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Pd-catalyzed Suzuki–Miyaura coupling reactions in the synthesis of 5-aryl-1-[2-(phosphonomethoxy)ethyl]uracils as potential multisubstrate inhibitors of thymidine phosphorylase

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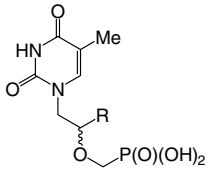
Abstract—5-Aryl-1-[2-(phosphonomethoxy)ethyl]uracils were prepared via Pd-catalyzed Suzuki–Miyaura coupling reaction of the appropriate 5-bromouracil derivative with a series of arylboronic acids followed by deprotection. These compounds were designed as potential multisubstrate inhibitors of thymidine phosphorylase based on an assumption that the potential hydrophobic effect of the aryl groups might modify the inhibitory effect towards this enzyme and they may also demonstrate cytostatic activity.

Pyrimidine acyclic nucleoside phosphonates (ANPs) possess a broad spectrum of biological activity[1] and have been investigated as multisubstrate and catabolically stable inhibitors[2] of thymidine phosphorylase (TP). This enzyme is crucial for phosphorolysis of thymidine to thymine and 2-deoxy-D-ribose-1-phosphate.[3a] The latter compound is then dephosphorylated to 2-deoxy-D-ribose which was recently identified as an endothelial-cell chemoattractant (PD-ECGF)[3] and an angiogenesis-inducing factor.[4] Therefore, development of multisubstrate TP inhibitors which block thymine (dThd) and phosphate-binding sites[2,5] may be useful as tumor growth[4c] suppressors.

In this study, we prepared 5-aryl substituted uracil ANPs in order to modify the bioactivity of 1-[2-(phosphonomethoxy)ethyl]pyrimidine derivatives (PME compounds) which display only a marginal inhibitory effect on human TP.[6–8] On the other hand, 1-[2-(phosphonomethoxy)ethyl]thymine demonstrates inhibitory activity towards TP from SD-lymphoma.[6] Therefore, we assumed that the active sites of both enzymes could be significantly different. In addition, the inhibitory effect towards TP from SD-lymphoma also decreased in the presence of other bioactive pyrimidine ANPs (e.g., FPMPT, HPMPT, PMPT, see Fig. 1). This is probably caused by the absence of alkyl substitutents on the side chain of the 2-(phosphonomethoxy)alkyl group as documented in a previous paper.[6] Therefore, our efforts were directed in particular to increasing the inhibitory effect by modification of the possible hydrophobic interaction of PME derivatives with TP near the C-5-position of the uracil moiety.



PMET, R = H, $K_i/^{dThd}K_m = 0.0175$
(*S*)-PMPT, R = Me, $K_i/^{dThd}K_m = 0.0082$
(*R*)-HPMPT, R = CH_2OH, $K_i/^{dThd}K_m = 0.0044$
(*R*)-FPMPT, R = F, $K_i/^{dThd}K_m = 0.0026$

Figure 1. Structure of 1-[2-(phosphonomethoxy)alkyl]thymines which inhibit thymidine phosphorylase from SD-lymphoma.

It is known, that some 5-aryl-6-chloro-substituted uracils demonstrate a significant impact on the inhibitory activity towards human TP in which the favoured hydrophobic interaction of the aryl substituents is probably directed by the halogen electron-withdrawing effect.[9] Based on this hypothesis we expected that the hydrophobic effect could also be influenced by the

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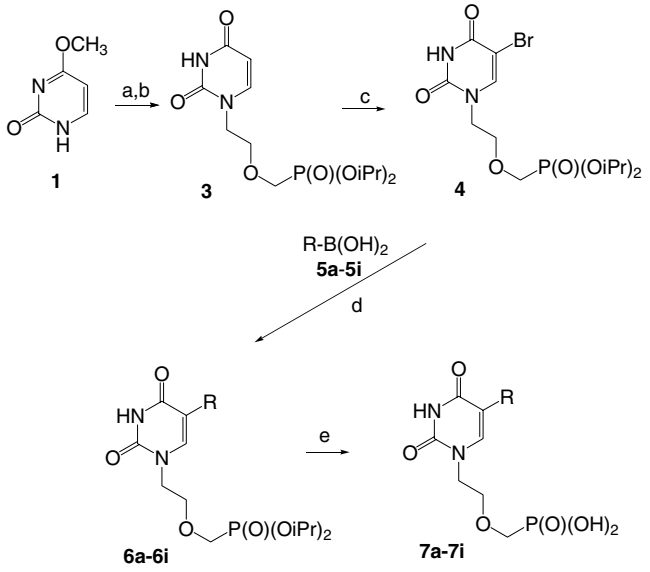
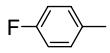
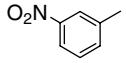
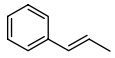
introduction of different aryl substituents bearing electron-withdrawing groups, heteroatoms or other conjugated moieties such as naphthyl, 2-phenylvinyl, 4-fluorophenyl, 3-nitrophenyl, 3-furyl, 3-thienyl, pyridin-3- and 4-yl. Efficient arylation methods are usually based on Pd-catalyzed Stille coupling reactions using toxic aryl(trialkyl)stannanes.[10] However, the convenience of this method is limited for biochemical or medicinal purposes owing to the difficulty in removing undesirable stannane by-products. In contrast, arylboronic acids used as arylating agents in Suzuki–Miyaura coupling reactions[10,11] have wide application due to their low toxicity. Arylation of the C-5 position of the uracil moiety has not been so far studied in detail via this process.[11] Therefore, we decided to introduce various aryl and heteroaryl groups to pyrimidine ANP derivatives using the Suzuki–Miyaura coupling reaction for the first time.

Firstly, we prepared the 5-bromouracil derivative **4** as a suitable building block by a simple three-step synthesis from 4-methoxypyrimidin-2(1*H*)-one[12] (**1**) (Scheme 1). Alkylation of the protected base with PME synthon **2** and further hydrolysis was carried out using our improved method[7] and afforded **3** in a good preparative yield. Likewise, bromination of the uracil moiety using *N*-bromosuccinimide in THF catalyzed with azobisisobutyronitrile gave **4** in quantitative yield. For coupling reactions we applied a number of commercial aryl and heteroaryl boronic acids **5a**–**i**. Simple transformation of 5-bromo derivative **4** to pyrimidinones **6a**–**i** took place in DMF–H_2O solution catalyzed by $Pd(PPh_3)_4$. Sodium carbonate was used for the activation of the boronic acids. Full conversion of **4** to the products took place in all cases at a temperature of ~130 °C (analysis by TLC). However, the products of arylation[13] were isolated in only 24–58% yields (see Table 1) probably due to losses caused by difficulties in purification. From this point of view, the presented cross-coupling reactions report only unoptimized yields.



Scheme 1. Reagents and conditions: (a) NaH, $CH_2ClCH_2OCH_2$-$P(O)(OiPr)_2$ (**2**), DMF, 80 °C, 49%; (b) Dowex 50 (H^+), 90% aq MeOH, 91%; (c) NBS, AIBN, THF, 60 °C, 99%; (d) $Pd(PPh_3)_4$, Na_2CO_3, DMF, H_2O, 130 °C; (e) $(CH_3)_3SiBr$, CH_3CN, rt.

Table 1. Reaction of 5-bromo derivative **4** with boronic acids **5a**–**i** followed by hydrolysis

Entry	R	Yield[a,b] of **6** (%)	Yield[b] of **7** (%)
a		24	75
b		44	59
c		34	48
d		30	80
e		30	63
f		58	34
g		24	72
h		38	78
i		35	58

[a] Conditions: 1.0 equiv **4**, 2 equiv **5**, 0.1 equiv Pd catalyst, base (3.3 equiv of Na_2CO_3), 6–9 h, 130 °C; 8:1 DMF/H_2O.
[b] All products were isolated and characterized by NMR and MS spectroscopy; yields are unoptimized.

On the other hand, this method can be considered as a significant tool with regard to its simplicity to prepare sufficient quantities of final products by further treatment with bromotrimethylsilane followed by hydrolysis[14] and the corresponding compounds **7a**–**i** were thus obtained in good preparative yields. Therefore, this method should be amenable for the introduction of various functionalized aryl substituents.

In conclusion, we have developed a simple alternative method for the rapid preparation of 5-aryl substituted pyrimidine ANPs as potential multisubstrate inhibitors of TP. The inhibitory potency of all the synthesized compounds together with their potential modified hydrophobic effects will be investigated in further research.

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13. *A typical procedure for Suzuki–Miyaura coupling of 2-bromouracil derivative* **4** *with arylboronic acids*: A mixture of compound **4** (700 mg, 1.69 mmol), aryl boronic acid **5b** (379 mg, 3.39 mmol), $Pd(PPh_3)_4$ (200 mg, 0.17 mmol) and sodium carbonate (593 mg, 5.59 mmol) in DMF (40 mL) and degassed H_2O (5 mL) was heated (~130 °C, oil bath) under argon for 6 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 (chloroform/methanol 20:1) to give 300 mg (44% yield) of **6b** as a yellow amorphous solid. IR: ν_{max} ($CHCl_3$) 3404, 3164, 2985, 1721, 1712, 1701, 1643, 1432, 1575, 1466, 1387, 1376, 1237, 1159, 1104, 1078, 1023, 1016, 999, 886 cm^{-1}. For $C_{17}H_{25}N_2O_7P$ (400.4) calcd: C, 51.00; H, 6.29; N, 7.00. Found: C, 50.82; H, 6.28; N, 6.97. FABHRMS: For $C_{17}H_{26}N_2O_7P$: Found: 401.1477. Calcd: 401.1481. FABMS, *m/z*: 401 $[MH]^+$ (61). ^1H NMR (400 MHz, $CDCl_3$): 1.23 and 1.26 (2 × d, 2 × 6H, $J_{vic} = 6.2$, $(CH_3)_2CH$); 3.75 (d, 2H, $J_{H,P} = 8.4$, CH_2P); 3.86 (t, 2H, $J_{vic} = 4.8$, CH_2O); 4.04 (m, 2H, CH_2N); 4.69 (dh, 2H, $J_{H,P} = 7.6$, $J_{vic} = 6.2$, $CH(CH_3)_2$); 6.45 (dd, 1H, $J_{4,3} = 3.4$, $J_{4,5} = 1.8$, H-4-fur); 7.03 (dd, 1H, $J_{3,4} = 3.4$, $J_{3,5} = 0.8$, H-3-fur); 7.35 (dd, 1H, $J_{5,4} = 1.8$, $J_{5,3} = 0.8$, H-5-fur); 7.80 (s, 1H, H-6); 8.78 (br s, 1H, NH). ^{13}C NMR (100.6 MHz, $CDCl_3$): 23.84 and 23.92 (d, $J_{C,P} = 4$, $(CH_3)_2CH$); 49.04 (CH_2N); 66.24 (d, $J_{C,P} = 168$, CH_2P); 70.78 (d, $J_{C,P} = 11$, CH_2O); 71.21 (d, $J_{C,P} = 7$, $CH(CH_3)_2$); 106.41 (C-5); 109.23 (CH-3-fur); 111.84 (CH-4-fur); 139.64 (CH-6); 141.01(CH-5-fur); 145.64 (C-2-fur); 149.67 (C-2); 160.21 (C-4).
14. *A typical procedure for the deprotection of 5-aryluracil-substituted ANPs*: A mixture of **6b** (269 mg, 0.67 mmol) and bromotrimethylsilane (1.03 g, 6.70 mmol) in acetonitrile (15 mL) was stirred overnight at room temperature. The mixture was concentrated and then codistilled with water (2 × 2 mL). The residue was heated with Dowex 50 × 8 (H^+form) (2 mL) in water (15 mL) at 70 °C for 1 h. The mixture was then cooled to room temperature and filtered. The filtrate was concentrated and the residue was purified on DEAE-Sephadex (Cl^-, 0–0.4 M TEAB) with subsequent deionization of the product on activated charcoal with water. The product was eluted with 12% aqueous ammonia/methanol (1:1). The residue was lyophilized to give 125 mg (59% yield) of **7b** as a white amorphous solid. IR: ν_{max} ($CHCl_3$) 3235, 1705, 1686, 1638, 1516, 1431, 3161, 1572, 1160, 1083, 1108 cm^{-1}. FABHRMS: For $C_{11}H_{14}N_2O_7P$: Found: 317.0544. Calcd: 317.0539. FABMS, *m/z*: 317 $[MH]^+$ (28). ^1H NMR (400 MHz, D_2O, $ref_{dioxane} = 3.75$ ppm): 3.66 (d, 2H, $J_{H,P} = 8.5$, CH_2P); 3.87 (t, 2H, $J_{vic} = 5.2$, CH_2O); 4.05 (m, 2H, CH_2N); 6.54 (dd, 1H, $J_{4,3} = 3.4$, $J_{4,5} = 1.9$, H-4-fur); 6.83 (dd, 1H, $J_{3,4} = 3.4$, $J_{3,5} = 0.8$, H-3-fur); 7.53 (dd, 1H, $J_{5,4} = 1.9$, $J_{5,3} = 0.8$, H-5-fur); 8.05 (s, 1H, H-6). ^{13}C NMR (100.6 MHz, D_2O, $ref_{dioxane} = 69.3$ ppm): 51.12 (CH_2N); 69.73 (d, $J_{C,P} = 156$, CH_2P); 72.72 (d, $J_{C,P} = 11$, CH_2O); 109.07 (C-5); 111.24 (CH-3-fur); 114.29 (CH-4-fur); 144.60 (CH-6); 144.95 (CH-5-fur); 148.33 (C-2-fur); 154.10 (C-2); 165.65 (C-4). ^{31}P NMR (100.6 MHz, D_2O): 16.36.