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Use of Pd-catalyzed Suzuki–Miyaura coupling reaction in the rapid synthesis of 5-aryl-6-(phosphonomethoxy)uracils and evaluation of their inhibitory effect towards human thymidine phosphorylase

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ABSTRACT

A number of new 5-aryl substituted pyrimidine acyclic nucleoside phosphonates were synthesized and tested for their ability to inhibit human TP. Their rapid synthesis using Pd-catalyzed Suzuki–Miyaura coupling reactions of various arylboronic acids with 5-bromo-4-(phosphonomethoxy)-2,6-dibutoxypyrimidine was successfully applied. For a series of 5-aryl-6-phosphonomethoxyuracils, an increased inhibitory effect was determined. This effect is supported by the results found for 4-fluorophenyl (K_i^{dThd}=4.89±0.62) and 3-nitrophenyl (K_i^{dThd}=3.98±0.46) substituents.

1. Introduction

Acyclic nucleoside phosphonates (ANPs) are known as compounds possessing various kinds of biological activities.[1] Currently, the biological effects for pyrimidine ANP derivatives could be also extended in context of their potential inhibitory potency towards thymidine phosphorylase (TP).[2,3] This enzyme catalyzes reversible phosphorolysis of 2′-deoxythymidine (dThd) to thymine and 2-deoxy-D-ribose 1-phosphate,[4] which is dephosphorylated to 2-deoxy-D-ribose. Regarding the identical principle of TP to platelet-derived endothelial cell growth factor (PD-ECGF), a potential inhibition of this enzyme may be important in tumour angiogenesis.[5] For ANPs designed as 'multisubstrate inhibitors' of TP with various interfered pyrimidine bases and phosphonoalkyl groups at thymine and phosphate-binding sites[2] there are several advantages compared to most single-substrate inhibitors. These compounds are catabolically stable and flexible in structure and there could be more points for interactions with enzyme through their functionalized acyclic linkage. Therefore, ANP inhibitors may find utility as efficient suppressors of tumour growth[5c] in future.

In our study, we have focused on the development of synthetic methods for the rapid preparation of novel ANPs, which were further tested for their ability to inhibit human TP. Recently, we published a series of ANPs, such as 1-[2-(phosphonomethoxy)ethyl]thymine (PMET), 1-[3-hydroxy-2-(phosphonomethoxy)propyl]thymine (HPMPT) and 1-[3-fluoro-2-(phosphonomethoxy)propyl]thymine (FPMPT) and many others,[6–9] which possess a considerable inhibitory effect newly tested on TP isolated from SD-lymphoma.[6] Unfortunately, these compounds showed a marginal activity on human enzymes expressed in V79 hamster cells as well as the enzyme isolated from human placenta. It seems the differences in recognition of active sites of both enzymes are probably appreciable and these findings may therefore exclude the utilization of TP from SD-lymphoma as a model enzyme.

In contrast, it is known that various 5-alkyl and 5-aryl-6-halogeno substituted uracils[10] as single-substrate inhibitors demonstrate a considerable impact on the inhibitory activity towards human TP probably due to their potential hydrophobic interaction of alkyl and aryl substituents directed by the halogen electron-withdrawing effect (Fig. 1a).

On the other hand, [5-chloro-6-(2-iminopyrrolidin-1-yl)methyl-2,4-(1*H*,3*H*)-pyrimidine] (TPI)[11] represents the most efficient inhibitor towards required TP (Fig. 1b). However, its interaction with active site of enzyme is probably energetically favoured through the

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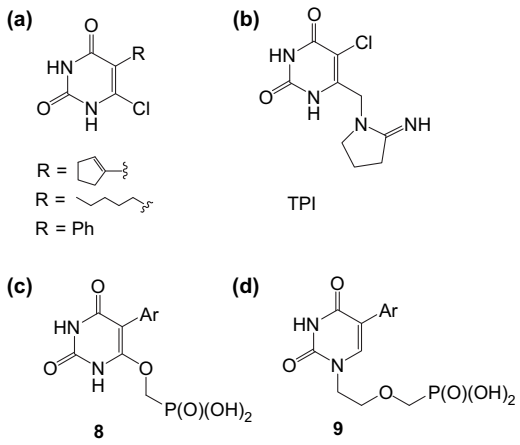
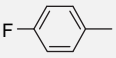
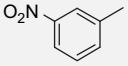
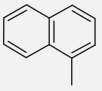
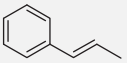


Figure 1. Pyrimidine derivatives as investigated inhibitors of human TP.

solvatation of its positively charged imino group with phosphate and/or H_2O located near the phosphate binding site. Thus activated TPI may be similar to the thymidine cleavage.[12] From this point of view, a mechanism of inhibitor binding with TP may be explained by several ways depending on their structure.[2,12] Based on this proposal, our strategy was to mimic a potential solvatation of TPI in conjunction with hydrophobic effect expected in those of 5-alkyl and 5-aryl-6-chlorouracils. That means we tried to introduce a catabolically stable phosphonomethoxy group to C-6-position and various aryl groups bearing electron-withdrawing substituents, heteroatoms or other conjugated moieties to the position C-5 of pyrimidine base such as phenyl, naphth-1-yl, (*E*)-2-(phenyl)vinyl, 4-fluorophenyl, 3-nitrophenyl, 3-furyl, 3-thienyl, pyridin-3-yl and -4-yl. Thus we have started the synthesis of 5-aryl-6-(phosphonomethoxy)uracils **8a–i** as a new class of ANPs aimed at positive influence of inhibitory effect towards human TP (Fig. 1c; Table 1).

Table 1
Suzuki-coupling reaction and deprotection for 5-aryl-C-6-subtituted uracil ANPs

Entry	R (Ar)[b]	Solvent	Reaction time (h)	Yield[a] of **7** (%)	Yield of **8** (%)
a		Toluene	23	45	33
b		Toluene (DMF)	12 (7)	—	—
c		DMF	6	77	23
d		DMF	6	72	53
e		DMF	4	66	68
f		DMF	4.5	32	61
g		DMF	4	78	29
h		DMF	5.5	43	57
i		DMF	6	25	—
j	H	—	—	—	58

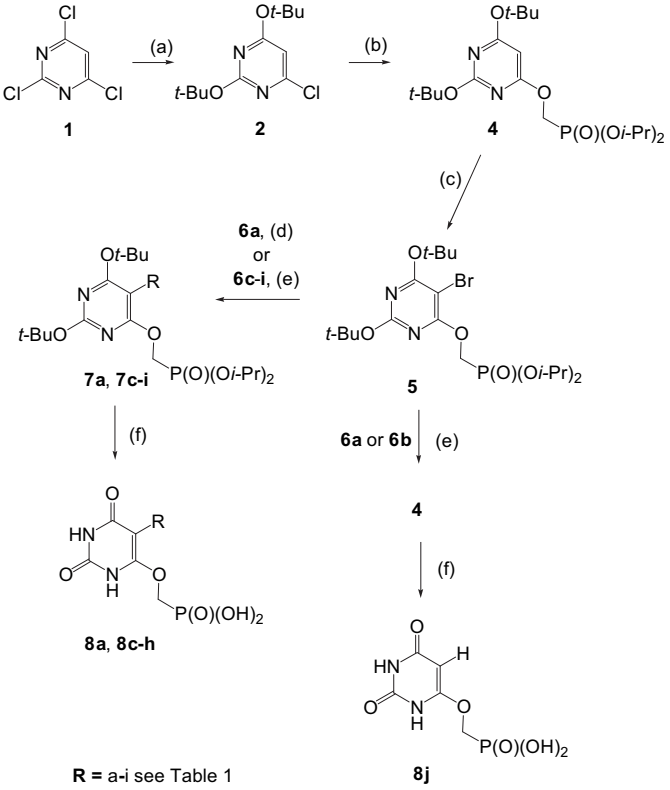
[a] Conditions: **5** (1.0 equiv), **6** (2 equiv), Pd catalyst (0.1 equiv), base (3.3–4.7 equiv of Na_2CO_3), 130 °C; solvent/H_2O 1:6 (entries **a** and **b**) and 8:1 (entries **c–i**).

[b] Aryl subtituents of ANPs **9a–i** correspond to entries **a–i**. The yields of these compounds are mentioned in Ref. 15.

2. Results and discussion

For the efficient arylation method of uracil moiety in some pyrimidine ANPs we utilized Suzuki–Miyaura cross-coupling,[13,14] which have generally been used widely due to their low-toxicity. As the arylation of pyrimidine ANPs has not been studied in detail via this process[14] we decided to introduce various aryl and heteroaryl groups to pyrimidine ANP derivatives using this synthetic method.

As indicated in our preliminary communication,[15] we have successfully employed the Suzuki–Miyaura cross-coupling for the preparation of N^1-substituted 5-arylpyrimidine ANPs **9a–i** (Fig. 1d; Table 1). We have extended this work by studying the arylation of a 5-bromo uracil derivative **5** (Scheme 1). In addition, we have introduced various functionalized aryl groups.



Scheme 1. Reagents and conditions: (a) *t*-BuONa (2 equiv), THF, 0 °C→reflux, 45%; (b) NaH, $HOCH_2P(O)(Oi\text{-}Pr)_2$ (**3**), THF, 0 °C→rt, 39%; (c) NBS, AIBN, THF, 60 °C, 99%; (d) $Pd(PPh_3)_4$ (0.10 equiv), Na_2CO_3, toluene, H_2O, 130 °C, 45%; (e) $Pd(PPh_3)_4$ (0.10 equiv), Na_2CO_3, DMF, H_2O, 130 °C, 43–78%; (f) $(CH_3)_3SiBr$, CH_3CN, rt, 0 °C, 23–68%.

First we prepared the 5-bromo derivative **5** by a three-step synthesis from commercially available 2,4,6-trichloropyrimidine **1**. In this case, we selectively protected **1** by reaction with 2 equiv of sodium *tert*-butoxide to give chloro derivative **2**. In a further reaction step, we chose hydroxymethylphosphonate **3** as a simple phosphonate building block with the assumption that the final structure of **8** could largely mimic a leading structure of TPI in interaction with additional phosphate or water, respectively.[12] The reaction of chloro derivative **2** with **3** converted quantitatively in the presence of sodium hydride into phosphonate **4** (monitoring by TLC). In contrast to a previous method of bromination,[15] we left a deprotection of methoxy groups in uracil moiety regarding to possible difficulty with unfavourable cleavage of phosphonomethoxy substituents. Instead,

we brominated C-5-position of the pyrimidine moiety in **4** with *N*-bromosuccinimide. Similarly to a previous procedure with N^1-substituted phosphonates, this reaction was initiated with azobisisobutyronitrile and afforded in quantitative yield the 5-bromopyrimidine derivative **5**. For coupling reactions we applied a number of commercial aryl and heteroaryl boronic acids **6a**–**i**. Simple transformation of 5-bromo derivative **5** to aryl derivatives **7a**–**i** took place in DMF–H_2O solution catalyzed by $Pd(PPh_3)_4$. Sodium carbonate was used for activation of the arylboronic acids. Full conversion of **5** to products proceeded in all cases at ~130 °C (monitored by TLC).

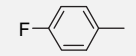
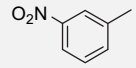
However, the application of Suzuki-coupling reaction conditions to bromo derivative **5** results in some different observations. While N^1-phosphonoalkyl-5-aryluracils as products of arylation were isolated in 24–58% yields (see Ref. 15), the reaction of **5** with a series of arylboronic acids **6c**–**i** afforded phosphonates **7c**–**i** with higher preparative yields (43–78%) (Table 1). In addition, we have found that 2-furyl and 3-thienyl boronic acids **6a** and **6b** surprisingly reacted with **5** to give no arylated product. Instead, the major product **4** of dehalogenation[16] in DMF was isolated in good preparative yield. This competitive dehalogenation may proceed in consequence of energetically unfavourable transmetallation[16,17] due to possible steric hindrance of bromo derivative **5**. Furthermore, arylboronic acids are disposed to hydrolysis[17] in the presence of base. Therefore, all these factors such as shielding and other electronic effects of heteroaryl substituents could play an important role during those of rate-determining step. The poor reactivity of thienylboronic acid **6b** with **5** confirmed by its treatment in benzene. After extending the reaction time (14 h), we isolated a mixture of **5**/**7a**/**4** in a ratio 13.6:1 (monitoring by NMR spectroscopy). Based on this observation, we carried out the arylation in toluene at temperature (~130 °C of bath) for an additional 23 h. For this reaction the hydrodehalogenated product **4** was formed as the minor component, and the ratio of **5**/**7a**/**4**=1:6:1. The corresponding thienyl derivative **7a** was isolated in acceptable yield (45%) after difficulties during purification by preparative TLC followed by HPLC. In contrast, the reaction of **5** with 2-furylboronic acid did not proceed in toluene (see Table 1, entry **b**).

Intermediates **4** and **7a**, **7c**–**i** obtained by cross-coupling were deprotected using bromotrimethylsilane followed by hydrolysis at low temperature (0 °C). Thus, we expected the deprotection of *tert*-butoxy groups under mild conditions could avoid a cleavage of ether group at C-6-position. While a deprotection of phosphonate **7i** afforded a complicated mixture of non-identified compounds and final phosphonates **8a**, **8c**, **8d**, **8g**, **8h** were obtained in lower yields by this hydrolysis; these preparative values are comparable to corresponding N^1-substituted uracil derivatives **9a**–**i** (see Ref. 15).

The determination of inhibitory activity in all final compounds was performed with TP isolated from human enzyme expressed in V79 Chinese hamster cells. Data obtained from the biochemical assay did not show any significant effect of phosphonates **8f**–**j** and **9a**–**i** by comparison with FPMPT, HPMPT, PMET and their previously synthesized analogues,[7–9] we have not found any influence of their aryl substituents on the inhibitory activity.

On the other hand, a series of 5-aryl-6-phosphonomethoxyuracils **8c**–**e** exhibits an increase in inhibitory activity against the enzyme. This effect seems to be supported for 3-nitrophenyl and 4-fluorophenyl groups (Table 2, compounds **8d** and **8e**). In this case, we suppose that a potential hydrophobic effect could be slightly influenced with electron-withdrawing groups as well as this effect is probably induced in 5-(alkyl)aryl-6-chlorouracils possessing a halogen at the C-6-position. The inhibitory effect determined by K_i^{dThd} for these two inhibitors with respect to 2′-deoxythymidine was comparable with most of 5-alkyl and 5-aryl-6-chlorouracils (K~1.03–5.81),[10] 5-halo 6-aminouracils (K_i~3.8–11.6)[18] and recently published sugar-containing phosphonates mimicking a lead structure of thymidine (K_i~1.05 and ~8.03).[19] However, the inhibitory activity observed for our 5-aryluracil phosphonates showed lower order values in comparisons, e.g., with most effective 5-chloro-6-cyclopentenyluracil (K_i=0.20±0.03) and 6-chloro-5-phenyluracil (K_i=0.40±0.04).[10]

Table 2
Comparison of the inhibitory effect on human TP for C-6-subtituted 5-arylpyrimidine ANPs

Entry	R	Inhibition of human TP expressed in V79	
		Compound **8**	
		K_i^{dThd} (μmol L^{-1})[a]	K_i^{Pi} (μmol L^{-1})[a]
d		4.89±0.62	5.55±0.67
e		3.98±0.46	2.43±0.33

[a] The K_m (thymidine) value for V79TP was 124±23 μmol L^{-1} and 83±12 μmol L^{-1} for phosphate as a substrate.

In contrast to the above-mentioned 5-alkyl(aryl)-6-chlorouracils and other single-substrate inhibitors, the inhibitory potency of our compounds was carried out with respect to the both substrates 2′-deoxythymidine and inorganic phosphate. Our data determined by K_i^{Pi} for compounds **8d** and **8e** also demonstrate a possible competition of phosphonate unity with inorganic phosphate (Table 2). It may be consequently postulated that these compounds could resemble the multisubstrate character of previously reported ANP inhibitors towards TP from *Escherichia coli*, such as 1-(8-phosphonooctyl)-6-amino-5-bromouracil (TP-64) and 1-(8-phosphonooctyl)-7-deazaxanthine (TP-65).[3]

Despite of the in vitro activity, none of the presented compounds (c=10 μmol L^{-1}) did not exhibit a significant cytostatic activity in tissue cultures estimated in mouse lymphocytic leukaemia L1210 cells (ATCC CCL 219), CCRF-CEM T lymphoblastoid cells (human acute lymphoblastic leukaemia, ATCC CCL 119), human promyelocytic leukaemia HL-60 cells (ATCC CCL 240) and human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2).

3. Conclusions

In summary, we have developed a simple method for the preparation of new 5-aryl-6-(phosphonomethoxy)uracils for biochemical study bearing some functionalized aryl substituents. In comparison with N^1-substituted uracil ANPs, this arylation method afforded higher yields of 5-aryluracil derivatives in DMF and this is limited for thienyl and furyl groups due to unfavourable hydrodehalogenation. In some cases, we have found that an incorporation of a phosphonoalkyl group to C-6-position of 5-aryluracil moiety results in an increase of inhibitory activity towards human TP. This inhibitory effect is supported for 4-fluorophenyl and 3-nitrophenyl groups. From this point of view, this new class of uracil ANPs could be promising for other structural modifications.

4. Experimental

4.1. General comments

Unless stated otherwise, solvents were evaporated at 40 °C/0.5–2 kPa and compounds were dried at 50 °C/13 Pa. Melting points were determined on a Kofler block and are uncorrected. IR spectra were recorded on an FTIR spectrometer Bruker IFS 55 (Equinox) in KBr and CCl_4. Analytical TLC was carried out on Silufol UV_{254} plates (Kavalier Votice, Czech Republic). Preparative TLC was carried out on

45×18×0.4 cm loose-layer silica gel containing UV indicator (system S1). Reversed-phase HPLC was performed on a Waters Delta 600; phenomenex®, Luna, preparative C_{18} column 10 μm 21.20×250 mm, gradient 0-8 min in 0–50% MeOH–H_2O (system S2). Purification of phosphonates **8a** and **8c–h** was performed on Sephadex A-25-120 DEAE (Cl^- form, activated with 0.02M triethylammonium hydrogencarbonate) and eluted in 0–0.4M triethylammonium hydrogencarbonate buffer (system S3). NMR spectra were recorded on a Bruker Avance 600, Bruker Avance 500 or Bruker Avance 400 (^{1}H at 600.1, 500.0, 499.8 or 400.1 MHz, ^{13}C at 150.9, 125.7 or 100.6 MHz, ^{31}P at 202.3 or 162.0 and ^{19}F at 470.3 MHz) in $CDCl_3$ (referenced to TMS), DMSO (referenced to residual solvent signal; 2.50 ppm for ^{1}H and 39.70 ppm for ^{13}C) or in D_2O solutions with dioxane[20] as an internal standard (3.75 ppm for ^{1}H and 69.3 ppm for ^{13}C). ^{31}P and ^{19}F NMR spectra were referenced to the signal of H_3PO_4 (0 ppm) and hexafluorobenzene (−163 ppm), respectively, which were used as an internal standards or as external standards in coaxial 2 mm capillary tube. Chemical shifts (δ, ppm) and coupling constants (J, Hz) were obtained by first-order analysis of the spectra. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (Xe ionization, accelerating voltage 8 kV, glycerol matrix) and Q-Tof micro (Waters, Milford, MA, USA) equipped with an ion source for atmospheric pressure chemical ionization (APCI probe temperature 400 °C, sample cone voltage 30 V, corona discharge current 5.0 μA).

4.2. Materials and chemicals

Standard chemicals, ion-exchange resin (Dowex 50WX8-200) and activated charcoal were purchased from Sigma–Aldrich (Czech Republic). Ion-exchange resin (Sephadex A-25-120 DEAE) was purchased from Fluka. Dimethylformamide, tetrahydrofuran and acetonitrile were dried by distillation from calcium hydride. Thymidine phosphorylase was used from human, expressed in V79 Chinese hamster cells (0.41 mU, Sigma T 9319). The phosphonates **9a–i** were prepared by our method reported in Ref. 15 (see Supplementary data).

4.3. Enzyme assay

The reaction mixture (50 μL, K_i^{dThd} estimation) contained 20 mM bisTris–HCl pH 6.4, 1 mM EDTA and 2 mM DTT, 200 μM potassium phosphate pH 6.7, various concentrations of [^{3}H-methyl]thymidine and tested compounds. K_i^{Pi} was determined in the presence of 200 mM [^{3}H-methyl]thymidine and various concentrations of phosphate. The reaction was started by the addition of 44 nU of enzyme, incubated at 37 °C for 8 min and stopped by spotting a 2 μL aliquot onto Silica gel 60 F_{254} plate that had been prespotted with 0.01 μmol of each thymine and thymidine. The plate was developed in the non-aqueous phase of the solvent system ethyl acetate–water–formic acid (60:35:5). The spots were visualized under UV light (254 nm) and cut out for radioactivity determination in the toluene-based scintillation cocktail (4 g PPO+0.2 g POPOP/1 L toluene (Sigma)). Kinetic constants K_m and K_i were determined from the Lineweaver–Burk and Dixon plots. Data based on results from at least four independent experiments were evaluated by the non-linear regression method using software GOSA, Bio-Log, France.

4.4. 2,6-Di-*tert*-butyl-4-chloropyrimidine (2)

A solution of trichloropyrimidine **1** (8 g, 43.6 mmol) in THF (50 mL) was stirred at 0 °C and sodium *tert*-butoxide powder (8.38 g, 87.2 mmol) was added. The mixture was allowed to warm to rt and stirred for 14 h. The mixture was concentrated in vacuo to a minimum volume and diluted with chloroform (100 mL). The mixture was washed with water (20 mL). Organic layer was dried with magnesium sulfate, filtered and evaporated in vacuo. The residue was chromatographed on silica gel (chloroform–light petroleum 1:1 followed by 5:1). Yield 5.1 g (45%) of colourless oil; ν_{max} (CCl_4) 1575, 1463, 1546, 1478, 1435, 1398, 1388, 1366; δ_H (400.1 MHz, $CDCl_3$) 1.60 and 1.61 (2×s, 2×9H, $(CH_3)_3C$), 6,26 (s, 1H, H-5); δ_C (100.6 MHz, $CDCl_3$) 28.3 and 28.4 ($(CH_3)_3C$), 81.4 and 82.2 ($C(CH_3)_3$), 102.2 (CH-5), 160.6 (C-6), 163.5 (C-2), 171.2 (C-4). ESI-MS m/z 579 $[MH+Na+K]^+$ (100). Anal. Calcd for $C_{12}H_{19}ClN_2O_2$: C, 55.70; H, 7.40; N, 10.83. Found: C, 55.67; H, 7.32; N, 10.61.

4.5. 2,6-Di-*tert*-butyl-4-[(diisopropoxyphosphoryl)-methoxy]pyrimidine (4)

A solution of hydroxymethylphosphonate **3** (6 g, 31.4 mmol) in dioxane (80 mL) was stirred at 10 °C and 60% sodium hydride (1.2 g, 31.4 mmol) was added. The mixture was allowed to warm to 80 °C and chloropyrimidine **2** (2.6 g, 10 mmol) was added. The mixture was vigorously refluxed for 14 h until the conversion of **2** finished. The mixture was concentrated in vacuo to a minimum volume and diluted with chloroform (80 mL). The mixture was washed with water (10 mL). Organic layer was dried with magnesium sulfate and evaporated in vacuo. The residue was chromatographed on silica gel (chloroform–light petroleum 4:1) followed by ethyl acetate–chloroform 1:1. Yield 1.63 g (39%) of slightly yellow oil; ν_{max} (CCl_4) 2981, 1592, 1586, 1459, 1398, 1365, 1380, 1259, 1173, 1161, 1108, 1009, 990, 890, 546; δ_H (400.1 MHz, $CDCl_3$) 1.32 and 1.35 (2×d, 2×6H, J_{vic}=6.2, $(CH_3)_2CH$), 1.57 and 1.61 (2×s, 2×9H, $(CH_3)_3C$), 4.55 (d, 2H, $J_{H,P}$=8.7, CH_2P), 4.79 (dh, 2H, $J_{H,P}$=7.6, J_{vic}=6.2, $CH(CH_3)_2$), 5.71 (s, 1H, H-5); δ_C (100.6 MHz, $CDCl_3$) 23.9 (d, $J_{C,P}$=4.7, $(CH_3)_2CH$), 24.1 (d, $J_{C,P}$=3.9, $(CH_3)_2CH$), 28.5 and 28.7 ($(CH_3)_3C$), 59.8 (d, $J_{C,P}$=172.0, CH_2P), 71.5 (d, $J_{C,P}$=6.5, $CH(CH_3)_2$), 80.4 and 80.9 ($C(CH_3)_3$), 86.6 (CH-5), 163.2 (C-2), 170.9 (d, $J_{C,P}$=12.5, C-6), 172.0 (C-4). FABMS m/z 419 $[MH]^+$ (38); HRMS (FAB) calcd for $C_{19}H_{36}N_2O_6P$ 419.2311, found 419.2316. Anal. Calcd for $C_{19}H_{35}N_2O_6P$: C, 54.53; H, 8.43; N, 6.69; P, 7.40. Found: C, 54.60; H, 8.46; N, 6.41; P, 7.27.

4.6. 5-Bromo-2,6-di-*tert*-butyl-4-[(diisopropoxyphosphoryl)-methoxy]pyrimidine (5)

A mixture of compound **4** (2.38 g, 7.12 mmol), *N*-bromosuccinimide (1.28 g, 7.19 mmol) and azobisisobutyronitrile (30 mg, 0.18 mmol) in THF (30 mL) was heated at 60 °C for 10 h. The mixture was concentrated in vacuo to a minimum volume. The residue was purified by chromatography on silica gel (chloroform–methanol 19:1) to give 2.9 g (99% yield) of **5** as a colourless oil; ν_{max} (CCl_4) 1576, 1550, 1478, 1416, 1397, 1366, 1259, 1177, 1136, 1107, 1006, 987; δ_H (400.1 MHz, $CDCl_3$) 1.35 and 1.36 (2×d, 2×6H, J_{vic}=6.2, $(CH_3)_2CH$), 1.61 and 1.62 (2×s, 2×9H, $(CH_3)_3C$), 4.63 (d, 2H, $J_{H,P}$=8.5, CH_2P), 4.85 (dh, 2H, $J_{H,P}$=7.4, J_{vic}=6.2, $CH(CH_3)_2$); δ_C (100.6 MHz, $CDCl_3$) 23.9 (d, $J_{C,P}$=4.8, $(CH_3)_2CH$), 24.0 (d, $J_{C,P}$=3.8, $(CH_3)_2CH$), 28.3 and 28.5 ($(CH_3)_3C$), 60.5 (d, $J_{C,P}$=171.1, CH_2P), 72.2 (d, $J_{C,P}$=6.6, $CH(CH_3)_2$), 80.9 ($C(CH_3)_3$), 82.3 (C-5), 82.9 ($C(CH_3)_3$), 161.2 (C-2), 166.0 (d, $J_{C,P}$=11.6, C-6), 167.3 (C-4). FABMS m/z 497 $[MH]^+$ (38); HRMS (FAB) calcd for $C_{19}H_{35}BrN_2O_6P$ 497.1416, found 497.1412. Anal. Calcd for $C_{19}H_{34}BrN_2O_6P$: C, 45.88; H, 6.89; Br, 16.07; N, 5.63; P, 6.23. Found: C, 45.86; H, 7.08; Br, 16.08; N, 5.41; P, 6.41.

4.7. 2,6-di-*tert*-Butyl-4-[(diisopropoxyphosphoryl)methoxy]-5-thienylpyrimidine (7a)

A mixture of compound **5** (151 mg, 0.30 mmol), arylboronic acid **6a** (180 mg, 1.41 mmol), $Pd(PPh_3)_4$ (97 mg, 0.08 mmol) and sodium carbonate (112 mg, 1.06 mmol) in toluene (1 mL) and degassed H_2O

(6 mL) was heated (~130 °C, oil bath) under argon for 23 h. The mixture was then concentrated and codistilled with toluene (2×20 mL). The residue was diluted with EtOAc (20 mL) and washed with aqueous EDTA saturated with NH_4Cl (5 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by preparative TLC on silica gel (S1) in toluene–EtOAC 1:1 followed by HPLC (S2). Yield 69 mg (45%) as a colourless oil; ν_{max} (CCl_4) 2981, 1593, 1553, 1528, 1478, 1457, 1428, 1400, 1385, 1365, 1253, 1177, 1168, 1132, 1105, 1009, 988, 890, 847, 465; δ_H (500.0 MHz, $CDCl_3$) 1.14 and 1.22 (2×d, 2×6H, J_{vic}=6.2, (CH_3)$_2$CH), 1.54 and 1.57 (2×s, 2×9H, (CH_3)$_3$C), 4.53 (d, 2H, $J_{H,P}$=8.3, CH_2P), 4.64 (dh, 2H, $J_{H,P}$=7.6, J_{vic}=6.2, $CH(CH_3)_2$), 7.16 (dd, 1H, $J_{5,4}$=5.0, $J_{5,2}$=3.1, H-5-thienyl), 7.39 (dd, 1H, $J_{4,5}$=5.0, $J_{4,2}$=1.3, H-4-thienyl), 7.52 (dd, 1H, $J_{2,5}$=3.1, $J_{2,4}$=1.3, H-2-thienyl); δ_C (125.7 MHz, $CDCl_3$) 23.8 (d, $J_{C,P}$=4.6, (CH_3)$_2$CH), 24.1 (d, $J_{C,P}$=3.5, (CH_3)$_2$CH), 28.5 and 28.7 ((CH_3)$_3$C), 60.1 (d, $J_{C,P}$=170.7, CH_2P), 71.5 (d, $J_{C,P}$=6.4, $CH(CH_3)_2$), 80.4 and 81.9 ($C(CH_3)_3$), 96.3 (C-5), 122.5 (CH-5-thienyl), 124.5 (CH-2-thienyl), 130 (CH-4-thienyl), 130.5 (C-3-thienyl), 160.8 (C-2), 166.8 (d, $J_{C,P}$=11.7, C-6), 167.9 (C-4). ESI-MS *m*/*z* 523 $[MNa]^+$ (100); HRMS (ESI) calcd for $C_{23}H_{37}N_2NaO_6PS$ 523.2002, found 523.2002.

4.8. Pd-catalyzed Suzuki–Miyaura coupling reactions of 5-bromopyrimidine 5 with boronic acids (compounds 4 and 7c–i). General procedure

A mixture of compound **5** (1 mmol), arylboronic acid **6a–i** (2 mmol), $Pd(PPh_3)_4$ (116 mg, 0.10 mmol), sodium carbonate (352 mg, 3.32 mmol) in DMF (20 mL) and degassed H_2O (4 mL) was heated (~130 °C, oil bath) under argon for 4–6 h (see Table 1). The mixture was then concentrated and codistilled with toluene (2×20 mL). The residue was diluted with EtOAc (20 mL) and washed with aqueous EDTA saturated with NH_4Cl (5 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by preparative TLC on silica gel (S1) in below mentioned systems (see Sections 4.8.1–4.8.8) to give compounds **4** and **7c–i**.

*4.8.1. 2,6-Di-tert-butyl-4-[(diisopropoxyphosphoryl)methoxy]pyrimidine (**4**).* (a) The reaction of **5** with boronic acid **6a** afforded 187 mg (42%) of pyrimidine **4**; (b) the reaction of **5** with boronic acid **6b** afforded 236 mg (57%) of pyrimidine **4**. ^1H NMR data were identical with those of compound prepared in Section 4.5.

*4.8.2. 2,6-Di-tert-butyl-4-[(diisopropoxyphosphoryl)methoxy]-5-phenylpyrimidine (**7c**).* Column chromatography was performed in toluene–ethyl acetate 1:1. Yield 383 mg (77%) as a colourless oil; ν_{max} (CCl_4) 1602, 1593, 1579, 1550, 1478, 1458, 1426, 1406, 1393, 1382, 1365, 1255, 1177, 1169, 1134, 1106, 1007, 995, 988, 848, 463; δ_H (500.0 MHz, $CDCl_3$) 1.12 and 1.21 (2×d, 2×6H, J_{vic}=6.2, (CH_3)$_2$CH), 1.53 and 1.65 (2×s, 2×9H, (CH_3)$_3$C), 4.57 (d, 2H, $J_{H,P}$=8.3, CH_2P), 4.58 (dh, 2H, $J_{H,P}$=7.6, J_{vic}=6.2, $CH(CH_3)_2$), 7.22 (m, 1H, H-*p*-Ph), 7.31 (m, 2H, H-*m*-Ph), 7.38 (m, 2H, H-*o*-Ph); δ_C (125.7 MHz, $CDCl_3$) 23.7 (d, $J_{C,P}$=4.7, (CH_3)$_2$CH), 24.0 (d, $J_{C,P}$=3.7, (CH_3)$_2$CH), 28.5 and 28.6 ((CH_3)$_3$C), 60.1 (d, $J_{C,P}$=170.1, CH_2P), 71.5 (d, $J_{C,P}$=6.5, $CH(CH_3)_2$), 80.4 and 81.4 ($C(CH_3)_3$), 100.9 (C-5), 126.5 (CH-*p*-Ph), 127.3 (CH-*m*-Ph), 130.9 (CH-*o*-Ph), 131.7 (C-*i*-Ph), 164.3 (C-2), 167.1 (d, $J_{C,P}$=11.0, C-6), 168.3 (C-4). FABMS *m*/*z* 497 $[MH]^+$ (12); HRMS (FAB) calcd for $C_{25}H_{40}N_2O_6P$ 495.2624, found 495.2636. Anal. Calcd for $C_{25}H_{39}N_2O_6P$: C, 60.71; H, 7.95; N, 5.66; P, 6.26. Found: C, 60.28; H, 7.99; N, 5.50; P, 6.41.

*4.8.3. 2,6-Di-tert-butyl-5-(4-fluorophenyl)-4-[(diisopropoxyphosphoryl)methoxy]-pyrimidine (**7d**).* Column chromatography was performed in toluene–ethyl acetate 1:1. Yield 370 mg (72%) as a white amorphous solid; ν_{max} (CCl_4) 1611, 1592, 1551, 1514, 1451, 1478, 1428, 1420, 1405, 1392, 1386, 1378, 1365, 1254, 1233, 1106, 1009, 1002, 989; δ_H (400.1 MHz, $CDCl_3$) 1.13 and 1.26 (2×d, 2×6H, J_{vic}=6.2, (CH_3)$_2$CH), 1.53 and 1.65 (2×s, 2×9H, (CH_3)$_3$C), 4.56 (d, 2H, $J_{H,P}$=8.3, CH_2P), 4.61 (dh, 2H, $J_{H,P}$=7.5, J_{vic}=6.2, $CH(CH_3)_2$), 7.01 (m, 2H, H-*m*-C_6H_4F), 7.36 (m, 2H, H-*o*-C_6H_4F); δ_C (100.6 MHz, $CDCl_3$) 23.7 (d, $J_{C,P}$=4.8, (CH_3)$_2$CH), 24.0 (d, $J_{C,P}$=3.7, (CH_3)$_2$CH), 28.5 and 28.6 ((CH_3)$_3$C), 60.1 (d, $J_{C,P}$=170.7, CH_2P), 71.4 (d, $J_{C,P}$=6.5, $CH(CH_3)_2$), 80.5 and 81.6 ($C(CH_3)_3$), 99.9 (C-5), 114.2 (d, $J_{C,F}$=21.3, CH-*m*-C_6H_4F), 127.6 (d, $J_{C,F}$=3.4, C-*i*-C_6H_4F), 132.5 (d, $J_{C,F}$=7.9, CH-*o*-C_6H_4F), 161.5 (C-2), 161.6 (d, $J_{C,F}$=245.2, C-*p*-C_6H_4F), 167.0 (d, $J_{C,P}$=11.6, C-6), 168.3 (C-4). FABMS *m*/*z* 513 $[MH]^+$ (12); HRMS (FAB) calcd for $C_{25}H_{39}FN_2O_6P$ 513.2530, found 513.2520.

*4.8.4. 2,6-Di-tert-butyl-4-[(diisopropoxyphosphoryl)methoxy]-5-(3-nitrophenyl) pyrimidine (**7e**).* Column chromatography was performed in toluene–ethyl acetate 1:1. Yield 357 mg (66%) as a yellow oil; ν_{max} (CCl_4) 1594, 1577, 1549, 1533, 1480, 1437, 1428, 1405, 1394, 1381, 1366, 1349, 1254, 1106, 1008, 990; δ_H (600.1 MHz, $CDCl_3$) 1.15 and 1.23 (2×d, 2×6H, J_{vic}=6.2, (CH_3)$_2$CH), 1.56 and 1.66 (2×s, 2×9H, (CH_3)$_3$C), 4.59 (d, 2H, $J_{H,P}$=8.3, CH_2P), 4.63 (dh, 2H, $J_{H,P}$=7.6, J_{vic}=6.2, $CH(CH_3)_2$), 7.50 (dd, 1H, $J_{5,4}$=8.3, $J_{5,6}$=7.7, H-5-$C_6H_4NO_2$), 7.81 (ddd, 1H, $J_{6,5}$=7.7, $J_{6,2}$=1.6, $J_{6,4}$=1.1, H-6-$C_6H_4NO_2$), 8.10 (ddd, 1H, $J_{4,5}$=8.3, $J_{4,2}$=2.4, $J_{4,6}$=1.1, H-4-$C_6H_4NO_2$), 8.33 (dd, 1H, $J_{2,4}$=2.4, $J_{2,6}$=1.6, H-2-$C_6H_4NO_2$); δ_C (150.9 MHz, $CDCl_3$) 23.7 (d, $J_{C,P}$=4.6, (CH_3)$_2$CH), 23.9 (d, $J_{C,P}$=3.7, (CH_3)$_2$CH), 28.5 and 28.6 ((CH_3)$_3$C), 60.2 (d, $J_{C,P}$=170.6, CH_2P), 71.4 (d, $J_{C,P}$=6.5, $CH(CH_3)_2$), 81.0 and 82.4 ($C(CH_3)_3$), 98.3 (C-5), 121.4 (CH-4-$C_6H_4NO_2$), 125.9 (CH-2-$C_6H_4NO_2$), 128.1 (CH-5-$C_6H_4NO_2$), 133.7 (C-1-$C_6H_4NO_2$), 137.7 (CH-6-$C_6H_4NO_2$), 147.6 (C-3-$C_6H_4NO_2$), 162.0 (C-2), 166.9 (d, $J_{C,P}$=11.2, C-6), 168.2 (C-4). FABMS *m*/*z* 540 $[MH]^+$ (12); HRMS (FAB) calcd for $C_{25}H_{38}N_3O_8P$ 540.2475, found 540.2480.

*4.8.5. 2,6-Di-tert-butyl-4-[(diisopropoxyphosphoryl)methoxy]-5-(1-naphthyl)pyrimidine (**7f**).* Column chromatography was performed in toluene–ethyl acetate 1:1. Yield 176 mg (32%) as a yellow oil; ν_{max} (CCl_4) 1602, 1589, 1553, 1509, 1478, 1415, 1393, 1385, 1365, 1256, 1007, 987; δ_H (600.1 MHz, $CDCl_3$) 0.82, 0.90, 0.96 and 1.01 (4×d, 2×3H, J_{vic}=6.2, (CH_3)$_2$CH), 1.38 and 1.70 (2×s, 2×9H, (CH_3)$_3$C), 4.24 (m, 2H, $CH(CH_3)_2$), 4.54 and 4.57 (2×dd, 2H, J_{gem}=14.2, $J_{H,P}$=8.4, CH_2P), 7.35 (dd, 1H, $J_{2,3}$=7.0, $J_{2,4}$=1.3, H-2-naphth), 7.39 (ddd, 1H, $J_{7,8}$=8.3, $J_{7,6}$=6.8, $J_{7,5}$=1.5, H-7-naphth), 7.43 (ddd, 1H, $J_{6,5}$=8.1, $J_{6,7}$=6.8, $J_{6,8}$=1.4, H-6-naphth), 7.46 (dd, 1H, $J_{3,4}$=8.2, $J_{3,2}$=7.0, H-3-naphth), 7.54 (m, 1H, $J_{8,7}$=8.3, $J_{8,6}$=1.4, $J_{8,4}$=1.0, $J_{8,5}$=0.7, H-8-naphth), 7.78 (ddd, 1H, $J_{4,3}$=8.2, $J_{4,2}$=1.3, $J_{4,8}$=1.0, H-4-naphth), 7.83 (ddd, 1H, $J_{5,6}$=8.1, $J_{5,7}$=1.5, $J_{5,8}$=0.7, H-5-naphth); δ_C (150.9 MHz, $CDCl_3$) 23.4 and 23.5 (d, $J_{C,P}$=4.5, (CH_3)$_2$CH), 23.73 and 23.79 (d, $J_{C,P}$=3.7, (CH_3)$_2$CH), 28.5 and 28.6 ((CH_3)$_3$C), 60.1 (d, $J_{C,P}$=168.9, CH_2P), 71.4 and 71.5 (d, $J_{C,P}$=6.5, $CH(CH_3)_2$), 80.5 and 81.3 ($C(CH_3)_3$), 98.9 (C-5), 125.2 (CH-3-naphth), 125.3 (CH-6-naphth), 125.4 (CH-7-naphth), 125.9 (CH-8-naphth), 127.5 (CH-4-naphth), 128.0 (CH-5-naphth), 128.6 (CH-2-naphth), 130.0 (C-1-naphth), 132.4 (C-8a-naphth), 133.5 (C-4a-naphth), 162.3 (C-2), 167.9 (d, $J_{C,P}$=11.2, C-6), 169.2 (C-4). FABMS *m*/*z* 545 $[MH]^+$ (15); HRMS (FAB) calcd for $C_{29}H_{42}N_2O_6P$ 545.2781, found 545.2773.

*4.8.6. 2,6-Di-tert-Butyl-4-[(diisopropoxyphosphoryl)methoxy]-5-(pyridin-4-yl)pyrimidine (**7g**).* Column chromatography was performed in ethyl acetate–chloroform–methanol 21:20:1. Yield 385 mg (78%) as a slightly yellow oil; ν_{max} (CCl_4) 1599, 1585, 1549, 1478, 1425, 1403, 1366, 1254, 1106, 1008, 996, 986; δ_H (400.1 MHz, $CDCl_3$) 1.16 and 1.26 (2×d, 2×6H, J_{vic}=6.2, (CH_3)$_2$CH), 1.56 and 1.65 (2×s, 2×9H, (CH_3)$_3$C), 4.57 (d, 2H, $J_{H,P}$=8.4, CH_2P), 4.65 (dh, 2H, $J_{H,P}$=7.6, J_{vic}=6.2, $CH(CH_3)_2$), 7.40 (m, 2H, H-3,5-py), 8.55 (m, 2H, H-2,6-py); δ_C (100.6 MHz, $CDCl_3$) 23.7 (d, $J_{C,P}$=4.7, (CH_3)$_2$CH), 24.0 (d, $J_{C,P}$=3.8, (CH_3)$_2$CH), 28.5 and 28.6 ((CH_3)$_3$C), 60.2 (d, $J_{C,P}$=171.0, CH_2P), 71.5 (d, $J_{C,P}$=6.5, $CH(CH_3)_2$); 80.9 and 82.3 ($C(CH_3)_3$); 98.0 (C-

5); 125.3 (CH-3,5-py); 140.4 (C-4-py); 148.8 (CH-2,6-py); 162.1 (C-2); 167.0 (d, $J_{C,P}$=11.3, C-6); 168.2 (C-4). FABMS *m*/*z* 496 $[MH]^+$ (39); HRMS (FAB) calcd for $C_{24}H_{39}N_3O_6P$ 496.2577, found 496.2592. Anal. Calcd for $C_{24}H_{38}N_3O_6P$: C, 58.17; H, 7.73; N, 8.48; P, 6.25. Found: C, 57.84; H, 7.85; N, 8.31; P, 6.04.

*4.8.7. 2,6-Di-tert-butyl-4-[(diisopropoxyphosphoryl)methoxy]-5-(pyridin-3-yl)pyrimidine (**7h**).* Column chromatography was performed in ethyl acetate–chloroform–methanol 21:20:2. Yield 212 mg (43%) as a slightly yellow oil; ν_{max} (CCl_4) 1598, 1587, 1557, 1539, 1497, 1478, 1438, 1429, 1406, 1394, 1366, 1105, 1009, 993, 987; δ_H (400.1 MHz, $CDCl_3$) 1.15 and 1.24 (2×d, 2×6H, J_{vic}=6.2, $(CH_3)_2CH$), 1.55 and 1.66 (2×s, 2×9H, $(CH_3)_3C$), 4.58 (d, 2H, $J_{H,P}$=8.3, CH_2P), 4.62 (dh, 2H, $J_{H,P}$=7.5, J_{vic}=6.2, $CH(CH_3)_2$), 7.26 (ddd, 1H, $J_{5,4}$=7.9, $J_{5,6}$=4.8, $J_{5,2}$=0.9, H-5-py), 7.75 (ddd, 1H, $J_{4,5}$=7.9, $J_{4,2}$=2.0, $J_{4,6}$=1.6, H-4-py), 8.46 (dd, 1H, $J_{6,5}$=4.8, $J_{6,4}$=1.6, H-6-py), 8.63 (br d, 1H, $J_{2,4}$=2.0, H-2-py); δ_C (100.6 MHz, $CDCl_3$) 23.7 (d, $J_{C,P}$=4.7, $(CH_3)_2CH$), 24.0 (d, $J_{C,P}$=3.9, $(CH_3)_2CH$), 28.5 and 28.6 ($(CH_3)_3C$), 60.1 (d, $J_{C,P}$=170.5, CH_2P), 71.5 (d, $J_{C,P}$=6.6, $CH(CH_3)_2$), 80.8 and 82.0 ($C(CH_3)_3$), 97.3 (C-5), 122.4 (CH-5-py), 128.1 (C-3-py), 138.1 (CH-4-py), 147.4 (CH-6-py), 151.6 (CH-2-py), 162.0 (C-2), 167.3 (d, $J_{C,P}$=11.1, C-6), 168.4 (C-4). FABMS *m*/*z* 496 $[MH]^+$ (27); HRMS (FAB) calcd for $C_{24}H_{39}N_3O_6P$ 496.2577, found 496.2572.

*4.8.8. 2,6-Di-tert-butyl-4-[(diisopropoxyphosphoryl)methoxy]-5-[(E)-2-phenylvinyl]pyrimidine (**7i**).* Column chromatography was performed in toluene–ethyl acetate 1:1. Yield 131 mg (25%) as a yellow oil; ν_{max} (CCl_4) 1648, 1583, 1549, 1497, 1450, 1478, 1425, 1402, 1387, 1377, 1365, 1252, 1107, 1009, 996, 991; δ_H (499.8 MHz, $CDCl_3$) 1.32 and 1.35 (2×d, 2×6H, J_{vic}=6.2, $(CH_3)_2CH$), 1.63 and 1.68 (2×s, 2×9H, $(CH_3)_3C$), 4.65 (d, 2H, $J_{H,P}$=8.2, CH_2P), 4.83 (dh, 2H, $J_{H,P}$=7.6, J_{vic}=6.2, $CH(CH_3)_2$), 7.18 (d, 1H, J_{trans}=16.7, C*H*=CH-Ph), 7.20 (m, 1H, H-*p*-Ph), 7.32 (m, 2H, H-*m*-Ph), 7.46 (m, 2H, H-*o*-Ph), 7.50 (d, 1H, J_{trans}=16.7, CH=C*H*–Ph); δ_C (125.7 MHz, $CDCl_3$) 24.0 (d, $J_{C,P}$=4.7, $(CH_3)_2CH$), 24.1 (d, $J_{C,P}$=3.9, $(CH_3)_2CH$), 28.6 and 28.8 ($(CH_3)_3C$), 60.2 (d, $J_{C,P}$=170.9, CH_2P), 71.5 (d, $J_{C,P}$=6.5, $CH(CH_3)_2$), 80.6 and 82.2 ($C(CH_3)_3$), 97.7 (C-5), 117.4 (CH=CH–Ph), 126.1 (CH-*o*-Ph), 126.9 (CH-*p*-Ph), 128.5 (CH-*m*-Ph), 130.5 (CH=CH–Ph), 139.1 (C-*i*-Ph), 160.5 (C-2), 167.3 (d, $J_{C,P}$=11.9, C-6), 168.3 (C-4). FABMS *m*/*z* 521 $[MH]^+$ (11); HRMS (FAB) calcd for $C_{27}H_{42}N_2O_6P$ 521.2781, found 521.2799.

4.9. Deprotection of 5-aryluracils 7a, 7c–h and 7j. General procedure

A solution of **7** (0.54 mmol) in acetonitrile (10 mL) was cooled to 0 °C. Bromotrimethylsilane (839 mg, 5.44 mmol) was added dropwise and the mixture was stirred overnight at room temperature. The mixture was concentrated and neutralized with 0.5M TEAB solution at 0 °C. The mixture was evaporated in vacuo and then codistilled with water (2×2 mL). The residue was purified on DEAE-Sephadex (Cl^-, 0–0.4M TEAB, S3) with subsequent deionization of the product on activated charcoal with water. The residue was dissolved in water (3 mL), applied onto a column of Dowex 50×8 (Li^+ form, 30 mL) and then the column was washed with water. The appropriate UV absorbing fraction containing product **8** was evaporated to dryness in vacuo. The residue was dissolved in water and lyophilized. The following compounds were obtained as dilithium salts:

*4.9.1. 6-(Phosphonomethoxy)-5-(3-thienyl)uracil (**8a**).* Yield 15 mg (33%) as a slightly brown amorphous solid; mp >300 °C; ν_{max} (KBr) 3270, 3110, 1718, 1708, 1630, 1492, 1426, 1354, 1125, 1086, 1001, 800; δ_H (499.8 MHz, D_2O) 4.14 (d, 2H, $J_{H,P}$=8.6, CH_2P), 7.40 (dd, 1H, $J_{4,5}$=5.0, $J_{4,2}$=1.3, H-4-thienyl), 7.45 (dd, 1H, $J_{5,4}$=5.0, $J_{5,2}$=3.0, H-5-thienyl), 7.54 (dd, 1H, $J_{2,5}$=3.0, $J_{2,4}$=1.3, H-2-thienyl); δ_C (125.7 MHz, D_2O) 66.6 (d, $J_{C,P}$=151.1, CH_2P), 91.8 (C-5), 126.5 (CH-2-thienyl), 127.3 (CH-5-thienyl), 132.9 (CH-4-thienyl), 135.1 (C-3-thienyl), 162.0 (C-2), 169.7 (C-4), 173.7 (d, $J_{C,P}$=10.8, C-6); δ_P (202.3 MHz, D_2O) 12.51; ESI-MS *m*/*z* 315 $[M-H]^-$ (24); HRMS (ESI) calcd for $C_9H_6Li_2N_2O_6PS$ 315.0010, found 315.0003.

*4.9.2. 5-Phenyl-6-(phosphonomethoxy)uracil (**8c**).* Yield 46 mg (23%) as a slightly yellow amorphous solid; mp >300 °C; ν_{max} (KBr) 1710, 1626, 1601, 1499, 1444, 1130, 1094, 1077, 1035, 996, 937, 755, 699, 573; δ_H (400.1 MHz, D_2O, ref(dioxane)=3.75 ppm) 4.21 (d, 2H, $J_{H,P}$=7.8, CH_2P), 7.35 (m, 1H, H-*p*-Ph), 7.39 (m, 2H, H-*o*-Ph), 7.45 (m, 2H, H-*m*-Ph); δ_C (100.6 MHz, D_2O, ref(dioxane)=69.3 ppm) 68.3 (d, $J_{C,P}$=149.5, CH_2P), 97.9 (C-5), 129.9 (CH-*p*-Ph), 131.2 (CH-*m*-Ph), 134.0 (CH-*o*-Ph), 134.6 (C-*i*-Ph), 159.2 (C-2), 169.2 (d, $J_{C,P}$=7.1, C-6), 170.0 (C-4); δ_P (162.0 MHz, D_2O) 12.62; ESI-MS *m*/*z* 311 $[MH]^+$ (70), HRMS (ESI) calcd for $C_{11}H_{10}Li_2N_2O_6P$ 311.0597, found 311.0605. Anal. Calcd for $C_{11}H_9Li_2N_2O_6P \cdot 3.5H_2O$: C, 35.41; H, 4.32; N, 7.51; P, 8.30. Found: C, 35.14; H, 4.21; N, 7.29; P, 7.88.

*4.9.3. 5-Fluorophenyl-6-(phosphonomethoxy)uracil (**8d**).* Yield 111 mg (53%) as a white amorphous solid; mp >300 °C; ν_{max} (KBr) 1717, 1624, 1559, 1509, 1406, 995, 934, 577; δ_H (500.0 MHz, D_2O, ref(dioxane)=3.75 ppm) 4.22 (d, 2H, $J_{H,P}$=7.8, CH_2P), 7.17 (m, 2H, H-*m*-C_6H_4F), 7.39 (m, 2H, H-*o*-C_6H_4F); δ_C (125.7 MHz, D_2O, ref(dioxane)=69.3 ppm) 68.1 (d, $J_{C,P}$=149.3, CH_2P), 96.9 (C-5), 117.9 (d, $J_{C,F}$=21.4, CH-*m*-C_6H_4F), 130.6 (d, $J_{C,F}$=3.2, C-*i*-C_6H_4F), 135.7 (d, $J_{C,F}$=8.2, CH-*o*-C_6H_4F), 159.5 (C-2), 164.3 (d, $J_{C,F}$=243.5, C-*p*-C_6H_4F), 169.5 (d, $J_{C,P}$=6.8, C-6), 170.0 (C-4), δ_P (202.3 MHz, D_2O) 12.73; δ_F (470.3 MHz, D_2O) −112.17. FABMS *m*/*z* 351 $[M+Na]^+$ (9); HRMS (FAB) calcd for $C_{11}H_8FLi_2NaN_2O_6P$ 351.0321, found 351.0337. Anal. Calcd for $C_{11}H_8FLi_2N_2O_6P \cdot 3.0H_2O$: C, 34.57; H, 2.69; N, 7.33; F, 4.97; P, 8.11. Found: C, 34.29; H, 2.42; N, 7.10; F, 4.83; P, 8.41.

*4.9.4. 5-(3-Nitrophenyl)-6-(phosphonomethoxy)uracil (**8e**).* Yield 150 mg (68%) as a yellow amorphous solid; mp >320 °C; ν_{max} (KBr) 1710, 1627, 1602, 1580, 1526, 1496, 1423, 1353, 1128, 1085, 990, 905; δ_H (499.8 MHz, D_2O, ref(dioxane)=3.75 ppm) 4.29 (d, 2H, $J_{H,P}$=8.0, CH_2P), 7.62 (dd, 1H, $J_{5,4}$=8.3, $J_{5,6}$=7.8, H-5-$C_6H_4NO_2$), 7.85 (ddd, 1H, $J_{6,5}$=7.8, $J_{6,2}$=1.6, $J_{6,4}$=1.1, H-6-$C_6H_4NO_2$), 8.14 (ddd, 1H, $J_{4,5}$=8.3, $J_{4,2}$=2.4, $J_{4,6}$=1.1, H-4-$C_6H_4NO_2$), 8.30 (dd, 1H, $J_{2,4}$=2.4, $J_{2,6}$=1.6, H-2-$C_6H_4NO_2$); δ_C (125.7 MHz, D_2O, ref(dioxane)=69.3 ppm) 66.8 (d, $J_{C,P}$=151.8, CH_2P), 95.5 (C-5), 124.2 (CH-4-$C_6H_4NO_2$), 128.6 (CH-2-$C_6H_4NO_2$), 131.8 (CH-5-$C_6H_4NO_2$), 136.9 (C-1-$C_6H_4NO_2$), 140.7 (CH-6-$C_6H_4NO_2$), 150.4 (C-3-$C_6H_4NO_2$), 160.6 (C-2), 169.7 (C-4), 170.7 (d, $J_{C,P}$=7.2, C-6); δ_P (202.3 MHz, D_2O) 13.34. ESI-MS *m*/*z* 342 $[MH-2Li]^+$ (100), HRMS (ESI) calcd for $C_{11}H_9N_3O_8P$ 342.0127, found 342.0128. Anal. Calcd for $C_{11}H_8Li_2N_3O_8P \cdot 2.75H_2O$: C, 32.66; H, 3.36; N, 10.38; P, 7.66. Found: C, 32.47; H, 3.40; N, 10.05; P, 8.02.

*4.9.5. 5-(Naphth-1-yl)-6-(phosphonomethoxy)uracil (**8f**).* Yield 121 mg (61%) as a white amorphous solid; mp >320 °C; ν_{max} (KBr) 1715, 1624, 1592, 1579, 1507, 1497, 1430, 1399, 1363, 1126, 1095, 1000, 939, 805, 780, 588; δ_H (600.0 MHz, D_2O, ref(dioxane)=3.75 ppm) 4.05 (dd, 1H, J_{gem}=13.7, $J_{H,P}$=7.8, CH_aH_bP), 4.22 (dd, 1H, J_{gem}=13.7, $J_{H,P}$=7.8, CH_aH_bP), 7.49 (dd, 1H, $J_{2,3}$=7.0, $J_{2,4}$=1.3, H-2-naphth), 7.54 (ddd, 1H, $J_{7,8}$=8.3, $J_{7,6}$=6.8, $J_{7,5}$=1.5, H-7-naphth), 7.57 (ddd, 1H, $J_{6,5}$=8.1, $J_{6,7}$=6.8, $J_{6,8}$=1.4, H-6-naphth), 7.60 (dd, 1H, $J_{3,4}$=8.3, $J_{3,2}$=7.0, H-3-naphth), 7.79 (m, 1H, $J_{8,7}$=8.3, $J_{8,6}$=1.4, $J_{8,4}$=1.1, $J_{8,5}$=0.7, H-8-naphth), 7.97 (ddd, 1H, $J_{4,3}$=8.3, $J_{4,2}$=1.3, $J_{4,8}$=1.1, H-4-naphth), 8.99 (ddd, 1H, $J_{5,6}$=8.1, $J_{5,7}$=1.5, $J_{5,8}$=0.7, H-5-naphth); δ_C (150.9 MHz, D_2O, ref(dioxane)=69.3 ppm) 68.3 (d, $J_{C,P}$=149.1, CH_2P), 95.6 (C-5), 128.0 (CH-8-naphth), 128.8 (CH-3-naphth), 128.9 (CH-6-naphth), 129.3 (CH-7-naphth), 131.0 (CH-4-naphth), 131.2 (CH-5-naphth), 132.2 (C-1-naphth), 132.7 (CH-2-naphth), 135.2 (C-8a-naphth), 136.3 (C-4a-naphth), 159.8 (C-2), 170.3 (C-4,6); δ_P (202.3 MHz, D_2O)

16.82. ESI-MS *m*/*z* 347 $[MH-2Li]^-$ (100); HRMS (ESI) calcd for $C_{15}H_{12}N_2O_6P$ 347.0433, found 347.0448.

*4.9.6. 6-(Phosphonomethoxy)-5-(pyridin-4-yl)uracil (**8g**).* Yield 48 mg (29%) as a white amorphous solid; mp >300 °C; ν_{max} (KBr) 1723, 1630, 1496, 1374, 1287, 1091, 1000, 594; δ_H (600.0 MHz, D_2O, ref(dioxane)=3.75 ppm) 4.34 (d, 2H, $J_{H,P}$=8.3, CH_2P), 8.23 (m, 2H, H-3,5-py), 8.35 (m, 2H, H-2,6-py); δ_C (150.9 MHz, D_2O, ref(dioxane)=69.3 ppm) 66.0 (d, $J_{C,P}$=153.4, CH_2P), 93.0 (C-5), 128.4 (CH-3,5-py), 142.1 (CH-2,6-py), 155.0 (C-4-py), 162.2 (C-2), 169.43 (C-4), 173.6 (d, $J_{C,P}$=10.4, C-6); δ_P (202.3 MHz, D_2O) 12.99. ESI-MS *m*/*z* 305 $[MH-Li]^+$ (32); HRMS (ESI) calcd for $C_{10}H_8LiN_3O_6P$ 304.0311, found 304.0318.

*4.9.7. 6-(Phosphonomethoxy)-5-(pyridin-3-yl)uracil (**8h**).* Yield 96 mg (57%) as a slightly yellow amorphous solid; mp >300 °C; ν_{max} (KBr) 1635, 1558, 1491, 1465, 1375, 1284, 1090, 810, 710, 791, 625; δ_H (500.0 MHz, D_2O, ref(dioxane)=3.75 ppm) 4.30 (d, 2H, $J_{H,P}$=8.2, CH_2P), 7.68 (br dd, 1H, $J_{5,4}$=8.1, $J_{5,6}$=4.9, H-5-py), 8.33 (br d, 1H, $J_{4,5}$=8.1, H-4-py), 8.39 (br s, 1H, H-6-py), 8.73 (br s, 1H, H-2-py); δ_C (125.7 MHz, D_2O, ref(dioxane)=69.3 ppm) 66.3 (d, $J_{C,P}$=153.4, CH_2P), 92.0 (C-5), 127.6 (CH-5-py), 134.4 (C-3-py), 144.1 (CH-6-py), 145.9 (CH-4-py), 148.9 (CH-2-py), 161.4 (C-2), 169.5 (C-4), 171.7 (d, $J_{C,P}$=8.8, C-6); δ_P (202.3 MHz, D_2O) 13.25. ESI-MS *m*/*z* 304 $[M-H-Li]^-$ (11); HRMS (ESI) calcd for $C_{10}H_8LiN_3O_6P$ 304.0311, found 304.0311.

*4.9.8. 6-(Phosphonomethoxy)uracil (**8j**).* Yield 139 mg (58%) as a white amorphous solid; mp >300 °C; ν_{max} (KBr) 1720, 1634, 1520, 1372, 1242, 1070, 1031, 925; δ_H (400.1 MHz, DMSO-d_6) 3.78 (d, 2H, $J_{H,P}$=9.1, CH_2P), 4.89 (s, 1H, H-5); δ_C (100.6 MHz, DMSO-d_6) 66.9 (d, $J_{C,P}$=151.4, CH_2P), 77.0 (CH-5), 150.8 (C-2), 164.3 (d, $J_{C,P}$=12.6, C-6), 165.2 (C-4).; δ_P (162.0 MHz, D_2O) 10.54. ESI-MS *m*/*z* 223 $[MH-2Li]^+$ (56); HRMS (ESI) calcd for $C_5H_8N_2O_6P$ 223.0120, found 223.0128.

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Supplementary data

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