Studies Related to Cephalosporins. Part 2 [1a]. Displacement Reactions on 3-Bromomethylcephems with Salts of Carboxylic Acids

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Received November 2, 1984

The reactivity of Δ^3- and Δ^2-3-bromomethylcephems toward carboxylate nucleophiles has been studied. The Δ^3-bromomethylcephem **1**, less reactive than the Δ^2-analogue **4**, is converted in high yields into 3-acyloxymethyl-3-cephems **2a-d**, generally with no isomerization of the double bond, only within a narrow range of conditions. In particular, the Δ^3-7-aminocephalosporanic acid (7-ACA) derivative **2a** has been obtained as the only product in 91% yield by treatment of **1** with triethylammonium acetate in acetic acid.

The Δ^2-bromomethyl-cephem **4** is easily converted into the Δ^2-acyloxymethyl-cephems **5a-d** without double bond isomerization, in very high yields.

J. Heterocyclic Chem., **22**, 1001 (1985).

In a previous paper, we have described some new reactions involving 3-bromomethyl-2-cephems and 3-bromomethyl-3-cephems. They react smoothly with alcohols and phenols and, in the latter case, only C-substitution products are obtained [1a].

Our study on the reactivity of 3-halomethylcephems takes its origin from the consideration that several commercially available 3'-substituted-cephalosporins [2] have been prepared from 3-acetoxymethylcephems by substitution of the acetoxy group with sulphur or nitrogen nucleophiles. Substitution products with carbon or oxygen nucleophiles seem to be less accessible [3]. Therefore, a better knowledge on the reactivity of 3-halomethylcephems, in principle more reactive than the 3-acetoxymethyl analogues [4] is highly desirable.

There are only few records in the periodicals or patent literature about the substitution reactions at the 3'-position of Δ^3- and Δ^2-3-bromomethylcephems given by carboxylate anions [5]. They occur in low yields and give mixtures of Δ^3- and Δ^2-derivatives, since a prominent isomerization of the double bond occurs during the reaction course. In order to avoid the formation of a mixture, silver acetate has been used [6]. Yields however, are not high (30%).

These results are not unexpected. Any base promotes the $\Delta^2 \rightleftharpoons \Delta^3$ equilibrium and, in addition, the β-lactam ring is sensitive to bases. On the other hand, it is well known that alkyl halides are not very reactive toward carboxylate anions, this being justified in terms of the soft-hard interaction [7].

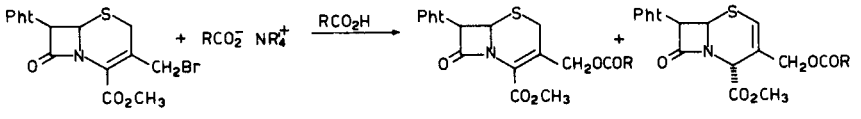
With these considerations in mind, we have studied the substitution reaction at the 3'-position of Δ^3- and Δ^2-3-bromomethylcephem-derivatives with carboxylate salts. The displacement reaction with acetate salts are discussed taking into consideration the effect of different cations and solvent systems. In particular in this paper the conditions under which these substitution reactions can be carried out in high yields, without concomitant isomerization of the double bond, are described.

Results.

Displacement Reactions on Methyl 3-Bromomethyl-7-phthalimido-3-cephem-4-carboxylate (1).

The synthesis of the starting material **1** has been already described [1a]. It should be taken into account that the 7-phthalimido group is removable in order to give access to useful 7-amidic substitution [1b].

Preliminary substitution experiments, carried out with



Pht = phthalimido

a ; R = Me
b ; R = $PhCH_2$
c ; R = n-Pr
d ; R = Ph

Scheme 1

Table 1

Conversion of the Bromomethyl-3-cephem **1** into Derivative **2a** by Means of Acetate Salts in Pure Acetic Acid [a]

Entry	Acetate counterion [c]	Reaction time (days)	Reaction temperature (°C)	Yield [c] (%)
1	triethylammonium	7	20	91
2	triethylammonium	3 (hours)	50	86
3	tetrabutylammonium	7	20	65
4 [b]	tetrabutylammonium	22	20	87
5	hexylammonium	7	20	34 [d]
6	potassium	7	20	53 [d]

[a] Substrate concentration = 0.2 *M*. [b] Substrate concentration = 0.05 *M*. [c] No Δ^2-isomer (**3a**) was present in the reaction mixture. [d] 20% of unreacted material was recovered.

Table 2

Conversion of the Bromomethyl-3-cephem **1** into Derivatives **2b-d** and **3c-d** by Means of Triethylammonium Carboxylates in the Pure Corresponding Carboxylic Acids.

Entry	Molar ratios **1**:salt:acid	Reaction time (hours)	Reaction temperature (°C)	Product	Yield (%)
1	1:2.5:7.5	3	50	**2b**	78
2	1:3[a]:50	12	20	**2c**	52
3	1:2.5:50	8 (days)	20	**2c**	43
				3c	25
4	1:2.5:50	6.5	50	**2c**	48
				3c	9
5	1:3:10 [b]	22	50	**2d**	46
				3d	19
6	1:3:5	2	50	**2d**	47
				3d	34

[a] *N*-Methylmorpholinium butyrate instead of the triethylammonium salt. [b] Chloroform was added (see Experimental).

potassium acetate under the same conditions used by Webber and co-workers [5a], also in our case afforded mixtures of Δ^2 (prevalent) and Δ^3-isomers. Other reactions were performed in acetonitrile solutions using triethylammonium acetate, where, at difference from potassium acetate, the carboxylate ion is supposed to be much less associated. Under these conditions, at different substrate **1** concentrations (0.5-1.5 *M*) and over a period of 20 hours, a mixture of Δ^3-**2a** and Δ^2-**3a** 3-acetoxymethylcephalosporin derivatives in 1:4 ratio was formed (see Scheme 1), along with 5% of methyl 3-bromomethyl-7-phthalimido-2-cephem-4-carboxylate, this product deriving from the substrate isomerization. The overall recovery was 75% at a low substrate concentration, but it was reduced (44%) at higher concentrations: the formation of highly polar side products was in fact increased (tlc analysis). No appreciable solvent influence (benzene, methylene chloride, tetrahydrofuran or hexamethylphosphoramide used instead of acetonitrile) was observed.

As to the counterion influence, when the reaction was carried out with tetrabutylammonium acetate, the reaction rate was dramatically increased (the substrate was completely converted after 10 minutes) but the reaction mixture composition was roughly the same as when the triethylammonium salt was used, the Δ^2-acetoxymethyl-derivative **3a** being the most prominent reaction product. Silver and mercury acetates in acetonitrile solution gave only the 3-cephem **2a**, although in very low yields (30% and 14% respectively).

When variable, small amounts of acetic acid were added to the reaction mixture (triethylammonium acetate as the reagent) a sharp decrease in the reaction rate was observed. By monitoring the reaction time (tlc analysis), the sole formation of the Δ^3-acetoxymethyl-derivative **2a** was observed at the beginning. Subsequently, the appearance and the relative rise of the Δ^2-isomer **3a** was found. A small amount of methyl 3-bromomethyl-7-phthalimido-2-cephem-4-carboxylate was revealed since the formation of the Δ^2-acetoxymethyl-derivative **3a**. These observations can be rationalized by considering possible substrate and product isomerizations, and taking into account the higher reactivity of the Δ^2-bromomethyl derivatives with respect to that of the Δ^3-isomers [1a]. Separate experiments showed that, actually, the Δ^3-acetoxymethyl deriva-

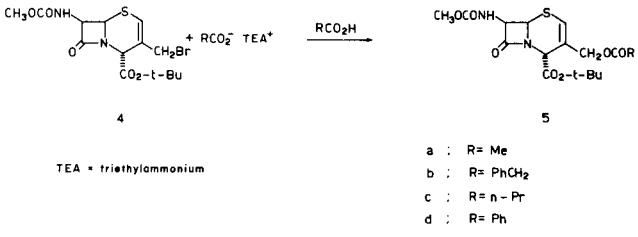
tive **2a** undergoes partial isomerization under the reaction conditions.

When the substitution reaction was carried out at room temperature in acetic acid as solvent, no isomerization was observed with acetates of different cations (see Table 1), the best results being obtained with triethylammonium acetate (entry 1) affording the 3-cephem **2a** in 91% yield. No reaction took place just dissolving the substrate in acetic acid.

It is worth pointing out that this reaction, combined with the Kukolja penam nucleus enlargement [1a,8] and Koppel bromination reaction of the 3'-position of the cephem nucleus [1a,6,9] provides for the first time a high yielding conversion of penicillin (6-APA) to 7-aminocephalosporanic acid (7-ACA) derivatives.

When the reaction with triethylammonium acetate was carried out at 50° (entry 2) in order to speed up the reaction rate, no significant decrease in the yield was observed. Tetrabutylammonium acetate (entry 3) gave poorer conversion with respect to the triethylammonium salt but the yield was higher at higher substrate dilution (entry 4). Hexylammonium and potassium acetates (entries 5 and 6) were also used but the reaction rates and yields were low.

Salts of different acids were also tried (see Scheme 1 and Table 2). When triethylammonium phenylacetate was added to phenylacetic acid (molar ratio 1:3) a liquid mixture was obtained in which a reasonable amount of the substrate was soluble at 50°. By operating under these conditions (entry 1) the Δ^3-phenylacetoxymethyl-derivative **2b**, free of the Δ^2-isomer, was obtained in 78% yield. Butyric acid was reacted as *N*-methylmorpholinium salt, using the acid itself as the reaction medium (entry 2). It gave the corresponding acyloxy-3-cephem derivative **2c** in 53% yield. On carrying out the same reaction using triethylammonium butyrate (entry 3), 25% of the Δ^2-butyrate **3c** was also formed, as well as 43% of the Δ^3-butyrate **2c**. Benzoic acid, under similar conditions (entry 5 and 6), failed to give a single product, variable amounts of Δ^2-derivative **3d** being always present in the reaction mixtures (see also Experimental).



Scheme 2

Displacement Reactions on *t*-Butyl 3-Bromomethyl-7-methoxycarbonylamino-2-cephem-4-carboxylate **4**.

The synthesis of the starting material **4** has already been described [1a].

In a preliminary experiment, carried out with 2 equivalents of triethylammonium acetate in benzene solution at room temperature, the substrate reacted over a period of 24 hours, giving a mixture of Δ^2- and Δ^3-derivatives. When the reaction was carried out with triethylammonium or hexylammonium acetate, in the presence of a small amount (0.1 equivalents) of acetic acid, no isomerization was observed according to the lack in the pmr spectrum of the crude reaction mixture of -CH_2-S- signals. The same reaction was also performed with salts of different acids, always in the presence of the free acid (0.1:1 molar ratio with the substrate): all the products were free (pmr spectra of the crude materials) from the isomeric Δ^3-materials and yields ranged from 35 to 65% (see experimental).

The substitution reaction was finally carried out with triethylammonium acetate in acetic acid as the solvent and the Δ^2-acetoxymethyl derivative **5a** was obtained in 90% yield (see Scheme 2). Potassium acetate gave similar results.

Owing to the high reactivity of the 3-bromomethyl-2-cephem-derivatives compared to that of the Δ^3-isomers [1a,10] it was easy to extend these reactions to other carboxylate salts (see Scheme 2). In the case of butyric acid, it was possible to reproduce the same conditions used in the reaction with triethylammonium acetate. Phenylacetic and benzoic acids were also reacted as triethylammonium salts, using large excess of the free acids as co-sovents in acetonitrile solution. Results are reported in Table 3.

Table 3

Conversion of the Bromomethyl-2-cephem **4** into Derivatives **5a-d** by Means of Triethylammonium Carboxylates at the Presence of the Corresponding Acids at Room Temperature Over a Period of 10 hours

Entry	Solvent	Product	Yields (%)
1	$MeCO_2H$	**5a**	90
2	$PhCH_2CO_2H/CH_3CN$	**5b**	85
3	*n*-pr-CO_2H	**5c**	97
4	$PhCO_2H/CH_3CN$	**5d**	93

Discussion.

The data presented above show that the bromine replacement in high yields by acyloxy residues is only possible within a narrow range of conditions. Side reactions which lower the yields are of two types: i) isomerization of the substrates as well as of the products giving rise to an equilibrium mixture ($\Delta^2 \rightleftharpoons \Delta^3$) of compounds, and ii) degradation of the substrates and/or of the products, as shown by the formation of polar products (tlc analysis), reasonably due to the cleavage of the β-lactam ring.

The reaction of the latter type can be partially controlled by lowering the substrate concentration and by using salts of weak bases. In this respect triethylammonium salts

give better results as compared to other salts.

The isomerization reaction appears to be easily controlled in the case of the Δ^2-isomers, since a slight free acid excess prevents this side reaction. This is not the case of the Δ^3-analogues. In fact any added solvent promotes isomerization and mixture of Δ^2- and Δ^3-isomers are obtained, unless the reaction is carried out in the acid itself as the reaction medium. Under these conditions the reaction generally gives high yields of the unisomerized product. However, it is not always possible to obtain these reaction conditions, since they require appropriate physical properties of the acid and of the corresponding salt.

We would like to add some more comments to this substitution reaction on the bromomethyl derivatives carried out by acyloxy residues. The cited side reactions are possible since the electrophilic reactivity of the bromide is low towards the acyloxy anions, especially in the case of the Δ^3-isomers. Moreover, the isomerization reaction exhibits requirements very similar to those of the substitution reaction, *i.e.* the nucleophile, acting as a base, can extract a proton at C-2, promoting the cephem isomerization. On the other hand, also the β-lactam ring opening is base promoted. Therefore it appears reasonable that all conditions which increase the nucleophilic activity of the reagent, also increase the isomerization process and the β-lactam ring opening. On the contrary, in acidic media, (where substitution generally occurs in high yields without double bond isomerization) the anion basicity seems to be reduced much more than its nucleophilicity.

Alternatively, the substitution of bromine without concurrent isomerization is also possible under conditions which favour the bromine departure (*e.g.* with silver [6] or mercury acetates), this increasing the rate of the desired reaction. However, under these conditions, the yields are drastically lowered because of the formation of very polar side products.

EXPERIMENTAL

The ir spectra were recorded on a Perkin-Elmer 257 instrument and pmr spectra on a Varian EM 360 60 MHz instrument. Low and high resolution (RP 10.000) mass spectra were recorded on an AEI MS 12 spectrometer and a VG ZAB 2F spectrometer respectively. Preparative and analytical tlcs were performed on pre-coated Merck-Kieselgel 60 F 254 plates. Visualization was performed with uv light and sulphuric acid plus heating. Column chromatography was performed using Merck-Kieselgel 60 (70-230 mesh ASTM) or Merck-Alumina aktiv basisch, Aktivitätsstufe I (70-230 mesh ASTM). All anhydrous solvents were distilled from phosphorus pentoxide, except triethylamine which was dried over potassium hydride and acetic acid which, before distillation, was stirred for one hour in the presence of 10% acetic anhydride.

All new compounds are supported by elemental analyses. Cephem derivatives **3c**, **5a**, **5b**, **5c** and **5d** were uncrystallizable oils, and satisfactory elemental analyses could not be obtained; attempted high vacuum distilation resulted in decomposition of the material. Their identity was supported by adequate ir, nmr and high resolution mass spectral data; purity was checked by tlc and confirmed by pmr spectroscopy.

Preparation of the Organic Salts.

Unless otherwise reported the organic salts used have been prepared as follows:

Tetrabutylammonium Acetate.

The title compound was prepared *via* a modified procedure of the literature method [11]. A mixture of tetrabutylammonium iodide (738 mg, 2 mmoles) and silver acetate (334 mg, 2 mmoles) in anhydrous methanol (5 ml) was stirred for 30 minutes at 40°. Filtration of the precipitate and methanol removal gave a gummy solid which was dissolved in dry ethyl acetate and treated with charcoal. After filtration, the solution was evaporated do dryness, the solid residue was dissolved with anhydrous benzene and then cooled to -80° (dry ice-acetone bath); solvent removal at this temperature under vacuum (0.2 mm Hg) afforded a white, very hygroscopic powdery material (mp 90-92°) which was stored in a desiccator.

Triethylammonium Benzoate.

Benzoic acid (124 mg, 1 mmole) and triethylamine (3 ml) were mixed with stirring at 0°. The white crystalline solid which formed was filtered off and stored in a desiccator.

n-Hexylammonium Acetate.

Acetic acid (0.3 ml, 5 mmoles) and *n*-hexylamine (0.6 ml, 6 mmoles) were mixed with stirring at 0°. The resulting viscous liquid was cooled to -80° for 10 minutes whereupon a white crystalline solid formed. The hydroscopic salt was stored in a desiccator.

n-Hexylammonium Benzoate.

Benzoic acid (132 mg, 1 mmole) and *n*-hexylamine (0.132 ml, 1 mmole) were dissolved in anhydrous benzene (3 ml). Evaporation of the solvent yielded white crystals which were stored in a desiccator.

Tetrabutylammonium Benzoate.

A solution of sodium benzoate (144 mg, 1 mmole) in anhydrous methanol (6 ml) was added, with stirring, to a solution of tetrabutylammonium chloride (278 mg, 1 mmole) in methanol (3 ml). The opalescent solution was evaporated to dryness; the residue dissolved in anhydrous benzene and filtered under vacuum. Solvent removal afforded a crystalline hygroscopic material (235 mg) which was stored in a desiccator.

Substitution Reaction of **1** with the Acetate Anion in Acetonitrile.

In a typical experiment the cephem **1** (200 mg, 0.44 mmole) was dissolved in anhydrous acetonitrile (0.3 mg, substrate concentration 1.5 *M*). The solution was cooled to -60° (dry ice-chloroform bath) and triethylammonium acetate (1.9 ml of 1:1 acetic acid-triethylamine) was added. The temperature was allowed to rise to -17°; the reaction mixture was then kept at this temperature in a freezer for 19 hours. After quenching with cold 0.1 *M* hydrochloric acid (final *p*H 3), the reaction mixture was extracted with chloroform, the organic layer was dried over sodium sulphate and evaporated to dryness. Separation by preparative tlc (benzene-ethyl acetate (8/2)) afforded **1** (mg 8, 4%), **2a** (mg 13, 7%), **3a** (mg 48, 25%) and methyl 3-bromomethyl-7-phthalimido-2-cephem-4-carboxylate (mg 16, 8%). Identification of the products was based on their spectroscopic properties.

Methyl 3-Acetoxymethyl-7-phthalimido-3-cephem-4-carboxylate (**2a**).

Crystallization from 95% ethyl alcohol gave pure **2a**, mp 141-142°; ir (potassium bromide): 1795 (lactam C=O), 1720 cm^{-1} (esters and imide C=O); pmr (deuteriochloroform): δ 2.07 (s, $OCOCH_3$, 3H), 3.12-3.72 (q, H-2, 2H, J = 16 Hz), 3.83 (s, CO_2CH_3, 3H), 5.05 (d, H-6, 1H, J = 5 Hz), 4.65-5.30 (q, H-3', 2H, J = 13 Hz), 5.73 (d, H-7, 1H, J = 5 Hz), 7.4-7.9 (m, aromatic 4H); ms: 416 (M^+, 7), 356 (8), 230 (55), 187 (100), 170 (58), 160 (38), 132 (69).

Anal. Calcd. for $C_{19}H_{16}N_2O_7S$: C, 54.81; H, 3.87; N, 6.73. Found: C, 54.45; H, 3.97; N, 6.42.

Methyl 3-Acetoxymethyl-7-phthalimido-2-cephem-4-carboxylate (**3a**).

Crystallization from ethyl acetate gave pure **3a**, mp 222-223°; ir

(nujol): 1783 (lactam C=O), 1740 (C-4 ester C=O), 1720 cm^{-1} (C-3' ester and imide C=O); pmr (deuteriochloroform): δ 2.07 (s, $OCOCH_3$, 3H), 3.83 (s, CO_2CH_3, 3H), 4.70 (s, H-3', 2H), 5.12 (s, H-4, 1H), 5.38 (d, H-6, 1H, J = 4 Hz), 5.71 (d, H-7, 1H, J = 4 Hz), 6.40 (s, H-2, 1-H), 7.6-7.9 (m, aromatic 4H); ms: 416 (M^+, 11), 357 (5), 230 (20), 187 (100), 170 (53), 160 (27), 132 (67).

Anal. Calcd. for $C_{19}H_{16}N_2O_7S$: C, 54.81; H, 3.87; N, 6.73. Found: C, 54.54; H, 3.99; N, 6.71.

Methyl 3-Bromomethyl-7-phthalimido-2-cephem-4-carboxylate.

The bromide was obtained as an unstable oil [9] which was identified on the basis of its pmr spectral characteristics; pmr (deuteriochloroform): δ 3.85 (s, CO_2CH_3, 3H), 4.25 (q, H-3', 2H, J = 10 Hz), 5.3 (broad, H-4, 1H), 5.40 (d, H-6, 1H, J = 4 Hz), 5.70 (d, H-7, 1H, J = 4 Hz), 6.45 (s, H-2, 1H), 7.7-8.0 (m, aromatic 4H).

The other reactions using different concentrations of **1** (200 mg, 0.44 mmole) in acetonitrile (0.5 and 0.7 *M*) were performed following the same procedure described above and gave **1** (mg 24, 12%), **2a** (mg 18, 10%), **3a** (mg 75, 41%), methyl 3-bromomethyl-7-phthalimido-2-cephem-4-carboxylate (mg 22, 12%) in the former case and **1** (mg 20, 10%), **2a** (mg 16.5, 9%), **3a** (mg 71.5, 39%), methyl 3-bromomethyl-7-phthalimido-2-cephem-4-carboxylate (mg 20, 10%) in the latter one.

The reaction using tetrabutylammonium acetate was carried out in the same way as that described using triethylammonium acetate. The reaction work-up (as reported before, including preparative tlc separation) after 10 minutes afforded **1** (mg 10, 5%), **2a** (mg 18.5, 10%), **3a** (mg 88, 48%) and methyl 3-bromomethyl-7-phthalimido-4-carboxylate (mg 10, 5%).

Reactions with silver or mercury acetate were run at room temperature, by using solvent, substrate and reagent concentrations identical to those described for triethylammonium acetate. Work-up after 90 minutes and preparative tlc separation afforded **1** (mg 16, 8%) and **2a** (mg 55, 30%) in the case of silver acetate and **1** (mg 8, 4%) and **2a** (mg 25.5, 14%) in the case of mercury acetate.

Reaction of **1** with Triethylammonium Acetate in Acetic Acid.

The cephem **1** (100 mg, 0.228 mmole) was dissolved in anhydrous acetic acid (1 ml) and anhydrous triethylamine (0.093 ml) was added. When the reaction was complete (7 days, at room temperature), the reaction mixture was poured into a becker containing an aqueous suspension of sodium bicarbonate (1.25 g in 5 ml of water) and then kept in a refrigerator for 3 hours. Precipitated methyl 3-acetoxymethyl-7-phthalimido-3-cephem-4-carboxylate **2a** was collected as white crystals (80 mg) by filtration. The mother liquors were extracted with chloroform, the organic layer was dried over sodium sulphate and evaporated to dryness. Preparative tlc purification (chloroform-ethylacetate (8/2)) afforded more of the same compound **2a** (6.5 mg): total yield 86.5 mg (91%). The same reaction was performed under different conditions of temperature and acetate counterion: yields and reaction conditions are reported in Table 1.

Reaction of **1** with Triethylammonium Phenylacetate in Phenylacetic Acid.

A mixture of the cephem **1** (100 mg, 0.228 mmole), phenylacetic acid (316 mg) and anhydrous triethylamine (0.08 mg) was stirred at 50° for 3 hours. When the reaction was finished (tlc: chloroform-ethyl acetate (8/2)) the mixture was poured into a cold saturated aqueous solution of sodium bicarbonate (10 ml) and extracted with chloroform. The organic extracts were washed with an aqueous solution of sodium bicarbonate and brine, then dried over sodium sulphate and evaporated to dryness. The residue was purified by preparative tlc (chloroform-ethyl acetate (8/2)) to afford methyl 3-phenylacetoxymethyl-7-phthalimido-3-cephem-4-carboxylate **3b** (mg 88, 78%) as pure material. Crystallization from 95% ethyl alcohol afforded white crystals, mp 128-129°; ir (potassium bromide) 1795 (lactam C=O), 1730 cm^{-1} (esters and imide C=O); pmr (deuteriochloroform): δ 2.96-3.56 (q, H-2, 2H, J = 16 Hz), 3.60 (s, $-OCOCH_2-$, 2H), 3.80 (s, CO_2CH_3, 3H), 4.96 (d, H-6, 1H, J = 5 Hz), 4.70-5.36 (q, H-3', 2H, J = 13 Hz), 5.70 (d, H-7, 1H, J = 5 Hz), 7.14 (s, aromatic 5H), 7.4-7.8 (m, phthalimido, 4H); ms: 492 (M^+, 6), 306 (16), 246 (13), 187 (48), 170 (17), 160 (17), 132 (26), 91 (100).

Anal. Calcd. for $C_{25}H_{20}N_2O_7S$: C, 60.97; H, 4.09; N, 5.69. Found: C, 60.70; H, 3.98; N, 5.44.

Reaction of **1** with *N*-Methylmorpholinium Butyrate in *n*-Butyric Acid.

To a solution of distilled *N*-methylmorpholine (0.016 ml) and distilled *n*-butyric acid (0.25 ml) the cephem **1** (100 mg, 0.228 mmole) was added. The reaction mixture was left 12 hours at room temperature and then poured into a cold solution of saturated aqueous sodium bicarbonate (10 ml) and extracted with chloroform. The organic extract was washed with saturated aqueous sodium bicarbonate, brine and dried over sodium sulphate. Evaporation to dryness and separation by preparative tlc (chloroform-ethyl acetate (8/2)) gave methyl 3-butyroxymethyl-7-phthalimido-3-cephem-4-carboxylate **2c** (54 mg, 53%) and methyl 3-butyroxymethyl-7-phthalimido-2-cephem-4-carboxylate **3c** (2 mg, 8%).

Product **2c** was crystallized from *n*-hexane, mp 51-52°; ir (chloroform): 1795 (lactam C=O), 1730 cm^{-1} (esters and imide C=O); pmr (deuteriochloroform): δ 0.93 (t, $-CH_2-CH_3$, 3H, J = 8 Hz), 1.20-1.80 (m, $-CH_2-CH_3$, 2H), 2.36 (t, $-OCOCH_2-$, 2H, J = 7 Hz), 3.14-3.80 (q, H-2, 2H, J = 16 Hz), 3.93 (s, CO_2CH_3, 3H), 4.80-5.02 (q, H-3', 2H, J = 13 Hz), 5.16 (d, H-6, 1H, J = 5 Hz), 5.84 (d, H-7, 1H, J = 5 Hz), 7.5-7.8 (m, aromatic 4H); ms: 444 (M^+, 11), 356 (10), 258 (37), 198 (21), 187 (100), 170 (28).

Anal. Calcd. for $C_{21}H_{20}N_2O_7S$: C, 56.75; H, 4.54; N, 6.30. Found: C, 56.72; H, 4.55; N, 6.28.

Product **3c** had ir (potassium bromide): 1783 (lactam C=O), 1740 (C-4 ester C=O), 1720 cm^{-1} (C-3' ester and imide C=O); pmr (deuteriochloroform): δ 0.96 (t, $-CH_2-CH_3$, 3H, J = 8 Hz), 1.20-1.80 (m, $-CH_2-CH_3$, 2H), 2.33 (t, $-OCOCH_2-$, 2H, J = 7 Hz), 3.76 (s, CO_2CH_3, 3H), 4.60 (s, H-3', 2H), 5.10 (s, H-4, 1H), 5.30 (d, H-6, 1H, J = 4 Hz), 5.60 (s, H-7, 1H, J = 4 Hz), 6.26 (s, H-2, 1H), 7.40-7.80 (m, aromatic 4H); ms: 444 (M^+, 10), 357 (5), 258 (16), 198 (18), 187 (67), 170 (18), 160 (100), 132 (39); Found: M^+, 444.0989; $C_{21}H_{20}N_2O_7S$ requires M^+, 444.0990. The same reaction was made at 50° for 6 hours and half and for 8 days at room temperature using triethylammonium as butyrate counterion, yields and reaction conditions are reported in Table 2.

Reaction of **1** with Triethylammonium Benzoate in Benzoic Acid/Chloroform.

To a mixture of triethylammonium benzoate (154.5 mg, 0.686 mmole), benzoic acid (284 mg, 2.28 mmoles) and anhydrous chloroform (0.57 ml), the cephem **1** (100 mg, 0.228 mmole) was added. The reaction was complete after 22 hours at 50°. The reaction mixture was poured into a cold saturated aqueous sodium bicarbonate solution (10 ml) and extracted with chloroform. The organic extracts were washed with saturated aqueous sodium bicarbonate solution, dried over sodium sulphate and evaporated to dryness. The residue was chromatographed by preparative tlc (chloroform-ethyl acetate (8/2)) to give methyl 3-benzoyloxymethyl-7-phthalimido-3-cephem-4-carboxylate **2d** (50 mg) in a yield of 46% and methyl 3-benzoyloxymethyl-7-phthalimido-2-cephem-4-carboxylate **3d** (21 mg, 19%).

Product **2d** crystallized from 95% ethyl alcohol, mp 164-165°; ir (potassium bromide): 1795 (lactam C=O), 1725 cm^{-1} (esters and imide C=O); pmr (deuteriochloroform): δ 3.27-3.80 (q, H-2, 2H, J = 16 Hz), 3.83 (s, CO_2CH_3, 3H), 5.03 (d, H-6, 1H, J = 5 Hz), 4.96-5.63 (q, H-3', 2H, J = 13 Hz), 5.71 (d, H-7, 1H, J = 5 Hz), 7.20-8.00 (m, aromatic 9H); ms: 478 (M^+, 8), 232 (7), 187 (27), 160 (15), 132 (17), 105 (100).

Anal. Calcd. for $C_{29}H_{18}N_2O_7S$: C, 60.25; H, 3.79; N, 5.86. Found: C, 60.20; H, 3.71; N, 5.73.

Product **3d** crystallized from ethyl acetate and had mp 205-206°; ir (potassium bromide): 1783 (lactam C=O), 1725 cm^{-1} (esters and imide C=O); pmr (deuteriochloroform): δ 3.68 (s, CO_2CH_3, 3H), 4.85 (s, H-3', 2H), 5.16 (s, H-4, 1H), 5.33 (d, H-6, 1H, J = 4 Hz), 5.61 (d, H-7, 1H, J = 4 Hz), 6.36 (s, H-2, 1H), 7.20-8.00 (m, aromatic 9H); ms: 478 (M^+, 9), 328 (8), 292 (13), 232 (17), 187 (73), 105 (100).

Anal. Calcd. for $C_{24}H_{18}N_2O_7S$: C, 60.25; H, 3.79; N, 5.86. Found: C, 60.15; H, 3.70; N, 5.75.

The reaction was repeated at 50° without any added solvent: for the reaction conditions and yields see Table 2, entry 6.

Reaction of **4** with Carboxylate Salts in the Presence of the Corresponding Carboxylic Acids.

In a typical experiment, **4** (100 mg, 0.24 mmole) was dissolved in anhydrous benzene (2 ml), the appropriate carboxylate salt (2 equivalents) (see the following table), and the corresponding carboxylic acid (0.1 equivalents) were then added. After 24 hours at room temperature, the crystalline precipitate formed in the course of the reaction was dissolved upon adding cold water; the reaction mixture was then extracted with ethyl acetate (×3). The combined organic extracts were washed with a 5% sodium bicarbonate solution, water, brine and dried over sodium sulphate. The crude product obtained after solvent evaporation was filtered on basic alumina in order to remove the excess of carboxylic acid. Preparative tlc purification afforded the substitution products. (Eluents: *n*-hexane-ethyl acetate (6/4) for **5a** and *n*-hexane-diethylether (1/1) for **5b**, **5c** and **5d**). Adopting this procedure the following products (as colourless oils), were obtained (for the yields see the following table).

t-Butyl 3-Acetoxymethyl-7-methoxycarbonylamino-2-cephem-4-carboxylate (**5a**).

Compound **5a** had ir (chloroform): 3450 (N-H), 1780 (lactam C=O), 1750-1720 cm^{-1} (esters and amide C=O); pmr (deuteriochloroform): δ 1.53 (s, $(CH_3)_3C$, 9H), 2.08 (s, $OCOCH_3$, 3H), 3.70 (s, CH_3OCO, 3H), 4.65 (d, H-3', 2H, J = 6 Hz), 4.92 (s, H-4, 1H), 5.30 (d, H-6, 1H, J = 4 Hz), 5.49 (q, H-7, 1H, J = 4 Hz), 6.40 (broad, H-2, 1H); ms: 386 (M^+, 7), 272 (13), 217 (60), 197 (20), 158 (10), 156 (66), 57 (100); Found: M^+, 386.1147; $C_{16}H_{22}N_2O_7S$ requires M^+ 386.1148.

t-Butyl 3-Phenylacetoxymethyl-7-methoxycarbonylamino-2-cephem-4-carboxylate (**5b**).

This compound had ir (chloroform): 3450 (N-H), 1780 (lactam C=O), 1750-1730 (esters and amide C=O); pmr (deuteriochloroform): δ 1.42 (s, $(CH_3)_3C$, 9H), 3.52 (s, $OCOCH_2$-, 2H), 3.62 (s, CH_3OCO, 3H), 4.66 (d, H-3', 2H, J = 6 Hz), 4.77 (s, H-4, 1H), 5.17 (d, H-6, 1H, J = 4 Hz); 5.47 (q, H-7, 1H, J = 4 Hz), 6.20 (broad, H-2, 1H), 7.15 (s, aromatic 5H); ms: 462 (M^+, 3), 365 (20), 291 (14), 258 (11), 202 (82), 197 (38), 156 (85), 91 (20), 57 (100); Found: M^+, 462.1450; $C_{22}H_{26}N_2O_7S$: requires M^+, 462.1460.

t-Butyl 3-Butyroxymethyl-7-methoxycarbonylamino-2-cephem-4-carboxylate **5c**.

This compound had ir (chloroform): 3450 (N-H), 175 (lactam C=O), 1740-1720 cm^{-1} (esters and amide C=O); pmr (deuteriochloroform): δ 0.92 (t, $-CH_2CH_3$, 3H, J = 8 Hz), 1.47 (s, $(CH_3)_3C$-, 9H), 1.8-1.6 (m, $-CH_2-CH_3$, 2H), 2.27 (t, $-OCOCH_2$-, 2H, J = 7 Hz), 3.67 (s, CH_3OCO, 3H), 4.57 (d, H-3', 2H, J = 6 Hz), 4.85 (s, H-4, 1H), 5.15 (d, H-6, 1H, J = 4 Hz), 5.57 (q, H-7, 1H, J = 4 Hz), 6.27 (broad, H-2, 1H); ms 414 (M^+, 9), 300 (22), 244 (72), 158 (55), 156 (88), 57 (100); Found: M^+, 414.1459; $C_{18}H_{26}N_2O_7S$ requires M^+, 414.1461.

t-Butyl 3-Benzoyloxymethyl-7-methoxycarbonylamino-2-cephem-4-carboxylate (**5d**).

This compound had ir (chloroform): 3450 (N-H), 1750 (lactam C=O), 1745-1720 cm^{-1} (esters and amide (C=O); pmr (deuteriochloroform): δ 1.45 (s, $(CH_3)_3C$, 9H), 3.67 (s, CH_3OCO, 3H), 4.64 (s, H-3', 2H), 4.89 (s, H-4, 1H), 5.20 (d, H-6, 1H, J = 4 Hz), 5.55 (q, H-7, 1H, J = 4 Hz), 6.45 (broad, H-2, 1H), 7.4-8.0 (m, aromatic 5 H); ms: 448 (M^+, 8), 334 (20), 278 (76), 158 (38), 156 (84), 105 (88), 57 (100); Found: M^+, 448.1313; $C_{21}H_{24}N_2O_7S$ requires M^+, 448.1304.

Product	Salt	Yield (%)
5a	triethylammonium acetate	63
5a	hexylammonium acetate	65
5b	triethylammonium phenylacetate	57
5c	triethylammonium butyrate	35
5d	triethylammonium benzoate	50
5d	hexylammonium benzoate	52
5d	tetrabutylammonium benzoate	58

Reaction of **4** with Triethylammonium Acetate in Acetic Acid.

To a solution of **4** (58 mg, 0.14 mmole) in acetic acid (0.5 ml) triethylamine (0.04 ml, 0.28 mmole) was added. The formation of a crystalline precipitate was immediately observed, its amount increasing during the time. Analysis (tlc, *n*-hexane-ethyl acetate (6/4)) showed that the reaction was complete after 10 hours at room temperature. The reaction mixture was poured into cold saturated sodium bicarbonate (10 ml) and extracted with ethyl acetate. The organic extracts were washed with water, 5% sodium bicarbonate, brine and dried over sodium sulphate. The crude 3-acetoxymethyl-2-cephem derivative **5a** obtained after solvent removal under reduced pressure was purified on preparative tlc with three chromatographic runs (*n*-hexane-ethyl acetate (6/4)). The yield of **5a** was 90% (48 mg).

Reaction of **4** with Potassium Acetate in Acetic Acid.

To a solution of **4** (97 mg, 0.24 mmole) in acetic acid (1 ml), potassium acetate (40 mg, 0.48 mmole) was added. The reaction mixture was allowed to stay at room temperature for 10 hours. After the work-up procedure and purification described above, 80 mg of **5a** were obtained (90%).

Reaction of **4** with Triethylammonium Phenylacetate in Phenylacetic Acid/Acetonitrile.

Cephem **4** (114 mg, 0.2 mmole) and phenylacetic acid (190 mg, 1.4 mmoles) were dissolved in anhydrous acetonitrile (2 ml) and triethylamine (0.115 ml, 0.84 mmole) was added. After 10 hours at room temperature, the resulting precipitate was filtered off under vacuum and the filtrate passed through basic alumina eluting with ethyl acetate in order to remove the excess of phenylacetic acid. Purification of the crude product by preparative tlc (four runs *n*-hexane-diethyl ether (1/1)) yielded 111 mg of **5b** (85%).

Reaction of **4** with Triethylammonium Butyrate in Butyric Acid.

To a solution of **4** (150 mg, 0.37 mmole) in butyric acid (0.33 ml, 3.7 mmoles), triethylamine (0.15 ml, 1.1 mmoles) were added. The reaction mixture was allowed to stay at room temperature for 10 hours. The resulting precipitate was filtered off under vacuum and the mother liquor passed through basic alumina eluting with ethylacetate in order to remove the excess of butyric acid. The crude 3-butyroxymethyl-2-cephem derivative was purified by preparative tlc chromatography (four runs *n*-hexane-diethyl ether (6/4)). The yield of **5c** was 97% (149 mg).

Reaction of **4** with Triethylammonium Benzoate in Benzoic Acid/Acetonitrile.

Cephem **4** (83 mg, 0.2 mmole) and benzoic acid (220 mg, 2 mmoles) were dissolved in anhydrous acetonitrile (1 ml) and triethylamine (0.12 ml, 0.6 mmole) was added. The reaction mixture was allowed to stay for 10 hours at room temperature. The resulting precipitate was filtered off under vacuum and the filtrate passed through basic alumina eluting with ethyl acetate. Purification of the crude product by preparative tlc (*n*-hexane-diethyl ether (1/1)) yielded 84 mg of **5d** (93%).

Acknowledgements.

Financial support of this work by the Italian CNR (Consiglio Nazionale delle Ricerche: Progetto Finalizzato Chimica Fine e Secondaria) is acknowledged. We also thank the ACRAF Research Laboratories, Rome, for providing elemental analyses. We also wish to thank Miss Concetta Pisani for carrying out some of the experiments described above.

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