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Reaction of Amino Acids with D-Glycosyl- and D-Gluconyl Isothiocyanates[1]

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Reaction of glycosyl isothiocyanate (**1a**) with amino acids such as ethyl L-phenylalaninate hydrochloride, ethyl L-leucinate hydrochloride and ethyl β-alaninate hydrochloride afforded glycosyl thioureides (**3a. b, c**) in good yields. Reaction of D-gluconyl isothiocyanate (**1c**) with amino acids (glycine, β-alanine, γ-aminobutyric acid, ε-aminocaproic acid) or *p*-aminobenzoic acid gave the corresponding D-gluconamides (**4a, b, c, d**) and/or D-gluconyl thioureides (**5a, b, 7, 8**). Treatment of **1a, b, c** with 6-aminopenicillanic acid (6-APA) or ampicillin in the presence of triethylamine gave triethylammonium 6-(substituted thioureido)penicillanates (**9a, b, c**) and triethylammonium 6-(D-glycosyl thioureido)benzylpenicillanates (**10a, b**). The reaction between **1c** and phenacyl 6-APA afforded phenacyl 6-(D-gluconyl thioureido)penicillanate (**9d**).

Keywords——glycosyl isothiocyanate; gluconyl isothiocyanate; N-glucopyranosyl-N′-carbethoxyalkyl thioureide; N-carboxyalkyl-D-gluconamide; D-gluconyl thioureide; amino acid; HSAB principle; triethylammonium 6-(substituted thioureido)penicillanate; triethylammonium 6-(glycosylthioureido)benzylpenicillanate; phenacyl 6-(D-gluconyl-thioureido)penicillanate

We have reported a synthesis of nucleoside analogs using glycosyl isothiocyanates as starting materials.[3] This paper describes a reaction of amino acids and related heterocycles with D-glycosyl- and D-gluconyl isothiocyanates (**1a, b, c**).

Although Micheel and Brunkherst[4] have reported that tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**1a**) can be treated with methyl DL-alaninate to give N-glucopyranosyl-N′-carbomethoxyalkyl-thioureide (**2**), the yield of **2** is very poor. We carried out the above reaction in benzene solution in the presence of pyridine and the corresponding thioureides (**3a, b, c**) were obtained in excellent yields. The structures of these compounds were confirmed by spectral and elemental analyses (Chart 1, Table I).

Treatment of 2,3,4,5,6-penta-*O*-acetyl-D-gluconyl isothiocyanate (**1c**) with glycine and β-alanine in tetrahydrofuran

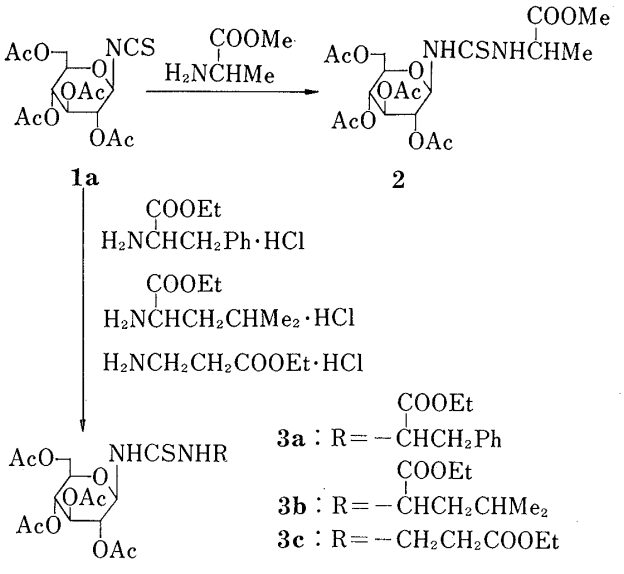

Chart 1

1) Previous paper: H. Takahashi, N. Nimura, and H. Ogura, *Chem. Pharm. Bull.* (Tokyo), **27**, 1130 (1979); This constitutes Part XXVII in a series entitled "Studies on Heterocyclic Compounds."
2) Location: *5-9-1 Shirokane Minato-ku, Tokyo 180, Japan.*
3) H. Ogura, H. Takahashi, K. Takeda, and N. Nimura, *Nucleic Acids Res.*, **S2**, 7 (1976); H. Ogura, H. Takahashi, K. Takeda, M. Sakaguchi, and H. Sakai, *Heterocycles*, **3**, 1129 (1977); H. Ogura and H. Takahashi, *Heterocycles*, **6**, 1633, **8**, 125 (1977); H. Ogura, H. Takahashi, and E. Kudo, *J. Carbohydr. Nucleosides Nucleotides*, **5**, 329 (1978).
4) F. Micheel and W. Brunkherst, *Chem. Ber.*, **88**, 481 (1955); A. Klemer and F. Micheel, *Chem. Ber.*, **89**, 1242 (1956); F. Micheel and W. Lengsfeld, *Chem. Ber.*, **89**, 1246 (1956).

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Table I. N-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-N′-carboethoxyalkylthioureides (**3a**, **b**, **c**)

Compd. No.	mp (°C)	Yield (%)	IR ν_{max}^{KBr} cm^{-1}	NMR (δ, $CDCl_3$) heterocyclic moiety	Analysis (%) Calcd.	Found
					$C_{25}H_{32}N_2O_{11}S$	
3a	Syrup[a]	90	3270, 1740, 1605, 1580, 1380, 1210, 740	1.20 (3H, t, Me), 3.20 (2H, q, CH_2), 5.65 (1H, bs, NH), 7.00 (1H, bs, NH), 7.20—7.40 (5H, m, Ph)	C 52.81	52.79
					H 5.67	5.65
					N 4.93	4.95
					$C_{23}H_{36}N_2O_{11}S$	
3b	143—144	83	3270, 1740, 1380, 1370, 1310, 1210	0.98, 1.02 (6H, s, *gem*-Me), 1.25 (3H, t, Me), 1.64 (2H, m, CH_2), 6.80, 6.95 (2H, bs, NH×2)	C 50.36	50.35
					H 6.61	6.55
					N 5.11	5.27
					$C_{20}H_{30}N_2O_{11}S$	
3c	Syrup[b]	93	3250, 1740, 1310, 1210	1.28 (3H, t, Me), (2H, q, CH_2)	C 47.42	47.45
					H 5.97	5.95
					N 5.53	5.50

a) TLC (silica gel) *Rf* 0.62 (benzene/acetone=3:2).
b) TLC (silica gel) *Rf* 0.57 (benzene/acetone=3:2).

(THF) solution gave the corresponding N-carboxyalkyl D-gluconamides (**4a**, **b**, **c**) in 70—80% yields. The physical data for these compounds are shown in Table II. Reaction of **1c** with *p*-aminobenzoic acid in THF, followed by treatment with diazomethane afforded D-gluconyl thioureide (**8**) in 80% yield. Similar treatment of **1c** with γ-aminobutyric acid afforded N-(D-gluconyl)-N′-(3-carboxypropyl)thioureide (**5a**) in 40% yield, and 53% of the starting material was recovered. Reaction between **1c** and ε-aminocaproic acid, followed by treatment with diazomethane yielded a mixture of N-(5-carbomethoxypentyl)-D-gluconamide (**4d**) and N-(5-carbomethoxypentyl)-N′-(D-gluconyl)thioureide (**5b**) in a 1:2 ratio (Chart 2, Table II).

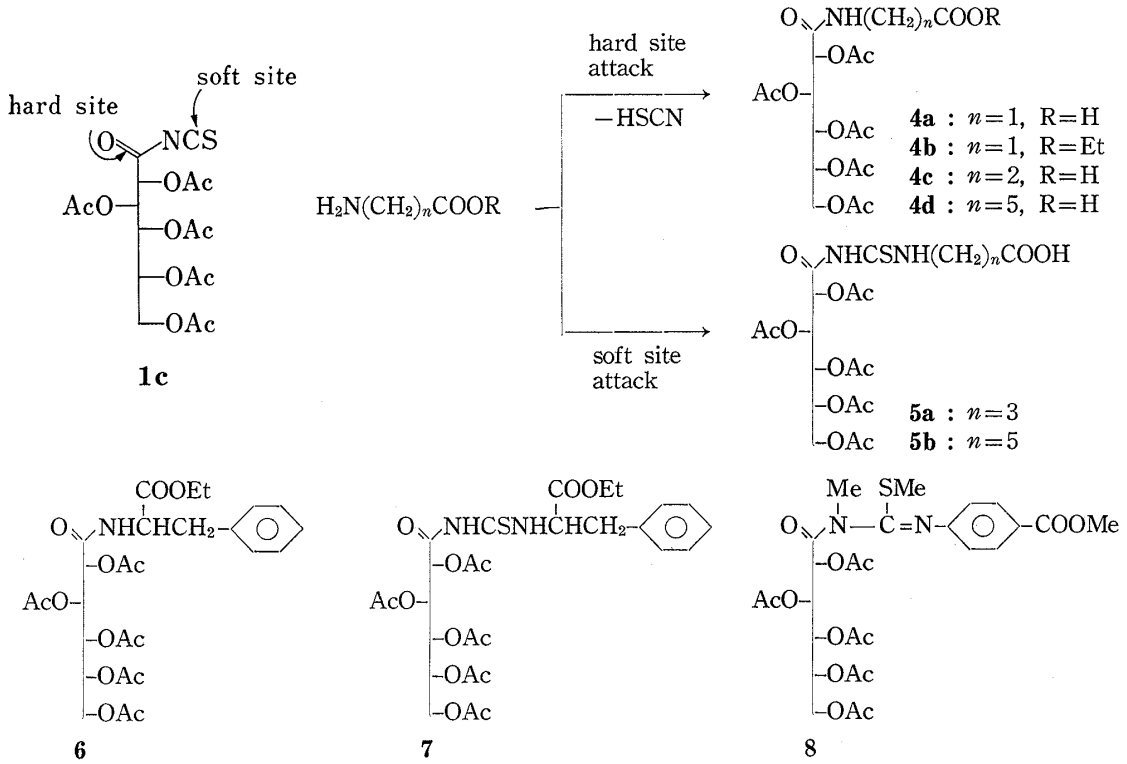

Chart 2

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Table II. N-Substituted 2,3,4,5,6-Penta-*O*-acetyl-D-gluconamides (**4a**, **b**, **c**, **d**) and N-(2,3,4,5,6-Penta-*O*-acetyl-D-gluconyl)-N′-substituted Thioureides (**5a**, **b**)

Compd. No.	mp (°C)	Yield (%)	IR ν_{max}^{KBr} cm^{-1}	NMR (δ, $CDCl_3$) heterocyclic moiety	Analysis (%) Calcd.	Found
					$C_{18}H_{25}NO_{13}\cdot H_2O$ (M^+. *m/e* 463)	
4a	123—125	80	3350, 1740, 1650, 1370, 1240	6.90 (1H, t, NH), 8.10 (1H, s, COOH)	C 44.90	44.71
					H 5.43	5.57
					N 3.02	2.85
					$C_{20}H_{29}NO_{13}$ (M^+, *m/e* 491)	
4b	147—149	90	3250, 1740, 1370, 1210	1.25 (3H, t, Me), 3.75 (2H, s, $NHC\underline{H}_2$, 6.60 (1H, bs, NH)	C 48.87	48.40
					H 5.94	5.76
					N 2.85	2.73
					$C_{19}H_{27}NO_{13}$	
4c	78— 79	85	3300, 1740, 1665, 1370, 1220	2.43—2.46 (2H, m, $C\underline{H}_2NH$), 3.26—3.65 (2H, m, $C\underline{H}_2CO$), 6.86—7.17 (1H, m, $N\underline{H}CO$), 7.55 (1H, bs, COOH)	C 44.77	44.76
					H 5.26	5.34
					N 5.22	4.48
					$C_{23}H_{35}NO_{13}$	
4d	70— 71	30	3250, 1740, 1360, 1210	1.10—1.82 (6H, m, $(CH_2)_3$), 3.10—3.40 (2H, m, CH_2CO), 3.70 (3H, s, Me), 6.30 (1H, bs, NH)	C 51.97	51.77
					H 6.54	6.61
					N 2.53	2.62
					$C_{21}H_{30}N_2O_{13}S$ ($M-2^+$, *m/e* 548)	
5a	113—115	40	3250, 1740, 1370, 1210	8.99—9.30 (2H, m, $(NH)_2$), 8.36 (1H, bs, COOH)	C 45.81	45.98
					H 5.49	5.47
					N 5.08	5.05
					$C_{25}H_{38}N_2O_{13}S$ ($M+1$, *m/e* 607)	
5b	Syrup[a]	60	3300, 1740, 1360, 1200	1.10—1.83 (6H, m, $(CH_2)_3$), 3.00—3.50 (2H, m, CH_2CO), 2.50 (3H, s, SMe), 3.70 (3H, s, Me), 10.56 (1H, bs, NH)	C 49.32	49.49
					H 6.19	6.31
					N 4.53	4.61

a) TLC (silica gel) *Rf* 0.53 ($CHCl_3$–MeOH=9:1).

Table III. N-(1-Carboxy-2-phenylethyl)-D-gluconamide (**6**), N-(1-Carboethoxy-2-phenylethyl)-N′-(D-gluconyl)thioureide (**7**) and N,N-Methyl(D-gluconyl)-N′-(*p*-carbomethoxyphenyl)-S-methylisourea (**8**)

Compd. No.	mp (°C)	Yield (%)	IR ν_{max}^{KBr} cm^{-1}	NMR (δ, $CDCl_3$) heterocyclic moiety	Analysis (%) Calcd.	Found
					$C_{25}H_{31}NO_{13}$ (M^+, *m/e* 553)	
6	158—159	80	3350, 1740, 1610, 1530, 1210, 695	3.15 (2H, d, J=5.8 Hz, $C\underline{H}_2Ph$), 6.40 (1H, J=8.0 Hz, NH), 6.90 (1H, s, COOH), 7.26 (5H, m, Ph)	C 54.24	53.96
					H 5.64	5.67
					N 2.53	2.65
					$C_{28}H_{36}N_2O_{13}S$ (M^+, *m/e* 640)	
7	Syrup[a]	78	3350, 1740, 1600, 1580, 1370, 1220, 700	1.20 (3H, t, $OCH_2C\underline{H}_3$), 3.23 (2H, d, J=6.0 Hz, $C\underline{H}_2Ph$), 4.15 (2H, q, $OC\underline{H}_2CH_3$), 7.20 (5H, m, Ph), 8.90 (1H, s, COOH), 10.40 (1H, d, J=8.0 Hz, NH)	C 52.50	52.82
					H 5.66	5.68
					N 4.37	4.28
					$C_{27}H_{34}N_2O_{13}S$ (M^+, *m/e* 627)	
8	Syrup[b]	80	3350, 1740, 1610, 1580, 1200, 700, 695	2.30 (3H, s, SMe), 3.39 (3H, s, NMe), 3.93 (3H, s, OMe), 7.73 (4H, m, Ph)	C 52.50	52.82
					H 5.46	5.42
					N 4.47	4.34

a) TLC (silica gel) *Rf* 0.70 (benzene–acetone=5:1).
b) TLC (silica gel) *Rf* 0.72 ($CHCl_3$–MeOH=9:1).

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Treatment of **1c** with L-phenylalanine in toluene solution under reflux, followed by treatment with ethyl bromide gave N-(1-carboethoxy-2-phenylethyl)-N′-(D-gluconyl)thioureide (**7**) in 75% yield. Similarly, reaction of **1c** with ethyl L-phenylalaninate hydrochloride in benzene under reflux afforded **7** in 78% yield. On the other hand, when the same reaction was carried out in THF, nitromethane or dioxane at room temperature, N-(carboxy-2-phenylethyl)-D-gluconamide (**6**) was obtained in 70—80% yield (Table III).

Schrefer *et al.*[5] and Matsui *et al.*[6] discussed the reaction between 2-aminothiazole, 2-aminopyrimidine, 2-aminopyridine or 4-aminopyridine, and an ambident electrophile, namely ethoxycarbonyl isothiocyanate. The results are readily explicable by the HSAB principle.[7] It is known that the reaction is influenced by numerous factors (the electrophile, solvent, temperature, and the inherent structural characteristics of the nucleophile). In our experiments, in the case of glycine (number of methylene groups: $n=1$) and β-alanine ($n=2$), the nucleophilic reaction of **1c** probably occurred at a hard site, and the corresponding D-gluconamides (**4a**, **b** and **c**) were obtained in fair yields with elimination of a molecule of HNCS. On the other hand, in the case of γ-aminobutyric acid ($n=3$), the reaction with **1c** occurred at a soft site. The steric effects probably modified the HSAB principle.

Condensation between isothiocyanates and 6-aminopenicillanic acid (6-APA) or α-aryloxyalkanoic acid was reported by Perron *et al.*[8] Treatments of **1a**, **b** and **c** with 6-APA or ampicillin in THF or dimethylformamide (DMF) in the presence of triethylamine afforded the corresponding triethylammonium 6-(substituted thioureido)penicillanates (**9a**, **b**, **c**) and 6-(glycosylthioureido)benzylpenicillanates (**10a**, **b**) in good yields. Reaction of **1c** with phenacyl 6-aminopenicillanate gave phenacyl 6-(D-gluconylthioureido)penicillanate (**9d**) in 89% yield.[9]

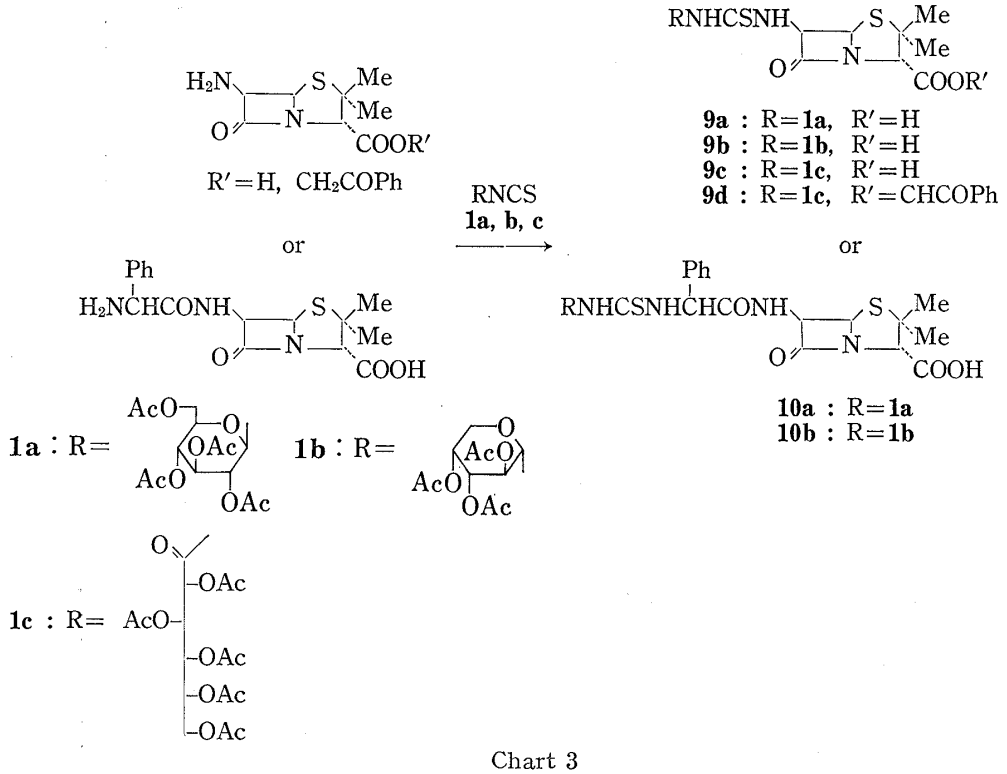

Chart 3

5) H.J. Schrefer, L. Capuano, and H-L. Schmidt, *Chem. Ber.*, **106**, 2118 (1973).
6) T. Matsui, M. Nagano, J. Tobitsuka, and K. Oyama, *Chem. Pharm. Bull.* (Tokyo), **22**, 2118 (1974); T. Matsui and M. Nagano, *Chem. Pharm. Bull.* (Tokyo), **22**, 2123 (1974).
7) R.G. Pearson, *J. Am. Chem. Soc.*, **85**, 3533 (1963); Tse-Lok Ho, "Hard and Soft Acids and Bases in Organic Chemistry," Academic Press., New York, 1977.
8) Y.G. Perron, W.F. Minor, L.B. Crast, and L.C. Cheney, *J. Org. Chem.*, **26**, 3365 (1961).
9) See, H. Ogura, and H. Takahashi, *Heterocycles*, **8**, 125 (1977). Some of these penicillin derivatives exhibited antibacterial activity.

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Table IV. Triethylammonium 6-(D-Glycosylthioureido)penicillanate (**9a, b**) and Triethylammonium 6-(D-Glycosylthioureido)benzylpenicillanate (**10a, b**)

Compd. No.	mp (°C)	Yield (%)	IR ν_{max}^{KBr} cm^{-1}	Analysis (%) Calcd.	Found
				$C_{29}H_{46}N_4O_{12}S_2$	
9a	160—161	87	3350, 3250, 1740, 1380, 1370, 1210	C 49.28	49.58
				H 6.56	6.62
				N 7.93	7.86
				$C_{26}H_{42}N_4O_{10}S_2$	
9b	Syrup[a]	76	3250, 1740, 1375, 1370, 1220	C 46.20	49.46
				H 6.67	6.57
				N 8.83	8.69
				$C_{37}H_{53}N_5O_{13}S_2$	
10a	Syrup[b]	90	3350, 1740, 1380, 1370, 1210, 760	C 52.91	53.06
				H 6.36	6.42
				N 8.34	8.30
				$C_{34}H_{49}N_5O_{11}S_2$	
10b	182—184	83	3350, 1740, 1375, 1370, 1220, 750	C 53.18	53.50
				H 6.43	6.27
				N 9.12	9.14

a) TLC (silica gel) *Rf* 0.52 (benzene/acetone=1:1).
b) TLC (silica gel) *Rf* 0.32 (benzene/acetone=1:1).

Experimental

All melting points are uncorrected. Infrared spectra (IR) were measured with a JASCO A-2 spectrometer and nuclear magnetic resonance (NMR) spectra on a Varian T-60 spectrometer; tetramethylsilane was used as an internal reference. Mass spectra were determined with a JMS-D-100 spectrometer using a direct inlet system at 75 eV.

N-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-N′-carboethoxyalkylthioureide (**3a, b, c**) (**Table I**)——A solution of 2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl isothiocyanate (389 mg, 0.001 mol) and amino acid (ethyl L-phenylalaninate hydrochloride, ethyl L-leucinate hydrochloride or ethyl β-alaninate hydrochloride) (0.001 mol) with dry pyridine (0.5 ml) in dry benzene (20 ml) was stirred for 5 hr at room temperature. The reaction solution was washed with H_2O, dried over $MgSO_4$ and evaporated to dryness under reduced pressure to give **2a, b** or **c** in 85—93% yield.

2,3,4,5,6-Penta-*O*-acetyl-D-gluconyl isothiocyanate (**1c**)——2,3,4,5,6-penta-*O*-acetyl-D-gluconyl chloride (9 g, 0.02 mol) prepared by the reported method[10] was dissolved in dry xylene (200 ml) under an N_2 atmosphere. Silver thiocyanate (AgNCS) (3.3 g, 0.02 mol) was added to the reaction solution with stirring. After warming the reaction mixture at 90—100° for 5 hr, AgNCS (1.7 g, 0.01 mol) was added gradually and stirring was continued at the same temperature for 3 hr. After cooling, the separated precipitate was removed by filtration and petroleum ether (300 ml) was added to the filtrate. The mixture was allowed to stand in a freezer overnight to form crystals. Colorless fine needles were collected by filtration to give 7.2 g (83%) of **1c**. mp 132—135°. IR ν_{max}^{KBr} cm^{-1}: 1980 (NCS), 1740 (ester). *Anal.* Calcd. for $C_{17}H_{21}NO_{11}S$: C, 45.64; H, 4.73; N, 3.13. Found: C, 45.59; H, 4.90; N, 3.05. NMR (C_6D_6) δ: 1.65, 1.66, 1.80 (15H, s, OCOMe×5), 4.04, 4.32 (2H, dd, 6-H_2), 5.22 (1H, m, 5-H), 5.43 (1H, d, J=2.0 Hz, 2-H), 5.78, 5.83 (2H, m, 3-H, 4-H).

N-Carboxymethyl-2,3,4,5,6-penta-*O*-acetyl-D-gluconamide (**4a**)——A mixture of **1c** (447 mg, 0.001 mol) and glycine (75 mg, 0.001 mol) in THF (10 ml) solution was stirred for 3 days at room temperature then evaporated to dryness under reduced pressure. The residue was crystallized from ether to afford crude **4a**. Recrystallization from MeOH gave **4a** as fine colorless needles.

N-Carboethoxymethyl-2,3,4,5,6-penta-*O*-acetyl-D-gluconamide (**4b**)——A mixture of **1c** (447 mg, 0.001 mol), ethyl glycinate hydrochloride (139 mg, 0.001 mol) and dry pyridine (0.2 ml) in THF (10 ml) was stirred for 24 hr at room temperature and then concentrated under reduced pressure. The residue obtained was extracted with $CHCl_3$ (100 ml), and the organic layer was washed with H_2O and dried over $MgSO_4$. Evaporation of the solvent left a syrup. ether (10 ml) was added to the syrup and the mixture was left in a freezer overnight. Separated crystals were collected by filtration and recrystallized from EtOH to give **4b** as colorless needles.

N-(2-Carboxyethyl)-2,3,4,5,6-penta-*O*-acetyl-D-gluconamide (**4c**)——A solution of **1c** (447 mg, 0.001 mol) and β-alanine (89 mg, 0.001 mol) in THF (10 ml) was stirred for 3 hr. Removal of the solvent by evaporation

10) R.T. Major and E.W. Cook, *J. Am. Chem. Soc.*, **58**, 2474 (1936).

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left a syrup which was chromatographed on silica gel with ether to give crystalline **4c**. Recrystallization from ether gave **4c** as fine colorless needles.

N-(3-Carboxypropyl)-N′-(2,3,4,5,6-penta-*O*-acetyl-D-gluconyl)thioureide (5a)——A solution of **1c** (447 mg, 0.001 mol) and γ-aminobutyric acid (103 mg, 0.001 mol) in THF (15 ml) was treated as described in **4a**. The yellow residue was dissolved in benzene (30 ml) and treated with active charcoal. The filtrate was concentrated under reduced pressure to afford a syrup which was chromatographed on silica gel with benzene. It was crystallized from ether to give **5a** as colorless needles.

N-(5-Carbomethoxypentyl)-2,3,4,5,6-penta-*O*-acetyl-D-gluconamide (4d) and N-(5-Carbomethoxypentyl)-N′-(2,3,4,5,6-penta-*O*-acetyl-D-gluconyl)thioureide (5b)——A mixture of **1c** (447 mg, 0.001 mol) and ε-aminocaproic acid (131 mg, 0.001 mol) in THF (10 ml) was stirred for 3 hr at room temperature then evaporated down under reduced pressure. The residue was dissolved in ether (50 ml) and treated with an excess of CH_2N_2–ether (15 ml) under cooling. The reaction solution was stirred for 2 hr, washed with H_2O and dried over $MgSO_4$. Removal of the solvent by evaporation gave a syrup which was chromatographed on silica gel with benzene–acetone. From the eluate with benzene, **4d** was obtained and crystallized from ether to give **4d** as colorless needles. Elution with benzene–acetone (5: 1) afforded **5b** as a colorless syrup.

N-(1-Carboxy-2-phenylethyl)-2,3,4,5,6-penta-*O*-acetyl-D-gluconamide (6)——A solution of **1c** (447 mg, 0.001 mol) and L-phenylalanine (165 mg, 0.001 mol) in THF, $MeNO_2$ or dioxane (10 ml) was stirred for 2 hr at room temperature and evaporated to dryness under reduced pressure. Recrystallization from ether–petroleum ether–EtOH (1: 1: 0.5) gave **6** as colorless needles.

N-(1-Carboethoxy-2-phenylethyl)-N′-(2,3,4,5,6-penta-*O*-acetyl-D-gluconyl)thioureide (7)——a) A solution of **1c** (447 mg, 0.001 mol), ethyl L-phenylalaninate hydrochloride (219 mg, 0.001 mol) and dry pyridine (0.5 ml) in dry benzene (10 ml) was refluxed for 30 min. The reaction mixture was washed with H_2O, dried over $MgSO_4$ and concentrated under reduced pressure to give **7** as a colorless syrup.

b) A suspension of **1c** (889 mg, 0.002 mol) and L-phenylalanine (330 mg, 0.002 mol) in dry toluene (10 ml) was refluxed for 8 hr and allowed to stand at room temperature. To the solution was added 200 mg of $NaHCO_3$, and the mixture was evaporated down under reduced pressure to give a solid. DMF (5 ml) was added to the solid and the reaction mixture was treated with EtBr (220 mg, 0.002 mol) with stirring at room temperature. After 4 hr, the mixture was poured into ice-H_2O (100 ml) and extracted with EtOAc. The organic layer was washed with H_2O, dried over $MgSO_4$ and evaporated down under reduced pressure to give a slightly brown residue. The residue was chromatographed on silica gel with benzene to afford **7** as a colorless syrup.

N,N-Methyl(2,3,4,5,6-penta-*O*-acetyl-D-gluconyl)-N′-(*p*-carbomethoxyphenyl)-S-methylisourea (8)——A mixture of **1c** (447 mg, 0.001 mol) and *p*-aminobenzoic acid (137 mg, 0.001 mol) in THF (10 ml) was stirred for 2 hr and evaporated to dryness. The residue was dissolved in ether and treated with CH_2N_2, followed by chromatographic separation as described for **5b** to give **8**.

Triethylammonium 6-(Glycosylthioureido)penicillanate (9a, b) and Triethylammonium 6-(Glycosylthioureido)benzylpenicillanate (10a, b)——To a solution of 6-APA (220 mg, 0.001 mol) or ampicillin (349 mg, 0.001 mol) in DMF (0.5—0.7 ml), glycosyl isothiocyanate (**1a** or **b**) (0.001 mol) was added at 0—5°. The reaction solution was stirred at 0° for 4 hr and then at room temperature for 20 hr. The reaction mixture was poured into ether (100 ml) and the separated precipitate was recrystallized from MeOH to give **9a** or **b** or **10a** or **b** as fine colorless needles.

6-(2,3,4,5,6-Penta-*O*-acetyl-D-gluconylthioureido)penicillanate (9c)——A solution of **1c** (447 mg, 0.001 mol) and 6-APA (216 mg, 0.001 mol) in DMF (15 ml) was stirred for 24 hr at room temperature. Insoluble material was filtered off and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in $CHCl_3$ (50 ml) and washed with saturated $NaHCO_3$. The basic layer was treated with 1 N HCl to render it acidic, with cooling. After extraction with $CHCl_3$ the organic layer was dried over $MgSO_4$ and removal of the solvent by evaporation gave crude **9c** as a syrup. This was chromatographed on silica gel with ether. Crystallization from DMF/ether (1: 1) afforded 670 mg (91%) of **9c** as fine colorless needles. mp 62—63°. IR ν_{max}^{KBr} cm^{-1}: 3500 (NH), 2900 (COOH), 1780 (β-lactam), 1740 (ester), 1650 (CO), 1380, 1210. *Anal.* Calcd. for $C_{25}H_{33}N_3O_{14}S_2 \cdot Me_2NCHO$: C, 45.64; H, 5.47; N, 7.60. Found: C, 45.17; H, 5.51; N, 7.64. NMR ($CDCl_3$) δ: 1.60 (6H, s, Me_2), 2.96 (6H, s, NMe_2), 8.06 (1H, s, CHO), 9.33 (1H, bs, NH), 9.73 (1H, s, COOH), 10.60 (1H, d, J=3.0 Hz, CSNH).

Phenacyl 6-(2,3,4,5,6-Penta-*O*-acetyl-D-gluconylthioureido)penicillanate (9d)——A mixture of **1c** (1.12 g, 0.0025 mol) and phenacyl 6-aminopenicillanate (830 mg, 0.0025 mol) in THF (10 ml) was stirred for 20 min at room temperature. The reaction solution was evaporated to dryness under reduced pressure to give 1.74 g (89%) of **9d** as colorless needles., mp 78—79°. IR ν_{max}^{KBr} cm^{-1}: 3500 (NH), 1785 (β-lactam), 1740 (ester), 1650 (CONH), 1380, 1210. NMR ($CDCl_3$) δ: 1.69 (6H, s, Me_2), 4.13—4.30 (2H, m, CH_2), 4.63 (1H, s, 3-H), 7.16—7.96 (4H, m, Ph), 9.30 (1H, s, COOH), 10.59 (1H, d, J=3.2 Hz, CSNH). *Anal.* Calcd. for $C_{33}H_{35}N_3O_{15}S_2 \cdot Me_2NCHO$: C, 50.57; H, 5.43; N, 6.55. Found: C, 50.85; H, 5.45; N, 6.65.

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