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Synthesis of N-β-D-glucopyranosyluronate derivatives of barbital, phenobarbital, metharbital, and mephobarbital

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Abstract

The synthesis and characterization of barbital, phenobarbital, metharbital, and mephobarbital glucuronides is reported. The condensation of per(trimethylsilyl)-barbital and -phenobarbital with methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate in the presence of trimethylsilyl trifluoromethanesulfonate gave moderate yields of the N^1-(β-D-glucopyranosyluronate) barbiturate derivatives. The diastereomers of the phenobarbital derivatives were resolved by use of C_{18} reversed-phase HPLC. The homologous N^3-methyl barbiturate N^1-glucuronates were prepared by reaction of the barbital and phenobarbital N^1-glucuronate derivatives with diazomethane. The absolute configuration of the phenobarbital N^1-β-D-glucopyranuronate epimers was determined by oxidative removal of the glycon from the mephobarbital N^1-β-D-glucopyranuronate epimers to give the optical isomers of mephobarbital. The spectroscopic data for this series of compounds will facilitate the characterization of N-glycosylated imide xenobiotics that may be detected as mammalian metabolites in biodisposition studies.

Keywords: D-Glucopyranosyluronate derivatives; Glucuronides; Barbiturates; Synthesis

1. Introduction

Glucuronidation is an important metabolic pathway in mammals and is responsible for the urinary and biliary excretion of a large number of endogenous and exogenous substances. It is apparent that glucuronidation can affect the pharmacology, toxicology,

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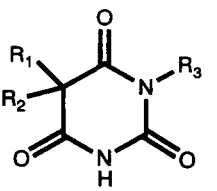
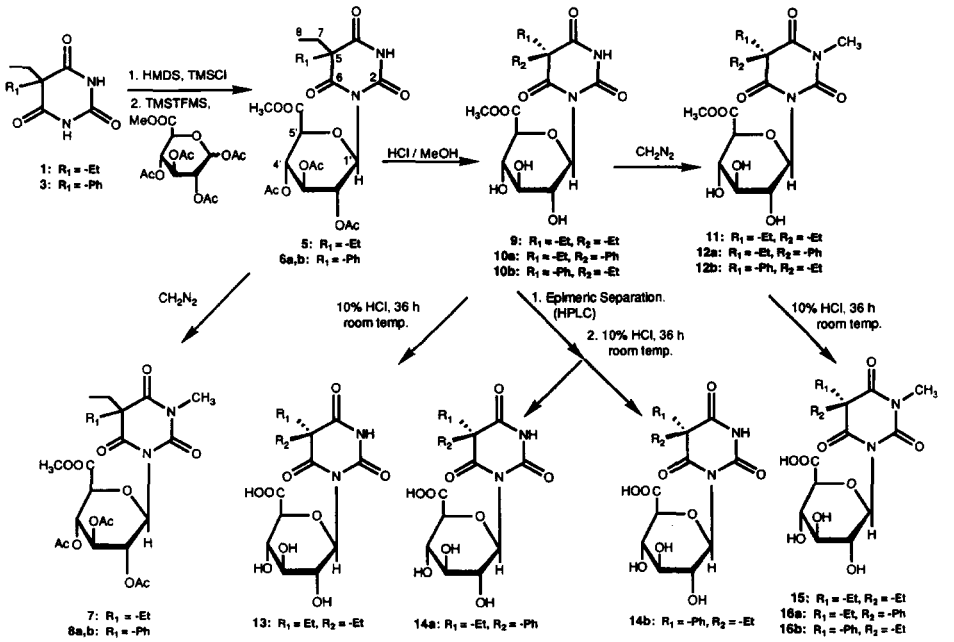
and pharmacokinetics of xenobiotic compounds and may have an important role in the therapeutic and toxic responses associated with drugs [1,2]. A limited number of nitrogen-containing heterocylic rings form glucuronide conjugates in vivo, and the structure–substrate relationships for this enzymatic reaction are not well understood. In humans, the clinically useful hydantoins, 5-ethyl-5-phenylhydantoin [3] and phenytoin (5,5-diphenylhydantoin) [4], form glucuronide conjugates via conjugation with the imide nitrogen. Based on the structural similarity of the hydantoin heterocycle to the barbiturate heterocycle, it would be anticipated that *N*-glucuronidation would occur for the structural homologues, phenobarbital (5-ethyl-5-phenylbarbituric acid) or mephobarbital (5-ethyl-1-methyl-5-phenylbarbituric acid). Although numerous studies have been done on the urinary excretion of phenobarbital and mephobarbital in a variety of species, the *N*-glucuronide of these barbiturates has not been identified. Since the coupling of D-glucuronic acid to N-1 or N-3 of phenobarbital confers asymmetry at C-5 of the barbiturate ring and generates two epimers, the synthetic methodology was developed using a barbiturate that does not form diastereomers upon conjugation with glucuronic acid. This report describes the use of a modification of the Hilbert–Johnson reaction using trimethylsilyl trifluoromethanesulfonate ($CF_3SO_3SiMe_3$) [5] for preparing the methyl *N*-glucopyranosyluronide derivatives of the compounds: barbital (**1**), metharbital (**2**), phenobarbital (**3**), and mephobarbital (**4**).



1: R_1 = -Et, R_2 = -Et, R_3 = -H
2: R_1 = -Et, R_2 = -Et, R_3 = -Me
3: R_1 = -Et, R_2 = -Ph, R_3 = -H
4: R_1 = -Et, R_2 = -Ph, R_3 = -Me

2. Results and discussion

The synthetic approach that was successful for the synthesis of the barbiturate *N*-glucuronides is shown in Scheme 1 and is comparable to that developed for the synthesis of the barbiturate *N*-glucosides [6]. The condensation of persilylated barbital with methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate with stannic chloride as the Lewis acid catalyst in either dichloromethane or acetonitrile was unsuccessful [7]. The reaction appeared to go in very low yield to a product or products that were most likely O-coupled glucuronides, since barbital was released when the product was treated with acid. Using $CF_3SO_3SiMe_3$ as the Lewis acid catalyst [5], a good yield of the desired barbital methyl tri-*O*-acetyl-β-D-glucuronate (**5**) was obtained. The synthesis of the



Scheme 1. Synthesis of N^1-glucopyranosyluronide barbiturates.

diastereomeric mixture of phenobarbital methyl tri-*O*-acetyl-β-D-glucuronate (**6a,b**) was also achieved using $CF_3SO_3SiMe_3$, although the time required for the reaction was longer and the yield was lower. The methyl tri-*O*-acetyl-β-D-glucopyranuronates of metharbital (**7**) and mephobarbital (**8a,b**) were readily prepared by reaction of **5** and **6a,b** with diazomethane. The barbiturate *N*-glucuronides were obtained by hydrolysis of the methyl tri-*O*-acetyl-β-D-glucopyranosyluronate barbiturate under strongly acidic conditions. Selective hydrolysis to the methylglucopyranosyluronate barbiturates (**9** and **10a,b**) using concentrated HCl in methanol gave useful intermediates for the chromatographic separation of the phenobarbital *N*-glucuronides. The inability to hydrolyze the *N*-glucopyranosyluronide imide conjugates under strongly acidic conditions is consistent with previous reports of acid stability of the *N*-glucopyranosyl imides [6,8–10].

The spectroscopic data for *N*-glucopyranosyluronide imides was consistent with that observed for the *N*-glucopyranosyl imides. In the ^{1}H NMR spectra, the chemical shift of the anomeric proton ranges from 5.7 to 6.0 ppm. The anomeric proton for the N^1-glucopyranosyluronide barbiturates and N^1-glucopyranosyluronate-N^3-methyl barbiturates appeared as two doublets in a 3:1 ratio due to the hindered rotation around the *N*-glycosylic bond [11]. The coupling constant ($J_{1,2}$) for the anomeric proton was 9–10 Hz, consistent with the assignment of the conjugates as β-glycosides [12]. Characteristic carbon absorbances observed for an N-linked glucuronide in the ^{13}C NMR spectra were for the anomeric carbon (82–86 ppm), urea carbonyl (149–151 ppm), and the malonate carbonyls (171–175 ppm). Both the ^{1}H and ^{13}C NMR spectra, as well as the stability to hydrolysis of the conjugate under strongly acidic conditions, were useful in verifying

that *N*-coupling versus *O*-coupling had occurred when characterizing imide *N*-glycosylic compounds.

After separation of **10a,b** by semipreparative HPLC, the absolute configuration of **10a** and **10b** was determined by converting them to methyl *N*-glucopyranosyluronides of mephobarbital, **12a** and **12b**, respectively, followed by the oxidative removal of the glucuronide to give optically active mephobarbital [13]. The specific rotations obtained for the optically active mephobarbital (**4**) samples were lower than the literature values for 'pure' enantiomer since the *N*-glucopyranosyluronide barbiturate used in the degradation studies were contaminated (~ 10%) with the other epimer. The purity of each glucuronide diastereomer was determined by HPLC analysis, and the only allowable contaminant was the opposite diastereomer. The mephobarbital (**4**) obtained from **10a** and **12a** had a negative rotation, indicating it was (*R*)-mephobarbital [14]. The mephobarbital obtained from **10b** and **12b** had a positive rotation, consistent with an absolute configuration of (*S*)-mephobarbital. Therefore, the absolute configuration at C-5 of **10a** and **12a** was (*S*), and the absolute configuration at C-5 for **10b** and **12b** was (*R*). The absolute configurations of the diastereomers of **14a,b** and **16a,b** were determined by converting the purified **10a,b** and **12a,b**, respectively, to these compounds through chemical transformations that did not affect the chirality at C-5 of the barbiturate ring. Therefore, the absolute configuration at C-5 of the barbiturate ring of phenobarbital and mephobarbital *N*-glucopyranosyluronides and derivatives has been established.

Using the spectral data obtained in this study, it appears that the structure of a barbiturate metabolite previously identified as *N*-glucopyranosyluronide bucolome (**17a**)

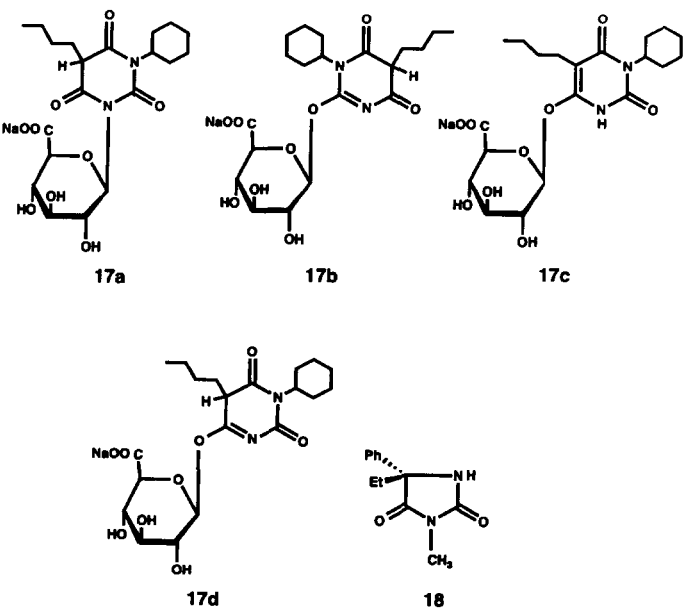


Fig. 1. (*S*)-Mephenytoin and possible structures of bucolome glucuronide conjugate previously characterized.

may have been incorrectly assigned [15] (Fig. 1). The characterization of the metabolite as an *N*-linked barbiturate was based primarily on the ^{13}C NMR carbonyl signals plus the absence of any prior reports of imides linked via the urea or malonyl oxygens. Analysis of the original spectroscopic data indicates that the structure depicted for **17d** is most likely the correct structure, i.e., an *O*-linked glucuronide was formed instead of a *N*-linked glucuronide. In the ^{1}H NMR spectrum, the doublet associated with the anomeric proton occurs at 5.1 ppm. In the ^{13}C NMR spectrum, the anomeric carbon absorbance occurs at 100.5 ppm. These chemical shifts are not consistent with a *N*-linked glucuronide, but rather with an O-linked glucuronide [16,17]. In addition, the observation that the bucolome glucuronide was hydrolyzed under acidic conditions supports an *O*-linkage. Conjugation of the C-4 oxygen as the imino tautomer instead of the enol tautomer (**17c**) is based on the original author's verification of the existence of a hydrogen atom at the 5-position of the barbiturate ring. The absorbance of the C-4 carbonyl at 157.5 ppm, and the urea carbonyl at 152.1 ppm is more consistent with the imino tautomer (**17d**) instead of an aminoenol tautomer (**17c**) or an amide carbonyl (**17b**). Based on this revised structure, no *N*-glucopyranosyluronide barbiturate has yet been reported as a metabolite of barbiturates in mammals. The identification of the bucolome metabolite as an *O*-glucuronide provides a precedent for *O*-linked glucuronide(s) as possible metabolites for imide drugs. (*S*)-Mephenytoin (**18**) has been frequently used in the genetic phenotyping of humans to characterize extensive and poor metabolizers of mephenytoin and structurally related imide drugs [18]. An unidentified metabolite of mephenytoin was reported that could influence the phenotyping analysis. The metabolite was a highly water-soluble compound which, on treatment with acid, released mephenytoin. It was proposed to be a conjugate of the hydantoin ring. Based on the revised structure of the bucolome conjugate and the reported acid instability, it is most likely an *O*-linked conjugate of the imino tautomer of the C-2 carbonyl of mephenytoin.

In conclusion, the general synthetic approach of using $CF_3SO_3SiMe_3$ as the catalyst for condensing silylated barbiturates with methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate was very effective for preparing the N^1-glucopyranosyluronide barbiturates. The chemical reactivity and spectroscopic data for this series of compounds will facilitate the isolation and identification of these and related imide *N*-glycosylic conjugates that may be present in metabolism studies. The ^{13}C NMR chemical shifts are reported in Table 1.

3. Experimental

Materials.—Acetonitrile (MeCN), ethyl acetate (EtOAc), and methanol (MeOH) used in chromatographic analysis were Mallinckrodt HPLC grade (Scientific Products, McGaw Park, IL). All other solvents were reagent grade unless otherwise noted. Dichloroethane was dried over CaH_2 and distilled from P_2O_5 under N_2. Silica Gel GHLF plates (250 μm, 2.5 × 10 cm) were used for routine thin-layer chromatography (TLC, Analtech Inc., Newark, DE). Phenobarbital, USP, was purchased from Merck

Table 1
^{13}C NMR chemical shifts of 5,5-disubstituted N^1-(β-D-glucopyranosyluronate)barbiturates

Atom	Compound number															
	5	**6a,b**	**7**	**8a,b**	**9**	**10a**	**10b**	**11**	**12a**	**12b**	**13**	**14a**	**14b**	**15**	**16a**	**16b**
2,4,6-(1*H*,3*H*,5*H*)-Primidinetrione residue																
C-2	146.9	146.6	147.5	147.8	149.9	149.7	149.7	149.8	150.5	150.4	149.8	149.3	149.3	150.4	150.1	150.2
(C-2)					151.2	151.0	151.0	151.4		152.5	150.7	150.5	150.5		151.6	151.6
C-4	170.5	170.6	170.7	169.8	174.9	173.6	173.6	173.1	171.3	172.1	174.1	173.4	173.1	173.6	172.9	172.5
C-6	170.5	169.9	171.4	169.9	174.3	172.9	172.9	173.4	171.9	171.1	174.7	172.7	172.7	173.9	172.5	171.7
(C-6)						172.7	172.5						172.3			
C-5	58.2	61.9	58.1	61.9	59.1	61.6	62.0	58.6	62.9	63.1	59.0	61.2	61.6	59.1	61.7	62.0
(C-5)	59.0	63.1			59.8	62.7	62.7	59.1	64.0	64.0	59.7	62.3	62.4	59.6	62.7	62.7
N-Me			28.2	28.7				28.0	29.0	29.0				28.6	29.0	28.8
(N-Me)				29.1				28.8	29.9	29.8				29.4	29.7	29.7
Substituents at C-5 of pyrimidine ring																
C-7	31.8	30.5	32.0	31.0	32.2	30.6	30.0	32.0	31.5	31.6	32.0	30.3	29.6	33.6	31.1	30.9
					33.4			31.3		31.2	31.6			32.5		
(C-7)	32.3	30.0	32.8	30.6	31.7	29.9	30.6	33.0			33.0	29.5	30.1	33.4	30.3	30.5
					33.2			32.9			33.2			31.9		
C-8	9.1	10.1	9.1	10.2	9.0	9.1	9.4	8.5	10.2	10.3	8.9	8.7	9.0	9.1	9.0	9.3
(C-8)	9.4	9.8	9.3	9.1	9.2	9.2	9.3	8.3		10.4	9.1	8.8	8.8	8.9	9.2	
ipso		137.0		137.7		137.4	137.4		139.7	139.6		136.9	137.0		137.6	136.2
o		125.9		125.8		127.0	126.7		127.3	127.4		126.8	126.3		127.0	126.6
(*o*)		126.0				126.9	127.1			127.3		126.5	126.8		126.7	
m		129.1		129.1		129.8	129.9		130.1	130.0		129.5	129.5		129.8	129.7
(*m*)		128.9		128.9											129.3	
p		128.5		128.4		129.4	129.6		129.5	129.5		129.2	129.2		129.4	129.3
(*p*)		128.4		128.3												

Sugar residue																
C-1′	79.1	79.7	79.2	79.8	82.1	82.2	82.6	81.7	83.9	84.4	82.0	81.7	82.1	82.3	82.3	82.3
(C-1′)	80.3	80.7	81.1	81.5	82.9	82.8	83.1	83.2	85.4	85.9	82.8	82.4	82.6	83.8	83.6	83.6
C-2′	68.9	68.1	68.7	68.2	69.0	68.9	69.3	68.3	69.9	70.6	71.5	68.5	68.8	71.4	68.8	69.3
(C-2′)	67.8	69.2		69.4	68.8	68.5	69.2			71.2	71.3	68.1		71.3	68.5	
C-3′	72.7	72.7	72.7	72.7	76.8	76.7	76.7	76.1	78.6	78.8	76.9	76.5	76.5	76.9	76.7	76.7
(C-3′)	73.0	72.4	73.0	72.3										76.8		
C-4′	68.3	68.7	68.4	68.7	71.5	71.4	71.4	70.8	72.8	72.8	68.9	71.0	71.0	68.9	71.3	71.3
					71.4						68.7				71.2	71.0
C-5′	74.8	74.8	74.8	74.8	77.7	77.6	77.6	77.1	79.5	79.4	77.7	77.3	77.4	77.8	77.5	77.0
(C-5′)	74.3	74.0	74.4	74.1	77.4	77.3		76.7		79.3	77.6			77.4		
C-6′	166.3	166.4	166.3	166.3	170.9	170.9	170.8	170.2	170.5	170.6	173.1	171.9	172.0	172.1	172.0	170.9
OMe	52.8	52.8	52.8	52.8	53.7	53.5	53.6	53.0	52.9	52.9						
Ac (C = O)	169.2	168.4	169.1	168.8												
	169.3	169.1	169.5	169.0												
	170.0	169.2	169.8	169.1												
Ac (Me)	20.3	20.3	20.3	20.3												
	20.4	20.4	20.4	20.4												

(Rahway, NJ). Barbital, USP, was purchased from Mallinckrodt Chemicals (St. Louis, MO).

General methods.—Melting points were determined on a Thomas–Hoover capillary melting-point apparatus (Philadelphia, PA) and are uncorrected. Infrared spectra were recorded on a Nicolet 5ZDX FT-IR spectrometer (Madison, WI). Elemental analyses were performed by Atlantic Microlab, Inc. (Norcoss, GA), and values are within 0.4% of theory. Optical rotations were determined on a Perkin–Elmer Model 141 polarimeter (Norwalk, CT) using a 1.0-mL ORD cell with a path length of 10 cm. Condensation reactions were conducted under N_2. ^1H NMR and ^{13}C NMR spectra were recorded on a General Electric QE-300 NMR spectrometer (Fremont, CA). Chemical shifts in ppm are relative to tetramethylsilane (Me_4Si) in deuterochloroform ($CDCl_3$), sodium 3-(trimethylsilyl)propionate in deuterium oxide (D_2O) for ^1H NMR spectra, and acetonitrile (MeCN) in D_2O for ^{13}C NMR spectra.

Chromatography.—Chromatographic purification of compounds and resolution of the diastereomers (wherever applicable) was done on a C_{18} reversed-phase column (Econosil, 250 × 10 mm i.d., particle size 10 μm, Alltech) with a pellicular ODS guard column (20 × 2 mm i.d., particle size 37–53 μm, Whatman). An Altex 110A pump (Berkeley, CA), a Rheodyne model 7125 syringe loading injector (Cotati, CA, 100 μL loop), and a Beckmann 166 detector module were used. Fractions were collected on a programmable Foxy 200 ISCO fraction collector.

HPLC analyses were performed on a Beckmann System Gold liquid chromatograph (San Ramon, CA) consisting of a Beckmann 110B solvent delivery module, a Beckmann 406 analogue interface module, a programmable Beckmann model 507 autosampler, and a Beckmann 167 diode array detector module. LC separation was achieved on a C_{18} reversed-phase column (Econosphere, 250 × 4.6 mm i.d., particle size 5 μm, Alltech) with a pellicular ODS guard column (20 × 2 mm i.d., particle size 37–53 μm, Whatman). A flow rate of 1.4 mL/min was used and the column effluent was monitored at 220 nm.

*5,5-Diethyl-1-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione* (**5**).—Methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosyuronate was synthesized by the procedure of Bollenbach et al. [19] In a minor modification of the procedure of Vorbrüggen and Krolikiewicz [20], persilylated barbital (6.75 g, 20.6 mmol) [6] was added to a solution of methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosyuronate (6.4 g, 17.0 mmol) in freshly distilled dichloroethane (80 mL). $CF_3SO_3SiMe_3$ (4.3 mL, 22.1 mmol) was added as catalyst to the ice-cooled reaction flask, and the mixture was stirred and allowed to slowly warm to room temperature. The reaction was monitored by TLC at 1, 3, 12, and 18 h (ether, R_f for **1** 0.74, R_f for **5** 0.54). After 18 h, the reaction appeared complete, 80 mL of CH_2Cl_2 was carefully added, and the mixture was poured into a beaker containing 100 mL of water and crushed ice and stirred vigorously for 10 min. The emulsion was filtered over a layer of sand–Celite, and the organic phase was separated and dried (Na_2SO_4, anhyd). The CH_2Cl_2 layer was filtered and evaporated under reduced pressure (72 torr) at 35°C to give a sticky yellow material which was crystallized from MeOH and water to give **5** (5.7 g, 11.4 mmol, 67%); mp 170–171°C; ^1H NMR ($CDCl_3$): δ 0.75 (t, *J* 8 Hz, 3 H, H-8), 0.85 (t, *J* 8 Hz, 3 H, H-8), 1.90–2.10 (m, 13 H, 3 $COCH_3$ and 2 H-7), 3.73 (s, 3

H, OCH_3), 4.18 (br d, 1 H, H-5′), 5.33–5.42 (m, 2 H, H-3′,4′), 5.98 (d, *J* 10 Hz, 1 H, H-1′), 6.05–6.15 (br t, 1 H, H-2′), 8.73 (s, 1 H, NH), 8.87 (minor s, NH); IR (KBr): 1757, 1743, 1721, 1707, 1250, 1222 cm^{-1}. Anal. Calcd for $C_{21}H_{28}N_2O_{12}$: C, 50.38; H, 5.64; N, 5.60. Found: C, 50.48; H, 5.67; N, 5.59.

*5-Ethyl-1-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-5-phenyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione* (**6a,b**).—Persilylated phenobarbital (9.54 g, 25.3 mmol), prepared as previously reported [6], was added to a solution of methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosyluronate (10.5 g, 27.9 mmol) followed by the addition of $CF_3SO_3SiMe_3$ (6.5 mL, 34.0 mmol). After the reaction appeared to be completed (TLC: ether, R_f for **3** 0.74, R_f for **6a,b** 0.62), the work up was carried out in a manner comparable to **5** to give a sticky yellow residue (13.0 g). The residue was treated with anhydrous ether to precipitate a mixture of diastereomers of phenobarbital methyl tri-*O*-acetyl-β-D-glucuronate, **6a,b** (4.9 g, 9.0 mmol, 36%). The precipitate was recrystallized from MeOH; mp 229–230°C; ^1H NMR ($CDCl_3$): δ 0.92–1.05 (m, 3 H, H-8), 1.92–2.12 (m, 9 H, 3 $COCH_3$), 2.30–2.45 (m, 1 H, H-7), 2.45–2.60 (m, 1 H, H-7), 3.73 (s, 3 H, OCH_3), 3.77 (minor s, OCH_3), 4.18 (d, *J* 13 Hz, 1 H, H-5′), 5.24–5.48 (m, 2 H, H-3′,4′), 5.86 (d, *J* 10 Hz, 1 H, H-1′), 5.93 (minor d, *J* 10 Hz, H-1′), 6.05 (t, *J* 10 Hz, 1 H, H-2′), 6.26 (minor t, *J* 10 Hz, H-2′), 7.27–7.48 (m, 5 H, ArH), 8.68 (s, 1 H, NH), 8.87 (minor s, NH); IR (KBr): 1764, 1757, 1728, 1707, 1250, 1229 cm^{-1}. Anal. Calcd for $C_{25}H_{28}N_2O_{12}$: C, 54.72; H, 5.15; N, 5.11. Found: C, 54.65; H, 5.18; N, 5.08.

*5,5-Diethyl-3-methyl-1-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione* (**7**).—Compound **5** (1.7 g, 3.4 mmol) was dissolved in EtOH (40 mL), and a freshly prepared ethereal alcoholic solution of diazomethane [21] (25 mL) was added. After bubbling had subsided, the mixture was stirred at room temperature for 20 min. The reaction was stopped by dropwise addition of dil AcOH until the solution was clear. The solvent was removed under reduced pressure to give a syrup. The residue was crystallized from 2-propanol–diisopropyl ether to give **7** (648 mg, 1.3 mmol, 38%); mp 124–126°C; ^1H NMR ($CDCl_3$): δ 0.72 (t, *J* 8 Hz, 3 H, H-8), 0.82 (t, *J* 8 Hz, 3 H, H-8), 1.92–2.10 (m, 13 H, 3 $COCH_3$ and 2 H-7), 3.33 (s, 3 H, NCH_3), 3.73 (br s, 3 H, OCH_3), 4.17 (br d, 1 H, H-5′), 5.30–5.40 (m, 2 H, H-3′,4′), 5.95–6.12 (m, 1 H, H-1′), 6.12–6.30 (m, 1 H, H-2′); IR (KBr): 1757, 1693, 1236, 1215 cm^{-1}. Anal. Calcd for $C_{22}H_{30}N_2O_{12}$: C, 51.34; H, 5.88; N, 5.45. Found: C, 51.29; H, 5.88; N, 5.42.

*5-Ethyl-3-methyl-1-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-5-phenyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione* (**8a,b**).—Diastereomers **6a,b** (2.0 g, 3.6 mmol) were dissolved in 50 mL of MeOH and treated with diazomethane as described for **7**. The solvent was removed under reduced pressure and the oily residue was crystallized from MeOH to give a mixture of diastereomers (**8a,b**, 1.4 g, 2.5 mmol, 69%); mp 155–157°C; ^1H NMR ($CDCl_3$): δ 0.88–1.02 (m, 3 H, H-8), 1.96–2.08 (m, 9 H, 3 $COCH_3$), 2.27–2.43 (m, 1 H, H-7), 2.43–2.60 (m, 1 H, H-7), 3.28 (minor s, NCH_3), 3.42 (s, 3 H, NCH_3), 3.75 (s, 3 H, OCH_3), 4.17 (d, *J* 10 Hz, 1 H, H-5′), 5.23–5.41 (m, 2 H, H-3′,4′), 5.91 (d, *J* 10 Hz, 1 H, H-1′), 6.07 (t, *J* 10 Hz, 1 H, H-2′), 6.25 (minor t, *J* 10 Hz, 1 H, H-2′), 7.17–7.42 (m, 5 H, ArH); IR (KBr): 1757, 1699, 1222, 1215 cm^{-1}. Anal. Calcd for $C_{26}H_{30}N_2O_{12}$: C, 55.49; H, 5.38; N, 4.98. Found: C, 55.42; H, 5.41; N, 4.95.

5,5-Diethyl-1-(methyl β-D-glucopyranosyluronate)-2,4,6-(1H,3H,5H)-pyrimidinetrione (**9**).—Compound **5** (906 mg, 1.8 mmol) was dissolved in MeOH (15 mL), and concd HCl (0.5 mL) was added. The mixture was heated at 59°C for 17 h, and the MeOH was removed under reduced pressure (77 mm Hg). The residue was dissolved in water (15 mL), and the solution was extracted with EtOAc (2 × 10 mL). The EtOAc fraction was dried (Na_2SO_4, anhyd), and evaporated under reduced pressure to give a white solid. The solid was recrystallized from EtOH to give **9** (366 mg, 0.98 mmol, 54%); mp 114–115°C; ^{1}H NMR (D_2O): δ 0.79–0.94 (m, 6 H, 2 H-8), 2.02 (br q, *J* 7 Hz, 4 H, 2 H-7), 3.61–3.80 (m, 2 H, H-3′,4′), 3.83 (s, 3 H, OCH_3), 4.17–4.29 (m, 1 H, H-5′), 4.42–4.58 (m, 1 H, H-2′), 5.78 (minor d, *J* 9 Hz, 1 H, H-1′), 5.87 (d, *J* 9 Hz, 1 H, H-1′); IR (KBr): 1728, 1707, 1250 cm^{-1}. Anal. Calcd for $C_{15}H_{22}N_2O_9 \cdot H_2O$: C, 45.92; H, 6.17; N, 7.14. Found: C, 45.55; H, 6.01; N, 6.60.

5-Ethyl-1-(methyl β-D-glucopyranosyluronate)-5-phenyl-2,4,6-(1H,3H,5H)-pyrimidinetrione (**10a,b**).—A mixture of diastereomers **6a,b** (5.0 g, 9.1 mmol) was dissolved in MeOH (55 mL), concd HCl (10 mL) was added, and the mixture was stirred at room temperature. The reaction was monitored by TLC (ether, R_f for **3** 0.74, R_f for **6a,b** 0.62, R_f for **10a,b** 0.15), which indicated incomplete hydrolysis after 17 h. The mixture was warmed to 50°C for 5 h, then cooled. The solvent was removed under reduced pressure to give a yellow oil. The oil was dissolved in 15 mL of water. The aqueous solution was extracted with CH_2Cl_2 (2 × 20 mL), and then with EtOAc (3 × 50 mL). The combined EtOAc extracts were dried (Na_2SO_4, anhyd), filtered, and evaporated to dryness under reduced pressure to give a mixture of diastereomers (**10a,b**, 2.57 g, 6.1 mmol, 67%).

The diastereomers **10a,b** were separated by semipreparative HPLC using a mobile phase of 20:80 MeCN–water at a flow rate of 4 mL/min. The effluent was monitored at 225 nm. Each injection contained ca. 60 mg of a crude mixture of the diastereomers **10a,b** dissolved in 100 μL of MeOH. Three fractions of effluent were collected: (5*S*)-**10a** from 19 to 23 min; (5*S*)-**10a** and (5*R*)-**10b** mixture from 23 to 27 min; and (5*R*)-**10b** from 27 to 31 min. The fractions were stored frozen until evaporated. The mobile phase was removed under reduced pressure, and the residue was dried at 56°C in vacuo.

Data for (5*S*)-**10a**: mp 84–85°C; ^{1}H NMR (D_2O): δ 0.83 (minor t, *J* 7 Hz, H-8), 0.95 (t, *J* 7 Hz, 3 H, H-8), 2.30–2.42 (minor m, 2 H, H-7), 2.42–2.58 (m, 2 H, H-7), 3.47–3.65 (m, 2 H, H-3′,4′), 3.75 (s, 3 H, OCH_3), 3.78 (minor s, OCH_3), 4.12–4.23 (m, 1 H, H-5′), 4.28 (minor t, *J* 10 Hz, 1 H, H-2′), 4.42 (t, *J* 10 Hz, 1 H, H-2′), 5.71 (minor d, *J* 10 Hz, 1 H, H-1′), 5.79 (d, *J* 10 Hz, 1 H, H-1′), 7.17–7.45 (br m, 5 H, ArH); IR (KBr): 1721, 1703, 1251 cm^{-1}. HPLC analysis with a mobile phase of 20:80 MeCN–water, t_R of (5*S*)-**10a** 15.4 min. Anal. Calcd for $C_{19}H_{22}N_2O_9$: C, 54.03; H, 5.21; N, 6.64. Found: C, 54.04; H, 5.23; N, 6.56.

Compound (5*S*)-**10a** (40 mg, 94 μmol) was treated with diazomethane as described for **7**, to form (5*S*)-**12a**. The reaction was stopped by addition of 3 drops of dil AcOH. The solvent was evaporated under a stream of N_2 and then in vacuo leaving a clear oil (50 mg). A solution of $Na_2Cr_2O_7$ (418 mg, 1.5 mmol) in 5.5 mL of 3.25 M H_2SO_4 was added to the oil, vortexed, and maintained at 93°C for 45 min. TLC (ether) indicated completion of the reaction based on formation of mephobarbital. The mixture was

cooled to room temperature, diluted with 5 mL of H_2O, and extracted with CH_2Cl_2 (5 × 10 mL). The combined organic extracts were dried (Na_2SO_4, anhyd), filtered, and evaporated under reduced pressure to give an oil. The oil was dissolved in 2 mL abs EtOH and transferred to a screw-cap test tube. The volume was reduced under a stream of N_2 to ca. 0.5 mL. A drop of water was added to the solution, and the mixture was left to crystallize in the refrigerator. The crystals were collected by centrifugation of the tube and decanting off the mother liquor. The white solid was dried in vacuo to give (*R*)-**4** (8 mg, 32 μmol, 34%); mp 100–101°C (lit. [14] mp 101°C); $[\alpha]_D^{21}$ −8.3° (*c* 0.4, abs EtOH) [lit. [14] $[\alpha]_D^{20}$ −9.0° (*c* 2.8, abs. EtOH)].

Data for (5*R*)-**10b**: mp 83–85°C; ^{1}H NMR (D_2O): δ 0.85 (t, *J* 7 Hz, 3 H, H-8), 0.93 (minor t, *J* 7 Hz, 3 H, H-8), 2.30–2.45 (m, 2 H, H-7), 3.45–3.65 (m, 2 H, H-3′,4′), 3.67 (minor s, 3 H, OCH_3), 3.74 (s, 3 H, OCH_3), 4.09 (minor d, *J* 10 Hz, 1 H, H-5′), 4.16 (d, *J* 10 Hz, 1 H, H-5′), 4.35–4.49 (m, 1 H, H-2′), 5.67 (minor d, *J* 10 Hz, H-1′), 5.76 (d, *J* 10 Hz, 1 H, H-1′), 7.21–7.48 (br m, 5 H, ArH); IR (KBr): 1721, 1703, 1251 cm^{-1}. Analysis by HPLC was conducted as described for (5*S*)-**10a**. The ratio of (5*R*)-**10b**:(5*S*)-**10a** = 94:6; t_R of (5*R*)-**10b** 17.8 min. Anal. Calcd for $C_{19}H_{22}N_2O_9$: C, 54.03; H, 5.21; N, 6.64. Found: C, 54.10; H, 5.11; N, 6.57.

Compound (5*R*)-**10b** (containing 10% (5*S*)-**10a**, 31 mg, 73 μmol) was treated with diazomethane as described for **7** to form (5*R*)-**12b**. Oxidation with a solution of $Na_2Cr_2O_7$ (278 mg, 1.06 mmol) in 5.0 mL of 3.25 M H_2SO_4 as described above for (5*S*)-**10a** gave (5*S*)-**4** (2 mg, 8 μmol, 11%); mp 100–101°C (lit. [14] 101°C); $[\alpha]_D^{21}$ +8.5 (*c* 0.2, abs. EtOH) [lit. [14] $[\alpha]_D^{20}$ +9.3° (*c* 3.1, abs EtOH)].

*5,5-Diethyl-3-methyl-1-(methyl β-*D*-glucopyranosyluronate)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione* (**11**).—Compound **7** (355 mg, 0.69 mmol) was dissolved in MeOH (4 mL), and concd HCl (0.25 mL) was added. The mixture was heated at 60°C for 18 h. The MeOH was removed under reduced pressure to give a viscous syrup that was dissolved in water (10 mL), and extracted with EtOAc (4 × 10 mL). The combined EtOAc fractions were dried (Na_2SO_4, anhyd) and concentrated under reduced pressure to give a white solid that was recrystallized from abs EtOH to give **11** (72 mg, 0.19 mmol, 27%); mp 101–103°C; ^{1}H NMR (D_2O): δ 0.75–0.89 (m, 6 H, 2 H-8), 1.94–2.10 (m, 4 H, 2 H-7), 3.31 (s, 3 H, NCH_3), 3.33 (minor s, NCH_3), 3.55–3.77 (m, 2 H, H-3′,4′), 3.83 (s, 3 H, OCH_3), 4.23 (minor d, *J* 10 Hz, H-5′), 4.26 (d, *J* 10 Hz, 1 H, H-5′), 4.40–4.54 (m, 1 H, H-2′), 5.79 (minor d, *J* 10 Hz, 1 H, H-1′), 5.88 (d, *J* 10 Hz, 1 H, H-1′); IR (KBr): 1728, 1707, 1251 cm^{-1}. Anal. Calcd for $C_{16}H_{24}N_2O_9$: C, 49.48; H, 6.19; N, 7.22. Found: C, 49.32; H, 6.10; N, 7.34.

*5-Ethyl-3-methyl-1-(methyl β-*D*-glucopyranosyluronate)-5-phenyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione* (**12a,b**).—Compounds (5*S*)-**10a** and (5*R*)-**10b** (each 1.0 g, 1.8 mmol) were each separately dissolved in MeOH (10 mL), and concd HCl (2.0 mL) was added. In each case the mixture was heated at reflux for 3 h. The solvent was removed under reduced pressure to give a yellow oil that was dissolved in water (10 mL). The aqueous solution was extracted with CH_2Cl_2 (2 × 10 mL), and then with EtOAc (3 × 20 mL). The combined EtOAc extracts for each were dried (Na_2SO_4, anhyd), and evaporated to a syrup that was crystallized from MeOH to give **12a** and **12b**, respectively (each 110 mg, 0.25 mmol, 14%).

Data for **12a**: mp 125–127°C; ^{1}H NMR ($CDCl_3$): δ 0.93 (t, *J* 7 Hz, 3 H, H-8),

2.35–2.52 (m, 2 H, H-7), 3.28 (s, 3 H, NCH_3), 3.30 (s, 3 H, NCH_3), 3.40–3.68 (m, 2 H, H-3′,4′), 3.75 (s, 3 H, OCH_3), 3.91–3.98 (m, 1 H, H-5′), 4.40–4.55 (m, 1 H, H-2′), 5.66 (minor d, *J* 10 Hz, 1 H, H-1′), 5.80 (d, *J* 10 Hz, 1 H, H-1′), 7.20–7.51 (br m, 5 H, ArH); IR (KBr): 1728, 1707, 1250, 1229 cm^{-1}.

Data for **12b**: mp 125–127°C; ^1H NMR ($CDCl_3$):δ 0.85–0.96 (m, 3 H, H-8), 2.22–2.50 (m, 2 H, H-7), 3.23 (s, 3 H, NCH_3), 3.28 (minor s, 3 H, NCH_3), 3.30–3.68 (m, 2 H, H-3′,4′), 3.72 (s, 3 H, OCH_3), 3.76 (minor s, 3 H, OCH_3), 3.87–4.02 (m, 1 H, H-5′), 4.43–4.63 (m, 1 H, H-2′), 5.66 (minor d, *J* 10 Hz, 1 H, H-1′), 5.73 (d, *J* 10 Hz, 1 H, H-1′), 7.21–7.51 (br m, 5 H, ArH); IR (KBr): 1728, 1707, 1250, 1229 cm^{-1}. Anal. Calcd for $C_{20}H_{24}N_2O_9$: C, 55.05; H, 5.50; N, 6.42. Found: C, 55.20; H, 5.56; N, 6.22.

5,5-Diethyl-1-(β-D-glucopyranosyluronate)-2,4,6-(1H,3H,5H)-pyrimidinetrione (**13**).—Compound **9** (100 mg, 267 μmol) was treated with 10 mL of aq 10% HCl for 48 h at room temperature. After 48 h, the reaction appeared complete by TLC (ether, R_f 0.74 for **1**, R_f 0.62 for **9**, R_f 0.00 for **13**), and on HPLC analysis with 15:85 MeCN–0.02 M phosphoric acid, t_R of **13** was 9.0 min. The mixture was concentrated and extracted with 3 × 10 mL of EtOAc. The EtOAc fractions were pooled together and dried over anhyd Na_2SO_4, followed by evaporation to dryness to yield crystalline **13** (80 mg, 222 μmol, 83%); mp 142–144°C; ^1H NMR (D_2O): δ 0.75–0.88 (m, 6 H, H-8), 1.90–2.05 (m, 4 H, H-7), 3.51–3.60 (m, 1 H, H-3′), 3.65 (dd, $J = J = 10$ Hz, 1 H, H-4′), 4.03–4.12 (m, 1 H, H-5′), 4.32–4.49 (m, 1 H, H-2′), 5.70 (minor d, *J* 10 Hz, 1 H, H-1′), 5.77 (d, *J* 10 Hz, 1 H, H-1′); IR (KBr): 1717, 1701 cm^{-1}. Anal. Calcd for $C_{14}H_{20}N_2O_9 \cdot 2.5H_2O$): C, 41.48; H, 6.22; N, 6.91. Found: C, 41.31; H, 5.86; N, 6.99.

5-Ethyl-1-(β-D-glucopyranosyluronate)-5-phenyl-2,4,6-(1H,3H,5H)-pyrimidinetrione (**14a,b**).—Each of the diastereomers **10a** and **10b** (1.93 g, 3.52 mmol) was individually suspended in 100 mL of aq 10% HCl, and the mixture was stirred at room temperature. After 48 h, the reaction appeared to be complete based on TLC (ether, R_f for **3** 0.74, R_f for **10a,b** 0.62, R_f for **14a,b** 0.00), and on HPLC analysis with 15:85 MeCN–0.02 M phosphoric acid, t_R of **14a** and **14b** 13 and 15 min, respectively. The mixture was cooled, concentrated under reduced pressure to a volume of 8 mL, and extracted with CH_2Cl_2 (2 × 20 mL), ether (2 × 20 mL), and EtOAc (4 × 20 mL). The EtOAc fractions were pooled, dried (Na_2SO_4, anhyd), filtered, and evaporated to dryness under reduced pressure to give a yellow oil (770 mg). The oil was triturated with ether (16 mL) and left to stand in a hood overnight. The diastereomers **14a** and **14b** precipitated (50 mg, 0.12 mmol, 3.4%).

Data for **14a**: mp 150–152°C; ^1H NMR (D_2O): δ 0.84–0.93 (m, 3 H, H-8), 2.38–2.47 (m, 2 H, H-7), 3.45–3.68 (m, 2 H, H-3′,4′), 4.02–4.12 (m, 1 H, H-5′), 4.34–4.43 (m, 1 H, H-2′), 5.67 (minor d, *J* 10 Hz, 1 H, H-1′), 5.74 (d, *J* 10 Hz, 1 H, H-1′), 7.42 (br d, 5 H, ArH); IR (KBr): 1721, 1703 cm^{-1}.

Data for **14b**: mp 150–152°C; ^1H NMR (D_2O): δ 0.83 (t, *J* 7 Hz, 3 H, H-8), 0.90 (minor t, *J* 7 Hz, 3 H, H-8), 2.4–2.6 (m, 2 H, H-7), 3.42–3.51 (m, 1 H, H-3′), 3.53–3.65 (m, 1 H, H-4′), 3.98–4.09 (m, 1 H, H-5′), 4.29–4.43 (m,1 H, H-2′), 5.60–5.70 (m, 1 H, H-1′), 7.30–7.43 (br m, 5 H, ArH); IR (KBr): 1721, 1703 cm^{-1}. Anal. Calcd for **14a,b** $C_{18}H_{20}N_2O_9 \cdot H_2O$): C, 50.71; H, 5.20; N, 6.57. Found: C, 51.00; H, 5.35; N, 6.35.

5,5-Diethyl-1-(β-D-glucopyranosyluronate)-3-methyl-2,4,6-(1H,3H,5H)-pyri-

midinetrione (**15**).—Compound **11** (100 mg, 258 μmol) was treated with 10 mL of aq 10% HCl for 48 h at room temperature. After 48 h, the reaction appeared complete by TLC (ether, R_f for **2** 0.74, R_f for **9** 0.62, R_f for **15** 0.00), and on HPLC analysis with 15:85 MeCN–0.02 M phosphoric acid, t_R of **15** 13.6 min. The mixture was concentrated and extracted with 3 × 10 mL of EtOAc. The EtOAc fractions were pooled together, dried over anhyd Na_2SO_4, and evaporated to dryness when crystalline **15** (80 mg, 213.9 μmol, 83%) was obtained; mp 60°C (softens), 143–145°C; ^{1}H NMR (D_2O): δ 0.70–0.84 (m, 6 H, H-8), 1.90–2.05 (m, 4 H, H-7), 3.24 (s, 3 H, N-CH_3), 3.29 (minor s, 3 H, N-CH_3), 3.51–3.70 (m, 2 H, H-3′,4′), 4.07–4.11 (minor d, *J* 10 Hz, 1 H, H-5′), 4.10–4.13 (d, *J* 10 Hz, 1 H, H-5′), 4.32–4.48 (m, 1 H, H-2′), 5.74 (minor d, *J* 10 Hz, 1 H, H-1′), 5.81 (d, *J* 10 Hz, 1 H, H-1′); IR (KBr): 1699, 1689 cm^{-1}. Anal. Calcd for $C_{15}H_{22}N_2O_9 \cdot 2H_2O$: C, 43.9; H, 6.39; N, 6.83. Found: C, 43.52; H, 6.19; N, 6.88.

5-Ethyl-1-(β-D-glucopyranosyluronate)-3-methyl-5-phenyl-2,4,6-(1H,3H,5H)-pyrimidinetrione (**16a,b**).—Each of the diastereomers **12a** and **12b** (each 100 mg, 0.24mmol) was individually suspended in 10 mL of aq 10% HCl, and the mixture was stirred at room temperature. After 36 h, the reaction appeared complete based on TLC (ether, R_f for **4** 0.74, R_f for **12a,b** 0.62, R_f for **16a,b** 0.00). The mixture was concentrated under reduced pressure, and extracted with 2dichloroethane (2 × 5 mL) and EtOAc (4 × 5 mL). In each case the EtOAc fractions were pooled, dried (Na_2SO_4, anhyd), filtered, and evaporated to dryness under reduced pressure to give a yellow oil. The diastereomers **16a** and **16b** precipitated (each 50 mg, 0.12 mmol, 34%). On analysis using HPLC with a mobile phase of 20:80 MeCN–water, t_R for **16a** and **16b** 6.5 and 9.5 min, respectively.

Data for **16a**: mp 150–152°C; ^{1}H NMR (D_2O): δ 0.85 (t, *J* 8 Hz, 3 H, H-8), 2.28–2.49 (m, 2 H, H-7), 3.20 (s, 1 H, N-CH_3), 3.25 (minor s, 1 H, N-CH_3), 3.45–3.68 (m, 2 H, H-3′,4′), 4.02–4.11 (m, 1 H, H-5′), 4.21–4.40 (m,1 H, H-2′), 5.69 (minor d, *J* 10 Hz, 1 H, H-1′), 5.79 (d, *J* 10 Hz, 1 H, H-1′), 7.43–7.58 (br m, 5 H, ArH); IR (KBr): 1721, 1703 cm^{-1}.

Data for **16b**: mp 150–152°C; ^{1}H NMR (D_2O): δ 0.85 (t, *J* 8 Hz, 3 H, H-8), 2.28–2.49 (m, 2 H, H-7), 3.20 (s, 1 H, N-CH_3), 3.25 (minor s, 1 H, N-CH_3), 3.45–3.68 (m, 2 H, H-3′,4′), 4.02–4.11 (m, 1 H, H-5′), 4.21–4.40 (m,1 H, H-2′), 5.69 (minor d, *J* 10 Hz, 1 H, H-1′), 5.79 (d, *J* 10 Hz, 1 H, H-1′), 7.05–7.28 (br m, 5 H, ArH); IR (KBr): 1721, 1703 cm^{-1}. Anal. Calcd for $C_{18}H_{20}N_2O_9 \cdot H_2O$: C, 50.71; H, 5.20; N, 6.57. Found: C, 50.32; H 5.10; N 6.65

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