Substituted benzimidazoles: antiviral activity and synthesis of nucleosides

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Dedicated to Professor Harri Lönnberg on the occasion of his 60th birthday

Abstract

The antiviral activity of a series of benzimidazole derivatives and substituted benzimidazole *β*-L- and *β*-D-2'-deoxyribonucleosides against selected RNA and DNA viruses including HIV-1, BVDV, YFV, DENV-2, WNV, HBV, HCV and human RSV was evaluated. In addition, the synthesis of several benzimidazole *β*-L-2'-deoxyribonucleosides (**1**-**4**) and 2'-deoxy-2'-fluoro-*β*-D-arabinofuranosyl nucleoside **5** is described. The stereoselective glycosylation of the anions of the functionalized benzimidazoles **6a**, **12a** and **28** with 3,5-di-*O*-(4-methylbenzoyl)-2-deoxy-α-L-*erythro*-pentofuranosyl chloride (**29**) furnished *β*-L-2'-deoxyribonucleosides **1**-**4** while the glycosylation of the anion of 2-bromobenzimidazole (**12a**) with 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide **34** gave the 2'-deoxy-2-fluoro-*β*-D-arabinofuranosyl nucleoside **5**. Moreover, the crystal structure of the benzoylated 2-bromobenzimidazole 2'-deoxy-2'-fluoro-*β*-D-arabinofuranosyl nucleoside **35** is reported.

Keywords: Benzimidazoles, L-nucleosides, antiviral screening, glycosylation

Introduction

The benzimidazole system is an integral part of numerous antiparasitic, fungicidal, anthelmintic and anti-inflammatory drugs.[1, 2] Among them, modified 2-trifluoromethyl-benzimidazoles are

particular promising as they show appreciable herbicidal activity due to the inhibition of photosynthesis.[3] Recently, potent anticancer, antibacterial, antifungal and antiprotozoal activities were reported for halogenated 2-trifluoromethyl- and 2-pentafluoroethyl-benzimidazoles.[4-6]

Not only the substituted benzimidazole heterocycles themselves but also a series of modified benzimidazole nucleosides are biologically active.[7-9] Originally, their synthesis was encouraged by the discovery that 5,6-dimethyl-1-(α-D-*erythro*-pentofuranosyl)-benzimidazole is a constituent of vitamin B_{12}.[10] 5,6-Dichlorobenzimidazole ribonucleoside (DRB) inhibits cellular and viral RNA synthesis.[11, 12] However, this activity is accompanied by a substantial cytotoxicity and therefore this compound has not found application as an antiviral drug.[13, 14]

Noteworthy is the recent finding that the L-ribonucleoside of 5,6-dichloro-2-isopropylaminobenzimidazole (1263W94) shows increased activity against the herpes virus HCMV (human cytomegalovirus) *in vitro* compared to its parent compound 2-bromo-5,6-dichloro-1-*β*-D-ribofuranosylbenzimidazole (BDCRB) and a low cytotoxicity.[15] This is in line with the general observation that several L-nucleosides exhibit an antiviral activity comparable and sometimes greater than their D-enantiomers, due to a more favourable toxicological profile and a greater metabolic stability.[16, 17] Thus, various L-nucleosides have been synthesized as potential antiviral and anticancer drugs such as 3TC (lamivudine), FTC (emtricitabine) and L-FMAU (clevudine).[16-20] The first synthesis of a L-nucleoside (*β*-L-dT) was reported in 1964 by Šmekal and Šorm[21] followed by the description of *β*-L-adenosine by Acton, Ryan and Goodman in the same year.[22] Furthermore, the anti-hepatitis B virus activity of the 2'-deoxyribonucleoside L-dT (Telbivudine, Tyzeka™, Sebivo™)[21b] against hepatitis B virus infection led to its approval as antiviral drug.

Herein, we report on the antiviral activity of benzimidazole derivatives against *Flaviviridae*, human immunodeficiency retrovirus (HIV-1), Hepatitis B virus (HBV), Hepatitis C virus (HCV) as well as Human Respiratory Syncytial Virus (human RSV) (Figure 1).

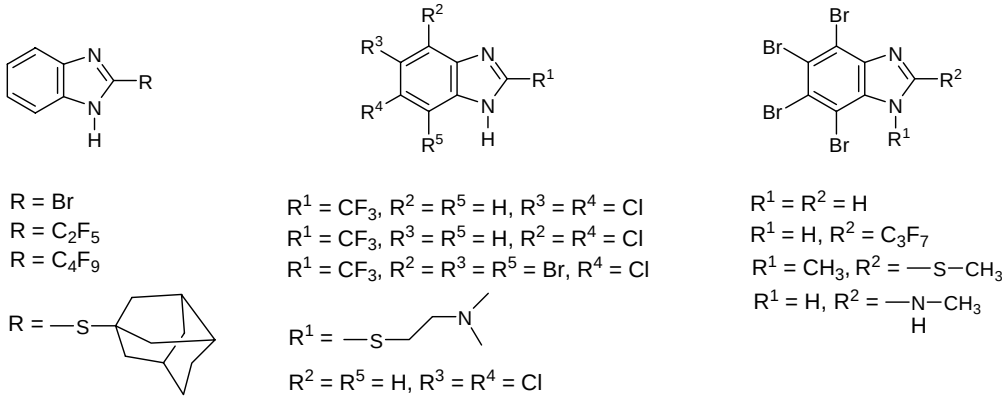
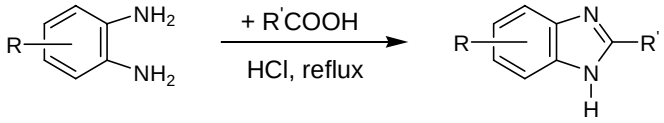


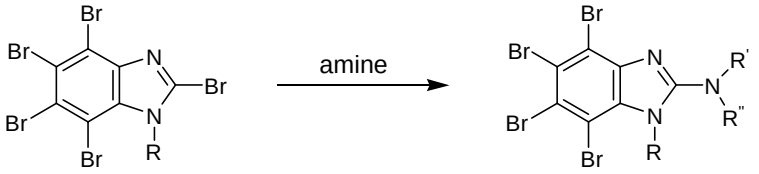
Figure 1. Selected benzimidazole derivatives for antiviral testing.

Some of them were already described by Baczynski and Niementowski in 1902 and Büchel in 1970, respectively,[23, 24] others were recently reported.[5, 6, 25, 26] The introduction of side chains

into position-2 of halogenated or non-halogenated benzimidazoles can be achieved by different routes.[5, 6, 25-27] One route uses non-halogenated or halogenated *o*-phenylenediamines as precursor which give after condensation with carboxylic acids and their derivatives (nitriles, imidates, orthoesters) the 2-substituted benzimidazoles (Scheme 1). In this manuscript compounds of Figure 4 were prepared employing this protocol.[5, 6] In another route 2,4,5,6,7-pentabrominated benzimidazole is subjected to a series of displacement reactions in position-2 using various nucleophiles (Scheme 2).[25] Compounds shown in Figure 3 were synthesized according to this route.



Scheme 1



Scheme 2

Furthermore, a series of substituted benzimidazole *β*-L- and *β*-D-2'-deoxyribonucleosides (**1**-**5**) were synthesized (Figure 2). They were evaluated for their antiviral activity against *Flaviviridae*, HIV-1, Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Human Respiratory Syncytial Virus (human RSV) and the results are discussed here.

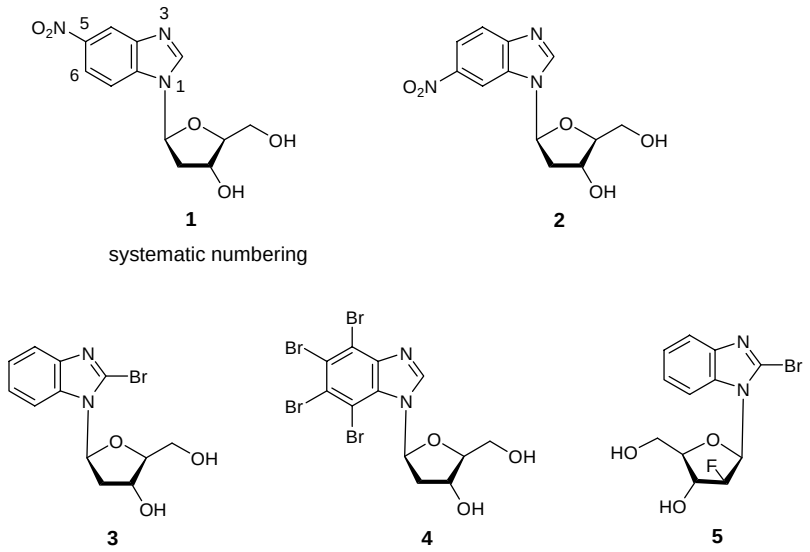


Figure 2. Substituted benzimidazole *β*-L- and *β*-D-2'-deoxyribonucleosides.

Results and Discussion

Antiviral activity and cytotoxicity

A series of benzimidazole derivatives and benzimidazole *β*-L- and *β*-D-2'-deoxyribonucleosides were evaluated in cell-based assays by determining the 50% cytotoxicity concentration (CC_{50}) and the 50% inhibitory concentration (EC_{50}) as previously described.[28] Among the tested viruses, representatives of three genera of the *Flaviviridae* which belong to the class of positive-sense single-stranded RNA ($ssRNA^+$) viruses were selected. These are *Pestivirus* (bovine viral diarrhoea virus, BVDV), *Flavivirus* including yellow fever virus (YFV), dengue virus type 2 (DENV-2) and West Nile virus (WNV) and *Hepacivirus* (hepatitis C virus; HCV). Moreover, activity against the representative member of the *Retroviridae* ($ssRNA^+$) the human immunodeficiency retrovirus (HIV-1) and the DNA virus Hepatitis B (HBV) was evaluated as well as the activity against the negative-sense single-stranded RNA virus ($ssRNA^-$) Human Respiratory Syncytial Virus (human RSV). The data are summarized in Tables 1-7 and the structures of compounds **1**-**27** are given in Figures 3-6.

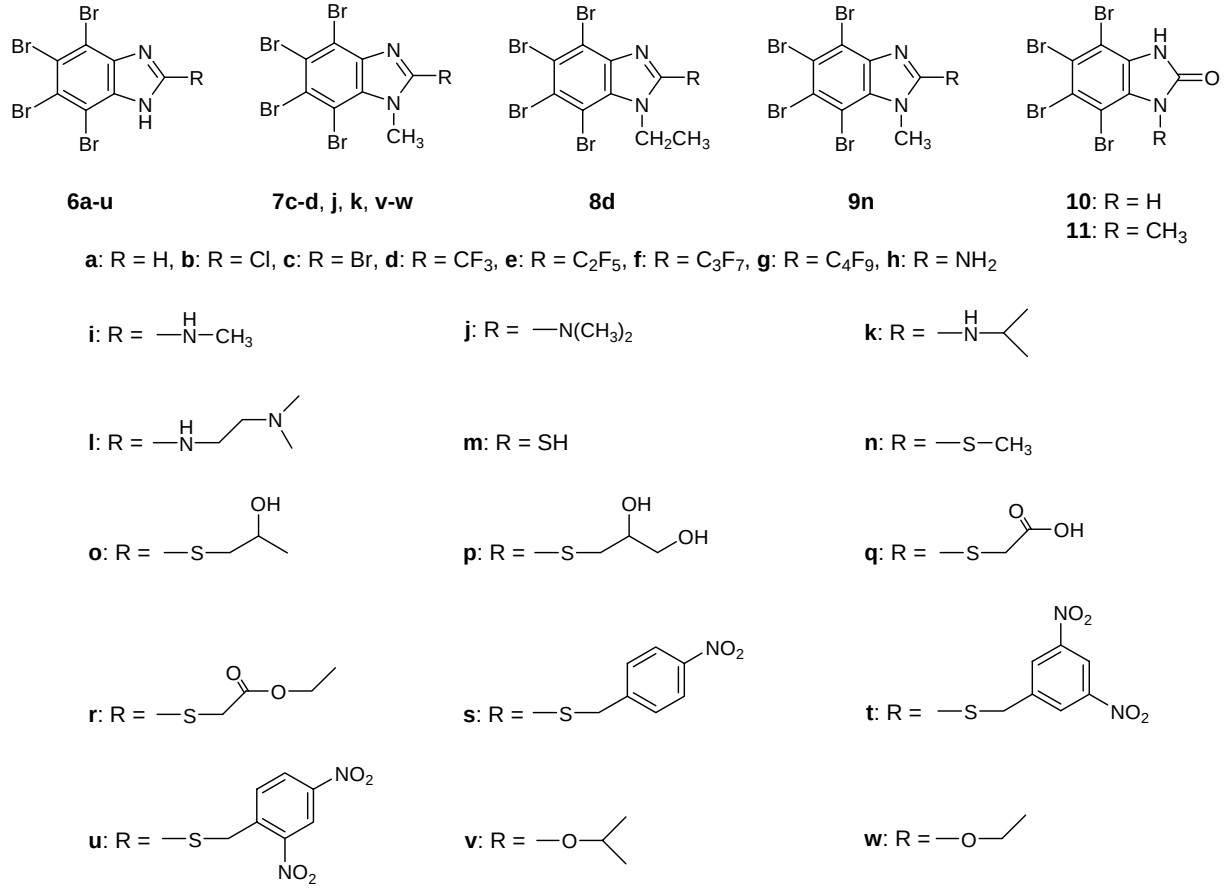


Figure 3. 4,5,6,7-Tetra-brominated benzimidazoles for antiviral testing.

Table 1. Antiviral activity of 4,5,6,7-tetrabrominated benzimidazoles against selected ssRNA$^+$ viruses (part 1)

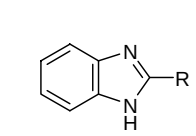
	HIV-1[a]		BVDV[b]		YFV[c]		DENV-2[c]		WNV[c]	
Comp.	CC_{50}[d]	EC_{50}[e]	CC_{50}[f]	EC_{50}[g]	CC_{50}[h]	EC_{50}[i]	CC_{50}[h]	EC_{50}[i]	CC_{50}[h]	EC_{50}[i]
6a	15	>15	13	>13	49	33	>100	>100	>100	>100
6b	8.0	>8.0	3.2	>3.2	3.0	>3.0	3.0	>3.0	3.0	>3.0
6c	9.0	>9.0	3.8	>3.8	2.6	>2.6	2.0	>2.0	2.0	>2.0
6d	<3.7	n.d.	2	>2	1	>1	1	>1	n.d.	>1
6e	<3.7	n.d.	0.8	>0.8	0.6	>0.6	0.5	>0.5	n.d.	>0.5
6f	1.0	>1.0	2.0	>2.0	0.8	>0.8	0.6	>0.6	0.6	>0.6
6g	1.2	>1.2	<3.7	n.d.	<3.7	n.d.	<3.7	n.d.	>3.7	n.d.
6h	4.0	>4.0	4.3	>4.3	58	41	66	46	66	31
6i	7.0	>7.0	65	>65	>100	>100	>100	>100	>100	>100
6j	17	>17	>100	>100	>100	>100	>100	>100	>100	>100
6k	4.0	>4.0	>100	>100	>100	>100	>100	>100	>100	>100
6l	5.0	>5.0	24	>24	56	23	18	>18	18	>18
6m	65	>65	>100	>100	>100	>100	>100	>100	>100	>100
6n	7.0	>7.0	15	>15	>100	>100	>100	>100	>100	>100
6o	15	>15	>100	>100	>100	>100	>100	>100	>100	>100
6p	17	7.0	14	>14	25	>25	>100	>100	>100	>100
6q	>100	>100	61	>61	39	>39	49	>49	49	>49
6r	≥100	>100	>100	>100	>100	>100	>100	>100	>100	>100
6s	4.0	>4.0	18	>18	3.0	>3.0	4.0	>4.0	4.0	>4.0
6t	3.7	>3.7	27	>27	4.0	>4.0	7.0	>7.0	7.0	>7.0
6u	3.0	>3.0	6.0	>6.0	3.0	>3.0	5.0	>5.0	5.0	>5.0
7c	≥100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7d	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7j	≥100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7k	68	>68	>100	>100	>100	>100	>100	>100	>100	>100
7v	76	>76	>100	>100	>100	>100	>100	>100	>100	>100
7w	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
8d	80	>80	>100	>100	>100	>100	>100	>100	>100	>100
9n	≥100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10	≥100	>100	77	>77	>100	>100	>100	>100	>100	>100
11	≥100	>100	>100	>100	>100	>100	>100	>100	>100	>100

[a] *Retroviridae*. [b] *Pestivirus*. [c] *Flavivirus*. [d] Compound concentration (μM) required to reduce MT-4 ($CD4^+$ human T-cells containing an integrated HTLV-1 genome) cell proliferation by 50%, as determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method, under conditions that allow untreated controls to undergo at least three consecutive

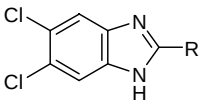
rounds of multiplication. [e] Compound concentration (μM) required to achieve 50% protection of MT-4 cell lines from virus-induced cytopathogenicity, as determined by the MTT method. [f] Compound concentration (μM) required to reduce the viability of mock-infected Madin Darby Bovin Kidney (MDBK) cells by 50%, as determined by the MTT method. [g] Compound concentration (μM) required to achieve 50% protection of preinfected MDBK cell lines from virus-induced cytopathogenicity, as determined by the MTT method. [h] Compound concentration (μM) required to reduce the viability of mock-infected baby hamster kidney (BHK) cells by 50%, as determined by the MTT method. [i] Compound concentration (μM) required to reduce the virus plaque number by 50% in BHK cell lines. n.d. not yet determined.

In the series of 4,5,6,7-tetrabrominated benzimidazole derivatives (Figure 3) no compound showing a significant selective activity against one of the ssRNA$^+$ viruses HIV-1, BVDV, YFV, DENV-2 or WNV could be identified as shown in Table 1. Among them, compounds **6h**, **6l** and **6p** exhibit a certain antiviral activity against HIV-1, YFV, DENV-2 or WNV. However, the antiviral activity is accompanied by cytotoxicity for the host cell lines within the same concentration range.

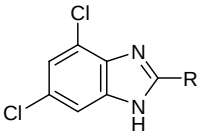
The closely related 4,5,6,7-tetrabromobenzimidazole derivatives **6d-g** having a trifluoromethyl, pentafluoroethyl, heptafluoropropyl or nonafluorobutyl side chain attached to position-2 of the benzimidazole ring exhibit strong cytotoxicity against the host cell lines in the low micromolar range (0.5-2.0 μM). On the other hand compounds **7d** and **7w** are completely inactive against all the tested ssRNA$^+$ viruses and are also not cytotoxic.



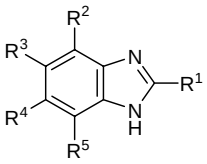
12a, **c-e**, **q**



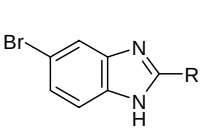
13b, **d-h**, **j**, **l**, **o-q**



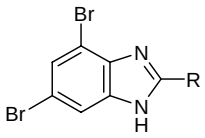
14b, **d**, **f-j**, **l**, **r**



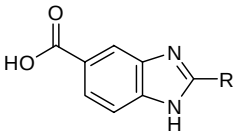
18: $R^1 = CF_3$, $R^2 = R^3 = R^4 = Br$, $R^5 = H$
19: $R^1 = CF_3$, $R^3 = R^4 = Br$, $R^2 = R^5 = H$
20: $R^1 = CF_3$, $R^2 = R^4 = R^5 = H$, $R^3 = Cl$
21: $R^1 = CF_3$, $R^2 = R^5 = Br$, $R^3 = R^4 = Cl$
22: $R^1 = CF_3$, $R^2 = R^3 = R^5 = Br$, $R^4 = Cl$
23: $R^1 = CF_3$, $R^2 = R^4 = Cl$, $R^3 = R^5 = Br$



15b



16b-c, **f-j**, **l-n**, **r**



17g, **k-l**, **p**, **s**

a: R = Br, **b**: R = CF_3, **c**: R = C_2F_5, **d**: R = C_3F_7, **e**: R = C_4F_9

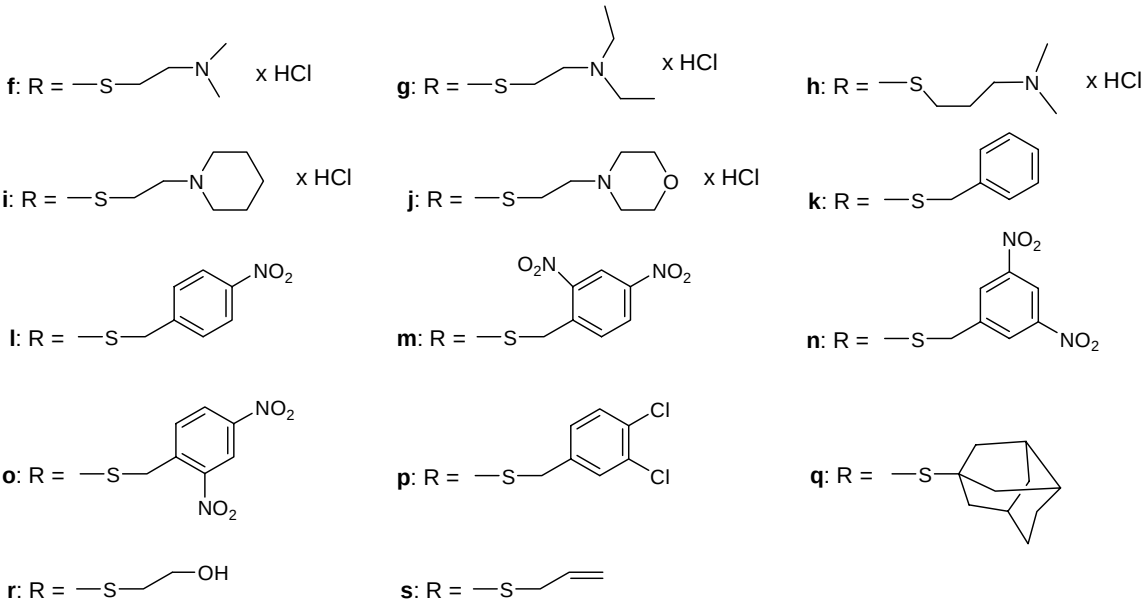


Figure 4. Halogenated and other benzimidazole derivatives for antiviral testing.

Table 2. Antiviral activity of halogenated benzimidazoles derivatives against selected ssRNA$^+$ viruses (part 2)[j]

	HIV-1[a]		BVDV[b]		YFV[c]		DENV-2[c]		WNV[c]	
Comp.	CC_{50}[d]	EC_{50}[e]	CC_{50}[f]	EC_{50}[g]	CC_{50}[h]	EC_{50}[i]	CC_{50}[h]	EC_{50}[i]	CC_{50}[h]	EC_{50}[i]
12a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12c	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12d	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12e	44	>44	>100	>100	>100	>100	>100	>100	>100	>100
12q	>100	64	>100	>100	>100	>100	>100	>100	>100	>100
13b	4.0	>4.0	3.0	>3.0	3.5	>3.5	12	>12	12	>12
13d	9	>9	2.5	>2.5	2.3	>2.3	1.3	>1.3	1.3	>1.3
13e	9	>9	11	>>11	9.5	>9.5	1	>1	1	>1
13f	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
13g	>100	>100	>100	100	100	29	≥100	≥100	>100	>100
13h	19	>19	8.8	>8.8	18	>18	49	>49	49	>49
13j	>100	>100	>100	>100	≥100	>100	47	>47	47	>47
13l	70	>70	37	>37	52	≥25	100	>100	100	>100
13o	18	>18	70	>70	≥100	>100	>100	>100	>100	>100
13p	>100	>100	65	>65	100	>100	59	>59	59	>59
13q	38	>38	>100	>100	>100	>100	>100	>100	>100	>100
14b	10	>10	4.2	>4.2	5.0	>5.0	4.2	>4.2	4.2	>4.2
14d	2.6	>2.6	2.9	>2.9	2.7	>2.7	2.4	>2.4	2.4	>2.4
14f	55	>55	72	32	35	>35	33	19	33	>33
14g	22	>22	48	>48	26	>26	31	>31	31	>31
14h	52	>52	44	32	20	>20	11	86	11	11
14i	25	>25	50	>50	51	>51	54	>54	54	>54
14j	70	>70	>100	>100	>100	>100	>100	>100	>100	>100
15b	9	>9	5.5	>5.5	11	>11	15	>15	15	>15
16b	17	>17	3.7	>3.7	5.5	>5.5	4.6	>4.6	4.6	>4.6
16c	6	>6	3.3	>3.3	3.3	>3.3	2.3	>2.3	2.3	>2.3
16f	47	>47	60	16	16	≥16	49	≥6.6	49	>49
16g	16	>16	35	18	18	>18	57	24	57	>57
16h	20	>20	70	>70	45	>45	30	>30	30	>30
16i	15	>15	44	>44	54	>54	>100	>100	>100	>100
16j	68	>68	>100	>100	>100	>100	>100	>100	>100	>100
16l	16	>16	12	>12	9	>9	8	>8	8	>8
16m	7	>7	14.5	10	17	9.5	20	>20	20	>20
16n	18	>18	>100	>100	>100	>100	>100	>100	>100	>100
16o	7.3	>7.3	14	10	17	9	20	>20	20	>20
16r	83	>83	100	>100	79	>79	>100	>100	>100	>100

Table 2. Continued

	HIV-1[a]		BVDV[b]		YFV[c]		DENV-2[c]		WNV[c]	
Comp.	CC_{50}[d]	EC_{50}[e]	CC_{50}[f]	EC_{50}[g]	CC_{50}[h]	EC_{50}[i]	CC_{50}[h]	EC_{50}[i]	CC_{50}[h]	EC_{50}[i]
17g	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
17k	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
17l	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
17p	>100	>100	99	>99	96	>96	83	>83	83	>83
17s	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
18	3.0	>3.0	1.3	>1.3	1.0	>1.0	2.3	>2.3	2.3	>2.3
19	4.0	>4.0	2.0	>2.0	2.3	>2.3	4.0	>4.0	4.0	>4.0
20	18	>18	13	>13	30	>30	34	>34	34	>34
21	4.0	>4.0	2.7	>2.7	1.7	>1.7	2.3	>2.3	2.3	>2.3
22	4.0	>4.0	3.5	>3.5	1.8	>1.8	2.0	>2.0	2.0	>2.0
23	4.0	>4.0	3.8	>3.8	2.0	>2.0	2.0	>2.0	2.0	>2.0

[a] *Retroviridae*. [b] *Pestivirus*. [c] *Flavivirus*. [j] For details of the antiviral assays see Table 1.

Next, various non-halogenated, chlorinated and brominated benzimidazole derivatives as well as 2-carboxybenzimidazoles (Figure 4) were evaluated for their antiviral activity against $ssRNA^+$ viruses (HIV-1, BVDV, YFV, DENV-2, WNV). Unfortunately, the compounds of this series do not show selective antiviral activity against any of the tested $ssRNA^+$ viruses. The moderate antiviral activities of **13g**, **14f**, **16f**, **16g**, **16m** and **16o** are accompanied by cytotoxicity against the host cell lines within the same concentration range as it was also observed for the 4,5,6,7-tetra-brominated benzimidazoles shown above.

However, from the data given in Table 2 it can be seen that compounds **14d**, **16c**, **18**, **19** and **21-23** are strongly cytotoxic within a range of 1.0-4.0 μM in all tested cell lines whereas compounds **12a**, **12c**, **12d**, **13f**, **17g**, **17k** and **17l** are completely inactive against all tested $ssRNA^+$ viruses and are also non-cytotoxic.

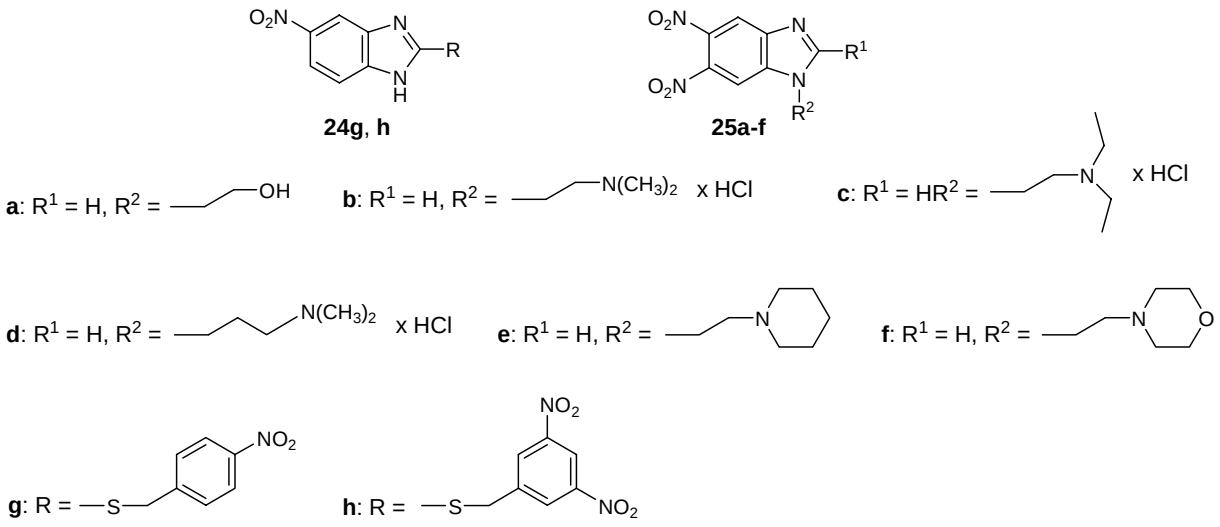


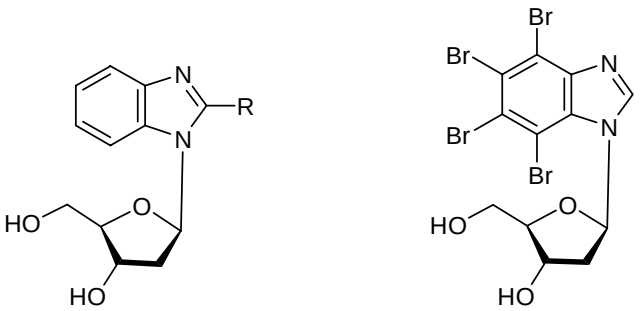
Figure 5. Nitrobenzimidazole derivatives for antiviral testing.

Table 3. Antiviral activity of nitrobenzimidazole derivatives against selected ssRNA$^+$ viruses (part 3)[j]

	HIV-1[a]		BVDV[b]		YFV[c]		DENV-2[c]		WNV[c]	
Comp.	CC_{50}[d]	EC_{50}[e]	CC_{50}[f]	EC_{50}[g]	CC_{50}[h]	EC_{50}[i]	CC_{50}[h]	EC_{50}[i]	CC_{50}[h]	EC_{50}[i]
24g	15	>15	>100	>100	>100	>100	>100	>100	>100	>100
24h	13	>13	9	>9	12	>12	19	>19	>19	>19
25a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
25b	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
25c	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
25d	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
25e	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
25f	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

[a] *Retroviridae*. [b] *Pestivirus*. [c] *Flavivirus*. [j] For details of the antiviral assays see Table 1.

The data summarized in Table 3 show that none of the nitrobenzimidazole derivatives (Figure 5) exhibit selective antiviral activity against ssRNA$^+$ viruses. Compound **24g** is cytotoxic against the cell line used in the HIV-1 assay while compound **24h** is toxic against all tested cell lines.



26a: R = Br
b: R = H

27

Figure 6. Benzimidazole *β*-D-2'-deoxyribonucleosides selected for antiviral testing.

Table 4. Antiviral activity of benzimidazole *β*-L- and *β*-D-2'-deoxyribonucleosides against selected ssRNA$^+$ viruses (part 4)[j]

	HIV-1[a]		BVDV[b]		YFV[c]		DENV-2[c]		WNV[c]	
Comp.	CC_{50}[d]	EC_{50}[e]	CC_{50}[f]	EC_{50}[g]	CC_{50}[h]	EC_{50}[i]	CC_{50}[h]	EC_{50}[i]	CC_{50}[h]	EC_{50}[i]
1	n.d.	n.d.	>100	>100	>100	>100	>100	>100	n.d.	>100
2	n.d.	n.d.	>100	>100	>100	>100	>100	>100	n.d.	>100
3	>100	>100	>100	>100	>100	>100	>100	>100	n.d.	>100
4	7.9	>7.9	5	>5	5	>5	6.5	>6.5	6.5	>6.5
5	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
26a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
27	4.0	>4.0	4	4	5.7	>5.7	45	24	45	18

[a] *Retroviridae*. [b] *Pestivirus*. [c] *Flavivirus*. [j] For details of the antiviral assays see Table 1. n.d. not yet determined.

Then, the benzimidazole *β*-L-2'-deoxyribonucleosides **1**-**4** as well as the fluoroarabino nucleoside **5** (Figure 2) and closely related *β*-D-2'-deoxyribonucleosides (**26a**, **27**; Figure 6) were screened for their antiviral activity against ssRNA$^+$ viruses as demonstrated by Table 4.[29] Among them, the 4,5,6,7-tetrabrominated benzimidazole *β*-L-2'-deoxyribonucleoside **4** as well as its corresponding *β*-D-enantiomer **27** show similar antiviral activity against the tested ssRNA$^+$ viruses. However, these compounds are also cytotoxic against the host cell lines within the same concentration range. For comparison, 4,5,6,7-tetrabromobenzimidazole **6a** also exhibits antiviral activity accompanied by toxicity (see Table 1). The 4,5,6,7-tetrabrominated benzimidazole 2'-deoxyribonucleosides **4** and **27** are more toxic than the heterocycle **6a** itself. It can be assumed that the nucleosides can enter the cells more efficiently and function as a release system delivering the active benzimidazole derivative **6a**. Unfortunately, the other benzimidazole L- and D-nucleosides show no antiviral activity and are non-toxic.

Table 5. Activity of benzimidazole derivatives against HBV

		HBV[a] RI	HBV[a] Vir			HBV[a] RI	HBV[a] Vir
Comp.	CC_{50}[b]	EC_{50}[c]	EC_{50}[d]	Comp.	CC_{50}[b]	EC_{50}[c]	EC_{50}[d]
1	>100	>10	>10	**13e**	1.8	>1	>1
2	>100	>10	>10	**13f**	>100	>10	>10
3	>100	>10	>10	**13g**	58	>10	>10
4	7.8	>10	>10	**13j**	64	>10	>10
5	>50	>10	>10	**13l**	17	>10	>10
6a	9.0	0.3	0.5	**13p**	5	>5	>5
6b	4.5	>10	>10	**14b**	12	>10	>10
6c	4.3	>10	>10	**14d**	2.0	>2.0	>2.0
6d	5	>1	>1	**14h**	58	>10	>10
6e	3	>1	>1	**15b**	27	>1	>1
6f	0.8	>0.8	>0.8	**16b**	17	>1	>1
6h	<3.7	0.3	0.3	**16c**	15	>1	>1
6i	6.6	0.5	0.2	**16f**	30	>10	>10
6j	100	5.0	1.0	**16g**	19	>10	>10
6k	5.8	0.7	0.4	**16m**	14	>10	>10
6l	6.0	>10	>10	**16n**	15	>10	>10
6m	n.d.	0.5	1.0	**16o**	14	>10	>10
6n	12	>10	>10	**17g**	>100	>10	>10
6o	9.0	0.4	0.6	**17k**	>100	>10	>10
6q	>100	>10	>10	**17l**	>100	>10	>10
6r	>100	>10	>10	**17p**	>100	>10	>10
6s	3.0	>3.0	>3.0	**17s**	>100	>10	>10
6t	3.6	>3.7	>3.7	**18**	2.7	>2.7	>2.7
6u	n.d.	>10	>10	**19**	2.8	>2.8	>2.8
7c	33	10	10	**20**	14	>10	>10
7d	>100	>10	>10	**21**	3.6	>10	>10
7j	>100	0.8	0.6	**22**	3.7	>3.0	>3.0
7k	>100	>10	>10	**23**	3.7	>7.5	>7.5
7v	>100	10	10	**25a**	>100	>10	>10
7w	>100	>10	>10	**25b**	>100	>10	>10
8d	>100	>10	>10	**25c**	>100	>10	>10
9n	100	>10	>10	**25d**	>100	>10	>10
10	n.d.	>10	>10	**25e**	>100	>10	>10

Table 5. Continued

		HBV[a] RI	HBV[a] Vir			HBV[a] RI	HBV[a] Vir
Comp.	CC_{50}[b]	EC_{50}[c]	EC_{50}[d]	Comp.	CC_{50}[b]	EC_{50}[c]	EC_{50}[d]
11	>100	>10	>10	**25f**	>100	>10	>10
12a	>100	>10	>10	**26**	>100	>10	>10
12e	52	>10	>10	**27**	4.7	>10	>10
13b	≤3	>3.0	>3.0	**3TC**		0.04	0.025
13d	>3.0	>1	>1				

[a] *Hepadnaviridae*. [b] Compound concentration (μM) required to reduce the viability of heptatocellular carcinoma (HepG2) cells by 50%, as determined by the MTT method. [c] Compound concentration (μM) required to reduce the intracellular HBV DNA (HBV replicative intermediates, RI). [d] Compound concentration (μM) required to reduce the intracellular HBV virion (HBV Vir).

Several benzimidazole derivatives were also tested for their activity against the Hepatitis B virus (HBV) which belongs to the DNA viruses. In this assay the 50% inhibitory concentration (EC_{50}) was determined for the virion (Vir) and the replicative intermediate (RI) of HBV. Table 5 shows that within the tested compounds the 4,5,6,7-tetrabrominated benzimidazole derivatives **6j** and **7j** both carrying a dimethylamino side chain attached to position-2 of the heterocycle show anti-HBV activity against the virion and the replicative intermediate in a low micromolar concentration range (**6j**: EC_{50} (RI) = 5.0 μM, EC_{50} (Vir) = 1.0 μM and **7j**: EC_{50} (RI) = 0.8 μM, EC_{50} (Vir) = 0.6 μM) while the cytotoxicity is less pronounced (**6j**: CC_{50} = 100 μM and **7j**: CC_{50} >100 μM). Interestingly, among the 4,5,6,7-tetrabrominated benzimidazoles having different side chains attached to position-2 (Figure 3) several compounds (**6a**, **h**, **i**, **k**, **o**) are toxic in low concentration (<1 μM). Apart from the L-nucleoside **4** which is cytotoxic the tested nucleosides **1-3**, **5** are without activity.

Table 6. Activity of benzimidazole derivatives in the HCV[a] replicon system

	HCV Replicon			HCV Replicon	
Compound	%INHIB[b]	%TOX[c]	Compound	%INHIB[b]	%TOX[c]
6a	47.4	1.6	**7w**	10.6	0
6c	29.9	0	**8d**	0	9.0
6h	78.6	42.5	**11**	0	0
6i	24.5	42.0	**12a**	0	1.6
6j	0	77.3	**12e**	21.9	0
6k	0	33.6	**13b**	66.6	68.0
6o	48.5	10.0	**14b**	15.0	28.0
6q	7.2	9.0	**16g**	7.3	14.1
6r	20.5	56.0	**16n**	10.4	19.0
7c	89.4	4.3	**18**	63.0	63.5
7d	0	0	**20**	22.3	48.0
7j	0	2.4	**21**	63.1	69.6
7k	0	0	**23**	53.8	69.0

[a] *Hepacivirus*. [b] The observed inhibition of HCV replicon replication at the test concentration of 15μM of drug as determined by the HCV replicon (ELISA) assay. [c] The observed cellular toxicity at the test concentration of 15μM of drug.

Regarding the activity in the HCV replicon system a series of benzimidazoles were selected for testing (Table 6). Their ability to inhibit the HCV replicon replication at the test concentration of 15μM was evaluated by an HCV replicon assay (ELISA) as previously described.[28] The cellular toxicity was determined at the test concentration of 15μM of drug.[28] For most compounds inhibition of the HCV replicon replication is accompanied by cytotoxicity. Only in case of **6c**, **7w** and **12e** inhibition was observed without cytotoxicity.

Table 7. Activity of benzimidazole derivatives against RSV[a]

	RSV			RSV			RSV	
Comp.	CC_{50}[b]	EC_{50}[c]	Comp.	EC_{50}[b]	EC_{50}[c]	Comp.	EC_{50}[b]	EC_{50}[c]
3	>100	>100	**6q**	100	>100	**13b**	3.4	>3.4
4	5	>5.0	**6r**	≥100	>100	**14b**	1.7	>1.7
5	>100	>100	**6s**	3.5	>3.5	**14h**	36	>36
6a	2.3	>2.3	**6t**	10	>10	**14d**	1.0	>1.0
6b	0.5	>0.5	**6u**	2.7	>2.7	**16g**	>100	>100
6c	1.3	>1.3	**7c**	>100	>100	**16n**	1.1	>1.1
6f	0.6	>0.6	**7d**	>100	>100	**16o**	6.0	>6.0
6h	1.0	>1.0	**7j**	>100	>100	**18**	0.8	>0.8
6i	5.0	>5.0	**7k**	>100	>100	**19**	1.2	>1.2
6j	>100	>100	**7w**	>100	>100	**20**	20	>20
6k	>100	>100	**8d**	>100	>100	**21**	0.7	>0.7
6l	6.0	>6.0	**9n**	>100	>100	**22**	0.7	>0.7
6m	>100	>100	**10**	100	>100	**23**	0.8	>0.8
6n	6.0	>6.0	**11**	≥100	>100	**26**	>100	>100
6o	23	>23	**12a**	>100	>100	**27**	2.0	>2.0
6p	72	>72	**12e**	>100	>100			

[a] *Paramyxoviridae*. [b] Compound concentration (μM) required to reduce the Vero cell number determined by methylene blue staining. [c] Compound concentration (μM) required to reduce the virus plaque number by 50% in Vero cell lines.

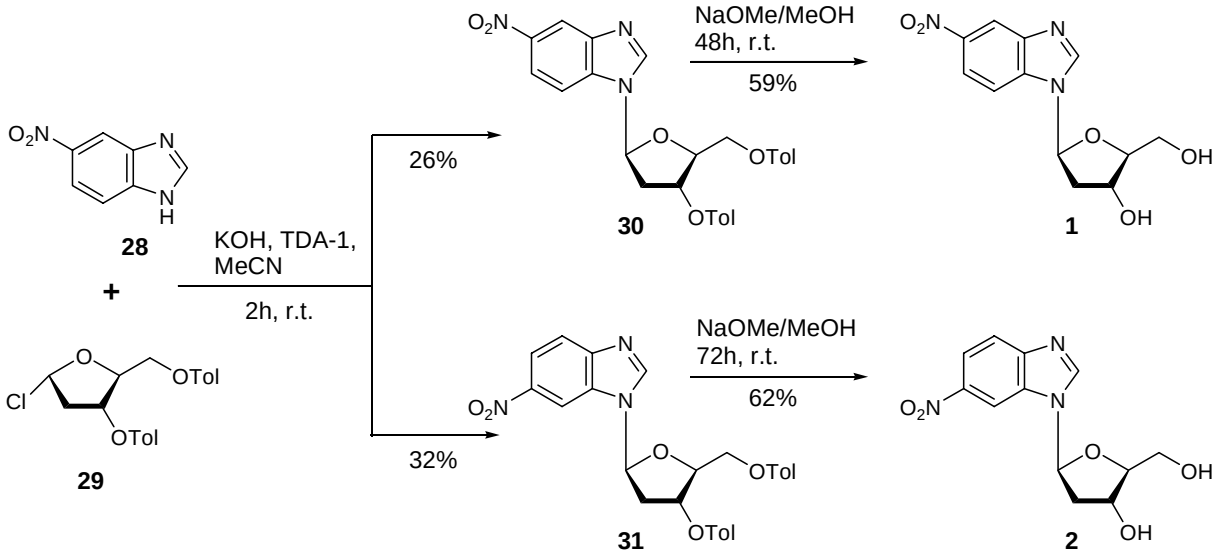
The Respiratory Syncytial Virus (RSV) is a major cause of respiratory illness in infants and young children. However, antiviral approaches are still required to treat this infection. From Table 7 it can be seen that none of the tested benzimidazole derivatives show selective antiviral activity against RSV. The compounds are either toxic or completely inactive. However, in the series of the halogenated benzimidazoles including the 4,5,6,7-tetrabrominated benzimidazole L-nucleoside **4** most compounds are cytotoxic.

Nucleoside Synthesis

Synthesis of benzimidazole β-L-2'-deoxyribonucleosides

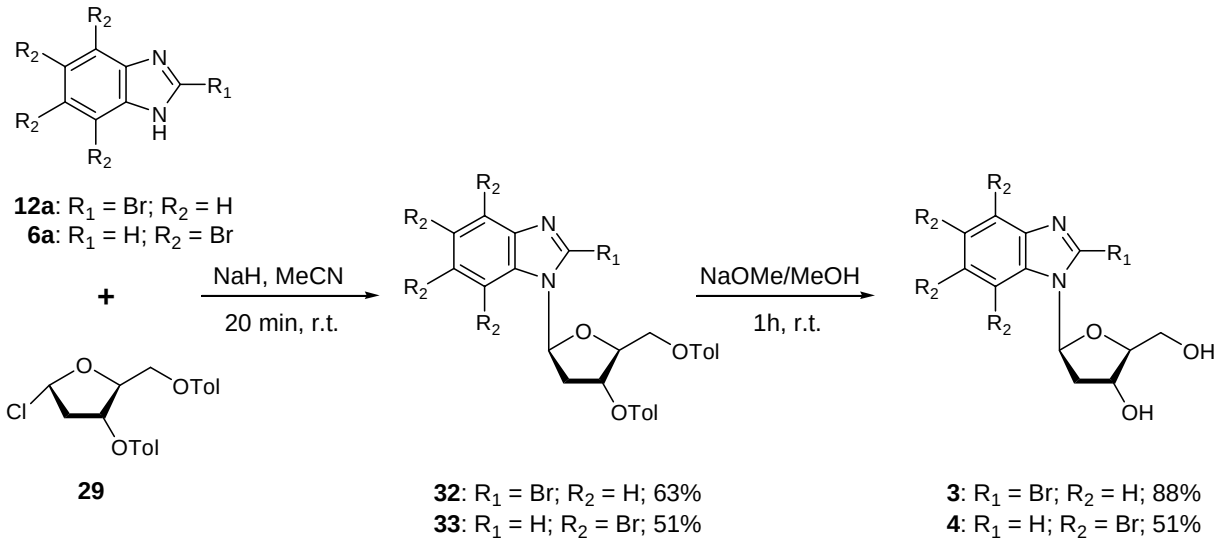
Considering the identical properties of L- and D-nucleosides and their precursors in a non-chiral environment, the protocols developed for the chemical synthesis of their D-counterparts can be employed for the preparation for the L-enantiomers.[30] Instead of 3,5-di-*O*-(4-methylbenzoyl)-2-deoxy-α-D-*erythro*-pentofuranosyl chloride the corresponding L-enantiomer is used as sugar component. 3,5-Di-*O*-(4-methylbenzoyl)-2-deoxy-α-L-*erythro*-pentofuranosyl chloride (**29**) was already described in 1964 [21] and was prepared according to the procedure for the corresponding D-halogenose.[31]

The glycosylation of 5(6)-nitrobenzimidazole[32] (**28**) with the α-L-2-deoxyribofuranosyl halide **29** was performed in MeCN in the presence of powdered KOH using TDA-1 (tris[2-(2-methoxyethoxy)ethyl]amine) as phase-transfer catalyst (nucleobase anion conditions[33]) (Scheme 3). The glycosylation led to a mixture of the N^1- and N^3-glycosylated regioisomers **30** and **31** which were separated by flash chromatography. The faster migrating compound was isolated in 26% (**30**) and the slower one (**31**) in 32% yield. Both compounds were deprotected with 1 M NaOMe/MeOH. Crystallization from methanol afforded the corresponding regioisomeric nucleosides **1** and **2** as colourless crystals in 59% and 62% yield, respectively.



Scheme 3

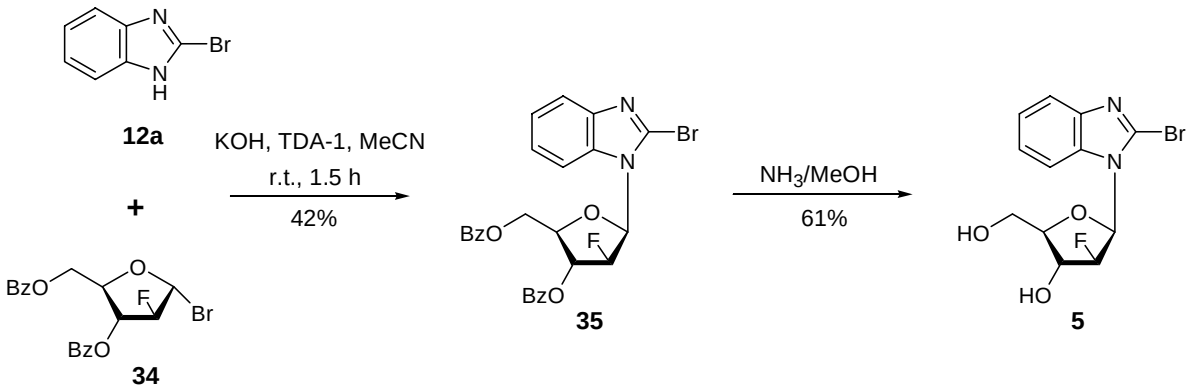
2-Bromobenzimidazole[25] (**12a**) and 4,5,6,7-tetrabromobenzimidazole[23] (**6a**) were prepared as described recently. Glycosylation of the brominated benzimidazoles **12a** or **6a** with halogenose **29** was achieved by employing the sodium salt glycosylation procedure as shown in Scheme 4. The sugar component **29** was added to a solution of 2-bromobenzimidazole **12a** or 4,5,6,7-tetrabromobenzimidazole **6a** in anhydrous MeCN in the presence of NaH. The glycosylation reaction afforded the toluoyl-protected *β*-L-nucleosides **32** or **33** in 63% and 51% yield, respectively. Deprotection of both nucleosides with 1 M NaOMe/MeOH furnished the brominated benzimidazole *β*-L-nucleosides **3** (88%) and **4** (51%).



Scheme 4

Synthesis of 2-bromo-1-[2-deoxy-2-fluoro-β-D-arabinofuranosyl]-benzimidazole (5). The convergent synthesis of a number of 2'-deoxy-2-fluoro-*β*-D-arabinofuranosyl nucleosides have already been reported.[34] The preparation of 2-bromo-2'-deoxy-2'-fluoro-*β*-D-arabinofuranosylbenzimidazole (**5**) started with the known 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide **34** which was obtained from the commercially available 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose by a three step procedure.[35]

The glycosylation reaction of nucleobase **12a** with fluoro sugar **34** was performed by nucleobase anion glycosylation (MeCN/KOH/TDA-1) in a similar way as described for the nitrobenzimidazole *β*-L-nucleosides above (Scheme 5). This reaction afforded the protected 2'-deoxy-2'-fluoro nucleoside **35** in 42% yield. Deprotection of **35** with methanolic ammonia resulted in the removal of the benzoyl groups and gave the free nucleoside **5** in 61%.



Scheme 5

Spectroscopic data and single crystal X-ray analysis. Compounds **1-5** as well as their intermediates were characterized by ^{1}H NMR spectra (experimental section) and ^{13}C-NMR spectra (Table 8) as well as by elemental analysis. For the benzimidazole *β*-L-2'-deoxyribonucleosides **1-2** and **30-31** the assignment of the ^{13}C-NMR chemical shifts were made according to the corresponding D-nucleosides.[36]

For compounds **4**, **33** the ^{13}C-NMR signals of the functionalized benzimidazole nucleobases were assigned according to ^{13}C-NMR chemical shifts of the non-brominated benzimidazole nucleoside (**26b**) (Figure 6).[37] Moreover, gated decoupled ^{1}H-^{13}C NMR spectra were measured to confirm the assignment of the ^{13}C-signals of nucleosides **5**, **32** and **35** (Table 9).

For the 2-bromobenzimidazole 2'-deoxy-2'-fluoro-*β*-D-arabinofuranosyl nucleosides **35** and **5** the presence of the fluorine atome in the sugar moiety was confirmed by ^{19}F-NMR spectra (**35**: -194.2 ppm and **5**: -194.0 ppm). Moreover, $J_{C,F}$ coupling constants are given in Table 9.

Table 8. ^{13}C NMR chemical shifts of benzimidazole *β*-L- and *β*-D-2'-deoxyribonucleosides[a]

	C(2)[b]	C(3a)[b]	C(4)[b]	C(5)[b]	C(6)[b]	C(7)[b]	C(7a)[b]
1	146.1	143.0	115.7	137.4[d]	118.2	112.1	143.0
2	147.2	143.0	119.9	117.6	132.2[d]	108.8	148.2
3	129.2	143.2	118.8	123.1	122.7	112.9	133.2
4	145.2[d]	144.1[d]	116.6[d]	122.9[d]	120.8[d]	106.6[d]	131.2[d]
5	128.3	142.7	118.4	123.1	122.6	114.0	135.2
30	145.9	143.2	115.9	137.3[d]	118.2	112.2	143.0
31	146.7	143.2	120.1	117.9	132.6	108.5	148.0
32	126.5	143.2	119.1	123.0[e]	123.0[e]	112.0	132.9
33	144.7[d]	143.9[d]	116.8[d]	123.5[d]	121.3[d]	106.9[d]	131.4[d]
35	133.9	142.7	118.6	122.8[e]	122.8[e]	133.6	135.0
26b[37]	142.2	143.8	119.6	122.7	122.1	111.4	133.0

Table 8. (continued)

	C-1'	C-2'	C-3'	C-4'	C-5'
1	85.0	- [c]	70.4	87.9	61.3
2	85.1	- [c]	70.3	87.8	61.2
3	86.4	38.4	70.2	87.4	61.3
4	85.7	41.8	69.1	87.8	60.5
5	86.0	97.4	73.8	82.9	59.9
30	81.8	36.5	74.6	85.2	63.9
31	81.9	36.6	74.8	85.1	64.1
32	80.7	35.4	73.5	85.9	63.6
33	81.9	37.8	74.3	85.1	63.7
35	86.2	94.9	76.3	77.3	62.6
26b[37]	84.5	-	70.6	87.6	61.6

[a] Measured in DMSO-d_6 at 298 K. [b] Systematic numbering. [c] Superimposed by the DMSO signal. [d] Tentative assignment. [e] The signals of C5 and C6 are superimposing.

Table 9. $J_{C,H}$ and $J_{C,F}$ values (in Hz) of β-L- and β-D-2'-deoxyribonucleosides[a, b]

	5	**32**	**35**
C2, H2	-	-	-
C2, H1'	3.5	8.3	m[e]
C3a, H4	14.1	15.5	15.5
C4, H4	165.0	162.1	162.6
C5, H5	160.4	162.7[c]	160.0[c]
C6, H6	160.1	162.7[c]	160.0[c]
C7, H7	168.7	165.5	168.4
C7a, H7	20.1	23.0	m[e]
C1'-H1'	161.0	151.2	161.5
C2'-H2'	193.5	135.8	193.8
C3'-H3'	149.0	156.6	156.2
C4', H4'	147.7	162.7	151.5
C5'-H5'	142.4	149.5	151.7
C1'-F2'	17.5	-	18.0
C2'-F2'	193.1	-	194.1
C3'-F2'	24.2	-	28.8
C4'-F2'	4.8	-	-

[a] Measured in DMSO-d_6 at 298 K. [b] Systematic numbering is used. [c] The signals of C5 and C6 are superimposing. [d] Superimposed by the DMSO signal. [e] Superimposing multipletts.

Furthermore, the solid state structure of the benzoylated 2-bromo-1-[2-deoxy-2-fluoro-β-D-arabinofuranosyl]-benzimidazole **35** was confirmed by single-crystal x-ray analysis.[38]
From the crystal structure of **35**, the orientation of the nucleobase relative to the sugar moiety was determined to be *syn* with χ = (O1'-C1'-N11-C18) = 39.7(9)°. The natural 2'-deoxyribonucleosides usually adopt an *anti* conformation. However, it is observed that the introduction of a bulky substituent in position-2 of the heterocyclic moiety shifts the conformation around the glycosylic bond from *anti* to *syn* as it was recently reported for 2'-deoxyimmunosine.[39] The sugar moiety of **35** shows a pseudorotation phase angle *P* of 118.8° and a maximum amplitude of puckering τ_m of 41.8° which indicates a south (*S*) conformation. This result is in contrast to many other 2'-fluoroarabino nucleosides that are in *N*-conformation. However, the conformation of the sugar residue is also strongly influenced by the benzoyl protecting groups.

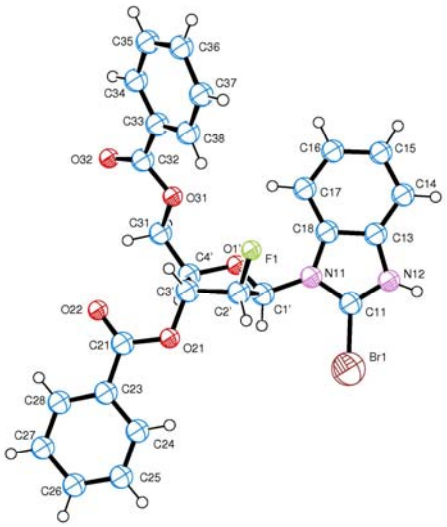


Figure 7. The crystal structure of compound **35** obtained from single crystal X-ray analysis.

Conclusions

The antiviral activity of a series of benzimidazole derivatives and substituted benzimidazole *β*-L- and *β*-D-nucleosides against selected RNA and DNA viruses including HIV-1, BVDV, YFV, DENV-2, WNV, HBV, HCV and human RSV was evaluated. Unfortunately, no selective antiviral activity was observed for the tested compounds. However, among the halogenated benzimidazoles including the 4,5,6,7-tetrabrominated benzimidazoles *β*-L-2'-deoxyribonucleoside **4** several derivatives were found to be cytotoxic against the tested cell lines.

These compounds are potential candidates for anti-cancer screening.

Furthermore, the stereoselective synthesis of benzimidazole *β*-L-2'-deoxyribonucleosides and the 2-bromobenzimidazole 2'-deoxy-2'-fluoro-*β*-D-arabinofuranosyl nucleoside was accomplished using nucleobase anion glycosylation. For that, 3,5-di-*O*-(4-methylbenzoyl)-2-deoxy-α-L-*erythro*-pentofuranosyl chloride or 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide were employed as sugar residues. The compounds were characterized by NMR spectroscopy and compound **35** by single-crystal X-ray analysis.

Experimental Section

General Procedures. All chemicals were purchased from Acros, Fluka or Sigma-Aldrich. Solvents were of technical grade and were distilled before use. Thin-layer chromatography (TLC) was carried out on TLC aluminium sheets covered with silica gel 60 F_{254}, 0.2 mm layer (VWR, Germany). Column flash chromatography (FC) was performed at 0.4 bar on silica gel 60 H (VWR, Germany). UV-spectra: U3200 spectrophotometer (Hitachi, Japan); λ_{max} in nm, ε in dm^3 mol^{-1}. NMR Spectra: Avance-DPX-250, Avance-DPX-300 or AMX-500 spectrometers (Bruker, Rheinstetten, Germany) at 250.13, 300.15 and 500 MHz for ^1H and ^{13}C; chemical shifts (δ) are in ppm rel. to internal $SiMe_4$ (^1H, ^{13}C). Microanalyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany).

1-[2-Deoxy-3,5-di-*O*-(4-methylbenzoyl)-*β*-L-*erythro*-pentofuranosyl]-5-nitrobenzimidazole (30) and 1-[2-Deoxy-3,5-di-*O*-(4-methylbenzoyl)-*β*-L-*erythro*-pentofuranosyl]-6-nitrobenzimidazole (31). To a suspension of powdered KOH (1.013 g, 18.0 mmol) and TDA-1 (178 mg, 0.6 mmol) in anhyd. CH_3CN (300 ml) 5(6)-nitrobenzimidazole (**28**; 980 mg, 6.0 mmol) was added at room temperature. After the mixture was stirred for 30 min, 3,5-di-*O*-(4-methylbenzoyl)-2-deoxy-α-L-*erythro*-pentofuranosyl chloride (**29**; 2.45 g, 6.3 mmol) was introduced and stirring was continued for 120 min. Insoluble material was filtered off and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 and purified by flash chromatography (FC) (silica gel, 50 x 5.5 cm) and the compounds **30** and **31** were eluted with CH_2Cl_2/EtOAc (8:2).

From the faster migrating zone, compound **30** was isolated. After evaporation of the solvent **30** was obtained as colorless crystals (0.8 g, 26%). M.p. 134-135 °C. TLC (silica gel, CH_2Cl_2/EtOAc 8:2): R_f 0.70. UV (MeOH): 238 (49400), 298 (8800). ^1H NMR (500 MHz, DMSO-d_6): δ 2.39, 2.42 (2s, 6H, 2x -CH_3), 2.87 (m, 1H, H-2'$_\beta$), 3.11 (m, 1H, H-2'$_\alpha$), 4.58 (m, 3H, H-4', H-5'), 5.75 (d, *J* = 5.7 Hz, 1H, H-3'), 6.69 ('t', *J* = 6.9 Hz, 1H, H-1'), 7.36 (dd, *J* = 7.8, 7.9 Hz, 4H, arom. H), 8.01 (m, 6H, arom. H, H-6, H-7), 8.56 (s, 1H, H-4), 8.81 (s, 1H, H-2). Anal. Calcd. for $C_{28}H_{25}N_3O_7$ (515.5) requires: C, 65.24; H, 4.89; N, 8.15. Found: C, 65.15; H, 4.87; N, 8.10.

From the slower migrating zone, compound **31** was isolated as colorless foam (1.0 g, 32%). M.p. 75-80 °C. TLC (silica gel, CH_2Cl_2/EtOAc, 8:2): R_f 0.62. UV (MeOH): 237 (52900), 296

(10400). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.36, 2.42 (2s, 6H, 2x -CH_3), 2.91 (m, 1H, H-2'$_\beta$), 3.13 (m, 1H, H-2'$_\alpha$), 4.57 (m, 2H, H-5'), 4.67 (m, 1H, H-4'), 5.73 (d, *J* = 5.6 Hz, 1H, H-3'), 6.78 ('t', *J* = 6.8 Hz, 1H, H-1'), 7.35 (dd, *J* = 7.8, 7.9 Hz, 4H, arom. H), 7.85 (m, 5H, arom. H, H-4), 8.15 (d, *J* = 8.9 Hz, 1H, H-5), 8.78 (s, 1H, H-7), 8.87 (s, 1H, H-2). Anal. Calcd. for $C_{28}H_{25}N_3O_7$ (515.5) requires: C, 65.24; H, 4.89; N, 8.15. Found: C, 65.26; H, 4.95; N, 8.11.

1-[2-Deoxy-*β*-L-*erythro*-pentofuranosyl)-5-nitrobenzimidazole (1). A solution of compound **30** (500 mg, 0.97 mmol), dissolved in a mixture of MeOH (130 ml) and 1 M NaOMe/MeOH (2.0 ml) was stirred at room temperature for 48 h. After evaporation of the solvent a solid residue was obtained which was applied to FC (silica gel, column 3 x 20 cm, $CHCl_3$/MeOH, 85:15). Crystallisation from MeOH afforded **1** as colorless crystals (160 mg, 59%). M.p. 157-160 °C. TLC (silica gel, $CHCl_3$/MeOH, 85:15): R_f 0.79. UV (MeOH): 238 (27100), 298 (9200). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 2.37 (m, 1H, H-2'$_\beta$), 2.62 (m, 1H, H-2'$_\alpha$), 3.88 (m, 2H, H-5'), 3.91 (m, 1H, H-4'), 4.42 (m, 1H, H-3'), 5.03 (m, 1H, 5'-OH), 5.40 (d, *J* = 3.9 Hz, 1H, 3'-OH), 6.47 ('t', *J* = 6.6 Hz, 1H, H-1'), 7.99 (d, *J* = 9.0 Hz, 1H, H-7), 8.19 (d, *J* = 9.1 Hz, 1H, H-6), 8.58 (s, 1H, H-4), 8.80 (s, 1H, H-2). Anal. Calcd. for $C_{12}H_{13}N_3O_5$ (279.3) requires: C, 51.61; H, 4.69; N, 15.05. Found: C, 51.59; H, 4.61; N, 14.89.

1-[2-Deoxy-*β*-L-*erythro*-pentofuranosyl)-6-nitrobenzimidazole (2). As described for **1**, with compound **31** (600 mg, 1.16 mmol), in MeOH (20 ml) and 1 M NaOMe/MeOH (2.5 ml) for 72 h. FC resulted in a colorless solid. Crystallisation from MeOH afforded **2** as colorless crystals (200 mg, 62%). M.p. 185-187 °C. TLC (silica gel, $CHCl_3$/MeOH, 8:2): R_f 0.66. UV (MeOH): 234 (16400), 297 (9800). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.38 (m, 1H, H-2'$_\beta$), 2.64 (m, 1H, H-2'$_\alpha$), 3.59 (m, 2H, H-5'), 3.92 (m, 1H, H-4'), 4.44 (m, 1H, H-3'), 5.05 (m, 1H, 5'-OH), 5.39 (d, *J* = 3.9 Hz, 1H, 3'-OH), 6.54 ('t', *J* = 6.5 Hz, 1H, H-1'), 7.88 (d, *J* = 8.9 Hz, 1H, H-4), 8.15 (d, *J* = 7.8 Hz, 1H, H-5), 8.80 (s, 1H, H-7), 8.85 (s, 1H, H-2). Anal. Calcd. for $C_{12}H_{13}N_3O_5$ (279.3) requires: C, 51.61; H, 4.69; N, 15.05. Found: C, 51.60; H, 4.59; N, 14.90.

2-Bromo-1-[2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-*β*-L-*erythro*-pentofuranosyl]-benzimidazole (32). NaH (80 mg, 2 mmol, 60% in oil) was added portionwise to a suspension of 2-bromobenzimidazole (**12a**; 295.5 mg, 1.5 mmol) in 25 ml anhyd. MeCN at room temperature. The reaction mixture was stirred for additional 10 min at rt. To this solution compound **29** (583.5 mg, 1.5 mmol) was added portionwise within 5 min and stirring was continued for 20 min at rt. The reaction mixture was diluted with CH_2Cl_2 (20 ml), filtered through a Celite pad, and the filtrate was evaporated. The residue was triturated with MeOH. The resulting suspension was filtered and compound **32** was obtained from MeOH as a white solid (520 mg, 63%). M.p. 117-118 °C. TLC (silica gel, $CHCl_3$/MeOH, 95:5): R_f 0.78. UV (MeOH): 242 (38800), 275 (8400), 282 (7600). ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.38, 2.40 (2s, 6H, 2x -CH_3), 2.66 (m, 1H, H-2'$_\beta$), 3.03 (m, 1H, H-2'$_\alpha$), 4.62 (m, 1H, H-4'), 4.75 (m, 2H, H-5'), 5.79 (m, 1H, H-3'), 6.53 ('t', *J* = 7.0 Hz, 1H, H-1'), 6.93 (t, *J* = 7.7 Hz, 1H, H-5), 7.22 (t, *J* = 7.4 Hz, 1H, H-6), 7.61 (d, *J* = 7.9 Hz, 1H, H-4), 7.70 (d, *J* = 8.0 Hz, 1H, H-7) and arom. H of the protecting groups. Anal. Calcd. for $C_{28}H_{25}N_2BrO_5$ (549.4) requires: C, 61.21; H, 4.59; N, 5.10. Found: C, 61.12; H, 4.50; N, 5.11.

2-Bromo-1-[2-deoxy-*β*-L-*erythro*-pentofuranosyl]-benzimidazole (3). A solution of compound **32** (420 mg, 0.76 mmol), dissolved in a mixture of MeOH (15 ml) and 1 M NaOMe/MeOH (2 ml) was stirred for 1 h at rt. After evaporation of the solvent a solid residue was obtained which was applied to FC (silica gel, column 2 x 20 cm, CH_2Cl_2/MeOH, 9:1). Evaporation of the solvent and crystallization from EtOH gave compound **3** as a colorless solid (210 mg, 88%). M.p. 150-152 °C. TLC (silica gel, CH_2Cl_2/MeOH, 9:1): R_f 0.69. UV (MeOH): 246 (7900), 267 (5100), 275 (6400), 282 (6200). ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.17 (m, 1H, H-2'$_\beta$), 2.62 (m, 1H, H-2'$_\alpha$), 3.68 (m, 2H, H-5'), 3.88 (m, 1H, H-4'), 4.43 (m, 1H, H-3'), 5.08 (m, 1H, 5'-OH), 5.44 (d, J = 3.1 Hz, 1H, 3'-OH), 6.37 (dd, J = 6.1, 6.0 Hz, 1H, H-1'), 7.25 (m, 2H, H-4, H-7), 7.63 (m, 1H, H-5), 7.93 (m, 1H, H-6). Anal. Calcd. for $C_{12}H_{13}N_2BrO_3$ (313.1) requires: C, 46.03; H, 4.18; N, 8.95. Found: C, 45.94; H, 4.16; N, 8.78.

1-[2-Deoxy-3,5-di-*O*-(4-methylbenzoyl)-*β*-L-*erythro*-pentofuranosyl]-4,5,6,7-tetrabromobenzimidazole (33). NaH (80 mg, 2 mmol, 60% in oil) was added portionwise to a suspension of 4,5,6,7-tetrabromobenzimidazole (**6a**; 651 mg, 1.5 mmol) in anhyd. MeCN (25 ml) at room temperature. The reaction mixture was stirred for additional 10 min at rt and then heated under reflux for 10 min. Compound **29** (583.5 mg, 1.5 mmol) was added, and stirring was continued for 20 min at rt. The reaction mixture was diluted with CH_2Cl_2 (20 ml), filtered through a Celite pad, and the filtrate was evaporated. The dry material was applied onto FC (silica gel, column 3.5 x 20 cm, CH_2Cl_2). Compound **33** was obtained from MeOH as a white solid (600 mg, 51%). M.p. 191-193 °C. TLC (silica gel, petroleum ether/ethyl acetate, 2:1): R_f 0.63. UV (MeOH): 230 (27500). ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.38, 2.41 (2s, 6H, 2x -CH_3), 2.97 (m, 1H, H-2'$_\beta$), 3.13 (m, 1H, H-2'$_\alpha$), 4.53 (m, 1H, H-4'), 4.57, 4.60 (2m, 2H, H-5'), 5.71 (m, 1H, H-3'), 7.16 ('t', J = 6.4, 1H, H-1'), 8.83 (s, 1H, H-2) and H arom. of the protecting groups. Anal. Calcd. for $C_{28}H_{22}N_2Br_4O_5$ (786.1) requires: C, 42.78; H, 2.82; N, 3.56. Found: C, 42.69; H, 2.74; N, 3.58.

1-[2-Deoxy-*β*-L-*erythro*-pentofuranosyl]-4,5,6,7-tetrabromobenzimidazole (4). A solution of compound **33** (500 mg, 0.64 mmol) in MeOH (20 ml) and 1 M NaOMe/MeOH (2.5 ml) was stirred under reflux for 10 min. Silica gel 60 (2 g) was added, and the solvent was evaporated. The dry material was applied onto FC (silica gel, column 2 x 10 cm, $CHCl_3$/MeOH, 9:1). Compound **4** was obtained from MeOH as a white solid (180 mg, 51%). M.p. 92-96 °C. TLC (silica gel, $CHCl_3$/MeOH, 9:1): R_f 0.39. UV (MeOH): 268 (10700). ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.48 (m, 1H, H-2'$_\beta$), 2.57 (m, 1H, H-2'$_\alpha$), 3.59, 3.63 (2m, 2H, H-5'), 3.89 (m, 1H, H-4'), 4.37 (m, 1H, H-3'), 5.10 (m, 1H, 5'-OH), 5.39 (m, 1H, 3'-OH), 7.01 (m, 1H, H-1'), 8.89 (s, 1H, H-2). Anal. Calcd. for $C_{12}H_{10}N_2Br_4O_3$ (549.8) requires: C, 26.21; H, 1.83; N, 5.09. Found: C, 26.25; H, 2.00; N, 5.16.

2-Bromo-1-[2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-*β*-D-arabinofuranosyl]-benzimidazole (35). Compound **12a** (230 mg, 1.2 mmol) was dissolved in 20 ml anhyd. MeCN. After addition of powdered KOH (200 mg, 3.6 mmol), the solution was stirred for 15 min at rt. Then TDA-1 (70 mg, 0.22 mmol) was added and stirring was continued for another 15 min at rt. After addition of the fluoro sugar **34** (400 mg, 0.945 mmol) in MeCN (5 ml), stirring was continued for

additional 1.5 h. Insoluble material was filtered off and the filtrate was evaporated to dryness (water bath temperature of the rotation evaporator not exceeding 35 °C). The resulting syrup was absorbed on silica gel and applied to FC (silica gel, column 2.5 x 20 cm, petroleum ether/ethyl acetate, 3:1). Compound **35** was obtained from MeOH as colorless crystals (270 mg, 42%). TLC (silica gel, petroleum ether/ethyl acetate, 1:1): R_f = 0.70. M.p. 60-63 °C. UV (MeOH): 267 (5200), 273 (6300), 281 (5700). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.74 (m, 2H, H-5'), 4.91 (m, 1H, H-4'), 5.76 (dd, $J_{2',F}$ = 32.5 Hz, 1H, H-2'), 5.89 (m, 1H, H-3'), 6.66 (dd, $J_{1',F}$ = 20.9 Hz, $J_{1',2'}$ = 3.8 Hz, 1H, H-1'), 6.82 (t, J = 7.7 Hz, 1H, H-5), 7.15 (t, J = 7.7 Hz, 1H, H-6), 7.7, 8.09 (2m, 12H, H-4, H-7 and arom. H of the protecting groups). Anal. Calcd. for $C_{26}H_{20}N_2FBrO_5$ (539.35) requires: C, 57.90; H, 3.74; N, 5.19. Found: C, 57.98; H, 3.81; N, 5.25.

2-Bromo-1-[2-deoxy-2-fluoro-*β*-D-arabinofuranosyl]-benzimidazole (5). Compound **35** (240 mg, 0.44 mmol) was dissolved in methanolic ammonia (saturated at 0 °C, 50 ml) and the soution was stirred at room temperature for 5 h. The solvent was evaporated and the residue applied to FC (silica gel, column 2 x 10 cm, CH_2Cl_2/MeOH, 95:5). The product containing fractions were collected and the solvent was evaporated to dryness to give compound **5** as a white solid (90 mg, 61%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1): R_f = 0.84. M.p. 177-179 °C. UV (MeOH): 245 (5700), 266 (4000), 273 (5000), 281 (4800). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.77 (m, 3H, H-5', H-4'), 4.40 (dd, $J_{3',F}$ = 23.5 Hz, 1H, H-3'), 5.10 (t, J = 3.1 Hz, 1H, H-2'), 5.28 (t, J = 3.2 Hz, 1H, 5'-OH), 6.01 (m, 1H, 3'-OH), 6.40 (dd, $J_{1',F}$ = 19.3 Hz, $J_{1'2'}$ = 3.7 Hz, 1H, H-1'), 7.22 (m, 2H, H-4, H-7), 7.60 (m, 1H, H-5), 7.85 (m, 1H, H-6). Anal. Calcd. for $C_{12}H_{12}N_2FbrO_3$ (331.14) requires: C, 43.53; H, 3.65; N, 8.46. Found: C, 43.49; H, 3.64; N, 8.44.

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References and Notes

1. Tunçbilek, M.; Göker, H.; Ertan, R.; Eryigit, R.; Kendi, E.; Altanlar, E. *Arch. Pharm.* **1997**, *330*, 372.
2. Preston, P. N. *Chem. Rev.* **1974**, *74*, 279 and references citied therein.

3. Burton, D. E.; Lambie, A. J.; Ludgate, J. C. L.; Newbold, G. T.; Percival, A.; Saggers, D. T. *Nature (London)* **1965**, *208*, 1166.
4. Navarrete-Vázquez, G.; Cedillo, R.; Hernández-Campos, A.; Yépez, L.; Hernández-Luis, F.; Valdez, J.; Morales, R.; Cortés, R.; Hernández, M.; Castillo, R. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 187.
5. Andrzejewska, M.; Yépez-Mulia, L.; Cedillo-Rivera, R.; Tapia, A.; Vilpo, L.; Vilpo, J.; Kazimierczuk, Z. *Eur. J. Med. Chem.* **2002**, *37*, 973.
6. Kazimierczuk, Z.; Andrzejewska, M.; Kaustova, J.; Klimešova, V. *Eur. J. Med. Chem.* **2005**, *40*, 203.
7. Stefańska, J. Z.; Gralewska, R.; Starościak, B. J.; Kazimierczuk, Z. *Pharmazie* **1999**, *54*, 879.
8. Townsend, L. B.; Devivar, R. V.; Turk, S. R.; Nassiri, M. R.; Drach, J. C. *J. Med. Chem.* **1995**, *38*, 4098.
9. North, T. W.; Sequar, G.; Townsend, L. B.; Drach, J. C.; Barry, P. A. *Antimicrob. Agents Chemother.* **2004**, *48*, 2760.
10. Townsend, L. B.; Revankar, G. R. *Chem. Rev.* **1970**, *70*, 389.
11. (a) Tamm, I. *Science* **1957**, *126*, 1235. (b) Sehgal, P. B.; Fraser, N. W.; Darnell, J. E. *Virology* **1979**, *94*, 185.
12. Tamm, I.; Kikuchi, T.; Darnell, Jr., J. E.; Salditt-Georgieff, M. *Biochemistry* **1980**, *19*, 2743.
13. Tamm, I.; Sehgal, P. B. *Adv. Virus Res.* **1978**, *22*, 187.
14. Bucknall, R. A. *J. Gen. Virol.* **1967**, *1*, 89.
15. Biron, K. K.; Harvey, R. J.; Chamberlain, S. C.; Good, S. S.; Smith III, A. A.; Davis, M. G.; Talarico, C. L.; Miller, W. H.; Ferris, R.; Dornsife, R. E.; Stanat, S. C.; Drach, J. C.; Townsend, L. B.; Koszalka, G. W. *Antimicrob. Agents Chemother.* **2002**, *46*, 2365.
16. Gumina, G.; Chong, Y.; Choo, H.; Song, G.-Y.; Chu, C. K. *Curr. Top. Med. Chem.* **2002**, *2*, 1065.
17. Gumina, G.; Song, G.-Y.; Chu, C. K. *FEMS Microb. Lett.* **2001**, *202*, 9.
18. Wang, P.; Hong, J. H.; Cooperwood, J. S.; Chu, C. K. *Antiviral Res.* **1998**, *40*, 19.
19. Bryant, M. L.; Bridges, E. G.; Placidi, L.; Faraj, A.; Loi, A.-G.; Pierra, C.; Dukhan, D.; Gosselin, G.; Imbach, J.-L.; Hernandez, B.; Juodawlkis, A.; Tennant, B.; Korba, B.; Cote, P.; Marion, P.; Cretton-Scott, E.; Schinazi, R. F.; Sommadossi, J.-P. *Antimicrob. Agents Chemother.* **2001**, *45*, 229.
20. Mathé, C.; Gosselin, G. *Antiviral Res.* **2006**, *71*, 276.
21. (a) Šmejkal, J.; Šorm, F. *Collect. Czech. Commun.* **1964**, *29*, 2809. (b) Gosselin, G.; Pierra, C.; Benzaria-Prad, S.; Dukhan, D.; Cretton-Scott, E.; Standring, D.; Sommadossi, J.-P. *Collection Symposium Series* (M. Hocek, Ed.), Vol. 10, p. 244. Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague 2008.
22. Acton, E. M.; Ryan, K. J.; Goodman, L. *J. Am. Chem. Soc.* **1964**, *86*, 5352.
23. Baczynski, W.; Niementowski, S. *Chem. Zentralbl.* **1902**, *73*, 940.
24. Büchel, K.-H. *Z. Naturforsch.* **1970**, *25b*, 934.
25. (a) Andrzejewska, M.; Pagano, M. A.; Meggio, F.; Brunati, A. M.; Kazimierczuk, Z. *Bioorg.*

Med. Chem. **2003**, *11*, 3997. (b) Pagano, M. A.; Andrzejewska, M.; Ruzzene, M.; Sarno, S.; Cesaro, L.; Bain, J.; Elliott, M.; Meggio, F.; Kazimierczuk, Z.; Pinna, L. A. *J. Med. Chem.* **2004**, *47*, 6239.
26. Andrzejewska, M.; Yepez-Mulia, L.; Tapia, A.; Cedillo-Rivera, R.; Laudy, A. E.; Starościak, B. J.; Kazimierczuk, Z. *Eur. J. Pharm. Sci.* **2004**, *21*, 323.
27. Xiangming, H.; Huiqiang, M.; Yulu, W. *ARKIVOC* **2007**, *13*, 150.
28. Benzaria, S.; Bardiot, D.; Bouisset, T.; Counor, C.; Rabeson, C.; Pierra, C.; Storer, R.; Loi, A. G.; Cadeddu, A.; Mura, M.; Musiu, C.; Liuzzi, M.; Loddo, R.; Bergelson, S.; Bichko, V.; Bridges, E.; Cretton-Scott, E.; Mao, J.; Sommadossi, J.-P.; Seifer, M.; Standring, D.; Tausek, M.; Gosselin, G.; La Colla, P. *Antiviral Chem. Chemother.* **2007**, *18*, 225.
29. Seela, F.; Lin, W.; Kazimierczuk, Z.; Rosemeyer, H.; Glaçon, V.; Peng, X.; He, Y.; Ming, X.; Andrzejewska, M.; Gorska, A.; Zhang, X.; Eickmeier, H.; La Colla, P. *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 859.
30. (a) Seela, F.; Peng, X. In *Current Protocols in Nucleic Acid Chemistry*; Beaucage, S. L.; Bergstrom, D. E.; Glick, G. D.; Jones, R. A.; Harkins, E. W.; Eds. 2005, 1.10.1-1.10.20. John Wiley & Sons, Inc., USA. (b) Seela, F.; Peng, X. *Collect. Czech. Chem. Commun.* **2006**, *71*, 956.
31. Hoffer M. *Chem. Ber.* **1960**, *93*, 2777.
32. Rabinowitz, J. L.; Wagner, E. C. *J. Am. Chem. Soc.* **1951**, *73*, 3030.
33. (a) Winkeler, H.-D.; Seela, F. *J. Org. Chem.* **1983**, *48*, 3119. (b) Seela, F.; Westermann, B.; Bindig, U. *J. Chem. Soc., Perkin Trans. 1* **1988**, 697.
34. (a) Seela, F.; Chittepu, P. *Org. Biomol. Chem.* **2008**, *6*, 596. (b) Pankiewicz, K. W. *Carbohydr. Res.* **2000**, *327*, 87. (c) Watanabe, K. A. *Collection Symposium Series* **2002**, *5*, 48.
35. (a) Howell, H. G.; Brodfuehrer, P. R.; Brundidge, S. P.; Benigni, D. A.; Sapino, Jr., C., *J. Org. Chem.* **1988**, *53*, 85. (b) Gudmundsson, K. S.; Freeman, G. A.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **2000**, *43*, 2473. (c) Tann, C. H.; Brodfuehrer, P. R.; Brundidge, S. P.; Sapino, Jr., C., Howell, H. G. *J. Org. Chem.* **1985**, *50*, 3644.
36. Seela, F.; Bourgeois, W. *Synthesis* **1989**, 912.
37. Seela, F.; Bourgeois, W.; Rosemeyer, H.; Wenzel, T. *Helv. Chim. Acta* **1996**, *79*, 488.
38. Crystal data: $C_{26}H_{20}N_2$ $FBrO_5$, MW = 539.36, T = 293 K, λ = 0.71073 Å, orthorhombic, space group P2 (1) 2 (1) 2 (1), a = 8.535 (3) Å, b = 14.165 (2) Å, c = 21.546 (3) Å, V = 2604.8 (9) Å^3, Z = 4, D = 0.362 Mg/m^3, μ = 0.407 mm^{-1}, F(000) = 289. CCDC 698585 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1 EZ, UK; fax: +44 1223 336033.
39. Seela, F.; Ming, X.; Budow, S.; Eickmeier, H.; Reuter, H. *Acta Cryst.* **2007**, C*63*, o549.