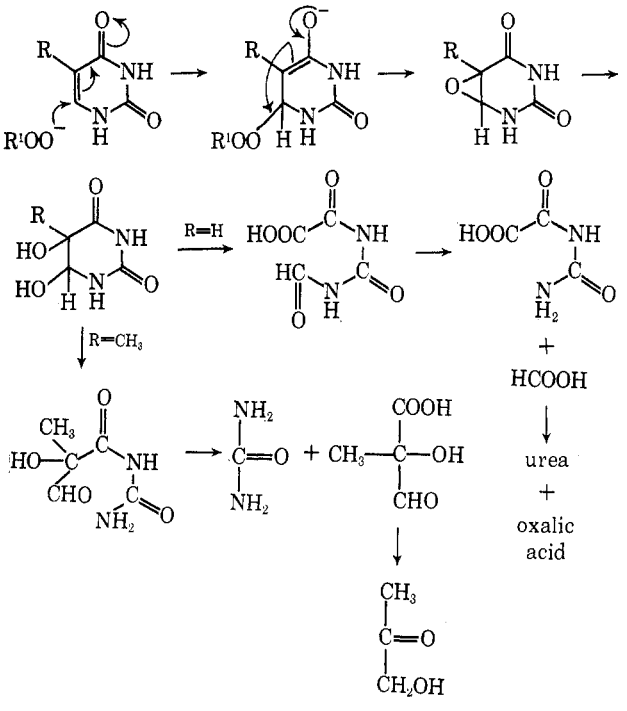
SCHEME I



rate maximum for a given substrate would be at the same pH for all of the hydroperoxides. (3) The observation of second-order kinetics, although conceivable for a radical pathway, is much commoner for nonradical pathways. For example, a nonchain radical mechanism involving rate-limiting homolysis of the peroxide and subsequent attack by the hydroxyl radical on the substrate would show no kinetic dependence on substrate concentration. In contrast to the evidence suggesting the predominance of polar reactions at alkaline pH values, the demonstration that allyl alcohol decreases the rate of loss of thymidine 5′-phosphate in the presence of hydrogen peroxide at pH 7.4 approximately sixfold is positive evidence that a radical pathway is involved under these conditions. There is some reaction of hydrogen peroxide with allyl alcohol, but the change in concentration of hydrogen peroxide due to this reaction is negligible (less than 10% at the highest concentration of allyl alcohol used) up to 30 hr and hence can only account for a small portion of the effect which we observe. The kinetics of the disappearance of thymidine 5′-phosphate under these conditions are complex since Rhaese, *et al.*,[9] have shown that several different reactions, all of which lead to decreases in the absorbancy, occur simultaneously.

Registry No.—Hydrogen peroxide, 7722-84-1; uracil, 66-22-8; thymine, 65-71-4; thymidine 5′-phosphate, 365-07-1; methyl hydroperoxide, 3031-73-0; *tert*-butyl hydroperoxide, 75-91-2; peroxyacetic acid, 79-21-0.

Acknowledgment.—We are grateful to the National Science Foundation (GB-7998) and the Frasch Foundation for support.

Chemistry of Cephalosporin Antibiotics. XXI. Conversion of Penicillins to Cephalexin[1]

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A laboratory synthesis from the biosynthetic penicillins is described for cephalexin (**7**), an orally active deacetoxycephalosporin antibiotic. Penicillins V and G were converted to sulfoxide trichloroethyl esters **3a** and **3b**, respectively, by an esterification to compounds **2a** and **2b** followed by sulfoxidation. The sulfoxide esters **3a** and **3b** were rearranged thermally to their corresponding deacetoxycephalosporin esters **4a** and **4b**. Proof of structure for **4a** and **4b** was supplied by their independent syntheses from 7-aminodeacetoxycephalosporanic acid (**9**). N-Deacylation of **4a** and **4b** afforded a common amino ester, 7-aminodeacetoxycephalosporanic acid trichloroethyl ester (**5d**). Compound **5d** was reacylated in mixed anhydride coupling reactions with *N*-trichloroethyloxycarbonyl-D-α-phenylglycine and with *N*-*tert*-butoxycarbonyl-D-α-phenylglycine. The doubly protected cephalexin derivatives **6** and **12** were deblocked yielding cephalexin in good yield.

Previous publications from these laboratories have disclosed the *in vitro* and *in vivo* biological,[2] toxicological,[3] and pharmacological[3,4] properties of the orally absorbed deacetoxycephalosporin antibiotic, cephalexin (**7**).

We have examined several synthetic routes to cephalexin. One already described by Ryan, *et al.*,[5] proceeds from cephalosporin C through 7-aminodeacetoxycephalosporanic acid (7-ADCA, **9**). Another, which forms the basis of this report, stems from the work of Morin, *et al.*,[6] on the conversion of penicillin sulfoxides to deacetoxycephalosporins. The latter demonstrated that phenoxymethylpenicillin sulfoxide methyl ester, when heated under reflux in toluene with *p*-toluenesulfonic acid, rearranged in about 20% yield to the corresponding deacetoxycephalosporin methyl ester. A plausible mechanism offered was a cleavage of the S–C bond in the thiazolidine ring of the penicillin sul-

(1) Cephalexin is the generic name for 7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid; cephalexin monohydrate; KEFLEX, Lilly.

(2) (a) W. E. Wick, *Appl. Microbiol.*, **15**, (4), 765 (1967); (b) W. E. Wick and W. S. Boniece, "Proceedings of the 6th International Congress of Chemotherapy," Vienna, Austria, June 26–July 1, 1967, p 717–734.

(3) J. S. Wells, R. O. Froman, W. R. Gibson, N. V. Owen, and R. C. Anderson, *Antimicrob. Ag. Chemother.*, 489 (1968).

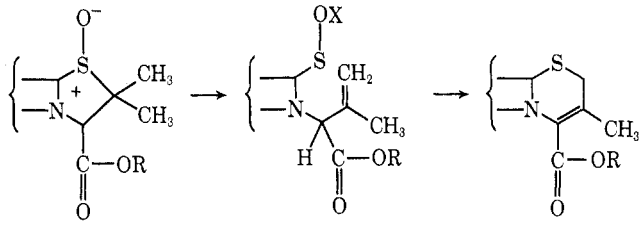
(4) R. S. Griffith and H. R. Black, *Clin. Med.*, **75** (11), 14 (1968).

(5) C. W. Ryan, R. L. Simon, and E. M. Van Heyningen, *J. Med. Chem.*, **12**, 310 (1969).

(6) (a) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Amer. Chem. Soc.*, **85**, 1896 (1963); (b) *ibid.*, **91**, 1401 (1969); (c) R. B. Morin and B. G. Jackson, U. S. Patent 3,275,626 (1966).

foxide to an unsaturated sulfenic acid (or anhydride) intermediate which recloses, with the sulfur adding to the terminal carbon of the double bond, to produce the more stable dihydrothiazine ring of the deacetoxycephalosporin (Scheme I).

SCHEME I



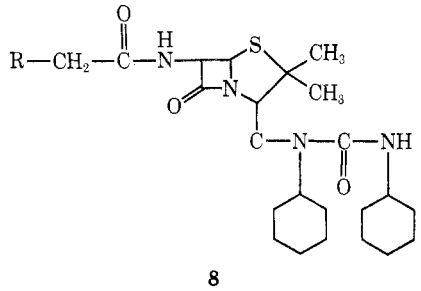
X = H, Ac, $(CH_2)_3SO_3H$

The potential utility of this transformation prompted an attempt to convert such a penicillin-derived deacetoxycephalosporin into cephalexin. Needed were (1) an improvement in yield in the penicillin sulfoxide rearrangement reaction, (2) a chemical or an enzymatic means of replacing the *N*-acyl function originally on the penicillins by the D-α-phenylglycyl moiety of cephalexin, and (3) an easily removable ester-protecting group for the carboxylic acid group in the starting penicillins. This paper describes solutions to these problems which led to a laboratory synthesis of cephalexin from the biosynthetic penicillins V and G (Scheme II).

Formation of Penicillin Sulfoxide Esters.—The earlier workers had found that the ring expansion of the free acid of phenoxymethylpenicillin sulfoxide was accompanied by extensive decarboxylation under the thermal and acidic requirements of the reaction.[6a] Further, the methyl ester employed in the original work could not be saponified satisfactorily without involvement of double-bond isomerization in the dihydrothiazine ring.[6b]

The trichloroethyl ester was a protecting group proven useful in the work of Woodward, *et al.*,[7] in a synthesis of cephalosporin C. Because it was anticipated that this ester might survive the working conditions of the rearrangement and subsequent side-chain cleavage reactions and could be removed without damage to the β-lactam dihydrothiazine ring system, it was deemed a suitable protecting group for our work.

Esterification of the penicillins **1a** and **1b** was achieved at first using trichloroethanol in the presence of *N*,*N*′-dicyclohexylcarbodiimide in methylene chloride solution containing dry pyridine. The yields of penicillin trichloroethyl esters **2a** and **2b** were reasonably good but were attended by a significant amount of the *N*,*N*′-dicyclohexylureido amide **8**.



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The occurrence of this by-product necessitated chromatographic separation or careful and repeated fractional crystallization of the desired esters. Because of this inconvenience and the relatively expensive condensing agent, we sought an alternative method of esterification. We had noted previously that cephalothin[8] reacts with chloroformates[9] to give stable carbonate esters which upon heating in anhydrous solutions undergo a quantitative decarboxylation to esters of the corresponding chloroformate. Accordingly, the penicillins **1a** and **1b** were allowed to react with trichloroethyl chloroformate in anhydrous acetone or tetrahydrofuran containing dry pyridine. The carbonate esters of the penicillins decarboxylated spontaneously at room temperature, affording satisfactory yields of the desired trichloroethyl esters **2a** and **2b**.

Because phenoxymethylpenicillin trichloroethyl ester (**2a**) is difficult to crystallize, it was used as an unpurified oil in the subsequent step.

Sulfoxidation[10] of these esters in chloroform solution using 85% *m*-chloroperbenzoic acid proceeded smoothly to penicillin sulfoxide esters **3a** and **3b**. No or only trace amounts of sulfones were observed in crude products by tlc.

Penicillin Sulfoxide Rearrangement.—As originally conceived, the penicillin sulfoxide rearrangement gave a low yield of the desired deacetoxycephalosporin along with non-β-lactam products[6b] that complicated the isolation procedure. The effects of a variety of acid catalysis and solvents,[11] and of time and temperature, on the course of the reaction were explored.

Ultimately, either acetic anhydride or propane sultone in combination with dimethylformamide or dimethylacetamide at temperatures below 135° was deemed most effective in promoting the desired transformation. Either phenoxymethyl (**3a**) or benzyl (**3b**) penicillin sulfoxide trichloroethyl ester was dissolved in dimethylformamide containing a fivefold excess of acetic anhydride and heated at 130° for 1 hr. The solvent and catalyst were removed under reduced pressure. The residual oil was redissolved in benzene and thoroughly washed with water. The benzene solution was dried and shown by thin layer chromatography and nmr spectroscopy to contain mainly deacetoxycephalosporin esters **4a** and **4b**, respectively.

These benzene solutions, when subjected to Florisil column chromatography, provided pure, crystalline samples of deacetoxycephalosporin esters that were identical (by elementary analyses, melting point, and ir spectra) with authentic phenoxy- (**4a**) and phenyl- (**4b**) acetamidodeacetoxycephalosporin trichloroethyl esters. The known esters were prepared by stepwise acylation of 7-ADCA (**9**) with phenoxy- and phenylacetyl chlorides to the respective acids **10a** and **10b** and esterification of these, using trichloroethyl chloroformate, with

(7) (a) R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, and H. Vorbüggen, *J. Amer. Chem. Soc.*, **88**, 852 (1966). (b) R. B. Woodward, South African Patent 65105 (Derwent No. 26,121) (1966). (c) The trichloroethyl ester was also successfully used as a protecting group for phosphates in peptide chemistry: F. Eckstein, *Angew. Chem., Int. Ed. Engl.*, **4**, 876 (1965).

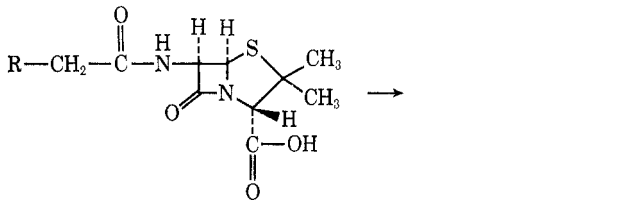
(8) Cephalothin is the generic name for 7-(thiophene-2-acetamido)-cephalosporanic acid; cephalothin sodium salt, KEFLIN, Lilly.

(9) R. R. Chauvette and E. H. Flynn, *J. Med. Chem.*, **9**, 741 (1966).

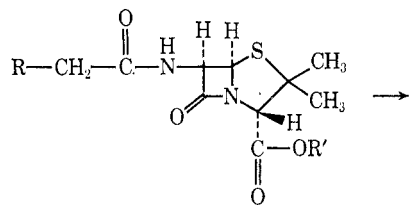
(10) C. J. Cavallito and J. H. Harley, *J. Org. Chem.*, **15**, 815 (1950).

(11) R. D. G. Cooper, Canadian Patent 817,883 (1969).

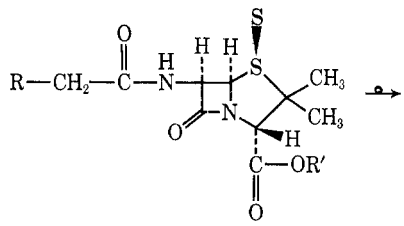
SCHEME II



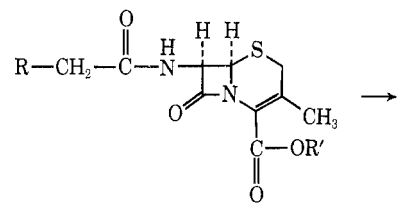
1a, b



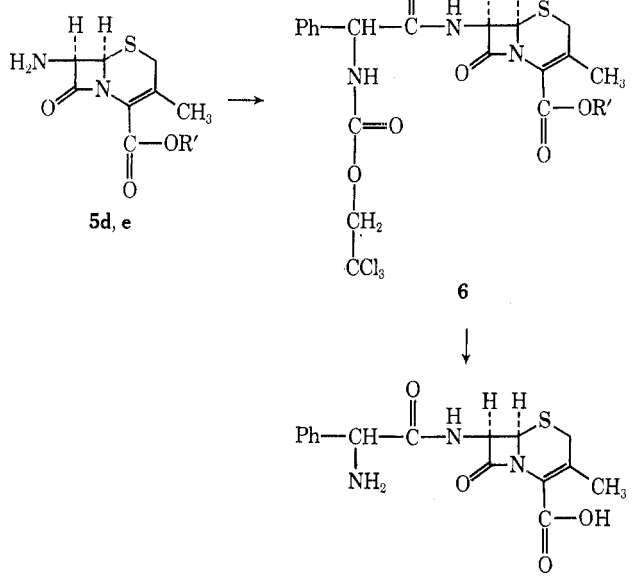
2a–c



3a–c



4a–c



5d, e

6

7

a, R = PhO; R′ = CH_2CCl_3

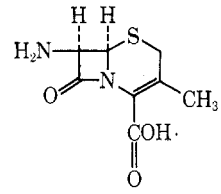
b, R = Ph; R′ = CH_2CCl_3

c, R = PhO; R′ = CH_2–C$_6$H$_4$–OCH_3

d, R′ = CH_2CCl_3

e, R′ = CH_2–C$_6$H$_4$–OCH_3

subsequent separation of the desired products from Δ^2 isomeric esters **11** by fractional crystallization.



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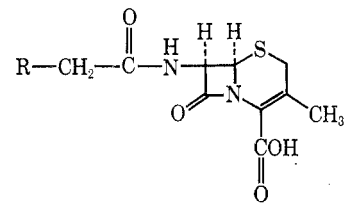
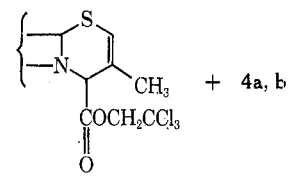


10a, b



11

A yield approaching 60% for the rearrangement reaction was realized when this reaction and the following N-deacylation step were carried out successively without isolation of the intermediate esters **4a** and **4b**.

Side-Chain Cleavage.—The utility of the foregoing reaction depended in large measure on our finding a convenient method—preferably a chemical one—for replacing the inherent *N*-acyl substituents by the D-α-phenylglycyl grouping. A known process[12] for removing the α-aminoadipoyl side chain of cephalosporin C (employing phosphorus oxychloride to produce an imino chloride intermediate of the 7-amide function) was inoperative with our compounds. Modifications of this procedure, however, resulted in a workable process for the removal of our phenoxy and phenylacetyl substituents.

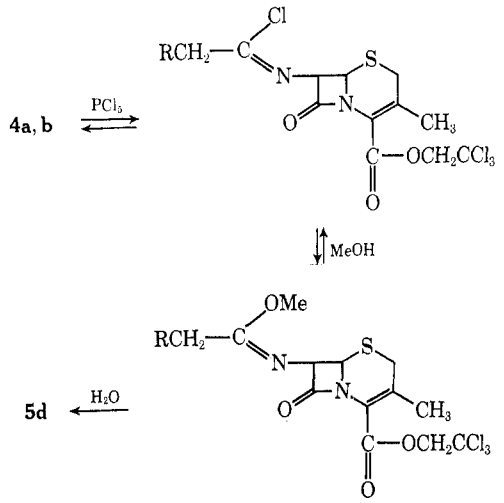
Phenoxy- (**4a**) and phenyl- (**4b**) acetamidodeacetoxycephalosporin trichloroethyl esters required heating to 60–80° in an anhydrous, nonpolar solvent such as benzene with phosphorus pentachloride and pyridine to form imino chlorides in good yield.

Methanol reacted with these at room temperature to form imido esters which hydrolyzed instantly in contact with water to liberate 7-aminodeacetoxycephalosporin trichloroethyl ester **5d**.

Benzene was the most satisfactory solvent in the PCl_5 reaction. The ratio of reactants in this step appeared to be very critical. The ratio of reactants in this step appeared to be very critical. Whereas the presence of 1 equiv of pyridine was essential, a large excess did not lead to the desired product. The use of a slight excess of PCl_5 and pyridine, each in equal molar ratio, over the amount of ester gave optimum yields of side-chain cleavage. The imido ester intermediate was

(12) (a) N. Rusting, J. C. Frielink, and C. F. van der Beek, Netherlands Patent 6,401,421 (Derwent No. 13,407) (1964). (b) The above process was also successfully applied to the side-chain cleavage of penicillin silyl esters: H. Wilhelm, O. Weissenberger, and M. G. van der Hoeven, Netherlands Patent 6,606,872 (Derwent No. 29,574) (1966).

hydrolyzed at room temperature, solubilized generally in a mixture of water–tetrahydrofuran at the existing pH (*ca.* 1.8). The resulting 7-ADCA trichloroethyl



ester (**5d**) so produced formed highly insoluble, crystalline salts with aromatic sulfonic acids, permitting its ready separation from the complex reaction mixtures. The yield of amido ester **5d**, isolated as a tosylate from either phenoxy- (**4a**) or phenyl- (**4b**) acetamidodeacetoxycephalosporanic acid trichloroethyl ester, was consistently 75–80%.

In the experiments in which the penicillin sulfoxide rearrangement was followed by side-chain cleavage without isolation of the first product, the overall yields ranged between 42 and 46%.

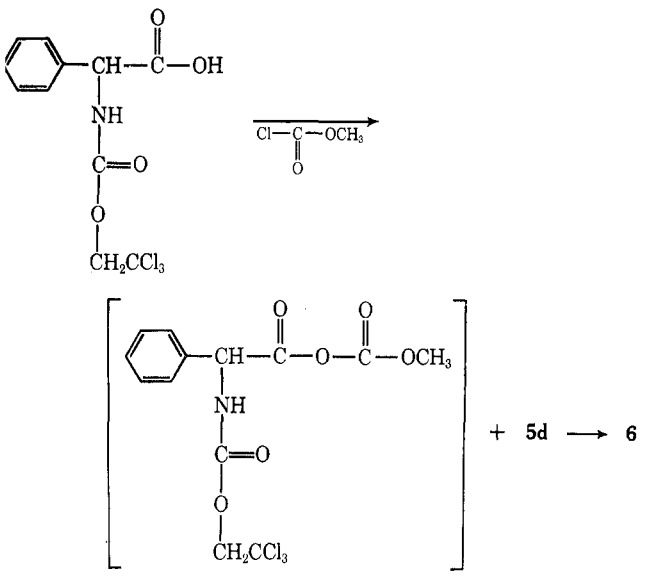
The preparation of 7-ADCA (**9**) from the trichloroethyl ester **5d** by removal of the ester grouping with zinc in acetic acid confirmed that the side-chain cleavage had occurred. The first reported preparation of 7-ADCA (**9**) was from a palladium-catalyzed hydrogenolysis of 7-ACA.[13]

Reacylation of this 7-ADCA trichloroethyl ester (**5d**) with phenoxy and phenyl acetyl chlorides in acetone (with urea in suspension to absorb HCl[14]) regenerated compounds **4a** and **4b**, respectively.

In the penicillin V series, we considered using the *p*-methoxybenzyl ester as a blocking group. Phenoxymethylpenicillin sulfoxide *p*-methoxybenzyl ester (**3c**) did not rearrange to a deacetoxycephalosporin (**4c**) as well as did the corresponding trichloroethyl ester. The relative lability of the *p*-methoxybenzyl group to acid was probably responsible for the lower yields experienced here and in the amide-cleavage reaction that followed. When compound **4c** was allowed to react with PCl_5 and cleaved in the manner described earlier, the expected 7-ADCA *p*-methoxybenzyl ester (**5e**) resulted in 47% yield. While we did not carry out the subsequent steps leading to cephalexin with this ester, we showed that the *p*-methoxybenzyl ester was indeed cleavable in anhydrous trifluoroacetic acid without adverse effect on the β-lactam. We generated the deacetoxycephalosporanic acid **10a** from **4c**.

Reacylation and "Deblocking."—In the reacylation of 7-aminodeacetoxycephalosporanic acid trichloroethyl ester (**5d**), the choice of the *N*-trichloroethyloxycarbonyl derivative of D-α-phenylglycine was one dictated by several considerations. It could be prepared from the same trichloroethyl chloroformate used for the protection of the carboxyl group in the penicillins, and could be removed simultaneously with the ester function in a final reductive "deblocking" step.

Consequently, D-α-phenylglycine was acylated, using trichloroethyl chloroformate in a Schotten–Baumann reaction. The N-protected amino acid was in turn used to acylate 7-ADCA trichloroethyl ester (**5d**) in a mixed anhydride coupling with methyl chloroformate. The resulting "doubly protected" cephalexin **6** was formed in near quantitative yield. This ease of acylation of amino ester **5d** is noteworthy compared with the difficulties commonly encountered in the acylation of 7-ACA[15] and 7-ADCA (**9**),[5] as their zwitterion forms in the same mixed anhydride reaction.



Compound **6** was recovered unchanged when treated with zinc dust in 90% aqueous acetic acid in the manner described for the formation of **9** from 7-ADCA trichloroethyl ester (**5d**). However, reductive cleavage of the protecting groups was effected using zinc dust in cold 90% aqueous formic acid, or using a zinc–copper couple in formic acid diluted ninefold with acetonitrile. In an experiment in which we followed the progress of the reaction at 15-min intervals by a biological assay for cephalexin (accurate to ±10%), the maximal yield of the antibiotic was reached within 45 min. The progress of the deblocking reactions could also be monitored by tlc and paper chromatograms.

Isolation of pure cephalexin was complicated by zinc ions that complexed with this compound. Components of the reaction mixture, such as zinc chloride or salts of formic acid, were shown to solubilize cephalexin in water–acetonitrile solutions from which it is usually precipitated at its isoelectric point.

The difficulties were alleviated either by removing the zinc ions as zinc sulfide or by passing these aqueous solutions at neutral pH through an imino diacetate resin (Bio-Rad, Chelex 100) to exchange sodium for zinc ions. Yields of cephalexin isolated from this "doubly protected" derivative (**6**) were approximately 60%.

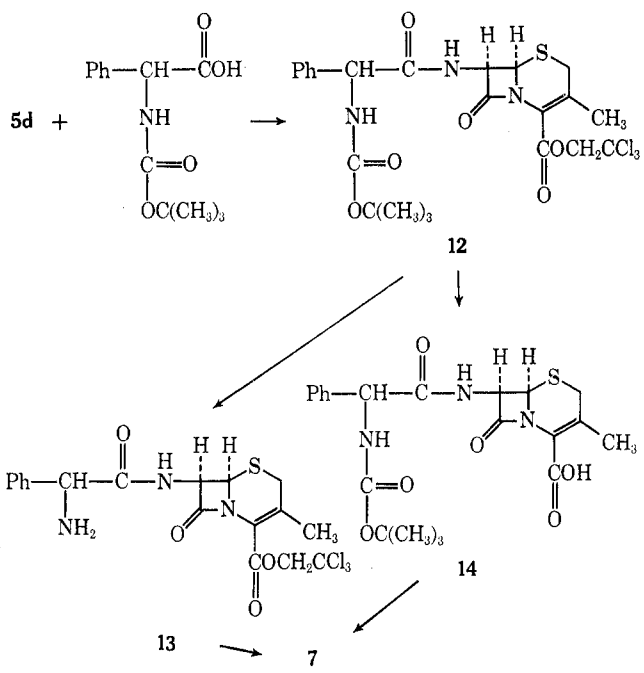
(13) R. J. Stedman, K. Swered, and J. R. E. Hoover, *J. Med. Chem.*, **7**, 117 (1964).

(14) H. M. Higgens, Jr., U. S. Patent 3,351,597 (1967).

(15) J. L. Spencer, E. H. F[illegible]nn, R. W. Roeske, F. Y. Siu, and R. R. Chauvette, *J. Med. Chem.*, **9**, 746 (1966).

The acylation of 7-ADCA trichloroethyl ester (**5d**) with *N*-*tert*-butoxycarbonyl-D-α- phenylglycine resulted in a "doubly protected" form of cephalexin (**12**) that necessitated stepwise deblocking (Scheme III). The

SCHEME III



acylation step proceeded in nearly quantitative yields in a wide variety of solvents (acetone, acetonitrile, dimethylformamide, ethyl acetate, methylene chloride). The *tert*-Boc group of **12** was removed by *p*-toluenesulfonic acid treatment in acetonitrile. Although thin layer chromatography indicated nearly complete reaction, cephalexin trichloroethyl ester (**13**) could not be crystallized as a tosylate salt and could only be crystallized with difficulty as the free amino ester. When the crude product was subjected to a zinc–HCl reduction in acetonitrile, the ensuing cephalexin was not pure. The alternate "stepwise-deblocking"scheme became clearly advantageous. We found that dimethylformamide was the best solvent for the deesterification step. Either glacial acetic acid or 98% formic acid, with or without water, in combination with dimethylformamide and zinc dust led to the isolation of *N*-*tert*-butoxycarbonylcephalexin (**14**) in greater than 90% yield. In this solvent a suspected competing reduction of trichloroethyl ester to dichloroethyl ester was apparently minimal.[16] One noteworthy advantage of this two-step process was that the resulting cephalexin derivative (**14**) was extractable into bicarbonate solution and back-titratable into organic solvent and could, therefore, be separated from troublesome inorganic and neutral organic[16] materials.

The action of *p*-toluenesulfonic acid on *N*-*tert*-butoxycarbonylcephalexin (**14**) was most facile in acetonitrile. Removal of the *tert*-Boc group was effected at room temperature within a few hours in this solvent. Under like conditions, compound **14** was either unchanged or little changed in solvents such as acetone, benzene, ethyl acetate, or ethanol, even after several days. The work-up and isolation of cephalexin from this reaction mixture was notably simple. As acetonitrile is an excellent antisolvent for cephalexin, the reaction mixture was diluted with water and adjusted to pH 4.5 with triethylamine or ammonium hydroxide. The cephalexin crystallized immediately in pure form in over 70% overall yield from 7-ADCA trichloroethyl ester (**5d**).

This synthesis, with suitable modifications, has been successfully implemented into a several-kilogram scale preparation of cephalexin.

(16) When this neutral fraction was chromatographed over silica, eluting with benzene–ethyl acetate (3:1), a cephalexin derivative was isolated and shown by nmr and mass spectra to be the dichloroethyl ester. The nmr, in $CDCl_3$, showed a triplet centered at τ 4.1 ascribable to the dichloromethine proton and a doublet centered at τ 4.55 for the methylene protons, shifted from τ 5.1 in the normal trichloroethyl ester. This neutral fraction represented less than 7% weight of the starting material.

Experimental Section[17]

2,2,2-Trichloroethyl 6-(Phenoxyacetamido)penicillanate (2a). Method A.—1a potassium salt (77.4 g, 200 mmol) was suspended in 1.5 l. of CH_2Cl_2, and pyridine hydrochloride (24 g, 200 mmol) was added. The suspension was cooled in an ice–H_2O bath for addition of trichloroethanol (30 g, 200 mmol) and then *N*,*N*′-dicyclohexylcarbodiimide (41.2 g, 200 mmol) in 250 ml of CH_2Cl_2, dropwise. The mixture was stirred at room temperature overnight and filtered. The filtrate was washed with 5% $NaHCO_3$ solution and then with H_2O, dried ($MgSO_4$), and concentrated to dryness *in vacuo*. The residual oil, weighing 85 g, gave one spot in tlc.

Nmr and ir spectra of this crude product were consistent with that of the penicillin ester prepared by method B.

A 5-g sample of this oil crystallized (with difficulty) from 10 ml of ether and 35 ml of petroleum ether: recovery, 4.4 g; mp 70–80°.

Anal. Calcd for $C_{18}H_{19}Cl_3N_2O_2S$: C, 44.87; H, 3.97; N, 5.82. Found: C, 45.01; H, 4.17; N, 5.95.

Method B.—1a (8.0 g, 22.8 mmol) was dissolved in 70 ml of CaH_2-dried THF containing dry pyridine (1.8 g, 22.8 mmol). While the reaction mixture was stirred and cooled at ice-bath temperature, trichloroethyl chloroformate (4.8 g, 22.8 mmol) in 30 ml of the same solvent was added dropwise. Stirring was continued at room temperature overnight. After a brief reflux, the solvent was removed *in vacuo*. The residue was dissolved in cold EtOAc, and this was washed with 5% $NaHCO_3$ solution and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residue (an oil weighing 9.8 g) did not crystallize, but gave satisfactory analysis and physical data, comparable to material prepared by method A.

Nmr (in $CDCl_3$) showed signals at τ 8.33 and 8.41 (2 s, 6 H, *gem*-di-CH_3), 5.40 (s, 2 H, α-CH_2), 5.35 (s, 1 H, C_3H), 5.19 (s, 2 H, ester CH_2), 4.28 (m, 2 H, C_5H and C_6H), and 3.17–2.4 (m, 6 H, aromatic and amide N–H). Ir (in $CHCl_3$) showed bands at 2.95 (amide NH), 5.6–5.7 (broad, β-lactam and ester carbonyls), 5.91 and 6.22 μ (amide carbonyl), and in the aromatic regions.

2,2,2-Trichloroethyl 6-(Phenoxyacetamido)penicillanate 1-Oxide (3a).—2a (25 g, 53 mmol) was dissolved in 250 ml of $CHCl_3$ and stirred in an ice–H_2O bath; 85% *m*-chloroperbenzoic acid (10 g, 50 mmol) in 150 ml of $CHCl_3$ was added dropwise over 30 min. Stirring and cooling were maintained for another 30 min. The reaction solution was washed with 5% $NaHCO_3$ solution and then with H_2O, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residual oil was redissolved in 100 ml of Et_2O (and a few drops of THF to clear the solution) and chilled for crystallization, yield 23.0 g (94%), mp 145–146°.

(17) All melting points were taken on a Mel-Temp apparatus and are uncorrected. All tlc was done using silica gel plates, C_6H_6–EtOAc (7:3) as eluent (unless otherwise stated), and an iodine chamber to develop the spots. Bioautographs (against *Bacillus subtilis* seeded agar plates) were made from paper chromatograms developed in *n*-BuOH–AcOH–H_2O. All evaporations were performed below 55° with a rotary vacuum evaporator. Nmr spectra were taken on a Varian Associates Model HR-60 spectrometer with TMS as internal standard. Uv spectra were recorded with a Cary spectrophotometer; ir spectra were recorded on Beckman IR-7 or Perkin-Elmer Models 21 or Infracord spectrophotometers.

Nmr (in $CDCl_3$) showed signals at τ 8.76 and 8.28 (2 s, 6 H, *gem*-di-CH_3), 5.51 (s, 2 H, α-CH_2), 5.23 (d, 2H, ester CH_2), 5.20 (s, 1 H, C_3H), 4.89 (d, 1 H, C_5H), 3.91 (q, 1 H, C_6H), 3.19–2.56 (m, 5 H, aromatic H), and 1.75 (d, 1 H, amide N–H). Ir (in $CHCl_3$) showed bands at 2.95 (amide NH), 5.50 and 5.61 (β-lactam and ester carbonyls, respectively), 5.90 and 6.65 μ (amide carbonyls), and in the aromatic regions.

Anal. Calcd for $C_{18}H_{19}Cl_3N_2O_6S$: C, 43.43; H, 3.85; N, N, 5.63; S, 6.44. Found: C, 43.66; H, 4.00; N, 5.78; S, 6.54.

2,2,2-Trichloroethyl 6-(Phenylacetamido)penicillanate (2b). Method A.—**1b** potassium salt (37.1 g, 100 mmol) was suspended in 1 l. of CH_2Cl_2. A slurry of pyridine hydrochloride (12 g, 100 mmol) in 50 ml of CH_2Cl_2 was added. A solution of 2,2,2-trichloroethanol (15 g, 100 mmol) in 50 ml of CH_2Cl_2 and then *N,N'*-dicyclohexylcarbodiimide (20.6 g, 100 mmol) in 100 ml of CH_2Cl_2 were added. The mixture was stirred at room temperature overnight and worked up as described for the preparation of **2a**. The product crystallized from Et_2O, yield 30.3 g (65%), mp 157–159°.

Nmr and ir spectra were consistent with that of material prepared by method B.

Method B.—**1b** potassium salt (8.5 g, 22.8 mmol) was suspended in 100 ml of molecular-sieve-dried Me_2CO containing dry pyridine (2.7 g, 34 mmol). The mixture was stirred at ice-H_2O temperature. 2,2,2-Trichloroethyl chloroformate (4.8 g, 22.8 mmol) in 30 ml of dry Me_2CO was added dropwise. Stirring and cooling were maintained overnight. Insoluble material was filtered. Addition of 50 ml of H_2O to the briefly warmed filtrate caused the product to crystallize, yield 7 g (66%), mp 160–161°.

Nmr (in $CDCl_3$) showed signals at τ 8.49 (s, 6 H, *gem*-di-CH_3), 6.41 (s, 2 H, α-CH_2), 5.55 (s, 1 H, C_3 H), 5.28 (s, 2 H, C_3 ester CH_2), 4.4 (m, 2 H, C_5 H and C_6 H), 3.75 (d, 1 H, amide NH), and 2.74 (s, 5 H, aromatic H). Ir (in $CHCl_3$) showed bands at 2.92 (amide NH), 5.6–5.7 (broad, β-lactam and ester carbonyls), 5.92 and 6.62 μ (amide carbonyl), and in the aromatic regions.

Anal. Calcd for $C_{18}H_{19}Cl_3N_2O_4S$: C, 46.41; H, 4.11; N, 6.02. Found: C, 46.75; H, 4.27; N, 5.99.

2,2,2-Trichloroethyl 6-(Phenylacetamido)pencillanate 1-Oxide (3b).—**2b** (4.7 g, 10 mmol) was dissolved in 50 ml of $CHCl_3$, The solution was stirred and cooled (to *ca.* 0°), and 85% *m*-chloroperbenzoic acid (2.2 g, 11 mmol) in 25 ml of $CHCl_3$ was added dropwise. The mixture was chilled for 2 hr. It was then washed with 5% $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residue was triturated with Et_2O and dried to give 4.05 g (84%), mp 167–169°. Recrystallization from THF–Et_2O raised the melting point to 174–176°.

Nmr (in $CDCl_3$) showed signals at τ 8.74 and 8.25 (2s, 6 H, *gem*-di-CH_3), 6.4 (s, 2 H, α-CH_2), 5.23 (s, 1 H, C_3 H), 5.16 (q, 2 H, ester CH_2), 4.99 (d, 1 H, C_5 H), 3.97 (q, 1 H, C_6 H), 2.81 (d, 1 H, amide NH), 2.70 (s, 5 H, aromatic H). Ir (in $CHCl_3$) showed bands at 2.95 (amide NH) and 5.50, 5.62, and 5.90 μ (β-lactam, ester, and amide carbonyls, respectively).

Anal. Calcd for $C_{18}H_{19}Cl_3N_2O_5S$: C, 44.88; H, 3.97; N, 5.81; Cl, 22.07. Found: C, 45.04; H, 4.20; N, 5.73; Cl, 21.75.

***p*-Methoxybenzyl 6-(Phenoxyacetamido)penicillanate 1-Oxide (3c).**—**1a** potassium salt (3.9 g, 10 mmol) was suspended in 50 ml of DMF containing *p*-methoxybenzyl bromide (2.0 g, 10 mmol). The mixture was stirred at room temperature for 90 min. The mixture was diluted with C_6H_6 and washed three times with H_2O and then with 5% $NaHCO_3$ solution. The C_6H_6 solution was dried ($MgSO_4$) and evaporated to a clear gum *in vacuo*. This was redissolved in 50 ml of $CHCl_3$, chilled in an ice-H_2O bath, and treated with *m*-chloroperbenzoic acid (1.7 g, 10 mmol). The mixture was cooled, stirred for 15 min, and extracted with 50 ml of saturated $NaHCO_3$ solution and then with 30 ml of H_2O. The $CHCl_3$ solution was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The residual glass was crystallized from THF–Et_2O to give 3.4 g (73% over-all yield from phenoxymethyl penicillin).

Nmr (in $CDCl_3$) showed signals at τ 8.96 and 8.39 (2s, 6 H, *gem*-di-CH_3), 6.2 (s, 3 H, OCH_3), 5.50 (s, 2 H, α-CH_2), 5.36 (s, 1 H, C_3 H), 5.0 (d, 1 H, C_5 H), 4.85 (d, 2 H, ester CH_2), 3.96 (q, 1 H, C_6 H), 3.2–2.62 (m, 9 H, aromatic H), and 1.75 (d, 1 H, amide NH). Ir (in $CHCl_3$) showed bands at 2.95 μ (amide NH), 5.55, 5.72, and 5.92 μ (β-lactam, ester, and amide carbonyls respectively), and in the aromatic region.

Anal. Calcd for $C_{24}H_{26}N_2O_7S$: C, 59.25; H, 5.39; N, 5.76; S, 6.59. Found: C, 59.49; H, 5.61; N, 5.97; S, 6.44.

2,2,2-Trichloroethyl 7-Phenoxyacetamido-3-methyl-3-cephem-4-carboxylate (4a). 1. Rearrangement of Penicillin Sulfoxide Ester.—**3a** (4.6 g, 9.2 mmol) was dissolved in a mixture of 260 ml of DMF and Ac_2O (4.9 g, 48 mmol), preheated to 130°. The mixture was maintained at this temperature for 1 hr. The solvent and excess catalyst were removed by evaporation *in vacuo*. The residue was taken up in 500 ml of C_6H_6 for several H_2O washes. The C_6H_6 solution was dried ($MgSO_4$), concentrated to a smaller volume *in vacuo*, and passed through a Florisil column. The C_6H_6 solution was again concentrated to dryness *in vacuo*. The residual oil did not crystallize but appeared to be a single material (tlc). This material was identical (nmr, ir) with the deacetoxycephalosporanic acid trichloroethyl ester prepared by the following alternative methods.

2. Acylation and Esterification of 7-ADCA (9). 7-Phenoxyacetamido-3-methyl-3-cephem-4-carboxylic Acid (10a). Method A.—**9** (10.7 g, 50 mmol) was dissolved in 400 ml of H_2O and 300 ml of Me_2CO containing $NaHCO_3$ (14 g, 166 mmol). With stirring and cooling in ice, phenoxyacetyl chloride (8.5 g, 50 mmol) in 100 ml of dry Me_2CO was added dropwise. The mixture was stirred in the cold overnight. Me_2CO was evaporated *in vacuo*. The aqueous solution was washed with EtOAc and then acidified to pH 2.5 in the cold and in the presence of EtOAc. The organic layer was separated, washed with cold H_2O, dried ($MgSO_4$), and concentrated to a smaller volume from which the product crystallized to give 12.4 g (71%). For characterization a small sample was recrystallized from EtOAc–petroleum ether, mp 186–188° dec.

Nmr (in DMSO-d_6) showed signals at τ 7.99 (s, 3 H, C_3 CH_3), 6.73 and 6.40 (2d, 2 H, C_2 H_2), 5.40 (s, 2 H, α-CH_2), 4.95 (d, 1 H, C_6 H), 4.40 (q, 1 H, C_7 H), 3.21–2.58 (m, 5 H, aromatic H), and 1.48 (d, 1 H, NH). Ir (in a Nujol mull) showed bands at 2.97 (amide NH), 5.7–6.0 μ (broad, β-lactam, acid and amide carbonyl, respectively), and in aromatic region. Uv (in EtOH) showed maxima at 215 mμ (ϵ 12,600), 2.67 (7210), and 272 (6100).

The electrometric titration (in 66% aqueous DMF) showed a titratable group at 5.8 and an average molecular weight of 333 (calcd 349).

Anal. Calcd for $C_{16}H_{16}N_2O_5S$: C, 55.17; H, 4.63; N, 8.04. Found: C, 55.13; H, 4.92; N, 8.01.

Method B.—**4c** (550 mg, 1.2 mmol) was dissolved in 20 ml of C_6H_6. Trifluoroacetic acid (3 ml) was added, and the mixture was stirred at room temperature for 30 min. The solvent and reagents were removed under reduced pressure. The residue was shaken with a C_6H_6–aqueous $NaHCO_3$ mixture. The aqueous portion was separated and acidified to pH 2. The crystalline precipitate, weighing 135 mg (33% yield), gave nmr and ir spectral data identical with those from the cephalosporanic acid **10a** prepared by the alternate procedure.

A mixture of this acid (**10a**) (85 g, 244 mmol), dry pyridine, (23.8 g, 300 mmol), and 2,2,2-trichloroethanol (36.6 g, 244 mmol) in 2.5 l. of CH_2Cl_2 was treated with *N,N'*-dicyclohexylcarbodiimide (55.6 g, 270 mmol) in 200 ml of the same solvent by dropwise addition. The mixture was stirred at room temperature overnight. The dicyclohexylurea was filtered. The solvent was replaced by cold EtOAc for successive cold washes with H_2O, 5% HCl, 5% $NaHCO_3$ solution, and H_2O. The EtOAc solution was dried ($MgSO_4$) and evaporated *in vacuo*. The residual oil was redissolved in 600 ml of anhydrous Et_2O. The ethereal solution was concentrated to about 300 ml and refrigerated for fractional crystallization of the Δ^3 ester from the more soluble isomeric Δ^2 product, yield 51.3 g (44%), mp 119°.

Nmr (in $CDCl_3$) showed signals at τ 7.86 (s, 3 H, C_3 CH_3), 6.89 and 6.51 (2 d, 2 H, C_2 H_2), 5.54 (s, 2 H, α-CH_2), 5.22 (d, 2 H, C_4 ester CH_2), 5.08 (d, 1 H, C_6 H), 4.29 (q, 1 H, C_7 H), and 3.30–2.45 (m, 6 H, aromatic H and amide NH). Ir (in $CHCl_3$) showed bands at 3.0 (amide NH) and 5.62, 5.79, and 5.94 μ (β-lactam, ester, and amide carbonyls, repectively), and in the aromatic region. Uv (in EtOH) showed maxima at 216 mμ (ϵ 13,100), 268 (6800), and 274 (6300).

Anal. Calcd for $C_{18}H_{17}Cl_3N_2O_5S$: C, 45.05; H, 3.57; N, 5.84. Found: C, 45.22; H, 3.77; N, 5.75.

3. Reacylation of 7-ADCA Ester (5d).—**5d** was acylated using phenoxyacetyl chloride in the manner described below for **4b**. The product, formed in 78% yield, was identical (nmr and ir) with material prepared by alternate methods.

2,2,2-Trichloroethyl 7-Phenylacetamido-3-methyl-3-cephem-4-carboxylate (4b). 1. Rearrangment of the Penicillin Sulfoxide Ester (3b).—**3b** (4.0 g, 8.5 mmol) dissolved in 200 ml of DMF containing Ac_2O (4 ml, 42 mmol) was placed in a preheated oil bath and heated for 1 hr at 130°. The solvent and catalyst were then removed by evaporation *in vacuo*. The residue was dis-

solved in 200 ml of C_6H_6, washed several times with H_2O, dried ($MgSO_4$), and concentrated to a smaller volume (about 50 ml). The concentrated C_6H_6 solution was passed through a Florisil column while eluting with additional C_6H_6 containing 5% EtOAc. The eluent was diluted with ether and refrigerated to induce crystallization. The pure product weighed 800 mg (20% yield), mp 162–164°.

Nmr (in $CDCl_3$) showed signals at τ 7.83 (s, 3 H, C_3 CH_3), 6.87 and 6.48 (2d, 2 H, C_2 H_2), 6.41 (s, 2 H, α-CH_2), 5.39–4.92 (three overlapping d, 3 H, ester CH_2 and C_6H), 4.25 (q, 1 H, C_7 H), 3.31 (d, 1 H, amide NH), and 2.70 (s, 5 H, aromatic H).

Anal. Calcd for $C_{18}H_{17}Cl_3N_2O_4S$: C, 46.62; H, 3.70; N, 6.04. Found: C, 46.87; H, 3.91; N, 6.01.

2. Acylation and Esterification of 7-ADCA (9). 7-Phenylacetamido-3-methyl-3-cephem-4-carboxylic Acid (10b).—9 (4.2 g, 20 mmol) was acylated with phenylacetyl chloride in the manner described for **10a**. The crude product was crystallized by trituration with petroleum ether and recrystallized from *i*-PrOH–petroleum ether, yield 3.5 g (53%), mp 198–200°.

The nmr and ir spectra were consistent with a deacetoxycephalosporanic acid.

Anal. Calcd for $C_{16}H_{16}N_2O_4S$: C, 57.81; H, 4.85; N, 8.43. Found: C, 57.92; H, 4.93; N, 8.33.

The acid product from above was esterified using *N*,*N*′-dicyclohexylcarbodiimide in the manner described with **10a**. The product was identical (melting point, nmr, and ir) with a sample prepared *via* the penicillin sulfoxide ester rearrangement.

3. Reacylation of 7-ADCA Ester (5d).—**5d**, regenerated from its tosylate (14 g, 27 mmol), was dissolved in 100 ml of molecular-sieve-dried Me_2CO. Urea (3.25 g, 54 mmol) was suspended to absorb the HCl from the acylation.[14] The reaction mixture was stirred during dropwise addition of phenylacetyl chloride (4.18 g, 27 mmol) in 50 ml of dry Me_2CO. The mixture was stirred for an additional hour and then filtered, and the Me_2CO was removed by evaporation. The residue was dissolved in cold EtOAc for successive cold washes with H_2O, 5% HCl, 5% $NaHCO_3$ solution, and H_2O. The EtOAc solution was dried ($MgSO_4$), concentrated to a smaller volume, and diluted with ether. The product crystallized at room temperature. The first crop weighed 5.35 g (43% yield). An additional crop raised the yield to 64%.

Nmr (in $CDCl_3$) showed signals at τ 7.82 (s, 3 H, C_3 CH_3), 6.90 and 6.50 (2d, 2 H, C_2 H_2), 6.42 (s, 2 H, α-CH_2), 5.17 (d, 2 H, C_4 ester CH_2), 5.01 (d, 1 H, C_6 H), 4.28 (q, 1 H, C_7 H), 3.30 (d, 1 H, amide NH), and 2.78 (s, 5 H, aromatic H). Ir (in CCl_4) showed bands at 2.95 (amide NH), 5.60, 5.75, and 5.95 μ (β-lactam, ester, and amide carbonyls, repectively), and in the aromatic region.

***p*-Methoxybenzyl 7-Phenoxyacetamido-3-methyl-3-cephem-4-carboxylate (4c).**—**3c** (3.4 g, 7.0 mmol) and 3.5 ml of Ac_2O were added to 200 ml of DMF preheated to 134°. The solution was maintained at this temperature for 1 hr. The volatile portion of the mixture was removed *in vacuo*. The residual oil crystallized on standing. The product, after trituration with CCl_4, weighed 860 mg (26% yield).

Nmr (in $CDCl_3$) showed all the signals expected for a phenoxyacetamidodeacetoxycephalosporin and, in addition, signals at τ 6.23 (s, 3 H, *p*-OCH_3), 4.81 (s, 2 H, ester CH_2), and 3.2–2.5 (m, four additional aromatic H).

Anal. Calcd for $C_{24}H_{24}N_2O_6S$: C, 61.53; H, 5.16; N, 5.98. Found: C, 59.92; H, 5.32; N, 6.36.

2,2,2-Trichloroethyl 7-Amino-3-methyl-3-cephem-4-carboxylate (5d). *p*-Toluenesulfonic Acid Salt. 1. Cleavage of the Phenoxyacetyl Side Chain. Method A.—**4a** (2.2 g, 4.6 mmol) was dissolved in 120 ml of CaH_2-dried C_6H_6 containing dry pyridine (540 mg, 6.8 mmol). The solution was placed in a water bath at 65°. While stirring, PCl_5 (1.4 g, 6.8 mmol) was added, and the mixture was stirred at this temperature and under nitrogen for 2 hr. The C_6H_6 was removed *in vacuo* and replaced by 240 ml of MeOH. The solution was stored at room temperature under nitrogen overnight. The alcohol was removed *in vacuo*. The residue was redissolved in a mixture of H_2O–THF at room temperature for 15 min to effect hydrolysis. The organic solvent was evaporated. The aqueous portion, with its oily precipitate, was slurried with EtOAc and adjusted to pH near 7 with 1 *N* NaOH. The EtOAc solution was separated, washed with H_2O, dried ($MgSO_4$), and concentrated to about 80 ml. The concentrate was treated with *p*-toluenesulfonic acid monohydrate (875 mg, 4.6 mmol) in 70 ml of the same solvent to precipitate the product as a crystalline salt, 1.9 g (80% yield).

Nmr (in DMSO-d_6) showed signals at τ 7.76 (s, 3 H, C_3 CH_3), 7.70 (s, 3 H, TSH CH_3), 6.40 (s, 2 H, C_2 H_2), 4.91 τ (s, 2 H, ester CH_2), 4.80 (s, 2 H, C_6 H and C_7 H), and 2.90–2.34 (4s, 4 H, aromatic H). Ir (in a Nujol mull) showed bands at 5.65 (β-lactam carbonyl), 5.81 (ester carbonyl), 8.1 μ (SO_3), and in the aromatic region. Electrometric titration (in 66% aqueous DMF) showed a basic pK_a of 3.9 and an average molecular weight of 380 (calcd 346). A sample was recrystallized from EtOH–ether, mp 193–194° dec.

Anal. Calcd for $C_{17}H_{19}Cl_3N_2O_6S_2$: C, 39.42; H, 3.69; N, 5.41. Found: C, 39.50; H, 3.84; N, 5.23.

Method B.—**3a** (4.6 g, 9.2 mmol) was dissolved in 260 ml of DMF containing Ac_2O (4.9 g, 48 mmol). The mixture was heated in an oil bath at 130° for 1 hr. The solvent was evaporated *in vacuo*. The residue was dissolved in 500 ml of C_6H_6 and washed three times with 400-ml portions of H_2O (saturated NaCl solution was used to break an emulsion that formed). The C_6H_6 solution was dried ($MgSO_4$) and concentrated *in vacuo* to about 200 ml. To the concentrate were added dry pyridine (1.1 g, 13.9 mmol) and PCl_5 (2.9 g, 13.9 mmol); the mixture, under nitrogen, was stirred and heated in water bath at 65° for 2 hr. The C_6H_6 was replaced by 400 ml of cold MeOH. The MeOH was removed and the residue redissolved in 100 ml of H_2O and 200 ml of THF for 20 min at room temperature to hydrolyze the intermediate. The organic solvent was evaporated and the aqueous layer adjusted to pH 6.5 in the presence of EtOAc. The EtOAc layer was separated, washed with H_2O, dried ($MgSO_4$), and concentrated *in vacuo* to about 125 ml. *p*-Toluenesulfonic acid monohydrate (1.75 g, 9.2 mmol) in 25 ml of the same solvent was added. The product immediately crystallized, 2.2 g (46% over-all yield).

Method C.—In the manner identical with method B, propane sultone (5.6 g, 46 mmol) was used in the place of Ac_2O (42% over-all yield).

The 7-ADCA trichloroethyl ester (**5d**) from both reactions B and C gave nmr spectra consistent with the expected structure.

2. Cleavage of the Phenylacetyl Side Chain.—**4b** (800 mg, 1.7 mmol) was allowed to react in the manner described for **4a**, giving 660 mg (75% yield) of the same product.

2,2,2-Trichloroethyl 7-Amino-3-methyl-3-cephem-4-carboxylate (5d). 2-Naphthalenesulfonic Acid Salt.—Naphthalenesulfonic acid was as effective as *p*-toluenesulfonic acid in isolating the product from the side-chain cleavage. This amino ester salt also precipitated from EtOAc and recrystallized from EtOH–ether, mp 192–193° dec.

Nmr and ir spectra were consistent with the proposed product.

Anal. Calcd for $C_{20}H_{19}Cl_3N_2O_6S_2$: C, 43.36; H, 3.45; N, 5.05. Found: C, 43.56; H, 3.65; N, 4.92.

2,2,2-Trichloroethyl 7-Amino-3-methyl-3-cephem-4-carboxylate (5d). Free Amino Ester. Method A.—The free amino ester may be obtained from evaporation of the final EtOAc solution in the PCl_5 reaction. The oily residue crystallized on refrigeration for several hours or from EtOAc–methylcyclohexane or cyclohexene–petroleum ether solutions.

Nmr (in $CDCl_3$) showed signals at τ 8.03 (broad s, 2 H, amide NH_2), 7.82 (s, 3 H, C_3 CH_3), 6.79 and 6.36 (2d, 2 H, C_2 H_2), 5.28–4.96 (m, 4 H, ester CH_2, C_6 H and C_7 H).

Method B.—The free amino ester **5d** was also recovered from its tosylate by suspending the salt in H_2O–ether and adjusting the pH to near 7 with 1 *N* NaOH. The ethereal solution was separated, dried ($MgSO_4$), and evaporated to dryness *in vacuo*.

The residual oil slowly crystallized under refrigeration. This recrystallized, with difficulty, from wet cyclohexene, mp 82–84°.

Anal. Calcd for $C_{10}H_{11}Cl_3N_2O_3S \cdot H_2O \cdot C_6H_{10}$: C, 43.10; H, 5.19; N, 6.28. Found: C, 43.50; H, 5.15; N, 6.35.

***p*-Methoxybenzyl 7-Amino-3-methyl-3-cephem-4-carboxylate (5e) *p*-Toluenesulfonic Acid Salt.**—**4c** (984 mg, 2.1 mmol) was dissolved in 30 ml of dry C_6H_6 containing dry pyridine (245 mg, 3.1 mmol) and heated to 50° for 2 hr with PCl_5 (645 mg, 3.1 mmol). The C_6H_6 was replaced by 60 ml of cold MeOH. The solution was stirred at room temperature overnight, treated with H_2O for 20 min, and then evaporated to dryness *in vacuo*. The residue was redissolved in EtOAc–H_2O for adjustment to pH near 7. The EtOAc layer was separated, dried ($MgSO_4$), and treated with *p*-toluenesulfonic acid monohydrate (400 mg, 2.1 mmol). A crystalline precipitate was filtered, washed with Me_2CO, and vacuum dried. The product weighed 500 mg (47% yield), mp 162–165°.

Nmr (in DMSO-d_6) showed signals at τ 7.93 (s, 3 H, C_3 CH_3), 7.75 (s, 3 H, TSA CH_3), 6.49 (s, 2 H, C_2 H_2), 6.32 (s, 3 H, OCH_3),

4.89 (s, 4 H, β-lactam and ester CH_2), and 3.21–2.42 (m, 8 H, aromatic H).

Recrystallization from EtOH–ether provided an analytical sample.

Anal. Calcd for $C_{23}H_{26}N_2O_7S_2$: C, 54.52; H, 5.17; N, 5.53. Found: C, 54.30; H, 5.25; N, 5.67.

7-Amino-3-methyl-3-cephem-4-carboxylic Acid (9).—**5d** (freed from its tosylate, 2.1 g, 4 mmol) was dissolved in 50 ml of 90% aqueous AcOH, cooled in an ice–H_2O bath, and treated with 2 g of zinc dust. The mixture was stirred at below room temperature for 3 hr. AcOH was removed *in vacuo*. The residue was dissolved in 50 ml of cold H_2O, slurried with 100 ml of cold EtOAc, and acidified to pH below 1 with concentrated HCl. After filtration, the aqueous layer was separated and readjusted in the cold to pH 3.6 using NH_4OH. The crystalline product was filtered, washed with cold H_2O, vacuum dried, and weighed, 690 mg (81% yield).

Nmr (in D_2O–$NaHCO_3$) showed signals at τ 8.09 (s, 3 H, C_3 CH_3), 6.82 and 6.34 (2 d, 2 H, C_2 H_2), 5.28 (d, 1 H, C_6 H), and 4.96 (d, 1 H, C_7 H) and corresponded exactly with that of another sample of this material prepared by the catalytic hydrogenolysis of 7-aminocephalosporanic acid.[13]

Electrometric titration (in 66% aqueous DMF) showed pK_a values of 3.3 and 6.2 and an average molecular weight of 217 (calcd 214).

2,2,2-Trichloroethyl Chloroformate.—To a solution of $COCl_2$ (40 g, 405 mmol) in 200 ml of Na-dried C_6H_6 were added dropwise trichloroethanol (15.8 g, 106 mmol) and dry pyridine (12.0 g, 152 mmol) in 200 ml of dry C_6H_6 and 400 ml of anhydrous ether with occasional cooling to keep the temperature slightly below 20°. The addition required about 2 hr. The pyridine hydrochloride was removed by filtration. The filtrate was cooled and then poured into 1 l. of ice–H_2O, shaken in a separatory funnel. The organic layer was quickly separated, dried ($MgSO_4$), and evaporated *in vacuo*. Distillation over $CaCO_3$ gave 15 g (67% yield) of the chloroformate, bp 43° (0.5 mm), $n^{25}D$ 1.4698.

Nmr (in $CDCl_3$) showed a lone signal at τ 5.12. Ir (in $CHCl_3$) showed bands at 5.63 and 8.85 μ (broad).

Redistillation afforded an analytical sample.

Anal. Calcd for $C_3H_2Cl_4O_2$: Cl, 66.94. Found: Cl, 66.74.

***N*-(2,2,2-Trichloroethyloxycarbonyl)-D-α-phenylglycine.**—To a solution of D-α-phenylglycine (22.7 g, 150 mmol), 300 ml of H_2O, 160 ml of 1 *N* NaOH, and 150 ml of ether were added dropwise, over a period of 1 hr, 2,2,2-trichloroethyl chloroformate (42.5 g, 200 mmol) in 200 ml of Na-dried dioxane and simultaneously 200 ml of 1 *N* NaOH, while cooling at ice–alcohol temperature and while stirring. The mixture was maintained cold for an additional hour and then washed with large volumes of ether. The aqueous mixture, slurried with EtOAc, was acidified in the cold to pH 2.5 with syrupy phosphoric acid. The EtOAc solution was separated, washed with H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residual oil crystallized when slurried with petroleum ether, yield 43 g (87%), mp 142–144°.

Nmr (in DMSO-d_6) showed signals at τ 5.17 (s, 2 H, *N*-carboxy CH_2), 4.74 (d, 1 H, α-CH), 2.56 (s, 5 H, aromatic H), and 1.82 (d, 1 H, amide NH).

Ir (in $CHCl_3$) showed bands at 2.92 (amide NH), 5.8 (broad, acid and carbamate carbonyls), and 6.67 μ (amide II and phenyl). Electrometric titration (in 66% aqueous DMF) showed a titratable group at 5.60 and an average molecular weight of 320 (calcd 327).

The sample was recrystallized from C_6H_6–petroleum ether.

Anal. Calcd for $C_{11}H_{10}Cl_3NO_4$: C, 40.45; H, 3.09; N, 4.29. Found: C, 40.60; H, 3.24; N, 4.55.

2,2,2-Trichloroethyl 7-[*N*-(2,2,2-Trichloroethyloxycarbonyl)-D-α-phenylglycylamido]-3-methyl-3-cephem-4-carboxylate (6).—To a solution of methyl chloroformate (2.1 g, 22 mmol) in 200 ml of CaH_2-dried THF, cooled in an ice–alcohol bath, was added dropwise a solution of *N*-(2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglycine (7.2 g, 22 mmol), triethylamine (2.2 g, 22 mmol), and dimethylbenzylamine (6 drops) in 100 ml of dry THF. Cooling and stirring were maintained for 20 min following addition. Then **5d**, freed from its tosylate (10.4 g, 20 mmol), in 100 ml of the same solvent was added dropwise. The reaction mixture was stirred at ice–alcohol temperature for 3 hr. The solvent was removed *in vacuo*. The residue was redissolved in cold EtOAc for successive cold washes with H_2O, 5% HCl, 5% $NaHCO_3$ solution, and H_2O. The solution was dried ($MgSO_4$) and evaporated *in vacuo*. The residual oil was redissolved in 60 ml of CCl_4 for crystallization, yield 12.2 g (93%), mp 95°.

Nmr (in $CDCl_3$) showed signals at τ 7.82 (s, 3 H, C_3 CH_3), 6.94 and 6.54 (2 d, 2 H, C_2 H_2), 5.35 (s, 2 H, *N*-carboxy CH_2), 5.19 and 5.11 (2 d, 3 H, ester CH_2 and C_6 H), 4.58 (d, 1 H, α-CH), 4.24 (q, 1 H, C_7 H), 3.42 (d, 1 H, amide NH), and 2.69 (s, 5 H, aromatic H). Ir (in $CHCl_3$) showed bands at 2.95 (amide NH), 5.62 (β-lactam carbonyl), 5.78 (ester and carbamate carbonyls), 5.93 μ (amide carbonyl), and in the aromatic region.

The sample recrystallized from the same solvent.

Anal. Calcd for $C_{21}H_{19}Cl_6N_3O_6S$: C, 38.55; H, 2.92; N, 6.42. Found: C, 38.30; H, 2.98; N, 6.21.

2,2,2-Trichloroethyl 7-[*N*-(*tert*-Butoxycarbonyl)-D-α-phenylglycylamido]-3-methyl-3-cephem-4-carboxylate (12).—To a solution of methyl chloroformate (2.1 g, 22 mmol) in 200 ml of CaH_2-dried THF cooled in an ice–alcohol bath were added dropwise and with stirring *N*-(*tert*-butoxycarbonyl-D-α-phenylglycine (5.5 g, 22 mmol), triethylamine (2.2 g, 22 mmol), and dimethylbenzylamine (6 drops), in 100 ml of dry THF. Twenty minutes following addition, **5d** (10.4 g, 20 mmol) in 100 ml of dry THF was added dropwise. The mixture was stirred in the cold for 3 hr. The precipitated triethylamine hydrochloride was filtered and air-dried (3.0 g). The filtrate was evaporated *in vacuo*. The residual oil was redissolved in EtOAc for successive cold washes with H_2O, 5% HCl, 5% $NaHCO_3$ solution, and H_2O. The EtOAc solution was then dried ($MgSO_4$) and evaporated *in vacuo*. The residue weighed 11.5 g. This crude product was one-spot material in tlc and could be used directly in the next ester reductive cleavage step without further purification.

Nmr (in $CDCl_3$) showed signals at τ 8.60 (s, 9 H, *tert*-Bu), 7.82 (s, 3 H, C_3 CH_3), 6.90 and 6.50 (2 d, 2 H, C_2 H_2), 5.22 and 5.00 (2 d, 2 H, ester CH_2), 5.08 (d, 1 H, C_6H), 4.72 (d, 1 H, α-CH), 4.2 (q and d, 2 H, C_7H and amide NH) and 2.78–2.62 τ (d and s, 6 H, amide NH and aromatic). Ir (in $CHCl_3$) showed bands at 2.90 (amide NH), 5.57 (β-lactam carbonyl), and 5.75–5.90 μ (broad, ester and amide carbonyls).

In an identical preparation, the product was better characterized following a purification by recrystallization from either EtOH–H_2O or ether–petroleum ether, mp 130°.

Anal. Calcd for $C_{23}H_{26}Cl_3N_3O_6S$: C, 48.18; H, 4.53; N, 7.26. Found: C, 47.98; H, 4.58; N, 7.31.

2,2,2-Trichloroethyl 7-(D-α-Phenylglycylamido)-3-methyl-3-cephem-4-carboxylate (13).—Crude **12** (5.0 g, 8.6 mmol) was dissolved in 40 ml of MeCN containing *p*-toluenesulfonic acid monohydrate (4.1 g, 21.5 mmol) and stored at room temperature overnight. The solvent was removed *in vacuo*. The residue contained no starting material as observed in tlc. The residue was dissolved in 100 ml of EtOAc, cooled, and washed successively with 5% $NaHCO_3$ solution and H_2O. The EtOAc solution was dried and evaporated *in vacuo*. The residue weighed 3.8 g and was used directly in the following ester reductive cleavage step.

In an identical preparation, the product was purified for characteriztion by crystallization from EtOAc, mp 150°.

Nmr (in $CDCl_3$) showed signals at τ 8.07 (s, 2 H, NH_2), 7.80 (s, 3 H, C_3 CH_3), 6.82 and 6.40 (2 d, 2 H, C_2 H_2), 5.49–4.90 (m, 4 H, C_6 H, ester CH_2 and α-CH), 4.25 (q, 1 H, C_7 H), 2.69 (s, 5 H, aromatic H), and 1.98 (d, 1 H, amide NH).

Anal. Calcd for $C_{18}H_{18}Cl_3N_3O_4S$: C, 45.15; H, 3.79; N, 8.78. Found: C, 45.10; H, 4.07; N, 8.68.

7-[*N*-(*tert*-Butoxycarbonyl)-D-α-phenylglycylamido]-3-cephem-4-carboxylic Acid (14).—Crude **12** (from a 10-mmol run of its preparation) was dissolved in a mixture made from 25 ml of molecular-sieve-dried DMF and 7.5 ml of glacial AcOH (or 98% formic acid). The solution was cooled in an ice–H_2O bath and stirred for 3 hr with zinc dust (5.8 g, 89 mmol). The mixture was filtered, and the filtrate was taken up in H_2O and EtOAc. The EtOAc solution was washed with 5% HCl and then with H_2O, dried over $MgSO_4$, and evaporated to an amorphous white solid. This crude product, on examination in tlc (using MeCN–H_2O 4:1 system) contained a major component representing *tert*-Boc cephalexin and a faint spot corresponding possibly to the starting material. This crude product can be used directly in the following deblocking step.

In an identical preparation, the product was better characterized following purification. The crude product was dissolved in a EtOAc–H_2O mixture and adjusted to pH near 7 with 1 *N* NaOH. The aqueous phase was separated and back-titrated to pH 2.5 with 1 *N* HCl in the presence of EtOAc. The EtOAc solution was dried ($MgSO_4$) and evaporated *in vacuo*. The residue single-spot material in tlc (using a MeCN–H_2O 4:1 system) was crystallized from ether–hexane, mp 135° dec.

Nmr (in $CDCl_3$) showed signals at τ 8.60 (s, 9 H, *tert*-Bu), 7.90 (s, 3 H, C_3 CH_3), 7.0 and 6.58 (2d, 2 H, H_3), 5.14 (d, 1 H, C_6H), 4.67 (d, 1 H, α-CH), 4.34 (q, 1 H, C_7 H), 3.91 (d, 1 H, amide NH), and 2.69 (s, 5 H, aromatic H). Electrometric titration (in 66% aqueous DMF) gave a pK_a of 5.7 and an apparent molecular weight of 500 (calcd 448).

Anal. Calcd for $C_{21}H_{25}N_3O_6S$: C, 56.37; H, 5.63; N, 9.39. Found: C, 56.18; H, 5.80; N, 9.10.

7-(D-Amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic Acid (7). Method A.—**6** (3.9 g, 6.0 mmol) was dissolved in 200 ml of 90% aqueous formic acid. The solution was cooled in an ice–H_2O bath. Zinc dust (3.9 g, 60 mg-atoms) was added, and the mixture was stirred for 55 min. The zinc was filtered and washed with 40 ml of aqueous formic acid. The filtrate and wash were combined and evaporated *in vacuo*, azeotroping with C_6H_6 to remove the last traces of formic acid. The residue was taken up in 80 ml of H_2O (pH 3.5) and treated with H_2S for 15 min. The precipitated zinc sulfide was filtered with the aid of Filter-Cel; the filtrate (pH 2) was concentrated to about 20 ml, cooled in ice, and adjusted to pH 7 with 50% NaOH. A slight amount of precipitate was removed by filtration. The solution was reacidified to pH 4.5 (isoelectric point of cephalexin) and diluted with 60 ml of MeCN. The crystallized product was pure cephalexin, 500 mg (24% yield).

Nmr (in D_2O–DCl) showed signals at τ 7.88 (s, 3 H, C_3 CH_3), 6.88 and 6.48 (2d, 2 H, C_2 H_2), 5.0 (d, 1 H, C_6 H), 4.53 (s, 1 H, α-CH), 4.29 (d, 1 H, C_7 H), and 2.32 (s, 5 H, aromatic H) and corresponded exactly with that of an authentic sample of cephalexin prepared according to Ryan, *et al.*[5]

In another run, the work-up was altered: The aqueous filtrate, following the zinc sulfide precipitation, was evaporated to dryness *in vacuo*. The residue was dissolved in 60 ml of MeCN by addition of triethylamine dropwise to pH 9. The mixture was filtered to remove insoluble impurities, and the filtrate was back-titrated to pH 6 with 1 *N* HCl. Cephalexin precipitated in 49% yield.

The bioautograph (*Bacillus subtilis* seeded agar plate of a paper chromatogram, developed in 1-butanol–AcOH–H_2O, 3:1:1) showed a single biologically active spot corresponding exactly in mobility and potency to authentic cephalexin at like concentration.

Method B.—Crude **13** was dissolved in 40 ml of MeCN and 6 ml of H_2O and stirred for 90 min in the cold with zinc dust (1.2 g, 18.4 mg-atoms) and 2 ml of concentrated HCl. The mixture was then filtered, and the filtrate was adjusted to pH 4.5 with NH_4OH. A white, crystalline precipitate developed. This was filtered, washed with MeCN, and vacuum dried, weight 2.5 g. Tlc (using MeCN–H_2O, 4:1 system) and an nmr spectrum of this material showed cephalexin as the major component.

Method C.—Crude **14** (from a 10-mmol run of its preparation) was dissolved in 50 ml of MeCN and treated with *p*-toluenesulfonic acid monohydrate (3.8 g, 20 mmol). The reaction solution was stored at room temperature overnight. The solution was cooled for the addition of 10 ml of H_2O and triethylamine to pH 4.8. After immediate precipitation, the product was filtered, washed with cold MeCN, and dried to constant weight in a vacuum dessicator. The over-all yield of cephalexin from **5d** has varied between 69 and 74%.

Anal. Calcd for $C_{16}H_{17}N_3O_4S$: C, 55.33; H, 4.93; N, 12.10. Found: C, 55.19; H, 5.19; N, 11.95.

Nmr, ir, and uv spectra were in agreement with those of authentic cephalexin.

Registry No.—**2a**, 19474-19-2; **2b**, 26774-86-7; **3a**, 19474-21-6; **3b**, 28180-78-1; **3c**, 28180-79-2; **4b**, 28180-80-5; **4c**, 28180-81-6; **5d**, 28180-82-7; **5d** *p*-toluenesulfonate salt, 28180-83-8; **5d** 2-naphthalenesulfonic acid salt, 28180-84-9; **5e** *p*-toluenesulfonate salt, 28180-85-0; **6**, 28292-01-5; **7**, 15686-71-2; **9**, 22252-43-3; **10a**, 10209-11-7; **10a** 2,2,2-trichloroethyl ester, 24647-47-0; **10b**, 27255-72-7; **12**, 28292-02-6; **13**, 28180-91-8; **14**, 28180-92-9; 2,2,2-trichloroethyl chloroformate, 17341-93-4; *N*-(2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglycine, 26553-34-4.

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Specific, Reversible Acylation of Free Peptides Containing Lysine[1]

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Differences in reactivity between α- and ε-amino groups makes possible specific N^{ϵ}-acylation of free peptides containing lysine, in good yield and under simple experimental conditions. Alanyllysylalanine and N^{α}-, N^{ϵ}-, and N^{α},N^{ϵ}-diacyl derivatives thereof were synthesized and used as standards. Reaction of the free tripeptide with *tert*-butylazidoformate at pH 7 was primarily at the N^{α} position. Reaction in pyridine–water–triethylamine was at the N^{ϵ} position. Reaction with trifluoroacetic anhydride in trifluoroacetic acid yielded only the N^{α}-acyl product. The two ε-amino groups of porcine β-melanotropin can be specifically acylated with *tert*-butylazidoformate in good yield either in water at pH 10.5 or in pyridine–water–triethylamine. Formation of triacyl-β-melanotropin, in which the terminal amino group is also acylated, required extended reaction times and larger excesses of reagent.

In a semisynthetic preparation of the lysine-10 analog of human β-melanotropin (β-MSH), a suitably blocked tetrapeptide azide was reacted with naturally occurring porcine β-MSH.[2] The latter compound contains two ε-amino as well as a terminal α-amino group. Although a solution pH of 6.5 was employed to maintain ε-amino sites in a protonated, unreactive form, considerable coupling at N^{ϵ} positions did occur. The present report describes methods to utilize this apparently very high N^{ϵ}-amino reactivity to effect specific N^{ϵ}-acylation of free peptides containing lysine.

Free lysine has been the subject of a number of specific derivatization studies. Bezas and Zervas prepared N^{ϵ}-benzylidine lysine by virtue of product insolubility and rapid precipitation from solution.[3] Weygand and Geiger synthesized N^{α}-trifluoroacetyllysine with trifluoroacetic anhydride in trifluoroacetic acid;[4] in this case, strong acid so repressed N^{ϵ}-ammonium–amino

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