Synthesis of HR 916 B: The First Technically Feasible Route to the 1-(Pivaloyloxy)ethyl Esters of Cephalosporins

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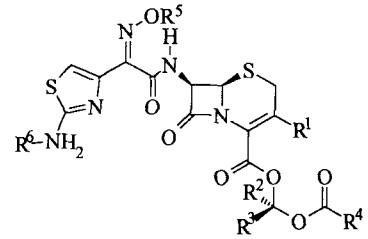
An efficient synthesis of HR 916 B (**4**), the orally active 1-(*RS*)-(pivaloyloxy)ethyl prodrug ester of the cephalosporin cefdaloxime, was developed and applied on a multi-kg scale. AMCA (**8**) was prepared by exchange of the acetoxy group of fermentation product ACA (**7**) with the nucleophile methanol under acidic conditions. Its 7-amino group was acylated with mixed anhydride **14** to give **15**. Carboxylic acid **15** was esterified with iodohydrin ester **27** or bromohydrin ester **30**, respectively, to provide the acylal **16**. Simultaneous removal of both the amino- and the oximo-protecting group furnished the prodrug HR 916 **3**, which was purified and stabilized by precipitation of its tosylate salt **4**. The overall yield of **4** (ratio **5/6** = 0.65) was 39% relative to AMCA (**8**) (four steps), 15% relative to ACA (**7**) (five steps).

Most of the therapeutically useful third-generation cephalosporins are orally not well absorbed. Conversion of the carboxylic acid to prodrug esters, that are metabolically cleaved after absorption from the bowl, has been successfully used to obtain orally absorbable cephalosporins. The 1-acyloxy- and 1-alkoxycarbonyloxy-substituted methyl and ethyl esters are especially useful. Typical examples for these types of esters are cefetamed pivoxil (**1**)[1] and cefpodoxime proxetil (**2**)[2]. In the case of the substituted ethyl esters an additional stereocentre is introduced into the molecule. If this centre is not controlled, the prodrug is a mixture of two diastereomers, because the cephem moiety is homochiral. Cefpodoxime proxetil (**2**) is on the market as a mixture of diastereomers.

In 1988, HR 916 (mixture of two diastereomeric prodrugs; free NH_2 group) **3**[3] was selected as a candidate for preclinical development, as its active metabolite cefdaloxime shows broad antibacterial activity against Gram-positive and Gram-negative bacteria[4], and the prodrug ester **3** exhibits good oral bioavailability in different animal species[5]. Soon, the development had to be switched to tosylate **4**[6] (HR 916 B), since pure **3** was too sensitive[7] in aqueous solution and even in the solid form. We observed that during recrystallization of **4** one of the two diastereomeric tosylates, HR 916 J **5**, was strongly enriched in the mother liquor, its diastereomer HR 916 K **6** in the crystals. Thus, repeated recrystallization of **4** led to the first significant amounts of **5** and **6**. They showed comparable bioavailabilities in mice and rats, but in dogs **6** was enterally absorbed to 68%, whereas **5** only to 20%[8]. Phase 1 clinical studies confirmed that **6** is enterally well absorbed[9]. Hence a diastereoselective synthesis of **6** had to be developed, that is described in the following paper[10].

Scheme 1. Orally absorbable prodrug esters of cephalosporins



	R^1	R^2	R^3	R^4	R^5	R^6
1	Me	H	H	CMe_3	Me	-
2	CH_2OMe	Me, H	H, Me	$OCHMe_2$	Me	-
3	CH_2OMe	Me, H	H, Me	CMe_3	H	-
4	CH_2OMe	Me, H	H, Me	CMe_3	H	H^+ *p*-TsO^-
5	CH_2OMe	H	Me	CMe_3	H	H^+ *p*-TsO^-
6	CH_2OMe	Me	H	CMe_3	H	H^+ *p*-TsO^-

Results and Discussion

All syntheses of HR 916 (**3**, **4**, **5**, or **6**) require (6*R*,7*R*)-7-amino-3-(methoxymethyl)-3-cephem-4-carboxylic acid (AMCA) (**8**) as the starting material. **8** was prepared by the exchange of the acetoxy group of (6*R*,7*R*)-7-aminocephalo-

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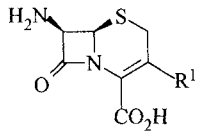
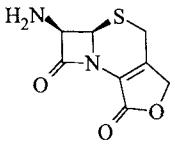
sporanic acid (ACA) (**7**) with the nucleophile methanol under strongly acidic conditions (Scheme 2)[11,12].

Scheme 2. Synthesis of (6*R*,7*R*)-7-amino-3-(methoxymethyl)-3-cephem-4-carboxylic acid (AMCA) (**8**)



	R^1
7	CH_2OAc
8	CH_2OMe



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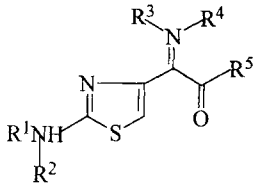
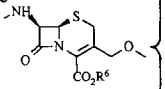
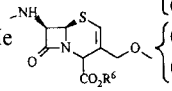
The formation of the principal by-product, lactone **9**, must be minimized by careful control of the reaction temperature and by HPLC monitoring of the reaction progress. The reaction must be interrupted by the addition of water as soon as the portion of **7** has diminished to 4–6%. Crude product **8** [59% yield (w/w); 78% purity (HPLC assay)] was purified by filtration of its acidic aqueous solution through the inexpensive resin HP 20®[13]. In this way, AMCA (**8**) was obtained in 35–38% yield [from ACA (**7**)] with 98–99% purity.

Retrosynthetic analysis suggested that HR 916 B **4** may be prepared by coupling of the 7-amino group of **8** with mixed anhydride **14** as the key step (Scheme 3).

Scheme 3. Convergent synthesis of HR 916 B



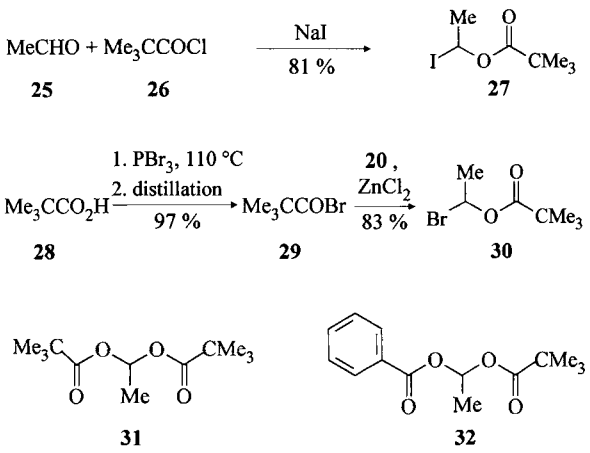
	R^1	R^2	R^3	R^4	R^5	R^6
10	Ph_3C	$H^+ Cl^-$	-	OH	OEt	
11	Ph_3C	-	-	OH	ONa	
12	Ph_3C	-	-	OH	OH	
13	Ph_3C	-	-	$OCMe_2OMe$	$O^- {}^+NEt_3H$	
14	Ph_3C	-	-	$OCMe_2OMe$	OSO_2-*p*-C_6H_4Me	
15	Ph_3C	-	-	$OCMe_2OMe$		H
16	Ph_3C	-	-	$OCMe_2OMe$		$CH(Me)O_2CtBu$
17	Ph_3C	-	-	OH		$CH(Me)O_2CtBu$
18	H	-	-	OH		H
19	Ph_3C	-	$OCMe_2OMe$	-		$CH(Me)O_2CtBu$
20	H	-	OH	-		$CH(Me)O_2CtBu$
21	Ph_3C	-	-	$OCMe_2OMe$		$CH(Me)O_2CtBu$
22	H	-	-	OH		$CH(Me)O_2CtBu$
23	Ph_3C	-	-	$OCMe_2OMe$	$CH(Me)O_2CtBu$	
24	H	-	-	OH	$CH(Me)O_2CtBu$	

To avoid homocondensation reactions, the amino and possibly also the oximo group in **14** should be protected. The protecting groups were demanded to survive the mildly basic conditions of both the coupling reaction and the esterification of carboxylic acid **15** to the acylal **16**, and they should be simultaneously removed under moderately acidic conditions. From those amino protecting groups R^1 that would satisfy these requirements[14a,15a], we favoured the triphenylmethyl (trityl) group, since corresponding derivatives are known to have excellent crystallinity, which was deemed helpful for purification of intermediates **10**–**13**, **15**, and notably **16**. It should be reemphasized that **16** is a 50:50 mixture of two diastereomers that, due to mutual melting point depression, needs this trityl assistance in order to crystallize[16]. The protecting group of the oxime shall be cleaved reliably under the conditions of *N*-trityl cleavage. Additionally, it shall not introduce a new stereocentre. This made 1-methoxy-1-methylethyl an obvious choice[14b,15b].

In practice, the synthesis of **4** led to the following results (Scheme 3): Ester **10** was saponified, sodium salt **11** was collected and acidified to furnish carboxylic acid **12** in 94% yield. The oxime was protected with 2-methoxypropene in acetone. Triethylammonium carboxylate **13** was precipitated in 93% yield. Mixed anhydride **14** was prepared by the reaction with *p*-tosyl chloride in acetone. A systematic investigation of the stability of **14** in the reaction medium indicated that the reaction temperature shall not exceed 15 °C, and the amount of *p*-tosyl chloride shall not exceed 0.95 equiv. relative to ammonium salt **13**. The water content of acetone must be <1%, preferably <0.2%. If these precautions are not obeyed, the thick white suspension of anhydride **14** tends to decompose exothermally and quantitatively to give a clear, yellow solution. It was advantageous not to isolate **14**, but to add a cold solution of the triethylammonium salt of AMCA (**8**) to the suspension of **14**. A solvate of **15** crystallized nicely (74% yield, 97.2% chemical purity) from 1-BuOAc/H_2O. Its esterification to **16** with 1-iodoethyl pivalate (**27**) proceeded best in acetone. Formation of the Δ^2-isomer **21** was suppressed by application of just 1.05 equivalents of the base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and careful control of a reaction temperature of 20 °C. Application of a larger excess of base or a higher temperature consistently produced considerable amounts of isomer **21** as an impurity that cannot be completely removed in the following steps. Screening of numerous organic and inorganic bases in several solvents indicated that these bases must be applied in a large excess (3–4 equiv.) in order to completely dissolve carboxylic acid **15**. DBU and its lower homologue 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) are special in the respect that this is already achieved with little more than one equiv. The physical reasons for this behaviour are not well understood. Ester **16** was furnished in quantitative yield and in 92% chemical purity as a 51:49 mixture of the two diastereomers.

Iodohydrin ester **27**, which is prepared from sodium iodide, acetaldehyde (**25**), and pivaloyl chloride (**26**) according to Scheme 4[17], is thermally and hydrolytically very sensitive. Since **27** cannot be purified by high-vaccum distillation and must be stored at <−20 °C (cf. Experimental), it was desirable to conduct the esterification with bromohydrin ester **30**[18], which is easily prepared according to Scheme 4, conveniently purified by high-vacuum distillation and stable on storage at ambient temperature. However, more than 5%

Scheme 4. Synthesis of 1-iodoethyl and 1-bromoethyl 2,2-dimethylpropanoate (**27** and **30**), the building blocks for the prodrug ester moiety of HR 916 B



of starting compound **15** remained unreacted when **30** was employed, while only 1.3% remained when **27** was used.

Both protecting groups were simultaneously removed in formic acid/water to provide **3** (50.5:49.5 ratio of the two diastereomers) in 90% yield. It contained 3.6% of HPLC-detectable impurities and additionally 6% of oligomers of **3**, that can only be detected by gel permeation chromatography (GPC) and a special low-resolution high-sensitivity MS technique (cf. Experimental)[19]. These impurities were removed by precipitation of *p*-toluenesulfonate[20] **4** (59% yield, >99% purity) from an 1-propanolic solution. HR 916 K **6** crystallizes preferentially in comparison to its diastereomer HR 916 J **5** and the diastereomeric ratio **5/6** in the crystals is shifted to 0.65. If four volumes of diisopropyl ether are added to the 1-propanolic suspension of **4** its precipitation is driven more to completion and **4** is obtained with 85–90% yield and with a virtually unshifted diastereomeric ratio (**5/6** = 49:51). In this case **24** and **22** still remain in the mother liquor, but the oligomers are inefficiently removed.

The overall yield of pure tosylate HR 916 B **4** (ratio **5/6** = 0.65) is 39% relative to AMCA (**8**) (4 steps), 15% relative to ACA (**7**) (5 steps), and 31% relative to protected aminothiazol ester **10** (6 steps). This route was scaled up and used to prepare 65 kg of HR 916 B **4** in the pilot plant. Its further utilization was terminated by the finding that HR 916 K **6** must be produced instead, due to superior resorption of **6**.

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Experimental

Our analytical and spectroscopy equipment has been described elsewhere[21]. – MS: a) "Fast atom bombardment" positive ionization (FAB): VG ZAB SEQ; NBA denotes *p*-nitrobenzyl alcohol. b) "Chemical ionization" (CI): Kratos MS 80. – Reagents and solvents were used as purchased from Aldrich, Fluka, Hoechst, Merck Darmstadt[20], and Riedel-de Haen. Compound **10** was purchased from LONZA Ltd. The acetone utilized in the preparation of **14** contained <0.2% H_2O, the CH_2Cl_2 used for the synthesis of **15** <0.05% H_2O, and the MeCN employed in the preparation of **27** <0.02% H_2O (Karl Fischer titration). All reactions were performed in dry glassware under nitrogen or argon.

1-(RS)-(Pivaloyloxy)ethyl (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-(methoxymethyl)-3-cephem-4-carboxylate (**3**): At 20 °C, 1.04 l of formic acid (98%) was added at once with stirring to 335 g (0.378 mol, corrected for 3.4 weight-% of solvents) of **16** [from procedure a)]. A progressive dissolution was noticed and a clear, brown solution was obtained after 15 min. Immediately thereafter, 210 ml of water was added at 20 °C within 5 min. The solution was heated to 24 °C within 3 min. Crystallization of triphenylcarbinol was noticed. The mixture was stirred for 2 h at 24 °C. The triphenylcarbinol was removed by filtration and washed with 350 ml of formic acid/water (5:1). [After drying in high vacuum at 30 °C for 1 d the triphenylcarbinol weighted 89.8 g (91%)]. At 20 °C 12.2 l of water was added to the clear, brown filtrate with stirring to give a milky orange solution with very small amounts of a precipitate and a pH of 1.7. At 20 °C, with external cooling and efficient stirring, a 28% aqueous solution of ammonia (approx. 1.44 l) was added within 30 min until a stable pH value of 4.0 was indicated. The resulting precipitate was somewhat gummy but still filterable. During the addition of ammonia the pH value should initially be measured by pH paper and then be controlled by a pH meter. Continuous immersion of the glass electrode should be avoided since it tends to produce erroneous values due to plugging by the precipitate. The suspension was stirred for 30 min at 20 °C and pH 4.0. It was then stirred for 30 min at 0 °C. The solid was suction-filtered, homogenized with a spatula, and washed with 3 × 1 l of water. The product was dried in high vacuum at 30 °C for 4 d to furnish 185.7 g of a yellow powder, which contained 0.6% of *n*-butyl acetate and 0.7% of water according to GC and Karl Fischer titration, respectively. The yield, corrected for 1.3% of solvents, was 183.3 g (90%), $[\alpha]_D^{25}$ = +48.2 (*c* = 1 in methanol). – IR (KBr): $\tilde{\nu}$ = 3420 and 3340 cm^{-1} (NH, OH), 2960 (CH, CH_2), 1780 (C=O, β-lactam), 1740 (C=O, ester), 1670 and 1520 (C=O, *sec.* amide), 1610 (C=C). – UV (MeOH): λ_{max} (lg ε) = 222 nm (4.30), 258 (4.14); both bands arise from overlapping absorptions of the aminothiazole and the cephem chromophores. – ^{1}H NMR (400 MHz, [D$_6$]DMSO; duplication of the signals is due to the presence of the 1:1 mixture of diastereomers): δ = 1.14/1.16 (s, 9H, CH_3), 1.47/1.48 (d, *J* ≈ 5 Hz, 3H, CH_3), 3.19/3.20 (s, 3H, OCH_3), 3.49/3.50 and 3.59/3.61 (AB system, J_{gem} ≈ 18 Hz, 2H, CH_2S), 4.12 (m, 2H, CH_2O), 5.18/5.20 (d, *J* ≈ 5 Hz, 1H, 6-CH), 5.81/5.84 (dd, *J* ≈ 8 and 5 Hz, 1H, 7-CH), 6.64/6.65 (s, 1H, 5'-CH), 6.86/6.92 (q, *J* ≈ 5 Hz, 1H, OCHO), 7.10 (br. s, 2H, NH_2), 9.45/9.46 (d, *J* ≈ 8 Hz, 1H, NH), 11.27 (s, 1H, NOH). – MS (FAB, matrix: 3-NBA + 10% PEG-2000), *m/z* (%): 542 (43) [M + H$^+$], 498 (22) [M + H$^+$ – CO_2], 396 (21) [M – OCH(Me)OCOCMe$_3$], 243 (19) [RC(=NOH)CONHC$^+$HCH=S], 227 (23) [RC(=NOH)CONHCH$_2$CO$^+$], 211 (34) [RC(=NOH)CONHCH=CH$^+$], 170 (26) [RC(=NOH)CO$^+$], 126 (100) [RC≡NH$^+$]; R stands for 2-aminothiazol-4-yl. – $C_{21}H_{27}N_5O_8S_2$ (541.6): calcd. C 46.57, H 5.02, N 12.93, S 11.84; found C 46.2, H 5.0, N 12.7, S 12.0. – HPLC (250 × 4.6 mm, 7-μm Nucleosil Phenyl; eluent: 1600 ml of H_2O, 900 ml of MeCN, 5.5 g of $NH_4H_2PO_4$, adjusted to pH 3.5 with conc. H_3PO_4; 1.5 ml/min, detection 254 nm) indicated 96.4% of **3** [t_{ret} 8.7 min (47.7%) and 9.3 min (48.7%)], 1.0% of Δ^2-isomer **22** (7.7 min), 0.7% of **24** (6.8 min), 0.6% of **18** (2.0 min), 0.8% of *anti*-isomer **20** [12.9 min (0.4%) and 13.8 min (0.4%)] and 0.5% of unidentified impurities. Comparison of the peak area of **3** (HPLC assay) with that of a highly purified reference standard (vide infra) indicated that the content of **3** was

89%. Since the solvent content and HPLC-visible impurities of **3** accounted for 4.9%, there were 6% of additional impurities not detected by ^{1}H NMR, IR, conventional MS, HPLC, or elemental analysis. They were identified as dimers, trimers, and tetramers of **3** by LR-MCA FAB-MS and visualized by GPC[19]. Similar oligomers were not detected by HPLC assay and GPC in the precursors **15** and **16**, indicating that they were formed in the formic acid/water solution. LR-MCA FAB-MS [mass range 300–3000 Da; NBA, PEG-2000), *m/z*: 542 [M + H$^+$ of **3**], 564 [M + Na$^+$ of **3**], 1052 [M – MeOH + H$^+$ of dimer], 1074 [M – MeOH + Na$^+$ of dimer], 1644 [M + H_2O + H$^+$ of trimer], 1662 [M + 2 H_2O + H$^+$ of trimer], 2185 [M + H_2O + H$^+$ of tetramer]. GPC (cross-linked polystyrene, eluent: THF, flow 1 ml/min, detection 254 nm): oligomers (t_{ret} 27.7, 28.7, 29.0, and 30.1 min, sum 6%), monomer **3** (t_{ret} 31.2 min, 94%).

1-(RS)-(Pivaloyloxy)ethyl (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-(methoxymethyl)-3-cephem-4-carboxylate p-Toluenesulfonate (**4**): 177.8 g (0.324 mol, corrected for 1.3% of solvents) of **3** was added continuously in small portions at 20°C within 1 h to 1.08 l of stirred 1-propanol. A faster introduction should be avoided since occasionally it causes the formation of a big gummy lump that subsequently is difficult to dissolve. A virtually clear solution with a few small gummy particles was formed. It was suction-filtered through a clarifying pad, and the filter was washed with 2 × 90 ml of 1-propanol. A solution of 66.6 g (0.350 mol) of *p*-toluenesulfonic acid monohydrate[20] in 180 ml of 1-propanol was added at 20°C within 30 min to the slowly stirred (200 min^{-1}) and cooled filtrate. 2–5 min later the crystallization commenced. The suspension was stirred for 5 h at 20°C. It was suction-filtered, the solid was washed with 3 × 150 ml of 1-propanol which had been precooled to 0°C. It was washed with 3 × 1 l of diisopropyl ether and then dried in high vacuum at 30°C for 3 d to furnish 135.9 g (59%) of a colorless powder, $[\alpha]_D^{25}$ = + 35.7 (*c* = 1 in MeOH). Karl Fischer titration indicated a H_2O content of 0.3%. GC indicated a content of <0.1% of solvents. HPLC (conducted as described for **3**) indicated 99.1% of **4** (39.1 and 60.0%, respectively; ratio **5/6** = 0.65), 0.1% of Δ^2-isomer **22**, 0% of **24**, 0.3% of **18**, 0.5% of *anti*-isomer **20** (0.2 and 0.3%, respectively). GPC indicated <0.5% of oligomers (detection limit). HPLC assay indicated a content of >99% of **4** relative to the reference standard. – ^{1}H NMR (270 MHz, [D$_6$]DMSO; duplication of the signals is due to the presence of the 40:60 mixture of diastereomers): δ = 1.13/1.15 (s, 9H, CH_3), 1.47/1.48 (d, *J* ≈ 5 Hz, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.19/3.20 (s, 3H, OCH_3), 3.51/3.52 and 3.62/3.63 (AB system, J_{gem} ≈ 18 Hz, 2H, CH_2S), 4.13 (m, 2H, CH_2O), 5.21/5.23 (d, *J* ≈ 5 Hz, 1H, 6-CH), 5.82/5.85 (m, 1H, 7-CH), 6.81/6.82 (s, 1H, 5'-CH), 6.86/6.92 (q, *J* ≈ 5 Hz, 1H, OCHO), 7.10 (d, *J* ≈ 8 Hz, 2H, aromatic H), 7.48 (d, *J* ≈ 8 Hz, 2H, aromatic H), 8.45 (br, 3H, NH_3^+), 9.66 (d, *J* ≈ 8 Hz, 1H, NH), 12.06 (br. s, 1H, NOH). – $C_{28}H_{35}N_5O_{11}S_3$ (713.8): calcd. C 47.12, H 4.94, N 9.81, S 13.48; found C 46.8, H 5.1, N 9.7, S 13.3.

Preparation of Reference Standards of **3** *and* **4**; *Preparation of* **6** *by Repeated Recrystallization of* **4**: At 20°C, 250 g (0.35 mol) of **4** was added at once to 250 ml of stirred *N,N*-dimethylacetamide (DMAA). A clear yellow solution was obtained after 50 min. 1.75 l of 1-propanol was added dropwise within 1 h. Precipitation commended when about half of the 1-propanol had been added. The suspension was stirred for 10 more min. The solid was suction-filtered, washed with 175 ml of 1-propanol, with 1.4 l of diisopropyl ether and then dried in high vacuum to afford 117 g (47%) of a colorless solid with a **5/6** ratio of 0.27. The product was recrystallized again (117 ml of DMAA, 900 ml of 1-propanol) to give 85 g (73%) of **4** with a **5/6** ratio of 0.17. The product was recrystallized again (85 ml of DMAA, 650 ml of 1-propanol) to furnish 70 g (82%) of **4** with a **5/6** ratio of 0.11. The product was recrystallized again (70 ml of DMAA, 540 ml of 1-propanol) to provide 65 g (93%) of **4** with a **5/6** ratio of 0.07. The product was recrystallized again (65 ml of DMAA, 500 ml of 1-propanol) to afford 60 g (92%) of **4** with a **5/6** ratio of 0.04. The product was recrystallized again (85 ml of DMAA, 470 ml of 1-propanol) to furnish 53 g (88%) of **4**, which consisted of 2.7% of HR 916 J **5** and 97.3% of HR 916 K **6**. This compound was used as a reference standard for tosylates **4**, **5**, and **6**. It was also used (with the respective molecular mass correction) as reference standard for HPLC assays of the free base **3**. The procedure (5 consecutive recrystallizations) was employed in the pilot plant to furnish 22.3 kg (70%) of HR 916 K **6** (containing <5% **5**) from 63.9 kg of HR 916 B **4**.

(6R,7R)-7-Amino-3-(methoxymethyl)-3-cephem-4-carboxylic Acid (AMCA) (**8**): To a mixture of 662 ml (10.2 mol) of methanesulfonic acid and 89 ml (1.0 mol) of trifluoromethanesulfonic acid was added with efficient cooling within 15 min 200 ml (4.9 mol) of anhydrous MeOH, such that the temp. remained at 10°C. At 13–14°C, 273 g (1.0 mol) of ACA (**7**) was added uniformly within 15 min to the vigorously stirred solution. The initial suspension turned into a solution. Every 15 min an aliquot (150 µl) was removed and diluted under ultrasonic irradiation to 50 ml with a 0.1% aqueous NH_4OAc solution. 4–5 ml of this turbid solution were filtered through a pad of 1 ml of LiChroprep RP 18, contained in a disposable plastic syringe. The filtrate was analyzed by HPLC (250 × 4.0 mm Nucleosil RP 18, 7 µm; eluent: H_2O + 0.1% NH_4OAc; detection 220 nm; t_{rel}: **8** 4.5 min, **7** 9.5 min, **9** 11.0 min), and the data depicted in Table 1 were measured. After 45 min, 1.0 l of ice/water was added to the solution, such that the reaction temp. remained <15°C. The mixture was stirred for 2.5 h at 20°C and then adjusted with cooling (10–20°C) to pH 2.5 by the addition of 40% aqueous NaOH. The suspension was stirred for 15 min at 10°C. The precipitate was suction-filtered, washed with water (5 × 200 ml), acetone (100 ml), diisopropyl ether (200 ml) and dried in vacuo to afford 144 g (59%) of a yellow powder. According to HPLC (conditions vide supra) **8** accounted for 97% of all detected peaks. By comparison ("assay") of the peak area of **8** with a highly purified standard, its purity was determined as 78%.

Table 1. Preparation of AMCA (**8**); HPLC monitoring of the reaction progress[a]

Rct. time (min)	AMCA (**8**) (%)	ACA (**7**) (%)	Lactone **9** (%)
15	64.3	25.9	6.6
30	74.1	13.3	9.6
45	78.5	5.8	11.7

[a] 100% method: The sum of the peak areas of all peaks was taken as 100%; peak areas are uncorrected.

The outlet of a 1-l "flash chromatography" column was filled with glass wool and topped by a porcelain perforated plate. 0.5 l of MeOH was filled into the column, followed by 400 g of HP 20®, such that the resin was completely covered by MeOH. The resin was allowed to swell overnight. The column was flushed with 1.0 l of H_2O. The upper surface of the resin was covered with a textile filter and a porcelain perforated plate. 143 g of crude **8** (content 78%, 0.45 mol) was largely dissolved with vigorous stirring in 420 ml of 2 N HCl. The thin suspension was suction-filtered through a Seitz filtering pad that had been topped by a layer of Celite (thickness 2 cm). The filter residue was stirred again with 160 ml, then again with 2 × 90 ml of 2 N HCl and suction-filtered each time.

The combined filtrates (≈850 ml) were given on top of and allowed to run through the column. The pH of the eluate was monitored with a glass electrode. From the moment when the eluate became strongly acidic (pH < 2), it was recycled to the head of the column by means of a laboratory pump. The column was run in this recycling mode for 30 min. Then the eluate was collected (1.5 l), and **8** was eluted by the continuous addition of 2 N HCl to the head of the column. At 10°C the eluate was adjusted to pH 2.5 with conc. aq. NH_3. (After the crystallization commenced at pH ≈ 0.5, the pH was increased in steps of 0.2 units and allowed to stabilize each time for 5 min in the stirred suspension, before the next portion of NH_3 was added.) The suspension was stirred for further 30 min at 7°C. The solid was suction-filtered, washed with 100 ml each of H_2O, acetone, and diisopropyl ether. It was dried in vacuo (25°C, 4 d) to give 92.3 g (38%) colourless crystals of 98.7% purity ("assay") and 1.6% water content (Karl Fischer titration). All spectra of **8** corresponded to those of a commercial sample purchased from Biochemie Kundl.

2-(Z)-(Hydroxyimino)-2-{2-[(triphenylmethyl)amino]thiazol-4-yl}acetic Acid (**12**): 780 ml (1.56 mol) of a 2 N aqueous NaOH solution was added at once to a stirred suspension of 300 g (0.61 mol) of ethyl 2-(*Z*)-(hydroxyimino)-2-{2-[(triphenylmethyl)amino]-thiazol-4-yl}acetate hydrochloride (**10**) in 1.14 l of THF. Due to a mildly exothermic reaction the temp. climbed to 30°C, and a clear two-phase solution was obtained. The crystallization of sodium salt **11** commenced after about 10 min. The mixture was stirred for 3.5 h at 50°C, then cooled to 5–10°C and maintained for 15 min. The precipitate was collected by suction-filtration and washed with 2 × 400 ml of THF. The solid was resuspended in a mixture of 660 ml of H_2O and 660 ml of EtOH. At 10°C, the pH of the stirred suspension was decreased by dropwise addition of 2 N aqueous HCl (≈440 ml), until it remained constant at pH 2.5. A change of the crystal form was noticed, and the pale yellow suspension was stirred for 30 min at 20°C. The precipitate was suction-filtered, washed with 2 × 250 ml of EtOH/H_2O (1:1) and dried at 50°C in vacuo to furnish 248.0 g (94%) of a colorless powder representing **12**. It was homogeneous by TLC ($CHCl_3$/CH_3OH/conc. NH_3, 80:20:2, detection 254 nm, R_f 0.20) and contained 0.1% of H_2O (Karl Fischer titration). – Thermal analysis (DSC, under 20 bar N_2, heating with 10°C/min) indicated broad maxima of exothermal heat flow (melting and/or decomposition) at 233 and 291°C. – ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 6.75 (s, 1H, thiazole H), 7.20–7.35 (m, 15H, aromatic H), 8.72 (s, 1H, NH), 11.60 (s, 1H, CO_2H), 13.06 (s, 1H, NOH). – $C_{24}H_{19}N_3O_3S$ (429.5): calcd. C 67.12, H 4.46, N 9.78, S 7.47; found C 66.8, H 4.6, N 9.7, S 7.2.

Triethylammonium 2-(Z)-[(1-Methoxy-1-methylethoxy)imino]-2-{2-[(triphenylmethyl)amino]thiazol-4-yl}acetate (**13**): 240 ml (*d* = 0.765, 2.55 mol) of 2-methoxypropene was added at once to the stirred suspension of 240 g (0.56 mol) of **12** in 1.2 l of acetone, contained in a flask with an efficient reflux condenser. The mixture was heated to 30–35°C (gentle reflux) for 1.5. A clear solution was obtained after about 40 min. Additional 120 ml (1.27 mol) of 2-methoxypropene was added at once, and the reflux was continued for additional 1.5 h, when TLC (conditions as described for **12**; R_f **13** 0.37) indicated the absence of **12**. The solution was cooled to 20°C and stirred for 1 h. At 20°C, with slight cooling, 96 ml (0.69 mol) of NEt_3 was added within 5 min. The suspension was stirred for 2 h. The precipitate was suction-filtered, washed with 120 ml of acetone/iPr_2O (1:1), then with 120 ml of iPr_2O, and dried at 20°C in vacuo to furnish 313.5 g (93%) of colorless crystals. Acidometric titration indicated 16.3% (by weight) of NEt_3 (calcd. 16.75%) and with two equivalents of acid 97% of **13**. – Thermal analysis (DSC, under 20 bar N_2, heating with 10°C/min) indicated a broad maximum of exothermal heat flow (melting and/or decomposition) with onset at 160°C and maximum at 173°C. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.31 (t, *J* = 7 Hz, 9H, CH_3 of HN^+Et_3), 1.52 [s, 6H, $OC(CH_3)_2O$], 3.09 (q, *J* = 7 Hz, 6H, CH_2 of HN^+Et_3), 3.27 (s, 3H, OCH_3), 4.73 (br. s, 1H, NH), 6.67 (s, 1H, thiazole H), 7.20–7.40 (m, 16H, 15 aromatic H and 1 NH). – $C_{34}H_{42}N_4O_4S$ (602.8): calcd. C 67.75, H 7.02, N 9.29, S 5.32; found C 67.3, H 7.2, N 9.0, S 5.1.

(6R,7R)-3-(Methoxymethyl)-7-{[2-(Z)-(1-methoxy-1-methylethoxy)imino]-2-[2-[(triphenylmethyl)amino]thiazol-4-yl]acetamido}-3-cephem-4-carboxylic Acid (**15**): At 13°C, 106 g (0.556 mol) of *p*-toluenesulfonyl chloride was added at once to a stirred suspension of 358 g (0.595 mol) of ammonium salt **13** in 920 ml of acetone. The suspension was stirred for 3 h at 13–14°C. Initially the suspension became more fluid, but later very viscous. This thick white suspension of the mixed anhydride **14** was then cooled to 0°C. In the meantime, in another flask, 275 ml (*d* = 0.725, 1.97 mol) of NEt_3 was added at once at 15°C to a stirred suspension of 133 g (0.536 mol) of AMCA (**8**) (98.5% purity) in 1.1 l of CH_2Cl_2. The mixture was stirred for 30 min and became a clear solution that was cooled to 0°C. This solution was transferred with a peristaltic pump at 0°C within 45 min into the cooled suspension of mixed anhydride **14**. After stirring for 30 min at 0–5°C, 110 ml of glacial acetic acid was added. Vacuo (20–30 Torr) was applied, and the solvents were evaporated at ≤15°C until the suspension became thick and difficult to stir. 800 ml of butyl acetate was added, and vacuo was applied again at 20°C until no more solvent distilled off (residual volume <900 ml). The mixture was cooled to 10°C, 900 ml of H_2O was added, and a two-phase emulsion of pH 5.5 was obtained. At 10°C it was acidified to pH 4.0 with 1 N HCl (≈640–670 ml). The suspension was stirred for 1 h at 10°C, and the precipitate was suction-filtered. It was washed with 500 ml of butyl acetate/H_2O (1:1). The solid was resuspended with stirring in 700 ml of acetone, stirred for 45 min at 10°C and then suction-filtered. It was washed with 250 ml of acetone, 1.2 l of iPr_2O and then dried in high vacuum for 3 d. No further significant weight loss was observed after the first two days. 344 g of **15** was obtained, that according to GC contained the following weight-% of solvents: 12.4% of butyl aceate, 2.4% of acetone, 0.6% of CH_2Cl_2, and 0.3% of H_2O. The yield corrected for 15.7% of solvents was 290.0 g **15** (74%). HPLC [250 × 4.0 mm 7-µm Nucleosil RP18; eluent A: MeOH/H_2O, 80:20, eluent B: H_2O + 0.1% NH_4OAc; ratio of eluents: A/B = 80:20; 25°C, flow 1.0 ml/min, detection 254 nm; t_{ret}: **8** (1.65 min), **12** (3.80), **13** (4.77), **15** (7.87)] indicated 97.2% of **15**, 2.0% of **13**, 0.5% of **12**, <0.1% of **8**. – IR (KBr): $\tilde{\nu}$ = 3300 cm^{-1} (NH, OH), 1780 (C=O, β-lactam), 1710 (C=O, carboxylic acid), 1680 and 1530 (C=O, *sec.* amide). – UV (MeOH): λ_{max} (lg ε) = 232 nm (4.43), 260 (4.23). – ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.37 [s, 6H, $NOC(CH_3)_2O$], 3.10 [s, 3H, $C(CH_3)_2OCH_3$], 3.19 (s, 3H, CH_2OCH_3), 3.45 and 3.55 (AB system, *J* ≈ 18 Hz, 2H, CH_2S), 4.16 (br. s, 2H, CH_2O), 5.12 (d, *J* ≈ 5 Hz, 1H, 6-CH), 5.67 (dd, *J* ≈ 8 and 5 Hz, 1H, 7-CH), 6.67 (s, 1H, 5'-CH), 7.19 (m, 3H, aromatic H), 7.27 (m, 6H, aromatic H), 7.34 (m, 6H, aromatic H), 8.87 (s, 1H, 2'-NH), 9.52 (d, *J* ≈ 8 Hz, 1H, 7-NH); additionally the signals of 1 equiv. of butyl acetate are indicated: δ = 0.88 (t, *J* ≈ 7 Hz, 3H, δ-CH_3), 1.33 (m, 2H, γ-CH_2), 1.54 (m, 2H, β-CH_2), 1.98 (s, 3H, $COCH_3$), 3.97 (t, *J* ≈ 7 Hz, 2H, α-CH_2). – MS (FAB; matrix: 3-NBA + 10% PEG-2000), *m/z* (%): 728 (3) [M + H$^+$], 656 (2) [M + H$^+$ of the free oxime], 243 (100) [Ph_3C^+], 165 (15) [243 – C_6H_6]. – MS (FAB, matrix: 3-NBA + LiCl), *m/z* (%): 734 (8) [M + Li$^+$], 662 (8) [M + Li$^+$ of the free oxime], 243 (100), 165 (21).

[(RS)-1-(Pivaloyloxy)ethyl] (6R,7R)-3-(Methoxymethyl)-7-{[2-(Z)-(1-methoxy-1-methylethoxy)imino]-2-[2-[(triphenylmethyl)amino]thiazol-4-yl]acetamido}-3-cephem-4-carboxylate (**16**). – a) *By Esterification of* **15** *with Iodohydrin Ester* **27**: At 20°C, 63.0 g (0.414 mmol) of DBU was added at once to the cooled, stirred suspension of 337.5 g (0.391 mol, corrected for 15.7 weight-% of solvents) of **15** in 3.25 l of acetone. Within 10 min a clear yellow-brown solution was obtained. At 20°C, 134.6 g (0.500 mol, corrected for a titer of 95%) of **27** was added at once, and the deep-brown solution was stirred for 1 h at 20°C. At 20°C it was added dropwise within 30 min into 20.0 l of vigorously stirred H_2O to furnish a yellow precipitate which was further stirred at 20°C for 2 h, while a stream of N_2 was bubbled through the suspension in order to evaporate the butyl acetate which formed a thin organic layer on top of the water layer and which renders the filtration of **16** more difficult by giving parts of it a gummy consistence. The solid was then suction-filtered, washed with 3 × 1 l of H_2O and dried in high vacuum at 25°C for 3 d to furnish 350 g of **16**, m.p. 120–123°C (dec.), that according to GC and Karl Fischer titration contained the following weight-% of solvents: 1.0% of butyl acetate, 1.1% of acetone, 1.3% of H_2O. The yield corrected for 3.4% of solvents was 338.1 g (101%). HPLC (250 × 4.0 mm 7-µm Nucleosil Phenyl; eluent: 50% MeOH, 50% 0.01 M aqueous NH_4OAc buffer, 0.5 ml/min; detection 254 nm) indicated 92% of **16** (t_{ret} 24.3 and 25.1 min, ratio of diastereomers 51:49), 2.0% of **23** (t_{ret} 22.2 min), 0.5% of Δ^2-isomer **21** (21.0 min), 1.2% of **17** (17.2 and 17.7 min, ratio of diastereomers 45:55), 1.3% of **15** (6.5 min), 3% of unidentified impurities. A better separation of the two diastereomers of **16** and **17** is achieved on 250 × 4.0 mm 7-µm Nucleosil RP 18 (eluent A: MeOH/H_2O 80:20; eluent B: 1600 ml of H_2O, 900 ml of MeCN, 5.5 g of $NH_4H_2PO_4$, adjusted to pH 3.5 with conc. H_3PO_4; eluent ratio A:B = 95:5, flow: 1.0 ml/min, detection 254 nm): **16** (t_{ret} 20.2 and 21.6 min, ratio 52:48), **17** (t_{ret} 12.1 and 13.0 min, ratio 56:44). HPLC assay (comparison with the peak area of a chromatographed sample of **16**) indicates a content of 86 weight-% **16**. – ^{1}H NMR (270 MHz, [D$_6$]DMSO; duplication of the signals is due to the presence of the 52:48 mixture of diastereomers): δ = 1.12/1.14 (s, 9H, CH_3), 1.37 [s, 6H, $OC(CH_3)_2O$], 1.47/1.48 (d, J = 5 Hz, 3H, CH_3), 3.12 (s, 3H, CMe_2OCH_3), 3.18/3.19 (s, 3H, CH_2OCH_3), 3.50–3.67 (m, 2H, CH_2S), 4.11/4.12 (s, 2H, CH_2O), 5.17/5.20 (d, J = 5 Hz, 1H, 6-CH), 5.72/5.75 (m, 1H, 7-CH), 6.72 (s, 1H, 5′-CH), 6.86/6.92 (q, J = 5 Hz, 1H, OCHO), 7.16–7.42 (m, 15H, aromatic H), 8.86 (s, 1H, N*H*trityl), 9.49/9.52 (d, J = 5 Hz, NHCO). – MS (FAB, matrix: 3-NBA + LiI), *m/z* (%): 862 (77) [M + Li$^+$], 790 (100) [M + Li$^+$ – CH_2=CMe–OMe].

b) *By Esterification of* **15** *with Bromohydrin Ester* **30**: At 20°C, 1.90 g (12.48 mmol) of DBU was added at once to the stirred suspension of 10.0 g (11.58 mmol, corrected for 15.7 weight-% of solvents) of **15** in 100 ml of acetone. An orange, slightly turbid solution was obtained. 3.65 g (17.45 mmol) of **30** was added at once, and the solution was stirred for 27 h at 20 ± 2°C. HPLC monitoring of aliquots (0.1 ml each) taken after 1, 3, 6, and 23 h indicated 16.5, 7.5, 5.9, and 5.5%, respectively, of **15** and less than 0.2% of the Δ^2-isomer **21**. At 15–20°C the bulk of acetone was evaporated in vacuo. The concentrated solution was poured into 200 ml of vigorously stirred water. The precipitate was filtered off, washed with water and dried in vacuo to furnish 10.1 g (102%) of a yellow powder, that contained 5% of **15** and had a content of 87% **16** according to HPLC assay.

1-Iodoethyl 2,2-Dimethylpropanoate (**27**): At 0°C, 1.37 kg (9.14 mol) of NaI that had been dried for 18 h at 125°C in vacuo, was added in portions with stirring and efficient cooling to 8.5 l of MeCN. The salt dissolved exothermally, and a clear solution was obtained. 401.2 g (9.11 mol) of acetaldehyde (**25**) was added within 5 min at 0°C, and the mixture was stirred for 5 min. 1.00 kg (8.29 mol) of pivaloyl chloride (**26**) was added within 45 min at 0°C with efficient cooling. The resulting colorless suspension was stirred for 2 h at 0°C and was poured at –2°C within 5 min into an unstirred, cooled mixture of 34 l of H_2O, 1.7 kg of $NaHCO_3$, and 0.53 kg of $Na_2S_2O_3$. The mixture was stirred slowly (50 min^{-1}) for 5 min while the reaction temp. dropped from –2 to –5°C. The (upper) aqueous layer was decanted within 5 min, and 23 l of H_2O (0°C) was added to the product layer. The mixture was stirred slowly for 5 min. The aqueous layer was decanted within 5 min. 0.20 kg of anhydrous Na_2SO_4 was added to the product layer. The mixture was stirred slowly for 10 min, and Na_2SO_4 was removed by suction-filtration. 1.81 kg of **27** with a titer of 95.0% (GC) was furnished as a pale yellow oil. GC (15 m × 0.25 mm fused silica gel column DB 1701, split 1:300, 0.5 bar He carrier gas; injector and oven: 110°C; detection (FID): 150°C): t_{ret} (%) [**25**: 0.79 min (0), MeCN: 0.83 (2.0), **26**: 0.94 (0.7), **28**: 1.18 (0.5), unknown: 1.33 (1.4), **27**: 2.66 (95.0), **31**: 3.87 (0.3), unknown: 4.05 (0.1)]. – The same titer was determined, when **27** was derivatized to **32** with benzoic acid and then analyzed by HPLC as described for bromo ester **30**. The yield, corrected for the titer, was 81%. – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.19 (s, 9H, CH_3), 2.21 (d, J = 6 Hz, 3H, CH_3), 6.86 (q, J = 6 Hz, 1H, CH). – $C_7H_{13}IO_2$ (256.1): calcd. C 32.83, H 5.12, I 49.56; found C 32.4, H 5.2, I 49.2. – The product must be stored with exclusion of light at –20 to –30°C under N_2. Even under these conditions it turned deep red-brown within a few days, but the titer did not significantly change within one month. *Note:* The yield was considerably lower at a higher reaction temp. (≈50% yield at 20°C), in the presence of water (0.5% of H_2O, 20°C: 32% yield), and in the case of a more intense or elongated time of contact with water during workup (55% yield, if the aqueous mixture was *stirred* while crude **27** was introduced). When 32 g of **27** was stirred at 0°C with 200 ml of water, hydrolysis to pivalic acid (**28**) and HI (12 and 9%, respectively, after 30 min; 22 and 14% after 1 h) was indicated by titration of aliquots.

1-Bromoethyl 2,2-Dimethylpropanoate (**30**): At –10°C, 15.1 g (0.11 mol) of $ZnCl_2$ was added to the stirred and cooled solution of 1827.8 g (11.08 mol) of pivaloyl bromide (**29**)[22] in 1.0 l of CH_2Cl_2. At –10 to 0°C the solution of 0.650 l (11.63 mol) of acetaldehyde (**25**) in 0.5 l of CH_2Cl_2 was added dropwise within 2 h. The mixture was stirred at –5 to 0°C for 0.5 h. It was poured into 1.5 l of ice/water, stirred for 2 min, and the aqueous layer was removed. The organic phase was washed three times with 0.5 l of a saturated $NaHCO_3$ solution and 150 g of ice each, and then dried ($MgSO_4$). The solvent was evaporated in vacuo, and the residue was distilled in vacuo through a 50-cm Vigreux column to afford 1924 g (83%) of a colorless liquid, b.p. 62°C/14 Torr. – Refractive index (20°C): 1.4360. – GC (conditions as described for **27**): purity 97–100%. – Thermal analysis (DSC, under 20 bar N_2): exothermal decomposition (507.9 J/g) with onset at 228°C and maximum at 239.3°C. – ^{1}H NMR (60 MHz, $CDCl_3$): δ = 1.22 (s, 9H, CH_3), 2.01 (d, J = 6.0 Hz, 3H, CH_3), 6.72 (q, J = 6.0 Hz, 1H, CH). – MS (CI, isobutane), *m/z* (%): 211 (28) [M + H$^+$, ^{81}Br], 209 (29) [M + H$^+$, ^{79}Br], 129 (21) [M$^+$ – Br], 103 (100) [M + H$^+$ of pivalic acid **28**]. – $C_7H_{13}BrO_2$ (209.1): calcd. C 40.21, H 6.27, Br 38.22; found C 39.7, H 6.3, Br 37.8. – The GC analysis had a standard deviation of 2%. For a highly reproducible analysis, **30** was derivatized with benzoic acid to acylal **32**, which was then analyzed by HPLC: 150 mg of **30** was added to the solution of 100 mg of benzoic acid and 0.15 ml of DBU in 2.5 ml of CH_2Cl_2, contained in a 25-ml volumetric flask. The mixture was shaken and allowed to stand stoppered at ambient temp. for 30 min. The solution was

diluted to the mark with CH_2Cl_2 and then washed twice with 10 ml of water each. 2 ml of the organic solution was pipetted into a 10-ml volumetric flask and diluted to the mark with the mobile phase for HPLC (250 × 4.6 mm 5-µm LiChrosorb Si 60; mobile phase: 95% heptane, 5% dioxane; flow: 1 ml/min; detection 274 nm; injection vol.: 20 µl): t_{ret} (%) [**32**: 6.05 min (99.2), **31**: 8.31 min (0.5), unknown: 11.59 min (0.3)].

1,1-Bis(pivaloyloxy)ethane (**31**) was isolated by distillation of the distillation residue of **30**; colorless liquid, b.p. 89–91 °C/10 Torr, GC: 99% purity. – ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.20 (s, 18H, CH_3), 1.47 (d, J = 6 Hz, 3H, CH_3), 6.82 (q, J = 6 Hz, 1H, CH). – MS (CI, NH_3), *m/z* (%): 248 (60) [M + NH_4^+], 186 (100) [M^+ – CH_3CHO], 85 (30) [tBuCO$^+$]. – $C_{12}H_{22}O_4$ (230.3): calcd. C 62.58, H 9.63; found C 62.3, H 9.4.

[1] Synthesis: A. Furlenmeyer, U. Weiss (Hoffmann-La Roche), EPA 231845, **1987** [*Chem. Abstr.* **1988**, *108*, 112064].

[2] Synthesis: K. Fujimoto, S. Ishihara, H. Yanagisawa, J. Ide, E. Nakayama, H. Nakao, S.-I. Sugawara, M. Iwata, *J. Antibiot.* **1987**, *40*, 370–384.

[3] F. Adam, J. Blumbach, W. Dürckheimer, G. Fischer, B. Mencke, D. Isert, G. Seibert (Hoechst AG), EPA 329008, **1989** [*Chem. Abstr.* **1990**, *112*, 98285].

[4] A. Bauernfeind, R. Jungwirth, E. Eberlein, N. Klesel, F. Adam, D. Isert, M. Limbert, A. Markus, E. Schrinner, G. Seibert, *J. Antibiot.* **1992**, *45*, 505–520.

[5] D. Isert, N. Klesel, M. Limbert, A. Markus, R. Lattrell, E. Schrinner, G. Seibert, Poster presented at 31st Intersci. Conf. Antimicr. Agents & Chemother., Chicago, Il., **1991**.

[6] F. Adam, W. Dürckheimer, B. Mencke, D. Isert (Hoechst AG), EPA 402806, **1990** [*Chem. Abstr.* **1991**, *115*, 78903].

[7] Significant decomposition was detected by HPLC in samples of pure, solid **3** after storage at 25 °C for one month and in aqueous solutions of **3** at 37 °C after 1 h (at pH 6.8) and 5 h (at pH 4.5), respectively.

[8] [8a] E. Defoßa, G. Fischer, D. Isert, H. Jendralla, R. Lattrell, T. Wollmann, EPA 514791, **1992** [*Chem. Abstr.* **1993**, *118*, 147401]. – [8b] D. Isert, N. Klesel, M. Limbert, A. Markus, G. Riess, G. Seibert, Poster presented at 32nd Intersci. Conf. Antimicr. Agents & Chemother., Anaheim, Ca., **1992**.

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[10] E. Defoßa, G. Fischer, U. Gerlach, R. Hörlein, D. Isert, N. Krass, R. Lattrell, U. Stache, T. Wollmann, *Liebigs Ann.* **1996**, 1743–1749; following paper.

[11] In 1991, AMCA (**8**) of excellent purity (98–99.5%) became commercially available from Biochemie Kundl, Austria.

[12] The preparation of AMCA (**8**) from ACA (**7**) under different sets of acidic conditions has been claimed in several patents: [12a] B. Prager, H. Sturm (Biochemie Kundl), EPA 204657, **1986** [*Chem. Abstr.* **1987**, *106*, 156152]. – [12b] J. Nishikido, K. Fukuzaki (Asahi Kasei Kogyo Kabushiki Kaisha), EPA 262744, **1988** [*Chem. Abstr.* **1988**, *109*, 190123]. – [12c] F. Adam, W. Dürckheimer, R. Hörlein, B. Mencke (Hoechst AG), Ger. Offen. DE 4004370, **1991** [*Chem. Abstr.* **1991**, *115*, 182950]. – [12d] Y. Hirayama, S. Sayama, K. Yamaguchi, S. Miyamoto, Y. Takahashi, K. Kato, T. Sasao, K. Iijima (Sankyo), EPA 485204, **1992** [*Chem. Abstr.* **1992**, *117*, 69659]. – [12e] M. Hirayama, S. Sayama, S. Watanabe, K. Yamaguchi, S. Miyamoto, K. Kato, T. Sasao, S. Iijima (Sankyo), Jpn. Kokai Tokkyo Koho JP 06145175, **1994** [*Chem. Abstr.* **1994**, *121*, 133861].

[13] This polystyrene resin is commercially available from Mitsubishi Chemicals.

[14] T. W. Greene, P. G. M. Wuts, *Protective Groups in Organic Synthesis*, 2nd ed., Wiley, New York, **1991**. – [14a] p. 364–368. – [14b] p. 39.

[15] P. J. Kocienski, *Protecting Groups*, Thieme Verlag, Stuttgart, **1994**; [15a] p. 220–232; [15b] p.84.

[16] For the same reason, it is advantageous to esterify the carboxylic acid functionality of **8** (to introduce the centre of diastereomerism) as late as possible in the synthetic sequence. When the acylal of **8** instead of the free carboxylic acid **8** was used in the coupling reaction with **14**, the crystalline intermediate **15** was circumvented, and purification from by-products was more difficult. Secondly, each crystallization of crude **16**, **3**, and **4** tended to shift the diastereomeric ratio away from 50:50, due to the pronouncedly different crystallization tendencies and (or) solubilities of the two diastereomers. Thirdly, the isomerization of the Δ^3-double bond into conjugation with the sulfur atom ($\Delta^3 \rightarrow \Delta^2$ isomerization) occurs most easily under basic conditions once the carboxylic acid is esterified. Since the coupling with mixed anhydride **14** requires basic conditions, formation of the Δ^2-isomer **21** is difficult to suppress if an ester (an acylal) of **8** is used.

[17] Alternatively, for a less efficient synthesis of **27** by the reaction of the corresponding chlorohydrin ester with sodium iodide see: Y. Yoshimura, N. Hamaguchi, T. Yashiki, *J. Antibiot.* **1986**, *39*, 1329–1342.

[18] Preparation of similar bromohydrin esters: [18a] M. Neuenschwander, P. Bigler, C. Christen, R. Kyburz, H. Mühle, *Helv. Chim. Acta* **1978**, *61*, 2047–2058. – [18b] P. Knochel, T.-S. Chou, C. Jubert, D. Rajagopal, *J. Org. Chem.* **1993**, *58*, 588–599.

[19] With the same method 4–17% of oligomers were detected in aqueous solutions of several commercial cephalosporin derivatives (Cefotiam®, Ceftazidime®, Cefpirome®) that had been allowed to stand 1 d at 25 °C.

[20] *p*-Toluenesulfonic acid monohydrate p.a. from Merck Darmstadt was used. Other qualities contain up to 10% of isomers and disulfonic acid. The reagent should be colorless to pale pink. If it is brownish, it must be recrystallized with active charcoal from boiling ethyl acetate.

[21] H. Jendralla, C.-H. Li, E. Paulus, *Tetrahedron: Asymmetry* **1994**, *5*, 1297–1320.

[22] Y. Yamase, *Bull. Chem. Soc. Jpn.* **1961**, *34*, 480–484.

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