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Synthesis and Biological Evaluation of an Electronically Activated Isooxacephem

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Abstract—New isooxacephem (±)-3-ethyl 2-hydrogen (6*RS*,7*RS*)-8-oxo-7-(phenylacetamido)-4-oxa-1-azabicyclo[4.2.0]oct-2-ene-2,3-dicarboxylate (**8**) was synthesized from (±)-dibenzyl 2-[*cis*-2-oxo-3-(phenylacetamido)-4-styryl-1-azetidinyl]-2-[*t*-butyldimethylsiloxy(methoxycarbonyl)methyl]malonate (**1**) in six steps. This bicyclic β-lactam was found to possess notable biological activities against several pathogenic microorganisms in vitro, including *Staphylococcus aureus* 95, *S. aureus* FDA 209P, *Escherichia coli* ATCC 39188, *Salmonella typhi* O-901, *Pseudomonas aeruginosa* 18S-H, *P. aeruginosa* 1101–75, and *Klebsiella pneumoniae* NCTC 418. The electronic activation of the β-lactam moiety by an ester group plays a prominent role in the biological activity of this novel isooxacephem.

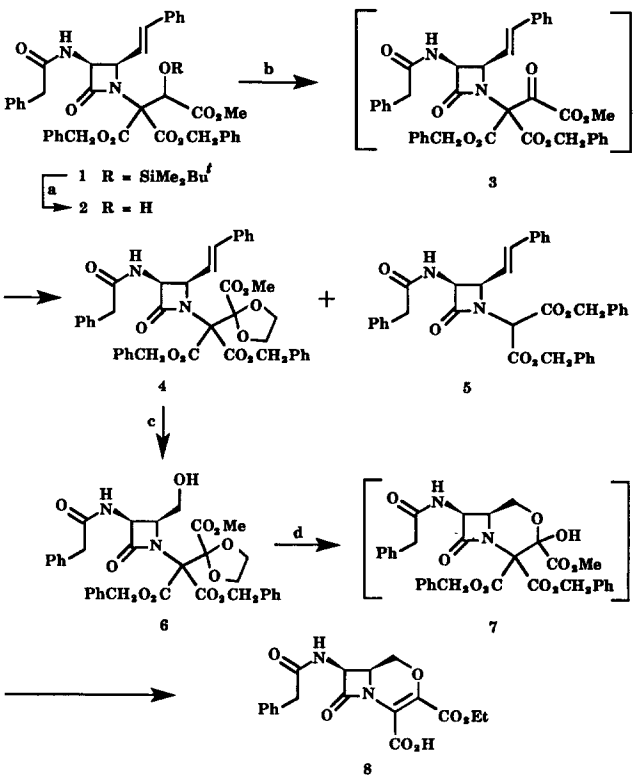
Introduction

Many β-lactams with a fused heterocyclic B-ring have been used in medicine and pharmacology.[1] Continuous efforts are needed in the development of new strategies for synthesis of novel β-lactams and in the exploration of structural modification for enhancement of their biological activities.

We synthesized a new isooxacephem (**8**) by utilizing the strategy involving construction of the 3,4-dihydro-2*H*-[1,4]oxazine ring (Scheme 1). We found that this isooxacephem possessed appealing antibacterial activity.

Results and Discussion

We desilylated silyloxy β-lactam (±)-**1**[2] with Bu_4NF in THF to give the corresponding alcohol **2** in 95% yield. Oxidation of **2** by use of 4-methylmorpholine *N*-oxide in the presence of a catalytic amount of tetrapropylammonium perruthenate[3] in CH_2Cl_2 afforded ketoester **3**. Upon treatment with a benzene solution of ethylene glycol and a catalytic amount of *p*-toluenesulfonic acid in situ, **3** was led to a mixture including the desired acetal **4** (23% overall yield) and the decarbonylated product **5**[4] (60% overall yield). Ozonolysis of **4** with an oxygen–ozone mixture in MeOH followed by reduction with $NaBH_4$ gave alcohol **6** in 10% yield only. We improved the yield to 46% by introducing ozone and nitrogen gases simultaneously;[5] the reaction mixture

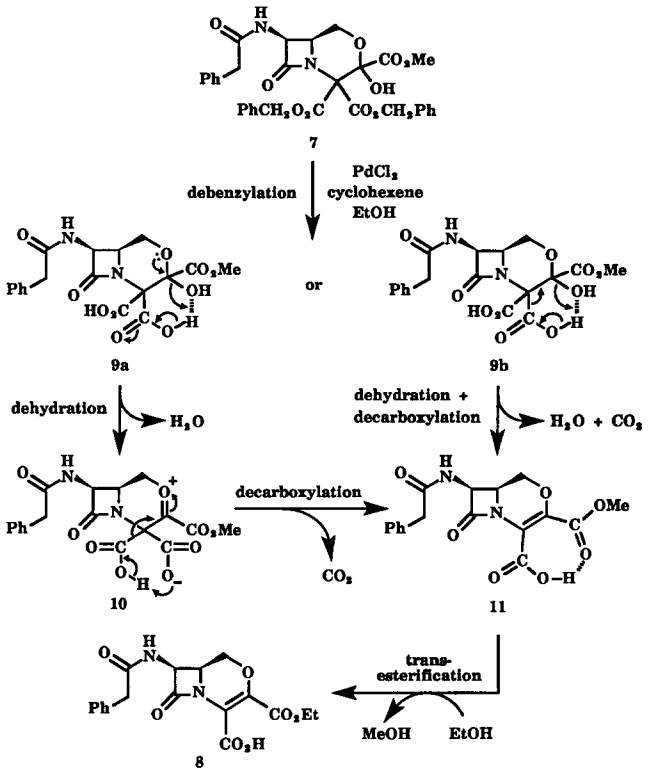


Scheme 1. (a) 95%: *n*-Bu_4NF, THF; (b) 83% overall yield for **2**→**3**→**4** (23% overall yield)+**5** (60% overall yield): (1) 4-methylmorpholine *N*-oxide, tetrapropylammonium perruthenate, CH_2Cl_2; (2) $HOCH_2CH_2OH$, *p*-TsOH, C_6H_6; (c) 46%: (1) O_3, N_2, CH_2Cl_2; (2) Me_2S; (3) $NaBH_4$–Al_2O_3; (d) 27% overall yield for **6**→**7**→**8**: (1) 95% aq CF_3CO_2H; (2) $PdCl_2$, cyclohexene, EtOH.

Key words: β-lactam, isooxacephem, antibiotic, antimicrobial, [1,4]oxazine.

was then treated with Me_2S and $NaBH_4$-impregnated alumina[6] in CH_2Cl_2. Hydrolysis of acetal **6** with 95% of aqueous CF_3CO_2H afforded lactol **7**, which was subsequently treated with $PdCl_2$ and cyclohexene in ethanol[7–10] to give the final product **8** as a racemic mixture in 27% overall yield.

We propose a mechanism for the conversion of lactol **7** to isooxacephem **8** in Scheme 2. Transformation of **7** to **8** by our 'one-flask' method involved debenzylations, dehydration, decarboxylation, and transesterification. After debenzylations occurred in **7**, the internal acid-catalysed dehydration of **9a** took place through a six-membered ring intermediate with anchimeric assistance from the lone pair electrons of the oxygen atom in the 3,4-dihydro-2*H*-[1,4]oxazine ring. Promoted by the oxonium center, the resultant intermediate **10** underwent decarboxylation to give ester **11**. Transesterification of **11** with the solvent, ethanol, produced the desired product **8**. Alternatively, the conversion of **7** to **8** may be achieved via intermediate **9b** through a concerted mechanism, involving simultaneous dehydration and decarboxylation (Scheme 2). The absence of regioisomeric carboxylic acid **13** in the final product indicates that the alternative route leading **11** to **8** via anhydride **12** did not take place (see Scheme 3).

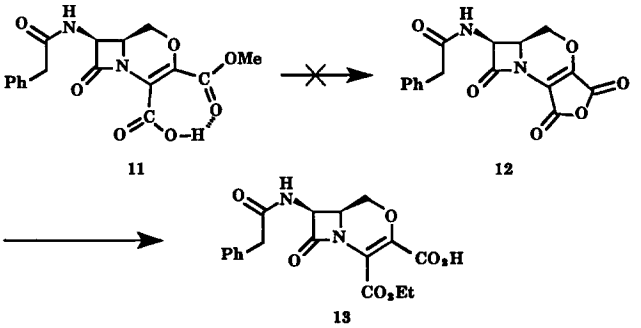


Scheme 2.

Biological activity of isooxacephem (8)

We tested the biological activity of the synthesized β-lactam (±)-**8** as well as naturally occurring penicillin (+)-**14** and reference cephalosporins (+)-**15**,[11,12] (+)-**16**,[11] and (+)-**17**[13,14] in vitro against several pathogenic microorganisms up to a level as high as 128 μg/mL (Table 1).[15] Our synthetic β-lactam **8** was in racemic form, whereas the comparative compounds (+)-**14**, (+)-**15**, (+)-**16**, and (+)-**17** are single enantiomers.[16] Thus, only one-half of the minimal inhibitory concentration may be necessary for the desired single enantiomer of **8**.

Isooxacephem **8** bearing an ethoxycarbonyl group exhibited notable antimicrobial activity. This might indicate that the electronic activation of the β-lactam moiety by an electron-withdrawing group (e.g., an ester functionality) plays an important role in the biological activity of bicyclic β-lactams.[17,18] In comparison with the reference penicillin and cephalosporins currently used in medicine, the novel isooxacephem **8** exhibited much stronger activity against methicillin-resistant *S. aureus* 95 and β-lactamase producer *P. aeruginosa* 18S-H (see Table 1).[19,20]

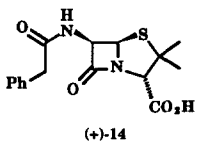


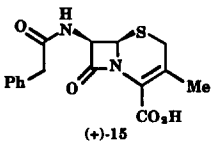
Scheme 3.

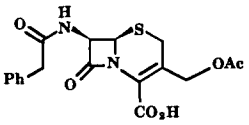
Table 1. Minimal inhibitory concentrations[a] (μg/mL) of β-lactams (±)-**8**, (+)-**14**, (+)-**15**, (+)-**16**, and (+)-**17**

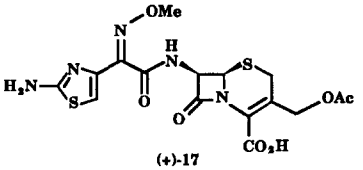
Microorganisms	(±)-**8**	(+)-**14**	(+)-**15**	(+)-**16**	(+)-**17**
S. aureus 95[b,c]	6.92	>128	>128	>128	38.00
S. aureus FDA 209P	0.02	0.40	0.64	0.72	0.08
E. coli ATCC 39188	0.37	2.30	13.13	98.95	0.25
S. typhi O-901	2.50	>128	24.50	25.32	20.30
P. aeruginosa 18S-H[c]	18.80	>128	>128	>128	128
P. aeruginosa 1101-75	15.00	>128	100.00	105.30	62.35
K. pneumoniae NCTC 418	0.27	>128	2.98	15.68	10.25

[a] They were obtained by use of a serial broth dilution method and represent the average of triplicate determinations.
[b] Methicillin-resistant.
[c] β-Lactamase producer.


(+)-14


(+)-15


(+)-16


(+)-17

Experimental

General

Chemicals were purchased from Fluka Chemical Co. Reagent-grade solvents were distilled and then stored over molecular sieves 4 Å. Products were isolated by use of column chromatography (Merck silica gel 60, particle size 230–400 mesh ASTM) packed in glass column (35 g of silica gel/gram of crude material). Analytical TLC analyses were performed on precoated plates (silica gel 60 F_{254}) purchased from Merck. Melting points were obtained by a Büchi 510 apparatus. Proton NMR spectra were obtained on a Bruker WH-90 or a Varian XL-200 spectrometer. IR spectra were measured on a Beckman-IR-8 spectrophotometer. Microanalysis data were obtained by use of a Perkin–Elmer 240B microanalyzer.

(±)-Dibenzyl 2-[*cis*-2-oxo-3-(phenylacetamido)-4-styryl-1-azetidinyl]-2-[hydroxy(methoxycarbonyl)methyl]-malonates (diastereomeric mixture; 2). To a solution of **1**[2] (0.79 g, 1.0 mmol) in THF (35 mL) was added *n*-Bu_4NF (1.0 M solution in THF, 1.0 mL, 1.0 mmol) at 0 °C in portions within 3 min. The solution was stirred at the same temperature for 1 h and was then partitioned between Et_2O (30 mL) and aq KH_2PO_4 buffer soln (pH 4.5, 30 mL). The ethereal layer was dried over $MgSO_4$ (s) and condensed under reduced pressure. The residue was purified by use of column chromatography (EtOAc as the eluant) to give **2** (0.64 g, 0.95 mmol) as a foam in 95% yield: ^{1}H NMR ($CDCl_3$): δ 3.49, 3.51 (2 s, 2H, CH_2CO), 3.65–3.66 (2 s, 3H, CH_3O), 4.21 (br, 1H, OH, exchanged with D_2O), 4.99 (br s, 1H, CH), 5.01–5.30 [m, 5H, $2\times CH_2O + HC(4)$], 5.59 [dd, 1H, $J=5.0$ and 8.5 Hz, HC(3)], 6.22–7.01 (m, 2H, CH=CH), 7.20–7.51 (m, 21H, $4\times C_6H_5 + NH$); IR (CH_2Cl_2) ν_{max}: 3350–3420 (NH, OH), 1778–1745 (β-lactam, ester), 1681 (amide) cm^{-1}. Anal. calcd for $C_{39}H_{36}N_2O_9$: C, 69.22; H, 5.36; N, 4.14. Found: C, 69.12; H, 5.28; N, 4.21.

(±)-Dibenzyl 2-[*cis*-2-oxo-3-(phenylacetamido)-4-styryl-1-azetidinyl]-2-[ethyleneketal(methoxycarbonyl)methyl]-malonate (4). To a solution of **2** (676 mg, 0.999 mmol) in CH_2Cl_2 (35 mL) containing molecular sieves 4 Å (2.00 g) and 4-methylmorpholine *N*-oxide (175 mg, 1.49 mmol) was added tetrapropylammonium perruthenate (87.7 mg, 0.250 mmol) at room temperature. The resultant green mixture was stirred for 2 h. Then the reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with 5% aq Na_2SO_3 soln (40 mL), brine (30 mL), and 3% aq $CuSO_4$ soln (40 mL). The organic layer was dried over $MgSO_4$ (s), filtered, and condensed under reduced pressure. The residue containing **3** was dissolved in dry benzene (50 mL) containing ethylene glycol (101 mg, 16.2 mmol) and a catalytic amount of *p*-toluenesulfonic acid. The solution was heated at 80 °C for 15 h and the water generated was removed by a Dean–Stark trap. The solution was then cooled, dried over $MgSO_4$ (s), filtered, and condensed under reduced pressure. After purification by use of a column chromatography ($CHCl_3$ followed by $CHCl_3$:EtOAc, 1:1, as the eluant), **4** (165 mg, 0.230 mmol, 23% yield) and **5**[4] (353 mg, 0.599 mmol, 60% yield) were obtained separately as a foam.

For **4**: ^{1}H NMR ($CDCl_3$): δ 3.55 (s, 2H, CH_2CO), 3.71–4.18 (m, 7H, CH_3 + OCH_2CH_2O), 5.00–5.29 [m, 5H, $2\times CH_2O + HC(4)$], 5.52 [dd, 1H, $J=5.0$ and 9.0 Hz, HC(3)], 6.12–6.95 (m, 3H, CH=CH+NH), 7.02–7.46 (m, 20H, $4\times C_6H_5$); IR (CH_2Cl_2) ν_{max}: 3405 (NH), 1779–1750 (β-lactam, ester), 1680 (amide) cm^{-1}. Anal. calcd for $C_{41}H_{38}N_2O_{10}$: C, 68.51; H, 5.33; N, 3.90. Found: C, 68.40; H, 5.31; N, 4.01.

(±)-Dibenzyl 2-[*cis*-4-hydroxymethyl-2-oxo-3-(phenylacetamido)-1-azetidinyl]-2-[ethyleneketal(methoxycarbonyl)methyl]malonate (6). β-Lactam **4** (718 mg, 0.999 mmol) in CH_2Cl_2 (70 mL) was saturated with N_2 gas at −78 °C. Then a mixed O_3 and N_2 gas was bubbled in until KI starch paper turned dark blue, which indicated the presence of excess O_3. The excess O_3 was removed by a stream of N_2 gas. Then Me_2S (620 mg, 9.98 mmol) was added at −30 °C. After 1 h, $NaBH_4$:Al_2O_3 (1:10 g/g) was added and the reaction mixture was stirred at room temperature for 1 h. Then it was filtered and condensed under reduced pressure. After purification by use of a column chromatography (EtOAc as the eluant), **6** (297 mg, 0.460 mmol) was obtained as a foam in 46% yield: ^{1}H NMR ($CDCl_3$): δ 3.50 (s, 2H, CH_2CO), 3.70–5.40 (m, 15H, $CH_3 + OCH_2CH_2O + CHCH_2OH + 2\times CH_2O$), 5.58 [dd, 1H, $J=5.0$ and 9.0 Hz, HC(3)], 6.92 (br, 1H, NH), 7.00–7.42 (m, 15H, $3\times C_6H_5$); IR (CH_2Cl_2) ν_{max}: 3300–3410 (NH, OH), 1778–1740 (β-lactam, ester), 1680 (amide) cm^{-1}. Anal. calcd for $C_{34}H_{34}N_2O_{11}$: C, 63.15; H, 5.30; N, 4.33. Found: C, 63.20; H, 5.29; N, 4.48.

(±)-3-Ethyl 2-hydrogen (6*RS*,7*RS*)-8-oxo-7-(phenylacetamido)-4-oxa-1-azabicyclo[4.2.0]oct-2-ene-2,3-dicarboxylate (8). Hydroxyl acetal **6** (2.58 g, 3.99 mmol) was dissolved in 95% aq CF_3CO_2H (50 mL) and the solution was stirred at room temperature for 7 h. It was then poured into a mixture of crushed ice and brine, and then was extracted with EtOAc. After the organic solution was washed with water (2×40 mL) and dried over $MgSO_4$ (s), the solvent was removed to afford **7** as an oil. The crude **7** was then dissolved in EtOH (60 mL), to which were added $PdCl_2$ (400 mg,

2.26 mmol) and cyclohexene (45 mL). The mixture was heated at reflux for 6 h, cooled, filtered, and the residue was washed with 80% aq EtOH (2 × 20 mL). The combined filtrates and washings were condensed to dryness under reduced pressure. After purification by use of column chromatography (EtOAc:MeOH, 9:1, as the eluant), **8** (403 mg, 1.08 mmol) was obtained in 27% yield: mp 138–140 °C (from ether); ^{1}H NMR (DMSO-d_6/$CDCl_3$/D_2O): δ 1.34 (t, 3H, J = 7.0 Hz, CH_3), 3.50 (s, 2H, CH_2CO), 3.45–3.89 [m, 2H, $H_2C(5)$], 4.20 (q, 2H, J = 7.0 Hz, CH_2O), 4.42 [m, 1H, HC(6)], 5.01 (*d*, 1H, J = 5.0 Hz, CHND), 7.40 (s, 5H, C_6H_5); IR (CH_2Cl_2) ν_{max}: 3050–3652 (COOH, NH), 1792 (β-lactam), 1748 (ester), 1715, 1708 (acid, C=C), 1680 (amide) cm^{-1}. Anal. calcd for $C_{18}H_{18}N_2O_7$: C, 57.75; H, 4.85; N, 7.48. Found: C, 57.68; H, 4.98; N, 7.34.

Antimicrobial activity tests

The serial broth dilution method was used to study the antibiotic activity.[15] The inocula were prepared by use of the heart infusion broth (Difco Laboratories) to make 10^{-4} dilutions of the overnight cultures. Tubes of the seeded antibiotic-containing media were incubated at 37 °C for 20 h. The lowest concentration of antibiotic that prevented visible growth of microorganisms was then determined.

Acknowledgments

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