was converted into 2,3-diamino-5-chloropyridine in 2 steps *via* the procedure of Vaughan, *et al.*[13] The following starting materials were obtd from Reilly Tar and Chemical Corporation: 2-aminopyridine, 2-amino-4-methylpyridine, 2-amino-6-methylpyridine, and 2-amino-4,6-dimethylpyridine. Bromination of 2-aminopyridine by the method described by Case[14] afforded 2-amino-5-bromopyridine, which was nitrated and reduced by means of the Vaughan procedure[13] to give 2,3-diamino-5-bromopyridine. 2,3-Diamino-5-bromo-4-methylpyridine and 2,3-diamino-5-bromo-6-methylpyridine were prepd according to the procedure of Israel and Day.[8] The method of Grayboyes and Day[15] was used to obtain 2,3-diamino-5-bromo-4,6-dimethylpyridine.

Condensation of Pyridinediamines with Alloxan Monohydrate. 1*H*,3*H*-2,4-Dioxopyrido[3,2-*g*]pteridines (1–6). The following procedure was used to prepare **1–6**, except that a full equiv of $B(OH)_3$ was used for the prepn of **1**.

A soln containing 4.8 g (0.03 mole) of alloxan monohydrate and 0.5 g (0.008 mole) of $B(OH)_3$ in 100 ml of glac AcOH was combined quickly with a soln of 0.03 mole of the pyridinediamine in 100 ml of glac AcOH and the resulting reaction soln was stirred at room temp for 2–3 hr. At the end of the reaction, the solid present was collected, washed well with cold H_2O, and then dried (NaOH pellets) The product was purified according to the method indicated in Table I. Purified samples showed single spots on paper chromatog in 2 solvent systems [1-BuOH–AcOH–H_2O (4:1:1) and 1-BuOH satd with H_2O].

As indicated by microchem analysis, **2** formed a hemihydrate on purification by base/acid pptn; the hemihydrate was stable to prolonged drying at 100° under vacuum. The existence of **2** in this sample was confirmed by mass spectral analysis which showed two peaks in a 3:1 relationship at *m/e* 249 and 251, consistent with the natural isotope distribution of ^{35}Cl and ^{37}Cl in **2**. Similarly, the mass spectrum of the methanolate of **5**, formed on crystn of **5** from MeOH, showed peaks of essentially equal intensity at *m/e* 307 and 309, consistent with the natural abundance of ^{79}Br and ^{81}Br in the parent compd.

2-Carboxyureido-3,4-dihydropyrido[2,3-*b*]pyrazin-3-one (7a). A soln of 1.81 g (0.017 mole) of 2,3-diaminopyridine in 75 ml of glac AcOH was combined with a soln of 2.7 g (0.017 mole) of alloxan monohydrate in 75 ml of glac AcOH. The reaction soln was stirred at room temp overnight. The yellow solid was sepd, washed with AcOH, and dried; mp 280–283° dec [lit.[2] mp 273–280° dec; 283–285° dec[4]]; uv: λ_{max}^{EtOH} 225, 277, and 361 nm; $\lambda_{max}^{pH\,10}$ 232, 260 (sh), 308, and 396 nm.

3-Keto-3,4-dihydropyrido[2,3-*b*]pyrazine-2-carboxylic Acids (9–14). Method A. Hydrolysis of 1–6. A sample of the corresponding azaalloxazine was boiled with 1 *N* NaOH (60 ml/g) for 5 hr. The pale yellow soln was cooled to below room temp and the pH was adjusted to 1 by the addn of 3 *N* HCl (CO_2 evoln). The pale yellow to green solid was purified by several pptns from 1 *N* NaOH soln (charcoal) by the addn of HCl.

Method B. Pyridinediamine and Disodio Ketomalonate in Base. A soln contg equimolar quantities of the pyridinediamine and the disodium salt of ketomalonic acid in 1 *N* NaOH was refluxed overnight. The soln was then acidified (to pH 1) and the product collected and purified by several base/acid pptns.

Method C. 2,3-Diaminopyridine and Alloxan in Base. Preparation of 9. A soln of 2.5 g (0.014 mole) of 2,3-diaminopyridine and 2.44 g (0.014 mole) of alloxan monohydrate in 100 ml of 1 *N* NaOH was boiled for 16 hr. The soln was cooled and a small quantity of inorganic material was sepd. The pale yellow filtrate was acidified to pH 1 by the addn of 4 *N* HCl and the ppt of **9** was collected.

References

(1) M. Israel, N. Muhammad, N. Tirosh, L. C. Jones, and E. J. Modest, *Int. Cong. Heterocycl. Chem., 3rd.*, 109 (1971).
(2) J. B. Ziegler, *J. Amer. Chem. Soc.*, 71, 1891 (1949).
(3) (a) R. Kuhn, K. Reinemund, F. Weygand, and R. Ströbele, *Ber.*, 68, 765 (1935); (b) R. Kuhn and F. Weygand, *ibid.*, 68, 1282 (1935).
(4) J. W. Clark-Lewis and M. J. Thompson, *J. Chem. Soc.*, 430 (1957).
(5) E. C. Taylor and H. M. Loux, *J. Amer. Chem. Soc.*, 81, 2474 (1959).
(6) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, Inc., San Francisco, Calif., 1962, p 47.
(7) H. G. Petering, U. S. Patent 2,973,359 (1961).
(8) M. Israel and A. R. Day, *J. Org. Chem.*, 24, 1455 (1959).
(9) M. Israel, L. C. Jones, and E. J. Modest, *J. Heterocycl. Chem.*, in press.
(10) (a) G. E. Foley and H. Eagle, *Cancer Res.*, 18, 1012 (1958); (b) H. Eagle and G. E. Foley, *ibid.*, 18, 1017 (1958).
(11) C. L. Maddock, G. J. D'Angio, S. Farber, and A. H. Handler, *Ann. N. Y. Acad. Sci.*, 89, 386 (1960).
(12) E. C. Wagner and J. F. Meyer, *Ind. Eng. Chem., Anal. Ed.*, 10, 584 (1938).
(13) J. R. Vaughan, Jr., J. Krapcho, and J. P. English, *J. Amer. Chem. Soc.*, 71, 1885 (1949).
(14) F. Case, *ibid.*, 68, 2576 (1946).
(15) H. Graboyes and A. R. Day, *ibid.*, 79, 6421 (1957).

Nucleoside Peptides. 4. Synthesis of Certain 1-(*N*-4-Aminoacyl-4-amino-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranuronic acid)cytosine Derivatives Related to Blasticidin S

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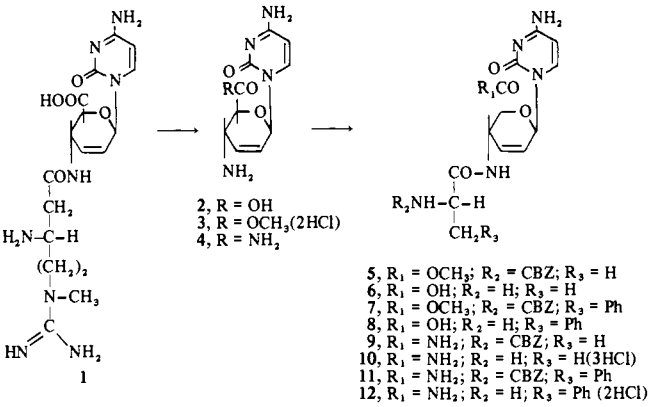
Received December 30, 1971

The antibiotic blasticidin S (**1**, Scheme I) has been studied extensively (for a recent review of blasticidin S see ref 1) and been found to be a potent inhibitor of protein biosynthesis.[2] The present study was initiated to investigate possible changes in biological properties of this antibiotic by the replacement of the amino acid (blasticidic acid) portion of blasticidin S with several commonly occurring amino acids. These studies follow a general program for the synthesis of nucleoside peptides as potential medicinal agents.[3a-c]

Scheme I



The starting material, cytosinine (**2**, Scheme I), was prepared by alkaline hydrolysis of **1** according to the procedure of Yonehara and coworkers.[4] In our hands cytosinine was not absorbed on an Amberlite IRC-50 column; therefore, this procedure was modified by the absorption of cytosinine (**2**) on an IRA-410 (OH^- form) column and eluting the desired product in 46% yield with acetic acid. The preparation of 1-(4-amino-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranuronic acid methyl ester)cytosine (**3**) has also been previously described[5] by treatment of cytosinine with methanol containing 3% hydrogen chloride. It was found, however, that 10% methanolic hydrogen chloride was necessary for the complete conversion of **2** to **3**.

The action of dicyclohexylcarbodiimide (DCC)[6] on a mixture of *N*-carbobenzyloxy(CBZ)-L-alanine *N*-hydroxysuccinimide (NHS),[7,3c] and **3** provided 1-(4-[(*S*)-2′-CBZ-aminopropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-eno-

pyranuronic acid methyl ester)cytosine (**5**). The CBZ-group of **5** was removed by treatment with hydrogen bromide while the methyl ester moiety was converted to a carboxylic acid by alkaline hydrolysis with an IRA-410 (OH^- form) column.[8] This provided the desired aminoacylcytosinine, 1-(4-[(*S*)-2′-aminopropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronic acid)cytosine (**6**).

In a similar manner *N*-CBZ-L-phenylalanine was coupled to **3** and the product **7** deblocked to afford 1-(4-[(*S*)-2′-amino-3′-phenylpropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronic acid cytosine (**8**).

Since the nucleoside portions of blasticidin S and gougerotin[9] were similar we investigated the possibility of converting the carboxyl function of these blasticidin S analogs to a carboxamide group as in the structure of gougerotin. A facile conversion of cytosinine methyl ester (**3**) to 1-(4-amino-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranuronamide)-cytosine (**4**) was accomplished with methanolic ammonia. Similarly 1-(4-[(*S*)-2′-CBZ-aminopropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronamide)cytosine (**9**) and 1-(4-[(*S*)-2′-CBZ-amino-3′-phenylpropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronamide)cytosine (**11**) were prepared from **5** and **7**, respectively. Removal of the CBZ blocking groups of **9** and **11** was accomplished by acid hydrolysis with hydrochloric acid and afforded 1-(4-[(*S*)-2′-aminopropionylamido]-2,3,4-trideoxy-β-D-*erythro*-pyranuronamide)cytosine (**10**) and 1-(4-[(*S*)-2′-amino-3′-phenylpropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronamide (**12**).

These analogs of blasticidin S were tested as inhibitors of protein biosynthesis in the cell-free protein synthesizing system of Ochoa, *et al.*,[10] utilizing an S-30 fraction from *Escherichia coli* Q-13 as the enzyme source. The assay system measured the poly(U)-dependent incorporation of [^{14}C]-phenylalanine into hot trichloroacetic acid (TCA) insoluble material. Under conditions of limiting S-30 and poly(U), blasticidin S was 50% inhibitory at a concentration of $3 \times 10^{-7} M$. Under these conditions compounds **8** and **12** were slightly inhibitory at $10^{-3} M$ (13% and 24% respectively), compounds **2**, **3**, **4**, **6**, and **9** showed no inhibition at $10^{-3} M$ and compounds **5**, **7**, **10**, and **11** were insoluble at $2 \times 10^{-3} M$ but also showed no inhibition when assayed from a saturated stock solution.

Experimental Section†

1-(4-Amino-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranuronic acid)cytosine (**2**). A suspension of **1** (HCl salt) (19.8 g, 0.043 mole) in aqueous NaOH (6.9 g, 400 ml) was stirred at 27–30° for 3 weeks. The resulting soln was neutralized with IRC-50 (H^+, *ca.* 120 ml (dry)) to pH 6.8–7. The resin and undissolved materials were removed by filtration with a Celite pad and washed with H_2O (300 ml). The filtrate was applied to an ion-exchange column (IRA-410, OH^-, 200 ml (wet), diameter = 2.6 cm) packed with water. The resin was washed with H_2O (1.6 l.), and the products were then eluted with 0.2 *N* HOAc; 100-ml fractions were collected. The fractions 10–17, which had uv absorption, were concentrated to dryness below 30° *in vacuo*. The residue was dissolved in H_2O (*ca.* 50 ml), and treated with charcoal. After removal of the charcoal, the soln was diluted with ethanol (100 ml) and stirred vigorously. After several minutes, crystals appeared. The soln was allowed to stand at 5° for 3 hr, and EtOH (50 ml) was then added with vigorous stirring. This mixture containing crystals was again allowed to stand at 5° overnight. The crystals were collected, washed with a small amount of cold H_2O, and dried (P_2O_5) to give 5.0 g (46%) of **2**. This compound could be used for further reactions.

Recrystallization from H_2O with charcoal treatment afforded an analytical sample, which was dried at room temp (P_2O_5) *in vacuo* overnight (Table I). *Anal.* Calcd for $C_{10}H_{12}N_4O_4$: C, H, N.

1-(4-Amino-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranuronic acid methyl ester)cytosine Dihydrochloride (**3**). A mixt of **2** (4.8 g, 0.019 mole) and 10% HCl–CH_3OH (90 ml) was gently refluxed at 90–95° (bath temp) for 1 hr. After the reaction mixt had been cooled, the resulting crystals were removed by filtration, washed with cold abs MeOH, and dried (P_2O_5–KOH), giving **3** (5.2 g, 75%).

Recrystallization from MeOH afforded an analytically pure sample, which was dried at room temp (P_2O_5) *in vacuo* for 2 hr (Table I). *Anal.* Calcd for $C_{11}H_{14}N_4O_4 \cdot 2HCl \cdot 1.5H_2O$: C, H, N.

1-(4-Amino-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranuronosyl amide)cytosine (**4**). To a soln of MeOH satd at 0° with NH_3 was added **3** (1.0 g, 2.7 mmoles), and the mixt was stirred at room temp overnight. The solvent was removed by repeated coevap with EtOH. The residue was dried over P_2O_5 in a desiccator and crystallized from EtOH–H_2O to give needles (700 mg, 89%). Recrystallization afforded an analytically pure sample, which was dried at 56° (P_2O_5) *in vacuo* for 4 hr (Table I). *Anal.* Calcd for $C_{10}H_{13}N_5O_3 \cdot 2H_2O$: C, H, N.

1-(4-[(*S*)-2′-CBZ-aminopropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronic acid methyl ester)cytosine (**5**). To a suspension of $3 \cdot 1.5H_2O$ (1.46 g, 4 mmoles) in DMF (16 ml) were added $(CH_3)_3N$ (848 mg, 8.4 mmoles), *N*-CBZ-L-Ala (892 mg, 4 mmoles), and *N*-hydroxysuccinimide (NHS) (460 mg, 4 mmoles) with stirring. After the mixture had been cooled with an ice-water bath, DCC (824 mg, 4 mmoles) was added. The mixture was then stirred at room temp for 2.5 hr. The crystals formed were removed by filtration and washed with a small amount of DMF. The combined filtrate and washings were poured dropwise into a cold soln of $NaHCO_3$ (0.5 g) in H_2O (200 ml) with vigorous stirring to give precipitation. After the mixt had been allowed to stand at 5° overnight, the precipitate was collected, washed with cold H_2O, and dried (P_2O_5) *in vacuo*, giving crude **5** (1.5 g, 79%), which could be used to run the later reactions.

This product (200 mg) was dissolved in a minimum amount of warm MeOH and the solution was applied to a silica gel column (40 g, 2.3 cm) which was packed with $CHCl_3$–MeOH (8:2, v/v). The product was eluted with the same solvents and 20-ml fractions were collected. Fractions 8–12 were combined and the solvent was removed *in vacuo* to give an analytical sample (120 mg) as a colorless solid, which was dried at 110° (P_2O_5) *in vacuo* for 2 hr: tlc (silica gel) R_f 0.56 ($CHCl_3$–MeOH (8:2, v/v)) (Table I). *Anal.* Calcd for $C_{22}H_{25}N_5O_7 \cdot 0.2H_2O$: C, H, N.

1-(4-[(*S*)-2′-Aminopropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronic acid)cytosine (**6**). To a 5.8% HBr–AcOH soln (5.2 ml) was added $5 \cdot 0.2H_2O$ (330 mg, 0.69 mmole) and the mixt was stirred at room temp for 1.3 hr. The solvent was removed below 35° *in vacuo* and the residue was taken up in MeOH (4 ml). This soln was dropwise added with stirring into diethyl ether (70 ml). The resulting precipitate was removed by filtration, washed with diethyl ether, and dried immediately (P_2O_5–KOH) *in vacuo*. The dried product was taken up in H_2O (10 ml) and the undissolved material was removed by filtration with a Celite pad and the filtrate was applied to a column (2 cm) of an ion-exchange resin (IRA-410, OH^- form, 40 ml). The column was washed slowly with water (70 ml) in order to complete the hydrolysis of the methyl ester, and then rapidly with an additional quantity of water (200 ml). The compound was eluted with 0.2 *N* acetic acid, 25-ml fractions were collected. The fractions 12–16 were combined and the solvent was removed *in vacuo* and gave crude **6** (200 mg, 84%), which was crystallized from EtOH–H_2O (Table I). *Anal.* Calcd for $C_{13}H_{17}N_5O_5 \cdot H_2O$: C, H, N.

1-(4-[(*S*)-2′-CBZ-amino-3′-phenylpropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronic acid methyl ester)cytosine (**7**). A suspension of $3 \cdot 1.5H_2O$ (1.46 g, 4 mmoles) in DMF (16 ml) was treated with $(C_2H_3)_3N$ (848 mg, 8.4 mmoles), *N*-CBZ-L-phenylalanine (1.2 g, 4 mmoles), NHS (460 mg, 4 mmoles), and DCC (824 mg, 4 mmoles) in a manner similar to that used in the preparation of **5**. The crude product was reprecipitated from refluxing isopropanol to give crude **7** as an amorphous solid (1.35 g, 62%).

Further reprecipitation from the same solvent afforded an analytical sample, which was dried (P_2O_5) at 110° *in vacuo* for 2 hr:

†Physical properties of these compounds were determined with the following instruments: melting points, Thomas-Hoover apparatus (uncorrected); uv spectra, Cary 15 uv spectrometer; specific rotations, Perkin-Elmer Model 141 polarimeter; nmr, Hitachi Perkin Elmer R20A high resolution nmr spectrometer (Me_4Si or DSS); and ir spectra, Perkin-Elmer Model 257 (KBr). The elemental analyses were performed by Heterocyclic Chemical Corporation, Harrisonville, Mo. Where indicated by elemental analyses, hydration was verified quantitatively by nmr spectroscopy in abs DMSO-d_6, then by exchange with addition of D_2O and reintegration of the spectral area where the D_2O peak had occurred.

Table I. Physical Constants of Blasticidin S Derivatives[d]

Compound	Mp, °C	$[\alpha]^{25}D$, deg	Ultraviolet spectra		
			$\lambda_{max}^{pH\ 1}$, nm (ϵ)	$\lambda_{max}^{pH\ 11}$, nm (ϵ)	λ_{max}^{MeOH}, nm (ϵ)
2	200 dec	−37.0[a]	274 (13,000)	268 (9,000)	268 (8400)
	(244-245)[e]	−20[b]		235 (8,000)	241 (8100)
			274 (13,800)[e]	267 (7,500)[e]	
3	198-199 dec	−24.3[a]	274 (13,600)	267 (9,600)	267 (8600)
	(200-223 dec)[e]				
4	235-237 dec	−48.5[a]	274 (13,000)	267 (9,200)	270 (8200)
				237 (8,400)	244 (9000)
5		+85.3[c]	274 (13,000)	267 (9,200)	267 (8700)
				237 (7,500)	239 (8700)
6	196-197 dec	+114[a]	274 (13,700)	267 (9,600)	269 (9200)
				236 (8,400)	238 (8300)
7		+107.5[c]	274 (14,500)	266 (10,500)	
				236 (9,400)	
8	195-197 dec	+140[a]	275 (12,600)	267 (8,900)	269 (6300)
				235 (7,700)	238 (5500)
9		+114[c]	274 (14,100)	266 (10,100)	266 (9200)
				242 (9,000)	248 (8700)
10	196-197 dec	+58.0[a]	274 (14,300)	267 (9,900)	268 (9400)
				237 (8,900)	243 (8600)
11	233-235 dec	+148[c]	275 (14,600)	266 (10,500)	265 (9800)
				(9,500)	
12	185-190 sinters		274 (12,800)	266 (8,900)	266 (8900)
				236 (7,900)	240 (8500)

[a] *c* 1, H_2O. [b] $[\alpha]^{18}D$ (*c* 1, H_2O). [c] *c* 1, DMSO. [d] See footnote †. [e] See ref 11.

tlc (silica gel) R_f 0.69 ($CHCl_3$–MeOH (8:2, v/v)) (Table 1). *Anal.* Calcd for $C_{28}H_{29}N_5O_7$: C, H, N.

1-(4-[(*S*)-2′-Amino-3′-phenylpropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronic acid)cytosine (8). To a 5% HBr–AcOH soln (7 ml) was added **7** (548 mg, 1.0 mmole), and the mixt was stirred at room temp for 1 hr. The solvent was removed *in vacuo* below 35°, and the residue was dissolved in MeOH (5 ml). The soln was added dropwise into stirring diethyl ether (100 ml). The resulting precipitate was collected, washed with diethyl ether, and dried immediately (P_2O_5–KOH) *in vacuo*. The dried compound was taken up in H_2O (8 ml), and the undissolved material was removed by filtration with charcoal. The filtrate was applied to an ion-exchange column (1RA-410, OH^- form, 40 ml, 1.9 cm), which was washed slowly with water (20 ml) in order to complete the hydrolysis of the methyl ester, and then rapidly with water (150 ml). The product was eluted with 0.2 *N* HOAc and 25-ml fractions were collected. The fractions 11–13 were combined and evaporated to dryness *in vacuo*. The residue was taken up in H_2O (10 ml), and the soln was treated with charcoal and filtered. The filtrate was freeze-dried to give crude **8** (300 mg, 75%), crystallized from water to give an analytically pure sample, and then dried at room temp *in vacuo* (P_2O_5, Table 1). *Anal.* Calcd for $C_{19}H_{21}N_5O_5$: C, H, N.

1-(4-[(*S*)-2′-CBZ-aminopropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronamide)cytosine (9). To a soln satd at 0° with MeOH–NH_3 (20 ml) was added **5**·0.2H_2O (780 mg, 1.6 mmoles), and the mixt was stirred at room temp for 2.5 hr. The solvent was removed *in vacuo* below 30° and the residue was dried over P_2O_5 *in vacuo* overnight. The residue was triturated with EtOAc (15 ml), collected by filtration, washed with EtOAc, and dried to yield crude **9** (720 mg, 93%).

The analytical sample was obtained by reprecipitation (twice) from hot H_2O to give an amorphous solid, which was dried at 110° (P_2O_5) *in vacuo* for 40 min: tlc (silica gel) R_f 0.20 ($CHCl_3$–MeOH, 8:2, v/v) (Table 1). *Anal.* Calcd for $C_{21}H_{24}N_6O_6 \cdot H_2O$: C, H, N.

1-(4-[(*S*)-2′-Aminopropionylamido]-2,3,4-trideoxy-β-D-*erythro*-pyranuronamide)cytosine Trihydrochloride (10). A mixt of **9**·H_2O (474 mg, 1.0 mmole) and 12 *N* HCl (10 ml) was stirred at room temp for 1 hr. The solvent was removed below 35° *in vacuo* to give a solid product, which was crystallized from EtOH containing a small amount of H_2O to afford **10** (210 mg, 49%).

Recrystallization from the same solvent gave an analytically pure sample, which was dried at 56° *in vacuo* (P_2O_5) for 4 hr (Table 1). *Anal.* Calcd for $C_{13}H_{18}N_6O_4 \cdot 3HCl$: C, H, N.

1-(4-[(*S*)-2′-CBZ-amino-3′-phenylpropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronamide)cytosine (11). To a saturated (at 0°) MeOH–NH_3 soln (30 ml) was added **7** (790 mg, 1.4 mmoles), and the mixt was stirred at room temp for 2.5 hr. The solvent was removed *in vacuo* below 30°. After being dried (P_2O_5) *in vacuo* overnight, the residue was taken in hot EtOH (25 ml). The soln was filtered and diluted with EtOAc (20 ml). The mixt was allowed to stand at −20° for 2 days to give crystalline **11** (570 mg, 72%).

Further crystallization gave an analytically pure sample, which was dried (P_2O_5) at 110 *in vacuo* for 2 hr (Table 1). *Anal.* Calcd for $C_{27}H_{28}N_6O_6 \cdot H_2O$: C, H, N.

1-(4-[(*S*)-2′-Amino-3′-phenylpropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-enopyranuronamide)cytosine Dihydrochloride (12). A mixt of **11**·H_2O (441 mg, 0.8 mmole) and concd HCl (10 ml) was stirred at room temp for 1 hr. The solvent was removed *in vacuo* and the residue was dried (P_2O_5–KOH) *in vacuo* overnight. The product was taken up in water (18 ml) and the undissolved material was filtered with charcoal. The filtrate was concentrated to dryness *in vacuo* and the residue was dissolved in 6 *N* HCl (2 ml). To this soln was added acetone (*ca.* 25 ml) to give a gummy precipitate, which was separated by decantation and triturated with acetone to afford **12** as an amorphous solid (210 mg, 40%).

Further reprecipitation from 1 *N* HCl–acetone gave an analytically pure sample, which was dried (P_2O_5) at 56° *in vacuo* for 5 hr (Table 1). *Anal.* Calcd for $C_{19}H_{23}N_6O_4 \cdot 2HCl$: C, H, N.

Acknowledgments. The authors would like to extend their appreciation to Drs. E. Akita, S. Fukatsu and T. Wakazawa of Meiji Seika Kaisha, Ltd., for their helpful discussions and generous gift of blasticidin S.

References

(1) R. J. Suhadolnik, "Nucleoside Antibiotics," Wiley-Interscience, New York, N. Y., 1970, p 189.
(2) H. Yamaguchi, C. Yamamoto, and N. Tanaka, *J. Biochem. (Tokyo)*, **57**, 667 (1965).
(3) (a) M. J. Robins, L. N. Simon, M. G. Stout, G. A. Ivanovics, M. P. Schweizer, R. J. Rousseau, and R. K. Robins, *J. Amer. Chem. Soc.*, **93**, 1474 (1971); (b) G. A. Ivanovics, R. J. Rousseau, and R. K. Robins, *J. Med. Chem.*, **14**, 1155 (1971); (c) M. Kawana, R. J. Rousseau, and R. K. Robins, *J. Org. Chem.*, **37**, 288 (1972).
(4) H. Yonehara, S. Takeuchi, N. Ōtake, T. Endō, Y. Sakagami, and Y. Sumiki, *J. Antibiot., Ser. A*, **16**, 195 (1963).
(5) N. Ōtake, S. Takeuchi, T. Endō, and H. Yonehara, *Tetrahedron Lett.*, 1405 (1965).
(6) J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, **77**, 1067 (1955).
(7) E. Wunsch and F. Drees, *Chem. Ber.*, **99**, 110 (1966).
(8) H. Yonehara and N. Ōtake, *Antimicrob. Ag. Chemother.*, **1965**, 855 (1966).
(9) J. J. Fox, Y. Kuwada, and K. A. Watanabe, *Tetrahedron Lett.*, 6029 (1968).

(10) K. Iwasaki, S. Sabol, A. Wahba, and S. Ochoa, *Arch. Biochem. Biophys.*, **125**, 542 (1968).
(11) N. Otake, S. Takeuchi, T. Endō, and H. Yonehara, *Agr. Biol. Chem.*, **30**, 126 (1966).

Mixed Thiolsulfonates and Sulfonamides from Polyfunctional Mercaptans Using Trifluoromethyl Thiosulfonates†

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Thiolsulfonates have shown biological interest both as antimicrobial agents and as protectants against ionizing radiations.[1,2] In a previous publication[3] we reported that the reaction of trifluoromethyl thiolsulfonates with mercaptans (eq 1) could be used to prepare S-polyfunctional

$$RSO_2SCF_3 + R'SH \longrightarrow RSO_2SR' + CF_3SH \quad (1)$$

thiolsulfonates which would be difficult to produce by the usual syntheses for mixed thiolsulfonates (eq 2 and 3)

$$RSO_2SK + R'Br \longrightarrow RSO_2SR' + KBr \quad (2)$$
$$RSO_2M + R'SCl \longrightarrow RSO_2SR' + MCl \quad (3)$$

where M is Na, Ag, etc.

Further, as will be demonstrated, these thiolsulfonates may be used for synthesis of asymmetrical disulfides (eq 4).

$$RSO_2SR' + R''SH \longrightarrow RSO_2H + R'SSR'' \quad (4)$$

This paper reports some results using these reactions to prepare compounds which were tested as radioprotectants and as bactericides.

The compounds fall into three groups: (1) asymmetrical thiolsulfonates from cysteine and related structures, (2) sulfonamides from mercaptopurines and similar compounds, (3) and asymmetrical disulfides.

The preparation of mixed thiolsulfonates RSO_2SR' (Table I) by any of the well-known methods is limited mainly by the reactivity of the SR' or R' moiety. If R' is an unsubstituted alkyl group in an alkyl halide one can generally alkylate the RSO_2SK (or Na) salt, eq 2. If R' is aryl and the SH group can be halogenated, one can prepare the sulfenyl halide and treat the salt RSO_2M (M = Na, Zn, etc.), eq 3. However, if the alkyl halide is unreactive or if the aryl mercaptan contains other groups that are sensitive to halogenation, this limits the scope of the method or prevents reaction altogether. Using trifluoromethyl thiolsulfonates, however, one can perform the reaction directly upon the SH group.

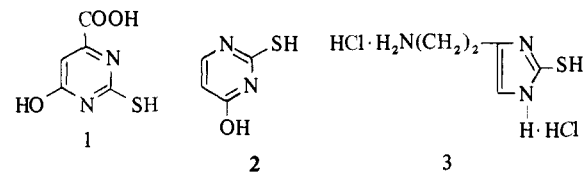
$$RSO_2SCF_3 + HSCH_2\underset{\displaystyle NH_2\cdot HCl}{\underset{|}{C}}HCOOH \longrightarrow RSO_2SCH_2\underset{\displaystyle NH_2\cdot HCl}{\underset{|}{C}}HCOOH + CF_3SH \quad (5)$$

It was discovered, during the several experiments preparing cysteine thiolsulfonates, that the main difficulty in using the RSO_2SCF_3 was prevention of the secondary reaction between the thiolsulfonate product and the starting mercaptan, eq 4. This not only reduced the yield but made purification of the product more difficult. By employing a 3–4 molar excess of the starting RSO_2SCF_3, disulfide formation was suppressed and a high yield of product was favored. Even under these conditions formation of some disulfide was noted and purification of the product sometimes was not easy. When the mercaptan was relatively unreactive, eq 1, as in thiosalicylic acid or *n*-dodecyl mercaptan, the mixed thiolsulfonate could be prepared in acid medium. This also helped to suppress disulfide formation and under these conditions a molar ratio of RSO_2SCF_3 and mercaptan was satisfactory.

The reaction of trifluoromethyl thiolsulfonates with mercaptopurines unexpectedly gave purinesulfonamides containing no -SH group. (This appears to be the first synthesis of this species as no previous report of purine sulfonamides has been found in the literature.) The reaction of RSO_2SCF_3 compounds with secondary amines was discussed earlier,[3] and was used to prepare sulfonamides. In mercaptopurines the thione structure exists in equilibrium with the mercapto form.[4] S is easily replaceable by H in purines and pyrimidines.[5] It would not be unusual, therefore, under the conditions of our reaction, to eliminate the mercapto S and obtain the sulfonamide. The nmr spectrum of the 6-mercaptopurine showed ring N but no NH peak, while in the ir there was strong SO absorption, and again no NH was observed. Both of the sulfonamides had strong ir absorption at 10.4 μ ($MeSO_2$).[6] Since mercaptopurines exist in tautomeric equilibrium between the thiol and thione forms, and this obviously involves the 7 and 9 N with the 8-SH position, we cannot say whether **12**, Table II, is the 7- or 9-methylsulfonamide. The compound derived from 6-mercaptopurine is likely to be the 7-methylsulfonamide since the 7-H would be nearer to the 6-SH group. Support for the 7 position was also obtained from nmr studies on the 6-mercaptopurine derivative and on purine itself. Comparison of the spectra shows a small shift in the values for hydrogens in the 4 and 6 positions and rather large shift for the 8-H. This suggests that the 7-sulfonamide is more likely since a 9-sulfonamide would probably cause a shift in only the 8-H. The hydrogens of the Me group absorb at δ 3.03 for aromatic systems and appear as a singlet. In the sulfonamide they are shifted only to δ 3.52, which is expected. The integration spectrum of the sulfonamide gave the ratio of H for the position 4, 6, 8, 10 as 1:1:1:3 which corresponds to the correct number and position.

The uv spectra were also obtained for the series of compounds, 6- and 8-mercaptopurines, and the sulfonamide derived from the 6 isomer.‡ The position of the $MeSO_2$ group cannot be distinguished by these spectra since corresponding values for other substituted purines are not available from the literature, and preparation of these compounds was felt to be beyond the scope of the present work.

Several pyrimidine and imidazole derivatives were treated with the RSO_2SCF_3 compounds. Repeated attempts were made to produce mixed thiolsulfonates using mercaptoorotic acid (**1**), thiouracil (**2**), and mercaptohistamine·2HCl (**3**). These reactions produced as the only identifiable products, sulfonyl sulfides, $RSO_2S_xSO_2R$ (where x = 1, 2, or 3), and high melting crystalline disulfides presumably from the



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†This work was supported by U. S. Public Health Service Research Grant 5 RO 1 CA 10753-03 from the National Cancer Institute.

‡The absorptions (λ) obtained were as follows: 6-mercaptopurine, 325, 217; 8-mercaptopurine, 318, 230.5; the sulfonamide from 6-mercaptopurine, 279.5, 210.