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Application of 4-Chlorobutyryl Group as Amino Protective Group in the Synthesis of 7-(D-2-Amino-2-phenylacetamido)-3--methyl-3-cephem-4-carboxylic Acid (Cephalexin)*[1,2]

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4-Chlorobutyryl group was used as amino protective group for D(-)-alpha-phenylglycine (I). *N*-Hydroxysuccinimide ester III was prepared in high yield but partly racemic. Reaction of III or IV with V afforded epimeric mixtures of N,O-protected cephalexins (VI), which can be separated by crystallisation. The 4-chlorobutyryl group in VII was removed under very mild conditions giving cephalexin (IX) in good yield and purity.

Of the several synthetic routes to cephalexin[3], the acylation of 7-aminodeacetoxycephalosporanic acid (7-ADCA; V, R=H) has been one of the widely used methods[4]. However, this method presents a problem in the selection of a convenient amino protecting group for D(-)-alpha-phenylglycine (I). Although many excellent amino protecting groups have been developed for use in the peptide synthesis[5], only few of them are available for use in beta-lactam chemistry, since usual methods of removal would disrupt the beta-lactam ring. The most useful groups for blocking beta-lactam antibiotics with an amino substituted side chain, have been discussed in recently published reviews[6]. The present paper is confined to the possible use of 4-chlorobutyryl group as acyl protective group for D(-)-alpha-phenylglycine during the acylation of 7-ADCA.

In general, simple acyl groups are not suitable as amino protective groups even in peptide synthesis, because they can form an additional amide bond that would be difficult to cleave selectively. Moreover, the possible formation of an oxazolone ring, common to all *N*-acyl-alpha-aminoacids, may lead to racemisation and therefore limits the application of an acyl protecting group for alpha-aminoacids[7].

The difficulty in selective removal of these groups can be eliminated in part by the use of an acyl group that can be removed by special methods, such as simple solvolytic reactions, or neighbouring group effects. One of such acyl groups is omega-halogenoacyl, which easily cyclises to iminoether in neutral medium, due to the participation of an amido nitrogen group during

* The authors dedicate this paper to Dr R. Seiwerth, for his pioneer work on the chemistry of penicillins in Pliva, on the occasion of his retirement.

internal dispalcement of halide ion. These iminoethers in most cases are rapidly hydrolised to give lactons and the free amino group[8]. The use of 4-chlorobutyryl (4-ClBu) as amino acyl protecting group was described by Peter[9] in the synthesis of some simple dipeptides. For the removal of this group silver tetrafluoroborate was used to form cyclic iminoether, which was further cleaved on coupling with the second aminoacid chloride.

In the preparation of some model dipeptides, with 4-ClBu as the amino protecting group, we detected easy removal of this group without silver tetrafluoroborate and under very mild reaction conditions[10]. On the other hand, in relation to the early observation of Peter[9], *N,O*-protected dipeptides gave in our case easily separable crystalline diastereoisomers. In such a way, the second difficulty in the use of an acyl function as a protecting group was largely dimminished.

These results stimulated us to study the possible application of 4-ClBu group, as an amino protecting group in the synthesis of semisynthetic cephalosporins. In the present paper we wish to report the use of 4-ClBu group as a protecting group for D(-)-alpha-phenylglycine (II) during the acylation of 7-ADCA in the course of the preparation of cephalexin.

N-Halogenoacyl derivatives of aminoacids can be prepared by standard methods of acylation, in this case the reaction is carried out with 4-chlorobutyryl chloride in aqueous solution and in the presence of base[9,11]. In some cases, higher yield and better purity of the product were obtained when the aminoacid was first protected with trimethylsilyl chloride[10]. Therefore, D(-)-alpha-phenylglycine was first silylated and then acylated with the acid chloride. The hydrolysis of *N*-4-ClBu-D(-)-alpha-phenylglycine (heating in water for 1 hour) gave D(-)-alpha-phenylglycine with the same value of optical rotation as the starting material.

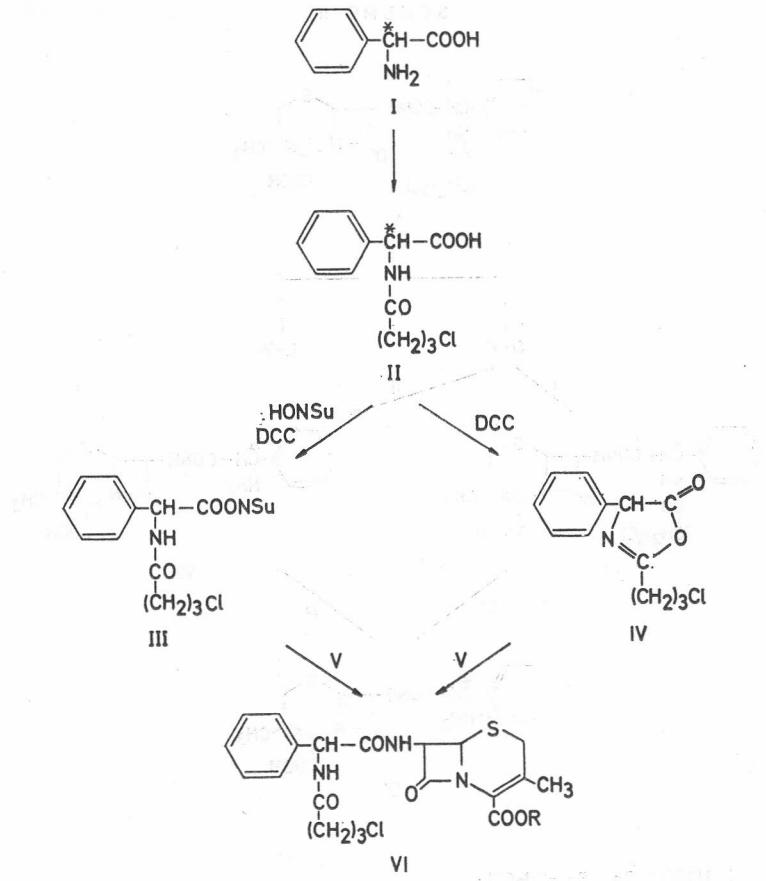
Of the several methods for the activation of the phenylglycine carboxyl group, the best results were obtained by the use of an active ester. *N*-Hydroxysuccinimide ester (III) was prepared by a standard DCC procedure, in 95% yield. The product was crystalline but partly recemic, due to the formation of oxazolone in the course of the reaction. The presence of oxazolone was indicated by the absorption band 1832 cm^{-1} in the IR spectrum of the sample taken from the reaction solution. The same absorption band in the IR spectrum was detected in a sample of 2-(3-chloropropyl)-4-phenyl-oxazol-5-on (IV) prepared from *N*-4-ClBu-alpha-phenylglicine with DCC.

Prior to acylation, the carboxyl group of 7-ADCA was also protected by a suitable ester. Several esters of 7-ADCA (V) were prepared by the procedure described[12,13], in order to detect which one would yield, upon acylation, the easy crystallisable and the best separable *N,O*-protected cephalexin (VI).

Sufficiently different R_f values of D- and L-isomers of VI on TLC, enabled us to follow the course of separation of isomers by crystallisation and to detect their purity before deprotection to give cephalexin.

The isolation of D- and L-isomers of VI was dependent on the ester of 7-ADCA, used in the acylation procedure. The D-isomers of 2',2',2'-trichloroethyl and diphenylmethyl ester (D-VI) crystallised from ethyl acetate solution; the L-isomers of the same esters (L-VI) were obtained from the mother liquor upon addition of benzene. The D-isomer of meta-methylbenzyl ester (D-VI) was obtained by crystallisation from methanol; the concentration of mother

SCHEME I


CH–COOH
NH2
I
CH–COOH
NH
CO
(CH2)3Cl
II
HONSu
DCC
DCC
CH–COONSu
NH
CO
(CH2)3Cl
III
CH–C
O
N
C
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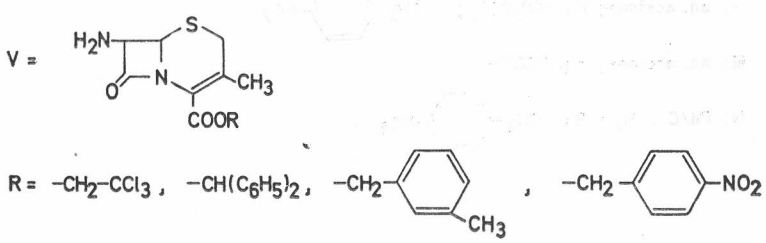
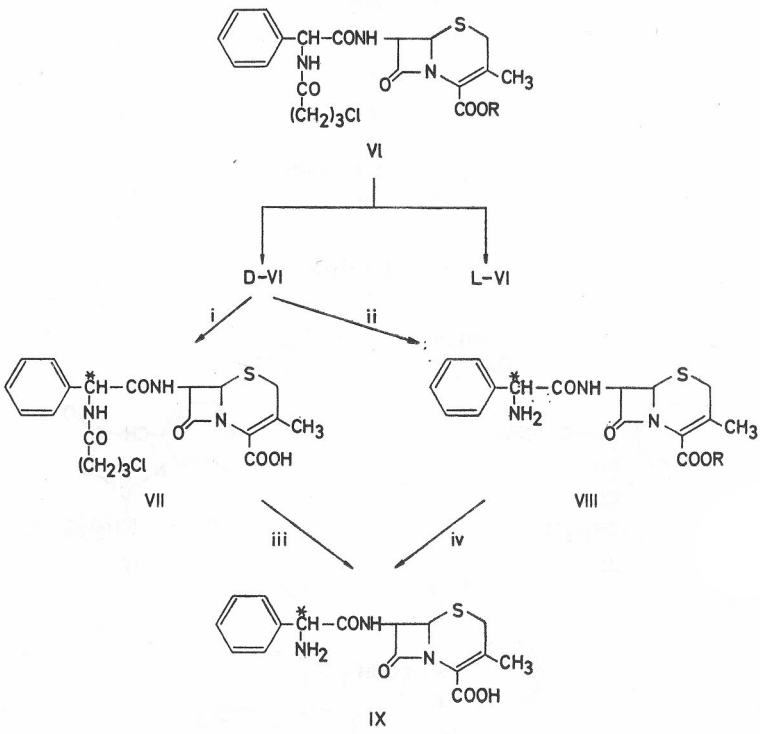
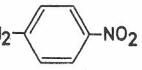
CO
(CH2)3Cl
S
O
N
CH3
COOR
VI


V =
H2N
S
O
N
CH3
COOR
R = –CH2–CCl3 , –CH(C6H5)2 , –CH2
CH3
, –CH2
NO2

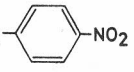
SCHEME II



i: HCOOH, Zn; R= $-CH_2CCl_3$
F_3CCOOH, 0°C; HCOOH, 50°C; R= $-CH(C_6H_5)_2$

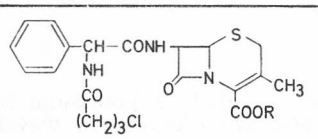
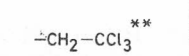
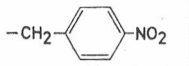
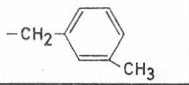
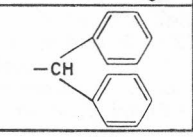
ii: aq. acetone; R= $-CH_2CCl_3$; 

iii: aq. acetone; aq. HCOOH

iv: Pd/C, H_2; R= 

liquor yielded the L-isomer (L-VI). On the contrary, the L-isomer of the para-nitrobenzyl ester (L-VI) crystallised directly from the reaction solution and the D-isomer was isolated by crystallisation of the residue from acetone solution. The excellent crystallisability of 2',2',2'-trichloroethyl ester of D-VI, enabled one to obtain the D-isomer in 40% yield (50% possible) even in the case when the ester V (R=CH_2—CCl_3) was acylated with oxazolone IV. The similar procedure for the separation of certain analogues of cephalexin from the mixture of D- and L-isomers of the free aminoacids, by the use of crystallisation was reported recently by Ryan et al.[4].

TABLE

		δ* (ppm)		
R	ISOMER	C_3-CH_3	C_6-H	C_7-H
	D	2.13	4.92	5.79
	L	2.18	5.01	5.64
	D	2.02	5.05	5.71
	L	2.06	5.13	5.63
	D	2.00	5.02	5.65
	L	2.03	5.02	5.67
	D	1.97	5.03	5.73
	L	2.03	5.12	5.62

* ^{1}H NMR spectra were taken in DMSO-d_6 except
** which was recorded in $CDCl_3$

For the purpose of identification of D- and L-isomers of VI and VII, cephalexin was silylated and then acylated with 4-chlorobutyryl chloride. The product obtained was compared with the sample of D- and L-isomers of VII, prepared by hydrolysis of the ester groups.

Analysis of the ^{1}H NMR spectra of the D-VI and L-VI isomers showed differences in the chemical shifts of C_3—CH_3 protons, as well as of the protons at positions 6 and 7 in the beta-lactam ring (Table). These data were similar to that of Ryan et al., who have noticed similar differences in the chemical shifts of the D- and L-isomers of certain analogues of IX[4].

For deprotection of the ester and *N*-acyl groups in VI »step-wise deblocking« procedure was applied. It was possible to hydrolyse the 2',2',2,-trichloroethyl and diphenylmethyl ester groups selectivelly in reaction conditions which

did not attack *N*-4-ClBu group. In the case of para-nitrobenzyl ester, selective hydrolysis of 4-ClBu group was only possible prior to hydrogenolysis procedure.

The hydrolysis of 4-ClBu group of VII, depends upon solvent and temperature used in the reaction. Heating of VII in 50% aqueous acetone gave cephalexin (IX) in 90 minutes; in diluted aqueous formic acid, hydrolysis was finished in 30 minutes. Protective groups in diphenylmethyl ester of VI could be removed succesively in diluted formic acid, depending only on temperature; at 0° C this ester was hydrolysed in 1 hour, and then 4-ClBu group, after being heated for 30 minutes.

Cephalexin, obtained by the all described ways, was identical to standard sample of cephalexin monohydrate, with biological activity claimed by B. P.[15].

EXPERIMENTAL

Melting points are uncorrected.

The IR spectra were recorded, unless otherwise stated, in potassium bromide plates on a Perkin-Elmer Infracord model 257 and are reported in wavelengths followed by relative intensities in brackets.

pH values were measured on a Radiometer pH-meter model PHM 61. The ^{1}H NMR spectra were recorded in DMSO-d_6, unless otherwise stated, with TMS as internal standard, on a Varian A-60 spectrometer and all chemical shifts are given in ppm downfield from TMS.

TLC was performed on original plates (Merck, Kieselgel HF_{254}) followed by detection with iodine vapors and UV absorption in solvent systems as stated:

(A) *n*-Butanol : acetic acid : water / 4 : 1 : 1
(B) Benzene : ethyl acetate / 3 : 1
(C) Chloroform : methanol / 9 : 1
(D) Dichloromethane : ether / 4 : 1

Optical rotations were measured on an Opton 372149 polarimeter at ambient temperature.

N-*(4-chlorobutyril)*-D-α-*phenylglycine (II)*

To a suspension of D-α-phenylglycine (I) (15.1 g, 0.1 mol; $[\alpha]_D^{23} = -153.6°$) in dichloromethane 230 ml), trimethylchlorsilane (28 ml, 0.22 mol) was added, reaction mixture was cooled to 0 °C and then triethylamine (31 ml, 0.22 mol) added dropwise. The reaction mixture was heated to 40 °C and stirred for 1 hour. Upon cooling to −10 °C, the solution of 4-chlorobutyryl chloride (11.2 ml, 0.1 mol) in dichloromethane (110 ml) was added dropwise to the reaction mixture and stirred first at −10 °C for 2 hours and then 1 hour at 25 °C. Separated triethylamin hydrochloride was filtered and the mother liquor evaporated to dryness. Acetone (70 ml) and water (270 ml) were added to the residue and stirred at +5 °C for 10 minutes. The separated crystals were filtered, washed with water and dried. Yield 21.2 g (83.2%).

Recrystallization from ethyl acetate gave analytical sample, m. p. 144—5 °C; $[\alpha]_D^{23} = -147.8$ °C (c = 1, MeOH); $R_F = 0.60$ (solvent system A).

Anal. $C_{12}H_{14}O_3NCl$ (255.68) calc'd: C 56.50; H 5.50; N 5.48%
found: C 56.49; H 5.61; N 5.20%

IR spectrum: 3340(s), 2750—3060(b), 2590(m), 1705(vs), 1620(vs), 1545(vs), 1232(vs), 720(s), 695(s) cm^{-1}.

^{1}H NMR spectrum δ: 1.95(t, J = 6 Hz, CH_2), 2.35(t, J = 6 Hz, $COCH_2$), 3.57(t, J = 6 Hz, CH_2Cl), 5.26(d, J = 7 Hz, CH), 7.31(s, C_6H_5), 8.56(d, J = 7 Hz, NH).

D-α-*phenylglycine (I)*

N-(4-Chlorobutyryl)-D-α-phenylglycine (II) (500 mg, 1.96 mmol), prepared according to the above procedure, was suspended in water (20 ml) and heated under reflux for 1 hour. Lyophilisation of the aqueous solution gave D-α-phenylglycine hydrochloride. Yield 310 mg (94.5%); m. p. 246—250 °C; R_F = 0.40 (solvent system A).

The obtained product (310 mg) was dissolved in water (10 ml) and the solution was adjusted to pH 6. Crystalline D-α-phenylglycine (I) was filtered and dried. Yield 260 mg (93.2%); m. p. 280 °C (Lit.[14] m. p. 300 °C); $[\alpha]_D^{23} = -153.2^0$ (c = 1, 1 N HCl) (Lit.[14] $[\alpha]_D^{23} = -154.5^0$); $R_F = 0.40$ (solvent system A).

N-(4-Chlorobutyril)-D-α-phenylglycine N-hydroxysuccinimido Ester (III)

To a solution of *N*-(4-chlorobutyryl)-D-α-phenylglycine (I) (25.5 g, 0.1 mol) in dioxane (450 ml) *N*-hydroxysuccinimide (11.5 g, 0.1 mol) and *N,N*-dicyclohexylcarbodiimide (22.7 g, 0.11 mol) were added and the reaction mixture stirred at 0 °C for 8 hours. The separated solid was removed and the filtrate evaporated at reduced pressure to dryness. The residue was recrystallized from dichloromethane *n*-hexane. Yield 34 g (96.3%); m. p. 139—140 °C; $[\alpha]_D^{23} = -28.23^0$ (c = 2, MeOH); $R_F = 0.75$ (solvent system A).

Anal. $C_{16}H_{17}N_2O_5Cl$ (352.76) calc'd: C 54.50; H 4.85; N 7.89%
found: C 54.72; H 4.97; N 7.94%

IR spectrum: 3320(m), 1815(s), 1785(s) 1745(vs), 1655(vs), 1530(s), 1200(vs), 1090(s), 900(m), 740(m) cm^{-1}.

^1H NMR spectrum (DMSO-d_6) δ: 2.02(tt, $J = 6$ Hz, CH_2), 2.35(t, $J = 6$ Hz, $COCH_2$), 2.82(s, CH_2—CH_2), 3.65(t, $J = 6$ Hz, CH_2Cl), 5.84(d, $J = 7$ Hz, CH), 7.45(s, C_6H_5), 9.00(d, $J = 7$ Hz, NH).

2-(3-Chloropropyl)-4-phenyloxazolon-5 (IV)

To a suspension of *N*-4-chlorobutyryl-D-α-phenylglycine (I) (0.750 g, 2.94 mmol) in dichloromethane (10 ml) a solution of *N,N'*-dicyclohexylcarbodiimide (0.678 g, 3.28 mmol) in dichloromethane (5 ml) was added dropwise within stirring at 0 °C and the reaction mixture stirred at 0 °C for additional 30 minutes. The separated *N,N'*-dicyclohexylurea was filtered and dichloromethane evaporated giving an oily product. Dichloromethane was added repeatedly to the obtained residue, traces of undissolved material were filtered and the solvent evaporated to yield the oily residue (0.690 g, 99.0%) with one spot on TLC $R_F = 0.60$ (solvent system B); $\alpha]_D^{23} = 0^0$ (c = 0.1, CH_2Cl_2).

IR spectrum (CH_2Cl_2): 1825(vs), 1670(s), 1105(m), 890(m) cm^{-1}.

Anal. $C_{12}H_{12}O_2ClN$ (237.681) calc'd: C 60.7; H 5.07; N 5.8%
found: C 59.9; H 5.00; N 6.0%

2',2',2'-Trichloroethyl 7-amino-3-methyl-3-cephem-4-carboxylate (V, R=CCl_3CH_2) was prepared according to the Chauvette's procedure[4].

Diphenylmethyl 7-amino-3-methyl-3-cephem-4-carboxylate (V, R=$(C_6H_5)_2CH$) and *p-Nitrobenzyl 7-amino-3-methyl-3-cephem-4-carboxylate (V, R=*p-NO_2—C_6H_4—CH_2) were prepared according to the procedure described in literature[12].

m-*Methylbenzyl 7-phenylacetamido-3-methyl-3-cephem-4-carboxylate*

To a solution of 7-phenylacetamido-3-methyl-3-cephem-4-carboxylic acid (4.81 g, 0.014 mol) in dimethylformamide (86 ml), triethylamine (1.45 g, 0.014 mol) and a solution of *m*-methylbenzylbromide (2.66 g, 0.014 mol) in dimethylformamide (30 ml) were added dropwise. Upon stirring for 4 hours at 20 °C the reaction mixture was poured into cold water (130 ml) with vigourous stirring. Separated solid was filtered, washed with water and dried. Yield 4.46 g (71%); m. p. 171—172 °C.

Recrystallization from acetone-petroleum ether gave analytical sample with m. p. 172—173 °C; $R_F = 0.8$ (solvent system D).

Anal. $C_{24}H_{24}N_2O_4S$ (436.51) calc'd.: C 66.03; H 5.54; N 6.42; S 7.34%
found: C 66.31; H 5.69; N 6.69; S 6.80%

IR spectrum: 3280(m), 1775(vs), 1705(s), 1645(vs), 1530(s), 1250(m), 1225(m), 1105(m), 780(m), 695(m) cm^{-1}.

^{1}H NMR spectrum ($CDCl_3$) δ: 2.09 (s, C_3-CH_3), 2.33 (s, Ph-CH_3), 3.07 (d, J = 17.5 Hz, C_2-H), 3.47 (d, J = 17.5, C_2-H), 3.62 (s, Ph-CH_2), 4.91 (d, J = 5 Hz, C_6-H), 5.21 (s, OCH_2), 5.73 (dd, J = 5 and 9 Hz, C_7-H), 6.63 (d, J = 9 Hz, NH), 7.10—7.40 (m, C_6H_5 and C_6H_4).

m-Methylbenzyl 7-amino-3-methyl-3-cephem-4-carboxylate hydrochloride (V, R=m—CH_3—C_6H_4—CH_2)

A solution of phosphoruspentachloride (3.02 g, 0.014 mol) in dichloromethane (60 ml) and pyridine (1.14 g, 0.0145 mol) was stirred at 20 °C for 40 minutes and *m*-methylbenzyl 7-phenylacetamido-3-methyl-3-cephem-4-carboxylate (4.36 g, 0.01 mol) was added at + 5 °C. The reaction mixture was stirred for 40 minutes and 1,3-butandiol (3.78 g) and diisopropyl ether (8.25 g) were added dropwise while cooling the reaction mixture to — 10 °C. Upon stirring the reaction mixture at — 10 °C for 2 hours and 1 hour at 20 °C, water (14 ml) and ether (140 ml) were added. Separated crystals were filtered and washed with water and ether. Yield 2.1 g (58.8%); m. p. 157—160 °C; R_F = 0.53 (solvent system D).

Recrystallization from ethyl acetate gave analytical sample, m. p. 168—170 °C.

Anal. $C_{16}H_{19}N_2O_3SCl$ (354.85) calc'd.: C 54.15; H 5.40; N 7.90%
found: C 53.90; H 5.45; N 8.12%

IR spectrum: 3505(w), 3340(m), 2850(b), 1775(s), 1690(w), 1615(m), 1310(m), 1220(m), 875(m), 785(w) cm^{-1}.

2',2',2'-Trichloroethyl-7-/N-(4-chlorobutyryl)-α-phenylglycylamido/-3-methyl-3-cephem-4-carboxylate (VI, R=CCl_3CH_2)

A) D-VI isomer

a) To a solution of 2',2',2'-trichloroethyl-7-amino-3-methyl-3-cephem-4-carboxylate (V, R=CCl_3CH_2) (3.45 g, 0.01 mol) in dichloromethane (40 ml) *N*-(4-chlorobutyryl)-D-α-phenylglycin *N*-hydroxysuccinimido ester (III) (3.53 g, 0.01 mol) was added and the reaction mixture stirred at 25 °C for 8 hours. Upon addition of water (40 ml) and stirring for further 5 minutes, organic layer was separated, dried ($MgSO_4$) and evaporated to dryness. The residue was dissolved in ethyl acetate and upon cooling the crystalline solid precipitated. Crystals were filtered and washed with cold ethyl acetate. Yield 3.4 g (59.0%); m. p. 200 °C; $[\alpha]_D^{23}$ = + 33.4° (c=0.2, MeOH); R_F = 0.30 (solvent system B).

Anal. $C_{22}H_{23}N_3O_5Cl_4S$ (583.32) calc'd.: C 45.4; H 3.96; N 7.22%
found: C 46.0; H 4.00; N 7.50%

IR spectrum: 3270(s), 1765(vs), 1725(s), 1635(vs) cm^{-1}.

^{1}H NMR spectrum ($CDCl_3$) δ: 2.13 (s, C_3-CH_3), 3.27 (dd, C_2H_2), 3.50 (t, J = 6.5 Hz, CH_2Cl), 4.92 (d, J = 5 Hz, C_6-H), 5.79 (dd, J = 5 and 8 Hz, C_7-H), 5.88 (d, J = 8.5 Hz, CH), 7.0—7.5 (b, NH), 7.97 (d, J = 8.5 Hz, NH), 4.88 (dd, OCH_2), 1.65—2.7 (m, CH_2CH_2).

b) To a solution of V (R=CCl_3CH_2) (3.45 g, 0.01 mol) in dichloromethane (40 ml) 2-(3-chloropropyl)-4-phenyloxazolon-5 (IV) (2.38 g, 0.01 mol) was added and the reaction solution heated under reflux for 5 hours. Dichloromethane was evaporated, the residue dissolved in ethyl acetate and cooled. The separated crystals were filtered and washed with cold ethyl acetate. Yield 2.33 g (40.0%); m. p.; $[\alpha]_D^{23}$; R_F; IR and ^{1}H NMR spectra were identical as for the product obtained according to the above described procedure.

B) L-VI isomer

The ethyl acetate mother liquor, after the separation of D-epimer, was evaporated to a shorter volume (10 ml) and benzene (1 ml) added. Upon cooling the crystals were separated and washed with the mixture of ethyl acetate-benzene. Yield 1.2 g (20.8%); m. p. 188 °C; $[\alpha]_D^{23}$ = + 121.6° (c = 0.2, MeOH); R_F = 0.25 (solvent system B).

IR spectrum was identical to the one of D-isomer.

^{1}H NMR spectrum ($CDCl_3$) δ: 2.18 (s, C_3-CH_3) 3.37 (dd, C_2H_2), 3.53 (t, J = 6 Hz, CH_2Cl), 5.01 (d, J = 4.7, C_6-H), 5.64 (dd, J = 4.7 Hz, C_7-H), 5.86 (d, J = 8 Hz, CH), 7.08 (d, J = 8 Hz, NH), 7.93 (d, J = 8 Hz), 4.87 (dd, O-CH_2), 1.65—2.7 (m, CH_2CH_2).

Diphenylmethyl 7-/N-(4-chlorobutyril)-D-α-phenylglycylamido/-3-methyl-3-cephem-4-carboxylate (VI, R=$(C_6H_5)_2CH$)

a) D-VI isomer

To a solution of diphenylmethyl 7-amino-3-methyl-3-cephem-4-carboxylate hydrochloride (V, R=$(C_6H_5)_2CH$) (2.08 g, 0.005 mol) in dichloromethane (50 ml) and water (20 ml) sodium hydrocarbonate (0.5 g, 0.005 mol) was added in small portions at 20 °C with stirring. The organic layer was then separated, dried ($MgSO_4$) and evaporated to a shorter volume. *N*-(4-Chlorobutyril)-D-α-phenylglycin *N*-hydroxysuccinimido ester (III) (1.46 g, 0.005 mol) was added to the dichloromethane solution and the reaction mixture stirred for 12 hours at 25 °C. Water (20 ml) was added and the mixture stirred for 5 minutes, the organic layer separated, dried ($MgSO_4$) and evaporated to a colourless oil. The residual oil was dissolved in a mixture of ethyl acetate-benzene / 3 : 1 and upon cooling separated crystals were filtered and washed with cold ethyl acetate. Yield 1.1 g (44.0%); m. p. 180—183 °C; $[\alpha]_D^{23} = -21.0^0$ (c = 0.2, CH_2Cl_2); $R_F = 0.37$ (solvent system B).

Anal. $C_{33}H_{32}N_3O_5SCl$ (618.20) calc'd.: C 64.11; H 5.22; N 6.79%
found: C 64.32; H 5.42; N 6.52%

IR spectrum: 3260(m), 1775(s), 1700(s), 1630(vs), 1520(s), 1360(m), 1220(s) cm^{-1}

^1H NMR spectrum δ: 1.97 (s, C_3-CH_3), 1.60—2.60 (m, CH_2-CH_2), 3.00—3.80 (m, C_2H_2), 3.62 (t, J = 6 Hz, CH_2Cl), 5.03 (d, J = 5 Hz, C_6-H), 5.73 (dd, J = 5 and 8 Hz, C_7-H), 5.72 (d, J = 8 Hz, CH), 6.89 (s, OCH), 7.10—7.70 (b, 3 C_6H_5), 8.54 (d, J = 8 Hz, NH), 9.23 (d, J = 8 Hz, NH).

a) L-VI isomer

The mother liquor after separation of D-isomer was evaporated to dryness. The residue (1.3 g) was passed over a column of silica gel and the column was eluated with benzene-ethyl acetate / 10 : 1. Appropriate fractions of the eluate were combined and evaporated to dryness.

Yield 0.95 g (38.0%); m. p. 203—205 °C; $[\alpha]_D^{23} = +64.2^0$ (c = 0.2, CH_2Cl_2); R_F = = 0.32 (solvent system B).

IR spectrum was identical to that of the product obtained under a).

^1H NMR spectrum δ: 2.03 (s, C_3-CH_3), 1.60—2.60 (m, CH_2-CH_2), 3.00—3.80 (m, C_2H_2), 3.62 (t, J = 6 Hz, CH_2Cl), 5.12 (d, J = 4.5 Hz, C_6-H), 5.62 (dd, J = 4.5 and 8.5 Hz, C_7-H), 5.63 (d, J = 8 Hz, CH), 6.88 (s, OCH), 7.20—7.70 (b, 3 C_6H_5), 8.52 (d, J = 8.5 Hz, NH), 9.23 (d, J = 8.0 Hz, NH).

p-Nitrobenzyl 7-/N(4-chlorobutyryl)-α-phenylglycylamido/-3-methyl-3-cephem-4-carboxylate (VI, R=p—NO_2—C_6H_4—CH_2—)

a) L-VI isomer

To a solution of *p*-nitrobenzyl 7-amino-3-methyl-3-cephem-4-carboxylate (V, R = p-NO_2-C_6H_4-CH_2-) (6.0 g, 0.0172 mol) in dichloromethane (300 ml) a solution of *N*-(4-chlorobutyryl)-D-α-phenylglycin *N*-hydroxysuccinimido ester (III) (6.6 g, 0.0187 mol) in dichloromethane (140 ml) was added and the reaction mixture stirred at 25 °C for 40 hours. The separated solid was filtered and washed with dichloromethane. Yield 2.79 g (27.9%); m. p. 163—165 °C; $R_F = 0.26$ (solvent system B).

IR spectrum: 3270(s), 3040(m), 2930(w), 1766(s), 1725(s), 1612(vs), 1515(s), 1345(m), 1220(s), 713(w) cm^{-1}.

^1H NMR spectrum δ: 1.70—2.60 (m, CH_2-CH_2), 2.06 (s, C_3-CH_3), 3.41 (dd, C_2H_2), 3.61 (t, J = 7 Hz, CH_2Cl), 5.65 (d, J = 8 Hz, CH), 5.38 (s, OCH_2), 5.13 (d, J = 5 Hz, C_6-H), 5.63 (dd, J = 5 and 8 Hz, C_7-H), 7.30—8.35 (m, C_6H_5 and C_6H_4), 8.53 (d, J = 8 Hz, NH), 9.20 (d, J = 8 Hz, NH).

b) D-VI isomer

The mother liquor after the separation of L-isomer was extracted with water (2 × 200 ml), dried ($MgSO_4$) and evaporated to dryness. The residue was dissolved in boiling acetone (740 ml) and cooled to 25 °C with stirring. Separated crystals were filtered and washed with cold acetone.

Yield 3.89 g (38.9%); m. p. 200—202 °C.

For analysis, a sample was recrystalized from acetone; m. p. 202—204 °C; R_F = = 0.31 (solvent system B); $[\alpha]_D^{23} = -2.22^0$ (c = 0.4, CH_2Cl_2).

Anal. $C_{27}H_{27}N_4O_7SCl$ (587.04) calc'd.: C 55.23; H 4.63; N 9.54; Cl 6.04; S 5.46%
found: C 55.36; H 4.80; N 9.46; Cl 6.50; S 5.50%

IR spectrum: 3270(s), 3045(w), 1767(s), 1716(m), 1630(s), 1520(s), 1343(s), 720(w) cm^{-1}.
^1H NMR spectrum δ: 1.80—2.60 (m, CH_2-CH_2), 2.02 (s, C_3-CH_3), 3.35 (dd, C_2H_2), 3.63 (t, J = 7 Hz, CH_2Cl), 5.05 (d, J = Hz, C_6-H), 5.4 (s, OCH_2), 5.71 (dd, J = 5 and 8 Hz, C_7-H), 5.68 (d, J = 8 Hz, CH), 7.25—8.35 (m, C_6H_5 and C_6H_4), 8.55 (d, J = 8 Hz, NH), 9.15 (d, J = 8 Hz, NH).

m-*Methylbenzyl-7-/N-(4-chlorobutyryl)-α-phenylglycylamido/-3-methyl-3-cephem-4-carboxylate (VI, R=m—CH_3—C_6H_4—CH_2)*

a) D-VI isomer

A solution of *m*-methylbenzyl-7-amino-3-methyl-3-cephem-4-carboxylate (V, R = *m*-CH_3-C_6H_4-CH_2) (4.2 g, 0.013 mol) in dichloromethane (5 ml) was added to a solution of *N*-(4-chlorobutyryl)-D-α-phenylglycin *N*-hydroxysuccinimido ester (III) (4.68 g, 0.013 mol) in dichloromethane (115 ml) and the reaction mixture was extracted with water (2 × 20 ml), the organic layer separated, dried ($MgSO_4$) and evaporated to dryness. The residue was dissolved in methanol and upon cooling separated solid was filtered and washed with cold methanol. Yield 3.32 g (46%); m. p. 208—212 °C; $[\alpha]_D^{23} = +53.5$ (c = 0.1, MeOH); R_F = 0.35 (solvent system B).

Anal. $C_{28}H_{30}N_3O_5SCl$ (556.07) calc'd.: C 60.47; H 5.44; N 7.56%
found: C 60.18; H 5.70; N 7.38%

IR spectrum: 3260(s), 1765(s), 1710(m), 1638(vs), 1525(m), 1220(m) cm^{-1}.
^1H NMR spectrum δ: 2.0 (s, C_3-CH_3), 2.3 (s, Ph-CH_3), 1.80—2.60 (m, CH_2-CH_2), 3.35 (dd, C_2H_2), 3.64 (t, J = 7 Hz, CH_2Cl), 5.02 (d, J = 5 Hz, C_6-H), 5.22 (b, OCH_2), 5.65 (dd, J = 5 and 8 Hz, C_7-H), 5.71 (d, J = 8.5 Hz, CH), 7.00—7.60 (m, C_6H_5 and C_6H_4).

b) L-VI isomer

The methanol filtrate after separation of D-epimer was evaporated to a reduced volume (30 ml). The crystals, separated upon standing at 25 °C for 8 hours were filtered and washed with cold methanol. Yield 2.28 g (31.5%); m. p. 170—173 °C; R_F = 0.4 (solvent system B); $[\alpha]_D^{23} = +140.1^0$ (c = 0.1, MeOH).

IR spectrum: 3270(s), 1763(s), 1718(s), 1638(vs), 1520(m) cm^{-1}.

^1H NMR spectrum δ: 2.03 (s, C_3-CH_3), 2.30 (s, Ph-CH_3), 1.67—2.65 (m, CH_2-CH_2), 3.32 (dd, C_2H_2), 3.64 (t, J = 6.5 Hz, CH_2Cl), 5.02 (d, J = 5 Hz, C_6-H), 5.22 (b, OCH_2), 5.67 (dd, J = 5 and 8.5 Hz, C_7-H), 5.72 (d, J = 8.5 Hz, CH), 7.09—7.60 (m, C_6H_5 and C_6H_4).

7-/N-*(4-chlorobutyryl)-D-α-phenylglycylamido/-3-methyl-3-cephem-4-carboxylic acid (VII)*

a) To a solution of 2',2',2'-trichloroethyl-7-/*N*-(4-chlorobutyryl)-D-α-phenylglycylamido/-3-methyl-3-cephem-4-carboxylate (D-VI, R = CCl_3CH_2-) (6.1 g, 0.01 mol) in 90% formic acid (300 ml) zinc powder (6.78 g, 0.1 g-atom) was added and the reaction mixture stirred for 1 hour at 0 °C. Undissolved material was filtered and the mother liquor evaporated to dryness. The residue was dissolved in water (300 ml) and the solution acidified with conc. hydrochloric acid to pH 0.5 while cooling at 2 °C. The separated product was filtered, washed with water and cold aceton. Yield 4.1 g (87%); m. p. 162—164 °C; $[\alpha]_D^{23} = +55.13^0$ (c = 0.2, MeOH); R_F = 0.80 (solvent system A).

Anal. $C_{20}H_{22}O_5N_3SCl$ (451.92) calc'd.: C 53.10; H 4.90; N 9.20%
found: C 53.40; H 5.20; N 8.80%

IR spectrum: 3285(s), 1765(vs), 1480—1540(s), 1350(s), 1220(vs), 692(m) cm^{-1}.
^1H NMR spectrum δ: 2.0 (s, C_3-CH_3), 1.65—2.5 (m, CH_2-CH_2), 3.61 (t, J = 6 Hz, CH_2Cl), 3.05—3.75 (m, C_2H_2), 4.93 (d, J = 5 Hz, C_6-H), 5.35—5.90 (m, C_7-H and CH), 7.40 (s, C_6H_5), 8.61 (d, J = 9 Hz, NH), 9.26 (d, J = 9 Hz, NH).

b) To a suspension of diphenylmethyl 7-/*N*(4-chlorobutyryl)-D-α-phenylglycylamido-3-methyl-3-cephem-4-carboxylate (D-VI, R = $(C_6H_5)_2CH$) (0.62 g, 0.001 mol) in dichloromethane (10 ml), trifluoroacetic acid (2 ml) was added at 0 °C and the solution stirred for 10 minutes. Upon addition of water (20 ml) and stirring for 30 minutes at 5 °C, separated solid was filtered and washed with water. The crude product was suspended in ether, the suspension was stirred for 5 minutes and the crystals were filtered and washed with ether. Yield 0.4 g (92%); m. p. 161—163 °C.
IR, ^{1}H NMR spectra and $[\alpha]_D^{23}$ were identical to that of the product obtained under (a).

A suspension of D-VI (R = $(C_6H_5)_2CH$) (0.62 g, 0.001 mol) in the mixture of 98% formic acid (6 ml) and water (2 ml) was stirred at 50 °C for 1 hour. Upon evaporation at reduced pressure the residue was suspended in water (5 ml) and dichloromethane (5 ml), the suspension stirred for 5 minutes, undissolved crystals separated and washed with ether. It was obtained 0.38 g (86%) of the product with the same m. p., $[\alpha]_D^{23}$, R_F, IR and ^{1}H NMR spectra as those in the above procedure.

c) To a suspension of cephalexin (2.6 g, 7.5 mmol) in dichloromethane (20 ml) trimethylchlorosilane (1.89 ml, 15 mmol) was added. To the reaction mixture, heated under reflux for 30 minutes, triethylamine (2.1 ml, 15 mmol) was added and it was stirred for further 30 minutes. The reaction mixture was cooled to — 10 °C and a solution of 4-chlorobutyrylchloride (0.84 ml, 7.5 mmol) in dichloromehtane (10 ml) added dropwise and stirred first at — 10 °C for 1 hour and then 1 hour at 25 °C. Water (3 × 25 ml) was added, separated crystals were filtered and dried over sodium hydroxide. Yield 2.4 g (70.8%); m. p. 160—163 °C; $[\alpha]_D^{23}$ = + 55.10° (c = 0.2, MeOH); R_F = 0.80 (solvent system A).

IR spectrum: 3285(s), 1765(vs), 1480—1540(s), 1350(s), 1220(vs), 695(m) cm^{-1}.

^{1}H NMR spectrum δ: 2.0 (s, C_3-CH_3), 1.65—2.5 (m, CH_2-CH_2), 3.61 (t, J = 6 Hz, CH_2Cl), 3.05—3.75 (m, C_2H_2), 4.93 (d, J = 5 Hz, C_6-H), 5.35—5.90 (m, C_7-H and CH), 7.40 (s, C_6H_5), 8.61 (d, J = 9 Hz ,NH), 9.26 (d, J = 9 Hz, NH).

p-*Nitrobenzyl 7-(D-α-phenylglycylamido)-3-methyl-3-cephem-4-carboxylate (VIII, R=*p*—NO_2—C_6H_4—CH_2)*

p-Nitrobenzyl 7-/*N*-(4-chlorobutyryl)-D-α-phenylglycylamido/-3-methyl-3-cephem-4-carboxylate (D-VI, R = *p*-NO_2-C_6H_4-CH_2) (1.43 g, 2.44 mmol) was added to a mixture of acetone (280 ml) and water (140 ml) and heated under reflux for 10 hours. Acetone was evaporated and the traces of unreacted starting material were separated. The mother liquor was adjusted to pH 7 with 1 N sodium hydroxide and the obtained solid separated and washed with water. Yield 0.89 g (95%); m. p. 151—153 °C; $[\alpha]_D^{23}$ = — 14.58° (c = 0.4, CH_2Cl_2); R_F = 0.51 (solvent system C).
IR spectrum: 3260(m), 3000(w), 1768(m), 1718(m), 1645(w), 1509(s), 1340(s), 1215(s), 728(w) cm^{-1}.

^{1}H NMR spectrum ($CDCl_3$) δ: 2.33 (s, CH_3), 3.50 (dd, C_2-H_2), 5.06 (d, C_6-H), 5.43 (s, CO_2CH_2), 5.83 (m, C_7-H, NH), 7.33—8.33 (m, C_6H_5 and C_6H_4), 8.03 (s, NH_2).

2',2',2'-Trichloroethyl-7-(D-α-phenylglycylamido)-3-methyl-3-cephem-4-carboxylate (VIII, R=—CH_2CCl_3)

2',2',2'-Trichloroethyl-7/*N*-(4-chlorobutyryl)-α-phenylglycylamido/-3-methyl-3-cephem-4-carboxylate (D-VI, R = — CH_2CCl_3) (0.1 g, 0.17 mmol) was added to a mixture of acetone (5 ml) and water (5 ml) and heated under reflux for 4 hours. Acetone was evaporated and the mother liquor was adjusted to pH 6 with 1 N sodium hydroxide. The obtained solid was separated and washed with water. Yield 0.060 g (72.6%); m. p. 150 °C (Lit.[4] m. p. 150 °C); R_F = 0.60 (solvent system A).

^{1}H NMR spectrum ($CDCl_3$) δ: 2.20 (s, C_3-CH_3), 3.20—3.60 (m, C_2H_2), 4.51—5.0 (m, C_6-H, ester CH_2, CH), 5.7 (m, C_7-H), 7.3 (s, C_6H_5), 8.02 (d, NH).

7-(D-α-phenylglycylamido)-3-methyl-3-cephem-4-carboxylic acid monohydrate (IX)

a) The suspension of 7-/*N*-(4-chlorobutyryl)-D-α-phenyl-glycylamido/-3-methyl-3-cephem-4-carboxylic acid (VII) (0.45 g, 1 mol) in acetone (5 ml) and water (5 ml) was stirred for 2 hours under reflux. Upon the evaporation of acetone the aqueous solution

was extracted with ethyl acetate (2 × 2 ml) and the aqueous layer was adjusted to pH 3.6 with triethylamine. While stirring at 0 °C, acetone (3 ml) was added dropwise to the obtained suspension. Cephalexin-monohydrate was separated and washed with the mixture acetone-water and then acetone. Yield 0.3 g (84.4%); $[\alpha]_D^{23} = +151^0$ ($c = 0.5$, H_2O) (Lit.[15] $[\alpha]_D^{23} = +149$ to $+158^0$) $R_F = 0.50$ (solvent system A); biopotency: 962 μg/mg (Lit.[15] biopotency: > 950 μg/mg).

Anal. $C_{16}H_{17}N_3O_4S$-H_2O (365.40) calc'd.: C 52.59; H 4.69; N 11.50; S 8.77; K. F. 4.93%
found: C 52.47; H 4.43; N 11.00; S 8.73; K. F. 5.60%

IR spectrum: 1770(s), 1690(s), 1580(s) cm^{-1}.
^1H NMR spectrum (D_2O) δ: 2.00 (s, C_3-CH_3), 3.37 (dd, $J = 18$ Hz, C_2H_2), 5.10 (d, $J = 4$ Hz, C_6-H), 5.33 (s, CH), 5.72 (d, $J = 4$ Hz, C_7-H), 7.62 (s, C_6H_5).

b) The suspension of VII (2.25 g, 5 mmol) in 98% formic acid (30 ml) and water (10 ml) was stirred for 30 minutes under reflux. The reaction mixture was evaporated to dryness and further elaborated as it is described in the example (a). Yield 1.42 g (77.5%).

c) The suspension of diphenylmethyl 7-/*N*-(4-chlorobutyryl)-D-α-phenylglycyl-amido/-3-methyl-3-cephem-4-carboxylate (D-VI, R = $(C_6H_5)_2CH$) (3.1 g, 0.005 mol) in the mixture of 98% formic acid (30 ml) and water (10 ml) was stirred for 30 minutes under reflux. The reaction mixture was evaporated to dryness and elaborated as it is described under (a). Yield 1.56 g (85.4%).

d) The mixture of *p*-nitrobenzyl 7-(D-α-phenylglycylamido)-3-methyl-3-cephem--4-carboxylate (VIII, R = *p*-NO_2-C_6H_4-CH_2) (1.02 g, 2.11 mol) and 10% palladium on carbon (160 mg) in methanol (110 ml) and 1 N hydrochloric acid (20 ml) was hydrogenated under pressure of 14 atm for 3 hours at 25 °C. Upon filtration of the catalyst, methanol was evaporated and the aqueous part adjusted to pH 4.5 with 1 N sodium hydroxide. Separated solid was filtered and cephalexin monohydrate was obtained by the addition of acetonitrile to the mother liquor. Yield 0.7 g (95.4%).

Obtained cephalexin monohydrate was found to be identical to the one prepared using procedures (a), (b) or (c).

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REFERENCES

1. Taken in part from the *M. Sc. Thesis* of Z. Mandić, University of Zagreb, 1980.
2. Presented in part at *VI Meeting of Chemists of Croatia*, Zagreb, 1979; and *VI Yugoslav Congress for Pure and Applied Chemistry*, Sarajevo, 1979.
3. Glaxo Lab. Ltd., *U. S. Pat.* 3957773 (1976); Eli Lilly & Co., *Brit. Pat.* 1336802 (1972), *Brit. Pat.* 1399349 (1971); Bristol-Myers Co., *Brit. Pat.* 1391838 (1975), *U. S. Pat.* 3890310 (1975); Takeda Chem. Ind., *Brit. Pat.* 1385943 (1975).
4. C. W. Ryan, R. J. Simon, and E. M. Van Heyningen, *J. Med. Chem.* **12** (1969) 310.
5. E. Schröder and K. Lübke, *The Peptides*, Vol. I, Academic Press, New York, 1965, pp. 3—51.
6. J. H. C. Nayler, *Advances in Penicillin Research*, in: *Adv. Drug Res.* **7** (1973) pp. 1—106; G. V. Kaiser and S. Kukolja, *Modifications of the beta-Lactam System*, in: E. H. Flynn (Ed.), *Cephalosporins and Penicillins*, Academic Press, New York (1972) pp. 74—94.
7. *Ibid.* ref. 5., p. 118.
8. C. J. M. Stirling, *J. Chem. Soc.* (1960) 255.
9. H. Peter, *Disertation*, ETH, Zürich 1961.
10. *Ibid.* ref. 1., p. 81.
11. H. Peter, M. Brugger, J. Schreiber, and A. Eschenmoser, *Helv. Chem. Acta* **46** (1963) 577.
12. Glaxo Lab. Ltd., *Offenlegungsschrift* 2 311 597 (1973)
13. R. R. Chauvette, P. A. Pennington, C. W. Ryan, R. D. G. Cooper, F. L. José, I. G. Wright, E. M. Van Heyningen, and G. W. Huffman, *J. Org. Chem.* **36** (1971) 1259.

14. C. S. Marvels, *J. Amer. Chem. Soc.* **42** (1920) 2264.
15. *British Pharmacopoeia,* Department of Health and Social Security — London (1973) 87; *Pharmacopoea Jugoslavica III,* Savezni zavod za zdravstvenu zaštitu, Beograd (1972) 1—3, 9, 100.

SAŽETAK

Primjena 4-klorbutiril grupe kao amino zaštitne grupe u sintezi cefaleksina

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U toku studija o mogućoj primjeni *N*-4-klorbutiril derivata aminokiselina u sintezi polusintetskih cefalosporina, razrađena je sinteza cefaleksina.

Kondenzacijom *N*-(4-klorbutiril)-α-fenilglicina s raznim esterima 7-amino-3--metil-3-cefem-4-karboksilne kiseline dobivena je smjesa epimera zaštićenog cefaleksina. Pokazano je da se navedeni esteri D- i L-epimera *N,O*-zaštićenog cefaleksina mogu jednostavno odvojiti kristalizacijom, a zaštitne skupine mogu ukloniti pri uvjetima kod kojih ne dolazi do cijepanja β-laktamskog prstena.

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