STUDIES ON IMPROVED SYNTHESIS OF 2′-DEOXYRIBONUCLEOSIDES OF PYRIDAZINE DERIVATIVES

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A number of 2′-deoxyribonucleosides of halogenated pyridazine derivatives were prepared by glycosylation of their respective potassium or DBU salts in acetone. The reaction yielded predominatly β-anomers that could be purified by simple crystallization or column chromatography. Of the studied pyridazines and deoxynucleosides, only 4-bromo-6-chloropyridazin-3-one and 6-chloro-2-(2′-deoxyribofuranosyl)pyridazin-3-one showed modest inhibition of CK2 kinase.

Keywords: Pyridazines; Nucleosides; Glycosidation; Glycosylation; CK2 inhibitors.

Pyridazine nucleosides were prepared by various methods that were discovered by 'nucleoside' chemists within the last few decades[1–5]. Studies on the synthesis of pyridazine nucleosides, and particularly of 1,2,4-triazine nucleosides, began over 40 years ago in Prof. Šorm research group. The first reported ribo- and 2′-deoxyribonucleosides of 6-hydroxypyridazin-3-one were prepared by the Hilbert–Johnson reaction of 3,6-dimethoxypyridazine with a protected halogenoribofuranose[6]. (The nomenclature of pyridazines differs between various literature sources. Some researchers describe 2-substituted pyridazin-3-ones as 1-substituted pyridazin-6-ones).

It should be noted that, in contrast with numerous publications on the synthesis and modifications of pyridazine-derived ribonucleosides, studies on the synthesis of the corresponding deoxynucleosides are very scarce[1,2,6].

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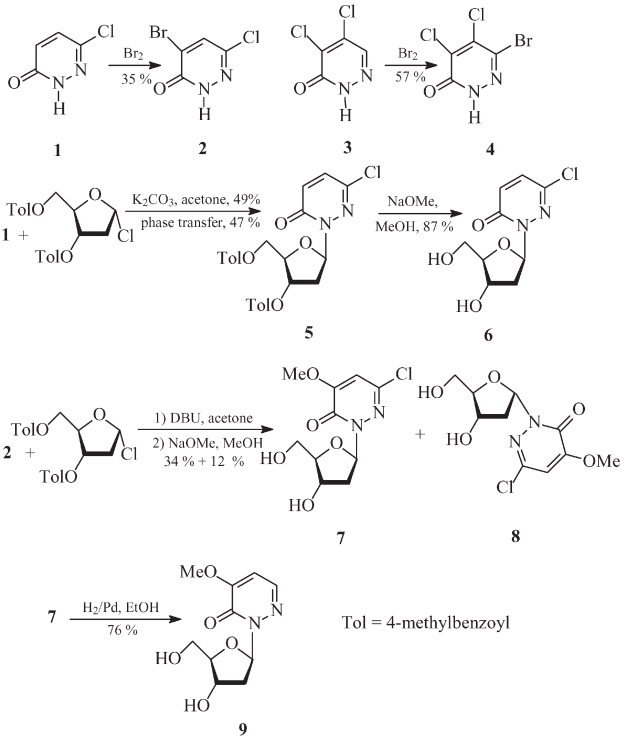
Besides the early Pliml and Šorm paper[6], only two reports have been published so far that dealt with this problem. One of the reports described the synthesis employing silver salts, which yielded a mixture of *O*- and *N*-deoxyribosides[1], and the other depicted the deoxyribonucleoside synthesis from the respective silylated bases according to the Vorbrüggen procedure[2]. The ratio of the anomers obtained was approximately 1:1 in both methods.

In this study we decided to test in the synthesis of pyridazine deoxynucleosides two methods that have been effectively used with other heterocyclic bases, namely the phase-transfer and 'sodium salt' protocols[7,8]. For the substrate bases we have chosen three known pyridazines, namely 6-chloropyridazin-3-one (**1**), 4,5-dichloropyridazin-3-one (**3**) and 4,5-dibromopyridazin-3-one (**10**), and two newly synthesized pyridazines, 4-bromo-6-chloropyridazin-3-one (**2**) and 6-bromo-4,5-dichloropyridazin-3-one (**4**). Bromination of **1** at elevated temperature for 3 days using excess of bromine in water led to 4-bromo derivative **2** as single product; its identity was confirmed in the course of the study. It should be noted that under the same conditions 4,5-dichloropyridazin-3-one (**3**) underwent bromination in position 6 to yield **4**, whereas the corresponding 4,5-dibromo derivative **10** remained unchanged even after a markedly longer reaction time. The reason for this dissimilar 'behavior' was presumably steric hindrance rather than electronic structure of the substrate (Scheme 1).

Of the aforementioned halopyridazines, only 6-chloro derivative **1** was sufficiently soluble in acetonitrile to generate the reactive anion necessary for the SN2 reaction with 3,5-di-*O*-*p*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranosyl chloride. In the phase transfer procedure the respective 2-(2′-deoxy-β-D-ribofuranoside) derivative **5** was obtained in good yield (method B). The salt formed in the reaction of **1** with sodium hydride under the sodium salt protocol was insufficiently soluble in acetonitrile and a prolonged reaction time would provide an enhanced amount of the undesired α-anomer due to anomerisation of the haloribose. However, a satisfactory result was obtained using acetone as solvent and an excess of anhydrous potassium carbonate for the glycosylation reaction. The yield of **5** was similar to that obtained by the phase transfer procedure (cf. methods A and B in Experimental). Notably, there was no *O*-glycosylated compounds in the final reaction mixtures. The probable reason was poorer stability of *O*-glycosylated compounds than that of the respective *N*-glycosylated derivatives. This assumption found support in the respective TLC patterns of the reaction mixture: some spots that appeared in the initial phase of the reaction were absent when the reaction was completed.

Removal of the protective 4-methylbenzoyl groups, which was achieved with methanolic sodium methanolate provided the respective β-deoxynucleoside **6**. No substitution of chlorine atom by methoxyl group was observed either at room temperature or under reflux conditions.

Poor solubility of the di- and trihalopyridazines **2**–**4** and **10** in acetonitrile and acetone precluded the synthesis of the corresponding deoxyribonucleosides by of the two mentioned methods. The problem was solved by using the respective DBU salts, which showed high solubility in acetone. This modification was successfully applied for deoxyribosylation of 4-bromo-6-chloropyridazin-3-one (**2**). Because of difficulties in separating the resulting protected anomers of the nucleoside, the reaction mixture was treated with methanolic solution of sodium methanolate. This resulted in deprotection and simultaneous exchange of the chlorine atom in position 5 for the methoxy group. Separation of the β- and α-anomers **7** and **8** (ca. 3:1 ratio) was achieved easily by column chromatography. The exchange of

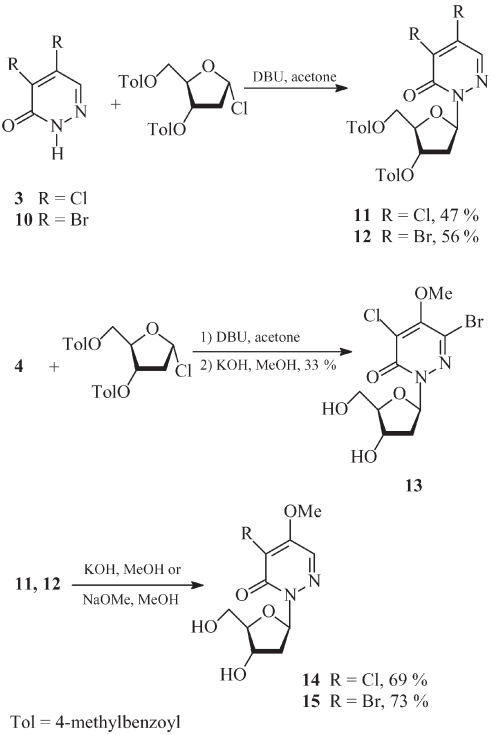


SCHEME 1

halogen in position 5 for the methoxy group was also observed in other di- and trihalosubstituted pyridazine nucleosides. In our hands, the substitution by methoxyl occured also when using KOH in methanol. Notably, in *N*-substituted 4,5-dihalopyridazines methoxylation was observed even in methanol with potassium carbonate or methanolic potassium cyanide[9,10]. Removal of chlorine from **7** using hydrogen and palladium on charcoal as catalyst led to 4-methoxy derivative **9**, which confirmed the exact position of bromine atom in the substrate base **2**.

The use of DBU salts in acetone appeared optimal for the synthesis of protected β-deoxynucleosides of 4,5-dichloropyridazin-3-one **11** and 4,5-dibromopyridazin-3-one **12**. The products were obtained in good yields and were successfully purified by simple crystallization from methanol (Scheme 2).

In the case of dichloro bromo derivative **4**, the deoxyribosylation product crystallized as a mixture of anomers (β/α 7:1, HPLC) which could not be separated either by crystallization or by column chromatography because of



SCHEME 2

similarity in their R_F values. Removal of protective groups and exchange of the chlorine atom in position 5 for methoxy group similarly to dihalonucleosides **11** and **12** yielded the methoxy halo-β-D-deoxynucleosides **13**, **14** and **15**. The position of the methoxy group in **13** was deduced from the respective hydrogenation product: the resulting compound was identical to that obtained by hydrogenation of **14** and was indistinguishable from 2-(2′-deoxy-β-D-ribofuranosyl)-5-methoxypyridazin-3-one described in the literature[2].

Anomeric configuration of the obtained deoxynucleosides was determined by the nuclear Overhauser effect (NOE). According to published data, this configuration can be immediately deduced from NOE values of H-4′ and H-2′a in the case of β-anomers and H-3′ and H-2′b in the case of α-nucleosides upon irradiation of H-1′ [11,12]. The protected nucleosides obtained are good substrates for various synthetic modifications which can yield numerous deoxynucleosides of substituted pyridazines. Further synthetic studies are under way and will be reported in due time.

The results of our previous investigations[13,14] on the inhibitor activity of various halosubstituted benzimidazoles against the antiapoptotic enzyme casein kinase 2 (CK2) encouraged us to explore the inhibitory properties of the newly obtained halopyridazines and their nucleosides, including several compounds not included in this report. Of the investigated compounds only 4-bromo-6-chloropyridazin-3-one (**2**) and 6-chloro-2-(2′-deoxy-β-D-ribofuranosyl)pyridazin-3-one (**6**) showed a modest CK2 inhibition (K_i = 14 and 20 μmol, respectively).

EXPERIMENTAL

All chemicals and solvents were purchased from Sigma-Aldrich. Melting points (uncorrected) were measured in open capillary tubes on a Gallenkamp-5 melting point apparatus. ^{1}H and ^{13}C NMR spectra were measured on a Bruker 200 MHz and a VarianUNITY 500 (499.8 MHz) spectrometers with TMS as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. The assignment of 13C signals of all compounds studied was made using results of 2D methods including 1H-13C gradient selected HSQC (Heteronuclear Single Quantum Correlation) and HMBC (Heteronuclear Multiple Bond Correlation). Ultraviolet absorption spectra (λ_{max} in nm) were recorded on a UV 8500 Techcomb spectrophotometer, and mass spectra (70 eV) were obtained with a ADM-604 Intectra spectrometer. Flash chromatography was performed on Merck silica gel 60 (200–400 mesh). Elemental analyses of the new compounds were within ±0.4% of the respective theoretical values. The procedure used for the determination of inhibitory activity of the heterocyclic derivatives against casein kinase type II (CK2) was reported previously[13,14].

4-Bromo-6-chloropyridazin-3(2*H*)-one (**2**)

To a stirred solution of **1** (6.0 g, 46.5 mmol) in water (130 ml) at 90 °C (bath temperature), bromine (5.0 ml, 97.5 mmol) was added portionwise over 2 days. An excess of bromine was present at all times. Next, the reaction mixture was cooled, and the precipitate formed was filtered off and washed with water. Crystallization from ethanol–water yielded the desired product. Yield 3.4 g (35%), m.p. 220–222 °C. MS-EI, *m/z* (%): 212 (24), 211 (6), 210 (100), 209 (5), 208 (78). ^{1}H NMR (DMSO-d_6): 8.20 s, 1 H (H-5); 13.5 s, 1 H (HN). ^{13}C NMR (DMSO-d_6): 130.1 (C-4), 136.0 (C-5), 136.5 (C-6), 156.8 (C-3). UV, pH 7, λ_{max} (ε): 223 (5900), 303 (2500). For $C_4H_2BrClN_2O$ (209.4) calculated: 22.94% C, 0.96% H, 13.38% N; found: 22.83% C, 1.05% H, 13.25% N.

6-Bromo-4,5-dichloropyridazin-3(2*H*)-one (**4**)

To a stirred suspension of **3** (3.4 g, 20.7 mmol) in water (100 ml) at 90 °C (bath temperature), bromine (4 ml, 78 mmol) was added portionwise over 2 days. An excess of bromine was present at all times. The reaction mixture was cooled and the precipitate formed was filtered off, washed with water and crystallized from ethanol–water to give the final product. Yield 2.9 g (57%), m.p. 246–248 °C. MS-EI, *m/z* (%): 248 (7), 246 (46), 245 (6), 244 (100), 242 (62). ^{1}H NMR (DMSO-d_6): 13.8 s, 1 H (HN). ^{13}C NMR (DMSO-d_6): 135.3 (C-6), 136.5 (C-5), 138.0 (C-4), 155.9 (C-3). UV, pH 7, λ_{max} (ε): 229 (14 500), 303 (3600). For $C_4HBrCl_2N_2O$ (243.9) calculated: 19.70% C, 0.41% H, 11.49% N; found: 19.56% C, 0.56% H, 11.38% N.

6-Chloro-2-[2′-deoxy-3′,5′-di-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-pyridazin-3(2*H*)-one (**5**)

Method A: To a stirred solution of **1** (1.31 g, 10 mmol) in acetone (70 ml), K_2CO_3 (6.9 g, 50 mmol) was added. After 30 min, 2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranosyl chloride (3.9 g, 10 mmol) was added portionwise to the solution over 30 min, and stirring was continued for another 30 min. Inorganic solids were filtered off on a pad of Cellite, washed with acetone, and the resulting filtrate was evaporated to an oil. The oil was purified on a silica gel column (4 × 20 cm) (elution with ethyl acetate–petroleum ether (1:3 to 1:1, 1 l). The fractions containing main product were evaporated to dryness, and the residue was crystallized from methanol to give the required product. Yield 2.4 g (49.5%), m.p. 108–110 °C (ref.[1] gives 104–106 °C). ^{1}H NMR (DMSO-d_6): 2.37 and 2.39, 6 H (2 CH_3); 2.50 m, 1 H (H-2′b); 2.88 m, 1 H (H-2′a); 4.50 m, 3 H (H-4′ and H-5′ and H-5″); 5.66 q, 1 H, J(3′,2′) = 4.2 (H-3′); 6.73 dd, 1 H, J(1′,2′a) = 1.2, J(1′2′b) = 5.4 (H-1′); 7.35 and 7.89 2m, 8 H (H-arom.); 7.06 d, 1 H, J(4,5) = 8.4 (H-4); 7.54 d, 1 H, J(5,4) = 8.3 (H-5).

An additional slower migrated product obtained, presumably the respective α-anomer (0.34 g, m.p. 95–97 °C; ref.[1] gives 94–96 °C). ^{1}H NMR (DMSO-d_6): 2.38 and 2.40, 6 H (2 CH_3); 2.65 m, 1 H (H-2′b); 3.10 m, 1 H (H-2′a); 4.60 m, 2 H (H-5′ and H-5″); 4.88 m, 1 H (H-4′); 5.60 m, 1 H (H-3′); 6.63 dd, 1 H, J(1′2′a) = 3.1, J(1′2′b) = 4.0 (H-1′); 7.35 and 7.89 2m, 8 H (H-arom.); 7.05 d, 1 H, J(4,5) = 8.3 (H-4); 7.56 d, 1 H, J(5,4) = 8.4 (H-5).

Method B: To a vigorously stirred solution of **1** (0.65 g, 5 mmol) in anhydrous acetonitrile (30 ml) containing powdered KOH (630 mg, 6 mmol) and the cryptand tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) (100 mg, 0.3 mmol), 2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranosyl chloride (1.95 g, 5 mmol) was added at room temperature and

stirring was continued for 20 min. Insoluble material was removed by filtration and the filtrate was evaporated. Separation of the final product **5** (1.15 g, 47%) was performed as above. The product was spectrally and chromatographically identical with that obtained by method A.

6-Chloro-2-(2′-deoxy-β-D-*erythro*-pentofuranosyl)pyridazin-3(2*H*)-one (**6**)

To a suspension of **5** (1.45 g, 3 mmol) in methanol (30 ml), 1 M methanolic MeONa (3.5 ml) was added and the mixture was refluxed for 1 h. The mixture was adsorbed on silica gel and placed on the top of a silica gel column (3 × 12 cm). Elution with 5% methanol in chloroform gave the desired product. Yield 0.64 g (87%), m.p. 127–129 °C (ethyl acetate) (ref.[1] gives 122–124 °C). MS-ESI, *m/z* (%) (M + Na^+): 269.2 (100), 271 (12). ^{1}H NMR (DMSO-d_6): 2.09 m, 1 H (H-2′a); 2.35 m, 1 H (H-2′b); 3.45 m, 2 H (H-5′ and 5″); 3.75 br q, 1 H (H-4′); 4.31 m, 1 H (H-3′); 4.68 t, 1 H, *J*(OH,5′) = 5.4 (5′-OH); 5.23 d, 1 H, *J*(OH,3′) = 4.5 (3′-OH); 6.56 dd, 1 H, *J*(1′,2′a) = 1.5, *J*(1′,2′b) = 5.4 (H-1′); 7.02 d, 1 H, *J*(4,5) = 9.6 (H-4); 7.53 d, 1 H, *J*(4,5) = 9.7 (H-5). NOE (irrad. H-1′): 5.9% (H-2′a); 1.4% (H-4′). ^{13}C NMR (DMSO-d_6): 37.8 (C-2′), 62.3 (C-5′), 70.7 (C-3′), 84.7 (C-1′), 87.8 (C-4′), 132.5 (C-4), 134.3 (C-5), 137.1 (C-6), 158.4 (C-3). UV, pH 7, λ_{max} (ε): 300 (3100).

6-Chloro-2-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-4-methoxypyridazin-3(2*H*)-one (**7**) and
6-Chloro-2-(2′-deoxy-α-D-*erythro*-pentofuranosyl)-4-methoxypyridazin-3(2*H*)-one (**8**)

To a stirred suspension of **2** (1.05 g, 5 mmol) in acetone (50 ml), DBU (0.91 g, 6 mmol) was added. The mixture became clarified in few minutes. After 10 min, 2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranosyl chloride (1.95 g, 5 mmol) was added portionwise to the solution over 20 min and stirring was continued for 30 min. The reaction mixture was then evaporated to an oil, which was dissolved in methylene chloride (60 ml). The solution was subsequently washed with water (30 ml), brine (2 × 30 ml) and again water (2 × 30 ml), dried with anhydrous magnesium sulfate, and evaporated to dryness. To the residue, methanol (25 ml) and 1 M methanolic MeONa (5 ml) were added and the mixture was stirred at room temperature overnight, evaporated to an oil and chromatographed on a silica gel column (3 × 25 cm). Elution was performed with chloroform (300 ml) and chloroform–methanol 95:5 (v/v). Two nucleoside-containing zones were identified and separated.

The faster-moving zone contained **8**. Yield 170 mg (12%), m.p. 153–155 °C (ethyl acetale). MS-ESI, *m/z* (%) (M + Na^+): 299.0 (100), 301.0 (11). ^{1}H NMR (DMSO-d_6): 2.24 m, 1 H (H-2′a); 2.56 m, 1 H (H-2′b); 3.40 and 3.60 2m, 2 H (H-5′ and 5″); 3.87 s, 3 H (OCH_3); 3.95 m, 1 H (H-4′); 4.07 m, 1 H (H-3′); 4.67 t, 1 H, *J*(OH,5′) = 4.8 (5′-OH); 5.13 d, 1 H, *J*(OH,3′) = 5.9 (3′-OH); 6.49 pt, 1 H, *J*(1′,2′b) = 6.9 (H-1′); 6.95 s, 1 H (H-5). NOE (irrad. H-1′): 4.9% (H-2′b); 1.1% (H-3′). UV, pH 7, λ_{max} (ε): 248 (4100), 281 (6800). For $C_{10}H_{13}ClN_2O_5$ (276.7) calculated: 43.41% C, 4.74% H, 10.12% N; found: 43.55% C, 4.84% H, 10.05% N.

The slower-moving zone contained **7**. Yield 475 mg (34%), m.p. 160–162 °C (ethyl acetale). MS-ESI, *m/z* (%) (M + Na^+): 299.0 (100), 301.0 (12). ^{1}H NMR (DMSO-d_6): 2.15 m, 1 H (H-2′a); 2.51 m, 1 H (H-2′b); 3.45 m, 2 H (H-5′ and 5″); 3.75 q, 1 H, *J*(4′,5′) = 5.5 (H-4′); 3.87 s, 3 H (OCH_3); 4.30 m, 1 H (H-3′); 4.62 t, 1 H, *J*(OH,5′) = 5.8 (5′-OH); 5.17 d, 1 H, *J*(OH,3′) = 4.8 (3′-OH); 6.58 dd, 1 H, *J*(1′,2′a) = 1.8, *J*(1′,2′b) = 5.3 (H-1′); 6.92 s, 1 H (H-5). NOE (irrad. H-1′): 5.1% (H-2′a); 1.2% (H-4′). ^{13}C NMR (DMSO-d_6): 37.9 (C-2′), 57.2 (OCH_3), 61.2 (C-5′), 70.0 (C-3′), 84.4 (C-1′), 86.3 (C-4′), 107.2 (C-5), 138.0 (C-6), 154.6 (C-3), 156.2

(C-4). UV, pH 7, λ_{max} (ε): 248 (4200), 281 (6850). For $C_{10}H_{13}ClN_2O_5$ (276.7) calculated: 43.41% C, 4.74% H, 10.12% N; found: 43.36% C, 4.80% H, 10.06% N.

2-(2′-Deoxy-β-D-*erythro*-pentofuranosyl)-4-methoxypyridazin-3(2*H*)-one (**9**)

A mixture containing **7** (200 mg, 0.73 mmol), palladium on charcoal (10%, 50 mg) and K_2CO_3 (136 mg, 1 mmol) in ethanol (20 ml) was hydrogenated under atmospheric pressure for 3 h (HPLC control). The insolubles were filtered off and the filtrate was evaporated to dryness. Crystallization from ethanol yielded colorless cubic crystals. Yield 135 mg (76%), m.p. 159–161 °C. MS-ESI, *m/z* (%) (M + Na^+): 265.1 (100). ^{1}H NMR (DMSO-d_6): 2.08 m, 1 H (H-2′a); 2.45 m, 1 H (H-2′b); 3.40 2m, 2 H (H-5′ and 5″); 3.74 q, 1 H, *J*(4′,5″) = 4.5 (H-4′); 3.81 s, 3 H (OCH_3); 4.30 m, 1 H (H-3′); 4.62 t, 1 H, *J*(OH,5′) = 5.8 (5′-OH); 5.17 d, 1 H, *J*(OH,3′) = 4.6 (3′-OH); 6.65 dd, 1 H, *J*(1′,2′a) = 1.2, *J*(1′,2b) = 5.6 (H-1′); 6.72 d, 1 H, *J*(5,6) = 4.9 (H-5); 7.85 d, 1 H, *J*(5,6) = 4.9. NOE (irrad. H-1′): 5.1% (H-2′a); 1.1% (H-4′). ^{13}C NMR (DMSO-d_6): 37.8 (C-2′), 56.3 (OCH_3), 62.5 (C-5′), 71.0 (C-3′), 84.6 (C-1′), 87.6 (C-4′), 105.0 (C-5), 137.4 (C-6), 155.1 (C-4), 156.2 (C-3). UV, pH 7, λ_{max} (ε): 240 (sh, 4000), 276 (8200). For $C_{10}H_{14}N_2O_5$ (242.2) calculated: 49.59% C, 5.83% H, 11.56 N; found: 49.55% C, 5.92% H, 11.44% N.

4,5-Dichloro-2-[2′-deoxy-3′,5′-di-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-pyridazin-3(2*H*)-one (**11**)

To a stirred suspension of **10** (1.64 g, 10 mmol) in acetone (50 ml), DBU (1.65 g, 11 mmol) was added. The mixture clarified in few minutes. After 10 min, 2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranosyl chloride (3.95 g, 10.1 mmol) was added portionwise to the solution over 20 min, and stirring was continued for 30 min. The reaction mixture was neutralized with acetic acid and evaporated to oil. This was dissolved in chloroform (70 ml), washed with water (2 × 50 ml) followed by brine (2 × 50 ml) and water (50 ml), dried with anhydrous magnesium sulfate, and evaporated to an oil. The residue was shortly refluxed with methanol (60 ml) and left in a refrigerator overnight. The chromatographically pure precipitate was separated and crystallized from methanol. Yield 2.45 g (47%), m.p. 153 °C (ref.[2] gives 150–151 °C). ^{1}H NMR (DMSO-d_6): 2.37 and 2.40, 6 H (2 CH_3); 2.50 m, 1 H (H-2′a); 2.90 m, 1 H (H-2′b); 4.42 m, 1 H (H-4′); 4.52 m, 2 H (H-5′ and H-5″); 5.68 q, 1 H, *J*(3′,2′) = 4.2 (H-3′); 6.73 dd, 1 H, *J*(1′,2′a) = 2.7, *J*(1′,2′b) = 4.4 (H-1′); 7.37 and 7.88 2m, 8 H (H-arom.); 8.18 s, 1 H (H-6).

4,5-Dibromo-2-[2′-deoxy-3′,5′-di-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-pyridazin-3(2*H*)-one (**12**)

To a stirred suspension of **10** (2.54 g, 10 mmol) in acetone (60 ml), DBU (1.65 g, 11 mmol) was added. The mixture clarified in few minutes. After 10 min, 2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranosyl chloride (3.95 g, 10.1 mmol) was added to the solution portionwise over 30 min, and stirring was continued for 20 min. The reaction mixture was then neutralized with acetic acid and evaporated to an oil. This was dissolved in chloroform (70 ml), washed with water (2 × 50 ml) followed by brine (2 × 50 ml) and water (50 ml), dried with anhydrous magnesium sulfate and evaporated. The oily residue formed was shortly refluxed with methanol (60 ml) and left in a refrigerator overnight. The chromatographically pure precipitate was separated and crystallized from methanol. Yield 3.4 g (56%),

m.p. 145–147 °C. ^{1}H NMR (DMSO-d_6): 2.37 and 2.39, 6 H (2 CH_3); 2.51 m, 1 H (H-2′b); 2.95 m, 1 H (H-2′a); 4.40 m, 1 H (H-4′); 4.48 m, 2 H (H-5′ and H-5″); 5.51 q, 1 H, *J*(3′,2′) = 4.2 (H-3′); 6.69 dd, 1 H, *J*(1′,2′a) = 2.9, *J*(1′2′b) = 4.3 (H-1′); 7.35 and 7.88 2m, 8 H (H-arom.); 8.12 s, 1 H (H-6). For $C_{25}H_{22}Br_2N_2O_6$ (606.3) calculated: 49.53% C, 3.66% H, 4.62% N; found: 49.49% C; 3.23% H, 4.55% N.

6-Bromo-4-chloro-2-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-5-methoxypyridazin-3(2*H*)-one (**13**)

To a stirred suspension of **4** (1.22 g, 5 mmol) in acetone (40 ml), DBU (0.91 g, 6 mmol) was added. The mixture clarified in few minutes. After 10 min, 2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranosyl chloride (1.95 g, 5 mmol) was added to the solution portionwise over 20 min, and stirring was continued for 30 min. The reaction mixture was evaporated to oil which was dissolved in methylene chloride (60 ml). The solution was washed with water (30 ml), brine (2 × 30 ml) and water again (2 × 30 ml), dried with anhydrous magnesium sulfate, and filtered. The filtrate after evaporation was treated with methanol (50 ml), and a white solid crystallized (1.35 g). This contained ca. 15% of α-anomer (HPLC). Attempts to separate the anomers by column chromatography using various solvent combinations were unsuccessful. The precipitate was treated with methanol (35 ml) containing KOH (0.56 g, 10 mmol); the mixture was stirred at room temperature overnight, then neutralized with acetic acid and adsorbed onto silica gel. This was placed on the top of a silica gel column (3 × 30 cm) and chromatographed with chloroform (300 ml) and chloroform–methanol 95:5 (v/v). The slower moving zone contained a mixture of dimethoxylated product and α-anomer (90 mg), and was not investigated further. The faster moving zone contained the title compound. Yield 590 mg (33 %) (ethyl acetate–petroleum ether), m.p. 126–128 °C. MS-ESI, *m/z* (%) (M + Na^+): 376.9 (55), 378.9 (100). ^{1}H NMR (DMSO-d_6): 2.18 m, 1 H (H-2′a); 2.48 m, 1 H (H-2′b); 3.45 m, 2 H (H-5′ and 5″); 3.77 q, 1 H, *J*(5′,4′) = 4.9 (H-4′); 4.04 s, 3 H (OCH_3); 4.31 m, 1 H (H-3′); 4.61 t, 1 H, *J*(OH,5′) = 5.4 (5′-OH); 5.21 d, 1 H, *J*(OH,3′) = 6.3 (3′-OH); 6.55 dd, 1 H, *J*(1′,2′a) = 1.8, *J*(1′,2′b) = 4.9 (H-1′). NOE (irrad. H-1′): 4.9% (H-2′a); 1.0% (H-4′). ^{13}C NMR (DMSO-d_6): 38.0 (C-2′), 58.3 (OCH_3), 62.2 (C-5′), 70.5 (C-3′), 86.1 (C-1′), 87.9 (C-4′), 122.9 (C-4), 125.9 (C-6), 153.9 (C-5), 157.5 (C-3). UV, pH 7, λ_{max} (ε): 264 (3050), 297 (3400). For $C_{10}H_{12}BrClN_2O_5$ (355.6) calculated: 33.78% C, 3.40% H, 7.88 N; found: 33.66% C, 3.53% H, 7.73% N.

4-Chloro-2-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-5-methoxypyridazin-3(2*H*)-one (**14**)

To a stirred suspension of **12** (1.0 g, 2 mmol) in methanol, potassium hydroxide (280 mg, 5 mmol) was added and the stirring was continued overnight. The resulting clear solution was neutralized, adsorbed on silica gel and placed on the top of a silica gel column (3 × 12 cm). Elution with 5% methanol in chloroform gave the desired product. Crystallization from ethyl acetate gave colorless crystals. Yield 0.38 g (69%), m.p 164–166 °C (ref.[2] gives 154–156 °C). MS-ESI, *m/z* (%) (M + Na^+): 299.0 (100), 301.0 (12). ^{1}H NMR (DMSO-d_6): 2.13 m, 1 H (H-2′a); 2.50 m, 1 H (H-2′b); 3.43 m, 2 H (H-5′ and 5″); 3.76 q, 1 H, *J*(5′,4′) = 4.5 (H-4′); 4.09 s, 3 H (OCH_3); 4.33 m, 1 H (H-3′); 4.61 t, 1 H, *J*(OH,5′) = 5.8 (5′-OH); 5.19 d, 1 H, *J*(OH,3′) = 4.7 (3′-OH); 6.64 dd, 1 H, *J*(1′,2′a) = 1.6, *J*(1′,2′b) = 5.3 (H-1′); 8.32 s, 1 H (H-6). NOE (irrad. H-1′): 5.0% (H-2′a); 1.2% (H-4′). ^{13}C NMR (DMSO-d_6): 37.8 (C-2′), 58.2 (OCH_3), 62.4 (C-5′), 70.8 (C-3′), 85.5 (C-1′), 87.7 (C-4′), 113.8 (C-4), 128.3 (C-6), 155.0 (C-5), 157.8 (C-3). UV, pH 7, λ_{max} (ε): 263 (6 100), 290 (6 200).

4-Bromo-2-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-5-methoxypyridazin-3(2*H*)-one (**15**)

To a stirred suspension of **12** (1.2 g, 2 mmol) in methanol, 1 M methanolic sodium methanolate (4 ml, 4 mmol) was added and the stirring was continued overnight. The resulting clear solution was neutralized, adsorbed onto silica gel and placed on the top of a silica gel column (3 × 14 cm). Elution with 5% methanol in chloroform gave the desired product. Crystallization from ethyl acetate with few drops of petroleum ether yielded colorless crystals. Yield 0.47 g (73%), m.p 170–172 °C. MS-ESI, *m/z* (%) (M + Na^+): 345.0 (95), 343.0 (100). ^{1}H NMR (DMSO-d_6): 2.14 m, 1 H (H-2′a); 2.50 m, 1 H (H-2′b); 3.43 m, 2 H (H-5′ and 5″); 3.76 q, 1 H, *J*(5′,4′) = 4.5 (H-4′); 4.09 s, 3 H (OCH_3); 4.33 m, 1 H (H-3′); 4.62 t, 1 H, *J*(OH,5′) = 5.8 (5′-OH); 5.19 d, 1 H, *J*(OH,3′) = 4.7 (3′-OH); 6.65 dd, 1 H, *J*(1′,2′a) = 1.4, *J*(1′,2b) = 5.5 (H-1′); 8.23 s, 1 H (H-6). NOE (irrad. H-1′): 5.2% (H-2′a); 1.3% (H-4′). ^{13}C NMR (DMSO-d_6): 37.9 (C-2′), 58.2 (OCH_3), 62.4 (C-5′), 70.8 (C-3′), 85.7 (C-1′), 87.7 (C-4′), 105.7 (C-4), 128.0 (C-6), 156.8 (C-5), 158.1 (C-3). UV, pH 7, λ_{max} (ε): 269 (5850), 291 (7000). For $C_{10}H_{13}BrN_2O_5$ (321.1) calculated: 37.40% C, 4.08% H, 8.72% N; found: 37.29% C, 4.14% H, 8.78% N.

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