of 45% based on the initial glycine incorporation on the resin (this yield is higher than any previously obtained in these laboratories and, as mentioned above, results from thorough washings of the AG 3-x4 resin): $[\alpha]^{22.0}D$ −78.0 (*c* 0.5, 1 *N* AcOH). This material was shown to be homogeneous by tlc, R_f 0.22 (BAW), and by paper electrophoresis. Only one spot in the direction of the cathode was observed in each of the buffer systems A and B. Amino acid analysis[22] gave Asp, 1.02; Arg, 1.05; Gly, 1.00; Phe, 0.98; Pro, 1.01; Tyr, 0.93; Val, 1.02; NH_3, 2.29. In addition, cysteine (0.41) and the mixed disulfide of cysteine and β-mercaptopropionic acid (0.59) were present.

Measurement of Duration of Antidiuretic Action (Figure 1). The duration of the antidiuretic action of DVDAVP was also tested in conscious rats of the Brattleboro strain, homozygous for the hereditary hypothalamic diabetes insipidus trait, by a method adapted from that of Kincl, *et al.*[27] DVDAVP and AVP were injected subcutaneously and spontaneous urine output was measured hourly thereafter. Six female diabetes insipidus rats weighing 185–200 g were used in a simple crossover design. The average rate of urine output for the 24 hr preceding injection in each rat was considered the control rate for that experiment.

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Synthetic Penicillins. Heterocyclic Analogs of Ampicillin. Structure–Activity Relationships

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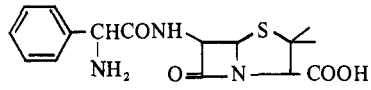
A number of glycines containing heterocyclic rings, DL-α-thienyl, DL-α-thiazolyl, and DL-α-isothiazolyl and their mono- or dimethyl derivatives, have been prepared as precursors of the title compounds. The two main preparative routes involved reduction of the glyoxalic acid oximes (or their esters) and application of the Baumgarten reaction to the cyanomethyl derivatives. The new penicillins have been prepared from these amino acids *via* a mixed anhydride, and their antibacterial activities against several microorganisms have been determined *in vitro*. Among these compounds, the unsubstituted series showed high, broad-spectrum antibacterial activity similar to that of ampicillin. Particularly, the penicillins derived from 4-thiazolylglycines are a new class of highly active antibacterials. Structure-activity relationships are discussed.

In an effort to develop semisynthetic penicillins having high broad-spectrum antibacterial activity, a number of penicillins containing a heterocyclic ring in the side chain have been synthesized (*e.g.*, cloxacillin).† However, little information was reported on the heterocyclic analogs of 6-[(*R*)-α-aminophenylacetamido)]penicillanic acid (ampicillin), except for a few examples such as 6-[(*R*)-α-amino-3- (or 2-) thienylacetamido]penicillanic acid[2,3] and 6-(α-amino-4-isothiazolylacetamido)penicillanic acid, reported by Raap[4] after completion of this work; they are comparable to ampicillin in activity.

We therefore undertook an extensive synthetic program in order to study the effect of the aryl group on the antibac-

† For recent reviews on penicillins and related compounds see ref 1.



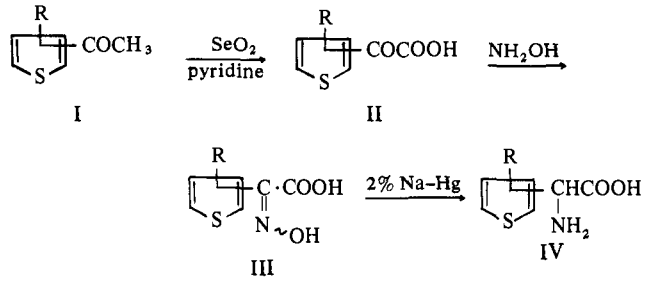
ampicillin

terial activities of heterocyclic analogs of ampicillin. In this paper, replacement of the phenyl ring of ampicillin with the thiophene, thiazole, or isothiazole ring and attendant changes in activity are described.

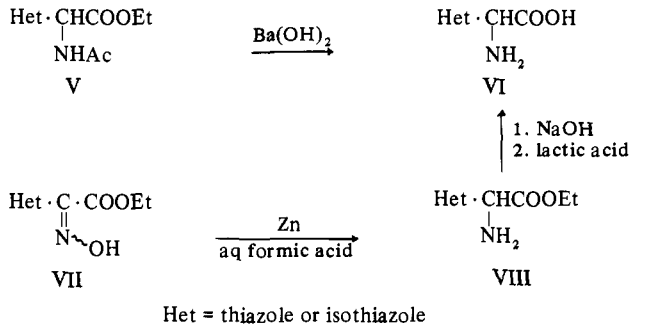
Synthesis. Prior to our work, some glycines containing heterocyclic rings (*e.g.*, thiophene,[5] imidazole,[6] and isoxazole[7]) were reported, and many of these were synthesized by the Strecker or the Bucherer reaction. We investigated the synthesis of DL-α-thienyl-, DL-α-thiazolyl-, and DL-α-isothiazolylglycines by two general methods (Scheme I), the

Scheme I

Sequence A

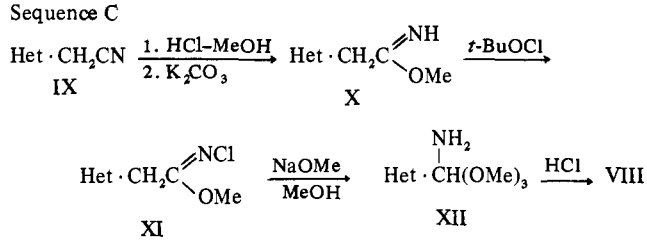


Sequence B



Het = thiazole or isothiazole

Sequence C

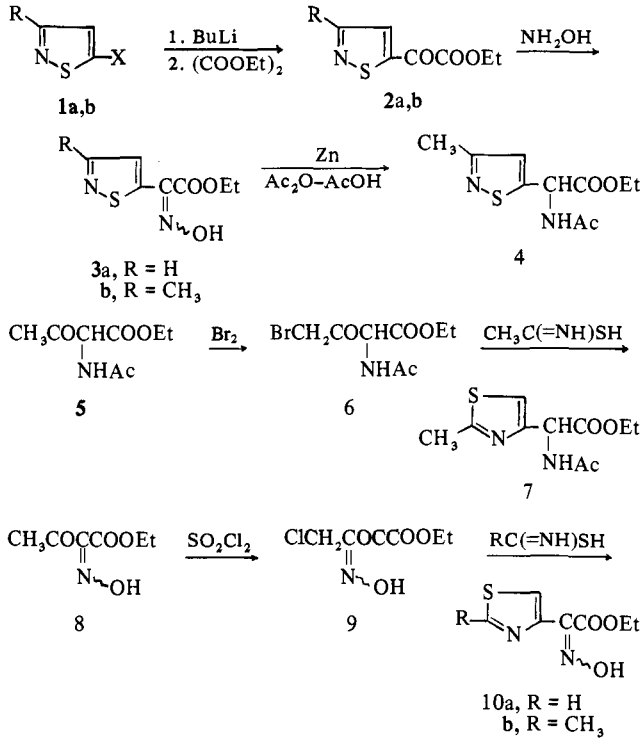


route involving reduction of the glyoxalic acid oxime (or its ester, sequence A and B) and the route *via* Baumgarten's method[8] from the cyanomethyl compounds (sequence C).

α-Thienylglycines (IV, **11-15**, Table I) were synthesized starting from acetylthiophenes I (sequence A) which were oxidized with SeO_2 in pyridine according to the procedure described in the patent[9] for 2-thienylglyoxalic acid to give the glyoxalic acids II in high yields. Treatment of II with NH_2OH in MeOH gave the oximes III. Reduction of III was carried out using 2% Na-Hg; this method is superior to the one reported by Bradley[10] and afforded quantitative yields of the desired amino acids IV.

Synthesis of 4-thiazolyl- and 5-isothiazolylglycines (**16**, **17**, **21**, and **22**, Table I) was initially attempted by the route (sequence B) *via* the acetamino ester V which was prepared

Scheme II



by the sequence outlined in Scheme II. Lithiation of isothiazole[11] (**1a**, X = H) or 5-bromo-3-methylisothiazole[12] (**1b**, X = Br) with BuLi, followed by addition of the 5-lithio compound to an excess of ethyl oxalate, afforded the glyoxalates‡ **2a** and **2b**, respectively, which on treatment with NH_2OH were readily converted to the oximes **3a** and **3b**, respectively. Reductive acetylation of **3b** using zinc dust in Ac_2O-AcOH gave **4** in high yield. Ethyl α-acetamino-2-methylthiazole-4-acetate (**7**) was also prepared starting from ethyl α-acetaminoacetoacetate (**5**)[14] which reacted with Br_2 in $CHCl_3$ to give the γ-bromo derivative **6**. Condensation of **6** with thioacetamide gave **7** in 20.6% yield. Hydrolysis of the acetamino esters V (**4** and **7**) was attempted under various conditions. Hot $Ba(OH)_2$ gave low yields of the desired amino acids VI (**22** and **17**), respectively; acid hydrolysis was unsuccessful and competed with decarboxylation.

In an effort to improve the yield of the amino acid, an alternate route (VII → VIII → VI, sequence B) was investigated. Reduction of VII to VIII was effected with zinc dust in aqueous AcOH or aqueous HCO_2H. Although the reduction proceeded more smoothly in aqueous AcOH than in aqueous HCO_2H, isolation of the product was simpler in the latter case because the resulting zinc salt was insoluble in this solvent and readily removable by filtration. Thus, the oximino esters **3a** and **3b** were treated with Zn-HCO_2H in aqueous MeOH to form the amino esters which on alkaline hydrolysis followed by neutralization with lactic acid afforded the amino acids **21** and **22**, respectively, in good yields. Ethyl α-oximino-4-thiazolylacetates (**10a** and **10b**) were also prepared by direct condensation of thioformamide or thioacetamide with ethyl γ-chloro-α-oximinoacetoacetate (**9**) which was obtained by γ-chlorination of ethyl α-oximinoacetoacetate (**8**)[14] with SO_2Cl_2 and used without purification because of its instability. The resulting **10a** was a mixture of geometrical isomers, separable by column chromatography; **10b** was a single isomer. Conver-

‡ After completion of this work, this method was reported by Micetich, *et al.*[13]

Table I. DL-α-Heterocyclic Glycines

No.	Compd	Mp,[a] °C dec	R_f[b]	Yield, %	Formula[f]
11	α-Amino-2-thienylacetic acid	210–212 (lit.[5] 223–224)	0.32	82[c]	$C_6H_7NO_2S$
12	α-Amino-3-thienylacetic acid	244–245 (lit.[5] 238–240)	0.42	71[c]	$C_6H_7NO_2S$
13	α-Amino-3,5-dimethylthiophene-2-acetic acid	163–165	0.63	78[c]	$C_8H_{11}NO_2S$
14	α-Amino-2,5-dimethylthiophene-3-acetic acid	203–205 (lit.[16] 203–204)	0.64	68[c]	$C_8H_{11}NO_2S \cdot 0.5H_2O$
15	α-Amino-2,4,5-trimethylthiophene-3-acetic acid	202–204	0.67	63[c]	$C_9H_{13}NO_2S \cdot H_2O$[g]
16	α-Amino-4-thiazolylacetic acid	155–156	0.43	68[d]	$C_5H_6N_2O_2S$
17	α-Amino-2-methylthiazole-4-acetic acid	157–158	0.61	51[d]	$C_6H_8N_2O_2S$
18	α-Amino-5-thiazolylacetic acid	155–157	0.37	26.6[e]	$C_5H_6N_2O_2S$
19	α-Amino-4-methylthiazole-5-acetic acid	202–203	0.45	46[e]	$C_6H_8N_2O_2S$
20	α-Amino-2,4-dimethylthiazole-5-acetic acid	197–199	0.63	32[e]	$C_7H_{10}N_2O_2S$
21	α-Amino-5-isothiazolylacetic acid	195–196	0.46	57[d]	$C_5H_6N_2O_2S$
22	α-Amino-3-methylisothiazole-5-acetic acid	192–193	0.56	68.3[d]	$C_6H_8N_2O_2S$
23	α-Amino-3-isothiazolylacetic acid	150–151	0.51	30[e]	$C_5H_6N_2O_2S$
24	α-Amino-3-methylisothiazole-4-acetic acid	191–192	0.59	68[e]	$C_6H_8N_2O_2S \cdot H_2O$
25	α-Amino-3-phenylisothiazole-4-acetic acid	182.5–183.5	1.03	55[e]	$C_{10}H_{10}N_2O_2S$

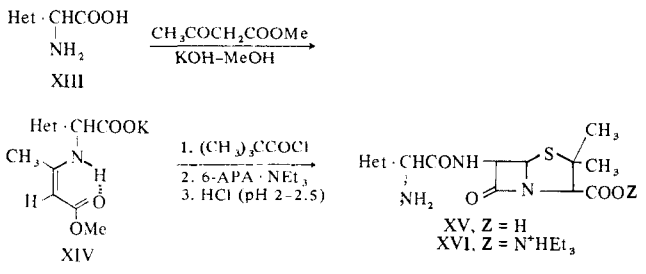
[a] All compounds were recrystallized from H_2O; all melting points are uncorrected. [b] Relative R_f values (R_f of leucine, 1) on paper chromatography; the solvent, *n*-BuOH–AcOH–H_2O (4:1:1). [c] From the corresponding acetylthiophenes. [d] From the corresponding oximino esters. [e] From the corresponding cyanomethyl compounds. [f] All compounds were analyzed for C, H, and N. [g] N: calcd, 49.76; found, 50.64.

sion of **10a** and **10b** to amino acids **16** and **17** was achieved as described above.

Synthesis of 3- and 4-isothiazolyl- and 5-thiazolylglycines (**18–20, 23–25**, Table I) was performed by application of the method of Baumgarten, *et al.*[8] (sequence C). All required intermediates, cyanomethyl compounds IX, were prepared from the corresponding carboxylate by $LiAlH_4$ reduction, chlorination with $SOCl_2$, and then cyanation with NaCN in DMSO or DMF. Preparation of the imino ether hydrochlorides was attended by the corresponding acetates and therefore required use of limited amount of MeOH under crucial anhydrous conditions. Optimum results were obtained by passing dry HCl for 30 min at −70° in absolute THF containing MeOH. Treatment of the free imino ethers with *t*-BuOCl in benzene gave the imino ether chlorides XI which were generally unstable and, particularly, thiazolyl derivatives decomposed even at room temperature. XI rearranged with NaOMe to produce the *o*-amino esters XII which on treatment with dilute HCl afforded the amino esters VIII. Hydrolysis of VIII gave the desired amino acids in considerable yields.

Condensation of the heterocyclic glycines with 6-aminopenicillanic acid (6-APA) was performed *via* a mixed anhydride using alkylidene (XIV) as the N-protecting group (Scheme III). Dane, *et al.*,[15] have first reported that in the

Scheme III



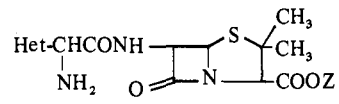
ampicillin synthesis, the use of the *N*-alkylidene protecting group was effective because it could be readily removed by dilute HCl at 0° after the condensation. In the present work Dane's method was found to be useful. Crystalline *N*-alkylidene derivatives XIV were generally obtained by heating the potassium salts of the amino acids XIII with methyl acetoacetate in absolute MeOH. The N-protected amino acids were in turn used to acylate the triethylammonium salt of 6-APA in a mixed anhydride coupling with pivaloyl chloride. After removal of the N-protecting group, isolation of the free penicillins in a crystalline form was very difficult, probably because they are diastereoisomeric mixtures. Ultimately, the compounds listed in Table III could be isolated as pure crystals having ir and nmr spectra consistent with the structure shown; the others did not crystallize. Partial purification of the latter series was effected by the procedure which is detailed in the Experimental Section, and these were isolated as triethylammonium salts XVI of sufficient purity for antibacterial testing. The ir spectra of the triethylammonium salts showed strong absorption at 1780–1770 cm^{-1}, characteristic of the β-lactam ring. Their purities were finally estimated at >80% by ir and nmr spectra and tlc.

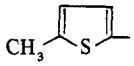
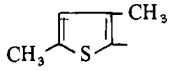
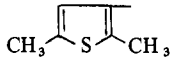
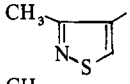
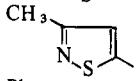
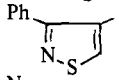
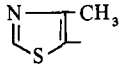
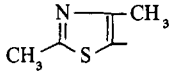
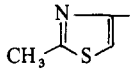
Antibacterial Activity. The new penicillins described herein were supplied to Dr. Y. Kimura and his associates for antibacterial testing. The minimum inhibitory concentrations (MIC) were determined as described in the Experimental Section, using the standard, twofold, agar-dilution technique. In Table II, the MIC values of these penicillins against several microorganisms are tabulated and compared with the values for ampicillin obtained under the same conditions.

As can be seen from Table II, six compounds, **26, 27, 31, 35, 38**, and **39**, exhibit high *in vitro* broad-spectrum antibacterial activity. Their inhibitory potency for both gram-positive and gram-negative organisms is similar to that of ampicillin. As they are diastereoisomeric mixtures, the high activity is significant; although **27** was slightly less active than ampicillin, the compound derived from D-α-amino-3-thienylacetic acid was reported by Price, *et al.*,[3] to possess activity comparable in all respects to that of ampicillin. Therefore, the penicillins (**38** and **39**) derived from α-amino-4-thiazolylacetic acids, the most active compounds among the 14 listed, represent a new class of highly active antibacterials.

These active members are not substituted on the heteroaromatic rings, with the exception of **39**. Substitution on the heteroaromatic rings tends to reduce activity particularly against gram-negative organisms. Definite differences in activity cannot be found among the three heteroaromatic species. However, the following comment on the position of substitution may be pertinent; the compounds in which the glycyl residue is located β to the

Table II. MIC Values (μg/ml) of New Penicillins



Compd no.	Het	Z	Gram +			Gram −		
			Staph. aureus (209P)	*Staph. epidermidis* (M-10)	*B. subtilis* (ATCC 6633)	*E. coli* (0-6)	*Sal. enteritidis* (No. 1)	*Shig. dysenteriae* (A-2)
26		H	0.075	0.62	0.15	5	5	1.25
27		H	0.32	1.25	0.075	5	10	1.25
28		$\overset{+}{N}HEt_3$	0.07	0.3	0.3	10	10	10
29		$\overset{+}{N}HEt_3$	0.15	0.31	0.31	10	10	10
30		H	0.31	0.15	0.31	10	2.5	10
31		H	0.07	0.15	0.31	10	2.5	0.65
32		H	0.62	1.25	0.15	>10	>10	10
33		$\overset{+}{N}HEt_3$	0.31	1.25	2.5	10	10	10
34		$\overset{+}{N}HEt_3$	0.62	0.62	0.62	10	10	10
35		$\overset{+}{N}HEt_3$	0.31	1.25	0.62	10	5	2.5
36		$\overset{+}{N}HEt_3$	0.15	0.62	2.5	10	10	5
37		$\overset{+}{N}HEt_3$	0.62	1.25	2.5	10	10	10
38		H	0.075	0.62	0.03	10	5	1.25
39		H	0.037	0.15	0.037	2.5	10	0.62
	Ampicillin		0.037	1.25	0.037	2.5	10	0.3

sulfur of the heteroaromatic ring are superior to those located at the α position.

Experimental Section

Melting points of the amino acids of Table I and the penicillins of Table III were determined in an open capillary, the remainder on a hot-stage (Yanagimoto melting-point apparatus); all are uncorrected The reaction products were checked routinely by ir and nmr spectroscopy and tlc (glc for the liquid samples). Ir spectra were determined on a Jasco Model IR-S spectrometer and nmr spectra on a 60-MHz Hitachi R-20 spectrometer (Me_4Si). Analyses are indicated only by symbols of the elements and were within ±0.4% of the calculated figures.

Acetylthiophenes. The following compounds were prepared according to literature methods: 2-acetylthiophene,[17] 3-acetylthiophene,[18] 2-acetyl-5-methylthiophene,[19] 3-acetyl-2,5-dimethylthiophene.[19]

The following two compounds were prepared from the corresponding alkylthiophenes according to the procedure for the preparation of 2-acetylthiophene: 2-acetyl-3,5-dimethylthiophene [bp 90–95° (4 mm), 65% yield]; 3-acetyl-2,4,5-trimethylthiophene [bp 96–98° (6 mm), 82% yield].

Thiopheneglyoxalic Acids. 2,4,5-Trimethylthiophene-3-glyoxalic Acid. A solution of 3-acetyl-2,4,5-trimethylthiophene (4.8 g, 0.0286 mol) in dry pyridine (20 ml) was warmed at 60°, and SeO_2 (4.6 g, 0.0415 mol) was added portionwise over 30 min with stirring. After an additional 4.5 hr, the cold mixture was filtered, and the filtrate was evaporated *in vacuo*. The residue was dissolved in H_2O and then steam distilled to remove the pyridine. The residual aqueous solution was acidified by addition of 40% H_3PO_4 (10 ml) and extracted with Et_2O. Drying ($MgSO_4$) and evaporation of the Et_2O gave a yellow solid (4.85 g, 85%) which was sufficiently pure for the subsequent reaction. Recrystallization from PhH–cyclohexane gave yellow prisms: mp 94.5–96.5°; ir ($CHCl_3$) 1780, 1730, and 1670 cm^{-1}. *Anal.* ($C_9H_{10}O_3S$) C, H.

By the same procedure, the other glyoxalic acids were prepared in good yields: 2-thienylglyoxalic acid (mp 85–87°, lit.[9] 88–90°), 3-thienylglyoxalic acid (mp 64–65°), 3,5-dimethylthiophene-2-glyoxalic acid (mp 130–135°), 2,5-dimethylthiophene-3-glyoxalic acid (mp 85–86.5°).

Thiopheneglyoxalic Acids Oximes. α-Oximino-2,4,5-trimethylthiophene-3-acetic Acid. The crude glyoxalic acid (4.7 g, 0.0238 mol) in 1 *N* NH_2OH–MeOH solution (50 ml) was refluxed for 30 min and then allowed to stand overnight at room temperature. After evaporation of the solvent the residue was dissolved in 1 *N* NaOH (60 ml) and filtered. The cold filtrate was acidified by addition of 20% HCl and extracted with Et_2O. Drying ($MgSO_4$) and evaporation of the Et_2O gave a white solid (4.95 g, 97%) which was sufficiently

pure for the next step. Recrystallization from PhH gave white prisms, mp 127–147°. *Anal.* ($C_9H_{11}NO_3S$) C, H, N.

By the same procedure, the other glyoxalic acids were converted to the oximes in quantitative yields: α-oximino-2-thienylacetic acid (mp 144°), α-oximino-3-thienylacetic acid (mp 145–146°), α-oximino-3,5-dimethylthiophene-2-acetic acid (mp 162–163.5°).

DL-α-Thienylglycines (11–15, Table I). α-Amino-2,4-5-trimethylthiophene-3-acetic Acid (15). To an ice-cooled solution of the crude oxime (4.7 g, 0.022 mol) in MeOH (20 ml) and H_2O (10 ml), 2% Na–Hg (160 g) was added gradually over 3 hr with vigorous stirring. The mixture was stirred for an additional 3 hr at room temperature and then allowed to stand overnight. The aqueous layer was acidified with 20% HCl, washed with Et_2O, and evaporated *in vacuo*. The residue was extracted with EtOH (50 ml). After filtration, the extract was again evaporated *in vacuo*. The residue was dissolved in dilute NH_4OH (30 ml), decolorized with charcoal, and concentrated *in vacuo*. The white precipitate was collected by filtration and dried *in vacuo* yielding the pure amino acid (3.7 g, 77%), mp 202–204° dec.

Ethyl 3-Methylisothiazole-5-glyoxalate (2b). A solution of 5-bromo-3-methylisothiazole[11] (68.8 g 0.38 mol) in absolute THF (200 ml) was cooled in a Dry Ice–acetone bath (−70°) and 15% BuLi–Et_2O solution (420 ml) was added with stirring at such a rate that the temperature was maintained below −65°. After the addition, the cold mixture was added through a glass tube to a cooled solution (−70°) of diethyl oxalate (80 g, 0.54 mol) in absolute Et_2O (300 ml). After an additional hour at −70°, the bath was removed, and the mixture was allowed to come to 0° during another hour of stirring. The mixture was again cooled at −50°, and 2 *N* HCl (300 ml) was added at once. The organic layer was washed with H_2O, dried ($MgSO_4$), and evaporated. The excess ethyl oxalate was distilled on an oil bath (100°) under reduced pressure (1 mm). The black crystalline residue was chromatographed on silica gel. The product of the eluates [PhH–petroleum ether (bp 30–70°)] on recrystallization from Et_2O–petroleum ether gave pale yellow needles (34.3 g, 45%): mp 77.5–79°; ir (Nujol) 1725 and 1690 cm^{-1}. *Anal.* ($C_5H_5NO_3S$) C, H, N.

Ethyl 5-Isothiazolylglyoxalate (2a). By a similar procedure, this was prepared from isothiazole[12] (17 g, 0.2 mol). The product was distilled yielding a yellow viscous liquid (12.4 g, 33.5%), bp 101–105° (0.4 mm).

Ethyl α-Oximino-3-methylisothiazole-5-acetate (3b). To a cooled mixture of **2b** (10 g, 0.05 mol), $NH_2OH \cdot HCl$ (5.25 g), and MeOH (60 ml), 1 *N* NaOH (52.5 ml) was added slowly with stirring over 3 hr. After stirring overnight at room temperature, the white precipitate was collected by filtration, washed with water, and dried *in vacuo*: white prisms (9.8 g, 91.5%); mp 172–173.5°. *Anal.* ($C_6H_{10}N_2O_3S$) C, H, N.

Ethyl α-Oximino-5-isothiazolylacetate (3a). By the same procedure this was obtained in quantitative yield as pale yellow prisms, mp 147–149°. *Anal.* ($C_7H_8N_2O_3S$) C, H, N.

Ethyl α-Acetamino-3-methylisothiazole-5-acetate (4). To a stirred solution of **3b** (730 mg, 3.4 mmol) in Ac_2O (3 ml), AcOH (10 ml), and CCl_4 (0.3 ml), zinc dust (1 g) was added portionwise at such a rate that the temperature was maintained below 45° After an additional hour, the mixture was poured into ice–water (50 ml), then stirred at room temperature for 1 hr, and extracted with $CHCl_3$. The extract was washed with 5% $NaHCO_3$, dried ($MgSO_4$), and evaporated *in vacuo* yielding yellow crystals (819 mg, 99.5%). Recrystallization from Et_2O gave white needles: mp 79.5–80.5°; ir (Nujol) 1738 and 1650 cm^{-1}. *Anal.* ($C_{10}H_{14}N_2O_3S$) C, H, N.

Ethyl α-Acetamino-γ-bromoacetoacetate (6). To an ice-cooled solution of ethyl α-acetaminoacetoacetate (5)[14] (94 g, 0.5 mol) in dry $CHCl_3$ (700 ml), Br_2 (82 g, 0.51 mol) was added dropwise with stirring. The mixture was stirred overnight at room temperature and then poured into ice water. The $CHCl_3$ layer was washed with water and dried ($MgSO_4$). Removal of the $CHCl_3$ *in vacuo* gave a pale brown solid which was sufficiently pure for the subsequent reaction.

Ethyl α-Acetamino-2-methylthiazole-4-acetate (7). A mixture of 6 (35 g, 0.13 mol), thioacetamide (12 g, 0.16 mol), absolute EtOH (50 ml), absolute Et_2O (20 ml), and dry pyridine (15 ml) was stirred at room temperature for 1 hr and then refluxed for 4 hr. After addition of EtOAc (100 ml), the cold mixture was filtered. The filtrate was washed with 1 *N* $NaHCO_3$ and dried ($MgSO_4$). Evaporation of the EtOAc gave a white solid (7 g, 20.6%) which on recrystallization from Me_2CO–Et_2O gave white prisms: mp 104–105°; ir (Nujol) 1740 and 1670 cm^{-1}. *Anal.* ($C_{10}H_{14}N_2O_3S$) C, H, N.

Ethyl γ-Chloro-α-oximinoacetoacetate (9). To an ice-cooled solution of 8[14] (22.5 g, 0.14 mol) in dry $CHCl_3$ (50 ml) was added SO_2Cl_2 (21 g, 0.155 mol) with stirring. After 14 hr at room temperature, the residue was washed with water and dried ($MgSO_4$). Evaporation of the solvent *in vacuo* gave a pale yellow liquid which was used without further purification.

Ethyl α-Oximino-2-methylthiazole-4-acetate (10b). A mixture of the crude 9 (10.7 g, 0.055 mol), thioacetamide (3.8 g, 0.05 mol), and dry PhH (50 ml) was heated gradually. When the temperature reached 70°, an exothermic reaction occurred (gas evolution). After the gas evolution ceased, the mixture was refluxed for 30 min. The white precipitate was collected by filtration, washed with PhH, and dried *in vacuo* yielding the hydrochloride (8.3 g). An additional crop was obtained by concentration of the combined solution: total yield 9.1 g (71.5%, based on thioacetamide); mp 175–182° dec. To a cooled suspension of the hydrochloride (8.3 g 0.033 mol) in PhH (50 ml), Et_3N (3.3 g) was added slowly. After stirring for 1 hr, $Et_3N \cdot HCl$ was filtered. The filtrate was evaporated *in vacuo* yielding the free oximino ester **10b** (6.7 g, 94.5%) which showed a single spot on tlc and on recrystallization from PhH gave white prisms: mp 95–98°; ir (Nujol) 1715 cm^{-1}. *Anal.* ($C_8H_{10}N_2O_3S$) C, H, N.

Ethyl α-Oximino-4-thiazolylacetate (10a). A mixture of the crude 9 (24 g, 0.125 mol) and crude thioformamide (16.1 g) in absolute EtOH (50 ml) and dry Et_2O (30 ml) was stirred overnight at room temperature. After addition of more thioformamide (13 g), the mixture was stirred for 2 days and then evaporated *in vacuo*. The residue was shaken with Et_2O (50 ml) and 2 *N* HCl (150 ml) and then filtered. The aqueous layer was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. Drying ($MgSO_4$) and removal of the $CHCl_3$ left a brown solid (16.6 g, 62%) which showed two spots on tlc. The solid was treated with $CHCl_3$–PhH, and the insoluble material was collected by filtration. Recrystallization from Me_2CO gave white prisms: mp 175–181°; ir (Nujol) 1735 cm^{-1}. *Anal.* ($C_7H_8N_2O_3S$) C, H, N. The filtrate was chromatographed on neutral alumina with PhH to give another isomer, which on recrystallization from $CHCl_3$ gave pale yellow prisms: mp 96–98°; ir (Nujol) 1717 cm^{-1}. *Anal.* ($C_7H_8N_2O_3S$) C, H, N.

4-Thiazolyl- and 5-Isothiazolylglycines (16, 17, 21, and 22, Table I). DL-α-Amino-2-methylthiazole-4-acetic Acid (17). From the Acetamino Ester 7. $Ba(OH)_2$ $8H_2O$ (11 g, 0.035 mol) was heated to 90–95°, and 7 (7 g, 0.029 mol) in MeOH (10 ml) was added slowly with stirring. After an additional 50 min at 90–95°, Celite (6.5 g) and H_2O (50 ml) were added, and the mixture was saturated with CO_2 gas at 85–90°. The precipitate was collected by filtration and washed well with hot water (3 × 50 ml). The combined aqueous solution was adjusted to pH 4.5 with dilute H_2SO_4, concentrated to 20 ml *in vacuo*, and filtered. After addition of EtOH (20 ml), the filtrate was allowed to stand overnight in the refrigerator. The white precipitate was collected by filtration yielding the crude 17 (1.2 g, 24%) which was identical with the product from **10b** (ir spectra and tlc).

From the Oximino Ester **10b.** To an ice-cooled solution of **10b** (2 g, 9.35 mmol) in MeOH (10 ml) and 50% formic acid (20 ml), zinc dust (1.5 g, 24 mmol) was added portionwise with stirring. The mixture was stirred at 0–5° for 5 hr and then filtered. The filtrate was evaporated *in vacuo*. The residue was dissolved in ice water, basified with K_2CO_3, and extracted well with $CHCl_3$. Evaporation of the solvent gave the oily amino ester (1.67 g). To an ice-cooled solution of this amino ester in MeOH (20 ml), 1 *N* NaOH (10 ml) was added dropwise with stirring. The mixture was stirred overnight at room temperature and then evaporated *in vacuo*. To a suspension of the resultant oil in EtOH (20 ml) was added lactic acid (1.0 g). After leaving overnight in the refrigerator, the white precipitate was collected by filtration yielding 1.08 g (67%). Pure 17 was obtained by recrystallization from H_2O, mp 157–158° dec.

Ethyl 3-Methylisothiazole-4-carboxylate. A solution of 4-cyano-3-methylisothiazole[20] (24.8 g, 0.2 mol) in absolute EtOH (100 ml) and 95% EtOH (87 ml) was saturated with dry HCl and then refluxed for 6 hr. After evaporation of the solvent *in vacuo*, ice water (300 ml) was added. The mixture was neutralized with K_2CO_3 and extracted with Et_2O. Drying ($MgSO_4$), evaporation of the Et_2O, and distillation of the residue gave a colorless liquid (27 g, 79%), bp 93–96° (6 mm).

4-Hydroxymethyl-3-methylisothiazole. To a solution of ethyl 3-methylisothiazole-4-carboxylate (27 g, 0.158 mol) in absolute Et_2O (120 ml), $LiAlH_4$ (3.6 g, 0.095 mol) in absolute Et_2O (150 ml) was added slowly at such a rate that gentle refluxing was maintained. After refluxing for an additional 40 min, H_2O (60 ml) was carefully added and the mixture was saturated with CO_2. Li_2CO_3 was filtered and washed well with $CHCl_3$–EtOH. The combined organic solution was dried ($MgSO_4$) and then evaporated. Distillation of the residue gave a colorless, viscous liquid (12.3 g, 60%), bp 128–130° (7 mm), and starting material (4.1 g).

5-Hydroxymethyl-4-methylthiazole. By a similar procedure this was prepared from ethyl 4-methylthiazole-5-carboxylate[21] (46.7 g, 0.27 mol) yielding 30.8 g (88.5%), bp 109–118° (1 mm).

5-Hydroxymethyl-2,4-dimethylthiazole. By a similar procedure this was prepared from ethyl 2,4-dimethylthiazole-5-carboxylate[22] (75 g, 0.405 mol) yielding 48.8 g (85%), bp 111–119° (1 mm).

5-Hydroxymethylthiazole. By a similar procedure this was prepared from ethyl 5-thiazolylcarboxylate[23] (15.4 g, 0.098 mol) yielding 8.9 g (77.5%), bp 105–106° (0.7 mm).

5-Cyanomethyl-4-methylthiazole. To an ice-cooled solution of 5-hydroxymethyl-4-methylthiazole (30.8 g 0.24 mol) in dry $CHCl_3$ (70 ml), $SOCl_2$ (33.8 g, 0.284 mol) in dry $CHCl_3$ (20 ml) was added slowly with stirring. The mixture was stirred overnight at room temperature and poured into ice water (100 ml) containing K_2CO_3 (43 g). The aqueous layer was extracted with $CHCl_3$. The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo* yielding the chloride as a dark brown liquid. The chloride was added slowly with stirring (below 50°) to a suspension of NaCN (15.5 g, 0.316 mol) in DMSO (65 ml). After an additional 6 hr the mixture was poured into ice water (200 ml) and extracted well with $CHCl_3$. Drying ($MgSO_4$), evaporation of the solvent, and distillation of the residue gave a colorless liquid (24.4 g, 70%), bp 110–114° (2 mm).

5-Cyanomethylthiazole. By a similar procedure this was prepared from 5-hydroxymethylthiazole (8.9 g, 0.0775 mol) yielding a colorless liquid (6.8 g, 71%), bp 91–98° (0.2 mm).

5-Cyanomethyl-2,4-dimethylthiazole. By a similar procedure this was prepared from 5-hydroxymethyl-2,4-dimethylthiazole (39.4 g, 0.275 mol). The product was purified by column chromatography on Florisil followed by recrystallization from Et_2O: pale yellow prisms (20.3 g, 49%); mp 88–89°. *Anal.* ($C_7H_8N_2S$) C, H, N.

4-Cyanomethyl-3-methylisothiazole. By a similar procedure this was prepared from 4-hydroxymethyl-3-methylisothiazole (13 g, 0.1 mol), but the intermediate chloride was purified on distillation [bp 100–104° (12 mm)] and DMF instead of DMSO was used as the solvent of cyanation, yielding 11.4 g (87%), bp 140–144° (13 mm).

4-Cyanomethyl-3-phenylisothiazole. 3-Phenylisothiazole-4-carboxylic acid[24] (16.3 g, 0.0795 mol) was esterified by heating for 2 days under reflux with EtOH (70 ml), $CHCl_3$ (70 ml), and 36 *N* H_2SO_4 (5 g) in a Soxhlet apparatus ($MgSO_4$); the mixture was washed with ice–water containing K_2CO_3 and evaporated *in vacuo*. The resulting crude ester was reduced ($LiAlH_4$), chlorinated ($SOCl_2$), and cyanated (NaCN–DMF) by a similar procedure without purification of the intermediates. The product was purified by column chromatography on Florisil with PhH followed by recrystallization from PhH–petroleum ether yielding yellow prisms (10.3 g, 64.7% from the carboxylic acid), mp 65.5–66.5°. *Anal.* ($C_{11}H_9N_2S$) C, H, N.

3-Bromomethylisothiazole. In a modification of the procedure of Slack, *et al.*,[25] a mixture of 3-methylisothiazole (14.3 g, 0.145 mol), 1,3-dibromo-5,5-dimethylhydantoin (21 g, 0.0734 mol), and dry CCl_4 (500 ml) was refluxed for 6 hr while bubbling through N_2 gas and with exposure to a 250-W lamp. Filtration and distillation of the filtrate gave a colorless liquid (14.2 g, 55%), bp 105–120° (15–20 mm), which crystallized at room temperature.

3-Cyanomethylisothiazole. By the procedure described for 4-cyanomethyl-3-methylisothiazole, this was prepared from 3-bromomethylisothiazole (14.2 g, 0.08 mol) yielding a colorless liquid (6.9 g, 70%), bp 125–129° (13 mm).

3- and 4-Isothiazolyl- and 5-Thiazolylglycines (18, 19, 20, 23–25, Table I). DL-α-Amino-3-methylisothiazole-4-acetic Acid (24). In a modification of the procedure of Baumgarten, *et al.*,[8] a solution of 4-cyanomethyl-3-methylisothiazole (2 g, 14.5 mmol) and absolute MeOH (2 g) in absolute THF (20 ml) was cooled in a Dry Ice–acetone bath (−70°) and then treated with dry HCl for 30 min. After removal of the bath, the mixture was left overnight and evaporated *in vacuo*. The white solid was washed with dry Et_2O and added portionwise with stirring to a cold 50% K_2CO_3 solution (20 ml). The mixture was extracted with $CHCl_3$. Drying (K_2CO_3) and evaporation of the $CHCl_3$ gave the imino ether as a colorless liquid, ir ($CHCl_3$) 1660 cm^{-1}.

To a cooled solution of the imino ether in dry PhH (20 ml) *tert*-butyl hypochlorite (1.78 g, 16 mmol) was added with stirring at such a rate that the temperature was maintained below 10°. The mixture was stirred at 10–15° for 1 hr, during which time the ir spectrum of the solution displayed C=N absorption at 1600 cm^{-1} instead of 1660 cm^{-1}. After cooling, a solution of Na (450 mg) in absolute MeOH (10 ml) was added.§ The mixture was stirred overnight at room temperature and then poured into ice water (20 ml). The aqueous layer was extracted with $CHCl_3$. The combined organic layers were dried (K_2CO_3) and evaporated *in vacuo*. The oily ortho ester was shaken with $CHCl_3$ (10 ml) and 2 *N* HCl (20 ml) for 1.5 hr. The mixture was adjusted to pH 2.7 with 20% NaOH. The aqueous layer was separated, washed with $CHCl_3$, basified with K_2CO_3, and extracted with $CHCl_3$. Drying (K_2CO_3) and evaporation of the solvent *in vacuo* gave the oily amino ester (2.1 g, 78%) which showed one spot on tlc, ir (liquid film) 1736 cm^{-1}.

To an ice-cooled solution of the crude amino ester in MeOH (30 ml) was added dropwise 1 *N* NaOH (14 ml) with stirring. The mixture was stirred overnight at room temperature and then evaporated *in vacuo*. The residue was suspended in EtOH (10 ml), and lactic acid (1.4 g) was added. After leaving overnight in the refrigerator the white precipitate was collected by centrifugation and dried *in vacuo* yielding a crystalline solid (1.7 g, 68%). A pure sample was obtained by recrystallization from water, mp 191–192° dec.

§ For 5-thiazolylglycines the inverse addition was used; otherwise poor yields resulted.

Table III. Properties of Crystalline Penicillins

Compd no.	Mp,[a] °C dec	Ir (Nujol), cm^{-1}	Yield, %	Formula[b]
26	186–189	1755 1710 1600	62.8	$C_{14}H_{17}N_3O_4S_2 \cdot H_2O$[c]
27	182–186	1750 1690 1600	61.5	$C_{14}H_{17}N_3O_4S_2 \cdot H_2O$
30	194–196	1770 1690 1640 1570	60	$C_{16}H_{21}N_3O_4S_2$
31	171	1760 1690 1600 1500	45	
32	196	1740 1690 1610	19.8	$C_{14}H_{18}N_4O_2S_2 \cdot H_2O$
38	187–190	1750 1690 1610	30.7	
39	146–149	1760 1690 1640 1570	37.4	$C_{14}H_{18}N_4O_4S_2 \cdot 2H_2O$[d]

[a] All melting points (capillary) are uncorrected. [b] All compounds were analyzed for C, H, and N. [c] C: calcd, 45.08; found, 45.94. [d] N: calcd, 13.79; found, 12.70.

***N*-Alkylidene Derivatives of α-Heterocyclic Glycines. General Procedure.** A mixture of the amino acid (2 mmol), methyl acetoacetate (2.2 mmol), and 1 *N* KOH–MeOH solution (2.2 ml) was refluxed for 30 min and evaporated to dryness *in vacuo*. The residue was dissolved in absolute EtOH–PhH, and the solvent was again distilled. The residue was dried *in vacuo* yielding solids or syrups which generally crystallized by treatment with dry Me_2CO. The *N*-alkylidene derivatives thus obtained were sufficiently pure for the next step. A pure sample was generally obtained on trituration in dry Me_2CO.

Penicillins. These were prepared by a modification of the procedure of Dane, *et al.*,[15] for the synthesis of ampicillin. The penicillins listed in Table III were obtained as crystals. The other compounds which did not crystallize were isolated as triethylammonium salts sufficiently pure for antibacterial tests. As examples, 39 and 37 are described in detail.

6-(DL-α-Amino-2-methylthiazolyl-4-acetamido)penicillanic Acid (39). The *N*-alkylidene derivative prepared from α-amino-2-methylthiazole-4-acetic acid (344 mg, 2 mmol) was suspended in absolute THF (5 ml) and cooled in an ice–salt bath. There was added, with stirring, pivaloyl chloride (247 mg, 2.05 mmol) in Me_2CO (1 ml) and then 1 drop of *N*-methylmorpholine. After stirring for an additional 2 hr at −15 to −10°, the mixture was added to a cooled solution of 6-APA (423 mg, 1.96 mmol), Et_3N (0.6 ml), and dry CH_2Cl_2 (5 ml). The mixture was stirred overnight at −20° and then filtered. The filtrate was extracted with H_2O (35 ml). The aqueous layer was stirred for 1 hr while keeping at pH 2.1–2.5 by addition of 10% HCl, then washed with $CHCl_3$, and filtered. The filtrate was adjusted to pH 5.0–5.2 by addition of Et_3N and then concentrated to 5 ml *in vacuo*. After leaving overnight in the re-

frigerator, the white precipitate was collected by filtration, carefully washed with a small amount of cold water, and dried at 45° *in vacuo* yielding 300 mg (37.4%), mp 146–149° dec.

6-(DL-α-Amino-2,4-dimethylthiazolyl-5-acetamido)penicillanic Acid (37). To a suspension of the *N*-alkylidene derivative [prepared from α-amino-2,4-dimethylthiazole-5-acetic acid (373 mg, 2 mmol)] in absolute THF (5 ml), cooled at −15 to −10°, was added with stirring pivaloyl chloride (247 mg 2.05 mmol) in absolute Me_2CO (1 ml) and then 1 drop of *N*-methylmorpholine. After an additional 2 hr, the mixture was added with stirring to a cooled solution of 6-APA (454 mg, 2.1 mmol), Et_3N (470 mg), and dry CH_2Cl_2 (5 ml). The mixture was stirred overnight at −20° and then washed quickly with a cold solution of 1 *N* HCl (4.5 ml) and H_2O (30 ml). The aqueous layer was separated and extracted with $CHCl_3$. The combined organic layers were stirred with H_2O (20 ml) for 1 hr, while keeping at pH 2.0–2.5 by addition of dilute HCl. After filtration the aqueous layer was adjusted to pH 5.0–5.1 with 1 *N* NaOH and evaporated to dryness *in vacuo*. The residue was stirred with a solution of Et_3N (1 ml) and CH_2Cl_2 (20 ml) for 1–2 hr, and the mixture was filtered. The filtrate was evaporated and dried *in vacuo* yielding the triethylammonium salt of 37 (650 mg). The ir spectrum (Nujol) displayed sharp bands at 1770, 1680, and 1600 cm^{-1}, attributed to the β-lactam, amide, and carboxylate carbonyl, respectively. The nmr spectrum (D_2O) showed 1.29 (s, 3 H) and 3.18 (q, 9 H, Et_3N^+), 1.51 (broad s, 6 H, the *gem*-dimethyl), 2.60, 2.37 (s, 3 H, 2- and 4-methyl of the thiazole ring).

Antibacterial Activities. Sensitivities of microorganisms of the penicillins thus obtained were determined in brain-heart-infusion agar medium. The penicillins were dissolved in sterile distilled water; DMF was used for the insoluble materials. Twofold dilutions of the penicillins were added to the above agar medium at a concentration of 0.1.

The microorganisms tested were cultured for 24 hr in brain-heart-infusion broth and inocula consisting of 10^{-5}–10^{-6} g of the organisms per milliliter of the medium were prepared. After incubation at 37° for 40 hr, end points were expressed as the minimum inhibitory concentrations (MIC) of the penicillins tested in micrograms per milliliter.

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Heterocyclic Thiosemicarbazones and Related Derivatives. Synthesis and Screening Data†

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New thiosemicarbazone derivatives of 6-formyl- and 8-hydroxy-6-formylpurine have been synthesized to evaluate their potential antitumor activity. To study the effect of the *N*-oxide function on the biological activity of 6-formylpurine thiosemicarbazone, the corresponding 1- and 3-oxides were prepared. Their synthesis involved the bromination of 6-methylpurine 1- and 3-oxide and treatment with carbonyl group reagents. The new thiosemicarbazone derivatives failed to improve the antitumor effect previously observed of 6-formylpurine thiosemicarbazone.

Substituted aromatic aldehyde thiosemicarbazones are known to possess bacteriostatic[1,2] and antiviral[3] activity. Moderate antileukemic activity of the heterocyclic aldehyde thiosemicarbazone, 2-formylpyridine thiosemicarbazone, was first observed by Brockman in 1956.[4] It was found later that 6-formylpurine thiosemicarbazone[5] exerted a marked effect against mouse leukemia L1210, but it was nephrotoxic.[6,7] Heterocyclic thiosemicarbazones have been found to exert an inhibitory activity on ribonucleotide diphosphate reductase.[8] 6-Formylpurine thiosemicarbazone is among those thiosemicarbazones with potent inhibition of this enzyme;[9] it also showed antiviral activity against herpes simplex and cytomegalovirus.[9,10]

In order to study the structure-activity relationship of several analogs of 6-formylpurine thiosemicarbazone, we have prepared the 8-hydroxy-6-formylpurine derivative and the N^4-phenyl- and -allylthiosemicarbazone of 6-formylpurine.

It has been shown that the introduction of an *N*-oxide

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