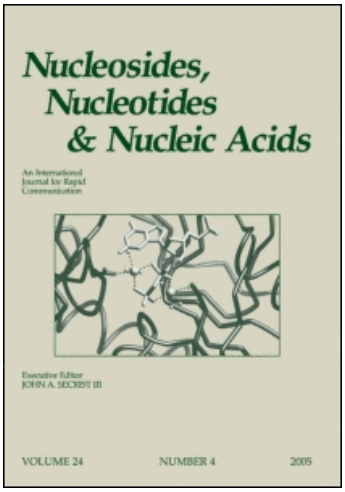

Nucleosides, Nucleotides and Nucleic Acids

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Solid-Phase Synthesis of 5'-Deoxy-5'-Amino-Clitocine Analogues

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SOLID-PHASE SYNTHESIS OF 5′-DEOXY-5′-AMINO-CLITOCINE ANALOGUES

Chamakura V. N. S. Varaprasad, Qazi Habib, Haoyun An, and Zhi Hong
□ *Department of Medicinal Chemistry, Valeant Pharmaceuticals International, Inc., Costa Mesa, California, USA*

□ *Several 6-substituted-amino-5′-deoxy-5′-amino-clitocine analogues were synthesized in a parallel fashion in solid phase. The desired scaffold was generated by coupling 2,3-*O*-bis-(t-butyldimethylsilyl)-5-N-(monomethoxytrityl-polystyrene-resin)-1,5-diamino-5-deoxy-β-D-ribofuranose and 4,6-dichloro-5-nitropyrimidine. The scaffold was then reacted with a variety of amines to generate a small library of 14 analogues of 5′-deoxy-5′-amino-clitocine following a protocol developed earlier.*

Keywords Exocyclic amino nucleosides; 5′-Deoxy-5′-amino-clitocine analogues; Polystyrene resin MMT-Cl

INTRODUCTION

Clitocine **1** (Figure 1), 6-amino-5-nitro-4-(β-D-ribofuranosylamino)-pyrimidine, is a naturally occurring exocyclic amino nucleoside isolated from the mushroom, *Clitocybe inversa.*[1] It shows strong insecticidal activity against the pink bollworm *Pectinophora gossyptella.*[1–3] Clitocine was found to inhibit, in vitro, growth of human lymphoblast derived WI-L2 cells (ID_{50}: 0.03 uM).[2] It is structurally congruous to adenosine and this makes it to be a potent competitor for the adenosine kinase binding site.[4] Because of this interesting relationship between them, several research groups have synthesized various acyclic[7,8] and carbocyclic[9–13] analogues of clitocine besides its total syntheses.[3–6] Recently, Lee *et al.*[4] have shown that several 5′-deoxy-clitocine analogues are potent inhibitors of adenosine kinase. Specifically, 5′-deoxy-5′-amino-clitocine **2** (Figure 1) (IC_{50}: 35 nM) was shown to be much more potent inhibitor than clitocine (∼2000 nM) itself. Undeniably, 5′-amino group is responsible for the potent activity of 5′-deoxy-5′-amino-clitocine **2**. Further, 5′-amino modification of adenosine also results

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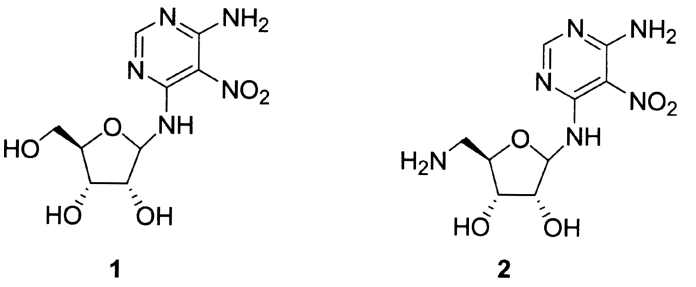
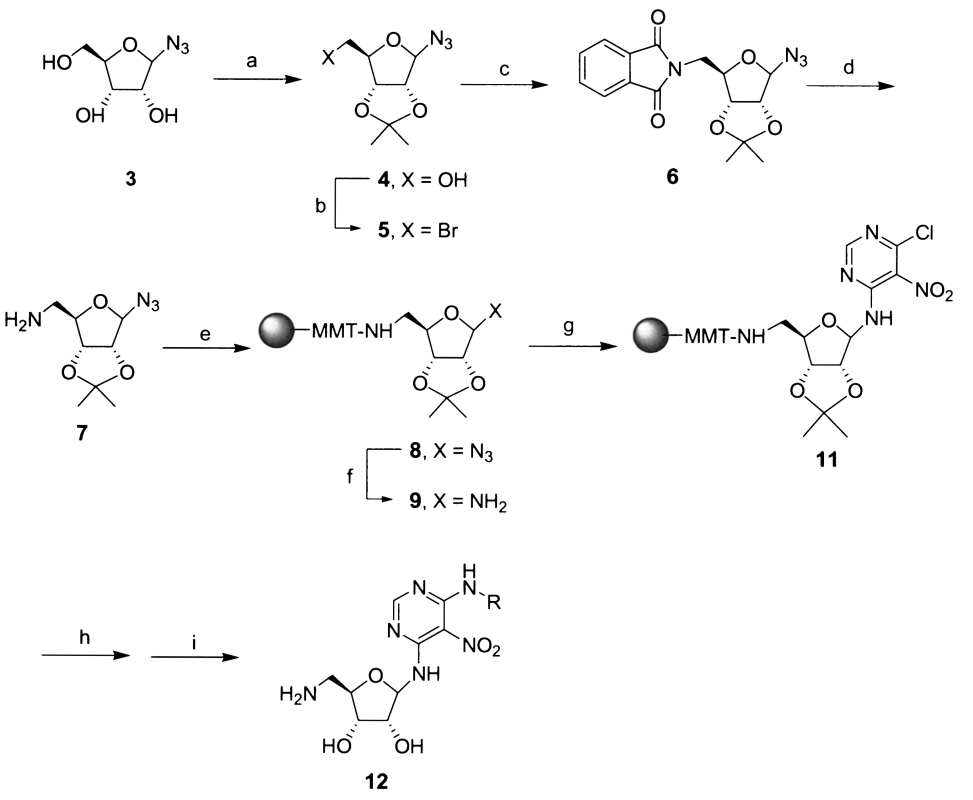
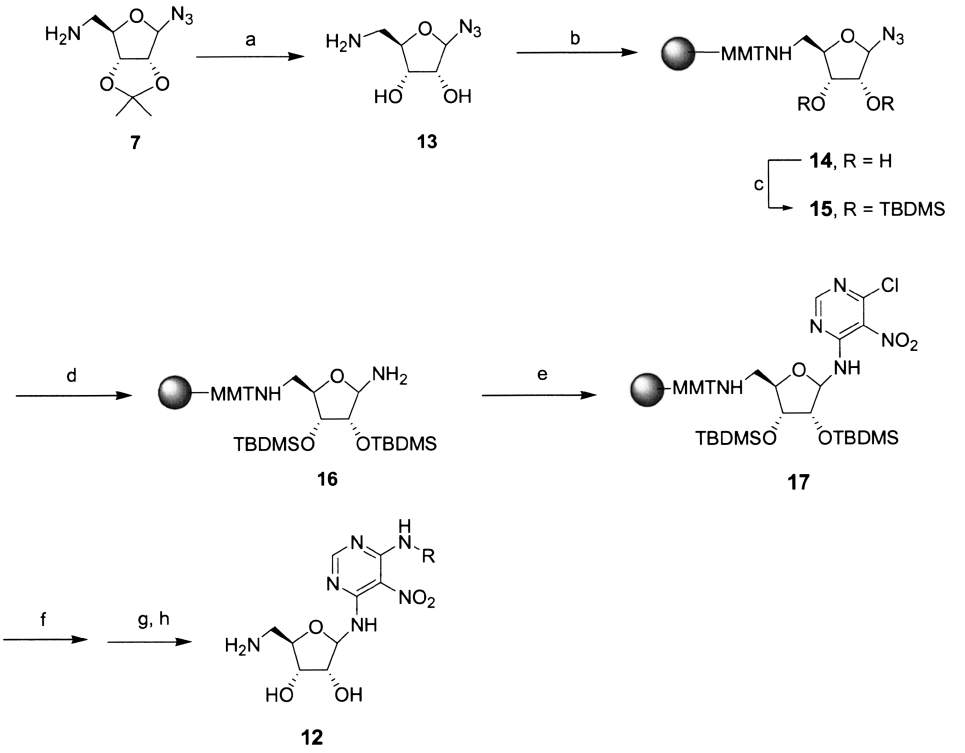


FIGURE 1

in the inhibition of adenosine kinase.[14] Our continued interest in the synthesis of novel nucleosides in solid phase in a parallel fashion[15] coupled with the interesting properties of 5′-amino derivatives of nucleosides led us to investigate further for novel compounds. Therefore, we synthesized a small library of 6-substituted-amino-5′-deoxy-5′-amino clitocine analogues



SCHEME 1 *Reagents*: a) acetone, H^+; b) Ph_3P, Br_2; c) potassium phthalimide, 1-methyl-2-pyrrolidinone, 80°C; d) anhydrous hydrazine, MeOH; e) polystyrene MMT-Cl resin, 4-dimethylaminopyridine, pyridine; f) Me_3P, THF-H_2O; g) 4,6-dichloro-5-nitropyrimdine, N,N-diisopropylethylamine; h) amine, N,N-diisopropylethylamine; i) 90% TFA/H_2O.



SCHEME 2 *Reagents*: a) 90% TFA/H_2O; b) polystyrene MMT-Cl resin, 4-dimethylaminopyridine, pyridine; c) t-butyldimethylsilyl chloride, imidazole, DMF; d) Me_3P, THF-H_2O; e) 4,6-dichloro-5-nitropyrimidine, N,N-diisopropylethylamine; f) amine, N,N-diisopropylethylamine; g) tetrabutylammonium fluoride, THF; h) 90% TFA/H_2O.

(Schemes 1 and 2) using an earlier developed solid-phase method[15] for in-house screening of various biological assays.

RESULTS AND DISCUSSION

The solid-phase synthesis of a library of monomeric nucleosides in a parallel[15] fashion is relatively undeveloped compared to the vast development of the solid-phase synthesis of their polymeric congeners RNA and DNA. However, the ease of assembly of clitocine skeleton in solution phase[3–6] is an added advantage in our pursuit of the synthesis of its library on solid phase. The desired scaffold was prepared by loading of the suitably protected amino-sugar **7** onto polystyrene monomethoxytrityl chloride (MMT-Cl) resin. Anchoring the 4,6-dichloro-5-nitropyrimidine **10** on the amino-sugar resin **9** resulted in a basic skeleton **11**. Substitution of chloro group in the scaffold **11** by various amines followed by deisopropylation and cleavage of the products from the resin afforded the desired library.

Thus, 1-azidoribose **3**[16–18] was selectively protected by isopropylidene to provide 2,3-isopropylidene-1-azidoribofuranose **4** which upon bromination

with Ph_3PBr_2 provided the corresponding bromide **5** (Scheme 1). Then **5** was reacted with potassium phthalimide in N-methyl-2-pyrrolidinone (NMP) to obtain compound **6**. Treatment of **6** with hydrazine generated the key intermediate 5-deoxy-5-amino derivative **7**, which was loaded onto the polystyrene MMT resin, as described earlier,[15] to afford the resin **8**.

The azido resin **8** was reduced under Staudinger[19] conditions in the presence of trimethylphosphine to give the amino resin **9**, which was reacted with 4,6-dichloro-5-nitropyrimidine **10** in the presence of N,N-diisopropylethylamine. The resultant scaffold **11** was then reacted with isopropylamine and *p*-methoxybenzylamine to validate the reaction conditions. The desired products (**12 e** and **12 n**, Table 1) were obtained, respectively, in 40% and 60% (LC-MS) purity after 2′,3′-ribose deprotection and release from resin by 90% trifluoroacetic acid (TFA) in water. This strong acid also caused partial deglycosylation resulting in overall low yield and purity. Therefore, a more appropriate protecting strategy was necessitated for obtaining a better quality library. The TBDMS protecting group had been verified to be suitable for our strategy[15] (Scheme 2).

Deisopropylation of derivative **7** by TFA gave the diol **13**, which was loaded onto the polystyrene MMT resin as described earlier to give the functionalized resin **14**. The diol **14** was protected as bis-TBDMS derivative **15**. The amino resin **16**, obtained from **15** under reduction conditions, upon treatment with **10** afforded the crucial scaffold **17**. The chloro-derivative **17** was then reacted with various amines. Then the TBDMS groups were removed by exposing the resin to tetrabutylammonium fluoride (TBAF) and followed by 1.5% TFA in dichloromethane to release the 6-substituted-amino-5′-deoxy-5′-amino clitocine analogs **12 a-n** (Table 1). The product's purity, determined by LC-MS, ranged from 60 to 93%. Products from the scaffold **17** having TBDMS protecting group gave much better purity of the final products compared to the scaffold **11**, wherein isoproplylidene was used as protective group. Further, based on the purity and yields of the products, the reactivity of various amines toward the scaffold **17** was found to be almost uniform and no large differences were seen among primary, secondary, or aromatic amines. The yields of the products are in the range of 20–30 mg. Preliminary screening of these novel nucleosides for various biological assays is currently in progress and will be reported elsewhere.

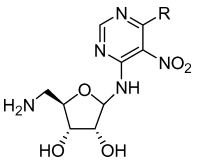
In conclusion, we have synthesized several new 6-substituted-amino-5′-deoxy-5′-amino clitocine derivatives by a novel strategy involving solid-phase synthesis.

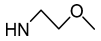
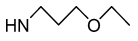
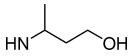
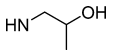
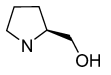
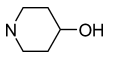
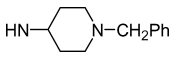
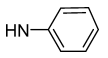
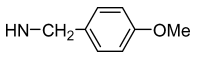
EXPERIMENTAL

General Methods

NMR spectra were recorded at 300 MHz (Varian) and the chemical shifts are expressed relative to the added tetramethylsilane. Fourier transform

TABLE 1 Library of 5′-Amino-5′-deoxy-clitocine Analogues



No.	R	MW (LC-MS)	Purity % (LC-MS)
12a	HN-CH_3	300	64
12b	NHOH	302	93
12c	$N(CH_3)_2$	314	70
12d	$N(CH_2CH_3)_2$	342	70
12e	$NHCH(CH_3)_2$	328	81
12f		344	64
12g		372	69
12h		344	62
12i		344	60
12j		370	70
12k		370	83
12l		459	62
12m		362	75
12n		406	77

infrared (FT-IR) spectra of the samples on solid support were obtained on a Perkin Elmer FT-IR spectrometer. The LC-MS system consists of Waters 2790 HPLC, Waters 996 photodiode array (PDA) detector and micromass/Waters 2Q mass spectrometer. Luna C_{18} column from Phenomenex was used for compound separation. The mass spectra at m/z 100–1000 were acquired using electrospray ionization with both positive and negative ion detections. UV spectra were recorded at 200–400 nm by the PDA and the compound purity was monitored based on the UV absorbancy at 220 nm. All reactions were monitored by thin-layer chromatography (tlc) carried out on silica gel alumina plates (0.25 mm). Detection of compounds by tlc was done either by UV light or by charring the plate after dipping it in an ethanol–conc. sulfuric acid mixture (9:1). Flash silica gel chromatography was performed on silica gel 60 (230–400 mesh) using the indicated solvents. Polystyrene

monomethoxytrityl chloride resin was purchased from Novabiochem. The required amines and reagents were purchased from Aldrich and other companies, and used directly. Elemental analysis of key intermediates was performed by NuMega Resonance Labs, Inc., San Diego, CA.

2,3-Isopropylidene-1-azido-β-D-ribofuranose (4). To a solution of 1-azidoribofuranose **3** (40.0 g, 228.5 mmol) in of acetone (400 mL) was added concentrated H_2SO_4 (2.0 mL). The reaction mixture was stirred at room temperature for 6 h and neutralized with aq. saturated sodium bicarbonate solution (100 mL). The reaction mixture was evaporated to dryness and the residue was extracted with CH_2Cl_2 (2 × 300 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated to give crude product **4** as colorless paste; ^{1}H NMR ($CDCl_3$) δ 5.54 (s, 1H), 4.77 (d, 1H, J = 6.0 Hz), 4.53 (d, 1H, J = 6.0 Hz), 4.41 (t, 1H, J = 3.6, 3.9 Hz), 3.74 (m, 2H), 2.29 (bs, 1H, D_2O ex), 1.45 (s, 3H), 1.32 (s, 3H). ES-MS: m/z 216 $(M + H)^+$; Anal. Calcd. for $C_8H_{13}N_3O_4$. 0.5 CH_3OH: C, 44.15; H, 6.49; N, 18.18. Found: C, 44.12; H, 6.20; N, 18.14.

5-Bromo-5-deoxy-2,3-isopropylidene-1-azido-β-D-ribofuranose (5). To a solution of compound **4** (32.0 g, 148.8 mmol) in CH_2Cl_2 (340 mL) was slowly added Ph_3PBr_2 (62.8 g, 148.8 mmol) at 0°C under N_2 atmosphere. The reaction mixture was allowed to warm to room temperature under stirring and continued to be stirred for 16 h. The reaction mixture was quenched with aq. saturated and cold sodium bicarbonate solution (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The organic layer was separated and washed with water (2 × 150 mL) and brine (300 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated. The resultant residue was purified by flash chromatography on a silica gel column ($CHCl_3$-MeOH, 9:1)), providing 34.0 g of product **5** (82.2%) as a colorless paste; ^{1}H NMR ($CDCl_3$) δ 5.58 (s, 1H), 4.79 (d, 1H, J = 6.0 Hz), 4.50 (d, 1H, J = 6.0 Hz), 4.48 (ddd, 1H, J = 10.2, 5.4, 1.1 Hz), 3.45 (ddt, 2H, J = 10.2, 5.4, 1.1 Hz), 1.49 (s, 3H), 1.32 (s, 3H). ES-MS: m/z 278 $(M + H)^+$.

5-(*N*-Phthalimido)-5-deoxy-2,3-isopropylidene-1-azido-β-D-ribofuranose (6). To a solution of compound **5** (34.0 g, 122.3 mmol) in 250 mL of N-methyl-2-pyrrolidinone (NMP) was added potassium phthalimide (45.25 g, 244.6 mmol). The resultant reaction mixture was heated at 80°C for 16 h and then diluted with of ice water (500 mL). The suspension was stirred at room temperature for 3 h. The solid was filtered and thoroughly washed with water (3 × 500 mL) to give crude compound **6** (34.0 g, 80.8%) as a white solid, which is sufficiently pure to take forward for the next reaction without further purification; ^{1}H NMR ($CDCl_3$) δ 7.87 (ddd, 2H, J = 39.9, 5.4, 3.3 Hz), 7.74 (ddd, 2H, J = 39.9, 5.4, 3.0 Hz), 5.57 (s, 1H), 4.78

(d, 1H, $J = 6.0$ Hz), 4.57 (t, 2H, $J = 5.9, 6.9$ Hz), 3.92 (m, 2H), 1.44 (s, 3H), 1.28 (s, 3H). ES-MS: m/z 345 $(M + H)^+$; Anal. Calcd. for $C_{16}H_{16}N_4O_5$: C, 55.81; H, 4.68; N, 16.27. Found: C, 55.55; H, 4.81; N, 16.48.

5-Amino-5-deoxy-2,3-isopropylidene-1-azido-β-D-ribofuranose (7). To a suspension of compound **6** (5.8 g, 16.86 mmol) in 100 mL of methanol was added anhydrous hydrazine (1.04 mL, 33.72 mmol). The reaction mixture was stirred at room temperature for 16 h. The precipitate was filtered and washed with methanol (3 × 25 mL). The combined filtrate was concentrated, and the residue was triturated with CH_2Cl_2 (100 mL). The filtrate was concentrated, and the residue was purified by flash chromatography on a silica gel column, which was pretreated with 5% triethylamine in hexane, to provide pure 3.0 g of product **7** (83.1%) as a colorless thick liquid. ^{1}H NMR (CD_3OD) δ 5.55 (s, 1H), 4.67 (dd, 1H, $J = 0.9, 6.0$ Hz), 4.50 (d, 1H, $J = 6.0$ Hz), 4.2 (t, 1H, $J = 7.8$ Hz), 2.77 (d, 2H, $J = 7.8$ Hz), 1.44 (s, 3H), 1.29 (s, 3H). ES-MS: m/z 252 $(M + H)^+$.

2,3-Isopropylidene-5-*N*-(monomethoxytrityl-polystyrene-resin)-5-amino-5-deoxy-β-D-ribofuranosyl-1-azide (8). To a suspension of polystyrene monomethoxytrityl chloride resin (1.0 g, 1.73 mmol/g) in pyridine (4 mL) were added a solution of compound **7** (0.4 g, 1.87 mmol) in pyridine (4 mL) and 4-*N,N*-dimethylaminopyridine (0.122 g, 1.0 mmol). The reaction mixture was shaken well at room temperature for 48 h. The resin was filtered and washed with CH_2Cl_2 (3 × 25 mL), and then a mixture of CH_2Cl_2-MeOH-*N,N*-diisopropylethylamine (8.5:1:0.5, 2 × 20 mL). The product resin **8** obtained was dried over KOH under vacuum for 16 h. Loading efficiency was 84%, calculation was based on the recovered starting material (1.52 mmol alcohol loaded). FT-IR (KBr) of resin: 2111.9 cm^{-1} (N_3 group). A small portion (50 mg) of the resin was treated with 1.5 mL of TFA solution in CH_2Cl_2 (1.5%) for 60 s, filtered, and washed with CH_2Cl_2. The filtrate was concentrated to give the starting azido compound **7**, which was confirmed by ^{1}H NMR.

2,3-Isopropylidene-5-*N*-(monomethoxytrityl-polystyrene-resin)-1(R,S),5-diamino-5-deoxy-β-D-ribofuranose (9). To a suspension of azido resin **8** (1.1 g) in a mixture of THF and water (7.5 mL, 9:1) was added a solution of trimethylphosphine in THF (2.5 mL, 1 M) and allowed the bubbling to slow down (~1 h). The flask was sealed with a septum and the mixture was shaken well at room temperature for 6 h (*Caution*: the pressure was released before opening the flask). The resin was filtered and then washed sequentially with a mixture of THF-H_2O (1:1, 3 × 10 mL), MeOH (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL). After drying over KOH under vacuum for 16 h, resin **9** was obtained. FT-IR (KBr): 2111.9 cm^{-1} (N_3 group) peak disappeared.

N^1-[(6-Chloro-5-nitro)pyrimidin-4-yl]-2′,3′-isopropylidene-5′-*N*-(monomethoxy-trityl-polystyrene resin)-1′(R, S),5′-diamino-5′-deoxy-β-D-ribofuranose (11). A suspension of resin **9** (1.0 g) suspended in a solution of *N,N*-diisopropylethylamine in NMP (8 mL, 20% v/v) was treated with 4,6-dichloro-5-nitropyrimidine **10** (1.0 g, 5.18 mmol). The reaction mixture was shaken well at room temperature for 4 h. The resultant brown suspension was filtered, and the resin was washed with NMP (3 × 25 mL) and CH_2Cl_2 (3 × 25 mL) and dried under vacuum over P_2O_5 for 16 h to obtain resin **11**.

N^1-[6-(Isopropylamino or benzylamino)-5-nitro-pyrimidin-4-yl]-1′-(R, S), 5′-diamino-5′-deoxy-β-D-ribofuranose (12 e or n). Resin **11** (50 mg) was suspended in a solution of *N,N*-diisoproplyethylamine in NMP (0.75 mL, 20% v/v), and an NMP solution of isopropylamine or benzylamine (0.75 mL, 1 M) was then added. The suspension was shaken well at room temperature for 16 h. The resin was filtered and washed sequentially with MeOH (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), an NMP-H_2O mixture (3:1, 3 × 10 mL), MeOH (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL). The resultant resin was then treated with a mixture of TFA-H_2O (9:1, 2 mL) and kept at room temperature for 1 h. The reaction mixture was filtered, and the resin washed with MeOH (2 × 5 mL). The combined filtrate was concentrated, and the residue was co-evaporated with toluene (2 × 5 mL) to provide the title compound **12e** or **12n** (15–20 mg) as the corresponding trifluoroacetate salt. The purity of these products was determined by LC-MS and found to be 40 and 60%, respectively. ES-MS of **12e**: m/z 329 $(M + H)^+$. ES-MS of **12n**: m/z 407 $(M + H)^+$.

5-Amino-5-deoxy-1-azido-β-D-ribofuranose (13). A solution of isopropylidene derivative **7** (0.50 g, 2.5 mmol) in a mixture of TFA-H_2O (9:1, 2 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated, and the residue was co-evaporated with toluene (2 × 5 mL) to provide product **13** in a quantitative yield (0.43 g) as the corresponding trifluoroacetate salt; ^{1}H NMR ($CDCl_3$) δ 5.33 (s, 1H), 4.10 (ddd, 1H, J = 2.7, 7.8, 9.6 Hz), 4.01 (dd, 1H, J = 4.2, 7.8 Hz), 3.87 (dd, 1H, J = 0.9, 4.2 Hz), 3.01 (dd, 2H, J = 9.6, 13.2 Hz). Anal. Calcd. for $C_5H_{10}N_4O_3$. 1 × CF_3CO_2H. 2 × H_2O: C, 25.93; H, 4.66; N, 17.28. Found: C, 26.19; H, 4.64; N, 16.92.

5-*N*-(Monomethoxytritylpolystyrene resin)-5-amino-5-deoxy-β-D-ribofuranosyl-1-azide (14). To a suspension of polystyrene monomethoxytrityl chloride resin (1.0 g, 1.73 mmol/g) in pyridine (4 mL) was added a solution of the trifluoroacetate salt of β-D-ribofuranosyl-1-azide **13** (0.40 g, 1.87 mmol) in pyridine (4 mL), followed by the addition of 4-*N,N*-dimethylaminopyridine (0.122 g, 1.0 mmol). The reaction mixture was shaken well at room temperature for 48 h. The resin was filtered and washed with CH_2Cl_2 (3 × 25 mL), a mixture of CH_2Cl_2-MeOH-*N,N*-diisopropylethylamine (8.5:1:0.5, 2 ×

20 mL). The product resin **14** was obtained after drying over KOH under vacuum for 16 h. Loading efficiency was 86%, calculation was based on the recovery of the starting material (1.57 mmol amino **13** loaded). FT-IR (KBr) 2107.7 cm^{-1} (N_3 group). A small portion (50 mg) of resin **14** was treated with 1.5 mL of TFA solution in CH_2Cl_2 (1.5%) for 60 seconds. The resultant resin was filtered and washed with CH_2Cl_2 (25 mL). The filtrate was concentrated to provide the starting azido compound **13**, which was confirmed by ^1H NMR.

2,3-*O*-*bis*(*t*-Butyldimethylsilyl)-5-*N*-(monomethoxytrityl-polystyrene-resin)-5-amino-5-deoxy-β-D-ribofuranosyl-1-azide (15). To a suspension of resin **14** (1.2 g) in 10 mL of DMF were added *t*-butyldimethylsilyl chloride (1.29 g, 8.65 mmol) and imidazole (1.17 g, 17.3 mmol). The reaction mixture was shaken well at room temperature for 16 h. The resin was filtered and washed sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL). Resin **15** was obtained after drying over KOH under vacuum for 16 h. A small portion (0.10 g) of resin **15** was treated with a solution of TFA in CH_2Cl_2 (1.5%) for 60 s, filtered, and washed with CH_2Cl_2 (2 × 5 mL). The combined filtrate was concentrated to give the 2,3-*O*-*bis*-(*t*-butyldimethylsilyl)-5-amino-5-deoxy-β-D-ribofuranosyl-1-azide (30 mg) as the corresponding trifluoroacetate salt, which was confirmed by ^1H NMR and MS. ^1H NMR (CD_3OD) δ 5.35 (s, 0.30 H), 5.28 (d, $J = 2.4$ Hz, 0.7 H), 4.18–3.85 (m, 3H), 3.28 (m, 1H), 3.02 (m, 1H), 0.94 (s, 18H), 0.16 (m, 12H). ES-MS: m/z 417 $(M + H)^+$.

2,3-*O*-*bis*-(*t*-Butyldimethylsilyl)-5-*N*-(monomethoxytrityl-polystyrene-resin)-1,5-diamino-5-deoxy-β-D-ribofuranose (16). To a suspension of resin **15** (1.1 g) in a mixture of THF-H_2O (9:1, 7.5 mL) was added a solution of trimethylphosphine in THF (2.5 mL, 1 M). The resultant mixture was shaken well at room temperature for 6 h and allowed the bubbling to slow down (~1 h). The flask was sealed with a septum and mixture was shaken well at room temperature for 6 h (*Caution*: the pressure was released before opening the flask). The resin was filtered and then washed with a THF and H_2O mixture (1:1, 3 × 10 mL), MeOH (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL). After drying over KOH under vacuum for 16 h, resin **16** was obtained. FT-IR (KBr) 2107.7 cm^{-1} (N_3 group) peak disappeared.

N^1-[(6-(Chloro)-5-nitro-pyrimidin-4-yl]-1′-(R,S)-amino-2′,3′-(*t*-butyl-dimethylsilyl)-5′-*N*-(mono-methoxytrityl-polystyrene resin)-1′,5′-diamino-5′-deoxy-β-D-ribofuranose (17). To a suspension of resin **16** (1.0 g) in a solution of *N,N*-diisopropylethylamine in NMP (8 mL, 20% v/v) was added 4,6-dichloro-5-nitropyrimidine (1.0 g, 5.18 mmol). The reaction mixture was shaken well at room temperature for 4 h. The brown suspension was filtered, and the resin was washed with NMP (3 × 25 mL) and CH_2Cl_2 (3 × 25 mL), and dried under vacuum over P_2O_5 for 16 h to provide resin **17**.

***N*^{1}-[6-(Alkyl or dialkyl or cycloalkyl-amino)-5-nitro-pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose (12 a-n).** To a suspension of resin **17** (50 mg) in a solution of *N,N*-diisopropylethylamine in NMP (0.75 mL, 20% v/v) was added an amine solution in NMP (0.75 mL, 1 M). The suspension was shaken well at room temperature for 16 h. The resin was filtered and washed sequentially with MeOH (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), an NMP-H_2O mixture (3:1, 3 × 10 mL), MeOH (3 × 10 ml), and CH_2Cl_2 (3 × 10 mL). The resin was then treated with a solution of TBAF (1M, THF) for 16 h at room temperature. It was followed by exposing the resin to DMF-AcOH-H_2O mixture (8:1:1, 1.5 mL) for 10 min and filtration. The resin was washed with a DMF-H_2O mixture (9:1, 3 × 10 mL), MeOH (3 × 10 mL), and CH_2Cl_2 (3 × 20 mL). After dried over KOH under vacuum for 16 h, a suspension of the resulted resin (50 mg) in 1.5 mL of TFA solution in CH_2Cl_2 (1.5%) was shaken well at room temperature for 60 s and filtered. The resin was further washed with MeOH (2 × 1 mL), and the combined filtrate was concentrated to give product (**12 a-n**, 20–30 mg) as the corresponding trifluoroacetate salt.

^{1}H NMR and ES-MS data for **12 a-n**:

N^1-[{6-(methylamino)-5-nitro}pyrimidin-4-yl]- 1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose**12a**. ^{1}H NMR (CD_3OD) δ 8.05 (s, 0.45H), 7.99 (s, 0.55H), 6.16 (d, J = 4.2 Hz, 0.45 H), 6.02 (d, J = 4.2 Hz, 0.55H), 4.14–3.94 (m, 3H), 3.44 (s, 3H), 3.26(dd, J = 2.4, 13.2 Hz, 1H), 3.01 (dd, J = 9.3, 12.9 Hz). ES-MS: m/z: 315 $(M + H)^+$.

N^1-[{6-(hydroxylamino)-5-nitro}pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose**12b**. ^{1}H NMR (CD_3OD) δ 8.02 (s, 0.45H), 7.96 (s, 0.55H), 6.18 (d, J= 4.0 Hz, 0.45 H), 6.05 (d, J= 4.4 Hz, 0.55H), 4.14–3.90 (m, 3H), 3.25(dd, J= 3.0, 13.2 Hz, 1H), 2.97 (dd, J = 8.7, 13.2 Hz). ES-MS: m/z: 303 $(M + H)^+$.

N^1-[{6-(dimethylamino)-5-nitro}pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose **12c**. ^{1}H NMR (CD_3OD) δ 8.06 (s, 0.45H), 7.97 (s, 0.55H), 6.15 (d, J= 4.0 Hz, 0.45 H), 5.98 (d, J= 4.5 Hz, 0.55H), 4.16–3.98 (m, 3H), 3.43 (s, 6H), 3.25(dd, J= 2.8, 13.2 Hz, 1H), 3.04 (dd, J= 9.6, 13.2 Hz). ES-MS: m/z: 315 $(M + H)^+$.

N^1-[{6-(diethylamino)-5-nitro}pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose **12d**. ^{1}H NMR (CD_3OD) δ 8.05 (s, 0.45H), 7.95 (s, 0.55H), 6.05 (d, J= 4.2 Hz, 0.45 H), 5.80 (d, J= 4.2 Hz, 0.55H), 4.10–3.92 (m, 3H), 3.50 (q, 4H), 3.24(dd, J= 3.0, 13.2 Hz, 1H), 3.02 (dd, J= 8.4, 13.2 Hz), 1.20(t, J= 6.9 Hz, 6H). ES-MS: m/z: 315 $(M + H)^+$.

N^1-[{6-(isopropylamino)-5-nitro}pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose **12e**. ^{1}H NMR (CD_3OD) δ 8.07 (s, 0.45H), 8.01 (s, 0.55H), 6.23 (d, J= 4.2 Hz, 0.45 H), 6.18 (d, J= 4.2 Hz, 0.55H), 4.15–3.90 (m, 3H), 3.24 (dd, J= 3.0 and 12.9 Hz, 2H), 3.09 (m, 1H), 1.30 (dd, J= 1.2 and 6.6 Hz). ES-MS: m/z: 329 $(M + H)^+$.

N^1-[{6-(2-methoxy-ethylamino)-5-nitro}pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose **12f**. ^{1}H NMR (CD_3OD) δ 8.02 (s, 0.45H), 7.92 (s, 0.55H), 6.12 (d, J = 4.0 Hz, 0.45 H), 5.98 (d, J = 4.4 Hz, 0.55H), 4.12–3.85 (m, 3H), 3.77 (br t, 3H), 3.28–3.01 (m, 6H). ES-MS: m/z: 345 $(M + H)^+$.

N^1-[{6-(3-ethoxy-propylamino)-5-nitro}-pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose **12g**. ^{1}H NMR (CD_3OD) δ 8.06 (s, 0.45H), 7.98 (s, 0.55H), 6.18 (d, J = 3.9 Hz, 0.45 H), 6.08 (d, J = 4.5 Hz, 0.55H), 4.13–3.98 (m, 3H), 3,37–3.41 (m, 4H), 3.21–2.98 (m, 4H), 1.65 (m, 2H), 1.12 (m, 3H). ES-MS: m/z: 372 $(M + H)^+$.

N^1-[{6-(3-hydroxy-1-methyl-propylamino-)-5-nitro}pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose **12h**. ^{1}H NMR (CD_3OD) δ 8.07 (s, 0.45H), 8.01 (s, 0.55H), 6.12 (d, J = 4.0 Hz, 0.45 H), 5.95 (d, J = 4.4 Hz, 0.55H), 4.12–3.70 (m, 5H), 3.24–2.97 (3H), 1.10–0.95 (m, 3H). ES-MS: m/z: 346 $(M + H)^+$.

N^1-[{6-(2-hydroxy-propylamino-)-5-nitro}pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose **12i**. ^{1}H NMR (CD_3OD) δ 8.02 (s, 0.45H), 7.92 (s, 0.55H), 6.14 (d, J = 4.0 Hz, 0.45 H), 5.98 (d, J = 4.4 Hz, 0.55H), 4.10–3.65 (m, 4H), 3.28–3.06 (m, 4H), 1.25–0.98 (m, 3H). ES-MS: m/z: 332 $(M + H)^+$.

N^1-[{6-(2-hydroxymethyl-1-pyrrolidin-1-yl)-5-nitro}pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose **12j**. ^{1}H NMR (CD_3OD) δ 8.06 (s, 0.45H), 7.98 (s, 0.55H), 6.19 (d, J = 4.0 Hz, 0.45 H), 6.02 (d, J = 4.4 Hz, 0.55H), 4.10–3.70 (m, 5H), 3.25–2.80 (m, 5H), 1.52 (m, 4H). ES-MS: m/z: 372 $(M + H)^+$.

N^1-[{6-(4-hydroxy-piperidin-1-yl)-5-nitro}-pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose **12k**. ^{1}H NMR (CD_3OD) δ 8.02 (s, 0.45H), 7.92 (s, 0.55H), 6.11 (d, J = 3.9 Hz, 0.45 H), 5.98 (d, J = 4.5 Hz, 0.55H), 4.15–3.82 (m, 4H), 3.30–3.01 (m, 6H), 1.54 (m, 4H). ES-MS: m/z: 371 $(M + H)^+$.

N^1-[{6-(1-benzyl-piperadin-4-yl)-amino-5-nitro}pyrimidin-4-yl]-1′-(R,S), 5′-diamino-5′-deoxy-β-D-ribofuranose **12l**. ^{1}H NMR (CD_3OD) δ 8.04 (s, 0.45H), 7.97 (s, 0.55H), 6.97 (m, 5H), 6.11 (d, J = 4.2 Hz, 0.45 H), 6.03 (d, J = 4.2 Hz, 0.55H), 4.20–3.98 (m, 5H), 3.29–2.95 (m, 7H), 1.52 (m, 4H). ES-MS: m/z: 460 $(M + H)^+$.

N^1-[{6-(phenylamino)-5-nitro}pyrimidin-4-yl]-1′-(R,S),5′-diamino-5′-deoxy-β-D-ribofuranose **12m**. ^{1}H NMR (CD_3OD) δ 8.01 (s, 0.45H), 7.92 (s, 0.55H), 6.98 (m, 5H), 6.12 (d, J = 4.2 Hz, 0.45 H), 6.06 (d, J = 4.2 Hz, 0.55H), 4.10–3.90 (m, 3H), 3.38 (s, 3H), 3.21(dd, J[=t] 2.4, 13.2 Hz, 1H), 2.98 (dd, J = 9.3, 12.9 Hz). ES-MS: m/z: 363 $(M + H)^+$.

N^1-[{6-(4-methoxy-benzylamino)-5-nitro}pyrimidin-4-yl]-1′-(R,S), 5′-diamino-5′-deoxy-β-D-ribofuranose **12n**. ^{1}H NMR (CD_3OD) δ 8.08 (s, 0.45H), 8.01 (s, 0.55H), 6.98–6.66 (m, 4H), 6.11 (d, J = 4.2 Hz, 0.45 H), 6.05 (d,

J = 4.2 Hz, 0.55H), 4.30–3.90 (m, 5H), 3.77 (s, 3H), 3.21 (dd, J = 2.4, 13.2 Hz, 1H), 2.96 (dd, J = 9.3, 12.9 Hz). ES-MS: m/z: 407 $(M + H)^+$.

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