the two hydroxyl groups for the estrogenic potency of diethylstilbestrol.[20,21] Replacement of one of the hydroxyl groups in diethylstilbestrol by an amino group had been shown to reduce the estrogenic activity 25-fold, and larger substitutions (methoxy or bromine) caused much greater reductions in activity.[10]

**Electrophilic Reactivity.** The reaction of esters of *N*-arylacethydroxamic acids with methionine-*methyl*-$^3H$ to yield sulfonium derivatives which decompose to benzene-soluble [$^3$H]methylmercaptoarylamides has served as a convenient assay for the electrophilic reactivity of these compounds.[14] Under our assay conditions 0.65% of the $^3H$ in methionine-*methyl*-$^3H$ was converted to a benzene–hexane-soluble form on incubation with *N*-acetoxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide. Under the same conditions reactions of 0.58 and 5.3% were obtained with *N*-acetoxy-*N*-4-*trans*-stilbenylacetamide or *N*-acetoxy-*N*-2-fluorenylacetamide, respectively. From the similar activity of **11** and *N*-acetoxy-*N*-4-stilbenylacetamide toward methionine, **11** might be expected to be reactive toward certain nucleophilic sites in cellular macromolecules. Carcinogenicity tests on *N*-hydroxy- and *N*-acetoxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide are in progress.

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# Total Synthesis of Bisnorpenicillin V

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Bisnorpenicillin V, an analog of penicillin V in which the two methyl groups are replaced by hydrogen atoms, was prepared by total synthesis. The reaction scheme was essentially that described by Sheehan, *et al.*, for the synthesis of penicillins. The antibacterial activity of bisnorpenicillin was lower than that observed for the parent penicillin V. Replacement of the two C-2 methyl groups of penicillin has no or little effect on its sensitivity to β-lactamase.

Several modifications at the C-2 position of penicillins have been reported recently.[1-4] These modifications concern the introduction of an acetoxy group or a halogen into one of the C-2 methyl groups. The present report deals with the synthesis of bisnorpenicillin V (**1a**), a penicillin V analog in which the two C-2 methyl groups are replaced by hydrogen atoms.† From earlier work[6] in the field of total synthesis of penicillins, one would expect bisnorpenicillins to be biologically active. Antibacterial activity was found for the reaction mixture of DL-cysteine with 2-benzyl-4-methoxymethylene-5(4)-oxazolone, a condensation which yields penicillin in very low yields.

For the preparation of pure **1a** the two reaction schemes reported by Sheehan, *et al.*,[7,8] for the synthesis of penicillin V (**1b**) were followed. Both reaction sequences, outlined in reaction schemes A and B, start with *tert*-butyl (3-carboxy-5-thiazolidine)phthalimidoacetate (**2a**).‡ In order to obtain a biologically active penicillin, it is necessary to use the isomer of **2a** with the configuration of natural penicilloate. This isomer can be obtained by condensation of *tert*-butyl phthalimidomalonaldehyde[9] (**3**) with D-cysteine in aqueous ethanol containing sodium acetate. The reaction proceeds in a similar way as reported for the condensation of **3** with D-penicillamine[7] and afforded a mixture of two diastereoisomers of **2a** in a ratio of about 5:1. In analogy to the synthesis of **2b**,[7] the natural configuration was assigned to the minor component, which will be designated as α isomer (the major component as γ isomer). The α and γ isomers can be differentiated by tlc (silica gel, solvent system I). In both cases the α isomer showed

† According to the nomenclature, proposed by Sheehan, *et al.*,[5] compound **1a** should be called 6-phenoxyacetamidopenam-3-carboxylic acid.

‡ To avoid confusion the penam numbering will be used in this paper for the thiazolidine nucleus of compounds related to penicillin.

**Table I.** Minimum Inhibitory Concentrations[a] ($\mu$g/ml) of **1a** and **1b**

| Microorganism | **1a**[b] | **1b**[b] |
|---|---|---|
| *Staphylococcus aureus* ATCC 6538P | 0.3 | 0.025 |
| *Staphylococcus aureus* (resistant strain) | >100 | >100 |
| *Micrococcus flavius* ATCC 10240 | 1.5 | 0.17 |
| *Sarcina lutea* ATCC 9341 | 1.5 | 0.08 |
| *Bacillus subtilis* NCTC 8236 | 0.35 | 0.17 |
| *Streptococcus faecalis* NICB 8192 | 1.56 | 3.12 |
| *Escherichia coli* 9661 | >100 | >100 |

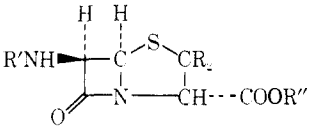
[a]Assayed by the agar-streak method on nutrient agar plates. [b]As potassium salt.

the highest $R_f$ value. In both series, the $\alpha$ isomer was the most dextrorotatory product. Analogy between the cysteine and penicillamine series was also observed in the nmr spectra of the benzyl esters of $\alpha$ and $\gamma$ isomers of **2a** and **2b**. In both series the H-3 signals of the $\gamma$ isomers were shifted upfield (0.23 ppm) with respect to the $\alpha$ isomers. From an nmr study[10] of various carboxythiazolidines‡ derived from penicillamine it can be concluded that the $\alpha$ series have a 5($R$) and the $\gamma$ series a 5($S$) configuration.§

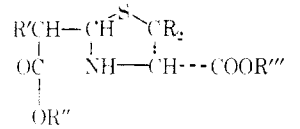
Higher yields of $\alpha$-**2a** and also of $\alpha$-**2b** were obtained when the condensation of **3** with cysteine or penicillamine was carried out in pyridine. Reaction temperatures ranging from 40 to 110° were investigated. Optimal temperatures were 55° for $\alpha$-**2a** and 80° for $\alpha$-**2b**. Almost pure $\alpha$ isomer crystallized upon evaporation of the pyridine solution. The $\gamma$ isomers, which are more soluble in pyridine, were isomerized into a mixture of $\alpha$ and $\gamma$ isomers by heating in pyridine. Thus, the total yield was 89.6% for $\alpha$-**2a** and 67.5% for $\alpha$-**2b**. It should be noted that the isomerization of $\alpha$-**2b** in pyridine has been reported by Sheehan, *et al.*[7]

Initially, the reaction given in scheme A, which proceeded in four steps, was investigated. It appeared that the intermediate $\alpha$-**4a** and especially the intermediates $\alpha$-**5a** and $\alpha$-**6a** were not stable (in contrast with the analogs in the penicillamine series) and could not be isolated in a pure state. Similar difficulties were reported with thiazane analog.[11] The yields observed for phenoxyacetylation of $\alpha$-**4a** and for removal of the *tert*-butyl group of $\alpha$-**5a** were extremely low. Therefore, this method was abandoned and the reaction sequence shown in scheme B was investigated. Thus, the isomer of *tert*-butyl (3-benzyloxycarbonyl-5-thiazolidino)phthalimidoacetate ($\alpha$-**7a**), obtained in a 76% yield by esterification of $\alpha$-**2a** with benzyl bromide in dimethylformamide, was subjected to hydrazinolysis as described for the parent $\alpha$-**7b**.[8] Reaction of $\alpha$-**8a** with anhydrous hydrogen chloride (for 4 hr at 0° instead of 27 hr as reported for $\alpha$-**8b**[8]) afforded the hydrochloride of $\alpha$-**9a** as an amorphous powder which decomposed at room temperature. When $\alpha$-**9a** was N-tritylated in aqueous 2-propanol or aqueous tetrahydrofuran,[8] extensive degradation of the tritylated compound $\alpha$-**10a** occurred. Therefore, the hydrochloride of $\alpha$-**9a** was tritylated by reaction at −18° with trityl chloride in $CH_2Cl_2$ in the presence of *N*-ethyldiisopropylamine. The crude reaction mixture, containing $\alpha$-**10a**, was freed from the amine and treated with diisopropylcarbodiimide (DICI) in nitromethane, yielding, after column chromatography over silica gel, crystalline benzyl 6-tritylaminobisnorpenicillanate (**11a**). The overall yield for the conversion of $\alpha$-**8a** to **11a** was 14.5%.‡ It was found that nitromethane was a better solvent for the cyclization of $\alpha$-**10a** than aqueous dioxane, which was reported for the $\beta$-lactam closure of $\alpha$-**11b**.

The reaction sequence used for the conversion of **11a** into **1a** was different from that reported[8] for the synthesis of penicillin V (scheme B). The benzyl ester **11a** was detritylated (84%) (*p*-toluenesulfonic acid in acetone[12]). N-Phenoxyacetylation of **12a** (phenoxyacetyl chloride in $CH_2Cl_2$ in the presence of triethylamine) afforded (87%) **13a**, which was debenzylated (Pd/C) and neutralized with KOH to yield **1a** (71.5%). Spectral data of compounds **1a**, **11a**, **12a**, and **13a** were consistent with the proposed structures; ir spectra showed the presence of a $\beta$-lactam carbonyl group (1769, 1785, 1782, and 1796 $cm^{-1}$, respectively); the coupling constants (4–4.5 Hz) between the C-5 and C-6 protons observed in the spectra of **1a**, **11a**, and **13a** were in agreement with a cis relationship between these protons.

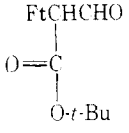


1. R′ = $PhOCH_2CO$; R″ = H
11. R′ = $Ph_3C$; R″ = Bz
12. R′ = H; R″ = Bz
13. R′ = $PhOCH_2CO$; R″ = Bz
14. R′ = $Ph_3C$; R″ = H
15. R′ = H; R″ = H



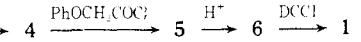
2. R′ = Ft; R″ = *t*-Bu; R‴ = H
4. R′ = $NH_2$; R′ = *t*-Bu; R‴ = H
5. R′ = $PhOCH_2CONH$; R″ = *t*-Bu, R‴ = H
6. R′ = $PhOCH_2CONH$; R″ = H; R‴ = H
7. R′ = Ft; R″ = *t*-Bu; R‴ = Bz
8. R′ = $NH_2$; R″ = *t*-Bu; R‴ = Bz
9. R′ = $NH_2$; R″ = H; R‴ = Bz
10. R′ = $PH_3CNH$; R″ = H; R‴ = Bz

series a, R = H; series b, R = Me; Ft = phthalimido

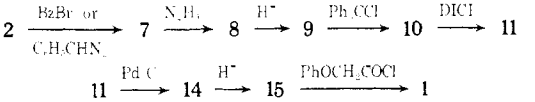


3

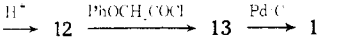
reaction sequence A



reaction sequence B



modification used in the present work



**Biological Activity.** The *in vitro* activity of bisnorpenicillin V was compared to that of the parent penicillin V. The data given in Table I indicate that replacement of the C-2 methyl groups by hydrogen atoms results in a marked decrease of the activity against penicillin-sensitive organisms. No activity was observed for **1a** against a penicillin-resistant *Staphylococcus aureus* strain and against *Escherichia coli*.

It has been pointed out that the structure of penicillin is very similar to that of D-ala-D-ala fragment, which is

§These authors concluded that the stereochemistry at C-5 affects the chemical shift of the C-3 proton. The latter appears at the highest field when C-3 and C-5 protons possess a cis configuration.

‡When the conversion of $\alpha$-**8b** to **11b** was carried out, under the reaction conditions described in the present report, the overall yield which has been reported to be 15%[8] was improved to 52%.

cleaved by a penicillin-sensitive transpeptidase during the biosynthesis of peptidoglycan of bacterial cell wall.[13] From an inspection of models one could conclude that the influence of the two C-2 methyl groups of penicillin would be negligible. These results indicate that their importance cannot be neglected.

It should be noted that the replacement of the two C-2 methyl groups of penicillin by hydrogen atoms has little or no effect on its sensitivity to β-lactamase. The rate of hydrolysis of **1a** by this enzyme is almost the same as that observed for **1b**.

## Experimental Section

Melting points were determined with a Büchi-Tottoli apparatus and are uncorrected. Solvents were evaporated under reduced pressure below 30° unless otherwise stated. Tlc was performed on silica gel F-254 plates (Merck) using the following solvent systems: I, $C_6H_6$–EtOAc–HCOOH (20:10:0.25); H, $C_6H_6$–$Me_2CO$ (95:5); III, *n*-BuOAc–*n*-BuOH–$H_2O$–MeOH–HOAc (80:15:24:5:40); IV, $C_6H_6$; V, $C_6H_6$–$Me_2CO$ (70:30). Spots were located by uv illumination and exposure to iodine vapor. Column chromatography was performed over silica gel (Merck, 0.05–0.2 mm). Petroleum ether refers to the fraction of boiling range 40–60°. Ir spectra were run on a Perkin-Elmer 257 spectrometer. Mass spectra were recorded on a A.E.I. MS12 apparatus and nmr spectra on a Varian A-60 or XL-100 spectrometer with tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSSA) as internal standard. The β-lactamase used was a penicillinase (Penase) obtained from Leo Pharmaceutical Products, Ballerup, Denmark.

*tert*-Butyl (3-Carboxy-5-thiazolidino)phthalimidoacetate α Isomer (α-2a). A slurry of D-cysteine hydrochloride (23.625 g, 150 mmol) and *tert*-butyl phthalimidomalonaldehydate (43.35 g, 150 mmol) in 250 ml of anhydrous pyridine was heated for 22 hr at 55° under $N_2$ atmosphere. The solution was cooled and concentrated until a crystalline precipitate was observed. After storage for 24 hr at room temperature the crystals were collected, washed ($Et_2O$ and $H_2O$), and dried, yielding 47.45 g (80.7%) of **α-2a**: mp 124–127° dec (after recrystallization from MeOH–$H_2O$); $[\alpha]^{25}D$ +67° (*c* 0.5, dioxane); mass spectrum $M^+$ 406; ir (KBr) 3280 (NH), 1780, 1715 (imide), 1740, 1155 (ester), 1715, 1380 (COOH), 715 $cm^{-1}$ (phenyl); tlc (system I) $R_f$ 0.21. The residue obtained on evaporation of the pyridine and $Et_2O$ filtrates (which contained the γ isomer) was converted into the α isomer by heating (55°) in pyridine for 22 hr. This afforded another 5.26 g (8.9%) of **α-2a**.

γ Isomer (γ-2a). To a solution of *tert*-butyl phthalimidomalonaldehydate (11.5 g, 40 mmol) in EtOH (80 ml) was added D-cysteine hydrochloride (6.3 g, 40 mmol) dissolved in 80 ml of $H_2O$ containing NaOAc (4.95 g). After storage for 12 hr at room temperature under $N_2$ atmosphere, crystals of **γ-2a** (10.3 g, 65.7%) were collected and recrystallized from MeOH: mp 161–163° dec; $[\alpha]^{25}D$ +6° (*c* 0.5, dioxane); mass spectrum $M^+$ 406; ir (KBr) 3260 (NH), 1785, 1720 (imide), 1745, 1160 (ester), 1720, 1380 (COOH), 715 $cm^{-1}$ (phenyl); tlc (system I) $R_f$ 0.12. Addition of $H_2O$ to the filtrate of the first fraction yielded 2.26 g (14.5%) of a mixture of the α and γ isomer which can be separated by fractional crystallization from methanol.

*tert*-Butyl (3-Carboxy-2,2-dimethyl-5-thiazolidine)phthalimidoacetate α Isomer (α-2b). D-Penicillamine (18.55 g, 100 mmol) and *tert*-butyl phthalimidomalonaldehyde (28.9 g, 100 mmol) in 500 ml of pyridine were heated for 22 hr at 80° under $N_2$ atmosphere. The reaction mixture was worked up as described for **α-2a**, yielding 28.87 g (67.5%) of **α-2b**: $[\alpha]^{25}D$ +11° (*c* 1, dioxane); tlc (system I) $R_f$ 0.34.

γ Isomer (γ-2b). This compound was prepared according to Sheehan, *et al.*:[7] $[\alpha]^{25}D$ −13° (*c* 1, dioxane); tlc (system I) $R_f$ 0.23. Other physical constants of both isomers were in agreement with the reported values.[7]

*tert*-Butyl (3-Carbobenzyloxy-5-thiazolidino)phthalimidoacetate α Isomer (α-7a). Freshly distilled $NEt_3$ (3.5 ml, 25 mmol) and freshly distilled benzyl bromide (4.5 ml, 38 mmol) were added to a solution of **α-2a** (7.84 g, 20 mmol) in 100 ml of dimethylformamide. The mixture was stirred for 26 hr at room temperature and poured into a mixture of 600 ml of ice–water, 100 ml of $C_6H_6$, and 4 ml of concentrated HCl. The $C_6H_6$ layer was separated and the aqueous layer extracted with $C_6H_6$. The combined organic layer was successively washed with $NaHCO_3$ (5%) and $H_2O$, dried ($Na_2SO_4$), and evaporated to a yellow oil which was crystallized from 50 ml of ether yielding 5.25 g (59.4%) of **α-7a**: mp 115–117°. Addition of petroleum ether to the ether filtrate afforded a second fraction (2.26 g, 16.7%). Recrystallization from $Et_2O$–petroleum ether increased the melting point to 117–118°: $[\alpha]^{25}D$ +45° (*c* 1, dioxane); mass spectrum $M^+$ 482; ir (KBr) 3365 (NH), 1780, 1715 (imide), 1730 (ester), 745, 710, 698 $cm^{-1}$ (phenyl); nmr ($CDCl_3$) δ 1.48 (s, *tert*-butyl), 3.06 (AB part of ABX pattern, $J_{AX}$ = 6 Hz, $J_{BX}$ = 9 Hz, $J_{AB}$ = 10 Hz, H-2), 4.06 (br, H-3), 4.80 (d, *J* = 10 Hz, FtCH<), 5.18 (s, $OCH_2C_6H_5$), 5.38 (d, *J* = 10 Hz, H-5), 7.33 (s, $C_6H_5$), 7.6–8.0 ppm (m, $C_6H_4$) (after addition of $D_2O$, the signal at 4.06 ppm showed the typical four lines for the X part with $J_{AX+BX}$ = 15 Hz); tlc (system II) $R_f$ 0.65.

γ Isomer. This compound was obtained in a similar way as described for the α isomer: mp 99.5–100.5°; $[\alpha]^{25}D$ −1° (*c* 1, dioxane); mass spectrum $M^+$ 482; ir (KBr) 3280 (NH), 1780, 1720 (imide), 1750, 1195 (ester), 745, 730, 698 $cm^{-1}$ (phenyl); nmr ($CDCl_3$) δ 1.48 (s, *tert*-butyl), 3.08 (AB part of ABX pattern, $J_{AX}$ = 6 Hz, $J_{BX}$ = 10 Hz, $J_{AB}$ = 10 Hz, H-2), 3.83 (br, H-3), 5.08 (d, *J* = 8.5 Hz, FtCH<), 5.21 (s, $OCH_2C_6H_5$), 5.24 (d, *J* = 8.5 Hz, H-5), 7.35 (s, $C_6H_5$), 7.6–8.0 ppm (m, $C_6H_4$) (after addition of $D_2O$, the signal at 3.83 ppm showed the typical four lines of the X part with $J_{AX+BX}$ = 16 Hz); tlc (system II) $R_f$ 0.60.

*tert*-Butyl (3-Carbobenzyloxy-2,2-dimethyl-5-thiazolidine)phthalimidoacetate (α- and γ-7b). Both α- and γ-**2b** were benzylated with $PhCHN_2$ as described by Sheehan, *et al.*:[14] nmr ($CDCl_3$) δ 1.12 (s, $CH_3$), 1.47 (s, *tert*-butyl), 1.62 (s, $CH_3$), 3.28 (br, NH), 3.82 (br, H-3), 4.9 (d, *J* = 10.5 Hz, FtCH<), 5.13 and 5.18 (AB pattern, $CH_2Ph$), 5.28 (d, *J* = 10.5 Hz, H-5), 7.35 (s, $C_6H_5$), 7.6–8.0 ppm (m, $C_6H_4$) for α-7b and 1.14 (s, $CH_3$), 1.46 (s, *tert*-butyl), 1.6 (s, $CH_3$), 3.36 (d, *J* = 12 Hz, NH), 3.59 (d, *J* = 12 Hz, H-3), 5.02 (d, *J* = 8 Hz, FtCH<), 5.14 and 5.18 (AB pattern, $CH_2Ph$), 5.34 (d, *J* = 8 Hz, H-5), 7.34 (s, $C_6H_5$), 7.6–8.0 ppm (m, $C_6H_4$) for γ-7b (after addition of $D_2O$, the H-3 signals gave a sharp singlet in both spectra, H-5 signals were also sharper). Other physical constants were in agreement with the reported values.

*tert*-Butyl (3-Carbobenzyloxy-5-thiazolidino)aminoacetate Hydrochloride (α-8a). Removal of the phthalyl group of **α-7a** was performed as described for the parent **α-7b**.[8] The product obtained on treatment of **α-7a** (9.24 g, 20 mmol) with hydrazine and decomposition of the resulting phthalhydrazide complex with HCl was crystallized from MeOH–$Et_2O$ yielding 6.98 g (76.3%) of crystalline **α-8a**: mp 141–142.5° dec; $[\alpha]^{25}D$ +84° (*c* 1, MeOH); ir (KBr) 3300 (NH), 2850 ($NH_3^+$), 1740, 1205, 1720, 1190 (ester), 755, 700 $cm^{-1}$ (phenyl); tlc (system III) $R_f$ 0.72.

Benzyl 6-Tritylaminobisnorpenicillanate (11a). A stream of anhydrous HCl was passed for 6 min through a cooled (0°) and stirred suspension of **α-8a** (3.495 g, 9 mmol) in $CH_3NO_2$ (60 ml). After a few minutes the solid material dissolved. The solution was stored for 4 hr at 0°, the major part of the HCl was removed under reduced pressure at 10°, and anhydrous $Et_2O$ (120 ml) was added to the stirred solution. After 45 min the precipitate of **α-9a** was filtered off, washed ($Et_2O$), and dried (3 hr) over $P_2O_5$ and KOH. $Ph_3CCl$ (7.56 g, 27 mmol) was added to the precipitate followed by a solution of *N*-ethyldiisopropylamine (9.36 g, 72 mmol) in $CH_2Cl_2$ (120 ml) and the reaction mixture was cooled (Dry Ice) immediately. After storage for 20 hr at −13° (until this stage, contact with humidity was avoided as much as possible) the solution was diluted with $CHCl_3$, poured into ice–water (150 ml), and adjusted to pH 6 with dilute $H_3PO_4$. The organic layer containing **α-10a** was washed ($H_2O$), dried ($Na_2SO_4$) for 0.5 hr, and evaporated at low temperature to an oil which was taken up in $CH_3NO_2$ (60 ml) containing diisopropylcarbodiimide (2.925 g, 22.5 mmol). After storage for 19 hr at room temperature the solvent was evaporated. Chromatography of the residue over silica gel (110 g) using benzene as eluent yielded after crystallization from $C_6H_6$–$CHCl_3$–$Et_2O$ 680 mg (14%) of **11a**: mp 196–197.5° dec; $[\alpha]^{25}D$ +117° (*c* 1, $CHCl_3$); mass spectrum $M^+$ 520; ir (KBr) 3290 (NH), 1785 (β-lactam), 1748, 1210 (ester), 740, 704, 695 $cm^{-1}$ (phenyl); nmr ($CDCl_3$) δ 3.22 (d, *J* = 5 Hz, H-2), 4.14 (d, *J* = 4 Hz, H-5), 4.52 (d, *J* = 4 Hz, H-6), 4.90 (t, *J* = 5 Hz, H-3), 5.08 (s, $CH_2C_6H_5$), 7.0–7.7 ppm (m, $C_6H_5$); tlc (system IV) $R_f$ 0.29.

Benzyl 6-Aminobisnorpenicillanate *p*-Toluenesulfonate (12a). A solution of *p*-toluenesulfonic acid monohydrate (760 mg, 4 mmol) in anhydrous $Me_2CO$ (15 ml) was added to a suspension of **11a** (2.08 g, 4 mmol) in $Me_2CO$ (15 ml). The reaction was stirred vigorously at room temperature for 2.5 hr. $CH_2Cl_2$ (4 ml) and $Me_2CO$ (20 ml) were added, and the precipitate was filtered off, washed ($Et_2O$), dried, and recrystallized from MeOH–$Et_2O$–$Me_2CO$ yielding **12a** (1.47 g, 81.5%): mp 169–170° dec; $[\alpha]^{25}D$

+118° (c 1, MeOH); ir (KBr) 2950, 1540 ($NH_3^+$), 1782 (β-lactam), 1740, 1190 (ester), 810, 745, 695 $cm^{-1}$ (phenyl); tlc (system V) $R_f$ 0.47.

**Benzyl Phenoxyacetamidobisnorpenicillanate (13a).** A suspension of **12a** (1.35 g, 3 mmol) in $CH_2Cl_2$ (120 ml) was neutralized with 1 equiv of $Et_3N$ in $CH_2Cl_2$ and cooled (0°). Solutions of $Et_3N$ (304 mg, 3.3 mmol) and phenoxyacetyl chloride (560 mg, 3.3 mmol) in $CH_2Cl_2$ (15 ml for each) were added gradually (in 1 hr) to the cooled solution of **12a.** After storage for 2 hr at 0° the reaction mixture was extracted successively with 0.05 *N* HCl, $NaHCO_3$ (5%), and $H_2O$. The organic layer was dried ($Na_2SO_4$) and evaporated and the residue was crystallized from $Et_2O$–petroleum ether, yielding **13a** (1.075 g, 87%): mp 113–114°; $[\alpha]^{25}D$ +145° (c 0.5, $CHCl_3$); mass spectrum $M^+$ 412; ir (KBr) 3370, 1685, 1520 (amide), 1796 (β-lactam), 1725, 1205 (ester), 745, 690 $cm^{-1}$ (phenyl); nmr ($CDCl_3$) δ 3.45 (d, *J* = 5 Hz, H-2), 4.54 (s, $OCH_2CO$), 5.03 (5, *J* = 5 Hz, H-3), 5.22 (s, $OCH_2C_6H_5$), 5.38 (d, *J* = 4.5 Hz, H-5), 5.72 (dd, *J* = 4.5 and 9 Hz, H-6), 6.7–7.6 ppm (m, $C_6H_5$); tlc (system II) $R_f$ 0.26.

**Bisnorpenicillin V Potassium Salt (1a).** A solution of **13a** (412 mg, 1 mmol) in EtOAc (30 ml) was hydrogenated over Pd/C (10%) (412 mg) for 5 hr at room temperature and at a pressure of 3 $kg/cm^2$. The catalyst was filtered off and washed with EtOAc. The combined filtrates were concentrated to 50 ml and $H_2O$ (50 ml) was added. The cooled mixture was adjusted to pH 6.3 with KOH (0.2 *N*). Freeze-drying of the aqueous layer yielded the potassium salt of **1a** (258 mg, 71.5%) which was crystallized from $H_2O$–$Me_2CO$: mp 175° dec; $[\alpha]^{25}D$ +185° (c 1, $H_2O$); ir (KBr) 3350 (NH), 1680, 1525 (amide), 1769 (β-lactam), 1610, 1405 ($COO^-$), 690, 750 $cm^{-1}$ (phenyl); nmr ($D_2O$, DSSA) δ 3.40 (AB part of ABX pattern, $J_{AX}$ = 4 Hz, $J_{BX}$ = 6 Hz, $J_{AB}$ = 11.5 Hz, H-2), 4.53 (s, $OCH_2C_6H_5$), 4.88 (X part of ABX pattern, $J_{AX+BX}$ = 10 Hz, H-3), 5.37 (d, *J* = 4 Hz, H-5), 5.48 (d, *J* = 4 Hz, H-6), 6.7–7.5 ppm (m, $C_6H_5$); tlc (system III) $R_f$ 0.66. *Anal.* ($C_{14}H_{13}N_2O_5SK$) C, H, N.

**Determination of the Sensitivity to β-Lactamase.** The rate of hydrolysis of **1a** and **1b** was determined at 30° and at pH 7 on 4-ml samples containing 12.5 μmol of penicillin and 13 units** of β-lactamase using the method described by Zyk.[16] Under these conditions the rate of hydrolysis was 17 μmol/hr for **1a** and 19.5 μmol/hr for **1b.**

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**One unit of β-lactamase is defined as the amount of enzyme that hydrolyzes 1 μmol of benzylpenicillin per hour at 30° and at pH 7.[15]

# New Streptozotocin Analogs with Improved Antileukemic Activity†

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The 3-methyl-3-nitrosoureido derivatives of the following amino sugars were prepared as analogs of streptozotocin with the anomeric carbon protected, by nitrosating the methylureas in water with $N_2O_3$: 3-amino-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose, methyl 3-amino-3-deoxy-β-D-xylopyranoside, methyl 3-amino-3-deoxy-α-D-altropyranoside, methyl 3-amino-3-deoxy-α-D-glucopyranoside, methyl 6-amino-6-deoxy-α-D-glucopyranoside, and methyl 3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside. Tests against murine leukemia L1210 show that the anticancer activity of streptozotocin not only was retained but was enhanced in most of these derivatives.

Streptozotocin (1) is an antibiotic with antileukemic and diabetogenic activity in animals.[1] In human patients it has been used with success for the treatment of malignant insulinoma[2] and is being tested against the whole range of common tumor types.[3] Kidney damage is the most common and most severe of various toxic side effects, so that analogs and derivatives of 1 are of interest for either enhanced anticancer activity or reduced toxicity. Recently observed separation of antileukemic, diabetogenic, and antibacterial activities in a series of new streptozotocin isomers and analogs[1,4] emphasized the promise of further structural variations. Analogs blocked at the anomeric carbon of the sugar moiety are of interest for their greater ease and convenience in preparation, relative to free reducing sugars. The methyl glycosides[4,5] of 1 have been studied *in vitro:* the β anomer was just as active as **1**, but the α anomer was twice as active.[4] The 6-

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