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CEPHALOSPORINS CONTAINING CARBONATE FUNCTIONS AT POSITIONS 3 AND 7

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Sulfones of 7β-alkoxycarbonyloxy-substituted cephalosporanic acid tert-butyl esters were synthesized by reduction of 7-oxocephalosporanic acid tert-butyl ester, acylation of the intermediate 7β-hydroxycephalosporanate with 2,2,2-trichloroethyl chloroformate or di-tert-butyl pyrocarbonate, and oxidation of the sulfur atom. Sulfones of 7α-chloro- and 7-alkylidene-substituted 3-alkoxycarbonyloxymethylcephalosporanic acid tert-butyl esters were obtained by replacement of the bromine atom in tert-butyl 3-bromomethylcephalosporanates with hydroxy group and acylation of the latter with chlorocarbonic esters. The cytotoxic activity of the synthesized substances was studied in vitro and also their ability to inhibit elastase.

Keywords: sulfones of 7β-alkoxycarbonyloxy-substituted cephalosporanic acids *tert*-butyl esters, sulfones of 7α-chloro- and 7-alkylidene-substituted 3-alkoxycarbonyloxymethylcephalosporanic acids *tert*-butyl esters, elastase inhibitors.

For many years the synthesis of cephalosporin analogs containing free hydroxyl groups in positions 3 or 7 and also products of their alkylation or acylation has been one of the priority areas for the structural modification of this antibiotic. Analysis of the literature shows that compounds of this type are key intermediates in reaction schemes for the preparation of antibacterial, anti-inflammatory, and cytotoxic substances [1-6].

The objective of this study was to obtain new *tert*-butyl esters of cephalosporanic acids containing carbonate functions at positions 7 or 3 of the cephem ring (Schemes 1, 2) and to study their inhibitory properties relative to leucocyte elastase and also their cytotoxic activity *in vitro*.

The synthesis of sulfones of 7β-alkoxycarbonyloxy-substituted cephalosporanates **5** is shown in Scheme 1.

Treatment of ester **1** with sodium borohydride or triethylsilane in the presence of palladium catalyst gave 7β-hydroxycephalosporanate **2**. As in the case of benzhydryl ester of 7-oxocephalosporine [1], reduction of the oxo group occurred stereospecifically. The 7β-configuration of the hydroxy group in compound **2**, which assumes the *cis*-orientation of the protons in positions 6 and 7, was confirmed by the corresponding spin-spin coupling constant, $J = 4.5$ Hz.

Acylation of the hydroxy group in compound **2** with 2,2,2-trichloroethyl chloroformate (**3a**) or di-*tert*-butyl pyrocarbonate (**3b**) in the presence of triethylamine gave cephalosporanates **4a,b** with carbonate functional groups at position 7 accompanied by partial isomerization of the double bond $\Delta^3 \rightarrow \Delta^2$. Oxidation of the 7-alkoxycarbonyloxy-substituted cephalosporanates **4a,b** with *meta*-chloroperbenzoic acid (MCPBA) at 0°C gave the corresponding sulfoxides **5a,c** (n = 1) and at 20°C gave the corresponding sulfones **5b,d** (n = 2). In both cases the reactions were accompanied by double bond migration so that Δ^3-cephalosporanates were the sole products.

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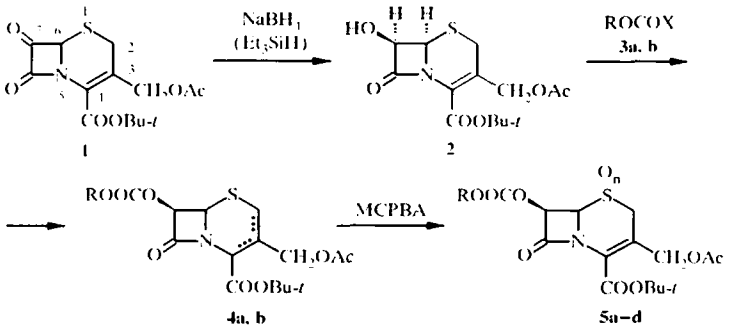
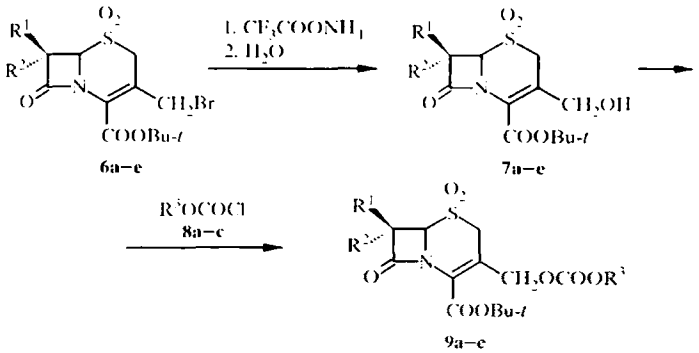
Scheme 1



3 a R = Cl_3CCH_2, X = Cl; **b** R = *t*-Bu, X = OCOOBu-*t*. **4 a** R = Cl_3CCH_2, **b** R = *t*-Bu.
5 a R = Cl_3CCH_2, *n* = 1; **b** R = Cl_3CCH_2, *n* = 2; **c** R = *t*-Bu, n = 1; **d** R = *t*-Bu, *n* = 2

The synthesis of 3-alkoxycarbonyloxymethylcephalosporanate sulfones **9** is shown in Scheme 2.

Scheme 2



6, 7 a R^1 = H, R^2 = Cl; **b** R^1 and R^2 = (*Z*)-*t*-BuOCOCH=; **c** R^1 and R^2 = (*Z*)-CH_3COCH=;
d R^1 and R^2 = (*Z*)-4-$O_2NC_6H_4CH$=; **e** R^1 and R^2 = (*E*)-4-$O_2NC_6H_4CH$= .
8 a R^3 = Cl_3CCH_2, **b** R^3 = $BrCH_2CH_2$, **c** R^3 = 4-$O_2NC_6H_4$. **9 a** R^1 = H, R^2 = Cl, R^3 = Cl_3CCH_2;
b R^1 = H, R^2 = Cl, R^3 = $BrCH_2CH_2$; **c** R^1 = H, R^2 = Cl, R^3 = 4-$O_2NC_6H_4$;
d R^1 и R^2 = (*Z*)-*t*-BuOCOCH=, R^3 = 4-$O_2NC_6H_4$; **e** R^1 and R^2 = (*E*)-4-$O_2NC_6H_4CH$= , R^3 = 4-$O_2NC_6H_4$

Replacement of the bromine atom in 3-bromomethylcephalonosporanates **6a-e*** by a hydroxy group was carried out with ammonium trifluoroacetate [7]. It was established that the nature of the solvent had a major influence on this reaction. The maximum yield (80%) of 3-hydroxymethylcephalosporanates **7a-e** was obtained with a mixture of acetone and dimethylformamide (30:1).

Optimization of the acylation of the 3-hydroxymethyl groups in compounds **7** with chlorocarbonic ester also required variation of the reaction conditions depending on the nature of the radical R^3 in reagents **8a-c**. In the case of 2,2,2-trichloroethyl chloroformate (**8a**) the reaction was carried out in dichloromethane in the presence of triethylamine at room temperature for thirty minutes. For 2-bromoethyl chloroformate the analogous result was achieved by boiling in benzene for 4 h and replacing triethylamine by 2,6-lutidine.

* The methods for the preparation and the physicochemical characteristics of *tert*-butyl 7-alkylidene-3-bromomethylcephalosporanates **6b-e** will be published in a separate paper.

Acylation of 3-hydroxymethylcephalosporanates **7a-c** with *para*-nitrophenyl chlorocarbonate (**8c**) in diethyl ether in the presence of 2,6-lutidine at room temperature appears to be more effective than the use of pyridine [5] or a combination of 2,6-lutidine with 4-dimethylaminopyridine in tetrahydrofuran [6] recommended for analogous compounds. Unlike the esters **9a,b**, cephalosporanates with R^3 = 4-$O_2NC_6H_4$ (**9c-e**) decompose rapidly in the presence of water, strong bases (triethylamine, DBU), or on increasing the temperature.

For some of the compounds synthesized their cytotoxic properties *in vitro* against two lines of cancer cells - HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma) - were studied, and also their ability to inhibit the amidolytic activity of elastase relative to the standard substrate, *para*-nitroanilide of N-methoxysuccinyl-Ala-Ala-Pro-Val.

The concentration of substance which provided 50% of tumor death effect *in vitro* (TD_{50}) (see Table 1) was determined by a standard methods based on the intensity of the cell membrane coloration with crystal violet and on the redox activity of mitochondrial enzymes with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

The concentration of substance which decreased to 50% the catalytic activity (IC_{50}) of Porcine Pancreas Elastase, Type III, against the substrate was determined photocolorimetrically by the method adapted for 96 chamber panels (see Table 1).

Of the substances tested, only 7α-chloro-3-hydroxymethylcephalosporanate **7a** showed a measurable cytotoxic activity towards the cancer cells. The high inhibitory activity towards elastases of 7α-chlorocephalosporanates **9a-c**, modified in position 3 by carbonate groups was very unexpected. Compounds **9b** (R^1 = H, R^2 = Cl, R^3 = $BrCH_2CH_2$) and **9c** (R^1 = H, R^2 = Cl, R^3 = 4-$O_2NC_6H_4$) were four times more effective than one of the most elastase inhibitors, *tert*-butyl ester of sulfone of 7α-chlorocephalosporanic acid [2].

EXPERIMENTAL

^{1}H NMR spectra of $CDCl_3$ solution with TMS as internal standard (δ, ppm; *J*, Hz) were recorded with Bruker WH-90/DS (90 MHz) spectrometer. IR spectra of nujol mulls were recorded with a Perkin-Elmer 580B spectrometer. Elemental analyses were carried out with a Carlo Erba 1108 instrument. HPLC data were obtained

TABLE 1. Biological Properties of Some Structural Analogs of Cephalosporin

Compound	Cytotoxic activity *in vitro*, TD_{50}, μg ml*				IC_{50}, μM*[4]
	HT-1080		MG-22A		
	CV*[2]	MTT*[3]	CV	MTT	
5b	>100	>100	>100	>100	
5d	>100	>100	>100	>100	
7a	9	25	18	33	24±2
9a	39	52	46	58	13±3
9b	37	56	45	42	0.040±0.006
9c	53	72	45	62	0.047±0.005
10*[5]					0.16±0.02 [9]

* Concentration causing 50% destruction of cells.

*[2] Coloration with crystal violet.

*[3] Coloration with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

*[4] 50% Inhibition of the amidolytic activity of Porcine Pancreas Elastase (Type III) relative to *para*-nitroanilide of N-methoxysuccinyl-Ala-Ala-Pro-Val substrate.

*[5] *tert*-Butyl ester of sulfone of 7α-chlorocephalosporanic acid.

with Du-Pont Model 8800 machine equipped with a UV detector (λ = 254 nm) and a column (4.6 × 250 mm) filled with Symmetry C_{18}, with 60:40 acetonitrile–0.1 N phosphate buffer with pH 2.5, and a flow rate of 0.8-1.5 ml/min. Optical densities in biological tests, conducted in 96 chamber panels, were measured with a Tetertek Multiscan MCC/340 horizontal spectrophotometer. Reactions were monitored by TLC on Merck Kieselgel strips with UV detection. Merck Kieselgel (0.063-0.230 mm) was used for preparative column chromatography. Reagents from Aldrich, Acros, and Sigma were used in the experiments.

***tert*-Butyl Ester of 7β-Hydroxycephalosporanic Acid (2). A.** The *tert*-butyl ester of 7-oxocephalosporanic acid **1** was reduced with sodium borohydride by a known method [1]. Yield 46%, containing 96% of the required substance according to HPLC; mp 115-117°C (diethyl ether). IR spectrum (nujol): 3350, 1740, 1720 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): 1.53 (9H, s, *t*-C_4H_9); 2.08 (3H, s, CH_3); 3.13 (1H, m, OH); 3.33 and 3.60 (2H, AB-system, J = 19, SCH_2); 4.80 and 5.08 (2H, AB-system, J = 14, CH_2O); 4.93 (1H, d, J = 4.5, 6-H); 5.31 (1H, m, 7-H).

B. Bis(triphenylphosphine)palladium(II) chloride (8.8 mg, 0.012 mol) and triphenylphosphine (4.8 mg, 0.018 mmol) were added to a solution of acid **1** (200 mg, 0.62 mmol) and triethylsilane (150 mg, 0.94 mmol) in DMF (10 ml). The mixture was heated for 2 h at 40-50°C, cooled, diluted with dichloromethane (40 ml), and poured into a solution of dilute hydrochloric acid. The organic layer was separated, washed with water, dried over Na_2SO_4, and evaporated. The residue was fractionated on a silica gel chromatographic column (eluent 1:2 ethyl acetate–petroleum ether). The fraction with R_f 0.11 was concentrated and evaporated to give a crystalline substance (20 mg, 40%) with physicochemical constants analogous to those of the product from method A.

***tert*-Butyl Ester of 7β-(2,2,2-Trichloroethoxycarbonyloxy)cephalosporanic Acid Sulfoxide (5a).** 2,2,2-Trichloroethyl chlorocarbonate (0.16 ml, 1.22 mmol) and triethylamine (0.17 ml, 1.22 mmol) were added to a solution of *tert*-butyl 7β-hydroxycephalosporanate (200 mg, 0.61 mol) in dichloromethane (10 ml), the mixture was stirred for 30 min at room temperature until the TLC spot at R_f 0.21 of starting material **2** disappeared and a new spot appeared at R_f 0.62 (eluent 1:1 hexane–ethyl acetate). The reaction mixture was washed with sodium carbonate solution and water, and dried over anhydrous Na_2SO_4. The solvent was removed at reduced pressure and the residue was fractionated on a silica gel column (eluent 1:1 hexane-ethyl acetate). The fraction with R_f 0.62 was collected and evaporated to give a 1:1 mixture of *tert*-butyl 3-acetoxymethyl-7β-(2,2,2-trichloroethoxycarbonyloxy)ceph-3-em-4-carbonate and *tert*-butyl 3-acetoxymethyl-7β-(2,2,2-trichloroethoxycarbonyloxy)ceph-2-em-4-carbonate **4a** (214 mg, 70%) based on the proton intensities in the ^1H NMR spectrum. ^1H NMR spectrum of the Δ^3 isomer ($CDCl_3$): 1.55 (9H, s, *t*-Bu); 2.09 (3H, s, CH_3); 3.33 and 3.60 (2H, AB-system, J = 19, SCH_2); 4.15 (2H, s CCl_3CH_2); 5.11 and 5.28 (2H, AB-system, J = 14, CH_2O); 4.93 (1H, d, J = 4.5, 6-H); 6.06 (1H, d, J = 4.5, 7-H). ^1H NMR spectrum of the Δ^2 isomer ($CDCl_3$): 1.49 (9H, s, *t*-Bu); 2.09 (3H, s, CH_3); 4.15 (2H, s, CCl_3CH_2); 4.55 and 4.80 (2H, AB-system, J = 13, CH_2O); 4.97 (1H, s, 4-H); 5.44 (1H, d, J = 4.5, 6-H); 5.77 (1H, d, J = 4.5, 7-H); 6.44 (1H, s, 2-H).

meta-Chloroperbenzoic acid (147 mg, 0.60 mmol) was added to a solution of a mixture of esters **4a** (100 mg, 0.19 mmol) in dichloromethane (10 ml), the mixture was stirred for 1 h at 0°C, diluted with dichloromethane (20 ml), washed with 5% Na_2SO_3 solution (50 ml) and 5% Na_2CO_3 solution, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was fractionated by chromatography on silica gel with ethyl acetate as eluent. The fraction with R_f 0.20 (1:1 hexane-ethyl acetate) was collected and evaporated to give *tert*-butyl ester of 7β-(2,2,2-trichloroethoxycarbonyloxy)-cephalosporanic acid sulfoxide **5a** as an amorphous powder (82 mg, 80%) containing >96% of the required material according to HPLC. ^1H NMR spectrum ($CDCl_3$): 1.51 (9H, s, *t*-Bu); 2.11 (3H, s, CH_3); 3.48 and 4.09 (2H, AB-system, J = 18, $SOCH_2$); 4.75 and 5.13 (2H, AB-system, J = 14, CH_2O); 4.77 (2H, s, CCl_3CH_2); 4.84 (1H, d, J = 5, 6-H); 6.26 (1H, d, J = 5, 7-H).

***tert*-Butyl Ester of 7β-(2,2,2-Trichloroethoxycarbonyloxy)cephalosporanic Acid Sulfone (5b).** *meta*-Chloroperbenzoic acid (147 mg, 0.60 mmol) was added to a solution of a mixture of esters **5a** (82 mg, 0.15 mol) in dichloromethane (10 ml) and the mixture was stirred for 3 h at room temperature, diluted with dichloromethane (20 ml), washed with 5% Na_2SO_3 solution (50 ml), 5% Na_2CO_3 solution, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was fractionated on a silica gel column (eluent ethyl acetate). The fraction with R_f 0.43 (1:1 hexane–ethyl acetate) was collected and

evaporated to give the *tert*-butyl ester of 7β-(2,2,2-trichloroethoxycarbonyloxy)cephalosporanic acid sulfone (**5b**) (75 mg, 91%), containing 95% of the required product according to HPLC; mp 84-87°C. ^{1}H NMR spectrum ($CDCl_3$): 1.60 (9H, s, *t*-Bu); 2.13 (3H, s, CH_3); 3.71 and 4.02 (2H, AB-system, J = 18, SO_2CH_2); 4.80 and 5.24 (2H, AB-system, J = 14, CH_2O); 4.86 (2H, s, CCl_3CH_2); 4.91 (1H, d, J = 5, 6-H); 6.11 (1H, d, J = 5, 7-H).

***tert*-Butyl Ester of 7β-(*tert*-Butoxycarbonyloxy)cephalosporanic Acid Sulfoxide (5c).** Di-*tert*-butyl pyrocarbonate (663 mg, 3.04 mmol) and triethylamine (0.42 ml, 3.04 mmol) were added to a solution of the *tert*-butyl ester of 7β-hydroxycephalosporanic acid (500 mg, 1.52 mmol) in dichloromethane (10 ml). The mixture was boiled until the TLC spot of the starting material at R_f 0.21 disappeared and a new spot appeared at R_f 0.57-0.65 (eluent 1:1 hexane–ethyl acetate). The mixture was washed with Na_2CO_3 solution, water, and dried over Na_2SO_4. The solvent was evaporated ar reduced pressure. The residue was fractionated by chromatography on column with silica gel (eluent 1:1 hexane–ethyl acetate). The fraction with R_f 0.60 was collected and evaporated to give 500 mg (76%) of a mixture of *tert*-butyl 7β-(*tert*-butoxycarbonyloxy)-3-acetoxymethylceph-3-em-4-carbonate and *tert*-butyl 7β-(*tert*-butoxycarbonyloxy)-3-acetoxymethylceph-2-em-4-carbonate **4b** in a ratio of 2:1 (according to proton signal intensities in the ^{1}H NMR spectra). ^{1}H NMR spectrum of the Δ^3 isomer ($CDCl_3$): 1.28-1.55 (18H, s, 2 *t*-Bu); 2.00 (3H, s, CH_3); 3.24 and 3.51 (2H, AB-system, J = 19, SCH_2); 4.68 and 5.00 (2H, AB-system, J = 14, CH_2O); 4.95 (1H, d, J = 4.0, 6-H); 5.84 (1H, d, J = 4.0, 7-H). ^{1}H NMR spectrum of the Δ^2 isomer ($CDCl_3$): 1.28-1.55 (18H, s, 2 *t*-Bu); 2.00 (3H, s, CH_3); 4.44 and 4.69 (2H, AB-system, J = 13, CH_2O); 4.82 (1H, s, 4-H); 5.28 (1H, d, J = 4.0, 6-H); 5.33 (1H, d, J = 4.0, 7-H); 6.35 (1H, s, 2-H).

The ester mixture **4b** was oxidized with *meta*-chloroperbenzoic acid in 84% yield, analogously to the synthesis of sulfoxide **5a**, to give *tert*-butyl ester of 7β-(*tert*-butoxycarbonyloxy)cephalosporanic acid sulfoxide **5c** with R_f 0.31 (eluent 1:2 hexane–ethyl acetate); mp 122-123°C. ^{1}H NMR spectrum ($CDCl_3$): 1.55 (18H, s, 2 *t*-Bu); 2.11 (3H, s, CH_3); 3.51 and 4.04 (2H, AB-system, J = 17, $SOCH_2$); 4.77 (1H, d, J = 5, 6-H); 4.80 and 5.13 (2H, AB-system, J = 14, CH_2O); 6.15 (1H, d, J = 5, 7-H). Found, %: C 51.04; H 6.14; N 3.09. $C_{19}H_{27}NO_6S$. Calculated, %: C 51.23; H 6.11; N 3.14.

***tert*-Butyl Ester of 7β-(*tert*-Butoxycarbonyloxy)cephalosporanic Acid Sulfone (5d)** was obtained in 60% yield by oxidation of cephalosporanate **5c** with *meta*-chloroperbenzoic acid analogously to the synthesis of sulfone **5b**. R_f 0.57 (eluent 1:2 hexane–ethyl acetate); mp 114-116°C. ^{1}H NMR spectrum ($CDCl_3$): 1.53 (9H, s, *t*-Bu); 1.57 (9H, s, *t*-Bu); 2.11 (3H, s, CH_3); 3.66 and 3.97 (2H, AB-system, J = 17, SO_2CH_2); 4.80 and 5.22 (2H, AB-system, CH_2O); 4.86 (1H, d, J = 5, 6-H); 6.04 (1H, d, J = 5, 7-H). Found, %: C 49.50; H 5.87; N 2.98. $C_{19}H_{27}NO_{10}S$. Calculated, %: C 49.45; H 5.90; N 3.04.

***tert*-Butyl Ester of 7α-Chloro-3-hydroxymethylceph-3-em-4-carbonic Acid Sulfone (7a).** Ammonium trifluoroacetate (500 mg, 3.80 mmol) was added to a solution of *tert*-butyl 7α-chloro-3-bromomethylceph-3-em-4-carbonate **6a** (300 mg, 0.75 mmol) in a mixture of acetone (6.0 ml) and DMF (0.2 ml). The mixture was boiled for 3.5 h, cooled, diluted with ethyl acetate (50 ml), and washed with water (10 ml). The solution was dried over anhydrous Na_2SO_4, and the solvent removed under reduced pressure. The residue was fractionated by chromatography on a silica gel column (eluent 1:2 hexane–ethyl acetate). The fraction with R_f 0.35 was collected and evaporated to give *tert*-butyl ester of sulfone **7a** (200 mg, 79%); mp 126-127°C. IR spectrum (nujol): 3500, 1810, 1720 cm^{-1}. ^{1}H NMR spectrum ($CDCl_3$): 1.57 (9H, s, *t*-Bu); 2.42 (1H, br. s, OH); 4.02 (2H, s, CH_2O); 4.09 and 4.51 (2H, AB-system, J = 14, SO_2CH_2); 4.77 (1H, d, J = 1, 6-H); 5.31 (1H, d, J = 1, 7-H). Found, %: C 42.53; H 4.67; N 4.08. $C_{12}H_{16}ClNO_6S$. Calculated, %: C 42.67; H 4.77; N 4.15.

***tert*-Butyl Ester of 7(*Z*)-(*tert*-Butoxycarbonyl)methylene-3-hydroxymethylceph-3-em-4-carbonic Acid Sulfone (7b)** was obtained from *tert*-butyl 7(*Z*)-(*tert*-butoxycarbonyl)methylene-3-bromomethylceph-3-em-4-carbonate **6b** analogously to **7a** with a yield of 71%, R_f 0.14 (eluent 1:2 hexane–ethyl acetate); mp 48-50°C, content of the required material 95% according to HPLC data. ^{1}H NMR spectrum ($CDCl_3$): 1.55 (18H, s, 2 *t*-Bu); 3.88 and 4.15 (2H, AB-system, J = 14, SO_2CH_2); 4.00 (1H, br. s, OH); 4.11 and 4.57 (2H, AB-system, J = 14, CH_2O); 6.53 (1H, d, J = 1, 6-H); 6.57 (1H, d, J = 1, 7-H).

***tert*-Butyl Ester of 7(*Z*)-Acetylmethylene-3-hydroxymethylceph-3-em-4-carbonic Acid Sulfone (7c)** was obtained from *tert*-butyl 7(*Z*)-acetylmethylene-3-bromomethylceph-3-em-4-carbonate **6c** analogously to **7a** as an oil with a yield of 67%, R_f 0.14 (eluent 1:2 hexane–ethyl acetate). Content of required material 95% according to HPLC. ^{1}H NMR spectrum ($CDCl_3$): 1.60 (9H, s, *t*-Bu); 2.44 (3H, s, CH_3COC=); 4.02 and 4.57 (2H, AB-system,

J = 14, SO_2CH_2); 4.04 (2H, s, CH_2O); 4.22 (1H, br. s, OH); 5.60 (1H, br. s, 6-H); 6.91 (1H, d, J = 1, 7-H).

***tert*-Butyl Ester of 7(*Z*)-4-Nitrobenzylidene-3-hydroxymethylceph-3-em-4-carbonic Acid Sulfone (7d)** was obtained from *tert*-butyl 7(*Z*)-nitrobenzylidene-3-bromomethylceph-3-em-4-carbonate **6d** analogously to **7a** in 40% yield as an amorphous substance, R_f 0.28 (eluent 1:1 hexane–ethyl acetate) containing 94% of the required material according to HPLC. ^{1}H NMR spectrum ($CDCl_3$): 1.60 (9H, s, *t*-Bu); 2.86 (1H, br. s, OH); 4.11 (2H, s, CH_2O); 4.13 and 4.55 (2H, AB-system, J = 14, SO_2CH_2); 5.62 (1H, br. s, 6-H); 7.44 (1H, br. s, –HC=); 7.86, 8.28 (4H, 2 d, J = 9, C_6H_4).

***tert*-Butyl Ester of 7(*E*)-4-Nitrobenzylidene-3-hydroxymethylceph-3-em-4-carbonic Acid Sulfone (7e)** was obtained from *tert*-butyl 7(*E*)-nitrobenzylidene-3-bromomethylceph-3-em-4-carbonate **6e** analogously to **7a** in 60% yield as an amorphous substance, R_f 0.17 (eluent 1:1 hexane–ethyl acetate) containing 95% of the required material according to HPLC. ^{1}H NMR spectrum ($CDCl_3$): 1.63 (9H, s, *t*-Bu); 3.35 (1H, br. s, OH); 4.04 (2H, s, CH_2O); 4.11 and 4.53 (2H, AB-system, J = 14, SO_2CH_2); 5.28 (1H, br. s, 6-H); 6.95 (1H, br. s, –HC=); 8.13, 8.26 (4H, 2 d, J = 9, C_6H_4).

***tert*-Butyl Ester of 7α-Chloro-3-(2,2,2-Trichloroethoxycarbonyloxy)methylceph-3-em-4-carbonic Acid Sulfone (9a)**. 2,2,2-Trichloroethyl chlorocarbonate (48 mg, 0.35 mmol) and triethylamine (0.1 ml, 0.70 mmol) were added to a solution of sulfone of *tert*-butyl 7α-chloro-3-hydroxymethylceph-3-em-4-carbonate **7a** (120 mg, 0.35 mmol) in dichloromethane (6 ml). The mixture was stirred for 30 min at room temperature. The end of the reaction was determined by disappearance of the spot of **7a** at R_f 0.28 and the appearance of a new spot at R_f 0.50 (eluent 2:1 hexane–ethyl acetate). The solution was diluted with dichloromethane (50 ml), washed with dilute HCl solution, dried over anhydrous Na_2SO_4, and the solvent was removed at low pressure. The residue was fractionated by chromatography on silica gel (eluent 2:1 hexane–ethyl acetate). The fraction with R_f 0.50 was collected and evaporated to give sulfone **9a** (118 mg, 65%) (98% pure by HPLC); mp 167-170°C. ^{1}H NMR spectrum ($CDCl_3$): 1.57 (9H, s, *t*-Bu); 3.77 and 4.13 (2H, AB-system, J = 18, SO_2CH_2); 4.71-4.88 (3H, m, CH_2Cl_3, 6-H); 4.86 and 5.33 (2H, AB-system, J = 14, CH_2OCO); 5.33 (1H, d, J = 1, 7-H).

***tert*-Butyl Ester of 7α-Chloro-3-(2-bromoethoxycarbonyloxy)methylceph-3-em-4-carbonic Acid Sulfone (9b)**. 2-Bromoethyl chlorocarbonate (0.063 ml, 0.60 mmol) and 2,6-lutidine (0.063 ml, 0.60 mmol) were added to a solution of **7a** (100 mg, 0.29 mmol) in benzene (6 ml). The mixture was boiled for 3.5 h. The end of the reaction was determined by disappearance of the spot of **7a** at R_f 0.28 and the appearance of a new spot at R_f 0.53 (eluent 2:1 hexane–ethyl acetate). The solution was diluted with dichloromethane (50 ml), washed with dilute HCl solution, dried over anhydrous Na_2SO_4, and the solvent was removed at low pressure. The residue was fractionated by chromatography on silica gel (eluent 2:1 hexane–ethyl acetate). The fraction with R_f 0.53 was collected and evaporated to give sulfone **9b** (100 mg, 70%) (97% pure by HPLC); mp 99-102°C. ^{1}H NMR spectrum ($CDCl_3$): 1.60 (9H, s, *t*-Bu); 3.57 (2H, t, J = 8, CH_2Br); 3.77 and 4.13 (2H, AB-system, J = 18, SO_2CH_2); 4.48 (2H, t, J = 8, $OCOCH_2$); 4.77 and 5.28 (2H, AB-system, J = 14, CH_2OCO); 4.84 (1H, br. s, 6-H); 5.33 (1H, d, J = 1, 7-H).

***tert*-Butyl Ester of 7α-Chloro-3-(4-nitrophenoxycarbonyloxy)methylceph-3-em-4-carbonic Acid Sulfone (9c).** 2,6-Lutidine (0.026 ml, 0.24 mmol) was added to a solution of sulfone **7a** (40 mg, 0.29 mmol) in dry diethyl ether (5 ml) at room temperature followed after 5 min by 4-nitrophenyl chlorocarbonate **8a** (24 mg, 0.24 mmol) in two portions 30 minutes apart. The end of the reaction was indicated by the disappearance of the spot of **7a** at R_f 0.28 and the appearance of a new spot at R_f 0.45 (eluent 2:1 hexane–ethyl acetate). The solvent was evaporated at reduced pressure, the residue was dissolved in methanol (3 ml), the solvent removed and the residue fractionated by chromatography on silica gel with ethyl acetate. The fraction with R_f 0.5 (2:1 hexane–ethyl acetate) was collected and evaporated to give sulfone **9c** (28 mg, 47%) (97% pure by HPLC); mp 52-54°C. ^{1}H NMR spectrum ($CDCl_3$): 1.57 (9H, s, *t*-Bu); 3.82 and 4.17 (2H, AB-system, J = 17, SO_2CH_2); 4.84 (1H, s, 6-H); 4.93 and 5.37 (2H, AB-system, J = 14, CH_2OCO); 5.33 (1H, d, J = 1, 7-H); 7.38 and 8.28 (4H, 2 d, J = 10, C_6H_4).

***tert*-Butyl Ester of 7(*Z*)-(*tert*-Butoxycarbonyl)methylene-3-(4-nitrophenoxycarbonyloxy)methylceph-3-em-4-carbonic Acid Sulfone (9d)** was prepared analogously to compound **9c** in 55% yield (95% pure by HPLC), R_f 0.40 (eluent 1:2 hexane–ethyl acetate); mp 67-70°C. IR spectrum (nujol): 1790, 1770, 1720, 1700 (sh); 1620, 1600 cm^{-1}. ^{1}H NMR spectrum ($CDCl_3$): 1.55 (18H, s, 2 *t*-Bu); 3.87 and 4.20 (2H, AB-system, J = 18, SO_2CH_2); 5.00 and 5.44 (2H, AB-system, J = 14, CH_2O); 5.62 (1H, br. s, 6-H); 6.62 (1H, d, J = 0.5, –CH=); 7.40 and 8.28 (4H, 2 d, J = 9, C_6H_4).

***tert*-Butyl Ester of 7(*E*)-4-Nitrobenzylidene-3-(4-nitrophenoxycarbonyloxy)methylceph-3-em-4-carbonic Acid Sulfone (9c)** was prepared analogously to compound **9c** in 63% yield (92% pure by HPLC), R_f 0.51 (eluent 1:2 hexane–ethyl acetate). ^{1}H NMR spectrum ($CDCl_3$): 1.64 (9H, s, *t*-Bu); 3.84 and 4.22 (2H, AB-system, J = 18, SO_2CH_2); 4.95 and 5.42 (2H, AB-system, J = 13, CH_2O); 5.42 (1H, br. s, 6-H); 6.98 (1H, br. s, –CH=); 7.40 and 8.31 (4H, 2 d, J = 9, C_6H_4–O); 8.18 and 8.30 (4H, 2 d, J = 9, C_6H_4–C=).

Biological Tests. The cytotoxic properties of the synthesized substances relative to monolayers of cancer cells and their inhibitory activity relative to Porcine Pancreas Elastase (Type III) were determined by methods described earlier [8].

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