Chemo-Enzymatic Synthesis and Biological Evaluation of 5,6-Disubstituted Benzimidazole Ribo- and 2′-Deoxyribonucleosides

Irina D. Konstantinova,[a] Olga M. Selezneva,[a] Ilja V. Fateev,[a] Tamara A. Balashova,[a] Svetlana K. Kotovskaya,[b] Zoya M. Baskakova,[b] Valery N. Charushin,[b] Alexander V. Baranovsky,[c] Anatoly I. Miroshnikov,[a] Jan Balzarini,*[d] Igor A. Mikhailopulo*[c]

[a] Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya 16/10, 117997 GSP, Moscow B-437, Russian Federation

[b] I. Ya. Postovsky Institute of Organic Synthesis, the Ural Branch of the Russian Academy of Sciences, Sophia Kovalevskaya/Academicheskaya St. 22/20, 620041 Yekaterinburg, Russian Federation

[c] Institute of Bioorganic Chemistry, National Academy of Sciences, Acad. Kuprevicha 5/2, 220141 Minsk, Belarus
Fax +375(17)2678761; E-mail: imikhailopulo@gmail.com

[d] Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

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Abstract: A number of new 5,6-disubstituted benzimidazoles have been prepared and their substrate properties for recombinant *E. coli* purine nucleoside phosphorylase (PNP; the product of the *deoD* gene) in the transglycosylation reaction were investigated. The heterocyclic bases showed good substrate activity for PNP and the ribo- and 2′-deoxyribonucleosides were synthesized. The predominant (OMe and OEt) or exclusive (O*i*-Pr, morpholino, and *N*-methylpiperazino) formation of the 5-substituted 6-fluoro-1-(β-D-ribofuranosyl)benzimidazoles was observed. The formation of the regioisomeric 6- methoxy-, 6-ethoxy-, or 6-isopropoxy-substituted 1-(2-deoxy-β-D-ribofuranosyl)-5-fluorobenzimidazoles was observed in the *trans*-2-deoxyribosylation reaction of the corresponding bases. The predominant or exclusive formation of the regioisomeric N^1-nucleosides with bulky 5-substituents of 6-fluorobenzimidazole points to a large hydrophobic pocket in the *E. coli* PNP active site that can accommodate these groups. The biological activity of the synthesized nucleosides was studied and revealed no inhibitory activity against a broad variety of DNA and RNA viruses. The compounds also lacked significant cytotoxicity.

Key words: 5,6-disubstituted benzimidazoles, purine nucleoside phosphorylase, substrate properties, transglycosylation reaction, nucleosides

Halogenated benzimidazole-based nucleosides have attracted much attention since the pioneering studies by Tamm and co-workers initiated in the early 1950s.[1] However, the most important findings from the viewpoint of the biochemical mechanism of antiviral activity of this class of modified nucleosides[2] as well as their possible practical application[3] were published over the last two decades.[4,5]

It was earlier shown that benzimidazole and its derivatives with substituents in the benzene ring are good substrates of *E. coli* PNP in transglycosylation reactions.[6–10] In the present study, we report on the synthesis of 5,6-difluorobenzimidazole and its derivatives, where one fluorine atom is replaced by a methoxy, ethoxy, isopropoxy, morpholino, or *N*-methylpiperazino group, and their use as acceptors of D-ribofuranose and 2-deoxy-D-ribofuranose residues in transglycosylation reactions. Uridine (or 2′-deoxyuridine) and thymidine are employed as the pentofuranose donors and recombinant *E. coli* uridine (UP; EC 2.4.2.3), thymidine (TP; 2.4.2.4), and purine (PNP; product of *deoD* gene; EC 2.4.2.1) nucleoside phosphorylases[11] were used as the biocatalysts (see Scheme 2).

Synthesis of 5,6-difluoro-1*H*-benzimidazole (**1**) was realized from 4,5-difluoro-2-nitroaniline (**2**)[12] by the catalytic reduction of the nitro group (H_2, Raney Ni),[13] followed by the well-documented imidazole ring-closure reaction by treatment with triethyl orthoformate. It was found that fluorine atoms of difluoride **1** are very resistant to nucleophilic substitution and attempts to replace one of them by an alkoxy group failed. Therefore, the sequence of reactions depicted in Scheme 1 was used to synthesize monofluorides **5**–**9** starting from the difluoride **2**. The physicochemical data for compounds **1**, **5**–**9** are given in the experimental section and Table 2.

The transglycosylation reaction was employed for the synthesis of β-D-ribo- and 2-deoxy-β-D-ribonucleosides **10**–**21** (Scheme 2). The reaction conditions were optimized depending on the ribofuranose donor, the donor/base ratio, quantity of the recombinant *E. coli* enzymes, and temperature of the reaction. The use of readily available natural purine ribonucleosides as donors of the ribofuranose residue in the transglycosylation reaction of benzimidazoles allows PNP to be employed as the sole biocatalyst. In contrast, the use of uridine (2′-deoxyuridine) or thymidine as donors of the pentofuranose residues requires two nucleoside phosphorylases for transglycosylation reactions, viz., recombinant *E. coli* UP or TP for the intermediary formation of α-D-ribofuranose 1-phosphate (α-D-Rib-1P) or 2-deoxy-α-D-ribofuranose 1-phosphate (α-D-dRib-1P) that is accepted by *E. coli* PNP for the synthesis of the benzimidazole nucleosides. The efficiency of natural purine vs. pyrimidine ribonucleosides as donors was tested in the transglycosylation of the difluoride **1** and it was found that the use of uridine (2′-deoxyuridine) or thymidine and the respective UP or TP

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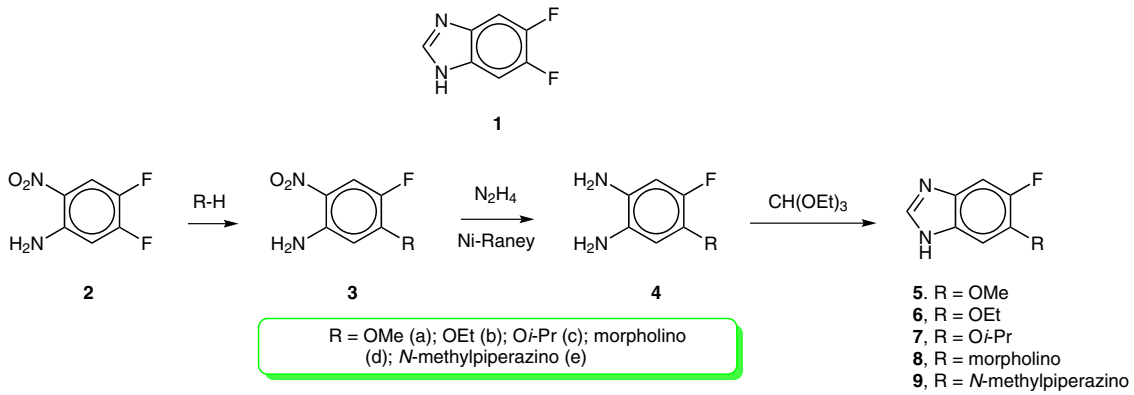
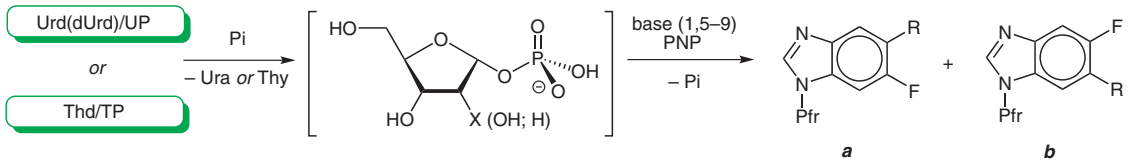


Scheme 1 Structure of 5,6-difluoro-1*H*-benzimidazole (**1**) and synthesis of 6(5)-substituted 5(6)-fluorobenzimidazoles **5**–**9**



Scheme 2 Enzymatic synthesis of 5,6-disubstituted benzimidazole ribo- and 2′-deoxyribo-nucleosides **10**–**21**

nucleoside phosphorylases in combination with PNP is preferable in terms of yield of the desired nucleosides (see, e.g., Figure 1).

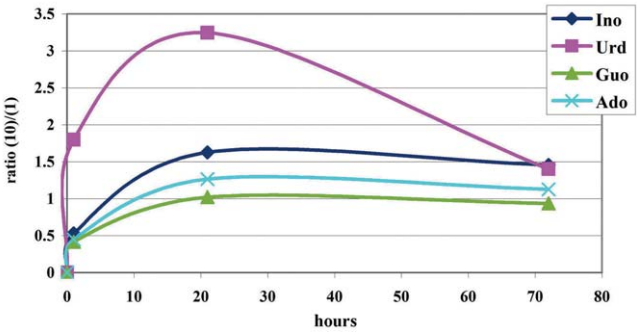


Figure 1 Progress of the synthesis of the riboside **10** depending on the type of D-ribofuranose donor (Ino = inosine; Urd = uridine; Guo = guanosine; Ado = adenosine)

Furthermore, the reaction conditions were optimized with regard to the donor/base ratio, quantity of the recombinant *E. coli* enzymes, and temperature of the reaction mixture. It was found that the 3:1 to 10:1 molar donor/base ratio and the use of 40 units of uridine phosphorylase (UP) per 1 mmol of uridine (60–160 UP units for 2′-deoxyuridine) and 155–400 units of PNP afforded the ribosides **10**–**21** in good yields calculated for the isolated products. In a similar way, thymidine was used for the synthesis of 2′-deoxyribosides **19** and **21** as a donor of the 2-deoxy-D-ribofuranose residue and thymidine phosphorylase (TP) for the generation of the intermediate 2-deoxy-α-D-ribofuranose 1-phosphate (Table 1, for experimental data on the enzymatic synthesis see Table 3, and physico-chemical properties of nucleosides synthesized, see Tables 4 and 5). Reactions were conducted at 52 °C in potassium phosphate buffer (5–20 mM; pH 7.0) monitoring the formation of the products by HPLC. The conversion of base into nucleoside(s) was ≥98.4%. Compounds **10** and **11** were crystallized directly from the reaction mixtures, and the other nucleosides were isolated by C18 RP silica gel column chromatography. It is noteworthy that the synthesis of 2′-deoxyribosides was completed in 1–3 hours, whereas the transribosylation reaction proceeded more slowly and required 22–28 hours to achieve high yields of products (Table 3). A similar trend was earlier observed in the case of the ribo- and 2′-deoxyribonucleoside synthesized using whole *E. coli* cells as the biocatalyst.[7]

The regioisomeric structure of all isolated nucleosides was proven by scrupulous analysis of the ^{1}H and ^{13}C NMR spectral data (including [^{1}H,^{1}H] and [^{1}H,^{13}C] 2D: COSY, HSQC, HMBC, and NOE spectra) (see Table 5).

Thus, for example in the [^{1}H,^{1}H] NOESY spectrum of the riboside **18a**, methylene $N(CH_2)_2$ protons (δ = 2.98) showed strong interaction with the proton at C4 (δ = 7.31, d), whereas the proton at C7 (δ = 7.70, d) interacted with the H2′, H1′, and H5′ protons (δ = 4.30, 5.79, and 3.65, respectively) (Figure 2). These observations allow the former doublet to be unequivocally assigned to H4 [$^{4}J_{H4,F6}$ = 7.9 Hz; C4 δ = 108.92 (s, $^{3}J_{C4,F6}$ < 1.0 Hz)] and the latter to H7 [$^{3}J_{H7,F6}$ = 12.4 Hz; C7 δ = (99.19, d, $^{2}J_{C7,F6}$ = 27.9 Hz)], as well as the position of the fluorine atom at C6. Moreover, the values of the *ortho* F,H7 ($^{3}J_{H7,F6}$ = 12.4 Hz) and

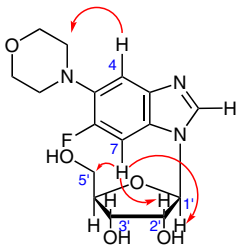
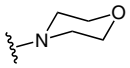
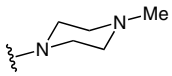


Figure 2 The observed NOE effects of protons H4 and H7 of the riboside **18a**

meta F,H4 ($^4J_{H4,F6}$ = 7.94 Hz) couplings are in favor of the suggested structure **18a** (cf. ref.[14]). The ^{13}C resonances of C4 and C7 carbon atoms arise from the [^1H,^{13}C] 2D spectra. A similar approach was used for the assignment of the structures of other nucleosides.

Table 1 Yields of the Isomeric Nucleosides

Compd	Pfr[a]	R	Yield[b] (%)	Isomer ratio	
				a	**b**
10	Rib	F	77	–	–
11	dRib		68	–	–
12	Rib	OMe	54	95	5
13	dRib		51	41	59
14	Rib	OEt	98	89	11
15	dRib		85	70	30
16	Rib	O*i*-Pr	75	100	–
17	dRib		65	96	4
18	Rib		75	100	–
19	dRib		80	100	–
20	Rib		79	100	–
21	dRib		68	100	–

[a] Pfr = β-D-pentofuranosyl (Rib = β-D-ribofuranosyl; dRib = 2′-deoxy-β-D-ribofuranosyl).
[b] Isolated yield.

The regioselectivity of the transglycosylation reaction in the case of bases **7**, **8**, and **9** is of interest from the viewpoint of the structure of the catalytic site of *E. coli* PNP. It is obvious that the bulkiness of the respective isopropoxy, morpholino, and *N*-methylpiperazino substituents is the main reason for the strict regioselectivity observed.

The predominant or exclusive formation of the regioisomeric N^1-nucleosides **16**–**21** with bulky 5-substituents on 6-fluorobenzimidazole points to a large hydrophobic pocket allowing the respective bases to bind in the *E. coli* PNP catalytic site and to realize the coupling of base and pentofuranose. We have previously shown that *E. coli* PNP accepts kinetin, N^6-benzoyl- and N^6-benzyl-adenines as well as N^2-acetyl-O^6-methyl- and N^2-acetyl-O^6-benzyl-guanines as substrates in the transglycosylation reactions.[15] In contrast, *E. coli* PNP cannot tolerate guanine derivatives with such bulky and voluminous functions as N^2-palmitoyl and O^6-diphenylcarbamoyl. More recently, we reported the use of N^2-acetyl-O^6-[2-(4-nitrophenyl)ethyl]-guanine as a good substrate for *E. coli* PNP in transglycosylation reactions.[16] These data taken together enable one to visualize the spatial dimensions of a hydrophobic pocket in vicinity of the C^6–N^1–C^2 segment of the pyrimidine ring of purines and the C5 atom of benzimidazole derivatives in the *E. coli* PNP active site that can accommodate these groups (Figure 3).

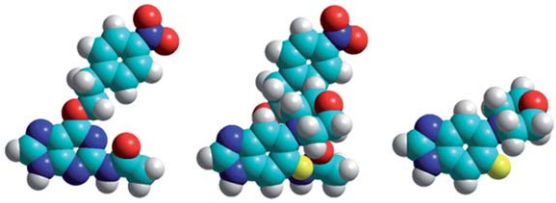


Figure 3 The geometry optimized (HyperChem, 8.1; the *ab initio* method, in vacuo, basis set; medium 6-31G* level) structures of N^2-acetyl-O^6-[2-(4-nitrophenyl)ethyl]guanine (left), 5-morpholino-6-fluoro-1*H*-benzimidazole (right), and an overlay of the imidazole fragments of both structures (middle)

Remarkably, the pocket in the *E. coli* PNP catalytic site exceeds the dimension of the purine base and allows a minimal contribution of the C^6 amino/carbonyl groups and N^1 nitrogen atom to the substrate binding to be suggested.

None of the compounds were cytostatic in cell culture, and they also did not show significant inhibitory activity against the replication of a broad variety of DNA and RNA viruses.

In conclusion, the efficient enzymatic method for the preparation of the 5-substituted 6-fluoro-1-(β-D-ribofuranosyl)- and 1-(2-deoxy-β-D-ribofuranosyl)-6-fluorobenzimidazoles is described. 5,6-Difluoro-1*H*-benzimidazole (**1**)[12,13] was found to be inert to nucleophilic substitution of a fluorine atom and another sequence of chemical transformations was, therefore, used for the synthesis of the desired heterocyclic bases **5**–**9**, viz., replacement of one of the fluorine atoms of the starting 4,5-difluoro-2-nitroaniline (**2**)[12] with a nucleophilic reagent, conventional reduction of the nitro group into an amine, and imidazole ring closure by treatment with triethyl orthoformate furnished the bases **5**–**9** in good yields.

The benzimidazole derivatives **1**, **5**–**9** showed good substrate activity for *E. coli* PNP in transglycosylation reactions. The use of uridine (2′-deoxyuridine)/UP or thymidine/TP is favorable vs. natural purine nucleosides and PNP for the production of intermediate 1-phosphates of α-D-ribo- or α-D-2-deoxyribofuranoses. The transglycosylation of bases **1**, **5**–**9** under optimal reaction conditions resulted in a more than 98.4% conversion of the base into nucleosides. It is notable that rather low rate of the

transribosylation (22–28 h) vs. that of trans-2-deoxyribosylation of benzimidazoles and arabinosylation of 2-fluoroadenine (1–3 h),[17] are in accord with results of the *ab initio* geometry optimization (HyperChem, 8.1 release; in vacuo, 6-31G* level) of the spatial structures of the corresponding 1-phosphates.[18] In chemical terms, the condensation of α-D-pentofuranose-1-phosphates with heterocyclic bases occurs as a result of nucleophilic attack of the base nitrogen atom on the electrophilic anomeric carbon atom of the 1-phosphate. Indeed, the calculated values of the partial positive charges of the C1 atoms of α-D-ribo- (0.425 *e*), α-D-2-deoxyribo- (0.454 *e*), and α-D-arabinofuranosyl 1-phosphates (0.464 *e*) (suggest increasing electrophilic properties of the anomeric carbon atom among these compounds.

One of the most important results of this work is that we were able to provide the visual size of the hydrophobic pocket of *E. coli* PNP. This result allows us to understand the mechanism of substrate binding in the active site of the enzyme, the subsequent activation of the substrate, and the formation of the glycosidic bond.[18]

Hydrazine hydrate and Raney Ni were purchased from Sigma-Aldrich. Silica gel C18 was supplied by Merck. NMR spectra: Bruker Avance-500-DRX and Bruker Avance-700-DRX (Bruker, Germany). Mass spectra: Agilent 6224, ESI-TOF, LC/MS (USA) and Esquire 3000 Plus (Bruker Daltonics, Germany) in positive ion mode (ESI^+). HPLC was performed on Waters system (Waters 1525, Waters 2487, Breeze 2; USA); column: Nova Pack C18, 4 μm, 4.6 × 150 mm; eluent A: 0.1% TFA–H_2O, eluent B: 70% MeCN in 0.1% TFA–H_2O; flow: 1 mL/min; UV detection: 254 and 280 nm. The UV spectra were recorded on the UV spectrophotometer Shimadzu UV-160 (Japan). Progress of the synthesis of compounds **3** and **4** and their purity was monitored and checked, respectively, by TLC [Sorbophil (Merck), $CHCl_3$–EtOH, 5:1]; satisfactory C, H and N (±0.40) elemental analyses were obtained for compounds **3**.

The preparation of recombinant *E. coli* UP, TP and PNP (the product of the *deoD* gene) was described previously.[11]

5-Alkoxy-4-fluoro-2-nitroanilines 3a–c; General Procedure
To a soln of 4,5-difluoro-2-nitroaniline[12] (**2**, 5.22 g, 30 mmol) in the alcohol (50 mL) was added a soln of NaOH (30 mmol) in the same alcohol and the mixture was stirred at r.t. for 1 h. The precipitate formed was filtered off, dried, and recrystallized (EtOH).

4-Fluoro-5-methoxy-2-nitroaniline (3a)
Yellow crystals; yield: 4.81 g (80%); mp 169–170 °C; R_f = 0.82.

5-Ethoxy-4-fluoro-2-nitroaniline (3b)
Yellow crystals; yield: 5.10 g (85%); mp 134–135 °C; R_f = 0.86.

4-Fluoro-5-isopropoxy-2-nitroaniline (3c)
Yellow crystals; yield: 1.93 g (30%); mp 150–152 °C; R_f = 0.78.

4-Fluoro-5-(morpholino)- (3d) and 4-Fluoro-5-(*N*-methylpiperazino)-2-nitroaniline (3e)
A mixture of 4,5-difluoro-2-nitroaniline (**2**; 30 mmol) and morpholine (60 mmol) or *N*-methylpiperazine (60 mmol) in EtOH (25 mL) was refluxed for 30–60 min and cooled to r.t. The precipitate formed was filtered off, dried and recrystallized (EtOH).

4-Fluoro-5-(morpholino)-2-nitroaniline (3d)
Yellow crystals; yield: 4.56 g (63%); mp 188–190 °C; R_f = 0.65.

4-Fluoro-5-(*N*-methylpiperazino)-2-nitroaniline (3e)
Yellow crystals; yield: 3.51 g (46%); mp 136–137 °C; R_f = 0.54.

5-Substituted 4-Fluorobenzene-1,2-diamines 4a–e; General Procedure
A mixture of compound **3** (30 mmol), hydrazine hydrate (98%, 4 mL) and Raney Ni (89%, 5% of the weight compound **3**) in EtOH (50 mL) was refluxed for 4–5 h. The hot reaction mixture was filtered, the residue on the filter was washed with EtOH (30 mL) and combined filtrate and washing was stored at +4 °C. The dark-brown precipitate was filtered off, washed with EtOH and dried.

4-Fluoro-5-methoxybenzene-1,2-diamine (4a)
Colorless crystalline powder; yield: 3.04 g (65%); mp 110–112 °C; R_f = 0.65.

5-Ethoxy-4-fluorobenzene-1,2-diamine (4b)
Colorless crystalline powder; yield: 3.21 g (63%); mp 80–82 °C; R_f = 0.56.

4-Fluoro-5-isopropoxybenzene-1,2-diamine (4c)
Brown powder; yield: 1.58 g (35%); mp 130–132 °C; R_f = 0.52

4-Fluoro-5-morpholinobenzene-1,2-diamine (4d)
Cream powder; yield: 3.42 g (54%); mp 126–127 °C; R_f = 0.49

4-Fluoro-5-(*N*-methylpiperazino)benzene-1,2-diamine (4e)
Cream powder; yield: 3.03 g (45%); mp 78–80 °C; R_f = 0.46.

5-Substituted 6-Fluorobenzimidazoles 5–9; General Procedure
Compound **4** (30 mmol) was refluxed in $CH(OEt)_3$ (60 mL) for 6–7 h, the mixture was evaporated to ca. 10 mL and residue poured out into cold (3–5 °C) H_2O. The precipitate formed was filtered off, dried and recrystallized (EtOH–H_2O). Physico-chemical and NMR data are collected in Table 2.

5,6-Difluoro-1*H*-benzimidazole (1)
Yellow powder; yield: 4.02 g (87%); mp 178–179 °C.

5-Fluoro-6-methoxy-1*H*-benzimidazole (5)
Colorless crystals; yield: 1.99 g (40%); mp 86–87 °C.

5-Ethoxy-6-fluoro-1*H*-benzimidazole (6)
Colorless crystals; yield: 3.24 g (60%); mp 98–100 °C.

5-Fluoro-6-isopropoxy-1*H*-benzimidazole (7)
Yellow oil; yield: 1.68 g (35%).

5-Fluoro-6-morpholino-1*H*-benzimidazole (8)
Cream crystalline powder; yield: 5.31 g (80%); mp 208–210 °C.

5-Fluoro-6-(*N*-methylpiperazino)-1*H*-benzimidazole (9)
Cream crystals; yield: 2.81 g (40%); mp 120–122 °C.

Enzymatic Reactions
The enzymatic reactions were performed in potassium phosphate buffers (5–20 mM; pH 7.0) at 52 °C monitoring the reaction progress by HPLC; the reactions were stopped by addition of EtOH when >98.5% of base was transformed into nucleoside. The mixture was evaporated to dryness and compounds **10** and **11** were recrystallized (H_2O). The remaining products were purified by column chromatography (silica gel C18, 20 × 120 mm, H_2O–MeCN). For details see Table 3 and physico-chemical and spectral data in Tables 4 and 5.

Antiviral Assays
The antiviral assays other than the anti-HIV assays, were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia virus, vesicular stomatitis virus, cytomegalovirus (HCMV) and varicella-zoster virus (VZV)], Vero (parainfluenza-3, reovirus-1, Sindbis virus and Coxsackie B4), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus), Crandel–Rees feline kidney

(CRFK) [feline coronavirus (FIPV) and feline herpes virus] or MDCK [influenza A (H1N1; H3N2) and influenza B] cell cultures. Confluent cell cultures (or nearly confluent for MDCK cells) in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) in the presence of varying concentrations of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the virus-infected cell cultures that were not treated with the test compounds.

Anti-HIV Assays

Human lymphocyte CEM cells (~3×10^5 cells/mL) cells were infected with 100 $CCID_{50}$ of $HIV(III_B)$ or HIV-2(ROD)/mL and seeded in 200 μL wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 d of incubation at 37 °C, virus-induced cytopathicity (giant cell formation) was recorded microscopically. The EC_{50} was defined as the compound concentration required to inhibit virus-induced cytopathogenicity by 50%. The CC_{50} was defined as the compound concentration required to inhibit CEM cell proliferation by 50%.

Table 2 Yields and ^{1}H NMR Data for Compounds **1**, **5**–**9**

Compd	Yield[a] (%)	^{1}H NMR (DMSO-d_6) δ, *J* (Hz)				
		H2 (s)	H4	H7	NH (br s)	Other
1	87	8.15	7.44 (m, 2 H)		12.11	–
5	40	7.95	7.16 (m)	7.28 (d, *J* = 11.0)	12.25	3.87 (s, 3 H, OCH_3)
6	60	7.94	7.13 (m)	7.27 (d, *J* = 10.8)	12.00	1.43 (t, 3 H, OCH_2CH_3), 4.08 (dd, 2 H, OCH_2CH_3)
7	35	7.95	7.16 (m)	7.27 (d, *J* = 11.0)	12.15	1.33 [d, 6 H, $OCH(CH_3)_2$], 4.50 [m, 1 H, $OCH(CH_3)_2$]
8	80	7.94	7.11 (d, *J* = 7.8)	7.24 (d, *J* = 12.5)	12.00	2.46–2.50 (t, 4 H, CH_2OCH_2), 2.98–3.01 (t, 4 H, CH_2NCH_2)
9	40	7.94	7.20 (m)	7.24 (d, *J* = 12.0)	12.00	2.26 (s, 3 H, NCH_3), 2.50 (m, 4 H, CH_2NCH_2), 2.99 (m, 4 H, CH_2NCH_2)

[a] Yields of isolated pure compounds; satisfactory C, H, and N (±0.40) elemental analyses were obtained.

Table 3 Experimental Data for the Enzymatic Synthesis of Benzimidazole β-D-Ribo- and β-D-2′-Deoxyribonucleosides[a]

Compd	Acceptor[b]	Donor[b]	Substrates			PNP[c] units (A)	UP[c] units (B)	TP[c] units (C)	Time (h)	Residual base (%)	MeCN[d] (%)
			Acceptor (mg) [mmol]	Donor (mg) [mmol]	Ratio A/D						
10	**1**	Urd	100 [0.65]	474 [1.94]	1:3	101 (155)	78 (40)	–	22	1.49	0
11	**1**	dUrd	200 [1.3]	887 [3.9]	1:3	207 (160)	233 (60)	–	1	1.09	0
12	**5**	Urd	180 [1.08]	2.647 [10.8]	1:10	169 (155)	432 (40)	–	26	0.26	0
13	**5**	dUrd	56 [0.34]	771 [3.38]	1:10	53 (155)	304 (90)	–	3	0.42	20
14	**6**	Urd	200 [1.1]	1.355 [5.5]	1:5	177 (160)	230 (40)	–	24	1.57	10
15	**6**	dUrd	180 [1.0]	2.282 [10.0]	1:10	160 (160)	460 (80)	–	3	0.86	10
16	**7**	Urd	40 [0.2]	503 [2.0]	1:10	80 (400)	80 (40)	–	24	0.78	21
17	**7**	dUrd	40 [0.2]	470 [2.0]	1:10	80 (400)	315 (160)	–	2	1.10	25
18	**8**	Urd	200 [0.9]	1.545 [6.3]	1:7	144 (160)	250 (40)	–	24	0.96	0
19	**8**	Thd	297 [1.3]	2.276 [9.4]	1:7	260 (200)	–	891 (95)	1	1.07	10
20	**9**	Urd	40 [0.17]	415 [1.7]	1:10	51 (300)	68 (40)	–	28	1.32	20
21	**9**	Thd	40 [0.17]	288 [1.2]	1:7	68 (400)	–	128 (107)	1	1.08	25

[a] For conditions, see the experimental procedures.
[b] MW used: **1** (154.12); **5** (166.16); **6** (180.19); **7** (194.21); **8** (221.24); **9** (234.25); Urd (244.2); dUrd (228.2); Thd (242.23).
[c] A: ratio of PNP (units)[11] per acceptor base (1 mmol), e.g., 101/0.65 = 155; B: ratio of UP (units)[11] per nucleoside [uridine (Urd) or 2′-deoxyuridine (dUrd)] (1 mmol), e.g., 78/1.94 = 40; C: ratio of TP (units)[11] per thymidine (Thd; 1 mmol), e.g., 891/9.4 = 95.
[d] Nucleosides were eluted from the C18-silica gel column with a MeCN–H_2O mixture, the percentage of MeCN is given.

Table 4 Physicochemical Properties of Nucleosides **10**–**21**

Compound	R	Pfr	Yield (%) [mg]	Purity (%) [HPLC][a]	Mp (°C) (solvent)[b]	UV[c] λ_{max} (nm) [ε]	Calcd MW	MS[d]
10	F	Rib	77 [143.1]	99.89 [t_R = 8.9 (II)]	186–190 (H_2O)[e]	278.4 [5,200], 243.4 [6,700]	286.233	286.838 [M + H]$^+$, 154.711 [M – Rib + 2 H]$^+$, 115.707 [M – Rib – 2 F + H]$^+$
11	F	dRib	68 [238.7]	99.67 [t_R = 9.4 (II)]	175–178 (H_2O)[e]	278.8 [5,700], 243.2 [7,100]	270.233	271.071 [M + H]$^+$, 154.9281 [M – dRib + 2 H]$^+$, 116.912 [M – B]$^+$
12a (ratio **12a**/**12b** 95:5)	OMe	Rib	54 [173.8]	97.62 [t_R = 14.05 (I)]		–	298.268	299.097 [M + H]$^+$
13a,**b** (ratio **13a**/**13b** 41:59)	OMe	dRib	51 [48.9]	**13a**: 40.90 [t_R = 15.37 (I)] **13b**: 59.10 [t_R = 14.07 (I)]		–	282.269	283.022 [M + H]$^+$
14a (ratio **14a**/**14b** 89:11)	OEt	Rib	98 [336.4]	100 [t_R = 9.24 (III)]		–	312.295	313.115 [M + H]$^+$, 180.976 [M – Rib + 2 H]$^+$
15a (ratio **15a**/**15b** 70:30)	OEt	dRib	85 [251.6]	100 [t_R = 8.00 (II)]		–	296.296	297.064 [M + H]$^+$, 180.963 [M – dRib + 2 H]$^+$, 115.889 [M – dRib – F – C_2H_5O + H]$^+$
16a	O*i*-Pr	Rib	75 [48.9]	97.88 [t_R = 9.57 (III)]	66–69	289.6 [4,800], 247.4 [5,200], 204.8 [32,400][g]	326.322	327.080 [M + H]$^+$, 194.880 [M – Rib + 2 H]$^+$
17a (ratio **17a**/**17b** 96:4)	O*i*-Pr	dRib	65 [40.3]	100 [t_R = 14.79 (V)]	–[f]	–	310.322	311.044 [M + H]$^+$
18a	morpholino	Rib	74 [235.2]	100 [t_R = 12.92 (IV)]	92–96	290.2 [5,550], 214.6 [20,700]	353.347	354.166 [M + H]$^+$, 221.026 [M – Rib + H]$^+$, 222.024 [M – Rib + 2 H$^+$], 334.144 [M – F]$^+$
19a	morpholino	dRib	80 [350.6]	98.86 [t_R = 14.08 (IV)]	154–158	290.8 [6,050], 213.8 [21,400] {295.2 [6,450], 219.0 [20,900][h]}	337.348	338.163 [M + H]$^+$, 318.136 [M – F]$^+$, 221.025 [M – dRib + H]$^+$, 222.016 [M – dRib + 2 H]$^+$
20a	*N*-methyl-piperazino	Rib	79 [49.2]	100 [t_R = 5.76 (III)]	104–108	288.2 [6,600], 214.0 [28,000]	336.389	367.175 [M + H]$^+$, 234.993 [M – Rib + 2 H]$^+$
21a	*N*-methyl-piperazino	dRib	68 [40.47]	94.58 [t_R = 6.35 (III)]	84–88	288.2 [6,500], 213.6 [26,600]	350.390	351.114 [M + H]$^+$, 234.958 [M – dRib + 2 H]$^+$, 213.779 [M – dRib – F]$^+$, 214.780 [M – dRib – F + H]$^+$

[a] HPLC (Waters 1525, Waters 2487, Breeze 2; column: Nova Pack C18, 4 μm, 4.6 × 150 mm; eluent A: 0.1 % TFA–H_2O, eluent B: 70% MeCN in 0.1 % TFA–H_2O; flow: 1 mL/min; UV detection: 254 and 280 nm). Elution: (I) 7% B; (II) 7% B over 5 min, 7% to 100% B over 15 min; (III) 0 to 35% B over 10 min, then 35% B over 10 min; (IV) 7% B over 5 min, 7% to 15% B over 1 min, then 15% B over 14 min; (V) 7% B over 5 min, 7% to 20% B over 2 min, then 20% B over 10 min.
[b] White amorphous powder unless otherwise stated.
[c] UV spectra recorded on a UV-spectrophotometer Shimadzu UV-160 (Japan) in H_2O, unless otherwise indicated.
[d] The mass spectra [electrospray ionization (ESI)] were recorded on Esquire 3000 Plus (Bruker Daltonics, Germany).
[e] Colorless crystals.
[f] Colorless oil.
[g] In EtOH.
[h] Aq 80% EtOH.

Table 5 Selected ^{1}H and ^{13}C NMR Spectroscopy Data of Benzimidazole Nucleosides (DMSO-d_6) δ, J (Hz)[a]

Compd	H1′ [C1′]	H2′a [C2′]	H2′b [C2′]	H3′ [C3′]	H4′ [C4′]	H5′a/H5′b [C5′]	Other [^{13}C]
10	5.86 (d, J = 6.6) [89.39]	4.33 (m, J = 6.6, 5.6) [73.84]	–	4.12 (m) [70.47]	4.00 (m) [86.16]	3.66 (m) [61.55]	8.52 (1 H, H2) [144.53], 8.01 (dd, $^{3}J_{H4,F5}$ = 10.8, $^{4}J_{H4,F6}$ = 7.5, 1 H, H4) [100.39], 7.75 (dd, $^{3}J_{H7,F6}$ = 11.0, $^{4}J_{H7,F5}$ = 7.5, 1 H, H7) [106.91], 5.41 (br s, 2′-OH), 5.19 (br s, 3′-OH), 5.26 (br s, 5′-OH)
11	6.34 (dd, J = 7.4, 6.2) [–[b]]	2.54 (m, ^{gem}J = 13.6, 7.5, 6.30 [–[b]]	2.26 (octet, ^{gem}J = 13.2, 5.9, 3.0) [–[b]]	4.40 (dt, J = 6.3, 3.2) [–[b]]	3.88 (q, J = 3.7) [–[b]]	3.58 (m) [–[b]]	8.54 (1 H, H2) [144.04], 7.98 (dd, $^{3}J_{H4,F5}$ = 10.8, $^{4}J_{H4,F6}$ = 7.4, 1 H, H4) [100.07 (d, $^{3}J_{C4,F5}$ = 23.5, C4)], 7.73 (dd, $^{4}J_{H7,F5}$ = 7.5, $^{3}J_{H7,F6}$ = 11.0, 1 H, H7) [106.83 (d, $^{3}J_{C7,F6}$ = 19.4)], 5.36 (d, J = 4.0, 1 H, 3′-OH), 5.07 (t, J = 5.2, 1 H, 5′-OH)
12a	5.79 (d, J = 6.4) [88.55]	4.31 (dd) [73.00]	–	4.11 (br s) [69.70]	3.97 (br m) [85.17]	3.63, 3.67 (dd, dd) [60.88]	8.36 (s, 1 H, H2) [142.46], 7.76 (d, $^{3}J_{H7,F6}$ = 11.4, 1 H, H7) [98.91], 7.41 (d, $^{4}J_{H4,F6}$ = 7.8, 1 H, H4) [103.11], 5.39 (br s, 2′-OH), 5.17 (br s, 3′-OH), 5.15 (br s, 5′-OH), 3.86 (s, 3 H, OCH_3) [55.99 (s)]
12b	5.85 (d, J = 6.5) [88.49]	4.38 (dd) [72.92]	–	4.13 (br s) [69.78]	3.99 (br m) [85.25]	3.63, 3.67 (dd, dd) [60.88]	8.33 (s, 1 H, H2) [142.22], 7.64 (d, $^{4}J_{H7,F5}$ = 7.7, 1 H, H7) [96.11], 7.50 (d, $^{3}J_{H4,F5}$ = 11.6, 1 H ,H4) [105.28], 5.44 (br s, 2′-O*H*), 5.19 (br s, 3′-OH), 5.16 (br s, 5′-OH), 3.86 (s, 3 H, OCH_3) [55.99]
13a	6.29 (dd, J = 7.3, 6.4) [84.43]	2.55 (m) [39.28]	2.25 (m)	4.40 (m) [70.28]	3.86 (m) [87.36]	3.62–3.58 (m) [61.12]	8.38 (s, 1 H, H2) [141.82], 7.70 (d, $^{3}J_{H7,F6}$ = 11.5, 1 H, H7) [98.67], 7.39 (d, $^{4}J_{H4,F6}$ = 7.9, 1 H, H4) [103.11], 5.30 (d, J = 4.1, 3′-OH), 4.98 (t, J = 5.1, 5′-OH), 3.87 (3 H, OCH_3) [56.07]
13b	6.36 (dd, J = 7.3, 6.5) [84.40]	2.56 (m) [39.31]	2.26 (octet, ^{gem}J = 13.1, 5.8, 2.7)	4.43 (m) [70.21]	3.88 (m) [87.15]	3.61–3.59 (m) [61.18]	8.36 (s, 1 H, H2) [141.56], 7.57 (d, $^{3}J_{H7,F5}$ = 7.9, 1 H, H7) [95.83], 7.49 (d, $^{4}J_{H4,F5}$ = 11.7, 1 H, H4) [105.26], 5.31 (d, J = 3.9 Hz, 3′-OH), 5.01 (t, J = 5.2, 5′-OH), 3.89 (3 H, OCH_3) [56.10]
14a	5.79 (d, J = 6.4) [88.75]	4.31 (dd) [73.12]	–	4.11 (br m, overlapping OCH_2CH_3 m) [69.85]	3.97 (m) [85.38]	3.61–3.69 (m, ^{gem}J = 12.0) [60.94]	8.35 (s, 1 H, H2) [142.5], 7.74 (d, J = 11.4, 1 H, H7) [99.15 (d, $^{2}J_{C7,F6}$ = 25.17)], 7.39 (d, J = 7.8, 1 H, H4) [104.1 (s, $^{3}J_{C4,F6}$ < 1)], [125.57 (d, $^{3}J_{7a,F6}$ = 12.51, C7a), 139.86 (s, C4a), 143.30 (d, $^{2}J_{C5,F6}$ = 13.12, C5)], 5.27 (br s, 3 OH), 4.12 (q, 2 H, CH_2CH$_3$) [64.53], 1.36 (t, J = 6.9 Hz, 3 H, CH$_2$CH_3) [14.24]
14b	5.84 (d, J = 6.4) [88.64]	4.36 (dd) [73.12]	–	4.14 (m) [69.85]	3.99 (m) [85.38]	3.61–3.69 (m) [60.94]	8.32 (s, 1 H, H2) [142.5], 7.62 (d, J = 7.8, 1 H, H7) [97.31 (s, $^{3}J_{C7,F5}$ < 1)], 7.49 (d, J = 11.6, 1 H, H4) [105.5 (d, $^{3}J_{C7,F5}$ = 20.96)], 4.13 (q, 2 H, CH_2CH$_3$) [64.53], 1.38 (t, 3 H, CH$_2$CH_3) [14.0]
15a	6.28 (dd, J = 7.5, 6.2) [85.30]	2.54 (m) [40.10]	2.26 (m)	4.38 (m) [70.93]	3.86 (m) [87.95]	3.57 (dd, dd, J = 3.8, 4.1, ^{gem}J = 11.7) [61.93]	8.37 (s, 1 H, H2) [142.78], 7.68 (d, $^{3}J_{H7,F6}$ = 11.4, 1 H, H7) [99.49 (d, $^{2}J_{C7,F6}$= 25.2)], 7.37 (d, $^{4}J_{H4,F6}$ = 7.9, 1 H, H4) [105.10 (s, $^{3}J_{C4,F6}$ < 1)], 5.05 (br s, 3 OH), 8.37 (s, 1 H, H2) [142.78], 4.11 (m ,2 H, CH_2CH$_3$) [65.22], 1.36 (t, 3 H, CH$_2$CH_3), [15.08]
15b	6.34 (dd, J = 7.7, 6.1) [85.23]	2.56 (m) [40.10]	2.26 (m)	4.41 (m) [70.98]	3.88 (m) [87.975]	3.59 (dd, dd) [61.93]	8.34 (s, 1 H, H2) [142.44], 7.56 (d, $^{3}J_{H7,F5}$ = 7.9, 1 H, H7) [97.62 (s, $^{3}J_{C7,F5}$ < 1.0, C7)], 7.47 (d, $^{4}J_{H4,F5}$ = 11.6, 1 H, H4) [106.07 (d, $^{2}J_{C4,F5}$ = 20.96, C4)], 5.30 (br s, 3 OH), 8.34 (s, 1 H, H2) [142.44], 4.13 (m, 2 H, CH_2CH$_3$) [65.22], 1.37 (t, 3 H, CH$_2$CH_3) [15.08]
16a	5.79 (d, J = 6.5) [88.63]	4.31 (m) [73.05]	–	4.10 (m) [69.78]	3.97 (m) [85.29]	3.64, 3.66 (m) [60.97]	8.36 (s, 1 H, H2) [142.74], 7.73 (d, $^{3}J_{H7,F6}$ = 12.4, 1 H, H7) [99.06], 7.40 (d, $^{4}J_{H4,F6}$ = 7.9, 1 H, H4) [107.37], 5.41 (br s, 2′-OH); 5.20 (br s, 3′-OH), 5.19 (br s, 5′-OH), 4.58 (m, 1 H, C*H*Me$_2$) [71.95], 1.29 (d, 6 H, 2 CH$_3$) [21.66]

Table 5 Selected ^{1}H and ^{13}C NMR Spectroscopy Data of Benzimidazole Nucleosides (DMSO-d_6) δ, *J* (Hz)[a] (continued)

Compd	H1′ [C1′]	H2′a [C2′]	H2′b [C2′]	H3′ [C3′]	H4′ [C4′]	H5′a/H5′b [C5′]	Other [^{13}C]
17a[c]	6.28 (dd, *J* = 7.6, 6.1) [85.24]	2.55 (m) [38.26]	2.26 (octet, *J* = 13.3, 6.0, 3.1)	4.39 (m) [70.17]	3.82 (m) [87.91]	3.52 (m) [61.17]	8.34 (s, 1 H, H2) [143.04], 7.68 (d, $^{3}J_{H7,F6}$ = 11.2, 1 H, H7) [99.61 (d, $^{2}J_{C7,F6}$ = 24.0)], 7.36 (d, $^{4}J_{H4,F6}$ = 7.8, 1 H, H4) [107.99 (s, $^{3}J_{C4,F6}$ < 1)], 5.33 (d, *J* = 3.8, 3′-OH), 5.01 (t, *J* = 5.1, 5′-OH), 4.58 (m, 1 H, C*H*CH$_3$) [72.56], 1.28 (d, *J* = 6, 6 H, CHC*H*$_3$) [21.56]
17b[d]	6.31 (dd, *J* = 7.8, 5.9) [85.26]	~2.55 (m) [–[b]]	~2.26 (m) [–[b]]	~4.39 (m) [–[b]]	~3.83 (m) [–[b]]	~3.52 (m) [–[b]]	8.31 (s, 1 H, H2) [142.55], 7.54 (d, $^{4}J_{H7,F5}$ = 7.8, 1 H, H7) [100.03], 7.44 (d, $^{3}J_{H4,F5}$ = 11.6, 1 H, H4) [106.18], 5.33 (br s, 3′-OH), 5.02 (br s, 5′-OH), 4.64 (m, 1 H, C*H*CH$_3$) [72.33], 1.30 (d, 6 H, CHC*H*$_3$)
18a	5.79 (d, *J* = 6.5) [88.64]	4.30 (m) [73.10]	–	4.10 (m) [69.79]	3.97 (m) [85.26]	3.65 (m) [60.99]	8.37 (s, 1 H, H2) [142.59], 7.70 (d, $^{3}J_{H7,F6}$ = 12.4, 1 H, H7) [99.19 (d, $^{2}J_{C7,F6}$ = 27.9)], 7.31 (d, $^{4}J_{H4,F6}$ = 7.9, 1 H, H4) [108.92 (s, $^{3}J_{C4,F6}$ < 1)], 5.40 (br s, 2′-OH), 5.18 (br s, 3′-OH), 5.15 (br s, 5′-OH), 3.76 (m, 4 H, O(C*H*$_2$)$_2$) [60.26], 2.98 (m, 4 H, NC*H*$_2$) [51.27]
19a	6.28 (dd, *J* = 7.1, 6.4) [84.41]	2.54 (m) [39.24]	2.25 (m)	4.38 (m) [70.05]	3.86 (m) [87.07]	3.55, 3.58 (dd, dd, ^{gem}J = 11.7, 4.0) [61.08]	8.37 (s, 1 H, H2) [142.07], 7.64 (d, $^{3}J_{H7,F6}$ = 12.4, 1 H, H7) [98.84 (d, $^{2}J_{C7,F6}$ = 27.72)], 7.30 (d, $^{4}J_{H4,F6}$ = 7.9, 1 H, H4) [108.83 (s, $^{3}J_{C4,F6}$ < 1)], 5.31 (br s, 3′-OH), 4.98 (br s, 5′-OH), 3.76 (m, 4 H, OC*H*$_2$CH$_2$) [65.97], 2.97 (m, 4 H, NC*H*$_2$CH$_2$) [51.16]
20a	5.78 (d, *J* = 6.3) [88.62]	4.30 (m) [73.06]	–	4.10 (m) [69.78]	3.97 (m) [85.20]	3.6–3.7 (m) [60.93]	8.34 (s, 1 H, H2) [142.47], 7.66 (d, $^{3}J_{H7,F6}$ = 12.4, 1 H, H7) [99.03], 7.29 (d, $^{4}J_{H4,F6}$ = 7.9, 1 H, H4) [108.97], 5.38 (d, *J* = 6.5, 1 H, 2′-OH), 5.15 (d, *J* = 4.7, 1 H, 3′-OH), 5.12 (t, *J* = 5.0, 1 H, 5′-OH), 2.98 (m, 4 H, MeNCH$_2$C*H*$_2$) [50.73], 2.50 (m, 4 H, MeNC*H*$_2$CH$_2$) [54.59], 2.24 (s, 3 H, NCH$_3$) [45.51]
21a	6.28 (dd, *J* = 7.3, 6.4) [84.60]	2.54 (m) [39.14]	2.26 (m) [39.14]	4.38 (m) [70.16]	3.86 (m) [87.37]	3.51–3.61 (m) [61.21]	8.36 (s, 1 H, H2) [142.30], 7.61 (d, $^{3}J_{H7,F6}$ = 12.4, 1 H, H7) [98.97], 7.28 (d, $^{4}J_{H4,F6}$ = 7.9, 1 H, H4) [109.12], 5.29 (d, *J* = 4.1, 1 H, 3′-OH), 4.96 (t, *J* = 5.2, 1 H, 5′-OH), 2.50 (m, 4 H, MeNC*H*$_2$CH$_2$) [54.50], 2.98 (m, 4 H, MeNCH$_2$C*H*$_2$) [50.79], 2.24 (s, 3 H, NCH$_3$) [45.48]

[a] ^{1}H, ^{13}C, and ^{15}N resonances were assigned using 2D homo- and heteronuclear experiments: $^{1}H/^{1}H$-COSY, $^{1}H/^{13}C$-HSQC, $^{1}H/^{13}C$-HMBC, $^{1}H/^{15}N$-HSQC, $^{1}H/^{15}N$-HMBC, and [^{1}H,^{1}H] NOE spectra.
[b] Not determined, for example, the quantity of isomer **17b** (4%) in the mixture with **17a** was not sufficient to determine its ^{13}C nuclear shifts in $^{1}H/^{13}C$-HSQC, $^{1}H/^{13}C$-HMBC spectra.
[c] Mixture: 96%.
[d] Mixture: 4%.

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