Acta Chemica Scandinavica B 38 (1984) 5–14

Synthesis of Analogs of D-Ala–D-Ala as Potential Inhibitors of Bacterial Cell Wall Biosynthesis

ERIK A. HAGEN,[a] TOM BERGAN[b] and ARNE J. AASEN[a]

[a] Department of Chemistry and [b] Department of Microbiology, Institute of Pharmacy, University of Oslo, Box 1068, Blindern, Oslo 3, Norway

The syntheses of the L,L- and D,D-stereoisomers of *N*-phenoxyacetyl-X-alanine in which X=Ser, Ala(βCl) or Arg, are described. The antibacterial activity of these peptides and some of their synthetic intermediates has been examined. Four of the intermediates in which X=Ala(βCl) and Arg(NO_2), which possess *C*-terminal benzyl ester groups, were active against viridans streptococci and *Streptococcus agalactiae*. The D,D-enantiomers were more active than the corresponding L,L-isomers. None of the compounds were active against β-lactamase producing bacteria or acted as β-lactamase inhibitors.

Antibiotics of the groups including penicillins, cephalosporins, cycloserine, and vancomycin inhibit the cross-linking of the D-Ala–D-Ala units of the peptide chains of the growing cell wall peptidoglycans.[1–3] Cycloserine by structural relationship to D-Ala interferes with the enzymes alanine racemase and D-Ala–D-Ala synthetase whereby the formation of the dipeptide D-Ala–D-Ala involved in completion of the pentapeptide side-chain of the polysaccharide backbone, is prevented.[3,4] Vancomycin forms complexes with cell wall precursors and thereby inhibits cell wall biosynthesis at the site of the carboxyl terminus of the oligopeptide side chain.[5–7] Probably by structural resemblance to the terminal D-Ala–D-Ala of the nascent peptidoglycan units, penicillins and cephalosporins inhibit transpeptidase and carboxypeptidase by covalent binding to these enzymes.[8,9]

Dipeptides which might be considered as D-Ala–D-Ala analogs have previously been synthesized and tested for antibacterial activity.[10,11] Moderate to good inhibition of bacterial growth was observed for dipeptides in which the *N*-terminal amino acid was D-Ala(βF).[10]

This paper deals with the syntheses of six D-Ala–D-Ala analogs with the intention that they might fit the active enzymatic sites which are normally occupied by alanine residues and thus interfere with the bacterial cell wall synthesis. These analogs consisting of three moieties are describable by the general formula

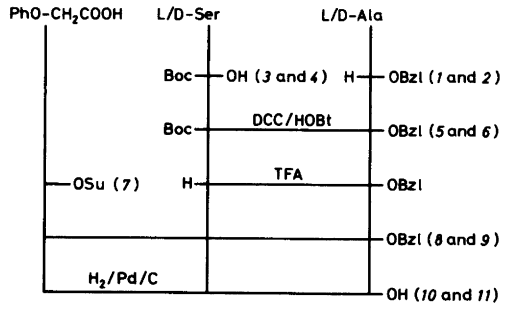
PhO–CH_2CO–X–L/D-Ala

in which PhO–CH_2CO=phenoxyacetyl and X=L- or D-serine, L- or D-arginine, or L- or D-3-chloroalanine. The side chains of the X-part would presumably assist in blocking active sites by ionic – and/or hydrogen bonds (Arg; Ser) – or by reacting with nucleophiles (3-chloroalanine). Phenoxyacetyl was chosen to resemble the side chain of phenoxymethylpenicillin.

RESULTS AND DISCUSSION

Synthesis. PhO–CH_2CO–Ser–Ala (*10*) and its enantiomer PhO–CH_2CO–D-Ser–D-Ala (*11*) were prepared as outlined in Scheme 1. The syntheses were initiated by condensing Boc-L/D-Ser ("L/D„ denotes L- or D-, or L,L- or D,D-derivatives, respectively.)(*3* and *4*) without side chain protection, with L/D-Ala–OBzl (*1* and *2*) employing DCC and HOBt[12] as coupling reagents to yield Boc–L/D-Ser–L/D-Ala–OBzl (*5* and *6*). The Boc groups of *5* and *6* were cleaved by TFA and the products, L/D-Ser–L/D-Ala–OBzl ·

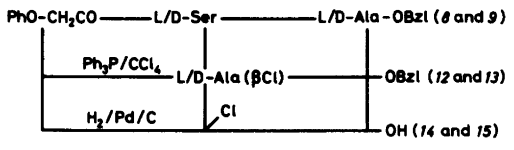
0302-4369/84 $2.50



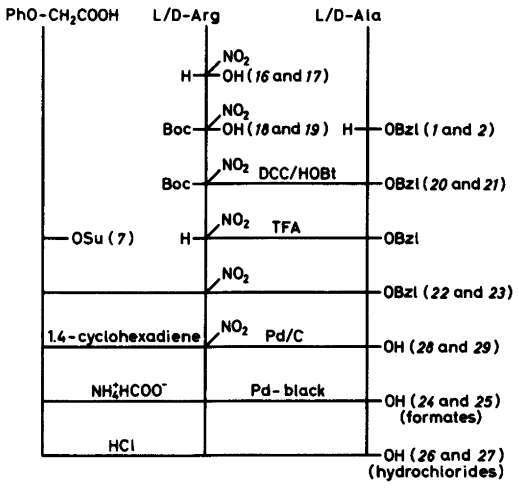
Scheme 1. The syntheses of PhO–CH_2CO–L/D-Ser–L/D-Ala (*10* and *11*).

TFA, were subsequently acylated using an active ester of phenoxyacetic acid, PhO–CH_2CO–OSu (*7*), as acylating reagent in the presence of *N*-ethylmorpholine to give PhO–CH_2CO–L/D-Ser–L/D-Ala–OBzl (*8* and *9*) in rather low yield (*ca.* 30 %). The L,L- and D,D-enantiomers (*8* and *9*) revealed deviating melting points and numerical values of rotations (112–114 °C, $[\alpha]_D$ −4.2° and 97–99 °C, $[\alpha]_D$ +3.3°, respectively) due to an impurity (*ca.* 5 %; TLC). The low yields of the intermediates *8* and *9* were apparently due to the lack of protection of the hydroxymethyl group of serine which had been acylated by the highly activated ester *7*.[13] Two by-products whose ^{1}H and ^{13}C NMR spectra indicated their structures as Ser(PhO–CH_2CO)–Ala–OBzl (*8a*) and PhO–CH_2CO–Ser(PhO–CH_2CO)–Ala–OBzl (*8b*), respectively, accounted for about 40 % of the product mixture implying that extensive *O*-acylation had occurred. The benzyl groups of compounds *8* and *9* were finally removed by catalytic hydrogenation furnishing PhO–CH_2CO–L/D-Ser–L/D-Ala (*10* and *11*) whose spectral properties were in agreement with their structures. Examination of the chiral purity of the amino acid residues of *10* and *11* indicated, however, that slight epimerization (3.8 % Ala) had occurred in the D-Ala moiety of PhO–CH_2CO–D-Ser–D-Ala (*11*); *cf.* Table 3. The optical activity of the starting material D-Ala did not reveal detectable amounts of Ala.

The preparation of the enantiomeric pair PhO–CH_2CO–L/D-Ala(βCl)–L/D-Ala (*14* and *15*; Scheme 2) was accomplished by converting the hydroxyl groups of PhO–CH_2CO– L/D-Ser–L/D-Ala–OBzl (*8* and *9*) to the corresponding chlorides using the Ph_3P/CCl_4 method.[14–16] This method, which takes place under essentially neutral conditions, was adopted to avoid elimination of HCl from the products *12* and *13*. The chloro derivatives *12* and *13* revealed characteristic upfield ^{13}C NMR shifts[17] of *ca.* 18.7 ppm (*cf.* Table 2) for the $-CH_2Cl$ groups relative to the $-CH_2OH$ groups in PhO–CH_2CO–L/D-Ser–L/D-Ala–OBzl (*8* and *9*). PhO–CH_2CO–Ala(βCl)–Ala–OBzl (*12*) was also obtained by allowing PhO–CH_2CO–Ser–Ala–OBzl (*8*) to react with thionyl chloride in CCl_4 in the presence of an equimolar amount of pyridine.[18,19] The two modes of syntheses of *12* yielded products exhibiting the same optical activities. The yield using the latter method was somewhat better (65 % *versus* 35–40 %). The benzyl groups of *12* and *13* were finally removed by hydrogenolysis furnishing PhO–CH_2CO–L/D-Ala(βCl)–L/D-Ala (*14* and *15*). Unsuccessful attempts were made to determine the optical purity of *14* and *15*; L- and



Scheme 2. The syntheses of PhO–CH_2CO–L/D-Ala(βCl)–L/D-Ala (*14* and *15*).



Scheme 3. The syntheses of PhO–CH_2CO–L/D-Arg–L/D-Ala · HCl (*26* and *27*).

D-Ala(βCl) were destroyed during the assay procedure, and the GLC-peaks corresponding to L- and D-Ala were too small to allow accurate integration.

The syntheses of the hydrochlorides of PhO–CH_2CO–L/D-Arg–L/D-Ala (*26* and *27*) were attained as outlined in Scheme 3. The intermediate dipeptides Boc–L/D-Arg(NO_2)–L/D-Ala–OBzl (*20* and *21*) were obtained by coupling Boc–L/D-Arg(NO_2) (*18* and *19*) to L/D-Ala–OBzl (*1* and *2*) applying the standard DCC-HOBt method.[12] Removal of the Boc groups with TFA was succeeded by reaction with the active ester *7* of phenoxyacetic acid and yielded PhO–CH_2CO–L/D-Arg(NO_2)–L/D-Ala–OBzl (*22* and *23*). The *C*-terminal protecting groups of *22* and *23* were selectively removed by catalytic transfer hydrogenation utilizing 1,4-cyclohexadiene as hydrogen donor in the presence of 10 % Pd/C rather than palladium black, which is reported to deprotect Arg(NO_2).[20] The products, *28* and *29*, which were Sakaguchi-negative,[21] displayed the same ^{13}C NMR chemical shifts (159.13 ppm) for the ζ-carbon in the Arg moiety as the corresponding carbon in PhO–CH_2CO–L/D-Arg(NO_2)–L/D-Ala–OBzl (*22* and *23*) confirming the presence of the nitro unit. In the next step of the present synthesis, removal of the nitro group in –Arg(NO_2)– created an upfield shift of about 2 ppm for the ζ-carbon of the Arg residue; *cf.* Table 2. The protected Arg and Ala moieties of *22* and *23* were both deprotected by catalytic transfer hydrogenation utilizing ammonium formate as hydrogen donor and palladium black as catalyst according to Anwer and Spatole.[22] However, fifteen hours were required to obtain complete conversion to PhO–CH_2CO–L/D-Arg–L/D-Ala (*24* and *25*) as compared to five minutes reported[22] for the analogous deprotection of Boc–Arg(NO_2)–Leu–O*t*Bu. The formates *24* and *25* and the corresponding hydrochlorides *26* and *27* were Sakaguchi-positive[21] and exhibited an upfield ^{13}C NMR chemical shift of about 2 ppm for the C_ζ of the Arg unit when compared to the Arg(NO_2) derivatives *20*, *22* and *28*; *cf.* Table 2. The formates (R_F 0.53 on reversed phase TLC) *24* and *25* were converted to the hydrochlorides *26* and *27* (R_F 0.75 on reversed phase TLC; same solvent system) on evaporation of aqueous solutions containing hydrochloric acid. The racemization tests of the amino acids of *26* and *27* revealed that extensive epimerization had occurred in the L/D-Ala moieties (8.5 % and 14.4 %, respectively; *cf.* Table 3); the extent of racemization in the L/D-Arg components could not be determined because these amino acids did not survive the test procedure. The amino acid sequence may under certain circumstances influence the extent of racemization under the strongly acidic conditions employed during peptide hydrolysis; *e.g.* cystein promotes complete racemization of an adjacent isoleucine residue.[23,24] Such effects have, to the best of our knowledge, not been reported for arginine and we are unable to offer an explanation for the present high degree of racemization.

Antibacterial effect. The minimum inhibitory concentration (MIC) was greater than 100 mg/l for *6*, *9*, *10*, *21*, *26* and *27*, and greater than 50 mg/l for *11*, *14*, *15* and *29* towards all organisms. Four of the compounds exhibited higher activity against two strains of viridans streptococci and one strain of *Streptococcus agalactiae*; *cf.* Table 1. The four compounds that possessed antibacterial activity PhO–CH_2CO–L/D-Ala(βCl)–L/D-Ala–OBzl (*12* and *13*) and PhO–CH_2CO–L/D-Arg(NO_2)–L/D-Ala–OBzl (*22* and *23*), carried *C*-terminal ester groups, while the corresponding

Table 1. Minimum inhibitory concentration (mg/l) of four D-Ala–D-Ala analogs against three bacterial strains.

Organism	Compound *12*	*13*	*22*	*23*
viridans streptococci,				
strain 2	50	0.19	1.56	0.39
strain 1470	50	0.19	1.56	0.39
Streptococcus agalactiae				
strain 1	50	25	0.19	0.19

free acids were inactive.

It is a matter for speculation whether the esters are active *per se*, or only after hydrolysis before the molecules reach the site of action. It is noteworthy, though, that the D,D-enantiomers were more antibacterially active than their corresponding L,L-isomers. These agents only inhibited Gram-positive organisms. This might be due to a more ready penetration of the substances to the site of action in these bacteria, which lack the lipophilic outer cell wall membrane possessed by Gram-negative species.

The fact that inhibition was obtained against some of the bacterial strains supports the rational behind the design of analogs of cell wall components. By structural similarity with D-Ala–D-Ala we intended the agents to interfere with the bacterial cell wall synthesis. The compounds, however, did not serve as β-lactamase inhibitors as do *e.g.* clavulanic acid and sulbactam, which have little antibacterial activity of their own.[25]

EXPERIMENTAL

General. Amino acids exhibiting optical activities in accordance with literature values were purchased from the Koch-Light Laboratories. Protecting group chemicals and coupling reagents were obtained from the Fluka AG and Koch-Light Laboratories. Column chromatography was performed on Merck's Kieselgel 60 (0.040–0.063 mm) and Merck's Lobar LiChroprep RP-8 prepacked columns, respectively. Analytical TLC was carried out on homemade (I) plates coated with silica gel containing fluorescence indicator (254 nm), or precoated plates from Merck: (II) DC Kieselgel 60 F_{254}, (III) HPTLC Kieselgel F_{254}, or (IV) HPTLC RP-18 F_{254s} using the following solvent systems: (A) $CH_3OH-CHCl_3$ 3:100, (B) $CH_3OH-CHCl_3$ 6:100, (C) $EtOH-H_2O$ 1:1, or (D) $CH_3OH-CHCl_3$ 1.5:100. The spots were visualized with Merck's molybdophosphoric acid spray, Merck's ninhydrin spray, 1-naphthol-hypobromite reagent (Sakaguchi) for monosubstituted guanidines[21] or UV-light (254 nm). Melting points (uncorrected) were determined on Reichert or Mettler FP61 instruments. Optical rotations were recorded on Perkin-Elmer 141 or 241 instruments. ^1H and ^{13}C NMR spectra were obtained with Jeol FX-90Q, Jeol FX-100, Bruker CXP-200 or Bruker WM-400 instruments. Mass spectra, electron impact (EI) and chemical ionization (CI), were recorded on a Micromass 7070 H instrument. CI mass spectra were obtained by the direct inlet method employing isobutane as ionizing gas. Combustion analyses were carried out by *Ilse Beetz Mikroanalytisches Laboratorium, Kronach,* West-Germany. Analyses of the chiral purity of the final products were accomplished at the Central Institute for Industrial Research, Oslo.

Abbreviations. Standard abbreviations for amino acids and protecting groups follow the tentative rules of the IUPAC-IUB Commission on Biochemical Nomenclature in *J. Biol. Chem. 247* (1972) 977 and *Biochemistry 14* (1975) 449. Additional abbreviations are used: DCC, *N,N'*-dicyclohexylcarbodiimide; DCU, *N,N'*-dicyclohexylurea; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; HOSu, *N*-hydroxysuccinimide; $PhO-CH_2CO-$, phenoxyacetyl.

Synthesis. L/D-Ala–OBzl tosylate[26] (*1* and *2*), Boc-L/D-Ser[27–29] (*3* and *4*), L/D-Arg(NO_2)[26,30] (*16* and *17*), Boc-L/D-Arg(NO_2),[27,28,31] (*18* and *19*), prepared according to known procedures, exhibited physical and spectral properties in agreement with data in the literature.

Boc–Ser–Ala–OBzl (5). DCC (10.3 g; 50 mmol) was added to a chilled (−15 °C) mixture of Boc–Ser (*3*; 5.0 g; 22.4 mmol), Ala–OBzl tosylate (*1*; 8.58 g; 26.7 mmol), HOBt (7.4 g; 55 mmol), and *N*-ethylmorpholine (3.07 g; 26.7 mmol) in CH_2Cl_2 (40 ml). After stirring for 1 h at −15 °C and 5 h at room temperature, DCU was removed by filtration and CH_2Cl_2 (100 ml) was added. The solution was washed once with H_2O, 5 % citric acid, 1 M $NaHCO_3$, and H_2O (100 ml each), respectively, dried over Na_2SO_4, filtered, and evaporated. The residue was chromatographed thrice on silica gel columns yielding pure Boc–Ser–Ala–OBzl (*5*; 5.30 g; 63 %) on elution with CH_2Cl_2 and 1.5 % CH_3OH in CH_2Cl_2. $[\alpha]_D^{20}$ −19.3° (*c* 1.6; DMF); m.p. 77–78 °C; R_F 0.3 (I, A); ^1H NMR (90 MHz, $CDCl_3$): δ 1.40 (3H, d, *J ca.* 5.3 Hz), 1.44 (9H, s), 3.58 (1H, broad s), *ca.* 3.6 (1H, m), *ca.* 4.0 (1H, m), *ca.* 4.2 (1H, m), 4.61 (1H, m), 5.16 (2H, s), 5.69 (1H, d, *J ca.* 7.6 Hz), 7.33 (5H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 28.11 (q), 67.14 (t), 80.25 (s), 128.02 (d), 128.31 (d), 128.45 (d), 135.08 (s), 155.80 (s) and signals given in Table 2; *m/z* (CI, %): 367 (M^++1, 25).

Boc–D-Ser–D-Ala–OBzl (6). *6* was synthesized as described above for *5*. Yield: 8.25 g (53 %); $[\alpha]_D^{20}$ +19.3° (*c* 1.3; DMF); m.p. 73–74 °C; R_F 0.3 (I, A); ^1H and ^{13}C NMR: see under *5*.

$PhO-CH_2CO-OSu$ (7). HOSu (6.91 g; 60 mmol) was added to a chilled solution (−10 °C) of phenoxyacetic acid (7.61 g; 50 mmol) and DCC (12.38 g; 60 mmol) in DMF (30 ml). The

Table 2. ^{13}C NMR chemical shifts (ppm) relative to tetramethylsilane (TMS). *5*: Boc–Ser–Ala–OBzl; *7*: PhO–CH_2CO–OSu; *8*: PhO–CH_2CO–Ser–Ala–OBzl; *10*: PhO–CH_2CO–Ser–Ala; *12*: PhO–CH_2CO–Ala(βCl)–Ala–OBzl; *14*: PhO–CH_2CO–Ala(βCl)–Ala; *20*: Boc–Arg(NO_2)–Ala–OBzl; *22*: PhO–CH_2CO–Arg(NO_2)–Ala–OBzl; *24*: PhO–CH_2CO–Arg–Ala·HCOOH; *26*: PhO–CH_2CO–Arg–Ala·HCl; *28*: PhO–CH_2CO–Arg(NO_2)–Ala.

Residue [a]	*5*	*7*	*8*	*10*	*12*	*14*	*20*	*22*	*24*	*26*	*28*
Ala											
C_α	48.19		48.57	49.56	48.62	49.68	48.40	47.64	49.20	47.47	49.97
C_β	17.63		17.66	18.68	18.12	18.54	17.53	16.60	18.41	16.86	17.36
CO	170.86[b]		170.05[b]	175.52[b]	172.08	175.10	172.68[b]	172.07[b]	175.21	173.70[b]	174.28
Ser											
C_α	54.96		53.85	56.48							
C_β	62.86		62.82	63.96							
CO	172.51[b]		172.57[b]	171.27[b]							
Ala(βCl)											
C_α					53.47	55.37					
C_β					44.07	46.45					
CO					167.61[b]	169.77[b]					
Arg											
C_α							53.36	51.28	51.44	51.22	51.42
C_β							30.35	29.44	29.11	29.29	29.37
C_γ							24.51	24.54	24.17	24.71	24.51
C_δ							40.68	[c]	[c]	[c]	[c]
C_ζ							159.60	159.13	157.17[b]	156.88[b]	159.13
CO							172.08[b]	170.98[b]	169.69	170.72[b]	170.48
PhO–CH_2CO–											
CH_2		63.10	67.39	68.84	67.37	68.74		65.85	66.59	66.48	66.52
C–1		157.06	157.24	159.61	157.19	159.72		157.60	157.62[b]	157.57[b]	157.55
C–3,C–5		129.58	129.80	131.08	129.88	131.05		129.40	129.40	129.40	129.35
C–2,C–6		114.61	114.90	116.44	114.90	116.42		114.58	114.61	114.53	114.49
C–4		122.26	122.80	122.96	122.43	122.95		121.11	121.09	121.06	121.04
CO		168.67	169.07	169.42	168.78[b]	169.54[b]		167.42	167.16	167.41	167.38
$HCOO^-$									165.23		

[a] See Experimental for shifts of activating – and protecting groups; [b] assignment uncertain; [c] coinciding with solvent peak.

temperature rose to room temperature and the mixture was stirred for 4 h. After filtration, the product was precipitated by addition of H_2O and recrystallized from ethyl acetate–pentane. Yield: 6.2 g (50 %); m.p. 102–103 °C; R_F 0.7 (I, A); ^{1}H NMR (100 MHz, $CDCl_3$): δ 2.85 (4H, s), 4.98 (2H, s), *ca.* 6.99 (3H, m), *ca.* 7.26 (2H, m); signals of impurity: δ 2.63 (0.32H, s), 4.53 (0.16H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 25.38 (t), 164.57 (s) and signals given in Table 2.

PhO–CH_2CO–Ser–Ala–OBzl (8). TFA (12 ml) was added to Boc–Ser–Ala–OBzl (*5*; 3.6 g; 9.84 mmol) and the mixture stirred at room temperature for 30 min. The TFA was distilled under reduced pressure and the product, Ser–Ala–OBzl, was dried *in vacuo* for 24 h. PhO–CH_2CO–OSu (*7*; 3 g; 12 mmol) and triethylamine (Et_3N; 1.0 g; 9.84 mmol) were added to a solution of the Ser–Ala–OBzl in CH_2Cl_2 (15 ml) and the mixture stirred for 24 h at room temperature. CH_2Cl_2 (50 ml) was added and the solution was washed once with H_2O, 5 % citric acid, 1 M $NaHCO_3$, 5 % citric acid and H_2O (40 ml each), respectively. The organic phase was dried over Na_2SO_4 and evaporated leaving an oil which was chromatographed four times on silica gel columns. On elution with CH_2Cl_2, 0.5, 1.0 and 1.5 % CH_3OH in CH_2Cl_2, pure PhO–CH_2CO–Ser–Ala–OBzl (*8*; 1.1 g; 28 %) was obtained. $[\alpha]_D^{20}$ −4.2° (*c* 1.1; DMF); m.p. 112–114 °C; R_F 0.3 (I, A); ^{1}H NMR (100 MHz, $CDCl_3$): δ 1.42 (3H, d, *J* 7.3 Hz), *ca.* 3.6 (2H, m), *ca.* 3.95 (1H, m), *ca.* 4.5 (1H, m), 4.52 (2H, s), 4.6 (1H, m), 5.14 (1H, d, *J* 12.2 Hz), 5.20 (1H, d, *J* 12.2 Hz), 6.96 (3H, m), 7.23 (2H, m), 7.34 (5H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 67.39 (t), 128.20 (d), 128.50 (d), 128.66 (d), 135.33 (s) and signals given in Table 2; *m/z* (CI; %): 401 (M^+ +1, 18). Two by-products (*8a* and *8b*) whose physical properties are described below, were isolated from the less polar fractions.

Ser(PhO–CH_2CO)–Ala–OBzl (8a). Yield: 0.4 g (10 %); $[\alpha]_D^{20}$ −24.0 °(*c* 1.0; DMF); m.p. 140–142 °C; R_F 0.7 (I, A); ^{1}H NMR (100 MHz, $CDCl_3$): δ 1.4 (3H, d, *J* 7.3 Hz), 4.4 (2H, m), 4.6 (1H, m), 4.68 (2H, s), 4.8 (1H, m), 5.14 (2H, s) 6.9 (3H, m), 7.3 (5H, s), *ca.* 7.3 (2H, m); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 17.80 (q), 48.84 (d), 52.17 (d), 63.84 (t), 65.17 (t), 67.53 (t), 114.80 (d), 122.03 (d), 128.20 (d), 128.64 (d), 128.72 (d), 129.67 (d), 135.19 (s), 157.78 (s), 166.42 (s), 168.94 (s), and 171.98 (s).

PhO–CH_2CO–Ser(PhO–CH_2CO)–Ala–OBzl (8b). Yield: 1.0 g (30 %); $[\alpha]_D^{20}$ −2.1° (*c* 1.4; DMF); m.p. 123–125 °C; R_F 0.5 (I, A); ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.38 (3H, d, *J* 7.1 Hz), 4.48 (2H, s), *ca.* 4.5 (2H, m), 4.59 (2H, s), *ca.* 4.9 (2H, m), 5.15 (2H, s), 6.95 (6H, m), 7.25 (4H, m), 7.32 (5H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 17.96 (q), 48.54 (d), 51.60 (d), 64.20 (t), 65.23 (t), 67.34 (2×t), 114.82 (d), 114.90 (d), 121.86 (d), 122.33 (d), 128.18 (d), 128.50 (d), 128.66 (d), 129.58 (d), 129.80 (d), 135.33 (s), 157.24 (s), 157.86 (s), 167.67 (s), 168.81 (2×s), 172.14 (s).

PhO–CH_2CO–D-Ser–D-Ala–OBzl (9). (*9*) was prepared as described above for *8*. Yield: 0.41 g (31 %); $[\alpha]_D^{20}$ +3.3° (*c* 1.1; DMF); m.p. 97–99 °C; R_F 0.3 (I, A); ^{1}H and ^{13}C NMR: see under *8*.

PhO–CH_2CO–Ser–Ala (10). PhO–CH_2CO–Ser–Ala–OBzl (*8*; 400 mg; 1.0 mmol) dissolved in CH_3OH (10 ml) was hydrogenated at room temperature by bubbling H_2 through the solution for 30 min in the presence of 10 % Pd/C (200 mg). The mixture was filtered, evaporated, and recrystallized from CH_3OH–H_2O. Yield: 237 mg (76 %); $[\alpha]_D^{20}$ +17.3° (*c* 0.9; DMF); m.p. 197–199 °C; R_F 0.87 (IV, C); ^{1}H NMR (400 MHz, DMF-d_7): δ 1.45 (3H, d, *J* 7.3 Hz), *ca.* 3.6 (1H, broad s), 3.91 (1H, dd, *J* 5.0 and 11.0 Hz), 3.95 (1H, dd, *J* 5.7 and 11.0 Hz), 4.51 (1H, m), 4.70 (2H, s), 4.71 (1H, m), *ca.* 7.1 (3H, m), *ca.* 7.4 (2H, m), 8.10 (1H, d, *J* 7.9 Hz), 8.36 (1H, d, *J* 7.1 Hz); ^{13}C NMR (100 MHz, DMF-d_7): see Table 2; *m/z* (EI; %): 310 (M^+, 1); found: C 53.67; H 5.97; N 8.55; calc. for $C_{14}H_{18}N_2O_6$: C 54.19; H 5.85; N 9.03.

PhO–CH_2CO–D-Ser–D-Ala (11). *11* was prepared as described above for *10*. Yield: 70 mg (45 %); $[\alpha]_D^{20}$ −15.3° (*c* 0.8; DMF); m.p. 198–200 °C; R_F 0.87 (IV, C); ^{1}H and ^{13}C NMR as for *10*; *cf.* Table 2; found C 54.06; H 5.78; N 8.10; calc. for $C_{14}H_{18}N_2O_6$: C 54.19; H 5.85; N 9.03.

PhO–CH_2CO–Ala(βCl)–Ala–OBzl (12). A solution of PhO–CH_2CO–Ser–Ala–OBzl (*8*; 400 mg; 1.0 mmol) and triphenylphosphine (289 mg; 1.1 mmol) in CCl_4 (10 ml) was refluxed for 24 h.[14–16] CCl_4 (50 ml) was added and the organic phase washed once with H_2O (50 ml), dried over Na_2SO_4, and evaporated. The residue was chromatographed twice on silica gel columns furnishing on elution with CH_2Cl_2 and 1 % CH_3OH in CH_2Cl_2, pure PhO–CH_2CO–Ala(βCl)–Ala–OBzl (*12*), which was recrystallized from ethyl acetate–light petroleum. Yield: 168 mg (40 %); $[\alpha]_D^{20}$ −5.8° (*c* 0.5; DMF); m.p. 122–123 °C; R_F 0.5 (I, D); ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.40 (3H, d, *J* 7.9 Hz), 3.85 (2H, m), 4.53 (2H, s), 4.6 (1H, m), 4.9 (1H, m), 5.17 (2H, s), 6.96 (3H, m), 7.25 (2H, m), 7.34 (5H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 67.37 (t), 128.23 (d), 128.53 (d), 128.66 (d), 135.33 (s) and signals given in Table 2; *m/z* (CI; %): 421 (20) and 419

(M^++1, 42). *12* was also prepared by reacting *8* with $SOCl_2$:[18,19] $SOCl_2$ (51 mg; 0.43 mmol) was added to a chilled (0 °C) solution of PhO–CH_2CO–Ser–Ala–OBzl (*8*; 69 mg; 0.17 mmol) in pyridine (13 mg; 0.17 mmol) and CCl_4 (8 ml). The solution was refluxed for 3 h and subsequently evaporated to dryness *in vacuo*. The residue was chromatographed once on a silica gel column using CH_2Cl_2, 0.3 %, and 0.6 % CH_3OH in CH_2Cl_2 as eluants. The product was recrystallized from ethyl acetate–light petroleum. Yield: 47 mg (65 %); $[\alpha]_D^{20}$ −5.8° (*c* 0.9; DMF); m.p. 121–122 °C; R_F 0.5 (I, E).

PhO–CH_2CO–D-Ala(βCl)–D-Ala–OBzl (13). *13* was prepared as described above for *12* using the Ph_3P/CCl_4 method. Yield: 73 mg (35 %); $[\alpha]_D^{20}$ +5.9° (*c* 0.5; DMF); m.p. 122–123 °C; R_F 0.5 (I, D); ^{1}H and ^{13}C NMR: as for *12*, see above and Table 2.

PhO–CH_2CO–Ala(βCl)–Ala (14). H_2 was bubbled through a solution of PhO–CH_2CO–Ala(βCl)–Ala–OBzl (*12*; 102 mg; 0.24 mmol) in CH_3OH (5 ml) in the presence of 10 % Pd/C (44 mg) at room temperature for 1 h. The mixture was filtered, evaporated, and recrystallized from CH_3OH–H_2O. Yield: 56 mg (71 %); $[\alpha]_D^{20}$ +8.1° (*c* 0.6; DMF); m.p. 188–190 °C; R_F 0.79 (IV, C); ^{1}H NMR (400 MHz, DMF-d_7): δ 1.46 (3H, d, *J* 7.3 Hz), 4.08 (1H, dd, *J* 7.1 and 11.2 Hz), 4.14 (1H, dd, *J* 4.3 and 11.2 Hz), 4.49 (1H, quintet, *J ca.* 7.3 Hz), 4.75 (2H, s), 5.01 (1H, m), 7.09 (3H, m), 7.42 (2H, m), 8.47 (1H, d, *J* 8.2 Hz), 8.61 (1H, d, *J* 7.3 Hz); ^{13}C NMR (100 MHz, DMF-d_7: see Table 2; *m/z* (CI; %): 331 (7) and 329 (M^++1, 10); found: C 51.05; H 5.35; N 8.25; Cl 10.40; calc. for $C_{14}H_{17}ClN_2O_5$: C 51.15; H 5.21; N 8.52; Cl 10.78.

PhO–CH_2CO–D-Ala(βCl)–D-Ala (15). *15* was prepared as described above for *14*. Yield: 29 mg (75 %); $[\alpha]_D^{20}$ −10.0° (*c* 0.7; DMF); m.p. 191–193 °C; R_F 0.79 (IV, C); ^{1}H and ^{13}C NMR: see *14* above and Table 2; found: C 51.27; H 5.51; N 8.32; calc. for $C_{14}H_{17}ClN_2O_5$: C 51.15; H 5.21; N 8.52.

Boc–Arg(NO_2)–Ala–OBzl (20). DCC (2.0 g; 9.9 mmol) was added to a chilled (−10 °C) mixture of Boc–Arg(NO_2) (*18*; 2.0 g; 6.6 mmol), Ala–OBzl tosylate (*1*; 2.3 g; 7.3 mmol), HOBt[12] 1.6 g; 12 mmol) and *N*-ethylmorpholine (0.8 g; 7.3 mmol) in CH_2Cl_2 (20 ml) which was stirred for 1 h at −10 °C and at ambient temperature overnight. DCU was removed by filtration and the solid washed with CH_2Cl_2 (30 ml). The combined solutions were washed once with H_2O, 5 % citric acid, 1 M $NaHCO_3$, and H_2O (50 ml each), respectively, dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed once on a silica gel column yielding pure Boc–Arg(NO_2)–Ala–OBzl (*20*; 1.83 g; 58 %) on elution with CH_2Cl_2, 1.0 %, and 2 % CH_3OH in CH_2Cl_2. $[\alpha]_D^{20}$ −26.9° (*c* 1.3; CH_3OH); lit.[32] $[\alpha]_D^{20}$ −26.5±0.5° (*c* 1.4; CH_3OH); the product did not crystallize; lit.[32] m.p. 118.5–120 °C; R_F 0.3 (I, B); ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.40 (9H, s), *ca.* 1.4 (3H, d, partly hidden by peak at δ 1.40), 1.68 (4H, m), 3.28 (2H, m) 4.35 (1H, m), 4.56 (1H, quintet with broad lines, *J ca.* 7 Hz), 5.13 (2H, s), 5.66 (1H, d, *J* 7.9 Hz), 7.32 (5H, s), 7.6 (3H, m); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 28.39 (q), 80.37 (s), 128.07 (d), 128.45 (d), 128.63 (d), 135.41 (s), 156.10 (s), and signals given in Table 2.

Boc–D-Arg(NO_2)–D-Ala–OBzl (21). *21* was prepared as described above for *20*. Yield: 1.95 g (62 %); $[\alpha]_D^{20}$ +25.7° (*c* 1.6; CH_3OH); R_F 0.3 (I, B); ^{1}H NMR and ^{13}C NMR: see under *20*.

PhO–CH_2CO–Arg(NO_2)–Ala–OBzl (22). TFA (10 ml) was added to Boc–Arg(NO_2)–Ala–OBzl (*20*; 1.6 g; 3.4 mmol) and the mixture stirred at room temperature for 45 min. The TFA was distilled under reduced pressure and the product, Arg(NO_2)–Ala–OBzl, dried *in vacuo* overnight. PhO–CH_2CO–OSu (*7*; 2.4 g; 9.6 mmol) and *N*-ethylmorpholine (0.4 g; 3.4 mmol) were added to a solution of the Arg(NO_2)–Ala–OBzl in CH_2Cl_2 (5 ml). The mixture was subsequently stirred overnight at ambient temperature. CH_2Cl_2 (70 ml) was added and the solution washed once with H_2O, 5 % citric acid, 1 M $NaHCO_3$, and H_2O (50 ml each), respectively, dried over Na_2SO_4, filtered, and evaporated. The residue was chromatographed twice on silica gel columns yielding pure PhO–CH_2CO–Arg(NO_2)–Ala–OBzl (*22*; 0.98 g; 56 %) on elution with CH_2Cl_2, 1 %, and 2 % CH_3OH in CH_2Cl_2. *22* was recrystallized from CH_2Cl_2. $[\alpha]_D^{20}$ −12.6° (*c* 0.9; DMF); m.p. 152–153 °C; R_F 0.37 (III, B); R_F 0.40 (IV, C); ^{1}H NMR (400 MHz, DMF-d_7): δ 1.46 (3H, d, *J* 7.3 Hz), 1.77 (3H, m), 1.98 (1H, m), 3.38 (2H, m), 4.54 (1H, quintet, *J* 7.2 Hz), 4.69 (1H, doublet of triplets, *J* 5.2 and 8.3 Hz), 4.71 (2H, s), 5.26 (1H, d, *J* 12.5 Hz), 5.28 (1H, d, *J* 12.5 Hz), 7.09 (3H, m), 7.41 (2H, m), 7.50 (5H, m), 8.15 (1H, d, *J* 7.7 Hz), 8.64 (1H, d, *J* 6.7 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 66.55 (t), 127.70 (d), 127.95 (d), 128.33 (d), 135.85 (s) and signals given in Table 2; *m/z* (CI; %): 515 (M^++1, 2); found: C 56.04; H 5.75; N 16.10; calc. for $C_{24}H_{30}N_6O_7$: C 56.02; H 5.88; N 16.33.

PhO–CH_2CO–D-Arg(NO_2)–D-Ala–OBzl (23). *23* was prepared as described for *22*. Yield: 1.36 g (76 %); $[\alpha]_D^{20}$ +14.0° (*c* 1.2; DMF); m.p. 152–153 °C; R_F 0.37 (III, B); R_F 0.40 (IV, C); ^{1}H NMR and ^{13}C NMR: see under *22*; found: C

56.02; H 5.69; N 16.22; calc. for $C_{24}H_{30}N_6O_7$: C 56.02; H 5.88; N 16.33.

PhO–CH_2CO–Arg–Ala · HCOOH (24). A mixture of PhO–CH_2CO–Arg(NO_2)–Ala–OBzl (*22*; 400 mg; 0.78 mmol), Pd-black (300 mg) and NH_4^+ $HCOO^-$ (200 mg; 3.17 mmol) in CH_3OH–HCOOH (1:1; 4 ml) was stirred overnight at room temperature.[22] The mixture was filtered, concentrated to dryness *in vacuo* and the residue chromatographed on a Lobar RP-8 column. On elution with CH_3OH–H_2O (1:1) followed by removal of the solvent under reduced pressure, *24* was obtained as a colourless oil which solidified on drying. Yield: 296 mg (90 %); $[\alpha]_D^{20}$ +7.9° (*c* 0.9; DMF); R_F 0.53 (IV, C); ^{1}H NMR: (400 MHz, DMF-d_7): δ 1.39 (3H, d, *J* 7.1 Hz), 1.73 (2H, m), 1.89 (1H, m), 2.03 (1H, m), 3.31 (2H, m), 4.29 (1H, quintet, *J* 7.1 Hz), 4.67 (1H, m), 4.71 (2H, s), 7.08 (3H, m), 7.41 (2H, m); ^{13}C NMR (50 MHz, DMSO-d_6): see Table 2.

PhO–CH_2CO–D-Arg–D-Ala · HCOOH (25). *25* was prepared as described above for *24*. Yield: 281 mg (85 %); $[\alpha]_D^{20}$ −7.3° (*c* 0.9; DMF); R_F 0.53 (IV, C); ^{1}H NMR and ^{13}C NMR: see under *24*.

PhO–CH_2CO–Arg–Ala · HCl (26). PhO–CH_2CO–Arg–Ala · HCOOH (*24*; 172 mg; 0.40 mmol) was dissolved in 0.01 N HCl (75 ml) and concentrated to dryness *in vacuo*. The residue was redissolved in 0.01 N HCl (25 ml) and the process repeated. The residue was chromatographed on a Lobar RP-8 column. The product was obtained as a colourless oil on elution with CH_3OH–H_2O (1:1) followed by removal of the solvent. The oil solidified on drying *in vacuo*. Yield: 152 mg (91 %); $[\alpha]_D^{20}$ −6.1° (*c* 1.1; DMF); R_F 0.75 (IV, C); ^{1}H NMR (100 MHz, DMSO-d_6): δ 1.28 (3 H, d, *J* 7.3 Hz), 1.6 (4H, m), 3.1 (2H, m), 4.20 (1H, quintet, *J* 7.3 Hz), 4.43 (1H, m), 4.56 (2H, s), 6.96 (3H, m), 7.31 (5H, m), 7.84 (1H, m), 8.14 (1H, d, *J* 7.8 Hz), 8.44 (1H, d, *J* 6.8 Hz); ^{13}C NMR (25 MHz, DMSO-d_6): see Table 2; a satisfactory combustion analysis was not obtained.

PhO–CH_2CO–D-Arg–D-Ala · HCl (27). *25* was converted to the hydrochloride *27* as described for *26*. Yield: 142 mg (86 %); $[\alpha]_D^{20}$ +4.8° (*c* 0.9; DMF); R_F 0.75 (IV, C); ^{1}H NMR and ^{13}C NMR; see under *26*; a satisfactory elemental analysis was not obtained.

PhO–CH_2CO–Arg(NO_2)–Ala (28). N_2 was bubbled through a mixture of PhO–CH_2CO–Arg(NO_2)–Ala–OBzl (*22*; 100 mg; 0.19 mmol), 1,4-cyclohexadiene (160 mg; 2 mmol), and 10 % Pd/C (100 mg) in ethanol (2 ml) according to the method by Felix *et al.*[20] The mixture was stirred overnight, filtered and concentrated *in vacuo* to yield an oil. Yield: 41 mg

Table 3. Enantiomeric composition of amino acids in the hydrolysates.[b]

Compound[a]	Ser	D-Ser	Ala	D-Ala	Arg	D-Arg
PhO–CH_2CO–Ser–Ala (10)	100	0	100	0		
PhO–CH_2CO–D-Ser–D-Ala (11)	0	100	3.8	96.2		
PhO–CH_2CO–Arg–Ala · HCl (26)			91.5	8.5	*a*	
PhO–CH_2CO–D-Arg–D-Ala · HCl (27)			14.4	85.6		*a*

[a] L/D-Ala(βCl) from *14* and *15*, and L/D-Arg from *26* and *27* were destroyed during the hydrolysis and/or derivatization procedure. [b] Values are corrected for racemization induced during acid hydrolysis according to average values (1.8 and 0.7 % for L/D-Ala and L/D-Ser, respectively) observed after hydrolysis of polypeptides under similar conditions.[33,36]

(50 %); $[\alpha]_D^{20}$ +4.7° (*c* 0.7; DMF); R_F 0.74 (IV, C); ^{1}H NMR (100 MHz, DMSO-d_6): δ 1.26 (3H, d, *J* 6.8 Hz), 1.55 (4H, m), 3.12 (2H, m), *ca.* 4.4 (*ca.* 1H, m, partly coinciding with solvent peak), 4.55 (2H, s), 6.99 (3H, m), 7.30 (3H, m), *ca.* 8.15 (4H, m); ^{13}C NMR (25 MHz, DMSO-d_6): see Table 2.

PhO–CH_2CO–D-Arg(NO_2)–D-Ala (29). 29 was prepared as described above for *28*. Yield: 48 mg (58 %); $[\alpha]_D^{20}$ −4.9° (*c* 0.4; DMF); R_F 0.74 (IV, C); ^{1}H NMR and ^{13}C NMR: see above under *28*.

Assay for chiral purity. The assay procedure was based on that described by Frank and co-workers.[33] The peptide derivatives (*ca.* 0.3 μmol) were sealed in 6 N HCl (1 ml) under vacuum and kept at 110 °C for 24 h. The hydrolysates were evaporated to dryness *in vacuo* in the presence of KOH-pellets. 2-Propanol (0.5 ml) containing 2 N HCl was added and the mixtures heated at 100 °C for 1 h. Excess reagent was removed by a stream of N_2. $CHCl_3$ (200 μl) and pentafluoropropionic anhydride (50 μl) were added and the reaction mixtures kept at 110 °C for 10 min. Excess reagent was removed by N_2, the derivatized samples were dissolved in a small amount of $CHCl_3$ and injected (1 μl) on a Carlo Erba gas chromatograph fitted with an "on-column" injection system. Separation of L- and D-amino acid derivatives was accomplished on a 18 m×0.3 mm glass capillary column coated with Chirasil-Val[34,35] using H_2 (0.35 kg cm^{-2}) as carrier gas and temperature programming from 60 °C to 200 °C at a rate of 2 °C min^{-1}. The results are presented in Table 3.

Antibacterial testing. Growth inhibition was examined by incorporating two-fold dilutions of the compounds at concentrations up to 100 mg/l in Mueller-Hinton Medium (Merck, Darmstadt, West-Germany) with 1.5 % Agar 3 (Oxoid, London, Great Britain). The final pH of the medium was 7.4. The growth was examined after 48 h at 37 °C and the minimum inhibitory concentration (MIC) noted.

The substances were dissolved and kept as stock solutions in water or ethanol. From these, dilutions were made in sterile water and added to the medium. The highest ethanol concentration in the final growth medium did not interfere with bacterial growth.

MIC of the substances *10–15, 22, 23, 26* and *27* was determined with the following 25 bacterial strains, which in part were recent clinical isolates: *Branhamella catarrhalis* strain No. 1, *Bacillus cereus* 1, *Citrobacter* sp. 1, *Corynebacterium diphtheriae* 1, *Escherichia coli* 645, 649, *Klebsiella aerogenes* 670, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* 187, 464, 681, 1718, 1771, ATCC 6538p, *S. epidermidis* 310, 462, *Streptococcus agalactiae* 1, *Str. pneumoniae* 1211, *Str. pyogenes* 186, 195, enterococci 3, 428, 639, and viridans streptococci 2 and 1470.

MIC of the compounds *6, 9, 21* and *29* was determined against the following 25 isolates: *Citrobacter* sp. 11, *Escherichia coli* 649, *Klebsiella aerogenes* 670, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* 464, 1718, 1771, 4242, *Streptococcus agalactiae* 1, B, 4242, 12506, *Str. pneumoniae* 12769, viridans streptococci 1, 12347, 12407, 4137, 12478, 12463, enterococci 3, 11255, 12478, 12473, *Acinetobacter calcoaceticus* 12769, and *Enterobacter* sp. 639.

Ampicillin was chosen as a partner for examination of possible synergy since its activity is potentiated by the β-lactamase inhibitors clavulanic acid and sulbactam.[25] For this purpose, synergy was tested with the 25 first listed bacteria and the potential of the substances *10–15, 22, 23, 26* and *27* as β-lactamase inhibitors against strains producing such enzymes: *E. coli* strains 2526, 1517, 2173, *K. aerogenes* 134, 135, and *E. coli* JT R^+, which carries a TEM^+ plasmid, and its plasmid deficient parallel JT R^-. These strains have previously been employed in the study of β-lactamase inhibitors.[37]

The bacterial inocula were prepared from overnight blood agar cultures grown at 37 °C. The growth was suspended in Mueller-Hinton broth and adjusted by optical density (OD) on Aminco Fluoro-Colorimeter model j4-7440 (American Instrument Company, Silver Spring, Maryland, USA) to 10^5 colony forming units (CFU) per ml. Per inoculate (by multiinoculator with 25 loops dispensing 0.01 ml each) this gave approximately 1000 CFU on an agar surface of 0.25 cm^2.

Acknowledgements. The authors are indebted to Frøydis Oreld, the Central Institute for Industrial Research, Oslo, and John H. Johansen, Department of Chemistry, University of Oslo. They assisted in determining the enantiomeric composition of the amino acids of the peptide derivatives. We appreciate the aid of Arnfinn Kvarsnes, University of Tromsø, in recording NMR and mass spectra. Grateful acknowledgement is made to the Norwegian Research Council for Science and the Humanities (NAVF) for grants.

REFERENCES

1. Ghuysen, J.-M., Strominger, J. L. and Tipper, D. J. In Florkin, M. and Stotz, E. H., Eds., *Comprehensive Biochemistry 26 A*, Elsevier, Amsterdam 1967.

2. Strominger, J. L. In Gunsalus, I. C. and Stanier, R. Y., Eds., *The Bacteria 3,* Academic, London 1962.
3. Gale, E. F., Cundcliffe, E., Reynolds, P. E., Richmond, M. H. and Waring, M. J. *The Molecular Basis of Antibiotic Action,* Wiley, London 1972.
4. Edwards, D. I. *Antimicrobial Drug Action,* MacMillan, London 1980.
5. Nieto, M. and Perkins, H. R. *Biochem. J. 123* (1971) 789.
6. Sheldrick, G. M., Jones, P. G., Kennard, O., Williams, D. H. and Smith, G. A. *Nature 271* (1978) 223.
7. Williamson, M. P. and Williams, D. H. *J. Am. Chem. Soc. 103* (1981) 6580.
8. Strominger, J. L., Izaki, K., Matsuhashi, M. and Tipper, D. J. In Perlman, D., Ed., *Topics in Pharmaceutical Sciences,* Interscience, New York 1968, Vol. 1.
9. Spratt, B. G. *Sci. Prog. Oxf. 65* (1978) 101.
10. Chu, D. T., Martin, J. R. and Thomas, A. M. *Antibacterial Peptides, U.S. Pat.* 4.309.342, 1982.
11. Smissman, E. E., Terada, A. and El-Antably, S. *J. Med. Chem. 19* (1976) 165.
12. König, W. and Geiger, R. *Chem. Ber. 103* (1970) 788.
13. Bodanszky, M., Fink, M. L., Klausner, Y. S., Natarajän, S., Tatemoto, K., Yiotakis, A. and Bodanszky, A. *J. Org. Chem. 42* (1977) 149.
14. Downie, I. M., Holmes, J. B. and Lee, J. B. *Chem. Ind. London (1966) 900.*
15. *Appel, R. Angew. Chem. 87* (1975) 863.
16. Tömösközi, I., Gruber, L. and Radics, L. *Tetrahedron Lett.* (1975) 2473.
17. Stothers, J. B. *Carbon-13 NMR Spectroscopy,* Academic, New York 1972.
18. Darzens, G. *Compt. Rend. 152* (1911) 1314, 1601.
19. Gerrard, W. and Hudson, H. R. *J. Chem. Soc.* (1963) 1059.
20. Felix, A. M., Heimer, E. P., Lambros, T. J., Tzougraki, C. and Meienhofer, J. *J. Org. Chem. 43* (1978) 4194.
21. Roche, J., Thoai, N. v. and Hatt, J. L. *Biochim. Biophys. Acta 14* (1954) 71.
22. Anwer, M. K. and Spatola, A. F. *Synthesis* (1980) 929.
23. Craig, L. C., Hausmann, W. and Weisiger, J. R. *J. Am. Chem. Soc. 76* (1954) 2839.
24. Manning, J. M. *J. Am. Chem. Soc. 92* (1970) 7449.
25. Wise, R., Andrews, J. M. and Bedford, K. A. *J. Antimicrobial Chemother. 6* (1980) 197.
26. Gibian, H. and Schröder, E. *Justus Liebigs Ann. Chem. 642* (1961) 145.
27. Moroder, L., Hallett, A., Wünsch, E., Keller, O. and Wersin, G. *Hoppe-Seyler's Z. Physiol. Chem. 357* (1976) 1651.
28. Schnabel, E. *Justus Liebigs Ann. Chem. 702* (1967) 188.
29. Schnabel, E., Stoltefuss, J., Offe, H. A. and Klauke, E. *Justus Liebigs Ann. Chem. 743* (1971) 57.
30. Hofmann, K., Peckham, W. D. and Rheiner, A. *J. Am. Chem. Soc. 78* (1956) 238.
31. Nagasawa, T., Kuroiwa, K., Narita, K. and Isowa, Y. *Bull. Chem. Soc. Jpn. 46* (1973) 1269.
32. Wünsch, E. and Zwick, A. *Chem. Ber. 97* (1964) 3312.
33. Frank, H., Woiwode, W., Nicholson, G. and Bayer, E. *Ann. Chem.* (1981) 354.
34. Frank, H., Nicholson, G. J. and Bayer, E. *J. Chromatogr. Sci. 15* (1977) 174.
35. Nicholson, G. J., Frank, H. and Bayer, E. *J. High Resolution Chromatogr. Chromatogr. Commun. 2* (1979) 411.
36. Liardon, R., Ledermann, S. and Ott, U. *J. Chromatogr. 203* (1981) 385.
37. Fuglesang, J. E. and Bergan, T. *Infection. In press.*

Received February 4, 1983.