2,4-Dinitrophenyl as *N*-Blocking Group in Pyrimidine Nucleoside Synthesis with 2-Amino Sugars

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The fusion of bis(trimethylsilyl)thymine and tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide (**1**), with subsequent alkaline treatment, yielded 1-(2-amino-2-deoxy-β-D-glucopyranosyl)thymine, whereas, unexpectedly, only the glycoside, with an oxygen attachment, was formed in the reaction of **1** with (dithyminyl)mercury.

There has been a need for an easily removable and conveniently prepared *N*-blocking group in nucleoside synthesis utilizing 2-amino-2-deoxy sugars, especially for those with a *trans* configuration on C-2 and C-3. For such purposes, this laboratory has employed the 2,4-dinitrophenyl group,[1] first introduced in the sugar series by Lloyd and Stacy.[2] Employment of this group was shown to offer the possibility of obtaining both anomeric forms in the Davoll–Lowy nucleoside synthesis[3] employing mercury complexes of the purine bases. In the work herein reported we have extended this procedure to a pyrimidine base,[4] thymine. We have found that the thymine nucleoside was sorbed on the resin used to remove the 2,4-dinitrophenyl group and could not be desorbed. This *N*-blocking group was, however, removed with hot barium hydroxide.

When the tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide[2,5] (**1**) was brought into reaction with dithyminylmercury, the only product isolated was one anomeric form of the acetylated 2-glycoside (**2**) which on deacetylation yielded 2-*O*-[2-deoxy-2-(2,4-dinitroanilino)-D-glucopyranosyl]thymine (**3**). That this product was the glycoside derivative, with the base attached to oxygen, was determined by its positive Fehling and Tollens tests and by its instability toward methanolic hydrogen chloride which split it into methyl 2-deoxy-2-(2,4-dinitroanilino)-D-glucopyranoside and thymine. Pyrimidine nucleosides with the base attached to nitrogen, unlike glycosides, have been reported to be characteristically resistant to mild acid hydrolysis.[6] It is known also that the pyrimidine nucleosides from ribonucleic acid (uridine and cytidine) cannot be hydrolyzed by dilute mineral acid.[6] Moreover, Fox and co-workers[4] have removed the acetyl groups in 1-(tetra-*O*-acetyl-β-D-glucopyranosyl)thymine, 1-(tri-*O*-acetyl-D-xylopyranosyl)thymine, and 1-(tri-*O*-acetyl-L-arabinopyranosyl)thymine by methanolic hydrogen chloride at room temperature without concomitant cleavage of the sugar–pyrimidine linkage.

The acetylated 1-nucleoside derivative (**4**), with the base attached to nitrogen, was obtained in one anomeric (β-D)[7] form by application of the fusion technique[8] with bis(trimethylsilyl)thymine.[9] Compound **4** was also obtainable by the O → N migration of the glycoside **2** with mercuric bromide in refluxing toluene according to the general procedure of Ulbricht.[10] This rearrangement provides further proof of the glycosidic nature of **2** and thus of the nucleoside nature of **4**. It is surprising that the glycoside was obtained in the nucleoside synthesis with dithiminylmercury and the glycosyl bromide. However, the O → N rearrangement proceeded in very low yield (7.5%) and required a large excess of mercuric bromide. The presence of the 2,4-dinitroanilino group probably influenced the course of the reaction. Deacylation of **4** with methanolic hydrogen chloride yielded 1-[2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]thymine (**6**), previously reported by Wolfrom and Bhat[11] through a synthesis utilizing the *N*-trifluoroacetyl blocking group.

Experimental Section[12]

2-*O*-[Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-D-glucopyranosyl]thymine (2).—Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide[2,5] (**1**, 36.0 g) was added to an azeotropically dried suspension of dithyminylmercury[4] (21.0 g), cadmium carbonate (28.0 g), and Celite in xylene (1000 ml) and the whole was refluxed for 4.5 hr with stirring. The mixture was filtered hot and the filter cake was extracted with hot toluene. The combined filtrate and extracts were cooled in ice and diluted with petroleum ether (bp 60–80°, 1 l.). The yellow precipitate that formed was separated and dissolved in chloroform. The chloroform solution was washed twice with 30% aqueous potassium iodide solution and then with water. The dried (sodium sulfate) solution was concentrated to yield 35 g (84%) of a yellow glass. This material was dissolved in a small volume of acetone and methanol was added to incipient crystallization. The solution was allowed to stand overnight in a refrigerator. Yellow crystals formed in a yield of 18.0 g (44%): mp 220°; $[\alpha]^{21}D$ +103° (*c* 3.02, chloroform); absorption spectra data, λ_{max}^{KBr} 3.1 (NH), 5.71 (OAc), 6.10, 6.25, 6.54, 7.43, (NO_2), and 13.51 μ (substituted benzene); λ_{max}^{EtOH} 264 mμ (ε 12,390) and 340 mμ (ε 16,980); X-ray powder diffraction data, 12.63 s, 9.21 m, 8.27 s, 6.86 vs (1), 5.44 vs (2), 4.96 m, 4.75 s, 4.48 m, 4.15 s, 3.74 m, 3.47 vs (3), 3.30 m, 3.20 m, 3.05 m, and 2.92 m. The compound was slowly reducing toward Fehling and Tollens reagents.

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(7) The nucleoside **6** and its derivatives have been established as β-D by the nmr spectrum of its tetraacetyl derivative: M. L. Wolfrom and M. W. Winkley, to be published. The anomeric form of **3** is not established.

(8) T. Nishimura, B. Shimizu, and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **12**, 1471 (1964); *Chem. Abstr.*, **62**, 9223 (1965).

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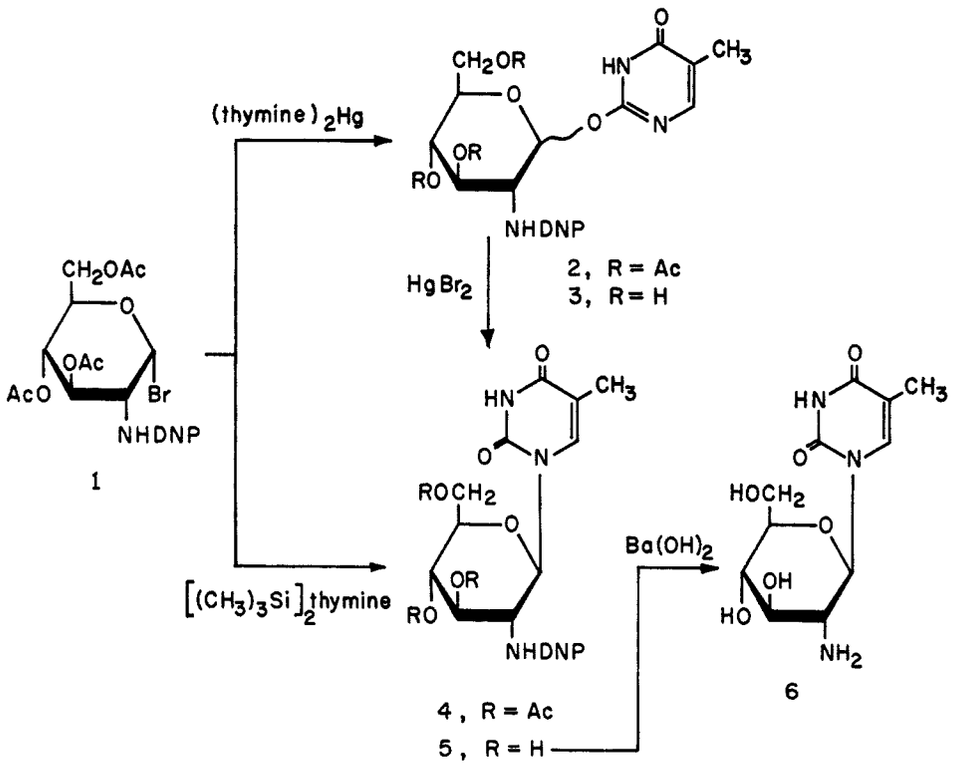
(12) All infrared spectra were measured with a Perkin-Elmer Infracord spectrophotometer and ultraviolet spectra were measured with a Bausch and Lomb Spectronic 505 spectrophotometer. X-Ray powder diffraction data refer to interplanar spacing in angstroms with Cu Kα radiation. Relative intensities were estimated visually: s, strong; m, medium; w, weak; v, very. The stronger lines are numbered in order (1, strongest). Polarimetric readings were taken in a 2-dm tube. All evaporations were performed under diminished pressure below 45°. Microanalyses were by W. N. Rond.



Anal. Calcd for $C_{23}H_{25}N_5O_{13}$: C, 47.66; H, 4.32; N, 12.09. Found: C, 47.63; H, 4.63; N, 11.74.

The mother liquor material from the crystallization was chromatographed on silica gel G with ethyl acetate–benzene (1:1, v/v) as developer and showed the presence of two components, R_f 0.5 and 0.7 (preponderant). The component with R_f 0.7 migrated at the same rate as the crystalline glycoside derivative isolated above. The material in the other zone was obtained as an acetone-soluble syrup which was not further characterized.

A solution of the above-described crystalline substance (2, 0.4 g) in methylene chloride (5 ml) was treated with anhydrous methanol (10 ml) nearly saturated with hydrogen chloride at 0°. The solution was maintained at room temperature and was examined at intervals by thin layer chromatography. After 6 hr, no starting material was detected. The solution was concentrated to dryness and again twice with benzene. Recrystallization of the dried product from methanol gave yellow crystals, identified as methyl 2-deoxy-2-(2,4-dinitroanilino)-D-glucopyranoside, in a yield of 0.2 g: mp 216°; $[\alpha]^{21}D$ +19° (*c* 1.0, acetone) (lit.[2] mp 216–218°; $[\alpha]^{19}D$ +13° (*c* 1.0, acetone)).

Anal. Calcd for $C_{13}H_{17}N_3O_9$: C, 43.45; H, 4.74; N, 11.69; OMe, 8.6. Found: C, 43.46; H, 4.98; N, 12.01; OMe, 7.7.

The mother liquor from the recrystallization was concentrated to dryness and the residue was sublimed at 0.1 mm and 210°. A colorless, crystalline sublimate was obtained with the same melting point and infrared spectrum as an authentic sample of thymine. In another experiment, 2 (0.4 g) was dissolved in a mixture of methylene chloride (5 ml) and anhydrous methanol (10 ml) and the solution was treated at room temperature with methanol nearly saturated with hydrogen chloride (10 drops). After 16 hr, methyl 2-deoxy-2-(2,4-dinitroanilino)-D-glucopyranoside and thymine were again isolated.

2-*O*-[2-Deoxy-2-(2,4-dinitroanilino)-D-glucopyranosyl]thymine (3).[13]—2-*O*-[Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-D-glucopyranosyl]thymine (2, 3.3 g) in methylene chloride (40 ml) and absolute methanol (100 ml) was cooled to 0° and a stream of ammonia was passed in for 30 min. After keeping it at room temperature for 2 hr, the solution was concentrated to 30 ml under diminished pressure whereupon crystallization occurred. The deep yellow, crystalline compound was removed by filtration to yield 2.30 g (86%): mp 185–187° dec; $[\alpha]^{26}D$ +104 ±2° (*c* 2.01, acetone); λ_{max}^{KBr} 2.90–3.10 (OH, NH), 6.20, 6.30, 6.60, 7.55 (NO_2), and 13.44 μ (substituted benzene); λ_{max}^{MeOH} 264 mμ (ε 13,000) and 346 mμ (ε 19,400); X-ray powder diffraction data, 10.40 s (1), 7.89 m, 6.75 w, 5.64 s (3), 3.90 s (2), 3.54 w, and 3.37 w. The compound was slowly reducing toward Fehling and Tollens reagents.

Anal. Calcd for $C_{17}H_{19}N_5O_{10}$: C, 45.03; H, 4.22; N, 15.45. Found: C, 44.64; H, 4.20; N, 15.14.

Experiments designed to remove the 2,4-dinitroanilino group with methanolic sodium methoxide were unsuccessful. On attempted removal with Dowex 1 (OH^-) resin, the material was adsorbed on the resin and could not be desorbed.

1-[Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]thymine (4).—A mixture of tri-*O*-acetyl-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide[2,5] (1, 10.9 g) and bis(trimethylsilyl)thymine (7.0 g) was evacuated with a water pump and the system was closed. The residue was heated slowly to 135° and so maintained for 45 min, whereupon the material was cooled and evacuated to dryness. The dark-colored residue was treated with 80% aqueous methanol and concentrated to a small volume. The residue was taken up in chloroform, the chloroform extract was filtered, and the solvent was removed from the dried filtrate. The residue was crystallized from acetone in lemon yellow, fluffy needles to yield 3.5 g: mp 219°; $[\alpha]^{22}D$ +37° (*c* 1.36, chloroform); λ_{max}^{EtOH} 263 mμ (ε 18,030) and 340 mμ (ε 15,870); λ_{max}^{KBr} 3.1 (NH), 5.8 (OAc), 5.9 (thymine), 6.22, 6.32, 6.6, 7.55 (NO_2), 8.1–8.25 (ester), 8.9, 9.7, 10.8, 12.0, and 13.5 μ (substituted benzene); X-ray powder diffraction data, 10.53 vs (1), 8.67 m, 8.04 w, 7.08 w, 5.68 s, 4.90 s, 4.33 vs (2), 3.93 vs (3), 3.71 m, 3.62 m, 3.40 m, and 3.14 m.

Anal. Calcd for $C_{23}H_{25}N_5O_{13}$: C, 47.66; H, 4.32; N, 12.09. Found: C, 47.22; H, 4.47; N, 12.40.

Chromatography of the mother liquor material, from the crystallization of 4, on silica gel G with ethyl acetate–benzene (1:1 v/v) as developer, showed the presence of two components with R_f 0.9 and 0.3. The former band contained unreacted sugar; the material from the other band was syrupy and was not characterized.

1-[2-Deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]thymine (5). A.—1-[Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]thymine (4, 300 mg) was dissolved in methylene chloride (10 ml) and methanol (30 ml) and was deacetylated with ammonia as described above for the deacetylation of 2-*O*-[tri-*O*-acetyl-2-deoxy-(2,4-dinitroanilino)-D-glucopyranosyl]thymine (2). The residue obtained on solvent removal was crystallized, as orange-yellow needles, from ethanol to yield 175 mg of 5: mp 249–250°; $[\alpha]^{26}D$ −125° (*c* 0.94, ethanol); λ_{max}^{EtOH} 264 mμ (ε 16,350) and 348 mμ (ε 15,900); λ_{max}^{KBr} 2.9–3.1 (OH, NH), 5.9 (thymine), 6.15, 6.25, 6.5, 7.5 (NO_2), 7.8, 8.8, 9.1, 10.8, and 13.5 μ (substituted benzene); X-ray powder diffraction data,

(13) This experiment was performed by M. W. Winkley of this laboratory.

11.19 m, 8.85 vs (1), 7.76 s, 6.66 vs (3), 6.19 w, 5.50 m, 4.44 s, 37.4 w, 3.43 vs (2), 3.27 m, 3.01 s, and 2.88 m.

Anal. Calcd for $C_{17}H_{19}N_5O_{10}$: C, 45.03; H, 4.22; N, 15.45. Found: C, 44.77; H, 4.36; N, 15.34.

Attempts to remove the 2,4-dinitroanilino group in this compound with Dowex 1 (OH^-) resin encountered the same difficulties as were found in like experiments with the glycoside derivative **3**.

B.—Compound **4** (300 mg) was dissolved in methylene chloride (10 ml) and methanol (30 ml). The cooled solution was nearly saturated with hydrogen chloride and was then maintained at room temperature for 25 hr. Solvent removal under diminished pressure left a crystalline residue which was recrystallized from ethanol to yield 190 mg, mp and mmp 249–250°; infrared spectrum and X-ray powder diffraction data are identical with those of **5** prepared by method A above.

O → N Rearrangement[10] of the Glycoside Derivative 2 into the Nucleoside Derivative 4.—Compound **2** (200 mg) was refluxed with toluene (10 ml) and mercuric bromide (500 mg) for 20 hr. The dark mixture was diluted with methylene chloride (50 ml), washed with water, dried, and concentrated to dryness. The residue was purified by thick layer (1.25 mm) chromatography on silica gel G with ethyl acetate–benzene (1:1 v/v) as developer. The band of R_f 0.5 was eluted with ethyl acetate to give a lemon yellow, crystalline product which was recrystallized from acetone to yield 15 mg (7.5%), mp and mmp 219°; infrared spectrum and X-ray powder diffraction data are identical with those of **4**.

1-(2-Amino-2-deoxy-β-D-glucopyranosyl)thymine (6).—A mixture of 1-[2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranoysl]thymine (**5**, 0.5 g), barium hydroxide octahydrate (3.0 g), and water (100 ml) was gently refluxed for 2 hr. After cooling, the mixture was treated with 1 *N* sulfuric acid to pH 3.0. After settling, the barium sulfate was filtered and the orange filtrate was extracted three times with ethyl acetate. The aqueous layer was stirred with barium carbonate (1.0 g) to pH 8.0 and filtered through Celite. The filtrate was evaporated to dryness; the residue was dissolved in dilute methanol (2 ml) and chromatographed on two silica gel G plates using ethyl acetate–methanol (1:1 v/v) as developer. The band of R_f 0.5 was extracted with 95% ethanol. Evaporation of the ethanol extract gave a colorless residue which was readily crystallized from methanol to yield 210 mg (80%): mp 240–242°; $[\alpha]^{22}D$ +6.0° (*c* 3.46, water); X-ray powder pattern identical with the sample prepared previously.[11]

Registry No.—2, 13388-99-3; **3**, 13389-00-9; **4**, 13421-40-4; **5**, 13428-21-2; **6**, 13389-01-0; methyl 2-deoxy-2-(2,4-dinitroanilino)-D-glucopyranoside, 13389-02-1.

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Optically Pure *N*-substituted Derivatives of Benzyl 2-Amino-2-deoxy-α- and -β-D-glucopyranoside[1-3]

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Treatment of 2-acetamido-2-deoxy-D-glucose with benzyl alcohol and hydrogen chloride at 70° gave both anomers of the benzyl glucopyranoside; they were separated as 3,4,6-tri-*O*-acetyl- or 3-*O*-acetyl-4,6-*O*-benzylidene derivatives. Treatment of both anomers of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranoside with potassium hydroxide in ethanol at 110° gave the 2-amino derivatives. Treatment of these two derivatives with acid chlorides or with acids in the presence of a carbodiimide derivative gave, in both series, the *N*-benzyloxycarbonyl, *N*-phenoxycarbonyl, and *N*-chloroacetyl derivatives and, in the α series, the *N*-benzoyl and *N*-bromoacetyl derivatives. In addition, the *N*-*p*-methoxybenzylidene Schiff bases of both 2-amino derivatives were obtained.

The study of a new synthesis of muramic acid [2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose][4] and of the conversion of 2-amino-2-deoxy-D-glucose into 2-amino-2-deoxy-D-galactose[5] required the preparation of optically pure glycosides of 2-amino-2-deoxy-D-glucose. The benzyl glycosides were selected for this purpose because removal of the aglycone by hydrogenolysis required only mild conditions. In addition, the aromatic nucleus facilitates the solubility in organic solvent and crystallization. The benzyl glycosides may be detected by the extinction of the ultraviolet fluorescence of thin layer plates coated with silicic acid containing zinc silicate, thus omitting the need for spray reagents. Since derivatives of 2-amino-2-deoxy-D-glucose are present in natural products as *N*-acetyl derivatives, the glycosidation of 2-acetamido-2-deoxy-D-glucose (**1**) was reinvestigated. Although both anomers of benzyl 2-acetamido-2-deoxy-D-glucopyranoside have been previously synthesized,[6,7] the method[6] for obtaining the α-D anomer is not completely satisfactory. This procedure involves heating under reflux **1** in benzyl alcohol in the presence of hydrogen chloride, thus causing extensive degradation, and low yields. Although samples of both anomers with optical rotations approaching the highest value could occasionally be obtained after several recrystallizations, the method was not always reproducible. Moreover, no solvent system could be found to separate both anomers by thin layer chromatography in order to control their purity.

When 2-acetamido-2-deoxy-D-glucose (**1**) was heated at 70° with benzyl alcohol in the presence of hydrogen

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