the reaction was carried out in the presence of various trapping reagents.

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Registry No. **4**, 91003-52-0; **8**, 117985-28-1; **9**, 87-52-5; **10**, 117985-29-2; **11**, 100208-13-7; **12**, 100208-14-8; **13**, 100208-15-9; **14**, 117985-30-5; **15**, 117985-31-6; **16**, 117985-32-7; **18**, 117985-33-8; **19**, 117985-34-9; **20**, 117985-35-0; **21**, 117985-36-1; **22**, 117985-37-2; **23**, 117985-38-3; **24**, 117985-39-4; **32**, 117985-40-7; **34**, 5833-50-1; **35**, 117985-41-8; **36**, 118017-03-1; **37**, 117985-42-9; **38**, 117985-43-0; **42**, 117985-44-1; **43**, 117985-45-2; **44**, 117985-46-3; **45**, 113334-35-3; **46**, 117985-47-4; **47**, 19291-02-2; **49**, 75475-91-1; **50**, 107124-28-7; DMAD, 762-42-5; (*E*)-$PhSO_2CH{=}CHSO_2Ph$, 963-16-6; (*Z*)-$PhSO_2CH{=}CHSO_2Ph$, 963-15-5; PhCHO, 100-52-7; indole, 120-72-9; 3-indolecarboxaldehyde, 487-89-8; skatale, 83-34-1; *N*-phenylmaleimide, 941-69-5; dimethyl fumarate, 624-49-7; dimethyl maleate, 624-48-6; acrylonitrile, 107-13-1; maleic anhydride, 108-31-6; pyrrole-2-carboxaldehyde, 1003-29-8; *N*-[(trimethylsilyl)methyl]pyrrole-2-carboxaldehyde, 117985-48-5; *N*-[(trimethylsilyl)methyl]pyrrole-2-carbinal, 117985-49-6; *N*-[(trimethylsilyl)methyl]-2-[(dimethylamino)methyl]pyrrole, 117985-50-9; *N*-[(trimethylsilyl)methyl]-3-[(dimethylamino)methyl]-pyrrole, 117985-51-0; *N*-[(trimethylsilyl)methyl]-2-[(dimethylamino)methyl]pyrrole methiodide, 117985-52-1; 2-methyl-3-cyano-*N*-[(trimethylsilyl)methyl]pyrrole, 117985-53-2; sodium *p*-toluenesulfinate, 824-79-3; cysteine hydrochloride, 52-89-1.

Studies on the Structures of Imipenem, Dehydropeptidase I Hydrolyzed Imipenem, and Related Analogues

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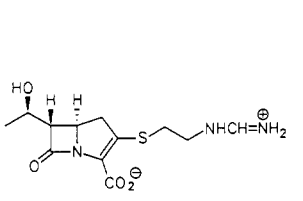
Dehydropeptidase I catalyzed hydrolysis of the carbapenem antibiotics imipenem (**1**) and the 3-methylthio analogue **14** was examined by spectroscopic methods. The principal product in both cases was a mixture of β-lactam ring-opened 1-pyrrolines epimeric at C3. A transient 2-pyrroline intermediate was observed by ^1H NMR in the methylthio carbapenem case. Nonenzymatic acid-catalyzed hydrolysis of each antibiotic under dilute conditions rapidly afforded a protonated 2-pyrroline product that isomerized to the diastereomeric 1-pyrroline mixture on neutralization. At higher carbapenem concentrations, the acid-catalyzed process also produced a diketopiperazine structure resulting from initial bimolecular attack of the carboxyl group of one carbapenem molecule on the β-lactam group of a second molecule. The analysis of the imipenem-derived products was complicated by the observation of side-chain formamidinium rotational isomers. Under mildly acidic conditions, the imipenem side-chain isomers were separable by high pressure liquid chromatography and the major form was identified as the *Z* isomer by X-ray crystallography.

Imipenem (**1**), the crystalline *N*-formimidoyl derivative[1,2] of thienamycin, is the first clinically available member of a new class of β-lactam antibiotics that possess the carbapenem ring system. Imipenem exhibits an extremely broad spectrum of activity[3] against gram-positive and gram-negative aerobic and anaerobic species, which is due in part to its high stability in the presence of β-lactamases[4] of both plasmid and chromosomal origin. Unlike the classical penicillins and cephalosporins, however, imipenem and related carbapenem antibiotics show varying degrees of susceptibility to the mammalian renal dipeptidase, dehydropeptidase I (DHP-I).[5] Localization of this enzyme in the kidney proximal tubule leads to poor urinary recovery of imipenem and requires coadministration of the DHP-I inhibitor[6] sodium cilastatin (**2**) to increase efficacy against urinary tract infections. The clinically used 1:1 combination of imipenem and sodium cilastatin is known as Primaxin.



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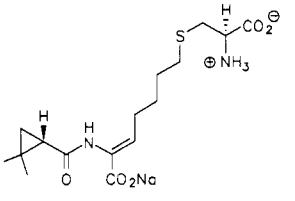

2

Our interest in the structure of DHP-I degraded imipenem arose from questions concerning the possible role of degradate in the toxicological evaluation of imipenem. Furthermore, it was of interest to determine whether enzymatically degraded antibiotic produced structures similar to those obtained by deliberate chemical hydrolysis or by

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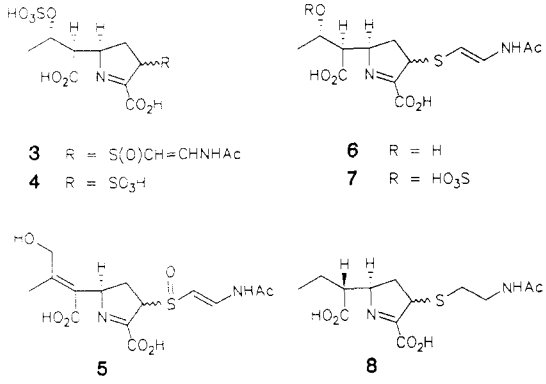
0022-3263/89/1954-0653$01.50/0

degradation on prolonged storage in aqueous solution. Early biochemical studies[5] with imipenem and other carbapenems revealed that DHP-I catalyzed hydrolysis of the β-lactam ring, but no structures were proposed for the products. The chemical stability of imipenem in aqueous solution as a function of pH and concentration has also been investigated.[7] The stability study revealed that rate-determining β-lactam ring opening preceded the formation of several products whose structures were not established.

Previous investigations involving other carbapenem antibiotics have shed some light on the structures of β-lactam ring-opened products. For example, chemical hydrolysis of the 3-sulfinyl and 3-sulfo substituted carbapenems MC696-SY2-A[8], asparenomycin A,[9] and SF-2103 A[10] afforded the 1-pyrroline structures **3–5**, which were useful in establishing the structures of the natural products. Easton and Knowles[11] have shown that the products derived from either β-lactamase-induced hydrolysis or nonenzymatic, base-catalyzed hydrolysis of the olivanic acids MM4550, MM22382, and MM13902 are diastereomeric mixtures of 1-pyrrolines **3**, **6** and **7**, respectively. In addition, the chemical structure of the primary DHP-I metabolite of antibiotic PS-5, a 6-ethyl-3-acetamidoethylthio carbapenem natural product, has been elucidated[12] as a mixture of 1-pyrrolines **8** epimeric at C3. The present paper extends and amplifies these observations by describing the results of a spectroscopic investigation into the structures of imipenem, its enzymatic and chemically derived degradates, and those of simpler, related analogues.



Results and Discussion

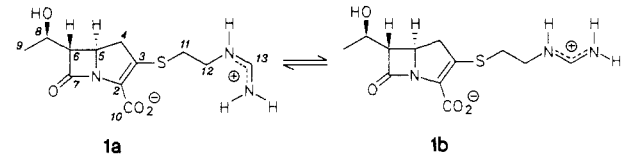
NMR probes into the structures of imipenem and its degradates are complicated by the observation of side-chain rotational isomers. This problem manifests itself as the appearance of two closely spaced formamidine methine resonances at δ 7.83 and 7.84 in the ^1H NMR spectrum of imipenem and by doubling of many of the ^{13}C NMR signals (see Table I).[13] The magnitude of the ^{13}C chemical shift differences and the location of the paired resonances point to the side-chain formamidinium group as the locus of isomerization, and the peak height ratios for the paired signals suggest approximately a 2:1 mixture. These results are readily interpreted in terms of interconverting *Z* and *E* formamidinium isomers **1a** and **1b** due to hindered rotation[14,15] about the partial NC double bonds. The major form is tentatively assigned the *Z* configuration on the basis of the upfield positions of its side-chain carbon resonances. Under physiological conditions of temperature and pH, this equilibrium is expected to be dynamic and, consequently, to have no effect on the biological properties of imipenem.

Table I. ^{13}C NMR Spectral Data for Imipenem (1) and Its Degradates

carbon[b]	^{13}C chemical shifts (ppm)[a]			
	1	**20a**	**20b**[c]	**21a**[d]
2	131.1 (131.6)	171.9 (171.8)[e]		126.2
3	139.8 (139.4)	50.4[f]		132.8 (132.5)
4	39.7 (39.9)	38.3 (35.8)	37.1 (35.6)	38.1
5	53.2	72.1 (71.1)		56.0
6	65.8	62.3	62.5	60.9
7	169.0	180.6 (180.4)		178.3
8	65.8	68.2	68.3	66.7
9	21.0	21.9	22.0	21.6
10	180.4 (180.3)	174.0 (173.3)[e]		156.2
11	30.1 (32.5)	31.8 (29.3)	31.3 (28.8)	30.1 (31.9)
12	42.1 (47.9)	41.6 (46.9)	41.8 (47.1)	42.1 (47.6)
13	155.2 (158.5)	155.2 (158.3)		155.5 (158.8)

[a] The spectra were determined in D_2O by using internal dioxane (67.4 ppm) as standard. Signals due to the minor *E* side-chain isomer are given in parentheses wherever they are resolved from the signals of the major *Z* isomer. [b] The numbering system is that shown in structure **1a**. [c] Only nondegenerate signals for **20b** are given. [d] Spectrum determined at pH 4.5. [e] Tentative assignments. [f] Deuterium exchange at C3 decreases the intensity of the C3 resonance, thereby allowing only the major isomer of the **20a,b** mixture to be detected.

Circumstantial evidence suggesting that the rotational isomers **1a** and **1b** are separable under acidic conditions (pH <6) has been obtained. For example, reverse-phase HPLC of imipenem at pH 4.8 (C_{18} μBondapak column, 0.01 M KH_2PO_4 eluant) reveals a 7:3 mixture of two closely moving species. Peak shaving gives mixtures enriched in each species that reequilibrate to the original mixture over a period of time. At 0 °C and pH 5.5, the equilibrium takes several hours to reach completion, whereas at pH 7 and room temperature equilibrium is reestablished within a few minutes.[16] While the individual isomers have not been spectroscopically characterized, we believe that the HPLC picture is best interpreted in terms of the rotational isomers **1a** and **1b** and, as such, represents the first reported example of isolable *N*-substituted formamidinium isomers. The pH dependence on the rate of isomer interconversion suggests that equilibration occurs through a neutral form-



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(13) The NMR samples of imipenem in D_2O should exhibit a pD near 5.5 since the pH of a 10 mg/mL solution of imipenem monohydrate in H_2O is 5.5.
(14) For excellent reviews on amidine and amidinium isomers, see: Hafelinger, G. In *The Chemistry of Amidines and Imidates*; Patai, S., Ed.; John Wiley and Sons: New York, 1975; Chapter 1, and Fodor, G.; Phillips, B. A., Chapter 2.
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(16) Imipenem is hydrolyzed by porcine DHP-I at 0.9× the rate of thienamycin (see ref 5). Since DHP-I susceptibilities are measured at pH 7.0 and 37 °C, conditions under which isomers **1a** and **1b** are rapidly interconverting, the relative rates of the two isomers have not been determined.

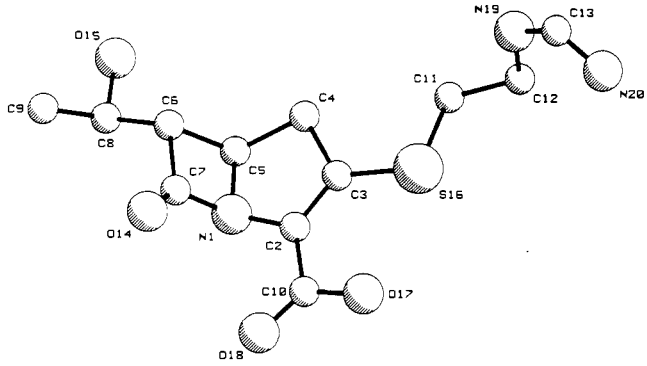


Figure 1. Computer-generated drawing of imipenem (1) derived from the X-ray coordinates.

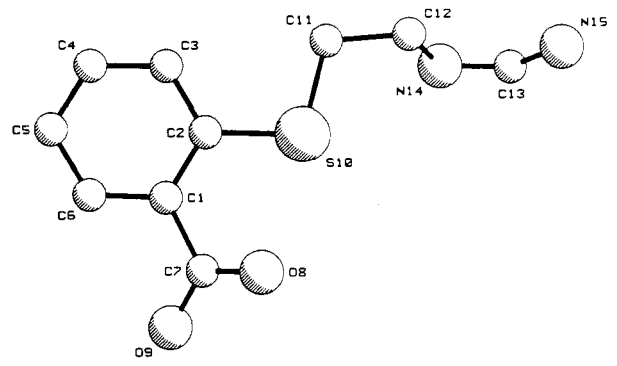
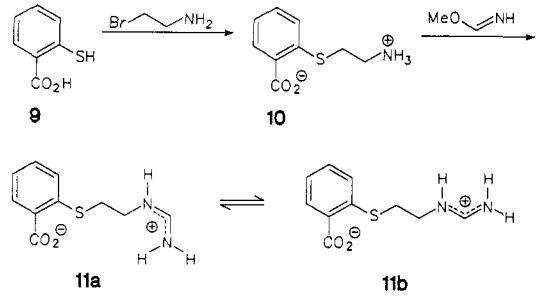


Figure 2. Computer-generated drawing of compound 11 derived from the X-ray coordinates.

amidine intermediate by rotation about a single NC bond.

The imipenem model compound 11 was prepared in order to investigate the formamidinium isomer phenomenon in a simpler system. Alkylation of thiosalicylic acid (9) with 2-bromoethylamine in liquid NH_3 gave amino acid 10, which was subsequently N-formimidoylated using excess methyl formimidate in H_2O at pH 9 to afford 11. The NMR and HPLC behavior of 11 were remarkably similar to that of imipenem. The ^{13}C spectrum exhibited signals for carbons 11, 12, and 13 (imipenem numbering) for the major and (minor) rotational isomers at δ 32.2 (34.4), 40.9 (46.6), and 155.1 (158.1). The peak height ratios were again in the 2:1 range. The ^1H NMR spectrum showed two formamidinium CH singlets at δ 7.73 (major form) and 7.67, as well as two distinct NCH_2 multiplets centered at δ 3.56 (major form) and 3.49. HPLC analysis at pH 4.8 revealed the presence of two peaks in a 7:3 ratio from which a sample enriched in the minor isomer was obtained by peak shaving. On storing at room temperature, the enriched sample gradually reequilibrated to the original 7:3 mixture. The similarity of model system 11 and imipenem with regard to side-chain isomerization removes any possible contributions of the carbapenem ring system to the isomer phenomenon and provides a potential tool for investigating the kinetic process in greater detail.



The single-crystal X-ray structures of imipenem monohydrate and the model compound 11 have been solved. The conformations in the solid state are those shown in Figures 1 and 2, respectively. Although both compounds are zwitterionic, there are no intramolecular interactions between the charged groups and only intermolecular hydrogen bonds. The sum of the three bond angles around the β-lactam nitrogen (322.5°) of imipenem and the distance of that nitrogen atom from the plane of its three attached carbon atoms (0.52 Å) compare closely with the corresponding values for *N*-acetylthienamycin methyl ester[17] and indicate a highly folded, bicyclic ring system.

Examination of the unit cell of imipenem reveals an extensive matrix of hydrogen bonds centered mainly on the 1 mol of solvated water. The hydroxyl group proton and the proton on the inner nitrogen atom of the formamidine group are directed toward the water molecule. The protons in the water molecule are then directed toward two imipenem carboxyl groups to form a tetrahedral hydrogen bond structure about the water oxygen.

The X-ray structures of 1 and 11 also reveal that the configuration of the formamidinium group for both compounds in the crystal state is *Z* and not *E*. Dissolution of crystalline 11 in ice-cold 0.01 M KH_2PO_4 followed by immediate HPLC analysis at pH 4.8 shows a greater than 95:5 ratio of isomers in which the major component has the same retention time as the major form at equilibrium. An identical experiment with crystalline imipenem gave an 83:17 mixture of isomers. These observations demonstrate that the major equilibrium isomer in solution also has the *Z* configuration, which is in accord with the preliminary assignments based on the ^{13}C NMR data.

Enzymatically degraded imipenem, hereafter referred to as metabolite I, was obtained[18] by passing an aqueous solution of imipenem, $NaHCO_3$, and $Zn(OAc)_2$ through a DHP reactor column prepared by covalently linking highly purified porcine DHP-I to Sepharose. The column effluent was ultra-filtered, passed through a chelating column to remove zinc, and lyophilized to yield metabolite I. HPLC analysis of the lyophilizate under neutral conditions revealed the presence of two closely moving products[19] exhibiting only end absorption in the UV. The IR absorbance band at 1780 cm^{-1}, assigned to the β-lactam carbonyl of imipenem, was absent in the spectrum of metabolite I. The ^1H and ^{13}C NMR spectra of the metabolite were very complicated, due in part to the mixture of products and to the presence of side-chain formamidinium isomers. Acidification of an aqueous solution of metabolite I to pH 1 produced a new product as evidenced by the appearance of a UV chromophore at 282 nm[20] and by significant changes in the NMR spectra. Subsequent neutralization of the acidic solution reestablished the original product mixture.

In order to simplify the NMR analysis and to remove the ambiguities associated with side-chain formamidinium isomers, we decided to investigate the enzymatic and

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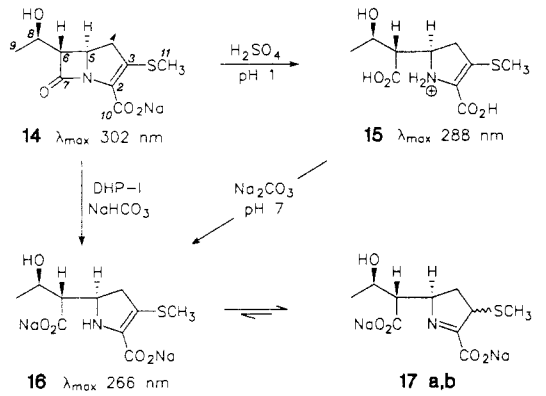
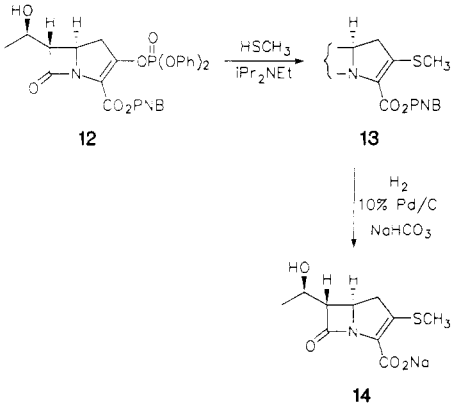
(19) The HPLC ratio of these products varies from 1:1 to 2:1 depending on the age and pH of the test solution.

(20) The observation that acidification of aqueous solutions hydrolyzed imipenem produces a UV chromaphore at 282 nm was originally made by Dr. D. G. Musson of these laboratories. This phenomenon has been developed into an HPLC method for analysis of metabolite I in biological fluids: Musson, D. G.; Hajdu, R.; Bayne, W. F.; Rogers, J. D. Manuscript in preparation.

Scheme I



chemical degradation of the simpler methylthio analogue **14**. This compound was prepared from the carbapenem vinyl phosphate **12** by using well-established procedures.[21] Treatment of **12** with CH_3SH and iPr_2NEt gave the crystalline intermediate **13**, which underwent hydrogenolytic deblocking of the *p*-nitrobenzyl ester in the presence of $NaHCO_3$ to provide **14** as an amorphous solid. Both **14** and imipenem exhibit similar stability in dilute solution at pH 7; however, **14** is consumed by DHP-I at approximately 25 times the rate of imipenem.



The hydrolysis of carbapenem **14** was dynamically monitored by ^1H NMR spectroscopy. Acidification of a dilute D_2O solution of **14** (5 mg/mL) to pH 1 with H_2SO_4 rapidly gave rise to a new species that was characterized by downfield and upfield shifts for H5 and H6 (see Table II). These shifts and a large $J_{5,6}$ coupling constant of 6.4 Hz are consistent with rapid β-lactam hydrolysis to the protonated 2-pyrroline structure **15** (see Scheme I). Neutralization of the acidic solution with Na_2CO_3 produced three major components, one of which disappeared over a period of approximately 2 h at room temperature. The NMR data presented in Table II are consistent with structures **16**, **17a**, and **17b** for these products, with the first-formed 2-pyrroline isomer **16** gradually tautomerizing to a 2:1 mixture of 1-pyrroline isomers **17a** and **17b**. The upfield H6 positions and large $J_{5,6}$ values for compounds **16** and **17** confirm the absence of the β-lactam ring, and the relative positions of the H4 and SCH_3 signals are in accord with the isomeric 2- and 1-pyrroline assignments.

It should be noted that the 1-pyrroline isomers **17** produced in this manner contain a deuterium atom at C3, since they are derived from **16** by deuteriation from the D_2O solvent. While this feature simplifies the spectral interpretation of the product mixtures, it provides no information regarding the vicinal couplings of H3 to H4a and H4b. Proton NMR studies in 95:5 H_2O/D_2O circumvented this problem; however, the C3 configuration remained unresolved since both couplings were equal ($J_{3,4a} = J_{3,4b} = 9.0$ Hz for **17a**). Irradiation of the SCH_3 signals of isomers **17a** and **17b** also failed to provide any stereochemical information as no NOE enhancements were observed at either H4 or H5.

Additional support for the NMR structural assignments was obtained by monitoring the pH-dependent transformations of **14** by UV spectroscopy. The carbapenem chromophore at 302 nm rapidly shifted to 288 nm on ring opening to **15**. Subsequent neutralization gave rise to a lower intensity chromophore at 266 nm associated with the 2-pyrroline **16** that gradually decreased in intensity with time. Imino isomers **17** have no characteristic UV.

The chemically produced degradates described above were related to the enzymatic process by observing the ^1H NMR spectrum of **14** in the presence of purified porcine DHP-I (5 mg of **14** and 5 μg of DHP-I in 1 mL of D_2O containing 1.5 equiv of $NaHCO_3$). The signals corresponding to **14** gradually diminished in intensity and were completely absent after 1 h at room temperature. As **14** disappeared, signals corresponding to the 2-pyrroline **16** appeared, and these gradually gave rise to signals corresponding to the 1-pyrroline isomers **17a** and **17b**. After 90 min at room temperature, the NMR spectrum showed primarily **17a** and **17b** along with minor amounts of **16**. As illustrated in Scheme I, enzymatic hydrolysis of carbapenem **14** is equivalent to treating **14** with acid followed by neutralization, and the latter process provides a convenient alternative for generating metabolite.

The composition of imipenem derived metabolite I was readily determined by comparing its ^1H NMR spectrum to that of the aged mixture of 1-pyrroline diastereomers **17**. Close similarities in the positions and multiplicites of the H4, H5, and H6 resonances in the two series revealed that the metabolite was approximately a 1:1 mixture of diastereomeric 1-pyrrolines **20** (see Scheme II). In the

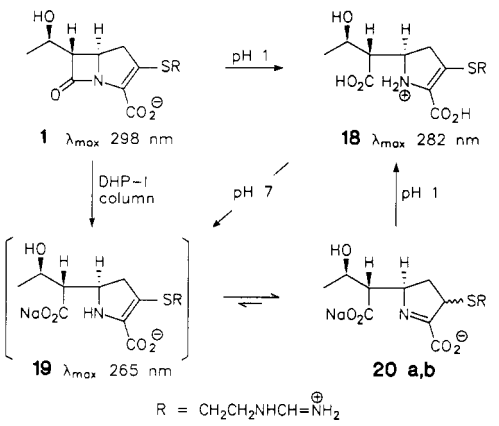
Table II. ^1H NMR Spectral Data for Carbapenem 14 and Its Degradates

	^1H chemical shifts (ppm) and coupling constants (Hz)[a]					
proton[b]	**14**	**15**	**16**	**17a**	**17b**	**21b**
4a (dd)	3.14	3.27	2.90	1.77[c]	2.25	3.02
4b (dd)	3.32	3.57	3.33	2.69[c]	*d*	3.33
5 (m)	4.20	4.54	4.25	4.28	4.45	5.11
6 (dd)	3.39	3.12	2.57	2.40	*d*	2.30
8 (dq)	4.23	4.33	4.06	4.16	*d*	3.94
9 (d)	1.30	1.32	1.29	1.26	1.25	1.26
11 (s)	2.35	2.48	2.38	2.06	2.07	2.64
J value	**14**	**15**	**16**	**17a**	**17b**	**21b**
$J_{4a,4b}$	17.8	17.9	17.0	13.7	14.4	17.8
$J_{4a,5}$	8.6	9.0	10.2	8.5	7.6	2.7
$J_{4b,5}$	9.6	9.6	9.3	7.7		10.4
$J_{5,6}$	2.4	6.4	4.4	7.3		1.9
$J_{6,8}$	6.2	4.9	7.8	8.9		10.5
$J_{8,9}$	6.4	6.6	6.5	6.2	6.5	6.0

[a] The spectra of **14**–**17** were determined in D_2O solutions by using HOD (δ 4.79) as an internal standard. The spectrum of **21b** was recorded in $CDCl_3$ solution. [b] The numbering system is that shown for compound **14** in Scheme I. Observed multiplicities are given in parentheses. [c] When the spectrum was determined in 5% D_2O/H_2O, H4a and H4b of **17a** appeared as ddd's with $J_{3,4a} = 9.0$ Hz and $J_{3,4b} = 9.0$ Hz. [d] The H4b, H6, and H8 resonances of **17b** are unresolved multiplets centered near 2.4, 2.4, and 4.1 ppm, respectively.

(21) (a) Salzmann, T. N.; Ratcliffe, R. W.; Christensen, B. G.; Bouffard, F. A. *J. Am. Chem. Soc.* **1980**, *102*, 6161. (b) Shinkai, I.; Reamer, R. A.; Hartner, F. W.; Liu, T.; Sletzinger, M. *Tetrahedron Lett.* **1982**, *23*, 4903.

Scheme II



imipenem series, the H3 proton of **20** was indirectly observed by noting its coupling to the upfield H4 signals in both isomers. Storing the NMR solution at room temperature for 24 h resulted in equilibration to a 2:1 mixture of 1-pyrroline isomers similar to that observed for **17** and resulted in disappearance of the $J_{3,4}$ coupling due to deuterium exchange at C3.

As shown in Scheme II, metabolite I (**20**) could also be produced by acidification of a D_2O solution of imipenem (5 mg/mL) to pH 1 followed by neutralization. The ^1H NMR spectrum of intermediate **18** exhibited characteristic H5, H6, and CH_3CH signals quite similar to those of the methylthio analogue **15**. Intermediate **18** is also available by acidification of metabolite I, but this method is not as clean as the direct conversion from imipenem. Although the presence of 2-pyrroline **19** was not observed by NMR in the base-induced transformation of **18** to **20**, its presence was detected in a parallel UV experiment as a low intensity absorption at 265 nm.

The ^1H NMR based structural assignment for metabolite I as a mixture of diastereomeric 1-pyrrolines **20** was corroborated by extensive ^{13}C NMR studies. Solutions of metabolite I in D_2O (40 mg/mL) at pH 7.0 (0.115 M phosphate buffer) and at pH 9.0 (unbuffered) were aged at room temperature over a 14-h period and periodically examined by ^{13}C NMR spectroscopy. Four discrete structures were observed corresponding to the *Z* and *E* side-chain rotomers of 1-pyrrolines **20a** and **20b** (see Table I for NMR assignments). While the metabolite showed no significant degradation under the neutral or basic conditions, the rate at which equilibrium was reached among the two forms of **20** was affected. At neutral pH it required between 10 and 14 h for the initial 3:1 **20a** to **20b** mixture to equilibrate to a 1:1 mixture, whereas under the basic conditions equilibrium was established approximately 15 min after dissolution. The *Z* to *E* formamidinium ratio for both pyrroline diastereomers remained at 2:1 throughout the experiment. There was no evidence that lowering the concentration or the pH would substantially shift the equilibrium to a single species.

A similar profile of rotomeric 1-pyrroline isomers was observed when imipenem itself was aged in pH 7.0 phosphate buffer (5 mg/mL) at ambient temperature and under a nitrogen atmosphere. Under these conditions,[22] imipenem degraded slowly with a half-life of approximately 80 h. Metabolite I appeared to be the sole product emerging over the 159-h period of the NMR experiment. A COSY 2D-NMR taken after 53 h clearly supported the structures of the four degradates.

(22) Phosphate anion has been shown to react with imipenem, thereby affecting the rate of degradation; see ref 7.

Table III. ^{13}C NMR Spectral Data for Carbapenem 14 and Its Degradates

	^{13}C chemical shifts (ppm)[a]			
carbon[b]	**14**	**15**	**17a,b**[c]	**21b**[d]
2	128.2	115.1	172.1	123.9
3	144.4	136.8	50.8	136.2
4	39.2	39.7	34.0 (35.6)	37.3
5	52.9	53.1	71.1	55.7
6	65.6	55.5	62.5	60.7
7	169.3	174.6[e]	180.5	178.5
8	65.3	66.9	68.3	66.5
9	20.3	20.0	21.7 (21.5)	21.4
10	180.4	175.0[e]	174.3	156.4
11	15.0	14.8	12.2	14.3

[a] The spectra were determined in D_2O by using internal dioxane (67.4 ppm) as standard. [b] The numbering system is that shown for structure 14 in Scheme I. [c] Signals due to the minor isomer are given in parentheses wherever they are resolved from the signals of the major isomer. [d] Spectrum determined at pH 6.8. [e] Tentative assignments.

Carbon NMR data were also obtained for the 3-methylthio carbapenem **14** and its degradates. The data collected in Table III are in excellent accord with the imipenem data and further support the structural assignments initially based on the proton spectra.

The high concentrations of substrate required for convenient accumulation of the ^{13}C NMR data lead to an interesting observation. Whereas degradation of imipenem in D_2O at pH 1 and 5 mg/mL concentration cleanly produced the protonated 2-pyrroline **18**, degradation at pH 1 and 20–30 mg/mL concentration gave approximately a 3:1 mixture of **18** and a new product. At pH 4.5, the new product was the major degradate. Once formed, the new product appeared quite stable in acid solution and persisted after neutralizing the solution to pH 7. Since the new species was formed at higher initial concentrations of imipenem, it was assumed to be a bimolecular product. A COSY 2D-NMR taken after neutralization clearly showed a new downfield H5 resonance at δ 4.57, which was partially obscured by the HOD peak in the 1-D spectrum. This observation, coupled with an upfield shift of the ^{13}C signal at 174.4 ppm (carboxylate) to 156.2 ppm (amide), lead to the proposed dimeric diketopiperazine structure **21a** for the new product.[23]

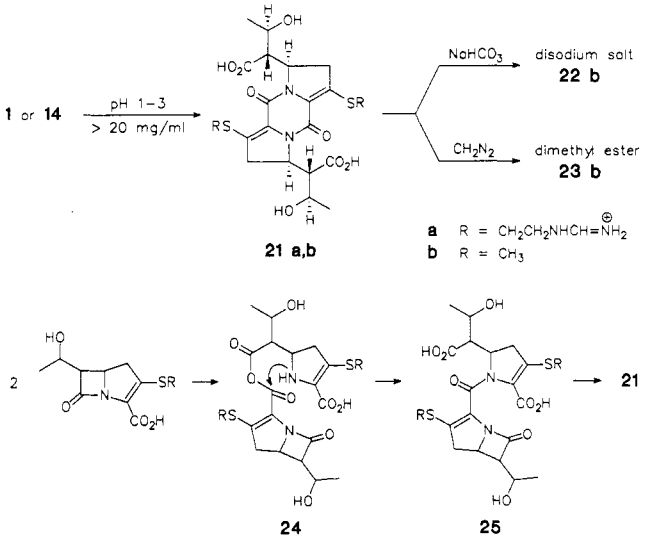
Similar behavior was observed for the 3-methylthio carbapenem **14**. When **14** was treated with pH 3.0 sulfuric acid at approximately 25 mg/mL concentration, the diketopiperazine **21b** was the predominant product, which in fact slowly precipitated out of solution. The structure of **21b** was confirmed by NMR, IR, UV, and mass spectrometry and by its conversion to the disodium salt **22b** and the dimethyl ester **23b**. As expected, the NMR data for **21a** and **21b** were quite similar (see Tables I and III).

A simple mechanistic proposal for diketopiperazine formation is shown in Scheme III. Initial attack by the carboxyl group of one carbapenem molecule at the β-lactam carbonyl group of a second carbapenem molecule[24] affords the dimeric mixed anhydride **24**. Intramolecular transacylation then affords the dimeric amide **25**, which ultimately yields the diketopiperazine. The ring-opening and intramolecular transacylation reactions have been

(23) Diketopiperazine **21a** has been isolated from neutralized aqueous solution by semipreparative, reverse phase HPLC (Whatman M9 Partisil 10/50 PAC column, mobile phase 55:45 H_2O/MeCN, flow rate 4 mL/min, UV detection at 210 and 320 nm, retention time 13.5 min). G. C. Dezeny, personal communication.

(24) Dr. G. B. Smith of these laboratories has developed kinetic evidence showing that the carbapenem carboxyl group is the principal second-order reactant under acidic conditions.

Scheme III



observed in the acetolysis of thienamycin[17] and in an attempted oxidation of a thienamycin derivative.[25]

The results of the present study extend to imipenem the observation that the major hydrolysis product, either enzymatically or chemically derived, of thio-substituted carbapenems is a mixture of diastereomeric 1-pyrroline structures. In addition, we have provided spectral information for the previously unknown 2-pyrroline intermediate, have demonstrated the pH-dependent relationship among the various pyrroline isomers, and have provided a simple, nonenzymatic method for generating metabolite. We have also shown that a bimolecular reaction leading to a diketopiperazine structure dominates the degradation pathway under acidic conditions and at higher concentrations.

The degradates described in this paper are the primary products resulting from pseudo-first-order and second-order decompositions. Numerous other minor products have been detected by HPLC in the decomposition mixtures derived from imipenem at both low and high concentration and at different pH's. The nature of these minor products and a kinetic analysis of the decomposition pathways will be the subject of a forthcoming communication[26] from these laboratories.

Experimental Section

General Comments. Uncorrected melting points were measured in capillary tubes with a Thomas-Hoover Uni-Melt apparatus. Infrared spectra were recorded with a Perkin-Elmer 1420 instrument. Ultraviolet spectra were taken with a Perkin-Elmer 552A UV/vis spectrophotometer coupled to a Houston Instrument Omnigraphic 2000 recorder. Fast atom bombardment (FAB-MS) and high resolution mass spectra (HR-MS) were measured with a MAT 731 instrument. High resolution data was determined in the EI mode, whereas FAB spectra were recorded by using an ion teck gun with xenon gas. Elemental analyses were performed by the Merck Microanalytical Laboratory.

Proton NMR spectra were measured with Varian XL-200 and XL-300 spectrometers and with a Bruker WM-250 spectrometer. Chemical shifts are given in δ units relative to internal TMS for $CDCl_3$ solutions and relative to internal TSP for D_2O solutions. Multiplicities are reported with the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, mc = multiplet centered. The 2D COSY 45 data[27] were obtained with the Bruker instrument by using a data matrix size of 1K × 512W and a digital resolution of 3.91 Hz per point in each dimension. Carbon NMR spectra of compounds 1 and 11 were recorded on Varian CFT-20 and XL-100A spectrometers operating at 20.0 and 25.2 MHz, respectively. All other carbon NMR spectra were recorded on a Bruker WM-250 spectrometer operating at 62.9 MHz. Chemical shifts are given in δ units relative to internal dioxane (δ 67.4) as the reference. Carbon multiplicities were determined by the attached proton test (APT) method.[28]

Crystalline imipenem monohydrate was obtained from the Merck Process Research Department and enzymatically degraded imipenem (metabolite I) was prepared as described in ref 18. Unless otherwise noted, all of the chemicals used were reagent grade and no additional purification was necessary. Acetonitrile was distilled from P_2O_5 and stored over Linde type 4A molecular sieves. Diisopropylethylamine was distilled from CaH_2 and stored under a N_2 atmosphere. Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately before use. MOPS buffer was prepared by titrating an aqueous solution of 3-(*N*-morpholino)propanesulfonic acid to pH 7.0 with concentrated aqueous NaOH.

2-[(2-Aminoethyl)thio]benzoic Acid (10). A mixture of 2-mercaptobenzoic acid (50.0 g, 0.324 mol), 2-bromoethylamine hydrobromide (66.4 g, 0.324 mol), and liquid NH_3 (ca. 500 mL) was heated at reflux. The initial suspension gave way to a light yellow solution within 10 min. After 5 h, the NH_3 was evaporated to give a grey solid, which was recrystallized from 4:1 iPrOH–H_2O to afford **10** (27.2 g, 43%) as white needles: mp 211–212 °C; IR (Nujol) 3410, 1585, 1570, 1545, 1520, 755 cm^{-1}; ^1H NMR (300 MHz, D_2O–TSP) δ 3.14 (mc, SCH_2), 3.26 (mc, NCH_2), 7.41 (mc, 3,4,5-*H*), 7.58 (mc, 6-*H*); ^{13}C NMR (20 MHz, D_2O) δ 32.2 (SCH_2), 38.9 (NCH_2), 127.8 (*C*-5), 128.6 (*C*-3), 129.2 (*C*-1), 130.4 (*C*-6), 132.2 (*C*-4), 143.6 (*C*-2). Anal. Calcd for $C_9H_{11}NO_2S$: C, 54.80; H, 5.62; N, 7.10; S, 16.25. Found: C, 54.35; H, 5.89; N, 7.21; S, 16.13.

2-[[2-[(Iminomethyl)amino]ethyl]thio]benzoic Acid (11). An ice-cold, stirred suspension of amino acid **10** (2.50 g, 12.7 mmol) in H_2O was brought to pH 9.0 by addition of 2.5 N NaOH. The resulting solution was treated with solid methyl formimidate hydrochloride (7.00 g, 73.3 mmol) added in two equal portions spaced 10 min apart. The pH was maintained near 9.0 throughout the reaction by periodic addition of 2.5 N NaOH. After 20 min, the reaction mixture was adjusted to pH 7.0 with 2.5 N HCl and added to a column of Bio-Rad AG 50W-X4 resin (5 × 54 cm, 200–400 mesh, Na form) that was eluted with ice-cold deionized H_2O at a flow rate of 10 mL/min. The product containing fractions (ca. 1.3–1.9 column volumes) were located by HPLC, combined, and concentrated in vacuo to ca. 30 mL to give a suspension. The solid was collected, washed with EtOH, and vacuum-dried to afford **11** (0.703 g, 25%) as white crystals. An analytically pure sample and crystals suitable for X-ray analysis were obtained by recrystallization from H_2O: mp 197–198 °C dec; IR (Nujol) 1585, 1570, 1535, 1340, 1290, 740 cm^{-1}; UV (H_2O) λ_{max} 255 (ϵ 5300) nm; ^1H NMR (300 MHz, D_2O–TSP) δ 3.15–3.26 (m, SCH_2), 3.49 (mc, NCH_2, *Z* isomer), 3.56 (mc, NCH_2, *E* isomer), 7.27–7.44 (m, 3,4,5-*H*), 7.52 (mc, 6-*H*), 7.67 (s, NC*H*N, *E* isomer), 7.73 (s, NC*H*N, *Z* isomer); ^{13}C NMR (25.2 MHz, D_2O) δ 32.2 (SCH_2, *Z* isomer), 34.4 (SCH_2, *E* isomer), 40.9 (NCH_2, *Z* isomer), 46.6 (NCH_2, *E* isomer), 127.8, 128.1, 130.1, 131.0, 131.6, 143.1 (*C*-2), 155.1 (NCHN, *Z* isomer), 158.1 (NCHN, *E* isomer). Anal. Calcd

(25) Oxidation of the hydroxyethyl side chain of *N*-(benzyloxycarbonyl)thienamycin benzyl ester with Ac_2O–DMSO afforded a 1-acetyl-5-(2-oxopropyl)-2-pyrroline derivative, presumable by addition of acetic acid to the β-lactam group of the desired ketone product followed by intramolecular transacylation with concomitant decarboxylation. R. W. Ratcliffe, unpublished results.

(26) Smith, G. B.; Dezeny, G. C.; Douglas, A. W. Manuscript in preparation.

(27) (a) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229. (b) Nagayama, K.; Kumar, A.; Wuthrich, K.; Ernst, R. R. *J. Magn. Reson.* **1980**, *40*, 321.

(28) Patt, S. L.; Shoolery, J. N. *J. Magn. Reson.* **1982**, *46*, 535.

(29) The following library of crystallographic programs were used: (a) Main, P.; et al. MULTAN-80, 1980, University of York, York, England. (b) Johnson, C. K. ORTEP-II, 1970, Oak Ridge National Laboratory, Oak Ridge, TN. (c) Okaya, Y.; Frenz, B. A. and associates SDP Plus V1.1, 1984, College Station, TX.

(30) Amorphous carbapenem **14** prepared in this manner is judged to be approximately 77% pure by weight. The degree of purity is based on an assumed NH_2OH extinguished $E^{1\%}$ value of 358 at λ_{max} 302 nm for 100% pure material. The $E^{1\%}$ value is derived from the corresponding value obtained for crystalline imipenem monohydrate at its absorption maximum.

for $C_{10}H_{12}N_2O_2S$: C, 53.55; H, 5.40; N, 12.49; S, 14.29. Found: C, 53.54; H, 5.34; N, 12.49; S, 14.63.

HPLC of Imipenem (1). The initial HPLC analysis of **1** was performed on a Waters 3.9 mm × 30 cm μBondapak C_{18} reverse-phase column using aqueous 0.01 M KH_2PO_4 as solvent at a flow rate of 1.5 mL/min. UV detection at 254 nm showed two peaks with retention times of 5.8 and 6.6 min corresponding to the *Z* and *E* isomers **1a** and **1b**. Dissolution of crystalline imipenem monohydrate in ice-cold, deionized H_2O (1 mg/mL) followed by immediate HPLC analysis gave a 70:30 *Z*/*E* mixture, whereas dissolution in ice-cold 0.01 M KH_2PO_4 (1 mg/mL) provided an 83:17 *Z*/*E* mixture. The same 83:17 ratio was observed after storing the acidic imipenem solution at 0 °C for 10, 24, and 40 min, indicating that no isomerization occurred on brief storage at pH 4.8. The acidic solution equilibrated to the 7:3 mixture on standing overnight at room temperature. Collection of eluant from the front end of the first peak gave a 90:10 mixture of **1a** to **1b** that instantly equilibrated to the 7:3 mixture on basification to pH 9.

The separation of imipenem isomers was improved by lowering the pH still further. The final system arrived at for isolating the two forms and studying their equilibration consisted of a 3.9 mm × 30 cm Waters 10-μm reverse-phase C_{18} column equilibrated at 24 °C with 0.1 M pH 3.5 potassium acetate/acetic acid buffer. Flow was maintained at 1.4 mL/min and the column eluate was monitored at 300 nm. A 20 mg/mL solution of amorphous imipenem was prepared and 10 μL chromatographed in the above system. The early half of the first peak and the late half of the second peak were collected at the detector output and reassayed after adjustment of the pH to 5.5. The two samples obtained in this manner had initial **1a** to **1b** ratios of 75:25 and 49:51. Both samples were maintained in an ice bath and periodically reassayed. The data revealed gradual convergence of both samples to the equilibrium mixture. At about 340 min, both samples were allowed to warm to room temperature and by 400 min both had reached the same 65:35 **1a** to **1b** composition. No further change in composition was observed after storing the solutions overnight.

HPLC of 11. HPLC analysis of **11** (Waters 3.9 mm × 30 cm μBondapak C_{18} column, mobile phase 0.01 M KH_2PO_4, flow rate 1.5 mL/min, UV detection at 254 nm) showed two peaks with retention times of 6.2 and 7.3 min corresponding to the *Z* and *E* isomers **11a** and **11b**. Dissolution of recrystallized **11** in ice-cold, deionized H_2O (1 mg/mL) followed by immediate HPLC analysis gave a 72:28 *Z*/*E* mixture, whereas dissolution in ice-cold 0.01 M KH_2PO_4 (1 mg/mL) provided ca. a 95:5 *Z*/*E* mixture that slowly equilibrated to the 7:3 mixture on standing overnight at room temperature. Collection of the eluant from the trailing end of the minor peak provided a mixture enriched in the *E* isomer (ca. 1:1 *Z*/*E*) that also slowly equilibrated to the 7:3 mixture.

HPLC of Metabolite I (20). DHP-I degraded imipenem was analyzed on a Waters 3.9 mm × 30 cm μBondapak C_{18} column by using a mobile phase made by mixing equal volumes of 0.5 mM tetrabutylammonium hydroxide adjusted to pH 7.0 with phosphoric acid and 0.5 mM KH_2PO_4 adjusted to pH 7.0 with KOH. Solvent flow was maintained at a rate of 1.5 mL/min. UV detection at 215 nm revealed a 3:2 mixture of components **20a** and **20b** having retention times of 9.1 and 10.2 min, respectively. Under these conditions, no separation of imipenem rotational isomers **1a** and **1b** was detected and imipenem appeared as a single peak having a retention time of 7.1 min.

X-ray Crystal Structure Analysis of Imipenem (1). Tiny crystals (75 μm × 75 μm × 175 μm) of **1** ($C_{12}H_{17}N_3O_4S \cdot H_2O$, FW = 317.4) for X-ray diffraction studies formed from H_2O with space group symmetry of $P2_12_12_1$ and cell constants of a = 8.268 (3) Å, b = 11.140 (6) Å, and c = 15.452 (9) Å for Z = 4 and a calculated density of 1.481 g/cm^3. Of the 1139 reflections measured with an automatic four circle diffractometer equipped with Cu radiation, 613 were observed ($|\geq 3\sigma|$). The structure was solved with a multisolution direct methods approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[29] Hydrogens could not be properly refined because of the small number of observed reflections. The function $\sum w(|F_o| - |F_c|)^2$ with $w = 1/(\sigma F_o)^2$ was minimized to give an unweighted residual of 0.075. The formamidinium group is in the *Z* configuration. The solvent molecule of H_2O (O21) appears to be involved with four hydrogen bonds: O21–N19 2.84 Å, O21–O17 2.72 Å, O21–O18 2.79 Å, and O21–O15 2.68 Å, while one additional potential hydrogen bond of length 2.86 Å connects N20 and O15. Tables IV, V, and VI containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as supplementary material. Figure 1 is a computer-generated perspective drawing of **1** from the X-ray coordinates.

X-ray Crystal Structure Analysis of 11. Suitable crystals of **11** ($C_{10}H_{12}N_2O_2S$, FW = 224.3) for X-ray diffraction studies formed from H_2O with space group symmetry of $P2_1/n$ and cell constants of a = 8.811 (2) Å, b = 16.340 (4) Å, c = 7.599 (1) Å, and β = 108.03 (2)° for Z = 4 and a calculated density of 1.432 g/cm^3. Of the 1410 reflections measured with an automatic four circle diffractometer equipped with Cu radiation, 1149 were observed ($|\geq 3\sigma|$). The structure was solved with a multisolution direct methods approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[29] Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\sum w(|F_o| - |F_c|)^2$ with $w = 1/(\sigma F_o)^2$ was minimized to give an unweighted residual of 0.032. The molecule is in a zwitterionic form as evidenced by the bond distances and positions of the hydrogens and the formamidinium group is in the *Z* configuration. Three intermolecular hydrogen bonds were noted: one between N14(H14)–O9 for total length 2.74 Å, the second between N15(H15B)–O8 of total length 2.76 Å, and the last between N15(H15A)–O9 for total length 2.91 Å. Tables VII, VIII, and IX containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as supplementary material. Figure 2 is a computer generated perspective of **11** from the X-ray coordinates.

***p*-Nitrobenzyl (5*R*,6*S*)-6-[1(*R*)-Hydroxyethyl]-3-(methylthio)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (13).** A 2.36 M solution of MeSH in dry MeCN (1.5 mL, 3.5 mmol) was added dropwise to an ice-cold, stirred solution of iPr_2NEt (0.61 mL, 3.5 mmol) and (diphenylphosphono)oxy carbapenem **12** (1.856 g, 3.2 mmol) in dry MeCN (15 mL). The resulting mixture was stirred in the cold under an N_2 atmosphere. Additional MeSH in MeCN was added after 50 min (1.2 mL, 2.8 mMol) and after 80 min (4.1 mL, 9.8 mMol). After 3 h, the mixture was filtered to remove the product, which was washed with cold MeCN and EtOAc and dried in vacuo to provide **13** (603 mg, 49.8%) as a pale yellow solid. The filtrate and washings from the first crop were evaporated in vacuo to ca. 5 mL, diluted with EtOAc, washed with H_2O, 5% $NaHCO_3$, and brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. The resulting mixture was cooled in an ice bath to afford additional **13** (238 mg, 19.7%) as an off-white solid. An analytically pure sample was obtained by recrystallization from EtOAc: mp 169-171 °C; IR ($CHCl_3$) 1775, 1700, 1525, 1350, 1320 cm^{-1}; UV (MeOH) λ_{max} 267 (ϵ 11690), 320 (12670) nm; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.37 (d, J = 6.4 Hz, C*H*$_3$CH), 3.09 (dd, J = 8.4, 18.0 Hz, CH*a*Hb), 3.20 (dd, J = 2.6, 6.8 Hz, 6-*H*), 3.32 (dd, J = 9.7, 18.0 Hz, CHa*H*b), 4.18-4.32 (m, 5-*H* and CH_3CH), 5.23 and 5.50 (two d, J = 14.5 Hz, CH_2Ar), 7.64 and 8.22 (two m, Ar *H*). Anal. Calcd for $C_{17}H_{18}N_2O_6S$: C, 53.96; H, 4.79; N, 7.40; S, 8.47. Found: C, 53.39; H, 4.78; N, 7.29; S, 8.51.

Sodium (5*R*,6*S*)-6-[1(*R*)-Hydroxyethyl]-3-(methylthio)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (14). A solution of carbapenem ester **13** (593 mg, 1.57 mmol) in THF (56 mL), EtOH (56 mL), and deionized H_2O (44 mL) containing $NaHCO_3$ (132 mg, 1.57 mMol) was treated with 10% Pd/C (590 mg) and stirred under an H_2 atmosphere for 130 min. The mixture was filtered through a Celite pad and the catalyst washed with H_2O (50 mL). The filtrate was washed with CH_2Cl_2 and Et_2O, concentrated in vacuo to 33 mL, and lyophilized to afford **14** (390 mg) as an amorphous, off-white solid:[30] IR (Nujol) 3340 (br), 1750, 1590, 1395, 1260, 1070 cm^{-1}; UV (0.05M pH 7.0 MOPS buffer) λ_{max} 302 ($E^{1\%}$ 275, 95% NH_2OH extinquished) nm; ^{1}H NMR (200 MHz, D_2O) see Table II; ^{13}C NMR (62.9 MHz, D_2O) see Table III.

Chemical Hydrolysis 14. A solution of carbapenem **14** (5.6 mg) in D_2O (0.5 mL) was acidified to pD = 1 by addition of 0.1 M D_2SO_4 in D_2O (0.5 mL). The resulting solution was stored at ambient temperature (ca. 22 °C) and periodically examined by ^{1}H NMR. After 40 min, the solution was neutralized by addition of solid Na_2CO_3 (8 mg) and then kept at room temperature an additional 120 min while periodically examining it by ^{1}H NMR. The NMR spectra revealed immediate acid-catalyzed hydrolysis

to the protonated 2-pyrroline **15**, which remained stable under the acidic conditions. Neutralization gave a mixture of 2-pyrroline **16** and 1-pyrrolines **17a** plus **17b**, which slowly isomerized to a 2:1 mixture of **17a** and **17b**. The ^{1}H NMR (200 MHz, D_2O) data for compounds **15**, **16**, **17a**, and **17b** appear in Table II.

In a parallel UV experiment, carbapenem **14** (0.828 mg) was dissolved in H_2O (20.0 mL) and 3.6 mL of this solution was acidified to pH 1 with concentrated H_2SO_4 (10 μL). The UV spectrum was recorded with time. After 12 min, the acid solution was brought to pH 7 by addition of solid Na_2CO_3 (29 mg) and the UV again monitored periodically. Acidification of the UV solution resulted in immediate formation of the protonated pyrroline **15** as evidenced by replacement of the starting carbapenem chromophore at 302 nm with a slightly more intense chromophore at 288 nm. Neutralization of the UV solution lead to a less intense chromophore at 266 nm attributed to the 2-pyrroline **16**, which gradually disappeared with time.

Enzymatic Hydrolysis of 14. A solution of carbapenem **14** (5 mg) in D_2O (1.0 mL) containing $NaHCO_3$ (2.4 mg) was kept at ambient temperature (ca. 22 °C) and periodically examined by UV (25-μL aliquots in 3.0 mL of H_2O). The absence of change in the intensity and position of the UV chromophore indicated carbapenem **14** was stable to the slightly basic reaction conditions. After 70 min, a solution of purified porcine DHP-I[18] (5 μg) in D_2O (10 μL) was added and the resulting solution was kept at ambient temperature and periodically examined by ^{1}H NMR and UV spectroscopy. After 100 min with DHP-I present, the solution was acidified to pH 0–1 with concentrated H_2SO_4 (4 μL) and the ^{1}H NMR spectrum recorded again. The NMR spectra revealed DHP-I mediated hydrolysis to the 2-pyrroline **16**, which isomerized to a mixture of 1-pyrrolines **17a** and **17b**. After 60 min in the presence of DHP-I, carbapenem **14** was absent and the products were ca. a 2:1:1 mixture of **17a**:**17b**:**16**. After 90 min, the ratio was ca. 2:1:0.5. Acidification gave a mixture of products containing the protonated 2-pyrroline **15**. The NMR results were supported by the UV spectra, which showed replacement of the carbapenem chromophore at 302 nm with a reduced intensity chromophore at 266 nm associated with the 2-pyrroline **16**.

Chemical Hydrolysis of Imipenem (1). A solution of imipenem monohydrate (5.0 mg) in 0.05 M D_2SO_4 in D_2O (1.0 mL) was stored at ambient temperature (ca. 22 °C) and periodically examined by ^{1}H NMR. After 60 min, the solution was neutralized with solid Na_2CO_3 (8 mg) and again examined by ^{1}H NMR. The NMR spectra revealed immediate acid-catalyzed hydrolysis to the protonated 2-pyrroline **18**, which underwent minor degradation on storage under the acidic conditions. Neutralization gave a mixture of 1-pyrrolines **20a** and **20b** as evidenced by comparison of its ^{1}H NMR spectrum to that of the **17a**,**b** mixture: ^{1}H NMR of **18** (200 MHz, D_2O) δ 1.29 (d, J = 6.4 Hz, CH_3CH), 3.09 (t, J = 5.6 Hz, 6-H), 3.18–3.56 (m, 4-CH_2), 3.26 (t, J = 6.2 Hz, SCH_2), 3.63 (t, J = 6.2 Hz, NCH_2), 4.31 (mc, CH$_3$CH), 4.45 (mc, 5-H), 4.79 (HOD), 7.83 (s, NCHN of major rotomer), 7.85 (shoulder on δ 7.85 peak, NCHN of minor rotomer); ^{1}H NMR of **20a**,**b** (200 MHz, D_2O) δ 1.20 (d, J = 6.2 Hz, CH_3CH of **20b**), 1.21 (d, J = 6.2 Hz, CH_3CH of **20a**), 1.70 (dd, J = 8.5, 13.8 Hz, 4-Ha of **20a**), 2.17 (dd, J = 7.2, 14.3 Hz, 4-Ha of **20b**), 2.28–2.44 (m, 6-H of **20a**,**b** and 4-Hb of **20b**), 2.72 (dd, J = 8.6, 13.8 Hz, 4-Hb of **20a**), 2.84 (mc, SCH_2 of **20a**,**b**), 3.52 (mc, NCH_2 of **20a**,**b**), 4.14 (mc, CH$_3$CH of **20a**,**b**), 4.28 (mc, 5-H of **20a**), 4.46 (mc, 5-H of **20b**), 4.79 (HOD), 7.79, 7.80, and 7.84 (three s, NCHN of **20a**,**b**); ^{13}C NMR of **20a**,**b** (62.9 MHz, D_2O) see Table I.

In a parallel UV experiment, imipenem monohydrate (0.491 mg) was dissolved in H_2O (10.0 mL) and 3.6 mL of this solution was acidified to pH 1 with concentrated H_2SO_4 (10 μL). After a few minutes, the acidic solution was neutralized by addition of solid Na_2CO_3 (28 mg). Acidification resulted in formation of the protonated 2-pyrroline **18** as evidenced by replacement of the initial carbapenem chromophore at 298 nm with a slightly less intense chromophore at 282 nm. Neutralization of the acid solution afforded a considerably less intense absorbance at 265 nm that is attributed to the 2-pyrroline **19**.

Diketopiperazines 21b, 22b, and 23b. A solution of methylthio carbapenem **14** (49 mg) in deionized H_2O (1.3 mL) was adjusted from pH 7.6 to pH 3 by addition of 1 M H_2SO_4 (0.083 mL). The resulting mixture was stirred at ambient temperature and under an N_2 atmosphere. The pale yellow solution gradually gave way to a yellow-orange suspension. After 30 min, the mixture was extracted with CH_2Cl_2 (3 × 5 mL) and the extracts were dried over $MgSO_4$, filtered, and evaporated in vacuo to give a yellow glass. This material was triturated with Et_2O to afford **21b** (6 mg) as a yellow powder: IR ($CHCl_3$) 3700, 3440 (br), 1700, 1645, 1600, 1420 cm^{-1}; IR (Nujol) 3400 (br), 1720, 1640, 1600, 1420 cm^{-1}; UV (dioxane) λ_{max} 366 ($E^{1\%}$ 437) nm; ^{1}H NMR (200 MHz, $CDCl_3$) see Table II; ^{13}C NMR (62.9 MHz, D_2O) see Table III; FAB-MS, m/e 487 (M^+ + H).

A sample of **21b** was dissolved in H_2O containing $NaHCO_3$ and the solution was lyophilized to afford disodium salt **22b** as a yellow, amorphous powder: IR (Nujol) 3380 (br), 1635, 1590 cm^{-1}; UV (0.05 M pH 7.0 MOPS buffer) λ_{max} 373 nm; ^{1}H NMR (200 MHz, D_2O) δ 1.27 (d, J = 6.2 Hz, CH_3CH), 2.49 (s, SCH_3), 2.67 (dd, J = 2.8, 9.8 Hz, CHCO$_2$Na), 3.31 (dd, J = 4.8, 18.5 Hz, CHaHb), 3.42 (dd, J = 9.5, 18.5 Hz, CHaHb), 4.08 (dq, J = 6.2, 9.8 Hz, CH$_3$CH), 4.87 (HOD masking the CHN resonance).

A solution of **21b** in EtOAc was treated with excess ethereal CH_2N_2 solution. After standing a few minutes at room temperature, the mixture was evaporated in vacuo and the residue was diluted with EtOAc, washed with 5% $NaHCO_3$ and brine, dried with $MgSO_4$, filtered, and evaporated in vacuo. The residual oil was chromatographed on an Analtech 0.25 mm × 20 × 20 cm silica gel GF plate using EtOAc as developing solvent. The major UV visible band at R_f 0.27–0.33 was extracted with EtOAc to provide the dimethyl ester **23b** as a yellow oil: IR ($CHCl_3$) 1725, 1640, 1415 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.19 (d, J = 6.0 Hz, CH_3CH), 2.36 (mc, CHCO$_2$CH$_3$), 2.39 (s, SCH_3), 3.06 (dd, J = 2.5, 17.2 Hz, CHaHb), 3.25 (dd, J = 9.8, 17.2 Hz, CHaHb), 3.70 (s, CO$_2$CH_3), 3.95 (mc, CH$_3$CH), 5.08 (mc, CHN); HR-MS, exact mass calcd for $C_{22}H_{30}N_2O_8S_2$ 514.1444, found 514.1444.

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Supplementary Material Available: Tables of final fractional coordinates, temperature parameters, bond distances, and bond angles for **1** and **11** (6 pages). Ordering information is given on any current masthead page.