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SYNTHESIS OF RIBOFURANOSYL GLYCOSIDES OF ECHIGUANINES A AND B, INHIBITORS OF PHOSPHATIDYLINOSITOL 4-KINASE

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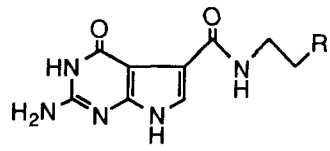
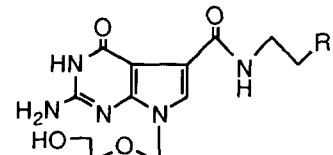
Abstract: The synthesis of ribofuranosyl glycosides of echiguanines A and B, PI 4-kinase inhibitors, was achieved from 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine and 2,3-*O*-isopropylidene-5-*O*-(*t*-butyl) dimethylsilyl-α-D-ribofuranosyl chloride. The ribofuranosyl echiguanine A weakly inhibited PI 4-kinase.

A variety of mitogens [1] and oncogenes [2,3] are known to activate intracellular phosphatidylinositol turnover. Phosphatidylinositol 4-kinase (PI 4-kinase) is involved in the phosphatidylinositol turnover pathway and may be important for the regulation of phosphatidylinositol 4,5-bisphosphate levels. Recently, one of us reported the isolation of two novel and potent inhibitors of PI 4-kinase derived from the A431 cell membrane, echiguanines A **1** and B **2**, from the fermentation broth of *Streptomyces*.[4] Although echiguanines are the naturally occurring aglycons of 7-deazaguanine nucleoside analogs, their ribofuranosyl glycosides have not yet been isolated. Also, echiguanines did not inhibit PI 4-kinase in cultured cells, possibly because of poor permeability, and their glycosides may be more easily transported. Therefore, we directed our attention to the preparation of glycosides of echiguanines A and B. In this report, we describe the synthesis of ribofuranosyl echiguanines A **3** and B **4**, and their inhibitory activities against PI 4-kinase.



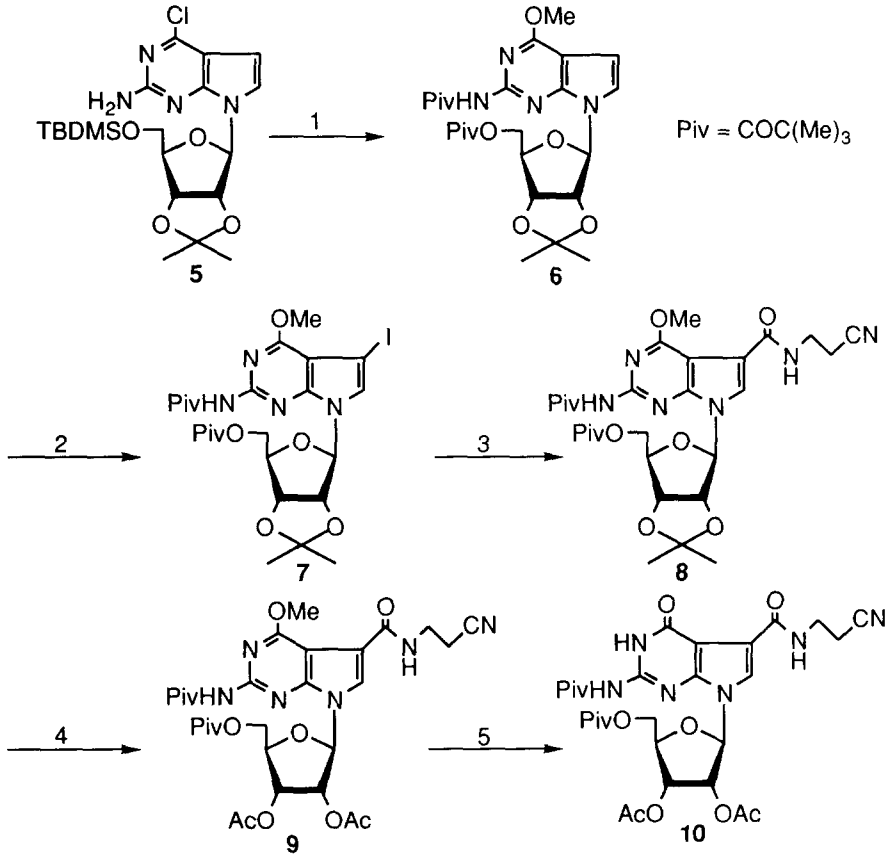
1. R = $C(=NH)NH_2$ Echiguanine A
2. R = CH_2NH_2 Echiguanine B



3. R = $C(=NH)NH_2$
4. R = CH_2NH_2

Chemistry:

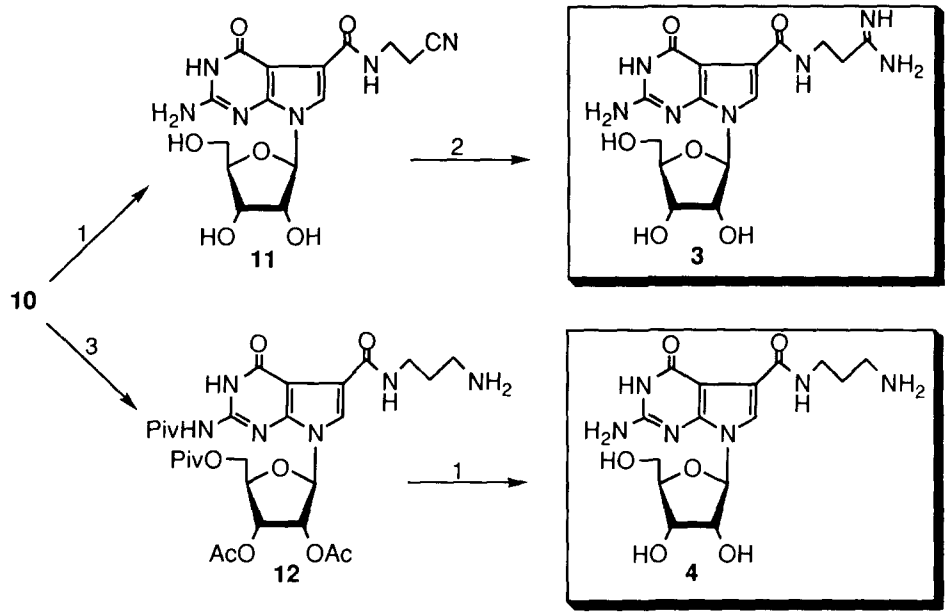
As shown in Scheme 1, the synthesis of ribofuranosyl glycosides of **1** and **2** began with the readily available 2-amino-4-chloro-7-[2,3-*O*-isopropylidene-5-*O*-(*t*-butyl)dimethylsilyl-β-D-ribofuranosyl]pyrrolo[2,3-*d*]pyrimidine **5** prepared from 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine and 2,3-*O*-isopropylidene-5-*O*-(*t*-butyl)dimethylsilyl -α-D-ribofuranosyl chloride.[5]



Scheme 1 *Reagents and Conditions*: 1) a: 1N-NaOMe, MeOH, 70 °C, 4.5 h; b: nBu_4NF, THF, rt, 1 h; c: pivaloyl chloride, pyridine, rt, 14 h; 2) NIS, DMF, rt, 25 h; 3) $H_2NCH_2CH_2CN$, CO, $PdCl_2(Ph_3P)_2$, DMF, 80 °C, 2 h; 4) a: CF_3COOH-H_2O (9:1), 0 °C, 1.5 h; b: Ac_2O, pyridine, rt, 20 h; 5) TMSI, CH_3CN, rt, 1 h, then reflux, 4 h.

Compound **5** was first converted to dipivalate **6** *via* a three-step sequence for an overall 78 % yield: 1) treatment with NaOMe in MeOH to change the C-4 chloro group into a methoxy group, 2) desilylation with nBu_4NF, 3) acylation with pivaloyl chloride. Reaction of **6** with *N*-iodosuccinimide in DMF afforded 7-iodo-7-deazapurine **7** in 81 % yield as the sole regioisomer.[6] The cyanoethylcarbamoylation of **7** by the palladium-catalyzed carbon-carbon bond-forming reaction was performed by use of the protocol of Shih and Hu.[7,8] When **7** was treated with cyanoethylamine under a carbon monoxide atmosphere in the presence of bis-(triphenylphosphine)palladium (II) chloride, cyanoethylamide **8** was obtained in 95 % yield. At this stage, the

isopropylidene protecting group of **8** was altered into two acetyl groups for the sake of future reactions. Hydrolysis of **8** with 90 % trifluoroacetic acid followed by acetylation with acetic anhydride gave diacetate **9** in 70 % overall yield. The key compound **10** as a common intermediate for the synthesis of **3** and **4** could be obtained in 95 % yield by cleavage of the ether linkage of **9** with trimethylsilyl iodide[9] in refluxing CH_3CN.



Scheme 2 *Reagents and Conditions*: 1) NH_3, MeOH, sealed tube, 70 °C, 72 h; 2) a: HCl gas, anhydrous EtOH, 0 °C, 20 h; b: NH_3, anhydrous EtOH, sealed tube, rt, 20 h; 3) PtO_2, H_2, AcOH, rt, 20 h.

In the preparation of ribofuranosyl echiguanine A **3**, compound **10** was first transformed into the deprotected cyanoethylamide **11** in 77 % yield by heating in methanolic ammonia at 70 °C for 72 h. Then, the cyano group of **11** was converted to the amidinoethyl side chain by treatment with anhydrous ethanolic hydrogen chloride at 0 °C to give the corresponding imino ethyl ether, which was then subjected to ammonolysis with anhydrous ethanolic ammonia to afford the target compound **3**[10] in 27 % overall yield. For the preparation of ribofuranosyl echiguanine B **4**, compound **10** was hydrogenated with PtO_2 as a catalyst in acetic acid to 3-aminopropylamide **12** in 74 % yield, which was subsequently deprotected by heating in methanolic ammonia to provide the target compound **4**[11] in 47 % yield (Scheme 2).

Biological activities:

The PI 4-kinase activity was assayed according to the protocol described earlier.[4] Although echiguanine A showed potent inhibition against PI 4-kinase with an IC_{50} value of 0.03 μg/ml as reported before[4], the ribofuranosyl compound **3** only weakly inhibited the enzyme, as shown in Fig. 1, and **4** did not, to any extent, inhibit the enzyme even at 100 μg/ml (data not shown). Toyokamycin and adenosine having ribofuranosides inhibit PI 4-kinase.[12] Several 7-substituted echiguanine analogs with or without ribofuranosides are being synthesized for increasing the activity and for study of the structure-activity relationship.

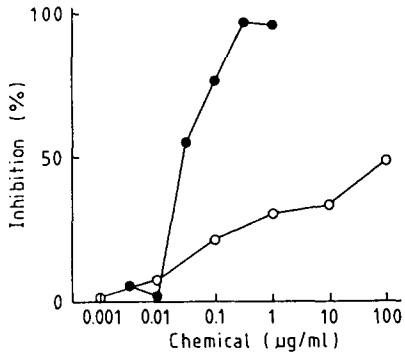


Fig. 1 Inhibition of PI 4-kinase by **3**. The membrane fraction of A431 cells was incubated with γ-^{32}P-ATP and **3** (○) or **1** (●) for 10 min at 20 °C. The values are means of duplicate determinations. Each difference was smaller than 10%.

In summary, we have devised methods for the synthesis of ribofuranosyl echiguanines A **3** and B **4** from the easily obtainable 2-amino-4-chloro-7-[2,3-*O*-isopropylidene-5-*O*-(*t*-butyl)dimethylsilyl-β-D-ribofuranosyl] pyrrolo[2,3-*d*]pyrimidine. Unexpectedly, ribofuranosylation of natural compounds **1** and **2** diminished their inhibitory activities against PI 4-kinase.

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10. Selected spectroscopic data for **3**: colorless foam as HCl salt, ^{1}H NMR (400 MHz, D_2O) d 2.64 (2H, t, J=6.6 Hz), 3.62 (2H, t, J=6.6 Hz), 3.68 (1H, dd, J=12.6 and 4.4 Hz), 3.76 (1H, dd, J=12.6 and 3.1 Hz), 4.07 (1H, ddd, J=3.7, 3.1 and 4.4 Hz), 4.23 (1H, dd, J=4.5 and 3.7 Hz), 4.46 (1H, dd, J=5.8 and 4.5 Hz), 5.86 (1H, d, J=5.8 Hz), and 7.52 (1H, s); ^{13}C NMR (100.5 MHz, D_2O) d 33.7, 37.4, 62.4, 71.3, 74.8, 85.8, 88.4, 97.9, 114.5, 125.8, 153.3, 154.0, 162.1, 166.1, and 170.0; HRMS m/z 431.1320 calcd for $C_{15}H_{22}N_7O_6Cl$ (M^++HCl), found 431.1340.
11. Selected spectroscopic data for **4**: colorless foam as free amine form, ^{1}H NMR (400 MHz, D_2O) d 1.67 (2H, m), 2.85 (2H, t, J=7.7 Hz), 3.17 (2H, t, J=6.4 Hz), 3.58 (1H, dd, J=12.0 and 4.0 Hz), 3.67 (1H, dd, J=12.0 and 3.2 Hz), 3.96 (1H, m), 4.11 (1H, dd, J=5.1 and 4.0 Hz), 4.31 (1H, dd, J=5.5 and 5.1 Hz), 5.68 (1H, d, J=5.5 Hz), and 7.31 (1H, s); ^{13}C NMR (100.5 MHz, D_2O + DCl) d 27.4, 37.2, 38.0, 62.0, 71.4, 75.0, 86.6, 90.8, 99.1, 115.3, 126.4, 151.7 (x2), 160.4, and 165.2; HRMS m/z (as HCl salt) 419.1446 calcd for $C_{15}H_{24}N_6O_6Cl$ (M^++1+HCl), found 419.1447.
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