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Benzimidazole nucleosides: antiviral and antitumour activities and methods of synthesis

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The review systematically covers experimental data on the synthesis of modified benzimidazole nucleosides and evaluation of their biological activity published mainly over the last two decades. Various synthetic approaches to benzimidazole nucleosides, from multistep chemical processes to up-to-date biotechnology-based methods, are considered. A historical background of the development and structural improvement of various benzimidazole nucleosides is given and the structure – activity relationships for the synthesized compounds are addressed. Data on the antiviral and antitumour activities of these derivatives are presented and the cellular targets of benzimidazole nucleosides are discussed.
The bibliography includes 160 references.

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1. Introduction

Benzimidazole nucleosides are non-natural nucleosides, which exhibit selective antiviral and antitumour activities and are characterized by low cytotoxicity.[1,2] It is known that therapy of human viral diseases with commonly used drugs, such as acyclovir, ganciclovir, cidofovir, foscarnet, *etc.*, leads to the emergence of drug resistance. Hence, there is an ongoing need for new antiviral drugs characterized by less frequent emergence of drug-resistant viral strains, showing lower systemic toxicity and having less adverse effects. Such drugs include benzimidazole nucleosides, which possess a unique mechanism of action and have structures suitable for different modifications.

Despite numerous benzimidazole nucleosides synthesized over the last 50 years, including compounds with significant antiviral activity, maribavir (its structure is described below) is currently the only drug approved and used in clinical practice in the United States. In Europe, this agent is in the final stage of clinical trials as a drug for prevention of cytomegalovirus infections.

Extensive data on the synthesis of benzimidazole nucleosides and different activities of these compounds were published in the open literature; however, comprehensive reviews dealing with these compounds are lacking. Data on activity of selected representatives of this class are available in general reviews[1,2] on modified nucleosides. There are interesting reviews[3–5] dealing with modified benzimidazoles containing no carbohydrate residues. An overview paper by Biron *et al.*[6] is devoted mainly to the drug maribavir and its structural precursors. The present review integrates relevant data on antiviral and antitumour activities of benzimidazole nucleosides and different approaches to their synthesis, including first attempts to synthesize benzimidazole nucleosides by a chemical method in the 1960s. This class of compounds has attracted interest in the mid-20th century after the determination of the structure of vitamin B_{12} containing the 5,6-dimethylbenzimidazole α-D-ribonucleotide moiety. Since that time, benzimidazole nucleosides, as well as analogues of natural purine and pyrimidine nucleosides, were generally prepared by multistep chemical synthesis. This approach is time-consuming, utilizes expensive reactants and requires the

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isolation of intermediates in each step, the separation of isomer mixtures, procedures for introduction and removal of protective groups and a labourious purification of target compounds.

In recent years, the more advanced chemoenzymatic synthesis was increasingly used instead of chemical methods. This is a simple and convenient method for the synthesis of β-D-modified nucleosides, which meets the requirements of modern bioindustry. In the present review, the shift from chemical to biotechnological processes is considered in a historical context in terms of increasing complexity of the structures of the resulting nucleosides and improvement of relevant methods for their synthesis. The review mainly summarizes data on synthetic approaches, which resulted in the preparation of the most active compounds of this class and were published in the last 15 years.

The following designations and abbreviations are used in the review:

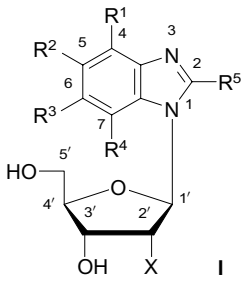
ABR — 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose,
ACV — acyclovir,
ACVMP — acyclovir 5′-monophosphate,
ACVTP — acyclovir 5′-triphosphate,
BDCRB — 2-bromo-5,6-dicloro-1-(β-D-ribofuranosyl)-benzimidazole,
BSA — *N*,*O*-bis(trimethylsilyl)acetamide,
BTCRB — 2-bromo-4,5,6-trichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole,
CD_{50} — 50% cytotoxic dose,
CDV — cidofovir,
CMV — cytomegalovirus,
Cl_4RB — 2,4,5,6-tetrachloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole
DAST — (diethylamino)sulfur trifluoride,
DMAP — 4-(dimethylamino)pyridine,
DRB — 5,6-dicloro-1-(β-D-ribofuranosyl)benzimidazole
dAdo — 2′-deoxyadenosine,
dCTP — 2′-deoxycytidine 5′-triphosphate,
dGTP – 2′-deoxyguanosine 5′-triphosphate,
dGuo — 2′-deoxyguanosine,
dUrd — 2′-deoxyuridine,
EC_{50} — 50% effective dose,
GCV — ganciclovir,
Guo — guanosine,
HBV — hepatitis B virus,
HCMV — human cytomegalovirus,
Hep-2 — human laryngeal carcinoma cell line,
HIV — human immunodeficiency virus,
HMDS — hexamethyldisilazane,
HSV-1 and HSV-2 — herpes simplex virus type 1 and type 2,
IC_{50} and IC_{95} — concentrations producing 50% and 95% inhibition of virus-induced cytopathic effect compared to the control, in which the cells are infected but not treated with the drug,
IDCRB — 5,6-dicloro-2-iodo-1-(β-D-ribofuranosyl)-benzimidazole
KB cells — human oral epidermoid carcinoma cells,
*Lr*NDT — *Lactobacillus reuteri N*-deoxyribosyltransferase,
MOM — methoxymethyl,
MSTFA — *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide,
NBS — *N*-bromosuccinimide,
NDT-1 and NDT-2 — class I and II *N*-deoxyribosyltransferases,
NDTs — *N*-deoxyribosyltransferases,
Ni-Ra — Raney nickel,
NPs — nucleoside phosphorylases,
PCV — penciclovir,
PFA — foscarnet (trisodium phosphonoformate),
Pi — inorganic phosphate,
PNP — purine nucleoside phosphorylase,
Pur — purine residue,
Pyr — pyrimidine residue,
PyNP — pyrimidine nucleoside phosphorylase,
pUL97 — HCMV-encoded protein kinase,
RSV — human respiratory syncytial virus,
SI — selectivity index equal to the CD_{50}/EC_{50} ratio,
TAR — 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose,
TCRB — 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole,
TDA-1 — tris(hydroxymethyl)aminomethane,
Thy — thymine,
TfO — trifluoromethanesulfonate,
TK — thymidine kinase,
TMS — trimethylsilyl,
Tol — toluyl,
Tr — trityl (2,4,6-triphenylmethyl),
TP — thymidine phosphorylase,
TSA — *p*-toluenesulfonic acid,
UP — uridine phosphorylase,
Ura — uracil,
Urd — uridine,
VZV — varicella – zoster virus,
UL54 — DNA polymerase gene.

2. Antiviral and antitumour activities of benzimidazole nucleosides

Nucleosides **I** containing the benzimidazole ring as a nitrogen base represent a class of modified nucleosides and exhibit antiviral and antitumour activities. Benzimidazole nucleoside derivatives have a rather broad antiviral activity spectrum and most often show an effect against herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2), human immunodeficiency virus (HIV) and various cytomegaloviruses (CMV), in particular, human cytomegalovirus (HCMV).[6–11]

In 1954, Tamm *et al.*[12] reported the synthesis of first halogenated benzimidazole nucleosides. 5,6-Dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB, **1a**) proved to be active against influenza A and B viruses and other RNA viruses.[6]

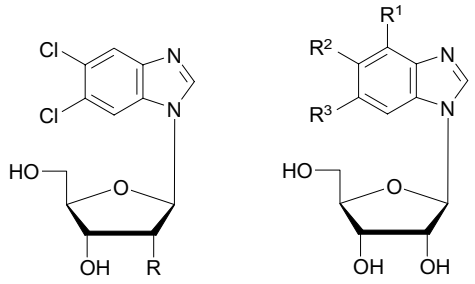
Structures I



I

R^1, R^2, R^3, R^4, R^5 = H, Cl, Br, Me, NH_2, *etc.*; X = H, OH

Structures 1, 2



1a,**b** **2a – f**

1: R = OH (**a**, DRB), H (**b**); **2**: $R^1 = R^2 = R^3 = Cl$ (**a**), Br (**f**); $R^1 = Br$, $R^2 = R^3 = Cl$ (**b**); $R^1 = R^2 = H$, $R^3 = Br$ (**c**); $R^1 = R^3 = H$, $R^2 = Br$ (**d**); $R^1 = H$, $R^2 = R^3 = Br$ (**e**)

Besides, this compound exhibits activity against DNA viruses (*e.g.*, cowpox virus),[13] although it has low activity against HCMV and HSV-1. Thus, the concentration of the compound producing 50% inhibition of the virus-induced cytopathic effect compared to the control, in which the cells are infected but not treated with the drug (IC_{50}), is 42 and 30 μmol litre^{-1}, respectively. Meanwhile, along with antiviral activity, DRB is characterized by high cytotoxicity, which precludes the use of this compound as a drug.[6] The data on high cytotoxicity to human foreskin fibroblasts and KB cells (human oral epidermoid carcinoma cells)[14] prompted researchers to assess antitumour activity of this compound. In 2007, the antitumour effect of this compound against the human laryngeal carcinoma Hep-2 cell line was reported.[15] It was shown that DRB inhibits the proliferation of Hep-2 cells in a dose-dependent manner (at a DRB concentration of 40 μmol litre^{-1}, the rate of cell growth decreases by 68%) and then induces the apoptosis. Antitumour activity of DRB may be assigned to its ability to inhibit Ser/Thr kinase CK2 that acts as a regulator of cell apoptosis and carcinogenesis.[16]

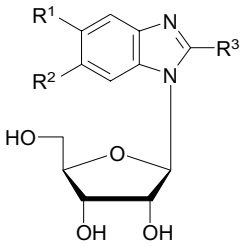
The attempts to improve the antiviral profile of DRB by introducing an additional chlorine (compound **2a**) or bromine (**2b**) atom at position 4 of the benzene ring failed.[17, 18] Brominated benzimidazoles **2c – f** were also synthesized. 4,5,6-Trichloro-1-(β-D-ribofuranosyl)benzimidazole (**2a**), although proved to be active against influenza A virus and parainfluenza virus type 1, exhibits considerable cytotoxicity, like DRB.[6] Meanwhile, 5(6)-monobromo (**2c**,**d**), 5,6-dibromo (**2e**), 4,5,6-tribromo (**2f**) and 4-bromo-5,6-dichloro derivatives (**2b**) of DRB show weaker antiviral activity compared to starting compound **1a**.[17, 18]

The majority of analogues modified at the ribose residue also exhibited weaker antiviral action compared to DRB.[17, 19] 5,6-Dichlorobenzimidazole 1-β-D-2′-deoxyriboside (**1b**) was found to inhibit *in vitro* replication of both the DNA-containing virus (herpes simplex virus) and the RNA-containing virus (papillomavirus).[20] Xylo and arabino derivatives have certain activity against HCMV at concentrations lower than cytotoxic doses. However, these compounds were not sufficiently selective and, consequently, it was unreasonable to consider them as antiviral drugs.[21]

In 1996, Townsend and co-workers[22] reported the synthesis of a new series of C(2)-substituted benzimidazole ribonucleosides as potential inhibitors of HCMV and HSV-1 replication. Two isomers, 1-(β-D-ribofuranosyl)-substituted 2,5-dichloro- (**3a**) and 2,6-dichlorobenzimidazoles (**3b**), and several disubstituted analogues **3c – h** were synthesized. The structure – activity relationship analysis of these compounds showed that the presence of a chlorine atom at position 2 of the heterocycle ($R^3 = Cl$) is essential for manifestation of low cytotoxicity but is insufficient for antiviral activity.

Compounds **3c – h** proved to be non-toxic and either exerted no activity, including against HSV-1, or exhibited weak activity against HCMV.[22]

Structures 3



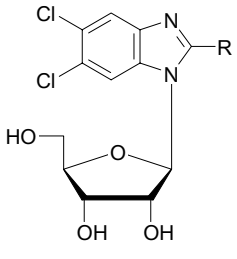
3a – h

Compound **3**	R^1	R^2	R^3
a	Cl	H	Cl
b	H	Cl	Cl
c	NH_2	H	Cl
d	H	SH	Cl
e	H	Cl	SH
f	H	Cl	SMe
g	H	Cl	SBn
h	H	Cl	OMe

Dichloro-substituted benzimidazole ribonucleosides **3a**,**b** also showed weak activity against HCMV, 2,6-dichloro isomer **3b** being much more active than 2,5-dichloro derivative **3a**. The assessment of activity and cytotoxicity of these compounds compared to DRB (**1a**) allowed researchers to draw important conclusions. Since equally low antiviral activity and cytotoxicity of isomers **3a**,**b** are in obvious contrast to the activity of DRB (5,6-dichloro isomer) and taking into account that the latter compound inhibits, at approximately equal concentrations, CMV and HSV-1, as well as the cell growth of human foreskin fibroblasts and KB cell lines, the authors attributed the antiviral activity of DRB to cytotoxicity rather than to the specific effect. On the contrary, weak activity of disubstituted analogues **3a**,**b** against HCMV is specific antiviral activity, because experiments were carried out in the non-cytotoxic concentration range.[22]

These conclusions are in agreement with the earlier data[14] on considerable specific activity against HCMV and low cytotoxicity of another analogue of DRB, namely, 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB, **4a**). Antiviral activity of TCRB was initially compared with that of DRB. Additional studies of both nucleosides showed that DRB has insignificant activity against HCMV and HSV-1 compared with TCRB, being highly cytotoxic to the noninfected cells.

Structures 4



4a – d

R = Cl (**a**, TCRB), Br (**b**, BDCRB), NH_2 (**c**), I (**d**)

Townsend *et al.*[14] concluded that the selective antiviral activity required the presence of a substituent (Cl or Br) at position 2 of the benzimidazole ring. Another important condition is the presence of the ribose residue. Thus, the activity profile of the starting base (2,5,6-trichlorobenzimidazole) is similar to that of DRB, but differs from the activity profile of TCRB. It is interesting that compound **4a** was initially synthesized as a potential antitumour agent, but it was found to be inactive and non-cytotoxic.[6,23] 2,5,6-Trichloro-1-(β-D-ribofuranosyl)benzimidazole also exhibited low activity against HSV-1 (IC_{50} = 102 μmol litre^{-1}). However, due to high selectivity against HCMV (IC_{50} = 2.9 μmol litre^{-1}, IC_{90} = 1.4 μmol litre^{-1}, where IC_{90} is the concentration producing 90% inhibition of virus-induced cytopathic effect compared to the control, in which the cells are infected but not treated with this agent) at non-cytotoxic concentrations.[14] Then TCRB was used for further modifications and the search for new potential antiviral compounds. A broad range of TCRB derivatives were synthesized and tested. However, only TCRB by itself, 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BDCRB, **4b**)[24] and their 5′-deoxy analogues had noticeable activity.[6,10] 2,3,5-Tri-O-acetyl derivatives of TCRB and BDCRB (**4b**) showed potential activity against HCMV at non-toxic concentrations. However, additional studies demonstrated that acyl groups of tri-O-acetyl derivatives are hydrolyzed in cells by serum esterases. Consequently, particularly TCRB and BDCRB ribonucleosides are active compounds. The replacement of a chlorine atom by bromine at position 2 of the benzimidazole ring (compound **4b**) leads to a fourfold increase in the activity against HCMV compared to that of TCRB, while no noticeable increase in cytotoxicity was observed. Meanwhile, BDCRB had weak activity against HSV-1.[14]

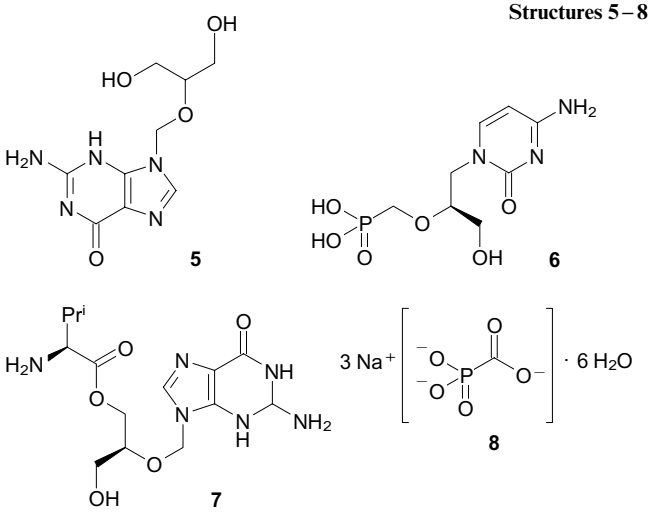
Compounds containing other substituents at position 2 of the imidazole ring exerted no antiviral activity. Thus, derivative **4d** (2-iodo analogue of TCRB) showed weaker activity against HCMV and lower selectivity. 2-Amino derivative **4c** proved to be more active against HCMV than 2-iodine-containing analogue **4d** but less active than 2-bromo (**4b**) and 2-chloro derivatives (**4a**).[14]

It should be noted that four main drugs are currently used for the treatment of HCMV: ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine, **5**], cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (CDV, **6**)], valganciclovir (valine ester prodrug of ganciclovir, **7**, which is metabolized in the body to ganciclovir) and foscarnet (trisodium phosphonoformate, PFA, **8**).[25,26] The first three drugs belong to acyclic nucleosides. Foscarnet is a non-nucleoside inhibitor of HSV DNA polymerase and, since it causes numerous side effects, it is rarely used in clinical practice (only when acyclic nucleosides **5** – **7** are not effective).[25–27]

Therefore, in a series of 5,6-dichloro-substituted benzimidazole ribofuranosides containing a substituent at position 2, only two compounds, TCRB and BDCRB, are superior in antiviral activity to ganciclovir (**5**) and foscarnet (**8**).[14,24]

Most approaches currently used to treat herpes infections are also based on modified nucleosides or their depot forms (prodrugs). Clinically approved antiherpetic drugs mainly block the activity of the key viral replication enzyme, DNA polymerase.[28] Second-generation antiherpetic drugs belonging to acyclic nucleosides (acyclovir, valacyclovir, penciclovir, famciclovir and the above-mentioned ganciclovir and valganciclovir) are commonly used to treat HSV. These drugs have a rather broad spectrum of activity and suppress infections caused by HSV-1, HSV-2, varicella – zoster virus (VZV) and HCMV.[29,30] This is the reason why ganciclovir (**5**) and foscarnet (**8**) are most often used as reference compounds to assess antiviral activity and toxicity of new compounds.

Structures 5 – 8



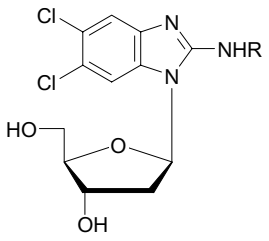
The evaluation of antiviral activity of TCRB (**4a**) and its bromine-containing analogue BDCRB (**4b**) showed that these compounds have a unique mechanism of action (have no effect on viral DNA polymerase), the spectrum of activity of these compounds being very specific and restricted to cytomegaloviruses. Nucleosides **4a**,**b** are equally active against rhesus and human cytomegaloviruses and are less active against guinea pig CMV.[31] It should be noted that neither TCRB nor BDCRB exhibited activity against other herpes viruses, including HSV-1 and HSV-2, varicella – zoster virus or human herpes virus type 6. These compounds are inactive against respiratory viruses (including influenza A and B viruses, respiratory syncytial virus and strains of adenovirus type 5 and type 7), measles virus, enteroviruses (coxsackieviruses A9 and B1, echoviruses 7 and 9 and poliovirus), human immunodeficiency virus (HIV) and human papillomavirus. It was found that TCRB and BDCRB do not inhibit the RNA, DNA or protein synthesis. It is assumed that these compounds act in the viral replication cycle, having impact on virus assembly and inhibiting the cleavage of DNA concatemers into monomeric genome-length DNA.[32]

2′-Deoxy-β-D-ribosyl-containing analogues of TCRB and BDCRB proved to be more cytotoxic and less active against HSV-1 and HCMV (compared to ganciclovir) and are comparable in activity to foscarnet.[33]

In addition to 2′-deoxy-TCRB and 2′-deoxy-BDCRB belonging to 2′-deoxy-β-D-ribosides, 2-amino (**9a**) and 2-isopropylamino derivatives of benzimidazole (**9b**) were studied. The latter compounds exhibited activity against HCMV. However, activity of these compounds was lower than that of TCRB. Consequently, it can be concluded that benzimidazole 2′-deoxy-β-D-ribosides are less active against HCMV than β-D-ribosides.[33]

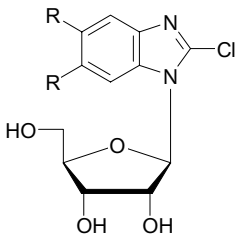
Later, it was found that, although BDCRB (**4b**) has high specific activity against HCMV, it cannot be used as a drug.

Structures 9



9a,**b** R = H (**a**), Pr^{i} (**b**)

Structures 11

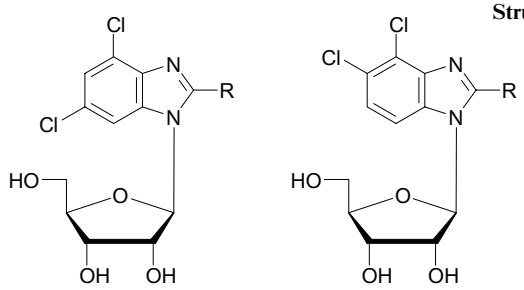


11a – **d** R = F (**a**), Br (**b**), I (**c**), Me (**d**)

This compound is rapidly metabolized in the cell by DNA glycosylases to form an inactive and toxic aglycone,[7, 34] and its further clinical trials were stopped.

Ongoing attempts to synthesize different TCRB and BDCRB derivatives modified at the heterocyclic base and(or) a carbohydrate residue were aimed at studying the structure – activity relationship in more detail. Benzimidazole nucleosides were mainly modified at the benzimidazole ring. The position of the chlorine atom in the benzene ring was varied, with the substituent at position 2 (chlorine or bromine atom) remaining unchanged. Thus, 4,6-dichloro and 4,5-dichloro analogues of TCRB and BDCRB were synthesized. Compounds **10a** – **d** were found to be active against HCMV at non-toxic concentrations. 2-Bromo derivatives **10b**,**d** exhibited higher activity compared to 2-chloro-containing analogues **10a**,**c**.[35]

Structures 10



10a,**b** **10c**,**d**

R = Cl (**a**, **c**), Br (**b**, **d**)

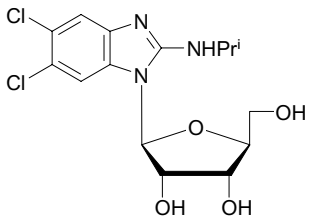
None of the synthesized compounds exhibited activity against HSV-1, and their activity profiles and cytotoxicity against HCMV were similar to those of TCRB (**4a**) and BDCRB (**4b**).[35]

In further structure – activity relationship studies of TCRB analogues, Zou *et al.*[36] synthesized 5,6-difluoro- (**11a**), 5,6-dibromo- (**11b**), 5,6-diiodo- (**11c**) and 2-chloro-5,6-dimethylbenzimidazole ribosides (**11d**).

5,6-Dimethyl- (**11d**) and 5,6-difluoro-containing (**11a**) ribonucleosides proved to be inactive against HCMV and HSV-1. 5,6-Dibromo derivative **11b** exhibited not only activity against HCMV but also higher cytotoxicity compared to TCRB (**4a**). Analogue **11c** containing two iodine atoms at positions 5 and 6 was active but simultaneously more toxic than TCRB (**4a**) and bromo derivative **11b**. The activity of this series of ribonucleosides changes depending on the nature of the substituent in the following order: I $\approx$ Br $\approx$ Cl $\gg$ F > H = Me. Cytotoxicity of halogen-substituted analogues of TCRB (most active compounds of this group) decreases in the following series of substituents: I > Br > Cl. These results provide additional evidence that the trichloro derivative of TCRB is the most active and selective compound in the series of halogen-substituted benzimidazole nucleosides containing a chlorine atom at position 2 of the heterocycle. It should be noted that the size of the substituent at positions 5 and 6 and its electronegativity are key factors responsible for activity of the compounds against HCMV and their cytotoxicity.[36]

In order to improve metabolic stability of benzimidazole nucleosides and enhance activity of this class of compounds against HCMV, L-riboside analogues of BDCRB (**4b**) were synthesized. In this series, 5,6-dichloro-2-isopropylamino-1-(β-L-ribofuranosyl)benzimidazole (maribavir, **12**) proved to be the most active compound.[6, 37, 38]

Structures 12

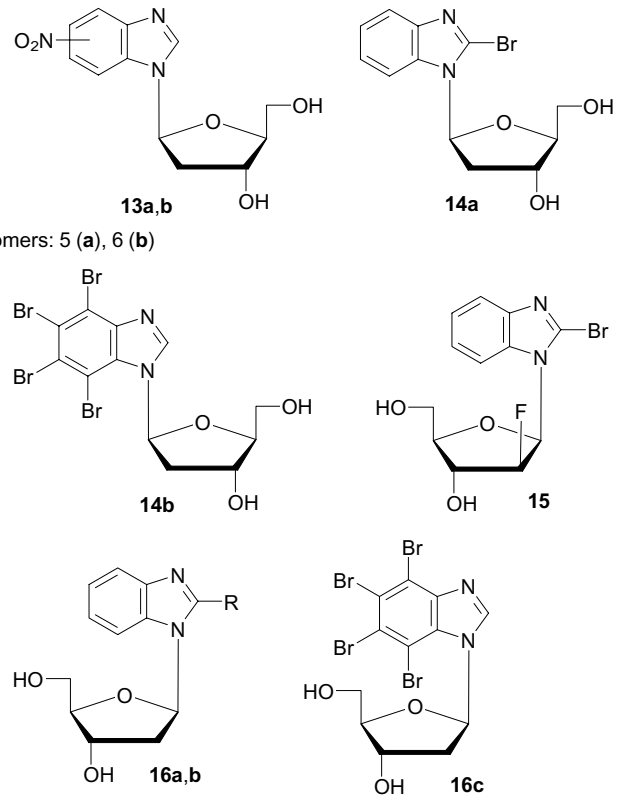


12 (Maribavir)

Maribavir has selective activity against HCMV,[6, 39] the mechanism of action being radically different from that of BDCRB. Maribavir inhibits viral DNA synthesis but has no inhibitory activity against viral DNA polymerase. The evaluation of antiviral activity of this nucleoside showed that maribavir also inhibits *in vitro* Epstein – Barr virus replication.[40, 41] However, this drug in inactive against HSV-1 and HSV-2, chickenpox, HIV and hepatitis B virus.[9, 37, 41] Maribavir (**12**) has received orphan drug status in the US for the prevention of CMV infection after bone marrow transplantation.[42] In Europe, maribavir is currently in additional clinical trials as a drug for the prevention and treatment of CMV in patients resistant to therapy with other medications, and its optimal dosage form and possible combinations with antacids and(or) antibiotics are being developed in order to enhance efficacy and oral bioavailability.[43]

Since active compounds were found both among β-D-ribonucleosides and β-L-epimers, it was interesting to compare the activity in the series of β-D- and β-L-2′-benzimidazole deoxyribonucleosides. For this purpose, compounds **13** – **16** containing different substituents in the benzimidazole ring were synthesized. The activity of these compounds was assessed against the following viruses: the genus *Flavivirus*, human immunodeficiency virus (HIV-1), hepatitis B

Structures 13 – 16



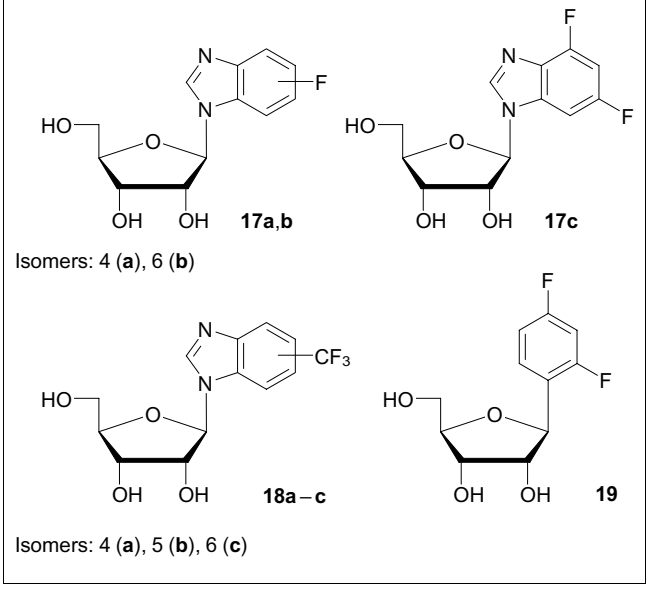
virus (HBV), hepatitis C virus (HCV) and human respiratory syncytial virus (human RSV).[44]

4,5,6,7-Tetrabromobenzimidazole β-L-2′-deoxyribonucleoside (**14b**) and its β-D-enantiomer (**16c**)[45] had equal activity against the tested viruses. However, these compounds were cytotoxic within the same concentration range. Other benzimidazole L- and D-nucleosides showed neither antiviral activity nor cytotoxicity. L-Nucleoside **14b** exhibited weak activity against hepatitis B virus (HBV, DNA virus) with high cytotoxicity. Compounds **13a**,**b**, **14a** and **16a**,**b** exerted no activity against HBV. None of L- and D-nucleosides were active against human respiratory syncytial virus.[44]

To study the ability of nucleosides modified at the heterocyclic base to form complementary pairs upon the formation of the RNA secondary structure, fluorinated (**17a** – **c**) and trifluoromethyl-substituted (**18a** – **c**) benzimidazole ribosides were synthesized.[46, 47] These nucleosides were subjected to phosphoramidation to prepare 12-mer oligonucleotides with single point mutations introduced into natural nucleosides, resulting in benzimidazole nucleosides **17** and **18**. The study of the hydrogen bonding between modified bases giving RNA duplexes demonstrated that nucleoside **17c** involved in the duplex can form weak hydrogen bonds with complementary 2,4-difluorobenzene riboside **19**.[46]

The activity of subsequently synthesized 6-fluoro-5-R-substituted benzimidazole β-D-ribosides and 2′-deoxyribonucleosides **20** was evaluated against a broad range of DNA and RNA viruses (including HIV, HSV-1, HSV-2, HCMV, coxsackievirus, human respiratory syncytial virus and influenza A and B viruses). None of the compounds were found to be active, but all compounds had low cytotoxicity (50% cytotoxic dose (CD_{50}) > 1 g litre^{-1}).[48]

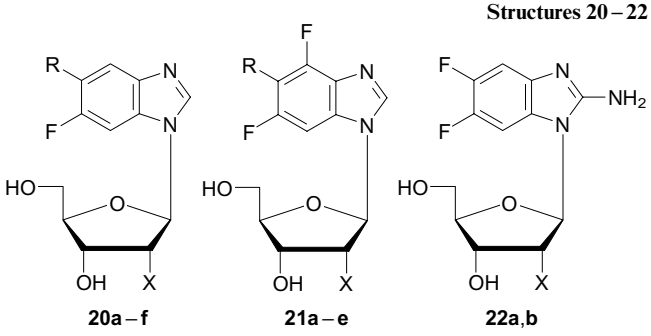
Structures 17 – 19



To examine the influence of an additional fluorine atom at position 4 of the benzimidazole ring on activity and toxicity of modified nucleosides, a series of derivatives **21** were synthesized.[49] Some compounds of this series showed relevant antiviral activity *in vitro* in the HSV-1 model. Thus, 4,5,6-trifluorobenzimidazole riboside **21a** has the selectivity index (SI = CD_{50}/EC_{50}, where EC_{50} is the median effective dose) equal to 9, and 4,6-difluoro-5-methoxybenzimidazole 2′-deoxyriboside (**21b**) has SI = 4. Despite the introduction of an additional fluorine atom into the molecule, all nucleosides exhibited low cytotoxicity (CD_{50} varied from 0.51 to 1 g litre^{-1}).[49]

Another modification of benzimidazole nucleosides was based on the introduction of a substituent at position 2 of fluorinated benzimidazole.[50] An attempt to synthesize a fluorinated analogue of maribavir resulted in the preparation of 2-amino-5,6-difluorobenzimidazole β-D-riboside (**22a**) and 2-amino-5,6-difluorobenzimidazole 2-deoxyriboside (**22b**). 2-Amino-5,6-difluoro-1-(β-D-ribofuranosyl)benzimidazole (**22a**) exhibited *in vitro* relevant selective

Structures 20 – 22

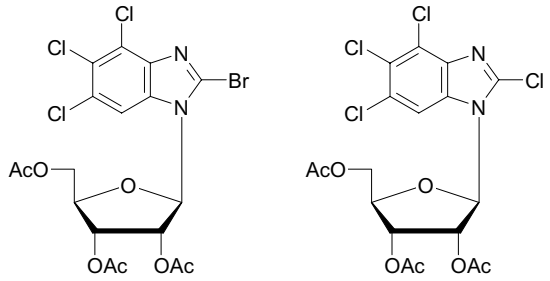


20: X = H, OH; R = F (**a**), Me (**b**), OEt (**c**), OPr^{i} (**d**), morpholino (**e**), *N*-methylpiperazino (**f**); **21**: X = H, OH; R = F (**a**), OMe (**b**), OEt (**c**), pyrrolidino (**d**), morpholino (**e**); **22**: X = OH (**a**), H (**b**)

antiviral activity against HSV-1, EC_{50} of this compound being much lower than CD_{50} (SI = 32). The EC_{50} value for compound **22a** was 104 μmol litre^{-1}, which is four times lower than CD_{50} of ribavirin and eight times lower than that of maribavir, which were used as reference compounds. Besides, riboside **22a** showed activity against HSV-1 strains resistant to acyclovir, CDV (**6**) and PFA (**8**).

Benzimidazole nucleosides were modified at the carbohydrate residue. Two nucleosides with the fully halogenated benzimidazole ring were found to be active: 2-bromo-4,5,6-trichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (BTCRB, **23**) and 2,4,5,6-tetrachloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (Cl_4RB, **24**).[51]

Structures 23, 24



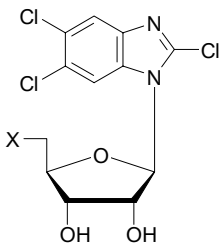
23 (BTCRB) **24** (Cl_4RB)

Nucleosides **23** and **24** were active against HCMV, and their mechanism of action is similar to that of BDCRB (**4b**). It should be noted that both compounds inhibit ganciclovir-sensitive (GCV) and GCV-resistant clinical isolates of the virus. All compounds, BTCRB (**23**), Cl_4RB (**24**), and BDCRB (**4b**) (control), were found to be equally active against the former isolate of the virus; EC_{50} was 0.35 μmol litre^{-1}. The compound Cl_4RB (**24**) was most active against the latter isolate (EC_{50} = 0.15 μmol litre^{-1}), the efficacy of BTCRB (**23**) was at the same level as BDCRB (**4b**) (EC_{50} = 0.5 μmol litre^{-1}). Besides, these compounds were active against rat cytomegalovirus and VZV. The compound BTCRB (**23**) had weak activity against HSV-1.[51, 52]

Gudmundsson and co-workers[53] synthesized nine 5′-modified analogues of TCRB (**25a**–**i**) and tested them against two types of herpes virus (HCMV and HSV-1). These compounds exhibited considerable activity against HCMV (EC_{50} = 0.5–14.2 μmol litre^{-1}), but low activity against HSV-1 (EC_{50} > 100 μmol litre^{-1}).

The antiviral activity profile of some compounds was similar to that of TCRB, while the activity does not correlate with cytotoxicity. 5′-Halogenated derivatives **25a**–**d** exhibited more pronounced activity against HCMV than other 5′-derivatives, their activity being even slightly higher than that of TCRB. Virtually no changes in activity were observed in the series of derivatives **25e**–**i** containing different substituents at position 5′ despite the differences in the electronegativity and size of the substituents. The activity of compounds **25e**–**g** containing alkoxy substituents decreases with increasing size of the alkyl group (from methyl to n-butyl). The presence of azido (compound **25h**) and methylsulfanyl substituents (**25i**) has no evident effect on antiviral properties. Thus, activity of these compounds against HCMV was comparable with that of TCRB. However, all compounds containing substituents at position 5′ proved to be twice as cytotoxic as TCRB. The highest selectivity was observed for fluorinated derivative **25a**. It was concluded that the design of compounds with activity against HCMV tolerates modifications at position 5′ of TCRB because the presence of substituents at this position does no decrease activity of the derivatives against this virus.[53]

Structures 25



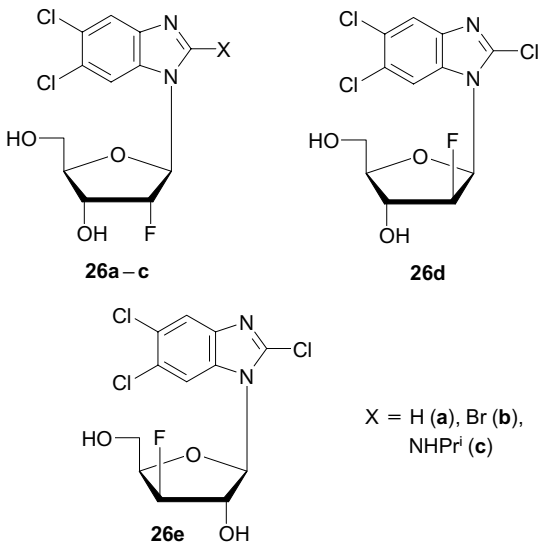
25a–**i**

X = F (**a**), Cl (**b**), Br (**c**), I (**d**), OMe (**e**), OEt (**f**), OBu^n (**g**), N_3 (**h**), SMe (**i**)

Another modification of nucleosides involves the introduction of a fluorine atom into a carbohydrate residue. Townsend and Gudmundsson with their co-workers[53, 54] used this modification and synthesized five benzimidazole nucleosides fluorinated at the carbohydrate residue: 2′-deoxy-2′-fluororibo- (**26a**–**c**), 2′-deoxy-2′-fluoroarabino- (**26d**) and 3′-deoxy-3′-fluoroxylo-β-D-furanosides (**26e**).

All nucleosides of this series proved to be inactive against HSV-1, but TCRB analogues, such as 2′-fluoroarabinoside **26d** and 3′-fluoroxyloside **26e**, like the BDCRB analogue 2′-fluororiboside **26b**, exhibit low activity against HCMV, which is lower than that of TCRB and BDCRB. Besides, these analogues were more toxic than the starting compounds, and further clinical trials of fluorinated nucleosides of this series were stopped.[6] Compounds **26a** and **26c**, which are 2′-fluororibo analogues of DRB and maribavir, respectively, were also inactive against HCMV. It is surprising that compound **26a** has no activity despite the fact that it is a direct 2′-fluororibofuranose analogue of 2′-deoxy-DRB,[12] which exhibits both antiviral activity and cytotoxicity. It should be noted that the synthesis of the target fluorinated nucleosides proceeds through intermediates

Structures 26



26a–**c** **26d** **26e**

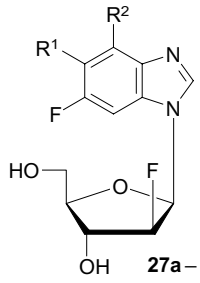
X = H (**a**), Br (**b**), $NHPr^i$ (**c**)

containing protected hydroxy groups in the carbohydrate residue (3′,5′-di-*O*-trityl-TCRB, 2′,3′,5′-tri-*O*-trityl-TCRB and 2′-F-3′,5′-di-*O*-benzoylarabino derivative of TCRB; trityl is 2,4,6-triphenylmethyl), which also had certain activity against HSV-1, but were totally inactive against HCMV.[55]

The synthesis of benzimidazole 2′-deoxy-2′-fluoroarabinosides **27** containing a different number of fluorine atoms in the benzimidazole ring was recently described.[56] The goal was to find out whether benzimidazole nucleosides containing three or four fluorine atoms (both in the base and the carbohydrate residue) are cytotoxic and whether their activity is higher than that of ribosides and 2′-deoxyribosides bearing fluorine atoms only in the base.

Surprisingly, these compounds were found to have low cytotoxicity to Vero E6 cells, and three of four nucleosides exhibited also relevant activity against HSV-1. The SI values for 2′-deoxy-2′-fluoroarabinosides **27a**,**c**,**d** were from 6 to 8. 6-Fluoro-5-methoxybenzimidazole deoxy-2′-fluoroarabinoside (**27a**) proved to be the most cytotoxic compound in this series. Difluorinated A benzimidazole 2′-fluoroarabinoside **27c** did not show an effect corresponding to CD_{50} even at the highest tested concentration (1000 μg litre^{-1}).[56] This low cytotoxicity is usually characteristic of nucleosides containing natural carbohydrate residues. Since active compounds were found among benzimidazole 2′-deoxy-2′-fluoroarabinosides **27**, the modification involving the replacement of a natural carbohydrate residue by 2-fluoroarabinose holds certain promise for further study.

Structures 27



27a – d

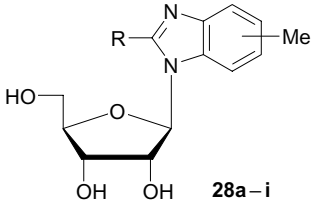
R^1 = OMe: R^2 = H (**a**), F (**b**);
R^2 = F: R^1 = H (**c**), F (**d**)

As mentioned above, all C(2)-substituted benzimidazole ribonucleosides synthesized by Biron *et al.*,[6] in particular 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB, **4a**), were initially considered as potential antitumour agents. However, these compounds showed low cytotoxicity and insufficient activity in antitumour tests.[23] In subsequent studies, 5′-modified derivatives of TCRB (compounds **25**) were tested as potential antitumour agents. These compounds were found to be much more cytotoxic in human foreskin fibroblasts than in KB cells. Since these compounds also had considerable antiviral activity, the researchers concluded that the modification is well-tolerated at position 5′ without obvious impact on activity against HCMV, but it leads to a decrease in the toxic effect on human cells.[6, 53]

In 2006, renewed attempts were made to synthesize analogues of benzimidazole nucleosides with antitumour activity. A series of 2-substituted 4(5)-methylbenzimidazole nucleosides **28a – i** were prepared.[57] These compounds are structural analogues of the effective antitumour agent 8-chloroadenosine.[58] However, data on their efficacy as cytostatic agents were not published in the open literature.

In 2009, monomethyl derivatives **28a**,**f** were employed to probe functional activity of HSV-1 DNA primase.[59] The authors examined the ability of this enzyme to form short RNA primers from modified nucleotides, which do not form complementary pairs with natural bases.

Structures 28



28a – i

Isomers: 4 (**a – e**),
5 (**h**, **i**), 7 (**f**, **g**);
R = H (**a**, **f**, **h**), Cl (**b**),
Me (**c**, **i**), OEt (**d**), OH (**e**, **g**)

In 2007, antitumour activity of DRB (**1a**) was evaluated in human laryngeal carcinoma cells (Hep-2 cell line).[16] *In vitro* experiments showed that DRB begins to inhibit Hep-2 cell invasion at a concentration of 5 μmol litre^{-1}, the inhibitory effect being increased with increasing concentration of the agent.

4,5,6,7-Tetrabromobenzimidazole deoxyriboside (**16c**) is a potent highly selective inhibitor of two kinases: casein kinase CK2 and serine/threonine protein kinase PIM1 (IC_{50} = 32 and 86 nmol litre^{-1}, respectively). Apparently, this property is responsible for high antitumour activity of this nucleoside compared to DRB (**1a**), which is a selective inhibitor only of kinase CK2.[16, 60]

3. Mechanism of action of benzimidazole nucleosides against human cytomegalovirus and herpes simplex virus

Although benzimidazole nucleosides have been known for more than 60 years, their mechanism of action, *in vitro* and *in vivo* cellular targets and metabolism are poorly understood. What is known with certainty is that their antiviral action differs from the mechanism of action of drugs currently used in clinical practice.

Most drugs used for the prevention and treatment of cytomegalovirus infection are inhibitors of HCMV replication and act on viral DNA polymerase. Drug resistance of cytomegalovirus to ganciclovir (**5**) and foscarnet (**8**) was demonstrated to be associated with mutations in the UL54 gene.[6, 61]

Foscarnet (**8**) directly reacts with the pyrophosphate-binding site of DNA polymerase. Ganciclovir (**5**) and cidofovir (**6**) have to be initially phosphorylated (ganciclovir in three steps and cidofovir in two steps; to triphosphate and diphosphate, respectively). Then these compounds interact as competitive inhibitors [substrates alternative to 2′-deoxyguanosine 5′-triphosphate (dGTP) and 2′-deoxycytidine 5′-triphosphate (dCTP), respectively] with the substrate-binding site of DNA polymerase. As alternative substrates, ganciclovir triphosphate and cidofovir diphosphate are incorporated (as ganciclovir monophosphate and cidofovir) into the growing DNA strand and block chain elongation. The first phosphorylation stage in the intracellular metabolism of ganciclovir is catalyzed by HCMV-encoded protein kinase (pUL97) produced by the UL97 gene, which is thereby responsible for predominant phos-

phorylation of ganciclovir in the cells infected with cytomegalovirus. Cidofovir is not phosphorylated by pUL97.[6,61]

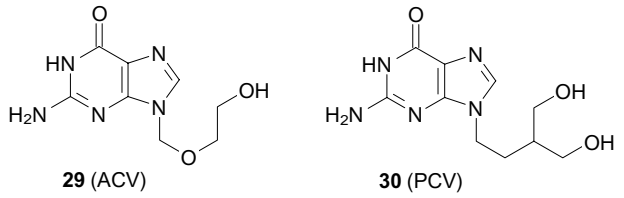
The UL97 gene is the main site for mutations, which can cause resistance of cytomegalovirus to ganciclovir. Evidently, these mutations do not lead to resistance of the virus to other anti-HCMV agents (*e.g.*, foscarnet and cidofovir) because their mechanism of action is not associated with UL97.[25] On the contrary, different mutations mediated by viral DNA polymerase can cause resistance of cytomegalovirus strains to both cidofovir (often accompanied by cross-resistance to ganciclovir) and foscarnet.

Mutations and resistance of the virus to drugs commonly used for the treatment of cytomegalovirus are serious threats. This is why scientists are constantly searching for new compounds with alternative mechanisms of action, which are more effective and less toxic. Some benzimidazole nucleosides with a unique mechanism of action hold promise. In 1998, Krosky *et al.*[61] showed that resistance of cytomegalovirus to TCRB (**4a**) is associated with mutations in the UL89 and UL56 genes encoding the small and large subunits of terminase.[62–64] Biron *et al.*[7] examined BDCRB (**4b**) and established that β-D-ribofuranosylbenzimidazoles target DNA maturation and processing. Besides, it was found that terminase is involved in the packaging process and is responsible for insertion of viral DNA into the capsid, and it is this enzyme that is a target for the benzimidazole nucleosides TCRB and BDCRB.[51] Consequently, the mechanism of inhibition of viral replication by BDCRB and TCRB does not impact DNA polymerase and viral DNA synthesis. Since the DNA maturation process and DNA insertion into the capsid are specific for viruses and are absent in mammals and taking into account that the compounds target the enzyme that is present only in the virus, benzimidazole nucleosides are evidently more safe than the drugs currently used in clinical practice.

Maribavir (**12**), which is the 2-isopropylamino derivative of BDCRB (L form), is the most active compound against cytomegalovirus (compared to BDCRB and ganciclovir) in the series of modified benzimidazole nucleosides. This agent is also less toxic *in vitro* to bone marrow cells than ganciclovir.[7] Maribavir exhibits activity against cytomegalovirus strains resistant to ganciclovir. The mechanism of action of maribavir is associated with inhibition of viral replication by targeting pUL97 protein kinase activity.[7,25,65–67] It is known that pUL97 protein kinase is not only able to phosphorylate ganciclovir to monophosphate, but also can undergo autophosphorylation. It should be noted that preliminary autophosphorylation of pUL97 is a necessary condition for phosphorylation of ganciclovir. Recently, the function of the UL97 gene product was elucidated. This product was found to be responsible for nuclear egress, *i.e.*, for the isolation of cytomegalovirus nucleocapsids from the nuclei. Consequently, maribavir can block encapsidation of viral DNA, acting in the cytomegalovirus replication cycle in later stages compared to BDCRB, because the egress of nucleocapsids from the nuclei occurs after the completion of DNA maturation and packaging.[7,68]

Agents active against HSV-1, like anti-CMV agents, mainly inhibit activity of the key viral replication enzyme, namely, DNA polymerase.[28] Acyclovir (ACV, **29**), which was synthesized back in 1977, remains the gold standard in the treatment of herpes infections. The mechanism of action of ACV is based on its phosphorylation by viral thymidine kinase (TK) to form the corresponding acyclovir 5′-monophosphate (ACVMP). The subsequent phosphorylation to triphosphate (ACVTP) is mediated by host cell kinases. The compound ACVMP serves as a substrate of viral DNA polymerase, which mediates the ACVMP incorporation into the viral DNA strand and blocks its synthesis.[6] Valacyclovir (acyclovir L-valine ester) is an effective and safe drug with enhanced oral bioavailability. There are currently numerous HSV strains resistant to ACV, main mutations being located in the genes encoding both DNA polymerase (UL30) and TK (UL23). In 95% of cases, the resistance to ACV is associated with mutations in the TK gene and only in 5% cases with mutations in the DNA polymerase gene.[28]

Structures 29, 30



29 (ACV) **30** (PCV)

Penciclovir (PCV, **30**) is another acyclic nucleoside structurally similar to acyclovir and ganciclovir. This agent and its depot form (famciclovir) are active against HSV-1. The drug PCV is used in the treatment of HSV-induced skin lesions; besides, this drug inhibits the development of a HSV-1 strain bearing the mutant DNA polymerase gene.[69] The conversion of PCV to monophosphate by viral thymidine kinase is required for this compound to exhibit activity against HSV-1.

The above-considered agent foscarnet is a non-nucleoside DNA polymerase inhibitor active against both HCMV and HSV. This compound blocks the active site of the enzyme in a non-competitive manner (with respect to nucleotides), preventing binding of the incoming nucleoside triphosphate.[70] Since foscarnet is highly toxic, it is used only if the treatment with acyclovir and other nucleoside analogues is inefficient or if the resistance is developed to these drugs.[71]

Alternative targets of these agents are, for example, the virus helicase primase complex (the UL5, UL52 and UL8 genes), which is responsible for DNA unwinding and DNA primer synthesis or (as in the case with CMV) acts in the late stages of virus replications (capsid assembly, genome packaging and egress of nucleocapsids from the nucleus), and the UL36 and UL37 proteins involved in this complex.[28]

2-Amino-5,6-difluoro-1-(β-D-ribofuranosyl)benzimidazole (**22a**) occupies a special place. The evaluation of antiviral activity of this compound in HSV-1 strains with different drug resistance showed that this compound has a specific mechanism of action. The mechanism of antiviral activity is poorly understood; however, it is known that neither DNA polymerase nor TK are targets of this compound.[50]

Modern biomedical studies showed that, apart from the effect on the main target enzymes (DNA polymerase and thymidine kinase), other mechanisms of action of benzimidazole nucleosides against HSV are also possible. It is assumed that DRB (**1a**), being a cyclin-dependent kinase-9 inhibitor, inhibits late γ2 gene expression, which generally occurs in the final stages of viral infections.[72]

4. Methods of chemical synthesis of benzimidazole nucleosides

First chemical syntheses of benzimidazole nucleosides date back to the mid-20th century.[12, 19, 73] Meanwhile, chemoenzymatic approaches to the synthesis of these compounds characterized by stereoselectivity and high efficiency started to be actively developed only in the last 20 years due to advances in genetic engineering techniques for the preparation of enzymes of nucleic metabolism.[48, 49, 56, 74]

There are two chemical approaches to the synthesis of benzimidazole nucleosides. One approach involves the condensation of benzimidazole with a protected carbohydrate residue based on the classical Vorbrüggen reaction.[75] The last step of this synthesis, after removal of protecting groups, generally results in the formation of a difficult-to-separate mixture of nucleosides (α- and β-epimers) in different ratios.[76,77] Known modifications of this approach, such as the use of silyl protection, selective catalysts, appropriate solvents and temperature conditions, enable the formation of a particular regio- and(or) stereoisomer as the major product.[76, 78 – 81]

Another approach represents a modification of the synthesized nucleoside at the base or carbohydrate residue. This approach is limited by the availability of the starting reagents.

4.1. Synthesis of benzimidazole β-D-ribosides by glycosylation

The method of nucleoside synthesis by the Vorbrüggen reaction is outlined in Scheme 1 (PG is a protecting group). This method can be employed to prepare β-N(1)- (**A**), β-N(3)- (**B**), α-N(1)- (**C**) and α-N(3)-isomers (**D**). Examples of compounds synthesized by this method are given in Table 1.

This approach is convenient for the synthesis of nucleosides containing identical substituents R^3 and R^4 in the benzimidazole ring. If these substituents are different,

Scheme 1

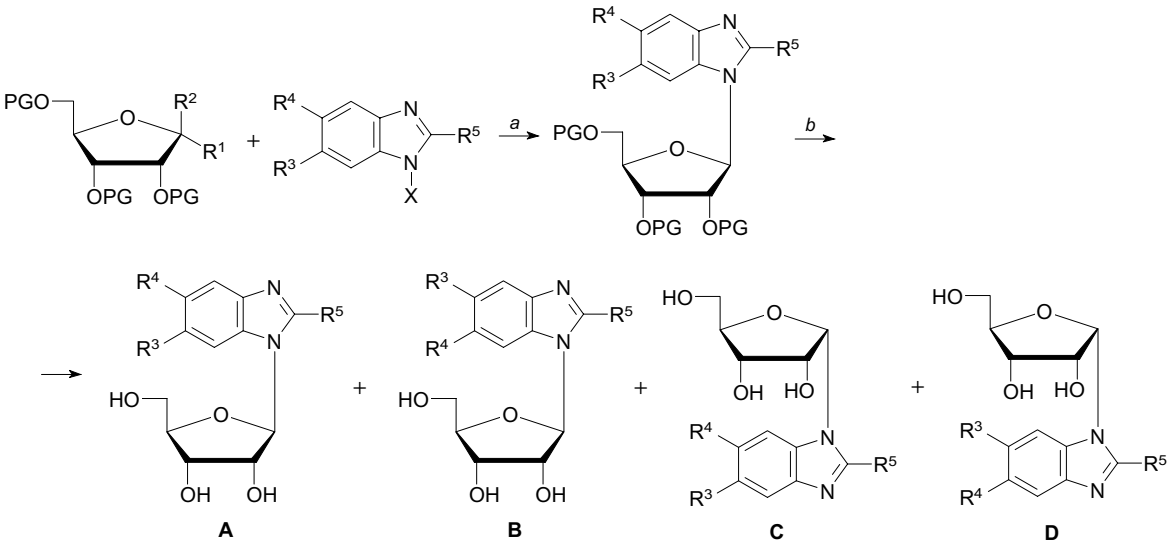
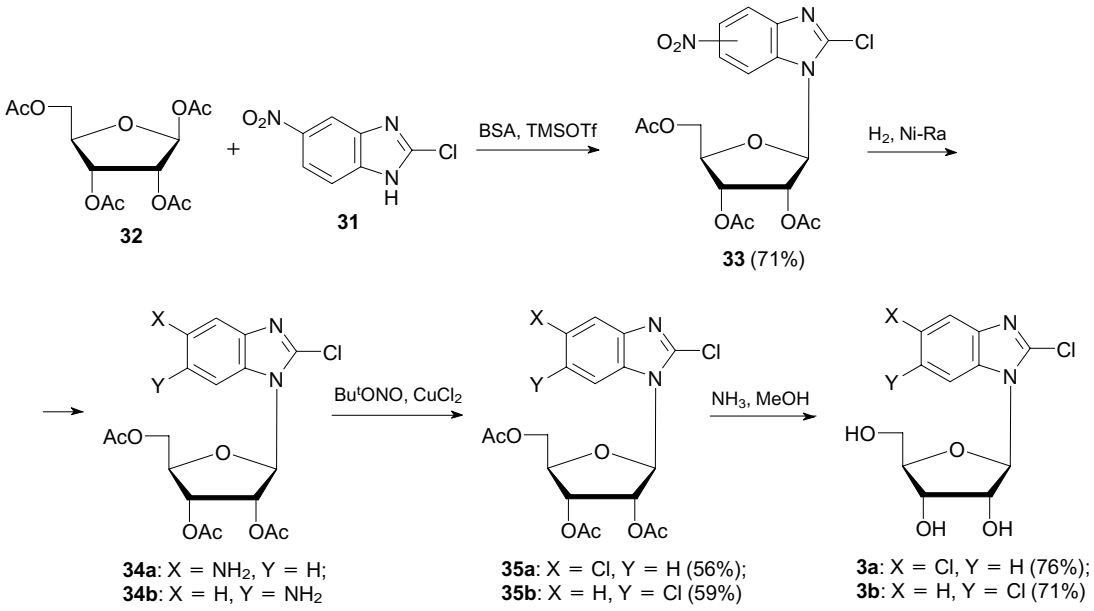


Table 1. Synthesis of benzimidazole nucleosides by the Vorbrüggen method (see Scheme 1).

Product	Starting compounds							Conditions and reagents	Reaction outcome	Yield of β-D-nucleoside (%)	Ref.
	PG	R^1	R^2	X	R^3	R^4	R^5				
1a (DRB)	Bz	See [a]	See [a]	HgCl	Cl	Cl	H	*a*) with xylene distilling off; *b*) MeONa, MeOH	–	68	19
	Bz	H	OAc	H	Cl	Cl	H	*a*) TSA, fusion at 170 – 180 °C, 1 h; *b*) NH_3, MeOH	Mixture of α- and β-isomers (1 : 1)	51	82
	Bz	H	OAc	H	Cl	Cl	H	*a*) BSA, TMSOTf, MeCN; *b*) NH_3, MeOH	–	51	21
11a	Ac	H	OAc	H	F	F	Cl	*a*) BSA, TMSOTf; *b*) NH_3, MeOH	Mixture of α- and β-isomers, with the latter predominating	50	36
11b	Ac	H	OAc	H	Br	Br	Cl	*a*) BSA, TMSOTf; *b*) Na_2CO_3	The same	64	36
11c	Ac	H	OAc	H	I	I	Cl	*a*) BSA, TMSOTf; *b*) Na_2CO_3	"	68	36

Note. A dash indicates that the isomer ratio was not determined in the original publication. The following abbreviations are used: TSA is *p*-toluenesulfonic acid; BSA is *N*,*O*-bis(trimethylsilyl)acetamide; TMSOTf is trimethylsilyl trifluoromethanesulfonate. [a] R^1 = H, R^2 = Cl or R^1 = Cl, R^2 = H.

Scheme 2

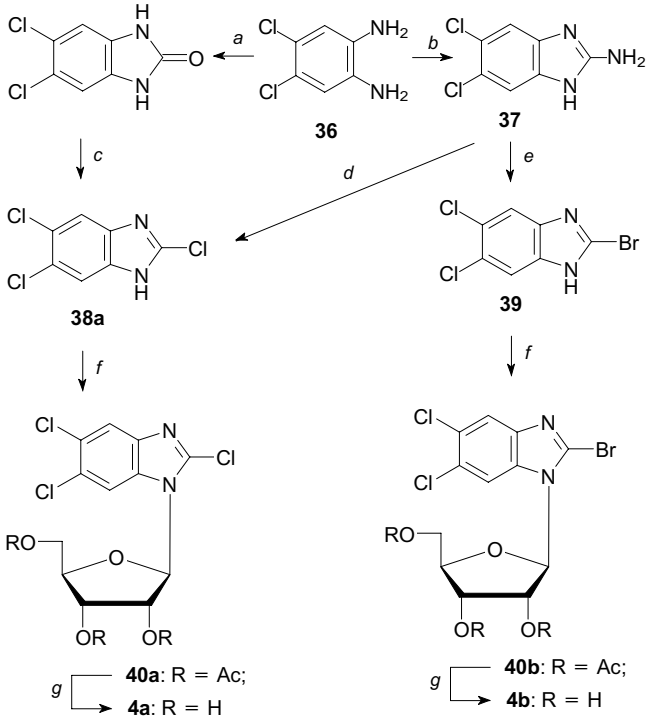


glycosylation affords a mixture of N(1)- and N(3)-regioisomers, and their isolation from the reaction mixture becomes difficult. Scheme 2 shows the synthesis of 2,5(6)-dichloro-1-(β-D-ribofuranosyl)benzimidazoles. The reaction of 2-chloro-5(6)-nitrobenzimidazole (**31**) with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**32**, TAR) gives product **33** as a mixture of two isomers, 2-chloro-5-nitro- and 2-chloro-6-nitro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazoles. These isomers are reduced by hydrogen on Raney nickel (Ni-Ra) to the corresponding amino derivatives **34a**,**b**, which are separated by silica gel column chromatography. In the next step, a chlorine atom is introduced at the appropriate position of the benzimidazole ring. 5-Amino isomer **34a** is diazotized by *tert*-butyl nitrite in the presence of copper chloride in acetonitrile to prepare pure 5-chloro derivative **35a**. The simultaneous diazotization of 6-amino isomer **34b** affords 6-chloro derivative **35b**. The removal of acetyl protecting groups by the treatment with ammonia in methanol gives 2,5-dichloro- (**3a**) and 2,6-dichloro-1-(β-D-ribofuranosyl)benzimidazoles (**3b**), respectively.[22]

An approach presented in Scheme 3 was developed for the synthesis of TCRB (**4a**) and BDCRB (**4b**). In the first step, the ring closure of commercially available 4,5-dichloro-1,2-phenylenediamine (**36**) with cyanogen bromide followed by diazotization of amino derivative **37** gave 2,5,6-trichlorobenzimidazole (**38a**).[14] This method of cyclization of 4,5-dichloro-1,2-phenylenediamine (**36**) with cyanogen bromide in methanol is successfully applied for the synthesis of various 2-aminobenzimidazoles.[83] After a sequence of transformations,[14] 2-amino-5,6-dichlorobenzimidazole (**37**) was isolated in 98% yields rather than in 22% yield reported earlier.[84] Then compound **37** was used to prepare 2,5,6-trichlorobenzimidazole (**38a**) or 2-bromo-5,6-dichlorobenzimidazole (**39**) in order to synthesize TCRB or BDCRB.

In the second step, 2,5,6-trichlorobenzimidazole (**38a**) was silylated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and than ribosylated with TAR (**32**) in the presence of TMSOTf to prepare target ribofuranoside.[85] This method gave 2,5,6-trichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (**40a**) with an insignificant admixture of the α-anomer. After removal of acetyl groups by the treatment with ammonia in methanol, target TCRB (**4a**) was isolated in 74% yield.[14]

Scheme 3



(*a*) $(NH_2)_2CO$, n-$C_5H_{11}OH$; (*b*) CNBr, MeOH; (*c*) $POCl_3$, HCl; (*d*) HCl, NaONO, $CuCl_2$; (*e*) HBr, NaONO, CuBr; (*f*) 1) BSA, MeCN; 2) TAR, TMSOTf; (*g*) NH_3, MeOH

A similar procedure was employed to synthesize BDCRB (**4b**) (see Scheme 3). 2-Amino-5,6-dichlorobenzimidazole (**37**) was diazotized with sodium nitrite in aqueous HBr. The diazonium salt was decomposed in the presence of

copper bromide to form 2-bromo-5,6-dichlorobenzimidazole (**39**), which was silylated with BSA. The ribosylation with TAR in the presence of TMSOTf produced 2-bromo-5,6-dichloro-l-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (**40b**). After removal of acetyl protecting groups, BDCRB (**4b**) was isolated in 37% yield.[14]

Since it appeared that it is impossible to prepare 5,6-dichloro-2-iodobenzimidazole by the Sandmeyer reaction, an alternative approach was developed for the synthesis of 5,6-dichloro-2-iodo-l-(β-D-ribofuranosyl)benzimidazole (**4d**, IDCRB).[14] The first step was required for investigation of the specificity of diazotization of 2-amino-5,6-disubstituted benzimidazole ribosides with tertiary alkynyl nitrites in a nonaqueous medium and resulted in the formation of 2-amino-5,6-dichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (**41**) (Scheme 4).

The silylation of 2-amino-5,6-dichlorobenzimidazole (**37**) followed by ribosylation with TAR (**32**) gave protected ribofuranoside **41** in 51% yield.[86] The latter product was treated with a tenfold excess of *tert*-butyl nitrite in CH_2I_2, the result of the transformation being unexpected. Thus, the product did not contain a iodine atom but had a nitro group in the benzene ring. The mechanism of this unusual reaction was established due to the isolation of 5,6-dichloro-l-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazol-2-one (**42**) as the key intermediate. The structure of the intermediate was confirmed by the independent synthesis of intermediate **42** and its unprotected analogue **43**. For this purpose, 5,6-dichlorobenzimidazol-2-one,[87] which was prepared by the ring closure of 4,5-dichloro-1,2-phenylenediamine (**36**) in the presence of urea (see Scheme 3), was subjected to ribosylation.[14]

The treatment of compound **42** with *tert*-butyl nitrite (10 equiv.) in diiodomethane at 100 °C resulted in its complete conversion into 5,6-dichloro-4-nitro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazol-2-one (**44**) over 2 h, no formation of by-products being observed. After crystallization, compound **44** was isolated in 86% yield. The deprotection of the latter afforded ribosylated 5,6-dichloro-4-nitrobenzimidazol-2-one **45**.

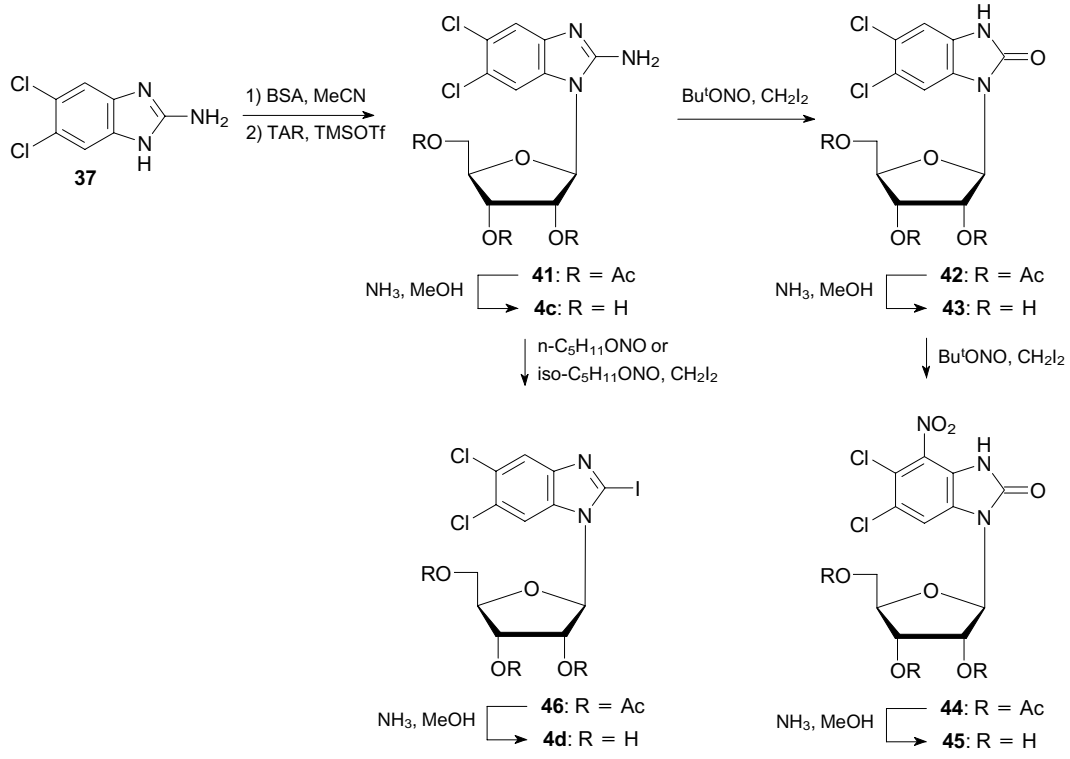
2-Iodo derivative **46** can be synthesized using primary alkynyl nitrite for diazotization (see Scheme 4). Compound **41** was treated with amyl or isoamyl nitrite under the same conditions as those giving undesired product **44**. As a result, the same compound, *viz*., 5,6-dichloro-2-iodo-l-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (**46**), was synthesized in 55% or 63% yield, respectively. Target 5,6-dichloro-2-iodo-l-(β-D-ribofuranosyl)benzimidazole (**4d**) was obtained after removal of protecting groups with ammonia in methanol (90% yield).[14]

Fluorinated benzimidazole ribosides were synthesized by the Vorbrüggen reaction (Scheme 5). The heating of 4-fluoro-1*H*-benzimidazole (**47**) with BSA under reflux followed by trans-silylation of the base with TAR (**32**) in the presence of TMSOTf gave 4-fluoro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (**48a**) in 65% yield. N(3)-Isomer **49a** was isolated by chromatography as a by-product in 8% yield.[88]

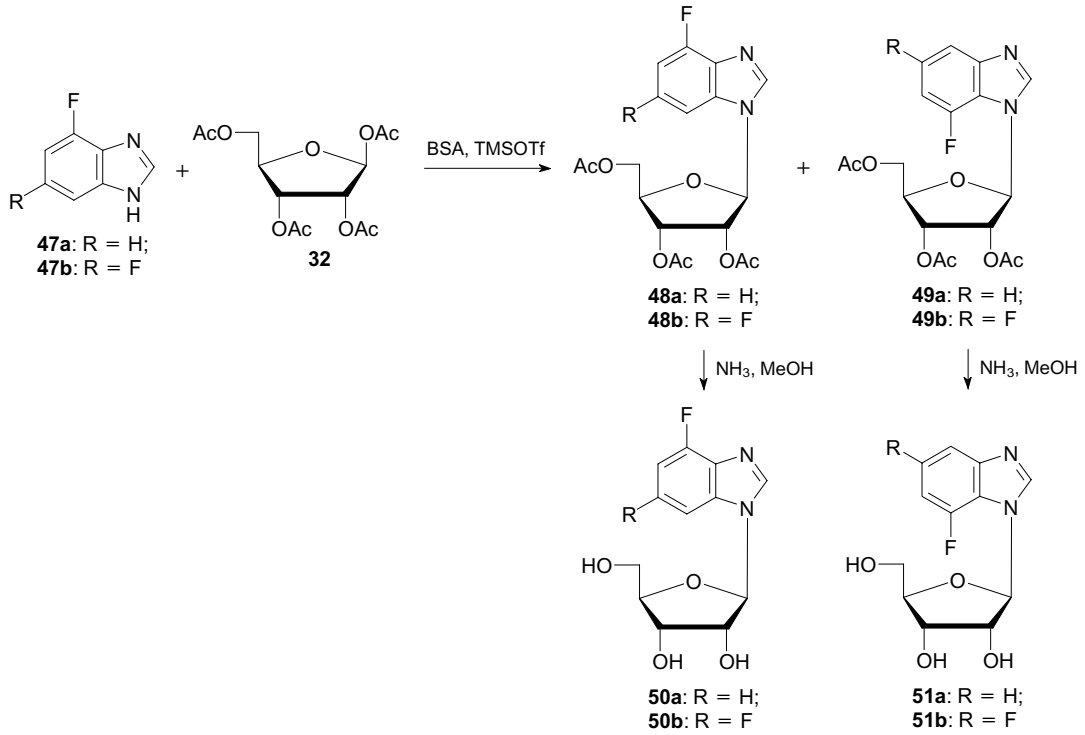
In the synthesis of 4,6-difluoro-1*H*-benzimidazole riboside (**50b**), protected β-D-ribofuranoside **48b** was generated in 67% yield (see Scheme 5), while the yield of N(3)-isomer **49b** was 11%. After the separation of the reaction products, acetyl protecting groups of nucleosides **48** and **49** were removed with methanolic ammonia. Free nucleosides **50a**,**b** were isolated in 89 and 94% yields, respectively; their N(3)-regioisomers **51a**,**b** were obtained in small amounts.[88]

Fluorinated benzimidazole ribosides **17a**–**c** and **18a**–**c** were synthesized in a similar fashion from the corresponding benzimidazoles **52a**–**c** and **53a**–**c**. However, the acetyl protecting groups of ribosides **54a**–**c** and **55a**–**c** were

Scheme 4

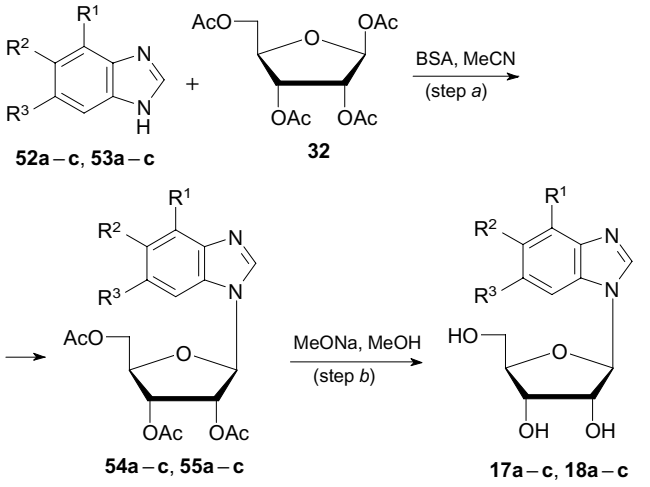


Scheme 5



removed in the last step by treatment with sodium methoxide in methanol (Scheme 6, Table 2).[46, 47]

Scheme 6



In 2016, 5-trifluoromethylbenzimidazole riboside (**18b**) and its thio analogue were synthesized in order to evaluate cytotoxicity and antitumour activity.[89] 2-Nitro-4-trifluoromethylaniline (**56**) was used as the starting compound. The synthesis was performed according to Scheme 7 employing classical Vorbrüggen glycosylation and the Zemplén method for deprotection. The imidazole ring closure of 4-trifluoromethylphenylene-1,2-diamine (**57**) with formic acid under reflux gave benzimidazole **53b** in quantitative yield, while the treatment of diamine **57** with carbon disulfide under reflux afforded 2-thioxo-5-trifluoromethyl-1*H*-benzimidazole (**58**) in high yield. After the preparation of silyl derivatives, bases **59** and **60** were subjected to the

Table 2. Synthesis of fluorinated benzimidazole nucleosides by the Vorbrüggen method (see Scheme 6).

Compounds	R^1	R^2	R^3	Yield (%)	
				step *a*	step *b*
17a, **52a**, **54a**	F	H	H	65	98
17b, **52b**, **54b**	H	H	F	42	94
17c, **52c**, **54c**	F	H	F	69	96
18a, **53a**, **55a**	CF_3	H	H	70	92
18b, **53b**, **55b**	H	CF_3	H	57	91
18c, **53c**, **55c**	H	H	CF_3	40	90

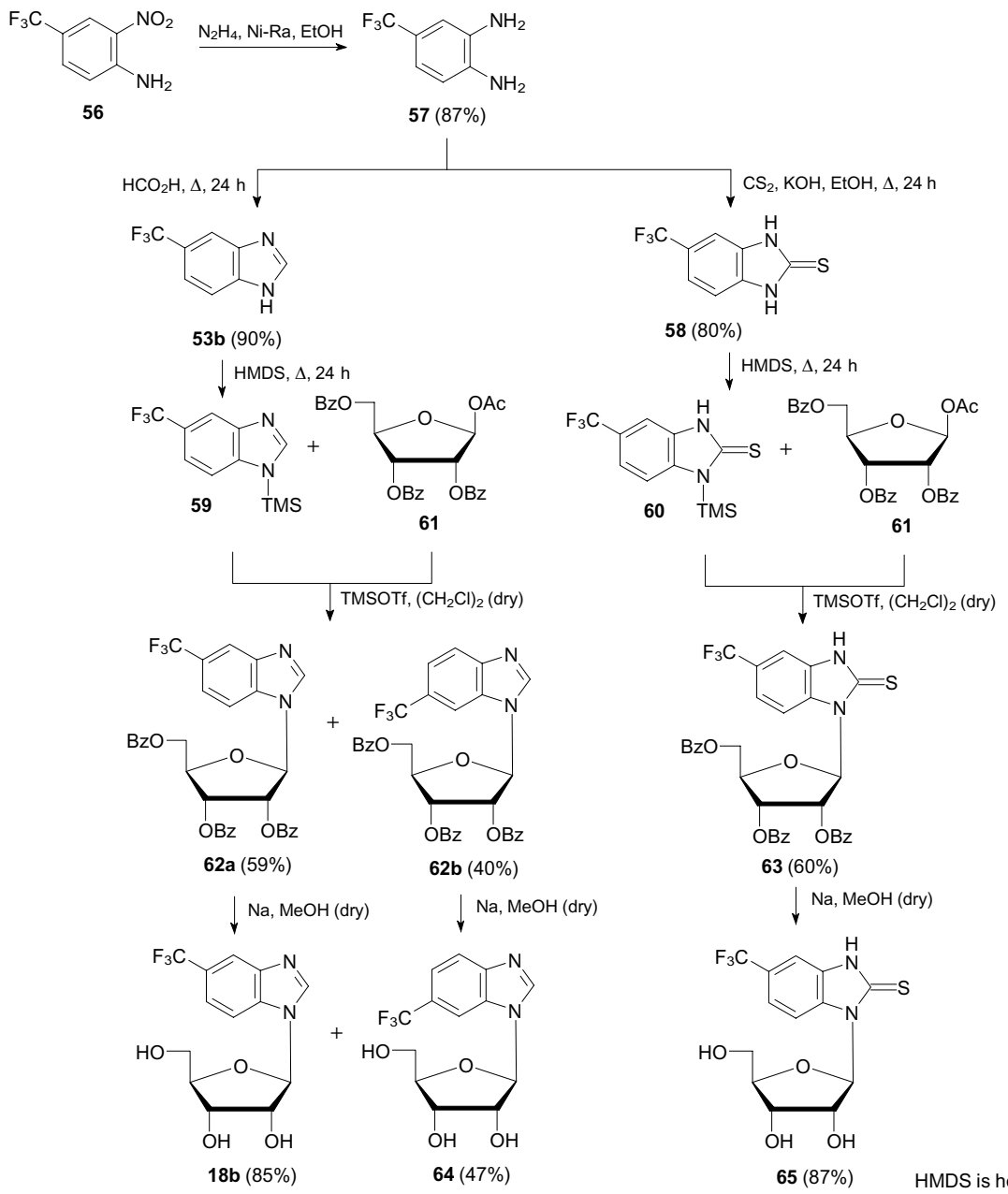
reaction with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (ABR, **61**) in the presence of TMSOTf in dry dichloroethane. Isomers **62a**,**b** were separated by column chromatography. In the last step, compounds **62a**,**b** and **63** were deprotected with sodium methoxide in dry methanol to form isomeric trifluoromethylbenzimidazole nucleosides **18b** and **64** and their 2-thione analogue **65**.

Silylated 4-(benzoylamino)- and 4-nitrobenzimidazoles, which were prepared by the treatment of the appropriate starting heterocyclic bases with a HMDS – TMSCl mixture at room temperature without intermediate isolation, were subjected to Vorbrüggen glycosylation with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**61**) (Scheme 8). Tin(IV) chloride was used as the catalyst.[85, 90] The ammonolysis of protecting groups of nucleosides **66a**,**b** with a 25% ammonia solution gave 4-amino- (**67a**) and 4-nitrobenzimidazole-containing ribosides (**67b**).[85, 91]

4.2. Synthesis of substituted benzimidazole 2′-deoxyribonucleosides

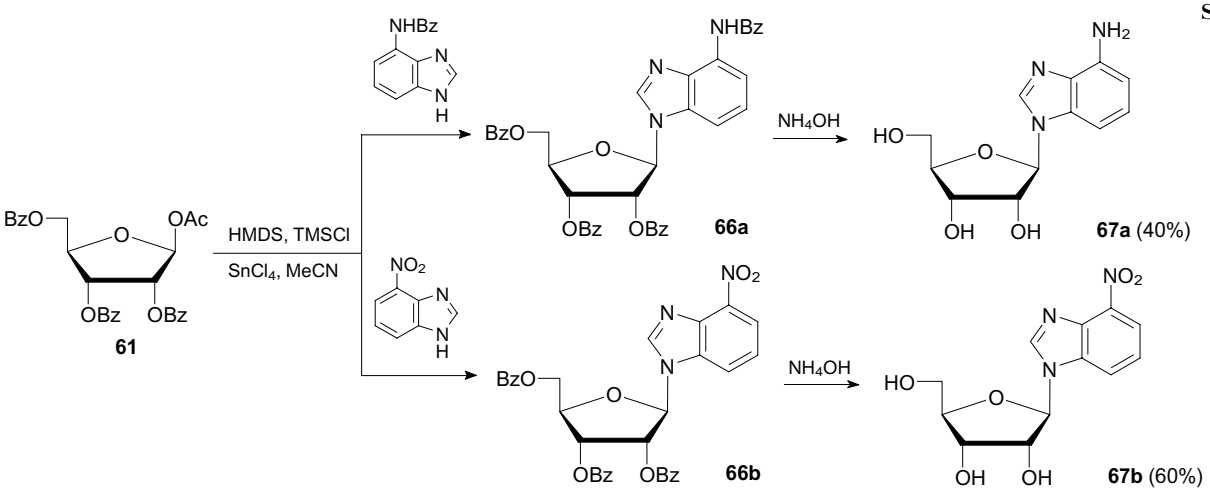
β-D-Ribosides comprise the main group of synthetic benzimidazole nucleosides. For C(2)-substituted 5,6-dichloro-

Scheme 7

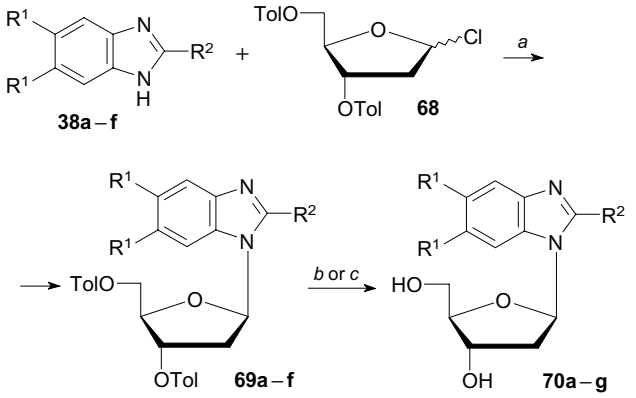


HMDS is hexamethyldisilazane

Scheme 8



Scheme 9



(*a*) BSA, TMSOTf; (*b*) NH_3, MeOH; (*c*) MeONa, MeOH

Compound	R^1	R^2	Yield (%)	
			69	**70**
a	Cl	Cl	89	70
b	Cl	Br	81	79
c	Cl	SH	33	82
d	Cl	SMe	71	68
e	Cl	SBn	64	40
f	Me	Cl	58	72
g	Cl	OMe	–	95

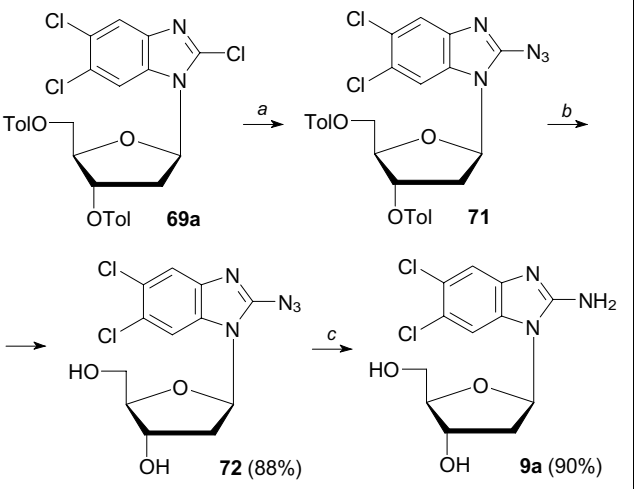
benzimidazoles, a series of 2′-deoxy analogues of β-D-ribosides were synthesized (Scheme 9).[33]

Zou *et al.*[33] used stereoselective glycosylation of the base, namely, 2,5,6-trichlorobenzimidazole (**38a**), with 2-deoxy-3,5-di-*O*-*p*-toluyl-α-D-*erythro*-pentofuranosyl chloride (**68**) giving β-anomers as the main products. Protected 2,5,6-trichloro-l-(2-deoxy-3,5-di-*O*-*p*-toluyl-D-*erythro*-pentofuranosyl)benzimidazole (**69a**) was synthesized in 89% yield. After deprotection, target 2,5,6-trichloro-l-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole nucleoside (**70a**) was isolated in 70% yield. Other β-D-2′-deoxyribosides (**69b – f** and **70b – f**) were synthesized from appropriate substituted benzimidazoles **38b – f**. Free 5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-methoxybenzimidazole (**70g**) was prepared by the treatment of compound **69a** with sodium methoxide in methanol (conditions *c*).[33]

The treatment of nucleoside **69a** with lithium azide followed by deprotection of azide **71** with methanolic ammonia produced 2-azido-5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**72**) (Scheme 10). 2-Amino-5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**9a**) was prepared by reduction of the azido group of nucleoside **72** by hydrogen on Raney nickel.[33, 35]

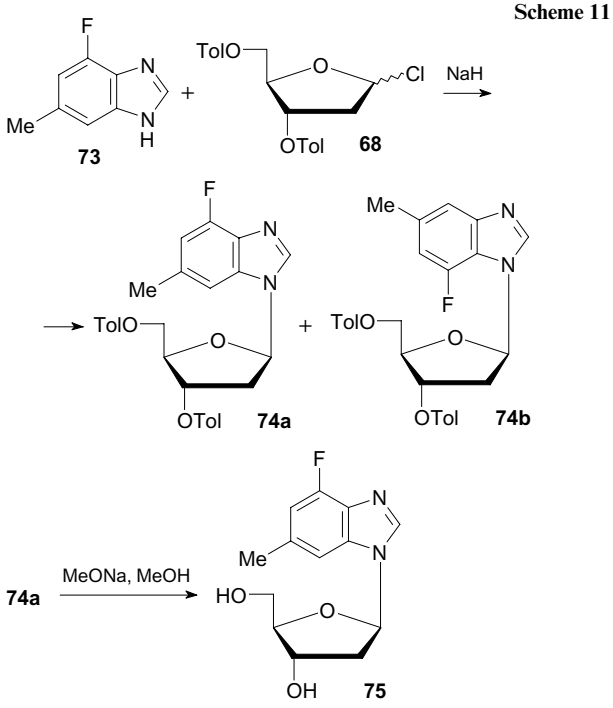
4-Fluoro-6-methylbenzimidazole (**73**) was synthesized from 2-fluoro-4-methylaniline in five steps in a total yield of 15%. This base was treated with sodium hydride and then subjected to the reaction with 2-deoxy-3,5-di-*O*-*p*-toluyl-α-D-erythro-pentofuranosyl chloride (**68**) (Scheme 11).[92] The mixture of isomers **74a**,**b** was separated by silica gel chromatography, and protected nucleoside **74a** was isolated in 46% yield. After deprotection with sodium methoxide in methanol and chromatographic separation,

Scheme 10



(*a*) LiN_3, EtOH; (*b*) NH_3, MeOH; (*c*) H_2, Ni-Ra

Scheme 11



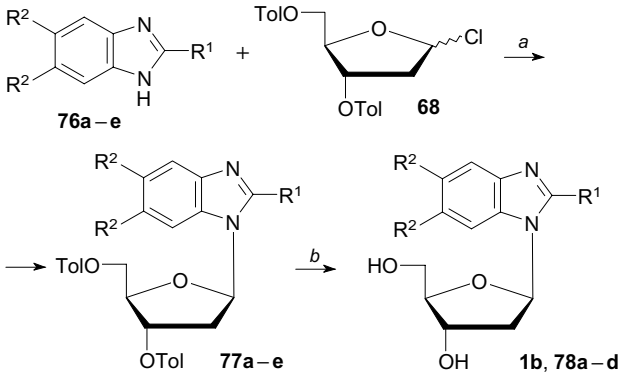
target 4-fluoro-6-methylbenzimidazole 2′-deoxyriboside (**75**) was isolated in 65% yield.[92]

A series of 5,6-disubstituted and 2,5,6-trisubstituted deoxyribosides were synthesized in a similar way through the formation of sodium salts of the starting compounds **76a – e** (Scheme 12). The removal of protecting groups from nucleosides **77a – e** gave products **1b** and **78a – d** in high yields based on the starting bases.[93]

4.3. Synthesis of benzimidazole arabinosides

Data on the synthesis of benzimidazole β-D-arabinosides are scarce despite the fact that there are several nucleosides of other heterocyclic bases with antiviral and antitumour activity, which have proven therapeutic efficacy (*e.g.*, vidarabine, nelarabine, clofarabine, fludarabine, cytarabine) . It

Scheme 12



(*a*) NaH, MeCN (anhydrous); (*b*) MeONa, MeOH

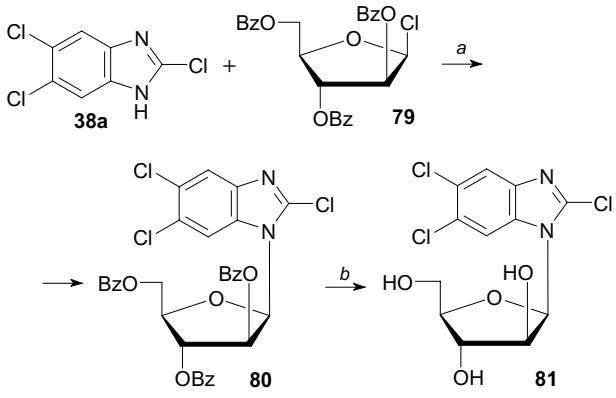
Benzimidazole	R^1	R^2	Product	Yield (%)
76a	H	F	**78a**	91
76b	H	Cl	**1b**	84
76c	CF_3	Cl	**78b**	57
76d	Me	Cl	**78c**	72
76e	Pr^i	Cl	**78d**	49

should be noted that the synthesis of benzimidazole α-D- and α-L-arabinosides was described in the literature,[17,94,95] whereas methods for the preparation of β-D-arabinosides are unknown.

Townsend patented[96] the synthesis of 2,5,6-trichloro-1-(2,3,5-tri-*O*-benzoyl-β-D-arabinofuranosyl)benzimidazole (**80**) starting from compound **38a** and 2,3,5-tri-*O*-benzoyl-D-arabinofuranosyl chloride (**79**) followed by removal of benzoyl protecting groups from compound **80** to form 1-(β-D-arabinofuranosyl)-2,5,6-trichlorobenzimidazole (**81**) in 78% yield (Scheme 13). However, the structure of target β-D-nucleoside **81** was not confirmed by spectroscopic data, and the biological activity and cytotoxicity of this nucleoside were not evaluated.

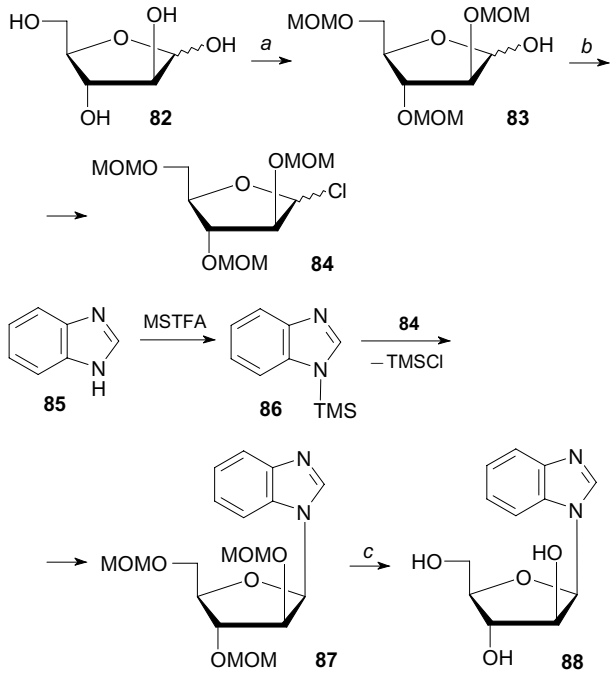
A method for the synthesis of benzimidazole arabinoside using methoxymethyl (MOM) protecting groups was described by Kharitonova *et al.*[56] The first step involves the alkylation of arabinose (**82**) with methoxymethyl chloride (3 equiv.) (Scheme 14) giving a complex mixture of methoxymethyl ethers of arabinofuranose and arabinopyranose (in Scheme 14, pyranosides are not shown), including MOM ether of arabinofuranose **83** with an unprotected OH group at position 1. The treatment of this mixture with the Vilsmeier reagent gave a mixture of 2,3,5-tri-*O*-methoxymethylarabinofuranosyl chloride (**84**) and the corresponding pyranosyl chloride. To use this mixture, without isolation, for glycosylation, the TMS group was preliminary introduced at the nitrogen atom of unprotected benzimidazole (**85**) (intermediate **86**). The synthesis of arabinoside **87** could, in principle, produce four possible arabinosyl nucleosides. However, the reaction of arabinofuranosyl chloride **84** with 1-trimethylsilylbenzimidazole (**86**) appeared to be stereospecific and afforded a mixture of two protected β-anomers of arabinosylbenzimidazole in furanose (**87**) and pyranose forms. The subsequent treatment of the reaction mixture with trifluoromethanesulfonic acid in methanol produced a mixture of β-D-furanosyl- (**88**) and β-D-arabinopyranosyl-substituted benzimidazoles, which can easily be separated by preparative HPLC.[56]

Scheme 13



(*a*) NaH, MeCN; (*b*) NH_3, MeOH

Scheme 14

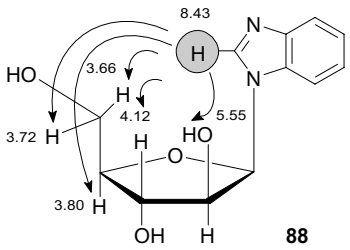


(*a*) MOMCl (3 equiv.), Pr^i_2NEt; (*b*) $(COCl)_2$, DMF; (*c*) TfOH; MSTFA is *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide

Since the alkylation of arabinose (**82**) with methoxymethyl chloride in dioxane and subsequent transformations produced a mixture of arabinopyranosyl- and arabinofuranosylbenzimidazoles in a ratio of 7 : 3, the first synthesis step was optimized in such a way as to diminish the amount of pyranose forms. The alkylation at reduced temperature in nonpolar solvents (benzene, dichloromethane) resulted in a considerable prevalence of furanose derivative **88** (7 : 1 ratio). The yield of arabinoside **88** was 30% based on the starting benzimidazole (**85**).

The structure of β-D-arabinofuranosylbenzimidazole (**88**) was confirmed by ^1H NMR spectroscopy and by 2D homo- and heteronuclear NMR spectroscopy ($^1H/^1H$-COSY, $^1H/^{13}C$-HSQC, $^1H/^{13}C$-HMBC, $^1H/^{15}N$-HSQC, $^1H/^{15}N$-HMBC, [^1H,^1H]-NOE experi-

Structure 88



88

ments). For example, the [^{1}H,^{1}H]-NOE spectrum showed cross-peaks between the proton at the C(2) atom of benzimidazole (8.43 ppm) and protons at positions 5′ (3.66 and 3.72 ppm) and 3′ (4.12 ppm), as well as the proton at the hydroxy group at position 2′ (5.55 ppm) of arabinose. The cross-peaks between the proton at the C(2) atom and the proton at position 4′ were absent. These data attest to the β-configuration of the synthesized nucleoside **88**.[56]

4.4. Synthesis of benzimidazole nucleosides fluorinated at the carbohydrate moiety

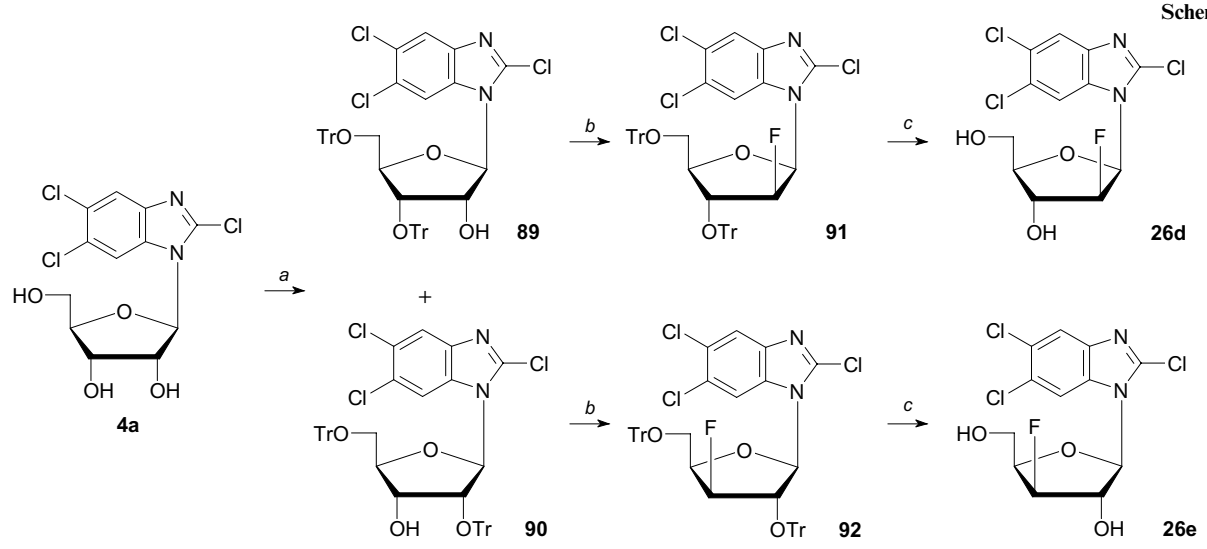
There are two different approaches to the synthesis of nucleosides fluorinated at the carbohydrate moiety. One approach is based on the introduction of a fluorine atom into the synthesized nucleoside containing protected hydroxy groups. Another approach involves the condensation of fluorinated carbohydrate with a heterocyclic base. Pankiewicz *et al.*[97] showed that some purine bases are difficult to glycosylate with 2′-deoxy-2′-fluoroarabinofuranosyl derivatives. Hence, Gudmundsson *et al.*[53] examined the possibility of fluorination of O-trityl-protected TCRB (**4a**) with (diethylamino)sulfur trifluoride (DAST). As reported earlier,[98] DAST is an efficient fluorinating agent for β-nucleosides. The attack by weakly nucleophilic fluorine is known to strongly depend on the conformation of the protected furanose residue of carbohydrate. Therefore, for leaving groups at position 2′ to be replaced, the furanose ring needs to adopt a conformation unfavourable for *trans*-elimination. This can be attained by using substrates with bulky protecting groups at positions 5′ and 3′, *e.g.*, 2,5,6-trichloro-1-(3,5-di-*O*-trityl-β-D-ribofuranosyl)benzimidazole (**89**) (Scheme 15).

Tritylation of the starting nucleoside **4a** with TrCl and 4-dimethylaminopyridine (DMAP) in pyridine at 80 °C for 4 days afforded a mixture of ditrityl derivatives **89** and **90** in a total yield of 38%. All attempts to increase the yield of reaction products by increasing the reaction time, rising the temperature or using other bases instead of DMAP did not improve the results. Attempts to separate di-O-trityl derivatives **89** and **90** by column flash chromatography were unsuccessful; a mixture in a ratio of 3 : 1 was obtained. These nucleosides were separated by fractional crystallization from Et_2O,[55,98] derivative **90** was precipitated from diethyl ether in 29% yield. Pure nucleoside **89** were obtained by repeated recrystallization from an ethyl acetate – hexane mixture (9% yield).

The fluorination of 3′,5′-ditrityl derivative **89** with DAST in the presence of pyridine in CH_2Cl_2 produced 2′-deoxy-2′-fluoroarabinoside **91** in 63% yield; under the same conditions, nucleoside **90** gave xylofuranosylbenzimidazole **92** in 71% yield. 2,5,6-Trichloro-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)- (**26d**) and 2,5,6-trichloro-1-(3-deoxy-3-fluoro-β-D-xylofuranosyl)benzimidazoles (**26e**) were obtained after removal of trityl protecting groups (10% CF_3CO_2H in chloroform) from compounds **91** and **92**, respectively (see Scheme 15). This sequence of transformations resulted in the formation of target fluorinated arabinofuranoside **26d** in a yield as low as 5%.[98]

Another method for the synthesis of benzimidazole arabinonucleosides fluorinated at the carbohydrate residue involves the condensation of fluorinated carbohydrate with a base. 2,5,6-Trichloro-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)benzimidazole (**26d**) was synthesized by the condensation of appropriate halogenose (*e.g.*, compound **93**) with 2,5,6-trichlorobenzimidazole (**38a**).[99] Convergent syntheses of a series of 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides were described by two research teams.[100,101] The first attempts to form a glycosidic bond by the Vorbrüggen method using alkali metal benzimidazolides generated from heterocycle **38a** failed. Howell *et al.*[102]

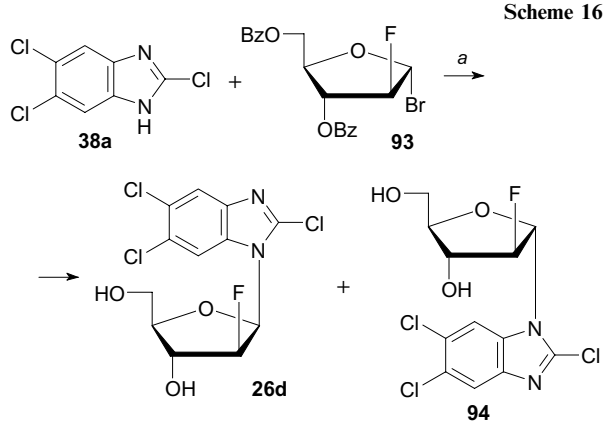
Scheme 15



(*a*) TrCl, DMAP, pyridine, 80 °C, 4 days; (*b*) DAST, CH_2Cl_2, pyridine; (*c*) CF_3CO_2H, $CHCl_3$; DMAP is 4-dimethylaminopyridine; Tr is trityl

succeeded in performing glycosylation of base **38a** with 3,5-di-*O*-benzoyl-1-bromo-2-deoxy-2-fluoro-α-D-arabinofuranose (**93**) under conditions similar to those described by Montgomery *et al.*[103] The condensation occurred in dichloroethane at 80 °C in the presence of molecular sieves (MS) 4 Å (Scheme 16) to give a mixture of anomers. After separation of this mixture and deprotection, product **26d**, 2′-deoxy-2′-fluoro-β-D-arabino analogue of TCRB, was obtained.

Scheme 16

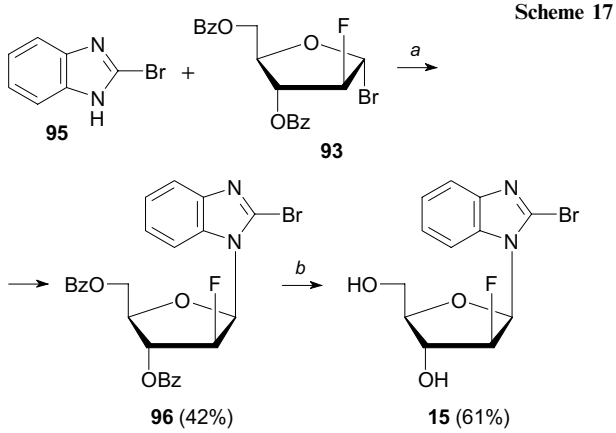


(*a*) 1) $(CH_2Cl)_2$, MS 4 Å, 80 °C; 2) NH_3, MeOH

Further studies of this reaction showed that the ratio of anomers in the mixture strongly depends on the conditions of condensation. The condensation in nonpolar solvents (*e.g.*, in dichloroethane and benzene) affords β-anomer **26d** as the major product (the ratio β : α varies from 5 : 1 to 10 : 1), while α-anomer **94** is dominant in mixtures produced in more polar solvents, such as acetonitrile and nitromethane. The total yields of the products in dichloroethane are higher (80%, β : α = 8 : 1) than those in acetonitrile (9%, β : α = 1 : 7). The reaction performed under the conditions presented in Scheme 16 gave target β-anomer **26d** in 50% yield.[102]

The glycosylation of base **95** with fluorinated furanoside **93** in the tris[2-(2-methoxyethoxy)ethyl]amine – KOH – MeCN system (Scheme 17) affords protected 2′-deoxy-2′-fluoronucleoside **96**.

Scheme 17



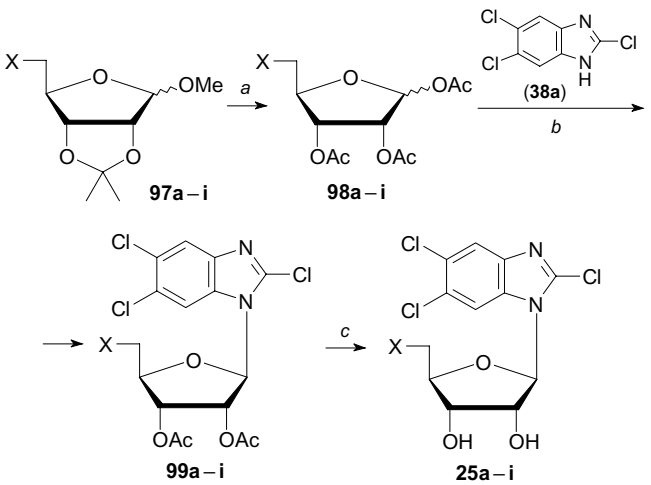
(*a*) KOH, tris[2-(2-methoxyethoxy)ethyl]amine, MeCN, 25 °C; (*b*) NH_3, MeOH

Nucleoside **15** was isolated after deprotection of **96** by ammonolysis and chromatographic separation of the mixture.[44]

4.5. Synthesis of 5′-derivatives of 2,5,6-trichlorobenzimidazole

Gudmundsson *et al.*[53] described the synthesis of a series of 5′-substituted 2,5,6-trichlorobenzimidazole nucleosides (**25a – i**) (Scheme 18).

Scheme 18



(*a*) 1) 0.04 M HCl, Δ; 2) Ac_2O, pyridine; (*b*) 1) BSA, MeCN; 2) TMSOTf, MeCN; (*c*) Na_2CO_3, aq. EtOH; X = F (**a**), Cl (**b**), Br (**c**), I (**d**), OMe (**e**), OEt (**f**), OBu^n (**g**), N_3 (**h**), SMe (**i**)

Methyl 5-*O*-alkyl-2,3-*O*-isopropylidene-D-ribofuranosides **97e – g** were synthesized by a procedure developed earlier.[104] Methyl glycosides **97e – g** were transformed into 1,2,3-tri-*O*-acetyl-5-*O*-alkyl-D-ribofuranoses **98e – g** by the treatment with dilute hydrochloric acid followed by acetylation with acetic anhydride in pyridine. Derivatives **98e – g** were used to glycosylate 2,5,6-trichlorobenzimidazole (**38a**) by the Vorbrüggen method.[105] Intermediate 1-(5-*O*-alkyl-2,3-di-*O*-acetyl-β-D-ribofuranosyl)-2,5,6-trichlorobenzimidazoles **99e – g** were prepared in high yields (up to 80%). After deprotection of compounds **99e – g** with sodium carbonate in aqueous ethanol, target 1-(5-*O*-alkyl-β-D-ribofuranosyl)-2,5,6-trichlorobenzimidazoles **25e – g** were obtained in 75%, 82% and 70% yields, respectively (see Scheme 18).

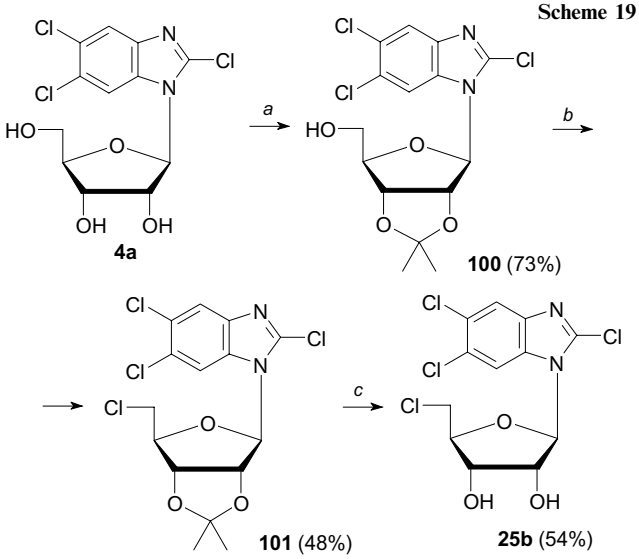
5′-Chloro derivatives **25b** were synthesized in four steps from 5-chloro-5-deoxy-2,3-*O*-isopropylidene-1-methyl-β-D-ribofuranoside (**97b**).[106] Compound **97b** was converted into 1,2,3-tri-*O*-acetyl-5-chloro-5-deoxy-β-D-ribofuranose (**98b**) and condensed with heterocycle **38a**. After deprotection, compound **25b** was obtained in 78% yield.

This procedure was used to synthesize 5′-halogenated derivatives **25a**,**c**,**d** in 80% – 81% yields,[53] because there was no risk of unwanted halogen exchange at position 2 of the benzimidazole moiety. The starting compounds [5-deoxy-5-fluoro-2,3-*O*-isopropylidene-1-methoxy-D-ribofuranoside (**97a**), 5-bromo-5-deoxy-2,3-*O*-isopropylidene-1-methoxy-D-ribofuranoside (**97c**) and 5-deoxy-2,3-*O*-isopropylidene-5-iodo-1-methoxy-D-ribofuranoside (**97d**)] were synthesized by a method proposed by Classon and Liu.[107]

2,5,6-Trichloro-1-(5-deoxy-5-methylsulfanyl-β-D-ribofuranosyl)benzimidazole (**25i**) was synthesized from 1,2,3-tri-*O*-acetyl-5-deoxy-5-(methylsulfanyl)ribofuranose (**98i**) using procedures described by Montgomery *et al.*[108] and by Kanazawa.[109]

An alternative method was proposed for the synthesis of 5′-chloro-5′-deoxy-containing analogue **25b** starting from TCRB (**4a**), the diol system of which was protected by the isopropylidene group. Then the 5′-OH group of compound **100** was replaced by a chlorine atom (Scheme 19). For this purpose, intermediate **100** was treated with tetrachloromethane and triphenylphosphine to obtain nucleoside **101**. The deprotection of the latter by acid hydrolysis afforded compound **25b**.[53]

Scheme 19



(*a*) acetone, 2,2-dimethoxypropane, Dowex 50 H^+; (*b*) PPh_3, CCl_4, MeCN; (*c*) 2 M HCl, THF

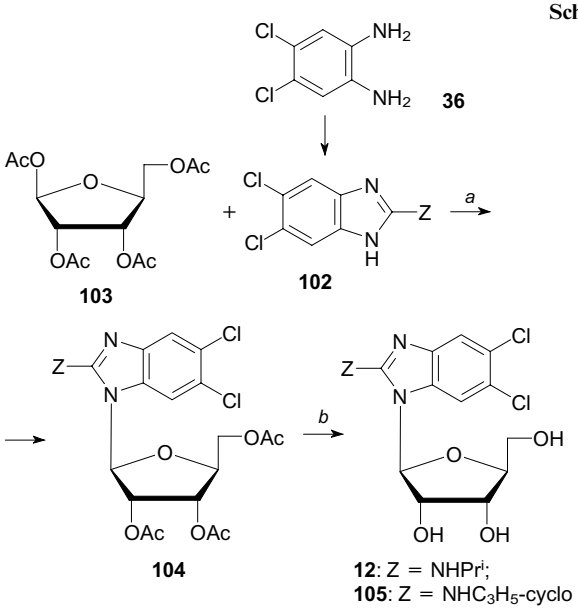
4.6. Synthesis of benzimidazole β-L-nucleosides

5,6-Dichloro-2-isopropylamino-1-(β-L-ribofuranosyl)benzimidazole (maribavir, **12**) and some its 2-alkylamino-containing analogues were prepared by a universal method for the synthesis of benzimidazole L-nucleosides described in a patent [110] granted to Chamberlain.

The general procedure for this synthesis involves three steps. The first step afforded 2-(alkylamino)benzimidazoles **102** (Scheme 20). The corresponding 1,2-phenylenediamine **36** was condensed with isothiosulfonate in anhydrous pyridine on heating of the reaction mixture to 80 °C followed by the addition of 3-cyclohexyl-1-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate.

The second step was a glycosylation reaction of 2-(alkylamino)benzimidazoles **102** with 1,2,3,5-tetra-*O*-acetyl-L-ribofuranose (**103**). Benzimidazoles **102** produced in the first step were heated with BSA in 1,2-dichloroethane at 80 °C, and then TMSOTf and 1,2,3,5-tetra-*O*-acetyl-L-ribofuranose (**103**) were added. The reaction mixture was kept at 80 °C for a few hours and then cooled to room temperature and neutralized with 5% aqueous sodium bicarbonate solution. Products **104** were isolated by silica gel flash chromatography.

Scheme 20

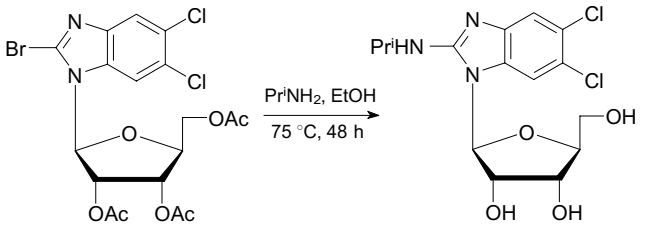


(*a*) 1) BSA, $(CH_2Cl)_2$, 80 °C; 2) TMSOTf, 80 °C; (*b*) Na_2CO_3

The third step involved the removal of protecting groups. Thus, 2-alkylamino-1-(2,3,5-tri-*O*-acetyl-β-L-ribofuranosyl)benzimidazole (**104**) was dissolved in ethanol and treated with an aqueous sodium carbonate suspension in methanol. Reaction products **12** and **105** were isolated by silica gel chromatography.[110] Maribavir (**12**) was isolated in 63% yield.

The second version of the synthesis of maribavir (**12**) is based on the condensation of 2-bromo-5,6-dichloro-1-(2,3,5-tri-*O*-acetyl-β-L-ribofuranosyl)benzimidazole (**106**) with isopropylamine in anhydrous ethanol at 75 °C for 48 h (Scheme 21). The target product was isolated from the reaction mixture by silica gel column chromatography; the yield was 60%.[111]

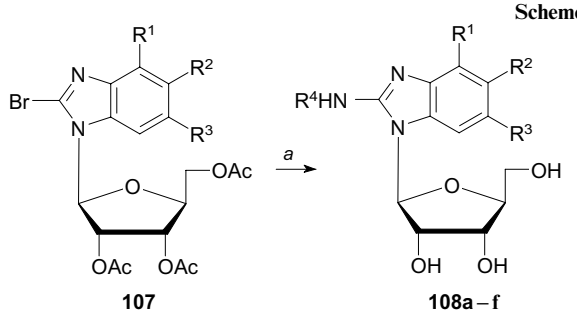
Scheme 21



This method was used to synthesize analogues of maribavir, compounds **108a**–**f** containing the isopropylamino or cyclopropylamino group at position 2 of the benzimidazole ring and bearing chlorine and(or) bromine atoms in the benzene ring, starting from nucleoside **107**. The yields of the products were 59% – 75% (Scheme 22).[111]

Deoxyribobenzimidazole β-L-2′-nucleosides **13a**,**b** were synthesized according to Scheme 23. In the first step, 5(6)-nitrobenzimidazole (**109**) was glycosylated with 1-α-chloro-2-deoxy-3,5-ditoluyl-α-L-ribofuranose (**110**) in acetonitrile in the presence of KOH using tris(hydroxymethyl)aminomethane (TDA-1) as the catalyst.[44]

Scheme 22



107 **108a – f**

(*a*) R^4NH_2, EtOH, 75 °C, 48 h; $R^1 = R^2 = R^3 = Cl$: $R^4 = Pr^i$ (**a**), cyclo-C_3H_5 (**b**); $R^1 = Br$, $R^2 = R^3 = Cl$: $R^4 = Pr^i$ (**c**), cyclo-C_3H_5 (**d**); $R^1 = R^3 = Cl$, $R^2 = H$, $R^4 = Pr^i$ (**e**); $R^1 = R^2 = Cl$, $R^3 = H$, $R^4 = Pr^i$ (**f**)

The reaction gave a mixture of N(1)- and N(3)-regioisomers **111a**,**b**, which were separated by flash chromatography. Protecting groups were removed by the treatment with a solution of sodium methoxide in methanol. After recrystallization from methanol, the corresponding regioisomers **13a**,**b** were isolated in 59% and 62% yields, respectively.[44]

Glycosylation of 2-bromobenzimidazole (**112a**) and 4,5,6,7-tetrabromobenzimidazole (**112b**) with halogenose **110** afforded products **113a**,**b** in rather high yields (63% and 51%, respectively) due to the use of sodium hydride (Scheme 24).

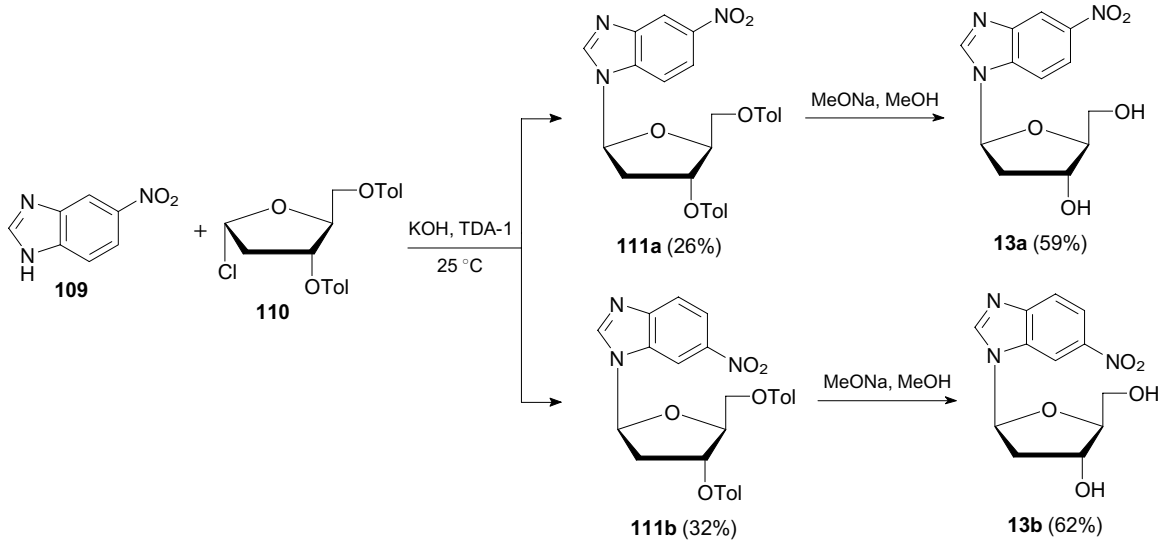
The toluyl protecting groups of nucleosides **113a**,**b** were removed with a 1 M solution of sodium methoxide in methanol to form bromobenzimidazole β-L-nucleosides **14a**,**b** in 89% and 51% yields, respectively.[44]

5. Chemoenzymatic synthesis of modified benzimidazole nucleosides

Enzymatic glycosylation (transglycosylation) is an alternative to chemical methods of synthesis of benzimidazole nucleosides. In this approach, the glycosidic bond formation is accomplished in an aqueous medium in one step (as opposed to multistep chemical methods).[112] This method does not require the introduction of protecting groups into either a heterocyclic base or a carbohydrate moiety and is characterized by stereoselectivity and relative regioselectivity. Enzymatic glycosylation is an environmentally friendly, safe, facile and highly specific method for nucleoside synthesis.

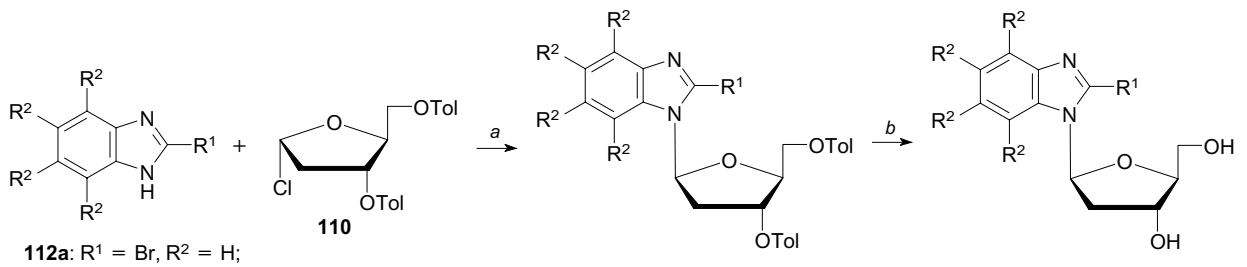
The transglycosylation is performed using bacterial glycosyltransferases: *N*-deoxyribosyltransferases (NDTs, EC 2.4.2.6)[113–116] and nucleoside phosphorylases (NPs). Nucleoside phosphorylases include uridine phosphorylase (UP, EC 2.4.2.3),[117,118] thymidine phosphorylase (TP,

Scheme 23



109 **110** **111a** (26%) **13a** (59%) **111b** (32%) **13b** (62%)

Scheme 24



112a: $R^1 = Br$, $R^2 = H$;
112b: $R^1 = H$, $R^2 = Br$

110

113a: $R^1 = Br$, $R^2 = H$ (63%);
113b: $R^1 = H$, $R^2 = Br$ (51%)

14a: $R^1 = Br$, $R^2 = H$ (89%);
14b: $R^1 = H$, $R^2 = Br$ (51%)

(*a*) NaH, MeCN, 25 °C; (*b*) MeONa, MeOH

EC 2.4.2.4)[118–120] and purine nucleoside phosphorylase (PNP, EC 2.4.2.1).[121–123]

5.1. Synthesis of benzimidazole nucleosides using N-deoxyribosyltransferases

Such microorganisms as *Lactobacilli* contain a sufficient amount of *N*-deoxyribosyltransferases. Two classes of these enzymes are currently known. Enzymes of one class (NDT-1) catalyze the transfer of 2-deoxyribose exclusively between purine bases [Pur(1) and Pur(2)], while enzymes of another class (NDT-2) catalyze the transfer of 2-deoxyribose both between purines and between pyrimidines (Pyr) (Scheme 25, dRib is a deoxyribose moiety).[114,124–126]

Scheme 25

For NDT-1

dRib—Pur(1) + Pur(2) ⇌ dRib—Pur(2) + Pur(1)

For NDT-2

dRib—Pur(1) + Pur(2) ⇌ dRib—Pur(2) + Pur(1)

dRib—Pur + Pyr ⇌ dRib—Pyr + Pur

dRib—Pyr(1) + Pyr(2) ⇌ dRib—Pyr(2) + Pyr(1)

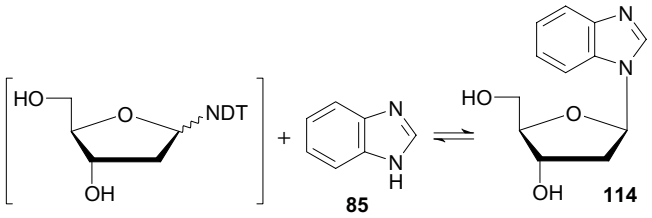
Transferases are mainly produced from *Lactobacillus helveticus* or *L. leichmanni*. Their kinetic mechanisms, as well as catalytic and substrate-binding sites are well-known and characterized.[127,128] These enzymes are specific to 2′-deoxyribosides and 2′,3′-dideoxyribosides. Enzyme-catalyzed reactions are highly stereospecific (produce only β-anomers of nucleosides[115]) and regioselective [glycosylation occurs at the N(1) atoms in pyrimidines and at the N(9) atoms in purines].[126] Transferase-catalyzed reactions proceed *via* a covalent oxyribosyl–enzyme intermediate (Scheme 26). A glutamine residue (Glu98) was shown to be essential for substrate binding in the active site of the enzyme.[129,130]

2′-Deoxycytidine and thymidine are the best donors of carbohydrate moieties in reactions with NDTs. These enzymes have tolerance towards acceptor bases allowing the use of different structural variants of the latter.[131] For example, the antileukemic agent 2-chloroadenine 2′-deoxynucleoside (cladribine) was synthesized in the presence of *L. leichmanni* NDT.[132] This enzyme can also be employed to prepare 2′,3′-dideoxynucleosides.[115] Thus, 2′,3′-dideoxynucleosides of natural bases[133] and 2-halogen-substituted adenosines[134] were synthesized using *L. helveticus* NDT as the catalyst. Transferases from *L. leichmanni* can catalyze the transfer of 2′,3′-dideoxyribose from 2′,3′-dideoxycytidine to N(6)-substituted adenines.[135]

N-Deoxyribosyltransferases are searched for not only in mesophilic microorganisms but also in extremophiles. Transferases from these sources make it possible to extend the applicability of biocatalytic methods. For instance, NDT from *Leishmania mexicana* can catalyze the nucleoside synthesis in alkaline media (pH up to 10) in a wide temperature range (up to 70 °C).[136] *N*-Deoxyribosyltransferases immobilized on different supports (functionalized agarose, sepharose, aminopropyl-Silochrome, *etc.*) are suitable for repeated use.[125,137]

The first data on the synthesis of benzimidazole deoxyribosides using NDT refer directly to benzimidazole 2′-deoxyriboside and 5,6-dimethylbenzimidazole 2′-deoxyriboside, which were prepared with 33% and 38% conversion, respectively (according to HPLC analysis; the products were not isolated and characterized).[124] The reactions were performed in phosphate buffer at 40 °C using thymidine as a deoxyribose donor. Other data on the enzymatic synthesis of benzimidazole deoxyriboside using NDT date back to 1990.[138] In this study, 2′-deoxycytidine served as a deoxyriboside donor, the reaction was performed in citrate buffer, and target nucleoside **114** was isolated from the reaction mixture (46% yield) and characterized (Scheme 27).

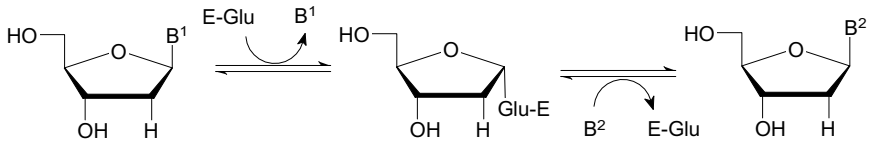
Scheme 27



More recently, it was shown that NDT can be employed to synthesize 2-aminobenzimidazole and 2-hydroxybenzimidazole 2′-deoxyribosides.[139] In these reactions, 2′-deoxyadenosine served as a donor of a carbohydrate moiety. However, the activity of this enzyme towards benzimidazole bases was as low as 4.8% (the activity of the enzyme towards deoxyadenosine and cytosine was taken to be 100%).

N-Deoxyribosyltransferases were found also in other microorganisms: in some species of *Bacillus*,[114] in *Crithidia luciliae*,[140,141] *Trypanosoma brucei*[142] and *Borrelia burgdorferi*.[143] All the above-described NDTs are specific to 2′-deoxyribose and do not accept natural ribonucleosides as ribose donors. Recently, Fernández-Lucas *et al.*[126] reported the cloning and expression of the *ndt* gene encoding NDT-2 from *Lactobacillus reuteri* (*Lr*NDT) in *Escherichia coli*. The enzyme *Lr*NDT is suitable for the synthesis of natural and non-natural nucleosides, it has lower substrate specificity than other enzymes described in the literature. It should be noted that *Lr*NDT can synthesize new non-natural nucleosides (2′-fluorodeoxyribonucleosides and -arabinonucleosides), but this enzyme also does not recognize natural ribosides as substrates. The enzyme *Lr*NDT is thermally stable and can function at temperatures up to 64 °C. The optimal conditions for the synthesis in the presence of this enzyme are 40 °C and pH 6.5. The enzyme

Scheme 26



E is enzyme,
B^1 and B^2 are purine or pyrimidine residues, Glu is glutamine residue

*Lr*NDT was employed to prepare benzimidazole 2′-deoxyriboside **114**; 2′-deoxyuridine (dUrd) or 2′-deoxyadenosine (dAdo) served as a donor of a carbohydrate moiety. Besides, 2′-fluorodeoxyriboside was synthesized in the presence of this enzyme (2′-FdUrd as the donor), but benzimidazole arabinoside was not detected in the reaction with 1-β-D-arabinofuranosyluracil.[126]

Some modified benzimidazole 2′-deoxyribosides were synthesized by the chemoenzymatic method using NDT.[76] 5,6-Dichlorobenzimidazole (**38h**) was shown to be a substrate for *N*-deoxyribosyltransferases. The reaction of base **38h** with deoxyuridine is catalyzed by NDT in citrate buffer at 50 °C and pH 6 (Scheme 28). The subsequent acetylation of compound **1b** gives 5,6-dichloro-1-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)benzimidazole (**115**) in good yield. The treatment of compound **115** with *N*-bromosuccinimide (NBS) affords 2-bromo derivative **116**.

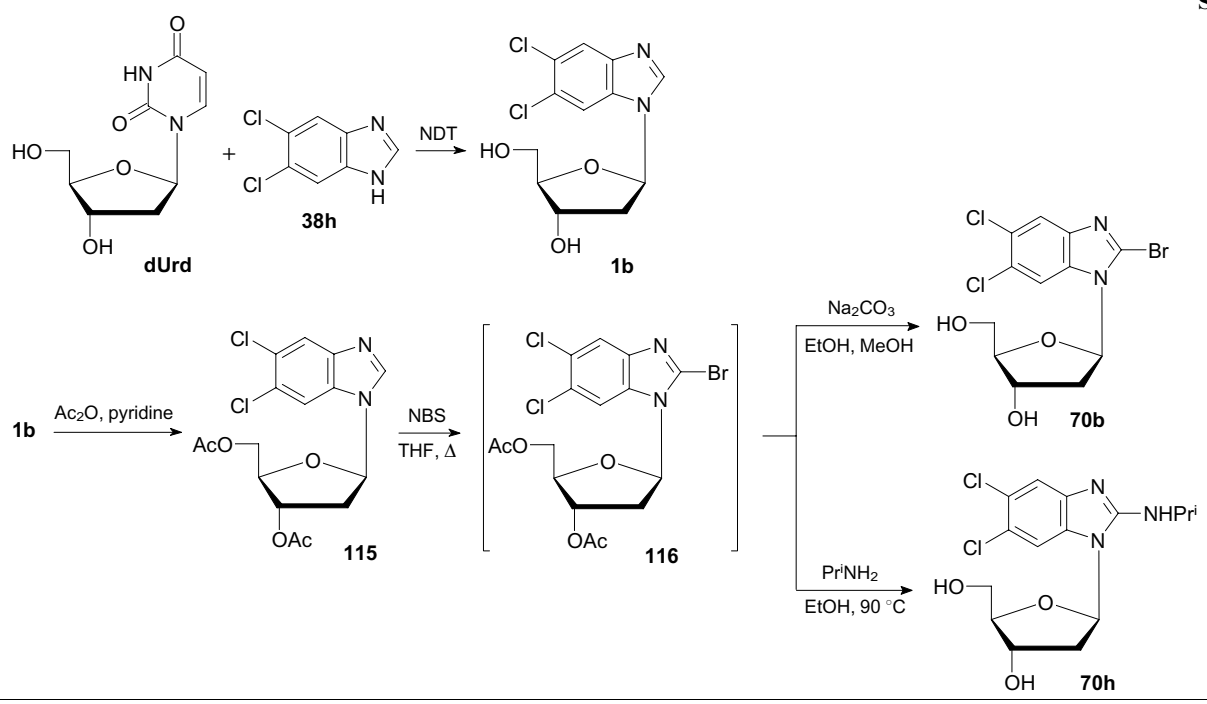
Bromo derivative **116** is converted into 2-bromo-5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**70b**) on treatment with sodium carbonate. 5,6-Dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-isopropylaminobenzimidazole (**70h**), which is the D-analogue of maribavir, was synthesized by the nucleophilic substitution of the bromine atom at position 2 of the base with isopropylamine accompanied by removal of protecting groups.[35]

Attempts were made to apply this approach for the synthesis of benzimidazole nucleosides fluorinated at the carbohydrate moiety. Townsend *et al.*[54] described an attempt of the enzymatic synthesis of 2′-deoxy-2′-fluororibonucleoside, a TCRB derivative. However, the enzymatic transfer of a carbohydrate moiety from 2′-deoxy-2′-fluorouridine (**117**) to 2,5,6-trichlorobenzimidazole (**38a**) did not take place.

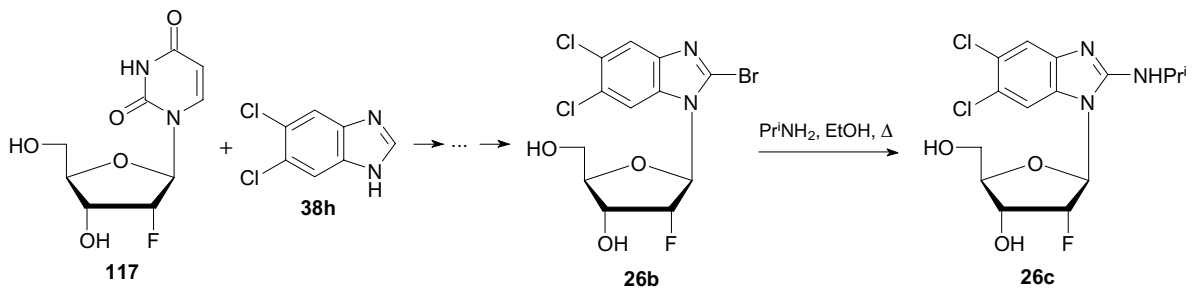
It should be noted that NDT was employed to transfer the carbohydrate moiety from compound **117** to 5,6-dichlorobenzimidazole (**38h**) (Scheme 29), because it has already been known that this base is a substrate for this enzyme. The protection of hydroxy groups in the nucleoside obtained in the first step of the synthesis, the introduction of a bromine atom at position 2 of the benzimidazole ring in the presence of NBS in dioxane and subsequent deprotection with sodium carbonate gave the fluorinated analogue of BDCRB, 2-bromo-5,6-dichloro-1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)benzimidazole (**26b**). 5,6-Dichloro-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-2-isopropylaminobenzimidazole (**26c**), as the fluorinated β-D-analogue of maribavir, was synthesized from compound **26b** in 57% yield (Scheme 29).[53]

Therefore, the use of NDT in the synthesis of benzimidazole nucleosides provides a very convenient and efficient route to 2′-deoxyribosides, but not to ribosides.

Scheme 28



Scheme 29

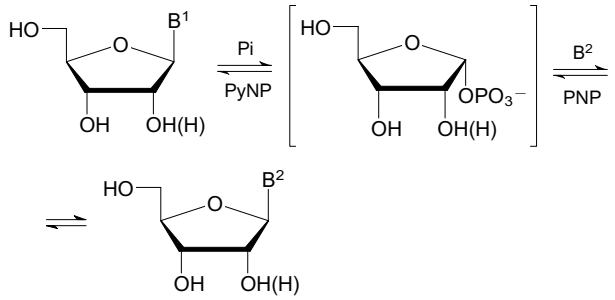


5.2. Synthesis of benzimidazole nucleosides using nucleoside phosphorylases

Another approach to the enzymatic synthesis of benzimidazole nucleosides is based on the use of NPs. This method allows the synthesis of not only benzimidazole 2′-deoxyribofuranosides but also benzimidazole ribosides [48, 49, 52, 57, 74] and benzimidazole 2-fluoroarabinosides.[56]

All three nucleoside phosphorylases (UP, TP and PNP) belong to key enzymes of nucleic acid metabolism and were found in more than 130 microorganisms; however, NPs from *E. coli* have found most use. The following two functions of nucleoside phosphorylases were established: the involvement in nucleoside synthesis [144 – 146] and catabolism of nucleosides.[147 – 149] The enzyme PNP has an essential role in purine metabolism.[150, 151] Generally, all PNPs are active in a wide pH range (from 6 to 8.5 depending on the source) and have a broad substrate specificity.[150, 152, 153] These enzymes are exploited as a tool for the synthesis of nucleosides with potential antiviral and antitumour activity.[145, 146, 150, 154, 155] The mechanism of transglycosylation involving these enzymes is shown in Scheme 30.

Scheme 30



Pi is inorganic phosphate, B^1 is pyrimidine base, B^2 is modified purine base

This reaction proceeds *via* the formation of the corresponding α-furanose 1-phosphate, which is then utilized by PNP as the substrate.

This strategy was applied to prepare a series of nucleosides with a modified carbohydrate residue and(or) a base. The synthetic approach presented in Scheme 30 involves the use of pyrimidine phosphorylases (PyNP), such as UP or TP, in the first synthesis step, because pyrimidine nucleosides most often serve as a source of a carbohydrate moiety due to their low cost. Besides, the preparation of pentose 1-phosphate from pyrimidine (but not purine) nucleosides is a rational strategy to synthesize nucleosides with non-natural carbohydrate residues, because these purine analogues are sometimes not available.[156 – 158] There are modifications of enzymatic synthesis of modified purine 2-fluoroarabinosides, which utilize chemically synthesized modified pentofuranoside 1-α-phosphates (*e.g.*, 2-deoxy-2-fluoro-α-D-arabinofuranose 1-phosphate).[122] The application of this strategy of enzymatic synthesis of modified nucleosides was described in detail [146, 150, 154, 157] for purine nucleosides (the yields of the target products were 70% – 80%).

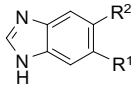
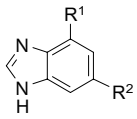
The preparation of benzimidazole nucleosides using PNP was described for the first time in 1995.[74] The study of substrate properties of the enzyme in transglycosylation showed that benzimidazole has high acceptor capacity due to good affinity for the active site of the enzyme. Besides, it was shown that benzimidazole β-D-riboside is a poor substrate for phosphorolysis mediated by *E. coli* PNP,[159] which is associated with higher stability of a glycosidic bond of benzimidazole riboside compared to that of purine nucleosides,[160] which interferes with the phosphorolysis of benzimidazole nucleoside that is formed in the active site of the enzyme. Consequently, this enzymatic method can be applied to synthesize different benzimidazole nucleosides.

Nine modified benzimidazoles, namely, compounds **38h**, **76a**, **85**, **109**, **118**, **119** and **120a – c**, were tested as substrates for PNP (Table 3).[74] Guanosine and 2′-deoxyguanosine were utilized as donors of carbohydrate moieties. It was agreed that it would not be appropriate to use adenine nucleosides in transglycosylation because the employment of *E. coli* whole cells (rather than isolated PNP) can lead to the biosynthesis of inosine from adenosine as a side reaction catalyzed by adenosine deaminases.

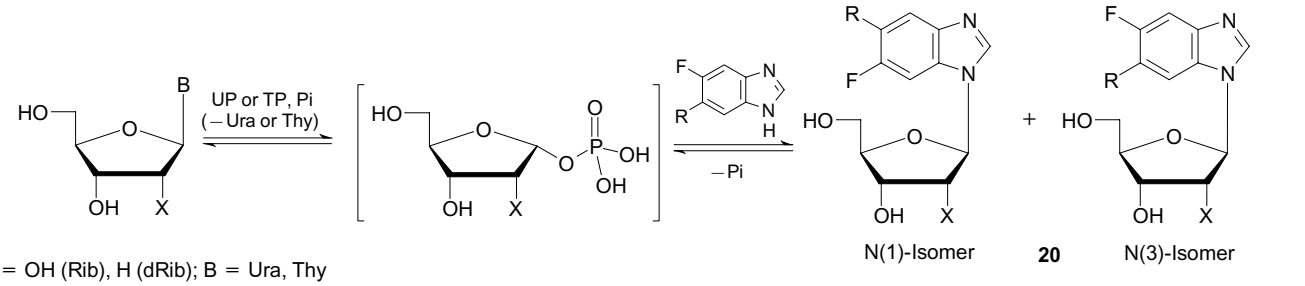
The highest yield of the product was observed in the synthesis of nucleosides from the following bases: 5,6-dichloro- (**38h**), 5,6-difluoro- (**76a**) and 5,6-dimethylbenzimidazoles (**118**), whereas the lowest yield was observed in the reactions with 4,6-dinitrobenzimidazole (**119**).[74]

Enzymatic trans-glycosylation using genetically engineered *E. coli* PNPs was employed to synthesize a series of 6-fluoro-5-substituted 1-(β-D-ribo)- and 1-(2-deoxyribofuranosyl)benzimidazoles **20** from modified bases (Scheme 31, Table 4).[48] Uridine, 2′-deoxyuridine and thymidine served as donors of carbohydrate moieties. The reaction was performed at 52 °C and pH 7 in phosphate buffer. The yield of nucleosides was from 51% to 98%. In this study, the authors proved for the first time (NMR spectroscopic data) that *E. coli* purine nucleoside phosphorylase synthesizes not only N(1)- but also N(3)-regioisomers of unsym-

Table 3. Efficiency of the synthesis of benzimidazole nucleosides using PNP-containing *E. coli* whole cells.[74]

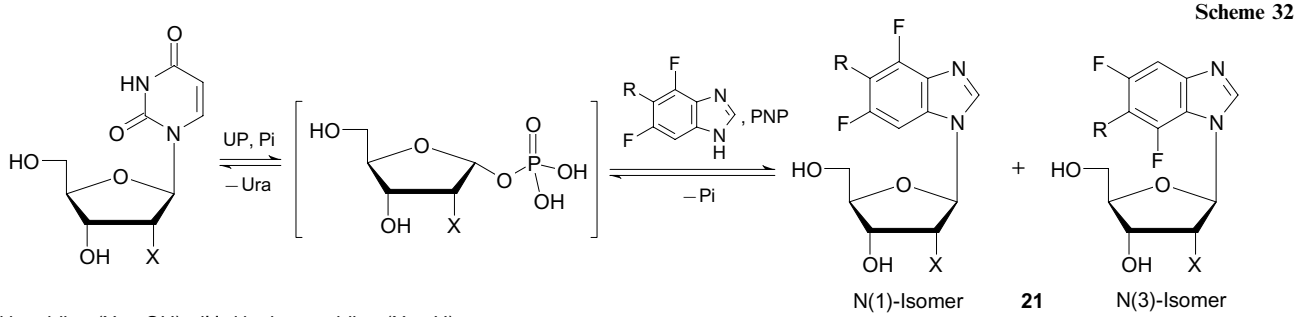
Base	Number	R^1	R^2	Donor	Time /h	Yield (%)	Nucleoside
	**85**	H	H	Guo	23	70	
				dGuo	3.5	90	**114**
	76a	F	F	Guo	24	75	**20a**
				dGuo	2	80	
	38h	Cl	Cl	Guo	3	80	**1a**
				dGuo	0.2	72	**1b**
	118	Me	Me	Guo	1.5	98	
				dGuo	1.5	98	
	119	NO_2	NO_2	Guo	48	32	
				dGuo	4	65	
	109	H	NO_2	Guo	24	75	
				dGuo	2.5	77	
	**120a**	Br	Br	Guo	3	50	
				dGuo	1.5	66	
	120b	NO_2	NO_2	Guo	48	< 1	
				dGuo	24	7	
	120c	NH_2	H	Guo	48	< 1	
				dGuo	24	57	

Scheme 31



X = OH (Rib), H (dRib); B = Ura, Thy

Scheme 32



Urd is uridine (X = OH), dUrd is deoxyuridine (X = H)

Table 4. Synthesis of 6-fluoro-5-substituted 1-(β-D-ribo)- and 1-(2-deoxyribofuranosyl)benzimidazoles **20** using genetically engineered *E. coli* PNPs (see Scheme 31).[48]

R	Carbohydrate moiety	Yield (%)	Isomer content (%)	
			N(1)	N(3)
F	Rib	77	–	–
	dRib	68	–	–
OMe	Rib	54	95	5
	dRib	51	41	59
OEt	Rib	98	89	11
	dRib	85	70	30
OPr^i	Rib	75	100	0
	dRib	65	96	4
Morpholino	Rib	75	100	0
	dRib	80	100	0
N-Methyl-piperazino	Rib	79	100	0
	dRib	68	100	0

Table 5. Synthesis of 4,5,6-trisubstituted 1-(β-D-ribo)- and 1-(2-deoxyribofuranosyl)benzimidazoles **21** using genetically engineered *E. coli* PNPs (see Scheme 32).[49]

R	Carbohydrate moiety	Yield (%)	Isomer content (%)	
			N(1)	N(3)
H	Rib	60	87	13
	dRib	94	88	12
F	Rib	89	90	10
	dRib	65	92	8
OMe	Rib	79	91	9
	dRib	83	94	6
OEt	Rib	70	91	9
	dRib	71	90	10
Morpholino	Rib	83	100	0
	dRib	93	100	0
Pyrrolidino	Rib	61	100	0
	dRib	63	89	11

metrical benzimidazole nucleosides in the active site. The amount of the N(3)-regioisomer in the mixture decreases with increasing bulkiness of the substituents R.

A similar strategy was employed to perform the *E. coli* NP-mediated synthesis of 4,5,6-trisubstituted benzimidazole ribo- and 2′-deoxyribosides **21** (Scheme 32, Table 5)[49] and 2-amino-substituted benzimidazole ribosides and 2′-deoxyribosides (Scheme 33, Table 6).[50] The yields of nucleosides **21** varied from 60% to 94%;[49] the yields of 2-aminobenzimidazole nucleosides **22** and **121** were from 35% to 87%.[50]

It was shown that substituted benzimidazole 2′-deoxy-2′-fluoroarabinosides **27a**–**d** can be prepared using *E. coli* PNP (Scheme 34, Table 7).[56]

It should be noted that N(1)- and N(3)-regioisomers of benzimidazole nucleosides were identified in all these studies,[48–50] like in the chemical glycosylation described by Parsch and Engels.[88] However, N(1)-isomers of trisubstituted benzimidazoles greatly predominated (up to 98% according to NMR spectroscopic data). The amount of N(3)-isomers was, on average, 10%. The exceptions were 5-fluoro-6-methoxybenzimidazole nucleosides[48] and 2-amino-6-fluoro-5-methoxybenzimidazole nucleosides.[50] The synthesis of the latter compounds gave regioisomers in an approximately equal ratio. All benzimidazole nucleosides prepared by the enzymatic method using PNP were isolated from reaction mixtures by reversed-phase column chromatography. The products were eluted with a methanol or acetonitrile gradient in water.[48–50,56]

Scheme 33

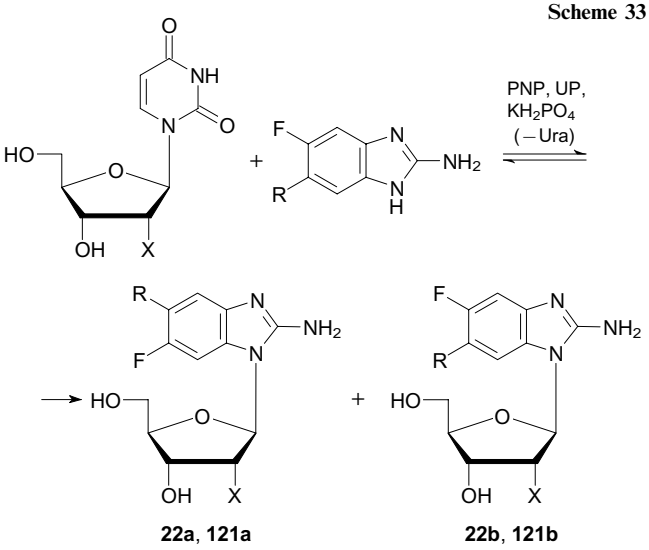
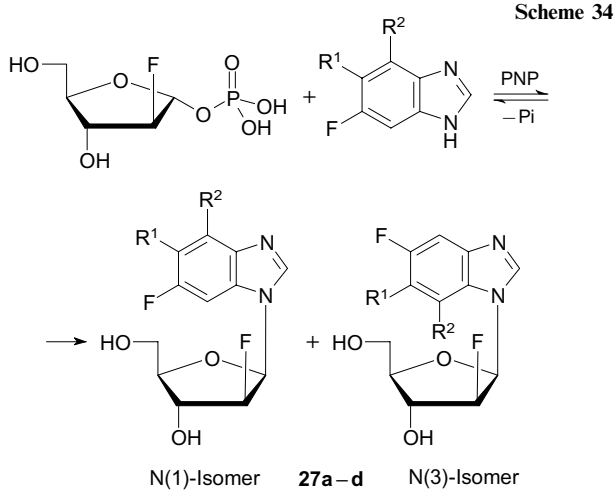


Table 6. Synthesis of 2-amino-substituted 1-(β-D-ribo)- and 1-(2-deoxyribofuranosyl)benzimidazoles **22** and **121** using genetically engineered *E. coli* PNPs (see Scheme 33).[50]

Compound	Carbohydrate moiety	R	Yield (%)	Isomer content (%)	
				N(1)	N(3)
22a	Rib	F	87	–	–
22b	dRib	F	50	–	–
121a	Rib	OMe	50	55	45
121b	dRib	OMe	35	75	25

Scheme 34



Hence, the use of enzymes in the glycosylation step provides a simple and efficient approach to the synthesis of various modified benzimidazole β-D-nucleosides.

* * *

This review shows that benzimidazole nucleosides represent a very interesting and promising class of modified nucleosides having a narrow specificity towards viral targets and high potential for further structural modifications. The elucidation of the mechanism of action in *in vitro* systems and the exact identification of cellular targets will allow researchers to design drugs with high antiviral activity and low systemic toxicity.

Table 7. Synthesis of substituted benzimidazole 2′-deoxy-2′-fluoro-arabinosides **27** using genetically engineered *E. coli* PNPs (see Scheme 34).[56]

Compound	R^1	R^2	Yield (%)	Isomer content (%)	
				N(1)	N(3)
27a	OMe	H	42	60	40
27b	OMe	F	55	96	4
27c	H	F	41	98	2
27d	F	F	40	96	4

Drugs based on these compounds would become indispensable in medical practice for the treatment of recurrent or very severe viral infections and resulting from the long-term use of standard drugs that causes the evolution of drug-resistant virus strains. Therefore, the discovery of new antiviral agents, such as modified benzimidazole nucleosides with an alternative mechanism of action and new targets is a challenging problem.

References

1. K.K.Biron. *Antivir. Res.*, **71**, 154 (2006)
2. E.De Clercq. *Adv. Virus Res.*, **73**, 1 (2009)
3. W.Ramanpreet, M.Hedaitullah, F.N.Syeda, I.Khalid, H.S.Lamba. *Int. J. Res. Pharm. Chem.*, **1**, 565 (2011)
4. B.Narasimhan, D.Sharma, P.Kumar. *Med. Chem. Res.*, **21**, 269 (2012)
5. Y.Geeta, G.Swastika. *Eur. J. Med. Chem.*, **97**, 419 (2015)
6. K.K.Biron, K.S.Gudmundsson, J.C.Drach, in *Antiviral Drugs: From Basic Discovery throught Clinical Trials.* (1st Edn). (Ed. W.M.Kazmierski). (Hoboken, NJ: Wiley, 2011). P. 417
7. K.K.Biron, R.J.Harvey, S.C.Chamberlain, S.S.Good, A.A.Smith III, M.G.Davis. *Antimicrob. Agents Chemother.*, **46**, 2365 (2002)
8. M.N.Prichard, S.L.Frederick, S.Daily, K.Z.Borysko, L.B.Townsend, J.C.Drach, E.R.Kern. *Antimicrob. Agents Chemother.*, **55**, 2442 (2011)
9. S.L.Williams, C.B.Hartline, N.L.Kushner, E.A.Harden, D.J.Bidanset, J.C.Drach, L.B.Townsend. *Antimicrob. Agents Chemother.*, **47**, 2186 (2003)
10. P.M.Krosky, K.Z.Borysko, M.R.Nassiri, R.V.Devivar, R.G.Pink, M.G.Davis, K.K.Biron, L.B.Townsend, J.C.Drach. *Antimicrob. Agents Chemother.*, **46**, 478 (2002)
11. J.S.Hwang, R.Schilf, J.C.Drach, L.B.Townsend, E.Bogner. *Antimicrob. Agents Chemother.*, **53**, 5095 (2009)
12. I.Tamm, K.Folkers, C.H.Shunk, F.L.Horsfall. *J. Exp. Med.*, **99**, 227 (1954)
13. P.Pothier, A.Dru, G.Beaud. *J. Gen. Virol.*, **55**, 87 (1981)
14. L.B.Townsend, R.V.Devivar, S.R.Turk, M.R.Nassiri, J.C.Drach. *J. Med. Chem.*, **38**, 4098 (1995)
15. J.Wang, S.Gong, Y.Fu, Q.Xue, G.Chen, Y.Liu. *J. Huazhong Univ. Sci. Technol.*, **27**, 104 (2007)
16. J.Raaf, E.Brunstein, O.-G.Issinger, K.Niefind. *Chem. Biol.*, **15**, 111 (2008)
17. Z.Kazimierczuk, L.Dudyu, R.Stolarski, D.Shugar. *Z. Naturforsch., Sect. C*, **35**, 30 (1980)
18. I.Tamm, P.B.Sehgal. *Biochem. Pharmacol.*, **27**, 2475 (1978)

19. H.M.Kissman, R.G.Child, M.J.Weiss. *J. Am. Chem. Soc.*, **79**, 1185 (1957)
20. A.Diwan, C.N.Gowdy, R.K.Robins, W.H.Prusoff. *J. Gen. Virol.*, **3**, 393 (1968)
21. G.Gosselin, C.Perigaud, M.-C.Bergogne, J.Balzarini, E.De Clercq, J.-L.Imbach. *Antiviral Chem. Chemother.*, **5**, 243 (1994)
22. R.Zou, K.R.Ayres, J.C.Drach, L.B.Townsend. *J. Med. Chem.*, **39**, 3477 (1996)
23. C.M.Smith, G.Zombor, J.F.Henderson. *Cancer Treat. Rep.*, **60**, 1567 (1976)
24. M.A.McVoy, D.E.Nixon. *J. Virol.*, **79**, 11115 (2005)
25. E.De Clercq. *J. Antimicrob. Chemother.*, **51**, 1079 (2003)
26. B.Pierce, C.L.Richardson, L.Lacloche, A.Allen, M.G.Ison. *Transplant. Infect. Dis.*, **20**, 12852 (2018)
27. M.Ogata, K.Takano, Y.Moriuchi, T.Kondo, T.Ueki, N.Nakano, T.Mori, N.Uoshima, K.Nagafuji, S.Yamasaki. *Biol. Blood Marrow Transplant.*, **24**, 1264 (2018)
28. M.K.Khukhanova, A.N.Korovina, S.N.Kochetkova. *Usp. Biol. Khim.*, **54**, 457 (2014)
29. D.M.Coen, P.A.Schaffer. *Nat. Rev. Drug Discov.*, **2**, 278 (2003)
30. E.De Clercq, H.J.Field. *Br. J. Pharmacol.*, **147**, 1 (2006)
31. T.W.North, G.Sequar, L.B.Townsend, J.C.Drach, P.A.Barry. *Antimicrob. Agents Chemother.*, **48**, 2760 (2004)
32. M.R.Underwood, R.G.Ferris, D.W.Selleseth, M.G.Davis, J.C.Drach, L.B.Townsend, K.K.Biron, F.L.Boyd. *Antimicrob. Agents Chemother.*, **48**, 1647 (2004)
33. R.Zou, E.Kawashima, G.A.Freeman, G.W.Koszalka, J.C.Drach, L.B.Townsend. *Nucleosides, Nucleotides Nucleic Acids*, **19**, 125 (2000)
34. P.L.Lorenzi, C.P.Landowski, A.Brancale, X.Song, L.B.Townsend, J.C.Drach, G.L.Amidon. *Drug Metab. Dispos.: Biol. Fate Chem.*, **34**, 1070 (2006)
35. R.Zou, J.C.Drach, L.B.Townsend. *J. Med. Chem.*, **40**, 802 (1997)
36. R.Zou, J.C.Drach, L.B.Townsend. *J. Med. Chem.*, **40**, 811 (1997)
37. Patent US 20120283210 (2012)
38. E.R.Kern, C.B.Hartline, R.J.Rybak, J.C.Drach, L.B.Townsend, K.K.Biron, D.J.Bidanset. *Antimicrob. Agents Chemother.*, **48**, 1749 (2004)
39. K.K.Biron. In *New Concepts of Antiviral Therapy*. (Eds E.Bogner, A.Holzenburg). (Dordrecht: Springer, 2006). P. 309
40. F.-Z.Wang, D.Roy, E.Gershburg, C.B.Whitehurst, D.P. Dittmer, J.S.Pagano. *J. Virol.*, **83**, 12108 (2009)
41. N.B.Price, M.N.Prichard. *Curr. Opin. Virol.*, **1**, 548 (2011)
42. *Drugs R&D*, **8**, 188 (2007); doi: 10.2165/00126839-200708030-00006
43. Patent US 8940707 (2015)
44. S.Budow, M.Kozlowska, A.Gorska, Z.Kazimierczuk, H.Eickmeier, P.Colla, G.Gosselin, F.Seela. *ARKIVOC*, (iii), 225 (2009)
45. P.Zień, M.Bretner, K.Zastapilo, R.Szyszka, D.Shugar. *Biochem. Biophys. Res. Commun.*, **306**, 129 (2003)
46. A.Zivkovic, J.W.Engels. *Nucleosides, Nucleotides Nucleic Acids*, **24**, 1023 (2005)
47. G.N.Lipunova, E.V.Nosova, V.N.Charushin. *Chem. Heterocycl. Compd.*, **50**, 764 (2014)
48. I.D.Konstantinova, O.M.Selezneva, I.V.Fateev, T.A.Balashova, S.K.Kotovskaya, Z.M.Baskakova, V.N.Charushin, A.V.Baranovsky, A.I.Miroshnikov, J.Balzarini, I.A.Mikhailopulo. *Synthesis*, **45**, 272 (2013)
49. M.I.Kharitonova, I.V.Fateev, A.L.Kaushin, I.D.Konstantinova, S.K.Kotovskaya, V.L.Leont'eva, G.A.Galegov, V.N.Charushin, A.I.Miroshnikov. *Synthesis*, **48**, 394 (2016)
50. M.I.Kharitonova, A.O.Denisova, V.L.Àndronova, A.L.Kayushin, I.D.Konstantinova, S.K.Kotovskaya, G.A.Galegov, V.N.Charushin, A.I.Miroshnikov. *Bioorg. Med. Chem. Lett.*, **27**, 2484 (2017)
51. J.-S.Hwang, R.Schilf, J.C.Drach, L.B.Townsend, E.Bogner. *Antimicrob. Agents Chemother.*, **12**, 5095 (2009)
52. A.Dittmer, I.Woskobojnik, R.Adfeldt, J.C.Drach, L.B.Townsend, S.Voigt, E.Bogner. *Antiviral Res.*, **137**, 102 (2017)
53. K.S.Gudmundsson, J.C.Drach, L.L.Wotring, L.B.Townsend. *J. Med. Chem.*, **40**, 785 (1997)
54. L.B.Townsend, K.S.Gudmundsson, S.M.Daluge, J.J.Chen, Z.Zhu, G.W.Koszalka, L.Boyd, S.D.Chamberlain, G.A.Freeman, K.K.Biron, J.C.Drach. *Nucleosides Nucleotides*, **18**, 509 (1999)
55. Patent US 5840743 (1998)
56. M.I.Kharitonova, I.V.Fateev, K.V.Antonov, A.L.Kaushin, I.D.Konstantinova, S.K.Kotovskaya, V.L.Andronova, G.A.Galegov, V.N.Charushin, A.I.Miroshnikov. *Synthesis*, **49**, 1043 (2017)
57. Q.Ji, J.Li, F.Ding, J.Han, M.Pang, S.Liu, J.Meng. *Tetrahedron*, **62**, 2529 (2006)
58. J.Shelton, X.Lu, J.A.Hollenbaugh, J.H.Cho, F.Amblard, R.F.Schinazi. *Chem. Rev.*, **116**, 14379 (2016)
59. M.Urban, N.Joubert, M.Hocek, R.E.Alexander, R.D.Kuchta. *Biochemistry*, **48**, 10866 (2009)
60. G.Cozza, S.Sarno, M.Ruzzene, C.Girardi, A.Orzeszko, Z.Kazimierczuk, G.Zagotto, E.Bonaiuto, M.L.DiPaolo, L.A.Pinna. *Biochim. Biophys. Acta – Proteins Proteomics*, **1834**, 1402 (2013)
61. P.M.Krosky, M.R.Underwood, S.R.Turk, K.W.Feng, R.K.Jain, R.G.Ptak, A.C.Westerman, K.K.Biron, L.B.Townsend, J.C.Drach. *J. Virol.*, **72**, 4721 (1998)
62. E.Bogner. *Rev. Med. Virol.*, **12**, 115 (2002)
63. G.Komazin, L.B.Townsend, J.C.Drach. *J. Virol.*, **78**, 710 (2004)
64. A.Dittmer, J.C.Drach, L.B.Townsend, A.Fischer, E.Bogner. *J. Virol.*, **79**, 14660 (2005)
65. S.Chou, L.C.Van Wechel, G.I.Marousek. *J. Infect. Dis.*, **196**, 91 (2007)
66. M.N.Prichard. *Rev. Med. Virol.*, **19**, 215 (2009)
67. J.M.Reitsma, J.P.Savaryn, K.Faust, H.Sato, B.D.Halligan, S.S.Terkune. *Cell Host Microbe*, **9**, 103 (2011)
68. D.G.Wolf, C.T.Courcelle, M.N.Prichard, E.S.Mocarski. *Proc. Natl. Acad. Sci. USA*, **98**, 1895 (2001)
69. M.R.Boyd, T.H.Bacon, D.Sutton, M.Cole. *Antimicrob. Agents Chemother.*, **31**, 1238 (1987)
70. B.Oberg. *Pharmacol. Ther.*, **40**, 213 (1989)
71. A.Canestri, J.Ghosn, M.Wirden, F.Marguet, N.Ktorza, I.Boubezari, S.Dominguez, P.Bossi, E.Caumes, V.Calvez, C.Katlama. *Antivir. Ther.*, **11**, 561 (2006)
72. Z.Zhao, K.-W.Tang, I.Muylaert, T.Samuelsson, P.Elias. *J. Biol. Chem.*, **292**, 15489 (2017)
73. F.Weygand, A.Wacker, F.Wirth. *Z. Naturforsch., Sect. B*, **6**, 25 (1951)
74. I.A.Mikhailopulo, Z.Kazimierczuk, A.I.Zinchenko, V.N.Barai, V.V.Romanova, L.A.Eroshevskaya. *Nucleosides Nucleotides*, **14**, 477 (1995)
75. H.Vorbrüggen, C.Ruh-Pohlenz. *Org. React.*, **55**, 1 (1999)
76. M.H.Zhong, I.Nowak, M.Robins. *Org. Lett.*, **7**, 4601 (2005)
77. X.G.Zhang, H.R.Xia, X.C.Dong, J.Jin, W.-D.Meng, F.-L.Qing. *J. Org. Chem.*, **68**, 9026 (2003)
78. T.Mochizuki, Y.Kondo, H.Abe, S.C.Tovey, S.G.Dedos, C.W.Taylor, M.Paul, B.V.L.Potter, A.Matsuda, S.Shuto. *J. Med. Chem.*, **49**, 5750 (2006)
79. B.C.Bookser, N.B.Raffaele. *J. Org. Chem.*, **72**, 173 (2007)
80. J.Boryski. *Curr. Org. Chem.*, **12**, 309 (2008)

81. J.P.Henschke, X.Zhang, X.Huang, L.Mei, G.Chu, K.Hu, Q.Wang, G.Zhu, M.Wu, C.Kuo. *Org. Proc. Res. Dev.*, **17**, 1419 (2013)
82. K.Ruf, W.Pfleiderer. *Carbohydr. Res.*, **216**, 421 (1991)
83. R.Rastogi, S.Sharma. *Synthesis*, 861 (1983)
84. J.R.Homer, D.W.Henry. *J. Med. Chem.*, **11**, 946 (1968)
85. H.Vorbrüggen, K.Krolikiewicz, B.Bennua. *Chem. Ber.*, **114**, 1234 (1981)
86. R.V.Devivar, J.C.Drach, L.B.Townsend. *Bioorg. Med. Chem. Lett.*, **2**, 1105 (1992)
87. R.L.Clark, A.A.Pessolano. *J. Am. Chem. Soc.*, **80**, 1657 (1958)
88. J.Parsch, J.W.Engels. *Helv. Chim. Acta*, **83**, 1791 (2000)
89. L.M.Break. *Int. J. Chem.*, **8**, 188 (2016)
90. F.Seela, W.Bourgeois, H.Rosemeyer, T.Wenzel. *Helv. Chim. Acta*, **79**, 488 (1996)
91. S.Sinha, R.Srivastava, E.DeClercq, R.K.Singh. *Nucleosides, Nucleotides Nucleic Acids*, **23**, 1815 (2004)
92. H.Wojtowicz-Rajchel. *J. Fluorine Chem.*, **143**, 11 (2012)
93. Z.Kazimierczuk, R.Stolarski, D.Z.Shugar. *Z. Naturforsch., Sect. C*, **40**, 715 (1985)
94. J.-L.Girardet, J.C.Drach, S.D.Chamberlain, G.W.Koslzalka, L.B.Townsend. *Nucleosides Nucleotides*, **17**, 2389 (1998)
95. Patent WO 1999051619 (1999)
96. Patent US 5360795 (1994)
97. K.W.Pankiewicz, J.Krzeminski, L.A.Ciszewski, W.Y.Ren, K.A.Watanabe. *J. Org. Chem.*, **57**, 553 (1992)
98. J.Krezeminski, B.Nawrot, K.W.Pankiewicz, K.A.Watanabe. *Nucleosides Nucleotides*, **19**, 781 (1991)
99. K.S.Gudmundsson, G.A.Freeman, J.C.Drach, L.B.Townsend. *J. Med. Chem.*, **43**, 2473 (2000)
100. K.W.Pankiewicz. *Carbohydr. Res.*, **327**, 87 (2000)
101. F.Seela, P.Chittepu. *Org. Biomol. Chem.*, **6**, 596 (2008)
102. H.G.Howell, P.R.Brodfuehrer, S.P.Brundige, D.A.Benigne, C.J.Sapino. *J. Org. Chem.*, **53**, 85 (1988)
103. J.A.Montgomery, A.T.Shortnacy, D.A.Carson, J.A.Secrist III. *J. Med. Chem.*, **29**, 2389 (1986)
104. A.Holy, J.Konig, J.Vesely, D.Cech, I.Votruba, E.De Clercq. *Collect. Czech. Chem. Commun.*, **52**, 1589 (1987)
105. H.Vorbruggen, G.Hofle. *Chem. Ber.*, **114**, 1256 (1981)
106. S.Hanessian, M.Kagotani, K.Komaglou. *Heterocycles*, **28**, 1115 (1989)
107. B.Classon, Z.Liu. *J. Org. Chem.*, **53**, 6126 (1988)
108. J.A.Montgomery, A.T.Shortnacy, H.J.Thomas. *J. Med. Chem.*, **17**, 1197 (1974)
109. T.A.Kanazawa. *Nippon Kagaku Zasshi (J. Chem. Soc. Jpn.)*, **81**, 516 (1960)
110. Patent US 6204249 (2001)
111. Patent US 7858773 (2010)
112. M.J.Lapponi, C.W.Rivero, M.A.Zinni, C.N.Britos, J.A.Trelles. *J. Mol. Catal. B: Enzym.*, **133**, 218 (2016)
113. J.Fernandez-Lucas, L.A.Condezo, F.Martinez-Lagos, J.V.Sinisterra. *Enzyme Microb. Technol.*, **40**, 1147 (2007)
114. A.Fresco-Taboada, I.de la Mata, M.Arroyo, J.Fernandez-Lucas. *Appl. Microbiol. Biotechnol.*, **97**, 3773 (2013)
115. P.A.Kaminski, P.Dacher, L.Dugué, S.Pochet. *J. Biol. Chem.*, **283**, 20053 (2008)
116. W.Ye, D.Paul, L.Gao, J.Seckute, R.Sangaiah, K.Jayaraj, Z.Zhang, P.A.Kaminski, S.E.Ealick, A.Gold, L.M.Ball. *PLoS One*, **9**, e115082 (2014)
117. R.G.Silva, V.L.Schramm. *Biochemistry*, **50**, 9158 (2011)
118. S.Xiong, Y.Wang, X.Wang, J.Wang, J.Li, G.Zhang, R.Zhang, L.Xie, H.Wang. *J. Biosci. Bioeng.*, **118**, 723 (2014)
119. L.Wang, S.R.Schmidl, J.Stuelke. *Nucleosides, Nucleotides Nucleic Acids*, **33**, 296 (2014)
120. I.Serra, T.Bavaro, D.Cecchini, S.Daly, A.Albertini, M.Terreni, D.Ubiali. *J. Mol. Catal. B: Enzym.*, **95**, 16 (2013)
121. L.F.Timmers, R.A.Caceres, L.A.Basso, D.S.Santos, W.F.De Azevedo. *Protein Pept. Lett.*, **15**, 843 (2008)
122. I.V.Fateev, K.V.Antonov, I.D.Konstantinova, T.I.Muravyova, F.Seela, R.S.Esipov, A.I.Miroshnikov, I.A.Mikhailopulo. *Beilstein J. Org. Chem.*, **10**, 1657 (2014)
123. B.Wielgus-Kutrowska, K.Breer, M.Hashimoto, S.Hikishima, T.Yokomatsu, M.Narczyk, A.Dyzma, A.Girstun, K.Staro, A.Bzowska. *Bioorg. Med. Chem.*, **20**, 6758 (2012)
124. J.Holguin, R.Cardinaud, C.A.Salemink. *Eur. J. Biochem.*, **54**, 515 (1975)
125. J.Fernández-Lucas, A.Fresco-Taboada, C.Acebal, I.de la Mata, M.Arroyo. *Appl. Microbiol. Biotechnol.*, **91**, 317 (2011)
126. J.Fernández-Lucas, C.Acebal, J.V.Sinisterra, M.Arroyo, I.de la Mata. *Appl. Environ. Microbiol.*, **76**, 1462 (2010)
127. J.Fernández-Lucas. *Appl. Microbiol. Biotechnol.*, **99**, 4615 (2015)
128. R.Anand, P.A.Kaminski, S.E.Ealick. *Biochemistry*, **43**, 2384 (2004)
129. Y.Miyamoto, T.Masaki, S.Chohnan. *Biochim. Biophys. Acta – Proteins Proteomics*, 1774, 1323 (2007)
130. D.J.T.Porter, S.A.Short. *J. Biol. Chem.*, **270**, 15557 (1995)
131. P.A.Kaminski, G.Labesse. *J. Biol. Chem.*, **288**, 6534 (2013)
132. D.A.Carson, D.B.Wasson, E.Beutler. *Proc. Natl. Acad. Sci. USA*, **81**, 2232 (1984)
133. D.A.Carson, D.B.Wasson. *Biochem. Biophys. Res. Commun.*, **155**, 829 (1988)
134. T.Haertle, C.J.Carrera, D.B.Wasson, L.C.Sowers, D.D.Richman, D.A.Carson. *J. Biol. Chem.*, **263**, 5870 (1988)
135. D.W.Hutchinson. *TIBTECH*, **8**, 348 (1990)
136. N.Crespo, P.A.Sánchez-Murcia, F.Gago, J.Cejudo-Sanches, M.A.Galmes, J.Fernández-Lucas, J.M.Mancheco. *Appl. Microbiol. Biotechnol.*, **101**, 7187 (2017)
137. C.N.Britos, M.J.Lapponi, V.A.Cappa, C.W.Rivero, J.A.Trelles. *J. Fluorine Chem.*, **186**, 91 (2016)
138. D.Betbeder, D.W.Hutchinson. *Nucleosides Nucleotides*, **9**, 569 (1990)
139. E.Pistotnik, H.Sakamoto, S.Pochet, A.Namane, O.Barzu. *Anal. Biochem.*, **271**, 192 (1999)
140. D.J.Steenkamp. *Eur. J. Biochem.*, **197**, 431 (1991)
141. D.J.Steenkamp, T.J.Halbich. *Biochem. J.*, **287**, 125 (1992)
142. J.Bosch, M.A.Robien, C.Mehlin, E.Boni, A.Riechers, F.S.Buckner, W.C.Van Voorhis, P.J.Müler, E.A.Worthey, G.DeTitta, J.R.Luft, A.Lauricella, S.Gulde, L.A.Anderson, O.Kalyuzhniy, H.M.Neely, J.Ross, T.N.Earnest, M.Soltis, L.Schoenfeld, F.Zucker, E.A.Merritt, E.Fan, C.L.Verlinde, W.G.Hol. *J. Med. Chem.*, **49**, 5939 (2006)
143. K.A.Lawrence, M.W.Jewett, P.A.Rosa, F.C.Gherardini. *Mol. Microbiol.*, **72**, 1517 (2009)
144. C.S.Alexeev, G.G.Sivets, T.N.Safonova, S.N.Mikhailov. *Nucleosides, Nucleotides Nucleic Acids*, **36**, 107 (2017)
145. I.A.Mikhailopulo, A.I.Miroshnikov. *Acta Naturae*, **2**, 36 (2010)
146. I.A.Mikhailopulo, A.I.Miroshnikov. *Mendeleev Commun.*, **21**, 57 (2011)
147. J.C.Lelr, K.Hammer-Jespersen, M.Schwartz. *Eur. J. Biochem.*, **75**, 217 (1977)
148. E.Lewkowicz, A.Iribarren. *Curr. Org. Chem.*, **10**, 1197(2006)
149. A.Fresco-Taboada, I.Serra, M.Arroyo, J.Fernández-Lucas, I.de la Mata, M.Terreni. *Catal. Today*, **259**, 197 (2015)
150. A.Bzowska, E.Kulikowska, D.Shugar. *Pharmacol. Ther.*, **88**, 349 (2000)
151. J.A.Montgomery. *Med. Res. Rev.*, **13**, 209 (1993)
152. G.Koellner, A.Bzowska, B.Wielgus-Kutrowska, M.Luic, T.Steiner, W.Saenger, J.Stepinski. *J. Mol. Biol.*, **315**, 35 (2002)
153. G.Mikleusević, Z.Stefanić, M.Narczyk, B.Wielgus-Kutrowska, A.Bzowska, M.Luić. *Biochimie*, **93**, 1610 (2011)
154. S.A.Taran, K.N.Verevkina, S.A.Feofanov, A.I.Miroshnikov. *Russ. J. Bioorg. Chem.*, **35**, 739 (2009)

155. C.W.Rivero, E.C.DeBenedetti, J.E.Sambeth, M.E.Lozano, J.A.Trelles. *Bioorg. Med. Chem. Lett.*, **22**, 6059 (2012)
156. W.-G.Chae, D.C.K.Chan, C.Chang. *Tetrahedron*, **54**, 8661 (1998)
157. W.R.Birmingham, C.A.Starbird, T.D.Panosian, D.P.Nannemann, T.Iverson, B.O.Bachmann. *Nat. Chem. Biol.*, **10**, 392 (2014)
158. A.O.Denisova, Yu.A.Tokunova, I.V.Fateev, A.A.Breslav, V.N.Leonov, E.V.Dorofeeva, O.I.Lutonina, I.S.Muzyka, R.S.Esipov, A.L.Kayushin, I.D.Konstantinova, A.I.Miroshnikov, V.A.Stepchenko, I.A.Mikhailopulo. *Synthesis*, **49**, 4853 (2017)
159. A.Bzowska, E.Kulikowska, D.Shugar. *Z. Naturforsch., Sect. B*, **45**, 59 (1990)
160. M.O.Oivanen, H.Lonnberg, Z.Kazimierczuk, D.Shugar. *Nucleosides Nucleotides*, **8**, 133 (1989)