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SYNTHESIS AND CYTOTOXIC PROPERTIES OF DERIVATIVES OF THE *tert*-BUTYL ESTER OF 7-ALKYLIDENE-3-METHYL-3-CEPHEME-4-CARBOXYLIC ACID

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Sulfones of the tert-butyl esters of 7-arylmethylene- and 7-(2-furyl)methylene-3-methyl-3-cepheme-4-carboxylic acid were obtained by the condensation of the tert-butyl ester of 3-methyl-7-oxo-3-cepheme-4-carboxylic acid with arylmethylene- and 2-furylidenetriphenylphosphoranes and subsequent oxidation of the intermediate products by meta-chloroperbenzoic acid. The combination of the tert-butyl esters of 7E-bromomethylene- and 7,7-dibromomethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acids with trimethylsilylacetylene under conditions of the Sonogashira reaction gave the tert-butyl esters of 3-methyl-1,1-dioxo-7E-(3-trimethylsilyl-2-propynylidene)-3-cepheme-4-carboxylic acid and 3-methyl-1,1-dioxo-7-[1,5-bis(trimethylsilyl)-1,4-pentadiyn-3-ylidene]-3-cepheme-4-carboxylic acid. The Vilsmeier reagent was used to incorporate the dimethylaminomethylene group at C-2 of the 7Z- and 7E-isomers of the tert-butyl ester of 7-(4-chlorophenyl)methylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid. The cytotoxic properties of the derivatives of the tert-butyl ester of 7-alkylidene-3-methyl-3-cepheme-4-carboxylic acid in regard to cancer and normal cells in vitro depends on the structure and 7Z- or 7E-isomerism of the substituent in the 7-alkylidene group as well as the presence of a dimethylaminomethylene group at C-2 of the 3-cepheme system.

Keywords: 7*Z*-acetylmethylene-3-methyl-1,1-dioxo-3-cepheme, *tert*-butyl esters of 7-arylmethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acids, *tert*-butyl ester of 7-(2-furyl)-methylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid, *tert*-butyl ester of 3-methyl-1,1-dioxo-7*E*-(3-trimethylsilyl-2-propynylidene)-3-cepheme-4-carboxylic acid, *tert*-butyl ester of 3-methyl-1,1-dioxo-7-[1,5-bis-(trimethylsilyl)-1,4-pentadiyn-3-ylidene]-3-cepheme-4-carboxylic acid, *tert*-butyl ester of 7*Z*-(4-chlorophenyl)methylene-2-(dimethylaminomethylene)-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid, *tert*-butyl ester of 7*E*-(4-chlorophenyl)methylene-2-dimethylaminomethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid, cytotoxic activity.

We have already shown that the introduction of alkylidene group at C-7 in the *tert*-butyl ester of 3-acetoxy-1,1-dioxo-3-cepheme-4-carboxylic acid **1a-g** is conducive to high cytotoxic activity relative to cancer cells *in vitro* [1]. In subsequent work [2], we synthesized and studied the physicochemical properties of

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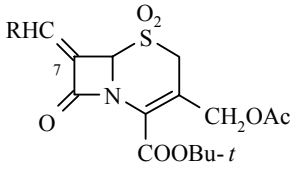
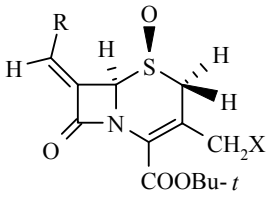
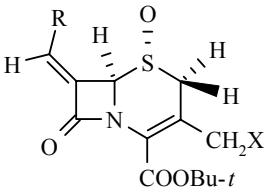
1*R*- and 1*S*-sulfoxides as well as the sulfones of the *tert*-butyl esters of 7*Z*-acetylmethylene-3-methyl-3-cepheme-4-carboxylic acid **2a,b** and 3-acetoxymethyl-7*Z*-(3-trimethylsilyl-2-propynylidene)-3-cepheme-4-carboxylic acid **3a,b**.



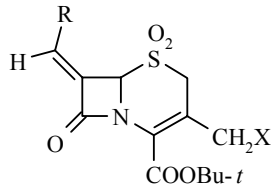
1a–g



1*S*, 7*Z* **- 2a,b**



1*R*,7*Z*-**2a,b**



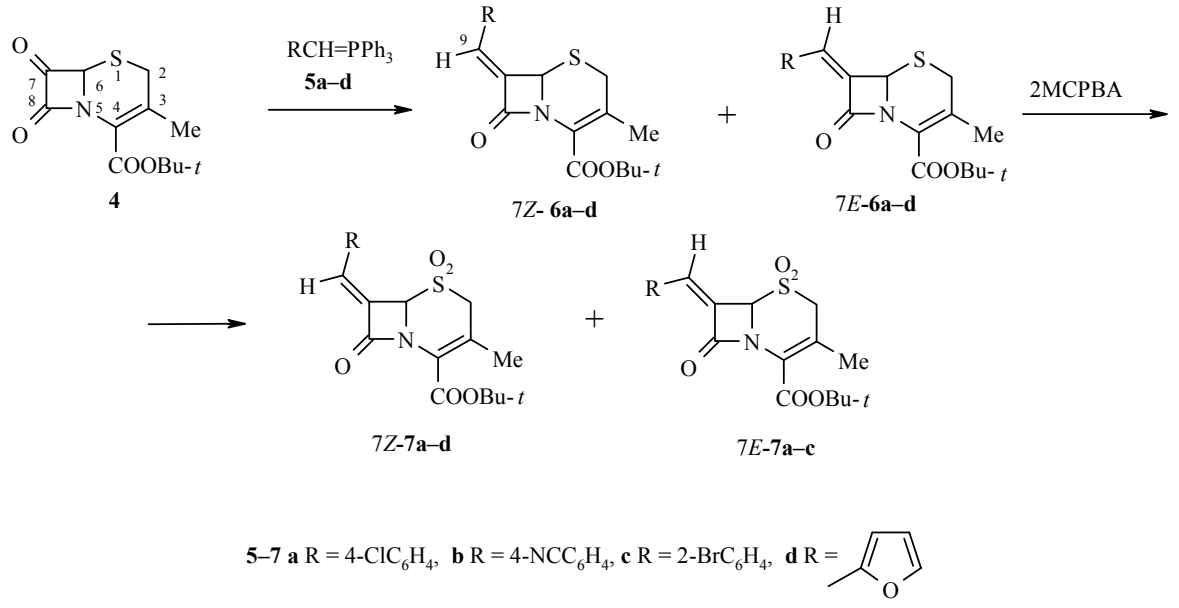
7*Z*-**3a,b**

1 a R = *t*-BuOCO, **b** R = MeOCO, **c** R = MeCO, **d** R = Ph, **e** R = 4-$O_2NC_6H_4$,
f R = 4-pyridyl, **g** R = 2-furyl; **2, 3 a** R = Ac, X = H; **b** R = $Me_3SiC{\equiv}C$, X = OAc

In a continuation of this investigation, we have synthesized new analogs of the *tert*-butyl ester of 7-alkylidene-1,1-dioxo-3-methyl-3-cepheme-4-carboxylic acid and subjected these products to cytotoxic screening, including **2,3a,b** relative to cancer and normal cells *in vitro* in order to establish a link between their structure and anti-carcinogenic properties.

7-Arylmethylene and 7-(2-furyl)methyl derivatives of *tert*-butyl esters of 3-cepheme-4-carboxylic acids **6a-d** as chromatographically inseparable mixtures of the 7*E*- and 7*Z*-isomers were synthesized according to methods described in previous work [1-3] by the condensation of the *tert*-butyl ester of 7-oxo-3-methyl-3-cepheme-4-carboxylic acid (**4**) with phosphoranes **5a-d**. The oxidation of the resultant cephemes **6a-d** to the corresponding sulfones **7a-d** was carried out using *meta*-chloroperbenzoic acid (MCPBA). The presence of an oxidized sulfur atom in the mixtures of sulfones **7a-d** facilitated their separation into pure 7*E*- and 7*Z*-isomers by column chromatography. Structural identification of these products by ^{1}H NMR spectroscopy was carried out in accord with the literature data [2, 3]. Thus, 3-cephemes 7*Z*-**7a-d** are characterized by a downfield shift of the signal for H-9 in comparison to the signal for 7*E*-**7a-d** due to deshielding by the β-lactam carbonyl.

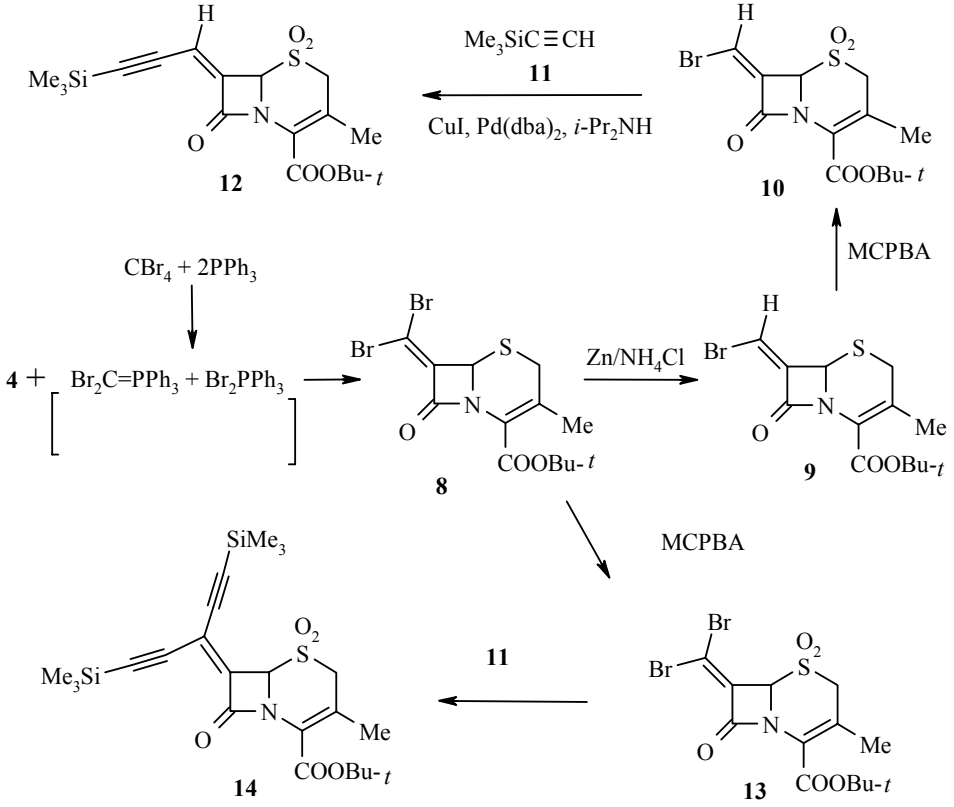
In our preceding work [2], we found that the sulfoxides and sulfone of the *tert*-butyl ester of 3-acetoxymethyl-7*Z*-(3-trimethylsilyl-2-propynylidene)-3-cepheme-4-carboxylic acid (**2b,3b**) are obtained predominantly as the 7*Z*-isomer due to stereospecific features of the Wittig reaction. Thus, in order to obtain the other isomer, we adapted a procedure for the synthesis of 7*E*-alkylidene derivatives of cephalosporin [3]. The *tert*-butyl ester of 3-methyl-7-oxo-3-cepheme-4-carboxylic acid (**4**) was converted to the *tert*-butyl ester of 7-dibromomethyl-3-methyl-3-cepheme-4-carboxylic acid (**8**) by the action of dibromomethylenephosphorane. Ester **8** was stereoselectively reduced by Zn/NH_4Cl to 7*E*-monobromide **9**. The conversion of bromide **9** to the *tert*-butyl ester of 3-methyl-1,1-dioxo-7*E*-(3-trimethylsilyl-2-propynylidene)-3-cepheme-4-carboxylic acid (7*E*-**12**) involved oxidation of the sulfur heteroatom and coupling of the 7*E*-bromomethylene group of



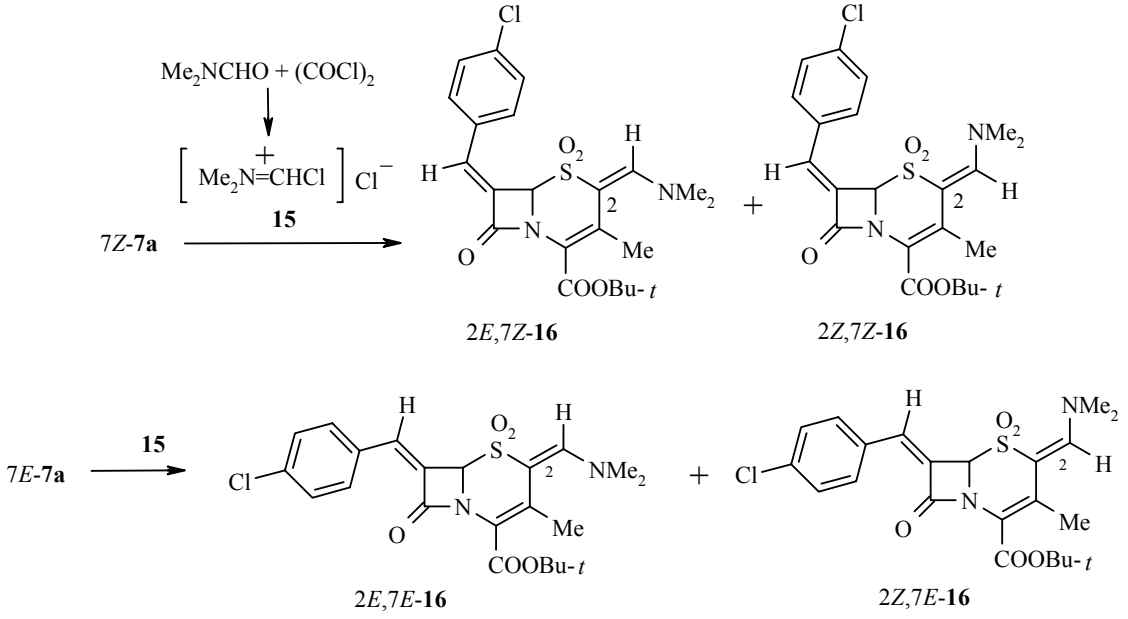
5–7 a R = 4-ClC$_6$H$_4$, **b** R = 4-NCC$_6$H$_4$, **c** R = 2-BrC$_6$H$_4$, **d** R =

intermediate sulfone **10** with trimethylsilylacetylene (**11**) under conditions of the catalytic Sonogashira reaction. The use of the sulfone of the 7-dibromo derivative of 3-cepheme **13** as the starting compound in this reaction with two equivalents of trimethylsilylacetylene (**11**) led to the *tert*-butyl ester of 3-methyl-1,1-dioxo-7-[1,5-bis-(trimethylsilyl)-1,4-pentadiyn-3-ylidene]-3-cepheme-4-carboxylic acid (**14**).

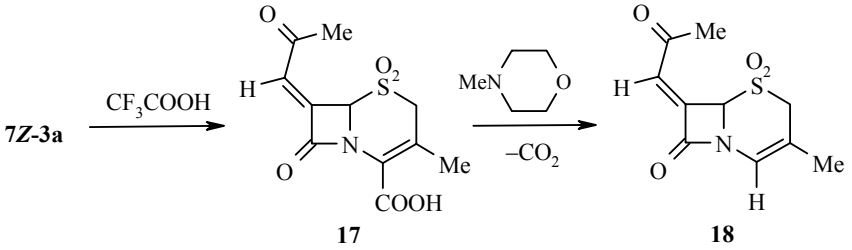
The introduction of a dimethylaminomethylene group at C-2 of the 7*Z*- and 7*E*-isomers of the *tert*-butyl ester of 7-(4-chlorophenyl)methylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid (7*Z*-**7a**, 7*E*-**7a**) was achieved using the Vilsmeier reagent according to the method given in our previous work [4].



Analysis of the ^{1}H NMR spectra of **16** showed that the dimethylaminomethylene group in these compounds exists as 2*E*- and 2*Z*-isomers. The deshielding effect of the sulfone oxygen atoms in 3-cephemes 2*E*,7*Z*-**16** and 2*E*,7*E*-**16** leads to a downfield shift of the signal for the =HCNMe$_2$ proton relative to the analogous signal for isomers 2*Z*,7*Z*-**16** and 2*Z*,7*E*-**16**. An analogous deshielding effect is observed for the signal of the six N(CH$_3$)$_2$ protons in the 2*Z*,7*Z*-**16** and 2*Z*,7*E*-**16** isomers.



The conversion of the *tert*-butyl ester of the sulfone of 7*Z*-acetylmethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid (7*Z*-**3a**) to 7*Z*-acetylmethylene-3-methyl-1,1-dioxo-3-cepheme (7*Z*-**18**) involved cleavage of the *tert*-butyl group using trifluoroacetic acid and decarboxylation of the intermediate carboxylic acid **17** in the presence of N-methylmorpholine.



Biological screening of these products *in vitro* included determination of their cytotoxic properties relative to monolayer lines of HT-1080 cancer cells (human fibrosarcoma) and MG-22A cancer cells (murine hepatoma) in comparison with those of normal 3T3 cells (embryonic muscle fibroblasts). Coloring of the 3T3 fibroblasts by Neutral Red permitted us to calculate the expected toxicity LD_{50} for the compounds tested using a special formula without *in vivo* experiments [5].

The screening data shown in Table 1 indicate that the structural variation including *Z*- and *E*-isomerism of the 7-alkylidene group, 1*S*- and 1*R*-isomerism of the sulfoxide oxygen, and inclusion of an N,N-dimethyl-aminomethylene group at C-2 in the 3-cepheme system has a significant effect on these parameters. Thus, the comparative analysis of the biological properties of the stereoisomeric sulfoxides of the *tert*-butyl ester of 7*Z*-acetylmethylene-3-methyl-3-cepheme-4-carboxylic acids 1*S*,7*Z*-**2a** and 1*R*,7*Z*-**2a** showed that the two

TABLE 1. Biological Properties of Structural Analogs of the *tert*-Butyl Ester of 7-Alkylidene-3-methyl-3-cepheme-4-carboxylic Acid*

Compound	Cytotoxic activity, LC_{50}, μkg/ml							LD_{50}, mg/kg
	HT-1080			MG-22A			3T3	
	CV	MTT	TG_{100}	CV	MTT	TG_{100}	NR	
1*S*,7*Z*-**2a**	2.3	4.8	350	1.5	1.6	450	11	316
1*R*,7*Z*-**2a**	2.3	3.3	450	2.8	2.8	450	33	521
7*Z*-**3a**	2.5	5.3	500	2.4	2.7	400	14	358
7*Z*-**3b**	0.05	0.07	1050	0.45	0.50	700	10*[2]	–
7*Z*-**18**	2.2	2.6	400	1.2	1	700	6	200
7*E*-**7a**	6	5	100	1.7	2	56	294	1476
7*Z*-**7a**	16	12	150	9	9	150	57	738
7*E*-**7b**	2	2	450	2	3	425	0.08	42
7*Z*-**7b**	2	1	175	2	3	433	14	392
7*E*-**7c**	3	2.5	233	2.5	3.2	400	7.1	318
7*Z*-**7c**	1.1	1.6	200	2.3	2.7	200	9.3	363
7*Z*-**7d**	21	19	250	19	16	250	142	1023
7*E*-**12**	19	9	550	23	26	300	3.2	206
14	2	2	350	4	0.3	550	4	256
7*E*-**16**	9	8	400	3	2	200	250	1488
7*Z*-**16**	10	17	150	8.0	10	150	84	930

* LC_{50} is the concentration providing for annihilation of 50% of cells, CV is coloration by Crystal Violet, MTT is coloration by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, TG_{50} is the specific NO generating activity of a compound, NR is coloration by Neutral Red, and LD_{50} is the dose of a substance providing for annihilation of 50% of cells.

*[2] Coloration by CV.

compounds have virtually identical cytotoxic activity *in vitro* relative to cancer cells but differ significantly in their cytotoxic activity relative to normal cells, reflected in a 1.5-fold greater LD_{50} value for the *R*-isomer. The oxidation of the sulfur atom in 1*S*,7*Z*-**2a** and 1*R*,7*Z*-**2a** to the sulfone did not alter the cytotoxic effect of 3-cepheme 7*Z*-**3a** but reduced its selectivity relative to cancer and normal cells. An analogous tendency was found for a structural analog of this compound, 7*Z*-**18**, with elimination of the *tert*-butoxycarbonyl fragment from C-4.

3-Cephemes 7*Z*-**3b**, 7*E*-**12**, and **14**, which differ in the structure of the substituent at C-3, 7*Z*- and 7*E*-isomerism of the trimethylsilyl fragments and their number in the 7-alkylidene group, have high toxicity relative to normal cells. Ester 7*Z*-**3b** suppressed the growth of cancer cells at much lower concentrations than 7*E*-**12** and **14**.

The biological properties of **7a-d** containing a monosubstituted aromatic or furan system at C-7, depend both on the isomerism of the alkylidene group and the structure of the aromatic system. Although the 7Z- and *7E*-isomers of 3-cepheme **7a** with a *para*-chlorophenyl substituent in the alkylidene group have similar cytotoxic activity relative to cancer cells, the 7*E*-**7a** isomer is only half as toxic as the 7*Z*-**7a** isomer. In the case of a *para*-cyanophenyl substituent, the toxicity of the 7*E*-**7b** isomer proved nine times greater than the toxicity of the 7*Z*-**7b** isomer. The cytotoxic properties of the 7*Z*-**7c** and 7*E*-**7c** isomers with an *ortho*-bromophenyl group relative to cancer and normal cells were found to be virtually identical. The introduction of a furan heterocycle into the alkylidene group of 7*Z*-**7d** led to a substantial decrease in its toxicity relative to cancer and normal cells.

In comparison with starting 3-cephemes 7*E*-**7a** and 7*Z*-**7a**, esters 7*E*-**16** and 7*Z*-**16,** containing an N,N-dimethylaminomethylene group at C-2, displayed similar cytotoxic activity relative to cancer cells and reduced cytotoxic activity relative to normal cells.

The cytotoxic effect of the compounds tested, similar to previously studied *tert*-butyl esters of 7-alkylidene derivatives of cephalosporins [1], is interrelated to their capacity to their capacity to generate NO radicals in the cell medium. High cytotoxicity of these compounds is accompanied, as a rule, by enhanced NO radical generation and *vice versa*.

EXPERIMENTAL

The ^{1}H NMR spectra were taken on a Bruker WH90/DS spectrometer at 90 MHz and Varian Mercury-200 spectrometer at 200 MHz in $CDCl_3$ as the internal standard. The elemental analysis was carried out on a Carlo Erba 1108 analyzer. The ESI (electrospray ionization) mass spectrometry was carried out on a Micromass Quatro Micro^{TM} API. The high-performance liquid chromatography was carried out on a Dupont Model 8800 chromatograph equipped with a UV detector (λ = 254. nm) and 4.6×250-mm column packed with μPorasil using 20:80 ethyl acetate–hexane as the eluent, Altima C18 using 50:50 acetonitrile–0.1 M phosphate buffer (pH 2.5) as the eluent, and Zorbax R×C_{18} with 60:40 acetonitrile-water as the eluent. The reaction course was monitored by thin-layer chromatography on Merck Kieselgel plates with UV detection. Merck Kieselgel (0.063-0.230 mm) was used for the preparative column chromatography. Reagents and materials supplied by Acros, Aldrich, and Sigma were used in the experiments. The optical density in the biological tests carried out on 96-well panels was determined using a horizontal Tetretek Multiscan MCC/340 spectrophotometer.

***tert*-Butyl Ester of 7*Z*-(4-Chlorophenyl)methylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic Acid (7*Z*-7a) and *tert*-Butyl Ester of 7*E*-(4-Chlorophenyl)methylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic Acid (7*E*-7a).** A sample of butyllithium in hexane (2.2 ml, 1.6 M) was added with stirring to a solution of *para*-chlorobenzyltriphenylphosphonium chloride (694 mg, 1.64 mmol) in absolute THF (15 ml) at 0°C. The solution was stirred for 30 min at 10°C, cooled to -78°C, and then, 479 mg (1.64 mmole) *tert*-butyl ester of 3-methyl-7-oxo-3-cepheme-4-carboxylic acid [5] in dichloromethane (10 ml) was added. The mixture obtained was stirred for 30 min at -78°C and poured into saturated aqueous NH_4Cl (100 ml) with ice. The mixture was stirred until the ice melted and extracted with two 50 ml portions of dichloromethane. The organic phase was washed with cold aq. NH_4Cl and dried over anhydrous sodium sulfate. the solvent was evaporated off at reduced pressure and the residue was fractionated on a silica gel column using 1:2.5 ethyl acetate–petroleum ether as the eluent. The fractions with R_f 0.68 were combined and evaporated off to give 500 mg (80%) of an oily product. HPLC analysis indicate that this product consisted of a 1:1 mixture of the *tert*-butyl ester of 7*Z*-(4-chlorophenyl)methylene-3-methyl-3-cepheme-4-carboxylic acid (7*Z*-**6a**) and the *tert*-butyl ester of 7*E*-(4-chlorophenyl)methylene-3-methyl-3-cepheme-4-carboxylic acid (7*E*-**6a**).

A sample of 70% 3-chloroperbenzoic acid (815 mg, 3.31 mmol) was added with stirring to a solution mixture of isomers 7*Z*-**6a** and 7*E*-**6a** (500 mg, 1.32 mmol) in dichloromethane (20 ml) at 0°C. The mixture was stirred at room temperature for 4 h, diluted by adding 20 ml dichloromethane, washed with 5% aq. Na_2SO_3 (50 ml) followed by two 50 ml portions of 5% aq. Na_2CO_3, and dried over anhydrous sodium sulfate. The solvent was evaporated off at reduced pressure and the residue was fractionated on a silica gel column using 1:2 ethyl acetate–petroleum ether as the eluent.

The fractions with R_f 0.28 gave 230 mg (42%) 7*Z*-**7a**, mp 40-41°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.51 (9H, s, C_4H_9); 2.06 (3H, s, CH_3); 3.63 and 3.98 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.51 (1H, br. s, H-6); 7.34 (1H, br. s, H-9); 7.38 and 7.76 (4H, two d, ^3J = 7, C_6H_4); Found, %: C 55.90; H, 5.08; N 3.53. $C_{19}H_{20}ClNO_5S$. Calculated, %: C 55.68; H 4.92; N 3.42.

The fractions with R_f 0.11 gave 187 mg (34%) 7*E*-**7a**, mp 185-186°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.53 (9H, s, C_4H_9); 2.04 (3H, s, CH_3); 3.58 and 3.95 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.17 (1H, br. s, H-6); 6.82 (1H, br. s, H-9); 7.95 and 8.08 (4H, two d, ^3J = 7, C_6H_4). Found, %: C 55.87; H 5.00; N 3.58. $C_{19}H_{20}ClNO_5S$. Calculated, %: C 55.68; H 4.92; N 3.42.

***tert*-Butyl Ester of 7*Z*-(4-Cyanophenyl)methylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic Acid (7*Z*-7b) and *tert*-Butyl Ester of 7*E*-(4-Cyanophenyl)methylene-3-Methyl-1,1-dioxo-3-cepheme-4-carboxylic acid (7*E*-7b).** A sample of butyllithium (2.2 ml, 1.6 M) in hexane was added with stirring to a solution of *para*-cyanobenzyltriphenylphosphonium chloride (559 mg, 1.35 mmol) in absolute THF (15 ml) at 0°C. The solution was stirred for 30 min at 10°C, cooled to -78°C, and then *tert*-butyl (479 mg, 1.64 mmol) ester of 3-methyl-7-oxo-3-cepheme-4-carboxylic acid in dichloromethane (10 ml) was added. The mixture obtained was stirred for 30 min at -78°C and poured into saturated aqueous NH_4Cl (100 ml) with ice. The mixture was stirred until the ice melted and extracted with two 50 ml portions of dichloromethane. The organic phase was washed with cold aq. NH_4Cl and dried over anhydrous sodium sulfate. The solvent was evaporated off at reduced pressure and the residue was fractionated on a silica gel column with 1:2.5 ethyl acetate-petroleum ether as the eluent. The fractions with R_f 0.68 were combined and evaporated to give 380 mg (76%) of an oily product. HPLC analysis indicated that this product consisted of a mixture of the *tert*-butyl ester of 7*Z*-(4-cyanophenyl)methylene-3-methyl-3-cepheme-4-carboxylic acid (7*Z*-**6b**) and the *tert*-butyl ester of 7*E*-(4-cyanophenyl)methylene-3-methyl-3-cepheme-4-carboxylic acid (7*E*-**6b**).

A sample of 70% 3-chloroperbenzoic acid (665 mg, 2.70 mmol) was added to a solution of the mixture of isomers 7*Z*-**6b** and 7*E*-**6b** (380 mg, 1.03 mmol) in dichloromethane (20 ml) at 0°C. The mixture was stirred at room temperature for 4 h, diluted by adding 20 ml dichloromethane, washed with 50 ml 5% aq. Na_2SO_3 followed by two 50 ml portions of 5% aq. Na_2CO_3,and dried over anhydrous sodium sulfate. The solvent was evaporated at reduced pressure and the residue was fractionated on a silica gel column with 1:4 ethyl acetate–petroleum ether as the eluent.

The fractions with R_f 0.42 gave 91 mg (22%) 7*Z*-**7b**, mp 153-154°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.55 (9H, s, C_4H_9); 2.16 (3H, s, CH_3); 3.68 and 3.95 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.53 (1H, br. s, H-6), 7.39 (1H, br. s, H-9); 7.74 and 8.00 (4H, two d, ^3J = 8, C_6H_4). Found, %: C 59.99; H 5.03; N 7.00. $C_{20}H_{20}N_2O_5S$. Calculated, %: C 60.09; H 5.08; N 7.11.

The fractions with R_f 0.24 gave 57 mg (14%) 7*E*-**7b**, mp 118-120°C. ^{1}H NMR spectrum (200 MHz), δ, ppm (*J*, Hz): 1.57 (9H, s, C_4H_9); 2.17 (3H, s, CH_3); 3.62 and 3.90 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.17 (1H, br. s, H-6); 6.88 (1H, br. s, H-9); 7.98 and 8.07 (4H, two d, AB system, ^3J = 7, C_6H_4). Found, %: C 60.13; H 5.23; N 7.12. $C_{20}H_{20}N_2O_5S$. Calculated, %: C 60.09; H 5.08; N 7.11.

***tert*-Butyl Ester of 7*Z*-(2-Bromophenyl)methylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic Acid (7*Z*-7c) and *tert*-Butyl Ester of 7*E*-(2-Bromophenyl)methylene-1,1-dioxo-3-cepheme-4-carboxylic Acid (7*E*-7c).** A sample of sodium *tert*-butylate (234 mg, 2.44 mmol) was added with stirring to a solution of 2-bromobenzyltriphenylphosphonium chloride (1140 mg, (2.44 mmol) in absolute THF (15 ml) at 0°C. The solution was stirred for 30 min at 10°C, cooled to -78°C, and, then, *tert*-butyl ester (479 mg, 1.64 mmol) of 3-methyl-7-oxo-3-cepheme-4-carboxylic acid in dichloromethane (10 ml) was added. The mixture obtained was stirred for 30 min at -78°C and poured into saturated aqueous NH_4Cl (100 ml) with ice. The mixture was stirred until the ice melted and then extracted with two 50 ml portions of dichloromethane. The organic phase was washed with cold aq. NH_4Cl and dried over anhydrous sodium sulfate. The solvent was evaporated off at reduced pressure and the residue was fractionated on a silica gel column with 1:4 ethyl acetate–petroleum ether as the eluent. The fractions with R_f 0.68 were combined and evaporated to give 400 mg (39%) of an oily product. HPLC indicated that this product was a 1:1 mixture of the *tert*-butyl ester of 3-methyl-7*Z*-(2-bromophenyl)methylene-3-cepheme-4-carboxylic acid (7*Z*-**6c**) and the *tert*-butyl ester of 3-methyl-7*E*-(2-bromophenyl)-3-cepheme-4-carboxylic acid (7*E*-**6c**).

A sample of 70% 3-chloroperbenzoic acid (566 mg, 2.30 mmol) was added with stirring to a solution of the mixture of isomers 7*Z*-**6c** and 7*E*-**6c** (400 mg) in dichloromethane (20 ml) at 0°C. The mixture was stirred at room temperature for 4 h, diluted by adding dichloromethane (20 ml), washed with 5% aq. Na_2SO_3 (50 ml) followed by two 50 ml portions of 5% aq. Na_2CO_3, and dried over anhydrous sodium sulfate. The solvent was evaporated at reduced pressure and the residue was fractionated on a silica gel column with 1:3 ethyl acetate–petroleum ether as the eluent.

The fractions with R_f 0.24 gave 84 mg (19%) 7*Z*-**7c**, mp 118-119°C. ^{1}H NMR spectrum (200 MHz), δ, ppm (*J*, Hz): 1.56 (9H, s, C_4H_9); 2.06 (3H, s, CH_3); 3.67 and 3.93 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.44 (1H, s, H-6), 7.21-7.44 (2H, m, C_6H_4), 7.61-7.68 (1H, m, C_6H_4), 7.81 (1H, s, H-9), 7.85-7.91 (1H, m, C_6H_4). Found, %: C 50.31; H 4.53; N 3.14. $C_{19}H_{20}BrNO_5S$. Calculated, %: C 50.23; H 4.44; N 3.08.

The fractions with R_f 0.14 gave 121 mg (28%) 7*E*-**7c**, mp 140-141°C. ^{1}H NMR spectrum (200 MHz), δ, ppm (*J*, Hz): 1.55 (9H, s, C_4H_9); 2.07 (3H, s, CH_3); 3.62 and 3.89 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.19 (1H, s, H-6); 7.20-7.46 (2H, m, C_6H_4); 7.32 (1H, s, H-9); 7.58-7.66 (1H, m, C_6H_4); 8.49-8.55 (1H, m, C_6H_4). Found, %: C 50.43; H 4.49; N 3.28. $C_{19}H_{20}BrNO_5S$. Calculated, %: C 50.23; H 4.44; N 3.08.

***tert*-Butyl Ester of 7*Z*-(2-Furyl)methylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic Acid (7*Z*-7d).** A sample of butyllithium in hexane (1 ml, 1.6 M) was added with stirring to a solution of 2-furyltriphenylphosphonium chloride (604 mg, 1.60 mmol) in THF (10 ml) at 10°C. The mixture was stirred for 30 min at 10°C, cooled to -78°C and then, *tert*-butyl ester (523 mg, 1.60 mmol) of 3-methyl-7-oxo-3-cepheme-4-carboxylic acid in absolute THF (5 ml) was added. The mixture was stirred for 30 min at -78°C and poured into saturated aqueous NH_4Cl (100 ml) with ice. The mixture was stirred until the ice melted and extracted with two 20 ml portions of dichloromethane. The organic phase was washed with cold aqueous $NH_4Cl$and dried over anhydrous sodium sulfate. The solvent was evaporated at reduced pressure and the residue was fractionated on a silica gel column using 1:3 ethyl acetate–petroleum ether as the eluent. The fractions with R_f 0.50 were combined and evaporated to give 350 mg (39%) of an oily product showed by HPLC analysis to consist of a 10:1 mixture of the *tert*-butyl ester of 7*Z*-(2-furyl)methylene-3-methyl-3-cepheme-4-carboxylic acid (7*Z*-**6d**) and the *tert*-butyl ester of 7*Z*-(2-furyl)methylene-3-methyl-3-cepheme-4-carboxylic acid (7*E*-**6d**).

A sample of 70% 3-chloroperbenzoic acid (815 mg, 3.31 mmol) was added with stirring to a solution of the mixture of isomers of 7*Z*-**6d** and 7*E*-**6d** (350 mg, 1.04 mmol) in dichloromethane (20 ml) at 0°C. The mixture was stirred at room temperature for 4 h, diluted by adding 20 ml dichloromethane, washed with 50 ml 5% aq. Na_2SO_3 followed by two 50 ml portions of 5% aq. Na_2CO_3, and dried over anhydrous sodium sulfate. The solvent was evaporated at reduced pressure and the residue was fractionated on a silica gel column using 1:2 ethyl acetate–petroleum ether as the eluent. The fractions with R_f 0.14 gave 87 mg (23%) 7*Z*-**7d**, mp 103-105°C.

^{1}H NMR spectrum (200 MHz); δ, ppm (*J*, Hz): 1.54 (9H, s, C_4H_9); 2.13 (3H, s, CH_3); 3.70 and 3.95 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.69 (1H, d, ^4J = 0.25, H-6); 6.64 (1H, two d, ^3J = 2, ^3J = 4, C-4 furan); 7.41 (1H, d, ^3J = 4, C-3 furan); 7.56 (1H, d, ^3J = 2, C-5 furan); 7.70 (1H, d, ^4J = 0.25, H-9). Found, %: C 56.01; H 5.43; N 3.94. $C_{17}H_{19}NO_6S$. Calculated, %: C 55.88; H 5.24; N 3.83.

***tert*-Butyl Ester of 7,7-Dibromomethylene-3-methyl-3-cepheme-4-carboxylic Acid (8).** A sample of tetrabromomethane (3.78 g, 11.4 mmol) was added in one portion to a solution of triphenylphosphine (6.00 g, 22.8 mmol) in dichloromethane (40 ml) cooled to -10°C in an argon atmosphere. The solution was stirred for 10 min at room temperature, cooled to -70°C and then, a solution of *tert*-butyl ester (3.00 g, 11.4 mmol) of 3-methyl-7-oxo-3-cepheme-4-carboxylic acid in dichloromethane previously (40 ml) cooled to -40°C was added. The solution was warmed to -40°C and stirred for 15 min. The solution was then warmed to -10°C and poured into saturated aqueous NH_4Cl (100 ml) with ice. The mixture was stirred until the ice melted and extracted with two 50 ml portions of dichloromethane. The organic phase was washed with cold aqueous NH_4Cl and dried over anhydrous sodium sulfate. The solvent was evaporated at reduced pressure and the residue was fractionated on a silica gel column using 1:4 ethyl acetate–petroleum ether as the eluent. The fractions with

R_f 0.61 were combined and evaporated to give 1.2 g (24%) **8**, mp 133-135°C. The product contained 90% major compound as indicated by HPLC analysis on a Zorbax R×C_{18} column. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.57 (9H, s, C_4H_9), 2.09 (3H, s, 3-CH_3), 3.13 and 3.49 (2H, two d, AB system, ^2J = 18, SCH_2), 5.17 (1H, s, H-6).

***tert*-Butyl Ester of 7*E*-Bromomethylene-3-methyl-3-cepheme-4-carboxylic Acid (9).** Ammonium chloride (50 mg, 0.94 mmol) and zinc powder (31 mg, 0.48 mmol) were added to a solution of *tert*-butyl ester (100 mg, 23.5 mmol) 7-dibromomethylene-3-cepheme-4-carboxylic acid (**8**) in 2:1 methanol-tetrahydrofuran cooled (6 ml) to 0°C. The solution was stirred for 30 min at 0°C for an additional 30 min at room temperature, filtered, and evaporated at reduced pressure. The residue was treated with diethyl ether (60 ml), washed with aqueous NaCl (30 ml), dried over anhydrous sodium sulfate, and evaporated at reduced pressure. The residue was fractionated on a silica gel column using 1:3 ethyl acetate–petroleum ether as the eluent. The fractions with R_f 0.17 were combined and evaporated to give 20 mg (24%) **9**, mp 106-108°C. The product contains 97% of the desired compound as indicated by HPLC analysis on a Zorbax×C_{18} column. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.53 (9H, s, C_4H_9);2.04 (3H, s, 3-CH_3); 3.11 and 3.49 (2H, two d, AB system, ^2J = 18, SCH_2); 5.13 (1H, br. s, H-6); 6.66 (1H, br.s, H-9).

***tert*-Butyl Ester of 7*E*-Bromomethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic Acid (10).** A sample of 70% 3-chloroperbenzoic acid (4.84 g, 19.65 mmol) was added with stirring to a solution of *tert*-butyl ester (1.7 g, 4.91 mmol) of 7*E*-bromomethylene-3-methyl-3-cepheme-4-carboxylic acid in dichloromethane (25 ml) at 0°C. The mixture was stirred at room temperature for 4 h, diluted by adding dichloromethane (25 ml), washed with 50 ml 5% aqueous Na_2SO_3 followed by two 50 ml portions of 5% aqueous Na_2CO_3, and dried over anhydrous sodium sulfate. The solvent was evaporated on a silica gel column using 1:1 ethyl acetate–petroleum ether as the eluent. The fractions with R_f 0.52 were combined and evaporated to give 1.30 g (70%) **10**, mp 88-90°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.51 (9H, s, C_4H_9); 2.04 (3H, s, 3-CH_3); 3.55 and 3.77 (2H, two d, AB system, ^2J = 18, SO_2CH_2);5.09 (1H, d, ^4J = 0.5, H-6); 6.93 (1H, d, ^4J = 0.5, H-9). Found, %: C 41.37; H 4.31, N 3.87. $C_{13}H_{16}BrNO_3S$. Calculated, %: C 41.28; H 4.26; N 3.70.

***tert*-Butyl Ester of 3-Methyl-1,1-dioxo-7-(3-trimethylsilylpropyn-2-ylidene)-3-cepheme-4-carboxylic Acid (7*E*-12).** A sample of trimethylsilylacetylene (31 µl, 0.22 mmol) and *i*-Pr_2NH (27 µl, 0.37 mmol) were added to a suspension of *tert*-butyl ester (70 mg, 18.5 mmol) of 7*E*-bromomethylene-1,1-dioxo-3-methyl-3-cepheme-4-carboxylic acid, CuI (70 mg, 0.37 mmol), and adduct of tris(dibenzylidenacetone)dipalladium(0) with chloroform (17 mg) in N-methylpyrrolidone (10 ml) in an argon atmosphere. The solution was stirred for 24 h at room temperature, diluted by adding diethyl ether (80 ml), and washed with 30 ml 5% aqueous K_2CO_3 and then 30 ml 5% NaCl, dried over anhydrous sodium sulfate, and evaporated at reduced pressure. The residue was fractionated on a silica gel column using 1:10 ethyl acetate–petroleum ether. The fractions with R_f 0.11 were combined and evaporated to give 20 mg (40%) 7*E*-**12**. The product contains 97% desired compound as indicated by HPLC analysis. ^{1}H NMR spectrum(90 MHz), δ, ppm (*J*, Hz): 0.20 (9H, s, $(CH_3)_3Si$); 1.55 (9H, s, C_4H_9); 2.04 (3H, s, 3-CH_3); 3.55 and 3.91 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.11 (1H, s, H-6); 6.09 (1H, s, H-9).

***tert*-Butyl Ester of 7,7-Dibromomethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic Acid (13).** A sample of 70% 3-chloroperbenzoic acid (1.62 g, 6.60 mmol) was added with stirring to a solution of *tert*-butyl ester (1.12 g, 2.64 mmol) of 7,7-dibromomethylene-3-methyl-3-cepheme-4-carboxylic acid in dichloromethane (25 ml) at 0°C. The mixture was stirred at room temperature for 4 h, diluted by adding dichloromethane (25 ml), washed with 50 ml 5% aqueous Na_2SO_3 followed by two 50-ml portions of 5% aqueous Na_2CO_3, and dried over anhydrous sodium sulfate. The solvent was evaporated at reduced pressure and the residue was fractionated on a silica gel column using 1:2 ethyl acetate–petroleum ether as the eluent. The fractions with R_f 0.27 were combined and evaporated to give 0.70 g (58%) **13**, mp 108-110°C. ^{1}H NMR spectrum (90 MHz), δ, ppm

(*J*, Hz): 1.53 (9H, s, C_4H_9); 2.06 (3H, s, 3-CH_3); 3.11 and 3.49 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.17 (1H, s, H-6). Found, %: C 34.25; H 3.46; N 3.15. $C_{13}H_{15}Br_2NO_5S$ Calculated, %: C 34.16; H 3.31; N 3.06.

***tert*-Butyl Ester of 3-Methyl-1,1-dioxo-7-[1,5-bis(trimethylsilyl)-1,4-pentadiyn-3-ylidene]-3-cepheme-4-carboxylic Acid (14).** A sample of trimethylsilylacetylene (62 µl, 0.44 mmol) and *i*-Pr_2NH (27 µl, 0.37 mmol) were added to a suspension of *tert*-butyl ester (100 mg, 22 mmol) of 7-dibromomethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid, CuI (130 mg, 0.68 mmol), and adduct of tris(dibenzylideneacetone)dipalladium(0) with chloroform (20 mg) in N-methylpyrrolidone (10 ml) in an argon atmosphere. The solution was stirred for 24 h at room temperature, diluted by adding diethyl ether (80 ml), and washed with 30 ml 5% aq. K_2CO_3, then 30 ml 5% aq. NaCl, dried over anhydrous sodium sulfate, and evaporated at reduced pressure. The residue was fractionated on a silica gel column using 1:10 ethyl acetate–petroleum ether as the eluent. The fractions with R_f 0.36 were combined and evaporated to give 31 mg (28%) **14**, mp 48-50°C. This product contained 97.5% desired compound as indicated by HPLC analysis. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 0.22 (18H, s, 2(CH_3)$_3$Si); 1.53 (9H, s, C_4H_9); 2.00 (3H, s, 3-CH_3); 3.55 and 3.88 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.17 (1H, s, H-6).

Mixture of *tert*-Butyl Ester of 7*Z*-(4-Chlorophenyl)methylene-2*E*-dimethylaminomethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic Acid (2*E*,7*Z*-16) and *tert*-Butyl Ester of 7*Z*-(4-Chlorophenyl)methylene-2*Z*-dimethylaminomethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid (2*Z*,7*Z*-16). A sample of *tert*-butyl ester (100 mg, 0.24 mmol) of 7*Z*-(4-chlorophenyl)methylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid was added to a stirred solution of DMF (76 µl, 0.97 mmol) and oxalyl chloride (85 µl, 0.97 mmol) in dichloromethane (10 ml) at 0°C in an argon atmosphere. After 30 min stirring at 0°C, pyridine (79 µl, 0.79 mmol) was added to the reaction mixture, which was then stirred at room temperature for 1 h. The reaction mixture was evaporated at reduced pressure. The residue was dissolved in 20 ml dichloromethane. The solution obtained was washed with 40 ml saturated aqueous NH_4Cl and dried over anhydrous sodium sulfate. The solvent was evaporated at reduced pressure. The residue was fractionated on a silica gel column using 2:1 ethyl acetate–hexane as the eluent. The fractions with R_f 0.61 were combined and evaporated to give 76 mg (68%) of a 3:1 mixture of the 2*E*- and 2*Z*-somers as indicated by ^{1}H NMR spectroscopy at 200 MHz. ^{1}H NMR spectrum (200 MHz), δ, ppm (*J*, Hz): 2*Z*,7*Z*-**16**) 1.58 (9H, s, C_4H_9); 2.22 (3H, s, 3-CH_3); 3.33 (6H, s, N(CH_3)$_2$); 5.17 (1H, d, ^4J = 0.2, H-6); 7.18 (1H,s, =$CHNMe_2$); 7.26 (1H, d, ^4J = 0.2, H-9); 7.38 and 7.60 (4H, two d, ^3J = 8, C_6H_4); 2*E*,7*Z*-**16**) 1.54 (9H, s, C_4H_9); 2.22 (3H, s, CH_3); 3.06 (6H, s, N(CH_3)$_2$); 5.17 (1H, d, ^4J = 0.2, H-6); 7.26 (1H, d, ^4J = 0.2, H-9); 7.28 (1H, s, =$CHNMe_2$); 7.38 and 7.60 (4H, two d, ^3J = 8, C_6H_4). ESI-MS (MeCN): 487 [MNa^+].

Mixture of *tert*-Butyl Ester of *E*-(4-Chlorophenyl)methylene-2*E*-dimethylaminomethylene-3-methyl-1,1-dioxo-73-cepheme-4-carboxylic Acid (2*E*,7*E*-16) and *tert*-Butyl Ester of 7*E*-(4-Chlorophenyl)methylene-2*Z*-dimethylaminomethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic Acid (2*Z*,7*E*-16) was obtained according to the above procedure using the *tert*-butyl ester of 7*Z*-(4-chlorophenyl)methylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid and separated from the fractions with R_f 0.48 upon chromatography using 2:1 ethyl acetate–hexane as the eluent. The yield of this mixture was 57 mg (51%). The ^{1}H NMR spectrum at 200 MHz indicated that the product was a 4:1 mixture of the indicated 2*E*- and 2*Z*-isomers. ^{1}H NMR spectrum (200 MHz), δ, ppm (*J*, Hz): 2*Z*,7*Z*-**16**) 1.55 (9H, s, C_4H_9); 2.26 (3H, s, 3-CH_3); 3.32 (6H, s, N(CH_3)$_2$); 5.12 (1H, s, H-6); 6.73 (1H, s, H-9); 7.17 (1H, s, =$CHNMe_2$); 7.40 and 7.94 (4H, two d, ^3J = 8, C_6H_4); 2*E*,7*Z*-**16**) 1.55 (9H, s, C_4H_9); 2.26 (3H, s, 3-CH_3); 3.02 (6H, s, N(CH_3)$_2$); 5.12 (1H, s, H-6); 6.73 (1H, s, H-9); 7.24 (1H, s, =$CHNMe_2$); 7.40 and 7.94 (4H, two d, ^3J = 8, C_6H_4). ESI-MS (MeCN): 487 [MNa^+].

7*Z*-Acetylmethylene-3-methyl-1,1-dioxo-3-cepheme (7*Z*-18). A sample of trifluoroacetic acid (2 ml, 38 mmol) was added to a solution of *tert*-butyl ester (341 mg, 1.00 mmol) of 7*Z*-acetylmethylene-3-methyl-1,1-dioxo-3-cepheme-4-carboxylic acid in dichloromethane (30 ml)at 0°C. The mixture was stirred for 1 h at room temperature, diluted by adding dichloromethane (100 ml) and water (100 ml). The organic phase was

separated and extracted with 40 ml 5% aqueous Na_2CO_3. The aqueous extract was brought to pH 2 by adding concentrated hydrochloric acid and extracted with 100 ml ethyl acetate. The extract was evaporated at reduced pressure. The residue was dissolved in a mixture of 20 ml acetone and 2 ml N-methylmorpholine. The solution was stirred at room temperature for 1 h and the solvent was evaporated at reduced pressure. The residue was fractionated on a silica gel column using 1:2 ethyl acetate–hexane as the eluent. The fractions with R_f 0.12 were combined and evaporated to give 181 mg (75%) 7*Z*-**18**, mp 135-137°C. ^{1}H NMR spectrum (200 MHz), δ, ppm (*J*, Hz): 1.82 (3H, s, 3-CH_3); 2.42 (2H, s, CH_3CO); 3.48 and 4.03 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 5.58 (1H, s, H-6), 6.57 (1H, s, H-9); 6.85 (1H, s, 4-H). Found, %: C 49.91; H 4.77; N 5.89. $C_{10}H_{11}NO_4S$. Calculated, %: C 49.78; H 4.60; N 5.81.

Determination of the *in vitro* Cytotoxic Activity. The cytotoxic activity of the products relative to cultures of monolayer cancer and normal cells at $c = (2\text{-}5)\cdot 10^4$ cells/ml [HT-1080 (human fibrosarcoma), MG-22A (murine hepatoma), 3T3 (embryonic muscle fibroblasts)] was determined on 96-well plastic panels. The NO radical concentrations (nmoles) in the culture medium with surviving cells after incubation for 72 h in the presence of the compound tested at c = 50 μg/ml in a 200 μl well were used to calculate the specific NO generating activity of the compounds (TG_{100}):

$$TG_{100} = G\ 100/C\ (\text{nmol}/\mu\text{l}),$$

where G is the NO concentration (nmoles) in 200 μl culture medium with surviving cells, while C is the percentage of surviving cells determined by their CV coloration.

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