Synthesis of *s*-Triazolo[4,3-*b*]pyridazine *C*-Nucleosides (1)

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A one step synthesis of *s*-triazolo[4,3-*b*]pyridazine using 3-chloro-6-hydrazinopyridazine and an appropriate thioformimidate is described. Applying this procedure to 5-*O*-benzoyl-1-benzylthio-1-formimidate-D-ribofuranose, 5' benzoyl-6-chloro-3β-D-ribofuranosyl-*s*-triazolo[4,3-*b*]pyridazine was obtained. Substitutions of chlorine by nucleophilic reagents afforded some derivatives of a new series of *C*-nucleosides. Structural determination including anomeric configuration assignment is discussed based mainly on ^{1}H nmr spectroscopy.

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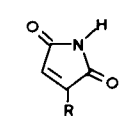
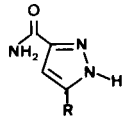
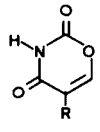
The isolation from various streptomyces (2-4) of several *C*-nucleoside antibiotics characterized by the presence of a carbon to carbon linkage between the sugar and heterocyclic moieties, has stimulated a great interest toward the synthesis of such compounds. Naturally occurring *C*-nucleosides such as showdomycin (**1**), pyrazofurin (**2**), oxazinomycin (**3**) and formycin (**4**), exhibit antitumor activity as well as very specific biochemical properties (5-7).



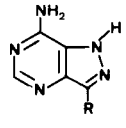
Showdomycin **1**



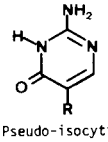
Pyrazofurin **2**



Oxazinomycin **3**



Formycin **4**



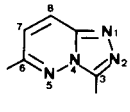
Pseudo-isocytidine **5**

R = β-D-ribofuranose

Pseudoisocytidine (**5**), a synthetic *C*-nucleoside, has been shown to be very effective in inhibiting the growth of P815 and L1210 leukemic cells *in vitro* and *in vivo* (8). Furthermore, these compounds seem to be resistant to catabolic reactions in cells, due to the resistance of the carbon-carbon glycosidic bond to nucleoside phosphorylases (8).

Numerous *C*-nucleosides analogs have been recently synthesized using different starting material (9-16). Ribofuranosylthioimidate has proved to be one useful starting compound leading to different *C*-ribosyl nucleosides including 8-amino-3(β-D-ribofuranosyl)-*s*-triazolo[4,3-*a*]pyrazine (16), 3-β-D-ribofuranosyl-1,2,4 triazolo-5-carboxamide (17) and 3-β-D-ribofuranosyl-*s*-triazolo-[4,3-*a*]pyridine (18).

The present paper reports a convenient synthesis of a new class of *C*-nucleosides, namely, 3-β-D-ribofuranosyl-*s*-triazolo[4,3-*b*]pyridazine derivatives.



Results and Discussion.

Although the *s*-triazolo[4,3-*b*]pyridazine system has already been studied (19), we first investigated conditions for its synthesis which would allow the introduction of a C-3 ribosyl moiety. The reaction of 3-chloro-6-hydrazinopyridazine (**6**) (20) with benzylthio acetimidate (**7**, R = CH_3) and benzylthiophenylacetimidate (**7**, R = $CH_2C_6H_5$) in pyridine afforded 3-substituted-6-chloro-*s*-triazolo-[4,3-*b*]pyridazines (**8**) and (**9**), respectively. However, compound **6** reacted with methyl orthoacetate under reflux giving triazolo pyridazine (**8**) in higher yields (93%). No difficulty had been reported previously for the preparation of 3-aminopyridazine from 3-chloropyridazine although it is known that during aminolysis side reactions may occur, the most common being the solvolytic displacement of the halogen with the formation of methoxypyridazine (21). In the *s*-triazolo[4,3-*b*]pyridazine series, this side reaction seems to be the most important one. Thus, preliminary experiments were performed with compound **8** and methanolic or ethanolic ammonia at 100° in a sealed vessel affording 6-methoxylated and 6-ethoxylated derivatives (**10**) and (**11**) in 82 and 73.8% yield respectively. At room temperature in methanol, a mixture of 6-methoxy derivative (**10**) and 3-methyl-6-amino-*s*-triazolo[4,3-*b*]pyridazine (**12**) was obtained in poor yield. Aqueous ammonia at 100° under pressure transformed the 6-chloro compound (**8**) into a mixture of corresponding 6-amino derivative (**12**) (31%) and 3-methyl-6-hydroxy-*s*-triazolo[4,3-*b*]pyridazine (**13**) (6%), respectively. This latter compound has also been obtained from **8** with a molar equivalent of aqueous potassium hydroxide. In contrast with aqueous methylamine at 100° in a sealed vessel, 6-methylamino derivative (**14**) was obtained in high yield (93%) and no hydroxylated compound (**13**) was detected. In the same way, benzylamine and pyrrolidine reacted with compound **8** in refluxing methyl cellosolve giving the expected corresponding amino derivatives (**15**) and (**16**).

Thus, it can be pointed out that substitution of the 6-chlorine atom in *s*-triazolo[4,3-*b*]pyridazine system

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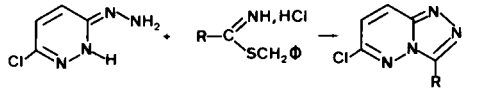
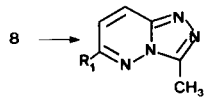
occurs normally with primary and secondary amines and that with alcoholic ammonia, solvolysis could frequently become the predominent reaction.



6 7

8 R = CH_3
9 R = $CH_2\Phi$



10 $R_1 = OCH_3$
11 $R_1 = OC_2H_5$
12 $R_1 = NH_2$
13 $R_1 = OH$
14 $R_1 = NHCH_3$
15 $R_1 = NHCH_2\Phi$
16 $R_1 = N(CH_2)_4$

Figure 1

In the carbohydrate series, condensation of 5-*O*-benzoyl-1-benzylthioformimidate ribofuranose hydrochloride (**17**) (22,23,24) with 6-chloro-3-hydrazinopyridazine (**6**) gave a mixture of 6-chloro-3-α-D-ribofuranosyl-*s*-triazolo[4,3-*b*]-pyridazine (**18α**) (3%) and 52% of the corresponding β-anomer (**18β**). This reaction was performed at room temperature to avoid substitution of 6-chlorine atom by the benzylmercaptan ion. Due to the small quantities of the α-anomer available, deprotection of the 5'-OH group as well as substitutions of the 6-chlorine atom were only studied with the β-anomer (**18β**), by action of several nucleophilic agents under various experimental conditions.

In methanolic ammonia, methoxylated derivative (**19**) was obtained exclusively and no amino derivative (**20**) could be detected. In another attempt performed at 5° under mild conditions, diluted methylamine in methanol to solubilize the starting material transformed **18β** yielding a mixture of four compounds. These are: a) 3-(5'-*O*-benzoyl-β-D-ribofuranoysl)-6-methoxy-*s*-triazolo[4,3-*b*]-pyridazine (**21**) (5.2%); b) the corresponding 6-methyl-

Table I

Relaxation Times (in seconds) for 18α and 18β Anomers

Compound	H1'	H2'	H3'4'5'5"	H7	H8	Hoo'	Hmm'
18α	0.85	—	0.5	1.89	1.88	1.43	1.41
18β	2.30	1.03	0.5	2.05	2.05	1.60	1.48

Hoo' and Hmm' represent *ortho* and *meta* protons, respectively, of the benzoyl group.

Table II

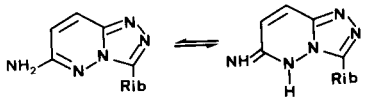
^{1}H Nmr Chemical Shifts (ppm) at 100 MHz in DMSO-d_6 (a)

Compound No.	H-1'	H-2'	H-3'	H-4'	H5'	H5"	OH-2'	OH-3'	OH-5'	H-7	H-8	CH_3	NH	$\varnothing_{oo'}$	$\varnothing_{mm'}$
18α	5.79 (4.4)	4.50 (4.8)	4.23 (8.2)	4.38	-	4.60 (b)	5.03 (4.5)	5.23 (7.1)	-	7.48 (9.7)	8.44	-	-	8.03	7.60
18β	5.32 (4.4)	4.73 (4.6)	4.39	4.25	4.52 (5.6)	4.42 (b)	5.43 (5.5)	5.31 (6.0)	-	7.50 (9.7)	8.45	-	-	8.23	7.88
19	5.19 (6.0)	4.68 (5.0)	4.16 (4.8)	3.89 (4.5)	3.57 (11.6)	3.45	5.17 (6)	5.00 (5.3)	4.68 (5.7)	7.08 (9.8)	8.25	4.00	-	-	-
20 (c)	5.15 (5.7)	4.61		3.98 (d)	3.46 (d)		-	-	-	7.05 (9.7)	8.17	-	-	-	-
21	5.29 (4.8)	4.74	4.49	4.39	4.22 (d)		5.36 (5.6)	5.28 (6.3)	-	7.05 (9.8)	8.23	3.98	-	7.90	7.57
22	5.25 (5)	4.71	4.11			4.51 (d)	5.30	4.80	-	6.77	7.89 (10)	2.81 (5)	7.18	7.94	7.54
23	5.13 (5.9)	4.66 (5.3)	4.15 (4.3)	3.86 (4.5)	3.58 (11.7)	3.45	5.11 (6)	4.94 (5.3)	4.70 (5.7)	6.79 (9.9)	7.89	2.81 (4.9)	7.38	-	-

(a) Numbers in parentheses represent coupling constants, in hertz, between vicinal protons and are given in the order: $J_{1'2'}$, $J_{2'3'}$, $J_{3'4'}$, $J_{4'5'}$, $J_{5'5''}$, $J_{4'5'}$, $J_{2'OH2'}$, $J_{3'OH3'}$, $J_{5'OH5'}$, J_{78}, J_{NHCH_3}. (b) Assignments not certain for these three protons. (c) In DMSO-d_6, broad signals were obtained, therefore nmr spectrum was carried out with **20** in DMSO-d_6 in the presence of 10μl of deuterium oxide and 10 μl of deuteriotrifluoroacetic acid. (d) Non resolved signals.

amino-5′-*O*-benzoyl derivative (**22**) (10.6%); c) the 5′-deprotected 6-methoxynucleoside (**19**) identical with the one already obtained (15.6%); and d) 6-methylamino-3-β-D-ribofuranosyl-*s*-triazolo[4,3-*b*]pyridazine (**23**) (10.7%).

Again these findings emphasize the solvolytic effect of the methanol and the easy substitution of the 6-chlorine atom. They explain why we failed in obtaining selective 5′-*O*-debenzoylation in order to prepare 6-chloro-*C*-nucleoside (**25**). With the goal of avoiding alcoholysis of the chlorine atom, compound **18**β was treated with aqueous ammonia under various conditions (100° for 18 hours, 60° for 4 hours or 18 hours at room temperature). In every case, a mixture of at least six compounds was obtained which separated by analytical thin layer chromatography on silical gel (ethyl acetate-methanol-water-formic acid: 100-25-20-1); (**20**) was isolated from this mixture in low yield (5.6%). Since no difficulty was encountered to synthesize the methylamino derivative (**23**), the problems to obtain the amino derivative (**20**) were rather surprising. On the other hand, the behavior of **20**, in the nmr was unusual. Broad signals were observed for all protons in DMSO-d_6 whereas good resolutions resulted from addition of a small amount of trifluoroacetic acid and water. This may be interpreted by the presence of an equilibrium between two prototropic forms:



This hypothesis, which is consistent with the possibility of hydrogen bonding between the base and cyclic oxygen of the sugar explains the unresolved peaks in nmr when performed in DMSO. Only one form is present under acidic conditions. The presence of two forms for **20** may also explain the additional fragmentation observed in mass spectrometry.

Surprisingly with sodium hydroxide in aqueous dioxane, no expected hydroxylated nucleoside (**24**) could be characterized. We have not found satisfactory explanation to this failure. However, aqueous methylamine at 100° in a sealed vessel afforded in satisfactory yield (55%), 6-methylamino-3-*C*-nucleoside (**23**) already obtained in low yield under milder conditions in methanol.

Structural assignments of nucleosides were based on elemental analysis, uv, ^{1}H nmr and mass spectra. In agreement with previous work using ribosylthioformimidate derivatives (16-18) condensation of 3-chloro-6-hydrazinopyridazine (**6**) with thioformimidate (**17**) afforded predominantly the β-anomer which was separated from the α-anomer by chromatography on a silice gel column. A β/α ratio of 95/5 was found.

The availability of the α and β anomers of the 5′ OH protected compounds (**18**) allowed an easy determination

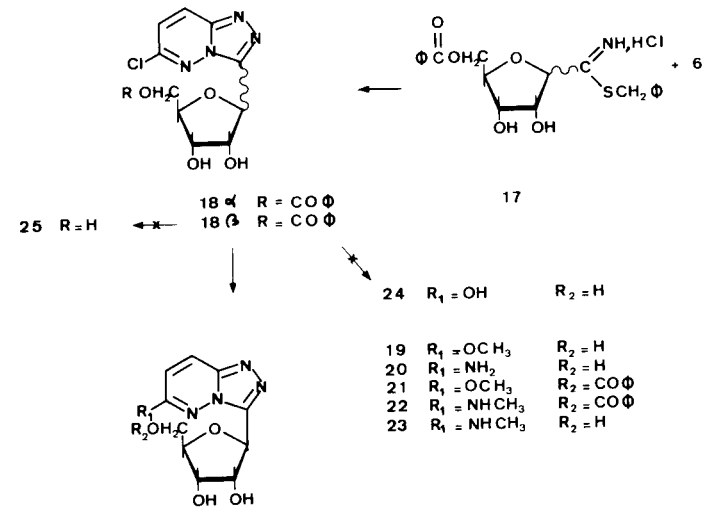


Figure 2

of their anomeric configuration by ^{1}H nmr spectroscopy. Thus, it has been shown previously (25) that the relaxation time of the anomeric H 1′ proton of the β anomer is about two and a half times longer than that of the α anomer which in turn is of the same order of magnitude than the other protons of the ribose ring. Therefore **18**α and **18**β have been unambiguously identified by the measurement of the relaxation times of ribose protons (Table I). Furthermore chemical shift assignments of the different protons is in full agreement with the proposed structures (Table II).

The structure of the nucleosides is also supported by their mass spectra which show characteristic peaks at B + 2H, B + 30 and B + 44 (Table III) as observed previously (18,23). As expected B + H and B + 2H fragments are absent or very low for all C nucleosides studied. This is in itself indicative of the carbon-carbon glycosidic bond structure. On the other hand, B + 30 and B + 44 are always observed as major fragmentations. Nevertheless B + 44 is the major peak (100% relative intensity) and this represents an exception to the proposed emperical rule which stipulates the B + 30 fragment as the major peak (100% relative intensity) in *C*-nucleosides (26,27). As a characteristic feature of the C-C glycosidic bond structure, the absence of fragmentation giving a peak at m/e 133 (ribose) should be pointed out.

Ultraviolet absorption spectra of all *C*-nucleosides presently described are similar to those of the corresponding heterocycle compound.

EXPERIMENTAL

Melting points were determined with a Kofler Apparatus and were uncorrected. Ultraviolet spectra were recorded with Cary 118C. Nmr spectra were obtained using a Varian XL-100 with tetramethylsilane as the internal reference. Mass spectra were obtained by M.C. Bosso, CERMAV, Grenoble, with a MS30 AEI Cratos apparatus equipped with a computer

Table III

Mass Spectrometry Data at 70 ev, 150°C (a)

Compound	B + 2H	B + 30	B + 44	M+	Other Peaks (b)
18α	155 (14)	183 (85)	197 (32)	390 (1.6)	105 (100)
18β	155 (18)	183 (85)	197 (100)	390 (4.3)	105 (66)
19	151 (13)	179 (37)	193 (100)	—	—
20	136 (32)	164 (48.2)	178 (100)	—	122 (29); 128 (23); 149 (36); 202 (11)
21	151 (14)	179 (100)	193 (47)	386 (5)	105 (60)
22	150 (15)	178 (35)	192 (100)	—	105
23	150 (17)	178 (54)	192 (100)	—	—

(a) Numbers in parentheses represent percentages of the base peak. (b) C_6H_5-C≡$\hat{O}$ ion: m/e = 105.

Table IV

Ultraviolet Absorption Spectral Data

Compound	λ max (ε) in ethanol	
8	216 (28200)	311 (2400)
9	220 (30000)	310 (2100)
10	212 (21200)	272 (3700)
12	224 (26900)	284 (5200)
13	216 (26400)	311 (2000)
14	229 (26800)	285 (4800)
18α	220 (38200)	310 (2500)
18β	220 (36600)	310 (1800)
19	216 (20000)	272 (3600)
20	224 (19900)	275 (4800)
21	214 (24500)	272 (2000)
22	230 (28000)	285 (3200)
23	230 (25000)	285 (3900)

Varian 100 MS. Chromatography were performed with Kieselgel 60, 230 mesh, Merck. Microanalyses were performed by the "Service Central de Microanalyse" CNRS-ICSN, 91190-Gif/Yvette, France.

6-Chloro-3-methyl-*s*-triazolo[4,3-*b*]pyridazine (**8**).

Method A.

Ten g (69 mmoles) of 3-chloro-6-hydrazinopyridazine (20) (**6**) and 13.9 g (69 mmoles) of benzylthiomethylacetamimidate (**7**) (R = CH_3) in 100 ml of pyridine were stirred overnight at room temperature and heated under reflux for an additional 2 hours. The residue, after evaporation under reduced pressure, was extracted twice with hot cyclohexane. Pure compound crystallized after cooling to yield 6.5 g (56%) of **8** mp 107-108°.

Method B.

3-Chloro-6-hydrazinopyridazine (20) (**6**) (10 g, 69 mmoles) in distilled methyl orthoacetate (60 ml) was heated under reflux overnight. After evaporation to dryness, the residue was recrystallized in cyclohexane to afford 10.8 g (93%) of **8** mp 107-108°; ms: m/e 168.0 (100% M+) and 170.0 (31% M+).

Anal. Calcd. for $C_6H_5ClN_4$ (168.5): C, 42.72; H, 2.96; N, 33.23. Found: C, 42.91; H, 3.08; N, 33.02.

3-Benzyl-6-chloro-*s*-triazolo[4,3-*b*]pyridazine (**9**).

A solution of 5.8 g (40.1 mmoles) of 3-chloro-6-hydrazinopyridazine (**6**) and 11.1 g (40 mmoles) of benzylthiophenylacetimidate (**7b**) (R, $CH_2\phi$) (28) in 100 ml of pyridine was stirred overnight at room temperature and refluxed for an additional 2 hours. The residue of evaporation was purified on a silica gel column (ethyl acetate-ethanol 9-1 v/v) yielding 1.4 g (14%) of **9** mp 128-130°; ms: m/e 244 (8% = M+) and 209 (100% = M − 35).

Anal. Calcd. for $C_{12}H_9ClN_4$ (244.5): C, 58.89; H, 3.68; N, 22.9; Cl, 14.51. Found: C, 59.13; H, 3.82; N, 22.47; Cl, 14.06.

3-Methyl-6-methoxy-*s*-triazolo[4,3-*b*]pyridazine (**10**).

6-Chloro-3-methyl-*s*-triazolo[4,3-*b*]pyridazine (**8**) (2 g, 11.8 mmoles) was heated in a steel vessel at 100° for 4 hours in 80 ml of methanol previously saturated with ammonia. After evaporation of the solvent, recrystallization from hot cyclohexane yielded 1.6 g (82%) of **10**, mp 170°.

Anal. Calcd. for $C_7H_8N_4O$ (164): C, 51.21; H, 4.91; N, 34.1. Found: C, 51.37; H, 4.91; N, 33.97.

6-Ethoxy-3-methyl-*s*-triazolo[4,3-*b*]pyridazine (**11**).

As described above for **10**, **11** (1.31 g, 73.8%) was obtained from **8** (1.68 g, 10 mmoles) in 80 ml of ethanol-ammonia for 4 hours at 100° in a steel vessel, mp 135°.

Anal. Calcd. for $C_8H_{10}N_4O$ (178): C, 53.92; H, 5.66; N, 31.45. Found: C, 53.85; H, 6.01; N, 31.25.

6-Amino-3-methyl-*s*-triazolo[4,3-*b*]pyridazine (**12**) and 3-Methyl-6-hydroxy-*s*-triazolo[4,3-*b*]pyridazine (**13**).

6-Chloro-3-methyl-*s*-triazolo[4,3-*b*]pyridazine (**8**) (1 g, 5.9 mmoles) was heated in 28% aqueous ammonia (100 ml) in a steel vessel for 4 hours at 120°. After addition of sodium hydroxide (236 mg, 5.9 mmoles) and evaporation, recrystallization from hot water afforded 273 mg (31.3%) of **12**, mp > 250°.

Anal. Calcd. for $C_6H_7N_5$ (149): C, 48.32; H, 4.69; N, 46.97. Found: C, 48.37; H, 4.65; N, 46.67.

Recrystallization of the residue from hot dioxanne afforded 54 mg (6.1%) of **13**, mp > 250°; ms: m/e 150.1 (100% M+).

Anal. Calcd. for $C_6H_6N_4O$ (150): C, 48.00; H, 4.02; N, 37.32. Found: C, 47.97; H, 3.98; N, 37.19.

6-Methylamino-3-methyl-*s*-triazolo[4,3-*b*]pyridazine (**14**).

6-Chloro-3-methyl-*s*-triazolo[4,3-*b*]pyridazine (**8**), 1 g (5.9 mmoles) was heated at 100° for 4 hours in a steel vessel with 100 ml of 33% aqueous methylamine.

After evaporation to dryness and solubilization in water, sodium hydroxide was added to neutralize the chlorhydrate. Recrystallization from hot water yielded 900 mg (93%) of pure **14**, mp 246-247°; ms: m/e 163.1 (100 M+).

Anal. Calcd. for $C_7H_9N_5$ (163): C, 51.53; H, 5.52; N, 42.94. Found: C, 51.27; H, 5.46; N, 42.84.

6-(Benzylamino)-3-methyl-*s*-triazolo[4,3-*b*]pyridazine (**15**).

Compound **8** (1.68 g, 10 mmoles) was heated under reflux for 1 hour with benzylamine (3.21 g, 30 mmoles) in 20 ml of methylcellosolve. The solvent was evaporated under diminished pressure and the residue recrystallized from ethanol yielding 1.5 g (62.7%) of **15**, mp 226-227°.

Anal. Calcd. for $C_{13}H_{13}N_5$ (239.27): C, 65.25; H, 5.48; N, 29.27. Found: C, 65.53; H, 5.78; N, 29.60.

3-Methyl-6(1 pyrrolidin)-*s*-triazolo[4,3-*b*]pyridazine (**16**).

As described above for **15** (1.6 g, 44.2%) of **16** was obtained from **8** (3 g, 17.8 mmoles), pyrrolidine (3.1 g, 43 mmoles) and 15 ml of methylcellosolve under reflux for 1 hour, mp 178° (ethanol).

Anal. Calcd. for $C_{10}H_{13}N_5$ (203): C, 59.0; H, 6.45; N, 34.46. Found: C, 58.82; H, 6.46; N, 34.31.

5-*O*-Benzoyl-1-benzylthioformimidate-D-ribofuranosyl, Chlorhydrate (**17**).

This compound was synthesized from 5-*O*-benzoyl-D-ribofuranosyl-1-cyanide (22,24) as described by Huynh-Dunh, *et al* (23) except that ether oxide was replaced by anhydrous dioxane.

3-(5′-*O*-Benzoyl-α and -β-D-ribofuranosyl)-6-chloro-*s*-triazolo[4,3-*b*]-pyridazine (**18**α) and (**18**β).

A solution of 8.1 g (19.1 mmoles) of freshly prepared thioformimidate **17** and 3 g (20.7 mmoles) of **6** in 70 ml of pyridine was stirred under anhydrous conditions for 24 hours at room temperature. The solution was then evaporated to dryness, and the residue was dissolved in aqueous methanol and neutralized with sodium hydroxide (1*N*). Silical gel column chromatography was then carried out in dichloromethane-ethanol (96-4) (50 × 4 cm) which allowed co-purification of the α and β anomers **18** (3.8 g, 51.3%). Compound **18**β recrystallized in ethanol and chromatography (50 × 2 cm) (ethyl acetate-ethanol: 8-2) (Rf **18**α = 0.51; Rf **18**β = 0.63) of the mother liquor afforded a total 3.61 g of **18**β (overall yield of 48.7%) and 0.19 g (overall yield of 2.6%) of **18**α. Compound **18**α had $[\alpha]_D^{20} = -53°$ (C = 0.45 ethanol) and **18**β had $[\alpha]_D^{20} = -98°$ (C = 1.1 ethanol). Compound **18**β had mp 152°.

Anal. Calcd. for $C_{17}H_{15}ClN_4O_5$ (390.5): C, 52.24; H, 3.84; N, 14.34; Cl, 9.09. Found: C, 51.90; H, 3.79; N, 14.05; Cl, 9.01.

Compound **18**α had mp 211°.

Anal. Calcd. for $C_{17}H_{15}ClN_4O_5$ (390.5): C, 52.25; H, 3.84; N, 14.34; Cl, 9.09. Found: C, 51.85; H, 3.97; N, 13.94; Cl, 9.24.

6-Methoxy 3-(β-D-ribofuranosyl)-*s*-triazolo[4,3-*b*]pyridazine (**19**).

A solution of 1 g (2.5 mmoles) of 3-(5′-*O*-benzoyl-β-D-ribofuranosyl)-6-chloro-*s*-triazolo[4,3-*b*]pyridazine (**18**) in methanolic ammonia (100 ml) was heated in a sealed tube overnight at 100°. The solvent was evaporated and the residue chromatographed on Sephadex G10 (water) (100 × 2 cm). Crystallization from ethanol yielded 0.4 g (56.7%) of **19**, mp 96°, Rf 0.19 (ethyl acetate-ethanol, 8/2); $[\alpha]_D^{20} = -45°$ (C = 0.35, ethanol).

Anal. Calcd. for $C_{11}H_{14}N_4O_5H_2O$: C, 44.00; H, 5.37; N, 18.66; O, 31.97. Found: C, 44.05; H, 5.17; N, 19.02; O, 31.76.

6-Amino-3-(β-D-ribofuranosyl)-*s*-triazolo[4,3-*b*]pyridazine (**20**).

A solution of 390,5 mg (1 mmole) of 3-(5′-*O*-benzoyl-β-D-ribofuranosyl)-6-chloro-*s*-triazolo[4,3-*b*]pyridazine (**18**β) in 34% aqueous ammonia (100 ml) was stirred overnight at room temperature. Thin layer chromatography of the resulting solution showed six different spots (silicagel, ethyl acetate-methanol-water-formic acid: 100-25-20-1). Only the product giving a Rf of 0.26 similar to the 6-methylamino derivative **23** was purified. Purification consisted of silica gel column chromatography with the solvent system mentionned above followed by chromatography with Sephadex G10 in water and recrystallization in ethanol which gave 15 mg (5.6%) of **20**, mp > 250°.

Anal. Calcd. for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.90. Found: C, 44.73; H, 4.77.

3(5′-*O*-Benzoyl-β-D-ribofuranosyl)-6-methoxy-*s*-triazolo[4,3-*b*]pyridazine (**21**) and 6-methylamino-3-(5′-*O*-benzoyl-β-D-ribofuranosyl)-*s*-triazolo-[4,3-*b*]pyridazine (**22**).

A solution of 390.5 mg (1 mmole) of **18**β in 30 ml of methanol and 3 ml of a 33% aqueous solution of methylamine was stirred (+5°) for 3 hours. After evaporation to dryness, the residue was subjected to a silica gel column (50 × 2 cm) (ethylacetate/methanol, 8:2) which allowed the separation of 4 compounds in the order **21** (20 mg, 5.2%); **22** (30 mg, 10.6%); **19** (60 mg, 15.6%) and **23** (30 mg, 10.7%) (**19** and **23** have been obtained in better yield in another experiment).

Compound **21** had mp 146-147°.

Anal. Calcd. for $C_{18}H_{18}N_4O_6 \cdot \frac{1}{2}H_2O$: C, 54.68; H, 4.81; N, 14.17. Found: C, 54.62; H, 4.69; N, 14.43.

Compound **22** had mp 72°.

Anal. Calcd. for $C_{18}H_{19}N_5O_5$: C, 56.10; H, 4.97; N, 18.17. Found: C, 56.26; H, 4.81; N, 18.07.

6-Methylamino-3-(β-D-ribofuranosyl)-*s*-triazolo[4,3-*b*]pyridazine (**23**).

A solution of 1.12 g (2.86 mmoles) of 3-(5′-*O*-benzoyl-β-D-ribofuranosyl)-6-chloro-*s*-triazolo[4,3-*b*]pyridazine (**8**) in 33% aqueous methylamine (100 ml) was heated in a sealed tube overnight at 100°. After evaporation to dryness, the residue was subjected to a Sephadex G10 column (water) (100 × 2 cm). Recrystallization from ethanol yielded 444 mg (55%) of **23**, mp 198°; $[\alpha]_D^{20} = -24°$ (C = 0.55, ethanol).

Anal. Calcd. for $C_{11}H_{15}N_5O_4,H_2O$ (299): C, 44.14; H, 5.73; N, 23.40. Found: C, 44.18; H, 5.80; N, 23.46.

REFERENCES AND NOTES

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