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Cephalosporanic Acid 7β-Alkylideneammonio Salts

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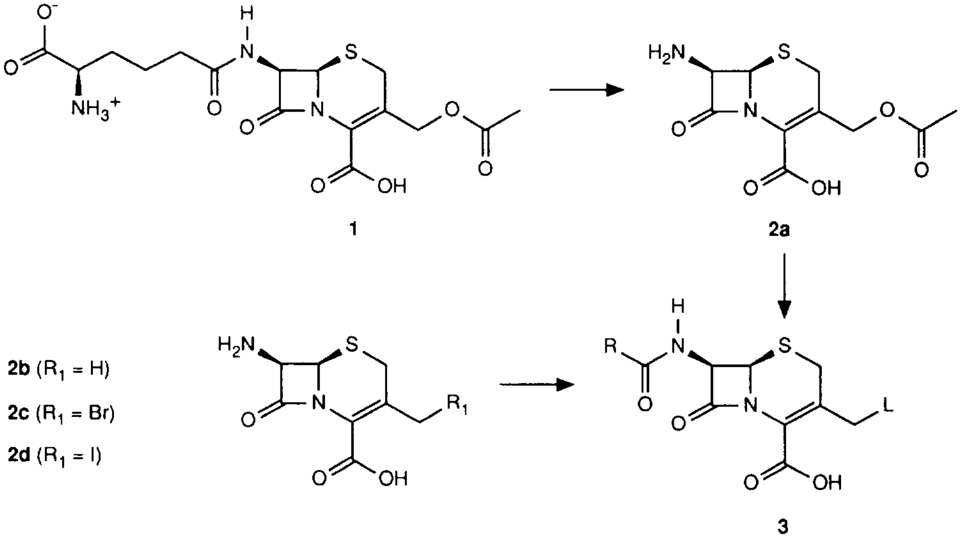
Abstract: Reaction of 7β-aminocephalosporanic acid derivatives **2** with ketones under acidic conditions leads to the unprecedented formation of alkylideneammonio salts **4**. 3'-Halogenated cephalosporanic acid derivatives bearing an unprotected carboxylgroup are normally considered to be sensitive intermediates that can easily undergo lactonization. However, these compounds are suprisingly stable when transformed into their 7β-alkylideneammonio salts and can thus be elegantly used as versatile intermediates in cephalosporin chemistry.

INTRODUCTION

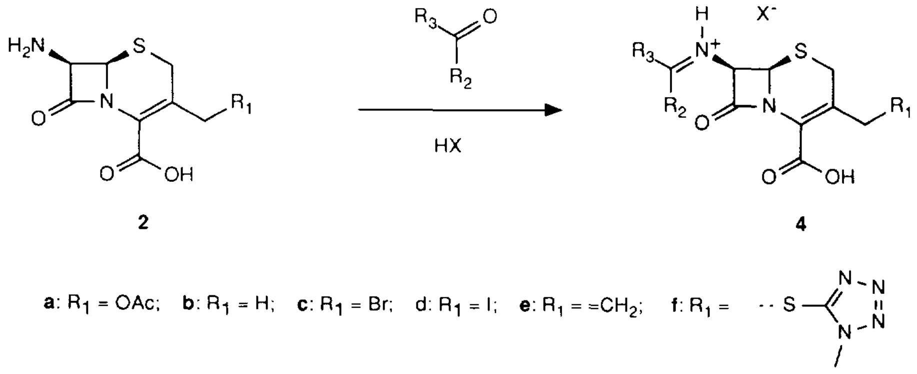
Many of the semi-synthetic cephalosporin antibiotics are equipped with a leaving group L at position 3' (**3** in Scheme 1). Typically, this leaving group is acetate, thioalkyl, or quaternary substituted nitrogen. The presence of this group facilitates nucleophilic attack at the β-lactam carbonyl,[1] the key step in the disruption of the bacterial cell wall assembly.[2]



Scheme 1

The synthesis of the majority of the above mentioned antibiotics involves introduction of the leaving group either by direct displacement of the acetoxy group in fermentatively produced Cephalosporin C (**1**), or its derivative 7β-aminocephalosporanic acid (7-ACA, **2a**), or through substitution of a synthetically derived 3'-halogen derivative (**2c**, **2d**), depending on the chemical nature of the incoming substituent. However, 3'-activated cephalosporins having an unprotected carboxyl function are prone to undergo lactonization under acidic conditions.[3] This phenomenon is particularly pronounced in 3'-halogenated derivatives, and therefore intermediates like **2c** and **2d** are prepared *in situ* and converted into the desired product without further purification.

In the course of a research programme directed at the synthesis of halogenated intermediates for ß-lactam antibiotics, we have found an unprecedented reaction leading to crystalline stable 3'-halogenated cephalosporins. In general, we established that 7-ACA and derivatives of 7β-amino-3'-desacetoxycephalosporanic acid (7-ADCA, **2b**) can react under acidic conditions with (cyclo)ketones to form (cyclo)alkylideneammonio salts (**4** in Scheme 2).



Scheme 2

RESULTS AND DISCUSSION

3'-Halogen substituted cephalosporins are important intermediates for the industrial production of antibiotics.[4] From an economic point of view, intermediates having an unprotected carboxyl moiety would be favourable since ultimate deprotection can be avoided. Examples of unprotected intermediates are 3'-bromo-7-ADCA **2c** and 3'-iodo-7-ADCA **2d**. However, to our knowledge **2c** has not been isolated yet, whereas **2d** can indeed be prepared from 7-ACA using trimethylsilyl iodide[5] or sodium iodide and trifluoromethanesulphonic acid,[6] but isolation is hampered by product lability. The above mentioned intermediates could well be used for several types of conversions leading to a broad range of antibiotics,[7] including the Wittig-type formation of 3-alkenyl-7β-aminocephalosporins. [8] Particularly these 3-alkenyl substituted cephalosporins can only be obtained from halogenated precursors.

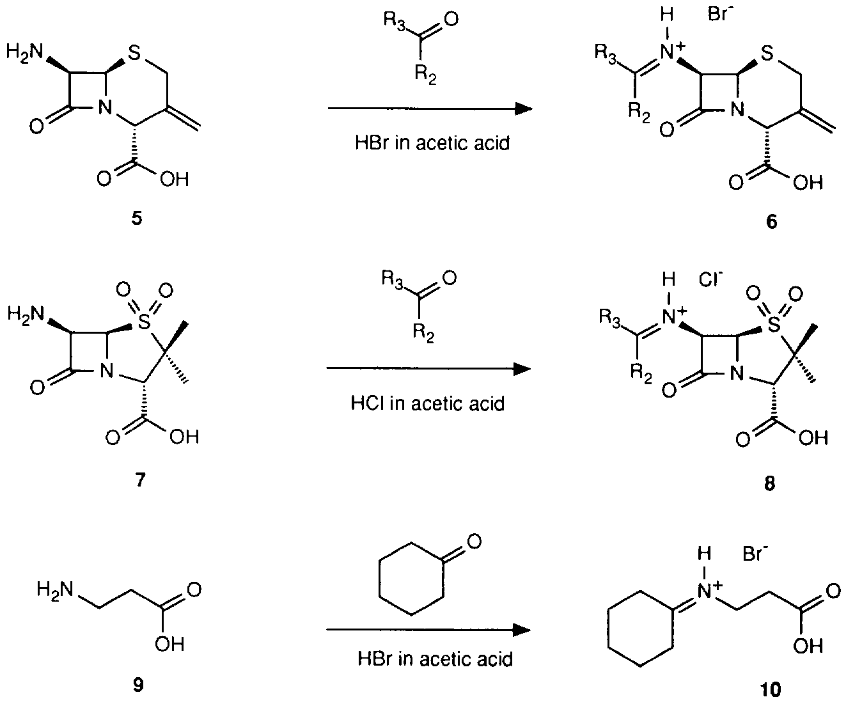
While investigating the synthesis of 3'-halogenated derivatives of 7-ADCA, we were confronted with product lactonization since unprotected carboxyl functionalities do not tolerate the presence of halogen γ to the carboxyl group. As a result of this phenomenon high yields could not be obtained and eventually a strategy was developed based on phenylacetyl protection of the amino group and *tert*-butyl protection of the carboxyl function.[4,9]

Table 1. Synthesis of Cephalosporin Derivatives.

Starting Material	Acid	Ketone	Product	R_2,R_3	X	Yield (%)
2a	Acetic acid satd. with HCl	Acetone	**4a**	CH_3,CH_3	Cl	97
2a	33% HBr in acetic acid	Acetone	**4a**	CH_3,CH_3	Br	91
2a	47% HBr in water	Acetone	**4a**	CH_3,CH_3	Br	78
2a	57% HI in water	Acetone	**4a**	CH_3,CH_3	I	34
2a	33% HBr in acetic acid	Cyclopentanone	**4a**	$-(CH_2)_4-$	Br	80
2a	37% HCl in water	Cyclohexanone	**4a**	$-(CH_2)_5-$	Cl	84
2a	47% HBr in water	Cyclohexanone	**4a**	$-(CH_2)_5-$	Br	92
2a	4% H_2SO_4 in acetic acid	Cyclohexanone	**4a**	$-(CH_2)_5-$	HSO_4	89
2b	Acetic acid satd. with HCl	Acetone	**4b**	CH_3,CH_3	Cl	95
2b	37% HCl in water	Acetone	**4b**	CH_3,CH_3	Cl	87
2b	47% HBr in water	Acetone	**4b**	CH_3,CH_3	Br	92
2b	47% HBr in water	Cyclohexanone	**4b**	$-(CH_2)_5-$	Br	67
2b	70% Perchloric acid in water	Cyclohexanone	**4b**	$-(CH_2)_5-$	ClO_4	71
2c[¶]	47% HBr in water	Acetone	**4c**	CH_3,CH_3	Br	85
2d	57% HI in water	Acetone	**4d**	CH_3,CH_3	I	76
2d	57% HI in water	Cyclohexanone	**4d**	$-(CH_2)_5-$	I	59
2d	Acetic acid satd. with HCl	Acetone	**4d**	CH_3,CH_3	Cl	71
2e	7% HCl in acetic acid	Acetone	**4e**	CH_3,CH_3	Cl	98
2e	33% HBr in acetic acid	Acetone	**4e**	CH_3,CH_3	Br	88
2e	47% HBr in water	Cyclohexanone	**4e**	$-(CH_2)_5-$	Br	70
2f	Acetic acid satd. with HCl	Acetone	**4f**	CH_3,CH_3	Cl	94
2f	33% HBr in acetic acid	Acetone	**4f**	CH_3,CH_3	Br	100
5	33% HBr in acetic acid	Acetone	**6**	CH_3,CH_3	Br	86
7	7% HCl in acetic acid	Acetone	**8**	CH_3,CH_3	Cl	85
9	13% HBr in acetic acid	Cyclohexanone	**10**	$-(CH_2)_5-$	Br	44

[¶] Obtained *in situ* from *tert*-butyl penicillin G-1-oxide[4]

An unexpected observation was made when penultimate experiments aimed at isolation of intermediate **2c** were conducted. A solution containing compound **2c**, obtained from penicillin G,[4] was treated with acetone and an aqueous solution of hydrogen bromide. Immediately, crystals were formed which were washed with acetone and isolated in high yield. IR and ^1H NMR analysis indicated the formation of a hitherto unknown compound resulting from acid-catalyzed condensation of acetone with the β-lactam amino group (Scheme 2). The scope of this reaction was successfully explored using different types of cephalosporins. First of all, 7-ACA (**2a**) and 7-ADCA (**2b**) were converted into their 7β-isopropylidene-ammonio salts, generally in high yields. Similarly, the vinyl substituted derivative **2e**, intermediate to the antibiotics Cefdinir and Cefixime, as well as the thio substituted derivative **2f**, intermediate to a wide range of antibiotics amongst which Cefamandole and Cefoperazone, were also smoothly derivatized. Finally, the exomethylene Cefaclor intermediate **6** (Scheme 3) was obtained from **5** using a 33% hydrogen bromide solution in acetic acid followed by the addition of acetone. Furthermore, this new method of protecting the aminogroup in β-lactams could be extended to cyclic ketones such as cyclopentanone and cyclohexanone as summarized in Table 1.[10,11]



Scheme 3

We then investigated the synthesis of 3'-iodinated derivatives of 7-ADCA, potentially powerful intermediates, however notorious for their intrinsic instability. The synthesis of **2d** is well documented, albeit that isolation leads to low yields.[5,6] We reasoned that the newly discovered ketone condensation could serve to prepare stable 3'-iodinated intermediates. Indeed, treatment of **2d** with hydrogen iodide in water in the presence of acetone or cyclohexanone led to the successful formation of derivatives **4d** (X = I). Likewise, **2d** was converted into **4d** (X = Cl) using hydrochloric acid in acetic acid.

The applicability of this newly found alkylideneammonio protection seems limited to cephalosporins, the more stable of the β-lactams. Extensive experimentation directed at converting penicillanic acid derivatives led to a single positive result in the case of 6β-aminopenicillanic acid-sulfone (**7** in Scheme 3), a β-lactam with an enhanced stability compared to 6β-aminopenicillanic acid itself. We were also interested in the behaviour of other compounds containing amino- and carboxyl groups, particularly amino acids. However, nearly all attempts to convert amino acids failed. In just one example we obtained a low yield (44%) conversion of the unnatural amino acid β-alanine (**9** in Scheme 3) into **10** using cyclohexanone in a 13% hydrogen bromide solution in acetic acid.

CONCLUSIONS

To the best of our knowledge, the formation of these (cyclo)alkylideneammonio carboxylic acid derivatives has not been described. The only other β-lactams referred to are those having an aldimine substituent obtained after reaction of a 7β-aminocephalosporin with a benzaldehyde derivative.[12]

We believe that 7β-alkylideneammonio derivatives may serve as versatile building blocks in cephalosporin chemistry. This is particularly the case with the new type of halogenated cephalosporins **4c** and **4d**. Preliminary experiments have indicated that these compounds are useful starting materials for various therapeutically useful antibiotics. For instance, 3'-thiosubstituted cephalosporins (*i.e.* Ceftriaxone) and derivatives containing a quarternary nitrogen at the 3'-position (*i.e.* Ceftazidime) were easily obtained from 7β-alkylideneammonio salts of halogenated cephalosporins.[10,11]

EXPERIMENTAL

Materials and methods

Acetic acid, acetone, acetonitrile, dichloromethane, diethylether and *N,N*-dimethylformamide were from Merck (*pro analysi*). Other chemicals were from Janssen Chimica. IR spectra were recorded on a Pye Unicam PU9714 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 360 MHz or a Bruker AMX 600 MHz instrument. Chemical shifts (δ) are in ppm relative to the internal standard TMS. Purities were determined using ^{1}H NMR using an internal reference.

*(6R,7R)-3-Acetoxymethyl-4-carboxy-7-isopropylideneammonioceph-3-em chloride (**4a**; $R_2=R_3=CH_3$; X=Cl)*

20.0 g (purity 90%; 70.5 mmoles) of (6*R*,7*R*)-3-acetoxymethyl-7-aminoceph-3-em-4-carboxylic acid was dissolved in 50 ml acetic acid saturated with hydrochloric acid. After 3 min, acetone (110 ml) was added and a precipitate was formed. After stirring for another 30 minutes and adding acetone (80 ml), the precipitate was filtered, washed with acetone and dried giving 26.5 g (purity 90%) of the white title product. Yield 97%. IR (KBr-disc, values in cm^{-1}): 3000, 2820, 2570, 1980, 1785, 1720, 1665, 1500, 1380, 1340, 1225, 1160, 1115, 1035, 975, 920, 875, 810, 720, 700, 440. ^{1}H NMR (360 MHz: CF_3COOD): 1.99 (s, 3H); 2.52 (s, 3H); 2.62 (s, 3H); 3.45, 3.55 (AB-q, 2H; J = 18 Hz); 4.98, 5.20 (AB-q, 2H; J = 14.4 Hz); 5.27 (d, 1H; J = 4.6 Hz); 5.84 (d, 1H; J = 4.6 Hz). ^{13}C NMR (90 MHz: CF_3COOD): 11.7; 15.1; 19.1; 19.4; 49.3; 56.2; 57.5; 116.8; 131.9; 152.7; 157.9; 168.8; 195.4 (C=N).

*(6R,7R)-3-Acetoxymethyl-4-carboxy-7-isopropylideneammonioceph-3-em bromide (**4a**; $R_2=R_3=CH_3$; X=Br)*

A: Using 33% hydrogen bromide in acetic acid: to a suspension of 10.0 g (purity 96%; 35.3 mmoles) of (6*R*,7*R*)-3-acetoxymethyl-7-aminoceph-3-em-4-carboxylic acid in 50 ml of acetone, 9.8 ml of a hydrogen bromide solution in acetic acid (33%) was added gradually at 0°C. The precipitate formed was filtrered off, washed with acetone and dried, giving 13.7 g (purity 93%) of the light brown title product. Yield 91%. IR (KBr-disc, values in cm^{-1}): 3185, 3050, 2940, 2905, 2715, 1735, 1710, 1665, 1625, 1520, 1415, 1405, 1375, 1335, 1230, 1215, 1195, 1165, 1110, 1060, 1040, 975, 920, 820, 790, 720, 700, 440. ^{1}H NMR (360 MHz: CF$_3$COOD): 1.99 (s, 3H); 2.53 (s, 3H); 2.63 (s, 3H); 3.49, 3.56 (AB-q, 2H; J = 18 Hz); 5.00, 5.19 (AB-q, 2H; J = 14.4 Hz); 5.33 (d, 1H; J = 4.6 Hz); 5.88 (d, 1H; J = 4.6 Hz). ^{13}C NMR (90 MHz: CF$_3$COOD): 11.7; 15.1; 19.1; 19.4; 49.3; 56.2; 57.5; 116.8; 131.9; 152.7; 157.9; 168.8; 195.4 (C=N).

B: Using 47% hydrogen bromide in water: to a suspension of 10.0 g (purity 96%; 35.3 mmoles) of (6*R*,7*R*)-3-acetoxymethyl-7-aminoceph-3-em-4-carboxylic acid in 100 ml of acetone and 20 ml of acetic acid, 6.7 ml of a hydrogen bromide solution in water (47%) were added. After stirring for 210 minutes at 0°C the precipitate formed was filtered off, washed with acetone and dried in vacuo, giving 10.77 g (purity 100%) of the brown title product. Yield 78%.

*(6R,7R)-3-Acetoxymethyl-4-carboxy-7-isopropylideneammonioceph-3-em iodide (**4a**; R_2=R_3=CH_3; X=I)*

To a suspension of 5 g (purity 97%; 17.8 mmoles) of (6*R*,7*R*)-3-acetoxymethyl-7-aminoceph-3-em-4-carboxylic acid , 4.0 ml of a hydrogen iodide solution in water (57%) were added. After stirring for about 90 minutes at 0°C, the crystals formed were filtered off, washed with acetone and dried giving 2.69 g of yellow title product. Yield 34%. IR (KBr-disc, values in cm^{-1}): 3180, 3040, 2900, 2760, 1795, 1740, 1715, 1675, 1630, 1510, 1415, 1400, 1380, 1230, 1205, 1165, 1115, 1065, 1045, 975, 920, 840, 815, 785, 715, 700, 440. ^{1}H NMR (360 MHz: CF$_3$COOD): 1.99 (s, 3H); 2.53 (s, 3H); 2.64 (s, 3H); 3.50, 3.57 (AB-q, 2H; J = 18 Hz); 5.00, 5.19 (AB-q, 2H; J = 14.4 Hz); 5.35 (d, 1H; J = 4.6 Hz); 5.89 (d, 1H; J = 4.6 Hz). ^{13}C NMR (90 MHz: CF$_3$COOD): 11.7; 15.1; 19.1; 19.4; 49.3; 56.2; 57.5; 116.8; 131.9; 152.7; 157.9; 168.8; 195.4 (C=N).

*(6R,7R)-3-Acetoxymethyl-4-carboxy-7-cyclopentylideneammonioceph-3-em bromide (**4a**; R_2,R_3=-$(CH_2)_4$-; X=Br)*

In a centrifuge tube 0.8 ml of a hydrogen bromide solution (33%) in acetic acid was added to 1.0 g (purity 96%; 3.53 mmoles) of (6*R*,7*R*)-3-acetoxymethyl-7-aminoceph-3-em-4-carboxylic acid at 0°C with stirring. After adding gradually 5 ml of cyclopentanone a crystalline precipitate was obtained after 30 minutes, which was filtered off, washed with acetone and cyclopentanone, and dried giving 1.17 g (80%) of light brown title product. IR (KBr-disc, values in cm^{-1}): 3180, 3000, 2900, 2630, 1795, 1740, 1715, 1680, 1630, 1500, 1415, 1380, 1340, 1235, 1210, 1160, 1110, 1070, 1045, 975, 920, 825, 790, 715, 455. ^{1}H NMR (360 MHz: CF$_3$COOD): 2.02 (s, 3H); 2.02 (m, 4H); 3.02 (m, 4H); 3.52, 3.58 (AB-q, 2H; J = 18.4 Hz); 5.01, 5.23 (AB-q, 2H; J = 14.1 Hz); 5.31 (d, 1H; J = 4.3 Hz); 5.75 (d, 1H; J = 4.3 Hz). ^{13}C NMR (90 MHz: CF$_3$COOD): 21.4; 26.1; 26.4; 28.9; 38.0; 40.3; 58.2; 59.0; 61.5; 65.9; 68.8; 126.5; 141.6; 178.5; 218.2 (C=N).

*(6R,7R)-3-Acetoxymethyl-4-carboxy-7-cyclohexylideneammonioceph-3-em chloride (**4a**; R_2,R_3=-$(CH_2)_5$-; X=Cl)*

A mixture of 165 mg (0.606 mmole) of (6*R*,7*R*)-3-acetoxymethyl-7-aminoceph-3-em-4-carboxylic acid, 0.08 ml of a concentrated hydrogen chloride solution and 0.10 ml (0.96 mmole) of cyclohexanone was stirred vigorously in an ultrasonic bath for 15 minutes. During the next 80 minutes more cyclohexanone (0.2 ml) was added in 2 portions. Collection and washing of the precipitate with acetonitrile and ether afforded 197 mg (84%) of light yellow title product. IR (KBr-disc, values in cm^{-1}): 3440, 1804, 1731, 1715, 1656, 1620, 1525, 1418, 1380, 1229, 1065, 1039, 876, 721, 704. ^{1}H NMR (360 MHz: CF$_3$COOD): 1.66 (m, 2H); 1.92 (m, 4H); 1.99 (s, 3H); 2.77 (m, 4H); 3.45, 3.57 (2xd, 2H; J = 18.0 Hz); 4.99, 5.19 (2xd, 2H; J = 14.4 Hz); 5.27 (d, 1H; J = 4.4 Hz); 5.87 (d, 1H; J = 4.4 Hz).

*(6R,7R)-3-Acetoxymethyl-4-carboxy-7-cyclohexylideneammonioceph-3-em bromide (**4a**; R_2,R_3=-$(CH_2)_5$-; X=Br)*

To 165 mg (purity 96%; 0.58 mmole) of (6*R*,7*R*)-3-acetoxymethyl-7-aminoceph-3-em-4-carboxylic acid was added 0.12 ml of a concentrated hydrogen bromide solution and 0.1 ml of cyclohexanone at 0°C with vigorous stirring. When stirring continued initially a clear solution mixture was obtained but after some time a thick crystalline precipitate was formed. After adding 0.5 ml of cyclohexanone and stirring for 30 minutes the crystalline precipitate was filtered off and dried giving 234 mg of the title product as an off-white solid with a purity of 93%. Yield 92%. IR (KBr-disc, values in cm^{-1}): 3440, 2945, 2850, 1810, 1731, 1651, 1625, 1510, 1417, 1230, 1196, 1067, 1041, 833, 702. ^{1}H NMR (360 MHz: CF_3COOD): 1.70 (m, 2H); 1.93 (m, 4H); 1.99 (s, 3H); 2.69 (m, 4H); 3.28, 3.36 (2xd, 2H; J = 17.0 Hz); 4.57, 4.74 (2xd, 2H; J = 12.0 Hz); 4.85 (d, 1H; J = 4.5 Hz); 5.36 (d, 1H; J = 4.5 Hz).

(6R,7R)-3-Acetoxymethyl-4-carboxy-7-cyclohexylideneammonioceph-3-em hydrogen sulphate ***(4a***; *R_2,R_3=-$(CH_2)_5$-; X=HSO_4)*

To 1.0 g (3.53 mmoles) of (6*R*,7*R*)-3-acetoxymethyl-7-aminoceph-3-em-4-carboxylic acid in acetic acid (5 ml) and sulfuric acid (0.2 ml), cyclohexanone (5 ml) was added. After standing overnight at 2°C the crystalline precipitate formed was filtered off, washed with cyclohexanone and acetone and dried in vacuo yielding 1.41 g (89%) of the title product as a white solid. IR (KBr-disc, values in cm^{-1}): 2960, 2880, 1810, 1725, 1710, 1665, 1535, 1410, 1390, 1360, 1310, 1280, 1230, 1165, 1050, 980, 850, 710, 580, 440. ^{1}H NMR (360 MHz: CF_3COOD): 1.68 and 1.94 (m, 6H); 1.93 (m, 4H); 2.02 (s, 3H); 2.79 (m, 4H); 3.45, 3.61 (AB-q, 2H; J = 17.7 Hz); 5.01, 5.20 (AB-q, 2H; J = 14.1 Hz); 5.31 (d, 1H; J = 4.3 Hz); 5.90 (d, 1H; J = 4.3 Hz).

(6R,7R)-4-Carboxy-7-isopropylideneammonio-3-methylceph-3-em chloride ***(4b***; *R_2=R_3=CH_3; X=Cl)*

A: Using acetic acid saturated with hydrogen chloride: to a mixture of 1.0 g (purity 98%; 4.57 mmoles) of (6*R*,7*R*)-7-amino-3-methylceph-3-em-4-carboxylic acid and 2.5 ml of acetic acid saturated with hydrogen chloride, 3 ml of acetone were gradually added with vigorous stirring. After stirring for 10 minutes another 5 ml of acetone were added, the reaction mixture was stored in the refrigerator for 2 hours and the precipitate formed was filtered off, washed with acetone and dried, giving 1.26 g off-white title product with a purity of 100% according to NMR assay. Yield 95%. IR (KBr, values in cm^{-1}): 3010, 2660, 1985, 1780, 1720, 1665, 1625, 1535, 1415, 1395, 1365, 1285, 1205, 1185, 1115, 1080, 1050, 1000, 970, 955, 910, 880, 800, 740, 715, 690, 565, 510. ^{1}H NMR (360 MHz: CF_3COOD): 2.18 (s, 3H); 2.51 (s, 3H); 2.62 (s, 3H); 3.24, 3.33 (AB-q, 2H; J = 18.0 Hz); 5.25 (d, 1H; J = 4.3 Hz); 5.71 (d, 1H; J = 4.3 Hz). ^{13}C NMR (90 MHz: DCOOD): 18.4; 21.7; 25.8; 29.6; 55.8; 63.5; 120.8; 144.6; 158.8; 163.6; 200.5 (C=N).

B: Using 37% hydrogen chloride in water: at 0°C, 1.0 g (purity 98%; 4.6 mmoles) of (6*R*,7*R*)-7-aminoceph-3-methylceph-3-em-4-carboxylic acid was added to a mixture of 10 ml of acetone, 10 ml of acetic acid, and 0.45 ml of a 37% solution of hydrogen chloride in water. After stirring for 45 minutes the white precipitate formed was filtered off, washed with acetone and dried in vacuo, giving 1.16 g off-white title product. Yield 87%.

(6R,7R)-4-Carboxy-7-isopropylideneammonio-3-methylceph-3-em bromide ***(4b***; *R_2=R_3=CH_3; X=Br)*

To a suspension of 1.0 g (purity 98.5%; 4.6 mmoles) of (6*R*,7*R*)-7-amino-3-methylceph-3-em-4-carboxylic acid in a mixture of 10 ml of acetic acid and 15 ml acetone, 0.86 ml of hydrogen bromide (47% in water) was added. The white crystals formed were filtrated and dried giving 1.47 g of the title product was obtained (purity 96%). Yield 92%. IR (KBr, values in cm^{-1}): 3120, 2820, 2740, 1785, 1725, 1660, 1630, 1525, 1395, 1365, 1285, 1205, 1185, 1115, 1080, 1050, 965, 955, 910, 865, 805, 780, 715, 690, 565, 505. ^{1}H NMR (360 MHz: CF_3COOD): 2.18 (s, 3H); 2.52 (s, 3H); 2.64 (s, 3H); 3.21, 3.37 (AB-q, 2H; J = 18.0 Hz); 5.28 (d, 1H; J = 4.3 Hz); 5.75 (d, 1H; J = 4.3 Hz). ^{13}C NMR (90 MHz: CF_3COOD): 12.1; 15.5; 19.6; 24.1; 49.7; 50.5; 51.5; 57.4; 114.4; 143.7; 148.5; 159.1; 194.8 (C=N).

(6R,7R)-4-Carboxy-7-cyclohexylideneammonio-3-methylceph-3-em bromide ***(4b***; *R_2,R_3=-$(CH_2)_5$-; X=Br)*

To a solution of 500 mg (2.33 mmoles) of (6*R*,7*R*)-7-amino-3-methylceph-3-em-4-carboxylic acid in 0.45 ml of a concentrated hydrogen bromide solution (47%), 0.50 ml of cyclohexanone was added at ambient temperature. After 20 minutes crystallization started and during the next 6 hours 2.0 ml of cyclohexanone were added in portions. After standing overnight, the crystals formed were filtered off, washed and dried, yielding 590 mg (67%) of white title product. IR (KBr, values in cm^{-1}): 3430, 2870, 1796, 1709, 1658, 1630, 1517, 1401, 1354, 1214, 1195, 828, 705. ^{1}H NMR (360 MHz: CF_3COOD): 1.63 (m, 2H); 1.92 (m, 4H); 2.17 (s, 3H); 2.78 (m, 4H); 3.25 (d, 1H; J = 16.2 Hz); 3.38 (d, 1H; J = 16.2 Hz); 5.28 (d, 1H; J = 4.5 Hz); 5.78 (d, 1H; J = 4.5 Hz). ^{13}C NMR (90 MHz: CF_3COOD): 21.3; 25.5; 29.8; 30.0; 33.4; 35.5; 39.6; 59.9; 66.0; 168.5; 209.1 (C=N).

*(6R,7R)-4-Carboxy-7-cyclohexylideneammonio-3-methylceph-3-em perchlorate (**4b**; $R_2,R_3=-(CH_2)_5-$; $X=ClO_4$)*

In an ultrasonic bath 130 mg (0.61 mmole) of (6*R*,7*R*)-7-amino-3-methylceph-3-em-4-carboxylic acid was dissolved in 0.175 ml of a perchloric acid solution at 0°C and 0.1 ml of cyclohexanone was added over a period of 30 minutes. After another 30 minutes the crystals were filtered off, washed and dried, giving 171 mg (71%) of white product. IR (KBr, values in cm^{-1}): 3430, 3205, 3110, 3010, 1790, 1716, 1658, 1635, 1521, 1410, 1357, 1218, 1196, 1140, 1100, 1050, 835, 703, 621. ^{1}H NMR (360 MHz: CF$_3$COOD): 1.58 (m, 2H); 1.87 (m, 4H); 2.13 (s, 3H); 2.73 (m, 4H); 3.21 (s, 2H); 5.20 (d, 1H; J = 4.5 Hz); 5.67 (d, 1H; J = 4.5 Hz).

*(6R,7R)-3-Bromomethyl-4-carboxy-7-isopropylideneammonioceph-3-em bromide (**4c**; $R_2=R_3=CH_3$; $X=Br$)*

390.65 g of a dichloromethane solution containing 23.17 mmol trimethylsilyl (6*R*,7*R*)-3-bromomethyl-7-phenylacetamidoceph-3-em-4-carboxylate 1β-oxide was cooled to -65°C and treated with 3.85 ml (14.2 mmoles) of tributylphosphite during 70 minutes at -60°±2°C. Thereafter the mixture was submitted to sulphoxide reduction using 4.2 ml (32.2 mmoles) of *cis*-cyclooctene, 0.5 ml (3.9 mmoles) of *N*,*N*-dimethylaniline, 0.8 ml (10.4 mmoles) of *N*,*N*-dimethylformamide and 7.65 g (36.6 mmoles) of phosphorus pentachloride during 65 minutes at about -66°C, and subjected to treatment with 28 ml (30.3 mmoles) of *iso*butanol. After 1 hour and 30 minutes stirring at about -65°C the reaction mixture was finally subjected to treatment with 345 ml (4.7 mole) of acetone and 19 ml (166 mmoles) of a solution of hydrogen bromide, 47%. The crystals were filtered and washed with dichloromethane and with acetone. After drying in vacuo at 40°C the white-pink coloured (6*R*,7*R*)-3-bromomethyl-4-carboxy-7-isopropylideneammonioceph-3-em bromide herewith obtained weighed 8.14 g (19.65 mmoles). Yield 85%. IR (KBr, values in cm^{-1}): 3420, 1790, 1692, 1650, 1610, 1512, 1397, 1347, 1215, 1180, 1091, 1059, 992, 820, 720, 697, 620. ^{1}H NMR (360 MHz: CF$_3$COOD): 2.54 (s, 3H); 2.63 (s, 3H); 3.53, 3.58 (AB-q, 2H; J = 17.3 Hz); 4.29, 4.33 (AB-q, 2H; J = 10.7 Hz); 5.33 (d, 1H; J = 4.5 Hz); 5.83 (d, 1H; J = 4.5 Hz).

*(6R,7R)-4-Carboxy-3-iodomethyl-7-isopropylideneammonioceph-3-em iodide (**4d**; $R_2=R_3=CH_3$; $X=I$)*

To a solution of 0.5 g (purity 60%; 0.9 mmole) of (6*R*,7*R*)-7-amino-3-iodomethylceph-3-em-4-carboxylic acid in 0.5 ml of a hydrogen iodide solution in water (57%) 1.0 ml of acetone was added in three minutes at 0°C. After stirring for 5 minutes at 0°C, the crystals formed were filtered off, washed with acetone and dried giving 0.57 g of orange title product (purity 60%). Yield 76%. IR (KBr, values in cm^{-1}): 3400, 3030, 2930 2850, 2750, 1795, 1710, 1655, 1610, 1510, 1425, 1400, 1390, 1365, 1340, 1210, 1180, 1155, 1150, 1130, 1085, 1060, 990, 950, 930, 840. 810, 785, 730, 720, 695. ^{1}H NMR (600 MHz: HCOOD): 2.93 (s, 3H); 3.02 (s, 3H); 3.93, 3.95 (AB-q, 2H; J = 18 Hz); 4.68, 4.77 (AB-q, 2H; J = 9.4 Hz); 5.65 (d, 1H, J = 4.4 Hz); 6.14 (d, 1H; J = 4.4 Hz). ^{13}C NMR (90 MHz: CF$_3$COOD): 20.5; 24.8; 27.8; 56.1; 63.1; 119.7; 144.6; 157.6; 200.2 (C=N).

*(6R,7R)-4-Carboxy-7-cyclohexylideneammonio-3-iodomethylceph-3-em iodide (**4d**; $R_2,R_3=-(CH_2)_5-$; $X=I$)*

0.5 g (purity 79%; 1.2 mmoles) of (6*R*,7*R*)-7-amino-3-iodomethylceph-3-em-4-carboxylic acid was dissolved in 0.5 ml of hydrogen iodide solution in water (57%). 1 ml of cyclohexanone was added at 0°C under vigorous stirring. After 5 minutes crystals were formed. The yellow crystalline precipitate was filtered off, washed and dried, giving 0.58 g of the title product. Purity is 65%. Yield 59%. IR (KBr, values in cm^{-1}): 3410, 3030, 2940, 2870, 1790, 1720, 1645, 1620, 1505, 1445, 1395, 1345, 1315, 1210, 1180, 1165, 1145, 1135, 1090, 1065, 980, 930, 850, 820, 780, 730, 695. ^{1}H NMR (600 MHz: HCOOD): 2.10 (m); 2.40 (m); 3.13 (m, 4H); 3.95 (AB-q, 2H; J = 17 Hz); 3.96 (AB-q, 2H; J = 17 Hz); 4.68, 4.78 (AB-q, 2H; J = 9.4 Hz); 5.66 (d, 1H, J = 4.4 Hz); 6.17 (d, 1H; J = 4.4 Hz).

*(6R,7R)-4-Carboxy-7-isopropylideneammonio-3-iodomethylceph-3-em chloride (**4d**; $R_2=R_3=CH_3$; $X=Cl$)*

After addition of 0.49 g (purity 74%; 1.1 mmoles) of (6*R*,7*R*)-7-amino-3-iodomethylceph-3-em-4-carboxylic acid to 1.25 ml of acetic acid saturated with hydrogen chloride at 0°C, the solution had to be stirred in an ultrasonic bath at room temperature before a clear solution was obtained. Then 2.0 ml of acetone were added, the mixture was cooled down and a precipitate was formed. The crystals formed were filtered off, washed with acetone and dried, giving 0.46 g of light yellow title product. Purity is 69%. Yield 71%. IR (KBr, values in cm^{-1}): 3400, 2800, 1790, 1690, 1650, 1600, 1515, 1400, 1340, 1220, 1175, 1145, 1125, 1080, 1050, 980, 920, 840, 740, 720, 690. ^{1}H NMR (360 MHz: CF_3COOD/DMSO-d_6): 2.92 (s, 3H); 3.01 (s, 3H); 3.98 (AB-q, 2H; J = 16 Hz); 4.70, 4.75 (AB-q, 2H; J = 9.4 Hz); 5.66 (d, 1H, J = 4.3 Hz); 6.13 (d, 1H; J = 4.3 Hz). ^{13}C NMR (90 MHz: CF_3COOD): 20.5; 24.8; 27.8; 56.1; 63.1; 119.7; 144.6; 157.6; 200.2 (C=N).

*(6R,7R)-4-Carboxy-7-isopropylideneammonio-3-vinylceph-3-em chloride (**4e**; R_2=R_3=CH_3; X=Cl)*

After addition of 0.5 g (2.1 mmoles) of (6*R*,7*R*)-7-amino-3-vinylceph-3-em-4-carboxylic acid to 2 ml of acetic acid with 7% of hydrogen chloride at 0°C, a yellow solution was obtained which solidified gradually. After adding acetone (5 ml) the precipitate dissolved and crystalline product was formed. After stirring for 30 min, the crystals were filtered off, washed with acetone and dried, giving 0.62 g off-white title product. Yield 98%. IR (KBr, values in cm^{-1}): 2980, 2820, 2500, 2000, 1780, 1710, 1660, 1420, 1400, 1370, 1350, 1200, 1175, 1150, 1130, 1060, 995, 935, 845, 720, 695. ^{1}H NMR (360 MHz: CF_3COOD): 2.50 (s, 3H); 2.60 (s, 3H); 3.46, 3.52 (AB-q, 2H; J = 18 Hz); 5.28 (d, 1H, J = 4.3 Hz); 5.48 (d, 1H; J = 10.8 Hz); 5.62 (d, 1H; J = 16.8 Hz): 5.74 (d, 1H; J = 4.3 Hz); 7.27 (dd, 1H; J = 10.8 and 16.8 Hz). ^{13}C NMR (90 MHz: CF_3COOD): 15.3; 17.8; 19.6; 50.8; 57.9; 114.2; 118.8; 137.4; 158.8; 194.9 (C=N).

*(6R,7R)-4-Carboxy-7-isopropylideneammonio-3-vinylceph-3-em bromide (**4e**; R_2=R_3=CH_3; X=Br)*

After addition of 0.5 g (2.1 mmoles) of (6*R*,7*R*)-7-amino-3-vinylceph-3-em-4-carboxylic acid to 0.4 ml of a 33% solution of hydrogen bromide in acetic acid at 0°C, solid material was formed which was pulverized. After addition of acetone (5 ml) the precipitate dissolved and crystalline product was formed. After stirring for 30 min, the crystals were filtered off, washed with acetone and dried, giving 0.62 g off-white product. Yield 88%. IR (KBr, values in cm^{-1}): 2940, 2810, 2740, 1795, 1715, 1655, 1610, 1580, 1515, 1395, 1340, 1190, 1170, 1120, 1055, 1020, 990, 935, 800, 775, 725, 690, 425. ^{1}H NMR (360 MHz: CF_3COOD): 2.54 (s, 3H); 2.66 (s, 3H); 3.54 (s, 2H), 5.29 (d, 1H; J = 4.3 Hz); 5.51 (d, 1H, J = 10.8 Hz); 5.63 (d, 1H; J = 16.8 Hz); 5.78 (d, 1H; J = 4.3 Hz); 7.32 (dd, 1H; J = 10.8 and 16.8 Hz). ^{13}C NMR (90 MHz: CF_3COOD): 15.3; 17.8; 19.6; 50.8; 57.9; 114.2; 118.8; 137.4; 158.8; 194.9 (C=N).

*(6R,7R)-4-Carboxy-7-cyclohexylideneammonio-3-vinylceph-3-em bromide (**4e**; R_2,R_3=-$(CH_2)_5$-; X=Br)*

After addition of 140 mg (purity 95%; 0.59 mmoles) of (6*R*,7*R*)-7-amino-3-vinylceph-3-em-4-carboxylic acid, cyclohexanone (1.4 mmol), and acetonitrile (0.1 ml) to 0.12 ml of a 47% solution of hydrogen bromide in water at 0°C, solid material was formed overnight. The precipitate was filtered off, washed with acetone and dried, giving 159 mg off-white title product. Yield 70%. IR (KBr, values in cm^{-1}): 3440, 2870, 1798, 1710, 1655, 1575, 1394, 1350, 1212, 978, 943, 702. ^{1}H NMR (360 MHz: CF_3COOD): 1.63 (m, 2H); 1.91 (m, 4H); 2.79 (m, 4H), 3.49 (d, 1H; J = 16.2 Hz); 3.57 (d, 1H, J = 16.2 Hz); 5.32 (d, 1H; J = 4.5 Hz); 5.49 (d, 1H; J = 10.8 Hz); 5.64 (d, 1H; J = 15.1 Hz); 5.82 (d, 1H; J = 4.5 Hz); 7.30 (dd, 1H; J = 10.8 and 15.1 Hz).

*(6R,7R)-4-Carboxy-7-isopropylideneammonio-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]ceph-3-em chloride (**4f**; R_2=R_3=CH_3; X=Cl)*

To 2 ml of acetic acid saturated with hydrochloric acid 1 g (purity 95%; 2.9 mmoles) of (6*R*,7*R*)-7-amino-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]ceph-3-em-4-carboxylic acid and 3 ml of acetone were added at 5°C. After stirring for 40 minutes at 0°C the crystalline material was filtered off, washed with acetone and dried giving 1.1 g of white title product. Yield 94%. IR (KBr, values in cm^{-1}): 2950, 2910, 2830, 1800, 1715, 1655, 1625, 1525, 1470, 1405, 1350, 1250, 1235, 1180, 1110, 1060, 835, 710, 695. ^{1}H NMR (360 MHz: CF_3COOD): 2.52 (s, 3H); 2.62 (s, 3H); 3.63, 3.74 (AB-q, 2H; J = 18 Hz); 3.97 (s, 3H); 4.01, 4.51 (AB-q, 2H, J = 13.2 Hz); 5.32 (d, 1H; J = 4.3 Hz); 5.83 (d, 1H; J = 4.3 Hz). ^{13}C NMR (90 MHz: CF_3COOD): 15.0; 19.4; 21.0; 27.0; 27.9; 49.2; 57.9; 117.9; 129.1; 145.9; 152.2; 157.4; 195.5 (C=N).

*(6R,7R)-4-Carboxy-7-isopropylideneammonio-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]ceph-3-em bromide (**4f**; R_2=R_3=CH_3; X=Br)*

To a suspension of 1 g (purity 95%; 2.9 mmoles) of (6*R*,7*R*)-7-amino-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]ceph-3-em-4-carboxylic acid in 7 ml of acetone was added 0.81 ml of a 33% hydrogen bromide solution in acetic acid. The reaction mixture was stirred for 30 minutes at 0°C and stored overnight at 4°C. After filtration the crystals were washed with acetone and dried to give 1.35 g off-white title product. Yield 100%. IR (KBr, values in cm^{-1}): 3180, 2945, 2900, 2730, 1795, 1720, 1655, 1625, 1515, 1455, 1395, 1370, 1345, 1230, 1175, 1105, 1055, 995, 930, 820, 770, 705. ^{1}H NMR (360 MHz: CF$_3$COOD): 2.52 (s, 3H); 2.64 (s, 3H); 3.64, 3.76 (AB-q, 2H; J = 18 Hz); 3.97 (s, 3H); 4.06, 4.57 (AB-q, 2H, J = 13.2 Hz); 5.37 (d, 1H; J = 4.3 Hz); 5.85 (d, 1H; J = 4.3 Hz). ^{13}C NMR (90 MHz: CF$_3$COOD): 15.2; 19.6; 21.1; 27.3; 27.9; 49.6; 57.9; 118.2; 129.1; 146.2; 152.4; 157.8; 195.4 (C=N).

*(6R,7R)-4-Carboxy-7-isopropylideneammonio-3-methylenecepham bromide (**6**; $R_2=R_3=CH_3$)*

At 0°C, 1.0 g (purity 96%; 4.5 mmoles) of (6*R*,7*R*)-7-amino-3-methylenecepham-4-carboxylic acid was added to a 33% solution of hydrogen bromide in acetic acid (0.8 ml). A yellow precipate formed and gradually 10 ml acetone was added. As a result of this, the syrupy material changed into a crystalline product. After stirring for 30 min, the precipitate was recovered by filtration and washed with acetone. Drying under vacuum gave 1.30 g off-white product. Yield 86%. IR (KBr, values in cm^{-1}): 2840, 2750, 1775, 1730, 1655, 1525, 1420, 1370, 1230, 1180, 1140, 1075, 935, 800, 790, 730, 590. ^{1}H NMR (360 MHz: CF$_3$COOD): 2.47 (s, 3H); 2.59 (s, 3H); 3.24 (AB-q, 2H; J = 14.4 Hz); 5.20 (s, 1H); 5.23 (s, 1H); 5.26 (d, 1H); 5.61 (d, 1H; J = 4.3 Hz); 5.71 (d, 1H; J = 4.3 Hz). ^{13}C NMR (90 MHz: CF$_3$COOD): 15.2; 19.7; 22.0; 47.8; 49.9; 57.8; 112.6; 123.7; 165.3; 194.7 (C=N).

*(5R,6R)-3-Carboxy-6-isopropylideneammonio-2,2-dimethyl-1,1-dioxopenam chloride (**8**; $R_2=R_3=CH_3$)*

A 7% hydrogen chloride solution in acetic acid (1.0 ml) was added to 0.5 g (2.0 mmoles) of (5R,6R)-6-amino-2,2-dimethyl-1,1-dioxopenam-3-carboxylic acid at 0°C. After adding 3 ml of acetone a crystalline precipitate was obtained, which was collected by filtration, washed with acetone, and dried to give 0.55 g off-white title product. Yield 85%. IR (KBr, values in cm^{-1}): 3000, 2900, 2620, 1815, 1760, 1665, 1525, 1465, 1430, 1400, 1325, 1210, 1190, 1150, 1115, 1085, 965, 855, 745, 645, 555. ^{1}H NMR (360 MHz: CF$_3$COOD): 1.68 (s, 3H); 1.81 (s, 3H); 2.88 (s, 3H); 2.90 (s, 3H); 4.90 (s, 3H); 5.54 (d, 1H, J = 4.2 Hz); 6.40 (d, 1H; J = 4.2 Hz). ^{13}C NMR (90 MHz: CF$_3$COOD): 11.9; 14.4; 17.9; 22.8; 57.9; 59.7; 60.3; 61.6; 163.0; 165.8; 199.0 (C=N).

*N-Cyclohexylidene-β-alaninium bromide (**10**)*

Cyclohexanone (10 ml) was added to a solution of β-alanine (1.0 g; 11.2 mmoles) in 5 ml 13% hydrogen bromide in acetic acid. The white precipitate was collected by filtration, washed with cyclohexanone and ether, and dried to give 1.23 g off-white product. Yield 44%. Mp 94-96. IR (KBr, values in cm^{-1}): 1730, 1680, 1525, 1460, 1370, 1325, 1210, 1095, 1075, 1065, 980, 950, 875, 855, 825. ^{1}H NMR (360 MHz: CF$_3$COOD): 1.67 (m, 2H); 1.87 (m, 4H); 2.72 (m, 4H); 2.98 (t, 2H); 4.03 (m, 2H). ^{13}C NMR (90 MHz: CF$_3$COOD): 19.1; 22.1; 22.9; 28.6; 32.7; 32.9; 37.8; 172.2; 195.2 (C=N).

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