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On the Stereochemical Purity of (+)-7-Aminocephalosporanic Acid

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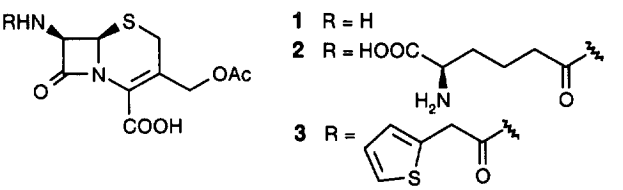
Abstract: (±)-7-Aminocephalosporanic acid (**18a**) and (±)-7-*epi*-aminocephalosporanic acid (**4a**) have been synthesized. A chiral HPLC method has been developed for the separation of the four stereoisomers. Natural (+)-7-aminocephalosporanic acid (**1**) was demonstrated to be enantiomerically (ee ≫ 99.95%) and diastereomerically (de ≫99.95%) pure. Copyright © 1996 Elsevier Science Ltd

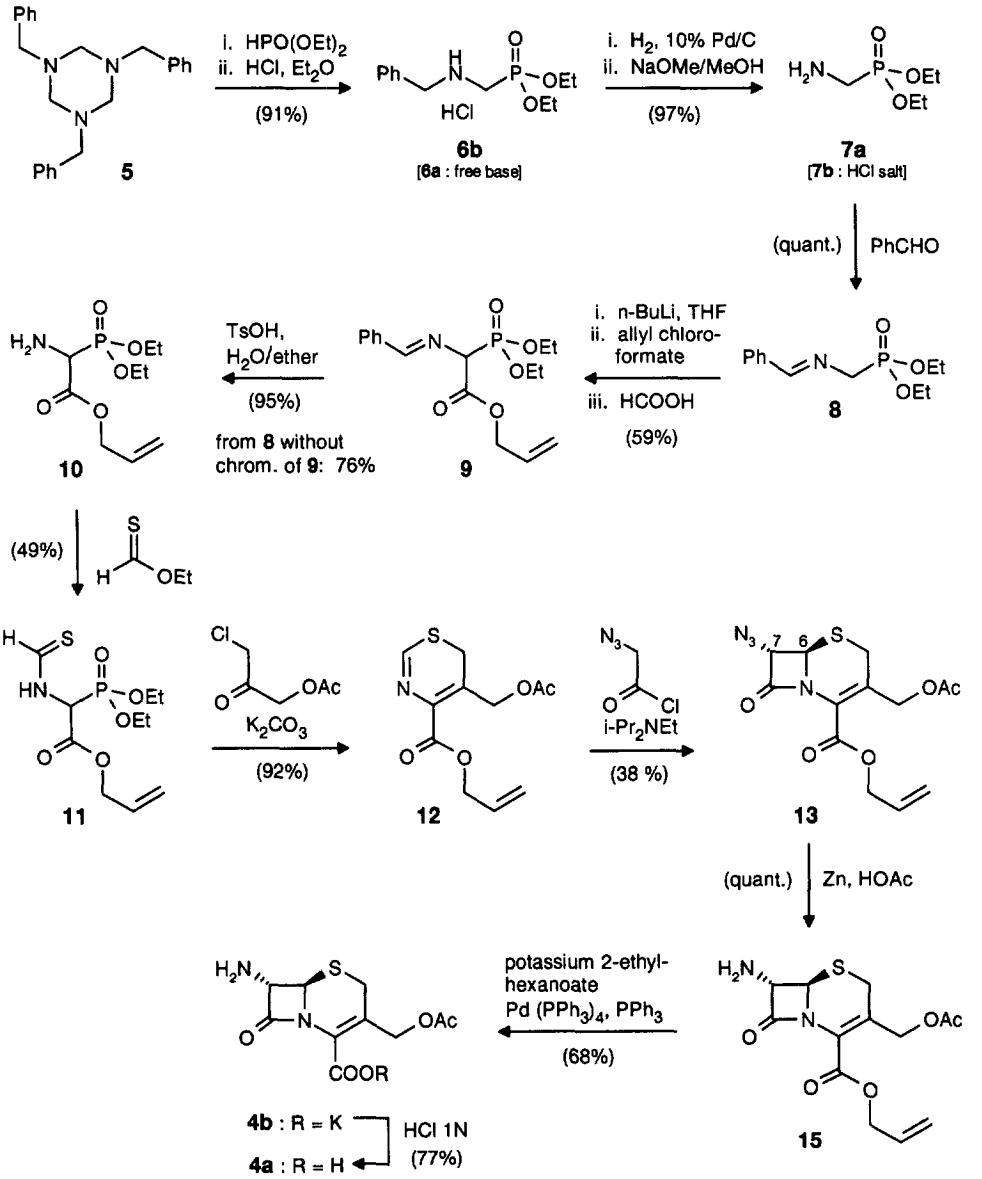
(+)-7-Aminocephalosporanic acid (7-ACA) (**1**) is the starting material for many semisynthetic cephalosporins. Most of the estimated 1500 tons/year are produced from cephalosporin C (**2**) by chemical cleavage of the D-2-aminoadipic acid side chain.[1]



The structure of cephalosporin C (**2**) was established through chemical[2] and X-ray crystallographic studies[3] as well as through total synthesis.[4] The relative[5] and absolute[6] configuration of cephalosporin derivatives was determined by crystal structure analysis. The biosynthetic precursor of penicillins, cephalosporins and cephamycins is the tripeptide δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine, which is oxidatively cyclized to isopenicillin N by isopenicillin N synthase. This enzyme tolerates the D-configuration of the aminoadipoyl terminus but is constrained to the L-configuration of the cysteinyl moiety and the D-configuration of the valinyl moiety.[7] However, to our knowledge the enantio- and diastereomeric purity of **1** has not yet been determined. Herein we report the total synthesis of both diastereomers of 7-ACA in racemic form as well as a chiral HPLC-method which permits the separation of the four stereoisomers.

RESULTS AND DISCUSSION

Our approach to 7-ACA is based on Christensen's synthesis of (±)-cephalothin (**3**)[8] and employs a Staudinger-Bose ketene-imine cycloaddition[9] to form the ß-lactam ring. We have modified and optimized the synthesis and here disclose our results with full experimental detail. The synthesis of (±)-7-*epi*-ACA **4a** is shown in Scheme 1.

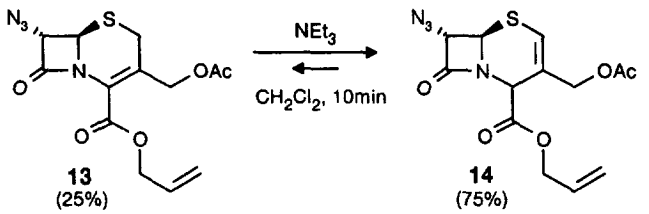


Scheme 1

Michaelis-Becker reaction of triazine **5**[8c, 10] with diethyl phosphite, according to the procedure of Christensen,[8a,c] gave the amine **6a**. Attempts to debenzylate crude **6a** with H_2/Pearlman catalyst[11] failed. It turned out that careful purification of **6a** as the HCl-salt **6b** was crucial for successful debenzylation. Thus, **6a** was dissolved in Et_2O and precipitation of **6b** was affected by the slow addition of 0.9 equiv HCl in Et_2O. Exhaustive hydrogenation of **6b** afforded the ammonium salt **7b**, which upon neutralization with NaOMe in MeOH provided the amine **7a**. Alternative workup procedures were less favorable: liberation of **7a** with NH_3 in $CHCl_3$[8a,c] gave a very fine precipitate of NH_4Cl which was difficult to remove by filtration. Workup of **7b** with aqueous NH_3 on the other hand, required a continuous extraction of **7a** with CH_2Cl_2 due it's high H_2O-solubility. Imine **8**, prepared from amine **7a** and benzaldehyde, was deprotonated with n-BuLi and carboxylated with allyl chloroformate. The Christensen synthesis employed 4-methoxybenzyl chloroformate[8a] which is less convenient to handle due to it's instability. Since allyl ester **9** is more acidic than imine **8**, at least 2 equiv n-BuLi are necessary to achieve complete conversion. The best results were obtained by the following procedure: addition of 1.15 equiv n-BuLi followed by 0.7 equiv allyl chloroformate at -70°C and twofold, sequential addition of 0.575 equiv n-BuLi and 0.35 equiv allylchloroformate was repeated twice. For optimum yields it was also essential to maintain strictly neutral conditions during the aqueous workup and to add 0.2% NEt_3 to the solvent mixture used for chromatography. The benzylidene group was removed with TsOH in a biphasic mixture of H_2O and Et_2O[8a,c] to give amine **10**. When crude allylester **9** was subjected to TsOH treatment without prior chromatographic purification, the yield of **10** from **8** could be raised from 56% to 76%. Thus, amine **10** was obtained in 65% combined yield from triazine **5** (as compared to 21% of the corresponding 4-methoxybenzyl protected amine in ref. 8a) without the need of any chromatography.

Thioformylation of **10** to **11** with ethylthioformate[12] in CCl_4 occurred without incident. In contrast, thioformylation of the corresponding 4-methoxybenzyl ester required the reaction to be carried out in H_2S under autogenous pressure.[8a] Thiazine **12** was prepared by reaction of thioamide **11** and 3-chloro-2-oxo-propyl acetate[13] in the presence of finely ground K_2CO_3 (coarse K_2CO_3 resulted in long and unpredictable reaction times). According to the literature,[8b,c] **11** is first alkylated at the sulfur atom and then cyclization takes place via an intramolecular Horner-Emmons-Wadsworth reaction. In fact we could detect a compound more polar than the starting material **11** by TLC. As the reaction proceeded, this compound disappeared in favor of the apolar product **12**. It is crucial to monitor the reaction closely and to work it up as soon as all of the starting material **11** and intermediate are consumed. Thiazine **12** is an unstable compound and was immediately taken on to the next step.

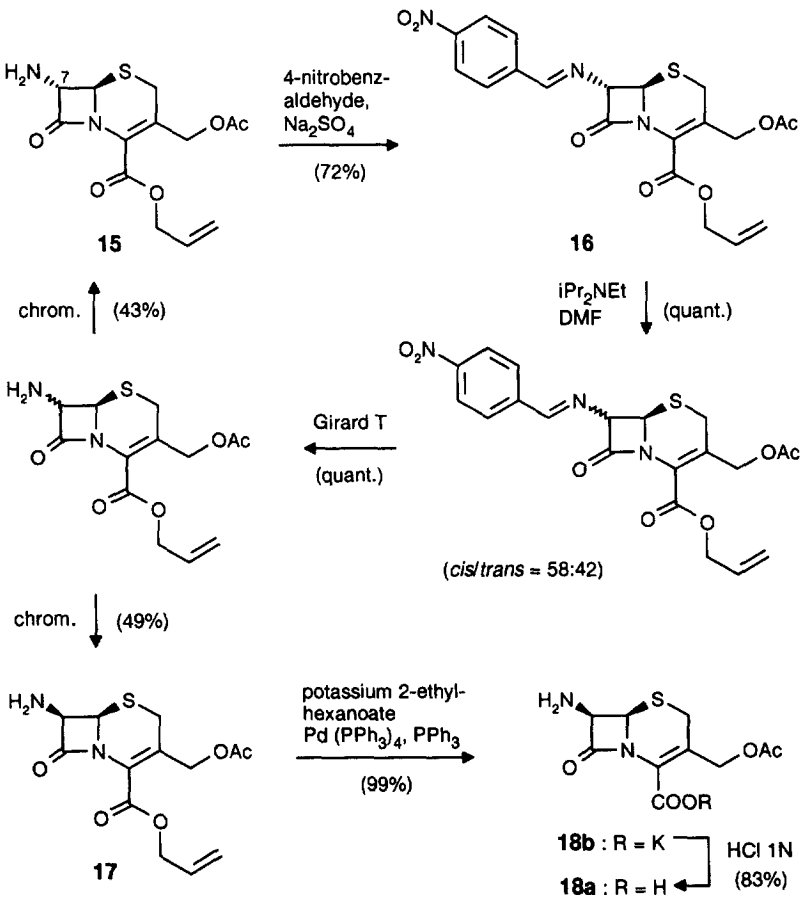
Extensive experimentation was required to find suitable conditions for the ketene-imine cycloaddition of **12** with azidoacetyl chloride. When a solution of azidoacetyl chloride[14] in CH_2Cl_2 was added to a solution of thiazine **12** and NEt_3 in CH_2Cl_2 at -30°C, only a 7% yield of a 80:20 mixture of **13** and it's Δ^3-isomer **14** (single diastereomer) was isolated. Addition of azidoacetyl chloride at -78°C and warming up the reaction mixture to room temperature[8b] resulted in even lower yields of **13** + **14**. Simultaneous addition of azidoacetyl chloride and NEt_3 to thiazine **12** suppressed the formation of **14** and resulted in a 21.5% yield of **13**. By replacing NEt_3 with *i*-Pr_2NEt[20], the yield could be further improved to 38%. The *trans*-configuration of **13** was deduced from the coupling constant $J_{H6,H7}$ of 1.9 Hz. The Δ^2-isomer **13** could be easily isomerized to the Δ^3-isomer **14** which is a common feature of cephem esters.[15] Brief treatment of **13** with NEt_3 in CH_2Cl_2 resulted in an equilibrium mixture of 25% **13** and 75% **14** (Scheme 2).



Scheme 2

Azidocephem **13** was reduced to amine **15** with Zn/HOAc in quantitative yield. Due to its instability, **15** was immediately subjected to a Pd(0) catalyzed cleavage of the allylic ester. The potassium 2-ethylhexanoate protocol[16] proved to be the best suited, since **4b** precipitated directly from the reaction mixture. No side products arising from N-allylation or interference of Pd(0) with the allylic acetate in the cephem ring system were detected. (±)-7-*epi*-ACA **4a** was prepared by neutralization of **4b** with HCl.

The synthesis of (±)-7-ACA **18a** is outlined in Scheme 3. Epimerization of C-7 was achieved by the method of Firestone.[17]



Scheme 3

Schiff base **16**, prepared from **15** and 4-nitrobenzaldehyde, renders the C-7 proton more acidic and allows inversion of this center by deprotonation with PhLi and kinetic reprotonation with aq. HOAc. We obtained a 2:1 *cis/trans*-mixture of the Schiff base isomers accompanied by a substantial amount of several decomposition products. With *i*-PrNEt$_2$ in DMF at -40°C, the reaction proceeded cleanly and resulted in a 3:2 *cis/trans* mixture. We discovered that even DMF (free of HNMe$_2$) itself was sufficient for the epimerization to take place. Equilibrium was reached after 6 d at 4°C: 2:3 *cis/trans*. This would imply that the two isomers are almost isoenergetic. Attempts to separate the isomeric imines by chromatography on silica gel failed. The Schiff base was therefore cleaved with Girard's reagent T[18] and amines **15** and **17** were separated by chromatography. In order to avoid excessive decomposition of these somewhat labile compounds, the silica gel was deactivated by the addition of 0.1% H_2O to the solvent-mixture (CH_2Cl_2 /*t*-BuOMe, 9:1). Amine **17** was converted to (±)-7-ACA **18a** ($J_{H6,H7}$ = 5.1 Hz) as outlined above.

With **18a** and **4a** in hand, a chiral HPLC method for the separation of the four isomers was developed. All four isomers could be separated on a Crownpak CR® column. The column employs a chiral crown ether-coated reversed phase packing[21] and is available in the (+)- and (-)-form. The critical pair to separate is natural (6*R*, 7*R*)-(+)-7-ACA and its enantiomer. The Crownpak CR(-) column elutes the potential impurity (6*S*, 7*S*)-(-)-7-ACA in front of the large (+)-peak and is therefore preferable over the (+) column. The HPLC trace of an approximately equimolar mixture of **18a** and **4a**[19] is displayed in Figure 1. Figure 2 shows the trace of natural (+)-7-ACA **1**. Natural (+)-7-ACA was also spiked with 0.05 % of **18a**. 0.025 % of (-)-7-ACA were clearly visible and could be integrated while the main peak still remained in the linear range of the detector. The detection limits for the diastereomeric isomers are at least as low as for (-)-7-ACA. None of the other three isomers could be detected in natural (+)-7-ACA. The enantiomeric and diastereomeric purity of **1** is thus greater than 99.95%.

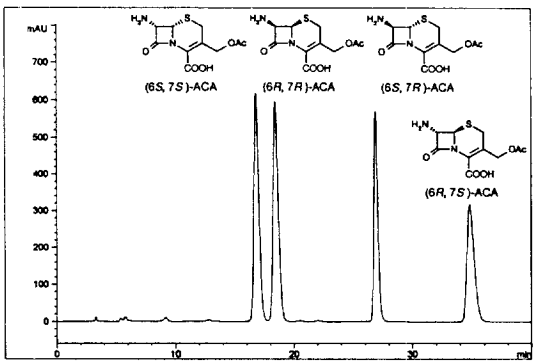


Figure 1. HPL-chromatogram of the four stereo-isomers of 7-ACA.

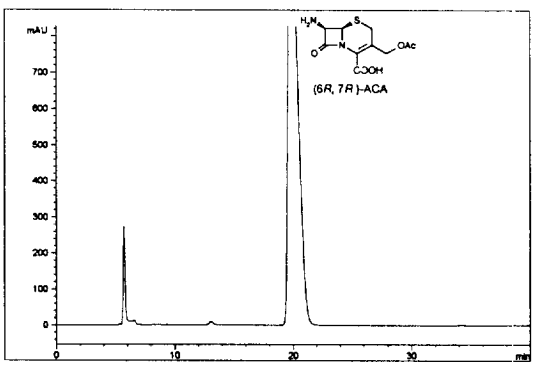


Figure 2. HPL-chromatogram of natural (+)-7-ACA (**1**).

EXPERIMENTAL SECTION

General. Unless otherwise indicated, all starting materials and reagents were obtained from commercial suppliers and used without further purification. (+)-7-ACA **1** was obtained from Biochemie, Kundl. Solvents for reactions, extraction and chromatography were analytical grade. All reactions were performed under an

inert atmosphere of argon unless performed in H_2O. After extractive workup, organic solutions were dried with anhydrous Na_2SO_4 or by filtration over cotton and concentrated at reduced pressure with a rotary evaporator. Column (flash) chromatography was performed using 230-400 mesh silica gel 60. Melting points were determined in Pyrex capillaries and are uncorrected. Chemical shifts in ^1H, ^{13}C and ^{31}P NMR spectra are given in ppm (δ); multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), qi (quintet), m (multiplet) or mm (superimposition of signals belonging to different H's). Coupling constants, *J*, are reported in Hertz. Peaks in IR spectra are reported in cm^{-1} with the following relative intensities: s (strong, 0-33 % transmittance), m (medium, 34-66 %), w (weak, greater than 67 %). Low resolution EI mass spectra were obtained with an ionization voltage of 70 eV. Data are reported in the form of *m/z* (intensity relative to base = 100). Analytical HPLC employed a Merck Superspher® 100 RP-18 endcapped column (250 x 4 mm) and a potassium phosphate buffer (0.03 M, pH 6)/CH_3CN gradient (1.2 ml/min, 50°C). TLC was performed on precoated Merck glass plates (0.25 mm) with silica gel 60 F_{254} unless otherwise specified. Compound visualization was affected by UV light (254 nm) unless otherwise indicated.

Diethyl-benzylaminomethyl-phosphonate hydrochloride (6b). A mixture of triazine **5** (80.00 g, 224 mmol) and diethyl phosphite (104 ml, 806 mmol, 3.6 equiv) was heated at 100°C for 15 h. The reaction mixture was taken up in ether (950 ml), cooled to 2°C and HCl in ether (~27%, 82.30 g, 609 mmol, 2.72 equiv) was added over 30 min with intensive stirring. The HCl salt **6b** started to precipitate after the addition of ~1/3 of the HCl solution. Filtration, thorough washing with Et_2O (4 x 150 ml) and drying at high vacuum (0.001 mbar, 45°C) afforded 179.60 g (91 %) **6b** as a white and extremely hygroscopic solid. Data for **6b**: mp 87-89°C; ^1H NMR (250 MHz, D_2O) 1.35 (t, *J* = 7.1, 6H), 3.56 (d, *J* = 14.2, 2H), 4.25 (dq, *J* = 8.5, 7.1, 4H), 4.36 (s, 2H), 7.48-7.55 (m, 5H); ^{31}P NMR (100 MHz, D_2O) 18.95; IR (KBr) 2982 (s), 2748 (s, br), 1240 (s), 1020 (s); MS (EI) 258 ($(M+H)^+$, 0.15), 228 (0.15), 120 (38), 91 (100); TLC R_f 0.34 (hexane/EtOAc/NEt_3, 5/4/1); Anal. Calcd for $C_{12}H_{21}NClO_3P$ (293.731): C, 49.07; H, 7.21; N, 4.77. Found C, 48.76; H, 7.47; N, 4.77.

Diethyl-aminomethyl-phosphonate (7a). To a solution of ammonium salt **6b** (165.70 g, 564 mmol) in EtOH (1100 ml) was added 10 % Pd/C (16.6 g). The well stirred mixture was exhaustively (1-24h) hydrogenated in an autoclave at 50°C and 5 bar pressure of H_2. The catalyst was filtered off and the solvent stripped whereby the crude HCl salt **7b** crystallized. Data for **7b**: ^1H NMR (250 MHz, D_2O) 1.36 (t, *J* = 7.1, 6H), 3.50 (d, *J* = 13.9, 2H), 4.26 (dq, *J* = 8.5, 7.1, 4H); ^{31}P NMR (100 MHz, D_2O) 20.57. The residual HCl salt was dissolved in CH_3OH (300 ml), cooled to 2°C and neutralized with CH_3ONa (30.50 g, 564 mmol, 1 equiv). Precipitated NaCl was removed by filtration and the filtrate concentrated. The residue was taken up in Et_2O (300 ml), again filtered, evaporated and dried at high vacuum (0.001 mbar, rt, stirring). 91.5 g (97 %) amine **7a** was obtained as a pale yellow liquid. Data for **7a**: bp 68°C (0.001 mbar, analytical sample); ^1H NMR (250 MHz, $CDCl_3$) 1.18 (br s, 2H), 1.35 (t, *J* = 7.1, 6H), 3.01 (d, *J* = 10.3, 2H), 4.15 (dq, *J* = 8.5, 7.1, 4H); ^{31}P NMR (100 MHz, $CDCl_3$) 28.26; IR (neat) 3456 (m), 3382 (m), 3309 (m), 2984 (m), 1615 (w, br), 1230 (s), 1055 (s), 1027 (s); MS (EI) 167 (M^+, 2), 138 (24), 111 (45), 82 (50), 30 (100); TLC (ninhydrin) R_f 0.09 (hexane/EtOAc/NEt_3, 3/5/2); Anal. Calcd for $C_5H_{14}NO_3P$ (167.145): C, 35.93; H, 8.44; N, 8.38. Found C, 36.15; H, 8.76; N, 8.41.

Diethyl-(*E*)-[(benzylideneamino)-methyl]-phosphonate (8). Amine **7a** (70.00 g, 419 mmol) was dissolved in toluene (200 ml), Na_2SO_4 (45 g) was added and the mixture was cooled to 2°C. After the addition

of benzaldehyde (45.80 g, 431 mmol, 1.03 equiv), stirring was continued at rt for 2h. Na_2SO_4 was filtered and the colorless filtrate concentrated. Traces of benzaldehyde were removed at high vacuum (0.001 mbar, 55°C, stirring). 106.5 g (99.5%) imine **8** was obtained as colorless oil. Data for **8**: ^{1}H NMR (250 MHz, $CDCl_3$) 1.35 (t, J = 7.1, 6H), 4.12 (d, J = 17.8, 2H), 4.20 (dq, J = 8.5, 7.1, 4H), 7.39-7.45 (m, 3H), 7.73-7.76 (m, 2H), 8.32 (d, J = 4.9, 1H); ^{31}P NMR (100 MHz, $CDCl_3$) 22.90; IR (neat) 2983 (m), 1640 (s), 1580 (w), 1392 (w), 1251 (s), 1032 (s), 758 (m), 694 (m); MS (EI) 256 ([M+H]$^+$, 18), 152 (90), 125 (100), 118 (46), 91 (77); TLC R_f 0.49 (hexane/EtOAc/NEt_3, 3/5/2), 0.18 (*t*-BuOMe); Anal. Calcd for $C_{12}H_{18}NO_3P$ (255.254): C, 56.47; H, 7.11; N, 5.49. Found C, 56.38; H, 7.33; N, 5.69.

Allyl-(*E*)-(*RS*)-benzylideneamino-diethoxyphosphoryl-acetate (9). To a cold (-70°C) solution of imine **8** (40.00 g, 157 mmol) in THF (200 ml) was added n-BuLi (113 ml ~1.6 M solution in hexane, 180 mmol, 1.15 equiv) over 20 min. A solution of allyl chloroformate (13.22 g, 110 mmol, 0.7 equiv) in THF (30 ml) was added dropwise to the cold (-70°C), dark red reaction mixture over 5 min resulting in a yellow solution. The addition of n-BuLi (56.5 ml, 90 mmol, 0.575 equiv) and allyl chloroformate (6.61 g, 55 mmol, 0.35 equiv) was repeated two times. Finally the reaction mixture was quenched with formic acid (7.7 ml, 204 mmol, 1.3 equiv) at -60°C and concentrated. The oily residue was taken up in *t*-BuOMe (200 ml) and washed with 0.5 M potassium phosphate buffer (pH 7, 2 x 200 ml). The aqueous layers were back-extracted with *t*-BuOMe (200 ml). The combined organic layers were dried and concentrated. The crude product (58.4 g) was purified by column chromatography on silica gel (700 g, hexane/*t*-BuOMe 2:3 → 2:5 with 0.2% NEt_3) to afford 31.2 g (59%) allyl ester **9** as a pale yellow liquid. Data for **9**: ^{1}H NMR (250 MHz, $CDCl_3$) 1.34 (m, 6H), 4.25 (m, 4H), 4.74 (dm, J = 5.7, 2H) 4.77 (d, J = 19.4, 1H), 5.27 (dq, J = 10.4, 1.2, 1H), 5.40 (dq, J = 18.0, 1.5, 1H), 5.88-6.04 (m, 1H), 7.38-7.47 (m, 3H), 7.80-7.84 (m, 2H), 8.39 (d, J = 4.6, 1H); ^{31}P NMR (100 MHz, $CDCl_3$) 15.67; IR (neat) 2985 (m), 1745 (s), 1637 (s), 1259 (s), 1201 (m), 1051 (s), 1022 (s), 759 (m), 695 (m); MS (EI) 281 (4), 254 (9), 221 (50), 138 (64), 41 (100); TLC R_f 0.65 (hexane/EtOAc/NEt_3, 3/5/2), 0.44 (*t*-BuOMe).

Allyl-(*RS*)-amino-(diethoxyphosphoryl)-acetate (10). To a solution of allyl ester **9** (30.00 g, 88.4 mmol) in diethyl ether (150 ml) was added TsOH (20.20 g, 106 mmol, 1.2 equiv) and H_2O (9 ml). The biphasic mixture was stirred vigorously at rt for 2h. Cyclohexane (90 ml) was added and stirring was continued for 15 min. The upper ether/cyclohexane layer which contained benzaldehyde was removed and the residue was thoroughly washed with ether/cyclohexane 2/1 (2 x 150 ml). CH_2Cl_2 (150 ml) and H_2O (150 ml) were added and the pH of the aqueous layer was adjusted to 7.5 with $NaHCO_3$ (9.10 g, 108 mmol). The organic layer was washed with H_2O (150 ml) and each aqueous layer was back-extracted with CH_2Cl_2 (2 x 100 ml). The combined organic layers were dried and concentrated. Drying at high vacuum (0.001 mbar, rt) afforded 21.1 g (95%) amine **10** as a pale yellow liquid. Data for **10**: ^{1}H NMR (250 MHz, $CDCl_3$) 1.35 (dt, J = 7.0, 1.9, 6H), 1.89 (br s, 2H), 3.97 (d, J = 20.1, 1H), 4.20 (dqi, J = 7.1, 1.2, 4H), 4.72 (dm, J = 5.3, 2H), 5.27 (dq, J = 10.4, 1.2, 1H), 5.40 (dq, J = 17.5, 1.5, 1H), 5.86-6.01 (m, 1H); ^{31}P NMR (100 MHz, $CDCl_3$) 20.07; IR (neat) 3388 (m), 3310 (w), 2984 (m), 1741 (s), 1600 (w, br), 1252 (s), 1163 (s), 1052 (s), 1025 (s); MS (ESI) 252 ([M+H]$^+$, 100); TLC (ninhydrin) R_f 0.35 (hexane/EtOAc/NEt_3, 3/5/2), 0.13 (*t*-BuOMe/EtOAc 4/1). This reaction was also carried out with crude allyl ester **9** instead of chromatographically purified material. Allylcarboxylation of 64.80 g (254 mmol) imine **8** and hydrolysis of the imine afforded 48.6 g (76 %) of pure amine **10**.

Allyl-(*RS*)-(diethoxyphosphoryl)-thioformylamino-acetate (11). To an ice-cold solution of ethyl thioformate (15.10 g, 167 mmol, 1.4 equiv) in CCl_4 (30 ml) was added dropwise a solution of amine **10** (30.00 g, 119 mmol) in CCl_4 (40 ml) over 30 min. Stirring was continued at rt for 7h. The malodorous solvent was distilled and trapped in a cooled (-78°C) flask. The crude yellow product was purified by column chromatography on silica gel (500 g, hexane/*t*-BuOMe 1:2) to afford 17.1 g (48.5%) thioamide **11** as a pale yellow solid. Data for **11**: mp 68-70°C (Et_2O/hexane), ^1H NMR (250 MHz, $CDCl_3$) 1.35 (dt, J = 7.0, 1.9, 6H), 4.15-4.29 (m, 4H), 4.73 (dm, J = 5.7, 2H), 5.29 (dq, J = 10.9, 1.2, 1H), 5.42 (dq, J = 17.9, 1.5, 1H), 5.85-6.01 (m, 1H), 6.04 (dd, J = 21.5, 8.6, 1H), 8.79 (br s, 1H), 9.51 (d, J = 5.5, 1H); ^{31}P NMR (100 MHz, $CDCl_3$) 14.75; IR (KBr) 3183 (m), 2993 (m), 1752 (s), 1650 (w), 1534 (m), 1437 (s), 1225 (s), 1022 (s); MS (EI) 295 (M^+, 40), 262 (14), 181 (34), 138 (60), 110 (60), 41 (100); TLC R_f 0.64 (*t*-BuOMe/EtOAc 4/1), 0.28 (*t*-BuOMe); Anal. Calcd for $C_{10}H_{18}NO_5PS$ (295.290): C, 40.68; H, 6.14; N, 4.74; S, 10.86; P, 10.49. Found C, 40.93; H, 6.30; N, 4.82; S, 10.74; P, 10.59.

Allyl-5-acetoxymethyl-6H-1,3-thiazine-4-carboxylate (12). To a solution of thioamide **11** (10.00 g, 33.9 mmol) in acetone (100 ml) was added well ground K_2CO_3 (14.04 g, 101.6 mmol, 3 equiv). The mixture was stirred at 20°C for 10 min. A solution of 3-chloro-2-oxo-propyl acetate (5.41 g, 35.6 mmol, 1.05 equiv) in acetone (60 ml) was added dropwise over 30 min. The reaction was closely monitored by TLC. Besides the spots corresponding to starting material and product, a third spot (R_f 0.18 with *t*-BuOMe; presumably the S-alkylated, not yet cyclized compound) was observed. As soon as all starting material had been consumed (3-5h), the reaction mixture was diluted with CH_2Cl_2 (60 ml), filtered and concentrated. The residue was taken up in CCl_4 (40 ml), if necessary filtered through cotton to remove a tarry, undissolved residue and washed with 0.3 M Na_2HPO_4 (50 ml) and H_2O (30 ml). The aqueous layers were back-extracted with CCl_4 (2 x 30 ml). The combined organic layers were dried and concentrated. The yellow residue was briefly dried at high vacuum (0.001 mbar, rt). Crude thiazine **12** (8.53 g, purity ~ 70% according to ^1H NMR) was immediately subjected to the next step. Data for **12**: ^1H NMR (250 MHz, $CDCl_3$) 2.11 (s, 3H), 3.40 (s, 2H), 4.77 (dt, J = 5.9, 1.4, 2H), 5.19 (s, 2H), 5.29 (dq, J = 10.2, 1.2, 1H), 5.39 (dq, J = 17.9, 1.5, 1H), 5.93-6.09 (m, 1H), 8.40 (s, 1H); TLC R_f 0.48 (*t*-BuOMe), 0.43 (CH_2Cl_2/*t*-BuOMe 19/1).

Allyl-(6*RS*, 7*SR*)-3-acetoxymethyl-7-azido-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate (13). To a cold (-30°C) solution of thiazine **12** (~ 34 mmol) in CH_2Cl_2 (40 ml) was simultaneously added a solution of *i*-Pr_2NEt (4.82 g, 37.3 mmol, 1.1 equiv) in CH_2Cl_2 (14 ml) and a solution of azido acetylchloride (4.45 g, 37.3 mmol, 1.1 equiv) in CH_2Cl_2 (14.5 ml) with two syringe pumps over 15 min. During the addition the two needles dipped into the solution and the temperature was maintained at -30°C (bath -45°C). Stirring was continued for 60 min at -30°C under argon. The brown reaction mixture was quenched with 0.3 M NaH_2PO_4 (20 ml). The organic layer was washed with 0.3 M NaH_2PO_4 (40 ml) and 18 % brine (2 x 40 ml). Each aqueous layer was back-extracted with CH_2Cl_2 (40 ml). The organic layers were filtered over cotton, combined and concentrated. The crude product was purified by column chromatography on silica gel (130 g, CH_2Cl_2/hexane 3:1) to afford 4.36 g (38%) azidocephem **13** as an off-white solid. Data for **13**: mp 69-71°C (MeOH), ^1H NMR (250 MHz, $CDCl_3$) 2.09 (s, 3H), 3.37, 3.61 (2d, J = 17.8, 2H), 4.57 (d, J = 1.9, 1H), 4.64 (d, J = 1.9, 1H), 4.77-4.82 (mm, 3H), 5.03 (d, J = 13.3, 1H), 5.31 (dq, J = 10.3, 1.2, 1H), 5.41 (dq, J = 17.2, 1.5, 1H), 5.92-6.08 (m, 1H); IR (KBr) 2144 (s), 2119 (s), 1774 (s), 1750 (s), 1721 (s), 1639 (m), 1381 (s), 1238 (s), 1115 (s), 1038 (m), 988 (m), 933 (w); MS (EI) 310 (0.9), 278 (1.5), 250 (1.7), 209 (13), 181 (12),

154 (20), 138 (18), 43 (77), 41 (100); TLC R_f 0.64 (CH_2Cl_2/*t*-BuOMe 19/1), 0.90 (CH_2Cl_2/*t*-BuOMe 4/1)., Anal. Calcd for $C_{13}H_{14}N_4O_5S$ (338.338): C, 46.15; H, 4.17; N, 16.56. Found C, 46.09; H, 4.29; N, 16.32.

Allyl-(2*RS*, 6*RS*, 7*SR*)- or (2*SR*, 6*RS*, 7*SR*)-3-acetoxymethyl-7-azido-8-oxo-5-thia-1-aza-bicyclo [4.2.0]oct-3-ene-2-carboxylate (14). A solution of azidocephem **13** (100 mg, 0.30 mmol) in CH_2Cl_2 (1 ml) and NEt_3 (13 µl, 0.10 mmol, 0.3 equiv) was stirred for 10 min at rt. The solvent was evaporated at reduced pressure. ^1H NMR analysis of the crude product revealed a 1:3 mixture of **13** and **14**. The two compounds were separated by HPLC (RP18, CH_3CN/H_2O gradient). Fractions containing **14** were collected, concentrated at reduced pressure and extracted with CH_2Cl_2. 60 mg (0.18 mmol) **14** was obtained as a colorless oil. Data for **14**: ^1H NMR (400 MHz, $CDCl_3$) 2.08 (s, 3H), 4.62 (m, 1H), 4.63, 4.71 (2d, J = 12.6, 2H), 4.69 (d, J = 6.0, 2H), 4.99 (s, 1H), 5.07 (s, 1H), 5.32 (dm, J = 10.4, 1H), 5.36 (dm, J = 17.2, 1H), 5.87-5.96 (m, 1H), 6.46 (s, 1H); MS (ESI) 361.2 ([M+Na]$^+$, 27), 356.2 ([M+NH_4]$^+$, 100), 339.2 ([M+H]$^+$, 5), 279 (51); TLC R_f 0.59 (CH_2Cl_2/*t*-BuOMe 19/1).

Allyl-(6*RS*, 7*SR*)-3-acetoxymethyl-7-amino-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate (15). To an ice-cold solution of azidocephem **13** (2.00 g, 5.9 mmol) in THF (20 ml) was added Zn powder (3.86 g, 59.1 mmol, 10 equiv). Acetic acid (13.5 ml, 236 mmol, 40 equiv) was added dropwise over 5 min and stirring under argon was continued for 90 min at 2°C. The reaction mixture was filtered and evaporated. The yellow residue was taken up in CH_2Cl_2 (30 ml) and washed with H_2O (20 ml), 0.5 M $NaHCO_3$ (20 ml) and 18 % brine (20 ml). Each aqueous layer was back-extracted with CH_2Cl_2 (2 x 30 ml). The combined organic layers were filtered through cotton, combined, concentrated and the yellow, oily residue was briefly dried at high vacuum (0.001 mbar, rt). Crude aminocephem **15** (1.84 g, ~100%) was used in next step without further purification. Data for **15**: ^1H NMR (250 MHz, $CDCl_3$) 2.08 (s, 3H), 3.32, 3.58 (2d, J = 18.3, 2H), 4.17 (d, J = 2.1, 1H), 4.50 (d, J = 2.1, 1H), 4.75, 4.97 (2d, J = 13.1, 2H), 4.78-4.84 (m, 2H), 5.31 (dq, 1.2, J = 10.3, 1H), 5.41 (dq, J = 18.6, 1.5, 1H), 5.93-6.09 (m, 1H); IR (neat) 3332 (m), 2942 (m), 1773 (s), 1734 (s) 1685 (m), 1646 (m), 1386 (s), 1234 (s); MS (ESI) 335 ([M+Na]$^+$, 57), 295 (7), 278 (22), 225 (100); TLC R_f 0.14 (CH_2Cl_2/*t*-BuOMe 4/1), 0.08 (hexane/EtOAc, 1/1).

Potassium-(6*RS*, 7*SR*)-3-acetoxymethyl-7-amino-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate (4b). To a solution of aminocephem **15** (600 mg, 1.92 mmol) in THF (12 ml) was added simultaneously a 0.5 M solution of potassium 2-ethylhexanoate in THF (5.8 ml, 2.88 mmol, 1.5 equiv) and a solution of $Pd(PPh_3)_4$ (55.5 mg, 0.048 mmol, 2.5 mol%) and PPh_3 (25.2 mg, 0.096 mmol, 5 mol%) in THF (4 ml). The color of the reaction mixture changed immediately from orange to dark purple. After some minutes, a white precipitate started to form. Stirring at rt under argon was continued for 90 min. The suspension was filtered and the precipitate thoroughly washed with THF (5 x 8 ml). After drying at high vacuum (0.001 mbar, rt) 405.4 mg (68%) of the potassium salt of (±)-7-*epi*-ACA was obtained as an off-white solid. Data for **4b**: mp 164°C (dec), ^1H NMR (400 MHz, D_2O) 2.10 (s, 3H), 3.34, 3.65 (2d, J = 17.8, 2H), 4.24 (d, J = 2.1, 1H), 4.65 (d, J = 2.1, 1H), 4.67, 4.83 (2d, J = 12.4, 2H); ^{13}C NMR (100 MHz, D_2O) 23.14, 27.94, 61.01, 65.09, 67.13, 118.60, 134.43, 171.77, 172.51, 176.98; IR (KBr) 3385 (m), 1755 (s), 1739 (s), 1610 (s), 1404 (m), 1232 (m); MS (ESI) 271.2([M-K]$^-$, 36), 211.2 (100); TLC (RP18) R_f 0.76 (CH_3CN/H_2O 1/9); Anal. Calcd for $C_{10}H_{11}N_2O_5SK$ (310.369): C, 38.70; H, 3.57; N, 9.03; S, 10.33; K 12.60. Found C, 38.53; H, 3.82; N, 8.48; S, 9.88; K, 13.16.

(6*RS*, 7*SR*)-3-Acetoxymethyl-7-amino-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (4a). Potassium salt **4b** (400 mg, 1.29 mmol) was dissolved in ice-cold H_2O (5 ml). The pH of the solution (7.4) was adjusted to 3.5 by the addition of 1 N HCl (1.29 ml, 1.0 equiv) over 8 min with a syringe pump. (±)-7-*epi*-ACA **4a** started to precipitate at pH 6.4. After the addition of HCl, stirring at 2°C was continued for 15 min. **4a** was collected by filtration, washed with H_2O (3 x 1 ml) and CH_3OH (3 x 1 ml) and dried at high vacuum (0.001 mbar, rt). 270 mg (77%) (±)-7-*epi*-ACA **4a** was obtained as an off-white solid. Data for **4a**: ^{1}H NMR (400 MHz, CF_3COOD) 2.29 (s, 3H), 3.63, 3.85 (2d, J = 18.6, 2H), 4.91 (d, J = 2.1, 1H), 5.19, 5.44 (2d, J = 14.2, 2H), 5.35 (d, J = 2.1, 1H); IR (KBr) 2600 (w, br), 1793 (s), 1731 (s), 1591 (s), 1412 (m), 1244 (m); MS (ESI) 295.3 ($[M+Na]^+$, 50), 290.3 ($[M+NH_4]^+$, 82), 279.3 ($[M+Li]^+$, 100), 273.2 ($[M+H]^+$, 55); HPLC t_R 3.23 min; Anal. Calcd for $C_{10}H_{12}N_2O_5S$ (272.275): C, 44.11; H, 4.44; N, 10.29. Found C, 43.80; H, 4.45; N, 10.10. The observed analytical data are in excellent agreement with those of (6*R*, 7*S*)-7-*epi*-ACA.[19]

Allyl-(*E*)-(6*RS*, 7*SR*)-3-acetoxymethyl-7-(4-nitro-benzylideneamino)-8-oxo-5-thia-1-aza-bicyclo [4.2.0]oct-2-ene-2-carboxylate (16). To a solution of aminocephem **15** (1.85 g, 5.91 mmol) in CH_2Cl_2 (10 ml) was added 4-nitrobenzaldehyde (0.89 g, 5.91 mmol) and Na_2SO_4 (2g). During the reaction (4h at rt) the color of the mixture changed from yellow to red. Na_2SO_4 was filtered and the filtrate evaporated. The crude product was purified by column chromatography on silica gel (40 g, 10 ml CH_2Cl_2 to dissolve the crude product, CH_2Cl_2 to elute unchanged 4-nitrobenzaldehyde, CH_2Cl_2 /*t*-BuOMe 99:1 to elute the product) to afford 2.106 g **16** as a yellow, tacky foam. Crystallization from CH_2Cl_2 / Et_2O yielded 1.895 g (72%) imine **16** as a beige solid. According to the ^{1}H NMR spectrum, the compound contained about 5% of the *cis*-imine. Data for **16**: mp 89-91°C; ^{1}H NMR (250 MHz, $CDCl_3$, signals corresponding to *cis*-imine not given) 2.10 (s, 3H), 3.41, 3.67 (2d, J = 18.3, 2H), 4.80-4.87 (mm, 4H), 4.98 (d, J = 1.9, 1H), 5.05 (d, J = 13.2, 1H), 5.30 (dq, J = 10.4, 1.2, 1H), 5.41 (dq, J = 17.2, 1.5, 1H), 5.92-6.08 (m, 1H), 7.96, 8.30 (2dm, J = 8.8, 4H), 8.58 (d, J = 1.2, 1H); IR (KBr) 1779 (s), 1732 (s), 1720 (s), 1637 (m), 1600 (m), 1523 (s), 1347 (s), 1235 (s), 850 (w); TLC R_f 0.63 (hexane/EtOAc, 1/1), 0.41 (toluene/*t*-BuOMe, 4/1).

Epimerization: *trans* ↔ *cis*-imine. a) To a cold (-40°C) solution of imine **16** (1.82 g, 4.09 mmol) in DMF (20 ml, filtered over acidic aluminum oxide act. I) was added iPr_2NEt (1.4 ml, 8.2 mmol, 2 equiv). The dark blue solution was stirred under argon for 30 min and then quenched with 1M KH_2PO_4 (25 ml). The yellow mixture was diluted with H_2O (20 ml) and extracted with CH_2Cl_2 (40 ml). The organic layer was washed with H_2O (2 x 30 ml). Each aqueous layer was back-extracted with CH_2Cl_2 (30 ml). The organic layers were filtered through cotton, combined and evaporated. 1.81 g (99.5%) of a 58 : 42 mixture of *cis*- and *trans*-imine (^{1}H NMR) was obtained as a pale yellow solid. Data for *cis*-imine: ^{1}H NMR (250 MHz, $CDCl_3$, selected signals only) 2.09 (s, 3H), 3.39, 3.61 (2d, J = 18.4, 2H), 5.10 (d, J = 13.3, 1H), 5.23 (d, J = 5.1, 1H), 5.51 (dd, J = 5.1, 1.9, 1H), 8.72 (d, J = 1.9, 1H); TLC R_f 0.51 (hexane/EtOAc, 1/1), 0.30 (toluene/*t*-BuOMe, 4/1). b) Imine **16** (50 mg) was dissolved in ice-cold DMF (0.5 ml, filtered over acidic aluminum oxide act. I). The pale blue solution was kept at 4°C (refrigerator). After 90 min the color had changed to pale yellow. Aliquots were taken after 2h, 1, 3, 6 and 15 days, evaporated and analyzed by ^{1}H NMR spectroscopy. The equilibrium of 40% *cis*- and 60% *trans*-imine was reached after 6 days.

Allyl-(6*RS*, 7*RS*)-3-acetoxymethyl-7-amino-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate (17). To a cold (2°C) suspension of the *cis*/*trans* imine-mixture (1.81 g, 4.06 mmol) in MeOH (20 ml) was added Girard reagent T (2.04 g, 12.2 mmol, 3 equiv) dissolved in MeOH (30 ml) in 5 min. The ice bath

was removed after 10 min and stirring was continued at rt for 4 h. The clear, yellow solution was concentrated. The solid residue was taken up in CH_2Cl_2 (100 ml) and washed with 1 M NaCl (100 ml) and H_2O (2 x 100 ml). Each aqueous layer was back-extracted with CH_2Cl_2 (100 ml). The organic layers were filtered over cotton, combined and evaporated. *Cis*-amine **17** and *trans*-amine **15** were separated by chromatography on silica gel (60g, CH_2Cl_2 /*t*-BuOMe 9:1 + 0.1% H_2O (!) to elute **17**, CH_2Cl_2 /*t*-BuOMe 5:1 + 0.1% H_2O (!) to elute **15**). 546 mg (1.748 mmol, 43%) amine **15** as an orange oil and 621 mg (1.99 mmol, 49 %) amine **17** as a beige solid were obtained. Data for **17**: mp 57-59°C (CH_2Cl_2/Et_2O); ^{1}H NMR (250 MHz, $CDCl_3$) 1.78 (s br, 2H), 2.09 (s, 3H), 3.39, 3.58 (2d, J = 18.4, 2H), 4.76-4.85 (mm, 4H), 4.96 (d, J = 5.1, 1H), 5.08 (d, J = 13.3, 1H), 5.29 (dq, J = 10.3, 1.2, 1H), 5.39 (dq, J = 18.6, 1.5, 1H), 5.89-6.03 (m, 1H); IR (KBr) 3422 (s), 1774 (s), 1737 (s), 1730 (s), 1644 (m), 1397 (s), 1353 (s), 1277 (s), 1229 (s); MS (ESI) 335 ($[M+Na]^+$, 16), 330.2 ($[M+NH_4]^+$, 10), 313.2 ($[M+H]^+$, 14), 225.2 (100); TLC R_f 0.26 (CH_2Cl_2/*t*-BuOMe 4/1), 0.13 (hexane/EtOAc, 1/1).

Potassium-(6*RS*, 7*RS*)-3-acetoxymethyl-7-amino-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate (18b). Allylester cleavage of **17** (520 mg, 1.665 mmol) as described for **15** afforded 509 mg (98.5%) of the potassium salt of (±)-7-ACA **18b** as a white solid. Data for **18b**: mp 187°C (dec), ^{1}H NMR (400 MHz, D_2O) 2.11 (s, 3H), 3.40, 3.66 (2d, J = 18.0, 2H), 4.71, 4.88 (2d, J = 12.4, 2H), 4.78 (d, J = 5.2, 1H), 5.07 (d, J = 5.2, 1H), ^{13}C NMR (100 MHz, D_2O) 23.13, 29.60, 60.98, 67.08, 69.27, 115.14, 135.20, 171.59, 176.96; IR (KBr) 3401 (m), 1741 (s), 1609 (s), 1399 (m), 1234 (m); MS (ESI) 271.1 ($[M-K]^-$, 30), 211.0 (100); Anal. Calcd for $C_{10}H_{11}N_2O_5SK$ (310.369): C, 38.70; H, 3.57; N, 9.03; S, 10.33; K, 12.60. Found C, 38.98; H, 4.09; N, 8.03; K, 13.08.

(6*RS*, 7*RS*)-3-Acetoxymethyl-7-amino-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (18a). Neutralization of potassium salt **18b** (450 mg, 1.450 mmol) as described for **4b** afforded 326 mg (83%) (±)-7-*epi*-ACA **18a** as a white solid. Data for **18a**: ^{1}H NMR (400 MHz, CF_3COOD) 2.30 (s, 3H), 3.74, 3.81 (2d, J = 17.6, 2H), 5.30, 5.50 (2d, J = 14.4, 2H), 5.44 (s, 2H); IR (KBr) 2600 (w, br), 1804 (s), 1739 (s), 1618 (s), 1544 (s), 1413 (s), 1238 (s); MS (ESI) 295.3 ($[M+Na]^+$, 47), 290.3 ($[M+NH_4]^+$, 76), 279.4 ($[M+Li]^+$, 100), 273.3 ($[M+H]^+$, 41); HPLC t_R 2.95 min; Anal. Calcd for $C_{10}H_{12}N_2O_5S$ (272.275): C, 44.11; H, 4.44; N, 10.29. Found C, 43.24; H, 4.40, N, 10.01. The observed analytical data are in excellent agreement with those of (+)-7-ACA **1**.

Chiral HPLC employed a Crownpak CR(-) column (Daicel Chemical Industries, 150 x 4 mm) and an 0.11 M $HClO_4$ (A) / H_2O (B) gradient [(t[min], A:B): (0, 100:0), (20, 100:0), (22, 10:90), (38, 10:90), (40, 100:0)]. The flow rate was 0.5 ml/min at rt and the detection wave length 260 nm. Retention times: 17.0 min for the (6*S*, 7*S*) isomer, 18.5 min for (6*R*, 7*R*), 26.5 min for (6*S*, 7*R*) and 34.8 min for (6*R*, 7*S*).[19]

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