RNA Polymerase Inhibition Test. DNA-dependent RNA polymerase from a rifampicin-sensitive and a rifampicin-resistant *E. coli* strain was purified by ammonium sulfate fractionation and DEAE cellulose chromatography, according to the procedure of Burgess.[29] Polymerase activity was assayed by determining the incorporation of [^{14}C]AMP into acid insoluble material using highly polymerized calf thymus DNA as a template. The assay conditions were as reported by Burgess,[29] the concentration of the enzyme in our tests being about 8 units per milliliter of final assay mixture.

Acknowledgments. RNA polymerase purification and inhibition tests have been performed by Dr. R. J. White. The authors are also indebted to Dr. V. Arioli for the experimental infections, to Dr. A. Fietta and Miss G. Carniti for MIC determinations, to Dr. M. G. Serralunga for the acute toxicity data, to Dr. E. Martinelli and Mr. A. Ripamonti and Mr. P. Radaelli for the physicochemical determinations, and to Mr. E. Petressi for the microanalyses. The authors also thank Mr. G. Cietto, L. Gastaldo, and L. Sala for skillful assistance.

References

(1) N. Maggi, C. R. Pasqualucci, R. Ballotta, and P. Sensi, *Chemotherapia*, 11, 285 (1966).
(2) N. Maggi, R. Pallanza, and P. Sensi, *Antimicrob. Ag. Chemother.*, 765 (1965).
(3) L. Thiry and G. C. Lancini, *Nature (London)*, **227**, 1048 (1970).
(4) N. Maggi, G. G. Gallo, and P. Sensi, *Farmaco, Ed. Sci.*, **22**, 316 (1967).
(5) E. L. Schumann, R. V. Heinzelman, M. E. Greig, and W. Veldkamp, *J. Med. Chem.*, **7**, 329 (1964), and references cited therein.
(6) W. Oppolzer, Konstitution der Rifamycine, Promotion Arbeit E.D.T.H., Zurich, 1963.
(7) N. Maggi, S. Furesz, and P. Sensi, *J. Med. Chem.*, 11, 368 (1968).
(8) G. Pelizza, G. C. Lancini, G. C. Allievi, and G. G. Gallo, *Farmaco, Ed. Sci.*, **28**, 298 (1973).
(9) G. C. Lancini and G. Sartori, *Experientia*, **24**, 1105 (1968).
(10) C. Gurgo, R. Ray, and M. Green, *J. Nat. Cancer Inst.*, **49**, 61 (1972).
(11) S. Riva, A. Fietta, and L. G. Silvestri, *Biochem. Biophys. Res. Commun.*, **49**, 1263 (1972).
(12) S. S. Yang, F. M. Herrera, R. G. Smith, M. S. Reitz, G. C. Lancini, R. C. Ting, and R. C. Gallo, *J. Nat. Cancer Inst.*, **49**, 7 (1972).
(13) M. Meilhac, Z. Tysper, and P. Chambon, *Eur. J. Biochem.*, **28**, 291 (1972).
(14) M. J. Chamberlin and J. Ring, *Biochem. Biophys. Res. Commun.*, **49**, 4 (1972).
(15) T. Onishi and M. Muramatsu, *Biochem. J.*, **128**, 1361 (1972).
(16) P. Juhasz, B. J. Benecke, and K. H. Seifart, *FEBS Lett.*, **27**, 30 (1972).
(17) H. W. Butterworth, R. F. Cox, and C. Chesterton, *Eur. J. Biochem.*, **23**, 229 (1971).
(18) R. Adman, L. D. Schultz, and B. D. Hall, *Proc. Nat. Acad. Sci. U. S.*, **69**, 1702 (1972).
(19) E. Di Mauro, C. P. Hollenberg, and B. D. Hall, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2818 (1972).
(20) H. Saito and S. Mitsuhushi, *J. Antibiot.*, **25**, 477 (1972).
(21) S. Kurashige and S. Mitsuhashi, *J. Antibiot.*, **25**, 619 (1972).
(22) C. E. Sekeris and W. Roewekamp, *FEBS Lett.*, **23**, 34 (1972).
(23) S. Barlati and P. Vigier, *FEBS Lett.*, **24**, 343 (1972).
(24) S. Barlati, A. Brega, R. Cricchio, G. C. Lancini, and L. G. Silvestri, *Biochem. Biophys. Res. Commun.*, submitted for publication.
(25) N. Maggi, A. Vigevani, and R. Pallanza, *Experientia*, **24**, 209 (1968).
(26) P. Sensi, N. Maggi, R. Ballotta, S. Furesz, R. Pallanza, and V. Arioli, *J. Med. Chem.*, 7, 596 (1964).
(27) V. Arioli, R. Pallanza, S. Furesz, and G. Carniti, *Arzneim.-Forsch.*, **17**, 523 (1967).
(28) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
(29) R. R. Burgess, *J. Biol. Chem.*, **244**, 6160 (1969).

Synthesis, Chemistry, and Biological Activity of 5-Thiocyanatopyrimidine Nucleosides as Potential Masked Thiols

Tomoako Nagamachi, Jean-Louis Fourrey, Paul F. Torrence,† James A. Waters, and Bernhard Witkop*

Laboratory of Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014. *Received October 23, 1973*

The reaction of chlorothiocyanogen (ClSCN) with various derivatives of uracil was investigated as a potential route to 5-mercaptouracils. Reaction of 1,3-dimethyluracil, 1-(β-D-ribofuranosyl)uracil, 1-(2′-deoxy-β-D-ribofuranosyl)uracil, 1-(β-D-arabinofuranosyl)uracil, 1-(2′,3′,5′-tri-*O*-chloroacetyl-β-D-ribofuranosyl)uracil, and 1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)uracil with ClSCN in acetic acid gave the corresponding 5-thiocyanato derivatives in fair to excellent yields. These 5-thiocyanatopyrimidine nucleosides can be reduced by dithiothreitol, sodium dithionite–2-mercaptoethanol, or glutathione to the biologically active 5-mercaptopyrimidine nucleosides. The intermediate 5-thiocyanatopyrimidine nucleosides themselves show significant biological activity, probably as the result of *in vivo* reduction.

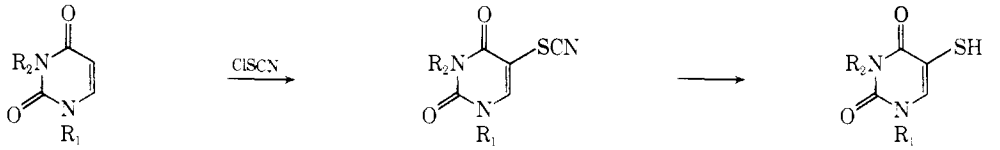
The chemical modification of preformed nucleosides has the advantage of convenience for the preparation of substituted purine and pyrimidine nucleoside analogs. Such an approach is more direct than the laborious condensation of a base with an appropriately protected sugar. A representative example is the direct fluorination of protected pyrimidine nucleosides by trifluoromethyl hypofluorite.[1,2]

5-Mercaptouridine (1) and 5-mercapto-2′-deoxyuridine (2), previously synthesized by a modification of the Hilbert–Johnson reaction,[3-5] both show significant antibacterial and antitumor properties.[6,7] Alternate approaches to 1 and 2 have included the addition of methyl hypobromite to uracil derivatives followed by reaction with sodium disulfide.[8] The latter procedure is suitable for the thiolation of polynucleotides.[9,10] Because of the success of the use of chlorothiocyanogen[11,12] (ClSCN) in the preparation of sulfur analogs of dopamine and norepinephrine, and because of our continuing interest in modified polynucleotides,[13-16] we have investigated the thiocyanation of pyrimidine nucleosides as a route to 5-mercaptopyrimidine nucleosides. (For a preliminary account of a portion of this work, see ref 17.)

Chemistry. Exploratory experiments with 1,3-dimethyluracil (3) revealed that the 2,4-dioxopyrimidine ring readily reacted with chlorothiocyanogen to give a moder-

† U. S. Public Health Service Senior Staff Fellow.

Scheme I



3, $R_1 = CH_3$; $R_2 = CH_3$
5, R_1 = 2′,3′,5′-tri-*O*-chloroacetyl-β-D-ribofuranosyl; R_2 = H
7, R_1 = β-D-ribofuranosyl; R_2 = H
8, R_1 = 2′-deoxy-β-D-ribofuranosyl; R_2 = H
9, R_1 = β-D-arabinofuranosyl; R_2 = H
12, R = 2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl; R_2 = H

4, $R_1 = CH_3$; $R_2 = CH_3$
6, R_1 = β-D-ribofuranosyl; R_2 = H
10, R_1 = 2′deoxy-β-D-ribofuranosyl; R_2 = H
11, R_1 = β-D-arabinofuranosyl; R_2 = H
13, R_1 = 2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl; R_2 = H

1, R_1 = β-D-ribofuranosyl; R_2 = H
2, R_1 = 2′-deoxy-β-D-ribofuranosyl; R_2 = H
14, R_1 = β-D-arabinofuranosyl; R_2 = H

ate yield of the 5-thiocyanato derivative **4**. In this instance, as well as for the reactions discussed below, 5-thiocyanation could be monitored by the appearance of the characteristic SCN ir band at 2160 cm^{-1}, retention of the uracil chromophore in the uv, and the presence of the uracil H_6 proton at 8–9 ppm in the nmr spectrum. In the case of uracil nucleosides, the position of substitution was further confirmed by reduction to authentic 5-mercaptouracils (*vide infra*).

Initial attempts to introduce the thiocyanato group into uridine made use of the chloroacetyl protecting group.[18,19] This group has the advantage of being readily displaced under mild conditions by reagents such as thiourea[18,19] or pyridine,[19] thus preventing decomposition of the newly formed thiocyanates to disulfides.[20] Nearly quantitative yields of the tri-*O*-chloroacetylated uridine **5** were obtained simply by heating uridine with excess chloroacetic anhydride on a steam bath for a short time. When **5** was dissolved in pyridine–water (1:1) and the resulting solution allowed to stand for several hours, uridine was recovered nearly quantitatively. Reaction of **5** with ClSCN followed by removal of the chloroacetyl protecting groups by pyridine treatment led to an acceptable yield (20%) of the desired 5-thiocyanatopyrimidine nucleoside **6**.

In subsequent experiments it was found that protecting groups were not necessary in this reaction and, in fact, yields were higher when no protecting group was used. Thus, direct thiocyanation of uridine (**7**), 2′-deoxyuridine (**8**), and 1-(β-D-arabinofuranosyl)uracil (**9**) gave the corresponding 5-thiocyanato nucleosides (**6**, **10**, and **11**, respectively) in 30–55% yields. In a similar fashion, 2′,3′,5′-tri-*O*-acetyluridine (**12**) was thiocyanated to give **13**. Substantial decomposition was noted when the triacetate (**12**) was treated with NH_3–CH_3OH to remove the acetyl groups. The addition of methanol at early stages of these workups resulted in substantially reduced yields of desired products (Scheme I).

While the thiocyanation of olefins,[21,22] aromatic compounds,[23] and pyrrole carboxylates[24] has been previously described, the addition to olefins containing electron-withdrawing substituents does not usually occur.[25] Like other pseudohalogens (*e.g.*, CF_3OF[1,2]), the addition of ClSCN to the uracil ring follows the Markovnikov rule. In the reactions investigated, yield and nature of the products were not significantly dependent on the presence or absence of the hydroxyl group at C-2′ of the ribose or on the stereochemistry at that position. The preparation of thiocyanatopyrimidine nucleosides by the action of cyanogen bromide (BrCN) on 4-thiouracil derivatives has been previously reported.[26,27] Additionally, there exists one other potential route to such 5-mercapto-substituted pyrimidines *via* the reaction of uracil derivatives with sulfur or disulfur dichloride, occasionally yielding disulfides[28] which could be reduced to the mercapto compounds.

Attempts to extend this thiocyanation reaction to cytidine and N^4-acetylcytidine have thus far proved unsuccessful.

The reduction of the 5-thiocyanatopyrimidine nucleosides **1**, **2**, and **14** to the corresponding 5-mercapto derivatives was achieved by either sodium dithionite–mercaptoethanol, dithiothreitol (DTT),[29] or glutathione (*vide infra*). Thus, the reaction sequence of thiocyanation followed by reduction provides a fast convenient method for the synthesis of modified 5-mercaptopyrimidine nucleosides for evaluation as antibacterial, antiviral, or antitumor agents.

Biological Evaluation. It was of interest to determine the biological activity of the synthesized 5-thiocyanatopyrimidine nucleosides since such analogs might behave in at least two different ways: (a) as pseudohalogens, with the SCN group mimicking the iodo group present in 5-iodo-2′-deoxyuridine, a clinically useful antiviral agent; (b) the thiocyanato nucleosides might be reduced *in vivo* to the corresponding 5-mercaptopyrimidine nucleosides which have a broad spectrum of biological activity. The latter pathway seemed probable in view of the facile reduction of 5-thiocyanato-2′-deoxyuridine (**10**) by glutathione, an ubiquitous constituent of biological systems.[30]

The results of such studies are presented in Table I. The only compound showing significant activity in KB or L5178 Y cells is 5-thiocyanato-2′-deoxyuridine (**10**). It was found that the effect of **10** on L5178 Y cells was *completely* reversed by 10^{-5} *M* thymidine and that its effect could be slightly reversed by 10^{-5} *M* 2′-deoxyuridine. This indicates that **10** may be inhibiting the enzyme thymidylate synthetase. It appears likely that **10** is reduced in such cellular systems to 5-mercapto-2′-deoxyuridine (**2**) which is subsequently phosphorylated by thymidine kinase to the corresponding 5′-monophosphate, a potent inhibitor of thymidylate synthetase.[7] Conversely, this sequence of events could also start with phosphorylation of 5-thiocyanato-2′-deoxyuridine. The rate of (enzymatic) reduction of nucleophilic replacement of the thiocyanato group may determine whether the concomitant formation of cyanide ion can reach toxic levels.

It is possible that such 5-thiocyanato nucleosides and other thiocyanato analogs in general may offer certain advantages over mercapto derivatives with respect to potential usage as drugs or biochemical tools. They can be prepared in high yield in a one-step synthesis, are reasonably stable, and not readily autoxidized to disulfides. The neutral thiocyanato analogs might be more readily taken up

Table I. Biological Activity of 5-Thiocyanatopyrimidine Nucleosides; Concentrations Causing 50% Inhibition of Growth

Cell type	6	10	13	11
KB	2×10^{-4} M	9×10^{-6} M	$>3 \times 10^{-4}$ M	
L5178Y	5×10^{-5} M	8×10^{-7} M	$>1 \times 10^{-4}$ M	$>1 \times 10^{-4}$ M
Vaccinia virus replication in HeLa Cells		1×10^{-4} M[a]		

[a] 75% reduction in plaque formation at 10^{-4} M, the lowest concentration tested.

by cells and subsequently reduced to the biologically active mercapto species. It is also possible that such a reduction may be used to increase drug selectivity based on different cellular environments. Similar reasoning served as the basis for the synthesis of certain *S*-acylmercaptopyrimidine nucleotides which are cleaved by aliphatic thiols to yield active mercaptans.[31]

In conclusion, the thiocyanation of pyrimidine nucleosides with ClSCN followed by reduction of the resulting thiocyanate provides a facile convenient synthesis of 5-mercaptopyrimidine nucleosides. The intermediates in the synthesis, the 5-thiocyanato analogs (especially **10**), show significant biological activity, most probably because they are easily reduced in the cell to 5-mercapto derivatives.

Experimental Section

Melting points were obtained with a Kofler hot-stage apparatus and are corrected. Where analyses are indicated only by symbols of the elements, the results obtained for those experiments are within ±0.4% of the theoretical values. Uv spectra were determined on a Cary 15 instrument, nmr were obtained on a Varian HA-100 spectrometer, and ir were recorded on either a Perkin-Elmer Model 237B or 20. For tlc, Analtech silica gel GF plates were used. Evaporations were carried out *in vacuo* at <40°.

Anhydrous conditions were absolutely necessary to produce high yields of thiocyanato reaction products. Dried KSCN was used in the preparation of ClSCN and the Cl_2 gas was passed through a $CaCl_2$ trap prior to use. Acetic acid was dried by freezing reagent grade acid at 4–5° and then decanting off the supernatant liquid which was enriched in H_2O. ClSCN was prepared in the following manner. Dry finely powdered KSCN (5.35 g, 55 mmol) was added to an ice-cold solution of Cl_2 (3.55 g, 50 mmol) in dry CH_3COOH. The resulting suspension was stirred for 30 min–2 hr at room temperature and then the desired uracil derivative was added.

1,3-Dimethyl-5-thiocyanatouracil (4). 1,3-Dimethyluracil (**3**, 280 mg, 2 mmol) was dissolved in dry HOAc (20 ml) which was 0.2 *M* in ClSCN. The solution was stirred for 5 hr at room temperature, filtered, and evaporated under reduced pressure. The residue was recrystallized two times from CH_3OH to give **4** (150 mg, 38%): mp 155–156°; M^+ 197; nmr (CD_3CN) δ 3.28 (s, 3, NCH_3), 3.37 (s, 3, NCH_3), and 7.92 (s, 1, H_6); ν_{max} 2180 cm^{-1} (SCN); uv λ_{max} 276 nm (ε 8700); *Anal.* ($C_7H_7N_3O_2S$) C, H, N, S.

1-(2′,3′,5′-Tri-*O*-chloroacetyl-β-D-ribofuranosyl)uracil (5). Uridine (**7**, 2.44 g, 0.01 mol) was intimately mixed with $(ClCH_2CO)_2O$ (20 g) and the entire mixture warmed on a steam bath. Tlc analyses of the molten reaction mixture showed total conversion to the desired product in *ca.* 1 hr. The mixture was cooled and poured into ice–H_2O and extracted three times with $CHCl_3$. The $CHCl_3$ extracts were washed three times with dilute $NaHCO_3$ solution and two times with H_2O. After drying over anhydrous Na_2SO_4, the $CHCl_3$ solution was evaporated to give **5** (4.5 g, 95%). This material was pure by tlc and was routinely used in further reactions. A small sample was recyrstallized with difficulty from EtOH–acetone to give an analytical sample: mp softens 45–50°. *Anal.* ($C_{15}H_{15}N_2O_9Cl_3$) C, H, N.

When **5** was treated with pyridine–H_2O (1:1) at room temperature for several hours, it was quantitatively converted to uridine (**7**).

1-(β-D-Ribofuranosyl)-5-thiocyanatouracil (6). Method A. The chloroacetylated uridine (**5**, 4.8 g, 10 mmol) was added to a solution of ClSCN (from 7.1 g of Cl_2 and 10.7 g of KSCN) in HOAc (150 ml). The reaction mixture was stirred at room temperature overnight and filtered free of insoluble material, and the filtrate was evaporated. The residue was extracted with benzene–acetone (1:1) and the insoluble material again removed by filtration. The filtrate was evaporated once more and the resulting residue taken up in pyridine (100 ml) to which H_2O (100 ml) was then added. The solution was stirred (at room temperature) overnight and evaporated under reduced pressure, and the resulting residue was dissolved in methanol–water (1:1) and applied to a Dowex 50 W-X4 ion-exchange resin. The eluted solution was evaporated and the residue dissolved in the minimum amount of MeOH. This solution was applied to a silica gel column which was eluted with $CHCl_3$–MeOH to give the 5-thiocyanatouridine (**6**, 250 mg, 18% based on recovered uridine) and uridine (1.2 g). Analytically pure **6** was obtained by EtOH recrystallization: mp 167–172°; ν_{max} 2160 cm^{-1} (SCN); uv λ_{max} (MeOH) 273 nm (ε 10,400); nmr (CD_3OD) δ 8.88 (s, 1, H_6). *Anal.* ($C_{10}H_{11}O_6N_3S$) C, H, N, S.

Method B. Uridine (**7**, 1.22 g, 5 mmol) was added to a solution of ClSCN (from 3.55 g of Cl_2 and 5.35 g of KSCN) in HOAc (300 ml) and the resulting mixture was stirred at room temperature for 1 hr. Cyclohexene (15 ml) was added and stirring continued for 20 min. After filtration the solution was evaporated to give a residue which was dissolved in acetone. To this solution, silica gel powder was added and the resulting mixture freed of acetone by evaporation on a rotary evaporator. This silica gel powder was then applied to the top of a silica gel column which was eluted with EtOAc–acetone (9:1) to give, after evaporation of the appropriate fractions, **6** (720 mg, 48%) homogenous by tlc in $CHCl_3$–MeOH (7:3) and identical with that obtained by method A.

1-(2′-Deoxy-β-D-ribofuranosyl)-5-thiocyanatouracil (10). Method B including the same purification procedure, applied to 2′-deoxyuridine (**8**, 1.14 g, 5 mmol), gave the corresponding 5-thiocyanato product **10** (780 mg, 55%). Recrystallization from EtOH gave an analytical sample: mp 183–184.5°; ν_{max} 2160 cm^{-1} (SCN); uv λ_{max} (MeOH) 274 nm; nmr (CD_3OD) δ 8.18 (s, 1, H_6). *Anal.* ($C_{10}H_{11}O_5N_3S$) C, H, N, S.

1-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-5-thiocyanatouracil (13). 2′3′5′-Triacetyluridine (Aldrich Chemical Co., **12**, 1.85 g, 5 mmol) was added to a solution of ClSCN (from 3.55 g of Cl_2 and 5.35 g of KSCN) in HOAc (300 ml) and the resulting mixture stirred at room temperature. After tlc indicated complete disappearance of starting material (~10 hr), cyclohexene (15 ml) was added and the mixture stirred for an additional 20 min. After removal of insoluble material by filtration, the solution was evaporated and the resulting residue applied to a silica gel column. Elution with $CHCl_3$–EtOAc (1:1) gave **13** (2.05 g, 95%) as an amorphous solid: uv λ_{max} (MeOH) 271 nm; ν_{max} 2160 cm^{-1} (SCN); nmr (CD_3OD) δ 8.31 (s, 1, H_6). *Anal.* ($C_{16}H_{17}N_3O_9S$) C, H, N, S.

1-(β-D-Arabinofuranosyl)-5-thiocyanatouracil (11). Under conditions described in method B above, 1-(β-D-arabinofuranosyl)-uracil (**9**, 488 mg, 2 mmol) was allowed to react with ClSCN. Tlc indicated complete reaction in 1.5 hr, after which time cyclohexene (5 ml) was added and the mixture stirred for another 20 min. After filtration and evaporation, the residue was applied to a silica gel column and elution accomplished with EtOAc–acetone to give **11** (192 mg, 32%). This arabinoside was recrystallized from EtOH to give an analytical sample: mp 183–185°; ν_{max} 2163 cm^{-1} (SCN); uv λ_{max} (pH 7) 268; nmr (CD_3OD) δ 8.56 (s, 1, H_6). *Anal.* ($C_{10}H_{11}O_6N_3S$) C, H, N.

1-(β-D-Ribofuranosyl)-5-mercaptouracil (1). Method I. Reduction by Dithiothreitol (DTT, Cleland's Reagent). To a solution (10 ml) of freshly prepared 10^{-3} *M* DTT in 0.1 *M* EDTA (pH 7.8) was added **6** (0.4 mg, 0.013 mmol). The progress of the reaction was monitored by the appearance of the 335-nm band in the uv. In this case the reaction was complete in less than 2 min. The resulting solution was evaporated (<40°) to a thick oil which was suspended in MeOH and then evaporated. A MeOH slurry of the resulting colorless residue was placed on a short (5 g) silica gel column which was eluted with MeOH (20 ml). The filtrate was evaporated to dryness and taken up in MeOH (1 ml). Insoluble material was removed by centrifugation and the resulting supernate used for tlc. On silica gel tlc with *n*-BuOH–MeOH–H_2O–

NH_4OH (6:2:2:1) as developing solvent, authentic **6** had R_f 0.60 and authentic **1** had R_f 0.44. Under these conditions the product of the DTT reaction had the same R_f as **1**. With *i*-PrOH–H_2O–NH_4OH (7:2:1) as developing solvent, authentic **6** had R_f 0.69 and authentic **1** had R_f 0.38. Again in this system the material isolated from the DTT reduction had R_f identical with authentic **1**. In both systems above, a trace impurity visible under uv light was noted at $R_f \sim 0.9$.

Method II. Reduction by Sodium Dithionite–2-Mercaptoethanol. To a stirred solution of K_2CO_3 (172 mg, 1.72 mmol) and sodium dithionite (16.7 mg, 0.096 mmol) in H_2O (0.65 ml) was added 2-mercaptoethanol (0.35 ml) followed immediately by **6** (2.4 mg, 0.08 mmol). After 30 min at room temperature, the reaction mixture was applied to a preparative tlc plate (1 mm, silica gel GF) and developed with acetone–EtOAc–MeOH (5:5:1). Two major zones were seen under uv light; one (R_f 0.64) was eluted with MeOH, and the other (R_f 0.0–0.1) was eluted with MeOH–H_2O (9:1). The slower moving zone had uv spectral properties identical with authentic **1**. The faster running zone had λ_{max} 256 nm in 10^{-3} *M* DTT–0.1 *M* EDTA (pH 7.8) or 0.1 *M* EDTA (pH 7.8). 1-(2′-Deoxy-β-D-ribofuranosyl)-5-mercaptouracil (**2**) and 1-(β-D-arabinofuranosyl)-5-mercaptouracil (**14**) were not isolated as such; however, when 10^{-4} *M* solution of either **10** or **11** in 0.1 *M* EDTA (pH 7.5) was made 10^{-3} *M* in DTT, in a reaction which was instantaneous, the λ_{max} 335 nm appeared, characteristic of the 5-thiolate anion. In addition, **10** [10^{-4} *M* in 0.1 *M* EDTA (pH 7.5)] was instantaneously reduced to **2** by 10^{-3} *M* glutathione.

Acknowledgments. We are deeply indebted to Dr. Thomas J. Bardos, State University of New York at Buffalo, for authentic samples of compounds **1** and **2**, to Dr. Florence White, Drug Evaluation Branch, National Cancer Institute, for evaluating compounds **6**, **10**, and **12** in KB cells, and to Dr. Charles Heidelberger, McArdle Laboratories, University of Wisconsin, for determining biological activities of the thiocyanatopyrimidine nucleosides in leukemia L5178Y cells and vaccinia virus replication in HeLa cells.

References

(1) M. J. Robins and S. R. Naik, *J. Amer. Chem. Soc.*, **93**, 5277 (1971).
(2) D. H. R. Barton, R. H. Hesse, H. T. Toh, and M. M. Pechet, *J. Org. Chem.*, **37**, 329 (1972).
(3) T. J. Bardos, M. P. Kotick, and C. Szantay, *Tetrahedron Lett.*, 1759 (1966).
(4) M. P. Kotick, C. Szantay, and T. J. Bardos, *J. Org. Chem.*, 34, 3806 (1969).
(5) G. L. Szekeres and T. J. Bardos, *J. Med. Chem.*, **13**, 708 (1970).
(6) K. Baranski, T. J. Bardos, A. Bloch, and T. I. Kalman, *Biochem. Pharmacol.*, **18**, 347 (1969).
(7) T. I. Kalman and T. J. Bardos, *Mol. Pharmacol.*, **6**, 621 (1970).
(8) L. Szabo, T. I. Kalman, and T. J. Bardos, *J. Org. Chem.*, **35**, 1434 (1970).
(9) T. J. Bardos, P. Chakrabarti, T. I. Kalman, A. J. Mikulski, and L. Novak, Abstracts of Papers, 163rd National Meeting of the American Chemical Society, Boston, Mass., April 9–14, 1972, MEDI 021.
(10) T. J. Bardos, K. Baranski, P. Chakrabarti, T. I. Kalman, and A. J. Mikulski, *Proc. Amer. Ass. Cancer Res.*, No. 359 (1972).
(11) W. B. Lutz, C. R. Creveling, J. W. Daly, B. Witkop, and L. I. Goldberg, *J. Med. Chem.*, **15**, 795 (1972).
(12) R. G. R. Bacon in "Organic Sulfur Compounds," N. Kharasch, Ed., Pergamon, New York, N. Y., 1961, p 320.
(13) P. F. Torrence and B. Witkop, *Biochemistry*, 11, 1737 (1972).
(14) P. F. Torrence, J. A. Waters, and B. Witkop, *J. Amer. Chem. Soc.*, **94**, 3638 (1972).
(15) P. F. Torrence, J. A. Waters, C. E. Buckler, and B. Witkop, *Biochem. Biophys. Res. Commun.*, **52**, 890 (1973).
(16) P. F. Torrence, A. M. Bobst, J. A. Waters, and B. Witkop, *Biochemistry*, **12**, 3962 (1973).
(17) T. Nagamachi, P. F. Torrence, J. A. Waters, and B. Witkop, *J. Chem. Soc., Chem. Commun.*, 1025 (1972).
(18) A. F. Cook and D. T. Maichuk, *J. Org. Chem.*, **35**, 1940 (1970).
(19) M. Bertolini and C. P. J. Glaudemans, *Carbohyd. Res.*, **15**, 263 (1970).
(20) J. L. Wood in "Organic Reactions," Vol. III, R. Adams, Ed., Wiley, New York, N. Y., 1946, Chapter 6.
(21) A. B. Angus and R. G. R. Bacon, *J. Chem. Soc.*, 774 (1958).
(22) R. G. Guy and I. Pearson, *J. Chem. Soc., Perkin Trans. 1*, 281 (1973).
(23) R. G. R. Bacon and R. G. Guy, *J. Chem. Soc.*, 318 (1958).
(24) R. K. Olsen and H. R. Snyder, *J. Org. Chem.*, **30**, 184 (1965).
(25) R. G. Guy and I. Pearson, *Chem. Ind. (London)*, 1255 (1967).
(26) R. T. Walker, *Tetrahedron Lett.*, **24**, 2145 (1971).
(27) M. Saneyoshi and S. Nishimura, *Biochim. Biophys. Acta*, **145**, 208 (1967).
(28) R. J. Badger, D. J. Brown, and N. V. Khromou-Borisou, *Aust. J. Chem.*, **25**, 2275 (1972).
(29) W. W. Cleland, *Biochemistry*, **3**, 480 (1964).
(30) P. C. Jocelyn, "Biochemistry of the SH Group," Academic Press, New York, N. Y., 1972.
(31) M. P. Kotick, T. I. Kalman, and T. J. Bardos, *J. Med. Chem.*, 13, 74 (1970).

Synthesis and Biological Activity of Some 8-Substituted Seleno Cyclic Nucleotides and Related Compounds

Shih-Hsi Chu,* Chyng-Yann Shiue, and Ming-Yu Chu

Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island 02912. *Received September 24, 1973*

8-Bromoadenosine 3′,5′-cyclic monophosphate and 8-bromoadenosine served as an intermediate for the chemical synthesis of a series of 8-substituted seleno cyclic nucleotides and their nucleosides. In an *in vitro* test using murine leukemic cells (L-5178Y) these 8-substituted derivatives of c-AMP showed some antitumor activities. A nucleic acid analyzer was used to study the hydrolysis of these cyclic nucleotides by phosphodiesterase. It was found that lengthening the side chain increased resistance to hydrolysis.

Adenosine 3′,5′-cyclic phosphate (cAMP) has been recognized as an intracellular second messenger of hormone action. At least some of its actions can be explained through the stimulation of a cAMP-dependent protein kinase. Recently, a large number of new cAMP analogs have been synthesized with modification of the purine,[1-9] carbohydrate,[10-12] and phosphate moieties.[13-16] Some of them were more active than the parent nucleotide in several biological systems. For example, all 8-substituted cAMPs except 8-amino were more resistant to phosphodiesterase than cAMP; 8-MeS-cAMP and 8-BzlS-cAMP were found to be inhibitors of cAMP phosphodiesterase.[6]

In view of these considerable changes in biological activity observed when 8-hydrogen of cAMP was replaced by sulfur, it is of interest to investigate the replacement of the 8-hydrogen by selenium as well. We have synthesized