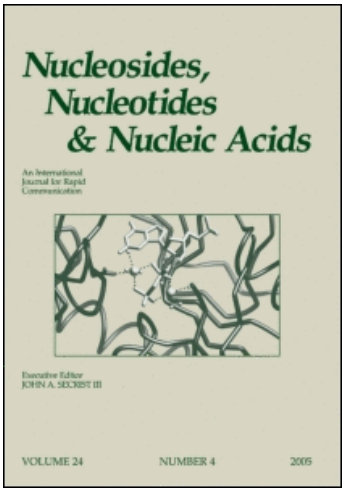

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Solid-phase Parallel Synthesis of 4-β-D-Ribofuranosylpyrazolo[4,3-d]pyrimidine Nucleosides

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SOLID-PHASE PARALLEL SYNTHESIS OF 4-β-D-RIBOFURANOSYLPYRAZOLO[4,3-d]PYRIMIDINE NUCLEOSIDES

Kanda S. Ramasamy, Roberto B. Amador, Qazi Habib, Frank Rong, Xiaogang Han, David Y. Li, Jingfan Huang, Zhi Hong, and Haoyun An □ *R&D Division, Valeant Pharmaceuticals, Costa Mesa, California, USA*

□ *The synthesis of pyrazolo[4,3-d]pyrimidine nucleoside library using solid-phase parallel synthesis methodology is described. Glycosylation of the trimethylsilyl (TMS) derivative of 1- and 2-(methyl)-1H and 2H-pyrazolo[4,3-d]pyrimidine-5,7-(4H,6H)-dione (***5***) with 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose in the presence of TMS triflate provided two novel protected nucleosides* **6** *and* **7**. *The structures of* **6** *and* **7** *were assigned by 1H and 2D NMR experiments. Nucleosides* **6** *and* **7** *were then transformed to the key intermediates* **12** *and* **15** *respectively. Reaction of* **12** *and* **15** *with MMTCl resin in the presence of 2,6-lutidine afforded the necessary scaffolds B and C. Different amines (96) were introduced selectively by nucleophilic substitution on scaffolds B and C using solid-phase parallel semi-automated synthesizer. Cleavage of the products from the solid support with 30% HFIP in a parallel fashion yielded nucleoside libraries simultaneously, and they were analyzed and characterized by high-throughput LC-MS.*

Keywords Solid phase; Parallel synthesis; Pyrazolo[4,3-d]pyrimidine nucleosides

INTRODUCTION

During the last few years, combinatorial chemistry has been used to generate huge numbers of heterocyclic compound libraries and identified lead candidate for different targets using high throughput screening. Thus, combinatorial synthesis of large diverse libraries of organic molecules and high throughput screening technologies are playing a key role in drug discovery.[1] If nucleosides could be made through combinatorial chemistry approach, a large number of nucleoside analogues could be synthesized

In honor and celebration of the life and career of John A. Montgomery.

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within months instead of decades, and large nucleoside libraries could be developed. A combinatorial approach to nucleosides may also encourage scientists to focus beyond the biological targets such as DNA or RNA polymerases, IMP dehydrogenase, protein kinases, and adenosine receptors. Thus, if a vast number of diverse nucleoside analogues could be created, their use extend far beyond these previously recognized biological targets and this would open a new avenue for the use of nucleoside analogues as human therapeutics.

Although, solid-phase combinatorial methodology has been applied to synthesize highly functionalized libraries of heterocyclic compounds[2] and oligonucleotides,[3] it has not been exploited in any significant fashion to the synthesis of nucleosides.[4,5] This is due to: (a) the glycosidic bond formation between a sugar moiety and a nucleoside base can be achieved only through selective reaction conditions; (b) the liability of the glycosidic bond to varying types of acids and reaction conditions; (c) most of the reaction conditions that are being used in combinatorial chemistry approaches may not be suitable to generate nucleoside libraries; (d) nucleosides have many reactive sites that need to be selectively protected. Recently, two methods[6,7] appeared in the literature: one utilized carboxypolystyrene solid support[6] and the other[7] used solid support containing 2′,3′-acetal linkages, which requires 5% TFA at 50°C deprotection condition. Thus, there is a growing interest to develop new solid-phase parallel synthesis methodologies for the production of nucleoside libraries using different solid supports coupled with mild cleavage condition from the solid support.

Pyrazolopyrimidine ring system forms a integral part of naturally occurring nucleoside antibiotics such as Formycin A and B[8] and medicinally useful drug such as allopurinol.[9] In addition, nucleosides having the pyrazolopyrimidine skeleton act as inhibitors of purine nucleoside phosphorylase.[10] Furthermore, newly discovered novel pyrazolopyrimidine derivatives have demonstrated promising antimicrobial activities against Gram-positive bacteria.[11] These interesting biological properties coupled with our ongoing effort[12] to develop solid-phase parallel synthesis methodologies and mild cleavage conditions to nucleosides prompted us to focus on nucleosides having the pyrazolopyrimidine ring system.

RESULTS AND DISCUSSION

First, a prototype scaffold **A** (Figure 1) was selected to identify the sites for diversity and the linking position on the solid support. Of course, the scaffold **A** has many sites for diversity and for the linkage to the solid support resin. However, a number of factors were considered in order to develop a solid-phase parallel synthesis methodology to a prototype nucleoside scaffold **A** (Figure 1): (a) identify a suitable resin and point of attachment

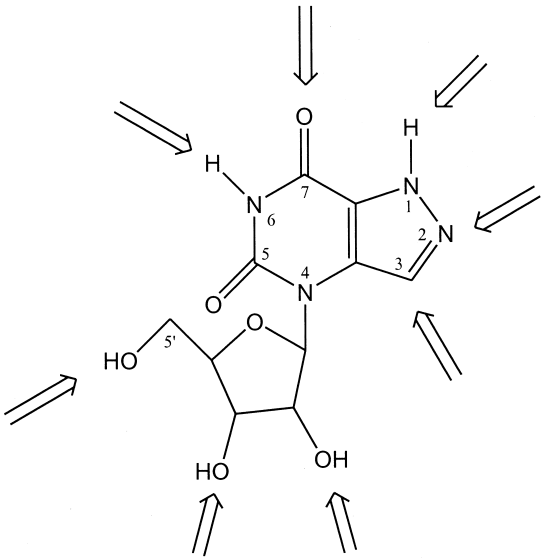


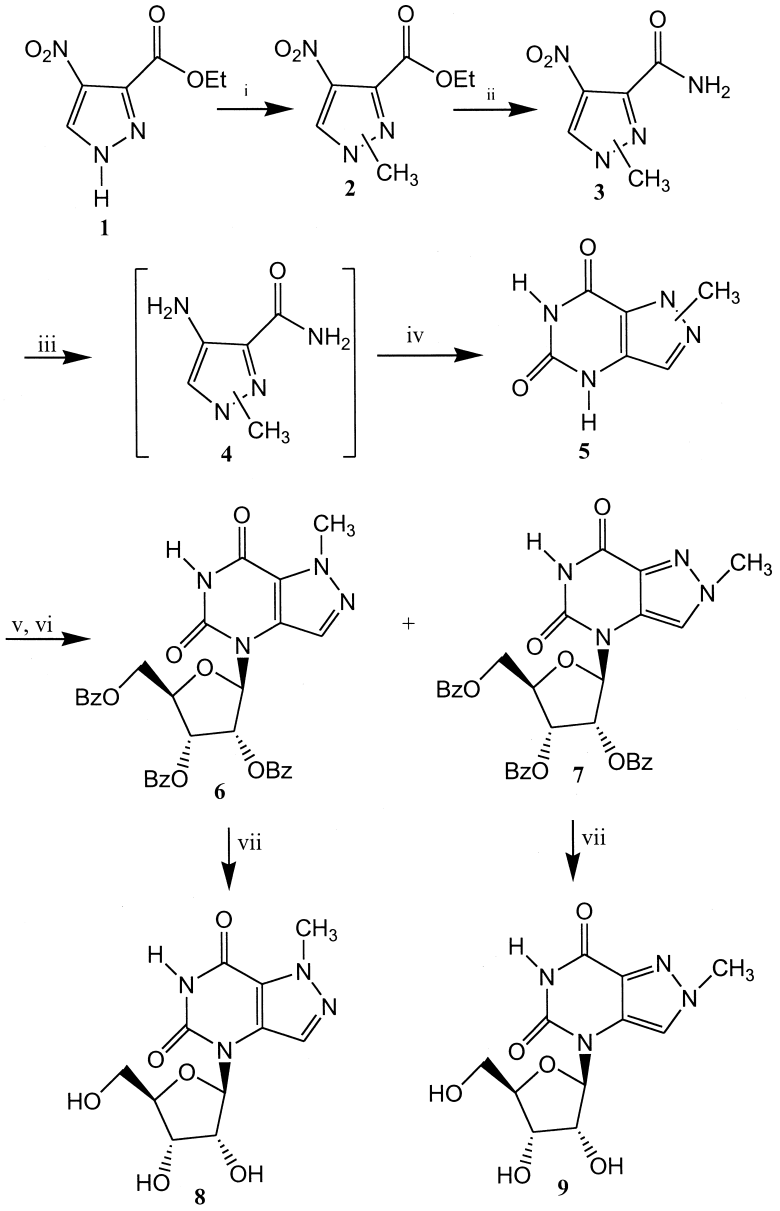
FIGURE 1 Prototype scaffold **A**.

for optimal nucleoside modifications and cleavage conditions that should not affect the glycosidic bond, (b) exploit the advantage of solid phase synthesis, and (c) design a strategy that could afford high quality products and are amenable to combinatorial library production. In order to explore the applicability of solid-phase chemistry to pyrazolopyrimidine scaffold **A**, the diversity profile was set to a minimum. Therefore, the objective was to introduce one site of diversity at the C_7 position on the heterocyclic portion of the scaffold **A**. To avoid N_1 or N_2 isomer complications, the pyrazole ring of scaffold **A** was alkylated and the isomers separated before adding on to the solid support. Also, the C_7 carbonyl group of scaffold **A** has to be converted to an "SCH_3" functionality so that nucleophilic substitution reaction at C_7 can be performed effectively. Since the 5′-hydroxyl group of the ribose moiety can be selectively protected[13] with trityl chloride, dimethoxytrityl chloride, or monomethoxytrityl chloride in the presence of unprotected 2′- and 3′-hydroxyl groups, the belief is that the corresponding resins can also be used to link nucleosides at the 5′-position of the sugar moiety. Interestingly, the 2′, 3′-hydroxyl groups of the sugar moiety **A** was kept open because these groups can be further functionalized to provide dideoxy nucleosides if needed. In addition, these solid support resins are stable enough to introduce diversification on the heterocyclic part, and cleavable under mild acidic or neutral conditions. Even though resins such as trityl (Trt), dimethoxytrityl (DMT) and monomethoxytrityl (MMT) can satisfy the above requirements, the MMT polystyrene resin was preferred. The reasoning was that MMT might be cleavable under neutral condition

rather than other trityl resins which require acidic conditions and more stable than DMT resin. Hence, the main strategy was to link the solid support on the 5′-position of the ribose moiety and introduce diversity at the C_7 position of pyrazolopyrimidine nucleoside scaffold **A** by keeping a fixed functionality either at N_1- or at N_2-positions. Furthermore, the solid-phase parallel synthesis methodology was applied to produce high-quality nucleosides in parallel by utilizing the ACT Vanguard semi-automated synthesizer combined with our in-house data processing techniques, which enables us to have a facile quality control during library production, purification, and high throughput screening.

Prior to exploring the solid phase chemistry, the key nucleosides **6** and **7** were synthesized starting from commercially available pyrazole derivative **1** as shown in Scheme 1. Alkylation of **1** with methyl iodide in the presence of sodium hydride provided a mixture of N_1 and N_2 alkylated pyrazoles **2**. The alkylated intermediates **2** were heated with methanolic ammonia at 110°C in a steel bomb to give the corresponding amide **3** quantitatively. Reduction of the nitro group of **3** followed by cyclization of the intermediate **4** with urea at high temperature afforded a mixture of N_1- and N_2-(methyl)-1H- and 2H-pyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dione (**5**) as greenish yellow solid. Silylation of the pyrazolopyrimidine **5** was accomplished by heating of **5** with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in the presence of a catalytic amount of ammonium sulfate in anhydrous pyridine for 16 h under an Argon atmosphere. The silyl derivative was then condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose in the presence of trimethylsilyl triflate (TMS triflate) in anhydrous acetonitrile at room temperature. A clean reaction was observed and the reaction products (**6** and **7**) were separated by flash chromatography over silica gel.

The structures of **6** and **7** were assigned based on NMR experiments carried out on the corresponding deblocked products **8** and **9**. For NMR studies, compounds **6** and **7** were heated separately with methanolic ammonia at 40°C in steel bomb for 12 h to provide **8** and **9** respectively in good yields. In ^{1}H NMR, N-CH_3 protons chemical shifts for **8** and **9** were appeared as singlet at 4.12 and 4.00 ppm, respectively. It is noteworthy that the presence of adjacent C_7 carbonyl to the N_1-CH_3 in compound **8** caused the N_1-CH_3 protons of **8** to shift 0.12 ppm downfield than the N_2-CH_3 of **9**. On the other hand, the C_3-H protons of **8** and **9** were resonated at 8.08 and 8.25 ppm, respectively. The downfield shift of C_3-H proton in **9** is expected,[14–16] because of the shielding effect induced by the N_2-CH_3 of **9**. Additional evidence for the assignment of structures was furnished by consideration of the 2D ROSEY experiments conducted on **8** and **9**. As anticipated, 2D ROSEY experiments of **8** and **9** in DMSO-d_6 with a short mixing time of 200 ms detected ROE between N_2-CH_3 and C_3-H protons in structure **9** but not for structure **8** (Figure 2).



SCHEME 1 Reagents and conditions: (i) NaH/MeI; (ii) MeOH/NH_3/110°C; (iii) Pd/C/MeOH/NH_4OH; (iv) Urea/220°C; (v) HMDS/135°C; (vi) 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose/TMS triflate; (vii) NaOMe/MeOH/Py.

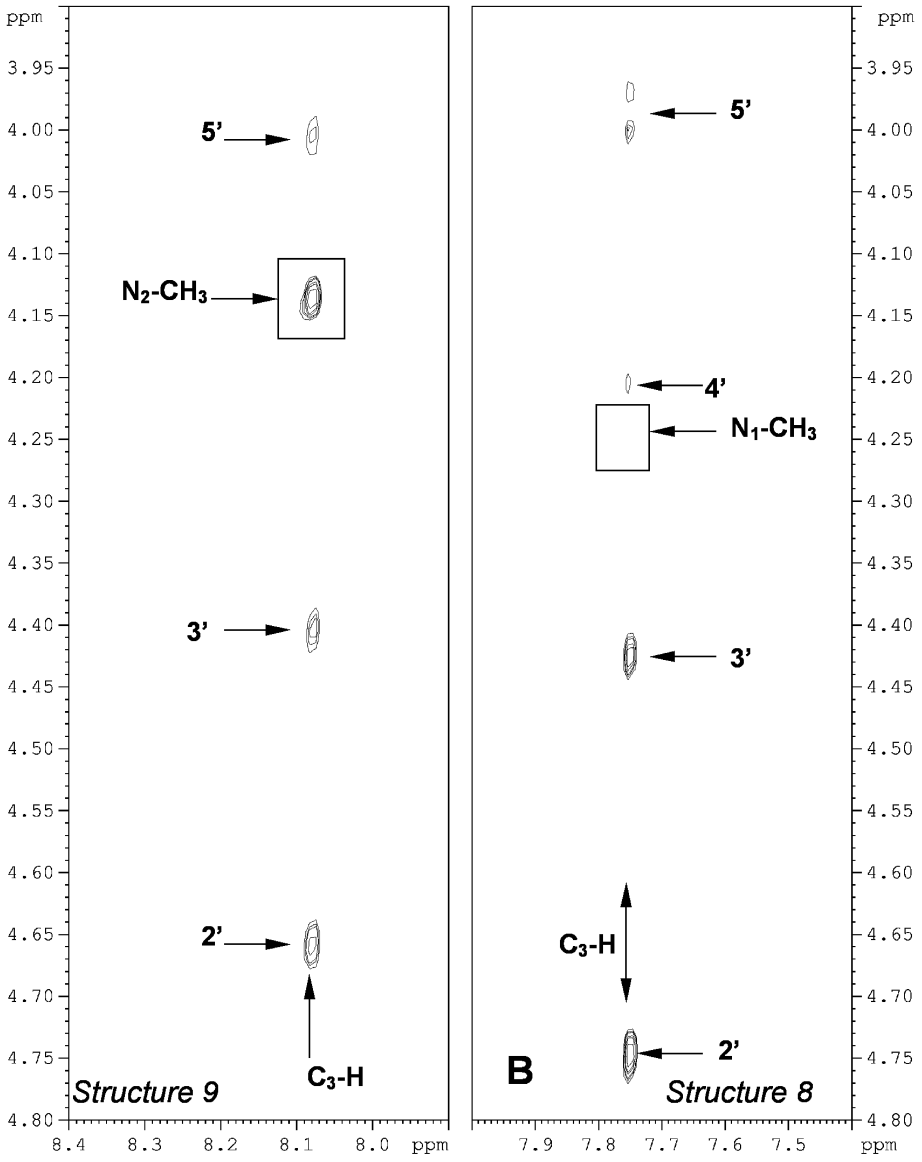
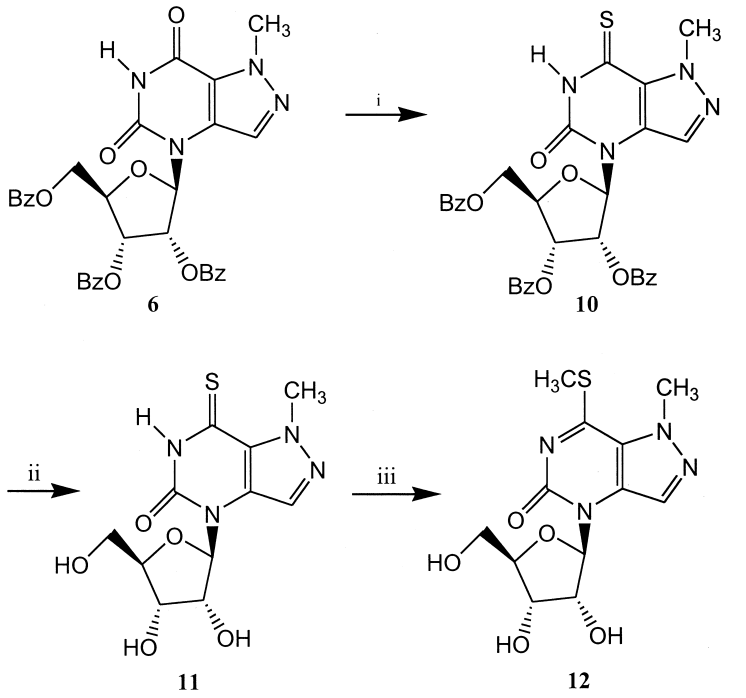
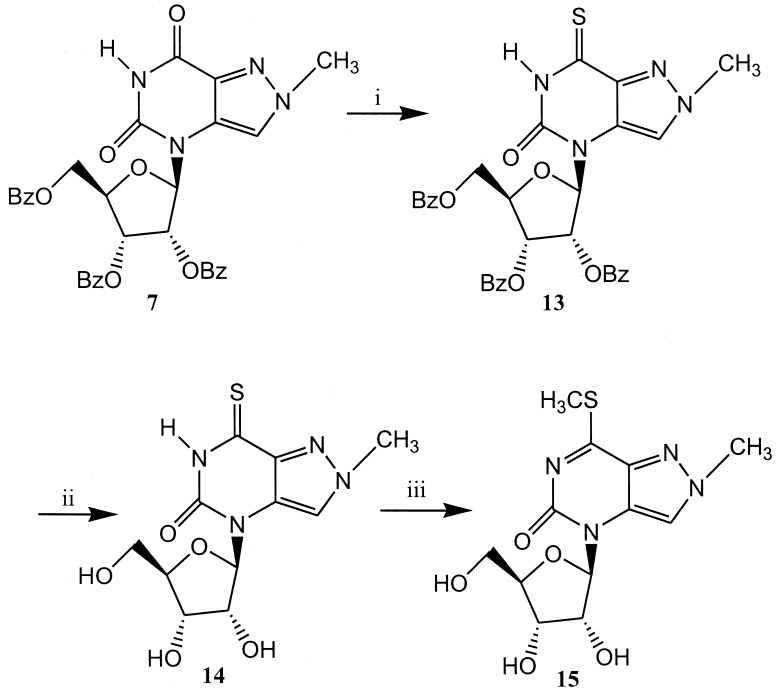


FIGURE 2 ROESY spectra were recorded at 32°C, with mixing time of 200 ms. ^{1}H assignments are also based on 2D TOCSY and ^{1}H-^{13}C HMQC experiments.

After establishing the structures of **6** and **7**, they were transformed to the key intermediates **12** and **15** as shown in Schemes 2 and 3, respectively. Treatment of **6** with phosphorus pentasulfide in anhydrous dioxane at reflux proceeded smoothly to afford 1-methyl-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5-oxo-pyrazolo[4,3-d]pyrimidine-7(1H,6H)-thione (**10**) in 60% yield. Deprotection of the benzoyl groups of **10** with sodium methoxide in



SCHEME 2 Reagents and conditions: (i) P_2S_5/Dioxane; (ii) NaOMe/MeOH; (iii) MeI/NNDIPEA.

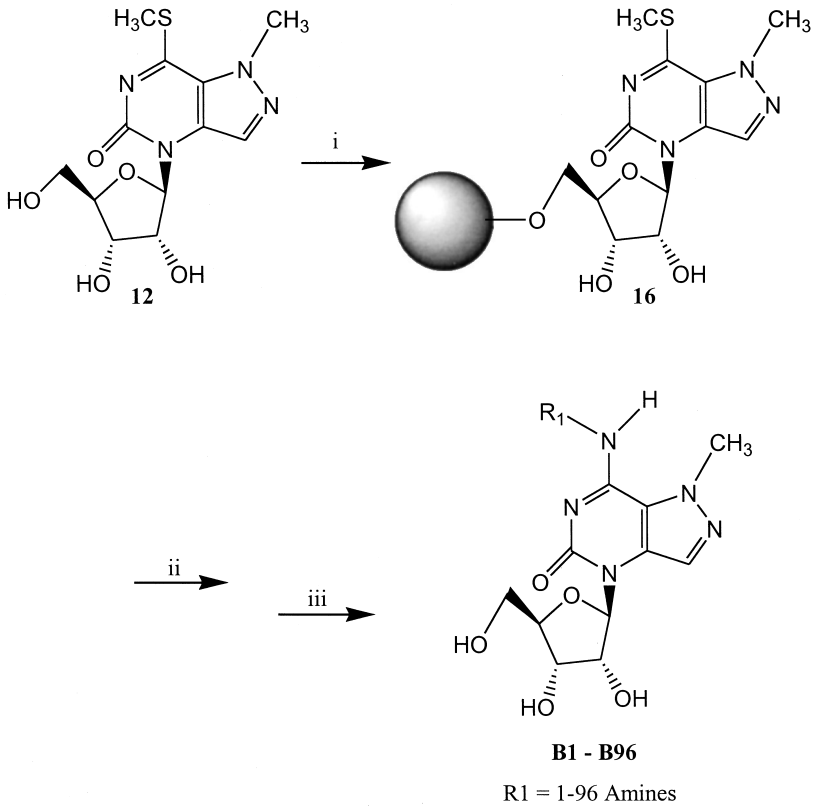


SCHEME 3 Reagents and conditions: (i) Lawesson's regent; (ii) NaOMe/MeOH; (iii) MeI/NNDIPEA.

methanol-pyridine mixture gave **11** as yellow crystals. Transformation of **11** into **12** was accomplished by a selective alkylation of the thiol group of **11** with methyl iodide in the presence of N,N-diisopropylethylamine. Similarly, compound **15** was prepared from **7** by following the reaction conditions used for the synthesis of **12** with a minor modification in the transformation of **13** from **7**. Accordingly, heating of 2-methyl-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine-5,7(1H,6H)-dione **7** with Lawesson's reagent[17] in anhydrous pyridine provided the corresponding thio derivative **13** in 80% yield. Deblocking of the benzoyl protecting groups in **13** with sodium methoxide followed by methylation of the corresponding intermediate **14** provided another scaffold **15** in good yield.

Loading of the Pyrazolopyrimidine Nucleoside Scaffolds on MMT Resin

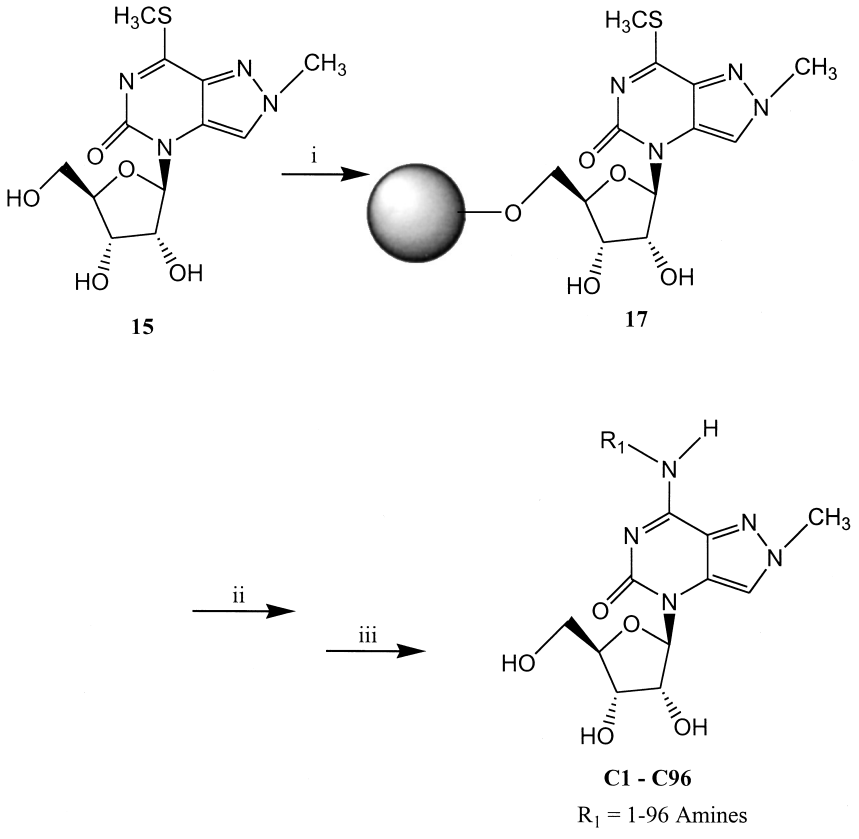
The synthesis of resin bound scaffolds **B (16)** and **C (17)** is outlined in Schemes 4 and 5, respectively. Polystrene MMT-Cl resin was stirred with **12** in



SCHEME 4 Reagents and conditions: (i) MMTCl resin/2,6-leutidine/DMF; (ii) 1.5 M amines in NMP/80°C; (iii) 30% HFIP/50°C.

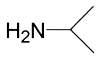
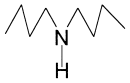
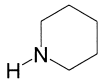
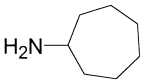
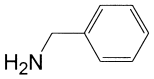
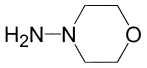
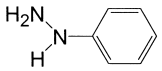
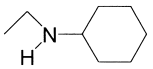
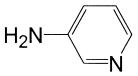
anhydrous DMF in the presence of excess 2,6-lutidine for 3 days under inert atmosphere. MMT resin was selectively loaded on the 5′-hydroxyl group of the scaffold **B**. The loading efficency (66%) was estimated based on the increase in weight of the starting resin. Similarly, the nucleoside **15** was also loaded on MMT resin in 74% yield.

Utilizing solid-phase chemistry the scaffold **B** was validated by synthesizing ten new library members which were then used as reference conditions during the production of actual library on the synthesizer. Table 1 shows the ten amine building blocks used for validation and the results. Fifty milligrams of the Scaffold **B** was placed in ten separate wells of a 48 reaction vessel (LabTech, Advanced ChemTech) and ten different amines were added. The reaction block was heated at 80°C for 24 h with gentle agitation. The reaction block was cooled to room temperature and the reagents were drained. The resin was washed with DMF, MeOH, and CH_2Cl_2, and dried. To the dried resin 30% hexafluoro isopropanol (HFIP, 1.5 mL) in CH_2Cl_2 was added to each well and heated at 50°C for 24 h. The reaction



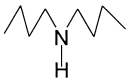
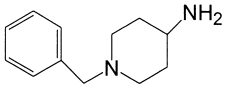
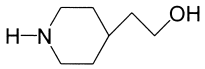
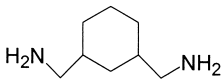
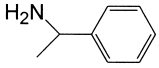
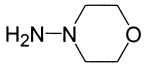
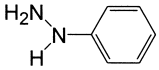
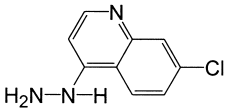
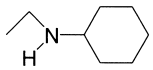
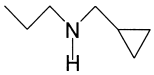
SCHEME 5 Reagents and conditions: (i) MMTCl resin/2,6-leutidine/DMF; (ii) 1.5 M amines in NMP/80°C; (iii) 30% HFIP/50°C.

TABLE 1 Validation of Scaffold **B** with Ten Different Amines

Entry	Amines	LC-MS purity of products (%)
a		90
b		71
c		3
d		81
e		75
f		92
g		36
h		0
i		0
j		0

mixture was filtered into ten different vials. The filtrates were evaporated, and the residues obtained were analyzed by LC-MS. Preliminary validation results indicated (Table 1) that high quality products were obtained when primary amines were used. Amines such as secondary amines, hydrazines, piperazines, and sterically hindered amines did not react well, and they were eliminated in the final production. Scaffold **C** behaved differently on validation with ten different amines (Table 2). N,N-dimethylformamide (DMF)

TABLE 2 Validation of Scaffold **C** with Ten Different Amines

Entry	Amines	LC-MS purity products of (%)
a		47
b		79
c		100
d		82
e		64
f		8
g		7
h		65
i		2
j		0

was found to be useful for these reactions, but, the formation of side products was noted. Therefore, DMF was not used for library production. After experimenting with different solvents, 1-methyl-2-pyrrolidinone (NMP) was found to be suitable for nucleoside library production using solid-phase chemistry.

Based on availability, diversity, lipophilicity, and reactivity of the amine building blocks, 96 primary amines were selected. Simultaneously, 96 wells of reactions were carried out for each scaffold (2 × 96) in one reaction block on the ACT Vanguard semi-automated synthesizer. The various amines selected for the final library production are shown in Figure 3. The 96 compounds in different wells were enumerated by the Afferent TeamWorks software to generate the molecular weight, structural and other related information for the desired library members, and the key impurities found

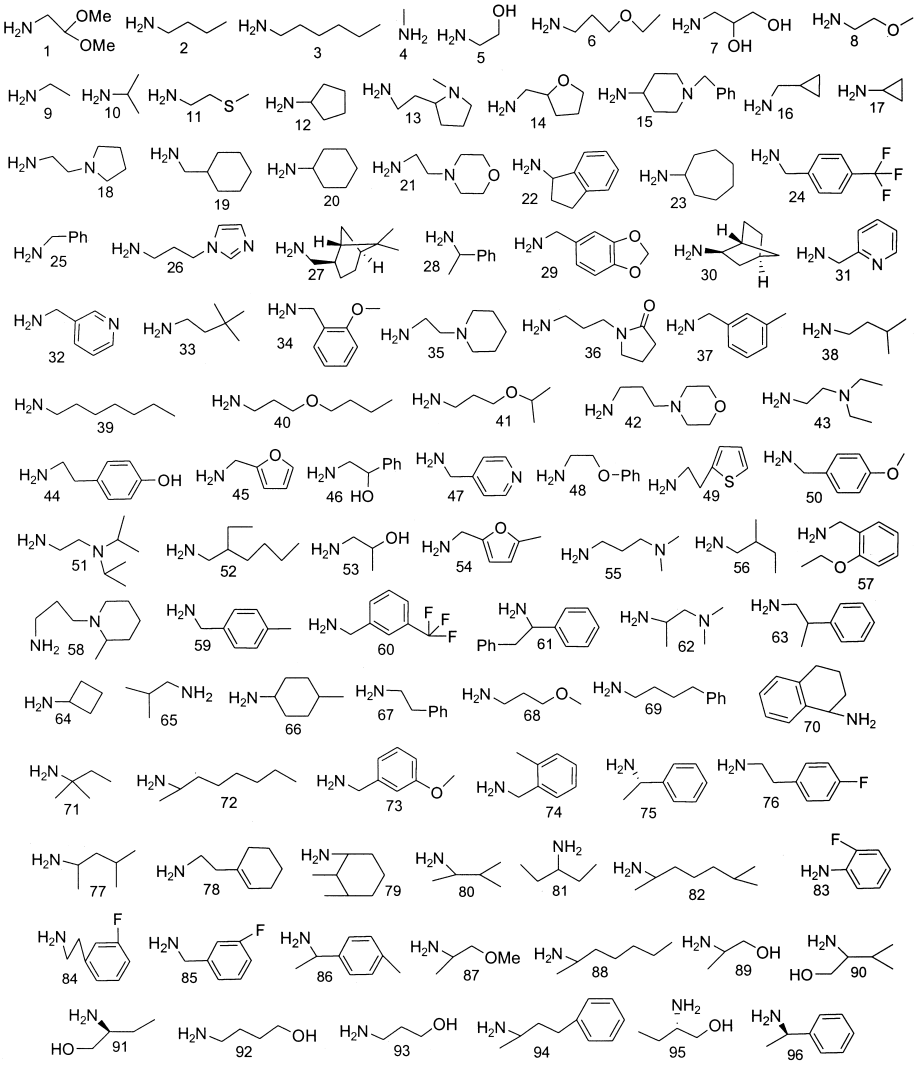


FIGURE 3 Building blocks: 96 amines used for the nucleophilic reactions with scaffolds **B** and **C** to provide the corresponding products **B1-B96** and **C1-C96**.

in each reaction well. Afferent TeamWorks transferred the data to Label Automador for automated sample bar code labeling and weighing. Libraries (Schemes 4 and 5) were synthesized in a parallel fashion utilizing the optimized procedures and conditions as well as using NMP as solvent. The washing procedures were automatically conducted and controlled by the pre-designed program.

Cleavage of Nucleosides from the MMT Resin

A variety of cleavage conditions were explored to obtain the highest purity of the cleaved nucleoside products. Although 2% TFA in CH_2Cl_2 had been utilized to cleave nucleosides from MMT resin,[12] application of this cleavage condition for pyrazolopyrimidine nucleosides resulted in glycosidic bond cleavage and therefore reduction in product purity. After exploration of a range of TFA/CH_2Cl_2 (0.5–2.0%) mixtures, it was found that 0.5% TFA in CH_2Cl_2 was the ideal reagent to cleave the pyrazolopyrimidine nucleosides from the MMT resin. However, application of the same cleavage condition (0.5% TFA/CH_2Cl_2) to the products derived from scaffold **C** again resulted in glycosidic bond cleavage of a few nucleosides. Hence, a different reagent to cleave the MMT resin containing pyrazolopyrimidine nucleosides was sought. After a few attempts with other reagents (data not shown), it was found that 30% hexafluoroisopropanol (HFIP) is sufficient to cleave the nucleosides from the MMT resin and provided good recovery of the products with acceptable purity. Remarkably, based on the results obtained the HFIP cleavage condition is the best to use for the acid sensitive nucleosides.

In all cleavage procedure, the wells containing the resin were treated with 30% HFIP (1.5 mL) and shaken at 50°C for 24 h. The mixture was cooled to room temperature, filtered and washed with MeOH (2 mL), and the combined filtrate was dried using Savant SpeedVac and the residue was analyzed by high-throughput LC-MS. The Afferent TeamWorks software generated compound ID numbers and molecular weights, which were input into the sample list for LC-MS data acquisition. The LC-MS data were acquired using MassLynx software, and the LC-MS data were processed using OpenLynx software. The LC-MS data and the compound purity summary were input into Afferent TeamWorks as a record for the database. Table 3 shows the quality of nucleoside libraries that were produced from 96 amines (Figure 3) with scaffolds B and C using solid-phase parallel synthesis methodology. LC-MS results (Table 3) indicate that 78% of library members (**B1**-**B96**)[18] are more than 80% purity and are registered in the data base directly without purification. The library with more than 80% purity was considered a good quality library and a successful demonstration of the use of solid phase chemistry to generate nucleoside libraries. Hence, the

TABLE 3 Quality of Libraries (**B1-B96** and **C1-C96**) that Obtained from Scaffolds **B** and **C** on Reaction with 96 Amines

Scaffolds	% of products 80–100% purity	% of products 60–80% purity	% of products >60% purity
Scaffold **B**	78.1	9.4	87.5
Scaffold **C**	93.7	4.1	97.8

96 Compounds for each library.

library with more than 80% purity was selected by the programmed software and automatically registered to the database for biological screening. 9.4% of library members (**B1-B96**)[18] have more than 60% purity and these members were purified by HPLC and submitted for testing. In total, 88% of the library production was considered a success from scaffold **B**. On the other hand the library (**C1-C96**)[18] produced from the scaffold **C** has a success rate of 98% (Table 3). In both cases, an average of 12 to 20 mg individual library member was obtained.

In conclusion, we have prepared new pyrazolopyrimidine nucleosides, assigned their structures by NMR studies, transformed them to the key intermediates (**12** and **15**) and loaded on MMT resin efficiently. The resulting scaffolds **B** and **C** were then subjected to nucleophilic substitution reactions with a variety of amines at the solid-phase level. Subsequently, the products were cleaved from the solid support under neutral condition, without cleavage of the glycosidic bond, to provide quality nucleoside libraries. Thus, the solid-phase parallel synthesis methodology discussed here is straightforward and opens a new avenue to generate a wide variety of nucleoside combinatorial libraries. Biological screening of these nucleoside libraries is in progress and will be reported elsewhere.

EXPERIMENTAL

All 1D NMR spectra were recorded at 300 MHz and the chemical shifts are referenced to trimethylsilane. All 2D NMR experiments were carried out on a Bruker Avance DRX 500 system with proton carrier frequency at 500.13 MHz. Data were acquired with 5000 Hz spectral width, 8 transients, 3 seconds delay and 512 data points in t1 dimension. Fourier-transform infrared (FT-IR) spectra of the samples on solid support were obtained on a Perkin-Elmer FT-IR spectrometer. The libraries were enumerated by Afferent TeamWorks 3.0, labeled, and weighed by Label Automador, and synthesized on the ACT Vanguard semi-automated synthesizer. Libraries were analyzed on a LC-MS system. The LC-MS system consists of Waters 2790 HPLC, Waters 996 photodiode array (PDA) detector, and Micromass/Waters ZQ mass spectrometer. Luna C18 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 absorbency at 220 nm. The LC-MS operation was controlled by MassLynx software, and the LC-MS data were processed by OpenLynx software. High resolution mass spectra (HRMS) were acquired using a Q-tof-2 mass spectrometer equipped with an electrospray ionization source. Polystyrene monomethoxytrityl chloride resin was purchased from Novabiochem. Other starting materials, building blocks, and reagents were purchased from Aldrich and other companies, and used directly.

Ethyl 4-Nitro-1- and -2-(Methyl)-1H- and 2H-Pyrazole-3-carboxylate (2). To a stirred solution of ethyl 4-nitro-1H-pyrazole-3-carboxylate (**1**, 25.00 g, 135.0 mmol) in anhydrous DMF (200 mL) under an argon atmosphere was added NaH (6.00 g, 150.0 mmol) for 30 min at 0°C. After the addition of NaH, the reaction mixture was stirred at room temperature for 1 h and cooled to 0°C. Methyl iodide (15 mL) was added slowly and the stirring continued at 0°C for 1 h and at room temperature for 6 h. The reaction mixture was evaporated to dryness. The residue was partitioned between EtOAc (500 mL) and water (300 mL), and extracted with EtOAc. The organic layer was washed with brine (150 mL), dried, and concentrated to dryness. The residue was used as such for the next reaction. Yield: 26.8 g (100%). UV (MeOH) λ_{max} 273 (5250) and 275.(5100). Anal. calcd. for $C_7H_9N_3O_4$: C, 42.21; H, 4.56; N, 21.09. Found: C, 42.41; H, 4.53; N, 21.25.

4-Nitro-1- and -2-(Methyl)-1H- and 2H-Pyrazole-3-carboxamide (3). A mixture of compound **2** and methanolic ammonia (prepared freshly at 0°C, 400 mL) was allowed to stir in a steel bomb at 110°C for 12 h. The steel vessel was cooled in dry ice/acetone bath for 1 h, opened carefully, and the content was evaporated to dryness. The residue was triturated with acetone and filtered. The solid was used as such for further reaction. Yield: 22.97 g (100%). ^{1}H NMR ($CDCl_3$): δ 3.84 and 3.88 (3H, 2 s, NC*H*$_3$), 7.72–8.8 (3H, m, CON*H*$_2$ and Ar*H*). UV (MeOH) λ_{max} 272 (5200) and 278 (5300). Anal. calcd. for $C_5H_6N_4O_3$: C, 35.29; H, 3.55; N, 32.93. Found: C, 35.40; H, 3.73; N, 32.95.

1- and -2-(Methyl)-1H- and 2H-Pyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dione (5). To a solution of **3** (22.97 g, 115.4 mmol) in a mixture of MeOH/dioxane (9:1, 300 mL) was added NH_4OH (20 mL) followed by Pd/C (5%, 1.00 g). The reaction mixture was hydrogenated at 45 psi of hydrogen for 12 h. The catalyst was filtered, washed with MeOH (100 mL), and the filtrate evaporated to dryness. The residue **4** that obtained was used as such for the next reaction without characterization.

The above crude product **4** (18.92 g, 135.0 mmol) and urea (25.00 g) were mixed together and heated at 250°C with stirring for 45 min. The product formed as a liquid and then solidified. The solid was dissolved in 2 M NaOH solution (100 mL) at 70°C and filtered while hot. The filtrate was acidified with AcOH and cooled. The precipitated solid was filtered, washed with water and dried over solid NaOH under vacuum. Yield: 22.41 g (100%). ^{1}H NMR (DMSO-d_6): δ 3.91 and 4.02 (3H, 2 s, NCH_3), 7.32 and 7.62 (1H, 2 s, Ar*H*), 10.86 (2H, br s, 2 × N*H*). UV (MeOH) λ_{max} 285 (5450) and 290 (5650).

1-Methyl-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dione (6) and 2-Methyl-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dione (7). A mixture of **5** (18.30 g, 110.0 mmol), ammonium sulfate (0.4 g), 1,1,1,3,3,3-hexamethyldisilazane (200 mL), and anhydrous pyridine (50 mL) was heated at 135°C for 12 h under argon and evaporated to dryness. The residue was co-evaporated with dry toluene (2 × 100 mL) and dissolved in dry CH_3CN (200 mL). 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (65.59 g, 130.0 mmol) was added and cooled to 0°C under argon. Trimethylsilyl trifluoromethanesulfonate (TMS triflate, 28.1 mL, 158.4 mmol) was added slowly during 30 min. The reaction mixture was stirred at 0°C for 1 h and at room temperature for 12 h. The solution was evaporated to dryness, partitioned between EtOAc (500 mL) and sat. $NaHCO_3$ (300 mL), and extracted with EtOAc. The organic extract was washed with water (200 mL) and brine (200 mL), dried and evaporated to dryness. The residue was purified by flash chromatography over silica gel using $CHCl_3 \rightarrow$ EtOAc as the eluent. Two products (**6** and **7**) were isolated. Yield of **6**: 27.0 g (40%); ^{1}H NMR (DMSO-d_6): δ 4.02 (3H, s, NCH_3), 4.69 (3H, m, C_5'-CH_2 and C_4'-*H*), 6.01 (2H, m, C_2'-*H* and C_3'-*H*), 6.34 (1H, d, C_1'-*H*), 7.40–8.02 (16H, m, Ar*H*), 11.66 (1H, s, N*H*). UV (MeOH) λ_{max} 282 (9200). Anal. calcd. for $C_{32}H_{26}N_4O_9$: C, 62.95; H, 4.29; N, 9.18. Found: C, 63.15; H, 4.33; N, 9.09. HRMS (ESI-Q-tof-2) *m/z* 633.1622 (633.1597 calcd for $C_{32}H_{26}N_4O_9Na$ $[M+Na]^+$). Yield of **7**: 36.93 g (56%); ^{1}H NMR (DMSO-d_6): δ 3.82 (3H, s, NCH_3), 4.66 (3H, m, C_5'-CH_2 and C_4'-*H*), 5.98 (2H, m, C_2'-*H* and C_3'-*H*), 6.28 (1H, d, C_1'-*H*), 7.30–8.12 (16H, m, Ar*H*), 11.38 (1H, s, N*H*). UV (MeOH) λ_{max} 280 (8000). Anal. calcd. for $C_{32}H_{26}N_4O_9$: C, 62.95; H, 4.29; N, 9.18. Found: C, 62.71; H, 4.13; N, 9.29. HRMS (ESI-Q-tof-2) *m/z* 633.1616 (633.1597 calcd for $C_{32}H_{26}N_4O_9Na$ $[M+Na]^+$).

1-Methyl-4-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5,7(1H,6H)-dione (8). Compound **6** (1.00 g, 1.6 mmol) was stirred in methanolic ammonia (saturated at 0°C, 70 mL) in a pressure bottle for 12 h at room temperature. The pressure bottle was cooled to 0°C, opened, and the

content was evaporated to dryness. The residue was triturated with EtOAc (20 mL) and the EtOAc extract was discarded. The residue on trituration with MeOH gave colorless powder, which was filtered. Yield: 0.42 g (86%). ^{1}H NMR (500 MHz; DMSO-d_6): δ 3.66 (2H, s), 3.86 (1H, s), 4.12 (3H, s, NCH_3), 4.30 (1H, s), 5.12–5.30 (3H, m, 3 × OH), 6.04 (1H, s, C_1'-H), 8.08 (1H, s, ArH), 11.54 (1H, s, NH). UV (MeOH) λ_{max} 283 (5200). Anal. calcd. for $C_{11}H_{14}N_4O_6$: C, 44.29; H, 4.73; N, 18.78. Found: C, 44.17; H, 4.41; N, 18.90. HRMS (ESI-Q-tof-2) *m/z* 321.0821 (321.0811 calcd for $C_{11}H_{14}N_4O_6Na$ $[M+Na]^+$).

2-Methyl-4-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5,7(1H,6H)-dione (9). Compound **7** (0.61 g, 1.0 mmol) was stirred in methanolic ammonia (saturated at 0°C, 70 mL) in a pressure bottle for 12 h at room temperature. The pressure bottle was cooled to 0°C, opened, and the content was evaporated to dryness. The residue was triturated with EtOAc (20 mL) and the EtOAc extract was discarded. The residue on trituration with MeOH gave a colorless powder, which was filtered. Yield: 0.27 g (90%). ^{1}H NMR (500 MHz; DMSO-d_6): δ 3.72 (2H, s), 3.86 (1H, s), 4.00 (3H, s, NCH_3), 4.08 (1H, s), 4.28 (1H, s), 5.08–5.36 (3H, m, 3 × OH), 6.02 (1H, s, C_1'-H), 8.25 (1H, s, ArH), 11.30 (1H, s, NH). UV (MeOH) λ_{max} 278 (4850). Anal. calcd. for $C_{11}H_{14}N_4O_6$: C, 44.29; H, 4.73; N, 18.78. Found: C, 44.07; H, 4.71; N, 18.59. HRMS (ESI-Q-tof-2) *m/z* 321.0827 (321.0811 calcd for $C_{11}H_{14}N_4O_6Na$ $[M+Na]^+$).

1-Methyl-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5-oxo-pyrazolo[4,3-d]pyrimidine-7(1H,6H)-thione (10). A mixture of **6** (10.0 g, 16.4 mmol) and P_2S_5 (10.0 g) in anhydrous dioxane (100 ml) was heated at reflux for 12 h under argon and evaporated to dryness. The residue was partitioned between EtOAc (300 mL) and water (200 mL), and extracted with EtOAc. The organic extract was washed with brine (200 mL), dried, and evaporated to dryness. The residue was purified by flash chromatography over silica gel using $CHCl_3$ → EtOAc as the eluent. The pure fraction were pooled and concentrated to dryness to give the titled compound **10** as yellow foam. Yield: 7.00 g (68%). ^{1}H NMR (DMSO-d_6): δ 4.27 (3H, s, NCH_3), 4.71 (3H, m, C_5'-CH_2 and C_4'-H), 6.04 (2H, m, C_2'-H and C_3'-H), 6.35 (1H, d, C_1'-H), 7.40–8.04 (16H, m, ArH), 12.89 (1H, s, NH). UV (MeOH) λ_{max} 266 (4250). Anal. calcd. for $C_{32}H_{26}N_4O_8S$: C, 61.34; H, 4.18; N, 8.94; S, 5.11. Found: C, 61.44; H, 4.31; N, 9.00; S, 5.26. HRMS (ESI-Q-tof-2) *m/z* 649.1354 (649.1369 calcd for $C_{32}H_{26}N_4O_8SNa$ $[M+Na]^+$).

1-Methyl-4-β-D-ribofuranosyl-5-oxo-pyrazolo[4,3-d]pyrimidine-7(1H,6H)-thione (11). Compound **10** (10.0 g, 15.9 mmol) was dissolved in anhydrous pyridine (100 mL) and stirred at room temperature. To this

stirred solution was added anhydrous MeOH (100 mL) followed by 25% wt. NaOMe solution (15 mL) in MeOH. The reaction mixture was stirred at room temperature for 2 h and neutralized with H^+ (Dowex) resin. The resin was filtered and washed with pyridine (50 mL) and MeOH (50 mL). The filtrate was concentrated to dryness. The residue was triturated with MeOH (minimum) and cooled to 0°C. The precipitated solid was filtered and washed with minimum amount of MeOH. Yield: 4.22 g (84%). ^{1}H NMR (DMSO-d_6): δ 3.62 (2H, m, C_5'-CH_2), 3.80 (1H, m), 4.02 (1H, m), 4.27 (3H, s, NCH_3), 5.02 (1H, m, O*H*), 5.10 (1H, m, O*H*), 5.14 (1H, m, O*H*), 6.00 (1H, m, C_1'-*H*), 8.13 (1H, s, Ar*H*), 12.70 (1H, s, N*H*). UV (MeOH) λ_{max} 258 (4650). Anal. calcd. for $C_{11}H_{14}N_4O_5S$: C, 42.04; H, 4.49; N, 17.83; S, 10.18. Found: C, 42.31; H, 4.39; N, 17.66; S, 10.06. HRMS (ESI-Q-tof-2) *m/z* 337.0594 (337.0583 calcd for $C_{11}H_{14}N_4O_5SNa$ $[M+Na]^+$).

1-Methyl-4-*β*-D-ribofuranosyl-5-oxo-7-thiomethyl-pyrazolo[4,3-d]pyrimidine (12). To a stirred solution of **11** (5.00 g, 15.9 mmol) in anhydrous DMF (100 mL) at 0°C was added N,N-diisopropylethylamine (2.58 g, 20.0 mmol). To this stirred solution was added MeI (5.0 mL) and the stirring continued at 0°C for 1 h and at room temperature for 12 h. The reaction mixture was evaporated to dryness. The residue was triturated with MeOH (minimum) and cooled to 0°C. The precipitated solid was filtered and washed with minimum amount of MeOH. Yield: 4.50 g (86%). ^{1}H NMR (DMSO-d_6): δ 2.78 (3H, s, SCH_3), 3.62 (2H, m, C_5'-CH_2), 3.80 (1H, m), 4.04 (1H, m), 4.16 (3H, s, NCH_3), 4.24 (1H, m), 5.04–5.24 (3H, m, 3 × O*H*), 6.06 (1H, d, C_1'-*H*), 8.08 (1H, s, Ar*H*). Anal. calcd. for $C_{12}H_{16}N_4O_5S$: C, 43.90; H, 4.91; N, 17.07; S, 9.75. Found: C, 43.91; H, 4.83; N, 17.26; S, 9.76. HRMS (ESI-Q-tof-2) *m/z* 351.0746 (351.0739 calcd for $C_{12}H_{16}N_4O_5SNa$ $[M+Na]^+$).

2-Methyl-4-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-5-oxo-pyrazolo[4,3-d] pyrimidine-7(1H,6H)-thione (13). A mixture of **7** (18.3 g, 30.0 mmol) and Lawessons' reagent (24.24 g, 60.0 mmol) in anhydrous pyridine (300 mL) was heated at reflux for 12 h under argon and evaporated to dryness. The residue was suspended in EtOAc (300 mL) and water (200 mL) and treated with sat. $NaHCO_3$ solution to pH 7. The aqueous layer was extracted with EtOAc (2 × 150 mL). The organic extract was washed with brine (200 mL), dried, and evaporated to dryness. The residue was purified by flash chromatography over silica gel using $CHCl_3$ → EtOAc as the eluent. The pure fraction were pooled and concentrated to dryness to give the titled compound **13** as yellowish foam. Yield: 18.7 g (99%). ^{1}H NMR (DMSO-d_6): δ 3.84 (3H, s, NCH_3), 4.70 (3H, m, C_5'-CH_2 and C_4'-*H*), 5.98 (2H, m, C_2'-*H* and C_3'-*H*), 6.32 (1H, d, C_1'-*H*), 7.34–8.12 (16H, m, Ar*H*), 12.68 (1H, s, N*H*). UV (MeOH) λ_{max} 262 (4050). Anal. calcd. for $C_{32}H_{26}N_4O_8S$: C, 61.34; H, 4.18; N, 8.94; S, 5.11. Found: C, 61.11; H, 4.39; N, 9.17; S,

5.05. HRMS (ESI-Q-tof-2) *m/z* 649.1360 (649.1369 calcd for $C_{32}H_{26}N_4O_8SNa$ $[M+Na]^+$).

2-Methyl-4-β-D-ribofuranosyl-5-oxo-pyrazolo[4,3-d]pyrimidine-7(1H, 6H)-thione (14). Compound **13** (18.7 g, 29.8 mmol) was dissolved in anhydrous pyridine (100 mL) and stirred at room temperature. To this stirred solution was added anhydrous MeOH (100 mL) followed by 25% wt NaOMe solution (20 mL) in MeOH. The reaction mixture was stirred at room temperature for 2 h and neutralized with H^+ (Dowex) resin. The resin was filtered and washed with pyridine (50 mL) and MeOH (50 mL). The filtrate was concentrated to dryness. The residue was triturated MeOH (minimum) and cooled to 0°C. The precipitated solid was filtered and washed with minimum amount of MeOH. Yield 8.5 g (91%). ^{1}H NMR (DMSO-d_6): δ 3.62 (2H, m, C_5'-CH_2), 3.82 (1H, m), 3.94 (3H, s, NCH_3), 4.00 (1H, m), 4.22 (1H, m), 5.07 (1H, m, O*H*), 5.24 (1H, m, O*H*), 5.27 (1H, m, O*H*), 5.98 (1H, d, C_1'-*H*), 8.21 (1H, s, Ar*H*), 12.58 (1H, s, N*H*). UV (MeOH) λ_{max} 256 (4350). Anal. calcd. for $C_{11}H_{14}N_4O_5S$: C, 42.04; H, 4.49; N, 17.83; S, 10.18. Found: C, 42.07; H, 4.12; N, 17.86; S, 10.23. HRMS (ESI-Q-tof-2) *m/z* 337.0591 (337.0583 calcd for $C_{11}H_{14}N_4O_5SNa$ $[M+Na]^+$).

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-thiomethyl-pyrazolo[4,3-d]pyrimidine (15). To a stirred solution of **14** (8.40 g, 26.7 mmol) in anhydrous DMF (120 mL) at 0°C was added N,N-diisopropylethylamine (3.87 g, 30.0 mmol). To this stirred solution was added MeI (10 mL) and the stirring continued at 0°C for 1 h and at room temperature for 12 h. The reaction mixture was evaporated to dryness. The residue was triturated with MeOH (minimum) and cooled to 0°C. The precipitated solid was filtered and washed with minimum amount of MeOH. Yield: 8.20 g (93%). ^{1}H NMR (DMSO-d_6): δ 2.54 (3H, s, SCH_3), 3.64 (2H, m, C_5'-CH_2), 3.82 (1H, m), 3.98 (3H, s, NCH_3), 4.02 (1H, m), 4.20 (1H, m), 5.06 (1H, d, O*H*), 5.20 (2H, m, 2 × O*H*), 6.04 (1H, d, C_1'-*H*), 8.20 (1H, s, Ar*H*). Anal. calcd. for $C_{12}H_{16}N_4O_5S$: C, 43.90; H, 4.91; N, 17.07; S, 9.75. Found: C, 43.84; H, 4.97; N, 17.31; S, 9.56. HRMS (ESI-Q-tof-2) *m/z* 351.0756 (351.0739 calcd for $C_{12}H_{16}N_4O_5SNa$ $[M+Na]^+$).

1-Methyl-4-(5)′-*O*-monomethoxytrityl-polystyrene Resin-β-D-ribofuranosyl)-5-oxo-7-thiomethyl-pyrazolo[4,3-d]pyrimidine (16). Scaffold B. To a suspension of polystyrene monomethoxytrityl chloride resin (7.5 g, 1.8 mmol/g) in anhydrous DMF (30 mL) was added compound **12** (5.02 g, 15.8 mmol) followed by 2,6-lutidine (3.68 g, 33.6 mmol). The reaction mixture was shaken well at room temperature for 3 days under an inert atmosphere. The resin was quenched with MeOH (5 mL) and filtered, and sequentially washed with anhydrous DMF (3 × 20 mL), CH_2Cl_2 (3 × 20 mL), MeOH

(3×20 mL), and CH_2Cl_2 (3×20 mL). The resin was dried under vacuum over solid KOH for 12 h. The loading efficiency (66%) was calculated based on increase in weight of the resin. FT-IR (KBr) υ 1701 (C=O), 1248 (S-CH_3) cm^{-1}.

2-Methyl-4-(5)′-*O*-monomethoxytrityl-polystyrene Resin-β-D-ribofuranosyl)-5-oxo-7-thiomethyl-pyrazolo[4,3-d]pyrimidine (17). Scaffold C. To a suspension of polystyrene monomethoxytrityl chloride resin (11.11 g, 1.8 mmol/g) in anhydrous DMF (25 mL) was added compound **15** (7.90 g, 24.1 mmol) followed by 2,6-lutidine (12.89 g, 120.5 mmol). The reaction mixture was shaken well at room temperature for 4 days under an inert atmosphere. The resin was quenched with MeOH (10 mL) and filtered, and sequentially washed with anhydrous DMF (3×20 mL), CH_2Cl_2 (3×20 mL), MeOH (3×20 mL) and CH_2Cl_2 (3×20 mL). The resin was dried under vacuum over solid KOH for 12 h. The loading efficiency (74%) was calculated based on increase in weight of the resin. FT-IR (KBr) υ 1700 (C=O), 1249 (S-CH_3) cm^{-1}.

General Procedure for the Synthesis of Pyrazolopyrimidine Libraries

Approximately (50 mg) of the resin **16** was dispensed in each of the 96 reaction wells using a spatula and funnel. The respective amines (1.5 M in NMP, 1.5 mL) were added to the corresponding wells. The reaction flask was covered and placed in the synthesizer. The reaction conditions were programmed and turned on. The reaction block was heated at 80°C with shaking for 24 h. The reaction block was cooled to room temperature and the solvent was drained. The resin was washed with DMF (3×1 mL), MeOH (3×1 mL) and CH_2Cl_2 (3×1 mL), and dried under nitrogen.

Cleavage of the Nucleosides from Resin

A 30% solution of HFIP in CH_2Cl_2 (1.5 mL) was added to the resin in each well. The reaction block was closed and shaken with heating at 50°C for 24 h. The 96 reaction wells were filtered into 96 pre-labeled and pre-weighed vials in 96 well format. The 96 wells were washed with MeOH (1.00 mL) and filtered into the corresponding 96 wells. The filtrates were concentrated under vacuum using Savant SpeedVac to dryness to provide 12–20 mg of products.

^{1}H NMR and mass spectral data of the selected library members are given below:

1-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(2,2-dimethoxy)ethylamino)]-pyrazolo[4,3-d]pyrimidine (B1). ^{1}H NMR (CD_3OD): δ 3.40–3.60 (5H, m),

3.64–3.84 (6H, m), 3.92–4.18 (5H, m), 4.22 (1H, m), 4.52 (1H, m), 6.07 (1H, d, *J* 6.6, C_1'-*H*), 7.93 (1H, m, Ar*H*); MS (ESI) *m/z* 386 $(M+H)^+$.

1-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(2-methoxy)ethylamino)]-pyrazolo[4,3-d]pyrimidine (B8). ^{1}H NMR (CD_3OD): δ 3.30 (2H, m), 3.38 (3H, s), 3.65 (2H, m), 3.81 (2H, m), 3.98 (1H, m), 4.18 (3H, s), 4.24 (1H, m), 4.58 (1H, m), 6.06 (1H, d, C_1'-*H*), 7.90 (1H, s, Ar*H*); MS (ESI) *m/z* 356 $(M+H)^+$.

1-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[2-(1-methylpyrrolidin-2-yl)ethylamino)]-Pyrazolo[4,3-d]pyrimidine (B13). ^{1}H NMR (CD_3OD): δ 1.70–2.20 (4H, m), 2.60 (3H, s), 2.78 (1H, m), 3.30 (2H, m), 3.42 (1H, m), 3.62 (2H, m), 3.82 (2H, m), 4.00 (1H, m), 4.18 (3H, s), 4.24 (1H, m), 4.56 (1H, m), 6.07 (1H, d, C_1'-*H*), 7.92 (1H, s, Ar*H*); MS (ESI) *m/z* 409 $(M+H)^+$.

1-Methyl-4-β-D-ribofuranosyl-5-oxo-7-(cyclopropylamino)-pyrazolo[4,3-d]pyrimidine (B17). ^{1}H NMR (CD_3OD): δ 0.74 (2H, m), 0.86 (2H, m), 3.78 (2H, m), 3.98 (1H, m), 4.04 (1H, m), 4.14 (3H, s), 4.24 (1H, m), 4.58 (1H, m), 6.40 (1H, d, C_1'-*H*), 7.92 (1H, s, Ar*H*); MS (ESI) *m/z* 338 $(M+H)^+$.

1-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(pyridine-4-yl)methylamino]-pyrazolo[4,3-d]pyrimidine (B32). ^{1}H NMR (CD_3OD): δ 3.80 (4H, m), 3.98 (1H, m), 4.22 (4H, m), 4.56 (1H, m), 6.06 (1H, d, *J* 6.6, C_1'-*H*), 7.50 (1H, m, Ar*H*), 7.92 (1H, s, Ar*H*), 8.08 (1H, d, Ar*H*), 8.46 (1H, d, Ar*H*), 8.69 (1H, s, Ar*H*); MS (ESI) *m/z* 389 $(M+H)^+$.

1-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[2-(N,N-diethylamino)ethylamino]-pyrazolo[4,3-d]pyrimidine (B43). ^{1}H NMR (CD_3OD): δ 1.10 (6H, m), 2.72 (4H, m), 2.83 (2H, m), 3.60–3.80 (4H, m), 3.98 (1H, m), 4.18 (3H, s), 4.24 (1H, m), 4.56 (1H, m), 6.07 (1H, d, *J* 6.6, C_1'-*H*), 7.90 (1H, s, Ar*H*); MS (ESI) *m/z* 397 $(M+H)^+$.

1-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[3-(N,N-dimethylamino)propylamino]-pyrazolo[4,3-d]pyrimidine (B55). ^{1}H NMR (CD_3OD): δ 1.95 (2H, m), 2.46 (6H, s), 2.69 (2H, m), 3.65 (2H, m), 3.80 (2H, m), 3.99 (1H, m), 4.18 (3H, s), 4.24 (1H, m), 4.56 (1H, m), 6.07 (1H, d, *J* 6.6, C_1'-*H*), 7.92 (1H, s, Ar*H*); MS (ESI) *m/z* 383 $(M+H)^+$.

1-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[3-(hydroxy)propylamino]-pyrazolo[4,3-d]pyrimidine (B93). ^{1}H NMR (CD_3OD): δ 1.85 (2H, m), 3.05 (2H, m), 3.68 (4H, m), 3.98 (1H, m), 4.18 (3H, s), 4.25 (1H, m), 4.54 (1H, m), 6.06 (1H, d, C_1'-*H*), 7.92 (1 H, s, Ar*H*); MS (ESI) *m/z* 356 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(2-hydroxy)ethylamino]-pyrazolo [4,3-d]pyrimidine (C5). ^{1}H NMR (CD_3OD): δ 3.64 (2H, m), 3.76 (2H, m), 3.82 (2H, m), 3.98 (4H, m), 4.22 (1H, m), 4.46 (1H, m), 6.07 (1H, d, *J* 6.9, C_1'-*H*), 8.06 (1H, s, Ar*H*); MS (ESI) *m/z* 342 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(2,3-dihydroxy)propylamino]-pyrazolo[4,3-d]pyrimidine (C7). ^{1}H NMR (CD_3OD): δ 3.50–3.80 (4H, m), 3.82 (3H, m), 3.99 (4H, m), 4.22 (1H, m), 4.45 (1H, m), 6.09 (1H, d, *J* 6.6, C_1'-*H*), 8.10 (1H, s, Ar*H*); MS (ESI) *m/z* 372.45 [372 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-(cyclopropylamino)-pyrazolo[4,3-d]pyrimidine (C17). ^{1}H NMR (CD_3OD): δ 0.68 (2H, m), 0.86 (2H, m), 3.04 (1H, m), 3.82 (2H, m), 3.98 (4H, m), 4.22 (1H, m), 4.46 (1H, m), 6.09 (1H, d, *J* 6.6, C_1'-*H*), 8.06 (1H, s, Ar*H*); MS (ESI) *m/z* 338 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[3-(imidazol-1-yl)propylamino]-pyrazolo[4,3-d]pyrimidine (C26). ^{1}H NMR (CD_3OD): δ 2.18 (2H, m), 3.58 (2H, m), 3.82 (2H, m), 3.98 (1H, m), 4.01 (3H, s), 4.14 (2H, m), 4.23 (1H, m), 4.44 (1H, m), 6.09 (1H, d, C_1'-*H*), 6.98 (1H, s, Im*H*), 7.22 (1H, s, Im*H*), 7.80 (1H, s, Im*H*), 8.08 (1H, s, Ar*H*); MS (ESI) *m/z* 406 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(3-methyl)benzylamino)-pyrazolo [4,3-d]pyrimidine (C37). ^{1}H NMR (CD_3OD): δ 2.31 (3H, s), 3.81 (2H, m), 3.97 (1H, m), 4.01 (3H, s), 4.22 (1H, m), 4.46 (1H, m), 4.72 (2H, m), 6.09 (1H, d, *J* 6.6, C_1'-*H*), 7.00–7.22 (4H, m, Ph*H*), 8.08 (1H, s, Ar*H*); MS (ESI) *m/z* 402 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[2-(N,N-diethylamino)ethylamino]-pyrazolo[4,3-d]pyrimidine (C43). ^{1}H NMR (CD_3OD): δ 1.52 (6H, m), 2.74–3.00 (6H, m), 3.72 (2H, m), 3.82 (2H, m), 3.99 (1H, m), 4.01 (3H, s), 4.22 (1H, m), 4.44 (1H, m), 6.08 (1H, d, C_1'-*H*), 8.08 (1H, s, Ar*H*); MS (ESI) *m/z* 397 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(furan-2-yl)methylamino]-pyrazolo[4,3-d]pyrimidine (C45). ^{1}H NMR (CD_3OD): δ 3.82 (2H, m), 3.98 (1H, m), 4.01 (3H, s), 4.24 (1H, m), 4.44 (1H, m), 4.74 (2H, m), 6.11 (1H, d, *J* 6.6, C_1'-*H*), 6.35 (2H, s, Ar*H*), 7.42 (1H, s, Ar*H*), 8.09 (1H, s, Ar*H*); MS (ESI) *m/z* 378 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(pyridine-4-yl)methylamino]-pyrazolo[4,3-d]pyrimidine (C47). ^{1}H NMR (CD_3OD): δ 3.82 (2H, m), 4.02 (4H, m), 4.46 (1H, m), 6.08 (1H, d, *J* 6.6, C_1'-*H*), 7.53 (2H, br s, Py*H*), 8.12 (1H, s, Ar*H*), 8.36–8.80 (2H, m, Py*H*); MS (ESI) *m/z* 389 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(2-hydroxy)propylamino]-pyrazolo[4,3-d]pyrimidine (C53). ^{1}H NMR (CD_3OD): δ 1.20 (3H, m), 3.44 (2H, m), 3.58 (1H, m), 3.82 (2H, m), 3.96–4.04 (3H, m), 4.23 (1H, m), 4.45 (1H, m), 6.08 (1H, d, C_1'-*H*), 8.10 (1H, s, Ar*H*); MS (ESI) *m/z* 356 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(4-methyl)benzylamino]-pyrazolo[4,3-d]pyrimidine (C59). ^{1}H NMR (CD_3OD): δ 2.29 (3H, s), 3.82 (2H, m), 3.99 (4H, m), 4.22 (1H, m), 4.48 (1H, m), 4.70 (2H, m), 6.09 (1H, d, *J* 6.6, C_1'-*H*), 7.10 (2H, d, Ph*H*), 7.25 (2H, d, Ph*H*), 8.06 (1H, s, Ar*H*); MS (ESI) *m/z* 402 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(4-hydroxy)butylamino]-pyrazolo[4,3-d]pyrimidine (C92). ^{1}H NMR (CD_3OD): δ 1.62 (2H, m), 1.73 (2H, m), 3.60 (4H, m), 3.82 (2H, m), 3.98 (4H, m), 4.22 (1H, m), 4.45 (1H, m), 6.07 (1H, d, *J* 6.6, C_1'-*H*), 8.06 (1H, s, Ar*H*); MS (ESI) *m/z* 370 $(M+H)^+$.

2-Methyl-4-β-D-ribofuranosyl-5-oxo-7-[(3-hydroxy)propylamino]-pyrazolo[4,3-d]pyrimidine (C93). ^{1}H NMR (CD_3OD): δ 1.84 (2H, m), 3.63 (4H, m), 3.82 (2H, m), 3.98 (1H, m), 4.00 (3H, s), 4.22 (1H, m), 4.45 (1H, m), 4.61 (2H, m), 6.06 (1H, d, C_1'-*H*), 8.06 (1H, s, Ar*H*); MS (ESI) *m/z* 356 $(M+H)^+$.

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18. **B1-B96** are the corresponding products obtained by the reaction 96 amines (Figure 3) with scaffold **B**. **C1-C96** are the corresponding products obtained by the reaction 96 amines (Figure 3) with scaffold **C**. **1-96** represents the corresponding amines from Figure 3.