THIOUREAS AND ISOTHIOURONIUM SALTS OF THE AGLYCONE OF TEICOPLANIN

I. SYNTHESIS AND BIOLOGICAL ACTIVITY

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A series of thiourea and isothiouronium salt derivatives of the aglycone of teicoplanin was prepared by reaction of the terminal amino group with isothiocyanates, followed by *S*-alkylation of the thiourea compounds.

Unexpectedly, the two classes of derivatives show a similar *in vitro* antibacterial activity against Gram-positive bacteria.

Thiourea compounds, due to the lack of a positively charged *N*-terminus group, have a 10-fold lower binding constant to Ac-D-Ala-D-Ala, a bacterial cell-wall model, than the parent antibiotic and isothiouronium salt derivatives.

Glycopeptide antibiotics have been the subject of intense investigation in recent years along with the appearance in hospitals of *Staphylococcus aureus* and *Staphylococcus epidermidis* that are resistant to methicillin treatment. Besides vancomycin, the new glycopeptide teicoplanin[1] (Targocid) has recently been introduced in therapeutic use in some countries. Teicoplanin (**I**) (Fig. 1) consists of five major closely related factors (A2/1~5) differing in the *N*-acyl chain linked with β-D-glucosamine at position C-56. It also contains one α-D-mannose and one *N*-acetyl-β-D-glucosamine at C-42 and C-34 positions, respectively. All three sugars can be removed by acidic hydrolysis under selected conditions obtaining the aglycone (**II**) (Fig. 1).[2]

From the first observation by CHATTERJEE and PERKINS[3] that the glycopeptide vancomycin formed a stoichiometric 1 : 1 complex with cell-wall precursors terminating with the peptide D-alanyl-D-alanine, much evidence has been obtained on the mechanism of action of glycopeptide antibiotics.[4,5] The role of the terminus amino group of glycopeptide antibiotics in the binding to bacterial cell-wall model *N*-acetyl-D-alanyl-D-alanine (Ac-D-Ala-D-Ala) has been studied.[6] Data indicate that *N*-terminus favors the initial Coulomb complex with the carboxylate ion of the peptide but is not essential for the binding.[6~8]

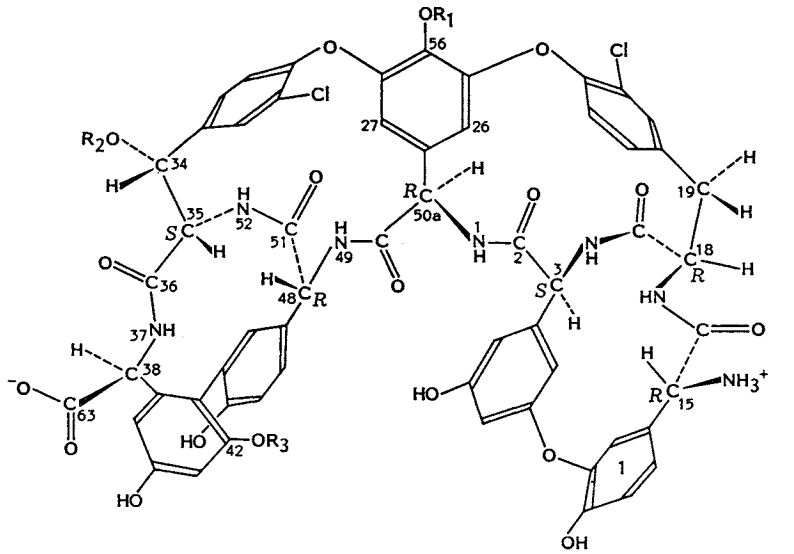
Continuing[8] our study on teicoplanins concerning the importance of the *N*-terminus group for antibacterial activity, we synthetized a series of thioureas **III** and isothiouronium salts **IV** of **I** and **II** (Scheme 1). In this paper, for conciseness, only the derivatives of **II** are reported.

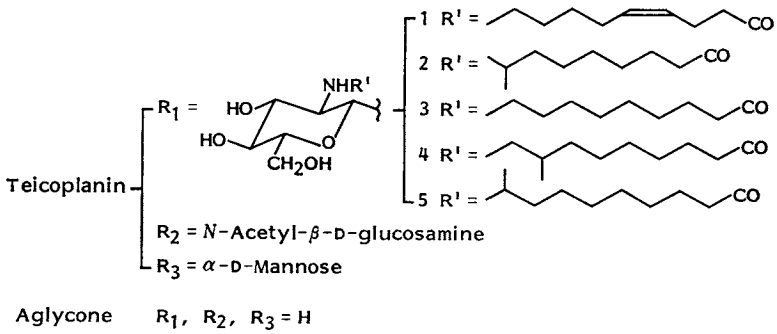
The binding constants (K_A, M^{-1}) of a few derivatives with Ac-D-Ala-D-Ala were measured by UV differential spectroscopy.

Chemistry

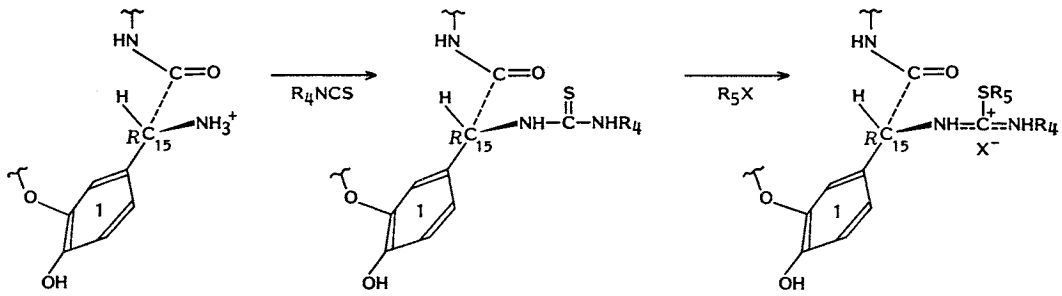
Nine thiourea derivatives of **II** (**1~9**, Table 1) were prepared according to Scheme 1 utilizing commercially available isothiocyanates as starting material in a CH_3CN - H_2O mixture. The synthesis of unsubstituted thiourea **III** (R_4=H, **2**) failed when potassium or ammonium thiocyanate in

Fig. 1. Structures of the components of teicoplanin (I) and the aglycone (II).





Scheme 1.



Aglycone (II) III IV $R_5=CH_3$, C_2H_5 X=I, Br

For R_4 see Table 1.

different reaction conditions were used. Then the two step synthesis, described by DOUGLASS and DAINS,[8] was followed. In the first step substrate **II** reacts with benzoyl thiocyanate (Method A) to obtain structure **III** ($R_4=COC_6H_5$, **1**). The second step is the alkaline hydrolysis of the benzoyl group. Because the hydrolysis conditions of the DOUGLASS method (10% NaOH boiling) are too drastic, and

Table 1. Thiourea compounds (**III**, Scheme 1).

No.	R_4	Method	Yield (%)	HPLC[a,b] t_R (minutes)	pK_{MCS}[a]	Formula	MW	FAB (M+H)
1	COC_6H_5	A	45.8	37.4	nd	$C_{68}H_{50}N_8O_{19}Cl_2S$	1,362.1	
2	H	B	82.0	19.0	nd	$C_{59}H_{46}N_8O_{18}Cl_2S$	1,258.0	
3	CH_3	C	74.2	23.6	5.3	$C_{60}H_{48}N_8O_{18}Cl_2S$	1,272.0	1,273
4	C_2H_5	C	72.5	27.2	nd	$C_{61}H_{50}N_8O_{18}Cl_2S$	1,286.0	
5	C_2H_5OCO	D	84.3	30.4	nd	$C_{62}H_{50}N_8O_{20}Cl_2S$	1,330.1	
6	C_6H_{11}	C	80.5	38.8	5.4	$C_{65}H_{56}N_8O_{18}Cl_2S$	1,340.2	
7	$C_6H_5CH_2$	C	45.1	36.8	4.8	$C_{66}H_{52}N_8O_{18}Cl_2S$	1,348.1	1,349
8	*p*-F-C_6H_4	C	69.8	34.2	4.8	$C_{65}H_{49}N_8O_{18}Cl_2FS$	1,352.1	
9	C_6H_5	C	75.3	34.4	4.8	$C_{65}H_{50}N_8O_{18}Cl_2S$	1,334.1	

[a] See Experimental section. [b] Aglycone (**II**) t_R 12.2.
nd: Not determined.

Table 2. Isothiouronium salts (**IV**, Scheme 1).

No.	R_4	R_5	X	Method	Yield (%)	HPLC[a,b] t_R (minutes)	pK_{MCS}[a]	Formula[c]	MW[c]	FAB (M+H)
10	H	CH_3	I	E	75.3	16.7	4.9~7.3	$C_{60}H_{48}N_8O_{18}Cl_2S$	1,272.1	
11	H	C_2H_5	Cl	F	25.0	19.4	5.0~7.5	$C_{61}H_{50}N_8O_{18}Cl_2S$	1,286.1	
12	CH_3	CH_3	I	E	85.2	20.0	4.8~7.5	$C_{61}H_{50}N_8O_{18}Cl_2S$	1,286.1	1,287
13	C_2H_5	CH_3	I	E	79.5	21.4	4.9~7.1	$C_{62}H_{52}N_8O_{18}Cl_2S$	1,300.1	
14	C_6H_{11}	CH_3	I	E	82.0	33.5	4.7~6.9	$C_{66}H_{58}N_8O_{18}Cl_2S$	1,354.2	
15	$C_6H_5CH_2$	CH_3	I	E	75.3	31.0	4.5~6.4	$C_{67}H_{54}N_8O_{18}Cl_2S$	1,362.2	1,363
16	*p*-F-C_6H_4	CH_3	I	E	72.5	42.3	4.9~3.5	$C_{65}H_{51}N_8O_{18}Cl_2FS$	1,354.1	
17	C_6H_5	CH_3	I	E	74.0	40.4	4.9~3.6	$C_{66}H_{52}N_8O_{18}Cl_2S$	1,348.1	

[a] See Experimental section. [b] Aglycone (**II**), t_R 12.2. [c] Formula and MW of the base.

epimerization at the C-3 position may occur, a use gentle or weak hydrolysis with K_2CO_3 in acetone at room temperature was used (Method B). The isothiouronium salts **IV** (Scheme 1), listed in Table 2 were obtained by *S*-alkylation of thioureas **III** with CH_3I (**10** and **12**~**17**) or with C_2H_5Br (**11**). As expected, isothiouronium salts **IV** are more soluble in water than the corresponding thioureas **III**, but their solubility does not significantly differ from parent antibiotic **II**. Compounds of structure **IV** are not stable as free bases in water and undergo a hydrolysis resulting in the formation of mercaptane. When a chromatographic purification was necessary, 1 N HCl was added (1%) to the eluting solvent to maintain the correct acidity of the mobile phase. Following this procedure the compounds were recovered as hydrochlorides.

The acid-base titration of **II** in methyl cellosolve (MCS) - H_2O (4:1) shows two ionizable functions, with the apparent p*K* values of 4.8 and 6.9 which are attributed to the terminal carboxyl and amino groups, respectively, which give rise to a zwitterion. Thioureas **III** exhibit only one potentiometric detectable function attributable to the terminal carboxyl group (Table 1) whereas isothiouronium salts show an acidic and a basic function (Table 2). The p*K* value of the isothiouronium group is affected by the electronic character of substituent R_4 (**IV**, Scheme 1). While alkyl groups give about p*K* 7 (Table 2), an electron withdrawing group decreases it to 3.5 (**16**).

The reactions and the purity of the compounds were carefully monitored by reverse phase HPLC. In the HPLC mobile phase conditions (pH 4.7 see Experimental section) isothiouronium derivatives **IV** were eluted before the corresponding thioureas **III** even though **IV** had the lipophilic contribution of the additional alkyl R_4 (Scheme 1). Compounds **16** and **17** have a low p*K* and remain non-ionized

Table 3. ^{1}H NMR assignments of selected spectral signals of the aglycone derivatives (labeling system Fig. 1).

Proton	II	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
15-H	4.66	5.90	5.99	6.06	6.06	6.18	6.07	6.09	6.16	6.15	ni	ni	5.79	ni	ni	ni	ni	ni
18-H	4.97	4.94	4.96	4.95	4.96	4.92	4.96	4.96	4.97	4.97	4.92	4.93	4.95	4.96	4.92	4.90	5.00	5.00
3-H	5.33	5.34	5.32	5.33	5.33	5.33	5.33	5.34	5.34	5.36	5.39	5.40	5.34	5.39	5.37	5.37	5.34	5.33
50a-H	5.67	5.63	5.64	5.66	5.66	5.59	5.64	5.68	5.63	5.64	5.63	5.64	5.62	5.59	5.61	5.61	5.62	5.61
48-H	4.33	4.32	4.34	4.35	4.36	4.28	4.35	4.35	4.35	4.35	4.34	4.35	4.33	4.38	4.35	4.35	4.35	4.35
35-H	4.11	4.12	4.11	4.13	4.12	4.12	4.12	4.13	4.13	4.11	4.11	4.12	4.13	4.12	4.12	4.12	4.12	4.12
38-H	4.39	4.40	4.39	4.42	4.41	4.38	4.43	4.42	4.43	4.40	4.43	4.44	4.43	4.47	4.44	4.44	4.43	4.43
34-H	5.08	5.12	5.10	5.10	5.10	5.13	5.09	5.10	5.09	5.12	5.10	5.10	5.12	5.12	5.10	5.10	5.11	5.10
26-H	5.50	5.53	5.53	5.55	5.54	5.51	5.55	5.55	5.53	5.54	5.52	5.54	5.53	5.59	5.54	5.54	5.53	5.53
27-H	5.10	5.12	5.13	5.14	5.14	5.13	5.13	5.14	5.13	5.13	5.10	5.11	5.10	5.14	5.10	5.10	5.10	5.10
C_6H_5		7.5						7.4	7.5	7.50						7.34	7.2	7.5
CH_2CH_3					3.40	4.10						4.22		3.51				
CH_2CH_3					1.08	1.20						1.25		1.20				
SCH_3											2.58		2.50	2.67	2.65	2.64	2.45	
$NHCH_3$													2.55					
C_6H_{11}							4.20								2.69			
							1.87								1.73			
							1.62								1.30			
$CH_2C_6H_5$								4.7								4.71		
CH_3			2.04															

ni: Not identified.

in HPLC buffer and hence show longer retention times (t_R) than the corresponding thioureas **8** and **9** as expected.

The IR spectra give mainly two pieces of information; one is general and is concerned with the maintaining of the peptide or glycopeptide structure in these compounds. The other is specific and is concerned with the ionization state of the terminal carboxyl group (ν C=O of COOH gives a shoulder at 1730 cm^{-1}).

^{1}H NMR spectral assignments (Table 3) confirm the overall structural features concerning the peptide core and the moiety chemically introduced. The chemical shift of the 15-H signal (α-proton of amino acid **1**) is sensitive to the nature of such a moiety. It undergoes a downfield shift with respect to the same 15-H signal in the parent compound. When it is not overlapped by other peaks, as generally happens in the case of the isothiouronium salts, this signal is found in the range 5.6~6.2 ppm.

Table 4. Association constants of selected aglycone derivatives with Ac-D-Ala-D-Ala.

Compound	K_A (M^{-1})[a]
10	2.4×10^5
12	2.4×10^5
Aglycone (**II**)	1.8×10^5
2	5.0×10^4
TDW[b]	1.1×10^4
6	0.8×10^4

Assay by UV difference spectroscopy in 0.02 M citrate at pH 5.

[a] Measured at 31°C.

[b] 15-Deamino aglycone.

Results and Discussion

The role of the terminal amino group in glycopeptide antibiotics seems to consist mainly of favoring the binding with peptides terminating in acyl-D-alanyl-D-alanine.[5] Moreover, the ionization of the *N*-terminus group affects the antibiotic water solubility.

The reason for the synthesis of derivatives **III** and **IV** of aglycone described here was to study

Table 5. *In vitro* antibacterial activity[a] (thiourea derivatives **III**).

Organism	MIC (μg/ml)										
	II	TDW	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Staphylococcus aureus TOUR	0.063	0.125	0.125	0.125	0.125	0.25	0.125	0.5	0.5	0.5	0.25
S. aureus TOUR[b]	0.125	0.125	0.5	0.125	0.125	0.25	0.125	1	1	0.5	0.5
S. epidermidis ATCC 12228	0.016	0.125	0.125	0.125	0.125	0.125	0.063	0.125	0.063	0.125	0.125
S. haemolyticus L 602[c]	0.25	nd	nd	nd	nd	0.5	0.5	0.5	1	0.25	nd
Streptococcus pyogenes C 203 SKF 13400	0.125	0.25	0.125	0.125	0.125	0.25	0.125	0.25	0.25	0.125	0.125
S. pneumoniae UC 41	0.125	0.25	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
S. faecalis ATCC 7080	0.125	1	1	0.25	0.5	0.5	0.5	2	1	2	2
S. mitis L 796[c]	0.125	0.5	0.125	0.25	1	0.25	0.125	0.125	0.125	0.125	0.125
Escherichia coli SKF 12140	64	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]
Proteus vulgaris HX 19 ATCC 881	128	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]
Pseudomonas aeruginosa ATCC 10145	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]

[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] Inoculum 10^6 cfu/ml.

[c] Clinical isolates.

[d] >128.

nd: Not determined.

how the degree of ionization of the *N*-terminus affects the binding with Ac-D-Ala-D-Ala and antibacterial activity. The binding strength of four compounds have been measured by UV spectroscopy and compared with those of the aglycone and of the 15-deamino compound (TDW).[8] The binding constants (K_A) with the bacterial cell-wall model are in accordance with our expectations. In fact, the isothiouronium compounds (**10** and **12**) that possess a terminal protonable group (**6**~**8**) show K_A of the same magnitude of the aglycone while the thiourea compounds (**2** and **6**) have a lower K_A, comparable to that of TDW.

The *in vitro* antibacterial activities of thiourea and isothiouronium derivatives (**III** and **IV**) are reported in Tables 5 and 6 in comparison with that of parent antibiotic **II**. Also reported is the activity of TDW which lacks the amino terminus and should represent the least active compound. The comparison of the thiourea derivative with the corresponding isothiouronium compound activities shows differences too small to suggest a correlation between degree of ionization of the 15-terminal group and antibacterial activity.

A 10-fold difference in the binding constant, measured by UV spectroscopy, cannot predict a significant variation in the *in vitro* activity probably because the UV assay does not consider the diffusion capability of compounds in cell-walls.

Corresponding teicoplanin (**I**) derivatives were synthesized with the same procedure utilized for preparing aglycone derivatives (**1**~**4**, and **13** and **14** see Experimental section). For these compounds as well the *in vitro* antibacterial activity was comparable to that of the parent antibiotic.

Table 6. *In vitro* antibacterial activity[a] (isothiouronium derivatives IV).

Organism	MIC (μg/ml)									
	II	TDW	10	11	12	13	14	15	16	17
Staphylococcus aureus TOUR	0.063	0.125	0.063	0.063	0.125	0.063	0.125	0.125	0.5	1
S. aureus TOUR[b]	0.125	0.125	0.125	0.125	0.125	0.125	0.5	0.25	0.5	1
S. epidermidis ATCC 12228	0.016	0.125	0.063	0.063	0.063	0.063	0.063	0.063	0.125	0.125
S. haemolyticus L 602[c]	0.25	nd	nd	0.125	nd	0.5	0.25	0.125	0.5	0.5
Streptococcus pyogenes C 203 SKF 13400	0.125	0.25	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.25
S. pneumoniae UC 41	0.125	0.25	0.063	0.125	0.125	0.063	0.063	0.125	0.125	0.125
S. faecalis ATCC 7080	0.125	1	0.125	0.125	0.125	0.125	0.5	0.5	1	1
S. mitis L 796[c]	0.125	0.5	0.125	0.125	0.125	0.125	0.063	0.125	0.125	0.25
Escherichia coli SKF 12140	64	[d]	64	64	[d]	[d]	[d]	[d]	[d]	[d]
Proteus vulgaris HX 19 ATCC 881	128	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]
Pseudomonas aeruginosa ATCC 10145	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]

[a] See note to Table 5. [b] Inoculum 10^6 cfu/ml. [c] Clinical isolates. [d] >128.
nd: Not determined.

Experimental

Evaporation of solvents was carried out, after adding BuOH to prevent foaming with a rotary evaporator at 45°C under vacuum. If not otherwise stated, the intermediates and the final products were washed with Et_2O and dried at 40°C under vacuum.

Column chromatography was performed 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 1090L chromatograph equipped with a UV detector at 254 nm and ODS-Hypersil C-18 (100 mm, 5 μm) column. Injection volume, 10 μl; flow rate, 1 ml/minute; mobile phases: (A) 0.02 aq NaH_2PO_4 (pH 4.7); (B) CH_3CN; linear step-gradient as follows:

Minutes	0	40	45	48	55	56
% B	10	33	47	47	10	Stop

All compounds were analyzed for C, H, N, and S on samples previously dried at 140°C under N_2 atmosphere. Weight loss was determined by TGA at 140°C; inorganic residue was determined after heating the samples at 900°C in O_2 atmosphere. The analytical results were in accordance with the theoretical values.

Fast atom bombardment mass spectra (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).

The binding constants were measured with Ac-D-Ala-D-Ala by UV differential spectroscopy as previously described.[8]

MIC was determined using the 2-fold dilution method in 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

was read as the lowest concentration which showed no visible growth after 18~24 hours incubation at 37°C.

Thioureas of the Aglycone

Method A

N^{15}-(Benzoylamino)thioxomethyl (**1**): Aglycone (**II**) (4.8 g, 4 mmol) and Na_2CO_3 (0.53 g, 5 mmol) were dissolved in DMF (150 ml) then benzoyl isothiocyanate (0.6 ml, 4.4 mmol) was added and the mixture stirred at room temperature for 5 hours. The mixture was diluted with 1.5 liters of water and the pH adjusted to 3, and the product was extracted with 800 ml of a mixture of BuOH - EtOAc (7 : 3). The organic solvent, washed with water (150 ml), was concentrated to a final volume of about 60 ml, and by adding Et_2O (300 ml) a solid separated which was collected. This crude material (5.8 g) containing a less lipophilic compound was purified by chromatography on a column packed with 500 g of silanized silica gel developed with a linear gradient from 15 to 40% of CH_3CN in water. By working out the appropriate fractions, 2.5 g of compound **1** were obtained.

Method B

N^{15}-(Aminothioxomethyl) (**2**): A solution of **1** (2.5 g, 1.8 mmol), K_2CO_3 (2 g, 14.4 mmol) in 100 ml of Me_2CO - H_2O (1 : 1) was stirred 2 hours at room temperature. Acetone was removed under vacuum and the aqueous solution, adjusted to pH 3, was extracted with BuOH (100 ml). The organic layer, washed with water (30 ml) was evaporated to dryness. The residue was triturated with Et_2O, filtered and washed with Et_2O, yielding 1.8 g of the title compound.

Method C

N^{15}-(Phenylmethyl)aminothioxomethyl (**7**): A solution of **II** (5 g, 4.1 mmol) in 300 ml of CH_3CN - H_2O (1 : 1) containing benzyl isothiocyanate (0.6 ml, 4.6 mmol) was stirred at 40°C for 5 hours mantaining the pH at 7.5 by adding 1 N NaOH. If after this time some starting material was still present (HPLC assay) additional isothiocyanate was added. The organic solvent was evaporated under vacuum, the water, brought to pH 3, was extracted with 300 ml of a mixture of BuOH - EtOAc (3 : 1) and the organic layer washed with water. The residue obtained after solvent evaporation was triturated with Et_2O filtered and dried under vacuum, yielding 5 g of a crude material, which was purified by column chromatography on silanized silica gel eluting with a linear gradient CH_3CN - H_2O from 10 to 40% of CH_3CN. By working out the appropriate fractions 2.5 g of title compound were obtained.

Method D

N^{15}-(Ethoxycarbonylamino)thioxomethyl (**5**): A solution of **II** (2 g, 1.66 mmol), in 100 ml of CH_3CN and 20 ml of H_2O, was treated with ethoxycarbonyl isothiocyanate (40 ml) and the mixture stirred at room temperature overnight. The solvents were evaporated under vacuum at 45°C. The residue was then treated with Me_2CO, filtered, washed with Et_2O and dried under vacuum, yielding 2 g of 90% pure (HPLC) title compound.

Isothiouronium Salts of the Aglycone

Method E

N^{15}-(Cyclohexylimino)(methylthio)methyl (**14**): A solution of compound **6** (2 g, 1.58 mmol) in 40 ml of MeOH was stirred at room temperature while a total of 1 ml (16 mmol) of CH_3I was added over 48 hours. The solvent was concentrated to a small volume and by adding Et_2O (100 ml) a precipitate formed which was filtered and dried under vacuum. Yield 1.8 g of title compound.

Method F

N^{15}-Amino(ethylthio)methylene (**11**): A solution of **2** (1.5 g, 1.2 mmol) and EtBr (0.4 ml, 5 mmol) in 30 ml of CH_3CN and 5 ml of H_2O was stirred at 40°C while additional EtBr (0.4 ml) was added over 24 hours. The reaction, cooled at room temperature, was diluted with 100 ml water and then loaded on the top of a chromatographic column containing 150 g of silanized silica gel. The product was eluted with a linear gradient from 10 to 30% of CH_3CN in water containing 0.1% 1 N

HCl. The fractions containing pure product (>90% HPLC) were pooled, BuOH was added and the solvents were evaporated to dryness. The residue was tritured with H_2O and filtered. Yield 400 mg of title compound.

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References

1) DEL FAVERO, A. & F. MENICHETTI: Teicoplanin: A new glycopeptide antibiotic. Drugs of Today 24: 641~648, 1988
2) MALABARBA, A.; P. FERRARI, G. G. GALLO, J. KETTENKING & B. CAVALLERI: Teicoplanin, antibiotics from *Actinoplanes teichomyceticus* nov. sp. VII. Preparation and NMR characteristics of the aglycone of teicoplanin. J. Antibiotics 39: 1430~1442, 1986
3) CHATTERJEE, A. N. & H. R. PERKINS: Compounds formed between nucleotides related to the biosynthesis of bacterial cell wall and vancomycin. Biochem. Biophys. Res. Commun. 24: 489~494, 1966
4) NIETO, M. & H. R. PERKINS: Modification of acyl-D-alanyl-D-alanine terminus affecting complex formation with vancomycin. Biochem. J. 123: 789~803, 1971
5) BARNA, J. C. J. & D. H. WILLIAMS: The structure and mode of action of glycopeptide antibiotics of the vancomycin group. Annu. Rev. Microbiol. 38: 339~357, 1984
6) BARNA, J. C. J.; D. H. WILLIAMS & M. P. WILLIAMSON: Structural features that affect the binding of teicoplanin, ristocetin A, and their derivatives to the bacterial cell-wall model *N*-acetyl-D-alanyl-D-alanine. J. Chem. Soc. Chem. Commun. 1985: 254~256, 1985
7) HERRIN, T. R.; A. M. THOMAS, T. J. PERUN, J. C. MAO & S. W. FESIK: Preparation of biologically active ristocetin derivatives: Replacements of the 1'-amino group. J. Med. Chem. 28: 1371~1375, 1985
8) TRANI, A.; P. FERRARI, R. PALLANZA & G. TARZIA: Deaminoteicoplanin and its derivatives. Synthesis, antibacterial activity and binding strength ot Ac-D-Ala-D-Ala. J. Med. Chem. 32: 310~314, 1989
9) DOUGLASS, I. B. & F. B. DAINS: The preparation and hydrolysis of mono- and disubstituted benzoylthioureas. J. Am. Chem. Soc. 56: 1408~1409, 1934