Synthesis and Biological Activity of Enantiomeric Oxapenemcarboxylic Acids

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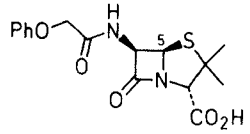
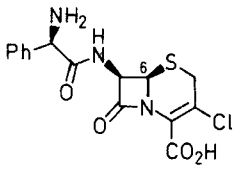
The synthesis of enantiomerically pure potassium (5*R*)- and (5*S*)-*trans*-2-*tert*-butyl-6-hydroxymethyloxapenem-3-carboxylates is reported. Surprisingly, both enantiomers were active antibiotics and β-lactamase inhibitors.

For more than twenty years the penicillins and the cephalosporins have been the most valuable antibiotics in the therapy of infectious diseases. These compounds possess two common structural features: a bicyclic β-lactam system and the 5*R* (for penicillins) or the 6*R* configuration (for cephalosporins). The corresponding *S*-isomers are generally considered to be inactive as antibacterials.

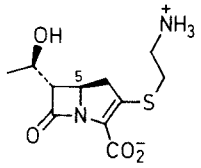


penicillin V
(5*R*)

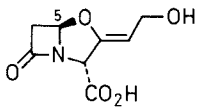


cefaclor (cephalosporins)
(6*R*)

The nonclassical β-lactams discovered later in nature, i.e. thienamycin[1] and clavulanic acid[2] proved to have also the 5*R*-configuration and within the synthetic class of penems antibacterial activity was inherent with the 5*R* configuration, whereas the 5*S* enantiomers were entirely[3] or almost[4] inactive.

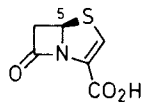


thienamycin
(5*R*)

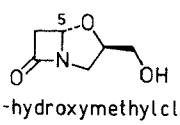


clavulanic acid
(5*R*)

A unique exception to the above-mentioned occurence of natural β-lactams with *R*-configuration was observed in the case of clavamycins,[5] which lack antibacterial activity. However, they possess potent antifungal properties.

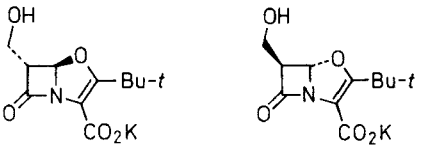


penem-3-carboxylic acid
(5*R*)



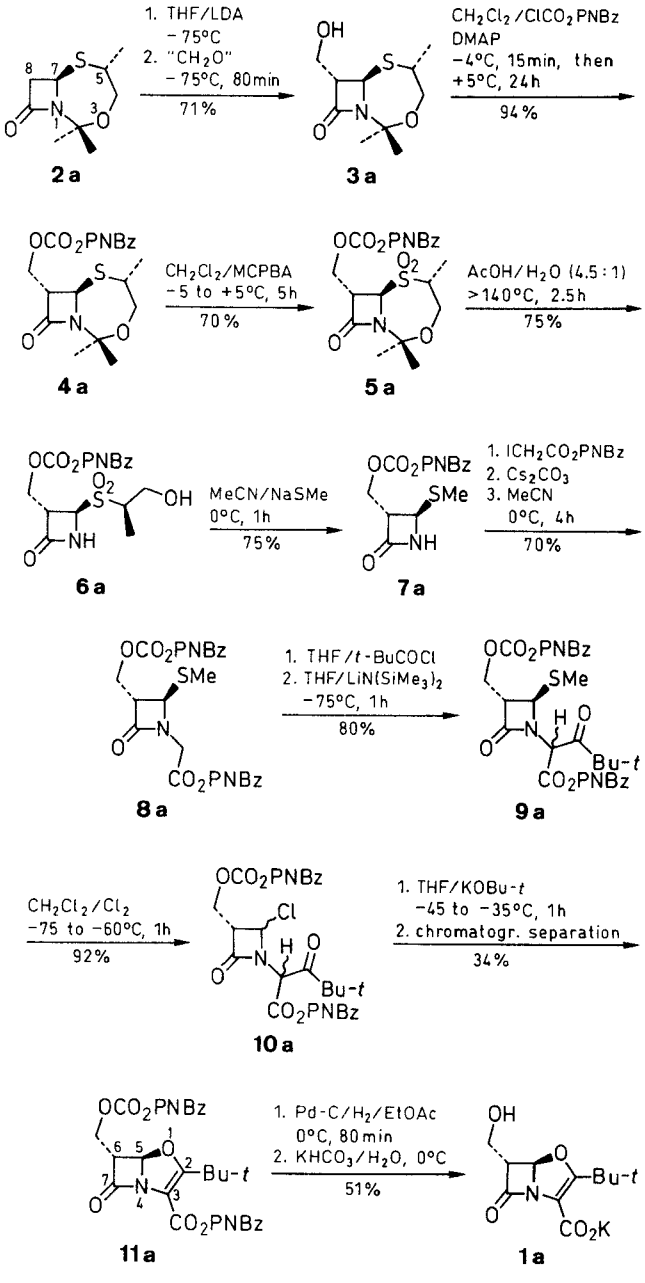
2-hydroxymethylclavam
(clavamycins)
(5*S*)

We wish to report here the preparation, antibacterial and β-lactamase inhibitory activities of (+)- and (−)-potassium *trans*-2-*tert*-butyl-6-hydroxymethyloxapenem-3-carboxylates (**1a** and **1b**). These model compounds were selected, after we had found that the corresponding racemic mixture was an effective antibacterial agent and β-lactamase inhibitor.[6,7]



1a **1b**

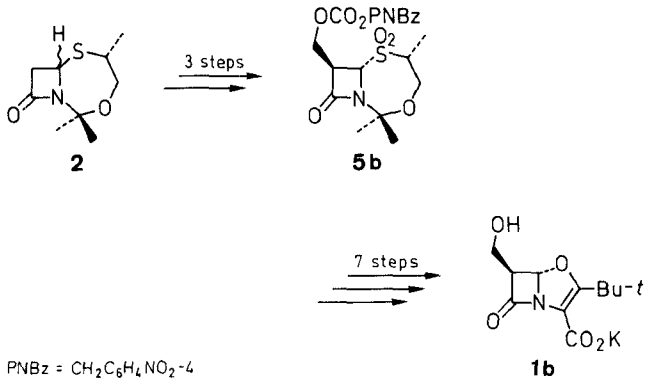
A proven way of preparing enantiomeric carboxylic acids is fractional crystallization of their diastereomeric salts with natural alkaloids. However, it was doubtful whether the sensitive racemic target compound **1** would have survived such handling.



PNBz = $CH_2C_6H_4NO_2$-4

Consequently, we chose a different strategy, allowing us to achieve resolution at an early stage of the reaction sequence, and thus used the far less reactive bicyclic β-lactam **2** as the starting material. Here, the chiral auxiliary derived from (*S*)-2-mercaptopropanol was covalently attached to the β-lactam ring. We have shown[8] that the separation of the "natural", (5*R*,7*R*)-diastereoisomer, the so called "4,7-lactam", could be achieved within a single crystallization from the (1 : 1) diastereomeric mixture.

For the preparation of the "natural" (+)-enantiomer **1a**, the above mentioned crystalline bicyclic (5*R*,7*R*)-**2a** (mp 85–86 °C) was suitable as the starting material. It was initially planned to obtain the "unnatural" enantiomer **1b** similarly from (5*R*,7*S*)-**2b**. However, the melting point of **2b** was lower (53–54 °C), and it was difficult to crystallize this stereoisomer to purity. Consequently, the synthesis of (−)-**1b** started with the diastereomerically enriched (5*R*,7*S*)-**2**, obtained from the mother liquor. Final resolution was accomplished later, by fractional crystallization of the intermediate **5b**.



The hydroxymethyl side chain was introduced stereospecifically (**2** → **3**) into the trans position of the β-lactam moiety, using monomeric formaldehyde generated from cyclohexanol/formaldehyde adduct.[9] The corresponding cis isomer of **3** was not found.

During the following substitution reaction with sodium methyl mercaptide (**6** → **7**) the chiral auxiliary was conveniently removed without any additional transformation.

The following construction of the oxazoline moiety of **11** (**8** → **11**) was a proven method[10] established in 1977. It has been successfully applied in the syntheses of 6-hydroxyethyl-2-isopropyloxapenemcarboxylic acids[11] and the more stable 2-*tert*-butyloxapenemcarboxylic acids.[12]

The cyclisation reaction (**10** → **11**) afforded a mixture of *trans*- and *cis*-oxapenem esters in a 2 : 1 ratio. They were separated by column chromatography on silica gel at −20 °C.

The fully protected *trans*-oxapenems **11a** and **11b** were carefully investigated regarding their enantiomeric purity. $[\alpha]_D$ values were + 54° (*c* = 1.6, EtOAc) for **11a** and − 56° (*c* = 0.8, EtOAc) for **11b**.

Finally, the two *p*-nitrobenzyl protecting groups in **11a** and **11b** were removed by catalytic hydrogenation, affording the title compounds **1a** and **1b**. ^{1}H NMR spectroscopy at 400 MHz indicated less then 3% of the corresponding *cis* isomers. The absolute configurations of the final compounds **1a** and **1b** were unequivocally established by two X-ray structure determinations,[13] by investigating single crystals of intermediates **3a** and **5b**. Determination of the biological acitvities (Table 1) revealed that both enantiomeric oxapenems (+)-**1a** and (−)-**1b** were active as antibacterials. Like the racemic form[7] of **1**, both enantiomers were more powerful inhibitors of staphylococcal β-lactamase than clavulanic acid[2] (Table 2).

Table 1. Minimal Inhibitory Concentration (μg/ml) of Penicillin V, Cefaclor and Enantiomeric Potassium Oxapenemcarboxylates **1a** and **1b**. Difco Nutrient Broth, 17 h, 37 °C.

	Pen V	Cefaclor	(+)-**1a**	(−)-**1b**
Staph. aureus DSM 1104	< 0.2	0.5	0.5	0.5
Staph. aureus 25466 (penicillin resistant)	> 128	16	0.2	2
E. coli DSM 1103	64	4	2	2
E. coli TEM 1	> 128	> 128	32	> 128
E. cloacae 30054	> 128	> 128	8	64

Table 2. β-Lactamase Inhibitory Activity (μg/mL) of Enantiomeric Potassium Oxapenemcarboxylates **1a** and **1b**. *Staph. aureus* 25466, Difco Nutrient Broth, 17 h, 37 °C.

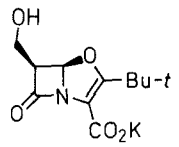
Compound	Activity[a]
Penicillin V	> 128
Penicillin V + **1a**	0.5 + 0.1
Penicillin V + **1b**	0.5 + 0.1
Cefaclor	16
Cefaclor + **1a**	1 + 0.2
Cefaclor + **1b**	1 + 0.2

[a] Determined by dilution assay. Minimal inhibitory concentration (MIC) of mixture, showing no turbidity.

The remarkably high antibacterial and β-lactamase inhibitory activity of a bicyclic β-lactam (−)**1b**, having the (5*S*)-configuration contrary to that of all known natural and synthetic β-lactam antibiotics, was most surprising. However, it is known[14] that the oxazoline ring of oxapenems can be opened and closed in the presence of nucleophiles.

With the optically active *trans*-(5*S*,6*R*)-**1b**, this reaction would lead to an equilibrium mixture of **1b** with its epimer *cis*-(5*R*,6*R*)-**1c**, having the natural configuration at C-5. Indeed we were able to detect such a very slow conversion in D_2O at 37 °C by NMR spectroscopy (new signal at 5.87 ppm; d, *J* = 2.7 Hz). It is conceivable that this epimerisation of **1b** into **1c** was faster in the nutrition medium or was enhanced by the bacterial enzymes during the biological tests (lasting 17 h at 37 °C). If this was true, the measured activities with **1b** could be attributed to the portion of natural cis isomer **1c** formed during the tests.

It has been stated[15] that, besides the reactive β-lactam unit, a leaving group at the 5-position of bicyclic β-lac-



1c

tams would be essential for their action as *irreversible* inhibitors of β-lactamases. Like clavulanic acid, the oxapenems possess such an additional reactive moiety. It is possible that the superior activity of the oxapenems against these bacterial enzymes is associated with the aforementioned opening reaction of the oxazoline ring.

Note Added in Proof:
With *isolated* penicillinase (type IV from Enterobacter cloacae) the (+)-enantiomer **1a** was found to be 50 times more active than the (−)-enantiomer **1b** (nitrocefin assay). This assay requires only 30 min for completion. After a preincubationat 37 °C for 15 min, the inhibition concentration (IC_{50}) was $2 \cdot 10^{-9}$ M (!) for **1a** and $1 \cdot 10^{-7}$ M for **1b**. This suggests that the observed activities for **1b** against intact bacteria (Tables 1 and 2) are indeed due to *cis*-isomer **1c**.

Reagents and solvents were of commercial quality from freshly opened containers and were purchased from Fluka Chemical Co. THF was refluxed under N_2 with $LiAlH_4$ and distilled immediately before use. The lithium bis[trimethylsilyl]amide solution in THF was prepared from equimolar amounts of hexamethyldisilazane and 2.5 M solution of BuLi in hexane (Janssen) at − 78 °C. The solvent was removed in vacuo and replaced by THF (under Ar). MeOH free, aqueous formaldehyde solution was obtained from BASF. *p*-Nitrobenzyl chloroformate was purified by recrystallization from diisopropyl ether at − 30 °C. Analytical silica gel TLC plates and silica gel were purchased from E. Merck. The progress of all reactions was monitored by TLC. Melting points were taken on a Büchi 535 apparatus and are uncorrected. Microanalyses were obtained using a Heraeus CHN Standard microanalyzer, IR spectra were obtained using a Perkin-Elmer 1420 IR-spectrometer, optical rotations on a Zeiss 0.005° polarimeter at r.t. and NMR spectra using a Varian VXR 400 S (400 MHz) spectrometer.

Cyclohexanol/Formaldehyde Adduct (40% Solution):
A mixture of cyclohexanol (474 g, 4.74 mol) and 30% aq (MeOH free) formaldehyde solution (237 g, 2.37 mol) was magnetically stirred under N_2 in a 1 L two-necked flask for 2 h at r.t. The flask was connected to a simple distillation apparatus and the volatile parts boiling at 45 °C (210 g) were removed at 30 Torr at a bath temperature of 100 °C. The pressure was then reduced to 15 Torr until the head temperature reached 66 °C and some decomposition of the adduct was indicated by deposition of paraformaldehyde in the condenser. The distillation residue was cooled to r.t. under N_2 and dried for 4 d using molecular sieves 4 Å (50 g) affording a viscous, colourless liquid, yield: 485 g. The solution can be stored over molecular sieves at r.t. for years without decomposition.

^{13}C NMR ($CDCl_3$/TMS): δ = 24.3 (t, CH_2, cyclohexanol), 25.6 (t, CH_2, cyclohexanol), 25.7 (t, CH_2, adduct), 32.6 (t, CH_2, adduct), 33.0 (t, CH_2, adduct), 35.5 (t, CH_2, cyclohexanol), 70.2 (d, CH, cyclohexanol), 75.0 (d, CH, adduct), 87.1 (t, CH_2, adduct). Additional very weak signals at δ = 75.6 (d, CH), 86.3 (t, CH_2), 89.6 (t, CH_2) and 90.1 (t, CH_2).

(+)-(5*R*,7*R*)-2,5,5-Trimethyl-9-oxo-3-oxa-6-thia-1-azabicyclo [5.2.0.^{1,7}]nonane (2a):
The "4,7-lactam" **2** was prepared[8] as a 1:1 diastereoisomeric mixture of **2a** and **2b** (yield: 60%). Product **2a** with natural configuration was obtained after a single crystallization from n-hexane; yield: 20%; mp 84.7–86.1 °C; $[\alpha]_D^{20}$ + 132.5° (*c* = 1.665, $CHCl_3$); TLC: R_f 0.40 (toluene/EtOAc, 2:1, I_2 staining).

$C_9H_{15}NO_2S$	calc.	C 53.70	H 7.51	N 6.96	S 15.93
(201.3)	found	53.99	7.54	6.77	16.03

IR (CH_2Cl_2): ν = 2980, 2960, 2930 (C−H), 1755 (C=O), 1235, 1110 cm^{-1}.

(+)-(5*R*,7*R*,8*S*)-8-(1-Hydroxymethyl)-2,2,5-trimethyl-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane (3a):
The reaction apparatus consisted of a 250 mL 3-necked flask, fitted with gas outlet bubbler (containing paraffin oil), septum and mechanical stirrer. To this flask was connected a 100 mL Schlenk flask via a Liebig condenser. The apparatus was carefully dried by passing dry N_2 and heating. The Schlenk flask was supplied with cyclohexanol/formaldehyde adduct (19 g, 40% solution) and into the three-necked flask dry THF (30 mL) was added and its content cooled to − 78 °C. Within 5 min, diisopropylamine (1.64 g, 2.3 mL, 16.23 mmol), within 5 further min, BuLi (2.5 M solution in hexane, 6.6 mL, 16.50 mmol) and finally, also within 5 min, a solution of **2a** (2.88 g, 14.29 mmol) in dry THF (30 mL) were added by a syringe. The Schlenk flask was immersed into a preheated (130 °C) oil bath, heated to 160 °C within 10 min, and kept at 160–180 °C during the reaction. Cold water was circulated through the Liebig condenser in order to condense cyclohexanol. During this procedure the liberated monomeric formaldehyde was passed over the surface of the cold (− 78 °C) and well stirred reaction mixture by passing a slow stream of N_2 (2–3 bubbles per sec) through the apparatus. After 1 h TLC indicated the absence of starting material **2a**. The oil bath was then removed and the N_2 flow stopped. EtOAc (95 mL) was slowly added within 30 min and the mixture transferred to a separatory funnel with a further portion of EtOAc (140 mL). The solution was subsequently washed with a mixture of brine (140 mL) and 2 N HCl (70 mL) and then with brine (2 × 140 mL). The aqueous phases were reextracted with EtOAc (140 mL) and the combined organic layers dried ($MgSO_4$). Filtration and evaporation in vacuo afforded crude product **3a** (3.06 g, 93%) as a colourless powder. It was chromatographed on silica gel (63–200 μm, 360 g) using toluene/EtOAc (2:1) as eluent (23 fractions, 300 mL each). Besides starting material **2a** in the first fractions, pure **3a** (2.33 g, 71%) was obtained from fractions 11–20, mp 135.7–136.6 °C (toluene); yield: 2.33 g (71%); $[\alpha]_D^{20}$ + 137.5° (*c* = 1.61, $CHCl_3$); TLC: R_f 0.12 (toluene/EtOAc, 2:1, I_2 staining); X-ray.[13]

$C_{10}H_{17}NO_3S$	calc.	C 51.93	H 7.41	N 6.06	S 13.86
(231.3)	found	52.13	7.20	5.96	13.86

IR (CH_2Cl_2): ν = 3600 (O−H), 2925, 2875, 1740 (C=O), 1450, 1380, 1350, 1220, 1110, 1070, 1040 cm^{-1}.

^{13}C NMR ($CDCl_3$/TMS): δ = 17.8 (q, CH_3), 24.2 (q, CH_3), 26.6 (q, CH_3), 40.7 (d, CH), 53.0 (d, CH), 57.8 (t, CH_2), 58.0 (d, CH), 72.1 (t, CH_2), 87.5 (s, quart. C), 165.8 (s, quart. C).

(+)-(5*R*,7*R*,8*S*)-2,2,5-Trimethyl-8-(1-*p*-nitrobenzyloxycarbonyloxymethyl)-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane (4a):
To a solution of **3a** (3.40 g, 14.69 mmol) and recrystallized *p*-nitrobenzyl chloroformate (3.81 g, 17.66 mmol) in anhydrous CH_2Cl_2 (60 mL, filtered through basic Al_2O_3) in a 250 mL Schlenk flask was added solid 4-dimethylaminopyridine (DMAP) (2.75 g, 22.53 mmol) in small portions at − 4 °C under N_2 atmosphere. During addition, a voluminous, colourless precipitate was formed. The mixture was then left at + 5 °C in a refrigerator for 24 h. It was diluted with EtOAc (300 mL) and the resulting solution subsequently washed with 2 N HCl (180 mL), 15% NaCl (160 mL) and 5% $NaHCO_3$ (160 mL) solutions. The aqueous phases were reextracted with EtOAc (150 mL) and the combined organic phases were dried ($MgSO_4$). Filtration and evaporation in vacuo gave the crude product **4a**. Chromatography on silica gel (63–200 μm, 200 g) using toluene/EtOAc (9:1) as eluent (30 fractions, 100 mL each) afforded pure **4a** (5.69 g, 94%) as pale yellow resin; yield: 5.69 (94%); $[\alpha]_D^{20}$ + 80.3° (*c* = 1.115, $CHCl_3$); TLC: R_f 0.14 (toluene/EtOAc, 9:1, UV active, I_2 staining).

IR (CH_2Cl_2): ν = 3060 (C−H_{arom}), 2980, 2945, 2880 (C−H_{aliph}), 1760 (C=O), 1615 (C=C), 1530 (NO_2), 1460, 1395, 1360 (NO_2), 1240 (C−O), 1080, 1040 cm^{-1}.

(+)-(5*R*,7*R*,8*S*)-2,2,5-Trimethyl-8-(1-*p*-nitrobenzyloxycarbonyloxymethyl)-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane-6,6-dioxide (5a):

To a solution of **4a** (1.00 g, 2.44 mmol) in CH_2Cl_2 (20 mL) was added in small portions *m*-chloroperbenzoic acid (MCPBA) (55%, 2.10 g, 6.56 mmol) within 20 min at −5 °C. The mixture was stirred for an additional 4.5 h at −5 to +5 °C, where upon TLC indicated the absence of starting material. It was diluted with CH_2Cl_2 (95 mL) and the resulting solution washed subsequently with sat. $NaHCO_3$ (35 mL), 10% $NaHSO_3$ and finally with sat. $NaHCO_3$ (35 mL). The aqueous layers were reextracted with CH_2Cl_2 (40 mL) and the combined organic phases were dried ($MgSO_4$). Filtration and removal of the solvent in vacuo afforded a noncrystalline, nearly colourless solid (1.12 g). It was recrystallized from toluene to give pure (5*R*,7*R*,8*S*)-isomer **5a**; yield: 0.72 g (70%); mp 124.0–125.1 °C; $[\alpha]_D^{20}$ + 18.4° (*c* = 1.78, $CHCl_3$), TLC: R_f 0.37 (toluene/EtOAc, 1:1, UV active and I_2 staining).

$C_{18}H_{22}N_2O_9S$	calc.	C 48.86	H 5.01	N 6.33	S 7.25
(442.45)	found	48.74	5.07	6.30	7.25

IR (CH_2Cl_2): *v* = 3050 (C−H_{arom}), 2980, 2945 (C−H_{aliph}), 1775 (C=O, β-lactam), 1755 (sh, C=O, carbonate), 1610 (C=C), 1525 (NO_2), 1450, 1390, 1375, 1350 (NO_2), 1330, 1305, 1235 (C−O) 1180, 1150, 1135, 1080 cm^{-1}.

^{13}C NMR ($CDCl_3$/TMS): δ = 11.5 (q, CH_3), 23.4 (q, CH_3), 26.8 (q, CH_3), 48.4 (d, CH), 60.4 (d, CH), 61.3 (t, CH_2), 62.7 (t, CH_2), 63.4 (d, CH), 68.4 (t, CH_2), 89.3 (s, quart. C), 123.9 (d, CH), 128.5 (d, CH), 142.0 (s, quart. C), 148.0 (s, quart. C),154.1 (s, quart. C), 163.4 (s, quart. C).

(−)-(5*R*,7*S*,8*R*)-2,2,5-Trimethyl-8-(1-*p*-nitrobenzyloxycarbonyloxymethyl)-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane-6,6-dioxide (5b):

Compound **4** (5.51 g, 13.43 mmol), consisting of 78% (5R,7S, 8R)-**4b** and 22% of (5*R*,7*R*,8*S*)-diastereomer **4a**, was converted analogously to the corresponding crude mixture of diastereomers **5**; yield: 6.59 g. The yellow crystalline solid was suspended in dry toluene (20 mL) and stirred at r. t. for 5 min and the solid collected on a glass filter; yield 3.47 g (58%). It consisted of nearly pure **5b** and was recrystallized from hot toluene to give pure (5*R*,7*S*,8*R*)-diastereomer **5b**; Yield 3.02 g (50%); mp 145.1–145.8 °C; $[\alpha]_D^{20}$ −20.8° (*c* = 1.815, $CHCl_3$); TLC: R_f 0.37 (toluene/EtOAc, 1:1, UV active and I_2 staining); X-ray.[13]

$C_{18}H_{22}N_2O_9S$	calc.	C 48.86	H 5.01	N 6.33	S 7.25
(442.5)	found	49.06	5.02	6.17	7.24

IR (CH_2Cl_2): identical to that of **5a**.

^{13}C NMR ($CDCl_3$/TMS): δ = 7.1 (q, CH_3), 23.2 (q, CH_3), 27.0 (q, CH_3), 48.6 (d, CH), 60.3 (d, CH), 62.8 (t, CH_2), 63.4 (t, CH_2), 66.1 (d, CH), 68.3 (t, CH_2), 88.7 (s, quart. C), 123.8 (d, CH), 128.4 (d, CH), 142.0 (s, quart. C), 148.0 (s, quart. C), 154.1 (s, quart. C), 163.3 (s, quart. C).

(+)-(3*S*,4*R*)-4-[(2*R*)-2-(1-Hydroxypropyl)-sulfonyl]-3-(*p*-nitrobenzyloxycarbonyloxymethyl)azetidin-2-one (6a):

Compound **5a** (3.0 g, 6.78 mmol) was dissolved in AcOH (165 mL) at 60 °C and to this solution H_2O (37 mL) was added. The mixture was refluxed under N_2 for 2.5 h at an oil bath temperature of ≥ 140 °C. After cooling, the solvent was removed in vacuo at 45–50 °C. The residue was treated with EtOAc and the solvent removed again in vacuo. The residue, a brown-yellow resin, was dried for 30 min at 0.001 Torr, yielding crude **6a** (3.14 g). It was chromatographed on silica gel (63–200 μm, 180 g) using toluene/EtOAc (1:1) as eluent (26 fractions, 150 mL each), affording some starting material **5a** from the early fractions and pure **6a** from fractions 10–26, as an extremely hygroscopic, noncrystalline solid; yield: 2.05 g (75%); $[\alpha]_D^{20}$ + 29.1° (*c* = 2.02, $CHCl_3$); TLC: R_f 0.06 (toluene/EtOAc, 1:1, UV active and I_2 staining).

IR (CH_2Cl_2): *v* = 3605 (O−H), 3400 (N−H), 3050 (C−H_{arom}), 2960, 2890 (C−H_{aliph}), 1795 (C=O, β-lactam, 1755 (C=O, carbonate), 1610 (C=C), 1525 (NO_2), 1455, 1395, 1350 (NO_2), 1330, 1310, 1240 (C−O), 1135 (SO_2), 1085, 1050, 1020, 980, 855 cm^{-1}.

(+)-(3*S*,4*R*)-4-Methylthio-3-(*p*-nitrobenzyloxycarbonyloxymethyl)-azetidin-2-one (7a):

To a solution of **6a** (1.97 g, 4.89 mmol) in MeCN (60 mL) was added a solution of NaSMe (0.45 g, 6.46 mmol) in H_2O (50 mL) within 20 min at 0 °C. The mixture was allowed to stir for an additional 40 min at 0 °C. It was then diluted with CH_2Cl_2 (180 mL) and the resulting solution was washed with H_2O (80 mL). The aqueous layer was reextracted with CH_2Cl_2 (2 × 80 mL) and the combined organic phases were dried ($MgSO_4$). Filtration and evaporation of the solvent in vacuo and drying of the residue at 0.001 Torr for 2.5 h, gave the crude product **7a** (1.60 g, 100%) as a yellow resin. It was purified by chromatography on silica gel (63–200 μm, 100 g) using toluene/EtOAc (9:1) (9 fractions, 200 mL each) and toluene/EtOAc (4:1) as eluents (20 fractions, 100 mL each). Evaporation of the fractions 14–25 afforded pure **7a**; yield: 1.20 g (75%), mp 67.9–69.5 °C, after drying at 0.001 Torr; $[\alpha]_D^{20}$ + 58.2° (*c* = 1.88, $CHCl_3$); TLC: R_f 0.34 (toluene/EtOAc, 1:1, UV active, I_2 staining).

(−)-(3*R*,4*S*)-**7b**: $[\alpha]_D^{20}$ − 55.2° (*c* = 1.60, $CHCl_3$).

$C_{13}H_{14}N_2O_6S$	calc.	C 47.85	H 4.32	N 8.58	S 9.83
(326.3)	found	48.12	4.43	8.52	9.81

IR (CH_2Cl_2): *v* = 3400 (N−H), 3060 (C−H_{arom}), 2960, 2930 (C−H_{aliph}), 1775 (C=O, β-lactam), 1755 (sh, C=O, carbonate), 1610 (C=C), 1525 (NO_2), 1390, 1350 (NO_2), 1240 (C−O), 1155, 970, 955, 855 cm^{-1}.

***p*-Nitrobenzyl (−)-(3*S*,4*R*)-[4-Methylthio-3-(*p*-nitrobenzyloxycarbonyloxymethyl)-2-oxazetidin-1-yl]acetate (8a):**

In a dried 100 mL Schlenk flask, fitted with a magnetic stirrer and a balloon filled with Ar, compound **7a** (125 mg, 0.382 mmol) was cooled to 0 °C and *p*-nitrobenzyl iodoacetate (184 mg, 0.573 mmol) and Cs_2CO_3 (187 mg, 0.573 mmol) (both dried for 1.5 h at 0.001 Torr just before use) were added. The dry solid mixture was stirred for 5 min until it became a fine white powder. Cold anhydrous MeCN (15 mL, stored over molecular sieves 4 Å) was added and the mixture was allowed to stir for 4 h at 0 °C. TLC indicated the absence of starting material. The cold mixture was diluted with toluene (75 mL), EtOAc (15 mL) was added and the resulting solution was washed subsequently with cold 1 N HCl (60 mL), 10% $NaHCO_3$ (50 mL) and 10% NaCl solutions. The aqueous phases were reextracted with EtOAc (2 × 25 mL). The combined organic solutions were dried ($MgSO_4$). Filtration and evaporation of the solvent in vacuo gave crude product **8a** (266 mg). It was rapidly chromatographed on silica gel (40–63 μm, 20 g) using toluene/EtOAc (4:1) as eluent (23 fractions, 10 mL each). Evaporation of fractions 11–19 afforded pure **8a** as a pale yellow resin, after drying for 8 h at 0.001 Torr; yield: 139 mg (70%); $[\alpha]_D^{20}$ − 16.2° (*c* = 1.65, $CHCl_3$); TLC: R_f 0.40 (toluene/EtOAc, 1:1, UV active and I_2 staining).

(+)-(3*R*,4*S*)-**8b**: $[\alpha]_D^{20}$ + 17.3° (*c* = 2.2, $CHCl_3$).

$C_{22}H_{21}N_3O_{10}S$	calc.	C 50.87	H 4.07	N 8.09	S 6.17
(519.5)	found	51.32	4.37	7.83	6.19

IR (CH_2Cl_2): *v* = 3060 (C−H_{arom}), 2990, 2960, 2930 (C−H_{aliph}), 1770 (C=O), β-lactam), 1755 (C=O, carbonate + ester), 1610 (C=C), 1525 (NO_2), 1410, 1395, 1350 (NO_2), 1240 (C−O), 1180, 1130, 970, 940, 855 cm^{-1}.

***p*-Nitrobenzyl (2*R*)- and (2*S*)-2-[(3*S*,4*R*)-4-Methylthio-3-(*p*-nitrobenzyloxycarbonyloxymethyl)-2-oxoazetidin-1-yl]-4,4-dimethyl-3-oxopentanoates (9a):**

In a dried 100 m Schlenk flask equipped with a rubber septum was charged a solution of **8a** (528 mg, 1.016 mmol) in dry THF (25 mL) under Ar atmosphere. To this was added with a syringe pivaloyl chloride (129 mg, 131 μL, 1.067 mmol) at −78 °C. Within 30 min a 1 N solution of lithium bis[trimethylsilyl]amide (2.032 mL, 2.032 mmol) was then added at −78 °C. The resulting yellow solution was allowed to stir for 30 min at −78 °C and it was diluted with toluene (130 mL). The resulting solution was subsequently washed with cold 2 N HCl (0 °C, 120 mL) and brine (2 × 120 mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo to give crude **9a**. It was chromatographed on silica gel (40–63 μm, 50 g) using toluene/EtOAc (9:1) as eluent (17 fractions, 50 mL each). From fractions 7–12, pure **9a** (0.490 g, 80%) was

obtained as a colourless crystalline solid, after drying for 8 h at 0.0005 Torr (diastereomeric mixture, ratio approx. 2:1); yield: 0.49 g (80%); mp 79.5–86.1 °C; TLC: R_f 0.60 (toluene/EtOAc, 1:1, UV active, I_2 staining).

$C_{27}H_{29}N_3O_{11}S$	calc.	C 53.73	H 4.84	N 6.96	S 5.31
(603.6)	found	53.83	4.90	6.76	5.31

IR (CH_2Cl_2): ν = 3050 (C–H_{arom}), 2970, 2930, 2870 (C–H_{aliph}), 1770 (sh, C=O, β-lactam), 1755 (C=O, carbonate + ester), 1715 (C=O, ketone), 1610 (C=C), 1525 (NO_2), 1460 (br), 1350 (NO_2), 1315, 1240 (C–O), 1180, 1110, 975 (br), 850 cm^{-1}.

p-Nitrobenzyl (2*R*)- and (2*S*)-2-[(3*S*,4*R*)- and (3*S*,4*S*)-4-Chloro-3-(*p*-nitrobenzyloxycarbonyloxymethyl)-2-oxoazetidin-1-yl]-4,4-dimethyl-3-oxopentanoates (10a):

To a solution of **9a** (393 mg, 0.651 mmol) in CH_2Cl_2 (40 mL, filtered through basic Al_2O_3) in a 100 mL Schlenk flask was added with a syringe anhydrous gaseous Cl_2 (38 mL, 1.583 mmol) at −78 °C. The needle was dipped into the solution during addition. The mixture was allowed to stir for an additional 40 min at −78 °C to −60 °C TLC indicated the absence of starting material. The cold solution was subsequently washed with a solution containing $NaHSO_3$ (2.10 g) and Na_2CO_3 (1.67 g) in H_2O (40 mL). The aqueous layer was reextracted with CH_2Cl_2 (10 mL), the combined organic phases were dried ($MgSO_4$) and the solvent was removed in vacuo. The viscous liquid residue was immediately chromatographed on silica gel (40–60 μm, 20 g) at −25 to −10 °C using toluene/EtOAc (9:1) as eluent (22 fractions, 10 mL each). The solvent was removed from fractions 7–21 and the residue reevaporated once with EtOAc and twice with CH_2Cl_2.

After drying at 0.0005 Torr for 2 h pure colourless, non-crystalline **10a** (356 mg, 92%) was obtained (diastereomeric mixture, ratio, approx. 5:3:2:1). It was stored at −80 °C under Ar; yield: 356 mg (92%); TLC: R_f 0.65 and 0.57 (toluene/EtOAc, 1:1, UV active and weak iodine staining).

IR (CH_2Cl_2): ν = 3050 (C–H_{arom}), 2960, 2930, 2855 (C–H_{aliph}), 1790 (C=O, β-lactam), 1755 (C=O, carbonate + ester), 1715 (C=O, ketone), 1610 (C=C), 1525 (NO_2), 1460 (br), 1370, 1350 (NO_2), 1320, 1240 (C–O), 1180, 970, 855 (br) cm^{-1}.

p-Nitrobenzyl (+)-(5*R*,6*S*)-3-*tert*-Butyl-6-(*p*-nitrobenzyloxy-carbonyloxymethyl)-7-oxo-3-oxa-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (11a):

To a solution of **10** (329 mg, 0.556 mmol) in dry THF (40 mL) in a 100 mL Schlenk flask was added a 0.92 M solution of KOBu-*t* (0.66 mL, 0.606 mmol) within 20 min at −45 to −35 °C. The mixture turned brown-yellow at the completion of addition. It was allowed to stir for an additional 60 min at −45 to −35 °C, till TLC indicated the absence of starting material. To the cold reaction mixture EtOAc (80 mL) was added and the resulting solution was washed subsequently with 10% aq NaCl (70 mL) and brine (35 mL). The aqueous layers were reextracted with EtOAc (40 mL) and the combined organic phases dried ($MgSO_4$). Filtration and evaporation of the solvent in vacuo and drying of the residue at 0.0005 Torr for 1.5 h afforded crude **11** (313 mg) as a viscous yellow liquid. Separation of the desired *trans*-isomer **11a** from the (5*S*,6*S*)-*cis*-isomer was achieved by medium pressure chromatography on silica gel (5–20 μm, 50 g) at −15 to −6 °C. The flow rate was 25 mL/45 min with toluene/EtOAc (30:1) as eluent (20 fractions, 25 mL each). From fractions 13 and 14 pure *trans*-isomer **11a** (105 mg, 34%) was obtained as a pale yellow noncrystalline solid (after drying at 0.0005 Torr for 8 h); yield: 105 mg (34%). Fractions 15–17 (61 mg, 20%) contained, besides **11a**, its (5*S*,6*S*)-*cis*-isomer. To obtain an analytically pure sample, the isomeric mixture (50 mg) was chromatographed on silica gel (15–40 μm, 5 g) at −20 to −10 °C using hexane/EtOAc (3:1). From fractions 16–22, 39 mg of an analytically pure, *cis*, *trans*-isomeric mixture was obtained; **11a** $[\alpha]_D^{20}$ + 54.2° (*c* = 1.57, EtOAc); TLC: R_f 0.27 (*trans*) and 0.21 (*cis*) (toluene/EtOAc, 19:1, UV active and I_2 staining).

$C_{26}H_{25}N_3O_{11}$	calc.	C 56.22	H 4.54	N 7.56	
(555.5)	found	56.21	4.79	7.51	

IR (CH_2Cl_2): ν = 3050 (C–H_{arom}), 2960, 2930, 2860 (C–H_{aliph}), 1805 (C=O, β-lactam), 1755 (C=O, carbonate), 1715 (C=O, ester), 1610 (C=C), 1580 (C=C, five-membered ring), 1525 (NO_2), 1350 (NO_2), 1320, 1240 (C–O), 1170, 1090 (C–O), 1015, 850 cm^{-1}.

^1H NMR (CD_3CN/TMS): δ = 1.31 (s, 9H, *t*-C_4H_9), 4.08 (ddd, *J* = 4.8, 3.5, 0.9 Hz, 1H, H-6, *trans*), 4.46 (dd, *J* = 12.1, 4.8 Hz, 1H, CH_2O), 4.54 (dd, *J* = 12.1, 3.5 Hz, 1H, CH_2O), 5.27 (s, 2H, Ar–CH_2), 5.22 and 5.43 (AB, J_{AB} = 14.1 Hz, 2H, Ar–CH_2), 5.85 (d, ^3J = 0.9 Hz, 1H, H-5, *trans*), 7.58 (d, *J* = 8.8 Hz, 2H_{arom}), 7.65 (d, *J* = 8.8 Hz, 2H_{arom}), 8.19 (d, *J* = 8.8 Hz, 4H_{arom}).

^{13}C NMR (CD_3CN/TMS): δ = 26.6 (q, CH_3), 33.7 (s, quart. C), 62.3 (t, CH_2), 62.7 (d, CH), 64.6 (t, CH_2), 68.2 (t, CH_2), 90.6 (d, CH), 123.5 (d, CH), 123.7 (d, CH), 128.1 (d, CH), 128.6 (d, CH), 142.9 (s, quart. C), 144.0 (s, quart. C), 154.2 (s, quart. C), 160.7 (s, quart. C), 176.3 (s, quart. C), 176.5 (s, quart. C).

p-Nitrobenzyl (−)-(5*S*,6*R*)-3-*tert*-Butyl-6-(*p*-nitrobenzyloxycarbonyloxymethyl)-7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (11b):

Following the procedure for **11a**, **10b** was converted to the pure *trans*-isomer (−)-**11b**. Spectral data of **11a** and **11b** are identical (enantiomers); $[\alpha]_D^{20}$ of pure *trans*-(5*S*,6*R*)-isomer **11b**: −56.0° (*c* = 0.82, EtOAc).

$C_{26}H_{25}N_3O_{11}$	calc.	C 56.22	H 4.54	
(555.5)	found	56.83	4.64	

Potassium (5*R*,6*S*)-3-*tert*-Butyl-6-hydroxymethyl-7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (1a):

In a hydrogenation apparatus equipped with a magnetic stirrer and a rubber septum, a suspension of Pd on C (10%, 80 mg) in EtOAc (6 mL) was prehydrogenated at 0 °C (consumption 4.2 mL of H_2 in 15 min). A solution of **11a** (77 mg, 0.139 mmol) in EtOAc (2 mL) was added with a syringe and the mixture hydrogenated at 0 °C until the reaction came to an end (65 min). The consumption was 28 mL (8.4 equiv instead of 8 equiv). The mixture was filtered through a small glass filter (No. 4) into a reagent flask which contained a cold solution of $KHCO_3$ (10.4 mg, 0.104 mmol) in H_2O (3.5 mL). The two-phase mixture was vigorously shaken under N_2 for 3 min at 0 °C, the organic layer removed with a pipette and the aqueous layer washed twice with portions (2 mL) of EtOAc at 0 °C. Residual EtOAc was removed from the aqueous phase, in vacuo (13 Torr) in a 10 mL round-bottomed flask and then by short evacuation (2 min) in high vacuum. UV spectroscopy indicated a yield of 19.67 mg (51%). For lyophilisation a completely tight dessicator was used, containing 100 g of "Siccapent" (Merck). The solution containing **1a** was distributed into several portions in glass ampoules. They were cooled to −78 °C and kept in the dessicator, which was immediately evacuated and stored in a refrigerator at −35 °C. After 1 d the vacuum was controlled (< 0.005 Torr). After 2 weeks at −35 °C and 0.001 Torr, H_2O was completely absorbed and **1a** was obtained as a voluminous white powder (content 92%); yield: 18.1 g (47%); $[\alpha]_D$ + 188.7° (*c* = 0.26, H_2O).

IR (KBr): ν = 2960, 2935 and 2875 (C–H_{aliph}), 1781 (C=O, β-lactam), 1602 (carboxylate), 1576 (sh), 1391 (carboxylate), 1306, 1200, 1092, 1054, 793 cm^{-1}.

UV (H_2O): λ_{max} = 262 nm (ε = 5000).

^1H NMR (D_2O/$Me_3SiCD_2CD_2CO_2Na$): δ = 1.25 (s, 9H, *t*-C_4H_9), 3.90 (dt, *J* = 4.1 Hz, 0.6 Hz, 1H, H-6, *trans*), 3.96 (dd, *J* = 4.1 Hz, 2.6 Hz, 2H, CH_2OH), 5.78 (d, *J* = 0.6 Hz, 1H, H-5, *trans*).

Potassium (5*S*,6*R*)-3-*tert*-Butyl-6-hydroxymethyl-7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-en-2-carboxylate (1b):

Following the procedure for the preparation of **1a**, **11b** was converted into **1b**. Spectral data of **1a** and **1b** were identical (enantiomers); $[\alpha]_D^{20}$ −190.2° (*c* = 0.295, H_2O).

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