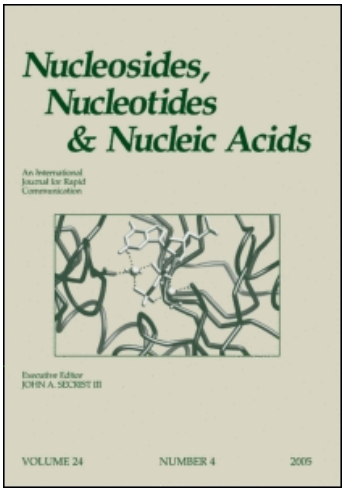

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Nucleosides, XLIV[1] Synthesis, Properties and Biological Activity of Indazole Nucleosides

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NUCLEOSIDES, XLIV[1]
SYNTHESIS, PROPERTIES AND BIOLOGICAL ACTIVITY OF INDAZOLE NUCLEOSIDES

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Abstract. Various new haloindazole-1-ß-D-ribofuranosides (10-17, 20, 21) and a 2-ß-D-ribofuranoside (18) have been synthesized by the fusion method and by direct halogenations, respectively. The new nucleosides have been characterized by UV and ^{1}H NMR spectra as well as pK_a determinations. Indazole ribofuranosides behave in aqueous acid like purine and benzimidazole nucleosides showing the same mechanism of cleavage of the glycosidic bonds. Toxicity studies against various cell populations indicate only little biological activities.

1-ß-D-Ribofuranosides of halogenated benzimidazoles show antiviral activity[2,3,4] and act as highly active inhibitors of cell RNA synthesis.[5,6] 5,6-Dichloro-1-ß-D-ribofuranosylbenzimidazole (DRB) in particular has extensively been studied and found to induce interferon production in human fibroblasts besides inhibiting hnRNA synthesis[7,8] by interfering with the incorporation of adenosine into RNA.[6] Considerations of structural analogy caused us to synthesize and investigate halogen ated indazoleribosides as potential antiviral and anticancer agents.

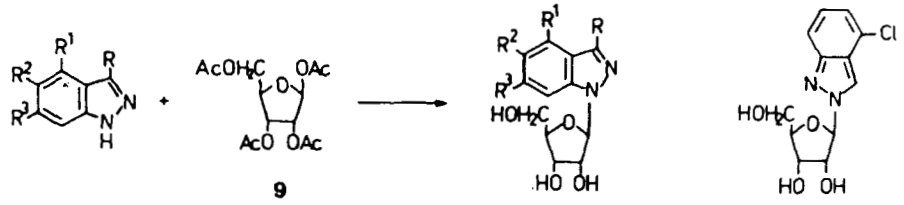
S Y N T H E S E S

Earlier studies on the glycosylation of indazoles indicated that the silyl- and $Hg(CN)_2$-nitromethane methods[9] respectively lead preferentially to the formation of 2-glycosyl indazoles.[10-13] The synthesis of the isomeric N-1-D-glucopyranosides and N-1-D-ribofuranosides[14], however, is achieved by the fusion method which is controlled thermodynamically and gives rise, in presence of Lewis acids as catalysts, to the most stable isomer. The dependence of the product formation, which includes also the α-nucleosides in minor amounts, has been studied under various conditions.[15-18]

Halogenations of unsubstituted indazoles proceed in aqueous and organic media, respectively, according to the sequence 3,5,7. This is the reason why most haloindazoles bear substituents at these positions of the nucleus. On the other hand, it was also observed that N-1 and N-2 subsituted indazoles are halogenated first at position 5.[19] To start the study from a broad variety of haloindazoles, we synthesized first 6-chloro-(7) and 5,6-dichloroindazole (8) as new derivatives from 4-chloro- and 4,5-dichloro-o-toluidine, respectively, by acetylation, N-nitrosation and subsequent rearrangement and ring closure.

The haloindazoles 1-8 were then ribosylated in a fusion reaction with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (9) and p-toluenesulfonic acid or iodine as catalysts at a temp. of 160°C for 20 min. The reaction mixture was treated directly with sodium methoxide in methanol according to Zemplen[20] and then the free 1-β-D-ribofuranosylindazoles 10-17 isolated chromatographically in moderate to good yield as the main reaction products. Milder fusion conditions (145-150°C, 15 min) led to preferential N-2 substitution as demonstrated by the conversion of 4-chloroindazole (4) into 4-chloro-2-β-D-ribofuranosylindazole (18) in 50 % yield.

Compound 15 was also obtained in 67 % yield by reaction of 1-β-D-ribofuranosylindazole (19) with bromine in water at room temp. Chlorination of 19 gave a more complex

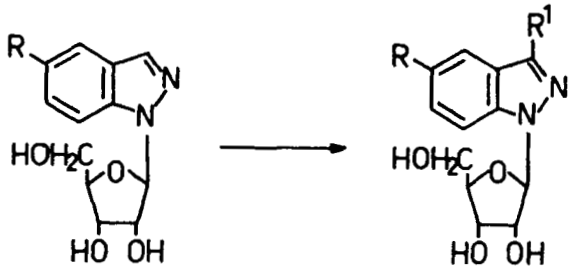


9

18

	R	R^1	R^2	R^3
1	Cl	H	H	H
2	Br	H	H	H
3	I	H	H	H
4	H	Cl	H	H
5	H	H	Cl	H
6	H	H	Br	H
7	H	H	H	Cl
8	H	H	Cl	Cl

	R	R^1	R^2	R^3
10	Cl	H	H	H
11	Br	H	H	H
12	I	H	H	H
13	H	Cl	H	H
14	H	H	Cl	H
15	H	H	Br	H
16	H	H	H	Cl
17	H	H	Cl	Cl



	R	R^1
14	Cl	H
15	Br	H
20	Cl	Br
21	Br	Br

	R
14	Cl
15	Br
19	H

mixture of compounds, from which 14 could be separated chromatographically and characterized by comparison with the fusion product 14. Extended bromination of 14 and 15 led to the isolation of 3-bromo-5-chloro-(20) and 3,5-dibromo-1-β-D-ribofuranosylindazole (21).

PHYSICAL AND CHEMICAL PROPERTIES

The structural assignment of the newly synthesized haloindazole nucleosides was achieved by UV and ^{1}H-NMR spectral comparisons. The UV spectra differentiate nicely betwenn N-1 and N-2 substitution, of which the latter type exhibits a different shape, absorbs at longer wave lengths and shows a higher extinction due to the quinonoid character of the molecule. Introduction of the halogen atoms Cl, Br, I into the 3-position is associated with a gradual bathochromic shift of the spectrum and an increase in extinction of the long wavelength band in this order. A comparison of the monochloroindazole-1-β-D-ribofuranosides indicated that the blue shift of the UV spectrum follows the sequence 6, 4, 3, 5 (Table 1 and 2).

The determination of the basic pK_a values of the indazole nucleosides by the spectrophotometric method[21] indicated that this group of nucleosides are weakly basic and, in general, are not protonated in the normal pH range. Cation formation is associated with a small bathochromic shift of the long wavelength absorption band as expected from the protonation of the N-2 ring atom. This fact is in agreement with the strongest base weakening effects of the 3-halo substituents, whereas substitutions at the benzene ring are of minor influence, as seen from comparisons with the unsubstituted 1-β-D-ribofuranosylindazole (19). It is also noteworthy to mention that the 4-chloro-2-β-D-ribofuranosylindazole (18) is a somewhat stronger base than its N-1 isomer (13) expressing nicely the differences in resonance stabilization of the quinonoid versa the benzenoid system.

The anomeric configuration of the glycosidic linkage of the 1-β-D-ribofuranosylhaloindazoles can be depicted from the ^{1}H-NMR spectra. The chemical shifts of the C-1' protons in D_6-DMSO/D_2O appear all in a small range of 5.99-6.12 ppm and are therefore in close agreement with the corresponding signal 1-β-D-ribofuranosylindazole.[17] The coup-

Table 1 - Physical Data of Haloindazole Nucleosides

-1-β-D-ribofuranosyl-indazole	pK_a in H_2O	UV Absorption Spectra λ_{max} (nm)	UV Absorption Spectra log ε	Ho pH
(19)	-1.5	203 256 [261] 299 249 [255] 287 [296]	4.59 3.96 [3.91] 3.74 3.73 [3.67] 3.66 [3.56]	-4.0 6.0
3-Chloro- (10)	-3.8	206 260 [266] 308 252 260 292 [300]	4.46 3.85 [3.82] 3.85 3.66 3.59 3.78 [3.69]	-6.2 6.0
3-Bromo- (11)	-3.8	206 261 [267] 310 253 [259] 292 [302]	4.46 3.85 [3.82] 3.88 3.62 3.55 3.79 [3.71]	-6.2 6.0
3-Iodo- (12)	-2.8	208 260 [268] 313 254 [260] 296 [303]	4.49 3.86 [3.79] 3.98 3.60 [3.52] 3.85 [3.78]	-4.9 6.0
4-Chloro- (13)	-2.0	208 261 268 306 254 262 291 [300]	4.45 3.84 3.81 3.83 3.76 3.73 3.72 [3.61]	-4.0 6.0
5-Chloro- (14)	-1.9	210 257 [264] 310 [244] 252 [258] 298 [308]	4.60 3.84 [3.77] 3.66 [3.60] 3.65 [3.58] 3.57 [3.46]	-4.0 6.0
5-Bromo- (15)	-1.9	210 258 [265] 311 242 252 [258] 298 [306]	4.52 3.83 [3.77] 3.61 3.64 3.65 [3.57] 3.54 [3.44]	-4.0 6.0
6-Chloro- (16)	-1.8	206 270 [272] 297 256 263 289 298	4.48 4.00 [4.00] 3.78 3.84 3.82 3.67 3.61	-4.0 6.0
5,6-Dichloro-(17)	-2.2	216 270 [277] 311 222 264 270 301 310	4.58 3.94 [3.90] 3.68 4.57 3.63 3.61 3.57 3.51	-4.3 6.0
3-Bromo-5-chloro- (20)	-4.3	215 262 [269] 321 257 [263] 303 [312]	4.55 3.75 [3.69] 3.78 3.56 [3.49] 3.70 [3.62]	-6.8 6.0
3,5-Dibromo-(21)	-4.3	219 263 [271] 323 245 258 [266] 303 [310]	4.53 3.83 [3.77] 3.81 3.58 3.60 [3.47] 3.72 [3.60]	-6.8 6.0
4-Chloro-2-β-D- (18)	-0.7	206 213 266 273 304 [270] 279 297	4.36 4.36 3.99 3.97 3.75 [3.77] 3.86 3.83	-3.0 6.0

[ ] = Shoulder

Table 2 - ^{1}H NMR Data for the Haloindazole Nucleosides

-1-β-D-Ribofurano-syl-indazole	^{1}H-NMR Spectra in D_6-DMSO/D_2O (δ-values in ppm)									
	3-H	Aromatic-H			1'-H	$J_{1',2'}$	2'-H	3'-H	4'-H	5'-H
(19)	8.16s	7.76m	7.42pt	7.17pt	6.08d	4.6	4.63pt	4.20pt	3.91m	3.45m
3-Chloro- (10)	-	7.74d	7.65d	7.51pt 7.28pt	6.02d	4.2	4.57pt	4.16pt	3.91m	3.48m
3-Bromo- (11)	-	7.80d	7.59d	7.52pt 7.29pt	6.03d	4.9	4.61pt	4.15pt	3.90m	3.45m
3-Iodo- (12)	-	7.64d	7.49d	7.40pt 7.23pt	6.02d	4.9	4.61pt	4.18pt	3.94m	3.48m
4-Chloro- (13)	8.26s	7.82d	7.43pt	7.28d	6.12d	4.6	4.64pt	4.23pt	3.94m	3.50m
5-Chloro- (14)	8.08s	7.74s	7.63d	7.34d	6.02d	5.2	4.62pt	4.21pt	3.95m	3.50m
5-Bromo- (15)	8.13s	7.98s	7.59d	7.51d	6.02d	4.9	4.60pt	4.19pt	3.92m	3.47m
6-Chloro- (16)	8.21s	8.00s	7.81d	7.22d	6.11d	4.6	4.61pt	4.21pt	3.92m	3.53m
5,6-Dichloro-(17)	8.24s	8.18s	8.08s		6.07d	4.9	4.55pt	4.17pt	3.91m	3.48m
3-Bromo-5-chloro- (20)	-	7.86d	7.64s	7.51d	6.02d	4.9	4.56pt	4.14pt	3.91m	3.47m
3,5-Dibromo-(21)	-	7.76s	7.69d	7.59d	5.99d	4.9	4.57pt	4.14pt	3.92m	3.51m
4-Chloro-2-β-D- (18)	8.78s	7.59d	7.26pt	7.13d	5.97d	3.6	4.37pt	4.18pt	4.10m	3.65m

s = Singlet; d = doublet; pt = pseudotriplet; m = multiplet.

ling constants of these doublets are also very similar between 4.2 and 5.2 Hz, indicating a close resemblance of the conformations of the sugar moieties.

Hydrolysis in aqueous acid.

The acid-catalyzed hydrolysis of purine nucleosides has been established to proceed by a rate-limiting formation of a cyclic glycofuranosyl oxocarbenium ion.[22] The same mechanism has also been applied to the hydrolysis of benzimidazole nucleosides[23] and (α-L-arabinofuranosyl)-bromoisoquinolinium salt.[24] In contrast, the nucleosides of 7-deazaadenine have been shown to be hydrolyzed via cationic Shiff base with concurrent isomerization of the sugar moiety.[25] Accordingly, the mechanism for the acidic hydrolysis of isosteric analogues of purine nucleosides cannot be predicted a priori and we have therefore performed measurements to clarify the kinetics and the mechanism of the hydrolytic degradation of the newly synthesized indazole nucleosides.

As seen from table 1, indazole nucleosides are extremely weakly basic compounds with pK_a values of monocation formation ranging from -0.7 to -4.3 at 298.2 K. Consistence with this observation are the first order rate constants for the hydrolysis which are proportional to the acidity of the reaction mixture at oxonium ion concentration less than 1.0 mol dm^{-3}, and show a marked levelling to a constant value only in several molar acid solutions. The rate of hydrolysis is rather insenstive to the polar properties of the base moiety, indicating that the influences on the pre-equilibrium protonation and rate-limiting heterolysis are opposite. The rate constants, $k(SH^+)$, calculated for the heterolysis of the monocations of the substrates studied are listed in table 3 together with the observed second-order rate constants, k(obs.).The dependence of log $[k(SH^+)/s^{-1}]$ on log $K(SH^+)$ is very similar to that reported for purine[26] and benzimidazole nucleosides[23] and the points fall on the same correlation as seen from fig. 1. This may be

Table 3 - Observed second-order rate constants, k(obs.), for the hydrolysis of indazole nucleosides in aqueous hydrogen chloride at 363.2 K, acidity constants, $K(SH^+)$, of their monocations and rate constants, $k(SH^+)$, for the heterolysis of the monocations under the same conditions.

1-β-D-ribofuranosylindazole		k(obs.)[a] / 10^{-3} dm^3 mol^{-1} s^{-1}	$K(SH^+)$[b] / 10^3 mol dm^{-3}	$k(SH^+)$ / s^{-1}
	(19)	9.5 (5)	0.063	0.60
3-Chloro-	(10)	1.4 (2)	17	23
3-Bromo-	(11)	1.6 (2)	16	26
3-Iodo-	(12)	2.8 (2)	1.6	4.5
4-Chloro-	(13)	1.1 (1)	0.23	0.25
5-Chloro-	(14)	3.4 (2)	0.17	0.58
5-Bromo-	(15)	2.9 (2)	0.72	0.64
6-Chloro-	(16)	2.3 (1)	0.15	0.35
5.6-Dichloro-	(17)	0.80(9)	0.42	0.34
3-Bromo-5-chloro-	(20)	0.60(5)	50	300
3,5-Dibromo-	(21)	0.53(5)	50	270
4-Chloro-2-β-D-	(18)	22.0 (2)	0.013	0.29

[a]The first-order rate constants obtained in aqueous HCl were proportional to the Ho value of the solution over the acidity range studied ($0.02 < [HCl]/mol\ dm^{-3} < 1.0$). [b] Extrapolated from 298.2 K to 363.2 K by assuming that the dependence of $K(SH^+)$ on temperature is similar to the reported for purine[26] and benzimidazole nucleosides.[23]

regarded as a strong evidence of the similarity of the mechanism of cleavage of the glycosidic linkage. Moreover, no sign of anomerization could be detected, when the hydrolysis of 3-chloro-1-β-D-ribofuranosylindazole was followed by ^{1}H-NMR spectroscopy.

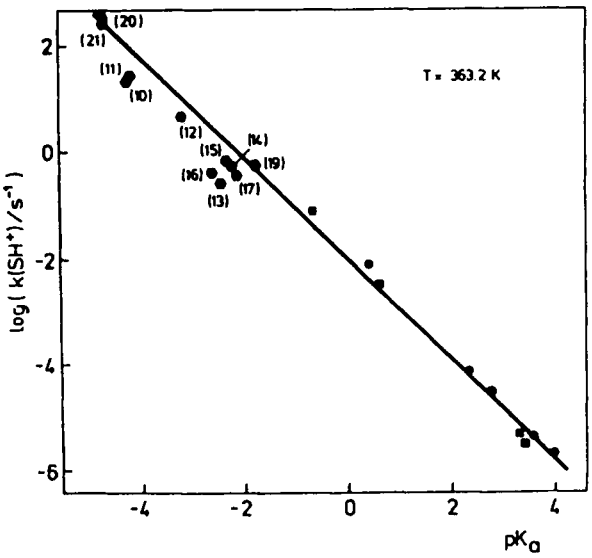


Fig. 1 - Logarithmic rate constants for the heterolysis of the monocations of indazole (●), benzimidazole (●) and purine ribonucleosides (■) plotted against the pK_a-values of the same species at 363.2 K.

In summary, the indazole nucleosides appear to behave in aqueous acid like purine or benzimidazole nucleosides.

BIOLOGICAL ACTIVITIES

The toxicity of the indazole nucleosides 10-16, 20 and 21 was tested against 10 different hematopoietic cell populations in vitro. The cell populations represented 7 established human leukemia/lymphoma lines, human peripheral blood mitogen-stimulated lymphocytes, an Epstein-Barr virus-infected monkey lymphoblastoid cell line, and a mouse leukemia (table 4). The cells were cultured for 3 days with the test compounds, and ^{14}C-leucine incorporation during the final 24 hours of culture was used as an end-point.[27] This system is well suited for the assessment of nucleoside toxicity and a good correlation has been noted between cell number and ^{14}C-leucine incorporation per culture.[28]

Table 4 - Toxicity of indazole nucleosides against normal and malignant hematopoietic cells in vitro.

1-β-D-ribofuranosyl-indazole	ID_{50}[a] (μg/ml)									
	HL-60[b]	U-937	K-562	MOLT-4	JM	NALL-1	Raji	PHA-LY	B-95-8	L1210
3-Chloro- (10)	29	6.3	34	7.0	49	44	>100	16	>100	>100
3-Bromo- (11)	>100	37	>100	50	85	>100	>100	88	>100	>100
3-Iodo- (12)	>100	60	>100	72	80	>100	85	>100	>100	65
4-Chloro- (13)	13	1.0	5.5	3.4	9.5	4.4	36	20	>100	45
5-Chloro- (14)	50	33	>100	32	55	>100	85	38	>100	70
5-Bromo- (15)	100	34	>100	60	68	64	54	60	>100	53
6-Chloro- (16)	35	90	>100	44	>100	45	>100	42	>100	>100
3-Bromo-5-chloro- (20)	44	26	57	33	28	14	14	31	60	37
3,5-Dibromo- (21)	n.t.[c]	28	55	64	44	95	52	36	40	n.t.

[a] = ID_{50} is the concentration of the compound, which caused a 50 % decrease in the ^{14}C-leucine incorporation. It was calculated from the dose-response curve determined with 0, 1.0, 10, and 100 μg/ml (in triplicate) of each analogue. [b] = HL-60, acute promyelocytic leukemia; U-937 = histiocytic lymphoma; K-562 = chronic myelogenous leukemia (blast crisis form having erythroid, myeloid and megakaryocytic features); MOLT-4 and JM = acute T-cell leukemias; Raji = Burkitt's lymphoma - all human cell lines. PHA-LY = phytohemagglutinin-stimulated human peripheral blood lymphocytes; B-95-8 = Epstein-Barr virus-transformed monkey lymphoblastoid cell lines; L1210 = mouse leukemia; [c] = not tested.

The results of cytotoxicity tests are illustrated in table 4. The susceptibility of individual cell populations varied; the most sensitive population appeared to be the histiocytic lymphoma (U-937), and the most resistant line was monkey lymphoblastoid line B-95-8. The 4-chloro derivative was the most toxic of the compounds tested; approximately 13 µg/ml was required to cause a 50 % inhibition in HL-60 cell cultures. This means only moderate toxicity. For comparison, a similar inhibition can be achieved with only 0.2 µg/ml of cytosine arabinoside, which is a well known antileukemic compound in clinical use.[27] Nevertheless, the compound merits further evaluation, particularly if it shows any selectivity towards malignant cells.

E X P E R I M E N T A L

UV Spectra were recorded on a Perkin Elmer spectrophotometer Lambda 5; ^{1}H-NMR spectra were measured with a Bruker WM-250 high resolution spectrometer with tetramethylsilane as an internal standard and on a δ-scale in ppm. The pK_a values were determined spectrophotometrically in aqueous perchloric acid solution, the H_o-values of which were taken from literature.[29] First-order rate constants for the hydrolysis were determined by HPLC.[22] Thin-layer chromatography was performed on silica-gel sheets F 1550 LS 254 of Schleicher & Schüll and column chromatography on Merck silica-gel 60 (particle size 0.036-0.2 mm). Drying of the substances was achieved in a vacuum desiccator or in a Büchi-TO 50 drying oven under vacuum at room temp. and slightly elevated temp. respectively. Melting points were determined in a Tottoli apparatus and are uncorrected.

6-Chloroindazole (7). The synthesis is done analogously to the indazole preparation[30] starting from 15 g (0.1 mol) of 4-chloro-o-toluidine in 100 mL of acetic anhydride. The resulting precipitate is treated in the reaction solu-

tion with nitrous gases at 0-5°C till a dark-green solution is obtained. It is then stirred for another 2 h and the mixture poured on 600 g of ice. The yellow precipitate is collected, washed with water, dried and then dissolved in 300 mL of toluene. The solution is kept at 40°C for 2 days. The reaction solution is then extracted twice with 100 mL of 3N HCl and thrice with 100 mL of 2 N HCl. The combined aqueous extracts are neutralized with ammonium hydroxide to pH 8 forming a yellowish precipitate (6.1 g, 38 %). The material is chromatographically pure, but a small amount was recrystallized from aqueous methanol to give colorless needles of 7 (m.p. 178-179°C).

UV (MeOH): 254 (3.67), 263 (3.69), 286 (3.63), 296 (3.57) nm.

Anal. calcd. for $C_7H_5ClN_2$ (152.6): C, 55.10; H, 3.30; N, 18.39. Found: C, 55.07; H, 3.33; N, 18.46.

5,6-Dichloroindazole (8). Analogously to the preceding procedure are treated 12 g (68 mmol) of 4,5-dichloro-o-toluidine[31] in 80 mL of acetic anhydride. After treatment with nitrous gases at 0-5°C, stirring for 2 h and pouring on ice, 12.3 g of a yellow precipitate are obtained. After drying the material is dissolved in 300 mL of dioxane and then kept at 40°C for 2 days. The reaction solution is evaporated to dryness, the residue dissolved in 400 mL of 3 N HCl, treated with charcoal, filtered and then neutralized by ammonium hydroxide to pH 8. The precipitate is collected and dried to yield yellowish crystals of 8 (4.8 g, 38 %, m.p. 204°C).

UV (MeOH): 262 (3.71), 268 (3.71), 297 (3.54), 308 (3.49) nm.

Anal. calcd. for $C_7H_4Cl_2N_2$ (187.0): C, 44.95; H, 2.16; N, 14.98. Found: C, 44.82; H, 2.21; N, 14.72.

3-Chloro-1-(β-D-ribofuranosyl)indazole (10). A mixture of 0.765 g (5 mmol) of 3-chloroindazole (1)[32], 1.91 g (6 mmol) of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (9)[33] and 95 mg (0.5 mmol) of p-toluenesulfonic acid is heated to

160°C for 20 min under vacuum (20 torr). The dark melt is dissolved in 50 mL of MeOH, 10 mL of 1 N sodium methoxide solution added and then heated under reflux for a few minutes. The solution is treated with charcoal, filtered and then the filtrate evaporated to dryness in the presence of some silica-gel. This material is put onto a silica-gel column (3x25 cm) and the product subsequently eluted with 300 mL of $CHCl_3$, 400 mL of $CHCl_3$/MeOH (95/5), and 500 mL of $CHCl_3$/MeOH (9/1). The product fraction is evaporated and the residue recrystallized from water to give colorless crystals of 10 (0.44 g, 31 %, m.p. 120-122°C).

Anal. calcd. for $C_{12}H_{13}ClN_2O_4$ (284.7): C, 50.63; H, 4.60, N, 9.83. Found: C, 50.51; H, 4.50; N, 9.81.

3-Bromo-1-(β-D-ribofuranosyl)indazole (11). Analogous to the preceding procedure 0.98 g (5 mmol) of 3-bromoindazole (2)[34] was treated with 1.91 g of 9 and 0.1 g of p-toluenesulfonic acid in a fusion reaction. After work-up and chromatography, colorless crystals of 11 (0.576 g, 35 %, m.p. 128°C) were obtained.

Anal. calcd. for $C_{12}H_{13}BrN_2O_4$ (329.2): C, 43.79; H, 3.98; N, 8.51. Found: C, 43.74; H, 4.02; N, 8.41.

3-Iodo-1-(β-D-ribofuranosyl)indazole (12). The fusion reaction with 1.22 g (5 mmol) of 3-iodoindazole (3)[32,35] is done analogously to the preceding procedure. After chromatographical work-up and recrystallization from water, 12 was obtained as colorless crystals (0.884 g, 47 %, m.p. 127-129°C).

Anal. calcd. for $C_{12}H_{13}I\,N_2O_4$ (376.2): C, 38.32; H, 3.48; N, 7.45. Found: C, 38.17; H, 3.52; N, 7.31.

4-Chloro-1-(β-D-ribofuranosyl) indazole (13). A well-grinded mixture of 0.765 g (5 mmol) of 4-chloroindazole (4),[33] 2.07 g (6.5 mmol) of 1,2,3,5-tetra-O-acetyl-β-D-ribofu-

ranose (9), 0.1 g p-toluenesulfonic acid, and 0.254 g (1 mmol) of iodine is heated to 165°C for 20 min in vacuum (20 torr). The reaction mixture is dissolved in 50 ml of MeOH, then 15 ml of 1 N sodium methoxide solution added and stirred over night. It is filtered, the filtrate evaporated together with some silica-gel. The dry material is put onto a silica-gel column (3x25 cm) and subsequently eluted with 300 mL of $CHCl_3$, 400 mL of $CHCl_3$/MeOH (95/5), and 400 mL of $CHCl_3$/MeOH (9/1). The product fraction is evaporated, the residue recrystallized from water to give 13 as colorless crystals (0.45 g, 32 %, m.p. 149-150°C).

Anal. calcld. for $C_{12}H_{13}Cl\ N_2O_4$ (284.7): C, 50.63; H, 4.60; N, 9.83. Found: C, 50.48; H, 4.66; N, 9.68.

5-Chloro-1-(β-D-ribofuranosyl)indazole (14). a) A well-grinded mixture of 0.915 g (6 mmol) of 5-chloroindazole (5),[34] 2.19 g (6.5 mmol) of 9, and 0.1 g of p-toluenesulfonic acid are treated and worked up analogously to the preceding procedure to give, on recrystallization from water, colorless needles of 14 (0.72 g, 42 %, m.p. 201-203°C).

b) In 35 mL of H_2O are dissolved 0.5 g of $Na_2HPO_4 \cdot H_2O$ and 0.275 g (1.1 mmol) of 1-(β-D-ribofuranosyl)indazole (19),[17] 15 mL of a saturated solution of chlorine in H_2O (0°C) are added dropwise within 30 min. The solution is put on a XAD-4 (100-200 µm) column (2.5x40 cm) and eluted with a gradient of H_2O/isopropanol (9/1) - H_2O/isopropanol (1/4). The product fraction is evaporated and the residue recrystallized from H_2O to give colorless needles (0.12 g, 45 %, m.p. 201-203°C).

Anal. calcd. for $C_{12}H_{13}ClN_2O_4$ (284.7): C, 50.63; H, 4.60; N, 9.83. Found: C, 50.45; H, 4.49; N, 9.99.

5-Bromo-1-(β-D-ribofuranosyl)indazole (15). a) In 30 mL of H_2O are dissolved 0.27 g KH_2PO_4 and 0.275 g (1.1 mmol) of 1-(β-D-ribofuranosyl)indazole (19). To this solution are

added slowly dropwise 10 mL of bromine water with stirring. After 2 h the precipitate is filtered off and the filtrate evaporated to a smaller volume to get a second crop. The substance is recrystallized from EtOH/H_2O (1/1) to give colorless needles of 15 (0.245 g, 67 %, m.p. 200-201°C).

Anal. calcd. for $C_{12}H_{13}BrN_2O_4$ (329.2): C, 43.97; H, 3.98; N, 8.51. Found: C, 43.87; H, 3.93; N, 8.60.

b) A mixture of 0.98 g (5 mmol) of 5-bromoindazole (6), 2.03 g (6.4 mmol) of 9, and 0.1 g of p-toluenesulfonic acid are heated to 160°C for 20 min in vacuum (20 torr). The mixture is dissolved in 50 mL of MeOH, then 10 mL of 1 N sodium methoxide solution added and heated under reflux for a few min. It is filtered and then the filtrate evaporated after addition of some silica-gel. The material is put onto a silica-gel column (3x25 cm). Elution is done first with 400 mL of CH_2Cl_2 and followed by 700 mL of CH_2Cl_2/MeOH (9/1). The product fraction is evaporated to dryness and the residue recrystallized from H_2O to give colorless needles of 15 (0.78 g, 47 %, m.p. 200-202°C).

6-Chloro-1-(β-D-ribofuranosyl)indazole (16). Analogous to the procedure of compound 10, 0.765 g (5 mmol) of 6-chloroindazole (7) was ribosylated with 1.91 g (6 mmol) of 9 in presence of 0.1 g of p-toluenesulfonic acid. After work-up and recrystallization from H_2O, 16 was obtained as colorless needles (0.67 g, 47 %, m.p. 188°C).

Anal. calcd. for $C_{12}H_{13}ClN_2O_4$ (284.7): C, 50.63; H, 4.60; N, 9.83. Found: C, 50.50; H, 4.49; N, 9.75.

5,6-Dichloro-1-(β-D-ribofuranosyl)indazole (17). A well-grinded mixture of 0.935 g (5 mmol) of 5,6-dichloroindazole (8), 2.07 g (6.5 mmol) of 9, 0.1 g of p-toluenesulfonic acid and 0.25 g (1 mmol) of iodine was heated under vacuum for 20 min to 165-170°C. The dark melt was dissolved in 50 mL of MeOH, 15 mL of 1 N sodium methoxide added and the resulting mixture stirred over night at room temp. Fol-

lowing filtration, the filtrate was evaporated in the presence of some silica-gel and this material put onto a silica-gel column (3x25 cm) for chromatography with 300 mL of CH_2Cl_2, 300 mL of CH_2Cl_2/MeOH (95/5), and 400 mL of CH_2Cl_2/MeOH (9/1). The product fraction is evaporated and the residue recrystallized from aqueous EtOH to give colorless needles of 17 (0.335 g, 21 %, m.p. 195°C).

Anal. calcd. for $C_{12}H_{12}Cl_2N_2O_4$ (319.1): C, 45.16; H, 3.97; N, 8.78. Found: C, 45.08; H, 3.81; N, 8.69.

4-Chloro-2-(β-D-ribofuranosyl) indazole (18). A mixture of 0.765 g (5 mmol) of 4-chloroindazole (4),[36] 1.75 g (5.5 mmol) of 9, 0.045 g (0.25 mmol) of p-toluenesulfonic acid is heated under vacuum for 15 min to 145-150°C. The dark melt is dissolved in 50 mL of MeOH, then 10 mL of 1 N sodium methoxide added and refluxed for a few min. It is filtered, the filtrate evaporated in presence of some silica-gel and this material then put onto a silica-gel column (3x25 cm) for subsequent chromatography with 300 mL of $CHCl_3$, 300 mL of $CHCl_3$/MeOH (95/5), and 400 mL of $CHCl_3$/MeOH (9/1). The product fraction is evaporated and the residue recrystallized from H_2O to yield 18 as colorless needles (0.71 g, 50 %, m.p. 157-159°C).

Anal. calcd. for $C_{12}H_{13}ClN_2O_4$ (284.7): C, 50.63; H, 4.60; N, 9.83. Found: C, 50.53; H, 4.50; H, 9.71.

3-Bromo-5-chloro-1-(β-D-ribofuranosyl)indazole (20). In 30 mL of H_2O and 10 mL of dioxane was dissolved 0.285 g (1 mmol) of 14 and 0.5 g of $Na_2HPO_4 \cdot H_2O$ with warming. The solution was kept at 50°C and then 0.22 g of bromine added under stirring. After 1 h the excess of bromine was destroyed by addition of $Na_2S_2O_4$ and then the reaction mixture cooled over night. The precipitate was collected and gave, after recrystallization from aqueous EtOH, 20 as colorless crystals (0.275 g, 76 %, m.p. 157-159°C).

Anal. calcd. for $C_{12}H_{12}BrClN_2O_4$ (364.6): C, 39.53; H, 3.59; N, 7.68. Found: C, 39.65; H, 3.37; N, 7.76.

3,5-Dibromo-1-(β-D-ribofuranosyl)indazole (21). a) In 40 mL of H_2O was dissolved 0.33 g (1 mmol) of 11 and 0.54 g of KH_2PO_4 by gentle warming. To this stirred solution was added, dropwise, 0.4 g (2.5 mmol) of bromine, which resulted in a precipitate after a few min. The excess bromine was destroyed by $Na_2S_2O_4$ after 3 h and the precipitate collected. Recrystallization from aqueous MeOH gave colorless needles of 21 (0.285 g, 70 %, m.p. 162°C).

b) In a mixture of 10 mL of H_2O and 10 mL of dioxane are dissolved 0.125 g (0.5 mmol of 19^{14} and 0.45 g of KH_2PO_4. To this solution is added 0.32 g (2 mmol) bromine dropwise under stirring. After 6 h, the excess of bromine is destroyed by addition of $Na_2S_2O_4$ and then the solution evaporated to dryness. The residue is recrystallized from aqueous MeOH to give colorless needles of 21 (0.18 g, 88 %, m.p. 162-163°C).

Anal. calcd. for $C_{12}H_{12}Br_2N_2O_4$ (408.5): C, 35.32; H, 2.96; N, 6.86. Found: C. 35.02; H, 3.11; N, 6.71.

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