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A solid-phase approach to novel purine and nucleoside analogs

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Abstract—This paper describes a method for the preparation of purine analogs using the solid-phase approach. Nucleoside bases were constructed on Merrifield resin by sequential displacement of purine dichloride with amines, and after detachment, the purine analogs were condensed with D,L-ribofuranoside compounds by the Vorbrüggen method. Thereof, L-ribofuranoside was prepared from L-arabinose via the selective oxidation–reduction procedure of the 2-OH group. Some compounds exhibited moderate activity against HIV-1 in PBM cells.

1. Introduction

Drug discovery for antiviral chemotherapy during the last 30 years has provided effective treatments for numerous viral diseases. In particular, the fight against AIDS has prompted the development of new classes of antiviral drugs, which have provided a considerable decrease in HIV-associated morbidity and mortality as well as improvement in the quality of life of AIDS patients. Although new, promising targets are being identified, nucleoside analogs remain the cornerstone of antiviral therapy. Currently, seven of the sixteen drugs available for the treatment of AIDS (AZT,[1] ddC,[2] ddI,[3] d4T,[4] 3TC,[5] abacavir,[6,7] and tenofovir[8]) belong to the category of nucleoside reverse transcriptase inhibitors (NRTIs). Among NRTIs for AIDS, 3TC is also the drug of choice for the treatment of hepatitis B virus infection.

The efficacy of nucleoside analogs depends on their ability to mimic natural nucleosides, thus interacting with viral and/or cellular enzymes and inhibiting critical processes in the metabolism of nucleic acids. For this reason, it had been believed that only D-enantiomer analogs, which possess the same stereochemistry as natural nucleosides, could effectively interact and inhibit metabolic enzymes. With the discovery of the antiviral activity of L-oxathiolane nucleosides, which eventually led to the 3TC as a therapeutic agent and the development of FTC and L-FMAU among others,[9] this assumption has been proven not to be true. Favorable features of L-nucleosides include an antiviral activity comparable to and sometimes even greater than that of their D-counterparts, a more favorable toxicological profile, and a greater metabolic stability.

This paper reports an efficient preparation of novel purine nucleoside analogs, which allows for the simultaneous modification of both purine and carbohydrate units. Purine can be synthesized on solid support for its potential inhibition of target nucleotide-binding proteins, which play a significant role in many biological processes.[10–14] Novel purine analogs with 2,6-disubstituents can react with D- or L-ribofuranoside to provide the corresponding nucleoside derivatives.

2. Results and discussion

The method for synthesis of novel purine analogs using solid-phase chemistry is shown in Scheme 1. The starting material for the THP linker used in Ellman's synthesis to prepare 3,4-dihydro-2*H*-pyran-2-methanol is no longer commercially available, but this highly versatile linker can be easily prepared.[15] 3,4-Dihydro-2*H*-pyran-2-methanol was converted to its sodium alkoxide

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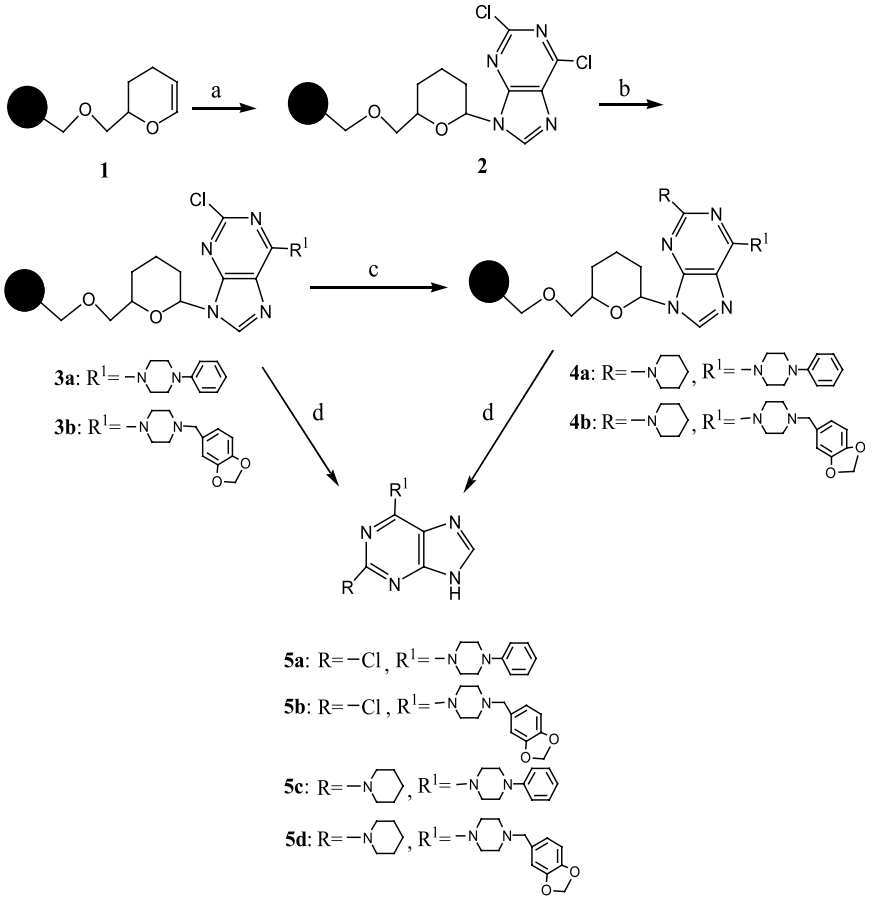
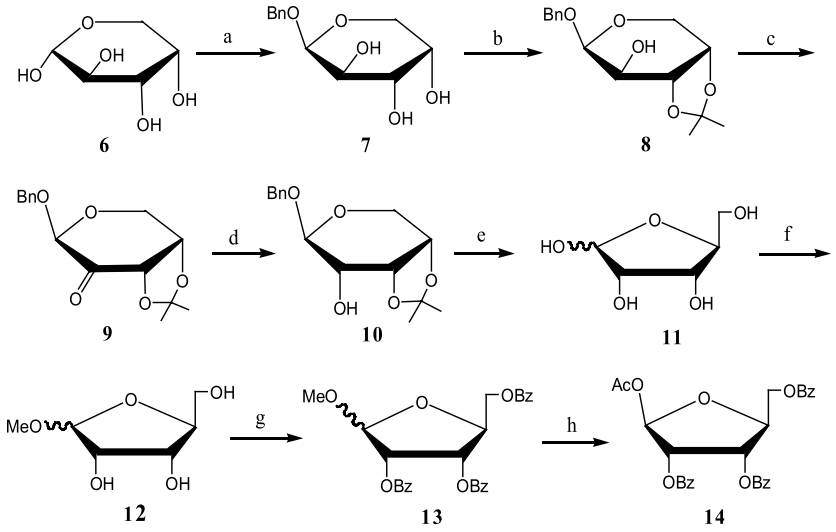


Scheme 1. Solid-phase synthesis of purine analogs. Reagents and conditions: (a) 2,6-dichloropurine, CSA, CH_2Cl_2, 60 °C; (b) 1-phenyl piperazine or 1-piperonylpiperazine, *n*-BuOH, Et_3N, 80 °C; (c) piperidine, 102 °C; (d) TFA/CH_2Cl_2.

using NaH in DMF at 25 °C for 30 min and then was treated with Merrifield chloride resin to give compound **1**. We routinely performed this reaction with excellent results regardless of the initial chlorine loading level. The attachment of 2,6-dichloropurine onto DHP resin **1** was accomplished using camphorsulfonic acid (CSA) in 1,2-dichloroethane at 60 °C for 30 h. The excess 2,6-dichloropurine was removed by filtration (the unreacted materials can be recovered simply by extraction and chromatography). Differential reactivity of the two chlorine atoms on 2,6-dichloropurine derivative **2** with nucleophilics allows for the sequential introduction of different substituents. The more reactive, 6-chloro position, was easily displaced with 1-phenyl piperazine or 1-piperonyl piperazine in triethylamine and *n*-butanol at 80 °C for 3 h to give **3a** and **3b**, respectively. The purity of the crude product was 98%. The 2-chloro substituent was less reactive and required more forcing reaction conditions. This was accomplished using the piperidine as solvent and heating the resin to 102 °C for 4 h to give **4a** and **4b**. The final product was cleaved from the resin using the standard protocol of acidic hydrolysis. The treatment of the resins **3a**, **3b**, **4a**, and **4b** with dichloromethane and trifluoroacetic acid (8:1) at room temperature for 10 min and subsequent washing with methanol gave compounds **5a–d** (Scheme 1).

L-Ribofuranoside was prepared from L-arabinose via the following steps according to Ref. 16. L-Arabinose reacted with benzyl alcohol (saturated with HCl (gas)) at rt for 10 h to give compound **7** as a white solid (94%). Compound **7** reacted with acetone in the presence of *p*-TsOH · H_2O at rt for 2 h to give compound **8** as a yellowish syrup, which was used for the next reaction without further purification. Compound **8** was oxidized with PDC in dichloromethane and acetic anhydride to give compound **9** as a syrup. Then the product was reduced with $NaBH_4$ at −20 °C over 3 h to give compound **10**. Compound **10** was hydrolyzed with 4% CF_3COOH for ~8 h to give L-ribose **11** as a yellowish syrup. The product was dissolved in 1% HCl (gas) in methanol at rt for 2 h to produce **12** as a yellowish syrup. Compound **12** reacted with benzoyl chloride in pyridine at rt for 8 h to give compound **13** as a yellowish syrup. Compound **13** was dissolved in acetic acid, acetic anhydride, and catalyzed by concd H_2SO_4 to give the product 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose (**14**) as a white solid, which was used in the following condensations with purines (Scheme 2).

A series of nucleoside analogs was then synthesized by coupling the disubstituted purines with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose or 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose by the Vorbrüggen method



Scheme 2. Synthesis of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose. Reagents and conditions: (a) HCl (g)/BnOH; (b) DMP, acetone; (c) PDC, Ac_2O, CH_2Cl_2, reflux; (d) $NaBH_4$, MeOH; (e) 4% CF_3COOH, reflux; (f) 1% HCl (g)/MeOH; (g) BzCl, pyridine; (h) AcOH, Ac_2O, concd H_2SO_4.

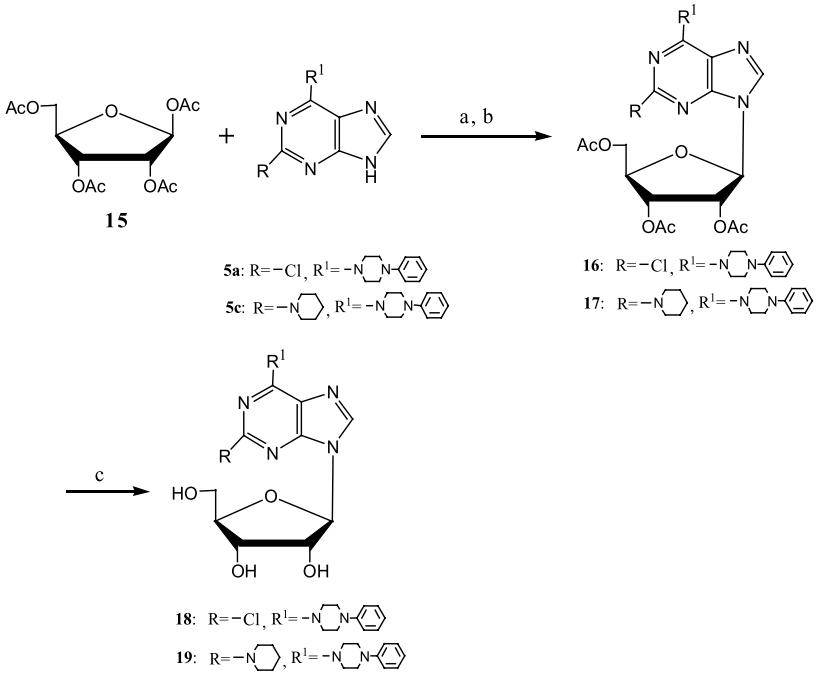
(Schemes 3 and 4). A Lewis acid can be used for the activation of the sugar portion in the condensation with easily prepared silylated bases. This one-step procedure produced the desired products in good yield.

All the synthesized nucleosides were evaluated for antiviral activity against human immunodeficiency virus type-1 (HIV-1) (Table 1). The compounds **22** and **23** showed moderate activity against HIV-1 in PBM cells (9.8, 3.6 μM) and no other compound showed any significant activity or toxicity up to 100 μM concentration against HIV-1.

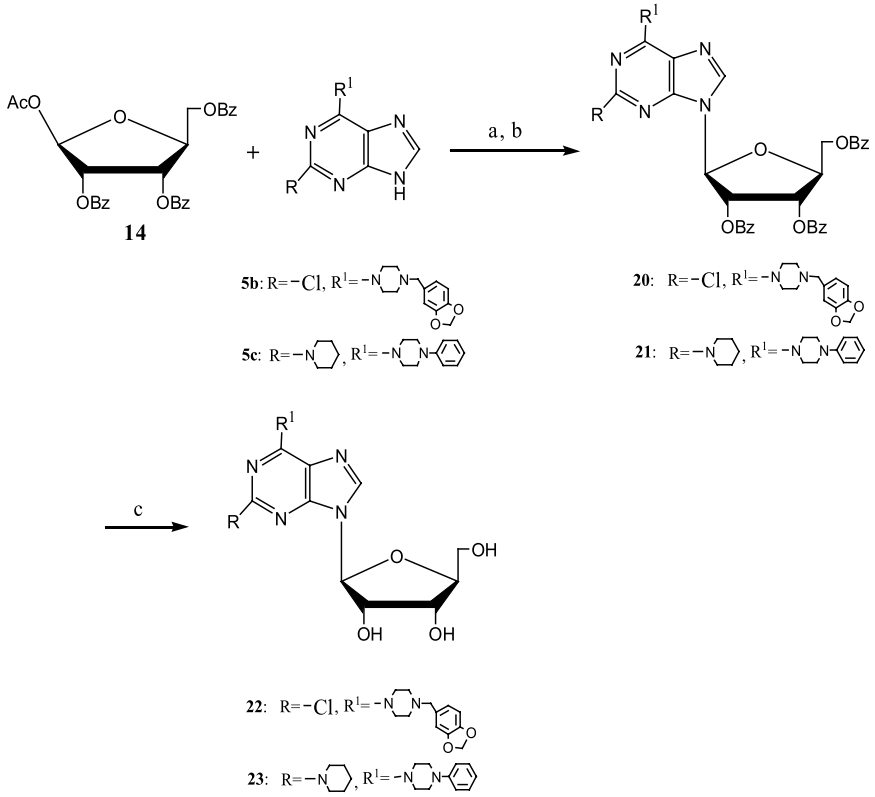
3. Experimental

3.1. Materials

Melting points were recorded with an XT-4 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a BrukerAV-300, and are referenced to internal tetramethylsilane (TMS) at 0.0 ppm, chemical shifts being reported in parts per million (δ). IR spectra were obtained on a Shimadzu-8700 infrared spectrometer (ν_{max} in cm^{-1}). Elemental analyses were performed with a Carlo-Erba 1106 C, H, and N ana-



Scheme 3. Synthesis of novel D-purine nucleoside analogs. Reagents and conditions: (a) HMDS, $(NH_4)SO_4$, reflux; (b) TMSOTf, DCE, rt; (c) NH_3/CH_3OH.



Scheme 4. Synthesis of novel L-purine nucleoside analogs. Reagents and conditions: (a) HMDS, $(NH_4)SO_4$, reflux; (b) TMSOTf, DCE, rt; (c) NH_3/CH_3OH.

Table 1. Anti-HIV-1 activities of L-nucleosides

Compound	HIV-1 EC_{50} (μM)	Toxicity (cell count) PBM (IC_{50}, μM)
AZT	0.004	>100
16	>100	>100
17	>100	>100
18	>100	>100
19	>100	>100
20	>100	>100
21	9.8	>100
22	>100	>100
23	3.6	>100

lyzer. UV spectra were obtained on a Shimadzu-2100 spectrophotometer. LC–MS spectra were measured on an Agilent MSD-1100 ESI-MS/MS System. TLC was performed on silica gel GF_{254} precoated plates.

3.1.1. ((3,4-Dihydro-2*H*-pyran-2-yl-methyl)oxy)-linked Merrifield resin (1). The Merrifield resin (**1**) was prepared (yield 92%) according to Ref. 15.

3.1.2. Support bound 2,6-dichloropurine (2). A solution of 2,6-dichloropurine (10 g, 53 mmol), resin **1** (17.7 g, 13.5 mmol), and camphorsulfonic acid (2.95 g, 12.5 mmol) in 1,2-dichloroethane (200 ml) was stirred at 60 °C for 30 h. The mixture was filtered, the resin was collected on a Buchner funnel, washed with CH_2Cl_2 (3 × 50 ml), DMF (3 × 50 ml), CH_2Cl_2 (3 × 50 ml), and dried in a vacuum oven at 60 °C for overnight to give the desired product **2** (22.3 g). The loading level of 2,6-dichloropurine was determined to be 1.37 mmol/g by gravimetric methods.

3.1.3. Support bound 2-chloro-6-(1-(4-phenyl)piperazine)-purine (3a) and support bound 2-chloro-6-(1-(4-piperonyl)piperazine)purine (3b). A suspension of resin **2** (5 g, 6.85 mmol), 1-phenyl piperazine (5.55 g, 34.25 mmol), and triethylamine (5.5 ml, 35 mmol) in *n*-butanol (1000 ml) was heated to 80 °C for 3 h. The mixture was cooled to rt and filtered, the resin was washed with methanol (3 × 30 ml), CH_2Cl_2 (3 × 30 ml), and dried in a vacuum oven at 60 °C for overnight to give the desired product **3a** (5.9 g).

Using the same procedure, resin **2** was made to react with 1-piperonyl piperazine in the presence of triethylamine and *n*-butanol to give the desired product **3b**.

3.1.4. Support bound 2-piperidine-6-(1-(4-phenyl)piperazine)purine (4a) and support bound 2-piperidine-6-(1-(4-piperonyl)piperazine)purine (4b). A suspension of **3a** (2.5 g) in piperdine (10 ml) was heated to 102 °C for 4 h. The mixture was then cooled and filtered, the resin was washed with methanol (3 × 30 ml), DMF (3 × 30 ml), methanol (3 × 30 ml), and CH_2Cl_2 (3 × 30 ml), and dried in a vacuum oven at 60 °C for overnight to give the desired product **4a** (4.6 g).

A procedure similar to that used for **4a** was used to get the desired product **4b**.

3.1.5. 2-Chloro-6-(1-(4-phenyl)piperazine)purine (5a). Resin **3a** (2.5 g) was suspended in CH_2Cl_2/TFA (8:1, 20 ml) and stirred for 10 min at rt. The solution was filtered and the resin was washed with methanol (2 × 10 ml). The filtrate was concentrated at reduced pressure to give the crude product **5a** as a white solid (1.02 g). An analytical sample was purified by silica gel chromatography (50% ethyl acetate/petroleum ether). Compound **5a**: mp 232–234 °C. UV (MeOH): λ_{max} 255.5 nm, ^{1}H NMR (acetone-d_6) δ 8.11 (s, 1H, H-8), 7.30–6.85 (m, 5H, Ar–H), 3.94 (m, 4H, CH_2CH_2), 3.38–3.31 (m, 4H, CH_2CH_2). MS m/z = 315 $[M+H]^+$. Anal. Calcd for $C_{15}H_{15}N_6Cl$: C, 57.32; H, 4.78; N, 26.75. Found: C, 57.24; H, 4.80; N, 26.70.

3.1.6. 2-Chloro-6-(1-(4-piperonyl)piperazine)purine (5b). Cleavage of resin **3b** by using a procedure similar to that used for **5a** gave product **5b** as a white solid. Mp 238–240 °C UV (MeOH) λ_{max} 278.5 nm; ^{1}H NMR (acetone-d_6) δ 7.98 (s, 1H, H-8), 7.17–6.90 (m, 3H, Ar–H), 6.07 (s, 2H, –CH_2–), 4.49 (s, 2H, –CH_2–), 3.84 (m, 4H, 2× –CH_2–), 3.58 (m, 4H, 2× –CH_2–); MS m/z = 373 $[M+H^+]$. Anal. Calcd for $C_{17}H_{17}N_6Cl$: C, 54.84; H, 4.57; N, 22.58. Found: C, 54.77; H, 4.60; N, 22.54.

3.1.7. 2-Piperidine-6-(1-(4-phenyl)piperazine)purine (5c). Cleavage of resin **4a** by using a procedure similar to that used for **5a** gave product **5c** as a white solid. Mp 183–185 °C; UV (MeOH) λ_{max} 277.0 nm; ^{1}H NMR (acetone-d_6) δ 7.94 (s, 1H, H-8), 7.30–6.83 (m, 5H, Ar–H), 4.55 (br s, 4H, 2× –CH_2–), 3.84 (m, 4H, 2× –CH_2–), 3.38–3.31 (4H, m, 2× –CH_2), 1.69–1.72 (6H, m, 3× –CH_2–); MS m/z = 364 $[M+H^+]$. Anal. Calcd for $C_{20}H_{25}N_7$: C, 66.11; H, 6.89; N, 27.00. Found: C, 66.23; H, 6.81; N, 26.96.

3.1.8. 2-Piperidine-6-(1-(4-piperonyl)piperazine)purine (5d). Cleavage of resin **4b** by using a procedure similar to that used for **5a** gave product **5d** as a white solid. Mp 222–224 °C. UV (MeOH): λ_{max} 258.5 nm, ^{1}H NMR (acetone-d_6) δ 7.72 (s, 1H, H-8), 6.88–6.74 (m, 3H, Ar–H), 6.00 (s, 2H, –CH_2–), 4.64 (s, 2H, –CH_2–), 4.13–4.03 (m, 4H, 2× –CH_2–), 3.64 (m, 4H, 2× –CH_2–), 2.52–2.42 (m, 4H, 2× –CH_2–), 1.55–1.18 (m, 6H, 3× –CH_2–). MS, m/z = 422 $[M+H]^+$. Anal. Calcd for $C_{22}H_{27}N_7$: C, 62.71; H, 6.41; N, 23.28. Found: C, 62.69; H, 6.46; N, 23.26.

3.1.9. 1-*O*-Benzyl-β-L-ribofuranoside (7). Benzyl alcohol (50 ml) was saturated with hydrogen chloride for 40 min at 0 °C, to which L-arabinose (10 g, 0.067 mol) was added and the mixture was stirred at rt for 10 h. During this time, compound **7** precipitated. EtOAc (75 ml) was slowly added while stirring for additional precipitation. The precipitate was filtered and washed with EtOAc, and dried in air, to give the product **7** as a white solid (15.36 g, 96%). Mp 170–171 °C; ^{1}H NMR (DMSO-d_6) δ 7.39–7.27 (m, 5H, Ar–H), 4.75 (d, J = 1.8 Hz, 1H, H-1), 4.68–4.43 (q, J = 12.44 Hz, 2H, $PhCH_2O$–), 3.72–3.43 (m, 5H, H-2, H-3, H-4, and H-5).

3.1.10. 1-*O*-Benzyl-3,4-*O*-isopropylidene-β-L-riboside (10). A mixture of 1-*O*-benzyl-β-L-riboside **7** (20 g, 0.083 mol), 2,2-dimethoxypropane (24 ml, 0.195 mol) and *p*-TsOH·H_2O (0.4 g, 2 mmol) in acetone (200 ml) was stirred at rt for 2 h. The reaction mixture was neutralized with triethylamine and evaporated under reduced pressure to give compound **8** as a yellowish syrup, which was used for the next reaction without further purification.

To a mixture of compound **8** and pyridinium dichromate (PDC, 24 g, 0.063 mol) in CH_2Cl_2 (200 ml), Ac_2O (24 ml, 0.254 mol) was added at 0 °C and then the mixture was refluxed until the starting material disappeared. The solvent was removed under reduced pressure to 1/3 of its original volume and the residue was poured into EtOAc (150 ml) with vigorous stirring using a mechanical stirrer, which was filtered through a Celite pad (20 cm). The filter cake was thoroughly washed with EtOAc. The blackish combined filtrate was filtered again through a silica gel column. The silica gel was washed with EtOAc until no more of compound **9** could be detected on TLC. The combined clear filtrate was evaporated to give compound **9** as a syrup.

The syrup **9** was dissolved in 200 ml methanol and cooled to −20 °C. To the solution, $NaBH_4$ (4 g, 0.106 mol) was slowly added over 3 h at −20 °C. After completion of the reaction, the solution was neutralized with acetic acid and evaporated under reduced pressure to a white solid residue, which was partitioned between EtOAc (100 ml). The combined organic layer was washed with brine (20 ml), dried with $MgSO_4$, and then evaporated to yield a white solid, which was recrystallized from hexane to give compound **10** (13.5 g, 58% from compound **7**) as white crystals. Mp 79–80 °C; $[\alpha]^{25}$ +143° (*c* 0.7, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.36–7.26 (m, 5H, Ar–H), 4.86 (d, J = 5.40 Hz, 1H, H-1), 4.56–4.28 (q, J = 11.8 Hz, 2H, $PhCH_2O$–), 4.50 (m, 1H, H-2), 3.86–3.37 (m, 2H, H-5), 3.74 (m, 2H, H-3 and H-4), 1.55 (s, 3H), 1.37 (s, 3H).

3.1.11. 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose (14). Compound **10** (20 g, 71.7 mmol) in 4% CF_3COOH (100 ml) was refluxed until the starting material and the intermediate (1-*O*-benzyl derivative) disappeared. The reaction mixture was cooled to rt and washed with CH_2Cl_2 (4 × 50 ml) to remove benzyl alcohol. The aqueous layer was evaporated in vacuo and coevaporated with toluene to give compound **11** as a yellowish syrup, which was completely dried under high vacuum to remove a trace amount of water.

Compound **11** was dissolved in 1% HCl (gas) of methanol solution (200 ml), the mixture was stirred at rt for 2 h, then neutralized with pyridine (18 ml), and concentrated in vacuo at 30–35 °C to give a yellowish syrup, which was coevaporated with pyridine to yield compound **12** as a yellowish syrup. Compound **12** was dissolved in pyridine (80 ml) and benzoyl chloride (22 ml) was added dropwise to the mixture at 0 °C. The mixture was stirred at rt for 8 h. After the reaction was almost completed, the mixture was heated at 45 °C for 1.5 h. The mixture was cooled to rt and ice was added to remove the remaining benzoyl chloride. Excess pyridine was evaporated to 1/2 volume at 35–40 °C and the

residue was dissolved in EtOAc (150 ml), which was washed with cold H_2O (50 ml), cold H_2SO_4 (3 N, 58 ml), satd $NaHCO_3$ (2 × 50 ml), and brine (2 × 50 ml). The organic layer was dried, filtered, and evaporated to afford compound **12** as a yellowish syrup.

To a solution of **12** in acetic acid (14.5 ml, 253 mol) and acetic anhydride (33 ml, 354 mmol), concd H_2SO_4 (5 ml, 90 mmol) was slowly added dropwise at 0 °C, during which crystallization occurred. The mixture was brought to rt and kept in a refrigerator overnight. The mixture was poured into ice water (70 ml) mixture and filtered, and the filter cake was washed twice with cold water. The solid was dissolved in EtOAc (200 ml), which was washed with water (50 ml), satd $NaHCO_3$ (50 ml), and brine (50 ml), respectively. The organic layer was dried and filtered, with the removal of the solvent and recrystallization of the residue from methanol to give compound **14** as a white solid (15 g, 41.5% from compound **10**). Mp 124–125 °C; ^{1}H NMR ($CDCl_3$) δ 8.09–7.32 (m, 15H, 3× Ar–H), 6.43 (s, 1H, H-1), 5.91 (dd, J = 4 and 8 Hz, 1H, H-3), 5.79 (d, J = 8 Hz, 1H, H-2), 4.49–4.81 (m, 3H, H-4 and H-5), 2.00 (s, 3H, CH_3COO).

3.1.12. 9-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-2-chloro-6-(1-(4-phenyl)piperazine)purine (16). A suspension of **5a** (0.16 g, 0.5 mmol) and ammonium sulfate (0.01 g) in hexamethyldisilazane (HMDS, 10 ml) was heated at reflux until a clear solution was obtained. The reaction mixture was cooled to room temperature, and the HMDS was removed under reduced pressure and anhydrous conditions. To the residue under nitrogen was added the 1,2,3,5-tetra-*O*-acetyl-D-ribofuranosyl (0.11 g, 0.35 mmol) in dry 1,2-dichloroethane. The reaction mixture was cooled to 0 °C, treated with trimethylsilyl triflate (TMSOTf) (0.2 ml, 1.03 mmol), and allowed to stir at 0 °C for 10 min and then 10 min at room temperature. The reaction mixture was poured into EtOAc and the organic layer was washed once with satd $NaHCO_3$ (3 × 20 ml), water (3 × 20 ml), and brine (3 × 20 ml), dried ($MgSO_4$), filtered, and concentrated. The residue was chromatographed over silica gel eluting with hexanes and hexanes/ethyl acetate (6:1 to 4:1) to give **16** (0.17 g, 82%) as a white solid. Mp 228–231 °C; UV (MeOH) λ_{max} 204.5 nm; ^{1}H NMR ($CDCl_3$) δ 7.89 (s, 1H, H-8), 7.33–6.90 (m, 5H, Ar–H), 6.20 (d, J = 5.7 Hz, 1H, 1′-H), 5.77 (t, J = 5.7 Hz, 1H, 2′-H), 5.58 (dd, 1H, J = 5.1 and 5.4 Hz, 3′-H), 4.48–4.23 (m, 7H, 4′-H, 5′-H, 2× –CH_2–), 3.30 (m, 4H, 2× –CH_2–), 2.16 (s, 6H, 2× Ac), 2.08 (s, 3H, Ac); ESI-MS/MS at m/z 595 [M+Na^+].

3.1.13. 9-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-2-piperidine-6-(1-(4-phenyl)piperazine)purine (17). Using a procedure similar to that used for **16**, the silylation of **5c** (0.18 g, 0.5 mmol) reacted with 1,2,3,5-tetra-*O*-acetyl-D-ribofuranosyl (0.11 g, 0.35 mmol) to give **17** (0.19 g, 86%) as a white solid. Mp 221–224 °C; UV(MeOH) λ_{max} 251.5 and 203.5 nm; ^{1}H NMR($CDCl_3$) δ 7.54 (s, 1H, 8-H), 7.32–6.91 (m, 5H, Ar–H), 6.13 (dd, 1H, J = 3.3, and 5.4 Hz, 2′-H), 5.95 (d, J = 3.3 Hz, 1H, 1′-H), 5.79 (t, J = 5.7 Hz, 1H, 3′-H), 4.48–4.23 (m, 7H, 4′-H, 5′-H, and 2× –CH_2–), 3.76 (m, 4H, 2× –CH_2–), 3.30 (m, 4H, 2× –CH_2–), 2.12 (s, 6H, 2× Ac), 2.07 (s, 3H, Ac); ESI-MS/MS at m/z 622 [M+H^+].

3.1.14. 9-(β-D-Ribofuranosyl)-2-chloro-6-(1-(4-phenyl)piperazine)purine (18). To a solution of **16** (0.15 g, 0.252 mmol) in MeOH (3 ml) was added saturated MeOH/NH_3 (5 ml), and the reaction mixture was stirred at rt for 24 h. After the solvent was removed under reduced pressure, the residue was purified by thin column chromatography to get the desired product **18** (99.1 mg, 88%) as a white solid. Mp 236–238 °C; UV(MeOH) λ_{max} 279.5 and 207.5 nm; ^{1}H NMR($CDCl_3$) δ: 7.81 (s, 1H, H-8), 6.95–6.89 (m, 5H, Ar–H), 6.32 (d, J = 6.6 Hz, 1H, 1′-H), 5.76 (t, J = 6.3 Hz, 1H, 2′-H), 5.02 (m, 1H, 3′-H), 4.37–4.28 (m, 4H, 2× –CH_2–), 4.15-4.03 (m, 1H, 4′-H), 3.76–3.69 (m, 2H, 5′-H), 3.27–3.22 (m, 4H, 2× –CH_2–); ESI-MS/MS at m/z 447 [M+H]$^+$; Anal. Calcd for $C_{20}H_{23}O_4N_6Cl$: C, 53.81; H, 5.16; N, 18.83. Found: C, 53.92; H, 5.25; N, 18.89.

3.1.15. 9-(β-D-Ribofuranosyl)-2-piperidine-6-(1-(4-phenyl)piperazine)purine (19). Using a procedure similar to that used for **18**, to get the product **19** (91%) as a white solid. Mp 245–248 °C; UV(MeOH) λ_{max} 297.0, 251.5 and 205.5 nm; ^{1}H NMR($CDCl_3$) δ 7.56 (s, 1H, H-8), 7.29–6.85 (m, 5H, Ar), 5.70 (d, J = 6.6 Hz, 1H, 1′-H), 4.86 (t, J = 6.3 Hz, 1H, 2′-H), 4.37 (m, 1H, 3′-H), 4.26–4.21 (m, 5H, 2× –CH_2–, 4′-H), 3.83–3.65 (m, 2H, 5′-H), 3.61 (m, 4H, 2× –CH_2–), 3.23 (m, 4H, 2× –CH_2–), 1.58 (m, 6H, 3× –CH_2–); ESI-MS/MS at m/z 496 [M+H^+]. Anal.Calcd for $C_{25}H_{33}O_4N_7$: C, 60.61; H, 6.67; N, 19.80. Found: C, 60.58; H, 6.62; N, 19.86.

3.1.16. 9-(2′,3′,5′-Tri-*O*-benzoyl-β-2-chloro-L-ribofuranosyl)-6-(1-(4-piperonyl)piperazine)purine (20). A suspension of **5b** (0.17 g, 0.5 mmol) and $(NH_4)_2SO_4$(0.01 g) in hexamethyldisilazane (HMDS, 10 ml) was heated at reflux until a clear solution was obtained. The reaction mixture was cooled to rt, and the HMDS was removed under reduced pressure and anhydrous conditions. To the residue under nitrogen was added the 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose **14** (0.177 g, 0.35 mmol) in dry 1,2-dichloroethane (DCE) (5 ml). The reaction mixture was cooled to 0 °C, treated with trimethylsilyl triflate (TMSOTf) (0.2 ml, 1.03 mmol), allowed to stir at 0 °C for 10 min, and then the mixture was brought to rt until the starting material (**14**) disappeared. The reaction mixture was poured into EtOAc and satd $NaHCO_3$ with stirring. The organic layer was washed once with satd $NaHCO_3$ (2 × 5 ml), water (2 × 5 ml), brine(2 × 5 ml), and then dried ($MgSO_4$), and concentrated. The residue was purified by TLC to give **20** (0.25 g, 88%) as a white syrup. UV (CH_3OH), λ_{max} 200.0, 225.0, 281.0 nm; ^{1}H NMR($CDCl_3$) δ 8.08–7.85 (m, 16H, H-8, and 3× Ar–H), 6.87–6.75 (m, 3H, Ar–H), 6.47 (d, J = 6.6 Hz, 1H, H-1′), 6.13 (m, 2H, H-2′, and H-3′), 5.94 (s, 2H, –OCH_2O–), 4.88–4.70 (m, 3H, H-4′, and H-5′), 4.25 (br s, 4H, 2× –CH_2–), 3.48 (s, 2H, –CH_2–), 2.56 (m, 4H, 2× –CH_2–), 2.00–1.56 (m, 6H, 3× –CH_2–); ESI-MS/MS m/z = 817 [M+H^+].

3.1.17. 9-(2′,3′,5′-Tri-*O*-benzoyl-β-L-ribofuranosyl)-2-piperidine-6-(1-(4-phenyl)piperazine)purine (21). Using a

procedure similar to that used for **20**, the silylation of **5c** (0.182 g, 0.5 mmol) reacted with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose (0.177 g, 0.35 mmol) to give **21** (0.231 g, 82%) as a white syrup. UV (CH_3OH), λ_{max} 201.0, 230.0 nm; ^1H NMR ($CDCl_3$) δ 7.99–6.85 (m, 21H, 4 × Ar–H and H-8), 6.54 (d, J = 6.3 Hz, 1H, H-1′), 6.39 (m, 1H, H-2′), 6.21 (m, 1H, H-3′), 4.81-4.64 (m, 3H, H-4′, and H-5′), 4.39 (m, 4H, 2× $–CH_2–$), 3.79 (m, 4H, 2× $–CH_2–$), 3.32 (m, 4H, 2× $–CH_2–$), 1.68–1.61 (m, 6H, 3× $–CH_2–$); ESI/MS/MS m/z = 808 [M+H$^+$].

3.1.18. 9-(β-L-Ribofuranosyl)-2-chloro-6-(1-(4-piperonyl)-piperazine)purine (22). To a solution of **20** (0.25 g, 0.306 mmol) in methanol (5 ml), which was saturated with ammonia at 0 °C, was added and stirred at rt for 48 h. After the reaction was completed, the solvent was removed under reduced pressure, the residue was purified by TLC to give the desired product **22** (139 mg, 90%) as a white solid. Mp 203–205 °C; UV (CH_3OH), λ_{max} 200.0, 218.0, 282.0 nm; ^1H NMR ($CDCl_3$) δ 7.74 (s, 1H, H-8), 6.87–6.75 (m, 3H, Ar–H), 5.96 (s, 2H, $–OCH_2O–$), 5.74 (d, J = 7.2 Hz, 1H, H-1′), 5.03 (m, 1H, H-2′), 4.37–4.22 (m, 6H, H-3′, H-4′, and 2× $–CH_2–$), 3.95–3.71 (m, 2H, H-5′), 3.48 (s, 2H, $–CH_2–$), 2.62 (br s, 4H, 2× $–CH_2–$); ESI-MS /MS m/z = 504.8 [M+H$^+$].

3.1.19. 9-(β-L-Ribofuranosyl)-2-piperidine-6-(1-(4-phenyl) piperazine)purine (23). Using a procedure similar to that used for **22** gave the desired product **23** (85%) as a white solid. Mp 245–247 °C; UV (CH_3OH), λ_{max} 201.0, 251.0, 295.0 nm; ^1H NMR (DMSO-d_6) δ 8.03 (s, 1H, H-8), 7.26–6.81 (m, 5H, Ar–H), 5.80 (d, J = 6.6 Hz, 1H, H-1′), 4.56 (m, 1H, H-2′), 4.27 (m, 4H, 2× $–CH_2–$), 4.14–3.87 (m, 2H, H-3′, and H-4′), 3.70 (m, 4H, 2× $–CH_2–$), 3.60–3.41 (m, 2H, H-5′), 3.24 (m, 4H, 2× $–CH_2–$), 1.59–1.52 (m, 6H, 3× $–CH_2–$); ESI-MS/MS m/z = 496 [M+H$^+$].

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