Synthesis and Chromatographic Separation of the Glucuronides of (*R*)- and (*S*)-Propranolol

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One of the major metabolites of propranolol (Inderal) is the *O*-glucuronide. In order to further study its disposition, possible metabolism, and contribution to the antihypertensive effect of propranolol, we have synthesized and separated the two diastereomeric propranolol *O*-β-D-glucuronides (**9a,b**). These compounds were prepared by reaction of naphthol with epichlorohydrin and treatment of the resulting (2*RS*)-1′-(2,3-epoxypropoxy)naphthalene (**2**) with sodium azide to give (2*RS*)-1-(1′-naphthoxy)-3-azido-2-propanol (**3**). Alkylation of **3** with methyl (2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranosid)uronate (**4**) gave methyl (2*RS*)-[1-(1′-naphthoxy)-3-azido-2-propyl-2″,3″,4″-tri-*O*-acetyl-β-D-glucopyranosid]uronate (**5a,b**). Reductive alkylation, followed by HPLC separation of the diastereomers, gave methyl (2*R*)- and (2*S*)-[1-(1′-naphthoxy)-3-(isopropylamino)-2-propyl-2″,3″,4″-tri-*O*-acetyl-β-D-glucopyranosid]uronate (**6a,b**). Hydrolytic removal of the acetyl and methyl protecting groups gave the free glucuronides, which were then converted to the sodium salts, **9a,b**. The stereochemistry of the glycoside linkage was deduced from the 400-MHz ^{1}H NMR spectra. The absolute configuration of the aglycon portion was determined after Glusulase hydrolysis by derivatization with (*R*)-(+)- or -(−)-α-methylbenzyl isocyanate and comparison of the HPLC retention volumes with those of derivatized reference (*R*)- and (*S*)-propranolols.

Propranolol is extensively metabolized in rat, dog, and man. In addition to both side chain and naphthalene ring oxidation, the parent drug is also converted to the *O*-glucuronide. Interestingly, in patients undergoing long-term propranolol therapy, the plasma concentration of the glucuronide is four times higher than the parent drug,[1] and in patients on dialysis, the glucuronide concentration is even higher.[2] In addition, the terminal elimination of both propranolol and its glucuronide is unexpectedly slow,[1] and the propranolol glucuronide present in both dog[3,4] and human[4,5] plasma is enriched in the *S* enantiomer. It has also been shown that in man (*S*)-propranolol is preferentially glucuronidated[5] and that both (*S*)-propranolol and its glucuronide have a slower elimination[5] than the corresponding *R* isomers. As pointed out by Walle et al.,[2] these observations on propranolol glucuronide are in opposition to the generally accepted disposition characteristics of drug glucuronides, i.e., that glucuronides are rapidly cleared from the body.[6] In addition, partially purified human propranolol glucuronide when injected intravenously into the dog is deconjugated to propranolol.[2] In order to explain the aforementioned cumulation, enrichment in the *S* enantiomer, deconjugation, and slow elimination, it was suggested that systemic and enteric deconjugation of the glucuronide, as well as enterohepatic recirculation, may be responsible[2] and that some or all of these processes may be stereoselective. In order to study these phenomena, as well as the possible role of the glucuronide in the antihypertensive action of propranolol, gram quantities are required—amounts not easily obtainable via the previously reported preparation methods of Fenseleau and Johnson[7] and Thompson et al.[8] In this paper we report the chemical synthesis and separation of the diastereomeric propranolol glucuronides.

Chemistry. A number of attempts to directly attach the glucuronide moiety to propranolol, *N*-(trifluoroacetamido)propranolol, or *N*-benzylpropranolol were unsuccessful.[9] These failures were likely due to steric and/or electrostatic factors in both the secondary alcohol and the bromoglucuronide (**4**). To circumvent the problem, we postulated that a smaller precursor group, which could be easily converted to the isopropylamino function, would allow the coupling to occur. The azido group fulfills these requirements. We have therefore developed a synthetic strategy in which the glucuronide is formed with an azido precursor of the propranolol aglycon. The azide function is subsequently coverted to the isopropylamino group after formation of the glycoside bond.

Compound **2**, obtained from the alkylation of 1-naphthol (Scheme I) with epichlorohydrin, was refluxed with sodium azide in aqueous ethanol to give azidohydrin **3**. Several catalysts were investigated for introducing the glucuronide moiety into **3**: silver carbonate,[10] silver oxide,[11] silver 4-hydroxyvalarate,[12] mercuric oxide–mercuric bromide,[13] and mercuric cyanide.[14] Mercuric cyanide gave the best yields. Accordingly a 2.5-fold molar excess of **3** was stirred with **4** and 1 molar equiv of mercuric cyanide in nitromethane to give a mixture the diastereomeric β-glucuronides (**5a,b**). Because the separation of **5a,b** could be effected only with great difficulty using HPLC, the large-scale separation required was deferred to next step in the synthesis.

The mixture of **5a,b** was reductively alkylated with hydrogen over platinum[15–17] in anhydrous acetone to give

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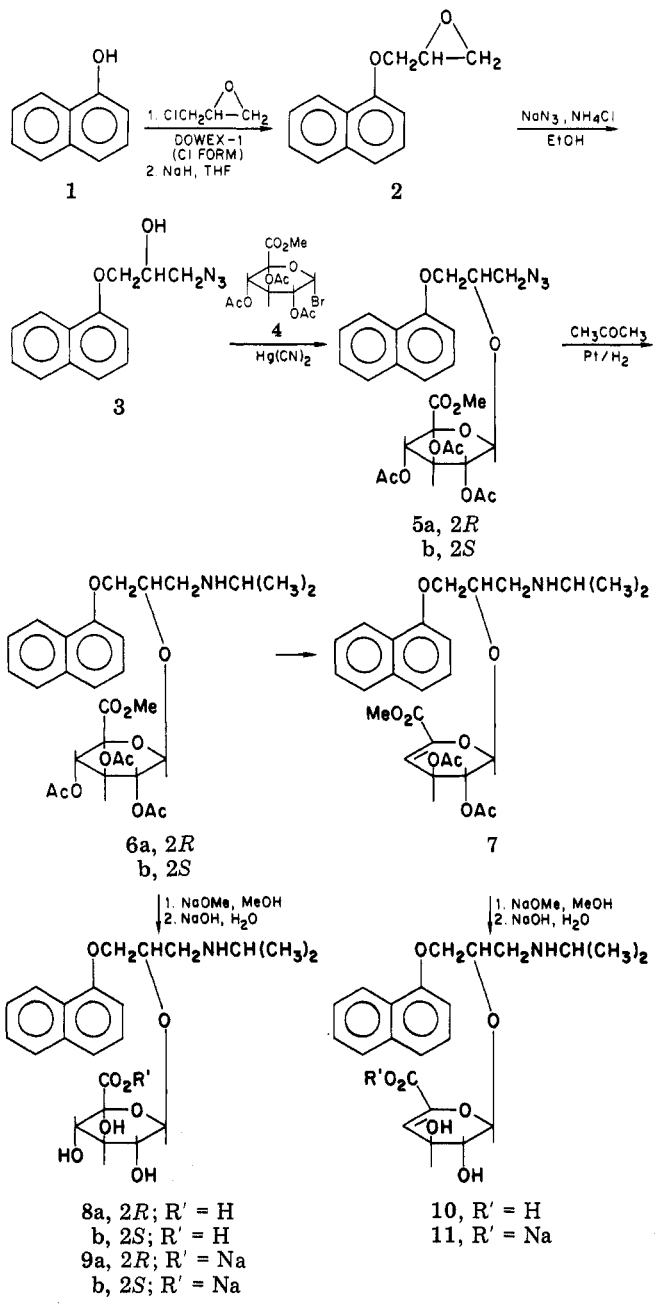
Scheme I



1
2
3
5a, 2R
b, 2S
6a, 2R
b, 2S
7
8a, 2R; R' = H
b, 2S; R' = H
9a, 2R; R' = Na
b, 2S; R' = Na
10, R' = H
11, R' = Na

6a,b. During the partial separation of **6a,b** by recrystallization of the mixture from ethanol, an interesting and facile decomposition occurred. This conversion of **6b** to **7** appears specific for the *S* diastereomer and occurs readily even at 0 °C. However, the amount of **7** produced is minimized if the **6a,b** and **7** mixture is either promptly separated and hydrolyzed or stored at -70 °C. Separation of the diastereomeric β-glucuronides and **7** was effected by HPLC on a Whatman M-9 PAC column. (For structure and stereochemical assignments of **6a,b** and **7**, see below.) Hydrolysis of the separated methyl acetates first with sodium methoxide and then with sodium hydroxide afforded **8a,b** and **10**. After initial purification by HPLC, each compound was converted to its sodium salt, **9a,b** and **11**, in order to facilitate further purification and characterization.

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Table I. *K'* for (*R*)-(+)- and (*S*)-(−)-Propranolol Derivatized with either (*R*)-(+)- or (*S*)-(−)-α-Methylbenzyl Isocyanate

	K' values for propranolols examined and sources			
	reference[a]		synthetic	
resolving agent	*R*	*S*	8a	8b
(*R*)-(+)-α-methylbenzyl isocyanate	6.13	5.45	6.12	5.38
(*S*)-(−)-α-methylbenzyl isocyanate	5.46	6.23	5.46	6.18

[a] Resolved enantiomers obtained from Ayerst.

Structure and Stereochemistry. Since the Koenigs–Knorr method of glycoside synthesis yields almost exclusively the β-anomer, it was assumed that this stereochemistry is present in **5a,b**. Subsequent analysis of the 400-MHz NMR spectra of **6a,b** showed the glycoside protons to have chemical-shift values (δ 3.9–5.3) similar to those reported (δ 4.0–5.4)[18] for other β-glucuronides. When the linkage at C-1″ is α, the glycoside protons are shifted slightly to lower field (δ 4.4–5.6).[18] A similar comparison of the glycoside chemical shifts in **8a,b** with literature values is difficult due to the paucity of the latter data. However, the H-1 signal in methyl β-D-glucopyransiduronic acid appears as a doublet at δ 4.40,[19] a value in close agreement to the δ 4.54 found for H-1″ in both **8a** and **8b**. Definitive evidence, however, for the β-linkage in **6a,b** and **8a,b** is provided by the magnitude of the H-1″–H-2″ coupling constants. If either **6a** or **6b** has an α-configuration at C-1″, $J_{1''-2''}$ is 3.5 Hz,[18] corresponding to an equatorial–axial orientation of the $C_{1''}$ and $C_{2''}$ protons, respectively. On the other hand, a typical coupling constant for the diaxially oriented H-1″ and H-2″ in the β-anomer is 7 Hz,[18] a value similar to the 7.70 and 8.00 Hz found in both **6a** and **6b**, respectively. Similar good agreement is obtained by comparing J_{1-2} of 7.5 Hz in methyl β-D-glucopyransiduronic acid[19] with $J_{1''-2''}$ of 7.73 and 7.70 Hz found in **8a** and **8b**, respectively.

Because the β-adrenergic blocking activity of propranolol resides almost exclusively in (*S*)-(−)-propranolol, assignment of the absolute stereochemistry of the aglycon portion of these glucuronides is necessary in order to interpret biological results. Recently it was shown by Thompson et al.[20] that derivatization of (*RS*)-propranolol with either (*R*)-(+)-α-methylbenzyl isocyanate [(+)-MBI] or (*S*)-(−)-α-methylbenzyl isocyanate [(−)-MBI] resulted in diastereomers that are separable by reverse-phase HPLC. In order to apply this method, **8a,b** were first hydrolyzed, with retention of aglycon stereochemistry,[21] employing Glusulase according to the method of Walle et al.[22] The resulting (*R*)- and (*S*)-propranolols were derivatized with either (+)- or (−)-MBI. As a basis for the assignment of absolute configuration, the resolved (*R*)- and (*S*)-propranolols were likewise derivatized.

Comparison of the *K'* values (Table I) for both (*R*)-(+)- and (*S*)-(−)-MBI derivatized (*R*)-(+)- and (*S*)-(−)-propranolols with the *K'* values of (*RS*)-propranolol and

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Table II. NMR Data of the Glycoside Portion of the 1-Substituted 4-Deoxy-α-L-*threo*-4-enohexopyranosiduronic Acids

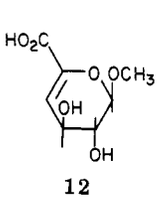
	chem shift, δ (J, Hz)			
compd	H-1 δ (Hz)	H-2 δ (Hz)	H-3 δ (Hz)	H-4 δ (Hz)
7	5.58 (J_{12} = 3.97)	5.15	5.28	6.18 (J_{34} = 4.27)
11	5.18 (J_{12} = 5.79)	3.57	4.02	5.64 (J_{34} = 3.46)
12	5.08 (J_{12} = 5.0)			5.89 (J_{34} = 3.5)
13	5.22 (J_{12} = 5.0)			5.89 (J_{34} = 3.5)

propranolol obtained from the enzymatically hydrolyzed glucuronides **8a,b** indicates that the aglycon portion of the faster eluting **8a** (K' = 9.5) has the *R* absolute configuration and that the aglycon portion of the slower eluting **8b** (K' = 12.8) has the *S* absolute configuration, a result consistent with that obtained by Thompson et al.[8]

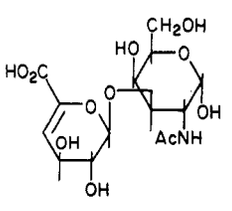
Additional evidence for these stereochemical assignments is provided by propranolol glucuronide isolated from dog urine. As has been shown in both dog[3,4] and human,[4,5] plasma propranolol glucuronide is enriched in the *S* enantioner by a factor of 4 to 1. A similar *S*/*R* ratio (3.1:1) was obtained by HPLC analysis of propranolol glucuronide isolated from dog urine. Although it is possible to have some stereoselectivity in renal clearance, a reversal of the relative enantiomer concentration is unlikely.

The structural assignments for methyl (2*S*)-[1-(1′-naphthoxy)-3-(isopropylamino)-2-propyl-2″,3″-di-*O*-acetyl-4-deoxy-α-L-*threo*-4-hexenopyranosid]uronate (**7**) and sodium (2*S*)-[1-(1′-napthoxy-3-(isopropylamino)-2-propyl-4-deoxy-α-L-*threo*-4-hexenopyransid]uronate (**11**) were based on their NMR, IR, and mass spectra, as well as elemental analysis. The mass spectrum of **7** has a parent ion *m*/*z* of 515, indicating a loss of acetic acid from **6b**. In addition, another major fragment in the mass spectrum of **6b**, *m*/*z* 432 (M − naphthoxy), has an analogous fragment at *m*/*z* 372 in **7**. On the other hand, fragments that are characteristic of the glucuronide portion[23] are either absent entirely or greatly diminished in **7**, while fragments, such as *m*/*z* 242 and 72, that arise from the aglycon portion are present in **6a,b** and **7**.

Although the aromatic and side-chain resonances in the NMR of **7** and **11** are the same as in **6a,b** and **9a,b**, the glycoside portion differs significantly, suggesting that the glucuronide portion has been altered. Such changes are consistent with the presence of a double bond. Based on the comparison (Table II) of the chemical shifts and coupling constants for H-1 and H-4 in **11** with the chemical shifts and coupling constants in methyl 4-deoxy-α-L-*threo*-4-hexenopyransiduronic acid[19] (**12**) and (4-deoxy-α-



12



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L-*threo*-hex-4-enopyranosiduronic acid)-(1→3)-2-acetamido-2-deoxy-α-D-glactopyranose[19] (**13**), the double bonds in **7** and **11** have been assigned to the 4″,5″ position.

Experimental Section

Both (*R*)-(+)- and (*S*)-(−)-propranolols were obtained from Robert Kyle of Ayerst Laboratories, New York, NY, and Sigma Chemical Co., St. Louis, MO.

Melting points, determined on a Thomas-Hoover capillary melting point apparatus, are uncorrected. UV spectra were recorded on a Cary 15 scanning spectrophotometer. NMR spectra were recorded on a Varian T-60 spectrometer employing Me_4Si as internal standard and on a Bruker WH-400 spectrometer interfaced with an Aspect computer located at the NSF Regional NMR Facility at the University of South Carolina (supported by NSF Grant CHE78-18723). Electron-impact mass spectra (EIMS) were obtained at 70 eV on a Finnigan 3200 GC/MS interfaced with a Finnigan 6000 data system. Fast atom bombardment mass spectra (FAB/MS) were obtained at the NSF Mid-Atlantic Regional Mass Spectrometry Facility at Johns Hopkins University on a Kratos MS-50 using xenon bombardment. Optical rotations were obtained with a Steeg and Reuter SR 6 polarimeter. NMR and MS data are consistent with assigned structure. Preparative HPLC was done on either an EM 310 × 25 mm LiChroprep Si60, a Whatman 50 cm M9 ODS-2, or a Whatman 50 cm M9 PAC column employing a Waters Associates M-6000A pump and a Varian variable-wavelength detector (320 nm). Analytical HPLC was done on a Waters Associates liquid chromatography equipped with a UV detector (280 nm) and a M-6000A pump using a 25 cm × 4.6 mm μBondapak C-18 column. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA, and are within ± 0.4% of theory. Analytical samples were dried in vacuo at ambient temperature and are free of significant impurities on TLC (EM silica gel 60 F_{254}) and HPLC.

Methyl (1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosid)uronate was prepared from D-glucurono-3,6-lactone in 32.3% yield according to the procedure of Bollenbach et al.,[10] mp 158.5–171 °C (lit.[10] mp 176.5–178 °C).

Methyl (2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranosid)uronate (4) was prepared according to the procedure of Bollenbach et al.[10] in 79.8% yield, mp 80–83 (lit.[10] mp 106–107 °C). Although further purification raised its melting point, the lower melting product was of sufficient purity for the next reaction.

(2*RS*)-1′-(2,3-Epoxypropoxy)naphthalene (2). A mixture of **1** (72 g, 0.5 mol) and 72 g of Dowex resin (chloride form) was refluxed for 1 h in epichlorohydrin (400 mL, 5.19 mol). The reaction mixture was cooled to room temperature, and the resin removed by filtration. After the excess epichlorohydrin was removed in vacuo, toluene (100 mL) was added and then distilled in vacuo. The process was repeated once. The red-brown residue was added dropwise to NaH (18 g of 50% mineral oil dispersion) suspended in cold dry THF (100 mL). After the addition was complete, the reaction mixture was filtered through Celite 545, and the filtrate was concentrated. The residue was dissolved in ether, and the resulting precipitate was removed by filtration through Celite. The filtrate was washed with 2 × 50 mL of NaOH, 1 × 50 mL of H_2O, and 2 × 50 mL of brine and dried (K_2CO_3). The solvent was removed with reduced pressure to yield 87.1 g of a yellow-orange liquid. Purification by Kugelrohr distillation 150–160 °C (~ 1 mm) gave 82.8 of colorless liquid (82.7%).

(2*RS*)-1-(1′-Naphthoxy)-3-azido-2-propanol (3). To a stirred solution of **2** (10.24 g, 51.1 mmol) in 60 mL of ethanol was added NaN_3 (5.12 g, 78.7 mmol) and a solution of NH_4^+ Cl^- (1.0 g, 18.7 mmol) in 20 mL of H_2O. The mixture was refluxed for 30 min, at which time all the starting epoxide was consumed. After cooling to room temperature, the reaction mixture was poured into 250 mL of saturated NaCl solution. The aqueous solution was extracted with 2 × 250 mL of CH_2Cl_2, the organic layers were combined, washed with 2 × 250 mL of H_2O, and dried ($MgSO_4$), and the solvent was evaporated to yield 12.17 g of orange liquid. Flash chromatography[24] on silica gel using benzene-ether (7:3) gave 9.0 g (72.4%) or pure **3**: NMR ($CDCl_3$) δ 3.1 (s, 1 OH), 3.45 (d, 2, CH_2N_3), 4.03 (m, 3, OCH_2CHO), 6.67 (dd, 1, H-2′), 7.1–8.0 (m, 5, H-4′, H-3′, H-5′, H-6′, H-7′), 8.17 (m, 1, H-8′); EIMS, *m*/*z*

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243 (M^+), 144 (naphthol). Anal. ($C_{13}H_{13}N_3O_2$) C, H, N.

Methyl (2*R*)- and (2*S*)-[1-(1′-Naphthoxy)-3-azido-2-propyl-2″,3″,4″-tri-*O*-acetyl-β-D-glucopyranosid]uronate (5a,b). To **3** (5.95 g, 24.5 mmol) dissolved in 8.6 mL of dry nitromethane was added 4 (3.90 g, 9.82 mmol), followed by $Hg(CN)_2$ (2.48 g, 9.82 mmol). The reaction mixture was stirred in the dark at ambient temperature for 24 h, and then the solvent was removed in vacuo. The residual tar was dissolved in benzene and filtered through Celite, and then the benzene was removed by rotary evaporation to leave an orange tar. Separation of **3** from **5b** and **5a** was effected by using the flash chromatography[24] system, eluting first with benzene and then with benzene-chloroform (40:60) to yield 2.98 g (54.3%) of **5a,b**, mp 109–130 °C. This mixture was used for the next step without further purification. For analysis, the mixture was recrystallized from absolute EtOH, mp 140–142.5 °C, and contains **5a,b** (2.6:1). Anal. ($C_{26}H_{29}N_3O_{11}$) C, H, N.

Small amounts of both diastereomers were separated by HPLC on a 25 × 1 cm silica gel HPLC column using CH_2Cl_2–hexane–EtOAc (1:8:1) as eluent. Compound **5a**: mp 146–147 °C; NMR ($CDCl_3$) δ 1.70, 2.03, 2.05 (s, 9, $COCH_3$), 3.67 (d, 2, CH_2N_3), 3.83 (s, 3, CO_2CH_3), 4.0–4.6 (m, 4, OCH_2CHO, H-5″), 4.9–5.6 (m, 4, H-1″, H-2″, H-3″, H-4″), 6.80 (2 d, 1, H-2′), 7.2–8.1 (m, 5, H-4′, H-3′, H-5′, H-6′, H-7′), 8.3 (m, 1, H-8′). Compound **5b**: mp 111–118 °C; NMR ($CDCl_3$) δ 2.00, 2.08 (s, 9, $COCH_3$), 3.63 (m, 5, CH_2N_3, CO_2CH_3), 4.1–4.7 (m, 4, OCH_2CHO, H-5″), 4.9–5.6 (m, 4, H-1″, H-2″, H-3″, H-4″), 6.8 (2 d, 1, H-2′) 7.3–8.1 (m, 5, H-4′, H-3′, H-5′, H-6′, H-7′), 8.2 (m, 1, H-8′). EIMS (both **5a** and **5b**), *m/z* 559 (M^+) 317, 257, 197, 155 (glucuronide portion), 144 (naphthol).

Methyl (2*R*)- and (2*S*)-[1-(1′-Naphthoxy)-3-(isopropylamino)-2-propyl-2″,3″,4″-tri-*O*-acetyl-β-D-glucopyranosid]uronate (6a,b). Methyl (2*S*)-[1-(1′-Naphthoxy)-3-(isopropylamino)-2-propyl-2″,3″-di-*O*-acetyl-4-deoxy-α-L-*threo*-hex-4-enopyranosid]uronate (7). A suspension of PtO_2 (900 mg) in 150 mL of absolute EtOH was hydrogenated under 30 psi of H_2 for 16 h at room temperature. The EtOH was removed by decantation, and 150 mL of anhydrous acetone was added, followed by the mixture **6a,b** (5.6 g, 10.0 mmol). The reductive alkylation was effected by shaking at 30 psi of H_2 for 8 h. The catalyst was filtered and rinsed with acetone, and the filtrate was concentrated to 6.02 g of a green, oil, which partially crystalized upon sitting at 0 °C. Separation of **6a** from **6b** was accomplished by HPLC on a Whatman Partisil M9 10/50 PAC column using $CHCl_3$–CH_3CN (9.95:0.05), to yield 1.74 g (60.5% based on a 1:1 mixture of diastereomers) of **6a** as a white solid: mp 140–140.5 °C; NMR ($CDCl_3$)[25] glycoside portion δ 3.90 (d, *J* = 9.9 Hz, 1, H-5″) 5.13 (t, 1, H-4″), 5.26 (t, 1, H-3″), 5.01 (t, 1, H-2″), 4.92 (d, *J* = 7.70 Hz, 1, H-1″); EIMS, *m/z* 575 (M^+), 560 ($M^+ - CH_3$), 515 (M – CH_3CO_2), 432 (M – naphthoxy), 317, 257, 197, 155 (glucuronide portion),[23] 242 (M – *O*-glucuronide), 144 (naphthol), 72 [CH_2=$NHCH(CH_3)_2$]. Anal. ($C_{29}H_{37}NO_{11}$) C, H, N. Also isolated was 1.35 g (46.9% based on a 1:1 mixture of diastereomers) of **6b** as a yellow gum: NMR ($CDCl_3$)[25] glycoside portion δ 4.22 (d, *J* = 10.4 Hz, 1, H-5″), 5.18 (t, 1, H-4″), 5.28 (t, 1, H-3″), 5.03 (t, 1, H-2″), 4.94 (d, *J* = 8.00 Hz, 1, H-1″). The MS is identical with **6a**. Anal. ($C_{29}H_{37}NO_{11}\cdot{}^1/_2H_2O$) C, H, N. Also isolated was 185 mg of **7** as a pale yellow gum: NMR ($CDCl_3$)[25] δ 1.05 [d, *J* = 6.41 Hz, 6, $CH(CH_3)_2$], 1.89, 2.00 (s, 6, acetate), 3.82 (s, 3, CO_2CH_3), 2.81 [m, 1, $CH(CH_3)_2$], 2.90 (m, 2, CH_2N), 4.48 (m, *CHO*), 4.24 (m, 2, OCH_2), 5.58 (d, *J* = 3.97 Hz, 1, H-1″), 5.15 (m, 1, H-2″), 5.28 (m, 1, H-3″), 6.18 (d, *J* = 4.27 Hz, H-4″), 6.79 (d, *J* = 7.3 Hz, 1, H-2′), 7.34 (m, 1, H-3′), 7.42 (d, *J* = 8 Hz, 1, H-4′), 7.78 (m, 1, H-5′), 7.46 (m, 2, H-6′, H-7′), 8.19 (m, 1, H-8′); EIMS, *m/z* 515 (M^+), 372 (M – naphthoxy), 242 (M – *O*-glucuronide), 197, 155 (glucuronide fragments[23]), 144 (naphthol), 72 [CH_2=$NH^+CH(CH_3)_2$]. Anal. ($C_{29}H_{37}NO_{11}$) C, H, N.

Sodium (2*R*)-[1-(Isopropylamino)-3-(1′-naphthoxy)-2-propyl-β-D-glucopyranosid]uronate (9a). To a suspension of **6a** (890 mg, 1.55 mmol) in 34 mL of anhydrous MeOH was added NaOMe (39 mg, 0.72 mmol); after the reaction mixture was stirred at 30 °C for 40 min, 5 drops of glacial HOAc was added. The MeOH was removed with reduced pressure, and the residue was stirred for 45 min in 22 mL of 0.5 M NaOH. The aqueous solution was washed with 2 × 20 mL of ether, acidified to pH 7.4 with 1 M H_2SO_4, washed with 1 × 20 mL of EtOAc, and then lyophilized to yield 1.49 g of solid. Initial purification was effected on a Whatman M9 ODS-2 HPLC column eluting with 0.2 M NH_4OAc–CH_3CN (8.5:1.5) to yield 441 mg of white solid, after lyophilization: FAB/MS, *m/z* 436 (MH^+) 260 (M – glucuronide).

In order to further purify and characterize the products, **8a** was converted to its sodium salt. A solution of **8a** (780 mg) dissolved in H_2O, which had been adjusted to pH 8.8 with 10 M NaOH, was put on a 200-g XAD-2 column, and the column was washed with 4 × 250 mL of H_2O and then with 2 × 250 mL of MeOH. The MeOH was removed with reduced pressure, and the residual aqueous solution was lyophilized to yield 592 mg of cream-colored solid. Recrystallization from *i*-PrOH (280 mL/g) gave 452 mg of **9a** as a white solid: mp >155 °C dec; $[\alpha]^{25}_D$ +3.5° (*c* 2.86, H_2O); NMR (Me_2SO-d_6–D_2O, 1:1)[25] glycoside portion δ 4.54 (d, *J* = 7.73 Hz, 1, H-1″), 3.23 (m, 1, H-2″), 3.33 (m, 2, H-3″, H-4″), 3.49 (m, 1, H-5″); UV (95% EtOH) λ_{max} 212 nm (ε 4300), 284 (600). Anal. ($C_{22}H_{28}NO_8Na$) C, H, N.

Sodium (2*S*)-[1-(Isopropylamino)-3-(1′-naphthoxy)-2-propyl-β-D-glucopyranosid]uronate (9b). To a suspension of **6b** (1.35 g, 8.03 mmol) in 38 mL of anhydrous MeOH was added NaOMe (70 mg, 1.30 mmol). After stirring at ambient temperature for 40 min, a second addition of NaOMe (90 mg, 1.67 mmol) was made, the solution was stirred at room temperature for 40 min, and then glacial HOAc (0.2 mL, 14 mmol) was added. The reaction mixture was concentrated at reduced pressure, and the residue was stirred in 25 mL of 0.5 M NaOH for 1.4 h. The aqueous solution was first washed with 2 × 20 mL of ether, acidified to pH 7.4 with 1 M H_2SO_4, and then washed with 2 × 25 mL of EtOAc. The aqueous layer was lyophilized to yield 1.92 g of white solid. Initial purification was effected on a Whatman M9 ODS-2 HPLC column eluting with 0.2 M NH_4OAc–CH_3CN (8.5:1.5) to yield 590 mg of **8b** as a white solid: FAB/MS, *m/z* 436 (MH^+), 260 (M – glucuronide).

In order to further purify and characterize the product, **8b** was converted to its sodium salt. Conversion of the acid to **9b** was accomplished by adjusting the pH of an aqueous solution to 9.0, adding the solution to an XAD-2 column, washing the column with 4 × 200 mL of H_2O, and then eluting the product with 3 × 200 mL of MeOH. The MeOH eluant was evaporated, and the residual aqueous solution was lyophilized to yield 404 mg of **9b**. The product was recrystallized from *i*-PrOH (50 mL/g) to yield 274 mg of a white solid: mp >170 °C dec; $[\alpha]^{25}_D$ −59.7° (*c* 2.43, H_2O); NMR (Me_2SO-d_6–D_2O, 1:1)[25] glycoside proton δ 4.54 (d, *J* = 7.70 Hz, 1, H-1″), 3.15 (m, 1, H-2″), 3.3 (m, 2, H-3″, H-4″), 3.51 (m, 1, H-5″); UV (95% EtOH) λ_{max} 213 nm (ε 4300), 284 (600). Anal. ($C_{22}H_{28}NO_8Na$) C, H, N.

Sodium (2*S*)-(1′-Naphthoxy)-3-(isopropylamino)-2-propyl-4-deoxy-α-L-*threo*-4-hexenopyranosid]uronate (11). To a solution of **7** (328 mg, 0.636 mmol) in 9 mL of MeOH was added NaOMe (19 mg, 0.35 mmol). After the reaction mixture was stirred for 40 min at ambient temperature, additional NaOMe (25 mg, 0.46 mmol) was added, and the solution was stirred for an additional 40 min. Glacial acetic acid (2 drops) was added, and the MeOH was removed with reduced pressure. The residue was stirred for 1.5 h in 7 mL of 0.5 M NaOH. The aqueous solution was washed with 2 × 10 mL of ether, acidified to pH 7.4 with 1 M H_2SO_4, washed with 2 × 10 mL of EtOAc, and then lyophilized to yield 420 mg of a pale yellow solid. Purification was effected on a Whatman M9 ODS-2 HPLC column eluting with 0.2 M NH_4OAc–CH_3CN (8.5:1.5) to yield 317 mg of a white solid.

A solution of **10** (317 mg) dissolved in H_2O and adjusted to pH 9.0 with 10 M NaOH was put on a 200-g XAD-2 column. The column washed with 4 × 200 mL of H_2O and then with 3 × 200 mL of MeOH. The MeOH was removed with reduced pressure, and the residual H_2O was lyophilized to yield **11**. The product was recrystallized from *i*-PrOH (100 mL/g) to yield 100 mg of a white solid: mp >150 °C dec; IR (KBr) 1600 (CO_2Na), 1200–1000 (CO); NMR (D_2O–Me_2SO-d_6, 1:1)[25] δ 1.17 [d, 6, $CH(CH_3)_2$], 3.26 [m, 1, $CH(CH_3)_2$], 3.30 (m, 2, CH_2N), 4.29 (m, 2, CH_2O), 4.46 (m, 1, *CHO*), 5.18 (d, *J* = 5.79 Hz, 1, H-1″), 3.57 (m, 1, H-2″), 4.02

(25) NMR spectra obtained at 400 MHz; d = doublet, t = triplet, m = multiplet. The triplets in these spectra are not true triplets but rather the AB portion of an ABX system.

(m, 1, H-3″), 5.64 (d, J = 3.46 Hz, 1, H-4″), 6.89 (m, 1, H-2′), 7.34 (m, 1, H-3′), 7.43 (m, 3, H-4′, H-6′, H-7′), 7.78 (m, 1, H-5′), 8.09 (m, 1, H-8′). Anal. ($C_{22}H_{26}NO_7Na$) C, H; N: calcd, 5.96; found, 6.46, 6.44.

Glusulase[21] hydrolysis and quantitative analysis (in duplicate) of the liberated propranolol by GC/MS[22] showed both **9a** and **9b** to contain 100.9% each of the theoretical amount of propranolol. Not surprisingly, **11** was only 14% hydrolized under identical conditions. In addition, the HPLC retention volumes for the glucuronides **9a** and **9b** are identical with the retention volumes of the propranolol glucuronides isolated from dog urine.

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Registry No. 1, 90-15-3; (*R*)-**2**, 87144-72-7; (*R*)-**3**, 87102-64-5; **4**, 21085-72-3; **5a**, 87102-65-6; **5b**, 87102-66-7; **6a**, 87102-67-8; **6b**, 87102-68-9; **7**, 87102-69-0; **8a**, 58657-79-7; **8b**, 87144-74-9; **9a**, 87102-70-3; **9b**, 87144-73-8; **10**, 87102-72-5; **11**, 87102-71-4; methyl (1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosid)uronate, 7355-18-2; D-glucurono-3,6-lactone, 32449-92-6; epichlorohydrin, 106-89-8.

Synthesis and Biological Activity of Various 3′-Azido and 3′-Amino Analogues of 5-Substituted Pyrimidine Deoxyribonucleosides

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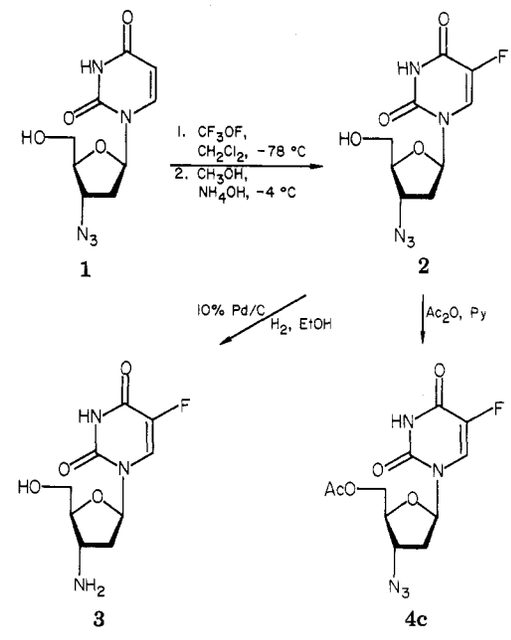
Various new 5-substituted 3′-azido- and 3′-amino derivatives of 2′-deoxyuridine and 2′-deoxycytidine have been synthesized and biologically evaluated. Among these compounds, 3′-amino-2′,3′-dideoxy-5-fluorouridine (**3**), 3′-amino-2′,3′-dideoxycytidine (**7a**), and 3′-amino-2′,3′-dideoxy-5-fluorocytidine (**7c**) were found to be the most active against murine L1210 and sarcoma 180 neoplastic cells in vitro, with an ED_{50} of 15 and 1 μM, 0.7 and 4 μM, and 10 and 1 μM, respectively. The 3′-azido derivatives, **2** and **6c**, were less active in comparison with their 3′-amino counterparts. In addition, the 5-fluoro-3′-amino nucleosides, **3** and **7c**, were tested against L1210 leukemia bearing CDF_1 mice. Our preliminary findings indicate that compound **7c** (6 × 200 mg/kg) was as active as the positive control, 5-fluorouracil (6 × 20 mg/kg), yielding a T/C × 100 of 146 and 129, respectively. However, **3** was found to be inactive in this experiment.

3′-Amino-3′-deoxythymidine was synthesized previously by Miller and Fox[2] and by Horwitz et al.[3] independently. This compound has been found to strongly inhibit the replication of L1210 murine leukemia both in vitro[4] and in mice.[5] The biochemical basis for its anticancer activity is under study, and the findings of Fischer et al.[6] suggest that interference with DNA synthesis is involved. Recently, 3′-amino-2′,3′-dideoxycytidine (**7a**), a new derivative of 2′-deoxycytidine, has been synthesized in this laboratory[7] and found to not only have excellent aqueous solubility but also to be a potent inhibitor of L1210 and P388 murine leukemias both in vitro and in mice.[7,8] These findings demonstrate that the structure modification of thymidine and 2′-deoxycytidine in the 3′-position with an amino group leads to compounds with significant antineoplastic activity. Furthermore, we have found that (**7a**) was resistant to deamination by partially purified cytidine–deoxycytidine deaminase from human KB cells.[8,9]

Scheme I



Based on these findings, various new 5-substituted 3′-azido and 3′-amino derivatives of 2′-deoxyuridine and 2′-deoxycytidine, including 3′-azido-2′,3′-dideoxy-5-fluorouridine (**2**), 3′-amino-2′,3′-dideoxy-5-fluorouridine (**3**), 3′-

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