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Synthesis and biological activity of benzo-fused 7-deazaadenosine analogues. 5- and 6-substituted 4-amino- or 4-alkylpyrimido[4,5-*b*]indole ribonucleosides

Michal Tichý [a], Radek Pohl [a], Eva Tloušťová [a], Jan Weber [a], Gina Bahador [b], Yu-Jen Lee [b], Michal Hocek [a,c,*]

[a] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Gilead Sciences & IOCB Research Center, Flemingovo nam. 2, CZ-16610 Prague 6, Czech Republic
[b] Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, USA
[c] Department of Organic and Nuclear Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, CZ-12843 Prague 2, Czech Republic

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ABSTRACT

Two series of new 4-aminopyrimido[4,5-*b*]indole ribonucleosides bearing phenyl or hetaryl group at position 5 or 6 have been prepared by Suzuki or Stille cross-coupling reactions employing X-Phos ligand with (het)arylboronic acids or stannanes. A series of 4-substituted nucleosides has been also prepared by Pd-catalyzed cross-couplings or nucleophilic substitution. Some of these compounds displayed moderate antiviral activities against HCV and dengue viruses.

1. Introduction

Systematic research in modified purine and deazapurine nucleosides in our group led to the discovery of two novel classes of cytostatic 7-deazapurine ribonucleosides. 6-Hetaryl-7-deazapurine ribonucleosides[1] bearing either H (**1**) or F (**2**) at the position 7 and five-membered hetaryl groups (furyl, thienyl, etc.) at the position 6 (Fig. 1) and 7-hetaryl-7-deazaadenosines **3**[2] showed nanomolar in vitro cytostatic activities against a broad panel of cancer or leukemia cell lines. Both of these groups of compounds also exerted some non-specific antiviral activities against HCV. On the other hand, sugar-modified derivatives[3] and phosphate prodrugs[4] derived from **1** were less active or inactive. Also other groups have reported biologically active deazapurine nucleosides as inhibitors of Polio and dengue viruses.[5,6] Very recently we have designed and prepared the first generation of benzo-fused analogues of 7-deazapurines, 4-arylpyrimido[4,5-*b*]indoleribonucleosides,[7] and found their promising antiviral activity against dengue virus. Here we report the synthesis and biological activity of other related benzo-fused adenosine analogues, that is, derivatives of 4-amino-5-aryl-, 4-amino-6-aryl- and 4-alkylpyrimido[4,5-*b*]indole ribonucleosides.

Besides our recent study,[7] some other types of pyrimido[4,5-*b*]indole heterocycles were reported as tyrosine kinase inhibitors,[8]

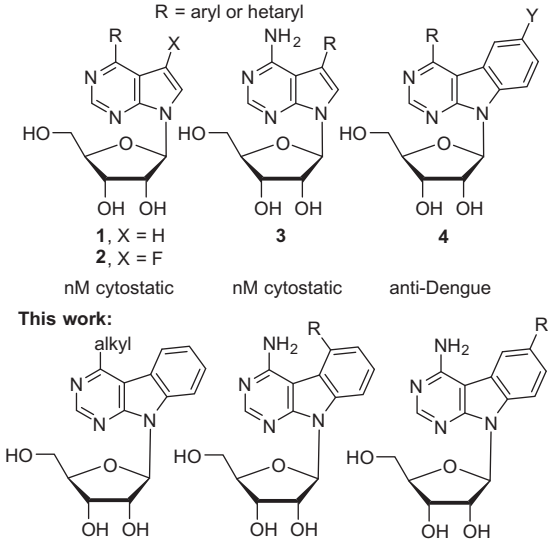

Figure 1. Structures of recently discovered 7-deazapurine ribonucleoside cytostatics and antivirals **1**–**4** and the target compounds for this study.

* Corresponding author. Tel.: +420 220183324; fax: +420 220183578.
E-mail address: hocek@uochb.cas.cz (M. Hocek).

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vascular endothelial growth factor receptor-2 inhibitors, antiangiogenic agents[9] and microtubule disrupting compounds effective against multidrug resistant cells.[10]

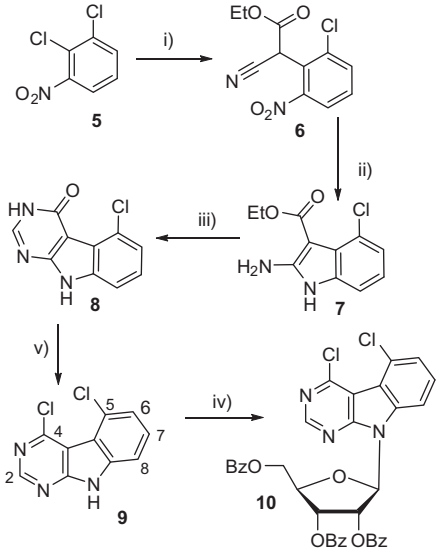
The pyrimido[4,5-*b*]indoles were previously prepared by Pd-catalyzed intramolecular arylation,[11] photolysis of tetrazoles[12] or by condensation of 2-amino-indole-3-carboxylates with formamide or orthoformate.[13] 4-amino-6-methoxypyrimido[4,5-*b*]indole 2′-deoxyribonucleoside was used[14] as fluorescent label for single-nucleotide polymorphism typing, for study of hole transport through DNA[15] and for the construction of DNA logic gates[16] and the corresponding nucleoside triphosphate was successfully incorporated to DNA by PCR.[17]

2. Results and discussion

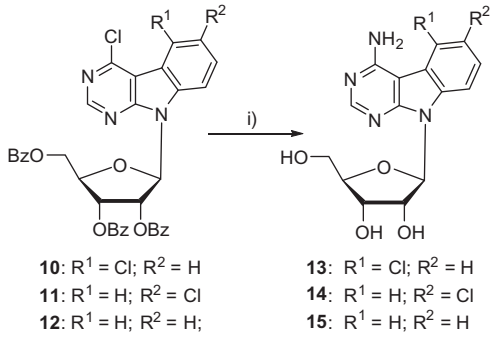
2.1. Chemistry

Our strategy for the synthesis of target 4-amino-6-hetaryl-, 4-amino-5-hetaryl- and 4-substituted pyrimidoindole ribonucleosides was based on palladium catalyzed cross-coupling reactions[18] or nucleophilic substitutions of the corresponding chloro-derivatives. Synthesis of the 4-chloro- and 4,6-dichloropyrimido[4,5-*b*]indoles and their ribonucleosides **11** and **12** has been reported[7] previously. The remaining key intermediate, 4,5-dichloropyrimido[4,5-*b*]indole was built up from 2,3-dichloronitrobenzene **5** (Scheme 1) in analogy to previous work.[7] The sequence started by arylation of cyanoacetate followed by reduction of the nitro group by Zn, cyclization, cyclocondensation with formamide and reaction with $POCl_3$ to give 4,5-dichloropyrimido[4,5-*b*]indole (**9**) in 39% overall yield. The corresponding ribonucleoside **10** was then prepared by glycosidation using *N*,*O*-bis(trimethylsilyl)acetamide and TMSOTf.

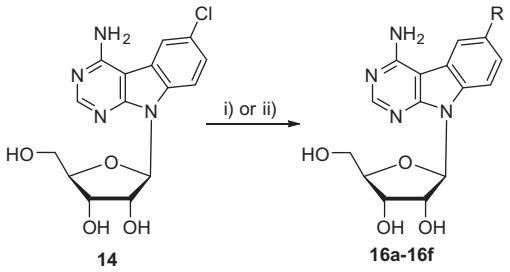
Benzoylated chloropyrimidoindole nucleosides **10**, **11** and **12**, were all easily converted to the corresponding 4-amino derivatives **13**, **14** and **15** by treatment with aqueous ammonia in dioxane (3:1) at 100 °C (Scheme 2). Simultaneously, the benzoyl protecting groups at the ribose were cleaved off. The desired 4-aminopyrimido[4,5-*b*]indole nucleosides **13**, **14** and **15** were isolated just by filtration in good yields (72–96%).



Scheme 1. Reagents and conditions: (i) $CNCH_2COOEt$, *t*-BuOK, THF, reflux, 48 h; (ii) Zn, AcOH, 55 °C, 150 min; (iii) formamide, 190 °C, 12 h; (iv) $POCl_3$, reflux, 4 h; (v) 1-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-ribofuranose, BSA, TMSOTf, 70 °C, 8 h.



Scheme 2. Reagents and conditions: (i) aq NH_3/dioxane, 100 °C, 24 h.

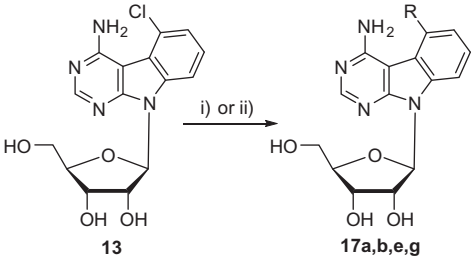


Scheme 3. Reagents and conditions: (i) RM, M = $B(OH)_2$ (1.5 equiv), $Pd(OAc)_2$ (0.05 equiv), X-Phos (0.1 equiv), K_2CO_3 (3 equiv), DMF, 100 °C, 12 h; (ii) M = $SnBu_3$ (1.2 equiv), $Pd(OAc)_2$ (0.05 equiv), X-Phos (0.1 equiv), DMF, 100 °C, 12 h.

Table 1
Synthesis of 4-amino-6-hetaryl nucleosides

Compd	Reagent	R–	Yield (%)
16a	Phenylboronic acid	Phenyl	93
16b	(Furan-2-yl)$SnBu_3$	2-Furyl	68
16c	(Furan-3-yl)$B(OH)_2$	3-Furyl	72
16d	(Thiophen-2-yl)$SnBu_3$	2-Thienyl	73
16e	(Thiophen-3-yl)$B(OH)_2$	3-Thienyl	77
16f	(Benzofuran-2-yl)$B(OH)_2$	2-Benzofuryl	75

The first class of the target compounds were 4-amino-6-(het)arylpyrimido[4,5-*b*]indole nucleosides which were envisaged to be prepared by the cross-coupling reactions of 6-chloro nucleoside **14**. Since the chlorine at the position 6 is unreactive, a thorough optimization of catalytic system and reaction conditions was necessary. Standard procedure using water soluble ligand TPPTS and $Pd(OAc)_2$ in acetonitrile/water mixture did not work. Therefore, we tested several Buchwald-type ligands (X-Phos, S-Phos and RuPhos) and another water soluble ligand Cataxcium F in mixtures of water and acetonitrile for the Suzuki reaction of free 4-amino-6-chloronucleoside **14** with phenyl boronic acid. All these reactions proceeded but only the use of X-Phos led to complete conversion of starting nucleoside to desired product **16a**, which was isolated in excellent yield 93%. Unfortunately, reaction with 3-furylboronic acid under the same conditions gave just traces of desired product probably due to decomposition of the boronic acid in aqueous mixture at high temperature. Therefore, the acetonitrile/water mixture was replaced by DMF and then a full conversion of starting nucleoside **14** in reactions with 3-furyl-, 3-thienyl- and 2-benzofurylboronic acid to products **16c**, **e**, **f** was observed (Scheme 3, Table 1). For the synthesis of 2-furyl and 2-thienyl derivatives **16b**, **d**, the Stille coupling with the corresponding hetarylstannanes was used to give the desired products in good yields.



Scheme 4. Reagents and conditions: (i) RM, M = $B(OH)_2$ (2.0 equiv), $Pd(OAc)_2$ (0.1 equiv), X-Phos (0.2 equiv), K_2CO_3 (3 equiv), DMF, 120 °C, 12 h; (ii) M = $SnBu_3$ (1.5 equiv), $Pd(OAc)_2$ (0.1 equiv), X-Phos (0.2 equiv), DMF, 120 °C, 12 h.

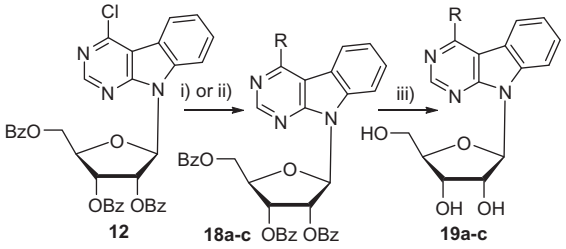
Table 2
Synthesis of 4-amino-5-substituted nucleosides

Entry	Compd	Reagent	R–	Yield (%)
1	**17a**	Phenylboronic acid	Phenyl	28
2	**17b**	(Furan-2-yl)SnBu3	2-Furyl	33
	17g		Butyl	34
3	**17g**	(Thiophen-2-yl)SnBu3	Butyl	42
4	**17e**	(Thiophen-3-yl)$B(OH)_2$	3-Thienyl	24

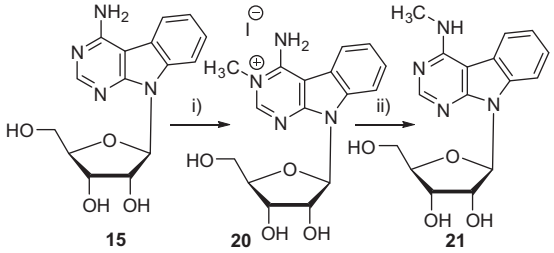
The same catalyst and conditions were used also for introduction of (het)aryl substituents into position 5. The reaction of 5-chloro nucleoside **13** with phenylboronic acid furnished just 28% of desired nucleoside **17a** even if 2 equiv of boronic acid were used and reaction temperature was increased to 120 °C. The Stille reaction with 2-thiophenyl(tributyl)stannane surprisingly gave only product of butyl transfer **17g** in 42% yield. In reaction with 2-furyl(tributyl)stannane, the 5-butyl nucleoside **17g** (34%) was isolated again, together with the desired 5-furyl nucleoside **17b** (33%). The Suzuki reaction of **13** with 3-thiophenylboronic acid provided just 5% of desired product **17e**. The yield was slightly improved by the addition of the boronic acid in two portions but still the desired nucleoside **17e** was isolated in low yield of 24% (Scheme 4, Table 2). Apparently, the reactivity of 5-chloro-derivative **13** in cross-coupling reactions is much lower compared to 6-chloro derivative **14** probably due to steric hindrance of the substituent at the position 6 with the amino group at position 4.

To examine the influence of amino group at position 4 of pyrimidoindole core to biological activity of these compounds, a series of derivatives either replacing the amino group with an alkyl or substituting the amino function by one or two methyl groups was designed and prepared. Methyl and cyclopropyl groups were introduced at position 4 by palladium catalyzed cross-coupling reaction of protected 4-chloro nucleoside **12** with trimethylaluminium or by Negishi reaction with cyclopropylzinc chloride under standard conditions in the presence of $Pd(PPh_3)_4$ in THF at 70 °C.[19] In both cases, desired nucleosides **18a** and **18b** were isolated in good yields (63% and 85%) (Scheme 5). *N*,*N*-Dimethylamino nucleoside **18c** was synthesized by simple nucleophilic substitution of **12** with dimethylamine at rt in good 74% yield. Deprotection of nucleosides **18a–c** under Zemplen conditions using NaOMe in MeOH afforded target free nucleosides **19a–c** in excelent yields 76–92%.

Synthesis of *N*-methyl nucleoside **21** by nucleophilic substitution of **12** with methyl amine failed even if 10 equiv of amine and higher temperature was used. Also the Pd-catalyzed Buchwald–Hartwig amination failed. Therefore, the target 4-(*N*-methylamino)nucleoside **21** was prepared in analogy to literature procedure[20] by methylation of aminonucleoside **15** by MeI in DMA at position 3 followed by sodium hydroxide induced rearrangement of the quaternary salt **20** to nucleoside **21** in 64% yield (Scheme 6).



Scheme 5. Reagents and conditions: (i) reagent (2 equiv, see Table 3), $Pd(PPh_3)_4$ (0.05 equiv), THF, rt; (ii) Me_2NH in THF (2 equiv), propan-2-ol, rt, 24 h; (iii) 1 M MeONa in MeOH (0.3 equiv), MeOH, rt, 24 h.



Scheme 6. Reagents and conditions: (i) MeI (2 equiv), DMA, rt, 24 h; (ii) 1 M NaOH, 100 °C, 1.5 h.

Table 3
Synthesis of 4-substituted nucleosides

Entry	Reagent	R	Prot. prod.	Yield (%)	Unprot. prod.	Yield (%)
1[a]	Me_3Al	Me	**18a**	63	**19a**	92
2[a]	Cyclopropyl-ZnCl	Cyclopropyl	**18b**	63	**19b**	89
3[b]	Me_2NH	Me_2N	**18c**	74	**19c**	88

[a] Conditions: (i) were used.
[b] Conditions: (ii) were used.

Since all the title pyrimidoindole nucleosides exhibited fluorescence, we have studied electronic spectra and photophysical properties in detail. Table 4 summarizes the results showing that the compounds exerted fluorescence with emission maxima at 341–485 nm with moderate quantum yields at 0.07–0.36.

2.2. Biological activity profiling

All the title nucleosides **13**, **14**, **15**, **16a–h**, **17a–c**, **19a–c**, **20** were subjected to biological activity screening. The cytotoxic activity in vitro was studied on the following cell cultures: (i) human promyelocytic leukemia HL60 cells (ATCC CCL 240); (ii) human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2); (iii) human T lymphoblastoid CCRF-CEM cell line (ATCC CCL 119), and (iv) hepatocellular carcinoma cells HepG2 (ATCC HB 8065). Cell viability was determined following a 3-day incubation using metabolic 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT) based method.[21] The title nucleosides did not show any significant cytotoxicity in these assays. The only three compounds with non-negligible activities (in 11–100 μM) are shown in Table 5. The most active compound was 4-amino-5-chloronucleoside **13**. All 5- or 6-hetaryl derivatives were inactive except for benzofuryl derivative **16f**. The 4-methylnucleoside **19a** was the only cytotoxic compound in series of 4-substituted derivatives.

All the title nucleosides were also tested against HCV genotype 1A, 1B and 2A replicons. The results are summarized in Table 6. Several nucleosides showed micromolar activity against HCV, but

Table 4
UV and fluorescence spectral data[a]

Compd	Absorption		Emission		
	λ_{max} (nm)	ε (L mol^{-1} cm^{-1})	λ_{exc} (nm)	φ	λ_{max} (nm)
13	321	13,769	300	0.10	447
	292	15,071			
	247	71,635			
14	320	12,786	310	0.12	395
	273	13,564			
15	315	10,212	310	0.19	341
	282	16,538			
	242	88,024			
16a	325	2876	330	0.22	361
	263	33,463			
16b	280	19,049	320	0.24	442
	229	13,557			
16c	324	1147	330	0.30	384
	257	18,736			
16d	282	30,297	320	0.07	435
	236	23,989			
16e	269	36,191	320	0.08	467
16f	314	35,291	340	0.36	427
17a	290	8993	320	0.20	452
	248	38,259			
17b	323	10,281	320	0.07	440
	248	51,832			
17e	320	9163	320	0.07	356
	294	12,085			
	248	86,990			
17g	289	23,021	300	0.09	454
	245	80,745			
19a	284	16,395	310	0.13	386
	255	45,473			
	235	44,282			
19b	288	26,360	300	0.13	485
	255	49,620			
	236	57,993			
19c	326	10,102	300	0.03	449
	298	13,086			
	250	31,625			
20	318	16,054	310	0.17	341
	290	15,592			
	245	63,821			

[a] UV spectra were measured in methanol at 25 °C. All 5×10^{-5} μM solutions.

Table 5
Cytotoxic activities of nucleosides

Compound	IC_{50} (μm)			
	HL-60	HeLa S3	CCRF-CEM	HepG2
13	17	21	12	21
16f	87	93	32	60
19a	54	11	82	15

the activity was usually accompanied by some cytotoxicity in MT-4 cells. The 4-amino-5-chloro derivative **13** was the most active but also the most cytotoxic. However, 4-methyl nucleoside **19a** showed submicromolar activities in the HCV 1A and 1B replicon assays and cytotoxicity higher than 44 μM.

All the nucleosides were also tested for activity against dengue virus in Vero cells. Several derivatives showed activity in micromolar concentrations, but only benzofuryl derivative **16f** was not cytotoxic to these cells. 4-Amino-5-chloronucleoside **13** was the most active compound, but with low selectivity index (Table 7).

3. Conclusions

A new series of 5- and 6-(het)aryl derivatives of 4-aminopyrimido[4,5-*b*]indole ribonucleosides were designed as substituted benzo-fused analogues of adenosine. They were prepared by the Suzuki or Stille coupling reactions of the corresponding 5- or 6-chloro derivatives with (het)arylboronic acids or -stannanes. The cross-coupling reactions using X-phos proceeded very well at position 6 but only low conversions were obtained at position 5 due to steric hindrance. Also a series of 4-alkyl, 4-methylamino and 4-dimethylamino derivatives was prepared to study the influence of the replacement of the amino group. The target 5- and 6-(het)aryl derivatives did not show any significant cytotoxicity or antiviral activity. However, the 4-amino-5-chloro nucleoside **13** showed significant anti-dengue and anti-HCV activity but accompanied by cytotoxicity. The 4-methyl nucleoside **19a** showed high activity against HCV with low cytotoxicity. All the title compounds are fluorescent and thus have some potential for fluorescent labeling of RNA.

Table 6
Anti-HCV activities of nucleosides

Compound	HCV replicon 1A		HCV replicon 1B		HCV replicon 2A	
	EC_{50} (μm)	CC_{50} (μm)	EC_{50} (μm)	CC_{50} (μm)	EC_{50} (μm)	CC_{50} (μm)
13	0.53	1.16	0.32	1.71	1.28	1.66
15	30.55	>44	11.05	>44	>44	>44
16d	10.03	18.10	8.75	>44	24.89	>44
16e	10.09	17.34	7.38	25.60	13.77	30.19
16f	6.13	14.47	4.83	28.0	8.77	18.00
17g	>44	>44	8.81	>44	>44	>44
19c	21.27	>44	7.07	>44	>44	>44
19a	0.56	>44	0.34	>44	>44	>44

Table 7
Anti-dengue activities of nucleosides

Compound	Vero cells, DENV-2 (μm)		
	EC_{50}	CC_{50}	SI
13	0.85	1.14	1.3
16e	18.8	46.3	2.5
17g	14.8	62.9	4.3
16f	15.4	>100	>6.5
15	39.6	43.5	1.1

4. Experimental part

NMR spectra were recorded using a 400 MHz (^{1}H at 400 MHz, ^{13}C at 100.6 MHz), 500 MHz (500 MHz for ^{1}H and 125.7 MHz for ^{13}C), or 600 MHz (600 MHz for ^{1}H and 151 MHz for ^{13}C) spectrometer. Melting points were determined using a Kofler block and are uncorrected. High resolution mass spectra were measured using electrospray ionization. Reverse phase high performance flash chromatography (HPFC) purifications were performed with Biotage SP1 apparatus on KP-C18-HS columns. Optical rotations were measured at 25 °C, $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. UV–vis and fluorescence spectroscopy measurements and quantum yields determinations were performed in the same way as in a previous case.[22] The purity of final compounds (>95%) was confirmed by elemental analyses and clean NMR spectra.

4.1. Ethyl 2-(6-chloro-2-nitrophenyl)-2-cyanoacetate (6)

Compound **6** was prepared from 2,3-dichloronitrobenzene (**5**) (20 g; 104 mmol) and ethyl cyanoacetate (26.12 ml; 208.4 mmol) according to literature conditions.[7] Compound **6** (21 g; 86%) was obtained as a dark oil. The crude material was used directly for the next step. For analysis, the oil was purified by column chromatography (hexane/EtOAc 0–10% EtOAc). ^{1}H NMR is in agreement with literature.[23]

4.2. Ethyl 2-amino-4-chloro-1*H*-indole-3-carboxylate (7)

Compound **7** was prepared according to literature conditions[7] from crude **6** (27 g; 115 mmol). Compound **7** (21.0 g; 66%) was obtained as brown solid and further purified by column chromatography (hexane/chloroform, 0–60% chloroform); mp 147–149 °C. IR (ATR): ν = 3 459, 3 347, 1 633, 1 596, 1 492, 1 426, 1 378, 1 330, 1 318, 1 232, 1 177, 1 143, 731 cm^{-1}. ^1H NMR is in agreement with literature.[23] Crude material was used directly for the next step.

4.3. 5-Chloro-3*H*-pyrimido[4,5-*b*]indol-4(9*H*)-one (8)

Compound **8** was prepared according to literature conditions[7] from crude **7** (20.5 g; 73.8 mmol). Desired product **8** (15.0 g; 93%) was obtained as brown powder. For analysis, it was purified by column chromatography (chloroform/MeOH, 3% MeOH); mp over 300 °C. IR: 3176, 3062, 2959, 2 920, 1669, 1635, 1623, 1587, 1551, 1422, 1354, 1343, 1313, 1084, 992, 766. ^1H NMR (499.8 MHz, DMSO-d_6): 7.23 (dd, 1H, $J_{6,5}$ = 7.8, $J_{6,8}$ = 1.1, H-6); 7.28 (t, 1H, $J_{7,6}$ = $J_{7,8}$ = 7.8, H-7); 7.40 (dd, 1H, $J_{8,7}$ = 7.8, $J_{8,6}$ = 1.1, H-8); 8.13 (d, 1H, J = 3.5, H-2); 12.19 (br s, 1H, NH-3); 12.51 (br s, 1H, NH-9). ^{13}C NMR (125.7 MHz, DMSO-d_6): 99.64 (C-4a); 1110.68 (CH-8); 120.93 (C-4b); 122.57 (CH-6); 125.26 (CH-7); 125.88 (C-5); 137.16 (C-8a); 148.68 (CH-2); 154.51 (C-9a); 157.16 (C-4). ESI MS m/z (rel%): 242 (100) [M+Na], 244 (33) [M+2+Na]. HR MS (ESI) for $C_{10}H_6ClN_3ONa$ [M+Na]: calcd 242.00916; found 242.00915.

4.4. 4,5-Dichloro-9*H*-pyrimido[4,5-*b*]indole (9)

Compound **9** was prepared according to literature conditions[7] from crude **8** (15.0 g; 68.3 mmol). Desired product **9** (12.0 g; 74%) was obtained as brown powder. For analysis, it was purified by column chromatography (chloroform/MeOH, 3% MeOH); mp 252 °C. IR: 3048, 2967, 2804, 1590, 1559, 1541, 1441, 1397, 1306, 1159, 988, 775. ^1H NMR (500.0 MHz, DMSO-d_6): 7.44 (m, 1H, H-6); 7.59 (m, 2H, H-7, H-8); 8.80 (s, 1H, H-2); 13.17 (br s, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 110.45 (C-4a); 111.50 (CH-8); 115.73 (C-4b); 123.91 (CH-6); 127.23 (C-5); 129.47 (CH-7); 140.42 (C-8a); 151.52 (C-4); 154.16 (CH-2); 156.45 (C-9a). ESI MS m/z (rel%): 238 (100) [M+H], 240 (66) [M+2+H], 242 (16) [M+4+H]. HR MS (ESI) for $C_{10}H_5Cl_2N_3$ [M+H]: calcd 236.9861; found 236.9860.

4.5. 4,5-Dichloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimido[4,5-*b*]indole (10)

Compound **10** was prepared according to literature conditions[7] from crude **9** (1.0 g; 4.2 mmol). Desired product **10** (1.4 g; 52%) was obtained as white crystals after flash chromatography in hexane/EtOAc 7:1; mp 169–173 °C. IR: 3071, 2925, 1732, 1720, 1560, 1451, 1279, 1261, 1244, 1109, 1088, 1068, 1051, 1034, 1023, 703. ^1H NMR (499.8 MHz, DMSO-d_6): 4.69 (dd, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.2, H-5′b); 4.85 (dd, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.3, H-5′a); 4.90 (ddd, $J_{4',3'}$ = 6.7, $J_{4',5'}$ = 4.2, 3.3, H-4′); 6.37 (t, $J_{3',2'}$ = $J_{3',4'}$ = 6.7, H-3′); 6.54 (dd, $J_{2',3'}$ = 6.7, $J_{2',1'}$ = 4.3, H-2′); 7.08 (d, $J_{1',2'}$ = 4.3, H-1′); 7.41 (m, 2H, H-*m*-Bz-2′); 7.49, 7.50 (2 × m, 2 × 2H, H-*m*-Bz-3′,5′); 7.53 (m, 2H, H-6,7); 7.62 (m, 1H, H-*p*-Bz-2′); 7.68 (m, 2H, H-*p*-Bz-3′,5′); 7.83 (m, 2H, H-*o*-Bz-2′); 7.92 (m, 2H, H-*o*-Bz-5′); 7.98 (m, 2H, H-*o*-Bz-3′); 8.10 (m, 1H, H-8); 8.81 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 63.05 (CH_2-5′); 70.17 (CH-03′); 72.40 (CH-2′); 78.80 (CH-4′); 86.52 (CH-1′); 110.99 (CH-8); 111.70 (C-4a); 116.19 (C-4b); 125.45 (CH-6); 127.66 (C-5); 128.64, 128.79 (C-*i*-Bz); 128.90, 128.95, 128.97 (CH-*m*-Bz); 129.33 (CH-*o*-Bz-5′, C-*i*-Bz); 129.51 (CH-*o*-Bz-2′); 129.61 (CH-*o*-Bz-3′); 129.84 (CH-7); 133.80, 134.10 (CH-*p*-Bz); 140.01 (C-8a); 152.14 (C-4); 153.91 (CH-2); 155.62 (C-9a); 164.81 (COPh-2′); 164.98 (COPh-3′); 165.52 (COPh-5′). ESI MS m/z (rel%): 682 (100) [M+H], 684 (66) [M+2+H], 686 (26) [M+4+H]. HR MS (ESI) for $C_{36}H_{26}Cl_2N_3O_7$ [M+H]: calcd 682.11480; found 682.11423; for $C_{36}H_{25}Cl_2N_3O_7Na$ [M+Na]: calcd 704.09660; found 704.09618.

4.6. 4-Amino-5-chloro-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (13)

Compound **13** was prepared as described for **14** from 4,5-dichloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimido[4,5-*b*]indole (**10**) (0.5 g; 1.4 mmol) to give pure nucleoside **13** (256 mg, 76%) as white crystals; mp 117–122 °C; $[\alpha]_D$ −32.4 (0.26). IR (ATR): ν = 3471, 3321, 3188, 2925, 2859, 1627, 1567, 1556, 1449, 1319, 1188, 1120, 1079, 1041, 986, 770. ^1H NMR (600.1 MHz, DMSO-d_6): 3.65 (ddd, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.1, $J_{5'b,4'}$ = 3.7, H-5′b); 3.71 (ddd, J_{gem} = 12.0, $J_{5'a,OH}$ = 4.9, $J_{5'a,4'}$ = 3.1, H-5′a); 3.96 (dt, $J_{4',5'}$ = 3.7, 3.1, $J_{4',3'}$ = 3.1, H-4′); 4.19 (ddd, $J_{3',2'}$ = 5.7, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 3.1, H-3′); 4.75 (ddd, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.7, H-2′); 5.13 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3′); 5.19 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2′); 5.33 (dd, 1H, $J_{OH,5'}$ = 6.1, 4.9, OH-5′); 6.43 (d, $J_{1',2'}$ = 7.3, H-1′); 7.26 (br s, 1H, NH_aH_b); 7.36 (dd, 1H, $J_{6,7}$ = 7.8, $J_{6,8}$ = 1.2, H-6); 7.39 (dd, 1H, $J_{7,8}$ = 8.0, $J_{7,6}$ = 7.8, H-7); 7.65 (br s, 1H, NH_aH_b); 7.94 (dd, 1H, $J_{8,7}$ = 8.0, $J_{8,6}$ = 1.2, H-8); 8.32 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 61.86 (CH_2-5′); 70.25 (CH-3′); 70.55 (CH-2′); 85.66 (CH-4′); 87.23 (CH-1′); 94.50 (C-4a); 111.66 (CH-8); 118.92 (C-4b); 122.78 (CH-6); 124.47 (C-5); 126.30 (CH-7); 137.98 (C-8a); 155.18 (CH-2); 155.80 (C-9a); 157.77 (C-4). ESI MS m/z (rel%): 351 (100) [M+H], 373 (40) [M+Na]. HR MS (ESI) for $C_{15}H_{16}Cl_1N_4O_4$ [M+H]: calcd 351.08546; found 351.08552. Anal. Calcd for $C_{15}H_{15}ClN_4O_4 \cdot 0.55\ CH_3OH$: C, 50.70; H, 4.71; N, 15.21. Found: C, 50.85; H, 4.76; N, 15.09.

4.7. 4-Amino-6-chloro-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (14)

4,6-Dichloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimido[4,5-*b*]indole (**11**) (2.8 g; 4.1 mmol) was dissolved in dioxane (10 ml) and 30% aqueous ammonia (30 ml) was added. Reaction mixture was stirred in screw-cap pressure glass tube at 100 °C for 24 h, then cooled to rt and filtered. After drying under reduced pressure, desired product **14** (1.38 g, 96%) was observed as white crystals; mp 174 °C. $[\alpha]_D$ −35.8 (0.29). IR (ATR): ν = 3426, 3325, 3159, 2956, 2931, 1721, 1703, 1663, 1644, 1597, 1569, 1451, 1433, 1288, 1269, 1177, 1131, 1096, 1062, 1024, 1008, 905, 848, 829, 794, 708. ^1H NMR (500.0 MHz, DMSO-d_6): 3.63 (ddd, J_{gem} = 12.1, $J_{5'b,OH}$ = 6.4, $J_{5'b,4'}$ = 3.7, H-5′b); 3.70 (ddd, J_{gem} = 12.1, $J_{5'a,OH}$ = 4.6, $J_{5'a,4'}$ = 3.3, H-5′a); 3.96 (ddd, $J_{4',5'}$ = 3.7, 3.3, $J_{4',3'}$ = 2.8, H-4′); 4.18 (ddd, $J_{3',2'}$ = 5.9, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 2.8, H-3′); 4.77 (ddd, $J_{2',1'}$ = 7.5, $J_{2',OH}$ = 6.7, $J_{2',3'}$ = 5.9, H-2′); 5.14 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3′); 5.20 (d, 1H, $J_{OH,2'}$ = 6.7, OH-2′); 5.42 (dd, 1H, $J_{OH,5'}$ = 6.4, 4.6, OH-5′); 6.33 (d, $J_{1',2'}$ = 7.5, H-1′); 7.39 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 7.46 (br s, 2H, NH_2); 7.88 (d, 1H, $J_{8,7}$ = 8.8, H-8); 8.30 (s, 1H, H-2); 8.51 (d, 1H, $J_{5,7}$ = 2.1, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.01 (CH_2-5′); 70.47 (CH-3′); 70.82 (CH-2′); 85.68 (CH-4′); 87.23 (CH-1′); 95.29 (C-4a); 113.32 (CH-8); 120.80 (CH-5); 121.76 (C-4b); 124.63 (CH-7); 126.06 (C-6); 134.72 (C-8a); 155.23 (CH-2); 155.88 (C-9a); 157.97 (C-4). ESI MS m/z (rel%): 373 (100) [M+Na], 351 (70) [M+H]. HR MS (ESI) for $C_{15}H_{16}O_4N_4Cl$ [M+H]: calcd 351.08546; found 351.08549. Anal. Calcd for $C_{15}H_{15}ClN_4O_4 \cdot 0.2\ CH_3OH$: C, 51.11; H, 4.46; N, 15.69. Found: C, 51.62; H, 4.56; N, 15.89.

4.8. 4-Amino-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (15)

Compound **15** was prepared as describe for **14** from protected nucleoside **12** (0.8 g; 1.24 mmol) to give pure nucleoside **15**

(282 mg, 72%) as white crystals; mp 237–240 °C; $[\alpha]_D$ −47.0 (0.30); IR (ATR): ν = 3467, 3444, 3341, 2373, 2169, 2039, 1656, 1596, 1583, 1567, 1449, 1316, 1198, 1089, 1075, 1024, 740. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.63 (ddd, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.9, $J_{5'b,4'}$ = 3.7, H-5′b); 3.71 (ddd, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 4.5, $J_{5'a,4'}$ = 3.2, H-5′a); 3.96 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.2, $J_{4',3'}$ = 3.0, H-4′); 4.19 (ddd, 1H, $J_{3',2'}$ = 5.8, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 3.0, H-3′); 4.83 (ddd, 1H, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.7, $J_{2',3'}$ = 5.8, H-2′); 5.15 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3′); 5.20 (d, 1H, $J_{OH,2'}$ = 6.7, OH-2′); 5.48 (dd, 1H, $J_{OH,5'}$ = 6.9, 4.5, OH-5′); 6.33 (d, 1H, $J_{1',2'}$ = 7.3, H-1′); 7.29 (ddd, 1H, $J_{6,5}$ = 7.8, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.0, H-6); 7.32 (br s, 2H, NH_2); 7.39 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.2, H-7); 7.82 (dd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.7, H-8); 8.34 (dd, 1H, $J_{5,6}$ = 7.8, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.7, H-5); 8.35 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.13 (CH_2-5′); 70.56 (CH-3′); 70.76 (CH-2′); 85.60 (CH-4′); 87.28 (CH-1′); 95.95 (C-4a); 111.67 (CH-8); 120.29 (C-4b); 121.22 (CH-6); 121.47 (CH-5); 124.90 (CH-7); 136.29 (C-8a); 154.53 (CH-2); 155.25 (C-9a); 157.93 (C-4). ESI MS m/z (rel%): 317 (11) [M+H] 339 (100) [M+Na]. HR MS (ESI) for $C_{15}H_{17}N_4O_4$ [M+H]: calcd 317.12443; found 317.12448; for $C_{15}H1_6N_4O_4Na$ [M+Na]: calcd 339.10638; found 339.10638. Anal. Calcd for $C_{15}H_{16}N_4O_4{\cdot}1\ H_2O$: C, 53.89; H, 5.43; N, 16.76. Found: C, 54.05; H, 5.28; N, 16.62.

4.9. General procedure for the Suzuki coupling

Free aminonucleoside **13** or **14** (0.5 mmol), boronic acid (1.5 equiv), K_2CO_3 (3 equiv), $Pd(OAc)_2$ (0.05 equiv) and X-Phos (0.1 equiv) were dissolved in DMF and heated to 100 °C for 12 h. The volatiles were removed in vacuo and the residue was purified by RP-HPFC on C-18 (0→100% MeOH in water). Products were obtained as white powders or crystals after recrystallization from MeOH/H_2O mixtures.

4.10. General procedure for the Stille coupling

Free aminonucleoside **13** or **14** (0.5 mmol), tributylstannane (1.5 equiv), $Pd(OAc)_2$ (0.05 equiv) and X-Phos (0.1 eqiuv) were dissolved in DMF and heated to 100 °C for 12 h. The volatiles were removed in vacuo and the residue was first put on silica column containing 15% of KF. Column was washed with 3 L of hexane, than with gradient of MeOH in DCM (2–40% MeOH). Fractions containing crude product were evaporated and purified by RP-HPFC on C-18 (0→100% MeOH in water). Products were obtained as white powders or crystals after recrystallization from MeOH/H_2O mixtures.

4.11. 4-Amino-6-phenyl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (16a)

Nucleoside **14** (50 mg, 0.14 mmol), phenylboronic acid (25 mg, 0.21 mmol), K_2CO_3 (58 mg, 0.42 mmol), $Pd(OAc)_2$ (1.6 mg, 0.007 mmol) and X-Phos (6.7 mg, 0.014 mmol) were dissolved in acetonitrile/water mixture (3:2, 5 ml) and heated to 100 °C for 16 h. The volatiles were removed in vacuo and the residue was purified by RP-HPFC on C-18 (0→100% MeOH in water). Product **16a** (52 mg, 93%) was obtained as white powder after recrystallization from MeOH/H_2O mixture; mp 216–218 °C; $[\alpha]_D$ −49.8 (0.20). IR (ATR): ν = 3437, 3329, 3156, 1651, 1629, 1594, 1570, 1468, 1405, 1316, 114, 1045, 1022, 988, 717. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.65 (ddd, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.8, $J_{5'b,4'}$ = 3.7, H-5′b); 3.73 (ddd, J_{gem} = 12.0, $J_{5'a,OH}$ = 4.5, $J_{5'a,4'}$ = 3.2, H-5′a); 3.98 (ddd, $J_{4',5'}$ = 3.7, 3.2, $J_{4',3'}$ = 2.9, H-4′); 4.20 (ddd, $J_{3',2'}$ = 5.7, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 2.9, H-3′); 4.84 (ddd, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.8, $J_{2',3'}$ = 5.7, H-2′); 5.15 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3′); 5.22 (d, 1H, $J_{OH,2'}$ = 6.8, OH-2′); 5.50 (dd, 1H, $J_{OH,5'}$ = 6.8, 4.5, OH-5′); 6.36 (d, $J_{1',2'}$ = 7.3, H-1′); 7.35 (m, 1H, H-*p*-Ph); 7.48 (m, 4H, NH_2, H-*m*-Ph); 7.71 (dd, 1H, $J_{7,8}$ = 8.7, $J_{7,5}$ = 1.9, H-7); 7.86 (m, 2H, H-*o*-Ph); 7.91 (dd, 1H, $J_{8,7}$ = 8.9, $J_{8,5}$ = 0.4, H-8); 8.29 (s, 1H, H-2); 8.62 (dd, 1H, $J_{5,7}$ = 1.9, $J_{5,8}$ = 0.4, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.15 (CH_2-5′); 70.61 (CH-3′); 70.89 (CH-2′); 85.69 (CH-4′); 87.32 (CH-1′); 96.13 (C-4a); 112.13 (CH-8); 119.36 (CH-5); 121.08 (C-4b); 123.81 (CH-7); 127.00 (CH-*p*-Ph); 127.25 (CH-*o*-Ph); 128.96 (CH-*m*-Ph); 133.82 (C-6); 135.83 (C-8a); 140.78 (C-*i*-Ph); 154.74 (CH-2); 155.77 (C-9a); 158.03 (C-4). ESI MS m/z (rel%): 393 (100) [M+H]. HR MS (ESI) for $C_{21}H_{21}N_4O_4$ [M+H]: calcd 393.15573; found 393.15563. Anal. Calcd for $C_{21}H_{20}N_4O_4$: C, 61.05; H, 5.44; N, 13.56. Found: C, 60.92; H, 5.35; N, 13.44.

4.12. 4-Amino-6-furan-2-yl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (16b)

Free aminonucleoside **14** (150 mg, 0.43 mmol) and 2-(tributylstannyl)furane (230 mg, 0.64 mmol) were used. Desired product **16b** (111 mg, 68%) was obtained as white powder; mp 219–222 °C; $[\alpha]_D$ −34.8 (0.28). IR (ATR): ν = 3343, 3215, 3128, 1633, 1597, 1570, 1463, 1311, 1084, 1043, 1012, 800, 736. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.64 (dd, J_{gem} = 11.9, $J_{5'b,4'}$ = 3.7, H-5′b); 3.72 (dd, J_{gem} = 11.9, $J_{5'a,4'}$ = 3.2, H-5′a); 3.97 (ddd, $J_{4',5'}$ = 3.7, 3.2, $J_{4',3'}$ = 2.9, H-4′); 4.20 (dd, $J_{3',2'}$ = 5.8, $J_{3',4'}$ = 2.9, H-3′); 4.82 (dd, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.8, H-2′); 5.45 (br s, 3H, OH-2′,3′,5′); 6.33 (d, $J_{1',2'}$ = 7.2, H-1′); 6.62 (dd, 1H, $J_{4,3}$ = 3.3, $J_{4,5}$ = 1.8, H-4-furyl); 7.04 (dd, 1H, $J_{3,4}$ = 3.3, $J_{3,5}$ = 0.8, H-3-furyl); 7.46 (br s, 2H, NH_2); 7.737 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.7, H-7); 7.743 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.8, H-5-furyl); 7.87 (d, 1H, $J_{8,7}$ = 8.6, H-8); 8.29 (s, 1H, H-2); 8.64 (d, 1H, $J_{5,7}$ = 1.7, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.11 (CH_2-5′); 70.58 (CH-3′); 70.94 (CH-2′); 85.67 (CH-4′); 87.38 (CH-1′); 95.93 (C-4a); 104.96 (CH-3-furyl); 112.01 (CH-8); 112.19 (CH-4-furyl); 116.58 (CH-5); 120.58 (CH-7); 120.80 (C-4b); 124.36 (C-6); 135.67 (C-8a); 142.33 (CH-5-furyl); 154.14 (C-2-furyl); 154.83 (CH-2); 155.79 (C-9a); 157.98 (C-4). ESI MS m/z (rel%): 383 (100) [M+H]. HR MS (ESI) for $C_{19}H_{19}N_4O_5$ [M+H]: calcd 383.13500; found 383.13516. Anal. Calcd for $C_{19}H_{18}N_4O_5{\cdot}1\ CH_3OH$: C, 57.97; H, 5.35; N, 13.52. Found: C, 58.06; H, 5.32; N, 13.60.

4.13. 4-Amino-6-furan-3-yl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (16c)

Free aminonucleoside **14** (100 mg, 0.29 mmol) and furan-3-yl boronic acid (48 mg, 0.43 mmol) were used. Desired product **16c** (111 mg, 72%) was obtained as white powder; mp 121–122 °C; $[\alpha]_D$ −41.6 (0.33). IR (ATR): ν = 3330, 3164, 1652, 1593, 1571, 1464, 1307, 1077, 1046, 1029, 794, 596. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.64 (bddd, J_{gem} = 11.8, $J_{5'b,OH}$ = 6.1, $J_{5'b,4'}$ = 3.3, H-5′b); 3.72 (bdt, J_{gem} = 11.8, $J_{5'a,OH}$ = $J_{5'a,4'}$ = 3.3, H-5′a); 3.97 (td, $J_{4',5'}$ = 3.3, $J_{4',3'}$ = 2.7, H-4′); 4.20 (ddd, $J_{3',2'}$ = 5.7, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 2.7, H-3′); 4.82 (ddd, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.7, $J_{2',3'}$ = 5.7, H-2′); 5.13 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3′); 5.19 (d, 1H, $J_{OH,2'}$ = 6.7, OH-2′); 5.47 (bdd, 1H, $J_{OH,5'}$ = 6.1, 3.3, OH-5′); 6.33 (d, $J_{1',2'}$ = 7.3, H-1′); 7.19 (dd, 1H, $J_{4,5}$ = 1.8, $J_{4,2}$ = 0.8, H-4-furyl); 7.43 (br s, 2H, NH_2); 7.67 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.7, H-7); 7.76 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,2}$ = 1.5, H-5-furyl); 7.83 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.4, H-8); 8.25 (dd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.8, H-2-furyl); 8.28 (s, 1H, H-2); 8.51 (dd, 1H, $J_{5,7}$ = 1.7, $J_{5,8}$ = 0.4, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.10 (CH_2-5′); 70.56 (CH-3′); 70.89 (CH-2′); 85.64 (CH-4′); 87.33 (CH-1′); 95.93 (C-4a); 109.30 (CH-4-furyl); 112.01 (CH-8); 117.99 (CH-5); 120.83 (C-4b); 122.64 (CH-7); 125.52 (C-6); 126.55 (C-3-furyl); 135.35 (C-8a); 138.94 (CH-2-furyl); 144.16 (CH-5-furyl); 154.68 (CH-2); 155.59 (C-9a); 157.90 (C-4). ESI MS m/z (rel%): 383 (30) [M+H]; 405 (100) [M+Na]. HR MS (ESI) for $C_{19}H_{19}N_4O_5$ [M+H]: calcd 383.13500; found 383.13495; for $C_{19}H_{18}N_4O_5Na$ [M+Na]: calcd 405.11694; found 405.11683. Anal. Calcd for

$C_{19}H_{18}N_4O_5 \cdot 1\ H_2O$: C, 57.00; H, 5.03; N, 13.99. Found: C, 56.85; H, 4.89; N, 13.96.

4.14. 4-Amino-6-thiophen-2-yl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (16d)

Free aminonucleoside **14** (100 mg, 0.29 mmol) and 2-(tributylstannyl)thiophene (160 mg, 0.43 mmol) were used. Desired product **16d** (83 mg, 73%) was obtained as white powder; mp 228–230 °C; $[\alpha]_D$-50.0 (0.18). IR (ATR): ν = 3396, 3301, 1688, 1674, 1659, 1545, 1511, 1502, 1401, 1381, 1243, 1160, 965, 762. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.64 (br d, J_{gem} = 12.4, H-5′b); 3.72 (bdd, J_{gem} = 12.4, $J_{5'a,4'}$ = 3.1, H-5′a); 3.97 (q, $J_{4',5'} = J_{4',3'}$ = 3.1, H-4′); 4.20 (dd, $J_{3',2'}$ = 5.7, $J_{3',4'}$ = 3.1, H-3′); 4.82 (dd, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.7, H-2′); 5.20 (br s, 2H, OH-2′,3′); 5.47 (br s, 1H, OH-5′); 6.33 (d, $J_{1',2'}$ = 7.3, H-1′); 7.17 (dd, 1H, $J_{4,5}$ = 5.1, $J_{4,3}$ = 3.6, H-4-thienyl); 7.51 (br s, 2H, NH_2); 7.51 (dd, 1H, $J_{5,4}$ = 5.1, $J_{5,3}$ = 1.2, H-5-thienyl); 7.63 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.8, H-7); 7.69 (dd, 1H, $J_{3,4}$ = 3.6, $J_{3,5}$ = 1.2, H-3-thienyl); 7.89 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.4, H-8); 8.29 (s, 1H, H-2); 8.63 (dd, 1H, $J_{5,7}$ = 1.8, $J_{5,8}$ = 0.4, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.11 (CH_2-5′); 70.57 (CH-3′); 70.89 (CH-2′); 85.68 (CH-4′); 87.28 (CH-1′); 95.91 (C-4a); 112.26 (CH-8); 117.93 (CH-5); 121.05 (C-4b); 122.92 (CH-7); 123.54 (CH-3-thienyl); 124.98 (CH-5-thienyl); 127.67 (C-6); 128.50 (CH-4-thienyl); 135.73 (C-8a); 144.41 (C-2-thienyl); 154.90 (CH-2); 155.85 (C-9a); 158.01 (C-4). ESI MS *m*/*z* (rel%): 399 (65) [M+H]; 421 (100) [M+Na]. HR MS (ESI) for $C_{19}H_{19}N_4O_4S$ [M+H]: calcd 399.11215; found 399.11219. Anal. Calcd for $C_{19}H_{18}N_4O_4S \cdot 1.1\ H_2O$: C, 54.56; H, 4.87; N, 13.40; S, 7.67. Found: C, 54.31; H, 4.78; N, 13.28; S, 7.58.

4.15. 4-Amino-6-thiophen-3-yl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (16e)

Nucleoside **16e** was prepared according to general procedure. Free aminonucleoside **14** (50 mg, 0.14 mmol) and thiophene-3-yl boronic acid (27.0 mg, 0.21 mmol) were used. Desired product **16e** (43 mg, 77%) was obtained as white powder; mp 167–169 °C; $[\alpha]_D$ −38.9 (0.30). IR (ATR): ν = 3373, 3364, 1640, 1630, 1469, 1080, 782. ^{1}H NMR (600.1 MHz, DMSO-d_6): 3.65 (ddd, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.7, $J_{5'b,4'}$ = 3.7, H-5′b); 3.72 (ddd, J_{gem} = 12.0, $J_{5'a,OH}$ = 4.5, $J_{5'a,4'}$ = 3.4, H-5′a); 3.99 (ddd, $J_{4',5'}$ = 3.7, 3.4, $J_{4',3'}$ = 2.8, H-4′); 4.21 (bdd, $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 2.8, H-3′); 4.83 (bdd, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.5, H-2′); 5.14 (br s, 1H, OH-3′); 5.20 (br s, 1H, OH-2′); 5.47 (dd, 1H, $J_{OH,5'}$ = 6.7, 4.5, OH-5′); 6.34 (d, $J_{1',2'}$ = 7.3, H-1′); 7.46 (br s, 2H, NH_2); 7.66 (dd, 1H, $J_{5,4}$ = 5.0, $J_{5,2}$ = 3.0, H-5-thienyl); 7.79 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.7, H-7); 7.82 (dd, 1H, $J_{4,5}$ = 5.0, $J_{4,2}$ = 1.4, H-4-thienyl); 7.85 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.5, H-8); 7.97 (dd, 1H, $J_{2,5}$ = 3.0, $J_{2,4}$ = 1.4, H-2-thienyl); 8.29 (s, 1H, H-2); 8.63 (dd, 1H, $J_{5,7}$ = 1.7, $J_{5,8}$ = 0.5, H-5). ^{13}C NMR (150.9 MHz, DMSO-d_6): 62.09 (CH_2-5′); 70.54 (CH-3′); 70.87 (CH-2′); 85.62 (CH-4′); 87.31 (CH-1′); 96.03 (C-4a); 111.99 (CH-8); 118.55 (CH-5); 119.96 (CH-2-thienyl); 120.90 (C-4b); 123.22 (CH-7); 126.74 (CH-5-thienyl); 126.93 (CH-4-thienyl); 128.93 (C-6); 135.46 (C-8a); 142.14 (C-3-thienyl); 154.69 (CH-2); 155.70 (C-9a); 157.93 (C-4). ESI MS *m*/*z* (rel%): 399 (100) [M+H]; 421 (95) [M+Na]. HR MS (ESI) for $C_{19}H_{19}N_4O_4S$ [M+H]: calcd 399.11215; found 399.11211. Anal. Calcd for $C_{19}H_{18}N_4O_4S \cdot 1.15\ H_2O$: C, 54.45; H, 4.88; N, 13.37. Found: C, 54.39; H, 4.92; N, 13.47.

4.16. 4-Amino-6-benzofuran-2-yl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (16f)

Nucleoside **16f** was prepared according to general procedure. Free aminonucleoside **14** (150 mg, 0.43 mmol) and benzofuran-2-yl boronic acid (140.0 mg, 0.86 mmol) were used. Desired product **16f** (138 mg, 75%) was obtained as white crystals; mp 265–268 °C; $[\alpha]_D$ −53.3 (0.30). IR (ATR): ν = 3342, 3202, 2940, 2372, 1632, 1594, 1569, 1452, 1084, 1042, 799, 750. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.66 (bddd, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.1, $J_{5'b,4'}$ = 3.6, H-5′b); 3.74 (bdt, J_{gem} = 12.0, $J_{5'a,OH} = J_{5'a,4'}$ = 3.6, H-5′a); 4.00 (td, $J_{4',5'}$ = 3.6, $J_{4',3'}$ = 2.8, H-4′); 4.22 (bdd, $J_{3',2'}$ = 5.6, $J_{3',4'}$ = 2.8, H-3′); 4.86 (bdd, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.6, H-2′); 5.16 (br s, 1H, OH-3′); 5.24 (br s, 1H, OH-2′); 5.47 (bdd, 1H, $J_{OH,5'}$ = 6.1, 3.6, OH-5′); 6.37 (d, $J_{1',2'}$ = 7.2, H-1′); 7.27 (ddd, 1H, $J_{5,4}$ = 7.5, $J_{5,6}$ = 7.2, $J_{5,7}$ = 1.0, H-5-benzofuryl); 7.31 (ddd, 1H, $J_{6,7}$ = 8.1, $J_{6,5}$ = 7.2, $J_{6,4}$ = 1.5, H-6-benzofuryl); 7.54 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 7.55 (br s, 2H, NH_2); 7.64 (dtd, 1H, $J_{7,6}$ = 8.1, $J_{7,3} = J_{7,5}$ = 1.0, $J_{7,4}$ = 0.7, H-7-benzofuryl); 7.68 (ddd, 1H, $J_{4,5}$ = 7.5, $J_{4,6}$ = 1.5, $J_{4,7}$ = 0.7, H-4-benzofuryl); 7.96 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.5, H-7); 7.98 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.6, H-8); 8.33 (s, 1H, H-2); 8.89 (dd, 1H, $J_{5,7}$ = 1.5, $J_{5,8}$ = 0.6, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.09 (CH_2-5′); 70.57 (CH-3′); 70.91 (CH-2′); 85.73 (CH-4′); 87.36 (CH-1′); 95.90 (C-4a); 101.03 (CH-3-benzofuryl); 111.14 (CH-7-benzofuryl); 112.17 (CH-8); 117.97 (CH-5); 120.94 (C-4b); 120.97 (CH-3-benzofuryl); 121.60 (CH-7); 123.32 (CH-5-benzofuryl); 123.44 (C-6); 124.42 (CH-6-benzofuryl); 129.37 (C-3a-benzofuryl); 136.60 (C-8a); 154.33 (C-7a-benzofuryl); 155.02 (CH-2); 155.97 (C-9a); 156.49 (C-2-benzofuryl); 158.03 (C-4). ESI MS *m*/*z* (rel%): 433 (92) [M+H]; 455 (100) [M+Na]. HR MS (ESI) for $C_{23}H_{21}N_4O_5$ [M+H]: calcd 433.15065; found 433.15073; calcd 455.13259; for $C_{23}H_{20}N_4O_5Na$ [M+H]: found 455.13266. Anal. Calcd for $C_{23}H_{20}N_4O_5$ 1.3 H_2O: C, 60.60; H, 5.00; N, 12.29. Found: C, 60.85; H, 4.89; N, 12.04.

4.17. 4-Amino-5-phenyl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (17a)

Nucleoside **13** (200 mg, 0.57 mmol), phenylboronic acid (139 mg, 1.14 mmol)), K_2CO_3 (236 mg, 1.14 mmol), $Pd(OAc)_2$ (12.0 mg, 0.05 mmol) and X-Phos (54.0 mg, 0.11 mmol) was dissolved in DMF (5 ml) and heated to 120 °C for 16 h. The volatiles were removed in vacuo and the residue was purified by RP-HPFC on C-18 (0→100% MeOH in water). Product **17a** (62 mg, 28%) was obtained as white powder after recrystallization from MeOH/H_2O mixture; mp 185–157 °C; $[\alpha]_D$ −28.2 (0.26). IR (ATR): ν = 3469, 3378, 2934, 1578, 1561, 1456, 1321, 1092, 1029, 794, 765, 705. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.66 (dd, J_{gem} = 12.0, $J_{5'b,4'}$ = 3.8, H-5′b); 3.73 (dd, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.1, H-5′a); 3.98 (dt, $J_{4',5'}$ = 3.8, 3.1, $J_{4',3'}$ = 3.1, H-4′); 4.22 (dd, 1H, $J_{3',2'}$ = 5.8, $J_{3',4'}$ = 3.1, H-3′); 4.86 (dd, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.8, H-2′); 5.28, 5.43 (2 × br s, 3H, OH-2′,3′,5′); 6.46 (d, $J_{1',2'}$ = 7.3, H-1′); 7.13 (dd, 1H, $J_{6,7}$ = 7.4, $J_{6,8}$ = 1.0, H-6); 7.45 (dd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.4, H-7); 7.51 (br m, 2H, H-*o*-Ph); 7.56 (m, 3H, H-*m*,*p*-Ph); 7.92 (dd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, H-8); 8.22 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.07 (CH_2-5′); 70.45 (CH-3′); 70.62 (CH-2′); 85.62 (CH-4′); 87.31 (CH-1′); 95.58 (C-4a); 111.20 (CH-8); 119.21 (C-4b); 123.39 (CH-6); 124.86 (CH-7); 128.61 (CH-*p*-Ph); 128.67 (CH-*m*-Ph); 130.04 (CH-*o*-Ph); 135.39 (C-5); 136.72 (C-8a); 141.73 (C-*i*-Ph); 154.37 (CH-2); 155.86 (C-9a); 157.37 (C-4). ESI MS *m*/*z* (rel%): 393 (100) [M+H]; 415 (89) [M+Na]. HR MS (ESI) for $C_{21}H_{21}N_4O_4$ [M+H]: calcd 393.15573; found 393.15573. Anal. Calcd for $C_{21}H_{20}N_4O_4$ 1 H_2O: C, 61.46; H, 5.40; N, 13.65. Found: C, 61.32; H, 5.52; N, 13.48.

4.18. 4-Amino-5-furan-2-yl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (17b)

Compound **17b** was prepared according to general procedure for Stille coupling. Free aminonucleoside **13** (150 mg, 0.43 mmol), $Pd(OAc)_2$ (12 mg, 0.05 mmol), X-Phos (50 mg, 0.1 mmol) and 2-(tributylstannyl)furane (230 mg, 0.64 mmol) were used. RP-HPFC purification furnished nucleoside **17b** (54 mg, 33%) as white solid; mp 112–113 °C; $[\alpha]_D$ −37.8 (0.21). IR (ATR): ν = 3329, 3285, 2373,

2351, 2170, 1557, 1451, 1073, 1040, 788, 739. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.66 (ddd, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.4, $J_{5'b,4'}$ = 3.7, H-5′b); 3.73 (ddd, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 4.7, $J_{5'a,4'}$ = 3.2, H-5′a); 3.98 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.2, $J_{4',3'}$ = 3.0, H-4′); 4.21 (ddd, 1H, $J_{3',2'}$ = 5.8, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 3.0, H-3′); 4.82 (ddd, 1H, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.9, H-2′); 5.15 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3′); 5.22 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2′); 5.41 (dd, 1H, $J_{OH,5'}$ = 6.4, 4.7, OH-5′); 6.44 (d, 1H, $J_{1',2'}$ = 7.3, H-1′); 6.74 (dd, 1H, $J_{3,4}$ = 3.2, $J_{3,5}$ = 0.8, H-3-furyl); 6.77 (dd, 1H, $J_{4,3}$ = 3.2, $J_{4,5}$ = 1.9, H-4-furyl); 7.31 (dd, 1H, $J_{6,7}$ = 7.4, $J_{6,8}$ = 1.0, H-6); 7.46 (dd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.4, H-7); 7.94 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,3}$ = 0.8, H-5-furyl); 8.01 (dd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, H-8); 8.27 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.00 (CH_2-5′); 70.41 (CH-3′); 70.65 (CH-2′); 85.66 (CH-4′); 87.22 (CH-1′); 95.56 (C-4a); 110.77 (CH-3-furyl); 111.92 (CH-4-furyl); 112.93 (CH-8); 119.61 (C-4b); 124.05 (CH-6); 125.22 (C-5); 124.78 (CH-7); 136.86 (C-8a); 144.01 (CH-5-furyl); 152.53 (C-2-furyl); 154.68 (CH-2); 155.93 (C-9a); 157.79 (C-4). ESI MS *m*/*z* (rel%): 383 (43) [M+H]; 405 (100) [M+Na]. HR MS (ESI) for $C_{19}H_{19}N_4O_5$ [M+H]: calcd 383.13500; found 383.13505. Anal. Calcd for $C_{19}H_{18}N_4O_5$: C, 59.68; H, 4.74; N, 14.65. Found: C, 59.96; H, 4.86; N, 14.89.

4.19. 4-Amino-5-thiophen-3-yl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (17e)

Free nucleoside **13** (200 mg, 0.57 mmol), K_2CO_3 (236 mg, 1.71 mmol), $Pd(OAc)_2$ (12.8 mg, 0.053 mmol), X-Phos (54.0 mg, 0.110 mmol) and one third of all amount of 3-furanboronic acid (146 mg, 1.14 mmol) were dissolved in anhydrous DMF (20 ml) and heated to 120 °C for 3 h. Second third of boronic acid was added and reaction was stirred at 120 °C for 3 h. Then, last third of boronic acid was added and reaction was heated for another 3 h at 120 °C. Solvent was evaporated under reduced pressure and crude product was purified by RP-HPFC (MeOH/H_2O, 0→100% MeOH). Nucleoside **17e** was obtained (54 mg, 24%) as white crystals; mp 141 °C; $[\alpha]_D$ −39.4 (0.32). IR (ATR): ν = 3464, 3313, 3200, 2919, 2870, 2372, 2346, 1629, 1577, 1559, 1454, 1320, 1077, 1042, 738. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.65 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 3.5, H-5′b); 3.73 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.2, H-5′a); 3.98 (dt, 1H, $J_{4',5'}$ = 3.5, 3.2, $J_{4',3'}$ = 3.2, H-4′); 4.21 (dd, 1H, $J_{3',2'}$ = 5.8, $J_{3',4'}$ = 3.2, H-3′); 4.84 (dd, 1H, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.8, H-2′); 5.20-5.50 (br m, 3H, OH-2′,3′,5′); 6.44 (d, 1H, $J_{1',2'}$ = 7.3, H-1′); 7.14 (dd, 1H, $J_{6,7}$ = 7.4, $J_{6,8}$ = 1.0, H-6); 7.36 (dd, 1H, $J_{4,5}$ = 4.8, $J_{4,2}$ = 1.3, H-4-thienyl); 7.42 (dd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.4, H-7); 7.74 (dd, 1H, $J_{2,5}$ = 2.9, $J_{2,4}$ = 1.3, H-2-thienyl); 7.80 (dd, 1H, $J_{5,4}$ = 4.8, $J_{5,2}$ = 2.9, H-5-thienyl); 7.91 (dd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, H-8); 8.23 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.04 (CH_2-5′); 70.43 (CH-3′); 70.60 (CH-2′); 85.60 (CH-4′); 87.27 (CH-1′); 95.71 (C-4a); 111.33 (CH-8); 119.75 (C-4b); 123.65 (CH-6); 124.71 (CH-7); 125.65 (CH-2-thienyl); 127.10 (CH-5-thienyl); 130.34 (C-5); 130.58 (CH-4-thienyl); 136.75 (C-8a); 142.07 (C-3-thienyl); 154.38 (CH-2); 155.76 (C-9a); 157.56 (C-4). ESI MS *m*/*z* (rel%): 399 (100) [M+H]. HR MS (ESI) for $C_{19}H_{19}N_4O_4S$ [M+H]: calcd 399.11215; found 399.11218. Anal. Calcd for $C_{19}H_{18}N_4O_4S$ 1.0 H_2O: C, 54.80; H, 4.84; N, 13.45; S, 7.70. Found: C, 54.68; H, 4.86; N, 13.55; S, 7.60.

4.20. 4-Amino-5-butyl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (17g)

Compound **17g** was prepared according to general conditions for Stille coupling. Free aminonucleoside **13** (150 mg, 0.43 mmol) and 2-(tributylstannyl)thiophene (230 mg, 0.64 mmol) were used. Unexpected product **17g** (66 mg, 42%) was obtained as white powder; mp 125–127 °C; $[\alpha]_D$ −31.2 (0.24). IR (ATR): ν = 3542, 3384, 3363, 2965, 2373, 2169, 1637, 1591, 1559, 1456, 1327, 1056, 1023, 991, 863. ^{1}H NMR (500.0 MHz, DMSO-d_6): 0.88 (t, 3H, J_{vic} = 7.4, $CH_3CH_2CH_2CH_2$); 1.33 (m, 2H, $CH_3CH_2CH_2CH_2$); 1.63 (m, 2H, $CH_3CH_2CH_2CH_2$); 3.22 (dd, 2H, J_{vic} = 8.4, 7.1, $CH_3CH_2CH_2CH_2$); 3.63 (ddd, J_{gem} = 11.9, $J_{5'b,OH}$ = 6.5, $J_{5'b,4'}$ = 4.0, H-5′b); 3.71 (ddd, J_{gem} = 11.9, $J_{5'a,OH}$ = 4.8, $J_{5'a,4'}$ = 3.2, H-5′a); 3.95 (dt, $J_{4',5'}$ = 4.0, 3.3, $J_{4',3'}$ = 3.3, H-4′); 4.20 (br m, 1H, H-3′); 4.83 (bddd, $J_{2',1'}$ = 7.1, $J_{2',OH}$ = 6.3, $J_{2',3'}$ = 4.3, H-2′); 5.12 (br s, 1H, OH-3′); 5.17 (d, 1H, $J_{OH,2'}$ = 6.3, OH-2′); 5.39 (dd, 1H, $J_{OH,5'}$ = 6.5, 4.8, OH-5′); 6.39 (d, $J_{1',2'}$ = 7.1, H-1′); 6.76 (br s, 2H, NH_2); 7.10 (dd, 1H, $J_{6,7}$ = 7.4, $J_{6,8}$ = 0.8, H-6); 7.31 (dd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.4, H-7); 7.68 (dd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 0.8, H-8); 8.26 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 13.99 ($CH_3CH_2CH_2CH_2$); 21.82 ($CH_3CH_2CH_2CH_2$); 33.27 ($CH_3CH_2CH_2CH_2$); 35.64 ($CH_3CH_2CH_2CH_2$); 62.04 (CH_2-5′); 70.37 (CH-3′); 70.44 (CH-2′); 85.46 (CH-4′); 87.34 (CH-1′); 96.82 (C-4a); 109.68 (CH-8); 119.33 (C-4b); 123.00 (CH-6); 125.27 (CH-7); 135.86 (C-5); 137.13 (C-8a); 153.68 (CH-2); 155.63 (C-9a); 158.24 (C-4). ESI MS *m*/*z* (rel%): 373 (100) [M+H]. HR MS (ESI) for $C_{19}H_{25}N_4O_4$ [M+H]: calcd 373.18703; found 373.18707. Anal. Calcd for $C_{19}H_{24}N_4O_4$ 0.85 CH_3OH: C, 59.66; H, 6.91; N, 14.02. Found: C, 59.86; H, 6.52; N, 13.65.

4.21. 4-Methyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimido[4,5-*b*]indole (18a)

$(Me)_3Al$ (310 μl, 2 M in toluene) was added to solution of nucleoside **12** (200 mg, 0.31 mmol) and $Pd(PPh_3)_4$ (17.9 mg, 0.015 mmol) in THF (8 ml) and the reaction mixture was stirred at 70 °C for 12 h. Volatiles were removed under reduced pressure and crude product was purified by HPFC (10–50% EtOAc in hexane) to give **18a** (120 mg, 63%) as white solid; mp 155–158 °C; IR (ATR): ν = 1733, 1497, 1457, 1420, 1278, 1263, 1136, 1113, 1091, 1072, 727, 707. ^{1}H NMR (499.8 MHz, DMSO-d_6): 2.94 (s, 3H, CH_3); 4.68 (dd, 1H, J_{gem} = 12.5, $J_{5'b,4'}$ = 4.1, H-5′b); 4.82 (dd, 1H, J_{gem} = 12.5, $J_{5'a,4'}$ = 3.2, H-5′a); 4.89 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.1, 3.2, H-4′); 6.36 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3′); 6.65 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.8, H-2′); 6.99 (d, 1H, $J_{1',2'}$ = 4.8, H-1′); 7.40 (m, 2H, H-*m*-Bz-2′); 7.44 (m, 1H, H-6); 7.45 (m, 1H, H-7); 7.49, 7.51 (2 × m, 2 × 2H, H-*m*-Bz-3′,5′); 7.61 (m, 1H, H-*p*-Bz-2′); 7.67, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3′,5′); 7.82 (m, 2H, H-*o*-Bz-2′); 7.95 (m, 2H, H-*o*-Bz-5′); 7.99 (m, 2H, H-*o*-Bz-3′); 8.01 (m, 1H, H-8); 8.20 (m, 1H, H-5); 8.78 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 23.00 (CH_3); 63.24 (CH_2-5′); 70.39 (CH-3′); 72.08 (CH-2′); 78.61 (CH-4′); 85.96 (CH-1′); 111.65 (CH-8); 112.77 (C-4a); 119.84 (C-4b); 122.69 (CH-6); 123.40 (CH-5); 127.71 (CH-7); 128.63, 128.87 (C-*i*-Bz); 128.97, 129.01, 129.03 (CH-*m*-Bz); 129.45 (CH-*o*-Bz-5′, C-*i*-Bz); 129.53 (CH-*o*-Bz-2′); 129.67 (CH-*o*-Bz-3′); 133.84, 134.17 (CH-*p*-Bz); 137.98 (C-8a); 154.01 (CH-2); 154.45 (C-9a); 160.64 (C-4); 164.84 (COPh-2′); 165.10 (COPh-3′); 165.66 (COPh-5′). ESI MS *m*/*z* (rel%): 628 (39) [M+H] 650 (100) [M+Na]. HR MS (ESI) for $C_{37}H_{30}N_3O_7$ [M+H]: calcd 628.20783; found 628.20790; for $C_{37}H_{29}N_3O_7Na$ [M+Na]: calcd 650.18977; found 650.18975.

4.22. 4-Cyclopropyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimido[4,5-*b*]indole (18b)

Nucleoside **18b** was prepared in analogy to literature conditions.[24] THF (2 ml) was added to dried zinc chloride (84.5 mg, 0.62 mmol) under argon atmosphere. Mixture was cooled to −10 °C and cyclopropylmagnesium chloride (1.24 ml, 0.5 M in THF) was added dropwise. After 40 min of stirring the solution of nucleoside **12** (200 mg, 0.31 mmol) and $Pd(PPh_3)_4$ (35.8 mg, 0.03 mmol) in THF (5 ml) was added. The mixture was stirred at 40 °C for 2 h. The reaction mixture was dilluted with water (20 ml) and extracted with ethyl-acetate (3 × 50 ml). The collected organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (20–40% EtOAc in hexane)

furnished **18b** (128 mg, 63%); mp 202–204 °C; $[\alpha]_D$ −51.8 (0.24); ^{1}H NMR (600.1 MHz, DMSO-d_6): 1.24–1.33 (m, 4H, CH_2-cyclopropyl); 2.91 (ttd, 1H, J_{vic} = 7.7, 4.7, ^5J = 0.5, CH-cyclopropyl); 4.68 (dd, 1H, J_{gem} = 12.4, $J_{5'b,4'}$ = 4.1, H-5′b); 4.82 (dd, 1H, J_{gem} = 12.4, $J_{5'a,4'}$ = 3.2, H-5′a); 4.89 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.1, 3.2, H-4′); 6.36 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3′); 6.66 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.7, H-2′); 6.99 (d, 1H, $J_{1',2'}$ = 4.7, H-1′); 7.41 (m, 2H, H-*m*-Bz-2′); 7.42 (m, 1H, H-6); 7.45 (m, 1H, H-7); 7.49, 7.50 (2 × m, 2 × 2H, H-*m*-Bz-3′,5′); 7.61 (m, 1H, H-*p*-Bz-2′); 7.67, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3′,5′); 7.83 (m, 2H, H-*o*-Bz-2′); 7.95 (m, 2H, H-*o*-Bz-5′); 7.98 (m, 2H, H-*o*-Bz-3′); 8.02 (m, 1H, H-8); 8.41 (m, 1H, H-5); 8.72 (d, 1H, ^5J = 0.5, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 11.22 (CH_2-cyclopropyl); 15.04 (CH-cyclopropyl); 63.24 (CH_2-5′); 70.38 (CH-3′); 72.05 (CH-2′); 78.55 (CH-4′); 85.94 (CH-1′); 111.64 (CH-8); 112.20 (C-4a); 119.75 (C-4b); 122.59 (CH-6); 123.01 (CH-5); 127.46 (CH-7); 128.62, 128.84 (C-*i*-Bz); 128.91, 128.95, 128.97 (CH-*m*-Bz); 129.40 (CH-*o*-Bz-5′); 129.42 (C-*i*-Bz); 129.49 (CH-*o*-Bz-2′); 129.62 (CH-*o*-Bz-3′); 133.77, 134.09 (CH-*p*-Bz); 137.92 (C-8a); 154.21 (CH-2); 154.23 (C-9a); 164.80 (COPh-2′); 165.03 (COPh-3′); 165.31 (C-4); 165.60 (COPh-5′). ESI MS *m*/*z* (rel%): 654 (62) [M+H] 676 (100) [M+Na]. HR MS (ESI) for $C_{39}H_{32}N_3O_7$ [M+H]: calcd 654.22348; found 654.22349.

4.23. 4-(*N*,*N*-Dimethylamino)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimido[4,5-*b*]indole (18c)

Dimethylamine (230 μl, 2 M in THF) was added to solution of nucleoside **12** (200 mg, 0.31 mmol) in propan-2-ol (10 ml) and the reaction mixture was stirred at rt for 24 h. Volatiles were removed under reduced pressure and crude product was purified by HPFC (15% EtOAc in hexane) to give **18c** (150 mg, 74%) as white solid; mp 64–67 °C; IR (ATR): *ν* = 1724, 1576, 1556, 1512, 1269, 1251, 1099, 1070, 710. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.24 (s, 6H, $(CH_3)_2$N); 4.67 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.2, H-5′b); 4.81 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.3, H-5′a); 4.86 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.2, 3.3, H-4′); 6.35 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3′); 6.64 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.8, H-2′); 6.97 (d, 1H, $J_{1',2'}$ = 4.8, H-1′); 7.29 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.3, H-7); 7.34 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.1, H-6); 7.42 (m, 2H, H-*m*-Bz-2′); 7.48, 7.51 (2 × m, 2 × 2H, H-*m*-Bz-3′,5′); 7.61 (m, 1H, H-*p*-Bz-2′); 7.67, 7.69 (2 × m, 2 × 1H, H-*p*-Bz-3′,5′); 7.84 (m, 2H, H-*o*-Bz-2′); 7.93 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.1, $J_{8,5}$ = 0.7, H-8); 7.95 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.3, $J_{5,8}$ = 0.7, H-8); 7.97 (m, 2H, H-*o*-Bz-3′); 7.98 (m, 2H, H-*o*-Bz-5′); 8.40 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.10 ($(CH_3)_2$N); 63.36 (CH_2-5′); 70.41 (CH-3′); 72.11 (CH-2′); 78.44 (CH-4′); 85.92 (CH-1′); 98.31 (C-4a); 111.10 (CH-8); 120.13 (C-4b); 121.99 (CH-6); 123.19 (CH-5); 125.23 (CH-7); 128.66, 128.85 (C-*i*-Bz); 128.92, 128.97, 128.98 (CH-*m*-Bz); 129.44 (CH-*o*-Bz-5′, C-*i*-Bz); 129.50 (CH-*o*-Bz-2′); 129.60 (CH-*o*-Bz-3′); 133.79, 134.08, 134.10 (CH-*p*-Bz); 136.48 (C-8a); 153.51 (CH-2); 156.13 (C-9a); 160.12 (C-4); 164.81 (COPh-2′); 165.03 (COPh-3′); 165.63 (COPh-5′). ESI MS *m*/*z* (rel%): 657 (79) [M+H] 679 (100) [M+Na]. HR MS (ESI) for $C_{38}H_{33}N_4O_7$ [M+H]: calcd 657.23438; found 657.23432; for $C_{38}H_{32}N_4O_7Na$ [M+Na]: calcd 679.21632; found 679.21619.

4.24. General procedure for deprotection of 4-substituted nucleosides

Protected nucleoside **18a**–**18c** (0.2 mmol) was disolved in methanol (10 ml) and 1 M solution of MeONa in MeOH (0.3 equiv) was added. Reaction mixture was stirred at rt overnight. Solvent was evaporated under reduced pressure and crude products were purified using RP-HPFC (0→100% of MeOH in H_2O).

4.25. 4-Methyl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (19a)

Deprotection of **18a** (80 mg, 0.12 mmol) according to the general procedure afforded compound **19a** (36 mg, 92%) as white solid: mp 212–215 °C; $[\alpha]_D$ −25.6 (0.21); IR (ATR): *ν* = 3341, 3253, 1598, 1457, 1433, 1111, 1056, 1039, 1012, 994, 749, 739. ^{1}H NMR (600.1 MHz, DMSO-d_6): 2.95 (s, 3H, CH_3); 3.66 (bdt, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = $J_{5'b,OH}$ = 4.0, H-5′b); 3.73 (bdt, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = $J_{5'a,OH}$ = 3.3, H-5′a); 3.98 (ddd, 1H, $J_{4',5'}$ = 4.0, 3.3, $J_{4',3'}$ = 3.1, H-4′); 4.23 (dd, 1H, $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 3.1, H-3′); 4.83 (dd, 1H, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.9, H-2′); 5.22 (br m, 1H, OH-5′); 5.27, 5.31 (2 × br s, 2 × 1H, OH-2′,3′); 6.47 (dd, 1H, $J_{1',2'}$ = 7.2, $J_{1',3'}$ = 0.4, H-1′); 7.43 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.0, H-6); 7.57 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.2, H-7); 8.04 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.7, H-8); 8.21 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.7, H-5); 8.84 (d, 1H, ^5J = 0.3, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 22.93 (CH_3); 61.88 (CH_2-5′); 70.30 (CH-3′); 70.57 (CH-2′); 85.56 (CH-4′); 87.08 (CH-1′); 112.32 (C-4a); 112.95 (CH-8); 119.93 (C-4b); 122.09 (CH-6); 123.07 (CH-5); 127.43 (CH-7); 137.77 (C-8a); 153.82 (CH-2); 154.83 (C-9a); 160.12 (C-4). ESI MS *m*/*z* (rel%): 316 (15) [M+H] 338 (100) [M+Na]. HR MS (ESI) for $C_{16}H_{18}N_3O_4$ [M+H]: calcd 316.12918; found 316.12924; for $C_{16}H_{17}N_3O_4Na$ [M+Na]: calcd 338.11113; found 338.11105. Anal. Calcd for $C_{16}H_{17}N_3O_4$ 0.6 H_2O: C, 58.92; H, 5.62; N, 12.88. Found: C, 58.67; H, 5.28; N, 12.58.

4.26. 4-Cyclopropyl-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (19b)

Deprotection of **18b** (80 mg, 0.12 mmol) according to the general procedure afforded compound **19b** (37 mg, 89%) as white solid: mp 230–231 °C, $[\alpha]_D$ −31.3 (0.26); IR (ATR): *ν* = 3389, 3228, 2185, 2000, 1597, 1085, 1053, 1020, 752 cm^{-1}. ^{1}H NMR (499.8 MHz, DMSO-d_6): 1.22-1.36 (m, 4H, CH_2-cyclopropyl); 2.92 (tt, 1H, J_{vic} = 8.0, 4.7, CH-cyclopropyl); 3.66, 3.73 (2 × br d, 2 × 2H, J_{gem} = 11.7, H-5′); 3.98 (td, 1H, $J_{4',5'}$ = 3.8, $J_{4',3'}$ = 3.0, H-4′); 4.23 (dd, 1H, $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 3.0, H-3′); 4.83 (dd, 1H, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.9, H-2′); 5.22 (br s, 3H, OH-2′,3′,5′); 6.46 (d, 1H, $J_{1',2'}$ = 7.3, H-1′); 7.42 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.0, H-6); 7.56 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.2, H-7); 8.03 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.7, H-8); 8.42 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.7, H-5); 8.77 (d, 1H, ^5J = 0.2, H-2). ^{13}C NMR (12.57 MHz, DMSO-d_6): 10.98, 11.03 (CH_2-cyclopropyl); 14.97 (CH-cyclopropyl); 61.89 (CH_2-5′); 70.30 (CH-3′); 70.51 (CH-2′); 85.55 (CH-4′); 87.07 (CH-1′); 111.86 (C-4a); 112.95 (CH-8); 119.86 (C-4b); 122.05 (CH-6); 122.77 (CH-5); 127.25 (CH-7); 137.76 (C-8a); 154.04 (CH-2); 154.63 (C-9a); 164.87 (C-4). ESI MS *m*/*z* (rel%): 342 (15) [M+H] 364 (100) [M+Na]. HR MS (ESI) for $C_{18}H_{20}N_3O_4$ [M+H]: calcd 342.14483; found 342.14486; for $C_{18}H_{19}N_3O_4Na$ [M+Na]: calcd 364.12678; found 364.12668. Anal. Calcd for $C_{18}H_{19}N_3O_4$ ·1.8 H_2O: C, 57.84; H, 6.09; N, 11.24. Found: C, 57.94; H, 6.12; N, 11.20.

4.27. 4-(*N*,*N*-Dimethylamino)-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (19c)

Deprotection of **18c** (80 mg, 0.12 mmol) according to the general procedure afforded compound **19c** (37 mg, 88%) as white solid: mp 98–101 °C; $[\alpha]_D$ −28.5 (*c* 0.29, DMSO). IR (ATR): *ν* = 3279, 3243, 1580, 1556, 1113, 1070, 1043, 749. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.24 (s, 6H, $(CH_3)_2$N); 3.64 (bdd, 1H, J_{gem} = 11.6, $J_{5'b,4'}$ = 4.0, H-5′b); 3.72 (bdd, 1H, J_{gem} = 11.6, $J_{5'a,4'}$ = 3.2, H-5′a); 3.96 (ddd, 1H, $J_{4',5'}$ = 4.0, 3.2, $J_{4',3'}$ = 3.1, H-4′); 4.21 (dd, 1H, $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 3.1, H-3′); 4.84 (dd, 1H, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.9, H-2′); 5.21 (br s, 2H, OH-2′,3′); 5.35 (br s, 1H, OH-5′); 6.42 (d, 1H, $J_{1',2'}$ = 7.3, H-1′); 7.34 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.1, H-6);

7.43 (ddd, 1H, $J_{7,8}$ = 8.1, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.3, H-7); 7.91 (ddd, 1H, $J_{8,7}$ = 8.1, $J_{8,6}$ = 1.1, $J_{8,5}$ = 0.5, H-8); 7.95 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.3, $J_{5,8}$ = 0.5, H-5); 8.43 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.12 ($(CH_3)_2N$); 62.04 (CH_2-5′); 70.41 (CH-3′); 70.66 (CH-2′); 85.53 (CH-4′); 87.28 (CH-1′); 98.19 (C-4a); 112.20 (CH-8); 120.03 (C-4b); 121.45 (CH-6); 122.94 (CH-5); 125.12 (CH-7); 136.49 (C-8a); 153.22 (CH-2); 156.33 (C-9a); 160.25 (C-4). ESI MS *m*/*z* (rel%): 345 (100) [M+H] 367 (75) [M+Na]. HR MS (ESI) for $C_{17}H_{21}N_4O_4$ [M+H]: calcd 345.15573; found 345.15577. Anal. Calcd for $C_{17}H_{20}N_4O_4$: C, 56.35; H, 6.12; N, 15.46. Found: C, 56.22; H, 6.01; N, 15.33.

4.28. 4-(*N*-Methylamino)-9-β-D-ribofuranosyl-pyrimido[4,5-*b*]indole (21)

Nucleoside **21** was prepared in analogy to literature procedure.[20] Aminonucleoside **15** (100 mg, 0.32 mmol) was dissolved in DMA (5 ml) and MeI (0.2 ml, 0.62 mmol) was added. The reaction mixture was stirred overnight at rt, poured into diethylether and precipitated hydroiodide **20** was filtered out and dried under vacuum. IR (ATR): *v* = 3397, 3302, 2985, 1667, 1502, 1462, 1411, 1243, 1119, 1034, 966, 786, 760. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.70 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 5.1, $J_{5'b,4'}$ = 3.9, H-5′b); 3.73 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 5.1, $J_{5'a,4'}$ = 3.2, H-5′a); 3.89 (s, 3H, CH_3); 4.00 (ddd, 1H, $J_{4',5'}$ = 3.9, 3.2, $J_{4',3'}$ = 2.8, H-4′); 4.21 (ddd, 1H, $J_{3',2'}$ = 5.7, $J_{3',OH}$ = 4.9, $J_{3',4'}$ = 2.8, H-3′); 4.69 (ddd, 1H, $J_{2',1'}$ = 7.5, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.7, H-2′); 5.19 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5′); 5.28 (d, 1H, $J_{OH,3'}$ = 4.9, OH-3′); 5.29 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2′); 6.44 (d, 1H, $J_{1',2'}$ = 7.5, H-1′); 7.51 (ddd, 1H, $J_{6,5}$ = 7.7, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.0, H-6); 7.57 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.2, H-7); 8.22 (d, 1H, $J_{8,7}$ = 8.3, H-8); 8.62 (d, 1H, $J_{5,6}$ = 7.7, H-5); 8.88 (s, 1H, H-2); 8.94 (br s, 2H, NH_2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 38.21 (CH_3); 61.62 (CH_2-5′); 70.08 (CH-3′); 71.03 (CH-2′); 86.07 (CH-4′); 87.08 (CH-1′); 95.58 (C-4a); 114.21 (CH-8); 119.26 (C-4b); 121.81 (CH-5); 123.21 (CH-6); 126.88 (CH-7); 136.37 (C-8a); 149.87 (CH-2); 151.11 (C-4); 152.32 (C-9a). ESI MS *m*/*z* (rel%): 331 (100) [M+H]. HR MS (ESI) for $C_{16}H_{19}O_4N_4$ [M+H]: calcd 331.14008; found 331.14006.

Crude hydroiodide salt **20** was dissolved in 1 M NaOH (5 ml) and heated to 100 °C for 1.5 h. Reaction mixture was cooled to rt, filtered and the filtrate was neutralized with 2 M HCl. The product crystallized from solution after standing at 4 °C for 48 h. Filtration furnished desired compound **21** (66 mg, 64%); mp 242–244 °C; $[\alpha]_D$ −61.8 (0.14); IR (ATR): *v* = 3395, 3119, 2261, 1626, 1608, 1576, 1027, 1011, 999, 740 cm^{-1}. ^{1}H NMR (600.1 MHz, DMSO-d_6): 3.10 (d, 3H, J_{vic} = 4.6, CH_3N); 3.63 (bddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 6.6, $J_{5'b,4'}$ = 3.8, H-5′b); 3.71 (bddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 4.8, $J_{5'a,4'}$ = 3.8, H-5′a); 3.96 (td, 1H, $J_{4',5'}$ = 3.8, $J_{4',3'}$ = 2.9, H-4′); 4.19 (dd, 1H, $J_{3',2'}$ = 5.7, $J_{3',4'}$ = 2.9, H-3′); 4.83 (bdd, 1H, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.7, H-2′); 5.15, 5.20 (2 × br s, 2 × 1H, OH-2′,3′); 5.47 (bdd, 1H, $J_{OH,5'b}$ = 6.6, 4.8, OH-5′); 6.35 (d, 1H, $J_{1',2'}$ = 7.3, H-1′); 7.29 (br q, 1H, J_{vic} = 4.6, NH); 7.32 (ddd, 1H, $J_{6,5}$ = 7.9, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.0, H-6); 7.40 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.84 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.6, H-8); 8.35 (ddd, 1H, $J_{5,6}$ = 7.9, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5); 8.39 (d, 1H, ^5J = 0.4, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 28.05 (CH_3N); 62.14 (CH_2-5′); 70.56 (CH-3′); 70.83 (CH-2′); 85.60 (CH-4′); 87.31 (CH-1′); 96.38 (C-4a); 111.79 (CH-8); 119.96 (C-4b); 121.18 (CH-5,6); 124.84 (CH-7); 136.09 (C-8a); 154.51 (C-9a); 154.53 (CH-2); 157.14 (C-4). ESI MS *m*/*z* (rel%): 331 (100) [M+H]. HR MS (ESI) for $C_{16}H_{19}O_4N_4$ [M+H]: calcd 331.14008; found 331.14005. Anal. Calcd for $C_{16}H_{18}N_4O_4$ 1 H_2O: C, 55.17; H, 5.79; N, 16.08. Found: C, 55.23; H, 5.71; N, 16.02.

4.29. Anti-dengue and cytotoxicity assays

The anti-dengue activity was measured by determining the extent to which the test compounds inhibited replication in Vero cells (European Collection of Cell Cultures). Briefly, two-fold serial dilutions of compounds were added in triplicate in a 96-well plate with 20,000 Vero cells plated day ago in DMEM with L-glutamine supplemented with 2% fetal bovine serum, 100 U of penicillin/mL, 100 μg of streptomycin/mL (all reagents Biotech). After 1 h incubation dengue type 2 virus (DENV-2, obtained from Dr. Jochen Bodem, University of Wurzburg) was added at multiplicity of infection 0.3 IU/cell. After three days incubation at 37 °C in 5% CO_2 incubator, cells were fixed with 4% paraformaldehyde and permeabilized in 0.2% Triton X-100 for 4 min. Cells were washed, incubated with DENV-2 specific antibody (harvested from HB-46 cells, obtained from ATCC) overnight at 4 °C, followed by 1.5 h incubation with Cy3-labeled donkey anti-mouse IgG (Jackson ImmunoResearch Europe) and documented using fluorescence microscope with camera (Carl Zeiss). Images were processed in ImageJ program (NIH) and EC_{50}s were calculated using nonlinear regression analysis with GraphPad Prism version 5.04 for Windows (GraphPad Software).

Cytotoxicity of the compounds was evaluated by incubating serial dilutions of each compound with Vero cell monolayers. After 48 h incubation XTT solution was added (Sigma) for 4 h and formation of orange formazan solution was spectrophotometrically quantified using Victor X3 plate reader (Perkin Elmer). The concentration of compound with 50% cytotoxic effect (CC_{50}) was calculated as above mentioned EC_{50}s.

4.30. UV–vis and fluorescence spectroscopy

The UV–vis spectra were measured on Varian CARY 100 Bio Spectrophotometer (ε is the molar extinction coefficient in L mol^{-1} cm^{-1}) in MeOH. The fluorescence measurements (in MeOH) were performed on a spectrofluorometer Aminco Bowman series 2 with 220–850 nm range, xenon source, excitation and emission wavelength scans, spectral bandwidth 1–16 nm, PMT detector, scan rate 3–6000 nm/min, Saya–Namioka grating monochromator. We used the comparative method of Williams et al. for recording fluorescence quantum yield of a sample Φ_{SA} (a 10 mM solution of quinine sulfate in 0.1 M H_2SO_4 (in H_2O) was chosen as a standard: Φ_{ST} = 0.54). Thus, the fluorescence quantum yield of a sample Φ_{SA} is calculated by the formula of Eq. 1, where the subscripts ST and SA denote standard and sample respectively, Φ is the fluorescent quantum yield, the terms $F_{(SA)}$ and $F_{(ST)}$ are the integrated fluorescence intensities of the sample and the standard, respectively; $A_{(SA)}$ and $A_{(ST)}$ are the optical densities of the sample and the standard solution at the wavelength of excitation, respectively; and $n_{(SA)}$ and $n_{(ST)}$ are the values of the refractive index for the solvents used for the sample, respectively.

$$\Phi_{F(SA)} = \Phi_{F(ST)} \times \left[F_{(SA)}/F_{(ST)}\right] \times \left[A_{(ST)}/A_{(SA)}\right] \times \left[n_{(SA)}/n_{(ST)}\right]^2 \quad (1)$$

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Supplementary data

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