mCi/μmol), and polymer. Polymer was preincubated with poly(A) for 10 min at 25 °C. After that S-100 and ribosomes were added and reaction was carried out for 20 min at 37 °C. Polylysine (200 μg) was added and reaction was stopped by adding 0.1 mL of 1 N KOH. The solution was incubated for 20 min at 37 °C after which 25 μL of 100% trichloroacetic acid and 3 mL of 5% trichloroacetic acid–0.25% sodium tungstate, pH 2.0, were added. Samples were filtered and counted as described for poly(U) translation.

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Synthesis and Antibacterial Activity of Some Derivatives of Tolypomycinone. Relationship between Structure and Activity in Ansamycins

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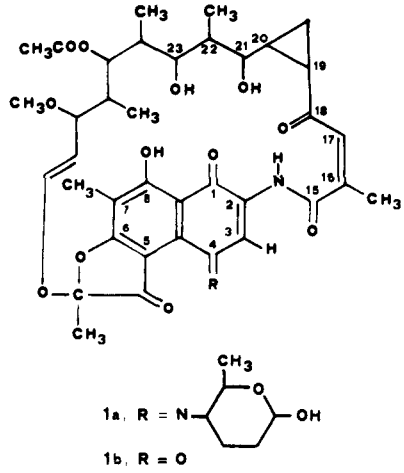
3-Aminotolypomycinones and 3,16-diamino-16,17-dihydrotolypomycinones are formed by the addition of primary and secondary amines to tolypomycinone, obtained by mild hydrolysis of the antibiotic tolypomycin Y. 3-Amino-16,17-dihydrotolypomycinones are formed by the addition of primary and secondary amines to 16,17-dihydrotolypomycinone. In vitro microbiological tests showed high antibacterial activity in compounds obtained by the addition of primary amines, which must be unbranched in the α position to the nitrogen atom, to position 3 of the naphthoquinone ring. The relationship between structure and activity is described, and evidence is presented that hydrogen bonding between the amino NH bonded to C_3 and the amide CO of tolypomycinone is very important for biological activity.

Tolypomycin Y (**1a**) is an antibiotic isolated from fermentation broths of *Streptomyces tolypophorus*.[1-3] The antibiotic has high antimicrobial activity against gram-positive bacteria and is active to some extent also against gram-negative bacteria.[4] Its ansa structure[5-7] is similar to that previously determined for the rifamycins[8,9] and streptovaricins.[10,11] It has also been demonstrated that activity of tolypomycin is due to inhibition of bacterial RNA polymerase as for the other naphthalenic ansamycins.[12,13]



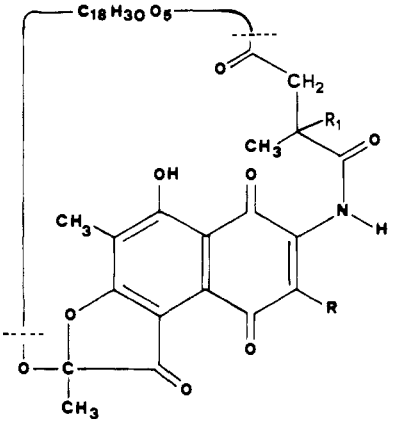
Tolypomycin Y is unstable both to acids and alkalies;[4] mild acid hydrolysis of tolypomycin Y leads to tolypomycinone (**1b**) that is structurally related to rifamycin S but differs from it in having a considerably lower antibacterial activity. We therefore undertook the task of preparing derivatives more stable than tolypomycin Y and with a higher antibacterial activity than tolypomycinone.

From structure–activity relationship (SAR) studies carried out on rifamycin derivatives,[14,15] the hypothesis was advanced that the presence of two free hydroxy groups on C_{21} and C_{23} and of aromatic nucleus with oxygen atoms on C_1 and C_8 is required for antibacterial activity. Also it was hypothesized that only if the hydroxyls on C_{21} and C_{23} have the proper orientation will the derivative be able to form, with bacterial RNA polymerase, the complex responsible for antibiotic activity.[15]

From crystallographic studies of rifamycin SV *p*-iodoanilide and rifampicin,[16] both very active rifamycin derivatives, it has been evidenced that the hydroxy groups on C_{21} and C_{23} are almost parallel to the plane of the naphthohydroquinone nucleus and oriented to the same direction as the phenolic hydroxyls on C_1 and C_8.

The 8,21,23-tri-*m*-bromobenzoate of tolypomycinone, the structure of which has also been determined by x rays,[7] is completely inactive because the hydroxy groups on C_8, C_{21}, and C_{23} are esterified, but the conformation of the ansa corresponding to the C_{21} and C_{23} atoms is very close to that

Table I. Antibacterial Activity of N-Substituted 3-Amino-16,17-dihydrotolypomycinones and N-Substituted 3,16-Diamino-16,17-dihydrotolypomycinones[a]

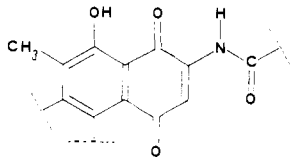


Compd	R	R_1	Formula	Analyses	Minimal inhibitory concn, $\mu g/mL$[b] A	B	C	D
Tolypomycin Y (1a)			$C_{43}H_{54}N_2O_{14}$	C, H, N	0.025	100	>100	>100
Tolypomycinone (1b)			$C_{37}H_{43}NO_{13}$	C, H, N	0.5			
2	-H	-H	$C_{37}H_{45}NO_{13}$	C, H, N	0.5			
3	$-NHCH_3$	-H	$C_{38}H_{48}N_2O_{13}$	C, H, N	0.5			
4	$-NHCH_3$	$-NHCH_3$	$C_{39}H_{51}N_3O_{13}$	C, H; N[c]	0.005	>100	>100	50
5	$-NHCH_2-C_6H_5$	$-NHCH_2-C_6H_5$	$C_{51}H_{59}N_3O_{13}$	C, H; N[d]	0.25			

[a] Melting points have not been included because most compounds decompose on heating. [b] A = *Staphylococcus aureus* 209 P (FDA). B = *Klebsiella pneumoniae* Ottaviani. C = *Escherichia coli* MC/35. D = *Salmonella paratyphi* B 0248 K (Sclavo). [c] N: calcd, 5.45; found, 5.01. [d] N: calcd, 4.55; found, 4.77.

found in the rifamycin SV *p*-iodoanilide and in rifampicin. We therefore set ourselves to prepare compounds from tolypomycinone that had the preferential ansa conformation seen in its tri-*m*-bromobenzoyl derivative but with the hydroxy groups on C_8, C_{21}, and C_{23} free.

If the structure of the ansa in the region from C_2 to C_{21} is considered, it can be seen that the bond where rotation is easiest is that between C_2 and N. The conformation at atom C_{21} depends therefore mainly on the conformation of the C_2–N linkage. In 8,21,23-tri-*m*-bromobenzoyltolypomycinone, the amidic CO has a conformation different from that of rifamycins;[9,16] it lies approximately in the plane of the naphthoquinone ring and on the opposite side of the quinone oxygen on C_1, as shown diagrammatically in partial formula A. In order that the tolypomycinone

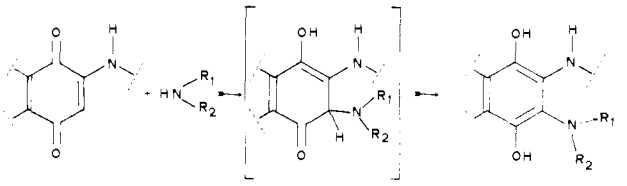


A

derivatives should have the preferential conformation at C_{21} and C_{23} found in the crystalline state for tolypomycinone tri-*m*-bromobenzoate, we have to stabilize the conformation of the amide group as shown in partial formula A. If the working hypothesis were valid, then we had to presume that, unlike the situation in the rifamycins,[17-19] the hydrazones of 3-formyltolypomycinone would be inactive, since the planar carbon introduced in position 3 would cause a significant change in the conformation of the amidic group. But, on the other hand, the introduction at position 3 of groups capable of forming a hydrogen bond with the amidic CO should give a high activity.

In this paper the derivatives prepared by the addition of primary amines at C_3 are described, in which the formation of a hydrogen bond between the aminic NH and the amidic CO can be anticipated.

Scheme I



Chemistry. The site on the naphthoquinone nucleus of tolypomycinone most easily attacked by nucleophilic reagents, such as amines, is C_3, according to the mechanism shown in Scheme I.

In tolypomycins, in contrast to the case of rifamycins, the amines can also add across the C_{16}–C_{17} double bond conjugated with the carbonyl group, leading to the formation of 16,17-dihydro-16-aminotolypomycinones. By the addition, at room temperature, of a large excess of amine to tolypomycinone, mixtures of 3-amino and 3,16-diamino derivatives were obtained, together with small amounts of other compounds that showed low polarity on TLC. These last probably derived from the formation of ketal bridges between the carbonyl group in position 18 and the hydroxyls on C_{21} and C_{23}, as has already been reported by Kishi et al.[6]

It was possible to separate the 3-amino and 16,17-dihydro-3,16-diamino derivatives in the reaction mixture by column chromatography. If the reaction mixture was cooled with ice and only a small excess of amine used, it was possible to restrict the addition of amine only to position 3; by this way it was possible to achieve moderate to good yields of the 3-amino derivative, according to the nature of the amine used. If the quinone nucleus was preliminarily reduced to hydroquinone with ascorbic acid, it was possible to direct the addition to position 16 only. By a preliminary catalytic hydrogenation of the C_{16}–C_{17} double bond, addition to position 3 only occurred with formation of 3-amino-16,17-dihydrotolypomycinones. The

catalytic hydrogenation led also to a reduction of the naphthoquinone which could be selectively reoxidated to the quinone form with potassium ferricyanide. Subsequent nucleophilic attack with the amine occurred only at C_3.

During the addition, the 3-amino derivatives obtained did not remain in the hydroquinone form because of the presence of tolypomycinone excess; a redox system was set up and the 3-amino-1,4-dihydrotolypomycinone formed was partly reoxidized to 3-aminotolypomycinone. The existence of this equilibrium reduced the yield of the reaction, because the 1,4-dihydrotolypomycinone formed cannot add the amine. In some cases the yields could be increased by adding manganese dioxide during the reaction to reoxidize hydroquinone to quinone. The manganese dioxide used was prepared according to Rosenkranz.[21]

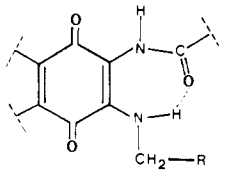
The reactions were carried out in solvents such as chloroform and, less frequently, dioxane, at room temperature or in an ice bath. The reaction products, after oxidation with potassium ferricyanide in aqueous solution, were isolated by extraction with ethyl acetate or chloroform and purified by column chromatography. Finally the 3-aminotolypomycinones were reduced to the corresponding hydroquinone with ascorbic acid. The products were obtained as crystalline solids. Their structure was confirmed by both UV–visible absorption spectra, where the appearance of a band between 520 and 540 nm was seen, as in the case of similar derivatives of the rifamycins,[20] and the disappearance of the aromatic proton signal in position 3 at δ 7.8 ppm in ^{1}H NMR spectra. In the ^{1}H NMR spectra of the 3,16-disubstituted derivatives there was the disappearance of both the C_3-H signal and an olefinic proton signal at δ 6.48 ppm.

Results and Discussion

Tables I and II show the data from the in vitro antibacterial tests against *Staphylococcus aureus*, giving the activities found for all the newly synthesized derivatives. In the more interesting cases, the derivatives were also tested against three strains of gram-negative bacteria. To complete the comparisons, the activities of tolypomycinone, tolypomycin Y, rifamycin SV, and rifampicin were included.

3-Methylamino-16,17-dihydrotolypomycinone (**3**) is less active than 3-methylaminotolypomycinone (**8**), in agreement with previous findings for rifamycins, where reduction of the double bonds C_{16}–C_{17} and C_{18}–C_{19} led to a fall of biological activity,[22] probably because of loss of rigidity of the ansa. On the other hand, 3,16-bis(methylamino)-16,17-dihydrotolypomycinone (**4**) shows a higher activity than compound **3** probably due to the steric hindrance on the ansa chain, caused by the presence of the methylamino group on C_{16}.

Among the 3-amino derivatives, the compounds formed by addition of a primary alkyl- or arylalkylamine, unbranched at the carbon on the α position to the amine group (partial formula B), almost invariably had high



B

antibacterial activity. In derivatives of this type the aminic NH has the ability to form a hydrogen bond with the amidic CO. In compound **7**, formed by the addition of dimethylamine to position 3 of tolypomycinone, where the formation of such a hydrogen bond is not possible, the antibacterial activity was relatively lower than in compound **8**, especially against gram-negative bacteria.

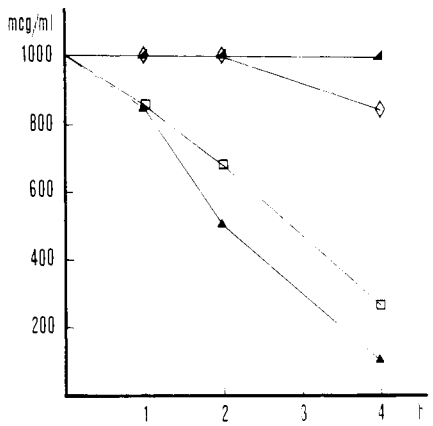


Figure 1. Residual inhibitory activity of 3-[2-(*N*-morpholyl)-ethylamino]tolypomycinone (**11**) and tolypomycin Y (**1a**) (for comparison) in simulated gastric (pH 1.3) and intestinal (pH 8.3) juices: compound **11** at pH 8.3 (◇) and pH 1.3 (◢); compound **1a** at pH 8.3 (□) and pH 1.3 (▲).

The formation of a very stable hydrogen bond between the amino group and amidic CO was confirmed by the ^{1}H NMR spectra of the 3-methylaminotolypomycinone. The signal of the methyl group bonded to the nitrogen atom at δ 3.35 ppm is a sharp doublet with J = 5.5 Hz, indicating its coupling with the aminic proton and therefore making chemical exchange very slow if not impossible. In 3-methylaminorifamycin S, the corresponding methyl signal is a singlet.[20]

The low activity found in the cyclohexylamine derivatives **6** is probably caused by steric hindrance due to the branching α to the amino group. As a consequence of this negative result, we synthesized the other derivatives (compounds **8–31**) without branching in position α to the amino group.

Changes in the antibacterial spectrum as a function of the chemical and physical characteristics of the amines added will be reported elsewhere.

Figure 1 shows the residual inhibitory activity of 3-[2-(*N*-morpholyl)ethylamino]tolypomycinone (**11**) against *S. aureus* after 1, 2, and 4 h in simulated gastric and intestinal juice (pH 1.3 and 8.3). The activity of tolypomycin Y under the same conditions is included for comparison. The ordinate values represent the residual antibacterial activity. The derivative **11** shows an enhanced stability and, consequently, a prolonged antibacterial activity in both acidic and alkaline solutions, as compared with tolypomycin Y (**1a**).

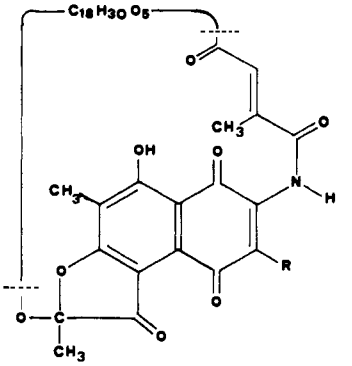
Since inactivation of tolypomycin Y takes place principally by hydrolysis to tolypomycinone in acidic solution and by formation of cyclic ketals between the carbonyl CO in position 18 and the hydroxyls at C_{21} and C_{23} in alkaline solution, we are led to the view that conformational stabilization slows or prevents ketalization.

Experimental Section

Electronic spectra were obtained on a double-beam Perkin-Elmer 124 spectrophotometer in methanol, while absorbances at different λ were taken on a Beckman DU-2. ^{1}H NMR spectra were obtained on a Jeol C-60HL at 60 MHz in $CDCl_3$. TLC was performed on silica gel plates 60 F_{254}, layer thickness 0.2 mm (Merck); preparative chromatographies were carried out over silica gel 60 F_{254} precoated plates (thickness 2 mm) (Merck) and column chromatographies over silica gel 60, 70-230 ASTM (Merck).

(1) **Synthesis of 3-Methylamino-16,17-dihydrotolypomycinones.** (a) **16,17-Dihydrotolypomycinone (2).** To a

Table II. Antibacterial Activity of N-Substituted 3-Aminotolypomycinones[a]



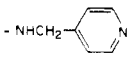
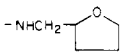
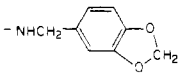
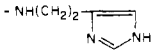
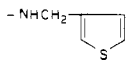
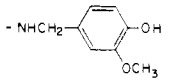
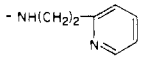
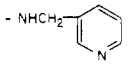
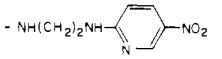
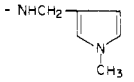
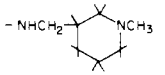
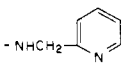
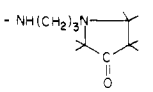
Compd	R	Formula	Analyses	Minimal inhibitory concn, μg/mL[b] A	B	C	D
Tolypomycin Y (1a)		$C_{43}H_{54}N_2O_{14}$	C, H, N	0.025	100	>100	>100
Tolypomycinone (1b)		$C_{37}H_{43}NO_{13}$	C, H, N	0.5			
6	-NH-c-C_6H_{11}	$C_{43}H_{54}N_2O_{13}$	C, H, N	0.25			
7	$-N(CH_3)_2$	$C_{39}H_{48}N_2O_{13}$	C, H, N	0.025	100	50	100
8	$-NHCH_3$	$C_{38}H_{46}N_2O_{13}$	C, H; N[c]	0.005	25	50	12
9	$-NH(CH_2)_3OH$	$C_{40}H_{50}N_2O_{14}$	C, H, N	0.025	25	12	12
10	$-NHCH_2-C_6H_5$	$C_{44}H_{50}N_2O_{13}$	C, H; N[d]	<0.005			
11	$-NH(CH_2)_2$-c-$N(CH_2CH_2)_2O$	$C_{43}H_{55}N_3O_{14}$	C, H; N[e]	<0.005	50	12	12
12	$-NH(CH_2)_3N(CH_3)_2$	$C_{42}H_{55}N_3O_{13}$	C, H, N	0.025	50	12	12
13		$C_{43}H_{49}N_3O_{13}$	C, H; N[f]	0.005	100	25	12
14		$C_{42}H_{52}N_2O_{14}$	C, H, N	0.005	25	6	12
15		$C_{45}H_{50}N_2O_{15}$	C, H, N	<0.005	>100	>100	100
16	$-NHCH_2-C_6H_4$-*p*-$N(CH_3)_2$	$C_{46}H_{55}N_3O_{13}$	C, H; N[g]	<0.005	12	23	12
17		$C_{42}H_{50}N_4O_{13}$	C, H, N	0.05	12	50	12
18	$-NH(CH_2)_3$-c-$N(CH_2CH_2)_2NCH_3$	$C_{45}H_{60}N_4O_{13}$	C, H, N	0.025	25	12	3
19		$C_{42}H_{48}N_2O_{13}S$	C, H, N, S	<0.005	100	12	12
20	$-NH(CH_2)_2-C_6H_4$-*p*-OH	$C_{45}H_{52}N_2O_{14}$	C, H, N	<0.005	>100	>100	>50
21		$C_{45}H_{52}N_2O_{15}$	C, H, N	<0.005	100	100	100
22		$C_{44}H_{51}N_3O_{13}$	C, H, N	<0.005	25	6	12
23		$C_{43}H_{49}N_3O_{13}$	C, H, N	<0.005	25	6	12
24		$C_{44}H_{51}N_5O_{15}$	C, H, N	<0.025	>50	>50	>50
25		$C_{43}H_{51}N_3O_{13}$	C, H, N	0.005	12	12	12
26		$C_{44}H_{57}N_3O_{13}$	C, H, N	0.025	12	12	12
27		$C_{43}H_{49}N_3O_{13}$	C, H, N	<0.005	12	6	25
28	$-NH(CH_2)_2$-c-$N(CH_2CH_2)_2NCH_3$	$C_{44}H_{58}N_4O_{13}$	C, H, N	0.025	12	25	6
29		$C_{44}H_{55}N_3O_{14}$	C, H, N	0.005		50	25
30	$-NH(CH_2)_2CH(OCH_3)_2$	$C_{42}H_{54}N_2O_{15}$	C, H, N	0.005	50	25	25
31	$-NH(CH_2)_2N(CH_3)_2$	$C_{41}H_{53}N_3O_{13}$	C, H, N	<0.005	25	6	12
Rifamycin SV				<0.005	100	100	100
Rifampicin				0.025	25	6	12

Table II (*Continued*)

[a] Melting points have not been included because most compounds decompose on heating. [b] A = *Staphylococcus aureus* 209 P(FDA). B = *Klebsiella pneumoniae* Ottaviani. C = *Escherichia coli* MC/35. D = *Salmonella paratyphi* B 0248 K (Sclavo). [c] N: calcd, 3.79; found, 3.87. [d] N: calcd, 3.43; found, 3.14. [e] N: calcd, 5.01; found, 4.94. [f] N: calcd, 5.15; found, 4.79. [g] N: calcd, 4.89; found, 5.20.

solution of 0.710 g (1 mmol) of tolypomycinone (**1b**) in 50 mL of ethanol 0.180 g of palladium on charcoal at 5% was added. The hydrogenation was carried out at normal pressure and room temperature. The reaction was stopped when 4 equiv of hydrogen had been absorbed. The suspension was filtered over diatomaceus earth (Celite) and the liquid concentrated to about 10 mL. Ethyl acetate (100 mL) was added and the organic phase was treated with a 3% aqueous solution of $FeCl_3$ (4 × 50 mL) and then washed many times with water, dried (Na_2SO_4), and evaporated to dryness. The residue was purified over a silica gel column (300 mL) using as eluent a mixture of ethyl acetate–methanol (24:1): UV (methanol) λ_{max}, nm (log ϵ), 228 (4.36), 277 (sh), 333 (3.91), 408 (3.58); yield 0.535 g (75%).

(b) **3-Methylamino-16,17-dihydrotolypomycinone (3).** To a solution of 0.710 g (1 mmol) of **2** in 5 mL of $CHCl_3$ 0.095 g (3 mmol) of methylamine in 2 mL of $CHCl_3$ at 10 °C was added. The mixture was stirred until all the starting product had disappeared, as seen by TLC. $CHCl_3$ (300 mL) was added to the mixture and washed once with a 0.5% aqueous solution of citric acid and three times with water. The organic phase was dried over Na_2SO_4 and brought to dryness. The residue was purified on a silica gel column: UV (methanol) λ_{max}, nm (log ϵ), 224 (4.22), 307 (4.06), 362 (3.84), 520 (3.36); yield 0.225 g (33%).

(2) **Synthesis of N-Substituted 3,16-Diamino-16,17-dihydrotolypomycinones.** All compounds shown on Table I were prepared according to the procedure described for compound **4** (see below).

(a) **3,16-Bis(methylamino)-16,17-dihydrotolypomycinone (4).** **1b** (0.710 g, 1 mmol) in 10 mL of $CHCl_3$ was stirred for 12 h at room temperature with 0.310 g (10 mmol) of methylamine. The mixture was oxidized with MnO_2 and after 20 min the MnO_2 was removed by filtration and another 2 equiv of methylamine was added. Six hours later the reaction was stopped by adding 200 mL of $CHCl_3$ and by washing several times with water. The organic layer was brought to dryness and purified over a silica gel column: UV (methanol) λ_{max}, nm (log ϵ), 234 (4.30), 262 (4.26), 318 (4.08), 373 (3.79), 530 (3.39); yield 0.350 g (45%).

(3) **Synthesis of N-Substituted 3-Aminotolypomycinones.** All compounds reported in Table II were prepared according to the method used for compound **8**.

(a) **3-Methylaminotolypomycinone (8).** To a solution containing 0.710 g (1 mmol) of **1b** in 10 mL of $CHCl_3$ 0.090–0.150 g (3–5 mmol) of methylamine was added. The mixture was stirred at room temperature until all the starting product disappeared. MnO_2 (0.300 g) was added and after 10 min removed by filtration. The mixture, diluted with 200 mL of $CHCl_3$, was washed several times with 0.5% citric acid and then with water. The dried organic phase was brought to dryness and the residue was purified on a silica gel column: UV (methanol) λ_{max}, nm (log ϵ), 232 (4.38), 263 (4.32), 318 (4.06), 360 (3.85), 530 (3.12); yield 30–45%.

Biological Tests. Antimicrobial Activity. MIC values were determined in a liquid medium, by means of the serial dilution method in test tubes. The medium employed was Brain Heart Infusion (BHI, Difco). The inoculum size was always 10^6 cells/mL. The MIC was defined as the lowest antibiotic concentration that prevented a visible growth after 24 h of incubation at 35 °C. In order to evaluate the stability of tolypomycin Y derivatives and to compare it with that of the starting material, solutions in both simulated gastric and intestinal juice (pH 1.3 and pH 8.3, respectively) were prepared. At regular intervals (1, 2, and 4 h) samples were withdrawn and their residual inhibitory activity against *S. aureus* 209 P (FDA) was determined. A comparison between the stabilities of compounds **11** and **1a** can be made on the basis of the results summarized in Figure 1, which reports the residual potency of the two compounds (μg/mL) as a function of time.

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