Acta Chemica Scandinavica B 38 (1984) 647–655

Synthesis of D-Ala–D-Ala Analogues with Postulated Antibacterial Activity

THRINA LOENNECHEN,[a] TOM BERGAN,[b] LEIV K. SYDNES [a] and ARNE J. AASEN[c]

[a] Department of Chemistry, University of Tromsø, P.O. Box 953, 9001 Tromsø, Norway.
[b] Department of Microbiology and [c] Department of Chemistry, Institute of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, Oslo 3, Norway

The syntheses of the L,L- and D,D-stereoisomers of *N*-phenoxyacetyl-X-alanine in which X=His, Tyr, or Lys, are described. The antibacterial activity of some of the peptide derivatives and their synthetic intermediates have been examined. Some of the intermediates exhibited moderate activity against viridans streptococci, enterococci and *Streptococcus agalactiae*. None of the compounds were active against *β*-lactamase producing bacteria or served as *β*-lactamase inhibitors.

In the search for drugs exhibiting antibacterial activity broader or different from that of established antibiotics[1-4] D-Ala–D-Ala analogues have emerged as interesting compounds due to their *β*-lactam feature.[5-7] The present paper describes the synthesis of various such dipeptides with an *N*-terminal phenoxyacetyl group (by analogy with phenoxymethylpenicillin) and their testing against some bacterial strains.

RESULTS AND DISCUSSION

Syntheses. The amino acids are numbered according to the nomenclature proposed in Ref. 8. "L/D" denotes L- or D-, or L,L- or D,D-derivatives, respectively. The preparations of PhO-CH_2CO-His-Ala (*9*) and its enantiomer PhO-CH_2CO-D-His-D-Ala (*16*) are outlined in Fig. 1.

0302-4369/84 $2.50

The syntheses were initiated by condensing Boc-His(Bzl) (*3*) and Boc-D-His(Bzl) (*10*) with Ala-OBzl (*5*) and D-Ala(OBzl) (*12*), respectively, to the protected dipeptides Boc-L/D-His(Bzl)-L/D-Ala-OBzl (*6* and *13*) utilizing the DCC method. The 1,4-disubstituted pattern of *3* and *10* was established on the basis of ^{1}H NMR data.[9] The L,L-isomer was also obtained, although in low yield (12 %), by applying the recently developed push-pull method.[10,11] The low yield of *6* might be due to steric hindrance of the nucleophilic attack of the histidyl derivative on the somewhat crowded push-pull acetylene. Analogous coupling to prepare Boc-His-Ala-OBzl was attempted by use of the less bulky nucleophile Boc-His. However, this procedure did not yield any dipeptide derivative. The Boc groups of the dipeptide derivatives *6* and *13* were cleaved when subjected to TFA and the products phenoxyacetylated to yield PhO-CH_2CO-L/D-His(Bzl)-L/D-Ala-OBzl (*7* and *14*) by utilizing various reactive derivatives of PhO-CH_2COOH. The L,L-isomer *7* was prepared by applying the corresponding acyl chloride (*1a*) in refluxing ethyl acetate, and by the more gentle (−10 to +20 °C) push-pull method. Both methods gave *7* with approximately the same optical activity, but the yield was more favourable (95 % versus 67 %) in the case of the latter method. The masking Bzl ester groups of *7* and *14* were removed by selective catalytic hydrogenolysis in CH_3OH. This gave the histidine-blocked derivatives *8* and *15*, which were ultimately subjected to catalytic hydrogenolysis in CH_3OH containing

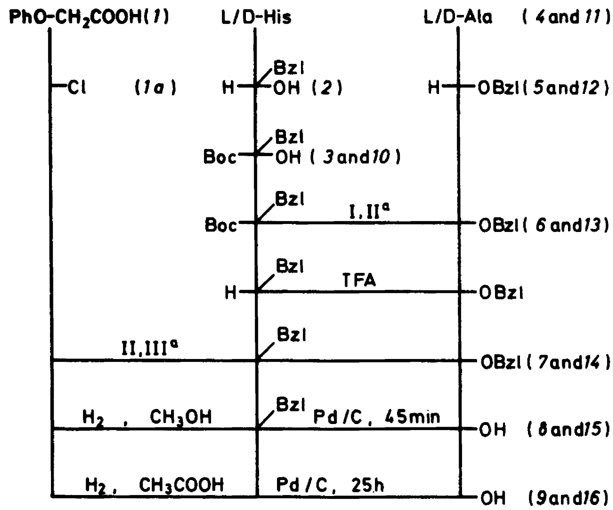

Fig. 1. The syntheses of PhO-CH_2CO-L/D-His-L/D-Ala (*9* and *16*). I, II, III and IV denote the DCC, the push-pull, the acid chloride and the active ester method, respectively. [a] L,L-isomer only. [b] D,D-isomer only.

AcOH. The structures of the crystalline products *9* and *16* were confirmed by ^{1}H and ^{13}C NMR as well as low and high resolution MS. Removal of the imidazole protecting group by a variety of other methods[12–14] was unsuccessful.

The enantiomeric pair PhO-CH_2CO-L/D-Tyr-L/D-Ala (*20* and *25*) was synthesized as summarized in Fig. 2. Boc-Tyr (*17*) and Boc-D-Tyr (*22*) were coupled to L/D-Ala-OBzl (*5* and *12*) employing both the push-pull and the DCC method. The amino groups of *18* and *23* were deprotected by treatment with TFA. The products were subsequently phenoxyacetylated by PhO-CH_2COOH activated as the *N*-hydroxysuccinimide ester *1b*, or as the acyl component in the push-pull process, to yield PhO-CH_2CO-L/D-Tyr-L/D-Ala-OBlz (*19* and *24*). Acylation with PhO-CH_2CO-OSu (*1b*) furnished the desired products in lower yields probably because of the lability of this ester. In order to permit a reliable comparison of the two methods, the active ester *1b* presumably has to be freshly prepared *in situ* prior to the coupling reaction. The Bzl groups of compounds *19* and *24* were removed by catalytic

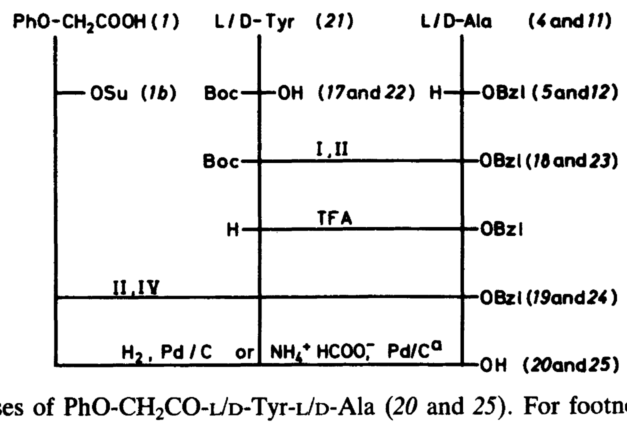

Fig. 2. The syntheses of PhO-CH_2CO-L/D-Tyr-L/D-Ala (*20* and *25*). For footnotes: see Fig. 1.

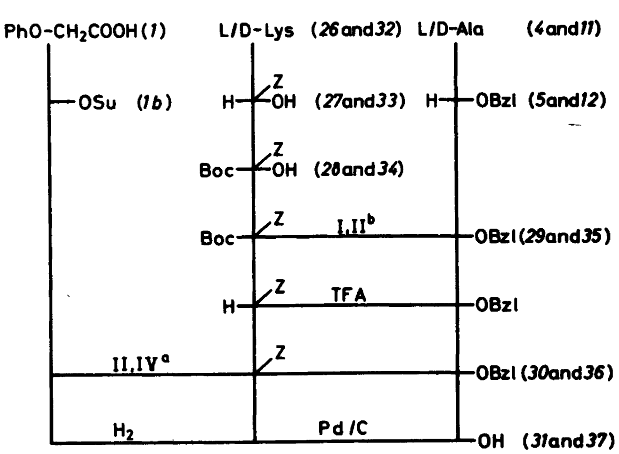

Fig. 3. The syntheses of PhO-CH_2CO-L/D-Lys-L/D-Ala (*31* and *37*). For footnotes: see Fig. 1.

hydrogenation to give *20* and *25*. The L,L-enantiomer (*20*) was also obtained in a good yield by catalytic transfer hydrogenation[12,13] employing ammonium formate[14] as hydrogen donor.

PhO-CH_2CO-Lys-Ala (*31*) and its enantiomer PhO-CH_2CO-D-Lys-D-Ala (*37*) were synthesized as outlined in Fig. 3. Boc-L/D-Lys(Z) (*28* and *34*) were condensed with L/D-Ala-OBzl (*5* and *12*) employing DCC as coupling reagent to yield the protected dipeptides Boc-L/D-Lys(Z)-L/D-Ala-OBzl (*29* and *35*). The D,D-enantiomer *35* was also obtained, although in a somewhat lower yield (80 % versus 94 %), by coupling according to the push-pull method. The Boc groups of *29* and *35* were cleaved by TFA to give L/D-Lys(Z)-L/D-Ala-OBzl·TFA which were subsequently acylated with PhO-CH_2COOH by the push-pull procedure to yield PhO-CH_2CO-L/D-Lys(Z)-L/D-Ala-OBzl (*30* and *36*). In the case of the L,L-enantiomer, the active ester PhO-CH_2CO-OSu (*1b*) was also utilized; however, the yield obtained was inferior. The Bzl and Z groups of derivatives *30* and *36* were removed by catalytic hydrogenation to give *31* and *37*, respectively.

Chiral purity of the peptide derivatives. The biological activity of peptides usually depends on their optical purity[15] and it was therefore of interest to examine the chiral purity of the D-Ala–D-Ala analogues. The analysis was carried out as outlined by Frank *et al.*[16] The results, summarized in Table 1, have been corrected for probable racemization during assay preparation.[16,17]

Table 1. Enantiomeric composition of the amino acids in the hydrolysates of the dipeptide derivatives.

	Compound PhO-CH_2CO-X-Y	Enantiomer[a] % L	% D
9	X=His[b]	–	–
	Y=Ala	96.1	3.9
16	X=D-His[b]	–	–
	Y=D-Ala	6.3	93.7
	Y=D-Ala[c]	9.9	90.1
20	X=Tyr	99.4	0.6
	Y=Ala	99.5	0.5
25	X=D-Tyr	3.4	96.6
	Y=D-Ala	0.7	99.3
31	X=Lys	98.9	1.1
	Y=Ala	100	0
37	X=D-Lys	0	100
	Y=D-Ala	0	100

[a] Values are corrected for probable racemization during acid hydrolysis according to investigations by Frank *et al.*[16]: 1.4, 1.2 and 1.6 % for L/D-Ala, L/D-Tyr and L/D-Lys, respectively. [b] Volatile derivatives of L/D-His were not obtained. [c] Treatment with alkali prior to the derivatization procedure, see Experimental.

Table 2. Minimum inhibitory concentration (mg/l) of three D-Ala–D-Ala analogues against five bacterial strains.

Organism	Compound 13	23	24
Streptococcus agalactiae, strain B	>100	>100	100
Streptococcus agalactiae, strain 1	50	>100	>100
Enterococci, strain 11255	>100	>100	100
Viridans streptococci, strain 12347	>100	>100	100
Viridans streptococci, strain 12478	100	100	50

A somewhat higher degree of racemization (3.4 % versus 0.6 %) was found for the D-Tyr moiety of PhO-CH_2CO-D-Tyr-D-Ala (*25*) than for its enantiomer *20*. Boc-D-Tyr (*22*) was prepared under strongly alkaline conditions (pH 10.2) which might have effected partial racemization. The procedure employed to prepare the commercially available Boc-Tyr (*17*) is unknown.

The slight differences in the chiral purity of the L/D-Lys moieties of PhO-CH_2CO-L/D-Lys-L/D-Ala (*31* and *37*) might follow from minor differences in the strongly alkaline conditions used when introducing the Boc groups into either Boc-Lys(Z) (*28*) or Boc-D-Lys(Z) (*34*).

The L/D-His moieties of PhO-CH_2CO-L/D-His-L/D-Ala (*9* and *16*) did not furnish the desired volatile derivatives excluding determination of the chiral purity of these building blocks. L- and D-Ala of *9* and *16* revealed some epimerization (3.9 and 6.3 %, respectively) which might have occurred during either the synthesis or the hydrolysis-derivatitization steps.

Antibacterial effect. The minimum inhibitory concentration (MIC) was greater than 100 mg/l for the compounds *7, 14, 15, 16, 20, 25, 31, 35, 36,* and *37* towards all bacterial strains. Three synthetic intermediates, Boc-D-His(Bzl)-D-Ala-OBzl (*13*), Boc-D-Tyr-D-Ala-OBzl (*23*) and PhO-CH_2CO-D-Tyr-D-Ala-OBzl (*24*), exhibited an antibacterial activity; *cf.* Table 2. These compounds were relatively lipophilic carrying a *C*-terminal benzyl ester group and an *N*-terminal PhO-CH_2CO or a Boc group. No activity was exhibited by the final synthetic products, which were carboxylic acids. Whether the esters are active *per se*, or they are hydrolyzed after passage through parts of the bacterial cell wall and at the target site exert their activity as free acids, which are structurally more similar to D-Ala–D-Ala, is a matter of conjecture. The fact that these agents inhibited only Gram-positive bacteria might be a consequence of an easier penetration to the site of action in these bacteria, which, unlike Gram-negative cells, are without a lipophilic outer cell wall membrane. The antibacterial activity supports the idea that D-Ala–D-Ala analogues might be able to interfere with bacterial cell wall synthesis. The compounds tested in this regard, however, did not serve as β-lactamase inhibitors, as do *e.g.* clavulanic acid and sulbactam, which have only little inherent antibacterial activity.[18]

EXPERIMENTAL

General. Melting points (uncorrected), optical rotations, and mass spectra were recorded on Mettler FP61 or Electrothermal, Perkin-Elmer 241 and Micromass 7070H instruments, respectively. Chemical ionization mass spectra (CI) were obtained by the direct inlet method using isobutane as ionizing gas. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol FX90Q instrument operating in the pulsed-Fourier transform mode. NMR data of the intermediates are available on request to the authors. ^{13}C NMR spectra were obtained using a pulse width of 5.5 μs (45° pulse), a spectral width of 5000 Hz (16K data points), an acquisition time of 0.998 s and a pulse repetition time of 3.0 s. The sodium salt of 3-(trimethylsilyl)propanesulfonic acid was used as an internal reference when spectra were obtained in D_2O; otherwise TMS was used. Unless stated otherwise, analytical TLC was performed on silica gel F_{254} plates (HPTLC pre-coated plates, Merck) using the following eluants: (A) BuOH–AcOH–pyridine–H_2O= 15:3:10:12, (B) 5 % CH_3OH in $CHCl_3$, (C) 10 % CH_3OH in $CHCl_3$, (D) CH_2Cl_2–THF=4:1, (E)

CH_2Cl_2–THF=8:1, (F) CH_2Cl_2–THF=20:1, (G) CH_3CH_2OH–H_2O=5:1, and (H) CH_3OH–benzene=1:1. When explicitly mentioned, reversed phase F_{254} plates (RP-18 HPTLC, Merck) were used. Spots were visualized with molybdophosphoric acid spray (Merck), UV-light (254 nm), or, for some His derivatives, Pauly-reagent.[19,20] Column chromatography was performed on Merck Kieselgel 60 (0.040–0.063 mm).

Elemental analyses were carried out at *Ilse Beetz Mikroanalytisches Laboratorium*, Kronach, West-Germany. Gas chromatographic determination of the enantiomeric composition of the amino acids in the hydrolysates of the final peptide derivatives was carried out at the Central Institute for Industrial Research, Oslo. All coupling reactions were performed under nitrogen.

Abbreviations. Standard abbreviations for amino acids and protecting groups follow the tentative rules of the IUPAC-IUB Commision on Biochemical Nomenclature.[8] Additional abbreviations are used: DCC, *N,N'*-dicyclohexylcarbodiimide; DCU, *N,N'*-dicyclohexylurea; NEM, *N*-ethylmorpholine; TFA, trifluoroacetic acid; HOBt, 1-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; DCHA, dicyclohexylammonium; PhO-CH_2CO-, phenoxyacetyl.

Synthesis. The amino acid derivatives were synthesized using the general methods outlined below.

DCC coupling. This procedure was employed to couple *N*-protected amino acids to Ala-OBzl tosylate. DCC (1.1 eq) in CH_2Cl_2 was added in portions to a cold (−20 °C) suspension of the amino acid derivative (1.0 eq), NEM (1.0 eq), and HOBt or HOSu (2.5 eq) in CH_2Cl_2. The mixture was stirred at −20 °C for 1 h, stored overnight while the temperature slowly rose to 20 °C, and then stirred at 20 °C for 1 h. In some cases AcOH was then added. When AcOH was added the mixture was filtered after 5 min. The solid material was washed with CH_2Cl_2; the combined filtrates were diluted with CH_2Cl_2 and washed with 0.2 M HCl, 1 M $NaHCO_3$ and H_2O, dried over Na_2SO_4 and evaporated. Pure product was obtained by eluting the residue on a silica gel column with CH_2Cl_2 and 1 % CH_3OH in CH_2Cl_2.

When AcOH was *not* added at the end of the coupling the solvent was removed by flushing N_2. The resulting oil was dissolved in EtOAc which was washed three times each with 0.2 M HCl, 5 % $NaHCO_3$, and H_2O and dried over Na_2SO_4. Filtration and evaporation left a residue which gave pure product when chromatographed twice on silica gel columns with CH_2Cl_2 and 1–2 % CH_3OH in CH_2Cl_2 as eluants.

Push-pull coupling. 1-(4-Chlorophenyl)-3-(4-methyl-1-piperazinyl)-2-propyn-1-one (1 eq) in CH_2Cl_2 was added in portions to a cold (−10 °C) solution of carboxylic acid (1 eq) in CH_2Cl_2. The mixture was stirred at −10 °C for 1 h and at ambient temperature for *x* h (t_1=(1+*x*)h). The temperature was lowered to −10 °C and a solution of amine (0.9–1 eq) and NEM (1 eq) in CH_2Cl_2 was added. The mixture was stirred at −10 °C for 1 h and at room temperature for *y* h (t_2=(1+*y*) h). The solvent was removed and the residue dissolved in EtOAc which was washed three times with 0.2 M HCl, 1 M $NaHCO_3$ and H_2O, respectively, and dried (Na_2SO_4). Evaporation of the solvent left a residue from which the product was obtained by silica gel chromatography with CH_2Cl_2 and CH_2Cl_2–THF=4:1 as eluants.

Boc-His(Bzl) (3).[21,22] $[\alpha]_D^{20}$+23.2° (*c* 1; CH_3OH); lit.[21] $[\alpha]_D^{20}$ +24.8° (*c* 1; CH_3OH).

Ala-OBzl tosylate (5).[23] $[\alpha]_D^{2}$ −6.2° (*c* 2.5; CH_3OH); lit.[23] $[\alpha]_D^{26}$ −6 (*c* 4; CH_3OH).

Boc-His(Bzl)-Ala-OBzl (6) was obtained in 65 % yield from *3* and *5* by DCC coupling; m.p. 101 °C (from EtOAc–light petroleum); R_f 0.4 (B); $[\alpha]_D^{20}$ −16.1° (*c* 1; CH_3OH); MS (IP 70 eV): mol.wt. obs. 506.2498, calc. for $C_{28}H_{34}N_4O_5$ 506.25290. The yield dropped to 12 % when push-pull coupling of *3* and *5* was performed (t_1=18 h, t_2=49 h); $[\alpha]_D^{20}$ −16.3° (*c* 1; CH_3OH).

PhO-CH₂CO-His(Bzl)-Ala-OBzl (7). Boc-His(Bzl)-Ala-OBzl (506 mg; 1.0 mmol; *6*) was treated with 50 % TFA in CH_2Cl_2 (3 ml) in the presence of anisole (0.5 ml) for 1 h. Excess TFA and CH_2Cl_2 was removed *in vacuo*. The TFA salt of His(Bzl)-Ala-OBzl was subsequently treated with NEM (117 mg; 1.0 mmol) and refluxed in EtOAc (25 ml) in the presence of PhO-CH_2COCl[24] (171 mg; 1.0 mmol) for 2 h. The mixture was diluted with EtOAc (100 ml) at ambient temperature and then washed (0.2 M HCl, 1 M $NaHCO_3$, H_2O) and dried (Na_2SO_4). Evaporation of EtOAc left a residue which was purified by chromatography (silica gel, CH_2Cl_2 and CH_2Cl_2–THF=8:1) to give 367 mg (68 %) of *7;* m.p. 125 °C (from EtOAc–light petroleum); R_f 0.9 (C); $[\alpha]_D^{20}$ −9.6° (*c* 1; CH_3OH). Anal. $C_{31}H_{32}N_4O_5$: C, H, N. The yield increased to 95 % when push-pull coupling of $PhOCH_2COOH$ to His(Bzl)-Ala-OBzl was carried out (t_1=24 h, t_2=25 h); $[\alpha]_D^{20}$ −10.5° (*c* 1; CH_3OH).

PhO-CH₂CO-His(Bzl)-Ala (8) was prepared in 84 % by hydrogenation (10 % Pd/C, room temperature, 1 atm) of *7* in CH_3OH; m.p. 217–218 °C (from CH_3OH–Et_2O); R_f 0.5 (H);

Pauly-negative;[19,20] $[\alpha]_D^{20}$ +11.5° (*c* 1; CH_3OH); MS (IP 70 eV): mol.wt. obs. 450.1880, calc. for $C_{24}H_{26}N_4O_5$ 450.19031.

PhO-CH₂CO-His-Ala (9) was isolated in 60 % yield (column chromatography, silica gel, benzene and benzene:CH_3OH=4:1) after hydrogenation (10 % Pd/C, room temperature, 1 atm) of *8*; m.p. 138 °C (from CH_3OH-Et_2O); R_f 0.3 (H); Pauly-positive;[19,20] $[\alpha]_D^{20}$ −5.2° (*c* 0.7; H_2O); MS [IP 70 eV; m/z (%)]: 360 (3, M); mol.wt., obs. 360.1436, calc. for $C_{17}H_{20}N_4O_5$: 360.14436; ^1H NMR (89.55 MHz, D_2O): δ 1.43 (3H, d, *J* 7 Hz), 3.1−3.3 (2H, m), 4.20 (1H, q, *J* 7 Hz), 4.5 (1H?, partly coinciding with the solvent signal), 4.67 (2H, s), 6.8−7.5 (6H, m), 8.3−8.5 (1H, m); ^{13}C NMR (22.50 MHz, D_2O): δ 19.5, 29.4, 53.4, 54.1, 68.9, 117.0, 119.9, 124.7, 130.9, 132.3, 136.3, 159.3, 172.4, 173.5, 181.9.

D-*Ala-OBzl tosylate* (12).[25] $[\alpha]_D^{20}$ +6.0° (*c* 2.3; CH_3OH); lit.[25] $[\alpha]_D^{27}$ +6.9° (2 % in H_2O).

*Boc-*D*-His(Bzl)-*D*-Ala-OBzl* (13) was prepared in 70 % by DCC coupling of *10* and *12*; m.p. 100 °C; $[\alpha]_D^{20}$ +15.0° (*c* 1; CH_3OH). Found: C 65.53; H 6.81; N 11.22. Calc. for $C_{28}H_{34}N_4O_5$: C 66.38; H 6.77; N 11.06. R_f 0.4 (B); MS [IP 70 eV; m/z (%)]: 506 (0.5, M); ^1H NMR (89.55 MHz, $CDCl_3$): δ 1.25 (3H, d, *J* 7 Hz), 1.44 (9H, s), 2.85−3.10 (2H, m), 4.25−4.65 (2H, m), 4.99 (2H, s), 5.13 (2H, s), 6.15−6.35 (1H, m), 6.68 (1H, m), 7.10−7.60 (3H, m), 7.30 (10H, s); ^{13}C NMR (22.50 MHz, $CDCl_3$): δ 18.1 (q), 28.3 (q), 30.5 (t), 48.2 (d), 51.0 (t), 54.8 (d), 66.9 (t), 78.8 (s), 117.3 (d), 127.5 (d), 128.0 (d), 128.3 (d), 135.6 (s), 135.8 (s), 136.6 (d), 138.4 (s), 155.6 (s), 172.4 (s), 173.4 (s).

*PhO-CH₂CO-*D*-His(Bzl)-*D*-Ala-OBzl* (14) was synthesized in 76 % yield by push-pull coupling of $PhOCH_2COOH$ and D-His(Bzl)-D-Ala-OBzl, obtained from *13* as described for compound *7*; m.p. 122 °C; $[\alpha]_D^{20}$ +10.4° (*c* 1; CH_3OH). Anal. $C_{31}H_{32}N_4O_5$: C, H, N.

*PhO-CH₂CO-*D*-His(Bzl)-*D*-Ala* (15) was prepared in 95 % yield by hydrogenation (10 % Pd/C, room temperature, 1 atm) of *14* in CH_3OH; m.p. 218−220 °C; $[\alpha]_D^{20}$ −10.0° (*c* 1; CH_3OH). Anal. $C_{24}H_{26}N_4O_5$: C, H, N.

*PhO-CH₂CO-*D*-His-*D*-Ala* (16) was synthesized in 57 % yield by hydrogenation (10 % Pd/C, room temperature, 1 atm) of *15* in CH_3OH:AcOH=5:1; m.p. 142−147 °C; MS (CI, *m/z* (%)): 361 (0.1, M+1); $[\alpha]_D^{20}$ +6.4° (*c* 0.7; H_2O).

Boc-Tyr-Ala-OBzl (18) was obtained in 62 % yield from Boc-Tyr[26] ($[\alpha]_D^{20}$ +3.7° (*c* 2; AcOH)) and *5* by DCC coupling; m.p. 120 °C; R_f 0.8 (C); $[\alpha]_D^{20}$ −15.8° (*c* 1; CH_3OH); MS [IP 70 eV; *m/z* (%)]: 442 (0.01, M). Anal. $C_{24}H_{30}N_2O_6$: C, H, N. The yield increased to 79 % when the push-pull method was employed (t_1=4 h, t_2=22 h).

PhO-CH₂CO-Tyr-Ala-OBzl (19) was prepared in 18 % yield from *18* and PhO-CH_2CO-OSu (*1b*) as described for compound *7*; m.p. 178−182 °C; R_f 0.6 (B); $[\alpha]_D^{20}$ −19.5° (*c* 1; CH_3OH); MS [IP 70 eV; *m/z* (%)]: 476 (0.5, M). When *19* was prepared by push-pull coupling of Tyr-Ala-OBzl·HCl and PhO-CH_2COOH (t_1=3 h, t_2=24 h) the yield was 96 %.

PhO-CH₂CO-Tyr-Ala (20) was prepared in 98 % yield by hydrogenation (10 % Pd/C, room temperature, 1 atm) of *19* in CH_3OH; m.p. 155 °C (from CH_3OH−EtOAc); R_f 0.8 (G); R_f 0.9 (G, reversed phase TLC); $[\alpha]_D^{20}$ −4.6° (*c* 1; DMF); MS [IP 70 eV; *m/z* (%)]: 386 (0.8, M); ^1H NMR (89.55 MHz, CD_3OD): δ 1.39 (3H, d, *J* 7 Hz), 2.7−3.2 (2H, m), 4.25−4.72 (2H, m), 4.85 (2H, s), 6.5−7.4 (9H, m); ^{13}C NMR (22.50 MHz, THF-d_8): δ 18.3, 38.2, 48.6, 54.5, 68.1, 115.7, 115.8, 122.1, 128.3, 130.1, 131.1, 157.2, 159.0, 168.1, 170.9, 174.3. Anal. $C_{20}H_{22}N_2O_6$: C, H, N. Catalytic transfer hydrogenation[14] of *19* using ammonium formate gave *20* in 91 % yield.

*Boc-*D*-Tyr-*D*-Ala-OBzl* (23) was synthesized in 40 % yield from Boc-D-Tyr DCHA (*22*)[22,27] ($[\alpha]_D^{20}$ −2.9°, (*c* 2; AcOH)) and *12* by DCC coupling; $[\alpha]_D^{20}$ +15.2° (*c* 1; CH_3OH). The compound was isolated in 64 % yield when push-pull coupling (t_1=21 h, t_2=32 h) was carried out; $[\alpha]_D^{20}$ +14.7° (*c* 1.2; CH_3OH); R_f 0.8 (C); MS [IP 70 eV; *m/z* (%)]: 442 (0.01, M); ^1H NMR (89.55 MHz, $CDCl_3$); δ 1.34 (3H, d, *J* 7 Hz), 1.41 (9H, s), 2.9−3.0 (2H, m), 4.2−4.5 (2H, m), 5.14 (2H, s), 6.5−6.7 (1H, m, N*H*), 6.70 (2H, m, J_{ortho} 8 Hz), 7.01 (2H, m, J_{ortho} 8 Hz), 7.33 (5H, s), 7.6−7.7 (1H, m, N*H*); ^{13}C NMR (22.50 MHz, $CDCl_3$): δ 17.8 (q), 28.2 (q), 37.6 (t), 48.3 (d), 56.3 (d), 67.1 (t), 80.4 (s), 115.6 (d), 127.5 (d), 128.1 (d), 128.3 (s), 128.5 (d), 130.3 (d), 135.3 (s), 155.6 (s), 155.7 (s), 171.2 (s), 171.9 (s).

*PhO-CH₂CO-*D*-Tyr-*D*-Ala-OBzl* (24) was prepared in 43 % yield by coupling PhO-CH_2CO-OSu to D-Tyr-D-Ala-OBzl (from *23* by TFA treatment) as described for compound *19*; m.p. 178 °C; $[\alpha]_D^{20}$ +21.0° (*c* 1; CH_3OH). Anal. $C_{27}H_{28}N_2O_6$: C, H, N. Push-pull coupling of PhO-CH_2COOH and *23* (t_1=26 h, t_2=49 h) gave *24* in 85 % yield; $[\alpha]_D^{20}$ +18.8° (*c* 1; CH_3OH); R_f 0.8 (D); MS [IP 70 eV; *m/z*(%)]; 476 (0.5, M); ^1H NMR (89.55 MHz, CD_3OD): δ 1.38 (3H, d, *J* 7 Hz), 2.63−3.15 (2H, m), 4.42 (2H, s), 4.25−4.75 (2H, m), 5.15 (2H, s), 6.64 (2H, m, J_{ortho} 8 Hz), 6.98 (2H, m, J_{ortho} 8 Hz), 6.7−7.3 (5H, m), 7.33 (5H, s), 7.4−7.6 (1H, m, N*H*); ^{13}C NMR (22.50 MHz, CD_3OD): δ 17.3 (q), 37.0 (t), 49.0 (d), 55.0 (d), 67.9 (t), 68.1 (t), 115.8 (d), 116.2 (2), 122.8 (d), 128.3 (s), 129.2 (d), 129.4 (s), 130.6 (d), 131.3 (d), 137.2 (s), 157.3 (s), 159.0 (s), 170.5 (s), 172.7 (s), 173.4 (s).

PhO-CH_2CO-D-Tyr-D-Ala (25) was prepared by hydrogenation (10 % Pd/C, room temperature, 1 atm) of *24* in CH_3OH in 95 % yield; m.p. 155 °C; $[\alpha]_D^{20}$ +4.2° (*c* 1; DMF).

Boc-Lys(Z)-Ala-OBzl (29) was synthesized in 79 % from Boc-Lys (Z) DCHA[22,26,28-30] ($[\alpha]_{578}^{20}$ −7.9° (*c* 1.1; AcOH)) and *5*, using DCC as coupling reagent in the presence of HOBt; m.p. 110 °C (from EtOAc−light petroleum); R_f 0.6 (B); $[\alpha]_D^{20}$ −30.7° (*c* 1; CH_3OH). Anal. $C_{29}H_{39}N_3O_7$: C, H, N.

PhO-CH_2CO-Lys(Z)-Ala-OBzl (30) was obtained in 20 % yield from *29* and PhO-CH_2CO-OSu in the same manner as compound *7* except that anisole was not used; m.p. 123−124 °C (from EtOAc−light petroleum); R_f 0.7 (E); $[\alpha]_D^{20}$ −13.5° (*c* 1; $CHCl_3$). Anal. $C_{32}H_{37}N_3O_7$: C, H, N. When the TFA salt of Lys(Z)-Ala-OBzl (from *29*) and PhO-CH_2COOH were coupled using the push-pull method (t_1=17 h, t_2=24 h) *30* was obtained in 90 % yield; $[\alpha]_D^{20}$ −14.3° (*c* 1.1; $CHCl_3$).

PhO-CH_2CO-Lys-Ala (31) was obtained in 84 % by hydrogenation (10 % Pd/C, room temperature, 1 atm) of *30* in CH_3OH; m.p. 200 °C (from CH_3OH−Et_2O); R_f 0.45 (G); $[\alpha]_D^{20}$ −24.0° (*c* 0.3; H_2O); MS [IP 70 eV; *m/z* (%)]: 351 (0.3, M); ^1H NMR (89.55 MHz, D_2O): δ 1.1−2.0 (6H, m), 1.31 (3H, d, *J* 7 Hz), 2.8−3.1 (2H, m), 4.13 (1H, q, *J* 7 Hz), 4.42 (1H, t, *J* 7 Hz), 4.7 (the signal was partly blurred by the HDO peak), 6.9−7.6 (5H, m); ^{13}C NMR (22.50 MHz, D_2O): δ 20.0, 24.3, 28.7, 33.1, 41.8, 53.5, 55.5, 69.2, 117.4, 124.4, 132.5, 159.6, 173.7, 174.6, 181.9. Anal. Found: C 57.34; H 7.21; N 11.46. Calc. for $C_{17}H_{25}N_3O_5$; C 58.10; H 7.17; N 11.96.

Boc-D-Lys(Z)-D-Ala-OBzl (35) was prepared in 94 % yield by DCC coupling of Boc-D-Lys(Z) to *12*; m.p. 109 °C. $[\alpha]_D^{20}$ +31.6° (*c* 1.1; CH_3OH). Anal. $C_{29}H_{39}N_3O_7$: C, H, N. Push-pull coupling of the same compounds (t_1=18 h, t_2=21 h) gave *35* in 80 % yield; $[\alpha]_D^{20}$ +31.0° (*c* 1; CH_3OH).

PhO-CH_2CO-D-Lys(Z)-D-Ala-OBzl (36) was synthesized in 80 % yield by push-pull coupling (t_1=17 h, t_2=24 h) of *1* to D-Lys(Z)-D-Ala-OBzl (obtained from *35* by TFA treatment); m.p. 124 °C; $[\alpha]_D^{20}$ +13.4° (*c* 1; CH_3OH). Anal. $C_{32}H_{37}N_3O_7$: C, H, N.

PhO-CH_2CO-D-Lys-D-Ala (37) was obtained in 97 % yield by hydrogenation (10 % Pd/C, room temperature, 1 atm) of *36* in CH_3OH; $[\alpha]_D^{20}$ +24.0° (*c* 0.3; H_2O). Anal. $C_{17}H_{25}N_3O_5$: C, H, N.

Assays for chiral purity analysis. The assay procedure of Frank *et al.*[16] was employed. In the case of *16*, complete derivatization of His was attempted both by additional treatment with isobutyl chloroformate (50 μl) at 110 °C for 10 min,[32] and addition of alkali (1 eq. $NaHCO_3$) prior to the derivatization procedure. The results are presented in Table 1.

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 H_2O or CH_3CH_2OH. From these, dilutions were made in sterile H_2O and added to the medium. The highest CH_3CH_2OH concentration present in the growth medium did not interfere with bacterial growth.

MIC of the compounds *7, 20, 25, 31* and *37* was determined with the following 25 bacterial strains, which were in part 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 substances *13, 14, 15, 16, 23, 24, 35* and *36* was determined against the following 25 isolates: *Citrobacter* sp. 11, *E. coli* 649, *K. aerogenes* 670, *M. luteus* ATCC 9341, *S. aureus* 464, 1718, 1771, 4242, *Str. 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 β-lactamase inhibitors like clavulanic acid and sulbactam.[18] For this purpose, synergy was tested with the substances *7, 20, 25, 31* and *37* against the 25 first listed bacteria and the five β-lactamase producing strains of *E. coli* 2526, 1517, 2173 and *K. aerogenes* 134, and 135. Synergy was also tested with *E. coli* JT R^+, which carries a TEM^+ plasmid, and strain JT R^-, which is its plasmid deficient parallel. The last seven strains have previously been employed in the study of β-lactamase inhibitors.[33]

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 area of 0.25 cm^2.

Acknowledgements. The authors are indebted to cand. real. John H. Johansen, University of Oslo, for valuable discussions. Grateful acknowledgement is made to the Norwegian Research Council for Science and the Humanities (NAVF) for grants.

REFERENCES

1. Sammes, P. G. *Chem. Rev. 76* (1976) 113.
2. Abraham, E. P. *Sci. Am. 244* (1981) 64.
3. Raff, M. J. and Summersgill, J. T. *Process Biochem. 16* (1981) 15.
4. Bremner, D. *New Scientist 91* (1981) 352.
5. Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H. and Waring, M. J. *The Molecular Basis of Antibiotic Action,* Wiley, London 1972.
6. Smissmann, E. E., Terada, A. and El-Antably, S. *J. Med. Chem. 19* (1976) 165.
7. Okada, Y., Okinaka, M., Yagyu, M., Watabe, K., Sano, K. and Kakiuchi, Y. *Chem. Pharm. Bull. 24* (1976) 3081.
8. IUPAC Commission on the Nomenclature of Organic Chemistry and IUPAC-IUB Commission on Biochemical Nomenclature *Nomenclature of α-Amino Acids, Biochem. 14* (1975) 449; Commission on Biochemical Nomenclature *Symbols for Amino-Acid Derivatives and Peptides, J. Biol. Chem. 247* (1972) 977.
9. Matthews, H. R. and Rapoport, H. *J. Am. Chem. Soc. 95* (1973) 2297.
10. Neuenschwander, M., Fahrni, H.-P. and Lienhard, U. *Helv. Chim. Acta 61* (1978) 2437.
11. Neuenschwander, M. and Stämpfli, U. *Chimia 33* (1979) 439.
12. Felix, A. M., Heimer, E. P., Lambros, T. J., Tzougraki, C. and Meienhofer, J. *J. Org. Chem. 43* (1978) 4194.
13. Sivanandaiah, K. M. and Gurusiddappa, S. *J. Chem. Res. (S)* (1979) 108.
14. Anwer, M. K. and Spatola, A. F. *Synthesis* (1980) 929.
15. Bodanszky, M., Klausner, Y. S. and Ondetti, M. A. *Peptide Synthesis,* 2nd Ed., . Wiley, New York 1976.
16. Frank, H., Woiwode, W., Nicholson, G. and Bayer, E. *Justus Liebigs Ann. Chem.* (1981) 354.
17. Liardon, R., Ledermann, S. and Ott, U. *J. Chromatogr. 203* (1981) 385.
18. Wise, R., Andrews, J. M. and Bedford, K. A. *J. Antimicrob. Chemother. 6* (1980) 197.
19. Von Arx, E. and Neher, R. *J. Chromatogr. 12* (1963) 329.
20. *Dying Reagents for Thin Layer and Paper Chromatography,* Merck, E., Darmstadt, West-Germany 1976, p. 94.
21. Nagasawa, T., Kuroiwa, K., Narita, K. and Isowa, Y. *Bull. Chem. Soc. Jpn. 46* (1973) 1269.
22. Schnabel, E. *Justus Liebigs Ann. Chem. 702* (1967) 188; Moroder, L., Hallett, A., Wünsch, E., Keller, O. and Wersin, G. *Hoppe-Seyler's Z. Physiol. Chem. 357* (1976) 1651.
23. Gibian, H. and Schröder, E. *Justus Liebigs Ann. Chem. 642* (1961) 145.
24. Rosenmund, K. W. and Zetzsche, F. *Ber. Dtsch. Chem. Ges. 56* (1923) 1481.
25. Winitz, M., Block-Frankenthal, L., Isumiya, N., Birnbaum, S. M., Baker, C. G. and Greenstein, J. P. *J. Am. Chem. Soc. 78* (1956) 2423.
26. Anderson, G. W. and McGregor, A. C. *J. Am. Chem. Soc. 79* (1957) 6180.
27. Broadbent, W., Morley, J. S. and Stone, B. E. *J. Chem. Soc. C* (1967) 2632.
28. Zahn, H. and Schmidt, F. *Makromol. Chem. 36* (1960) 1.
29. Polzhofer, K. P. *Tetrahedron 28* (1972) 855.
30. Zahn, H. and Falkenburg, H. R. *Justus Liebigs Ann. Chem. 636* (1960) 117.
31. Eckstein, H., Sievers, R. E. and Bayer, E. *Justus Liebigs Ann. Chem.* (1973) 1467.
32. Liardon, R. and Ledermann, S. *J. High Resolut. Chromatogr. Chromatogr. Commun. 3* (1980) 475.
33. Fuglesang, J. E. and Bergan, T. *Infection. In press.*
34. Voelter, W., Fuchs, St., Seuffer, R. H. and Zech, K. *Monatsh. Chem. 105* (1974) 1110.
35. Deslauriers, R. and Smith, I. C. P. In Berliner, L. J. and Reuben, J., Eds., *Biological Magnetic Resonance,* Plenum, New York 1980, vol. 2, p. 243.
36. Deslauriers, R., Garrigou-Lagrange, C., Bellocq, A.-M. and Smith, I. C. P. *FEBS Lett. 31* (1973) 59.

37. Pasaribu, S. J. *Aust. J. Chem. 32* (1979) 1575.
38. Margetson, S. A. and Moore, W. J. *Aust. J. Chem. 33* (1980) 2411.
39. Pasaribu, S. J. *Aust. J. Chem. 33* (1980) 2427.

Received October 26, 1983.