The Journal of Antibiotics (2019) 72:702–708
https://doi.org/10.1038/s41429-019-0190-z

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

ARTICLE

Isolation and characterization of side-products formed through Δ2-isomerization in the synthesis of cefpodoxime proxetil

Matthias Pieper[1] · Herbert Schleich[2] · Harald Gröger[1]

Received: 12 November 2018 / Revised: 5 March 2019 / Accepted: 12 March 2019 / Published online: 19 June 2019

Abstract

In the synthesis of cephalosporin antibiotics, esterified in 4-position, the Δ2-isomerization is a well-known side reaction proceeding under basic conditions. In this work, we investigated the Δ2-isomerization of the esterified cefpodoxime proxetil. Due to the *R*-configuration and *S*-configuration of the stereogenic center in the side chain in 4-position, there are two starting materials being diastereomeric to each other. Furthermore, an additional stereogenic center is formed in the isomerization step, thus leading to four possible products. To the best of our knowledge, in this work for the first time the Δ2-isomerization of the two isolated diastereomers of AMCA proxetil, a precursor of cefpodoxime proxetil, as a starting material is reported. It has been shown, that each diastereomer only reacts to one of the two possible Δ2-diastereomers. The synthesis, isolation and characterization of (*R*)-diastereomers as well as (S)-diastereomers of Δ2-AMCA proxetil and cefpodoxime proxetil, respectively, are presented.

Introduction

Cefpodoxime is a third-generation semi-synthetic β-lactam antibiotic [1]. Previous work shows a higher oral activity for cephalosporin antibiotics esterified in 4-position [2]. An example for this type of prodrug is cefpodoxime proxetil, a broad spectrum β-lactam antibiotic for oral application [2–6]. Cefpodoxime proxetil itself is an antibacterial inactive substance, which is de-esterified by intestinal wall esterases to the metabolite cefpodoxime [3, 4]. Beside the advantage of possible oral application the esterification in 4-position leads to instability against basic conditions. Under these conditions the double bond in Δ3-position isomerizes to Δ2-position in a reversible reaction [7, 8]. With respect to the nomenclature, it has to be stated that by using IUPAC nomenclature the double bond in cefpodoxime proxetil is in Δ2-position and in Δ3-position in the isomerized form. However, in the traditional nomenclature used by the cephalosporin community and which therefore has been used also in this work, numbering starts at the sulfur, thus leading to the double bond in cefpodoxime proxetil being in Δ3-position and in Δ2-position in the isomerized form. A possible mechanism for this isomerization was suggested by Bently et al. and Saab et al. and confirmed by Richter et al. (Fig. 1) [8–10]. After a deprotonation in 2-position, the free electron pair moves to the 3-position and the resulting free electron pair in 4-position is then protonated. It is known that the Δ2-compound is pharmaceutically inactive, thus representing an unfavorable side-product [7, 9].

The side chain in 4-position of cefpodoxime proxetil contains a stereogenic center, which can possess the *R*-configuration as well as *S*-configuration. Taking into account the additional two stereogenic centers in 6-position and 7-position with a fixed absolute configuration, this results in two possible diastereomers of cefpodoxime proxetil. Both isomers are reported to exhibit similar biological activities, but showing differences in their physicochemical properties [11, 12]. As a process for the isolation of (*R*)-AMCA and (*S*)-AMCA proxetil (with *R*-configuration and *S*-configuration in the side chain, respectively; AMCA: 7-amino-3-methoxymethyl-3-cephem-4-carboxylic

Supplementary information The online version of this article (https://doi.org/10.1038/s41429-019-0190-z) contains supplementary material, which is available to authorized users.

✉ Harald Gröger
harald.groeger@uni-bielefeld.de

[1] Chair of Industrial Organic Chemistry and Biotechnology, Faculty of Chemistry, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany

[2] Sandoz GmbH, Business Unit Anti-Infectives, Biochemiestrasse 10, 6250 Kundl, Austria

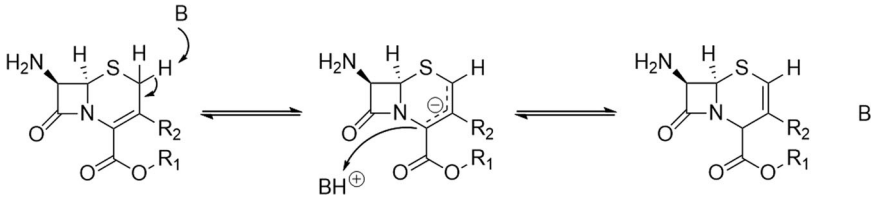

Fig. 1 Mechanism of the Δ2-isomerization of cephalosporins esterified in 4-position [10]

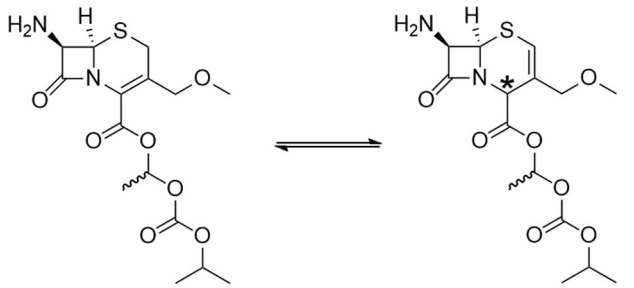

Fig. 2 Δ2-isomerization of AMCA proxetil leading to the formation of a new stereogenic center [10]

acid) as a starting material for the synthesis of (*R*)-cefpodoxime and (*S*)-cefpodoxime proxetil, crystallization with *p*-toluenesulfonic acid is known [13]. These two diastereomers were isolated and examined previously; however, to the best of our knowledge the Δ2-isomerization was not investigated starting from the (*R*)-diastereomers and (*S*)-diastereomers up to now. This Δ2-isomerization is an interesting reaction, because a new stereogenic center with an unknown absolute configuration is formed at the carbon atom in 4-position (Fig. 2). In the following, we report this Δ2-isomerization starting from the two isolated diastereomers of AMCA proxetil, a precursor of cefpodoxime proxetil, as a starting material.

Experimental part

Experimental setup

Automatic flash chromatography: For automatic flash chromatography *Isolera One* System from Biotage (Biotage, Box 8, 75103 Uppsala, Sweden) was used. For chromatography *Snap Ultra* cartridges of Biotage with 10 or 25 g silica gel were used.

NMR Spectroscopy: ^{1}H and ^{13}C NMR-spectra were measured with an *Avance III 500 HD* spectrometer (^{1}H: 500 MHz, ^{13}C: 125 MHz) from Bruker (Bruker Corporation, 40 Manning Road, Billerica, MA 01821, USA). All samples were measured as solution in deuterated chloroform and calibrated to the solvent signal (^{1}H: 7.26 ppm, ^{13}C: 77.16 ppm).

HPLC-chromatography: A HPLC-System from JASCO (JASCO Deutschland GmbH, Robert-Bosch-Strasse 14, 64319 Pfungstadt, Germany) was used, which includes pumps *PU-2080Plus*, column thermostat *CO-2060Plus*, detector *MD-2010Plus*, autosampler *AS-2059-SFPlus* and degaser *DG-2080-53*. The used column is a *NUCLEODUR C18 HTec*, 5 µm, 150, 4.6 mm form MACHEREY-NAGEL (MACHEREY-NAGEL GmbH & Co. KG, Neumann Neander Str. 6-8, 52355 Düren, Germany).

Accurate mass spectrometry: The accurate masses were determined with an *Agilent 6220* time-of-flight mass spectrometer from Agilent Technologies (Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA 95051, USA).

Isolation of AMCA proxetil diastereomers

A mixture of both AMCA proxetil diastereomers (200 mg; for preparation, see ref. [14]) was separated using automatic flash chromatography (cyclohexane/ethyl acetate, 12–100% ethyl acetate). The (*R*)-diastereomer (40 mg) and the (*S*)-diastereomer (56 mg) were obtained in addition to a mixed fraction.

Mixture of (*R*)- and (*S*)-AMCA proxetil diastereomers

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention times 8.45, 9.05 min.

(*R*)-AMCA proxetil diastereomer

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention time 8.96 min.

(*S*)-AMCA proxetil diastereomer

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention time 8.45 min.

Preparation of cefpodoxime proxetil diastereomeric mixture

A solution of AMCA proxetil (512 mg, 1.37 mmol) in ethyl acetate (13.5 ml) was cooled to 0 °C. After adding MAEM (582 mg, 1.66 mmol; MAEM: *S*-(benzo[d]thiazol-2-yl) (*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)ethanethioate) the suspension was stirred for 18 h in an ice bath. After filtration and washing the residue with ethyl acetate (2 ml) the solvent was removed in vacuum. The residue of filtrate was separated using automatic flash chromatography (cyclohexane/ethyl acetate, 12–100% ethyl acetate). The resulting cefpodoxime proxetil (as a diastereomeric mixture) was isolated as slightly yellow solid (538 mg, 0.965 mmol, 70% isolated yield).

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention times 11.53, 12.88 min.

Preparation of (*R*)-cefpodoxime proxetil diastereomer

A solution of (*R*)-AMCA proxetil diastereomer (50 mg, 0.13 mmol) in ethyl acetate (1 ml) was cooled to 0 °C. After adding MAEM (57 mg, 0.16 mmol) the suspension was stirred for 18 h in an ice bath. After filtration and washing the residue with ethyl acetate (0.5 ml) the solvent was removed in vacuum. The residue of filtrate was separated using automatic flash chromatography (cyclohexane/ethyl acetate, 12–100% ethyl acetate). (*R*)-Cefpodoxime proxetil diastereomer was isolated as slightly yellow solid (63 mg, 0.11 mmol, 69% isolated yield).

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention time 12.96 min.

Preparation of (*S*)-cefpodoxime proxetil diastereomer

A solution of (*S*)-AMCA proxetil diastereomer (42 mg, 0.11 mmol) in ethyl acetate (1 ml) was cooled to 0 °C. After adding MAEM (57 mg, 0.14 mmol) the suspension was stirred for 18 h in an ice bath. After filtration and washing the residue with ethyl acetate (0.5 ml) the solvent was removed in vacuum. The residue of filtrate was separated using automatic flash chromatography (cyclohexane/ethyl acetate, 12–100% ethyl acetate). (*S*)-Cefpodoxime proxetil diastereomer was isolated as slightly yellow solid (41 mg, 0.074 mmol, 66% isolated yield).

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention time 11.55 min.

Study of the equilibrium of Δ2-AMCA and Δ3-AMCA proxetil

AMCA proxetil (20 mg, 0.053 mmol) was solved in ethyl acetate (1 ml). After addition of triethylamine (0–74 µL, 0–0.533 mmol) the mixture was stirred for 0.5–4 h at 0 °C or room temperature. After removing all volatile components in vacuum, the residue was analyzed using ^{1}H NMR. For quantification the proton at the stereogenic center in the side chain was used. Detailed weighing and results can be found in the supplementary information.

Isomerization of Δ2-AMCA proxetil

Δ2-AMCA proxetil (19.5 mg, 0.052 mmol) was solved in ethyl acetate (1 ml). After addition of triethylamine (74 µl, 0.534 mmol) the mixture was stirred for 2 h at room temperature. After removing all volatile components in vacuum the residue was analyzed using ^{1}H NMR. For quantification the proton at the stereogenic center in side chain was used. For Δ2-AMCA/Δ3-AMCA proxetil a ratio of 63:37 could be determined.

Preparation of Δ2-AMCA proxetil diastereomeric mixture

Triethylamine (2.15 ml, 15.5 mmol) was added to a solution of AMCA proxetil (581 mg, 1.55 mmol) in ethyl acetate (10 ml). After 2 h of stirring at room temperature all volatile components were removed in vacuum. The residue was separated using automatic flash chromatography (cyclohexane/ethyl acetate, 12–100% ethyl acetate). The Δ2-AMCA proxetil diastereomeric mixture was isolated as slightly yellow solid (201 mg, 0.537 mmol, 35% isolated yield).

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention time 7.00 min.

Preparation of (*R*)-Δ2-AMCA proxetil diastereomer

Triethylamine (0.414 ml, 2.99 mmol) was added to a solution of (*R*)-AMCA proxetil (113 mg, 0.303 mmol) in ethyl acetate (5.6 ml). After 2 h of stirring at room temperature all volatile components were removed in vacuum. The residue was separated using automatic flash chromatography (cyclohexane/ethyl acetate, 12–100% ethyl acetate). (*R*)-Δ2-AMCA proxetil diastereomer was isolated as slightly yellow solid (34 mg, 0.091 mmol, 30% isolated yield).

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention time 6.99 min.

Preparation of (*S*)-Δ2-AMCA proxetil diastereomer

Triethylamine (0.414 ml, 2.99 mmol) was added to a solution of (*S*)-AMCA proxetil (112 mg, 0.301 mmol) in ethyl acetate (5.6 ml). After 2 h of stirring at room temperature all volatile components were removed in vacuum. The residue was separated using automatic flash chromatography (cyclohexane/ethyl acetate, 12–100% ethyl acetate). (*S*)-Δ2-AMCA proxetil diastereomer was isolated as slightly yellow solid (55 mg, 0.148 mmol, 49% isolated yield).

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention time 7.03 min.

Preparation of Δ2-cefpodoxime proxetil diastereomeric mixture

A solution of Δ2-AMCA proxetil diastereomer (58.0 mg, 0.155 mmol) in ethyl acetate (3 ml) was cooled to 0 °C. After adding MAEM (67.7 mg, 0.193 mmol) the suspension was stirred for 18 h in an ice bath. After stirring all volatile

compounds were removed in vacuum. The residue was separated using automatic flash chromatography (cyclohexane/ethyl acetate, 12–100% ethyl acetate). The Δ2-cefpodoxime proxetil diastereomeric mixture was isolated as slightly yellow solid (85.8 mg, 0.154 mmol, 99% isolated yield).

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention time 10.12 min.

Preparation of (*R*)-Δ2-cefpodoxime proxetil diastereomer

A solution of (*R*)-Δ2-AMCA proxetil diastereomer (21 mg, 0.056 mmol) in ethyl acetate (1 ml) was cooled to 0 °C. After adding MAEM (27 mg, 0.077 mmol) the suspension was stirred for 18 h in an ice bath. After stirring all volatile compounds were removed in vacuum. The residue was separated using automatic flash chromatography (cyclohexane/ethyl acetate, 12–100% ethyl acetate). (*R*)-Δ2-cefpodoxime proxetil diastereomer was isolated as slightly yellow solid (28 mg, 0.050 mmol, 89% isolated yield).

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention time 10.03 min.

Preparation of (*S*)-Δ2-cefpodoxime proxetil diastereomer

A solution of (*S*)-Δ2-AMCA proxetil diastereomer (15 mg, 0.036 mmol) in ethyl acetate (1 ml) was cooled to 0 °C. After adding MAEM (19.2 mg, 0.055 mmol) the suspension was stirred for 18 h in an ice bath. After stirring all volatile compounds were removed in vacuum. The residue was separated using automatic flash chromatography (cyclohexane/ethyl acetate, 12–100% ethyl acetate). (*S*)-Δ2-cefpodoxime proxetil diastereomer was isolated as slightly yellow solid (14 mg, 0.025 mmol, 65% isolated yield).

HPLC (C18, ammonium acetate buffer (pH 5.0, 20 mM)/acetonitrile, 60/40, 1 ml min^{-1}): retention time 10.23 min.

Results and discussion

Diastereomers of AMCA proxetil and cefpodoxime proxetil

As a first step, both diastereomers of AMCA proxetil were prepared in isolated form in order to subsequently transform them into the corresponding cefpodoxime proxetil diastereomers. Towards this end, an automatic flash chromatography system has been used for this separation, leading to both diastereomers of AMCA proxetil in isolated form. Then, both isolated diastereomers of AMCA proxetil as well as a mixture thereof were converted with *S*-(benzo[d]thiazol-2-yl) (*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino) ethanethioate acid to amidate the 7-position of AMCA. By means of this reaction, isolated cefpodoxime proxetil diastereomers as well as a mixture of them were obtained. The application of activated thioesters is a well-known concept for the derivatization of the amino group in the 7-position of 7-aminocephalosporonic acid or derivatives thereof [15–17]. By using HPLC chromatography together with the information that the more soluble *S*-isomer elutes first [11], it was possible to determine the absolute configuration of the stereogenic center in the side chain for the isolated cefpodoxime proxetil diastereomers within our study and this information has then been used for an analogous determination of absolute configuration of the AMCA proxetil diastereomers (Fig. 3).

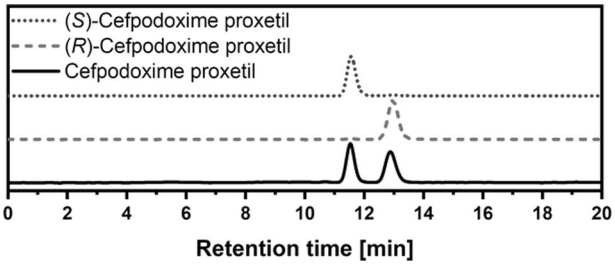

Fig. 3 HPLC chromatogram from (*R*)-cefpodoxime proxetil and (*S*)-cefpodoxime proxetil and a mixture of them

Diastereomers of Δ2-AMCA proxetil and Δ2-cefpodoxime proxetil

In the next step the equilibrium of Δ2-AMCA proxetil and Δ3-AMCA proxetil was determined by means of a screening study. Therefore, Δ3-AMCA proxetil was stirred under different reaction conditions (varying the amount of base, temperature, and reaction time) and the amount of formed Δ2-AMCA proxetil was subsequently determined (Fig. 4). As expected, the amount of Δ2-AMCA proxetil can be increased by higher amounts of base, longer reaction times, and higher temperatures. All the reactions of the screening approach led to an equilibrium containing an amount of 62% of Δ2-AMCA proxetil. In addition, the back reaction from Δ2-AMCA proxetil to Δ3-AMCA proxetil was analyzed. In this study, the isolated Δ2-AMCA proxetil was stirred at room temperature for 2 h with 10 equivalents of triethylamine, leading to an amount of 63% Δ2-AMCA proxetil in equilibrium, which also confirms the previously determined equilibrium with an amount of Δ2-AMCA proxetil being in the range of slightly above 60%.

In order to synthesize the (*R*)-Δ2-AMCA proxetil and (*S*)-Δ2-AMCA proxetil diastereomers in isolated and purified form, the isolated (*R*)-AMCA proxetil and (*S*)-AMCA proxetil diastereomers were stirred under the same conditions as the back reaction described before (room temperature, 2 h, 10

equivalents of triethylamine). By means of this approach, (*R*)-Δ2-AMCA proxetil and (*S*)-Δ2-AMCA proxetil could be synthesized with a yield of 30% and 49%, respectively. By analyzing (*R*)-Δ2-AMCA and (*S*)-Δ2-AMCA proxetil by HPLC, no difference in retention times could be observed. With the used HPLC system it was not possible to separate the mixture of both diastereomers. In the ^{1}H NMR spectra (Fig. 5) both diastereomers in the side chain have one set of signals each, which underlines that only one of the two diastereomers with the stereogenic center in the six-membered ring was formed. Comparing the ^{1}H NMR spectra of the (*R*)-diastereomers and (*S*)-diastereomers with that of the corresponding diastereomeric mixture, it can be unambiguously shown that they represent the expected two diastereomers. Most of the signals are very similar, but the signals in a range between 4.50 and 5.50 ppm show some differences (compare Fig. 5). The most significant difference is the shift of 0.04 ppm of the doublet from the proton in 6-position at nearly 5.20 ppm. A second clear difference is the shift of the signal of the 4-position in the region of 5.00 ppm. Both sets of signals are visible in the green curve, as there are two (different) isolated signals each for these protons underlining the presence of the two diastereomers. The double set of a heptet in the region between 4.85 and 4.93 ppm and the double set of a doublet at 4.61 ppm in the mixture further underline the presence of diastereomers.

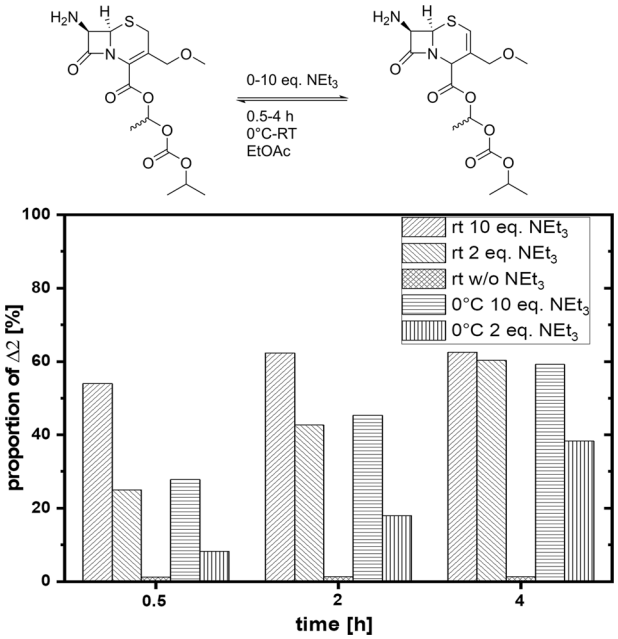

Fig. 4 Isomerization from Δ3-AMCA proxetil to Δ2-AMCA proxetil under different conditions

The (*R*)-Δ2-AMCA proxetil and (*S*)-Δ2-AMCA proxetil diastereomers as well as the mixture thereof were used to synthesize the corresponding different cefpodoxime-type products. In analogy to the synthesis of the diastereomers of cefpodoxime proxetil, *S*-(benzo[d]thiazol-2-yl) (*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)ethanethioate (MAEM) was used to form the desired amide function in 7-position. In these transformations, (*R*)-Δ2-cefpodoxime proxetil and (*S*)-Δ2-cefpodoxime proxetil and the mixture thereof were obtained in yields of 89%, 65% and 99%, respectively. In analogy to the HPLC data of the two Δ2-AMCA proxetil diastereomers, the corresponding two Δ2-cefpodoxime proxetil diastereomers again show an identical retention time in our HPLC analysis. Thus, likewise with the used HPLC-system it was not possible to separate the mixture of both diastereomers. In the ^{1}H NMR spectra of (*R*)-cefpodoxime proxetil and (*S*)-cefpodoxime proxetil most signals are similar (compare Fig. 6). However, again in accordance with the analogous spectra of the two

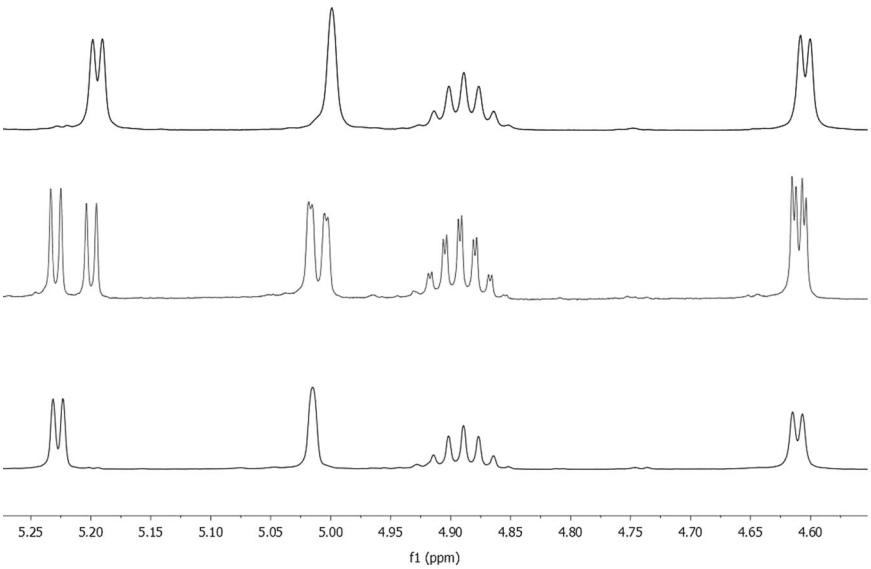

Fig. 5 Excerpt of ^{1}H NMR-spectra of (*R*)-Δ2-AMCA proxetil (top), mixture of (*R*)-Δ2-AMCA proxetil and (*S*)-Δ2-AMCA proxetil (middle), and (*S*)-Δ2-AMCA proxetil (bottom)

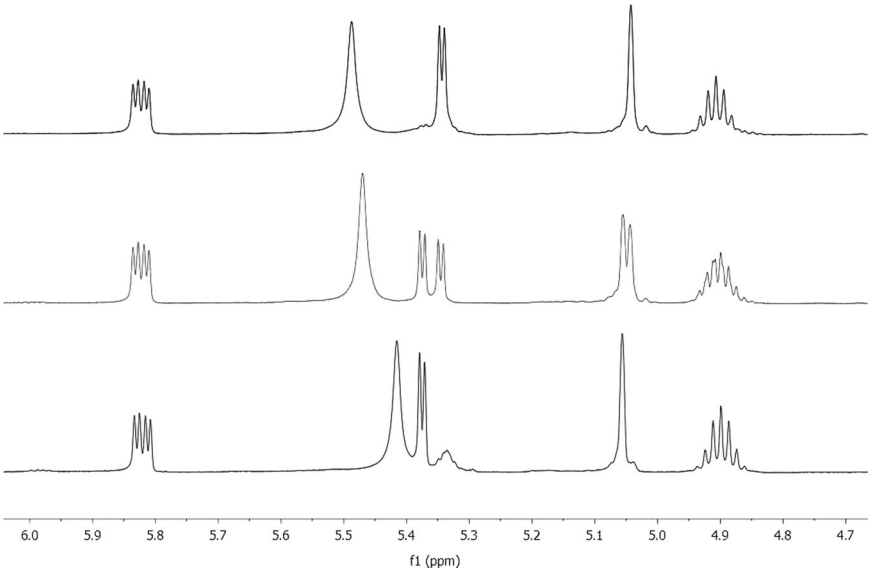

Fig. 6 Excerpt of ^{1}H NMR-spectra of (*R*)-Δ2-cefpodoxime proxetil (top), mixture of (*R*)-Δ2-cefpodoxime proxetil and (*S*)-Δ2-cefpodoxime proxetil (middle), and (*S*)-Δ2-cefpodoxime proxetil (bottom)

Δ2-AMCA proxetil diastereomers (see Fig. 5) there is a difference in the signal of the protons in position 4 and 6. In the mixture (green curve) a double set of a doublet from position 4 is observable in a range of 5.35 ppm. For the single diastereomers (blue and red curves) there is one doublet each. The signals in the region of 5.05 ppm indicate that there are two singlets in the mixture (green curve), one belonging to each diastereomer. The broad signal left of the signal of the 4-position (5.45–5.50 ppm) belongs to the amine group and shows a variability in terms of the chemical shift.

Conclusion

In conclusion, we have successfully synthesized, isolated, and characterized the two different diastereomers from AMCA proxetil and cefpodoxime proxetil having an (*R*)-configuration or (*S*)-configuration in the side chain of the substituent at the 4-position. In addition, the Δ2-isomerization of the two different AMCA proxetil diastereomers was investigated. According to the ^{1}H NMR spectra, this isomerization leads to only one of the two possible diastereomers for each AMCA proxetil diastereomer. These two Δ2-AMCA proxetil diastereomers were characterized and could be distinguished by means of ^{1}H NMR spectra. Furthermore, we successfully converted the Δ2-AMCA proxetil diastereomers to the Δ2-cefpodoxime proxetil diastereomers, which were also isolated and characterized.

Acknowledgements The authors thank the German Federal Environmental Foundation (Deutsche Bundesstiftung Umwelt, DBU) for generous support within the funding initiative "Sustainable Pharmacy" (Project AZ 31723-32) and Ingo Kröger for experimental assistance.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Dabernat H, Avril JL, Boussougant Y. In-vitro activity of cefpodoxime against pathogens responsible for community-aquired respiratory tract infections. J Antimicrob Chemother. 1990; 26E:1–6.
2. Fujimoto K, Ishihara S, Yanagisawa H, Ide J, Nakayama E, et al. Studies on orally active cephalosporin esters. J Antibiot. 1987;40: 370–84.
3. Chocas EC, Paap CM, Godley PJ. Cefpodoxime proxetile: a new broad-spectrum, oral cephalosporin. Ann Pharmacother. 1993;27: 1369–77.
4. Saathoff N, Lode H, Neider K, Depperman KM, Borner K, et al. Phamacokinetics of cefpodoxime proxetil and interactions with an antacid and an H_2 receptor antagonist. AAC. 1992;36:796–800.
5. Fulton B, Perry CM. Cefpodoxime proxetile. Pediatr Drugs. 2001;3:137–58.
6. Nakayama E, Fujimoto K, Muramatsu S, Ide J. Syntesis and antibacterial activity of 7-[2-(2-Aminooxazol-4-yl)-(*Z*)-2-alkoxyiminoacetamido]-cephalosporin antibiotics. J Antibiot. 1992;45: 1193–4.
7. Stoeckel K, Hofheinz W, Laneury JP, Duchene P, Shedlofsky S, et al. Stability of cephalosporin prodrug esters in human intestinal juice: implications for oral bioavailability. AAC. 1998;42:2602–6.
8. Bentley PH, Brooks G, Zomaya II. Phthalidyl esters of cephalosporins. Tetrahedron Lett. 1976;41:3739–42.
9. Saab AN, Dittert LW, Hussain AA. Isomerization of cephalosporin esters: implication for the prodrug ester approach to

enhancing the oral bioavailabilities of cephalosporins. J Pharm Sci. 1988;77:906–7.
10. Richter WF, Chong YH, Stella VJ. On the mechanism of isomerization of cephalosporin esters. J Pharm Sci. 1990;79:185–6.
11. Kakumanu VK, Arora VK, Bansal AK. Development and validation of isomer specific RP-HPLC method for quantification of cefpodoxime procetil. J Chromatogr B. 2006;835:16–20.
12. Kakumanu VK, Arora V, Bansal AK. Investigation on physicochemical and biological differences of cefpodoxime proxetil enantiomers. Eur J Pharm Biopharm. 2006;64:255–9.
13. Fischer G, Defossa E, Gerlach U, Hörlein R, Krass N, et al. Diastereomere des 3-Cephem-4-carbonsäure-1-(-isopropoxycarbonyloxy) ethylesters und Verfahren zu deren Herstellung. EU Patent Application EP0531875A2; 1992.
14. Greil J, Ludescher J, Totschnig K, Wolf S. Process for the preparation of cefpodoxime proxetil diastereomers. PCT International Application WO9935149; 1999.
15. Zuodi W. Synthesis method of cefpodoxime proxetil intermediate. CN Patent Application CN105669701A; 2016.
16. Miao D, Wang L, Xu J, Zeng X. Preparation technology of cefotaxime. CN Patent Application CN101560217; 2009.
17. Zhang J, Liu Q, Wang Y, Gao J. Method for preparing cefotaxime acid. CN Patent Application CN102702230; 2012.