Semisynthetic Derivatives of Purpuromycin as Potential Topical Agents for Vaginal Infections

Aldo Trani,* Clelia Dallanoce, Gianbattista Panzone, Franca Ripamonti, Beth P. Goldstein,† and Romeo Ciabatti

Biosearch Italia S.p.A., via R. Lepetit 34, 21040 Gerenzano (VA), Italy

Received September 26, 1996[⊗]

Purpuromycin (**1**) is an antibiotic with a broad spectrum of antimicrobial activity, encompassing bacteria, fungi, and protozoa, particularly those involved in vaginal infections. With the aim of enhancing the solubility and reducing the serum binding, a chemical program of modifications was undertaken on the natural compound, and a new interesting series of derivatives at the naphthoquinone system was synthesized and evaluated as potential topical agents for vaginal infections. In particular three semisynthetic derivatives, 7′-amino (**8a**), 7′-methylamino (**8b**), 7′-ethylamino (**8c**), of 7′-demethoxypurpuromycin seemed to be the most promising. They were tested for *in vitro* activity against three of the most important vaginal pathogens and showed activity similar to that of purpuromycin against *Candida* isolates while they were significantly more active against *Trichomonas vaginalis* and *Gardnerella vaginalis*, which are cultured in media containing blood or serum. This is probably due to the fact that the activity of the derivatives is less antagonized by these supplements than that of purpuromycin.

Introduction

A variety of different microorganism coexist in the normal flora of the vagina, and several other organisms (including bacteria, fungi, and protozoa) can also colonize the vagina, causing pathologies known as vaginitis and vaginosis. Vaginosis is generally due to infection with *Gardnerella vaginalis*, probably acting synergistically with obligate anaerobes such as *Bacteroides* and *Mobiluncus* spp. Organisms commonly causing vaginitis include the yeast *Candida albicans* and the protozoan *Trichomonas vaginalis*. Topical and/or systemic antifungal agents may be prescribed to treat these *Candida* infections, while systemic or topical metronidazole and topical disinfectants are used against *Trichomonas* and anaerobic bacteria.[1−3]

Purpuromycin (**1**), an antibiotic produced by filamentous bacterium *Actinoplanes ianthinogenes*,[4] contains naphthazarin and isocoumarin moieties linked through a spiroketal system. It has a broad spectrum of antimicrobial activity, including fungi, bacteria, and protozoa.[5] However, in systemic animal model infections, purpuromycin showed poor activity, attributable to low solubility and little absorption, and perhaps also to a strong affinity for protein, as revealed by an antagonism of its *in vitro* activity by serum. Because topical therapy, including with nonspecific disinfectants, is still widely used as an adjunct to more targeted therapy of vaginal infections, we have been evaluating the potentiality of purpuromycin and semisynthetic derivatives of this antibiotic for such an indication, while seeking to improve its bioavailability through chemical modification.

In general, reduction in lipophilicity and an increase in positive charge reduce the interaction of compounds with serum proteins.[6] With the aim of increasing solubility and reducing serum binding, a chemical program of derivatization was undertaken on the structure of purpuromycin. The derivatives obtained by modification of the ester group in position 7 and the hydroxyl group in position 4 did not show interesting activity.[7,8] This paper deals with the synthesis and the antimicrobial activities of the most promising series of derivatives, obtained by introducing an amine residue on the naphthoquinone system.

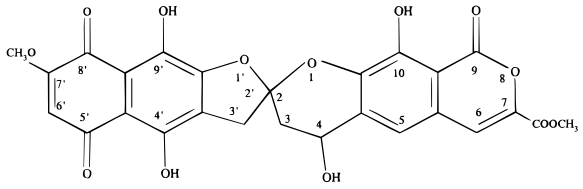


Figure 1. Structure of purpuromycin (**1**).

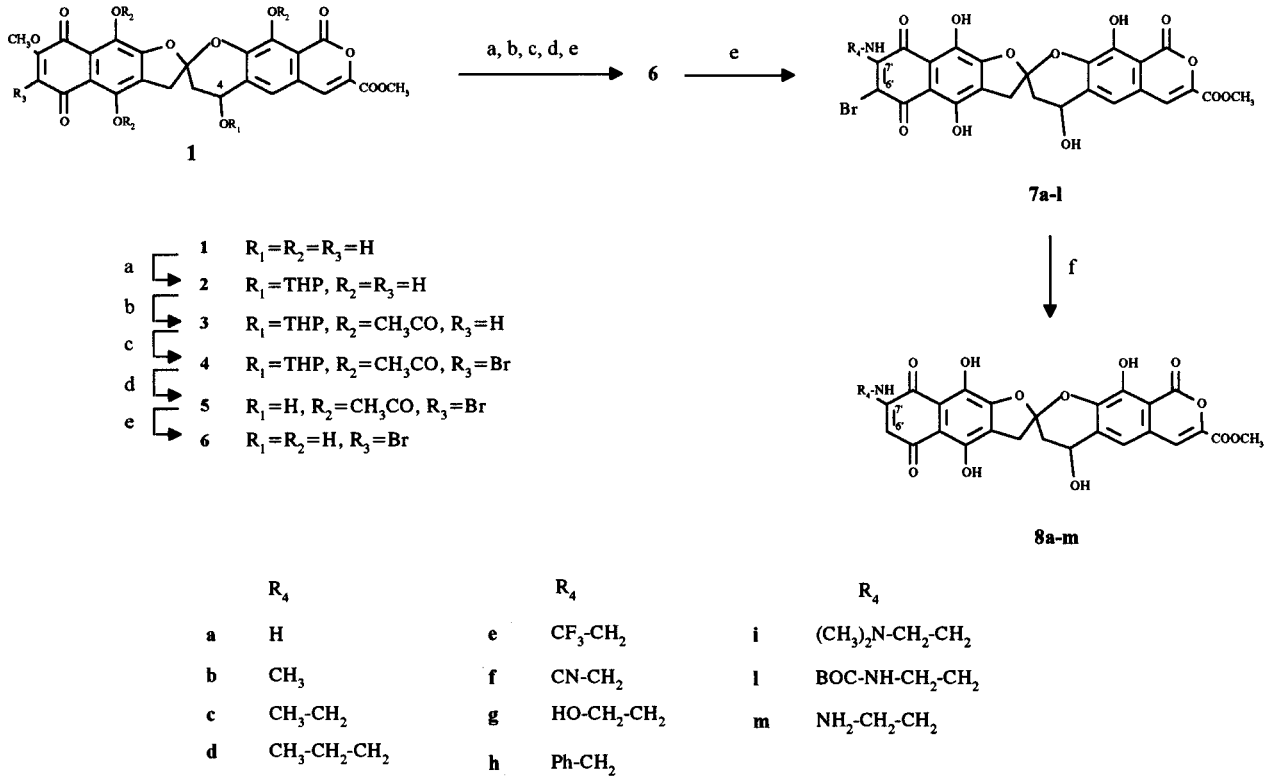
Chemistry

The common reactions which usually occur on quinone rings [9−11] turned out not to be feasible for purpuromicin (**1**) (Figure 1). This is probably due to the rapid tautomerism of the naphthazarin system resulting in the simultaneous existence of benzenoid and quinoid properties in both rings.[12] For this reason the synthesis of 7′-amino-7′-demethoxy derivatives of purpuromycin was accomplished by employing a route (Scheme 1) in which **1** is first protected at the 4-hydroxyl group with a tetrahydropyranyl ether, and this species **2** is converted into the corresponding 4′,9′,10-triacetyl derivative **3** with acetic anhydride. Treatment of **3** with pyridinium bromide perbromide yielded the 6′-bromo derivative **4**, which was deprotected at position 4 with a catalytic amount of *p*-toluenesulfonic acid in methanol to provide compound **5**. 4′,9′,10-Triacetyl-6′-bromopurpuromycin (**5**) was used as starting material for the preparation of the series of amine derivatives **8a**−**m** (Scheme 1). The nucleophilic substitution of the 7′-methoxy group with amines is possible due to the presence in the *ortho* position of the bromine, which is then removed by catalytic hydrogenation. This nucleophilic displacement works only with primary amines. When the key intermediate **5** was submitted to reaction with amines in wet THF or DMF at room temperature,

* Corresponding author.
† Current address: Applied Microbiology Inc., 771 Old Saw Mill River Rd, Tarrytown, NY 10591.
⊗ Abstract published in *Advance ACS Abstracts,* February 1, 1997.

S0022-2623(96)00672-3 CCC: $14.00

Scheme 1[a]



[a] Reagents: (a) DHP/H^+, THF; (b) $(CH_3CO)_2O$, py; (c) $PyBr_2HBr$, CH_2Cl_2; (d) MeOH/H^+; (e) R_4-NH_2, THF or DMF; (f) H_2−5% Pd/C, CH_3COONa, THF−MeOH.

Table 1. *In vitro* Antimicrobial Activity of Purpuromycin Derivatives (MIC, μg/mL)

strains	1	8a	8b	8c	8d	8e	8f	8g	8h	8i	8l	8m
Staphylococcus aureus L165	0.03	0.03	0.016	0.06	0.03	0.016	0.13	0.06	0.5	0.13	0.13	1
Staphylococcus aureus L165 10^6cfu/ml	0.06	0.06	0.06	0.06	0.03	0.03	0.13	0.13	1	0.13	0.13	1
Staphylococcus aureus L165 30% bovine serum	8	1	0.25	1	2	2	2	1	32	0.25	2	8
Staphylococcus epidermidis ATCC12228	0.03	0.03	0.008	0.06	0.016	0.016	0.13	0.06	0.13	0.13	0.06	1
Staphylococcus haemolyticus L602	0.03	0.06	0.016	0.06	0.06	0.016	0.13	0.13	0.13	0.5	0.06	1
Streptococcus pyogenes L49	0.25	0.03	0.03	0.06	0.13	0.13	0.13	0.03	1	0.13	0.13	2
Streptococcus pneumoniae L44	0.06	0.016	0.016	0.06	0.03	0.06	0.03	0.016	0.06	0.06	0.06	1
Enterococcus faecalis ATCC7080	0.06	0.06	0.03	0.06	0.06	0.03	0.13	0.06	4	0.13	0.13	2
Propionibacterium acnes ATCC6919	0.03	0.008	0.008	0.06	nd[a]	0.008	0.016	0.004	nd[a]	0.06	0.016	0.5
Bacteroides fragilis ATCC25285	0.25	0.06	0.03	0.06	0.03	0.06	0.5	0.13	0.5	0.5	0.5	>128
Neisseria gonorrhoeae L997	0.06	0.03	0.06	0.06	0.06	0.06	0.06	0.03	0.25	0.13	0.13	64
Haemophilus influenzae ATCC19418	0.03	0.06	0.13	0.06	0.13	0.06	0.13	0.06	2	0.13	1	32
Escherichia coli L47	8	>128	>128	>128	>128	>128	>128	≥128	>128	16	>128	>128
Pseudomonas aeruginosa ATCC10145	>128	>128	>128	>128	>128	>128	>128	≥128	>128	128	>128	>128
Proteus vulgaris ATCC881	32	>128	>128	>128	>128	>128	>128	≥128	>128	128	>128	>128
Candida albicans L145	1	32	2	2	>128	8	>128	≥128	>128	64	>128	>128
Trichophyton mentagrophytes L634	0.25	32	≤0.13	≤0.13	64	>128	128	≥128	≥128	128	>128	>128

[a] nd not determined.

we observed an immediate color change of the reaction mixture from yellow to violet due to basic hydrolysis of the acetyl groups with formation of 6′-bromopurpuromycin (**6**). Subsequently the nucleophilic displacement of the 7′-methoxy group with the amine residue occurred to yield the 6′-bromo-7′amine derivatives **7a**−**l**. By using 36% ammonia in THF a mixture of **6** with **7a** was recovered after 48 h at room temperature. By contrast, when more nucleophilic amines, e.g. methylamine, were used, the reaction was very fast, lasting minutes, and in this case we preferred to stop the reaction, even if **6** was still present, to avoid the subsequent opening of the lactone ring. Finally dehalogenation of **7a**−**l** was accomplished by hydrogenolysis with 5% Pd/C at atmospheric pressure to produce the amine derivatives **8a**−**m**.

Antimicrobial Activity

The antimicrobial activity of purpuromycin derivatives **8a**−**m** were determined in comparison with the parent compound **1** (Table 1). The derivatives as well as purpuromycin had excellent activity against Gram-positive bacteria and fastidious Gram-negative species (*Bacteroides*, *Neisseria*, *Haemophilus*). Compounds **8a**−**m** lost the activity that **1** had against Enterobacteriaceae (*Escherichia coli* and *Platanus vulgaris*). None of the compounds were active against *Pseudomonas aeruginosa*. The derivatives, with the exception of **8h** and **8m**, had somewhat better activity than **1** against *Streptomyces aureus* in the presence of 30% bovine serum. Only three derivatives, **8a**−**c** were active against *C. albicans* and *Trichophyton mentagrophytes*. These three compounds were evaluated, in comparison with

Table 2. *In vitro* anti-*Candida* Activity of Selected Compounds (MIC, μg/mL)

species (n. isolates)	**1**	**8a**	**8b**	**8c**
C. tropicalis (2)	128	128	64	64−128
C. glabrata (1) 1		32	128	32
C. albicans (24)	0.25−2	2−128[a]	0.25−32	0.25−8
MIC_{50}	1	64	2	2
MIC_{90}	2	128	2	4

[a] Partial inhibition of growth at lower concentrations.

Table 3. Activity of Selected Compounds against 12 Strains of *Gardnerella vaginalis*

compd	MIC range	MIC_{50}	MIC_{90}
1	8−16	8	8
8a	0.06−13	0.06	0.06
8b	1−2	1	2
8	0.5−2	2	2

1, for their *in vitro* activity against clinical isolates of *Candida* (Table 2), against *G. vaginalis* (Table 3), and against *T. vaginalis* (Table 4), three of the most important vaginal pathogens. Compound **1** had excellent activity against clinical isolates of *C. albicans* and was also active against a single isolate of *Candida glabrata* but not against isolates of *Candida tropicalis*. Among the derivatives, **8b** and **8c** had anti-*Candida* activities similar to that of **1**, although the MICs of the derivatives were higher for some isolates. MICs of **8a** covered a wider range, and the growth of most of the isolates was not completely inhibited even at high concentrations of this compound.

Against *G. vaginalis*, all three derivatives had activity significantly better than that of **1**. Two of the compounds (**8a** and **8b**) also had activity significantly better than that of **1** against *T. vaginalis*; their MICs were similar to that of the standard anti-*Trichomonas* drug, metronidazole.

8b and purpuromycin were tested for activity against a large number of clinical isolates of anaerobic bacteria, many of them associated with bacterial vaginosis, as reported by Goldstein *et al.*[13] **8b** was generally more active than purpuromycin, with MICs often $^1/_{16}$ those of **1**, whose MICs ranged from 1 to 8 μg/mL for most species. Both **8b** (MICs $\leq$ 0.03 μg/mL) and purpuromycin (MICs 0.25−0.5 μg/mL) were particularly active against 20 isolates of *Mobiluncus*.

The nitrogen-containing compounds **8**−**c** were the most active among all of the derivatives previously obtained by chemical modification of **1** in positions 4 and 7.[7,8] **8**−**8c** were insoluble in water but showed a reduced serum antagonism (Table 1), satisfying one of our aims, and have potentially useful activity against pathogens associated with bacterial vaginosis, trichomoniasis, and candidiasis. These compounds have been selected for further study as potential topical agents in vaginal infections.

Experimental Section

Microbiological Materials and Methods. For the organisms listed in Tables 1 and 2, the minimal inhibitory concentrations (MIC) were determined by broth microdilution methodology.[15] Inocula were approximately 10^4 colony-forming units per milliliter (cfu/mL) for bacteria and *Candida* sp. except for anaerobes (5×10^5 cfu/mL) and 1% (v/v) of a suspension of mycelia and conidia for *T. mentagrophytes*. Incubation was at 37 °C, except for *T. mentagrophytes* (30 °C). *Neisseria gonorrhoeae* and *Haemophils influenzae* were incubated in 5% CO_2 in air; *Propionibacterium acnes* and *Bacteroides fragilis* in nitrogen−carbon dioxide−hydrogen (80:10:10); other organisms in air. Incubation times were 48 h for *N. gonorrhoeae*, *H. influenzae*, *P. acnes*, and *B. fragilis*; 72 h for *T. mentagrophytes*; 20−24 h for other organisms. The growth media were as follows: Oxoid Iso-Sensitest broth for staphylococci, *E. fecalis*, *E. coli*, *P. vulgaris*, and *P. aeruginosa*; Difco Todd Hewitt broth for streptococci; Difco GC Base broth + 1% (v/v) BBL IsoVitaleX for *N. gonorrhoeae*; Difco Brain Heart Infusion broth + 1% (v/v) Difco supplement C for *H. influenzae*; Wilkins-Chalgren broth (Difco) for *P. acnes* and *B. fragilis;* phosphate-buffered Yeast Nitrogen Base broth (Difco) supplemented with glucose (1% w/v) and L-asparagine (0.15% w/v) for *Candida* sp.; Difco YM broth for *T. mentagrophytes*. MICs for *G. vaginalis*, shown in Table 3, were determined by an agar dilution method in Difco Casman medium supplemented with 5% (v/v) whole rabbit blood and 0.15% (v/v) lysed rabbit blood. Inocula were approximately 10^5 colony-forming units. Incubation was for 48 h at 37 °C in $N_2-CO_2-H_2$ (80:10:10). MICs for *T. vaginalis* (Table 4) were determined by a broth microdilution method[14] in 0.2 mL of Trichomonas Culture Medium Base (Merck) + 10% horse serum in flat-bottomed microtiter wells. The inoculum was approximately 10^5 cells/mL. After 48 h of incubation at 37 °C in $N_2-CO_2-H_2$, the wells were viewed directly with a microscope to detect the presence of viable (motile) protozoa.

Table 4. Activity of Selected Compounds against *Trichomonas vaginalis*

compd	MIC, μg/mL	compd	MIC, μg/mL
1	8−16	**8c**	4−8
8a	0.5	metronidazole	0.25−1
8b	1		

Chemistry: Materials and Methods. Evaporation of the solvents was carried out with a rotary evaporator at 50 °C under vacuum. HPLC analysis was used to monitor the progress of reactions, homogeneity of chromatographic fractions, and purity of the compounds using a Hewlett-Packard 1090L instrument equipped with a UV detector at 254 nm and a Hibar LiChroCart 125-4 column, packed with LiChrospher 100 RP-18 (5 μm) injection volume, 10 μL; flow rate, 1 mL/min; mobile phases, (A) 0.02 M NaH_2PO_4 (pH 4.7); (B) CH_3-CN].

gradient 1	time (min)	0	2	25	35	36	
	%B in A	40	40	60	40	stop	
gradient 2	time (min)	0	2	20	25	30	31
	%B in A	35	35	50	50	35	stop

The reported t_R values are relative to the t_R value of purpuromycin obtained in the same analysis. Thin layer chromatography (TLC) was performed on precoated plates of 0.25 mm thickness and preparative plates of 1 mm thickness (silica gel 60 F_{254}, Merck). Column chromatography was performed using silica gel 60 (70−230 mesh) (Merck) buffered with KH_2PO_4.[14] Melting points were determined on a Buchi apparatus and are uncorrected. The compounds were analyzed for C, H, N, and halogens using samples previously dried at 140 °C under N_2. Weight loss was determined by thermogravimetric analysis (TGA) at 140 °C, and inorganic residue was determined after the samples were heated at 900 °C in O_2. The results were ±0.4% of the theoretical values. LC/MS negative and positive ion spectra were obtained on an HP 5985 B instrument; (source 250 °C, 0.9 Torr). HPLC Hewlett-Packard 1090L equipped with Hewlett-Packard ODS Hypersil 100 × 4.6 mm, 5 μm column; eluent $CH_3CN/THF/H_2O$ (70:10:30 v/v). FAB/MS positive ion spectra were obtained on a Kratos MS-50 instrument fitted with a standard FAB source and a high-field magnet; the sample (10 μmol) was dispersed in a few microliters of 2-thioglycerol−diglycerol (1:1) matrix and bombarded with a 6−9 keV beam of Xe atoms. IR spectra were recorded as a Nujol mull with a Perkin-Elmer 580 spectrophotometer. ^{1}H NMR spectra were obtained with a Bruker AM 500 instrument equipped with an Aspect 3000

Table 5. Reaction Conditions and Physical−Chemical Data for Compounds **7a**−**l**

compd	amine	molar ratio: amine/**5**	solvent	time (h)	yield (%)	anal. (C, H, Br, N)	HPLC (t_R rel)[a]	MS, *m*/*z*[a]
7a	NH_4OH, 36% aq	50	THF	48	37	$C_{25}H_{16}BrNO_{12}$	1.16[b]	601, $[M]^-$ [d]
7b	CH_3NH_2, 35% aq	1.5	THF	2	33	$C_{26}H_{18}BrNO_{12}$	1.12[b]	615, $[M]^-$ [d]
7c	$C_2H_5NH_2$, 70% aq	1.9	THF	15	84	$C_{27}H_{20}BrNO_{12}$	1.34[b]	629, $[M]^-$ [d]
7d	$C_3H_7NH_2$	7.4	THF	20	96	$C_{28}H_{22}BrNO_{12}$	2.54[c]	643, $[M]^-$ [d]
7e	$CF_3CH_2NH_2$	14.8	DMF	96	70	$C_{27}H_{17}BrF_3NO_{12}$	1.52[b]	684, $[MH]^+$ [e]
7f	$CNCH_2NH_2$	5	DMF	18	96	$C_{27}H_{17}BrN_2O_{12}$	0.91[b]	641, $[MH]^+$ [e]
7g	$HOC_2H_4NH_2$	4	DMF	6	98	$C_{27}H_{20}BrNO_{13}$	0.56[b]	646, $[MH]^+$ [e]
7h	$PhCH_2NH_2$	5.5	THF	2	87	$C_{32}H_{22}BrNO_{12}$	2.89[c]	691, $[M]^-$ [d]
7i	$(CH_3)_2NC_2H_4NH_2$	2	THF	2	92	$C_{29}H_{25}BrN_2O_{12}$	0.73[c]	672, $[M]^-$ [d]
7l	$BOCNHC_2H_4NH_2$	5	DMF	1	78	$C_{32}H_{29}BrN_2O_{14}$	1.58[b]	689, $[MH]^+$ [e]

[a] See the Experimental Section for HPLC and MS conditions. [b] Gradient 2. [c] Gradient 1. [d] LC/MS. [e] FAB/MS.

Table 6. Workup Conditions and Physical−Chemical Data for Compounds **8a**−**l**

compd	reaction time, h	workup	yield (%)	anal. (C, H, Br, N)	HPLC (t_R rel)[a]	MS, *m*/*z*[a]
8a	2	C. c.[a] ($CHCl_3$/AcOH, 99:1, v/v)	48	$C_{25}H_{17}NO_{12}$	0.74[b]	523, $[M]^-$ [d]
8b	3	C. c. ($CHCl_3$/MeOH, 99.5:0.5, v/v)	50	$C_{26}H_{19}NO_{12}$	0.80[b]	538, $[MH]^+$ [e]
8c	4	C. c. ($CHCl_3$/MeOH/AcOH, 98:2:0.1, v/v)	40	$C_{27}H_{21}NO_{12}$	0.92[b]	551, $[M]^-$ [d]
8d	2	C. c. ($CHCl_3$/MeOH/AcOH, 98:2:0 to 96:4:0.1, v/v)	34	$C_{28}H_{23}NO_{12}$	1.72[c]	565, $[M]^-$ [d]
8e	1.5	C. c. ($CHCl_3$/MeOH/AcOH, 98:2:0 to 96:4:0.1, v/v)	20	$C_{27}H_{18}F_3NO_{12}$	1.61[b]	606, $[MH]^+$ [e]
8f	3	C. c. ($CHCl_3$/MeOH/AcOH, 100:0.5:0.05 to 80:20:0.1, v/v)	35	$C_{27}H_{18}N_2O_{12}$	0.68[b]	563, $[MH]^+$ [e]
8g	4	C. c. (CH_2Cl_2/MeOH, 99:1, v/v)	23	$C_{27}H_{21}NO_{13}$	0.44[b]	568, $[MH]^+$ [e]
8h	1.5	C. c. ($CHCl_3$/MeOH/AcOH, 100:0:0 to 95:5:0.1, v/v)	62	$C_{32}H_{23}NO_{12}$	2.15[c]	613, $[M]^-$ [d]
8i	2	the residue is taken up in absolute EtOH, filtered, and washed with Et_2O	45	$C_{29}H_{26}N_2O_{12}$	0.55[c]	594, $[M]^-$ [d]
8l	3	the same as **8i**	74	$C_{32}H_{30}N_2O_{14}$	1.29[b]	667, $[MH]^+$ [e]

[a] See Experimental Section for C. c. (column chromatography), HPLC, and MS conditions. [b] Gradient 2. [c] Gradient 1. [d] LC/MS. [e] FAB/MS.

Table 7. ^1H NMR (δ, ppm) Data for Purpuromycin and Compounds **7a**−**l** and **8a**−**l**

compd	3-H (m), (m)	3′-H (d), (d)	7,7′OCH_3 (s), (s)	4-H (m)	6′-H (s)	5,6-H (s), (s)	10-H (s)	9′-H (s)	4′-H (s)	substituents
1	2.52, 2.79	3.43, 3.62	3.87, 3.89	4.95	6.39	7.50, 7.75	10.66	11,87	13.10	
7a	2.51, 2.78	3.43, 3.63	3.88	4.95		7.51, 7.76	10.66	11.50	13.50	7.95 (2H, s)
7b	2.51, 2.76	3.42, 3.62	3.86	4.94		7.49, 7.74	10.62	11.40	13.61	3.13 (3H, d); 7.70 (1H, q)
7c	2.50, 2.77	3.42, 3.62	3.87	4.95		7.50, 7.74	10.54	11.38	13.60	1.22 (3H, t); 3.81 (2H, q); 7.61 (1H, t)
7d	2.50, 2.77	3.41, 3.61	3.87	4.95		7.50, 7.74	nd	12.70	13.63	0.89 (3H, t); 1.62 (2H, q); 3.75 (2H, q); 7.60 (1H, t)
7e	2.51, 2.77	3.43, 3.63	3.88	4.96		7.50, 7.75	8.30	10.65	13.22	4.65 (2H, m); 5.85 (1H, t)
7f	2.52, 2.75	3.42, 3.64	3.86	4.95		7.51, 7.77	10.66	11.41	13.21	4.65 (2H, d); 7.73 (1H, t)
7g	2.49, 2.78	3.42, 3.59	3.85	4.94		7.50, 7.75	nd	nd	13.55	3.61 (2H, m); 3.65 (2H, m); 7.33 (1H, t)
7h	2.46, 2.73	3.32, 3.53	3.87	4.94		7.49, 7.73	nd	nd	nd	5.05 (2H, s); 7.24−7.41 (5H, m); 7.87 (1H, t)
7i	2.52, 2.78	3.44, 3.63	3.86	4.96		7.50, 7.74	nd	nd	nd	2.84 (3H, s); 2.86 (3H, s); 3.36 (2H, m); 4.10 (2H, m); 7.58 (1H, t)
7l	2.50, 2.79	3.42, 3.65	3.87	4.96		7.51, 7.78	10.68	11.47	13.55	1.34 (9H, s); 3.22 (2H, m); 3.87 (2H, m); 7.00 (1H, t); 7.55 (1H, t)
8a	2.49, 2.77	3.39, 3.60	3.87	4.96	5.73	7.50, 7.74	10.70	11.57	14.04	7.45 (2H, broad)
8b	2.50, 2.78	3.41, 3.61	3.88	4.97	5.53	7.51, 7.76	10.68	11.55	14.19	2.82 (3H, d); 7.93 (1H, q)
8c	2.49, 2.77	3.40, 3.60	3.87	4.96	5.59	7.50, 7.74	nd	nd	14.15	1.16 (3H, t); 3.32 (2H, q); 7.79 (1H, t)
8d	2.48, 2.76	3.40, 3.60	3.87	4.96	5.60	7.50, 7.74	10.61	11.55	14.13	0.90 (3H, t); 1.59 (2H, m); 3.16 (2H, q); 7.82 (1H, t)
8e	2.50, 2.77	3.41, 3.61	3.86	4.95	6.02	7.50, 7.75	10.64	11.53	13.77	4.14 (2H, m); 7.93 (1H, t)
8f	2.50, 2.76	3.42, 3.62	3.88	4.96	5.90	7.50, 7.75	10.65	nd	nd	4.47 (2H, d); nd (1H, t)
8g	2.49, 2.76	3.40, 3.60	3.87	4.95	5.66	7.50, 7.75	10.65	11.50	14.14	3.28 (2H, m); 3.58 (2H, m); 7.61 (1H, t)
8h[a]	2.50, 2.77	3.39, 3.59	3.86	4.95	5.50	7.50, 7.74	nd	nd	nd	4.47 (2H, d); 8.39 (1H, t)
8i	2.50, 2.78	3.42, 3.61	3.87	4.97	5.82	7.51, 7.75	10.55	11.50	13.93	2.80 (6H, s); 3.31 (2H, m); 3.63 (2H, m); 7.86 (1H, t)
8l	2.49, 2.78	3.43, 3.60	3.87	4.97	5.69	7.51, 7.77	10.65	11.55	14.12	1.36 (9H, s); 3.14 (2H, m); 3.23 (2H, m); 7.01 (1H, t);7.83 (1H, t)

[a] CD_3SOCD_3 + CF_3COOD.

console at 500 MHz. The spectra were recorded at 40 °C in DMSO-d_6 or $CDCl_3$ solution (internal standard TMS, δ 0.00 ppm).

4-*O*-(Tetrahydro-2*H*-pyran-2-yl)purpuromycin (2). Dihydropyran (50 mL, 0.548 mol), camphorsulfonic acid (500 mg, 2.15 mmol), and molecular sieve 4Å (50 g) were added to a stirred solution of purpuromycin (**1**) (10 g, 0.0186 mol) in anhydrous CH_2Cl_2, and the mixture was heated at reflux for 2 h. After the reaction mixture was cooled to 0 °C, it was washed with 0.5% chilled aqueous $NaHCO_3$ (1 L) and water (1 L) and dried (Na_2SO_4). The solvent was concentrated under reduced pressure, and the residue was suspended in ether, filtered, and washed with the same solvent to afford the product (8.2 g, 70% yield): TLC R_f 0.35 (chloroform−methanol, 98:2, v/v); HPLC t_R rel 2.7 (gradient 2); IR 3600−3100, 1735, 1690, 1605, 1250 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.45−1.75 (m, 6H), 2.70 and 2.82 (2m, 2H), 3.41 and 3.61 (2d, 2H), 3.59−3.63 (m, 2H), 3.87 and 3.89 (2s, 6H), 4.95−5.05 (m, 1H), 5.01 (m, 1H), 6.39 (s, 1H), 7.50 and 7.80 (2s, 2H), 10.68 (s, 1H), 11.88 (s, 1H), 13.12 (s, 1H) anomeric mixture; MS (LC) *m*/*z* 622 $[M]^+$. Anal. ($C_{31}H_{26}O_{14}$) C, H.

4′,9′,10-Triacetyl-4-*O*-(tetrahydro-2*H*-pyran-2-yl)purpuromycin (3). A mixture of the tetrahydropyranyl ether **2** (2.07 g, 3.33 mmol), anhydrous pyridine (3 mL), and acetic anhydride (50 mL) was stirred overnight at room temperature under an atmosphere of argon. The reaction mixture was

poured into ice water (300 mL) with stirring and extracted with ethyl acetate (3 × 200 mL). The ethyl acetate extract was washed with 0.5% aqueous $NaHCO_3$ (1 × 200 mL) and water (3 × 200 mL) and dried over anhydrous Na_2SO_4. After removal of solvent, the solid residue was washed with ether, filtered, and dried *in vacuo* to give pure **3** (2.1 g, 85% yield): mp 160 °C dec; HPLC t_R rel 1.9 (gradient 2); ^{1}H NMR (DMSO-d_6) δ 1.45−1.75 (m, 6H), 2.18 (s, 3H), 2.26 (s, 3H), 2.35 (s, 3H), 2.75 and 2.88 (2m, 2H), 3.50−3.60 (m, 2H), 3.51 and 3.72 (2d, 2H), 3.79 and 3.86 (2s, 6H), 4.95−5.12 (m, 1H), 5.03 (m, 1H), 6.12 (s, 1H), 7.76 and 7.94 (2s, 2H) anomeric mixture; MS (LC) m/z 748 $[M]^+$. Anal. ($C_{37}H_{32}O_{17}$) C, H.

4′,9′,10-Triacetyl-6′-bromo-4-*O*-(tetrahydro-2*H*-pyran-2-yl)purpuromycin (4). To compound **3** (4.15 g, 5.54 mmol) in methylene chloride (500 mL) were added pyridinium bromide perbromide (3.5 g, 0.011 mmol) and pyridine (2.5 mL). The reaction mixture was stirred overnight at 30 °C, washed with aqueous $Na_2S_2O_5$ (2 × 200 mL) and water (2 × 200 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated by adding toluene (50 mL) to eliminate pyridine. To the solid residue was added ether, and the precipitate was filtered, rinsed with ether, and vacuum dried to afford **4** (4.3 g, 94% yield): HPLC t_R rel 2.73 (gradient 2); ^{1}H NMR (DMSO-d_6) δ 1.45−1.80 (m, 6H), 2.19 (s, 3H), 2.26 (s, 3H), 2.38 (s, 3H), 2.65 and 2.79 (2m, 2H), 3.42 and 3.59 (2d, 2H), 3.50−3.60 (m, 2H), 3.87 and 4.09 (2s, 6H), 4.96 (m, 1H), 5.04 (m, 1H), 7.75 and 7.94 (2s, 2H) anomeric mixture; MS (LC) m/z 825 $[M]^-$. Anal. ($C_{37}H_{31}BrO_{17}$) C, H, Br.

4′,9′,10-Triacetyl-6′-bromopurpuromycin (5). A mixture of **4** (30 g, 36 mmol) and catalytic *p*-toluenesulfonic acid (300 mg) in methanol (3 L) was heated to reflux for 5 h. The reaction solution was concentrated to a small volume, and the resulting precipitate was filtered, washed with ether, and vacuum dried to give **5** (24.2 g, 90% yield): 2.19 (s, 3H), 2.26 (s, 3H), 2.38 (s, 3H), 1.15 (gradient 2); ^{1}H NMR (DMSO-d_6) δ 2.15 (s, 3H), 2.32 (s, 3H), 2.35 (s, 3H), 2.78 and 2.81 (2m, 2H), 3.47 and 3.71 (2d, 2H), 3.88 and 4.10 (2s, 6H), 5.01 (m, 1H), 7.78 and 7.94 (2s, 2H). Anal. ($C_{32}H_{23}BrO_{16}$) C, H, Br.

6′-Bromopurpuromycin (6) and 6′-Bromo-7′-demethoxy-7′-aminopurpuromycin (7a). To a stirred solution of **5** (300 mg, 0.403 mmol) in tetrahydrofuran (100 mL) was added 36% NH_4OH (1 mL), and the mixture was stirred at room temperature for 48 h. The reaction mixture was then neutralized with 1 N HCl and the solvent evaporated *in vacuo*. The crude material obtained was purified by flash column chromatography eluted with chloroform−methanol (99:1 v/v) to afford pure **6** (100 mg, 40% yield) and **7a** (90 mg, 37% yield). **6**: HPLC t_R rel 1.83 (gradient 2); ^{1}H NMR (DMSO-d_6) δ 2.51 and 2.79 (2m, 2H), 3.45 and 3.64 (2d, 2H), 3.87 and 4.17 (2s, 6H), 4.95 (m, 1H), 7.50 and 7.75 (2s, 2H), phenolic protons not determined; MS (LC) m/z 616 $[M]^-$. Anal. ($C_{26}H_{17}BrO_{13}$) C, H, Br. **7a**: HPLC t_R rel 1.16 (gradient 2); ^{1}H NMR (DMSO-d_6) δ 2.51 and 2.78 (2m, 2H), 3.43 and 3.63 (2d, 2H), 3.88 (s, 3H), 4.95 (m, 1H), 7.51 and 7.76 (2s, 2H), 7.95 (s, 2H), 10.66 (s, 1H), 11.50 (s, 1H), 13.50 (s, 1H); MS (LC) m/z 601 $[M]^-$. Anal. ($C_{25}H_{16}BrNO_{12}$) C, H, N, Br.

General Method A for Derivatives 7b−l. To a stirred solution of **5** (743 mg, 1 mmol) in the solvent (100 mL) was added the amine, and the mixture was stirred at room temperature until the reaction was complete. The mixture was treated with 1 N HCl up to color change from violet to red, and then water was added under stirring until complete precipitation of the product. The suspension was centrifuged (4500 rpm, 10 °C, 10 min), and the precipitate was separated from the supernatant and dissolved in acetone. The solvent was evaporated, and the residue was taken up in ethanol absolute, filtered, washed with ether, and vacuum dried to afford compounds **7a−l** (Table 5, reaction conditions and physical−chemical data; Table 7, ^{1}H NMR data).

General Method B for Derivatives 8a−l. To a stirred solution of compound **7** (1 mmol) in a mixture of tetrahydrofuran (100 mL) and methanol (25 mL) were added under an argon atmosphere sodium acetate (0.5 mmol) and 5% palladium on activate carbon (300 mg). This reaction mixture was hydrogenated at room temperature and atmospheric pressure until the starting material disappeared and then was filtered on Celite (filter aid) and acidified with 0.1 N HCl. After removal of solvent, the residue was worked up to afford compounds **8a−l** (Table 6, workup conditions and physical−chemical data; Table 7, ^{1}H NMR data).

7′-[(2-Aminoethyl)amino]-7′-demethoxypurpuromycin Trifluoroacetate (8m). A solution of **8l** (175 mg, 0.262 mmol) and trifluoroacetic acid (1.75 mL) in dichloromethane (35 mL) was stirred at room temperature for 3 h with progressive precipitation of the product. The solvent was evaporated, and the residue was taken up in ether, filtered, and vacuum dried to afford **8m** (170 mg, 95% yield): HPLC t_R rel 0.62 (gradient 1); IR 3500−3100, 1710 (shoulder), 1682, 1589, 1522, 1331, 1229, 1204, 1144, 1003, 943, 798, 721 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.50 and 2.77 (2m, 2H), 3.02 (m, 2H), 3.41−3.49 (m, 2H), 3.42 and 3.62 (2d, 2H), 3.87 (s, 3H), 4.97 (m, 1H), 5.79 (s, 1H), 7.51 and 7.76 (2s, 2H), 7.78 (broad, 4H), phenolic protons not determined; MS (FAB) m/z 567 $[MH]^+$. Anal. ($C_{27}H_{22}N_2O_{12}\cdot CF_3CO_2H$) H; C: calcd, 51.18; found, 51.58; N: calcd, 4.12; found, 4.05.

Acknowledgment. The authors thank Dr. Pietro Ferrari, Dr. Marina Galimberti, and Dr. Marino Nebuloni, of the Analytical Sciences Department, for determination of ^{1}H NMR, mass spectra, and elemental analysis, respectively.

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JM960672T