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Phosphoramidate pronucleotides of cytostatic 6-aryl-7-deazapurine ribonucleosides

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ABSTRACT

A series of *O*-phenyl methyl-, ethyl- and benzylalanyl phosphoramidate pronucleotides derived from cytostatic 6-aryl-7-deazapurine ribonucleosides were prepared by the cross-coupling reactions of the 2′,3′-isopropylidene protected 6-chloro-7-deazapurine ribonucleoside phosphoramidates with (het)aryl-boronic acids or -stannanes followed by deprotection. Most of the prepared prodrugs exerted in vitro cytostatic effects against both solid tumor and lymphoid cancer cells within low micromolar range of concentrations. These activities were in general weaker or comparable to the activities of the parent nucleosides. Additional testing of selected prodrugs suggests that the lack of activity improvement over parent nucleosides is not due to the lack of permeability or inefficient catabolism of alanyl-ester by intracellular hydrolases. More likely, active efflux of prodrugs may play a role in their weak cytotoxic activity.

1. Introduction

Nucleoside cytostatics are an important class of antitumor therapeutics.[1] Although they have been extensively studied, there is still a space for new derivatives and analogues, especially for the treatment of drug-resistant tumors. In the past, we have reported a strong cytostatic effect of 6-aryl- and 6-hetarylpurine ribonucleosides.[2] Recently, we found[3] that 6-hetaryl-7-deazapurines **1** (Chart 1) and -7-fluro-7-deazapurines bearing small hetaryl groups (i.e., furyl and thienyl) at position 6 possess nanomolar in vitro anti-proliferative activities against diverse solid tumor and leukemia cell lines. The potency of these new nucleosides was comparable to that of clinically used cytostatics such as clofarabine. Preliminary metabolism study showed that the nucleosides are phosphorylated to triphosphates and inhibit RNA synthesis. As the phosphorylation to nucleoside monophosphates by adenosine kinase might be the crucial step in their activation, we have decided to study the biological activity of selected phosphate-prodrugs.[4] Recently, we have reported the synthesis of *cyclo*Sal-phosphate pronucleotides **2**.[5] In most cases, their cytostatic effects were comparable or slightly lower than those of the parent nucleosides (nanomolar to micromolar).

Therefore, we now focused on the phosphoramidate pronucleotides (ProTides)[6,7] that are known to be enzymatically hydrolyzed by intracellular proteases or esterases and, in many cases, they are the superior type of prodrug for nucleoside antivirals or cytostatics.

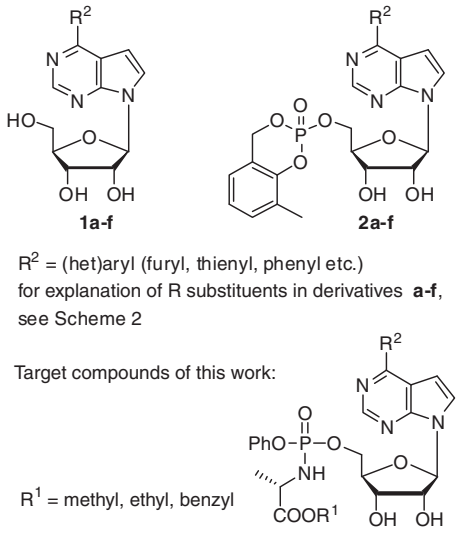

Chart 1. Structures of cytostatic 7-deazapurine nucleosides and pronucleotides.

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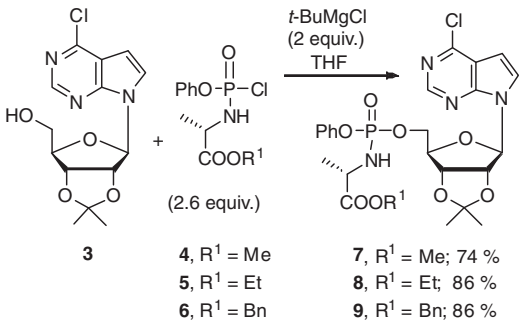
2. Results and discussion

2.1. Chemistry

Previous works on nucleoside ProTides indicated that the *O*-phenyl esters alanyl-phosphoramidates are the most versatile type of prodrug and that there is a strong influence of the alkyl group at alanine ester (more bulky alkyl esters tend to be more active).[6,7] Therefore, we decided to prepare three types of alanyl esters (methyl, ethyl and benzyl). Our strategy relied on the cross-coupling reactions of 2′,3′-protected 6-chloro-7-deazapurine ribonucleoside phosphoramidate intermediates with (het)aryl organometallics. The protected intermediates were prepared in one step from the 2′,3′-isopropylidene protected 6-chloro-7-deazapurine nucleoside **3** by the reactions with alanine-ester-phophorochloridates **4–6** (2.6 equiv) in the presence of *t*-BuMgCl (2 equiv) in good yields in analogy to the literature[7] (Scheme 1). Compounds **7**–**9** were obtained as mixtures of diastereomers due to introduction of another centre of chirality on phosphorus atom.

Palladium-catalyzed Suzuki–Miyaura cross-coupling reactions of 6-chloro-7-deazapurine pronucleotide intermediates **7–9** with (hetero)arylboronic acids **10a–f** in presence of potassium carbonate (K_2CO_3) in toluene at 85 °C were used for the introduction of the (het)aryl group at position 6. Most of the reactions proceeded smoothly to afford a series of 6-(hetero)aryl-7-deazapurine alanine–methylester–phophoramidate pronucleotides **12a**,**b**,**d–f**, alanine–ethylester–phophoramidate pronucleotides **13a**,**b**,**d–f** and alanine–benzylester–phophoramidate pronucleotides **14b**, **d–f**, that were obtained as mixtures of diastereomers (epimers at the phosphorus atom) in moderate to excellent yields (41–95%) (Scheme 2). 2-Thienylboronic acid **10c** was not sufficiently reactive in any of these reactions and the 2-furylboronic acid **10a** gave low yield in coupling with benzyl intermediate **9**. Therefore, the Stille couplings with the corresponding hetarylstannanes were used in those cases. Thus the Pd-catalyzed cross-coupling reactions of intermediates **7–9** with 2-thienyltributylstannane (**11c**) in DMF (105 °C) gave the desired protected 6-(2-thienyl)-7-deazapurine pronucleotides derivatives **12c**, **13c** and **14c** in good yields (64–92%) (Scheme 2). Similarly, the Stille cross-coupling of **9** with stannane **11a** under the same conditions provided the 2-furyl derivative **14a** in 88% yield (Scheme 2). The phosphoramidate group showed good stability under the conditions of both Suzuki and Stille cross-coupling reactions. This is important for the further development of this masked phosphate groups in other types of biologically active compounds.

The isopropylidene protecting group was removed by treatment with 90% aqueous trifluoroacetic acid (TFA). In all cases, the target 6-(het)aryl-7-deazapurine ribonucleoside phosphoroamidates **15a**–**f**, **16a–f** and **17a–f** were obtained in moderate to good yields (40-87%) (Scheme 2). The isolated yields of the final nucleosides were lowered by partial hydrolysis of ester groups during the deprotection. The final products were purified by reverse-phase flash chromatography on C18 solid phase or by silica gel column chromatography.



3

4, R^1 = Me
5, R^1 = Et
6, R^1 = Bn

7, R^1 = Me; 74 %
8, R^1 = Et; 86 %
9, R^1 = Bn; 86 %

Scheme 1. Synthesis of protected phosphoramidate intermediates **7**–**9**.

2.2. Biological activity

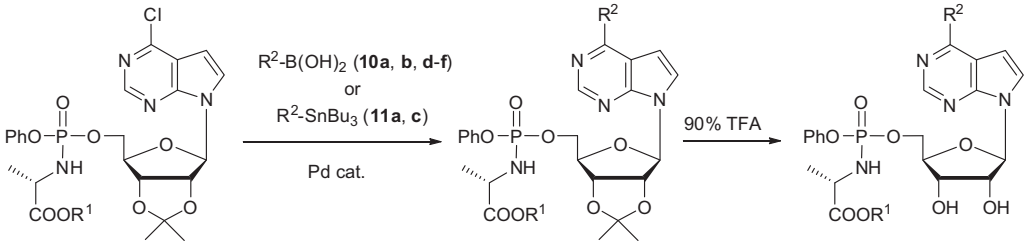
Cytostatic activity of all 18 prepared phosphoramidate ProTides **15–17a–f** was evaluated against four different cell lines derived from various human solid tumors including lung (NCI-H23 cells), prostate (DU-145 cells), colon (HCT-116 cells), and breast (HS-578 cells) carcinomas. Concentrations inhibiting the cell growth by 50% (GIC_{50}) were determined using a quantitative cellular staining with sulforhodamine B (SRB)[8] following a 5-day treatment. The SRB method allowed for determining a net effect on cell growth by subtracting background signal generated by the cell culture inoculum at the beginning of treatment. The title pronucleotides were also tested for their cytotoxic activity in human T-lymphoid (CCRF-CEM), promyelocytic leukemia (HL60), cervical carcinoma (HeLa) and human hepatocellular carcinoma (Hep G2) cell lines using XTT-based cell viability assay[8] following a shorter 72-h incubation. The results (Table 1) were compared with parent nucleosides **1a–f**.

In general, the in vitro cytostatic activities of the phoshoramidate prodrugs of 7-deazapurine ribonucleosides **15**–**17a–f** are lower (up to two orders of magnitude) and occasionally comparable to those of parent nucleosides **1a**–**f**. The differences in the 72-h assays are generally smaller than the differences in the longer 5-days tests. Comparison of methyl (**15**), ethyl (**16**) and benzyl (**17**) esters of alanine phosphoramidates shows that the most bulky benzyl esters exert highest activities compared to the smaller ones. The benzyl esters **17a** and **17b** in HeLa cells and **17c** in CCRF-CEM cells were the only cases when the activity of the pronucleotides was slightly higher than that of the parent nucleosides **1a–c**. Prodrugs **15**–**17f** bearing very bulky dibenzofuryl groups (apparently too big for any cytostatic activity in nucleosides) were completely inactive.

Cell-cycle analysis of selected ProTides in CCRF-CEM cells shows the accumulation of the cells in *S* phase and a decrease in the proportion of cells in G1 phase (Table 2), a profile consistent with targeting the RNA synthesis. The increased levels of cellular debris are indicative of a cytotoxic effect of the compounds.

Reduced cellular permeability and/or high active efflux of the explored prodrugs could at least in part explain their weak cytostatic activity compared to parent nucleosides. Therefore, one subgroup of the prodrugs **15c**, **16c**, **17c** and the corresponding nucleoside **1c** have been characterized for bi-directional cell permeability in Caco-2 cells. The data indicate similar permeability of three tested prodrugs and the parent nucleoside (Table 3). However, the ratio of bi-directional permeability is consistently higher for the prodrugs than for the nucleoside, indicating faster active efflux of the prodrugs, potentially via P-glycoprotein present at high levels in Caco2 cells. The efflux mediated by P-glycoprotein would likely be relevant for multiple cancer cell lines including those employed for the testing of cytotoxic activity.

Enzymatic hydrolysis of the alanyl esters (the first step of the pronucleotide activation in most cases followed by spontaneous hydrolysis of phenyl-ester of the phosphoramidate) by relevant hydrolases (cathepsin A and carboxyesterases 1 and 2) previously shown[9] to be able of catalyzing this activation step was studied with four selected compounds (**15–17a** and **15d**, Table 4). All tested prodrugs were readily hydrolyzed by cathepsin A and carboxyesterase 1, suggesting that the first activation step might not be limiting for the intracellular activation of these phosphoramidate prodrugs. As observed previously with some other phosphoramidates, carboxyesterase 2 did not recognize these prodrugs as substrates. There is no apparent correlation between the hydrolysis and cytostatic effects of these compounds, which further



10-17	Starting compound	R^1	R^2	Reagent	Products (yields) Cross-coupling	Deprotection
a	**7**	Me	2-furyl	**10a**	**12a** (81 %)	**15a** (52 %)
b	**7**	Me	3-furyl	**10b**	**12b** (81 %)	**15b** (60 %)
c	**7**	Me	2-thienyl	**11c**	**12c** (64 %)	**15c** (61 %)
d	**7**	Me	3-thienyl	**10d**	**12d** (80 %)	**15d** (52 %)
e	**7**	Me	phenyl	**10e**	**12e** (65 %)	**15e** (54 %)
f	**7**	Et	4-dibenzofuryl	**10f**	**12f** (41 %)	**15f** (42 %)
a	**8**	Et	2-furyl	**10a**	**13a** (57 %)	**16a** (40 %)
b	**8**	Et	3-furyl	**10b**	**13b** (63 %)	**16b** (40 %)
c	**8**	Et	2-thienyl	**11c**	**13c** (82 %)	**16c** (47 %)
d	**8**	Et	3-thienyl	**10d**	**13d** (62 %)	**16d** (75 %)
e	**8**	Et	phenyl	**10e**	**13e** (60 %)	**16e** (87 %)
f	**8**	Et	4-dibenzofuryl	**10f**	**13f** (91 %)	**16f** (60 %)
a	**9**	Bn	2-furyl	**11a**	**14a** (88 %)	**17a** (41 %)
b	**9**	Bn	3-furyl	**10b**	**14b** (58 %)	**17b** (69 %)
c	**9**	Bn	2-thienyl	**11c**	**14c** (94 %)	**17c** (59 %)
d	**9**	Bn	3-thienyl	**10d**	**14d** (95 %)	**17d** (62 %)
e	**9**	Bn	phenyl	**10e**	**14e** (94 %)	**17e** (65 %)
f	**9**	Bn	4-dibenzofuryl	**10f**	**14f** (92 %)	**17f** (64 %)

Scheme 2. Cross-coupling reactions of 6-chloro-7-deazapurine intermediates followed by deprotection.

supports the notion that the alanyl-ester hydrolysis might not be limiting for the activity of these prodrugs.

3. Conclusions

In conclusion, the results of this work (together with the previous study[5] on CycloSal-prodrugs) demonstrate that the explored pronucleotide approach that was successfully applied to many other nucleoside antivirals and cytostatics[4,6,7] is not effective in further improving the potent (nanomolar) in vitro cytostatic activity[3] of 6-hetaryl-7-deazapurine ribonucleosides. There could be several possible explanations: (i) the transport across plasma membrane and subsequent intracellular phosphorylation to 5′-*O*-monophosphate by adenosine kinase are not the limiting steps for the activity of parent nucleosides. Indeed, the bi-directional cell permeability studies indicate that the amidate prodrugs might be more efficient substrates for active cellular efflux than the parent nucleosides. Finally, some intermediates of the prodrug catabolism might be non-productively trapped in intracellular compartments such as endosomes or lysosomes where the initial activation step may occur.[9] Despite the lack of improvement in in vitro cytostatic activities, some pronucleotides still can be considered for in vivo delivery of these compounds and/or for the development of orally available formulations.

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. High performance flash chromatography (HPFC) purifications were performed using C-18 columns with a H_2O–MeOH gradient. All final compounds were more than 97% pure (HPLC). For compounds **15a**–**e**, **16a**–**e** and **17a**–**e** gradient of acetonitrile in water was used: 0% acetonitrile (1 min), 0–50% acetonitrile (9 min), 50–100% acetonitrile (20 min). For compounds **15f**, **16f** and **17f** gradient of acetonitrile in water was used: 0% acetonitrile (1 min), 0–75% acetonitrile (14 min), 50–100% acetonitrile (15 min). All reactions were performed under anhydrous conditions in an argon atmosphere.

Table 1
Cytostatic activities of ProTides **15–17**

	GIC_{50}[a] (μM)					IC_{50}[b] (μM)		
	HCT 116	NCI-H23	HS-578	DU-145	HL-60	CCRF-CEM	HeLa S3	HepG2
15a	1.03	4.63	2.31	1.19	3.31	3.44	1.40	1.20
16a	2.50	2.87	2.18	2.29	4.86	3.34	1.02	1.42
17a	1.08	1.91	1.04	1.36	2.19	1.13	0.58	0.62
1a[c]	0.078	ND	0.049	0.007	0.81	0.31	0.77	0.47
15b	7.95	>10	>10	3.86	>10	>10	7.84	3.61
16b	9.06	8.28	5.97	9.05	>10	>10	4.89	2.96
17b	1.42	5.19	1.10	1.09	>10	3.11	1.31	2.01
1b[c]	0.092	ND	0.26	0.036	1.5	1.9	4.6	1.72
15c	6.22	6.79	6.59	3.23	>10	8.47	2.51	1.41
16c	>10	10.91	9.60	>10	>10	>10	5.93	1.53
17c	0.68	5.12	1.42	0.989	ND	2.09	1.56	0.88
1c[c]	0.049	ND[d]	0.10	0.009	1.2	8.8	0.29	1.19
15d	>10	>10	>10	>10	>10	>10	>10	>10
16d	>10	>10	>10	>10	>10	>10	>10	>10
17d	7.51	7.44	7.56	3.57	>10	>10	5.45	4.58
1d[c]	0.22	ND	0.71	0.032	>40	4.0	ND	3.22
15e	>10	>10	>10	>10	>10	>10	>10	>10
16e	>10	ND	>10	ND	>10	>10	>10	>10
17e	>10	ND	>10	ND	5.65	>10	>10	>10
1e[c]	5.7	ND	3.1	0.075	ND	1.0	4.1	>10
15f	>10	ND	>10	ND	>10	>10	>10	>10
16f	>10	ND	>10	ND	>10	>10	>10	>10
17f	>10	ND	>10	ND	>10	>10	>10	>10
1f[c]	>10	ND	>10	ND	>10	>10	>10	>10

[a] Cytostatic activity (GIC_{50}) was determined by SRB assay following a 5-day incubation with tested compounds. Values represent means from two independent experiments.
[b] Cytostatic activity was determined by XTT assay following a 3-day incubation. Values represent means from four independent experiments.
[c] Data from Ref. 3.
[d] ND, not determined.

Table 2
Cell cycle analysis of selected ProTides

Compound	[%]			
	G1	S	G2/M	Debris
16a	26.6	55.3	18.06	42.5
17a	31.6	54.7	13.7	48.8
17b	37.5	54.0	8.4	21.5
17c	30.7	57.9	11.5	49.5
17d	35.9	54.2	9.9	22.2
Control	47.5	40.6	11.9	6.3

Table 3
Bi-directional permeablity of a parent nucleaoside and its phosphoramidate prodrugs

Compound	P_{app} (10^{-6} cm/s)			Efflux ratio[c]
	Cell-free	A-to-B[a]	B-to-A[b]	
1c	34.11	1.57	9.31	5.9
15c	35.12	0.74	9.75	13.3
16c	33.82	0.76	9.62	12.7
17c	29.93	1.10	13.19	12.0

[a] Cellular permeability for apical to basolateral direction.
[b] Cellular permeability for basolateral to apical direction.
[c] Ratio of permeability for the two directions.

Table 4
Enzymatic hydrolysis of selected ProTides

Compound	SA[a] (pmol min^{-1} μg^{-1})		
	CatA[b]	Ces1[c]	Ces2[d]
15a	1249	86	0
16a	11,989	149	0
17a	14,727	133	0
15d	1496	167	0

[a] specific activity, the decrease of the amount of the alanyl ester per minute and μg of the enzyme.
[b] Cathepsin A.
[c] Carboxyesterase 1.
[d] Carboxyesterase 2.

4.1. General procedure for the synthesis of 6-chloro-7-deazapurine ribonucleoside ProTides 7–9

Nucleoside **3** (1.22 mmol) was dissolved in THF (15 ml). Solution of *t*-BuMgCl in THF (1 M, 2 equiv) was added drop-wise and the reaction mixture was stirred for 15 min. Then solution of a phosphochloridate **4–6** (2.6 equiv) in THF (15 ml) was added and the reaction mixture was stirred overnight at room temperature. Saturated NH_4Cl solution (2.5 ml) was added and reaction mixture was extracted with EtOAc and water. Organic layer was dried with $MgSO_4$ and evaporated under reduced pressure. Crude product was purified using column chromatography (SiO_2, hexane/EtOAc 2:1) Products were obtained as oils. Mixtures of diastereomers were obtained, ratios were determined by NMR spectra.

4.1.1. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)] phosphate (7)

Nucleoside **3** (385 mg, 1.18 mmol) and phosphochloridate **4** (853 mg, 2.6 equiv) were used. Yield: 495 mg, 74% (mixture of diastereomers 1:1.25). ^{1}H NMR (600.1 MHz, $CDCl_3$): 1.28, 1.30 (2 × d, 2 × 3H, J_{vic} = 7.2, CH_3-Ala); 1.35, 1.37, 1.62, 1.63 (4 × s, 4 × 3H, $(CH_3)_2C$); 3.53, 3.57 (2 × br t, 2 × 1H, $J_{H,P}$ = J_{vic} = 10.3, NH-Ala); 3.67, 3.69 (2 × s, 2 × 3H, CH_3O); 3.96 (m, 2H, CH-Ala); 4.26, 4.33 (2 × m, 4H, H-5′); 4.43, 4.45 (2 × m, 2 × 2H, H-4′); 4.98 (m, 2H, H-2′,3′); 5.01 (m, 1H, H-3′); 5.17 (m, 1H, H-2′); 6.30, 6.32 (2 × br s, 2 × 1H, H-1′); 6.62, 6.64 (2 × d, 2 × 1H, $J_{5,6}$ = 3.2, H-5); 7.13–7.20 (m, 6H, H-*o*,*p*-Ph); 7.29, 7.31 (2 × m, 2 × 2H, H-*m*-Ph); 7.38, 7.39 (2 × d, 2 × 1H, $J_{6,5}$ = 3.2, H-6); 8.66 (s, 2H, H-2). ^{13}C NMR (150.9 MHz, $CDCl_3$): 20.91, 20.97 (d, $J_{C,P}$ = 4.9, CH_3-Ala); 25.32,

25.40, 27.20, 27.22 ((CH_3)$_2$C); 50.05, 50.18 (d, $J_{C,P}$ = 1.4, CH-Ala); 52.56, 52.58 (CH_3O); 61.18, 66.29 (d, $J_{C,P}$ = 5.2, CH_2-5′); 80.71, 80.87 (CH-3′); 83.94, 84.13 (d, $J_{C,P}$ = 8.0, CH-4′); 84.25, 84.43 (CH-2′); 90.70, 90.87 (CH-1′); 100.83 (CH-5); 114.75, 114.83 (*C*(CH_3)$_2$); 118.35, 118.46 (C-4a); 119.99, 120.05 (d, $J_{C,P}$ = 4.9, CH-*o*-Ph); 125.06, 125.14 (CH-*p*-Ph); 127.46, 127.75 (CH-6); 129.72, 129.74 (CH-*m*-Ph); 150.49, 150.51 (d, $J_{C,P}$ = 6.8, C-*i*-Ph); 150.75 (C-7a); 150.85, 150.92 (CH-2); 150.93 (C-7a); 152.17, 152.21 (C-4); 173.72, 173.82 (d, $J_{C,P}$ = 7.4, CO-Ala). ^{31}P NMR (202.3 MHz, $CDCl_3$): 2.82, 3.03. ESI MS *m*/*z* (rel.%): 591 (37) [M+2+Na], 589 (100) [M+Na], 569 (15) [M+2+H], 567 (40) [M+H]. HR MS (ESI) for $C_{24}H_{28}O_8N_4NaP$ [M+Na]: calcd 589.12255; found 589.12242.

4.1.2. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)] phosphate (8)

Nucleoside **3** (550 mg, 1.67 mmol) and phosphochloridate **5** (1.28 g, 2.6 equiv) were used. Yield: 1.02 g, 86% (mixture of diastereomers 1:1.29). ^{1}H NMR (500.0 MHz, $CDCl_3$): 1.22 and 1.24 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH_2O); 1.28 and 1.30 (2 × dd, 2 × 3H, J_{vic} = 6.8, $J_{H,P}$ = 0.7, CH_3-Ala); 1.35, 1.37, 1.62 and 1.63 (4 × s, 4 × 3H, (CH_3)$_2$C); 3.54 and 3.58 (2 × br t, 2 × 1H, $J_{H,P}$ = J_{vic} = 10.0, NH-Ala); 3.95 (m, 2H, CH-Ala); 4.06–4.20 (m, 4H, CH_3CH_2O); 4.25 (ddd, 1H, J_{gem} = 11.4, $J_{H,P}$ = 6.7, $J_{5'b,4'}$ = 4.8, H-5′b); 4.30–4.36 (m, 3H, 2 × H-5′a and H-5′b); 4.44 (m, 2H, H-4′); 4.97 (m, 2H, H-2′,3′); 5.01 (dd, 1H, $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 3.5, H-3′); 5.16 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.9, H-2′); 6.30 (d, 1H, $J_{1',2'}$ = 2.9, H-1′); 6.32 (d, 1H, $J_{1',2'}$ = 2.4, H-1′); 6.62 and 6.64 (2 × d, 2 × 1H, $J_{5,6}$ = 3.7, H-5); 7.14 (m, 1H, H-*p*-Ph); 7.15 (m, 2H, H-*o*-Ph); 7.16 (m, 1H, H-*p*-Ph); 7.19 (m, 2H, H-*o*-Ph); 7.28 and 7.31 (2 × m, 2 × 2H, H-*m*-Ph); 7.388 and 7.393 (2 × d, 2 × 1H, $J_{6,5}$ = 3.7, H-6); 8.657 and 8.659 (2 × s, 2 × 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 14.06 and 14.09 (*C*H_3CH$_2$O); 20.93 and 21.00 (d, $J_{C,P}$ = 4.8, CH_3-Ala); 25.30, 25.37, 27.20 and 27.22 (($\mathbf{C}H_3$)$_2$C); 50.13 and 50.26 (d, $J_{C,P}$ = 1.5, CH-Ala); 61.62 and 61.63 (CH_3CH_2O); 66.10 and 66.19 (d, $J_{C,P}$ = 5.2, CH_2-5′); 80.72 and 80.86 (CH-3′); 83.96 and 84.14 (d, $J_{C,P}$ = 8.0, CH-4′); 84.22 and 84.37 (CH-2′); 90.71 and 90.85 (CH-1′); 100.84 (CH-5); 114.75 and 114.83 (C(CH_3)$_2$); 118.33 and 118.44 (C-4a); 119.99 and 120.04 (d, $J_{C,P}$ = 5.0, CH-*o*-Ph); 125.03 and 125.11 (CH-*p*-Ph); 127.46 and 127.76 (CH-6); 129.71 and 129.72 (CH-*m*-Ph); 150.54 (d, $J_{C,P}$ = 6.6, C-*i*-Ph); 150.55 (d, $J_{C,P}$ = 7.0, C-*i*-Ph); 150.73 (CH-2); 150.77 (C-7a); 150.79 (CH-2); 150.94 (C-7a); 152.06 and 152.10 (C-4); 173.25 and 173.33 (d, $J_{C,P}$ = 7.7, CO-Ala). ^{31}P NMR (202.3 MHz, $CDCl_3$): 2.85 and 3.04. ESI MS *m*/*z* (rel.%): 605 (40) [M+2+Na], 603 (100) [M+Na], 583 (13) [M+2+H], 581 (37) [M+H]. HR MS (ESI) for $C_{25}H_{30}O_8N_4NaP$ [M+Na]: calcd, 603.1382; found 603.1384.

4.1.3. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)] phosphate (9)

Nucleoside **3** (600 mg, 1.84 mmol) and phosphochloridate **6** (1.69 g, 2.6 equiv) were used. Yield: 862 mg, 86% (mixture of diastereomers 1:1.03). ^{1}H NMR (499.8 MHz, $CDCl_3$): 1.29, 1.31 (2 × dd, 2 × 3H, J_{vic} = 7.4, $J_{H,P}$ = 1.0, CH_3-Ala); 1.34, 1.37, 1.62, 1.63 (4 × s, 4 × 3H, (CH_3)$_2$C); 3.61, 3.65 (2 × dd, 2 × 1H, $J_{H,P}$ = 11.0, J_{vic} = 9.5, NH-Ala); 4.02 (m, 2H, CH-Ala); 4.22 (ddd, 1H, J_{gem} = 11.1, $J_{H,P}$ = 6.8, $J_{5'b,4'}$ = 4.9, H-5′b); 4.28–4.34 (m, 3H, 2 × H-5′a, H-5′b); 4.40, 4.42 (2 × m, 2 × 1H, H-4′); 4.95 (m, 2H, H-2′,3′); 4.99 (dd, 1H, $J_{3',2'}$ = 6.5, $J_{3',4'}$ = 3.6, H-3′); 5.06 (d, 1H, J_{gem} = 12.2, CH_aH_bPh); 5.115 (s, 2H, CH_2Ph); 5.12 (d, 1H, J_{gem} = 12.2, CH_aH_bPh); 5.15 (dd, 1H, $J_{2',3'}$ = 6.5, $J_{2',1'}$ = 2.9, H-2′); 6.29 (d, 1H, $J_{1',2'}$ = 2.9, H-1′); 6.31 (d, 1H, $J_{1',2'}$ = 2.5, H-1′); 6.58, 6.64 (2 × d, 2 × 1H, $J_{5,6}$ = 3.7, H-5); 7.12 (m, 1H, H-*p*-Ph); 7.14 (m, 4H, H-*o*-Ph); 7.17 (m, 1H, H-*p*-Ph); 7.24–7.37 (m, 16H, H-6, H-*m*-Ph, H-*o*,*m*,*p*-Bn); 8.641 and 8.644 (2 × s, 2 × 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 20.81, 21.88 (d, $J_{C,P}$ = 5.0, CH_3-Ala); 25.26, 25.35, 27.17, 27.20 (($\mathbf{C}H_3$)$_2$C); 50.17, 50.31 (d, $J_{C,P}$ = 1.5, CH-Ala); 66.07, 66.17 (d, $J_{C,P}$ = 5.2, CH_2-5′); 67.25 (CH_2Ph); 80.67, 80.81 (CH-3′); 83.87, 84.05 (d, $J_{C,P}$ = 8.0, CH-4′); 84.16, 84.31 (CH-2′); 90.60, 90.73 (CH-1′); 100.72, 100.73 (CH-5); 114.69, 114.79 ($\mathbf{C}$(CH_3)$_2$); 118.30, 118.41 (C-4a); 119.98, 120.02 (d, $J_{C,P}$ = 5.1, CH-*o*-Ph); 125.01, 125.09 (CH-*p*-Ph); 127.18, 127.50 (CH-6); 128.14, 128.22 (CH-*o*-Bn); 128.50 (CH-*p*-Bn); 128.61, 128.62 (CH-*m*-Bn); 129.67, 129.69 (CH-*m*-Ph); 135.05, 135.10 (C-*i*-Bn); 150.48, 150.49 (d, $J_{C,P}$ = 6.7, C-*i*-Ph); 150.74, 150.92 (C-7a); 150.96, 151.03 (CH-2); 152.31, 152.36 (C-4); 173.08, 173.16 (d, $J_{C,P}$ = 7.3, CO-Ala). ^{31}P NMR (202.3 MHz, $CDCl_3$): 2.81, 3.02. ESI MS *m*/*z* (rel.%): 67 (40) [M+2+Na], 665 (100) [M+Na], 643 (10) [M+H]. HR MS (ESI) for $C_{30}H_{32}O_8N_4ClNaP$ [M+Na]: calcd, 665.1538; found 665.1539.

4.2. General procedure for the Stille coupling

A 4-chloroProTide **7**–**9** (200 mmol), a tributylstannane **11a**,**c** (1.5 equiv) and $PdCl_2(PPh_3)_2$ (0.05 equiv) were dissolved in anhydrous DMF (2 ml) and heated to 105 °C. The reaction mixture was stirred for 1–3 h (TLC analysis) and then solvent was removed under reduced pressure. Crude product was purified using column chromatography (SiO_2, hexane/EtOAc 2:1 or 3:2). Products were obtained as oils. Mixtures of diastereomers were obtained, ratios were determined by NMR spectra.

4.2.1. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(2-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)]phosphate 12c

4-ChloroProTide **7** (128 mg, 0.2 mmol) and 2-thienyltributylstanne (**11c**) (135 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1). Yield: 89 mg, 64% (mixture of diastereomers 1:1.02). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.24 and 1.26 (2 × d, 2 × 3H, J_{vic} = 8.4, CH_3-Ala); 1.35, 1.37, 1.62 and 1.63 (4 × s, 4 × 3H, (*C*H_3)$_2$C); 3.12 and 3.63 (2 × s, 2 × 3H, CH_3O); 3.66 and 3.68 (2 × bt, 2 × 1H, $J_{H,P}$ = J_{vic} = 10.4, NH-Ala); 3.94 (m, 2H, CH-Ala); 4.25–4.36 (m, 4H, 2 × H-5′a and 2 × H-5′b); 4.43 (m, 2H, H-4′); 4.99 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.03 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{3',4'}$ = 3.6, H-3′); 5.07 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{3',4'}$ = 3.2, H= H-3′); 5.24 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.4, H-2′); 6.34 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.35 (d, 1H, $J_{1',2'}$ = 3.2, H-1′); 6.87 (d, 1H, $J_{5,6}$ = 3.6, H-5); 6.90 (d, 1H, $J_{5,6}$ = 4.0, H-5); 7.11–7.28 (m, 12H, 2 × H-*p*-Ph, 4 × H-*o*-Ph, 4 × H-*m*-Ph, 2 × H-4-thienyl); 7.385 and 7.394 (2 × d, 2 × 1H, $J_{6,5}$ = 3.6, H-6); 7.56 (m, 2H, 2 × H-3-thienyl); 7.97 (m, 2H, 2 × H-5-thienyl); 8.33 and 8.34 (2 × s, 2 × 1H, 2 × H-2). ESI MS *m*/*z* (rel.%): 637 (100) [M+Na], 615 (70) [M+H]. HR MS (ESI) for $C_{28}H_{31}O_8N_4NaPS$ [M+Na]: calcd 637.1492; found 637.1501.

4.2.2. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(2-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)] phosphate 13c

4-ChloroProTide **8** (120 mg, 0.2 mmol) and 2-thienyltributylstanne (**11c**) (121 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1). Yield: 106 mg, 82% (mixture of diastereomers 1:1.23). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.18 and 1.20 (2 × t, 2 × 3H, J_{vic} = 7.2, *C*H_3CH$_2$O); 1.26 and 1.27 (2 × d, 2 × 3H, *J* = 7.2, CH_3-Ala); 1.35, 1.38, 1.626 and 1.631 (4 × s, 4 × 3H, (CH_3)$_2$C); 3.60 and 3.62 (2 × bt, 2 × 1H, $J_{H,P}$ = J_{vic} = 9.6, NH-Ala); 3.94 (m, 2H, CH-Ala); 4.07–4.13 (m, 4H, CH_3CH_2O); 4.27 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.8, $J_{5'b,4'}$ = 5.2, H-5′b); 4.32–4.38 (m, 3H, 2 × H-5′a and H-5′b); 4.44 (m, 2H, 2 × H-4′); 4.99 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.02–5.07 (m, 2H, 2 × H-3′); 5.23 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.35 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.37 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.89 (d, 1H, $J_{5,6}$ = 4.0, H-5); 6.92 (d, 1H, $J_{5,6}$ = 4.0, H-5); 7.11–7.30 (m, 12H, 2 × H-*p*-Ph, 4 × H-*o*-Ph, 4 × H-*m*-Ph, 2 × H-4-thienyl); 7.41 (m, 2H, 2 × H-6); 7.59 (m, 2H,

2 × H-3-thienyl); 8.03 (m, 2H, 2 × H-5-thienyl); 8.85 and 8.86 (2 × s, 2 × 1H, 2 × H-2). ESI MS *m*/*z* (rel.%): 651 (100) [M+Na], 629 (63) [M+H]. HR MS (ESI) for $C_{29}H_{34}O_8N_4PS$ [M+H]: calcd 629.1829; found 629.1828.

4.2.3. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(2-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)] phosphate 14a

4-ChloroProTide **9** (200 mg, 0.3 mmol) and 2-furyltributylstanne (**11a**) (171 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 3:2).Yield: 185 mg, 88% (mixture of diastereomers 1:1.33). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.27 and 1.29 (2 × d, 2 × 3H, J_{vic} = 7.6, CH_3-Ala); 1.35 and 1.37 (2 × s, 2 × 3H, $(CH_3)_2C$); 1.63 (s, 6H, $(CH_3)_2C$); 3.54 and 3.57 (2 × t, 2 × 1H, $J_{H,P} = J_{vic}$ = 9.6, NH-Ala); 4.03 (m, 2H, CH-Ala); 4.24 (ddd, 1H, J_{gem} = 12.0, $J_{H,P}$ = 6.4, $J_{5'b,4'}$ = 5.2, H-5′b); 4.29–4.33 (m, 3H, 2 × H-5′a and H-5′b); 4.41 (m, 2H, H-4′); 4.96 (dd, 2H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.01–5.04 (m, 3H, 2 × H-3′, CH_2-Pha); 5.08–5.11 (m, 3H, CH_2-Pha, 2 × CH_2-Phb); 5.22 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.4, H-2′); 6.33 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.35 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.64 (m, 2H, H-4-furyl); 7.03 and 7.05 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, H-5); 7.12–7.18 (m, 6H, 6 × H-Ph); 7.23–7.38 (m, 8H, 14 × H-Ph, 2 × H-3-furyl, 2 × H-6); 7.71 (m, 2H, H-5-furyl); 8.84 and 8.85 (2 × s, 2 × 1H, 2 × H-2). ESI MS *m*/*z* (rel.%): 697 (100) [M+Na], 675 (56) [M+H]. HR MS (ESI) for $C_{34}H_{36}O_9N_4P$ [M+H]: calcd 675.2214; found 675.2215.

4.2.4. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(2-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)]phosphate 14c

4-ChloroProTide **9** (200 mg, 0.3 mmol) and 2-thienyltributylstanne (**11c**) (180 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 3:2). Yield: 201 mg, 94% (mixture of diastereomers 1:1.10). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.28 and 1.29 (2 × d, 2 × 3H, J_{vic} = 7.6, CH_3-Ala); 1.36 and 1.38 (2 × s, 2 × 3H, $(CH_3)_2C$); 1.63 (s, 6H, $(CH_3)_2C$); 3.54 and 3.56 (2 × t, 2 × 1H, $J_{H,P} = J_{vic}$ = 10.4, NH-Ala); 4.03 (m, 2H, CH-Ala); 4.24 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.4, $J_{5'b,4'}$ = 5.2, H-5′b); 4.30–4.34 (m, 3H, 2 × H-5′a and H-5′b); 4.42 (m, 2H, H-4′); 4.97 (dd, 2H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.01–5.04 (m, 3H, 2 × H-3′, CH_2-Pha); 5.08–5.11 (m, 3H, CH_2-Pha, 2 × CH_2-Phb); 5.22 (dd, 1H, $J_{2',3'}$ = 6.0, $J_{2',1'}$ = 2.4, H-2′); 6.34 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.36 (d, 1H, $J_{1',2'}$ = 3.2, H-1′); 6.88 and 6.90 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, H-5); 7.12–7.18 (m, 6H, 6 × H-Ph); 7.24–7.32 (m, 16H, 14 × H-Ph, 2 × H-4-thienyl); 7.39 (m, 2H, 2 × H-6); 7.59 (m, 2H, 2 × H-3-thienyl); 8.01 (m, 2H, 2 × H-5-thienyl); 8.85 and 8.86 (2 × s, 2 × 1H, 2 × H-2). ESI MS *m*/*z* (rel.%): 713 (100) [M+Na], 691 (92) [M+H]. HR MS (ESI) for $C_{34}H_{36}O_8N_4PS$ [M+H]: calcd 691.1986; found 691.1987.

4.3. General procedure for the Suzuki coupling

A 4-ChloroProTide **7–9** (200 mmol), a boronic acid **10a**,**b**,**d–f** (1.5 equiv), K_2CO_3 (2 equiv) and $Pd(PPh_3)_4$ (0.05 equiv) were dissolved in anhydrous toluene (2 ml) and heated to 85 °C. The reaction mixture was stirred for 1–3 h (TLC analysis). Then, 1 M HCl (3 ml) was added and reaction mixture was extracted with EtOAc and water. Organic layer was dried with $MgSO_4$ and evaporated under reduced pressure. Crude product was purified using column chromatography (SiO_2, hexane/EtOAc 2:1 or 3:2). Products were obtained as oils. Mixtures of diastereomers were obtained, ratios were determined by NMR spectra.

4.3.1. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(2-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)] phosphate (12a))

4-ChloroProTide **7** (98 mg, 0.17 mmol) and 2-furylboronic acid (**10a**) (31 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1). Yield: 83 mg, 81% (mixture of diastereomers 1:1). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.24 and 1.26 (2 × d, 2 × 3H, J_{vic} = 8.4, CH_3-Ala); 1.36, 1.38, 1.629 and 1.634 (4 × s, 4 × 3H, $(CH_3)_2C$);); 3.56 and 3.59 (2 × bt, 2 × 1H, $J_{H,P} = J_{vic}$ = 9.6, NH-Ala); 3.63 and 3.65 (2 × s, 2 × 3H, CH_3O); 3.95 (m, 2H, CH-Ala); 4.27 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.4, $J_{5'b,4'}$ = 4.8, H-5′b); 4.31–4.37 (m, 3H, 2 × H-5′a and H-5′b); 4.44 (m, 2H, H-4′); 4.99 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.02–5.07 (m, 2H, H-2′,3′); 5.23 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.34 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.36 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.65 (m, 2H, H-4-furyl); 7.05 and 7.08 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.10–7.20 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 7.25–7.32 (m, 6H, 4 × H-*m*-Ph, 2 × H-3-furyl); 7.38 and 7.39 (2 × d, 2 × 1H, $J_{6,5}$ = 3.6, H-6); 7.76 (m, 2H, 2 × H-5-furyl); 8.348 and 8.353 (2 × s, 2 × 1H, 2 × H-2). ESI MS *m*/*z* (rel.%): 621 (77) [M+Na], 599 (100) [M+H]. HR MS (ESI) for $C_{28}H_{32}O_9N_4P$ [M+H]: calcd 599.1901; found 599.1899.

4.3.2. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(3-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)] phosphate (12b)

4-ChloroProTide **6** (85 mg, 0.15 mmol) and 3-furylboronic acid (**10b**) (25 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1). Yield: 73 mg, 81% (mixture of diastereomers 1:1.08). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.24 and 1.26 (2 × d, 2 × 3H, J_{vic} = 8.4, CH_3-Ala); 1.36, 1.38 (2 × s, 2 × 3H, $(CH_3)_2C$)); 1.63 (s, 6H, $(CH_3)_2C$)); 3.54 and 3.56 (2 × br t, 2 × 1H, $J_{H,P} = J_{vic}$ = 10.0, NH-Ala); 3.64 and 3.66 (2 × s, 2 × 3H, CH_3O); 3.96 (m, 2H, CH-Ala); 4.25 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.4, $J_{5'b,4'}$ = 4.8, H-5′b); 4.30–4.36 (m, 3H, 2 × H-5′a and H-5′b); 4.44 (m, 2H, H-4′); 5.00 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.02–5.09 (m, 2H, H-2′,3′); 5.24 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.34 and 6.36 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.8, H-1′); 6.72 and 6.74 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.10–7.21 (m, 8H, 2 × H-*p*-Ph, 4 × H-*o*-Ph, 2 × H-4-furyl); 7.24–7.32 (m, 4H, 4 × H-*m*-Ph); 7.38 and 7.40 (2 × d, 2 × 1H, $J_{6,5}$ = 3.6, H-6); 7.59 (m, 2H, H-5-furyl); 8.30 (br s, 2H, H-2-furyl); 8.88 (s, 2H, H-2). ESI MS *m*/*z* (rel.%): 621 (100) [M+Na], 599 (56) [M+H]. HR MS (ESI) for $C_{28}H_{32}O_9N_4P$ [M+H]: calcd 599.1901; found 599.1890.

4.3.3. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(3-thienyl)-7H-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)]phosphate (12d)

4-ChloroProTide **7** (98 mg, 0.17 mmol) and 3-thienylboronic acid (**10d**) (33 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1). Yield: 85 mg, 80% (mixture of diastereomers 1:1.18). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.25 and 1.27 (2 × d, 2 × 3H, J_{vic} = 8.0, CH_3-Ala); 1.36, 1.38, 1.63 and 1.64 (4 × s, 4 × 3H, $(CH_3)_2C$); 3.59 and 3.60 (2 × br t, 2 × 1H, $J_{H,P} = J_{vic}$ = 10.0, NH-Ala); 3.63 and 3.65 (2 × s, 2 × 3H, CH_3O); 3.96 (m, 2H, CH-Ala); 4.27 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.8, $J_{5'b,4'}$ = 5.2, H-5′b); 4.32–4.38 (m, 3H, 2 × H-5′a and H-5′b); 4.45 (m, 2H, H-4′); 5.00 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.04 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{3',4'}$ = 3.6, H-3′); 5.08 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{3',4'}$ = 3.2, H-3′); 5.25 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.35 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.38 (d, 1H, $J_{1',2'}$ = 3.2, H-1′); 6.83 and 6.86 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.10–7.20 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 7.25–7.31 (m, 4H, 4 × H-*m*-Ph); 7.41 and 7.42 (2 × d, 2 × 1H, $J_{6,5}$ = 3.6, H-6); 7.49 (m, 2H, H-4-thienyl); 7.88 (m, 2H, H-5-thienyl); 8.23 (br s, 2H, H-2-thienyl); 8.907 and 8.912 (2 × s, 2 × 1H, H-2). ESI MS *m*/*z* (rel.%): 637 (100) [M+Na], 615 (94) [M+H]. HR MS (ESI) for $C_{28}H_{32}O_8N_4PS$ [M+H]: calcd 613.1673; found 613.1673.

4.3.4. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)] phosphate (12e)

4-ChloroProTide **7** (120 mg, 0.2 mmol) and phenylboronic acid (**10e**) (40 mg, 1.5 equiv) were used. Column chromatography

(SiO_2, hexane/EtOAc 2:1). Yield: 84 mg, 65% (mixture of diastereomers 1:1). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.26 and 1.28 (2 × d, 2 × 3H, J_{vic} = 7.6, CH_3-Ala); 1.37, 1.39, 1.64 and 1.65 (4 × s, 4 × 3H, (CH_3)$_2$C); 3.53 and 3.55 (2 × br t, 2 × 1H, $J_{H,P}$ = J_{vic} = 9.6, NH-Ala); 3.64 and 3.65 (2 × s, 2 × 3H, CH_3O); 3.97 (m, 2H, CH-Ala); 4.29 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.4, $J_{5'b,4'}$ = 4.8, H-5′b); 4.33–4.38 (m, 3H, 2 × H-5′a and H-5′b); 4.46 (m, 2H, H-4′); 5.01 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.04–5.09 (m, 2H, H-3′); 5.25 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.38 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.40 (d, 1H, $J_{1',2'}$ = 3.2, H-1′); 6.85 and 6.88 (2 × d, 2 × 1H, $J_{5,6}$ = 4.0, 2 × H-5); 7.13–7.21 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 7.26–7.32 (m, 4H, 4 × H-*m*-Ph); 7.43 and 7.44 (2 × d, 2 × 1H, $J_{6,5}$ = 4.0, H-6); 7.55–7.60 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 8.10 (m, 4H, 4 × H-*m*-Ph); 8.998 and 9.001 (2 × s, 2 × 1H, H-2). ESI MS *m*/*z* (rel.%): 631 (100) [M+Na], 609 (40) [M+H]. HR MS (ESI) for $C_{30}H_{34}O_8N_4P$ [M+H]: calcd 609.2109; found 609.2106.

4.3.5. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(4-dibenzofuryl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)]phosphate (12f)

4-ChloroProTide **7** (120 mg, 0.2 mmol) and 4-dibenzofurylboronic acid (**10f**) (69 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1). Yield: 61 mg, 41% (mixture of diastereomers 1:2.05). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.27 and 1.29 (2 × d, 2 × 3H, J_{vic} = 7.6, CH_3-Ala); 1.38 and 1.41 (4 × s, 4 × 3H, (CH_3)$_2$C); 1.67 (s, 6H, (CH_3)$_2$C); 3.55 and 3.58 (2 × br t, 2 × 1H, $J_{H,P}$ = J_{vic} = 9.6, NH-Ala); 3.63 (s, 6H, CH_3O); 4.00 (m, 2H, CH-Ala); 4.33 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.8, $J_{5'b,4'}$ = 5.2, H-5′b); 4.37–4.43 (m, 3H, 2 × H-5′a and H-5′b); 4.48 (m, 2H, H-4′); 5.04 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.07–5.09 (m, 2H, H-3′); 5.28 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.4, H-2′); 6.43 and 6.45 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.78 and 6.80 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.11–7.24 (m, 6H, 6 × H-Ar); 7.25–7.32 (m, 4H, 4 × H-Ar); 7.39–7.52 (m, 5H, 4 × H-Ar, H-6); 7.55–7.60 (m, 4H, 4 × H-Ar); 8.03–8.06 (m, 4H, 4 × H-Ar); 8.14–8.17 (m, 2H, 2 × H-Ar); 9.13 (s, 2H, H-2). ESI MS *m*/*z* (rel.%): 721 (100) [M+Na], 699 (31) [M+H]. HR MS (ESI) for $C_{36}H_{36}O_9N_4P$ [M+H]: calcd 699.2214; found 699.2212.

4.3.6. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(2-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)] phosphate (13a)

4-ChloroProTide **8** (120 mg, 0.2 mmol) and 2-furylboronic acid (**10a**) (37 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1). Yield: 73 mg, 57% (mixture of diastereomers 1:1.27). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.19 and 1.20 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH_2O); 1.25 and 1.27 (2 × d, 2 × 3H, J = 6.8, CH_3-Ala); 1.35, 1.38, 1.626 and 1.631 (4 × s, 4 × 3H, (CH_3)$_2$C); 3.60 (m, 2H, NH-Ala); 3.94 (m, 2H, CH-Ala); 4.05–4.13 (m, 4H, CH_3CH_2O); 4.27 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.4, $J_{5'b,4'}$ = 5.2, H-5′b); 4.31–4.36 (m, 3H, 2 × H-5′a and H-5′b); 4.43 (m, 2H, 2 × H-4′); 4.98 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.02–5.05 (m, 2H, 2 × H-3′); 5.23 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.34 and 6.36 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.8, H-1′); 6.64 (m, 2H, H-4-furyl); 7.04 and 7.06 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.12–7.20 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 7.25–7.31 (m, 6H, 4 × H-*m*-Ph, 2 × H-3-furyl); 7.38 and 7.39 (2 × d, 2 × 1H, $J_{6,5}$ = 3.6, H-6); 7.72 (m, 2H, 2 × H-5-furyl); 8.345 and 8.350 (2 × s, 2 × 1H, H-2). ESI MS *m*/*z* (rel.%): 635 (100) [M+Na], 613 (8) [M+H]. HR MS (ESI) for $C_{29}H_{33}O_9N_4NaP$ [M+Na]: calcd 635.1877; found 635.1877.

4.3.7. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(3-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)] phosphate (13b)

4-ChloroProTide **8** (120 mg, 0.2 mmol) and 3-furylboronic acid (**10b**) (37 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1). Yield: 79 mg, 63% (mixture of diastereomers 1:1.44). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.18 and 1.20 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH_2O); 1.26 and 1.27 (2 × d, 2 × 3H, J = 7.2, CH_3-Ala); 1.35, 1.37 (2 × s, 2 × 3H, (CH_3)$_2$C); 1.63 (s, 6H, (CH_3)$_2$C); 3.62 (m, 2H, NH-Ala); 3.94 (m, 2H, CH-Ala); 4.06–4.13 (m, 4H, CH_3CH_2O); 4.26 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.4, $J_{5'b,4'}$ = 5.2, H-5′b); 4.31–4.37 (m, 3H, 2 × H-5′a and H-5′b); 4.44 (m, 2H, H-4′); 4.99 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.02–5.07 (m, 2H, 2 × H-3′); 5.23 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.34 and 6.36 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.8, H-1′); 6.70 and 6.73 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.12–7.20 (m, 8H, 2 × H-*p*-Ph, 4 × H-*o*-Ph, 2 × H-4-furyl); 7.24–7.31 (m, 4H, 4 × H-*m*-Ph); 7.39 (d, 2H, $J_{6,5}$ = 3.6, H-6); 7.58 (m, 2H, H-5-furyl); 8.27 and 8.29 (2 × br s, 2 × 1H, H-2-furyl); 8.86 and 8.87 (2 × s, 2 × 1H, H-2). ESI MS *m*/*z* (rel.%): 635 (100) [M+Na], 613 (12) [M+H]. HR MS (ESI) for $C_{29}H_{33}O_9N_4NaP$ [M+Na]: calcd 635.1877; found 635.1879.

4.3.8. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(3-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)] phosphate (13d)

4-ChloroProTide **8** (120 mg, 0.2 mmol) and 3-thienylboronic acid (**10d**) (40 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1).Yield: 81 mg, 62% (mixture of diastereomers 1:1.53). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.18 and 1.20 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH_2O); 1.26 and 1.27 (2 × d, 2 × 3H, J = 7.2, CH_3-Ala); 1.36, 1.38, 1.63 and 1.64 (4 × s, 4 × 3H, (CH_3)$_2$C); 3.63 (m, 2H, NH-Ala); 3.95 (m, 2H, CH-Ala); 4.05–4.13 (m, 4H, CH_3CH_2O); 4.27 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.8, $J_{5'b,4'}$ = 5.2, H-5′b); 4.32–4.36 (m, 3H, 2 × H-5′a and H-5′b); 4.44 (m, 2H, H-4′); 4.99 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.03–5.07 (m, 2H, 2 × H-3′); 5.23 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.36 and 6.38 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.8, H-1′); 6.83 and 6.86 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.11–7.20 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 7.24–7.31 (m, 4H, 4 × H-*m*-Ph); 7.41 and 7.42 (2 × d, 2 × 1H, $J_{6,5}$ = 3.6, H-6); 7.49 (m, 2H, H-4-thienyl); 7.88 (m, 2H, H-5-thienyl); 8.23 (br s, 2H, H-2-thienyl); 8.907 and 8.912 (2 × s, 2 × 1H, H-2). ESI MS *m*/*z* (rel.%): 651 (100) [M+Na], 629 (12) [M+H]. HR MS (ESI) for $C_{29}H_{33}O_8N_4NaPS$ [M+Na]: calcd 651.1649; found 651.1648.

4.3.9. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)] phosphate (13e)

4-ChloroProTide **8** (120 mg, 0.2 mmol) and phenylboronic acid (**10e**) (38 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1). Yield: 77 mg, 60% (mixture of diastereomers 1:1.48). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.18 and 1.20 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH_2O); 1.266 and 1.274 (2 × d, 2 × 3H, J = 6.8, CH_3-Ala); 1.36 and 1.38 (2 × s, 2 × 3H, (CH_3)$_2$C); 1.64 (s, 6H, (CH_3)$_2$C); 3.59 (m, 2H, NH-Ala); 3.95 (m, 2H, CH-Ala); 4.04–4.13 (m, 4H, CH_3CH_2O); 4.28 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.4, $J_{5'b,4'}$ = 5.2, H-5′b); 4.33–4.37 (m, 3H, 2 × H-5′a and H-5′b); 4.45 (m, 2H, H-4′); 5.01 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.05–5.08 (m, 2H, 2 × H-3′); 5.26 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.37 and 6.40 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.8, H-1′); 6.83 and 6.86 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.12–7.20 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 7.25–7.31 (m, 4H, 4 × H-*m*-Ph); 7.399 and 7.404 (2 × d, 2 × 1H, $J_{6,5}$ = 3.6, H-6); 7.53–7.58 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 8.09 (m, 4H, 4 × H-*m*-Ph); 8.976 and 8.981 (2 × s, 2 × 1H, H-2). ESI MS *m*/*z* (rel.%): 645 (100) [M+Na], 623 (37) [M+H]. HR MS (ESI) for $C_{31}H_{35}O_8N_4NaP$ [M+Na]: calcd 645.2085; found 645.2086.

4.3.10. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(4-dibenzofuryl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)]phosphate (13f)

4-ChloroProTide **8** (120 mg, 0.2 mmol) and 4-dibenzofurylboronic acid (**10f**) (68 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 2:1). Yield: 154 mg, 91% (mixture of

diastereomers 1:1.02). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.17 and 1.18 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH_2O); 1.29 and 1.30 (2 × d, 2 × 3H, J = 7.2, CH_3-Ala); 1.38, 1.40, 1.66 and 1.67 (4 × s, 4 × 3H, $(CH_3)_2C$); 3.59 and 3.60 (2 × br t, 2 × 1H, $J_{H,P}$ = J_{vic} = 10.0, NH-Ala); 3.99 (m, 2H, CH-Ala); 4.04–4.13 (m, 4H, CH_3CH_2O); 4.32 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.6, $J_{5'b,4'}$ = 5.2, H-5′b); 4.37–4.43 (m, 3H, 2 × H-5′a and H-5′b); 4.48 (m, 2H, H-4′); 5.02 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.05–5.08 (m, 2H, 2 × H-3′); 5.26 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.43 and 6.46 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.8, H-1′); 6.78 and 6.80 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.10–7.14 (m, 2H, 2 × H-Ar); 7.16–7.22 (m, 4H, 4 × H-Ar); 7.24–7.31 (m, 4H, 4 × H-Ar); 7.38–7.43 (m, 2H, 2 × H-Ar); 7.47–7.53 (m, 5H, 4 × H-Ar, H-6); 7.55–7.60 (m, 4H, 4 × H-Ar); 8.02–8.08 (m, 4H, 4 × H-Ar); 8.14–8.17 (m, 2H, 2 × H-Ar); 9.13 (s, 2H, H-2). ESI MS *m*/*z* (rel.%): 735 (100) [M+Na], 713 (46) [M+H]. HR MS (ESI) for $C_{37}H_{37}O_9N_4NaP$ [M+Na]: calcd 735.2196; found 735.2200.

4.3.11. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(3-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)]phosphate (14b)

4-ChloroProTide **9** (150 mg, 0.2 mmol) and 3-furylboronic acid (**10b**) (42 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 3:2). Yield: 91 mg, 58% (mixture of diastereomers 1:1.14). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.280 and 1.284 (2 × d, 2 × 3H, J_{vic} = 7.2, CH_3-Ala); 1.36, 1.38, 1.631 and 1.634 (4 × s, 4 × 3H, $(CH_3)_2C$); 3.54 and 3.56 (2 × t, 2 × 1H, $J_{H,P}$ = J_{vic} = 9.2, NH-Ala); 4.02 (m, 2H, CH-Ala); 4.23 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.8, $J_{5'b,4'}$ = 5.2, H-5′b); 4.29–4.36 (m, 3H, 2 × H-5′a and H-5′b); 4.42 (m, 2H, H-4′); 4.97 (dd, 2H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.01–5.05 (m, 3H, 2 × H-3′, CH_2-Pha); 5.08–5.11 (m, 3H, CH_2-Pha, 2 × CH_2-Phb); 5.22 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.4, H-2′); 6.34 and 6.36 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.8, H-1′); 6.69 and 6.71 (2 × d, 2 × 1H, $J_{5,6}$ = 4.0, H-5); 7.12–7.20 (m, 8H, 2 × H-*p*-Ph, 4 × H-*o*-Ph, 2 × H-4-furyl); 7.22–7.38 (m, 16H, 14 × H-Ph, 2 × H-6); 7.59 (m, 2H, H-5-furyl); 8.28 (br s, 2H, H-2-furyl); 8.87 and 8.88 (2 × s, 2 × 1H, H-2). ESI MS *m*/*z* (rel.%): 697 (100) [M+Na], 675 (67) [M+H]. HR MS (ESI) for $C_{34}H_{36}O_9N_4P$ [M+H]: calcd 675.2214; found 675.2215.

4.3.12. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(3-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)]phosphate (14d)

4-ChloroProTide **9** (200 mg, 0.3 mmol) and 3-thienylboronic acid (**10d**) (60 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 3:2). Yield: 204 mg, 95% (mixture of diastereomers 1:1.07). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.29 (d, 6H, J_{vic} = 7.2, CH_3-Ala); 1.36, 1.38, 1.634 and 1.637 (4 × s, 4 × 3H, $(CH_3)_2C$); 3.56 and 3.58 (2 × t, 2 × 1H, $J_{H,P}$ = J_{vic} = 9.6, NH-Ala); 4.02 (m, 2H, CH-Ala); 4.24 (ddd, 1H, J_{gem} = 12.00, $J_{H,P}$ = 6.8, $J_{5'b,4'}$ = 5.6, H-5′b); 4.30–4.37 (m, 3H, 2 × H-5′a and H-5′b); 4.42 (m, 2H, H-4′); 4.98 (dd, 2H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.01–5.05 (m, 3H, 2 × H-3′, CH_2-Pha); 5.08–5.11 (m, 3H, CH_2-Pha, 2 × CH_2-Phb); 5.22 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.4, H-2′); 6.35 and 6.38 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.8, H-1′); 6.81 and 6.83 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.11–7.18 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 7.24–7.34 (m, 14H, 14 × H-Ph); 7.39 (m, 2H, H-6); 7.49 (m, 2H, H-4-thienyl); 7.87 (m, 2H, H-5-thienyl); 8.23 (br s, 2H, H-2-thienyl); 8.91 and 8.92 (2 × s, 2 × 1H, H-2). ESI MS *m*/*z* (rel.%): 713 (100) [M+Na], 691 (88) [M+H]. HR MS (ESI) for $C_{34}H_{36}O_8N_4PS$ [M+H]: calcd 691.1986; found 691.1988.

4.3.13. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)] phosphate (14e)

4-ChloroProTide **9** (200 mg, 0.3 mmol) and phenylboronic acid (**10e**) (57 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 3:2). Yield: 200 mg, 94% (mixture of diastereomers 1:1.21).^{1}H NMR (400.0 MHz, $CDCl_3$): 1.28 (d, 6H, J_{vic} = 7.2, CH_3-Ala); 1.36 and 1.38 (2 × s, 2 × 3H, $(CH_3)_2C$); 1.64 (s, 6H, $(CH_3)_2C$); 3.57 and 3.59 (2 × t, 2 × 1H, $J_{H,P}$ = J_{vic} = 9.6, NH-Ala); 4.03 (m, 2H, CH-Ala); 4.25 (ddd, 1H, J_{gem} = 10.8, $J_{H,P}$ = 6.4, $J_{5'b,4'}$ = 5.2, H-5′b); 4.30–4.38 (m, 3H, 2 × H-5′a and H-5′b); 4.42 (m, 2H, H-4′); 4.99 (dd, 2H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.2, H-2′); 5.01–5.07 (m, 3H, 2 × H-3′, CH_2-Pha); 5.08–5.10 (m, 3H, CH_2-Pha, 2 × CH_2-Phb); 5.25 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.8, H-2′); 6.36 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.38 (d, 1H, $J_{1',2'}$ = 3.2, H-1′); 6.80 and 6.81 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.12–7.19 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 7.25–7.36 (m, 14H, 14 × H-Ph); 7.36 and 7.37 (2 × d, 2 × 1H, $J_{6,5}$ = 4.0, H-6); 7.53–7.56 (m, 6H, 2 × H-*p*-Ph, 4 × H-*o*-Ph); 8.07 (m, 4H, 4 × H-*m*-Ph); 8.968 and 8.973 (2 × s, 2 × 1H, H-2). ESI MS *m*/*z* (rel.%): 707 (100) [M+Na], 685 (28) [M+H]. HR MS (ESI) for $C_{36}H_{37}O_8N_4NaP$ [M+Na]: calcd 707.22412; found 707.22446.

4.3.14. (2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-4-(4-dibenzofuryl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)]phosphate (14f)

4-ChloroProTide **9** (200 mg, 0.3 mmol) and 4-dibenzofurylboronic acid (**10f**) (98 mg, 1.5 equiv) were used. Column chromatography (SiO_2, hexane/EtOAc 3:2). Yield: 221 mg, 92% (mixture of diastereomers 1:1.19). ^{1}H NMR (400.0 MHz, $CDCl_3$): 1.30 (d, 6H, J_{vic} = 7.2, CH_3-Ala); 1.338 and 1.40 (2 × s, 2 × 3H, $(CH_3)_2C$); 1.66 (s, 6H, $(CH_3)_2C$); 3.57 and 3.59 (2 × t, 2 × 1H, $J_{H,P}$ = J_{vic} = 9.6, NH-Ala); 4.06 (m, 2H, CH-Ala); 4.28 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.8, $J_{5'b,4'}$ = 5.2, H-5′b); 4.33–4.41 (m, 3H, 2 × H-5′a and H-5′b); 4.45 (m, 2H, H-4′); 5.00–5.03 (m, 2H, H-2′, CH_2-Pha); 5.06–5.11 (m, 5H, H-3′, 2 × CH_2-Pha, 2 × CH_2-Phb); 5.28 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 2.4, H-2′); 6.40 (d, 1H, $J_{1',2'}$ = 2.8, H-1′); 6.43 (d, 1H, $J_{1',2'}$ = 3.2, H-1′); 6.73 and 6.74 (2 × d, 2 × 1H, $J_{5,6}$ = 3.6, 2 × H-5); 7.09–7.30 (m, 10H, 10 × H-Ar); 7.38–7.43 (m, 6H, 5 × H-Ar, H-6); 7.45–7.50 (m, 4H, 4 × H-Ar); 7.52–7.61 (m, 6H, 6 × H-Ar); 7.93–8.08 (m, 8H, 8 × H-Ar); 8.12–8.14 (m, 4H, 4 × H-Ar); 9.098 and 9.101 (2 × s, 2 × 1H, H-2). ESI MS *m*/*z* (rel.%): 797 (100) [M+Na], 775 (63) [M+H]. HR MS (ESI) for $C_{42}H_{40}O_9N_4P$ [M+H]: calcd 775.25274; found 775.25316.

4.4. General procedure for the deprotection and synthesis of the final ProTides

Protected pronucleotides (100 μmol) were dissolved in 90% TFA (1 ml) and stirred for 1 h at room temperature. Then the solvent was repeatedly coevaporated with methanol under reduced pressure. Products were purified using Biotage flash chromatography (C-18 reverse phase, gradient of methanol in water) or by column chromatography (SiO_2, 2% of methanol in chloroform). Products were obtained as lyofilizates (benzene/*t*-butanol 1:1). Mixture of diastereomers was obtained, ratios were determined by NMR spectra.

4.4.1. β-D-Ribofuranosyl-4-(2-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)]phosphate (15a)

Protected ProTide **12a** (83 mg, 0.14 mmol) was used. Flash chromatography (C-18 reverse phase, gradient of methanol in water). Yield: 40 mg, 52% (mixture of diastereomers 1:1.05). mp: 61–65 °C; ^{1}H NMR (499.8 MHz, CD_3OD): 1.23, 1.30 (2 × d, 2 × 3H, J_{vic} = 7.2, CH_3-Ala); 3.62, 3.63 (2 × s, 2 × 3H, CH_3O); 3.85 (dq, 1H, $J_{H,P}$ = 9.2, J_{vic} = 7.2, CH-Ala); 3.94 (dq, 1H, $J_{H,P}$ = 9.9, J_{vic} = 7.2, CH-Ala); 4.24, 4.26 (2 × m, 2 × 1H, H-4′); 4.30–4.45 (m, 6H, H-3′,5′); 4.52, 4.55 (2 × t, 2 × 1H, $J_{2',3'}$ = $J_{2',1'}$ = 5.3, H-2′); 6.37, 6.38 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 6.726, 6.730 (2 × dd, 2 × 1H, $J_{4,3}$ = 3.9, $J_{4,5}$ = 1.7, H-4-furyl); 7.09, 7.13 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.17, 7.18 (2 × m, 2 × 1H, H-*p*-Ph); 7.21, 7.24 (2 × m, 2 × 2H, H-*o*-Ph); 7.34 (m, 4H, H-*m*-Ph); 7.47, 7.48 (2 × d, 2 × 1H, $J_{3,4}$ = 3.9, H-3-furyl); 7.63, 7.67 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 7.89, 7.90

(2 × d, 2 × 1H, $J_{5,4}$ = 1.7, H-5-furyl); 8.711 and 8.712 (2 × s, 2 × 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 20.27 (d, $J_{C,P}$ = 7.6, CH_3-Ala); 20.42 (d, $J_{C,P}$ = 6.5, CH_3-Ala); 51.41, 51.46 (d, $J_{C,P}$ = 1.3, CH-Ala); 52.69, 52.75 (CH_3O); 67.30, 67.67 (d, $J_{C,P}$ = 5.4, CH_2-5′); 71.69, 71.77 (CH-3′); 75.62 (CH-2′); 83.91, 83.94 (d, $J_{C,P}$ = 8.5, CH-4′); 89.02, 89.20 (CH-1′); 103.52, 103.54 (CH-5); 113.59, 113.61 (CH-4-furyl); 114.76 (CH-3-furyl); 114.81, 114.83 (C-4a); 121.34, 121.37 (d, $J_{C,P}$ = 4.3, CH-*o*-Ph); 126.20 (CH-*p*-Ph); 128.74, 128.86 (CH-6); 130.81 (CH-*m*-Ph); 147.27 (CH-5-furyl); 148.10, 148.12 (C-4); 151.82, 151.84 (CH-2); 152.13 (d, $J_{C,P}$ = 6.7, C-*i*-Ph); 153.61, 153.64 (C-2-furyl); 153.75 (C-7a); 175.31 (d, $J_{C,P}$ = 4.8, CO-Ala); 175.51 (d, $J_{C,P}$ = 4.0, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.87, 5.00. IR (ATR): ν = 3398, 2952, 1738, 1598, 1565, 1489, 1448, 1247, 1022 cm^{-1}; ESI MS *m*/*z* (rel.%): 557 (100) [M−H]. HR MS (ESI) for $C_{25}H_{26}O_9N_4P$ [M−H]: calcd 557.1443; found 557.1446.

4.4.2. β-D-Ribofuranosyl-4-(3-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)]phosphate (15b)

Protected ProTide **12b** (73 mg, 0.12 mmol) was used. Flash chromatography (C-18 reverse phase, gradient of methanol in water). Yield: 41 mg, 60% (mixture of diastereomers 1:1.12). Mp: 60–69 °C; ^{1}H NMR (500.0 MHz, CD_3OD): 1.23 (dd, 3H, J_{vic} = 7.1, $J_{H,P}$ = 1.2, CH_3-Ala); 1.29 (dd, 3H, J_{vic} = 7.1, $J_{H,P}$ = 1.0, CH_3-Ala); 3.362 (s, 6H, CH_3O); 3.87 (dq, 1H, $J_{H,P}$ = 9.3, J_{vic} = 7.1. CH-Ala); 3.93 (dq, 1H, $J_{H,P}$ = 10.0, J_{vic} = 7.1. CH-Ala); 4.22–4.28 (m, 2H, H-4′); 4.29–4.44 (m, 6H, H-3′,5′); 4.52 and 4.55 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 6.37 and 6.38 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 6.91 and 6.95 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.17 and 7.18 (2 × m, 2 × 1H, H-*p*-Ph); 7.19 and 7.20 (2 × dd, 2 × 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl); 7.21 and 7.23 (2 × m, 2 × 2H, H-*o*-Ph); 7.33 and 7.34 (2 × m, 2 × 2H, H-*m*-Ph); 7.63 and 7.67 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 7.718 and 7.722 (2 × dd, 2 × 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.6, H-5-furyl); 8.44 and 8.46 (2 × dd, 2 × 1H, $J_{2,5}$ = 1.6, $J_{2,4}$ = 0.9, H-2-furyl); 8.73 and 8.74 (2 × s, 2 × 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 20.27 (d, $J_{C,P}$ = 7.4, CH_3-Ala); 20.42 (d, $J_{C,P}$ = 6.5, CH_3-Ala); 51.41 and 51.48 (d, $J_{C,P}$ = 1.1, CH-Ala); 52.72 and 52.76 (CH_3O); 67.34 and 67.68 (d, $J_{C,P}$ = 5.4, CH_2-5′); 71.70 and 71.77 (CH-3′); 75.60 and 75.61 (CH-2′); 83.90 and 83.94 (d, $J_{C,P}$ = 8.3, CH-4′); 89.08 and 89.24 (CH-1′); 102.46 and 102.48 (CH-5); 110.33 (CH-4-furyl); 116.76 and 116.77 (C-4a); 121.35 and 121.39 (d, $J_{C,P}$ = 4.1, CH-*o*-Ph); 126.11 and 126.14 (C-3-furyl); 126.21 (CH-*p*-Ph); 128.43 and 128.53 (CH-6); 130.82 (CH-*m*-Ph); 145.62 and 145.63 (CH-5-furyl); 145.92 and 145.93 (CH-2-furyl); 152.02 and 152.04 (CH-2); 152.14 (d, $J_{C,P}$ = 6.8, C-*i*-Ph); 152.22 and 152.23 (C-4); 153.225 and 153.234 (C-7a); 173.31 (d, $J_{C,P}$ = 5.2, CO-Ala); 175.52 (d, $J_{C,P}$ = 4.3, CO-Ala). ^{31}P NMR (202.3 MHz, CD_3OD): 4.89 and 5.01. IR (ATR): ν = 3313, 2952, 1739, 1589, 1567, 1489, 1455, 1210, 1010 cm^{-1}; ESI MS *m*/*z* (rel.%): 557 (100) [M−H]. HR MS (ESI) for $C_{25}H_{28}O_9N_4P$ [M+H]: calcd 559.1588; found 559.1585.

4.4.3. β-D-Ribofuranosyl-4-(2-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)]phosphate (15c)

Protected ProTide **12c** (89 mg, 0.14 mmol) was used. Flash chromatography (C-18 reverse phase, gradient of methanol in water). Yield: 51 mg, 61% (mixture of diastereomers 1:1). mp: 57–65 °C; ^{1}H NMR (600.1 MHz, CD_3OD): 1.23, 1.29 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.3, CH_3-Ala); 3.61, 3.62 (2 × s, 2 × 3H, CH_3O); 3.85 (dq, 1H, $J_{H,P}$ = 9.3, J_{vic} = 7.2, CH-Ala); 3.93 (dq, 1H, $J_{H,P}$ = 9.9, J_{vic} = 7.2, CH-Ala); 4.25, 4.27 (2 × m, 2 × 1H, H-4′); 4.30–4.45 (m, 6H, H-3′,5′); 4.52, 4.55 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 6.37, 6.38 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 7.02, 7.06 (2 × d, 2 × 1H, $J_{5,6}$ = 3.9, H-5); 7.17, 7.18 (2 × m, 2 × 1H, H-*p*-Ph); 7.21, 7.24 (2 × m, 2 × 2H, H-*o*-Ph); 7.280, 7.285 (2 × dd, 2 × 1H, $J_{4,5}$ = 5.0, $J_{4,3}$ = 3.8, H-4-thienyl); 7.33, 7.34 (2 × m, 2 × 2H, H-*m*-Ph); 7.65, 7.70 (2 × d, 2 × 1H, $J_{6,5}$ = 3.9, H-6); 7.730, 7.734 (2 × dd, 2 × 1H, $J_{5,4}$ = 5.0, $J_{5,3}$ = 1.2, H-5-thienyl); 8.06, 8.08 (2 × dd, 2 × 1H, $J_{3,4}$ = 3.8, $J_{3,5}$ = 1.2, H-3-thienyl); 8.698 and 8.700 (2 × s, 2 × 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 20.27 (d, $J_{C,P}$ = 7.5, CH_3-Ala); 20.42 (d, $J_{C,P}$ = 6.4, CH_3-Ala); 51.40, 51.45 (d, $J_{C,P}$ = 1.1, CH-Ala); 52.72, 52.77 (CH_3O); 67.28, 67.65 (d, $J_{C,P}$ = 5.3, CH_2-5′); 71.67, 71.75 (CH-3′); 75.64 (CH-2′); 83.89, 83.92 (d, $J_{C,P}$ = 8.5, CH-4′); 89.10, 89.28 (CH-1′); 102.67, 102.69 (CH-5); 115.32, 115.34 (C-4a); 121.34, 121.37 (d, $J_{C,P}$ = 4.6, CH-*o*-Ph); 126.21 (CH-*p*-Ph); 128.77, 128.89 (CH-6); 129.67, 129.68 (CH-4-thienyl); 130.75, 130.81, 130.82 (CH-3-thienyl, CH-*m*-Ph); 131.33 (CH-5-thienyl); 142.92, 142.95 (C-2-thienyl); 151.79, 151.82 (CH-2); 152.12 (d, $J_{C,P}$ = 6.7, C-*i*-Ph); 152.24, 152.25 (C-4); 153.60, 153.61 (C-7a); 175.37 (d, $J_{C,P}$ = 5.3, CO-Ala); 175.52 (d, $J_{C,P}$ = 4.1, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.89, 5.01. IR (ATR): ν = 3282, 2921, 1739, 1561, 1490, 1438, 1210, 1012 cm^{-1}; ESI MS *m*/*z* (rel.%): 597 (100) [M+Na], 575 (11) [M+H]. HR MS (ESI) for $C_{25}H_{27}O_8N_4NaPS$ [M+Na]: calcd 597.1179; found 597.1178.

4.4.4. β-D-Ribofuranosyl-4-(3-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)]phosphate (15d)

Protected ProTide **12d** (74 mg, 0.12 mmol) was used. Flash chromatography (C-18 reverse phase, gradient of methanol in water). Yield: 36 mg, 52% (mixture of diastereomers 1:1.07). mp: 57–60 °C; ^{1}H NMR (500.0 MHz, $CDCl_3$): 1.27 and 1.30 (2 × d, 2 × 3H, J_{vic} = 7.0, CH_3-Ala); 3.619 and 3.621 (2 × s, 2 × 3H, CH_3O); 3.90–4.00 (m, 2H, CH-Ala); 4.09 (bdd, 1H, $J_{H,P}$ = 12.0, J_{vic} = 10.2, NH-Ala); 4.29–4.47 (m, 10H, H-2′,4′,3′,5′); 6.27 and 6.29 (2 × d, 2 × 1H, $J_{1',2'}$ = 4.9, H-1′); 6.71 and 6.74 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.08 and 7.09 (2 × m, 2 × 1H, H-*p*-Ph); 7.13 and 7.15 (2 × m, 2 × 2H, H-*o*-Ph); 7.23 (m, 4H, H-*m*-Ph); 7.40 (d, 1H, $J_{6,5}$ = 3.8, H-6); 7.42–7.46 (m, 3H, H-5-thienyl and H-6); 7.78 and 7.80 (2 × dd, 2 × 1H, $J_{4,5}$ = 5.0, $J_{4,2}$ = 1.3, H-4-thienyl); 8.10 and 8.12 (2 × dd, 2 × 1H, $J_{2,5}$ = 2.9, $J_{2,4}$ = 1.3, H-2-thienyl); 8.76 (s, 2H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 20.73 and 20.76 (d, $J_{C,P}$ = 5.2, CH_3-Ala); 50.13 (d, $J_{C,P}$ = 15.0, CH-Ala); 52.47 and 52.55 (CH_3O); 66.05 and 66.36 (d, $J_{C,P}$ = 5.1, CH_2-5′); 70.77 and 70.89 (CH-3′); 74.95 and 75.05 (CH-2′); 82.77 and 82.80 (d, $J_{C,P}$ = 7.1, CH-4′); 89.91 and 88.99 (CH-1′); 101.23 and 101.32 (CH-5); 115.54 and 115.59 (C-4a); 119.93 and 120.03 (d, $J_{C,P}$ = 4.9, CH-*o*-Ph); 125.01 and 125.11 (CH-*p*-Ph); 126.48 (CH-5-thienyl); 126.57 and 126.72 (CH-6); 127.36 (CH-4-thienyl); 127.82 and 127.84 (CH-2-thienyl); 129.69 (CH-*m*-Ph); 139.66 and 139.71 (C-3-thienyl); 150.43 (d, $J_{C,P}$ = 6.8, C-*i*-Ph); 150.73 and 150.84 (CH-2); 151.35 and 151.45 (C-7a); 152.37 and 152.42 (C-4); 173.87 (d, $J_{C,P}$ = 6.9, CO-Ala). ^{31}P NMR (202.3 MHz, $CDCl_3$): 3.18 and 3.40. IR (ATR): ν = 3251, 2922, 1739, 1564, 1490, 1455, 1209, 1020 cm^{-1}; ESI MS *m*/*z* (rel.%): 573 (100) [M−H]. HR MS (ESI) for $C_{25}H_{26}O_8N_4PS$ [M−H]: calcd 573.1214; found 573.1217.

4.4.5. β-D-Ribofuranosyl-4-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)]phosphate (15e)

Protected ProTide **12e** (82 mg, 0.13 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 41 mg, 54% (mixture of diastereomers 1:1.05). Mp: 52–58 °C; ^{1}H NMR (600.1 MHz, CD_3OD): 1.23, 1.29 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.2, CH_3-Ala); 3.61, 3.62 (2 × s, 2 × 3H, CH_3O); 3.87 (dq, 1H, $J_{H,P}$ = 9.3, J_{vic} = 7.2, CH-Ala); 3.94 (dq, 1H, $J_{H,P}$ = 10.0, J_{vic} = 7.2, CH-Ala); 4.26, 4.28 (2 × m, 2 × 1H, H-4′); 4.31–4.45 (m, 6H, H-3′,5′); 4.54, 4.56 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 6.41, 6.42 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 6.87, 6.91 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.16, 7.17 (2 × m, 2 × 1H, H-*p*-OPh); 7.21, 7.23 (2 × m, 2 × 2H, H-*o*-OPh); 7.33 (m, 4H, H-*m*-OPh); 7.55–7.62 (m, 6H, H-*m*,*p*-Ph); 7.67, 7.72 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 8.03, 8.05 (2 × m, 2 × 2H, H-*o*-Ph); 8.841, 8.843 (2 × s, 2 × 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 20.27 (d, $J_{C,P}$ = 7.2, CH_3-Ala); 20.41 (d, $J_{C,P}$ = 6.3, CH_3-Ala); 51.44, 51.51 (CH-Ala); 52.70, 52.75 (CH_3O);

67.37, 67.69 (d, $J_{C,P}$ = 5.3, CH_2-5′); 71.71, 71.76 (CH-3′); 75.64 (CH-2′); 83.92, 83.98 (d, $J_{C,P}$ = 8.3, CH-4′); 89.32, 89.45 (CH-1′); 102.87, 102.90 (CH-5); 117.85 (C-4a); 121.32, 121.36 (d, $J_{C,P}$ = 5.0, CH-*o*-OPh); 126.17 (CH-*p*-OPh); 128.96, 129.04 (CH-6); 129.96, 130.00 (CH-*o*,*m*-Ph); 130.78 (CH-*m*-OPh); 131.54 (CH-*p*-Ph); 138.45 (C-*i*-Ph); 151.94, 151.97 (CH-2); 152.14 (d, $J_{C,P}$ = 7.0, C-*i*-Ph); 153.46, 153.48 (C-7a); 158.81 (C-4); 175.30 (d, $J_{C,P}$ = 4.2, CO-Ala); 175.49 (d, $J_{C,P}$ = 3.3, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.89, 5.02. IR (ATR): ν = 3219, 2921, 1737, 1560, 1490, 1438, 1207, 1015 cm^{-1}; ESI MS *m*/*z* (rel.%): 591 (100) [M+Na], 569 (38) [M+H]. HR MS (ESI) for $C_{27}H_{30}O_8N_4P$ [M+H]: calcd 569.1796; found 569.1798.

4.4.6. β-D-Ribofuranosyl-4-(4-dibenzofuryl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(methyloxy-L-alaninyl)]phosphate (15f)

Protected ProTide **12f** (61 mg, 0.09 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 24 mg, 42% (mixture of diastereomers 1:1.91). Mp: 77–84 °C; ^{1}H NMR (499.8 MHz, CD_3OD): 1.25, 1.30 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.1, CH_3-Ala); 3.58, 3.61 (2 × s, 2 × 3H, CH_3O); 3.89 (dq, 1H, $J_{H,P}$ = 9.2, J_{vic} = 7.2, CH-Ala); 3.95 (dq, 1H, $J_{H,P}$ = 10.2, J_{vic} = 7.2, CH-Ala); 4.28 (m, 2H, H-4′); 4.32–4.47 (m, 6H, H-3′,5′); 4.58, 4.60 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 6.46, 6.47 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 6.68, 6.72 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.14 (m, 2H, H-*p*-Ph); 7.21, 7.23 (2 × m, 2 × 2H, H-*o*-Ph); 7.31 (m, 4H, H-*m*-Ph); 7.43 (m, 2H, H-8-dibenzofuryl); 7.52, 7.53 (2 × ddd, 2 × 1H, $J_{7,6}$ = 8.4, $J_{7,8}$ = 7.1, $J_{7,9}$ = 1.3, H-7-dibenzofuryl); 7.57 (m, 2H, H-6-dibenzofuryl); 7.589, 7.593 (2 × t, 2 × 1H, $J_{2,1} = J_{2,3}$ = 7.7, H-2-dibenzofuryl); 7.66, 7.72 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 7.92, 7.94 (2 × dd, 2 × 1H, $J_{3,2}$ = 7.7, $J_{3,1}$ = 1.3, H-3-dibenzofuryl); 8.134, 8.135 (2 × dd, 2 × 1H, $J_{9,8}$ = 7.7, $J_{9,7}$ = 1.3, H-9-benzofuryl); 8.253, 8.255 (2 × dd, 2 × 1H, $J_{1,2}$ = 7.7, $J_{1,3}$ = 1.3, H-1-benzofuryl); 8.936, 8.938 (2 × s, 2 × 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 20.28 (d, $J_{C,P}$ = 7.3, CH_3-Ala); 20.44 (d, $J_{C,P}$ = 6.5, CH_3-Ala); 51.43, 51.51 (d, $J_{C,P}$ = 1.2, CH-Ala); 52.69, 52.76 (CH_3O); 67.38, 67.69 (d, $J_{C,P}$ = 5.4, CH_2-5′); 71.74, 71.80 (CH-3′); 75.71 (CH-2′); 83.96, 84.01 (d, $J_{C,P}$ = 8.4, CH-4′); 89.22, 89.38 (CH-1′); 103.64 (CH-5); 112.72 (CH-6-dibenzofuryl); 119.44, 119.47 (C-4a); 121.34, 121.37 (d, $J_{C,P}$ = 4.8, CH-*o*-Ph); 122.05 (CH-9-dibenzofuryl); 123.15, 123.17 (C-4-dibenzofuryl); 123.83 (CH-1-dibenzofuryl); 124.48 (CH-2-dibenzofuryl); 124.52 (CH-8-dibenzofuryl); 124.96 (C-9a-dibenzofuryl); 126.19 (CH-*p*-Ph); 126.74 (C-9b-dibenzofuryl); 128.69, 128.81 (CH-6); 128.99 (CH-7-dibenzofuryl); 129.41 (CH-3-dibenzofuryl); 130.81 (CH-*m*-Ph); 152.00, 152.01 (CH-2); 152.14 (d, $J_{C,P}$ = 6.9, C-*i*-Ph); 153.29 (C-7a); 154.60 (C-4a-dibenzofuryl); 155.57, 155.59 (C-4); 157.53 (C-5a-dibenzofuryl); 175.31 (d, $J_{C,P}$ = 4.9, CO-Ala); 175.31 (d, $J_{C,P}$ = 3.6, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.90, 5.03. IR (ATR): ν = 3284, 2925, 1740, 1567, 1412, 1211, 1156, 1021 cm^{-1}; ESI MS *m*/*z* (rel.%): 681 (100) [M+Na], 659 (18) [M+H]. HR MS (ESI) for $C_{33}H_{32}O_9N_4P$ [M+H]: calcd 659.1901; found 659.1902.

4.4.7. β-D-Ribofuranosyl-4-(2-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)]phosphate (16a)

Protected ProTide **13a** (123 mg, 0.14 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 46 mg, 40% (mixture of diastereomers 1:1.03). mp: 47–53 °C; ^{1}H NMR (500.0 MHz, CD_3OD): 1.17, 1.18 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH$_2$O); 1.23, 1.30 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.2, CH_3-Ala); 3.84 (dq, 1H, $J_{H,P}$ = 9.1, J_{vic} = 7.2, CH-Ala); 3.91 (dq, 1H, $J_{H,P}$ = 9.9, J_{vic} = 7.2, CH-Ala); 4.03–4.13 (m, 4H, CH_3CH_2O); 4.24, 4.26 (2 × m, 2 × 1H, H-4′); 4.30–4.45 (m, 6H, H-3′,5′); 4.52, 4.54 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 6.36, 6.37 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 6.728, 6.731 (2 × dd, 2 × 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.8, H-4-furyl); 7.09, 7.13 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.17, 7.18 (2 × m, 2 × 1H, H-*p*-Ph); 7.21, 7.24 (2 × m, 2 × 2H, H-*o*-Ph); 7.34 (m, 4H, H-*m*-Ph); 7.47, 7.48 (2 × dd, 2 × 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.8, H-3-furyl); 7.63, 7.67 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 7.89, 7.90 (dd, 2H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.8, H-5-furyl); 8.713 and 8.715 (2 × s, 2 × 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 14.39, 14.43 (CH_3CH$_2$O); 20.31 (d, $J_{C,P}$ = 7.4, CH_3-Ala); 20.46 (d, $J_{C,P}$ = 6.4, CH_3-Ala); 51.50, 51.56 (d, $J_{C,P}$ = 1.3, CH-Ala); 62.31, 62.36 (CH_3CH_2O); 67.30, 67.70 (d, $J_{C,P}$ = 5.5, CH_2-5′); 71.69, 71.77 (CH-3′); 75.60, 75.62 (CH-2′); 83.90, 83.93 (d, $J_{C,P}$ = 8.4, CH-4′); 89.07, 89.23 (CH-1′); 103.53, 103.55 (CH-5); 113.61, 113.62 (CH-4-furyl); 114.80 (CH-3-furyl); 114.82 (C-4a); 121.34, 121.36 (d, $J_{C,P}$ = 4.8, CH-*o*-Ph); 126.20 (CH-*p*-Ph); 128.80, 128.87 (CH-6); 130.81 (CH-*m*-Ph); 147.29 (CH-5-furyl); 148.06, 148.08 (C-4); 151.78, 151.81 (CH-2); 152.14 (d, $J_{C,P}$ = 6.7, C-*i*-Ph); 153.56, 153.60, 153.74 (C-7a, C-2-furyl); 174.87 (d, $J_{C,P}$ = 5.3, CO-Ala); 175.07 (d, $J_{C,P}$ = 4.3, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.91, 5.04. IR (ATR): ν = 3215, 2920, 1734, 1598, 1565, 1488, 1455, 1206, 1008 cm^{-1}; ESI MS *m*/*z* (rel.%): 611 (13) [M+K], 595 (56) [M+Na], 573 (100) [M+H]. HR MS (ESI) for $C_{26}H_{30}O_9N_4P$ [M+H]: calcd 573.1745; found 573.1747.

4.4.8. β-D-Ribofuranosyl-4-(3-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)]phosphate (16b)

Protected ProTide **13b** (86 mg, 0.14 mmol) was used. Flash chromatography (C-18 reverse phase, gradient of methanol in water). Yield: 32 mg, 40% (mixture of diastereomers 1:1.60). mp: 60–69 °C; ^{1}H NMR (600.1 MHz, CD_3OD): 1.17, 1.18 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH$_2$O); 1.24, 1.29 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.2, CH_3-Ala); 3.85 (dq, 1H, $J_{H,P}$ = 9.1, J_{vic} = 7.2, CH-Ala); 3.91 (dq, 1H, $J_{H,P}$ = 9.9, J_{vic} = 7.2, CH-Ala); 4.03–4.12 (m, 4H, CH_3CH_2O); 4.24, 4.26 (2 × m, 2 × 1H, H-4′); 4.30–4.44 (m, 6H, H-3′,5′); 4.52, 4.54 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 6.37, 6.38 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 6.92, 6.96 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.16–7.25 (m, 8H, H-4-furyl, H-*o*,*p*-Ph); 7.34 (m, 4H, H-*m*-Ph); 7.63, 7.68 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 7.728, 7.730 (2 × dd, 2 × 1H, $J_{5,4}$ = 2.0, $J_{5,2}$ = 1.5, H-5-furyl); 8.46, 8.47 (2 × dd, 2 × 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 1.0, H-2-furyl); 8.742 and 8.744 (2 × s, 2 × 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 14.40, 14.44 (CH_3CH$_2$O); 20.30 (d, $J_{C,P}$ = 7.5, CH_3-Ala); 20.46 (d, $J_{C,P}$ = 6.4, CH_3-Ala); 51.50, 51.59 (d, $J_{C,P}$ = 1.3, CH-Ala); 62.33, 62.36 (CH_3CH_2O); 67.33, 67.70 (d, $J_{C,P}$ = 5.3, CH_2-5′); 71.70, 71.78 (CH-3′); 75.60, 75.61 (CH-2′); 83.90, 83.95 (d, $J_{C,P}$ = 8.2, CH-4′); 89.09, 89.23 (CH-1′); 102.50, 102.52 (CH-5); 110.31, 110.32 (CH-4-furyl); 116.75, 116.76 (C-4a); 121.36, 121.38 (d, $J_{C,P}$ = 4.8, CH-*o*-Ph); 126.02, 126.05 (C-3-furyl); 126.21, 126.22 (CH-*p*-Ph); 128.52, 128.53 (CH-6); 130.82, 130.83 (CH-*m*-Ph); 145.65 (CH-5-furyl); 145.97 (CH-2-furyl); 151.94, 151.97 (CH-2); 152.14 (d, $J_{C,P}$ = 6.7, C-*i*-Ph); 152.15, 152.16 (C-4); 153.22, 153.23 (C-7a); 174.87 (d, $J_{C,P}$ = 5.6, CO-Ala); 175.07 (d, $J_{C,P}$ = 4.0, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.91, 5.04. IR (ATR): ν = 3275, 2924, 1736, 1591, 1568, 1490, 1456, 1209, 1022 cm^{-1}; ESI MS *m*/*z* (rel.%): 571 (100) [M−H]. HR MS (ESI) for $C_{26}H_{28}O_9N_4P$ [M−H]: calcd 571.1599; found 571.1602.

4.4.9. β-D-Ribofuranosyl-4-(2-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)]phosphate (16c)

Protected ProTide **13c** (90 mg, 0.14 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 40 mg, 47% (mixture of diastereomers 1:1.31). mp: 51–57 °C; ^{1}H NMR (499.8 MHz, CD_3OD): 1.169, 1.174 (2 × t, 2 × 3H, J_{vic} = 7.1, CH_3CH$_2$O); 1.24, 1.30 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.2, CH_3-Ala); 3.85 (dq, 1H, $J_{H,P}$ = 9.1, J_{vic} = 7.2, CH-Ala); 3.91 (dq, 1H, $J_{H,P}$ = 9.9, J_{vic} = 7.2, CH-Ala); 4.02–4.13 (m, 4H, CH_3CH_2O); 4.25, 4.27 (2 × m, 2 × 1H, H-4′); 4.30–4.45 (m, 6H, H-3′,5′); 4.52, 4.55 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 6.37, 6.38 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 7.02, 7.06 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.16, 7.18 (2 × m, 2 × 1H, H-*p*-Ph); 7.21, 7.24 (2 × m, 2 × 2H, H-*o*-Ph); 7.28, 7.29 (2 × dd, 2 × 1H, $J_{4,5}$ = 5.0, $J_{4,3}$ = 3.8, H-4-thienyl); 7.33 (m, 4H, H-*m*-Ph); 7.66, 7.70 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 7.737,

7.742 (2 × dd, 2 × 1H, $J_{5,4}$ = 5.0, $J_{5,3}$ = 1.2, H-5-thienyl); 8.07, 8.08 (2 × dd, 2 × 1H, $J_{3,4}$ = 3.8, $J_{3,5}$ = 1.2, H-3-thienyl); 8.706 and 8.707 (2 × s, 2 × 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 14.40, 14.44 (CH_3CH_2O); 20.31 (d, $J_{C,P}$ = 7.3, CH_3-Ala); 20.46 (d, $J_{C,P}$ = 6.6, CH_3-Ala); 51.50, 51.57 (d, $J_{C,P}$ = 1.4, CH-Ala); 62.33, 62.36 (CH_3CH_2O); 67.31, 67.69 (d, $J_{C,P}$ = 5.3, CH_2-5′); 71.68, 71.76 (CH-3′); 75.64 (CH-2′); 83.90, 83.94 (d, $J_{C,P}$ = 8.5, CH-4′); 89.19, 89.33 (CH-1′); 102.70, 102.72 (CH-5); 115.34, 115.35 (C-4a); 121.35, 121.36 (d, $J_{C,P}$ = 4.8, CH-*o*-Ph); 126.20 (CH-*p*-Ph); 128.89, 128.97 (CH-6); 129.68 (CH-4-thienyl); 130.80, 130.82 (CH-3-thienyl, CH-*m*-Ph); 131.41 (CH-5-thienyl); 142.77, 142.81 (C-2-thienyl); 151.71, 151.73 (CH-2); 152.14, 152.15 (d, $J_{C,P}$ = 6.7, C-*i*-Ph); 152.16, 152.17 (C-4); 153.60, 153.61 (C-7a); 174.87 (d, $J_{C,P}$ = 5.1, CO-Ala); 175.07 (d, $J_{C,P}$ = 4.6, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.91, 5.04. IR (ATR): ν = 3255, 2926, 1735, 1563, 1490, 1449, 1209, 1022 cm^{-1}; ESI MS *m*/*z* (rel.%): 587 (100) [M−H]. HR MS (ESI) for $C_{26}H_{28}O_8N_4PS$ [M−H]: calcd 587.1371; found 587.1372.

4.4.10. β-D-Ribofuranosyl-4-(3-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)]phosphate (16d)

Protected ProTide **13d** (70 mg, 0.11 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 49 mg, 75% (mixture of diastereomers 1:1.56). mp: 48–52 °C; ^{1}H NMR (600.1 MHz, CD_3OD): 1.169, 1.174 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH_2O); 1.24, 1.29 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.2, CH_3-Ala); 3.85 (dq, 1H, $J_{H,P}$ = 9.1, J_{vic} = 7.2, CH-Ala); 3.91 (dq, 1H, $J_{H,P}$ = 9.8, J_{vic} = 7.2, CH-Ala); 4.03–4.12 (m, 4H, CH_3CH_2O); 4.25, 4.27 (2 × m, 2 × 1H, H-4′); 4.30–4.45 (m, 6H, H-3′,5′); 4.53, 4.55 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 6.39, 6.40 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 6.99, 7.03 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.17, 7.18 (2 × m, 2 × 1H, H-*p*-Ph); 7.21, 7.24 (2 × m, 2 × 2H, H-*o*-Ph); 7.33 (m, 4H, H-*m*-Ph); 7.64, 7.65 (2 × dd, 2 × 1H, $J_{5,4}$ = 5.1, $J_{5,2}$ = 2.9, H-5-thienyl); 7.66, 7.71 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 7.87, 7.88 (2 × dd, 2 × 1H, $J_{4,5}$ = 5.1, $J_{4,2}$ = 1.3, H-4-thienyl); 8.33, 8.34 (2 × dd, 2 × 1H, $J_{2,5}$ = 2.9, $J_{2,4}$ = 1.3, H-2-thienyl); 8.777 and 8.779 (2 × s, 2 × 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 14.41, 14.44 (CH_3CH_2O); 20.31 (d, $J_{C,P}$ = 7.3, CH_3-Ala); 20.46 (d, $J_{C,P}$ = 6.5, CH_3-Ala); 51.50, 51.59 (d, $J_{C,P}$ = 1.3, CH-Ala); 62.33, 62.36 (CH_3CH_2O); 67.33, 67.69 (d, $J_{C,P}$ = 5.3, CH_2-5′); 71.71, 71.78 (CH-3′); 75.64, 75.66 (CH-2′); 83.93, 83.98 (d, $J_{C,P}$ = 7.7, CH-4′); 89.13, 89.28 (CH-1′); 102.94, 102.96 (CH-5); 116.90, 116.91 (C-4a); 121.36, 121.37 (d, $J_{C,P}$ = 4.8, CH-*o*-Ph); 126.21, 126.22 (CH-*p*-Ph); 127.93 (CH-5-thienyl); 128.39, 128.40 (CH-4-thienyl); 128.88, 128.95 (CH-6); 129.55 (CH-2-thienyl); 130.81, 130.83 (CH-*m*-Ph); 140.35, 140.38 (C-3-thienyl); 151.66, 151.68 (CH-2); 152.14 (d, $J_{C,P}$ = 6.9, C-*i*-Ph); 153.51, 153.57, 153.58 (C-4,7a); 174.87 (d, $J_{C,P}$ = 5.3, CO-Ala); 175.07 (d, $J_{C,P}$ = 4.1, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.91, 5.04. IR (ATR): ν = 3269, 2922, 1734, 1562, 1489, 1455, 1205, 1016 cm^{-1}; ESI MS *m*/*z* (rel.%): 611 (100) [M+Na], 589 (65) [M+H]. HR MS (ESI) for $C_{26}H_{30}O_8N_4PS$ [M+H]: calcd 589.1516; found 589.1516.

4.4.11. β-D-Ribofuranosyl-4-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)]phosphate (16e)

Protected ProTide **13e** (66 mg, 0.11 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 54 mg, 87% (mixture of diastereomers 1:1.60). mp: 48–50 °C; ^{1}H NMR (600.1 MHz, CD_3OD): 1.169, 1.173 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH_2O); 1.24, 1.29 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.2, CH_3-Ala); 3.85 (dq, 1H, $J_{H,P}$ = 9.3, J_{vic} = 7.2, CH-Ala); 3.91 (dq, 1H, $J_{H,P}$ = 9.8, J_{vic} = 7.2, CH-Ala); 4.03–4.12 (m, 4H, CH_3CH_2O); 4.25, 4.28 (2 × m, 2 × 1H, H-4′); 4.31–4.45 (m, 6H, H-3′,5′); 4.54, 4.55 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 6.41, 6.42 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 6.88, 6.92 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.16, 7.18 (2 × m, 2 × 1H, H-*p*-OPh); 7.21, 7.24 (2 × m, 2 × 2H, H-*o*-OPh); 7.33 (m, 4H, H-*m*-OPh); 7.55–7.62 (m, 6H, H-*m*,*p*-Ph); 7.67, 7.72 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 8.03, 8.05 (2 × m, 2 × 2H, H-*o*-Ph); 8.844 and 8.846 (2 × s, 2 × 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 14.41, 14.44 (CH_3CH_2O); 20.31 (d, $J_{C,P}$ = 7.4, CH_3-Ala); 20.46 (d, $J_{C,P}$ = 6.5, CH_3-Ala); 51.50, 51.59 (d, $J_{C,P}$ = 1.5, CH-Ala); 62.32, 62.35 (CH_3CH_2O); 67.34, 67.68 (d, $J_{C,P}$ = 5.4, CH_2-5′); 71.72, 71.78 (CH-3′); 75.65, 75.67 (CH-2′); 83.93, 83.99 (d, $J_{C,P}$ = 8.8, CH-4′); 89.18, 89.31 (CH-1′); 102.89, 102.92 (CH-5); 117.82 (C-4a); 121.35, 121.37 (d, $J_{C,P}$ = 4.7, CH-*o*-OPh); 126.20 (CH-*p*-OPh); 128.99, 129.05 (CH-6); 129.97, 129.98, 130.03 (CH-*o*,*m*-Ph); 130.81, 130.82 (CH-*m*-OPh); 131.59 (CH-*p*-Ph); 138.41, 138.42 (C-*i*-Ph); 151.93, 151.94 (CH-2); 152.13, 152.14 (d, $J_{C,P}$ = 6.9, C-*i*-Ph); 153.46, 153.47 (C-7a); 158.77 (C-4); 174.86 (d, $J_{C,P}$ = 5.2, CO-Ala); 175.06 (d, $J_{C,P}$ = 4.7, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.92, 5.04. IR (ATR): ν = 3269, 2958, 1735, 1561, 1490, 1440, 1205, 1019 cm^{-1}; ESI MS *m*/*z* (rel.%): 621 (16) [M+K], 605 (20) [M+Na], 583 (100) [M+H]. HR MS (ESI) for $C_{28}H_{32}O_8N_4P$ [M+H]: calcd 583.1952; found 583.1957.

4.4.12. β-D-Ribofuranosyl-4-(4-dibenzofuryl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(ethyloxy-L-alaninyl)]phosphate (16f)

Protected ProTide **13f** (114 mg, 0.16 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 65 mg, 60% (mixture of diastereomers 1:1.31). mp: 71–76 °C; ^{1}H NMR (600.1 MHz, CD_3OD): 1.11, 1.14 (2 × t, 2 × 3H, J_{vic} = 7.2, CH_3CH_2O); 1.24, 1.30 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.1, CH_3-Ala); 3.87 (dq, 1H, $J_{H,P}$ = 9.2, J_{vic} = 7.2, CH-Ala); 3.93 (dq, 1H, $J_{H,P}$ = 9.8, J_{vic} = 7.2, CH-Ala); 4.01–4.10 (m, 4H, CH_3CH_2O); 4.28, 4.30 (2 × m, 2 × 1H, H-4′); 4.33–4.47 (m, 6H, H-3′,5′); 4.57, 4.59 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 6.46, 6.47 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 6.67, 6.71 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.13, 7.14 (2 × m, 2 × 1H, H-*p*-Ph); 7.21, 7.24 (2 × m, 2 × 2H, H-*o*-Ph); 7.31 (m, 4H, H-*m*-Ph); 7.43 (m, 2H, H-8-dibenzofuryl); 7.51, 7.52 (2 × ddd, 2 × 1H, $J_{7,6}$ = 8.4, $J_{7,8}$ = 7.1, $J_{7,9}$ = 1.3, H-7-dibenzofuryl); 7.56 (m, 2H, H-6-dibenzofuryl); 7.576, 7.580 (2 × t, 2 × 1H, $J_{2,1} = J_{2,3}$ = 7.7, H-2-dibenzofuryl); 7.65, 7.71 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 7.91, 7.92 (2 × dd, 2 × 1H, $J_{3,2}$ = 7.7, $J_{3,1}$ = 1.4, H-3-dibenzofuryl); 8.13 (dd, 2H, $J_{9,8}$ = 7.8, $J_{9,7}$ = 1.3, H-9-benzofuryl); 8.242, 8.243 (2 × dd, 2 × 1H, $J_{1,2}$ = 7.7, $J_{1,3}$ = 1.4, H-1-benzofuryl); 8.93 (s, 2H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 14.39 (CH_3CH_2O); 20.33 (d, $J_{C,P}$ = 7.4, CH_3-Ala); 20.48 (d, $J_{C,P}$ = 6.5, CH_3-Ala); 51.50, 51.60 (d, $J_{C,P}$ = 1.3, CH-Ala); 62.29, 62.35 (CH_3CH_2O); 67.38, 67.71 (d, $J_{C,P}$ = 5.4, CH_2-5′); 71.74, 71.80 (CH-3′); 75.71, 75.73 (CH-2′); 83.95, 84.01 (d, $J_{C,P}$ = 9.4, CH-4′); 89.19, 89.32 (CH-1′); 103.66, 103.67 (CH-5); 112.72 (CH-6-dibenzofuryl); 119.41, 119.42 (C-4a); 121.35, 121.36 (d, $J_{C,P}$ = 4.8, CH-*o*-Ph); 122.06 (CH-9-dibenzofuryl); 123.09, 123.11 (C-4-dibenzofuryl); 123.85 (CH-1-dibenzofuryl); 124.47 (CH-2-dibenzofuryl); 124.52 (CH-8-dibenzofuryl); 124.94 (C-9a-dibenzofuryl); 126.19 (CH-*p*-Ph); 126.73 (C-9b-dibenzofuryl); 128.70, 128.79 (CH-6); 128.99 (CH-7-dibenzofuryl); 129.41, 129.42 (CH-3-dibenzofuryl); 130.82 (CH-*m*-Ph); 151.96, 151.97 (CH-2); 152.14, 152.15 (d, $J_{C,P}$ = 7.0, C-*i*-Ph); 153.28 (C-7a); 154.57 (C-4a-dibenzofuryl); 155.51 (C-4); 157.50 (C-5a-dibenzofuryl); 174.86 (d, $J_{C,P}$ = 5.4, CO-Ala); 175.05 (d, $J_{C,P}$ = 4.4, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.93, 5.05. IR (ATR): ν = 3350, 2916, 1736, 1567, 1490, 1451, 1209, 1150, 1022 cm^{-1}; ESI MS *m*/*z* (rel.%): 671 (100) [M−H]. HR MS (ESI) for $C_{34}H_{32}O_9N_4P$ [M−H]: calcd 671.1912; found 671.1911.

4.4.13. β-D-Ribofuranosyl-4-(2-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)]phosphate (17a)

Protected ProTide **14a** (173 mg, 0.26 mmol) was used. Flash chromatography (C-18 reverse phase, gradient of methanol in water). Yield: 67 mg, 41% (mixture of diastereomers 1:1.32). mp: 50–55 °C; ^{1}H NMR (500.0 MHz, CD_3OD): 1.24, 1.30 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.2, CH_3-Ala); 3.92 (dq, 1H, $J_{H,P}$ = 9.3, J_{vic} = 7.2, CH-Ala); 3.98 (dq, 1H, $J_{H,P}$ = 10.0, J_{vic} = 7.2, CH-Ala); 4.21

(m, 2H, H-4′); 4.26–4.40 (m, 6H, H-3′,5′); 4.50, 4.51 (2 × t, 2 × 1H, $J_{2',3'} = J_{2',1'} = 5.3$, H-2′); 5.03, 5.06, 5.08, 5.09 (4 × d, 4 × 1H, $J_{gem} = 12.2$, CH_2Ph); 6.35, 6.36 (2 × d, 2 × 1H, $J_{1',2'} = 5.3$, H-1′); 6.71, 6.72 (2 × dd, 2 × 1H, $J_{4,3} = 3.4$, $J_{4,5} = 1.7$, H-4-furyl); 7.06, 7.09 (2 × d, 2 × 1H, $J_{5,6} = 3.8$, H-5); 7.14–7.23 (m, 6H, H-*o*,*p*-Ph); 7.24–7.34 (m, 14H, H-*m*-Ph, H-*o*,*m*,*p*-Bn); 7.44, 7.45 (2 × dd, 2 × 1H, $J_{3,4} = 3.4$, $J_{3,5} = 0.8$, H-3-furyl); 7.60, 7.64 (2 × d, 2 × 1H, $J_{6,5} = 3.8$, H-6); 7.87 (dd, 2H, $J_{5,4} = 1.7$, $J_{5,3} = 0.8$, H-5-furyl); 8.698 and 8.703 (2 × s, 2 × 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 20.23 (d, $J_{C,P} = 7.5$, CH_3-Ala); 20.36 (d, $J_{C,P} = 6.6$, CH_3-Ala); 51.55, 51.64 (d, $J_{C,P} = 1.2$, CH-Ala); 67.28, 67.75 (d, $J_{C,P} = 5.4$, CH_2-5′); 67.86, 67.92 (CH_2Ph); 71.65, 71.75 (CH-3′); 75.58, 75.62 (CH-2′); 83.86, 83.90 (d, $J_{C,P} = 8.3$, CH-4′); 89.09, 89.18 (CH-1′); 103.52, 103.54 (CH-5); 113.56, 113.58 (CH-4-furyl); 114.69 (CH-3-furyl); 114.83 (C-4a); 121.36, 121.38 (d, $J_{C,P} = 4.8$, CH-*o*-Ph); 126.18, 126.20 (d, $J_{C,P} = 1.1$, CH-*p*-Ph); 128.72, 128.76 (CH-6); 129.21, 129.23 (CH-*o*-Bn); 129.26, 129.29 (CH-*p*-Bn); 129.52, 129.55 (CH-*m*-Bn); 130.80 (CH-*m*-Ph); 137.14, 137.22 (C-*i*-Bn); 147.21 (CH-5-furyl); 148.15, 148.18 (C-4); 151.86, 151.90 (CH-2); 152.12 (d, $J_{C,P} = 6.9$, C-*i*-Ph); 153.68, 153.71, 153.73, 153.75 (C-7a, C-2-furyl); 174.62 (d, $J_{C,P} = 5.0$, CO-Ala); 174.82 (d, $J_{C,P} = 4.0$, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.84, 5.02. IR (ATR): ν = 3233, 2922, 1740, 1600, 1566, 1488, 1455, 1209, 1146, 1009 cm^{-1}; ESI MS *m*/*z* (rel.%): 673 (10) [M+K], 657 (100) [M+Na], 635 (17) [M+H]. HR MS (ESI) for $C_{31}H_{32}O_9N_4P$ [M+H]: calcd 635.1901; found 635.1900.

4.4.14. β-D-Ribofuranosyl-4-(3-furyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)]phosphate (17b)

Protected ProTide **14b** (85 mg, 0.13 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 55 mg, 69% (mixture of diastereomers 1:1.15). mp: 51–57 °C; ^{1}H NMR (600.1 MHz, CD_3OD): 1.24, 1.29 (2 × d, 2 × 3H, $J_{vic} = 7.2$, CH_3-Ala); 3.92 (dq, 1H, $J_{H,P} = 9.8$, $J_{vic} = 7.2$, CH-Ala); 3.97 (dq, 1H, $J_{H,P} = 9.1$, $J_{vic} = 7.2$, CH-Ala); 4.22 (m, 2H, H-4′); 4.26–4.40 (m, 6H, H-3′,5′); 4.50, 4.51 (2 × dd, 2 × 1H, $J_{2',3'} = 5.6$, $J_{2',1'} = 5.1$, H-2′); 5.03, 5.08 (2 × d, 2 × 1H, $J_{gem} = 12.4$, CH_2Ph); 5.09 (s, 2H, CH_2Ph); 6.36, 6.37 (2 × d, 2 × 1H, $J_{1',2'} = 5.1$, H-1′); 6.88, 6.90 (2 × d, 2 × 1H, $J_{5,6} = 3.9$, H-5); 7.14–7.22 (m, 8H, H-4-furyl, H-*o*,*p*-Ph); 7.25–7.34 (m, 14H, H-*m*-Ph, H-*o*,*m*,*p*-Bn); 7.61, 7.65 (2 × d, 2 × 1H, $J_{6,5} = 3.9$, H-6); 7.72 (br s, 2H, H-5-furyl); 8.42 (br s, 2H, H-2-furyl); 8.728 and 8.732 (2 × s, 2 × 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 20.22 (d, $J_{C,P} = 7.5$, CH_3-Ala); 20.35 (d, $J_{C,P} = 6.8$, CH_3-Ala); 51.54, 51.65 (d, $J_{C,P} = 1.3$, CH-Ala); 67.29, 67.73 (d, $J_{C,P} = 5.3$, CH_2-5′); 67.88, 67.93 (CH_2Ph); 71.65, 71.73 (CH-3′); 75.58, 75.62 (CH-2′); 83.87, 83.92 (d, $J_{C,P} = 8.1$, CH-4′); 89.09, 89.17 (CH-1′); 102.54 (CH-5); 110.30, 110.31 (CH-4-furyl); 116.73 (C-4a); 121.37, 121.38 (d, $J_{C,P} = 4.8$, CH-*o*-Ph); 125.90, 125.93 (C-3-furyl); 126.20, 126.22 (CH-*p*-Ph); 128.53, 128.55 (CH-6); 129.23 (CH-*o*-Bn); 129.28, 129.31 (CH-*p*-Bn); 129.53, 129.56 (CH-*m*-Bn); 130.82 (CH-*m*-Ph); 137.13, 137.20 (C-*i*-Bn); 145.64, 145.66 (CH-5-furyl); 145.99 (CH-2-furyl); 151.85, 151.88 (CH-2); 152.06, 152.07 (C-4); 152.09 (d, $J_{C,P} = 6.7$, C-*i*-Ph); 153.17, 153.19 (C-7a); 174.65 (d, $J_{C,P} = 5.0$, CO-Ala); 174.84 (d, $J_{C,P} = 3.9$, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.82, 5.02. IR (ATR): ν = 3297, 2923, 1738, 1590, 1568, 1490, 1456, 1210, 1146, 1023 cm^{-1}; ESI MS *m*/*z* (rel.%): 657 (100) [M+Na], 635 (22) [M+H]. HR MS (ESI) for $C_{31}H_{32}O_9N_4P$ [M+H]: calcd 635.1901; found 635.1900.

4.4.15. β-D-Ribofuranosyl-4-(2-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)]phosphate (17c)

Protected ProTide **14c** (203 mg, 0.29 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 113 mg, 59% (mixture of diastereomers 1:1.11). mp: 51–54 °C; ^{1}H NMR (600.1 MHz, CD_3OD): 1.24, 1.29 (2 × dd, 2 × 3H, $J_{vic} = 7.2$, $J_{H,P} = 1.2$, CH_3-Ala); 3.91 (dq, 1H, $J_{H,P} = 9.3$, $J_{vic} = 7.2$, CH-Ala); 3.97 (dq, 1H, $J_{H,P} = 10.0$, $J_{vic} = 7.2$, CH-Ala); 4.22 (m, 2H, H-4′); 4.26–4.40 (m, 6H, H-3′,5′); 4.50, 4.51 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'} = 5.3$, H-2′); 5.03, 5.06, 5.07, 5.09 (4 × d, 4 × 1H, $J_{gem} = 12.3$, CH_2Ph); 6.36, 6.37 (2 × d, 2 × 1H, $J_{1',2'} = 5.3$, H-1′); 6.98, 7.00 (2 × d, 2 × 1H, $J_{5,6} = 3.8$, H-5); 7.14–7.22 (m, 6H, H-*o*,*p*-Ph); 7.24–7.33 (m, 16H, H-4-thienyl, H-*m*-Ph, H-*o*,*m*,*p*-Bn); 7.62, 7.66 (2 × d, 2 × 1H, $J_{6,5} = 3.8$, H-6); 7.717, 7.722 (2 × dd, 2 × 1H, $J_{5,4} = 5.0$, $J_{5,3} = 1.1$, H-5-thienyl); 8.026, 8.033 (2 × dd, 2 × 1H, $J_{3,4} = 3.9$, $J_{3,5} = 1.1$, H-3-thienyl); 8.686 and 8.690 (2 × s, 2 × 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 20.22 (d, $J_{C,P} = 7.5$, CH_3-Ala); 20.35 (d, $J_{C,P} = 6.7$, CH_3-Ala); 51.53, 51.63 (d, $J_{C,P} = 1.2$, CH-Ala); 67.24, 67.73 (d, $J_{C,P} = 5.3$, CH_2-5′); 67.87, 67.93 (CH_2Ph); 71.62, 71.72 (CH-3′); 75.60, 75.63 (CH-2′); 83.83 (d, $J_{C,P} = 8.3$, CH-4′); 83.87 (d, $J_{C,P} = 8.6$, CH-4′); 89.14, 89.23 (CH-1′); 102.64, 102.65 (CH-5); 115.33 (C-4a); 121.36, 121.38 (d, $J_{C,P} = 4.8$, CH-*o*-Ph); 126.19, 126.21 (CH-*p*-Ph); 128.71, 128.75 (CH-6); 129.21, 129.24 (CH-*o*-Bn); 129.27, 129.30 (CH-*p*-Bn); 129.52, 129.56 (CH-*m*-Bn); 129.65, 129.66 (CH-4-thienyl); 130.68 (CH-3-thienyl); 130.81 (CH-*m*-Ph); 131.22, 131.24 (CH-5-thienyl); 137.12, 137.20 (C-*i*-Bn); 143.08, 143.12 (C-2-thienyl); 151.89, 151.92 (CH-2); 152.10 (d, $J_{C,P} = 6.9$, C-*i*-Ph); 152.34, 152.35 (C-4); 153.58, 153.61 (C-7a); 174.65 (d, $J_{C,P} = 5.0$, CO-Ala); 174.85 (d, $J_{C,P} = 4.1$, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.82, 5.01. IR (ATR): ν = 3257, 2937, 1739, 1563, 1490, 1453, 1212, 1146, 1023 cm^{-1}; ESI MS *m*/*z* (rel.%): 689 (11) [M+K], 673 (100) [M+Na], 651 (18) [M+H]. HR MS (ESI) for $C_{31}H_{32}O_8N_4PS$ [M+H]: calcd 651.1673; found 651.1671.

4.4.16. β-D-Ribofuranosyl-4-(3-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)]phosphate (17d)

Protected ProTide **14d** (234 mg, 0.34 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 136 mg, 62% (mixture of diastereomers 1:1). mp: 55–62 °C; ^{1}H NMR (500.0 MHz, CD_3OD): 1.24, 1.29 (2 × dd, 2 × 3H, $J_{vic} = 7.2$, $J_{H,P} = 1.2$, CH_3-Ala); 3.92 (dq, 1H, $J_{H,P} = 9.3$, $J_{vic} = 7.2$, CH-Ala); 3.97 (dq, 1H, $J_{H,P} = 10.0$, $J_{vic} = 7.2$, CH-Ala); 4.22 (m, 2H, H-4′); 4.26–4.40 (m, 6H, H-3′,5′); 4.51, 4.52 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'} = 5.2$, H-2′); 5.03, 5.06, 5.07, 5.09 (4 × d, 4 × 1H, $J_{gem} = 12.3$, CH_2Ph); 6.38, 6.39 (2 × d, 2 × 1H, $J_{1',2'} = 5.2$, H-1′); 6.95, 6.96 (2 × d, 2 × 1H, $J_{5,6} = 3.8$, H-5); 7.13–7.22 (m, 6H, H-*o*,*p*-Ph); 7.24–7.33 (m, 14H, H-*m*-Ph, H-*o*,*m*,*p*-Bn); 7.626, 7.631 (2 × dd, 2 × 1H, $J_{5,4} = 5.2$, $J_{5,2} = 2.9$, H-5-thienyl); 7.64, 7.68 (2 × d, 2 × 1H, $J_{6,5} = 3.8$, H-6); 7.84, 7.86 (2 × dd, 2 × 1H, $J_{4,5} = 5.2$, $J_{4,2} = 1.3$, H-4-thienyl); 8.28, 8.29 (2 × dd, 2 × 1H, $J_{2,5} = 2.9$, $J_{2,4} = 1.3$, H-2-thienyl); 8.764 and 8.767 (2 × s, 2 × 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 20.22 (d, $J_{C,P} = 7.6$, CH_3-Ala); 20.35 (d, $J_{C,P} = 6.8$, CH_3-Ala); 51.55, 51.66 (d, $J_{C,P} = 1.2$, CH-Ala); 67.29 (d, $J_{C,P} = 5.1$, CH_2-5′); 67.75 (d, $J_{C,P} = 5.5$, CH_2-5′); 67.87, 67.92 (CH_2Ph); 71.66, 71.74 (CH-3′); 75.61, 75.66 (CH-2′); 83.89, 83.94 (d, $J_{C,P} = 8.3$, CH-4′); 89.19, 89.26 (CH-1′); 102.92, 102.93 (CH-5); 116.91 (C-4a); 121.37, 121.38 (d, $J_{C,P} = 4.8$, CH-*o*-Ph); 126.19 (CH-*p*-Ph); 127.86, 127.88 (CH-5-thienyl); 128.41, 128.42 (CH-4-thienyl); 128.82 (CH-6); 129.22 (CH-*o*-Bn); 129.27, 129.30 (CH-*p*-Bn); 129.47 (CH-2-thienyl); 129.53, 129.56 (CH-*m*-Bn); 130.81 (CH-*m*-Ph); 137.14, 137.22 (C-*i*-Bn); 140.44, 140.47 (C-3-thienyl); 151.71, 151.75 (CH-2); 152.11 (d, $J_{C,P} = 6.8$, C-*i*-Ph); 152.55, 152.56, 153.57 (C-4,7a); 174.63 (d, $J_{C,P} = 4.7$, CO-Ala); 174.83 (d, $J_{C,P} = 3.7$, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.82, 5.00. IR (ATR): ν = 3252, 2941, 1740, 1566, 1490, 1455, 1205, 1144, 1023 cm^{-1}; ESI MS *m*/*z* (rel.%): 673 (100) [M+Na], 651 (31) [M+H]. HR MS (ESI) for $C_{31}H_{32}O_8N_4PS$ [M+H]: calcd 651.1673; found 651.1672.

4.4.17. β-D-Ribofuranosyl-4-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)]phosphate (17e)

Protected ProTide **14e** (180 mg, 0.26 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield:

110 mg, 65% (mixture of diastereomers 1:1.23). mp: 60–62 °C; ^{1}H NMR (600.1 MHz, CD_3OD): 1.24, 1.29 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.2, CH_3-Ala); 3.92 (dq, 1H, $J_{H,P}$ = 9.2, J_{vic} = 7.2, CH-Ala); 3.97 (dq, 1H, $J_{H,P}$ = 9.9, J_{vic} = 7.2, CH-Ala); 4.23 (m, 2H, H-4′); 4.27–4.40 (m, 6H, H-3′,5′); 4.517, 4.522 (2 × t, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 5.03, 5.07 (2 × d, 2 × 1H, J_{gem} = 12.2, CH_2Ph); 5.08 (s, 2H, CH_2Ph); 6.40, 6.41 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.1, H-1′); 6.83, 6.85 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.14–7.22 (m, 6H, H-*o*,*p*-OPh); 7.24–7.33 (m, 14H, H-*m*-OPh, H-*o*,*m*,*p*-Bn); 7.54–7.61 (m, 6H, H-*m*,*p*-Ph); 7.64, 7.67 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 8.01, 8.03 (2 × m, 2 × 2H, H-*o*-Ph); 8.826, 8.830 (2 × s, 2 × 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 20.22 (d, $J_{C,P}$ = 7.7, CH_3-Ala); 20.35 (d, $J_{C,P}$ = 6.7, CH_3-Ala); 51.54, 51.66 (CH-Ala); 67.29, 67.75 (d, $J_{C,P}$ = 5.4, CH_2-5′); 67.88, 67.93 (CH_2Ph); 71.66, 71.74 (CH-3′); 75.61, 75.66 (CH-2′); 83.87, 83.92 (d, $J_{C,P}$ = 8.4, CH-4′); 89.19, 89.24 (CH-1′); 102.84, 102.87 (CH-5); 117.84 (C-4a); 121.37 (d, $J_{C,P}$ = 4.8, CH-*o*-OPh); 126.19, 126.21 (CH-*p*-OPh); 128.82 (CH-6); 129.24, 129.25 (CH-*o*-Bn); 129.29, 129.32 (CH-*p*-Bn); 129.54, 129.57 (CH-*m*-Bn); 129.96, 129.99, 130.01 (CH-*o*,*m*-Ph); 130.82 (CH-*m*-OPh); 131.48, 131.50 (CH-*p*-Ph); 137.13, 137.21 (C-*i*-Bn); 138.64, 138.68 (C-*i*-Ph); 152.11 (d, $J_{C,P}$ = 5.2, C-*i*-OPh); 152.12, 152.15 (CH-2); 153.44, 153.47 (C-7a); 158.95, 158.96 (C-4); 174.66 (d, $J_{C,P}$ = 4.8, CO-Ala); 174.84 (d, $J_{C,P}$ = 4.0, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.83, 5.02. IR (ATR): ν = 3224, 2922, 1739, 1559, 1490, 1454, 1207, 1145, 1022 cm^{-1}; ESI MS *m*/*z* (rel.%): 667 (89) [M+Na], 645 (100) [M+H]. HR MS (ESI) for $C_{33}H_{34}O_8N_4P$ [M+H]: calcd 645.21088; found 645.21131.

4.4.18. β-D-Ribofuranosyl-4-(4-dibenzofuryl)-7*H*-pyrrolo[2,3-*d*] pyrimidine-5′-*O*-[phenyl(benzyloxy-L-alaninyl)]phosphate (17f)

Protected ProTide **14f** (200 mg, 0.26 mmol) was used. Column chromatography (SiO_2, 2% of methanol in chloroform). Yield: 121 mg, 64% (mixture of diastereomers 1:1.24). mp: 62–66 °C; ^{1}H NMR (600.1 MHz, CD_3OD): 1.24, 1.29 (2 × dd, 2 × 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.2, CH_3-Ala); 3.91 (dq, 1H, $J_{H,P}$ = 9.1, J_{vic} = 7.2, CH-Ala); 3.98 (dq, 1H, $J_{H,P}$ = 9.8, J_{vic} = 7.2, CH-Ala); 4.25 (m, 2H, H-4′); 4.29–4.42 (m, 6H, H-3′,5′); 4.552, 4.554 (2 × dd, 2 × 1H, $J_{2',1'} = J_{2',3'}$ = 5.3, H-2′); 5.00, 5.01. 5.03, 5.05 (4 × d, 4 × 1H, J_{gem} = 12.4, CH_2Ph); 6.45, 6.46 (2 × d, 2 × 1H, $J_{1',2'}$ = 5.3, H-1′); 6.62, 6.63 (2 × d, 2 × 1H, $J_{5,6}$ = 3.8, H-5); 7.11–7.24 (m, 16H, H-*o*,*p*-Ph, H-*o*,*m*,*p*-Bn); 7.29 (m, 4H, H-*m*-Ph); 7.429, 7.432 (2 × ddd, 2 × 1H, $J_{8,9}$ = 7.7, $J_{8,7}$ = 7.1, $J_{8,6}$ = 0.9, H-8-dibenzofuryl); 7.52 (ddd, 2H, $J_{7,6}$ = 8.2, $J_{7,8}$ = 7.1, $J_{7,9}$ = 1.3, H-7-dibenzofuryl); 7.54, 7.55 (2 × dt, 2 × 1H, $J_{6,7}$ = 8.2, $J_{6,8} = J_{6,9}$ = 0.9, H-6-dibenzofuryl); 7.57, 7.59 (2 × t, 2 × 1H, $J_{2,1} = J_{2,3}$ = 7.7, H-2-dibenzofuryl); 7.63, 7.67 (2 × d, 2 × 1H, $J_{6,5}$ = 3.8, H-6); 7.89, 7.91 (2 × dd, 2 × 1H, $J_{3,2}$ = 7.7, $J_{3,1}$ = 1.3, H-3-dibenzofuryl); 8.13, 8.14 (2 × ddd, 2 × 1H, $J_{9,8}$ = 7.7, $J_{9,7}$ = 1.3, $J_{9,6}$ = 0.9, H-9-benzofuryl); 8.24, 8.26 (2 × dd, 2 × 1H, $J_{1,2}$ = 7.7, $J_{1,3}$ = 1.3, H-1-benzofuryl); 8.921, 8.924 (2 × s, 2 × 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 20.23 (d, $J_{C,P}$ = 7.7, CH_3-Ala); 20.37 (d, $J_{C,P}$ = 6.6, CH_3-Ala); 51.54, 51.64 (d, $J_{C,P}$ = 1.0, CH-Ala); 67.26, 67.77 (d, $J_{C,P}$ = 5.4, CH_2-5′); 67.84, 67.92 (CH_2Ph); 71.66, 71.76 (CH-3′); 75.68, 75.73 (CH-2′); 83.90, 83.94 (d, $J_{C,P}$ = 8.4, CH-4′); 89.21, 89.26 (CH-1′); 103.65, 103.70 (CH-5); 112.74 (CH-6-dibenzofuryl); 119.44 (C-4a); 121.36 (d, $J_{C,P}$ = 4.8, CH-*o*-Ph); 122.05, 122.07 (CH-9-dibenzofuryl); 123.23, 123.28 (C-4-dibenzofuryl); 123.79, 123.80 (CH-1-dibenzofuryl); 124.46, 124.49 (CH-2-dibenzofuryl); 124.51, 124.53 (CH-8-dibenzofuryl); 124.97, 124.99 (C-9a-dibenzofuryl); 126.18, 126.20 (CH-*p*-Ph); 126.73, 126.75 (C-9b-dibenzofuryl); 128.60, 128.62 (CH-6); 128.98, 128.99 (CH-7-dibenzofuryl); 129.18, 129.20, 129.23, 129.24 (CH-*o*,*p*-Bn); 129.42, 129.43 (CH-3-dibenzofuryl); 129.47, 129.49 (CH-*m*-Bn); 130.82 (CH-*m*-Ph); 137.09, 137.11 (C-*i*-Bn); 152.07, 152.10 (CH-2); 152.12 (d, $J_{C,P}$ = 6.1, C-*i*-Ph); 153.26, 153.28 (C-7a); 154.59, 154.61 (C-4a-dibenzofuryl); 155.636, 155.643 (C-4); 157.51 (C-5a-dibenzofuryl); 174.64 (d, $J_{C,P}$ = 5.0, CO-Ala); 174.84 (d, $J_{C,P}$ = 4.0, CO-Ala). ^{31}P NMR (162.0 MHz, CD_3OD): 4.90, 5.03. IR (ATR): ν = 3231, 2924, 1739, 1566, 1412, 1186, 1146, 1011 cm^{-1}; ESI MS *m*/*z* (rel.%): 757 (100) [M+Na], 735 (55) [M+H]. HR MS (ESI) for $C_{39}H_{36}O_9N_4P$ [M+H]: calcd 735.22144; found 735.22198.

4.5. Cytostatic activity assays

All cell lines were obtained from ATCC (Manassas, VA). Colon (HCT116), breast (HS 578) and lung (NCI-H23) cell lines were maintained in the RPMI cultivation medium (Invitrogen; Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS). Prostate cell line (Du145) was cultivated in MEM and F12K medium containing 10% FBS, respectively. Trichloro acetic acid (TCA), and sulforhodamine B (SRB) were from Sigma–Aldrich (St. Louis, MO).

A modified protocol of sulforhodamine B colorimetric assay was used for the cytostatic activity screening.[8] Cells were distributed into the 96-well plates in 150 μl of media (HCT116 and Du145 5300 cell/ml; Hs578 26,600 cells/ml; NCI-H23 40,000 cells/ml), and incubated overnight in humidified CO_2 incubator at 37 °C. Next day, one plate of each cell line was fixed with TCA by removing media and adding 100 μl cold 10% (vol/vol) TCA to each well. After 1 h incubation at 4 °C, TCA was discarded and plates were washed four times with tap water. These plates represented cell counts at day zero. The tested compounds were fivefold serially diluted and distributed to cells in 50 μl of media. After five day incubation, the plates were fixed with TCA as mentioned above and 100 μl of 0.057% SRB solution in 1% (vol/vol) acetic acid was added to each well. After 30 min incubation at room temperature SRB was removed, and the plates were rinsed four times 1% (vol/vol) acetic acid. Next, 200 μl of 10 mm Tris base solution (pH 10.5) was added to each well of completely dried plates and absorbance of cell associated SRB was read at 500 nm. The percentage of cell-growth inhibition was calculated using the following formula: % of control cell growth = 100 × (OD_{sample} − mean OD_{day0})/($OD_{neg\ control}$ − mean OD_{day0}). For GIC_{50} determination, plot a dose–response curves between the compound concentration and percent of growth inhibition. GIC_{50} values can be derived by fitting dose–response curves using sigmoidal dose–response equation.

4.6. Cell cycle analysis

CCRF-CEM cells were cultivated in RPMI 1640 medium supplemented with 10% calf fetal serum in the presence of tested compounds at concentrations corresponding to IC_{50} values. The endpoint of the cell growth was 72 h following the drug addition. The viability of cells was quantified using XTT standard spectrophotometric assay (Roche Molecular Biochemicals).The cell cycle analysis by flow cytometry (BD FACSAria) was performed by using ethanol-fixed cells stained with propidium iodide in buffer containing RNase A.

4.7. Cellular permeability

Caco-2 cells were maintained in DMEM media with sodium pyruvate, non-essential amino acids, and 10% fetal bovine serum. Cells were seeded at 2100 cells/well and grown for 21 days on 24-well polyethylene-terephthalate plates (BD Biosciences). Experiments were run using an apical (A) buffer containing HBSS with additional 10 mM HEPES, 15 mM glucose and pH adjusted to 6.5. The basolateral (B) compartment contained HBSS buffer supplemented with 1% BSA and the pH adjusted to 7.4. Test compounds (10 μM) were added to either the A or B compartment. Samples from both compartments were collected after 60 and 120 min of incubation. Permeability through a cell-free trans-well was also determined under the same conditions. Samples were analyzed by HPLC combined with tandem mass spectrometry. The apparent

permeability, P_{app}, was calculated as $P_{app} = (dR/dt) \times V_r/(A \times D_0)$, where dR/dt is the slope of the cumulative concentration in the receiver compartment versus time in μM/s, V_r and V_d is the volume in the receiver and donor compartment in cm^3, respectively, A is the area of the cell monolayer (0.33 cm^2), and D_0 is the initial donor concentration.

4.8. Enzymatic hydrolysis

Purified Cathepsin A (1 μg/ml)[9] or Carboxyesterases 1 and 2 (R&D Systems, Minneapolis, MN; 5 μg/ml) were incubated with 30 μM **15–17a** and **15d** in a reaction buffer containing 25 mM 2-[*N*-morpholino]ethanesulfonic acid (MES), pH 6.5, 100 mM NaCl, 1 mM DTT, 0.1% NP40 (Cat A) or 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4 (Ces 1 and 2) at 37 °C. At different time points, 65 μl aliquots were collected from the reaction mixture and 180 μl ice-cold 100% methanol was added to stop the reaction. Samples were incubated at −20 °C for 30 min followed by centrifugation at 13,000 g for 30 min at 4 °C to remove denatured proteins. The supernatants were evaporated, re-suspended in 100 μl buffer A (25 mM potassium phosphate, pH 6.0, 5 mM tetrabutylammonium bromide (TBAB)) and injected onto a C18 reverse phase column (5 μm, 2.1 × 100 mm, ODS(2), Phenomenex, Torrace, CA) equilibrated with buffer A. Reaction substrates and products were eluted using a linear gradient of acetonitrile (0–65%, 10 min, 0.25 ml/min) in buffer A.

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References and notes

1. (a) Johnson, S. A. *Expert Opin. Pharmacother.* **2001**, *2*, 929; (b) Parker, W. B.; Secrist, J. A. I. I. I.; Waud, W. R. *Curr. Opin. Invest. Drugs* **2004**, *5*, 592; (c) Robak, T.; Korycka, A.; Kasznicki, M.; Wrzesien-Kus, A.; Smolewski, P. *Curr. Cancer Drug Targets* **2005**, *5*, 421; (d) Jordheim, L.; Galmarini, C. M.; Dumontet, C. *Curr. Drug Targets* **2003**, *4*, 443; (e) Jordheim, L. P.; Galmarini, C. M.; Dumontet, C. *Recent Patents Anti-Cancer Drug Discov.* **2006**, *1*, 163; (f) Parker, W. B. *Chem. Rev.* **2009**, *109*, 2880.
2. (a) Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *J. Med. Chem.* **2000**, *43*, 1817; (b) Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *Collect. Czech. Chem. Commun.* **2001**, *66*, 483; (c) Hocek, M.; Nauš, P.; Pohl, R.; Votruba, I.; Furman, P. A.; Tharnish, P. M.; Otto, M. J. *J. Med. Chem.* **2005**, *48*, 5869.
3. Nauš, P.; Pohl, R.; Votruba, I.; Džubák, P.; Hajdúch, M.; Ameral, R.; Birkus, G.; Wang, T.; Ray, A. S.; Mackman, R.; Cihlar, T.; Hocek, M. *J. Med. Chem.* **2010**, *53*, 460.
4. Reviews: (a) Schultz, C. *Bioorg. Med. Chem.* **2003**, *11*, 885; (b) Peyrottes, S.; Egron, D.; Lefebre, I.; Gosselin, G.; Imbach, J. L.; Perigaud, C. *Mini-Rev. Med. Chem.* **2004**, *4*, 395; (c) Hecker, S. J.; Erion, M. D. *J. Med. Chem.* **2008**, *51*, 2328; (d) Meier, C. *Eur. J. Org. Chem.* **2006**, 1081.
5. Spáčilová, P.; Nauš, P.; Pohl, R.; Votruba, I.; Snášel, J.; Zábranská, H.; Pichová, I.; Ameral, R.; Birkuš, G.; Cihlář, T.; Hocek, M. *ChemMedChem* **2010**, *5*, 1386.
6. Review: (a) Mehellou, Y.; Balzarini, J.; McGuigan, C. *ChemMedChem* **2009**, *4*, 1779; Recent examples: (b) McGuigan, C.; Derudas, M.; Bugert, J. J.; Andrei, G.; Snoeck, R.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4364; (c) McGuigan, C.; Perrone, P.; Madela, K.; Neyts, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4316.
7. (a) Perrone, P.; Luoni, G. M.; Kelleher, M. R.; Daverio, F.; Angell, A.; Mulready, S.; Congiatu, C.; Rajyaguru, S.; Martin, J. A.; Levêque, V.; Le Pogam, S.; Najera, I.; Klumpp, K.; Smith, D. B.; McGuigan, C. *J. Med. Chem.* **2007**, *50*, 1840; (b) Perrone, P.; Daverio, F.; Valente, R.; Rajyaguru, S.; Martin, J. A.; Lévêque, V.; Le Pogam, S.; Najera, I.; Klumpp, K.; Smith, D. B.; McGuigan, C. *J. Med. Chem.* **2007**, *50*, 5463.
8. Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827.
9. (a) Birkus, G.; Wang, R.; Liu, X.; Kutty, N.; MacArthur, H.; Cihlar, T.; Gibbs, C.; Swaminathan, S.; Lee, W.; McDermott, M. *Antimicrob Agents Chemother.* **2007**, *51*, 543; (b) Birkus, G.; Kutty, N.; He, G. X.; Mulato, A.; Lee, W.; McDermott, M.; Cihlar, T. *Mol. Pharmacol.* **2008**, *74*, 92.