(1 H, d, $J_{1,2}$ = 7 Hz), 8.54 (1 H, s), 9.32 (1 H, d, $J_{1,2}$ = 9 Hz), 9.66 (1 H, s). IR: 1766.5 cm^{-1} (β-lactam carbonyl). MS (FAB): *m/e* (M + 1) 497.

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-6,7-dihydro-5*H*-1-pyrindinium Hydroxide, Inner Salt (27). Yield: 57%. ^1H NMR (DMSO-d_6): 1.80–2.27 (4 H, m), 2.43–2.73 (2 H, m), 3.07–3.40 (4 H, m), 3.83 (3 H, s), 3.87–4.00 (1 H, m), 5.40–5.50 (1 H, m), 6.77 (1 H, s), 7.20 (2 H, s), 7.81 (0.5 H, d, $J_{1,2}$ = 8 Hz), 7.86 (0.5 H, d, $J_{1,2}$ = 8 Hz), 8.34–8.40 (1 H, m), 0.5 H, d, $J_{1,2}$ = 6 Hz), 8.61 (0.5 H, d, $J_{1,2}$ = 6 Hz), 9.31 (0.5 H, d, $J_{1,2}$ = 8 Hz), 9.40 (0.5 H, d, $J_{1,2}$ = 8 Hz). IR: 1765 cm^{-1} (β-lactam carbonyl); MS (FAB): *m/e* (M + 1) 483.

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-5,6,7,8-tetrahydroquinolinium Hydroxide, Inner Salt (28). Yield: 56%. ^1H NMR (90 MHz, DMSO-d_6): 3.8–4.0 (1 H, m), 5.40 (1 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.70 (1 H, s), 7.12 (2 H, br s), 7.72 (1 H, br t, $J_{1,2}$ = 7 Hz), 8.20 (1 H, br d, $J_{1,2}$ = 7 Hz), 8.53 (1 H, br d, $J_{1,2}$ = 7 Hz), 9.18 (1 H, br d, $J_{1,2}$ = 9 Hz). IR: 1769.4 cm^{-1} (β-lactam carbonyl). MS (FAB): *m/e* (M + 1) 497.

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-2,5-dimethylpyridinium Hydroxide, Inner Salt (29). Yield: 31%. ^1H NMR (DMSO-d_6): 1.83–2.17 (2 H, m), 2.40–2.43 (3 H, s), 2.63–2.68 (3 H, s), 2.73–2.80 (2 H, m), 4.03–4.20 (2 H, m), 4.88 (3 H, s), 5.39 (0.3 H, dd, $J_{1,2}$ = 5 and 9 Hz), 5.45 (0.7 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.80 (1 H, s), 7.20 (2 H, s), 7.90 (0.7 H, d, $J_{1,2}$ = 8 Hz), 7.93 (0.3 H, d, $J_{1,2}$ = 8 Hz), 8.32 (1 H, br d, $J_{1,2}$ = 8 Hz), 8.72 (0.7 H, s), 8.79 (0.3 H, s), 9.32 (0.7 H, d, $J_{1,2}$ = 9 Hz), 9.39 (0.3 H, d, $J_{1,2}$ = 9 Hz). IR: 1771.4 cm^{-1} (β-lactam carbonyl). MS (FAB): *m/e* (M + 1) 471. UV: λ_{max} 234 nm (ε 17006).

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-3,4-dimethylpyridinium Hydroxide, Inner Salt (30). Yield: 47%. ^1H NMR (DMSO-d_6): 1.73–2.07 (2 H, m), 2.35–2.77 (8 H, m), 3.77–3.90 (4 H, m), 5.42 (1 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.77 (1 H, s), 7.20 (1 H, s), 7.87 (1 H, d, $J_{1,2}$ = 8 Hz), 8.63 (1 H, d, $J_{1,2}$ = 8 Hz), 8.77 (1 H, s), 9.38 (1 H, d, $J_{1,2}$ = 9 Hz). IR: 1772.5 cm^{-1} (β-lactam carbonyl). MS (FAB): *m/e* (M + 1) 471.

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-4-(dimethylamino)pyridinium Hydroxide, Inner Salt (33). To 175 mg of **15b** was added at 0 °C a mixture of 1.2 mL of trifluoroacetic acid, 90 μL of triethylsilane, and 1.2 mL of methylene chloride. After stirring at 0 °C for 30 min, the external cooling bath was removed for 5 min and diethyl ether added to precipitate the product. The solid was recovered by centrifugation, washed three times with diethyl ether, and dried to provide 81 mg of crude title product. The material was dissolved in a small amount of water, filtered, and chromatographed by reverse-phase HPLC employing 8% acetonitrile and 1% acetic acid in water as the eluant. Lyophilization of the desired fractions provided 41 mg of the desired product. ^1H NMR (DMSO-d_6): 1.70–2.00 (2 H, m), 2.38–2.66 (2 H, m), 3.20 (6 H, s), 3.70–3.86 (2 H, m), 5.32 (1 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.73 (1 H, s), 6.95 (2 H, d, $J_{1,2}$ = 8 Hz), 7.68 (2 H, s), 8.10 (2 H, d, $J_{1,2}$ = 8 Hz), 9.33 (1 H, d, $J_{1,2}$ = 9 Hz). IR: 1767.7 cm^{-1} (β-lactam carbonyl). MS (FAB): *m/e* (M + 1) 486.

1-[7β-[[(2-Amino-4-thiazolyl)(benzyloximino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-3,4-dimethylpyridinium Hydroxide, Inner Salt (32). Fifty milligrams of **15c** was added to 0.3 mL of trifluoroacetic acid and 9.7 μL of thiophenol under a nitrogen atmosphere and cooled by means of an external ethanol/ice bath. After stirring for 30 min at 0 °C and after 10 min with the ice bath removed, diethyl ether was added and the solid was worked up in the same manner as above. Chromatography with a continuous gradient of water to 12% acetonitrile in water and lyophilization provided 12 mg of the desired material. ^1H NMR (DMSO-d_6): 1.72–2.01 (2 H, m), 2.45 (3 H, s), 2.61 (3 H, s), 2.72–2.88 (2 H, m), 3.98–4.08 (1 H, m), 5.18 (2 H, s), 5.63 (1 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.84 (1 H, s), 7.24–7.36 (7 H, m), 8.08 (1 H, d, $J_{1,2}$ = 7 Hz), 8.94 (1 H, d, $J_{1,2}$ = 9 Hz), 9.00 (1 H, s), 9.56 (1 H, d), 7.94 (1 H, d, $J_{1,2}$ = 9 Hz). IR: 1769.3 cm^{-1} (β-lactam carbonyl), MS (FAB): *m/e* (M + 1) 547. UV: λ_{max} 234 (ε 21900), 295 nm (ε 8350).

Synthesis and Biological Properties of N^{63}-Carboxamides of Teicoplanin Antibiotics. Structure–Activity Relationships

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The condensation of the carboxyl function of teicoplanin A2 (CTA) and its acidic hydrolysis pseudoaglycons (TB, TC) and aglycon (TD) with amines carrying various functional groups and chains produced amide derivatives with different isoelectric points and lipophilicities. Amide formation did not affect the ability of these compounds to bind to Ac_2-L-Lys-D-Ala-D-Ala, a model for the natural peptide binding site in bacterial cell walls. The antimicrobial activities of teicoplanin amides were found to depend mostly on their ionic and lipophilic character and on the type and number of sugars present. Positively charged amides were generally more in vitro active than the respective unmodified antibiotics against Gram-positive organisms. In particular, most basic amides of CTA were markedly more active than teicoplanin against coagulase-negative staphylococci. A few amides of TC and most of those of TD also showed a certain activity against Gram-negative bacteria. In experimental *Streptococcus pyogenes* septicemia in the mouse, some basic amides were more active than the parent teicoplanins when administered subcutaneously. Some of those of CTA were also slightly more effective than teicoplanin by oral route.

The glycopeptide antibiotic teicoplanin[1] was recently introduced in therapeutic use for the parenteral treatment of severe infections caused by aerobic and anaerobic Gram-positive bacteria, including methicillin-resistant (MR) staphylococci.[2] It is very poorly absorbed when administered orally and is inactive in vitro against Gram-

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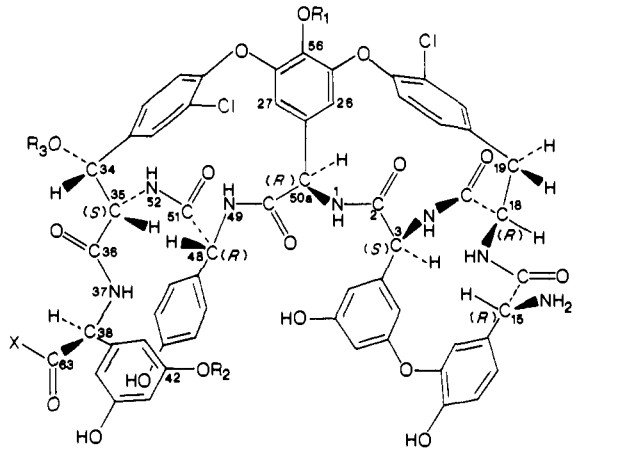
(1) (a) Coronelli, C.; Gallo, G. G.; Cavalleri, B. *Farmaco, Ed. Sci.* 1987, *10*, 767. (b) Parenti, F. *J. Hosp. Infect.* 1986, *7*, 79 (Suppl. A).

0022-2623/89/1832-2450$01.50/0



CTA

N-acyl (NHCOR) side chains

R_1 = *N*-acyl-β-D-glucosaminyl
- component A2-1: R = $(CH_2)_2CH{=}CH(CH_2)_4CH_3$
- component A2-2: R = $(CH_2)_8CH(CH_3)_2$
- component A2-3: R = *n*-C_9H_{19}
- component A2-4: R = $(CH_2)_8CH(CH_3)CH_2CH_3$
- component A2-5: R = $(CH_2)_7CH(CH_3)_2$

R_2 = α-D-mannosyl; R_3 = *N*-acetyl-β-D-glucosaminyl

TB: R_1 = H; R_2 = α-D-mannosyl; R_3 = *N*-acetyl-β-D-glucosaminyl
TC: R_1 = R_2 = H; R_3 = *N*-acetyl-β-D-glucosaminyl
TD: R_1 = R_2 = R_3 = H

Figure 1. The structures of teicoplanin antibiotics (X = OH) and their amides (X = NR'R"; see tables).

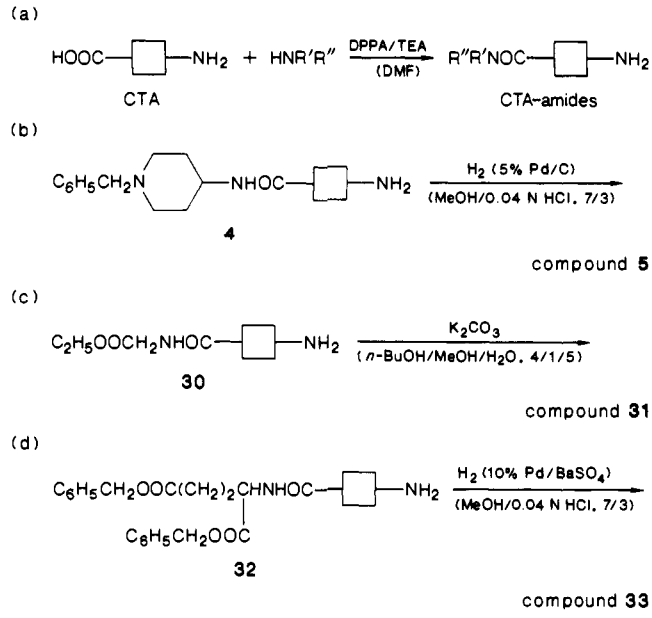
negative organisms. Lately, some clinical isolates of coagulase-negative staphylococci (CNST), in particular, strains of *Staphylococcus hemolyticus* (*Staph. hemolyticus*), have been found to be somewhat resistant to teicoplanin.[3] These strains may be of clinical importance because of their pattern of resistance to currently used antibiotics.[4]

Like that of the other antibiotics of the vancomycin family, the mechanism of action of teicoplanin consists in the inhibition of the biosynthesis of the bacterial cell wall through a complex formation with terminal peptide D-alanyl-D-alanine of peptidoglycan.[5]

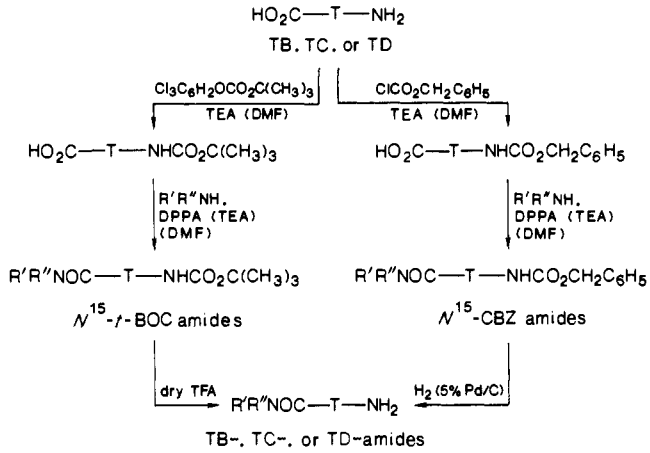
The program of chemical transformation of teicoplanin aims at broadening the antibacterial spectrum of activity and improving the oral absorption. As a preliminary approach, we opted for the synthesis of derivatives possessing net positive charge and lipophilic character by modifying the carboxyl group, a function that does not seem to be directly involved in the binding to the antibiotic's target peptide.

Preparation of a series of ester derivatives[6] of the *N*-acetylglucosaminyl aglycon and of the aglycon of teicoplanin provided compounds more active in vitro against CNST and with a certain activity against Gram-negative bacteria, but less effective in the experimental infection in the mouse. The drop in in vivo activity was interpreted as due to either the low solubility in water of these derivatives or a possible hydrolysis of the ester bond.

Scheme I



Scheme II. General Procedure e



On the basis of the above results and considering the lower lipophilicity and higher stability of the amide linkage, we synthesized different series of amide derivatives of teicoplanin A2 complex (CTA), its pseudoaglycons T-A3-1 (TB) and T-A3-2 (TC),[7] and aglycon (TD)[8] (Figure 1). In order to establish a relationship between isoelectric point, lipophilicity and hydrosolubility at the physiological pH, and antimicrobial activity, we introduced through the amide bond various lipophilic chains bearing appropriate functional groups.

The structures of teicoplanin amides were determined by ^1H NMR and FAB-MS spectroscopies.

The products were tested for their in vitro antibacterial activity and in vivo efficacy in experimental *Streptococcus pyogenes* (*Strep. pyogenes*) septicemia in the mouse. We

(2) (a) Gruneberg, R. N.; Ridgway, G. L.; Cremer, A. W. F.; Felmingham, D. *Drugs Exp. Clin. Res.* **1983**, *9*, 139. (b) Guentner, S. H.; Wenzel, R. P. *Antimicrob. Agents Chemother.* **1984**, *36*, 268. (c) Workshop on "Teicoplanin, a new clinical candidate for treatment of severe gram-positive infections"; Abstract of the 14th International Congress of Chemotherapy, 1985, Kyoto, Japan. (d) Durrande, J. B.; Dumas, Y.; Danglas, P. *J. Pharm. Clin.* **1988**, *7*, 225.

(3) Arioli, V.; Pallanza, R. *Lancet* **1987**, *1*, 39.

(4) Brumfitt, W.; Hamilton-Miller, J. M. T.; Neville, L. O. *Lancet* **1987**, *2*, 328.

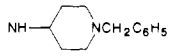
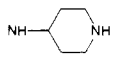
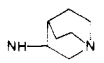
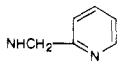
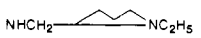
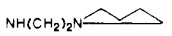
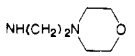
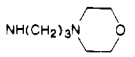
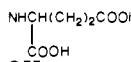
(5) Somma, S.; Gastaldo, L.; Corti, A. *Antimicrob. Agents Chemother.* **1984**, *26*, 917.

(6) (a) Malabarba, A.; Trani, A.; Ferrari, P.; Pallanza, R.; Cavalleri, B. *J. Antibiot.* **1987**, *40*, 1572. (b) U.S. Patent 839,320, June, 1985. (c) Unpublished results, these laboratories. ED_{50} values (in experimental *S. pyogenes* septicemia in the mouse, upon subcutaneous administration) were 3–40 times higher than that (0.95 mg/kg) of TD.

(7) Malabarba, A.; Strazzolini, P.; Depaoli, A.; Landi, M.; Berti, M.; Cavalleri, B. *J. Antibiot.* **1984**, *37*, 988.

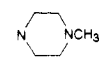
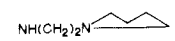
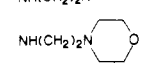
(8) Malabarba, A.; Ferrari, P.; Gallo, G. G.; Kettenring, J.; Cavalleri, B. *J. Antibiot.* **1986**, *39*, 1430.

Table I

compd	NR′R″	yield, %	HPLC[a] t_R, min	HPLC[a] rel t_R	log *P*	IEF, p*I*	titration, EW	MW[b]
1	$N(CH_3)_2$	90	30.56	1.15		7.8	1912	1910
2		62	32.05	1.20	−0.5	7.7	1980	1968
3		47	31.26	1.17	−1.5	8.2	1015	1965
4		53	43.75	1.65		nd[e]	1046	2055
5[d]		48[c]	28.85	1.08		8.5	1038	1965
6		84	31.74	1.19		8.7	1020	1991
7		58	31.92	1.20		7.9	1005	1973
8		67	36.89	1.39		nd	1014	1993
9		61	32.25	1.21		8.6	990	1979
10		66	31.97	1.20		8.0	1023	1995
11		82	36.09	1.36		8.1	1043	2009
12	$NH(CH_2)_2NH_2$	43	30.90	1.16	−2.4	8.6	981	1925
13	$NH(CH_2)_3NH_2$	49	35.12	1.32		8.7	990	1939
14	$NH(CH_2)_4NH_2$	46	35.30	1.33		8.8	987	1953
15	$NH(CH_2)_6NH_2$	21	35.63	1.34	−2.1	8.8	996	1981
16	$NH(CH_2)_2NHCH_3$	51	32.21	1.21		8.6	1000	1939
17	$NH(CH_2)_3NHCH_3$	54	35.40	1.35	−2.3	8.7	1002	1953
18	$NH(CH_2)_2NHC_2H_5$	43	33.42	1.26		8.5	1014	1953
19	$NH(CH_2)_3NHC_2H_5$	49	35.59	1.34	−2.0	8.5	1006	1967
20	$NH(CH_2)_2N(CH_3)_2$	48	32.47	1.22		8.5	985	1953
21	$NH(CH_2)_3N(CH_3)_2$	46	33.64	1.26	−2.4	8.7	981	1967
22	$NH(CH_2)_4N(CH_3)_2$	71	35.84	1.35	−2.0	8.8	1010	1981
23	$NH(CH_2)_5N(CH_3)_2$	66	35.89	1.35	−2.0	8.8	1005	1995
24	$NH(CH_2)_7N(CH_3)_2$	58	37.36	1.40	−1.9	8.8	1042	2023
25	$NH(CH_2)_3N(C_2H_5)_2$	50	34.99	1.32	−1.6	8.8	1010	1995
26	$NH(CH_2)_3N(n\text{-}C_4H_9)_2$	42	39.03	1.47	+0.5	8.6	1015	2051
27	$N(CH_3)(CH_2)_2NHCH_3$	59	34.12	1.28		8.7	1004	1953
28	$N(CH_3)(CH_2)_3NHCH_3$	64	36.18	1.36		8.8	999	1967
29	$N(CH_3)(CH_2)_2N(CH_3)_2$	57	36.20	1.36		8.6	1001	1967
30	$NHCH_2COOC_2H_5$	80	32.59	1.22		7.6	1960	1968
31	$NHCH_2COOH$	64[c]	27.31	1.03		5.5	981	1940
33[d]		58[c]	26.43	0.99		4.3	702	2012
CTA	OH		26.51	1.00	−2.7	5.8	1021	1883

[a] Data are referred to component A2-2 of each "complex" compound, HPLC method A. [b] Weighted average values, dealing with mixtures of five components. [c] Overall yields calculated from CTA. [d] Derivatives of component A2-1 free CTA. [e] nd = not determined.

Table II. Amides of TB

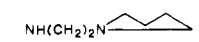
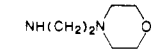
compd	NR′R″	yield,[a] % B	yield,[a] % C	HPLC[b] t_R, min	HPLC[b] rel t_R	IEF, p*I*	titration, EW	MW	formula
34		48	96	16.7	1.62	7.2	1671	1649	$C_{76}H_{75}N_9O_{27}Cl_2S$
35		52	94	14.2	1.38	7.7	840	1646	$C_{77}H_{78}N_{10}O_{27}Cl_2$
36		52	97	14.7	1.43	8.0	852	1646	$C_{77}H_{78}N_{10}O_{27}Cl_2$
37		64	89	14.3	1.39	7.6	862	1676	$C_{78}H_{80}N_{10}O_{28}Cl_2$
38	$NH(CH_2)_3N(CH_3)_2$	38	91	14.5	1.41	8.3	839	1648	$C_{77}H_{80}N_{10}O_{27}Cl_2$
39	$NH(CH_2)_3N(C_2H_5)_2$	45	95	14.8	1.44	8.4	846	1677	$C_{79}H_{84}N_{10}O_{27}Cl_2$
40	$NH(CH_2)_3N(n\text{-}C_4H_9)_2$	30	93	20.1	1.95	8.1	870	1733	$C_{83}H_{92}N_{10}O_{27}Cl_2$
TB	OH			10.3	1.00	5.7	1610	1564	$C_{72}H_{68}N_8O_{28}Cl_2$

[a] From TB (B, method a); from the corresponding amide of CTA (C, method f_1). [b] Rel t_R referred to TB, HPLC method B.

also investigated the influence of the sugar moieties on the activity against Gram-negative bacteria by comparing the in vitro activity of corresponding amides of CTA, TB, TC, and TD.

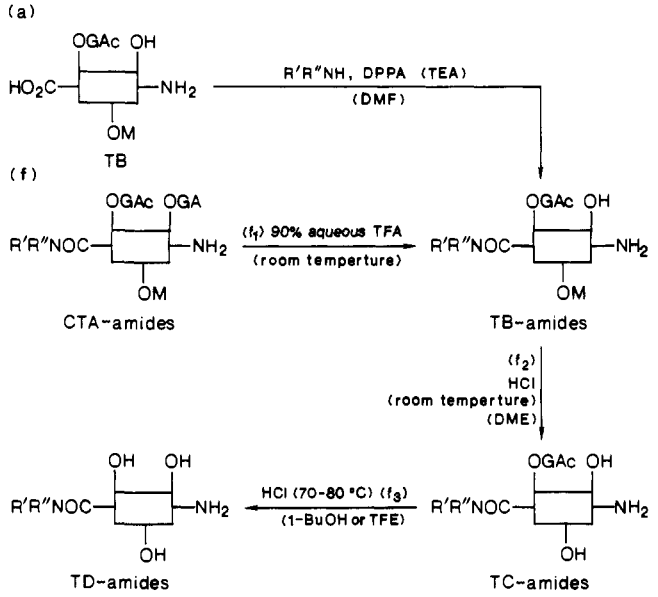
The ability of some representative amides of CTA, TB, TC, and TD to bind to Ac_2-L-Lys-D-Ala-D-Ala, a synthetic analogue of the antibiotic's target peptide, was also determined.

Table III. Amides of TC

compd	NR'R''	yield,[a] % A	yield,[a] % C	HPLC[b] t_R, min	HPLC[b] rel t_R	IEF, pI	titration, EW[c]	MW	formula
41		31	44	16.2	1.41	8.0	750	1484	$C_{71}H_{68}N_{10}O_{22}Cl_2$
42		60	36	16.1	1.40	7.3	763	1514	$C_{72}H_{70}N_{10}C_{23}Cl_2$
43	$NH(CH_2)_3N(CH_3)_2$	22	61	15.5	1.35	7.9	745	1486	$C_{71}H_{70}N_{10}O_{22}Cl_2$
44	$NH(CH_2)_3N(C_2H_5)_2$	38	57	16.9	1.47	8.1	752	1514	$C_{73}H_{74}N_{10}O_{22}Cl_2$
45	$NHCH_2COOCH_3$		80[d]	16.4	1.43	7.2	1521	1473	$C_{69}H_{63}N_9O_{24}Cl_2$
46	$NHCH_2COOC_2H_5$	91		18.3	1.59	7.2	1526	1487	$C_{70}H_{65}N_9O_{24}Cl_2$
TC	OH			11.5	1.00	5.6	1473	1402	$C_{66}H_{58}N_8O_{23}Cl_2$

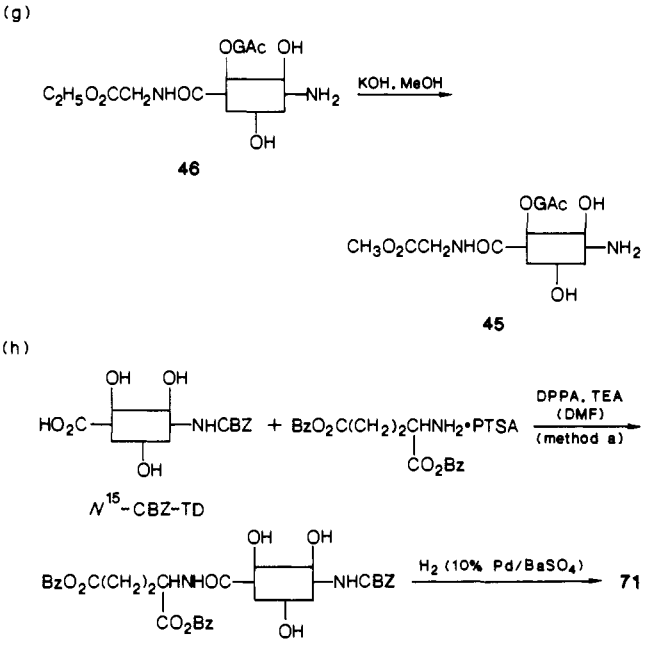
[a] From TC (A, method e); from the corresponding amide of CTA (C, method f_2). [b] Rel t_R referred to TC, HPLC method B. [c] Equivalent weight. [d] From **46**.

Scheme III[a]



[a] GA = *N*-acyl-β-D-glucosaminyl; GAc = *N*-acetyl-β-D-glucosaminyl; M = α-D-mannosyl.

Scheme IV[a]



[a] GAc = *N*-acetyl-β-D-glucosaminyl; Bz = $CH_2C_6H_5$.

Chemistry. The N^{63}-carboxamides described in this paper (Tables I–IV) were synthesized according to different procedures (Schemes I–IV), mostly depending on the characteristics of the starting materials.

The amides of CTA were prepared (method a) by reaction of CTA with a proper amine at room temperature in DMF, in the presence of diphenyl phosphorazidate (DPPA), as the condensing agent, and TEA. Compounds **5**, **31**, and **33** were obtained from the corresponding suitably protected derivatives by removing the protecting groups. In particular, compound **5** was prepared by catalytic (5% Pd/C) hydrogenation of compound **4** at 1 atm and room temperature (method b). Saponification of the ester group of compound **30** to give compound **31** was carried out with potassium carbonate in a heterogeneous hydroalcoholic (1-BuOH/MeOH/H_2O, 4/1/5) mixture at 10–15 °C (method c). These last reaction conditions were critical since concurrent epimerization at C-3[9] might occur with bases when higher temperatures or stronger alkalies are used. Compound **33** was obtained by catalytic (10% Pd/$BaSO_4$) hydrogenolysis (1 atm, 20 °C, method d) of the benzyl groups of the intermediate peptide with glutamic acid dibenzyl ester (**32**). Compounds **5** and **33** are derivatives of factor A2-1 free CTA (CTA'), since the double bond of the acylglucosaminyl side chain of this component of the complex is invariably reduced under hydrogenation conditions to give component A2-3.[10]

The amides of TB, TC, and TD were prepared according to a general procedure (method e) which consisted in the protection of the primary amino group at C-15 as *tert*-butyloxycarbonyl (*t*-BOC) or benzyloxycarbonyl (CBZ).[6] The resulting N^{15}-protected teicoplanin intermediates were then allowed to react with a proper amine in DMF, in the presence of DPPA and TEA. Deprotection of N^{15}-*t*-BOC amides with dry TFA or N^{15}-CBZ derivatives by hydrogenolysis (1 atm, 5% Pd/C) freed the final amides. The amides of TB were also obtained with good yields (method a) without protecting the amino group, thus allowing one-step reactions as described above for CTA-amides. All the amides of TB and the majority of those of TC and TD were alternatively synthesized (method f) by selective removal of the sugars from the corresponding amides of CTA, under proper acidic conditions.[7,8] Transesterification (method g) of compound **46** with methanol, in the presence of potassium hydroxyde, produced derivative **45**. Compound **71** was prepared (method h) by condensation of the amino group of glutamic acid dibenzyl ester *p*-tosylate with the carboxyl function of N^{15}-CBZ-TD according to procedure *e*, followed by the displacement of the three pro-

(9) Barna, J. C. J.; Williams, D. H.; Strazzolini, P.; Malabarba, A.; Leung, T.-W. C. *J. Antibiot.* **1984**, *37*, 1204.

(10) European Patent Appl., Publ. No. 152,902, Aug 1985.

tecting groups by catalytic hydrogenolysis (1 atm, 10% $Pd/BaSO_4$).

The progress of the reactions and the homogeneity of the final products were checked by HPLC. The ratio (rel t_R) between the retention time (t_R) at pH 6.9[11] of each amide of CTA, TB, TC, and TD and that of the corresponding unmodified teicoplanin was used as the relative parameter of lipophilicity vs CTA, TB, TC, and TD, respectively. Though to a different extent, the majority of amides were more lipophilic than the respective teicoplanins at neutral pH.

The values (log P's) of lipid–water partition coefficients, determined at pH 6[12] between 1-butanol[13] and water, indicated that almost all of the products, with the exception of the amide **26** of CTA, the amide **47** of TD, and TD, possess hydrophilic character. It is noteworthy that, at slightly acidic pH, the amides of CTA are still more lipophilic than CTA while those of TD are more hydrophilic than TD and that TD is about twice as soluble in 1-butanol as in water.

Preliminary data[14] of solubility in water showed that these amides are generally more soluble than parent teicoplanins at acidic pH, but less soluble at pH 7, and that the amides of CTA and TB are about 10 times more soluble than the corresponding amides of TC and TD.

The isoelectrofocusing (IEF) technique coupled with bioautography detection gave the isoelectric points (pI's) of the most representative compounds, with values ranging from 3.6 to 8.8.

The ^1H NMR spectra of teicoplanin amides, obtained at 250 MHz, showed that both the amidic and teicoplanin moieties are present. A common feature in TD-amides, which do not contain acetylglucosamine, consists in the downfield shift (~0.2 ppm) of the signal due to the proton at C-34, while the overall pattern of the spectra that resulted was unmodified compared to that of TD.[8] The presence of the acetylglucosamine in CTA, TB, and TC causes a similar (~0.2 ppm) downfield shift of H-34 that is interpreted as due to both inductive and anisotropic effects of the sugar on this proton. In the case of terminal amide groups, an anisotropic effect on H-34, due to a conformational change in the surroundings induced by an hydrogen bond between the amidic NH and the 36-C=O, is suggested. When both the sugar and amide moieties are present, only one of these exerts its deshielding effect on H-34. In this case, the influence of the amide is excluded since the amide group is prevented from assuming the suitable conformation to affect the chemical shift of H-34 by the steric hindrance of the acetylglucosamine. Hence, in order to confirm their structures, some amides of CTA, TB, and TC were transformed into the respective amides of TD under the appropriate acidic conditions described above (method f).

Acid–base titrations and, except for compounds **31**, **33**, and **71**, IR spectra indicated that the original free carboxyl group was modified. The molecular weights of some compounds were also confirmed by FAB mass spectrometry.

Biological Activity. The majority of CTA-amides (Table V) was in vitro at least as active as teicoplanin against *Staphylococcus aureus* Tour and streptococci; they were generally more active than teicoplanin in vivo, in the murine model of *Strep. pyogenes* septicemia, upon subcutaneous administration. Compounds **5**, **13**, **14**, **21**, **22**, **25**, **27**, and **29** were also effective by oral route at doses lower than 100 mg/kg. None of these derivatives was active against Gram-negative bacteria up to 128 μg/mL.

In the preliminary screening, most basic amides of CTA were more active than teicoplanin in vitro against two strains (*Staphylococcus epidermidis* ATCC 12228 and *Staph. hemolyticus* 602) of CNST. In particular, compound **21**, one of the most active derivatives in experimental septicemia, was also tested against a series of clinical isolates of CNST, including MR strains. Data reported in Table VI show that amide **21** was more active than teicoplanin against most of these organisms. Its activity was particularly good (MIC range 1–2 μg/mL) against clinical isolates of *Staph. hemolyticus*, which were only inhibited by relatively high concentrations (up to 32 μg/mL) of teicoplanin.

The amides of TB and TC (Tables VII and VIII) were generally less active than those of CTA and TD (Table IX) against Gram-positive organisms, reflecting the in vitro activities of TB, TC, CTA, and TD. The amides of TB did not show any activity against Gram-negative organisms. Some TC-amides had a modest in vitro activity on *Escherichia coli*, but only one (compound **44**) was also active against *Proteus vulgaris*.

The amides of TD exhibited the most interesting in vitro activity against Gram-negative bacteria. The majority of TD-amides inhibited *E. coli* between 2 and 8 μg/mL, and *P. vulgaris* and *Pseudomonas* Aeruginosa, between 16 and 64 μg/mL. Against Gram-positive organisms, the amides of TD were almost as active as those of CTA.

In experimental *Strep. pyogenes* septicemia in the mouse, the amides of CTA were the most effective upon both oral and subcutaneous administration. Though markedly lower than that of CTA-amides, good in vivo activity was also exhibited by most amides of TB and TD, those of TB generally being less effective. None of the derivatives of TB, TC, and TD was orally active up to 300 mg/kg.

Peptide Binding Studies. In order to verify whether the improvement of the antimicrobial properties of the amides with respect to those of the corresponding unmodified teicoplanins was related to an increased ability to complex with the antibiotic's target peptide D-Ala-D-Ala, we measured their binding to the synthetic analogue Ac_2-L-Lys-D-Ala-D-Ala in comparison with CTA, TB, TC, nd TD. The differential UV assay[15] was used. The values of the association constants (K_a's, Table X), determined at pH 9 for a few representative compounds, show that amide formation does not significantly influence the

(11) For the amides of CTA, HPLC was carried out at pH 4.7. The use of phosphate buffer at pH 6.9 was unsuitable to obtain reproducible chromatograms for most basic derivatives of CTA. Nevertheless, the ratios between the t_R of each amide of CTA and those of the other CTA-amides were approximately the same at both neutral and acidic pHs.

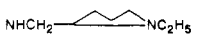
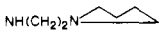
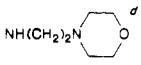
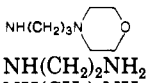
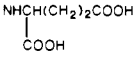
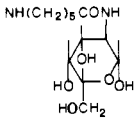
(12) For the amides of CTA and TD, the values of log P determined at pH 7.0 were not reliable, because of the high variability of results obtained under our experimental conditions. This variability was essentially related to the ability of most of these amides to create colloidal dispersions which give rise to gel formation in water at neutral pH. With some of them, the same aggregation phenomena were also observed in 1-butanol at pH 7.0 and at their isoelectric point. In general, CTA- and TD-amides precipitated in the interphase between 1-butanol and water when a 1/1 mixture of these solvents was used. The log P of TB- and TC-amides was not calculated due to the negligible solubility of these compounds in organic solvents at all pHs.

(13) Commonly used organic solvents, such as 1-octanol or isoamyl alcohol, were unsuitable for our purposes, due to the insolubility of most products in these alcohols.

(14) Unpublished data, these laboratories.

(15) Nieto, M.; Perkins, H. R. *Biochem. J.* **1971**, *124*, 845.

Table IV. Amides of TD

compd	NR′R″	yield,[a] % A	yield,[a] % C	HPLC[b] t_R, min	HPLC[b] rel t_R	IEF, pI	log P^c	titration, EW	MW	formula
47		35		6.6	1.49	7.0	+0.1	1253	1268[d]	$C_{62}H_{52}N_8O_{18}Cl_2$
48		26	38	6.1	1.38	7.6		640	1281	$C_{63}H_{55}N_9O_{17}Cl_2$
49		60	43	6.0	1.36	8.1	−0.8	636	1293	$C_{64}H_{55}N_9O_{17}Cl_2$
50		50	49	9.2	2.10	nd[g]		661	1309	$C_{65}H_{50}N_9O_{17}Cl_2$
51		24	47	7.6	1.72	7.8		645	1293	$C_{64}H_{55}N_9O_{17}Cl_2$
52		39		7.3	1.65	7.4		660	1311	$C_{64}H_{57}N_9O_{18}Cl_2$
53		45		7.9	1.80	7.4	−0.8	672	1325	$C_{65}H_{59}N_9O_{18}Cl_2$
54	$NH(CH_2)_2NH_2$	32		7.0	1.59	8.1	−3.0	643	1241	$C_{60}H_{51}N_9O_{17}Cl_2$
55	$NH(CH_2)_3NH_2$	41		7.2	1.63	8.2	−2.4	645	1255	$C_{61}H_{53}N_9O_{17}Cl_2$
56	$NH(CH_2)_4NH_2$	67	32	7.3	1.65	8.3	−1.9	643	1269[d]	$C_{62}H_{55}N_9O_{17}Cl_2$
57	$NH(CH_2)_6NH_2$	81		7.8	1.78	8.3	−1.8	665	1297	$C_{64}H_{59}N_9O_{17}Cl_2$
58	$NH(CH_2)_2NHCH_3$	46		7.6	1.72	7.9	−2.0	656	1255	$C_{61}H_{53}N_9O_{17}Cl_2$
59	$NH(CH_2)_3NHCH_3$	55		7.8	1.77	8.0	−2.0	664	1269	$C_{62}H_{55}N_9O_{17}Cl_2$
60	$NH(CH_2)_2NHC_2H_5$	58		7.7	1.75	7.9		649	1269	$C_{62}H_{55}N_9O_{17}Cl_2$
61	$NH(CH_2)_2N(CH_3)_2$	33	52	7.8	1.77	7.9	−1.9	650	1269	$C_{62}H_{55}N_9O_{17}Cl_2$
62	$NH(CH_2)_3N(CH_3)_2$	28	63	8.0	1.81	8.0	−2.4	638	1283[d]	$C_{63}H_{57}N_9O_{17}Cl_2$
63	$NH(CH_2)_4N(CH_3)_2$	31	57	8.1	1.85	8.1	−2.4	649	1297	$C_{64}H_{59}N_9O_{17}Cl_2$
64	$NH(CH_2)_5N(CH_3)_2$	35	49	8.4	1.91	8.1	−1.8	655	1311	$C_{65}H_{61}N_9O_{17}Cl_2$
65	$NH(CH_2)_7N(CH_3)_2$	60	49	10.9	2.48	nd	−1.5	675	1399	$C_{67}H_{65}N_9O_{17}Cl_2$
66	$NH(CH_2)_3N(C_2H_5)_2$	22	64	8.3	1.89	8.1	−1.4	658	1311[d]	$C_{65}H_{61}N_9O_{17}Cl_2$
67	$NH(CH_2)_3N(n\text{-}C_4H_9)_2$	24	52	10.9	2.48	8.0	−0.2	700	1367	$C_{69}H_{69}N_9O_{17}Cl_2$
68	$N(CH_3)(CH_2)_2NHCH_3$	46		7.8	1.77	8.0		671	1269	$C_{62}H_{5N9}O_{17}Cl_2$
69	$N(CH_3)(CH_2)_3NHCH_3$	54		7.9	1.79	8.1	−1.0	657	1283	$C_{63}H_{57}N_9O_{17}Cl_2$
70	$N(CH_3)(CH_2)_2N(CH_3)_2$	39	51	8.0	1.82	7.9		650	1283	$C_{63}H_{57}N_9O_{17}Cl_2$
71		70[e]		4.1	0.93	3.6		445	1328	$C_{63}H_{52}N_8O_{21}Cl_2$
72[f]		76		5.2	1.19	6.9		744	1473	$C_{70}H_{67}N_9O_{23}Cl_2$
TD	OH			4.4	1.00	5.5	+0.3	1280	1199	$C_{58}H_{45}N_7O_{18}Cl_2$

[a] From TD (A, method e); from the corresponding amide of CTA (C, method f_3). [b] Rel t_R referred to TD, HPLC method C. [c] Partition coefficient determined between 1-BuOH and H_2O at pH 6.0. [d] FAB-MS, $(M + H)^+$: 1269, compound **47**; 1270, **56**; 1284, **62**; 1312, **66**. [e] From N^{15}-CBZ-TD (method h). [f] Obtained by condensation (method e) with the N_ϵ-aminocaproyl-β-D-galactopyranosylamine (Sigma Chemical Co.). [g] nd = not determined.

binding strength of teicoplanin antibiotics. In fact, though small differences can be seen in favor of most amides, they are not such as to indicate a stronger binding.

Conclusions

The conversion of the carboxyl group into an amide does not affect the ability of teicoplanin antibiotics to bind to Ac_2-L-Lys-D-Ala-D-Ala but generally improves the antimicrobial activity and in vivo efficacy of the resulting teicoplanin amides to a different extent, depending on their ionic and lipophilic characters and on the number and structure of sugars present.

In general, the combined effect of a moderate basicity (p*I* 7.9–8.8) and a slightly increased (vs the respective unmodified teicoplanins) lipophilicity at neutral pH has a positive influence on the in vitro activity, in particular, of CTA-amides against CNST, that is likely related to an increased penetrability through the bacterial cell wall.

The activity against Gram-negative bacteria is inversely correlated with the number of sugars in the molecule. In fact, sugar-free TD-amides are the most active teicoplanin derivatives against these organisms, while compounds bearing one sugar (TC-amides and TD-amide **72**) are only slightly active. When two or more sugars are present (TB- and CTA-amides), the activity is absent.

The most effective compounds in the experimental *Strep. pyogenes* septicemia in the mouse are some CTA-amides slightly more lipophilic than CTA and with p*I* 8.3–8.5. Though still poorly absorbed by oral route, resulting from the ratio between the values of oral and subcutaneous ED_{50}, most of them are even somewhat active orally. The majority of the basic amides of TB and TD also possesses a good in vivo efficacy when administered subcutaneously, but it is lower than that of the corresponding amides of CTA.

The higher in vivo activity against *Strep. pyogenes* of the amides of CTA, compared to that of the corresponding amides of TB, TC, and TD, is likely due to the concomitant positive effects of the *N*-acylglucosamine and mannose.

The positive influence of mannose is evident by comparing the in vivo activity of the amides of TB with that of parent amides of TC and seems to be strictly related to the higher hydrosolubility of TB-amides at neutral pH. The presence of the *N*-acetylglucosamine has less effect on the solubility in water, since TC-amides are only slightly more soluble than the corresponding amides of TD. In this case, the higher in vivo activity of the amides of TD may

Table V. In Vitro (MIC) and in Vivo (ED_{50}, Mice Infected with *Strep. pyogenes* C 203) Activity

compd	MIC, μg/mL: *Staph. aureus* Tour	*Staph. epidermidis* ATCC 12228	*Staph. hemolyticus* 602[a]	*Strep. pyogenes* C 203	*Strep. pneumoniae* UC 41	*Strep. faecalis* ATCC 7080	ED_{50}, mg/kg: po	sc
1	0.12	0.25	nd[d]	0.06	0.06	0.12	139	0.08
2	0.12	0.25	nd	0.06	0.12	0.12	220	0.08
3	0.12	0.12	nd	0.06	0.12	0.06	115	<0.03
4	0.12	0.12	4	0.06	0.06	0.12	>300	0.08
5	0.12	0.12	1	0.06	0.06	0.12	89.6	0.08
6	0.12	0.12	nd	0.06	0.06	0.12	173	0.07
7	0.12	0.12	8	0.06	0.06	0.12	139	0.08
8	0.12	0.06	0.12	0.06	0.06	0.12	140	0.10
9	0.12	0.12	nd	0.06	0.12	0.12	173	0.06
10	0.12	0.12	nd	0.06	0.12	0.12	300	0.08
11	0.12	0.12	0.5	0.06	0.06	0.12	>300	0.18
12	0.12	0.06	0.12	0.06	0.12	0.12	139	0.18
13	0.12	0.06	0.12	0.03	0.12	0.12	89.6	0.08
14	0.12	0.06	0.12	0.06	0.12	0.12	90	0.07
15	0.12	0.06	0.12	0.06	0.12	0.12	>300	0.14
16	0.12	0.12	0.12	0.06	0.12	0.12	173	0.08
17	0.12	0.06	0.12	0.06	0.12	0.12	112	0.14
18	0.12	0.06	0.12	0.06	0.12	0.12	300	0.18
19	0.12	0.06	0.12	0.06	0.12	0.12	112	0.10
20	0.12	0.06	0.12	0.06	0.06	0.12	140	0.09
21	0.12	0.06	0.12	0.06	0.06	0.12	70.7	0.05
22	0.12	0.06	0.25	0.06	0.12	0.12	90	0.10
23	0.12	0.12	0.5	0.06	0.06	0.12	112	0.12
24	0.12	0.12	0.5	0.06	0.12	0.12	>300	0.18
25[b]	0.12 (0.25)	0.12 (0.12)	0.12 (nd)	0.06 (0.06)	0.06 (0.12)	0.12 (0.06)	90 (58)	0.05 (0.10)
26	0.25	0.06	nd	0.06	0.06	0.12	300	0.10
27	0.12	0.06	1	0.06	0.12	0.12	72	0.10
28	0.12	0.06	0.25	0.06	0.12	0.12	>300	0.13
29	0.12	0.06	0.25	0.06	0.06	0.06	90	0.15
30	0.25	0.12	nd	0.06	0.06	0.12	>300	0.10
31	0.12	0.12	nd	0.12	0.12	0.25	>300	0.23
33	0.5	0.12	4	0.06	0.12	0.25	nd	nd
teicoplanin	0.12	0.25	4–8[c]	0.06	0.06	0.12	170–300[c]	0.12

[a] Clinical isolate. [b] Values between brackets refer to single component A2-2 which was separated from "complex" compound **25** by reverse-phase column chromatography. [c] Range of values found for teicoplanin in several experiments. [d] nd = not determined.

Table VI. In Vitro Activity of Compound **21** against 36 Clinical Isolates of CNST, Including 23 MET-R Strains, in Comparison with Teicoplanin

CNST (no. of strains)	compd	MIC range, μg/mL	MIC_{50}, μg/mL	MIC_{90}, μg/mL
Staph. epidermidis (12)	**21**	0.125–1	0.25	1
	teicoplanin	0.25–2	0.5	2
Staph. hemolyticus (8)	**21**	1–2	1	2
	teicoplanin	2–32	16	32
others[a] (16)	**21**	0.25–1	0.5	1
	teicoplanin	0.25–4	1	4

[a] Including *Staph. saprophyticus* (4), *Staph. hominis* (3), *Staph. capitis* (2), *Staph. cohnii* (2), *Staph. simulans* (2), *Staph. warneri* (2), and *Staph. xylosus* (1).

be ascribed to their relatively higher lipophilic character.

On the basis of these findings, it is quite difficult to establish which parameter, between hydrosolubility and lipophilicity, plays the leading role for the in vivo activity, also considering that structurally related amides of TB and TD possess comparable efficacy in experimental *Strep. pyogenes* septicemia in the mouse.

The best compromise between hydrosolubility and lipophilicity is obtained with CTA-amides which benefit by the presence of the three sugars, in particular, mannose and *N*-acylglucosamine. The former only contributes to the hydrosolubility of these compounds while the long aliphatic chain of the latter is responsible for their lipophilicity. Also, the *N*-acetyl- and acylglucosamine probably contribute to the solubility in water but certainly less than mannose. For these reasons, the amides of CTA, nearly as soluble as TB-amides, are more soluble in water than those of TC and TD, and although still hydrophilic at the physiological pH, they are the most lipophilic teicoplanin amides.

Hydrosolubility and lipophilicity more than likely promote the drug absorption and distribution in animals and may justify the higher in vivo efficacy of CTA-amides that is also probably related to a longer half-life. In fact, it is known that a strong correlation exists between physicochemical and pharmacokinetic properties of the glyco-

Table VII. In Vitro (MIC) and in Vivo (ED_{50}, Mice Infected with *Strep. pyogenes* C 203) Activity of TB-Amides

compd	MIC, μg/mL: *Staph. aureus* Tour	*Staph. epidermidis* ATCC 12228	*Strep. pyogenes* C 203	*Strep. pneumoniae* UC 41	*Strep. faecalis* ATCC 7080	Gram⁻	ED_{50}, mg/kg sc
34	0.5	0.12	0.5	0.5	2	>128	1.6
35	0.25	0.12	0.5	1	2	>128	0.95
36	0.25	0.25	0.12	0.5	0.5	>128	0.41
37	0.25	0.06	0.12	0.5	1	>128	1.6
38	0.5	0.12	0.12	0.5	1	>128	0.81
39	0.25	0.06	0.12	0.5	0.5	>128	0.3
40	1	0.06	0.12	0.5	0.5	>128	0.18
TB	0.25	0.25	0.5	0.5	2	>128	2.64

Table VIII. In Vitro (MIC) and in Vivo (ED_{50}, Mice Infected with *Strep. pyogenes* C 203) Activity of TC-Amides[a]

	MIC, μg/mL							
compd	*Staph. aureus* Tour	*Staph. epidermidis* ATCC 12228	*Strep. pyogenes* C 203	*Strep. pneumoniae* UC 41	*Strep. faecalis* ATCC 7080	*E. coli* SKF 12140	*P. vulgaris* X 19 H[b]	ED_{50}, mg/kg sc
41	0.12	0.06	0.25	0.25	0.5	128	>128	2.2
42	0.12	0.12	0.5	1	0.5	>128	>128	nd[c]
43	0.12	0.06	0.12	0.25	0.25	64	>128	2.2
44	0.12	0.06	0.12	0.5	0.25	64	128	0.95
45	0.25	0.06	0.25	2	0.5	>128	>128	7
46	0.5	0.12	0.25	1	1	128	>128	5
TC	0.5	0.12	0.5	1	1	>128	>128	2.46

[a] None of TC amides was active against *Pseudomonas aeruginosa* ATCC 10145 up to 128 μg/mL. [b] ATCC 881. [c] nd = not determined.

Table IX. In Vitro (MIC) and in Vivo (ED_{50}, Mice Infected with *Strep. pyogenes* C 203) Activity of TD-Amides

	MIC, μg/mL									
compd	*Staph. aureus* Tour	*Staph. epidermidis* ATCC 12228	*Staph. hemolyticus* 602[a]	*Staph. pyogenes* C 203	*Staph. pneumoniae* UC 41	*Staph. faecalis* ATCC 7080	*E. coli* SKF 12140	*Proteus vulgaris* ATCC 881	*P. aeruginosa* ATCC 10145	ED_{50}, mg/kg sc
47	0.06	0.016	nd[b]	0.12	0.12	0.12	16	64	64	1.02
48	0.06	0.03	nd	0.12	0.12	0.12	16	64	32	0.95
49	0.12	0.06	nd	0.06	0.06	0.12	8	64	32	0.46
50	0.06	0.06	0.12	0.06	0.06	0.12	4	32	64	1.25
51	0.06	0.016	nd	0.06	0.12	0.12	8	64	64	1.6
52	0.06	0.016	nd	0.12	0.12	0.12	16	128	64	2.2
53	0.06	0.06	0.12	0.06	0.12	0.12	8	64	32	1.4
54	0.06	0.03	0.12	0.06	0.12	0.12	4	16	16	1.25
55	0.12	0.06	0.12	0.06	0.12	0.12	4	32	32	1.25
56	0.06	0.06	0.12	0.06	0.12	0.12	8	32	32	0.54
57	0.06	0.06	0.12	0.06	0.06	0.12	4	64	64	0.54
58	0.06	0.03	0.06	0.06	0.12	0.12	4	32	32	2.2
59	0.06	0.06	0.25	0.06	0.12	0.12	4	16	16	0.95
60	0.06	0.06	0.12	0.06	0.12	0.12	8	64	32	1.25
61	0.06	0.06	0.06	0.06	0.12	0.12	4	32	16	1.25
62	0.06	0.016	nd	0.06	0.12	0.12	8	16	32	0.31
63	0.06	0.06	0.12	0.06	0.12	0.12	8	32	32	0.54
64	0.06	0.06	0.12	0.06	0.12	0.12	8	128	64	0.72
65	0.06	0.06	0.12	0.06	0.12	0.12	16	128	128	0.95
66	0.12	0.03	nd	0.06	0.12	0.12	8	32	32	0.18
67	0.12	0.06	nd	0.06	0.12	0.12	8	32	64	0.72
68	0.12	0.06	0.12	0.12	0.12	0.12	16	>128	64	1.25
69	0.06	0.06	0.06	0.06	0.12	0.12	2	32	16	0.95
70	0.06	0.06	0.06	0.06	0.06	0.12	8	64	64	1.25
71	0.12	0.12	nd	0.12	0.12	0.5	>128	>128	>128	5
72	0.12	0.06	nd	0.12	0.25	0.25	64	>128	>128	2.9
TD	0.06	0.016	0.25	0.12	0.12	0.12	64	128	>128	0.95

[a] Clinical isolate. [b] nd = not determined.

Table X. Association Constants with Ac_2-L-Lys-D-Ala-D-Ala

compd	K_a, M^{-1}	compd	K_a, M^{-1}
13	6.5×10^4	**42**	3.4×10^4
17	6.6×10^4	**43**	5.4×10^4
20	8.3×10^4	TC	3.2×10^4
21	2.5×10^5	**55**	2.6×10^4
30	1.2×10^5	**62**	5.5×10^4
31	7.4×10^4	**65**	5.0×10^4
33	4.6×10^5	**71**	8.2×10^4
CTA	2.1×10^5	TD	3.0×10^4
34	1.2×10^5		
35	1.0×10^5		
TB	0.9×10^5		

peptide antibiotics.[16] In particular, for compounds that have identical p*I*'s, the half-life increases with increasing lipophilicity.

Experimental Section

IR spectra (Nujol) were recorded on a Perkin-Elmer 580 spectrometer.

^1H NMR spectra were recorded on a Bruker WH-250 spectrometer at 250 MHz, with Me_4Si (δ 0.00 ppm) as internal reference.

Isoelectrofocusing (IEF) was performed on slabs of 24.5 × 11.5 cm and 1-mm thickness prepared on a sheet of Gel Fix (Serva Feinbiochemica), using an LKB Multiphor 2117 cell and a Bio-Rad power supply, Model 1420 A.

Acid–base titrations were carried out under the following conditions: the sample was dissolved in MCS/H_2O (4/1). After addition of an excess of 0.01 M HCl in the same solvent mixture, the resulting solution was titrated with 0.01 N NaOH.

The products were purified by reverse-phase column chromatography on silanized silica gel (0.063–0.2 mm; Merck). Reactions, column eluates, and final products were checked by HPLC analyses, which were performed on a column Hibar (120 × 4.5 mm; Merck) prepacked with LiChrosorb RP-8 (5 μm), using a Hewlett-Packard Model 1084 automatic apparatus. Elutions were carried out according to one of the following methods, depending on the structure of the starting teicoplanin antibiotic. In method A (CTA derivatives), a linear step gradient from 8% to 40% of 0.02 M aqueous NaH_2PO_4/CH_3CN (25/75) in 0.02 M aqueous NaH_2PO_4/CH_3CN (95/5),[11] in 40 min at the rate of 1 mL/min, was used. In methods B (TB- and TC-amides) and C (TD-amides), eluent a, 0.02 M aqueous Na_2HPO_4 buffer adjusted at pH 6.9 with 1 N H_3PO_4, and eluent b, CH_3CN, were used. The flow rate was 2 mL/min. Linear step gradients were programmed as follows:

min:	0	10	25	30
method B, % of eluent b in eluent a:	6	22	30	6
method C, % of eluent b in eluent a:	20	40	60	20

(16) Pitkin, D. H.; Mico, B. A.; Sitrin, R. D.; Nisbet, L. J. *Antimicrob. Agents Chemother.* **1986**, *29*, 440.

Chromatograms were recorded by using CTA factor A2-2, TB, TC, and TD as internal references for obtaining the relative t_R values of the respective amides.

Partition coefficients, expressed as log P's, were obtained from the maximum solubilities of each compound in 1-BuOH and in water. Samples were prepared by dissolving the products (2.5 mg/mL) in 0.01 M aqueous NaH_2PO_4 at pH 6 and subsequent freeze-drying of the resulting solutions. The maximum solubilities were determined by the internal standard HPLC method on the saturated solutions at room temperature.

All derivatives were analyzed for C, H, N, and Cl, on samples previously dried at 140 °C under N_2 atmosphere. Weight loss was determined by thermogravimetry (TG), at 140 °C. Inorganic residue was determined after heating the samples at 900 °C in O_2 atmosphere. The analytical results obtained for C, H, N, and Cl were within ±0.4% of the theoretical values. Solvent content (in general H_2O, with traces of 1-BuOH) and inorganic residue were always less than 10% and 0.3%, respectively.

When teicoplanin[1] was used as starting material instead of purified CTA, the composition was the following: CTA 80%, TB 10%,[1] and H_2O 10%. The composition of CTA and of its amide derivatives, expressed as percentages of the areas of peaks (HPLC) for each component of the complex, was approximately as follows: factor T-A2-1 10%, factor T-A2-2 50%, factor T-A2-3 15%, factor T-A2-4 12%, and factor T-A2-5 13%.

Amides of CTA (General Procedure, Method a). A solution of 1.1 mmol of DPPA in 5 mL of DMF was added dropwise at 0–5 °C to a stirred solution of 1 mmol of teicoplanin (or CTA) and 2 mmol of the appropriate amine[17] in 20 mL of DMF. The reaction mixture was stirred at 5 °C for 6 h and at room temperature overnight; afterward, a solution of 0.5 mmol of DPPA in 2.5 mL of DMF was added and stirring was continued at room temperature for additional 8 h. After 125 mL of EtOAc was added, the precipitated solid was collected and chromatographed on 50 g of silanized silica gel. Elution was performed with a linear gradient from 10% to 80% of CH_3CN in 0.05 N AcOH, in 15 h at the rate of 200 mL/h, while collecting 20-mL fractions. The appropriate ones were combined to obtain a solution of the final complex amide with the predetermined (see above) composition in its five components. After adjusting at pH 8.6 with 1 N NaOH, the resulting solution was concentrated to a small volume at 30 °C under reduced pressure and the precipitated solid was collected, washed with H_2O, and dried in the air at room temperature overnight to yield CTA-amides, as the free bases (Table I).

N^{63}-[1-(Phenylmethyl)-4-piperidinyl]teicoplanin A2 Amide (4). To a stirred solution of 15 g (~8 mmol) of CTA in 150 mL of DMF, 2.1 mL (~15 mmol) of TEA and 2 mL (~9.8 mmol) of 4-amino-1-benzylpiperidine were added followed by 2.1 mL (~9.7 mmol) of DPPA, while cooling at 5 °C. After 6 h, further 1.05 mL (~4.9 mmol) of DPPA was added and the reaction mixture was stirred at room temperature overnight; afterward, 1 L of 1-BuOH was added. The resulting solution was extracted with H_2O (3 × 500 mL), and the organic layer was concentrated at 40 °C in vacuo to a small volume. On addition of Et_2O, the precipitated solid (~10 g) was collected and chromatographed on a column of 500 g of silanized silica gel, as described above, to yield 8.9 g (53%) of compound 4.

N^{63}-(4-Piperidinyl)teicoplanin A2′ Amide (5). A solution of 2.1 g (~1 mmol) of compound 4 in 150 mL of a MeOH/0.04 N HCl (7/3) mixture was hydrogenated at 1 atm and room temperature over 2 g of 5% Pd/C. About 320 mL of H_2 was absorbed within 2 h. The catalyst was filtered off, the filtrated solution was adjusted at pH 8.3 with 1 N NaOH, and then it was concentrated at 35 °C in vacuo to a small volume. The precipitated solid was collected by centrifugation, washed with 25 mL of H_2O, and filtered, yielding 1.8 g (90%) of compound 5.

N^{63}-Amide of CTA with Glycine Ethyl Ester (30). To a stirred solution of 9.5 g (~5 mmol) of CTA in 100 mL of DMF were added 1.6 mL (~11 mmol) of TEA, 0.77 g (~5.5 mmol) of glycine ethyl ester hydrochloride, and 1.35 mL (~6.3 mmol) of DPPA in the order given at 0–5 °C. After stirring at room temperature overnight, 400 mL of EtOAc was added and the precipitated solid was collected and purified on a column of 500 g of silanized silica gel, as described above, to give 8 g (80%) of the title compound.

N^{63}-Amide of CTA with Glycine (31). To a stirred solution of 2 g (~1 mmol) of compound 30 in 150 mL of a H_2O/MeOH (5/1) mixture were added 100 mL of 1-BuOH and 2 g of K_2CO_3 at room temperature. Stirring was continued vigorously at 10–15 °C for 6 h, and then the aqueous phase was separated, adjusted at pH 3.1 with 1 N HCl, and extracted with 100 mL of 1-BuOH. The organic layer was washed twice with H_2O (2 × 50 mL) and concentrated at 45 °C under reduced pressure. On addition of EtOAc, a solid separated, which was collected, washed with Et_2O, and dried in the air at room temperature overnight, to yield 1.6 g (~80%) of compound 31.

N^{63}-Amide of CTA′ with D,L-Glutamic Acid (33). A solution of 30 g (~15 mmol) of teicoplanin, 8.25 g (16.5 mmol) of D,L-glutamic acid dibenzyl ester p-tosylate, and 4.5 mL (~32 mmol) of TEA in 300 mL of DMF was stirred at 0–5 °C while adding 3.75 mL (~17.4 mmol) of DPPA. After 20 h at room temperature, 700 mL of EtOAc was added and the precipitated solid was collected (~41 g, as crude compound 32, i.e., the dibenzyl ester of compound 33) and redissolved in 1.8 L of a MeOH/0.04 N HCl (7/3) mixture. The resulting solution was hydrogenated at 1 atm and room temperature in the presence of 25 g of 10% Pd/$BaSO_4$, while absorbing 1.2 L of H_2 in 4 h. The catalyst was filtered off, and the filtrated solution was adjusted at pH 6 with 1 N NaOH; then most of the methanol was evaporated at 30 °C in vacuo. The resulting aqueous solution was loaded at the top of a column of 1.5 kg of silanized silica gel which was developed as previously described. Fractions containing compound 33 were pooled, and enough 1-BuOH was added to obtain, after concentration of the resulting solution at 40 °C in vacuo to a small volume, a dry butanolic suspension. On addition of 3 volumes of Et_2O, a solid separated which was collected and dried at 35 °C in vacuo overnight, to yield 18 g (58%) of the title compound.

Amides of TB. Procedure a. A solution of 1.5 mmol of DPPA in 5 mL of DMF was added dropwise at 0–5 °C to a stirred solution of 1 mmol of TB and of 2 mmol of the proper amine[17] in 25 mL of DMF. The reaction mixture was stirred at 5 °C for 6–8 h and at room temperature overnight. On addition of 170 mL of EtOAc, the precipitated solid was collected and chromatographed on 100 g of silanized silica gel by eluting with a linear gradient from 1% to 30% of CH_3CN in H_2O, in 15 h at the rate of 150 mL/h, while collecting 10-mL fractions. Those containing pure amides of TB were combined, and solvents were evaporated at 45 °C under reduced pressure (in the presence of 1-BuOH to avoid foaming). The solid residue was collected, washed with 100 mL of Et_2O, and dried in vacuo at room temperature overnight. The title compounds were thus obtained as the free bases (Table II).

Procedure f_1. A solution of 1 mmol of a selected amide of CTA in 200 mL of 90% aqueous TFA was stirred at room temperature for 2 h, and then the solvent was evaporated at room temperature under reduced pressure. The oily residue was dissolved in H_2O, and the resulting solution was adjusted at pH 8 with 1 N NaOH. Purification by column chromatography, carried out as described above, yielded the amide of TB.

Amides of TC. Procedure e. A solution of 1.5 g (1 mmol) of N^{15}-t-BOC-TC,[6] 2 mmol of the appropriate amine,[17] and 1.2 mmol of DPPA in 20 mL of DMF was stirred at room temperature overnight. On addition of 100 mL of EtOAc, the precipitated solid was collected and redissolved in 50 mL of dry TFA. The solvent was evaporated at 30 °C under reduced pressure, and the oily residue was redissolved in a minimum amount of H_2O. The resulting solution was loaded on a column of 100 g of silanized silica gel in H_2O. The column was eluted with a linear gradient from 5% to 35% of CH_3CN in H_2O, in 20 h at the rate of 100 mL/h, while collecting 10-mL fractions. Those containing pure amides of TC were pooled, and the resulting solution was adjusted at pH 8.5 with 1 N NaOH. By concentration at 35 °C under reduced pressure, a solid separated, which was collected, washed with 10 mL of H_2O, and dried in vacuo at 30 °C overnight, to yield the title compounds, as the free bases (Table III).

Procedure f_2. A suspension of 1 mmol of a selected amide of CTA (or of TB) in 50 mL of dimethoxyethane (DME) was

(17) Alternatively, 1.2 mmol of reactant amine and 1 mmol of TEA can be used. When acid addition salts of the reactant amine were used, a suitable amount of TEA was required to free the proper amino group to be condensed with the carboxyl function of teicoplanin antibiotics.

stirred for 12–48 h while bubbling in dry HCl at room temperature; afterward, it was poured into 250 mL of Et_2O. The precipitated solid was collected and purified by reverse-phase column chromatography to give the product.

Procedure g. N^{63}-Amide of TC with Glycine Methyl Ester (45). To a stirred solution of 1.05 g (~0.7 mmol) of amide **46** in 60 mL of MeOH was added dropwise 6 mL of 1 M methanolic KOH at room temperature in 1 h. After 30 min, the reaction mixture was diluted with 200 mL of H_2O, neutralized with 1 N HCl, and concentrated to a small volume (~20 mL) at 30 °C under reduced pressure. A solid separated which was collected, washed with 10 mL of H_2O, and dried in vacuo at 40 °C overnight, to yield 0.84 g (80%) of the title compound. The mother liquors contained TC (as shown by HPLC analysis in comparison with an authentic sample), as the only byproduct of the reaction.

Amides of TD. Procedure e. A solution of 1.3 g (1 mmol) of N^{15}-*t*-BOC-TD,[6] 2 mmol of the appropriate amine,[17] and 1.4 mmol of DPPA in 25 mL of DMF was stirred at 0–5 °C for 8 h and at room temperature overnight. On addition of 150 mL of Et_2O, a solid separated which was collected and redissolved in 25 mL of dry TFA. After 10–15 min, the solvent was evaporated at 40 °C under reduced pressure and the oily residue was dissolved in the minimum amount of CH_3CN/H_2O (1/9). The resulting solution was loaded on a column of 60 g of silanized silica gel in H_2O. The column was developed with a linear gradient from 5% of CH_3CN in H_2O to 60% of CH_3CN in 0.001 N HCl, in 10 h at a flow rate of 150 mL/h, while collecting 15-mL fractions. Those fractions containing pure amides of TD were pooled and worked up as described above for the recovery of TC amides, thus obtaining the title compounds, as the free bases (Table IV).

Procedure f_3. A suspension of 1 mmol of a selected amide of CTA (or of TB or TC) in 100 mL of 2–3 M butanolic HCl[18] was stirred at 70–75 °C for 6–12 h; then the solvent was evaporated and the residue was purified by reverse-phase column chromatography to give the product.

Procedure h. N^{63}-Amide of TD with Glutamic Acid (71). A solution of 3.1 g (~11 mmol) of DPPA in 10 mL of DMF was added dropwise, in 30 min, to a stirred solution of 13.5 g (~10 mmol) of N^{15}-CBZ-TD,[6] 3 mL (~22 mmol) of TEA, and 6.0 g (~12 mmol) of D,L-glutamic acid dibenzyl ester *p*-tosylate in 240 mL of DMF, while cooling at 4–7 °C. Stirring was continued at 10 °C for 3 h and at room temperature overnight; afterward, 800 mL of Et_2O was added. The precipitated solid was collected and redissolved in 600 mL of a MeOH/0.04 N HCl (7/3) mixture. The resulting solution was hydrogenated (1 atm, room temperature) over 10 g of 10% $Pd/BaSO_4$, while absorbing 1 L of H_2 in 6 h. The catalyst was filtered off, and 500 mL of 1-BuOH was added to the filtered solution. The solvents were evaporated at 35 °C under reduced pressure, and the solid residue was dissolved in 350 mL of a CH_3CN/H_2O (2/8) mixture. The resulting solution was loaded on a column of 750 g of silanized silica gel. The column was developed with a linear gradient from 20% to 40% of CH_3CN in H_2O in 10 h, at the rate of 250 mL/h, while collecting 20-mL fractions. Those containing pure title compound were pooled, and the solvents were evaporated at 40 °C in vacuo. The solid residue was collected and dried in vacuo at 45 °C overnight, to give 9.5 g (70%) of compound **71**, as the internal salt.

Microbiological Activity Determination. Antibacterial activity expressed as MIC (minimal inhibitory concentration in μg/mL) was determined by a microdilution method in Difco Todd–Hewitt broth (streptococci) or Oxoid Iso-Sensitest broth (staphylococci and Gram-negative organisms). Iso-Sensitest agar was used with the clinical isolates of CNST. The final inoculum was about 10^4 cfu/mL. MIC was read as the lowest concentration that showed no visible growth after 18–24-h incubation at 37 °C.

Experimental septicemia was induced in five mice groups (five mice/treatment) by intraperitoneal injection of about 10^5 cells of *Strep. pyogenes* C 203, a challenge corresponding to about 100 times the lethal dose for 50% infected animals. Mice were treated once immediately after infection by subcutaneous (sc) or oral (po) route. On the 7th day, the ED_{50} (effective dose, expressed in mg/kg, for 50% infected animals) was calculated[19] on the basis of the percentage of surviving mice at each dose.

Binding Assays. The interaction of Ac_2-L-Lys-D-Ala-D-Ala with CTA, TB, TC, TD, and a few selected amides was determined by UV differential spectroscopy.[15] Experiments were run on a Perkin-Elmer 320 double-beam spectrophotometer with 4-cm path length not thermostated cells. The temperature was 24 ± 2 °C. The initial volume of antibiotic solution was 10 mL at 30 μM concentration in 10% MeOH in sodium phosphate buffer (pH 9). The difference in absorbance (ΔA) developed on addition of the test peptide was monitored at the wavelength (294 nm) that showed the maximum change. The association constant (K_a) for complex formation of each derivative was obtained from the slope of the straight line resulting from a Scatchard plot, $\Delta A/(\Delta A_{max})(C)$ vs $\Delta A/\Delta A_{max}$, of the data. Binding constants were obtained with a standard deviation of about 20%.[20]

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Registry No. 1 (A2-1), 122173-13-1; 1 (A2-2), 122173-14-2; 1 (A2-3), 122172-66-1; 1 (A2-4), 122188-92-5; 1 (A2-5), 122173-15-3; **2** (A2-1), 122188-93-6; **2** (A2-2), 122173-16-4; **2** (A2-3), 122172-67-2; **2** (A2-4), 122188-94-7; **2** (A2-5), 122173-17-5; **3** (A2-1), 122173-18-6; **3** (A2-2), 122173-19-7; **3** (A2-3), 122172-69-6; **3** (A2-4), 122173-20-0; **3** (A2-5), 122173-21-1; **4** (A2-1), 122188-95-8; **4** (A2-2), 122212-45-7; **4** (A2-3), 122188-70-9; **4** (A2-4), 122212-46-8; **4** (A2-5), 122212-47-9; **5** (A2-2), 122188-96-9; **5** (A2-3), 122188-71-0; **5** (A2-4), 122188-97-0; **5** (A2-5), 122188-98-1; **6** (A2-1), 122188-99-2; **6** (A2-2), 122189-00-8; **6** (A2-3), 122172-68-3; **6** (A2-4), 122189-01-9; **6** (A2-5), 122207-65-2; **7** (A2-1), 122173-22-2; **7** (A2-2), 122173-23-3; **7** (A2-3), 122188-72-1; **7** (A2-4), 122189-02-0; **7** (A2-5), 122189-03-1; **8** (A2-1), 122173-24-4; **8** (A2-2), 122173-25-5; **8** (A2-3), 122172-69-4; **8** (A2-4), 122173-26-6; **8** (A2-5), 122173-27-7; **9** (A2-1), 122189-04-2; **9** (A2-2), 122173-28-8; **9** (A2-3), 122172-70-7; **9** (A2-4), 122173-29-9; **9** (A2-5), 122189-05-3; **10** (A2-1), 122173-30-2; **10** (A2-2), 122173-31-3; **10** (A2-3), 122172-71-8; **10** (A2-4), 122173-32-4; **10** (A2-5), 122173-33-5; **11** (A2-1), 122173-34-6; **11** (A2-2), 122173-35-7; **11** (A2-3), 122172-72-9; **11** (A2-4), 122173-36-8; **11** (A2-5), 122173-37-9; **12** (A2-1), 122173-38-0; **12** (A2-2), 122173-39-1; **12** (A2-3), 122173-73-0; **12** (A2-4), 122173-40-4; **12** (A2-5), 122173-41-5; **13** (A2-1), 122173-42-6; **13** (A2-2), 122173-43-7; **13** (A2-3), 122172-74-1; **13** (A2-4), 122173-44-8; **13** (A2-5), 122173-45-9; **14** (A2-1), 122173-46-0; **14** (A2-2), 122173-47-1; **14** (A2-3), 122188-73-2; **14** (A2-4), 122173-48-2; **14** (A2-5), 122173-49-3; **15** (A2-1), 122173-50-6; **15** (A2-2), 122173-51-7; **15** (A2-3), 122172-75-2; **15** (A2-4), 122173-52-8; **15** (A2-5), 122173-53-9; **16** (A2-1), 122173-54-0; **16** (A2-2), 122173-55-1; **16** (A2-3), 122172-76-3; **16** (A2-4), 122173-56-2; **16** (A2-5), 122212-48-0; **17** (A2-1), 122173-57-3; **17** (A2-2), 122173-58-4; **17** (A2-3), 122172-77-4; **17** (A2-4), 122173-59-5; **17** (A2-5), 122173-60-8; **18** (A2-1), 122173-61-9; **18** (A2-2), 122173-62-0; **18** (A2-3), 122172-78-5; **18** (A2-4), 122173-63-1; **18** (A2-5), 122173-64-2; **19** (A2-1), 122173-65-3; **19** (A2-2), 122173-66-4; **19** (A2-3), 122172-79-6; **19** (A2-4), 122173-67-5; **19** (A2-5), 122173-68-6; **20** (A2-1), 122173-69-7; **20** (A2-2), 122173-70-0; **20** (A2-3), 122172-80-9; **20** (A2-4), 122173-71-1; **20** (A2-5), 122173-72-2; **21** (A2-1), 122173-73-3; **21** (A2-2), 122173-74-4; **21** (A2-3), 122172-81-0; **21** (A2-4), 122173-75-5; **21** (A2-5), 122173-76-6; **22** (A2-1), 122173-77-7; **22** (A2-2), 122173-78-8; **22** (A2-3), 122188-74-3; **22** (A2-4), 122173-79-9; **22** (A2-5), 122173-80-2; **22** (A2-1), 122173-81-3; **23** (A2-2), 122212-49-1; **23** (A2-3), 122172-82-1; **23** (A2-4), 122173-82-4; **23** (A2-5), 122173-83-5; **24** (A2-1), 122173-84-6; **24** (A2-2), 122173-85-7; **24** (A2-3), 122172-83-2; **24** (A2-4), 122173-86-8; **25** (A2-5), 122173-87-9; **25** (A2-1), 122173-88-0; **25** (A2-2), 122173-89-1; **25**

(18) When the starting amide contained free carboxyl groups, the reaction was carried out in suspension in 2,2,2-trifluoroethanol (TFE), under reflux, while bubbling dry HCl and, in general, the crude reaction product was collected by filtration from the reaction mixture previously chilled with ice bath.

(19) Finney, D. J. In *Statistical Method in Biological Assay*; Griffin, G., Ed.; London, 1952; p 524.

(20) Harris, C. M.; Fesik, S. W.; Thomas, A. M.; Kannan, R.; Harris, T. M. *J. Org. Chem.* **1986**, *51*, 1509.

(A2-3), 122172-84-3; **25** (A2-4), 122173-90-4; **25** (A2-5), 122173-91-5; **26** (A2-1), 122173-92-6; **26** (A2-2), 122173-93-7; **26** (A2-3), 122172-85-4; **26** (A2-4), 122173-94-8; **26** (A2-5), 122173-95-9; **27** (A2-1), 122173-96-0; **27** (A2-2), 122173-97-1; **27** (A2-3), 122188-75-4; **27** (A2-4), 122173-98-2; **27** (A2-5), 122173-99-3; **28** (A2-1), 122174-00-9; **28** (A2-2), 122174-01-0; **28** (A2-3), 122172-86-5; **28** (A2-4), 122174-02-1; **28** (A2-5), 122174-03-2; **29** (A2-1), 122174-04-3; **29** (A2-2), 122174-05-4; **29** (A2-3), 122188-76-5; **29** (A2-4), 122174-06-5; **29** (A2-5), 122174-07-6; **30** (A2-1), 120562-31-4; **30** (A2-2), 120538-93-4; **30** (A2-3), 122188-77-6; **30** (A2-4), 120538-95-6; **30** (A2-5), 120538-96-7; **31** (A2-1), 120562-32-5; **31** (A2-2), 120538-97-8; **31** (A2-3), 120538-98-9; **31** (A2-4), 120562-52-9; **31** (A2-5), 120562-53-0; **32** (A2-2), 122174-16-7; **32** (A2-3), 122172-88-7; **32** (A2-4), 122174-17-8; **32** (A2-5), 122174-18-9; **33** (A2-2), 120562-55-2; **33** (A2-3), 120562-56-3; **33** (A2-4), 122189-06-4; **33** (A2-5), 120562-58-5; **34**, 122172-90-1; **35**, 122188-78-7; **36**, 122188-79-8; **37**, 122172-91-2; **38**, 122188-80-1; **39**, 122172-92-3; **40**, 122172-93-4; **41**, 122188-81-2; **42**, 122188-82-3; **43**, 122188-83-4; **44**, 122188-84-5; **45**, 122188-85-6; **46**, 122188-86-7; **47**, 122188-87-8; **48**, 122188-88-9; **49**, 117251-07-7; **50**, 122172-94-5; **51**, 122172-95-6; **52**, 122172-96-7; **53**, 122172-97-8; **54**, 122172-98-9; **55**, 122188-89-0; **56**, 122188-90-3; **57**, 122172-99-0; **58**, 122173-00-6; **59**, 122173-01-7; **60**, 122173-02-8; **61**, 122173-03-9; **62**, 117226-72-9; **63**, 122173-04-0; **64**, 122173-05-1; **65**, 122173-06-2; **66**, 122173-07-3; **67**, 122173-08-4; **68**, 122173-09-5; **69**, 122188-91-4; **70**, 122173-10-8; **71**, 122173-11-9; **72**, 122173-12-0; CTA (A2-1), 91032-34-7; CTA (A2-2), 91032-26-7; CTA (A2-3), 91032-36-9; CTA (A2-4), 91032-37-0; CTA (A2-5), 91032-38-1; TB, 93616-27-4; TC, 91032-39-2; TD, 89139-42-4; TD (N^{15}-Cbz derivative), 104581-72-8; $HNMe_2$, 124-40-3; $H_2NCH_2CH_2NH_2$, 107-15-3; $H_2N(CH_2)_3NH_2$, 109-76-2; $H_2N(CH_2)_4NH_2$, 110-60-1; $H_2N(CH_2)_6NH_2$, 124-09-4; $H_2NCH_2CH_2NHMe$, 109-81-9; $H_2N(CH_2)_3NHMe$, 6291-84-5; $H_2NCH_2CH_2NHEt$, 110-72-5; $H_2N(CH_2)_3NHEt$, 10563-23-2; $H_2NCH_2CH_2NMe_2$, 108-00-9; $H_2N(CH_2)_3NMe_2$, 109-55-7; $H_2N(CH_2)_4NMe_2$, 3529-10-0; $H_2N(CH_2)_5NMe_2$, 3209-46-9; $H_2N(CH_2)_7NMe_2$, 22078-09-7; $H_2N(CH_2)_3NEt_2$, 104-78-9; $H_2N(CH_2)_3NBu_2$, 102-83-0; $MeNHCH_2CH_2NHMe$, 110-70-3; $MeNH(CH_2)_3NHMe$, 111-33-1; $MeNHCH_2CH_2NMe_2$, 142-25-6; H-Gly-OEt·HCl, 623-33-6; H-DL-Glu(OCH_2Ph)-OCH_2Ph·TsOH), 120538-51-4; thiomorpholine, 123-90-0; *N*-methylpiperazine, 109-01-3; *N*-benzyl-4-piperidinamine, 50541-93-0; 3-amino-1-azabicyclo[2.2.2]octane, 6238-14-8; 2-(aminomethyl)pyridine, 3731-51-9; *N*-ethyl-2-(aminomethyl)pyrrolidine, 26116-12-1; *N*-(2-aminoethyl)pyrrolidine, 7154-73-6; *N*-(2-aminoethyl)morpholine, 2038-03-1; *N*-(3-aminopropyl)morpholine, 123-00-2; morpholine, 110-91-8; N^{ϵ}-aminocaproyl-β-D-galactopyranosylamine, 122211-76-1.

Design, Synthesis, and Testing of Potential Antisickling Agents. 7. Ethacrynic Acid Analogues

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In search of a drug to treat sickle cell anemia, several analogues of the diuretic ethacrynic acid (ECA) have been synthesized and found equivalent in antigelling potency to ECA, but they have moderate or little diuretic activity. Structure–activity studies revealed that most of the highly active derivatives contain an acryloyl moiety. The latter functionality reacts covalently with protein sulfhydryl groups via a Michael addition reaction. Other derivatives, which lack the acryloyl moiety, showed notably lower antigelling activity. Since the antigelling assay is run under anaerobic conditions, activity implies a stereochemical inhibition of polymerization of deoxyhemoglobin S. The solubility ratios obtained from [HbS drug]/[HbS control] of several compounds (Table I) are near those expected for a drug with clinical potential (1.06–1.20 at tolerable doses in vivo).

Our discovery of the potent antigelling and antisickling activity of ethacrynic acid (ECA)[1] (compound **1**, Table I) suggested the exploitation of this lead by extensive structure–activity studies since the strong diuretic properties exhibited by ECA preclude its use as an oral therapeutic agent for the treatment of sickle cell anemia. Therefore, we set out to find ECA analogues which might retain strong antigelling activity but would lack the diuretic properties.

We now report the synthesis and antigelling activity of 32 ECA analogues. Some of the new compounds are equivalent in antigelling potency to ECA but have moderate or little diuretic activity. Structural modification of ECA included both the phenoxyacetic acid and the acryloyl moieties. Table I lists 9 derivatives of ECA with the same acryloyl group but different phenoxy acid substituents, while Table II lists 12 ECA derivatives varied in either or both moieties. Table III lists five analogues with the same phenoxyacetic acid moiety, but the vinyl moieties have been saturated, and Table IV lists six miscellaneous structurally related cyclic analogues of ECA.

Chemistry

The compounds listed in Table I–IV can be grouped into three categories with regard to their source: (1) compounds previously prepared at Merck Sharp and Dohme Co.; these include compounds **20** and **21**, (Table II), compounds **22–26** (Table III), and compounds **27**, **28**, and **31** (Table IV); (2) compounds synthesized as previously reported; these include compound **1** (ECA)[2] (Table I), compounds **10**,[3] **12**,[2] **15**,[4] **16**,[5] **17**,[5] and **19**,[5] (Table II), and compounds **29**,[6] **30**,[7] and **32**[8] (Table IV); (3) newly synthesized com-

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(1) Kennedy, P. E.; Williams, F. S.; Abraham, D. J. *J. Med. Chem.* **1984**, *27*, 103.
(2) Schultz, E. M.; Cragoe, E. J., Jr.; Bicking, J. B.; Bolhofer, W. A.; Sprague, J. M. *J. Med. Pharm. Chem.* **1962**, *5*, 66.
(3) Cragoe, E. J., Jr. U.S. Patent 4,390,537, June 28, 1983.
(4) Bicking, J. B.; Holtz, W. J.; Watson, L. S.; Cragoe, E. J., Jr. *J. Med. Chem.* **1976**, *19*, 530.
(5) Schultz, E. M.; Bicking, J. B.; Deana, A. A.; Gould, N. P.; Strobaugh, T. P.; Watson, L. S.; Cragoe, E. J., Jr. *J. Med. Chem.* **1976**, *19*, 783.
(6) de Solms, S. J.; Woltersdorf, O. W., Jr.; Cragoe, E. J., Jr. *J. Med. Chem.* **1978**, *21*, 437.
(7) Cragoe, E. J., Jr.; Schultz, E. M.; Schneeberg, J. D.; Stokker, G. I.; Woltersdorf, O. W., Jr.; Fanelli, G. M.; Watson, L. S. *J. Med. Chem.* **1975**, *18*, 225.

0022-2623/89/1832-2460$01.50/0