SYNTHESIS AND BIOLOGICAL ACTIVITY OF O56-SUBSTITUTED CARBOXYESTERS AND CARBOXAMIDES OF TEICOPLANIN AGLYCONE

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A series of O56-substituted carboxyester or carboxyamide derivatives of deglucoteicoplanin (TD) was prepared by condensation of the 56-hydroxyl function with various alkylating agents of general formula RBr, where R represents functional groups with different physico-chemical properties.

The modifications at position 56 influenced the antimicrobial activity of the new derivatives; activity depended on the structure of various R groups, their ionic properties, and their steric hindrance.

The activity of the new compounds did not show any significant improvement when compared with TD.

The physico-chemical and antibacterial properties of the synthesized compounds are reported.

Teicoplanin,[1~3] which recently became available for therapeutic uses, is a glycopeptide antibiotic suitable for treatment of severe Gram-positive infections.[4,5]

The mechanism of action of teicoplanin is similar to that of other glycopeptide antibiotics. It binds strongly to the terminal D-alanyl-D-alanine of the muramylpentapeptide during the biosynthesis of bacterial cell wall peptidoglycan.[2,6]

The chemical work on teicoplanin complex (CTA) and on derivatives obtained from partial or total hydrolysis of the glycosidic bonds (TB, TC and TD, see Fig. 1),[7,8] coupled with *in vitro* binding assays,[9] has indicated which functional groups of teicoplanin are important in the binding to the target. Other parts of the antibiotic, such as the carboxyl in position 63,[10~12] can be modified to obtain compounds with different physico-chemical properties, and possibly better antimicrobial activity, without affecting the binding strength.

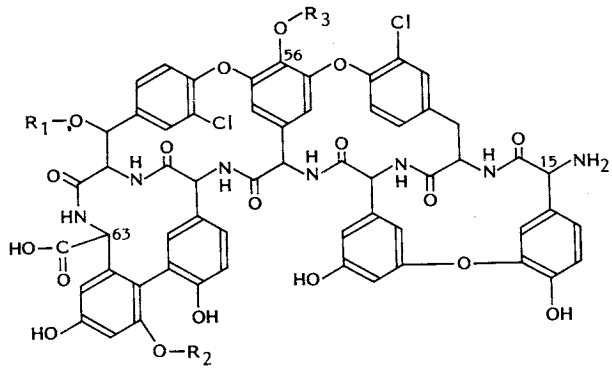
The chemical derivatizations carried out in the past were mainly directed toward broadening the antibacterial spectrum of activity of teicoplanin (in particular against Gram-negative bacteria and coagulase-negative staphylococci) and toward the preparation of orally active derivatives.[10~12]

The product obtained from total glycosidic hydrolysis of teicoplanin, deglucoteicoplanin (TD), shows some *in vitro* activity against Gram-negative bacteria[7] that are not sensitive to teicoplanin. However, TD has poor efficacy as compared with teicoplanin (CTA) in experimental infections.[3]

The acylglucosamine in position 56 of CTA thus appears to be important for *in vivo* activity of the molecule while its removal (together with the other sugars) increases penetration into Gram-negative bacteria.

We decided to explore the influence of various substituent introduced in position 56 (Fig. 1) replacing the acylglucosamine moiety of the natural antibiotic. We thought that these modifications might produce aglycone derivatives having favourable physico-chemical properties and, consequently, improved

Fig. 1.



TD: $R_1=R_2=R_3=H$
TC: $R_1=N$-Acetyl glucosamine, $R_2=R_3=H$
TB: $R_1=N$-Acetyl glucosamine, $R_2=$mannose, $R_3=H$
CTA: $R_1=N$-Acetyl glucosamine, $R_2=$mannose, $R_3=N$-acyl glucosamine

antimicrobial activity. The introduction of new functional groups, such as amines or carboxyls, at position 56 might also create additional binding positions for the terminal pentapeptide of the bacterial peptidoglycan.

In this paper the synthesis, physico-chemical properties and the antimicrobial activity of a series of OH-56 ethers, OH-56 ether 63-carboxyesters and OH-56 ether 63-carboxyamides of TD are described.

Chemistry

A series of OH-56 substituted carboxymethyl esters (CMEs) of TD were prepared according to Scheme 1: compounds **6** and **7** (Method A) come from protection of the 15-amino group of TD as *t*-butyl carbamate (Boc) **1**, esterification of the 63-carboxyl group with MeI-$KHCO_3$ to give **3**,[11] alkylation of the OH-56 with RBr to give **4** and **5** and deprotection of NH-15 Boc with trifluoroacetic acid (TFA).

Compounds **13**~**16** were prepared in a similar way (Method B) except for NH_2-15 protection as benzyl carbamate (CBz) **2** and deprotection of intermediates **9**~**12** by catalytic hydrogenation.

In Scheme 2 the synthesis of substituted CME compounds containing alkylamino chains in 56 is reported: **18** from **11** by reaction with ethylenediamine through intermediate **17**; **19** from **14** after hydrolysis of the phthalimido group with hydrazine hydrate; **22** from **10** after hydrolysis of the phthalimido group to give **20** followed by reductive coupling with dimethylaminoacetaldehyde hydrochloride through intermediate **21**; **25** and **26** from **12** after coupling with pyrrolidine or morpholine through intermediates **23** and **24**.

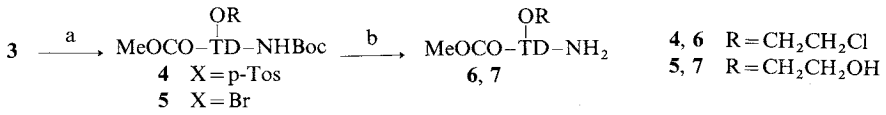
Compounds bearing 63-esters other than methyl were also prepared; in Scheme 3 the intermediate **27**[12] gives rise to the O-56 cyanomethyl derivative **29** through intermediate **28**, and to two 63-carboxyamides through amidation with methylamine (intermediates **30** and **31**) or ethylenediamine (intermediates **33** and **34**) producing compounds **32** and **35**, respectively.

Key intermediate **37** (Scheme 4) gives rise to 56-substituted 63-carboxyesters of TD by simple deprotection to give **38**, by reaction with glycylglycine methyl ester through intermediate **39** to give **40**, or by hydrolysis with NaOH through intermediate **41** to give **42**.

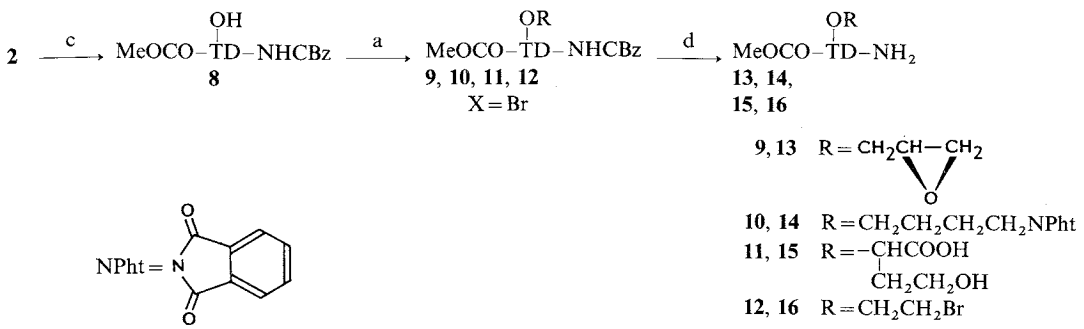
Intermediate **43** (Scheme 5) gives rise to 56-substituted 63-benzyl esters of TD after coupling with 1,2-dibromoethane (**47**, intermediate **44**), with benzyl bromide (**48**, intermediate **45**) or with

Scheme 1.

Method A

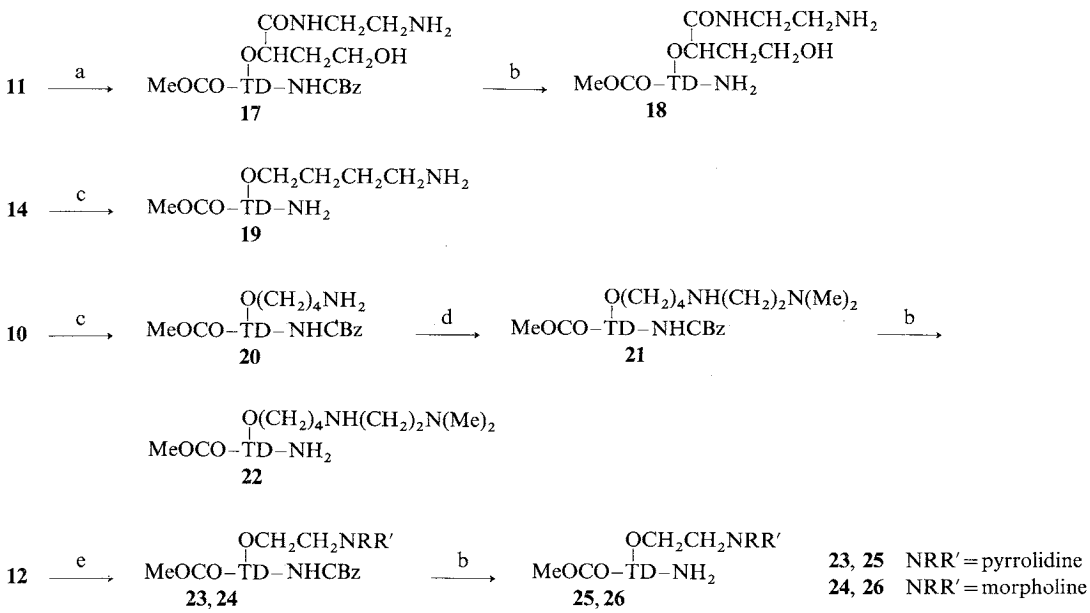


Method B



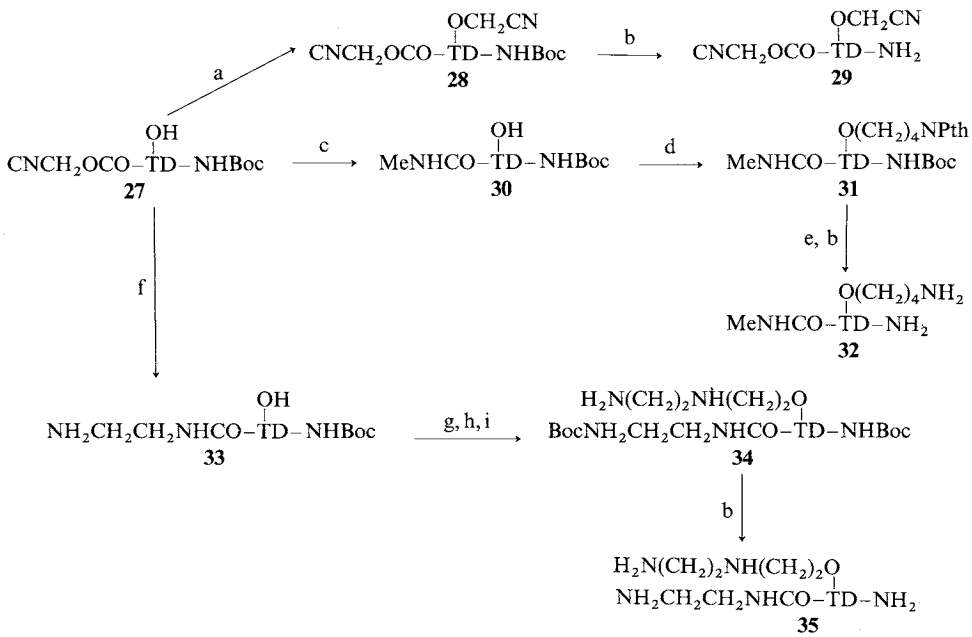
a) RX, K_2CO_3, DMSO, 40°C; b) TFA/CH_2Cl_2, R.T., 15 minutes; c) CH_3I, $KHCO_3$, DMSO, R.T., 16 hours; d) H_2, 5% Pd/C, MeOH/HCl aq., 4 hours.

Scheme 2.



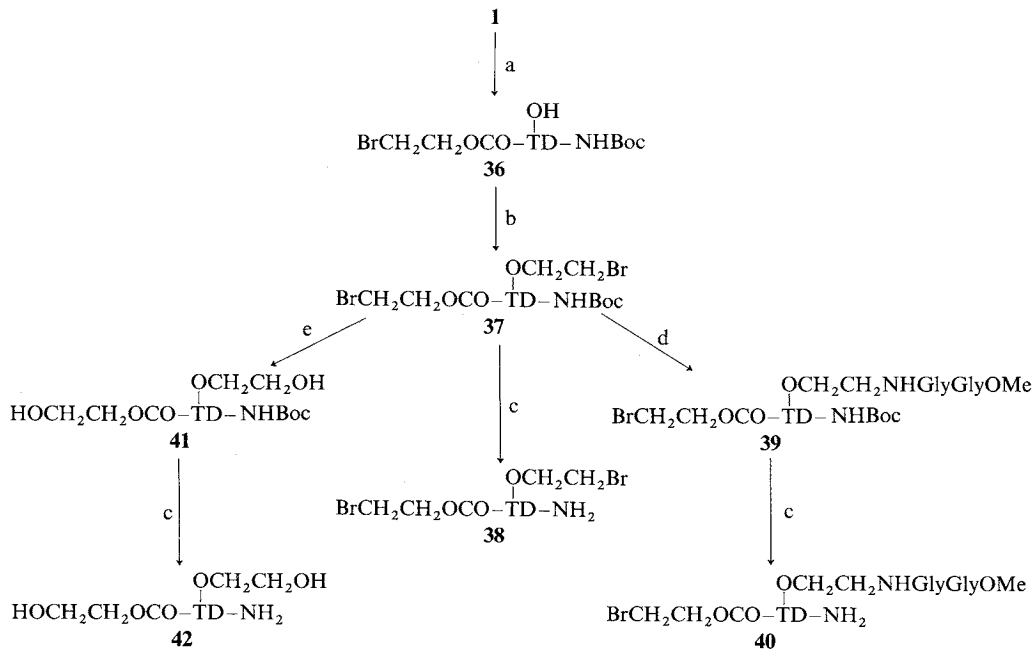
a) $NH_2CH_2CH_2NH_2$, EtOH/CH_3CN, R.T., 16 hours; b) H_2, 5% Pd/C, MeOH/HCl aq.; c) $N_2H_4 \cdot H_2O$, EtOH/CH_3CN, R.T., 16 hours; d) $(Me)_2NCH_2CHO \cdot HCl$, MeOH/CH_3CN, $NaCNBH_3$, R.T., 4 hours; e) NRR′, EtOH/DMSO, 50°C.

Scheme 3.



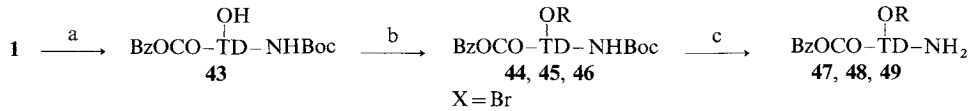
a) $ClCH_2CN$, K_2CO_3, DMF, R.T.; b) see b of Sch. 1; c) $MeNH_2$, EtOH, R.T., 2 hours; d) $Br(CH_2)_4NPth$, DMF, R.T.; e) see c of Sch. 2; f) $NH_2CH_2CH_2NH_2$, EtOH, R.T.; g) *t*-BuOCOO-(2,3,5-Cl-phenyl), DMF, TEA; h) $BrCH_2$–CH_2Br, DMSO, K_2CO_3, R.T.; i) $NH_2CH_2CH_2NH_2$, DMSO/EtOH, 50°C, 8 hours.

Scheme 4.



a) $BrCH_2CH_2Br$, $KHCO_3$, DMSO, R.T., 2 hours; b) see h of Sch. 3; c) see b of Sch. 1; d) GlyGlyOMe·HCl, K_2CO_3, DMSO, 40°C; e) NaOH, DMSO, 40°C, 1 hour.

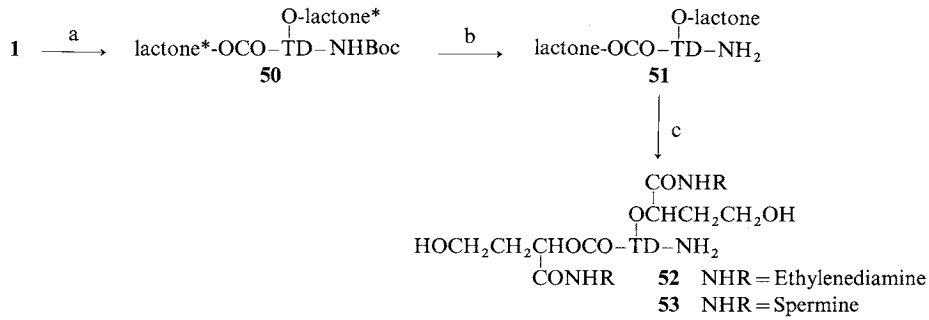
Scheme 5.



44, **47** R=CH_2CH_2Br
45, **48** R=Benzyl
46, **49** R=α-(γ-butyrolactone)

a) BzBr, $KHCO_3$, DMSO, R.T., 2 hours; b) see a of Sch. 1; c) see b of Sch. 1.

Scheme 6.



52 NHR=Ethylenediamine
53 NHR=Spermine

a) alfa-Br-gamma-butyrolactone*, K_2CO_3, DMSO, 40°C, 3 hours; b) see b of Sch. 1; c) RNH_2, DMSO, R.T., 1 hour.

Scheme 7.

a) DBU, $ClCH_2CH_2OCH_2CH_3$, 40°C, 48 hours; b) see b of Sch. 1; c) see d of Sch. 1.

α-bromo-γ-butyrolactone (**49**, intermediate **46**).

The 63-lactone ester **51** is prepared as in Scheme 6 through intermediate **50**, then reacted with ethylenediamine or spermine to give the 56-substituted 63-carboxyamides **52** or **53**, respectively.

Scheme 7 shows the preparation of two simple 56-substituted carboxy TD compounds by alkylation of **1** (**54**) or hydrogenolysis of **49** (**55**).

All 25 final products are listed in Table 1 together with some of their properties; NMR data of significant protons are reported in Table 2. For the spectrum of TD core structure see ref 7.

All the reactions were monitored by HPLC and the products were characterized by HPLC, IR, MS and NMR (see the Experimental for details).

Results and Discussion

Table 3 shows the *in vitro* antibacterial activity of all the O-56 substituted derivatives that were

Table 1. Teicoplanin aglycone (TD) derivatives.

OA
RCO–TD–NH_2

No.	R	A	Formula	MW	t_R (Method)[a]
6	OCH_3	2-Chloroethyl	$C_{61}H_{50}N_7O_{18}Cl_3$	1,275.50	15.2 (A)
7	OCH_3	2-Hydroxyethyl	$C_{61}H_{51}N_7O_{19}Cl_2$	1,257.08	3.8 (A)
13	OCH_3	2,3-Epoxypropyl	$C_{62}H_{51}N_7O_{19}Cl_2$	1,269.33	8.2 (A)
14	OCH_3	4-Phthalimido-butyl	$C_{71}H_{58}N_8O_{20}Cl_2$	1,414.19	20.0 (A)
15	OCH_3	1-Carboxy-3-hydroxypropyl	$C_{63}H_{53}N_7O_{21}Cl_2$	1,315.05	12.5 (A)
16	OCH_3	2-Bromoethyl	$C_{61}H_{50}N_7O_{18}BrCl_2$	1,319.91	12.5 (A)
18	OCH_3	1((2-Aminoethylamino)carbonyl)-3-hydroxypropyl	$C_{65}H_{59}N_9O_{20}Cl_2$	1,357.14	2.5 (A)
19	OCH_3	4-Aminobutyl	$C_{63}H_{56}N_8O_{18}Cl_2$	1,284.10	3.4 (A)
22	OCH_3	4(Bis(2(dimethylamino)ethyl)-amino)butyl	$C_{71}H_{74}N_{10}O_{18}Cl_2$	1,426.37	11.4 (A)
25	OCH_3	2-(1-Pyrrolidinyl)ethyl	$C_{65}H_{58}N_8O_{18}Cl_2$	1,310.12	5.4 (A)
26	OCH_3	2-(1-Morpholinyl)ethyl	$C_{65}H_{58}N_8O_{19}Cl_2$	1,326.12	4.2 (A)
29	OCH_2CN	Cyanomethyl	$C_{62}H_{47}N_9O_{18}Cl_2$	1,309.00	11.6 (A)
32	$NHCH_3$	4-Aminobutyl	$C_{63}H_{57}N_9O_{17}Cl_2$	1,283.05	2.6 (A)
35	$NHCH_2CH_2NH_2$	2((2-Aminoethyl)amino)ethyl	$C_{64}H_{61}N_{11}O_{18}Cl_2$	1,343.24	n.d.
38	OCH_2CH_2Br	2-Bromoethyl	$C_{62}H_{51}N_7O_{18}Br_2Cl_2$	1,412.80	26.5 (C)
40	OCH_2CH_2Br	2-((2-((2-Methoxy-2-oxoethyl)amino)-2-oxoethyl)amino)ethyl	$C_{67}H_{60}N_9O_{21}Br_2Cl_2$	1,478.07	25.0 (C)
42	OCH_2CH_2OH	2-Hydroxyethyl	$C_{62}H_{53}N_7O_{20}Cl_2$	1,287.04	25.0 (C)
47	OCH_2Ph	2-Bromoethyl	$C_{72}H_{62}N_7O_{18}BrCl_2$	1,464.13	31.0 (C)
48	OCH_2Ph	Phenylmethyl	$C_{72}H_{57}N_7O_{18}Cl_2$	1,379.18	10.0 (C)
49	OCH_2Ph	Tetrahydro-2-oxo-3-furyl	$C_{69}H_{55}N_7O_{20}Cl_2$	1,373.13	13.5 (C)
51	OCHCOO	Tetrahydro-2-oxo-3-furyl	$C_{66}H_{53}N_7O_{22}Cl_2$	1,367.08	22.5 (C)
52	O-AECP[b]	AECP[b]	$C_{70}H_{69}N_{11}O_{22}Cl_2$	1,487.28	19.0 (C)
53	O-APAB[c]	APAB[c]	$C_{86}H_{105}N_{15}O_{22}Cl_2$	1,771.78	18.5 (C)
54	OH	2-Ethoxyethyl	$C_{62}H_{53}N_7O_{19}Cl_2$	1,385.13	3.4 (C)
55	OH	Tetrahydro-2-oxo-3-furyl	$C_{62}H_{49}N_7O_{20}Cl_2$	1,283.01	13.0 (C)

[a] See Experimental section.
[b] 1(((2-Aminoethyl)amino)carbonyl)-3-hydroxypropyl.
[c] 1(((3-((4-((3-Aminopropyl)amino)butyl)amino)propyl)amino)carbonyl)-3-hydroxypropyl.

synthesized in comparison with TD.

The antibacterial activity of these derivatives is generally good against streptococci, while it goes from moderate to good against staphylococci and against *Enterococcus faecalis*. No one among them showed any improvement in *in vitro* activity when compared with TD.

Some structure-activity relationships can be extracted from the examination of these data. Bulky esters in position 63 (benzyl, butyrolactone) caused the changing from good to moderate activity against staphylococci and enterococci; bulky groups in position 56 (phthalimidobutyl) caused a similar effect in antibacterial activity on staphylococci and enterococci; a basic chain in position 56 (pyrrolidine, ethylenediamine, aminobutyl) produced derivatives with good antimicrobial activity.

The binding constants for D-Ala-D-Ala, determined for some of the derivatives bearing different functional groups in position 56, resulted to be similar among basic (pyrrolidine, morpholine) and neutral (hydroxyl, chloro) 56-substituents (Table 4), and comparable with the binding constant of TD. This did not indicate the presence of additional binding groups for the target terminal pentapeptide at the 56 position of teicoplanin derivatives.

Table 2.

Compound No.	^{1}H NMR spectra (δ, ppm) in DMSO-d_6 (proton attribution).
6	10.20～9.03 (phenolic OH's); 6.08 (C_{39}-H); 5.72 (C_{50a}-H); 5.52 (C_{25}-H); 5.33 (C_3-H); 5.17 (C_{27}-H); 5.11 (C_{34}-H); 4.91 (C_{18}-H); 4.50 (C_{38}-H); 4.50 (OCH_2); 4.00 (CH_2Cl); 3.71 (OCH_3)
7	9.48～8.81 (phenolic OH's); 6.10 (C_{39}-H); 5.65 (C_{50a}-H); 5.59 (C_{15}-H); 5.50 (C_{25}-H); 5.38 (C_3-H); 5.16 (C_{27}-H); 5.11 (C_{34}-H); 4.91 (C_{18}-H); 4.53 (C_{38}-H); 4.34 (C_{48}-H); 4.12 (C_{35}-H); 4.25 (OCH_2); 3.81 (CH_2OH); 3.70 (OCH_3)
13	7.81 (C_{54}-H); 6.10 (C_{39}-H); 5.70 (C_{50a}-H); 5.55 (C_{25}-H), (C_{15}-H); 5.38 (C_3-H); 5.17 (C_{27}-H); 5.12 (C_{34}-H); 4.91 (C_{18}-H); 4.53 (C_{38}-H); 4.35 (C_{48}-H); 3.72 (OCH_3); 4.29～3.30 (CH_2) and (CH) of epoxide, (CH_2O-C_{56})
14	10.0～9.00 (phenolic OH's); 7.80 (phthalimide CH's); 6.07 (C_{39}-H); 5.67 (C_{50a}-H); 5.52 (C_{25}-H); 5.49 (C_{15}-H); 5.36 (C_3-H); 5.11 (C_{27}-H), (C_{34}-H); 4.89 (C_{18}-H); 4.49 (C_{38}-H); 4.35～4.20 (C_{48}-H), (CH_2O-C_{56}); 3.69 (OCH_3); 1.83 (CH_2) of the aliphatic chain
15	8.55 (N_{49}-H), (N_{37}-H); 7.78 (C_{54}-H); 6.06 (C_{39}-H); 5.68 (C_{50a}-H); 5.48 (C_{25}-H); 5.33 (C_3-H); 5.09 (C_{27}-H), (C_{34}-H); 4.89 (C_{18}-H); 4.47 (C_{38}-H); 4.30 (C_{48}-H); 4.09 (–CH–); 3.7 (–CH_2OH): 2.08 (CH_2–); 3.68 (OCH_3)
16	6.05 (C_{39}-H); 5.75 (C_{50a}-H); 5.70 (C_{25}-H); 5.32 (C_3-H); 5.12 (C_{27}-H); 5.10 (C_{34}-H); 4.97 (C_{18}-H); 4.54 (C_{38}-H), (OCH_2); 4.32 (C_{48}-H); 4.12 (C_{35}-H); 3.84 (CH_2Br); 3.68 (OCH_3)
18	10.5～8.98 (phenolic OH's); 6.06 (C_{39}-H); 5.66 (C_{50a}-H); 5.51 (C_{25}-H); 5.34 (C_3-H); 5.11 (C_{27}-H), (C_{34}-H); 5.10 (–CH_2OH); 3.7 (–CH_2OH); 2.10 (–CH_2); 4.83 (C_{18}-H); 4.48 (C_{38}-H); 4.33 (C_{48}-H); 4.12 (C_{35}-H); 3.68 (OCH_3); 2.83 (CH_2–NH_2)
19	9.92～8.88 (phenolic OH's); 6.10 (C_{39}-H); 5.68 (C_{50a}-H); 5.56 (C_{25}-H); 5.39 (C_3-H); 5.19 (C_{27}-H); 5.12 (C_{34}-H); 4.54 (C_{38}-H); 4.34 (C_{48}-H); 4.13 (C_{35}-H); 4.25 (CH_2-1); 1.89 (CH_2-1, CH_2-3); 2.92 (CH_2-4); 3.71 (OCH_3)
22	6.08 (C_{39}-H); 5.68 (C_{50a}-H); 5.55 (C_{25}-H); 5.38 (C_3-H); 5.17 (C_{27}-H); 5.11 (C_{34}-H); 4.88 (C_{18}-H); 4.52 (C_{38}-H); 4.26 (1-CH_2); 1.85 (2-CH_2), (3-CH_2); 2.78 (N-CH_2); 2.51 (N-CH_3); 4.13 (C_{35}-H); 3.70 (OCH_3)
25	10.0～9.45 (phenolic OH's); 7.80 (C_{54}-H); 6.07 (C_{39}-H); 5.69 (C_{50a}-H); 5.60 (C_{25}-H); 5.57 (C_{15}-H); 5.37 (C_3-H); 5.17 (C_{27}-H); 5.10 (C_{34}-H); 4.86 (C_{18}-H); 4.60 (C_{56}-OCH_2); 4.50 (C_{38}-H); 4.33 (C_{48}-H); 4.11 (C_{35}-H); 3.70 (OCH_3); 3.65 (N-CH_2 pyrrolidine); 2.92 (C_{19}-H); 1.99, 1.88 (CH_2 pyrrolidine)
26	9.98～8.96 (phenolic OH's); 6.06 (C_{39}-H); 5.70 (C_{50a}-H); 5.56 (C_{25}-H); 5.37 (C_3-H); 5.16 (C_{27}-H); 5.10 (C_{34}-H); 4.86 (C_{18}-H); 4.65 (CH_2O-C_{56}); 4.50 (C_{38}-H); 4.32 (C_{48}-H); 4.10 (C_{35}-H); 4.0～3.6 (morpholine CH_2); 3.68 (OCH_3); 2.90 (C_{19}-H)
29	9.96～9.07 (phenolic OH's); 7.78 (C_{54}-H); 6.07 (C_{39}-H); 5.75 (C_{50a}-H); 5.55 (C_{25}-H); 5.40～5.09 (C_3-H), (C_{27}-H), ($COOCH_2$-CN), (C_{56}-OCH_2CN); 4.93 (C_{18}-H); 4.53 (C_{38}-H); 4.32 (C_{48}-H); 4.12 (C_{35}-H); 2.86 (C_{19}-H)
32	6.23 (C_{39}-H); 5.77 (C_{50a}-H); 5.58 (C_{25}-H); 5.39 (C_3-H); 5.29 (C_{34}-H); 5.21 (C_{27}-H); 4.95 (C_{18}-H); 4.38 (C_{38}-H); 4.26 (OCH_2); 1.88 (CH_2-2), (CH_2-3); 2.91 ($CH_2(NH_2)$); 2.70 ($CH_3(NH)$)
35	7.79 (C_{54}-H); 6.20 (C_{39}-H); 5.71 (C_{50a}-H); 5.54 (C_{25}-H); 5.43 (C_{15}-H); 5.39 (C_3-H); 5.25 (C_{34}-H); 5.18 (C_{27}-H); 4.92 (C_{18}-H); 4.24 (C_{56}-OCH_2), (C_{38}-H); 4.36 (C_{48}-H); 4.15 (C_{35}-H); 3.5～2.9 (various NCH_2)
38	9.90～9.0 (phenolic OH's); 8.56～6.10 (aromatic protons, peptidic NH's); 4.13～5.65 (peptidic CH's); 4.53, 4.44, 3.84, 3.66 (CH_2, side chains)
40	9.85～9.0 (phenolic OH's); 8.52～6.13 (aromatic protons, peptidic NH's); 5.62～4.13 (peptidic CH's); 4.38, 4.10 (CH_2–CH_2–R_2); 4.42, 4.95, 3.5 (CH_2-side chains)
42	8.52～6.09 (aromatic protons, peptidic NH's), 5.53～4.12 (peptidic CH's); 4.23, 3.78, 3.58, (CH_2 side chains)
47	9.98～9.90 (phenolic OH's); 8.5～6.1 (aromatic protons, peptidic NH's); 5.70～4.05 (peptidic CH's); 5.18 (CH_2 benzyl); 4.55, 3.85 (CH_2 side chains)
48	9.98～9.0 (phenolic OH's); 8.5～6.14 (aromatic protons, peptidic NH's); 5.65～4.08 (peptidic CH's); 5.32, 5.14 (CH_2 benzyl)
49	8.5～6.12 (aromatic protons, peptidic NH's); 5.68～4.08 (peptidic CH's); 5.12 (CH_2-benzyl); 5.72, 4.53 (CH, CH_2-lactone)
51	9.98～9.0 (phenolic OH's); 8.53～6.11 (aromatic protons, peptidic NH's); 5.68～4.08 (peptidic CH's); 5.65, 5.15, 4.43, 4.35 (CH, CH_2-acetone)
52	8.55～6.08 (aromatic protons, peptidic NH's); 5.75～4.10 (peptidic CH's); 3.78, 3.12, 3.08, 2.18 (CH_2-side chains)
53	8.52～6.05 (aromatic protons, peptidic NH's); 5.58～4.13 (peptidic CH's); 3.85, 3.72, 3.28, 2.12, 1.75, 1.55 (CH_2 side chains)
54	10.01～8.96 (phenolic OH's); 7.79 (C_{54}-H); 6.24 (C_{39}-H); 5.71 (C_{50a}-H); 5.52 (C_{25}-H); 5.37 (C_3-H); 5.14 (C_{27}-H); 5.09 (C_{34}-H); 4.89 (C_{18}-H); 4.41 (C_{38}-H); 4.33 (C_{48}-H, C_{56}-O–CH_2–); 4.12 (C_{35}-H); 3.79 (O–CH_2–(CH_2)); 3.55 (CH_2–(CH_3)); 2.93 (C_{19}-H); 1.12 (CH_3–(CH_2))
55	8.48～6.21 (aromatic protons, peptidic NH's); 5.72～4.11 (peptidic CH); 5.82, 4.39 (CH, CH_2-lactone)

Table 3. *In vitro* activity of O56-substituted teicoplanin aglycone derivatives.

Organism	MIC (μg/ml)					
	TD	**6**	**7**	**13**	**14**	**15**
Staphylococcus aureus Tour L 165	0.06	0.13	0.13	0.13	2	0.5
S. haemolyticus L 602	0.25	1	2	1	4	2
S. epidermidis ATCC 12228	0.02	0.5	0.13	0.06	2	0.3
Streptococcus pyogenes L 49	0.13	0.13	0.06	0.06	0.13	0.13
S. pneumoniae L 44	0.13	0.13	0.13	0.13	0.13	0.13
Enterococcus faecalis ATCC 7080	0.13	2	0.13	0.5	4	0.13
Neisseria gonorrhoeae L 997	8	>128	32	64	>128	64
Haemophilus influenzae ATCC 19418	16	64	64	64	>128	>128
Escherichia coli L 47	64	>128	32	32	>128	>128
Proteus vulgaris ATCC 881	128	>128	>128	>128	>128	>128
Pseudomonas aeruginosa ATCC 10145	>128	>128	>128	64	>128	>128

Organism	MIC (μg/ml)					
	16	**18**	**19**	**22**	**25**	**26**
Staphylococcus aureus Tour L 165	0.5	0.13	0.13	0.3	0.13	0.3
S. haemolyticus L 602	2	0.5	0.5	0.5	0.5	2
S. epidermidis ATCC 12228	0.13	0.13	0.13	0.3	0.13	0.13
Streptococcus pyogenes L 49	0.13	0.06	0.06	0.03	0.06	0.3
S. pneumoniae L 44	0.3	0.3	0.13	0.3	0.13	0.13
Enterococcus faecalis ATCC 7080	1	0.3	0.13	0.5	0.13	0.13
Neisseria gonorrhoeae L 997	>128	64	n.d.	128	32	64
Haemophilus influenzae ATCC 19418	64	128	n.d.	>128	32	64
Escherichia coli L 47	64	16	8	16	16	32
Proteus vulgaris ATCC 881	>128	64	32	>128	64	>128
Pseudomonas aeruginosa ATCC 10145	>128	128	32	>128	64	>128

Organism	MIC (μg/ml)					
	29	**32**	**35**	**38**	**40**	**42**
Staphylococcus aureus Tour L 165	0.13	0.13	0.13	0.3	0.13	0.06
S. haemolyticus L 602	1	0.3	0.3	4	2	0.5
S. epidermidis ATCC 12228	0.06	0.13	0.13	0.13	n.d.	n.d.
Streptococcus pyogenes L 49	0.13	0.06	0.06	0.13	0.13	0.13
S. pneumoniae L 44	0.13	0.13	0.13	0.5	n.d.	n.d.
Enterococcus faecalis ATCC 7080	1	0.13	0.3	0.13	n.d.	n.d.
Neisseria gonorrhoeae L 997	>128	32	64	>128	n.d.	n.d.
Haemophilus influenzae ATCC 19418	64	32	128	>128	n.d.	n.d.
Escherichia coli L 47	>128	16	8	>128	>128	32
Proteus vulgaris ATCC 881	>128	32	64	>128	n.d.	n.d.
Pseudomonas aeruginosa ATCC 10145	>128	16	64	>128	n.d.	n.d.

Organism	MIC (μg/ml)						
	47	**48**	**49**	**51**	**52**	**53**	**55**
Staphylococcus aureus Tour L 165	1	0.5	2	0.5	0.3	0.3	0.13
S. haemolyticus L 602	4	4	4	8	0.3	0.3	4
S. epidermidis ATCC 12228	0.5	0.3	1	0.3	0.13	0.13	0.3
Streptococcus pyogenes L 49	0.3	0.13	0.13	2	0.13	0.13	0.5
S. pneumoniae L 44	0.5	0.3	0.3	0.5	0.3	0.13	0.3
Enterococcus faecalis ATCC 7080	4	0.5	2	1	0.5	1	0.5
Neisseria gonorrhoeae L 997	>128	>128	>128	>128	128	>128	64
Haemophilus influenzae ATCC 19418	>128	>128	>128	128	>128	>128	>128
Escherichia coli L 47	>128	>128	>128	>128	32	32	>128
Proteus vulgaris ATCC 881	>128	>128	>128	>128	>128	>128	>128
Pseudomonas aeruginosa ATCC 10145	>128	>128	>128	>128	128	64	>128

Table 4. Association constants[a] for O56-substituted teicoplanin aglycone derivatives with Ac-D-Ala-D-Ala at 31°C.

Compound	K_A, M^{-1}
TD	1.8×10^5
6	1.1×10^5
7	1.0×10^5
25	3.4×10^5
26	4.6×10^4

[a] Measured by differential UV spectrophotometry in 10% methanol - 90% 0.02 M sodium citrate solution (pH 5).

Experimental

All the compounds coupled with TD and the reagents utilized in each synthetic step were commercially available.

The preparation of compounds **1**~**3** and **27** has been previously reported.[11,12]

Evaporation of solvents with a rotary evaporator was always carried out below 50°C to prevent decomposition of TD derivatives; water solutions were evaporated in the presence of antifoaming agents, *e.g.* *n*-BuOH.

The reactants, the intermediates and the final compounds were analyzed by HPLC using a Hibar column (100 × 4.6 mm, Merck) pre-packed with LiChrospher RP-18 (5 μm) installed on a Hewlett-Packard 1090L instrument equipped with a 10 μl loop injector and a 254 nm UV detector. Elution was performed at a flow rate of 1 ml/minute by mixing eluent a) (0.02 M NaH_2PO_4, pH 4.8) with b) (acetonitrile). Three different methods were used:

Time (minutes):	0	2	25	30	35
Method A, % of b in a:	26	26	40	47	26
Method B, % of b in a:	35	35	54	54	35
Method C, % of b in a:	20		60	75	20

All compounds were analyzed for C, H, N and Cl using samples dried at 140°C under N_2 atmosphere. Weight loss was determined after heating the sample at 900°C in O_2 atmosphere. The analytical results were in accordance with theoretical values.

IR spectra (nujol) were recorded with a Perkin-Elmer 850 spectrometer.

^1H and ^{13}C NMR spectra were obtained at 250 MHz with a Bruker AM 250 instrument equipped with an Aspect 3000 console. The spectra were recorded at 40°C in deuterated DMSO solution using tetramethylsilane (TMS, 0.00 ppm) as internal reference.

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 micromoles) was dispersed in a few microliters of 2-thioglycerol - diglycerol (1 : 1) matrix and bombarded with a 6~9 keV beam of Xe atoms.

Binding constants of peptide to teicoplanin aglycone or its derivatives were measured essentially as described by NIETO and PERKINS[9] except that tandem arrangement of cuvettes was not required, since the peptide used in this work has no significant absorption in the range 250~340 nm. Experiments were carried out with a Perkin-Elmer 580 spectrophotometer, using cuvettes with 4-cm light path. Solutions (10 ml) containing antibiotic (25 μm in 10% methanol: 90% 0.02 M sodium citrate, pH 5) were placed in the sample and reference cuvettes, and the difference in absorbance that developed upon addition of Ac-D-Ala-D-Ala (10~50 μl of 4 mM solution) to the sample cuvette and the same volume of buffer to the reference cuvette was measured at 282~285 nm. The temperature was 31°C. Association constants were determined by Scatchard plots. The antibiotic concentrations were determined by UV absorption, with the extinction coefficient of teicoplanin at 279 nm ($E_{1\,cm} = 59.4$) as reference.[13] The peptide titer was first determined in distilled water by potentiometric titration, and then the desired concentration was prepared by dilution.

MIC were determined using the microbroth dilution method. The media used were: Todd-Hewitt broth (Difco) for streptococci; Iso-Sensitest broth (Oxoid) for staphylococci, *E. faecalis*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*; GC base broth (Difco) + 1% v/v BBL Isovitalex for *Neisseria gonorrhoeae*; brain-heart infusion broth (Difco) + 1% v/v Difco supplement C for *Haemophilus influenzae*. The inoculum was about 10^4 cfu/ml. Incubation was at 37°C in 5% CO_2 for 48 hours for *N. gonorrhoeae* and *H. influenzae*, in air for 18~24 hours for other species.

O-56-(2-Hydroxyethyl) TD Methyl Ester (**7**)

To a solution of **3**[11] (2 g, 1.52 mmol) in 80 ml of DMSO containing K_2CO_3 (414 mg, 3 mmol) and molecular sieves 4A (3 g), 2-bromoethanol (0.6 ml, 7 mmol) was added and the mixture stirred at room temperature for 6 hours. A second portion of 2-bromoethanol was added and the reaction was allowed to stand overnight at room temperature. The mixture was then diluted with 400 ml of water and the suspension, adjusted to pH 4 with 1 N HCl, was extracted with 200 ml of ethyl acetate - *n*-butanol 4 : 1. The organic layer was concentrated *in vacuo* and the residue was purified on a silica gel column (130 g) eluting with a mixture methylene chloride - methanol 9 : 1 yielding 1.04 g of compound **5**. The protecting group was removed by treatment of **5** with TFA (3 ml) for 15 minutes. After concentration *in vacuo*, purification of the crude material was done on a silanized silica gel column, eluting with acetonitrile - water 15 : 85 and obtaining 550 mg of pure title compound.

N-15-CBz TD Methyl Ester (**8**)

Starting from **2**[11] and using substantially the same esterification procedure described for **3**[11] 35 g of pure title compound were obtained.

O-56-(4-(*N*-Phthalimido)-butyl)TD Methyl Ester Hydrochloride (**14**)

The reaction between **8** (2 g, 1.48 mmol) and *N*-(4-bromobutyl) phthalimide (420 mg, 1.48 mmol) was performed as for compound **5** producing 1 g of pure intermediate **10**. This product was dissolved in 80 ml of MeOH containing 1 ml of 1 N HCl and hydrogenated with 5% Pd/C (400 mg) at room temperature and atmospheric pressure. The catalyst was filtered on filter aid (Celite), the solvent was removed *in vacuo* from the filtrate and the solid was treated with ethyl ether, collected by filtration and dried, yielding 860 mg of pure title compound.

O-56-(4-Aminobutyl) TD Methyl Ester Dihydrochloride (**19**)

Compound **14** (800 mg, 0.56 mmol) was dissolved in a mixture of absolute ethanol (80 ml) and acetonitrile (20 ml), then 0.5 ml of hydrazine hydrate were added and the reaction was stirred overnight at room temperature. The solvents were removed *in vacuo* and the resulting crude material was purified on a silanized silica gel column (60 g) eluting first the impurities with a gradient from 35 to 45% of MeCN in water (600 ml), then the desired product with 300 ml of MeOH containing 1% of 1 N HCl. The pooled fractions were concentrated to dryness yielding 260 mg of pure title compound.

N-15-CBz-O-56-(2-bromoethyl) TD Methyl Ester (**12**)

Intermediate **8** (6 g, 4.4 mmol) was dissolved in DMSO (60 ml) and K_2CO_3 (620 mg, 4.5 mmol) was added while stirring the mixture at 40°C for 15 minutes; 1.5 ml (17 mmol) of 1,2-dibromoethane were then added. The mixture was stirred at 40°C for 4 hours and overnnight at room temperature, then it was diluted with 400 ml of water, adjusted to pH 5 with 1 N HCl; the resulting suspension was centrifuged. The solid was recovered, washed with water and dried *in vacuo* at 40°C, yielding 6 g of pure title compound.

O-56-(2-(1-Pyrrolidinyl)-ethyl)TD Methyl Ester Dihydrochloride (**25**)

700 mg (0.5 mmol) of **12** were dissolved in absolute ethanol (40 ml) and DMSO (20 ml); pyrrolidine (0.7 ml, 8.4 mmol) was added and the solution was heated at 50°C for 8 hours. The mixture was then poured into 300 ml of chilled water and the solid was recovered by centrifugation. The solid was dissolved in 40 ml of 30% THF in water at pH 6.5 (1 N HCl) and loaded onto a silanized silica gel column; it was eluted using MeCN - water 3 : 7 producing 400 mg of compound **23**. Deprotection in MeOH (45 ml) and 0.1 N HCl (15 ml) with 5% Pd/C for 3 hours produced 280 mg of pure title compound.

N-15-Boc TD Ethylenediamine Amide (**33**)

Compound **27**[12] (2 g, 1.6 mmol) was reacted at room temperature with a strong excess of ethylenediamine for 2 hours in 200 ml of absolute ethanol; after cooling, the solution was concentrated to a crude material that was chromatographed with a reversed phase silica gel column, eluting with a gradient of 20 to 50% of MeCN in water. After concentration to dryness of the pooled fractions, 1.4 g of pure title compound were obtained.

O-56-(2-(1-Ethylenediaminyl)-ethyl)TD Ethylenediamine Amide (**35**)

Compound **33** (3.5 g, 2.6 mmol) was protected at the primary amino group of the amide moiety with Boc by reaction with *t*-butyl 2,4,5-trichlorophenylcarbonate in DMF (30 ml) with TEA (0.6 ml); after purification on a silanized silica gel column, pure protected compound (2 g) was obtained. This compound was reacted with 1,2-dibromoethane (0.425 ml) in DMSO (17 ml) in the presence of K_2CO_3 (175 mg); after 16 hours at room temperature a second portion of 1,2-dibromoethane (0.45 ml) was added with K_2CO_3 (200 mg) and the reaction was stirred for 12 hours. The mixture was then poured into chilled water (300 ml), the pH adjusted to 5, and the product recovered by centrifugation. This material was dissolved in 200 ml of ethyl acetate, washed with water, and the solvent was removed *in vacuo*. The residue was dissolved in *n*-butanol and the mixture was concentrated under vacuum to a final volume of 5 ml. Ethyl ether was added (400 ml) and the solid was recovered by filtration, yielding an intermediate from which crude **34** was prepared following the procedure used for compound **23** just replacing pyrrolidine with ethylenediamine (1.5 g). It was purified on a silanized silica gel column, eluting with MeCN - water 3 : 7 with a pH gradient of 0 to 1.5% of 1 N HCl, obtaining 650 mg of pure intermediate **34**. This was fully deprotected following the procedure used for **7** yielding 500 mg of pure title compound.

N-15-Boc TD 2-Bromoethyl Ester (**36**)

To a solution of **1**[11] (5 g, 3.87 mmol) in 50 ml of DMSO, $KHCO_3$ (500 mg, 5 mmol) and 1,2-dibromoethane (5 ml, 58 mmol) were added with stirring at room temperature. After 2 hours the reaction mixture was poured into water (500 ml), the pH was adjusted to 5 and the precipitate was filtered, washed with water and collected as a crude material. It was then purified on a reverse-phase chromatographic column eluting with a gradient of 10 to 50% of MeCN in water at about pH 3 (1 N HCl) obtaining 4.85 g of pure title compound.

O-56-(2-((2-((2-Methoxy-2-oxoethyl)amino)-2-oxoethyl)amino)ethyl)TD 2-Bromoethyl Ester (**40**)

To a solution of **36** (5 g, 3.56 mmol) in 50 ml of DMSO, K_2CO_3 (550 mg, 4 mmol) and 1,2-dibromoethane (5 ml, 58 mmol) were added with stirring and the resulting solution was heated at 50°C for 2 hours. After cooling, it was poured into 300 ml of water, the pH was adjusted to about 6 and the precipitate was filtered, washed with water and dried obtaining 5.47 g of intermediate **37**. To a solution of **37** (1 g, 0.66 mmol) in 10 ml of DMSO, K_2CO_3 (220 mg, 1.6 mmol) and glycylglycine methyl ester hydrochloride (260 mg, 1 mmol) were added with stirring and the solution was kept at 40°C for 6 hours. After cooling, the mixture was poured into 50 ml of water, adjusting the pH to 6; the precipitate was filtered, washed with water and dried obtaining 0.93 g of intermediate **39** which was deprotected as for compound **7** to give 0.52 g of pure title compound.

N-15-Boc TD Phenylmethyl Ester (**43**)

This compound was prepared similarly to **36**, using benzyl bromide (5 ml, 20 mmol) instead of 1,2-dibromoethane and obtaining 5.04 g of pure title compound.

O-56-(Tetrahydro-2-oxo-3-furanyl) TD Phenylmethyl Ester (**49**)

To a solution of **43** (2 g, 1.45 mmol) in 20 ml of DMSO, K_2CO_3 (220 mg, 1.6 mmol) and α-bromo-γ-butyrolactone (2 ml, 24 mmol) were added with stirring and the mixture was kept at 50°C for 2 hours. After a workup similar to that used for **39**, 2.07 g of compound **46** were obtained and deprotected as for **7** to give 1.66 g of pure title compound.

O-56-(Tetrahydro-2-oxo-3-furanyl) TD Tetrahydro-2-oxo-3-furanyl Ester (**51**)

To a solution of **1** (5 g, 3.87 mmol) in 50 ml of DMSO, K_2CO_3 (1.1 g, 8 mmol) was added with α-bromo-γ-butyrolactone (10 ml, 120 mmol) with stirring and kept for 3 hours at 40°C. After a workup as for **39** 5.76 g of intermediate **50** were obtained. It was then treated as described for **7** obtaining 4.14 g of pure title compound.

O-56-((1-(2-Aminoethyl)amino)carbonyl-3-hydroxypropyl) TD (1-(2-Aminoethyl)amino)carbonyl-3-hydroxypropyl Ester (**52**)

To a solution of **51** (1 g, 0.731 mmol) in 10 ml of DMSO, ethylenediamine was added (0.25 ml,

3.74 mmol) with stirring at room temperature. After an hour a workup as for **39** followed, giving 0.86 g of pure title compound.

O-56-(Tetrahydro-2-oxo-3-furanyl) TD (**55**)

A solution of 100 mg of **49** (0.078 mmol) in 10 ml of MeOH - water 7 : 3 adjusted to pH 3 with 1 N HCl was hydrogenated at room temperature and atmospheric pressure with 50 mg of 5% Pd/C for 2 hours with vigorous stirring. Then the pH was djusted to about 6 and the MeOH removed *in vacuo*. The resulting suspension was centrifuged and the solid, collected by filtration, was washed with water and dried giving 55 mg of pure title compound.

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