A Galactoside Derivative of a Nitrosoisocytosine Inhibitor of Dihydropteroate Synthase: Synthesis and Biological Evaluation

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The synthesis of the *O*-β-D-galactosyl derivative **3** of the dihydropteroate synthase inhibitor 6-(3-(4-hydroxyphenoxy)propylamino)-5-nitrosoisocytosine (**2**) was accomplished through coupling of *N*-(3-(4-hydroxyphenoxy)propyl)phthalimide (**4**) with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide followed by complete deblocking of the resulting β-glycoside **9** with hydrazine and then methanolic ammonia to provide amine **10**, and subsequent condensation of **10** with 6-methylthio-5-nitrosoisocytosine (**11**). Glycoside **3** inhibited the synthase with moderate potency (I_{50} = 11 μ*M*) but did not exhibit antibacterial activity.

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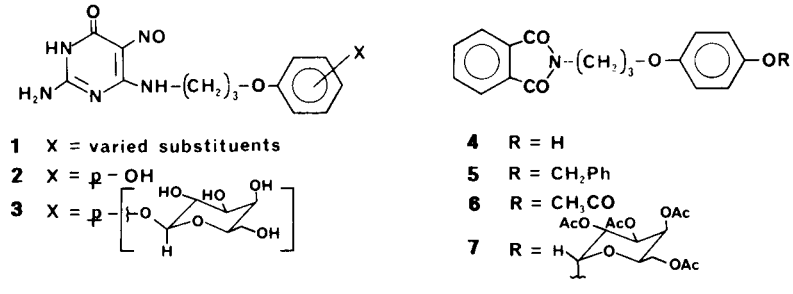
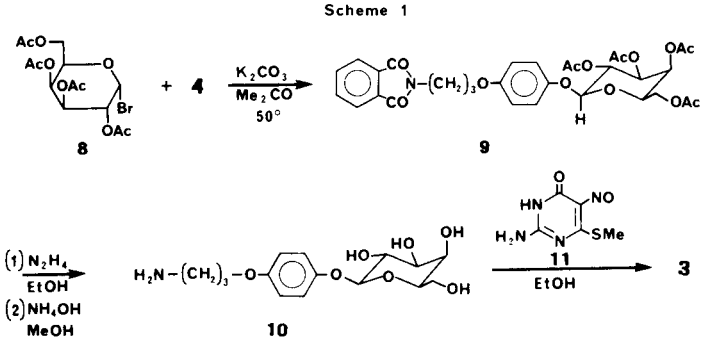
We have recently reported that a variety of 6-alkylamino-5-nitrosoisocytosines, including the aryloxy analogues **1**, inhibit the partially purified dihydropteroate synthase of *Escherichia coli B* with potencies equivalent to the clinically effective sulfonamide inhibitors [2,3]. In contrast with the sulfonamide drugs, however, the nitrosoisocytosines do not inhibit the growth of whole bacterial cells, which may reflect poor transport of these inhibitors through the bacterial cell wall. One approach to cellular delivery involves smuggling a drug into the cell by covalent attachment to a carrier molecule which normally enters the cell by an active transport process [4,5]. The bacterial lactose permease system has been shown to be relatively insensitive to the substituent at C-1 of the galactopyranosyl moiety [6]. Accordingly, we synthesized a β-D-galactopyranosyl derivative **3** of the synthase inhibitor **2** and evaluated the effects of glycoside **3** on synthase activity and on cell growth of several *E. coli* strains which differ in their transport and β-galactosidase responses to galactosides.

The synthesis of **3** was accomplished as outlined in Scheme 1. Phthalimido phenol **4**, available from reductive debenzylation of **5** [3], was coupled with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**8**) in acetone in the presence of potassium carbonate. In this fashion, pure β-anomer **9** was obtained (14%) after preparative hplc



Scheme 1



(silica gel). Acetylated phenol **6** and both α and β anomers of galactose pentaacetate were also isolated, which indicates that acetyl transfers are partially responsible for the overall low conversion to **9**. Presumably non-peracetylated galactose derivatives were also formed but were lost in the aqueous work-up. Coupling of **4** and **8** with mercuric and silver salts [7] in acetonitrile did not provide superior results; both the β-anomer **9** and a minor amount of the undesired α-anomer **7** were isolated from chromatography of the reaction mixture.

Treatment of **9** with hydrazine followed by treatment with methanolic ammonia removed the phthalimide protecting group and deacetylated the hydroxyl groups of the carbohydrate moiety to provide the fully deblocked intermediate **10** (62%). Amine **10** was then condensed with 6-methylthio-5-nitrosoisocytosine (**11**) [2,3] in ethanol to afford, after chromatography on Biogel P-2 in water, the target β-glycoside **3**.

Glycoside **3** was a moderately effective inhibitor (I_{50} = 11 μM) of *E. coli* dihydropteroate synthase [8], although the presence of the carbohydrate moiety reduced the inhibitory potency relative to the parent phenol **2** (I_{50} = 2.2 μM, [3]). At a concentration of 100 μM, compound **3** did not inhibit the specific growth rate [9] of *E. coli* strains ML-30 (an inducible strain possessing a functional permease and β-galactosidase when induced), ML-308 (a strain not requiring induction and constitutively possessing a functional permease and β-galactosidase), and ML-308-225 (a strain not requiring induction and possessing a permease but no β-galactosidase). These observations may be due to lack of transport of **3** into the bacterial cell despite the presence of the galactose moiety, or could be due to ineffective *in vivo* competition of **3** for dihydropteroate synthase, which may result from *in vivo* metabolic conversion to an inactive compound.

EXPERIMENTAL

Melting points were taken on a Buchi melting point apparatus and are uncorrected. The nmr spectra were obtained on Varian CFT-20, HA-100 and T-60 spectrometers and chemical shifts are reported in δ units downfield from tetramethylsilane as internal standard. Ultraviolet spectra were obtained with a Varian SuperScan 3 spectrophotometer. Thin layer chromatographic analyses were carried out on silica gel with solvents coded as follows: A (chloroform:ethyl ether/1:1), B (ethyl acetate:hexane/1:1), C (chloroform: methanol:ammonia:water/40:30:10:3). Microanalyses were performed by Atlantic Microlabs, Inc., Atlanta, Georgia.

N-[3-(4-Hydroxyphenoxy)propyl]phthalimide (**4**).

Benzyloxy compound **5** [3] (19.35 g, 50 mmoles) was hydrogenated in ethyl acetate (250 ml) over 20% palladium on charcoal (1.5 g) at 40 psi in a Parr shaker. Hydrogen uptake was complete after 1 hour and the catalyst was removed by filtration through celite. The solvent was removed under reduced pressure and the residue was recrystallized from ethyl acetate to give 11.3 g (76%) of phenol **4** (tlc: solvent A, R_f 0.64), mp 130-132°; nmr (deuteriochloroform): 100 MHz, δ 2.15 (quintet, 2H), 3.90 and 3.97 (two overlapping t, 4H), 4.52 (s, 1H, exchangeable), 6.71 (s, 4H), 7.77 (complex A_2B_2 pattern, 4H).

Anal. Calcd. for $C_{17}H_{15}NO_4$: C, 68.67; H, 5.08; N, 4.71. Found: C, 68.45; H, 5.04; N, 4.65.

N-{3-[4-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)phenoxy]propyl}phthalimide (**9**) and Acetoxy Compound **6**.

Phenol **4** (44.59 g, 0.15 mole) was added to a stirred mixture of anhydrous potassium carbonate (82.9 g, 0.60 mole) and dry acetone (300 ml) which had been heated to 58°. An initial portion of bromide **8** was then added and three additional portions were added after 1.5, 2.0, and 19.5 hours; the four portions of **8** totaled 82.20 g (0.20 mole). After an overall reaction period of 48 hours at 58°, the mixture was stirred at room temperature overnight and then filtered. The filtrate was evaporated under reduced pressure and the residue was dissolved in 1 ℓ of ethyl acetate. This solution was washed twice with 500 ml portions of water, twice with 200 ml portions of 4% aqueous sodium hydroxide, and finally twice with 250 ml portions of water. The solution was dried over anhydrous sodium sulfate and then evaporated *in vacuo* to give a viscous oil. The oil was triturated with ethyl acetate/hexane (45:55) to provide a solid which was collected and chromatographed on silica gel with chloroform to give 4.8 g of acetoxy compound **6**, mp 144-147°; nmr (deuteriochloroform): 60 MHz, δ 2.17 (quintet, 2H) partially obscured by 2.23 (s, 3H), 3.90 and 4.00 (two overlapping t, 4H total), 6.85 (A_2B_2 m, 4H), 7.75 (A_2B_2 m, 4H).

Anal. Calcd. for $C_{19}H_{17}NO_5$: C, 67.25; H, 5.05; N, 4.13. Found: C, 67.45; H, 5.10; N, 4.14.

The filtrate from the above trituration was diluted to a volume of 210 ml with additional ethyl acetate/hexane (45:55) and this solution was chromatographed in 5 portions on silica gel with ethyl acetate/hexane (45:55) using a Waters Prep-500 chromatograph. The first band eluted (12.6 g) primarily consisted of **6** as judged by tlc analysis. The next band provided 20 g of a viscous oil which was shown by nmr and tlc comparison with authentic samples (Sigma Chemical Co.) to be predominately the α and β anomers of galactose pentaacetate. The third major band consisted of slightly impure **9** which was rechromatographed under the above conditions to provide a pure sample (12.87 g, 14%) of β-anomer **9** as a white powdery solid (tlc: solvent B, R_f 0.48), mp 58-64°; nmr (DMSO-d_6): 80 MHz, δ 1.94 (s, 3H), 2.00 (s, 3H), 2.04 (s, 3H), 2.14 (s, 3H) [the preceding singlets are superimposed over a m (2H) at *ca.* δ 2.0], 3.60-4.15 (m, 6H), 4.32 (br m, 1H), 5.23 (m, 4H, includes anomeric proton), 6.78 (A_2B_2 pattern, 4H), 7.80 (s, 4H).

Anal. Calcd. for $C_{31}H_{33}NO_{13}$: C, 59.32; H, 5.30; N, 2.23. Found: C, 59.11; H, 5.33; N, 2.23.

N-{3-[4-(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyloxy)phenoxy]propyl}phthalimide (**7**).

A mixture of phenol **4** (6.6 g, 20 mmoles), bromide **8** (8.22 g, 20 mmoles), mercuric cyanide (5.05 g, 20 mmoles), mercuric bromide (7.21 g, 20 mmoles) and silver carbonate (5.51 g, 20 mmoles) was stirred in dry acetonitrile (70 ml) for 23 hours. The mixture was filtered and the solid was washed with acetonitrile and then with chloroform. The combined filtrates were washed with aqueous potassium bromide (100 ml, 1 molar) and then twice with water (50 ml portions). The solution was diluted with ethyl acetate (50 ml), dried over sodium sulfate, and then evaporated under reduced pressure. The residual oil was dissolved in methylene chloride and subsequent addition of petroleum ether caused precipitation of a solid. The mixture was filtered and the filtrate was concentrated to provide a foamy residue which was chromatographed on silica gel with methylene chloride/ethyl acetate (90:10) to give a white foam (4.53 g) which consisted of **7** (minor isomer) and **9** (major isomer). A portion (2.08 g) of this mixture was then chromatographed on silica gel with hexane/ethyl acetate (10:7) to provide pure **9** (0.926 g, mp 60-64°) and pure α-anomer **7** (0.439 g), (tlc: solvent B, R_f 0.57) mp 118-122°; nmr (DMSO-d_6): 80 MHz, δ 1.90 (s, 3H), 1.97 (s, 3H), 2.07 (s, 3H), 2.14 (s, 3H) [the preceding singlets are superimposed over a m (2H) at *ca.* δ 2.0], 3.55-4.10 (m, 6H), 4.38 (br t, J *ca.* 6 Hz, 1H), 4.9-5.4 (m, 3H), 5.60 (d, J = 3.5 Hz, anomeric proton), 6.82 (A_2B_2 pattern, 4H), 7.78 (s, 4H).

Anal. Calcd. for $C_{31}H_{33}NO_{13}$: C, 59.32; H, 5.30; N, 2.23. Found: C, 59.09; H, 5.39; N, 2.19.

3-[4-(β-D-Galactopyranosyloxy)phenoxy]propylamine (**10**).

A solution of galactoside **9** (10.04 g, 16 mmoles) and hydrazine hydrate (85%, 5.56 g, 96 mmoles) was refluxed for 3 hours in 95% ethanol (100 ml). The solvent was removed under reduced pressure and the residue was treated for several minutes with a mixture of concentrated ammonium hydroxide (5 ml) and methanol (20 ml). After removal of solvents *in vacuo*, the residue was treated with aqueous sodium hydroxide (1*N*, 16 ml). The mixture was filtered and the solid was washed with a small amount of cold water and then dried *in vacuo* to afford amine **10** (3.25 g, 62%) as the monohydrate (tlc: solvent C, R_f 0.28), mp 187-189°; nmr (DMSO-d_6): 80 MHz, δ 1.71 (quintet, 2H), 2.63 (t, J = 7 Hz, 2H), 2.9-3.75 (m, 14H, includes eight exchangeable protons), 3.91 (t, J = 7 Hz, 2H), 4.60 (d, J = 7 Hz, 1H), 6.84 (A_2B_2 pattern, 4H).

Anal. Calcd. for $C_{15}H_{23}NO_7 \cdot H_2O$: C, 51.86; H, 7.25; N, 4.03. Found: C, 51.90; H, 7.28; N, 4.00.

2-Amino-6-{3-[4-(β-D-galactopyranosyloxy)phenoxy]propylamino}-5-nitrosopyrimidin-4(3*H*)-one (**3**).

A mixture of amine **10** (1.74 g, 5 mmoles) and methylthiopyrimidine **11** (0.84 g, 4.5 mmoles) was heated to reflux for two days using an oil bath which was partially immersed in an ultrasonic bath to provide agitation. The mixture was filtered and the solid was extracted with several portions of water. The aqueous extracts were then chromatographed in water on Biogel P-2 (200-400 mesh). Appropriate fractions were pooled, lyophilized, and recrystallized from water to provide **3** (0.35 g, 17%) as a pink powder: mp 168-172°; nmr (DMSO-d_6): 80 MHz, δ 1.97 (br quintet, 2H), 3.31-3.81 (m, 8H), 3.97 (t, J = 6 Hz, 2H), 4.43 (d, J = 4.5 Hz, 1H, exchangeable), 4.50-4.91 (m, 3H, which on exchange with deuterium oxide collapses to δ 4.66, d, J = 6.7 Hz, 1H, 1'-CH), 5.07 (d, J = 4.3 Hz, 1H, exchangeable), 6.95 (s, 4H) superimposed over a broad exchangeable band at *ca.* δ 6.9 (1H), 7.5-8.5 (v br, 1H, exchangeable), 12.6 (br, 1H, exchangeable), 14.5 (v br t, 1H, exchangeable, pyrimidine 6-N*H*); uv (methanol): λ max nm (ε) 322.5 (18400), 290 (sh, 9700), 222.5 (27600).

Anal. Calcd. for $C_{19}H_{25}N_5O_9$: C, 48.82; H, 5.39; N, 14.98. Found: C, 48.66; H, 5.43; N, 14.81.

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[9] Bacterial growth rates were measured in glucose minimal medium at 35° in the presence **3** (100 μ*M*). The specific growth rate (*k*) in hours^{-1} was computed by linear regression.