CARBOXYHYDRAZIDES OF THE AGLYCONE OF TEICOPLANIN

SYNTHESIS AND ANTIBACTERIAL ACTIVITY

ALDO TRANI, ADRIANO MALABARBA, PIETRO FERRARI, ROSETTA PALLANZA, MARISA BERTI and ROMEO CIABATTI

Lepetit Research Center,
21040 Gerenzano (VA), Italy

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The condensation of the terminal carboxyl group of the deglucoteicoplanin (TD) with various substituted hydrazines produced hydrazide derivatives having different physico-chemical properties. This chemical modification of the carboxyl function does not affect the ability of teicoplanin antibiotics to interfere in bacterial cell-wall synthesis.

The antibacterial activity of deglucoteicoplanin hydrazides (**V**) were found to depend mostly on their ionic character. All the hydrazides were slightly more active than TD on *Escherichia coli*. Those possessing an additional basic group were more *in vitro* active than TD against Gram-negative microorganisms. In Experimental *Streptococcus pyogenes* septicemia in the mouse, basic hydrazides were more active than other derivatives when administered subcutaneously although they are as potent as TD.

The glycopeptide antibiotic teicoplanin (Targocid)[1,2] was recently introduced in some countries for the parenteral treatment of severe infections caused by aerobic and anaerobic Gram-positive bactera.[3,4]

Teicoplanin is a complex consisting of five major closely related fractors (CTA) differing in the *N*-acyl chain linked with β-D-glucosamine at position C-56. CTA also contains one α-D-mannose and one *N*-acetyl-β-D-glucosamine at positions C-42 and C-34, respectively.[5] All three sugars can be removed by acidic hydrolysis under selected conditions obtaining the related aglycone (deglucoteicoplanin: TD) (Fig. 1).[6] Teicoplanin is very poorly absorbed when administered orally and is inactive *in vitro* against Gram-negative organism.

Our program of synthetic modifications of teicoplanin antibiotics[7,8] is directed to extend the antibacterial activity against Gram-negative organism and also to improve the bioavailability after oral administration. There is much evidence that modifications of the terminal carboxyl group do not change the intrinsic capability of teicoplanin antibiotics to inhibit the biosynthesis of bacterial cell wall.[7,9]

The ester derivatives of teicoplanin, previously synthesized,[7] possessed good *in vitro* activity even if they showed a general reduction of the *in vivo* antibacterial activities probably related to a decreased water solubility caused by an increase in the lipophilic character of the derivatives.

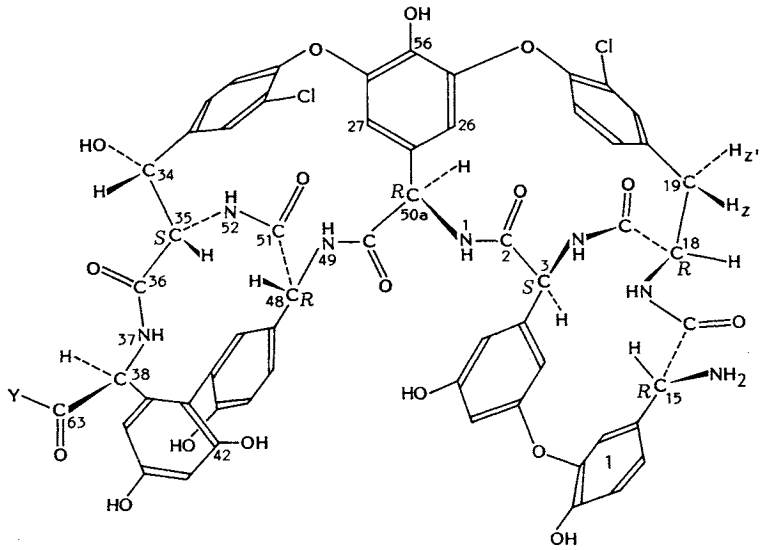
Instead, the more hydrophilic and more water soluble N^{63}-carboxamide derivatives of teicoplanin antibiotics[9] were found more potent *in vitro* and more active *in vivo* after systemic administration than the parent teicoplanins and some of them even slightly more effective by the oral route.

Proceeding on with this promising approach and following the same strategy, we replaced the amide with the similar but more hydrophilic hydrazide group. To verify the influence of such modification on the *in vitro* and *in vivo* antibacterial activity, a series of N^{63}-carboxylhydrazides of TD have been synthesized.

Chemistry

The formation of carboxamides by condensation of amines with the carboxyl group of the teicoplanin

Fig. 1. Structure of TD and hydrazides V.



TD Y=OH

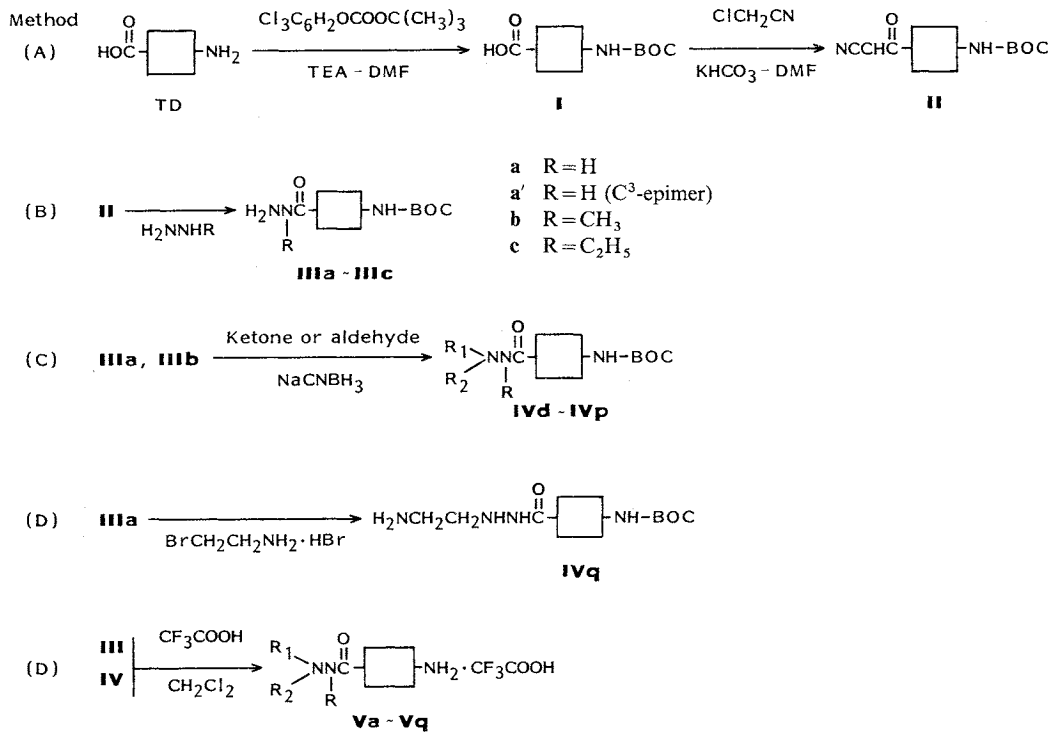
Hydrazides V $Y=N(R)-N<^{R_1}_{R_2}$

Table 1. Hydrazides of TD (Fig. 1).

Compound	R	R_1	R_2	Method[a]	HPLC[b,c] Rt (minutes)		Formula[d]					MW[e]
					(1)	(2)	C	H	Cl	N	O	
Va	H	H	H	B	9.0	2.9	58	47	2	9	17	1,212.96
Va'	H	H	H	B		5.6	58	47	2	9	17	1,212.96
Vb	CH_3	H	H	B	9.6	2.7	59	49	2	9	17	1,226.99
Vc	C_2H_5	H	H	B	10.2		60	51	2	9	17	1,241.02
Vd	H	H	$CH_2-C_6H_5$	C		7.8	65	53	2	9	17	1,303.09
Ve	H	H	n-C_3H_7	C	10.5		61	53	2	9	17	1,255.04
Vf	H	n-C_3H_7	n-C_3H_7	C		6.8	64	59	2	9	17	1,297.12
Vg	CH_3	H	n-C_3H_7	C	10.6		62	55	2	9	17	1,269.07
Vh	H	CH_3	CH_3	C	8.8		60	51	2	9	17	1,241.02
Vi	H	H	$CH_2-CHOH-CH_2OH$	C		7.4	61	53	2	9	19	1,287.04
Vl	H	$=CH-CH=CH-CH_3$		C	12.8	4.7	62	51	2	9	17	1,265.04
Vm	H	H	C_5H_9	C	13.4		63	55	2	9	17	1,281.08
Vn	H	H	$CH(CH_3)CH_2CH(CH_3)_2$	C		8.9	64	59	2	9	17	1,297.12
Vo	H	$=C(CH_3)C_6H_5$		C		7.5	66	53	2	9	17	1,315.10
Vp	H	H	$CH_2CH_2N^+(CH_3)_2$	C		5.6[f]	62	56	2	10	17	1,284.08
Vq	H	H	$CH_2CH_2N^+H_2$	D		5.0[f]	60	52	2	10	17	1,256.03

[a] See Scheme 1.
[b] See Experimental section.
[c] TD (1) Rt 6.5 minutes; (2) Rt 2.0 minutes.
[d] Obtained as CF_3COOH salt; **Vp** and **Vq** as $2CF_3COOH$ salts.
[e] Confirmed by FAB-MS.
[f] Broad peak.

Scheme 1.



For R_1, R_2 and **Vd**~**Vq** see Table 1.

antibiotics[9] was accomplished in good yield with diphenylphosphoryl azide as the condensing agent.[10] Conversely the same reagent proved to be ineffective for the synthesis of the carboxylhydrazides of TD described in this paper (Table 1). These derivatives were prepared according to the methods outlined in Scheme 1.

The 15-amino group of TD (Fig. 1) was protected as *N-tert*-butyloxycarbonyl (*N*-BOC)[9] (Scheme 1, **I**) then the 63-carboxyl group was activated[11] forming the cyanomethyl ester **II** by adding chloroacetonitrile to a solution of **I** dissolved in *N*,*N*-dimethylformamide containing $KHCO_3$. Compounds **III** were obtained from **II** and hydrazines (Scheme 1, B) in ethanol at 50°C. By using methanol as the solvent the undesired *trans* esterification of the active ester was obtained. The alkaline conditions used for this reaction must be carefully controlled to avoid epimerization at position 3 of TD (Fig. 1).[12] In fact, reacting **II** with hydrazine, a mixture of **IIIa** and of the corresponding epimer **IIIa'** was obtained. The epimers of TD derivatives show a characteristic peak shape in reversed phase HPLC, a higher Rt and a lower solubility than the corresponding parent compounds. For these reasons they are easily monitored (HPLC) and eliminated by filtration from the reaction mixture. Pure **IIIa** was instead quantitatively prepared from **II** by reaction with hydrazine monoacetate. Reaction of **II** with methylhydrazine and ethylhydrazine oxalate, according to Method B, led only to the corresponding 1-substituted hydrazides **IIIb** and **IIIc**. These last reactions were faster and more selective than that with hydrazine and did not form the corresponding epimers. Under the same reaction conditions 1,1-dimethylhydrazine and 1,2-dimethylhydrazine did not react. This failure can be explained by the steric hindrance of the methyl groups.

Hydrazides **IIIa** and **IIIb** were transformed into derivatives **IV** (Scheme 1) according to Method C by reaction with proper aldehydes and ketones in the presence of $NaCNBH_3$. Although the adopted procedure is common, the solvent varies from compound to compound, utilizing preferably a mixture of organic solvent and water. In particular, the reaction of **IIIa** with propionaldehyde gave 2-propylhydrazide (**IVe**) in BuOH - H_2O - AcOH and 2,2-dipropylhydrazide (**IVf**) in MeOH - AcOH while the reaction with formaldehyde led only to 2,2-dimethylhydrazide (**IVh**) in both solvents. The reaction of **IIIb** with propionaldehyde gave only the mono alkyl **IVg**. The reaction intermediate Schiff bases were detected by HPLC only for compounds **IVe** and **IVm** and isolated as stable compounds with crotonaldehyde (**IVl**) and acetophenone (**IVo**). In the other cases the equilibrium $-NH_2+C=O \leftrightarrows -N=C-$ is such that to Schiff base is detectable or this is hydrolyzed under the HPLC analysis conditions. Adding $NaCNBH_3$ the equilibrium is forced to the right by reduction of the C=N double bond. The two compounds isolated as Schiff bases **IVl** and **IVo**, were stable to the reducing action of $NaCNBH_3$. No attempts with other reducing agents were tried. The two hydrazides **IVp** and **IVq**, bearing an amino group on the side chain were synthesized by condensation of **IIIa** with *N*,*N*-dimethylamino acetaldehyde according to Method C and by *N*-alkylation with 2-bromoethylamine hydrobromide, respectively (Method D).

Final products **V** (Table 1), obtained by deprotection of **IV** with CF_3COOH (TFA) at room temperature, were isolated as TFA salt.

All the hydrazides and their intermediates show a UV absorption maximum at 280 nm in MeOH and in acidic medium like the starting compound TD.

The IR spectra are in accordance with the assigned structures.

The ^1H NMR spectral data of hydrazides **V** were compared with those assigned in the detailed analysis of the spectrum of TD HCl.[6] The spectra showed that both the hydrazidic and TD moieties were present. The 15-H signal (see Fig. 1) of the reported derivatives is at 5.4 ppm instead of 4.6 ppm[6] because of the deshielding effect of the 15-NH_3^+ group confirming that hydrazides **V** are as TFA salts. Table 2 shows only the significative assignments of the hydrazide moieties and of those protons showing different chemical shifts with respect to starting TD.

Acid-base titration of hydrazides **V** in methyl cellosolve (MCS) - H_2O (4:1) shows the presence of ionizable functions with the apparent constant values ranging between 6.4 and 6.7 attributable to the terminal amino group. The found values were similar to the value of 6.9 obtained for TD.[12] The pK_{MCS} values of the hydrazide moiety are not detectable under these analytical conditions while the amino group at the end of the hydrazidic chain, as in **Vp** and **Vq**, shows a pK_{MCS} in the range 7.5~8.5. It is not possible to calculate an exact value due to the partial overlapping with the titration curve of the phenolic groups of TD.[13]

Results and Discussion

The rational for transforming the terminal carboxyl group into an hydrazide was to reduce the lipophilicity, to modify the ionization bahavior and to improve water solubility of TD which was chosen because it is a single product obtained fairly easily from the teicoplanin complex.

Compounds **IV** and **V** were tested for their *in vitro* antibacterial activity (MIC). The intermediates **IV** were found, as expected, poorly active.[14] The antibacterial activities of **V** and of the parent compound TD are reported in Table 3.

The *in vitro* activities of hydrazides **V** are comparable to that of TD against Staphylococci and

Table 2. ^{1}H NMR assignments[a] (δ ppm) of selected spectral signals of the hydrazides **V** (labeling system Fig. 1).

Compound	R	R_1	R_2	50a-H	3-H	19-H_z	19-$H_{z'}$
Va				5.60	5.35[b]	2.88	3.35
Va'				5.83	5.39[b]	2.73	[c]
Vb	2.67 (CH_3N)						
Vc	1.05 (CH_3)						
	2.96 (CH_2N)						
Vd			5.12 (CH_2N)				
			n.i. (C_6H_5)				
Ve			0.88 (CH_3)				
			1.49 (CH_2)				
			2.87 (CH_2N)				
Vf		0.88 ($2CH_3$)					
		1.44 ($2CH_2$)					
		2.77 ($2CH_2N$)					
Vg	2.54 (CH_3N)		0.88 (CH_3)				
			1.37 (CH_2)				
			2.63 (CH_2N)				
Vh		2.52 ($2CH_3N$)					
Vi			2.89 (CH_2N)				
			3.34 (CH_2O)				
			3.61 (CHO)				
Vl		1.77, 1.85 (CH_3 *trans*/*cis*)					
		5.08 (CH=N)					
		7.45, 7.95 (CH=CH)					
Vm			1.50 ($2CH_2$ to N)				
			1.65 ($2CH_2$ to N)				
			3.56 (CH–N)				
Vn			0.93~0.98 ($3CH_3C$)				
			1.33 (CH_2)				
			1.68 (CHC)				
			2.94 (CHN)				
Vo		1.30 (CH_3)					
		n.i. (C_6H_5)					
Vp			2.85 (CH_2N)				
			3.04 ($(CH_3)_2N^+$)				
			3.15 (CH_2N^+)				
Vq			2.87 (CH_2N)				
			[c] (CH_2N^+)				

[a] The spectra were recorded at 40°C in DMSO-d_6 solution (internal standard; TMS, δ 0.00 ppm).
[b] **Va**: J (Hz) 10; **Va'** J (Hz) 8.
[c] Hidden under H_2O peak about 3.35 ppm.
n.i.: Not identified.

Streptococci while some exhibit better activity against *E. coli*. Compounds **Vp** and **Vq**, bearing a further basic group, have a slightly broader spectrum than TD against Gram-negative showing also activities against *Pseudomonas aeruginosa* and *Proteus vulgaris*. The epimer compound **Va'** has significantly lost, as expected, the antibacterial activity because in this molecule the unique configuration of teicoplanin antibiotics is destroyed.[12]

The *in vivo* efficacy of selected hydrazides **V** was determined in experimental septicemia in the mouse (Table 4). As expected, taking into consideration amide derivatives previously prepared,[9] the more basic compounds **Vp** and **Vq** show a better *in vivo* activity with respect to other derivatives but similar to that of starting TD. The lipophilicity parameter of the non basic hydrazides, measured as Rt in reverse phase

Table 3. *In vitro* antibacterial activity[a] (hydrazide compounds **V**).

Organism	MIC (μg/ml)																
	Va	**Va'**	**Vb**	**Vc**	**Vd**	**Ve**	**Vf**	**Vg**	**Vh**	**Vi**	**Vl**	**Vm**	**Vn**	**Vo**	**Vp**	**Vq**	TD
Staphylococcus aureus TOUR	0.12	2	0.12	0.06	0.06	0.06	0.12	0.06	0.12	0.12	0.12	0.06	0.12	0.06	0.06	0.06	0.06
S. epidermidis ATCC 12228	0.06	1	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.02
S. haemolyticus L 602[b]	0.25	8	0.12	0.12	0.06	0.12	0.12	0.25	0.12	0.12	0.12	0.12	0.12	0.25	0.12	0.12	0.25
Streptococcus pyogenes C 203	0.12	4	0.12	0.12	0.06	0.12	0.06	0.06	0.12	0.12	0.12	0.12	0.12	0.06	0.12	0.12	0.12
S. pneumoniae UC 41	0.12	8	0.12	0.12	0.06	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.06	0.12	0.12	0.12
S. faecalis ATCC 7080	0.12	4	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
S. mitis L 796[b]	0.12	4	0.12	0.12	0.06	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.06	0.12	0.12	0.12
Neisseria gonorrhoeae ISM68/126	64	[c]	16	32	32	32	16	32	64	64	64	32	16	16	32	32	8
Escherichia coli SKF 12140	16	[c]	32	32	[c]	32	32	32	32	32	16	32	32	16	8	8	64
Proteus vulgaris ATCC 881	64	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	32	[c]	[c]
Pseudomonas aeruginosa ATCC 10145	64	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	32	32	[c]

[a] MIC was determined using the 2-fold dilution method in microtiter system. The media used were: Iso-Sensitest broth (Oxoid) for Staphylococci, *S. faecalis* and Gram-negative bacteria: Todd-Hewitt broth (Difco) for Streptococci. The final inoculum was about 10^4 cfu/ml. MIC was read as the lowest concentration which showed no visible growth after 18~24 hours incubation at 37°C.

[b] Clinical isolates.

[c] > 128 μg/ml.

HPLC, were found to be roughly correlated to their *in vivo* activity. Among the tested derivatives the less lipophilic **Vb** (Rt 2.7 minutes) is more active than the most lipophilic **Vo** (Rt 7.5 minutes). The presence of a basic moiety as in **Vp** and **Vq** increases the *in vivo* antibacterial activity much more than predictable from the corresponding Rt values.

Table 4. *In vivo*[a] activity of hydrazides **V** and HPLC Rt[b].

Compound	ED_{50} mg/kg (sc)	Rt (minutes)
TD	0.95	2.0
Vb	3.8	2.7
Vl	5	4.7
Vf	6.6	6.8
Vo	>10	7.5
Vp	0.95	5.6
Vq	0.95	5.0

[a] Experimental septicemia in the mouse caused by *Streptococcus pyogenes* C 203.
[b] See Table 1.

In conclusion, the transformation of the terminal carboxyl of TD into a hydrazide group provides some derivatives exhibiting in general a better activity against *E. coli*, in particular those compounds carrying an amino group in addition to the hydrazide moiety. Also the *in vivo* activity is favorably influenced by the presence of a basic amino group. This effect was also previously verified in the amide derivatives of teicoplanin antibiotics.[9] These results agree with the well known correlations[15] between physico-chemical and pharmacokinetic properties. In fact, positively charged derivatives of teicoplanin antibiotics generally showed lower serum binding and better *in vivo* activities than parent compounds and, moreover, activity against Gream-negative bacteria was obtained.

Experimental

Evaporation of solvents was carried out, after adding BuOH to prevent foaming with a rotary evaporator at 45°C under vacuum.

All compounds **IV** were purified by reverse-phase column chromatography using silanized Silica gel 60 (0.06~0.2 mm; Merck).

HPLC was applied to monitor reactions, chromatographic fractions, and purity of the compounds using a Hewlett-Packard 1084 equipped with a UV detector at 254 nm and a Hibar Li-ChroCart packet with Li-Chrosorb RP-8 (150 × 4 mm, 5 μm) column. Injection volume, 20 μl; flow rate, 1 ml/minute; mobile phases: (A) CH_3CN - 0.02 M aq NaH_2PO_4 (pH 4.7), 5:95; (B) CH_3CN - 0.02 M aq NaH_2PO_4, 75:25; step gradients as follows:

(1)	Minutes	0	15	20	25	27	28.
	%B	15	35	45	60	15	Stop.
(2)	Minutes	0	2	25	30	35	36.
	%B	30	30	50	60	30	Stop.

All compounds were analyzed for C, H, N, using samples previously dried at 140°C under N_2 atmosphere. Weight loss was determined by thermogravimetric analysis (TGA) at 140°C and inorganic residue was determined after heating the samples at 900°C in O_2 atmosphere. The analytical results were $\pm$0.3% the theoretical values.

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 μl of 2-thioglycerol - diglycerol (1:1) matrix and bombarded with a 6~9 KeV beam of Xe atoms.

The pK_{MCS} values were determined potentiometrically in MCS - H_2O (4:1) solution. An excess of 0.01 N HCl in the same solvent mixture was added and the resulting solution was titrated with 0.01 N NaOH in the same solvent mixture.

The IR spectra were obtained in a Nujol mull with a Perkin-Elmer 850 instrument. The ^1H NMR spectra were obtained with a Bruker instrument AM 250 equipped with an Aspect 3000 console at 250 MHz. The spectra were recorded at 40°C in DMSO-d_6 solution (internal standard TMS, δ 0.00 ppm).

MIC was determined using the 2-fold dilution method in a microtiter system. The media used were: Todd-Hewitt broth (Difco) for Streptococci and Iso-Sensitest broth (Oxoid) for Staphylococci, *Streptococcus faecalis* and Gram-negative bacteria. The final inoculum was about 10^4 cfu/ml. MIC (expressed in μg/ml) was read as the lowest concentration which showed no visible growth after 18~24 hours incubation at 37°C.

Experimental septicemia was induced in male and female CD-1 mice (Charles River; weight 18~22 g; group five mice/treatment) by intraperitoneal injection of about 10^5 cells of *Streptococcus pyogenes* C 203, a challenge corresponding to about 100 times the LD_{50} infected animals. Mice were treated once immediately after infection by sc route. On the 7th day, the ED_{50} (infected animals), expressed in mg/kg, was calculated[16] on the basis of the percentage of surviving mice at each dose.

Method A

N^{15}-BOC-TD (**I**): Teicoplanin aglycone (TD) (7 g, 5.8 mmol) was dissolved in 150 ml of DMF at room temperature, and 2,4,5-trichlorophenyl *tert*-butyl carbonate (2.55 g, 8.5 mmol) and triethylamine (TEA) (1.1 ml) were added. The reaction mixture was stirred at 40°C overnight. The solvent was evaporated under reduced pressure at 50°C, and the residue obtained was dissolved in BuOH-EtOAc (2:1), washed with acid water (pH 3) and then washed twice with distilled water. The solvent was removed under reduced pressure and the resulting concentrated solution (about 30 ml) was diluted with 600 ml of ethyl ether. A solid was separated and collected by filtration and washed with ethyl ether, yielding 5.5 g of compound **I** (HPLC (2), Rt 12.3 minutes).

N^{15}-BOC-TD Cyanomethyl Ester (**II**): Chloroacetonitrile (1 ml) was added to a solution of **I** (3 g, 2.3 mmol) in 25 ml of DMF containing $KHCO_3$ (450 mg). The reaction was stirred for about 3 hours at room temperature after which a second measure of $ClCH_2CN$ (0.5 ml) was added and stirring continued for a further 3 hours. When the reaction was considered terminated (HPLC assay), the reaction mixture was worked up by diluting it with ethyl ether (200 ml). The solid which formed was collected and dried yielding 3 g of crude **II** that was used as such in the next reactions. IR ν_{max} (Nujol) cm^{-1} 1755 (CO) (HPLC (2), Rt 23.6 minutes).

Preparation of N^{15}-BOC Hydrazides **III** and **IV**

Method B

N^{15}-BOC-TD Hydrazide (**IIIa**): To a solution of **II** (10 g, 7.55 mmol) in 500 ml of abs EtOH, CH_3COOH (4.3 ml, 75 mmol) and $NH_2NH_2 \cdot H_2O$ (3.7 ml, 76 mmol) were added. The reaction mixture was stirred at room temperature for 12 hours, then a second measure of CH_3COOH (0.21 ml) and hydrazine monohydrate (0.185 ml) was added and the reaction continued for 3 hours. The solvent was evaporated under reduced pressure. The residue was diluted with water (200 ml) and the pH corrected to 3 with 1 N HCl and extracted with 500 ml BuOH and 200 ml of EtOAc. The organic layer, was washed three times with water, concentrated to 60 ml and then it was diluted with 350 ml of ether. After chilling the suspension for about 1 hour, the precipitate was filtered, washed with ether and dried under vacuum overnight yielding 8.2 g of the title compound (HPLC (2), Rt 15.6 minutes).

N^{15}-BOC-TD Hydrazide Epimer (**IIIa'**): A solution of **II** (1 g, 0.75 mmol) in 30 ml of abs EtOH was added with 5 ml of $NH_2NH_2 \cdot H_2O$ and the cloudy solution which formed was stirred at 40°C for 3 hours. The reaction HPLC profile showed the corresponding peak of compound **IIIa** (40%) and a new peak with a higher Rt (**IIIa'**, 60%). The solvent was removed under vacuum and the residue, suspended in water, was filtered and then dissolved in a minimum amount of a mixture of H_2O-CH_3CN (1:1). The resulting solution was applied at the top of a chromatographic column prepared with 100 g of silanized silica gel, pre-equilibrated with 150 ml of 1% aq $HCOONH_4$ at pH 5 and stabilized with 200 ml of a mixture H_2O-CH_3CN (9:1). The column was washed with 150 ml of the same mixture, and then developed with a stepwise gradient from 10 to 50% of CH_3CN in H_2O. Fractions of about 10 ml each were collected and assayed by HPLC. Fractions containing the desired compound were pooled, BuOH was added and the solution evaporated under vacuum. The residue was treated with ethyl ether, filtered and dried over P_2O_5 yielding 400 mg of the title compound (HPLC (2), Rt 20.2 minutes).

N^{15}-BOC-TD 1-Methylhydrazide (**IIIb**): A solution of **II** (1 g, 0.7 mmol) in 80 ml of abs EtOH was added with methyl hydrazine (1 ml) and the mixture stirred at 40°C for 1 hour. A second portion of methyl

hydrazine (1 ml) was added and the reaction continued for 1 hour. The mixture was then concentrated to about 10 ml under reduced pressure and ethyl ether (100 ml) was added. A precipitate formed which was filtered, dissolved in the minimum amount of a mixture of H_2O - CH_3CN (1 : 1) and chromatographed on silanized silica gel as reported. Fractions containing the pure product (HPLC area > 92%) were worked up as usual, yielding 320 mg of the title compound (HPLC (2), Rt 17.7 minutes).

N^{15}-BOC-TD 1-Ethylhydrazide (**IIIc**): To a solution of **II** (1 g, 0.7 mmol) in 100 ml of abs EtOH, ethylhydrazine oxalate (115 mg, 1.15 mmol) and TEA (0.3 mg, 2.3 mmol) were added. The mixture was stirred at 50 °C for 4 hours after which it was worked up and purified, as in the previous example, yielding 250 mg of **IIIc** (HPLC (2), Rt 18.5 minutes).

Method C

N^{15}-BOC-TD 2-Benzylhydrazide (**IVd**): To a solution of compound **IIIa** (1.5 g, 1.1 mmol) in a mixture of CH_3COOH (22 ml) and MeOH (11 ml), benzaldehyde (1.5 ml) and a catalytic amout of *p*-toluensulfonic acid (1 mg) were added. After about 15 minutes of stirring at room temperature, the HPLC analysis (gradient profile 2) showed a peak at Rt 25.5 minutes (2-benzylidenehydrazide derivative). Two portions of $NaCNBH_3$ (0.5 g each) were then added at 20 minute intervals. The compound with Rt 25.5 minutes completely transformed into a compound having Rt 24.2 minutes. The solvent was removed under reduced pressure, then the residue was treated with distilled water (50 ml) and the solid which formed was filtered. The wet crude material was suspended in CH_3CN - H_2O (7 : 3) and mixed with silanized silica gel (10 g); after removing the solvents under reduced pressure, the solid was loaded on the top of a chromatographic column containing 120 g of the same silica gel prepared with a 10% CH_3CN in H_2O. Impurities were removed by eluting with CH_3CN - H_2O (3 : 7), then the compound having Rt 24.2 minutes was eluted with a linear gradient from 40 to 60% CH_3CN in H_2O. Fractions containing the pure compound were pooled, BuOH was added and the solvents removed under vacuum. The residue, treated with ethyl ether was filtered and dried, yielding 500 mg of the title compound (HPLC (2), Rt 24.2 minutes).

N^{15}-BOC-TD 2-Propylhydrazide (**IVe**): A solution of **IIIa** (0.7 g, 0.5 mmol) was added to a mixture of BuOH (60 ml), H_2O (5.5 ml), CH_3COOH (1.2 ml) and propionic aldehyde (0.6 ml, 8.33 mmol). The mixture was stirred for 20 minutes at room temperature then a second portion of aldehyde (0.6 ml) was added and the reaction continued for 1 hour. HPLC analysis (program 2) showed the starting material (**IIIa**, Rt 15.6 minutes) and its Schiff base (Rt 18.78 minutes) with a ratio in the range 30 : 70, respectively. $NaCNBH_3$ (0.5 g) was added under stirring at 25°C internal temperature. Aftet 2 hours a second measure of propionic aldehyde (0.6 ml) was added and the reaction continued for 1 hour. $NaCNBH_3$ (0.3 g) was then added and stirring was continued until disappearance of the starting material. Thereafter the reaction mixture was acidified by adding diluted hydrochloric acid. The precipitate which formed was collected by filtration, dissolved in a mixture of H_2O - CH_3CN (7 : 3), loaded on a column of silanized silica gel and eluted with a linear gradient from 30 to 65% of CH_3CN in H_2O. Working up fractions containing the pure compound, 330 mg of the title compound was obtained (HPLC (2), Rt 18.8 minutes).

N^{15}-BOC-TD 2,2-Dipropylhydrazide (**IVf**): To a solution of **IIIa** (1.2 g, 0.9 mmol) in MeOH (30 ml) and CH_3COOH (15 ml), propionaldehyde (1.5 ml, 20.7 mmol) and *p*-toluensulfonic acid (1.2 mg) were added. The mixture was stirred at room temperature for 30 minutes, and then $NaCNBH_3$ (0.5 g, 7.9 mmol) was added. After about 2 hours of reaction the reaction was adjusted to pH 2 by adding 1 N HCl and the solvent was evaporated under reduced pressure. The residue, dissolved in a mixture of CH_3CN - H_2O (2 : 3) was loaded on a chromatographic column of silanized silica gel (150 g) and eluted with 25% CH_3CN in water (500 ml) and then with a linear gradient from 25 to 40% of CH_3CN in water. Fractions containing the pure compound were pooled, evaporated and treated with ethyl ether. The precipitated solid was collected and dried at 50°C under reduced pressure yielding 550 mg of title compound (HPLC (2), Rt 22.9 minutes).

N^{15}-BOC-TD 1-Methyl-2-propylhydrazide (**IVg**): To a solution of methylhydrazide **IIIb** (2 g, 1.5 mmol) in 60 ml of MeOH, propionaldehyde (1.5 ml, 20.7 mmol) and *p*-toluensulfonic acid (15 mg) were added and the mixture stirred at room temperature for 2 hours. The $NaBH_4$ (100 mg, pellets) was added and the reaction continued overnight. The solvent was removed under reduced pressure and the residue, suspended in 40 ml of water at pH 4, was purified by column chromatography as previously reported. Working up the fractions containing pure compound, 320 mg of **IVg** was obtained (HPLC (2), Rt 10.4

minutes).

N^{15}-BOC-TD 2,2-Dimethylhydrazide (**IVh**): A 40% aq HCHO (1.2 ml) was added to a solution of hydrazide **IIIa** (1.2 g, 0.9 mmol) in 72 ml of CH_3CN. The mixture was stirred at room temperature for 15 minutes, and then two portions of $NaCNBH_3$ (100 mg) were added at 2 hourly intervals. The reaction was continued overnight, then the organic solvent was removed under reduced pressure and the residue diluted with 50 ml of water. The suspension was adjusted to pH 3 with 1 N HCl and the product extracted with BuOH (100 ml). The organic layer was washed with water (30 ml) and evaporated under reduced pressure. The residue was treated with EtOAc, filtered, washed with ethyl ether and dried under vacuum, yielding 800 mg of **IVh** (HPLC (2), Rt 16.3 minutes).

N^{15}-BOC-TD 2-(2,3-Dihydroxy)propylhydrazide (**IVi**): To a solution of hydrazide **IIIa** (1.2 g, 0.9 mmol) in BuOH (120 ml) and H_2O (100 ml), D,L-glyceraldehyde (600 mg, 6.6 mmol) and *p*-toluensulfonic acid (60 mg) were added. The reaction was stirred at room temperature for 20 minutes, then $NaCNBH_3$ (300 mg, 127 mmol) was added and the reaction continued. After 2 hours, a second measure of D,L-glyceraldehyde (600 mg) was added and followed after 30 minutes by $NaCNBH_3$ (700 mg). Stirring was continued for 3 hours and then the pH was adjusted to 8 with $NaHCO_3$. Ethyl acetate (50 ml) was added and the mixture was washed with water (40 ml × three times). The organic solvent was removed under reduced pressure and the residue was mixed with Celite (a filter aid) and loaded on a chromatographic column prepared with 150 g of silanized silica gel in H_2O-CH_3CN (9 : 1). This column was eluted with 1 liter of 30% aq CH_3CN and then with 1 liter of 40% aq CH_3CN. Fractions containing pure product were collected and worked up as previously reported, yielding 600 mg of title compound (HPLC (2), Rt 15.1 minutes).

N^{15}-BOC-TD 2-(2-Butenylidene)hydrazide (**IVl**): To a solution of hydrazide **IIIa** (1.2 g, 0.9 mmol) in CH_3CN (72 ml) and H_2O (10 ml), 0.6 ml of crotonaldehyde (7.3 mmol) was added. The solution was stirred at room temperature for 15 minutes then $NaCNBH_3$ (100 mg) was added followed by a second measure after 1 hour and stirring continued for 3 hours. The solvent was removed, water (80 ml) was added and the resulting mixture was adjusted to pH 3 with 1 N HCl and extracted with 200 ml of BuOH-EtOAc (3 : 1). The organic layer was washed with 30 ml of water and evaporated under reduced pressure. The residue was suspended in EtOAc, filtered and washed with ethyl ether, yielding 1.2 g of title compound (HPLC (2), Rt 20.8 minutes).

N^{15}-BOC-TD 2-Cyclopentylhydrazide (**IVm**): To a solution of hydrazide **IIIa** (1.2 g, 0.9 mmol) in 60 ml of BuOH, 10 ml of H_2O cyclopentanone (1 ml, 11.3 mmol) and *p*-toluensulfonic acid (25 mg) were added. The mixture was stirred at room temperature for 1 hour. The HPLC analysis (gradient profile 2) showed the formation of the corresponding Schiff base (Rt 19.5 minutes). $NaCNBH_3$ (1 g) was added in small amounts during 1 hour. A second measure of cyclopentanone (0.2 ml) was added followed after 30 minutes by an additional amount of $NaCNBH_3$ (100 mg). The reaction was continued for 3 hours, the solvent was then removed and the residue purified by column chromatography on silanized silica gel with a linear gradient from 25 to 40% of CH_3CN in water. Working up fractions containing pure product 600 mg of title compound was obtained (HPLC (2), Rt 20.5 minutes).

N^{15}-BOC-TD 2-/(1,3-Dimethyl)butyl/hydrazide (**IVn**): To a solution of hydrazide **IIIa** (1.2 g, 0.9 mmol) in 108 ml of BuOH and 12 ml of H_2O, methyl isobutylketone (1.2 ml, 9.6 mmol) was added. The solution was stirred at room temperature for 1 hour, then $NaCNBH_3$ (0.8 g) was added. After 2 hours, EtOAc (30 ml) was added and the organic phase separated, washed with water (50 ml) and evaporated under reduced pressure. The residue was treated with 10 ml of EtOAc and diluted with 100 ml of ethyl ether. The solid which separated was filtered washed with Et_2O and dried under vacuum, yielding 650 mg of **IVn** (HPLC (2), Rt 23.2 minutes).

N^{15}-BOC-TD 2-(1-Phenylethylidene)hydrazide (**IVo**): To a solution of 1.2 g (0.9 mmol) of hydrazide **IIIa** in 140 ml of BuOH and 12 ml of H_2O, acetophenone (1.2 ml, 10 mmol) was added and the solution was allowed to stand for 2 hours at room temperature. Solvents were evaporated under reduced pressure and the crude material was purified on a silica gel column eluting with abs EtOH. Fractions containing pure product were pooled, evaporated and the residue taken up with Et_2O, collected by filtration and dried under reduced pressure, yielding 0.9 mg of **IVo** (HPLC (2), Rt 23.7 minutes).

N^{15}-BOC-TD 2-(2-Dimethylamino)ethylhydrazide (**IVp**): To a solution of **IIIa** (1.2 g, 0.9 mmol) in 80 ml of BuOH and 10 ml of H_2O, dimethylamino acetaldehyde hydrochloride (200 mg, 1.36 mmol)

(obtained from dimethylamino acetaldehyde diethylacetal)[17] and CH_3COONa (246 mg, 3 mmol) were added under nitrogen. The mixture was stirred at room temperature for 1 hour, then $NaCNBH_3$ (30 mg, 0.4 mmol) was added. After 2 hours, a second measure of the same aldehyde (200 mg) was added followed, after 1 hour, by 30 mg of $NaCNBH_3$. The reaction was continued overnight then the pH adjusted to 3 with 1 N HCl and the solvent evaporated under reduced pressure. The residue was purified on a silanized silica gel (150 g) chromatographic column developed with a linear gradient from 25 to 60% of CH_3CN in water, yielding 500 mg of title compound (HPLC (2), Rt 21.3 minutes).

Method D

N^{15}-BOC-TD 2-(2-Aminoethyl)hydrazide (**IVq**): To a solution of hydrazide **IIIa** (1.15 g, 0.89 mmol) in DMF (70 ml), $Br(CH_2)_2NH_2 \cdot HBr$ (0.2 g, 0.9 mmol) was added. The reaction was maintained at 50°C for 4 hours and then a second portion of bromoethylamine (0.2 g) was added followed, after 2 hours, by TEA (150 ml, 1.07 mmol). The reaction was continued overnight at room temperature (HPLC analysis showed a 70% transformation). The crude material, after solvent evaporation under reduced pressure, was dissolved in the minimum amount of a mixture CH_3CN-H_2O (3:7) and loaded on the top of a column containing 150 g of silanized silica gel. The column was eluted with a linear gradient from 20 to 35% of CH_3CN in H_2O. Fractions containing the desired compound were combined, the solvent evaporated to dryness and the solid, treated with Et_2O, was filtered off and dried under vacuum, yielding 350 mg of **IVq** (HPLC (2), Rt 20.8 minutes).

Preparation of Hydrazides **V**

Method E

The BOC protective group of all hydrazides **III** and **IV** was removed according to the procedure reported here:

EX: CF_3COOH (4 ml) was dropped into a suspension of a purified N^{15}-BOC-TD hydrazide (1 mmol) in 20 ml of CH_2Cl_2 and 10°C. The resulting solution was stirred for 15 minutes at room temperature then Et_2O was added and the solid which formed was filtered, washed with Et_2O and dried under vacuum yielding the hydrazide **V** as mono-trifluoro acetate salt (**Vp** and **Vq** were obtained as di-trifluoro acetate salts).

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