Synthesis and Biological Activity of Substituted [[3(S)-(Acylamino)-2-oxo-1-azetidinyl]oxy]acetic Acids. A New Class of Heteroatom-Activated β-Lactam Antibiotics

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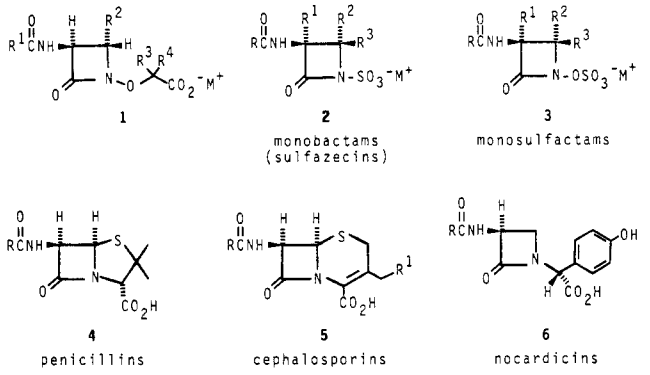
The synthesis of substituted [[3(S)-(acylamino)-2-oxo-1-azetidinyl]oxy]acetic acids (1) is described. 3-[(Carbobenzyloxy)amino]-*N*-hydroxy-2-azetidinones (**13a,b**), prepared from serine and threonine, were alkylated with 2-(trimethylsilyl)ethyl bromoacetate in the presence of potassium carbonate in THF/H_2O. Alkylation with secondary α-bromo esters was accomplished with potassium hydroxide in dimethyl sulfoxide. The Cbz group was replaced with the 2-(2-amino-4-thiazolyl)-2(*Z*)-(methoxyimino)acetamido side chain by catalytic hydrogenation followed by treatment with **21**. Removal of the 2-(trimethylsilyl)ethyl ester with fluoride ion provided derivatives suitable for antimicrobial evaluation. In vitro tests showed that the title compounds possess significant activity predominantly against Gram-negative bacteria.

Recently we reported the preparation of the [[3(*S*)-(acylamino)-2-oxo-1-azetidinyl]oxy]acetic acids (1),[1] a new

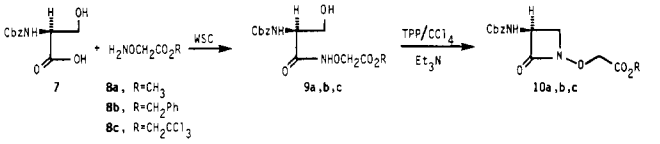


class of monocyclic β-lactam antibiotics. These β-lactams are activated toward nucleophilic attack at the β-lactam carbonyl by the presence of the electronegative oxygen atom on the ring nitrogen. Both the naturally occurring monobactams **2**[2] (sulfazecin)[3] and the related monosulfactams **3**[4] possess similar heteroatom activation. But unlike these monocyclic β-lactam antibiotics, derivatives of **1** possess the more common carboxylic acid moiety as the requisite ionizable group. Various acyl derivatives of the parent structure **1** (R^3, R^4 = H) possess significant biological activity even though the presence of the oxygen atom places the ionizable group one atom further from the β-lactam nitrogen than in the penicillins **4**, cephalosporins **5**, and nocardicins **6**. Herein we report, in full detail, our synthesis of forms of **1** that allows for substitution (R^1, R^2, R^3, R^4) about the parent nucleus with full control of stereochemistry.

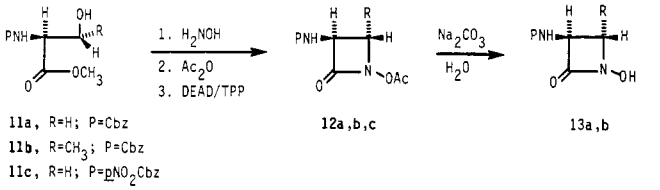
Our original approach toward the preparation of **1** utilized the hydroxamate-mediated synthesis of β-lactams developed earlier in our laboratory.[5] As shown in Scheme I, Cbz-L-serine was coupled to α-(aminooxy)acetate esters with a water-soluble carbodiimide (WSC). The resulting hydroxamates **9** were cyclized under modified Mitsunobu conditions.[6] Although this method provided the protected nucleus **10** in good yields, we sought a more versatile method that would allow for modifications of the oxyacetic acid moiety without necessitating the need for the preparation of individual α-(aminooxy)alkyl esters.

Due to their unusual acidity (pK = 5–7),[5] the utility of *N*-hydroxy-2-azetidinones (**13**), prepared from serine and threonine[7] (Scheme II), as precursors to the title compounds was explored. Reaction of **13a** with (*S*)-(+)-*p*-nitrobenzyl mandelate under Mitsunobu conditions[6] failed

Scheme I



Scheme II



Scheme III

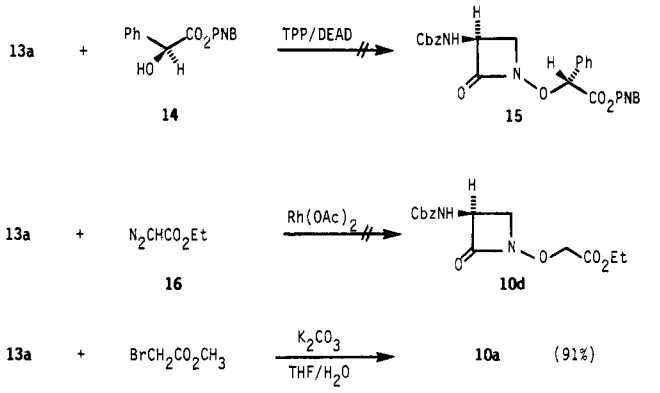


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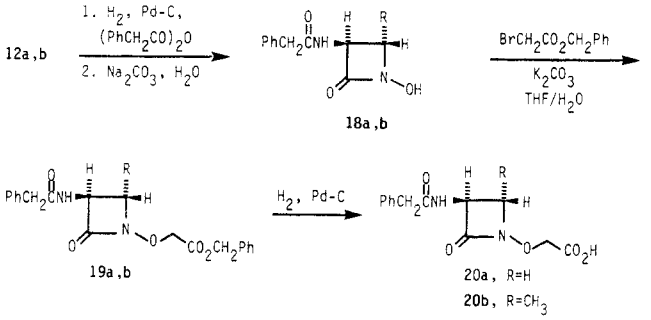
Table I. Broad-Spectrum Agar Disk Plate Assay[a]

organism concn, mg/mL	zone of inhibn, mm								
	20a 10	**20b** 10	**25** 10	**26** 10	**31** 10	**37a** 1	**37b** 1	**27** 10	**27** 1
Staphococcus aureus X1	23	13	tr	19	18			22	tr
Bacillus subtilis X12	16	11	tr	13	12			23	15
Bacillus subtilis X12M	20	18	13	19	21			30	16
Bacillus stearothermophilus C451	tr		24	24				30	25
Sarcina lutea X186	23	23	21	22	15	tr	tr	27	25
Proteus vulgaris X45	10	10	21	36	19	25	28	46	40
Salmonella gallinarum X142	tr	tr	22	30	22	17	25	36	48
Escherichia coli X161		tr	20	35	24	26	17	40	40
Escherichia coli X161M			19	44	25	28	30	54	40
Escherichia coli X580	11	26	37	56	36	38	46	66	54
Pseudomonas aeruginosa X48				15				25	tr
Serratia marcescens X99			16	33	23	18	24	40	33
Pseudomonas solanacearum X185			16	20				40	29

[a] Performed at Eli Lilly and Co. using standard methods.

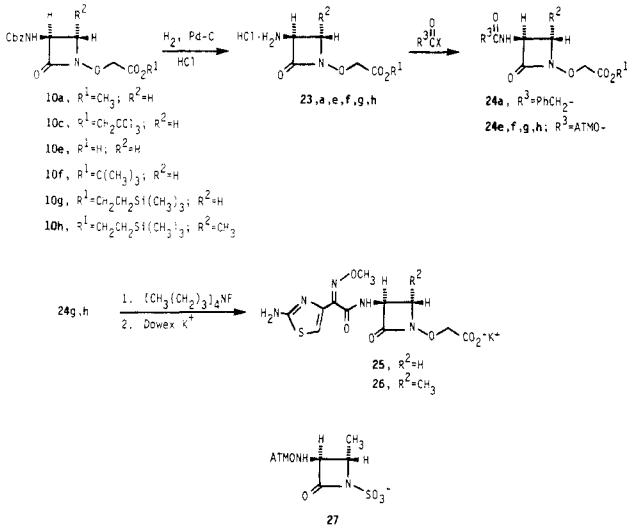
Scheme IV



to give any of the coupled product **15** (Scheme III). Intermolecular carbene insertion reactions into the O–H bond were also inefficient as the reaction of **13a** with ethyl diazoacetate in the presence of a catalytic amount of rhodium acetate[8] gave a complex mixture of products. Fortunately, we found that the *N*-hydroxy-2-azetidinones could be simply alkylated with alkyl halides in the presence of base. Thus, alkylation of **13a** with methyl bromoacetate in THF/H_2O with 1 equiv of potassium carbonate gave **10a** in 91% yield.

The β-lactams **20a,b**, with the phenylacetyl side chain, were then prepared by the alkylation of **18a,b**[7] with benzyl bromoacetate followed by catalytic hydrogenation of the benzyl ester (Scheme IV). Preliminary antibacterial tests of **20a,b** (Table I) prompted us to replace the phenylacetyl group with the more biologically responsive 2-(2-amino-4-thiazolyl)-2(*Z*)-(methoxyimino)acetamido (ATMO) side chain. Introduction of the ATMO side chain by catalytic hydrogenation of **12a** in the presence of the *N*-hydroxybenzotriazole ester of 2-(2-amino-4-thiazolyl)-2(*Z*)-(methoxyimino)acetic acid (**21**) was attempted, but the Cbz group could not be removed (eq 1). Presumably the sulfur-containing ATMO active ester was poisoning the catalyst. Replacement of the Cbz group with the *p*-nitrobenzyl carbamate did not facilitate the hydrogenation. We therefore turned our attention to introduction of the side chain after the parent nucleus had been prepared.

Scheme V



The Cbz–amino free acid **10e** (Scheme V), prepared by saponification of methyl ester **10a**, was hydrogenated in the presence of 1 equiv of 1.2 N HCl. Although the hydrochloride salt **23e** was not isolated, treatment with benzyl chloroformate gave the original starting material **10e** back in a 40% yield. Unfortunately, we were unable to isolate any of **24e** by treatment of **23e** with the ATMO active ester **21** (Scheme V). When the free acid was protected as an ester, this acylation step proceeded in good yield. Hy-

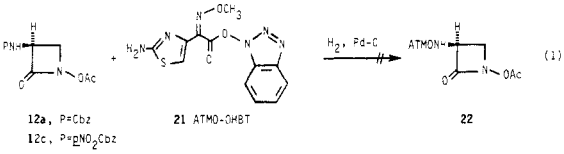


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Table II. Minimum Inhibitory Concentrations[a]

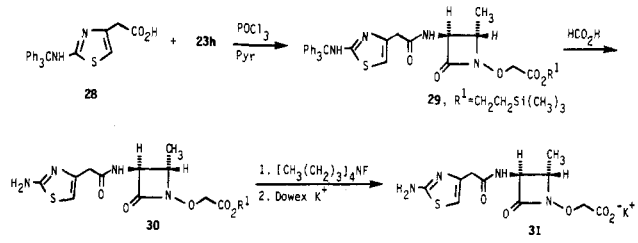
organism	MIC, μg/mL 25	26	27
Staphylococcus aureus (4 strains)	>64	>64	>64
Staphyloccus epidermidis (2 strains)	>64	>64	>64
Streptococcus pyogenes C203	16	16	4
Streptococcus pneumoniae Park I	32	32	4
Streptococcus sp. Group D (2 strains)	>64	>64	>64
Haemophilus influenzae C.L.	16	1	0.5
Haemophilus influenzae 76	16	1	0.25
Escherichia coli N10	8	0.25	0.125
Escherichia coli EC14	4	0.25	0.06
Escherichia coli TEM	32	0.5	0.125
Klebsiella pneumoniae X26	8	0.25	0.06
Klebsiella pneumoniae KAE	>64	32	>64
Klebsiella pneumoniae X68	8	0.25	0.125
Enterobacter aerogenes C32	8	0.25	0.125
Enterobacter aerogenes EB 17	8	0.25	0.125
Enterobacter cloacae EB5	16	1	0.125
Enterobacter cloacae 265A	>64	>64	>64
Salmonella typhi X514	4	0.125	0.06
Salmonella typhi 1335	8	0.25	0.125
Pseudomonas aeruginosa (4 strains)	>64	>64	>64
Serratia marcescens X99	16	0.5	0.25
Serratia marcescens SE3	16	1	0.25
Proteus morganii PR15	32	2	0.25
Proteus inconstans PR33	2	0.125	0.03
Proteus rettgeri C24	2	0.125	0.03
Citrobacter freundii CF17	16	2	0.25
Acinetobacter calcoaceticus AC12	>64	>64	4

[a] Performed at Eli Lilly and Co. using the standard agar dilution method.[21]

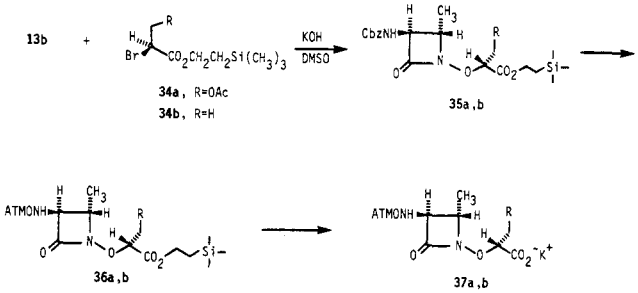
drogenation of methyl ester **10a** followed by acylation with phenylacetic anhydride gave **24a** in a 56% yield. However, the methyl ester could not be saponified without destruction of the β-lactam ring. The use of esters that could be removed under nonbasic, nonhydrogenolytic conditions was therefore examined. The Cbz group of the 2,2,2-trichloroethyl ester[9] **10c** proved resistant to hydrogenation, but the Cbz group of both the *tert*-butyl ester **10f** and the 2-(trimethylsilyl)ethyl ester[10] **10g** underwent hydrogenation in the presence of HCl to give the unstable hydrochloride salts **23f** and **23g**. Separate reaction of **23f** and **23g** with the ATMO active ester **21** gave the acylated products **24f** and **24g** in overall yields of 77% and 76% from the carbamates **10f** and **10g**, respectively. All attempts to cleave the *tert*-butyl ester under acidic conditions gave predominant opening of the β-lactam ring. Fortunately, treatment of **24g** with tetra-*n*-butylammonium fluoride[10] cleanly removed the 2-(trimethylsilyl)ethyl ester to give the tetra-*n*-butylammonium salt. Ion-exchange chromatography provided the potassium salt **25** (93%). The 4-methyl derivative **26** was prepared in the same manner from L-threonine. Both **25** and **26** possess significant antimicrobial activity predominantly against Gram-negative bacteria (Tables I and II). The 4-methyl group increases the activity such that the minimum inhibitory concentrations (MICs) of **26** are only twice as large as the MICs of the corresponding monobactam **27**, with the same ATMO side chain.

In order to study the scope of the biological activity of **1** (R^3, R^4 = H), the ATMO side chain was replaced with the aminothiazoleacetic acid side chain (Scheme VI). Thus, hydrochloride salt **23h** was coupled to *N*-trityl-2-aminothiazole-4-acetic acid (**28**) with $POCl_3$/pyridine

Scheme VI

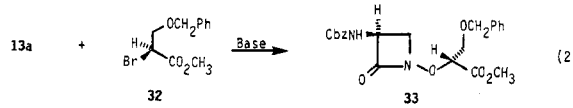


Scheme VII



(74%). Removal of the trityl group with formic acid (43%) followed by the usual deprotection of the 2-(trimethyl)ethyl ester gave potassium salt **31** (91%). No significant increase in activity against Gram-positive bacteria was seen in **31**. In fact, overall **31** was less active than **26** (Table I).

As part of a study to determine the effects that substituents on the oxyacetic acid moiety have on activity, we examined the alkylation of *N*-hydroxy-2-azetidinones with secondary α-bromo esters. Reaction of methyl 2(*S*)-bromo-3-(benzyloxy)propionate (**32**) (prepared from *O*-benzyl-L-serine by deaminative bromination[11] followed by esterification) with **13a** under the aforementioned alkylation conditions (K_2CO_3, THF/H_2O) failed to give any **33** (eq 2). Alkylation under phase-transfer-catalyzed con-



(2)

ditions in a homogeneous solution[12] (Et_3N, $(n\text{-Bu})_4NBr$, CH_3CN) provided **33** in a low yield (22%). The best yield was obtained when potassium hydroxide was used as the base in dimethyl sulfoxide[13] (75%). The alkylation proceeded with complete inversion to give only the one diastereomer as seen by ^1H NMR and HPLC. In this manner, alkylation of **13b** with secondary α-bromo esters **34a** and **34b** provided **35a** (57%) and **35b** (50%) (Scheme VII). Deblocking (H_2, Pd–C, HCl), acylation (Et_3N, **21**; 50–75%), and deprotection ($(n\text{-Bu})_4NF$; Dowex K^+; 55–73%) provided **37a** and **37b**. Substitution on the oxyacetic acid moiety has little effect on biological activity. In fact, the unsubstituted parent compound **26** is more active (Table I).

Conclusions. Representative forms of **1** were prepared by the alkylation of *N*-hydroxy-2-azetidinones with α-bromo esters in good yields under specific conditions. Due to the activating effect of the N–O bond, these compounds show good to potent activity against Gram-negative bac-

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teria even though the ionizable carboxyl group is displaced one atom further from the β-lactam nitrogen than in the penicillins, cephalosporins, and nocardicins. Simple alkyl substituents on the oxyacetic acid moiety appear to decrease the biological activity.

Experimental Section

General Comments. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 727b spectrometer. ^{1}H NMR spectra were obtained in chloroform-*d* with tetramethylsilane as a reference, unless otherwise stated, on a Varian EM 390 or XL-100 or Nicolet NB 300 spectrometer. Field desorption mass spectra were recorded by John Occolwitz at Eli Lilly and Co. Electron impact mass spectra were recorded on an AEI Scientific Apparatus MS 902. Elemental analyses were determined by Midwest Microlabs, Indianapolis, IN, or M-H-W Laboratories, Phoenix, AZ. High-pressure liquid chromatography was carried out on a Beckman/Altex Model 332 chromatograph. Radial chromatography was performed on a Chromatotron Model 7924 purchased from Harrison Research Inc., Palo Alto, CA. Optical rotations were obtained with a Rudolf 574 polarimeter. TLC was carried out with use of aluminum-backed silica gel 60 F-254, 0.2-mm plates purchased from MCB Reagents. Opti-up C_{12} glass-backed plates purchased from Fluka Chemical Corp. were used for reversed-phase TLC. Whatman #1 filter paper was used for paper chromatography. Solvents used were dried and purified by standard methods. Biological testing was done at Eli Lilly and Co. by standard methods.

Methyl (aminooxy)acetate (8a) was prepared according to literature procedures.[14] A solution of 3.0 g (24 mmol) of (aminooxy)acetic acid hydrochloride[15] in 25 mL of anhydrous methanol was saturated at room temperature with hydrogen chloride and then allowed to stand for 24 h. The solution was concentrated under reduced pressure, cooled in an ice bath, dissolved in 10 mL of water, and then rendered alkaline with sodium carbonate. The known free base[14] was extracted with ethyl acetate, dried over magnesium sulfate, filtered, and evaporated to leave 1.9 g (18 mmol, 77%) of oil.[14] ^{1}H NMR: δ 3.75 (s, 3 H), 4.2 (s, 2 H), 6.0 (br s, 2 H).

Benzyl (aminooxy)acetate hydrochloride (8b) was prepared similarly from (aminooxy)acetic acid and benzyl alcohol. Treatment of the free base with hydrogen chloride provided the hydrochloride salt: mp 86–87 °C; ^{1}H NMR (D_2O) δ 4.95 (s, 2 H), 5.45 (s, 2 H), 7.7 (s, 5 H).

2,2,2-Trichloroethyl (aminooxy)acetate (8c) was prepared by the general procedure of Carson.[16] A 2.0-g (15.7 mmol) portion of (aminooxy)acetate acid, 21.1 g (142 mmol) of 2,2,2-trichloroethanol (Aldrich), and 7.5 g (39.3 mmol) of *p*-toluenesulfonic acid monohydrate in 100 mL of carbon tetrachloride was refluxed for 48 h in a flask fitted with a Soxhlet extractor containing anhydrous sodium sulfate. The solvent was evaporated, and trituration with ether followed by recrystallization from ethanol gave 4.3 g (10.9 mmol, 70%) of product as the *p*-toluenesulfonic acid salt: mp 164–166 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.3 (s, 3 H), 5.0 (s, 2 H), 5.1 (s, 2 H), 7.2–7.8 (dd, 4 H), 9.6 (br s, 3 H); IR (KBr) 3450 br, 1760 cm^{-1}. Anal. ($C_{11}H_{14}Cl_3NO_6S$) C, H, N. Treatment with sodium carbonate provided the free base as a clear oil: ^{1}H NMR δ 4.5 (s, 2 H), 5.0 (s, 2 H), 6.3 (br s, 2 H).

Formation of the hydroxamates was accomplished by the procedure reported earlier.[5] Thus, a solution of Cbz-L-serine and 1 equiv of the (aminooxy)acetate ester free base or hydrochloride salt in H_2O or H_2O/THF was adjusted to pH 4–5, and an aqueous solution of the water-soluble carbodiimide (WSC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride, 2.5 equiv, Sigma) was added. The pH was maintained at 4–5 by addition of 1 N HCl. After 30 min the reaction mixture was extracted with three portions of ethyl acetate. The combined ethyl acetate was washed with 1 N citric acid, 5% $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was recrystallized from ethyl acetate/hexanes.

Methyl [[[(carbobenzyloxy)-L-seryl]amino]oxy]acetate (9a) was prepared from Cbz-L-serine and methyl (aminooxy)acetate in this manner in 88% yield: mp 89.5–91.5 °C; ^{1}H NMR: δ 3.7 (s, 3 H), 3.8–4.3 (m, 4 H, α-CH, β-CH_2, OH), 4.45 (s, 2 H), 5.1 (s, 2 H), 6.0 (br d, 1 H), 7.2 (s, 5 H), 9.5 (br s, 1 H); IR (in $CDCl_3$) 3100–3700, 1750, 1680–1720 cm^{-1}. Anal. ($C_{14}H_{18}N_2O_7$) C, H, N. TLC (silica, ethyl acetate) R_f 0.37.

Benzyl [[[(carbobenzyloxy)-L-seryl]amino]oxy]acetate (9b) was prepared from Cbz-L-serine and benzyl (aminooxy)acetate hydrochloride in 80% yield: mp 111–113 °C; ^{1}H NMR δ 3.5–4.3 (m, 4 H, α-CH, β-CH_2, OH), 4.4 (s, 2 H), 5.05 (s, 2 H), 5.1 (s, 2 H), 6.15 (br d, 1 H), 7.25 (s, 5 H), 7.3 (s, 5 H), 10.5 (br s, 1 H); IR (in $CDCl_3$) 3100–3700, 1740, 1680–1720 cm^{-1}. Anal. $C_{20}H_{22}N_2O_7$) C, H, N. TLC (silica, ethyl acetate) R_f 0.46.

2,2,2-Trichloroethyl [[[(carbobenzyloxy)-L-seryl]amino]oxy]acetate (9c) was prepared from Cbz-L-serine and 2,2,2-trichloroethyl (aminooxy)acetate in 59% yield: mp 48–51 °C dec; ^{1}H NMR δ 3.5–4.5 (m, 4 H, α-CH, β-CH_2, OH), 4.6 (s, 2 H), 4.75 (s, 2 H), 5.1 (s, 2 H), 6.1 (br d, 1 H), 7.3 (s, 5 H), 10.3 (br s, 1 H); IR (in $CDCl_3$) 3150–3700, 1760, 1680–1730 cm^{-1}; TLC (silica, ethyl acetate) R_f 0.41.

Cyclization to the β-lactams was accomplished by utilizing the procedure previously reported.[5] Treatment of the hydroxamate with 1 equiv of triphenylphosphine, carbon tetrachloride, and triethylamine in acetonitrile, followed by chromatography and recystallization, gave the 2-azetidinones.

Methyl [[3(*S*)-[(benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]acetate (10a) was prepared by cyclization of **9a** in 60% yield: mp 59–61 °C; ^{1}H NMR δ 3.55 (dd, 1 H), 3.7 (s, 3 H), 3.8 (t, 1 H), 4.45 (s, 2 H), 4.55 (m, 1 H), 5.05 (s, 2 H), 6.3 (br d, 1 H), 7.3 (s, 5 H); IR (KBr) 1770, 1740, 1690 cm^{-1}. Anal. ($C_{14}H_{16}N_2O_6$) C, H, N. TLC (silica, ethyl acetate) R_f 0.52.

Benzyl [[3(*S*)-[(benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]acetate (10b) was prepared by cyclization of **9a** in 60% yield: mp 84–86 °C; ^{1}H NMR δ 3.6 (dd, 1 H), 3.9 (t, 1 H), 4.5 (s, 2 H), 4.6 (m, 1 H), 5.1 (s, 2 H), 5.2 (s, 2 H), 5.6 (br d, 1 H), 7.3 (s, 5 H), 7.4 (s, 5 H); IR (KBr) 1770, 1750, 1690 cm^{-1}. Anal. ($C_{20}H_{20}N_2O_6$) C, H, N. TLC (silica, ethyl acetate) R_f 0.67.

2,2,2-Trichloroethyl [[3(*S*)-[(benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]acetate (10c) was prepared by cyclization of **9c** in 56% yield: mp 82–85 °C; ^{1}H NMR δ 3.7 (dd, 1 H), 3.9 (t, 1 H), 4.6 (m, 1 H), 4.65 (s, 2 H), 4.8 (s, 2 H), 5.1 (s, 2 H), 6.3 (br s, 1 H), 7.35 (s, 5 H); IR (KBr) 1780, 1720, 1700 cm^{-1}; TLC (silica, ethyl acetate) R_f 0.68.

3-[(Carbobenzyloxy)amino]-*N*-hydroxy-2-azetidinones 13a and 13b and 3-(phenylacetamido)-*N*-hydroxy-2-azetidinones 18a and 18b were prepared as reported earlier.[7]

Alkylation of *N*-Hydroxy-2-azetidinones with α-Bromoacetates Using Potassium Carbonate in THF/H_2O. To a solution of the *N*-hydroxy-2-azetidinone in (1:1) THF/H_2O (0.05–0.1 M) were added 1 equiv of both potassium carbonate and the α-bromoacetate with stirring at room temperature. The reaction was monitored for disappearance of starting material by TLC (silica, ethyl acetate/hexanes). Upon completion (2–8 h), the reaction mixture was taken up into ethyl acetate, washed with 5% $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed on the chromatotron with silica gel plates (ethyl acetate/hexanes). Solids were recrystallized from ethyl acetate/hexanes.

Methyl [[3(*S*)-[(benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]acetate (10a), identical with that prepared by methods above, was prepared in this manner from **13a** and methyl α-bromoacetate (Aldrich) in a 91% yield.

Benzyl [[3(*S*)-(phenylacetamido)-2-oxo-1-azetidinyl]oxy]acetate (19a) was prepared from **18a** and benzyl α-bromoacetate in 35% yield as a colorless oil: ^{1}H NMR δ 3.5 (br s, 3 H total), 3.9 (t, 1 H), 4.5 (s, 2 H), 4.6 (m, 1 H), 5.2 (s, 2 H), 6.5 (br d, 1 H), 7.3 (s, 5 H), 7.4 (s, 5 H), IR (in $CDCl_3$) 1760, 1650, cm^{-1}; homogeneous on TLC (silica, 1:1 ethyl acetate/hexanes).

Benzyl [[4(*S*)-methyl-3(*S*)-(phenylacetamido)-2-oxo-1-azetidinyl]oxy]acetate (19b) was prepared from **18b** and benzyl α-bromoacetate in 52% yield as a colorless oil: ^{1}H NMR δ 1.45 (d, 3 H), 3.55 (s, 2 H), 3.85 (m, 1 H), 4.2 (dd, 1 H), 4.55 (s, 2 H),

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5.2 (s, 2 H), 6.95 (br d, 1 H), 7.3 (s, 5 H), 7.4 (s, 5 H); IR (in $CDCl_3$) 1760, 1650 cm^{-1}; homogeneous on TLC (silica, 1:1 ethyl acetate/hexanes).

1,1-Dimethylethyl [[3(S)-[(benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]acetate (10f) was prepared from **13a** and *tert*-butyl α-bromoacetate (Aldrich) in 55% yield as a white solid: mp 84–86 °C; ^1H NMR δ 1.4 (s, 9 H), 3.6 (dd, 1 H), 3.85 (t, 1 H), 4.3 (s, 2 H), 4.55 (m, 1 H), 5.1 (s, 2 H), 6.4 (br d, 1 H), 7.35 (s, 5 H); IR (in $CDCl_3$) 1780, 1730 cm^{-1}. Anal. ($C_{17}H_{22}N_2O_6$) C, H, N.

2-(Trimethylsilyl)ethyl [[3(S)-[(benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]acetate (R^1 = H, 10g) was prepared from **13a** and 2-(trimethylsilyl)ethyl α-bromoacetate in 84% yield as a colorless oil: ^1H NMR δ 0.0 (s, 9 H), 1.0 (t, 2 H), 3.7 (dd, 1 H), 3.95 (t, 1 H), 4.35 (t, 2 H), 4.6 (s, 2 H), 4.7 (m, 1 H), 5.2 (s, 2 H), 6.55 (br d, 1 H), 7.65 (s, 5 H); IR (neat) 1785, 1720 cm^{-1}; homogeneous on TLC (silica, 7:3 ethyl acetate/hexanes).

2-(Trimethylsilyl)ethyl [[4(S)-methyl-3(S)-[(benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]acetate (R^1 = CH_3, 10h) was prepared from **13b** and 2-(trimethylsilyl)ethyl α-bromoacetate in 62% yield as a colorless oil: ^1H NMR δ 0.0 (s, 9 H), 1.0 (t, 2 H), 1.5 (d, 3 H), 4.1 (m, 1 H), 4.4 (m, 3 H total), 4.6 (s, 2 H), 5.2 (s, 2 H), 6.2 (br d, 1 H), 7.55 (s, 5 H); IR (neat) 1790, 1720 cm^{-1}; homogeneous on TLC (silica, 1:1 ethyl acetate/hexanes).

[[3(S)-(Phenylacetamido)-2-oxo-1-azetidinyl]oxy]acetic Acid (20a). Benzyl ester **19a** (90 mg, 0.245 mmol) was added to 25 mL of THF, and the flask was flushed with N_2. Pd–C (15 mg of 10%) was added, and H_2 was bubbled through the suspension. After 30 min, starting material had disapeared by TLC (silica, (7:3) ethyl acetate/hexanes). The catalyst was removed by filtration. The THF was evaporated and the residue recystallized from acetone/hexanes to give 48 mg (0.173 mmol, 70%) of white powder: mp 108–110 °C; ^1H NMR (acetone-d_6) δ 3.5 (s, 2 H), 3.6 (dd, 1 H), 3.9 (t, 1 H), 4.55 (s, 2 H), 4.8 (m, 1 H), 6.8 (br s, 1 H), 7.45 (s, 5 H), 8.1 (br d, 1 H); IR (KBr) 3700–2900, 1770 cm^{-1}; FD mass spectrum m/e 279 (M + 1): reversed-phase TLC (1:1 2-propanol/H_2O) R_f 0.70; paper chromatography (2-propanol/H_2O (7.5:2.5)) R_f 0.74. Anal. ($C_{13}H_{14}N_2O_5$) H, N; C: calcd, 56.11; found, 54.97.

[[4(S)-Methyl-3(S)-(phenylacetamido)-2-oxo-1-azetidinyl]oxy]acetic acid 20b was prepared in the same manner from **19b** in 75% yield as a colorless oil: ^1H NMR (acetone-d_6) δ 1.4 (d, 3 H), 3.6 (s, 2 H), 4.0 (m, 1 H), 4.35 (m, 1 H), 4.6 (s, 2 H), 7.5 (s, 5 H), 8.1 (br, 3 H); IR (neat) 3700–2900, 1770 cm^{-1}; reversed-phase TLC (2-propanol/H_2O (1:1)) R_f 0.55; paper chromatography (2-propanol/H_2O (7.5:2.5)) R_f 0.63.

***N*-Acetoxy-3-[[[(*p*-nitrobenzyl)oxy]carbonyl]amino]-2-azetidinone (12a)** was prepared following the procedure reported earlier:[7] mp 165–166 °C; ^1H NMR (Me_2SO-d_6/acetone-d_6/$CDCl_3$) δ 2.1 (s, 3 H), 3.7 (dd, 1 H), 4.0 (t, 1 H), 4.9 (m, 1 H), 5.2 (s, 2 H), 7.6–8.3 (m, 4 H); IR (KBr) 1780, 1700 cm^{-1}. Anal. ($C_{13}H_{13}N_3O_7$) C, H, N.

[[3(S)-[(Benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]-acetic Acid (10e). Compound **10a** (411 mg, 1.33 mmol) was dissolved in 20 mL of ice-cold THF/H_2O (1:1). Exactly 0.719 mL of 1.849 M KOH (1.33 mmol) was added. The reaction was monitored for disappearance of starting material on TLC (silica, ethyl acetate). After 30 min the aqueous solution was extracted with ethyl acetate and then acidified to pH 2.5 with 1.2 N HCl. The acid was extracted into ethyl acetate. The ethyl acetate layers were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. Recrystallization from ethyl acetate/hexanes gave 215 mg (0.731 mmol, 55%) of an analytical sample: mp 111–113 °C; ^1H NMR (Me_2SO-d_6) δ 3.6 (dd, 1 H), 3.9 (t, 1 H), 4.1 (br, 1 H), 4.5 (s, 2 H), 4.6 (m, 1 H), 5.15 (s, 2 H), 7.5 (s, 5 H), 8.2 (br d, 1 H); IR (KBr) 3700–2900, 1760, 1700 cm^{-1}. Anal. ($C_{13}H_{14}N_2O_6$) C, H, N.

Methyl [[3(S)-(Phenylacetamido)-2-oxo-1-azetidinyl]-oxy]acetate (24a). Compound **10a** (100 mg, 0.325 mmol) and 82 mg 0.325 mmol) of phenylacetic anhydride were dissolved in 20 mL of ethyl acetate, and the flask was flushed with N_2. Pd–C (10 mg of 10%) was added, and H_2 was bubbled through the suspension. After 3 h, starting material had disappeared by TLC analysis (silica, 7:3 ethyl acetate/hexanes). The catalyst was removed by filtration and the solvent evaporated. The residue was chromatographed on the chromatotron using a 1-mm silica gel plate (7:3 ethyl acetate/hexanes) to give 53 mg (0.182 mmol, 56%) of product. Recrystallization from ethyl acetate/hexanes provided an analytical sample: mp 128.5–130 °C; ^1H NMR ($CDCl_3$/CD_3OD) δ 3.5 (s, 2 H), 3.6 (dd, 1 H), 3.8 (s, 3 H), 3.9 (t, 1 H), 4.55 (s, 2 H), 4.65 (m, 1 H), 7.3 (s, 5 H); IR (KBr) 3300, 1760, 1730, 1650 cm^{-1}. Anal. ($C_{14}H_{16}N_2O_5$) C, H, N. TLC (silica, 7:3 ethyl acetate/hexanes) R_f 0.20.

2-(Trimethylsilyl)ethyl [[3(S)-[2-(2-Amino-4-thiazolyl)-2(Z)-(methoxyimino)acetamido]-2-oxo-1-azetidinyl]oxy]-acetate (R^2 = H) (24g). Compound **10g** (55 mg, 0.139 mmol) and 0.116 mL of 1.2 N HCl (0.139 mmol) were added to 15 mL of absolute ethanol. The flask was purged with N_2, and 10 mg of 10% Pd–C was added. H_2 was bubbled through the solution. The reaction was followed by disappearance of starting material by TLC (silica, 7:3 ethyl acetate/hexanes). After 45 min, the catalyst was filtered off and the solvent evaporated. A 15-mL portion of THF/ethanol (1:1) was added to the residue. To this ice-cold solution were added 0.039 mL (0.278 mmol) of triethylamine and 45 mg (0.139 mmol) of **21**. The ice bath was removed, and after 24 h the solution was taken up into ethyl acetate, washed with H_2O, 5% $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, evaporated, and chromatographed on the chromatotron using a 1-mm silica gel plate (ethyl acetate). The product was recrystallized from ethyl acetate/hexanes without heating to give 48 mg (0.108 mmol, 76%) of light yellow solid: mp >90 °C dec; ^1H NMR δ 0.0 (s, 9 H), 1.0 (t, 2 H), 3.9 (dd, 1 H), 4.1 (s, 3 H), 4.25 (t, 1 H), 4.35 (t, 2 H), 4.7 (s, 2 H), 5.3 (m, 1 H), 6.0 (br s, 2 H), 6.9 (s, 1 H), 8.9 (br d, 1 H); IR (in $CDCl_3$) 1780, 1750 cm^{-1}; FD mass spectrum m/e 444 (M + 1). Anal. ($C_{16}H_{25}N_5O_6SSi$) C, H, N.

2-(Trimethylsilyl)ethyl [[4(S)-methyl-3(S)-[2-(2-amino-4-thiazolyl)-2(Z)-(methoxyimino)acetamido]-2-oxo-1-azetidinyl]oxy]acetate (R^2 = CH_3) (24h) was prepared in the same manner from **10h** in 50% yield as a light yellow solid: mp > 80 °C dec; ^1H NMR δ 0.0 (s, 9 H), 0.9 (t, 2 H), 1.55 (d, 3 H), 3.9 (s, 3 H), 4.2 (m, 3 H total), 4.5 (s, 2 H), 4.6 (m, 1 H), 5.6 (br s, 2 H), 6.7 (s, 1 H), 8.5 (br d, 1 H); IR (KBr) 1770 br, 1660 cm^{-1}; FD mass spectrum m/e 458 (M + 1). Anal. ($C_{17}H_{27}N_5O_6SSi$) C, H, N.

1,1-Dimethylethyl [[3(S)-[2-(2-amino-4-thiazolyl)-2(Z)-(methoxyimino)acetamido]-2-oxo-1-azetidinyl]oxy]acetate (24f) was prepared in the same manner from **10f** in 77% yield as a yellow solid: mp >100 °C dec; ^1H NMR δ 1.4 (s, 9 H), 3.4–4.2 (m, 5 H total), 4.4 (s, 2 H), 5.2 (m, 1 H), 6.0 (br s, 2 H), 6.65 (s, 1 H), 8.7 (br d, 1 H); IR (in $CDCl_3$) 1780, 1740 cm^{-1}; FD mass spectrum m/e 400 (M + 1).

2-(Trimethylsilyl)ethyl 2(R)-[[4(S)-methyl-3(S)-[2-(2-amino-4-thiazolyl)-2(Z)-(methoxyimino)acetamido]-2-oxo-1-azetidinyl]oxy]-3-acetoxypropionate (R = OAc) (36a) was prepared from **35a** in 49% yield as a light yellow oil: ^1H NMR δ 0.0 (s, 9 H), 1.05 (t, 2 H), 1.6 (d, 3 H), 2.1 (s, 3 H), 3.9 (s, 3 H), 4.05 (m, 1 H), 4.2 (t, 2 H), 4.4 (dd, 1 H), 4.5 (dd, 1 H), 4.65 (m, 3 H total), 6.0 (br s, 2 H), 6.9 (s, 1 H), 8.9 (br d, 1 H); IR (in $CDCl_3$) 1780, 1750 cm^{-1}; FD mass spectrum m/e 530 (M + 1); high-pressure liquid chromatography (Alltech silica 5 μm, 25 cm × 4.6 mm) 3% 2-propanol/97% methylene chloride, flow rate 4.5 mL/min, retention time 1.96 min.

2-(Trimethylsilyl)ethyl 2(R)-[[4(S)-methyl-3(S)-[2-(2-amino-4-thiazolyl)-2(Z)-(methoxyimino)acetamido]-2-oxo-1-azetidinyl]oxy]propionate (R = H) (36b) was prepared from **35b** in 74% yield as a light yellow solid: mp 75–85 °C dec; ^1H NMR δ 0.0 (s, 9 H), 1.0 (t, 2 H), 1.5 (pair of d, 6 H total), 3.9 (s, 3 H), 4.05 (m, 1 H), 4.2 (t, 2 H), 4.6 (m, 2 H total), 6.0 (br s, 2 H), 6.6 (s, 1 H), 9.0 (br d, 1 H); IR (in $CDCl_3$) 1770, 1740, 1660 cm^{-1}; FD mass spectrum m/e 472 (M + 1). Anal. ($C_{18}H_{29}N_5O_6SSi$) C, H, N.

2-(Trimethylsilyl)ethyl [[4(S)-Methyl-3(S)-[[2-[(triphenylmethyl)amino]-4-thiazolyl]acetamido]-2-oxo-1-azetidinyl]oxy]acetate (29). Compound **10h** (103 mg, 0.252 mmol) and 0.210 mL (0.252 mmol) of 1.2 N HCl were added to 20 mL of absolute ethanol. The flask was purged with N_2, and 10 mg of 10% Pd–C was added. H_2 was bubbled through the solution. The reaction was followed by disappearance of starting material by TLC (silica, 1:1 ethyl acetate/hexanes). After 30 min, the catalyst was filtered off and the solvent evaporated. Methylene

chloride (10 mL) was added to the residue. Pyridine (0.10 mL, 1.25 mmol), 97 mg (0.252 mmol) of **28**, and 0.023 mL (0.252 mmol) of phosphorus oxychloride were added with stirring at room temperature. The reaction was followed by TLC (silica, 7:3 ethyl acetate/hexanes). After 1 h the reaction mixture was taken up into ethyl acetate, washed with H_2O, 1.2 N HCl, 5% $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, evaporated, and chromatographed on the chromatotron using a 2-mm silica gel plate (7:3 ethyl acetate/hexanes) to give 122 mg (0.186 mmol 74%) of clear oil: ^1H NMR δ 0.0 (s, 9 H), 1.0 (t, 2 H), 1.5 (d, 3 H), 3.5 (s, 2 H), 4.1 (m, 2 H), 4.4 (t, 2 H), 4.7 (s, 2 H), 6.3 (s, 1 H), 6.9 (br s, 1 H), 7.6 (s, 15 H), 7.8 (br d, 1 H); IR (in $CDCl_3$) 1790, 1770 cm^{-1}; FD mass spectrum m/e 656 (M), 657 (M + 1).

2-(Trimethylsilyl)ethyl [[4(*S*)-methyl-3(*S*)-[(2-amino-4-thiazolyl)acetamido]-2-oxo-1-azetidinyl]oxy]acetate (30). Compound **29** (62 mg, 0.095 mmol) was stirred in 3 mL of anhydrous formic acid at room temperature for 1 h. The formic acid was evaporated, and 25 mL of ether was added to the residue. This solution was sonicated for 15 min to aid in solubility, filtered, evaporated, and chromatographed on the chromatotron using a 1-mm silica gel plate (ethyl acetate) to give 17 mg (0.041 mmol, 43%) of colorless oil: ^1H NMR δ 0.0 (s, 9 H), 1.0 (t, 2 H), 1.5 (d, 3 H), 3.5 (s, 2 H), 4.0 (m, 1 H), 4.25 (dd, 1 H), 4.35 (t, 2 H), 4.6 (s, 2 H), 5.3 (br s, 2 H), 6.4 (s, 1 H), 7.9 (br d, 1 H); IR (in $CDCl_3$) 3100–3600, 1770 cm^{-1}; FD mass spectrum m/e 415 (M + 1).

Secondary α-bromo esters were prepared from α-amino acids by the procedure of Testa.[11]

(*S*)-Methyl 2-Bromo-3-(benzyloxy)propionate (32). *O*-Benzyl-L-serine (1.85 g, 9.49 mmol, Chemical Dynamics) and 3.95 g (33.2 mmol) of potassium bromide were dissolved in 19 mL of ice-cold 2.5 N H_2SO_4. An aqueous solution of 0.98 g (14.2 mmol) of sodium nitrite was allowed to add dropwise to this stirred 0 °C solution over a 15-min period. After 45 min the ice bath was removed and the solution was stirred for an additional hour at room temperature. The solution was extracted with ethyl acetate, and the combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to leave 2.2 g (8.49 mmol, 89%) of (*S*)-2-bromo-3-(benzyloxy)propionic acid as a yellow oil: ^1H NMR δ 3.85 (m, 2 H), 4.35 (dd, 1 H), 4.6 (s, 2 H), 7.3 (s, 5 H), 10.5 (br s, 1 H); IR (neat) 2800–3600, 1720 cm^{-1}; $[\alpha]^{20}_D$ −17.5° (*c* 3.15, $CHCl_3$), lit.[20] $[\alpha]_{578}$ −14.7° (*c* 2.14, MeOH); TLC (silica, 15:3:1 ethyl acetate/acetic acid/water) R_f 0.66; EI mass spectrum m/e 260 (M + 2), 259 (M + 1), 258 (M), 257 (M − 1).

Stirring this acid with 1 equiv of thionyl chloride in methanol overnight provided the methyl ester **32** as a light yellow oil in 91% yield: ^1H NMR δ 3.9 (s, 3 H), 4.0 (m, 2 H), 4.5 (dd, 1 H), 4.75 (s, 2 H), 7.6 (s, 5 H); IR (neat) 1730 cm^{-1}; $[\alpha]_D^{20}$ −22.5° (*c* 2.5, $CHCl_3$), lit.[20] $[\alpha]_{578}$ +9.6° (*c* 2.0, $CHCl_3$) for the opposite (*R*) enantiomer. Although there is a large discrepancy in the optical rotation of **32** prepared by us and that reported by Armarego,[20] alkylation of **13a** with **32** apparently proceeded with complete inversion since a 75% yield of only the one diastereomer **33** was obtained as determined by ^1H NMR and HPLC; TLC (silica, 1:1 ethyl acetate/hexanes) R_f 0.55; EI mass spectrum m/e 274 (M + 2), 272 (M).

(*S*)-2-(Trimethylsilyl)ethyl 2-Bromo-3-acetoxypropionate (34a). Deaminative bromination of *O*-acetyl-L-serine[17] provided (*S*)-2-bromo-3-acetoxypropionic acid as a clear oil in 70% yield: ^1H NMR δ 2.1 (s, 3 H), 4.5 (s, 3 H total), 9.6 (br s, 1 H); IR (neat) 1730 br cm^{-1}. Esterification with 2-(trimethylsilyl)ethanol[10] was accomplished by the method of Stadler.[18] Thus, 0.583 mL (7.50 mmol) of *N*,*N*-dimethylformamide was added to 30 mL of acetonitrile. This solution was stirred at −20 °C under a $CaCl_2$ drying tube, and 0.218 mL (2.5 mmol) of oxalyl chloride was added slowly. After 15 min of stirring, 530 mg (2.5 mmol) of (*S*)-2-bromo-3-acetoxypropionic acid was added. After an additional 15 min, 296 mg (2.5 mmol) of 2-(trimethylsilyl)ethanol and 0.25 mL (3.09 mmol) of pyridine were added. The solution was then allowed to warm to room temperature. After 1.5 h the reaction mixture was taken up into ethyl acetate, washed with H_2O, 5% $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and evaporated to leave 641 mg (2.06 mmol, 82%) of oil: ^1H NMR δ 0.0 (s, 9 H), 1.0 (t, 2 H), 2.1 (s, 3 H), 4.3 (t, 2 H), 4.5 (m, 3 H total); IR (neat) 1730 cm^{-1}.

(*S*)-2-(Trimethylsilyl)ethyl 2-Bromopropionate (34b). 2-(Trimethylsilyl)ethanol (500 mg, 4.24 mmol) and 0.55 mL (4.24 mmol) of *N*,*N*-dimethylaniline were added to 25 mL of ice-cold methylene chloride. A solution of 725 mg (4.24 mmol) of (*S*)-2-bromopropionic acid chloride[19] in 3 mL of methylene chloride was added dropwise. The ice bath was removed, and after 1 h the reaction mixture was taken up into ethyl acetate, washed with 1.2 N HCl and brine, dried over $MgSO_4$, filtered, and evaporated to leave 1.07 g (4.24, mmol, 100%) of light yellow oil: ^1H NMR δ 0.0 (s, 9 H), 1.0 (t, 2 H), 1.8 (d, 3 H), 4.2 (t, 2 H), 4.3 (m, 1 H); IR (neat) 1740 cm^{-1}; $[\alpha]^{20}_D$ −19.4 (*c* 2.85, $CHCl_3$); TLC (silica, 6:4 hexanes/ethyl acetate) R_f 0.60.

Alkylation of *N*-Hydroxy-2-azetidinones with Secondary α-Bromo Esters Using Potassium Hydroxide in Dimethyl Sulfoxide.[13] To a suspension of 1 equiv of powdered potassium hydroxide in dimethyl sulfoxide were added 1 equiv of the *N*-hydroxy-2-azetidinone, and 1 equiv of the α-bromo ester. The reaction was monitored by TLC (silica, ethyl acetate/hexanes). Upon completion (15–45 min) the reaction was taken up into ethyl acetate, washed with H_2O, 5% $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, evaporated, and chromatographed on the chromatotron using silica gel plates (ethyl acetate/hexanes). Solids were recrystallized from ethyl acetate/hexanes.

Methyl 2(*R*)-[[3(*S*)-[(benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]-3-(benzyloxy)propionate (33) was prepared in this manner from **13a** and **32** as a white solid in a 75% yield: mp 91.5–93 °C; ^1H NMR δ 3.6 (dd, 1 H), 3.75 (s, 3 H), 3.85 (m, 3 H total), 4.5 (s, 2 H), 4.65 (m, 2 H), 5.1 (s, 2 H), 5.7 (br d, 1 H), 7.3 (pair of s, 10 H total); IR (in $CDCl_3$) 1790, 1720 cm^{-1}. Anal. ($C_{22}H_{24}N_2O_7$) C, H, N. High pressure liquid chromatography (Alltech silica 5 μm, 25 cm × 4.6 mm) 2% 2-propanol/98% methylene chloride, flow rate 3.0 mL/min, retention time 2.90 min; $[\alpha]^{20}_D$ +34.0° (*c* 0.60, $CHCl_3$).

2-(Trimethylsilyl)ethyl 2(*R*)-[[4(*S*)-methyl-3(*S*)-[(benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]-3-acetoxypropionate (35a) was prepared in the same manner from **13b** and **34a** in 50% yield as a yellow oil: ^1H NMR δ 0.0 (s, 9 H), 1.0 (t, 2 H), 1.4 (d, 3 H), 2.05 (s, 3 H), 3.95 (m, 1 H), 4.3 (t, 2 H), 4.5 (m, 4 H total), 5.2 (s, 2 H), 6.3 (br d, 1 H), 7.55 (s, 5 H); IR (in $CDCl_3$) 1790, 1720–1760 cm^{-1}.

2-(Trimethylsilyl)ethyl 2(*R*)-[[4(*S*)-methyl-3(*S*)-[(benzyloxy)formamido]-2-oxo-1-azetidinyl]oxy]propionate (35b) was prepared from **13b** and **34b** in 57% yield as a light yellow oil: ^1H NMR δ 0.0 (s, 9 H), 1.0 (t, 2 H), 1.45 (pair of d, 6 H total), 3.9 (m, 1 H), 4.1 (m, 1 H), 4.2 (t, 2 H), 4.5 (q, 1 H), 5.05 (s, 2 H), 5.4 (br d, 1 H), 7.3 (s, 5 H); IR (in $CDCl_3$) 1780, 1720 cm^{-1}; TLC (silica, 1:1 ethyl acetate/hexanes) R_f 0.47.

Alkylation under Phase-Transfer-Catalyzed Conditions[12] in a Homogenous Solvent. Compound **13a** (179 mg, 0.758 mmol), 207 mg (0.758 mmol) of **32**, 0.106 mL (0.758 mmol) of triethylamine, and 24 mg (0.076 mmol) of tetra-*n*-butylammonium bromide were added to 25 mL of acetonitrile and stirred at room temperature. The reaction was followed by TLC (silica, 7:3 ethyl acetate/hexanes). After 15 h the solution was taken up into ethyl acetate, washed with H_2O, 5% $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, evaporated, and chromatographed on the chromatotron using a 2-mm silica gel plate (7:3 ethyl acetate/hexanes) to give 70 mg (0.164 mmol, 22%) of product that was recrystallized from ethyl acetate/hexanes. Characterization data were identical with **33** prepared above.

Deprotection of the 2-(Trimethylsilyl)ethyl Esters with Tetra-*n*-butylammonium Fluoride.[10] Equimolar amounts of the 2-(trimethylsilyl)ethyl ester and 1.0 N tetra-*n*-butylammonium fluoride were added to THF at room temperature with stirring. The reaction was followed by TLC (silica, 15:3:1 ethyl acetate/acetic acid/H_2O). Upon completion (2–3 h) the reaction mixture

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was taken up into ethyl acetate and washed with water. The water layers were passed through an ion-exchange column (3.4 × 25 cm, Dowex 50W-8X, K^+ form, 50–100 mL of eluant). Freeze-drying left the potassium salts as yellow flaky residues. We were unable to obtain satisfactory field desorption or fast atom bombardment mass spectral data on any of these potassium salts.

Potassium [[3(*S*)-[2-(2-amino-4-thiazolyl)-2(*Z*)-(methoxyimino)acetamido]-2-oxo-1-azetidinyl]oxy]acetate (25) was prepared from **24g** by this method in 92% yield: ^{1}H NMR (D_2O) δ 3.9 (dd, 1 H), 4.0 (s, 3 H), 4.2 (t, 1 H), 4.5 (s, 2 H), 5.0 (m, 1 H), 7.1 (s, 1 H); IR (KBr) 3700–2800, 1760 cm^{-1}; reversed-phase TLC (2-propanol/water (1:1)) R_f 0.76; paper chromatography (2-propanol/water (7.5:2.5)) R_f 0.40.

Potassium [[4(*S*)-methyl-3(*S*)-[(2-amino-4-thiazolyl)-2-(*Z*)-(methoxyimino)acetamido]-2-oxo-1-azetidinyl]oxy]-acetate (26) was prepared from **24h** in 72% yield: ^{1}H NMR (D_2O) δ 1.5 (d, 3 H), 4.0 (s, 3 H), 4.35 (m, 1 H), 4.55 (s, 2 H), 4.6 (m, 1 H), 7.1 (s, 1 H); IR (KBr) 3700–2800, 1770 cm^{-1}.

Potassium [[4(*S*)-methyl-3(*S*)-[(2-amino-4-thiazolyl)-acetamido]-2-oxo-1-azetidinyl]oxy]acetate (31) was prepared from **30** in 91% yield: ^{1}H NMR (D_2O) δ 1.45 (d, 3 H), 3.55 (s, 2 H), 4.2 (m, 1 H), 4.35 (d, 1 H), 4.5 (s, 2 H), 6.6 (s, 1 H); IR (KBr) 3600–2800, 1760 cm^{-1}.

Potassium 2(*R*)-[[4(*S*)-methyl-3(*S*)-[2-(2-amino-4-thiazolyl)-2(*Z*)-(methoxyimino)acetamido]-2-oxo-1-azetidinyl]-oxy]-3-acetoxypropionate (37a) was prepared from **36a** in 55% yield: ^{1}H NMR (D_2O) δ 1.5 (d, 3 H), 2.1 (s, 3 H), 4.0 (s, 3 H), 4.35 (m, 1 H), 4.65 (d, 1 H), 4.70 (m, 3 H total), 7.1 (s, 1 H); IR (KBr) 3700–2800, 1760, 1730 cm^{-1}; reversed-phase TLC (2-propanol/water (1:1)) R_f 0.81; paper chromatography (2-propanol/water (7.5:2.5)) R_f 0.72.

Potassium 2(*R*)-[[4(*S*)-methyl-3(*S*)-[2-(2-amino-4-thiazolyl)-2(*Z*)-(methoxyimino)acetamido]-2-oxo-1-azetidinyl]-oxy]propionate (37b) was prepared from **36b** in 72% yield: ^{1}H NMR (D_2O) δ 1.6 (pair of d, 6 H total), 4.05 (s, 3 H), 4.3 (m, 1 H), 4.6 (d, 1 H), 4.8 (1 H under H_2O peak), 7.0 (s, 1 H); IR (KBr) 3600–2800, 1755 cm^{-1}; reversed-phase TLC (2-propanol/water (1:1)) R_f 0.70; paper chromatography (2-propanol/water (7.5:2.5)) R_f 0.75.

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Registry No. **7**, 1145-80-8; **8** (R = H), 645-88-5; **8a**, 25184-48-9; **8b**, 46230-31-3; **8b**·HCl, 97486-16-3; **8c**, 97486-17-4; **8c** tosylate, 97486-18-5; **9a**, 97486-19-6; **9b**, 97486-20-9; **9c**, 97486-21-0; **10a**, 97486-22-1; **10b**, 97486-23-2; **10c**, 97486-24-3; **10e**, 90849-46-0; **10f**, 90848-81-0; **10g**, 93589-32-3; **10h**, 97486-25-4; **13a**, 83105-75-3; **13b**, 93589-31-2; **18a**, 82933-25-3; **18b**, 82933-31-1; **19a**, 93589-28-7; **19b**, 93589-29-8; **20a**, 93589-30-1; **20b**, 93712-27-7; **21**, 71445-20-0; **23f**, 93589-33-4; **23g**, 93589-34-5; **23h**, 97486-32-3; **24a**, 97486-26-5; **24f**, 90857-56-0; **24g**, 93589-35-6; **24h**, 97486-27-6; **25**, 90849-05-1; **25** (acid), 97486-43-6; **26**, 93712-30-2; **26** (acid), 90898-90-1; **28**, 64220-26-4; **29**, 97486-33-4; **30**, 97486-34-5; **31**, 97486-40-3; **31** (acid), 97589-46-3; **32**, 97486-35-6; **32** (acid), 62076-21-5; **33**, 97486-36-7; **34a**, 97486-38-9; **34a** (acid), 97486-37-8; **34b**, 97486-39-0; **34b** (acid chloride), 22592-73-0; **35a**, 97486-29-8; **35b**, 97486-31-2; **36a**, 97486-28-7; **36b**, 97486-30-1; **37a**, 97486-41-4; **37a** (acid), 97588-27-7; **37b**, 97486-42-5; **37b** (acid), 97588-28-8; $BrCH_2CO_2CH_3$, 96-32-2; $BrCH_2CO_2CH_2Ph$, 5437-45-6; $BrCH_2CO_2C(CH_3)_3$, 5292-43-3; $BrCH_2CO_2CH_2CH_2Si(CH_3)_3$, 79414-13-4; $HOCH_2CH_2Si(CH_3)_3$, 2916-68-9; $(PhCH_2CO)_2O$, 1555-80-2; *O*-benzyl-L-serine, 4726-96-9; *O*-acetyl-L-serine, 5147-00-2.

Substituent Effects on the Bioactivation of 2-(*N*-Hydroxyacetamido)fluorenes by *N*-Arylhydroxamic Acid *N*,*O*-Acyltransferase

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A series of 7-substituted analogues of 2-(*N*-hydroxyacetamido)fluorene (1) was subjected to bioactivation by a partially purified preparation of hamster hepatic AHAT, and the rates of methylthio adduct formation resulting from the reaction of the activated intermediates with *N*-acetylmethionine were determined. Electronegative substituents enhanced the amount of adduct formed; this finding contrasted with the results of a previous study in which it was found that electron-donating substituents facilitated the mechanism-based inactivation of AHAT by analogues of 1. The structures of the adducts formed from reaction of the activated forms of several of the 7-substituted compounds with *N*-acetylmethionine and with 2′-deoxyguanosine were determined; the types of adducts formed were similar to those formed with electrophiles generated by the AHAT-catalyzed activation of 1. Electronegative substituents enhanced the amount of adducts formed in the reaction with 2′-deoxyguanosine as well as with *N*-acetylmethionine.

The relationship between the mutagenicity and/or carcinogenicity of numerous organic compounds and the covalent binding of such agents to cellular macromolecules has become well established during the past 25 years.[1] In addition to the role of covalent interactions in the production of genotoxicity, a variety of other toxic effects may be attributed to reactions between xenobiotics and critical functional groups on cellular macromolecules.[2]

A number of arylamines and arylamides are included in that group of organic molecules that produce at least some of their untoward effects subsequent to covalent binding to cellular constituents. The metabolism and toxicological properties of arylamines and arylamides are of interest because of human exposure due to their industrial applications and to their presence as structural components of herbicides, pesticides, drugs, and hair dyes.[3-6] The

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