The Penems, a New Class of β-Lactam Antibiotics. 4. Syntheses of Racemic and Enantiomeric Penem Carboxylic Acids

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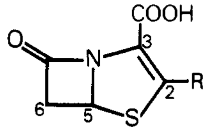
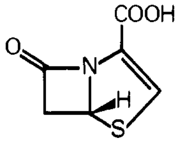
Abstract: The racemic parent penem-3-carboxylic acid **2** as well as its two enantiomers **2a** and **2b** has been synthesized.

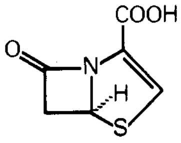
Recently the biologically active racemic penems **1**, prepared by total synthesis, have been reported from our laboratories.[1] Those bearing a small substituent on C-2, such as the 2-methylpenem-3-carboxylic acid **1** (R = CH_3), were effective in vitro against both Gram-positive and Gram-negative bacteria. In this paper we wish to report the total synthesis of the racemic parent penem carboxylic acid **2**, as well as its component enantiomers **2a** and **2b**.



1
2 R=H

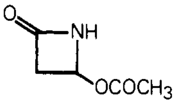


2a



2b

In the already mentioned syntheses of racemic 2-substituted penem carboxylic acids **1**,[1] 4-acylthio-2-azetidinones **4**, prepared from 4-acetoxy-2-azetidinone **3** by reaction with salts of thiocarboxylic acids, served as intermediates.



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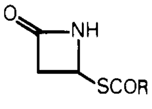

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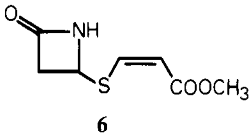
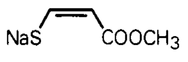


6

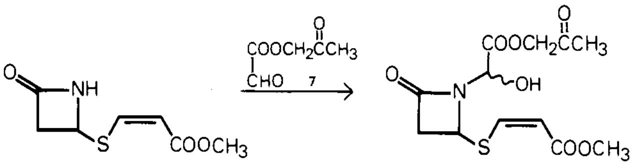
The preparation of the unsubstituted penem carboxylic acid **2** following the above-mentioned route would involve the azetidinone **4** (R = H). However, our experience with a similar (3-acylamino-substituted) 4-formylthioazetidinone suggested that compound **4** (R = H) would probably be very unstable and unsuitable for use as an early intermediate in a synthetic scheme. Therefore, as in the synthesis of 6*R*-phenoxyacetamido-(5*R*)-penem-3-carboxylic acid,[2] instead of the labile formylthio compound we preferred an equivalent β-mercapto acrylate (compound **6**)—prepared by a novel sequence—which at a later stage of the reaction sequence could be converted into a formylthio derivative.



5

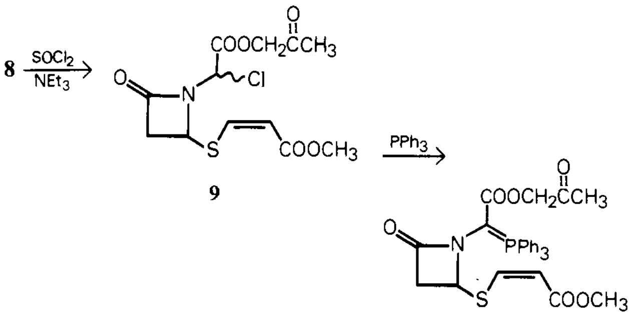
The crystalline azetidinone **6** was obtained from the readily available acetoxy-β-lactam **3**[3] by a displacement reaction using the sodium salt **5** of methyl *cis*-β-mercaptoacrylate (preparation described in the Experimental Section) in aqueous solution at −10 °C in 80% yield.

The success of the subsequent reaction steps is chiefly due to the selection and use of a known but nevertheless uncommon protection method for the carboxylic acid group: the acetonyl ester.[4] We have found these versatile esters to be easily crystallized or chromatographed on silica. They are stable toward acidic reagents, but smoothly and efficiently cleaved by mild alkaline hydrolysis.



6 **8**

Thus, racemic 4-(*cis*-β-carbomethoxyvinylmercapto)azetidin-2-one (**6**) reacted with acetonyl glyoxylate (**7**) (preparation described in the Experimental Section) in a mixture of toluene and DMF at room temperature to afford two epimeric carbinolamides **8**. The mixture reacted with thionyl chloride and triethylamine in tetrahydrofuran at −10 °C to afford the expected chloride epimers **9** which, after reaction with triphenylphosphine, furnished one single compound, the amorphous phosphorane **10**, in 60% yield (based on **6**).



9

10

Ozonolysis of **10**, carried out in a mixture of methylene chloride and trifluoroacetic acid to convert the ozone-sensitive carbon–phosphorus double bond into an inert single bond of the phosphonium salt **11**, selectively cleaved the double bond of the β-mercaptoacrylate moiety, to give the desired thioformate **12** which subsequently was deprotonated and then

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[†] Deceased July 8, 1979.

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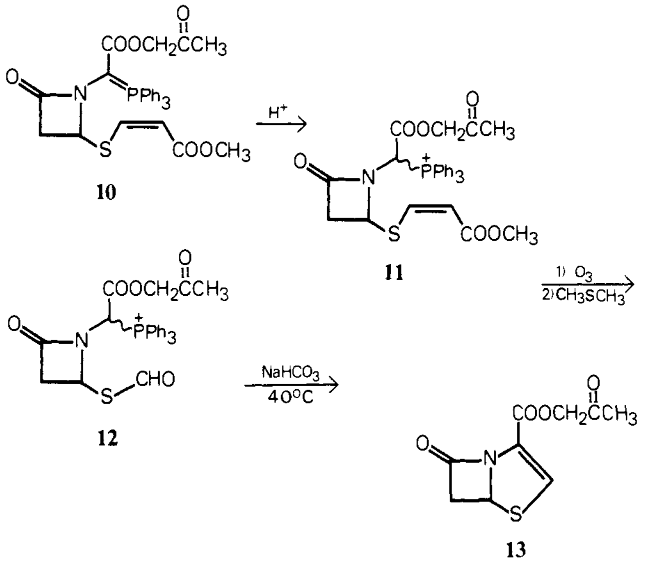
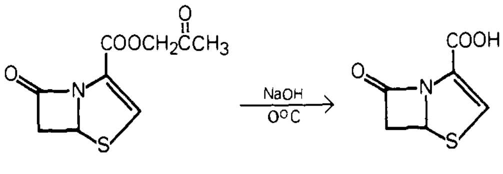
underwent an intramolecular Wittig condensation at 40 °C, yielding the racemic acetonyl penem-3-carboxylate **13** (81% based on **10**), mp 116 °C, along with triphenylphosphine oxide and methyl glyoxylate, the expected byproducts.



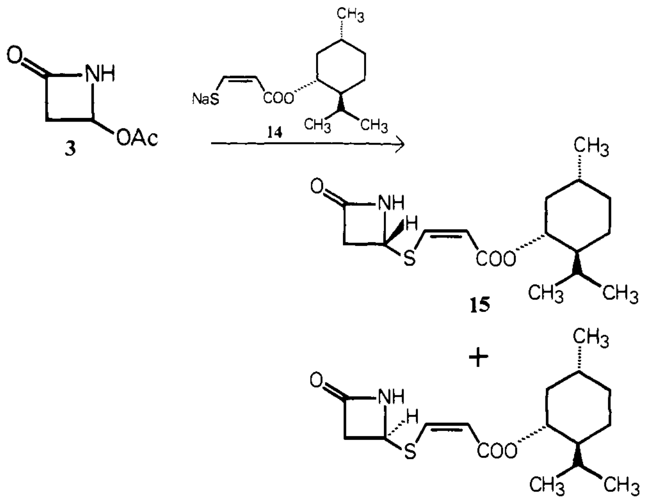
10 **11** **12** **13**

Finally, the acetonyl protecting group was removed from **13** by titration with 1 equiv of aqueous sodium hydroxide at 0 °C, yielding (65%) the racemic and crystalline penem-3-carboxylic acid **2** after neutralization. It exhibited broad in vitro antibiotic activity against Gram-positive and Gram-negative bacteria.



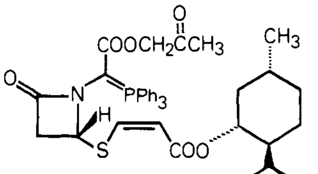
13 **2**

The aforementioned use of the β-mercaptoacrylate function as the synthetic equivalent of a formylthio group opened the possibility of the preparation of *optically active* penem-3-carboxylic acids **2a** and **2b**, thus allowing an answer to the question of whether only one or both of the enantiomers of the penem acid **2** are biologically active. Through reaction of acetoxy-β-lactam **3** with the sodium salt **14** of the β-mercaptoacrylate ester of *optically active* (−)-menthol, a 1:1 mixture of the two *diastereomeric* compounds, the 4(*R*)-(*cis*-β-carbomethyloxyvinylmercapto)azetidin-2-one (**15**) and its 4(*S*) isomer **16**, was obtained and its components were *separated* by fractional crystallization.



3 **15** **16**

The independent conversion of the two diastereomers **15** and **16** into the optically active phosphoranes **17** and **18** involved the same reagents and reaction conditions as mentioned in the scheme for the racemic series, and gave similar yields.



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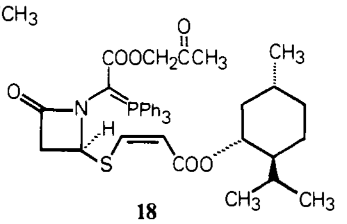
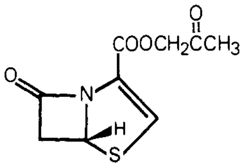
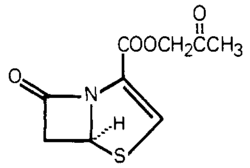


18

At this stage the (−)-menthyl residue, having been introduced for racemate resolution, was no longer needed. It was removed without any additional procedure from phosphoranes **17** and **18** during the already outlined ozonolysis step, in the presence of trifluoroacetic acid, leading, after treatment with aqueous bicarbonate and cyclization, to the enantiomeric (and dimorphous) acetonyl (5*R*)-penem-3-carboxylate (**19**), mp 94 °C, $[\alpha]^{20}_D$ + 251° ($CHCl_3$, *c* 1), and its mirror image **20**, mp 107 °C, $[\alpha]^{20}_D$ −249° ($CHCl_3$, *c* 1), as efficiently as in the racemic series.

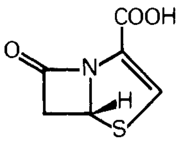


19

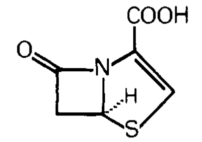


20

Finally both enantiomeric esters **19** and **20** were hydrolyzed to the crystalline target compounds, the (5*R*)-penem-3-carboxylic acid **2a**, $[\alpha]^{20}_D$ + 384° (CH_2Cl_2, *c* 1), and its 5*S* enantiomer **2b**, $[\alpha]^{20}_D$ −383° (CH_2Cl_2, *c* 1), respectively, by short treatment with 1 equiv of sodium hydroxide.



2a



2b

The absolute configurations were assigned to compounds **16**, **18**, **20**, and **2b** (*S* series) and consequently to **15**, **17**, **19**, and **2a** (*R* series) by an X-ray analysis of a crystal of the optically active penem ester **20**.

Biological investigation of the racemate **2** and the enantiomers **2a** and **2b** revealed (Table I) that the penem acid **2a**, having the 5*R* configuration as in penicillin, is approximately twice as active as the racemic penem acid **2**, a finding which is in good agreement with the previously reported biological data on racemic 2-methylpenem-3-carboxylic acid **1** (R = CH_3) and its penicillin derived 5*R* enantiomer.[5]

Moreover, enantiomer **2b**, whose 5*S* configuration has no equivalent among naturally occurring β-lactams, was *completely inactive*. This lack of activity conclusively shows that the 5*R* configuration in penems is the sole essential stereochemical requirement for the antibiotic activity of this class of novel compounds, a conclusion which can possibly be extended to all other bicyclic β-lactam antibiotics. Whereas in

Table I. Antibacterial in Vitro Activities of the Optically Active 5*R*, 5*S*, and Racemic Forms of Penem-2-carboxylic Acid

	MIC, µg/mL[a]		
microorganism	**2a** (5*R*)	**2** (rac)	**2b** (5*S*)
Staphylococcus aureus Smith	4	8	inactive
Staphylococcus aureus 2999 (resistant)	2	8	inactive
Escherichia coli 205	2	4	inactive
Pseudomonas aeruginosa ATCC 12055	4	16	inactive

[a] Minimal inhibitory concentrations in VST agar; inoculum ca. 10^4 cells; pH 7.4.

the latter, more complicated, compounds this requirement may be obscured by the presence of other chiral centers, it becomes very clear in the described penem acids **2.** In fact, the optically active penem-3-carboxylic acid **2a** with the 5*R* configuration as in penicillin is considered to be structurally the simplest β-lactam antibiotic at present known.

Experimental Section

Melting points are uncorrected. All rotations were determined in $CHCl_3$, and all IR spectra in CH_2Cl_2 as solvents unless otherwise mentioned. NMR spectra were recorded ($CDCl_3$ with Me_4Si as internal standard) on a Varian HA-100D spectrometer; all chemical shifts are reported in δ values. All R_f values were determined on Merck silica gel 60 F_{254} TLC plates.

A. Auxiliary Materials. *cis*-β-Carbomethoxyvinylisothiuronium Chloride.[6a] Thiourea (7.6 g, 0.1 mol) was dissolved in 2.0 N HCl (50 mL, 0.1 mol) and to this solution at room temperature methyl propiolate (8.4 g, 0.1 mol) in methanol (20 mL) was added slowly at a rate which allowed the reaction temperature to be maintained at 50 °C (exothermic reaction). After completed addition the reaction mixture was stirred at room temperature for 2 h, the solvent removed in vacuo (13 mm), and the residue dried under high vacuum. The crystalline residue (20 g) was recrystallized slowly from hot 2-propanol (200 mL), finally at −20 °C, to yield 12.0 g (61%) of pure *cis*-β-carbomethoxyvinylisothiuronium chloride, mp 166–167 °C. By repeated crystallization of the residue from the mother liquor more of the title compound (4.5 g, 23%) was secured. Anal. Calcd for $C_5H_9N_2O_2SCl$ (196.65): C, 30.54; H, 4.61; N, 14.24. Found: C, 30.69; H, 4.74; N, 14.36.

Sodium *cis*-β-Carbomethoxyvinylmercaptide (5) (in Solution).[6b] To a stirred solution of *cis*-β-carbomethoxyvinylisothiuronium chloride (1.96 g, 10 mmol) in 95% ethanol (40 mL) at −10 °C a precooled (0 °C) solution of NaOH in H_2O (1.0 N, 20 mL, 20 mmol) was added within 2 min and the temperature maintained at −10 °C by occasional cooling in a dry ice-acetone bath of −30 °C. A white precipitate was formed instantaneously. The obtained suspension, also containing urea and NaCl as byproducts, was *immediately* used for further transformations.

(−)-Menthyl Propiolate. (−)-Menthol (62.4 g, 0.4 mol) and propiolic acid (containing 4% H_2O, 42 g, ca. 0.55 mol) were dissolved in benzene (120 mL); concentrated sulfuric acid (8 drops, 200 mg) was added and the solution was boiled at reflux with a Dean-Stark separator for 2 days. Benzene (120 mL) was added, the solution washed with saturated $NaHCO_3$ solution (2 × 300 mL) followed by NaCl solution (100 mL), and the organic layer dried over Na_2SO_4. Evaporation of the solvent in vacuo gave a pale yellow, crystalline solid. It was chromatographed on Merck silica gel (1000 g) with toluene-AcOEt (19:1) (five 800-mL fractions) to give (−)-menthyl propiolate (63 g, 77%). Recrystallization from hot hexane (350 mL) gave white needles (60.5 g, 74%): mp 90–91 °C; R_f 0.65, toluene-AcOEt (9:1); $[\alpha]^{20}{}_D$ −82° (*c* 1); IR 3.1, 3.45, 4.75, 5.85, 8.1 µ; NMR in $CDCl_3$ δ 0.8–2.1 (m, 18), 2.9 (s, 1), 4.8 (m, 1). Anal. Calcd for $C_{13}H_{20}O$ (208.30): C, 74.96; H, 9.68. Found: C, 75.23; H, 9.56.

(−)-*cis*-β-Carbomenthyloxyvinylisothiuronium Chloride. Thiourea (7.6 g, 0.1 mol) was dissolved in a mixture of aqueous HCl (50 mL, 2.0 N, 0.1 mol) and ethanol (50 mL), and to this solution at room temperature a solution of (−)-menthyl propiolate (20.8 g, 0.1 mol) in ethanol (100 mL) was slowly added with stirring in order that the temperature did not exceed 40 °C. The formed suspension was stirred at 40 °C for 2 h. The white precipitate had dissolved during this time. The solvent was removed in vacuo (13 mm) and the residue dried under high vacuum (31 g). It was washed with acetone (250 mL), collected on a glass filter, and then recrystallized from hot water (filtration of the hot solution) with cooling to 0 °C. Pure (−)-*cis*-β-carbomenthyloxyvinylisothiuronium chloride (22 g, 70%), mp 176–179 °C, was obtained: $[\alpha]^{20}{}_D$ −74° (ethanol, *c* 1); IR (KBr) 3.0, 3.5, 6.05, 8.1 µ; UV in ethanol 262 nm (ε 16 300). Anal. Calcd for $C_{14}H_{25}N_2O_2SCl$ (320.88): C, 52.41; H, 7.85; N, 8.73; S, 9.99. Found: C, 52.17; H, 7.69; N, 8.70; S, 9.89.

Sodium (−)-*cis*-β-Carbomenthyloxyvinylmercaptide (14) (in Solution). To a solution of (−)-*cis*-β-carbomenthyloxyvinylisothiuronium chloride (32.1 g, 0.1 mol) in ethanol (400 mL), precooled aqueous NaOH (200 mL, 1.0 N, 0.2 mol) was added at −10 °C and the formed suspension, also containing NaCl and urea as byproducts, was immediately used for further transformations.

Bis(acetonyl) Fumarate. Disodium fumarate (32 g, 0.2 mol) was suspended in dry DMF (300 mL), chloroacetone (40.6 g, 35 mL, 0.44 mol) added slowly, and the mixture heated at 100 °C with mechanical stirring for 15 h. After cooling, methylene chloride (1.5 L) was added, and the resulting solution was washed with 1 N aqueous HCl (400 mL) and with water (400 mL). The organic layer was dried over Na_2SO_4 and the solvent removed in vacuo (13 mm) and then under high vacuum. The residue was crystallized from methylene chloride-ether and the obtained crystals washed with ether. The yield of pure bis(acetonyl) fumarate, mp 121–123 °C, was 29 g (64%): IR 5.7, 5.8, 7.3, 7.7, 8.7, 10.2 µ; NMR δ 2.1 (s, 6), 4.8 (s, 4), 7.0 (s, 2). Anal. Calcd for $C_{10}H_{12}O_6$ (228.20): C, 52.64; H, 5.30; O, 42.07. Found: C, 52.7; H, 5.5; O, 42.0.

Acetonyl Glyoxylate (7). Into a solution of bis(acetonyl) fumarate (22.8 g, 0.1 mol) in methylene chloride (400 mL) and methanol (200 mL), ozone was introduced at −15 °C at a rate of 0.33 mmol/min. After 9 h TLC indicated the absence of starting material (toluene-AcOEt (1:1)). Dimethyl sulfide (100 mL) was added and the mixture stirred at room temperature for 15 h. Evaporation in vacuo and distillation under high vacuum yielded a mixture of partially hydrated and polymerized[7] acetonyl glyoxylate (20 g, 77%) as a viscous liquid, bp 70–80 °C (0.05 mm). It was stored at −20 °C: R_f 0.3 (AcOEt); IR 5.7, 5.8 µ. Anal. Calcd for $C_5H_6O_4$ (130.10): C, 46.16; H, 4.65; O, 49.19. Found: C, 45.56; H, 5.26; O, 49.12.

B. Synthesis of Racemic Penem-3-carboxylic Acid (2). 4-(*cis*-β-Carbomethoxyvinylmercapto)azetidin-2-one (6). To a freshly prepared suspension of sodium *cis*-β-carbomethoxyvinylmercaptide **5** (from 10 mmol of the corresponding isothiuronium salt) in ethanol-water at −10 °C, a cold solution of 4-acetoxyazetidin-2-one **3** (1.29 g, 10 mmol) in ethanol (20 mL) was added within 2 min with stirring. The temperature was maintained at −10 °C by occasional cooling in a dry ice-acetone bath of −30 °C and then the reaction mixture was stirred for 30 min at −10 °C and 15 min at 0 °C. It was diluted with methylene chloride (150 mL), saturated aqueous NaCl solution (50 mL) added, the organic layer separated, and the aqueous layer extracted with methylene chloride (50 mL). The combined organic fractions were dried over Na_2SO_4 and the solvent removed in vacuo, leaving a noncrystalline solid. It was chromatographed on Merck silica gel (70 g deactivated with 10% H_2O) with toluene-AcOEt (2:1) to give the pure and crystalline title compound (1.50 g, 80%). An analytical sample was obtained by recrystallization from hot benzene: mp 92–93 °C; R_f 0.27 (toluene-AcOEt (1:1)); UV in ethanol λ_{max} 277 nm (ε 13 300); IR 5.6, 5.9, 6.3, 8.2, 8.5 µ; NMR δ 3.0–3.7 (m, 2), 3.77 (s, 3), 4.59 (m, 1), 6.0 (d, 1, *J* = 10 Hz), 6.8 (broad s, 1), 7.15 (d, 1, *J* = 10 Hz). Anal. Calcd for $C_7H_9NO_3S$ (187.21): C, 44.91; H, 4.85; N, 7.48; S, 17.13; O, 25.64. Found: C, 44.97; H, 4.92; N, 8.07; S, 16.70; O, 25.70.

Acetonyl [4-(*cis*-β-Carbomethoxyvinylmercapto)azetidin-2-on-1-yl] hydroxyacetates (8). 4-(*cis*-β-Carbomethoxyvinylmercapto)azetidin-2-one **6** (1.87 g, 10 mmol) was dissolved in dry DMF (10 mL) and toluene (20 mL) and distilled acetonyl glyoxylate (**7**) (3.25 g, 25 mmol) were added. The mixture was stirred with molecular sieves (3 Å, 30 g) at 40 °C for 20 h and filtered and the sieves were washed carefully with AcOEt. The combined filtrates were evaporated first at 13 mm and finally under high vacuum (0.01 mm) with magnetic stirring at 60 °C. Residual acetonyl glyoxylate was removed by repeated evaporation under high vacuum with dry DMF at 60 °C until the weight of product was 3.3 g (3.17 g = 100%) and the residue became an amorphous solid which was used for further transformations without additional purification: R_f 0.1 (toluene-AcOEt (1:1)); IR 5.65, 5.8, 5.9 µ.

Acetonyl [4-(*cis*-β-Carbomethoxyvinylmercapto)azetidin-2-on-1-yl]chloroacetates (9). The crude acetonyl [4-(*cis*-β-carbomethoxyvinylmercapto)azetidin-2-on-1-yl]hydroxyacetates **8** (epimeric, from 10 mmol of **6**) were dissolved in dry THF (50 mL) and at −15 °C thionyl chloride (0.86 mL, 12 mmol) followed by a solution of triethylamine (1.66 mL, 12 mmol) in dry THF (4 mL) were added within 5 min. When the reaction mixture was stirred at 0 °C for 15 min, a white precipitate was produced. Ice-cold methylene chloride (300 mL) was added and the solution carefully washed with 0.1 N HCl solution (100 mL). The organic layer was dried over Na_2SO_4 and the solvent removed in vacuo, leaving yellow, oily product (4.0 g). Chromatography on Merck silica gel (120 g, deactivated with 10% H_2O) with toluene–AcOEt (1:1) (25 fractions, 30 mL each) gave an epimeric mixture of the pure chlorides **9** (2.6 g, 74% based on **6**) as an amorphous solid: R_f 0.27 (toluene–AcOEt (1:1)); IR 5.65, 5.8, 5.9, 7.35, 8.25, 8.55 μ.

Acetonyl [4-(*cis*-β-Carbomethoxyvinylmercapto)azetidin-2-on-1-yl]triphenylphosphoranylideneacetate (10). The chromatographed acetonyl [4-(β-carbomethoxyvinylmercapto)azetidin-2-on-1-yl]-chloroacetates **9** (epimeric, 2.3 g, 6.6 mmol) were dissolved in dry THF (6 mL), triphenylphosphine (3.46 g, 13.2 mmol) was added, and the dissolved mixture was left under nitrogen for 48 h at room temperature. Methylene chloride (100 mL) was added, the solution washed carefully with cold saturated $KHCO_3$ solution, the organic layer dried over Na_2SO_4, and the solvent removed in vacuo, leaving an amorphous solid (5.4 g). Chromatography on acid-washed silica gel (80 g) with toluene–AcOEt (1:1) (six fractions, 40 mL each) gave triphenylphosphine and elution with AcOEt (12 fractions, 40 mL each) afforded the title phosphorane (3.10 g, 81%) as a noncrystalline solid: R_f 0.3 (AcOEt); IR 5.7, 5.85, 6.2, 6.85 μ. Anal. Calcd for $C_{30}H_{28}NO_6SP$ (561.59): C, 64.16; H, 5.03; N, 2.49; S, 5.71. Found: C, 64.39; H, 5.26; N, 2.61; S, 5.49.

Acetonyl Penem-3-carboxylate (13). Acetonyl [4-(*cis*-β-carbomethoxyvinylmercapto)azetidin-2-on-1-yl]triphenylphosphoranylideneacetate (**10**, 1.15 g, 2 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and at −20 °C trifluoroacetic acid (1 mL) was added. At −20 °C ozone was passed through the solution for 10 min at a rate of 0.33 mmol/min and the solution was kept at this temperature for 15 min. Nitrogen was passed through the solution to remove excess ozone and then dimethyl sulfide (1.5 mL) was added and the mixture was allowed to reach 0 °C. After stirring of the solution for 5 min at 0 °C the KI starch iodide test became negative. Ice-cold CH_2Cl_2 (30 mL) and saturated $NaHCO_3$ solution (60 mL) were added and the resulting mixture was shaken thoroughly. The organic layer was washed again with cold saturated $NaHCO_3$ solution (20 mL) and dried over Na_2SO_4 and the solvent was removed in vacuo, leaving the crude thioformate derivative as a noncrystalline, pale yellow solid (0.90 g) after drying under high vacuum for 5 min. It was redissolved in dry CH_2Cl_2 (50 mL) and the solution refluxed for 90 min. The solvent was removed in vacuo and the crude penem ester immediately separated from triphenylphosphine oxide by chromatography on acid-washed silica gel (20 g) using toluene–ethyl acetate (1:1) as eluant (20 fractions, 10 mL each), yielding pure, crystalline title compound (368 mg, 81%). A sample for analysis was recrystallized from CH_2Cl_2–ether: mp 115–116 °C; R_f 0.5 (AcOEt). UV in EtOH λ_{max} 317 nm (ϵ 7700); IR 5.6, 5.75, 5.85 μ; NMR δ 2.2 (s, 3), 3.5–4.0 (m, 2), 4.76 (s, 2), 5.83 (m, 1), 7.38 (s, 1). Anal. Calcd for $C_9H_9NO_4S$ (227.23): C, 47.57; H, 3.99; N, 6.17; S, 14.11. Found: C, 47.51; H, 4.08; N, 6.23; S, 13.83.

Penem-3-carboxylic Acid (2). Acetonyl penem-3-carboxylate **13** (227 mg, 1 mmol) was dissolved in THF (45 mL) and the resulting solution was diluted with water (15 mL) at 0 °C. Aqueous NaOH (0.1 N, 10 mL, 1 mmol) was added within 20 min while stirring under nitrogen, and stirring at 0 °C was continued for another 5 min. The reaction solution was then washed twice with cold methylene chloride (2 × 100 mL) and these washings were discarded. The same solvent (100 mL) was added again. A cold aqueous solution (4 mL) of 20% citric acid was added. The whole mixture was extracted twice with 50-mL portions of cold methylene chloride, the combined organic extracts were dried over Na_2SO_4, and the solvent was removed in vacuo, leaving a pale yellow, crystalline solid (135 mg). It was washed with cold (−10 °C) methylene chloride (2 mL) and collected on a filter (112 mg, 65%): mp >230 °C dec; R_f 0.16 (AcOH–toluene–H_2O (5:5:1)); UV in ethanol λ_{max} 258 nm (ϵ 2900), 311 (6500); IR (KBr) 5.65, 6.0, 6.5, 7.0 μ; NMR in Me_2SO-d_6 δ 3.4–4.0 (m, 2), 5.8 (m, 1) 7.75 (s, 1). Anal. Calcd for $C_6H_5NO_3S$ (171.17): C, 42.10; H, 2.95; N, 8.18; S, 18.73. Found: C, 42.08; H, 2.94; N, 8.16; S, 18.26.

C. Synthesis of Enantiomeric Penem-3-carboxylic Acids (2a and 2b). 4(*R*)- and 4(*S*)-(*cis*-β-Carbomenthyloxyvinylmercapto)azetidin-2-ones (15 and 16). To a freshly prepared suspension of sodium *cis*-β-carbomenthyloxyvinylmercaptide (**14**) in ethanol–water (in situ preparation from 0.1 mol of the corresponding isothiuronium salt) at −10 °C, a precooled solution of 4-acetoxyazetidin-2-one (12.9 g, 0.1 mol) in 95% ethanol (200 mL) was added in one portion; temperature was maintained at −10 °C by occasional cooling in a dry ice–acetone bath of −30 °C. After addition the mixture was stirred for an additional 30 min at −10 °C and 15 min at 5 °C. Methylene chloride (1.5 L) and saturated NaCl solution (500 mL) were added and the mixture was transferred to a separatory funnel. The organic layer was separated and the aqueous layer extracted with methylene chloride (500 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was removed in vacuo, leaving a crystalline solid (32 g). Chromatography on acid-washed silica gel (800 g) with toluene–AcOEt (4:1) (15 fractions, 800 mL each) gave a colorless, crystalline solid (23.8 g, 76%), consisting of an approximately 1:1 mixture of the two epimeric title compounds. Six crystallizations from methylene chloride–pentane afforded, in several crops, the pure 4*S* isomer **16** in a total yield of 3.7 g, i.e., 12%, based on **3**; mp 134–135 °C; R_f 0.2 (toluene–AcOEt (1:1)); $[\alpha]^{20}_D$ −82° (*c* 1); UV in ethanol λ_{max} 277 nm (ϵ 15 000); IR 5.62, 5.9, 8.2, 8.55 μ; NMR δ 0.5–2.2 (m, 18), 2.9–3.6 (m, 2), 4.7 (m, 1), 4.92 (m, 1), 5.95 (d, 1, J = 10 Hz), 6.95 (broad s, 1), 7.13 (d, 1, J = 10 Hz). Anal. Calcd for $C_{16}H_{25}NO_3S$ (311.44): C, 61.71; H, 8.09; N, 4.50; S, 10.30. Found: C, 61.8; H, 8.0; N, 4.5; S, 9.8. The pure 4*R* isomer **15** (2.8 g, 9%) was obtained by fivefold recrystallization of the residue from the mother liquor from methanol (with cooling at −70 °C). After one additional recrystallization from methylene chloride–pentane the melting point was 139–141 °C: R_f 0.2 (toluene–AcOEt (1:1)); $[\alpha]^{20}_D$ −91° (*c* 1); UV in ethanol λ_{max} 278 nm (ϵ 14 800); IR 5.62, 5.9, 8.2, 8.55 μ; NMR δ 0.5–2.2 (m, 18), 2.9–3.6 (m, 2), 4.7 (m, 1), 4.92 (m, 1), 5.95 (d, 1, J = 10 Hz), 6.95 (broad s, 1), 7.13 (d, 1, J = 10 Hz). Anal. Calcd for $C_{16}H_{25}NO_3S$ (311.44): C, 61.71; H, 8.09; N, 4.50; S, 10.30. Found: C, 61.85; H, 8.11; N, 4.74; S, 10.19.

Acetonyl [4(*R*)-(*cis*-β-Carbomenthyloxyvinylmercapto)azetidin-2-on-1-yl]triphenylphosphoranylideneacetate (17). A solution of pure 4(*R*)-(*cis*-β-carbomenthyloxyvinylmercapto)azetidin-2-one (**15**) (935 mg, 3 mmol) and acetonyl glyoxylate (1.0 g, 7.7 mmol) in dry DMF (3 mL) and toluene (6 mL) was stirred with molecular sieves (3 Å, 12 g) for 20 h at 40 °C. The molecular sieves were filtered off and washed with AcOEt. The combined filtrates were evaporated in vacuo (13 mm) and finally at 0.01 mm. The residue was evaporated several times with DMF under high vacuum with magnetic stirring at 60 °C until the weight was 1.40 g (1.34 g = 100%) and the residue became an amorphous solid (epimeric mixture). The epimeric mixture of the carbinolamides thus obtained was used for further transformation without any additional purification: R_f 0.2 (toluene–AcOEt (1:1)); IR 3.45, 5.65, 5.8, 5.9 μ.

The crude mixture of the two epimeric carbinolamides was dissolved in dry THF (15 mL) at −15 °C and thionyl chloride (0.26 mL, 3.6 mmol) was added. At this temperature, with stirring, triethylamine (0.5 mL, 3.6 mmol) in THF (1.5 mL) was added within 5 min and then the mixture was stirred at 0 °C for 15 min. Dilution with cold methylene chloride (100 mL), washing with 0.1 N aqueous HCl (30 mL), separation, and drying of the organic layer over Na_2SO_4 gave, after evaporation of the solvent in vacuo, a noncrystalline solid which was chromatographed on Merck silica gel (40 g) with toluene–AcOEt (1:1) (ten fractions, 20 mL each) to afford a mixture of epimeric chlorides as a pale, noncrystalline solid (1.40 g, 100%): R_f 0.52 (AcOEt); IR 3.45, 5.6, 5.75, 5.9 μ.

The chromatographed mixture of both epimeric chlorides was dissolved in dry THF (2.5 mL), triphenylphosphine (1.57 g, 6 mmol) was added, and the resulting solution was kept under nitrogen at room temperature for 24 h. The mixture was diluted with methylene chloride (50 mL) and thoroughly shaken with a saturated aqueous $KHCO_3$ solution (20 mL). The organic layer was dried over Na_2SO_4 and the solvent removed in vacuo. The residue was chromatographed on Merck silica gel (40 g) with toluene–AcOEt (1:1) (eight fractions, 20 mL each) to remove triphenylphosphine. Elution with toluene–AcOEt (1:2) (ten fractions, 40 mL each) gave the title compound as a colorless, noncrystalline solid (1.50 g, 73% based on **15**): R_f 0.33 (AcOEt); IR 3.45, 5.7, 5.9, 6.15 μ. Anal. Calcd for $C_{39}H_{44}NO_6SP$ (685.82): C, 68.30; H, 6.47; N, 2.04; S, 4.67. Found: C, 67.83; H, 6.61;

N, 2.30; S, 5.23.

Acetonyl [4(*S*)-(*cis*-*β*-Carbomenthyloxyvinylmercapto)azetidin-2-on-1-yl]triphenylphosphoranylideneacetate (18), Following the above-mentioned procedure from pure 4(*S*)-(*cis*-*β*-carbomenthyloxyvinylmercapto)azetidin-2-one (**16,** 935 mg, 3 mmol), through the corresponding intermediates having the same R_f values and IR data as those in the 4*R* series, the amorphous title compound (1.42 g, 69%) was prepared: R_f 0.33 (AcOEt); IR 3.45, 5.7, 5.9, 6.15 *μ*. Anal. Calcd for $C_{39}H_{44}NO_6SP$ (685.82): C, 68.30; H, 6.47: N, 2.04; S, 4.67. Found: C, 67.75; H, 6.59; N, 2.47; S, 4.92.

Acetonyl (5*R*)-Penem-3-carboxylate (19), Acetonyl [4(*R*)-(*cis*-*β*-carbomenthyloxyvinylmercapto)azetidin-2-on-1-yl]triphenylphosphoranylideneacetate (**17**, 1.37 g, 2 mmol) was dissolved in methylene chloride (30 mL) and at −20 °C trifluoroacetic acid (1 mL) was added; at the same temperature a stream of ozone in oxygen was introduced at a rate of 0.33 mmol/min for exactly 10 min. The mixture was left at −20 °C for another 15 min and then excess ozone was removed by passing nitrogen through the solution for 1 min. Dimethyl sulfide (1.5 mL) was then added and the mixture warmed up to 10 °C. After 5 min at 10 °C the starch–iodide test became negative. Ice-cold methylene chloride (30 mL) was added and the solution washed with ice-cold 10% aqueous $NaHCO_3$ solution (50 mL). The organic layer was washed again with $NaHCO_3$ solution (15 mL) and dried over Na_2SO_4. The solvent was removed in vacuo and the residue containing the crude 4-formylthioazetidinone was dried under high vacuum for 5 min. It was redissolved in methylene chloride (50 mL filtered through basic Alox) and refluxed for 90 min under nitrogen. The solvent was removed in vacuo and the residue immediately chromatographed on acid-washed silica gel (20 g) with toluene–AcOEt (3:1) (20 fractions, 20 mL each). After the first five fractions containing hydrated (−)-menthylglyoxylate, the pure title compound (373 mg) was eluted. It was recrystallized from methylene chloride–ether (342 mg, 75%): mp 93–94 °C; $[\alpha]^{20}{}_D$ +251° (*c* 1); R_f 0.5 (AcOEt); UV in ethanol λ_{max} 317 nm (ϵ 7800); IR 5.6, 5.75, 8.85, 7.6, 8.3, 8.55 *μ*; NMR δ 2.2 (s, 3), 3.5–4.0 (m, 2), 4.76 (s, 2), 5.83 (m, 1), 7.38 (s, 1). Anal. Calcd for $C_9H_9NO_4S$ (227.23): C, 47.57; H, 3.99: N, 6.17; S, 14.11. Found: C, 47.78; H, 4.07; N, 6.32; S, 13.78.

Acetonyl (5*S*)-Penem-3-carboxylate (20), Following the above-mentioned procedure from acetonyl [4(*S*)-(*cis*-*β*-carbomenthyloxyvinylmercapto)azetidin-2-on-1-yl]triphenylphosphoranylideneacetate (**18**) the pure title compound (318 mg, 70%), mp 106–107 °C (after two recrystallizations from methylene chloride–ether), was obtained: $[\alpha]^{20}{}_D$ −249° (*c* 1); UV in ethanol λ_{max} 318 nm (ϵ 7900); IR 5.6, 5.75, 5.85, 7.6, 8.3, 8.55 *μ*; NMR δ 2.2 (s, 3), 3.5–4.0 (m, 2), 4.76 (s, 2), 5.83 (m, 1), 7.38 (s, 1). Anal. Calcd for $C_9H_9NO_4S$ (227.23): C, 47.57; H, 3.99; N, 6.17; S, 14.11. Found: C, 47.66; H, 3.93; N, 6.16; S, 13.91. The structure and absolute configuration of **20** were determined by X-ray crystallography.

(5*R*)-Penem-3-carboxylic Acid (2a), Pure acetonyl (5*R*)-penem-3-carboxylate (**19**) (113.5 mg, 0.5 mmol) was dissolved in THF (22.5 mL) and the resulting solution was diluted with water (7.5 mL). At 0 °C with stirring under nitrogen, an aqueous solution of NaOH (0.1 N, 5 mL, 0.5 mmol) was added within 20 min and the mixture stirred for 5 min at 0 °C. The reaction solution was then washed twice with cold (−10 °C) portions (50 mL) of methylene chloride and the same solvent (100 mL) was added again. A cold aqueous solution (2 mL) of 20% citric acid was added and the mixture shaken immediately. The organic layer was separated and the aqueous layer extracted twice with 25-mL portions of cold methylene chloride. The combined organic extracts were dried over Na_2SO_4 and the solvent was removed in vacuo, leaving a noncrystalline solid (71 mg), which was immediately chromatographed on Merck silica gel (5 g) with CH_2Cl_2–AcOH (9:1) (20 fractions, 2.5 mL each). The fractions containing the title compound were evaporated under high vacuum and finally repeatedly with toluene to remove traces of acetic acid. Pure crystalline title compound (50 mg, 58%), mp >230 °C dec, was thus obtained: $[\alpha]^{20}{}_D$ + 383° (CH_2Cl_2, *c* 1); R_f 0.16 (AcOH–toluene–H_2O (5:5:1)); UV in ethanol λ_{max} 257 nm (ϵ 2800), 310 (6400); IR 5.55, 5.9, 6.45 *μ*; NMR in Me_2SO-d_6 δ 3.4–4.0 (m, 2), 5.8 (m, 1), 7.75 (s, 1). Anal. Calcd for $C_6H_5NO_3S$ (171.17): C, 42.10; H, 2.95; N, 8.18. Found: C, 41.78; H, 3.15; N, 8.08.

(5*S*)-Penem-3-carboxylic Acid (2b), Following the above-mentioned procedure from pure acetonyl (5*S*)-penem-3-carboxylate (**20**) (91 mg, 0.4 mmol), 41 mg (60%) of the crystalline title compound was obtained: mp >230 °C dec; $[\alpha]^{20}{}_D$ −383° (CH_2Cl_2, *c* 1); R_f 0.16 AcOH–toluene–H_2O (5:5:1)); UV in ethanol λ_{max} 256 nm (ϵ 2800), 310 (6300); IR 5.55, 5.9, 6.45 *μ*; NMR in Me_2SO-d_6 δ 3.4–4.0 (m, 2), 5.8 (m, 1), 7.57 (s, 1). Anal. Calcd for $C_6H_5NO_3S$ (171.17): C, 42.10; H, 2.95; N, 8.18. Found: C, 41.90; H, 3.15; N, 7.70.

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