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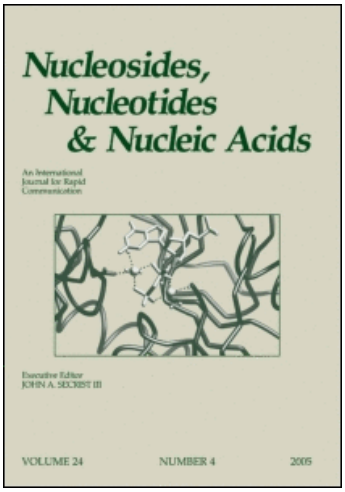

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Synthesis of, and Conformational Studies on, 2-Trifluoromethyl, Substituted Benzimidazole Ribofuranosides

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NUCLEOSIDES & NUCLEOTIDES, 1(3), 275-287 (1982)

SYNTHESIS OF, AND CONFORMATIONAL STUDIES ON, 2-TRIFLUOROMETHYL SUBSTITUTED BENZIMIDAZOLE RIBOFURANOSIDES

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Abstract. The 1-β-D-ribofuranosides of several 2-trifluoromethyl benzimidazoles were prepared by the fusion method, and their conformations, particularly about the glycosidic bond, determined by ^{1}H NMR spectroscopy.

Derivatives of 2-trifluoromethylbenzimidazole are known inhibitors of photosynthetic processes,[1] and some of them exhibit appreciable herbicidal and insecticidal activities.[2] Substituted 2-trifluoromethylbenzimidazoles are also potent decouplers of oxidative phosphorylation in mitochondria.[3] On the other hand, nucleosides of halogeno-substituted benzimidazoles, in particular 5,6-dichloro-1-β-D--ribofuranosyl benzimidazole (DRB), are specific and reversible inhibitors of nuclear hnRNA synthesis[4,5] and superinductors of interferon production in human fibroblasts.[6] In a continuation of a program for the synthesis of halogenobenzimidazole nucleosides with potential biological activity,[7] we describe here the synthesis of several new nucleosides of 2-trifluoromethylbenzimidazole, and some of their physico-chemical, particularly conformational, properties.

Results and Discussion

Syntheses of analogues of 2-trifluoromethylbenzimidazole have hitherto been based largely on the method of Phillips,[8] involving

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the reaction of an appropriate o-phenylinediamine with trifluoroacetic acid. We have applied this procedure to the synthesis of the as yet unknown 5,6-difluoro-2-trifluoromethylbenzimidazole, which was obtained by simultaneous reduction with tin, and cyclization with trifluoroacetic acid, in an atmosphere of argon, of 1,2-difluoro-4,5-dinitrobenzene,[7] as shown in Scheme 1.

Application of the fusion reaction[9] to various 2-trifluoromethylbenzimidazoles with 1,2,3,4-tetracetyl-β-D-ribofuranose gave the corresponding 1-β-D-ribofuranosides in yields ranging from 8 to 35% (Scheme 2). In each instance the reaction mixture was subjected to deacetylation with sodium methoxylate in methanol, and the desired nucleoside isolated by chromatography on a silica gel column. This also led to recuperation of unreacted base. The procedure gave exclusively the 1-β-D- anomers. The differences in overall condensation yields may be ascribed, at least in part, to the differences in sublimation properties of the bases.

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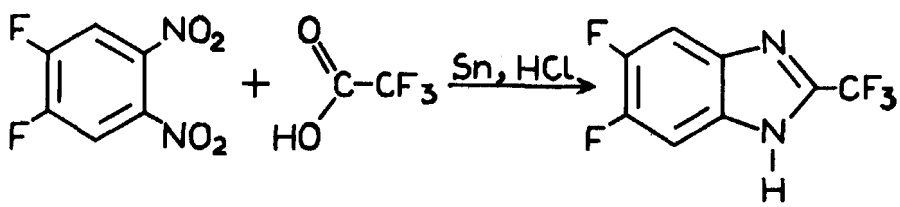
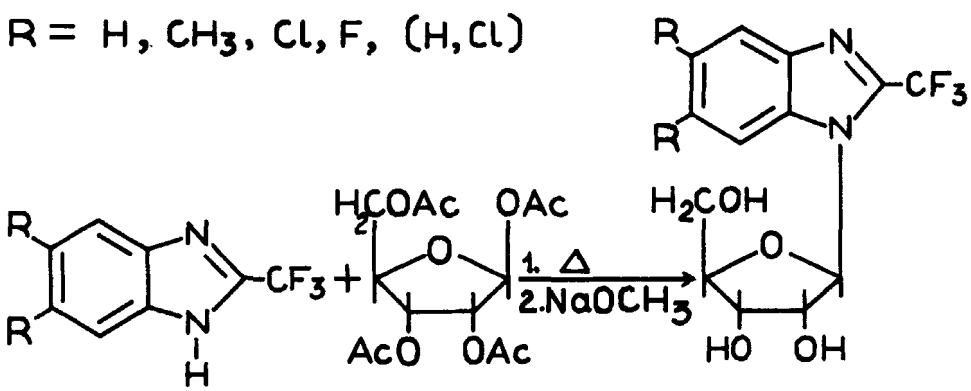


SCHEME 1



SCHEME 2

Of particular interest was the observation that the reaction of 5(6)-chloro-2-trifluoromethylbenzimidazole with the acetylated sugar yielded a single isomer, 6-chloro-2-trifluoromethyl-1-β-D-ribofuranosylbenzimidazole, the structure of which was unequivocally established by means of ^{1}H NMR spectroscopy. The chlorine substituent in the monochloro derivative of this nucleoside must be exclusively at C(5) or C(6), in accordance with the appearance in the NMR spectrum of a single system of signals of three protons, as for the monobromo derivative of benzimidazole-α-D-arabinoside, and in contrast to the two groups of signals observed with the non-separable mixture of the 5- and 6-bromo derivatives of benzimidazole-β-D-riboside.[10] Differentation between the two possible isomers was hindered by the existence of identical systems of coupled protons for both. The location of the chlorine substituent was finally established by analysis and comparison of the proton chemical shifts in benzimidazole-β-riboside, 2-trifluoromethylbenzimidazole-β-riboside, benzimidazole-α-arabinoside, and their mono- and di- bromo and chloro derivatives (ref. 11, and Table 2). The effects of a 5- or 6-substituent on the chemical shifts of the protons ortho and meta to the substituent showed that the monochloro derivative must be the 6-chloro.

During the course of this investigation, some difficulty was encountered in the unequivocal interpretation of some of the ^{1}H signals in the nucleosides. This difficulty was surmounted by preparing the corresponding N(1)-methyl analogues of 5,6-dichloro- and 5,6-dichloro-2-trifluoromethylbenzimidazoles (see Experimental). The use of these methyl derivatives is described below in connection with analyses of the conformations of the nucleosides about the glycosidic bond.

Table 1 lists the UV spectral data for the various compounds.

Solution conformations of nucleosides

Table 2 lists the chemical shift data for the various compounds, and Table 3 the corresponding coupling constants.

Sugar rings. The conformation of the ribofuranose ring in all of the nucleosides was determined from the values of the vicinal proton-proton coupling constants J(1',2'), J(2',3'), J(3',4') (see Table 3), in accordance with the two-state equilibrium model N ⇌ S, or

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TABLE 1. Spectral data for 2-trifluoromethyl substituted analogues of benzimidazole-1-β-D-ribofuranoside in aqueous medium over the pH range 2 - 12; and for 1-methyl-5,6-dichloro-2-trifluoromethylbenzimidazole in methanol.

Analogue	λ_{max} (ε_{max})	λ_{max} (ε_{max})	λ_{max} (ε_{max})
Parent nucleoside	252 (8700),	278 (5100),	285[a] (4000)
5,6-Dimethyl-	258 (7500),	285 (5500),	290[a] (4800)
5,6-Dichloro-	260 (7200),	291 (5300),	301 (4700)
6-Chloro-	258 (8000),	282 (6400),	291 (5500)
5,6-Difluoro-	249 (7200),	280 (5800),	289 (4800)
.....			
1-Methyl-5,6-dichloro--2-CF_3-benzimidazole	267 (6500),	293 (7200),	301 (6500)

[a]Shoulder

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C(3')endo ⇌ C(2')endo, of Altona and Sundaralingam.[12] The resulting populations of the conformer S are listed in Table 3 (% population N = 100% - S). It will be noted that, for all the nucleosides, there is a marked preference for the S type conformer (70-80%), typical for all halogeno benzimidazole nucleosides with a C(2)-substituent hitherto examined.[11] Similarly the values of J(1',2') + J(3',4'), and of J(2',3'), for all 2-substituted benzimidazole nucleosides, are close to each other, pointing to similar angles of pseudorotation and puckering parameters of the ring for both conformations. With the aid of the graphical model of Guschlbauer[13] the parameters are as follows: $^{N}P = 20^{\circ}$; $^{S}P = 160^{\circ}$; $^{N}\tau_m = {}^{S}\tau_m = 38^{\circ}$. For comparison purposes, the values for nucleosides without a 2-substituent are: $^{N}P = 10^{\circ}$; $^{S}P = 170^{\circ}$; $^{N}\tau_m = {}^{S}\tau_m = 40^{\circ}$.[14]

Exocyclic carbinol groups. The conformation of these groups about the C(4')-C(5') bond was based on the accepted assumption of the existence of three classical conformers, gauche-gauche, gauche-trans and trans-gauche, as in the case of purine nucleosides.[15] The population of the gauche-gauche conformer in each instance was derived from the parametrization of the Karplus relationship[15] applied to purine nucleosides. The closely similar values of the chemical shifts of H(5') and H(5'') made it impractical, with the resolution of our

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TABLE 2. Values for the chemical shifts (in ppm vs internal Me_4Si) for various 2-trifluoromethyl-benzimidazole and 2-trifluoromethylbenzimidazole-β-riboside analogues in $(C^2H_3)_2SO$.

	Chemical shifts										
	H(4)	H(5)	H(6)	H(7)	CH_3	H(1')	H(2')	H(3')	H(4')	H(5')	H(5'')[a]
Benzimidazole	7.59	7.18	7.18	7.59	—	—	—	—	—	—	—
5,6-DiCl-benzimidazole	7.86	—	—	7.86	—	—	—	—	—	—	—
5,6-DiCl-1-CH_3-benzimidazole	7.93	—	—	7.97	3.85	—	—	—	—	—	—
2-CF_3-benzimidazole	7.74	7.37	7.37	7.74	—	—	—	—	—	—	—
2-CF_3-5,6-diCl-benzimidazole	8.00	—	—	8.00	—	—	—	—	—	—	—
2-CF_3-5,6-diCl-1-CH_3-benzimidazole	8.14	—	—	8.25	3.99	—	—	—	—	—	—
2-CF_3-5,6-diF-benzimidazole	7.79	—	—	7.79	—	—	—	—	—	—	—
1-β-D-ribofuranosyl-benzimidazole	7.65	7.21	7.24	7.72	—	5.86	4.36	4.11	3.97	3.66	
1-β-D-ribofuranosyl-2-CF_3-benzimidazole	7.85	7.41[b]	7.41[b]	8.19	—	5.85	4.52	4.18	4.01	3.72	
1-β-D-ribofuranosyl-2-CF_3-6-Cl-benzimidazole	7.86	7.42	—	8.52	—	5.84	4.47	4.18	4.04	3.74	
1-β-D-ribofuranosyl-2-CF_3-5,6-diCl-benzimidazole	8.19	—	—	8.79	—	5.84	4.44	4.19	4.06	3.75	
1-β-D-ribofuranosyl-2-CF_3-5,6-diF-benzimidazole	7.98	—	—	8.55	—	5.85	4.43	4.18	4.06	3.75	
1-β-D-ribofuranosyl-2-CF_3-5,6-diCH_3-benzimidazole	7.58	—	—	8.01	2.32	5.81	4.51	4.18	4.00	3.74	

[a] Centre of the band H(5'), H(5'')

[b] Centre of the band H(5), H(6)

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TABLE 3. Values for the vicinal coupling constants (in Hz) for various trifluoromethylbenzimidazole-β-riboside analogues in $(C^2H_3)_2SO$ and conformational parameters for the sugar rings and exocyclic groups derived from these data.

Analogue of 1-β-D-ribofuranosyl-2-trifluoromethyl-benzimidazole	Coupling constants				Conformation	
	J(1',2')	J(2',3')	J(3',4')	J(4',5')[a]	C(2')endo (%)	gauche-gauche (%)
Parent nucleoside	7.9	5.5	2.4	3.1	78	73
6-Chloro-	7.7	5.4	2.5	2.8	75	80
5,6-Dichloro-	7.7	5.4	2.5	2.8	75	80
5,6-Difluoro-	7.9	5.6	2.3	2.8	78	80
5,6-Dimethyl-	7.0	5.8	3.0	3.0	70	75

[a]Average of J(4',5') and J(4',5'')

instrument, to determine the values of J(4',5') and J(4',5''), but only their sum. Hence only the gauche-gauche populations could be established; and these were predominant, 75-80%, as for other benzimidazole nucleoside analogues.[7,11]

Conformation about glycosidic bond. As in the case of purine nucleosides with an 8-substituent,[16] a 2-substituent in benzimidazole nucleosides sterically favours the syn conformation about the glycosidic bond. For 2-trifluoromethyl nucleoside analogues one would anticipate a strong preference for, or even exclusively, the syn conformation. The procedure we have previously developed for evaluation of the conformer populations about the glycosidic bond in purine nucleosides,[16,17] based on a comparison of the chemical shifts of the sugar protons relative to those displayed by model analogues fixed in the syn and anti conformations, has also been applied to benzimidazole nucleosides.[7,11] In contrast to purine nucleosides, benzimidazole nucleosides exhibit a very marked preference for the conformation syn; however, because of the small differences between the chemical shifts of H(2') for the syn and anti conformations, quantitative evaluations of populations were not feasible. Supplementary evidence for preponderance of the form syn was furnished by analyses of the chemical shifts of the benzimidazole protons, principally H(7).[11]

As shown in Table 2, the chemical shifts of H(2') in 2-trifluoromethyl analogues of benzimidazole nucleosides differ from the corresponding values for other benzimidazole nucleosides with 2-substituents by about 0.10 - 0.15 ppm, and are therefore in the range characteristic for the model analogue fixed in the form anti, 5,7-dibromobenzimidazole-1-β-D-ribofuranose, for which δH(2') is 4.45 ppm. Furthermore, the changes in chemical shift of H(1') for various halogenobenzimidazole analogues, resulting from insertion of a 2-CF_3 substituent, do not exceed 0.04 ppm. While these facts point to a preference for the form anti, it is not supported by analysis of the chemical shifts of the benzimidazole protons, previously applied to analogues without a 2-trifluoromethyl substituent,[11] and based on a comparison of the changes in chemical shifts of H(4), H(5), H(6) and H(7), with particular attention to H(4) and H(7), following attachment of the pentose ring. This leads to small changes in chemical shifts of H(4), H(5) and H(6), not exceeding 0.1 ppm. By contrast the chemical shift

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of H(7) increases by up to 0.68 ppm, depending on the substituents at C(5) and C(6). The difference in chemical shift between H(7) and H(4) may be as large as 0.5 ppm. This is not a consequence of a modification in the symmetry of the molecule, since introduction of a methyl group at N(1) of 5,6-dichlorobenzimidazole leads to a difference between the chemical shifts of H(7) and H(4) of only 0.04 ppm (see Table 2). It follows that, in the nucleoside, there is an additional marked effect of the sugar on H(7), possible only with the conformation syn. A methyl substituent at N(1) of 5,6-dichloro-2-trifluoromethylbenzimidazole leads to slightly enhanced deshielding of H(7) and H(4) by comparison with the corresponding analogue without the 2-CF_3 substituent, and the difference between the chemical shifts of H(7) and H(4) is then 0.11 ppm (Table 2). But, following attachment of a sugar moiety to the 2-trifluoromethyl analogues of benzimidazole, the foregoing difference in chemical shifts varies from 0.44 to 0.60 (for the 5,6 dichloro analogue). This effect, as previously observed for benzimidazole nucleosides without the 2-trifluoromethyl substituent,[11] points to a preference for the form syn in the 2-trifluoromethyl nucleosides. The differences between the influence of the sugar ring on the benzimidazole protons in the various analogues with C(5) and C(6) substituents are likely due (as in the case of other benzimidazole nucleosides) to differences in values of the glycosidic torsion angles of the syn conformers.

The non-typical values of the chemical shifts of H(2') and H(1') in the trifluoromethyl nucleoside analogues are most likely due to the very pronounced inductive effect of the trifluoromethyl substituent, which is particularly significant with the small differences in chemical shifts between the forms syn and anti. For example, substituents such as benzyl or hydroxybenzyl at C(2) do not affect the chemical shifts of H(5) and H(6), distal from C(2), whereas the trifluoromethyl substituent leads to a change of 0.2 ppm.

The benzimidazole nucleosides with a 2-trifluoromethyl substituent exhibit other conformational properties similar to those with a 2-methyl substituent, viz. C(2')endo ~75%, and gauche-gauche ~80%. The values of the individual coupling constants do not differ by more than 0.5 Hz, resulting from minor differences in conformer populations and angles of pseudorotation ^{N}P and ^{S}P, as well as τ_m. It may like-

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wise be concluded that both classes of nucleosides are predominantly, if not exclusively, in the syn conformation about the glycosidic bond, and resulting from the steric effect of the 2-substituent on the parent nucleosides which exhibit a preference for the syn conformer even in the absence of the substituent.

An examination of the CD spectra of some benzimidazole nucleosides by Miles et al.[18] led to the conclusion that 1-β-D-ribofuranosylbenzimidazole, and some 2-substituted derivatives, are in the syn conformation. However a 2-methyl substituent was claimed to alter the conformation to anti. This was based on an observed positive Cotton effect in the vicinity of the 250 nm absorption band of the 2-methyl derivative, in contrast to the negative CD band exhibited by other analogues, e.g. 2-chloro-, 2-dimethylamino- and the parent benzimidazole nucleoside itself (the CD results were based on the use of 1-β-D--2'-deoxyribofuranosyl-2-methylbenzimidazole, and not the ribose derivative as for the other compounds in the series). The proposal that the positive Cotton effect for the 250 nm band unequivocally establishes the conformation as anti is not convincing, the more so in that for a derivative with such a fixed conformation, viz. 2,5'-O-cyclobenzimidazole riboside, the observed Cotton effect was zero. To this must be added the frequently cited conclusions (e.g. refs. 19,20) regarding the unreliability of CD spectra for establishement of the conformation of purine nucleosides about the glycosidic bond.

To remove any possible doubts in the present instance, we examined the CD spectra of 2-methyl- and 2-trifluoromethyl- 5,6-dichlorobenzimidazole ribofuranosides, and found that both exhibited negative Cotton effects in the vicinity of the 250-260 nm bands. It follows that the conformation of various benzimidazole nucleosides with a 2-methyl substituent is, in fact, syn, as is also the case with a 2-trifluoromethyl substituent. Apart from conformational effects, the shape and magnitude of the CD spectra are also dependent on the nature of substituents in the benzimidazole ring.

Experimental

Melting temperatures (uncorr.) were measured on a Boetius microscope hot stage. UV absorption spectra were run with a Zeiss (Jena, GDR) VSU-2 spectrophotometer. CD spectra were recorded on a

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Jasco ORD-UV-5 instrument fitted with a CD attachment, at concentrations of 2 x 10^{-4} M in 10-mm cuvettes. Column chromatography was carried out with Merck (Darmstadt, GFR) 70-230 mesh silica gel; and thin-layer chromatography with Merck silica gel 60 F_{254}. Elementary microanalyses were performed on a Perkin-Elmer Model 240 instrument by Dr. I. Celler of the Institute of Organic Chemistry. ^1H NMR spectra were recorded on a Varian-100 at room temperature, on 0.2 M solutions in $(C^2H_3)_2SO$, with $(CH_3)_4Si$ as internal standard.

1-(β-D-ribofuranosyl)-2-trifluoromethylbenzimidazole. A finely ground mixture of 0.93 g (5 mmole) of 2-trifluoromethylbenzimidazole[21] and 1.92 g (6 mmole) of 1,2,3,4-tetraacetyl-β-D-ribofuranoside (Pharma Waldhof, Dusseldorf, GFR) was heated for one hour at 175-180°C under reduced pressure (20 mm Hg, water pump). The mixture was taken up in 50 ml methanol, to which was added 30 ml of a 1 M methanolic solution of sodium methoxylate, and the solution brought to the boil for 5 min. The cooled solution was brought to neutrality with acetic acid and brought to dryness under reduced pressure. The residue was deposited on a 35 x 3 cm column of silica gel and eluted with 300 ml methylene chloride, 300 ml of 2% methanol in methylene chloride, and 300 ml of 10% methanol in methylene chloride. The fractions containing the nucleoside were pooled, brought to dryness, and crystallized from 20 ml water to yield 480 mg (30%) of needles, m.p. 170-171°C. Elem. anal.: Calculated for $C_{13}H_{13}N_2O_4F_3$: C, 49.22%; H, 3.81%; N, 8.83%; Found: C, 49.22%; H, 3.95%; N, 8.69%.

5,6-dichloro-1-methylbenzimidazole. To 930 mg (5 mmole) of 5,6-dichlorobenzimidazole in 10 ml of 1 N NaOH was added 130 mg (0.5 mmole) triethylbenzylamine chloride and 25 ml ethylene chloride. The mixture was stirred for 1 hr, followed by addition of 850 mg (6 mmole) of methyl iodide. Stirring was continued for 20 hr. The organic phase was washed several times with water, dried over anhydrous Na_2SO_4, and brought to dryness under reduced pressure. Crystallization of the residue from 50% aqueous methanol yielded 820 mg (82%) in the form of needles, m.p. 179°C.

5,6-dichloro-2-trifluoromethyl-1-methylbenzimidazole. The procedure applied was identical to that in the preceding paragraph, and the product was obtained in 75% yield in the form of needles, m.p. 167°C.

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1-(β-D-ribofuranose)-5,6-dimethyl-2-trifluoromethylbenzimidazole. A mixture of 1.28 g (6 mmole) of 5,6-dimethyl-2-trifluoromethylbenzimidazole[21] and 2.24 g (7 mmole) of 1,2,3,4-tetraacetyl-β-D-ribofuranose was heated for 1 hr at 180-185°C under reduced pressure (20 mm Hg). The mixture was taken up in 50 ml of 0.5 M sodium methoxylate in methanol and heated under reflux for 10 min. It was brought to neutality with acetic acid and deposited on a 25 x 3 cm column of silica gel. The nucleoside was isolated by elution with 400 ml chloroform, 400 ml of 3% methanol in chloroform, and 400 ml of 10% methanol in chloroform. The fractions containing the nucleoside were pooled, brought to dryness under reduced pressure, and crystallized from 10 ml of 30% aqueous methanol to yield 530 mg (25%) in the form of needles, m.p. 166-167°C. Elem. anal.: Calculated for $C_{15}H_{17}N_2O_4F_3$: C, 52.05%; H, 4.94%; N, 8.09%; Found: C, 51.88%; H, 5.00%; N, 7.88%.

1-(β-D-ribofuranosyl)-5,6-dichloro-2-trifluoromethylbenzimidazole. This was prepared from 1.27 g (5 mmole) 5,6-dichloro-2-trifluoromethylbenzimidazole[22] and 1.92 g (6 mmole) of the acetylated ribofuranose as reported for the 5,6-dimethyl analogue, above, to yield 710 mg (35%) of the nucleoside monohydrate in the form of needles, m.p. 95-97°C. Elem. anal.: Calculated for $C_{13}H_{13}N_2O_5F_3Cl_2$: C, 38.53%; H, 3.23%; N, 6.91%; Found: C, 38.70%; H, 3.23%; N, 6.84%.

5,6-difluoro-2-trifluoromethylbenzimidazole. To a solution of 4.1 g (20 mmole) of 1,2-difluoro-4,5-dinitrobenzene[7] in 80 ml of conc. HCl and 14 ml trifluoroacetic acid, at 70°C under an atmosphere of argon, was added 12.5 g of tin granules over a period of 1 hr, following which the mixture was heated under reflux for 1 hr. It was then cooled, 100 ml isopropanol added, and brought to pH 8-9 with the aid of conc.NH_4OH. The mixture was passed through a Celite pad, and the resulting clear filtrate brought to dryness under reduced pressure. The residue was taken up in 100 ml water and clarified by filtration. The clear filtrate was brought to dryness and the residue crystallized from 50 ml of 50% aqueous ethanol to give 2.84 g (64%) of the desired benzimidazole derivative in the form of needles, m.p. 224°C (sublimes at 200°C). Elem. anal.: Calculated for $C_8H_3N_2F_5$: N, 12.61%; Found: N, 12.42%.

1-(β-D-ribofuranosyl)-5,6-difluoro-2-trifluoromethylbenzimidazole. A mixture of 1.11 g (5 mmole) of 5,6-difluoro-2-trifluoromethyl-

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benzimidazole and 1.92 g (6 mmole) of 1,2,3,4-tetraacetyl-β-D-ribofuranose was heated for 1 hr at 170-180°C under reduced pressure. The mixture was then taken up in 50 ml of 0.5 M sodium methoxylate in methanol and heated under reflux for 10 min. It was brought to neutrality with acetic acid, brought to dryness, and deposited on a 40 x 2 cm column of silicagel. The column was eluted with 200 ml of ethylene chloride, 500 ml of 1% methanol in ethylene chloride, and 500 ml of 10% methanol in ethylene chloride. The fractions containing the nucleoside were brought to dryness, and the residue crystallized from water to give 150 mg (8%) of the semi-hydrate of the nucleoside in the form of needles, m.p. 174-176°C. Elem. anal.: Calculated for $C_{13}H_1N_2O_4F_5 \cdot 1/2\ H_2O$: C, 42.98%; H, 3.32%; N, 7.71%; Found: C, 42.81%; H, 3.28%; N, 7.81%.

1-(β-D-ribofuranosyl)-6-chloro-2-trifluoromethylbenzimidazole. A mixture of 0.96 g (4.36 mmole) of 5(6)-chloro-2-trifluoromethylbenzimidazole[22] and 1.76 g (5.5 mmole) of 1,2,3,4-tetraacetyl-β-D-ribofuranose was heated for 30 min at 140-150°C under reduced pressure. The mixture was taken up in 30 ml of 0.5 M sodium methoxylate in methanol and heated under reflux for 10 min. It was then brought to neutrality with acetic acid and solvent removed under reduced pressure. The residue was deposited on a 40 x 2 cm column of silica gel and eluted succesively with 200 ml ethylene chloride, 700 ml of 5% isopropanol in ethylene chloride, and 300 ml of 10% isopropanol in ethylene chloride. The fractions containing the nucleoside were pooled, brought to dryness, and crystallized from 10% aqueous isopropanol to yield 0.25 g (16%) of the nucleoside in the form of needles, m.p. 203-204°C. Elem. anal.: Calculated for $C_{13}H_{12}N_2O_4F_3Cl$: C, 44.26%; H, 3.42%; N, 7.94%; Found: C, 44.09%; H, 3.52%; N, 7.78%.

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