Synthesis and Biological Evaluation of New Fragments from Kirromycin Antibiotic

P. TAVECCHIA*, A. MARAZZI, C. DALLANOCE, A. TRANI, I. CICILIATO,
P. FERRARI, E. SELVA and R. CIABATTI

Lepetit Research Center,
Via R. Lepetit 34, 21040 Gerenzano (VA), Italy

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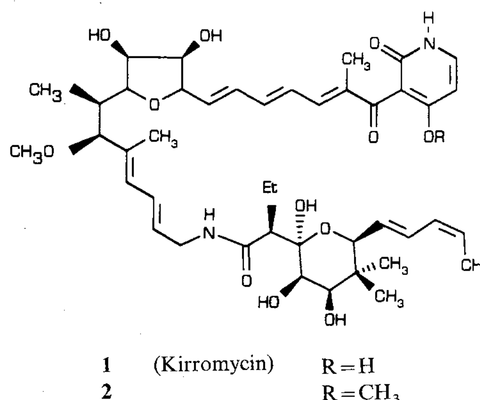
New *N*-acyl derivatives of 1-*N*-desmethyl goldinamine were obtained from degradation of kirromycin. Periodate-oxidation of these derivatives provided new aldehydic fragments that were further elaborated. Both *N*-phenyl ureido and *N*-phthalimido derivatives of 1-*N*-desmethyl goldinamine are able to inhibit bacterial protein synthesis in cell-free assay and are active against whole microorganisms, although with lower potency than kirromycin. The derivatives from the aldehydic fragments are totally inactive.

Kirromycin **1** (Fig. 1) is a natural microbial product, produced by fermenting *Streptomyces* sp. and *Actinoplanes* sp. strains. Compound **1** belongs to the "elfamycin" class of antibiotics. Elfamycins inhibit bacterial protein synthesis by binding elongation factor Tu (EF-Tu) to form a non-dissociable ribosome/EF-Tu/antibiotic complex[1,2]. They are characterized by a restricted spectrum of antibacterial activity. These antibiotics are selectively active against Streptococci, anaerobes and some Gram-negative pathogens such as *Neisseria gonorrhoeae* and *Haemophilus influenzae*[3,4]. Membrane permeability is a critical factor in *Escherichia coli* which is insensitive to **1** but has a kirromycin-sensitive EF-Tu[5,6]. On the contrary, *Staphylococcus aureus* is insensitive because its EF-Tu is kirromycin-resistant[7]. Extensive and elegant chemical degradation work was performed on kirromycin and related microbial products to elucidate the structure[8]. Information on structure-activity relationships has been derived from degradation studies, from the comparison of the antimicrobial activity of different elfamycins produced by microorganisms and from derivatives prepared from aurodox and kirromycin[9,10]. In particular, an aldehydic fragment obtained from periodate oxidation of kirromycin was claimed to possess all the effects of the natural antibiotic in cell-free experiments although endowed with lower potency than kirromycin[11]. This aldehydic fragment lacks the pyridone-trienic-tetrahydro furane moiety suggesting that this part of the molecule is not strictly essential for the binding and for the biological activity. Indeed, NMR studies showed that the pyridone ring of kirromycin is not intimately involved in the binding to EF-Tu[12]. Additional indications derive from naturally occuring elfamycins. The polyketide core structure of kirromycin is conserved in aurodox, heneicomycin, SB22484 and efrotomycin. Structures of antibiotics factumycin, A73A, UK69753 and kirrothricin appear to result from the opening of the tetrahydrofuran ring. Antibiotics L-681,217, phenelfamycins, ganefromycin (LL-E19020) and A83016F lack the pyridone moiety. All these antibiotics show comparable antimicrobial profile and mode of action. These facts suggest that the pyridone moiety and the tetrahydrofuran ring are not essential for the activity. We prepared degradation products of kirromycin and some of their derivatives and evaluated their biological activities. The aim was to acquire information about the minimal structural requirement of elfamycins essential to maintain their biological activity.

Chemistry

We used for our chemical work kirromycin **1** that was obtained by fermenting a kirromycin producer microorganism *Actinoplanes* sp. A8924[13]. It was reported[8] that kirromycin 4-*O*-methyl ether **2** underwent a selective

Fig. 1. Molecular structure of kirromycin (**1**) and its 4-*O*-methyl derivative (**2**).

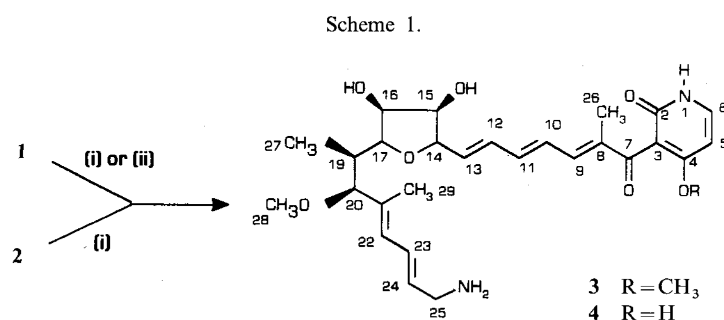


1 (Kirromycin) R=H
2 R=CH_3

cleavage of the amide bond when exposed to acetic acid at room temperature for 4 days, or when heated on the steam bath for 30 minutes. This reaction provided 4-*O*-methyl *N*-desmethyl goldinamine **3** as its acetic acid salt (Scheme 1) with a yield of about 20% for the two chemical steps (protection/hydrolysis). We studied the amidic cleavage of the unprotected natural compound **1** to generate *N*-desmethyl goldinamine **4** (Scheme 1). The best results were achieved when **1** reacted in acetic acid at room temperature for 114 hours or in dioxane at 35°C for 22 hours in presence of formic acid 99%. In both cases compound **4** was recovered in good yield and acceptable purity (75% HPLC) after a simple "work up". Prolonged reaction time or higher temperatures gave lower yields or more side products. Reaction of **1** with either potassium hydrogen sulfate, ammonium chloride, citrate buffer, or camphorsulfonic acid in dioxane, or aqueous dioxane at 25~60°C afforded low yields (0~26%) of **4**. The ^{1}H and ^{13}C NMR spectra of compound **4** formate salt are in agreement with the previously reported data[14] (Table 1).

The amino group of **4** was then derivatized. Acetylation of **4** (pyridine/acetic anhydride/4-dimethylamino pyridine) followed by overnight treatment with metha-

Scheme 1.



(i) Acetic acid, R.T.; (ii) dioxane-HCOOH 99%, 35°C.

Table 1. ^{1}H and ^{13}C NMR data of compound **4**.

Position	Coupling	^{1}H NMR[a,b]	*J* (Hz)	^{13}C NMR[a]	Long range
1	$HCOO^-$	8.3 s		163.9	
2	C=O			165.0	H-6
3	C			111.5	H-5
4	C			162.0	H-6
5	CH	5.95 d	7.2	99.2	H-6
6	CH	7.23 d	7.2	136.2	H-5
7	C=O			196.0	H-26, H-9
8	C			136.4	H-26, H-10
9	=CH	6.83 d	10.1	140.0	H-26, H-10
10	=CH	6.62 dd	15.2~10.1	128.5	H-12
11	=CH	6.59 dd	15.2~9.7	139.9	H-13, H-10, H-9
12	=CH	6.34 dd	15.2~9.7	131.6	H-14, H-10
13	=CH	5.96 m		136.5	H-15
14	CH	4.16 dd	7.6~6.1	79.8	H-16, H-13, H-12
15	CH	4.12 dd	6.1~4.6	73.2	H-13
16	CH	4.08 dd	6.1~4.6	72.6	H-19, H-17, H-14
17	CH	3.80 dd	5.1~4.6	80.3	H-27, H-20, H-16, H-15
19	CH	2.06 ddq	7.2~9.3~4.6	35.0	H-27, H-20
20	CH	3.35 d	9.3	89.3	H-29, H-28, H-27, H-22
21	C			137.9	H-29, H-23
22	=CH	6.03 d	11.0	128.1	H-29, H-24, H-20
23	=CH	6.64 dd	15.1~11.0	130.6	H-25
24	=CH	5.71 dt	15.1~6.4	125.5	H-25, H-22
25	CH_2	3.50 m		40.6	H-24, H-23
26	CH_3	1.86 s		11.5	
27	CH_3	0.74 d	7.2	12.6	
28	CH_3	3.06 s		55.8	
29	CH_3	1.64 s		11.3	

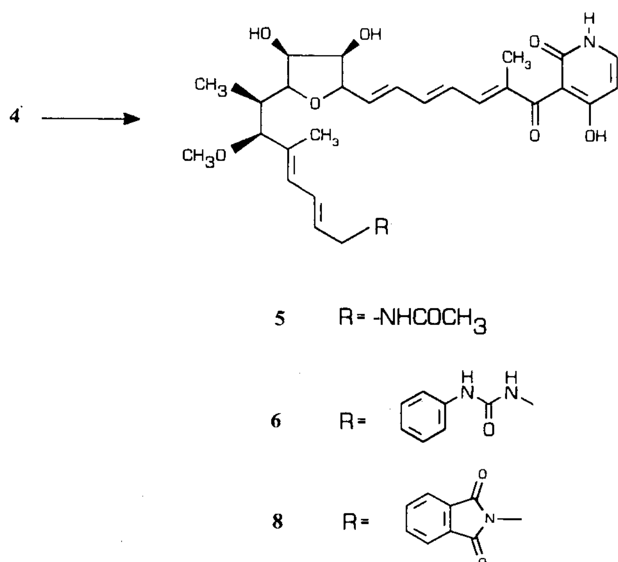
[a] Chemical shifts (ppm) in DMSO-d_6.
[b] The OH, NH_2 peaks and the pyridone NH peak are not observed.

nolic-ammonia solution gave the *N*-acetyl derivative **5** in poor yield.

The reaction of compound **4** with phenyl isocyanate in dimethylformamide gave in good yield the *N*-phenylureido derivative **6**. Attempts to obtain *N*-phthalimido derivative **8** according to the classical methodology[15] (phthalic anhydride, $CHCl_3$, 70°C) yielded a complex reaction mixture. Instead, compound **8** was obtained under mild conditions from **4** by using *N-tert*-butoxycarbonyl phthalimide **7** in the presence of triethylamine at room temperature. Reagent **7** was prepared from phthalimide, di-*tert*-butyldicarbonate and 4-dimethylaminopyridine as a catalyst in dioxane.

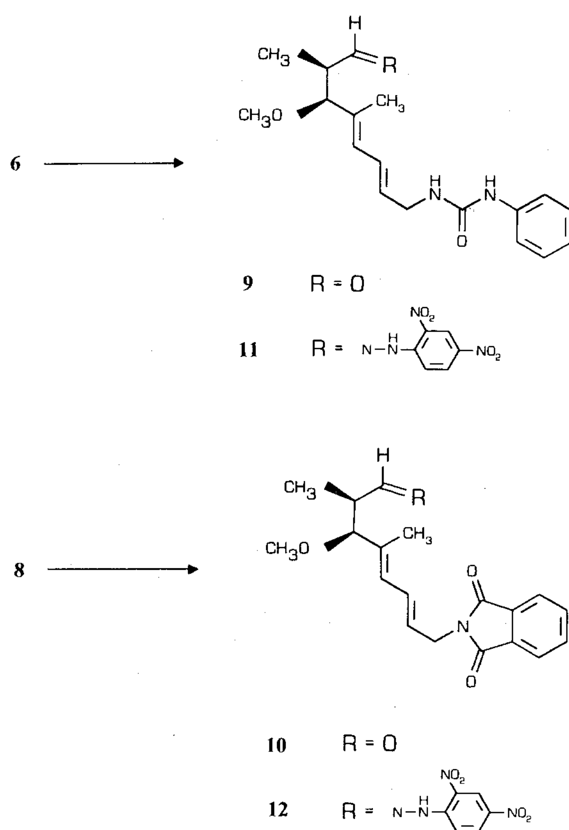
The periodate-oxidation of compound **6** was, at first, performed following the procedure reported for goldinamine acetate salt[8]. A mixture of products was obtained containing mainly the compound cleaved at the tetrahydrofuran glycol moiety as indicated by mass spectroscopy analyses. Additional evidence that the periodate-oxidative step did not occur according to HESSE-MIX[16] criteria, was the lack of the transient red-wine color, a marker for the over oxidation after the initial glycol cleavage. It appeared that the *N*-protected goldinamine behaved differently from the goldinamine acetate salt. Considering that the main difference was the "ionization status" of the amino group, we were led to speculate that goldinamine acetate salt could have behaved as a buffer in the reaction mixture. Indeed, when compound **6** was submitted to the periodate-oxidation in the presence of ammonium acetate, an immediate a transient red-wine color appeared and the aldehydic fragment **9** was isolated. The periodate-oxidation of **6** worked also in the presence of ammonium formate and acetic acid. On the contrary, the periodate-oxidation in the presence of potassium acetate, or phosphate buffer (pH = 7) did not work. This suggests that the pH of the reaction mixture is critical in forming the enol necessary for further oxidation. The *N*-phtalimido aldehyde **10** was obtained from **8** under the same conditions. Both compounds **9** and **10** were smoothly transformed into their 2,4-dinitrophenylhydrazones **11** and **12**. The reduction of compound **9** with sodium borohydride and the reaction with 4-methyl-1-aminopiperazine gave the corresponding alcohol **13** and hydrazone **14** respectively in good yields. Two carbon homologation of aldehydes **9** and **10** by a Wittig-Horner reaction with triethyl phosphonacetate produced the corresponding α-β unsaturated esters **15** and **17**, respectively. The two new olefinic hydrogens at 7.02 ppm (dd) and 5.84 ppm (d) show a coupling constant of 15 ~ 16 Hz indicating an *E* (*trans*) configuration. Compound **15** was then treated with NaOH 1 N in dioxane

Scheme 2.



5, Pyridine-acetic anhydride, DMAP (cat.) then NH_3/ethanol; **6**, DMF-dioxane, NEt_3, phenyl isocyanate; **8**, DMF-dioxane, NEt_3, *N-tert*-butoxycarbonylphthalimide (**7**).

Scheme 3.



9 and **10**, Dioxane-MeOH-water, ammonium acetate, sodium metaperiodate; **11** and **12**, 2,4-dinitrophenylhydrazine, EtOH.

Scheme 4.

13, Sodium borohydride, EtOH; 14, THF, 4-methyl-1-aminopiperazine.

15 R=Et

16 R=H

15, THF, NaH 60%, triethyl phosphonacetate; 16, dioxane, NaOH 1 N.

to provide the corresponding free carboxylic acid **16**.

Biological Evaluation

Activities of the kirromycin derivatives against microorganisms and on poly(U)-directed protein synthesis are reported in Tables 2 and 3, respectively.

The *N*-desmethyl goldinamine **4** and its acetyl derivative **5** were completely inactive in both systems. These results are in line with literature data[9]. Compounds **6** and **8**, however, showed significant activity against test microorganisms, although lower than that of **1**. These compounds also showed detectable cross-resistance with **1** on *Enterococcus hirae* ATCC 8043 mutant strain resistant to kirromycin[17] (Table 3). This suggests that these compounds maintain the EF-Tu molecular target. In cell-free experiments, compounds **6** and **8** inhibited bacterial protein synthesis with a potency about ten and one hundred time less than that of **1**. This is likely to be due to a decreased affinity of compounds **6** and **8** for EF-Tu. It was reported[18] that kirromycin altered the rate of EF-Tu cleavage by trypsin. Fig. 2 shows that compound **6** did not posses the kirromycin effects on the kinetics of proteolytic cleavage. A low affinity for EF-Tu is indeed consistent with this behaviour.

Compounds **6** and **8** were further cleaved at the tetrahydrofuran ring yielding the corresponding aldehydes that were then transformed. The aldehydic derivatives **13**, **14**, **15**, **16** and **17** showed no biological activity. Only compound **11** was marginally active on the cell free system of protein synthesis.

Scheme 5.

(i) THF, NaH 60%, triethyl phosphonacetate.

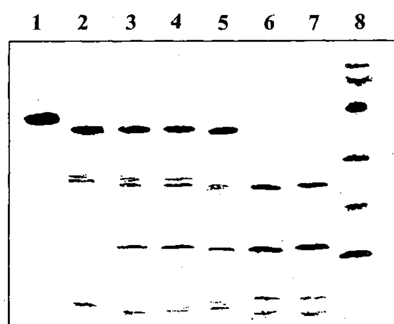
Table 2. Cell free protein synthesis of kirromycin and related derivatives.

Compounds	1	4	5	6	8	11	12	13	14	15	16	17
IC_{50} (μg/ml)	0.14	$\gg$125	$\gg$125	1.76	17	117	$\gg$128	$\gg$128	$\gg$128	$\gg$128	$\gg$128	$\gg$128

Table 3. Antibacterial activity (MIC, μg/ml) of selected compounds.

Strains	1	4	5	6	8	11	13	14	15	16	17
S. aureus Smith	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
S. pyogenes C203	4	>128	>128	64	>128	>128	>128	>128	>128	>128	>128
S. pneumoniae UC41	0.25	>128	>128	8	>128	>128	>128	>128	>128	>128	>128
E. hirae ATCC 8043	1	>128	>128	16	64	>128	>128	>128	>128	>128	>128
E. hirae ATCC 8043 res.	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
E. faecium NCTC 7171	2	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
N. gonorrhoeae ISM68/126	0.06	>128	>128	1	4	>128	>128	>128	>128	>128	>128
H. influenzae type B ATCC 19418	4	>128	>128	128	>128	>128	>128	>128	>128	>128	>128
M. catarrhalis ATCC 8176	0.06	>128	>128	8	4	>128	>128	64	32	>128	>128
E. coli SKF 12140	≥128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
E. coli K12 hyperp.	8	>128	>128	128	128	>128	>128	>128	>128	>128	>128

Fig. 2. Effect of kirromycin and compound **6** on trypsin cleavage of EF-Tu.



1) EF-Tu undegested; 2) EF-Tu/kirromycin/trypsine (2 minutes); 3) EF-Tu/compound **6**/trypsine (2 minutes); 4) EF-Tu/trypsine (2 minutes); 5) EF-Tu/kirromycin/trypsine (30 minutes); 6) EF-Tu/compound **6**/trypsine (30 minutes); 7) EF-Tu/trypsine (30 minutes); 8) low molecular weight standard.

Conclusion

N-Acyl derivatives of **4** were prepared to evaluate the contribution to the activity of some of these substituents. The *N*-acetyl derivative **5** was completely inactive whereas compounds **6** and **8**, bearing an aromatic substituent still maintained some antimicrobial activity. This unexpected result indicates that the pyranosidic moiety (goldinoic acid) present in all the naturally occurring elfamycins can be substituted by other suitable moieties. Additional cleavage at the furane ring of these bioactive derivatives produced microbiologically inactive compounds.

Experimental

MPs were determined with a Büchi Model 510 capillary apparatus and are uncorrected. The IR absorption spectra were recorded with an I.F.S. 48 Bruker F.T.I.R. spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. All NMR experiments were performed on a Bruker AM 500 or Bruker AMX 600 spectrometer equipped with a X32 computer. The MS spectra were obtained with a triple stage quadrupole spectrometer TSQ 700 Finningan or Perkin-Elmer API PLUS III SCIEX. The elemental analyses were carried out with a Carlo Erba Mod. 1106 equipped with Eager computer. HPLC analysis for compound **4**: Hewlett Packard 1090 Liquid Chromatograph equipped with a 3390A Hewlett Packard integrator. Detection UV at 254 nm. Column Lichrocart 125-4 (Merck)—Lichrospher 100 RP18 (5 μm). Mobile phase A: NaH_2PO_4 0.02 M; mobile phase B: acetonitrile. Flow-rate: 1 ml/minute; gradient profile time (minutes) 0 (B 15%), 1 (B 15%), 30 (B 75%), 32 (B 75%), 35 (B 15%). The reactions were monitored by TLC on Silica Gel 60 F254 (Merck) and detected by UV (254 nm).

1-*N*-Desmethylgoldinamine (**4**)

Kirromycin (**1**) (20 g; 25.12 mmol) in 200 ml of dioxane and 50 ml of formic acid 99% was stirred at 35°C for 22 hours. After cooling, the reaction mixture was concentrated to half of its volume and then 250 ml of Et_2O were added. The solvent was decanted and the gummy residue was stirred in Et_2O - EtOAc (1 : 1 v/v). The solid material obtained was washed with Et_2O and dried at room temperature to give 12 g of **4** formic acid salt (21.97 mmol, yield 87%) as a yellow solid that was used without further purification. An analytical sample was obtained from 200 mg by chromatographic purification on silanized silica gel (33 g; Merck-70 ~ 230 mesh) eluting with H_2O - CH_3CN 9 : 1 (300 ml); H_2O - CH_3CN 8 : 2 (200 ml) and H_2O - CH_3CN 6 : 4 (100 ml). After evaporation of the acetonitrile and freeze-drying, 60 mg of pure formic acid salt **4** was obtained as a pale yellow amorphous solid: Rt (HPLC) 8.0/8.3 minutes; ^1H NMR and ^{13}C see Table 2; IR (Nujol) cm^{-1} 1691, 1650, 1599; $[\alpha]_D^{25}$ −37.9° (*c* 0.08, MeOH); ESI-MS *m/z* 501 (MH^+, 55%), 484 (10%), 445 (100%), 429 (15%), 371 (30%).

Anal Calcd for $C_{27}H_{36}N_2O_7$: C 64.8, H 7.2, N 5.6.
Found: C 64.3, H 7.15, N 5.56.

1-*N*-Desmethyl-25-*N*-acetylgoldinamine (**5**)

1-*N*-Desmethyl goldinamine formate (**4**) (302 mg, 0.55 mmol) was dissolved in 5 ml anhydrous pyridine at room temperature and acetic anhydride (3.8 ml, 40.2 mmol) and catalytic amounts of dimethylaminopyridine were added. After stirring for 3 hours, the reaction was diluted with 30 ml of 1 N HCl and extracted with $CHCl_3$ (2 × 40 ml). The organic phase was washed with 50 ml of 1 N HCl, dried on Na_2SO_4 and evaporated. The crude product was treated with 2 ml of 12% ammonia in methanol at room temperature overnight. The reaction was acidified with 8% HCl in MeOH and then evaporated. The residue was dissolved in water and extracted with *n*-BuOH. The organic phase was evaporated under vacuum to give 290 mg of brown solid material that was purified on silanized silica gel eluting with water, water - acetonitrile from 95 : 5 to 75 : 25. The fractions containing the product were collected, the solvent eliminated under vacuum and the resulting solid washed with Et_2O to obtain pure compound **5** (18.3 mg): Rt (HPLC) 11.4/12.01 minutes, gradient profile as for compound **4**; IR (Nujol) cm^{-1} 1691, 1616, 1540, 1212; $[\alpha]_D^{25}$ −16.98° (*c* 0.53, MeOH); FAB-MS *m/z* 543 (MH^+, 35%), 511 (70%), 262 (100%); ^1H NMR (DMSO-d_6) δ 7.98 (1H, br s), 7.17 (1H, br s), 6.79 (1H, d 8.3 Hz), 6.60 (1H, m), 6.40 (2H, m), 5.98 (2H, m), 5.66 (1H, m), 4.49 (2H, m), 4.40 (1H, m), 4.12 (1H, m), 4.09 (1H, m), 3.74 (2H, m), 3.05 (3H, s), 2.06 (1H, m), 1.86 (3H, s), 1.81 (3H, s), 1.76 (3H, s), 0.74 (3H, d 6.8 Hz).

Anal Calcd for $C_{29}H_{38}N_2O_8$: C 64.2, H 7.01, N 5.16.
Found: C 63.8, H 6.90, N 5.02.

1-*N*-Desmethyl-25-*N*-phenylureidogoldinamine (**6**)

To a solution of compound **4** formic acid salt (500 mg; 0.91 mmol) in 3 ml of DMF, triethylamine (0.32 ml; 2.29 mmol) and phenyl isocyanate (99 μl; 0.91 mmol) were added at 0°C. After stirring at room temperature for 1 hour, the reaction was diluted with ice cold water (20 ml) and acidified with 2.75 ml of 1 N HCl. The precipitate was filtered, washed with water and then with Et_2O. The crude solid material was purified by flash chromatography ($CHCl_3$ - MeOH 95 : 5) to give 282 mg of **6** (0.45 mmol; yield 50%) as a yellow solid. An analytical sample was obtained by reversed phase chromatography (water - acetonitrile from 90 : 10 to 70 : 30): Rt (HPLC) 15.8 minutes, gradient profile as for compound **4**; IR (Nujol) cm^{-1} 1691, 1650, 1597, 1549; ^1H NMR (DMSO-d_6) δ 11.1 (1H, br s), 10.9 (1H, br s), 8.42 (1H, s), 7.38 (2H, d 9.5 Hz), 7.28 (1H, d 7.1 Hz), 7.22 (2H, t 8.3 Hz), 6.88 (1H, m), 6.83 (1H, d 9.4 Hz), 6.63 (2H, m), 6.44 (1H, dd 11, 14.9 Hz), 6.35 (1H, dd), 6.25 (1H, t 5.6 Hz), 6.01 (2H, m), 5.93 (1H, d 7.3 Hz), 5.72 (1H, m), 4.47 (2H, m), 4.14 (2H, m), 4.05 (1H, m), 3.77 (3H, m), 3.32 (1H, d 9.5 Hz), 3.06 (3H, s), 2.09 (1H, m), 1.87 (3H, s), 1.61 (3H, s), 0.75 (3H, d 6.9 Hz); $[\alpha]_D^{25}$ −39.7° (*c* 0.095, MeOH); ESI-MS *m/z* 620 (MH^+, 80%), 331 (35%), 310 (100%), 279 (38%).

Anal Calcd for $C_{34}H_{41}N_3O_8$: C 65.89, H 6.67, N 6.78.
Found: C 65.42, H 6.59, N 6.67.

N-(*tert*-Butoxycarbonyl)phthalimide (**7**)

To a suspension of phthalimide (3 g; 20.4 mmol), 3 ml of triethylamine (22 mmol) and 244 mg of 4-dimethylaminopyridine (2 mmol) in 40 ml of dioxane was added a solution of di-*tert*-butyldicarbonate (4.58 g, 21 mmol) in 15 ml of dioxane. After two hours, the reaction mixture was poured into 100 ml of ice cold water and stirred for 15 minutes. The precipitate was then filtered, washed with water and dissolved in $CHCl_3$. The organic phase was dried over Na_2SO_4 and concentrated to a small volume. Compound **7** (4 g, 16.19 mmol, yield 80.9%) was obtained as white crystals by addition of *n*-hexane: TLC (silica gel) Rf = 0.42 (*n*-hexane - acetone 7 : 3); mp 97.4°C; ^1H NMR ($CDCl_3$) δ 7.95 (2H, m), 7.80 (2H, m), 1.63 (9H, s); IR (Nujol) cm^{-1} 1802, 1776, 1720; FAB-MS *m/z* 248 (MH^+, 100%).

Anal Calcd for $C_{13}H_{13}NO_4$: C 63.15, H 5.26, N 5.66.
Found: C 62.19, H 5.32, N 5.57 (about 1.5% of phtalimide cocrystallizes with **7**)[19]

1-*N*-Desmethyl-25-*N*-phthaloylgoldinamine (**8**)

To a solution of **4** formic acid salt (546 mg, 1 mmol) in 5 ml of DMF, triethylamine (0.35 ml, 2.5 mmol) and *N*-*tert*-butoxycarbonylphthalimide (**7**) (247 mg, 1 mmol) were added at 0°C. After stirring at room temperature for 2 hours, the reaction mixture was treated with 30 ml of ice cold water and then with 3 ml of 1 N HCl. The yellow precipitate was filtered, washed with water (3 × 20 ml) and Et_2O (3 × 20 ml). The crude solid material was purified by flash chromatography ($CHCl_3$ - MeOH 95 : 5; $CHCl_3$ - MeOH 92 : 8) to give 350 mg of **8** (0.55 mmol) as a yellow solid. An analytical sample was obtained by reversed phase chromatography (water - acetonitrile from 70 : 30 to 65 : 35): Rt (HPLC) 20.3/21.1 minutes, gradient profile as for compound **4**; IR (Nujol) cm^{-1} 1770, 1713, 1610; ^1H NMR (DMSO-d_6) δ 11.4 (1H, br s), 11.1 (1H, br s), 7.85 (4H, m), 7.27 (1H, d 7.1 Hz), 6.83 (1H, d 9.3 Hz), 6.61 (2H, m), 6.48 (1H, dd 11.0, 14.5 Hz), 6.34 (1H, m), 5.97 (2H, m), 5.91 (1H, d 7.2 Hz), 5.72 (1H, m), 4.48 (2H, m), 4.26 (2H, d 5.8 Hz), 4.13 (2H, m), 4.04 (1H, m), 3.05 (3H, s), 2.05 (1H, m), 1.86 (3H, s), 1.58 (3H, s), 0.73 (3H, d 6.8 Hz); $[\alpha]_D^{25}$ −42.4° (*c* 0.85, MeOH); ESI-MS *m/z* 631 (MH^+, 100%), 599 (30%), 449 (15%).

Anal Calcd for $C_{35}H_{38}N_2O_9$: C 66.65, H 6.07, N 4.44.
Found: C 66.12, H 5.95, N 4.39.

3-Methoxy-2,4-dimethyl-8-(*N'*-phenylureido)-4*E*,6*E*-octadienal (**9**)

A solution of sodium metaperiodate (5.1 g, 24 mmol) in 75 ml of water was added dropwise to a well stirred solution of **6** (2.5 g, 4 mmol) and ammonium acetate (462 mg, 6 mmol) in a mixture of 50 ml of dioxane and 15 ml of MeOH. A transient wine-red color developed immediately indicating the liberation of iodine. The

mixture was stirred overnight, the precipitate was filtered off and washed with dioxane. The solution was diluted with water (100 ml) and extracted with Et_2O (5 × 50 ml). The organic phase was washed with 0.1 N NaOH (100 ml), 100 ml of brine, dried over Na_2SO_4 and evaporated *in vacuo* giving 760 mg of **9** (2.4 mmol) as a straw colored syrup. This material showed a single spot in TLC (silica gel) with Rf = 0.3 (*n*-hexane - acetone 6 : 4) and was used without further purification; Rt (HPLC) 18.0 minutes, gradient profile as for compound **4**; ^1H NMR ($CDCl_3$) δ 9.7 (1H, d 3.1 Hz), 7.4 (4H, m), 7.0 (1H, t 7.2 Hz), 6.38 (1H, dd 10.9, 15.2 Hz), 5.97 (1H, d 10.8 Hz), 5.67 (2H, m), 3.88 (2H, m), 3.59 (1H, d 9.5 Hz), 3.14 (3H, s), 2.55 (1H, m), 1.63 (3H, s), 0.85 (3H, d 7.6 Hz).

3-Methoxy-2,4-dimethyl-8-(*N'*-phthalimido)-4*E*,6*E*-octadienal (**10**)

To a solution of compound **8** (3.0 g, 4.95 mmol) and ammonium acetate (14.85 mmol; 1.14 g) in 100 ml of dioxane - methanol (3 : 1 v/v), was added dropwise over about 20 minutes, a solution of sodium metaperiodate (29.7 mmol; 6.32 g) in 100 ml of water. A transient red-wine color developed immediately. After stirring for 5 hours, the resulting orange suspension was filtered and the precipitate washed with a small amount of dioxane. The solution was concentrated under vacuum, diluted with 200 ml of water and extracted with Et_2O (5 × 70 ml). The organic phase was then washed with 100 ml of 0.1 N NaOH, water (2 × 100 ml), dried over Na_2SO_4 and evaporated yielding 700 mg of compound **10** (2.1 mmol; yield 49%) as a straw colored syrup that was used without further purification; TLC (silica gel) Rf = 0.45 (*n*-hexane - acetone 6 : 4); Rt (HPLC): 10.2 minutes gradient profile: time (minute) 0 (B 40%), 1 (B 40%), 15 (B 75%); ^1H NMR ($CDCl_3$) δ 9.7 (1H, d 2.9 Hz), 7.85 (2H, m), 7.35 (2H, m), 6.5 (1H, dd 10.7, 14.9 Hz), 6.0 (1H, d 10.7 Hz), 5.8 (1H, m), 4.36 (2H, m), 3.60 (1H, d 9.6 Hz), 3.15 (3H, s), 2.55 (1H, m), 1.68 (3H, s), 0.86 (3H, d 7.3 Hz).

2,4-Dinitrophenylhydrazone (**11**)

Compound **9** (38.3 mg, 0.12 mmol) and 2,4-dinitrophenylhydrazine (24 mg, 0.121 mmol) were stirred at room temperature for 72 hours. After filtration, the solvent was evaporated and the resulting crude solid material crystallized from EtOAc-hexane to give 18 mg of compound **11** as an yellow-orange solid; Rt (HPLC) 27.1 minutes, gradient profile as for compound **4**; mp 158 ~ 160°C; ^1H NMR ($CDCl_3$) δ 11.0 (1H, s), 9.13 (1H, d 2.3 Hz), 8.31 (1H, dd 2.4, 9.5 Hz), 7.94 (1H, d 9.6 Hz), 7.61 (1H, d 5.6 Hz), 7.35 (4H, m), 7.15 (1H, m), 6.47 (1H, dd 10.9, 15.2 Hz), 6.30 (1H, s), 6.05 (1H, d 10.8 Hz), 5.78 (1H, m), 4.81 (1H, t 5.7 Hz), 3.99 (2H, m), 3.44 (1H, d 9.0 Hz), 3.19 (3H, s), 2.77 (1H, m), 1.78 (3H, s), 1.06 (3H, d 7.0 Hz); ESI-MS *m/z* 497 (MH^+, 100%), 465 (60%), 259 (35%).

Anal Calcd for $C_{24}H_{28}N_6O_6$: C 58.05, H 5.68, N 16.92.
Found: C 57.7, H 5.62, N 16.58.

2,4-Dinitrophenylhydrazone (**12**)

Compound **10** (35 mg, 0.10 mmol) and 2,4-dinitrophenylhydrazine (21.9 mg, 0.11 mmol) were stirred at room temperature for 3 hours in 2 ml of abs. EtOH. The solid was filtered, washed with abs. EtOH and crystalized from abs. EtOH to give 20 mg of pure **12** as a yellow-orange solid; Rt (HPLC) 30.5 minutes, gradient profile as for compound **4**; mp 157.5°C; $[\alpha]_D^{25}$ +38.8° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 11.0 (1H, s), 9.13 (1H, d 2.5 Hz), 8.31 (1H, dd 2.5, 9.6 Hz), 7.94 (1H, d 9.6 Hz), 7.88 (2H, m), 7.75 (2H, m), 7.60 (1H, d 5.7 Hz), 6.57 (1H, dd 10.9, 15.1 Hz), 6.02 (1H, d 10.7 Hz), 5.80 (1H, m), 4.39 (2H, d 6.4 Hz), 3.43 (1H, d 8.9 Hz), 3.18 (3H, s), 2.77 (1H, m), 1.57 (3H, s), 1.05 (3H, d 6.9 Hz); FAB-MS *m/z* 507 (M^+, 11%), 506 (23.5%), 476 (26.5%), 460 (88%); 429 (23.5%), 401 (35.2%), 327 (100%).

Anal Calcd for $C_{25}H_{25}N_5O_7$: C 59.16, H 4.96, N 13.8.
Found: C 59.52, H 4.81, N 13.23.

3-Methoxy-2,4-dimethyl-8-*N*-phenylureido-2*E*,4*E*-octadienol (**13**)

Sodium borohydride (22.7 mg; 0.6 mmol) was added to a solution of compound **9** (190 mg; 0.60 mmol) in 3 ml of abs. EtOH at 0°C. After 45 minutes, the reaction was quenched with acetone (few drops), diluted with water (20 ml), the pH was corrected to 2 (1 N HCl) and extracted with Et_2O (2 × 20 ml). The organic phase was dried over Na_2SO_4 and evaporated to give a crude material that was purified on silica gel (*n*-hexane - acetone 6 : 4). The desired compound **13** (140 mg; 0.44 mmol; yield 73%) was obtained as a glassy syrup: IR ($CDCl_3$) cm^{-1} 3626, 2964, 2934, 1674, 1526, 1499; ^1H NMR ($CDCl_3$) δ 7.3 (4H, m), 7.13 (1H, m), 6.43 (1H, dd 10.8, 15.1 Hz), 6.40 (1H, br s), 5.96 (1H, d 10.7 Hz), 5.72 (1H, m), 4.86 (1H, m), 3.97 (2H, m), 3.61 (2H, m), 3.36 (1H, d 6.8 Hz), 3.30 (1H, br s), 3.15 (3H, s), 1.90 (1H, m), 1.65 (3H, s), 0.68 (3H, d 7.0 Hz); FAB-MS *m/z* 319 (MH^+, 40%), 287 (100%), 257 (30%).

Anal Calcd for $C_{18}H_{26}N_2O_3$: C 67.92, H 8.17, N 8.80.
Found: C 67.5, H 8.02, N 8.56.

N-(3-Methoxy-2,4-dimethyl-8-*N*-phenylureido-4*E*,6*E*-octadienylidene)-4-methyl-1-piperazinamine (**14**)

4-Methyl-1-aminopiperazine (86 mg; 0.75 mmol) in 1 ml of THF were added to a solution of compound **9** (240 mg; 0.75 mmol) in 3 ml of THF. After 3 hours at room temperature, the solvent was evaporated and the crude material purified by flash chromatography ($CHCl_3$ - MeOH from 98 : 2 to 95 : 5) to give 185 mg (0.44 mmol; yield 59%) of a pure compound **14** as an orange-yellow syrup: Rt (HPLC) 14.1 minutes, gradient profile as for compound **4**; IR ($CDCl_3$) cm^{-1} 2974, 2943, 2885, 2808, 1674, 1599, 1528, 1499; ^1H NMR ($CDCl_3$) δ 7.31 (4H, m), 7.0 (1H, m), 6.93 (1H, d 6.3 Hz), 6.76 (1H, br s), 6.42 (1H, dd 10.3, 15.2 Hz), 5.96 (1H, d 10.7 Hz), 5.70 (1H, m), 5.16 (1H, m), 3.93 (2H, m), 3.37 (1H, d 8 Hz), 3.15 (3H, s), 3.01 (4H, m), 2.56 (6H, m), 2.31 (3H, s), 1.65 (3H, s), 0.95 (3H, d 6.9 Hz); FAB-MS *m/z*

414 (MH^+, 100%).

Anal Calcd for $C_{23}H_{35}N_5O_2$: C 66.82, H 8.47, N 16.94.
Found: C 66.25, H 8.25, N 16.34.

5-Methoxy-4,6-dimethyl-10-(N'-phenylureido)-2*E*,6*E*,8*E*-decatrienoic Acid Ethyl Ester (**15**)

Triethyl phosphonoacetate (536 mg; 2.39 mmol) was added to a suspension of sodium hydride (60% oil dispersion; 83.6 mg; 2.09 mmol) in 5 ml of dry THF under argon at 0°C. After 20 minutes the pale yellow solution was cooled to −50°C and a solution of compound **9** (630 mg; 1.99 mmol) in 8 ml of dry THF was added. The reaction was left stirring for 2 hours at −10°C, diluted with Et_2O (15 ml), washed with water and the aqueous phase extracted with Et_2O (2 × 10 ml). The organic phase was washed with a saturated solution of NaCl, dried over Na_2SO_4 and evaporated. Purification by flash chromatography (*n*-hexane - acetone 7 : 3) gave 480 mg of compound **15** (1.51 mmol, yield 75%) as a syrup: TLC (silica gel) Rf = 0.32 (*n*-hexane - ethyl acetate 8 : 2); Rt (HPLC) 23.7 minutes, gradient profile as for compound **4**; IR ($CDCl_3$) cm^{-1} 3434, 2981, 2934, 1700, 1684, 1653, 1524; ^1H NMR ($CDCl_3$) δ 7.31 (4H, m), 7.15 (1H, m), 7.02 (1H, dd 7.4, 15.8 Hz), 6.59 (1H, br s), 6.45 (1H, dd 10.7, 15.0 Hz), 5.96 (1H, d 10.7 Hz), 5.84 (1H, d 15.8 Hz), 5.72 (1H, m), 4.99 (1H, t 5.4 Hz), 4.19 (2H, q 7 Hz), 3.95 (2H, m), 3.26 (1H, d 8.6 Hz), 3.14 (3H, s), 2.52 (1H, m), 1.66 (3H, s), 1.29 (3H, t 7 Hz), 0.90 (3H, d 6.9 Hz); $[\alpha]_D^{25}$ +28.13° (*c* 0.16, $CDCl_3$); FAB-MS *m/z* 387 (MH^+, 100%), 355 (30%), 259 (55%).

Anal Calcd for $C_{22}H_{30}N_2O_5$: C 65.65, H 7.51, N 6.96.
Found: C 65.15, H 7.41, N 6.78.

5-Methoxy-4,6-dimethyl-10-(N'-phenylureido)-2*E*,6*E*,8*E*-decatrienoic Acid (**16**)

Compound **15** (414 mg; 1.07 mmol) in 5 ml of dioxane and 2.5 ml of 1 N NaOH were reacted at room temperature for 23 hours. The reaction was cooled to 0°C, brought to pH 2 with 1 N HCl, diluted with water and extracted with CH_2Cl_2. The organic phase was washed with saturated solution of NaCl, dried over Na_2SO_4 and evaporated to give 353 mg of compound **16** (0.98 mmol; yield 91%) as a pale yellow solid; Rt (HPLC) 12.7 minutes, gradient profile as for compound **4**; IR (Nujol) cm^{-1} 3330, 1693, 1650, 1597, 1552; ^1H NMR ($CDCl_3$) δ 7.29 (4H, m), 7.12 (2H, m), 6.98 (1H, br s), 6.42 (1H, dd 10.8, 14.9 Hz), 5.96 (1H, d 10.8 Hz), 5.84 (1H, d 15.6 Hz), 5.70 (1H, m), 4.95 (1H, m), 3.95 (2H, m), 3.28 (1H, d 8.4 Hz), 3.15 (3H, s), 2.54 (1H, m), 1.66 (3H, s), 0.92 (3H, d 6.9 Hz); FAB-MS *m/z* 359 (MH^+, 100%), 327 (50%).

Anal Calcd for $C_{20}H_{26}N_2O_4$: C 67.03, H 7.26, N 7.82.
Found: C 66.48, H 7.06, N 7.55.

5-Methoxy-4,6-dimethyl-10-(N'-phthalimido)-2*E*,6*E*,8*E*-decatrienoic Acid Ethyl Ester (**17**)

To a suspension of sodium hydride (60% oil dispersion; 30.8 mg; 0.77 mmol) in 2 ml of dry THF under argon, triethyl phosphonacetate (0.168 ml; 0.84 mmol) was added at 0°C. After 30 minutes the pale yellow solution was cooled to −50°C and compound **10** (230 mg; 0.7 mmol) in 3 ml of dry THF was added. The reaction was stirred for 2 hours at −10°C, diluted with Et_2O (10 ml) and washed with water. The acqueous phase was extracted with Et_2O (2 × 10 ml), the combined organic phases were washed with a saturated solution of NaCl, dried over Na_2SO_4 and evaporated. The crude was purified by flash chromatography on silica gel (*n*-hexane - ethyl acetate 8 : 2) to give 230 mg of compound **17** (0.57 mmol; yield 82.7%) as a syrup; Rf = 0.28 (*n*-hexane - ethyl acetate 8 : 2); Rt (HPLC): 15.0 minutes gradient profile: time (minute) 0 (B 40%), 1 (B 40%), 15 (B 75%), 20 (B 40%); IR (Neat) cm^{-1} 2979, 2931, 2821, 1772, 1713, 1652; ^1H NMR ($CDCl_3$) δ 7.87 (2H, m), 7.72 (2H, m), 7.0 (1H, dd 7.56, 15.6 Hz), 6.54 (1H, dd 10.8, 15.1 Hz), 5.94 (1H, d 10.9 Hz), 5.82 (1H, d 16 Hz), 5.75 (1H, m), 4.36 (2H, d 6.6 Hz), 4.18 (2H, q 7.1 Hz), 3.23 (1H, d 8.8 Hz), 3.1 (3H, s), 2.49 (1H, m), 1.67 (3H, s), 1.28 (3H, t 7.3 Hz), 0.87 (3H, d 6.9 Hz); $[\alpha]_D^{25}$ +32.06° (*c* 0.97, $CHCl_3$); ESI-MS 398 (MH^+, 30%), 366 (25%), 320 (100%), 292 (15%), 173 (60%).

Anal Calcd for $C_{23}H_{27}NO_5$: C 69.5, H 6.84, N 3.52.
Found: C 68.86, H 6.77, N 3.43.

Bacterial Protein Synthesis Cell Free System

L-[^{14}C]Phenylalanine (513 mCi/mmol) was from the Radiochemical Centre, Amersham, Bucks., UK. Poly(*U*) was from Boeringer Mannheim, Germany. Nucleotides and all other reagents were from Sigma Chemical Company, St. Louis, MO, U.S.A. Crude extracts were prepared from *E. coli* and the polyphenylalanine synthesis was performed according to the literature[20]. The compound was dissolved in DMSO : H_2O 1 : 9 (v/v) and 5 μl of the mother-solution were stirred for 3 minutes with 65 μl of S30 preparation in a microtiter well. 30 μl of a mixture of t-RNA, poliU and L-phenylalanine ^3H were added and the mixture was incubated for 1 hour at room temperature. The reaction was then quenched by incubating at 30°C with 6 N NaOH (50 μl/well) and then neutralized with 6 N HCl (50 μl/well). Finally, 20% trichloroacetic acid (100 μl/well) was sequentially added and the mixture was maintained overnight at 4°C. The precipitate was filtered and the radioactivity was measured.

Measurement of *In Vitro* Antibacterial Activity

The MICs determinations were performed by standard methods with strains taken from frozen stock cultures maintained at −80°C. Iso-Sensitest broth was used for all bacteria except Streptococci (Todd-Hewitt broth), *N. gonorrhoeae* (GC broth) and *H. influenzae* (brain heart infusion broth; Oxoid plus 1% supplement C). All tests were incubated aerobically; *N. gonorrhoeae* and *H. influenzae* were incubated in a 5% CO_2 atmosphere. All tests were incubated at 35°C.

Digestion of EF-Tu, EF-Tu-kirromycin and EF-Tu-compound **6** by Trypsin

EF-Tu-GDP (50 μg) was incubated with 10 μg of kirromycin or alternatively with 10 μg of compound **6** for 1 hour at room temperature in buffer Tris 20 mM, $MgCl_2$ 10 mM and dithiotreitol 2.5 mM at pH 7.7. Proteolitic cleavage by trypsin of EF-Tu, EF-Tu/kirromycin and EF-Tu/compound **6** was performed as described[21]. As depicted in Fig. 2 lines 2, 3 and 4 referred to aliquots removed after 2 minutes while lines 5, 6 and 7 after 30 minutes. In both cases the reaction was terminated by boiling for 5 minutes in the gel sample buffer. The protein was analyzed by SDS-PAGE on 20% acrilamide phast Gel with PHAST SYSTEM (Pharmacia).

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