Synthesis of a New Class of Uridine Phosphorylase Inhibitors

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Dedicated to the memory of Roland K. Robins

A new series of potent uridine phosphorylase inhibitors have been prepared from barbituric acid. Among them, 1-[(2-hydroxyethoxy)methyl]-5-(*m*-benzyloxy)benzylbarbituric acid (**37**, BBBA) is the most promising having a K_i value of 1.1 ± 0.2 n*M* with uridine phosphorylase from human liver. The new inhibitors are easily synthesized and are better inhibitors of human uridine phosphorylase than their uracil counterparts.

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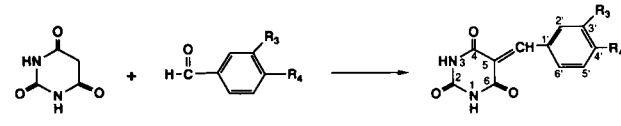
Two distinct pyrimidine nucleoside phosphorylases occur in mammalian cells [1,2]. One of them, uridine phosphorylase (UrdPase) primarily cleaves pyrimidine ribosides (except cytidines), but will also cause phosphorolysis of pyrimidine 2′- and 5′-deoxyribosides [1-8]. The other enzyme, thymidine phosphorylase (dThdPase) is generally specific and cleaves only pyrimidine 2′- and 5′-deoxyribosides [3-11].

UrdPase plays a critical role in the chemotherapy of cancer and AIDS. In cancer chemotherapy, UrdPase is responsible for the activation and deactivation of certain 5-fluoropyrimidines, *e.g.*, 5-fluorouracil, 5-fluoro-2′-deoxyuridine, and 5-fluoro-5′-deoxyuridine [3,5-7,9-14], since most human tumors are apparently devoid of dThdPase activity [1,3,7,12,15]. Recently, UrdPase was shown to exhibit a circadian rhythm in mice which is opposite to that observed for the anticancer efficacy of 5-fluoro-2′-deoxyuridine [16,17]. In addition, host-toxicity of 5-fluorouracil [18,19,20] and 3′-azido-3′-deoxythymidine (AZT) [21] is reversed by uridine. The bioavailability and concentration of this riboside is controlled by UrdPase [16]. Inhibitors of this enzyme should enhance the chemotherapeutic efficacy of the aforementioned agents by preventing their degradation and/or host-toxicity.

The 5-benzylacyclouridines were developed for this purpose, *i.e.*, as specific inhibitors of UrdPase [4,6,8,12,22]. This class of inhibitors potentiated the antineoplastic activity of 5-fluoro-2′-deoxyuridine *in vitro* and *in vivo* [12,23,24], increased the level of uridine and its duration in plasma [25,26,27], and reduced the host-toxicity of 5-fluorouracil [26,27], 5-fluoro-2′-deoxyuridine [28], and AZT [29,30]. The 5-benzylacyclouridines are not without fault; however, their potency, overall cost, and poor water solubility limit their clinical usefulness. Such problems prompted us to search for UrdPase inhibitors which would be more potent, synthetically cost-effective, and have greater water solubility. We have now prepared a series of 5-monosubstituted barbituric acid derivatives and identified several as excellent inhibitors of UrdPase.

The starting materials for the 5-arylidene barbituric acids **11-19** depicted in Scheme 1 were barbituric acid (**1**) and the appropriate aromatic aldehyde, **2-10**. These

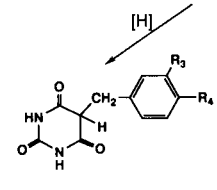
Scheme 1



1

2; R_3,R_4 = H
3; R_3 = OCH_3, R_4 = H
4; R_3 = $OCH_2C_6H_5$, R_4 = H
5; R_3 = Br, R_4 = H
6; R_3 = Cl, R_4 = H
7; R_3 = NO_2, R_4 = H
8; R_3 = $OCH_2C_6H_5$, R_4 = OCH_3
9; R_3 = OCH_3, R_4 = $OCH_2C_6H_5$
10; R_3,R_4 = OCH_3

11; R_3,R_4 = H
12; R_3 = OCH_3, R_4 = H
13; R_3 = $OCH_2C_6H_4$, R_4 = H
14; R_3 = Br, R_4 = H
15; R_3 = Cl, R_4 = H
16; R_3 = NO_2, R_4 = H
17; R_3 = $OCH_2C_6H_5$, R_4 = OCH_3
18; R_3 = OCH_3, R_4 = $OCH_2C_6H_5$
19; R_3,R_4 = OCH_3



20; R_3,R_4 = H
21; R_3 = OCH_3, R_4 = H
22; R_3 = $OCH_2C_6H_5$, R_4 = H
23; R_3 = Br, R_4 = H
24; R_3 = Cl, R_4 = H
25; R_3 = NO_2, R_4 = H
26; R_3 = $OCH_2C_6H_5$, R_4 = OCH_3
27; R_3 = OCH_3, R_4 = $OCH_2C_6H_5$
28; R_3,R_4 = OCH_3

materials are commercially available and the preparation of the 5-arylidenebarbituric acids **11-19** followed known synthetic methodology [31-33]. An aqueous solution of barbituric acid was heated at reflux for approximately 1 hour with a slight excess of the desired aldehyde. The 5-arylidene barbituric acids were obtained in good yield and could be used without further purification. They are highly colored and all exhibit a characteristic =C*H*- resonance at approximately δ 8.2 in their ^{1}H nmr spectra. Reduction of the exocyclic double bond at C5 was carried out with sodium borohydride [33] in cold ethanol. Triethylammonium formate (TEAF) has also been employed as a reducing agent for this step and was shown to reduce only the C5 exocyclic double bond of certain substituted arylidenes [32]. *N*-Unsubstituted 5-arylidenebarbituric acids underwent reduction at elevated temperatures in TEAF to provide the reduced product in good yield, but they were isolated as their triethylammonium salts. A drawback of this reducing agent is that it is not commerically available and it must be prepared [34], thus for convenience, sodium borohydride is the preferred reagent for the preparation of **20-28**.

Of the 5-benzylbarbituric acids, **22** exhibited the best inhibitory activity toward human liver UrdPase (K_i = 2.77 ± 0.53) μM [35]). Based on this promising result, we decided to prepare the acyclonucleoside **37** of **22**. This decision was patterned after the 5-benzyluracil series where the *N*1-acyclonucleoside enhanced inhibitory activity over that of the parent heterocycle [4,6]. The synthesis of the targeted acyclonucleosides was approached by two different routes as illustrated in Scheme 2. The first pathway involved alkylation of persilylated barbituric acid (**29**) with (2-acetoxyethoxy)methyl bromide (**30**) [36] in dry acetonitrile to provide 1-[(2-acetoxyethoxy)methyl]barbituric acid (**31**) in 72% yield after purification by silica gel column chromatography. Deprotection of **31** with sodium methoxide furnished **32** in near-quantitative yield. Either **31** or **32** could be used in the next step leading to the *meta*-substituted arylidene, but we selected **31** for ease of workup due to better organic solubility. Reacting **31** with either **3** or **4** afforded the arylidenes **33** (88%) and **34** (87%), respectively, as intimate mixtures of *E*- and *Z*-isomers. Reduction (sodium borohydride) of the arylidene acyclonucleosides provided **35** and **36** in good yield. Deprotection with sodium methoxide afforded the targeted analogue **37** (BBBA). An alternate pathway to **37** involved direct alkylation of the persilylated *meta*-substituted 5-benzylbarbituric acid **38** with **30**. Treatment of **38** with **30** in anhydrous 1,2-dichloroethane in the presence of a catalytic amount of aluminum chloride furnished BBBA acetate **36** (50%)

Scheme 2

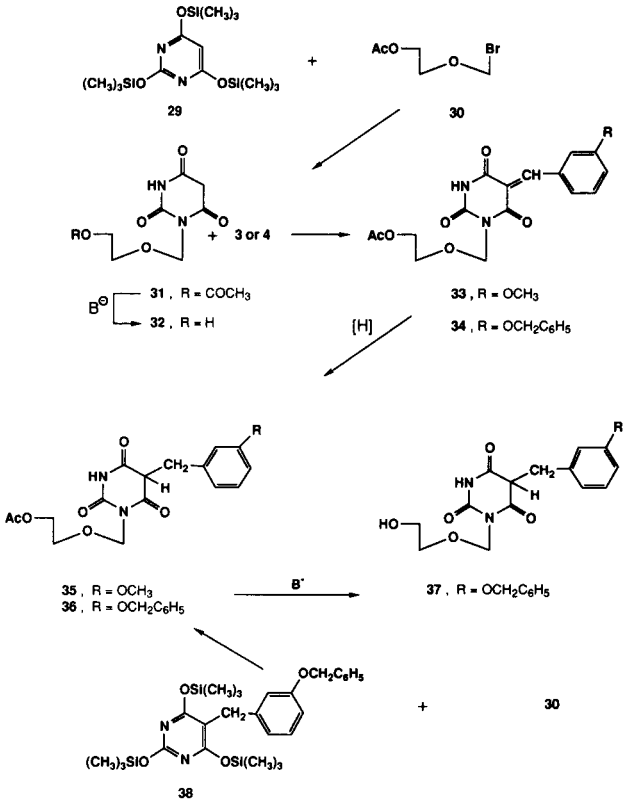
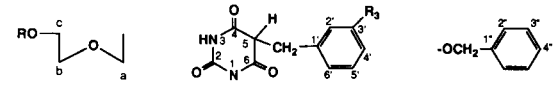


Table 1. ^{1}H and ^{13}C nmr Chemical Shifts (δ) of Certain Barbituric Acid Acyclonucleosides.



Compound (type, solvent)	Chemical Shifts
31: ^{1}H ($CDCl_3$);	2.06 (s, 3, $COCH_3$), 3.80 (s, 2, H5), 3.85 (t, 2, Hb), 4.21 (t, 2, Hc), 5.40 (s, 2, Ha), 9.13 (br s, 1, N*H*).
^{13}C ($CDCl_3$);	21.0 ($COCH_3$), 39.9 (C5), 63.3 (Cc), 68.9 (Cb), 71.8 (Ca), 151.9 (C2), 165.1 (C4), 165.1 (C6), 171.5 (C = O).
32: ^{1}H (DMSO-d_6);	3.42 (s, 4, Hb, Hc), 3.93 (br s, 3, O*H*, H5), 5.09 (s, 2, Ha), 10.42 (br s, 1, N*H*).
35: ^{1}H ($CDCl_3$);	2.02 (s, 3, $COCH_3$), 3.23 (s, 2, $CH_2C_6H_4$-), 3.65-3.78 (m, 5, OCH_3, Hb), 4.15 (dd, 2, Hc), 4.70 (br s, 1, H5), 5.13 (dd, 2, Ha), 6.63-6.69 (m, 2, H4',H2'), 6.75-6.82 (m, 1, H6'), 7.15 (t, 1, H5'), 9.41 (br s, 1, N*H*).
^{13}C ($CDCl_3$);	20.8 ($COCH_3$), 35.7 ($CH_2C_2H_4$-), 47.8 (C5), 55.3 (OCH_3), 63.3 (Cc), 68.4 (Cb), 71.5 (Ca), 113.3 (C4'), 116.2 (C2'), 122.4 (C6'), 129.6 (C5'), 133.1 (C1'), 149.3 (C2), 159.5 (C3'), 169.9 (C4), 171.0 (C6), 171.4 (C=O).

which after deprotection provided BBBA **37**. BBBA exhibited significant activity against human liver UrdPase. This acyclonucleoside had a K_i of 1.1 ± 0.2 n*M* [35] and represents the most potent inhibitor known of this enzyme.

Presently, we are exploring new synthetic routes to BBBA and its analogues. Modification of the alkyl side chain, *i.e.*, the "acyclo tail", is under investigation in an effort to find multisubstrate activity. We are also examining analogues which can serve as ligands for affinity chromatography to purify uridine phosphorylase so it can be crystallized for future X-ray crystallographic studies.

EXPERIMENTAL

Melting points were determined with a Buchi 535 apparatus or a Fargo apparatus and are uncorrected. The ^{1}H nmr spectra were recorded with either a Varian EM-390, Varian XL-GEM 200, or a Bruker AM-300 spectrometer. The ^{13}C nmr spectra were run on either the Varian XL-GEM 200 or Bruker AM-300 spectrometers. The chemical shifts are expressed in parts per million (δ) with respect to TMS. Low resolution mass spectra were obtained with a VG Quattro mass spectrometer and high resolution mass spectra were recorded on a VG 70-250 mass spectrometer. Thin layer chromatography was run on precoated (0.2 mm) silica gel 60 F-254 plates manufactured by EM Laboratories, Inc., and short-wave ultaviolet light (254 nm) was used to detect the uv-absorbing spots. Silica gel (Merck, 230-400 mesh, 60 Å) was employed for column chromatography. A chromatotron Model 7924T by Harrison Research was also employed for preparative separations. The 1 mm plates used were coated with silica gel PF-254 with a calcium sulfate binder. All chromatograms were run under inert conditions with a nitrogen flow rate of 20 ml/minute. All solvent proportions are by volume unless started otherwise. The aldehydes were purchased from Aldrich and, with the exception of benzaldehyde, were used without purification. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

General Method for the Preparation of the 5-Arylidene Barbituric Acids **11-19** [31].

Barbituric acid (99%, Aldrich) was added to a 3-neck round bottomed flask containing water. The mixture was stirred (magnetically or mechanically depending on the reaction scale) and heated until the barbituric acid dissolved. At this point, the appropriate aldehyde (slight excess) was added, in one portion, and the mixture stirred and heated at reflux. During the addition of the aldehyde a color change is observed and the product begins to precipitate. The reaction can be monitored by tlc, but usually after one hour at reflux, the reaction is complete. The reaction mixture was allowed to cool and the precipitated product removed by filtration. This material was washed several times with cold water and then air-dried. The product is usually pure enough for use in the reduction step.

5-Benzylidenebarbituric Acid (**11**).

This compound had mp 263-265° (95%, from absolute ethanol) (lit [31] 254-256°); ^{1}H nmr (DMSO-d_6): δ 7.2-7.6 (m, 3, H3′,4′,5′), 7.9-8.2 (m, 2, H2′,6′), 8.31 (s, 1, =C*H*-), 11.2 (br s, 1, N*H*), 11.25 (br s, 1, N*H*) {lit [37] (DMSO-d_6): δ 7.46 (m, 3, H3′,4′,5′), 8.08 (m, 2, H2′,6′), 8.30 (s, 1, =C*H*-)}; ^{13}C nmr (DMSO-d_6): δ 119.0 (=*C*H-), 127.9 (C3′), 132.1 (C4′), 132.6 (C1′), 133.0 (C2′), 150.1 (C2), 154.6 (C5), 161.4 (C4), 163.3 (C6) [38].

5-(*m*-Methoxy)benzylidenebarbituric Acid (**12**).

This compound had mp 245-247° (94%, from absolute ethanol); ^{1}H nmr (DMSO-d_6): δ 3.79 (s, 3, OC*H*$_3$), 6.96-7.8 (m, 4, C$_6$*H*$_4$), 8.23 (s, 1, =C*H*-), 11.1 (br s, 1, N*H*), 11.28 (br s, 1, N*H*); ^{13}C nmr (DMSO-d_6): δ 55.2 (O*C*H$_3$), 117.6, 118.4, 119.2, 126.0, 129.0, 133.8, 150.1, 154.6, 158.6, 161.5, 163.3.

Anal. Calcd. for $C_{12}H_{10}N_2O_4$: C, 58.54; H, 4.10; N, 11.38. Found: C, 58.36; H, 4.21; N, 11.35.

5-(*m*-Benzyloxy)benzylidenebarbituric Acid (**13**).

This compound had mp 243-245° (92%, absolute ethanol); ^{1}H nmr (DMSO-d_6): δ 5.14 (s, 2, OC*H*$_2$C$_6$H$_5$), 7.18-7.64 (m, 7, OCH$_2$C$_6$*H*$_5$ and H4′,5′,6′), 7.91 (s, 1, H2′), 8.24 (s, 1, =C*H*-), 11.26 (s, 1, N*H*), 11.40 (s, 1, N*H*); ^{13}C nmr (DMSO-d_6): δ 69.4, 118.7, 119.1, 119.3, 126.3, 127.8, 128.0, 128.5, 129.2, 133.9, 136.8, 150.2, 154.4, 157.7, 161.6, 163.4.

Anal. Calcd. for $C_{18}H_{14}N_2O_4$: C, 67.08; H, 4.38; N, 8.69. Found: C, 66.95; H, 4.51; N, 8.59.

5-(*m*-Bromo)benzylidenebarbituric Acid (**14**).

This compound had mp 278-280° (80%, from absolute ethanol); ^{1}H nmr (DMSO-d_6): δ 7.38 (t, 1, H5′), 7.64 (d, 1, H4′), 7.87 (d, 1, H6′), 8.20 (s, 1, H2′), 8.27 (s, 1, =C*H*-), 11.29 (s, 1, N*H*), 11.45 (s, 1, N*H*); ^{13}C nmr (DMSO-d_6): δ 120.7, 121.4, 130.3, 132.0, 134.4, 134.6, 135.4, 150.5, 153.0, 161.8, 163.3.

Anal. Calcd. for $C_{11}H_7N_2O_3Br$: C, 44.77; H, 2.39; N, 9.49; Br, 27.08. Found: C, 44.73; H, 2.41; N, 9.45; Br, 27.24.

5-(*m*-Chloro)benzylidenebarbituric Acid (**15**).

This compound had mp 268-270° (71%, from absolute ethanol); ^{1}H nmr (DMSO-d_6): δ 7.47 (t, 1, H5′), 7.55 (d, 1, H4′), 7.85 (d, 1, H6′), 8.15 (s, 1, H2′), 8.22 (s, 1, =C*H*-), 11.29 (s, 1, N*H*), 11.44 (s, 1, N*H*); ^{13}C nmr (DMSO-d_6): δ 120.6, 129.8, 131.3, 131.4, 131.5, 132.6, 134.9, 150.3, 152.6, 161.5, 163.1.

Anal. Calcd. for $C_{11}H_7N_2O_3Cl$: C, 52.71; H, 2.82; N, 11.18; Cl, 14.14. Found: C, 52.60; H, 2.84; N, 11.16; Cl, 14.25.

5-(*m*-Nitro)benzylidenebarbituric Acid (**16**).

This compound had mp 254-255° (67%, from acetic acid) (lit [39] 254-255°); ^{1}H nmr (DMSO-d_6): δ 7.68 (t, 1, H5′), 8.19 (d, 1, H4′), 8.25 (d, 1, H6′), 8.29 (s, 1, =C*H*-), 8.88 (s, 1, H2′), 11.34 (s, 1, N*H*), 11.48 (s, 1, N*H*); ^{13}C nmr (DMSO-d_6): δ 121.6, 125.7, 126.3, 129.5, 134.6, 138.7, 147.3, 150.4, 151.7, 161.7, 163.0 [38].

5-(3′-Benzyloxy-4′-methoxy)benzylidenebarbituric Acid (**17**).

This compound had mp 280-282° (90%, from absolute ethanol); ^{1}H nmr (DMSO-d_6): δ 3.89 (s, 3, OC*H*$_3$), 5.12 (s, 2, OC*H*$_2$C$_6$H$_5$), 7.14 (d, 1, H5′), 7.3-7.5 (m, 5, CH$_2$C$_6$*H*$_5$), 7.96 (d, 1, H6′), 8.24 (s, 1, =C*H*-), 8.47 (s, 1, H2′), 11.20 (s, 1, N*H*); 11.31 (s, 1, N*H*); ^{13}C nmr (DMSO-d_6): δ 55.9 (O*C*H$_3$), 69.9 (O*C*H$_2$C$_6$H$_5$), 111.4, 115.4, 118.6, 121.2, 128.0, 128.4, 131.7, 136.6, 146.8, 150.1, 153.9, 155.3, 162.3, 163.9; ms: (m/z) 352 (M^+). Mass. Calcd. for $C_{19}H_{16}N_2O_5$: 352.1059. Found: 352.1064.

Anal. Calcd. for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.58; N, 7.95. Found: C, 64.60; H, 4.57; N, 7.87.

5-(3′-Methoxy-4′-benzyloxy)benzylidenebarbituric Acid (**18**).

This compound had mp 247-248° (94%, from 95% ethanol); ^{1}H

nmr (DMSO-d_6): δ 3.82 (s, 3, OCH_3), 5.23 (s, 2, OCH_2C$_6$H$_5$), 7.19 (d, 1, H5'), 7.3-7.5 (m, 5, CH$_2$C$_6$$H_5$), 7.87 (d, 1, H6'), 8.25 (s, 1, =CH-), 8.41 (s, 1, H2'); ^{13}C nmr (DMSO-d_6): δ 55.5 (OCH_3), 70.0 (OCH_2C$_6$H$_5$), 112.4, 115.4, 117.1, 125.5, 128.0, 128.1, 128.3, 128.5, 131.4, 136.3, 148.0, 150.2, 152.6, 155.4, 162.3, 164.0; ms: (m/z) 352 (M$^+$). Mass Calcd. for $C_{19}H_{16}N_2O_5$: 352.1059. Found: 352.1060.

Anal. Calcd. for $C_{19}H_{16}N_2O_5 \cdot H_2O$: C, 61.62; H, 4.90; N, 7.56. Found: C, 61.63; H, 4.89; N, 7.58.

5-(3',4'-Dimethoxy)benzylidenebarbituric Acid (**19**).

This compound had mp >290° (quantitative, from 95% ethanol); ^{1}H nmr (DMSO-d_6): δ 3.80 (s, 3, OCH_3), 3.88 (s, 3, OCH_3), 7.10 (d, 1, H5'), 7.90 (d, 1, H6'), 8.25 (s, 1, =CH-), 8.41 (s, 1, H2'); ^{13}C nmr (DMSO-d_6): δ 55.4 (OCH_3), 55.9 (OCH_3), 111.1, 115.3, 116.8, 125.3, 131.7, 147.8, 150.2, 153.7, 155.5, 162.4, 164.0; ms: (m/z) 276 (M$^+$). Mass Calcd. for $C_{13}H_{12}N_2O_5$: 276.0746. Found: 276.0745.

Anal. Calcd. for $C_{13}H_{12}N_2O_5$: C, 56.52; H, 4.38; N, 10.14. Found: C, 56.43; H, 4.37; N, 10.12.

General Method for the Reduction of the 5-Arylidene Barbituric Acids **11-19**.

The arylidene barbituric acid derivatives were reduced with sodium borohydride (Aldrich, 98% powder) in ethanol using a modification of the procedure reported by Yoneda and coworkers [33]. The arylidene was suspended in ethanol (approximately 50 ml/g of arylidene) and the colored suspension was cooled (ice-bath) and stirred. Sodium borohydride [two (2) to five (5) moles of sodium borohydride per mole of arylidene] was added portionwise to the cool suspension. During the addition of sodium borohydride, a rapid loss of color was observed (in certain cases, the intensity of color becomes lighter). After the addition of sodium borohydride was finished, the cooling bath was removed and the stirred reaction mixture was allowed to warm to room temperature. The reduction can be monitored by tlc, but usually the process is complete after 1.5 to 2 hours of stirring. Next, the reaction mixture was concentrated to remove most of the ethanol and then a small portion of water was added. The reaction mixture was again cooled and then carefully acidified, with a 10% hydrochloric acid solution, to a *p*H of 3.5-4.0 (*p*H meter). During this process, solution is achieved and, on standing, the product begins to precipitate out of solution. At this point, the flask and contents are allowed to stand at 4° (refrigerator) overnight. The solid is removed by filtration, washed with two portions of ice water, and recrystallized.

5-Benzylbarbituric Acid (**20**).

This compound had mp 210-212° (61%, from 95% ethanol) (lit [40] 212.5-214°); ^{1}H nmr (DMSO-d_6): δ 3.25 (d, 2, J = 4.7 Hz, CH_2C$_6$H$_5$), 3.90 (t, 1, J = 4.8 Hz, H5), 7.06-7.30 (m, 5, CH$_2$C$_6$$H_5$), 11.17 (br s, 2, N$H$); ^{13}C nmr (DMSO-$d_6$): δ 33.3 (C$H_2C_6H_5$), 49.3 (C5), 127.0 (C4'), 128.6, 129.1 (C2', C3'), 137.6 (C1'), 150.9 (C2), 170.4 (C4, C6).

Anal. Calcd. for $C_{11}H_{10}N_2O_3$: C, 60.55; H, 4.61; N, 12.83. Found: C, 60.35; H, 4.56; N, 12.59 [38].

5-(*m*-Methoxy)benzylbarbituric Acid (**21**).

This compound had mp 174-175° (80%, from absolute ethanol); ^{1}H nmr (DMSO-d_6): δ 3.21 (d, 2, J = 4.7 Hz, CH_2C$_6$H$_4$-), 3.68 (s, 3, OCH_3), 3.88 (t, 1, J = 4.7 Hz, H5), 6.6-6.8 (m, 3, H2',4',6'), 7.16 (t, 1, H5'), 11.18 (s, 1, NH); ^{13}C nmr (DMSO-d_6): δ 33.4 (CH_2C$_6$H$_4$-), 49.2 (C5), 54.9 (OCH_3), 112.2 (C4'), 114.9 (C2'), 121.3 (C6'), 129.7 (C5'), 139.1 (C1'), 150.9 (C2), 159.4 (C3'), 170.4 (C4, C6).

Anal. Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.28. Found: C, 57.63; H, 5.24; N, 11.50.

5-(*m*-Benzyloxy)benzylbarbituric Acid (**22**).

This compound had mp 187-188° (82%, from absolute ethanol); ^{1}H nmr (DMSO-d_6): δ 3.21 (d, 2, J = 4.8 Hz, CH_2C$_6$H$_4$-), 3.90 (t, 1, J = 4.8 Hz, H5), 5.02 (s, 2, OCH_2C$_6$H$_5$), 6.65 (d, 1, H4'), 6.73 (s, 1, H2'), 6.85 (dd, 1, H6'), 7.16 (t, 1, H5'), 7.29-7.45 (m, 5, OCH$_2$C$_6$$H_5$), 11.19 (s, 2, N$H$); ^{13}C nmr (DMSO-$d_6$): δ 33.3 (C$H_2C_6H_4$-), 49.2 (C5), 69.1 (O$CH_2C_6H_5$), 112.9 (C4'), 115.8 (C2'), 121.5 (C6'), 128.0 (C4''), 128.1 (C3''), 128.7 (C2''), 129.7 (C5'), 137.2 (C1''), 139.2 (C1'), 150.5 (C2), 158.5 (C3'), 170.4 (C4, C6); ms: (m/z) 324 (M$^+$).

Anal. Calcd. for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.48; H, 5.04; N, 8.46.

5-(*m*-Bromo)benzylbarbituric Acid (**23**).

This compound had mp 204-206° (70%, from absolute ethanol) (lit [41] 205-207°); ^{1}H nmr (DMSO-d_6): δ 3.24 (br s, 2, CH_2C$_6$H$_4$-), 3.99 (br s, 1, H5), 7.0-7.5 (m, 4, H2', H4', H5', H6'), 11.12 (s, 2, NH); ^{13}C nmr (DMSO-d_6): δ 32.4 (CH_2C$_6$H$_4$-), 49.4 (C5), 121.7 (C3'), 128.2 (C6'), 129.8 (C4'), 130.7 (C3'), 132.0 (C2'), 141.0, (C1'), 150.8 (C2), 170.0 (C4, C6).

Anal. Calcd. for $C_{11}H_9N_2O_3Br$: C, 44.47; H, 3.05; N, 9.43. Found: C, 44.85; H, 3.11; N, 9.43 [38].

5-(*m*-Chloro)benzylbarbituric Acid (**24**).

This compound had mp 203-205° (76%, from absolute ethanol) (lit [41] 204-205°); ^{1}H nmr (DMSO-d_6): δ 3.25 (br s, 2, CH_2C$_6$H$_4$-), 3.99 (br s, 1, H5), 7.05-7.35 (m, 4, H2', H4', H5', H6'), 11.23 (s, 2, NH); ^{13}C nmr (DMSO-d_6): δ 32.5 (CH_2C$_6$H$_4$-), 49.4 (C5), 126.9 (C4'), 127.8 (C6'), 129.1 (C2'), 130.4 (C5'), 133.0 (C3'), 140.7 (C1'), 150.8 (C2), 170.0 (C4, C6).

Anal. Calcd. for $C_{11}H_9N_2O_3Cl$: C, 52.29; H, 3.59; N, 11.09. Found: C, 52.29; H, 3.59; N, 11.08 [38].

5-(*m*-Nitro)benzylbarbituric Acid (**25**).

This compound had mp 201-202° (81%, from absolute ethanol) (lit [32] 205-206°); ^{1}H nmr (DMSO-d_6): δ 3.38 (br s, 2, CH_2C$_6$H$_4$-), 4.13 (br s, 1, H5), 7.5-7.7 (m, 2, H5', H6'), 7.95-8.20 (m, 2, H2', H4'), 11.33 (br s, 2, NH); ^{13}C nmr (DMSO-d_6): δ 32.1 (CH_2C$_6$H$_4$-), 49.6 (C5), 122.0 (C4'), 124.0 (C2'), 130.1 (C5'), 136.3 (C6'), 141.0 (C1'), 148.0 (C3'), 151.0 (C2), 170.0 (C4, C6) [38].

5-(3'-Benzyloxy-4'-methoxy)benzylbarbituric Acid (**26**).

This compound had mp 155-156° (65%, from absolute ethanol); ^{1}H nmr (DMSO-d_6): δ 3.20 (br s, 2, CH_2C$_6$H$_3$-), 3.71 (s, 3, OCH_3), 3.84 (br s, 1, H5), 4.97 (s, 2, OCH_2C$_6$H$_5$), 6.33 (d, 1, H6'), 6.80 (s, 1, H2'), 6.86 (d, 1, H5'), 7.3-7.48 (m, 5, OCH$_2$C$_6$$H_5$), 11.19 (br s, 2, N$H$); ^{13}C nmr (DMSO-$d_6$): δ 33.4 (C$H_2CH_6H_3$-), 49.6 (C5), 55.5 (O$CH_3$), 70.2 (O$CH_2C_6H_5$), 112.0 (C5'), 114.7 (C2'), 121.6 (C6'), 127.9 (C2'', C4''), 128.4 (C3''), 129.4 (C1'), 137.0 (C1''), 147.5 (C3'), 148.1 (C4'), 150.6 (C2), 170.1 (C4, C6); ms: (m/z) 354 (M$^+$).

Mass Calcd. for $C_{19}H_{18}N_2O_5$: 354.1216. Found: 354.1219.

Anal. Calcd. for $C_{19}H_{18}N_2O_5$: C, 64.40; H, 5.12; N, 7.90. Found: C, 64.03; H, 5.38; N, 7.68.

5-(3'-Methoxy-4'-benzyloxy)benzylbarbituric Acid (**27**).

This compound had mp 184-185° (69%, from absolute ethanol); ^{1}H nmr (DMSO-d_6): δ 3.21 (br s, 2, $CH_2C_6H_3$-), 3.71 (s, 3, OCH_3), 3.83 (br s, 1, H5), 5.01 (s, 2, $OCH_2C_6H_5$), 6.58 (d, 1, H6'), 6.70 (s, 1, H2'), 6.91 (d, 1, H5'), 7.30-7.45 (m, 5, $OCH_2C_6H_5$), 11.18 (s, 2, NH); ^{13}C nmr (DMSO-d_6): δ 33.5 ($CH_2C_6H_3$-), 49.5 (C5), 55.4 (OCH_3), 69.9 ($OCH_2C_6H_5$), 113.0 (C2'), 113.4 (C5'), 120.9 (C6'), 128.1 (C2''), 128.4 (C3'', C4''), 129.9 (C1'), 137.1 (C1''), 146.7 (C4'), 148.7 (C3'), 150.6 (C2), 170.2 (C4, C6); ms: (m/z) 354 (M^+).

Anal. Calcd. for $C_{19}H_{18}N_2O_5$: C, 64.40; H, 5.12; N, 7.90. Found: C, 64.24; H, 5.31; N, 7.83.

5-(3',4'-Dimethoxy)benzylbarbituric Acid (**28**).

This compound had mp 162-163° (75%, from absolute ethanol); ^{1}H nmr (DMSO-d_6): δ 3.20 (br s, 2, $CH_2C_6H_3$-), 3.68 (s, 3, OCH_3), 3.69 (s, 3, OCH_3), 3.81 (br s, 1, H5), 6.59 (d, 1, H5'), 6.66 (s, 1, H2'), 6.82 (d, 1, H6'), 11.16 (br s, 2, NH); ^{13}C nmr (DMSO-d_6): δ 33.6 ($CH_2C_6H_3$-), 49.5 (C5), 55.3 (OCH_3), 55.4 (OCH_3), 111.7 (C5'), 112.7 (C2'), 120.9 (C6'), 129.3 (C1'), 147.6 (C4'), 148.3 (C3'), 150.5 (C2), 170.2 (C4, C6); ms: (m/z) 278 (M^+). Mass Calcd. for $C_{13}H_{14}N_2O_5$: 278.0903. Found: 278.0902.

Anal. Calcd. for $C_{13}H_{14}N_2O_5$: C, 56.11; H, 5.07; N, 10.07. Found: C, 55.71; H, 5.13; N, 10.09.

1-[(2-Acetoxyethoxy)methyl]barbituric Acid (**31**).

Dry barbituric acid (99% Aldrich, 5.0 g, 39.0 mmoles) and chlorotrimethylsilane (10 ml) were heated in hexamethyldisilazane (HMDS, 100 ml) at reflux overnight under anhydrous conditions [42]. The excess HMDS was removed by distillation and the resulting crystalline persilylated barbituric acid derivative **29** was dissolved in **dry** acetonitrile (100 ml). This solution was cooled to 0° and to it was added (2-acetoxyethoxy)methyl bromide (**30**, 7.7 g, 39.1 mmoles) which had been previously dissolved in **dry** acetonitrile (40 ml). The reaction mixture was allowed to warm to room temperature and it was stirred for six hours with the exclusion of moisture. At this point, the reaction had reached completion (tlc), the solvent was removed *in vacuo*, and the resulting residue was dissolved in a minimal amount of dichloromethane. To this flask was added an equivalent amount of silica gel and the mixture taken to dryness on a rotary evaporator. The uniform mixture was applied to a silica gel column and the column was eluted with dichloromethane-methanol (98:2) to furnish pure **31** (6.96 g, 73%), mp 104° (see Table 1).

Anal. Calcd. for $C_9H_{12}N_2O_6$: C, 44.27; H, 4.95; N, 11.47. Found: C, 44.19; H, 5.45; N, 11.35.

1-[(2-Hydroxyethoxy)methyl]barbituric Acid (**32**).

Compound **31** (300 mg, 1.22 mmoles) was dissolved in dry methanol (10 ml) and to this stirred solution was added a fresh 0.1*N* sodium methoxide solution (15 ml, 1.5 mmoles). The reaction was stirred at room temperature under a dry N_2 atmosphere for two hours. Next, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin to a *p*H of 6 (*p*H paper). The resin was filtered off, washed with methanol (3 x 10 ml), and the filtrate and wash were concentrated under diminished pressure. The resulting residue was crystallized from 95% ethanol to give **32** (250 mg, 93%), mp 220° dec.

Anal. Calcd. for $C_7H_{10}N_2O_5 \cdot H_2O$: C, 38.19; H, 5.49; N, 12.72. Found: C, 38.44; H, 5.10; N, 12.51.

1-[(2-Acetoxyethoxy)methyl]-5-(*m*-methoxy)benzylidenebarbituric Acid (**33**).

Dry acyclonucleoside **31** (1.104 g, 4.51 mmoles) was dissolved in warm, distilled water (25 ml) and to this solution was added freshly distilled *m*-anisaldehyde (0.60 ml, 4.93 mmoles). The mixture was heated at reflux with stirring for one hour and then allowed to cool to room temperature. The precipitate which formed was collected by filtration, washed with ice water (2 x 10 ml), and dried in a vacuum dessicator (Drierite) to provide **33** (1.432 g, 88%), mp 130-131°.

Anal. Calcd. for $C_{17}H_{18}N_2O_7$: C, 56.35; H, 5.01; N, 7.73. Found: C, 56.58; H, 5.17; N, 7.78.

1-[(2-Acetoxyethoxy)methyl]-5-(*m*-benzyloxy)benzylidenebarbituric Acid (**34**).

The synthesis of **34** was conducted in a similar manner as **33**. **Dry 31** (4.0 g, 16.3 mmoles) was dissolved in warm distilled water (50 ml) and *m*-benzyloxybenzaldehyde (3.72 g, 17.4 mmoles) was added to this solution. The reaction mixture was heated and stirred for four hours. The yellow precipitate was purified by silica gel column chromatography using dichloromethane-methanol (97:3) as eluent. This procedure afforded pure **34** (6.2 g, 87%), mp 128-130°.

Anal. Calcd. for $C_{23}H_{22}N_2O_7$: C, 63.00; H, 5.06; N, 6.39. Found: C, 62.94; H, 5.17; N, 6.25.

1-[(2-Acetoxyethoxy)methyl]-5-(*m*-methoxy)benzylbarbituric Acid (**35**).

The arylidene **33** (2.042 g, 5.64 mmoles) was suspended in cool, absolute ethanol (50 ml) and to the reaction flask was added sodium borohydride (1.07 g, 28.2 mmoles) portionwise. Once the addition was complete, the reaction was stirred for two hours at room temperature. The reaction was quenched with water (20 ml) and, with cooling, carefully acidified with a 5% hydrochloric acid solution to a *p*H of 5. The excess solvent was removed *in vacuo* (40°) and the residue was dissolved in a minimal amount of methanol and applied to a silica gel column. The column was eluted with dichloromethane-methanol (97:3) and the fractions (25 ml) containing product were pooled to furnish pure **35** (1.85 g, 91%). An analytical sample was recrystallized from 95% ethanol, mp 55-57°.

Anal. Calcd. for $C_{17}H_{20}N_2O_7 \cdot 1.5H_2O$: C, 52.17; H, 5.92; N, 7.16. Found: C, 52.50; H, 5.34; N, 6.81.

1-[(2-Acetoxyethoxy)methyl]-5-(*m*-benzyloxy)benzylbarbituric Acid (**36**).

Method A.

Compound **34** (4.021 g, 9.17 mmoles) was reduced in the same manner as **33**. Like **33**, five equivalents of sodium borohydride (1.735 g, 45.86 mmoles) were used. The workup was changed, however, because the water quench step in a preliminary run caused deacetylation, Thus, Amberlite IR-120 H^+ was added directly to the reaction mixture until it was slightly acidic (*p*H paper). The resin was removed, washed with ethanol (3 x 30 ml), and the filtrate combined with the reaction mixture. The ethanol was removed under diminished pressure (40°) and the resulting residue purified on a silica gel column using dichloromethane-methanol (9:1) as eluent. The title compound **36** was obtained as a gum (3.43 g, 82%).

Anal. Calcd. for $C_{23}H_{24}N_2O_7 \cdot H_2O$: C, 60.26; H, 5.72; N, 6.11. Found: C, 60.52; H, 5.48; N, 5.84.

Method B.

5-(*m*-Benzyloxy)benzylbarbituric acid (**22**, 2.0 g, 6.17 mmoles) was silylated with chlorotrimethylsilane (1.6 ml) and hexamethyldisilazane (100 ml) as **31**. Silyl **38** was dissolved in **dry** 1,2-dichloroethane (130 ml) and to this solution was added (2-acetoxyethoxy)methyl bromide (1.22 g, 6.17 mmoles) which had been dissolved in **dry** 1,2-dichloroethane (20 ml). A catalytic amount of anhydrous aluminum chloride (40 mg) was added to the reaction flask and the reaction mixture was allowed to stir 48 hours at room temperature under anhydrous conditions. Next, the reaction mixture was cooled (ice bath) and carefully neutralized (approximately *p*H 7) with a saturated sodium bicarbonate solution. The organic layer was separated and the aqueous phase was extracted with chloroform (3 x 100 ml). The organic layer and extracts were combined and dried over anhydrous magnesium sulfate. The dried organic layer was evaporated to dryness and the resulting residue was dissolved in a minimal amount of dichloromethane and applied to a silica gel column. The column was eluted with dichloromethane-methanol (98:2) to give **36** (1.36 g, 50%). This material was identical in all respects to **36** from Method A.

1-[(2-Hydroxyethoxy)methyl]-5-(*m*-benzyloxy)benzylbarbituric Acid (**37**).

Acyclonucleoside **36** was deprotected in the same manner as described for the preparation of **32**. BBBA acetate **36** (610 mg, 1.38 mmoles) gave **37** (450 mg, 82%), mp 193-195°.

Anal. Calcd. for $C_{21}H_{22}N_2O_6$: C, 63.31; H, 5.57; N, 7.03. Found: C, 63.24; H, 5.68; N, 6.81.

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