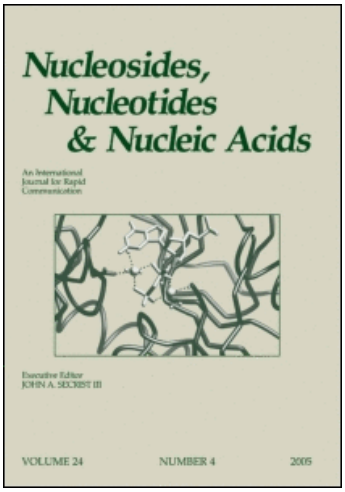

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Synthesis of Nucleoside Libraries on Solid Support. II. 2,6,8-Trisubstituted Purine Nucleosides Using 8-Bromoguanosine as Precursor†

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ABSTRACT

A series of 2,6,8-trisubstituted purine nucleoside libraries was prepared by parallel solid-phase synthesis using 8-bromoguanosine as a common synthetic precursor. Polystyrene-methoxytrityl chloride resin was linked to the N^2 or O5′ position of the guanosine analogues. 8-Bromoguanosine was derivatized at the C8 position via carbon-carbon bond formation. Nucleophilic aromatic substitution at C2 and/or C6 positions with various amines produced two series of purine nucleoside libraries with very diverse substitution.

Key Words: Combinatorial library; Nucleoside; Purine.

†In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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INTRODUCTION

Nucleoside analogues are an important class of chemotherapeutic agents and many of them have been approved for treating both cancer and viral infections.[1,2] Testing a large number of diverse sets of nucleoside analogues, using high throughput screening techniques, would greatly increase the chance to find more efficient drug candidates. Therefore, there is a strong interest in synthesizing a large number of nucleoside analogues with a large variety of substituents. Recently, solid-phase synthesis has become an important tool for generating diverse compound libraries of pharmaceutical interest.[3,4] However, it has not been applied to the synthesis of nucleoside analogues until recently when our group reported the synthesis of a triazine nucleoside library.[5] We have reported in this journal the synthesis of a N^2,N^6-diaminopurine nucleoside library,[6] and in this present article, we report a solid-phase parallel synthesis of two 2,6,8-trisubstituted purine nucleoside libraries using the same approach.

RESULTS AND DISCUSSION

Scheme 1 illustrates the synthesis of 2,8-disubstituted guanosine libraries. Derivatization at the C8 position is interesting because this position is known to have an effect on adenosine A_1 receptors[7] and to strongly influence the syn/anti conformation of nucleosides.[8] Both alkylation and arylation of the C8 position of 8-bromo-2′,3′,5′-*O*-triacetylguanosine (**1**) with organostannane reagents through palladium-catalyzed coupling reactions were carried out using the reported procedure.[9] Compounds **2a–e** were obtained in moderate to good yields (60–85%) while compound **2f** was obtained from commercial sources. To derivatize the C2 position, it was necessary to protect the O^6 position with a base-labile group.[10] Under Mitsunobu conditions, compounds **2a–f** were protected with 2-(4-nitrophenyl)ethyl (Npe) group to give the corresponding orthogonally protected compounds, and the subsequent removal of the acetyl groups with methanolic ammonia gave **3a–f**. These reaction conditions did not affect the Npe protection on O^6. Diazotization and fluoride displacement of **3a–f** gave 2-fluoroinosine derivatives **4a–f**. The scaffold **4** was linked to polystyrene methoxytrityl chloride (PS-MMTrCl) resin in pyridine through the O5′ position of the sugar moiety to afford resins **5a–f**. Displacement of the fluoro atom at C2 position of **5a–f** with various primary and secondary amines was achieved at 80°C in 1-methyl-2-pyrrolidone (NMP). We discovered during the development research that most aliphatic amines gave high yields, while most substituted anilines were unreactive, and therefore they were excluded from our library synthesis. The guanosine libraries **6a–f** were obtained from **5a–f** by deprotection of the Npe group with DBU, followed by cleavage of the nucleosides from the resin. As expected, the glycosidic bond of the purine nucleosides **6a–f** was extremely susceptible to various acidic conditions, such as trichloroacetic or dichloroacetic acid in dichloromethane. Successful cleavage of the nucleoside libraries from our PS-MMTr resin without deglycosylation was achieved by using a 30% solution of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in dichloroethane, which was originally developed for peptide synthesis.[11] During our first experiments, we noticed that our compounds were contaminated by a noticeable amount of DBU, which indicated that a standard washing method for the resin (dimethylformamide, methanol,

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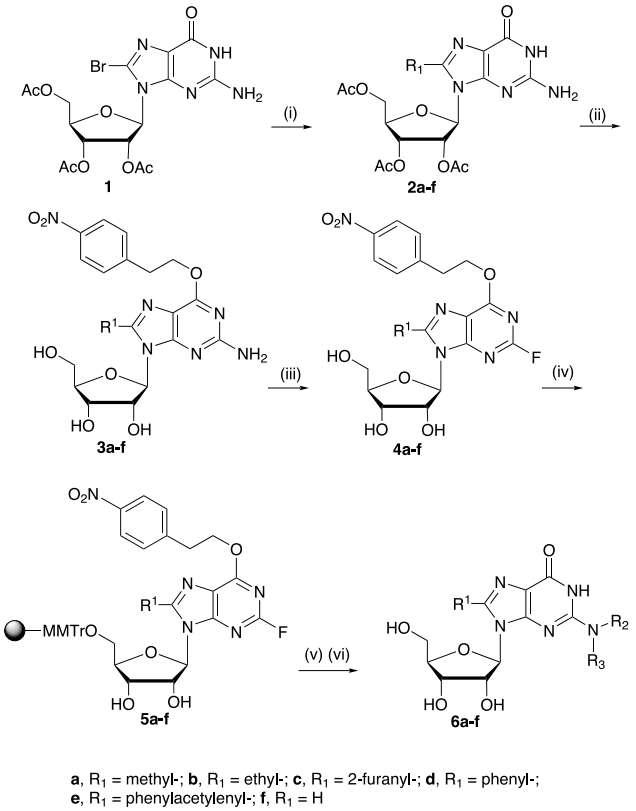
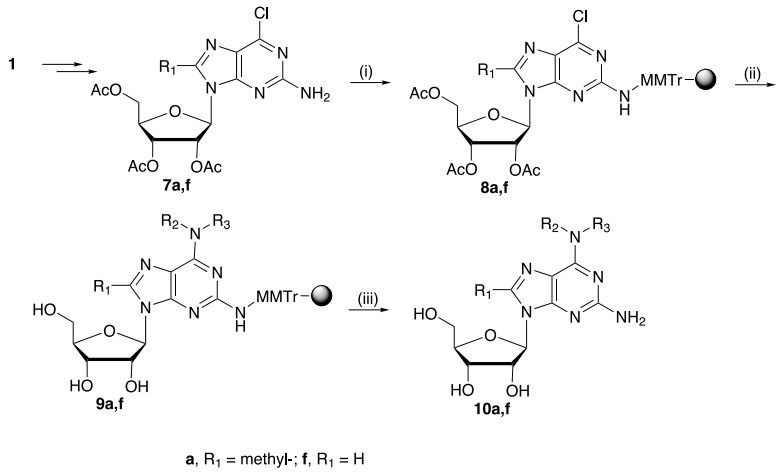


a, R_1 = methyl-; **b**, R_1 = ethyl-; **c**, R_1 = 2-furanyl-; **d**, R_1 = phenyl-; **e**, R_1 = phenylacetylenyl-; **f**, R_1 = H

Scheme 1. Reagents and conditions: (i) $(R_1)_4Sn$ (for **a** and **b**), R_1SnBu_3 (for **c** and **d**), R_1H (for **e**), $Cl_2Pd(PPh_3)_2$, DMF; (ii) *p*-$NO_2PhCH_2CH_2OH$, DEAD, PPh_3 dioxane; NH_3, MeOH; (iii) HF-pyridine, *t*-butyl nitrite; (iv) PS-MMTrCl, pyridine; (v) R_2R_3NH, NMP, 6 h at 60°C, then 24 h at 80°C; DBU, NMP; (vi) HFIP, DCE, 24 h at 50°C.

followed by methylene chloride) was not sufficient to wash away the excess of base. An additional washing step with a 10% solution of acetic acid in dimethylformamide was needed to completely remove DBU, and this extra step was implemented before the cleavage by HFIP. Six 96-well plates allowed the production of more than 570 compounds based on structure **6a–f** by a Vanguard automated synthesizer. Characterization by LC-MS showed that 87% of the compounds synthesized had a purity over 60%.

Scheme 2 illustrates the synthetic strategy for 2-amino-6,8-disubstituted purine nucleoside libraries **10a,f** starting from 8-bromoguanosine. Chlorination of compounds **2a,f** with $POCl_3$ produced their corresponding 6-chloro derivatives **7a,f**.[12] The amino group at the C2 position of **7a,f** was reacted with PS-MMTrCl resin using 2,6-lutidine as a base in tetrahydrofuran. The resulting resins **8a,f** were subjected to nucleophilic aromatic substitution with various amines in NMP, followed by subsequent deacetylation with methylamine in methanol. The reactivity of this halogen toward various amines was comparable to that of the fluoro atom of the scaffold **5**. Cleavage of the nucleoside libraries



Scheme 2. Reagents and conditions: (i) PS-MMTrCl, 2,6-lutidine, THF; (ii) R_2R_3NH, NMP, 6 h at 60°C, then 24 h at 80°C; $MeNH_2$, MeOH, 24 h at 50°C; (iii) HFIP, DCE, 24 h at 50°C.

from the resin without deglycosylation was achieved by using the same method described earlier (HFIP, DCE). More than 190 compounds in this series of nucleoside were synthesized based on the structure **10**.

In conclusion, parallel solid-phase strategies for the synthesis of nucleoside libraries have been developed and successfully applied to the preparation of more than 760 2,6,8-trisubstituted purine nucleoside analogues for a wide range of biological screenings.

EXPERIMENTAL

General methods. NMR spectra were recorded at 300 MHz and the chemical shifts are expressed relative to TMS. The libraries were enumerated by Afferent TeamWorks 3.0, labeled and weighted by Label Automador, and synthesized on ACT Vanguard semi-automated synthesizers. Libraries were analyzed on a LC-MS system, consisting of Waters 2790 HPLC, Waters 996 photodiode array (PDA) detector, and micromass/Waters ZQ mass spectrometer. Luna C18 columns from Phenomenex were used for compound separation. Mass spectra from 100 to 1000 were acquired using electrospray ionization with positive and negative ion detections. UV spectra were recorded at 200–400 nm by the PDA, and the compound purity was monitored based on UV absorbency at 220 nm. The LC/MS operation was controlled by MassLynx software, and the LC/MS data were processed by OpenLynx software. Polystyrene methoxytrityl chloride resin was purchased from Novabiochem. Other reagents were purchased from Aldrich, Fluka, Acros and other companies, and used directly.

2-Amino-6-[2-(4-nitrophenyl)ethoxy]-8-phenyl-9-(β-D-ribofuranosyl)purine (3d). To a stirred solution of **2d**[9] (4.4 g, 9.1 mmol), 4-nitrophenethyl alcohol (1.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) in anhydrous dioxane (150 mL) under argon was added a solution of diethyl azodicarboxylate (1.7 mL, 11 mmol) in

anhydrous dioxane (10 mL) dropwise for 1 h. After stirring at room temperature for 22 h, the mixture was concentrated to dryness. Silica gel chromatography (CH_2Cl_2:EtOAc = 75:25) yielded 2′,3′,5′-tri-*O*-acetyl-2-amino-6-[2-(4-nitrophenyl)-ethoxy]-8-phenyl-purine-riboside as yellow solids. The solids were dissolved in methanolic ammonia (50 mL, saturated at 0°C) and the solution was stirred at room temperature in a sealed bomb for 15 h. The bomb was cooled to 0°C before opening to an air and the mixture was concentrated to dryness. Silica gel chromatography (CH_2Cl_2:MeOH = 90:10) yielded 3.1 g of **3d** (66% for 2 steps) as a pale foam. ^{1}H NMR (DMSO-d6): δ 8.17 (d, *J* = 8.7 Hz, 2H), 7.69–7.59 (m, 7H), 6.43 (bs, 2H), 5.65 (d, *J* = 6.6 Hz, 1H), 5.38 (d, *J* = 6.0 Hz, 1H), 5.22 (m, 1H), 5.14 (m, 1H), 5.04 (d, *J* = 4.8 Hz, 1H), 4.69 (t, *J* = 6.9 Hz, 2H), 4.08(m, 1H), 3.85 (m, 1H), 3.70–3.50 (m, 2H), 3.26 (t, *J* = 6.9 Hz, 2H).

2-Fluoro-6-[2-(4-nitrophenyl)ethoxy]-8-phenyl-9-(β-D-ribofuranosyl)purine (4d). In a polypropylene flask, **3d** (1.6 g, 3.2 mmol) was dissolved in 60% HF/pyridine (130mL) at −50°C under argon. To the solution was added *tert*-butyl nitrite (0.56 mL, 4.7 mmol) via syringe over 10 min, while the temperature was maintained at −50°C. After stirring at −40°C for additional 30 min, the mixture was diluted with $CHCl_3$ (100 mL) and poured into K_2CO_3 (30 g). To the mixture was added H_2O (100 mL) carefully. The aqueous layer was extracted with $CHCl_3$ (2 × 200 mL) and the combined organic solution was washed with brine (1 × 100 mL), dried with Na_2SO_4, and concentrated to dryness. Silica gel chromatography (CH_2Cl_2:MeOH = 95:5) yielded 1.28 g of **4d** (79%) as a yellow solid. ^{1}H NMR (DMSO-d6): δ 8.17 (d, *J* = 8.7 Hz, 2H), 7.76–7.59 (m, 7H), 5.71 (d, *J* = 6.6 Hz, 1H), 5.44 (d, *J* = 6.0 Hz, 1H), 5.23 (d, *J* = 5.1 Hz, 1H), 5.10 (m, 1H), 4.85 (m, 3H), 4.18(m, 1H), 3.88 (m, 1H), 3.72–3.49 (m, 2H). ^{19}F NMR (DMSO-d6): δ − 47.5. MS(ES) *m/e* 512.5 (M + H).

5′-*O*-PSMMTr-2-fluoro-6-[2-(4-nitrophenyl)ethoxy]-8-phenyl-9-(β-D-ribofuranosyl)purine (5d). A solution of **4d** (7.0 g, 14 mmol) in anhydrous pyridine (45 mL) was added to a reaction vessel containing MMTrCl resin (5.6 g, 1.73 mmol/g, 9.7 mmol). The reaction mixture was shaken at room temperature for 3 days. The mixture was quenched by the addition of methanol (6 mL), followed by shaking for 30 min. The resin was filtered, and washed with DMF (3 × 15 mL), MeOH (3 × 15 mL), and CH_2Cl_2 (3 × 15 mL). The washed resin was dried in vacuo at 45°C overnight to yield 9.8 g of **5d** (91%).

2-(*N*-Alkyl)-8-phenyl-guanosine library (6d). To each reaction vessel containing 70 mg of resin nucleoside **5d** was added a 0.5 *M* solution of various amines in anhydrous 1-methyl-2-pyrrolidinone (1.6 mL). The vessels were shaken at 60°C for 4 h and then shaken at 80°C for 20 h to make amination complete. The vessels were cooled down, filtered and washed with DMF (3 × 1 mL), MeOH (3 × 1 mL), and CH_2Cl_2 (3 × 1 mL). To each reaction vessel was added a 0.2 *M* solution of 1,8-diazabicyclo[5,4,0]undec-7-ene in anhydrous pyridine (1.5 mL). The vessels were shaken at room temperature for 16 h, filtered and washed with DMF (3 × 1 mL), 10% AcOH in DMF (3 × 1 mL), MeOH (3 × 1 mL), and CH_2Cl_2 (3 × 1 mL). For cleavage of nucleosides from the resin, a 30% solution of 1,1,1,3,3,3-hexafluoro-2-propanol in

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anhydrous dichloroethane (1.5 mL) was added to each reaction vessel. The vessels were shaken at 50°C for 24 h and the solution was pushed down into the receiving vessels while keeping the temperature at 50°C. The reaction vessels were washed with a 1:1 mixture of MeOH:CH_2Cl_2 (1.5 mL). The combined solution (3 mL) was concentrated to yield **6d**.

2′,3′,5′-Tri-*O*-acetyl-2-*N*-PSMMTr-6-chloro-8-methyl-9-(β-D-ribofuranosyl)-purine (8a). A solution of **7a** (6.8 g, 15 mmol) and 2,6-lutidine (2.2 mL, 19 mmol) in anhydrous THF (45 mL) was added to a reaction vessel containing MMTrCl resin (6.16 g, 1.80 mmol/g, 11 mmol). The reaction mixture was shaken at room temperature for 5 days. The mixture was quenched by the addition of methanol (6 mL), followed by shaking for 30 min. The resin was filtered, and washed with DMF (3 × 15 mL), MeOH (3 × 15 mL), and CH_2Cl_2 (3 × 15 mL). The washed resin was dried in vacuo at 45°C overnight to yield 9.8 g of **8a** (86%).

2-Amino-6-(*N*-alkyl)-8-methyl-9-(β-D-ribofuranosyl)purine (10a). To each reaction vessel containing resin nucleoside **8a** (70 mg) was added a 1 *M* solution (1.5 mL) of various amines in anhydrous 1-methyl-2-pyrrolidinone (NMP). The vessels were shaken at 60°C for 6 h and then shaken at 80°C for 24 h. The vessels were cooled down and washed with DMF (3 × 1 mL), MeOH (3 × 1 mL), and CH_2Cl_2 (3 × 1 mL). To each reaction vessel was added a 2 *M* solution (1.5 mL) of CH_3NH_2 in anhydrous MeOH. The vessels were shaken at 50°C for 24 h and washed with DMF (3 × 1 mL), MeOH (3 × 1 mL), and CH_2Cl_2 (3 × 1 mL) to yield **9a**. To each reaction vessel containing the resin **9a** was added a 30% solution (1.5 mL) of 1,1,1,3,3,3-hexafluoro-2-propanol in anhydrous dichloroethane. The vessels were shaken at 50°C for 24 h and the solution was pushed down into the receiving vessels while keeping the temperature at 50°C. The reaction vessels were washed with a 1:1 mixture of MeOH:CH_2Cl_2 (1.5 mL). The combined solution (3 mL) was concentrated to yield **10a**.

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