The Penems, a New Class of β-Lactam Antibiotics. 5. Total Synthesis of Racemic 6-α-Hydroxyethylpenemcarboxylic Acids

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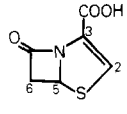
Abstract: The novel racemic *threo-trans*-6-α-hydroxyethylpenem-3-carboxylic acid **3t** and its erythro-trans isomer **3e** have been prepared over 13 steps from acetoxyazetidinone **4.**

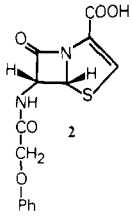
The influence of the side chains on the biological activity of β-lactam antibiotics remains a puzzle in spite of the numerous analogues which have been prepared and evaluated. Whereas acylamino substituents seem to be indispensable for antibacterial properties of the penams and cephems, in the recently disclosed thienamycin derivatives enhancement of antibiotic potency was obtained with an α-hydroxyethyl side chain.[1]

Our experience in the penem field suggested that the incorporation of an acylamino side chain into the parent penemcarboxylic acid **1**[2] was not desirable. The 6-acylamino substituted penemcarboxylic acid **2**[3] was markedly less active[4] than the 6-unsubstituted compound **1,** obviously as a result of its *chemically* too reactive β-lactam, which made even its testing under physiological conditions difficult. Would on the other hand an α-hydroxyethyl group, the natural side chain of thienamycin, improve the antibacterial potency of the parent penem **1?**

To answer this question we report here the total synthesis and in vitro antibacterial testing of the racemic *threo-trans*-α-hydroxyethylpenemcarboxylic acid **3t** and its erythro-trans isomer **3e.**



1



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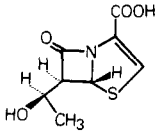
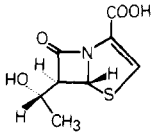
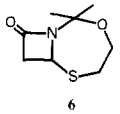


3t



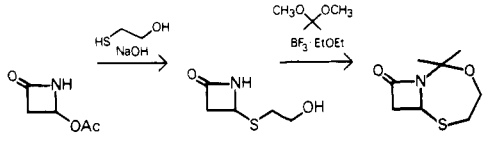
3e

It was intended to introduce the α-hydroxyethyl side chain by a known[5,6] aldol-type reaction. However, the low chemical stability of the penems, especially toward strong base, suggested that this process should take place at an early stage of the reaction scheme, before the labile penem system was formed. We thought that the novel bicyclic lactam **6** could become the key intermediate in our synthesis.



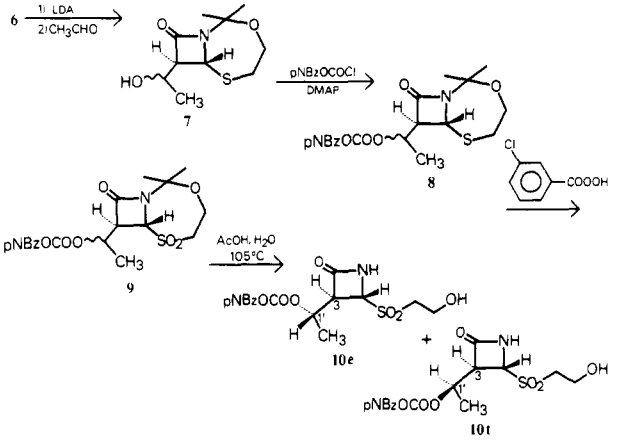
6

It was prepared from the readily accessible[7] 4-acetoxyazetidin-2-one **4.** First the acetoxy group was displaced with 2-mercaptoethanol in the presence of sodium hydroxide (leading to **5**), then simultaneous protection of the OH and NH functions by condensation with acetone dimethyl ketal and boron trifluoride etherate at room temperature furnished **6** in 83% overall yield from **4.**



4 **5** **6**

Using lithium diisopropylamide as base, the aldol reaction of **6** with acetaldehyde led to the introduction of the desired side chain stereoselectively in a trans position to the attached seven-membered ring. An epimeric mixture of the α-hydroxyethyl derivatives **7** was formed in 86% yield. Without separation of the isomers, the hydroxyl group of **7** was protected using *p*-nitrobenzyl chloroformate and 4-dimethylaminopyridine at 40 °C; a mixture of the epimeric bicyclic carbonates **8** was thus obtained in 80% yield after chromatographic purification.



7 **8** **9** **10e** **10t**

The bicyclic carbonates **8** were oxidized with *m*-chloroperbenzoic acid at −10 °C to the isomeric sulfones **9.** The following hydrolysis of the aminoketal grouping with aqueous acetic acid at 105 °C efficiently furnished a mixture of the isomers **10e** and **10t.**

The predominant (two parts) erythro compound **10e** crystallized readily from the isomeric mixture and was easily separated from the *noncrystalline* threo isomer (one part) **10t,** which, in its turn, was obtained pure by chromatography of the mother liquors. The threo and erythro assignments were based on the NMR proton coupling constants between H-3 and H-1′, which were 5.0 and 4.0 Hz, respectively.[8]

Alkylsulfonylazetidin-2-ones have been reported[7] to undergo smooth displacement reactions with various nucleophiles.

† Deceased July 8, 1979.

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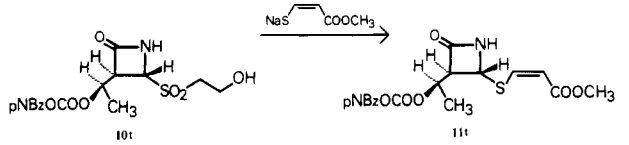
Table I. Antibacterial in Vitro Activities of the *Racemic* Parent Penemcarboxylic Acid **1** and the *Racemic* 6-α-Hydroxyethylpenemcarboxylic Acids **3t** and **3e** and Their Chemical Stabilities in Phosphate Buffer (pH 7.4) at 37 °C

	MIC, μg/mL[a]		
microorganism	**1** (parent)	**3t** (threo)	**3e** (erythro)
Staphylococcus aureus 10 B	8	1	64
Staphylococcus aureus 2999i+ p+ (penicillin resistant)	8	1	64
Neisseria gonorrhoeae	8	0.5	32
Escherichia coli 205	4	8	128
Escherichia coli 205 Richmond +TEM (β-lactamase producing)	32	8	128
Salmonella typhimurium 277	4	4	64
Enterobacter cloacae P 99	>128	8	>128
Pseudomonas aeruginosa ATCC 12055	16	64	>128
half-live values at pH 7.4 and 37 °C, h	20	105	150

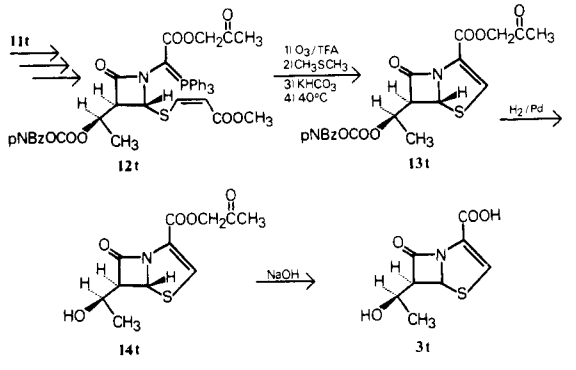
[a] Minimal inhibitory concentration in VST agar; inoculum ca. 10^4 cells; pH 7.4.

Similarly the β-hydroxyethylsulfonyl group in **10t** (threo isomer) was easily displaced by methyl *cis*-β-mercaptoacrylate[2] in the presence of sodium hydroxide at 0 °C, affording the crystalline **11t** in 97% yield after chromatography.

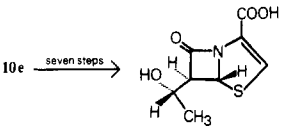


10t 11t

As depicted in the following scheme and in analogy to the reported[2] synthesis of **1**, the azetidinone **11t** was converted into the phosphorane **12t** in 60% yield via a three-step sequence involving addition to acetonyl glyoxylate,[2] reaction with thionyl chloride and triethylamine, and treatment with triphenylphosphine followed by sodium carbonate. Selective ozonolysis of the C=C double bond in the presence of trifluoroacetic acid, reduction of the intermediates with dimethyl sulfide, regeneration of the C=P double bond by $KHCO_3$ treatment, and finally Wittig-type condensation afforded the protected penem ester **13t** in 83% yield (based on **12t**). The *p*-nitrobenzyl carbonate ester was then cleaved by hydrogenation in the presence of a Pd on carbon catalyst affording in 80% yield the crystalline acetonyl 6-α-hydroxyethylpenem-3-carboxylate (**14t**), which was hydrolyzed to give the sodium salt of *threo-trans*-6-α-hydroxyethylpenem-3-carboxylic acid (**3t**) in 60% yield after reverse phase chromatography. Finally, the pure crystalline **3t** (UV max 311 nm, ε 6400) was obtained from the salt using a strongly acidic ion exchange resin (overall yield 23%, based on **10t**).



Using the same sequence of reactions, the crystalline *erythro-trans*-α-hydroxyethylpenem-3-carboxylic acid (**3e**) (UV max 311 nm, ε 6300) was prepared from the *erythro*-α-hydroxyethylazetidinone **10e** (in an overall yield of 25%).



The in vitro biological evaluation (Table I) of the two novel racemic 6-α-hydroxyethylpenemcarboxylic acids **3t** and **3e** showed a remarkable difference in antibacterial activities between the two isomers. The acid **3t**, which has the threo-trans configuration of the side chain as in thienamycin, is the more active. Compared to the racemic parent penem-3-carboxylic acid **1**, a distinct increase in biological activity became evident with **3t** against *Gram-positive bacteria* as well as against *enterobacteria* and *β-lactamase producing strains*. Its activity against *sensitive Gram-negative bacteria* was slightly decreased.

The introduction of the *trans*-α-hydroxyethyl side chain into the parent penem **1** increased the *chemical stability* of the β-lactam, as expressed in half-life values in neutral solution (Table I), a feature observed with both isomers **3t** and **3e**.

An inspection of models suggested that the erythro isomer **3e** should have a somewhat better chance than **3t** to form an intramolecular hydrogen bond between the hydroxyl group and the oxygen atom of the β-lactam carbonyl. We thought that such a structural difference might play a role in the observed, very different biological behavior of the two isomers. However, the IR spectra of both the isomeric acids **3t** and **3e** and those of their ester precursors **14t** and **14e** indicate that there is no *intramolecular* bonding in any of the four compounds.[9]

Thus, in the absence of any apparent difference in their chemical reactivities (Table I), it remains an open question whether the enhanced antibacterial activity of **3t** over **3e** arises from a *direct* interaction of its chiral side chain with an active site of an enzyme involved in the bacterial growth.

Experimental Section

Melting points (Kofler) are uncorrected. All IR spectra were recorded in CH_2Cl_2 on a Perkin-Elmer 710B or 580B spectrometer, and all NMR spectra in $CDCl_3$ (with Me_4Si as internal standard) on a Varian XL-100 or a Bruker HX-360 spectrometer (chemical shifts are reported in δ values) unless otherwise mentioned. UV spectra were recorded on a Beckman DB-GT spectrometer. All R_f values were determined on Merck silica gel 60 F_{254} TLC plates.

A. Syntheses of 6-α-Hydroxyethylpenemcarboxylic Acids 3t and 3e. 4-(β-Hydroxyethylthio)azetidin-2-one (5). To a solution of 4-acetoxyazetidin-2-one (12.9 g, 0.1 mol) and 2-mercaptoethanol (11.14 g, 10 mL, 0.142 mol) in 95% ethanol (75 mL) with constant stirring under nitrogen and at −10 °C, an aqueous solution of 2.0 N sodium hydroxide (55 mL) was added dropwise within 30 min. The solution was then stirred at 0 °C for an additional 1 h and neutralized by addition of trifluoroacetic acid (2 mL) and the solvent removed in a rotary evaporator in vacuo. The residue was dried *under high vacuum*. It was extracted several times with 200-mL portions of chloroform upon addition of sodium sulfate (100 g). The combined extracts were filtered, the solvent was removed in vacuo, and the residue was dried *under high vacuum*, affording a viscous liquid (14.7 g, 100%). A sample was purified by column chromatography on Merck silica gel using ethyl acetate as an eluant: mp 36 °C; R_f 0.12 (AcOEt); IR 3580, 3380, 1770 cm^{-1}; NMR δ 7.3 (s, 1), 4.8 (dd, 1, J = 5, 2.5 Hz), 3.7–3.9 (m, 2), 3.25–3.5 (m, 2), 2.7–3.0 (m, 2), 2.6 (s, 1). Anal. Calcd for $C_5H_9NO_2S$ (147.19): C, 40.80; H, 6.16; N, 9.52. Found: C, 40.90; H, 6.35; N; 9.41.

2,2-Dimethyl-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane (6). Crude 4-(β-hydroxyethylthio)azetidin-2-one **5** (14.7 g, 0.1 mol) and acetone dimethyl ketal (26.2 g, 30.5 mL, 0.25 mmol) were dissolved in dry, ethanol-free methylene choride (100 mL) and with stirring at

−10 °C boron trifluoride etherate (2 mL) was added to the mixture, which was then stirred at −10 °C for 30 min and at room temperature for 2 h. Dilution of the reaction mixture with methylene chloride (150 mL), washing with ice-cold saturated aqueous sodium bicarbonate solution (350 mL), drying of the organic layer with sodium sulfate, filtration, and removal of the solvent gave a residue which was dried *under high vacuum*. Recrystallization from ether–*n*-hexane, finally at 0 °C, gave pure **6** (13.4 g, 71%). By chromatography of the mother liquors on Merck silica gel with toluene–EtOAc (3:1) more of the pure **6** (2.3 g, 12%) was secured: mp 67–68 °C; R_f 0.5 (EtOAc); IR 1750, 1345, 1240, 1080 cm^{-1}; NMR δ 5.0 (dd, 1, J = 2.5, 5 Hz), 4.1–4.2 (m, 2), 2.9–3.4 (m, 2), 2.6–2.8 (m, 2), 1.75 (s, 3), 1.5 (s, 3). Anal. Calcd for $C_8H_{13}NO_2S$ (187.26): C, 51.31; H, 7.00; N, 7.48. Found: C, 51.34; H, 6.79; N, 7.53.

2,2-Dimethyl-*trans*-8-(α-hydroxyethyl)-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane (Mixture of Two Isomers) (7). To a stirred mixture of diisopropylamine (11 g, 15.5 mL, 0.11 mol) in dry (distilled over $LiAlH_4$) tetrahydrofuran (200 mL) at −65 °C under nitrogen a solution of *n*-butyllithium in *n*-hexane (55 mL, 2 N) was added dropwise over 15 min and the mixture stirred for an additional 15 min at the same temperature. Over 15 min at −65 °C a solution of 2,2-dimethyl-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane (18.7 g, 0.1 mol) in dry tetrahydrofuran (80 mL) was added dropwise and the mixture allowed to stir for an additional 10 min at −65 °C. Over 15 min at −65 °C a solution of dry acetaldehyde (13.2 g, 17 mL, 0.3 mol) in dry tetrahydrofuran (80 mL) was added dropwise and the mixture slowly allowed to reach 0 °C. After additional stirring at 0 °C for 1 h the reaction mixture was poured on ice–water (1 L) and then extracted with methylene chloride (2.5 L). The organic layer was dried over sodium sulfate and filtered and the solvent removed in a vacuum rotary evaporator. The residue was chromatographed on Merck silica gel (1 kg) using toluene–EtOAc (2:1) as eluant (20 fractions, 500 mL each). The fractions containing **7** were combined and the solvent was removed in vacuo, yielding a viscous liquid (19.5 g, 84%). It consists of *threo-trans*-hydroxyethyl compound (one part) and its erythro isomer (two parts): R_f 0.4 (EtOAc); IR 3550, 1740, 1220, 1080 cm^{-1}. Anal. Calcd for $C_{10}H_{17}NO_3S$ (231.31): C, 51.93; H, 7.41; N, 6.06. Found: C, 51.34; H, 7.75; N, 6.02.

2,2-Dimethyl-*trans*-8-(α-*p*-nitrobenzyloxycarbonyloxyethyl)-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane (Mixture of Two Isomers) 8. To a stirred solution of 2,2-dimethyl-*trans*-8-(α-hydroxyethyl)-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane (mixture of two isomers) (17.4 g, 75 mmol) in ethanol-free methylene chloride (200 mL) *p*-nitrobenzyl chloroformate (21.6 g, 0.1 mol) was added and then solid 4-*N*,*N*-dimethylaminopyridine (12.2 g, 0.1 mol) added in small portions over a period of 30 min at −10 °C. The reaction mixture was then stirred at room temperature for 1 h and heated under reflux for 6 h. After cooling of the reaction mixture, dilution with methylene chloride (1.5 L), washing with cold aqueous (10%) sodium chloride solution (1 L), drying of the organic layer over sodium sulfate, filtration, and evaporation of the solvent, the residue was chromatographed on Merck silica gel (1 kg) using toluene–EtOAc (9:1) as eluant (20 fractions, 500 mL each). The fractions containing **8** were combined and the solvent was evaporated in vacuo, leaving **8** as an isomeric mixture (noncrystalline solid, 24.6 g, 80%): R_f 0.4 (toluene–EtOAc, 1:1); IR 1760, 1750, 1610, 1530, 1355 cm^{-1}; UV in ethanol λ_{max} 263 nm (ε 10 100). Anal. Calcd for $C_{18}H_{22}N_2O_7S$ (410.20): C, 52.68; H, 5.41; N, 6.83. Found: C, 52.82; H, 5.34; N, 6.97.

2,2-Dimethyl-*trans*-8-(α-*p*-nitrobenzyloxycarbonyloxyethyl)-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane 6-Dioxide (Mixture of Two Isomers) (9). To a stirred solution of 2,2-dimethyl-*trans*-8-(α-*p*-nitrobenzyloxycarbonyloxyethyl)-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane (mixture of two isomers, 16.4 g, 40 mmol) in methylene chloride (200 mL) at −10 °C within 30 min solid (90%) *m*-chloroperbenzoic acid (19.4 g, 0.1 mol) was added in small portions. The reaction mixture was then stirred at 0 °C for 1 h, diluted with methylene chloride (1.5 L), and subsequently washed with saturated aqueous sodium bicarbonate solution (600 mL), 10% aqueous sodium bisulfite solution (300 mL), and again with sodium bicarbonate solution (450 mL). The organic layer was dried over sodium sulfate and the solvent removed in vacuo, leaving pure noncrystalline **9** (17.7 g, 100%): R_f 0.6 (EtOAc); IR 1780, 1750, 1610, 1325, 1135 cm^{-1}; UV in ethanol λ_{max} 263 nm (ε 9800). Anal. Calcd for $C_{18}H_{22}N_2O_9S$ (442.45): C, 48.86; H, 5.01; N, 6.33. Found: C, 49.04; H, 5.23; N, 6.15.

***trans*-3-(α-*p*-Nitrobenzyloxycarbonyloxyethyl)-4-(β-hydroxyethylsulfonyl)azetidin-2-one (Mixture of Two Isomers) (10).** Crude 2,2-dimethyl-*trans*-8-(α-*p*-nitrobenzyloxycarbonyloxyethyl)-9-oxo-3-oxa-6-thia-1-azabicyclo[5.2.0^{1,7}]nonane 6-dioxide (17.7 g, 40 mmol) was dissolved in acetic acid (260 mL) and diluted with water (60 mL) and the resulting mixture heated under reflux for 105 min. After cooling of the reaction mixture the solvents were removed in vacuo and the residue, consisting of **10,** was dried *under high vacuum* (16.5 g, 100%).

Separation of *erythro-trans*-3-(α-*p*-Nitrobenzyloxycarbonyloxyethyl)-4-(β-hydroxyethylsulfonyl)azetidin-2-one (10e) and Its Threo-Isomer 10t. The isomeric mixture **10** (16.5 g, 40 mmol) was dissolved in hot methylene chloride (60 mL) and left at −20 °C, whereupon the main isomer **10e** crystallized. The crystals were filtered and recrystallized from ethyl acetate–pentane to give pure **10e** (4.8 g). The combined mother liquors were chromatographed on Merck silica gel (550 g) with toluene–ethyl acetate (2:1) (30 fractions, 800 mL each). The fractions with R_f 0.4 (EtOAc) were combined and the solvent was removed in vacuo, leaving the pure threo isomer **10t** (2.5 g). Mixed fractions, having R_f 0.4 and 0.35 (EtOAc), were combined, the solvent was removed in vacuo, and the residue was crystallized from methylene chloride, yielding more of **10e** (1.0 g). The mother liquors were again chromatographed, furnishing more of **10t** (0.5 g). The total yield of crystalline **10e** was thus 5.8 g (36%): mp 154 °C; R_f 0.35 (EtOAc); IR 3570, 3370, 1790, 1750, 1520, 1350 cm^{-1}; UV in ethanol λ_{max} 262 nm (ε 8200); NMR in Me_2SO-d_6 9.1 (s, 1), 7.6–8.3 (dd, 4), 5.3 (s, 2), 4.8–5.5 (broad s, 1), 5.1 (dd, 1, J = 4, 6.5 Hz), 4.9 (d, 1, J = 2.5 Hz), 3.7–3.9 (m, 3), 3.3–3.4 (m, 2), 1.5 (d, 3, J = 6.5 Hz). Anal. Calcd for $C_{15}H_{18}N_2O_9S$ (402.37): C, 44.78; H, 4.51; N, 6.96. Found: C, 44.78; H, 4.63; N, 6.95. The total yield of the noncrystalline **10t** was 3.0 g (19%): R_f 0.4 (EtOAc); IR 3570, 3370, 1790, 1750, 1520, 1350 cm^{-1}; UV in ethanol λ_{max} 263 nm (ε 9500); NMR in Me_2SO-d_6 δ 9.1 (s, 1), 7.6–8.4 (dd, 4), 5.3 (s, 2), 5.0–5.5 (broad s, 1), 5.1 (dd, 1, J = 5, 6.5 Hz), 4.0 (d, 1, J = 2.5 Hz), 3.7–3.9 (m, 3), 3.3 (m, 2), 1.45 (d, 3, J = 6.5 Hz). Anal. Calcd for $C_{15}H_{18}N_2O_9S$ (402.37): C, 44.78; H, 4.51; N, 6.95. Found: C, 44.75; H, 4.61; N, 7.18.

***threo-trans*-3-(α-*p*-Nitrobenzyloxycarbonyloxyethyl)-4-(*cis*-β-carbomethoxyvinylmercapto)azetidin-2-one (11t).** To a stirred solution of *cis*-β-carbomethoxyvinylisothiuronium chloride[2] (4.73 g, 24 mmol) in 95% ethanol (100 mL) at −15 °C and under nitrogen, aqueous sodium hydroxide (48 mL, 1.0 N) was added dropwise within 5 min. During the addition the reaction temperature was maintained at −15 °C by external cooling. After additional stirring at −10 °C for 5 min a solution of *threo-trans*-3-(α-*p*-nitrobenzyloxycarbonyloxyethyl)-4-(β-hydroxyethylsulfonyl)azetidin-2-one (**10t,** 8.1 g, 20 mmol) in 95% ethanol (60 mL) was added within 5 min and the mixture stirred at 2 °C for 80 min. Addition of methylene chloride (1 L), washing twice with saturated aqueous sodium chloride solution (500 mL per portion), drying of the organic layer over sodium sulfate, filtration, and evaporation of the solvent gave a solid residue which was chromatographed on Merck silica gel (300 g) using toluene–ethyl acetate (2:1) as eluant. The fractions having R_f 0.15 (toluene–EtOAc, 1:1) were combined and the solvent was removed in vacuo, leaving pure crystalline **11t** (7.95 g, 97%): mp 164.5–165.5 °C; IR 3500, 1790, 1760, 1710, 1530, 1360 cm^{-1}; UV in ethanol λ_{max} 270 nm (ε 21 400); NMR in Me_2SO-d_6 δ 8.8 (s, 1), 7.6–8.3 (dd, 4), 7.5 (d, 1, J = 10 Hz), 6.0 (d, 1, J = 10 Hz), 5.3 (s, 2), 5.1 (d, 1, J = 2.5 Hz), 5.0–5.2 (m, 1), 3.6 (s, 3), 3.5 (dd, 1, J = 6, 2.5 Hz), 1.35 (d, 3, J = 6.5 Hz). Anal. Calcd for $C_{17}H_{18}N_2O_8S$ (410.40): C, 49.76; H, 4.42; N, 6.83. Found: C, 49.68; H, 4.52; N, 6.80.

***erythro-trans*-3-(α-*p*-Nitrobenzyloxycarbonylethyl)-4-(*cis*-β-carbomethoxyvinylmercapto)azetidin-2-one (11e).** Following the procedure for the threo isomer, the title compound was obtained from **10e** (yield 97%): mp 135–137.5 °C (from EtOAc–pentane); R_f 0.15 (toluene–EtOAc, 1:1); IR 3400, 1790, 1760, 1710, 1530, 1630 cm^{-1}; UV in ethanol λ_{max} 270 nm (ε 21 300); NMR in Me_2SO-d_6 δ 8.8 (s, 1), 7.6–8.3 (dd, 4), 7.5 (d, 1, J 10 Hz), 6.0 (d, 1, J = 10 Hz), 5.3 (s, 2), 5.05 (d, 1, J = 2.5 Hz), 5.0–5.2 (m, 1), 3.65 (s, 3), 3.55 (dd, 1, J = 2.5, 5 Hz), 1.4 (d, 3, J = 6.5 Hz). Anal. Calcd for $C_{17}H_{18}N_2O_8S$ (410.40): C, 49.76; H, 2.42; N, 6.83. Found: C, 49.65; H, 4.45; N, 6.90.

Acetonyl 2-[*threo-trans*-3-(α-*p*-Nitrobenzyloxycarbonyloxyethyl)-4-(*cis*-β-carbomethoxyvinylmercapto)-2-oxoazetidin-1-yl]-2-triphenylphosphoranylidene Acetate (12t). A solution containing *threo-trans*-3-(α-*p*-nitrobenzyloxycarbonyloxyethyl)-4-(*cis*-β-carbomethoxyvinylmercapto)azetidin-2-one (**11t,** 2.05 g, 5 mmol), ac-

etonyl glyoxylate (2 g, 15 mmol) in toluene (10 mL), and DMF (5 mL) was stirred at 30 °C for 16 h with molecular sieves (3 Å, 20 g). After cooling, the sieves were removed by filtration and washed with ethyl acetate and from the combined washings and filtrate the solvent was removed in vacuo, leaving a viscous liquid, which was evaporated several times with DMF *under high vacuum* (0.02 mm) at 60 °C until its weight was not more than 3. 1 g. The resulting residue was chromatographed on Merck silica gel (100 g) using toluene–EtOAc (2:1) as eluant (20 fractions, 100 mL each). The fractions having R_f 0.6 (EtOAc) were combined and the solvent was removed in vacuo, *leaving a noncrystalline solid (2.7 g, 100%). To a solution containing* this solid (2.7 g, 5 mmol) and thionyl chloride (0.43 mL, 6 mmol) in dry tetrahydrofuran (25 mL) at −10 °C and under nitrogen with stirring, a solution of triethylamine (0.83 mL, 6 mmol) in dry tetrahydrofuran (3 mL) was added within 5 min. The mixture was stirred at 0 °C for 30 min and diluted with cold methylene chloride (150 mL), and the resulting solution was washed with cold aqueous HCl solution (50 mL, 0.1 N) followed by saturated NaCl solution (50 mL). After drying of the organic layer over sodium sulfate and filtration the solvent was removed in vacuo, leaving a noncrystalline solid (2.8 g, 100%). A solution of this solid (2.8 g, 5 mmol) and triphenylphosphine (2.9 g, 12 mmol) in dry tetrahydrofuran (4 mL) was kept under nitrogen for 2 days at room temperature. The reaction mixture was then diluted with methylene chloride (100 mL) and treated twice with portions (30 mL) of 10% aqueous sodium carbonate solution. The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo, leaving a noncrystalline solid. It was chromatographed on Merck silica gel (100 g) using toluene–ethyl acetate (3:1) as eluant (20 fractions, 100 mL each). The fractions having R_f 0.5 (EtOAc) were combined and the solvent was removed in vacuo, leaving **12t** as a noncrystalline, colorless solid (2.35 g, 60%): IR 1755, 1695, 1630, 1525 cm^{-1}; UV in ethanol λ_{max} 266 nm (ϵ 22 100). Anal. Calcd for $C_{40}H_{37}O_{11}N_2SP$ (784.77): C, 61.22; H, 4.75; N, 3.57. Found: C, 60.84; H, 4.87; N, 3.47.

Acetonyl 2-[*erythro-trans*-3-(α-*p*-Nitrobenzyloxycarbonyloxyethyl)-4-(*cis*-β-carbomethoxyvinylmercapto)-2-oxoazetidinyl]-2-triphenylphosphoranylidene Acetate (12e). Following the procedure for the threo isomer, from **11e** pure noncrystalline title compound **12e** was obtained (yield 65%): R_f 0.5 (EtOAc); IR 1755, 1695, 1630, 1525 cm^{-1}; UV in ethanol λ_{max} 266 nm (ϵ 22 100). Anal. Calcd for $C_{40}H_{37}N_2O_{11}SP$ (784.77): C, 61.22; H, 4.75; N, 3.57. Found: C, 61.26; H, 5.02; N, 3.35.

Acetonyl *threo-trans*-6-(α-*p*-Nitrobenzyloxycarbonyloxyethyl)-penem-3-carboxylate (13t). Into a solution containing acetonyl 2-[*threo-trans*-3-(α-*p*-nitrobenzyloxycarbonyloxyethyl)-4-(*cis*-β-carbomethoxyvinylmercapto)-2-oxoazetidin-1-yl]-2-triphenylphosphoranylidene acetate (**12t,** 1.96 g, 2.5 mmol) and trifluoroacetic acid (1.25 mL) in methylene chloride (40 mL) at −20 °C a stream of ozone in oxygen was introduced during 15 min at a rate of 0.33 mmol ozone/min. After nitrogen was passed through the solution for 2 min, dimethyl sulfide (2 mL) was added to the cold solution and the mixture stirred at 10 °C for 10 min. Subsequent dilution with ice-cold methylene chloride (50 mL), washing twice with portions (25 mL) of aqueous 10% $KHCO_3$ solution, drying the organic layer over sodium sulfate, and, after filtration, evaporation of the solvent gave a noncrystalline solid, which was dried *under high vacuum* for 2 min and then redissolved in dry (filtered through Alox) methylene chloride (60 mL). This solution was refluxed under nitrogen for 90 min and after cooling the solvent removed again in vacuo. The residue was chromatographed on Merck silica gel (25 g) using toluene–ethyl acetate (3:1) as eluant. Fractions having R_f 0.55 (EtOAc) were combined and the solvent was removed in vacuo, leaving pure **13t** as a pale yellow, noncrystalline solid (940 mg, 83%): IR 1795, 1750, 1745, 1720, 1530, 1350 cm^{-1}; UV in ethanol λ_{max} 261 nm (ϵ 12 600); λ_{max} 315 nm (ϵ 8000); NMR in acetone-d_6 δ 7.6–8.3 (dd, 4), 7.7 (s, 1), 5.95 (d, 1, J = 2.5 Hz), 5.3 (s, 2), 5.1–5.4 (m, 1), 4.8 (s, 2), 4.2 (dd, 1, J = 6, 2.5 Hz), 2.1 (s, 3), 1.45 (d, 3, J = 6.5 Hz). Anal. Calcd for $C_{19}H_{18}N_2O_9S$ (450.43): C, 50.67; H, 4.03; N, 6.22. Found: C, 50.47; H, 4.21; N, 6.13.

Acetonyl *erythro-trans*-6-(α-*p*-Nitrobenzyloxycarbonyloxyethyl)penem-3-carboxylate (13e). Following the procedure for the threo isomer, from **12e** pure crystalline title compound **13e** was obtained (yield 84%): mp 154–155 °C from EtOAc–pentane; R_f 0.55 (EtOAc); IR 1795, 1745, 1720, 1530, 1350 cm^{-1}; UV in ethanol λ_{max} 261 nm (ϵ 12 600), 315 (8000); NMR in acetone-d_6 δ 7.6–8.4 (dd, 4), 7.6 (s, 1), 5.8 (d, 1, J = 2.5 Hz), 5.35 (s, 2), 5.25 (dd, 1, J = 6.5, 3.5 Hz), 4.8 (s, 2), 4.2 (m, 1), 2.1 (s, 3), 1.5 (d, 3, J = 6.5 Hz). Anal. Calcd for $C_{19}H_{18}N_2O_9S$ (450.43): C, 50.67; H, 4.03; N, 6.22. Found: C, 50.76; H, 4.25; N, 6.26.

Acetonyl *threo-trans*-6-(α-Hydroxyethyl)penem-3-carboxylate (14t). Acetonyl *threo-trans*-6-(α-*p*-nitrobenzyloxycarbonyloxyethyl)penem-3-carboxylate (**13t,** 901 mg, 2 mmol) was hydrogenated in a mixture of acetonitrile (40 mL) and 95% ethanol (40 mL) using a 10% Pd on carbon catalyst (800 mg). After the mixture was stirred at room temperature for 2.5 h, H_2 was replaced by N_2 and the catalyst removed by filtration. The solvent was removed from the filtrate and *the residue chromatographed on Merck silica gel (40 g) using* toluene–ethyl acetate (2:1) as eluant (20 fractions, 40 mL each). The fractions with R_f 0.4 (EtOAc) were combined and the solvent was removed in vacuo, leaving crystalline **14t** (434 mg, 80%): mp 115.5–116.5 °C from EtOAc–pentane; IR 3610, 1795, 1740, 1720, 1560, 1175 cm^{-1}; IR (KBr) 3500, 1770, 1730, 1710 cm^{-1}; UV in ethanol λ_{max} 253 nm (ϵ 3000), 318 (7450); NMR in acetone-d_6 7.6 (s, 1), 5.85 (d, 1, J = 2 Hz), 4.8 (s, 2), 4.4 (m, 1), 4.0–4.4 (s, 1), 3.85 (dd, 1, J = 7, 2 Hz), 2.15 (s, 3), 1.3 (d, 3, J = 6.5 Hz). Anal. Calcd for $C_{11}H_{13}O_5NS$ (271.29): C, 48.70; H, 4.83; N, 5.16. Found: C, 48.95; H, 4.91; N, 5.30.

Acetonyl *erythro-trans*-6-(α-Hydroxyethyl)penem-3-carboxylate (14e). Following the procedure for the threo isomer from **13e** pure crystalline title compound was obtained in 80% yield: mp 120–121 °C from EtOAc–pentane; R_f 0.4 (EtOAc); IR 3610, 1795, 1740, 1720, 1560, 1175 cm^{-1}; IR (KBr) 3530, 1785, 1730, 1705 cm^{-1}; UV in ethanol λ_{max} 253 nm (ϵ 3020), 320 (7580); NMR in acetone-d_6 δ 7.55 (d, 1, J = 1 Hz), 5.8 (d, 1, J = 2 Hz), 4.8 (s, 2), 4.0–4.4 (s, 1), 4.2–4.4 (m, 1), 4.0 (m, 1), 2.15 (s, 3), 1.35 (d, 3, J = 6.5 Hz). Anal. Calcd for $C_{11}H_{13}O_5NS$ (271.29): C, 48.70; H, 4.83; N, 5.16. Found: C, 48.95; H, 4.91; N, 5.30.

***threo-trans*-6-(α-Hydroxyethyl)penem-3-carboxylic Acid (3t).** To a stirred solution of acetonyl *threo-trans*-6-(α-hydroxyethyl)-penem-3-carboxylate (**14t,** 271 mg, 1 mmol) in a mixture of acetonitrile (40 mL) and water (10 mL) at 0 °C under nitrogen within 15 min an aqueous solution of sodium hydroxide (10 mL, 0.1 N) was added and the reaction mixture stirred at 0 °C for another 15 min. Then the solvent was partly removed in vacuo, leaving approximately 10 mL of an aqueous solution. It was distributed over ten TLC plates L 254, OPTI-UP C 12, Antecgel Dodecyltrichlorsilan, 20 × 20 cm (supplier ANTEC Ltd., 4431 Bennwil, Switzerland), and the crude sodium salt chromatographed at 5 °C with water. The mobile, UV-active product was collected and washed from the adsorbant with acetonitrile–water (4:1) (100 mL). After partial evaporation in vacuo, the resulting aqueous solution was lyophilized *under high vacuum,* leaving the sodium salt of **3t** as a colorless, noncrystalline solid (143 mg, 60%): R_f 0.1 (AcOH–toluene–H_2O, 5:5:1); NMR in D_2O δ 7.25 (s, 1), 5.95 (d, 1, J = 2 Hz), 4.45 (m, 1), 4.15 (dd, 1, J = 6, 2 Hz), 1.48 (d, 3, J = 6.5 Hz). A sample (12 mg) was dissolved in water (0.5 mL) and the solution passed slowly through a column of prewashed (H_2O), strongly acidic cation exchange resin (Merck ion exchange I, 0.5 g) and rinsed with water until the eluant was no longer acidic. The acidic fractions were combined and the solution was lyophilized *under high vacuum,* leaving pure noncrystalline acid **3t** (10 mg). Crystallization from hot acetonitrile afforded analytically pure acid **3t:** mp >230 °C, solid–solid change at 155–156 °C; R_f 0.1 (AcOH–toluene–H_2O, 5:5:1); IR (KBr) 3480, 1795, 1670, 1545, 1435, 1255, 1160 cm^{-1}; UV in ethanol λ_{max} 260 nm (ϵ 3850), 311 (6400). Anal. Calcd for $C_8H_9NO_4S$ (215.22): C, 44.65; H, 4.22; N, 6.51. Found: C, 45.1; H, 4.5; N, 7.1.

***erythro-trans*-6-(1-Hydroxyethyl)penem-3-carboxylic Acid (3e).** Following the procedure for the threo isomer, from **13e** pure crystalline title compound was obtained (yield 60%). An analytical sample was crystallized from hot acetonitrile: mp >230 °C, solid–solid change at 147–151 °C; R_f 0.1 (AcOH–toluene–H_2O, 5:5:1); IR (KBr) 3510, 1785, 1670, 1550, 1435, 1260, 1180, 1035, 835 cm^{-1}; UV in ethanol λ_{max} 260 nm (ϵ 3650), 311 (6300); NMR of sodium salt in D_2O δ 7.3 (s, 1), 5.9 (d, 1, J = 2 Hz), 4.4 (m, 1), 4.2 (dd, 1, J = 4, 2 Hz), 1.52 (d, 3, J = 6.5 Hz). Anal. Calcd for $C_8H_9NO_4S$ (215.22): C, 44.65; H, 4.22; N, 6.51. Found: C, 45.2; H, 4.6; N, 7.2.

B. Stability Determinations of Penemcarboxylic Acids. Preparation of the "Biological Buffer Solution". $Na_2HPO_4 \cdot 2H_2O$ (9.62 g, 0.054 mol) and KH_2PO_4 (1.72 g, 0.013 mol) were dissolved in water and the volume was adjusted to 1 L at 20 °C. The buffer had pH 7.4.

Stability Determinations of 1, 3t, and 3e. A freshly prepared solution of the penemcarboxylic acid (~10^{-4} M) in "biological buffer" was

kept at 37 °C in a thermostat. At appropriate time intervals the UV spectrum was recorded (1-cm quartz cells) during approximately 7 half-lives, until the extinction at 311 nm remained constant. The exact half-life values were determined graphically by plotting the extinction values at 311 nm against the reaction time. First-order kinetics were usually observed during the first 3 half-lives. All calculations were based on the experimentally determined (after 7 half-lives) value at infinite time.

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(8) Throughout the synthetic scheme the threo compounds show larger coupling constants than their erythro isomers. The threo and erythro assignment has been confirmed, since the submission of this publication, by an X-ray structure determination on a crystal of the *threo*-hydroxyethylpenem ester **14t** (coupling constant 5.8 Hz compared to 3.5 Hz for the erythro isomer **14e**). The X-ray analysis, performed by Mrs. G. Rihs, Physical Research Laboratories, Ciba-Geigy Ltd., Basel, will be published elsewhere.
(9) The KBr spectra of **3t** and **3e** show OH absorptions at ca. 3500 cm^{-1}, consistent with *intermolecular* but *not* with *intramolecular* hydrogen bonding. The solution spectra in CH_2Cl_2 of both esters **14t** and **14e** (erythro isomer) show absorptions of free OH at 3610 cm^{-1} and associated OH at 3520 cm^{-1} at high (10%) concentrations. In dilute (1%) solutions the broad band at 3520 cm^{-1} disappears completely. This feature, which is observed with **14t** and **14e**, is *incompatible* with *intramolecular* hydrogen bonding: L. J. Bellamy, "The Infrared Spectra of Complex Molecules", 2nd ed., Wiley, New York, 1958, p 96. The X-ray structure analysis of **14t** also confirmed the absence of any intramolecular hydrogen bonding in the crystalline state.

Host–Guest Complexation. 22. Reciprocal Chiral Recognition between Amino Acids and Dilocular Systems

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Abstract: The direction of configurational bias and the extent of chiral recognition have been determined for complexation between seven amino acid perchlorate guests and four macrocyclic polyether hosts containing chiral elements. In solution experiments, racemic guest in D_2O was extracted by optically pure host in 0.45 mole fraction CD_3CN in $CDCl_3$. From the signs and magnitudes of the rotations of the guests recovered from each layer, the directions of the configurational bias and differences in free energies between the diastereomeric complexes ($\Delta(\Delta G°)$ values) were estimated. The hosts were 22-membered ring systems containing six ether oxygens attached to one another through four ethylene units (E) and two chiral 1,1′-binaphthyl (D) units of the same configuration attached to oxygens at their 2,2′ positions, or one D unit and one 1,1′-diphenyl (P) unit attached to oxygens at its 2,2′ positions. Methyl groups, when attached at the 3,3′ positions of the D units or one of the 3 positions of the P unit, extended the steric barriers. With D,L-$C_6H_5CH(CO_2H)NH_3ClO_4$ as the standard guest, hosts (*SS*)-$(CH_3)_2$D(OEOEO)$_2$D, (*S*)-$(CH_3)_2$D(OEOEO)$_2$P, (*RR*)-D(OEOEO)$_2$D, and (*S*)-$(CH_3)_2$D(OEOEO)$_2PCH_3$ at 0 °C gave complexes favoring the (*SS*)(L), (*S*)(L), (*RR*)(D), and (*S*)(L) configurations by $-\Delta(\Delta G°)$ values of 1.4, 0.8, 0.4, and 0.3 kcal/mol, respectively. The fraction of the total free energy of complexation which represents chiral recognition is termed the chiral efficiency. The chiral efficiencies of these hosts binding the standard chiral guest decreased in the same order from 0.33 to 0.17. With (*SS*)-$(CH_3)_2$D(OEOEO)$_2$D as the standard host at 0 °C, (*SS*)(L) complexes with $RCH(CO_2H)NH_3ClO_4$ of different R groups were favored by these respective $-\Delta(\Delta G°)$ values (kcal/mol): C_6H_5, 1.4; *p*-HOC_6H_4, 1.3; $C_6H_5CH_2$, 0.7; $(CH_3)_2CH$, 0.5; $C_8H_6NCH_2$(β-indolylmethyl), 0.4; $CH_3SCH_2CH_2$, 0.45; CH_3, 0.45. Chemical-shift differences in the ^1H NMR spectra of the (*SS*)(L) and (*SS*)(D) complexes of $(CH_3)_2$D(OEOEO)$_2$D with $C_6H_5CH(CO_2H)NH_3ClO_4$ were compatible with structural predictions based on CPK molecular model comparisons. The diphenyl unit of $(CH_3)_2$D(OEOEO)$_2$P was found by ^1H NMR temperature-dependent spectra to be conformationally equilibrating with an activation energy of about 19.4 kcal/mol at 89 °C. The direction of the chiral bias observed in solution was also observed in the crystalline state. The efficient and total enantiomeric resolution of (*RR*)(*SS*)-$(CH_3)_2$D(OEOEO)$_2$D was realized by crystallizing the (*RR*)(D) and (*SS*)(L) complexes prepared with D- and L-$C_6H_5CH(CO_2H)NH_3ClO_4$, respectively. The other diastereomeric complexes composed from these components failed to crystallize. A new synthesis of (*RR*)- and (*SS*)-$(CH_3)_2$D(OEOEO)$_2$D is reported.

Parts 7[3a] and 8[3b] of this series reported the syntheses, optical stabilities, absolute configurations, and maximum rotations of host compounds **1** and **2**, each of which contained two chiral elements (dilocular systems). Host compound **2** exhibited the highest chiral recognition in complexation of amino esters of a number of dilocular systems studied.[3c,d] Thus (*RR*)- or (*SS*)-**2** in $CDCl_3$ at 0 °C were found to extract preferentially the respective *R* or *S* enantiomers of amino esters, $RCH(CO_2CH_3)NH_3PF_6$, from D_2O by factors that ranged from 30 with R = C_6H_5 to 2.2 with R = $CH_3SCH_2CH_2$. The diastereomeric complexes, (*RR*)(D) and (*RR*)(L), differed from one another in $CDCl_3$ at 0 °C by $-\Delta(\Delta G°)$ values that

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