141. Syntheses of Novel Isopenam and Isocephem Antibiotics. Preparation of a Retinamido Derivative of a Highly Strained β-Lactam as Potent Anticancer Agent

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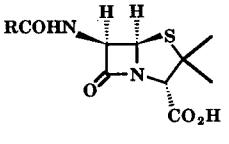
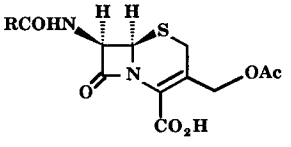
Syntheses of the *cis*-configurated isopenam **9** (*Scheme 1*), isocephem **14** (*Scheme 2*), and isocephem **19** (*Scheme 3*) are described. The key step in the preparation of **14** and **19** involved a *Pummerer*-type rearrangement of the corresponding sulfoxides **12** and **18.** These β-lactams were found to possess biological activity against several pathogenic microorganisms *in vitro*. The electronic activation of the lactam moiety of **19** remarkably enhanced its biological activity. A retinoic moiety was attached to **19** *via* an amino linker. The resultant retinamido-β-lactam **21** showed significant cytostatic activity in tracheal organ cultures obtained from vitamin-A-deficient hamsters.

Introduction. – Essential features of the classical β-lactam antibiotics penicillin (**I**) and cephalosporin (**II**) include *a*) a *cis*-fused β-lactam ring, *b*) an acylamino side chain, which can be varied considerably, *c*) an acidic function, and *d*) a five-membered ring or a six-membered ring containing a double bond conjugated with the β-lactam N-atom, thus conferring enough ring strain to raise the β-lactam frequency in the IR spectrum to $\geqslant 1765$ cm^{-1}. The S-atom, however, can be replaced by an O- or a C-atom without substantial loss of antimicrobial activity [1]. The IR absorption frequency of the carbonyl group of a β-lactam can also be considered as a measure of its reactivity towards nucleophilic attack [2]. Therefore, higher frequency might indicate the potential for higher biological activity.



I



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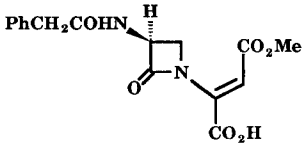


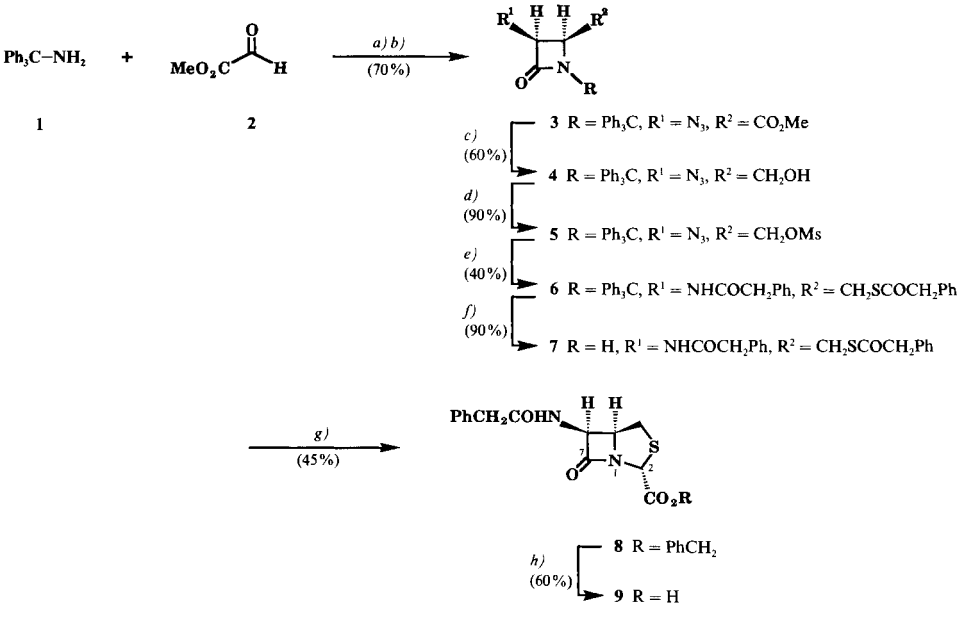
III

The syntheses of several monocyclic analogues of β-lactam antibiotics were reported [3] [4], in which the ring strain of fused β-lactams was replaced by an electron-withdrawing group (*e.g.* **III**). Being susceptible to nucleophilic attack, β-lactam **III** does not exhibit antimicrobial activity. Therefore, the enamine fragment might have to be prevented from being coplanar with the β-lactam nucleus for biological activity. Because fused β-lactams

meet this requirement, we prepared isopenam **9** (*Scheme 1*) and isocephem **14** (*Scheme 2*). We also report a synthesis of isocephem **19** (*Scheme 3*) in which the lactam moiety is activated electronically by an ester function. Furthermore, we attached a retinoic-acid moiety to **19** *via* an amino linker to afford compound **21** (*Scheme 4*), which exhibited anticancer activity.

Results and Discussion. – We treated trityl amine (**1**) with methyl glyoxylate (**2**) to produce the corresponding *Schiff* base, which upon reaction with azidoacetyl chloride gave β-lactam **3** in 70% yield. The coupling constant (5.0 Hz) of the two H-atoms on the β-lactam ring indicated the *cis*-relationship of the two substituents [5] [6]. Reduction of the methoxycarbonyl group of **3** with $NaBH_4$ in wet THF gave alcohol **4** (60%) [7] [8], which was mesylated to afford **5** in 90% yield [9].

Scheme 1



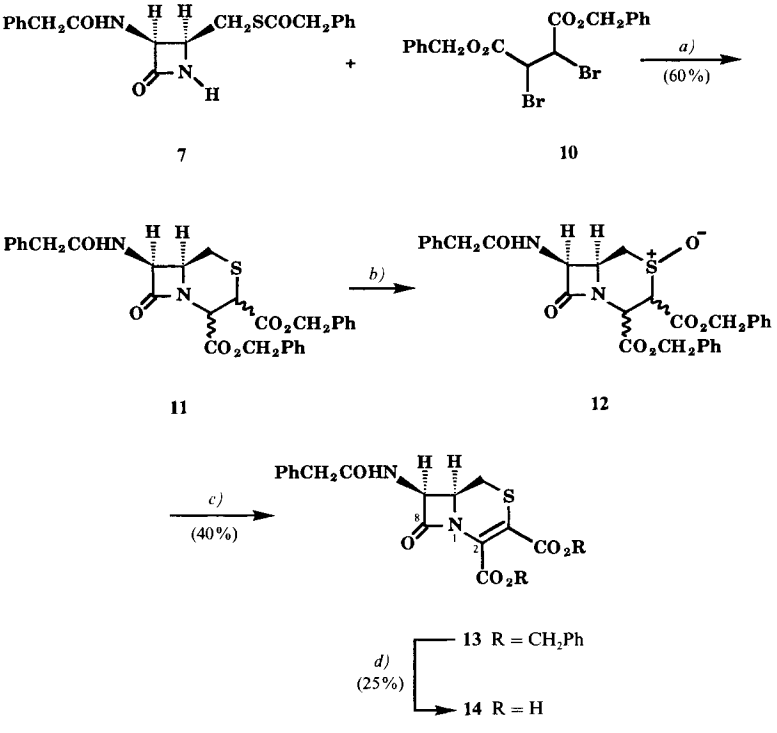
a) *Dean-Stark* trap, benzene. *b*) N_3CH_2COCl, Et_3N. *c*) $NaBH_4$ wet THF. *d*) $MeSO_2Cl$, Et_3N. *e*) $KSCOCH_2Ph$, butanone. *f*) CF_3CO_2H, $KClO_4$. *g*) $Br_2CHCO_2CH_2Ph$, piperidine. *h*) Pd/C, EtOH.

A novel effect of KSAc was reported [10] [11], achieving the one-pot reduction/acylation of the azide function to the amide group. By this method and using $KSCOCH_2Ph$, we obtained the *cis*-configurated phenylacetamide **6** in 40% yield from **5**. Removal of the trityl group from the N-atom of the β-lactam ring of **6** was more difficult than the corresponding detritylation of ordinary amides, amines, ethers, and esters [12] [13], probably because of the spatial arrangement of the lone-pair electrons of the β-lactam N-atom. We found that the addition of a trace of $KClO_4$ significantly accelerated the rate of deprotection of **6** with CF_3COOH at 25°, giving **7** in 90% yield after 1 h instead of 30 h;

this rate enhancement may be attributed to the special salt effect that caused the rate of ionization of the trityl function equal the rate of product formation [14]. Monocyclic β-lactam **7** was then treated with benzyl dibromoacetate in the presence of piperidine in boiling *t*-BuOH to give **8** (45%). Hydrogenolysis of **8** with Pd/C in EtOH at 45 psi afforded isopenam **9** (60% yield).

Isocephem **14** was obtained from the key intermediate **7**, which was reacted with dibenzyl 2,3-dibromosuccinate (**10**) and piperidine in DMF to give a diastereoisomeric isocepham mixture **11** in 60% overall yield. Treatment of **11** with 3-chloroperbenzoic acid (3-$ClC_6H_4CO_3H$) yielded the corresponding sulfoxide **12**. Reaction of **12** with AcCl and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded isocephem **13** (40% overall yield from **11**). In the presence of Pd/C in EtOH at 45 psi, **13** was resistant to hydrogenolysis. By using $PdCl_2$ as the catalyst, we were able to achieve the reductive cleavage to isocephem **14** in 25% yield.

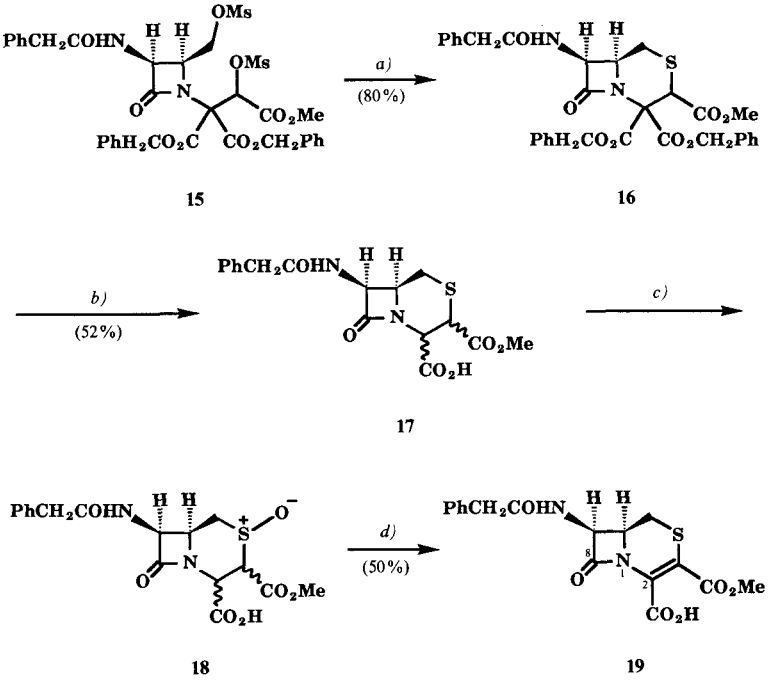
Scheme 2



a) Piperidine/DMF, 80°. *b*) 3-$ClC_6H_4CO_3H$, CH_2Cl_2, 0°. *c*) AcCl, DBU. *d*) $PdCl_2$, AcOEt.

We started our synthesis of isocephem **19** with β-lactam **15** [4] [15] (*Scheme 3*). Reaction of **15** with bis(trimethyltin) sulfide and Bu_4NF afforded isocepham **16** as a mixture of two diastereoisomers in 80% overall yield. Hydrogenolysis of **16** with Pd/C in MeOH containing 1% aq. $NaHCO_3$ solution at 60 psi followed by decarboxylation gave a diastereoisomeric mixture **17** of mono-carboxylic acids (52%) upon acidification with

Scheme 3



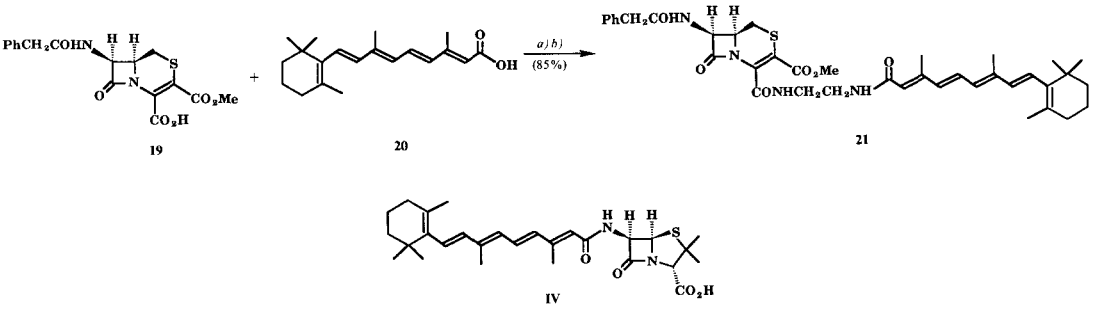
15 16 17 18 19

a) $(Me_3Sn)_2S$, Bu_4NF. *b*) Pd/C, MeOH/1% aq. $NaHCO_3$ soln., 60 psi. *c*) 3-$ClC_6H_4CO_3H$, THF, 0°. *d*) AcCl, DBU.

AcOH. Treatment of **17** with 3-$ClC_6H_4CO_3H$ gave the sulfoxide intermediate **18**, which reacted with AcCl and DBU *in situ* to **19** through a *Pummerer*-type rearrangement. This one-pot conversion of **17** to isocephem **19** was accomplished in 50% overall yield.

Recently, we reported antileukemic effects of some azetidinone derivatives which contain a retinoic-acid chain such as **IV** [16] [17] (see *Scheme 4*). Retinoids exhibit

Scheme 4



19 20 21 IV

a) For **19**: 1. ClCOOEt, pyridine; 2. $H_2N(CH_2)_2NH_2$. *b*) For **20**: ClCOOEt, pyridine.

anticancer properties for prophylaxis and can differentiate leukemic cells [18] [19]. On the other hand, β-lactams may act in a similar manner to alkylating agents to inhibit proliferation of the rapidly growing cells [20]. Therefore, we considered to combine a β-lactam with retinoic acid to give a new compound, which may have a special affinity for rapidly growing cells and thus may act as an effective anticancer agent. To this end, isocephem **19** was treated with ethyl chloroformate in pyridine to give the corresponding anhydride which upon reaction with ethylenediamine (amino linker) afforded an amide (*Scheme 4*). Retinoic acid (**20**) was also reacted with ethyl chloroformate in pyridine, and the resultant anhydride was added to the amide obtained from **19** to give retinamido-β-lactam **21** in 85% yield. Lactam **21** was stable under neutral conditions at 25–37° for one month.

Biological Activity. – Lactams **9**, **14**, **19**, and **21** as well as **IV**, ampicillin, cloxacillin, and penicillin G were tested *in vitro* against five pathogenic microorganisms up to level as high as 128 μg/ml. The results are summarized in *Table 1*. The β-lactam moiety in compounds **III** and **14** is activated electronically by a C=C–COOR functionality. In

Table 1. *Minimal Inhibitory Concentration* [μg/ml]

	S. aureus	*E. coli*	*S. typhi*	*Ps. aeruginosa*	*K. pneumonia*
Isopenam **9**	0.10	0.76	35.00	[a])	[a])
Isocephem **14**	0.85	13.00	26.00	50.00	20.00
Isocephem **19**	0.07	0.65	1.50	13.00	2.15
Retinoid **21**	[a])	[a])	[a])	[a])	[a])
Retinoid **IV**	30.00	20.00	13.00	80.00	50.00
Ampicillin	0.33	2.51	[a])	[a])	[a])
Cloxacillin	0.18	1.70	[a])	[a])	[a])
Penicillin G	0.40	2.30	[a])	[a])	[a])

[a]) Not active up to 128 μg/ml.

contrast to the freedom offered by the monocyclic β-lactam in **III**, the π-electrons of the C=C bond of the strained, bicyclic β-lactam **14** cannot not be aligned perfectly with the unshared electron pair of the N-atom. This discrepancy can account for the biological-activity difference between monocyclic and bicyclic β-lactams **III** and **14** (no antimicrobial activity of **III**). Results from our biological tests revealed the pronounced antimicrobial effect of β-lactams **9** and **14**. Consequently, we conclude that the electronic activation of β-lactams, although important, is not enough to secure the antibacterial effect of the compounds. However, the profound antimicrobial effect of the highly strained isocephem **19**, with respect to **14**, indicates that the electronic activation of the β-lactam moiety by an electron-withdrawing group plays an important role in biological activity of bicyclic β-lactams.

We studied the carcinostatic property of retinoid **21**, which was evaluated according to its ability to inhibit squamous metaplasia and keratinization in organ cultures of trachea derived from vitamin-A-deficient hamsters [21] [22]. Organ cultures were used according to a method described previously [22]. We found that retinoid **21** was more active than β-retinoic acid (**20**) and **IV** (*Table 2*). The potent carcinostatic property of retinoid **21** might also reflect the ring strain of β-lactam.

Table 2. *Activity of Retinoids in Tracheal Organ Cultures Obtained from Vitamin-A-Deficient Hamsters*

Retinoid	**21**	**IV**	β-Retinoic acid (**20**)
ED_{50}[a]) [M]	$1.0 \cdot 10^{-11}$	$7.8 \cdot 10^{-11}$	$1.15 \cdot 10^{-10}$

[a]) Mean effective doses (ED_{50}) for the reversal of keratinization of 50% of the explants, as determined by probit analysis [23] [24].

Furthermore, we determined the LD_{50} of the biologically active compounds in rats: thus, β-lactam **19** was administered at different doses intravenously (*i.v.*) and retinoid **21** subcutaneously (*s.c.*). Compounds **19** and **21** did not show any toxicity at concentration levels as high as 500 and 100 mg/kg, respectively. All rats were controlled and were in good conditions after 6 months of administration. Nevertheless, an LD_{50} (*i.v.*) of *ca.* 800 mg/kg was determined for isocephem **19** and an LD_{50} (*s.c.*) of 160 mg/kg for retinamido-β-lactam **21**.

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Experimental Part

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 column chromatography (CC; *Merck* silica gel *60* (230–400 mesh), packed in glass column (20 g of silica gel/g of crude material)). TLC: *Merck* silica gel *60 F 254* anal. sheets. M.p.: *Büchi 510*. IR Spectra: *Beckman-IR-8* spectrophotometer. ^{1}H-NMR Spectra: *Bruker-WH-90*, *Varian-XL-200*, and *Varian-T-60A* spectrometers.

Methyl cis-*3-Azido-4-oxo-1-tritylazetidine-2-carboxylate* (**3**). To a soln. of trityl amine (**1**; 2.28 g, 0.01 mol) in dry benzene (350 ml) was added methyl glyoxylate (**2**; 4.5 g, 0.05 mol) in portions within 8 h at 80° (*Dean-Stark* trap). After H_2O was all removed (*ca.* 10 h), the soln. was cooled, anh. $MgSO_4$ added, the mixture filtered after 2 h, and the filtrate evaporated. Then dry CH_2Cl_2 (150 ml) and Et_3N (2.02 g, 0.02 mol) were added, followed by dropwise addition of azidoacetyl chloride (1.20 g, 0.01 mol) at reflux temp. After stirring at the same temp. for 7 h, the soln. was washed with H_2O, dried ($MgSO_4$), and evaporated. The crude product was purified by CC (silica gel, CH_2Cl_2): **3** (2.7 g, 70%). Oil. IR (CH_2Cl_2): 2100 (N_3), 1778 (β-lactam), 1750 (ester). ^{1}H-NMR ($CDCl_3$): 3.81 (*s*, Me); 4.51 (*d*, *J* = 5, H–C(3)); 4.89 (*d*, *J* = 5, H–C(4)); 7.23 (br. *s*, 3 Ph). Anal. calc. for $C_{24}H_{20}N_4O_3$ (412.22): C 69.90, H 4.85, N 13.59; found: C 69.83, H 4.81, N 13.50.

cis-*3-Azido-4-(hydroxymethyl)-1-tritylazetidin-2-one* (**4**) was obtained from **3** in 60% yield as described in [7] [8]. IR (CH_2Cl_2): 3300–3370 (OH), 2100 (N_3), 1770 (β-lactam). ^{1}H-NMR ($CDCl_3$): 3.25–3.50 (*m*, CH_2OH); 3.71–4.20 (*m*, H–C(4)); 4.43 (*d*, *J* = 5, H–C(3)); 7.31 (br. *s*, 3 Ph). Anal. calc. for $C_{23}H_{20}N_4O_2$ (384.12): C 71.87, H 5.21, N 14.58; found: C 71.80, H 5.11, N 14.60.

cis-*3-Azido-4-[(mesyloxy)methyl]-1-tritylazetidin-2-one* (**5**) was prepared from **4** in 90% yield according to [9]. IR (CH_2Cl_2): 2100 (N_3), 1777 (β-lactam). ^{1}H-NMR ($CDCl_3$): 2.80 (*s*, $MeSO_3$); 3.66 (br., $MsOCH_2$); 4.10–4.33 (*m*, H–C(4)); 4.49 (*d*, *J* = 5, H–C(3)); 7.20 (*s*, 3 Ph). Anal. calc. for $C_{24}H_{22}N_4O_4S$ (462.32): C 62.34, H 4.76, N 12.12; found: C 62.31, H 4.77, N 12.21.

S-[cis-*4-Oxo-3-(phenylacetamido)-1-tritylazetidine-2-methyl] (Phenyl)thioacetate* (**6**). To a soln. of **5** (4.62 g, 0.01 mol) in butanone (100 ml) was added $KSCOCH_2Ph$ (7.64 g, 0.04 mol). The mixture was heated at reflux for 24 h, the solvent evaporated, and the resultant red-brown syrup taken up in AcOEt and washed with H_2O (3×). The org. layer was dried ($MgSO_4$), filtered, and evaporated. CC (silica gel, $CHCl_3$) gave **6** (2.4 g, 40%). Foam. IR (CH_2Cl_2): 3410 (NH), 1780 (β-lactam), 1730 (thioester), 1681 (amide). ^{1}H-NMR ($CDCl_3$): 3.11–3.33 (*m*, CH_2S); 3.39 (*s*, CH_2COS); 3.43 (*s*, CH_2CO); 3.90–4.21 (*m*, H–C(2)); 5.27 (*dd*, *J* = 5, 9, H–C(3)); 6.81 (br., NH); 6.82–7.75 (*m*, 5 Ph). Anal. calc. for $C_{39}H_{34}N_2O_3S$ (610.42): C 76.72, H 5.57, N 4.59; found: C 76.71, H 5.52, N 4.60.

S-[cis-*4-Oxo-3-(phenylacetamido)azetidine-2-methyl] (Phenyl)thioacetate* (**7**). To a soln. of **6** (3.0 g, 0.005 mol) in CF_3COOH (30 ml), a trace amount of $KClO_4$ was added and the soln. stirred at 25° for 1 h. Evaporation

and CC (silica gel, AcOEt) gave 1.60 g (90%) of **7**. Foam. IR (CH_2Cl_2): 1763 (β-lactam), 1720 (thioester), 1680 (amide). ^{1}H-NMR ($CDCl_3$): 3.01–3.42 (*m*, CH_2S); 3.41 (*s*, CH_2COS); 3.42 (*s*, CH_2CO); 4.10–4.31 (*m*, H–C(2)); 5.32 (*dd*, J = 4.5, 9, H–C(3)); 6.70–6.82 (br., 2 NH); 7.20, 7.31 (2*s*, 2 Ph). Anal. calc. for $C_{20}H_{20}N_2O_3S$ (368.32): C 65.22, H 5.43, N 7.61; found: C 65.19, H 5.43, N 7.57.

*Benzyl (2*RS,*5*SR,*6*SR*)-7-Oxo-6-(phenylacetamido)-3-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate* (**8**). To a soln. of **7** (3.68 g, 0.01 mol) in *t*-BuOH (50 ml) containing benzyl dibromoacetate (3.1 g, 0.01 mol), piperidine (8.50 g, 0.1 mol) was added and the soln. heated at reflux for 2 h. The solvent was evaporated under reduced pressure and the residue taken up in AcOEt and washed with 1% aq. HCl soln. (2×) and H_2O. The org. layer was dried ($MgSO_4$), filtered, and evaporated. CC (silica gel, $CHCl_3$) afforded **8** (1.80 g, 45%). Foam. IR (CH_2Cl_2): 3415 (NH); 1783 (β-lactam), 1742 (ester), 1670 (amide). ^{1}H-NMR ($CDCl_3$): 2.77–3.21 (*m*, CH_2S); 3.52 (*s*, CH_2CO); 4.32–4.58 (*m*, H–C(5)); 5.01 (*s*, CHCOO); 5.20 (*s*, CH_2O); 5.23–5.59 (*dd*, J = 5, 9.5, H–C(6)); 6.80–7.00 (br., NH); 7.31 (br. *s*, 2 Ph). Anal. calc. for $C_{21}H_{20}N_2O_4S$ (396.22): C 63.63, H 5.05, N 7.07; found: C 63.60, H 5.13, N 7.13.

*(2*RS,*5*SR,*6*SR*)-7-Oxo-6-(phenylacetamido)-3-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid* (**9**). A mixture of (1.32 g, 0.0033 mol), EtOH (60 ml), and 10% Pd/C (20 mg) was hydrogenated at 25° and 45 psi for 2 h, during which the pressure dropped to 41 psi. The soln. was then filtered and evaporated to give 0.70 g (70%) of **9**, m.p. 122–126°. CC (silica gel, AcOEt) gave 60% of **9**. M.p. 141–143°. IR (nujol): 3300–3500 (NH, COOH), 1779 (β-lactam), 1705 (acid), 1669 (amide). ^{1}H-NMR ((D$_6$)DMSO): 2.72–3.12 (*m*, CH_2S); 3.50 (*s*, CH_2CO); 4.21–4.28 (*m*, H–C(5)); 4.88 (*s*, CHCOO); 5.02–5.31 (*dd*, J = 4.5, 9, H–C(6)); 6.91 (br., NH); 7.38 (*s*, Ph); 7.80–8.50 (br., COOH). Anal. calc. for $C_{14}H_{14}N_2O_4S$ (306.14): C 54.90, H 4.57, N 9.15; found: C 55.01, H 4.48, N 9.18.

*Dibenzyl (6*RS,*7*RS*)-8-Oxo-7-(phenylacetamido)-4-thia-1-azabicyclo[4.2.0]octane-2,3-dicarboxylate* (**11**). To a soln. of **7** (3.68 g, 0.01 mmol) in DMF (40 ml), dibenzyl 2,3-dibromosuccinate (**10**; 4.56 g, 0.01 mol) and piperidine (8.5 g, 0.1 mol) were added. The soln. was heated at 80° for 3.5 h, then cooled, and after addition of AcOEt, the mixture was washed with H_2O (3×), dried ($MgSO_4$), and evaporated and the residue purified by CC ($CHCl_3$): 3.26 g (60%) of **11** as an oily mixture of diastereoisomers. IR (CH_2Cl_2): 3421 (NH), 1770 (β-lactam), 1735 (ester), 1680 (amide). ^{1}H-NMR ($CDCl_3$): 2.81–3.32 (*m*, CH_2S); 3.50, 3.52 (2*s*, CH_2CO); 3.71–3.95 (*m*, H–C(3)); 4.36–4.68 (*m*, H–C(2), H–C(6)); 5.01, 5.03, 5.20, 5.21 (4*s*, 2 CH_2O); 5.30–5.72 (*m*, H–C(7)); 6.85–7.23 (br., NH); 7.45 (br. *s*, 3 Ph). Anal. calc. for $C_{30}H_{28}N_2O_6S$ (544.62): C 66.15, H 5.18, N 5.14; found: C 66.25, H 5.20, N 5.16.

*Dibenzyl (6*RS,*7*RS*)-8-Oxo-7-(phenylacetamido)-4-thia-1-azabicyclo[4.2.0]oct-2-ene-2,3-dicarboxylate* (**13**). At 0°, 3-Cl$C_6H_4CO_3H$ (1.72 g, 0.01 mol) was added to **11** (5.44 g, 0.01 mol) in dry CH_2Cl_2 (100 ml). After stirring at 0° for 1 h and at 25° for 30 min, 1% $NaHCO_3$ soln. (100 ml) was added, the org. layer dried ($MgSO_4$) and evaporated, and the crude sulfoxide **12** dissolved in dry CH_2Cl_2 (100 ml) at 0° and treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (1.52 g, 0.01 mol) and AcCl (1.0 g, 0.013 mol). The soln. was stirred at 0° for 1 h and at reflux temp. for 2 h, then washed with 5% HCl, 5% $NaHCO_3$, and NaCl soln., dried ($MgSO_4$), and evaporated and the residue chromatographed (silica gel, CH_2Cl_2 (→impurities), then $CHCl_3$): 2.0 g (40%) of **13**. Foam. IR (CH_2Cl_2): 3423 (NH), 1796 (β-lactam), 1750 (ester), 1730 (C=C), 1682 (amide). ^{1}H-NMR ($CDCl_3$): 2.85–3.40 (*m*, CH_2S); 3.51 (*s*, CH_2CO); 4.28 (br., H–C(6)); 5.10, 5.11 (2*s*, 2 CH_2O); 5.12–5.45 (*dd*, J = 4.5, 9, H–C(7)); 6.82 (br., NH); 7.39, 7.25 (2*s*, 3 Ph). Anal. calc. for $C_{30}H_{26}N_2O_6S$ (542.62): C 66.42, H 4.80, N 5.16; found: C 66.44, H 4.92, N 5.06.

*(6*RS,*7*RS*)-8-Oxo-7-(phenylacetamido)-4-thia-1-azabicyclo[4.2.0]oct-2-ene-2,3-dicarboxylate* (**14**). A mixture of **13** (2.71 g, 0.005 mol), AcOEt (200 ml) and $PdCl_2$ (600 mg) was hydrogenated at 25° and 40 psi for 4 h. After filtration and evaporation the crude foam was crystallized from Et_2O: 0.45 g (25%) of **14**. M.p. 192–194°. IR (nujol): 3150–3680 (2 COOH, NH), 1780 (β-lactam), 1710, 1700 (2 C=O, C=C), 1675 (amide). ^{1}H-NMR ((D$_6$)DMSO/D_2O): 2.39–2.76 (*m*, CH_2S); 3.49 (*s*, CH_2CO); 4.32 (br. *m*, H–C(6)); 5.01 (*d*, J = 5, CHND); 7.38 (*s*, Ph). Anal. calc. for $C_{16}H_{14}N_2O_6S$ (362.24): C 53.04, H 3.87, N 7.73; found: C 53.25, H 3.69, N 8.00.

*2,2-Dibenzyl 3-Methyl (6*RS,*7*RS*)-8-Oxo-7-(phenylacetamido)-4-thia-1-azabicyclo[4.2.0]octane-2,2,3-tricarboxylate* (**16**). To a soln. of **15** (7.60 g, 0.01 mol) and bis(trimethyltin) sulfide (3.60 g, 0.01 mol) in dry THF (300 ml) at 0° was added dropwise, within 2 h, Bu_4NF (0.02 mol) in dry THF (15 ml). The soln. was stirred at 25° for 1 h and then partitioned between Et_2O and H_2O, the Et_2O layer dried ($MgSO_4$) and evaporated, and the residue chromatographed (silica gel, $CHCl_3$/AcOEt 1:1): 4.8 g (80%) of **16**. Oil. IR (CH_2Cl_2): 3350 (NH), 1774 (β-lactam), 1730–1750 (ester), 1680 (amide). ^{1}H-NMR ($CDCl_3$): 2.70–3.42 (*m*, CH_2S); 3.50 (br. *s*, CH_2CO); 3.52, 3.53 (2*s*, MeO); 4.01–4.39 (*m*, H–C(3), H–C(6)); 5.07–5.30 (*m*, 2 CH_2O); 5.31–5.69 (*m*, H–C(7)); 7.00–7.46 (*m*, 3 Ph, NH). Anal. calc. for $C_{32}H_{30}N_2O_8S$ (602.54): C 63.79, H 4.98, N 4.65; found: C 63.68, H 4.96, N 4.67.

*3-Methyl 2-Hydrogen (6*RS,*7*RS*)-8-Oxo-7-(phenylacetamido)-4-thia-1-azabicyclo[4.2.0]octane-2,3-dicarboxylate* (**17**). A soln. of **16** (3.01 g, 0.005 mol) in MeOH (100 ml) containing 1% aq. $NaHCO_3$ soln. (15 ml) was hydrogenated over 10% Pd/C (1.5 g) at 45° and 60 psi for 5 h. The mixture was filtered, and AcOH (20 ml) added, the solvent evaporated, and the residue purified by CC (silica gel, AcOEt/MeOH 9:1): 0.97 g (52%) of **17**. M.p. 130–133°. IR (nujol): 3200–3650 (COOH, NH), 1770 (β-lactam), 1730 (ester), 1700 (acid), 1668 (amide). ^{1}H-NMR

($CDCl_3$/(D_6)DMSO/D_2O): 2.50–2.91 (*m*, CH_2S); 3.51 (br. *s*, CH_2CO); 3.56 (br. *s*, MeO); 4.03–4.50 (*m*, H–C(2), H–C(3), H–C(6)); 5.20–5.51 (*m*, H–C(7)); 7.41 (*s*, Ph). Anal. calc. for $C_{17}H_{18}N_2O_6S$ (378.32): C 53.97, H 4.76, N 7.41; found: C 53.86, H 4.65, N 7.52.

3-Methyl 2-Hydrogen (6RS,7RS)-8-Oxo-7-(phenylacetamido)-4-thia-1-azabicyclo[4.2.0]oct-2-ene-2,3-dicarboxylate (**19**). At 0°, 3-$ClC_6H_4CO_3H$ (1.72 g, 0.01 mol) was added to **17** (3.78 g 0.01 mol) in dry THF (100 ml). After 2 h of stirring (TLC: no starting materials left), 1,8-diazabicyclo[5.4.0]undec-7-ene (3.04 g, 0.02 mol) and AcCl (3.0 g, 0.039 mol) were added. The soln. was stirred at 0° for 1 h and at reflux temperature for 4 h and then partitioned between AcOEt and H_2O. The org. layer was washed with 5% HCl soln. and H_2O, dried ($MgSO_4$), and evaporated. CC (silica gel, AcOEt/MeOH 9:1) gave 1.88 g (50%) of **19**. M.p. 150–153°. IR (nujol): 3150–3655 (COOH, NH), 1790 (β-lactam), 1750 (ester), 1710, 1703 (acid, C=C), 1675 (amide). ^{1}H-NMR ((D_6)DMSO/D_2O): 2.45–3.05 (*m*, CH_2S); 3.48 (*s*, CH_2CO); 3.95 (*s*, MeO); 4.33 (*m*, H–C(6)); 5.03 (*d*, J = 5, CHND); 7.39 (*s*, Ph). Anal. calc. for $C_{17}H_{16}N_2O_6S$ (376.32): C 54.25, H 4.25, N 7.45; found: C 54.23, H 4.22, N 7.50.

Methyl (6RS,7RS)-8-Oxo-7-(phenylacetamido)-2-{[2-(retinamido)ethylamino]carbonyl}-4-thia-1-azabicyclo[4.2.0]octa-2-ene-3-carboxylate (**21**). To a suspension of **19** (0.376 g, 1.0 mmol) in dry CH_2Cl_2 (10 ml) containing pyridine (0.20 g, 2.5 mmol), ethyl chloroformate (0.12 g, 1.1 mmol) was added dropwise under a stream of N_2 at −5°. The soln. was stirred for 15 min, then ethylenediamine (0.060 g, 1.0 mmol) was added through a syringe. Similarly, retinoic acid (**20**; 0.30 g, 1.0 mmol) in dry CH_2Cl_2 (10 ml) containing pyridine (0.2 g, 2.5 mmol) was treated with ethyl chloroformate (0.12 g, 1.1 mmol) under N_2 at −5° for 15 min. The resulting soln. was transferred by syringe to the above mixture obtained from **19**. After 1 h, the soln. was washed with H_2O (3×), dried (Na_2SO_4), filtered, and evaporated. Crystallization from Et_2O/hexane 1:1 gave 0.60 g (85%) of **21**. M.p. 71–74°. UV (EtOH): 350 (1897). IR (CH_2Cl_2): 3300–3420 (3 NH), 1793 (β-lactam), 1704 (ester), 1700 (C=C), 1680, 1645 (amides). ^{1}H-NMR ($CDCl_3$): 1.15 (*s*, Me_2C); 1.31–2.40 (*m*, 3 Me, 3 CH_2); 2.61–3.10 (*m*, CH_2S); 3.05 (br. *s*, NCH_2CH_2N); 3.58 (*s*, CH_2CO); 3.90 (*s*, MeO); 4.20–5.11 (*m*, H–C(6), H–C(7)); 5.60–6.71 (*m*, 6 CH, 3 NH); 7.40 (*s*, Ph). Anal. calc. for $C_{39}H_{48}N_4O_6S$ (700.64): C 66.86, H 6.90, N 7.99; found: C 66.98, H 6.79, N 8.21.

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