Pyrrolopyridazine Nucleosides. The Synthesis of Certain 1-β-D-Ribofuranosyl-1*H*-pyrrolo[2,3-*d*]pyridazin-4(5*H*)-ones

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Nucleosides of pyrrolo[2,3-*d*]pyridazin-4(5*H*)-ones were prepared by the single-phase sodium salt glycosylation of appropriately functionalized pyrrole precursors. The glycosylation of the sodium salt of ethyl 4,5-dichloro-2-formyl-1*H*-pyrrole-3-carboxylate (**4**), or its azomethino derivative **7**, with 1-bromo-2,3,5-tri-*O*-benzoyl-D-ribofuranose in acetonitrile afforded the corresponding substituted pyrrole nucleosides ethyl 4,5-dichloro-2-formyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrrole-3-carboxylate (**5**) and ethyl 4,5-dichloro-2-phenylazomethino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrrole-3-carboxylate (**8**), respectively. The latter, upon treatment with hydrazine, afforded the annulated product 2,3-dichloro-1-β-D-ribofuranosyl-1*H*-pyrrolo[2,3-*d*]pyridazin-4(5*H*)-one (**6**), in good yield. The unsubstituted analog 1-β-D-ribofuranosyl-1*H*-pyrrolo[2,3-*d*]pyridazin-4(5*H*)-one (**9**), was obtained upon catalytic dehalogenation of **6**. This report represents the first example of the synthesis of nucleosides of pyrrolopyridazines.

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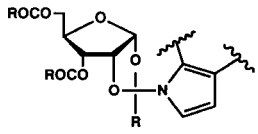
We have recently reported [1,2] the use of the stereospecific single-phase sodium salt procedure for the glycosylation of various halogeno-substituted purine analogs, which served as precursors for the synthesis of adenosine, inosine and guanosine analogs through simple chemical transformations. In the present work, we report the successful utilization of the versatile sodium salt glycosylation procedure for the synthesis of pyrrolo[2,3-*d*]-pyridazine nucleosides, *via* the glycosylation of appropriately functionalized pyrroles followed by ring closure with hydrazine.

Quite recently [2] we described a requirement necessary for successful glycosylation of pyrroles and fused pyrroles in the sodium salt procedure when using the convenient ester-protected sugars; namely, the presence of an electron withdrawing group (relative to hydrogen in terms of field effects) such as a cyano or halogen substituent [3,4] adjacent to the pyrrole ring nitrogen, in order to avoid the formation of an "orthoamide" product **1**. This product results from bond formation between the salt of the pyrrole derivative and the stabilized acyloxonium intermediate at the carbonyl carbon of the ester participating group instead of at the *C*-1 carbon of the glycon. Accordingly, a good candidate for the synthesis of pyrrolo-[2,3-*d*]pyridazine nucleosides would possibly be 2,3,4-trichloropyrrolo[2,3-*d*]pyridazine (**3**), which through glycosylation and simple chemical transformation could lead to the desired nucleosides. Compound **3** would seem easily accessible through simple chlorination of the available 2,3-dichloro-1*H*-pyrrolo[2,3-*d*]pyridazin-4(5*H*)-one (**2**) [5]. However, Bisagni and co-workers reported [6] that chlorination of pyrrolo[2,3-*d*]pyridazin-4-ones bearing no substituents at *N*-1, was unsuccessful and no chlorination products could be isolated. A similar observation was reported by Carbon [7] in connection with imidazo[2,3-*d*]-pyridazin-4-ones. Our own attempts to chlorinate **2** using a variety of reagents and conditions, including phosphoryl chloride, phenylphosphonic dichloride or thionyl chloride and methanesulfonic acid, were unsuccessful.



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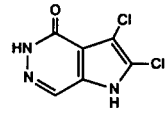

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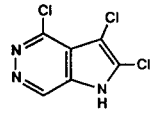

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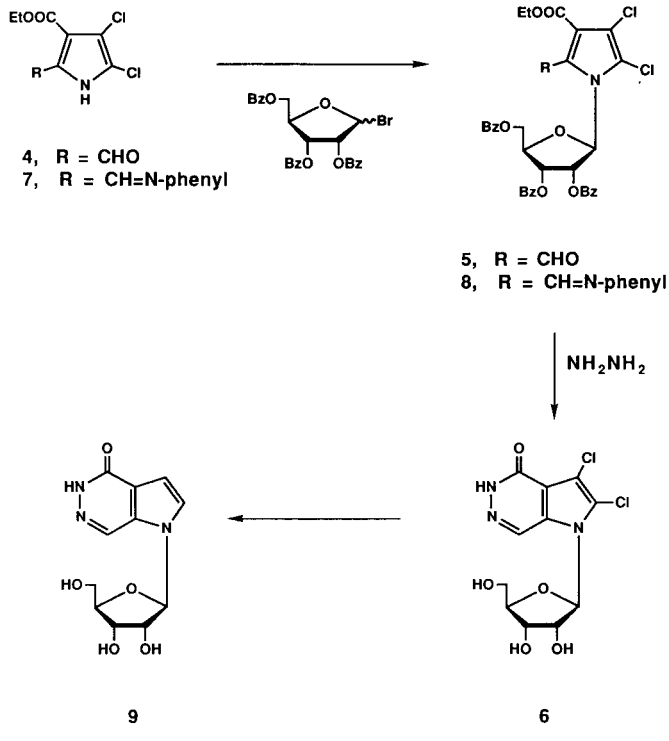
In view of the foregoing observations, an alternative route to the desired nucleosides was pursued through the glycosylation of an appropriately functionalized pyrrole intermediate with subsequent ring closure to form the pyridazine ring. Thus, ethyl 4,5-dichloro-2-formyl-1*H*-pyrrole-3-carboxylate (**4**) [6] was selected as the starting material in this study. This same strategy was employed by Ramesh and Panzica [8] recently to synthesize the ribofuranoside of imidazo[4,5-*d*]pyridazin-4(5*H*)-one [9]. Thus, treatment of the sodium salt of **4** with 1-bromo-2,3,5-tri-*O*-benzoyl-D-ribofuranose [10] at room temperature in acetonitrile stereospecifically yielded the intermediate ethyl 4,5-dichloro-2-formyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrrole-3-carboxylate (**5**) in 50% yield. Reaction of **5** with anhydrous hydrazine in refluxing ethanol effected direct cyclization with simultaneous deblocking of the ribofuranose moiety to provide the desired 2,3-dichloro-1-(β-D-ribofuranosyl)-1*H*-pyrrolo[2,3-*d*]pyridazin-4(5*H*)-one (**6**), in 78% yield. However, the relatively low yield observed for the glycosylation step leading to compound **5** prompted us to investigate the glycosylation of a derivative of **4**, in which the aldehyde function was protected by reaction with aniline to form the azomethine derivative ethyl 4,5-dichloro-2-phenylazomethino-1*H*-pyrrole-3-carboxylate (**7**). The glycosylation procedure using **7** then afforded the blocked nucleoside ethyl 4,5-di-

chloro-2-phenylazomethino-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1*H*-pyrrole-3-carboxylate (**8**) in superior yield (92% compared to 50%). Subsequent ring closure by treating **8** with anhydrous hydrazine in refluxing 1-butanol provided **6** in comparable yield to that obtained from **5**.

Scheme I



Finally, the 2,3-unsubstituted derivative 1-β-D-ribofuranosyl-1*H*-pyrrolo[2,3-*d*]pyridazin-4(5*H*)-one (**9**) was readily obtained by catalytic dehalogenation of **6** using palladium on carbon in a hydrogen atmosphere. The assignment of anomeric configuration of compounds **6** and **9** as β was made on the basis of ^{1}H nmr studies. The spectra of **6** and **9** in DMSO-d_6 revealed the anomeric doublet centered at 5.87 and 5.90 ppm, respectively, with respective coupling constants of 7.0 and 6.0 Hz. These constants are within the acceptable limits established for β-ribonucleosides [11].

All compounds were evaluated for immunological activity in vitro. Compounds **2** and **6** were found to potentiate the response of human lymphocytes toward the B-cell mitogen *Staphylococcus aureus* Cowan (SAC) by factors of 2.4 and 3.8 over controls, respectively.

This report constitutes the first example of the synthesis of ribofuranosides in the pyrrolo[2,3-*d*]pyridazine ring system and in the other pyrrolopyridazine systems in general. It also substantiates the versatility and usefulness of the single-phase sodium salt glycosylation procedure.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus or on a Haake-Buchler digital melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H nmr) spectra were determined at 300.1 MHz with an IBM NR300AF spectrometer. The chemical shifts are expressed in δ values (parts per million relative to the residual proton of DMSO-d_6 set at 2.50 ppm; br = broad singlet). Ultraviolet spectra were recorded on a Beckman DU-50 spectrophotometer. Elemental analyses were performed by Robertson Laboratory, Madison, NJ. Evaporations were carried out under reduced pressure with the bath temperature below 40. Thin layer chromatography (tlc) was run on silica gel 60 F-254 plates (EM reagents). E. Merck silica gel (230-400 mesh) was used for flash column chromatography.

Ethyl 4,5-Dichloro-2-phenylazomethino-1*H*-pyrrole-3-carboxylate (**7**).

To a solution of ethyl 4,5-dichloro-2-formyl-1*H*-pyrrole-3-carboxylate (**4**) [6] (4.7 g, 20 mmoles) in dry methylene chloride (150 ml) containing molecular sieves (4 Å, 5 g), was added aniline (1.8 g, 20 mmoles). The mixture was stirred at room temperature for 1 hour. It was then filtered. The filtrate was evaporated to dryness, adsorbed on silica and purified by flash silica gel column chromatography using hexane/ethyl acetate (5:1) as eluent to give 4.7 g (77%) of **7** as a yellow crystalline solid, mp 79-80°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.8 (s, 1H, methine-H), 7.43 (m, 4 H, aromatic), 4.30 (q, 2 H, ethyl CH_2), 1.31 (t, 3 H, ethyl CH_3).

Anal. Calcd. for $C_{14}H_{12}Cl_2N_2O_2$: C, 54.04; H, 3.89; N, 9.00; Cl, 22.79. Found: C, 53.91; H, 4.04; N, 8.73; Cl, 22.43.

Ethyl 4,5-Dichloro-2-formyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrrole-3-carboxylate (**5**).

To a solution of **4** (2.36 g, 10 mmoles) in dry acetonitrile (100 ml) was added sodium hydride (0.48 g, 12 mmoles, 60% in oil) and the mixture was stirred at room temperature for 30 minutes. A solution of 1-bromo-2,3,5-tri-*O*-benzoylribofuranose (12 mmoles, prepared from the corresponding 1-*O*-acetyl derivative [8]) in dry acetonitrile (25 ml) was added and the whole was stirred overnight at room temperature. The mixture was then filtered through a celite pad and the filtrate was evaporated to dryness under reduced pressure. The product was purified by flash silica gel column chromatography using toluene/ethyl acetate (9:1) as eluent to give **5** as a colorless syrup, 3.4 g (50%); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 9.90 (s, 1 H, CHO), 8.00-7.35 (m, 15 H, aromatic), 5.70 (d, J = 4.7 Hz, C_1H), 4.46 (q, 2 H, ethyl CH_2), 1.27 (t, 3 H, ethyl CH_3) and other sugar protons.

Anal. Calcd. for $C_{34}H_{27}Cl_2NO_{10}$: C, 60.01; H, 4.00; N, 2.06; Cl, 10.42. Found: C, 59.89; H, 4.12; N, 2.03; Cl, 10.17.

Ethyl 4,5-Dichloro-2-phenylazomethino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrrole-3-carboxylate (**8**).

To a solution of **7** (4.65 g, 15 mmoles) in dry acetonitrile (150 ml) was added sodium hydride (0.72 g, 18 mmoles; 60% in oil) and the mixture stirred at room temperature for 30 minutes. 1-bromo-2,3,5-tri-*O*-benzoyl-D-ribofuranose (18 mmoles; prepared from the corresponding 1-*O*-acetyl derivative [10]) dissolved in dry acetonitrile (50 ml) was added and the mixture stirred overnight at room temperature. It was then filtered through a celite

pad and the filtrate separated by flash silica gel column chromatography using toluene/ethyl acetate (10:1) to give 10.4 g (92%) of **8** as a yellow syrup; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.73 (s, 1 H, methine-H), 7.99-6.99 (m, 20 H, aromatics), 5.66 (d, J = 4.80 Hz, 1 H, C_1·H), 4.24 (q, 2 H, ethyl CH_2), 1.26 (t, 3 H, ethyl CH_3), and other sugar protons.

Anal. Calcd. for $C_{40}H_{32}Cl_2N_2O_9$: C, 63.58; H, 4.27; N, 3.71; Cl, 9.38. Found: C, 63.38; H, 4.39; N, 3.52; Cl, 9.09.

2,3-Dichloro-1-(β-D-ribofuranosyl)-1*H*-pyrrolo[2,3-*d*]pyridazin-4(5*H*)-one (**6**).

A. From **5**.

A mixture of **5** (6.8 g, 10 mmoles) and anhydrous hydrazine (1.5 g, 50 mmoles) in absolute ethanol (100 ml) was heated under reflux for 6 hours. The volatiles were evaporated under reduced pressure and the residue was purified by flash silica gel column chromatography using methylene chloride/acetone (4:1) followed by methylene chloride/methanol (5:1) to give 2.6 g (78%) of **6** as a colorless crystalline solid, mp 229-230° (decomp.); uv: λ max (*p*H 1,7,11) 296 nm (ε 14000), 265 nm (7000); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 12.64 (br, 1 H, NH), 8.83 (s, 1 H, C_7H), 5.87 (d, J = 7.0 Hz, 1 H, C_1·H), and other sugar protons.

Anal. Calcd. for $C_{11}H_{11}Cl_2N_3O_5$: C, 39.31; H, 3.30; N, 12.50; Cl, 21.10. Found: C, 39.39; H, 3.35; N, 12.47; Cl, 21.00.

B. From **8**.

A mixture of **8** (7.55 g, 10 mmoles) and anhydrous hydrazine (1.5 g, 50 mmoles) in 1-butanol (100 ml) was heated under reflux for 6 hours. The solution was evaporated to dryness under reduced pressure and the product was purified following the same steps as described in procedure A to give 3.8 g (81%) of **6** as a colorless crystalline solid, mp 229-230° dec (undepressed on admixture with a sample prepared by procedure A). The two products also have identical nmr and uv spectra.

1-β-D-Ribofuranosyl-1*H*-pyrrolo[2,3-*d*]pyridazin-4(5*H*)-one (**9**).

To a suspension of **6** (1.7 g, 5 mmoles) in water (100 ml) was added palladium on carbon (500 mg, 5%) and the mixture was hydrogenated at 30 psi at room temperature for 24 hours. The reaction mixture was filtered through a celite pad and the filtrate was evaporated to dryness under reduced pressure. The solid residue was purified by flash silica gel column chromatography using methylene chloride/methanol (3:1) as eluent to give 0.87 g (65%) of **9** as a colorless crystalline solid, mp 264°; uv: λ max (*p*H 1,7,11) 283 nm (ε 8100); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 12.38 (br, 1 H, NH, exchangeable), 8.53 (s, 1 H, C_7H), 7.69 (d, J = 2.9 Hz, 1 H, C_2H), 6.69 (d, J = 2.9 Hz, 1 H, C_3H), 5.90 (d, J = 6.0, 1 H, C_1·H), and other sugar protons.

Anal. Calcd. for $C_{11}H_{13}N_3O_5$: C, 49.44; H, 4.90; N, 15.72. Found: C, 49.19; H, 4.83; N, 15.47.

REFERENCES AND NOTES

[§] Present address: Beckman Instruments, 200 South Kraemer Bld., Brea, California 92621.

[1] N. S. Girgis, H. B. Cottam, S. B. Larson and R. K. Robins, *J. Heterocyclic Chem.*, **24**, 821 (1987).

[2] N. S. Girgis, R. K. Robins and H. B. Cottam, *J. Heterocyclic Chem.*, **27**, 171 (1990).

[3] Z. Kazimierczuk, G. R. Revankar and R. K. Robins, *Nucleic Acids Res.*, **12**, 1179 (1984).

[4] J. D. Anderson, R. J. Bontems, S. Geary, H. B. Cottam, S. B. Larson, S. S. Matsumoto, D. F. Smee and R. K. Robins, *Nucleosides Nucleotides*, **8**, 1201 (1989).

[5] J. P. Marquet, E. Bisagni and J. A. Louisfert, *Chim. Therap.*, **3**, 348 (1968).

[6] J. P. Marquet, J. A. Louisfert and E. Bisagni, *C. R. Acad. Sc. Paris*, **265**, 1271 (1967).

[7] J. C. Carbon, *J. Amer. Chem. Soc.*, **80**, 6083 (1958).

[8] K. Ramesh and R. Panzica, *J. Chem. Soc., Perkin Trans. I*, 1769 (1989).

[9] R. P. Gagnier, M. J. Halat and B. A. Otter, *J. Heterocyclic Chem.*, **21**, 481 (1984).

[10] J. D. Stevens, R. K. Ness and H. G. Fletcher Jr., *J. Org. Chem.*, **33**, 1800 (1968).

[11] L. B. Townsend in Synthetic Procedures in Nucleic Acid Chemistry, W. W. Zorbach and R. S. Tipson, eds, Vol **2**, Wiley-Interscience, New York, 1973, Chapter 7.