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The Chemistry of Ascomycin: Structure Determination and Synthesis of Pyrazole Analogues

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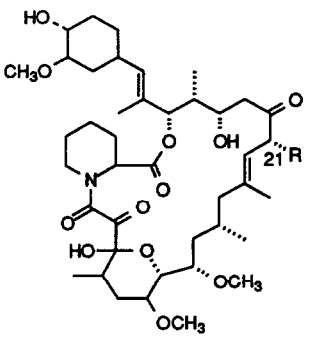
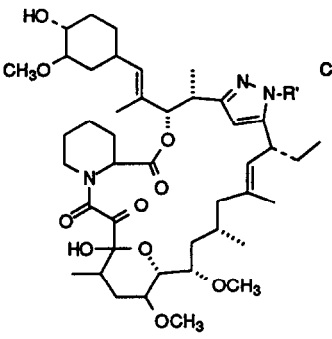
Abstract: Ascomycin (**1**) is a close analogue of the immunosuppressant FK-506 (**2**) which differs slightly in the side chain at position 21 (ethyl vs allyl). Structurally unique ascomycin and FK-506 are the subjects of intense chemical research because of their application in organ transplantation and autoimmune disease. We have been interested in studying the effect of structural modification and conformational change on biological activity. This paper reports the fermentation and structural determination of ascomycin as well as the synthesis and structure confirmation of a series of pyrazole analogues (**3** and **4**) derived from ascomycin.

Introduction

Ascomycin has been known for more than 30 years. The compound was first described as an antifungal antibiotic isolated from *Streptomyces hygroscopicus var. ascomyceticus* (ATCC 14891) by Arai *et al* in 1962. However, the structure of ascomycin was not determined in the 1962 report.[1] Recently, Arai *et al* published NMR spectra of samples of ascomycin and FR900520 from ATCC 14891 and *Streptomyces hygroscopicus* subsp. *yakushimaensis No. 7238* respectively.[2] Although no spectroscopic assignments were made, they showed that the two compounds appeared to be spectroscopically as well as chromatographically identical. FR-900520 was one of the several compounds structurally related to FK-506 isolated in fermentations by workers at Fujisawa.[4] We independently prepared ascomycin by fermentation using *Streptomyces hygroscopicus var. ascomyceticus* (ATCC 14891) and determined by x-ray crystallography that it has the same structure as FR900520 reported by Fujisawa. This unambiguous assignment is the conclusive demonstration that ascomycin and FK-506 differ only by one carbon and the degree of saturation in the side chain at position 21. A complete assignment of ^{13}C and ^{1}H NMR spectra was also made. FK-506, a 23-membered tricyclo-macrolactam, was discovered in 1984 by Fujisawa while screening *Streptomyces tsukubaensis* cultures for compounds with specific immunosuppressive activities.[5] The immunosuppressive activity of ascomycin is comparable to FK-506 because of the high degree of structural similarity. Both ascomycin and FK-506 are subjects of intense chemical research because of their application in organ transplantation and their unique structural features. We have been interested in studying the effect of structural modification and conformational change on the ability of analogues to interact with target proteins and cause immunosuppression. This paper reports the fermentation and structural determination of ascomycin as well as the synthesis of a series of pyrazole analogues (**3** and **4**) derived from ascomycin.



1. Ascomycin, R = Et
2. FK-506, R = Allyl



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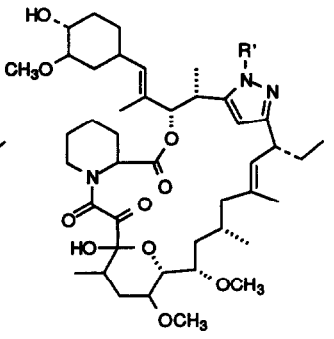

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Results and Discussion

Ascomycin was produced by fermentation of *Streptomyces hygroscopicus* subsp. *ascomyceticus* ATCC 14891 as previously described.[1] The compound was obtained from acetonitrile as colorless plates with m.p. = 163-165 °C. X-ray analysis was performed on single crystals obtained from acetonitrile by slow evaporation. A perspective drawing of the X-ray structure of ascomycin is shown in Fig 1. The acetonitrile molecule is included.

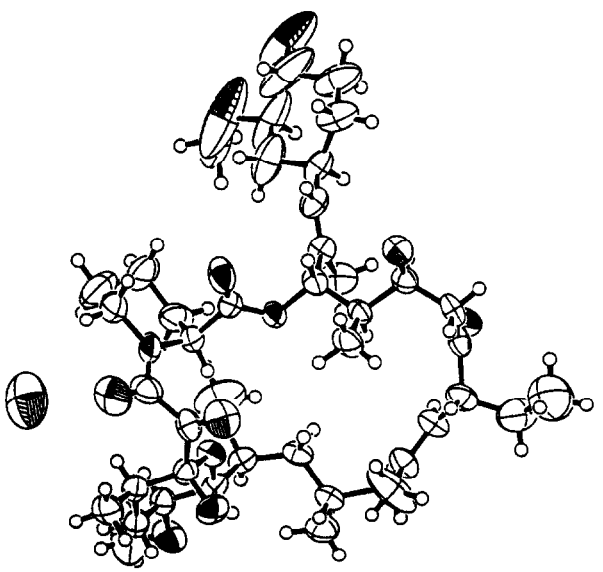


Figure 1. X-Ray Structure of Ascomycin

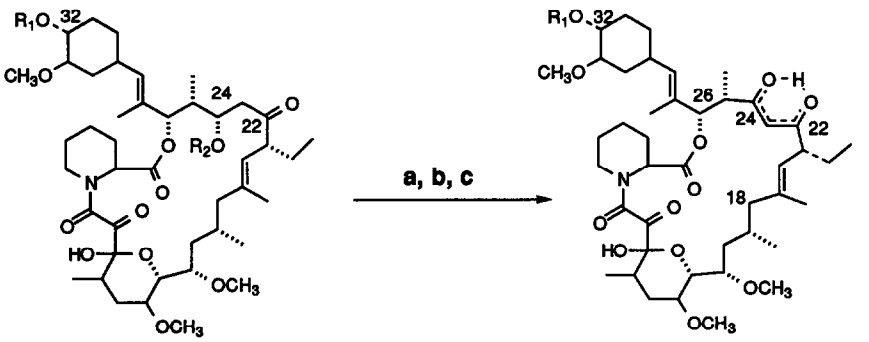
Table I. ^{13}C and ^{1}H Chemical Shift Data and Resonance Assignments for Ascomycin in $CDCl_3$.[a]

Positions	Groups	Major[b] ^{13}C (ppm)	Minor[b] ^{13}C (ppm)	Major[b] ^{1}H (ppm)	Minor[b] ^{1}H (ppm)
1	C=O	169.0	168.7	-	-
2	CH	56.6	52.7	4.61	5.00
3	CH_2	27.6	26.2	2.09, 1.99	2.33, 1.80
4	CH_2	21.1	20.8	1.78, 1.43	1.78, 1.43
5	CH_2	24.2	24.5	1.78, 1.43	1.78, 1.43
6	CH_2	39.2	43.9	4.43, 3.02	3.73, 3.29
8	C=O	164.7	165.8	-	-
9	C=O	196.1	192.6	-	-
10	C-O	97.0	98.6	-	-
10-OH	OH	-	-	4.26	4.83
11	CH	34.6	33.6	2.19	2.31
11	CH_3	16.2	16.0	1.00	0.97
12	CH_2	32.7	32.5	2.18, 1.48	2.13, 1.54
13	CH	73.7	73.7	3.40	3.45
13	OCH_3	56.3	56.0	3.39	3.38
14	CH	72.9	72.3	3.68	3.88
15	CH	75.2	76.6	3.58	3.58
15	OCH_3	57.0	57.5	3.31	3.34
16	CH_2	33.0	35.5	1.59, 1.06	1.59, 1.35
17	CH	26.3	26.0	1.70	1.70
17	CH_3	20.4	19.5	0.94	0.84
18	CH_2	48.7	48.4	2.18, 1.82	2.18, 1.95
19	C-	138.7	139.6	-	-
19	CH_3	15.8	15.7	1.60	1.63
20	=CH	123.1	123.3	5.02	5.02
21	CH	54.7	55.0	3.21	3.18
22	C=O	213.4	213.4	-	-
23	CH_2	43.2	43.6	2.79, 2.09	2.73, 2.33
24	CH	70.0	69.0	3.92	3.96
24-OH	OH	-	-	3.09	3.50
25	CH	39.8	40.4	1.91	1.91
25	CH_3	9.5	9.8	0.88	0.92
26	CH	77.3	77.9	5.33	5.21
27	C-	132.3	131.8	-	-
27	CH_3	14.1	14.2	1.63	1.67
28	=CH	129.7	129.6	5.10	5.06
29	CH	34.9	34.9	2.29	2.29
30	CH_2	34.9	34.8	2.06, 0.97	2.06, 0.97
31	CH	84.2	84.2	3.02	3.02
31	OCH_3	56.6	56.6	3.41	3.41
32	CH	73.5	73.5	3.40	3.40
32-OH	OH	-	-	2.70	2.70
33	CH_2	31.2	31.2	2.01, 1.37	2.01, 1.37
34	CH_2	30.6	30.6	1.63, 1.06	1.63, 1.06
35	CH_2	24.5	24.5	1.78, 1.48	1.75, 1.49
36	CH_3	11.7	11.7	0.87	0.87

a: The ^{13}C and ^{1}H 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

Like FK-506, ascomycin exists in solution as a mixture of two conformational isomers. DEPT and two-dimensional spectroscopy, including TOCSY, HMQC, and HMBC experiments were used to assign the ascomycin NMR spectra, including the resonances of the three hydroxyl protons. The resonances corresponding to the major and minor conformational isomers were unambiguously assigned and are listed in Table I.

One of the methods for pyrazole synthesis consists of the condensation of a 1,3-dicarbonyl compound with a hydrazine.[6] Examination of the structure of ascomycin reveals the presence of a β-hydroxyl keto system at positions 24 to 22. Oxidation of the 24-hydroxyl group would produce the necessary 1,3-dicarbonyl precursor for pyrazole formation. Given the two secondary hydroxyl groups present in ascomycin, the Swern oxidation of ascomycin with one equivalent of oxidizing agent produced a mixture of 32-keto and 24, 32-diketo ascomycin analogues in approximately a two to one ratio. In order to oxidize only the 24-hydroxyl group it proved necessary to protect the apparently more accessible 32-OH of ascomycin. Reaction of ascomycin with *tert*-butyldimethylsilyl chloride and imidazole in dry methylene chloride gave 32-TBS (**5a**) and 32,24-bis-TBS ascomycin (**5b**) in 81% and 18% yield, respectively. Swern oxidation of **5a** with 2 equivalents of oxalyl chloride-methylsulfoxide in dichloromethane followed by triethylamine gave **6** in good yield. The use of triethylamine in this particular case generated a small amount of side products and made the purification process difficult. Substituting triethylamine with diisopropylethylamine in the Swern oxidation eliminated the impurities and produced **6** in 92% yield. Compound **6** was deprotected with 2 equivalents of 48% hydrofluoric acid in acetonitrile to give precursor **7** in quantitative yield. The preparation of **7** is an efficient process that scales up well; 10g of the compound has been prepared from 14g of ascomycin (71% overall yield) in a single run.



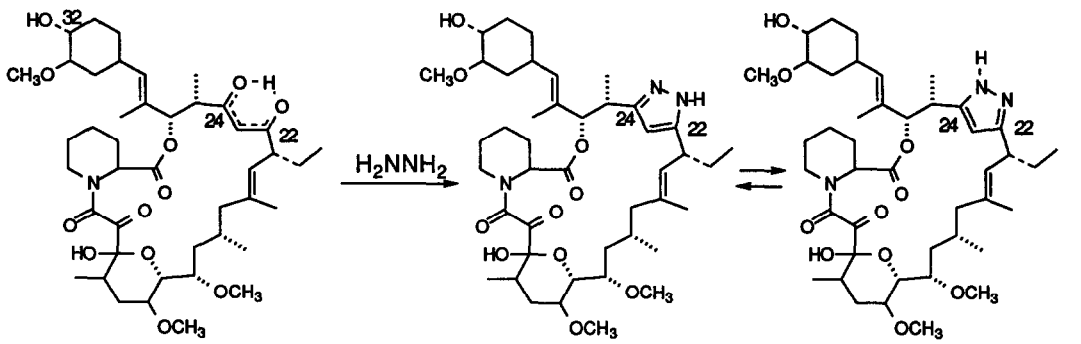
1. $R_1 = H, R_2 = H$
5a. $R_1 = TBS, R_2 = H$
5b. $R_1 = R_2 = TBS$

6. $R_1 = TBS$
7. $R_1 = H$

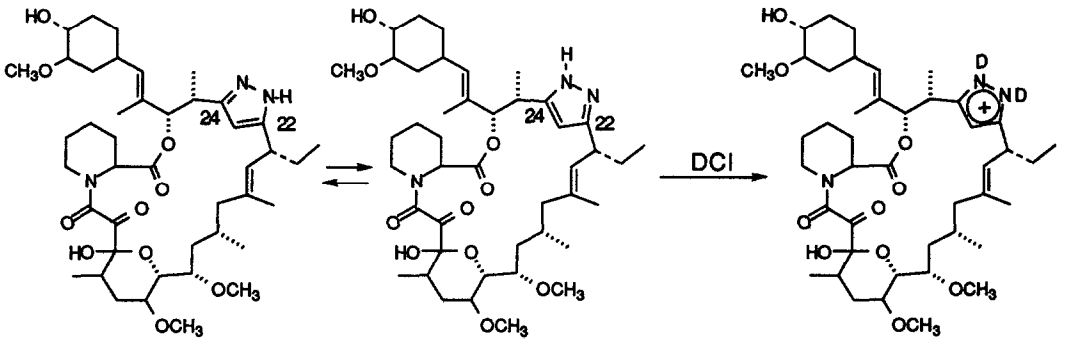
Reagents and Conditions: (a) t-$BuMe_2SiCl$, Imidazole, CH_2Cl_2, 81% of **5a** from **1**; (b) Oxalyl Chloride-DMSO, Diisopropylamine, 92% of **6** from **5a**; (c) HF, CH_3CN, 99% of **7** from **6**.

Although the 1,3-diketone system in **7** can exist either in diketo or two possible enolized forms, the ^{1}H and ^{13}C NMR analysis of **7** in deuterated chloroform indicated the existence of only one enol-keto form. Compound **7**, like ascomycin and FK-506, exists in solution as a *trans-cis* amide rotamer mixture.[3] As a result, every hydrogen and carbon atom gives two sets of NMR resonance signals. For example, the chemical shifts of H-23 are d 5.60 and 5.80 ppm, respectively. The C-18 to C-26 carbon resonance signals of the major and minor rotamers in $CDCl_3$ were assigned by 2-D homo- and heteronuclear correlation.[7] The DEPT experiment showed that C-23 (resonance signal at 97.6 and 96.0 ppm), is attached to one hydrogen, consistent with a single enol-keto form. The chemical shifts of C-22 (201.8 and 198.3 ppm) and C-24 (193.3 and 189.3 ppm) suggest that the two carbons are in very similar environments. The NMR data suggest the structure as shown in Scheme I with the enolic hydrogen delocalized between the oxygens attached to C-22 and C-24.

Treatment of **7** with 1.6 equivalent of hydrazine in tetrahydrofuran produced the parent pyrazoles (**3a, 4a**) in 54% yield. It is known that N-unsubstituted pyrazoles in solution exists as a mixture of annular tautomers in different proportions, depending on the nature of the substituents on the ring.[8,9] Indeed, the presence of a tautomeric pyrazole mixture is indicated by 2-D TLC analysis and further verified by ^{13}C NMR.

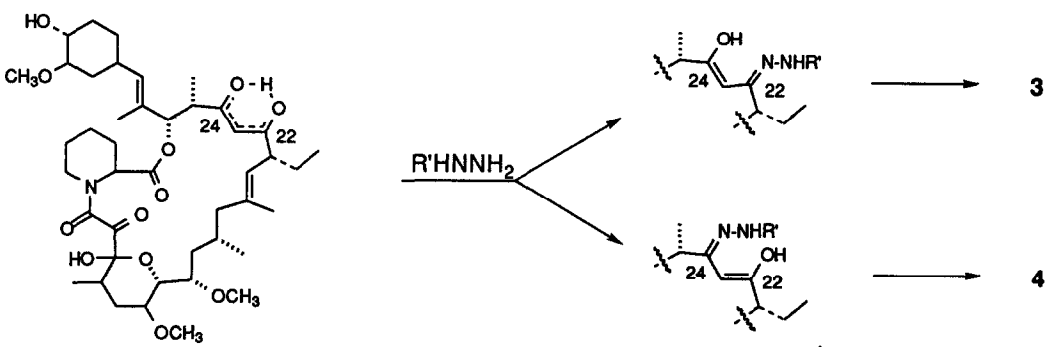


The pyrazole analogues of ascomycin, like the parent, exist in solution as a *trans-cis* amide rotamer mixture. Every hydrogen and carbon atom in the molecule gives two resonance signals, and the resulting ^{1}H and ^{13}C NMR spectra are rather complicated. For the tautomeric mixture **3a/4a**, every carbon atom shows four resonances in unequal intensities, making interpretation of the spectrum exceedingly difficult. Thus, we sought a way to simplify spectral interpretation. The basicity of pyrazoles **3a/4a** is estimated to be pK = 4 based on literature values.[10] Under acidic pH, **3a** and **4a** would be expected to be protonated to give a symmetrically-protonated species. Indeed, the ^{1}H and ^{13}C NMR spectra of the pyrazolium ion (**8**) in DCl-deuterated methanol are simplified to a mixture of two rotamers and become readily interpretable.



3a **4a** **8**

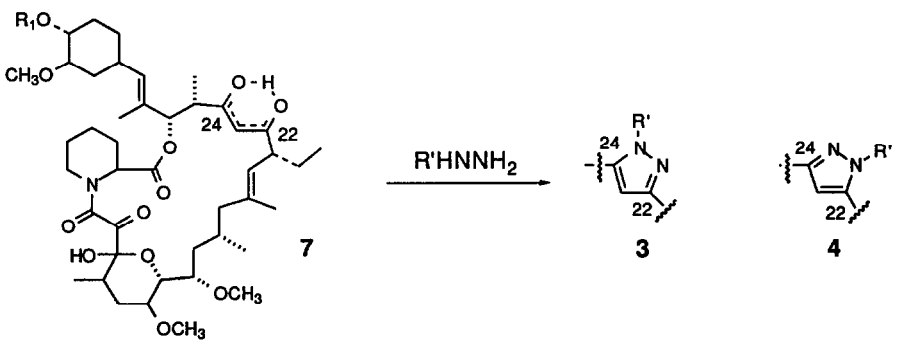
The reaction of **7** with substituted hydrazines was studied next. There are many ways that a hydrazine can initiate the reaction with **7**, but only two regio-isomers **3** and **4** are possible. Although the synthesis of pyrazoles using 1,3-dicarbonyl compounds and hydrazines is a well established process, and numerous publications have been devoted to this method, the structural reasons that favor a particular isomer are not well understood.[11] The complexity of the problem is simplified in this case because of the steric hindrance of the dicarbonyl system in **7**. Although the alkylhydrazines have two nucleophilic centers, only the unsubstituted nitrogen is less hindered and likely to react first. The phenylhydrazines are known to react at the terminal nitrogen first in neutral or acidic medium in the pyrazole formation reaction.[11] Addition of the terminal nitrogen at C-22 in **7** followed by cyclization gives isomer **3**. On the other hand, addition at C-24 followed by cyclization produces isomer **4**.



7

In practice, the reaction of **7** with methylhydrazine in ethanol produced predominantly one of the two possible isomers. The other isomer was present only in trace amount. From a medicinal chemist's point of view,

it is desirable to have both pyrazole isomers available for biological testing. Furthermore, having both isomers would aid in unambiguous structural determination. Two different approaches were carried out in order to produce both isomers. One is to produce larger quantities of both isomers by alkylating the parent pyrazoles **3a** and **4a**. The 32-TBS protected pyrazole **3c/4c** was prepared from **6** and hydrazine in 80% yield. Reaction of **3c/4c** with methylsulfate and diisopropylethylamine in THF produced equal amounts of **3d** and **4d** determined from the NMR of the product mixture. However, because it is generally difficult to predict and control the ratio of position isomers in the formation of N-alkyl derivatives from unsymmetrical pyrazoles,[12] another approach to generate the N-methyl pyrazoles directly using methylhydrazine under different reaction conditions was taken. In general, reaction of **7** with methylhydrazine in different solvents produced the same predominant isomer observed earlier. The other isomer was present only in trace amounts shown in ^{1}H NMR of the crude products but not enough for isolation. However, when **7** and 1.5 equivalent of methylhydrazine in dry tetrahydrofuran was stirred at room temperature in the presence of powdered magnesium sulfate, a 1:1 mixture of N-methyl pyrazoles was obtained. Pyrazole **3b**, which is the predominant product observed previously, and the other isomer **4b**, were purified by HPLC. The N-alkylpyrazoles and all other ascomycin pyrazole derivatives reported here, like ascomycin, exist in solution as a mixture of *trans-cis* amide rotamers. Most of the proton and carbon chemical shifts of the major and minor rotamers of N-methyl pyrazoles were determined by 2-D homo- and heteronuclear correlations. The NMR data in deuterated chloroform suggested that the methyl group in **3b** is attached to the nitrogen on C-24. This is supported by the chemical shifts of carbons 22 and 24[13] and the nOe observed between the N-methyl and proton 26. Similarly, the N-methyl group in **4b** was found to be connected to the nitrogen on C-22 and is supported by the chemical shifts of carbons 22 and 24[13] and the nOe observed between N-methyl and proton 21.



Compound	R_1	R'	Yield %
3a, 4a	H	H	54
3b	H	Me	56
4b	H	Me	30
3c, 4c	TBS	H	80
3d, 4d	TBS	Me	90
3e	H	Et	81
3f	H	CH_2COOEt	24
3g	H	CH_2CH_2OH	44
3h	H	Ph	48
3i	H	$p(MeSO_2)Ph$	30
4i	H	$p(MeSO_2)Ph$	10
3j	H	CH_2Ph	52
3k	H	CH_2CH_2Ph	50

The pyrazole formation reaction of **7** with hydrazines is a general and good reaction as shown in the above scheme. Since most of the hydrazines are commercially available as acid salts, the reaction is more conveniently carried out in protic solvents such as ethanol in the presence of a tert-amine base. The moderate purified yields reported were the result of thorough purifications to remove small amounts of close running impurities. For the N-benzyl pyrazole **3k**, the connectivity of Bn-N to C-22 was also determined by the 2-D homo-, heteronuclear correlation and nOe studies. In all cases, the predominant isomer is **3** based on comparison of the proton NMR pattern of H-23 to those of **3b** and **4b**. Regio- isomer **4** was produced only in trace amount but not isolated. When *p*-methylsufonylphenyl hydrazine was used, a significant amount (5%) of isomer **4i** was present in the product. Reaction of **7** with carbazides in refluxing ethanol produced only the parent pyrazoles **3a** and **4a** presumably through the intermediate N-acylpyrazoles and *in situ* hydrolysis.

Conclusions

The structure of ascomycin was unambiguously determined by x-ray crystallography, and complete NMR assignments for the major and minor rotamers were made. A practical four step synthesis of pyrazole analogues of ascomycin has been developed. The structures of isomeric N-methyl pyrazoles were also determined by detailed NMR studies. However, the reaction conditions that favor the production of one isomer over the other remains unpredictable. Biological characterization of these novel pyrazole analogues of ascomycin is in progress.

Experimental Section

General Methods. Unless otherwise specified, proton magnetic resonance spectra were run in deuteriochloroform solution at 500 MHz and carbon-13 nuclear magnetic resonance in deuteriochloroform solution at 300 MHz. Since pmr spectra always show very complicated overlapping multiplets, only relevant absorptions are reported. Deuteriochloroform 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.

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; similar but larger (10x20 cm) glass plates precoated with 2 mm thick silica gel were used for preparative purpose. 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, 230-400 mesh) from E. Merck. The term *in vacuo* refers to solvent removal via a rotary evaporator at water aspirator pressure followed by evaporation at 0.5 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 and dichloromethane (DCM) was distilled from calcium hydride under nitrogen before use.

Production of Ascomycin

Streptomyces hygroscopicus subsp. *ascomyceticus* ATCC 14891 was grown in a 42 liter LH fermentor charged to 30 liters in a stirred medium consisting of soluble starch (Staclipse JUB) 4.5%, corn steep liquor 0.5%, dried yeast (Red Star) 1% and $CaCO_3$ 0.1% from a single passage seed inoculum grown in a 2 liter shaken erlenmeyer flask charged to 600 ml with a medium consisting of glycerin 1%, soluble starch 1%, glucose 0.5%, cotton seed meal (Proflo) 0.5% dried yeast 0.5%, corn steep liquor 0.25% and $CaCO_3$ 0.2% which had been inoculated with 1% of a frozen vegetative stock. At harvest, *ca.* 96 hours, XAD-16 resin (4 liters, Rohm and Haas) was added to the beer and stirred for 1 hour. The resin and mycelia were filtered off, washed with water and extracted twice with acetone (5 liter portions). The combined acetone extracts were concentrated to an aqueous slurry, diluted with water (2 liters) and extracted twice with toluene (1 liter portions). The combined toluene extracts were concentrated to a semisolid residue which was partitioned in a mixture of hexane (2 liters), ethyl acetate (500 ml), methanol (1.5 liters), and water (500 ml). The lower layer was separated and concentrated to an aqueous slurry, diluted with water (1 liter) and extracted with toluene (1 liter). The toluene extract was concentrated to dryness and the residue was chromatographed over silica gel (E. Merck Kiesel gel 60, 70-230 mesh) in a hexane to acetone gradient. Fractions showing bioactivity against *Aspergillus niger* were pooled and concentrated and the residue was recrystallized from ether to give ascomycin as white crystals and recrystallized from acetonitrile as colorless plates, m.p.: 164-166 °C (Lit.[3] 148-152 °C). FABMS: $(M + K)^+$ Calcd for $C_{43}H_{69}NO_{12}K$ m/e 830, measured 830. Optical rotation: $[\alpha]_D^{23} = -80.5°$ (c 0.503, $CHCl_3$). ^1H and ^{13}C

NMR: Table I. IR (KBr) ν_{max}: 3450 (br), 2940, 1745, 1725, 1700, 1650 and 1450 cm^{-1}. Anal: Calcd for $C_{43}H_{69}NO_{12}$: C, 65.22; H, 8.78; N, 1.76. Found: C, 65.20; H, 8.87; N, 1.66.

X-ray Crystallography. Single crystal suitable for X-ray crystallographic study were obtained from acetonitrile by slow evaporation. The crystals were orthorhombic, space group $P2_12_12_1$: a = 15.680 (6)Å, b = 26.953 (7)Å, c = 10.980 (2)Å. The calculated density 1.159 g/cm^3 for Z = 4. The data was collected on a Rigaku A CF5 diffractometer with Cu K_α (λ = 1.5418Å) radiation using the ϖ/θ scan mode up to a θ limit of $\theta \leq 60.0^o$. A total of 3914 non-equivalent reflections were collected of which 2522 reflections were significant [$|F| \geq 3\sigma(|F_o|)$]. An empirical absorption correction was applied using the program DIFABA[14]. The structure was solved using the direct methods program SHELX 76[15]. The refinement of positional and anisotropic thermal paramenters of non-hydrogen atoms with full-matrix least-squares method TEXSAN[16] converged to a final R = 0.064 and Rw = 0.077, where $R = \Sigma \, | \, |F_o| - |F_c| \, | \, / \, \Sigma \, |F_o|$ and $Rw = [\, \Sigma \, w \, (\, |F_o| - |F_c| \,)^2 \, / \, \Sigma \, w \, F_o2]^{1/2}$. The final difference Fourier map was featureless with $\Delta\rho_{min}$ = -0.20 e/Å3.

32-TBS-ascomycin (5a) and **24, 32-Bis-TBS-ascomycin (5b).** Imidazole (20.7 g, 0.300 mole) was dissolved in DCM (100 mL) and added into a stirred solution of ascomycin (30 g, 0.038 mole) in DCM (60 mL) at 0 °C. *tert*-Butyldimethylsilyl chloride (22.9 g, 0.15 mole) in DCM (110 mL) was added to the stirred reaction mixture containing asomycin and imidazole at 0 °C and refrigerated (-5 to 0 °C) for 23 h. The reaction mixture was poured on a silica gel column containing 80 g of silica gel packed in ether and eluted with ether. Solvent was removed in vacuo to give a crude product (50 g). The crude solid was purified by silica gel chromatography (700 g) eluting with 20% acetone in hexanes. Yield: **5a**, 27.6 g, 81%. FABMS: $(M + K)^+$ Calcd for $C_{49}H_{83}NO_{12}SiK$ m/e 944, measured 944. Yield: **5b**, 7.0 g, 18%. FABMS: $(M + K)^+$ Calcd for $C_{55}H_{97}NO_{12}Si_2K$ m/e 1058, measured 1058.

32-TBS-24-oxo-ascomycin (6). Dimethylsulfoxide (2.94 mL, 41 mmol) was added into a solution of oxalyl chloride (2.1 mL, 23.8 mmol) in DCM (30 mL) cooled in dry ice-isopropanol bath. A solution of **5a** (11 g, 12 mmol) in DCM (13 mL) was added and stirred for 35 min. Diisopropylethylamine (8.8 mL, 50.7 mmol in 50 ml of DCM) was added dropwise. Stirring was continued for additional 25 min. and the cooling bath was replaced with ice-water bath. After TLC analysis (20% acetone-hexanes) showed the total disappearance of starting material, the reaction mixture was poured on a silica gel column containing 100 g of silica gel in ether and eluted with ether. Solvent was removed in vacuo, and the crude purified by silica gel chromatography (500 g) eluting with 10% acetone-hexanes. Yield: 10.2 g, 92%. FABMS: $(M + K)^+$ Calcd for $C_{49}H_{81}NO_{12}SiK$ m/e 942, measured 942.

24-oxo-ascomycin (7). A solution containing 48% hydrofluoric acid (0.25 mL) in acetonitrile (5 mL) was added dropwise into a stirred solution of **6** (6 g, 6.6 mmol) in acetonitrile (25 mL) at 0 °C. After being stirred at 0 °C for 15 min., the cooling bath was removed. TLC analysis (35% acetone-hexanes) showed the total disappearance of starting material within 30 min. The reaction mixture was cooled in ice-water bath. Powdered sodium bicarbonate (2 g) was added into the stirred reaction mixture followed by magnesium sulfate (5 g) 1h later. Solids were filtered off and solvent removed in vacuo to give 5.84 g of crude solid. Immediate purification by silica gel (200g) chromatography eluting with 35% acetone-hexanes give the product as white solid. Yield: 5.18 g, 99%; mp: 114-118 °C. FABMS: $(M + K)^+$ Calcd for $C_{43}H_{67}NO_{12}K$, m/e 828, found 828. IR (KBr) ν_{max}: 3420 (br), 2930, 1720, 1650 and 1430 cm^{-1}. Anal: Calcd for $C_{43}H_{67}NO_{12}$: C, 65.38; H, 8.55; N, 1.77. Found: C, 65.50; H, 9.00; N, 1.74. ^1H NMR: δ 5.60 (s, H_{23}), 5.80 (s, H_{23}). ^{13}C NMR: δ 202.1, 198.3,

194.9, 193.3, 189.1, 169.1, 168.9, 165.1, 164.6, 137.9, 137.2, 130.5, 130.4, 129.7, 129.5, 124.5, 124.1, 98.6, 98.0, 97.6, 95.9, 84.2, 84,1, 78.2, 77.3, 76.0, 75.9, 74.5, 74.2, 73.9, 73.7, 73.6, 72.3, 57.7, 56.6, 56.5, 56.4, 56.3, 56.0, 55.9, 52.9, 51.6, 50.0, 48.8, 48.5, 44.2, 42.9, 41.3, 39.1, 35.0, 34.9, 34.8, 34.6, 34.4, 33.5, 32.5, 32.4, 32.2, 31.2, 30.6, 30.4, 27.7, 27.6, 27.2, 26.2, 25.9, 25.8, 25.0, 24.3, 22.6, 21.5, 21.2, 21.1, 20.1, 15.9, 15.8, 15.7, 15.0, 14.1, 13.9, 11.6, 11.5, 10.33 and 9.89 ppm.

Pyrazoles 3a and 4a. Hydrazine (15 mL) in THF (2 mL) was added into a stirred solution of **7** (0.24 g) in THF (10 mL) at room temperature and refluxed under nitrogen for 3 h. Solvent was removed *in vacuo* and the solid residue purified by silica gel chromatography (50 g) eluting with 50% acetone-hexanes. The semi-pure product was further purified by preparative TLC. Yield: 0.13 g, 54%, mp: 110-116°C. FABMS: Calcd $(M + H)^+$ Calcd for $C_{43}H_{68}N_3O_{10}$ m/e 786, found 786; Calcd $(M + K)^+$ Calcd for $C_{43}H_{67}N_3O_{10}K$ m/e 824, found 824. Anal. Calcd for $C_{43}H_{67}N_3O_{10}\cdot H_2O$: C, 64.24; H, 8.65; N, 5.23. Found: C, 64.12; H, 8.29; N, 5.00. IR (KBr) ν_{max} 3420, 2930, 1740, 1645 and 1450 cm^{-1}. ^1H NMR (DCl-CD_3OD): δ 5.95 (s, H_{23}), 6.15 (s, H_{23}). ^{13}C NMR (DCl-CD_3OD): δ 198.5, 198.1, 170.5, 168.0, 167.3, 153.3, 152.9, 152.7, 152.3, 137.3, 136.6, 131.8, 131.7, 131.3, 130.8, 128.2, 127.6, 104.3, 102.8, 99.6, 99.3, 85.3, 85.2, 81.9, 81.0, 77.9, 76.8, 75.5, 75.2, 75.0, 74.7, 74.6, 73.7, 58.4, 58.2, 57.5, 57.4, 57.1, 56.8, 56.3, 40.4, 38.8, 38.7, 36.9, 36.8, 36.1, 35.0, 34.8, 34.5, 33.5, 32.1, 31.8, 30.5, 28.4, 28.0, 27.3, 21.4, 20.7, 16.3, 16.2, 15.7, 14.8, 14.4, 12.7, 12.0, 11.8 and 11.7 ppm.

N-Methyl-pyrazole (3b). A solution of **7** (0.50 g, 0.63 mmol) and methylhydrazine (51 mL, 0.95 mmol) in absolute ethanol (10 mL) was refluxed under nitrogen for 3 h. Solvent was removed in vacuo and product purified by silica gel chromatography (25 g) eluting with 2% methanol in DCM. Yield: 0.28 g, 56%, mp: 143-146 °C. FABMS: Calcd $(M + H)^+$ for $C_{44}H_{70}N_3O_{10}$ m/e 800, found 800; Calcd $(M + K)^+$ for $C_{44}H_{69}N_3O_{10}K$ m/e 838, found 838. Anal. Calcd for $C_{44}H_{69}N_3O_{10}$: C, 66.06; H, 8.69; N, 5.25. Found: C, 66.18; H, 8.78; N, 4.95. IR (KBr) ν_{max} 3420, 2930, 1740, 1645 and 1450 cm^{-1}. ^1H NMR: δ 5.78 (s, H_{23}), 5.97 (s, H_{23}); 3.82 (s, N-Me), 3.80 (s, N-Me). ^{13}C NMR: δ 195.9, 193.8, 169.0, 168.9, 165.2, 164.8, 155.0, 144.0, 143.9, 134.5, 133.9, 130.8, 130.3, 129.8, 129.7, 129.0, 128.9, 103.4, 102.1, 98.4, 97.6, 84.2, 84.1, 78.0, 77.7, 76.1, 75.4, 74.0, 73.5, 73.3, 72.5, 57.5, 56.8, 56.7, 56.6, 56.5, 56.3, 56.0, 52.6, 49.0, 48.9, 44.2, 39.2, 39.1, 38.7, 36.1, 34.9, 34.8, 34.7, 34.6, 34.5, 34.3, 32.7, 32.6, 32.4, 32.2, 31.2, 30.6, 30.5, 29.7, 28.5, 27.0, 26.5, 26.2, 24.8, 24.4, 21.7, 21.2, 20.0, 15.9, 15.3, 15.2, 14.9, 14.5, 12.8, 12.4, 11.8 and 11.5 ppm.

N-Methyl-pyrazole (4b). A mixture of **7** (0.5 g, 0.63 mmol), methylhydrazine (70 mL, 1.34 mmol) and magnesium sulfate (0.6 g) in dry tetrahydrofuran was stirred at room temperature overnight. The whole reaction mixture was purified by silica gel chromatography (10 g) eluting with 20% acetone in hexanes. Yield of crude products: 0.38 g. The isomers were then separated by HPLC. Yield **4b**: 0.16 g, 30%, m.p. 120-125 °C. FABMS: Calcd $(M + H)^+$ for $C_{44}H_{70}N_3O_{10}$ m/e 800, found 800; Calcd $(M + K)^+$ for $C_{44}H_{69}N_3O_{10}K$ m/e 838, found 838. Anal. Calcd for $C_{44}H_{69}N_3O_{10}$: C, 66.06; H, 8.69; N, 5.25. Found: C, 66.17; H, 8.76; N, 4.90. IR (KBr) ν_{max} 3420, 2910, 2830, 1740, 1645, 1450, 1380 and 1100 cm^{-1}. ^1H NMR: δ 5.80 (s, H_{23}), 5.90 (s, H_{23}); 3.83 (s, N-Me), 3.73 (s, N-Me). ^{13}C NMR: δ 195.8, 192.8, 168.7, 168.6, 165.9, 164.7, 152.8, 152.4, 147.1, 146.3, 135.3, 134.1, 131.7, 131.2, 129.0, 128.4, 127.4, 127.3, 101.3, 100.8, 98.0, 97.6, 96.1, 84.3, 84.2, 80.8, 80.7, 76.2, 75.3, 74.0, 73.9, 73.6, 72.1, 57.1, 56.8, 56.6, 56.5, 56.3, 51.9, 49.2, 48.6, 43.4, 39.2, 36.6, 36.5, 36.1, 36.0, 35.0, 34.9, 34.8, 34.7, 34.6, 34.5, 34.4, 33.6, 32.8, 32.6,

32.4, 32.2, 31.3, 30.7, 29.7, 29.6, 27.3, 27.1, 26.4, 26.0, 24.6, 24.5, 22.6, 21.6, 21.3, 21.2, 20.3, 16.1, 16.0, 15.7, 15.4, 15.0, 14.4, 12.4, 12.1, 11.5 and 11.3 ppm.

Pyrazoles 3c and 4c. Hydrazine (0.064 mg, 2 mmol) was added into a stirred solution of **6** (1.0 g, 1.1 mmole, in 50 mL THF) at room temperature. After being stirred at room temperature for 6 hours, THF was removed *in vacuo*. The solid residue was purified by silica gel chromatography (75 g) eluting with 2% isopropanol in dichloromethane. Yield **3c** and **4c**: 0.80 g, 80% yield. FABMS: Calcd $(M + H)^+$ Calcd for $C_{49}H_{82}N_3O_{10}Si$ m/e 900, found 900; Calcd $(M + K)^+$ Calcd for $C_{49}H_{81}N_3O_{10}SiK$ m/e 938, found 938. The compounds were used in next step without further purification.

Pyrazoles 3d and 4d. A solution of pyrazoles 3c and 4c (0.025 g, 0.028 mmol), diisopropylethylamine (0.007 g, 0.056 mmol) and dimethylsulfate (0.008 g, 0.06 mmol) in THF (0.5 mL) was refluxed under nitrogen for 5 hours. The products were purified by silica gel chromatography (5 g) eluting with ether. Yield: 0.023 g. FABMS: Calcd $(M + H)^+$ Calcd for $C_{50}H_{85}N_3O_{10}Si$ m/e 914, found 914; Calcd $(M + K)^+$ Calcd for $C_{50}H_{84}N_3O_{10}SiK$ m/e 952, found 952. ^1H NMR: δ 5.74 (s, H_{23}), 5.78 (s, H_{23}), 5.87 (s, H-23), 5.95 (s, H-23); 3.71 (s, N-Me), 3.78 (s, N-Me), 3.79 (s, N-Me), 3.82 (s, N-Me).

N-Ethyl-pyrazole (3e). A solution of **7** (0.50 g, 0.63 mmol), ethylhydrazine oxalate (0.143 g, 0.95 mmol) and 4-methylmorpholine (200 mL, 1.80 mmol) in absolute ethanol (10 mL) was refluxed under nitrogen for 45 min. Solvent was removed *in vacuo* and product purified by silica gel chromatography (50 g) eluting with 20% acetone-hexanes. Yield: 0.41 g, 81%. An analytical sample was purified by preparative TLC (3% methanol-DCM), mp: 95-105 °C. FABMS: Calcd $(M + H)^+$ Calcd for $C_{45}H_{72}N_3O_{10}$ m/e 814, found 814; Calcd $(M + K)^+$ for $C_{45}H_{71}N_3O_{10}K$ m/e 852, found 852. Anal. Calcd for $C_{45}H_{71}N_3O_{10}\cdot 0.5H_2O$: C, 65.67; H, 8.82; N, 5.09. Found: C, 65.62; H, 8.58; N, 5.13. IR (KBr) ν_{max} 3420, 2930, 1740, 1645, 1450 and 1390 cm^{-1}. ^1H NMR: δ 5.76 (s, H_{23}), 5.95 (s, H_{23}); 4.05-4.15 (m, CH_2 of N-Et), 1.45 (t, J = 7.5 Hz, CH_3 of N-Et), 1.25 (t, J = 7.5 Hz, CH_3 of N-Et). ^{13}C NMR: δ 195.7, 193.5, 168.9, 168.8, 165.2, 164.8, 155.1, 155.0, 141.7, 134.2, 134.0, 130.9, 130.4, 129.9, 129.7, 129.0, 128.7, 103.1, 102.0, 98.4, 97.5, 84.4, 84.1, 78.6, 78.4, 76.2, 75.4, 74.0, 73.6, 73.5, 73.3, 72.4, 57.5, 56.8, 56.6, 56.5, 56.3, 56.1, 52.6, 49.0, 44.1, 43.7, 43.6, 39.2, 39.1, 38.6, 35.0, 34.9, 34.7, 34.6, 34.3, 32.6, 32.5, 32.4, 32.2, 32.1, 31.2, 30.7, 30.5, 29.4, 28.7, 26.9, 26.6, 26.5, 26.3, 24.8, 24.4, 22.6, 21.8, 21.1, 19.9, 16.0, 15.9, 15.2, 15.1, 15.0, 14.6, 14.1, 13.1, 12.8, 11.8 and 11.5 ppm.

N-Ethyl carboxymethyl-pyrazole (3f). The title compound was prepared from **7**, ethylhydrazinoacetate hydrochloride and 4-methylmorpholine in absolute ethanol as described in the procedure for **3e**. Product was purified by HPLC. Yield: 0.13 g, 24%, mp: 116-122 °C. FABMS: Calcd $(M + H)^+$ Calcd for $C_{47}H_{74}N_3O_{12}$ m/e 872, found 872; Calcd $(M + K)^+$ for $C_{47}H_{73}N_3O_{12}K$ m/e 910, found 910. Anal. Calcd for $C_{47}H_{73}N_3O_{12}$: C, 64.73; H, 8.44; N, 4.82. Found: C, 64.47; H, 8.45; N, 4.58. IR (KBr) ν_{max} 3420, 2930, 1745, 1645, 1450 and 1380 cm^{-1}. ^1H NMR: δ 5.78 (s, H_{23}), 5.98 (s, H_{23}); 4.98, 5.02 (AB quartet, J = 7 Hz, N-CH_2); 5.04, 5.21 (AB quartet, J = 15 Hz, N-CH_2); 4.35-4.48 (m, CH_2 of COOEt); 1.40-1.50 (m, CH_3 of COOEt). ^{13}C NMR: δ 196.0, 193.0, 168.9, 168.7, 168.3, 168.1, 165.2, 164.8, 156.4, 156.2, 145.3, 145.2, 135.0, 133.9, 130.9, 130.5, 130.2, 129.6, 129.1, 128.9, 103.9, 103.0, 98.5, 97.6, 84.2, 84.1, 78.3, 78.2, 76.3, 75.5, 74.1, 73.6, 73.5, 73.3, 73.2, 61.8, 57.5, 56.8, 56.6, 56.5, 56.3, 56.1, 52.5, 51.0, 50.8, 48.9, 44.1, 39.2, 38.8, 35.2, 35.0, 34.9, 34.8, 34.6, 34.5, 34.1, 32.6, 32.4, 32.2, 31.6, 31.2, 30.6, 30.5,

28.8, 28.7, 27.0, 26.6, 26.5, 26.4, 24.8, 24.4, 22.6, 21.7, 21.2, 21.1, 19.7, 16.0, 15.2, 15.1, 14.9, 14.6, 14.1, 13.6, 12.9, 11.8 and 11.5 ppm.

N-(2-Hydroxyethyl)-pyrazole (3g). The title compound was prepared from **7** (0.50 g) and 2-hydroxyethylhydrazine in absolute ethanol as described in the procedure for **3e**. Yield: 0.23 g, 44%, mp: 150-155 °C. FABMS: Calcd (M + H)$^+$ Calcd for $C_{45}H_{72}N_3O_{11}$ m/e 830, found 830; Calcd (M + K)$^+$ for $C_{45}H_{71}N_3O_{11}K$ m/e 868, found 868. Anal. Calcd for $C_{45}H_{71}N_3O_{11}$: C, 65.11; H, 8.62; N, 5.06. Found: C, 64.82; H, 8.71; N, 5.00. IR (KBr) ν_{max} 3440, 2940, 1740, 1645, 1450 and 1380 cm^{-1}. ^{1}H NMR: δ 5.79 (s, H_{23}), 6.00 (s, H_{23}); 4.00-4.15 (m, N-CH_2). ^{13}C NMR: δ 196.7, 193.5, 169.1, 168.9, 165.4, 165.1, 155.7, 155.6, 144.9, 144.8, 134.7, 134.3, 130.8, 130.3. 130.0, 129.6, 128.7, 128.5, 103.2, 101.9, 98.2, 97.9, 84.2, 84.1, 78.6, 78.1, 76.1, 75.4, 74.0, 73.5, 73.4, 72.5, 61.6, 61.1, 57.4, 56.8, 56.6, 56.5, 56.3, 56.0, 52.5, 50.1, 50.0, 49.1, 49.0, 44.2, 39.2, 39.0, 38.7, 35.0, 34.9, 34.8, 34.7, 34.6, 34.2, 32.5, 32.4, 32.3, 32.2, 31.5, 31.1, 30.6, 30.5, 29.6, 28.5, 27.0, 26.6, 26.4, 26.1, 25.2, 24.7, 24.3, 22.6, 21.8, 21.2, 21.1, 20.1, 15.9, 15.8, 15.3, 15.2, 14.9, 14.5, 14.1, 13.2, 12.6, 11.7 and 11.5 ppm.

N-Phenylpyrazole (3h). The title compound was prepared from **7** (0.50 g) and phenylhydrazine in absolute ethanol as described in the procedure for **3e**. Yield: 0.26 g, 48%, mp: 150-155 °C. FABMS: Calcd (M + H)$^+$ Calcd for $C_{49}H_{72}N_3O_{10}$ m/e 862, found 862; Calcd (M + K)$^+$ for $C_{49}H_{71}N_3O_{10}K$ m/e 900, found 900. Anal. Calcd for $C_{49}H_{71}N_3O_{10}$: C, 68.27; H, 8.30; N, 4.87. Found: C, 67.86; H, 8.25; N, 4.78. IR (KBr) ν_{max} 3440, 2930, 1742, 1645, 1500, 1450 and 1380 cm^{-1}. ^{1}H NMR: δ 7.40-7.52 (m, Ph); 5.90 (s, H_{23}), 6.17 (s, H_{23}). ^{13}C NMR: δ 195.5, 193.4, 168.6, 165.3, 164.6, 156.6, 156.4, 145.0, 144.7, 139.9, 139.8, 134.9, 134.3, 130.9, 130.4, 129.3, 129.2, 129.0, 128.8, 128.4, 128.2, 126.6, 126.4, 104.3, 103.2, 98.4, 97.4, 84.2, 84.1, 78.0, 77.9, 76.2, 75.2, 73.9, 73.5, 73.3, 72.4, 57.5, 56.9, 56.7, 56.6, 56.5, 56.1, 52.5, 49.1, 49.0, 44.2, 39.3, 39.2, 38.6, 35.0, 34.9, 34.8, 34.7, 34.6, 34.2, 32.7, 32.6, 32.4, 32.0, 31.9, 31.1, 30.6, 30.5, 29.8, 28.8, 26.8, 26.6, 26.3, 24.8, 24.5, 21.9, 21.3, 21.1, 20.0, 16.0, 15.3, 15.1, 14.2, 14.0, 12.2, 12.1, 11.8 and 11.4 ppm.

N-(4-methylsulfonylphenyl)-pyrazole (3i and 4i). A solution of **7** (0.50 g, 0.63 mmol) and 4-methylsulfonylphenylhydrazine (0.27 g, 1.4 mmol) in THF (10 mL) was refluxed under nitrogen for 4 days. The reaction mixture was partitioned between water and ethyl acetate, the organic phase was washed with 1 N HCl (aq), saturated brine, dried over magnesium sulfate and solvent removed *in vacuo*. The products (**3i** and **4i**) were purified by silica gel chromatography (60 g) eluting with 3.5% methanol-DCM, 0.36 g, 61%. The isomers were separated by RPHPLC. Yield **3i**, 0.15 g, mp: 199 °C. FABMS: Calcd (M + H)$^+$ Calcd for $C_{50}H_{74}N_3O_{12}S$ m/e 940, found 940; Calcd (M + K)$^+$ for $C_{50}H_{73}N_3O_{12}SK$ m/e 978, found 978. Anal. Calcd for $C_{50}H_{73}N_3O_{12}S$: C, 63.87; H, 7.83; N, 4.47. Found: C, 63.18; H, 7.90; N, 4.12. IR (KBr) ν_{max} 3440, 2930, 1740, 1645, 1600, 1500, 1450 and 1380 cm^{-1}. ^{1}H NMR: δ 7.73 (d, J = 10 Hz, Ar-H), 7.72 (d, J = 10 Hz, Ar-H); 8.04 (d, J = 10 Hz, Ar-H), 8.08 (d, J = 10 Hz, Ar-H); 6.04 (s, H_{23}), 6.24 (s, H_{23}); 3.08 (bs, SO_2-CH_3). ^{13}C NMR: δ 195.7, 193.1, 168.6, 168.4, 165.6, 164.6, 158.1, 157.7, 145.3, 145.1, 144.5, 144.4, 139.7, 139.5, 135.3, 134.7, 130.4, 129.8, 129.7, 129.6, 128.7, 128.6, 128.1, 128.0, 126.6, 126.3, 105.9, 104.9, 98.2, 97.3, 84.1, 84.0, 77.5, 77.4, 77.0, 76.6, 76.2, 75.2, 73.9, 73.5, 73.3, 72.5, 57.4, 56.8, 56.7, 56.6, 56.5, 56.3, 56.1, 53.4, 52.2, 49.0, 48.8, 44.6, 44.0, 39.2, 38.6, 34.8, 34.6, 34.0, 32.7, 32.6, 32.1, 31.9, 31.1, 30.6, 30.5, 29.6, 29.1, 26.8, 26.6, 26.2, 25.3, 24.6, 24.4, 21.8, 21.2, 20.1, 16.0, 15.3, 15.2, 14.5, 14.2, 12.5, 12.4, 11.7 and 11.4 ppm.

Yield **4i**, 0.05 g, mp: 140 °C. FABMS: Calcd $(M + H)^+$ Calcd for $C_{50}H_{74}N_3O_{12}S$ m/e 940, found 940; Calcd $(M + K)^+$ for $C_{50}H_{73}N_3O_{12}SK$ m/e 978, found 978. Anal. Calcd for $C_{50}H_{73}N_3O_{12}S \cdot H_2O$: C, 62.67; H, 7.89; N, 4.39. Found: C, 62.31; H, 7.69; N, 4.38. IR (KBr) ν_{max} 3440, 2930, 1740, 1645, 1600, 1500, 1450 and 1380 cm^{-1}. ^1H NMR: δ 7.70 (d, J = 10 Hz, Ar-H), 7.71 (d, J = 10 Hz, Ar-H); 8.02 (d, J = 10 Hz, Ar-H), 8.12 (d, J = 10 Hz, Ar-H); 6.05 (s, H_{23}), 6.19 (s, H_{23}); 3.10 (s, SO_2-CH_3), 3.07 (s, SO_2-CH_3). ^{13}C NMR: δ 195.8, 192.5, 168.4, 168.3, 166.2, 164.5, 155.8, 155.4, 148.6, 148.1, 144.7, 144.5, 139.4, 139.1, 136.0, 135.0, 131.6, 131.0, 129.7, 129.1, 128.8, 128.6, 128.5, 127.1, 126.8, 126.2, 126.1, 103.8, 103.6, 97.9, 97.0, 84.2, 80.5, 80.2, 76.1, 74.9, 73.8, 73.6, 73.5, 73.2, 72.0, 57.1, 56.9, 56.6, 56.5, 56.3, 51.6, 49.0, 48.3, 44.6, 43.5, 39.2, 37.0, 36.3, 34.9, 34.8, 34.7, 34.6, 34.2, 33.6, 32.8, 31.9, 31.2, 30.7, 30.2, 28.0, 26.9, 26.5, 26.1, 24.5, 21.8, 21.2, 20.9, 20.3, 16.3, 16.1, 16.0, 15.3, 14.9, 14.4, 11.8, and 11.4 ppm.

N-Benzylpyrazole (3j). The title compound was prepared from **7** (0.40 g) and benzylhydrazine in absolute ethanol as described in the procedure for **3e**. Yield: 0.23 g, 52%, mp: 153-158 °C. FABMS: Calcd $(M + H)^+$ Calcd for $C_{50}H_{74}N_3O_{10}$ m/e 876, found 876. Anal. Calcd for $C_{50}H_{73}N_3O_{10} \cdot H_2O$: C, 67.16; H, 8.45; N, 4.70. Found: C, 67.54; H, 8.35; N, 4.35. IR (KBr) ν_{max} 3440, 2940, 1740, 1450, and 1380 cm^{-1}. ^1H NMR: δ 6.98 (bd, J = 7.5 Hz, Ph-H); 7.20-7.35 (m, Ph-H); 5.72 (s, H_{23}), 6.00 (s, H_{23}). ^{13}C NMR: δ 196.0, 192.7, 168.8, 168.5, 165.3, 164.8, 155.7, 155.4, 144.5, 135.0, 133.9, 131.0, 130.5, 129.6, 129.3, 128.9, 128.6, 128.4, 127.5, 126.1, 103.7, 103.1, 98.5, 97.5, 84.3, 84.1, 78.3, 78.1, 76.3, 75.4, 74.0, 73.6, 73.5, 73.4, 72.2, 71.9, 57.4, 57.1, 56.9, 56.6, 56.5, 56.3, 56.1, 53.4, 52.4, 48.9, 44.0, 39.2, 38.7, 34.8, 34.6, 32.6, 32.5, 32.2, 32.1, 31.1, 30.6, 29.6, 29.0, 26.9, 26.3, 24.8, 24.4, 21.8, 21.2, 20.1, 19.8, 16.0, 15.2, 14.5, 14.3, 13.3, 13.0, 11.8 and 15.5 ppm.

N-(2-Phenylethyl)pyrazole (3k). The title compound was prepared from **7** (0.50 g), 2-phenylethylhydrazine sulfuric acid salt and 4-methylmorpholine in absolute ethanol as described in the procedure for **3e**. Yield: 0.28 g, 50%, mp: 95-103 °C. FABMS: Calcd $(M + H)^+$ for $C_{51}H_{76}N_3O_{10}$ m/e 890, found 890; Calcd $(M + K)^+$ for $C_{51}H_{75}N_3O_{10}K$ m/e 928, found 928. Anal. Calcd for $C_{51}H_{75}N_3O_{10} \cdot H_2O$: C, 67.44; H, 8.54; N, 4.63. Found: C, 67.13; H, 8.31; N, 4.54. IR (KBr) ν_{max} 3440, 2940, 1740, 1450, and 1380 cm^{-1}. ^1H NMR: δ 6.80-7.02 (m, Ph-H); 7.20-7.30 (m, Ph-H); 5.70 (s, H_{23}), 5.85 (s, H_{23}). ^{13}C NMR: δ 195.9, 191.5, 168.9, 168.7, 165.3, 164.8, 156.0, 155.4, 138.8, 138.6, 130.8, 130.3, 129.5, 129.3, 128.8, 128.6, 128.5, 126.6, 126.5, 102.6, 101.7, 98.4, 97.6, 84.2, 84.1, 78.3, 78.2, 76.2, 75.5, 74.1, 73.6, 73.5, 73.4, 72.2, 58.4, 57.4, 56.8, 56.7, 56.6, 56.5, 56.3, 56.1, 52.4, 50.6, 50.5, 49.0, 48.9, 44.0, 39.2, 38.7, 37.2, 37.1, 35.1, 34.9, 34.8, 34.6, 34.5, 34.1, 32.6, 32.5, 32.2, 32.1, 31.9, 31.6, 31.2, 30.6, 30.5, 29.8, 29.1, 27.0, 26.5, 26.1, 24.7, 24.4, 21.8, 21.2, 21.1, 19.8, 17.1, 16.0, 15.9, 15.2, 14.8, 14.5, 14.1, 12.8, 12.5, 11.8 and 11.5 ppm.

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References:

1. Arai, T; Koyama, Y.; Suenaga, T.; Honda, H. *J. Antibiot.*, **1962**, *Ser. A. 15*, 231-232.
2. Morisaki, M.; Arai, T. *J. Antibiot.*, **1992**, *45*, 126-128.

3. Hatanaka, H.; Kino, T.; Miyata, S.; Inamura, N.; Kuroda, A.; Goto, T.; Tanaka, H.; Okuhara, M. *J. Antibiot.*, **1988**, *41*, 1592-1601.
4. Hatanaka, H.; Kino, T.; Asano M. *J. Antibiot (Tokyo)*, **1989**, *42*, 620.
5. (a) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M.; Taga, T. *J. Am. Chem. Soc.*, **1987**, *109*, 5031-5033.
(b) Goto, T.; Kino, T.; Hatanaka, H.; Okuhara, M.; Kohsaks, M.; Aoki, H.; Imanaka, H. *Tranaplantation Proceedings*, **1991**, *23*(6), 2713.
6. Coispeau G.; Elguero, *J. Bull. Soc. Chim. Fr.*, **1970**, 2717.
7. Compound **7** exists as a mixture of rotamers in $CDCl_3$. ^{13}C-NMR ($CDCl_3$), assigned resonances of major rotamer: δ 198.3 (C-22), 189.3 (C-24), 137.2 (C-19), 124.5 (C-20), 96.0 (C-23), 78.3 (C-26), 51.5 (C-21), 48.8 (C-18), 41.1 (C-25). Minor rotamer: δ 201.8 (C-22), 193.3 (C-24), 137.9 (C-19), 124.2 (C-20), 97.6 (C-23), 77.3 (C-26), 48.5 (C-21), 49.9 (C-18), 42.9 (C-25).
8. Elguero J.; Martin, C.; Katritzky, A.R.; Linda, P. *Adv. Heterocycl. Chem., Suppl. 1*, **1976**, 266-296.
9. (a) Hecht, S.M.; Werner, D.; Traficante, D.D.; Sundaralingam, M.; Prusiner, P.; Ito, T.; Sakurai, T. *J. Org. Chem.*, **1975**, *40*, 1348; Levy, G.C.; Pekh, T.
(b) Srinivasan, P.R. *Org. Magn. Reson.*, **1980**, *14*, 129.
10. Elguero, J.; Gonzalez, E.; Jacquier, R. *Bull. Soc. Chim. Fr.*, **1968**, 4003; 5009.
11. Lazaro, R.; Mathiew, D.; Fhan Tan Luu, R.; Elquero, J. *Bull. Soc. Chim. Fr.*, **1977**, 1163.
12. Katritzky, A.R.; Rees, C.W.; Potts, K. T. *Comprehensive Heterocyclic Chemistry, 5*, p.229; Pergamon Press: Oxford, 1984.
13. Compounds **3b** exists as a mixture of rotamers in $CDCl_3$. ^{13}C-NMR ($CDCl_3$), assigned resonances of major rotamer: δ 169.0 (C-1), 56.7 (C-2), 26.1 (C-3), 21.7 (C-4), 24.3 (C-5), 39.1 (C-6), 164.8 (C-8), 195.9 (C-9), 97.5 (C-10), 34.5 (C-11), 15.9 (11-CH_3), 32.5 (C-12), 26.9 (C-17), 21.1 (17-CH_3), 48.9 (C-18), 133.9 (C-19), 15.1 (19-CH_3), 128.7 (C-20), 154.9 (C-22), 102.0 (C-23), 144.0 (C-24), 32.3 (C-25), 12.8 (25-CH_3), 77.8 (C-26), 130.7 (C-27), 14.8 (27-CH_3), 129.8 (C-28), 34.8 (C-29), 34.8 (C-30), 84.1 (C-31), 31.2 (C-33), 30.6 (C-34), 29.6 (C-35), 11.5 (C-36), 36.0 (N-CH_3). Minor rotamer: δ 168.8 (C-1), 52.5 (C-2), 26.5 (C-3), 21.1 (C-4), 24.7 (C-5), 44.1 (C-6), 165.2 (C-8), 193.7 (C-9), 98.3 (C-10), 34.2 (C-11), 15.9 (11-CH_3), 32.5 (C-12), 72.4 (C-14), 76.0 (C-15), 34.7 (C-16), 26.5 (C-17), 20.0 (17-CH_3), 48.9 (C-18), 134.4 (C-19), 15.2 (19-CH_3), 128.7 (C-20), 154.9 (C-22), 103.4 (C-23), 144.1 (C-24), 32.6 (C-25), 12.3 (25-CH_3), 130.2 (C-27), 14.5 (27-CH_3), 129.6 (C-28), 84.1 (C-31), 31.2 (C-33), 30.5 (C-34), 28.5 (C-35), 11.7 (C-36), 36.0 (N-CH_3). ^1H-NMR ($CDCl_3$), assigned resonances of major rotamer: δ 4.03 (H-2), 2.08 (H-3), 1.75 (H-4), 1.68, 1.43 (H-5), 4.37, 2.62 (H-6), 2.29 (H-11), 0.91 (11-CH_3), 2.08, 1.43 (H-12), 3.32 (H-13), 3.48 (H-14), 3.57 (H-15), 1.64, 0.93 (H-16), 1.52 (H-17), 0.98, (17-CH_3), 2.24, 1.80 (H-18), 1.63 (19-CH_3), 5.17 (H-20), 3.47 (H-21), 5.76 (H-23), 3.08 (H-25), 1.22 (25-CH_3), 5.18 (H-26), 1.77 (27-CH_3), 5.17 (H-28), 2.33 (H-29), 2.06, 1.02 (H-30), 3.04 (H-31), 3.41 (H-32), 2.01, 1.38 (H-33), 1.64, 1.09 (H-34), 1.82, 1.44 (H-35), 0.84 (H-36), 3.82 (N-CH_3). Minor rotamer: δ 5.00 (H-2), 2.30, 1.64 (H-3), 1.56, 1.48 (H-5), 3.51, 2.88 (H-6), 2.35 (H-11), 0.93 (11-CH_3), 2.10, 1.50 (H-12), 3.48 (H-13), 3.80 (H-14), 3.58 (H-15), 1.64, 1.23 (H-16), 1.71 (H-17), 0.90 (17-CH_3), 2.10, 1.96 (H-18), 1.54 (19-CH_3), 5.16 (H-20),

3.38 (H-21), 5.96 (H-23), 3.08 (H-25), 1.24 (25-CH_3), 5.25 (H-26), 1.74 (27-CH_3), 5.17 (H-28), 3.04 (H-31), 1.90, 1.48 (H-35), 0.87 (H-36), 3.80 (N-CH_3).

Compounds **4b** also exists as a mixture of rotamers in $CDCl_3$. ^{13}C-NMR ($CDCl_3$), assigned resonances of major rotamer: δ 168.5 (C-1), 51.9 (C-2), 26.2 (C-3), 21.1 (C-4), 24.6 (C-5), 43.4 (C-6), 165.9 (C-8), 195.9 (C-9), 98.0 (C-10), 33.5 (C-11), 16.0 (11-CH_3), 32.7 (C-12), 73.6 (C-13), 56.3 (13-OCH_3), 72.0 (C-14), 76.2 (C-15), 57.1 (15-OCH_3), 34.9 (C-16), 27.2 (C-17), 20.2 (17-CH_3), 48.5 (C-18), 135.4 (C-19), 15.7 (19-CH_3), 127.2 (C-20), 36.6 (C-21), 147.4 (C-22), 101.4 (C-23), 152.7 (C-24), 34.5 (C-25), 11.9 (25-CH_3), 80.7 (C-26), 131.1 (C-27), 14.4 (27-CH_3), 128.4 (C-28), 34.8 (C-29), 35.0 (C-30), 84.2 (C-31), 56.7 (31-OCH_3), 73.6 (C-32), 31.2 (C-33), 30.6 (C-34), 29.6 (C-35), 11.5 (C-36), 35.9 (N-CH_3). Minor rotamer: δ 168.5 (C-1), 21.6 (C-4), 164.6 (C-8), 192.8 (C-9), 97.5 (C-10), 75.3 (C-15), 27.3 (C-17), 49.2 (C-18), 134.3 (C-19), 15.4 (19-CH_3), 127.1 (C-21), 146.4 (C-22), 100.8 (C-23), 152.3 (C-24), 34.3 (C-25), 12.0 (25-CH_3), 80.7 (C-26), 131.7 (C-27), 15.0 (27-CH_3), 128.9 (C-28), 84.2 (C-31), 30.7 (C-34), 29.6 (C-35), 11.3 (C-36), 36.1 (N-CH_3). ^{1}H-NMR ($CDCl_3$), assigned resonances of major rotamer: δ 4.71 (H-2), 2.33, 1.63 (H-3), 1.73, 1.27 (H-4), 1.56, 1.46 (H-5), 3.50, 2.89 (H-6), 2.17 (H-11), 0.98 (11-CH_3), 2.07, 1.46 (H-12), 3.43 (H-13), 3.38 (13-OCH_3), 3.73 (H-14), 3.51 (H-15), 3.31 (15-OCH_3), 1.61, 1.17 (H-16), 1.62 (H-17), 0.92 (17-CH_3), 2.09, 2.00 (H-18), 1.58 (19-CH_3), 5.30 (H-20), 3.48 (H-21), 5.89 (H-23), 3.20 (H-25), 1.17 (25-CH_3), 5.48 (H-26), 1.73 (27-CH_3), 5.07 (H-28), 2.33 (H-29), 2.07, 0.99 (H-30), 3.03 (H-31), 3.42 (31-OCH_3), 3,41 (H-32), 2.00, 1.38 (H-33), 1.62, 1.08 (H-34), 0.86 (H-36), 3.73 (N-CH_3). Minor rotamer: δ 3.49 (H-15), 1.52 (H-17), 0.98 (17-CH_3), 2.25, 1.82 (H-18), 1.62 (19-CH_3), 5.19 (H-20), 3.49 (H-21), 5.79 (H-23), 3.18 (H-25), 1.17 (25-CH_3), 5.49 (H-26), 1.76 (27-CH_3), 5.10 (H-28), 2.43 (H-29), 0.84 (H-36), 3.82 (N-CH_3).

14. Walker and Stuart, *Acta Cryst.*, **1982**, *A39*, 158-166.
15. Sheldrich, G.M. SHELX 76, *A program for Crystal Structure Determination*; University of Cambridge, **1976**.
16. TEXRAY Structure Analysis Package, Molecular Structure Corporation **1985**.