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Concise Large-Scale Synthesis of the Highly Active Cephalosporin Cefdaloxime

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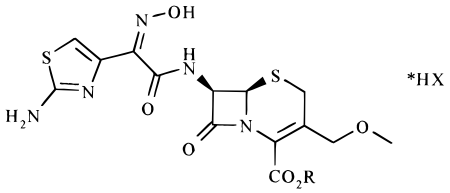
Abstract:
Cefdaloxime (1a) is the bioactive principle of the 1-(*S*)-(pivaloyloxy)ethyl ester prodrug HR916K (1b). To provide material for biological investigations a short and efficient large-scale synthesis of 1a was developed, which avoids chromatographic purification steps. Commercially available (6*R*,7*R*)-7-amino-3-(methoxymethyl)-3-cephem-4-carboxylic acid (AMCA) (2) is acylated with trityl-protected mercaptobenzothiazole thioester in the presence of bis(trimethylsilyl)acetamide to yield tritylated cefdaloxime. The trityl group is then removed by treatment with formic acid, followed by pH-adjusted precipitation of 1a. For a final purification, which has to consider cefdaloxime specific side reactions, crude 1a is dissolved in dimethyl sulfoxide and precipitated with methanol to obtain 1a in 66% overall yield on a kilogram scale.

HR916K (**1b**)[1,2] (Chart 1) belongs to a therapeutically interesting group of AMCA-derived[3] prodrug esters, synthesized by acylation with protected mercaptobenzothiazole thioester **3b**.[4] Similar procedures lead to the cefdaloxime precursor **4**. Cephalosporins of the core unit **2** (Scheme 1) have been shown in the past to be only moderate orally absorbable β-lactam antibiotics. However, in the early 1980s Heymes and Pronine[6] at Roussel Uclaf synthesized bioactive compounds of the general formula **5** with different substituents R, R′, and A and screened them very successfully against Gram-positive and Gram-negative bacteria. To improve absorption, prodrug esters of the free carboxylic acids have been used as interesting chemical modifications of the β-lactam drug unit. In particular the racemic prodrug ester HR916B (**1c**) exhibits good oral bioavailability in different animal species.[7] But in a further biological screening the diastereomer, HR916K (**1b**), appeared much better enterically absorbable than its diastereomer HR916J (**1d**)[2,8−10] and was therefore chosen as a candidate for additional clinical investigations. For comparisons with **1b** in clinical studies it was necessary to synthesize the free acid **1a** on a multi kilogram scale.

Chart 1. **Bioactive compounds 1 and 5 and protecting group intermediates 3, used in the synthesis of 1a**

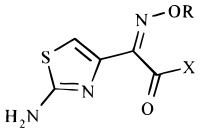


1a: R = H, without HX

1b: R = (*S*)-CH(CH$_3$)OC(O)tBu, HX = p-TsOH

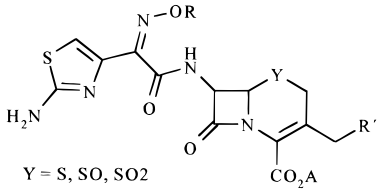
1c: R = *rac*-CH(CH$_3$)OC(O)tBu, HX = p- TsOH

1d: R = (*R*)-CH(CH$_3$)OC(O)tBu, HX = p-TsOH



3a: R = trityl, X = OH

3b: R = trityl, X = mercaptobenzothiazole



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A short large-scale synthesis of **1a** uses advantageously a strategy avoiding extensive and time-consuming chromatographic purification procedures at all reaction stages. A corresponding production method has to take into consideration the nature of emerging by-products and the mode of their removal. For that reason the introduction of the oxime moiety by amidation of the AMCA NH_2 group should be carried out with an activated ester, whose activating part can easily be cleaved and does not interfere with the subsequent reaction step. Furthermore, additional coupling reagents and protecting groups should be used sparingly. Last, but not least, the final purification process has to utilize the different

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(1) Defossa, E.; Fischer, G.; Gerlach, U.; Hörlein, R.; Isert, D.; Krass, N.; Lattrell, R.; Stache, U.; Wollmann, T. *Liebigs Ann. Chem.*, submitted.
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(3) (6*R*,7*R*)-7-Amino-3-(methoxymethyl)-3-cephem-4-carboxylic acid (**2**) (AMCA) from Biochemie Kundl with a purity of 96.5% (HPLC) was used.
(4) The synthesis of the corresponding free acid **3a** was published by Kamachi et al.,[5] whereas Defossa et al.[1] modified the preparation of activated derivatives of **3a** due to safety concerns during scale-up investigations.
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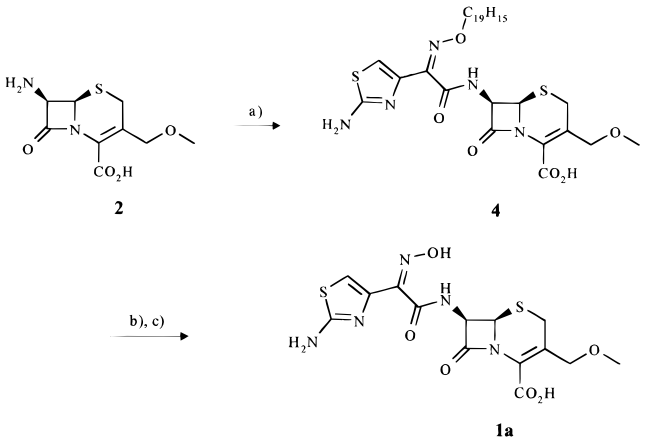
(10) Mendes, P.; Meyer, B. H.; Müller, F. O.; Scholl, T.; Luus, H.; de la Rey, N. Poster presented at *32nd Interscience Conference on Antimicrobial Agents & Chemotherapy*, Anaheim, CA, 1992.

Scheme 1. **Two-step synthesis of 1a via amidation of 2, subsequent removal of trityl protecting group, and purification**[a]



[a] (a) NMP, bis(trimethylsilyl)acetamide, **3b**; (b) formic acid; (c) purification by precipitation of a DMSO solution of crude **1a** in MeOH; 66% overall yield.

solubility properties of **1a** and its impurities and take into account the chemical reactivity of the β-lactam backbone.

The formation of amide **4** from **2** and trityl-protected oxime **3** was investigated using different combinations of solvents and amidation conditions. With samples provided by Wollmann et al.,[1] who have given detailed experimental procedures for the independent synthesis of both Δ^2 and Δ^3 cephalosporin and *syn* and *anti* isomers of the oxime moiety, and analytical data of Jendralla et al.,[2] who have developed analytical methods, especially HPLC, for a simultaneous discrimination of all isomers in any combination, we were able to determine product ratios of our reaction mixtures by HPLC comparison with respect to all isomers of **1a**, **3**, and **4**. Several in situ amine activations, such as treatment with chlorotrimethylsilane and tertiary nitrogen bases, needed increased reaction temperatures up to about 70 °C and afforded only moderate yields, not exceeding 60%. The auxiliary base hexamethyldisilazane induced up to 20% cephalosporin double bond shift, as could be detected by HPLC comparison of the reaction mixtures with reference material. Different mixed anhydrides, as, for example, the methanesulfonic anhydride of the corresponding acid **3a** of the oxime, produced partly unknown impurities, presumably β-lactam oligomers and ring-opened products, which could only be removed by chromatographic methods. However, the thioester **3b**[11] showed good results as an amidation agent with bis(trimethylsilyl)acetamide assistance. The reaction components **3b** and **2** were mixed at room temperature in *N*-methylpyrrolidin-2-one and stirred until the initially yellow colour changed to dark green. The mixture was poured into cold brine, and the precipitate, consisting of the amide **4**, mercaptobenzothiazole, and small amounts (<5%) of triphenylmethanol (from undesired trityl oxime cleavage), was collected by centrifugation, without further purification.

The subsequent cleavage of the trityl protecting group in formic acid was not hampered by the detected impurities in the raw material, nor did they catalyse undesired side reactions such as Δ^2 to Δ^3 rearrangement of the cephalosporin skeleton or *syn*−*anti* isomerization of the oxime moiety. HPLC monitoring of the deprotection showed smooth reaction at room temperature within a few hours. Due to their low solubilities in formic acid at about 5 °C, the by-products, mainly triphenylmethanol and residual mercaptobenzothiazole, could be removed by simple filtration. After pH adjustment of the remaining mother liquor to pH 2.5, **1a** precipitated with a purity of 92% area ratio. Wollmann et al.[1] reported an alternative cleavage procedure of the trityl-protected **1b** with *p*-TsOH. However, they observed increasing amounts of the oxime *anti* isomer depending on the water content of starting amides. The trityl cleavage with *p*-TsOH and scrupulously dried educt **4**[12] in 1-propanol at increased temperature resulted in low yields of product **1a** with a poor quality and confirmed earlier observations[1] with respect to the by-product profile. For a final purification of crude **1a** we had to take care particularly of three aspects: the reactivity of the β-lactam ring, the double-bond isomerization in the six-membered thiazine ring sytem, and the oxime *syn*−*anti* isomerization. First approaches were carried out with HP 20 adsorption chromatography and water as liquid phase, but the runs were difficult to evaluate with sometimes severe loss of yield. Conversion of the free acid **1a** into its water soluble sodium salt and subsequent precipitation seemed to be a more reliable method. **1a** was treated with equimolar amounts of 1 N NaOH, separated from insoluble residues, and finally precipitated by the addition of acetone. But for a further improvement of the HPLC purity, a second conversion back to the free acid had to be done. Solubility experiments showed, however, good purification results by repeated treatment with 2-propanol or methanol. Therefore, crude **1a** was dissolved in dimethyl sulfoxide, methanol added to the mixture, and the temperature lowered continuously. Final filtration and drying afforded a nearly colourless powder with a purity of >97.5% and an overall yield of 66% with less than 0.06 mass % residual solvents content.

With the reported synthesis strategy, we were able to provide **1a** on a multikilogram scale, pure enough for clinical studies. We could avoid chromatographic purification and did not need any special activating reagents for the amide coupling. The amounts of β-lactam ring opened products, *syn*−*anti* isomerization of the oxime moiety, and Δ^2 to Δ^3 double bond isomerization with less than 0.7% of any single impurity component were negligible with the procedure used.

Experimental Section

Bis(trimethylsilyl)acetamide (99%) was obtained from Sigma-Aldrich-Fluka. Formic acid (>99%) was purchased from Riedel-de Haen. 2-Benzothiazolyl (*Z*)-(2-aminothiazol-4-yl)[(triphenylmethoxy)imino]thioacetate (**3b**) was generated according to the method described in detail by Wollmann et al.[1] *N*-Methylpyrrolidin-2-one (NMP, >99%), dimethyl sulfoxide (DMSO, >99%) and methanol (MeOH, >99%) were used without further purification. All reactions were carried out under a nitrogen atmosphere and monitored with Merck precoated TLC plates (Kieselgel 60 F_{254}, 0.2 mm). HPLC (Kontron Instruments MT2): (6*R*,7*R*)-7-[(*Z*)-(2-Aminothiazol-4-yl)(hydroxyimino)acetamido]-3-(methoxymethyl)-3-cephem-4-carboxylic acid (**1a**) was analyzed

(11) For a synthesis of **3b** from **3a** and bis(benzothiazol-2-yl) disulfide and different alternatives, see ref 1 and literature cited therein.

(12) **4** was dried to a water content of <1%, as determined by Karl Fischer titration.

on a LiChrospher 100 RP-18 (5 μm) column (125 mm × 4 mm, Merck) using 0.1% NH_4OAc in water as solvent A and 0.1% NH_4OAc in acetonitrile/water (4:1) as solvent B with a linear gradient method (0 min/100% A, 20 min/0% A) and a flow rate of 1 mL/min (detection: 260 nm); percent values are basically given as peak area ratios unless otherwise characterized. After orientating laboratory experiments to a maximum reaction volume of 4 L with glass-based equipment determining the basic reaction course, the following scale-up was carried out in a pilot plant using glass vessels with agitation in a range of 25−70 L. Working-up procedures exceeding a 70 L volume were divided into fitting portions. Melting points were determined on a Büchi 535 melting point apparatus and are uncorrected. Safety thermal analysis measurements were done on DuPont 9900 thermal analysis DSC with a heating up speed of 3 °C/min. ^{1}H-NMR spectra (internal standard TMS) were recorded on a Varian Gemini 200 and a Bruker AM 270 spectrometer, respectively, and are reported in parts per million. Mass spectra were determined at 70 eV on a Fisons Instrument VG TRIO 2000 mass spectrometer.

(6*R*,7*R*)-7-[(*Z*)-(2-Aminothiazol-4-yl)[(triphenylmethoxy)-imino]acetamido]-3-(methoxymethyl)-3-cephem-4-carboxylic Acid (4). To a well-stirred mixture of 5.8 kg (10 mol) of 2-benzothiazolyl (*Z*)-(2-aminothiazol-4-yl)[(triphenylmethoxy)imino]thioacetate (**3b**) and 45 L of NMP were added 2.2 kg (9 mol) of AMCA[3] (**2**) and subsequently 2.48 L of bis(trimethylsilyl)acetamide at room temperature. The initially yellow suspension slowly changed colour to give a dark green solution. After 2 h the reaction solution was poured into a mixture of 150 L of saturated brine and 80 kg of ice, stirred for a further 30 min, and then centrifuged. The wet product was dried under vacuum at 30 °C[12] to afford 6.6 kg (>100% theory; contains mercaptobenzothiazole as the principal by-product and small amounts of triphenylmethanol) of crude **4**. This was used for the next reaction step without further purification. IR (KBr): ν 3440 cm^{-1} (OH), 3061 (=CH), 1785 (β-lact C=O), 1660. ^{1}H-NMR (200 MHz, d_6-DMSO) δ 9.90 (d, 1H, J = 8 Hz), 7.40−7.15 (m, 15H), 6.60 (s, 1H), 5.90 (dd, 1H, J = 8, 5 Hz), 5.25 (d, 1H, J = 5 Hz), 4.20 (s, 2H), 3.60 (d, 1H, J = 18 Hz), 3.47 (d, 1H, J = 18 Hz), 3.22 (s, 3H). MS (70 eV): *m*/*z* (relative intensity) 678 (10) [M + Na$^+$], 656 (15) [M + H$^+$], 243 (50) [$C_{19}H_{15}^+$].

(6*R*,7*R*)-7-[(*Z*)-(2-Aminothiazol-4-yl)(hydroxyimino)-acetamido]-3-(methoxymethyl)-3-cephem-4-carboxylic Acid (1a). To a suspension of 6.6 kg of crude **4** in 20 L of formic acid was added 4 L of H_2O, and then the mixture was stirred at room temperature for 2 h. The reaction mixture was cooled to 5 °C and filtered by suction, and the residue was washed three times with 2.5 L portions of formic acid/H_2O (5:1). The mother liquor was added with stirring to 130 L of H_2O of 5 °C, and the resulting diluted solution was adjusted to pH 2.5 with 25% aqueous ammonia and stirred for a further 1.5 h at 5 °C. The mixture was filtered by suction and the residue washed once with 3 L of H_2O and once with 3 L of MeOH. The precipitate was suspended in 13 L of MeOH at room temperature, stirred for 1 h, and filtered a second time. The residue was dried under vacuum at 25 °C to constant weight to yield 2.7 kg (73% over two steps) of a pale yellow powder with an HPLC content of 92%. This material was subjected to a final purification procedure. Mp: 168 °C dec. IR (KBr): ν 3298 cm^{-1} (OH), 1756 (β-lact C=O), 1642. ^{1}H-NMR (27 MHz, d_6-DMSO): δ = 11.30 (s, 1H), 9.45 (d, 1H, J = 8 Hz), 7.15 (s, 2H), 6.65 (s, 1H), 5.75 (dd, 1H, J = 8, 5 Hz), 5.15 (d, 1H, J = 5 Hz), 4.17 (s, 2H), 3.55 (d, 1H, J = 18 Hz), 3.42 (d, 1H, J = 18 Hz), 3.20 (s, 3H). MS (70eV): *m*/*z* (relative intensity) 414 (100) [M + H$^+$], 227 (20) [M$^+$ − $C_5H_6N_4O_2S$].

Purification of 1a. Crude **1a** (2.7 kg with 92%) was suspended in 4.2 L of DMSO at 30 °C and stirred until the mixture became a clear dark red solution. Thereafter 40 L of methanol was added continuously over 1 h, while the temperature was lowered continuously to a final temperature of 20 °C. The mixture was stirred for a further 30 min at 20 °C and then filtered by suction. The filter cake was washed four times with methanol (15 L total) and dried under vacuum at 40 °C to yield 2.4 kg (89%) of a nearly colourless powder with a purity of >97.5% (HPLC), less than 0.06 mass % residual solvents content as measured by drying loss at 70 °C in vacuum, and less than 0.7% (HPLC) of any single component impurity. Mp: 173 °C dec. A safety thermal analysis scanning 25−250 °C showed no exothermic reaction below the melting point. Spectroscopic data correspond with those mentioned above.

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