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Selective Epimerization and Skeletal Resection in the Ascomycin Framework: A Study of the Biological Consequences of Lactam Rotamer Selection

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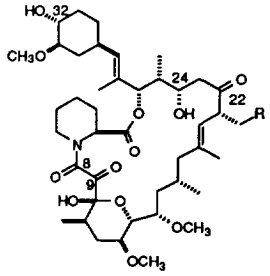
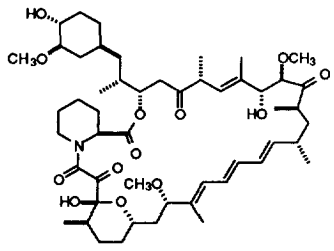
Abstract: Ascomycin (**1a**), a macrolactam antifungal antibiotic disclosed by Arai in 1962,[1] was found to display immunosuppressive activity more than 2 decades later by Okuhara and coworkers at Fujisawa.[2] Ascomycin (**1a**) and FK506 (**1b**) bind to a peptidyl-prolyl-isomerase, FKBP, a necessary but insufficient condition for drug activity. Both FK506 and ascomycin exist as a mixture of slowly interconverting *cis* and *trans* amide rotamers. It has also been shown that only the *trans* amide rotamer binds to FKBP. 24-epi-Ascomycin (**3**), 24-oxo-22-norascomyin (**9a**), and 22-norascomycin (**9b**), obtained by semisynthesis from ascomycin, exist as single rotamers on the NMR time scale. Their synthesis and the biological consequences of this observation are discussed.

Introduction

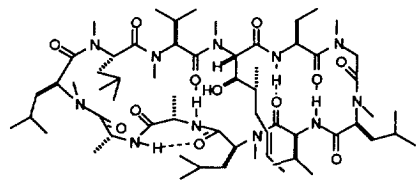
FK506 (**1b**) and rapamycin (RAP, **2**), exerting immunosuppressive effects in T-lymphocytes, bind to common peptidyl-prolyl-isomereases (PPIase's), in particular FKBP-12.[3] Interaction of FK506 and RAP with FKBP is necessary for inhibition of downstream T-cell proliferation, but is not a sufficient condition.[4] Cyclosporin A (CsA) binds to Cyclophilin (CyP), another PPIase, an event that likewise is necessary but alone inadequate for immunosuppression.[5] Structural similarities between FK506 and RAP notwithstanding, the fundamental mechanisms of action of FK506 and RAP are different, though FK506 and CsA operate by converging mechanisms. Whereas the RAP/FKBP complex binds to a unique effector protein,[6] the CsA/Cyp and FK506/FKBP complexes inhibit a common target, calcineurin.[7] Thus, T-cell proliferation is inhibited by formation of three unique complexes, with the mechanism being defined by the last protein being bound.



1a: R = -CH_3 *Ascomycin*
1b: R = -CH=CH_2 *FK506*



2 *Rapamycin*



Cyclosporin A

Although FK506 has elicited much hope as a less neurotoxic and nephrotoxic immunosuppressant than CsA for use in organ transplantation, a significant similarity in the toxicity/biological profile of FK506 and CsA has emerged.[8] Indeed, it has been reported that the toxic and immunosuppressive effects may be mechanistically related, implicating calcineurin inhibition as the origin of toxicity.[9] The eastern zone (as drawn) of FK506 is the portion of the drug that interacts with calcineurin. The notion that alteration of this region would change the mode of action and/or toxicity of the ensuing analogue is supported, if rapamycin is

viewed as an analogue of FK506. While published structural modifications of this "effector" region of FK506 and RAP have ranged from simple to complex, most examples resulted in reduced or abolished immunosuppressive activity.[4, 9-11] Herein we report modifications to the region spanning carbons 22 to 24 in macrocycle **1**.

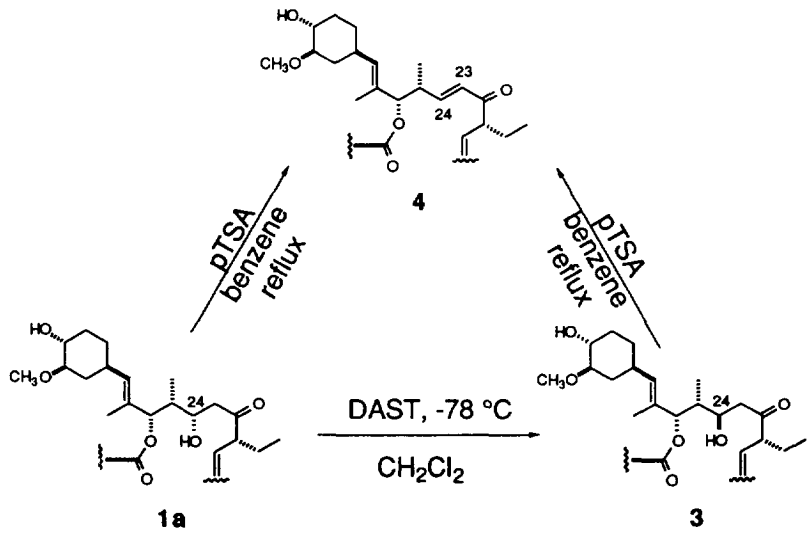
Results

Chemistry.

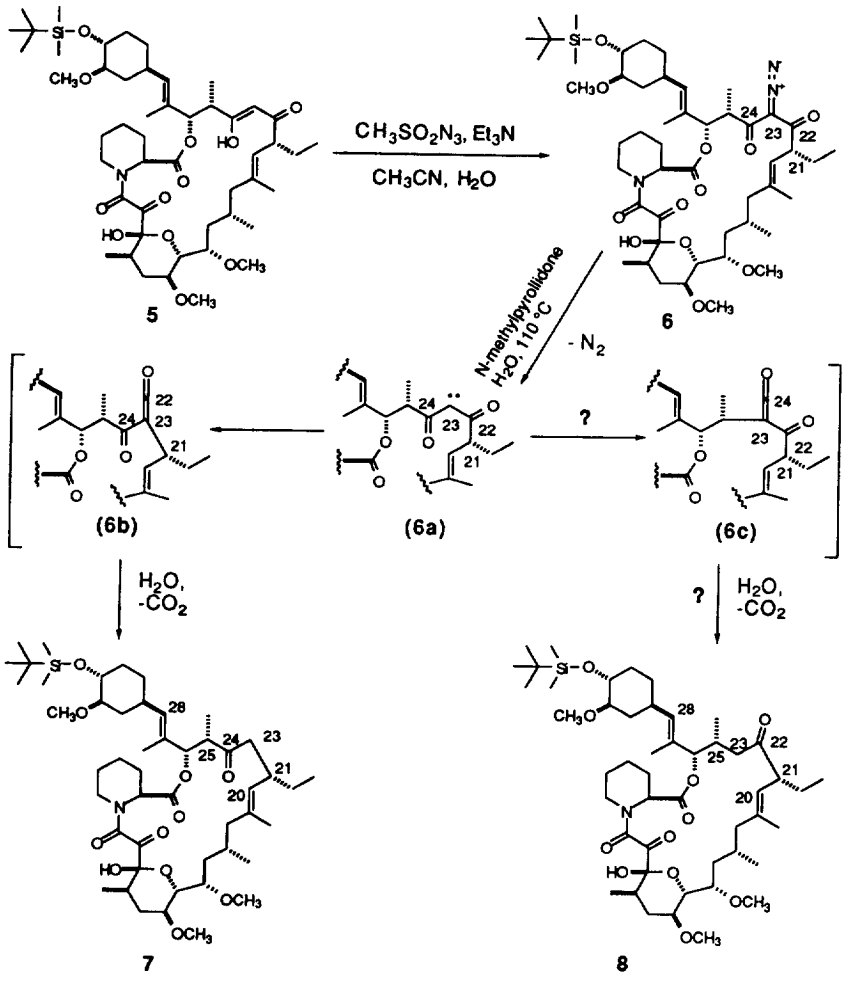
24-Epi-Ascomycin (**3**, Scheme 1)

We initiated our synthetic efforts with ascomycin (**1a**), since it demonstrates nearly identical biological activity with respect to FK506.[12] Inversion of the 24-hydroxyl group was easily accomplished in one step, without protecting the 32-hydroxyl functionality. In the event, ascomycin was treated with diethylaminosulfur trifluoride (DAST) at -78 °C (Scheme I), and after aqueous workup, a new product was isolated. The IR and mass spectra were virtually indistinguishable from those of ascomycin, however, ^{1}H NMR and ^{13}C NMR revealed that only signals due to a single conformer were present in the new compound. This was noteworthy since the NMR spectra of ascomycin and related analogues reflect two distinct rotamer populations.[13] We entertained the notion that perhaps lactone carbonyl assisted epimerization of the 24-hydroxyl group would provide 24-epi-ascomycin (**3**). Indeed, dehydration of ascomycin (**1a**) or 24-epi-ascomycin (**3**), by heating either **1a** or **3** in refluxing benzene with catalytic *p*-toluenesulfonic acid provided 23,24-dehydro-ascomycin (**4**), thereby proving the structure of **3**.

Scheme 1



Scheme 2



22-Norascomycin (9a, Scheme 2)

Excision of carbon-22 allows probing of the structure-function relationship in another way. Treatment of **5**[13] with mesyl azide[14] provided the unstable diazo-diketone **6**. Thermolysis in aqueous 1-methyl-2-pyrrolidinone generated the putative carbene (**6a**), which underwent Wolff rearrangement. As illustrated in Scheme 2, ketene (**6b**) was trapped by water, and the resulting β-keto-carboxylic acid smoothly underwent decarboxylation to provide 32-O-TBS-24-oxo-22-norascomycin (**7**) in 41% yield.[15] The remaining mass balance of the reaction consisted of numerous unidentified degradation products, preventing us from ruling out the intermediacy of (**6c**). Again, the ^{1}H NMR spectrum was significantly simplified due to the presence of only one rotamer. Proton assignments in Table 1 were obtained by correlation with the spectrum for ascomycin and

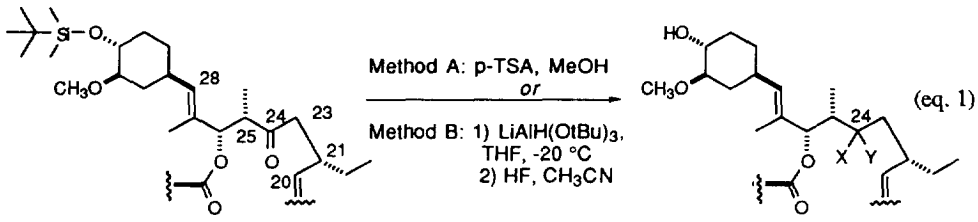
corroborated by COSY and TOCSY experiments. Relative to the ^1H NMR data for ascomycin, the following signals in the spectrum for **7** spanning the region from C-20

Table I. Comparative ^{13}C and ^1H NMR Data and Resonance Assignments for 1b and 7 in $CDCl_3$.[a]

Positions	Groups	1b Major[b] ^{13}C (ppm)	7 ^{13}C (ppm)	1b Major[b] ^1H (ppm)	7 ^1H (ppm)
1	C=O	169.0	168.7	-	-
2	CH	56.6	56.3	4.61	4.60
3	CH_2	27.6	28.1	2.09, 1.99	2.09, 1.3
4	CH_2	21.1	21.6	1.78, 1.43	
5	CH_2	24.2	24.6	1.78, 1.43	
6	CH_2	39.2	39.1	4.43, 3.02	4.46, 2.96
8	C=O	164.7	165.1	-	-
9	C=O	196.1	196.0	-	-
10	C-O	97.0	96.9	-	-
10-OH	OH	-	-	4.26	4.08
11	CH	34.6	34.7	2.19	2.22
11	CH_3	16.2	16.2	1.00	1.01
12	CH_2	32.7	32.9	2.18, 1.48	2.15, 1.48
13	CH	73.7	74.9	3.40	3.37
13	OCH_3	56.3	56.9	3.39	3.39
14	CH	72.9	72.4	3.68	3.68
15	CH	75.2	75.2	3.58	3.62
15	OCH_3	57.0	58.1	3.31	3.28
16	CH_2	33.0	33.8	1.59, 1.06	1.45, ?
17	CH	26.3	26.1	1.70	1.71
17	CH_3	20.4	20.4	0.94	0.90
18	CH_2	48.7	49.2	2.18, 1.82	2.25, 1.85
19	C-	138.7	134.3	-	-
19	CH_3	15.8	14.8	1.60	1.58
20	=CH	123.1	129.7	5.02	*4.76*
21	CH	54.7	34.9	3.21	*2.64*
22	C=O	213.4	-	-	-
23	CH_2	43.2	48.4	2.79, 2.09	2.55, 2.27
24	CH	70.0	209.5	3.92	-
24-OH	OH	-	-	3.09	-
25	CH	39.8	46.2	1.91	*2.61*
25	CH_3	9.5	10.3	0.88	*1.03*
26	CH	77.3	77.0	5.33	5.37
27	C-	132.3	130.0	-	-
27	CH_3	14.1	14.3	1.63	1.58
28	=CH	129.7	131.4	5.10	5.09
29	CH	34.9	35.0	2.29	2.97
30	CH_2	34.9	36.6	2.06, 0.97	
31	CH	84.2	84.1	3.02	3.4
31	OCH_3	56.6	56.7	3.41	3.42
32	CH	73.5	73.8	3.40	3.4
32-OH	OH	-	-	2.70	-
33	CH_2	31.2	31.6	2.01, 1.37	
34	CH_2	30.6	30.8	1.63, 1.06	
35	CH_2	24.5	27.5	1.78, 1.48	*1.32, 1.13*
36	CH_3	11.7	11.5	0.87	0.81

a: The ^{13}C and ^1H chemical shifts are referenced from the solvent resonances as 77.0 and 7.27 ppm, respectively.
b: Ascomycin exists in solution as a mixture of major and minor conformational isomers

to C-25, displayed the most dramatic shifts: 2.64 (C-21 CH, upfield shift 0.57), 2.61 (C-25 CH, downfield shift 0.7). Of structures **7** and **8**, only **7** with the ketone adjacent to C-25, was consistent with the data. This established product formation through the transient intermediates (**6a**) and (**6b**). The silyl ether was removed by acid hydrolysis (Method A) to provide **9a**. Alternatively, the 24-ketone was reduced using $LiAlH(OtBu)_3$, to



7

9a: X = Y = O
9b: X = H, Y = OH

provide a single C-24 alcohol of undetermined stereochemistry. Fluoride mediated deprotection then produced **9b**.

Ascomycin (**1a**) and FK506 (**1b**) exist as a mixture of slowly interconverting major (*cis*) and minor (*trans*) amide rotamers. Through an elegant ^{13}C NMR study, where ^{13}C-labeled FK506 was bound to FKBP-12, Schreiber and coworkers showed that only one rotamer was capable of binding FKBP.[16] Subsequent experiments proved that this isomer was due to the *trans* amide conformer in FK506 as well as ascomycin.[17] By spectral correlation, the C-9 carbonyl signals of compounds **3**, **9a** and **9b** were all similar to the chemical shift due to the major *cis*-amide conformer of the C-9 carbonyl in ascomycin (**1a**). Since these signals coincided with the non-FKBP-binding conformer of ascomycin, we predicted attenuated FKBP binding.

Biology.

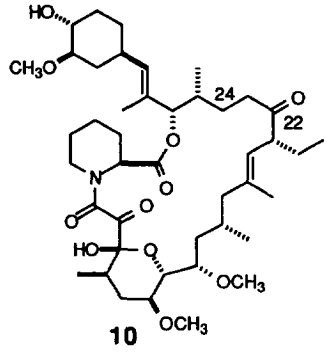
Table 2 portrays the *in vitro* characterization of ascomycin (**1a**), FK506 (**1b**), **3**, **9a** and **9b**. Binding affinity for FKBP-12 and immunosuppressive potency (mixed lymphocyte response, MLR) were evaluated as previously described.[11]

Table 2. FKBP-12 Binding Affinities and MLR Inhibition

Compound	FKBP-12 IC50 (nM)	MLR IC50 (nM)
1a	1.37	0.14
1b	1.24	0.06
3	4200	116.
9a	3000	>1000
9b	9600	>1000

Discussion

Considering the modest changes imposed on the molecular framework, either by an epimerization or a skeletal resection, the biological consequences of these actions are impressive. The basis for the loss of biological activity likely hinges on the inability of the new analogues to provide a significant *trans*-amide rotamer population which can bind to FKBP. Indeed, the C-24-desoxy-ascomycin analogue **10** exists as two well-populated *cis-trans* -amide rotamers at room temperature, binds avidly to FKBP, and potently inhibits the MLR, establishing that significant alteration of the "northeastern" section of the molecule is possible without compromising bioactivity.[18] Thus, native C-24-*S*-chirality of the hydroxyl (**1a**, **1b**), exchange of the C-24 hydroxyl with hydrogen (**10**), but not inversion of this center to a C-24-***R***-chirality (**3**) is compatible with strong FKBP binding. In contrast with some remaining measurable ability for **3** to inhibit the MLR, which suggests the attainment of a small *trans*-rotamer population when bound, compounds **9a** and **9b** appear devoid of all immunosuppressive effects. In the event the C-24 carbonyl of **9a** was responsible for the loss of activity, the single stereoisomer **9b**, was produced. The relative stereochemistry of the **9b** C-24 carbinol is uncertain. Since it has now been established that ***R***-chirality for a C-24 carbinol attenuates immunosuppressive activity, as in **3**, it would be surprising if ***R*-chirality** in **9b** wouldn't further decrease FKBP binding affinity. Alternatively, if the C-24 carbinol of **9b** bore the natural *S*-configuration, as in ascomycin (**1a**) or FK506 (**1b**), one would conclude that the C-22 ketone in ascomycin and FK-506 plays a critical role in skeletal dynamics, and consequently servicable presentation of the "effector domain" to calcineurin.



10

Petros and coworkers have shown that a water soluble C-32-modified-ascomycin analog exists as a 1:1 ratio of *cis:trans* amide rotamers in D_2O, which is comparable to the 1:2 *cis:trans* amide rotamer ratio of ascomycin in $CDCl_3$.[19] There are profound differences, however, in the overall conformations of the *cis*- or *trans*- ascomycin framework in aqueous versus $CDCl_3$ solution. While the aqueous solution structure of the *trans*-rotamer resembles the FKBP bound conformation, the *trans*-rotamer structure in $CDCl_3$ is strikingly different. Thus, the attainment of a *trans*-rotamer does not guarantee the formation of a macrocyclic geometry that is recognizable by FKBP. While the $CDCl_3$ structures of compounds **3**, **9a** and **9b** exist as non-FKBP-binding *cis*-amide conformers, one cannot rule out the possibility of achieving a *trans*-amide geometry in aqueous media. This aspect was not examined due to the inability of these agents to dissolve in water. Yet, even if amide isomerization were possible, the work of Petros shows that amide geometry alone is insufficient to guarantee high FKBP affinity, and the reduced binding energy may be due to other nonproductive interactions with FKBP.

Conclusion

We have discovered efficient routes to "northeastern" modified ascomycin analogues. While the changes imposed on the structure appear minimal, the effect on the amide rotamer distributions were not

predicted to be so dramatic. It appears that the changes wrought on the ascomycin framework prevents the attainment of an FKBP-servicable conformation, perhaps by impeding the ability of the drugs to assume a *trans*-lactam geometry, thereby greatly weakening FKBP affinity, and ultimately immunosuppressive efficacy.

Experimental Section

General Methods. Unless otherwise specified, proton magnetic resonance spectra were run as chloroform-*d* solutions at 500 MHz and carbon-13 nuclear magnetic resonance 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. Elemental analyses, mass spectra and infrared spectra and the above determinations were performed by the Analytical Research Department, Abbott Laboratories. Elemental analysis of **9a** was performed by Robertson Microlit Laboratories, Inc., Madison, N.J.

Analytical thin layer chromatography was done on 2x6 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 molydate (50 g) and ceric sulfate (20 g) in 10% sulfuric acid (2 L), or with iodine vapor, or with short wavelength ultraviolet light. Column chromatography was performed on silica gel (Kieselgel 60, 70-230 mesh) from E. Merck. HPLC purification was performed on a 20 x 300 mm column packed with YMC 15 μm 60-Å spherical silica gel. The term *in vacuo* refers to solvent removal via a rotary evaporator at 30 mm Hg. Analytical samples were further dried by final evacuation at 0.1 mm Hg for several hours.

Solvents and reagents were purchased from Aldrich Chemical Co. and were used without further purification unless otherwise specified. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl.

24-Epi-ascomycin (3). A 15 mL conical centrifuge tube was charged with ascomycin (**1a**) (1 g, 1.26 mmol) dissolved in dichloromethane (2 mL). The solution was gelled under nitrogen at -78 °C (10 min). Diethylamino sulfurtrifluoride (2 mL, 14.9 mmol) was carefully added as a layer over the gel and left unstirred for 15 minutes. The solid upper layer was then broken up with a spatula and the mixture agitated vigorously out of the ice bath with a pipette to mix the contents to homogeneity (< 1 min). The cold mixture was then immediately quenched by carefully adding to ice water (30 mL) and ethyl acetate (30 mL). The biphasic mixture was agitated and the organic layer was washed with brine (2 x 30 mL). Organics were dried (Na_2SO_4) and concentrated *in vacuo* producing a nearly colorless foam (0.98 g), which was filtered through silica gel (75 mL) eluting with hexane:acetone (2:1). The product containing fractions were collected, pooled and concentrated (0.4 g). Further purification by HPLC eluting with hexane:acetone (2:1) provided pure 24-epi-ascomycin (0.25 g, 0.32 mmol) as a colorless foam in 25% yield; IR ($CDCl_3$) ν 3584, 3472, 1748, 1724 (sh), 1696, 1650 cm^{-1}; ^1H NMR (500 MHz)(one rotamer) δ 5.62 (s, 1H), 4.95 (mult, 2H), 4.74 (d, 1H, J=5.0 Hz), 4.62 (s, 1H), 4.38 (dd, 1H, J=14.5, 4.5 Hz), 3.45-3.18 (mult, 8H), 3.41 (s, 3H), 3.37 (s, 3H), 3.28 (s, 3H), 3.02 (ddd, 1H, J=10.0, 7.5, 5.0 Hz), 2.84 (d, 1H, J=17.0 Hz), 0.83-2.30 (mult, 26H est.), 1.63 (s, 3H), 1.61 (s, 3H), 1.04 (d, 3H, J=7.0 Hz), 0.98 (d, 3H, J=7.0 Hz), 0.88 (dd, 3H, J=7.5, 7.5 Hz), 0.70 (d, 3H, J=7.5 Hz); ^{13}C NMR (125 MHz) (one rotamer) 213.3, 196.6, 169.4, 165.6, 140.4, 131.5, 128.0, 123.4, 96.9, 84.2, 75.3, 75.2, 74.3, 74.1, 73.6, 69.4, 56.9, 56.4, 56.3, 56.3, 55.7, 48.6, 41.8, 39.2, 39.1, 35.1, 34.7, 34.0, 32.9, 31.2, 30.7, 27.6, 24.4, 24.2, 21.0, 20.7, 16.3, 15.8, 14.3, 11.8, 9.3; mass spectrum (FAB), 792 (M+H), 830 (M+K).

Anal. calcd. for $C_{43}H_{69}NO_{12}$: C, 65.21; H, 8.78; N, 1.77. Found: C, 65.51; H, 8.74; N, 1.70.

32-O-t-Butyldimethylsilyl-23-diazo-24-oxo-ascomycin (6). 32-O-t-Butyldimethylsilyl-24-oxo-ascomycin[13] (16.9 g, 18.7 mmol) in CH_3CN containing water (0.34 mL, 18.7 mmol) and triethylamine (3.9 mL, 28.1 mmol, 1.5 eq) was treated with a portion of methanesulfonylazide (4.5 mL, 56.2 mmol) at ambient temperature (7 h). The mixture was concentrated *in vacuo* and filtered through a plug of silica gel (300 mL) eluting with hexane:EtOAc (1L, 2:1). Fractions containing product were pooled and concentrated. This was purified further by HPLC on silica gel (50mm x 500 mm, 230-400 mesh) eluting with hexane:EtOAc (6L, 5:1). The appropriate fractions were combined and concentrated to provide the product as a yellow foam (13.8 g, 14.8 mmol) in 79% yield. IR ($CDCl_3$) ν 2130, 1740, 1645 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ (rotamers) 5.45 (d, 0.5H, J=4 Hz), 5.17 (s, 0.5H), 5.12 (d, 0.5H, J=10 Hz), 5.00 (mult, 1.5H), 4.79 (d, 0.4H, J=5 Hz), 4.45 (d, 0.5H, J=12 Hz), 4.23 (s, 0.3H), 3.85 (br, 0.5H), 3.82 (dd, 0.5H, J=10, 3 Hz), 3.71 (d, 0.5H, J=10 Hz), 3.62 (dd, 0.5H, J=12, 5 Hz), 3.52 (mult, 1H), 3.42 (s, 1.5H), 3.41 (s, 1.5H), 3.39 (s, 1.5H), 3.38 (s, 1.5H), 3.37 (mult, 1H), 3.33 (s, 1.5H), 3.27 (s, 1.5H), 3.13 (br, 1H), 3.02 (dd, 1H, J=10, 10 Hz), 2.95 (dddd, 1H, J=13, 7, 4, 4 Hz), 1.30-2.5 (mult, 22H est.), 1.65 (s, 1.5H), 1.61 (s, 1.5H), 1.54 (s, 3H), 1.16 (d, 1.5H, J=7 Hz), 1.06 (d, 1.5H, J=7 Hz), 1.02 (d, 1.5H, J=6 Hz), 0.98 (d, 1.5H, J=6 Hz), 0.89 (s, 4.5H), 0.88 (s, 4.5H), 0.87 (d, 1.5H, J=6 Hz), 0.77 (d, 1.5H, J=6 Hz), 0.08 (s, 1.5H), 0.07 (s, 1.5H), 0.06 (s, 1.5H), 0.05 (s, 1.5H); ^{13}C NMR (125 MHz) $CDCl_3$ δ (rotamers) 196.2, 194.4, 193.6, 192.9, 168.9, 168.8, 166.7, 164.9, 142.7, 139.5, 132.4, 130.2, 130.1, 129.6, 123.0, 122.9, 98.2, 96.8, 84.1, 84.0, 80.4, 77.5, 76.2, 75.1, 73.7, 73.5, 72.5, 72.2, 58.0, 57.1, 57.0 , 56.3, 56.2, 55.3, 52.3, 52.0, 48.7, 48.2, 44.9, 44.8, 43.7, 42.8, 39.2, 36.5, 35.1, 34.9, 34.6, 33.7, 32.9, 32.7, 32.6, 30.9, 30.7, 27.9, 26.3, 25.8, 25.6, 24.6, 24.5, 21.6, 21.2, 20.2, 19.0, 18.1, 16.2, 16.0, 15.6, 15.3, 14.1, 13.9, 12.2, 11.9, 11.5, 8.70, -4.5, -4.7; MS (FAB) *m/z* 968 (M+K), 940 (M+K-N_2).

32-O-t-Butyldimethylsilyl-24-oxo-22-norascomycin (7). Diazo-diketone **6** (13.8 g, 14.8 mmol) in 1-methyl-2-pyrrolidinone (600 mL) containing water (96 mL) was heated at 110 °C for 80 min after gas evolution commenced. The mixture was cooled and partitioned between EtOAc (1 L) and water (1 L). The aqueous layer was extracted with additional EtOAc (1 L). The organic layers were each washed sequentially with water (1 L) and brine (500 mL), and were then combined and dried ($NaSO_4$). The solvent was removed *in vacuo* and the residue was passed through a pad of silica gel (300 mL) eluting with a mixture of hexane:EtOAc (2:1, 2 L). The fractions containing product were combined and concentrated to a yellow oil (10 g) which was further purified by HPLC on silica gel (1L, 230-400 mesh) eluting with hexane:EtOAc (5:1). Pure product was obtained (5.3 g, 6.1 mmol) in 41% yield. IR ($CDCl_3$) ν 2930, 1750, 1722, 1705 (sh), 1645 cm^{-1}; ^1H NMR (500 MHz) $CDCl_3$ δ 5.37 (s, 1H), 5.09 (d, 1H, J=10 Hz), 4.76 (d, 1H, J=10 Hz), 4.60 (mult, 1H), 4.46 (d, 1H, J=15 Hz), 4.08 (s, 0.7H), 3.68 (d, 1H, J=10 Hz), 3.62 (dd, 1H, J=12, 3 Hz), 3.42 (s, 3H), 3.39 (s, 3H), 3.28 (s, 3H), 3.33-3.45 (mult, 2H), 2.90-3.00 (mult, 2H), 2.68-2.80 (mult, 2H), 2.55 (dd, 1H, J=15, 7 Hz), 2.19-2.31 (mult, 4H), 2.15 (ddd, 1H, J=13, 4, 4 Hz), 2.05-2.12 (mult, 2H), 1.95 (br d, 1H, J=13 Hz), 1.85 (mult, 1H), 1.63-1.79 (mult, 4H), 1.58 (s, 6H), 1.25-1.58 (mult, 9H), 1.08-1.17 (mult, 2H), 1.03 (d, 3H, J=7 Hz), 1.01 (d, 3H, J=7 Hz), 0.95-1.05 (mult, 2H), 0.90 (s, 13H), 0.81 (dd, 3H, J=7, 7 Hz), 0.10 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (125 MHz) $CDCl_3$ δ 209.5, 196.0, 168.7, 165.1, 134.3, 131.4, 130.0, 129.7, 96.9, 84.1, 77.0, 75.2, 74.9, 73.8, 72.4, 58.1, 56.9, 56.7,

56.3, 49.2, 48.4, 46.2, 39.1, 36.6, 35.0, 34.9, 34.7, 33.8, 32.9, 31.6, 30.8, 28.1, 27.5, 26.1, 25.9, 24.6, 21.6, 20.4, 18.1, 16.2, 14.8, 14.3, 11.5, 10.3, -4.5, -4.7; MS (FAB) *m/z* 914 (M+K).

Anal. Calcd. for $C_{48}H_{81}NO_{11}Si$: C, 65.79; H, 9.32; N, 1.60. Found: C, 65.57; H, 9.08; N, 1.56.

24-Oxo-22-norascomycin (9a). Silyl ether **7** (100 mg, 0.11 mmol) in MeOH (1.2 mL) was stirred in the presence of *p*-toluenesulfonic acid monohydrate (32.5 mg, 0.17 mmol) for 1h. The solution was concentrated *in vacuo* and the residue purified by chromatography on silica gel (15 mL). The silica pad was eluted with a mixture of hexane:acetone (9:1, 100 mL) and (3:1, 200 mL) collecting 10 mL fractions throughout. Fractions containing pure product were pooled and concentrated *in vacuo* providing pure product (60 mg, 0.079 mmol) in 72% yield. IR ($CDCl_3$) ν 2950, 1750, 1712, 1705 (sh), 1645 cm $^{-1}$; ^{1}H NMR (500 MHz) $CDCl_3$ δ 5.37 (d, 1H, J=2 Hz), 5.10 (d, 1H, J=10 Hz), 4.77 (d, 1H, J=10 Hz), 4.57 (mult, 1H), 4.45 (d, 1H, J=15 Hz), 4.08 (d, 0.7H, J=2 Hz), 3.68 (d, 1H, J=10 Hz), 3.62 (dd, 1H, J=12, 3 Hz), 3.42 (s, 3H), 3.40 (s, 3H), 3.29 (s, 3H), 3.33-3.45 (mult, 2H), 3.01 (ddd, 1H, J=13, 9, 5 Hz), 2.95 (ddd, 1H, J=12, 12, 3 Hz), 2.70-2.80 (mult, 2H), 2.63 (d, 0.7H, J=2 Hz), 2.56 (dd, 1H, J=15, 7 Hz), 2.19-2.31 (mult, 4H), 2.15 (ddd, 1H, J=13, 4, 4 Hz), 1.98-2.12 (mult, 4H), 1.61 (s, 3H), 1.58 (s, 3H), 1.25-1.79 (mult, 13H), 0.85-1.17 (mult, 5H), 1.04 (d, 3H, J=7 Hz), 1.01 (d, 3H, J=7 Hz), 0.90 (s, 3H), 0.80 (dd, 3H, J=7, 7 Hz); ^{13}C NMR (125 MHz) $CDCl_3$ δ 209.6, 196.0, 168.7, 165.1, 134.3, 131.3, 130.4, 129.7, 97.0, 84.1, 77.2, 74.9, 73.7, 73.5, 72.5, 56.8, 56.6, 56.3, 49.2, 48.6, 46.2, 39.1, 35.0, 34.8, 34.7, 32.8, 31.6, 31.2, 30.5, 28.0, 27.4, 27.0, 26.1, 24.6, 21.6, 20.4, 16.2, 14.7, 14.4, 11.4, 10.5; MS (FAB) *m/z* 800 (M+K).

Anal. Calcd. for $C_{42}H_{67}NO_{11}$: C, 66.20; H, 8.86; N, 1.83. Found: C, 65.93; H, 8.58; N, 1.79.

22-Norascomycin: *Part A.* A 1.0M solution of $LiAlH(Ot\text{-}Bu)_3$ in THF (4.0 mL, 4.0 mmol) was added dropwise to a solution of ketone **7** (877 mg, 1.0 mmol) in THF (4.0 mL) at -20 °C and allowed to stir for 5h. The reaction was quenched by the addition of acetone (0.5 mL) and then stirred vigorously at RT for 15 minutes with a saturated aqueous solution of sodium, potassium tartrate (20 mL). The mixture was partitioned between water (60 mL) and EtOAc (100 mL), whereupon the organic layer was washed with 1N HCl (20 mL) and brine (2 x 30 mL). Aqueous layers were extracted with additional EtOAc (100 mL), and the organics were combined, dried (Na_2SO_4) and concentrated *in vacuo*. Purification by chromatography on silica gel (16 mL) eluting with toluene:EtOAc (4:1) provided intermediate product (389 mg, 0.44 mmol) as a colorless foam in 44% yield. IR ($CDCl_3$) ν 1735, 1720 (sh), 1670, 1645 cm $^{-1}$; ^{13}C NMR (125 MHz) $CDCl_3$ δ 195.7, 169.3, 165.2, 134.6, 132.2, 131.9, 129.1, 96.9, 84.1, 79.8, 75.2, 74.7, 73.7, 73.2, 72.4, 58.1, 57.5, 56.7, 56.3, 49.8, 40.5, 39.4, 37.3, 36.6, 34.9, 34.6, 33.8, 33.0, 31.9, 30.9, 28.6, 26.2, 25.9, 24.7, 21.8, 20.4, 16.3, 15.0, 14.8, 11.4, 7.7.

Part B. A 1M solution of HF (0.35 mL, 0.35 mmol (0.42 mL 48% aqueous HF in 9.58 mL CH_3CN provides a 1M solution)) was added dropwise to a solution of the product from Part A (290 mg, 0.33 mmol) in acetonitrile (3.2 mL) at 0 °C. The mixture was warmed to RT and stirred for 45 minutes, whereupon it was cooled to 0 °C and solid pulverized $NaHCO_3$ (244 mg, 2.9 mmol) was added. After 30 minutes anhydrous $MgSO_4$ (250 mg) was added and the mixture stirred for 15 minutes, when it was diluted with methylene chloride (15 mL), centrifuged and passed thru a plug of silica gel. The silica was eluted with hexane:acetone (1:1), and the fractions containing product were pooled, concentrated and purified by HPLC eluting with hexane:acetone (2:1) to provide desired product (163 mg, 0.21 mmol) in 64% yield. IR ($CDCl_3$) ν 1735, 1645

cm $^{-1}$; ^{1}H NMR (500 MHz) $CDCl_3$ δ 5.35 (s, 1H), 5.13 (d, 1H, J=10 Hz), 4.95 (d, 1H, J=10 Hz), 4.43 (mult, 2H), 4.13 (s, 1H), 3.81 (ddd, 1H, J=10, 3, 3 Hz), 3.69 (d, 1H, J=9 Hz), 3.65 (dd, 1H, J=12, 3 Hz), 3.42 (s, 3H), 3.39 (s, 3H), 3.37 (mult, 1H), 3.30 (s, 3H), 3.02 (ddd, 1H, J=13, 7, 4, Hz), 3.02 (ddd, 1H, J=12, 12, 4 Hz), 1.97-2.35 (mult, 8H), 0.9-1.85 (mult, 16H est.), 1.68 (s, 3H), 1.56 (s, 3H), 1.02 (d, 3H, J=6 Hz), 0.90 (d, 3H, J=7 Hz), 0.88 (d, 3H, J=7 Hz), 0.83 (dd, 3H, J=6, 6 Hz); ^{13}C NMR (125 MHz) $CDCl_3$ δ 195.7, 169.4, 165.2, 134.6, 132.4, 132.1, 128.9, 96.9, 84.2, 79.8, 74.7, 73.7, 73.5, 73.2, 72.4, 57.5, 56.7, 56.6, 56.3, 49.8, 40.6, 40.5, 39.4, 37.3, 34.9, 34.6, 33.0, 32.0, 31.2, 30.7, 28.6, 26.2, 26.0, 24.6, 21.8, 20.4, 16.3, 15.0, 14.8, 11.4, 7.8; MS (FAB) *m/z* 802 (M+K).

Anal. Calcd. for $C_{42}H_{69}NO_{11}$: C, 66.03; H, 9.10; N, 1.83. Found: C, 65.92; H, 8.93; N, 1.76.

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