Synthesis and Biological Evaluation of Optically Active 3-H-1-Carbacephem Compounds

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3-H-1-Carbacephem nuclei with or without a 2α- or 2β-methyl group were prepared *via* 2+2 cycloaddition followed by intramolecular Horner–Emmons cyclization.

Optically active 3-H-1-carbacephem compounds were efficiently prepared by employing a penicillin acylase-producing microorganism in two ways. That is, the 7-phenylacetamide of a racemic carbacephem nucleus was hydrolyzed enantioselectively with the enzyme to afford the optically pure nucleus, which was then acylated to give antimicrobial compounds. Alternatively, a racemic carbacephem nucleus was directly and enantioselectively phenylglycylated with the enzyme. 3-H-1-Carbacephem nuclei appeared to be better substrates for penicillin acylase than penam or cephem nuclei of natural origin.

3-H-1-Carbacephem compounds showed potent antimicrobial activity; compound 32a exhibited activity comparable to that of ceftizoxime, a cephem analog with the same acyl group. It is of interest that the 3-H-1-carbacephem compound turned out to have more potent antimicrobial activity than its 3-substituted methyl analog.

Keywords 1-carbacephem; 2+2 addition; β-lactam; Horner–Emmons; optically active; enzymatic reaction; penicillin acylase; structure–activity relationship

A 1-carbacephem compound was initially synthesized together with 1-oxacephem by Christensen *et al.*[1] This was a very important achievement, especially in that it demonstrated for the first time that the sulfur atom in the cephem nucleus is not essential for antimicrobial activity and can be replaced by carbon (methylene) or oxygen without substantial loss of activity. However, their compounds were racemic and were structurally confined to those with a conventional substituted methyl group at C-3; the antimicrobial activity of these compounds was slightly inferior to that of the corresponding cephalosporins. Later, Doyle *et al.*[2] reported several types of carbacephems with substituents at C-2. They were chemically of great interest, but their antimicrobial activity remained low because of the common methyl group at C-3 (cephalexin type). Few other studies on 1-carbacephem compounds have been reported[3] due mainly to the difficulty in their preparation and the lack of attractive biological activity in the compounds reported.

In the course of extensive work on 1-carbacephem antibiotics[4] we have been focussing our effort on the 3-H-carbacephem nucleus instead of conventional 3'-substituted carbacephem. This is the simplest carbacephem nucleus and yet turned out to possess interesting features as follows: 1) it can be readily prepared in large quantities by an efficient procedure that we have developed; 2) it exhibits potent antimicrobial activity upon acylation with a suitable acyl group; 3) it serves as a parent nucleus for versatile modification at C-2 and C-3; 4) it is a good substrate for penicillin acylase for optical resolution and phenylglycylation. Uyeo and Ona also elaborated 3-H-1-carbacephem compounds[3a] with the racemic nucleus unresolved. We would like to describe herein the first synthesis and the antimicrobial activity of optically active 3-H-carbacephem compounds with or without a methyl group at C-2.

Synthesis of the 3-H-Carbacephem Nucleus (Charts 1 and 2) The schiff base (**3**) formed by condensation of *tert*-butyl phosphonoglycinate[5] (**1**) and 4-hexenal (**2**) was condensed without purification with azidoacetyl chloride in the presence of triethylamine at 0 °C to give a *cis* and *trans* mixture of a single β-lactam (**4**, **5**) in moderate yield with a ratio of up to 6 to 1 in favor of the *cis* isomer. Each isomer was separated by silica gel chromatography and oxidatively cleaved to afford the corresponding unstable aldehyde (**6**, **7**), followed by intramolecular Horner–Emmons cyclization to give rise to the 3-H-carbacephem compound (**8a**, **9**).

The *cis* isomer was more efficiently prepared according to the scheme shown in Chart 2. Condensation of the amine (**1**) with 4,4-dimethoxy-2-butenal (**10**) in methylene chloride in the presence of molecular sieves at room temperature gave the Schiff base (**11**), which was reacted with azidoacetyl chloride and triethylamine to afford only the *cis* isomer of the β-lactam (**12**) in fairly good yield. Hydrolysis of the acetal group with diluted acid gave the aldehyde (**13**) quantitatively, and **13** was treated (without purification) with diazabicyclooctane to achieve facile formation of the

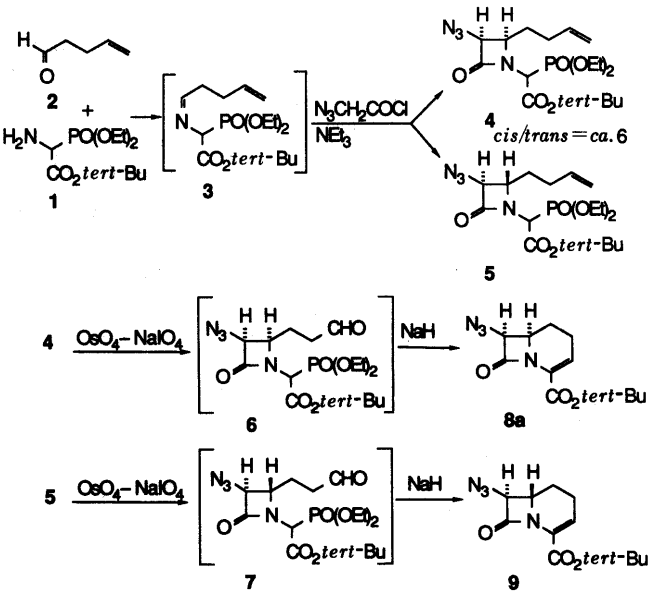


Chart 1

1,2-dehydrocarbacephem compound (**14**).[6] The one-pot reaction from the amine (**1**) to the diene (**14**) proceeded in 44% overall yield.

Smooth and selective reduction of the diene (**14**) and concomitant azide reduction were achieved by catalytic hydrogenation with palladium-on-carbon quantitatively to afford the aminoester (**15a**), which was identical with that derived by catalytic hydrogenation of the azido compound (**8a**). This enabled us to obtain 3-H-1-carbacephem compounds in fairly large quantity without much difficulty.

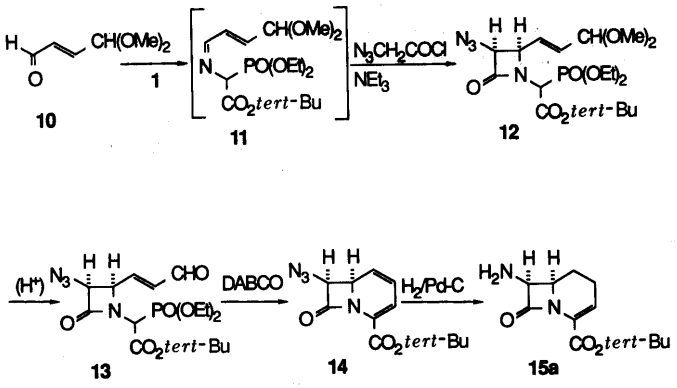


Chart 2

Synthesis of 2-CH_3-3-H-Carbacephem Compounds (Chart 3) A procedure similar to that shown in Chart 1 for 3-H-1-carbacephem was employed, starting with 3-methyl-4-hexenal (**16**) this time. *cis*-Predominance was less evident in the formation of the β-lactam (**18**, **19**). The *cis*-isomer (**19**) was formed alternatively from azetidinone acetate (**21**) with a strong base such as lithium diisopropylamide and diethyl phosphorochloridate. The *cis* isomer was submitted to Lemieux–Johnson oxidation to give the unstable aldehyde (**20**), followed by NaH-catalyzed cyclization to afford an oily mixture, which after chromatographic separation gave rise to the 2α-CH_3-3-H-1-carbacephem compound (**8b**) and 2β-CH_3 isomer (**8c**) in the ratio of 4 to 1. The configuration of 2-CH_3 was assigned from the $C_{2H,3H}$ coupling constant in the nuclear magnetic resonance (NMR) spectra (5.0 Hz for the 2α-CH_3 isomer and 2.7 Hz for the 2β-CH_3 isomer).

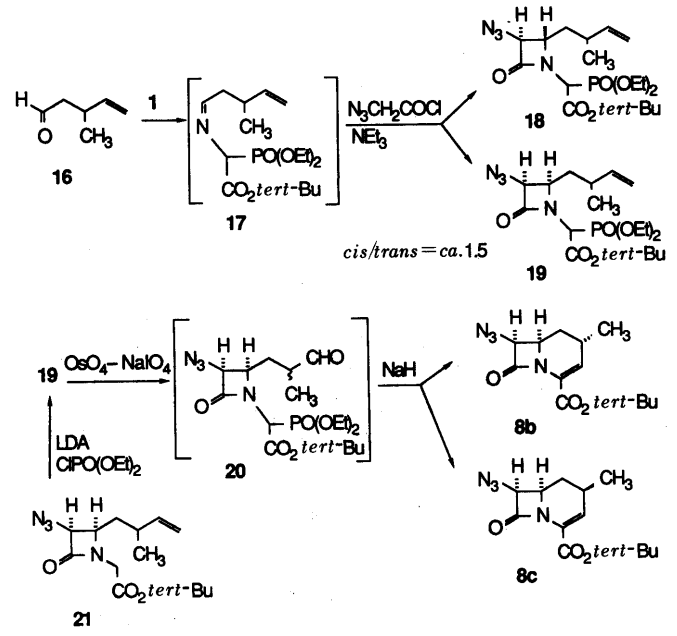


Chart 3

Reduction of the azidoester (**8**) either by catalytic hydrogenation or with hydrogen sulfide-triethylamine followed by deesterification with trifluoroacetic acid (TFA) gave the amino acid (**23**). The reaction order could be reversed. The amino acid (**23**) was acylated by a general method to obtain antimicrobial compounds (**24**, **25**) with typical acyl groups for cephalosporins. Alternatively, the amino ester (**15**) could be acylated first followed by the treatment with TFA to afford the acylated compounds (**24**, **25**).

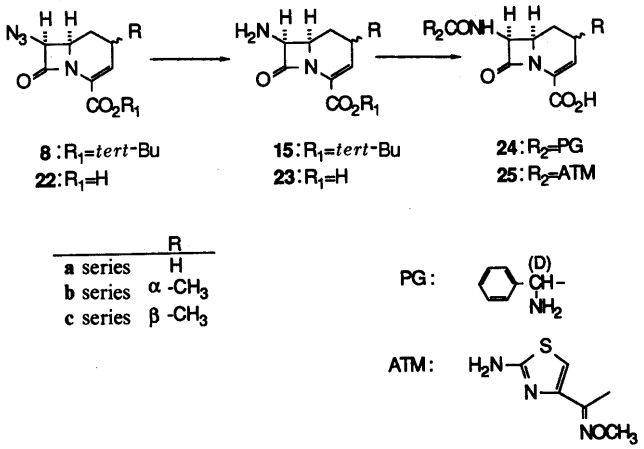


Chart 4

Synthesis of Otically Active Carbacephem Compounds We wished to obtain an optically active 3-H-1-carbacephem compound with (6*R*, 7*S*) absolute configuration that corresponds to the (6*R*, 7*R*) configuration of the cephem nucleus of natural origin so that we could directly compare the antimicrobial activity of the 1-carbacephem compound with that of the corresponding cephem compound for the first time. (Chart 5).

First, physicochemical optical resolution was attempted to separate the diastereomeric mixture of the (D)-phenylglycyl-3-H-1-carbacephem compound (**24a**) by preparative reverse-phase high performance liquid chromatography (HPLC), giving the isomers (**26a** and **28a**). Their absolute structures have not been determined but that of the former with positive $[\alpha]_D$ value was assigned as (6*R*, 7*S*) on the basis of its significant antimicrobial activity (Table II), whereas the diastereomeric isomer (**28a**) showed no antimicrobial activity.

The active isomer (**26a**) was alternatively prepared enantioselectively by enzymatic acylation of the racemic 1-carbacephem nucleus in high conversion yield. That is, the racemic amino acid (**23**) was incubated with D-phenylglycine methyl ester and intact cells of *Pseudomonas melanogenum* KY-8541, a penicillin acylase-producing β-lactamase-deficient mutant,[7] to afford the antimicrobial diastereoisomer (**26a**) selectively. The fact that compound **26a** was the sole product of this enzymatic acylation also supports the assigned absolute structure, assuming that the enzyme recognized the same absolute configuration at C_6

and C_7 of 1-carbacephem as of cephem.

Surprisingly, the 3-H-1-carbacephem nucleus served as a better substrate for penicillin acylase than the natural penam or cephem nucleus, as shown in the comparative rate study (Table I).

Kluyvera citrophila KY-7844, which has been selected for its high production of penicillin acylase[8] was found to hydrolyze the racemic phenylacetyl-3-H-1-carbacephem compounds (**30**) enantioselectively to afford the optically pure 3-H-1-carbacephem nucleus (**31**). The optically resolved 3-H-1-carbacephems (**31**) were furnished with the same acyl group as that of cefotaxime to give antimicrobial carbacephems **32**.

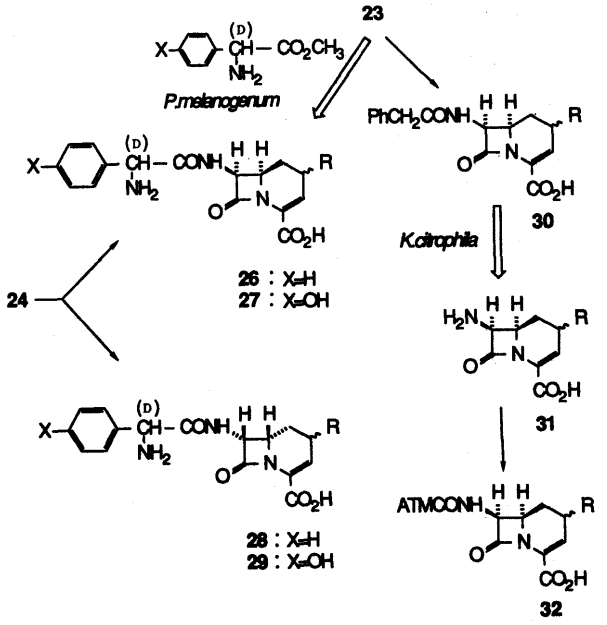


Chart 5

Antibacterial Activity The minimal inhibitory concentration (MIC) values of 3-H-1-carbacephem compounds against *S. aureus* and a variety of gram-negative bacteria were determined by the Mueller Hinton agar dilution method and are shown in Table II.

It is of great interest that the optical resolution of the carbacephem nucleus resulted in a remarkable improvement in antibacterial activities, to a greater extent than expected. This is especially evident in the case of compounds with optically resolved nuclei (**26a**, **32a** and **32b**), the antibacterial potencies of which were 4 fold or more greater than those of the corresponding recemates (**24a**, **25a** and **25b** respectively).

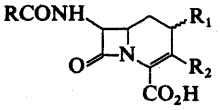
Among optically resolved nuclei, introduction of a 2α-CH_3 group (**26b**, **27b** and **32b**) did not alter the activities significantly, but that of 2β-CH_3 group (**26c**, **27c**) led to considerable diminution of activities against most microorganisms except *S. aureus*, which was actually most susceptible to the 2β-CH_3 compound. Similar comparative

TABLE I. Comparative Enzymatic Phenylglycylation of Various β-Lactam Nuclei

β-Lactam nucleus	Formed phenylglycyl compound (mmol/l)	Relative rate[a]
Carbacephem		
23a	0.50	140
23b	0.83	240
23c	0.72	200
6-APA	0.35	100
7-ACA	0.42	120

a) Calculated based on the rate of 6-APA as 100. Abbreviations: 6-APA, 6-aminopenicillanic acid; 7-ACA, 7-aminocephalosporanic acid.

TABLE II. Comparative Antimicrobial Activities [MIC (μg/ml)] of 3-H-Carbacephem Compounds



RCO	R_1	R_2	Compd. No.	S.a. 209P	E.c. NIHJJC-2	K.p. 8045	S.m. T-26	P.mir. 1287	P.v. 6897	P.mor. 4298	P.ret. 4289	P.a. #1
Racemic carbacephems												
PG	H	H	**24a**	0.4	12.5	3.13	a)	25	25	100	50	a)
	α-CH_3	H	**24b**	0.78	12.5	3.13	a)	50	50	100	a)	a)
	β-CH_3	H	**24c**	0.4	50	6.25	a)	a)	a)	a)	a)	a)
ATM	H	H	**25a**	12.5	≦0.01	≦0.01	0.78	≦0.01	≦0.01	≦0.01	≦0.01	25
	α-CH_3	H	**25b**	12.5	≦0.01	≦0.01	1.56	≦0.01	≦0.01	≦0.01	≦0.01	50
	H	CH_2OAc	**33**	25	0.2	0.05	1.56	0.2	≦0.01	0.05	0.4	6.25
Optically resolved carbacephems												
PG	H	H	**26a**	0.4	3.13	0.78	a)	12.5	25	50	12.5	a)
	α-CH_3	H	**26b**	0.78	12.5	0.78	a)	25	25	25	12.5	a)
	β-CH_3	H	**26c**	0.2	12.5	3.13	a)	50	a)	a)	100	a)
HO-PG	H	H	**27a**	0.4	3.13	0.78	100	50	100	100	12.5	a)
	α-CH_3	H	**27b**	0.4	6.25	3.13	50	25	100	100	a)	a)
	β-CH_3	H	**27c**	0.2	12.5	3.13	a)	50	a)	a)	a)	a)
ATM	H	H	**32a**	3.13	0.02	≦0.01	0.2	≦0.01	≦0.01	0.05	≦0.01	6.25
	α-CH_3	H	**32b**	1.56	0.02	≦0.01	0.4	≦0.01	≦0.01	0.02	≦0.01	25
	Ceftizoxime			0.78	0.02	≦0.01	0.2	≦0.01	≦0.01	0.1	≦0.01	6.25
	Cefotaxime			0.78	0.1	≦0.01	0.4	0.05	≦0.01	0.05	0.02	6.25

a) MIC >100 μg/ml. Abbreviations: PG, D-phenylglycyl; ATM, 2-(2-amino)thiazol-4-yl-2(*Z*)-methoxyiminoacetyl; HO-PG, *p*-hydroxy-D-phenylglycyl; S.a., *Staphylococcus aureus*; E.c., *Escherichia coli*; K.p., *Klebsiella pneumoniae*; S.m., *Serratia marcescens*; P.mir., *Proteus mirabilis*; P.v., *Proteus vulgaris*; P. ret., *Proteus rettgeri*; P.a., *Pseudomonas aeruginosa*.

activities were observed among corresponding cephem analogs.[9]

It should be noted that the 3-H-1-carbacephem compound (**25a**) exhibited quite high antimicrobial potency compared with the carbacephem compound (**33**) with a conventional 3-acetoxymethyl group. Its optically active form (**32a**) showed an antibacterial potency and breadth of spectrum comparable to those of the corresponding cephem antibiotics, ceftizoxime and cefotaxime.

These findings on 3-H-1-carbacephem compounds encouraged us to carry out further extensive studies of the chemistry and biology of this unique nuclear analog of cephem antibiotics. The result of these studies will be the subject of a series of forthcoming papers.

Experimental

Infrared (IR) spectra were measured with a JASCO IR-810, and ^{1}H-NMR spectra were measured on Varian T-60 and JEOL GNM PS-100 spectrometers. Optical rotations were measured on a Perkin Elmer model 141 polarimeter.

For column chromatography, silica gel (SiO_2, Wako C-200) or highly porous polymer resin (Mitsubishi Kasei Diaion HP 10) were used unless otherwise specified. Thin layer chromatography (TLC) was performed on Silica gel 60 F_{254} plates (Merck). All organic solvent extracts were dried over anhydrous sodium sulfate.

(±)-*tert*-Butyl 2-[*cis*-3-Azido-4-(3-butenyl)-2-oxoazetidin-1-yl]-2-diethylphosphonoacetate (4) and (±)-*tert*-Butyl 2-[*trans*-3-Azido-4-(3-butenyl)-2-oxoazetidin-1-yl]-2-diethylphosphonoacetate (5) A mixture of **1** (447 mg) and **2** (164 mg, 1.94 mmol) in anhydrous ether (25 ml) was stirred at room temperature for 1 h. Then molecular sieves 4A (200 mg) and anhydrous $MgSO_4$ were added to the reaction mixture, and stirring was continued for 1 h. Filtration and concentration *in vacuo* followed by azeotropic evaporation with benzene gave **3**. Azidoacetyl chloride (319 mg, 2.66 mmol) in cyclohexane (12.5 ml) was added dropwise to a stirred solution of **3** and triethylamine (0.369 ml, 2.66 mmol) in the presence of molecular sieves 4A (1 g) in cyclohexane (12.5 ml) and benzene (12.5 ml) at room temperature over 1.5 h. After 30 min, the reaction mixture was diluted with benzene (10 ml), washed with saturated NaCl, dried, and then concentrated *in vacuo*. The residue was separated by column chromatography (SiO_2, *n*-hexane–EtOAc, 2:1), giving **4** (345 mg, 46.4%) and **5** (58 mg, 7.8%) as colorless solids. **4**: IR ($CHCl_3$): 2120, 1775, 1770 (sh) 1750, 1740 (sh) 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.13—6.33 (1H, m), 4.93—5.17 (2H, m), 4.50—4.93 (2H, m), 3.80—4.40 (5H, m), 1.93—2.17 (4H, m), 1.50 (9H, s), 1.33 (6H, m). **5**: IR ($CHCl_3$): 2120, 1780, 1755, 1750 (sh), 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.43—6.20 (1H, m), 4.80—5.30 (2H, m), 3.75—4.75 (7H, m), 2.0—2.50 (4H, m), 1.50 (9H, s), 1.17 (6H, m).

***tert*-Butyl (6*R**,7*S**)-7-Azido-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylate (8a)** Osmium tetroxide (30 mg) was added to a stirred solution of **4** (298 mg, 0.72 mmol) in dioxane (8.5 ml) and H_2O (2.5 ml) at room temperature. After 30 min, powdered $NaIO_4$ (496 mg, 2.32 mmol) was added portionwise, and stirring was continued for 1.5 h, then the reaction mixture was extracted with ether (50 ml × 3). The organic layer was combined, washed with saturated NaCl, dried and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, benzene–EtOAc, 1:2), giving **6** (235 mg) as a 2,4-dinitrophenylhydrazine-positive spot on TLC.

Oil-dispersed NaH (50%, 27.1 mg, 0.56 mmol) was added to a stirred solution of **6** (235 mg) in anhydrous CH_3CN (15 ml) at room temperature under an N_2 stream. After 20 min, the reaction mixture was poured into 2% aqueous acetic acid, and extracted with ether (50 m × 4). The organic extracts were combined, washed with saturated NaCl, dried, filtered, and then concentrated *in vacuo*. The oily product was purified by column chromatography (SiO_2, *n*-hexane–EtOAc, 3.5:1), giving **8a** (91 mg, 51%) as white crystals. **8a**: mp 64.5—65.5 °C. IR ($CHCl_3$): 2130, 1790, 1730, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.30 (1H, t, J=4 Hz), 4.93 (1H, d, J=5 Hz), 3.80 (1H, m), 1.6—2.6 (4H, m), 1.52 (9H, s). EI-MS m/z: 264 (M^+).

***tert*-Butyl (6*S**,7*S**)-7-Azido-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylate (9)** The same treatment of **5** (767 mg, 1.84 mol) as that described for **8a** gave **9** (218 mg, 44.9%) as white crystals: mp 80.5—81.5 °C. IR ($CHCl_3$): 2110, 1780, 1720, 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.27 (1H, t), 4.28 (1H, d, J=2 Hz), 3.53 (1H, m), 2.0—2.6 (4H, m), 1.63 (9H, s). EI-MS m/z: 264 (M^+).

(±)-*tert*-Butyl 2-[*cis*-4-(3,3-Dimethoxy-1-propenyl)-3-azido-2-oxoazetidin-1-yl]-2-diethylphosphonoacetate (12) A mixture of **1** (1.08 g, 4.0 mmol) and 4,4-dimethoxy-*trans*-2-butenal (**10**) (580 mg, 4.4 mmol) was stirred at room temperature for 1 h in anhydrous CH_2Cl_2 (100 ml). Then anhydrous $MgSO_4$ (600 mg) was added and stirring was continued for 1 h. Filtration and concentration *in vacuo* gave **11** (1.63 g) as an oil.

Azidoacetyl chloride (580 mg, 4.8 mmol) in cyclohexane (40 ml) was added dropwise to a stirred solution of **11** (1.63 g) and triethylamine (0.84 ml, 7.0 mmol) in anhydrous benzene (30 ml) and cyclohexane (30 ml) at room temperature over 1.5 h. After 1 h, the reaction mixture was diluted with benzene (100 ml), washed successively with saturated $NaHCO_3$, and saturated NaCl, dried, filtered, and then concentrated *in vacuo*. The residue was separated by column chromatography (SiO_2, *n*-hexane–EtOAc, 1:2), giving **12** (550 mg, 28.2%) and **13** (220 mg, 11.3%). **12**: IR ($CHCl_3$): 2120, 1780, 1745 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.83—6.07 (2H, m), 4.50—5.00 (3H, m), 4.23 (4H, m), 3.33 (6H, s), 1.50 (9H, s), 1.37 (6H, m). **13**: IR ($CHCl_3$): 2120, 1785, 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 9.62 (1H, d, J=8.0 Hz), 7.00 (1H, dd, J=8.0, 15.0 Hz), 6.26 (1H, dd, J=7.0, 15.0 Hz), 4.84 (1H, d, J=24 Hz), 4.80—5.02 (2H, m), 4.16 (4H, m), 1.46 (9H, s), 1.26 (6H, m). EI-MS m/z: 417 ($M+1$).

(±)-*tert*-Butyl 2-[*cis*-4-(3-Oxo-1-propenyl)-3-azido-2-oxoazetidin-1-yl]-2-diethylphosphonoacetate (13) *p*-Toluenesulfonic acid monohydrate (155 mg) was added to a stirred solution of **12** (1.4 g, 3.0 mmol) in acetone (30 ml). Stirring was continued for 4 h at room temperature, then the reaction mixture was diluted with EtOAc (100 ml), washed successively with saturated $NaHCO_3$ (20 ml × 3) and saturated NaCl (20 ml × 2), dried, filtered, and then concentrated *in vacuo*. The residue (1.25 g, 100%) was identified as **13**.

***tert*-Butyl (6*R**,7*S**)-7-Azido-8-oxo-1-azabicyclo[4.2.0]oct-2,4-dien-2-carboxylate (14)** 1,4-Diazabicyclo[2.2.2]octane (224 mg, 2.0 mmol) was added to a stirred solution of **13** (832 mg, 2.0 mmol) in benzene (4 ml) at room temperature. After 4 h, the reaction mixture was diluted with EtOAc (20 ml), washed successively with saturated NH_4Cl, and saturated NaCl, dried, filtered, and then concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, *n*-hexane–EtOAc, 3:1), giving **14** (352 mg, 67.2%): mp 68.0—69.2 °C. IR ($CHCl_3$): 2130, 1790, 1720, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.64 (1H, d, J=6 Hz), 6.24 (1H, ddd, J=2.5, 6.0, 6.0 Hz), 6.04 (1H, dd, J=2.0, 10.0 Hz), 5.26 (1H, d, J=5.0 Hz), 4.64 (1H, m), 1.50 (9H, s).

***tert*-Butyl (6*R**,7*S**)-7-Amino-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylate (15a)** Compound **14** (210 mg, 0.80 mmol) in EtOAc (17 ml) was hydrogenated in the presence of 10% Pd–C (150 mg) under an atmosphere of H_2 at room temperature. The catalyst was removed by filtration and washed with EtOAc (40 ml). The filtrate and washing were combined and concentrated *in vacuo* to afford **15a** (115 mg, 60.8%): mp 103.0—107.5 °C. IR ($CHCl_3$): 1775, 1725, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.27 (1H, t, J=4.0 Hz), 4.49 (1H, d, J=5.0 Hz), 4.2—3.1 (3H, m), 2.60—1.70 (4H, m), 1.51 (9H, s).

(±)-*tert*-Butyl 2-[*cis*-4-(2-Methyl-3-butenyl)-3-azido-2-oxoazetidin-1-yl]-2-diethylphosphonoacetate (19). Method A A mixture of **1** (2.13 g, 8 mmol) and **16** was treated in a similar manner to that described for the preparation of **4** and **5**, to obtain **19** (380 mg, 11.0%), the *trans* isomer **18** (570 mg, 16.7%) and their mixture (201 mg, 5.8%). **18** IR ($CHCl_3$): 2110, 1765, 1745 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.45—6.13 (1H, m), 4.83—5.20 (2.5H, m), 4.67 (0.5H, m), 3.97—4.45 (6H, m), 1.77—2.55 (3H, m), 1.59 (9H, s), 1.33 (6H, t, J=7.0 Hz), 1.08 (3H, d, J=6.0 Hz). **19**: IR ($CHCl_3$): 2110, 1770, 1745 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.40—6.10 (1H, m), 5.27—4.90 (2.5H, m), 4.68 (0.5H, d, J=5.0 Hz), 4.23 (6H, m), 2.60—1.77 (3H, m), 1.53 (9H, s), 1.37 (6H, t, J=7.0 Hz), 1.10 (3H, d, J=6.0 Hz).

Method B. (±)-*tert*-Butyl 2-[*cis*-4-(2-Methyl-3-butenyl)-3-azido-2-oxoazetidin-1-yl]acetate (21) a) A mixture of *tert*-butyl glycinate (4.3 g, 32.8 mmol) and **16** (3.24 g, 33 mmol) in absolute benzene (100 ml) and cyclohexane (100 ml) was stirred with molecular sieves 4A (2 g) and anhydrous $MgSO_4$ at room temperature for 2.5 h. The reaction mixture was treated with triethylamine (6.83 ml, 49.2 mmol) followed by a solution of azidoacetyl chloride (5.88 g, 49.2 mmol) in dry cyclohexane (100 ml) and the mixture was stirred for 3 h. The solution was washed with 0.5 N HCl (50 ml × 3), saturated NaCl, 10% $NaHCO_3$ (50 ml × 3) and saturated NaCl, and dried.

Evaporation of the solvent gave a brown oil (7.85 g), which was chromatographed (SiO_2 460 g, *n*-hexane–EtOAc, 5:1) to afford oily **21** (2.82 g, 29.2%) along with the isomer (0.47 g, 4.9%). **21**: IR ($CHCl_3$): 2120, 1771, 1743 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.53—5.96 (1H, m), 4.83—5.20 (3H, m), 4.17 (1H, m), 3.53—4.00 (2H, m), 1.57—2.50 (3H, m), 1.47 (9H, s), 1.07 (3H, d, J=6 Hz).

b) Compound **21** (110 mg, 0.37 mmol) was added to a cold solution of di-isopropylcyclohexylamine (130.7 mg, 0.93 mmol), 1.4 M *n*-BuLi (0.66 ml, 0.93 mmol) and hexamethylphosphoramide (HMPA) (166 mg, 0.93 mmol) kept at −75 °C. The resulting deep orange-red solution was treated with diethylphosphorochloridate (65.6 mg, 0.38 mmol) and the mixture was stirred for 30 min at the same temperature. The reaction mixture was worked up and chromatographed on silica gel in the same manner as in method A to afford **19** (18 mg, 11%), the physicochemical data for which agreed with those of the oil prepared by method A.

***tert*-Butyl (4*S**,6*R**,7*S**)-4-Methyl-7-azido-8-oxo-1-azabicyclo[4.2.0]-oct-2-en-2-carboxylate (8b) and *tert*-Butyl (4*R**,6*R**,7*S**)-4-Methyl-7-azido-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylate (8c)** Compound **19** dissolved in distilled dioxane (370 ml) and water (113 ml) was treated with OsO_4 (1.0 g) in the dark and stirred at room temperature for 15 min. Pulverized $NaIO_4$ (18 g) was added with stirring, keeping the reaction temperature at 15 °C. After another 15 min of stirring, ether (1.0 l) was added and the organic layer was decanted. This was repeated three times and the combined organic layer was washed with saturated NaCl, dried and evaporated *in vacuo*. The residual oil was adsorbed on a short silica gel column (300 g) and eluted with benzene and AcOEt (1 : 2). The fraction positive in the 2,4-dinitrophenylhydrazine test was collected and concentrated to afford an oil (9.02 g).

The above oil (8.02 g) dissolved in anhydrous dimethoxyethane was treated with 60% NaH (800 mg) with stirring for 1 h. Ether (800 ml) was added and the mixture was washed successively with 2% AcOH, saturated NaCl, saturated $NaHCO_3$ and saturated NaCl, dried and evaporated *in vacuo* to give a colorless oil (5.14 g).

The oil was separated on a SYSTEM 500® preparative HPLC using *n*-hexane and AcOEt (10 : 1) as eluting solvents at an elution speed of 0.05 l per min. The less polar isomer **8b** (α-Me form, 1.82 g, 20.8% yield from the olefin **19**) and the more polar isomer **8c** (β-Me form, 0.76 g, 8.6% from **19**) were obtained together with a mixture of both isomers (0.404 g in 4.6% from **19**). **8b**: mp 82.0—84.0 °C. IR (KBr): 2120, 1790, 1721, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.33 (1H, d, J=5.0 Hz), 5.00 (1H, d, J=5.5 Hz), 3.89—3.68 (1H, m), 2.66 (1H, m), 1.82—1.57 (2H, m), 1.53 (9H, s), 1.12 (3H, d, J=7.0 Hz) **8c**: mp 84.0—86.5 °C. IR (KBr): 2135, 1783, 1715, 1622 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.13 (1H, d, J=2.7 Hz), 4.90 (1H, d, J=5.0 Hz), 3.93—3.73 (1H, m), 2.53 (1H, m), 2.16—1.75 (2H, m), 1.53 (9H, s), 1.20 (3H, d, J=6.0 Hz).

(6*R,7*S**)-7-Azido-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (22a)** The *tert*-butyl ester (**8a**) (0.22 mmol) was dissolved in TFA (2 ml), and this solution was stirred for 10 min. Concentration *in vacuo*, trituration with ether and collection by filtration gave the carboxylic acid (**22a**) quantitatively. **22a**: IR ($CHCl_3$): 2120, 1770 sh, 1715, 1635 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 6.48 (1H, t, J=4 Hz), 5.10 (1H, d, J=5 Hz), 3.83 (1H, q), 1.1—2.5 (4H, m).

Homologs **22b** and **22c** were obtained in the same manner. **22b**: mp 121.5—123.0 °C. IR ($CHCl_3$): 2110, 1769, 1750, 1716, 1630 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 6.47 (1H, d, J=5.6 Hz), 5.22 (1H, d, J=5.0 Hz), 4.2—3.7 (1H, m), 2.3—2.9 (1H, br), 1.11 (3H, d, J=7.2 Hz). **22c**: ^{1}H-NMR (CD_3OD) δ: 6.28 (1H, d, J=2.0 Hz), 5.12 (1H, d, J=5.0 Hz), 4.2—3.7 (1H, m), 2.3—2.9 (1H, br), 1.20 (3H, d, J=7.0 Hz).

***tert*-Butyl (4*S**,6*R**,7*S**)-7-Amino-4-methyl-8-oxo-1-azabicyclo[4.2.0]-oct-2-en-2-carboxylate (15b)** Compound **8b** (255 mg, 0.67 mmol) was dissolved in 10 ml of EtOH and 100 mg of 10% Pd–C was added thereto. The mixture was subjected to catalytic hydrogenation for 1.5 h and then filtered to remove the catalyst. The catalyst was washed with MeOH. The filtrate and the washings were combined and concentrated under reduced pressure to leave a pale yellow oily product. The product was dissolved in 8 ml of EtOAc and the solution was extracted five times with 3 ml of 10% citric acid. The water layer was adjusted to a pH of 6 to 7 with K_2CO_3 to obtain a white suspension. The suspension was then extracted twice with 5 ml of EtOAc and washed with saturated NaCl. The solution were dried and concentrated to obtain 177 mg (76.6%) of an oily product having the following properties. IR ($CHCl_3$): 3400, 1770, 1720, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.23 (1H, d, J=5.0 Hz), 4.53 (1H, d, J=5.8 Hz), 3.93—3.47 (1H, m), 2.56 (1H, m), 1.92 (2H, br), 1.80—1.60 (2H, m), 1.50 (9H, s), 1.31 (3H, d, J=7.0 Hz).

In the same manner, compounds **15a** and **15c** were prepared. **15c**: (quantative yield): mp 216—221 °C (dec.). IR (KBr): 3430, 2590, 1780, 1762, 1712, 1630 cm^{-1}.

(6*R,7*S**)-7-Amino-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (23a)** The *tert*-butyl ester (**15a**) (300 mg, 1.26 mmol) was dissolved in 3.0 ml of CH_2Cl_2 and 3.0 ml of TFA was added. The mixture was allowed to stand at room temperature for 1 h and 20 min. The reaction mixture was then concentrated and benzene was added to the residue. The solution was again concentrated to obtain 250 mg of the desired compound as a yellow powder having the following properties. IR (KBr): 1780, 1680, 1630 cm^{-1}. The above trifluoroacetate was dissolved in 2 ml of water and adjusted to pH 7.0 with saturated $NaHCO_3$ to form crystals. Then, 129 mg of compound **23a** was recovered by filtration. **23a**: IR (KBr): 1800, 1770 (sh), 1760 (sh), 1740, 1680, 1630 cm^{-1}. ^{1}H-NMR (100 M Hz, D_2O–DSS) δ: 6.16 (1H, d, J=5.1 Hz), 4.52 (1H, d, J=4.9 Hz), 3.86 (1H, m), 2.64 (1H, m), 1.9—1.4 (2H, m), 1.10 (3H, d, J=7.3 Hz). The 4α-methyl analog **23b** was similarly obtained. **23b** trifluoroacetate (quantative yield): IR (KBr): 3460, 2980—2500, 1780, 1685, 1630 cm^{-1}. ^{1}H-NMR (D_2O–DSS) δ: 6.77 (1H, d, J=5.8 Hz), 5.00 (1H, d, J=5.6 Hz), 4.10 (1H, m), 2.83 (1H, m), 1.86 (2H, m), 1.15 (3H, d, J=8.0 Hz).

(6*R,7*S**)-7(*R*)-Phenylglycinamido-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (24a)** a) A 1 N isobutyl chloroformate–tetrahydrofuran (THF) solution was added to a stirred solution of (*R*)-*N*-*tert*-butyloxycarbonyl phenylglycine (297.3 mg, 1.18 mmol) and 1 N methylmorpholine (1.18 mmol) in anhydrous THF (6.18 ml) at −30 °C. After 30 min, **15a** (234 mg, 0.983 mmol) in anhydrous THF (5 ml) was added, then the stirring was continued for 45 min at −30 °C and for about 4 h at 0 °C. The reaction mixture was diluted with CH_2Cl_2 (15 ml) and washed succesively with water, 1 N HCl and saturated NaCl, dried, filtered, and then concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, *n*-hexane–EtOAc, 1 : 1) to obtain the *tert*-butyl ester (322 mg, 69.4%). IR (KBr): 1770, 1750, 1720, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.32 (5H, s), 6.31 (1H, m), 5.90 (1H, m), 2.50—1.70 (4H, m), 1.50 (9H, s), 1.40 (9H, s).

b) TFA (5.0 ml) was added to a solution of this *tert*-butyl ester in anhydrous CH_2Cl_2 (2.5 ml) and anisole (2.5 ml) under ice cooling. After about 5 h, the reaction mixture was concentrated *in vacuo* and triturated with ether. The precipitate was collected by filtration to obtain the desired compound **24a** (202 mg, 70.9%). IR (KBr): 1765, 1680, 1630 cm^{-1}. ^{1}H-NMR (D_2O–DSS) δ: 7.51 (5H, s), 6.08 (1H, t, J=4.2 Hz), 5.41 (1H, d, J=4.9 Hz), 3.83 (1H, ddd, J=3.7, 4.9, 8.6 Hz), 1.01—2.28 (4H, m).

In a similar manner, the 2-methyl compounds **24b** and **24c** were obtained. **24b**: IR (KBr): 3430, 3200, 3060, 2960—2650, 1780 (sh), 1770, 1695 (sh), 1680 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 9.36 (1H, d, J=8 Hz), 7.47 (5H, s), 6.28 (1H, d, J=6 Hz), 5.40 (1H, m), 4.98 (1H, m), 3.70 (1H, br), 2.45 (br, partly overlapping with the signal of DMSO-d_6), 1.80 (2H, m), 1.06, 0.95 (3H, d, respectively, J=7.5 Hz) **24c**: IR (KBr): 3450 (sh), 3230 (sh), 3060, 2960—2800, 1769, 1695 (sh), 1681 (sh), 1673 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 9.29 (1H, t, J=8.0 Hz), 7.49 (5H, s), 6.11, 6.04 (each 1H, d, each J=2.0 Hz), 5.30 1H, m), 4.97 (1H, d, J=4 Hz), 3.82 (1H, br), 2.44 (br, partly overlapping with the signal of DMSO-d_6), 1.82 (2H, br), 1.14, 0.91 (each 3H, each d, J=7.5 Hz).

(6*R,7*S**)-7-[2-(2-Amino-4-thiazolyl)-2-*syn*-methoxyiminoacetamido]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic acid (25a)** 2-Chloroacetylamino-4-thiazolyl-2-*syn*-methoxyiminoacetic acid (54.2 mg, 0.195 mmol) was suspended in 0.98 ml of anhydrous CH_2Cl_2 and 23.48 mg (0.195 mmol) of triethylamine was added. Phosphorus pentachloride (40.8 mg, 0.195 mmol) was added under ice cooling. After stirring of the reaction mixture for 20 min, 3.92 ml of *n*-hexane was added and the supernatant was removed by decantation. The residue was dissolved in 1.96 ml of THF to obtain an acid chloride solution.

In a separate flask, **23a** (trifluoroacetate 45.9 mg, 0.155 mmol) was dissolved in 2 ml of 50% THF–water with 47.4 mg (0.469 mmol) of triethylamine. To this solution, the above acid chloride solution was added under ice cooling and the mixture was stirred for 2 h, adjusted to a pH of 2.0 with 10% HCl and extracted three times with EtOAc. The extracts were washed with saturated NaCl solution. The solution was dried and concentrated to obtain 80 mg of chloroacetamide as a pale yellow powder. The powder was dissolved in 0.96 ml of dimethylacetamide and 27.5 mg of thiourea was added at room temperature with stirring. The mixture was stirred for 14 h. After addition of ether, the supernatant was removed by decantation to obtain a red oily residue. The residue was purified by chromatography using Diaion HP-10 to obtain **25a** (19.2 mg). **25a**: IR (KBr): 1760, 1670, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 9.26 (1H, d, J=8.6 Hz), 7.11 (2H, br), 6.75 (1H, s), 6.30 (1H, m), 5.47 (1H, dd, J=5.4, 8.8 Hz), 3.89 (3H, s), 2.5—1.0 (4H, m).

The 4α-methyl analog **25b** was obtained in a similar manner. **25b**: IR (KBr): 1760, 1680, 1655 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 9.27 (1H, d, J=9.0 Hz), 7.15 (2H, br), 6.75 (1H, s), 6.31 (1H, d, J=4.2 Hz), 5.58 (1H, br), 3.85 (3H, s), 2.60 (1H, m), 1.67 (2H, br), 1.08 (3H, d, J=8 Hz).

Separation of Diastereomeric Mixture 24a by HPLC The diastereomeric mixture (**24a**) (50 mg) was separated by HPLC (Bondapak C-18

column, 7% MeOH containing 0.2 N potassium hydrogen phosphate buffer). Each fraction was concentrated *in vacuo* to remove MeOH, and lyophilized. The dried matter was dissolved in water, and absorbed on a column (HP10 20 ml), which was washed with water and eluted with 20% aqueous MeOH. The fraction was concentrated to remove MeOH and lyophilized to obtain **26a** (24.6 mg) and **28a** (14.0 mg). **26a**: $[\alpha]_D^{22}$ +57.2° (c=0.5, H_2O). IR (KBr): 1760, 1690, 1640 cm^{-1}. ^{1}H-NMR (D_2O) δ: 7.51 (5H, s), 6.08 (1H, t, J=4.2 Hz), 5.41 (1H, d, J=4.9 Hz), 3.83 (1H, ddd, J=8.6, 3.7, 4.9 Hz), 2.28—1.01 (4H, m). **28a**: $[\alpha]_D^{15}$ −74.2° (c=0.5, H_2O). IR (KBr): 1750, 1690, 1640 cm^{-1}. ^{1}H-NMR (D_2O) δ: 7.51 (5H, s), 6.15 (1H, t, J=3.9 Hz), 5.20 (1H, d, J=4.9 Hz), 5.19 (1H, s), 3.88 (1H, ddd, J=8.6, 3.7, 4.9 Hz), 2.41—1.41 (4H, m).

In a similar manner, **26b** was obtained. **26b**: $[\alpha]_D^{15}$ +4.23° (c=0.52, H_2O). IR (KBr): 3420, 1760, 1695, 1633 cm^{-1}. ^{1}H-NMR (D_2O) δ: 7.51 (5H, s), 6.10 (1H, d, J=5.1 Hz), 5.47 (1H, d, J=4.7 Hz), 5.19 (1H, s), 3.89 (1H, m), 2.45 (1H, m). 1.44—1.04 (2H, m), 1.00 (3H, d, J=7.4 Hz).

Enzymatic Phenylglycylation A disrupted cell suspension of *Pseudomonas melanogenum* KY8541 (200 mg as dry cell weight) was added to a solution of **23a** (120 mg) and methyl *R*-phenylglycinate hydrochloride (500 mg) in 1/30 M potassium phosphate buffer (pH 6.5) (10 ml). After 5 h at 30 °C, cell bodies were removed by centrifugation. The supernatant was concentrated *in vacuo* and separated by column chromatography (HP-10, 100 ml). The column was washed with water (200 ml) and eluted with 30% aqueous MeOH, then the eluate was concentrated *in vacuo* to 0.5 ml. The concentrate was purified by column chromatography (Sephadex LH20, 50% aqueous MeOH), concentrated to remove MeOH, and lyophilized to afford the desired compound **26a** (81 mg).

Physicochemical data coincided with those of the levo-rotatory isomer **26a** separated from the diastereomeric mixture. The 4-methyl analogs **26b**, **26c** and *p*-hydroxyphenylglycyl analogs **27a**, **27b** and **27c** were also obtained by similar procedures. **26c** $[\alpha]_D^{22}$ +120.8° [c=0.5, 1 M phosphate buffer (pH 7.0)] IR (KBr): 3450, 1780 (sh), 1770, 1760 (sh), 1700, 1640 cm^{-1}. ^{1}H-NMR (D_2O) δ: 7.51 (5H, s), 5.88 (1H, J=2.2 Hz), 5.34 (1H, d, J=4.9 Hz), 5.16 (1H, s), 3.79—3.99 (1H, m), 2.36—2.47 (1H, m), 1.43—1.65 (1H, m), 0.92 (3H, d, J=7.1 Hz), 0.43—0.82 (1H, m). **27a**: $[\alpha]_D^{15}$ +107.5° [c=0.5, 1 M phosphate buffer (pH 7.0)]. IR (KBr): 3450, 3290, 3090, 1700 (sh), 1685, 1640 cm^{-1}. ^{1}H-NMR (D_2O) δ: 7.36 (2H, d, J=8.8 Hz), 6.95 (2H, d, J=8.8 Hz), 6.06 (1H, t, J=3.9 Hz), 5.40 (1H, d, J=4.6 Hz), 5.12 (1H, s), 3.84 (1H, m), 2.22 (2H, m), 1.62 (1H, m), 1.12 (1H, m). **27b**: $[\alpha]_D^{15}$ +12.8° [c=0.5, 1 M phosphate buffer (pH 7.0)]. IR (KBr): 3420, 3260, 1760, 1685 cm^{-1}. ^{1}H-NMR (D_2O) δ: 7.35 (2H, d, J=8.0 Hz), 6.96 (2H, d, J=8.0 Hz), 6.10 (1H, d, J=5.1 Hz), 5.45 (1H, d, J=4.9 Hz), 5.11 (1H, s), 3.92 (1H, m), 2.45 (1H, m), 1.50—1.08 (2H, m), 1.01 (3H, d, J=7.1 Hz). **27c**: $[\alpha]_D^{22}$ +133.4° [c=0.5, 1 M phosphate buffer (pH 7.0)]. IR (KBr): 3450, 3230, 1780 (sh), 1770, 1760, 1690, 1620 cm^{-1}. ^{1}H-NMR (D_2O) δ: 7.37 (2H, d, J=8.8 Hz), 6.95 (2H, d, J=8.8 Hz), 5.88 (1H, J=2.2 Hz), 5.31 (1H, d, J=4.9 Hz), 5.09 (1H, s), 3.77—3.98 (1H, m), 2.29—2.47 (1H, m), 1.39—1.61 (1H, m), 0.93 (3H, d, J=7.1 Hz), 0.38—0.75 (1H, m).

Comparative Enzymatic Phenylglycylation of Various β-Lactam Nuclei A reaction mixture containing 3.2 mmol/l of racemic carbacephem compound **23** or 1.6 mmol of 6-APA or 7-ACA and 50 mmol/l of D-phenylglycine methyl ester was incubated with 5 g/l (as dry cell weight) of *Pseudomonas melanogenum* KY8541 in 1/30 M phosphate buffer (pH 6.5) at 30 °C for 10 min. The formation of phenylglycylated β-lactam compound was monitored by reversed phase HPLC and the results are shown in Table I.

Optical Resolution of Racemic Nucleus (23) a) Phenylacetylation (4*S,6*R**,7*S**)-7-Phenylacetamido-4-methyl-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (30b)** Phenylacetyl chloride (258.5 mg) was added to a stirred solution of **23** (399 mg, 1.28 mmol) and $NaHCO_3$ (432 mg, 5.14 mmol) in water (6.4 ml) and acetone (12.8 ml) under ice cooling. After 3 h, the reaction mixture was adjusted to pH 3 with 0.1 N HCl and extracted with EtOAc (10 ml × 5). The combined organic layer was washed with saturated NaCl, dried, and concentrated *in vacuo*. The residue was crystallized by addition of ether and filtered to obtain **30b** (254 mg). Then the water layer was extracted with EtOAc (10 ml × 10). The organic layers was combined, dried, and concentrated *in vacuo* to afford **30b** (49 mg). Total yield of the desired compound (**30b**) was 294 mg (72.6%). **30b**: IR (KBr): 1775, 1698, 1655 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 7.31 (5H, s), 6.48 (1H, d, J=5.0 Hz), 5.42 (1H, d, J=5.2 Hz), 3.85 (1H, m), 3.60 (2H, s), 2.62 (1H, m), 1.60 (2H, m), 1.10 (3H, d, J=7.5 Hz).

Compound **30a** was similarly obtained. mp 192.0—193.0 °C. IR (KBr): 1770, 1690, 1650, 1614 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 7.22 (5H, s), 6.40 (1H, t, J=4.0 Hz), 5.33 (1H, d, J=5.0 Hz), 3.57 (2H, s), 2.5—1.5 (9H, m).

b) Enzymatic Dephenylacetylation A disrupted cell suspension of *Kluyvera citrophila* KY7844 (200 mg as dry cell weight) was added to a solution of **30a** (200 mg) in 10 ml of 1/30 M phosphate buffer (pH 6.5) under shaking at 30 °C. After 80 min, the cell bodies were removed by centrifugation, then the supernatant was adjusted to pH 3.0 with 2 N HCl and separated by column chromatography (HP-10) with water. The desired fractions were concentrated *in vacuo* and lyophilized. The product was dissolved in a small amount of 50% aqueous MeOH. The solution was purified by column chromatography (Sephadex LH20 50% aqueous MeOH), concentrated to remove MeOH, and lyophilized to obtain **31a** (48 mg). **31a**: $[\alpha]_D^{15}$ +48.0° [c=0.5 in 1 M phosphate buffer solution (pH 7.0)]. Other physicochemical data coincided with those of the racemate **22a**.

Compound **31b** was obtained by the same procedure. **31b**: $[\alpha]_D^{15}$ −30.0° (c=0.5 in 1 M phosphate buffer). IR (KBr): 1800, 1770 (sh), 1760 (sh), 1740, 1680, 1630 cm^{-1}. ^{1}H-NMR (D_2O–DSS) δ: 6.16 (1H, d, J=5.1 Hz), 4.52 (1H, d, J=4.9 Hz), 3.86 (1H, m), 2.64 (1H, m), 1.9—1.4 (2H, m), 1.10 (3H, d, J=7.3 Hz).

(6*R*,7*S*)-7-[2-(2-Amino-4-thiazol)-2-(*syn*-methoxyiminoacetamido]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (32a) Phosphorus pentachloride (61.7 mg, 0.30 mmol) was added to a stirred solution of 2-(2-tritylamino-4-thiazoyl)-2-*syn*-methoxyiminoacetic acid (131.3 mg, 0.30 mmol) and triethylamine (4.1 ml, 0.30 mmol) in anhydrous THF (1 ml) at −20 °C. After 30 min, the reaction mixture was added dropwise to a solution of **31a** (40.2 mg, 0.17 mmol) in 50% aqueous THF (2 ml), and triethylamine (116 μl) was added portionwise to maintain pH 7.5. After 1 h, the reaction mixture was adjusted to pH 2.0 with 5% HCl and extracted with EtOAc (10 ml × 3). The organic layers were combined, washed with saturated NaCl, dried, and concentrated *in vacuo*. The residue was dissolved in 50% AcOH (10 ml) and stirred at 50 °C for 1.5 h. The solution was cooled to room temperature, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (Diaion HP 20). The fractions were eluted stepwise with water to 66% aqueous MeOH. Fractions showing a spot at *Rf* 0.3 (silica gel TLC, *n*-BuOH: AcOH: H_2O=4:1:1) were combined and concentrated *in vacuo* to obtain **32a** (13.5 mg, 22.4%) as white crystals. **32a**: mp 172 °C (dec.). $[\alpha]_D^{15}$ +32.6° (c=0.5, DMSO). Compound **32b** was prepared in the same manner. **32b**: mp 180 °C (dec.). $[\alpha]_D^{15}$ −27° (c=0.5, DMSO). IR (KBr): 1770, 1672, 1633, 1540 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 9.26 (1H, d, J=8.3 Hz), 7.18 (2H, s), 6.75 (1H, s), 6.31 (1H, d, J=5.1 Hz), 5.51 (1H, dd, J=8.3, 5.0 Hz), 3.83 (3H, s), 1.67 (2H, m), 1.07 (3H, d, J=7.3 Hz).

Determination of MICs MICs were determined by means of the usual twofold serial dilution method with Mueller Hinton agar (Difco). One loopful of diluted overnight culture in Mueller Hinton broth (Difco) of each test organism (about 10^6 colony-forming units per ml) was inoculated onto assay media containing graded concentration of the test drug. MICs were determined after incubation at 37 °C for 18 h. Type strains stocked in our laboratory were used for the experiments. S.m. T-26, P.mir. 1287, P.v. 6897, P.mor. 4298, P.ret. 4289 and P.a. #1 are standard strains and constitutive β-lactamase-producers.

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