Preparation and Biological Activity of Some Aminoacyl and Peptidyl Derivatives of 2′-Amino-2′-deoxyuridine

R. A. Sharma,* M. Bobek, and A. Bloch

Department of Experimental Therapeutics and Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14263. *Received April 4, 1975*

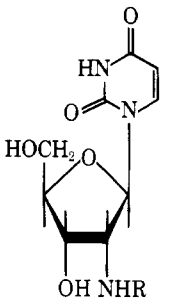
Several aminoacyl and peptidyl derivatives of 2′-amino-2′-deoxyuridine have been synthesized. 2′-Amino-2′-deoxyuridine (I) and 2′-*N*-glycyl-2′-amino-2′-deoxyuridine (IIIa) were condensed with *N*-Cbz blocked *p*-nitrophenyl esters of the appropriate amino acids to afford the protected aminoacyl and peptidyl nucleosides. Removal of the protecting groups gave the title compounds in good yields. The biological effects of these derivatives are discussed.

Aminoacyl and peptidyl nucleosides, such as the antibiotics puromycin, blasticidin S, gougerotin, and amicetin wherein the heterocycle is adenine or cytosine and wherein an amino acid or peptide is attached to the ribofuranose or hexopyranose moiety, have long been shown to interfere with the growth of microbial and mammalian cells by inhibiting protein synthesis.[1-4] In contrast, a group of structurally related nucleoside peptides, the polyoxins, wherein the heterocyclic moieties uracil, thymine, 5-hydroxymethyluracil, or uracil-5-carboxylic acid are attached to a 5-aminoribofuranosyl moiety, are active only against certain fungi whose growth they inhibit by interfering with chitin synthesis by virtue of their structural similarity to UDP-*N*-acetylglucosamine, a cofactor in that synthesis.[5-8]

Because of these biological effects, the preparation of some aminoacyl and peptidyl derivatives of 2′-amino-2′-deoxyuridine has been undertaken, preliminary to the analogous synthesis of the corresponding 2′-amino-2′-deoxycytidine derivatives. The compounds were evaluated for the growth inhibitory activity against *Escherichia coli* B and leukemia L1210 cells in vitro and the results of this evaluation together with the synthetic procedures are reported in this communication.

Chemical. Since the condensation of amino acids with 2′-amino-2′-deoxyuridine[9] (I) by use of the DCC method[10] proceeded very slowly and gave numerous side products, the active ester method of Bodanszky[11,12] was chosen for coupling the amino group of I with the *N*-carbobenzyloxy-*p*-nitrophenyl esters of the appropriate amino acids. Active esters have been previously utilized to react selectively with the amino group in the carbohydrate moiety of nucleosides.[13,14]

Thus, condensation of the *N*-carbobenzyloxyglycine *p*-nitrophenyl ester with I in dry DMF at room temperature afforded 2′-*N*-(*N*-carbobenzyloxyglycyl)-2′-amino-2′-deoxyuridine (IIa). Catalytic hydrogenolysis of IIa over 5% Pd/C in MeOH provided 2′-*N*-glycyl-2′-amino-2′-deoxyuridine (IIIa) in good yield. Similarly, 2′-*N*-(L-alanyl)- and 2′-*N*-(L-phenylalanyl)-2′-amino-2′-deoxyuridine (IIIb and IIIc) were obtained in excellent yields from IIb and IIc. The synthesis of the nucleoside peptides Va–c was done utilizing IIIa as the starting material. Treatment of IIIa with *N*-carbobenzyloxy-L-phenylalanine *p*-nitrophenyl ester in DMF at room temperature gave 2′-*N*-(*N*-carbobenzyloxy-L-phenylalanylglycyl)-2′-amino-2′-deoxyuridine (IVa) in 75% yield. Using identical experimental conditions, 2′-*N*-(*N*-carbobenzyloxy-L-leucylglycyl)- and 2′-*N*-(*N*-carbobenzyloxy-L-lysylglysyl)-2′-amino-2′-deoxyuridine (IVb and IVc) were obtained in approximately the same yields. These *N*-carbobenzyloxy blocked nucleoside peptides (IVa–c) were purified by chromatography on a dry silica gel column using EtOAc–MeOH (9:1) as the eluent. Catalytic hydrogenation of IVa–c over 5% Pd/C in MeOH afforded the peptidyl derivatives of 2′-amino-2′-deoxyuridine (Va–c) in good yields.



I, R = H
IIa, R = $-COCH_2NHCbz$
b, R = $-COCH(CH_3)NHCbz$
c, R = $-COCH(CH_2Ph)NHCbz$
IIIa, R = $-COCH_2NH_2$
b, R = $-COCH(CH_3)NH_2$
c, R = $-COCH(CH_2Ph)NH_2$
IVa, R = $-COCH_2NHCOCH(CH_2Ph)NHCbz$
b, R = $-COCH_2NHCOCH[CH_2CH(CH_3)_2]NHCbz$
c, R = $-COCH_2NHCOCH[(CH_2)_4NHCbz]NHCbz$
Va, R = $-COCH_2NHCOCH(CH_2Ph)NH_2$
b, R = $-COCH_2NHCOCH[CH_2CH(CH_3)_2]NH_2$
c, R = $-COCH_2NHCOCH[(CH_2)_4NH_2]NH_2$

The ir spectra of the newly prepared aminoacyl nucleosides showed the absence of an ester linkage, thus ruling out the possibility of a linkage of the amino acids to the hydroxyl group.

Biological. The effects which the newly prepared compounds exert on the in vitro growth of *E. coli* B are shown in Table I.[15,16] Whereas none of the compounds, at 1×10^{-4} *M*, inhibited the growth of leukemia L1210 cells by 50%, some of the compounds were weakly active against the bacteria where the concentration of inhibitor used extended to 10^{-3} *M*. Despite this weak activity, certain structure–activity relationships can be discerned. The compounds which carry a free amino group in their aminoacyl or peptidyl moiety (IIIa–c and Va–c) show some inhibitory activity; those in which this function is esterified (IIa–c and IVa–c) are inactive, suggesting that this "basic center" might make a contribution to activity. On the other hand, the hydrophobic carbobenzyloxy group may preclude effective binding. An increase in activity results when a dipeptide moiety (Va–c) replaces an aminoacyl residue (IIIa–c), suggesting that the length of the chain may affect the extent of binding.

As shown by an inhibition analysis, the inhibitory effects of compounds Va–c were readily reversed by uracil, cytosine, and their nucleosides, as well as by thymine and thymidine. It may well be that a second basic center at the opposite end of the molecule (e.g., an amino group at position 4 of the pyrimidine) is required for the compounds to interfere with protein synthesis, as is the case with other aminoacyl or peptidyl derivatives of cytidine or adenosine,[1] and that in the absence of such a function the uracil deriva-

Table I. Effect of Various Aminoacyl and Peptidyl Derivatives of 2′-Amino-2′-deoxyuridine on the Growth of *E. coli* B[a]

Compd	Derivative	Molar concn for 50% inhibn of growth
IIIa	Gly	9×10^{-4}
IIIb	L-Ala	1×10^{-3}
IIIc	L-Phe	1×10^{-3}
IVa	*N*-Cbz-L-Phe-Gly	$>10^{-3}$
IVb	*N*-Cbz-L-Leu-Gly	$>10^{-3}$
IVc	N^{α},N^{ϵ}-Di-Cbz-L-Lys-Gly	$>10^{-3}$
Va	L-Phe-Gly	6×10^{-4}
Vb	L-Leu-Gly	5×10^{-4}
Vc	L-Lys-Gly	5×10^{-4}

[a] At 1×10^{-4} these compounds did not cause 50% inhibition of leukemia L1210 growth.

tives, like the polyoxins, affect metabolic sites different from those involved in protein synthesis. The projected synthesis of aminoacyl and peptidyl derivatives of 2′-amino-2′-deoxycytidine will show whether attachment of the residues to the 2′ position of the cytosine nucleoside would give rise to inhibition of protein synthesis.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Uv spectra were recorded on a Cary Model 14 spectrometer and ir on a Perkin-Elmer 457 infrared spectrometer, and NMR were recorded using Varian XL-100 and JOEL MH-100 instruments. Satisfactory analyses (C, H, and N within ±0.4% of the theoretical values) were obtained from Robertson Laboratory, Florham Park, N.J. Evaporations were carried out under reduced pressure in a rotary evaporator. Thin-layer chromatography was run on precoated plastic sheets (silica gel N-HR/UV$_{254}$, Brinkman Instruments, Inc.) in the following solvent systems: (A) EtOAc–MeOH (9:1), (B) EtOAc–MeOH (8:2). Column chromatography was run on silica gel 60–200 mesh, J. T. Baker No. 3405. Paper chromatography was run on Whatman 3MM paper by the ascending technique in solvent C, 2-PrOH–NH_4OH–H_2O (7:1:2). Where indicated by elemental analysis, solvation was verified by NMR spectroscopy in DMSO-d_6. The *N*-carbobenzyloxyamino acid *p*-nitrophenyl esters were purchased from Sigma Chemical Co., St. Louis, Mo.

2′-*N*-(*N*-Carbobenzyloxyglycyl)-2′-amino-2′-deoxyuridine (IIa). To a solution of I (0.243 g, 0.001 mol) in 30 ml of dry DMF was added *N*-carbobenzyloxyglycine *p*-nitrophenyl ester (0.36 g, 0.0011 mol). The mixture was stirred at room temperature for 18–20 hr, after which TLC in EtOAc–MeOH (9:1) revealed the reaction to be complete. The mixture was then evaporated to dryness and coevaporated with MeOH. The residue was triturated with anhydrous ether to afford 0.394 g (92%) of chromatographically homogeneous (R_f 0.32 in solvent A) IIa. A sample to be used for analysis was recrystallized from an EtOAc–EtOH mixture: mp 179–180°. Anal. ($C_{19}H_{22}N_4O_8$) C, H, N.

2′-*N*-Glycyl-2′-amino-2′-deoxyuridine (IIIa). Compound IIa (0.7 g) was dissolved in 80 ml of MeOH and was hydrogenated at room temperature and atmospheric pressure for 2 hr, using 0.2 g of 5% Pd/C. The mixture was filtered through Celite, washed with MeOH, filtered through fine filter paper, and evaporated to dryness. The product was purified by recrystallization from EtOH and was obtained as a colorless crystalline material: yield 0.396 g (82%); mp 158–160°; λ_{max}^{MeOH} 260 nm (ϵ 8100); R_f 0.41 in solvent C. Anal. ($C_{11}H_{16}N_4O_6 \cdot 0.5EtOH \cdot 0.25H_2O$) C, H, N.

2′-*N*-(*N*-Carbobenzyloxy-L-alanyl)-2′-amino-2′-deoxyuridine (IIb). The procedure described for IIa was followed using I (0.243 g, 0.001 mol) and *N*-carbobenzyloxy-L-alanine *p*-nitrophenyl ester (0.375 g, 0.0011 mol) in 30 ml of DMF. The reaction mixture after evaporation and trituration with ether yielded 0.408 g (92%) of (TLC one spot, R_f 0.39 in solvent A) IIb. Further purification was achieved by recrystallization from EtOH: mp 223–224°. Anal ($C_{20}H_{24}N_4O_8$) C, H, N.

2′-*N*-(L-Alanyl)-2′-amino-2′-deoxyuridine (IIIb). Hydrogenation of IIb (0.8 g) in MeOH was carried out as described for the preparation of IIIa. Evaporation of the solvent furnished crude IIIb which was purified by recrystallization from EtOH: 0.502 g (89%); mp 155–156°; λ_{max}^{MeOH} 260 nm (ϵ 8321); R_f 0.51 in solvent C. Anal. ($C_{12}H_{18}N_4O_6 \cdot EtOH \cdot 1.5H_2O$) C, H, N.

2′-*N*-(*N*-Carbobenzyloxy-L-phenylalanyl)-2′-amino-2′-deoxyuridine (IIc). Compound I (0.243 g, 0.001 mol) and *N*-carbobenzyloxy-L-phenylalanine *p*-nitrophenyl ester (0.47 g, 0.0011 mol) in 50 ml of DMF were treated in the same manner as IIb. After trituration with ether, IIc was obtained (TLC homogeneous; R_f 0.53 in solvent A) in 0.5-g (95%) yield. Recrystallization from the EtOAc–EtOH mixture gave an analytical sample: mp 155–156°. Anal. ($C_{26}H_{28}N_4O_8 \cdot 0.5H_2O$) C, H, N.

2′-*N*-(L-Phenylalanyl)-2′-amino-2′-deoxyuridine (IIIc). A solution of 0.9 g of IIc in 100 ml of MeOH was hydrogenated as described above. Evaporation of the solvent and crystallization of the crude material from EtOH gave IIIc in 0.561-g (86%) yield: mp 206–207°; λ_{max}^{MeOH} 260 nm (ϵ 8095); R_f 0.64 in solvent C. Anal. ($C_{18}H_{22}N_4O_6$) C, H, N.

2′-*N*-(*N*-Carbobenzyloxy-L-phenylalanylglycyl)-2′-amino-2′-deoxyuridine (IVa). To a solution of IIIa (0.6 g, 0.002 mol) in 70 ml of DMF was added *N*-carbobenzyloxy-L-phenylalanine *p*-nitrophenyl ester (0.94 g, 0.0022 mol) and the reaction mixture was stirred at room temperature for 24 hr, the disappearance of IIIa being monitored by TLC in EtOAc–MeOH (9:1). The mixture was evaporated to dryness and was coevaporated with MeOH. The crude product was purified on a dry silica gel column (2 × 60 cm) eluting first with EtOAc and then with EtOAc–MeOH (9:1). The product obtained after evaporation was taken up in Me_2CO, and on standing at room temperature, IVa was obtained as a microcrystalline material: yield 0.9 g (78%); mp 119–120°; R_f 0.68 in solvent B. Anal. ($C_{28}H_{31}N_5O_9 \cdot 0.5H_2O$) C, H, N.

2′-*N*-(L-Phenylalanylglycyl)-2′-amino-2′-deoxyuridine (Va). Compound IVa (0.7 g) was hydrogenated in MeOH in the presence of 5% Pd/C. After filtration and evaporation of the solvent, the product Va was obtained as essentially one spot (R_f 0.69 in solvent C) in 73% (0.425 g) yield. A sample for analysis was prepared by dissolving the product in EtOH and precipitating it with ether: mp 178–180°; λ_{max}^{MeOH} 260 nm (ϵ 8046). Anal. ($C_{20}H_{25}N_5O_7 \cdot 0.5EtOH \cdot 0.5H_2O$) C, H, N.

2′-*N*-(*N*-Carbobenzyloxy-L-leucylglycyl-2′-amino-2′-deoxyuridine (IVb). The procedure employed for IVa was utilized for the preparation of IVb starting from 0.85 g (0.0028 mol) of IIIa and 1.3 g (0.0034 mol) of *N*-carbobenzyloxy-L-leucine *p*-nitrophenyl ester in 70 ml of DMF. Purification was carried out on a dry silica gel column (2 × 60 cm), eluting with EtOAc and then with EtOAc–MeOH (9:1). Evaporation and trituration with ether gave chromatographically homogeneous (R_f 0.73 in solvent B) IVb, 1.143 g (74% yield). An analytical sample was prepared by dissolving the product in EtOH and precipitating it with ether: mp 128–129°. Anal. ($C_{25}H_{33}N_5O_9$) C, H, N.

2′-*N*-(L-Leucylglycyl)-2′-amino-2′-deoxyuridine (Vb). A solution of IVb (0.9 g) in MeOH was hydrogenated as described in the previous experiments. Evaporation of MeOH and trituration with ether afforded Vb. The analytical sample was prepared by dissolving the residue in EtOH and precipitating the product with ether: 0.475 g (70%); mp 165–166°; λ_{max}^{MeOH} 260 nm (ϵ 8156). Anal. ($C_{17}H_{27}N_5O_7$) C, H, N.

2′-*N*-(N^{α},N^{ϵ}-Dicarbobenzyloxy-L-lysylglycyl)-2′-amino-2′-deoxyuridine (IVc). Compound IIIa (0.6 g, 0.002 mol) was dissolved in 70 ml of DMF and treated with N^{α},N^{ϵ}-dicarbobenzyloxy-L-lysine *p*-nitrophenyl ester (1.12 g, 0.0021 mol) according to the method described for IVb. The reaction mixture, after evaporation, was purified on a dry silica gel column (2 × 60 cm) using EtOAc–MeOH (9:1) as the eluent. Yield of TLC pure (R_f 0.68 in solvent B) IVc was 1.076 g (77%). The product was further purified by dissolving in EtOH and precipitating it with ether: mp 103–104°. Anal. ($C_{30}H_{40}N_6O_{11}$) C, H, N.

2′-*N*-(L-Lysylglycyl)-2′-amino-2′-deoxyuridine (Vc).[17] A solution of 0.9 g of IVc in 100 ml of MeOH was hydrogenated at atmospheric pressure in the presence of 0.4 g of 5% Pd/C for 2.5–3 hr. It was then filtered through Celite, washed with MeOH, filtered through a fine filter paper, and evaporated to dryness. Trituration of the product with ether afforded crude Vc. A sample for analysis was prepared by dissolving the product in EtOH and precipitating with ether: 0.360 g (65%); mp 175–177°; λ_{max}^{MeOH} 260 nm (ϵ 8132); NMR (D_2O using Me_4Si as external standard) δ 8.15 (d, 1, $J_{5,6}$ = 8 Hz, H-6), 6.50 (d, 1, $J_{1',2'}$ = 6 Hz, H-1′), 6.27 (d, 1, $J_{5,6}$ = 8 Hz, H-5), 5.01 (t, 1, $J_{1',2'} = J_{2',3'}$ = 6 Hz, H-2′), 4.24–4.84

(m, H-3′, H-4′, H-5′), 3.39 (br, glycyl protons), 1.72–2.14 (br, lysyl protons). Anal. Calcd for $C_{17}H_{28}N_6O_7$: C, 47.60; H, 6.53; N, 19.59. Found: C, 48.96; H, 6.71; N, 13.54.

Acknowledgments. This study was supported by Grants CA-12585 and CA-13038 from the National Cancer Institute, U.S. Public Health Service, and by Grant CI-124 from the American Cancer Society.

References and Notes

(1) J. J. Fox, K. A. Watanabe, and A. Bloch, *Prog. Nucl. Acid Res. Mol. Biol.*, **5**, 251 (1966).
(2) R. J. Suhadolnik, "Nucleoside Antibiotics", Wiley-Interscience, New York, N.Y., 1970.
(3) A. Bloch in "Drug Design", Vol. IV, E. J. Ariëns, Ed., Academic Press, New York, N.Y., 1973, p 285.
(4) A. Bloch and C. Coutsogeorgopoulos, *Biochemistry*, **5**, 3345 (1966).
(5) K. Isono, J. Nagatsu, Y. Kawashima, and S. Suzuki, *Agr. Biol. Chem.*, **29**, 848 (1965).
(6) S. Sasaki, N. Ohta, J. Eguchi, Y. Furukawa, T. Akashiba, T. Tsuchiyama, and S. Suzuki, *Nippon Shokubutsu Byori Gakkaiho*, **34**, 272 (1968).
(7) J. Eguchi, S. Sasaki, N. Ohta, T. Akashiba, T. Tsuchiyama, and S. Suzuki, *Nippon Shokubutsu Byori Gakkaiho*, **34**, 280 (1968).
(8) A. Endo and T. Misato, *Biochem. Biophys. Res. Commun.*, **37**, 718 (1969).
(9) J. P. H. Verheyden, D. Wagner, and J. G. Moffatt, *J. Org. Chem.*, **36**, 250 (1971).
(10) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
(11) M. Bodanszky, M. Szelke, E. Tomorkeny, and E. Weiss, *Chem. Ind. (London)*, 1517 (1955).
(12) M. Bodanszky and V. DuVigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
(13) M. J. Robins, L. N. Simon, M. G. Stout, G. A. Ivanovics, M. P. Schweizer, R. J. Rousseau, and R. K. Robins, *J. Am. Chem. Soc.*, **93**, 1474 (1971).
(14) K. A. Watanabe and J. J. Fox, *J. Org. Chem.*, **37**, 1198 (1972).
(15) A. Bloch and C. Coutsogeorgopoulos, *Biochemistry*, **10**, 4394 (1971).
(16) A. Bloch, G. Dutschman, B. L. Currie, R. K. Robins, and M. J. Robins, *J. Med. Chem.*, **16**, 294 (1973).
(17) Satisfactory elemental analyses could not be obtained. However, the structural assignment was made on the basis of NMR spectroscopy.

Book Reviews

Physiological Pharmacology. A Comprehensive Treatise. Volume V. Blood. Edited by Walter S. Root and Nathaniel I. Berlin with 24 contributors. Academic Press, New York, N.Y. 1974. xv + 588 pp. $44.00.

The purpose of this book is to interrelate present knowledge in hematology and pharmacology of drugs which affect components of the blood. The content of the book is organized along hematological rather than pharmacological classification. This type of classification is very beneficial to the person interested in the physiology and biochemistry of the blood because different classes of drugs are grouped as to their effect on hematology and, therefore, the information is readily available. Most chapters emphasize the physiology or pathophysiology of the blood which is done extremely well; however, the pharmacology is not as detailed in some chapters. The book does not include research methodology.

The book is a comprehensive treatise as the title indicates and therefore the subject matter is not an exhaustive review as one would find in a monograph. The book is only intended for the more advanced in the field of hematology. There is no uniform format to this book because each contributor (24 in all) presented his chapter in his unique style.

The content of the book is divided into five sections. The first section, coagulation, starts with a chapter on the physiology of blood coagulation, which is brief and to the point with factual information about the events leading to thrombin formation in the plasma. The author focuses on many unanswered questions. The emphasis for the second chapter, anticoagulants, is on the clinical indication for the use of these drugs and the mechanism of drug interactions with anticoagulants. Thrombolytic therapy is briefly discussed. The chapter on thrombogenesis is discussed from the experimental and clinical point of view while the last chapter, thrombolysis and thrombolytic agents, is presented from the clinical point of view with many therapeutic experiments cited.

The second section, platelets, starts with the biochemistry and physiology of platelets which only includes current information. The second chapter, thrombocytosis and thrombocythemia, includes clinical information and ends with the approach to the patient with a high platelet count. There is very little information on drug-induced thrombocytopenia; however, the third chapter, mechanisms of immunologic drug effects on blood cells, mentions a partial list of drugs that produce thrombocytopenia. The only drugs included are those which are known to produce this effect through antibody formation.

The section on hematopoietic stem cell discusses the origin, cellular basis, regulation, and interrelation of the hematopoietic stem cell.

The section of the book, white cells, interrelates the physiology and pharmacology of the blood the best. This section starts with a chapter on the physiology of myelopoiesis and ends with an excellent chapter on the effects of drugs on myelopoiesis. The last chapter is on the physiological and immunologic activities of lymphocytes.

The last section, red cells, starts with erythropoietic cellular proliferation and mainly considers the control of erythropoiesis from the kinetic point. The chapter on iron mainly includes information on iron deficiency, therapy, and drugs interfering with iron therapy. A brief outline on methods to study absorption of iron is included. A chapter on the biochemistry, physiology, and deficiency of vitamin B_{12} and folic acid is included. Red cell and hyperoxia includes the mechanisms of how lysis is caused by hyperoxia. Many descriptions of experiments are cited. The chapter on erythropoientin presents current information about the influence of drugs and hormones on the regulation of erythropoientin production. The last chapter, transferrin, presents the highlights of transferrin physicochemical properties, physiology, and genetics.

College of Pharmacy **M. Moldowan**
University of Florida
Gainesville, Florida 32610

Transport Phenomena in Aqueous Solutions. By T. Erdey-Gruz. Adam Hilger, Ltd, London. 1974. 512 pp. 16 × 24 cm. $37.50.

A life process which does not involve the movement of chemical species in aqueous media is rather difficult to imagine. Any monograph dealing with the structure of liquid water, the structure of aqueous solutions, and the nature of the transport phenomena occurring therein is thus of interest to a number of disciplines ranging from solution physical chemistry, through molecular biology and medicinal chemistry, to physiology and environmental science.

The first section of the present volume examines the structure of "normal" liquids, the structure of water, and the effect of solutes, both ionic and nonpolar, on the structure of water. The second section examines theories of viscosity and the effects of ions and nonelectrolytes on viscosity, including concentrated solutions, and the effects of temperature and hydrostatic pressure. The third