, 2.78.

4-Aminobenzotrifluoride−(32-Ascomycinyloxyacetic acid)amide (26). 4-Aminobenzotrifluoride was coupled to AOAA (**4**) on a 0.74 mmol scale according to the method used for the synthesis of **21**. The silica pad (40 g, 70−230 mesh) was eluted with CH_2Cl_2 (300 mL) followed by 2:1 hexane: acetone (300 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 0.77 g of pale green foam which was further purified by HPLC eluting with 5:2 hexane:acetone to give 0.66 g of a semipure material. Further purification by HPLC eluting with 3:1 CH_2Cl_2: CH_3CN produced pure **26** (0.50 g, 68% yield): IR (KBr) ν 3425, 3280 (sh), 2960 (sh), 2920, 2850, 2820, 1740, 1700, 1643, 1620 (sh), 1600 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.83 (s, 0.33H), 9.81 (s, 0.67H), 7.74 (d, 2H, $J = 8.5$ Hz), 7.59 (d, 2H, $J = 8.5$ Hz), 5.34 (d, 0.67H, $J = 3.0$ Hz), 5.22 (d, 0.33H, $J = 3.0$ Hz), 5.09−4.97 (mult, 2H), 4.88 (d, 1H, $J = 2.5$ Hz), 4.62 (d, 1H, $J = 6.0$ Hz), 4.45 (br d, 0.67H, $J = 15.0$ Hz), 4.27 (d, 0.67H, $J = 16.5$ Hz), 4.26 (d, 0.33H, $J = 16.0$ Hz), 4.24 (br s, 1H), 4.02 (d, 0.67H, $J = 17.0$ Hz), 4.01 (d, 0.33H, $J = 17.5$ Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, $J = 14.5$ Hz), 3.68 (d, 0.67H, $J = 10.0$ Hz), 3.59 (mult, 1H), 3.54−3.08 (comp, 4.33H), 3.57 (s, 3H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.01 (comp, 0.67H), 2.79 (dd, 0.67H, $J = 16.0$, 4.0 Hz), 2.74 (dd, 0.33H, $J = 16.0$, 4.0 Hz), 2.37−0.74 (comp, 26H), 1.70 (s, 1H), 1.67 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, $J = 6.0$ Hz), 0.98 (d, 1H, $J = 6.0$ Hz), 0.95 (d, 2H, $J = 7.5$ Hz), 0.92 (d, 1H, $J = 7.5$ Hz), 0.89 (d, 2H, $J = 7.5$ Hz), 0.88 (dd, 3H, $J = 7.5$, 7.5 Hz), 0.85 (d, 1H, $J = 7.5$ Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 169.6, 169.0, 168.7, 165.8, 164.7, 141.0, 139.7, 138.8, 133.0, 132.4, 128.9, 128.9, 126.6, 126.4, 126.2, 126.2, 125.9, 125.6, 125.2, 123.3, 123.1, 119.4, 98.7, 97.1, 82.3, 82.2, 77.9, 77.2, 76.6, 75.2, 73.7, 73.6, 72.9, 72.3, 70.0, 68.9, 67.9, 57.6, 57.0, 56.8, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.1, 40.4, 39.8, 39.2, 35.6, 35.5, 35.5, 34.6, 34.5, 33.7, 33.0, 32.7, 32.6, 30.4, 29.0, 27.5, 26.4, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.9, 15.7, 14.3, 14.2, 11.7, 9.8, 9.5; mass spectrum (FAB), 1031 $[M + K]^+$. Anal. Calcd for $C_{52}H_{75}N_2O_{13}F_3$: C, 62.89; H, 7.61; N, 2.82. Found: C, 63.29; H, 7.69; N, 2.82.

3-Fluoroaniline−(32-Ascomycinyloxyacetic acid) amide (27). 3-Fluoroaniline was coupled to AOAA (**4**) on a 0.59 mmol scale according to the method used for the synthesis of **9**. The silica pad was eluted with CH_2Cl_2 (60 mL) followed by 3:1 hexane:acetone (250 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 0.55 g of white foam which was further purified by HPLC eluting with 5:2 hexane:acetone to provide **27** (0.47 g, 85% yield): IR (KBr) ν 3420, 3290, 2960 (sh), 2940, 2880, 2820, 1740, 1695 (sh), 1690, 1645, 1610, 1540 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.70 (s, 0.33H), 9.69 (s, 0.67H), 7.52 (ddd, 1H, $J = 11.0$, 1.0, 1.0 Hz), 7.29 (comp, 2H), 6.82 (dddd, 1H, $J = 8.0$, 8.0, 1.0, 1.0 Hz), 5.34 (d, 0.67H, $J = 3.0$ Hz), 5.22 (d, 0.33H, $J = 3.0$ Hz), 5.09−4.97 (mult, 2H), 4.88 (d, 1H, $J = 2.5$ Hz), 4.62 (d, 1H, $J = 5.0$ Hz), 4.45 (br d, 0.67H, $J = 12.5$ Hz), 4.25 (s, 1H), 4.25 (d, 0.67H, $J = 16.0$ Hz), 4.24 (d, 0.33H, $J = 16.0$ Hz), 4.02 (d, 0.67H, $J = 16.0$ Hz), 4.01 (d, 0.33H, $J = 16.0$ Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, $J = 13.5$ Hz), 3.68 (d, 0.67H, $J = 10.0$ Hz), 3.59 (mult, 1H), 3.57 (s, 3H), 3.54−3.06 (comp, 4.33H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.02 (comp, 0.67H), 2.79 (dd, 0.67H, $J = 15.5$, 3.5 Hz), 2.74 (dd, 0.33H, $J = 15.5$, 3.5 Hz), 2.37−0.74 (comp, 26H), 1.70 (s, 1H), 1.67 (s, 2H), 1.64 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, $J = 6.0$ Hz), 0.98 (d, 1H, $J = 6.0$ Hz), 0.95 (d, 2H, $J = 7.5$ Hz), 0.92 (d, 1H, $J = 7.5$ Hz), 0.89 (d, 2H, $J = 7.5$ Hz), 0.88 (dd, 3H, $J = 7.5$, 7.5 Hz), 0.85 (d, 1H, $J = 7.5$ Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 169.3, 169.0, 168.7, 165.8, 164.7, 164.0, 162.0, 139.7, 139.5, 139.4, 138.8, 132.9, 132.3, 130.0, 129.9, 128.9, 128.9, 123.3, 123.0, 115.0, 110.9, 110.8, 107.3, 107.1, 98.7, 97.0, 82.2, 82.2, 77.8, 77.1, 76.6, 75.2, 73.7, 73.6, 72.9, 72.2, 70.0, 69.0, 67.8, 57.5, 57.0, 56.8, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7,

48.5, 43.9, 43.4, 43.1, 40.3, 39.8, 39.2, 35.6, 35.6, 35.5, 34.6, 34.5, 33.6, 32.9, 32.7, 32.6, 30.4, 29.0, 27.5, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.6, 14.3, 14.2, 11.7, 9.8, 9.4; mass spectrum (FAB), 981 $[M + K]^+$. Anal. Calcd for $C_{51}H_{75}N_2O_{13}F$: C, 64.95; H, 8.02; N, 2.97. Found: C, 64.82; H, 8.05; N, 2.91.

4-Fluoroaniline−(32-Ascomycinyloxyacetic acid) amide (28). 4-Fluoroaniline was coupled to AOAA (**4**) on a 0.88 mmol scale according to the method used for the synthesis of **21**. The silica pad (40 g, 70−230 mesh) was eluted with CH_2Cl_2 (250 mL) followed by 2:1 hexane:acetone (300 mL). Fractions containing the desired product were combined and concentrated in vacuo to give 0.57 g of yellow foam which was further purified by HPLC eluting with 2:1 hexane:acetone to give 0.41 g of a semipure material. Further purification by HPLC eluting with 5:2 CH_2Cl_2:CH_3CN provided **28** (0.27 g, 32% yield): IR (KBr) ν 3425, 3310 (sh), 2920, 2850, 2820, 1740, 1695, 1643, 1610 (sh), cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.63 (s, 0.33H), 9.62 (s, 0.67H), 7.58 (m, 2H), 7.03 (m, 2H), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.87 (d, 1H, J = 2.5 Hz), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.25 (br s, 1H), 4.24 (d, 0.67H, J = 16.0 Hz), 4.23 (d, 0.33H, J = 16.0 Hz), 4.00 (d, 0.67H, J = 16.0 Hz), 3.99 (d, 0.33H, J = 16.0 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.61−3.08 (comp, 4.33H), 3.59 (mult, 1H), 3.55 (s, 3H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.31 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 1.69 (s, 1H), 1.67 (s, 2H), 1.64 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.5, 213.4, 196.1, 192.5, 169.0, 169.0, 168.7, 165.8, 164.7, 159.3 (d, ^1J(CF) = 237.5 Hz) 139.7, 138.8, 134.0, 132.9, 132.3, 129.0, 128.9, 123.3, 123.0, 121.4 (d, ^3J(CF) = 12.5 Hz), 115.5 (d, ^2J(CF) = 25.0 Hz), 98.7, 97.1, 82.2, 77.8, 77.1, 76.6, 75.2, 73.7, 73.6, 72.9, 72.2, 70.0, 68.9, 67.9, 57.5, 57.0, 56.9, 56.6, 56.3, 56.0, 55.0, 54.7, 52.7, 48.7, 48.5, 43.9, 43.5, 43.1, 40.4, 39.8, 39.2, 35.7, 35.6, 35.5, 34.6, 34.5, 33.7, 32.9, 32.7, 32.6, 30.4, 29.0, 27.5, 26.3, 26.2, 26.0, 24.6, 24.5, 24.2, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0, 15.8, 15.6, 14.3, 14.2, 11.7, 9.8, 9.5; mass spectrum (FAB), 981 $[M + K]^+$. Anal. Calcd for $C_{51}H_{75}N_2O_{13}F$: C, 64.95; H, 8.02; N, 2.97. Found: C, 65.25; H, 8.21; N, 2.78.

4-Methylaniline−(32-Ascomycinyloxyacetic acid) amide (29). A solution of 4-methylaniline (1.0 g, 9.6 mmol) in dry THF (1 mL) was added dropwise to ethylmagnesium bromide (1 M in THF, 9.6 mL) at 0 °C. The mixture was stirred for 15 min and cooled to −78 °C, whereupon a solution of **3b** (0.8 g, 0.8 mmol) in dry THF (2 mL) was added dropwise. After 1 h the mixture was added dropwise to a stirring biphasic solution of 1 N H_3PO_4 (75 mL) and EtOAc (75 mL). The organic layer was washed with saturated $NaHCO_3$ solution (75 mL) and brine (75 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and was then passed through a pad of silica gel (20 g) eluting with CH_2Cl_2 (100 mL) and then 1:1 hexane:acetone (200 mL). The filtrate containing the desired product was concentrated in vacuo to give 0.87 g of dark yellow foam. Further purification by HPLC eluting with 2:1 hexane:acetone provided 537 mg of semipure material. Final purification by HPLC eluting with 4:1 CH_2Cl_2:CH_3CN afforded pure **29** (0.39 g, 51% yield): IR (KBr) ν 3440, 3300, 2960 (sh), 2925, 2870, 2820, 1740, 1695, 1650 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers 2:1) 9.52 (s, 0.33H), 9.50 (s, 0.67H), 7.49 (dd, 2H, J = 9.5, 3.0 Hz), 7.14 (d, 2H, J = 9.0 Hz), 5.34 (d, 0.67H, J = 3.0 Hz), 5.22 (d, 0.33H, J = 3.0 Hz), 5.09−4.97 (mult, 2H), 4.85 (s, 1H), 4.62 (d, 1H, J = 6.0 Hz), 4.45 (br d, 0.67H, J = 15.0 Hz), 4.26 (s, 1H), 4.23 (d, 0.67H, J = 16.0 Hz), 4.22 (d, 0.33H, J = 16.0 Hz), 4.01 (d, 0.67H, J = 16.0 Hz), 4.00 (d, 0.33H, J = 17.5 Hz), 3.98−3.87 (mult, 1.33H), 3.75 (br d, 0.33H, J = 14.5 Hz), 3.68 (d, 0.67H, J = 10.0 Hz), 3.58 (mult, 1H), 3.50−3.12 (comp, 4.33H), 3.55 (s, 3H), 3.39 (s, 2H), 3.38 (s, 1H), 3.35 (s, 1H), 3.30 (s, 2H), 3.03 (comp, 0.67H), 2.79 (dd, 0.67H, J = 17.5, 4.5 Hz), 2.74 (dd, 0.33H, J = 17.5, 4.5 Hz), 2.37−0.74 (comp, 26H), 2.33 (s, 3H), 1.67 (s, 1H), 1.65 (s, 2H), 1.63 (s, 1H), 1.61 (s, 2H), 1.01 (d, 2H, J = 6.0 Hz), 0.98 (d, 1H, J = 6.0 Hz), 0.95 (d, 2H, J = 7.5 Hz), 0.92 (d, 1H, J = 7.5 Hz), 0.89 (d, 2H, J = 7.5 Hz), 0.88 (dd, 3H, J = 7.5, 7.5 Hz), 0.85 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz) δ (rotamers) 213.3, 213.2, 196.1, 192.6, 168.9, 168.8, 168.6, 165.7, 164.6, 139.5, 138.6, 135.3, 133.6, 132.7, 132.2, 129.3, 129.1, 129.0, 123.2, 123.0, 119.7, 98.6, 97.0, 82.1, 82.0, 77.7, 77.3, 76.5, 75.1, 73.6, 73.6, 72.8, 72.2, 69.9, 68.9, 67.9, 57.4, 56.9, 56.7, 56.5, 56.2, 55.9, 54.9, 54.6, 52.6, 48.6, 48.3, 43.8, 43.4, 43.2, 40.2, 39.7, 39.1, 35.6, 35.5, 35.4, 34.5, 34.4, 33.6, 32.9, 32.6, 32.5, 30.3, 29.0, 27.5, 26.2, 26.1, 26.0, 24.5, 24.4, 24.1, 21.1, 20.8, 20.3, 19.4, 16.1, 15.9, 15.7, 15.6, 14.2, 14.0, 11.6, 9.7, 9.4; mass spectrum (FAB), 977 $[M + K]^+$. Anal. Calcd for $C_{52}H_{78}N_2O_{13}\cdot H_2O$: C, 65.24; H, 8.42; N, 2.92. Found: C, 65.13; H, 8.11; N, 2.93.

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(21) Ascomycin (**1b**) was recrystallized from diethyl ether or acetonitrile and is then stored as a crystalline solid. This material is exceptionally difficult to redissolve in acetonitrile unless it is returned to a solid "glass-like" state by dissolution in CH_2Cl_2 followed by removal of the solvent in vacuo. Preventing rapid crystallization upon addition of the acetonitrile is made possible only by premixing **1b** with benzyl iodoacetate.

(22) Unless the Ag_2O is added in small portions at 0 °C over the indicated time period, a vigorous exothermic reaction ensues which results in immediate and complete decomposition of the ascomycin (**1b**). It is presumed that iodide is released under these uncontrolled conditions, and this then adds to the C-9 carbonyl, promoting the well-documented benzylic acid rearrangement.

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